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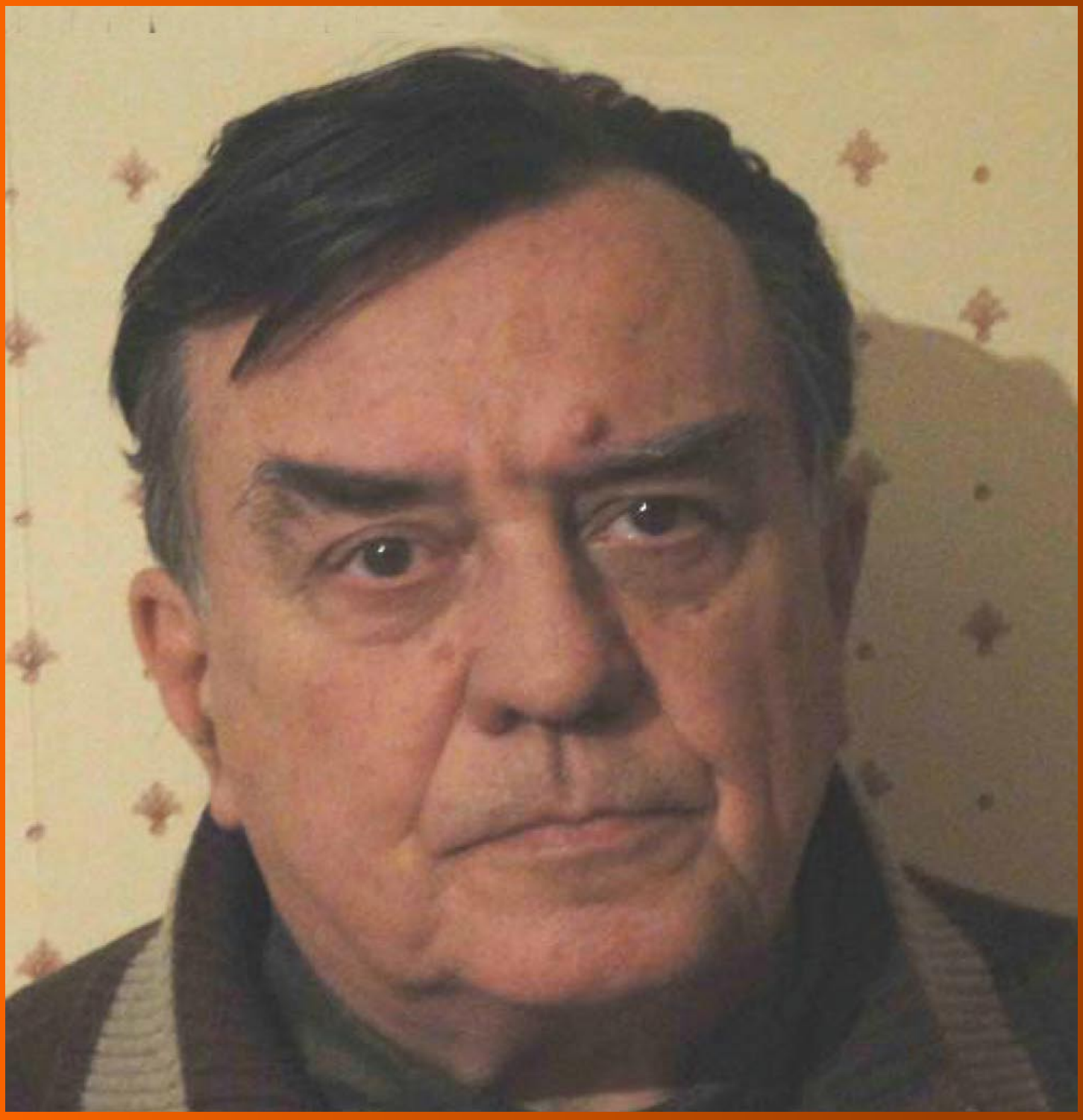

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EDITORIAL

Enhanced permeability and retention effect based nanomedicine, a solution for cancer

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Abstract

Tumor-targeting is becoming more and more important for cancer chemotherapy. Though many molecular-target drugs have been developed in the past two decades which shed some light on targeted tumor therapy, clinical results of those molecular-target drugs are not so encouraging especially for solid tumors, problems mostly relating to the heterogeneity and mutations of target molecules in human solid tumors. More general tumor-targeting strategy is thus anticipated. In this regard, the enhanced permeability and retention (EPR) effect which is a unique phenomenon of solid tumors based on the anatomical and pathophysiological nature of tumor blood vessels, is receiving more and more attentions. This EPR effect now served as a standard for tumor-targeted macromolecular anticancer therapy, namely nanomedicine. Many nanoplatforms have been developed as targeted drug delivery systems, including liposome, polymeric micelles, polymer conjugate, nanoparticles. Ample macromolecular drugs are now approved for clinical use or in clinical stage development, all of which by taking advantage of EPR effect, show superior *in vivo* pharmacokinetics and remarkable tumor selectivity, resulting in improved antitumor effects with less adverse effects. We thus believe EPR-based nanomedicine will be a solution for cancer in the future, whereas further consideration of factors involved in EPR effect and strategies to augment/improve EPR effect are warranted.

Key words: Enhanced permeability and retention effect; Tumor targeting; Nanomedicine; Cancer; Chemotherapy; Polymeric therapeutics; Macromolecular drugs

Core tip: Current cancer chemotherapy is less effective with adverse side effects, mostly due to lack of tumor-selectivity. Thus tumor-targeting is known the key for successful chemotherapy. Molecular-target therapy is such a strategy but the clinical results are disappointing probably due to the diversity of cancer-related molecules and enormous mutations. A more general tumor-targeting strategy is based on the unique

physiophathological and anatomical features of solid tumors - enhanced permeability and retention (EPR) effect. Accordingly nanomedicine has been developed, with promising therapeutic potential and very less side effects. We thus believe EPR-based nanomedicine will be a solution for cancer in the future.

