ISSN 1948-5204 (online)

World Journal of *Gastrointestinal Oncology*

World J Gastrointest Oncol 2015 November 15; 7(11): 263-374

Published by Baishideng Publishing Group Inc

WJGO World Journal of Gastrointestinal Oncology

A peer-reviewed, online, open-access journal of gastrointestinal oncology

Editorial Board

2011-2015

The *World Journal of Gastrointestinal Oncology* Editorial Board consists of 428 members, representing a team of worldwide experts in gastrointestinal oncology. They are from 40 countries, including Argentina (2), Australia (10), Belgium (5), Brazil (2), Canada (4), Chile (2), China (56), Czech Republic (1), Denmark (1), Finland (3), France (7), Germany (24), Greece (13), Hungary (2), India (9), Iran (2), Ireland (2), Israel (4), Italy (41), Japan (47), Kuwait (2), Mexico (1), Netherlands (7), New Zealand (2), Norway (1), Poland (3), Portugal (5), Romania (1), Saudi Arabia (1), Serbia (2), Singapore (4), South Korea (27), Spain (10), Sweden (5), Switzerland (2), Syria (1), Thailand (1), Turkey (6), United Kingdom (15), and United States (95).

Siu Tsan Yuen, *Hong Kong*
Wei-Hui Zhang, *Harbin*
Li Zhou, *Beijing*
Yong-Ning Zhou, *Lanzhou*

Czech Republic

Ondrej Slaby, *Brno*

Denmark

Hans Jørgen Nielsen, *Hvidovre*

Finland

Riyad Bendardaf, *Turku*
Pentti Ilmari Sipponen, *Espoo*
Markku Voutilainen, *Jyväskylä*

France

Bouvier Anne-Marie, *Cedex*
Stéphane Benoist, *Boulogne*
Ouaissi Mehdi, *Marseille*
Jean-François Rey, *Jean-François Rey*
Karem Slim, *Clermont-Ferrand*
David Tougeron, *Poitiers*
Isabelle Van Seuningen, *Lille*

Germany

Hajri Amor, *Freiburg*
Han-Xiang An, *Marburg*
Karl-Friedrich Becker, *München*
Stefan Boeck, *Munich*
Dietrich Doll, *Marburg*
Joachim Drevs, *Freiburg*
Volker Ellenrieder, *Marburg*
Ines Gütgemann, *Bonn*
Jakob Robert Izbicki, *Hamburg*
Gisela Keller, *München*
Jörg H Kleeff, *Munich*
Axel Kleespies, *Munich*
Hans-Joachim Meyer, *Solingen*
Lars Mueller, *Kiel*
Martina Müller-Schilling, *Heidelberg*
Joachim Pfannschmidt, *Heidelberg*
Marc André Reymond, *Bielefeld*
Robert Rosenberg, *München*
Ralph Schneider, *Marburg*
Helmut K Seitz, *Heidelberg*
Nikolas Hendrik Stoecklein, *Düsseldorf*
Oliver Stoeltzing, *Mainz*
Ludwig G Strauss, *Heidelberg*

Greece

Ekaterini Chatzaki, *Alexandroupolis*
Eelco de Bree, *Heraklion*
Maria Gazouli, *Athens*
Vassilis Georgoulias, *Heraklion*
John Griniatsos, *Athens*
Ioannis D Kanellos, *Thessaloniki*
Vaios Karanikas, *Larissa*
Georgios Koukourakis, *Athens*
Michael I Koukourakis, *Alexandroupolis*
Gregory Kouraklis, *Athens*
Kostas Syrigos, *Athens*
Ioannis A Voutsadakis, *Larissa*

Hungary

László Herszényi, *Budapest*
Zsuzsa Schaff, *Budapest*

India

Uday Chand Ghoshal, *Lucknow*
Ruchika Gupta, *New Delhi*
Kalpesh Jani, *Vadodara*
Ashwani Koul, *Chandigarh*
Balraj Mittal, *Lucknow*
Rama Devi Mittal, *Lucknow*
Susanta Roychoudhury, *Kolkata*
Yogeshwer Shukla, *Lucknow*
Imtiaz Ahmed Wani, *Kashmir*

Iran

Reza Malekezdeh, *Tehran*
Mohamad Amin Pourhoseingholi, *Tehran*

Ireland

Aileen Maria Houston, *Cork*
Colm Ó'Moráin, *Dublin*

Israel

Nadir Arber, *Tel Aviv*
Eytan Domany, *Rehovot*
Dan David Hershko, *Haifa*
Yaron Niv, *Patch Tikva*

Italy

Massimo Aglietta, *Turin*
Domenico Alvaro, *Rome*
Azzariti Amalia, *Bari*
Marco Braga, *Milan*
Federico Cappuzzo, *Rozzano*
Lorenzo Capussotti, *Torino*
Fabio Carboni, *Rome*
Vincenzo Cardinale, *Rome*
Luigi Cavanna, *Piacenza*
Massimo Colombo, *Milan*
Valli De Re, *Pordenone*
Ferdinando De Vita, *Naples*
Riccardo Dolcetti, *Aviano*
Pier Francesco Ferrucci, *Milano*
Francesco Fiorica, *Ferrara*
Gennaro Galizia, *Naples*
Silvano Gallus, *Milano*
Milena Gusella, *Trecenta*
Carlo La Vecchia, *Milano*
Roberto Francesco Labianca, *Bergamo*
Massimo Libra, *Catania*
Roberto Manfredi, *Bologna*
Gabriele Masselli, *Viale del Policlinico*
Simone Mocellin, *Padova*
Gianni Mura, *Arezzo*
Gerardo Nardone, *Napoli*
Gabriella Nesi, *Florence*
Francesco Perri, *San Giovanni Rotondo*
Francesco Recchia, *Avezzano*
Vittorio Ricci, *Pavia*
Fabrizio Romano, *Monza*
Antonio Russo, *Palermo*
Daniele Santini, *Rome*
Claudio Sorio, *Verona*
Cosimo Sperti, *Padova*
Gianni Testino, *Genova*
Giuseppe Tonini, *Rome*
Bruno Vincenzi, *Rome*
Zoli Wainer, *Forlì*
Angelo Zullo, *Rome*

Japan

Suminori Akiba, *Kagoshima*
Keishiro Aoyagi, *Kurume*
Narikazu Boku, *Shizuoka*
Yataro Daigo, *Tokyo*
Itaru Endo, *Yokohama*
Mitsuhiro Fujishiro, *Tokyo*
Osamu Handa, *Kyoto*
Kenji Hibi, *Yokohama*
Asahi Hishida, *Nagoya*
Eiso Hiyama, *Hiroshima*
Atsushi Imagawa, *Okayama*
Johji Inazawa, *Tokyo*
Terumi Kamisawa, *Tokyo*
Tatsuo Kanda, *Niigata*
Masaru Katoh, *Tokyo*
Takayoshi Kiba, *Hyogo*
Hajime Kubo, *Kyoto*
Hiroki Kuniyasu, *Kashihara*
Yukinori Kurokawa, *Osaka*
Chihaya Maesawa, *Morioka*
Yoshinori Marunaka, *Kyoto*
Osam Mazda, *Kyoto*
Shinichi Miyagawa, *Matsumoto*
Eiji Miyoshi, *Suita*
Toshiyuki Nakayama, *Nagasaki*
Masahiko Nishiyama, *Saitama*
Koji Oba, *Kyoto*
Masayuki Ohtsuka, *Chiba*
Masao Seto, *Aichi*
Tomoyuki Shibata, *Aichi*
Mitsugi Shimoda, *Tochigi*
Haruhiko Sugimura, *Hamamatsu*
Tomomitsu Tahara, *Aichi*
Shinji Takai, *Osaka*
Satoru Takayama, *Nagoya*
Akio Tomoda, *Tokyo*
Akihiko Tsuchida, *Tokyo*
Yasuo Tsuchiya, *Niigata*
Takuya Watanabe, *Niigata*
Toshiaki Watanabe, *Tokyo*
Yo-ichi Yamashita, *Hiroshima*
Hiroki Yamaue, *Wakayama*
Hiroshi Yasuda, *Kanagawa*
Hiroshi Yokomizo, *Kumamoto*
Yutaka Yonemura, *Osaka*
Reigetsu Yoshikawa, *Hyogo*

Kuwait

Fahd Al-Mulla, *Safat*

Salem Alshemmari, *Safat*

Mexico

Oscar G Arrieta Rodriguez, *Mexico City*

Netherlands

Jan Paul De Boer, *Amsterdam*
Bloemena Elisabeth, *Bloemena Elisabeth*
Peter JK Kuppen, *Leiden*
Gerrit Albert Meijer, *Amsterdam*
Anya N Milne, *Utrecht*
Godefridus J Peters, *Amsterdam*
Cornelis FM Sier, *Leiden*

New Zealand

Lynnette Robin Ferguson, *Auckland*
Jonathan Barnes Koea, *Auckland*

Norway

Kjetil Søreide, *Stavanger*

Poland

Andrzej Szkaradkiewicz, *Poznan*
Michal Tenderenda, *Polskiego*
Jerzy Wydmański, *Gliwice*

Portugal

Maria de Fátima Moutinho Gärtner, *Porto*
Celso Albuquerque Reis, *Porto*
Lucio Lara Santos, *Porto*
Maria Raquel Campos Seruca, *Porto*
Manuel António Rodrigues Teixeira, *Porto*

Romania

Marius Raica, *Timisoara*

Saudi Arabia

Ragab Hani Donkol, *Abha*

Serbia

Milos M Bjelovic, *Belgrade*
Goran Zoran Stanojevic, *Nis*

Singapore

Peh Yean Cheah, *Singapore*
Si-Shen Feng, *Singapore*
Zhi-Wei Huang, *Singapore*
Qi Zeng, *Singapore*

South Korea

Seungmin Bang, *Seoul*
Daeho Cho, *Seoul*
Byung Ihn Choi, *Seoul*
Hyun Cheol Chung, *Seoul*
Sang-Uk Han, *Suwon*
Jun-Hyeog Jang, *Incheon*
Seong Woo Jeon, *Daegu*
Dae Hwan Kang, *Mulgeum-Gigu*
Gyeong Hoon Kang, *Seoul*
Dong Yi Kim, *Gwangju*
Jae J Kim, *Seoul*
Jin Cheon Kim, *Seoul*
Jong Gwang Kim, *Daegu*
Min Chan Kim, *Busan*
Samyong Kim, *Daejeon*
Inchul Lee, *Seoul*
Jung Weon Lee, *Seoul*
Kyu Taek Lee, *Seoul*
Kyung Hee Lee, *Daegu*
Na Gyong Lee, *Seoul*
Suk Kyeong Lee, *Seoul*
Jong-Baeck Lim, *Seoul*
Young Joo Min, *Ulsan*
Sung-Soo Park, *Seoul*
Young Kee Shin, *Seoul*
Hee Jung Son, *Seoul*
Si Young Song, *Seoul*

Spain

Manuel Benito, *Madrid*
Ignacio Casal, *Madrid*
Antoni Castells, *Barcelona*
Jose JG Marin, *Salamanca*
Joan Maurel, *Barcelona*
Emma Folch Puy, *Barcelona*
Jose Manuel Ramia, *Guadalajara*
Margarita Sanchez-Beato, *Madrid*
Laura Valle, *Barcelona*
Jesus Vioque, *San Juan*

Sweden

Nils Albiin, *Stockholm*
Samuel Lundin, *Göteborg*
Haile Mahteme, *Uppsala*
Richard Palmqvist, *Umea*
Ning Xu, *Lund*

Switzerland

Paul M Schneider, *Zurich*
Luigi Tornillo, *Basel*

Syria

Zuhir Alshehabi, *Lattakia*

Thailand

Sopit Wongkham, *Khon Kaen*

Turkey

Uğur Coşkun, *Ankara*
Sukru Mehmet Erturk, *Istanbul*
Vedat Goral, *Diyarbakir*
Yavuz Selim Sari, *Istanbul*
Mesut Tez, *Ankara*
Murat H Yener, *Istanbul*

United Kingdom

Shrikant Anant, *Oklahoma City*
Runjan Chetty, *Scotland*
Chris Deans, *Edinburgh*
Dipok Kumar Dhar, *London*
Thomas Ronald Jeffry Evans, *Glasgow*
Giuseppe Garcea, *Leicester*
Oleg Gerasimenko, *Liverpool*
Neena Kalia, *Birmingham*
Anthony Maraveyas, *East Yorkshire*
Andrew Maw, *North Wales*
Kymberley Thorne, *Swansea*
Chris Tselepis, *Birmingham*
Nicholas Francis Scot Watson, *Nottingham*
Ling-Sen Wong, *Coventry*
Lu-Gang Yu, *Liverpool*

United States

Mohammad Reza Abbaszadegan, *Phoenix*
Gianfranco Alpini, *Temple*
Seung Joon Baek, *Knoxville*
Jamie S Barkin, *Miami Beach*
Carol Bernstein, *Arizona*
Paolo Boffetta, *New York*
Kimberly Maureen Brown, *Kansas City*
De-Liang Cao, *Springfield*
Weibiao Cao, *Providence*
Chris N Conteas, *Los Angeles*
Pelayo Correa, *Nashville*
Joseph John Cullen, *JCP*
James Campbell Cusack, *Boston*
Ananya Das, *Scottsdale*
Juan Dominguez-Bendala, *Miami*
Wafik S El-Deiry, *Philadelphia*
Laura Elnitski, *Rockville*
Guy Douglas Eslick, *Boston*
Thomas Joseph Fahey III, *New York*
James W Freeman, *San Antonio*
Bruce Joseph Giantonio, *Philadelphia*
Ajay Goel, *Dallas*
Karen Gould, *Omaha*
Nagana Gowda A Gowda, *West Lafayette*
Stephen Randolph Grobmyer, *Florida*
Young S Hahn, *Charlottesville*
John W Harmon, *Maryland*
Paul J Higgins, *New York*
Steven Norbit Hochwald, *Gainesville*
Jason L Hornick, *Boston*
Qin Huang, *Duarte*
Su-Yun Huang, *Houston*
Jamal A Ibdah, *Columbia*
Yihong Jiang-Cao Kaufmann, *Little Rock*
Temitope Olubunmilayo Keku, *Chapel Hill*
Saeed Khan, *Silver Spring*
Vijay Pranjivan Khatri, *Sacramento*

WJGO World Journal of Gastrointestinal Oncology

Contents

Monthly Volume 7 Number 11 November 15, 2015

World Journal of Gastrointestinal Oncology
Volume 7 Number 11 November 15, 2015

Contents

NAME OF JOURNAL
World Journal of Gastrointestinal Oncology

ISSN
ISSN 1948-5204 (online)

LAUNCH DATE
October 15, 2009

FREQUENCY
Monthly

EDITORIAL OFFICE
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Gastrointestinal Oncology
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLICATION DATE
November 15, 2015

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/2222-0682/g_info_20100722180909.htm.

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Gastrointestinal Oncology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4251/wjgo.v7.i11.263

World J Gastrointest Oncol 2015 November 15; 7(11): 263-270
ISSN 1948-5204 (online)

EDITORIAL

Novel therapy for advanced gastric cancer

Yue Zhang, Shenhong Wu

Yue Zhang, Shenhong Wu, Division of Hematology and Oncology, Stony Brook University, Stony Brook, NY 11794-8151, United States

Author contributions: Zhang Y and Wu S contributed the same to this paper.

Conflict-of-interest statement: None to declare.

Correspondence to: Yue Zhang, MD, MPH, Assistant Professor, Division of Hematology and Oncology, Stony Brook University, HSC 15-040, 101 Nicholls Rd, Stony Brook, NY 11794-8151, United States. yue.zhang@stonybrookmedicine.edu
Telephone: +1-631-6381000
Fax: +1-631-6380915

Received: June 18, 2015
Peer-review started: June 20, 2015
First decision: July 27, 2015
Revised: August 14, 2015
Accepted: September 16, 2015
Article in press: September 18, 2015
Published online: November 15, 2015

Abstract

Gastric cancer (GC) is a common lethal malignancy. Gastroesophageal junction and gastric cardia tumors are the fastest rising malignancies due to increasing prevalence of obesity and acid reflex in the United States. Traditional chemotherapy remains the main treatment with trastuzumab targeting human epidermal growth factor receptor 2 positive disease. The median overall survival (OS) is less than one year for advanced GC patients; thus, there is an urgent unmet need to develop novel therapy for GC. Although multiple targeted agents were studied, only the vascular endothelial growth factor receptor inhibitor ramucirumab was approved recently by the United States Food and Drug Administration because of its 1.4 mo OS benefit (5.2 mo *vs* 3.8 mo, $P = 0.047$) as a single agent; 2.2 mo improvement of survival (9.6 mo *vs* 7.4 mo, $P = 0.017$) when combined with paclitaxel in previously treated advanced GC patients. It is the first single agent approved for previously treated GC and the second biologic agent after trastuzumab. Even with limited success, targeted therapy may be improved by developing new biomarkers. Immune therapy is changing the paradigm of cancer treatment and is presently under active investigation for GC in clinical trials. More evidence supports GC stem cells existence and early stage studies are looking for its potential therapeutic possibilities.

Key words: Gastric cancer; Novel therapy; Targeted therapy; Immune therapy; Gastric cancer stem cell

Core tip: Advanced gastric cancer (GC) has very poor outcome with chemotherapy remains the main treatment. There is an urgent unmet need to develop novel therapy for GC. Limited success is achieved for targeted therapy after trastuzumab for human epidermal growth factor receptor 2 positive disease. Ramucirumab was recently approved by Food and Drug Administration as a single agent or combined with paclitaxel in refractory advanced GC patients. Immune therapy and GC stem cell research are on the horizon.

Zhang Y, Wu S. Novel therapy for advanced gastric cancer. *World J Gastrointest Oncol* 2015; 7(11): 263-270 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i11/263.htm
DOI: http://dx.doi.org/10.4251/wjgo.v7.i11.263

INTRODUCTION

Gastric cancer (GC) is a common malignancy and the second leading cause of cancer death worldwide[1]. In the United States, there were approximately 22220 new cases and 10990 death in 2014[2]. With overweight and obesity being a more serious epidemiologic issue in the United States, gastroesophageal junction and gastric cardia adenocarcinoma have been the fastest rising cancer. Majority of GCs are present at advanced stages with either metastatic or extensive local/regional disease. It is a group of heterogeneous diseases with different anatomy, epidemiology, etiology, pathogenesis, and behavior. Chemotherapy using fluoropyrimidine or platinum as backbone is the main treatment for advanced GCs. The median survival is limited to 7 to 12 mo in clinical trial setting[3,4]. There is an urgent demand for new therapy to improve its treatment and outcome.

DIFFICULTY AND PROGRESS IN TARGETED THERAPY

Targeted therapy has been the main focus in clinical trials, even though majority of the targeted agents were tested in an unselected "off target" patient population and there was a lacking of biomarkers. It has led to the failure of multiple large phase Ⅲ clinical trials in different pathways. Trastuzumab is approved for human epidermal growth factor receptor 2 (HER2) positive GCs. Ramucirumab has recently gained its label as a single agent or in combination with paclitaxel for refractory GCs patients following fluoropyrimidine or platinum containing chemotherapy.

Epidermal growth factor receptor targeting therapy

Epidermal growth factor receptor (EGFR) has been studied extensively. EXPAND and REAL 3 are the two recent phase Ⅲ clinical trials with EGFR antibodies: cetuximab and panitumumab. Both of them failed to show survival benefit and were concerning for worse toxicity in the EGFR inhibitor study arms. In the EXPAND trial, median progression-free survival (PFS) (4.4 mo *vs* 5.6 mo, $P = 0.32$) and overall survival (OS) (9.4 mo *vs* 10.7 mo, $P = 0.95$) favored the chemotherapy only group, overall response rates (RR) were similar 30% *vs* 29%[5]. Grade 3-4 toxicities were substantially higher in the cetuximab-containing regimen than in the control regimen[5]. REAL 3 trial demonstrated inferior OS in the panitumumab study group when compared to control group (11.3 mo *vs* 8.8 mo, $P = 0.013$) with more toxicities[6]. Biomarker was not used to select patient in both studies. Only 6% screened patients were positive for Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation, a potential association of benefit was found in KRAS mutated group although not significant[6]. This result is contrary to KRAS mutated colon cancer[7].

Phosphatidylinositol 3-kinase /Akt/ mammalian target of rapamycin targeting therapy

The phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin signaling pathway was studied with everolimus in 656 previous treated advanced GC patients in a phase Ⅲ trial: GRANITE-1. Primary endpoint was not reached (OS: 5.4 mo *vs* 4.3 mo, $P = 0.12$), even though PFS was improved (1.7 mo *vs* 1.4 mo, $P < 0.001$)[8]. No biomarker was required for this study entry.

HER2 targeting therapy

HER2 overexpression by immunohistochemistry or gene amplification by fluoresecnence *in situ* hybridization was required for patients' recruitment for the phase Ⅲ ToGA trial. This pivotal trial led to trastuzumab approval with all the outcomes better in the study group (median OS: 13.8 mo *vs* 11.1 mo, $P = 0.0046$; PFS: 6.7 mo *vs* 5.5 mo, $P = 0.0002$; RR: 47% *vs* 35%, $P = 0.0017$)[9]. A post-hoc analysis grouped HER2 status and suggested that larger survival benefit in patients with tumor HER 2 IHC 3+ or 2+ and FISH positive group (OS: 16.0 mo *vs* 11.8 mo, $P = 0.036$)[9]. Lapatinib is a dual tyrosine kinase inhibitor (TIK) inhibitor of HER2 and EGFR. It failed to meet OS benefit in two large phase Ⅲ trials: TRIO-013/Logic in the first line and TyTan in the second line settings (TRIO-013/Logic: 12.2 mo *vs* 10.5 mo, $P = 0.35$; TyTan: 11.0 mo *vs* 8.9 mo, $P = 0.1044$)[10,11]. Lapatinib failure in GC trials might partially relate to its EGFR inhibition effect. Pertuzumab is another humanized monoclonal antibody that binds HER2. Its combination with trastuzumab and chemotherapy is established as first line treatment for metastatic HER2 positive breast cancer[12]. This combination is being evaluated in a phase Ⅲ clinical trial for HER 2 positive advanced GCs (NCT01774786). Trastuzumab emtansine (T-DM1) is an antibody–drug conjugate with monoclonal antibody trastuzumab lined to cytotoxic agent emtansine. A randomized phase Ⅲ trial is ongoing with T-DM1 *vs* taxane for previously treated advanced GCs (NCT01641939).

Antiangiogenic pathway targeting therapy

Vascular endothelial growth factor (VEGF) pathway (angiogenesis) is of great interest in advanced GCs with recent success in ramucirumab, although VEGF-A neutralizing antibody bevacizumab did not reach its primary endpoint in phase Ⅲ AVAGAST trial (OS: 12.1 mo *vs* 10.1 mo, $P = 0.1002$; PFS: 6.7 mo *vs* 5.3 mo, $P = 0.0037$; RR: 46% *vs* 37.4%, $P = 0.0315$)[13]. Ramucirumab is a vascular endothelial growth factor receptor 2 (VEGFR2) monoclonal antibody inhibiting VEGF binding. Two pivotal phase Ⅲ clinical trials REGARD and RAINBOW have led to the approval of ramucirumab in 2014 for advanced GCs after progression on fluropyrimidine or platinum containing chemotherapy. In REGARD trial, ramucirumab was compared to placebo in previously treated advanced GC patients. Survival was significant better as a single agent (OS: 5.2 mo *vs* 3.8 mo, $P = 0.047$)[14]. Ramucirumab was investigated in combination with paclitaxel compared to paclitaxel alone in RAINBOW trial. It demonstrated survival benefit again (OS: 9.6 mo *vs* 7.4 mo, $P = 0.017$)[15]. Advanced GC patients in both trials have been treated previously

and the OS benefits were impressive. Ramucirumab has become the standard second line treatment for advanced GC. In the first line setting, ramucirumab was studied together with FOLFOX in a phase Ⅱ trial. It did not add much improvement (PFS: 6.4 mo *vs* 6.7 mo, *P* = 0.89; OS: 11.7 mo *vs* 11.5 mo)[16]. No biomarker has been established for ramucirumab either. A global phase Ⅲ trial RAINFALL (NCT 02314117) is ongoing comparing fluropyrimidine/Cisplatin with or without ramucirumab in HER2 negative advanced GC patients as first line treatment[17]. Apatinib is an oral small molecular TKI of VEGFR-2. In a phase Ⅲ clinical trial of advanced GC patients who failed second-line chemotherapy, the OS was significantly prolonged in the apatinib group when compared to the placebo group (6.5 mo *vs* 4.7 mo, *P* < 0.016; PFS: 2.6 mo *vs* 1.8 mo, *P* < 0.0001; RR 2.84% and 0.00%)[18]. This study further confirmed the efficacy of VEGFR-2 inhibitor for the patients with advanced GC[18]. Regorafenib, an oral multi kinase inhibitor with antiangiogenic effect by VEGFR-2 inhibition, showed PFS benefit over placebo for refractory advanced GC patients in a global phase Ⅱ trial (INTEGRATE, PFS: 11.1 wk *vs* 3.9 wk, *P* < 0.0001; OS: 25 wk *vs* 19.4 wk, *P* = 0.11)[19]. Another phase Ⅱ PaFLO trial (NCT 01503372) examined chemotherapy with or without the antiangiogenic TKI pazopanib as first line in HER2 negative patients. The study did not meet its predefined PFS rate of minimum of 40% at 6 mo (PFS rate: 31.4% *vs* 25.9%). Marginal efficacy in the pazopanib group was observed with median PFS 5.1 mo compared to 3.9 mo in the control group (HR: 0.93, 95%CI: 0.56-1.54)[20].

Mesenchymal-epithelial transition factor receptor/ hepatocyte growth factor targeting therapy

Mesenchymal-epithelial transition factor receptor (c-MET) and its ligand hepatocyte growth factor (HGF) were also evaluated. Rilotumumab is an antibody to HGF, and it was tested in the frontline with chemotherapy in MET-positive advanced GC patients in two phase Ⅲ clinical trials RILOMET-1 (NCT01697072) and RILOMET-2 (NCT02137343) based on the positive phase Ⅱ study[21]. Chemotherapies with or without the drug were examined. These studies have to stop early due to increased fatal adverse events for advanced GC patients. RILOMET-1 study recently reports significantly worse OS in the study group (OS: 9.6 mo *vs* 11.5 mo, HR: 1.37, *P* = 0.016)[22]. Onartuzumab is an antibody against c-MET being studied in combination chemotherapy in advanced GC patients with HER2-negative, MET-positive disease (MetGastric) in the frontline setting (NCT01662869). The study was negative with the addition of onartuzumab to chemotherapy favored placebo group (OS ITT: 11.3 mo *vs* 11.0 mo, *P* = 0.24; OS: MET 2+/3+ 9.7 mo *vs* 11.0 mo, *P* = 0.062)[23].

Poly (ADP-ribose) polymerase targeting therapy

Poly (ADP-ribose) polymerase (PARP) inhibitor in combination with paclitaxel was studied in a second line phase Ⅱ advanced GC study (NCT01063517). The study was enriched for patients with low ATM tumors by IHC based on preclinical data of responsiveness of GC cell lines to olaparib association with low ATM protein level. Of the 124 randomized patients, olaparib plus paclitaxel was well tolerated. Although the primary endpoint of PFS was not met (All patients: 3.9 mo *vs* 3.6 mo, *P* = 0.261; ATM patients: 5.3 mo *vs* 3.7 mo, *P* = 0.35), the OS was statistically significant improved in the study for both all patients and ATM patients (All patients: 13.1 mo *vs* 8.3 mo, *P* = 0.010; ATM patients: NC *vs* 8.2 mo, *P* = 0.003)[24]. A large phase Ⅲ study is ongoing in Asian patients (NCT01924533).

Hedgehog pathway targeting therapy

Hedgehog pathway inhibitor vismodegib combined with FOLFOX was examined in a phase Ⅱ study for advanced GC patients. Hedgehog pathway is over-expressed in GE tumors and pre-clinical data suggested hedgehog inhibitors control tumor growth, cell motility and invasiveness. Median PFS was 11.5 mo *vs* 9.3 mo (*P* = 0.34) and median OS was 12.2 mo *vs* 13.9 mo (*P* = 0.48)[25]. It is another negative trial in an unselected advanced GC population.

Fibroblast growth factor receptor targeting therapy

Fibroblast growth factor receptor (FGFR) pathway is required for driving growth and survival of GC carrying *FGFR2* gene amplification. Dovitinib (TKI258) and AZD4547 are evaluated in this pathway for GCs. Dovitinib is currently being studied as monotherapy or combined with docetaxel in the second or third line setting. One trial (NCT01719549) required patients to have FGFR2 gene amplification and the other two trials (NCT01576380, NCT01921673) were performed in the unselected patient population. The SHINE study (NCT01457846) of AZD4547 monotherapy *vs* paclitaxel for patients with FGFR2 polysomy or gene amplification recently reported to be negative. The PFS was 1.8 mo in the AZD group compared to 3.5 mo in the paclitaxel group[26].

No biomarkers except HER2 are available for clinical practice. The difficulty to identify predictive biomarkers for targeted therapy remains, and warrants further investigation. Majority of the above mentioned large phase Ⅱ or Ⅲ trials were done in unselected patient populations with negative results. The cancer genome atlas project recently proposed to divide GC into four subtypes: Epstein–Barr virus positive tumor, microsatellite unstable tumors, genomically stable tumor, and chromosomally unstable tumor[27]. This classification is based on comprehensive molecular characterization. The advance in technology and understanding of its heterogeneity will potentially lead to identify key targets and pathways for treatments. The laboratory testing to establish positive markers need to be standardized. Future clinical trial design should consider both predictive and prognostic biomarkers to direct targeted therapies.

ERA OF IMMUNE THERAPY

Immune therapy has gained tremendous interest in cancer research and starts a new era for cancer treatment in recent years. Immune checkpoint pathway has made significant progress with several new agents approved for clinical use recently. Suppressing this pathway allows T cell activation and use human immune system to attack tumor cells. High RR and possible durable response have been seen in melanoma and lung cancer with relative low toxicities[28-31]. There are two classes of agents which are under evaluation including inhibitors for cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) and program cell death 1 (PD-1) or its ligand (PD-L1) inhibitors. Multiple agents are in early development and some have been tested in clinical trials. CTLA-4 inhibitors such as ipilimumab (MDX-010) and tremelimumab (CP-675,206) regulate the amplitude of early stage T cell activation. PD-1 and PD-L1 inhibitors such as nivolumab (ONO-4538), pembrolimumab (MK-3475), MEDI4736 and MPDL3280A act on the T cell activity in the peripheral tissues. Seven GC patients were included in a safety study for anti-PD-L1 antibody BMS 936559[32]. Multiple early phase clinical trials are presently ongoing to evaluate their safety and efficacy in advanced solid tumors including GC (for example: NCT01375842, NCT01693562).

CTLA-4 inhibitor tremelimumab was studied in 18 advanced GC patients as a second line treatment. One patient achieved partial response (PR) and four patients had stable disease (SD). Improved survival was observed in patients experiencing a post treatment carcinoembryonic antigen proliferative response (OS: 17.1 mo *vs* 4.7 mo, $P = 0.004$) despite the objective RR was low[33]. Another phase Ⅱ trial of sequential ipilimumab *vs* best supportive care as a second line therapy has completed with results pending (NCT01585987).

PD-1 inhibitor pembrolizumab (MK-3475) demonstrated encouraging results in the phase 1b KEYNOTE-012 study for GC with 67% patients received ⩾ 2 prior therapies. PD-L1+ was used as the biomarker with 65 out of 162 (40%) screened patient being positive, and 39 patients enrolled eventually. ORR was 22% by central review and 33% by investigator review[34]. Median time to response was 8 wk with a median response duration of 24 wk. The 6-mo PFS and OS rate were 24% and 69%[34]. Four patients experienced high-grade drug-related adverse events: peripheral sensory neuropathy, fatigue, decreased appetite, hypoxia, and pneumonitis[34]. This promising result has led to further investigation. A phase Ⅱ KEYNOTE-059 (NCT02335411) study has been launched with pembrolizumab monotherapy or in combination with cisplatin plus 5-fluorouracil for advanced GC. Phase Ⅲ KEYNOTE-061 (NCT02370498) is planned with pembrolizumab *vs* paclitaxel after the first line therapy with platinum and fluropyrimidine. Another phase Ⅲ study with nivolumab (ONO-4538) is recruiting patients with advanced GC (NCT02267343) in Asian countries and PD-L1 positivity was not required.

Combining checkpoint pathway inhibitors are studied in advanced solid tumors with the hope to generate stronger immunogenicity. A phase Ⅰb/Ⅱ study is ongoing to assess the safety and efficacy of PD-L1 inhibitor MEDI4736 in combination with CTLA-4 inhibitor tremelimumab *vs* monotherapy for patients with advanced GC (NCT02340975). Another Phase Ⅰb/Ⅱ study of advanced solid tumor included GC is evaluating nivolumab monotherapy *vs* nivolumab combined with ipilimumab (NCT01928394).

Immune therapy is currently opening a new page for cancer treatment. Harness human immune system to fight for GC may become a reality very soon. Many obstacles and challenges warrant further investigation such as standardization of laboratory testing, biomarkers, tumor immune response criteria, management of immune related adverse events, safety and efficacy of re-exposure.

GC STEM CELL

Hematopoietic stem cell transplant has been well established and widely used in clinical practice to save lives. With more accumulative evidence in recent years, the questionable solid tumor stem cells hypothesis becomes more believable. GC stem cells are thought to be responsible for tumor self-renewal, metastasis, chemotherapy resistance and tumor recurrence[35]. *In vitro* sphere-forming assays and *in vivo* tumor formation in immune-deficient mice have been employed for solid tumor stem cell research. The gastric stem cell was thought to be existed in gastric epithelium initially. Bone marrow derived cells were also identified in mouse models of Helicobacter-induced GC[36,37]. However majority of the studies are still *in vitro* or using mice model[38]. One oral first in class cancer stemness inhibitor called BBI608 was studied plus weekly paclitaxel in a phase Ⅰb trial in refractory solid tumors. Two out of the five refractory GC patients had a partial response (48% and 45% regressions), one had stable disease (25% regression) and two had prolonged stable disease ⩾ 24 wk[39]. A phase Ⅲ clinical trial is ongoing (BRIGHTER: NCT02178956) with this cancer cell stemness inhibitor for previously treated advanced GC patients[40]. One GC patient demonstrated minor regression or SD ⩾ 16 wk in another phase Ⅰ cancer stem cell inhibitor BBI503 trial (NCT01781455)[41].

FUTURE PERSPECTIVE

GC is a common malignancy with poor outcomes. There is an urgent unmet need to improve treatment and outcome for this lethal disease. Understanding the heterogeneous nature of this cancer and incorporate genomic atlas to develop biomarkers as well as newer target agents are important. Develop precision medicine and tailor optimal therapies to individual patient based on

Table 1 Summary of selected targeted agents for advanced gastric cancer

Target	Study agent	Trial	Treatments	Phase	Biomarker	Results primary end point
EGFR	Cetuximab	EXPAND NCT00678535	Arm1: CX + cetuximab Arm 2: CX	Ⅲ	No	Negative PFS: 4.4 mo *vs* 5.6 mo (P = 0.32)
EGFR	Panitumumab	REAL3 NCT00824785	Arm1: EOC+ Panitumumab Arm2: EOC	Ⅱ / Ⅲ	No	Negative OS: 8.8 mo *vs* 11.3 mo (P = 0.013)
mTOR	Everolimus	GRANITE-1 NCT00879333	Arm1: Everolimus Arm2: Placebo	Ⅲ	No	Negative OS: 5.4 mo *vs* 4.3 mo (P = 0.124)
HER2	Trastuzumab	ToGA NCT01041404	Arm1: CF + Trastuzumab Arm2: CF	Ⅲ	Yes HER2	Positive OS: 13.8 mo *vs* 11.1 mo (P = 0.0046)
HER2/EGFR	Lapatinib	TRIO-013/Logic NCT00680901	Arm1: CX + Lapatinib Arm2: CX	Ⅲ	Yes HER2	Negative OS: 12.2 mo *vs* 10.5 mo (P = 0.35)
HER2/EGFR	Lapatinib	TyTAN NCT00486954	Arm1: Paclitaxel + Lapatinib Arm2: Paclitaxel	Ⅲ	Yes HER2	Negative OS: 11.1 mo *vs* 8.9 mo (P = 0.1044)
HER2	Pertuzumab	JACOB NCT0177486	Arm1: CF + Trasuzumab + Pertuzumab Arm2: CF + Trastuzumab	Ⅲ	Yes HER2	Ongoing
HER2	T-DM1	GATSBY NCT01641939	Arm1: Taxane Arm2: T-DM1 2.4 mg/kg once a week Arm3: T-DM1 3.6 mg/kg every 3 wk	Ⅱ / Ⅲ	Yes HER2	Ongoing
VEGF	Bevacizumab	AVAGAST NCT00548548	Arm1: CF + Bevacizumab Arm2: CF	Ⅲ	No	Negative OS: 12.1 mo *vs* 10.1 mo (P = 0.1002)
VEGFR	Ramucirumab	REGARD NCT00917384	Arm1: Ramucirumab Arm2: Placebo	Ⅲ	No	Positive OS: 5.2 mo *vs* 3.8 mo (P = -0.047)
VEGFR	Ramucirumab	RAINBOW NCT01170663	Arm1: Paclitaxel + Ramucirumab Arm2: Paclitaxel	Ⅲ	No	Positive OS: 9.6 mo *vs* 7.4 mo (P = 0.017)
VEGFR	Ramucirumab	RAINFALL NCT02314117	Arm1: CF + Ramucirumab Arm2: CF	Ⅲ	Yes HER2 negative	Ongoing
VEGFR	Apatinib	NCT0152745	Arm1: Apatinib Arm2: Placebo	Ⅲ	No	Positive OS: 6.5 mo *vs* 4.7 mo (P < 0.016), PFS: 2.6 mo *vs* 1.8 mo (P < 0.0001)
VEGFR (multi-kinase)	Regorafenib	INTEGRATE	Arm1: Regorafenib Arm2: Placebo	Ⅱ	No	Positive PFS: 11.1 wk *vs* 3.9 wk (P < 0.0001)
VEGFR, PDGFR c-Kit	Pazopanib	PaFLO	Arm1: FLO + Pazopanib Arm2: FLO	Ⅱ	Yes HER2 negative	Negative PFS rate at 6 mo 31.4% *vs* 25.9% (Did not meet predefined 40%)
MET/HGF	Rilotumumab	RILOMET-1 NCT01697072	Arm1: ECX + Rilotumumab Arm2:	Ⅲ	Yes MET	Terminated due to increased death signal Negative (Detrimental) OS: 9.6 *vs* 11.5 mo (HR 1.37, P = 0.016)
MET/HGF	Rilotumumab	RILOMET-2 NCT02137343	Arm1: CX + Rilotumumab Arm2: CX	Ⅲ	Yes MET	Terminated due to increased death signal
MET	Onartuzumab	METGastric NCT01662869	Arm1: FOLFOX Arm2: FOLFOX + Onartuzumab	Ⅲ	Yes MET+, HER2-	Negative ITT OS: 11.3 mo *vs* 11.0 mo (P = 0.24) MET2+/3+ OS: 9.7 mo *vs* 11.0 mo (P = 0.06)
PARP	Olaparib	NCT01063517	Arm1: Paclitaxel + Olaparib Arm2: Paclitaxel	Ⅱ	Yes ATM	Negative PFS: 3.9 mo *vs* 2.6 mo (P = 0.261) All patients PFS: 5.3 mo *vs* 3.7 mo (P = 0.315) ATM- patients Positive for secondary endpoints OS: 13.1 mo *vs* 8.3 mo (P = 0.010) All Patients OS: NR mo *vs* 8.2 mo (P = 0.003) ATM- patients
PARP	Olaparib	NCT01924533	Arm1: Paclitaxel + Olaparib Arm2: Paclitaxel	Ⅲ	No	Ongoing
Hedgehog	Vismodegib	NCT00982592	Arm1: FOLFOX + Vismodegib Arm2: FOLFOX	Ⅱ	No	Negative PFS: 7.3 mo *vs* 9.0 mo (P = 0.64)
FGFR	Dovitinib	NCT01719549	Dovitinib monotherapy	Ⅱ	Yes FGFR	Ongoing
FGFR	Dovitinib	NCT01576380	Dovitinib monotherapy	Ⅱ	No	Completed, waiting for result
FGFR	Dovitinib	NCT01921673	Docetaxel + Dovitinib	Ⅰ / Ⅱ	No	Ongoing
FGFR/VEGFR	AZD4547	SHINE NCT1457846	Arm1: AZD4547 Arm2: Paclitaxel	Ⅱ	Yes FGFR	Negative PFS: 1.8 (AZD) *vs* 3.5 mo

EOC: Epirubicin, oxalilatin, capecitabine; CF: Fluoropyrimidine, cisplatin; T-DM1: Trastuzumab emtansine; ECX: Epirubicin, csiplatin, capecitabine; CX: Cisplatin, capecitabine; FOLFOX: 5-Fluorouracil, folinic acid, oxaliplatin; NR: Not reached; FLO: 5-FU, leucovorine, oxaliplatin; EGFR: Epidermal growth factor receptor; mTOR: Mammalian target of rapamycin; HER2: Human epidermal growth factor receptor 2; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor; PDGFR: Platelet-derived growth factor receptor; MET: Mesenchymal-epithelial transition factor; HGF: Hepatocyte growth factor; PARP: Poly ADP-ribose polymerase; FGFR: Fibroblast growth factor receptor.

information including molecular study results will be the future focus. With the recent breakthrough in immune therapy in other solid tumors and promising early phase clinical trial results in GC, immune checkpoint pathway inhibitors are undergoing evaluation. In order to generate stronger immunogenicity, combining different checkpoint pathway inhibitors or chemotherapy or targeted therapy might be needed. GC stem cell research was initially cluttered with skepticism until more evidence accumulated recently. It is an exciting field warrants further evaluation.

CONCLUSION

Ramucirumab is the second biologic agent after trastuzumab approved with statistically significant but marginal survival benefit for GC patients in spite of multiple negative phase Ⅲ clinical trials of other targeted agents (as summarized in Table 1). Better understanding and use of genomic atlas/biomarkers will potentially lead to development of targeted agents with better efficacy. Immune therapy especially checkpoint pathway inhibition is a promising field and being studied in multiple clinical trials. GC stem cell therapy is finally moving from bench work to early phase clinical investigation. Targeted therapy, immune therapy and cancer stem cell therapy are promising fields and may meet the urgent demand for novel therapy to treat GC in near future.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

2 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015; **65**: 5-29 [PMID: 25559415 DOI: 10.3322/caac.21254]

3 **Cunningham D**, Starling N, Rao S, Iveson T, Nicolson M, Coxon F, Middleton G, Daniel F, Oates J, Norman AR. Capecitabine and oxaliplatin for advanced esophagogastric cancer. *N Engl J Med* 2008; **358**: 36-46 [PMID: 18172173 DOI: 10.1056/NEJMoa073149]

4 **Van Cutsem E**, Moiseyenko VM, Tjulandin S, Majlis A, Constenla M, Boni C, Rodrigues A, Fodor M, Chao Y, Voznyi E, Risse ML, Ajani JA. Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V325 Study Group. *J Clin Oncol* 2006; **24**: 4991-4997 [PMID: 17075117 DOI: 10.1200/jco.2006.06.8429]

5 **Lordick F**, Kang YK, Chung HC, Salman P, Oh SC, Bodoky G, Kurteva G, Volovat C, Moiseyenko VM, Gorbunova V, Park JO, Sawaki A, Celik I, Götte H, Melezínková H, Moehler M. Capecitabine and cisplatin with or without cetuximab for patients with previously untreated advanced gastric cancer (EXPAND): a randomised, open-label phase 3 trial. *Lancet Oncol* 2013; **14**: 490-499 [PMID: 23594786 DOI: 10.1016/s1470-2045(13)70102-5]

6 **Waddell T**, Chau I, Cunningham D, Gonzalez D, Okines AF, Okines C, Wotherspoon A, Saffery C, Middleton G, Wadsley J, Ferry D, Mansoor W, Crosby T, Coxon F, Smith D, Waters J, Iveson T, Falk S, Slater S, Peckitt C, Barbachano Y. Epirubicin, oxaliplatin, and capecitabine with or without panitumumab for patients with previously untreated advanced oesophagogastric cancer (REAL3): a randomised, open-label phase 3 trial. *Lancet Oncol* 2013; **14**: 481-489 [PMID: 23594787 DOI: 10.1016/s1470-2045(13)70096-2]

7 **Van Cutsem E**, Köhne CH, Láng I, Folprecht G, Nowacki MP, Cascinu S, Shchepotin I, Maurel J, Cunningham D, Tejpar S, Schlichting M, Zubel A, Celik I, Rougier P, Ciardiello F. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J Clin Oncol* 2011; **29**: 2011-2019 [PMID: 21502544 DOI: 10.1200/jco.2010.33.5091]

8 **Ohtsu A**, Ajani JA, Bai YX, Bang YJ, Chung HC, Pan HM, Sahmoud T, Shen L, Yeh KH, Chin K, Muro K, Kim YH, Ferry D, Tebbutt NC, Al-Batran SE, Smith H, Costantini C, Rizvi S, Lebwohl D, Van Cutsem E. Everolimus for previously treated advanced gastric cancer: results of the randomized, double-blind, phase III GRANITE-1 study. *J Clin Oncol* 2013; **31**: 3935-3943 [PMID: 24043745 DOI: 10.1200/jco.2012.48.3552]

9 **Bang YJ**, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010; **376**: 687-697 [PMID: 20728210 DOI: 10.1016/s0140-6736(10)61121-x]

10 **Hecht JR,** Bang YJ, Qin S, Chung H-C, Xu JM, Park JO, Jeziorski K, Shparyk Y, Hoff PM, Sobrero AF, Salman P, Li J, Protsenko S, Buyse ME, Afenjar K, Kaneko T, Kemner A, Santillana S, Press MF, Slamon DJ. Lapatinib in combination with capecitabine plus oxaliplatin (CapeOx) in HER2-positive advanced or metastatic gastric, esophageal, or gastroesophageal adenocarcinoma (AC): The TRIO-013/LOGiC Trial. *J Clin Oncol* 2013; **31** Suppl: abstr LBA4001

11 **Satoh T**, Xu RH, Chung HC, Sun GP, Doi T, Xu JM, Tsuji A, Omuro Y, Li J, Wang JW, Miwa H, Qin SK, Chung IJ, Yeh KH, Feng JF, Mukaiyama A, Kobayashi M, Ohtsu A, Bang YJ. Lapatinib plus paclitaxel versus paclitaxel alone in the second-line treatment of HER2-amplified advanced gastric cancer in Asian populations: TyTAN--a randomized, phase III study. *J Clin Oncol* 2014; **32**: 2039-2049 [PMID: 24868024 DOI: 10.1200/jco.2013.53.6136]

12 **Baselga J,** Cortés J, Kim SB, Im SA, Hegg R, Im YH, Roman L, Pedrini JL, Pienkowski T, Knott A, Clark E, Benyunes MC, Ross G, Swain SM. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *N Engl J Med* 2012; **366**: 109-119 [PMID: 22149875 DOI: 10.1056/NEJMoa1113216]

13 **Ohtsu A**, Shah MA, Van Cutsem E, Rha SY, Sawaki A, Park SR, Lim HY, Yamada Y, Wu J, Langer B, Starnawski M, Kang YK. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a randomized, double-blind, placebo-controlled phase III study. *J Clin Oncol* 2011; **29**: 3968-3976 [PMID: 21844504 DOI: 10.1200/jco.2011.36.2236]

14 **Fuchs CS**, Tomasek J, Yong CJ, Dumitru F, Passalacqua R, Goswami C, Safran H, dos Santos LV, Aprile G, Ferry DR, Melichar B, Tehfe M, Topuzov E, Zalcberg JR, Chau I, Campbell W, Sivanandan C, Pikiel J, Koshiji M, Hsu Y, Liepa AM, Gao L, Schwartz JD, Tabernero J. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial. *Lancet* 2014; **383**: 31-39 [PMID: 24094768 DOI: 10.1016/s0140-6736(13)61719-5]

15 **Wilke H**, Muro K, Van Cutsem E, Oh SC, Bodoky G, Shimada Y, Hironaka S, Sugimoto N, Lipatov O, Kim TY, Cunningham D, Rougier P, Komatsu Y, Ajani J, Emig M, Carlesi R, Ferry D, Chandrawansa K, Schwartz JD, Ohtsu A. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomised phase 3 trial. *Lancet Oncol* 2014; **15**: 1224-1235 [PMID: 25240821 DOI: 10.1016/s1470-2045(14)70420-6]

16 **Yoon HH,** Bendell JC, Braiteh FS, Firdaus I, Philip PA, Cohn AL, Lewis N, Anderson DM, Arrowsmith E, Schwartz JD, Xu Y, Koshiji M, Alberts SR, Wainberg ZA. Ramucirumab (RAM) plus FOLFOX as front-line therapy (Rx) for advanced gastric or esophageal adenocarcinoma (GE-AC): Randomized, double-blind, multicenter

phase 2 trial. *J Clin Oncol* 2014; **32**: abstr4004
17 **Fuchs CS,** Tabernero J, Al-Batran SE, Chau I, Ilson DH, Van Cutsem E, Ferry D, Emig M, Melemed AS, Vanvoorden V, Hsu Y, Xu Y, Sashegyi A, Das M, Shah MA. A randomized, double-blind, placebo-controlled phase III study of cisplatin plus a fluoropyrimidine with or without ramucirumab as first-line therapy in patients with metastatic gastric or gastroesophogeal junction (GEJ) adenocarcinoma (RAINFALL, NCT02314117). *J Clin Oncol* 2015; **33** Suppl: abstrTPS4131
18 **Qin S.** Phase III study of apatinib in advanced gastric cancer: A randomized, double-blind, placebo-controlled trial. *J Clin Onco*l 2014; **32** Suppl: abstr4003
19 **Pavlakis N,** Sjoquist KM, Tsobanis E, Martin AJ, Kang YK, Bang YJ, O'Callaghan CJ, Tebbutt NC, Rha SY, Lee J, Cho JY, Lipton LR, Burnell MJ, Alcindor T, Strickland A, Kim JW, Yip S, Simes J, Zalcberg JR, Goldstein D. INTEGRATE: A randomized, phase II, double-blind, placebo-controlled study of regorafenib in refractory advanced oesophagogastric cancer (AOGC): A study by the Australasian Gastrointestinal Trials Group (AGITG)--Final overall and subgroup results. *J Clin Oncol* 2015; **33**: abstr4003
20 **Thuss-Patience PC,** Al-Batran SE, Siveke JT, Homann N, Malfertheiner P, Glaeser D, Stein A, Tamm I, Daum S, Potenberg J, Florschutz A, Vogel A, Ridwelski K, Ritgen M, Geissler M, Schmalenberg H, Schlattmann P, Lorenz M, Breithaupt K, Pichlmeier U. Pazopanib and 5-FU/oxaliplatin as first-line treatment in advanced gastric cancer: PaFLO, a randomized phase II study from the AIO (Arbeitsgemeinschaft Internistische Onkologie). *J Clin Oncol* 2015; **33** Suppl: abstr4003
21 **Iveson T**, Donehower RC, Davidenko I, Tjulandin S, Deptala A, Harrison M, Nirni S, Lakshmaiah K, Thomas A, Jiang Y, Zhu M, Tang R, Anderson A, Dubey S, Oliner KS, Loh E. Rilotumumab in combination with epirubicin, cisplatin, and capecitabine as first-line treatment for gastric or oesophagogastric junction adenocarcinoma: an open-label, dose de-escalation phase 1b study and a double-blind, randomised phase 2 study. *Lancet Oncol* 2014; **15**: 1007-1018 [PMID: 24965569 DOI: 10.1016/s1470-2045(14)70023-3]
22 **Cunningham D,** Tebbutt NC, Davidenko I, Murad AM, Al-Batran SE, Ilson DH, Tjulandin S, Gotovkin E, Karaszewska B, Bondarenko I, Tejani MA, Udrea AA, Tehfe MA, Baker N, Oliner KS, Zhang Y, Hoang T, Sidhu R, Catenacci DVT. Phase III, randomized, double-blind, multicenter, placebo (P)-controlled trial of rilotumumab (R) plus epirubicin, cisplatin and capecitabine (ECX) as first-line therapy in patients (pts) with advanced MET-positive (pos) gastric or gastroesophageal junction (G/GEJ) cancer: RILOMET-1 study. *J Clin Oncol* 2015; **33** Suppl: abstr4000
23 **Shah MA,** Bang Y-J, Lordick F, Tabernero J, Chen M, Hack SP, Phan SC, Shames DS, Cunningham D. MET Gastric: A phase III study of onartuzumab plus mFOLFOX6 in patients with metastatic HER2-negative (HER2-) and MET-positive (MET) adenocarcinoma of the stomach or gastroesophageal junction (GEC). *J Clin Oncol* 2015; **33** Suppl: abstr4012
24 **Bang YJ,** Im SA, Lee KW, Cho JY, Song EK, Lee KH, Kim YH, Park JO, Chun HG, Zang DY, Fielding A, Rowbottom J, Kim WH. Olaparib plus paclitaxel in patients with recurrent or metastatic gastric cancer: A randomized, double-blind phase II study. *J Clin Oncol* 2015; **33** Suppl: abstr4013
25 **Cohen DJ,** Christos PJ, Kindler HL, Catenacci DVT, Bekaii-Saab TB, Tahiri S, Janjigian YY, Gibson MK, Chan E, Rajdev L, Urba S, Wade JL, Kozuch P, Love E, Vandris K, Takebe N, Hochster HS, Sparano JA, New York Cancer Consortium. Vismodegib (V), a hedgehog (HH) pathway inhibitor, combined with FOLFOX for first-line therapy of patients (pts) with advanced gastric and gastroesophageal junction (GEJ) carcinoma: A New York Cancer Consortium led phase II randomized study. *J Clin Oncol* 2015; **31** Suppl: abstr4011
26 **Bang YJ,** Van Cutsem E, Mansoor W, Petty RD, Chao Y, Cunningham D, Ferry D, Landers D, Stockman P, Smith NR, Geh C, Kilgour E. A randomized, open-label phase II study of AZD4547 (AZD) versus Paclitaxel (P) in previously treated patients with advanced gastric cancer (AGC) with Fibroblast Growth Factor Receptor 2 (FGFR2) polysomy or gene amplification (amp): SHINE study. *J Clin Oncol* 2015; **33** Suppl: abstr4014
27 **Cancer Genome Atlas Research Network.** Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; **513**: 202-209 [PMID: 25079317 DOI: 10.1038/nature13480]
28 **Hamid O**, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, Wolchok JD, Hersey P, Joseph RW, Weber JS, Dronca R, Gangadhar TC, Patnaik A, Zarour H, Joshua AM, Gergich K, Elassaiss-Schaap J, Algazi A, Mateus C, Boasberg P, Tumeh PC, Chmielowski B, Ebbinghaus SW, Li XN, Kang SP, Ribas A. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N Engl J Med* 2013; **369**: 134-144 [PMID: 23724846 DOI: 10.1056/NEJMoa1305133]
29 **Hodi FS**, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbé C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010; **363**: 711-723 [PMID: 20525992 DOI: 10.1056/NEJMoa1003466]
30 **Robert C**, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, Hassel JC, Rutkowski P, McNeil C, Kalinka-Warzocha E, Savage KJ, Hernberg MM, Lebbé C, Charles J, Mihalcioiu C, Chiarion-Sileni V, Mauch C, Cognetti F, Arance A, Schmidt H, Schadendorf D, Gogas H, Lundgren-Eriksson L, Horak C, Sharkey B, Waxman IM, Atkinson V, Ascierto PA. Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med* 2015; **372**: 320-330 [PMID: 25399552 DOI: 10.1056/NEJMoa1412082]
31 **Rizvi NA**, Mazières J, Planchard D, Stinchcombe TE, Dy GK, Antonia SJ, Horn L, Lena H, Minenza E, Mennecier B, Otterson GA, Campos LT, Gandara DR, Levy BP, Nair SG, Zalcman G, Wolf J, Souquet PJ, Baldini E, Cappuzzo F, Chouaid C, Dowlati A, Sanborn R, Lopez-Chavez A, Grohe C, Huber RM, Harbison CT, Baudelet C, Lestini BJ, Ramalingam SS. Activity and safety of nivolumab, an anti-PD-1 immune checkpoint inhibitor, for patients with advanced, refractory squamous non-small-cell lung cancer (CheckMate 063): a phase 2, single-arm trial. *Lancet Oncol* 2015; **16**: 257-265 [PMID: 25704439 DOI: 10.1016/s1470-2045(15)70054-9]
32 **Brahmer JR,** Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A, Wigginton JM. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med* 2012; **366**: 2455-2465 [PMID: 22658128 DOI: 10.1056/NEJMoa1200694]
33 **Ralph C**, Elkord E, Burt DJ, O'Dwyer JF, Austin EB, Stern PL, Hawkins RE, Thistlethwaite FC. Modulation of lymphocyte regulation for cancer therapy: a phase II trial of tremelimumab in advanced gastric and esophageal adenocarcinoma. *Clin Cancer Res* 2010; **16**: 1662-1672 [PMID: 20179239 DOI: 10.1158/1078-0432.ccr-09-2870]
34 **Muro K,** Bang YJ, Shankaran V, Geva R, Catenacci DVT, Gupta S, Eder JP, Berger R, Gonzalez EJ, Ray A, Dolled-Filhart M, Emancipator K, Pathiraja K, Lunceford JK, Cheng JD, Koshiji M, Chung HC. Relationship between PD-L1 expression and clinical outcomes in patients (Pts) with advanced gastric cancer treated with the anti-PD-1 monoclonal antibody pembrolizumab (Pembro; MK-3475) in KEYNOTE-012. *J Clin Oncol* 2015; **33** Suppl 3; abstr3
35 **Singh SR**. Gastric cancer stem cells: a novel therapeutic target. *Cancer Lett* 2013; **338**: 110-119 [PMID: 23583679 DOI: 10.1016/j.canlet.2013.03.035]
36 **Houghton J**, Stoicov C, Nomura S, Rogers AB, Carlson J, Li H, Cai X, Fox JG, Goldenring JR, Wang TC. Gastric cancer originating from bone marrow-derived cells. *Science* 2004; **306**: 1568-1571 [PMID: 15567866 DOI: 10.1126/science.1099513]
37 **Takaishi S**, Okumura T, Wang TC. Gastric cancer stem cells. *J*

Clin Oncol 2008; **26**: 2876-2882 [PMID: 18539967 DOI: 10.1200/jco.2007.15.2603]

38 **Zhao Y**, Feng F, Zhou YN. Stem cells in gastric cancer. *World J Gastroenterol* 2015; **21**: 112-123 [PMID: 25574084 DOI: 10.3748/wjg.v21.i1.112]

39 **Hitron M,** Stephenson J, Chi KN, Edenfield WJ, Leggett D, Li Y, Li W, Gada K, Li C. A phase 1b study of the cancer stem cell inhibitor BBI608 administered with paclitaxel in patients with advanced malignancies. *J Clin Oncol* 2014; **32** Suppl: abstr2530

40 **Shah MA,** Muro K, Shitara K, Tebbutt NC, Bang YJ, Lordick F, Borodyansky L, Li C. The BRIGHTER trial: A phase III randomized double-blind study of BBI608 weekly paclitaxel versus placebo (PBO) weekly paclitaxel in patients (pts) with pretreated advanced gastric and gastro-esophageal junction (GEJ) adenocarcinoma. *J Clin Oncol* 2015: **33** Suppl; abstr TPS4139

41 **Laurie SA,** Jonker DJ, Edenfield WJ, Stephenson J, Keller D, Hitron M, Li W, Li Y, Gada K, Gao Y, Li C. A phase 1 dose-escalation study of BBI503, a first-in-class cancer stemness kinase inhibitor in adult patients with advanced solid tumors. *J Clin Oncol* 2014: **32** Suppl: abstr 2527

P- Reviewer: Kim Y, Tuosto L
S- Editor: Tian YL **L- Editor**: A **E- Editor**: Jiao XK

World Journal of Gastrointestinal Oncology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4251/wjgo.v7.i11.271

World J Gastrointest Oncol 2015 November 15; 7(11): 271-284
ISSN 1948-5204 (online)

TOPIC HIGHLIGHT

2015 Advances in Colorectal Cancer

Autophagy in colorectal cancer: An important switch from physiology to pathology

Florin Burada, Elena Raluca Nicoli, Marius Eugen Ciurea, Daniel Constantin Uscatu, Mihai Ioana, Dan Ionut Gheonea

Florin Burada, Marius Eugen Ciurea, Mihai Ioana, Dan Ionut Gheonea, Research Center of Gastroenterology and Hepatology, University of Medicine and Pharmacy of Craiova, 200638 Craiova, Romania

Elena Raluca Nicoli, Department of Pharmacology, University of Oxford, Oxford, OX13QT London, United Kingdom

Elena Raluca Nicoli, Daniel Constantin Uscatu, Human Genomics Laboratory, University of Medicine and Pharmacy of Craiova, 200638 Craiova, Romania

Author contributions: Burada F, Nicoli ER, Ciurea ME and Uscatu DC performed the literature research; Burada F, Nicoli RE and Gheonea DI wrote the paper; Uscatu DC and Ioana M created the Figures; Burada F, Ioana M, Ciurea M and Gheonea DI critically revised the paper.

Supported by Grant POSDRU/159/1.5/S/133377, from European Social Found, Human Resources Development Operational Programme 2007-2013 (to Burada F).

Conflict-of-interest statement: The authors declare no conflicts of interest.

Correspondence to: Florin Burada, MD, PhD, Research Center of Gastroenterology and Hepatology, University of Medicine and Pharmacy of Craiova, 1 Mai 66, 200638 Craiova, Romania. buradaflorin@gmail.com
Telephone: +40-745-683949
Fax: +40-251-593077

Received: May 14, 2015
Peer-review started: May 15, 2015
First decision: June 2, 2015
Revised: June 20, 2015
Accepted: September 30, 2015
Article in press: October 9, 2015
Published online: November 15, 2015

Abstract

Colorectal cancer (CRC) remains a leading cause of cancer death in both men and women worldwide. Among the factors and mechanisms that are involved in the multifactorial etiology of CRC, autophagy is an important transformational switch that occurs when a cell shifts from normal to malignant. In recent years, multiple hypotheses have been considered regarding the autophagy mechanisms that are involved in cancer. The currently accepted hypothesis is that autophagy has dual and contradictory roles in carcinogenesis, but the precise mechanisms leading to autophagy in cancer are not yet fully defined and seem to be context dependent. Autophagy is a surveillance mechanism used by normal cells that protects them from the transformation to malignancy by removing damaged organelles and aggregated proteins and by reducing reactive oxygen species, mitochondrial abnormalities and DNA damage. However, autophagy also supports tumor formation by promoting access to nutrients that are critical to the metabolism and growth of tumor cells and by inhibiting cellular death and increasing drug resistance. Autophagy studies in CRC have focused on several molecules, mainly microtubule-associated protein 1 light chain 3, beclin 1, and autophagy related 5, with conflicting results. Beneficial effects were observed for some agents that modulate autophagy in CRC either alone or, more often, in combination with other agents. More extensive studies are needed in the future to clarify the roles of

autophagy-related genes and modulators in colorectal carcinogenesis, and to develop potential beneficial agents for the prognosis and treatment of CRC.

Key words: Colorectal cancer; Autophagy; Gene; Protein; Carcinogenesis

Core tip: This review describes the role of autophagy in cancer, focusing on the involvement of autophagy in colorectal cancer (CRC). Initially, we describe the steps and components of autophagy, and we then further highlight the dual role of autophagy in cancer, where it can potentially act as both a promoter and an inhibitor during the transformation from normal to malignant cell. In particular, we emphasize the major autophagy genes involved in CRC pathogenesis along with autophagy-modulating agents and their modes of action in the context of CRC therapy.

Burada F, Nicoli ER, Ciurea ME, Uscatu DC, Ioana M, Gheonea DI. Autophagy in colorectal cancer: An important switch from physiology to pathology. *World J Gastrointest Oncol* 2015; 7(11): 271-284 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i11/271.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i11.271

INTRODUCTION

Despite advances in diagnosis and treatment, colorectal cancer (CRC) remains one of the major causes of cancer death in both sexes worldwide: It is the third most common diagnosed cancer in males and the second most common in females[1]. It is well known that many risk factors, including multiple genes and environmental influences, are involved in malignant transformation. Recent research provides new data regarding the complex mechanisms involved in colorectal carcinogenesis. Among these mechanisms, autophagy is important in the switch from normal to malignant colorectal cells. The involvement of autophagy in cancer appears to be context specific, with evidence suggesting that it can have a dual role in both tumor suppressing and tumor promoting activities. Moreover, autophagy performs important functions in different processes that are connected to carcinogenesis, including inflammation, immune response and genome stability.

Here, we describe the involvement of autophagy in carcinogenesis, with a particular emphasis on CRC. We summarize the components and steps of macroautophagy (herein referred to as autophagy), and we emphasize the conflicting roles of autophagy in cancer, indicating that it has both promoter and suppressor mechanisms during malignant transformations. The second part of this study is focused on the autophagy genes and proteins that are associated with CRC. Finally, the effects of autophagy-based drugs in CRC treatment are discussed.

AUTOPHAGY STEPS AND REGULATION

Autophagy is an evolutionarily conserved catabolic process that is characterized by cellular self-digestion and the removal of excessive, long-lived or dysfunctional organelles and proteins[2]. Autophagy occurs as a physiological process in normal cells at a basal level to assure cellular homeostasis, or as a strategic survival mechanism that recycles energy and nutrients under special conditions. Hypoxia, stress and nutrient deprivation trigger autophagy as a critical adaptive response during starvation[3]. Three morphologically distinct forms of autophagy can be distinguished: macroautophagy, microautophagy and chaperone-mediated autophagy[4]. Macroautophagy is identified by the presence of double membrane vesicles known as an autophagosomes, which engulf cytoplasmic components that include damaged organelles and deliver them to lysosomes for degradation. The other two forms, microautophagy and chaperone-mediated autophagy, involve a direct membrane invagination to engulf damaged proteins and the translocation of soluble cytosolic proteins by chaperone-dependent selection across the lysosomal membrane, respectively[5,6].

Autophagy-related genes (ATGs) play a critical role in facilitating the regulation of well-orchestrated autophagy. To date, thirty-six ATGs have been identified[7]. Autophagosome formation is initiated by unc-51-like kinase (ULK) and class Ⅲ phosphatidylinositol 3-kinase (PI3K) complexes. The ULK complex consists of ATG13, ATG101, ULK1/2 and family-interacting protein FIP200[8,9]. Under normal growth conditions, the mammalian target of rapamycin (mTOR) complex inhibits the formation of the ULK complex, in effect blocking autophagy, and the ULK components are dissociated. Various stimuli (*e.g.*, hypoxia, starvation) inhibit mTOR, allowing the ULK kinase complex to be activated, which initiates the formation of an isolation membrane (Figure 1) called a phagophore[10,11]. The origin of phagophores has not been explained, but the plasma membrane, endoplasmic reticulum, Golgi apparatus and mitochondria are all possible sources[12]. The completion of this critical step is driven by vacuolar sorting protein 34, a class Ⅲ PI3K that is bound to beclin-1, and other ATG proteins (*e.g.*, ATG14), which generate PI3K, the second complex, that catalyzes the production of phosphatidylinositol-3-phosphate[10,13].

Autophagosome elongation and closure steps and the further conversion to a nascent closed autophagosome are controlled by two ubiquitin-like conjugates. First, ATG12 forms a conjugate with ATG5 under the control of ATG7 and ATG10, which have E1 and E2-like enzyme activity, respectively. The resulting ATG12-ATG5

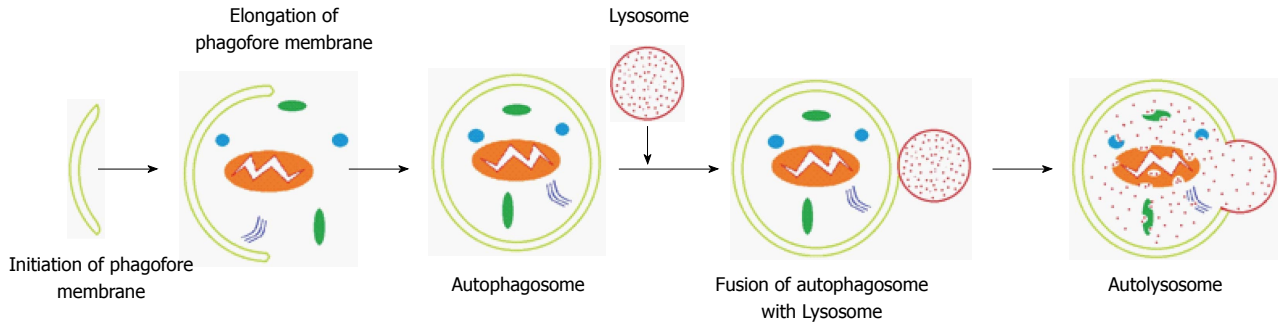


Figure 1 Morphological steps of the autophagy process. Autophagy is initiated with the formation of a phagophore, which sequesters cellular material in a double-membrane vesicle called an autophagosome. The autophagosome fuses with lysosomes to form an autolysosome.

complex interacts with ATG16L1 to form a multimeric ATG12-ATG5-ATG16L1 conjugate that is located on the outer surface of the autophagosomal membrane. It will dissociate from the membrane upon completion of the autophagosome[14,15]. The second ubiquitin-like pathway involves the conjugation of the microtubule-associated protein 1-light chain 3 (LC3-Ⅰ) to the lipid phosphatidylethanolamine (PE) by ATG7 and ATG3, which is an E2-like enzyme, to form the membrane-bound LC3-Ⅱ. LC3 is initially synthesized as a precursor protein, proLC3, and is immediately processed to LC3-Ⅰ by ATG4 through cleavage of its C-terminal amino acid. The membrane-bound form of LC-3, LC-3Ⅱ, is recruited to both sides of the autophagosomal membrane[16,17]. After fusion with lysosomes, LC3-Ⅱ on the cytoplasmic face of the autolysosome can be delipidated by ATG4 and recycled, whereas proteins located on internal surface of the autophagosome are processed for degradation by lysosomal enzymes in autolysosomes. During the maturation process, lysosomal-associated membrane protein 2 and the Ras-related protein Rab-7a facilitate autophagosome fusion with endocytic and lysosomal compartments to form an autolysosome. Autophagic cargo is then degraded through the activity of lysosomal proteases[18-21].

AUTOPHAGY: AN IMPORTANT SWITCH IN CANCER PATHOGENESIS

Autophagy plays crucial roles in the pathogenesis of various human diseases, including cancer, neurodegenerative diseases, infection, and cardiovascular, metabolic, and pulmonary diseases, and aging[22]. The currently accepted hypothesis is that autophagy has dual, contradictory roles in carcinogenesis (Figure 2). First, autophagy is a surveillance mechanism in normal cells, where it acts to protect cells from malignant transformations by removing damaged organelles and aggregated proteins and reducing DNA damage, reactive oxygen species (ROS) and mitochondrial abnormalities. However, autophagy also supports tumor formation by providing access to nutrients that are critical to the metabolism and growth of tumor cells, and by inhibiting cellular death and increasing drug resistance[7,23]. The response of cells to autophagy during cancer metastasis is stage dependent. Autophagy may help to reduce cancer metastasis in the early steps of tumor cell dissemination by promoting inflammatory responses against tumors. Furthermore, autophagy limits tumor necrosis and the expansion of dormant cancer cells into micrometastases, in tandem with impairing oncogene-induced senescence[24]. Autophagy seems to support metastasis during advanced stages of cancer by increasing the survival of detached metastatic cells in the absence of extracellular matrix, and by supporting the dissemination of cancer cells to distant organ sites by triggering tumor cells that lack a connection with the extracellular matrix in the new environment to shift to a dormant state until appropriate conditions occur[24,25].

Autophagy as a suppressor during early stages

Autophagy can prevent the transformation from normal to malignant through several suppressive mechanisms. An appropriate autophagic response is necessary for genome stability and for the clearance of mutagens because it acts to prevent the accumulation of the genetic defects that accompany malignant transformations. Damaged mitochondria and the redox-active aggregates of ubiquitinated proteins are removed by autophagy, resulting in avoidance of the overproduction of highly genotoxic ROS[26]. Inhibition of autophagy switches off this protection and can expose cells to ROS cytotoxicity, which promotes the activation of oncogenes[27,28]. In addition to mitophagy, autophagy supports genomic stability by enabling the discarding of micronuclei that are produced by cell cycle anomalies[29], and it may also promote autophagic cell death, known as type Ⅱ programmed cell death, under certain conditions[30,31].

The impact of autophagy on tumor progression exhibits a significant degree of context dependence[23]. BECN1 gene studies in hormone-related cancers unmasked, for the first time, the possible tumor suppressing role of autophagy[32,33]. There remains significant debate regarding the role of BECN1 as a tumor suppressor due to the proximity of BECN1 to BRCA1, a well-known tumor suppressor gene. Both of these genes are located on

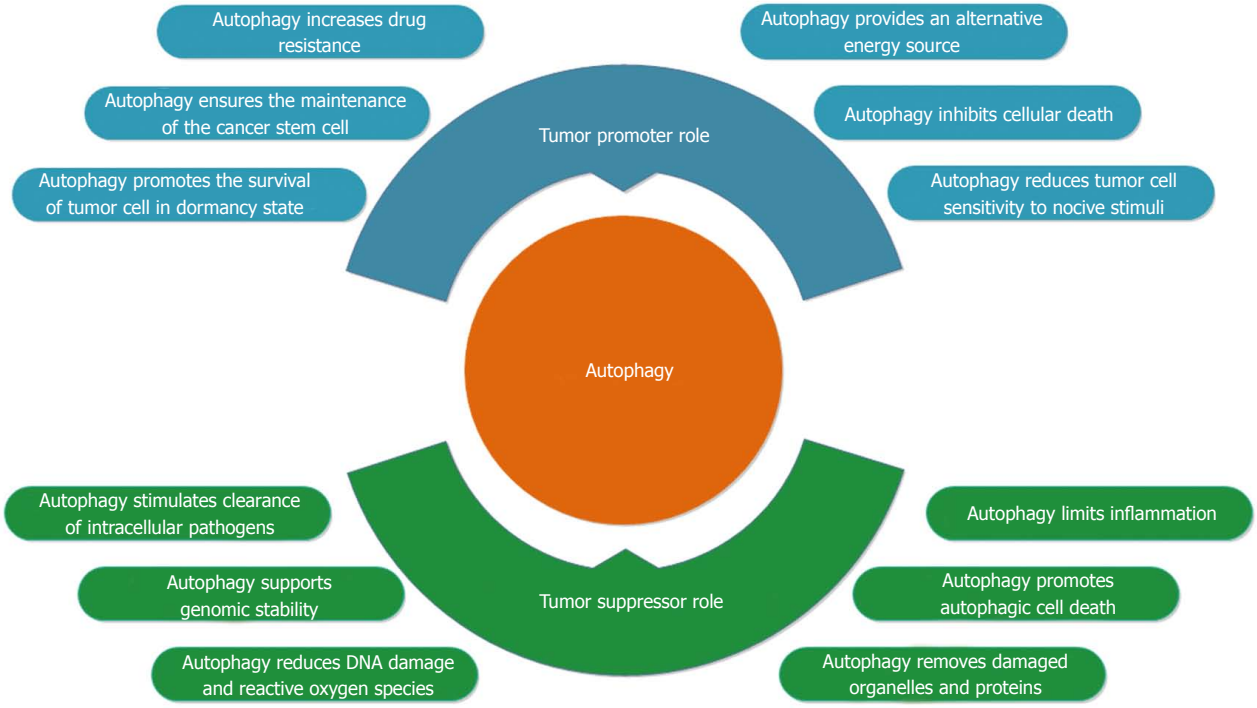


Figure 2 The dual and contradictory roles of autophagy in cancer. Autophagy can potentially act as either a promoter or an inhibitor during the transformation from normal cell to malignant cell. Autophagy supports tumor formation by providing an alternative energy source, increasing drug resistance, inhibiting cell death, promoting the survival of tumor cells in a dormant state and ensuring the maintenance of cancer stem cell compartments. Autophagy protects normal cells from malignant transformation by removing damaged organelles and proteins, reducing DNA damage and reactive oxygen species, supporting genomic stability, promoting autophagic cell death, limiting inflammation and stimulating the clearance of intracellular pathogens.

human chromosome 17q21[34]. The role of autophagy as an important tumor suppressive process that has been demonstrated in murine experiments. Lack of BECN1 gene in embryoid bodies leads to embryonic death[35], and mice with a heterozygotic deletion of BECN1 demonstrate increased susceptibility to tumorigenesis in multiple tissues[36,37]. Similarly, mice deficient for ATG5 and ATG7 died after birth[38,39], while mice with mosaic deletion of ATG5 and liver-specific ATG7-deficient mice developed only benign liver adenomas[40]. Mice lacking autophagy genes ATG5 or ATG7 acquired premalignant pancreatic cancer, while the progression to pancreatic cancer driven by KRasG12D was blocked[41]. ATG7 deletion in a murine model (BrafV600E-induced lung cancer) initially accelerated the proliferation of tumor cells, but at later stages of tumorigenesis it reduced tumor burden, blocked conversion to a more malignant phenotype and increased the life spans of experimental mice[42]. In the absence of autophagy, the advance to cancer can be arrested, resulting in protection from conversion into malignant cells. Progression to a malignant phenotype may require additional genetic alterations[43].

In addition, autophagy is involved in both innate and adaptive immune responses, by which it prevents the establishment and proliferation of malignant cells[44]. Malignant transformation can be stimulated by an inflammatory microenvironment, which contains high amounts of potentially genotoxic ROS as well as various mitogenic cytokines[45]. Autophagy limits inflammation by efficiently disposing of inflammasomes, thereby inhibiting the pro-inflammatory signals that are delivered by some pattern recognition receptors, such as RIG-I-like receptors[46], and limiting the abundance of B-cell CLL/lymphoma 10, a protein that is involved in pro-inflammatory NF-κB signaling[47]. Autophagy ensures a well-coordinated and appropriate response, enabling crucial cells in the immune system to develop properly and to produce interferon, secrete antimicrobial peptides or present antigens to stimulate adaptive immunity. Dying malignant cells may determine innate and/or adaptive antitumor immune responses by recruiting antigen-presenting cells and other cellular components of the immune system. Thus, defects in autophagy may prevent the host immune system from properly recognizing and eliminating premalignant and malignant cells. Moreover, autophagy mediates potent anti-inflammatory effects[48,49].

Autophagy plays a key role in the first line of defense against pathogens and thus has anticarcinogenic effects that combat viral and bacterial infections. A xenophagic response is required for the stimulation of pathogen-specific immune responses and for the rapid clearance of intracellular pathogens[48]. Some of these processes are associated with digestive cancers (*e.g.*, *Helicobacter pylori*, which is associated with gastric carcinoma, or *Streptococcus bovis*, which may cause colorectal carcinoma)[50,51].

Autophagy as a promoting factor during late stages

Autophagy seems to promote malignant progression and resistance to therapy following the initiation of tumor growth[2,27]. As a conserved cellular survival mechanism, tumor cells can use autophagy to provide a backup energy source for survival and expansion[52]. During the progression of tumors, malignant cells are under metabolic stress as a result of a high proliferation rate and exposure to hypoxia, and nutrient deprivation due to inadequate blood supply or selective pressure from therapeutic intervention[53]. Tumor cells usually have a high proliferation rate, which demands more energy and resources than normal cells, and both ATP and metabolites can be obtained by increasing autophagy[54]. Although angiogenesis does occur in tumors, the availability of glucose and glutamine is reduced in some tumor regions due to the leakiness of tumor-associated vessels and continued hypovascularization[55].

Autophagy is activated in the hypoxic areas of tumors, and the inhibition of autophagy by AKT activation or by monoallelic disruption of BECN1 promotes cell death specifically in those regions. These results support hypothesis that tumor cells can use autophagy as a surveillance mechanism under metabolic stress conditions, to provide an alternative energy source for the survival and proliferation of malignant cells[52].

The pro-malignant role of autophagy has been demonstrated in tumor studies in which the inhibition of autophagy was linked to reduced tumor processes. Moreover, down-regulating the expression of essential autophagy proteins impaired tumor growth and led to the accumulation of abnormal mitochondria and reduced oxygen consumption, and autophagy was necessary to support the growth of Ras-driven tumors[56]. However, increased autophagy has also been associated with poor outcomes and short disease-free periods in human pancreatic cancers[57]. *In vitro* studies have shown that the survival of Ras-driven cancer cells requires autophagy and that gaining autophagy results in a marked increase in the survival of malignant cells under conditions of metabolic stress[28]. Inhibiting autophagy by deleting ATG5 prevents the progression of premalignant lesions to cancer in either a p53-independent or p53-dependent manner[41,58]. Furthermore, deletion of ATG7 decreases the tumor growth rate and induces nonmalignant tumor formation. In addition, non-Ras-driven tumoral cell types also need autophagy for survival, and the loss of autophagy has been shown to inhibit malignant tumor development. For example, FIP200 deletion significantly reduced proliferation and suppressed mammary tumor initiation and progression in a mouse model of breast cancer driven by the PyMT oncogene[59]. In a Palb2 knockout mouse model, heterozygous deletion of the autophagy gene BECN1 reduced Palb2-associated mammary tumorigenesis in a p53-dependent manner, indicating that in the presence of DNA damage and oxidative stress, autophagy can support tumor development by suppressing p53[60].

Autophagy can improve the resistance of cancer cells to detachment from the basal membrane, resulting in transformed cells that are less sensitive to therapy-induced cell death. Moreover, this activity sustains the survival of cancer cells that enter a state of dormancy or senescence in response to therapy and ensures the maintenance of the cancer stem cell compartment[23].

Autophagic responses favor the growth and progression of established tumors by reducing their sensitivity to different stimuli that would normally promote their death[61]. KRasG12D-driven pancreatic adenocarcinoma cells that enter a state of dormancy in response to oncogene ablation have recently been shown to activate autophagy to efficiently counteract metabolic stress[62], demonstrating the functional and phenotypic features of cancer stem cells. In addition, mammary cancer stem cells are often characterized by elevated autophagic flux, and their ability to efficiently form tumors *in vivo* appears to rely on autophagy, as tumor formation can be abolished through the genetic inhibition of BECN1 or ATG4A[63,64]. Thus, autophagy may also sustain tumor progression by preserving the viability of the cancer stem cell compartment and/or by promoting the persistence of dormant cancer cells.

Moreover, autophagy is required not only for the emission of immunostimulatory signals by malignant cells succumbing to specific anticancer agents but also for the activation of tumor-targeting innate and adaptive immune responses[49]. Cancer cells that have been isolated from established tumors where autophagy was inhibited were less resistant to exogenous stimuli than their wild-type counterparts[61]. In line with these data, autophagy-deficient tumors are often more sensitive to several chemotherapeutic agents and radiation therapy than their autophagy-proficient counterparts[65,66]. Cancer cells that are exposed to therapeutic interventions can also undergo senescence. Although senescent cells do not proliferate, they may support disease relapse by releasing a wide panel of pro-inflammatory and mitogenic cytokines into the microenvironment[67].

AUTOPHAGY GENE SWITCHES TO CRC

The autophagy machinery involves multiple genes and proteins that have critical functions in complex autophagic pathways, and these genes may be involved in the important switch from normal to colorectal pathology under specific conditions (Table 1).

LC3 gene

The *LC3* gene family encodes three isoforms (*LC3A*, *LC3B*, and *LC3C*) and is the mammalian homologue of yeast ATG8[68]. The isoform *LC3B* is cleaved into the soluble form LC3B-Ⅰ, which is conjugated with PE to generate the lipidated form (LC3B-Ⅱ). LC3B-Ⅱ accumulates specifically on nascent autophagosomes and is one of the most widely and reliably used markers for autophagy[69]. *LC3* was the first autophagy marker proposed to be involved in human CRC[70]. LC3-Ⅱ is overexpressed in CRC compared to normal tissue,

Table 1 Autophagy-related genes in colorectal cancer

Gene/protein	Expression level in colorectal cancer
LC3/LC3-Ⅱ	Higher expression, especially in advanced stages[20]
	Higher expression associated with aggressiveness[71]
	Higher perinuclear expression associated with positive prognosis[77]
	Higher levels in DLD-1 and SW480 CRC lines treated with autophagy inhibitors[72]
	Higher levels in CRC cell lines treated with 5-FU[73]
	Higher levels in CRC cell lines treated with 5-FU and radiotreated[74]
	Lower levels associated with good outcome and treatment response[75,76]
	Negative expression associated with poor clinical outcome and survival[87]
BECN1/ Beclin-1	Higher expression, negatively linked to metastasis[82]
	Higher expression associated with favorable outcome[83]
	Higher expression associated with longer survival in patients treated with 5-FU[84]
	Higher expression associated with a worse survival in patients treated with 5-FU[85]
	Higher expression associated with metastasis and worse prognosis[86]
	Lower levels associated with increased survival in advanced CRC patients treated with cetuximab[75,76]
	Lower levels associated with poor clinical outcome and survival[87]
	Lower levels associated with a good response after chemoradiation in patients with rectal cancer[88]
ATG5	Higher levels associated with lymphovascular invasion[92]
	Lower levels[91]
	Lower expression associated with poor clinical outcome survival[87]
	Lower expression enhanced sensitivity to oxaliplatin[93]
ATG10	Higher expression associated with tumor lymph node metastasis and poor survival[95]
ATG16L1	ATG16L1T300A polymorphism improved overall survival in human CRC patients[116]
BCL2/Bcl-2	Higher levels associated with migration and invasion[105]
	Higher levels associated with resistance to paclitaxel[106]
Bif-1	Lower levels[109]

LC3: Microtubule-associated protein 1 light chain 3; CRC: Colorectal cancer; 5-FU: 5-fluorouracil; Bif-1: Bax-interacting factor 1; BECN1: Beclin 1; ATG5: Autophagy related 5; BCL2: B-cell CLL/lymphoma 2.

especially in advanced stages[20]. Zheng *et al*[71] reported that LC3B-Ⅱ was overexpressed in cancer cells and that autophagy enhanced the aggressiveness of CRC. LC3B expression in the peripheral areas of CRC tissues was correlated with tumor differentiation, growth pattern at the tumor margin, pN and pStage, as well as vessel and nerve plexus invasion. An increased level of LC3-Ⅱ protein was found in DLD-1 and SW480 CRC-derived cell lines that were treated with a combination of autolysosome inhibitors. Association with 3-methyl adenine (3-MA), an inhibitor of PI3K, blocks autophagosome formation and led to increased apoptosis in treated CRC cell lines[72]. The treatment of CRC cell lines with 5-fluorouracil (5-FU) activated the autophagic process as a protective mechanism in cancerous cells, increased LC3-II levels and reduced the rate of apoptosis compared with untreated cell lines, and an increase in the apoptotic rate was induced by adding 3-MA to 5-FU[73]. Similar results were reported by Schonewolf *et al*[74], who reported that both 5-FU treated and radiotreated CRC cell lines showed an increase in autophagy. After adding chloroquine (CQ) to the treatment, these authors reported an increase in the sensitivity of malignant cells to apoptosis. However, in early stages, LC3-Ⅱ expression levels were decreased compared with normal tissue[20]. A low LC3 value has been associated with a good response to treatment and a good survival prognosis, especially in patients with advanced CRC[75,76]. Perinuclear LC3A expression has been shown to be a positive predictor in patients with stage ⅡA-Ⅲ colorectal adenocarcinomas who were treated with only surgery, whereas an increased autophagic response was linked to metastasis and a worse prognosis[77].

BECN1 gene

BECN1, the mammalian orthologue of yeast ATG6, encodes the beclin-1 protein, which exerts its biological activities through three identified structural domains: A Bcl-2 homology domain, a central coiled-coiled domain and an evolutionarily conserved domain[78]. Beclin-1 plays a pivotal role in autophagy as a component of the autophagy class Ⅲ PI3K complex. By interacting with different factors, it regulates autophagy pathways, resulting in the gain (*e.g.*, AMBRA 1, UVRAG) or loss (*e.g.*, Bcl-2) of autophagy. Moreover, beclin-1 dysfunction has been linked to immune disorders, neurodegenerative diseases and cancer[79].

BECN1 plays a controversial role in colorectal carcinomas in that it supports tumorigenesis[80] but may also inhibit CRC cell growth[81]. Higher expression levels of BECN1 have been reported in malignant colorectal tissue than in normal colorectal mucosa[82], with overexpression being especially associated with advanced stages of CRC[75,83-85]. Using immunohistochemistry, Ahn *et al*[80], showed increased BECN1 expression in 95% of colorectal carcinoma samples compared to normal mucosal epithelial tissue, but they found no significant association with invasion, metastasis or stage. High BECN1 expression has been linked to a good prognosis and longer survival in patients with stage ⅢB colorectal carcinoma[83]. Consistent with these findings, an

increased level of BECN1 expression was strongly associated with longer 5-year survival in patients with locally advanced colon carcinomas who were treated with 5-FU chemotherapy for six months after surgery[84]. Overexpression of BECN1 in patients with resected stage Ⅱ and Ⅲ colon carcinomas who were treated with 5-FU-based adjuvant therapy was associated with worse overall survival, supporting a role for autophagy in drug resistance[85]. Moreover, in a meta-analysis, overexpression of BECN1 was associated with a poor prognosis and metastasis in patients with CRC[86]. Furthermore, low levels of BECN1 were correlated with a longer survival in advanced CRC patients who were treated with cetuximab-containing chemotherapy[75,76]. Supporting this hypothesis, a lack of the expression of the autophagy-related proteins LC3B, ATG5 and beclin-1 is associated with poor clinical outcomes and poor survival in CRC patients[87]. Rectal adenocarcinoma patients exhibiting low expression levels of BECN1 were more likely to experience a good response to chemoradiation than patients with increased expression levels of BECN1[88]. Moreover, the expression levels of BECN1 were reduced in a panel of human neoplasms, including brain tumors and gastric and colorectal carcinomas[89].

ATG5 gene

ATG5 protein is encoded by the *ATG5* gene and forms a complex with ATG12 that participates in autophagosome membrane elongation[22]. Mutations in the ATG2B, ATG5, ATG9B, and ATG12 genes have been associated with CRC and gastric cancer[90]. An association between mutations in the ATG5 gene and reduced levels of ATG5 protein expression has been shown in gastrointestinal cancers, including CRC[91]. ATG5 expression was down-regulated in 95% of CRC patients and, interestingly, increased ATG5 expression was associated with lymphovascular invasion[92]. Other research showed that ATG5 is down-regulated in colorectal carcinoma, in both tissue samples and cell lines, and that down-regulation of ATG5 in CRC enhanced sensitivity to oxaliplatin[93]. Heterozygous deletion of ATG5 predisposed mice to intestinal adenoma growth and enhanced the antitumor effect of interferon gamma. In CRC mouse models, treatment with ursolic acid promoted autophagic cell death through a path mediated by ATG5[94].

ATG10 gene

The *ATG10* gene has been mapped to chromosome 5 and encodes an E2 ubiquitin ligase-like enzyme that has essential functions in vesicle elongation, where it catalyzes the conjugation of ATG5 and ATG12[22]. ATG10 was found to be upregulated in CRC tissues and high protein expression of ATG10 was associated with tumor lymph node metastasis and invasion. Moreover, the presence of ATG10 was correlated with poor survival, indicating that ATG10 may be a potential prognostic marker for CRC[95].

AMBRA1 gene

The *AMBRA1* gene encodes the activating molecule in beclin-1-regulated autophagy (Ambra1) protein, which has roles in autophagy, cell growth, cell death, embryonic development and carcinogenesis[96]. AMBRA1 is mutated in a subset of colorectal neoplasms[97].

UVRAG gene

The UV radiation resistance-associated gene (UVRAG) encodes a tumor suppressor protein that induces autophagy by interacting with BECN1. In addition to its function in autophagy, UVRAG is also involved in endocytic trafficking, DNA damage repair and apoptosis[98]. UVRAG, in association with BECN1, supports the maintenance of genomic stability by protecting established CRC cells against radiation-induced DNA damage[99]. UVRAG is heterozygous mutated in a high proportion of gastric and colonic tumors[100,101].

BCL2 gene

The *BCL2* gene encodes the antiapoptotic B-cell lymphoma 2 (Bcl-2) protein, which inhibits autophagy by directly binding to the BH3 domain of beclin-1 and blocking its activity[102]. A recent report suggested that the prosurvival Bcl-2 protein modulates autophagy only indirectly, by inhibiting the apoptosis mediators Bax and Bak[103]. Bcl-2 has been associated with migration and invasion of malignant cells and with the prevention of apoptosis in pT3 CRC patients[104,105]. In addition, the overexpression of Bcl-2 in CRC was correlated with resistance to paclitaxel[106]. Furthermore, the role of Bcl-2 in modulating autophagy has been investigated in different cancer cell lines, including colon carcinoma, where the deletion of the BH4 domain in the Bcl-2 protein in HT29 colon carcinomas was not found to affect tumorigenicity[107].

Bif-1 gene

The *Bif-1* gene encodes Bax-interacting factor (Bif-1), also known as endophilin B1, which is involved in the control of membrane dynamics in cytosolic organelles, such as the Golgi complex and mitochondria, as well as in autophagosomes. Bif-1 induces the formation of autophagosomes and modulates autophagy-enhancing PI3K lipid kinase activity by interaction with beclin-1 through UVRAG[108]. The expression of Bif-1 was found to be reduced in colorectal carcinomas and the loss of Bif-1 suppressed programmed cell death and promoted colon adenocarcinomas. Bif-1 null mice developed normally, with the exception of an enlarged spleen, but they had an increased incidence of spontaneous tumor formation: 82.8% of Bif-1 null mice developed lymphoma compared with 14.3% of their wild-type counterparts[109].

IBD susceptibility genes

Autophagy has also been linked to CRC through inflammatory bowel disease (IBD). In the complex pathogenesis leading to colitis-associated cancer, the

severity of inflammation is a risk factor for CRC[110]. Cytokines released by epithelial and immune cells play an important role, and autophagy can affect the regulation of both inflammation and immune system functions[22]. Autophagy contributes to intestinal homeostasis by ensuring intracellular defenses against microbes, by maintaining the integrity of secretory granules in Paneth cells, and by regulating the inflammasome or mediating antigen presentation[111]. Genome-wide association studies provided the first link between autophagy and IBD by showing that the ATG16L1 T300A polymorphism is associated with an increased risk of Crohn's disease (CD)[112-114]. In addition, IRGM, NOD2, and LRRK2 have been identified as additional markers of CD risk, and autophagy and DAP1 were associated with ulcerative colitis[115]. Recently, the ATG16L1T300A polymorphism was found to improve overall survival in human CRC patients and to enhance the production of type Ⅰ interferon[116].

AUTOPHAGY DRUGS IN CRC

Recent data indicate that only tumors that utilize excessive levels of autophagy, even in nutrient-rich conditions and in the absence of stressful stimuli, respond to autophagy inhibitors *in vivo*[117]. This suggests that only a fraction of cancer patients may benefit from the administration of autophagy inhibitors. Along similar lines, autophagy has been shown to underlie, at least in part, the therapeutic activity of some anticancer regimens[118,119].

Autophagy promotes cancer cell survival under stressful conditions or nutrient deprivation and thus may contribute to chemoresistance. The drugs targeting various autophagy pathways can either induce gain or loss of autophagy. The exaggerated and sustained autophagy that is trigged by anticancer therapies can lead to type Ⅱ cell death in various cancers, including CRC. Increased autophagy in the early stages of cancers can induce protection by suppressing tumorigenesis, necrosis, and chronic inflammation[13]. On the contrary, inhibition of autophagic influx may accelerate the initial steps of tumorigenesis and reduce protein degradation, and as a consequence, the reduced protein turnover might induce the early tumor progression.

In advanced stages, tumor cells use autophagy to survive cellular metabolic stress and to provide essential nutrients to tumor cells that are experiencing ischemia. Therefore, inhibiting autophagy in late-stage cancers can suppress tumor progression by blocking this prosurvival mechanism in nutrient-deprived tumor cells and by preventing protein recycling and cellular growth[120]. On the other hand, inhibition of autophagy can also lead to a decrease in the antitumorigenic activity achieved by promoting non-apoptotic cell death.

This prosurvival autophagy mechanism can be overcome by inhibition. Autophagy-inhibiting compounds include lysosomotropic agents[121]. These agents target acidic compartments, such as lysosomes, but are not specific to tumor cells and therefore have a range of effects on other cells. Lysosomotropic agents cross the lysosomal membrane and are then protonated within the acidic vesicle[122]. This results in an increased pH, which prevents cellular degradation and indirectly inhibits autophagy. Preclinical studies have demonstrated the effects of lysosomotropic agents, including CQ, which include the indirect modulation of late-stage autophagy[123]. Furthermore, CQ inhibits phospholipase A2 and lysophospholipid acylhydrolase, enzymes that are required for the acidification of lysosomes[124].

Treating human colon carcinoma HT29 cells with CQ sensitized mouse colon cancers to antiangiogenic and cytotoxic therapy[93]. Moreover, the combination of CQ and 5-FU displayed a significant advantage over treatment with 5-FU alone in inhibiting tumor growth in colon 26 cells, which are a CRC cell line[125]. A combination of the autophagy inhibitor CQ and vorinostat, a histone deacetylase inhibitor, was shown to significantly reduce tumor growth and induce apoptosis in a colon cancer xenograft model[126]. Notably, the combination of CQ with saracatinib, an inhibitor of Src nonreceptor tyrosine kinase, enhanced apoptotic cell death and resulted in 64% tumor growth inhibition compared with saracatinib alone[127]. Autophagy inhibitors shown synergy with proteasome inhibitors; for example, the simultaneous use of bortezomib and CQ in a colon cancer xenograft model decreased tumor growth to a greater extent than the use of either of these drugs alone[128].

Interestingly, treatment of human HCT-15 colon adenocarcinoma culture cells with B-group soyasaponins induced autophagy and suppressed proliferation through a marked increase in autophagic cell death[129]. In addition to its effects on cell viability and anchorage-independent growth inhibition, the flavonoid quercetin induced autophagic processes in Ha-Ras transformed human colon cells and has been proposed to have anticancer properties[130]. Vitamin D can trigger autophagy by enhancing BECN1 expression and inducing PI3KC3 expression[131]. Cetuximab (an antibody for EGFR) generates autophagy and it is currently used to treat *K-Ras* mutation-negative, EGFR-expressing, metastatic CRC[121]. Moreover, MS-275, a synthetic benzamide derivative of HDAC, promoted Atg7 protein expression and induced autophagy to switch to apoptosis through the modulation of p38 in human colon cancer cells[132].

Curcumin is a natural polyphenolic compound that is isolated from the plant *Curcuma longa*. In addition to apoptosis, curcumin also promotes autophagic cell death type Ⅱ[133] by inhibiting the Akt/mTOR/p70S6K pathway or by activating the ERK1/2 pathway[134]. The proliferation of HT-29 and HCT-15 human colon cancer cell lines was inhibited by curcumin treatment, which arrested the cell cycle in the G2/M phase with no detected apoptosis[135]. Curcumin administered in combination with 5-FU plus oxaliplatin resulted in increased inhibition of growth and enhanced apoptosis

in HCT-116 and HT-29 colon cancer cells compared to each of these drugs alone, and these effects were attained mainly through the attenuation of the EGFR and IGF-1R signaling pathways[136]. The induction of autophagy activation and ROS production was observed in HCT116 human colon cancer cells that were treated with curcumin, and they showed higher mRNA and protein LC3 levels[137].

Autophagy facilitates cancer cell resistance to chemotherapy treatments, and the inhibition of autophagy may resensitize resistant tumor cells to anticancer therapy, thus enhancing the efficacy of the treatment. For example, imatinib induces nonapoptotic autophagic cell death, while the inhibition of autophagy enhances its cytotoxicity, but only at a late stage[138]. Autophagy activation was observed in colon cancer stem cells by analysis of the expression of the intestine-specific transcription factor Cdx1, which plays a crucial role in chemoresistance to paclitaxel[106]. Similarly, autophagy increased resistance to photodynamic therapy-induced apoptosis in CRC stem-like cells[139]. However, this report did not address whether the protective autophagy that was induced in cancer stem cells was due to a drug-mediated response to stress or to the inherent ability of cancer stem cells to maintain a high threshold for autophagy. Suppression of protective autophagy by 3-MA was reported to enhance the therapeutic efficacy of cisplatin and 5-FU in digestive cancers, including colon cancer[140].

Many mTOR inhibitors with effective antitumor activity have been developed. However, they also have downstream effects that include the activation of autophagy, which is linked to prosurvival mechanisms in tumor cells through the recycling of damaged cellular contents. The addition of an autophagy inhibitor could solve this complication by excluding this alternate recovery pathway and sensitizing malignant cells to anticancer therapies[141,142].

Taken together, these observations suggest that autophagy supports the progression of established neoplasms through several mechanisms and that pharmacological inhibitors of autophagy may exert robust antineoplastic effects, at least in some settings.

Future research aimed at exploring the context specific role of autophagy in particular cancer types can provide new opportunities to develop personalized therapeutic strategies based on the regulation of autophagy, and autophagy modulators may become a targetable option for enhancing the efficacy of anticancer therapies used alone or, more likely, in combination with other chemotherapeutic drugs[120].

CONCLUSION

Multiple genes and proteins are involved in the complex steps of autophagy. Recent evidence has suggested that autophagy plays an important role in all stages of carcinogenesis, by influencing initiation, progression and metastatic capacity in tumors. The precise mechanisms that involve autophagy in cancer are not yet defined, and they seem to be context dependent, having both promoting and inhibiting roles. During the first steps of cancer, autophagy may have a suppressive effect, whereas it may alternatively act as tumor promoter during advanced cancer stages. It is necessary to determine how these dual roles of autophagy in CRC are regulated and identify the signals, molecules, and mechanisms that enable autophagy to play a dominant pro-malignant role in one situation and the opposite role in another. The most important research on CRC has been focused on several molecules, mainly LC3, BECN1, ATG5, and these studies have produced conflicting results. Several therapeutic agents that modulate autophagy in CRC have been developed and show promising results supporting their use either alone or, more likely, in combination with other drugs. Further research is required to better understand the relationship between CRC and autophagy, and to produce potentially beneficial agents for the prognosis and therapy of CRC.

REFERENCES

1 **Torre LA**, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87-108 [PMID: 25651787 DOI: 10.3322/caac.21262]

2 **Levine B**, Kroemer G. Autophagy in the pathogenesis of disease. *Cell* 2008; **132**: 27-42 [PMID: 18191218 DOI: 10.1016/j.cell.2007.12.018]

3 **Mizushima N**, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature* 2008; **451**: 1069-1075 [PMID: 18305538 DOI: 10.1038/nature06639]

4 **Maiuri MC**, Zalckvar E, Kimchi A, Kroemer G. Self-eating and self-killing: crosstalk between autophagy and apoptosis. *Nat Rev Mol Cell Biol* 2007; **8**: 741-752 [PMID: 17717517 DOI: 10.1038/nrm2239]

5 **Mizushima N**, Komatsu M. Autophagy: renovation of cells and tissues. *Cell* 2011; **147**: 728-741 [PMID: 22078875 DOI: 10.1016/j.cell.2011.10.026]

6 **Klionsky DJ**. The molecular machinery of autophagy: unanswered questions. *J Cell Sci* 2005; **118**: 7-18 [PMID: 15615779 DOI: 10.1242/jcs.01620]

7 **Panda PK**, Mukhopadhyay S, Das DN, Sinha N, Naik PP, Bhutia SK. Mechanism of autophagic regulation in carcinogenesis and cancer therapeutics. *Semin Cell Dev Biol* 2015; **39**: 43-55 [PMID: 25724561 DOI: 10.1016/j.semcdb.2015.02.013]

8 **Jung CH**, Jun CB, Ro SH, Kim YM, Otto NM, Cao J, Kundu M, Kim DH. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol Biol Cell* 2009; **20**: 1992-2003 [PMID: 19225151 DOI: 10.1091/mbc.E08-12-1249]

9 **Mizushima N**. The role of the Atg1/ULK1 complex in autophagy regulation. *Curr Opin Cell Biol* 2010; **22**: 132-139 [PMID: 20056399 DOI: 10.1016/j.ceb.2009.12.004]

10 **Pattingre S**, Espert L, Biard-Piechaczyk M, Codogno P. Regulation of macroautophagy by mTOR and Beclin 1 complexes. *Biochimie* 2008; **90**: 313-323 [PMID: 17928127 DOI: 10.1016/j.biochi.2007.08.014]

11 **Chan EY**. mTORC1 phosphorylates the ULK1-mAtg13-FIP200 autophagy regulatory complex. *Sci Signal* 2009; **2**: pe51 [PMID: 19690328 DOI: 10.1126/scisignal.284pe51]

12 **Lamb CA**, Yoshimori T, Tooze SA. The autophagosome: origins unknown, biogenesis complex. *Nat Rev Mol Cell Biol* 2013; **14**: 759-774 [PMID: 24201109 DOI: 10.1038/nrm3696]

13 **Yang ZJ**, Chee CE, Huang S, Sinicrope FA. The role of autophagy in cancer: therapeutic implications. *Mol Cancer Ther* 2011;

10: 1533-1541 [PMID: 21878654 DOI: 10.1158/1535-7163.MCT-11-0047]
14 **Tanida I**, Minematsu-Ikeguchi N, Ueno T, Kominami E. Lysosomal turnover, but not a cellular level, of endogenous LC3 is a marker for autophagy. *Autophagy* 2005; **1**: 84-91 [PMID: 16874052]
15 **Geng J**, Klionsky DJ. The Atg8 and Atg12 ubiquitin-like conjugation systems in macroautophagy. 'Protein modifications: beyond the usual suspects' review series. *EMBO Rep* 2008; **9**: 859-864 [PMID: 18704115 DOI: 10.1038/embor.2008.163]
16 **Ren F**, Shu G, Liu G, Liu D, Zhou J, Yuan L, Zhou J. Knockdown of p62/sequestosome 1 attenuates autophagy and inhibits colorectal cancer cell growth. *Mol Cell Biochem* 2014; **385**: 95-102 [PMID: 24065390 DOI: 10.1007/s11010-013-1818-0]
17 **Kabeya Y**, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, Kominami E, Ohsumi Y, Yoshimori T. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J* 2000; **19**: 5720-5728 [PMID: 11060023 DOI: 10.1093/emboj/19.21.5720]
18 **Hanna RA**, Quinsay MN, Orogo AM, Giang K, Rikka S, Gustafsson ÅB. Microtubule-associated protein 1 light chain 3 (LC3) interacts with Bnip3 protein to selectively remove endoplasmic reticulum and mitochondria via autophagy. *J Biol Chem* 2012; **287**: 19094-19104 [PMID: 22505714 DOI: 10.1074/jbc.M111.322933]
19 **Bhutia SK**, Mukhopadhyay S, Sinha N, Das DN, Panda PK, Patra SK, Maiti TK, Mandal M, Dent P, Wang XY, Das SK, Sarkar D, Fisher PB. Autophagy: cancer's friend or foe? *Adv Cancer Res* 2013; **118**: 61-95 [PMID: 23768510 DOI: 10.1016/B978-0-12-407173-5.00003-0]
20 **Chen Z**, Li Y, Zhang C, Yi H, Wu C, Wang J, Liu Y, Tan J, Wen J. Downregulation of Beclin 1 and impairment of autophagy in a small population of colorectal cancer. *Dig Dis Sci* 2013; **58**: 2887-2894 [PMID: 23812859 DOI: 10.1007/s10620-013-2732-8]
21 **Kimura S**, Noda T, Yoshimori T. Dynein-dependent movement of autophagosomes mediates efficient encounters with lysosomes. *Cell Struct Funct* 2008; **33**: 109-122 [PMID: 18388399]
22 **Choi AM**, Ryter SW, Levine B. Autophagy in human health and disease. *N Engl J Med* 2013; **368**: 651-662 [PMID: 23406030 DOI: 10.1056/NEJMra1205406]
23 **Galluzzi L**, Pietrocola F, Bravo-San Pedro JM, Amaravadi RK, Baehrecke EH, Cecconi F, Codogno P, Debnath J, Gewirtz DA, Karantza V, Kimmelman A, Kumar S, Levine B, Maiuri MC, Martin SJ, Penninger J, Piacentini M, Rubinsztein DC, Simon HU, Simonsen A, Thorburn AM, Velasco G, Ryan KM, Kroemer G. Autophagy in malignant transformation and cancer progression. *EMBO J* 2015; **34**: 856-880 [PMID: 25712477 DOI: 10.15252/embj.201490784]
24 **Kenific CM**, Thorburn A, Debnath J. Autophagy and metastasis: another double-edged sword. *Curr Opin Cell Biol* 2010; **22**: 241-245 [PMID: 19945838 DOI: 10.1016/j.ceb.2009.10.008]
25 **Su Z**, Yang Z, Xu Y, Chen Y, Yu Q. Apoptosis, autophagy, necroptosis, and cancer metastasis. *Mol Cancer* 2015; **14**: 48 [PMID: 25743109 DOI: 10.1186/s12943-015-0321-5]
26 **Green DR**, Galluzzi L, Kroemer G. Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. *Science* 2011; **333**: 1109-1112 [PMID: 21868666 DOI: 10.1126/science.1201940]
27 **Mathew R**, Kongara S, Beaudoin B, Karp CM, Bray K, Degenhardt K, Chen G, Jin S, White E. Autophagy suppresses tumor progression by limiting chromosomal instability. *Genes Dev* 2007; **21**: 1367-1381 [PMID: 17510285 DOI: 10.1101/gad.1545107]
28 **Ávalos Y**, Canales J, Bravo-Sagua R, Criollo A, Lavandero S, Quest AF. Tumor suppression and promotion by autophagy. *Biomed Res Int* 2014; **2014**: 603980 [PMID: 25328887 DOI: 10.1155/2014/603980]
29 **Rello-Varona S**, Lissa D, Shen S, Niso-Santano M, Senovilla L, Mariño G, Vitale I, Jemaá M, Harper F, Pierron G, Castedo M, Kroemer G. Autophagic removal of micronuclei. *Cell Cycle* 2012; **11**: 170-176 [PMID: 22185757 DOI: 10.4161/cc.11.1.18564]
30 **Levine B**, Yuan J. Autophagy in cell death: an innocent convict? *J Clin Invest* 2005; **115**: 2679-2688 [PMID: 16200202 DOI: 10.1172/JCI26390]
31 **Kroemer G**, Levine B. Autophagic cell death: the story of a misnomer. *Nat Rev Mol Cell Biol* 2008; **9**: 1004-1010 [PMID: 18971948 DOI: 10.1038/nrm2529]
32 **Mizushima N**, Kuma A, Kobayashi Y, Yamamoto A, Matsubae M, Takao T, Natsume T, Ohsumi Y, Yoshimori T. Mouse Apg16L, a novel WD-repeat protein, targets to the autophagic isolation membrane with the Apg12-Apg5 conjugate. *J Cell Sci* 2003; **116**: 1679-1688 [PMID: 12665549]
33 **Liang XH**, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, Levine B. Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature* 1999; **402**: 672-676 [PMID: 10604474 DOI: 10.1038/45257]
34 **Laddha SV**, Ganesan S, Chan CS, White E. Mutational landscape of the essential autophagy gene BECN1 in human cancers. *Mol Cancer Res* 2014; **12**: 485-490 [PMID: 24478461 DOI: 10.1158/1541-7786.MCR-13-0614]
35 **Qu X**, Zou Z, Sun Q, Luby-Phelps K, Cheng P, Hogan RN, Gilpin C, Levine B. Autophagy gene-dependent clearance of apoptotic cells during embryonic development. *Cell* 2007; **128**: 931-946 [PMID: 17350577 DOI: 10.1016/j.cell.2006.12.044]
36 **Yue Z**, Jin S, Yang C, Levine AJ, Heintz N. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proc Natl Acad Sci USA* 2003; **100**: 15077-15082 [PMID: 14657337 DOI: 10.1073/pnas.2436255100]
37 **Qu X**, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, Rosen J, Eskelinen EL, Mizushima N, Ohsumi Y, Cattoretti G, Levine B. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *J Clin Invest* 2003; **112**: 1809-1820 [PMID: 14638851 DOI: 10.1172/JCI20039]
38 **Kuma A**, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, Ohsumi Y, Tokuhisa T, Mizushima N. The role of autophagy during the early neonatal starvation period. *Nature* 2004; **432**: 1032-1036 [PMID: 15525940 DOI: 10.1038/nature03029]
39 **Komatsu M**, Waguri S, Ueno T, Iwata J, Murata S, Tanida I, Ezaki J, Mizushima N, Ohsumi Y, Uchiyama Y, Kominami E, Tanaka K, Chiba T. Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. *J Cell Biol* 2005; **169**: 425-434 [PMID: 15866887 DOI: 10.1083/jcb.200412022]
40 **Takamura A**, Komatsu M, Hara T, Sakamoto A, Kishi C, Waguri S, Eishi Y, Hino O, Tanaka K, Mizushima N. Autophagy-deficient mice develop multiple liver tumors. *Genes Dev* 2011; **25**: 795-800 [PMID: 21498569 DOI: 10.1101/gad.2016211]
41 **Rosenfeldt MT**, O'Prey J, Morton JP, Nixon C, MacKay G, Mrowinska A, Au A, Rai TS, Zheng L, Ridgway R, Adams PD, Anderson KI, Gottlieb E, Sansom OJ, Ryan KM. p53 status determines the role of autophagy in pancreatic tumour development. *Nature* 2013; **504**: 296-300 [PMID: 24305049 DOI: 10.1038/nature12865]
42 **Strohecker AM**, Guo JY, Karsli-Uzunbas G, Price SM, Chen GJ, Mathew R, McMahon M, White E. Autophagy sustains mitochondrial glutamine metabolism and growth of BrafV600E-driven lung tumors. *Cancer Discov* 2013; **3**: 1272-1285 [PMID: 23965987 DOI: 10.1158/2159-8290.CD-13-0397]
43 **Zhi X**, Zhong Q. Autophagy in cancer. *F1000Prime Rep* 2015; **7**: 18 [PMID: 25750736 DOI: 10.12703/P7-18]
44 **Ma Y**, Galluzzi L, Zitvogel L, Kroemer G. Autophagy and cellular immune responses. *Immunity* 2013; **39**: 211-227 [PMID: 23973220 DOI: 10.1016/j.immuni.2013.07.017]
45 **Coussens LM**, Zitvogel L, Palucka AK. Neutralizing tumor-promoting chronic inflammation: a magic bullet? *Science* 2013; **339**: 286-291 [PMID: 23329041 DOI: 10.1126/science.1232227]
46 **Jounai N**, Takeshita F, Kobiyama K, Sawano A, Miyawaki A, Xin KQ, Ishii KJ, Kawai T, Akira S, Suzuki K, Okuda K. The Atg5 Atg12 conjugate associates with innate antiviral immune responses. *Proc Natl Acad Sci USA* 2007; **104**: 14050-14055 [PMID: 17709747 DOI: 10.1073/pnas.0704014104]
47 **Paul S**, Kashyap AK, Jia W, He YW, Schaefer BC. Selective autophagy of the adaptor protein Bcl10 modulates T cell receptor activation of NF-κB. *Immunity* 2012; **36**: 947-958 [PMID:

22658522 DOI: 10.1016/j.immuni.2012.04.008]
48 **Deretic V**, Saitoh T, Akira S. Autophagy in infection, inflammation and immunity. *Nat Rev Immunol* 2013; **13**: 722-737 [PMID: 24064518 DOI: 10.1038/nri3532]
49 **Kroemer G**, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. *Annu Rev Immunol* 2013; **31**: 51-72 [PMID: 23157435 DOI: 10.1146/annurev-immunol-032712-100008]
50 **Zhang L**, Sung JJ, Yu J, Ng SC, Wong SH, Cho CH, Ng SS, Chan FK, Wu WK. Xenophagy in Helicobacter pylori- and Epstein-Barr virus-induced gastric cancer. *J Pathol* 2014; **233**: 103-112 [PMID: 24633785 DOI: 10.1002/path.4351]
51 **Nakagawa I**, Amano A, Mizushima N, Yamamoto A, Yamaguchi H, Kamimoto T, Nara A, Funao J, Nakata M, Tsuda K, Hamada S, Yoshimori T. Autophagy defends cells against invading group A Streptococcus. *Science* 2004; **306**: 1037-1040 [PMID: 15528445 DOI: 10.1126/science.1103966]
52 **Degenhardt K**, Mathew R, Beaudoin B, Bray K, Anderson D, Chen G, Mukherjee C, Shi Y, Gélinas C, Fan Y, Nelson DA, Jin S, White E. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. *Cancer Cell* 2006; **10**: 51-64 [PMID: 16843265 DOI: 10.1016/j.ccr.2006.06.001]
53 **Naumov GN**, Folkman J, Straume O. Tumor dormancy due to failure of angiogenesis: role of the microenvironment. *Clin Exp Metastasis* 2009; **26**: 51-60 [PMID: 18563595 DOI: 10.1007/s10585-008-9176-0]
54 **White E**. Deconvoluting the context-dependent role for autophagy in cancer. *Nat Rev Cancer* 2012; **12**: 401-410 [PMID: 22534666 DOI: 10.1038/nrc3262]
55 **Jiang X**, Overholtzer M, Thompson CB. Autophagy in cellular metabolism and cancer. *J Clin Invest* 2015; **125**: 47-54 [PMID: 25654550 DOI: 10.1172/JCI73942]
56 **Guo JY**, Chen HY, Mathew R, Fan J, Strohecker AM, Karsli-Uzunbas G, Kamphorst JJ, Chen G, Lemons JM, Karantza V, Coller HA, Dipaola RS, Gelinas C, Rabinowitz JD, White E. Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes Dev* 2011; **25**: 460-470 [PMID: 21317241 DOI: 10.1101/gad.2016311]
57 **Fujii S**, Mitsunaga S, Yamazaki M, Hasebe T, Ishii G, Kojima M, Kinoshita T, Ueno T, Esumi H, Ochiai A. Autophagy is activated in pancreatic cancer cells and correlates with poor patient outcome. *Cancer Sci* 2008; **99**: 1813-1819 [PMID: 18616529 DOI: 10.1111/j.1349-7006.2008.00893.x]
58 **Yang A**, Rajeshkumar NV, Wang X, Yabuuchi S, Alexander BM, Chu GC, Von Hoff DD, Maitra A, Kimmelman AC. Autophagy is critical for pancreatic tumor growth and progression in tumors with p53 alterations. *Cancer Discov* 2014; **4**: 905-913 [PMID: 24875860 DOI: 10.1158/2159-8290.CD-14-0362]
59 **Wei H**, Wei S, Gan B, Peng X, Zou W, Guan JL. Suppression of autophagy by FIP200 deletion inhibits mammary tumorigenesis. *Genes Dev* 2011; **25**: 1510-1527 [PMID: 21764854 DOI: 10.1101/gad.2051011]
60 **Huo Y**, Cai H, Teplova I, Bowman-Colin C, Chen G, Price S, Barnard N, Ganesan S, Karantza V, White E, Xia B. Autophagy opposes p53-mediated tumor barrier to facilitate tumorigenesis in a model of PALB2-associated hereditary breast cancer. *Cancer Discov* 2013; **3**: 894-907 [PMID: 23650262 DOI: 10.1158/2159-8290.CD-13-0011]
61 **Kroemer G**, Mariño G, Levine B. Autophagy and the integrated stress response. *Mol Cell* 2010; **40**: 280-293 [PMID: 20965422 DOI: 10.1016/j.molcel.2010.09.023]
62 **Viale A**, Pettazzoni P, Lyssiotis CA, Ying H, Sánchez N, Marchesini M, Carugo A, Green T, Seth S, Giuliani V, Kost-Alimova M, Muller F, Colla S, Nezi L, Genovese G, Deem AK, Kapoor A, Yao W, Brunetto E, Kang Y, Yuan M, Asara JM, Wang YA, Heffernan TP, Kimmelman AC, Wang H, Fleming JB, Cantley LC, DePinho RA, Draetta GF. Oncogene ablation-resistant pancreatic cancer cells depend on mitochondrial function. *Nature* 2014; **514**: 628-632 [PMID: 25119024 DOI: 10.1038/nature13611]
63 **Gong C**, Bauvy C, Tonelli G, Yue W, Deloménie C, Nicolas V, Zhu Y, Domergue V, Marin-Esteban V, Tharinger H, Delbos L, Gary-Gouy H, Morel AP, Ghavami S, Song E, Codogno P, Mehrpour M. Beclin 1 and autophagy are required for the tumorigenicity of breast cancer stem-like/progenitor cells. *Oncogene* 2013; **32**: 2261-2272, 2261-2272, [PMID: 22733132 DOI: 10.1038/onc.2012.252]
64 **Wolf J**, Dewi DL, Fredebohm J, Müller-Decker K, Flechtenmacher C, Hoheisel JD, Boettcher M. A mammosphere formation RNAi screen reveals that ATG4A promotes a breast cancer stem-like phenotype. *Breast Cancer Res* 2013; **15**: R109 [PMID: 24229464 DOI: 10.1186/bcr3576]
65 **Ko A**, Kanehisa A, Martins I, Senovilla L, Chargari C, Dugue D, Mariño G, Kepp O, Michaud M, Perfettini JL, Kroemer G, Deutsch E. Autophagy inhibition radiosensitizes in vitro, yet reduces radioresponses in vivo due to deficient immunogenic signalling. *Cell Death Differ* 2014; **21**: 92-99 [PMID: 24037090 DOI: 10.1038/cdd.2013.124]
66 **Levy JM**, Thompson JC, Griesinger AM, Amani V, Donson AM, Birks DK, Morgan MJ, Mirsky DM, Handler MH, Foreman NK, Thorburn A. Autophagy inhibition improves chemosensitivity in BRAF(V600E) brain tumors. *Cancer Discov* 2014; **4**: 773-780 [PMID: 24823863 DOI: 10.1158/2159-8290.CD-14-0049]
67 **López-Otín C**, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell* 2013; **153**: 1194-1217 [PMID: 23746838 DOI: 10.1016/j.cell.2013.05.039]
68 **Wild P**, McEwan DG, Dikic I. The LC3 interactome at a glance. *J Cell Sci* 2014; **127**: 3-9 [PMID: 24345374 DOI: 10.1242/jcs.140426]
69 **Klionsky DJ**, Cuervo AM, Seglen PO. Methods for monitoring autophagy from yeast to human. *Autophagy* 2007; **3**: 181-206 [PMID: 17224625]
70 **Tanida I**, Ueno T, Kominami E. LC3 conjugation system in mammalian autophagy. *Int J Biochem Cell Biol* 2004; **36**: 2503-2518 [PMID: 15325588 DOI: 10.1016/j.biocel.2004.05.009]
71 **Zheng HY**, Zhang XY, Wang XF, Sun BC. Autophagy enhances the aggressiveness of human colorectal cancer cells and their ability to adapt to apoptotic stimulus. *Cancer Biol Med* 2012; **9**: 105-110 [PMID: 23691463 DOI: 10.3969/j.issn.2095-3941.2012.02.004]
72 **Sato K**, Tsuchihara K, Fujii S, Sugiyama M, Goya T, Atomi Y, Ueno T, Ochiai A, Esumi H. Autophagy is activated in colorectal cancer cells and contributes to the tolerance to nutrient deprivation. *Cancer Res* 2007; **67**: 9677-9684 [PMID: 17942897 DOI: 10.1158/0008-5472.CAN-07-1462]
73 **Li J**, Hou N, Faried A, Tsutsumi S, Kuwano H. Inhibition of autophagy augments 5-fluorouracil chemotherapy in human colon cancer in vitro and in vivo model. *Eur J Cancer* 2010; **46**: 1900-1909 [PMID: 20231086 DOI: 10.1016/j.ejca.2010.02.021]
74 **Schonewolf CA**, Mehta M, Schiff D, Wu H, Haffty BG, Karantza V, Jabbour SK. Autophagy inhibition by chloroquine sensitizes HT-29 colorectal cancer cells to concurrent chemoradiation. *World J Gastrointest Oncol* 2014; **6**: 74-82 [PMID: 24653797 DOI: 10.4251/wjgo.v6.i3.74]
75 **Guo GF**, Jiang WQ, Zhang B, Cai YC, Xu RH, Chen XX, Wang F, Xia LP. Autophagy-related proteins Beclin-1 and LC3 predict cetuximab efficacy in advanced colorectal cancer. *World J Gastroenterol* 2011; **17**: 4779-4786 [PMID: 22147978 DOI: 10.3748/wjg.v17.i43.4779]
76 **Yang M**, Zhao H, Guo L, Zhang Q, Zhao L, Bai S, Zhang M, Xu S, Wang F, Wang X, Zhao B. Autophagy-based survival prognosis in human colorectal carcinoma. *Oncotarget* 2015; **6**: 7084-7103 [PMID: 25762626]
77 **Giatromanolaki A**, Koukourakis MI, Harris AL, Polychronidis A, Gatter KC, Sivridis E. Prognostic relevance of light chain 3 (LC3A) autophagy patterns in colorectal adenocarcinomas. *J Clin Pathol* 2010; **63**: 867-872 [PMID: 20876316 DOI: 10.1136/jcp.2010.079525]
78 **Kang R**, Zeh HJ, Lotze MT, Tang D. The Beclin 1 network regulates autophagy and apoptosis. *Cell Death Differ* 2011; **18**: 571-580 [PMID: 21311563 DOI: 10.1038/cdd.2010.191]
79 **Sahni S**, Merlot AM, Krishan S, Jansson PJ, Richardson DR. Gene of the month: BECN1. *J Clin Pathol* 2014; **67**: 656-660 [PMID: 24811486 DOI: 10.1136/jclinpath-2014-202356]
80 **Ahn CH**, Jeong EG, Lee JW, Kim MS, Kim SH, Kim SS, Yoo NJ,

Lee SH. Expression of beclin-1, an autophagy-related protein, in gastric and colorectal cancers. *APMIS* 2007; **115**: 1344-1349 [PMID: 18184403 DOI: 10.1111/j.1600-0463.2007.00858.x]
81 **Koneri K**, Goi T, Hirono Y, Katayama K, Yamaguchi A. Beclin 1 gene inhibits tumor growth in colon cancer cell lines. *Anticancer Res* 2007; **27**: 1453-1457 [PMID: 17595761]
82 **Zhang MY**, Gou WF, Zhao S, Mao XY, Zheng ZH, Takano Y, Zheng HC. Beclin 1 expression is closely linked to colorectal carcinogenesis and distant metastasis of colorectal carcinoma. *Int J Mol Sci* 2014; **15**: 14372-14385 [PMID: 25196438 DOI: 10.3390/ijms150814372]
83 **Yang Z**, Ghoorun RA, Fan X, Wu P, Bai Y, Li J, Chen H, Wang L, Wang J. High expression of Beclin-1 predicts favorable prognosis for patients with colorectal cancer. *Clin Res Hepatol Gastroenterol* 2015; **39**: 98-106 [PMID: 25130795 DOI: 10.1016/j.clinre.2014.06.014]
84 **Li BX**, Li CY, Peng RQ, Wu XJ, Wang HY, Wan DS, Zhu XF, Zhang XS. The expression of beclin 1 is associated with favorable prognosis in stage IIIB colon cancers. *Autophagy* 2009; **5**: 303-306 [PMID: 19066461]
85 **Park JM**, Huang S, Wu TT, Foster NR, Sinicrope FA. Prognostic impact of Beclin 1, p62/sequestosome 1 and LC3 protein expression in colon carcinomas from patients receiving 5-fluorouracil as adjuvant chemotherapy. *Cancer Biol Ther* 2013; **14**: 100-107 [PMID: 23192274 DOI: 10.4161/cbt.22954]
86 **Han Y**, Xue XF, Shen HG, Guo XB, Wang X, Yuan B, Guo XP, Kuang YT, Zhi QM, Zhao H. Prognostic significance of Beclin-1 expression in colorectal cancer: a meta-analysis. *Asian Pac J Cancer Prev* 2014; **15**: 4583-4587 [PMID: 24969889]
87 **Choi JH**, Cho YS, Ko YH, Hong SU, Park JH, Lee MA. Absence of autophagy-related proteins expression is associated with poor prognosis in patients with colorectal adenocarcinoma. *Gastroenterol Res Pract* 2014; **2014**: 179586 [PMID: 24723943 DOI: 10.1155/2014/179586]
88 **Zaanan A**, Park JM, Tougeron D, Huang S, Wu TT, Foster NR, Sinicrope FA. Association of beclin 1 expression with response to neoadjuvant chemoradiation therapy in patients with locally advanced rectal carcinoma. *Int J Cancer* 2015; **137**: 1498-1502 [PMID: 25708267 DOI: 10.1002/ijc.29496]
89 **Rubinsztein DC**, Codogno P, Levine B. Autophagy modulation as a potential therapeutic target for diverse diseases. *Nat Rev Drug Discov* 2012; **11**: 709-730 [PMID: 22935804 DOI: 10.1038/nrd3802]
90 **Kang MR**, Kim MS, Oh JE, Kim YR, Song SY, Kim SS, Ahn CH, Yoo NJ, Lee SH. Frameshift mutations of autophagy-related genes ATG2B, ATG5, ATG9B and ATG12 in gastric and colorectal cancers with microsatellite instability. *J Pathol* 2009; **217**: 702-706 [PMID: 19197948 DOI: 10.1002/path.2509]
91 **An CH**, Kim MS, Yoo NJ, Park SW, Lee SH. Mutational and expressional analyses of ATG5, an autophagy-related gene, in gastrointestinal cancers. *Pathol Res Pract* 2011; **207**: 433-437 [PMID: 21664058 DOI: 10.1016/j.prp.2011.05.002]
92 **Cho DH**, Jo YK, Kim SC, Park IJ, Kim JC. Down-regulated expression of ATG5 in colorectal cancer. *Anticancer Res* 2012; **32**: 4091-4096 [PMID: 22993366]
93 **Selvakumaran M**, Amaravadi RK, Vasilevskaya IA, O'Dwyer PJ. Autophagy inhibition sensitizes colon cancer cells to antiangiogenic and cytotoxic therapy. *Clin Cancer Res* 2013; **19**: 2995-3007 [PMID: 23461901 DOI: 10.1158/1078-0432.CCR-12-1542]
94 **Wang L**, Wang Y, Lu Y, Zhang Q, Qu X. Heterozygous deletion of ATG5 in Apc(Min/+) mice promotes intestinal adenoma growth and enhances the antitumor efficacy of interferon-gamma. *Cancer Biol Ther* 2015; **16**: 383-391 [PMID: 25695667 DOI: 10.1080/15384047.2014.1002331]
95 **Jo YK**, Kim SC, Park IJ, Park SJ, Jin DH, Hong SW, Cho DH, Kim JC. Increased expression of ATG10 in colorectal cancer is associated with lymphovascular invasion and lymph node metastasis. *PLoS One* 2012; **7**: e52705 [PMID: 23285162 DOI: 10.1371/journal.pone.0052705]
96 **Cianfanelli V**, De Zio D, Di Bartolomeo S, Nazio F, Strappazzon F, Cecconi F. Ambra1 at a glance. *J Cell Sci* 2015; **128**: 2003-2008 [PMID: 26034061 DOI: 10.1242/jcs.168153]
97 **Cianfanelli V**, Fuoco C, Lorente M, Salazar M, Quondamatteo F, Gherardini PF, De Zio D, Nazio F, Antonioli M, D'Orazio M, Skobo T, Bordi M, Rohde M, Dalla Valle L, Helmer-Citterich M, Gretzmeier C, Dengjel J, Fimia GM, Piacentini M, Di Bartolomeo S, Velasco G, Cecconi F. AMBRA1 links autophagy to cell proliferation and tumorigenesis by promoting c-Myc dephosphorylation and degradation. *Nat Cell Biol* 2015; **17**: 20-30 [PMID: 25438055 DOI: 10.1038/ncb3072]
98 **Liang C**, Lee JS, Inn KS, Gack MU, Li Q, Roberts EA, Vergne I, Deretic V, Feng P, Akazawa C, Jung JU. Beclin1-binding UVRAG targets the class C Vps complex to coordinate autophagosome maturation and endocytic trafficking. *Nat Cell Biol* 2008; **10**: 776-787 [PMID: 18552835 DOI: 10.1038/ncb1740]
99 **Park JM**, Tougeron D, Huang S, Okamoto K, Sinicrope FA. Beclin 1 and UVRAG confer protection from radiation-induced DNA damage and maintain centrosome stability in colorectal cancer cells. *PLoS One* 2014; **9**: e100819 [PMID: 24956373 DOI: 10.1371/journal.pone.0100819]
100 **Kim MS**, Jeong EG, Ahn CH, Kim SS, Lee SH, Yoo NJ. Frameshift mutation of UVRAG, an autophagy-related gene, in gastric carcinomas with microsatellite instability. *Hum Pathol* 2008; **39**: 1059-1063 [PMID: 18495205 DOI: 10.1016/j.humpath.2007.11.013]
101 **Liang C**, Feng P, Ku B, Dotan I, Canaani D, Oh BH, Jung JU. Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. *Nat Cell Biol* 2006; **8**: 688-699 [PMID: 16799551 DOI: 10.1038/ncb1426]
102 **Green DR**, Levine B. To be or not to be? How selective autophagy and cell death govern cell fate. *Cell* 2014; **157**: 65-75 [PMID: 24679527 DOI: 10.1016/j.cell.2014.02.049]
103 **Lindqvist LM**, Heinlein M, Huang DC, Vaux DL. Prosurvival Bcl-2 family members affect autophagy only indirectly, by inhibiting Bax and Bak. *Proc Natl Acad Sci USA* 2014; **111**: 8512-8517 [PMID: 24912196 DOI: 10.1073/pnas.1406425111]
104 **Sadowska A**, Car H, Pryczynicz A, Guzińska-Ustymowicz K, Kowal KW, Cepowicz D, Kędra B. Expression of apoptotic proteins in human colorectal cancer and metastatic lymph nodes. *Pathol Res Pract* 2014; **210**: 576-581 [PMID: 24939147 DOI: 10.1016/j.prp.2014.04.023]
105 **Koehler BC**, Scherr AL, Lorenz S, Urbanik T, Kautz N, Elssner C, Welte S, Bermejo JL, Jäger D, Schulze-Bergkamen H. Beyond cell death - antiapoptotic Bcl-2 proteins regulate migration and invasion of colorectal cancer cells in vitro. *PLoS One* 2013; **8**: e76446 [PMID: 24098503 DOI: 10.1371/journal.pone.0076446]
106 **Wu S**, Wang X, Chen J, Chen Y. Autophagy of cancer stem cells is involved with chemoresistance of colon cancer cells. *Biochem Biophys Res Commun* 2013; **434**: 898-903 [PMID: 23624503 DOI: 10.1016/j.bbrc.2013.04.053]
107 **Trisciuoglio D**, De Luca T, Desideri M, Passeri D, Gabellini C, Scarpino S, Liang C, Orlandi A, Del Bufalo D. Removal of the BH4 domain from Bcl-2 protein triggers an autophagic process that impairs tumor growth. *Neoplasia* 2013; **15**: 315-327 [PMID: 23479509]
108 **Takahashi Y**, Coppola D, Matsushita N, Cualing HD, Sun M, Sato Y, Liang C, Jung JU, Cheng JQ, Mulé JJ, Pledger WJ, Wang HG. Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis. *Nat Cell Biol* 2007; **9**: 1142-1151 [PMID: 17891140 DOI: 10.1038/ncb1634]
109 **Coppola D**, Khalil F, Eschrich SA, Boulware D, Yeatman T, Wang HG. Down-regulation of Bax-interacting factor-1 in colorectal adenocarcinoma. *Cancer* 2008; **113**: 2665-2670 [PMID: 18833585 DOI: 10.1002/cncr.23892]
110 **Rutter M**, Saunders B, Wilkinson K, Rumbles S, Schofield G, Kamm M, Williams C, Price A, Talbot I, Forbes A. Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. *Gastroenterology* 2004; **126**: 451-459 [PMID: 14762782]
111 **Gardet A**, Xavier RJ. Common alleles that influence autophagy and the risk for inflammatory bowel disease. *Curr Opin Immunol* 2012;

24: 522-529 [PMID: 23041451 DOI: 10.1016/j.coi.2012.08.001]
112 **Hampe J**, Franke A, Rosenstiel P, Till A, Teuber M, Huse K, Albrecht M, Mayr G, De La Vega FM, Briggs J, Günther S, Prescott NJ, Onnie CM, Häsler R, Sipos B, Fölsch UR, Lengauer T, Platzer M, Mathew CG, Krawczak M, Schreiber S. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. *Nat Genet* 2007; **39**: 207-211 [PMID: 17200669 DOI: 10.1038/ng1954]
113 **Rioux JD**, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A, Green T, Kuballa P, Barmada MM, Datta LW, Shugart YY, Griffiths AM, Targan SR, Ippoliti AF, Bernard EJ, Mei L, Nicolae DL, Regueiro M, Schumm LP, Steinhart AH, Rotter JI, Duerr RH, Cho JH, Daly MJ, Brant SR. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat Genet* 2007; **39**: 596-604 [PMID: 17435756 DOI: 10.1038/ng2032]
114 **Barrett JC**, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, Brant SR, Silverberg MS, Taylor KD, Barmada MM, Bitton A, Dassopoulos T, Datta LW, Green T, Griffiths AM, Kistner EO, Murtha MT, Regueiro MD, Rotter JI, Schumm LP, Steinhart AH, Targan SR, Xavier RJ, Libioulle C, Sandor C, Lathrop M, Belaiche J, Dewit O, Gut I, Heath S, Laukens D, Mni M, Rutgeerts P, Van Gossum A, Zelenika D, Franchimont D, Hugot JP, de Vos M, Vermeire S, Louis E, Cardon LR, Anderson CA, Drummond H, Nimmo E, Ahmad T, Prescott NJ, Onnie CM, Fisher SA, Marchini J, Ghori J, Bumpstead S, Gwilliam R, Tremelling M, Deloukas P, Mansfield J, Jewell D, Satsangi J, Mathew CG, Parkes M, Georges M, Daly MJ. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet* 2008; **40**: 955-962 [PMID: 18587394 DOI: 10.1038/ng.175]
115 **Anderson CA**, Boucher G, Lees CW, Franke A, D'Amato M, Taylor KD, Lee JC, Goyette P, Imielinski M, Latiano A, Lagace C, Scott R, Amininejad L, Bumpstead S, Baidoo L, Baldassano RN, Barclay M, Bayless TM, Brand S, Buning C, Colombel JF, Denson LA, De Vos M, Dubinsky M, Edwards C, Ellinghaus D, Fehrmann RS, Floyd JA, Florin T, Franchimont D, Franke L, Georges M, Glas J, Glazer NL, Guthery SL, Haritunians T, Hayward NK, Hugot JP, Jobin G, Laukens D, Lawrance I, Lemann M, Levine A, Libioulle C, Louis E, McGovern DP, Milla M, Montgomery GW, Morley KI, Mowat C, Ng A, Newman W, Ophoff RA, Papi L, Palmieri O, Peyrin-Biroulet L, Panes J, Phillips A, Prescott NJ, Proctor DD, Roberts R, Russell R, Rutgeerts P, Sanderson J, Sans M, Schumm P, Seibold F, Sharma Y, Simms LA, Seielstad M, Steinhart AH, Targan SR, van den Berg LH, Vatn M, Verspaget H, Walters T, Wijmenga C, Wilson DC, Westra HJ, Xavier RJ, Zhao ZZ, Ponsioen CY, Andersen V, Torkvist L, Gazouli M, Anagnou NP, Karlsen TH, Kupcinskas L, Sventoraityte J, Mansfield JC, Kugathasan S, Silverberg MS, Halfvarson J, Rotter JI, Mathew CG, Griffiths AM, Gearry R, Ahmad T, Brant SR, Chamaillard M, Satsangi J, Cho JH, Schreiber S, Daly MJ, Barrett JC, Parkes M, Annese V, Hakonarson H, Radford-Smith G, Duerr RH, Vermeire S, Weersma RK, Rioux JD. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nat Genet* 2011; **43**: 246-252 [PMID: 21297633 PMCID: 3084597 DOI: 10.1038/ng.764]
116 **Grimm WA**, Messer JS, Murphy SF, Nero T, Lodolce JP, Weber CR, Logsdon MF, Bartulis S, Sylvester BE, Springer A, Dougherty U, Niewold TB, Kupfer SS, Ellis N, Huo D, Bissonnette M, Boone DL. The Thr300Ala variant in ATG16L1 is associated with improved survival in human colorectal cancer and enhanced production of type I interferon. *Gut* 2015 Feb 2; Epub ahead of print [PMID: 25645662 DOI: 10.1136/gutjnl-2014-308735]
117 **Maycotte P**, Aryal S, Cummings CT, Thorburn J, Morgan MJ, Thorburn A. Chloroquine sensitizes breast cancer cells to chemotherapy independent of autophagy. *Autophagy* 2012; **8**: 200-212 [PMID: 22252008 DOI: 10.4161/auto.8.2.18554]
118 **Salazar M**, Carracedo A, Salanueva IJ, Hernández-Tiedra S, Lorente M, Egia A, Vázquez P, Blázquez C, Torres S, García S, Nowak J, Fimia GM, Piacentini M, Cecconi F, Pandolfi PP, González-Feria L, Iovanna JL, Guzmán M, Boya P, Velasco G. Cannabinoid action induces autophagy-mediated cell death through stimulation of ER stress in human glioma cells. *J Clin Invest* 2009; **119**: 1359-1372 [PMID: 19425170]
119 **Vara D**, Salazar M, Olea-Herrero N, Guzmán M, Velasco G, Díaz-Laviada I. Anti-tumoral action of cannabinoids on hepatocellular carcinoma: role of AMPK-dependent activation of autophagy. *Cell Death Differ* 2011; **18**: 1099-1111 [PMID: 21475304 DOI: 10.1038/cdd.2011.32]
120 **Radogna F**, Dicato M, Diederich M. Cancer-type-specific crosstalk between autophagy, necroptosis and apoptosis as a pharmacological target. *Biochem Pharmacol* 2015; **94**: 1-11 [PMID: 25562745 DOI: 10.1016/j.bcp.2014.12.018]
121 **Duffy A**, Le J, Sausville E, Emadi A. Autophagy modulation: a target for cancer treatment development. *Cancer Chemother Pharmacol* 2015; **75**: 439-447 [PMID: 25422156 DOI: 10.1007/s00280-014-2637-z]
122 **Kaufmann AM**, Krise JP. Lysosomal sequestration of amine-containing drugs: analysis and therapeutic implications. *J Pharm Sci* 2007; **96**: 729-746 [PMID: 17117426 DOI: 10.1002/jps.20792]
123 **Rosich L**, Xargay-Torrent S, López-Guerra M, Campo E, Colomer D, Roué G. Counteracting autophagy overcomes resistance to everolimus in mantle cell lymphoma. *Clin Cancer Res* 2012; **18**: 5278-5289 [PMID: 22879389 DOI: 10.1158/1078-0432.CCR-12-0351]
124 **Sotelo J**, Briceño E, López-González MA. Adding chloroquine to conventional treatment for glioblastoma multiforme: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 2006; **144**: 337-343 [PMID: 16520474]
125 **Sasaki K**, Tsuno NH, Sunami E, Kawai K, Hongo K, Hiyoshi M, Kaneko M, Murono K, Tada N, Nirei T, Takahashi K, Kitayama J. Resistance of colon cancer to 5-fluorouracil may be overcome by combination with chloroquine, an in vivo study. *Anticancer Drugs* 2012; **23**: 675-682 [PMID: 22561420 DOI: 10.1097/CAD.0b013e328353f8c7]
126 **Carew JS**, Medina EC, Esquivel JA, Mahalingam D, Swords R, Kelly K, Zhang H, Huang P, Mita AC, Mita MM, Giles FJ, Nawrocki ST. Autophagy inhibition enhances vorinostat-induced apoptosis via ubiquitinated protein accumulation. *J Cell Mol Med* 2010; **14**: 2448-2459 [PMID: 19583815 DOI: 10.1111/j.1582-4934.2009.00832.x]
127 **Wu Z**, Chang PC, Yang JC, Chu CY, Wang LY, Chen NT, Ma AH, Desai SJ, Lo SH, Evans CP, Lam KS, Kung HJ. Autophagy Blockade Sensitizes Prostate Cancer Cells towards Src Family Kinase Inhibitors. *Genes Cancer* 2010; **1**: 40-49 [PMID: 20811583 DOI: 10.1177/1947601909358324]
128 **Ding WX**, Ni HM, Gao W, Chen X, Kang JH, Stolz DB, Liu J, Yin XM. Oncogenic transformation confers a selective susceptibility to the combined suppression of the proteasome and autophagy. *Mol Cancer Ther* 2009; **8**: 2036-2045 [PMID: 19584239 DOI: 10.1158/1535-7163.MCT-08-1169]
129 **Ellington AA**, Berhow M, Singletary KW. Induction of macroautophagy in human colon cancer cells by soybean B-group triterpenoid saponins. *Carcinogenesis* 2005; **26**: 159-167 [PMID: 15471899 DOI: 10.1093/carcin/bgh297]
130 **Psahoulia FH**, Moumtzi S, Roberts ML, Sasazuki T, Shirasawa S, Pintzas A. Quercetin mediates preferential degradation of oncogenic Ras and causes autophagy in Ha-RAS-transformed human colon cells. *Carcinogenesis* 2007; **28**: 1021-1031 [PMID: 17148506 DOI: 10.1093/carcin/bgl232]
131 **Wang J**, Lian H, Zhao Y, Kauss MA, Spindel S. Vitamin D3 induces autophagy of human myeloid leukemia cells. *J Biol Chem* 2008; **283**: 25596-25605 [PMID: 18628207 DOI: 10.1074/jbc.M801716200]
132 **Zhan Y**, Gong K, Chen C, Wang H, Li W. P38 MAP kinase functions as a switch in MS-275-induced reactive oxygen species-dependent autophagy and apoptosis in human colon cancer cells. *Free Radic Biol Med* 2012; **53**: 532-543 [PMID: 22634147 DOI: 10.1016/j.freeradbiomed.2012.05.018]
133 **Reuter S**, Eifes S, Dicato M, Aggarwal BB, Diederich M. Modulation of anti-apoptotic and survival pathways by curcumin as

a strategy to induce apoptosis in cancer cells. *Biochem Pharmacol* 2008; **76**: 1340-1351 [PMID: 18755156 DOI: 10.1016/j.bcp.2008.07.031]

134 **Aoki H**, Takada Y, Kondo S, Sawaya R, Aggarwal BB, Kondo Y. Evidence that curcumin suppresses the growth of malignant gliomas in vitro and in vivo through induction of autophagy: role of Akt and extracellular signal-regulated kinase signaling pathways. *Mol Pharmacol* 2007; **72**: 29-39 [PMID: 17395690 DOI: 10.1124/mol.106.033167]

135 **Hanif R**, Qiao L, Shiff SJ, Rigas B. Curcumin, a natural plant phenolic food additive, inhibits cell proliferation and induces cell cycle changes in colon adenocarcinoma cell lines by a prostaglandin-independent pathway. *J Lab Clin Med* 1997; **130**: 576-584 [PMID: 9422331]

136 **Patel BB**, Sengupta R, Qazi S, Vachhani H, Yu Y, Rishi AK, Majumdar AP. Curcumin enhances the effects of 5-fluorouracil and oxaliplatin in mediating growth inhibition of colon cancer cells by modulating EGFR and IGF-1R. *Int J Cancer* 2008; **122**: 267-273 [PMID: 17918158 DOI: 10.1002/ijc.23097]

137 **Lee YJ**, Kim NY, Suh YA, Lee C. Involvement of ROS in Curcumin-induced Autophagic Cell Death. *Korean J Physiol Pharmacol* 2011; **15**: 1-7 [PMID: 21461234 DOI: 10.4196/kjpp.2011.15.1.1]

138 **Shingu T**, Fujiwara K, Bögler O, Akiyama Y, Moritake K, Shinojima N, Tamada Y, Yokoyama T, Kondo S. Inhibition of autophagy at a late stage enhances imatinib-induced cytotoxicity in human malignant glioma cells. *Int J Cancer* 2009; **124**: 1060-1071 [PMID: 19048625 DOI: 10.1002/ijc.24030]

139 **Wei MF**, Chen MW, Chen KC, Lou PJ, Lin SY, Hung SC, Hsiao M, Yao CJ, Shieh MJ. Autophagy promotes resistance to photodynamic therapy-induced apoptosis selectively in colorectal cancer stem-like cells. *Autophagy* 2014; **10**: 1179-1192 [PMID: 24905352 DOI: 10.4161/auto.28679]

140 **Li J**, Hou N, Faried A, Tsutsumi S, Takeuchi T, Kuwano H. Inhibition of autophagy by 3-MA enhances the effect of 5-FU-induced apoptosis in colon cancer cells. *Ann Surg Oncol* 2009; **16**: 761-771 [PMID: 19116755 DOI: 10.1245/s10434-008-0260-0]

141 **Xie X**, White EP, Mehnert JM. Coordinate autophagy and mTOR pathway inhibition enhances cell death in melanoma. *PLoS One* 2013; **8**: e55096 [PMID: 23383069 DOI: 10.1371/journal.pone.0055096]

142 **Vignot S**, Faivre S, Aguirre D, Raymond E. mTOR-targeted therapy of cancer with rapamycin derivatives. *Ann Oncol* 2005; **16**: 525-537 [PMID: 15728109 DOI: 10.1093/annonc/mdi113]

P- Reviewer: Haerian BS, Yin PH
S- Editor: Yu J **L- Editor**: A **E- Editor**: Jiao XK

World Journal of Gastrointestinal Oncology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4251/wjgo.v7.i11.285

World J Gastrointest Oncol 2015 November 15; 7(11): 285-291
ISSN 1948-5204 (online)

TOPIC HIGHLIGHT

2015 Advances in Gastric Cancer

Breakthrough therapy for peritoneal carcinomatosis of gastric cancer: Intraperitoneal chemotherapy with taxanes

Hironori Yamaguchi, Joji Kitayama, Hironori Ishigami, Shinsuke Kazama, Hiroaki Nozawa, Kazushige Kawai, Keisuke Hata, Tomomichi Kiyomatsu, Toshiaki Tanaka, Junichiro Tanaka, Takeshi Nishikawa, Kensuke Otani, Koji Yasuda, Soichiro Ishihara, Eiji Sunami, Toshiaki Watanabe

Hironori Yamaguchi, Joji Kitayama, Hironori Ishigami, Shinsuke Kazama, Hiroaki Nozawa, Kazushige Kawai, Keisuke Hata, Tomomichi Kiyomatsu, Toshiaki Tanaka, Junichiro Tanaka, Takeshi Nishikawa, Kensuke Otani, Koji Yasuda, Soichiro Ishihara, Eiji Sunami, Toshiaki Watanabe,
Department of Surgical Oncology, the University of Tokyo, Tokyo 113-8655, Japan

Author contributions: Yamaguchi H, Kazama S, Nozawa H, Kawai K, Hata K, Kiyomatsu T, Tanaka T, Tanaka J, Nishikawa T, Otani K and Yasuda K performed the literature research; Yamaguchi H, Ishihara S and Sunami E reviewed the paper; Kitayama J, Ishigami H and Watanabe T supervised the project; Yamaguchi H wrote the paper.

Conflict-of-interest statement: The authors declare no conflict of interest.

Correspondence to: Hironori Yamaguchi, MD, PhD, Department of Surgical Oncology, the University of Tokyo, 7-3-1 Hongo Bunkyoku, Tokyo 113-8655, Japan. yamaguchih-tky@umin.net
Telephone: +81-3-58008653
Fax: +81-3-38116822

Received: May 4, 2015
Peer-review started: May 9, 2015
First decision: July 17, 2015
Revised: July 28, 2015
Accepted: September 10, 2015
Article in press: September 16, 2015
Published online: November 15, 2015

Abstract

The effect of chemotherapy on peritoneal carcinomatosis (PC) of gastric cancer remains unclear. Recently, the intraperitoneal (IP) administration of taxanes [*e.g.*, paclitaxel (PTX) and docetaxel (DOC)] during the perioperative period has shown promising results. Herein, we summarized the rationale and methodology for using IP chemotherapy with taxanes and reviewed the clinical results. IP administered taxanes remain in the IP space at an extremely high concentration for 48-72 h. The drug directly infiltrates peritoneal metastatic nodules from the surface and then produces antitumor effects, making it ideal for IP chemotherapy. There are two types of perioperative IP chemotherapy with taxanes: neoadjuvant intraperitoneal and systemic chemotherapy and sequential perioperative intraperitoneal chemotherapy (SPIC). In SPIC, patients receive neoadjuvant IP chemotherapy and the same regimen of IP chemotherapy after cytoreductive surgery (CRS) until disease progression. Usually, a taxane dissolved in 500-1000 mL of saline at ordinary temperature is administered through an IP access port on an outpatient basis. According to phase Ⅰ studies, the recommended doses (RD) are as follows: IP DOC, 45-60 mg/m^2; IP PTX [without intravenous (IV) PTX], 80 mg/m^2; and IP PTX (with IV PTX), 20 mg/m^2. Phase Ⅱ studies have reported a median survival time of 14.4-24.6 mo with a 1-year overall survival of 67%-78%. A phase Ⅲ study comparing S-1 in combination with IP and IV PTX to S-1 with IV cisplatin started in 2011. The prognosis of patients who underwent CRS was better than that of those who did not; however, this was partly due to selection bias. Although several phase Ⅱ studies have shown promising results, a randomized controlled study is needed to validate the effectiveness of IP chemotherapy with taxanes for PC of gastric cancer.

Key words: Taxane; Paclitaxel; Docetaxel; Carcinoma;

Gastric cancer; Intraperitoneal infusions; Cytoreduction surgical procedures

Core tip: Herein, we provided an overview on the recent advances in intraperitoneal (IP) chemotherapy using taxanes (*e.g.*, paclitaxel and docetaxel) for peritoneal carcinomatosis of gastric cancer. In particular, we focus on the rationale of IP chemotherapy with taxanes, treatment methodology, and results of current clinical studies. Intraperitoneally administered taxanes remain in the IP cavity for a long time, and they directly infiltrate the peritoneal metastatic nodule from the surface. Therefore, the repeated intra-abdominal administration of taxanes through an IP access port is needed to increase the antitumor effect of IP chemotherapy.

Yamaguchi H, Kitayama J, Ishigami H, Kazama S, Nozawa H, Kawai K, Hata K, Kiyomatsu T, Tanaka T, Tanaka J, Nishikawa T, Otani K, Yasuda K, Ishihara S, Sunami E, Watanabe T. Breakthrough therapy for peritoneal carcinomatosis of gastric cancer: Intraperitoneal chemotherapy with taxanes. *World J Gastrointest Oncol* 2015; 7(11): 285-291 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i11/285.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i11.285

INTRODUCTION

Gastric cancer is the fourth most common cancer worldwide, and it is the second leading cause of cancer-related deaths[1]. Gastric cancer may disseminate along the inside surface of the peritoneal cavity, leading to peritoneal carcinomatosis (PC). PC is the most frequent mode of metastasis and recurrence in patients with gastric cancer. According to the national registry database of Japan, PC accounted for 51% of deaths in 355 patients with non-curable primary gastric cancer[2]. The same database also revealed that PC was the most frequent cause of death in 13002 patients who underwent gastrectomy for primary gastric cancer[2]. Yoo *et al*[3] reported that in 508 patients who underwent radical gastrectomy for gastric cancer, the first recurrence site was the peritoneum (43.9%) and then a local site (32.5%) followed by the liver (16.9%).

Despite recent advances in chemotherapy regimens for gastric cancer, the effect of systemic chemotherapy on PC remains unclear. Clinical trials on methotrexate + 5-fluorouracil (5-FU), FOLFOX-4, and continuous 5-FU for PC of gastric cancer showed that the median survival time (MST) was 5.2-10.6 mo, and the 1-year overall survival (OS) was 16.2%-40.7%[4-7].

In alternative treatment modalities, cytoreductive surgery (CRS) combined with hyperthermic intraperitoneal chemotherapy (HIPEC) has been used for treating PC of gastric cancer. Reportedly, the MST and 1-year survival were 9.2-11.5 mo and 35.5%-48.1% respectively[8-11]. However, CRS + HIPEC should be performed in specialized facilities, because these demanding procedures are associated with a high mortality and morbidity[12].

The intraperitoneal (IP) administration of anticancer drugs is a reasonable method for treating PC, because an IP administered cytotoxic drug acts directly on the peritoneal metastatic nodules at a high concentration. In HIPEC procedures, mitomycin C (MMC) and/or cisplatin (CDDP) dissolved in heated saline at 42 ℃-43 ℃ are usually administered into the peritoneal cavity[13].

Recently, the IP administration of taxanes such as paclitaxel (PTX) or docetaxel (DOC) without heating them at the ordinary temperature during the perioperative period in gastric cancer patients with PC has been performed mainly in Japan. Several clinical trials using IP chemotherapy with taxanes have shown promising results[14-18].

Based on the literature published in the last decade, we summarized the rationale for using IP chemotherapy with taxanes, methodology used for IP chemotherapy, and clinical results of IP chemotherapy in gastric cancer patients with PC.

RATIONALE FOR USING IP CHEMOTHERAPY WITH TAXANES

Taxanes such as PTX and DOC produce cytotoxic effects by inducing excessive polymerization of tubulin and dysfunctional microtubules, which leads to mitotic arrest and cell death[19,20]. PTX and DOC are water insoluble, and for clinical use, they are solubilized with Cremophor EL (Taxol®; Bristol-Myers Squibb Co.) and Polysorbate 80 (Taxotere®; Aventis Pharma SA), respectively.

Since taxanes are hydrophobic, high-weight molecular materials, IP administered taxanes are gradually drained from the peritoneum through lymphatic stomata that open directly into the pleural space[21,22]. In contrast, hydrophilic, low-weight molecular materials such as MMC or CDDP are rapidly absorbed through the peritoneal mesothelial layer and into the capillary vessels.

The area under the curve ratios of the intra-abdominal space to the plasma after IP administration of the drug are about 1000 for PTX, 207-552 for DOC, 10-24 for MMC, and 12-21 for CDDP[23-28]. The prolonged retention of IP administered taxanes within the IP space allows the taxanes to directly penetrate into peritoneal disseminated tumors[23,29-31], which leads to the destruction of peripheral microvessels of tumor nodules[32]. However, the depth of infiltration from the surface of the peritoneal disseminated nodules after the one time IP administration of a taxane is limited[33,34]. In a previous study, we showed that the distance of PTX infiltration reached approximately 100-200 μm from the surface of the tumor[35]. Therefore, to improve the antitumor effects of taxanes against PC, repeated IP administration is necessary.

Table 1 Phase Ⅰ studies on intraperitoneal chemotherapy using taxanes for the treatment of gastric cancer with peritoneal carcinomatosis

Ref.	*n*	Intraperitoneally administered taxanes	Initial dose (mg/m^2)	MTD (mg/m^2)	RD (mg/m^2)	DLT
Kodera *et al*[42]	4	PTX	60	-	-	-
Fushida *et al*[26]	24	DOC	25	60	45	Abdominal pain and diarrhea
Ishigami *et al*[45]	9	PTX	20	30	20	Febrile neutropenia and diarrhea
Fujiwara *et al*[43]	12	DOC	40	-	60	-
Kurita *et al*[44]	18	PTX	40	90	80	Leukocytopenia
Fushida *et al*[16]	12	DOC	35	50	45	Febrile neutropenia and diarrhea

MTD: Maximum tolerated dose; RD: Recommended dose; DLT: Dose-limiting toxicities; PTX: Paclitaxel; DOC: Docetaxel.

From the perspective of pharmacokinetics and tissue penetration, taxanes are ideal drugs for IP chemotherapy. Moreover, even if taxanes are repeatedly administered intraperitoneally, they rarely cause adhesion of organs in the peritoneal cavity because of their antiproliferative effect. Thus, the distribution of IP administered taxanes across the intra-abdominal space is not hampered by drug-induced peritonitis.

METHODOLOGY OF USING IP CHEMOTHERAPY WITH TAXANES

Perioperative IP chemotherapy with taxanes

There are two types of perioperative IP chemotherapy with taxanes for treating PC of gastric cancer: neoadjuvant intraperitoneal and systemic chemotherapy (NIPS)[36] and sequential perioperative intraperitoneal chemotherapy (SPIC)[37]. In NIPS, patients receive 1-6 courses of IP chemotherapy with a taxane as a neoadjuvant therapy; however, they do not receive IP chemotherapy after CRS[17,38,39]. In SPIC, patients receive several courses of IP chemotherapy preoperatively, and they receive the same regimen of IP chemotherapy after CRS until disease progression[14-16].

Peritoneal access port system

In most reported studies, a peritoneal access port system was used for IP chemotherapy. However, this device was not used when patients received a single IP administration during staging laparoscopy[28,39], or if patients received IP administration two times *via* a catheter as neoadjuvant chemotherapy[17]. A peritoneal access port is implanted into the subcutaneous space of the lower abdomen, and a catheter is placed usually in the pelvic cavity. Taxane dissolved in 500-1000 mL of saline at the ordinary temperature is administered through the peritoneal access port. Thus, using this method, taxanes can be repeatedly administered on an outpatient basis.

Complications associated with the port system occurred in 20.6% of 131 patients at our institution[40]. Inflow obstruction and infection were the main complications that occurred in 7.6% and 6.9% of patients, respectively. The median period of IP chemotherapy using the peritoneal port system was 12.9 mo (range, 0.8-61.5 mo). Compared to previous studies on ovarian cancer[41], the course of IP chemotherapy performed at our institution was much longer, but the complication rate was lower.

The use of a peritoneal port system can facilitate IP administration and reduce the patients' burden of receiving IP chemotherapy. Moreover, the device can provide another benefit to patients, because the peritoneal lavage sample, which is essential for evaluating the effect of IP chemotherapy on PC, can be obtained noninvasively through the peritoneal access port.

CLINICAL STUDIES ON IP CHEMOTHERAPY WITH TAXANES

Phase Ⅰ study

The findings from six phase Ⅰ studies on IP chemotherapy with taxanes are summarized in Table 1. PTX was used for intraperitoneally administering agents in three studies, and DOC was used in the other three studies. PTX or DOC was IP administered without other anticancer drugs in two studies[26,42], DOC was IP administered with S-1 in two[16,43], PTX was IP administered with S-1 in one[44], and intravenous (IV) PTX and S-1 was administered in one[45].