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Table 1 Unique characteristics and factors involved in enhanced permeability and retention effect

Active angiogenesis and high vascular density
Extensive production of vascular mediators that facilitate extraravasation
Bradykinin
Nitric oxide
Vascular permeability factor/vascular endothelial growth factor
Prostaglandins
Collagenase (matrix metalloproteinases, or MMPs)
Peroxynitrite
Defective vascular architecture, for example, lack of smooth muscle layer cells, lack of or fewer receptors for angiotensin II, large gap in endothelial cell-cell junctions, anomalous conformation of tumor vasculature (*e.g.*, branching or stretching)
Impaired lymphatic clearance of macromolecules and lipids from interstitial tissue (→ prolonged retention of these substances)

INTRODUCTION

Cancer remains the major threat to human health in most advanced countries in the world. While surgical removal is effective to small and confined early-stage tumors, use of anticancer drugs (chemotherapy) is a less invasive option for cancer patients. Though there is more than 70-year history of chemotherapy, the clinical results of conventional chemotherapy is far from successful. The major problem is the lack of tumor selectivity of conventional anticancer drugs which are mostly small molecular drugs, namely non-selective delivery of cytotoxic drugs to normal vital organs and tissues results in less antitumor effect and severe adverse side effects. Thus, it is an urgent need to develop therapeutic strategies to selectively target tumors.

Development of molecular-target drugs is a remarkable progress in the past two decades, which usually focuses on specific genes or molecules that are highly expressed in tumors and essential for tumor growth. A successful example is imatinib, an inhibitor of the *BCR/ABL* oncogene product, which shows high efficacy in patients with chronic myeloid leukemia (CML) though it is not curative[1]. However, many recent clinical results using those molecular-target drugs are disappointing especially for solid tumors[2,3]. The problems probably relate to the intrinsic heterogeneity and mutations of cancer-related molecules in human solid tumors[4,5]. Namely, in most solid tumors, multiple mutated genes (10 to > 100) exist[4], different cells have distinct genetic lesions even in the same tumor[5], and the critical mutation is not always clear. Thus such a highly specific molecular approach seems to be premature or imperfect, not mentioning the toxic effects as well as enormous and inappropriate expense of these drugs.

A more general tumor-targeting strategy is focusing on the unique anatomical and pathophysiological features of solid tumors leading to high vascular permeability (Table 1, Figure 1), which facilitates delivery of macromolecules (*i.e.*, larger than 40 kDa) selectively into tumor tissues but with very less distribution in normal tissues[6]. This phenomenon is coined enhanced permeability and retention (EPR) effect that was first reported by Matsumura and Maeda in 1986[7], and is now considered a landmark principle in the development of anticancer drugs.

In this concept of EPR based tumor-targeted therapy, nanotechnology is introduced in cancer chemotherapy, namely nanomedicine. Many nanoplatforms have been developed as targeted drug delivery systems, including liposome, polymeric micelles, polymer conjugate, nanoparticles. For example, Doxil, a PEGylated liposome formulation of doxorubicin, is an FDA approved drug for the treatment of Kaposi sarcoma and other cancers. Other clinically used nanomedicine includes DaunoXome (nonpegylated liposomal daunorubicin), DepoCyt (nonpegylated liposomal cytarabine), Myocet (nonpegylated liposomal doxorubicin), Oncaspar (pegylated L-asparaginase), Abraxane (albumin-based paclitaxel), and Genexol-PM (paclitaxel-containing polymeric micelles, approved in South Korea). Much more liposome, polymeric or micellar drugs are in clinical stage development[8,9]. All these macromolecular drugs, by taking advantage of EPR effect, show superior *in vivo* pharmacokinetics and remarkable tumor selectivity, resulting in improved antitumor effects with less adverse effects[8,9].

It should be noted that EPR effect is the first and necessary step for successful anticancer chemotherapy, however many factors are involved in EPR effect, by which the EPR based tumor drug delivery could be further augmented, such as angiotensin II induced hypertension, nitroglycerin/nitric oxide, carbon monoxide[6,10]. Combination of these factors with macromolecular drugs may become useful strategies for more effective antitumor nanomedicine. In addition, another important issue for satisfied nanomedicine is the fate of nano-drugs after accumulation in tumor tissues by EPR effect. The ideal condition is the active drug component in nano-drugs should be released gradually in tumor tissues, otherwise the intact nano-drugs will show less antitumor effect tough they accumulate in tumor

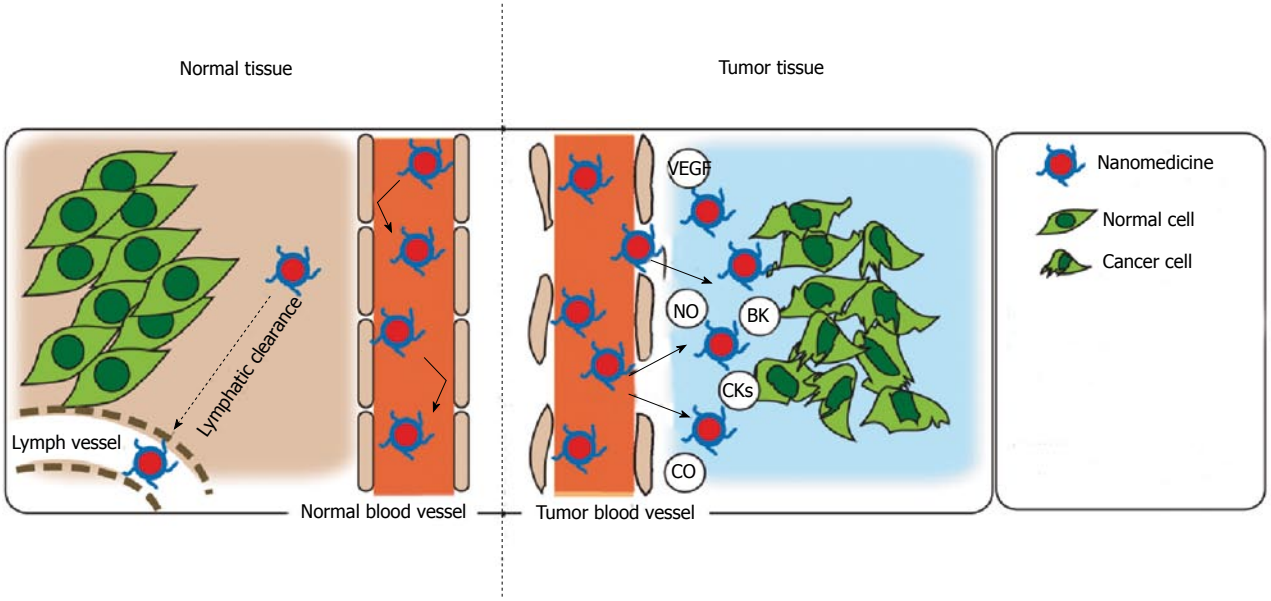

Figure 1 Abnormal characteristics and factors of solid tumors that influence the enhanced permeability and retention effect. Graphical image is from ref.[14] with permission. NO: Nitric oxide; BK: Bradykinin; CO: Carbon monoxide; CKs: Cytokines; VEGF: Vascular endothelial growth factor.