The recommended dose (RD) of DOC IP administration was 45-60 mg/m^2. The RD of PTX IP administration was 80 mg/m^2 when PTX was not IV administered, and it was 20 mg/m^2 when PTX was IV administered. Although the RD of 20 mg/m^2 in our phase Ⅰ study was relatively low because we used a combination of IV PTX, the IP PTX concentration remained extremely high for > 72 h.

Dose-limiting toxicities of these phase Ⅰ studies included grade 3 febrile neutropenia, leukopenia, and diarrhea for the PTX IP regimen; and grade 3 febrile neutropenia, abdominal pain, and diarrhea for the DOC IP regimen.

Phase Ⅱ study

The findings of six phase Ⅱ studies on IP chemotherapy with taxanes are summarized in Table 2. PTX was used for IP administered agents in three studies[14,15,39], and DOC was used in the other three studies[16,17,38]. The

Table 2 Phase Ⅱ studies on intraperitoneal chemotherapy using taxanes for the treatment of gastric cancer with peritoneal carcinomatosis

Ref.	*n*	Method	Intraperitoneally administered agents	MST (mo)	1-yr OS (%)	2-yr OS (%)	5-yr OS (%)
Yonemura *et al*[38]	61	NIPS	DOC (40 mg) + CBDCA (150 mg)	14.4	67		
Ishigami *et al*[14]	40	SPIC	PTX (20 mg/m^2)	22.6	78		
Fujiwara *et al*[17]	18	NIPS	DOC (40-60 mg/m^2)	24.6	76	54	
Imano *et al*[39]	35	NIPS	PTX (80 mg/m^2)	21.3	69	46	14
Yamaguchi *et al*[15]	35	SPIC	PTX (20 mg/m^2)	17.6	77	45	
Fushida *et al*[16]	27	SPIC	DOC (35-50 mg/m^2)	16.2	70	33	

MST: Median survival time; OS: Overall survival; DOC: Docetaxel; CBDCA: Carboplatin; PTX: Paclitaxel; NIPS: Neoadjuvant intraperitoneal and systemic chemotherapy; SPIC: Sequential perioperative intraperitoneal chemotherapy.

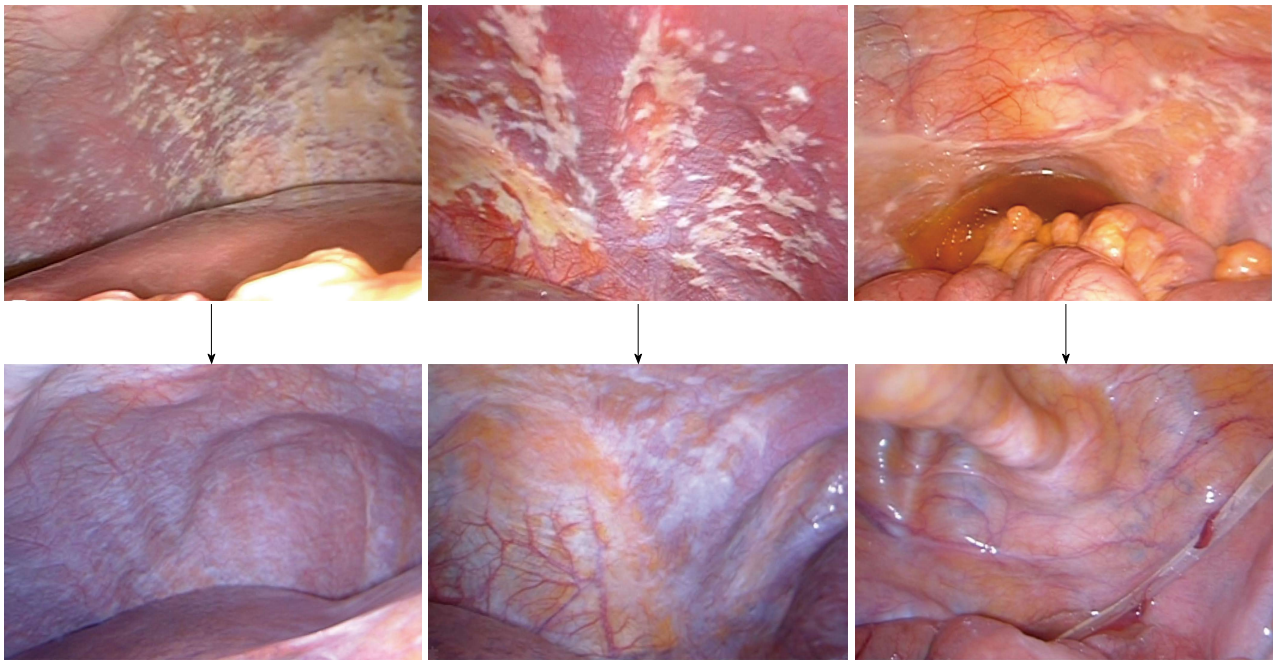


Figure 1 Laparoscopy before and after treatment. Staging laparoscopy (upper) showing peritoneal metastatic nodules in the right subphrenic peritoneum (left), left subphrenic peritoneum (middle), and Douglas pouch (left). The second laparoscopy (lower) revealing that the metastatic nodules have disappeared after 12 courses of the intravenous and intraperitoneal administration of paclitaxel and oral S-1 chemotherapy.

overall response rate among these phase Ⅱ studies ranged from 55%-71%. The MSTs and 1-year OS were 14.4-24.6 mo and 67%-78%, respectively. The main toxicities were hematologic (*e.g.*, anemia, neutropenia, and leukopenia), and the non-hematological toxic effects were relatively mild. Regarding CRS, gastrectomy with D2 dissection was usually performed. In addition to D2 gastrectomy, peritonectomy was performed only by Yonemura *et al*[38]. Post-operative complications, ranging 9%-22%, were reported in four studies[16,17,38,39]. Surgery-related mortality was found in one patient, and the cause of death was sepsis from an abdominal abscess[38].

In three of six phase Ⅱ studies, patients received 1-6 courses of NIPS. The MSTs of patients who underwent CRS after NIPS were 20.4-29.8 mo. In the other phase Ⅱ studies, patients received SPIC. In 2010, we reported on a phase Ⅱ study on SPIC in 40 gastric cancer patients with PC, which included six cytology positive (CY1) and macroscopically negative (P0) patients[14]. Sixteen patients underwent CRS. According to recently updated survival data, the MST was 23.6 mo and the 1-, 2-, and 5-year OS were 78%, 50%, and 18%, respectively.

We performed another phase Ⅱ study with the same regimen in 35 gastric cancer patients with PC[15]. However, in this study, CY1P0 patients were excluded, because they may have a better prognosis compared to macroscopic PC (P1) patients. CRS was performed in 21 patients. Patients with peritoneal cancer index (PCI) scores ≥ 20 had a lower survival rate than those with PCI scores < 20. According to recently updated data, the MST was 18.0 mo, and the 1-, 2-, and 4-year OS were 77%, 42%, and 10%, respectively. The findings from staging laparoscopy and second-look laparoscopy are shown from a representative case (Figure 1).

Fushida *et al*[16] performed a phase Ⅰ/Ⅱ study on SPIC with IP DOC in 27 patients. Fourteen patients underwent CRS and received postoperative IP chemotherapy.

The 1- and 2-year OS of patients who underwent CRS were 92.8% and 62.5%, respectively.

Phase Ⅲ study

In Japan, a randomized, multicenter, phase Ⅲ trial (the PHOENIX-GC trial, UMIN000005930) compared S-1 in combination with IV and IP PTX to S-1 with IV CDDP in 180 gastric cancer patients with P1. This study began in 2011, and the final analysis will be obtained in November 2015.

IP chemotherapy with taxanes combined with CRS

If PC can be controlled by IP chemotherapy with a taxane, gastrectomy as CRS is considered to be a reasonable treatment. Because IP chemotherapy as a localized therapy for peritoneal cavity may not have intensive antitumor effects on primary gastric tumors and metastatic lymph nodes. Other than the aforementioned phase Ⅱ studies, two studies have reported on the treatment results of IP chemotherapy combined with CRS.

Kitayama *et al*[18] treated 64 gastric cancer patients with PC who had malignant ascites with IP and IV PTX combined with S-1. CRS without peritonectomy was performed in 34 patients. After CRS, chemotherapy with the same regimen was continued (*i.e.*, SPIC). The MST of these patients and the 1-year OS were 26.4 mo and 82%, respectively. Those of the 30 patients who did not undergo gastrectomy were 12.1 mo and 26%, respectively.

Yonemura *et al*[46] performed NIPS with IP DOC and CDDP combined with S-1 in 96 patients. After two cycles of NIPS, 82 patients underwent CRS (gastrectomy with D2 dissection and peritonectomy). Complete cytoreduction was achieved in 58 patients. The MST and 1-year OS of patients who underwent CRS was 14.4 mo and 61%, respectively. The MST of patients who underwent complete cytoreduction and those who did not undergo CRS were 21.1 mo and 9 mo, respectively.

In these reports, the prognosis of patients who underwent CRS was better than that of those who did not. However, this survival difference was partly due to a strong selection bias since CRS was performed only in good responders. A randomized controlled study will need to be performed in order to determine the significance of CRS.

DISCUSSION

It is important whether IP chemotherapy with taxanes is needed after CRS. Yonemura *et al*[46] reported that 22 of 61 patients who received NIPS with complete CRS had recurrence in the peritoneum. Fujiwara *et al*[17] suggested that IP chemotherapy may have been needed in their patients, because 8 of 14 patients who had curative surgery following NIPS died from peritoneal recurrence. It is reasonable to consider that IP chemotherapy with a taxane should be continued as long as possible even after CRS to suppress the development of microscopic cancer cells that may still exist in the whole peritoneal cavity. Therefore, we consider that SPIC is better suited for treating PC of gastric cancer.

Another important issue is how the criteria for performing CRS are determined. If patients do not respond to IP chemotherapy, CRS should not be performed. We have performed CRS in patients who have met the following criteria: (1) no distant metastasis, except in the peritoneum; (2) a negative peritoneal lavage cytology; and (3) a second-look laparoscopy reveals that the peritoneal metastatic nodules are reduced. To select eligible patients for CRS more precisely, novel and useful biomarkers that reflect a good response to IP chemotherapy are needed.

Phase Ⅲ studies on IP chemotherapy with taxanes have been reported in the gynecological field, especially for PC of ovarian cancer. IP PTX with systemic chemotherapy for PC of ovarian cancer showed a significant survival benefit[47]. Based on the findings from these phase Ⅲ studies[47-49], the National Cancer Institute has recommended IP chemotherapy in patients with optimally debulked ovarian cancer[50].

Regarding the treatment of PC from gastric cancer, there are promising findings from several phase Ⅱ studies with IP chemotherapy using taxanes. However, it is difficult to draw any definitive conclusions about the overall clinical usefulness of this treatment method until we obtain the findings from the PHOENIX-GC phase Ⅲ trials.

In conclusion, IP administered taxanes remain in the IP cavity for a long period, and they produce antitumor effects by infiltrating peritoneal metastatic nodules from the surface. In addition, repeated IP administration of taxanes through an IP access port before and after CRS seems necessary for improving the effect of IP chemotherapy. Lastly, IP chemotherapy with taxanes for PC from gastric cancer is safe and feasible. Although several phase Ⅱ clinical studies have shown promising results, further randomized phase Ⅲ clinical trials are needed to validate IP chemotherapy with taxanes for gastric PC.

REFERENCES

1 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]

2 **Nashimoto A**, Akazawa K, Isobe Y, Miyashiro I, Katai H, Kodera Y, Tsujitani S, Seto Y, Furukawa H, Oda I, Ono H, Tanabe S, Kaminishi M. Gastric cancer treated in 2002 in Japan: 2009 annual report of the JGCA nationwide registry. *Gastric Cancer* 2013; **16**: 1-27 [PMID: 22729699 DOI: 10.1007/s10120-012-0163-4]

3 **Yoo CH**, Noh SH, Shin DW, Choi SH, Min JS. Recurrence following curative resection for gastric carcinoma. *Br J Surg* 2000; **87**: 236-242 [PMID: 10671934 DOI: 10.1046/j.1365-2168.2000.01360.x]

4 **Yamao T**, Shimada Y, Shirao K, Ohtsu A, Ikeda N, Hyodo I, Saito H, Iwase H, Tsuji Y, Tamura T, Yamamoto S, Yoshida S. Phase II study of sequential methotrexate and 5-fluorouracil chemotherapy against

peritoneally disseminated gastric cancer with malignant ascites: a report from the Gastrointestinal Oncology Study Group of the Japan Clinical Oncology Group, JCOG 9603 Trial. *Jpn J Clin Oncol* 2004; **34**: 316-322 [PMID: 15333683 DOI: 10.1093/jjco/hyh063]

5 **Oh SY**, Kwon HC, Lee S, Lee DM, Yoo HS, Kim SH, Jang JS, Kim MC, Jeong JS, Kim HJ. A Phase II study of oxaliplatin with low-dose leucovorin and bolus and continuous infusion 5-fluorouracil (modified FOLFOX-4) for gastric cancer patients with malignant ascites. *Jpn J Clin Oncol* 2007; **37**: 930-935 [PMID: 18211984 DOI: 10.1093/jjco/hym131]

6 **Shirao K,** Boku N, Yamada Y, Yamaguchi K, T. D, Takiuchi H, Nasu J, Nakamura K, Fukuda H, Ohtsu A. Randomized phase III study of 5-fluorouracil continuous infusion (5FUci) versus methotrexate and 5-FU sequential therapy (MF) in gastric cancer with peritoneal metastasis (JCOG0106). *J Clin Oncol* 2009; **27** Suppl: abstr 4545

7 **Imazawa M**, Kojima T, Boku N, Onozawa Y, Hironaka S, Fukutomi A, Yasui H, Yamazaki K, Taku K. Efficacy of sequential methotrexate and 5-fluorouracil (MTX/5FU) in improving oral intake in patients with advanced gastric cancer with severe peritoneal dissemination. *Gastric Cancer* 2009; **12**: 153-157 [PMID: 19890695 DOI: 10.1007/s10120-009-0517-8]

8 **Glehen O**, Schreiber V, Cotte E, Sayag-Beaujard AC, Osinsky D, Freyer G, François Y, Vignal J, Gilly FN. Cytoreductive surgery and intraperitoneal chemohyperthermia for peritoneal carcinomatosis arising from gastric cancer. *Arch Surg* 2004; **139**: 20-26 [PMID: 14718269 DOI: 10.1001/archsurg.139.1.20]

9 **Yonemura Y**, Kawamura T, Bandou E, Takahashi S, Sawa T, Matsuki N. Treatment of peritoneal dissemination from gastric cancer by peritonectomy and chemohyperthermic peritoneal perfusion. *Br J Surg* 2005; **92**: 370-375 [PMID: 15739249 DOI: 10.1002/bjs.4695]

10 **Yang XJ**, Huang CQ, Suo T, Mei LJ, Yang GL, Cheng FL, Zhou YF, Xiong B, Yonemura Y, Li Y. Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy improves survival of patients with peritoneal carcinomatosis from gastric cancer: final results of a phase III randomized clinical trial. *Ann Surg Oncol* 2011; **18**: 1575-1581 [PMID: 21431408 DOI: 10.1245/s10434-011-1631-5]

11 **Glehen O**, Gilly FN, Arvieux C, Cotte E, Boutitie F, Mansvelt B, Bereder JM, Lorimier G, Quenet F, Elias D. Peritoneal carcinomatosis from gastric cancer: a multi-institutional study of 159 patients treated by cytoreductive surgery combined with perioperative intraperitoneal chemotherapy. *Ann Surg Oncol* 2010; **17**: 2370-2377 [PMID: 20336386 DOI: 10.1245/s10434-010-1039-7]

12 **Gill RS**, Al-Adra DP, Nagendran J, Campbell S, Shi X, Haase E, Schiller D. Treatment of gastric cancer with peritoneal carcinomatosis by cytoreductive surgery and HIPEC: a systematic review of survival, mortality, and morbidity. *J Surg Oncol* 2011; **104**: 692-698 [PMID: 21713780 DOI: 10.1002/jso.22017]

13 **González-Moreno S**, González-Bayón LA, Ortega-Pérez G. Hyperthermic intraperitoneal chemotherapy: Rationale and technique. *World J Gastrointest Oncol* 2010; **2**: 68-75 [PMID: 21160924 DOI: 10.4251/wjgo.v2.i2.68]

14 **Ishigami H**, Kitayama J, Kaisaki S, Hidemura A, Kato M, Otani K, Kamei T, Soma D, Miyato H, Yamashita H, Nagawa H. Phase II study of weekly intravenous and intraperitoneal paclitaxel combined with S-1 for advanced gastric cancer with peritoneal metastasis. *Ann Oncol* 2010; **21**: 67-70 [PMID: 19605503 DOI: 10.1093/annonc/mdp260]

15 **Yamaguchi H**, Kitayama J, Ishigami H, Emoto S, Yamashita H, Watanabe T. A phase 2 trial of intravenous and intraperitoneal paclitaxel combined with S-1 for treatment of gastric cancer with macroscopic peritoneal metastasis. *Cancer* 2013; **119**: 3354-3358 [PMID: 23798046 DOI: 10.1002/cncr.28204]

16 **Fushida S**, Kinoshita J, Kaji M, Hirono Y, Goda F, Yagi Y, Oyama K, Sudo Y, Watanabe Y, Fujimura T. Phase I/II study of intraperitoneal docetaxel plus S-1 for the gastric cancer patients with peritoneal carcinomatosis. *Cancer Chemother Pharmacol* 2013; **71**: 1265-1272 [PMID: 23423490 DOI: 10.1007/s00280-013-2122-0]

17 **Fujiwara Y**, Takiguchi S, Nakajima K, Miyata H, Yamasaki M, Kurokawa Y, Mori M, Doki Y. Intraperitoneal docetaxel combined with S-1 for advanced gastric cancer with peritoneal dissemination. *J Surg Oncol* 2012; **105**: 38-42 [PMID: 21882194 DOI: 10.1002/jso.22057]

18 **Kitayama J**, Ishigami H, Yamaguchi H, Yamashita H, Emoto S, Kaisaki S, Watanabe T. Salvage gastrectomy after intravenous and intraperitoneal paclitaxel (PTX) administration with oral S-1 for peritoneal dissemination of advanced gastric cancer with malignant ascites. *Ann Surg Oncol* 2014; **21**: 539-546 [PMID: 23975319 DOI: 10.1245/s10434-013-3208-y]

19 **Rowinsky EK**, Cazenave LA, Donehower RC. Taxol: a novel investigational antimicrotubule agent. *J Natl Cancer Inst* 1990; **82**: 1247-1259 [PMID: 1973737 DOI: 10.1093/jnci/82.15.1247]

20 **Ringel I**, Horwitz SB. Studies with RP 56976 (taxotere): a semisynthetic analogue of taxol. *J Natl Cancer Inst* 1991; **83**: 288-291 [PMID: 1671606 DOI: 10.1093/jnci/83.4.288]

21 **Flessner MF**, Fenstermacher JD, Blasberg RG, Dedrick RL. Peritoneal absorption of macromolecules studied by quantitative autoradiography. *Am J Physiol* 1985; **248**: H26-H32 [PMID: 3155917]

22 **Wang ZB**, Li M, Li JC. Recent advances in the research of lymphatic stomata. *Anat Rec* (Hoboken) 2010; **293**: 754-761 [PMID: 20186966 DOI: 10.1002/ar.21101]

23 **Markman M**. Intraperitoneal antineoplastic drug delivery: rationale and results. *Lancet Oncol* 2003; **4**: 277-283 [PMID: 12732164 DOI: 10.1016/s1470-2045(03)01074-x]

24 **Ceelen WP**, Flessner MF. Intraperitoneal therapy for peritoneal tumors: biophysics and clinical evidence. *Nat Rev Clin Oncol* 2010; **7**: 108-115 [PMID: 20010898 DOI: 10.1038/nrclinonc.2009.217]

25 **Yan TD**, Cao CQ, Munkholm-Larsen S. A pharmacological review on intraperitoneal chemotherapy for peritoneal malignancy. *World J Gastrointest Oncol* 2010; **2**: 109-116 [PMID: 21160929 DOI: 10.4251/wjgo.v2.i2.109]

26 **Fushida S**, Kinoshita J, Yagi Y, Funaki H, Kinami S, Ninomiya I, Fujimura T, Nishimura G, Kayahara M, Ohta T. Dual anti-cancer effects of weekly intraperitoneal docetaxel in treatment of advanced gastric cancer patients with peritoneal carcinomatosis: a feasibility and pharmacokinetic study. *Oncol Rep* 2008; **19**: 1305-1310 [PMID: 18425392 DOI: 10.3892/or.19.5.1305]

27 **Yonemura Y**, Elnemr A, Endou Y, Hirano M, Mizumoto A, Takao N, Ichinose M, Miura M, Li Y. Multidisciplinary therapy for treatment of patients with peritoneal carcinomatosis from gastric cancer. *World J Gastrointest Oncol* 2010; **2**: 85-97 [PMID: 21160926 DOI: 10.4251/wjgo.v2.i2.85]

28 **Imano M**, Peng YF, Itoh T, Nishikawa M, Satou T, Yasuda A, Inoue K, Kato H, Shinkai M, Tsubaki M, Yasuda T, Imamoto H, Nishida S, Furukawa H, Takeyama Y, Okuno K, Shiozaki H. A preliminary study of single intraperitoneal administration of paclitaxel followed by sequential systemic chemotherapy with S-1 plus paclitaxel for advanced gastric cancer with peritoneal metastasis. *Anticancer Res* 2012; **32**: 4071-4075 [PMID: 22993363]

29 **Yonemura Y**, Endou Y, Bando E, Kuno K, Kawamura T, Kimura M, Shimada T, Miyamoto K, Sasaki T, Sugarbaker PH. Effect of intraperitoneal administration of docetaxel on peritoneal dissemination of gastric cancer. *Cancer Lett* 2004; **210**: 189-196 [PMID: 15183534 DOI: 10.1016/j.canlet.2004.03.018]

30 **Soma D**, Kitayama J, Ishigami H, Kaisaki S, Nagawa H. Different tissue distribution of paclitaxel with intravenous and intraperitoneal administration. *J Surg Res* 2009; **155**: 142-146 [PMID: 19328496 DOI: 10.1016/j.jss.2008.06.049]

31 **Soma D**, Kitayama J, Konno T, Ishihara K, Yamada J, Kamei T, Ishigami H, Kaisaki S, Nagawa H. Intraperitoneal administration of paclitaxel solubilized with poly(2-methacryloxyethyl phosphorylcholine-co n-butyl methacrylate) for peritoneal dissemination of gastric cancer. *Cancer Sci* 2009; **100**: 1979-1985 [PMID: 19604244 DOI: 10.1111/j.1349-7006.2009.01265.x]

32 **Kitayama J**, Emoto S, Yamaguchi H, Ishigami H, Watanabe T. Intraperitoneal paclitaxel induces regression of peritoneal metastasis partly by destruction of peripheral microvessels. *Cancer Chemother Pharmacol* 2014; **73**: 605-612 [PMID: 24464356 DOI: 10.1007/

s00280-014-2393-0]
33 **Kuh HJ**, Jang SH, Wientjes MG, Weaver JR, Au JL. Determinants of paclitaxel penetration and accumulation in human solid tumor. *J Pharmacol Exp Ther* 1999; **290**: 871-880 [PMID: 10411604]
34 **Kyle AH**, Huxham LA, Yeoman DM, Minchinton AI. Limited tissue penetration of taxanes: a mechanism for resistance in solid tumors. *Clin Cancer Res* 2007; **13**: 2804-2810 [PMID: 17473214 DOI: 10.1158/1078-0432.CCR-06-1941]
35 **Kamei T**, Kitayama J, Yamaguchi H, Soma D, Emoto S, Konno T, Ishihara K, Ishigami H, Kaisaki S, Nagawa H. Spatial distribution of intraperitoneally administrated paclitaxel nanoparticles solubilized with poly (2-methacryloxyethyl phosphorylcholine-co n-butyl methacrylate) in peritoneal metastatic nodules. *Cancer Sci* 2011; **102**: 200-205 [PMID: 20942868 DOI: 10.1111/j.1349-7006.2010.01747.x]
36 **Yonemura Y**, Endou Y, Sasaki T, Hirano M, Mizumoto A, Matsuda T, Takao N, Ichinose M, Miura M, Li Y. Surgical treatment for peritoneal carcinomatosis from gastric cancer. *Eur J Surg Oncol* 2010; **36**: 1131-1138 [PMID: 20933363 DOI: 10.1016/j.ejso.2010.09.006]
37 **Cashin PH**, Graf W, Nygren P, Mahteme H. Cytoreductive surgery and intraperitoneal chemotherapy for colorectal peritoneal carcinomatosis: prognosis and treatment of recurrences in a cohort study. *Eur J Surg Oncol* 2012; **38**: 509-515 [PMID: 22475555 DOI: 10.1016/j.ejso.2012.03.001]
38 **Yonemura Y**, Bandou E, Sawa T, Yoshimitsu Y, Endou Y, Sasaki T, Sugarbaker PH. Neoadjuvant treatment of gastric cancer with peritoneal dissemination. *Eur J Surg Oncol* 2006; **32**: 661-665 [PMID: 16621433 DOI: 10.1016/j.ejso.2006.03.007]
39 **Imano M**, Yasuda A, Itoh T, Satou T, Peng YF, Kato H, Shinkai M, Tsubaki M, Chiba Y, Yasuda T, Imamoto H, Nishida S, Takeyama Y, Okuno K, Furukawa H, Shiozaki H. Phase II study of single intraperitoneal chemotherapy followed by systemic chemotherapy for gastric cancer with peritoneal metastasis. *J Gastrointest Surg* 2012; **16**: 2190-2196 [PMID: 23099736 DOI: 10.1007/s11605-012-2059-3]
40 **Emoto S**, Ishigami H, Hidemura A, Yamaguchi H, Yamashita H, Kitayama J, Watanabe T. Complications and management of an implanted intraperitoneal access port system for intraperitoneal chemotherapy for gastric cancer with peritoneal metastasis. *Jpn J Clin Oncol* 2012; **42**: 1013-1019 [PMID: 22872745 DOI: 10.1093/jjco/hys129]
41 **Walker JL**, Armstrong DK, Huang HQ, Fowler J, Webster K, Burger RA, Clarke-Pearson D. Intraperitoneal catheter outcomes in a phase III trial of intravenous versus intraperitoneal chemotherapy in optimal stage III ovarian and primary peritoneal cancer: a Gynecologic Oncology Group Study. *Gynecol Oncol* 2006; **100**: 27-32 [PMID: 16368440 DOI: 10.1016/j.ygyno.2005.11.013]
42 **Kodera Y**, Ito Y, Ito S, Ohashi N, Mochizuki Y, Yamamura Y, Koike M, Fujiwara M, Nakanishi H, Nakao A. Intraperitoneal paclitaxel: a possible impact of regional delivery for prevention of peritoneal carcinomatosis in patients with gastric carcinoma. *Hepatogastroenterology* 2007; **54**: 960-963 [PMID: 17591103]
43 **Fujiwara Y**, Nishida T, Takiguchi S, Nakajima K, Miyata H, Yamasaki M, Yamamoto K, Moon JH, Mori M, Doki Y. Feasibility study of S-1 and intraperitoneal docetaxel combination chemotherapy for gastric cancer with peritoneal dissemination. *Anticancer Res* 2010; **30**: 1335-1339 [PMID: 20530449]
44 **Kurita N**, Shimada M, Iwata T, Nishioka M, Morimoto S, Yoshikawa K, Higashijima J, Miyatani T, Nakao T. Intraperitoneal infusion of paclitaxel with S-1 for peritoneal metastasis of advanced gastric cancer: phase I study. *J Med Invest* 2011; **58**: 134-139 [PMID: 21372498]
45 **Ishigami H**, Kitayama J, Otani K, Kamei T, Soma D, Miyato H, Yamashita H, Hidemura A, Kaisaki S, Nagawa H. Phase I pharmacokinetic study of weekly intravenous and intraperitoneal paclitaxel combined with S-1 for advanced gastric cancer. *Oncology* 2009; **76**: 311-314 [PMID: 19299904 DOI: 10.1159/000209277]
46 **Yonemura Y**, Elnemr A, Endou Y, Ishibashi H, Mizumoto A, Miura M, Li Y. Effects of neoadjuvant intraperitoneal/systemic chemotherapy (bidirectional chemotherapy) for the treatment of patients with peritoneal metastasis from gastric cancer. *Int J Surg Oncol* 2012; **2012**: 148420 [PMID: 22900159 DOI: 10.1155/2012/148420]
47 **Armstrong DK**, Bundy B, Wenzel L, Huang HQ, Baergen R, Lele S, Copeland LJ, Walker JL, Burger RA. Intraperitoneal cisplatin and paclitaxel in ovarian cancer. *N Engl J Med* 2006; **354**: 34-43 [PMID: 16394300 DOI: 10.1056/NEJMoa052985]
48 **Alberts DS**, Liu PY, Hannigan EV, O'Toole R, Williams SD, Young JA, Franklin EW, Clarke-Pearson DL, Malviya VK, DuBeshter B. Intraperitoneal cisplatin plus intravenous cyclophosphamide versus intravenous cisplatin plus intravenous cyclophosphamide for stage III ovarian cancer. *N Engl J Med* 1996; **335**: 1950-1955 [PMID: 8960474 DOI: 10.1056/NEJM199612263352603]
49 **Markman M**, Bundy BN, Alberts DS, Fowler JM, Clark-Pearson DL, Carson LF, Wadler S, Sickel J. Phase III trial of standard-dose intravenous cisplatin plus paclitaxel versus moderately high-dose carboplatin followed by intravenous paclitaxel and intraperitoneal cisplatin in small-volume stage III ovarian carcinoma: an intergroup study of the Gynecologic Oncology Group, Southwestern Oncology Group, and Eastern Cooperative Oncology Group. *J Clin Oncol* 2001; **19**: 1001-1007 [PMID: 11181662]
50 **National Cancer Institute.** Clinical Announcement: Intraperitoneal chemotherapy for ovarian cancer. [accessed 2006 Jan 5]. Available from: URL: http://ctep.cancer.gov/highlights/clin_annc_010506.pdf

P- Reviewer: Arsenijevic T
S- Editor: Tian YL **L- Editor**: A **E- Editor**: Jiao XK

World Journal of Gastrointestinal Oncology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4251/wjgo.v7.i11.292

World J Gastrointest Oncol 2015 November 15; 7(11): 292-302
ISSN 1948-5204 (online)

TOPIC HIGHLIGHT

2015 Advances in Gastric Cancer

Individualized treatment of gastric cancer: Impact of molecular biology and pathohistological features

Yves Dittmar, Utz Settmacher

Yves Dittmar, Utz Settmacher, Department of General, Visceral and Vascular Surgery, University Hospital Jena, 07745 Jena, Germany

Author contributions: Dittmar Y compiled and analyzed data and wrote the paper; Settmacher U reviewed the paper and provided valuable scientific input.

Conflict-of-interest statement: The authors declare no conflict of interest.

Correspondence to: Yves Dittmar, MD, Department of General, Visceral and Vascular Surgery, University Hospital Jena, Erlanger Allee 101, 07745 Jena, Germany. yves.dittmar@med.uni-jena.de
Telephone: +49-3641-9322601
Fax: +49-3641-9322602

Received: April 29, 2015
Peer-review started: May 7, 2015
First decision: June 25, 2015
Revised: July 23, 2015
Accepted: September 30, 2015
Article in press: October 10, 2015
Published online: November 15, 2015

Abstract

Gastric cancer is one of the most common malignancies worldwide. The overall prognosis remains poor over the last decades even though improvements in surgical outcomes have been achieved. A better understanding of the molecular biology of gastric cancer and detection of eligible molecular targets might be of central interest to further improve clinical outcome. With this intention, first steps have been made in the research of growth factor signaling. Regarding morphogens, cell cycle and nuclear factor-κB signaling, a remarkable count of target-specific agents have been developed, nevertheless the transfer into the field of clinical routine is still at the beginning. The potential utility of epigenetic targets and the further evaluation of microRNA signaling seem to have potential for the development of novel treatment strategies in the future.

Key words: Gastric cancer; Molecular biology; Targeted therapy; Personalized medicine; Signaling pathway

Core tip: Advanced gastric cancer remains a frequent malignancy with poor prognosis despite multimodal treatment options. Surgery alone has been demonstrated not to be the optimal strategy and is predominantly limited to cases without distant metastases. About one half of gastric cancer patients cannot be cured. Due to its individual heterogeneity on the molecular level these tumors frequently do not respond to systemic treatment. The implementation of the growing knowledge about the molecular behavior of gastric cancer in the development or improvement of target-specific treatment strategies might be one of the major challenges for the next decades.

Dittmar Y, Settmacher U. Individualized treatment of gastric cancer: Impact of molecular biology and pathohistological features. *World J Gastrointest Oncol* 2015; 7(11): 292-302 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i11/292.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i11.292

GENERAL CLINICAL ASPECTS

Gastric cancer is still one of the leading oncologic challenges due to its frequent occurrence as well as its poor prognosis[1]. The ongoing improvement of surgical techniques and perioperative care over the past decades have not only extended the repertoire of treatment options with curative intent but also have contributed to the reduction of perioperative morbidity. Thus, currently about 50% of all gastric cancer patients can be treated curatively and the majority of these patients undergo the surgical treatment without severe complications[2]. But still one half of all gastric cancer patients have to be regarded as palliative cases with no chance for long term survival and even the curatively resected patients face an overall recurrence rate of 50%[3].

In view of this development it can be assumed that further evolvement of surgical treatment will not improve tumor-related survival substantially. The molecular biology of the individual tumor might be one important key to a better understanding of the disease and an advancement in the prognosis of gastric cancer patients.

The knowledge about the molecular biology of gastric cancer is of high interest for several reasons: (1) aberrations at the genomic as well as at the proteomic level might be useful as biomarkers for exact classification; (2) molecular markers may further improve and refine tumor staging; (3) knowledge about the individual molecular signature may enable a personalized and target specific treatment; and (4) molecular presentation of the tumor and target specific treatment may lead to an improved prognosis.

UNDERSTANDING OF THE MOLECULAR BIOLOGY: GENERAL CHALLENGES

The understanding of molecular biology of gastric cancer is crucial for the appraisal of its clinical behavior and to control the tumor growth with all its consequences. As in almost all other tumor entities the following characteristics may challenge the establishment of an effective treatment: (1) every individual tumor presents with a unique pattern of molecular variance, comparable with an individual fingerprint; (2) in a certain manner every tumor can be regarded as an autonomous organism which in fact means that tumors do not consist of a homogenous tissue mass but show a regional heterogeneity; (3) over time every tumor changes spontaneously in its molecular biological behaviour; and (4) every tumor reacts in a distinct manner to treatment attempts.

These aspects are basically important in un-targeted treatment approaches as the application of conventional cytostatic substances or surgery but are even more important for target-specific treatment strategies. In view of the multidimensional complexity of molecular tumor biology it becomes clear that it is unlikely to find "the single one agent" to achieve a safe and sustainable tumor control.

CURRENT STATE OF THE ART IN MOLECULAR TARGETED TREATMENT

Growth factors, growth factor receptors and downstream features

Epithelial growth factor: To date, four different types of epithelial growth factor receptors (EGFR) have been identified, also called as ErbB1-4[4]. Once activated, they form homo- or heterodimers and then become internalized within the cell. From there three different pathways (MAP-kinase pathway, STAT pathway and PI3K pathway) can be activated, subsequently leading to the transmission of the signal into the nucleus and specific regulation of gene expression by activated cyclinD1, iNOS, B-myb, COX2 and Aurora kinase 2. With the exception of ErbB2, in addition to the original epidermal growth factors multiple other ligands can bind and activate EGFR: transforming growth factor alpha, epiregulin, amphiregulin and βcellulin. ErbB2 in contrast, can not be activated directly by any growth factor, but can be heterodimerized by other members of the EGFR family[5].

It has been reported that EGFR overexpression occurs in 60% to 70% of gastric cancer cases, however gene amplification seems to be rather uncommon[6,7]. EGFR2 measured by fluorescence *in situ* hybridisation was detected in 22% of gastric cancers[8,9], while it was more frequent in intestinal than in diffuse type gastric cancer according to the Lauren classification (32% and 20%)[9,10]. EGFR overexpression in gastric cancer was related to poorer survival and poorer response to chemotherapy[11].

Due to its central role in epithelial signaling as well as its biological properties EGFR became an interesting target for molecular-based treatment and thus there is now a remarkable variety of EGFR-targeted molecules available.

Three main target points have been proposed: the inactivation of the receptor, the stimulation of antibody-dependent cell cytotoxity and the inhibition of the tyrosine kinase activity by multityrosin kinase inhibitors.

To date, seven monoclonal antibodies targeting EGFR are available: cetuximab, trastuzumab, matuzutumab, panitumumab, nimetuzumab, perluzumab and T-DM1[10].

Cetuximab inhibits the binding of EGF and TGFalpha to EGFR, furthermore it promotes the internalization of the receptor[12]. The application of cetuximab is well established in stage 4 colorectal cancer (with k-ras wild type)[13] and in several head and neck malignancies[14,15].

Several phase 2 and 3 trials showed a positive effect of the administration of cetuximab combined with standard chemotherapy protocols as a first line therapy with response rates up to 58% and 69% in advanced gastroesophageal junction and gastric cancer (overall survival up to 9.5 mo)[10,16]. In contrast, cetuximab in combination with cisplatin or irinotecan as a second line

therapy revealed only a marginal benefit on the overall survival (7.1 mo)[17]. Moreover, cetuximab as a single-agent administration for second line therapy resulted in even lower impact on the overall survival (3.6 to 4 mo) with poor response (9%)[18].

Cetuximab in combination with several cytostatic substances for neoadjuvant chemotherapy showed response rates up to 70%[19,20].

Trastuzumab is known to have a broad variety of molecular effects: Binding to the extracellular part of the her-2/*neu* molecule und thus suppressing the intracellular localised tyrosine kinase activity, antibody dependent cell toxicity (ADCC)[21], activation of natural killer cells, inhibition of angiogenesis and the phosphoinositol-3-kinase signaling pathway (PI3K) as well as cell cycle arrest[22-24]. The administration of trastuzumab as adjuvant treatment has been approved for node positive breast cancer[25].

The most important study with respect to gastric cancer is the ToGA trial. It has been shown that those patients who were positive for the her-2/*neu* receptor (22% of all cases) had a significant improvement in tumor response and overall survival when standard chemotherapy was combined with trastuzumab (47% *vs* 34%, 13.8 mo *vs* 11.1 mo)[26]. An innovative and promising further development of trastuzumab, named T-DM1 is currently undergoing clinical testing. In the T-DM1 molecule the trastuzumab antibody is coupled to maytansine, a microtubule polymerization inhibitor which unfolds its effect after internalization of the antibody-receptor complex within the cytosol[27].

Recently it has been published that *in vitro* the cytotxic effect of trastuzumab on gastric cancer cell lines significantly increased when the cancer cells were pre-treated by incubation with reovirus serotype 3[28].

Matuzutumab is an IgG1 antibody with ADCC. Unlike cetuximab and nimotuzumab it is a fully humanized molecule. Unfortunately, it has been shown that combination treatment of matuzutumab with cytostatic substances is not beneficial for overall survival and response rates[29].

Panitumumab is an IgG2 antibody. It is routinely used in the treatment of metastatic colorectal cancer. The comparison of combined chemotherapy with or without panitumumab yielded disappointing results with a poorer outcome in the the panitumumab group in terms of overall survival and overall response rate (8.8 mo *vs* 11.3 mo and 42% *vs* 46%, respectively). Surprisingly, in the subgroup of patients with severe rash the overall survival of patients who received panitumumab-including treatment was significantly improved (10.2 mo *vs* 4.3 mo)[30].

Nimotuzumab is similar to matuzutumab a fully humanized antibody, known to exhibit ADCC. There is some evidence in the literature that nimotuzumab in combination with cytostatic substances might be effective in squamous cell carcinoma of the esophagus and in glioma. To date, there are two studies available investigating the effect of nimotuzumab plus cytostatic substances in metastatic gastric cancer. In one study, the overall response rate was improved (63% *vs* 50%) with similar progression free survival, the other study showed the progression free survival to be slightly improved with similar response rates (5.5 mo *vs* 3 mo)[10].

Pertuzumab is an inhibitor of homo - as well as heterodimerization of the EGF receptor. Therefore, it seems to be reasonable to combine pertuzumab with different EGF receptor antagonists like trastuzumab. It is also known to exhibit ADCC. The administration of pertuzumab is approved for metastatic breast cancer[31]. The combination of pertuzumab and trastuzumab seems to be effective in advanced gastric cancer with overall response rates up to 86%[32].

Vascular endothelial growth factor: The recruitment of new blood vessels for the supply of the growing tumor with nutrients and oxygen is known to be one of the crucial steps in tumor progression, especially in the development of distant metastases[33]. Although neoangiogenesis in the tumor environment and physiological angiogenesis partly have similar pathways there are remarkable differences in vessel architecture, vascular permeability as well as a different interplay of endothelial cells and perivascular cells. In this context, vascular growth factors play an crucial role. Vascular growth factors are expressed when tissue hypoxia is present. Several other changes can result in vascular endothelial growth factor (VEGF) up-regulation too, *e.g.*, low pH or silenced tumor suppressor genes like p53[34].

To date, we know five important factors of angiogensis: VEGF A-D and placenta derived growth factor. Furthermore, three targets for these growth factors have been detected: vascular endothelial growth factor receptor (VEGFR) 1-3. VEGFR2 seems to be the most important subtype. It is localized on the cell surface of endothelial cells and bone marrow derived endothelial progenitor cells[35]. VEGFR2 binds to VEGF A, C and D, leading to activation of the PI3K signaling pathway as well as MAP kinase signaling pathway[36]. Some of the most important down stream effects are the inhibition of apoptosis, the proliferation of endothelial cells and increased endothelial cell migration[35]. The binding of the mediator molecule to its receptor is substantially increased in the presence of the co-receptors neuropilin 1 and 2. The application of these co-receptors as possible targets for molecular based treatment is currently under development[37].

Overexpression of VEGF and its downstream molecules is common in numerous malignancies. Interestingly, Takahashi *et al*[38] already demonstrated in 1996 that VEGF is more frequently dysregulated in intestinal type than in diffuse type gastric cancer (36% and 16%, respectively). Two different antibodies targeting the VEGF signalling pathway have been shown to be effective and eligible in the treatment of advanced gastric cancer: Bevacizumab and ramucirumab.

Bevacizumab binds to VEGF-A and thus interrupts

the activation of VEGFR1 and VEGFR2[33]. Whereas different phase 1 and 2 trials revealed promising effects of bevacizumab on gastric cancer progression, the results of phase 3 studies were disappointing. Although in the AVAGAST study overall median survival was slightly longer in patients who received bevacizumab plus standard chemotherapy, these results did not reach a statistically significant level (12.1 mo and 10.1 mo, P = 0.1002). Merely progression free survival was significantly longer in the intervention group (6.7 mo and 5.3 mo, P = 0.0301)[39]. The subsequently performed AVATAR study did not show any benefit of treatment with bevacizumab in combination with standard chemotherapy as compared to standard chemotherapy only (median overall survival 10.5 and 11.4 mo, progression free survival 6.3 and 6.0 mo)[40]. Based on these results bevacizumab currently is not routinely used in the treatment of advanced gastric cancer.

Ramuzirumab is a competitive inhibitor of VEGFR2 with a 8fold higher affinity to the receptor as compared to natural ligands[41]. Two phase 3 studies revealed ramucirumab to have positive effects on the containment of gastric cancer progression. The REGARD study investigated the impact of ramucirumab as a second line therapy on advanced gastric cancer. In comparison to the placebo group as well overall survival, disease control rate and overall response rate were significantly better (3.8 mo *vs* 5.2 mo, 49% *vs* 23%, 3.4% *vs* 2.6%). Interestingly, among male patients these effects were even more distinct[42]. The RAINBOW study compared the outcomes after administration of paclitaxel with or without ramucirumab to a similar target audience. Overall survival and disease control rate both were better in the intervention group (9.6 mo *vs* 7.4 mo, 80% *vs* 64%)[43].

In summary, currently ramucirumab seems to be the only one option to treat advanced gastric cancer with a VEGF-R specific antibody.

Platelet derived growth factor receptor: The Platelet derived growth factor (PDGF) family consists of 4 homodimers A-D and the heterodimer AB. Due to its dimeric structure it binds to receptor molecules which subsequently activate each other. Two different subtypes of PDGF receptors have been identified (alpha and β)[44]. Under physiological conditions PDGF is released when platelets are damaged. Furthermore, PDGF signalling is known to play an important role in the embryonic development of kidney, blood vessels, lung and several components of the central nervous system[45,46].

In several aspects the importance of the PDGFs as well as its corresponding receptors have to be regarded as being closely connected with the VEGF system. Whereas activation of VEGF signalling leads to recruitment of new blood vessels, one important downstream effect of PDGF signaling is the maintenance of microvessels. The regulation of the tumor environment - especially activities of fibrocytes and pericytes - as well is partly realized by the PDGF signalling pathway[46].

Up-regulation of PDGF signaling has been demonstrated for prostate cancer, breast cancer, lung cancer as well as colorectal cancer. In gastric cancer it has been shown that PDGF is frequently overexpressed in tumor cells whereas its corresponding receptor is overexpressed in several cell types of the microenvironment. It has been postulated that the tumor cell derived PDGF signal selectively leads to the up-regulation of PDGFR expression in environmental non-tumour cells[46].

To date, there are no PDGF specific antibodies available for clinical use regarding gastric cancer.

Fibroblast growth factor: The fibroblast growth factor family constists of 23 molecule subtypes, targeting four different FGF receptor subtypes. In addition, several co-factors like Klotho-type co-receptors and heparan sulfat proteoglycans are involved in the initiation of the FGF signaling pathway[47]. Binding of the growth factor to its receptors leads to autophosphorylation of the receptor molecule which subsequently activates different signal cascades. Activation of the MAP kinase or WNT signaling pathway terminally regulates the transcription programming, whereas PI3K-AKT, Hedgehog, Notch and noncanonical WNT signaling pathway promote the epithelial-mesenchymal transition. Overall, the FGF signaling is involved in numerous biological processes, such as stemness, anti-apoptosis, proliferation, drug resistance, angiogenesis and invasion[47].

As for many other tumor entities, overexpression of FGF components has been described for gastric cancer, too. The FGFR-2 for instance is known to be up-regulated in 2%-9% of all gastric cancer cases, but is overexpressed in 50% in poorly differentiated and diffuse type gastric cancer[48].

Currently, there are several experimental studies in progress which evaluate the impact of monoclonal antibodies against FGF-19, FGFR-2 and FGFR-3 at the level of animal models.

Hepatocellular growth factor: Under physiological conditions, Hepatocellular growth factor (HGF) and its corresponding receptor MET play a central role in the embryonic development, wound healing and organ regeneration. Therefore, HGF is normally secreted by surrounding mesenchymal cells[49,50]. The physiological HGF signal can be altered by numerous molecular disorders, such as gene amplification, mutation and abnormal gene splicing[51]. Aberrant HGF signaling can be observed in a broad variety of different tumors, among them lung cancer, colorectal cancer, hepatocellular cancer and - as well - gastric cancer. The receptor is activated by receptor dimerization which is induced by binding of HGF. Activation of MAPK and PI3K-AKT signalings are typical subsequent downstream features which lead to cell proliferation, prolonged cell survival and cell mobilisation[52]. Whereas overexpression of MET seems to be a common feature in gastric cancer (22%-24%), gene amplification is infrequent (2%-10%). Aberrant

HGF signaling is related to poorer overall survival[53].

Currently, three different monoclonal antibodies targeting the HGF system are available: onartuzumab, rilotumumab and ficlatuzumab[52].

Onartuzumab has been demonstrated to be beneficial on the level of case reports but did not influence the clinical course in unselected patient populations.

Gastric cancer patients treated with rilotumumab in combination with chemotherapy following the ECX protocol showed a better overall survival as compared with those who received ECX only (5.7% and 4.2%)[54]. Global phase 3 studies dedicated to the impact of onartuzumab and rilotumumab on advanced gastric cancer are currently underway[52].

The benefit of ficlatuzumab combined with chemotherapy has been investigated for non-small cell lung cancer but did not have a statistically significant effect on overall survival[52].

Targeting the growth factor pathways by small molecules

During the last decades two main molecular approaches have been asserted to target growth factor receptors which in fact are complex proteins: Monoclonal antibodies which bind to selected regions on the molecule surface and receptor tyrosine kinase inhibitors (RTKI) which are small molecules. These molecules mimick a metabolite that binds to the active center of the kinase. Two main categories of RTKI can be (more or less) distinguished: RTKIs which bind selectively to one or more related receptor types, and so-called multi-tyrosine kinase receptor inhibitors which have a more pluripotent spectrum of potential receptor targets.

Essentially, RTKI are available for every growth factor receptor. However, clinical outcomes in particular regarding advanced or metastasized gastric cancer show at best moderate improvements in terms of tumor control and survival.

For EGFR gefitinib, erlotinib, lapatinib and dacomitinib have been developed. Gefitinib showed moderate improvement of overall survival in several phase 2 studies. Administration of erlotinib in combination with cytostatic substances led to significant improvement of tumor control in two phase 2 studies. Lapatinib did not show any improvement when administered to patients with advanced, unresectable or metastasized gastric cancer. The benefit of dacomitinib is not clearly evaluated to date[10].

For VEGFR apatinib is a selective inhibitor. Several studies showed a significant improvement for overall and progression free survival in patients with heavily pre-treated unresectable gastric cancer (OS 6.5 mo *vs* 4.7 mo, $P = 0.01$)[12].

Imatinib is a RTKI which targets PDGFR. It is well established in the treatment of gastrointestinal stroma tumors for over 10 years now. A phase 1 study in 2012 showed that imatinib was well tolerated in patients with advanced gastric cancer but did not show significant clinical improvement regarding survival and tumor control. Dasatinib, a novel PDGFR specific molecule is effective in the treatment of chronic lymphatic leukaemia, the benefit of dasatinib in the treatment of solid tumours is currently investigated[46].

For the FGFR family a broad variety of small molecules is presented in the literature: dovitinib, brivanib, intendanib and ponatinib to name only a few. However, none of them is established in the treatment of gastric cancer at present[47].

HGF specific small molecules can be subdivided in three categories: Type 1, 2 and 3.

Type 1 inhibitors are most specific to HGFR, for instance crizotinib. Type 2 inhibitors target a wider spectrum of receptors (AXL, RON, VEGFR2): foretinib, cabozantinib. Type 3 inhibitors bind as well to multiple receptor subtypes and different sites of the respective receptor: tivantinib. For gastric cancer only foretinib reached the level of a phase 2 study but unfortunately without significant benefit on an unselected patient group regarding HGFR expression[52].

Proteinase-activated receptors

Proteinase-activated receptors (PAR) is a subgroup in the family of G-protein-coupled receptors. Receptor activation is realized by specific serine-proteases, such as trypsine and thrombin, which subsequently leads to further activation of the PI3K signaling pathway. Interestingly, one downstream effect of upregulated PAR2 signaling is the trans-activation of EGF receptors with the known subsequent effects. There is some evidence that prostaglandin-2 may inhibit the PAR2 signaling pathway which could be a potential target for specific molecular treatment approaches, but to date there is no PAR-associated treatment introduced in to the clinical routine[55].

Morphogens and embryonic signaling pathways

Sonic hedgehog signaling: The Sonic hedgehog signaling (SHH) signaling pathway is one of the key players in the embryonic development, especially in defining body axes and segmental forming. The SHH signal is transduced within the cell *via* patched (PTCH), a transmembranous receptor which subsequently leads to the activation of smoothened and further to the deactivation of a protein complex which normally abolishes Gli, a nuclear factor that can initiate the expression of components of different other pathways, such as WNT, bone morphogenic protein (BMP) and Transforming growth factor β (TGF-β)[56].

Vismodegib, sonidegib and saredegib are small molecule drugs which inhibit smoothened and thus interrupt the intracellular transmitted SHH signal. Thereby, these molecules mimic the effect of cyclopamine, a naturally occurring SHH inhibitor. The effectiveness of vismodegib in targeted treatment has been described for different tumor entities: With a pilot study on metastatic pancreatic cancer patients it was shown that

vismodgib down-regulates the SHH activity but without statistical significance on survival so far[57]. Vismodegib has been proven as the very first SHH antagonist for the treatment of basal cell carcinoma in 2013[58].

Phase 1 studies to verify the clinical eligibility of sonidegib are currently underway. The evaluation of saridegib is at present in the stage of experimental studies.

Another interesting molecular approach towards SHH signaling might be the application of HMG reductase inhibitors, such as statins. The attachment of a cholesterin residue to the SHH molecule is known to be essential to initiate the SHH signaling pathway by SHH. Although to date there are no clinical trials available which introduced statins to clinical use for certain tumor entities, there is some evidence that statins influence the clinical and biological behavior of malignant tumors. Recently, it has been published that statins significantly decrease cancer-specific mortality, particularly in colorectal, prostate and breast cancer.

WNT signaling

WNT signaling is known to be evolutionary highly conserved. During the embryonic development it is mainly involved in cellular differentiation. But also in adults WNT signaling is indeed important, particularly in the stem cell niches of the gastrointestinal tract. Likewise the SHH signaling pathway, the WNT signal starts by binding of WNT ligands to its receptor frizzled which in turn co-acts with LRP and transduces the signal towards the cytosol. To date four different subpathways have been described. In the classical or also called the canonical WNT pathway a multiprotein complex consisting of Axin, GSK3B and APC is being destabilized. This multiprotein complex normally abolishes β-catenin by phosphorylation. The disintegration of the multiprotein complex in turn leads to an accumulation of active non-phosphorylized β-catenin, which subsequently moves to the nucleus and binds to components of transcription (TCF-LEF complex). Interestingly, WNT signaling is coupled to EGFR signaling by at least two mechanisms: First the activation of EGFR signaling leads to internalization of E-cadherin-β-catenin complexes which in turn promotes WNT-dependent gene expression and second E-cadherin inhibits EGFR signaling by preventing receptor dimerization[59,60].

The following targets have been defined to be eligible to suppress WNT activity: Porcupine (an enzyme that modifies the WNT ligands which is essential for their activity), the frizzled-LRP-dishevelled complex, axin, cyclooxygenase-2, GSK3β and the TCF-β-catenin complex. Different small molecules targeting porcupine are currently under experimental evaluation, most of them act as competitive ligands to porcupine. They are also called "inhibitors of WNT production"[61].

Aberrant WNT signaling is frequently observed in gastric cancer. B-catenin is overexpressed in up to 30% of gastric cancer cases, whereas the loss of APC function occurs in 20% of all gastric cancer cases. SFRP loss, a physiological down-regulation of WNT signaling, is as well frequently to be found in gastric cancer tissue[62,63].

At the moment there is no WNT associated treatment available for clinical routine, in particular not for gastric cancer.

Notch signaling

As another morphogenic signaling pathway Notch is known to be involved in embryonic organ development as well as in adult stem cell niche regulation. Notch promotes its cellular effects *via* regulation of proliferation, differentiation and apoptosis. The basic molecular mechanism is that one membrane-bound ligand (two subgroups: Jagged 1-2 and Delta like 1-4) binds to its receptor which is membrane-bound, too, but is belonging to a different cell. Thereafter the intracellular component of the receptor is cleaved. The Notch intracellular domain then moves to the nucleus and up-regulates expression of several genes, among them c-myc (oncogene), cyclin D1 (cell cycle promotion), p21 (cell cycle arrest) and bcl-2 (apoptosis)[64-66].

Notch activity has been described to be involved in several tumor entities and among them in gastric cancer. Particularly Notch 1, Jagged 1 and DLL 4 were found to be frequently dys-regulated in gastric cancer tissues. Furthermore, there were statistically significant differences in the incidence of their up-regulation when stratifying tumor tissues to the classification according to Lauren as well as tumor location and tumor size[66].

To date, there are no substances available which target at the Notch signaling pathway.

TGF-β and BMP

TGF-β and BMP constitute a super family of morphogens and regulate a broad variety of cellular activities. Up-regulation of the signal cascade may result in antidromic biological effects: At early tumor stages cell differentiation and apoptosis are promoted whereas proliferation is inhibited, leading finally to anti-tumor signals. On the other hand, the up-regulation of TGF-β and BMP in advanced tumor stages may result in the promotion of tumor angiogenesis, cell motility and aberrant interplay with the interstitium[67-69].

Several subtypes of the TGF-β/BMP family are frequently up-regulated in gastric cancer, for instance BMP7 can be verified in 55% of specimen, whereas BMP2 is up-regulated in almost all cases of gastric cancer and BMP4 up-regulation is a frequently occurring event in un-differentiated gastric cancer.

Dalantercept is an inhibitor of BMP9 and BMP10 which has been shown to suppress effectively tumor angiogenesis. It has been proven to be eligible in a phase 1 study and is now under evaluation as a palliative second line treatment for renal cell carcinoma. DMH-1, a novel small molecule which inhibits the intracellular component of BMP-1 has been shown to have anti-tumor effects in the animal model[70].

Nuclear factor κB and interleukin receptors

Nuclear factor κB (NF-κB) as well as interleukin signaling are known to be involved in cancer development and cancer progression. NF-κB can be regarded as a quick time transcription factor that regulates immune reaction as well as proliferation and apoptosis. Extracellular signals like bacterial or viral antigens, interleukin 1β and tumor necrosis factor initiate a signal which enters the nucleus within few minutes. This is realized by storing NF-κB in the cytosol which there is inactivated by forming a complex inhibitor of NF-κB (IκB). IKK, the IκB kinase inactivates IκB, which leads to a NF-κB release. Rapid movement of NF-κB to the nucleus in turn leads to up-regulated expression of different genes like cytokines, chemokines and adhesion molecules.

Upregulated NF-κB signaling in gastric cancer is associated with elevated proliferation, genomic instability and drug resistance.

Two different molecular approaches targeting NF-κB signaling are at the present time available: Phytochemicals: silibinin (Silybum marianum): Prostate cancer; resveratol (red grapes, red wine): Prostate cancer, mesothelioma; catechins (green tea): Prevention against numerous tumor entities.

The abovementioned agents are partly a domain of alternative medicine but not an integral part of the clinical routine. Systematic studies and randomized trials are needed to shed more light on the actual clinical impact of these treatment options.

Denosumab is an inhibitor of RANKL (receptor activator of NF-κB) and thus can down-regulate NF-κB signaling. It has been shown to be effective in giant cell tumor of bone in pre-clinical studies.

To our knowledge currently there is no molecular treatment available targeting the NF-κB signaling pathway in gastric cancer.

Furthermore, there is an abundance of inflammatory-associated molecular markers which are up-regulated in gastric cancer, including those which are associated with significantly poorer survival, such as different interleukins, HIF-1alpha, chemokine receptors as well as matrix metallo proeinases (MMP-3, -7, -9, -11).

Components and regulators of cell cycle

Cell cycle up-regulation is one of the most central mechanisms of tumor cell proliferation and tumor growth. It is strictly regulated by different controlling factors. The cell cycle can be sectioned into different cell cycle phases which only can be entered by passing the respective checkpoints. Under physiological conditions the entry of a cell into the cell cycle needs growth factors, whereas in tumor cells the cell cycle can be started at lower levels of growth factors or even at their complete absence[71,72]. Cyclin D1 and 2 as well as CDK 4 and 6 are the most important factors that promote the entry into the S phase of the cell cycle. Cyclin D1 and 2 are frequently up-regulated in gastric cancer. Furthermore, cyclin D is an important downstream target of different signaling pathways, such as SHH, WNT and Notch. In 15% of gastric cancer cases an up-regulated cyclin E can be observed[62,73,74]. The protein complexes formed by cyclin plus its corresponding CDK are inhibited by different factors, such as p21, which is down-regulated in 60% of gastric cancer cases[75].

Another major cell cycle associated key player is p53, the so-called "guardian of the genome", which is responsible for arresting the cell when DNA is severely damaged. Over 50% of all malignant tumors show a loss of p53, in gastric cancer these are at least 40%. Loss of p53 is known to be particularly frequent in advanced stages of gastric cancer and in those cases when tumor differentiation is low[76,77].

Cell cycle and its regulators are investigated intensively for several decades to find clinical eligible bonds which inhibit cell cycle activity and promote cycle arrest or apoptosis.

Flavipiridol (also known as alvocidib) as well as roscovitin (also known as seliciclib) can be regarded as CDK inhibitors of the first generation, both of them being relatively unspecific.

After promising results of phase 1 studies with inhibitory effects on multiple different CDK subtypes, the clinical outcomes in phase 2 studies were disappointing failing significant clinical activity. After all, there was a measurable clinical activity in some haematological neoplasms, such as chronic lymphatic leukaemia and mantle cell lymphoma.

Roscovitin, a purine based molecule failed to have clinical effects in as well phase 1 and phase 2 studies[78,79].

Dinaciclib as a CDK inhibitor of the second generation revealed remarkable activity on numerous tumor cell lines as well as in several tumor mouse models. In the subsequent phase 1 studies dinaciclib resulted in stable disease in different solid tumors, but again the positive results could not be confirmed with phase 2 studies with the exception of palliative treatment in refractory chronic lymphatic leukaemia, so that now a phase 3 study in this field is underway[78].

The impact of down-regulation of cyclin D1 by using adenoviral vectors is currently explored.

Currently the abovementioned drugs are not approved for clinical use in the treatment of gastric cancer.

SOME FUTURE PERSPECTIVES

Beside the further development of target-specific molecules against components of the abovementioned signaling pathways two categories of molecular tumor biology might be of interest: the clinical importance of micro RNAs and effectors of epigenetic regulation.

MicroRNAs are small molecules without coding function and with a usual length of 18 to 25 nucleotids. To date, more than 2000 different sequences have been detected in the human genome. It is postulated that microRNA molecules are involved in 30% of gene expression. Interestingly they are frequently to be found

at so-called fragile chromosomal sites and typically in intergenic regions. The signature of microRNAs changes from normal tissue to malignant tumor tissue. Micro RNAs can as well be down- and up-regulated.

For example miR-139 has been shown to be frequently down-regulated in gastric cancer. In contrast, overexpression leads to inhibited cell proliferation in gastric cancer cell lines. It seems to be involved in the regulation of the chemokine receptor CXCR4.

The individual signature of microRNAs might be used as a biomarker in predicting the biological behavior of tumors. Furthermore, antagonization of oncogenic microRNAs and the restoration of down-regulated microRNAs with tumorsuppressive activity might be promising targets in the future[80].

To a certain degree, the function of microRNA molecules is associated to epigenetic mechanisms, another challenging future perspective towards better understanding of the molecular biology of gastric cancer. Epigenetics means methylation of the DNA strand as well as different modifications of the histone molecules. DNA methylation is realized by DNMT 1 and 2 which place the methyl residues predominantly at so-called CpG rich regions. Hypermethylation of promoter regions upstream of tumor suppressor genes is a commonly observed phenomenon in different solid tumors. Histone molecules can be acetylated by HAT and deacetylated by HDACs at lysine sites, furthermore lysine as well as arginine sites can be methylated or demethylated. A broad variety of dys-regulated histone modification has been described for gastric cancer, for instance the hyperacetylation of histones neighboring the myc oncogene. Restoration of dyregulated histone and DNA modification might be another promising target to anticancer treatment[81].

Considering the variety of target specific therapeutics in relation to the clinical impact on the population of gastric cancer patients and the individual complexity of the "cancer organism" it becomes clear, that molecular targeted approaches generate their best effects on respective subgroups which harbour the suitable molecular signature. Therefore, the knowledge about the individual presence of molecular markers might become essential and of paramount interest in the future.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855]

2 **Meyer L**, Steinert R, Nowak L, Gellert K, Ludwig K, Saeger D, Gastinger I, Lippert H. [Prospective multicenter trial of gastric cancer surgery--a contribution to clinical research on quality control]. *Zentralbl Chir* 2005; **130**: 97-105 [PMID: 15849650]

3 **Cunningham SC**, Schulick RD. Palliative management of gastric cancer. *Surg Oncol* 2007; **16**: 267-275 [PMID: 17881220]

4 **Franklin WA**, Veve R, Hirsch FR, Helfrich BA, Bunn PA. Epidermal growth factor receptor family in lung cancer and premalignancy. *Semin Oncol* 2002; **29**: 3-14 [PMID: 11894009]

5 **Salomon DS**, Brandt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit Rev Oncol Hematol* 1995; **19**: 183-232 [PMID: 7612182]

6 **Hanawa M**, Suzuki S, Dobashi Y, Yamane T, Kono K, Enomoto N, Ooi A. EGFR protein overexpression and gene amplification in squamous cell carcinomas of the esophagus. *Int J Cancer* 2006; **118**: 1173-1180 [PMID: 16161046]

7 **Gibault L**, Metges JP, Conan-Charlet V, Lozac'h P, Robaszkiewicz M, Bessaguet C, Lagarde N, Volant A. Diffuse EGFR staining is associated with reduced overall survival in locally advanced oesophageal squamous cell cancer. *Br J Cancer* 2005; **93**: 107-115 [PMID: 15986037]

8 **Rüschoff J**, Dietel M, Baretton G, Arbogast S, Walch A, Monges G, Chenard MP, Penault-Llorca F, Nagelmeier I, Schlake W, Höfler H, Kreipe HH. HER2 diagnostics in gastric cancer-guideline validation and development of standardized immunohistochemical testing. *Virchows Arch* 2010; **457**: 299-307 [PMID: 20665045]

9 **Rüschoff J**, Hanna W, Bilous M, Hofmann M, Osamura RY, Penault-Llorca F, van de Vijver M, Viale G. HER2 testing in gastric cancer: a practical approach. *Mod Pathol* 2012; **25**: 637-650 [PMID: 22222640]

10 **Ayyappan S**, Prabhakar D, Sharma N. Epidermal growth factor receptor (EGFR)-targeted therapies in esophagogastric cancer. *Anticancer Res* 2013; **33**: 4139-4155 [PMID: 24122977]

11 **Galizia G**, Lieto E, Orditura M, Castellano P, Mura AL, Imperatore V, Pinto M, Zamboli A, De Vita F, Ferraraccio F. Epidermal growth factor receptor (EGFR) expression is associated with a worse prognosis in gastric cancer patients undergoing curative surgery. *World J Surg* 2007; **31**: 1458-1468 [PMID: 17516110]

12 **Waksal HW**. Role of an anti-epidermal growth factor receptor in treating cancer. *Cancer Metastasis Rev* 1999; **18**: 427-436 [PMID: 10855786]

13 **Karapetis CS**, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, Simes RJ, Chalchal H, Shapiro JD, Robitaille S, Price TJ, Shepherd L, Au HJ, Langer C, Moore MJ, Zalcberg JR. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med* 2008; **359**: 1757-1765 [PMID: 18946061]

14 **Bonner JA**, Harari PM, Giralt J, Cohen RB, Jones CU, Sur RK, Raben D, Baselga J, Spencer SA, Zhu J, Youssoufian H, Rowinsky EK, Ang KK. Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. *Lancet Oncol* 2010; **11**: 21-28 [PMID: 19897418]

15 **Vermorken JB**, Trigo J, Hitt R, Koralewski P, Diaz-Rubio E, Rolland F, Knecht R, Amellal N, Schueler A, Baselga J. Open-label, uncontrolled, multicenter phase II study to evaluate the efficacy and toxicity of cetuximab as a single agent in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck who failed to respond to platinum-based therapy. *J Clin Oncol* 2007; **25**: 2171-2177 [PMID: 17538161]

16 **Lorenzen S**, Schuster T, Porschen R, Al-Batran SE, Hofheinz R, Thuss-Patience P, Moehler M, Grabowski P, Arnold D, Greten T, Müller L, Röthling N, Peschel C, Langer R, Lordick F. Cetuximab plus cisplatin-5-fluorouracil versus cisplatin-5-fluorouracil alone in first-line metastatic squamous cell carcinoma of the esophagus: a randomized phase II study of the Arbeitsgemeinschaft Internistische Onkologie. *Ann Oncol* 2009; **20**: 1667-1673 [PMID: 19549707]

17 **Schønnemann KR**, Yilmaz M, Bjerregaard JK, Nielsen KM, Pfeiffer P. Phase II study of biweekly cetuximab in combination with irinotecan as second-line treatment in patients with platinum-resistant gastro-oesophageal cancer. *Eur J Cancer* 2012; **48**: 510-517 [PMID: 22244801]

18 **Chan JA**, Blaszkowsky LS, Enzinger PC, Ryan DP, Abrams TA, Zhu AX, Temel JS, Schrag D, Bhargava P, Meyerhardt JA, Wolpin BM, Fidias P, Zheng H, Florio S, Regan E, Fuchs CS. A multicenter phase II trial of single-agent cetuximab in advanced esophageal and gastric adenocarcinoma. *Ann Oncol* 2011; **22**: 1367-1373 [PMID: 21217058]

19 **Lee MS**, Mamon HJ, Hong TS, Choi NC, Fidias PM, Kwak EL, Meyerhardt JA, Ryan DP, Bueno R, Donahue DM, Jaklitsch MT, Lanuti M, Rattner DW, Fuchs CS, Enzinger PC. Preoperative

cetuximab, irinotecan, cisplatin, and radiation therapy for patients with locally advanced esophageal cancer. *Oncologist* 2013; **18**: 281-287 [PMID: 23429739]

20 **Ruhstaller T**, Pless M, Dietrich D, Kranzbuehler H, von Moos R, Moosmann P, Montemurro M, Schneider PM, Rauch D, Gautschi O, Mingrone W, Widmer L, Inauen R, Brauchli P, Hess V. Cetuximab in combination with chemoradiotherapy before surgery in patients with resectable, locally advanced esophageal carcinoma: a prospective, multicenter phase IB/II Trial (SAKK 75/06). *J Clin Oncol* 2011; **29**: 626-631 [PMID: 21205757]

21 **Clynes RA**, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. *Nat Med* 2000; **6**: 443-446 [PMID: 10742152]

22 **Nagata Y**, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin AA, Klos KS, Li P, Monia BP, Nguyen NT, Hortobagyi GN, Hung MC, Yu D. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell* 2004; **6**: 117-127 [PMID: 15324695]

23 **Lane HA**, Motoyama AB, Beuvink I, Hynes NE. Modulation of p27/Cdk2 complex formation through 4D5-mediated inhibition of HER2 receptor signaling. *Ann Oncol* 2001; **12** Suppl 1: S21-S22 [PMID: 11521716]

24 **Klos KS**, Zhou X, Lee S, Zhang L, Yang W, Nagata Y, Yu D. Combined trastuzumab and paclitaxel treatment better inhibits ErbB-2-mediated angiogenesis in breast carcinoma through a more effective inhibition of Akt than either treatment alone. *Cancer* 2003; **98**: 1377-1385 [PMID: 14508823]

25 **Smith I**, Procter M, Gelber RD, Guillaume S, Feyereislova A, Dowsett M, Goldhirsch A, Untch M, Mariani G, Baselga J, Kaufmann M, Cameron D, Bell R, Bergh J, Coleman R, Wardley A, Harbeck N, Lopez RI, Mallmann P, Gelmon K, Wilcken N, Wist E, Sánchez Rovira P, Piccart-Gebhart MJ. 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial. *Lancet* 2007; **369**: 29-36 [PMID: 17208639]

26 **Bang YJ**, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Ruschoff J, Kang Y-K, To GATI, Parnis F, McKendrick J, Price T, Mainwaring P, Van Cutsem E, Forones N, Olivatto L, Miziara JE, Li J, Wang J, Wang Y, Zheng L, Wang L, Feng-Yi F, Shen L, Xu JM, Jiao S, Guan Z, Yu SY, Pan L, Jin Y, Tao M, Qin S, Reyes DO, Valladares R, Landaverde D, Pfeiffer P, Vestlev PM, Tanner M, Bouche O, Michel P, Jacob J, Husseini F, Metges JP, Dominguez S, Viret F, Clemens M, zum Buschenfelde COM, Moehler M, Hoefeler H, Lordick F, Toache LMZ, Castro-Salguero H, Prasad S*VS*S, Gangadharan VP, Advani S, Julka PK, Aprile G, Barone C, Cascinu S, Di Costanzo F, Stefania S, Hamamoto Y, Sasaki Y, Yamaguchi K, Ohtsu A, Hatake K, Satoh A, Boku N, Sawaki A, Takiuchi H, Tamura T, Baba E, Nishina T, Miyata Y, Satoh T, Omuro Y, Saito H, Bang YJ, Kang YK, Hong DS, Jeung HC, Lim HY, Chung HC, Kim JG, Kim YH, Lee K, Park S, Leon E, Trevino SA, Sahui TS, Rodriguez AL, Sanchez RIL, Alvarez-Barreda R, de Mendoza FH, Leon-Chong J, Philco M, Sanches E, Quintela A, Sa A, Damasceno M, Coutinho C, Pinto AM, Teixeira MM, Braga S, Topuzov E, Cheporov S, Garin A, Gorbunova V, Lichinitser M, Khasanov RS, Manikhas GM, Biakhov M, Moiseenko V, Gotovkin E, Kulikov E, Lipatov O, Gladkov O, Landers G, Robertson B, Gravalos C, Queralt B, Galan MC, Gallego R, Aguilera MJS, Martin M, Fernandez-Martos C, Chao Y, Chang C, Yalcin S, Yilmaz U, Evans J, Falk S, Mansoor W, Ferry D, Thompson J, Gollins S. Trastuzumab in combination with chemotherapy *vs* chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010; **376**: 687-697 [PMID: 20728210]

27 **Barok M**, Tanner M, Köninki K, Isola J. Trastuzumab-DM1 is highly effective in preclinical models of HER2-positive gastric cancer. *Cancer Lett* 2011; **306**: 171-179 [PMID: 21458915]

28 **Hamano S**, Mori Y, Aoyama M, Kataoka H, Tanaka M, Ebi M, Kubota E, Mizoshita T, Tanida S, Johnston RN, Asai K, Joh T. Oncolytic reovirus combined with trastuzumab enhances antitumor efficacy through TRAIL signaling in human HER2-positive gastric cancer cells. *Cancer Lett* 2015; **356**: 846-854 [PMID: 25444894]

29 **Rao S**, Starling N, Cunningham D, Sumpter K, Gilligan D, Ruhstaller T, Valladares-Ayerbes M, Wilke H, Archer C, Kurek R, Beadman C, Oates J. Matuzumab plus epirubicin, cisplatin and capecitabine (ECX) compared with epirubicin, cisplatin and capecitabine alone as first-line treatment in patients with advanced oesophago-gastric cancer: a randomised, multicentre open-label phase II study. *Ann Oncol* 2010; **21**: 2213-2219 [PMID: 20497967]

30 **Waddell T**, Chau I, Cunningham D, Gonzalez D, Okines AF, Okines C, Wotherspoon A, Saffery C, Middleton G, Wadsley J, Ferry D, Mansoor W, Crosby T, Coxon F, Smith D, Waters J, Iveson T, Falk S, Slater S, Peckitt C, Barbachano Y. Epirubicin, oxaliplatin, and capecitabine with or without panitumumab for patients with previously untreated advanced oesophagogastric cancer (REAL3): a randomised, open-label phase 3 trial. *Lancet Oncol* 2013; **14**: 481-489 [PMID: 23594787]

31 **Dawood S**, Sirohi B. Pertuzumab: a new anti-HER2 drug in the management of women with breast cancer. *Future Oncol* 2015; **11**: 923-931 [PMID: 25760974]

32 **Kang YK**, Rha SY, Tassone P, Barriuso J, Yu R, Szado T, Garg A, Bang YJ. A phase IIa dose-finding and safety study of first-line pertuzumab in combination with trastuzumab, capecitabine and cisplatin in patients with HER2-positive advanced gastric cancer. *Br J Cancer* 2014; **111**: 660-666 [PMID: 24960402]

33 **Sullivan LA**, Brekken RA. The VEGF family in cancer and antibody-based strategies for their inhibition. *MAbs* 2010; **2**: 165-175 [PMID: 20190566]

34 **Kerbel RS**. Tumor angiogenesis. *N Engl J Med* 2008; **358**: 2039-2049 [PMID: 18463380]

35 **Fontanella C**, Ongaro E, Bolzonello S, Guardascione M, Fasola G, Aprile G. Clinical advances in the development of novel VEGFR2 inhibitors. *Ann Transl Med* 2014; **2**: 123 [PMID: 25568876]

36 **Ebos JM**, Bocci G, Man S, Thorpe PE, Hicklin DJ, Zhou D, Jia X, Kerbel RS. A naturally occurring soluble form of vascular endothelial growth factor receptor 2 detected in mouse and human plasma. *Mol Cancer Res* 2004; **2**: 315-326 [PMID: 15235107]

37 **Aprile G**, Bonotto M, Ongaro E, Pozzo C, Giuliani F. Critical appraisal of ramucirumab (IMC-1121B) for cancer treatment: from benchside to clinical use. *Drugs* 2013; **73**: 2003-2015 [PMID: 24277700]

38 **Takahashi Y**, Cleary KR, Mai M, Kitadai Y, Bucana CD, Ellis LM. Significance of vessel count and vascular endothelial growth factor and its receptor (KDR) in intestinal-type gastric cancer. *Clin Cancer Res* 1996; **2**: 1679-1684 [PMID: 9816116]

39 **Ohtsu A**, Shah MA, Van Cutsem E, Rha SY, Sawaki A, Park SR, Lim HY, Yamada Y, Wu J, Langer B, Starnawski M, Kang YK. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a randomized, double-blind, placebo-controlled phase III study. *J Clin Oncol* 2011; **29**: 3968-3976 [PMID: 21844504]

40 **Shen L**, Li J, Xu J, Pan H, Dai G, Qin S, Wang L, Wang J, Yang Z, Shu Y, Xu R, Chen L, Liu Y, Yu S, Bu L, Piao Y. Bevacizumab plus capecitabine and cisplatin in Chinese patients with inoperable locally advanced or metastatic gastric or gastroesophageal junction cancer: randomized, double-blind, phase III study (AVATAR study). *Gastric Cancer* 2015; **18**: 168-176 [PMID: 24557418]

41 **Miao HQ**, Hu K, Jimenez X, Navarro E, Zhang H, Lu D, Ludwig DL, Balderes P, Zhu Z. Potent neutralization of VEGF biological activities with a fully human antibody Fab fragment directed against VEGF receptor 2. *Biochem Biophys Res Commun* 2006; **345**: 438-445 [PMID: 16682007]

42 **Fuchs CS**, Tomasek J, Yong CJ, Dumitru F, Passalacqua R, Goswami C, Safran H, dos Santos LV, Aprile G, Ferry DR, Melichar B, Tehfe M, Topuzov E, Zalcberg JR, Chau I, Campbell W, Sivanandan C, Pikiel J, Koshiji M, Hsu Y, Liepa AM, Gao L, Schwartz JD, Tabernero J, Investigators RT, Mendez G, Maldonado D, Bartoli M, Guminski A, Ransom D, Price T, Jefford M, Karapetis C, Zalcberg J, Young R, Eek R, Beslija S, Barrios C, Franke F, Brust

L, Murad A, Andrade A, Nascimento Y, Liberatti M, Azambuja A, Skare NS, Schwartsmann G, Vieira dos Santos L, Lunardon Padilha S, Alberto Schlittler L, Spratlin J, Tehfe M, del Castillo C, Enrique Gonzalez Fernandez M, Bilic A, Mihaljevic S, Boric Z, Trivanovic D, Stahalova V, Brychta M, Vanasek J, Jakesova J, Lazarov P, Petera J, Zemanova M, Deeb N, Avendano Flores O, Chakravarthy S, Dassappa L, Ramanan S, Deshmukh C, Sivanandan C, Almel S, Kumar R, Prayogo N, Rudiman R, Bilancia D, Ravaioli A, Frustaci S, Bari M, Amoroso V, Amoroso D, Martoni A, Kim Y-H, Hong YS, Chung H, Cho Cho JY, Chehade I, Brincat S, Gibbs D, Querol J, Koralewski P, Rozamowski P, Ganea-Motan D, Filip D, Udrea A, Gorbunova V, Protsenko S, Gladkov O, Vladimirov V, Severtsev A, Akopov A, Orlov S, Robertson B, Fernandez Parra E, Rivera Herrero F, Longo F, Visa L, Hurtado A, Gallego Plazas J, Wang J-Y, Kok V, Thongprasert S, Erkisi M, Sevinc A, Turhal S, Gokmen E, Smith D, Ferry D, Hanna W, Leslie W, Barnhill M, Thomas M, Cescon T, Langdon R, Patel R, Kozuch P, Ajani J, Reid T, Malik I, Gravenor D, Fuchs C. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial. *Lancet* 2014; **383**: 31-39 [PMID: 24094768]

43 **Wilke H**, Muro K, Van Cutsem E, Oh SC, Bodoky G, Shimada Y, Hironaka S, Sugimoto N, Lipatov O, Kim TY, Cunningham D, Rougier P, Komatsu Y, Ajani J, Emig M, Carlesi R, Ferry D, Chandrawansa K, Schwartz JD, Ohtsu A. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomised phase 3 trial. *Lancet Oncol* 2014; **15**: 1224-1235 [PMID: 25240821]

44 **Heldin CH**. Targeting the PDGF signaling pathway in tumor treatment. *Cell Commun Signal* 2013; **11**: 97 [PMID: 24359404]

45 **Li X**, Eriksson U. Novel PDGF family members: PDGF-C and PDGF-D. *Cytokine Growth Factor Rev* 2003; **14**: 91-98 [PMID: 12651221]

46 **Suzuki S**, Dobashi Y, Hatakeyama Y, Tajiri R, Fujimura T, Heldin CH, Ooi A. Clinicopathological significance of platelet-derived growth factor (PDGF)-B and vascular endothelial growth factor-A expression, PDGF receptor-β phosphorylation, and microvessel density in gastric cancer. *BMC Cancer* 2010; **10**: 659 [PMID: 21118571]

47 **Katoh M**, Nakagama H. FGF receptors: cancer biology and therapeutics. *Med Res Rev* 2014; **34**: 280-300 [PMID: 23696246]

48 **Hattori Y**, Itoh H, Uchino S, Hosokawa K, Ochiai A, Ino Y, Ishii H, Sakamoto H, Yamaguchi N, Yanagihara K, Hirohashi S, Sugimura T, Terada M. Immunohistochemical detection of K-sam protein in stomach cancer. *Clin Cancer Res* 1996; **2**: 1373-1381 [PMID: 9816310]

49 **Chmielowiec J**, Borowiak M, Morkel M, Stradal T, Munz B, Werner S, Wehland J, Birchmeier C, Birchmeier W. c-Met is essential for wound healing in the skin. *J Cell Biol* 2007; **177**: 151-162 [PMID: 17403932]

50 **Huh CG**, Factor VM, Sánchez A, Uchida K, Conner EA, Thorgeirsson SS. Hepatocyte growth factor/c-met signaling pathway is required for efficient liver regeneration and repair. *Proc Natl Acad Sci USA* 2004; **101**: 4477-4482 [PMID: 15070743]

51 **Gherardi E**, Birchmeier W, Birchmeier C, Vande Woude G. Targeting MET in cancer: rationale and progress. *Nat Rev Cancer* 2012; **12**: 89-103 [PMID: 22270953]

52 **Smyth EC**, Sclafani F, Cunningham D. Emerging molecular targets in oncology: clinical potential of MET/hepatocyte growth-factor inhibitors. *Onco Targets Ther* 2014; **7**: 1001-1014 [PMID: 24959087]

53 **Kuniyasu H**, Yasui W, Kitadai Y, Yokozaki H, Ito H, Tahara E. Frequent amplification of the c-met gene in scirrhous type stomach cancer. *Biochem Biophys Res Commun* 1992; **189**: 227-232 [PMID: 1333188]

54 **Iveson T**, Donehower RC, Davidenko I, Tjulandin S, Deptala A, Harrison M, Nirni S, Lakshmaiah K, Thomas A, Jiang Y, Zhu M, Tang R, Anderson A, Dubey S, Oliner KS, Loh E. Rilotumumab in combination with epirubicin, cisplatin, and capecitabine as first-line treatment for gastric or oesophagogastric junction adenocarcinoma: an open-label, dose de-escalation phase 1b study and a double-blind, randomised phase 2 study. *Lancet Oncol* 2014; **15**: 1007-1018 [PMID: 24965569]

55 **Caruso R**, Pallone F, Fina D, Gioia V, Peluso I, Caprioli F, Stolfi C, Perfetti A, Spagnoli LG, Palmieri G, Macdonald TT, Monteleone G. Protease-activated receptor-2 activation in gastric cancer cells promotes epidermal growth factor receptor trans-activation and proliferation. *Am J Pathol* 2006; **169**: 268-278 [PMID: 16816379]

56 **Yang L**, Xie G, Fan Q, Xie J. Activation of the hedgehog-signaling pathway in human cancer and the clinical implications. *Oncogene* 2010; **29**: 469-481 [PMID: 19935712]

57 **Kim EJ**, Sahai V, Abel EV, Griffith KA, Greenson JK, Takebe N, Khan GN, Blau JL, Craig R, Balis UG, Zalupski MM, Simeone DM. Pilot clinical trial of hedgehog pathway inhibitor GDC-0449 (vismodegib) in combination with gemcitabine in patients with metastatic pancreatic adenocarcinoma. *Clin Cancer Res* 2014; **20**: 5937-5945 [PMID: 25278454]

58 **Meiss F**, Zeiser R. Vismodegib. *Recent Results Cancer Res* 2014; **201**: 405-417 [PMID: 24756807]

59 **Katoh Y**, Katoh M. Hedgehog target genes: mechanisms of carcinogenesis induced by aberrant hedgehog signaling activation. *Curr Mol Med* 2009; **9**: 873-886 [PMID: 19860666]

60 **Hu T**, Li C. Convergence between Wnt-β-catenin and EGFR signaling in cancer. *Mol Cancer* 2010; **9**: 236 [PMID: 20828404]

61 **Takada R**, Satomi Y, Kurata T, Ueno N, Norioka S, Kondoh H, Takao T, Takada S. Monounsaturated fatty acid modification of Wnt protein: its role in Wnt secretion. *Dev Cell* 2006; **11**: 791-801 [PMID: 17141155]

62 **Wu WK**, Cho CH, Lee CW, Fan D, Wu K, Yu J, Sung JJ. Dysregulation of cellular signaling in gastric cancer. *Cancer Lett* 2010; **295**: 144-153 [PMID: 20488613]

63 **Tahara E**. Molecular biology of gastric cancer. *World J Surg* 1995; **19**: 484-488; discussion 489-490 [PMID: 7676688]

64 **Borggrefe T**, Oswald F. The Notch signaling pathway: transcriptional regulation at Notch target genes. *Cell Mol Life Sci* 2009; **66**: 1631-1646 [PMID: 19165418]

65 **Ronchini C**, Capobianco AJ. Induction of cyclin D1 transcription and CDK2 activity by Notch(ic): implication for cell cycle disruption in transformation by Notch(ic). *Mol Cell Biol* 2001; **21**: 5925-5934 [PMID: 11486031]

66 **Du X**, Cheng Z, Wang YH, Guo ZH, Zhang SQ, Hu JK, Zhou ZG. Role of Notch signaling pathway in gastric cancer: a meta-analysis of the literature. *World J Gastroenterol* 2014; **20**: 9191-9199 [PMID: 25083094]

67 **Mishra L**, Derynck R, Mishra B. Transforming growth factor-beta signaling in stem cells and cancer. *Science* 2005; **310**: 68-71 [PMID: 16210527]

68 **Miyazono K**, Suzuki H, Imamura T. Regulation of TGF-beta signaling and its roles in progression of tumors. *Cancer Sci* 2003; **94**: 230-234 [PMID: 12824914]

69 **Kim YI**, Lee HJ, Khang I, Cho BN, Lee HK. Selective inhibition of cell growth by activin in SNU-16 cells. *World J Gastroenterol* 2006; **12**: 3000-3005 [PMID: 16718778]

70 **Gupta S**, Gill D, Pal SK, Agarwal N. Activin receptor inhibitors--dalantercept. *Curr Oncol Rep* 2015; **17**: 14 [PMID: 25708802]

71 **O'Connor PM**. Mammalian G1 and G2 phase checkpoints. *Cancer Surv* 1997; **29**: 151-182 [PMID: 9338101]

72 **Schwartz GK**, Shah MA. Targeting the cell cycle: a new approach to cancer therapy. *J Clin Oncol* 2005; **23**: 9408-9421 [PMID: 16361640]

73 **Boonstra J**. Progression through the G1-phase of the on-going cell cycle. *J Cell Biochem* 2003; **90**: 244-252 [PMID: 14505341]

74 **Akama Y**, Yasui W, Yokozaki H, Kuniyasu H, Kitahara K, Ishikawa T, Tahara E. Frequent amplification of the cyclin E gene in human gastric carcinomas. *Jpn J Cancer Res* 1995; **86**: 617-621 [PMID: 7559076]

75 **Yasui W**, Akama Y, Kuniyasu H, Yokozaki H, Semba S, Shimamoto F, Tahara E. Expression of cyclin-dependent kinase inhibitor

p21WAF1/CIP1 in non-neoplastic mucosa and neoplasia of the stomach: relationship with p53 status and proliferative activity. *J Pathol* 1996; **180**: 122-128 [PMID: 8976868]

76 **Menendez D**, Inga A, Resnick MA. The expanding universe of p53 targets. *Nat Rev Cancer* 2009; **9**: 724-737 [PMID: 19776742]

77 **Fenoglio-Preiser CM**, Wang J, Stemmermann GN, Noffsinger A. TP53 and gastric carcinoma: a review. *Hum Mutat* 2003; **21**: 258-270 [PMID: 12619111]

78 **Asghar U**, Witkiewicz AK, Turner NC, Knudsen ES. The history and future of targeting cyclin-dependent kinases in cancer therapy. *Nat Rev Drug Discov* 2015; **14**: 130-146 [PMID: 25633797]

79 **Whittaker SR**, Te Poele RH, Chan F, Linardopoulos S, Walton MI, Garrett MD, Workman P. The cyclin-dependent kinase inhibitor seliciclib (R-roscovitine; CYC202) decreases the expression of mitotic control genes and prevents entry into mitosis. *Cell Cycle* 2007; **6**: 3114-3131 [PMID: 18075315]

80 **Tong F**, Cao P, Yin Y, Xia S, Lai R, Liu S. MicroRNAs in gastric cancer: from benchtop to bedside. *Dig Dis Sci* 2014; **59**: 24-30 [PMID: 24114043]

81 **Kang C**, Song JJ, Lee J, Kim MY. Epigenetics: an emerging player in gastric cancer. *World J Gastroenterol* 2014; **20**: 6433-6447 [PMID: 24914365]

P- Reviewer: Bologna M
S- Editor: Yu J **L- Editor**: A **E- Editor**: Jiao XK

World Journal of Gastrointestinal Oncology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4251/wjgo.v7.i11.303

World J Gastrointest Oncol 2015 November 15; 7(11): 303-316
ISSN 1948-5204 (online)

TOPIC HIGHLIGHT

2015 Advances in Gastric Cancer

Gastric cancer: The times they are a-changin'

Maria Antonietta Satolli, Lucio Buffoni, Rosella Spadi, Ilaria Roato

Maria Antonietta Satolli, Department of Oncology, University of Turin, 10126 Torino, Italy

Lucio Buffoni, Rosella Spadi, Department of Onco-ematology, Medical Oncology, AOU Città della Salute e della Scienza, Molinette, 10126 Turin, Italy

Ilaria Roato, CeRMS, AOU Città della Salute e della Scienza di Torino, Department of Medical Science, University of Turin, 10126 Torino, Italy

Author contributions: Satolli MA designed and wrote the article; Spadi R and Buffoni L analysed literature data; Roato I wrote and revised the article.

Supported by CRT Foudation; and by the Italian Ministry of Health: Ricerca Sanitaria Finalizzata e Giovani Ricercatori 2009, No. GR 2009-1584485.

Conflict-of-interest statement: The authors declare no conflict of interest.

Correspondence to: Ilaria Roato, PhD, CeRMS, AOU Città della Salute e della Scienza di Torino, Department of Medical Science, University of Turin, Via Santena 5, 10126 Torino, Italy. roato78@libero.it
Telephone: +39-11-6334672
Fax: +39-11-6334672

Received: May 13, 2015
Peer-review started: May 15, 2015
First decision: July 1, 2015
Revised: July 15, 2015
Accepted: August 13, 2015
Article in press: August 14, 2015
Published online: November 15, 2015

Abstract

Gastric cancer is the third leading cause of cancer death worldwide. Even though during these last decades gastric cancer incidence decreased in Western countries, it remains endemic and with a high incidence in Eastern countries. The survival in advanced and metastatic stage of gastric cancer is still very poor. Recently the Cancer Genoma Atlas Research Network identified four subtypes with different molecular profiles to classify gastric cancer in order to offer the optimal targeted therapies for pre-selected patients. Indeed, the key point is still the selection of patients for the right treatment, on basis of molecular tumor characterization. Since chemotherapy reached a plateau of efficacy for gastric cancer, the combination between cytotoxic therapy and biological agents gets a better prognosis and decreases chemotherapeutic toxicity. Currently, Trastuzumab in combination with platinum and fluorouracil is the only approved targeted therapy in the first line for c-erbB2 positive patients, whereas Ramucirumab is the only approved targeted agent for patients with metastatic gastric cancer. New perspectives for an effective treatment derived from the immunotherapeutic strategies. Here, we report an overview on gastric cancer treatments, with particular attention to recent advances in targeted therapies and in immunotherapeutic approach.

Key words: Targeted therapy; Chemotherapy; Gastric cancer; Immunotherapy

Core tip: Gastric cancer, despite its decrease in West Countries, remains one of the most common malignancies worldwide. The prognosis in the advanced setting is often poor even with a multidisciplinary approach, which aims to increase the patients' survival. The molecular classification of four subtypes of gastric adenocarcinomas (The Cancer Genome Atlas project) allowed a better stratification of patients in clinical trials for targeted

therapies. Biologic agents, modulating the immune checkpoints, seem to be the best promising therapeutic approach, opening new perspective for advanced gastric cancer treatment.

Satolli MA, Buffoni L, Spadi R, Roato I. Gastric cancer: The times they are a-changin'. *World J Gastrointest Oncol* 2015; 7(11): 303-316 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i11/303.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i11.303

INTRODUCTION

During these last decades gastric cancer incidence decreased, but it still remains he third most frequent cause of cancer-related mortality worldwide[1,2]. At diagnosis, about half of gastric cancer patients show an advanced disease, with a 5-year survival rate lower than 30%[3,4]. Even though gastric cancer incidence decreased in Western countries, it remains endemic and with a high incidence in Eastern countries. The incidence in Eastern Asia was 24.2/100000; in Latin America and Caribben was 15.8-23.7/100000; in Africa and Northern America there was the lowest incidence (http://globocan.iarc.fr, accessed on 16/01/2015). In the United States the estimated number of new cases of gastric cancer in 2014 overtook 22000 cases[2], with differences among several ethnic groups. In Europe gastric cancer holds the 5th place for male sex and the 6th place for female sex for incidence[5,6].

Gastric cancer can be hereditary and associated to specific mutations[7]. Often Gastric cancer are sporadic and depends on progressive accumulations of genotypic and phenotypic modifications due to different etiological factors such as wrong diets, presence of gastritis, infection by *H. pylori*, smoking, obesity, elevated body mass index (BMI) and reflux[8,9]. Indeed, combinations of smoking, elevated BMI, and reflux may account for almost 70% of total cases[10,11]. Untreated gastritis induces a chronic mucosal inflammation, that causes structural changes of gastric mucosa, leading to metaplastic transformation and structural changes of the glandular tissue, that can undergo to a neoplastic differentiation[9,12].

Many efforts have been done in order to prevent gastric cancer: recognition and treatment of *Helicobacter pylori* (*H. pylori)* infections; diet changes like lower use of salted foods, and the use of refrigerators are factors which contributed to reduce the incidence of gastric cancer[13]. Nonetheless, the incidence of the cancers of gastro-esophageal junction (GEJ) and gastric cardia increased in western country[14]. To explain these epidemiological data there are several interpretations, such as problems related to a correct subdivision among esophageal, junctional and cardia adenocarcinomas, that may have cloud the issue leading to a misclassification[14,15].

MOLECULAR CLASSIFICATION: "THERE'S A BATTLE OUTSIDE AND IT IS RAGING"

The most common classification systems, such as the Laurén and the World Health Organization classifications, are essential for therapeutic decision, but are unable to predict response to targeted therapies. Recent studies on molecular profiling of upper gastrointestinal (GI) tumors increased our knowledge on the biology of gastric cancer and developed a molecular classification, identifying dysregulated pathways in different subgroups of gastric cancer.

The Cancer Genoma Atlas (TCGA) analysis uncovered four main genotypes of gastric cancer based on the molecular characterization of 295 primary adenocarcinomas[16]: Epstein-Barr virus (EBV) positive; microsatellite unstable (MSI); genomically stable (GS); and tumors with chromosomal instability (CIN). The EBV-associated tumors are about 10% of the cancers; they display CDKN2A promoter hypermethylation and in 80% of the cases they have PIK3CA mutations and amplification of JAK2 and CD274 and PDCD1LG2. This subset of gastric cancer can benefit of targeted immunotherapy. MSI tumors represent approximately the 20% of the cases and show mutations in PIK3CA, HER2, HER3, and EGFR. GS gastric tumors represent about 20% of the adenocarcinomas, they show newly described mutations in RHOA, which are relevant to control actin-myosin-dependent cell contractility and motility. Almost 50% of gastric tumors showed CIN, with a marked aneuploidy and focal amplification of receptor tyrosine kinases, such as VEGFA. This subtype is frequently found in GEJ cancer. This study provides a guide to test new agents against new molecular targets specific for a gastric cancer subtype, enabling clinicians to make a better selection of patients for future trials with targeted therapy and immunotherapy in gastric cancer.

SURGICAL TREATMENT

Radical surgery is still the only one curative treatment, but gastric cancer is mostly diagnosed in local advanced or metastatic stage, when the survival still remains poor[17]. Surgical resection for gastric or GEJ cancer combined with D1/D2 lymph node dissection should be performed by experienced team to reduce mortality and morbidity[18]. Surgery with curative intent has to provide free-margin and at least D1 resection combined with removal at minimum of 15 lymph nodes[19]. The extent of lymph node dissection is a significant surgical procedure that specifies the lymph node involvement, because preoperative lymph node staging is considered highly unreliable. The results of many randomized studies have not agreed to demonstrate superiority of D2 resection *vs* the D1 resection; to conclude the standard recommended surgery could be at least D1 resection, while D2 resection could be indicated in some

particular young patients[20-22].

A combine approach of surgery and chemotherapy can improve outcomes of gastric cancer patients, with potentially resectable tumors. The Magic trial conducted in United Kingdom[23] and the ACCORD trial conducted in France[24] showed a statistically significant longer 5-year survival for patients treated with perioperative chemotherapy. Decisions were less clear for adjuvant setting: chemotherapy alone or with radiotherapy should be recommended for patients underwent to a less than optimal lymph node resection, R1 or with lymph node involvement[25].

CYTOTOXIC CHEMOTHERAPY: "YOUR OLD ROAD IS RAPIDLY AGING"

The only treatment for patients with metastatic disease is the systemic chemotherapy. Currently there is no first-line standard single chemotherapeutic regimen but cisplatin based regimens, which able to improve the overall survival (OS) because a cytotoxic combination is superior to a single-agent regimen[26]. The physician's choice of platinum-based doublets or triplets is taken after careful assessment of the patients' performance status. Currently, standard first-line options include FOLFOX [5-fluorouracil (5-FU, oxaliplatin)], S1/cisplatin or 5-FU/cisplatin, DCF (docetaxel, cisplatin, and 5-FU), ECF/EOX (epirubicin, cisplatin/oxaliplatin, and 5-FU/capecitabine). In the platinum-based doublets oxaliplatin could substitute cisplatin, while capecitabine and S1 are equivalent in terms of effectiveness to 5-FU[27,28].

A third drug, usually epirubicin or taxotere, can be added with the aim to obtain a high response rate (RR) and a better control of the disease[29,30].

Although most patients receive a first-line chemotherapy, in clinical practice only less than half of patients progressing after treatment receive a salvage treatment, mostly in western countries. Only recently a second-line chemotherapy has shown to be superior to the best supportive care in advanced disease: Two distinct trials proved that irinotecan and docetaxel, in monochemotherapy, control the metastatic disease[31,32].

It's evident that chemotherapy reached a plateau of efficacy for gastric cancer, thus in an attempt to improve it, getting a better prognosis and decreasing chemotherapeutic toxicity, the combination between cytotoxic therapy and biological agents is useful. Indeed, results of ToGA trial allow to approve the first biologic drug for stomach cancer. Today, trastuzumab is indicated for first-line in patients HER2-positive in combination with 5-FU or capecitabine and cisplatinum[33].

Even more recently, two randomized trials demonstrated that Ramucirumab, a monoclonal antibody directed against VEGFR-2, is effective both alone or in combination with a second line chemotherapy with paclitaxel, in patients with metastatic gastric cancer[34,35].

BIOMARKERS FOR GASTRIC CANCER

Since chemotherapy is not effective in all patients, who are resistant to cytotoxic treatment, it's mandatory to develop new anticancer regimens and to identify biomarkers able to predict the patients' responses to different citotoxic drugs in gastric cancer. One of the molecules currently under investigation is the alpha-1 Microglobulin/Bikunin Precursor (AMBP), because its high level in serum could predict poor response to paclitaxel- capecitabine regimen[36]. Thus AMBP could be a potential biomarker to identify patients who would benefit from this specific chemotherapeutic regimen.

Forkhead box transcription factor 1 (FoxM1) could be an other potential biomarker and target for gastric cancer. Indeed, FoxM1 overexpression is correlated with the pathogenesis of a variety of human malignancies such as breast cancer, non-small-cell lung cancer and ovarian cancer, and it is a critical molecule for chemoresistance to a microtubule-stabilizing anticancer agent as docetaxel[37-42]. FoxM1 overexpression was significantly associated with resistance in chemotherapy of docetaxel in addition to 5-FU, S-1 and cisplatin (CDDP) for patients with advanced gastric cancer[43,44]. Taken together, these results suggest that FoxM1 is involved in the mechanisms of resistance to cytotoxic drugs and its inhibition might be a promising therapeutic strategy for is a pleiotropic protein affecting a wide range of molecular and cellular processes.

Accumulating data, derived by different studies on the role of ANXA2 in tumorigenesis, suggest that ANXA2 is aberrantly expressed in a wide spectrum of tumors, affecting tumor cell adhesion, proliferation, apoptosis, invasion, metastasis and the interaction between immune cells and cancer cells in the microenvironment[45,46]. The expression of ANXA2 in gastric cancer tissue is associated to a poor prognosis[47,48]. A recent study reported that ANXA2 might be a good diagnostic and predictive marker for response to chemotherapy, indeed the chemotherapy-unresponsive patients show higher serum ANXA2 levels than the chemotherapy-responsive ones[49].

Several studies have consistently demonstrated that miRNAs, short noncoding RNA molecules involved in post-translational regulation of gene expression, contribute significantly to human carcinogenesis by modulating the expression of both proto-oncogenes and tumor suppressor genes[50]. Studies on gastric cancer allowed to identify up- and down-regulated miRNAs, which can be associated to clinical-pathological features of gastric cancer[51,52]. Moreover, many data report that the expression of different miRNA patterns is also associated with premalignant stages or even risk conditions to develop gastric cancer, such as *H. pylori* infection[53,54].

TARGETED THERAPY: "FOR THE LOSER NOW, WILL BE LATER TO WIN"

Advances in knowledge of the cancer biology led to the

discover of specific oncogenic signalling pathways of different driver mutations, resulting in the development of many new target agents. The prevalence of genomic alterations in gastric cancer patients has been recently assessed. Indeed, five distinct gastric cancer patient subgroups have been identified, according to the genomic alterations: FGFR2 (9% of tumours), KRAS (9%), epidermal growth factor receptor (EGFR) (8%), ERBB2 (7%) and MET (4%). Therefore, about 37% gastric cancer patients could be treated with anti-RTK/RAS agents[55]. Many new target therapies were tested in clinical trials in gastric cancer patients, but without great results, thus we need further molecular studies to identify right patients for the right drugs.

EGFR1 inhibitors

EGFR is a trans-membrane glycoprotein receptor expressed in about 60% of gastric cancer patients. A meta-analysis on 1600 gastric cancer patients evaluated the survival according to the EGFR expression, showing that positive EGFR expression does not significantly predict the poor survival of gastric cancer[56].

Cetuximab is an immunoglobulin G1 type chimeric monoclonal antibody targeting EGFR. Thanks to the successes achieved by the cetuximab in colorectal cancer, it was also tested in gastric cancer in combination with chemotherapy in phase Ⅱ studies: FOLFIRI[57]; cisplatin plus docetaxel[58]; oxaliplatin plus 5-FU[59,60] with encouraging results regarding ORR in all trials. However, the expected results from the combination of chemotherapy and cetuximab were not confirmed by the phase Ⅲ EXPAND study (cetuximab in combination with capecitabin and cisplatin), that failed both in terms of OS and of progression-free survival (PFS)[61]. The analysis of potential biomarkers such as KRAS mutations, EGFR expression, HER2 expression, did not identify the patients group responsive to cetuximab.

The REAL3 randomised study tested the efficacy of panitumumab in combination with EOX (epirubicin, oxaliplatin, capecitabine). In October 2011, trial recruitment was halted and panitumumab withdrawn because did not show any benefit at interim analysis. In multivariate OS analysis with performance status and disease stage, both *KRAS* mutation and *PIK3CA* mutation were negatively prognostic. No prognostic effect was associated with *HER2* or *PTEN* status, and no *BRAF* mutations were identified[62].

The phase Ⅲ COG trial evaluated Gefitinib *vs* placebo in patients with metastatic esophageal or types Ⅰ/Ⅱ junctional adeno or squamous cell carcinoma, progressing after prior chemotherapy. This study did not improve OS; however, there was significant improvement in PFS, quality of life and palliation of symptoms[63].

Some trials of several novel EGFR agents are still ongoing. The phase Ⅲ ENRICH trial of nimotuzumab in combination with irinotecan in the second-line setting is pre-selecting patients with high EGFR expression (NCT01813253). Finally, before defining EGFR inhibitors as ineffective in gastric cancer, we absolutely identify predictive biomarker for response, in order to avoid repeating the mistakes done with gefitinib in lung cancer[64,65].

HER2 inhibitors

All members of the HER family of receptor tyrosine kinases, whose members include HER1 (or EGFR), HER2, HER3, and HER4, are expressed in gastric cancer. HER2 is a protoncogene encoded by ERBB2 found on chromosome 17. The percentage of gastric cancer patients positive to HER2 ranges from 7% to 42% due to tumor heterogeneity and the different methods and scoring systems used for evaluating HER2[66]. HER2-positivity also depends on histologic type: It is frequent in patients with intestinal histology (34%), rare in those with diffuse-type histology (6%); it also depends on disease site: It's frequent in GEJ (32%) and rare in gastric cancer (18%)[67]. It remains unclear whether HER2 positivity is a negative prognostic factor because there are studies both for and against this hypothesis[68,69]. The ToGA trial is a randomized Phase Ⅲ study which brought to the approval of Herceptin as the only targeted agent for patients with HER2 positive metastatic gastric and GEJ cancer. Three thousand six hundred patients were assessed for HER2 positivity, and the 594 patients HER2-positive were recruited in the clinical trial[33], which evaluated efficacy of anti-HER2 trastuzumab in combination with 5-FU or capecitabine and cisplatin *vs* chemotherapy alone in HER2 patient. Median OS in control arm was 11.1 mo compared with 13.8 mo in experimental arm with a statistically significant increase in RR. Every 3 wk for six cycles, the treatment was administered, whereas trastuzumab was continued every 3 wk until disease progression, or unacceptable toxicity, or withdrawal of consent. One of the most interesting result of this study was that the survival advantage was greatest in patients with IHC 3+ tumors (HR = 0.66, 95%CI: 0.50-0.87), less effective in patients with IHC 2+ tumors (HR = 0.78, 95%CI: 0.55-1.10), and ineffective in those with HER2 gene-amplified, but not protein expressing (IHC 0 or 1+) tumors. Grade 3 or 4 adverse events (AEs) occurred in similar percentages in both arms. Now all patients with advanced or metastatic gastric or GEJ cancer, and suitable for combination chemotherapy with fluoropyrimidine and cisplatin, should be assessed for the expression of HER2 and therefore can be treated with additional trastuzumab.

The phase Ⅲ HELOISE trial, combining trastuzumab with cisplatin and capecitabine (NCT01450696), and the TEX regimen, combining trastuzumab with Taxotere, Eloxatin and Xeloda as treatment for HER2 positive non-resectable cancer (NCT01295086) are ongoing to improve the efficacy of combination chemotherapy. Heloise trial aims to assess whether trastuzumab maintenance is able to increase the gastric cancer patients' survival. The second trial evaluates the safety

and efficacy of three drugs combination in addition to trastuzumab.

Development of resistance to trastuzumab urged investigators to test new drugs target HER2, but not all HER2-targeting agents have had such an unequivocal success.

The dual HER2/EGFR inhibitor lapatinib (Tykerb) is an orally drug. Lapatinib is a very interesting TK1 inhibitor, able to interfere with cell proliferation, to sensitize gastric cancer cells to the irinotecan metabolite SN-38[70] and to have a synergic effect combined with chemotherapy[71].

Lapatinib was evaluated in the first setting in combination with capecitabine/oxaliplatin (LOGiC trial). 545 patients were randomized and 487 had HER2+ centrally confirmed, but combination treatment failed to improve the median OS (12.2 mo *vs* 10.5 mo, HR = 0.91, 95%CI: 0.73-1.12) compared with chemotherapy alone. No correlation was found between intensity of staining for HER2 by IHC and outcomes**.** However, the LOGiC trial did suggest that Asian patients and those under age 60 years might benefit of this combination[72].

The TyTAN trial is a phase Ⅲ study second-line therapy of paclitaxel. Investigators enrolled 261 HER2-amplified Asian patients and they observed statistically significant improvements in OS and PFS among a pre-specified subgroup of patients with strong HER2 positivity. However, addition of lapatinib did not produce any significant benefit on PFS (5.4 mo *vs* 4.4 mo) or OS (11.0 mo *vs* 8.9 mo) with significant gastrointestinal (diarrhoea 20%) and bone marrow toxicity (febrile neutropenia, 7%)[73]. Several other HER2-targeting agents were also evaluated in clinical trials, including trastuzumab emtansine (T-DM1; Kadcyla) and pertuzumab (Perjeta).

T-DM1 is a conjugate molecule that combine a cytotoxic agent with an antibody targeted specific tumor cells. Due to positive results in breast cancer (EMILIA trial)[74], is now ongoing a randomized, multicenter, adaptive phase Ⅱ/Ⅲ study to study the efficacy and safety of trastuzumab emtansine (T-DM1) *vs* taxane (docetaxel or paclitaxel), in patients with previously treated locally advanced or metastatic HER2-positive gastric cancer, including adenocarcinoma of the GEJ (GATSBY trial, NCT01641939). Another phase Ⅰ/Ⅱ study was designed to assess T-DM1 in combination with capecitabine in patients with metastatic gastric cancer (NCT01702558). The ongoing phase Ⅲ JACOB trial is evaluating the combination of pertuzumab, trastuzumab, and chemotherapy (NCT01774786). The combination of two antibodies aims to amplify the trastuzumab antitumor efficacy in HER2-positive patients. Again with the aim of overcoming resistance to trastuzumab, it is also ongoing a phase Ⅱ trial with afatinib, an irreversible panHER TK1 (NCT01522768). A better and more accurate knowledge of the mechanisms of cellular resistance to trastuzumab is essential for the future. Certainly, the intra-tumor heterogeneity in HER2 expression/amplification is very important, but other mechanisms have been implicated as PI3K/Akt pathway, m-TOR inhibitors, MET-inhibitors (when c-MET is overexpressed), overexpression of IGF-1 receptor (IGF-1R), SRC inhibitors. From these pre-clinical studies will emerge the right molecules to be tested in the next clinical trials.

Another HER2-directed strategy is represented by vaccines. Despite the great success of HER2 vaccine strategies in animal models, effective clinical results have not yet been obtained[75].

HER2 vaccines, DNA or peptide-based, are studied mainly for breast cancer, often in combination with other HER2 targeted therapies[76]. Regional treatments are another possible application. Radio-immunotherapy is now evaluating 212Pb immunoconjugates with trastuzumab in intraperitoneal treatment[77].

Angiogenesis inhibitors

Angiogenesis is crucial for tumor growth, thus anti-angiogenic drugs are now a standard of care for many solid tumors of the adult. In gastric cancer VEGF is overexpressed in 40% and VEGFR in 36% of cases. Some studied reported that VEGF overexpression correlates with advanced and aggressive disease[78-80]. We recently showed that even though VEGF serum levels were higher in gastric patients than in controls, they were not correlated to the OS[81].

Bevacizumab is a recombinant humanized monoclonal antibody anti-VEGF-A, a strong driver of angiogenesis in tumorigenesis. Phase Ⅱ studies conducted with bevacizumab in chemotherapy combination, showed encouraging RR, time to disease progression (TTP), and OS[82,83], but not confirmed by phase Ⅲ trials. The phase Ⅲ trial AVAGAST evaluated effects of bevacizumab in combination with cisplatin and capecitabine as a first-line therapy in 774 patients with advanced gastric carcinoma[84]. Addition of bevacizumab failed to improve OS, with median OS 12.1 mo *vs* 10.1 mo, even though it achieved a significant increase in PFS (6.7 mo *vs* 5.3 mo) and overall RR (46.0% *vs* 37.4%). To evaluate the hypothesis that angiogenic markers may be predictive for bevacizumab efficacy, correlations between pre-specified biomarkers (VEGF-A, protein expression of neuropilin-1, and VEGFR-1 and VEGFR-2) and clinical outcomes were assessed too. High plasma VEGF-A levels and low expression of neuropilin-1 showed a trend toward improved OS. These are strong biomarker candidates that aim to predict the response to bevacizumab in gastric cancer patients from non-Asian regions[85]. Moreover, the sub-group analysis by geographical regions, tumor site and histology concluded that the highest survival benefits are for non-Asian patients with distal gastric non-diffuse type cancer (OS 11.4 mo *vs* 7.3 mo).

MAGIC-B trial with bevacizumab in combination with chemotherapy (ECX regimen) in perioperative setting is ongoing[86]. The study results could provide relevant information on antiangiogenic efficacy in the early stages of disease.

In this complex and rather disappointing background,

results of ramucirumab in the treatment of advanced gastric cancer have been published. Ramucirumab (IMC-1121B) is a fully human IgG1 monoclonal antibody direct aganist VEGFR-2. The phase Ⅲ REGARD trial was conducted to assess efficacy and safety of ramucirumab as second-line treatment *vs* supportive care in advanced gastric cancer. Three hundred and fifty-five patients were enrolled. Ramucirumab significantly improved OS (OS 5.2 mo *vs* 3.8 mo) and PFS (2.1 mo *vs* 1.3 mo), with good tolerability. Most frequent grade 3-4 AEs were hypertension (7.3% in experimental arm *vs* 2.6% in placebo arm), anemia (6.4% *vs* 7.8%), abdominal pain (51.% *vs* 2.6%), ascites effusion (4.2% *vs* 4.3%), asthenia (42.% *vs* 3.5%), hyponatremia (3.4% *vs* 0.9%) and anorexia (3.4% *vs* 3.5%). No grade 4 hypertension has been observed[34].

The phase Ⅲ RAINBOW was conducted in 665 patients with the aim to evaluate efficacy and safety of ramucirumab plus paclitaxel combination in second-line treatment in advanced gastric cancer patients. The study reached its primary objective of increasing OS, indeed the combination resulted superior in median OS (9.7 mo *vs* 7.3 mo), median PFS (4.4 mo *vs* 2.8 mo) and RR (28% *vs* 16%). Hypertension, fatigue and neutropenia were the most frequent toxicities in experimental arm, whereas febrile neutropenia had comparable incidence.

Gaining the results of ramucirumab in second-line, we would have expected a good success also in first-line. However, the study combination of FOLFOX6 plus ramucirumab has not demonstrated to increase OS and PFS in patients with metastatic gastric cancer (23%), GEJ (31%) and esophageal (46%). 168 patients were enrolled, median PFS 6.4 mo *vs* 6.7 mo, OS 11.7 mo *vs* 11.5 mo. Addition of RAM to FOLFOX6 showed PFS difference at 3 mo and improved disease control rate (DCR); longer PFS in RAM *vs* placebo was observed in gastric/GEJ cancer patients[87].

Apatinib is a tyrosine kinase inhibitor (TKI) agent targeting VEGFR-2 (VEGFR). A phase Ⅱ randomised trial tested apatinib *vs* placebo in 144 pre-treated gastric cancer patients. Apatinib was taken orally in two different ways: 850 mg once and 450 mg twice a day. Median OS times were 2.50 mo (in the placebo arm), 4.83 mo (apatinib 850 mg once a day arm) and 4.27 mo (apatinib 450 mg twice a day arm). Median PFS times were 1.40 mo, 3.67 mo, and 3.20 mo, respectively. The differences between apatinib and placebo groups were statistically significant for both PFS ($P < 0.001$) and OS ($P < 0.001$ and 0.0017).Toxicities were tolerable and manageable[88]. The multicenter, randomized, double-blind, placebo-controlled phase 3 trial tested Apatinib 850 mg, po, qd, 28 d as one cycle or matching placebo. The study was planned to enroll 270 cases, stratified to the number of metastatic sites ($\leqslant 2$ or > 2). Median overall survival (mOS) was significantly prolonger in the apatinib group compare with in the placebo group. The results confirmed the efficacy and safety of apatinib in the patients with advanced gastric cancer[89].

Sunitinib and sorafenib are multi-target TKIs also studied in order to suppress angiogenesis in gastric cancer. Phase Ⅱ open-label randomized trial evaluated the combination of sunitinib plus docetaxel *vs* docetaxel monotherapy in second-line treatment in 107 patients with metastatic gastric cancer. Sunitinib arm was associated with a significantly higher ORR (41.1% *vs* 14.3%), but there was no significant difference in TTP (3.9 mo *vs* 2.6 mo) [90].

Sorafenib targets BRAF, VEGF, and PDGFR[91]. Combination of sorafenib plus chemotherapy (docetaxel and cisplatin) was assessed in a phase Ⅱ trial, first-line setting, in 44 patients with metastatic gastric cancer. The combination demonstrated a PFS of 5.8 mo, median OS of 13.6 mo, and ORR 41%; grade 3-4 EAs toxicity was neutropenia[92].

Pazopanib is an oral second-generation multitargeted TKI, which showed antiangiogenic and antitumor activity. There are two phase Ⅱ trials now ongoing in order to evaluate efficacy and safety of pazopanib as first-line treatment in metastatic gastric cancer. The first one, a phase Ⅱ PaFLO trial, wants to examine FLO (5-FU, leukovorin and oxaliplatin) + pazopanib used in combination for advanced gastric cancer (ClinicalTrials.gov Identifier: NCT01503372). The second one, a phase Ⅱ non-randomized open label trial, evaluates Pazopanib in combination with Capecitabine and Oxaliplatin in patients with advanced gastric cancer. The primary end-point is RR, the second end-points are PFS, OS and metabolic response rate by PET-CT (ClinicalTrials.gov Identifier: NCT01130805).

Hepatocyte growth factor-mesenchymal-epithelial transition factor axis

Mesenchymal-epithelial transition factor (c-MET) is the TK receptor of hepatocyte growth factor (HGF)[93]. c-MET expression or amplification was documented in many solid tumors and was correlated with poor prognosis in gastric cancer too. IHC analysis in gastric cancer specimens showed c-MET expression in 65% of cases with high-intensity staining in about 20% of cases[94]. However, the real activation of c-MET mutations and its resulting amplification, is a rare event: c-MET amplification occurs in 5%-10% of cases[95]. This discrepancy between expression and amplification of c-MET has important consequences when we design clinical trials with HGF-c-MET pathway inhibitors.

Rilotumumab (AMG 102) is human monoclonal antibody (IgG2) against HGF. A phase Ⅱ double-blind randomized study, evaluated the efficacy and safety of rilotumumab with ECX regimen in gastric cancer patients in first-line treatment. Rilotumumab associated to chemotherapy improved the median PFS from 4.2 to 5.6 mo, and the OS from 8.9 to 11.1 mo. In the rilotumumab plus ECX arms, the most common adverse observed eventswere: neutropenia, anemia, peripheral edema, thrombocytopenia, and deep vein thrombosis[96]. MET protein levels and gene copy

numbers were measured in archival tumor samples by immunohistochemistry (IHC) and fluorescence *in situ* hybridization, respectively. Rilotumumab in combination with ECX improved the median OS from 5.7 to 11.1 mo in patients with gastric tumors with high MET expression.

The RILOMET-01 phase Ⅲ trial evaluated the efficacy and safety of Rilotumumab + ECX in MET-pos by IHC, previously untreated G/GEJ cancer. Primary endpoint was OS. 609 patients were randomized, but the study was stopped early bacause an imbalance in deaths (data cutoff: Nov 2014). OS, PFS and ORR were statistically worse in the exsperimental arm. The subgroup with higher percentages of cells with ⩾ 1+ MET expression does not seem to benefit with ramucirumab. PK and MET biomarker analyses are pending, thus we don't know whether they will offer any answers to this failure[97].

Onartuzumab is a humanized, monovalent (one-armed) monoclonal antibody against MET. One phase Ⅲ trial (randomized multicenter double-blind placebo-controlled studies), currently ongoing (but it's not recruiting participants) is evaluating the efficacy and safety of onartuzumab (MetMAb) in combination with mFOLFOX6 in patients with metastatic HER2-negative and Met-positive adenocarcinoma of the stomach or GEJ (NCT01662869).

Crizotinib is a small MET kinase inhibitor. Phase Ⅰ study showed promising activity in c-MET amplified gastric cancer patients[98].

Tivatinib is a selective non-ATP competitive small-molecule inhibitor of c-MET. Phase Ⅱ single-arm study evaluated the efficacy of tivantinib monotherapy in Asian patients with previous treatment for MGC (ARQ-197). Tivantinib was administered orally daily. The primary end-point was the DCR. Thirty patients were enrolled and no objective responses were observed, and DCR was 36.7%. There was not relationship between efficacy and gene amplification of c-MET, expression of c-MET, p-MET and HGF[99]. New clinical trials with c-MET inhibitors were restricted to patients defined as a "MET positive" to identify selected patients for a special genetic/molecular profile. However, the HGF/c-MET axis is involved in multiple pathways that operate at different levels[100]. The anti-HGF compounds may not be sufficient to complitely inhibit HGF/c-MET axis[101]. Hereafter it will be necessary to define with much more precision what "MET positive" gastric cancer means.

m-TOR inhibitors - PI3K pathway inhibition

m-TOR regulates angiogenesis, cellular metabolism, proliferation, and cell growth. Its activation is done through the PI3K pathway (*via* Akt/protein kinase B and tuberous sclerosis complex). In gastric cancer, mTOR and p-mTOR (its activated form) overexpression were respectively 50.8% and 46.5%. Overexpression of total mTOR protein significantly correlated with tumor differentiation, T1/T2 tumors, and stage Ⅰ/Ⅱ/Ⅲ disease. p-mTOR overexpression significantly correlated with lymph node metastasis and all stage disease[102].

Everolimus is an oral m-TOR inhibitor, approved for the treatment of renal cell carcinoma, breast cancer, and progressive NET of pancreatic origin. A phase Ⅱ study, in 53 patients with previously treated metastatic gastric cancer, reported a median PFS of 2.7 mo and OS of 10.1 mo. Common grade 3/4 AEs included anemia, hyponatremia, increased gamma-glutamyltransferase, and lymphopenia. Grade 1/2 pneumonitis was reported in 15.1% of patients[103]. Another phase Ⅱ trial assessed the efficacy and safety of combination regimen of capecitabine plus everolimus in patients with refractory gastric cancer who have failed at least two cytotoxic regimens. Forty seven patients were enrolled in this trial. Everolimus in combination with capecitabine achieved an ORR of 10.6% and a DCR of 48.9%, with respectively a median PFS and OS of 2.3 mo and 5.1 mo[104]. The phase Ⅲ GRANITE-1 evaluated everolimus or BSC plus placebo in 656 previously treated advanced gastric cancer patients. The results of this trial showed median OS of 5.39 mo in the everolimus arm and an OS of 4.3 mo in the placebo arm, with an advantage in PFS statistically significant but clinically irrelevant (1.7 mo *vs* 1.4 mo)[105]. Phase Ⅲ study in advanced gastroesophageal adenocarcinoma patients comparing everolimus combined with paclitaxel *vs* paclitaxel alone (NCT01248403) is ongoing.

IGF family

The IGF family plays an important role in growth and metabolism. Deregulation of IGFs/IGF-1R system promotes metastases diffusion, proliferation and invasion in gastric cancer. A number of antibodies targeting IGF-1R have been studied. Ganitumab (AMG 479) and figitumumab (CP 751) have been evaluated in phase Ⅰ study in patients with solid tumors, including gastric cancer. They showed promising results[106].

PARP inhibitors

PARP inhibitors (Poly-ADP-Ribose-Polymerase) have been studied in breast cancer with a know history of deficient BRCA1/2. The activity of PARPS inhibitors is improved in presence of drugs that cause double-strand breaks in DNA such as platinum compounds.

Olaparib activity has been proven in a phase Ⅱ trial with paclitaxel (Bang YJ Im SA J ClinOncol 2013 31(sup). The study failed to increase the PFS, but it improved OS. A randomized phase Ⅲ with paclitaxel in gastric cancer patient second-line is ongoing (NCT019245337).

IMMUNOTHERAPY: "…AND KEEP YOUR EYES WIDE"

Until few years ago, the more validated hypothesis was that epithelial tumors originate from tissue stem cells. A large intra-tumoral heterogeneity exists and cancer stem cells are part of it, indeed they are in the primary tumors, but they also disseminate to different

organs, remaining dormant or originating metastases and often are responsible to chemo-resistance[107,108]. To date, it's evident that tumor growth depends on the interactions among cancer cells, microenvironment and immune system cells. Tumor and cancer stem cells express receptors for antigens on specific cell type, thus determining the capability of one tumor to metastasize to a specific organ, such as for breast, lung and prostate cancer which commonly metastasize to bone[109-112]. The importance of tumor microenvironment in promoting cancer progression is even more recognized, because its cellular components release a series of factors which constitute a favourable soil for cancer cell homing and growth[113,114]. Looking at the immune system, a variable number of immune cells infiltrate tumors: mast cells, lymphocytes, macrophages and myeloid derived suppressor cells (MDSCs), with a deep impact on tumor progression[115]. For instance, MDSCs are a heterogeneous population of immature myeloid cells driving the progression of cancer disease by suppressing both the innate and adaptive immune response. Indeed they suppress CD4 and CD8 T cell populations, and promote the activation and expansion of regulatory T cells, which mediate immunosuppression[116-118].

A strong rationale exists to adopt the immunotherapy for gastric cancer, because inflammation has been recognised as an hallmark of cancer[119] and gastric cancer, particularly the upper GI tumors are an inflammatory-mediated disease[120]. Here we will describe the last frontiers of immunotherapy in gastric cancer treatment, but a comprehensive overview of immunotherapy in gastric cancer has been recently published my Murphy *et al*[121].

Encouraging results derive from the combination of cellular immunotherapy and chemotherapy, that improves the quality of life and might prevent the recurrence in patients with advanced gastric carcinoma[122]. The TCGA network identified elevated programmed death ligand-1 (PD-L1) expression in the EBV subtype in gastric cancer[16]. PD-1 is an immune checkpoint, involved in tumor suppression and in tumor microenvironment, because it regulates T cell pathways. New frontiers of immunotherapy are focalized on targeting the immune checkpoints, in order to remove inhibitory pathways that block an effective T cell response against the tumor[123]. Two antibodies against PD-1 (Pembrolizumab and Nivolumab) have been approved in 2014 form United States Food and Drug Administration. The checkpoint therapy could be useful for gastroesophageal cancer, which express PD-L1 in 18% to 42 % of cases[124]. Phase Ⅱ and phase Ⅲ clinical trials involving either single agent PD-1/PD-L1 inhibition or combined with CTLA-4 inhibitors (ipilimumab) are ongoing. In KEYNOTE-012 trial 39 patients PD-L1-positive with advanced gastric cancer received pembrolizumab, which showed a positive anti-cancer activity with an objective response of 22.2%, the median time to response was 8 wk (range 7-16 wk), with a median duration of response of 24 wk (range 8+ to 33+ wk). At 6 mo, 24% of patients showed no signs of disease progression, and 69% remained alive; the median PFS reached 1.9 mo. The most common AEs included fatigue (17.9%), decreased appetite (12.8%), hypothyroidism (12.8%), and arthralgia (10.3%). Four patients showed severe AEs associated with pembrolizumab, particularly, one of these patients died for treatment-associated hypoxia[125]. The OS data were presented at 2015 ASCO Annual meeting: The 6-mo OS rate was 69%. These results support the ongoing development of pembrolizumab for gastric cancer[126]. The phase Ⅱ KEYNOTE-059 study will soon be initiated to evaluate pembrolizumab as monotherapy or in combination with cisplatin and 5-FU in patients with advanced gastric or GEJ adenocarcinoma[127].

On May 2015 the phase Ⅲ KEYNOTE-061 study started. This is a Randomized trial of Pembrolizumab *vs* Paclitaxel in Advanced Gastric or GEJ adenocarcinoma patients who progressed after first-line therapy with platinum and fluoropyrimidine (NCT02370498).

In the near future, ipilimumab and nivolumab, two immunostimulatory monoclonal antibodies with antineoplastic effects, might offer new therapeutic options for patients with advanced gastric cancer[128]. In particular, Nivolumab, a fully human IgG4 anti-PD-1 monoclonal antibody, resulted active and generally well tolerated in patients with advanced solid tumors in a phase Ⅰ trial[129,130]. A Japanese randomized phase Ⅲ study started in october 2014 to evaluate Nivolumab (ONO-4538) *vs* BSC in patients with unresectable advanced or recurrent GC patients (NCT02267343).

CONCLUSION: "...AS THE PRESENT NOW, WILL LATER BE PAST"

Gastric cancer is one of the most common causes of cancer death in the world. Healing can only be guaranteed by an optimal surgery and still in the early stages of the disease. However, especially in Western countries, diagnosis is too late and the survival of patients with metastatic disease rarely exceeds 12 mo of diagnosis.

The multidisciplinary approach is always mandatory: The perioperative treatment, when indicated, has shown to be effective in increasing the survival of these patients and, in advanced disease, the total care by nutritionist, surgeon and oncologist has positive impact on the quality of life of these patients.

Chemotherapy in metastatic disease is the only chance of cure, but brings with it side effects also important and poor response rates. "... Your old road is rapidly aging" sang Bob Dylan (www.bobdylan.com), but it is true that at the moment that is the way we know best. Perhaps times are changing. As for lung and colorectal cancer, the targeted therapies are revolutionizing the clinical practice, but we also learned that to achieve maximum efficacy of these new molecules we have to change tumors classification.

New drugs and new classification: the genomic and molecular classification given by TCGA network will help

us to characterize with greater precision our patients. "... There's a battle outside and it is raging" but we will be armed with new knowledge.

Some clinical trials have led to the registration of drugs such as trastuzumab and ramucirumab. For EGFR inhibitors, lapatinib or everolius, the phase Ⅲ studies represented a setback.

However, the key is still patients selection on basis of molecular tumor characterization. Gefitinib in lung cancer reminds us "... for the loser now, will be later to win".

Which is the best cytotoxic combination for target therapies? Which is the best setting for using the new molecules? We do not know yet. In deed, it's possible that gastric cancer during progression disease and under evolutionary pressure of cytotoxic treatment can transform molecularly into a different phenotype.

Moreover, ethnic differences may cause different responses to the same molecules. Even this finding will lead to a personalized cancer medicine.

Finally, immunotherapy opens a vast and fascinating scenery for gastric cancer treatment. Some etiological factors such as viral and bacterial infections *via* EBV and *H. pylori* suggests that gastric cancer can be treated with new drugs such as immunotherapy checkpoint inhibitors.... And keep your eyes wide.

REFERENCES

1 **Parkin DM**. The global health burden of infection-associated cancers in the year 2002. *Int J Cancer* 2006; **118**: 3030-3044 [PMID: 16404738 DOI: 10.1002/ijc.21731]

2 **Siegel R**, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin* 2014; **64**: 9-29 [PMID: 24399786 DOI: 10.3322/caac.21208]

3 **Suzuki R**, Yamamoto E, Nojima M, Maruyama R, Yamano HO, Yoshikawa K, Kimura T, Harada T, Ashida M, Niinuma T, Sato A, Nosho K, Yamamoto H, Kai M, Sugai T, Imai K, Suzuki H, Shinomura Y. Aberrant methylation of microRNA-34b/c is a predictive marker of metachronous gastric cancer risk. *J Gastroenterol* 2014; **49**: 1135-1144 [PMID: 23942619 DOI: 10.1007/s00535-013-0861-7]

4 **Bria E**, De Manzoni G, Beghelli S, Tomezzoli A, Barbi S, Di Gregorio C, Scardoni M, Amato E, Frizziero M, Sperduti I, Corbo V, Brunelli M, Bersani S, Tortora G, Scarpa A. A clinical-biological risk stratification model for resected gastric cancer: prognostic impact of Her2, Fhit, and APC expression status. *Ann Oncol* 2013; **24**: 693-701 [PMID: 23131390]

5 **Ferlay J**, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, Forman D, Bray F. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. *Eur J Cancer* 2013; **49**: 1374-1403 [PMID: 23485231]

6 **Botterweck AA**, Schouten LJ, Volovics A, Dorant E, van Den Brandt PA. Trends in incidence of adenocarcinoma of the oesophagus and gastric cardia in ten European countries. *Int J Epidemiol* 2000; **29**: 645-654 [PMID: 10922340]

7 **Wang K**, Yuen ST, Xu J, Lee SP, Yan HH, Shi ST, Siu HC, Deng S, Chu KM, Law S, Chan KH, Chan AS, Tsui WY, Ho SL, Chan AK, Man JL, Foglizzo V, Ng MK, Chan AS, Ching YP, Cheng GH, Xie T, Fernandez J, Li VS, Clevers H, Rejto PA, Mao M, Leung SY. Whole-genome sequencing and comprehensive molecular profiling identify new driver mutations in gastric cancer. *Nat Genet* 2014; **46**: 573-582 [PMID: 24816253]

8 **Pizzi M**, Saraggi D, Fassan M, Megraud F, Di Mario F, Rugge M. Secondary prevention of epidemic gastric cancer in the model of Helicobacter pylori-associated gastritis. *Dig Dis* 2014; **32**: 265-274 [PMID: 24732192]

9 **Rugge M**, Capelle LG, Cappellesso R, Nitti D, Kuipers EJ. Precancerous lesions in the stomach: from biology to clinical patient management. *Best Pract Res Clin Gastroenterol* 2013; **27**: 205-223 [PMID: 23809241]

10 **Engel LS**, Chow WH, Vaughan TL, Gammon MD, Risch HA, Stanford JL, Schoenberg JB, Mayne ST, Dubrow R, Rotterdam H, West AB, Blaser M, Blot WJ, Gail MH, Fraumeni JF. Population attributable risks of esophageal and gastric cancers. *J Natl Cancer Inst* 2003; **95**: 1404-1413 [PMID: 13130116]

11 **Olsen CM**, Pandeya N, Green AC, Webb PM, Whiteman DC. Population attributable fractions of adenocarcinoma of the esophagus and gastroesophageal junction. *Am J Epidemiol* 2011; **174**: 582-590 [PMID: 21719746]

12 **Rugge M**, Fassan M, Pizzi M, Farinati F, Sturniolo GC, Plebani M, Graham DY. Operative link for gastritis assessment vs operative link on intestinal metaplasia assessment. *World J Gastroenterol* 2011; **17**: 4596-4601 [PMID: 22147965 DOI: 10.3748/wjg.v17.i41.4596]

13 **Bagnardi V**, Rota M, Botteri E, Tramacere I, Islami F, Fedirko V, Scotti L, Jenab M, Turati F, Pasquali E, Pelucchi C, Galeone C, Bellocco R, Negri E, Corrao G, Boffetta P, La Vecchia C. Alcohol consumption and site-specific cancer risk: a comprehensive dose-response meta-analysis. *Br J Cancer* 2015; **112**: 580-593 [PMID: 25422909]

14 **Corley DA**, Kubo A. Influence of site classification on cancer incidence rates: an analysis of gastric cardia carcinomas. *J Natl Cancer Inst* 2004; **96**: 1383-1387 [PMID: 15367571 DOI: 10.1093/jnci/djh265]

15 **Ekström AM**, Signorello LB, Hansson LE, Bergström R, Lindgren A, Nyrén O. Evaluating gastric cancer misclassification: a potential explanation for the rise in cardia cancer incidence. *J Natl Cancer Inst* 1999; **91**: 786-790 [PMID: 10328109]

16 Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; **513**: 202-209 [PMID: 25079317]

17 **Crew KD**, Neugut AI. Epidemiology of gastric cancer. *World J Gastroenterol* 2006; **12**: 354-362 [PMID: 16489633]

18 **Rausei S**, Dionigi G, Sano T, Sasako M, Biondi A, Morgagni P, Garofalo A, Boni L, Frattini F, D'Ugo D, Preston S, Marrelli D, Degiuli M, Capella C, Sacco R, Ruspi L, De Manzoni G, Roviello F, Pinotti G, Rovera F, Noh SH, Coit D, Dionigi R. Updates on surgical management of advanced gastric cancer: new evidence and trends. Insights from the First International Course on Upper Gastrointestinal Surgery--Varese (Italy), December 2, 2011. *Ann Surg Oncol* 2013; **20**: 3942-3947 [PMID: 23838909 DOI: 10.1245/s10434-013-3082-7]

19 **Maruyama K**, Okabayashi K, Kinoshita T. Progress in gastric cancer surgery in Japan and its limits of radicality. *World J Surg* 1987; **11**: 418-425 [PMID: 3630186]

20 **Hartgrink HH**, van de Velde CJ, Putter H, Bonenkamp JJ, Klein Kranenbarg E, Songun I, Welvaart K, van Krieken JH, Meijer S, Plukker JT, van Elk PJ, Obertop H, Gouma DJ, van Lanschot JJ, Taat CW, de Graaf PW, von Meyenfeldt MF, Tilanus H, Sasako M. Extended lymph node dissection for gastric cancer: who may benefit? Final results of the randomized Dutch gastric cancer group trial. *J Clin Oncol* 2004; **22**: 2069-2077 [PMID: 15082726 DOI: 10.1200/JCO.2004.08.026]

21 **McCulloch P**, Niita ME, Kazi H, Gama-Rodrigues JJ. Gastrectomy with extended lymphadenectomy for primary treatment of gastric cancer. *Br J Surg* 2005; **92**: 5-13 [PMID: 15635680 DOI: 10.1002/bjs.4839]

22 **Degiuli M**, Sasako M, Ponti A, Vendrame A, Tomatis M, Mazza C, Borasi A, Capussotti L, Fronda G, Morino M. Randomized clinical trial comparing survival after D1 or D2 gastrectomy for gastric cancer. *Br J Surg* 2014; **101**: 23-31 [PMID: 24375296 DOI: 10.1002/bjs.9345]

23 **Cunningham D**, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, Scarffe JH, Lofts FJ, Falk SJ, Iveson TJ, Smith DB, Langley RE, Verma M, Weeden S, Chua YJ.

Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *N Engl J Med* 2006; **355**: 11-20 [PMID: 16822992]

24 **Boige V**, Pignon J, Saint-Aubert B, Lasser P, Conroy T, Bouché O, Segol P, Bedenne L, Rougier P, Ychou M. Final results of a randomized trial comparing preoperative 5-fluorouracil (F)/cisplatin (P) to surgery alone in adenocarcinoma of stomach and lower esophagus (ASLE): FNLCC ACCORD07-FFCD 9703 trial. *J Clin Oncol* 2007; **25** (18 Suppl)

25 **Lutz MP**, Zalcberg JR, Ducreux M, Ajani JA, Allum W, Aust D, Bang YJ, Cascinu S, Hölscher A, Jankowski J, Jansen EP, Kisslich R, Lordick F, Mariette C, Moehler M, Oyama T, Roth A, Rueschoff J, Ruhstaller T, Seruca R, Stahl M, Sterzing F, van Cutsem E, van der Gaast A, van Lanschot J, Ychou M, Otto F. Highlights of the EORTC St. Gallen International Expert Consensus on the primary therapy of gastric, gastroesophageal and oesophageal cancer - differential treatment strategies for subtypes of early gastroesophageal cancer. *Eur J Cancer* 2012; **48**: 2941-2953 [PMID: 22921186]

26 **Wagner AD**, Grothe W, Haerting J, Kleber G, Grothey A, Fleig WE. Chemotherapy in advanced gastric cancer: a systematic review and meta-analysis based on aggregate data. *J Clin Oncol* 2006; **24**: 2903-2909 [PMID: 16782930]

27 **Lordick F**, Lorenzen S, Yamada Y, Ilson D. Optimal chemotherapy for advanced gastric cancer: is there a global consensus? *Gastric Cancer* 2014; **17**: 213-225 [PMID: 24048758 DOI: 10.1007/s10120-013-0297-z]

28 **Ajani JA**, Rodriguez W, Bodoky G, Moiseyenko V, Lichinitser M, Gorbunova V, Vynnychenko I, Garin A, Lang I, Falcon S. Multicenter phase III comparison of cisplatin/S-1 with cisplatin/infusional fluorouracil in advanced gastric or gastroesophageal adenocarcinoma study: the FLAGS trial. *J Clin Oncol* 2010; **28**: 1547-1553 [PMID: 20159816]

29 **Ross PJ**, Hill ME, Norman A, Cunningham D. ECF in gastric cancer. *J Clin Oncol* 2000; **18**: 3874-3875 [PMID: 11078502]

30 **Van Cutsem E**, Moiseyenko VM, Tjulandin S, Majlis A, Constenla M, Boni C, Rodrigues A, Fodor M, Chao Y, Voznyi E, Risse ML, Ajani JA. Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V325 Study Group. *J Clin Oncol* 2006; **24**: 4991-4997 [PMID: 17075117]

31 **Thuss-Patience PC**, Kretzschmar A, Bichev D, Deist T, Hinke A, Breithaupt K, Dogan Y, Gebauer B, Schumacher G, Reichardt P. Survival advantage for irinotecan versus best supportive care as second-line chemotherapy in gastric cancer--a randomised phase III study of the Arbeitsgemeinschaft Internistische Onkologie (AIO). *Eur J Cancer* 2011; **47**: 2306-2314 [PMID: 21742485]

32 **Ford HE**, Marshall A, Bridgewater JA, Janowitz T, Coxon FY, Wadsley J, Mansoor W, Fyfe D, Madhusudan S, Middleton GW, Swinson D, Falk S, Chau I, Cunningham D, Kareclas P, Cook N, Blazeby JM, Dunn JA. Docetaxel versus active symptom control for refractory oesophagogastric adenocarcinoma (COUGAR-02): an open-label, phase 3 randomised controlled trial. *Lancet Oncol* 2014; **15**: 78-86 [PMID: 24332238]

33 **Bang YJ**, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010; **376**: 687-697 [PMID: 20728210]

34 **Fuchs CS**, Tomasek J, Yong CJ, Dumitru F, Passalacqua R, Goswami C, Safran H, dos Santos LV, Aprile G, Ferry DR, Melichar B, Tehfe M, Topuzov E, Zalcberg JR, Chau I, Campbell W, Sivanandan C, Pikiel J, Koshiji M, Hsu Y, Liepa AM, Gao L, Schwartz JD, Tabernero J. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial. *Lancet* 2014; **383**: 31-39 [PMID: 24094768]

35 **Wilke H**, Muro K, Van Cutsem E, Oh SC, Bodoky G, Shimada Y, Hironaka S, Sugimoto N, Lipatov O, Kim TY, Cunningham D, Rougier P, Komatsu Y, Ajani J, Emig M, Carlesi R, Ferry D, Chandrawansa K, Schwartz JD, Ohtsu A. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomised phase 3 trial. *Lancet Oncol* 2014; **15**: 1224-1235 [PMID: 25240821]

36 **Huang H**, Han Y, Gao J, Feng J, Zhu L, Qu L, Shen L, Shou C. High level of serum AMBP is associated with poor response to paclitaxel-capecitabine chemotherapy in advanced gastric cancer patients. *Med Oncol* 2013; **30**: 748 [PMID: 24135868 DOI: 10.1007/s12032-013-0748-8]

37 **Gormally MV**, Dexheimer TS, Marsico G, Sanders DA, Lowe C, Matak-Vinković D, Michael S, Jadhav A, Rai G, Maloney DJ, Simeonov A, Balasubramanian S. Suppression of the FOXM1 transcriptional programme via novel small molecule inhibition. *Nat Commun* 2014; **5**: 5165 [PMID: 25387393]

38 **Yu G**, Zhou A, Xue J, Huang C, Zhang X, Kang SH, Chiu WT, Tan C, Xie K, Wang J, Huang S. FoxM1 promotes breast tumorigenesis by activating PDGF-A and forming a positive feedback loop with the PDGF/AKT signaling pathway. *Oncotarget* 2015; **6**: 11281-11294 [PMID: 25869208]

39 **Bergamaschi A**, Madak-Erdogan Z, Kim YJ, Choi YL, Lu H, Katzenellenbogen BS. The forkhead transcription factor FOXM1 promotes endocrine resistance and invasiveness in estrogen receptor-positive breast cancer by expansion of stem-like cancer cells. *Breast Cancer Res* 2014; **16**: 436 [PMID: 25213081]

40 **Peake BF**, Nahta R. Resistance to HER2-targeted therapies: a potential role for FOXM1. *Breast Cancer Manag* 2014; **3**: 423-431 [PMID: 25598845 DOI: 10.2217/bmt.14.33]

41 **Kong FF**, Qu ZQ, Yuan HH, Wang JY, Zhao M, Guo YH, Shi J, Gong XD, Zhu YL, Liu F, Zhang WY, Jiang B. Overexpression of FOXM1 is associated with EMT and is a predictor of poor prognosis in non-small cell lung cancer. *Oncol Rep* 2014; **31**: 2660-2668 [PMID: 24715097 DOI: 10.3892/or.2014.3129]

42 **Chiu WT**, Huang YF, Tsai HY, Chen CC, Chang CH, Huang SC, Hsu KF, Chou CY. FOXM1 confers to epithelial-mesenchymal transition, stemness and chemoresistance in epithelial ovarian carcinoma cells. *Oncotarget* 2015; **6**: 2349-2365 [PMID: 25537512]

43 **Li X**, Yao R, Yue L, Qiu W, Qi W, Liu S, Yao Y, Liang J. FOXM1 mediates resistance to docetaxel in gastric cancer via up-regulating Stathmin. *J Cell Mol Med* 2014; **18**: 811-823 [PMID: 24628949 DOI: 10.1111/jcmm.12216]

44 **Okada K**, Fujiwara Y, Takahashi T, Nakamura Y, Takiguchi S, Nakajima K, Miyata H, Yamasaki M, Kurokawa Y, Mori M, Doki Y. Overexpression of forkhead box M1 transcription factor (FOXM1) is a potential prognostic marker and enhances chemoresistance for docetaxel in gastric cancer. *Ann Surg Oncol* 2013; **20**: 1035-1043 [PMID: 23054116 DOI: 10.1245/s10434-012-2680-0]

45 **Xu XH**, Pan W, Kang LH, Feng H, Song YQ. Association of annexin A2 with cancer development (Review). *Oncol Rep* 2015; **33**: 2121-2128 [PMID: 25760910 DOI: 10.3892/or.2015.3837]

46 **Zhang F**, Liu Y, Wang Z, Sun X, Yuan J, Wang T, Tian R, Ji W, Yu M, Zhao Y, Niu R. A novel Anxa2-interacting protein Ebp1 inhibits cancer proliferation and invasion by suppressing Anxa2 protein level. *Mol Cell Endocrinol* 2015; **411**: 75-85 [PMID: 25917452]

47 **Emoto K**, Sawada H, Yamada Y, Fujimoto H, Takahama Y, Ueno M, Takayama T, Uchida H, Kamada K, Naito A, Hirao S, Nakajima Y. Annexin II overexpression is correlated with poor prognosis in human gastric carcinoma. *Anticancer Res* 2001; **21**: 1339-1345 [PMID: 11396210]

48 **Zhang Q**, Ye Z, Yang Q, He X, Wang H, Zhao Z. Upregulated expression of annexin II is a prognostic marker for patients with gastric cancer. *World J Surg Oncol* 2012; **10**: 103 [PMID: 22681645]

49 **Tas F**, Tilgen Yasasever C, Karabulut S, Tastekin D, Duranyildiz D. Circulating annexin A2 as a biomarker in gastric cancer patients:

correlation with clinical variables. *Biomed Pharmacother* 2015; **69**: 237-241 [PMID: 25661364]
50 **Fassan M**, Croce CM, Rugge M. miRNAs in precancerous lesions of the gastrointestinal tract. *World J Gastroenterol* 2011; **17**: 5231-5239 [PMID: 22219591 DOI: 10.3748/wjg.v17.i48.5231]
51 **Wang JL**, Hu Y, Kong X, Wang ZH, Chen HY, Xu J, Fang JY. Candidate microRNA biomarkers in human gastric cancer: a systematic review and validation study. *PLoS One* 2013; **8**: e73683 [PMID: 24040025 DOI: 10.1371/journal.pone.0073683]
52 **Ueda T**, Volinia S, Okumura H, Shimizu M, Taccioli C, Rossi S, Alder H, Liu CG, Oue N, Yasui W, Yoshida K, Sasaki H, Nomura S, Seto Y, Kaminishi M, Calin GA, Croce CM. Relation between microRNA expression and progression and prognosis of gastric cancer: a microRNA expression analysis. *Lancet Oncol* 2010; **11**: 136-146 [PMID: 20022810]
53 **Petrocca F**, Visone R, Onelli MR, Shah MH, Nicoloso MS, de Martino I, Iliopoulos D, Pilozzi E, Liu CG, Negrini M, Cavazzini L, Volinia S, Alder H, Ruco LP, Baldassarre G, Croce CM, Vecchione A. E2F1-regulated microRNAs impair TGFbeta-dependent cell-cycle arrest and apoptosis in gastric cancer. *Cancer Cell* 2008; **13**: 272-286 [PMID: 18328430]
54 **Shrestha S**, Hsu SD, Huang WY, Huang HY, Chen W, Weng SL, Huang HD. A systematic review of microRNA expression profiling studies in human gastric cancer. *Cancer Med* 2014; **3**: 878-888 [PMID: 24902858 DOI: 10.1002/cam4.246]
55 **Deng N**, Goh LK, Wang H, Das K, Tao J, Tan IB, Zhang S, Lee M, Wu J, Lim KH, Lei Z, Goh G, Lim QY, Tan AL, Sin Poh DY, Riahi S, Bell S, Shi MM, Linnartz R, Zhu F, Yeoh KG, Toh HC, Yong WP, Cheong HC, Rha SY, Boussioutas A, Grabsch H, Rozen S, Tan P. A comprehensive survey of genomic alterations in gastric cancer reveals systematic patterns of molecular exclusivity and co-occurrence among distinct therapeutic targets. *Gut* 2012; **61**: 673-684 [PMID: 22315472]
56 **Hong L**, Han Y, Yang J, Zhang H, Jin Y, Brain L, Li M, Zhao Q. Prognostic value of epidermal growth factor receptor in patients with gastric cancer: a meta-analysis. *Gene* 2013; **529**: 69-72 [PMID: 23954221]
57 **Pinto C**, Di Fabio F, Siena S, Cascinu S, Rojas Llimpe FL, Ceccarelli C, Mutri V, Giannetta L, Giaquinta S, Funaioli C, Berardi R, Longobardi C, Piana E, Martoni AA. Phase II study of cetuximab in combination with FOLFIRI in patients with untreated advanced gastric or gastroesophageal junction adenocarcinoma (FOLCETUX study). *Ann Oncol* 2007; **18**: 510-517 [PMID: 17164226]
58 **Pinto C**, Di Fabio F, Barone C, Siena S, Falcone A, Cascinu S, Rojas Llimpe FL, Stella G, Schinzari G, Artale S, Mutri V, Giaquinta S, Giannetta L, Bardelli A, Martoni AA. Phase II study of cetuximab in combination with cisplatin and docetaxel in patients with untreated advanced gastric or gastro-oesophageal junction adenocarcinoma (DOCETUX study). *Br J Cancer* 2009; **101**: 1261-1268 [PMID: 19773760]
59 **Han SW**, Oh DY, Im SA, Park SR, Lee KW, Song HS, Lee NS, Lee KH, Choi IS, Lee MH, Kim MA, Kim WH, Bang YJ, Kim TY. Phase II study and biomarker analysis of cetuximab combined with modified FOLFOX6 in advanced gastric cancer. *Br J Cancer* 2009; **100**: 298-304 [PMID: 19127259]
60 **Lordick F**, Luber B, Lorenzen S, Hegewisch-Becker S, Folprecht G, Wöll E, Decker T, Endlicher E, Röthling N, Schuster T, Keller G, Fend F, Peschel C. Cetuximab plus oxaliplatin/leucovorin/5-fluorouracil in first-line metastatic gastric cancer: a phase II study of the Arbeitsgemeinschaft Internistische Onkologie (AIO). *Br J Cancer* 2010; **102**: 500-505 [PMID: 20068568]
61 **Lordick F**, Kang YK, Chung HC, Salman P, Oh SC, Bodoky G, Kurteva G, Volovat C, Moiseyenko VM, Gorbunova V, Park JO, Sawaki A, Celik I, Götte H, Melezínková H, Moehler M. Capecitabine and cisplatin with or without cetuximab for patients with previously untreated advanced gastric cancer (EXPAND): a randomised, open-label phase 3 trial. *Lancet Oncol* 2013; **14**: 490-499 [PMID: 23594786]
62 **Waddell T**, Chau I, Cunningham D, Gonzalez D, Okines AF, Okines C, Wotherspoon A, Saffery C, Middleton G, Wadsley J, Ferry D, Mansoor W, Crosby T, Coxon F, Smith D, Waters J, Iveson T, Falk S, Slater S, Peckitt C, Barbachano Y. Epirubicin, oxaliplatin, and capecitabine with or without panitumumab for patients with previously untreated advanced oesophagogastric cancer (REAL3): a randomised, open-label phase 3 trial. *Lancet Oncol* 2013; **14**: 481-489 [PMID: 23594787]
63 **Dutton SJ**, Ferry DR, Blazeby JM, Abbas H, Dahle-Smith A, Mansoor W, Thompson J, Harrison M, Chatterjee A, Falk S, Garcia-Alonso A, Fyfe DW, Hubner RA, Gamble T, Peachey L, Davoudianfar M, Pearson SR, Julier P, Jankowski J, Kerr R, Petty RD. Gefitinib for oesophageal cancer progressing after chemotherapy (COG): a phase 3, multicentre, double-blind, placebo-controlled randomised trial. *Lancet Oncol* 2014; **15**: 894-904 [PMID: 24950987]
64 **Giaccone G**, Herbst RS, Manegold C, Scagliotti G, Rosell R, Miller V, Natale RB, Schiller JH, Von Pawel J, Pluzanska A, Gatzemeier U, Grous J, Ochs JS, Averbuch SD, Wolf MK, Rennie P, Fandi A, Johnson DH. Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial--INTACT 1. *J Clin Oncol* 2004; **22**: 777-784 [PMID: 14990632 DOI: 10.1200/JCO.2004.08.001]
65 **Herbst RS**, Giaccone G, Schiller JH, Natale RB, Miller V, Manegold C, Scagliotti G, Rosell R, Oliff I, Reeves JA, Wolf MK, Krebs AD, Averbuch SD, Ochs JS, Grous J, Fandi A, Johnson DH. Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial--INTACT 2. *J Clin Oncol* 2004; **22**: 785-794 [PMID: 14990633 DOI: 10.1200/JCO.2004.07.215]
66 **Asioli S**, Maletta F, Verdun di Cantogno L, Satolli MA, Schena M, Pecchioni C, Botta C, Chiusa L, Molinaro L, Conti L, Viale G, Ingravallo G, Maiorano E, Sapino A. Approaching heterogeneity of human epidermal growth factor receptor 2 in surgical specimens of gastric cancer. *Hum Pathol* 2012; **43**: 2070-2079 [PMID: 22658277]
67 **Gravalos C**, Jimeno A. HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target. *Ann Oncol* 2008; **19**: 1523-1529 [PMID: 18441328]
68 **Fisher SB**, Fisher KE, Squires MH, Patel SH, Kooby DA, El-Rayes BF, Cardona K, Russell MC, Staley CA, Farris AB, Maithel SK. HER2 in resected gastric cancer: Is there prognostic value? *J Surg Oncol* 2014; **109**: 61-66 [PMID: 24122802 DOI: 10.1002/jso.23456]
69 **Jørgensen JT**, Hersom M. HER2 as a Prognostic Marker in Gastric Cancer - A Systematic Analysis of Data from the Literature. *J Cancer* 2012; **3**: 137-144 [PMID: 22481979 DOI: 10.7150/jca.4090]
70 **LaBonte MJ**, Manegold PC, Wilson PM, Fazzone W, Louie SG, Lenz HJ, Ladner RD. The dual EGFR/HER-2 tyrosine kinase inhibitor lapatinib sensitizes colon and gastric cancer cells to the irinotecan active metabolite SN-38. *Int J Cancer* 2009; **125**: 2957-2969 [PMID: 19536776 DOI: 10.1002/ijc.24658]
71 **Kim JW**, Kim HP, Im SA, Kang S, Hur HS, Yoon YK, Oh DY, Kim JH, Lee DS, Kim TY, Bang YJ. The growth inhibitory effect of lapatinib, a dual inhibitor of EGFR and HER2 tyrosine kinase, in gastric cancer cell lines. *Cancer Lett* 2008; **272**: 296-306 [PMID: 18774637]
72 **Hecht JR**, Bang YJ, Shukui Q, Chung HC, Xu JM, Park JO, Jeziorski K, Shparyk Y, Hoff PM, Sobrero AF, Salman P, Li J, Protsenko S, Buyse ME, Afenjar K, Kaneko T, Kemner A, Santillana S, Press MF, Slamon DJ. Lapatinib in combination with capecitabine plus oxaliplatin (CapeOx) in HER2-positive advanced or metastatic gastric, esophageal, or gastroesophageal adenocarcinoma (AC): The TRIO-013/LOGiC Trial. *J Clin Oncol* 2013; **31** (suppl; abstr LBA4001)
73 **Satoh T**, Bang YJ. Interim safety analysis from TYTAN: A phase III Asian study of lapatinib in combination with paclitaxel as second-line therapy in gastric cancer. *J Clin Oncol* 2010; **28** (suppl; abstr 4057)
74 **Verma S**, Miles D, Gianni L, Krop IE, Welslau M, Baselga J,

Pegram M, Oh DY, Diéras V, Guardino E, Fang L, Lu MW, Olsen S, Blackwell K. Trastuzumab emtansine for HER2-positive advanced breast cancer. *N Engl J Med* 2012; **367**: 1783-1791 [PMID: 23020162 DOI: 10.1056/NEJMoa1209124]

75 **Occhipinti S**, Sponton L, Rolla S, Caorsi C, Novarino A, Donadio M, Bustreo S, Satolli MA, Pecchioni C, Marchini C, Amici A, Cavallo F, Cappello P, Pierobon D, Novelli F, Giovarelli M. Chimeric rat/human HER2 efficiently circumvents HER2 tolerance in cancer patients. *Clin Cancer Res* 2014; **20**: 2910-2921 [PMID: 24668647]

76 **Hamilton E**, Blackwell K, Hobeika AC, Clay TM, Broadwater G, Ren XR, Chen W, Castro H, Lehmann F, Spector N, Wei J, Osada T, Lyerly HK, Morse MA. Phase 1 clinical trial of HER2-specific immunotherapy with concomitant HER2 kinase inhibition [corrected]. *J Transl Med* 2012; **10**: 28 [PMID: 22325452]

77 **Yong KJ**, Milenic DE, Baidoo KE, Brechbiel MW. (212)Pb-radioimmunotherapy induces G(2) cell-cycle arrest and delays DNA damage repair in tumor xenografts in a model for disseminated intraperitoneal disease. *Mol Cancer Ther* 2012; **11**: 639-648 [PMID: 22238365]

78 **Fondevila C**, Metges JP, Fuster J, Grau JJ, Palacín A, Castells A, Volant A, Pera M. p53 and VEGF expression are independent predictors of tumour recurrence and survival following curative resection of gastric cancer. *Br J Cancer* 2004; **90**: 206-215 [PMID: 14710231 DOI: 10.1038/sj.bjc.6601455]

79 **Maeda K**, Chung YS, Ogawa Y, Takatsuka S, Kang SM, Ogawa M, Sawada T, Sowa M. Prognostic value of vascular endothelial growth factor expression in gastric carcinoma. *Cancer* 1996; **77**: 858-863 [PMID: 8608475 DOI: 10.1002/(SICI)1097-0142(19960301)77:5<858: : AID-CNCR8>3.0.CO; 2-A]

80 **Vidal O**, Metges JP, Elizalde I, Valentíni M, Volant A, Molina R, Castells A, Pera M. High preoperative serum vascular endothelial growth factor levels predict poor clinical outcome after curative resection of gastric cancer. *Br J Surg* 2009; **96**: 1443-1451 [PMID: 19918848 DOI: 10.1002/bjs.6780]

81 **D'Amico L**, Satolli MA, Mecca C, Castiglione A, Ceccarelli M, D' Amelio P, Garino M, De Giuli M, Sandrucci S, Ferracini R, Roato I. Bone metastases in gastric cancer follow a RANKL-independent mechanism. *Oncol Rep* 2013; **29**: 1453-1458 [PMID: 23404437 DOI: 10.3892/or.2013.2280]