with high concentration[10,11]. One successful strategy regarding this issue is the utilization of the acidic pH (*e.g.*, 6.5-6.7) of tumors. Maeda *et al*[10] recently reported a tumor environment/pH responsive poly(N-(2-hydroxypropyl)methacrylamide) conjugated pirarubicin (P-THP), which behaves as polymeric conjugate/micelle in circulation, but liberates free THP in acidic tumor environment, resulting a remarkable antitumor effect[11]. This P-THP therapy was also translated into clinic successfully; in a patient with advanced prostate cancer with multiple lung metastasis, P-THP treatment resulted in complete remission of metastatic tumor nodules in the lung, with significantly decreased levels of prostate specific antigen (PSA, from 1472 ng/mL to 0.067 ng/mL); no severe side effects were observed and no evidence of disease relapse has been recorded for 12 mo since the administration of P-THP (unpublished data).

Another issue should be addressed is that, EPR effect is the phenomenon of blood vessels, so it may varies depending on the patient/tumor's pathological characteristics and conditions. Namely tumors with less blood vessels, *e.g.*, pancreatic cancer, always show less EPR effect. The EPR effect is heterogeneous even in a single tumor nodule. Thus further augmentation of EPR effect is important or necessary for treating such tumors, which could be achieved by modulating the vascular mediators in tumor such as using angiotensin Ⅱ, nitric oxide/nitroglycerin, angiotensin Ⅱ converting enzyme inhibitor and carbon monoxide, all of which increase EPR effect by 2-10 times and some of them (*i.e.*, angiotensin Ⅱ) were proven in clinic[6,10,12,13].

EPR effect is now becoming the "gold standard" for design and development of cancer drug, we believe EPR-based nanomedicine that is becoming a promising paradigm of anticancer strategy, will be a solution for cancer in the future.

REFERENCES

1 **Druker BJ**, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers CL. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* 2001; **344**: 1031-1037 [PMID: 11287972]

2 **Sosman JA**, Kim KB, Schuchter L, Gonzalez R, Pavlick AC, Weber JS, McArthur GA, Hutson TE, Moschos SJ, Flaherty KT, Hersey P, Kefford R, Lawrence D, Puzanov I, Lewis KD, Amaravadi RK, Chmielowski B, Lawrence HJ, Shyr Y, Ye F, Li J, Nolop KB, Lee RJ, Joe AK, Ribas A. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *N Engl J Med* 2012; **366**: 707-714 [PMID: 22356324 DOI: 10.1056/NEJMoa1112302]

3 **Tol J**, Koopman M, Cats A, Rodenburg CJ, Creemers GJ, Schrama JG, Erdkamp FL, Vos AH, van Groeningen CJ, Sinnige HA, Richel DJ, Voest EE, Dijkstra JR, Vink-Börger ME, Antonini NF, Mol L, van Krieken JH, Dalesio O, Punt CJ. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. *N Engl J Med* 2009; **360**: 563-572 [PMID: 19196673 DOI: 10.1056/NEJMoa0808268]

4 **Vogelstein B**, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Kinzler KW. Cancer genome landscapes. *Science* 2013; **339**: 1546-1558 [PMID: 23539594 DOI: 10.1126/science.1235122]

5 **Gerlinger M**, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, Martinez P, Matthews N, Stewart A, Tarpey P, Varela I, Phillimore B, Begum S, McDonald NQ, Butler A, Jones D, Raine K, Latimer C, Santos CR, Nohadani M, Eklund AC, Spencer-Dene B, Clark G, Pickering L, Stamp G, Gore M, Szallasi Z, Downward J, Futreal PA, Swanton C. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med* 2012; **366**: 883-892 [PMID: 22397650 DOI: 10.1056/NEJMoa1113205]

6 **Fang J**, Nakamura H, Maeda H. The EPR effect: Unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. *Adv Drug Deliv Rev* 2011; **63**: 136-151 [PMID: 20441782 DOI: 10.1016/j.addr.2010.04.009]

7 **Matsumura Y**, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Res* 1986; **46**: 6387-6392 [PMID: 2946403]

8 **Torchilin V**. Tumor delivery of macromolecular drugs based on the EPR effect. *Adv Drug Deliv Rev* 2011; **63**: 131-135 [PMID:

20304019 DOI: 10.1016/j.addr.2010.03.011]
9 **Duncan R**, Vicent MJ. Polymer therapeutics-prospects for 21st century: the end of the beginning. *Adv Drug Deliv Rev* 2013; **65**: 60-70 [PMID: 22981753 DOI: 10.1016/j.addr.2012.08.012]
10 **Maeda H**, Nakamura H, Fang J. The EPR effect for macromolecular drug delivery to solid tumors: Improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo. *Adv Drug Deliv Rev* 2013; **65**: 71-79 [PMID: 23088862 DOI: 10.1016/j.addr.2012.10.002]
11 **Nakamura H**, Etrych T, Chytil P, Ohkubo M, Fang J, Ulbrich K, Maeda H. Two step mechanisms of tumor selective delivery of N-(2-hydroxypropyl)methacrylamide copolymer conjugated with pirarubicin via an acid-cleavable linkage. *J Control Release* 2014; **174**: 81-87 [PMID: 24269967 DOI: 10.1016/j.jconrel.2013.11.011]
12 **Nagamitsu A**, Greish K, Maeda H. Elevating blood pressure as a strategy to increase tumor-targeted delivery of macromolecular drug SMANCS: cases of advanced solid tumors. *Jpn J Clin Oncol* 2009; **39**: 756-766 [PMID: 19596662 DOI: 10.1093/jjco/hyp074]
13 **Fang J**, Liao L, Yin H, Nakamura H, Shin T, Maeda H. Enhanced bacterial tumor delivery by modulating the EPR effect and therapeutic potential of Lactobacillus casei. *J Pharm Sci* 2014; **103**: 3235-3243 [PMID: 25041982 DOI: 10.1002/jps.24083]
14 **Nakamura H**, Jun F, Maeda H. Development of next-generation macromolecular drugs based on the EPR effect: challenges and pitfalls. *Expert Opin Drug Deliv* 2015; **12**: 53-64 [PMID: 25425260 DOI: 10.1517/17425247.2014.955011]

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REVIEW

Lipoprotein based drug delivery: Potential for pediatric cancer applications

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Abstract

While survival rates for patients with childhood cancers have substantially improved, the quality of life of the survivors is often adversely impacted by the residual effects of chemo and radiation therapy. Because of the existing metabolic and physiological disparities between pediatric and adult patients, the treatment of pediatric cancer patients poses special challenges to oncologists. While numerous clinical trials being conducted, to improve treatment outcomes for pediatric cancer patients, new approaches are required to increase the efficacy and to minimize the drug related toxic side effects. Nanotechnology is a potentially effective tool to overcome barriers to effective cancer therapeutics including poor bioavailability and non-specific targeting. Among the nano-delivery approaches, lipoprotein based formulations have shown particularly strong promise to improve cancer therapeutics. The present article describes the challenges faced in the treatment of pediatric cancers and reviews the potential of lipoprotein-based therapeutics for these malignancies.

Key words: Drug delivery; Lipoprotein; Nanoparticles; Pediatric cancers; High density lipoprotein

Core tip: While survival rates for patients with childhood cancers have improved, the quality of life of survivors is often adversely impacted by the residual effects of therapy. Consequently, new approaches will be required to increase the efficacy and to minimize the drug related toxic side effects of pediatric cancer therapy. Nanotechnology is a potentially effective tool to improve cancer chemotherapy *via* enhanced bioavailability and specific targeting. Lipoprotein based formulations have shown particularly strong promise to improve cancer therapeutics. The present article describes the challenges faced in the treatment of pediatric cancers and reviews the potential of lipoprotein-based therapeutics for these malignancies.

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INTRODUCTION

Although cancer is the leading cause of death in children above 1 year of age in Europe and the Unites States, more than 80% of the children diagnosed with cancer are expected to survive, subsequent to treatment, though 40% will suffer through adulthood from the long term consequences of the treatment administered during childhood[1,2]. While advances in the chemotherapy of pediatric malignancies have produced major improvements in survival over the last several years, treatment-related side effects remain a major concern.

The recently developed nanotechnology-based drug delivery vehicles (nano-DDVs) are directed toward overcoming the shortcomings of the currently employed chemotherapeutic agents, including poor solubility, limited bioavailability and inadequate stability[3-6]. Additionally most of these nano DD systems target specific sites by either passive or active transport mechanisms[7-10] and thus minimize the systemic exposure of normal tissues to the drug. Nanotechnology has also been shown to improve localized drug delivery by selective administration routes in order to overcome anatomical or physiological barriers, such as the blood brain barrier in the central nervous system[11-13]. Currently available treatment modalities for pediatric malignancies involve chemotherapy, surgery, radiation, bone marrow transplant and immune based therapy. These treatments are often accompanied by short and long-term side effects, resulting in deterioration of physiological functions among the survivors that impact the quality of life well into adulthood[14]. While current therapeutic approaches have markedly improved the prognosis for survival of pediatric cancer patients, a significant portion of childhood malignancies remain resistant to current regimens, leading to progressive disease and death[15]. Hence there is an urgent need to develop novel therapeutic strategies for pediatric cancers, in addition to reducing the residual toxicities. This review aims to focus on the challenges involved in treating pediatric cancers and the potential for overcoming these barriers *via* nanotechnology in general, utilizing lipoprotein based nano DDV in particular.

PEDIATRIC CANCERS ARE DIFFERENT FROM ADULT CANCERS

Pediatric cancers are different from adult malignancies because they often originate from cellular populations that have not completed the process of terminal differentiation[16-18]. Childhood cancers are often the result of genetic changes that take place very early in life, sometimes even before birth. Unlike many cancers in adults, childhood cancers are thus not strongly linked to lifestyle or environmental risk factors. Accordingly, children are very rarely diagnosed with ovarian, breast, colon or lung carcinomas that frequently occur in adults. Although childhood cancers are often more aggressive and remain undetected until an advanced stage is reached, due to the advances in therapeutics over past decades pediatric cancers tend to be more easily curable than adult cancers. The most common cancers diagnosed in children are given in Table 1.

According to the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute a 5-year relative survival rate for all cancers combined has increased from 61.7% in 1975-1977 to 81.4% in 1999-2006, among children from 0 to 19 years of age (NCI SEERS 2010)[20]. Between 1975 and 2007 the mortality rates for non-Hodgkin lymphoma decreased by 75% followed by 60% reduction in mortality statistics for acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML)[20].

As a result, non-Hodgkin lymphomas and ALL are now among the most curable childhood cancers. These improvements in the prognoses of selected malignancies can be attributed to the improved risk assessment, supportive care, the development of new drugs directed at specific targets and most importantly, enrollment of large numbers of patients in well-designed prospective clinical trials. However, the survival rates for children with other solid tumors, including most bone and soft tissue sarcomas and brain tumors have not improved as dramatically over past four decades.

THERAPEUTIC CHALLENGES IN PEDIATRIC ONCOLOGY

The differences between the metabolic capacity, drug bio-distribution, organ function and absorption in response to drug therapy of children and that of adults are well known[21-23]. In addition, pediatric patients are less likely to have underlying health related issues as compared to adult populations undergoing treatment. The developmental changes profoundly affect the responses of children to medications and to related therapies[24]. All these factors affect the way in which treatment modalities are designed and applied to pediatric populations.