82 **Shah MA**, Ramanathan RK, Ilson DH, Levnor A, D'Adamo D, O' Reilly E, Tse A, Trocola R, Schwartz L, Capanu M, Schwartz GK, Kelsen DP. Multicenter phase II study of irinotecan, cisplatin, and bevacizumab in patients with metastatic gastric or gastroesophageal junction adenocarcinoma. *J Clin Oncol* 2006; **24**: 5201-5206 [PMID: 17114652]

83 **Shah MA**, Jhawer M, Ilson DH, Lefkowitz RA, Robinson E, Capanu M, Kelsen DP. Phase II study of modified docetaxel, cisplatin, and fluorouracil with bevacizumab in patients with metastatic gastroesophageal adenocarcinoma. *J Clin Oncol* 2011; **29**: 868-874 [PMID: 21189380]

84 **Ohtsu A**, Shah MA, Van Cutsem E, Rha SY, Sawaki A, Park SR, Lim HY, Yamada Y, Wu J, Langer B, Starnawski M, Kang YK. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a randomized, double-blind, placebo-controlled phase III study. *J Clin Oncol* 2011; **29**: 3968-3976 [PMID: 21844504]

85 **Van Cutsem E**, de Haas S, Kang YK, Ohtsu A, Tebbutt NC, Ming Xu J, Peng Yong W, Langer B, Delmar P, Scherer SJ, Shah MA. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a biomarker evaluation from the AVAGAST randomized phase III trial. *J Clin Oncol* 2012; **30**: 2119-2127 [PMID: 22565005]

86 **Okines AF**, Langley RE, Thompson LC, Stenning SP, Stevenson L, Falk S, Seymour M, Coxon F, Middleton GW, Smith D, Evans L, Slater S, Waters J, Ford D, Hall M, Iveson TJ, Petty RD, Plummer C, Allum WH, Blazeby JM, Griffin M, Cunningham D. Bevacizumab with peri-operative epirubicin, cisplatin and capecitabine (ECX) in localised gastro-oesophageal adenocarcinoma: a safety report. *Ann Oncol* 2013; **24**: 702-709 [PMID: 23108952]

87 **Yoon JH**, Bendell JC, Braiteh FS, Firdaus I, Philip PA, Lee Cohn AL, Lewis N, Anderson DM, Arrowsmith E, Schwartz JD, Xu Y, Koshiji M, Alberts SR, Wainberg ZA. Ramucirumab (RAM) plus FOLFOX as front-line therapy (Rx) for advanced gastric or esophageal adenocarcinoma (GE-AC): Randomized, double-blind, multicenter phase 2 trial. *J Clin Oncol* 2014; **32** (5 suppl; abstr 4004)

88 **Li J**, Qin S, Xu J, Guo W, Xiong J, Bai Y, Sun G, Yang Y, Wang L, Xu N, Cheng Y, Wang Z, Zheng L, Tao M, Zhu X, Ji D, Liu X, Yu H. Apatinib for chemotherapy-refractory advanced metastatic gastric cancer: results from a randomized, placebo-controlled, parallel-arm, phase II trial. *J Clin Oncol* 2013; **31**: 3219-3225 [PMID: 23918952]

89 **Qin S**. Phase III study of apatinib in advanced gastric cancer: A randomized, double-blind, placebo-controlled trial. *J Clin Oncol* 2014; **32** (5 suppl; abstr 4003)

90 **Yi JH**, Lee J, Lee J, Park SH, Park JO, Yim DS, Park YS, Lim HY, Kang WK. Randomised phase II trial of docetaxel and sunitinib in patients with metastatic gastric cancer who were previously treated with fluoropyrimidine and platinum. *Br J Cancer* 2012; **106**: 1469-1474 [PMID: 22460270]

91 **Moggio A**, Pittatore G, Cassoni P, Marchino GL, Revelli A, Bussolati B. Sorafenib inhibits growth, migration, and angiogenic potential of ectopic endometrial mesenchymal stem cells derived from patients with endometriosis. *Fertil Steril* 2012; **98**: 1521-30.e2 [PMID: 22981172]

92 **Sun W**, Powell M, O'Dwyer PJ, Catalano P, Ansari RH, Benson AB. Phase II study of sorafenib in combination with docetaxel and cisplatin in the treatment of metastatic or advanced gastric and gastroesophageal junction adenocarcinoma: ECOG 5203. *J Clin Oncol* 2010; **28**: 2947-2951 [PMID: 20458043]

93 **Trusolino L**, Bertotti A, Comoglio PM. MET signalling: principles and functions in development, organ regeneration and cancer. *Nat Rev Mol Cell Biol* 2010; **11**: 834-848 [PMID: 21102609]

94 **Ha SY**, Lee J, Kang SY, Do IG, Ahn S, Park JO, Kang WK, Choi MG, Sohn TS, Bae JM, Kim S, Kim M, Kim S, Park CK, Ignatius Ou SH, Kim KM. MET overexpression assessed by new interpretation method predicts gene amplification and poor survival in advanced gastric carcinomas. *Mod Pathol* 2013; **26**: 1632-1641 [PMID: 23807774]

95 **Graziano F**, Galluccio N, Lorenzini P, Ruzzo A, Canestrari E, D' Emidio S, Catalano V, Sisti V, Ligorio C, Andreoni F, Rulli E, Di Oto E, Fiorentini G, Zingaretti C, De Nictolis M, Cappuzzo F, Magnani M. Genetic activation of the MET pathway and prognosis of patients with high-risk, radically resected gastric cancer. *J Clin Oncol* 2011; **29**: 4789-4795 [PMID: 22042954]

96 **Oliner KS**, Tang R, Anderson A, Lan Y, Iveson T, Donehower RC, Jiang Y, Dubey D, Loh E. Evaluation of MET pathway biomarkers in a phase II study of rilotumumab (R, AMG 102) or placebo (P) in combination with epirubicin, cisplatin, and capecitabine (ECX) in patients (pts) with locally advanced or metastatic gastric (G) or esophagogastric junction (EGJ) cancer. *J Clin Oncol* 2012; **30** (suppl; abstr 4005)

97 **Cunningham D**, Tebbutt NC, Davidenko I, Murad AM, Al-Batran S, Ilson DH, Tjulandin S, Gotovkin E, Karaszewska B, Bondarenko I, Tejani MA, Udrea AA, Tehfe MA, Baker N, Smith Oliner KM, Zhang Y, Hoang T, Sidhu R, Catenacci DVT. Phase III, randomized, double-blind, multicenter, placebo (P)-controlled trial of rilotumumab (R) plus epirubicin, cisplatin and capecitabine (ECX) as first-line therapy in patients (pts) with advanced MET-positive (pos) gastric or gastroesophageal junction (G/GEJ) cancer: RILOMET-1 study. *J Clin Oncol* 2015; **33** (suppl; abstr 4000)

98 **Lennerz JK**, Kwak EL, Ackerman A, Michael M, Fox SB, Bergethon K, Lauwers GY, Christensen JG, Wilner KD, Haber DA, Salgia R, Bang YJ, Clark JW, Solomon BJ, Iafrate AJ. MET amplification identifies a small and aggressive subgroup of esophagogastric adenocarcinoma with evidence of responsiveness to crizotinib. *J Clin Oncol* 2011; **29**: 4803-4810 [PMID: 22042947]

99 **Kang YK**, Muro K, Ryu MH, Yasui H, Nishina T, Ryoo BY,

Kamiya Y, Akinaga S, Boku N. A phase II trial of a selective c-Met inhibitor tivantinib (ARQ 197) monotherapy as a second- or third-line therapy in the patients with metastatic gastric cancer. *Invest New Drugs* 2014; **32**: 355-361 [PMID: 24337769 DOI: 10.1007/s10637-013-0057-2]

100 **Lai AZ**, Abella JV, Park M. Crosstalk in Met receptor oncogenesis. *Trends Cell Biol* 2009; **19**: 542-551 [PMID: 19758803]

101 **Olwill SA**, Joffroy C, Gille H, Vigna E, Matschiner G, Allersdorfer A, Lunde BM, Jaworski J, Burrows JF, Chiriaco C, Christian HJ, Hülsmeyer M, Trentmann S, Jensen K, Hohlbaum AM, Audoly L. A highly potent and specific MET therapeutic protein antagonist with both ligand-dependent and ligand-independent activity. *Mol Cancer Ther* 2013; **12**: 2459-2471 [PMID: 24002935]

102 **Yu G**, Wang J, Chen Y, Wang X, Pan J, Li G, Jia Z, Li Q, Yao JC, Xie K. Overexpression of phosphorylated mammalian target of rapamycin predicts lymph node metastasis and prognosis of chinese patients with gastric cancer. *Clin Cancer Res* 2009; **15**: 1821-1829 [PMID: 19223493]

103 **Doi T**, Muro K, Boku N, Yamada Y, Nishina T, Takiuchi H, Komatsu Y, Hamamoto Y, Ohno N, Fujita Y, Robson M, Ohtsu A. Multicenter phase II study of everolimus in patients with previously treated metastatic gastric cancer. *J Clin Oncol* 2010; **28**: 1904-1910 [PMID: 20231677]

104 **Lee SJ**, Lee J, Lee J, Park SH, Park JO, Park YS, Lim HY, Kim KM, Do IG, Jung SH, Yim DS, Kang WK. Phase II trial of capecitabine and everolimus (RAD001) combination in refractory gastric cancer patients. *Invest New Drugs* 2013; **31**: 1580-1586 [PMID: 24013904 DOI: 10.1007/s10637-013-0022-0]

105 **Ohtsu A**, Ajani JA, Bai YX, Bang YJ, Chung HC, Pan HM, Sahmoud T, Shen L, Yeh KH, Chin K, Muro K, Kim YH, Ferry D, Tebbutt NC, Al-Batran SE, Smith H, Costantini C, Rizvi S, Lebwohl D, Van Cutsem E. Everolimus for previously treated advanced gastric cancer: results of the randomized, double-blind, phase III GRANITE-1 study. *J Clin Oncol* 2013; **31**: 3935-3943 [PMID: 24043745]

106 **Molife LR**, Fong PC, Paccagnella L, Reid AH, Shaw HM, Vidal L, Arkenau HT, Karavasilis V, Yap TA, Olmos D, Spicer J, Postel-Vinay S, Yin D, Lipton A, Demers L, Leitzel K, Gualberto A, de Bono JS. The insulin-like growth factor-I receptor inhibitor figitumumab (CP-751,871) in combination with docetaxel in patients with advanced solid tumours: results of a phase Ib dose-escalation, open-label study. *Br J Cancer* 2010; **103**: 332-339 [PMID: 20628389]

107 **Marusyk A**, Almendro V, Polyak K. Intra-tumour heterogeneity: a looking glass for cancer? *Nat Rev Cancer* 2012; **12**: 323-334 [PMID: 22513401]

108 **Chen J**, Li Y, Yu TS, McKay RM, Burns DK, Kernie SG, Parada LF. A restricted cell population propagates glioblastoma growth after chemotherapy. *Nature* 2012; **488**: 522-526 [PMID: 22854781]

109 **Mundy GR**. Metastasis to bone: causes, consequences and therapeutic opportunities. *Nat Rev Cancer* 2002; **2**: 584-593 [PMID: 12154351 DOI: 10.1038/nrc867]

110 **D'Amico L**, Patanè S, Grange C, Bussolati B, Isella C, Fontani L, Godio L, Cilli M, D'Amelio P, Isaia G, Medico E, Ferracini R, Roato I. Primary breast cancer stem-like cells metastasise to bone, switch phenotype and acquire a bone tropism signature. *Br J Cancer* 2013; **108**: 2525-2536 [PMID: 23801032]

111 **Keller ET**, Zhang J, Cooper CR, Smith PC, McCauley LK, Pienta KJ, Taichman RS. Prostate carcinoma skeletal metastases: cross-talk between tumor and bone. *Cancer Metastasis Rev* 2001; **20**: 333-349 [PMID: 12085970]

112 **Roato I**. Bone metastases: When and how lung cancer interacts with bone. *World J Clin Oncol* 2014; **5**: 149-155 [PMID: 24829862 DOI: 10.5306/wjco.v5.i2.149]

113 **Casey SC**, Amedei A, Aquilano K, Azmi AS, Benencia F, Bhakta D, Bilsland AE, Boosani CS, Chen S, Ciriolo MR, Crawford S, Fujii H, Georgakilas AG, Guha G, Halicka D, Helferich WG, Heneberg P, Honoki K, Keith WN, Kerkar SP, Mohammed SI, Niccolai E, Nowsheen S, Vasantha Rupasinghe HP, Samadi A, Singh N, Talib WH, Venkateswaran V, Whelan RL, Yang X, Felsher DW. Cancer prevention and therapy through the modulation of the tumor microenvironment. *Semin Cancer Biol* 2015; **pii**: S1044-579X(15)00015-2 [PMID: 25865775 DOI: 10.1016/j.semcancer.2015.02.007]

114 **Joyce JA**, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. *Science* 2015; **348**: 74-80 [PMID: 25838376 DOI: 10.1126/science.aaa6204]

115 **Fridman WH**, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer* 2012; **12**: 298-306 [PMID: 22419253 DOI: 10.1038/nrc3245]

116 **Kusmartsev S**, Nefedova Y, Yoder D, Gabrilovich DI. Antigen-specific inhibition of CD8+ T cell response by immature myeloid cells in cancer is mediated by reactive oxygen species. *J Immunol* 2004; **172**: 989-999 [PMID: 14707072]

117 **Liu Y**, Van Ginderachter JA, Brys L, De Baetselier P, Raes G, Geldhof AB. Nitric oxide-independent CTL suppression during tumor progression: association with arginase-producing (M2) myeloid cells. *J Immunol* 2003; **170**: 5064-5074 [PMID: 12734351]

118 **Mazzoni A**, Bronte V, Visintin A, Spitzer JH, Apolloni E, Serafini P, Zanovello P, Segal DM. Myeloid suppressor lines inhibit T cell responses by an NO-dependent mechanism. *J Immunol* 2002; **168**: 689-695 [PMID: 11777962]

119 **Hanahan D**, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**: 57-70 [PMID: 10647931]

120 **Houghton J**, Wang TC. Helicobacter pylori and gastric cancer: a new paradigm for inflammation-associated epithelial cancers. *Gastroenterology* 2005; **128**: 1567-1578 [PMID: 15887152]

121 **Murphy A**, Kelly RJ. Immunotherapy in upper GI malignancies. *Curr Treat Options Oncol* 2015; **16**: 20 [PMID: 25859830 DOI: 10.1007/s11864-015-0336-6]

122 **Cui J**, Li L, Wang C, Jin H, Yao C, Wang Y, Li D, Tian H, Niu C, Wang G, Han W, Xu J, Chen J, Li W. Combined cellular immunotherapy and chemotherapy improves clinical outcome in patients with gastric carcinoma. *Cytotherapy* 2015; **17**: 979-988 [PMID: 25890480]

123 **Sharma P**, Allison JP. The future of immune checkpoint therapy. *Science* 2015; **348**: 56-61 [PMID: 25838373]

124 **Sun J**, Xu K, Wu C, Wang Y, Hu Y, Zhu Y, Chen Y, Shi Q, Yu G, Zhang X. PD-L1 expression analysis in gastric carcinoma tissue and blocking of tumor-associated PD-L1 signaling by two functional monoclonal antibodies. *Tissue Antigens* 2007; **69**: 19-27 [PMID: 17212704]

125 **Muro K**, Bang Y, Shankaran V, Geva R, Catenacci DVT, Gupta S, Eder JP, Berger R, Gonzalez EJ, Ray A, Dolled-Filhart M, Emancipator K, Pathiraja K, Lunceford JK, Cheng DJ, Koshiji M, Chung H. Relationship between PD-L1 expression and clinical outcomes in patients (Pts) with advanced gastric cancer treated with the anti-PD-1 monoclonal antibody pembrolizumab (Pembro; MK-3475) in KEYNOTE-012. *J Clin Oncol* 2015; **33** (suppl 3; abstr 3)

126 **Bang Y**, Chung H, Shankaran V, Geva R, Catenacci DVT, Gupta S, Eder JP, Berger R, Gonzalez EJ, Ray A, Dolled-Filhart M, Emancipator K, Pathiraja K, Lunceford JK, Cheng DJ, Koshiji M, Muro K. Relationship between PD-L1 expression and clinical outcomes in patients with advanced gastric cancer treated with the anti-PD-1 monoclonal antibody pembrolizumab (MK-3475) in KEYNOTE-012. *J Clin Oncol* 2015; **33** (Suppl; abstr 4001)(15)

127 **Muro K**, Bang Y-J, Shankaran V, Ravit G, Catenacci DVT, Gupta S, Eder JP, Berger R, Gonzalez EJ, Ray A, Dolled-Filhart M, Emancipator K, Pathiraja K, Lunceford JK, Cheng JD, Koshiji M, Chung HC. Relationship between PD-L1 expression and clinical outcomes in patients (Pts) with advanced gastric cancer treated with the anti-PD-1 monoclonal antibody pembrolizumab (Pembro; MK-3475) in KEYNOTE-012. *J Clin Oncol* 2015; **33** (3 suppl; abstr 3)

128 **Venerito M**, Nardone G, Selgrad M, Rokkas T, Malfertheiner P. Gastric cancer--epidemiologic and clinical aspects. *Helicobacter*

2014; **19** Suppl 1: 32-37 [PMID: 25167943 DOI: 10.1111/hel.12164]

129 **Gettinger SN**, Horn L, Gandhi L, Spigel DR, Antonia SJ, Rizvi NA, Powderly JD, Heist RS, Carvajal RD, Jackman DM, Sequist LV, Smith DC, Leming P, Carbone DP, Pinder-Schenck MC, Topalian SL, Hodi FS, Sosman JA, Sznol M, McDermott DF, Pardoll DM, Sankar V, Ahlers CM, Salvati M, Wigginton JM, Hellmann MD, Kollia GD, Gupta AK, Brahmer JR. Overall Survival and Long-Term Safety of Nivolumab (Anti-Programmed Death 1 Antibody, BMS-936558, ONO-4538) in Patients With Previously Treated Advanced Non-Small-Cell Lung Cancer. *J Clin Oncol* 2015; **33**: 2004-2012 [PMID: 25897158]

130 **Galluzzi L**, Kroemer G, Eggermont A. Novel immune checkpoint blocker approved for the treatment of advanced melanoma. *Oncoimmunology* 2014; **3**: e967147 [PMID: 25941597 DOI: 10.4161/21624011.2014.967147]

P- Reviewer: Hironaka S
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Jiao XK

World Journal of Gastrointestinal Oncology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4251/wjgo.v7.i11.317

World J Gastrointest Oncol 2015 November 15; 7(11): 317-327
ISSN 1948-5204 (online)

TOPIC HIGHLIGHT

2015 Advances in Gastric Cancer

Clinical significance of MET in gastric cancer

Mikito Inokuchi, Sho Otsuki, Yoshitaka Fujimori, Yuya Sato, Masatoshi Nakagawa, Kazuyuki Kojima

Mikito Inokuchi, Sho Otsuki, Yoshitaka Fujimori, Yuya Sato, Masatoshi Nakagawa, Department of Gastrointestinal Surgery, Tokyo Medical and Dental University, Tokyo 113-8519, Japan

Kazuyuki Kojima, Department of Minimally Invasive Surgery, Tokyo Medical and Dental University, Tokyo 113-8519, Japan

Author contributions: Inokuchi M and Kojima K contributed to writing this manuscript; Nakagawa M, Otsuki S, Fujimori Y and Sato Y contributed to gathering, reviewing, and analyzing the related literature.

Conflict-of-interest statement: The authors have declared that they have no competing financial interests.

Correspondence to: Mikito Inokuchi, MD, PhD, Department of Gastrointestinal Surgery, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8519, Japan. m-inokuchi.srg2@tmd.ac.jp
Telephone: +81-35-8035261
Fax: +81-35-8030139

Received: February 24, 2015
Peer-review started: February 26, 2015
First decision: March 30, 2015
Revised: April 6, 2015
Accepted: August 25, 2015
Article in press: August 28, 2015
Published online: November 15, 2015

Abstract

Chemotherapy has become the global standard treatment for patients with metastatic or unresectable gastric cancer (GC), although outcomes remain unfavorable. Many molecular-targeted therapies inhibiting signaling pathways of various tyrosine kinase receptors have been developed, and monoclonal antibodies targeting human epidermal growth factor receptor 2 or vascular endothelial growth factor receptor 2 have become standard therapy for GC. Hepatocyte growth factor and its receptor, c-MET (MET), play key roles in tumor growth through activated signaling pathways from receptor in GC cells. Genomic amplification of *MET* leads to the aberrant activation found in GC tumors and is related to survival in patients with GC. This review discusses the clinical significance of MET in GC and examines MET as a potential therapeutic target in patients with GC. Preclinical studies in animal models have shown that MET antibodies or small-molecule MET inhibitors suppress tumor-cell proliferation and tumor progression in *MET*-amplified GC cells. These drugs are now being evaluated in clinical trials as treatments for metastatic or unresectable GC.

Key words: MET; Gastric cancer; Genomic amplification; Immunohistochemistry; Clinical trial

Core tip: MET protein overexpression or *MET* gene amplification was associated with tumor progression and survival in gastric cancer (GC), although the definition of MET overexpression remains to be standardized. In preclinical studies, MET antibodies or small-molecule MET inhibitors suppressed cell proliferation and tumor progression in *MET*-amplified GC cells. Therefore, MET-targeting therapy is promising, and MET overexpression might be a useful biomarker of the response to chemotherapy inhibiting MET. Some clinical trials of MET inhibitors were conducted in metastatic GC, but sufficient benefits have not been demonstrated yet.

K. Clinical significance of MET in gastric cancer. *World J Gastrointest Oncol* 2015; 7(11): 317-327 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i11/317.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i11.317

INTRODUCTION

Gastric cancer (GC) is the fourth most common cancer, with 989600 cases newly diagnosed in the world in 2008, accounting for about 8% of all newly diagnosed cancers[1]. The effectiveness of chemotherapy remains very limited in patients with unresectable or metastatic GC, and overall survival (OS) was 10 to 13 mo in patients who received combination chemotherapy with multiple cytotoxic agents[2,3].

Receptor tyrosine kinases (RTKs) are growth factor receptors associated with various physiological responses to embryogenesis and homeostasis. RTK activity is strictly regulated in normal cells, although dysregulation or constitutive activation of RTKs has been found in various types of cancer cells[4]. Aberrant or oncogenic activation of RTKs augments tumor-cell proliferation, anti-apoptosis, vascularization, metastasis, and resistance to anticancer agents. RTKs are the most intensively pursued target molecules for anticancer drugs, because tumor cells with activated RTK signaling pathways are sensitive to appropriate RTK inhibitors[5]. Trastuzumab, a monoclonal antibody against p185 human epidermal growth factor receptor 2 (HER2), was first used clinically to treat GCs with HER2 overexpression. However, only 12% of patients who received trastuzumab had tumors that overexpressed HER2 in that trial[6]. Ramucirumab is a monoclonal antibody against vascular endothelial growth factor receptor 2 (VEGFR2). Second-line treatment with ramucirumab significantly prolonged survival in two phase Ⅲ trials in GC[7,8]. Many inhibitors of RTKs have been investigated to identify potential targets for the treatment of GC.

Proto-oncogene c-MET (MET), a member of the RTK family, is a known hepatocyte growth factor (HGF) receptor that is encoded by the *MET* gene. MET has a primary single-chain precursor protein made of alpha and beta subunits, the latter of which contains a cytoplasmic kinase domain and a docking site[9]. Binding of HGF to the extracellular domain activates the kinase activity that phosphorylates the tyrosines at the carboxy terminal docking site. Phosphorylated MET (p-MET) can recruit a variety of proteins, including growth factor receptor-bound protein 2 (GRB2), GRB2-associated binding protein 1 (GAB1), phospholipase C (PLC)-gamma, SRC, and SHP2, and activates downstream signaling molecules such as phosphatidylinositol-3-kinase (PI3K)/AKT and extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathways[10,11]. Similar to other RTKs, MET plays key roles in tumor survival, growth, angiogenesis, and metastasis. The aberrant signaling of MET by overexpression or gene amplification has been detected and correlated with tumor progression or patients' survival in GC[12-15]. Alternative activation of the MET pathway is considered an important mechanism causing resistance to treatments targeting HER family members[16,17]. Unfortunately, a phase Ⅲ study of rilotumumab, an HGF monoclonal antibody inhibiting MET pathway, has been recently discontinued because of high treatment-related mortality. However, inhibition of MET must undoubtedly be an important treatment for GC.

In this article, we reassess the clinical significance of MET in GC and summarize currently available results of preclinical studies and clinical trials of MET inhibitors.

CLINICAL OUTCOMES OF MET EXPRESSION IN GC

Protein expression on immunohistochemistry

Studies examining the relation between MET protein expression and clinical outcomes in GC specimens are summarized in Table 1. MET protein expression on immunohistochemistry (IHC) is predominantly detected in cytoplasm of tumor cells, but is also found in the cell membrane[12,18-20]. Lee *et al*[12] assessed membranous MET expression according to a standardized technique, similar to that used to evaluate HER2 expression. MET expression was observed even in stromal cells in tumors[18]. Moreover, MET overexpression was more frequently detected in dysplasia and precancerous gastric lesions than in intestinal metaplasia[21].

MET overexpression has frequently been found in intestinal type or differentiated type cancers[12,14,22,23], although one study reported a correlation with diffuse type[13]. Retterspitz reported that MET was overexpressed in 51% (45 of 88) of diffuse type tumors[24]. MET overexpression has been significantly associated with tumor invasion depth[12,13,23], lymph-node metastasis[12,13,19,20,25,26], distant metastasis[12,13,25], tumor stage[12,20,23,26], and recurrence[14], although several studies found no relation to any clinicopathological factors[24,27,28]. MET overexpression correlated with liver metastasis only in stage Ⅳ disease[29]. Some studies showed that MET overexpression was an independent prognostic factor that was significantly related to poor survival[12-14,19,20,25,26,30-32].

In one study, p-MET was detected in 59% (72 of 121) of GC tumors and was significantly associated with lymph-node metastasis, disease stage, and outcomes[20]. In another study, however, only 7% (2 of 30) of tumors overexpressed p-MET in spite of the fact that 63% (24 of 38) overexpressed MET[22]. In another study using a new technique, collaborative enzyme enhanced reactive-immunoassay, p-MET was detected in 24% (103 of 434) of GC tumors, including 31% of intestinal type, 24% of diffuse type, and 0% of mixed type[33].

Gene expression

Studies assessing *MET* gene expression are summarized

Table 1 MET protein expressions on immunohistochemistry and clinical outcomes in gastric cancer

	n	Definition of overexpression	%	Relation to clinicopathological factors	Relation to survival	Ref.
Usual IHC	495	2+/3+, > 10%	22	Intestinal type, recurrence	Worse[3]	[14]
	170	Cytoplasmic, 2+/3+	13	ND	ND	[38]
	121	⩾ 5%	66	N, stage	Worse	[20]
	114	> 30%	74	NA	Worse[3]	[30]
	98	Intensity and extensity scoring system	59	N, M	Worse	[25]
	50		78	NA	NA	[28]
	38	2+/3+, ⩾ 25%	63	Intestinal type	ND	[22]
	94[1]	⩾ 50%	50	NA	NA	[24]
	121[2]	Any staining	98	Liver metastasis	ND	[29]
TMA	438	Membranous, 2+/3+, > 10%	24	T, N, M, stage, intestinal type	Worse	[12]
	436	Intensity and extensity scoring system	44	T, N, M, diffuse type	Worse[3,4]	[13]
	215	Cytoplasmic, > 10%	69	NA	NA	[27]
	212	2+/3+	12	ND	Worse[3]	[32]
	182	Intensity and extensity scoring system	66	N, intestinal type, differentiated type	Worse	[19]
	163	Cytoplasmic 2+/3+ ⩾ 10%, and positive > 75%	4	ND	Worse[3]	[31]
	124	Cytoplasmic, 3+	71	T, stage, intestinal type	ND	[23]
	114	Intensity and extensity scoring system	82	N, stage	Worse	[26]
	35		43	ND	Likely worse	[18]

[1]Limited to diffuse or mixed type; [2]Only stage IV; [3]An independent prognostic factor on multivariate analysis; [4]Only IHC3+. IHC: Immunohistochemistry; TMA; Tissue micro array; T: Tumor invasion depth; N: Lymph-node metastasis; M: Distant metastasis; ND: Not described; NA: Not associated.

Table 2 *MET* mRNA expressions and clinical outcomes in gastric cancer

	n	Overexpression		Relation to clinicopathological factors	Relation to survival	Ref.
		Cut-off value	%			
Tumor	100	Value determined by nonparametric receiver operating characteristics	11	M	Worse	[34]
	100	ND	24	ND	ND	[43]
	45			N, stage, differentiated type	ND	[35]
	43	Value of mean + 2 SD in noncancerous tissue	70	NA	ND	[36]
	15			Intestinal type	ND	[22]
Serum	52	Detected	62	T, N, M, stage, recurrence, v	Worse	[37]

T: Depth of tumor invasion; N: Lymph-node metastasis; M: Distant metastasis; v: Venous invasion; ND: Not described; NA: Not associated.

in Table 2. *MET* mRNA expression in GC tissue has been reported to significantly correlate with lymph-node metastasis, distant metastasis, and disease stage[34,35], although one study found no clinical significance[36]. Higher levels of *MET* mRNA expression were frequently detected in intestinal or differentiated type cancers[22,35]. Serum *MET* mRNA expression in peripheral blood has been detected and was significantly associated with tumor progression and short survival[37].

Studies of *MET* gene alterations are summarized in Table 3. On fluorescence *in situ* hybridization (FISH) or silver *in situ* hybridization, *MET* gene amplification was detected in 3.4% to 7.1% of tumors[12,32,38]. In a study of esophagogastric adenocarcinoma, *MET* amplification was observed in 2.2% (10 of 460) of patients[39]. However, overexpression has been defined according to two patterns, *i.e.*, both amplification and high polysomy, or amplification alone. Gene amplification has been found to be significantly related to distant metastasis and tumor stage[12,39]. On copy number assay using reverse transcription polymerase chain reaction (RT-PCR), *MET* gene amplification was observed in 1.5% to 30% of tumors, although the definition of *MET* amplification somewhat differed among studies[15,18,40-42]. In a study using single nucleotide polymorphism array, *MET* amplification was detected in 3% to 4% of patients[43,44]. Wang *et al*[43] reported that *MET* amplification was found in 7% (3 of 41) of intestinal type cancers, but not in other types.

In many studies using FISH or RT-PCR, patients with *MET*-amplified tumors had significantly poorer survival than those with non-amplified tumors[12,15,18,32,39,41,42]. Only a Japanese study, with the lowest incidence of gene amplification, reported no relation of *MET* amplification to survival or any clinicopathological characteristic[40].

Gene mutation

A mutation of *MET* exon 14 coding for the juxta-membrane domain with a regulatory site was detected, and all other mutations were found in MET exons 16 to 20[45]. *MET* exon 2 skipping was found in 30% (82 of 272) of GC cases and was associated with increased *MET* gene expression. In addition, novel variants of *MET* exon 18 and/or 19 skipping were observed in 42% (47

Table 3 *MET* gene alterations and clinical outcomes in gastric cancer

	n	Definition of positive expression	%	Relation to clinicopatho-logical factors	Relation to survival	Ref.
FISH	460[1]	GA	2.2	Stage	Worse	[39]
	196	GA	6.1	ND	Worse	[32]
	170	GA or HP	15 (GA7.1 HP7.6)	ND	ND	[38]
SISH	381	GA or HP	19 (GA3.4, HP16)	Intestinal (HP), M (GA), stage (GA)	Worse[2] (GA)	[12]
RT-PCR	472	> 4 copies	21	NA	Worse[2]	[33]
	266	> 4 copies	1.5	NA	NA	[40]
	216	⩾ 5 copies	10	Unknown	Worse[2]	[41]
	128	⩾ 4 copies	30	T, stage	Worse[2]	[42]
	45	⩾ 7 copies	7	ND	Worse	[18]
SNP array	193	GA	4	ND	ND	[44]
	100	GA	3	ND	ND	[43]
Polymorphism analysis	34 (tumor)	Any alterations	59	T, N, M	ND	[47]
	34 (serum)	Any alterations	41	N, M	ND	[47]

[1]Esophagogastric adenocarcinoma; [2]An independent prognostic factor on multivariate analysis. FISH: Fluorescence *in situ* hybridization; SISH: Silver *in situ* hybridization; RT-PCR: Reverse transcription polymerase chain reaction; SNP: Single nucleotide polymorphism; GA: Gene amplification; HP: High polysomy; ND: Not described; NA: Not associated; T: Tumor invasion depth; N: Lymph-node metastasis; M: Distant metastasis.

of 272) of GC patients[46]. In another study, alterations of the *MET* gene were detected in both cancer tissue and peripheral blood of GC patients, and such alterations significantly correlated with tumor depth, lymph-node metastasis, and distant metastasis[47]. *MET* polymorphism (A/G or G/G genotype of *MET* rs40239) was significantly associated with favorable survival in a Japanese cohort, although no significant association was found in American or Austrian cohorts[48].

PRECLINICAL STUDIES OF MET INHIBITORS FOR GC

Several GC cell lines (Hs746T, GTL16, MKN45, SNU5, SNU620, HSC58, 58As9, and 58As1) have *MET* amplification and were used in preclinical studies of MET inhibition.

Selective tyrosine kinase inhibitors for MET

Volitinib (HMPL-504/AZD6094) is a small, potent adenosine triphosphate (ATP)-competitive tyrosine kinase inhibitor (TKI) of MET. Volitinib showed higher antiproliferative activity against GC cell lines with gains of *MET* gene copy number (SNU5, Hs746T, SNU620, GTL16, *etc.*) than against those without such gains (MKN1, MKN74, AZ521, KATO Ⅲ, AGS, *etc.*). The expressions of p-MET, phosphorylated AKT (p-AKT), and phosphorylated ERK (p-ERK) were down-regulated by volitinib in Hs746T cells. In a GC patient-derived tumor xenograft model with *MET* amplification, volitinib inhibited tumor growth; furthermore, the antitumor activity of volitinib was enhanced by concurrent treatment with docetaxel[38].

SU11274 is a small molecule TKI of MET. SU11274 blocked HGF-induced epithelial-mesenchymal transition, inducing down-regulation of Snail-2 and vimentin and up-regulation of E-cadherin in MKN45 cells, but not in non-amplified GC cells (MKN74). SU11274 suppressed proliferation of tumor cells regardless of the presence of HGF and also inhibited migratory potential. In a mouse model of peritoneal dissemination established from MKN45, SU11274 reduced the numbers and sizes of peritoneal tumors[34]. SU11274 treatment combined with SN38 synergistically suppressed proliferation of GC cells (side population cells of OCUM-2M) and tumor volume in a xenograft model[49].

PHA-665752 is a specific TKI for MET. In GTL16 cells, PHA-665752 inhibited growth in soft agar as well as cell proliferation and induced apoptosis regardless of the presence of HGF. PHA-665752 treatment decreased expression of MET-dependent signaling pathways, including p-MET, p-AKT, p-ERK, phosphorylated focal adhesion kinase (p-FAK), p-PLC-gamma, or phosphorylated signal transducer and activator of transcription, in GTL-16 or MKN45 cells[50,51]. Inhibition efficacy was higher in MKN45 cells than in non-amplified GC cells (MKN1, MKN28, and AGS)[51]. PHA-665752 significantly inhibited an increase in tumor volume in a GTL16 xenograft model[50]. PHA-665752 induced autophagy, and combined treatment with PHA-665752 and an autophagy inhibitor acted synergistically in GTL16 cells[52]. Furthermore, PHA-665752 restored growth inhibition in GC cells (SNU216) resistant to lapatinib (anti-EGFR and HER2)[16].

SGX523 is a selective, ATP-competitive MET inhibitor. Tyr 1248 is essential for high-affinity binding of SGX523 to MET. SGX523 inhibited p-MET and downstream signal pathways (p-GAB1, p-AKT, and p-ERK) in GTL16 cells. SGX523 inhibited tumor growth in a GTL16 xenograft model[53].

BAY-853474 is a highly selective, ATP-competitive MET inhibitor. It suppressed tumor growth in an Hs746T xenograft model and reduced plasma biomarkers, such as soluble MET ectodomain and IL-8[54].

KRC-408 is a small-molecule TKI that inhibits MET by occupying the ATP binding site. KRC inhibited p-MET and its constitutive downstream effectors (p-AKT, p-MEK,

p-ERK, phosphorylated mammalian target of rapamycin (mTOR), and p-p70S6K in MKN45 cells. KRC-408 induced apoptosis as represented by increased levels of caspase-3 and PARP. MKN45 cells in G2/M phase accumulated and those in S phase decreased after KRC-408 treatment. KRC-408 significantly delayed tumor growth in an MKN45 xenograft model, accompanied by decreased expression of p-MET, p-AKT, p-ERK, and CD34[55].

AMG 337 is a small-molecule ATP-competitive TKI of MET. Treatment with AMG 337 affected the viability of only two GC cell lines (SNU5 and Hs746T). Administration of AMG 337 resulted in dose-dependent antitumor efficacy in MET-amplified GC xenograft models[56].

Multikinase TKI

Crizotinib (PF-2341066) is an ATP-competitive, small-molecule TKI of MET and anaplastic lymphoma kinase. Crizotinib inhibited GTL16 cell growth and induced apoptosis in GTL16 cells. Crizotinib treatment reduced p-MET expression and inhibited tumor growth in a GTL16 xenograft model. These effects were accompanied by a decrease in tumor mitotic index (Ki67 expression), induction of apoptosis (caspase-3 expression), and a reduction in microvessel density (CD31 expression)[57]. Crizotinib induced apoptosis and reduced expression of p-AKT and p-ERK in *MET*-amplified GC cells (SNU5, HSC58, 58As9, and 58As1), but not in non-amplified GC cells (MKN28 and MKN1). Crizotinib treatment up-regulated the expression of a proapoptotic member of the Bcl-2 family (BIM), whereas it down-regulated the expression of members of the inhibitor of apoptosis protein (IAP) family, such as survivin, X-linked IAP, and c-IAP1. Crizotinib exhibited marked antitumor activity in 58As9 and SNU5 xenografts, but not in other xenografts derived from non-amplified GC cells (AZ521 and MKN28)[58]. In another study, crizotinib effectively inhibited the growth of *MET*-amplified GC cells (SNU620, SNU5, Hs746T, and GLT16) or MET-overexpressed GC cells (SNU638). MET-positive patient-derived GC xenografts responded to crizotinib and showed down-regulation of p-MET, p-AKT, and p-ERK[32].

Forenitib (GSK1363089) is an ATP-competitive multikinase inhibitor of MET, RON, AXL, tunica internal endothelial cell kinase 2 (TIE2), and VEGFR2. Forenitib inhibited the growth of MKN45 cells and FGFR2-amplified GC cells (KATO-Ⅲ) more strongly than that of non-amplified GC cells (MKN1, MKN7, and MKN74). Foretinib suppressed phosphorylation of EGFR, HER3, and FGFR3 *via* MET inhibition in MKN45 cells, while it inhibited phosphorylation of EGFR, HER3 and MET *via* FGFR2 inhibition in KATO-Ⅲ cells[59].

Cabozantinib (XL184) is an ATP-competitive, small-molecule multikinase inhibitor against MET, VEGFR2, and RET. SNU5 and Hs746T cells markedly responded to cabozantinib[60].

S49076 is a potent ATP-competitive multikinase inhibitor of MET, AXL/MER, and FGFR1-3. S49076 decreased p-MET expression and cell viability in GTL16 cells. S49076 down-regulated p-MET, p-AKT, and phosphorylated p70S6K and inhibited tumor growth in a GTL16 xenograft model[61].

T-1840383 is a potent inhibitor that targets MET, VEGFR1-3, RET, RON, RSE, TIE2, and TRKA. T-1840383 inhibited tumor growth in association with reduced p-MET, p-AKT, and p-ERK expression in an MKN45 xenograft model. In a peritoneal dissemination model generated from GC cells (NUGC4 expressing luciferase), T-1840383 treatment significantly prolonged survival in mice[62].

MK-2461, an ATP-competitive multitargeted inhibitor of activated MET, FGFR2, and platelet-derived growth factor receptor, potently inhibited the phosphorylation of three tyrosine residues of MET (Y1003 in the juxtamembrane domain, and Y1349 and Y1365 in the COOH-terminal docking site) in GTL16 cells. The antiproliferative potencies of MK-2461 were higher in GC cells with amplification of *MET* or *FGFR2* (GLT16, SNU5, SNU16, KATO Ⅲ) than in non-amplified GC cells (MKN74, AGS, SNU1, *etc*.). In GTL16 xenograft models, MK-2461 effectively suppressed MET signaling and tumor growth[63].

Other drugs

K252a is a potent small molecule inhibitor of the TRK family and reduced MET-driven proliferation in GTL16 cells. After K252a treatment, GTL16 cells lost the ability to form lung metastases in mice[64].

Oridonin, a diterpenoid isolated from the plant Rabdosia rubescens, has been used in traditional Chinese medicine for the treatment of human cancer, such as esophageal and prostate carcinomas. Oridonin potently inhibited MET phosphorylation and MET-dependent cell proliferation in SNU5 cells. Oridonin inhibited tumor growth and down-regulated p-AKT, p-ERK, p-c-RAF in an SNU5 xenograft model. Expression levels of Ki67 and CD31 on IHC also decreased in that model[65].

Resistance to MET inhibitors

HER kinase activation has been shown to play a role in the acquisition of resistance to MET inhibitor in GC cells. Phosphorylation of EGFR and HER3, which are activated *via* MET-driven receptor cross-talk, were suppressed by a MET inhibitor (PHA-665752) in GTL-16 and MKN-45 cells. However, EGF or heregulin-beta1 (HRG) treatment activated MET-independent EGFR or HER3 and restimulated PI3K/AKT or MEK/MAPK pathway. EGF or HRG treatment increased expression of cyclin D1, which had been reduced by a MET inhibitor, and promoted the cell cycle from arrest phase to synthetic phase. Therefore, combined treatment with an MET inhibitor plus an MEK or AKT inhibitor suppressed cell proliferation that had been promoted by HER family activation[66]. In the other study, activation of HER family members induced resistance to MET inhibitor.

Table 4 Development of MET-targeting agents for gastric cancer

Type	Agent	Other targets	Phase	Line	Combined therapy	Results or status	Ref.
MET selective non-ATP competitive TKI	Tivantinib (ARQ197)	None	Ⅱ	2nd/3rd	None	No CR/PR Median PFS 1.4 mo	[72]
MET-selective ATP-competitive TKI	AMG 337	None	Ⅱ	Any	None	Ongoing	[74]
			Ⅰ	2nd/3rd	None	1 CR and 4 PR in 10 patients with MET -amplified tumor	[73]
Multitargeted ATP-competitive TKI	Foretinib (GSK1363089)	VEGFR2, RON, AXL, TIE2	Ⅱ	1st (95%)	Docetaxel, Cisplatin	No CR/PR Median OS 7.4	[75]
	Crizotinib (PF-2341066)	ALK	Ⅰ			Tumor shrinkage in 2 patients with PFS 3.5 and 3.7 mo	[39]
MET mAb	Onartuzumab (MetMab)	None	Ⅲ	1st	mFOLFOX	Ongoing	[77]
HGF mAb	Rilotumumab (AMG 102)	None	Ⅲ	1st	ECX	Suspended	[79]
		None	Ⅲ	1st	CX	Suspended	[80]
		None	Ⅱ	1st	ECX	Median PFS 4.2 mo Median OS 5.6 mo	[78]

ATP: Adenosine triphosphate; TKI: Tyrosine kinase inhibitor; mAb: Monoclonal antibody; VEGFR: Vascular endothelial growth factor receptor; ALK: Anaplastic lymphoma kinase; TIE: Tunica internal endothelial cell kinase; CR: Complete response; PR: Partial response; RFS: Relapse-free survival; OS: Overall survival; FOLFOX: Folinic acid + fluorouracil + oxaliplatin; ECX: Epirubicin + oxaliplatin + capecitabine; CX: Oxaliplatin + capecitabine.

GTL16 cells that had acquired constitutive activation of EGFR by EGFR-L858R mutation did not respond to anti-MET treatment, such as MET silencing or MET inhibitor (PHA-665752). mRNA levels of HER family members significantly increased in the resistant GTL16 cells[67]. Qi *et al*[68] reported two mechanisms of resistance to the MET inhibitors PHA-665752 and PF-2341066. One mechanism was the activation of EGFR signaling. In GC cells acquiring resistance to MET inhibitors, EGFR signaling (EGFR, AKT, and ERK) was activated *via* an increase in transforming growth factor alpha. The other mechanism involved a gene mutation in the MET activation loop (Y1230). That mutation destabilizes the autoinhibitory conformation of MET on structural analysis and abrogates interaction with the inhibitor[68]. Increased copy numbers of *MET* or *KRAS* and increased expression of p-ERK or p-AKT were detected in GTL16 cells resistant to the MET inhibitor PHA-665752[69]. In addition, a novel *SND1-BRAF* fusion was detected in GTL16 cells that were resistant to the MET inhibitor RF-04217903 and was proven to be responsible for the resistance[70].

CLINICAL STUDIES OF MET INHIBITORS IN GC

Published and ongoing clinical studies of MET inhibitors in GC are summarized in Table 4. Tivantinib (ARQ197) is a non-ATP-competitive, selective MET inhibitor. In a phase Ⅰ trial in 51 patients with GC, 14 patients had stable disease (SD) for 4 mo or longer, and circulating endothelial cells decreased in 58% (25 of 43) of patients. Tivantinib decreased p-MET, MET, and phosphorylated focal adhesion kinase and increased terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick-end labeling (TUNEL) staining in tumor biopsy specimens[71]. In a phase Ⅱ study of tivantinib as second- or third-line therapy in GC, no objective response was observed in the 30 patients enrolled; the disease control rate was 37%, and median progression-free survival (PFS) was only 43 d. Tivantinib seemed to have modest antitumor efficacy and mild toxicity. As for adverse effects, severe (grade 3 or higher) neutropenia and anemia were most common, each occurring in 13% (4 of 30) of the patients[72].

Recently, favorable outcomes of treatment with ANG 337 have been reported in a phase Ⅰ study in 10 patients with MET-amplified esophago GC[73]. One patient had a complete response, and 4 had partial responses, even when ANG 337 was given as second-line or subsequent chemotherapy. An ongoing phase Ⅱ study is expected to explore whether the levels of MET amplification and expression or the presence of mutation in tumor specimens correlates with the response to AMG 337[74].

Foretinib lacked efficacy against metastatic GC in a phase Ⅱ study enrolling 74 patients. The best response was SD in 23% (10 of 44) of patients who received intermittent dosing and 20% (5 of 25) of those who received daily dosing. Only 4% (3 of 67) of the patients had *MET* amplification in tumor specimens, and one of them had SD. OS was 7.4 mo with intermittent dosing and 4.3 mo with daily dosing. Severe (grade 3 or higher) treatment-related adverse events occurred in 44% (21 of 48) of the patients who received intermittent dosing and 35% (9 of 26) of those who received daily dosing. Elevated aspartate aminotransferase levels (10%) and fatigue (15%) were the most frequent adverse events in patients who received intermittent dosing and daily dosing, respectively. Plasma levels of MET, HGF, VEGFR2, and VEGF-A were measured at baseline and during treatment, but these markers did not correlate with response[75].

Crizotinib was administered to 4 patients with *MET*-amplified esophagogastric adenocarcinomas in part of a phase Ⅰ study. Two patients had tumor shrinkage (16% and 30%) with PFS of 3.5 and 3.7 mo, respectively[39].

Onartuzumab (formally called MetMAb and PRO 143966) is an anti-MET receptor monoclonal antibody. In a phase Ⅰ clinical trial, one patient with metastatic GC had a complete response for approximately 2 and a half years[76]. A phase Ⅲ study of onartuzumab combined with modified FOLFOX (5-fluorouracil + leucovorin + oxaliplatin) is ongoing[77].

Rilotumumab (AMG 102) is a monoclonal antibody against HGF. In a phase Ⅰb/Ⅱ study of rilotumumab combined with epirubicin, cisplatin, and capecitabine (ECX) as first-line chemotherapy, 121 patients were randomly assigned to treatment (40 to rilotumumab 15 mg/kg; 42 to rilotumumab 7.5 mg/kg; 39 to placebo). Median PFS was significantly longer in both rilotumumab groups combined than in the placebo group (5.7 and 4.2 mo, respectively). The response rate was 39%, and the disease control rate was 80% in the combined rilotumumab group. MET status was evaluated on IHC in that study, and MET positivity was defined as at least 25% membrane staining of tumor cells at any intensity. In the MET-positive group, median OS was much longer in the combined rilotumumab group than in the placebo group (10.6 mo *vs* 5.7 mo). In the MET-negative group, patients had better survival than those in the MET-positive group, and rilotumumab was not significantly effective. As for adverse effects, severe (grade 3 or higher) venous thromboembolism occurred in 20% (16 of 81) of the patients[78]. However, the management of thromboembolism might be the most critical issue. Two phase Ⅲ trials of rilotumumab plus ECX and rilotumumab plus cisplatin and capecitabine have been suspended because of increased treatment-related mortality[79,80].

CONCLUSION

Many studies have suggested that MET protein overexpression or *MET* amplification plays a critical role in the progression of GC and negatively affects survival in patients with GC. However, the criteria used to define overexpression of MET protein have differed among many studies, and the assessment of MET protein expression is unlikely to be standardized as strictly as that of HER2 or EGFR. It remains unclear whether staining intensity of the membrane or the cytoplasm of tumor cells should be assessed. Differences in staining intensity associated with the use of different antibodies and different IHC procedures used to assess MET expression remain a problem that must be solved before techniques for assessing MET status can be standardized. The use of different assessment techniques by different investigators is another problem. The evaluation of p-MET expression might provide the most objective measure of MET status; however, the fact that different antibodies recognize different phosphorylated sites might be a major obstacle to the standardization of techniques for assessing p-MET expression. On the other hand, *MET* amplification on FISH may be appropriate for standardized assessment, similar to *HER2* amplification. Several studies have used consistent criteria to define *MET* amplification on FISH, and it is more objective assessment than that of protein expression on IHC, although the cost- and time-effectiveness of gene analysis may be poor. Deng *et al*[44] reported that *MET* amplification was mutually exclusive from amplification of other genes, such as *EGFR*, *HER2*, *FGFR2*, and *KRAS*. Therefore, MET-targeting therapy is considered a promising treatment for GC with *MET*-amplification as well as GC with amplification of other RTKs.

Preclinical studies have suggested that MET inhibitors are most promising against *MET*-amplified or MET-overexpressed cancers. Various MET inhibitors have been developed and studied in clinical trials; however, several trials showed insufficient efficacy and unexpected outcomes. These results might have been caused by lack of identification of specific biomarkers. Methodological differences in the evaluation of MET status remain an important problem in conducting clinical trials. In an ongoing study of monoclonal antibodies of MET, patients with MET expression on IHC are being recruited[77]. As mentioned above, the assessment of MET protein expression on IHC remains to be standardized. The same procedure for assessment of MET status on IHC is needed for clinical studies. Many TKIs of MET have produced favorable results in *MET*-amplified GC in many preclinical studies, and AMG 337 and crizotinib were effective in some patients with *MET*-amplified GC in preliminary clinical studies[39,73]. MET TKIs thus may be a promising treatment for patients with *MET*-amplified GC.

Resistance to MET inhibitors is another critical issue. Several lines of evidence from preclinical studies suggest that activation of the HER family is involved in resistance to MET inhibitors, and treatment against HER family pathways may overcome this issue. Owing to the diversity of RTKs, treatment with a multitargeted TKI or combined therapy with single-targeted TKIs might be a promising approach to enhance efficacy. However, potential benefits of treatment with multiple inhibitors of RTKs have yet to be demonstrated in clinical trials in GC.

MET is considered a promising target in GC, although the results of phase Ⅲ trials of rilotumumab have been disappointing. It is essential to identify specific subgroups of patients most likely to benefit from treatment with MET inhibitors. Future studies should attempt to define biomarkers that would optimize the selection of patients who respond to MET inhibitors.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

2 **Cunningham D**, Starling N, Rao S, Iveson T, Nicolson M, Coxon F, Middleton G, Daniel F, Oates J, Norman AR. Capecitabine and oxaliplatin for advanced esophagogastric cancer. *N Engl J Med* 2008; **358**: 36-46 [PMID: 18172173 DOI: 10.1056/NEJMoa07

3149]
3 **Koizumi W**, Narahara H, Hara T, Takagane A, Akiya T, Takagi M, Miyashita K, Nishizaki T, Kobayashi O, Takiyama W, Toh Y, Nagaie T, Takagi S, Yamamura Y, Yanaoka K, Orita H, Takeuchi M. S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. *Lancet Oncol* 2008; **9**: 215-221 [PMID: 18282805 DOI: 10.1016/S1470-2045(08)70035-4]
4 **Schlessinger J**. Cell signaling by receptor tyrosine kinases. *Cell* 2000; **103**: 211-225 [PMID: 11057895 DOI: 10.1016/S0092-8674(00)00114-8]
5 **Weinstein IB**, Joe AK. Mechanisms of disease: Oncogene addiction--a rationale for molecular targeting in cancer therapy. *Nat Clin Pract Oncol* 2006; **3**: 448-457 [PMID: 16894390 DOI: 10.1038/ncponc0558]
6 **Bang YJ**, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010; **376**: 687-697 [PMID: 20728210 DOI: 10.1016/S0140-6736(10)61121-X]
7 **Fuchs CS**, Tomasek J, Yong CJ, Dumitru F, Passalacqua R, Goswami C, Safran H, dos Santos LV, Aprile G, Ferry DR, Melichar B, Tehfe M, Topuzov E, Zalcberg JR, Chau I, Campbell W, Sivanandan C, Pikiel J, Koshiji M, Hsu Y, Liepa AM, Gao L, Schwartz JD, Tabernero J. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial. *Lancet* 2014; **383**: 31-39 [PMID: 24094768 DOI: 10.1016/S0140-6736(13)61719-5]
8 **Wilke H**, Muro K, Van Cutsem E, Oh SC, Bodoky G, Shimada Y, Hironaka S, Sugimoto N, Lipatov O, Kim TY, Cunningham D, Rougier P, Komatsu Y, Ajani J, Emig M, Carlesi R, Ferry D, Chandrawansa K, Schwartz JD, Ohtsu A. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomised phase 3 trial. *Lancet Oncol* 2014; **15**: 1224-1235 [PMID: 25240821 DOI: 10.1016/S1470-2045(14)70420-6]
9 **Birchmeier C**, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. *Nat Rev Mol Cell Biol* 2003; **4**: 915-925 [PMID: 14685170 DOI: 10.1038/nrm1261]
10 **Liu X**, Newton RC, Scherle PA. Developing c-MET pathway inhibitors for cancer therapy: progress and challenges. *Trends Mol Med* 2010; **16**: 37-45 [PMID: 20031486 DOI: 10.1016/j.molmed.2009.11.005]
11 **Gherardi E**, Birchmeier W, Birchmeier C, Vande Woude G. Targeting MET in cancer: rationale and progress. *Nat Rev Cancer* 2012; **12**: 89-103 [PMID: 22270953 DOI: 10.1038/nrc3205]
12 **Lee HE**, Kim MA, Lee HS, Jung EJ, Yang HK, Lee BL, Bang YJ, Kim WH. MET in gastric carcinomas: comparison between protein expression and gene copy number and impact on clinical outcome. *Br J Cancer* 2012; **107**: 325-333 [PMID: 22644302 DOI: 10.1038/bjc.2012.237]
13 **Ma J**, Ma J, Meng Q, Zhao ZS, Xu WJ. Prognostic value and clinical pathology of MACC-1 and c-MET expression in gastric carcinoma. *Pathol Oncol Res* 2013; **19**: 821-832 [PMID: 23812675 DOI: 10.1007/s12253-013-9650-0]
14 **Ha SY**, Lee J, Kang SY, Do IG, Ahn S, Park JO, Kang WK, Choi MG, Sohn TS, Bae JM, Kim S, Kim M, Kim S, Park CK, Ignatius Ou SH, Kim KM. MET overexpression assessed by new interpretation method predicts gene amplification and poor survival in advanced gastric carcinomas. *Mod Pathol* 2013; **26**: 1632-1641 [PMID: 23807774 DOI: 10.1038/modpathol.2013.108]
15 **Lee J**, Seo JW, Jun HJ, Ki CS, Park SH, Park YS, Lim HY, Choi MG, Bae JM, Sohn TS, Noh JH, Kim S, Jang HL, Kim JY, Kim KM, Kang WK, Park JO. Impact of MET amplification on gastric cancer: possible roles as a novel prognostic marker and a potential therapeutic target. *Oncol Rep* 2011; **25**: 1517-1524 [PMID: 21424128 DOI: 10.3892/or.2011.1219]
16 **Chen CT**, Kim H, Liska D, Gao S, Christensen JG, Weiser MR. MET activation mediates resistance to lapatinib inhibition of HER2-amplified gastric cancer cells. *Mol Cancer Ther* 2012; **11**: 660-669 [PMID: 22238368 DOI: 10.1158/1535-7163.MCT-11-0754]
17 **Kneissl J**, Keller S, Lorber T, Heindl S, Keller G, Drexler I, Hapfelmeier A, Höfler H, Luber B. Association of amphiregulin with the cetuximab sensitivity of gastric cancer cell lines. *Int J Oncol* 2012; **41**: 733-744 [PMID: 22614881 DOI: 10.3892/ijo.2012.1479]
18 **Catenacci DV**, Cervantes G, Yala S, Nelson EA, El-Hashani E, Kanteti R, El Dinali M, Hasina R, Brägelmann J, Seiwert T, Sanicola M, Henderson L, Grushko TA, Olopade O, Karrison T, Bang YJ, Kim WH, Tretiakova M, Vokes E, Frank DA, Kindler HL, Huet H, Salgia R. RON (MST1R) is a novel prognostic marker and therapeutic target for gastroesophageal adenocarcinoma. *Cancer Biol Ther* 2011; **12**: 9-46 [PMID: 21543897 DOI: 10.4161/cbt.12.1.15747]
19 **Zhao J**, Zhang X, Xin Y. Up-regulated expression of Ezrin and c-Met proteins are related to the metastasis and prognosis of gastric carcinomas. *Histol Histopathol* 2011; **26**: 1111-1120 [PMID: 21751142]
20 **Wu JG**, Yu JW, Wu HB, Zheng LH, Ni XC, Li XQ, Du GY, Jiang BJ. Expressions and clinical significances of c-MET, p-MET and E2f-1 in human gastric carcinoma. *BMC Res Notes* 2014; **7**: 6 [PMID: 24393368 DOI: 10.1186/1756-0500-7-6]
21 **Sun Y**, Tian MM, Zhou LX, You WC, Li JY. Value of c-Met for Predicting Progression of Precancerous Gastric Lesions in Rural Chinese Population. *Chin J Cancer Res* 2012; **24**: 18-22 [PMID: 23359758 DOI: 10.1007/s11670-012-0018-x]
22 **Janjigian YY**, Tang LH, Coit DG, Kelsen DP, Francone TD, Weiser MR, Jhanwar SC, Shah MA. MET expression and amplification in patients with localized gastric cancer. *Cancer Epidemiol Biomarkers Prev* 2011; **20**: 1021-1027 [PMID: 21393565 DOI: 10.1158/1055-9965.EPI-10-1080]
23 **Sotoudeh K**, Hashemi F, Madjd Z, Sadeghipour A, Molanaei S, Kalantary E. The clinicopathologic association of c-MET overexpression in Iranian gastric carcinomas; an immunohistochemical study of tissue microarrays. *Diagn Pathol* 2012; **7**: 57 [PMID: 22640970 DOI: 10.1186/1746-1596-7-57]
24 **Retterspitz MF**, Mönig SP, Schreckenberg S, Schneider PM, Hölscher AH, Dienes HP, Baldus SE. Expression of {beta}-catenin, MUC1 and c-met in diffuse-type gastric carcinomas: correlations with tumour progression and prognosis. *Anticancer Res* 2010; **30**: 4635-4641 [PMID: 21115917]
25 **Guo T**, Yang J, Yao J, Zhang Y, Da M, Duan Y. Expression of MACC1 and c-Met in human gastric cancer and its clinical significance. *Cancer Cell Int* 2013; **13**: 121 [PMID: 24325214 DOI: 10.1186/1475-2867-13-121]
26 **Li Y**, Chen CQ, He YL, Cai SR, Yang DJ, He WL, Xu JB, Zan WH. Abnormal expression of E-cadherin in tumor cells is associated with poor prognosis of gastric carcinoma. *J Surg Oncol* 2012; **106**: 304-310 [PMID: 22231933 DOI: 10.1002/jso.23008]
27 **Tang Z**, Zhao M, Ji J, Yang G, Hu F, He J, Shen H, Gao Z, Zhao A, Li J, Lu Y. Overexpression of gastrin and c-met protein involved in human gastric carcinomas and intestinal metaplasia. *Oncol Rep* 2004; **11**: 333-339 [PMID: 14719064 DOI: 10.3892/or.11.2.333]
28 **Han SU**, Lee HY, Lee JH, Kim WH, Nam H, Kim H, Cho YK, Kim MW, Lee KU. Modulation of E-cadherin by hepatocyte growth factor induces aggressiveness of gastric carcinoma. *Ann Surg* 2005; **242**: 676-683 [PMID: 16244541 DOI: 10.1097/01.sla.0000186171.85804.fe]
29 **Amemiya H**, Kono K, Itakura J, Tang RF, Takahashi A, An FQ, Kamei S, Iizuka H, Fujii H, Matsumoto Y. c-Met expression in gastric cancer with liver metastasis. *Oncology* 2002; **63**: 286-296 [PMID: 12381909 DOI: 10.1159/000065477]
30 **Drebber U**, Baldus SE, Nolden B, Grass G, Bollschweiler E, Dienes HP, Hölscher AH, Mönig SP. The overexpression of c-met as a prognostic indicator for gastric carcinoma compared to p53 and p21 nuclear accumulation. *Oncol Rep* 2008; **19**: 1477-1483 [PMID:

18497953]
31 **Betts G**, Valentine H, Pritchard S, Swindell R, Williams V, Morgan S, Griffiths EA, Welch I, West C, Womack C. FGFR2, HER2 and cMet in gastric adenocarcinoma: detection, prognostic significance and assessment of downstream pathway activation. *Virchows Arch* 2014; **464**: 145-156 [PMID: 24306956 DOI: 10.1007/s00428-013-1517-y]
32 **Liu YJ**, Shen D, Yin X, Gavine P, Zhang T, Su X, Zhan P, Xu Y, Lv J, Qian J, Liu C, Sun Y, Qian Z, Zhang J, Gu Y, Ni X. HER2, MET and FGFR2 oncogenic driver alterations define distinct molecular segments for targeted therapies in gastric carcinoma. *Br J Cancer* 2014; **110**: 1169-1178 [PMID: 24518603 DOI: 10.1038/bjc.2014.61]
33 **Lee J**, Kim S, Kim P, Liu X, Lee T, Kim KM, Do IG, Park JO, Park SH, Jang J, Hoe N, Harvie G, Kuller A, Jain A, Meyer G, Leesman G, Park YS, Choi MG, Sohn TS, Bae JM, Lim HY, Singh S, Kang WK. A novel proteomics-based clinical diagnostics technology identifies heterogeneity in activated signaling pathways in gastric cancers. *PLoS One* 2013; **8**: e54644 [PMID: 23372746 DOI: 10.1371/journal.pone.0054644]
34 **Toiyama Y**, Yasuda H, Saigusa S, Matushita K, Fujikawa H, Tanaka K, Mohri Y, Inoue Y, Goel A, Kusunoki M. Co-expression of hepatocyte growth factor and c-Met predicts peritoneal dissemination established by autocrine hepatocyte growth factor/c-Met signaling in gastric cancer. *Int J Cancer* 2012; **130**: 2912-2921 [PMID: 21796631 DOI: 10.1002/ijc.26330]
35 **Chi F**, Fu D, Zhang X, Lv Z, Wang Z. Expression of the c-Met proto-oncogene and Integrin α5β1 in human gastric cardia adenocarcinoma. *Biosci Biotechnol Biochem* 2012; **76**: 1471-1476 [PMID: 22976495]
36 **Heideman DA**, Snijders PJ, Bloemena E, Meijer CJ, Offerhaus GJ, Meuwissen SG, Gerritsen WR, Craanen ME. Absence of tpr-met and expression of c-met in human gastric mucosa and carcinoma. *J Pathol* 2001; **194**: 428-435 [PMID: 11523050]
37 **Uen YH**, Lin SR, Wu CH, Hsieh JS, Lu CY, Yu FJ, Huang TJ, Wang JY. Clinical significance of MUC1 and c-Met RT-PCR detection of circulating tumor cells in patients with gastric carcinoma. *Clin Chim Acta* 2006; **367**: 55-61 [PMID: 16403482]
38 **Gavine PR**, Ren Y, Han L, Lv J, Fan S, Zhang W, Xu W, Liu YJ, Zhang T, Fu H, Yu Y, Wang H, Xu S, Zhou F, Su X, Yin X, Xie L, Wang L, Qing W, Jiao L, Su W, Wang QM. Volitinib, a potent and highly selective c-Met inhibitor, effectively blocks c-Met signaling and growth in c-MET amplified gastric cancer patient-derived tumor xenograft models. *Mol Oncol* 2015; **9**: 323-333 [PMID: 25248999 DOI: 10.1016/j.molonc.2014.08.015]
39 **Lennerz JK**, Kwak EL, Ackerman A, Michael M, Fox SB, Bergethon K, Lauwers GY, Christensen JG, Wilner KD, Haber DA, Salgia R, Bang YJ, Clark JW, Solomon BJ, Iafrate AJ. MET amplification identifies a small and aggressive subgroup of esophagogastric adenocarcinoma with evidence of responsiveness to crizotinib. *J Clin Oncol* 2011; **29**: 4803-4810 [PMID: 22042947 DOI: 10.1200/JCO.2011.35.4928]
40 **Kawakami H**, Okamoto I, Arao T, Okamoto W, Matsumoto K, Taniguchi H, Kuwata K, Yamaguchi H, Nishio K, Nakagawa K, Yamada Y. MET amplification as a potential therapeutic target in gastric cancer. *Oncotarget* 2013; **4**: 9-17 [PMID: 23327903 DOI: 10.18632/oncotarget.718]
41 **Graziano F**, Galluccio N, Lorenzini P, Ruzzo A, Canestrari E, D' Emidio S, Catalano V, Sisti V, Ligorio C, Andreoni F, Rulli E, Di Oto E, Fiorentini G, Zingaretti C, De Nictolis M, Cappuzzo F, Magnani M. Genetic activation of the MET pathway and prognosis of patients with high-risk, radically resected gastric cancer. *J Clin Oncol* 2011; **29**: 4789-4795 [PMID: 22042954 DOI: 10.1200/JCO.2011.36.7706]
42 **Shi J**, Yao D, Liu W, Wang N, Lv H, He N, Shi B, Hou P, Ji M. Frequent gene amplification predicts poor prognosis in gastric cancer. *Int J Mol Sci* 2012; **13**: 4714-4726 [PMID: 22606006 DOI: 10.3390/ijms13044714]
43 **Wang K**, Yuen ST, Xu J, Lee SP, Yan HH, Shi ST, Siu HC, Deng S, Chu KM, Law S, Chan KH, Chan AS, Tsui WY, Ho SL, Chan AK, Man JL, Foglizzo V, Ng MK, Chan AS, Ching YP, Cheng GH, Xie T, Fernandez J, Li VS, Clevers H, Rejto PA, Mao M, Leung SY. Whole-genome sequencing and comprehensive molecular profiling identify new driver mutations in gastric cancer. *Nat Genet* 2014; **46**: 573-582 [PMID: 24816253 DOI: 10.1038/ng.2983]
44 **Deng N**, Goh LK, Wang H, Das K, Tao J, Tan IB, Zhang S, Lee M, Wu J, Lim KH, Lei Z, Goh G, Lim QY, Tan AL, Sin Poh DY, Riahi S, Bell S, Shi MM, Linnartz R, Zhu F, Yeoh KG, Toh HC, Yong WP, Cheong HC, Rha SY, Boussioutas A, Grabsch H, Rozen S, Tan P. A comprehensive survey of genomic alterations in gastric cancer reveals systematic patterns of molecular exclusivity and co-occurrence among distinct therapeutic targets. *Gut* 2012; **61**: 673-684 [PMID: 22315472 DOI: 10.1136/gutjnl-2011-301839]
45 **Lee JH**, Han SU, Cho H, Jennings B, Gerrard B, Dean M, Schmidt L, Zbar B, Vande Woude GF. A novel germ line juxtamembrane Met mutation in human gastric cancer. *Oncogene* 2000; **19**: 4947-4953 [PMID: 11042681]
46 **Cancer Genome Atlas Research Network**. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; **513**: 202-209 [PMID: 25079317 DOI: 10.1038/nature13480]
47 **Wang JY**, Hsieh JS, Chen CC, Tzou WS, Cheng TL, Chen FM, Huang TJ, Huang YS, Huang SY, Yang T, Lin SR. Alterations of APC, c-met, and p53 genes in tumor tissue and serum of patients with gastric cancers. *J Surg Res* 2004; **120**: 242-248 [PMID: 15234219]
48 **Sunakawa Y**, Wakatsuki T, Yang D, Zhang W, Ning Y, Stintzing S, Stremitzer S, Yamauchi S, Sebio A, El-khoueiry R, Iqbal S, Barzi A, Gerger A, Stotz M, Azuma M, Watanabe M, Koizumi W, Lenz HJ. Prognostic impact of the c-MET polymorphism on the clinical outcome in locoregional gastric cancer patients. *Pharmacogenet Genomics* 2014; **24**: 588-596 [PMID: 25203738 DOI: 10.1097/FPC.0000000000000091]
49 **Yashiro M**, Nishii T, Hasegawa T, Matsuzaki T, Morisaki T, Fukuoka T, Hirakawa K. A c-Met inhibitor increases the chemosensitivity of cancer stem cells to the irinotecan in gastric carcinoma. *Br J Cancer* 2013; **109**: 2619-2628 [PMID: 24129235 DOI: 10.1038/bjc.2013.638]
50 **Christensen JG**, Schreck R, Burrows J, Kuruganti P, Chan E, Le P, Chen J, Wang X, Ruslim L, Blake R, Lipson KE, Ramphal J, Do S, Cui JJ, Cherrington JM, Mendel DB. A selective small molecule inhibitor of c-Met kinase inhibits c-Met-dependent phenotypes in vitro and exhibits cytoreductive antitumor activity in vivo. *Cancer Res* 2003; **63**: 7345-7355 [PMID: 14612533]
51 **Smolen GA**, Sordella R, Muir B, Mohapatra G, Barmettler A, Archibald H, Kim WJ, Okimoto RA, Bell DW, Sgroi DC, Christensen JG, Settleman J, Haber DA. Amplification of MET may identify a subset of cancers with extreme sensitivity to the selective tyrosine kinase inhibitor PHA-665752. *Proc Natl Acad Sci USA* 2006; **103**: 2316-2321 [PMID: 16461907]
52 **Humbert M**, Medová M, Aebersold DM, Blaukat A, Bladt F, Fey MF, Zimmer Y, Tschan MP. Protective autophagy is involved in resistance towards MET inhibitors in human gastric adenocarcinoma cells. *Biochem Biophys Res Commun* 2013; **431**: 264-269 [PMID: 23313490 DOI: 10.1016/j.bbrc.2012.12.120]
53 **Buchanan SG**, Hendle J, Lee PS, Smith CR, Bounaud PY, Jessen KA, Tang CM, Huser NH, Felce JD, Froning KJ, Peterman MC, Aubol BE, Gessert SF, Sauder JM, Schwinn KD, Russell M, Rooney IA, Adams J, Leon BC, Do TH, Blaney JM, Sprengeler PA, Thompson DA, Smyth L, Pelletier LA, Atwell S, Holme K, Wasserman SR, Emtage S, Burley SK, Reich SH. SGX523 is an exquisitely selective, ATP-competitive inhibitor of the MET receptor tyrosine kinase with antitumor activity in vivo. *Mol Cancer Ther* 2009; **8**: 3181-3190 [PMID: 19934279 DOI: 10.1158/1535-7163.MCT-09-0477]
54 **Klotz M**, Schmid E, Steiner-Hahn K, Rose T, Laube J, Roese L, Henderson D, Krahn T, von Ahsen O. Preclinical evaluation of biomarkers for response monitoring to the MET inhibitor BAY-853474. *Biomarkers* 2012; **17**: 325-335 [PMID: 22452362 DOI: 10.3109/1354750X.2012.670865]
55 **Hong SW**, Jung KH, Park BH, Zheng HM, Lee HS, Choi MJ, Yun

JI, Kang NS, Lee J, Hong SS. KRC-408, a novel c-Met inhibitor, suppresses cell proliferation and angiogenesis of gastric cancer. *Cancer Lett* 2013; **332**: 74-82 [PMID: 23348694 DOI: 10.1016/j.canlet.2013.01.015]

56 **Hughes PE**, Yang Y, Rex K, Zhang Y, Kaplan-Lefko PJ, Caenepeel S, Moriguchi J, Broome M, Choquette D, Radinsky R, Kendall R, Coxon A, Dussault I. AMG 337, a novel, potent and selective MET kinase inhibitor, has robust growth inhibitory activity in MET-dependent cancer models. [Proceedings: AACR Annual Meeting 2014; San Diego, CA] *Cancer Res* 2014; **74**: 728 [DOI: 10.1158/1538-7445.AM2014-728]

57 **Zou HY**, Li Q, Lee JH, Arango ME, McDonnell SR, Yamazaki S, Koudriakova TB, Alton G, Cui JJ, Kung PP, Nambu MD, Los G, Bender SL, Mroczkowski B, Christensen JG. An orally available small-molecule inhibitor of c-Met, PF-2341066, exhibits cytoreductive antitumor efficacy through antiproliferative and antiangiogenic mechanisms. *Cancer Res* 2007; **67**: 4408-4417 [PMID: 17483355]

58 **Okamoto W**, Okamoto I, Arao T, Kuwata K, Hatashita E, Yamaguchi H, Sakai K, Yanagihara K, Nishio K, Nakagawa K. Antitumor action of the MET tyrosine kinase inhibitor crizotinib (PF-02341066) in gastric cancer positive for MET amplification. *Mol Cancer Ther* 2012; **11**: 1557-1564 [PMID: 22729845 DOI: 10.1158/1535-7163.MCT-11-0934]

59 **Kataoka Y**, Mukohara T, Tomioka H, Funakoshi Y, Kiyota N, Fujiwara Y, Yashiro M, Hirakawa K, Hirai M, Minami H. Foretinib (GSK1363089), a multi-kinase inhibitor of MET and VEGFRs, inhibits growth of gastric cancer cell lines by blocking inter-receptor tyrosine kinase networks. *Invest New Drugs* 2012; **30**: 1352-1360 [PMID: 21655918 DOI: 10.1007/s10637-011-9699-0]

60 **Roy S**, Narang BK, Rastogi SK, Rawal RK. A novel multiple tyrosine-kinase targeted agent to explore the future perspectives of anti-angiogenic therapy for the treatment of multiple solid tumors: cabozantinib. *Anticancer Agents Med Chem* 2015; **15**: 37-47 [PMID: 25181996]

61 **Burbridge MF**, Bossard CJ, Saunier C, Fejes I, Bruno A, Léonce S, Ferry G, Da Violante G, Bouzom F, Cattan V, Jacquet-Bescond A, Comoglio PM, Lockhart BP, Boutin JA, Cordi A, Ortuno JC, Pierré A, Hickman JA, Cruzalegui FH, Depil S. S49076 is a novel kinase inhibitor of MET, AXL, and FGFR with strong preclinical activity alone and in association with bevacizumab. *Mol Cancer Ther* 2013; **12**: 1749-1762 [PMID: 23804704 DOI: 10.1158/1535-7163.MCT-13-0075]

62 **Awazu Y**, Nakamura K, Mizutani A, Kakoi Y, Iwata H, Yamasaki S, Miyamoto N, Imamura S, Miki H, Hori A. A novel inhibitor of c-Met and VEGF receptor tyrosine kinases with a broad spectrum of in vivo antitumor activities. *Mol Cancer Ther* 2013; **12**: 913-924 [PMID: 23548264 DOI: 10.1158/1535-7163.MCT-12-1011]

63 **Pan BS**, Chan GK, Chenard M, Chi A, Davis LJ, Deshmukh SV, Gibbs JB, Gil S, Hang G, Hatch H, Jewell JP, Kariv I, Katz JD, Kunii K, Lu W, Lutterbach BA, Paweletz CP, Qu X, Reilly JF, Szewczak AA, Zeng Q, Kohl NE, Dinsmore CJ. MK-2461, a novel multitargeted kinase inhibitor, preferentially inhibits the activated c-Met receptor. *Cancer Res* 2010; **70**: 1524-1533 [PMID: 20145145 DOI: 10.1158/0008-5472.CAN-09-2541]

64 **Morotti A**, Mila S, Accornero P, Tagliabue E, Ponzetto C. K252a inhibits the oncogenic properties of Met, the HGF receptor. *Oncogene* 2002; **21**: 4885-4893 [PMID: 12118367]

65 **Liu H**, Qian C, Shen Z. Anti-tumor activity of oridonin on SNU-5 subcutaneous xenograft model via regulation of c-Met pathway. *Tumour Biol* 2014; **35**: 9139-9146 [PMID: 24916572 DOI: 10.1007/s13277-014-2178-4]

66 **Bachleitner-Hofmann T**, Sun MY, Chen CT, Tang L, Song L, Zeng Z, Shah M, Christensen JG, Rosen N, Solit DB, Weiser MR. HER kinase activation confers resistance to MET tyrosine kinase inhibition in MET oncogene-addicted gastric cancer cells. *Mol Cancer Ther* 2008; **7**: 3499-3508 [PMID: 18974395 DOI: 10.1158/1535-7163.MCT-08-0374]

67 **Corso S**, Ghiso E, Cepero V, Sierra JR, Migliore C, Bertotti A, Trusolino L, Comoglio PM, Giordano S. Activation of HER family members in gastric carcinoma cells mediates resistance to MET inhibition. *Mol Cancer* 2010; **9**: 121 [PMID: 20500904 DOI: 10.1186/1476-4598-9-121]

68 **Qi J**, McTigue MA, Rogers A, Lifshits E, Christensen JG, Jänne PA, Engelman JA. Multiple mutations and bypass mechanisms can contribute to development of acquired resistance to MET inhibitors. *Cancer Res* 2011; **71**: 1081-1091 [PMID: 21266357 DOI: 10.1158/0008-5472.CAN-10-1623]

69 **Cepero V**, Sierra JR, Corso S, Ghiso E, Casorzo L, Perera T, Comoglio PM, Giordano S. MET and KRAS gene amplification mediates acquired resistance to MET tyrosine kinase inhibitors. *Cancer Res* 2010; **70**: 7580-7590 [PMID: 20841479 DOI: 10.1158/0008-5472.CAN-10-0436]

70 **Lee NV**, Lira ME, Pavlicek A, Ye J, Buckman D, Bagrodia S, Srinivasa SP, Zhao Y, Aparicio S, Rejto PA, Christensen JG, Ching KA. A novel SND1-BRAF fusion confers resistance to c-Met inhibitor PF-04217903 in GTL16 cells through [corrected] MAPK activation. *PLoS One* 2012; **7**: e39653 [PMID: 22745804 DOI: 10.1371/journal.pone.0039653]

71 **Yap TA**, Olmos D, Brunetto AT, Tunariu N, Barriuso J, Riisnaes R, Pope L, Clark J, Futreal A, Germuska M, Collins D, deSouza NM, Leach MO, Savage RE, Waghorne C, Chai F, Garmey E, Schwartz B, Kaye SB, de Bono JS. Phase I trial of a selective c-MET inhibitor ARQ 197 incorporating proof of mechanism pharmacodynamic studies. *J Clin Oncol* 2011; **29**: 1271-1279 [PMID: 21383285 DOI: 10.1200/JCO.2010.31.0367]

72 **Kang YK**, Muro K, Ryu MH, Yasui H, Nishina T, Ryoo BY, Kamiya Y, Akinaga S, Boku N. A phase II trial of a selective c-Met inhibitor tivantinib (ARQ 197) monotherapy as a second- or third-line therapy in the patients with metastatic gastric cancer. *Invest New Drugs* 2014; **32**: 355-361 [PMID: 24337769 DOI: 10.1007/s10637-013-0057-2]

73 **Kwak EL**, LoRusso P, Hamid O, Janku F, Kitaneh M, Catenacci DV, Chan E, Bekaii-Saab TS, Amore B, Hwang YC, Tang R, Ngarmchamnanrith G, Hong DS. Clinical activity of AMG 337, an oral MET kinase inhibitor, in adult patients with MET-amplified gastroesophageal junction, gastric, or esophageal cancer. *J Clin Oncol* 2015; **33** (suppl 3; abstr 1)

74 Phase 2 study of AMG 337 in MET amplified gastric/esophageal adenocarcinoma or other solid tumors. Accessed Feb 16, 2015. Available from: URL: https://www.clinicaltrials.gov/ct2/show/NCT02016534

75 **Shah MA**, Wainberg ZA, Catenacci DV, Hochster HS, Ford J, Kunz P, Lee FC, Kallender H, Cecchi F, Rabe DC, Keer H, Martin AM, Liu Y, Gagnon R, Bonate P, Liu L, Gilmer T, Bottaro DP. Phase II study evaluating 2 dosing schedules of oral foretinib (GSK1363089), cMET/VEGFR2 inhibitor, in patients with metastatic gastric cancer. *PLoS One* 2013; **8**: e54014 [PMID: 23516391 DOI: 10.1371/journal.pone.0054014]

76 **Catenacci DV**, Henderson L, Xiao SY, Patel P, Yauch RL, Hegde P, Zha J, Pandita A, Peterson A, Salgia R. Durable complete response of metastatic gastric cancer with anti-Met therapy followed by resistance at recurrence. *Cancer Discov* 2011; **1**: 573-579 [PMID: 22389872 DOI: 10.1158/2159-8290.CD-11-0175]

77 A study of onartuzumab (MetMAb) in combination with mFOLFOX6 in patients with metastatic HER2-negative and Met-positive gastroesophageal cancer (MetGastric). [Accessed Accessed Feb 16, 2015] Available from: URL: https://www.clinicaltrials.gov/ct2/show/NCT01662869

78 **Iveson T**, Donehower RC, Davidenko I, Tjulandin S, Deptala A, Harrison M, Nirni S, Lakshmaiah K, Thomas A, Jiang Y, Zhu M, Tang R, Anderson A, Dubey S, Oliner KS, Loh E. Rilotumumab in combination with epirubicin, cisplatin, and capecitabine as first-line treatment for gastric or oesophagogastric junction adenocarcinoma: an open-label, dose de-escalation phase 1b study and a double-blind, randomised phase 2 study. *Lancet Oncol* 2014; **15**: 1007-1018 [PMID: 24965569 DOI: 10.1016/S1470-2045(14)70023-3]

79 First-line treatment for locally advanced or metastatic mesenchymal epithelial transition factor (MET) - positive gastric, lower esophageal, or gastroesophageal junction (GEJ) adenocarcinoma

(RILOMET-1). [Accessed Accessed Feb 16, 2015] Available from: URL: https://www.clinicaltrials.gov/ct2/show/NCT01697072
80 A phase 3 study of rilotumumab (AMG 102) with cisplatin and capecitabine (CX) as first-line therapy in gastric cancer (RILOMET-2). [Accessed Feb 16, 2015] Available from: URL: https://www.clinicaltrials.gov/ct2/manage-recs/fdaaa

P- Reviewer: Alshehabi Z, Figura N, Goral V, Kim JJ, Maurel J
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Jiao XK

World Journal of Gastrointestinal Oncology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4251/wjgo.v7.i11.328

World J Gastrointest Oncol 2015 November 15; 7(11): 328-337
ISSN 1948-5204 (online)

TOPIC HIGHLIGHT

2015 Advances in Gastric Cancer

Polymorphisms in mucin genes in the development of gastric cancer

Rong Wen, Fang Gao, Cheng-Jiang Zhou, Yan-Bin Jia

Rong Wen, Fang Gao, Cheng-Jiang Zhou, Yan-Bin Jia, School of Basic Medicine, Baotou Medical College, Baotou 014060, Inner Mongolia Autonomous Region, China

Yan-Bin Jia, Inner Mongolia Institute of Digestive Diseases, the Second Affiliated Hospital of Baotou Medical College, Baotou 014030, Inner Mongolia Autonomous Region, China

Author contributions: All authors contributed to this work.