Designing formulations for pediatric patients is often complex because this age group is further subdivided into different groups, based on differences in biology and metabolic capacity. These groupings represent preterm newborn infants, term newborn infants (0-27 d), infants and toddlers (28 d-23 mo), preschool children (2-5 years), school children (6-11 years) and adolescents (12-18 years)[25]. Each subcategory displays different biochemical functions and capabilities[18,24] while the level of cognitive development may also impact the effectiveness of drug formulations for cancer therapy[26]. Because most pediatric cancers

Table 1 Frequently encountered pediatric malignancies[19]

Type of cancer	Definition/characteristics	% Incidence 2005
Leukemia	Leukemia is cancer of the body's blood-forming tissues, including the bone marrow and the lymphatic system	34
Brain and central nervous system tumors	Normal cells in the brain or the spinal cord change and grow uncontrollably, forming a mass	23
Neuroblastoma	It is a neuroendocrine tumor, most frequently originating in one of the adrenal glands, in addition to nerve tissues in the neck, chest, abdomen, or pelvis	7
Wilm's tumor or nephroblastoma	Cancer of kidney that occurs in children	5
Lymphoma (Hodgkins and Non-Hodgkins)	Blood cell tumor that develops from lymphocytes	12
Rhabdomyosarcoma	Cancer of soft tissues where the cancer cells originate from skeletal muscle progenitor	3
Bone cancer	Osteosarcoma and Ewing's sarcoma are the most common malignancies of bone	4
Germ cell tumors	Germ cells tumors typically emerge from gonads but may also originate in other parts of the body, while arising from embryonic germ cell "rests"	N/A

N/A: Statistics not available.

are rare; hence sample size is often a major concern regarding the design and performance of clinical trials.

Clinical trials involving pediatric patients are further restricted by the hesitancy of ethical review committees toward drug trials in children and the reluctance of pharmaceutical companies to invest in these costly ventures in view of the limited children's pharmaceutical market. Another challenge faced by pediatric oncologists while designing clinical trials, is determining the appropriate dosages of a drug for administration, especially as they apply to combination therapy. Even though the mechanism of action and the effective dose of most drugs in adults are known, a linear dose-per-kg correlation may not be appropriate for small children. Kearns *et al*[24] reviewed key maturational changes that account for differences in drug metabolism and disposition of drug formulations in pediatric populations *vs* those in adults. Gastric emptying time, gastric and duodenal pH, intestinal transit time, secretion and activity of bile and pancreatic fluid, bacterial colonization and transporters, such as P-glycoprotein (P-gp) are important factors for drug absorption[24], whereas key factors explaining differences in drug distribution between the pediatric population and adults are organ size, membrane permeability, plasma protein concentration and characteristics, endogenous substances in plasma, total body and extracellular water, fat content, regional blood flow and transporters such as P-gp, which is present not only in the gut, but also in liver, kidney, brain and other tissues[23].

Cancer therapeutics *via* nano drug delivery vehicle (DDV) is an emerging field that is yet to be fully investigated in children. The toxicological aspects of the exposure to nanoparticles will need to be thoroughly assessed to establish their safety for children, before the application of these formulations in pediatric oncology. These challenges notwithstanding, the application of nano DDVs in cancer therapeutics represents one of the most promising and rapidly expanding approaches based on the number of research reports and clinical trials in progress. Consequently, it is likely that, in due time, nano DDVs will be broadly applied in pediatric oncology.

Nanomedicine based therapeutics in children

The multiple advantageous features of nano DDVs, including high payload capacity, favorable biodistribution and pharmacokinetic profiles make them ideal candidates. Another advantage of most nano DDVs is their multimodal loading capability. The surface or core of the DDV may be loaded with multiple agents, so that treatment and monitoring of treatment *via* imaging can occur simultaneously (theranostics). Metals, chelators and/or radioisotopes may be included for CT and MRI or PET/SPECT imaging or *in vivo* imaging[27-29]. The ease of tracking nano DDVs *in vivo*, presents a unique opportunity for monitoring drug distribution on a patient by patient basis to determine whether drug accumulation is sufficient for a desirable therapeutic effect.

The potential of using nanomedicine to improve the diagnosis and the treatment of pediatric cancers has been extensively documented[30-32]. Several biologically based formulations have been applied in the form of nano DDVs[33-38] (including cross-linked liposomes, lipids, chitosan, lactic acid conjugates, *etc.*[36-38]) and chemical constructs (including polymer based, dendrimers, flo dots, quantum dots, ceramic, metal based, *etc.*).

As a result of research and development in nano DDV over past decade, several nano DDV formulations already made their way to the market including polymer-based poly(lactide-co-glycolide) nanocarriers, liposomes and abraxane[39,40]. However, all of these formulations are designated for use in adults. Similar formulations are currently in different phases of clinical trials in pediatric populations (Table 2); however, none has reached the clinic yet.

LIPOPROTEIN BASED NANO DDVS

An ideal DDV is expected to have excellent loading capacity, therapeutic shielding, biocompatibility and selective targeting capability. An effective DDV formulation should also be able to accommodate multimodal

Table 2 Drug delivery formulations currently undergoing clinical trials for pediatric cancers

FDA approved Formulations	Drug	Phase of pediatric clinical trial	Type of cancer	Ref.
Abraxane	Paclitaxel	Preclinical	Rhabdomyosarcoma Osteosarcoma Neuroblastoma	[30]
Nab paclitaxel	Paclitaxel	Phase I and II	Rhabdomyosarcoma, neuroblastoma	[41]
Doxil	Doxorubicin	Phase I and II	Refractory or recurrent Rhabdomyosarcoma, Neuroblastoma, Pontine glioma	[31]
DaunoXome	Daunorubicin	Phase III	AIDS related Kaposi Sarcoma, pediatric in acute myloid leukemia refractory/ relapsed	[32]
L-Annamycin	L-Annamycin	Phase I	Acute lymphocytic and acute myloid Leukemia	[42]
Depocyte (liposomalformulation)	Cytarabine	Phase I	Acute lymphocytic leukemia Recurrent brain tumor	[43] [44]
Marquibo	Vincristine sulfate	Phase I Phase II	Sarcoma Neuroblastoma	[45]
CPX 351	Cytarabine and daunorubicin	Phase I	Relapsed leukemia or lymphoma	[46]

FDA: United States Food and Drug Administration.

anti-cancer and /or contrast agents (for tumor imaging) and exhibit minimum undesirable side reactions by avoiding interactions with off target sites. Lipoprotein-inspired DDVs possess most of these desirable features and thus represent a promising platform for pediatric cancer therapeutics[35,47-50].

Lipoproteins are natural transport vehicles for shuttling lipids and lipophilic molecules in an aqueous milieu to organs of the body in mammals[51]. Although there are several classes of lipoproteins differing in size, buoyant density and the constituent apolipoproteins present, they exhibit common chemical characteristics that include a hydrophobic core surrounded by an amphiphilic shell of a phospholipid/cholesterol monolayer and several apolipoproteins. There are four major classes of lipoproteins present in the human/ mammalian circulation (Figure 1), including chylomicron (75-1000 nm/ApoB-48), very low density lipoprotein (30-80 nm/ApoB-1000), low density lipoprotein (LDL) (18–25 nm/ApoB-100) and high density lipoprotein (HDL) (5-12 nm/ApoA-I, A-II, -E and -C)[47,52,53]. Due to their unique structural/functional properties lipoproteins are considered an excellent model DDVs for transporting and delivering chemotherapeutic agents[47].

Lipoprotein DDVs may be artificially assembled in different ways to transport drugs or imaging agents to desired sites[34,35]. Depending on the chemical nature of the payload and the method of formulation these DDVs may be loaded either by covalent modification of the phospholipid or protein component, intercalation of the agent into phospholipid or encapsulation into the hydrophobic core of the DDV[47,54,55].

Drug delivery *via* LDL and HDL receptors: Carcinogenesis is a multifaceted process that involves immense reorganization of signaling pathways, genetic information, structural constituents and energy metabolism of the cell[56,57]. As a result, cancer cells exhibit markedly elevated metabolic/energy requirements to sustain the tumor proliferation and migration functions[58]. These changes are induced and facilitated by mutating growth factor receptors resulting in constitutive signaling to key metabolic pathways[50,59]. In addition to basic nutrients, cancer cells have an excessive need for many other substances including cholesterol for membrane biogenesis[60]. One of the mechanisms that cancer cells use to meet this requirement is by over-expressing the LDL and HDL lipoprotein receptors[59,61-63]. Drug delivery strategies have been developed using both LDL and HDL receptor targeting DDVs[64-67] as well as liposome DDVs modified by LDL receptor ligands[68,69]. The drug carrying reconstituted HDL (rHDL) nanoparticles targeted to Scavenger receptor B-1 (SR-B1) function as a "magic bullet" and enhance the therapeutic efficacy of the enclosed drugs toward malignant tumors[70]. The over-expression of the SR-B1 receptor in malignant tissues has the potential to facilitate the enhanced selective delivery of anti-cancer agents to tumors thus providing a marked improvement of the current chemotherapy regimens, including the limiting of off-target toxicity[59,61,62].

Why use the rHDL nanoparticles for drug delivery of anti-cancer drugs in pediatric oncology?

While numerous studies employed liposomes to produce improved solubility and bioavailability of anti-cancer agents, due to their small size, rHDL nanoparticles accrue substantial additional therapeutic benefits (Figure 1) *via* their enhanced capability to penetrate the tumor microenvironment, including its vasculature and stroma. This is anticipated to be a major advantage when treating pediatric cancers since these tumors are often associated with stroma. The rHDL DDVs have

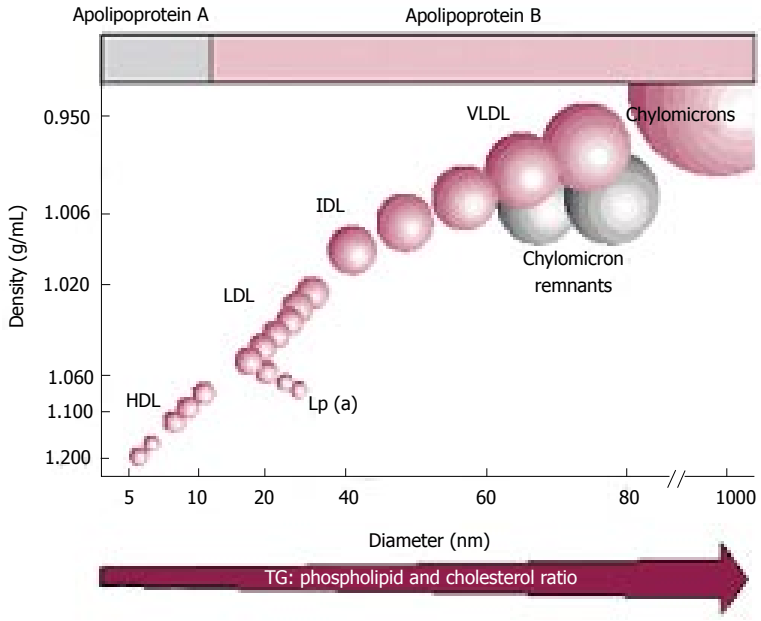

Figure 1 Size and density distribution of lipoproteins. HDL: High density lipoprotein; IDL: Intermediate density lipoprotein; VLDL: Very low density lipoprotein; LDL: Low density lipoprotein.

been evaluated regarding their efficacy and capacity to perform targeted delivery of cancer drugs[61,62,71]. In addition, the rHDL DDVs are comprised of endogenous biocompatible ingredients that have already been injected into human subjects during cholesterol metabolism trials[72].

Due to their structural similarity to their natural counterparts, rHDLs effectively avoid recognition by the reticuloendothelial system that clears foreign substances, and thus fail to trigger immune responses in contrast to other synthetic DDVs including liposomes[73]. Additional advantages of the rHDL DDVs include extended retention time in circulation, stability and cytoplasmic drug delivery to circumvent drug resistance that may develop during chemotherapy. Also lesser amounts of drug are likely to be required for achieving the same cytotoxic effect compared with the drug used in its free form[67]. Although these advantages of lipoprotein based nano DDV could be beneficial to all types of cancer patients, pediatric patients are anticipated to benefit the most by the extended safety, long drug retention time and enhanced therapeutic efficacy.

Our laboratory has focused on studies of targeted drug delivery, including optimization of the rHDL nanoparticle *via* attachment of targeting molecules. Mooberry *et al*[61] have shown that the uptake of paclitaxel by ovarian cancer cells from rHDL DDVs could thus be substantially enhanced by covalently attaching a folic acid residue to the apolipoprotein component of the nanoparticle. Similarly, Parker *et al*[74] exploited the overexpression of folate receptors in tumor cells by conjugating folic acid to the apolipoprotein B component of an LDL-like DDV and thus specifically targeted drugs, transported by the lipoprotein vehicle. These studies suggest that lipoprotein DDVs could be specifically functionalized for targeting surface antigens (including receptors) that are overexpressed by malignant tumors[48,60]. Overall, as described above, lipoproteins possess many desirable characteristics that enable them to serve as natural or synthetic drug transporters. While lipoproteins were proposed as efficient DDVs over thirty years ago, perhaps surprisingly, no lipoprotein formulation has so far been approved for clinical application to date. The recent upsurge in interest to develop lipoprotein DDVs will perhaps spawn the needed energy and investment to fully take advantage of this robust, natural drug carrier for therapeutic purposes in general and pediatric formulations in particular.