Supported by National Natural Science Foundation of China, No. 30960169 and No. 81250024; Natural Science Foundation of Inner Mongolia, No. 2011MS1103; and Inner Mongolian Committee of Science and Technology, China, No. 20110501.

Conflict-of-interest statement: The authors declare that there is no conflict of interest related to this paper.

Correspondence to: Yan-Bin Jia, Professor, School of Basic Medicine, Baotou Medical College, 31 Jianshe Road, Donghe District, Baotou 014060, Inner Mongolia Autonomous Region, China. jyb690318@hotmail.com
Telephone: +86-472-7167832
Fax: +86-472-7167739

Received: April 25, 2015
Peer-review started: April 26, 2015
First decision: June 2, 2015
Revised: July 1, 2015
Accepted: August 30, 2015
Article in press: September 7, 2015
Published online: November 15, 2015

Abstract

Gastric cancer (GC) is the third leading cause of cancer-related death worldwide. In areas of high prevalence, such as Japan, South Korea and China, most cases of GC are related to *Helicobacter pylori* (*H. pylori*), which involves well-characterized sequential stages, including infection, atrophic gastritis, intestinal metaplasia, dysplasia, and GC. Mucins are the most abundant high-molecular-weight glycoproteins in mucus, which is the first line of defense and plays a major role in blocking pathogenic factors. Normal gastric mucosa shows expression of MUC1, MUC5AC and MUC6 that is specific to cell type. However, the specific pattern of MUC1, MUC5AC and MUC6 expression is changed in gastric carcinogenesis, accompanied by *de novo* expression of secreted MUC2. Recent studies have provided evidence that variations in these mucin genes affect many steps of GC development, such as *H. pylori* infection, and gastric precancerous lesions. In this review, we focus on studies of the association between polymorphisms in mucin genes and development of GC. This information should be helpful for the early detection, surveillance, and treatment of GC.

Key words: Gastric cancer; *Helicobacter pylori*; Genetic polymorphism; Mucin; Risk; Association study; Atrophic gastritis

Core tip: *Helicobacter pylori* (*H. pylori*) infection is the single most important risk factor in the development of gastric cancer (GC), however the etiology of GC involves host and other environmental factors. Genetic and biological evidence highlights the important roles of variations in mucin genes in the development and progression of GC. In this review, we summarize studies

of the association between polymorphisms in *MUC1*, *MUC5AC*, *MUC6* and *MUC2* and development of GC, which should be helpful for the early detection, surveillance, and treatment of GC.

Wen R, Gao F, Zhou CJ, Jia YB. Polymorphisms in mucin genes in the development of gastric cancer. *World J Gastrointest Oncol* 2015; 7(11): 328-337 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i11/328.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i11.328

INTRODUCTION

Although gastric cancer (GC) incidence and mortality rates are declining in most countries, it is still the fifth most common cancer and the third leading cause of cancer-related death worldwide[1]. Epidemiological studies have shown that a high intake of salt, tobacco smoking, and *Helicobacter pylori (H. pylori)* infection increase the risk of GC[2-4]. In areas of high prevalence of GC, such as Japan, Korea and China, most cases of GC are related to *H. pylori*. GC is the result of a long complex multifactorial and multistep process that involves well-characterized sequential stages. The initial lesion is inflammatory and is usually caused by *H. pylori* infection, which results in chronic superficial gastritis. The following pathological model of GC progression includes atrophic gastritis, intestinal metaplasia, dysplasia and GC[5,6]. *H. pylori* infection is the most important risk factor for GC and it was classified as a class Ⅰ carcinogen by the World Health Organization in 1994, nevertheless, the etiology of GC also involves host and other environmental factors. This is demonstrated by the fact that only 1%-3% of patients with *H. pylori* infection develop GC[7,8]. The hypothesis that genetic susceptibility or predisposition plays an important etiological role in GC is supported by many case-control studies and genome-wide association studies (GWASs)[9-14].

H. pylori initiates colonization of the gastric mucosa by crossing the gastric mucus layer and adhering to the gastric epithelium[15]. Mucus is the first line of defense and plays a major role in blocking pathogenic factors, and mucins are the major components in mucus and are responsible for its biochemical and biophysical properties[16]. The mucin family comprises 21 members. The mucins are high-molecular-weight glycoproteins characterized by a heavily O-glycosylated tandem repeat region rich in proline, threonine and serine, which is encoded by a variable number of tandem repeats (VNTRs)[17-20]. Mucins are categorized into two subgroups according to their physiological and structural characteristics: membrane-bound, such as MUC1, and secreted, including MUC2, MUC5AC and MUC6[17]. *In situ* hybridization and immunohistochemistry have demonstrated the cell-type-specific expression of mucins in epithelial tissues[21,22]. Normal gastric mucosa shows cell-type-specific expression of MUC1, MUC5AC and MUC6[21-23]. Apical MUC1 is expressed in the gastric mucosa in the superficial and foveolar epithelium and mucous neck zone cells[24]. Secreted mucin MUC5AC is detected in the superficial epithelium, whereas MUC6 is found in the deep glands[25,26]. This specific pattern of MUC1, MUC5AC and MUC6 expression is changed in gastric carcinogenesis, accompanied by *de novo* expression of secreted MUC2[26-30]. Recent genetic and biological evidence highlights the important roles of variations in these mucin genes in the development and progression of GC. In this review, we focus on studies of the association between polymorphisms in *MUC1*, *MUC5AC*, *MUC6* and *MUC2* genes and development of GC (Table 1). Details of the studied single nucleotide polymorphisms (SNPs) in mucin genes are described in Table 2.

POLYMORPHISMS IN *MUC1* IN THE DEVELOPMENT OF GC

MUC1 is a highly polymorphic membrane-associated mucin that is often aberrantly expressed in cancer[31]. *MUC1* gene is located on chromosome 1q21 and contains a highly conserved VNTR of 20 amino acids, varying from 25 to 125 repeats, depending on the allele[32]. In recent decades, some studies were performed to investigate the potential roles of genetic variations in *MUC1* in gastric carcinogenesis, but most of them were focused on the VNTRs, with inconsistent results. Costa *et al*[33] observed that polymorphism in the *MUC1* VNTRs influenced the binding of *H. pylori* to gastric cells. Vinall *et al*[28] reported that small *MUC1* VNTR alleles were correlated with *H. pylori*-associated gastritis in European populations. Two studies from Portugal (which has the higher risk of GC in Europe) showed that small *MUC1* VNTR alleles were significantly associated with gastric carcinoma[34], as well as chronic atrophic gastritis and incomplete intestinal metaplasia, which are two well-established precursor lesions of GC[35]. However, another study from Denmark indicated that small *MUC1* VNTR alleles are more frequent in the Danish population (which has the lower risk of GC in Europe) than in Portugal[36].

GWASs have recently been important in identifying potential genetic variations related to cancer susceptibility. In 2010, Abnet *et al*[37] conducted a GWAS in 1625 patients with GC and 2100 controls. They identified a significant SNP of rs4072037 A/G in the *MUC1* gene for GC. The A allele was correlated with increased susceptibility to GC in Chinese patients during initial scanning, however, this association was not maintained in the second phase, or when the results of the two phases were combined. A GWAS on GC in Japan revealed the top 10 SNPs that were significantly related to the diffuse type of GC, which included two located in chromosome 1q22[38]. Subsequently, Saeki *et al*[39] performed high-density mapping to explore the

Table 1 List of association studies between polymorphisms in mucin genes and development of gastric cancer

Gene	Ref.	Population	Disease	Study design	Sample (case/control)	Polymorphism	Association
MUC1	Vinall *et al*[28]	European	*H. pylori* related gastritis	Case–control study	57 gastritis patients	VNTR	Yes
	Carvalho *et al*[34]	Portuguese	GC	Case–control study	159/324	VNTR	Yes
	Silva *et al*[35]	Portuguese	CAG, IM	Case–control study	174 patients	VNTR	Yes
	Abnet *et al*[37]	Chinese	GC	GWAS	1625/2100	rs4072037	Yes
					Replication: 615/1202		No
					Combined: 2240/3302		No
	Saeki *et al*[39]	Japanese	DGC	Case–control study	606/1264/	rs4072037, rs2070803	Yes
		Japanese			304/1465	rs4072037, rs2070803	Yes
		South Korean			452/372	rs4072037, rs2070803	Yes
	Xu *et al*[40]	Chinese	GC	Case–control study	138/241	rs4072037	Yes
	Jia *et al*[43]	Polish	GC	Case–control study	273/377	rs6427184	Yes
				(tag SNP approach)		rs4971052	Yes
						rs4276913	Yes
						rs4971088	Yes
						rs4971092	Yes
						rs4072037	Yes
	Jia *et al*[43]	Polish	*H. pylori* infection	Case–control study	320/57	rs6427184	No
				(tag SNP approach)		rs4971052	No
						rs4276913	No
						rs4971088	No
						rs4971092	No
						rs4072037	No
	Zhang *et al*[44]	Chinese	GC	Case–control study	1681/1858	rs4072037	Yes
	Palmer *et al*[45]	Caucasian	GC	Case–control study	596/587	rs4072037	Yes
	Li *et al*[46]	Chinese	GC	Case–control study	300/300	rs2070803	Yes
	Zhang *et al*[47]	Chinese	Non-cardia GC	Case–control study	288/281	rs4072037	No
				(tag SNP approach)		rs2990245	No
						rs9628662	No
						rs9426886	No
	Zhang *et al*[47]	Chinese	*H. pylori* infection	Case–control study	122/159	rs4072037	No
				(tag SNP approach)		rs2990245	No
						rs9628662	No
						rs9426886	No
	Frank *et al*[48]	German	CAG	Case–control study	533/1054	rs4072037	No
	Marín *et al*[49]	Spanish	GCPLs	Case–control study	387 patients	rs3814316	No
				(tag SNP approach)		rs9426886	No
						rs1045253	No
	Sun *et al*[50]	Hispanic American	GC	Case–control study	132/125	rs4072037	No
	Duan *et al*[51]	-	GC	Meta-analysis	4220/6384	rs4072037	Yes
	Zheng *et al*[52]	-	GC	Meta-analysis	6580/10324	rs4072037	Yes
	Mocellin *et al*[42]	Asian	DGC	Meta-analysis	7279 subjects	rs2070803	Yes
MUC5AC	Jia *et al*[43]	Polish	GC	Case–control study	273/377	rs1541314	No
				(tag SNP approach)		rs2014486	Yes
						rs2075859	No
						rs2672785	No
						rs2735733	Yes
						rs7118568	No
						rs868903	Yes
						rs4963049	No
	Jia *et al*[43]	Polish	*H. pylori* infection	Case–control study	320/57	rs1541314	No
				(tag SNP approach)		rs2014486	No
						rs2075859	No
						rs2672785	No
						rs2735733	No
						rs7118568	No
						rs868903	No
						rs4963049	No
	Zhou *et al*[61]	Chinese	Non-cardia GC	Case–control study	288/281	rs3793966	No
				(tag SNP approach)		rs7118568	No
						rs868903	No
						rs3793964	Yes
						rs3750919	No
						rs5743942	No
						rs4963062	No
						rs885454	Yes
						rs6578810	No
						rs11040869	Yes
						rs7118481	No
						rs7105198	No

	Zhou *et al*[62]	Chinese	*H. pylori* infection	Case–control study (tag SNP approach)	122/159	rs3793966	No
						rs7118568	No
						rs868903	No
						rs3793964	No
						rs3750919	No
						rs5743942	No
						rs4963062	No
						rs885454	No
						rs6578810	No
						rs11040869	No
						rs7118481	No
						rs7105198	No
	Wang *et al*[63]	Chinese	GC	Case–control study	230/328	VNTR	Yes
MUC6	Nguyen *et al*[68]	-	*H. pylori* infection	Case–control study	92/68	VNTR	Yes
	Garcia *et al*[69]	Portuguese	GC	Case–control study	157/376	VNTR	Yes
	Kwon *et al*[70]	South Korean	GC	Case–control study	470/1103	VNTR	Yes
	Jia *et al*[43]	Polish	GC	Case–control study (tag SNP approach)	273/377	rs1128413	No
						rs4077293	No
						rs7483870	No
						rs7943115	No
						rs11602663	No
						rs11605303	No
						rs10902076	No
						rs2071174	No
						rs11245936	No
						rs10794359	No
						rs7112267	No
						rs12574439	No
						rs7119740	No
						rs11601642	No
	Jia *et al*[43]	Polish	*H. pylori* infection	Case–control study (tag SNP approach)	320/57	rs1128413	No
						rs4077293	No
						rs7483870	No
						rs7943115	No
						rs11602663	No
						rs11605303	No
						rs10902076	No
						rs2071174	No
						rs11245936	No
						rs10794359	No
						rs7112267	No
						rs12574439	No
						rs7119740	No
						rs11601642	No
	Marín *et al*[49]	Spanish	GCPLs	Case–control study (tag SNP approach)	387 patients	rs4076950	No
						rs7481521	No
						rs11246384	No
						rs6597947	No
						rs9794921	No
	Frank *et al*[48]	German	CAG	Case–control study	533/1054	rs7481521	No
MUC2	Jeong *et al*[72]	South Korean	GC	Case–control study	455/457	VNTR	Yes
	Marín *et al*[49]	Spanish	GCPLs	Case–control study (tag SNP approach)	387 patients	rs10902073	Yes
						rs10794281	Yes
						rs2856082	No
						rs2071174	Yes
						rs7396030	No
						rs11245936	No
						rs7944723	Yes
						rs6421972	No
						rs10794293	Yes
						rs11245954	No
						rs7480563	No
						rs7126405	No
						rs3924453	Yes
						rs4077759	Yes
	Frank *et al*[48]	German	CAG	Case–control study	533/1054	rs2856111	No
						rs11825977	No

CAG: Chronic atrophic gastritis; DGC: Diffuse gastric cancer; GCPLs: Gastric cancer precursor lesions; *H. pylori*: *Helicobacter pylori*; IM: Intestinal metaplasia; SNP: Single nucleotide polymorphism; GC: Gastric cancer.

Table 2 Description of the studied single nucleotide polymorphisms in mucin genes

Gene	Chromosome	SNPs	Wild alleles	Mutated alleles	Contig position[1]	Location[2]
MUC1	1q21	rs4072037	A	G	12007689	T22T
		rs2070803	C	T	12000652	3′ flanking region
		rs6427184	A	G	11965720	3′ flanking region
		rs4971052	C	T	11968955	3′ flanking region
		rs4276913	A	G	11974610	3′ flanking region
		rs4971088	T	A	11985820	3′ flanking region
		rs4971092	T	C	11986883	3′ flanking region
		rs2990245	T	C	12043084	5′ flanking region
		rs9628662	T	G	12051963	5′ flanking region
		rs9426886	T	A	11994691	3′ flanking region
		rs3814316	C	T	11992655	3′ flanking region
		rs1045253	T	C	12046857	5′ flanking region
MUC5AC	11p15.5	rs1541314	G	A	1182293	3′ flanking region
		rs2014486	A	G	1177573	3′ flanking region
		rs2075859	C	T	1169258	3′ flanking region
		rs2672785	C	T	1165711	3′ flanking region
		rs2735733	C	T	1180410	3′ flanking region
		rs7118568	C	G	1162850	3′ flanking region
		rs868903	T	C	1161460	3′ flanking region
		rs4963049	A	G	1155197	3′ flanking region
		rs3793966	C	T	1221718	3′ flanking region
		rs3793964	C	T	1220752	3′ flanking region
		rs3750919	G	A	1211601	3′ flanking region
		rs5743942	C	T	1232798	3′ flanking region
		rs4963062	G	A	1245411	3′ flanking region
		rs885454	C	T	1162161	3′ flanking region
		rs6578810	T	G	1209349	3′ flanking region
		rs11040869	G	A	1203382	3′ flanking region
		rs7118481	G	C	1267108	3′ flanking region
		rs7105198	G	C	1086133	5′ flanking region
MUC6	11p15.5	rs1128413	C	T	950694	3′ flanking region
		rs4077293	C	T	936522	3′ flanking region
		rs7483870	C	T	916019	3′ flanking region
		rs7943115	G	A	913885	3′ flanking region
		rs11602663	C	T	960778	Intronic
		rs11605303	G	A	978110	5′ flanking region
		rs10902076	G	C	1006044	5′ flanking region
		rs2071174	C	T	1013712	5′ flanking region
		rs11245936	G	A	1026266	5′ flanking region
		rs10794359	C	T	991715	5′ flanking region
		rs7112267	C	T	996981	5′ flanking region
		rs12574439	G	C	997948	5′ flanking region
		rs7119740	C	G	1000419	5′ flanking region
		rs11601642	C	A	1002509	5′ flanking region
		rs4076950	C	T	955021	Intronic
		rs7481521	G	A	967811	V619M
		rs11246384	C	T	970448	Intronic
		rs6597947	G	T	977029	5′ flanking region
		rs9794921	G	T	979867	5′ flanking region
MUC2	11p15.5	rs10902073	C	A	1000934	5′ flanking region
		rs10794281	C	T	1003149	5′ flanking region
		rs2856082	C	G	1011562	5′ flanking region
		rs2071174	C	T	1013712	5′ flanking region
		rs7396030	C	T	1025368	Intronic
		rs11245936	G	A	1026366	G832S
		rs7944723	C	G	1039802	P1832P
		rs6421972	G	A	1042586	I2154T
		rs10794293	C	T	1045031	Intron
		rs11245954	A	G	1047170	V2459V
		rs7480563	G	A	1047741	T2524P
		rs7126405	G	A	1049388	Q2653P
		rs3924453	G	A	1051898	3′ flanking region
		rs4077759	C	T	1052068	3′ flanking region
		rs2856111	T	C	1015747	L58P
		rs11825977	A	G	1015920	V116M

[1]Based on contig NT_004487.20 for *MUC1* gene, and contig NT_009237.19 for *MUC5AC*, *MUC6* and *MUC2* genes; [2]SNP location relative to each gene in the region. SNPs: Single nucleotide polymorphisms.

susceptibility locus of GC at chromosome 1q22 and reported that two SNPs of rs2070803 and rs4072037 were significantly related to susceptibility to diffuse GC in Japan, and the results were validated in other Japanese and Korean studies. SNP rs4072037 is located in exon 2 of the *MUC1* gene and controls alternative splicing at the boundary between exons 1 and 2[39-41]. This SNP affects promoter activity and disrupts the physiological function of MUC1[41,42]. The rs4072037 G allele is correlated with higher VNTRs and the A allele with lower VNTRs[41]. However, the VNTRs are unlikely to be the causal polymorphism for GC susceptibility because the TRs are not translated in normal or malignant gastric epithelial cells[39]. This suggests that the VNTRs are a tagging polymorphism for other genetic variations, such as rs4072037, related to risk of gastric carcinogenesis. It is particularly interesting that rs4072037 A is a major allele in Chinese, Japanese and Korean populations, which have a high incidence of GC, but a minor allele in Caucasians, who have a low incidence of GC. SNP rs2070803 G/A is downstream of the *MUC1* and *TRIM46* genes and its functional effects are unknown. *MUC1* is located downstream of the *TRIM46* gene. These two genes are part of a cluster, which also includes *KRTCAP2*, *THBS3*, *MTX1*, *PKLR* and *HCN3*, located in a region of strong linkage disequilibrium (LD) and are transcribed in opposite directions[42]. *TRIM46* is not expressed in gastric mucosa[39], therefore, SNP rs2070803 might also be a tag for variants in other genes located in this LD region, such as *MUC1*, which are involved in gastric carcinogenesis.

In addition to GWASs, the association of *MUC1* SNPs with GC has been investigated in many case-control studies using a candidate gene approach. An association study in China showed that patients with rs4072037 AA genotype had a significantly increased risk of GC[40]. Jia *et al*[43] conducted a population-based, case-control study in the Polish population. Each of the tested tag SNPs (including rs6427184, rs4971052, rs4276913, rs4971088, rs4971092 and rs4072037) across the *MUC1* region had significant associations with increased risk of GC. This association remained significant after adjusting for multiple tests, which also demonstrated that rs4072037 AA genotype was related to increased risk of GC. However, the study showed that *MUC1* tag SNPs were not associated with *H. pylori* infection, suggesting that the effects of *MUC1* polymorphisms on risk of GC are not mediated by *H. pylori* infection. The association between rs4072037 A allele and increased GC risk was further replicated in Chinese and Caucasian populations[44,45]. Another study demonstrated that rs2070803 GA/AA genotypes were protective against GC, with > 50% risk reduction in Chinese individuals[46]. However, other studies have shown conflicting results. A case-control study conducted by our group showed that four tag SNPs (including rs4072037) in *MUC1* were not associated with the risk of non-cardia GC, or *H. pylori* infection in the Han population in Northwest China[47]. Another study showed no association between rs4072037 and risk of chronic atrophic gastritis, a well-defined precursor of GC in the German population[48]. Marín *et al*[49] reported that three tag SNPs (rs3814316, rs9426886 and rs1045253) in *MUC1* were not associated with precursor lesions of GC in a high-risk area of Spain. Another study demonstrated that rs4072037 was not associated with GC risk in Hispanic Americans[50]. To clarify the current limited and conflicting evidence, and to establish the true impact of *MUC1* variations on gastric carcinogenesis, several meta-analyses have been performed. Duan *et al*[51] conducted an analysis of 10 case-control studies comprising 4220 cases and 6384 controls. They found that rs4072037 G allele was associated with a decreased risk of GC progression, especially in Asians. This result is consistent with the study of Zheng *et al*[52] of 6580 cases and 10324 controls, which suggested the involvement of *MUC1* rs4072037 polymorphism in gastric carcinogenesis among Asian individuals. A further meta-analysis showed that the rare rs2070803 A allele was associated with reduced risk of diffuse-type GC[42]. All the evidence suggests that *MUC1* polymorphisms, such as rs4072037, are promising biological markers for predicting GC risk, especially in Asian populations.

POLYMORPHISMS IN *MUC5AC* IN THE DEVELOPMENT OF GC

MUC5AC is a major secreted mucin in healthy gastric mucosa and is the major receptor for *H. pylori* in the human stomach. BabA and SabA adhesins on *H. pylori* bind to Lewis B blood group antigens on MUC5AC, facilitating colonization[53-55]. In chronic *H. pylori* infection, normally expressed MUC5AC and MUC5AC-producing cells may gradually decrease[56,57]. *MUC5AC* is located on chromosome 11p15.5[58], which often has loss of heterozygosity in patients with GC[59,60]. Studies on the association between *MUC5AC* polymorphisms and GC development are limited at present. Jia *et al*[43] investigated the relationship between eight tag SNPs of *MUC5AC* and GC in a Polish study. The three tag SNPs rs868903, rs2014486 and rs2735733 in the 3' flanking region of *MUC5AC* were related to the risk of GC. Their minor allele homozygotes were significantly associated with increased risk of GC. However, none of the eight tested tag SNPs were associated with risk of *H. pylori* infection. Our group also performed a case-control study to evaluate the association of 12 tag SNPs of *MUC5AC* with risk of non-cardia GC in the Han population in Northwest China. We observed that three tag SNPs, rs3793964, rs11040869 and rs885454, were significantly associated with the risk of non-cardia GC. The minor allele homozygotes of rs3793964 and rs11040869, as well as the heterozygote of rs885454 had a protective effect on risk of non-cardia GC[61]. These three tag SNPs are all located in the 3' flanking region of *MUC5AC*. The discrepancies between the

two studies may have been due to racial differences in variant frequencies. However, few biological studies on genetic variations in *MUC5AC* have been reported. Similarly, our results also suggested that polymorphisms of *MUC5AC* gene were not associated with the risk of *H. pylori* infection, suggesting *MUC5AC* polymorphisms are involved in other processes besides bacterial binding in developing GC[62]. Wang *et al*[63] conducted a case-control study in the Chinese population, which reported that some variations in an upstream repetitive region of *MUC5AC* were associated with GC susceptibility and progression. Their findings highlight the importance of *MUC5AC* polymorphisms in risk of GC.

POLYMORPHISMS IN *MUC6* IN THE DEVELOPMENT OF GC

The secreted mucin, MUC6, is highly expressed in normal gastric mucosa. One study has shown that MUC6 has antimicrobial properties against *H. pylori*. Unique glycan residues on MUC6 inhibit biosynthesis of major cell wall component cholesteryl-α-D-glucopyranoside[64]. MUC6 is aberrantly expressed in response to *H. pylori* infection[65], and MUC6 expression is lower in GC compared with normal mucus[66]. *MUC6* is also located on chromosome 11p15.5, which is a region rich in recombination[59]. *MUC1* and *MUC6* have a large number of VNTRs[67]. Several studies have focused on the relationship between VNTR polymorphisms of *MUC6* and GC development. In one of these, small VNTR alleles of *MUC6* gene were associated with increased risk of *H. pylori* infection[68]. Others showed that small *MUC6* VNTR alleles were more frequent in patients with GC than in healthy blood donors[69], and short rare *MUC6* minisatellite 5 alleles had an effect on susceptibility to GC by regulating gene expression[70]. However, Jia *et al*[43] investigated the relationship between *MUC6* polymorphisms and GC, using a tag SNP approach. Fourteen of the tag SNPs tested across the *MUC6* region were not associated with risk of GC or *H. pylori* infection. The authors inferred that VNTR polymorphisms had many alleles, which might have divided the study population into several classes, thus making statistical analysis difficult. Similarly, Marín *et al*[49] observed that five tag SNPs in *MUC6* were not associated with GC precursor lesions. Furthermore, Frank *et al*[48] investigated the association between polymorphism in *MUC6* and the risk of chronic atrophic gastritis, using a candidate SNP approach. However, there was no association between the putative functional SNP rs7481521 (*MUC6* V619M) and chronic atrophic gastritis. Further studies are needed to elucidate the roles of *MUC6* polymorphisms in the gastric carcinogenesis pathway.

POLYMORPHISMS IN *MUC2* IN THE DEVELOPMENT OF GC

Normal gastric mucosa shows little or no expression of MUC2. However, in intestinal metaplasia and GC, the level of MUC2 is increased[27,29,30]. MUC2 might be activated by proinflammatory cytokines expressed after *H. pylori* infection, leading to its overexpression[71]. *MUC2* gene is clustered on chromosome 11p15.5 with *MUC5AC*, *MUC5B* and *MUC6*[58]. Only three studies have evaluated the relationship between *MUC2* polymorphisms and development of GC. Jeong *et al*[72] reported that the short rare minisatellite 6 alleles of *MUC2* gene are associated with GC. Marín *et al*[49] have investigated the association of 14 tag SNPs in *MUC2* with evolution of GC precursor lesions in 387 patients with 12.8 years follow-up. According to the diagnosis at recruitment and after follow-up, the patients were divided into three groups, that is, those with no change in lesions, progression of lesions, and regression of lesions. The results indicated that three SNPs (rs10794293, rs3924453 and rs4077759) at the 3′ moiety in *MUC2* were associated with a decreased risk of lesion progression. In contrast, another four SNPs (rs10902073, rs10794281, rs2071174 and rs7944723) at the 5′ moiety were significantly associated with lesion regression. The association of SNPs with GC precursor lesions was stronger in patients with *H. pylori* infection. However, it was also shown that functional SNP rs11825977 (V116M) in *MUC2*, which might influence *MUC2* mRNA expression[73], as well as the potentially functional SNP rs2856111 (L58P), were not associated with the risk of chronic atrophic gastritis[48].

CONCLUSION

GC is the third leading cause of cancer mortality and a serious global problem. Many studies have tried to identify the factors responsible for GC, but the exact sequence of molecular events involved in the development of GC remains unclear. In areas of high GC prevalence, most cases are related to *H. pylori* infection, and GC develops through several stages, including infection, gastric atrophy, intestinal metaplasia and dysplasia. There is a lot of evidence to support the key role of mucins in development of GC. This review focused on studies of the association between polymorphisms in mucin genes and development of GC. The strength of such an association varied among the studies. The diversity in study populations and lifestyle, as well as sample size may account for this inconsistency. For example, functional SNP rs4072037 in *MUC1* gene may affect the development of GC, but the effects seem to be stronger in Asian populations. Future association studies need global collaboration to expand sample size and identify more susceptibility polymorphisms. However, lifestyle factors should be taken into account to ensure accurate and significant results. Such studies will identify useful biomarkers for early detection of GC, with the potential for better disease prevention through selective treatment and surveillance of individuals harboring high-risk genetic profiles.

REFERENCES

1 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]

2 **Lee SA**, Kang D, Shim KN, Choe JW, Hong WS, Choi H. Effect of diet and Helicobacter pylori infection to the risk of early gastric cancer. *J Epidemiol* 2003; **13**: 162-168 [PMID: 12749604]

3 **Shikata K**, Doi Y, Yonemoto K, Arima H, Ninomiya T, Kubo M, Tanizaki Y, Matsumoto T, Iida M, Kiyohara Y. Population-based prospective study of the combined influence of cigarette smoking and Helicobacter pylori infection on gastric cancer incidence: the Hisayama Study. *Am J Epidemiol* 2008; **168**: 1409-1415 [PMID: 18945691 DOI: 10.1093/aje/kwn276]

4 **Helicobacter and Cancer Collaborative Group**. Gastric cancer and Helicobacter pylori: a combined analysis of 12 case control studies nested within prospective cohorts. *Gut* 2001; **49**: 347-353 [PMID: 11511555]

5 **Correa P**. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992; **52**: 6735-6740 [PMID: 1458460]

6 **Yuasa Y**. Control of gut differentiation and intestinal-type gastric carcinogenesis. *Nat Rev Cancer* 2003; **3**: 592-600 [PMID: 12894247]

7 **Wang F**, Meng W, Wang B, Qiao L. Helicobacter pylori-induced gastric inflammation and gastric cancer. *Cancer Lett* 2014; **345**: 196-202 [PMID: 23981572 DOI: 10.1016/j.canlet.2013.08.016]

8 **Wroblewski LE**, Peek RM, Wilson KT. Helicobacter pylori and gastric cancer: factors that modulate disease risk. *Clin Microbiol Rev* 2010; **23**: 713-739 [PMID: 20930071 DOI: 10.1128/CMR.00011-10]

9 **Xie Y**, Wang Y, Zhao Y, Guo Z. Single-nucleotide polymorphisms of microRNA processing machinery genes are associated with risk for gastric cancer. *Onco Targets Ther* 2015; **8**: 567-571 [PMID: 25784816 DOI: 10.2147/OTT.S79150]

10 **Ismaili A**, Yari K, Moradi MT, Sohrabi M, Kahrizi D, Kazemi E, Souri Z. IL-1B (C+3954T) gene polymorphism and susceptibility to gastric cancer in the Iranian population. *Asian Pac J Cancer Prev* 2015; **16**: 841-844 [PMID: 25684535 DOI: 10.7314/APJCP.2015.16.2.841]

11 **Shao A**, Zheng L, Chen S, Gu H, Jing H. p21, p53, TP53BP1 and p73 polymorphisms and the risk of gastric cardia adenocarcinoma in a Chinese population. *Biomarkers* 2015; **20**: 109-115 [PMID: 25532599]

12 **Ferrer-Ferrer M**, Malespín-Bendaña W, Ramírez V, González MI, Carvajal A, Une C. Polymorphisms in genes coding for HSP-70 are associated with gastric cancer and duodenal ulcer in a population at high risk of gastric cancer in Costa Rica. *Arch Med Res* 2013; **44**: 467-474 [PMID: 24051039 DOI: 10.1016/j.arcmed.2013.08.008]

13 **Shi Y**, Hu Z, Wu C, Dai J, Li H, Dong J, Wang M, Miao X, Zhou Y, Lu F, Zhang H, Hu L, Jiang Y, Li Z, Chu M, Ma H, Chen J, Jin G, Tan W, Wu T, Zhang Z, Lin D, Shen H. A genome-wide association study identifies new susceptibility loci for non-cardia gastric cancer at 3q13.31 and 5p13.1. *Nat Genet* 2011; **43**: 1215-1218 [PMID: 22037551 DOI: 10.1038/ng.978]

14 **Sala N**, Muñoz X, Travier N, Agudo A, Duell EJ, Moreno V, Overvad K, Tjonneland A, Boutron-Ruault MC, Clavel-Chapelon F, Canzian F, Kaaks R, Boeing H, Meidtner K, Trichopoulos A, Tsiotas K, Zylis D, Vineis P, Panico S, Palli D, Krogh V, Tumino R, Lund E, Bueno-de-Mesquita HB, Numans ME, Peeters PH, Quirós JR, Sánchez MJ, Navarro C, Ardanaz E, Dorronsoro M, Hallmans G, Stenling R, Manjer J, Allen NE, Travis RC, Khaw KT, Jenab M, Offerhaus GJ, Riboli E, González CA. Prostate stem-cell antigen gene is associated with diffuse and intestinal gastric cancer in Caucasians: results from the EPIC-EURGAST study. *Int J Cancer* 2012; **130**: 2417-2427 [PMID: 21681742 DOI: 10.1002/ijc.26243]

15 **Ruggiero P**. Helicobacter pylori and inflammation. *Curr Pharm Des* 2010; **16**: 4225-4236 [PMID: 21184659 DOI: 10.2174/138161210794519075]

16 **Rachagani S**, Torres MP, Moniaux N, Batra SK. Current status of mucins in the diagnosis and therapy of cancer. *Biofactors* 2009; **35**: 509-527 [PMID: 19904814 DOI: 10.1002/biof.64]

17 **Hollingsworth MA**, Swanson BJ. Mucins in cancer: protection and control of the cell surface. *Nat Rev Cancer* 2004; **4**: 45-60 [PMID: 14681689 DOI: 10.1038/nrc1251]

18 **Moniaux N**, Escande F, Porchet N, Aubert JP, Batra SK. Structural organization and classification of the human mucin genes. *Front Biosci* 2001; **6**: D1192-D1206 [PMID: 11578969]

19 **Hanisch FG**. O-glycosylation of the mucin type. *Biol Chem* 2001; **382**: 143-149 [PMID: 11308013 DOI: 10.1515/BC.2001.022]

20 **Carraway KL**, Hull SR. O-glycosylation pathway for mucin-type glycoproteins. *Bioessays* 1989; **10**: 117-121 [PMID: 2658987 DOI: 10.1002/bies.950100406]

21 **Audie JP**, Janin A, Porchet N, Copin MC, Gosselin B, Aubert JP. Expression of human mucin genes in respiratory, digestive, and reproductive tracts ascertained by in situ hybridization. *J Histochem Cytochem* 1993; **41**: 1479-1485 [PMID: 8245407 DOI: 10.1177/41.10.8245407]

22 **Gendler SJ**, Spicer AP. Epithelial mucin genes. *Annu Rev Physiol* 1995; **57**: 607-634 [PMID: 7778880 DOI: 10.1146/annurev.ph.57.030195.003135]

23 **Perez-Vilar J**, Hill RL. The structure and assembly of secreted mucins. *J Biol Chem* 1999; **274**: 31751-31754 [PMID: 10542193 DOI: 10.1074/jbc.274.45.31751]

24 **Jass JR**. Mucin core proteins as differentiation markers in the gastrointestinal tract. *Histopathology* 2000; **37**: 561-564 [PMID: 11122439]

25 **De Bolós C**, Garrido M, Real FX. MUC6 apomucin shows a distinct normal tissue distribution that correlates with Lewis antigen expression in the human stomach. *Gastroenterology* 1995; **109**: 723-734 [PMID: 7657100]

26 **Reis CA**, David L, Nielsen PA, Clausen H, Mirgorodskaya K, Roepstorff P, Sobrinho-Simões M. Immunohistochemical study of MUC5AC expression in human gastric carcinomas using a novel monoclonal antibody. *Int J Cancer* 1997; **74**: 112-121 [PMID: 9036879]

27 **Babu SD**, Jayanthi V, Devaraj N, Reis CA, Devaraj H. Expression profile of mucins (MUC2, MUC5AC and MUC6) in Helicobacter pylori infected pre-neoplastic and neoplastic human gastric epithelium. *Mol Cancer* 2006; **5**: 10 [PMID: 16545139 DOI: 10.1186/1476-4598-5-10]

28 **Vinall LE**, King M, Novelli M, Green CA, Daniels G, Hilkens J, Sarner M, Swallow DM. Altered expression and allelic association of the hypervariable membrane mucin MUC1 in Helicobacter pylori gastritis. *Gastroenterology* 2002; **123**: 41-49 [PMID: 12105832 DOI: 10.1053/gast.2002.34157]

29 **Reis CA**, David L, Carvalho F, Mandel U, de Bolós C, Mirgorodskaya E, Clausen H, Sobrinho-Simões M. Immunohistochemical study of the expression of MUC6 mucin and co-expression of other secreted mucins (MUC5AC and MUC2) in human gastric carcinomas. *J Histochem Cytochem* 2000; **48**: 377-388 [PMID: 10681391]

30 **Ho SB**, Shekels LL, Toribara NW, Kim YS, Lyftogt C, Cherwitz DL, Niehans GA. Mucin gene expression in normal, preneoplastic, and neoplastic human gastric epithelium. *Cancer Res* 1995; **55**: 2681-2690 [PMID: 7780985]

31 **Nath S**, Mukherjee P. MUC1: a multifaceted oncoprotein with a key role in cancer progression. *Trends Mol Med* 2014; **20**: 332-342 [PMID: 24667139 DOI: 10.1016/j.molmed.2014.02.007]

32 **Fowler J**, Vinall L, Swallow D. Polymorphism of the human muc genes. *Front Biosci* 2001; **6**: D1207-D1215 [PMID: 11578959 DOI: 10.2741/Fowler]

33 **Costa NR**, Mendes N, Marcos NT, Reis CA, Caffrey T, Hollingsworth MA, Santos-Silva F. Relevance of MUC1 mucin variable number of tandem repeats polymorphism in H pylori adhesion to gastric epithelial cells. *World J Gastroenterol* 2008; **14**: 1411-1414 [PMID: 18322957 DOI: 10.3748/wjg.14.1411]

34 **Carvalho F**, Seruca R, David L, Amorim A, Seixas M, Bennett E,

Clausen H, Sobrinho-Simões M. MUC1 gene polymorphism and gastric cancer--an epidemiological study. *Glycoconj J* 1997; **14**: 107-111 [PMID: 9076520]

35 **Silva F**, Carvalho F, Peixoto A, Seixas M, Almeida R, Carneiro F, Mesquita P, Figueiredo C, Nogueira C, Swallow DM, Amorim A, David L. MUC1 gene polymorphism in the gastric carcinogenesis pathway. *Eur J Hum Genet* 2001; **9**: 548-552 [PMID: 11464247 DOI: 10.1038/sj.ejhg.5200677]

36 **Carvalho F**, Peixoto A, Steffensen R, Amorim A, David L, Sobrinho-Simões M. MUC1 gene polymorphism does not explain the different incidence of gastric cancer in Portugal and Denmark. *Ann Hum Genet* 1999; **63**: 187-191 [PMID: 10738530 DOI: 10.1046/j.1469-1809.1999.6330187.x]

37 **Abnet CC**, Freedman ND, Hu N, Wang Z, Yu K, Shu XO, Yuan JM, Zheng W, Dawsey SM, Dong LM, Lee MP, Ding T, Qiao YL, Gao YT, Koh WP, Xiang YB, Tang ZZ, Fan JH, Wang C, Wheeler W, Gail MH, Yeager M, Yuenger J, Hutchinson A, Jacobs KB, Giffen CA, Burdett L, Fraumeni JF, Tucker MA, Chow WH, Goldstein AM, Chanock SJ, Taylor PR. A shared susceptibility locus in PLCE1 at 10q23 for gastric adenocarcinoma and esophageal squamous cell carcinoma. *Nat Genet* 2010; **42**: 764-767 [PMID: 20729852 DOI: 10.1038/ng.649]

38 **Study Group of Millennium Genome Project for Cancer**, Sakamoto H, Yoshimura K, Saeki N, Katai H, Shimoda T, Matsuno Y, Saito D, Sugimura H, Tanioka F, Kato S, Matsukura N, Matsuda N, Nakamura T, Hyodo I, Nishina T, Yasui W, Hirose H, Hayashi M, Toshiro E, Ohnami S, Sekine A, Sato Y, Totsuka H, Ando M, Takemura R, Takahashi Y, Ohdaira M, Aoki K, Honmyo I, Chiku S, Aoyagi K, Sasaki H, Ohnami S, Yanagihara K, Yoon KA, Kook MC, Lee YS, Park SR, Kim CG, Choi IJ, Yoshida T, Nakamura Y, Hirohashi S. Genetic variation in PSCA is associated with susceptibility to diffuse-type gastric cancer. *Nat Genet* 2008; **40**: 730-740 [PMID: 18488030 DOI: 10.1038/ng.152]

39 **Saeki N**, Saito A, Choi IJ, Matsuo K, Ohnami S, Totsuka H, Chiku S, Kuchiba A, Lee YS, Yoon KA, Kook MC, Park SR, Kim YW, Tanaka H, Tajima K, Hirose H, Tanioka F, Matsuno Y, Sugimura H, Kato S, Nakamura T, Nishina T, Yasui W, Aoyagi K, Sasaki H, Yanagihara K, Katai H, Shimoda T, Yoshida T, Nakamura Y, Hirohashi S, Sakamoto H. A functional single nucleotide polymorphism in mucin 1, at chromosome 1q22, determines susceptibility to diffuse-type gastric cancer. *Gastroenterology* 2011; **140**: 892-902 [PMID: 21070779 DOI: 10.1053/j.gastro.2010.10.058]

40 **Xu Q**, Yuan Y, Sun LP, Gong YH, Xu Y, Yu XW, Dong NN, Lin GD, Smith PN, Li RW. Risk of gastric cancer is associated with the MUC1 568 A/G polymorphism. *Int J Oncol* 2009; **35**: 1313-1320 [PMID: 19885554]

41 **Ng W**, Loh AX, Teixeira AS, Pereira SP, Swallow DM. Genetic regulation of MUC1 alternative splicing in human tissues. *Br J Cancer* 2008; **99**: 978-985 [PMID: 19238635 DOI: 10.1038/sj.bjc.6604617]

42 **Mocellin S**, Verdi D, Pooley KA, Nitti D. Genetic variation and gastric cancer risk: a field synopsis and meta-analysis. *Gut* 2015; **64**: 1209-1219 [PMID: 25731870 DOI: 10.1136/gutjnl-2015-309168]

43 **Jia Y**, Persson C, Hou L, Zheng Z, Yeager M, Lissowska J, Chanock SJ, Chow WH, Ye W. A comprehensive analysis of common genetic variation in MUC1, MUC5AC, MUC6 genes and risk of stomach cancer. *Cancer Causes Control* 2010; **21**: 313-321 [PMID: 19924550 DOI: 10.1007/s10552-009-9463-3]

44 **Zhang H**, Jin G, Li H, Ren C, Ding Y, Zhang Q, Deng B, Wang J, Hu Z, Xu Y, Shen H. Genetic variants at 1q22 and 10q23 reproducibly associated with gastric cancer susceptibility in a Chinese population. *Carcinogenesis* 2011; **32**: 848-852 [PMID: 21427165 DOI: 10.1093/carcin/bgr051]

45 **Palmer AJ**, Lochhead P, Hold GL, Rabkin CS, Chow WH, Lissowska J, Vaughan TL, Berry S, Gammon M, Risch H, El-Omar EM. Genetic variation in C20orf54, PLCE1 and MUC1 and the risk of upper gastrointestinal cancers in Caucasian populations. *Eur J Cancer Prev* 2012; **21**: 541-544 [PMID: 22805490 DOI: 10.1097/CEJ.0b013e3283529b79]

46 **Li F**, Zhong MZ, Li JH, Liu W, Li B. Case-control study of single nucleotide polymorphisms of PSCA and MUC1 genes with gastric cancer in a Chinese. *Asian Pac J Cancer Prev* 2012; **13**: 2593-2596 [PMID: 22938426]

47 **Zhang B**, Hao GY, Gao F, Zhang JZ, Zhou CJ, Zhou LS, Wang Y, Jia YB. Lack of association of common polymorphisms in MUC1 gene with H. pylori infection and non-cardia gastric cancer risk in a Chinese population. *Asian Pac J Cancer Prev* 2013; **14**: 7355-7358 [PMID: 24460302 DOI: 10.7314/APJCP.2013.14.12.7355]

48 **Frank B**, Weck MN, Müller H, Klopp N, Illig T, Raum E, Brenner H. Polymorphisms in MUC1, MUC2, MUC5B and MUC6 genes are not associated with the risk of chronic atrophic gastritis. *Eur J Cancer* 2012; **48**: 114-120 [PMID: 21596555 DOI: 10.1016/j.ejca.2011.04.016]

49 **Marín F**, Bonet C, Muñoz X, García N, Pardo ML, Ruiz-Liso JM, Alonso P, Capellà G, Sanz-Anquela JM, González CA, Sala N. Genetic variation in MUC1, MUC2 and MUC6 genes and evolution of gastric cancer precursor lesions in a long-term follow-up in a high-risk area in Spain. *Carcinogenesis* 2012; **33**: 1072-1080 [PMID: 22402132 DOI: 10.1093/carcin/bgs119]

50 **Sun Y**, Gu J, Ajani JA, Chang DW, Wu X, Stroehlein JR. Genetic and intermediate phenotypic susceptibility markers of gastric cancer in Hispanic Americans: a case-control study. *Cancer* 2014; **120**: 3040-3048 [PMID: 24962126 DOI: 10.1002/cncr.28792]

51 **Duan F**, Song C, Dai L, Cui S, Zhang X, Zhao X. The effect of MUC1 rs4072037 functional polymorphism on cancer susceptibility: evidence from published studies. *PLoS One* 2014; **9**: e95651 [PMID: 24755768 DOI: 10.1371/journal.pone.0095651]

52 **Zheng L**, Zhu C, Gu J, Xi P, Du J, Jin G. Functional polymorphism rs4072037 in MUC1 gene contributes to the susceptibility to gastric cancer: evidence from pooled 6,580 cases and 10,324 controls. *Mol Biol Rep* 2013; **40**: 5791-5796 [PMID: 24072653 DOI: 10.1007/s11033-013-2682-4]

53 **Van de Bovenkamp JH**, Mahdavi J, Korteland-Van Male AM, Büller HA, Einerhand AW, Borén T, Dekker J. The MUC5AC glycoprotein is the primary receptor for Helicobacter pylori in the human stomach. *Helicobacter* 2003; **8**: 521-532 [PMID: 14535999 DOI: 10.1046/j.1523-5378.2003.00173.x]

54 **Kocer B**, Ulas M, Ustundag Y, Erdogan S, Karabeyoglu M, Yldrm O, Unal B, Cengiz O, Soran A. A confirmatory report for the close interaction of Helicobacter pylori with gastric epithelial MUC5AC expression. *J Clin Gastroenterol* 2004; **38**: 496-502 [PMID: 15220684]

55 **Kobayashi M**, Lee H, Nakayama J, Fukuda M. Roles of gastric mucin-type O-glycans in the pathogenesis of Helicobacter pylori infection. *Glycobiology* 2009; **19**: 453-461 [PMID: 19150806 DOI: 10.1093/glycob/cwp004]

56 **Van De Bovenkamp JH**, Korteland-Van Male AM, Büller HA, Einerhand AW, Dekker J. Infection with Helicobacter pylori affects all major secretory cell populations in the human antrum. *Dig Dis Sci* 2005; **50**: 1078-1086 [PMID: 15986858]

57 **de Bolos C**, Real FX, Lopez-Ferrer A. Regulation of mucin and glycoconjugate expression: from normal epithelium to gastric tumors. *Front Biosci* 2001; **6**: D1256-D1263 [PMID: 11578953]

58 **Pigny P**, Guyonnet-Duperat V, Hill AS, Pratt WS, Galiegue-Zouitina S, d'Hooge MC, Laine A, Van-Seuningen I, Degand P, Gum JR, Kim YS, Swallow DM, Aubert JP, Porchet N. Human mucin genes assigned to 11p15.5: identification and organization of a cluster of genes. *Genomics* 1996; **38**: 340-352 [PMID: 8975711 DOI: 10.1006/geno.1996.0637]

59 **Baffa R**, Negrini M, Mandes B, Rugge M, Ranzani GN, Hirohashi S, Croce CM. Loss of heterozygosity for chromosome 11 in adenocarcinoma of the stomach. *Cancer Res* 1996; **56**: 268-272 [PMID: 8542579]

60 **Moskaluk CA**, Rumpel CA. Allelic deletion in 11p15 is a common occurrence in esophageal and gastric adenocarcinoma. *Cancer* 1998; **83**: 232-239 [PMID: 9669804 DOI: 10.1002/(SICI)1097-0142(19980715)83: 2<232: : AID-CNCR5>3.0.CO; 2-S]

61 **Zhou CJ**, Zhang LW, Gao F, Zhang B, Wang Y, Chen DF, Jia YB. Association analysis of common genetic variations in MUC5AC gene with the risk of non-cardia gastric cancer in a Chinese

population. *Asian Pac J Cancer Prev* 2014; **15**: 4207-4210 [PMID: 24935372 DOI: 10.7314/APJCP.2014.15.9.4207]
62 **Zhou CJ**, Zhang LW, Gao F, Zhang B, Wang Y, Chen DF, Jia YB. Common genetic variations in the MUC5AC gene are not related to helicobacter pylori serologic status. *Asian Pac J Cancer Prev* 2014; **15**: 10719-10722 [PMID: 25605164 DOI: 10.7314/APJCP.2014.15.24.10719]
63 **Wang C**, Wang J, Liu Y, Guo X, Zhang C. MUC5AC upstream complex repetitive region length polymorphisms are associated with susceptibility and clinical stage of gastric cancer. *PLoS One* 2014; **9**: e98327 [PMID: 24887023 DOI: 10.1371/journal.pone.0098327]
64 **Kawakubo M**, Ito Y, Okimura Y, Kobayashi M, Sakura K, Kasama S, Fukuda MN, Fukuda M, Katsuyama T, Nakayama J. Natural antibiotic function of a human gastric mucin against Helicobacter pylori infection. *Science* 2004; **305**: 1003-1006 [PMID: 15310903 DOI: 10.1126/science.1099250]
65 **Byrd JC**, Yan P, Sternberg L, Yunker CK, Scheiman JM, Bresalier RS. Aberrant expression of gland-type gastric mucin in the surface epithelium of Helicobacter pylori-infected patients. *Gastroenterology* 1997; **113**: 455-464 [PMID: 9247464]
66 **Zheng H**, Takahashi H, Nakajima T, Murai Y, Cui Z, Nomoto K, Tsuneyama K, Takano Y. MUC6 down-regulation correlates with gastric carcinoma progression and a poor prognosis: an immunohistochemical study with tissue microarrays. *J Cancer Res Clin Oncol* 2006; **132**: 817-823 [PMID: 16807756 DOI: 10.1007/s00432-006-0135-3]
67 **Lancaster CA**, Peat N, Duhig T, Wilson D, Taylor-Papadimitriou J, Gendler SJ. Structure and expression of the human polymorphic epithelial mucin gene: an expressed VNTR unit. *Biochem Biophys Res Commun* 1990; **173**: 1019-1029 [PMID: 2268309 DOI: 10.1016/S0006-291X(05)80888-5]
68 **Nguyen TV**, Janssen M, Gritters P, te Morsche RH, Drenth JP, van Asten H, Laheij RJ, Jansen JB. Short mucin 6 alleles are associated with H pylori infection. *World J Gastroenterol* 2006; **12**: 6021-6025 [PMID: 17009402]
69 **Garcia E**, Carvalho F, Amorim A, David L. MUC6 gene polymorphism in healthy individuals and in gastric cancer patients from northern Portugal. *Cancer Epidemiol Biomarkers Prev* 1997; **6**: 1071-1074 [PMID: 9419405]
70 **Kwon JA**, Lee SY, Ahn EK, Seol SY, Kim MC, Kim SJ, Kim SI, Chu IS, Leem SH. Short rare MUC6 minisatellites-5 alleles influence susceptibility to gastric carcinoma by regulating gene. *Hum Mutat* 2010; **31**: 942-949 [PMID: 20506113 DOI: 10.1002/humu.21289]
71 **Mejías-Luque R**, Lindén SK, Garrido M, Tye H, Najdovska M, Jenkins BJ, Iglesias M, Ernst M, de Bolós C. Inflammation modulates the expression of the intestinal mucins MUC2 and MUC4 in gastric tumors. *Oncogene* 2010; **29**: 1753-1762 [PMID: 20062084 DOI: 10.1038/onc.2009.467]
72 **Jeong YH**, Kim MC, Ahn EK, Seol SY, Do EJ, Choi HJ, Chu IS, Kim WJ, Kim WJ, Sunwoo Y, Leem SH. Rare exonic minisatellite alleles in MUC2 influence susceptibility to gastric carcinoma. *PLoS One* 2007; **2**: e1163 [PMID: 18000536 DOI: 10.1371/journal.pone.0001163]
73 **Moehle C**, Ackermann N, Langmann T, Aslanidis C, Kel A, Kel-Margoulis O, Schmitz-Madry A, Zahn A, Stremmel W, Schmitz G. Aberrant intestinal expression and allelic variants of mucin genes associated with inflammatory bowel disease. *J Mol Med* (Berl) 2006; **84**: 1055-1066 [PMID: 17058067 DOI: 10.1007/s00109-006-0100-2]

P- Reviewer: Chiurillo MA
S- Editor: Ma YJ **L- Editor**: A **E- Editor**: Jiao XK

World Journal of Gastrointestinal Oncology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4251/wjgo.v7.i11.338

World J Gastrointest Oncol 2015 November 15; 7(11): 338-346
ISSN 1948-5204 (online)

Immunotherapeutic approaches in biliary tract carcinoma: Current status and emerging strategies

Eric I Marks, Nelson S Yee

Eric I Marks, Department of Medicine, Penn State Milton S. Hershey Medical Center, Hershey, PA 17033, United States

Nelson S Yee, Division of Hematology-Oncology, Program of Experimental Therapeutics, Department of Medicine, Penn State Hershey Medical Center, Penn State Hershey Cancer Institute, Hershey, PA 17033-0850, United States

Author contributions: Marks EI and Yee NS conceived and designed the study, reviewed the literature, collected and analyzed the data, and wrote the paper.

Conflict-of-interest statement: The authors declare no conflict of interest in this manuscript.

Correspondence to: Nelson S Yee, MD, PhD, Division of Hematology-Oncology, Program of Experimental Therapeutics, Department of Medicine, Penn State Hershey Medical Center, Penn State Hershey Cancer Institute, 500 University Drive, Hershey, PA 17033-0850, United States. nyee@hmc.psu.edu
Telephone: +1-717-5310003
Fax: +1-717-5315076

Received: June 28, 2015
Peer-review started: July 11, 2015
First decision: July 28, 2015
Revised: August 17, 2015
Accepted: September 16, 2015
Article in press: September 18, 2015
Published online: November 15, 2015

Abstract

For biliary tract carcinoma (BTC), complete surgical resection of tumor is only feasible in a minority of patients, and the treatment options for patients with unresectable or metastatic disease are limited. Advances in cancer immunology have led to identification of tumor-infiltrating immune cells as indicators of prognosis and response to treatment in BTC. This has also facilitated development of immunotherapy that focuses on enhancing the immune system against biliary tumors. This includes peptide- and dendritic cell-based vaccines that stimulate *in-vivo* immune responses against tumor-specific antigens. Adoptive immunotherapy, which entails the *ex-vivo* expansion of tumor-infiltrating immune cells for subsequent reintroduction, and cytokine-based therapies have been developed in BTC. Clinical studies indicate that this type of therapy is generally well tolerated. Combination therapy with dendritic cell-based vaccines and adoptive immunotherapy has shown particularly good potential. Emerging strategies through discovery of novel antigen targets and by reversal of tumor-associated immunosuppression are expected to improve the efficacy of immunotherapy in BTC. Collaborative efforts by integration of targeted immunotherapeutics with molecular profiling of biliary tumor will hopefully make a positive impact on advancing towards the goal of developing precision treatment of patients with this highly lethal disease.

Key words: Adoptive immunotherapy; Cancer vaccines; Biliary tract carcinoma; Cholangiocarcinoma; Gallbladder carcinoma; Immunotherapy; Precision treatment

Core tip: Advances in cancer immunology have led to development of novel therapeutics that focuses on enhancing the immune system against biliary tract cancer. These include peptide- or dendritic cell-based vaccines, adoptive immunotherapy, and immunostimulatory cytokines. Immunotherapy is generally well tolerated with good potential for developing into treatment. The efficacy of immunotherapy may be improved by

reversal of tumor-associated immunosuppression and through discovery of novel antigen targets. Integration of targeted immunotherapeutics with molecular profiling of biliary tumor is expected to make a positive impact on advancing towards the goal of developing precision treatment of patients with this highly lethal disease.

Marks EI, Yee NS. Immunotherapeutic approaches in biliary tract carcinoma: Current status and emerging strategies. *World J Gastrointest Oncol* 2015; 7(11): 338-346 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i11/338.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i11.338

INTRODUCTION

Cholangiocarcinoma and gallbladder adenocarcinoma are the most common primary malignancies of the biliary tract. Collectively referred to as biliary tract carcinoma (BTC), these diseases are a cause of substantial morbidity and mortality. Each year in the United States alone, approximately 11000 patients are diagnosed with BTC and 3700 lives are claimed by the disease[1].

Until recently, the treatment options available to patients with BTC primarily involved surgery, radiation, and systemic chemotherapy. Complete surgical resection is potentially curative, but it can only be achieved in the 10% of patients who present with localized disease without vascular invasion[2]. Patients with BTC that is locally advanced, metastatic, or recurrent are typically offered single agent or combination chemotherapy, depending upon performance status. Typical regimens consist of gemcitabine, 5-fluorouracil, and platinum-based agents[3]. Despite these interventions, clinical outcomes in BTC are generally poor. Fewer than 5% of patients with cholangiocarcinoma[2] and 13% with gallbladder cancer[4] survive longer than two years following diagnosis.

Advances in cancer immunology and immunotherapy have facilitated the development of additional treatment options that bring new hope to patients with BTC. This new generation of therapeutics seeks to strengthen the patient's immune system in combating malignancy, typically by priming it against tumor-specific antigens. Such treatments are more selective against malignant cells and therefore tend to be less toxic than traditional chemotherapy. Furthermore, by exerting an antitumor effect indirectly through the immune system rather than *via* direct activity against malignant cells, these therapeutic approaches can produce durable responses that persist long after the drug itself has been metabolized.

In this article, we concisely review cancer immunology as it relates to malignancies of the biliary tract. The immunotherapeutic approaches that are being investigated for use in BTC will be described, along with the data from clinical trials that have been completed thus far. We will also discuss ongoing clinical trials and emerging strategies for immunotherapy in BTC.

CANCER IMMUNOLOGY IN BILIARY TRACT CANCER

Focusing and enhancing the antineoplastic effects of the immune system as treatment for BTC has only recently become a subject of concerted investigation. Evidence suggests that at the earliest stages of tumor development, the host immune system is capable of both detecting and controlling the disease. Over time, however, this generates evolutionary pressure that favors the proliferation of cancer cells that are less immunogenic or otherwise capable of suppressing the host immune response[5-9]. Despite this, there often persists a small cohort of immune cells that remain able to identify and invade the tumor. The characteristics of this immune infiltrate are of prognostic value in a variety of malignancies, including BTC[10,11]. The frequency and clinical significance of tumor infiltration by the cellular mediators of the host immune response is summarized in Table 1.

Tumor infiltration by the innate immune system

The innate immune system, consisting of the complement cascade, natural killer (NK) cells, granulocytes, and phagocytes, mounts an initial non-specific defense against infections and malignancy. The frequency of tumor infiltration by the cellular components of the innate immune system is highly variable. While fewer than half of biliary tumors are penetrated by NK cells[12,13] or mast cells[13], macrophages are observed in the majority of BTC[13].

Despite correlating with outcomes in a host of other malignancies[16-20], infiltration of BTC by the innate immune system appears to be of little clinical significance. Neither the presence of intratumoral NK cells nor mast cells is correlated with clinical outcomes[12]. The density of tumor-infiltrating macrophages, however, appears to increase as lesions progress from pre-malignant precursors to invasive malignancy and later to metastatic disease[13]. This is believed to be the result of activated macrophages releasing pro-inflammatory and pro-angiogenic cytokines that facilitate tumor growth. These include tumor necrosis factor-α, vascular endothelial growth factor A, and granulocyte macrophage colony-stimulating factor[21,22].

Tumor infiltration by the adaptive immune system

The adaptive immune response is initiated by the consumption of foreign material by antigen presenting cells, most often dendritic cells. After processing the antigen for presentation, dendritic cells migrate to lymph nodes where they stimulate the proliferation of antigen-specific lymphocytes and recruit $CD4^+$ T-helper cells. Activated $CD4^+$ cells release cytokines that induce the differentiation of B-lymphocytes into antibody-releasing plasma cells, and activate cytotoxic $CD8^+$

Table 1 Cellular mediators of innate and adaptive immune system in biliary tract carcinoma

Cell type	Frequency of infiltration	Clinical significance	Ref.
Natural killer cells	19.1%-33% overall 20% of ICC, 21% of ECC, 16% of GBC	No correlation with disease stage, grade, or survival	[12,13]
Mast cells	2% of ICC, 2.5% of ECC, 8.5% of GBC	No correlation with survival	[13]
Macrophages	87% of ICC, 70% of ECC, and 71% of GBC	Associated with more advanced disease	[13]
Dendritic cells	Not determined	Associated with improved survival	[12,14]
$CD4^+$ helper T-lymphocytes	43% of ICC, 30% of ECC, and 34%-51% of GBC	Associated with reduced probability of metastases and improved survival in ECC	[12,13]
$CD8^+$ cytotoxic T-lymphocytes	46% of ICC, 49%-55% of ECC, and 38%-51% of GBC	Associated with reduced probability of metastases and improved survival in ECC	[12,13,15]
B-lymphocytes /plasma cells	4.5% of ICC, 6.7% of ECC, and 10.1% of GBC	Associated with improved survival	[13]

ECC: Extrahepatic cholangiocarcinoma; GBC: Gallbladder carcinoma; ICC: Intrahepatic cholangiocarcinoma.

T-lymphocytes (CTL). After clearing the antigen, both $CD4^+$ and $CD8^+$ T cells may differentiate into memory T-cells that organize an expedited secondary immune response if the offending antigen is encountered again. It is these memory cells that form the physiologic basis for vaccination.

Like the innate immune system, there is considerable variability in the frequency of tumor infiltration by cells of the adaptive immune system. Although the exact percentage of BTC that contains dendritic cells is not clear, their presence appears to be nearly universal in both GBC[12] and cholangiocarcinoma[14]. Approximately 30%-50% of BTC is infiltrated with $CD4^+$ or $CD8^+$ T-lymphocytes[12,13]. Tumor infiltration by B-lymphocytes or plasma cells is seldom observed[13], which may be attributed to the tendency for these cells to rarely migrate outside of lymph nodes.

Tumor infiltration by the cellular mediators of the adaptive immune response is generally correlated with improved outcomes in BTC. The presence of dendritic cells[12,14], $CD4^+$ T-cells[12], $CD8^+$ T-cells[12,15], or plasma cells[13] within a biliary tumor is predictive of improved OS. This trend towards more favorable prognosis is consistent with findings in other malignancies, such as colorectal[23] and esophageal carcinoma[24]. Though it has not been reported in BTC, the subset of $CD3^+$ T-cells in colorectal cancer suggests that these cells are possibly involved in vitamin D-mediated immunoprevention[25].

IMMUNOTHERAPEUTIC APPROACHES IN BTC

While the endogenous immune response is initially successful in slowing the growth of BTC, the malignancy eventually becomes capable of evading the immune system. This occurs through intense evolutionary pressure that confers a survival advantage to cancer cells that lack foreign antigens, secrete immunosuppressive substances, or otherwise limit the effectiveness of the host immune system[5-9]. Several approaches for potentiating or redirecting the immune response to BTC are being investigated. Vaccines based upon either peptides or dendritic cells seek to sensitize the immune system against tumor-specific antigens. The extraction, amplification, and reintroduction of a patient's own tumor-infiltrating immune cells *via* adoptive immunotherapy is being evaluated. Treatment using immunostimulatory cytokines has been attempted.

Targets of vaccination

Through the controlled presentation of a particular antigen, vaccination primes the immune system to respond swiftly and accurately to repeat exposures in the future. This occurs, in part, through the production of memory T-cells that orchestrate this secondary response. As a result, the effectiveness of vaccination is a function of both the immune system's strength and the selection of a proper target antigen. Ideally, the target should be highly specific to malignant cells and strictly conserved within the tumor. This ensures that collateral damage to normal tissues will be minimized, while also reducing the likelihood that an antigen-negative cancer cell will arise to repopulate the tumor.

One antigen that largely fulfills these criteria is Wilm's Tumor protein 1 (WT1)[10], a transcription factor that is normally involved in urogenital development. This protein also functions as a tumor suppressor through interactions with platelet derived growth factor receptor, epithelial growth factor receptor, c-MYC, and B-cell lymphoma 2[26]. Approximately 68%-80% of biliary tumors harbor mutations of WT1[26]. While the clinical significance of mutated WT1 in BTC remains unclear, similar mutations are known to correlate with poor prognosis in testicular cancer[27], breast cancer[28], and squamous cell carcinoma of the head and neck[29].

Another potential target for immunization is the glycoprotein, mucin protein 1 (MUC1)[10]. Consisting of a large and heavily glycosylated extracellular domain, MUC1 forms the hydrophilic barrier that is characteristic of BTC and other types of adenocarcinoma. This mucinous shell repels hydrophobic chemotherapeutics and obstructs immune cells, while also allowing the tumor to immerse itself in growth factors[30]. MUC1 is over-expressed in 90% of gallbladder carcinoma[31] and 59%-77% of cholangiocarcinoma[31-34]. Excessive production of MUC1 in BTC is typically indicative of more

advanced disease[32] and impaired OS[31-33].

Peptide-based vaccines and personalized peptide vaccination

Peptide-based vaccines are among the most investigated class of cancer immunotherapy. The vaccine typically contains one or more antigens that are heavily expressed by malignant cells and often emulsified in Freund's adjuvant to increase immunogenicity. The goal of immunization is to stimulate mass-production of memory lymphocytes that can generate a strong secondary immune response against cancer cells that bear the particular antigen.

The efficacy of any single peptide-based vaccine is intrinsically limited, however, by the heterogeneity of BTC. Although the overall expression of certain antigens, such as WT1 and MUC1, is often increased within biliary tumors, the distribution of these antigens is non-uniform. While some cells over-express the antigen, there are often others from which it is entirely absent. Furthermore, the tenacity with which the immune system responds to these antigens varies widely between patients, even among those with similar HLA types[35]. This is due, in part, to differences in the number of lymphocyte precursors that are maximally sensitive to the particular antigen[36].

Personalized peptide vaccination seeks to overcome these limitations by immunizing patients against multiple antigens simultaneously. While it is likely that a tumor will harbor cells that lack any single antigen, the odds are exponentially less that any single cell will lack each of 3 to 4 antigens that are individually quite common. This has the additional benefit of theoretically counteracting the pressure of selection for tumor cells that lack the target antigens[35]. To bypass individual differences in sensitivity to particular antigens, it is possible to measure the frequency of antigen-sensitive CTL precursors within each patient. They may then be vaccinated against only the antigens to which they will most likely respond[36].

Dendritic cell-based vaccines

Similar to their peptide-based counterparts, dendritic cell-based vaccines expose the immune system to an antigen with the goal of generating memory lymphocytes that will produce a robust secondary immune response. Rather than simply introducing a peptide that requires subsequent processing and presentation to the adaptive immune system, these vaccines contain dendritic cells that are already loaded with antigen. These vaccines may be prepared against a particular antigen or more generally against a tumor lysate. While the latter approach stimulates the immune system against a larger number of antigens and theoretically produces a greater antitumor response, it may also carry a risk of autoimmunity. While the use of dendritic cells-based vaccines against BTC remains in its infancy, the success of sipuleucel-T in treating prostate cancer[37] demonstrates the promise that these therapeutics may someday fulfill.

Adoptive immunotherapy

Unlike the treatments described previously, adoptive immunotherapy is not intended to produce an *in-vivo* immune response. Instead, a patient's own tumor-infiltrating lymphocytes are extracted, modified, and induced to clonally proliferate *ex-vivo.* This expanded population of tumor-specific immune cells is then reintroduced, and they migrate back to the tumor and continue to combat its growth. The effectiveness of this treatment may be further increased by depleting the patient's existing lymphocyte population with cytotoxic chemotherapy in advance of returning the grafted lymphocytes. This is believed to prolong the lifespan of the transplanted cells.

Immunostimulating cytokines

The cytokine, interleukin-2 (IL2) is a potent anti-neoplastic agent due to its ability to stimulate the proliferation and cytotoxic effects of $CD8^+$ T-lymphocytes[38-40]. Administering IL2 as a monotherapy or in combination with adoptive immunotherapy is an effective treatment for certain malignancies, such as melanoma[41,42] and renal cell carcinoma[42,43]. Treatment with IL2 is associated with a substantial side effect profile that includes nephrotoxicity, extravasation of fluid secondary to increased vascular permeability, and rarely transient myocarditis[40,41].

CLINICAL STUDIES OF IMMUNOTHERAPY IN BTC

Each type of immune-based approach described above has been evaluated for therapeutic efficacy in patients with BTC. Many of these agents have been studied as monotherapy as well as in combination with traditional chemotherapy or targeted therapeutics. The completed clinical trials of immunotherapy in BTC are described below and the compiled data are summarized in Table 2.

Peptide-based vaccines

To date, most clinical studies of immunotherapy in BTC have focused on peptide-based vaccines, often targeted against WT1 or MUC1. This type of treatment is generally well tolerated; however it appears to exert only a modest anti-neoplastic effect when administered as monotherapy.

Vaccines against WT1 are often administered in combination with gemcitabine based chemotherapy. Preclinical studies suggest that gemcitabine upregulates the expression of WT1, thereby theoretically enhancing the effect of immunization[53]. In a phase I trial, anti-WT1 vaccination and gemcitabine were administered to patients with unresectable gallbladder cancer, cholangiocarcinoma, or pancreatic adenocarcinoma[44]. This regimen increased the number of WT1-specific

Table 2 Trials of immunotherapy in biliary tract carcinoma

Immunotherapy	Treatment regimens	Phase	*n*	Types of BTC	OS (mo)	PFS (mo)	Ref.
Peptide-based vaccine (WT1)	Peptide vaccine + gemcitabine	I	25	Pancreatic, GBC, ICC, ECC	9.3	--	[44]
Peptide-based vaccine (WT1)	Peptide vaccine monotherapy	I	9	Pancreatic, CC	--	--	[45]
Peptide-based vaccine (NUF2, CDH3, KIF20A)	Peptide vaccine triple therapy	I	9	GBC, ICC, ECC	9.7	3.4	[46]
Peptide-based vaccine (LY6K, TTK, IGF2BP3, DEPDC1)	Peptide vaccine quadruple therapy	I	9	GBC, ICC, ECC	12.3	5	[47]
Peptide-based vaccine (Many)	Personalized peptide vaccination +/- chemotherapy	II	25	GBC, ICC, ECC	6.7	--	[48]
Dendritic cell-based vaccine (MUC1)	Dendritic cell vaccination +/- chemotherapy +/- radiotherapy	I / II	12	Pancreatic, CC	26	--	[49]
Dendritic cell-based vaccine (WT1, MUC1)	Peptide vaccine +/- chemotherapy	--	65	GBC, ICC, ECC	--	--	[50]
Dendritic cell-based vaccine, adoptive immunotherapy	Surgery + dendritic cell vaccine + T-cell transfer *vs* surgery alone	--	36	ICC	31.9	18.3	[51]
Interleukin-2	Induction cisplatin + gemcitabine, consolidation capecitabine + radiation, and maintenance IL-2 + 13-cis-retinoic acid	II	54	Pancreatic, GBC, CC	> 27.5	16.2	[52]

CC: Cholangiocarcinoma; OS: Overall survival; PFS: Progression-free survival; WT1: Wilm's tumor 1; NUF2: Cell division cycle associated protein 1; CDH3: Cadherin 3; DEPDC1: DEP domain containing 1; ECC: Extrahepatic cholangiocarcinoma; GBC: Gallbladder cancer; ICC: Intrahepatic cholangiocarcinoma; IGF2BP3: Insulin-like growth factor-II mRNA binding protein 3; KIF20A: Kinesin family member 20A; LY6K: Lymphocyte antigen 6 complex locus K; MUC1: Mucin 1.

lymphocytes in circulation, but it did not improve clinical outcomes or increase toxicity over that which is expected from gemcitabine monotherapy. At the present time, a phase Ⅱ study of WT1 vaccination as an adjunct to combination chemotherapy with gemcitabine plus cisplatin is underway[53]. This study aims to establish the 1-year OS rate for patients receiving treatment.

Similar to WT1, peptide-based immunization against MUC1 is well tolerated but it lacks definite proof of clinical efficacy. In a phase Ⅰ trial of nine patients with advanced stage cholangiocarcinoma or pancreatic adenocarcinoma, monotherapy with peptide-based vaccines against MUC1 produced only a single instance of stable disease[45]. Despite failing to influence outcomes, vaccination did generate a robust anti-MUC1 IgG response in 78% of patients with negligible toxicity. In the future, vaccination against MUC1 could fill a niche in addition to gemcitabine or fluorouracil-based chemotherapy. This is because preclinical studies have found that these agents increase the expression of MUC1 in cholangiocarcinoma cells[53]. Further research is indicated to determine the safety and efficacy of such regimens.

The prospect of combination therapy with multiple peptide-based vaccines has been explored. Triple therapy with vaccines against cell division cycle associated protein 1 (NUF2), cadherin 3 (CDH3), kinesin family member 20A in patients with GBC, ICC, and ECC was investigated in a phase Ⅰ clinical trial[46]. This treatment stimulated peptide-specific T-cell responses in all patients and 55% achieved stable disease. A four vaccine regimen against lymphocyte antigen 6 complex locus K (LY6K), TTK protein kinase, insulin-like growth factor-Ⅱ mRNA binding protein 3, and DEP domain containing 1 has also been tested in a phase Ⅰ trial of nine patients with BTC[47]. Peptide specific T-cell responses were generated in 78% of patients receiving this regimen and clinical responses were observed in 67%. In both trials of combination therapy with peptide-based vaccines, the presence of an injection site reaction correlated with OS[46,47]. This underscores the reliance of this treatment upon provoking a strong immune response to generate an anti-tumor effect. Aside from these local dermatologic reactions, treatment-associated toxicity was minimal.

The efficacy of combination vaccination may be refined by individualizing the process by which targets are selected. This approach of personalized peptide-based vaccination was assessed in a phase Ⅱ trial of 25 patients with either gallbladder adenocarcinoma or cholangiocarcinoma[48]. Patients received as many as 4 of 31 possible vaccines in addition to systemic chemotherapy, if their performance status could support such treatment. This regimen produced stable disease in 80% of patients and negligible toxicity beyond that which is typically associated with chemotherapy.

Dendritic cell-based vaccines

Immunotherapy with antigen-pulsed dendritic cells is exceptionally well tolerated, and it appears to be efficacious against BTC. In a combined phase Ⅰ / Ⅱ trial, 12 patients with BTC or pancreatic adenocarcinoma received an anti-MUC1 dendritic cell-based vaccine following tumor resection and, in some instances, chemoradiation[49]. A median OS of 26 mo was observed, while 33% of patients survived longer than 50 mo without evidence of disease recurrence. While this study was not designed to differentiate between durable responses that occur due to vaccination and those that arise from complete surgical resection, it is conceivable

Table 3 Ongoing clinical trials of immunotherapy in biliary tract carcinoma

Agent	Treatment regimen	Phase	Estimated date of completion	Sponsoring Institution	Identification number
Cytokine induced killer cells	Cytokine induced killer cell monotherapy	Ⅰ / Ⅱ	May, 2016	Siriraj Hospital	NCT01868490
Tumor infiltrating lymphocytes	Tumor infiltrating lymphocytes + IL-2 + cyclophosphamide + fludarabine	Ⅱ	December, 2019	National Cancer Institute	NCT01174121
Poly-ICLC	Cyclophosphamide + radiation therapy + TACE + poly-ICLC	Ⅰ / Ⅱ	July, 2014	Rutgers, the State University of New Jersey	NCT00553683

IL-2: Interleukin-2; Poly-ICLC: Polyinosinic-polycytidylic acid polylysine carboxymethylcellulose; TACE: Transcatheter arterial chemoembolization.

that the combination of adjuvant chemotherapy, radiation therapy, and immunotherapy eliminated microscopic residual disease after surgery.

In another trial, dendritic cell-based vaccines against WT1 and/or MUC1 in combination with chemotherapy was evaluated in 65 patients with unresectable, metastatic, or recurrent BTC[50]. This regimen was well tolerated and 15% of patients had stable disease following 6 mo of treatment. Although the response rate did not differ between patients who were vaccinated against one or both targets, the correlation between post-immunization fever and improved survival does suggest the responses generated by this regimen may be at least partially attributed to immune activation.

Adoptive immunotherapy

Direct transfer of cellular immunity *via* adoptive immunotherapy has also been investigated for use in BTC. In a study of 36 patients with intrahepatic cholangiocarcinoma, surgery alone was compared to surgery followed by combination adoptive immunotherapy with tumor-lysate pulsed dendritic cells and transfer of activated T-cells[51]. Patients who received adjuvant immunotherapy experienced nearly double the OS of those treated with surgery alone with minimal toxicity. Among the 16 patients who produced the largest injection site reaction, median OS was 95.5 mo.

Similar durable and dramatic responses to combined immunotherapy with dendritic cell-based vaccines and activated T cell transfer have been described in case reports of patients with cholangiocarcinoma[54] and gallbladder cancer[55]. Anecdotal evidence also suggests that combining T-cell based adoptive immunotherapy with cetuximab may have activity against malignant ascites and peritoneal carcinomatosis due to metastatic cholangiocarcinoma[56].