FUTURE PERSPECTIVE FOR PEDIATRIC CANCER CHEMOTHERAPY

Conventional cancer chemotherapy has traditionally been associated with undesirable side effects that are especially troublesome during the treatment of pediatric patients. Researchers have drawn attention to the multidimensional benefits of lipoprotein based DDVs including their biocompatibility and stability that enable them to minimize these side effects *via* specifically targeting malignant cells and tumors while avoiding normal tissues[48,59,61,63,75]. Several clinical studies have demonstrated that HDL-type formulations have been safely administered to human subjects[76-78]. Selection of patients for rHDL driven chemotherapy could be based on the SR-B1 expression levels of each specific tumor involved; thus, provide a new bio-marker for eventual personalized therapy. There are numerous additional membrane proteins which could be used as targets for functionalized rHDL. This feature of rerouting DDVs from their endogenous receptors and steering them to specific sites[71] could further enhance the potential of the rHDL nanoparticles to facilitate the development of a robust personalized therapy regimen for pediatric cancers. Despite the major advances in

pediatric cancer research, there are several malignancies afflicting children that remain resistant to therapy. In addition, extension of 5 year survival or even producing permanent remission is often accompanied by harmful long lasting and debilitating side effects in pediatric cancer patients. Perhaps improved treatment modalities developed *via* novel nanoparticle formulations and specifically involving lipoprotein type carriers will provide the needed tools to overcome the current barriers to successful pediatric cancer therapy.

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REFERENCES

1 **Vassal G**, Zwaan CM, Ashley D, Le Deley MC, Hargrave D, Blanc P, Adamson PC. New drugs for children and adolescents with cancer: the need for novel development pathways. *Lancet Oncol* 2013; **14**: e117-e124 [PMID: 23434337 DOI: 10.1016/S1470-2045(13)70013-5]

2 **Kaatsch P**. Epidemiology of childhood cancer. *Cancer Treat Rev* 2010; **36**: 277-285 [PMID: 20231056 DOI: 10.1016/j.ctrv.2010.02.003]

3 **Felice B**, Prabhakaran MP, Rodríguez AP, Ramakrishna S. Drug delivery vehicles on a nano-engineering perspective. *Mater Sci Eng C Mater Biol Appl* 2014; **41**: 178-195 [PMID: 24907751 DOI: 10.1016/j.msec.2014.04.049]

4 **Rastogi V**, Yadav P, Bhattacharya SS, Mishra AK, Verma N, Verma A, Pandit JK. Carbon nanotubes: an emerging drug carrier for targeting cancer cells. *J Drug Deliv* 2014; **2014**: 670815 [PMID: 24872894 DOI: 10.1155/2014/670815]

5 **Matsumura Y**. The drug discovery by nanomedicine and its clinical experience. *Jpn J Clin Oncol* 2014; **44**: 515-525 [PMID: 24755547 DOI: 10.1093/jjco/hyu046]

6 **Bottini M**, Sacchetti C, Pietroiusti A, Bellucci S, Magrini A, Rosato N, Bottini N. Targeted nanodrugs for cancer therapy: prospects and challenges. *J Nanosci Nanotechnol* 2014; **14**: 98-114 [PMID: 24730253]

7 **Petkar KC**, Chavhan SS, Agatonovik-Kustrin S, Sawant KK. Nanostructured materials in drug and gene delivery: a review of the state of the art. *Crit Rev Ther Drug Carrier Syst* 2011; **28**: 101-164 [PMID: 21663574]

8 **Chakraborty M**, Jain S, Rani V. Nanotechnology: emerging tool for diagnostics and therapeutics. *Appl Biochem Biotechnol* 2011; **165**: 1178-1187 [PMID: 21847590 DOI: 10.1007/s12010-011-9336-6]

9 **Chandra S**, Nigam S, Bahadur D. Combining unique properties of dendrimers and magnetic nanoparticles towards cancer theranostics. *J Biomed Nanotechnol* 2014; **10**: 32-49 [PMID: 24724497]

10 **Dobbelstein M**, Moll U. Targeting tumour-supportive cellular machineries in anticancer drug development. *Nat Rev Drug Discov* 2014; **13**: 179-196 [PMID: 24577400 DOI: 10.1038/nrd4201]

11 **Vidu R**, Rahman M, Mahmoudi M, Enachescu M, Poteca TD, Opris I. Nanostructures: a platform for brain repair and augmentation. *Front Syst Neurosci* 2014; **8**: 91 [PMID: 24999319 DOI: 10.3389/fnsys.2014.00091]

12 **Dinda SC**, Pattnaik G. Nanobiotechnology-based drug delivery in brain targeting. *Curr Pharm Biotechnol* 2013; **14**: 1264-1274 [PMID: 24910011]

13 **Muthu MS**, Leong DT, Mei L, Feng SS. Nanotheranostics - application and further development of nanomedicine strategies for advanced theranostics. *Theranostics* 2014; **4**: 660-677 [PMID: 24723986 DOI: 10.7150/thno.8698]

14 **van Ginkel PR**, Sareen D, Subramanian L, Walker Q, Darjatmoko SR, Lindstrom MJ, Kulkarni A, Albert DM, Polans AS. Resveratrol inhibits tumor growth of human neuroblastoma and mediates apoptosis by directly targeting mitochondria. *Clin Cancer Res* 2007; **13**: 5162-5169 [PMID: 17785572 DOI: 10.1158/1078-0432.CCR-07-0347]

15 **Kersey JH**. Fifty years of studies of the biology and therapy of childhood leukemia. *Blood* 1998; **92**: 1838 [PMID: 9716617]

16 American cancer society's website. What are the differences between cancers in adults and children? [cited 2014 Feb 03]. Available from: URL: http://www.cancer.org/cancer/leukemiainchildren/detailedguide/childhood-leukemia-differences-children-adults