IL2 maintenance therapy

The use of IL2 as a maintenance therapy was explored in a multicenter phase Ⅱ trial of 54 patients with pancreatic adenocarcinoma or BTC[52]. These patients initially received 3 cycles of combination chemotherapy with cisplatin and gemcitabine as induction therapy. Patients who remained progression-free were subsequently treated with concurrent capecitabine and radiotherapy as consolidation, followed by maintenance IL2 and 13-cis-retinoic acid. The progression-free survival (PFS) and overall survival (OS) for all patients enrolled in this study was 6.8 and 12.1 mo, respectively. Outcomes were notably better when considering only the subset of patients who were able to complete the entire course of treatment, however, with median PFS of 16.2 mo and OS that had not yet been reached after a median follow-up of 27.5 mo. Further investigation will be needed to determine whether this differential survival is truly due to a response to treatment, or if those patients simply had more indolent disease independent of therapy.

ONGOING CLINICAL TRIALS OF IMMUNOTHERAPY IN BTC

Currently, several clinical trials of immunotherapy in malignancies of the biliary tract are ongoing and as listed in Table 3. These studies utilize different immunotherapeutic approaches. In one study, cytokine induced killer cells are employed as monotherapy. In another study, adoptive transfer of tumor-infiltrating lymphocytes is combined with IL2 and chemotherapy. In attempt to reverse systemic immunosuppression, the immunomodulatory agent, polyinosinic-polycytidylic acid polylysine carboxymethylcellulose, is used in combination with chemotherapy and radiation therapy. In those two studies involving chemotherapy, low-dose metronomic cyclophosphamide is used to eliminate the immunosuppressive regulatory T lymphocytes (T_{reg}) and prevent tumor-associated angiogenesis.

CONCLUSION

Immunotherapy in BTC has been under active investigation and tremendous opportunities exist for developing it into a safe and effective treatment of patients with this disease. Clinical studies indicate that this type of therapy is generally well tolerated. The efficacy of immune-based treatment of BTC is improving as the complex interactions between the immune system and biliary tumors are better understood. Combination therapy with dendritic cell-based vaccines and adoptive immunotherapy has shown particularly good potential. Several directions for future investigation of immunotherapy that may improve the clinical

outcomes of patients with this disease are described as follows.

Preliminary studies suggest that the distribution and types of immune cells that infiltrate biliary tumors may be used to predict the likelihood that an individual tumor will respond to a particular chemotherapy regimen[57]. Further characterizing these associations could be clinically beneficial, as it would provide a physiologic basis for selecting therapy as an adjunct to the current paradigm that relies upon tumor histology and stage. On the other hand, application of mass spectrometry and genomic sequencing to discover new antigens[58] may help facilitate development of novel strategies for targeted immunotherapy in BTC. Furthermore, evidence suggests that increased inflammatory signaling *via* IL6 is associated with reduced response to vaccination[36,48]. The hypothesis that addition of the IL6 receptor antagonist tocilizumab enhances the effects of vaccination remains to be tested.

Besides, tumor evasion of the immune system is often mediated by cytotoxic T-lymphocytes associated antigen 4 (CTLA4) or the interaction between programmed cell death 1 (PDCD1, also known as PD1 or CD279) and its ligand (PDCD1LG1, also known as PDL1 or CD274)[9]. It will be important to investigate the potential of blocking these immunosuppressive pathways with monoclonal antibodies in conjunction with the currently used immunotherapeutic approaches in BTC. The anti-CTLA4 antibody ipilimumab has shown great promise in other malignancies such as melanoma[59], but it has not yet been studied in BTC. Similarly, pembrolizumab and nivolumab, monoclonal antibodies that target PD1/CD279 signaling have been found to improve anti-tumor T-cell response and induce tumor regression in subsets of patients with melanoma, renal cell carcinoma, and non-small-cell lung cancer[8,60,61]. Preclinical studies suggest that immunohistochemical analysis for PDL1/CD274 in biliary tumors may help identify the patients who are likely to benefit from such therapeutics[62].

The synergistic relationships between cytotoxic chemotherapy and immunotherapy deserve further investigation for treatment of BTC. In one study, gemcitabine, which is a mainstay of treatment in BTC, was found to enhance cell-mediated immunity *via* increased expression of HLA on malignant cells[63]. Platinum-based agents have a similar effect on HLA expression, while also reducing PDL2/CD273-mediated suppression of antigen-specific T-lymphocytes[64]. It is plausible that the addition of gemcitabine and cisplatin to immunotherapy could further improve the treatment responses.

Ultimately, the goal is to combine the advances in cancer immunotherapy with those of targeted therapy and molecular profiling to develop precision treatment for improving the clinical outcomes of patients with this highly lethal disease.

REFERENCES

1 **American Cancer Society.** What are the key statistics about gallbladder cancer? 2014. Available from: URL: http: //www.cancer.org/cancer/gallbladdercancer/detailedguide/gallbladder-key-statistics

2 **Mihalache F**, Tantau M, Diaconu B, Acalovschi M. Survival and quality of life of cholangiocarcinoma patients: a prospective study over a 4 year period. *J Gastrointestin Liver Dis* 2010; **19**: 285-290 [PMID: 20922193]

3 **Benson A**, D'Angelica M, Abrams T, Are C, Bloomston PM, Chang D, Clary B, Covey A, Ensminger W, Iyer R, Kelley RK, Linehan D, Malafa M, Meranze S, Park J, Pawlik T, Posey J, Scaife C, Schefter T, Sigurdson E, Tian GG, Vauthey JN, Venook A, Yen Y, Zhu A. Hepatobiliary cancers. NCCN clinical practice guidelines in oncology (NCCN Guidelines®). National Comprehensive Cancer Network, 2014

4 **Smith GC**, Parks RW, Madhavan KK, Garden OJ. A 10-year experience in the management of gallbladder cancer. *HPB* (Oxford) 2003; **5**: 159-166 [PMID: 18332977 DOI: 10.1080/13651820304287]

5 **Rabinovich GA**, Gabrilovich D, Sotomayor EM. Immunosuppressive strategies that are mediated by tumor cells. *Annu Rev Immunol* 2007; **25**: 267-296 [PMID: 17134371 DOI: 10.1146/annurev.immunol.25.022106.141609]

6 **Shankaran V**, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, Schreiber RD. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* 2001; **410**: 1107-1111 [PMID: 11323675 DOI: 10.1038/35074122]

7 **Dunn GP**, Old LJ, Schreiber RD. The three Es of cancer immunoediting. *Annu Rev Immunol* 2004; **22**: 329-360 [PMID: 15032581 DOI: 10.1146/annurev.immunol.22.012703.104803]

8 **Tumeh PC**, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V, West AN, Carmona M, Kivork C, Seja E, Cherry G, Gutierrez AJ, Grogan TR, Mateus C, Tomasic G, Glaspy JA, Emerson RO, Robins H, Pierce RH, Elashoff DA, Robert C, Ribas A. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014; **515**: 568-571 [PMID: 25428505]

9 **Gubin MM**, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, Ivanova Y, Hundal J, Arthur CD, Krebber WJ, Mulder GE, Toebes M, Vesely MD, Lam SS, Korman AJ, Allison JP, Freeman GJ, Sharpe AH, Pearce EL, Schumacher TN, Aebersold R, Rammensee HG, Melief CJ, Mardis ER, Gillanders WE, Artyomov MN, Schreiber RD. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature* 2014; **515**: 577-581 [PMID: 25428507 DOI: 10.1038/nature13988]

10 **Takahashi R**, Yoshitomi M, Yutani S, Shirahama T, Noguchi M, Yamada A, Itoh K, Sasada T. Current status of immunotherapy for the treatment of biliary tract cancer. *Hum Vaccin Immunother* 2013; **9**: 1069-1072 [PMID: 23376808 DOI: 10.4161/hv.23844]

11 **Sasada T**, Suekane S. Variation of tumor-infiltrating lymphocytes in human cancers: controversy on clinical significance. *Immunotherapy* 2011; **3**: 1235-1251 [PMID: 21995574 DOI: 10.2217/imt.11.106]

12 **Nakakubo Y**, Miyamoto M, Cho Y, Hida Y, Oshikiri T, Suzuoki M, Hiraoka K, Itoh T, Kondo S, Katoh H. Clinical significance of immune cell infiltration within gallbladder cancer. *Br J Cancer* 2003; **89**: 1736-1742 [PMID: 14583778 DOI: 10.1038/sj.bjc.6601331]

13 **Goeppert B**, Frauenschuh L, Zucknick M, Stenzinger A, Andrulis M, Klauschen F, Joehrens K, Warth A, Renner M, Mehrabi A, Hafezi M, Thelen A, Schirmacher P, Weichert W. Prognostic impact of tumour-infiltrating immune cells on biliary tract cancer. *Br J Cancer* 2013; **109**: 2665-2674 [PMID: 24136146 DOI: 10.1038/bjc.2013.610]

14 **Takagi S**, Miyagawa S, Ichikawa E, Soeda J, Miwa S, Miyagawa Y, Iijima S, Noike T, Kobayashi A, Kawasaki S. Dendritic cells, T-cell infiltration, and Grp94 expression in cholangiocellular carcinoma. *Hum Pathol* 2004; **35**: 881-886 [PMID: 15257553 DOI: 10.1016/j.humpath.2004.03.016]

15 **Oshikiri T**, Miyamoto M, Shichinohe T, Suzuoki M, Hiraoka K, Nakakubo Y, Shinohara T, Itoh T, Kondo S, Katoh H. Prognostic value of intratumoral CD8+ T lymphocyte in extrahepatic bile duct carcinoma as essential immune response. *J Surg Oncol* 2003; **84**:

224-228 [PMID: 14756433 DOI: 10.1002/jso.10321]
16 **Ishigami S**, Natsugoe S, Tokuda K, Nakajo A, Che X, Iwashige H, Aridome K, Hokita S, Aikou T. Prognostic value of intratumoral natural killer cells in gastric carcinoma. *Cancer* 2000; **88**: 577-583 [PMID: 10649250]
17 **Coca S**, Perez-Piqueras J, Martinez D, Colmenarejo A, Saez MA, Vallejo C, Martos JA, Moreno M. The prognostic significance of intratumoral natural killer cells in patients with colorectal carcinoma. *Cancer* 1997; **79**: 2320-2328 [PMID: 9191519]
18 **Nielsen HJ**, Hansen U, Christensen IJ, Reimert CM, Brünner N, Moesgaard F. Independent prognostic value of eosinophil and mast cell infiltration in colorectal cancer tissue. *J Pathol* 1999; **189**: 487-495 [PMID: 10629548]
19 **Welsh TJ**, Green RH, Richardson D, Waller DA, O'Byrne KJ, Bradding P. Macrophage and mast-cell invasion of tumor cell islets confers a marked survival advantage in non-small-cell lung cancer. *J Clin Oncol* 2005; **23**: 8959-8967 [PMID: 16219934 DOI: 10.1200/JCO.2005.01.4910]
20 **Elpek GO**, Gelen T, Aksoy NH, Erdoğan A, Dertsiz L, Demircan A, Keleş N. The prognostic relevance of angiogenesis and mast cells in squamous cell carcinoma of the oesophagus. *J Clin Pathol* 2001; **54**: 940-944 [PMID: 11729214 DOI: 10.1136/jcp.54.12.940]
21 **Lin EY**, Li JF, Gnatovskiy L, Deng Y, Zhu L, Grzesik DA, Qian H, Xue XN, Pollard JW. Macrophages regulate the angiogenic switch in a mouse model of breast cancer. *Cancer Res* 2006; **66**: 11238-11246 [PMID: 17114237 DOI: 10.1158/0008-5472.CAN-06-1278]
22 **Pollard JW**. Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer* 2004; **4**: 71-78 [PMID: 14708027 DOI: 10.1038/nrc1256]
23 **Naito Y**, Saito K, Shiiba K, Ohuchi A, Saigenji K, Nagura H, Ohtani H. CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. *Cancer Res* 1998; **58**: 3491-3494 [PMID: 9721846]
24 **Schumacher K**, Haensch W, Röefzaad C, Schlag PM. Prognostic significance of activated CD8(+) T cell infiltrations within esophageal carcinomas. *Cancer Res* 2001; **61**: 3932-3936 [PMID: 11358808]
25 **Song M**, Nishihara R, Wang M, Chan AT, Qian ZR, Inamura K, Zhang X, Ng K, Kim SA, Mima K, Sukawa Y, Nosho K, Fuchs CS, Giovannucci EL, Wu K, Ogino S. Plasma 25-hydroxyvitamin D and colorectal cancer risk according to tumour immunity status. *Gut* 2015; Epub ahead of print [PMID: 25591978 DOI: 10.1136/gutjnl-2014-308852]
26 **Nakatsuka S**, Oji Y, Horiuchi T, Kanda T, Kitagawa M, Takeuchi T, Kawano K, Kuwae Y, Yamauchi A, Okumura M, Kitamura Y, Oka Y, Kawase I, Sugiyama H, Aozasa K. Immunohistochemical detection of WT1 protein in a variety of cancer cells. *Mod Pathol* 2006; **19**: 804-814 [PMID: 16547468 DOI: 10.1038/modpathol.3800588]
27 **Harada Y**, Nonomura N, Nishimura K, Tamaki H, Takahara S, Miki T, Sugiyama H, Okuyama A. WT1 Gene Expression in Human Testicular Germ-Cell Tumors. *Mol Urol* 1999; **3**: 357-364 [PMID: 10851296]
28 **Miyoshi Y**, Ando A, Egawa C, Taguchi T, Tamaki Y, Tamaki H, Sugiyama H, Noguchi S. High expression of Wilms' tumor suppressor gene predicts poor prognosis in breast cancer patients. *Clin Cancer Res* 2002; **8**: 1167-1171 [PMID: 12006533]
29 **Oji Y**, Inohara H, Nakazawa M, Nakano Y, Akahani S, Nakatsuka S, Koga S, Ikeba A, Abeno S, Honjo Y, Yamamoto Y, Iwai S, Yoshida K, Oka Y, Ogawa H, Yoshida J, Aozasa K, Kubo T, Sugiyama H. Overexpression of the Wilms' tumor gene WT1 in head and neck squamous cell carcinoma. *Cancer Sci* 2003; **94**: 523-529 [PMID: 12824878 DOI: 10.1111/j.1349-7006.2003.tb01477.x]
30 **Hollingsworth MA**, Swanson BJ. Mucins in cancer: protection and control of the cell surface. *Nat Rev Cancer* 2004; **4**: 45-60 [PMID: 14681689 DOI: 10.1038/nrc1251]
31 **Park SY**, Roh SJ, Kim YN, Kim SZ, Park HS, Jang KY, Chung MJ, Kang MJ, Lee DG, Moon WS. Expression of MUC1, MUC2, MUC5AC and MUC6 in cholangiocarcinoma: prognostic impact. *Oncol Rep* 2009; **22**: 649-657 [PMID: 19639217]
32 **Boonla C**, Sripa B, Thuwajit P, Cha-On U, Puapairoj A, Miwa M, Wongkham S. MUC1 and MUC5AC mucin expression in liver fluke-associated intrahepatic cholangiocarcinoma. *World J Gastroenterol* 2005; **11**: 4939-4946 [PMID: 16124042]
33 **Matsumura N**, Yamamoto M, Aruga A, Takasaki K, Nakano M. Correlation between expression of MUC1 core protein and outcome after surgery in mass-forming intrahepatic cholangiocarcinoma. *Cancer* 2002; **94**: 1770-1776 [PMID: 11920540 DOI: 10.1002/cncr.10398]
34 **Higashi M**, Yonezawa S, Ho JJ, Tanaka S, Irimura T, Kim YS, Sato E. Expression of MUC1 and MUC2 mucin antigens in intrahepatic bile duct tumors: its relationship with a new morphological classification of cholangiocarcinoma. *Hepatology* 1999; **30**: 1347-1355 [PMID: 10573510 DOI: 10.1002/hep.510300609]
35 **Noguchi M**, Sasada T, Itoh K. Personalized peptide vaccination: a new approach for advanced cancer as therapeutic cancer vaccine. *Cancer Immunol Immunother* 2013; **62**: 919-929 [PMID: 23197273 DOI: 10.1007/s00262-012-1379-1]
36 **Itoh K**, Yamada A. Personalized peptide vaccines: a new therapeutic modality for cancer. *Cancer Sci* 2006; **97**: 970-976 [PMID: 16984371 DOI: 10.1111/j.1349-7006.2006.00272.x]
37 **Kantoff PW**, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, Xu Y, Frohlich MW, Schellhammer PF. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med* 2010; **363**: 411-422 [PMID: 20818862 DOI: 10.1056/NEJMoa1001294]
38 **Kim MH**, Lee SS, Lee SK, Lee SG, Suh CW, Gong GY, Park JS, Kim YH, Kim SH. Interleukin-2 gene-encoded stromal cells inhibit the growth of metastatic cholangiocarcinomas. *World J Gastroenterol* 2006; **12**: 1889-1894 [PMID: 16609995]
39 **Liao W**, Lin JX, Leonard WJ. IL-2 family cytokines: new insights into the complex roles of IL-2 as a broad regulator of T helper cell differentiation. *Curr Opin Immunol* 2011; **23**: 598-604 [PMID: 21889323 DOI: 10.1016/j.coi.2011.08.003]
40 **Muhitch JB**, Schwaab T. High-dose IL-2 for metastatic renal cell carcinoma: can the first antitumor immunotherapy be reinvented? *Immunotherapy* 2014; **6**: 955-958 [PMID: 25341116 DOI: 10.2217/imt.14.78]
41 **Rosenberg SA**, Yannelli JR, Yang JC, Topalian SL, Schwartzentruber DJ, Weber JS, Parkinson DR, Seipp CA, Einhorn JH, White DE. Treatment of patients with metastatic melanoma with autologous tumor-infiltrating lymphocytes and interleukin 2. *J Natl Cancer Inst* 1994; **86**: 1159-1166 [PMID: 8028037 DOI: 10.1093/jnci/86.15.1159]
42 **Rosenberg SA**, Lotze MT, Yang JC, Aebersold PM, Linehan WM, Seipp CA, White DE. Experience with the use of high-dose interleukin-2 in the treatment of 652 cancer patients. *Ann Surg* 1989; **210**: 474-484; discussion 484-485 [PMID: 2679456]
43 **Klapper JA**, Downey SG, Smith FO, Yang JC, Hughes MS, Kammula US, Sherry RM, Royal RE, Steinberg SM, Rosenberg S. High-dose interleukin-2 for the treatment of metastatic renal cell carcinoma : a retrospective analysis of response and survival in patients treated in the surgery branch at the National Cancer Institute between 1986 and 2006. *Cancer* 2008; **113**: 293-301 [PMID: 18457330 DOI: 10.1002/cncr.23552]
44 **Kaida M**, Morita-Hoshi Y, Soeda A, Wakeda T, Yamaki Y, Kojima Y, Ueno H, Kondo S, Morizane C, Ikeda M, Okusaka T, Takaue Y, Heike Y. Phase 1 trial of Wilms tumor 1 (WT1) peptide vaccine and gemcitabine combination therapy in patients with advanced pancreatic or biliary tract cancer. *J Immunother* 2011; **34**: 92-99 [PMID: 21150717 DOI: 10.1097/CJI.0b013e3181fb65b9]
45 **Yamamoto K**, Ueno T, Kawaoka T, Hazama S, Fukui M, Suehiro Y, Hamanaka Y, Ikematsu Y, Imai K, Oka M, Hinoda Y. MUC1 peptide vaccination in patients with advanced pancreas or biliary tract cancer. *Anticancer Res* 2005; **25**: 3575-3579 [PMID: 16101182]
46 **Aruga A**, Takeshita N, Kotera Y, Okuyama R, Matsushita N, Ohta T, Takeda K, Yamamoto M. Phase I clinical trial of multiple-peptide vaccination for patients with advanced biliary tract cancer. *J Transl Med* 2014; **12**: 61 [PMID: 24606884 DOI: 10.1186/1479-5876-12-61]
47 **Aruga A**, Takeshita N, Kotera Y, Okuyama R, Matsushita N, Ohta

T, Takeda K, Yamamoto M. Long-term Vaccination with Multiple Peptides Derived from Cancer-Testis Antigens Can Maintain a Specific T-cell Response and Achieve Disease Stability in Advanced Biliary Tract Cancer. *Clin Cancer Res* 2013; **19**: 2224-2231 [PMID: 23479678 DOI: 10.1158/1078-0432.CCR-12-3592]

48 **Yoshitomi M**, Yutani S, Matsueda S, Ioji T, Komatsu N, Shichijo S, Yamada A, Itoh K, Sasada T, Kinoshita H. Personalized peptide vaccination for advanced biliary tract cancer: IL-6, nutritional status and pre-existing antigen-specific immunity as possible biomarkers for patient prognosis. *Exp Ther Med* 2012; **3**: 463-469 [PMID: 22969912]

49 **Lepisto AJ**, Moser AJ, Zeh H, Lee K, Bartlett D, McKolanis JR, Geller BA, Schmotzer A, Potter DP, Whiteside T, Finn OJ, Ramanathan RK. A phase I/II study of a MUC1 peptide pulsed autologous dendritic cell vaccine as adjuvant therapy in patients with resected pancreatic and biliary tumors. *Cancer Ther* 2008; **6**: 955-964 [PMID: 19129927]

50 **Kobayashi M**, Sakabe T, Abe H, Tanii M, Takahashi H, Chiba A, Yanagida E, Shibamoto Y, Ogasawara M, Tsujitani S, Koido S, Nagai K, Shimodaira S, Okamoto M, Yonemitsu Y, Suzuki N, Nagaya M. Dendritic cell-based immunotherapy targeting synthesized peptides for advanced biliary tract cancer. *J Gastrointest Surg* 2013; **17**: 1609-1617 [PMID: 23877328 DOI: 10.1007/s11605-013-2286-2]

51 **Shimizu K**, Kotera Y, Aruga A, Takeshita N, Takasaki K, Yamamoto M. Clinical utilization of postoperative dendritic cell vaccine plus activated T-cell transfer in patients with intrahepatic cholangiocarcinoma. *J Hepatobiliary Pancreat Sci* 2012; **19**: 171-178 [PMID: 21874278 DOI: 10.1007/s00534-011-0437-y]

52 **Recchia F**, Sica G, Candeloro G, Bisegna R, Bratta M, Bonfili P, Necozione S, Tombolini V, Rea S. Chemoradioimmunotherapy in locally advanced pancreatic and biliary tree adenocarcinoma: a multicenter phase II study. *Pancreas* 2009; **38**: e163-e168 [PMID: 19531969 DOI: 10.1097/MPA.0b013e3181abe222]

53 **Koido S**, Kan S, Yoshida K, Yoshizaki S, Takakura K, Namiki Y, Tsukinaga S, Odahara S, Kajihara M, Okamoto M, Ito M, Yusa S, Gong J, Sugiyama H, Ohkusa T, Homma S, Tajiri H. Immunogenic modulation of cholangiocarcinoma cells by chemoimmunotherapy. *Anticancer Res* 2014; **34**: 6353-6361 [PMID: 25368235]

54 **Higuchi R**, Yamamoto M, Hatori T, Shimizu K, Imai K, Takasaki K. Intrahepatic cholangiocarcinoma with lymph node metastasis successfully treated by immunotherapy with CD3-activated T cells and dendritic cells after surgery: report of a case. *Surg Today* 2006; **36**: 559-562 [PMID: 16715430]

55 **Khan JA**, Yaqin S. Successful immunological treatment of gallbladder cancer in India--case report. *J Zhejiang Univ Sci B* 2006; **7**: 719-724 [PMID: 16909473 DOI: 10.1631/jzus.2006.B0719]

56 **Kan N**, Yoshikawa K, Matsushita N, Fujii T. [The case of a patient with peritoneal metastasis from cholangiocarcinoma who responded to adoptive immunotherapy and cetuximab]. *Gan To Kagaku Ryoho* 2013; **40**: 1759-1761 [PMID: 24393913]

57 **Galluzzi L**, Senovilla L, Zitvogel L, Kroemer G. The secret ally: immunostimulation by anticancer drugs. *Nat Rev Drug Discov* 2012; **11**: 215-233 [PMID: 22301798 DOI: 10.1038/nrd3626]

58 **Yadav M**, Jhunjhunwala S, Phung QT, Lupardus P, Tanguay J, Bumbaca S, Franci C, Cheung TK, Fritsche J, Weinschenk T, Modrusan Z, Mellman I, Lill JR, Delamarre L. Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. *Nature* 2014; **515**: 572-576 [PMID: 25428506 DOI: 10.1038/nature14001]

59 **Vanneman M**, Dranoff G. Combining immunotherapy and targeted therapies in cancer treatment. *Nat Rev Cancer* 2012; **12**: 237-251 [PMID: 22437869 DOI: 10.1038/nrc3237]

60 **Topalian SL**, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012; **366**: 2443-2454 [PMID: 22658127 DOI: 10.1056/NEJMoa1200690]

61 **Herbst RS**, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, Sosman JA, McDermott DF, Powderly JD, Gettinger SN, Kohrt HE, Horn L, Lawrence DP, Rost S, Leabman M, Xiao Y, Mokatrin A, Koeppen H, Hegde PS, Mellman I, Chen DS, Hodi FS. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 2014; **515**: 563-567 [PMID: 25428504 DOI: 10.1038/nature14011]

62 **Ha H**, Nam AR, Bang JH, Choi Y, Oh DY, Kim TY, Lee KH, Han SW, Im SA, Kim TY, Bang YJ. Measurement of soluble programmed death-ligand 1 (soluble PD-L1) to predict survival in biliary tract cancer patients treated with chemotherapy. *J Clin Oncol* 2015; **33** (suppl): abstr 11094

63 **Liu WM**, Fowler DW, Smith P, Dalgleish AG. Pre-treatment with chemotherapy can enhance the antigenicity and immunogenicity of tumours by promoting adaptive immune responses. *Br J Cancer* 2010; **102**: 115-123 [PMID: 19997099 DOI: 10.1038/sj.bjc.6605465]

64 **Lesterhuis WJ**, Punt CJ, Hato SV, Eleveld-Trancikova D, Jansen BJ, Nierkens S, Schreibelt G, de Boer A, Van Herpen CM, Kaanders JH, van Krieken JH, Adema GJ, Figdor CG, de Vries IJ. Platinum-based drugs disrupt STAT6-mediated suppression of immune responses against cancer in humans and mice. *J Clin Invest* 2011; **121**: 3100-3108 [PMID: 21765211 DOI: 10.1172/JCI43656]

P- Reviewer: Harmanci O, Kassir R, Ogino S
S- Editor: Gong XM **L- Editor**: A **E- Editor**: Jiao XK

World Journal of Gastrointestinal Oncology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4251/wjgo.v7.i11.347

World J Gastrointest Oncol 2015 November 15; 7(11): 347-355
ISSN 1948-5204 (online)

Current status of familial gastrointestinal polyposis syndromes

Ioan Jung, Simona Gurzu, Gligore Sabin Turdean

Ioan Jung, Simona Gurzu, Gligore Sabin Turdean, Department of Pathology, University of Medicine and Pharmacy of Tirgu-Mures, 540139 Tirgu Mures, Romania

Author contributions: Jung I designed research and approval of the final variant of the article; Gurzu S designed research and drafted the article; Turdean GS analysed and interpreted the literature data.

Supported by The University of Medicine and Pharmacy of Tirgu-Mures, Romania, team research projects frame: UMFTGM-PO-CC-02-F01, No. 19/2014.

Conflict-of-interest statement: None declared.

Correspondence to: Dr. Simona Gurzu, MD, PhD, Professor in Pathology, Department of Pathology, University of Medicine and Pharmacy of Tirgu-Mures, 38 Ghe Marinescu Street, 540139 Tirgu Mures, Romania. simonagurzu@yahoo.com
Telephone: +40-745-673550
Fax: +40-265-210407

Received: June 1, 2015
Peer-review started: June 3, 2015
First decision: July 27, 2015
Revised: August 24, 2015
Accepted: September 16, 2015
Article in press: September 18, 2015
Published online: November 15, 2015

Abstract

Because of the rarity of familial gastrointestinal cancer-predisposing syndromes, their exploration in literature is not extensive. In this review, an update of the clinicopathological and molecular criteria of gastrointestinal familial polyposis syndromes with potential malignant transformation is performed. In addition, a guide for screening and surveillance was synthesized and a distribution of gene mutations according to the specific syndromes and geographic distribution was included. The following inherited polyposes syndromes were analyzed: familial adenomatous polyposis, the hamartomatous familial polyposes (Juvenile polyposis, Peutz-Jeghers syndrome, Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, hereditary mixed polyposis syndrome, Gorlin syndrome, Birt-Hogg-Dube syndrome, neurofibromatosis type I and multiple endocrine neoplasia syndrome 2B), Li-Fraumeni syndrome, and MUTYH-associated adenomatous polyposis. For proper medical care, subspecialization of gastroenterologists, pathologists, and geneticists in the field of familial diseases should be introduced in the medical curriculum.

Key words: Inherited polyposis syndromes; Hereditary cancer; Stomach; Intestine; Colorectal

Core tip: In this review the clinicopathological and histological aspects of inherited polyposes syndromes of the gastrointestinal tract are explored in detail. In addition, a guide for surveillance is proposed.

Jung I, Gurzu S, Turdean GS. Current status of familial gastrointestinal polyposis syndromes. *World J Gastrointest Oncol* 2015; 7(11): 347-355 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i11/347.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i11.347

INTRODUCTION

The familial cancer-predisposing syndromes of the

gastrointestinal tract are heterogeneous groups of diseases with the lifetime risk of gastrointestinal cancer generally low but their associated morbidities should be very attentively examined for developing specific programs of familial screening. Because these syndromes are relatively rare in the daily activity, management of their diagnosis and therapy is difficult.

These syndromes include, in particular, the following inherited polyposes syndromes: familial adenomatous polyposis (FAP), hamartomatous polyposis syndromes (Juvenile polyposis, Peutz-Jeghers syndrome, Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, hereditary mixed polyposis syndrome, Gorlin syndrome, Birt-Hogg-Dube syndrome, neurofibromatosis type Ⅰ, and multiple endocrine neoplasia syndrome 2B), Li-Fraumeni syndrome, and MUTYH-associated adenomatous polyposis. They are usually diagnosed from the stomach to the rectum, the esophagus and anal canal being only secondarily involved[1-30]. Although Cronkhite-Canada- and Proteus syndrome[22] are also polyposis syndromes of the gastrointestinal tract, they do not present familial predisposition and are not included in this paper.

In this review, an update of clinicopathological criteria used for diagnosis of the inherited cancer-predisposing syndromes of the gastrointestinal tract and identification of eligible families was performed, followed by revision of criteria of screening and surveillance in the daily practice. A synthesis of data regarding the molecular profile of hereditary syndromes and their geographic particularities are synthesized in Table 1, based on our experience and literature data[1-36].

CLINICOPATHOLOGICAL AND MOLECULAR FEATURES

FAP

FAP is a rare autosomal dominant syndrome (1:8300 live births), that is characterized by the presence of hundreds to thousands of adenomatous polyps scattered throughout colorectal mucosa[36] (Figure 1). It is produced through mutations of the adenomatous polyposis coli (*APC*) gene that was firstly described in 1991[1]. The risk for rectal adenocarcinomas is 87% up to 45 years of age and rise by 100% in older ages, but other colorectal segments can also be affected[1,28]. FAP-related colorectal cancer (CRC) represent < 1% of all CRC cases[36].

Other extracolonic associated lesions include small bowel, periampullary and gastric adenomatous polyps, adrenal adenomas and carcinomas[32]. The lifetime risk of occurrence of duodenal polyps is almost 100%[28]. The second and third portion of duodenum, including the periampullary region, are more predisposped to present adenomas[28].

Regarding the stomach, the adenomatous polyps were reported to occur in 12%-84% of patients with FAP but less than half of them are focally dysplastic and below 1% present malignant transformation[2,3]. They are located mostly in the antrum, followed by gastric fundus[2,28]. However, fundic gland polyps can also occur sporadically not only within FAP[2]. The reported incidence of sporadic fundic gland polyps is about 1%-2% of all middle-aged healthy females who underwent upper endoscopy, more rare in males (30% of all cases) while the familial ones are usually multiple, occur at younger ages, and have an equal gender distribution[3]. Microscopically, the fundic gland polyps consist of cystically dilated oxyntic glands lined by parietal cells, chief cells, and neck cells, with apical mucin bubbles[2,4,5]. Dysplasia occurs in the covering neck cells and/or foveolar epithelium and dysregulation of epithelial proliferation is immunohistochemically (IHC) proved by loss of the normal inverse topographic distribution of Ki-67 proliferation marker and the cyclin-dependent kinase inhibitor p21 (WAF1/CIP1)[2,4-6]. In these cases, for unknown reasons, a more increased risk for gastric intestinal-type adenocarcinomas have been reported in Japanese and Korean populations (four-fold) while no significant risk, when compared with the general population, was encountered in the Western countries (two-fold)[2,4-6]. Although FAP syndrome is not rare in Romanian patients, we did not have cases with associated gastric lesions (personal communication).

Gardner's syndrome is a variant of FAP characterized by APC mutation-related gastrointestinal polyps and associated osteomas, dental abnormalities (supranumerary teeth), epithelial and mesenchymal tumors of the skin (epidermoid cysts, lipoma, fibroma, leiomyoma), desmoid tumors (most frequently in the abdominal wall or intra-abdominal), congenital hypertrophy of the retinal pigment epithelium and tumors of the thyroid gland[28,32,34]. Congenital hypertrophy of the retinal pigment epithelium is the commonest extracolonic manifestation of FAP that occurs in 70%-80% of patients[28]. It is characterized by occurrence of gray-brown round lesions in the retina, the clinical significance being not known yet[28].

In Turcot's syndrome, the FAP is associated with tumors of the central nervous system, especially medulloblastoma[32].

The attenuated *FAP* (AFAP) is a less severe form of FAP that is characterized by predominance of proximally located polyps of the colon (10-99 adenomatous polyps), a later age of onset and a lower risk (lifetime cumulated risk < 70%) for developing CRC[7,32].

MUTYH-associated polyposis

It is an autosomal recessive syndrome produced through mutations of the mutY homolog (*MUTYH*) gene that was firstly described in 2002 in three members of a British family[27,28,35]. MUTYH-associated polyposis (MAP) is clinically similar to the AFAP, being characterized by the early-onset of multiple adenomatous polyps of the colorectal segments (10-99 adenomatous or serrated polyps), with risk for malignant transformation,

Table 1 The molecular profile and geographic particularities of inherited gastrointestinal cancer-predisposing syndromes[1-36]

Name of the syndrome	Mutated genes	Type of mutation	Geographic particularities
FAP	*APC*: Exon 15 - first half (54% of patients with FAP)	Classic phenotype: mutations between codons 178 and 309, and between 409 and 1580 (exons 5-8 and 9-14) Germline truncation (C > T), especially at codons 1309 and 1061: Nonsense mutations (28%) Small insertions (10%) Small deletions (46%)	NS
	APC: Chromosome arms 5q, 8p, 17p and 18q	LOH	NS
	β-catenin: Exon 3 (15%)	NS	NS
	APC/*β-catenin* (28%)	NS	NS
	K-ras: Codon 12 (3%) - associated mutation	GGT to TGT/GTT	NS
Gardner syndrome	*APC*: Long arm of chromosome 5	Interstitial deletion	NS
	APC: Patients with congenital hypertrophy of the retinal pigment epithelium	Truncating mutations between codons 311 and 1465	NS
	APC: Patients with desmoid tumor	Downstream codon 1400 (1445-2011)	NS
	APC: Patients with gastro-duodenal adenomas	Mutations at the 3′ before codon 1395 and between codons 564 and 1493	NS
	APC: Patients with hepatoblastomas	Mutations at the 5′ to the mid region between codons 141 and 1751	NS
	APC: Patients with thyroid tumors	Mutations between codons 140 and 1309	NS
AFAP	*APC*	Somatic G:C→T:A	NS
	APC: Exons 3 and 4 (5′ end of the gene), exon 9, and the very 3′ end of the gene beyond codon 1595	Truncating mutation	NS
	APC: Variants	Missense mutations I1307 K N1026S E1317Q	I1307K: almost exclusively in Ashkenazi Jewish descendents - detected in 6% of all family members, with 10%-20% lifetime risk of developing CRC N1026S: Identified in one Spanish AFAP family (all members) E1317Q: NS
MUTYH-associated polyposis	*MUTYH*: Located on the chromosome 1p34.3-p32.1, contains 16 exons	Germline biallelic inactivation	Absent in Asia (Japan, Taiwan, South Korea)
	MUTYH variants	Missense mutations: p.Y179C - exon 7 (c.536A > G; p.Tyr179Cys) p.G396D - exon 13 (c.1187g > A;p.Gly396Asp)	Specific for Eastern, Southern, and Central Europe, North America, European inhabitants from Canada, and Sephardi Jews Absent in Finland, India, Pakistan, Tunisia, Singapore, and Ashkenazi Jewish
		Missense mutation p.Ala385ProfsX23 p.E410GfsX43 Missense mutation p.Y104X Missense mutation p.E480X Heterozygous mutations	Specific for Northern Europe Specific for Tunisia Specific for Pakistan Specific for India Asia (Japan, Taiwan, South Korea): p.Arg19; p.Arg109Trp; p.Gly286Glu Southern Europe: p.Glu480del Pakistan: p.Tyr104 India: p.Glu480
	K-ras: Codon 12 - associated mutation (64%), usually in patients with sessile serrated adenomas	c.34G > T	NS
Juvenile polyposis syndrome (pure type)	*MADH4*/*SMAD4*/*DPC4*: Chromosome 18q21.1 (30%)	NS	NS
	BMPR1A: Chromosome 10q23 (20%-30%)	Large deletions	NS
	Other genes (49%)	NS	NS
	ENG: exons 11, 12		
	PTEN: chromosome 10q23.3		
Juvenile polyposis + hemorrhagic telangiectasia	*MADH4*/*SMAD4*/*DPC4*: Chromosome 18q21.1	NS	NS
	STK11: Chromosome 19p13.3 or 19q13.4 (50%-94%)	NS	NS
Peutz-Jeghers syndrome	*TGF-β*	NS	NS
	PTEN: Chromosome 10q23.3		

Peutz-Jeghers syndrome	*ALK1/ACVRL1*	NS	NS
+ primary pulmonary hypertension		NS	NS
Cowden syndrome	*PTEN*: Chromosome 10q23.3 (13-85%)	Nonsense mutations missense mutations frameshift mutations Large deletions	NS
Bannayan-Riley-Ruvalcaba syndrome	*PTEN*: Chromosome 10q23.3 (60%-65%)	NS	NS
Hereditary mixed polyposis syndrome	*BMPR1A*: Chromosome 10q23	NS	NS
	GREM1	NS	NS
Li-Fraumeni syndrome – classic type	*p53*: Exons 4-9 (23%-50%)	NS	NS
Unclassified/ unexplained polyposis syndromes (50%)	*PTEN*: Chromosome 10q23.3	Nonsense mutations missense mutations frameshift mutations	NS
	Other genes: *BMPR2*, *ACRV1*, *SMAD1*, *SMAD2*, *SMAD3*, *SMAD5*, *SMAD7* (22%)	NS	NS

FAP: Familial adenomatous polyposis; BMPR: Bone morphogenetic protein receptor; CRC: Colorectal cancer; ENG: Endoglin; FAP: Familial adenomatous polyposis syndrome; LOH: Loss of heterozygosity; NS: Non-specified; TGF: Transforming growth factor.

Figure 1 Macroscopic aspect of the colonic mucosa in a 43 years old male with classic Familial adenomatous polyposis.

and infrequent extracolonic manifestations[25-28]. The phenotype of MAP is less severe than classic FAP[36]. In some of the cases, MAP-related CRC can be developed without the polyposis background, the differential diagnosis with Lynch syndrome being difficult[35].

Juvenile polyposis syndrome

It is a rare autosomal dominant hereditary syndrome (1:100000-160000 live births) characterized by identification of 1-100 hamartomatous polyps throughout the gastrointestinal tract, mostly in the colorectal segments, diagnosed in young patients[8-12]. Microscopically, these polyps are covered by normal columnar epithelium and present mucus-filled tortuous dilated glands lined by columnar epithelium in the lamina propria; the dense stroma is edematous and rich in inflammatory infiltrate predominantly composed of plasma cells[8,11,13]. The clinical diagnosis is based on at least one of the following Jass's modified criteria[6,12]: (1) Multiple juvenile polyps throughout the gastrointestinal tract; (2) At least five colorectal juvenile polyps; or (3) Any number of juvenile polyps identified in patients with a family history of juvenile polyps. These polyps can present malignant transformation, the lifetime risk being about 34%-38% for colorectal segments and 21% for stomach[9,10,12]. Juvenile polyposis-related gastric cancers are rather produced through *SMAD4* than *BMPR1A* mutation genes[12]. Association with hereditary hemorrhagic telangiectasia also known as Osler-Weber-Rendu syndrome have been reported in about 20% of the cases; protein-losing enteropathy can also be associated[9,13].

Peutz-Jeghers syndrome

This syndrome is a rare autosomal dominant inherited disorder (1:8300-200000 live births) associated with a lifetime hazard for cancer up to 93%, which occurs as a consequence of a germline mutation in the *STK11* gene[12,14-16]. It is characterized by familial gastrointestinal hamartomatous polyposis and 1-5 mm mucocutaneous melanic spots around the mouth, in the buccal mucosa, on the fingertips and toes, and, infrequently, on the eyelid and sole of the foot[16]. The spots occur in first years of life; the skin spots spontaneously disappear at puberty but mucosal spots remains visible per life[16].

Regarding the polyps, the upper jejunum is most frequently involved (78%), followed by colon and stomach (24%)[15-19]. Solitary gastric polyps can occur rarely, less than 30 cases being reported to 2012[17]. Microscopically, the gastrointestinal hamartomatous polyps, that can undergo focal or total malignant transformation, are characterized by hyperplastic mucosal glands with periglandular proliferation of smooth muscle fibers[16,17]. Arborizing pattern of smooth muscle proliferation is characteristic[15,16]. In solitary polyps of the stomach, it was suggested that the branching of

the muscularis mucosae are not so well developed in the subsequent layers[15,17]. Gallbladder, bronchi, urinary bladder, and the ureter can also present hamartomatous polyps with similar histological architecture and further possible malignization[12].

Multiple synchronous or metachronous colonic and extra-colonic carcinomas of different organs like breast (54%), pancreas (36%), stomach (29%), ovary (21%), small bowel (13%), or other organs (cervix, uterus, testes, lung, appendix), can be associated in the same patient or his first-degree relatives, with a cumulative risk over 90%[12,15-18]. Associated lymphomas and sex-cord tumors were also encountered[16].

For a final diagnosis, one of the following criteria should be filled[12,14-19]: (1) At least two histologically proved Peutz-Jeghers polyps; (2) At least one histologically proved Peutz-Jeghers polyp in a patient with specific mucocutaneous spots; (3) Identification of at least one Peutz-Jeghers polyp in a patient with at least one relative with confirmed diagnosis of Peutz-Jeghers syndrome; and (4) Specific mucocutaneous spots in a patient with at least one relative with confirmed diagnosis of Peutz-Jeghers syndrome.

Cowden syndrome

It is an autosomal dominant hereditary syndrome that occur in 1:200000 live births (more frequent in Asian population). It is characterized by synchronous or metachronous tumors in multiple organs that occur in one patient or in members of his family. This familial gastrointestinal hamartomatous polyposis occurs as a result of mutations in the phosphatase and tensin (*PTEN*) gene.

The clinical diagnosis is based on the following International Cowden Cosortium major criteria, modified by the National Comprehensive Cancer Network Cowden syndrome[9,12,14,19,20]: macrocephaly (75%-97% of the cases - 58 cm for women and 60 cm for men), multiple (at least 3) gastrointestinal hamartomas including ganglioneuromas but excluding hyperplastic polyps (50%), dysplastic ganglyocytomas of the cerebellum associated with seizures, tremors, and disorders of coordination (Lhermitte-Duclos syndrome), breast cancer (37%), nonmedullary (follicular) thyroid carcinoma (16%), endometrial cancer, and macular pigmentation of the glans penis. The mucocutaneous lesions are considered as pathognomonic (major criteria) only if the following associations are identified[12,20]: At least three trichilemmomas (at least one being biopsically proved), at least three acral keratoses, at least three mucocutaneous neuromas, or oral papillomas (at least three without biopsy or at least one biopsically proved). The minor criteria are presence of benign lesions of the breast (fibrocystic change, benign epithelial tumors), thyroid (multinodular goiter, adenoma, papillary carcinoma), single lesion of the gastrointestinal tract (adenoma, lipoma, hamartoma), at least three lipomas, testicular lipomatosis, malformations or tumors of the urogenital tract, vascular malformations, and mental retardation (IQ $\leqslant$ 75)[12,19,20]. Recently, the autism spectrum disorders, colon/renal cancer, and esophageal glycogenic acanthosis (at least three) were included in the minor criteria[12]. For a final diagnosis, the following associations are necessary: at least three major criteria [at least one being macrocephaly, Lhermitte-Duclos syndrome (in adults), or gastrointestinal hamartomas], two major and three minor, or three minor criteria[12,19,20]. Absence of one of the associated criteria allows the diagnosis of the "Cowden syndrome-like family"[19].

Gastrointestinal hamartomas occur in 50% of patients with Cowden syndrome, being currently considered the second most common feature, after macrocephaly[19]. The estimated lifetime risk for malignancy at the age of 70 is 85% for any cancer, 77%-85% for breast and 35%-38% for thyroid cancer, 33% for renal cancer, 28% for endometrial, 7%-15% for CRC and 6% for melanoma[12,15,20,21]. Gastric malignancy is rarely associated, 1/100 patients with Cowden syndrome being affected[20].

Other hamartomatous polyposis syndromes

Besides Cowden syndrome, *PTEN* gene mutations were described in patients with Bannayan-Riley-Ruvalcaba- and hereditary mixed polyposis syndrome[7,12].

Bannayan-Riley-Ruvalcaba syndrome is an autosomal dominant disorder characterized by hamartomatous polyps of the small intestine and colon (25% of the cases) along with genital spots, macrocephaly, subcutaneous/visceral lipomas including lipomatosis of the glans penis, hemangiomas, and mental retard[7].

In some cases, identification of the specific genetic syndrome is very difficult, the recommended diagnosis being hereditary mixed polyposis syndrome. In this category, association of atypical juvenile polyps, hyperplastic polyps, sessile serrated adenomas, and adenomatous polyps can be associated with increased risk for CRC[7].

Other very rare familial hamartomatous syndromes that can include hamartomatous polyps of the gastrointestinal tract are the following[7,12]: Gorlin syndrome (consequence of *PTCH1* mutations), characterized by hyperkeratosis of palms, soles, and jaw, skeleton abnormalities, macrocephaly, frontal bossing, and associated medulloblastoma and basal-cell carcinomas; multiple endocrine neoplasia syndrome 2B (consequence of *RET* mutations), characterized by neuromas of the lips and tongue, and associated pheochromocytoma and medullary thyroid cancer; neurofibromatosis type Ⅰ (consequence of *NF1* mutations), characterized by café au lait spots, axillaries and inguinal freckling, and associated neurofibromas, gliomas, malignant peripheral nerve sheath tumors, and tumors of the breast; and Birt-Hogg-Dube syndrome (consequence of *FLCN* mutations), characterized by spontaneous pneumothorax and associated fibrofolliculomas of the skin, and renal tumors.

Li-Fraumeni syndrome

It is an autosomal dominant hereditary cancer syndrome characterized by mutations in the *p53* gene that determines occurrence of leukemia, carcinomas of the breast and adrenal glands, brain tumors, sarcomas of the soft tissues and bone, *etc*[19-21,23-26]. The classic Li-Fraumeni syndrome criteria of eligible families include one family member diagnosed with sarcoma before 45 years of age, a first-degree relative with any type of cancer before 45 years of age, and a first/second relative with any cancer diagnosed before 45 years of age or a sarcoma at any age[19,20]. Similar to Cowden syndrome, absence of one of the associated criteria allows the diagnosis of the "Li-Fraumeni syndrome-like family"[19,23,24].

Gastric carcinoma, preponderantly located in the proximal stomach, is reported to occur in about 2%-5% of carriers with *p53* mutations at the median age of 36 years, ranging between 12 and 74 years[24]. Association of early-onset gastric carcinoma and CRC can involve in 10%-28% of the families with classic Li-Fraumeni syndrome, but carcinomas of the lung, melanomas, lymphomas, and germ cell tumors have also been reported[24]. The incidence of Li-Fraumeni-related gastric cancer is higher in Asian population (Japan and South Korea), when compared with people from United States, being supposed that *p53* mutation could enhance the carcinogenic effect of *H. pylori*[24].

GENETIC COUNSELING AND CRITERIA FOR SURVEILLANCE

In patients with *FAP* and *FAP-variants* including *Gardner syndrome, Turcot syndrome,* and *AFAP,* the main goal of surveillance is to detect the CRC in early stages[28], combining molecular and clinical approaches[33].

The clinico-genetic screening should be performed in all first degree relatives of a patient with FAP and should be started, when it is possible, from the mid adolescence[28].

The genetic screening consists in attentively examination of the *APC* gene, according to the particularities presented in Table 1, after a proper genetic counseling of the patient who should be asked for the informed consent. The gold standard method is the full sequencing of the *APC* gene, to examine all the 15 exons[28]. The mutation cluster region (mutational hotspot of *APC* gene) is the 5'part of exon 15 from codon 1250 to 1464[28]. If no mutations are detected, the current guidelines recommend to continue testing for large gene rearrangements[28,35].

From colonoscopy point of view, it is worthy noticing that the small polyps are mostly limited to the recto-sigmoid at the time of adolescence and only thereafter increase in size and number[28]. However, because half of patients develop adenomatous polyps before puberty and 95% by 35 years, sigmoidoscopy screening is recommended starting at age 12-14 years old and performed every two years in mutation carriers. Identification of adenomas is an indicator for annually total colonoscopy, with biopsies from the suspect areas, until colectomy will be performed, depending on the individual endoscopic features[1,28]. Profilactic colectomy is recommended for multiple ulcerated polyps larger than 1 cm that shows high-grade dysplasia[28]. The type of resection depends on the patient's age and personal decision, number and extension of polyps, and also by the macroscopic aspect of the tumors[28].

At risk family members carrying germline mutations near codon 1300 can present early-onset CRC in their childhood and colonoscopy surveillance should also begin before puberty[32,33]. On the other hand, if the carrying germline mutations suggest risk for AFAP, screening should be carried out every two years from the age of 18-20 years, with focused attention on identification of the right-sided distribution of adenomas. Once adenomatous polyps are identified, endoscopic polypectomy followed by annually total colonoscopy is recommended, followed by colectomy in case of large ulcerated polyps with high-grade dysplasia[28,32]. Postoperative endoscopic follow-up is necessary in patients with rectal remnant, to detect the possible carcinoma of the ileo-anal pouch[28].

For classic FAP, flexible sigmoidoscopy remains the standard of care, whereas in patients with FAP variants the proximal colon should also be explored through total colonoscopy. Modern imagistic methods such as capsule endoscopy and/or entero-CT-scan or entero-MRI can also be used for complex investigations. Because duodenal cancer is the second cause of death of patients with FAP, with 5% lifetime risk[28], gastrointestinal endoscopy is recommended to be carried out every 5 years after identification of the colorectal polyps[28].

Besides the risk for gastrointestinal cancer, the protocol of surveillance should also take into account the extraintestinal manifestations, including papillary carcinoma of the thyroid (the third commonest tumor in patients with FAP, with a risk of about 160 times higher than in general population, and a male to female ratio of 1:17), pancreatic carcinoma but also the central nervous system tumors and neuroblastomas[14,28], based on the genetic particularities shown in Table 1.

Annually thyroid palpation, eventually completed by cervical ultrasonography, is recommended starting at the age 25 years[28,36]. Because patients with FAP present 1000-fold increased risk developing desmoid tumor, compared to the general population[34], diagnosis of such tumors, mostly in the abdominal wall, should be followed by a total colonoscopy, especially in young people. Although benign, due to highly recurrence rate, desmoids tumor represents one of the main causes of death of patients with FAP[28].

For patients diagnosed with *MAP,* the surveillance is identically to those used for AFAP. The colonoscopy surveillance begins at 18-20 years old being carried out every two years and annually after adenomas detection. Upper endoscopy is also recommended every five years

starting at the age of 25-30 years old, to explore the duodenal segments[28,36]. Screening for extra-intestinal manifestations is not recommended. Biallelic *MUTYH* gene mutations should be suspected and explored in patients with colorectal polyposis diagnosed before the age of 50 years, especially in associated serrated adenomas. In first degree relatives the two most common mutations, p.G396D and p.Y179C, should be determined. Identification of at least one of the two missense mutations should be follow up by full gene sequencing[28]. Sequencing should also be done in non-Caucasian suspected patients, focusing on the specific geographic and ethnic particularities shown in Table 1.

For juvenile polyposis syndrome, annual upper and lower endoscopies are recommended to be performed in the *MADH4/SMAD4* carriers by the mid-teens or at the time of initial symptoms, most of the cases being diagnosed around the age of 40 years[8-13]. Modern imagistic methods such as capsule endoscopy and/or entero-CT-scan or entero-MRI can also be used[37].

In the bioptic specimens of gastrointestinal polyps, loss or partial loss of the epithelial expression of SMAD4 protein, with or without retained stromal expression, can be a first sign of suspected *SMAD4* mutation[11]. Proctocolectomy or subtotal colectomy should be considered in patients with multiple polyps, severe symptoms, and/or history of familial CRC, but a specific guideline does not exist[12]. According to the British Society of Gastroenterology and Association of Coloproctology for Great Britain and Ireland, in asymptomatic family at-risk members, including the proved *SMAD4/ BMPR1A* mutations, every 1-2 years colonoscopy is recommended from age 15-18 years until age 70 years and gastroduodenoscopy from the age of 25 years[12,29].

In *SMAD-4* mutation-carriers, investigation for a possible associated hereditary telangiectasia is also recommended[13]. Because severe gastrointestinal bleeding can be associated in these syndromes, long-time intravenous using of low doses of the antiangiogenic (anti-VEGF) drugs such as bevacizumab (2 mg/kg per course, every 3 wk) have been recently proposed[30]. Identification of a pulmonary associated vascular malformation and a dilated thoracic aorta is mandatory to avoid bleeding complications[12].

Decreased SMAD4 expression can also activate the transforming growth factor-β and, as a consequence, breast epithelial malignant proliferation can occur, as in one of the previously reported cases[31]. Duodenal and pancreatic tumors can also occur in these patients[14].

In patients with Peutz-Jeghers syndrome, surveillance for tumors of the colorectum, small intestine, breast, pancreas, and sex-cord tumors should be performed[12,14]. Endoscopic examination of the gastrointestinal tract is recommended to be performed every 3 years beginning from the age of 18 years (and every 1-2 years after the age of 50 years) while suspicion for breast cancer should be excluded based on annual ultrasound examinations from the age of 25-30 years completed by annual mammography from the age of 50 years[12,15]. In symptomatic children, periodic gastrointestinal endoscopy should be done[12]. In patients with Peutz-Jeghers syndrome, the capsule endoscopy proved to have a higher diagnostic sensitivity than the Barium-contrast X-Ray and entero-MRI but the size and location of polyps are difficult to be evaluated[37].

No guidelines for screening of other cancers have been implemented to date.

For Cowden syndrome, being known that breast cancer and thyroid cancer occurs in 25%-50% of females and 3%-10% of all patients, respectively, a personal and familial cancer surveillance for these associated malignancies and also for endometrial cancer in females would be necessary[12,19]. Currently, the gastrointestinal tract surveillance is not routinely recommended below 50 years of age, although an earlier endoscopic colonic and gastric surveillance beginning at the age of 30-35 years with follow-up every 1-2 years was recently suggested, especially for Asian population[20]. However, annual mammogram and vaginal ultrasound with endometrial sampling should be done from age 30 years for women and biannual colonoscopy and renal ultrasound examination from age 35-40 years in both males and females are recommended in the most recent studies[12]. Annual thyroid examination should begin from age 18 or 5-10 years before the earliest thyroid tumor in the family[12].

For the other previously nominated hamartomatous polyposis syndromes, the childhood surveillance should take into account the gastrointestinal and extra-gastrointestinal complications such as bleeding, severe anemia, intussusception, whereas the adults should be examined to detect malignancies in early stages, similar to patients with Cowden syndrome[7,12].

In patients with Li-Fraumeni syndrome, although germline *p53* mutations can be identified in the family members, it is difficult to establish the rules of surveillance, because tumors can occur in every organs[19]. In these "p53 families", screening program is recommended to begin at earlier ages including investigations for breast, colorectal, and gastric cancer detection[19]. However, the guidelines of the National Comprehensive Cancer Network Surveillance recommend colonoscopy as part of the surveillance protocol in these carriers[20].

Because some of the inherited polyposis syndromes remain unexplained/unclassified, the genetic screening should take into account, after a meticulous histological examination, a minimal number of gene mutations, respectively the genes *SMAD4, BMPR1A, STK11,* and *PTEN*[14]. The surveillance protocol should also take into consideration the other nontumor complications such as intussusceptions, ileus, gastrointestinal hemorrhage, and anemia[21].

CONCLUSION

Despite the well-conducted screening programs worldwide, the accurate diagnosis of inherited cancer-

predisposing syndromes of gastrointestinal tract remains difficult. Lack of experience of both gastroenterologists and pathologists, due to rare occurence of these syndromes, increases the difficulty. Subspecialization in the field of familial malignancies and founded of specialized medical centers in this field is essential for future proper medical care.

Because of geographic and ethnic particularities of gene mutations, national and international guidelines of screening and surveillance in these risk families should be elaborated. Development of the IHC markers that could predict specific gene mutation is a cheaper method that can be routinely used to detect these familial cases. Although rare, association of multiple tumors in the same patient is a time- and money-consuming management, the reason why a proper screening and surveillance could benefit both the patient and medical care system.

ACKNOWLEDGMENTS

The English language manuscript was polished by SPI Global Professional Editing Service.

REFERENCES

1 **Buturovic S**. Multiple colon polyposis. *Med Arch* 2014; **68**: 221-222 [PMID: 25568540 DOI: 10.5455/medarh.2014.68.221-222]

2 **Abraham SC**, Park SJ, Mugartegui L, Hamilton SR, Wu TT. Sporadic fundic gland polyps with epithelial dysplasia : evidence for preferential targeting for mutations in the adenomatous polyposis coli gene. *Am J Pathol* 2002; **161**: 1735-1742 [PMID: 12414520 DOI: 10.1016/S0002-9440(10)64450-1]

3 **Koornstra JJ**, Mourits MJ, Sijmons RH, Leliveld AM, Hollema H, Kleibeuker JH. Management of extracolonic tumours in patients with Lynch syndrome. *Lancet Oncol* 2009; **10**: 400-408 [PMID: 19341971 DOI: 10.1016/S1470-2045(09)70041-5]

4 **Abraham SC**, Nobukawa B, Giardiello FM, Hamilton SR, Wu TT. Fundic gland polyps in familial adenomatous polyposis: neoplasms with frequent somatic adenomatous polyposis coli gene alterations. *Am J Pathol* 2000; **157**: 747-754 [PMID: 10980114 DOI: 10.1016/S0002-9440(10)64588-9]

5 **Utsunomiya J**, Maki T, Iwama T, Matsunaga Y, Ichikawa T. Gastric lesion of familial polyposis coli. *Cancer* 1974; **34**: 745-754 [PMID: 4852134 DOI: 10.1002/1097-0142(197409)34: 3<745: : AID-CNCR2820340333>3.0.CO; 2-Y]

6 **Park JG**, Park KJ, Ahn YO, Song IS, Choi KW, Moon HY, Choo SY, Kim JP. Risk of gastric cancer among Korean familial adenomatous polyposis patients. Report of three cases. *Dis Colon Rectum* 1992; **35**: 996-998 [PMID: 1327683 DOI: 10.1007/BF02253505]

7 **Krishnan V**, Chawla A, Wee E, Peh WC. Clinics in diagnostic imaging. 159. Jejunal intussusception due to Peutz-Jeghers syndrome. *Singapore Med J* 2015; **56**: 81-88; quiz 86 [PMID: 25715854 DOI: 10.11622/smedj.2015022]

8 **Howe JR**, Sayed MG, Ahmed AF, Ringold J, Larsen-Haidle J, Merg A, Mitros FA, Vaccaro CA, Petersen GM, Giardiello FM, Tinley ST, Aaltonen LA, Lynch HT. The prevalence of MADH4 and BMPR1A mutations in juvenile polyposis and absence of BMPR2, BMPR1B, and ACVR1 mutations. *J Med Genet* 2004; **41**: 484-491 [PMID: 15235019 DOI: 10.1136/jmg.2004.018598]

9 **Stadler ZK**, Salo-Mullen E, Zhang L, Shia J, Bacares R, Power DG, Weiser M, Coit D, Robson ME, Offit K, Schattner M. Juvenile polyposis syndrome presenting with familial gastric cancer and massive gastric polyposis. *J Clin Oncol* 2012; **30**: e229-e232 [PMID: 22826269 DOI: 10.1200/JCO.2012.41.7949]

10 **Howe JR**, Roth S, Ringold JC, Summers RW, Järvinen HJ, Sistonen P, Tomlinson IP, Houlston RS, Bevan S, Mitros FA, Stone EM, Aaltonen LA. Mutations in the SMAD4/DPC4 gene in juvenile polyposis. *Science* 1998; **280**: 1086-1088 [PMID: 9582123 DOI: 10.1126/science.280.5366.1086]

11 **Johansson J**, Sahin C, Pestoff R, Ignatova S, Forsberg P, Edsjö A, Ekstedt M, Stenmark Askmalm M. A Novel SMAD4 Mutation Causing Severe Juvenile Polyposis Syndrome with Protein Losing Enteropathy, Immunodeficiency, and Hereditary Haemorrhagic Telangiectasia. *Case Rep Gastrointest Med* 2015; **2015**: 140616 [PMID: 25705527 DOI: 10.1155/2015/140616]

12 **Jelsig AM**, Qvist N, Brusgaard K, Nielsen CB, Hansen TP, Ousager LB. Hamartomatous polyposis syndromes: a review. *Orphanet J Rare Dis* 2014; **9**: 101 [PMID: 25022750 DOI: 10.1186/1750-1172-9-101]

13 **Gallione CJ**, Repetto GM, Legius E, Rustgi AK, Schelley SL, Tejpar S, Mitchell G, Drouin E, Westermann CJ, Marchuk DA. A combined syndrome of juvenile polyposis and hereditary haemorrhagic telangiectasia associated with mutations in MADH4 (SMAD4). *Lancet* 2004; **363**: 852-859 [PMID: 15031030 DOI: 10.1016/S0140-6736(04)15732-2]

14 **Sweet K**, Willis J, Zhou XP, Gallione C, Sawada T, Alhopuro P, Khoo SK, Patocs A, Martin C, Bridgeman S, Heinz J, Pilarski R, Lehtonen R, Prior TW, Frebourg T, Teh BT, Marchuk DA, Aaltonen LA, Eng C. Molecular classification of patients with unexplained hamartomatous and hyperplastic polyposis. *JAMA* 2005; **294**: 2465-2473 [PMID: 16287957 DOI: 10.1001/jama.294.19.2465]

15 **Song SH**, Kim KW, Kim WH, Kwon CI, Ko KH, Hahm KB, Park PW, Hong SP. Gastrointestinal cancers in a peutz-jeghers syndrome family: a case report. *Clin Endosc* 2013; **46**: 572-575 [PMID: 24143323 DOI: 10.5946/ce.2013.46.5.572]

16 **Hofmann S**, Barth TF, Kornmann M, Henne-Bruns D. Appendix carcinoid associated with the Peutz-Jeghers syndrome. *Int J Surg Case Rep* 2014; **5**: 964-967 [PMID: 25460448 DOI: 10.1016/j.ijscr.2014.06.024]

17 **Jin JS**, Yu JK, Tsao TY, Lin LF. Solitary gastric Peutz-Jeghers type stomach polyp mimicking a malignant gastric tumor. *World J Gastroenterol* 2012; **18**: 1845-1848 [PMID: 22553412 DOI: 10.3748/wjg.v18.i15.1845]

18 **Giardiello FM**, Brensinger JD, Tersmette AC, Goodman SN, Petersen GM, Booker SV, Cruz-Correa M, Offerhaus JA. Very high risk of cancer in familial Peutz-Jeghers syndrome. *Gastroenterology* 2000; **119**: 1447-1453 [PMID: 11113065 DOI: 10.1053/gast.2000.20228]

19 **Marsh DJ**, Dahia PL, Caron S, Kum JB, Frayling IM, Tomlinson IP, Hughes KS, Eeles RA, Hodgson SV, Murday VA, Houlston R, Eng C. Germline PTEN mutations in Cowden syndrome-like families. *J Med Genet* 1998; **35**: 881-885 [PMID: 9832031 DOI: 10.1136/jmg.35.11.881]

20 **Heald B**, Mester J, Rybicki L, Orloff MS, Burke CA, Eng C. Frequent gastrointestinal polyps and colorectal adenocarcinomas in a prospective series of PTEN mutation carriers. *Gastroenterology* 2010; **139**: 1927-1933 [PMID: 20600018 DOI: 10.1053/j.gastro.2010.06.061]

21 **Offerhaus GJ**, Giardiello FM, Krush AJ, Booker SV, Tersmette AC, Kelley NC, Hamilton SR. The risk of upper gastrointestinal cancer in familial adenomatous polyposis. *Gastroenterology* 1992; **102**: 1980-1982 [PMID: 1316858]

22 **Turner JT**, Cohen MM, Biesecker LG. Reassessment of the Proteus syndrome literature: application of diagnostic criteria to published cases. *Am J Med Genet A* 2004; **130A**: 111-122 [PMID: 15372514 DOI: 10.1002/ajmg.a.30327]

23 **Birch JM**, Hartley AL, Tricker KJ, Prosser J, Condie A, Kelsey AM, Harris M, Jones PH, Binchy A, Crowther D. Prevalence and diversity of constitutional mutations in the p53 gene among 21 Li-Fraumeni families. *Cancer Res* 1994; **54**: 1298-1304 [PMID: 8118819]

24 **Masciari S**, Dewanwala A, Stoffel EM, Lauwers GY, Zheng H, Achatz MI, Riegert-Johnson D, Foretova L, Silva EM, Digianni

L, Verselis SJ, Schneider K, Li FP, Fraumeni J, Garber JE, Syngal S. Gastric cancer in individuals with Li-Fraumeni syndrome. *Genet Med* 2011; **13**: 651-657 [PMID: 21552135 DOI: 10.1097/GIM.0b013e31821628b6]

25 **Shinmura K**, Goto M, Tao H, Kato H, Suzuki R, Nakamura S, Matsuda T, Yin G, Morita M, Kono S, Sugimura H. Impaired 8-hydroxyguanine repair activity of MUTYH variant p.Arg109Trp found in a Japanese patient with early-onset colorectal cancer. *Oxid Med Cell Longev* 2014; **2014**: 617351 [PMID: 24799981 DOI: 10.1155/2014/617351]

26 **Aretz S**, Tricarico R, Papi L, Spier I, Pin E, Horpaopan S, Cordisco EL, Pedroni M, Stienen D, Gentile A, Panza A, Piepoli A, de Leon MP, Friedl W, Viel A, Genuardi M. MUTYH-associated polyposis (MAP): evidence for the origin of the common European mutations p.Tyr179Cys and p.Gly396Asp by founder events. *Eur J Hum Genet* 2014; **22**: 923-929 [PMID: 23361220 DOI: 10.1038/ejhg.2012.309]

27 **Lux A**, Gallione CJ, Marchuk DA. Expression analysis of endoglin missense and truncation mutations: insights into protein structure and disease mechanisms. *Hum Mol Genet* 2000; **9**: 745-755 [PMID: 10749981 DOI: 10.1093/hmg/9.5.745]

28 **Leoz ML**, Carballal S, Moreira L, Ocaña T, Balaguer F. The genetic basis of familial adenomatous polyposis and its implications for clinical practice and risk management. *Appl Clin Genet* 2015; **8**: 95-107 [PMID: 25931827 DOI: 10.2147/TACG.S51484]

29 **Cairns SR**, Scholefield JH, Steele RJ, Dunlop MG, Thomas HJ, Evans GD, Eaden JA, Rutter MD, Atkin WP, Saunders BP, Lucassen A, Jenkins P, Fairclough PD, Woodhouse CR. Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). *Gut* 2010; **59**: 666-689 [PMID: 20427401 DOI: 10.1136/gut.2009.179804]

30 **Wee JW**, Jeon YW, Eun JY, Kim HJ, Bae SB, Lee KT. Hereditary hemorrhagic telangiectasia treated with low dose intravenous bevacizumab. *Blood Res* 2014; **49**: 192-195 [PMID: 25325040 DOI: 10.5045/br.2014.49.3.192]

31 **Armstrong RW**, Borman B. Trends in incidence rates of adenocarcinoma of the oesophagus and gastric cardia in New Zealand, 1978-1992. *Int J Epidemiol* 1996; **25**: 941-947 [PMID: 8921478 DOI: 10.1093/ije/25.5.941]

32 **Heinen CD**. Genotype to phenotype: analyzing the effects of inherited mutations in colorectal cancer families. *Mutat Res* 2010; **693**: 32-45 [PMID: 19766128 DOI: 10.1016/j.mrfmmm.2009.09.004]

33 **Gebert JF**, Dupon C, Kadmon M, Hahn M, Herfarth C, von Knebel Doeberitz M, Schackert HK. Combined molecular and clinical approaches for the identification of families with familial adenomatous polyposis coli. *Ann Surg* 1999; **229**: 350-361 [PMID: 10077047 DOI: 10.1097/00000658-199903000-00008]

34 **Gurbuz AK**, Giardiello FM, Petersen GM, Krush AJ, Offerhaus GJ, Booker SV, Kerr MC, Hamilton SR. Desmoid tumours in familial adenomatous polyposis. *Gut* 1994; **35**: 377-381 [PMID: 8150351 DOI: 10.1136/gut.35.3.377]

35 **Hegde M**, Ferber M, Mao R, Samowitz W, Ganguly A. ACMG technical standards and guidelines for genetic testing for inherited colorectal cancer (Lynch syndrome, familial adenomatous polyposis, and MYH-associated polyposis). *Genet Med* 2014; **16**: 101-116 [PMID: 24310308 DOI: 10.1038/gim.2013.166]

36 **Vasen HF**, Möslein G, Alonso A, Aretz S, Bernstein I, Bertario L, Blanco I, Bülow S, Burn J, Capella G, Colas C, Engel C, Frayling I, Friedl W, Hes FJ, Hodgson S, Järvinen H, Mecklin JP, Møller P, Myrhøi T, Nagengast FM, Parc Y, Phillips R, Clark SK, de Leon MP, Renkonen-Sinisalo L, Sampson JR, Stormorken A, Tejpar S, Thomas HJ, Wijnen J. Guidelines for the clinical management of familial adenomatous polyposis (FAP). *Gut* 2008; **57**: 704-713 [PMID: 18194984 DOI: 10.1136/gut.2007.136127]

37 **Kovács M**, Pák P, Pák G, Fehér J. [Screening and surveillance for hereditary polyposis and non-polyposis syndromes with capsule endoscopy]. *Orv Hetil* 2008; **149**: 639-644 [PMID: 18375363 DOI: 10.1556/OH.2008.28349]

P- Reviewer: Riccioni ME
S- Editor: Tian YL **L- Editor**: A **E- Editor**: Jiao XK

World Journal of Gastrointestinal Oncology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4251/wjgo.v7.i11.356

World J Gastrointest Oncol 2015 November 15; 7(11): 356-360
ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Observational Study

Colorectal cancer screening in an academic center compared to the national average

Manuel O Gonzalez, Lilly M Sadri, Alfred B Leong, Smruti R Mohanty, Parag Mehta

Manuel O Gonzalez, Smruti R Mohanty, Department of Gastroenterology, New York Methodist Hospital, New York, NY 11215, United States

Lilly M Sadri, Department of Medicine, Stony Brook University Hospital, New York, NY 11215, United States

Alfred B Leong, Parag Mehta, Department of Medicine, New York Methodist Hospital, New York, NY 11215, United States

Author contributions: Gonzalez MO analyzed data, wrote the paper, performed research; Sadri LM analyzed data, wrote the paper; Leong AB designed research, performed research; Mehta P analyzed data, performed research; Mohanty SR analyzed data and made critical revisions.

Institutional review board statement: This study received approval by New York Methodist Hospital Institutional Review Board Committee (IRB reference No. 518027).

Informed consent statement: Waiver of informed consent was approved by the New York Methodist Hospital Institutional Review Board Committee as the study was an observational study and demonstrated minimal risk.

Conflict-of-interest statement: There are no conflicts of interests to be disclosed by any of the authors.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at New York Methodist Hospital, who will provide a permanent, citable and open-access home for the dataset.

Correspondence to: Manuel O Gonzalez, MD, Department of Gastroenterology, New York Methodist Hospital, 506 Sixth Street, Brooklyn, New York, NY 11215, United States. drmgonzalez@aol.com
Telephone: +1-305-5622807

Received: April 14, 2015
Peer-review started: April 16, 2015
First decision: June 2, 2015
Revised: June 22, 2015
Accepted: September 2, 2015
Article in press: September 7, 2015
Published online: November 15, 2015

Abstract

AIM: To investigate if the increased emphases on training and education on current colorectal cancer (CRC) screening guidelines has resulted in improved national CRC screening rates in an internal medicine training program, and to determine if the doctor's post graduate year (PGY) level of training affected CRC screening rates.

METHODS: We conducted a cross sectional study of every patient who presented to the outpatient clinic of New York Methodist Hospital, Brooklyn, NY, over the span of six continuous weeks in 2011. A questionnaire was integrated into every patient's medical interview that helped determine that patient's current CRC screening status, screening mammography status if applicable, Papanicolaou smear status if applicable, and current pneumococcal vaccination status. At the same time, patient demographics were also obtained. All of the questionnaire data was collected at the end of each medical visit and was compiled by a designated researcher. After all the data points were collected, it was ensured that the patient has been seen by his or her continuity care resident at least twice in the past. Data was then compiled into a secure, encrypted database to then be analyzed by our statistician.

RESULTS: Data from 547 consecutive clinic visits were obtained. Of these, we reviewed 483 charts that met all of the inclusion criteria and did not meet the exclusion criteria. The data was then analyzed for differences between PGY levels, patient's sex, race, and educational level. The study population consisted of 138 men and 345 women. 35 patients were white (7.40%), 174 were black (39.79%) and 264 were Hispanic (55.81%). Our CRC screening rates were: 66% for PGY-1's, 72% for PGY-2's and 77% for PGY-3's. There was no statistical difference noted between the three groups ($P \leqslant 0.05$) or was there any difference sex, insurance status or educational level. Overall CRC screening rate was 72% which was not different from the New York State average ($P < 0.05$). There was a statistically significant higher rate of CRC screening amongst Hispanics 76% ($P = 0.034$) and in people within the ages of 70-79, 82% ($P = 0.015$).

CONCLUSION: Patients that are followed by internal medicine residents at our urban outpatient teaching clinic did not receive higher rates of CRC screening nor did rates of screening vary with their PGY level.

Key words: Screening; Colorectal cancer; Post graduate year; Colorectal cancer; Residency; Urban

Core tip: It is assumed that greater seniority and experience amongst medical residents can equal improved colorectal cancer screening percentage in an outpatient academic center. We not only compare screening rates between different post graduate years but also compare the medical resident's screening rates to the national average.

Gonzalez MO, Sadri LM, Leong AB, Mohanty SR, Mehta P. Colorectal cancer screening in an academic center compared to the national average. *World J Gastrointest Oncol* 2015; 7(11): 356-360 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i11/356.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i11.356

INTRODUCTION

Despite established screening guidelines, national colorectal cancer (CRC) screening rates vary between 54%-75% of the at risk population[1]. CRC is the third leading cause of cancer-related deaths in the United States when men and women are considered separately, and the second leading cause when both sexes are combined[2]. CRC is expected to cause approximately 49700 deaths during 2015[2]. The American Cancer society estimates that there will be 93090 new cases of colon cancer and 39610 new cases of rectal cancer in 2015[2]. When diagnosed early, CRC is typically curable. Screening guidelines have been developed to help reduce the mortality of CRC. For a person without increased risk factors, starting at the age of 50 years, it has been generally accepted that a colonoscopy every 10 years, flexible sigmoidoscopy (FS) every 5 years or annual fecal occult blood test (FOBT) would be considered a sufficient screening technique[3].

Despite these screening strategies and increased efforts by governing bodies to increase awareness of CRC screening in both the medical community and general public, in 2010 only 54.1%-75.2% of the United States population responded that they were "up to date" with their CRC screening, with the state of New York averaging 69%-75.2%[1].

It is assumed that clinical guidelines are observed and followed more often in an academic training setting like a residency program due to the fact that there is more emphasis on education in an academic setting and the medical residents are under constant supervision. However, we have observed that a majority of resident training involves acute disease management in the inpatient setting and little research has attempted to assess the quality of ambulatory education and resident competence especially for disease prevention and health maintenance[4].

We assessed the CRC screening rates at New York Methodist Hospital in 2010 and compared them to the 2010 New York state screening rates as recognized by the Center for Disease Control (CDC). Furthermore, we wanted to try to recognize possible barriers to CRC screening in our community hospital and try to identify ways that we could improve our CRC screening rates. We felt it was important to ascertain if current efforts to educate physicians in training are effective and to help identify ways to improve education efforts.

MATERIALS AND METHODS

Ambulatory care resident education

The New York Methodist Hospital internal medicine residency program is a traditional, accredited 3 year program consisting of both inpatient and ambulatory based training. At the time of this study there were 106 medical residents providing longitudinal care for patients in the ambulatory clinic. All resident physicians provide patient care in the ambulatory clinic two half days every week throughout all three years of their training. Additionally, residents do 4 to 5 mo solely of ambulatory care without any inpatient responsibilities. During those 4 to 5 mo, residents have a weekly morning rotation in the clinic's gastroenterology clinic and work under the supervision of board certified gastroenterologist. Formal lectures addressing preventive care cancer screening are interspersed throughout the academic year including one lecture focused on colorectal cancer screening in the average risk patient. Throughout their training, residents are given monthly exams; in two of which the primary focus is to test the resident's knowledge on primary prevention and screening strategies.

Table 1 Study population breakdown

Population	Number of patients	Percentage of patients
PGY-level		
PGY-1	170	35.20%
PGY-2	160	33.13%
PGY-3	153	31.68%
Sex		
Female	345	71.43%
Male	138	28.57%
Race		
Blacks	174	36.02%
Whites	35	7.25%
Hispanics	264	54.66%
Other	10	2.07%
Highest educational level		
Elementary school	28	5.80%
Middle school	63	13.04%
High school	186	38.51%
College or University	43	8.90%
Unknown	163	33.75%
Insurance type		
Medicare/Medicaid	288	59.63%
Private Insurance	32	6.63%
Unknown	163	33.75%
Age of patient (yr)		
50-59	179	37.06%
60-69	177	36.65%
70-79	90	18.63%
80-89	34	7.04%
90-99	3	0.62%

PGY: Post graduate year.

Study population

A cross sectional study was taken from patients who received their care at the internal medicine clinic of New York Methodist Hospital over a 6 wk period. Residents were given a questionnaire and integrated it into their clinical data gathering during the patient's clinic visit session. Data was collected after every clinic encounter throughout the six weeks. Exclusion criteria included patients under the age of 50, patients with an increased risk for developing colorectal cancer (family or personal history of adenomatous polyps, CRC, or polyposis syndromes) patients who had previous CRC screening in last 5 years and patients who have been followed by an internal medicine resident for less than 8 mo and had less than 2 clinic visits in which the patient had been seen by their designated resident.

Data collection

Data from 547 consecutive office visits in the internal medicine resident ambulatory clinic over a span of 6 wk was collected. Four hundred and eighty-three of those charts met the inclusion criteria and were selected and reviewed in further detail. The investigators confirmed that there had been a minimum of two clinic visits with their assigned medical resident. Data recorded included patient demographics, patient's level of education, type of medical insurance, data on the use of screening colonoscopy (SC), fecal occult blood testing (FOBT), FS, and other preventative health measures such as influenza vaccination, screening mammography and Pap smear. For the purposes of this study, only the data relevant to CRC screening was analyzed. A patient's CRC screening was considered "up to date" if it met any of the following criteria: (1) the patient has had a SC within the last 10 years;(2) the patient has had a screening FS within the last 5 years; and (3) a FOBT within the last 12 mo. These screening modalities are readily available at our institution and generally accepted as appropriate screening tools[3]. FS, though a well-accepted screening modality, was not included in our survey as the procedure is not offered at our institution. Finally, the data was also then stratified between the resident's level of training (PGY1, PGY2, and PGY3). This study received IRB approval; IRB reference No. 518027.

Statistical analysis

Data was analyzed using the binomial test and the χ^2 distribution test. The binomial statistical test was used to compare the medical resident's screening rate to the New York state's 2010 CDC average of 70.1% and to determine if insurance status, patient's level of education, race, age or sex influenced the results. The χ^2 distribution test was used to determine if there were any statistical differences between the post graduate year level of training, age groups, sex, educational level, insurance status, or race. Statistical significance was defined as $P = 0.05$.

RESULTS

Four hundred and eighty three patients were considered appropriate for inclusion into the study. Table 1 depicts our patient characteristics. The study population consisted of 138 men with a mean age of 63.5 years (range, 50-88 years) and 345 women with a mean age of 64.17 years (range, 50-92 years). Thirty five patients were white (7.40%), one hundred and seventy four were black (39.79%) and two hundred and sixty four were Hispanic (55.81%). Two hundred and twenty nine (47.41%) responded that they had a high school education or above, ninety one (18.84%) responded that their educational level was below high school level and one hundred and sixty three (33.75%) did not provide their educational level. Table 2 depicts our statistical findings. The overall CRC screening rate at our hospital was 72%. We did not observe statistical difference between the CRC screening rates of our hospital compared to the 2010 United States or New York state screening rates as provided by the CDC[1] ($P = 0.05$). There was no observed statistical difference between the screening rates of PGY-1's, PGY-2's, and PGY-3's ($P = 0.096$), sex, insurance status or educational level. There was a statistically significant higher rate of CRC screening amongst Hispanics of 76% ($P = 0.034$) and in people within the ages of 70-79 years of 82% ($P = 0.015$).

Table 2 Statistical analysis comparing our colorectal cancer screening rates to the 2010 New York State screening rates as determined by the Center for Disease Control

Variable	Screening rate	*P* value	*P* value of the χ^2 distribution test comparing variability within groups
PGY-level			
PGY-1	0.66	0.3	
PGY-2	0.72	0.735	0.096
PGY-3	0.77	0.061	
Age of patient (yr)			
50-59	0.64	0.07	
60-69	0.77	0.58	
70-79	0.82	0.015[1]	0.006[1]
80-89	0.61	0.255	
90-99	0.67	1	
Sex			
Female	0.7	0.953	0.33
Male	0.75	0.26	
Race			
Black	0.68	0.508	
Hispanic	0.76	0.034[1]	0.023[1]
Other	0.8	0.733	
White	0.54	0.063	
Highest educational level			
College	0.72	0.869	
Elementary	0.75	0.682	
High School	0.74	0.336	0.888
Middle School	0.72	0.888	
Undisclosed	0.69	0.73	
Insurance type			
Medicare/Medicaid	0.73	0.245	
Private insurance	0.72	1	0.514
Undisclosed	0.68	0.607	
Overall screening rate	0.72	0.48	

[1]Statistical significance is defined as $P = 0.05$. New York State screening rate was standardized to a base rate of 0.701 for comparison. Data was analyzed by binomial statistical analysis. PGY: Post graduate year.

DISCUSSION

Our study did not support the assumption that CRC screening would be offered more frequently at an institution with a residency training program when compared to the state and national average screening rates which include non-teaching outpatient practices. There was a numerical difference between the screening rates of PGY-1 compared to PGY-3 (11%) however statistical significance, possibly due to function of power, was not achieved. Willett *et al*[5] had similar findings in 2005 when they compared PGY-1 and PGY-2 residents in their adherence rates to national guidelines for outpatient preventive health services and found no difference between the two groups for breast and colon cancer screening amongst others.

Despite didactics, emphasis on practicing evidence based medicine, and importance of implementing preventative measures with the use of well accepted screening measures CRC screening in our internal medicine residency training program was still found to be comparable to the national and state average CRC screening rates.

Prior studies have indeed shown poor CRC screening rates amongst internal medicine residents[6]. Numerous studies have elucidated the deficiency in knowledge of and compliance with CRC screening recommendations amongst internal medicine residents[6-9]. Our study however is unique in that we were able to compare the rates of CRC screening at an outpatient clinic of an urban teaching program to state and national rates which include non-teaching practices.

These results highlight the important fact that though we expect and anticipate that teaching programs ingrain the importance of screening and prevention in medicine, for reasons unknown, either fail to do this or just do not seem to reflect this in clinical training practice. If well accepted and proven screening techniques such as CRC screening are not offered more so by physicians in training who are assumed to be "up-to-date" with current screening guidelines and practices through their mandated hours of didactics, this raises the concern that perhaps there needs to be a change in the way both residents and their mentors are trained.

In the future, it is vital that efforts be made to improve education amongst physicians in training regarding CRC guidelines and the importance of CRC screening. A prior study by Gennarelli *et al*[10] showed that knowledge of CRC screening guidelines amongst medical professions is low for both average and high risk patients. Internal medicine residents in our program like most others receive weekly didactics in the form of lectures by attending physicians, fellows, and visiting professors averaging approximately 7 h/wk however these lectures span a wide variety of topics and are not focused on primary prevention or screening. Perhaps physicians in training would benefit from a teaching series focused specifically on preventative measures and screening techniques. A retrospective chart review done by Borum showed that internal medicine residents who had increased exposure to and reinforcement of surveillance recommendations through lectures and required documentation as well as formal FS training adhered to guidelines far more than other resident physicians[7].

Additionally, now that medical records are for the most part transitioning to electronic records across the country, clinical prompts incorporated into the standard outpatient note template may help as a reminder tool for physicians who have adequate knowledge of the topic but for the sake of time and other factors may not necessarily remember to ask their patients regarding their screening status. Seres *et al*[11]. showed that clinical prompts are superior to evidence based lectures when it comes to improving physician CRC screening rates.

Another aspect that must be considered is the patient's role in compliance with recommended screening. 1.5% of our patients had refused CRC screening when offered in the past and it is unknown if they were educated regarding the potential long term consequences of their decision. Residents in training

should learn early on the importance of patient education in both disease prevention and treatment. The realm of primary prevention and screening is one in which patient education regarding the importance of screening and potential dire outcomes of lack of screening become vital. Perhaps implementing use of patient educational tools such as easy-to-read brochures and pamphlets explaining current rates of CRC and screening modalities effect on prevention will help patient's make more educated decisions when it comes to screening. Rowe *et al*[12] even implemented use of an educational video while patients were waiting to be seen by residents.

In assessing the need for further investigations and future direction we will review the limitations of our study. Generalizability of our study, which included only residents from our primarily categorical internal medicine residency program, and if our findings are representative of other residency programs especially those which include family medicine or primary care tracks is of concern. Another limitation of the study is that it was conducted over the span of 6 wk and may not be an adequate representation of overall practice. In addition, the patient population was not a good representation of the different races; with 54.66% of patients were Hispanic and 7.25% Whites, this may explain the perception of higher screening rates in Hispanics as compared to Whites.

COMMENTS

Background

Routine screening has been proven to be an effective tool at preventing colorectal cancer (CRC). Many efforts have been put forth to educate medical professionals on proper CRC screening. The authors investigate if current efforts on CRC screening education are producing improved CRC screening rates.

Research frontiers

The Center for Disease Control has been providing a big push in CRC prevention. Current studies center on methods of improving education towards not only patients but health care providers as well.

Innovations and breakthroughs

This is the only article comparing the screening rate of medical residents compared to the national average and one of the few manuscripts comparing the screening rates between post graduate years (PGY).

Applications

The study results demonstrate that there is no appreciable difference between PGY or compared to the national average. This exposes potential weaknesses in current educational strategies and opens up some proven ideas that may help increase CRC screening rates.

Peer-review

This is a well-designed observational study that was tailored to minimize selection bias. The results can be applied to family medicine and internal medicine training programs alike.

REFERENCES

1 **US Cancer Statistics Working Group**. United States Cancer Statistics: 1999–2010 Incidence and Mortality Web-based Report. Atlanta: US Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute, 2013

2 **American Cancer Society**. Cancer Facts and Figures 2015. Atlanta: American Cancer Society, 2015

3 **Levin B**, Lieberman DA, McFarland B, Andrews KS, Brooks D, Bond J, Dash C, Giardiello FM, Glick S, Johnson D, Johnson CD, Levin TR, Pickhardt PJ, Rex DK, Smith RA, Thorson A, Winawer SJ. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *Gastroenterology* 2008; **134**: 1570-1595 [PMID: 18384785 DOI: 10.1053/j.gastro.2008.02.002]

4 **An PG**, Ashburner JM, Fosburgh BW, Atlas SJ. Performance on preventive cancer screening tests in the ambulatory setting by internal medicine resident physicians. *Teach Learn Med* 2010; **22**: 45-49 [PMID: 20391283 DOI: 10.1080/10401330903446362]

5 **Willett LL**, Palonen K, Allison JJ, Heudebert GR, Kiefe CI, Massie FS, Wall TC, Houston TK. Differences in preventive health quality by residency year. Is seniority better? *J Gen Intern Med* 2005; **20**: 825-829 [PMID: 16117750]

6 **Zack DL**, DiBaise JK, Quigley EM, Roy HK. Colorectal cancer screening compliance by medicine residents: perceived and actual. *Am J Gastroenterol* 2001; **96**: 3004-3008 [PMID: 11693339]

7 **Borum ML**. Medical residents' colorectal cancer screening may be dependent on ambulatory care education. *Dig Dis Sci* 1997; **42**: 1176-1178 [PMID: 9201080]

8 **Sharma VK**, Corder FA, Raufman JP, Sharma P, Fennerty MB, Howden CW. Survey of internal medicine residents' use of the fecal occult blood test and their understanding of colorectal cancer screening and surveillance. *Am J Gastroenterol* 2000; **95**: 2068-2073 [PMID: 10950059]

9 **Barrison AF**, Smith C, Oviedo J, Heeren T, Schroy PC. Colorectal cancer screening and familial risk: a survey of internal medicine residents' knowledge and practice patterns. *Am J Gastroenterol* 2003; **98**: 1410-1416 [PMID: 12818289]

10 **Gennarelli M**, Jandorf L, Cromwell C, Valdimarsdottir H, Redd W, Itzkowitz S. Barriers to colorectal cancer screening: inadequate knowledge by physicians. *Mt Sinai J Med* 2005; **72**: 36-44 [PMID: 15682261]

11 **Seres KA**, Kirkpatrick AC, Tierney WM. The utility of an evidence-based lecture and clinical prompt as methods to improve quality of care in colorectal cancer screening. *Am J Gastroenterol* 2009; **104**: 420-425 [PMID: 19190610 DOI: 10.1038/ajg.2009.73.Epub]

12 **Rowe S**, Goldsmith G, Price R, Brooks A, Harvey A. Health care providers' perspectives of an intervention designed to improve colorectal cancer screening rates in family medicine residency clinics: a qualitative study. *J Cancer Educ* 2012; **27**: 695-702 [PMID: 22826203 DOI: 10.1007/s13187-012-0393-5]

P- Reviewer: Chua AHL, Lee SY
S- Editor: Yu J **L- Editor**: A **E- Editor**: Jiao XK

World Journal of Gastrointestinal Oncology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4251/wjgo.v7.i11.361

World J Gastrointest Oncol 2015 November 15; 7(11): 361-368
ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Prospective Study

Tumor neoangiogenesis detection by confocal laser endomicroscopy and anti-CD105 antibody: Pilot study

Adriana Ciocâlteu, Adrian Săftoiu, Daniel Pirici, Claudia-Valentina Georgescu, Tatiana Cârţână, Dan Ionuţ Gheonea, Lucian Gheorghe Gruionu, Cosmin Gabriel Cristea, Gabriel Gruionu

Adriana Ciocâlteu, Adrian Săftoiu, Tatiana Cârţână, Dan Ionuţ Gheonea, Lucian Gheorghe Gruionu, Cosmin Gabriel Cristea, Gabriel Gruionu, Research Center of Gastroenterology and Hepatology, University of Medicine and Pharmacy of Craiova, 200349 Craiova, Romania

Daniel Pirici, Department of Research Methodology, University of Medicine and Pharmacy of Craiova, 200349 Craiova, Romania

Claudia-Valentina Georgescu, Department of Pathology, Emergency County Hospital, 200642 Craiova, Romania

Lucian Gheorghe Gruionu, Department of Mechanical Engineering, University of Craiova, 200585 Craiova, Romania

Gabriel Gruionu, Edwin Steele Laboratory of Tumor Biology, Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, United States

Author contributions: Ciocâlteu A, Săftoiu A and Gruionu G designed the research; Săftoiu A, Ciocâlteu A, Cârţână T and Gheonea DI performed the research; Pirici D, Gruionu LG and Georgescu CV contributed newreagents/analytic tools; Ciocâlteu A, Cristea CG, Pirici D and Georgescu CV analyzed the data; Ciocâlteu A wrote the paper; Săftoiu A, Gruionu G, Pirici D and Cristea CG revised and edited the paper.

Supported by National Research Council (CNCS), Romania, entitled "Clinical and Biomathematical Modeling of Vascular Changes Following Anti-Angiogenic Therapy in Advanced Colorectal Carcinoma", contract number PN-II-ID-PCE-2011-3-0664, and the European Social Found, Human Resources Development Operational Programme 2007- 2013, No. POSDRU/159/1.5/S/136893.

Institutional review board statement: The current study was conducted according to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004) and approved by the local Ethics Committee (No. 71/29.05.2014).

Clinical trial registration statement: Not applicable.

Informed consent statement: All study participants provided informed written consent prior to study enrollment.

Conflict-of-interest statement: The authors have no competing interests.

Data sharing statement: No additional data are available.

Correspondence to: Adrian Săftoiu, MD, PhD, MSc, FASGE, Professor, Research Center of Gastroenterology and Hepatology Craiova, University of Medicine and Pharmacy Craiova, Romania, 2 Petru Rares str., Craiova, Dolj 200349, Romania. adriansaftoiu@aim.com
Telephone: +40-744-823355
Fax: +40-251-310287

Received: April 3, 2015
Peer-review started: April 4, 2015
First decision: May 18, 2015
Revised: June 9, 2015
Accepted: August 25, 2015
Article in press: September 7, 2015
Published online: November 15, 2015

Abstract

AIM: To evaluate neoangiogenesis in patients with colon cancer by two fluorescently labeled antibodies on fresh biopsy samples imaged with confocal laser endomicroscopy (CLE).

METHODS: CLE is an imaging technique for gastrointestinal endoscopy providing *in vivo* microscopy at subcellular resolution. An important question in validating tumor angiogenesis is what proportion of the tumor vascular network is represented by pre-existing parent tissue vessels and newly formed vessels. CD105 (endoglin) represents a proliferation-associated endothelial cell adhesion molecule. In contrast to pan-endothelial markers, such as CD31, CD105 is preferentially expressed in activated endothelial cells that participate in neovascularization. Thus, we evaluated CD105 and CD31 expression from samples of ten patients with primary rectal adenocarcinoma, using a dedicated endomicroscopy system. A imaging software was used to obtain the Z projection of the confocal serial images from each biopsy sample previously combined into stacks. Vascular density and vessel diameters were measured within two 50 μm x 475 μm rectangular regions of interest centered in the middle of each image in the horizontal and vertical direction. The results were averaged over all the patients and were expressed as the mean ± SE.

RESULTS: The use of an anti-CD105 antibody was found to be suitable for the detection of blood vessels in colon cancer. Whereas anti-CD31 antibodies stained blood vessels in both normal and pathologic colon equally, CD105 expression was observed primarily in malignant lesions, with little or no expression in the vessels of the normal mucosa (244.21 ± 130.7 vessels/mm^3 in only four patients). The average diameter of anti-CD105 stained vessels was 10.97 ± 0.6 μm in tumor tissue, and the vessel density was 2787.40 ± 134.8 vessels/mm^3. When using the anti-CD31 antibody, the average diameter of vessels in the normal colon tissue was 7.67 ± 0.5 μm and the vessel density was 3191.60 ± 387.8 vessels/mm^3, while in the tumors we obtained an average diameter of 10.88 ± 0.8 μm and a vessel density of 4707.30 ± 448.85 vessels/mm^3. Thus, there were more vessels stained with CD31 than CD105 ($P < 0.05$). The average vessel diameter was similar for both CD31 and CD105 staining. A qualitative comparison between CLE *vs* immunohistochemistry lead to similar results.

CONCLUSION: Specific imaging and quantification of tumor microvessels are feasible in human rectal cancer using CLE examination and CD105 immunostaining of fresh tissue samples.

Key words: Rectal cancer; Neoangiogenesis; Confocal laser endomicroscopy; Panendothelial markers; Anti-CD105 antibody

Core tip: We evaluated CD105 expression from fresh tissue samples of human rectal adenocarcinoma, using confocal laser endomicroscopy (CLE). While vessels marked with fluorescent CD31 were visible in both normal and malignant tissue, CD105 was predominantly expressed in tumor lesions, having reduced affinity for normal rectal mucosa. Our data showed that CLE using CD105 antibody for tumor vascular network imaging is feasible and that CD105 represents a more specific marker for rectal cancer neoangiogenesis than panendothelial markers. To our knowledge, this is the first study to report the use of fluorescently-labeled CD105 antibody in conjunction with CLE in patients with rectal tumor.

Ciocâlteu A, Săftoiu A, Pirici D, Georgescu CV, Cârțână T, Gheonea DI, Gruionu LG, Cristea CG, Gruionu G. Tumor neoangiogenesis detection by confocal laser endomicroscopy and anti-CD105 antibody: Pilot study. *World J Gastrointest Oncol* 2015; 7(11): 361-368 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i11/361.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i11.361

INTRODUCTION

Tumor neoangiogenesis, defined as the neo-formation of blood vessels from pre-existing microvessels, represents an attractive target for both imaging and therapeutic strategies. It is thought that neovascularization is first activated by an "angiogenic switch" during premalignant phases of carcinogenesis, before tumors emerge (Folkman *et al*[1]; Bolontrade *et al*[2]; Huss *et al*[3]). An important question in validating tumor neoangiogenesis is what proportion of tumor vascular network is represented by pre-existing *vs* newly formed vessels. In this respect, new imaging and diagnostic techniques which differentiate tumors vascularization at different stages are desired[4].

Antihuman panendothelial cells antibodies are used to identify all types of blood vessels in a given tissue sample, irrespective of being mature or immature. Commonly used panendothelial markers such as CD31, CD34 or von Willebrand factor detect the parent vessels as well as the tumor vasculature, but they are not always expressed in all tumor blood vessels. Moreover, these antibodies seem to have a higher affinity for large than for microvessels[5].

Endoglin (CD105) is a co-receptor for various TGF-β family members and therefore a target for tumor vasculature[6]. The role of endoglin and the indispensable role for the TGF-β signaling pathway in developmental angiogenesis has been studied on genetically modified mice[7-9]. Unlike all other markers, endoglin mediates direct pro-angiogenic effects of TGF-β on endothelial cells and is specifically overexpressed in tumor vessels, on proliferating endothelial cells, at sites of active angiogenesis. Its expression has also been associated with metastasis and patient survival[6,10,11]. Recent reports suggest that elevated plasma levels of endoglin in patients with colorectal cancer correlate with poor prognosis (Li *et al*[7]; Duff *et al*[12]). As a result, endoglin

Table 1 Patient characteristics

Patient	Gender	Age	Tumor grading	Preoperative stage	RT	CTX
1	F	67	G1	T3N0M0	No	No
2	M	65	G2	T3N0M0	Neoadj	No
3	M	47	G2	T3N0M0	Neoadj	No
4	M	66	G2	T4N0M0	Adj	Adj
5	M	54	G2	T3N0M0	No	No
6	M	67	G1/G2	T3N1M0	Neoadj	Neoadj
7	F	80	G1 + Mucinous areas	T3N0M0	Neoadj	Neoadj
8	F	78	G2	T3N2M0	Neoadj	No
9	M	59	G1	T3N1M0	No	No
10	M	69	G1/G2	T3N0M0	Neoadj	No

RT: Radiotherapy; CTX: Chemotherapy; Neoadj: Neoadjuvant therapy; Adj: Adjuvant therapy; F: Female; M: Male.

could represent a valuable tool for the diagnosis, tumor vasculature visualization and targeted treatment of solid cancers[4].

Since endoglin is highly and specifically expressed on tumor endothelial cells, in the present study we hypothesized that it could be used as an appropriate marker to assess the vascularization of a tumor.

Confocal laser endomicroscopy (CLE) gained an important role in the study and real-time histopathological diagnosis of various gastrointestinal diseases, such as celiac disease, Barrett esophagus, microscopic colitis, inflammatory bowel disease, and recently Clostridium Difficile associated colitis[13]. Recent meta-analyses performed to determine the diagnostic accuracy of CLE in the detection of colorectal neoplasia showed high sensitivity and specificity of the method[14,15].

Recently, we have used CLE to assess tumor vasculature by fluorescence labelled antibodies targeted against endothelial markers[16,17]. In the present feasibility study, we used CLE to compare the selective expression of fluorescently labeled anti-CD105 antibodies in newly-formed vessels to fluorescently labeled anti-CD31 total vessel staining, and the gold standard of histopathology. More specifically, we aimed to answer the following questions: (1) Can the use of CLE in association with CD105 offer a more adequate quantitative and qualitative analysis of newly formed vessels than the commonly used panendothelial markers in human rectal cancer? and (2) Can this method be used *in vivo* for a rapid characterization of tumor microvascularization?

MATERIALS AND METHODS

Subjects

The current study was conducted according to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004) and approved by the local Ethics Committee. All the patients included read and accepted the written informed consent prior to study entry.

Tissue specimens from ten patients 47-80 years old (mean age of 65.2 ± 9.9 years), with histologically diagnosed rectal cancer, were collected during colonoscopy before undergoing surgical resection or neoadjuvant therapy to avoid artifacts (*e.g.*, false positive resulted from fibrosis or inflammation increased in case of radio-chemotherapy). Fresh tissue samples from these patients were immediately processed for both CLE and immunohistochemistry assessment.

The ten patient population contained stage Ⅱ-Ⅲ (according to AJCC staging system) rectal adenocarcinomas without metastatic spread.

The main clinical signs the patients presented at admission in the hospital were alternating diarrhea and constipation, accelerated intestinal transit, recent constipation, unintended weight loss, rectal bleeding, abdominal pain or discomfort. Only three patients accused rectal bleeding as a single symptom, also confirmed by the physical examination (digital rectal examination). Seven patients had nonspecific findings for the laboratory tests such as moderate elevated hematological values of erythrocyte sedimentation rate (three patients), slightly elevated white blood cells count (two patients) and moderate anemia (two patients). Two patients presented slightly elevated values of both tumor markers CEA and CA19-9, while three of them had only slightly elevated CEA value. Computed tomography scan excluded the presence of metastases in all ten patients and described rectal wall thickening in four cases. Histological examination findings from endoscopic samples are summarized in Table 1.

CLE

The biopsy samples collected with a standard colonoscope (CFQ160ZL, Olympus, Tokyo, Japan) were processed following a standardized protocol. During the endoscopic procedure, for every patient, six biopsies were taken from tumor, avoiding the ulcerated areas (paired biopsies for CLE assessment, standard immunohistochemistry and histopathological examination, respectively), as well as four biopsies from macroscopically normal surrounding tissue samples (paired biopsies for both CLE processing and standard immunohistochemistry). The biopsies were immersed immediately in 10% neutral buffered formalin for histopathological analysis, as well as in saline solution

for the *ex vivo* immunohistochemical processing. Samples from saline solution were thoroughly washed and incubated for one hour in the dark, at 37 ℃, with Alexa-Fluor 488-labeled anti-CD31 (PECAM) antibody (mouse anti-human IgG1, Exbio, Prague, Czech Republic) or respectively FITC-labeled anti-CD105/ Endoglin antibody (mouse anti-human IgG2a, Exbio), diluted as 1:15 and 1:5 in saline with 1% bovine serum albumin (BSA, Sigma-Aldrich, Munich, Germany). Afterwards, the excess antibodies were washed away in saline and the samples were immediately visualized in CLE imaging to assess the microvascularization *ex vivo* up to a maximum depth of 250 μm. CLE images were acquired using Pentax EC-3870 CIFK, Tokyo, Japan, a dedicated endomicroscopy system with an excitation wavelength of 488 nm and with a maximum laser power output of ≤ 1 mW at the surface of the tissue[16,17].

To assess both endothelial markers more accurately, we used the color overlay function in the ImageJ image processing software (National Institutes of Health, United States). This software was used to obtain the Z projection of the confocal serial image stacks from each biopsy sample (60-250 images per biopsy sample). The vascular density and the vessel diameters were measured from the Z projections within two 50 μm × 475 μm rectangular regions of interest (ROI) centered in the middle of each image in the horizontal and vertical direction as before[17].

Statistical analysis

The results were averaged over all the patients and were expressed as the mean ± SE. We used unpaired two-tailed Student's *t*-test, with the level of significance set at $P \leq 0.05$ to evaluate the variation of CD105 expression *vs* CD31 expression in microvessels from the normal mucosa tissue and from the rectal tumors.

Immunohistochemistry

To confirm the role of CD105 *vs* CD31 in tumor neo-angiogenesis, adjacent samples from the same patient were processed for immunohistochemistry, for normal and tumor samples as described previously[16,17]. Briefly, after formaldehyde fixation and paraffin embedding, 4 μm tissue sections were sliced from these blocks, deparaffinized, re-hydrated and processed for antigen retrieval by microwaving for 20 min in citrate buffer pH 6. Endogenous peroxidase was next blocked utilizing 1% H_2O_2 for 30 min, and the false antigenic sites were further blocked by incubating the slides in 5% skimmed milk (Bio-rad, München, Germany). Paraffin-certified antibodies were next incubated alternatively on the slides overnight at 4 ℃ (rabbit anti-human CD105 polyclonal antibody diluted as 1:50, LabVision, Fremont, CA, United States; and mouse anti-human CD31, IgG1, clone JC70A, Dako, Glostrup, Denmark). Next day the sections were washed in saline, signal amplified with a multi-species polymeric HRP system (EnVision, Dako), and finally vessels were visualized by adding the 3-3' diaminobenzidine substrate (DAB, Dako). Afterwards, the sections were counterstained with Hematoxylin and 3-4 hotspot high vessel density areas were captured using a Nikon Eclipse 55i microscope equipped with a 5 Megapixel CCD color camera (Nikon, Tokyo, Japan). There were selected images from the regions with the highest vascular density ("hot-spots"- according to Weidner *et al*[18]). Under constant illumination conditions, images were obtained using the 40 × objective, and saved as uncompressed TIF files using the Image ProPlus AMS 6 software (Media Cybernetics Inc., Bethesda, Maryland, United States). The contour for each microvessel was drawn separately with a dedicated hand tool in Adobe Photoshop software, and these ROI were filled with black RGB color and saved as layers. Images were brought back in Image ProPlus and after distance-to-pixel calibration, they were utilized for automated measurements. Total vascular area, and total vessel count were normalized to 1 mm^2 and automatically measured, considering a total area of the field of 36527.48 μm^2. Inflammatory plasma cells or tumor cells picking up the signal have been excluded from this interpretation by two pathologists (DP and CG).

RESULTS

Targeted anti-CD31 antibodies expression on the confocal laser images

To analyze CD31 expression in rectal cancer, we evaluated tumor rectal cancer tissue and normal rectal mucosa for the vascular morphometric assessment. The CD31 antibody stained blood vessels in both normal and tumor rectal mucosa. In normal mucosa, the average diameter of vessels was of 7.67 ± 0.5 μm and the vessel density was 3191.6 ± 387.8 vessels/mm^3. In the tumor sample, we obtained an average diameter of 10.88 ± 0.8 μm and a vessel density of 4707.3 ± 448.8 vessels/mm^3 (Figure 1A and B).

Targeted anti-CD105 antibodies for CLE imaging of normal colorectal tissue and tumor microvasculature

In the CLE samples that were fluorescently labeled with both CD31 and CD105 antibodies, the typical tumor vasculature pattern was observed, with tortuous, dilated and branched vessels, but the expression of CD105 in tumor tissue was generally lower compared to CD31 vessel staining (Figure 1C and D).

Staining for CD105 was low or absent in normal mucosa (244.21 ± 130.7 vessels/mm^3 in only four patients), whereas the microvascular network was visualized using CD31 as a control on samples from the same patients. The average diameter of anti-CD105 antibody stained vessels was 10.97 ± 0.6 μm in tumor tissue, and average density was 2787.4 ± 134.8 vessels/mm^3.

Next we analyzed the relationship between the vascular expression with CD31 and CD105 in colorectal

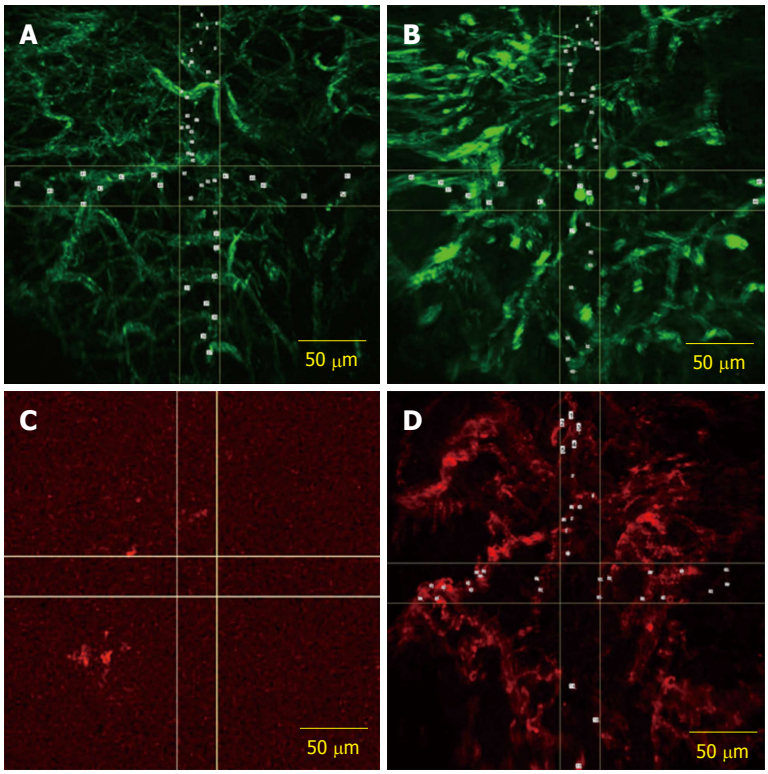


Figure 1 Confocal laser endomicroscopy. A: CLE images with AF488 anti-CD31 antibodies expression on vascular network from both normal; B: Tumor rectal mucosa; C: CLE image showing low expression of the fluorescently labeled anti-CD105 antibodies in normal rectal mucosa; D: Image from the same patient showing microvessels in rectal adenocarcinoma visualized by using CD105 staining as a specific endothelial marker. CLE: Confocal laser endomicroscopy.

Table 2 Quantitative results of vascular parameters from confocal laser endomicroscopy images

		CD31	CD105	*P*-value
Vascular Diameter (μm)	Normal Tissue	7.67 ± 0.5	3.46 ± 1.5	0.01
	Tumor	10.88 ± 0.8	10.97 ± 0.6	0.9
Vascular Density (vessels/mm^3)	Normal Tissue	3191.6 ± 387.8	244.21 ± 130.7	< 0.001
	Tumor	4707.3 ± 448.8	2787.4 ± 133.8	0.001

tumors. There were more vessels stained with CD31 than CD105 (P = 0.0006 for vascular density) in tumor. The average vessel diameter was similar for both CD31 and CD105 staining (P = 0.018 in normal samples, and P = 0.932 in malignant tissue).

The vascular density and the average diameter in tumor samples were significantly higher than the control in the 3D confocal reconstruction and in immunohistochemistry images. This fact was demonstrated by using both markers. In contrast, CD105 expression in colorectal tissues from the same patients was strongly enhanced in tumor vessels suggesting detection of the endoglin is an indication of angiogenesis particularly in malignant disease (Table 2).

Immunohistochemistry results

The CD105 and CD31 vascular expressions were studied in normal rectal mucosa and rectal cancer specimens. The immunohistochemical analysis revealed that the samples from normal tissue showed low detectable CD105 expression. CD105 was rarely expressed in normal mucosa, while in tumor specimens, CD105-positive vascular endothelial cells were clearly identified (Figure 2).

In normal tissue images CD31-stained we measured an average of 202.9 ± 91.8 vessels/mm^2, with a significantly lower density of 56.5 ± 35.1 vessels/mm^2 for the vascular network stained with CD105 (P = 0.00017). The intratumoral MVD average was about 298.04 ± 132.6 vessels/mm^2 on CD31 stained images and on CD105 images - 205.7 ± 100.06 vessels/mm^2 (P = 0.048) (Figure 3).

The values for the vascular area when using the panendothelial marker CD31 were 3.4% ± 1.3% in normal rectum and 9.4% ± 3.3% in tumors (P < 0.001). On CD105 stained sections, the total vascular area was 1.3% ± 1.4% in healthy tissue and 6.9% ± 3.1% in malignant tissue (P < 0.001).

DISCUSSION

Rectal cancer is one of the cancers which can benefit from antiangiogenic therapy with high chances of curability when the treatment is applied at an early stage. To date, no appropriate tissue biomarkers exist for staging, prediction or monitoring of the clinical response to a therapeutic intervention (*e.g.*, antiangiogenic therapy).

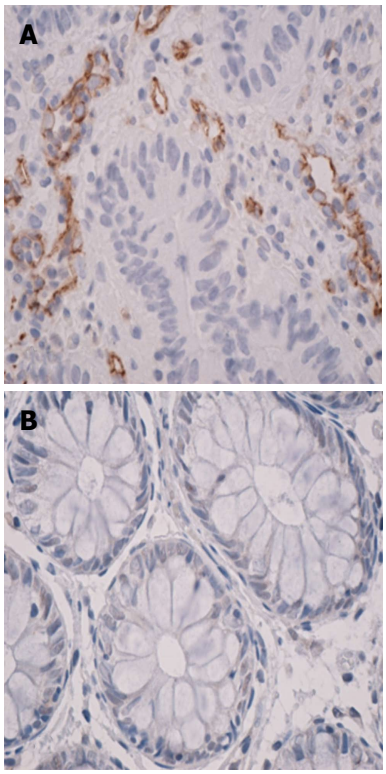
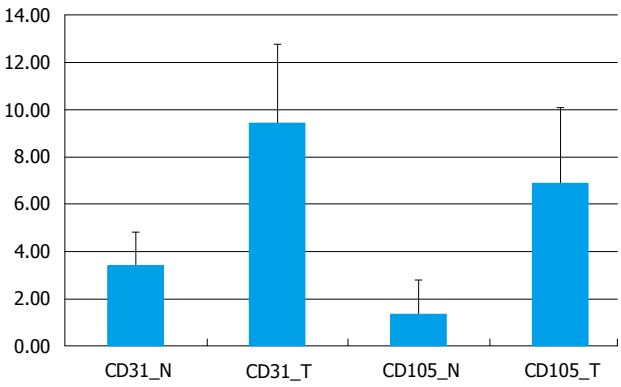


Figure 2 Immunohistochemistry on CD105 stained sequential sections from rectal cancer tissue samples (magnification 40 ×), CD105-positive vascular endothelial cells were clearly identified by their brown staining (A) and normal rectal mucosa displays the absence of endoglin expression (B).



CD31_N- MVD in normal mucosa stained with anti-CD31 antibodies
CD31_T- MVD in tumor mucosa stained with anti-CD31 antibodies
CD105_N- MVD in normal mucosa stained with anti-CD105 antibodies
CD105_T- MVD in tumor mucosa stained with anti-CD105 antibodies

Figure 3 Graphic representation of vascular density (microvessel density) obtained from CD31-immunostained images and CD105-immunostained images of normal mucosa in comparison with tumor mucosa (vessels/mm^2). MVD: Microvessel density.

Beyond its already presumed roles (higher affinity for microvascularization, prognostic role), recent *in vitro* studies suggested that endoglin targeting could improve treatment and could reverse resistance to bevacizumab in some refractory cancer patients[19].

We hypothesized that the use of fluorescently-labeled CD105 antibodies will be suitable for identifying microvessels specific to tumor tissue. Indeed, while vessels marked with fluorescent CD31 were visible in both normal and malignant tissue, CD105 was predominantly expressed in tumor lesions, having reduced affinity for normal rectal mucosa. Thus, specific imaging and quantification of tumor microvessels were feasible using CLE examination and CD105 immunostaining of samples.

Our study proves that fluorescently labeled endoglin antibodies stained intensively intratumoral vessels, whereas vessels in non-neoplastic tissue did not or weakly expressed CD105. These results are consistent with previous observations that endoglin reacts specifically with angiogenic endothelial cells from the malignant tissues[5]. Though, the endoglin expression on macroscopically normal mucosa in four of the patients could be explained by either the existent inflammation, or the tumor spread to normal surrounding tissue.

Endoglin, as a specific marker for activated endothelium, mainly reacts with fresh or frozen tissue, while its activity in paraffin-embedded specimens is dependent on fixation[17]. In the present study, a qualitative comparison between the two methods (CLE *vs* IHC) lead to similar results. The major advantage of the CLE method is time efficacy and less artifacts in comparison to common IHC regarding the processing techniques[20].

Due to CD105 specific overexpression in malignant vessels, the endoglin antibodies for tumor imaging have the potential of becoming an optimal target for anticancer treatment, to improve rectal cancer diagnosis and to monitor the therapy[4]. As there are already studies regarding tumor aggressiveness and the prognostic value of vascular density on IHC when using anti-CD105 antibodies, CLE opens the possibility of applying CD105 targeted therapy, which until now was only tested *in vitro* and on animal models, to *in vivo* human subjects. Its luminal distribution on newly formed vessels makes CD105 readily accessible for the antibodies and, consequently, an interesting candidate for CLE *in vivo*[11].

CLE monitoring of the relationship between endothelial presence of CD105 and survival of patients would be of great interest. In our group of patients, we observed an inter-patients variation in MVD endoglin expression in tumor tissue. On one hand, this could be related to the tumor grading or staging, as an increase in MVD was demonstrated by using CD105 during progressive stages of colorectal carcinogenesis[21]. On the other hand, reduced endoglin expression could also be caused by a decreased tumor vascularization in endoglin haploinsufficiency cases[22]. There are also differences in reactivity to endothelial cells depending on tumor localization[22-24]. However, in colorectal cancer, other studies showed that, with cancer progression, endoglin signaling was lost in most of the epithelial cancer cells which became refractory to the TGF-β growth inhibiting properties[25-29]. All these factors could lead to differences in diagnostic, prognostic and therapeutic efficacy.

To our knowledge, no other studies using fluorescently-labeled CD105 with CLE imaging in patients with rectal cancer have been reported prior to this study. A larger number of patients is needed to study the correlation between MVD and tumor differentiation grade and stage, with great potential for CD105 staining combined with CLE analysis to provide a more reliable evaluation of the angiogenetic status of patients with colorectal cancer. Other studies are needed to investigate if the same CLE method could be applied to other tumor types.

In conclusion, our data showed that CLE using CD105 targeted antibodies for tumor vascular network imaging is feasible and, moreover, that this proangiogenic molecule represents a more specific marker for rectal cancer neoangiogenesis than commonly used panendothelial markers.

COMMENTS

Case characteristics

The main clinical signs the patients showed were alternating diarrhea and constipation, accelerated intestinal transit, recent constipation, unintended weight loss, rectal bleeding, abdominal pain or discomfort.

Clinical diagnosis

Only three patients accused rectal bleeding as a single symptom, also confirmed by the physical examination (digital rectal examination).

Differential diagnosis

Other common digestive diseases such as hemorrhoidal disease, inflammatory bowel disease or irritable bowel syndrome were excluded.

Laboratory diagnosis

Seven patients presented nonspecific laboratory tests findings such as moderate elevated hematological values of erythrocyte sedimentation rate (three patients), slightly elevated white blood cells count (two patients) and moderate anemia (two patients); two patients presented slightly elevated values of both tumor markers CEA and CA19-9, while three of them had only slightly elevated CEA values.

Imaging diagnosis

Computed tomography scan excluded the presence of metastases in all ten patients and described rectal wall thickening in four cases.

Pathological diagnosis

Histological examination of endoscopic samples revealed moderately differentiated adenocarcinoma (G2) in five cases, well differentiated adenocarcinoma in two cases (G1), mixed subtypes in three cases (G1/G2- two cases, G1 with mucinous areas - one case).

Treatment

Tissue samples from patients with histological diagnosis of rectal cancer were collected during colonoscopy before undergoing surgical resection or neoadjuvant therapy.

Term explanation

Immunoendoscopy: Targeting markers of angiogenesis in association with confocal laser endomicroscopy (CLE) examination; Panendothelial markers: Present equal staining intensity in both small and large vessels and comparable reactivity in both frozen and paraffin sections, with obvious disadvantages regarding antigen specificity and sensitivity. They can identify all types of blood vessels in a given tissue sample, irrespective of being mature or immature.

Experiences and lessons

Specific imaging and quantification of tumor microvessels are feasible in human rectal cancer using CLE examination and CD105 immunostaining of fresh tissue samples. A larger number of patients is needed to study the correlation between MVD and tumor differentiation grade and staging, with great potential for CD105 staining combined with CLE analysis to provide a more reliable evaluation of the angiogenetic status of patients with colorectal cancer. CLE monitoring of the relationship between endothelial presence of CD105 and survival of patients would be of great interest.

Peer-review

The manuscript has original results. This is an interesting study on "Tumor neoangiogenesis detection by confocal laser endomicroscopy and anti-CD105 antibody: Pilot study". The research is limited to a small number of patients and, for this reason, this study should be considered pilot.

REFERENCES

1 **Folkman J**, Watson K, Ingber D, Hanahan D. Induction of angiogenesis during the transition from hyperplasia to neoplasia. *Nature* 1989; **339**: 58-61 [PMID: 2469964]

2 **Bolontrade MF**, Stern MC, Binder RL, Zenklusen JC, Gimenez-Conti IB, Conti CJ. Angiogenesis is an early event in the development of chemically induced skin tumors. *Carcinogenesis* 1998; **19**: 2107-13 [PMID: 9886564]

3 **Huss WJ**, Hanrahan CF, Barrios RJ, Simons JW, Greenberg NM. Angiogenesis and prostate cancer: identification of a molecular progression switch. *Cancer Res* 2001; **61**: 2736-43 [PMID: 11289156]

4 **Paauwe M**, ten Dijke P, Hawinkels LJ. Endoglin for tumor imaging and targeted cancer therapy. *Expert Opin Ther Targets* 2013; **17**: 421-435 [PMID: 23327677 DOI: 10.1517/14728222.2013.758716]

5 **Hasan J**, Byers R, Jayson GC. Intra-tumoural microvessel density in human solid tumours. *Br J Cancer* 2002; **86**: 1566-1577 [PMID: 12085206]

6 **Burrows FJ**, Derbyshire EJ, Tazzari PL, Amlot P, Gazdar AF, King SW, Letarte M, Vitetta ES, Thorpe PE. Up-regulation of endoglin on vascular endothelial cells in human solid tumors: implications for diagnosis and therapy. *Clin Cancer Res* 1995; **1**: 1623-1634 [PMID: 9815965]

7 **Li DY**, Sorensen LK, Brooke BS, Urness LD, Davis EC, Taylor DG, Boak BB, Wendel DP. Defective angiogenesis in mice lacking endoglin. *Science* 1999; **284**: 1534-1537 [PMID: 10348742]

8 **Arthur HM**, Ure J, Smith AJ, Renforth G, Wilson DI, Torsney E, Charlton R, Parums DV, Jowett T, Marchuk DA, Burn J, Diamond AG. Endoglin, an ancillary TGFbeta receptor, is required for extraembryonic angiogenesis and plays a key role in heart development. *Dev Biol* 2000; **217**: 42-53 [PMID: 10625534 DOI: 10.1006/dbio.1999.9534]

9 **Bourdeau A**, Dumont DJ, Letarte M. A murine model of hereditary hemorrhagic telangiectasia. *J Clin Invest* 1999; **104**: 1343-1351 [PMID: 10562296 DOI: 10.1172/JCI8088]

10 **Miller DW**, Graulich W, Karges B, Stahl S, Ernst M, Ramaswamy A, Sedlacek HH, Müller R, Adamkiewicz J. Elevated expression of endoglin, a component of the TGF-beta-receptor complex, correlates with proliferation of tumor endothelial cells. *Int J Cancer* 1999; **81**: 568-572 [PMID: 10225446]

11 **Fonsatti E**, Jekunen AP, Kairemo KJ, Coral S, Snellman M, Nicotra MR, Natali PG, Altomonte M, Maio M. Endoglin is a suitable target for efficient imaging of solid tumors: in vivo evidence in a canine mammary carcinoma model. *Clin Cancer Res* 2000; **6**: 2037-2043 [PMID: 10815930]

12 **Duff SE**, Li C, Garland JM, Kumar S. CD105 is important for angiogenesis: evidence and potential applications. *FASEB J* 2003; **17**: 984-992 [PMID: 12773481 DOI: 10.1096/fj.02-0634rev]

13 **Neumann H**, Günther C, Vieth M, Grauer M, Wittkopf N, Mudter J, Becker C, Schoerner C, Atreya R, Neurath MF. Confocal laser endomicroscopy for in vivo diagnosis of Clostridium difficile associated colitis - a pilot study. *PLoS One* 2013; **8**: e58753 [PMID:

23527018 DOI: 10.1371/journal.pone.0058753]
14 **Dong YY**, Li YQ, Yu YB, Liu J, Li M, Luan XR. Meta-analysis of confocal laser endomicroscopy for the detection of colorectal neoplasia. *Colorectal Dis* 2013; **15**: e488-e495 [PMID: 23810105 DOI: 10.1111/codi.12329]
15 **Su P**, Liu Y, Lin S, Xiao K, Chen P, An S, He J, Bai Y. Efficacy of confocal laser endomicroscopy for discriminating colorectal neoplasms from non-neoplasms: a systematic review and meta-analysis. *Colorectal Dis* 2013; **15**: e1-12 [PMID: 23006609 DOI: 10.1111/codi.12033]
16 **Cârțână T**, Săftoiu A, Gruionu LG, Gheonea DI, Pirici D, Georgescu CV, Ciocâlteu A, Gruionu G. Confocal laser endomicroscopy for the morphometric evaluation of microvessels in human colorectal cancer using targeted anti-CD31 antibodies. *PLoS One* 2012; **7**: e52815 [PMID: 23285192 DOI: 10.1371/journal.pone.0052815]
17 **Ciocâlteu A**, Săftoiu A, Cârțână T, Gruionu LG, Pirici D, Georgescu CC, Georgescu CV, Gheonea DI, Gruionu G. Evaluation of new morphometric parameters of neoangiogenesis in human colorectal cancer using confocal laser endomicroscopy (CLE) and targeted panendothelial markers. *PLoS One* 2014; **9**: e91084 [PMID: 24614504 DOI: 10.1371/journal.pone.0091084]
18 **Weidner N**, Carroll PR, Flax J, Blumenfeld W, Folkman J. Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. *Am J Pathol* 1993; **143**: 401-409 [PMID: 7688183]
19 **Rosen LS**, Gordon MS, Robert F, Matei DE. Endoglin for targeted cancer treatment. *Curr Oncol Rep* 2014; **16**: 365 [PMID: 24445497 DOI: 10.1007/s11912-013-0365-x]
20 **Romani AA**, Borghetti AF, Del Rio P, Sianesi M, Soliani P. The risk of developing metastatic disease in colorectal cancer is related to CD105-positive vessel count. *J Surg Oncol* 2006; **93**: 446-455 [PMID: 16615157]
21 **Akagi K**, Ikeda Y, Sumiyoshi Y, Kimura Y, Kinoshita J, Miyazaki M, Abe T. Estimation of angiogenesis with anti-CD105 immunostaining in the process of colorectal cancer development. *Surgery* 2002; **131**: S109-S113 [PMID: 11821796]
22 **Tsujie M**, Tsujie T, Toi H, Uneda S, Shiozaki K, Tsai H, Seon BK. Anti-tumor activity of an anti-endoglin monoclonal antibody is enhanced in immunocompetent mice. *Int J Cancer* 2008; **122**: 2266-2273 [PMID: 18224682 DOI: 10.1002/ijc.23314]
23 **Tsujie M**, Uneda S, Tsai H, Seon BK. Effective anti-angiogenic therapy of established tumors in mice by naked anti-human endoglin (CD105) antibody: differences in growth rate and therapeutic response between tumors growing at different sites. *Int J Oncol* 2006; **29**: 1087-1094 [PMID: 17016638]
24 **Nassiri F**, Cusimano MD, Scheithauer BW, Rotondo F, Fazio A, Yousef GM, Syro LV, Kovacs K, Lloyd RV. Endoglin (CD105): a review of its role in angiogenesis and tumor diagnosis, progression and therapy. *Anticancer Res* 2011; **31**: 2283-2290 [PMID: 21737653]
25 **Dales JP**, Garcia S, Bonnier P, Duffaud F, Andrac-Meyer L, Ramuz O, Lavaut MN, Allasia C, Charpin C. CD105 expression is a marker of high metastatic risk and poor outcome in breast carcinomas. Correlations between immunohistochemical analysis and long-term follow-up in a series of 929 patients. *Am J Clin Pathol* 2003; **119**: 374-380 [PMID: 12645339]
26 **Zijlmans HJ**, Fleuren GJ, Hazelbag S, Sier CF, Dreef EJ, Kenter GG, Gorter A. Expression of endoglin (CD105) in cervical cancer. *Br J Cancer* 2009; **100**: 1617-1626 [PMID: 19352388 DOI: 10.1038/sj.bjc.6605009]
27 **Seon BK**, Haba A, Matsuno F, Takahashi N, Tsujie M, She X, Harada N, Uneda S, Tsujie T, Toi H, Tsai H, Haruta Y. Endoglin-targeted cancer therapy. *Curr Drug Deliv* 2011; **8**: 135-143 [PMID: 21034418]
28 **Dallas NA**, Samuel S, Xia L, Fan F, Gray MJ, Lim SJ, Ellis LM. Endoglin (CD105): a marker of tumor vasculature and potential target for therapy. *Clin Cancer Res* 2008; **14**: 1931-1937 [PMID: 18381930 DOI: 10.1158/1078-0432.CCR-07-4478]
29 **Bredow S**, Lewin M, Hofmann B, Marecos E, Weissleder R. Imaging of tumour neovasculature by targeting the TGF-beta binding receptor endoglin. *Eur J Cancer* 2000; **36**: 675-681 [PMID: 10738134]

P- Reviewer: Baba H, Gu J, Santoro GA, Wittmann T
S- Editor: Ma YJ **L- Editor**: A **E- Editor**: Jiao XK

World Journal of Gastrointestinal Oncology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4251/wjgo.v7.i11.369

World J Gastrointest Oncol 2015 November 15; 7(11): 369-374
ISSN 1948-5204 (online)

CASE REPORT

Does St. John's Wort cause regression in gastrointestinal system adenocarcinomas?

Serap Karaarslan, Suna Cokmert, Atilla Cokmez

Serap Karaarslan, Department of Pathology, Sifa University Faculty of Medicine, 35100 Izmir, Turkey

Suna Cokmert, Department of Medical Oncology, Sifa University Faculty of Medicine, 35100 Izmir, Turkey

Atilla Cokmez, Department of General Surgary, Sifa University Faculty of Medicine, 35100 Izmir, Turkey

Author contributions: All authors contributed to this case report.

Institutional review board statement: Institutional Review Board statement was waived because of the retrospective characteristics of this report.

Informed consent statement: All study participants, or their legal guardians, provided informed written consent prior to case reports enrollment.

Conflict-of-interest statement: We declare that we have no conflict of interest.

Correspondence to: Serap Karaarslan, MD, Associate Professor, Department of Pathology, Sifa University Faculty of Medicine, Sanayi Caddesi No. 7, Bornova, 35100 Izmir, Turkey. serapkaraarslan@gmail.com
Telephone: +90-232-3434445
Fax: +90-232-3435656

Received: March 4, 2015
Peer-review started: March 10, 2015
First decision: May 19, 2015
Revised: August 1, 2015
Accepted: September 10, 2015
Article in press: September 16, 2015

Published online: November 15, 2015

Abstract

St. John's Wort (SJW) is an old herb which has long been consumed widely for its anti-inflammatory, antiviral, and anti-depressive properties. Here we present a detailed clinical evaluation of three cases (two colon and one duodenal adenocarcinoma) with remarkable and intensive lymphoplasmocytic host reaction, at the basal part of tumor, intensive fibrosis, giant cells, plasma cell increase in lymph nodes and few giant cells in germinal centers in resection specimens. The observation of similar host reaction in those tumors having otherwise usual appearance was interesting. None of the cases received neoadjuvant chemoradiotherapy or additional treatment before surgery but only SJW. These cases are presented to increase the awareness about such cases. Further research is needed to reveal the possible effect of SJW, which has long been consumed for different treatment purposes, on human tumors.

Key words: St. John's Wort; Adenocarcinoma; Giant cell

Core tip: St. John's Wort (SJW) is a well known herb that was used in treatment of many diseases during centuries. In this article we offer a perspective about the anti-tumoral effect of SJW with possible mechanisms and pathological data in three gastrointestinal cancer cases, where usage of SJW was identified in history questioning because of tumor regression and intensive inflammatory host reaction following pathological examination.

Karaarslan S, Cokmert S, Cokmez A. Does St. John's Wort cause regression in gastrointestinal system adenocarcinomas? *World J Gastrointest Oncol* 2015; 7(11): 369-374 Available from: URL:

http://www.wjgnet.com/1948-5204/full/v7/i11/369.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i11.369

INTRODUCTION

St. John's Wort (SJW) is a substance widely used for its anti-inflammatory, antiviral, antidepressant and anticancer effects[1-3]. It contains two active compounds: Firstly, hyperforin is responsible for anti-depressant activity and has supplied to be also a good inhibitor of leukocyte elastase, exerting forceful inhibition of *in vitro* tumor cell chemoinvasion and reduction of neovascularization and metastasis formation *in vivo*[4]. Secondly, hypericin is responsible for photocytotoxic effects *in vivo* and *in vitro*. The *in vivo* and *in vitro* photodynamic activities of hypericin as a photosensitizer mainly to induce a very potent anti-tumoral effect[5]. Also, the anti-retroviral feature of hypericin has beeen demonstrated *in vitro* and in animal models[6].

CASE REPORT

Case 1

A fifty-nine years old male patient has undergone colonoscopy for anemia evaluation, which revealed a tumoral mass in the cecum. The histological diagnosis of the biopsy was adenocarcinoma and no distant metastasis was detected in further clinic radiological investigation. Right hemicolectomy was performed and a pathological examination of surgical material revealed a cecal ulcero-vegetative mass which was 7 cm × 6 cm × 5 cm in size. The tumor invaded through muscularis propria to subserosal fat tissue and was consistent with a moderately differentiated adenocarcinoma. Notably, it showed fibrosis and inflammatory cell infiltration in the transitional zone between deep intestinal layers and normal mucosa, which was easily detectable even under low magnification (Figure 1A). Under higher magnifications, inflammatory cell infiltration was rich in plasma cells and lymphocytes. scattered eosinophils, polymorphonuclear leucocytes and few giant cells were also noted focally (Figure 1B). The inflammatory reaction and fibrosis were surrounding the tumor, as if they were trying to prevent the penetration of the tumor into deep tissue. Most of these lymphocytes were T lymphocytes and showed cytotoxic T cell ($CD8^+$) phenotype on immunohistochemical examination (Figure 1C). CD20 and CD4 stains were almost negative. Plasma cells were stained positive with CD138 and polytypic with kappa/lambda. Two of 18 lymph nodes dissected from mesentery showed few tumor cells located in subcapsular sinuses while no gross metastasis was detected. Notably, germinal centers of some lymph nodes had giant cells and increased number of plasma cells in inter-follicular areas (Figure 2A and B). Giant cells were CD68 positive on immunohistochemical examination (Figure 2C). These features were suggestive of changes developed secondary to neoadjuvant chemotherapy/radiotherapy, but the patient's past medical history did not reveal such treatment. His detailed medical history was taken and when he was also asked for the usage of some alternative treatments, he mentioned usage of SJW for other complaints such as diabetes, dyspepsia. He has been consuming SJW tea in the morning for five years, then he had used SJW oil regularly (one teaspoon in the morning) for two years and he has been using it regularly (one teaspoon in the morning and evening) for the last three years. Medical records of the patient revealed that he had chemotherapy for six months after surgery (FOLFOX-4 protocole once every 14 d) and no recurrence or metastasis were detected during two years of follow up.

Case 2

A fifty-eight years old female patient has undergone colonoscopy for anemia evaluation, which revealed a tumoral mass in the transverse colon. No distant metastasis was detected and the patient had undergone colectomy. On macroscopic examination of colectomy specimen, an ulcerovegetative tumor infiltrating all layers of intestinal wall was detected, measuring 3.5 cm × 2.5 cm × 2 cm in size. Microscopic examination revealed moderately differentiated adenocarcinoma with mixed inflammatory cell infiltration rich in lymphoplasmocytes on the background (Figure 3). Eosinophils were also prominent with a few giant cells. Fourteen lymph nodes, dissected from mesentery, were reactive. However, one of the lymph nodes had an increased number of plasma cells and giant cells in germinal center of the follicle. Immunohistochemical characteristics were similar to that of the first case. Based on the experience of the morphology of the first case, the patient was also asked for usage of alternative treatments. To our surprise she has also mentioned usage of SJW oil (one teaspoon in the morning on an empty stomach) for 1.5 mo. Her medical records revealed that she has refused chemotherapy and followed-up without treatment. No recurrence or metastases were detected during the first six months of follow-up period.

Case 3

A duodenal mass was detected in a 73 years old male patient with the complaints of abdominal pain and weight loss. The biopsy was reported as adenocarcinoma. Since there was no distant metastasis, surgery was recommended. Although, he initially refused surgery he agreed to an operation three months later. On his second admission to hospital it was seen that the tumor size had somewhat reduced during this three months period. When a detailed medical history was taken, it was also revealed there was daily use of SJW oil of one teaspoon for the last three months. On macroscopic examination, an ulcero-vegetative ampullary tumor was observed measuring 3.8 cm × 2.5 cm × 2.5 cm in size, involving all layers of duodenum and infiltrating the pancreas.

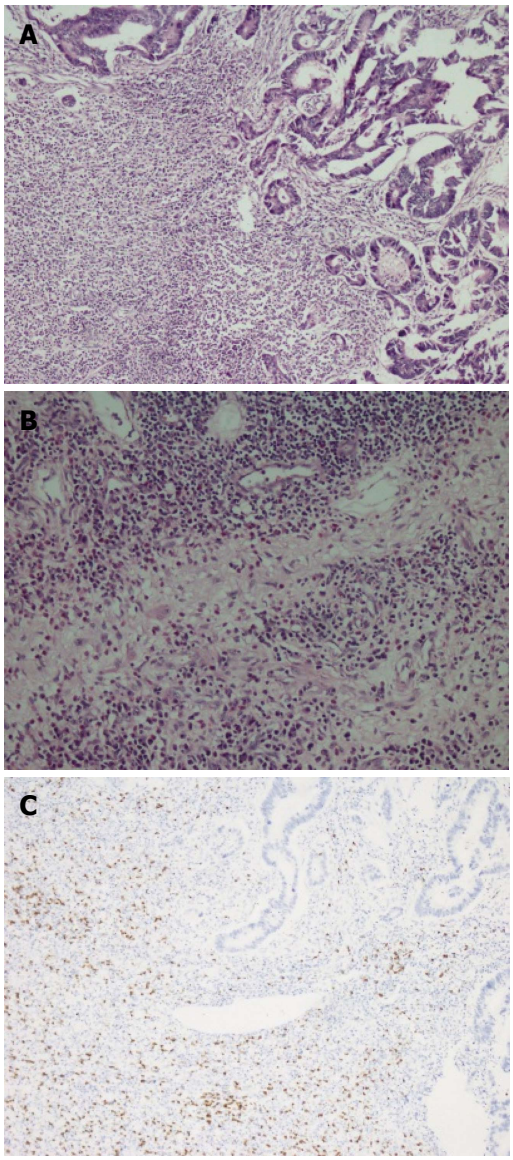


Figure 1 Adenocarcinoma. A: Adenocarcinoma showing fibrosis and inflammatory cell infiltration in the tumor base (HE × 10); B: Inflammatory cell infiltration consisting of plasma cells, lymphocytes, eosinophils and PNLs was seen in these areas (HE × 20); C: Inflammatory cell infiltration observed in the basis of tumors was rich in CD8 positive T lymphocytes (anti-CD8, × 5).

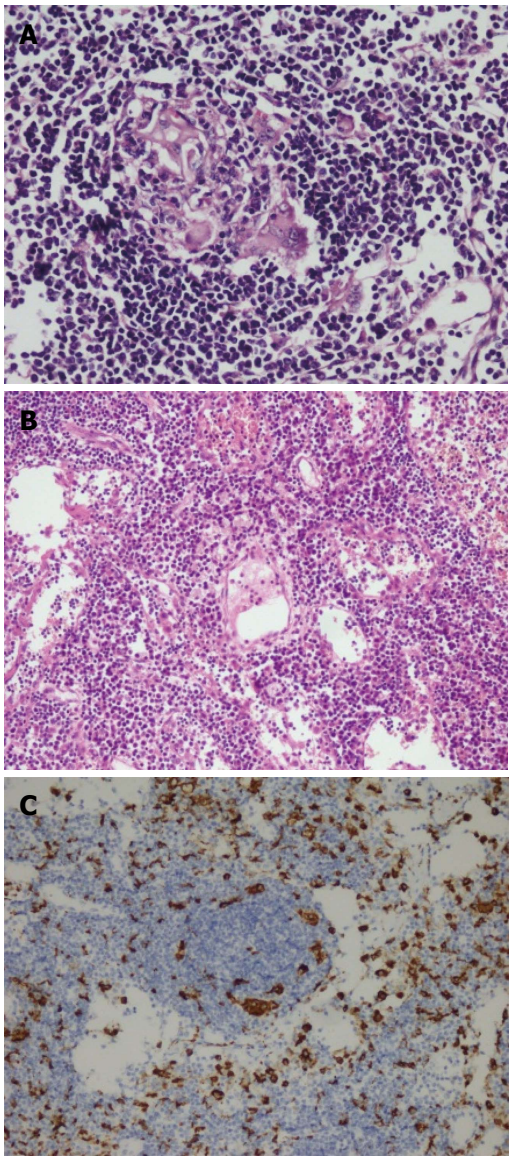


Figure 2 Germinal centers of some lymphoid follicles had giant cells and increased number of plasma cells in inter-follicular areas. A: Giant cells were detected in germinal centers of some lymph nodes (HE × 20); B: Interfollicular areas of some lymph nodes had increased number of plasma cells (HE × 10); C: Giant cells were stained with CD68 immunohistochemically (anti-CD68 × 10).

Areas showing the characteristics of moderately differentiated adenocarcinoma and mixed inflammatory cell infiltration rich in PNLs were observed. Similar to the previous two cases, eosinophils were also present and most prominent in the basilar parts of these areas (Figure 4A). The most common lymphocytic component was again CD8 positive T cells immunohistochemically (Figure 4B). Giant cells were seen in all layers, being more prominent in the areas in the vicinity of serosal surfaces (Figure 5A and B). These cells were stained with CD68 immunohistochemically (Figure 5C). Additionally, extensive perineural infiltration and intra-lymphatic tumoral thrombi were present. Four of 12 lymph nodes dissected from surrounding adipose tissue showed metastasis. The patient died due to anastomosis leakage and bleeding complications after surgery.

DISCUSSION

Hypericum perforatum, known as SJW, is a plant of the genus Hypericum and a herb with antidepressant feature and effective anti-inflammatory characteristics as an arachidonic acide/5-lipoxygenase inhibitor and COX-1 inhibitor[7]. In many countries, its drug form is available and sold out as an over the counter drug without prescription. It is most commonly used for the treatment of depression. Hyperforin is responsible for anti-depressant activity. The hyperforin constituent of SJW is TRPC6 receptor agonist and therefore, it causes noncompetitive reuptake inhibition of monoamines (especially, dopamine, norepinephrine, and serotonin), gamma-aminobutyric acid and glutamate[8]. Hyperforin

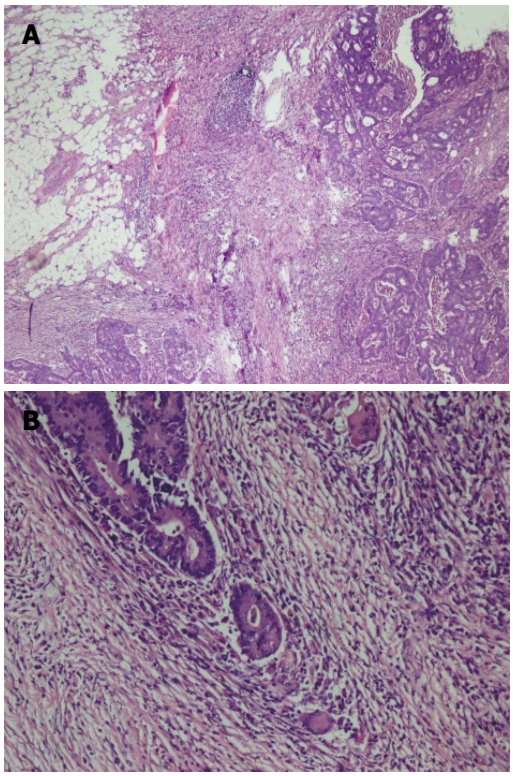


Figure 3 Moderately differentiated adenocarcinoma with mixed inflammatory cell infiltration rich of lymphoplasmocytes, eosinophils and few giant cells (A and B) (HE × 5, HE × 20).

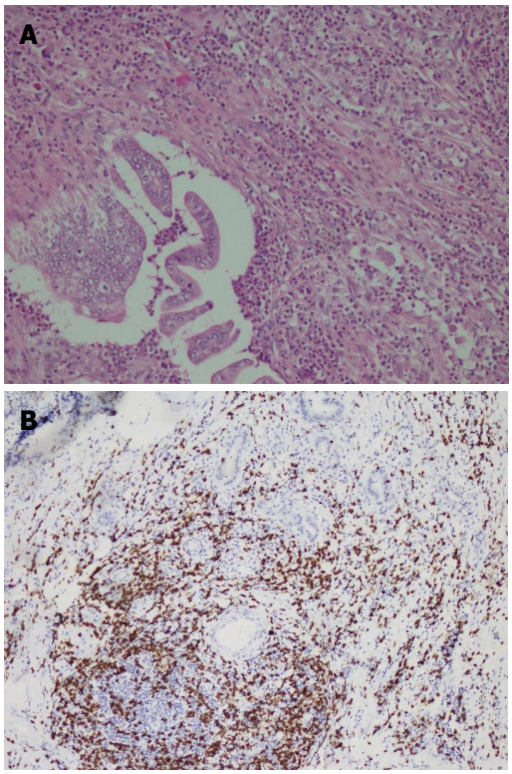


Figure 4 Adenocarcinoma showing mixed inflammatory cell infiltration rich in eosinophils and T - lymphocytes. A: Moderately differentiated adenocarcinoma showing mixed inflammatory cell infiltration rich in eosinophils and T - lymphocytes (HE × 20); B: The most prominent cellular component on immunohistochemical examination was CD8 positive T - lymphocytes (CD8 × 10).

inhibits reuptake of these neurotransmitters by increasing intra-cellular sodium ion amounts. Furthermore, SJW is known to downregulate the β_1 adrenoceptor and upregulate postsynaptic 5-HT_{1A} and 5-HT_{2A} receptors which are serotonin receptor[9]. A 2008 Cochrane review of 29 clinical trials inferred that it was superior to placebo in cases with major depression[10]. With respect to the National Center for Complementary and Integrative Health of the National Institutes of Health, it "may help some types of depression, though the evidence is not definitive"[11]. Hyperforin is also an anti-inflammatory complex with anti-angiogenic, antibiotic, and neurotrophic estates[12]. Moreover, it prevents neutrophil activation of matrix metalloproteinase-9 (MMP9) mobility and recruitment. Anti-proliferative and anti-metastatic feature has also been associated to down-regulation of NF-jB and its regulated molecules for example survivin and MMP9[13].

Hypericin is a photosensitive compound synthesized by SJW, and possesses properties suitable for photodynamic therapy (PDT). PDT is a carcinoma treatment methodology abusing non-toxic photosensitizer specifically localized in tumor tissue and its targeted activation with light. Thus, it leads to reactive oxygen kinds production and causes photochemically caused cell death[14]. The response to PDT depends on the photosensitizer's features, the illumination circumstances and the oxygenation conditions of the tissue[15]. It was also observed that hypericin blocks cell cycle at G2/M control point in colon cancer cell culture[16] Another colon cancer cell culture study showed re-localisation of apoptosis-inducing factor on the nucleus after hypericin treatment. Thus the anti-tumor effect of hypericins likely resulted from its apoptosis stimulating effect and its anti-proliferative effect by decreasing Ras protein[17].

Besides its many benefits there are also some studies in the literature showing its undesired adverse effects. Development of hepatotoxicity, cirrhosis and alteration of dosage properties and bioavailability of some drugs are some of its important adverse effects[18]. SJW has been displayed to cause a lot of drug interactions. Its effects are due to cytochrom P4503A enzyme activation and P-glycoprotein. This drug metabolizing enzyme induction effects in the raised metabolism of some drugs, such as indinavir, cyclosporine and oral contraceptives leading to reduced plasma density and possible clinical impact[19]. The main constituent thought to be responsible is hyperforin.In an other study it has been shown that the amount of intestinal and hepatic cytochrome P4503A and intestinal P-glycoprotein are increased by the short term usage of SJW in humans and rats[20]. Bone marrow necrosis, orofacial dystonia and radiation recall dermatitis are reported as less often adverse effects[21-23].

In an experimental study by Martarelli *et al*[24], on hormone independent human prostate cancer cells, it was shown that Hypericum perforatum extract decreased tumor cell proliferation by inhibiting serotonin

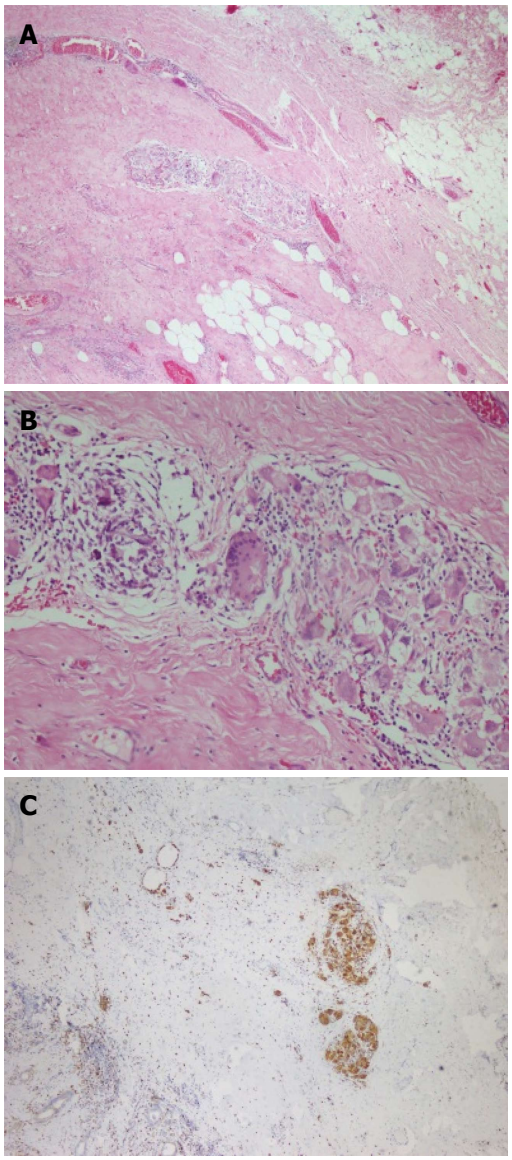


Figure 5 Giant cells in the areas beneath serosal surface and stained with CD68 immunohistochemically. A, B: Giant cells were seen in the areas beneath serosal surface (HE × 5, HE × 20); C: CD68 positivity in giant cells (anti -CD68 × 10).

reuptake and showed cytotoxic effects. In addition, it decreased frequency of local lymph node metastasis when compared to the control group[24]. There are experimental studies on the effects of SJW on colon, bladder and prostate carcinomas. In an experimental study by Dongre *et al*[25], the effect of Hypericum hookerianum on carcinomas was evaluated and it was found that serum neutrophil, lymphocyte, eosinophil, hemoglobin and erythrocyte values were closer to normal range when compared to control group[25]. In our cases, neutrophils and histiocytes-giant cells were more prominent early in the course (2nd and 3rd cases), while plasma cells, histiocytes and lymphocytes (cytotoxic CD8+) took over during chronic usage (1st case). Similar to the study by Dongre *et al*[25], morphological properties of our 2nd and 3rd cases may be due to acute effects (15 d) of Hypericum. In our case with long term SJW use, extensive host reaction and tendency to form barrier against tumor were remarkable and we interpreted it as a morphological sign of its anti-tumor response. Although the exact mechanism of these events is unknown, it may be a result of a chain of events triggered immunologically.

The aim of this presentation is not recommending SJW as a substitute for cancer treatment. The observations presented herein reflect the histological findings of only three cases and not enough to make a precise conclusion on its effects. We don't know yet either whether all cases using SJW present similar morphology or whether any other substances also induce a similar tumor-host reaction. We present these cases only to share our observations and draw attention to its possible effects on human tumor-host interaction. Further dedicated research is needed to unveil these questions.

ACKNOWLEDGMENTS

The authors thank Professor Dr. Basak Doganavsargil, MD, for critical reading the manuscript.

COMMENTS

Case characteristics

The authors present a detailed clinical evaluation of three intestinal adenocarcinoma cases which used St. John's Wort (SJW).

Clinical diagnosis

Patients have undergone colonoscopy for anemia, abdominal pain and weight loss evaluation, which revealed a tumoral mass in the colon and duodenum.

Pathological diagnosis

Biopsy and resection materials of all three cases were evaluated morphologically and immunohistochemically. Inflammatory cell population was rich in plasma cells and lymphocytes. In patients that used SJW in early stages polymorphonuclear leucocytes were significant. In patient those who used SWJ for long periods fibrosis and lymphoplasmositic cell infiltration was remarkable. Lymphocytes stained predominantly CD8 positive phenotype immnuhistochemically. Plasma cells were found to be kappa/lambda polytypic nature.

Treatment

Case revealed that he had chemotherapy for six months after surgery (FOLFOX-4 1 protocole once every 14 d).

Experiences and lessons

The aim in this study is not about to recommend usage of SJW. The authors only want to indicate their awareness of SJW usage after pathologic examination. The authors thing that these pathologic features might flash the benefits of SJW that had been discussed for ages.

Peer-review

This manuscript reports the clinico-pathological findings of three adenocarcinoma cases treated with SJW.

REFERENCES

1 **Tian R**, Koyabu N, Morimoto S, Shoyama Y, Ohtani H, Sawada Y. Functional induction and de-induction of P-glycoprotein by St. John's wort and its ingredients in a human colon adenocarcinoma

cell line. *Drug Metab Dispos* 2005; **33**: 547-554 [PMID: 15640377]
2 **Šemeláková M**, Mikeš J, Jendželovský R, Fedoročko P. The pro-apoptotic and anti-invasive effects of hypericin-mediated photodynamic therapy are enhanced by hyperforin or aristoforin in HT-29 colon adenocarcinoma cells. *J Photochem Photobiol B* 2012; **117**: 115-125 [PMID: 23099482 DOI: 10.1016/j.jphotobiol.2012.09.003]
3 **Lavie G**, Valentine F, Levin B, Mazur Y, Gallo G, Lavie D, Weiner D, Meruelo D. Studies of the mechanisms of action of the antiretroviral agents hypericin and pseudohypericin. *Proc Natl Acad Sci USA* 1989; **86**: 5963-5967 [PMID: 2548193]
4 **Hostanska K**, Reichling J, Bommer S, Weber M, Saller R. Hyperforin a constituent of St John's wort (Hypericum perforatum L.) extract induces apoptosis by triggering activation of caspases and with hypericin synergistically exerts cytotoxicity towards human malignant cell lines. *Eur J Pharm Biopharm* 2003; **56**: 121-132 [PMID: 12837490]
5 **Agostinis P**, Vantieghem A, Merlevede W, de Witte PA. Hypericin in cancer treatment: more light on the way. *Int J Biochem Cell Biol* 2002; **34**: 221-241 [PMID: 11849990]
6 **Lavie G**, Mazur Y, Lavie D, Prince AM, Pascual D, Liebes L, Levin B, Meruelo D. Hypericin as an inactivator of infectious viruses in blood components. *Transfusion* 1995; **35**: 392-400 [PMID: 7740610]
7 **Wölfle U**, Seelinger G, Schempp CM. Topical application of St. John's wort (Hypericum perforatum). *Planta Med* 2014; **80**: 109-120 [PMID: 24214835 DOI: 10.1055/s-0033-1351019]
8 Pharmacology. Hyperforin. Drugbank. University of Alberta. Retrieved 5, December 2013
9 **Nathan PJ**. Hypericum perforatum (St John's Wort): a non-selective reuptake inhibitor? A review of the recent advances in its pharmacology. *J Psychopharmacol* 2001; **15**: 47-54 [PMID: 11277608]
10 **Linde K**, Berner MM, Kriston L. St John's wort for major depression. *Cochrane Database Syst Rev* 2008; **(4)**: CD000448 [PMID: 18843608 DOI: 10.1002/14651858]
11 National Institute for Complementary and Integrative Health: St. John's Wort. Retrieved 2015-02-05
12 **Zanoli P**. Role of hyperforin in the pharmacological activities of St. John's Wort. *CNS Drug Rev* 2004; **10**: 203-218 [PMID: 15492771]
13 **Butterweck V**. Mechanism of action of St John's wort in depression: what is known? *CNS Drugs* 2003; **17**: 539-562 [PMID: 12775192]
14 **Barathan M**, Mariappan V, Shankar EM, Abdullah BJ, Goh KL, Vadivelu J. Hypericin-photodynamic therapy leads to interleukin-6 secretion by HepG2 cells and their apoptosis via recruitment of BH3 interacting-domain death agonist and caspases. *Cell Death Dis* 2013; **4**: e697 [PMID: 23807226 DOI: 10.1038/cddis.2013.219]
15 **Kleemann B**, Loos B, Scriba TJ, Lang D, Davids LM. St John's Wort (Hypericum perforatum L.) photomedicine: hypericin-photodynamic therapy induces metastatic melanoma cell death. *PLoS One* 2014; **9**: e103762 [PMID: 25076130 DOI: 10.1371/journal.pone.0103762]
16 **Sacková V**, Fedorocko P, Szilárdiová B, Mikes J, Kleban J. Hypericin-induced photocytotoxicity is connected with G2/M arrest in HT-29 and S-phase arrest in U937 cells. *Photochem Photobiol* 2006; **82**: 1285-1291 [PMID: 16740057]
17 **Sačková V**, Kuliková L, Kello M, Uhrinová I, Fedoročko P. Enhanced antiproliferative and apoptotic response of HT-29 adenocarcinoma cells to combination of photoactivated hypericin and farnesyltransferase inhibitor manumycin A. *Int J Mol Sci* 2011; **12**: 8388-8405 [PMID: 22272079 DOI: 10.3390/ijms12128388]
18 **Lampri ES**, Ioachim E, Harissis H, Balasi E, Mitselou A, Malamou-Mitsi V. Pleomorphic hepatocellular carcinoma following consumption of hypericum perforatum in alcoholic cirrhosis. *World J Gastroenterol* 2014; **20**: 2113-2116 [PMID: 24587684 DOI: 10.3748/wjg.v20.i8.2113]
19 **Henderson L**, Yue QY, Bergquist C, Gerden B, Arlett P. St John's wort (Hypericum perforatum): drug interactions and clinical outcomes. *Br J Clin Pharmacol* 2002; **54**: 349-356 [PMID: 12392581 DOI: 10.1046/j.1365-2125.2002.01683.x]
20 **Dürr D**, Stieger B, Kullak-Ublick GA, Rentsch KM, Steinert HC, Meier PJ, Fattinger K. St John's Wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4. *Clin Pharmacol Ther* 2000; **68**: 598-604 [PMID: 11180019]
21 **Demiroglu YZ**, Yeter TT, Boga C, Ozdogu H, Kizilkilic E, Bal N, Tuncer I, Arslan H. Bone marrow necrosis: a rare complication of herbal treatment with Hypericum perforatum (St. John's wort). *Acta Medica* (Hradec Kralove) 2005; **48**: 91-94 [PMID: 16259319]
22 **Milton JC**, Abdulla A. Prolonged oro-facial dystonia in a 58 year old female following therapy with bupropion and St John's Wort. *Br J Clin Pharmacol* 2007; **64**: 717-718 [PMID: 17578477]
23 **Putnik K**, Stadler P, Schäfer C, Koelbl O. Enhanced radiation sensitivity and radiation recall dermatitis (RRD) after hypericin therapy -- case report and review of literature. *Radiat Oncol* 2006; **1**: 32 [PMID: 16948841]
24 **Martarelli D**, Martarelli B, Pediconi D, Nabissi MI, Perfumi M, Pompei P. Hypericum perforatum methanolic extract inhibits growth of human prostatic carcinoma cell line orthotopically implanted in nude mice. *Cancer Lett* 2004; **210**: 27-33 [PMID: 15172117]
25 **Dongre SH**, Badami S, Natesan S, H RC. Antitumor Activity of the Methanol Extract of Hypericum hookerianum Stem Against Ehrlich Ascites Carcinoma in Swiss Albino Mice. *J Pharmacol Sci* 2007; **103**: 354-359 [PMID: 17443057]

P- Reviewer: Chiba T, Merino G
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Jiao XK

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com