17 **Sosnik A**, Seremeta KP, Imperiale JC, Chiappetta DA. Novel formulation and drug delivery strategies for the treatment of pediatric poverty-related diseases. *Expert Opin Drug Deliv* 2012; **9**: 303-323 [PMID: 22257003 DOI: 10.1517/17425247.2012.655268]

18 **Bowles A**, Keane J, Ernest T, Clapham D, Tuleu C. Specific aspects of gastro-intestinal transit in children for drug delivery design. *Int J Pharm* 2010; **395**: 37-43 [PMID: 20478372 DOI: 10.1016/j.ijpharm.2010.04.048]

19 Childhood Cancer overview from American Society of Clinical Oncology (ASCO). Cancer in Children from Centers for Disease Control and Prevention [cited 2014 June; updated 2009 Jul 30]. Available from: URL: http://www.cancer.org/cancer/cancerinchildren/detailedguide/cancer-in-children-types-of-childhood-cancers

20 **Kaatsch P**, Sikora E, Pawelec G. Types of childhood cancers statistics: (June 2010). "Epidemiology of childhood cancer". *Cancer treatment reviews* 2010; **36**: 277-285

21 **Ginsberg G**, Hattis D, Sonawane B, Russ A, Banati P, Kozlak M, Smolenski S, Goble R. Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. *Toxicol Sci* 2002; **66**: 185-200 [PMID: 11896285]

22 **Yanni S**. Disposition and interaction of biotherapeutics in pediatric populations. *Curr Drug Metab* 2012; **13**: 882-900 [PMID: 22475271]

23 **Strolin Benedetti M**, Whomsley R, Baltes EL. Differences in absorption, distribution, metabolism and excretion of xenobiotics between the paediatric and adult populations. *Expert Opin Drug Metab Toxicol* 2005; **1**: 447-471 [PMID: 16863455]

24 **Kearns GL**, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology--drug disposition, action, and therapy in infants and children. *N Engl J Med* 2003; **349**: 1157-1167 [PMID: 13679531]

25 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH E 11, Clinical Investigation of Medicinal Products in the Paediatric Population (CPMP/ICH/2711/99) (London, 2000, The US: International Conference on Harmonisation, Guidance on E 11 Clinical Investigation of Medicinal Products in Pediatric Population; Notice. Federal Register, 2000, 65). Cambridge: Cambridge University Press, 2003: 78493-78494

26 **Committee for medicinal products for human use (chmp)**. Reflection paper: formulations of choice for the paediatric population. European medicines agency pre-authorization evaluation of medicines for human use (London, 28 July 2006. EMEA/CHMP/PEG/194810/2005). Available from: URL: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003782.pdf

27 **Pérez-Medina C**, Abdel-Atti D, Zhang Y, Longo VA, Irwin CP, Binderup T, Ruiz-Cabello J, Fayad ZA, Lewis JS, Mulder WJ, Reiner T. A modular labeling strategy for in vivo PET and near-infrared fluorescence imaging of nanoparticle tumor targeting. *J Nucl Med* 2014; **55**: 1706-1711 [PMID: 25060196]

28 **Rangger C**, Helbok A, Sosabowski J, Kremser C, Koehler G, Prassl R, Andreae F, Virgolini IJ, von Guggenberg E, Decristoforo C. Tumor targeting and imaging with dual-peptide conjugated multifunctional liposomal nanoparticles. *Int J Nanomedicine* 2013; **8**: 4659-4671 [PMID: 24353415 DOI: 10.2147/IJN.S51927]

29 **Lee J**, Lee TS, Ryu J, Hong S, Kang M, Im K, Kang JH, Lim SM, Park S, Song R. RGD peptide-conjugated multimodal NaGdF4: Yb3+/Er3+ nanophosphors for upconversion luminescence, MR, and PET imaging of tumor angiogenesis. *J Nucl Med* 2013; **54**: 96-103 [PMID: 23232276 DOI: 10.2967/jnumed.112.108043]

30 **Zhang L**, Marrano P, Kumar S, Leadley M, Elias E, Thorner P, Baruchel S. Nab-paclitaxel is an active drug in preclinical model of pediatric solid tumors. *Clin Cancer Res* 2013; **19**: 5972-5983 [PMID: 23989978 DOI: 10.1158/1078-0432.CCR-13-1485]

31 **Marina NM**, Cochrane D, Harney E, Zomorodi K, Blaney S, Winick N, Bernstein M, Link MP. Dose escalation and pharmacokinetics of pegylated liposomal doxorubicin (Doxil) in children with solid tumors: a pediatric oncology group study. *Clin Cancer Res* 2002; **8**: 413-418 [PMID: 11839657]

32 **Kaspers GJ**, Zimmermann M, Reinhardt D, Gibson BE, Tamminga RY, Aleinikova O, Armendariz H, Dworzak M, Ha SY, Hasle H, Hovi L, Maschan A, Bertrand Y, Leverger GG, Razzouk BI, Rizzari C, Smisek P, Smith O, Stark B, Creutzig U. Improved outcome in pediatric relapsed acute myeloid leukemia: results of a randomized trial on liposomal daunorubicin by the International BFM Study Group. *J Clin Oncol* 2013; **31**: 599-607 [PMID: 23319696 DOI: 10.1200/JCO.2012.43.7384]

33 **Yang Y**, Wang S, Wang Y, Wang X, Wang Q, Chen M. Advances in self-assembled chitosan nanomaterials for drug delivery. *Biotechnol Adv* 2014; **32**: 1301-1316 [PMID: 25109677 DOI: 10.1016/j.biotechadv.2014.07.007]

34 **Ng KK**, Lovell JF, Zheng G. Lipoprotein-inspired nanoparticles for cancer theranostics. *Acc Chem Res* 2011; **44**: 1105-1113 [PMID: 21557543]

35 **Sabnis N**, Lacko AG. Drug delivery via lipoprotein-based carriers: answering the challenges in systemic therapeutics. *Ther Deliv* 2012; **3**: 599-608 [PMID: 22834404]

36 **Winter PM**. Perfluorocarbon nanoparticles: evolution of a multimodality and multifunctional imaging agent. *Scientifica* (Cairo) 2014; **2014**: 746574 [PMID: 25024867 DOI: 10.1155/2014/746574]

37 **Bazak R**, Houri M, El Achy S, Kamel S, Refaat T. Cancer active targeting by nanoparticles: a comprehensive review of literature. *J Cancer Res Clin Oncol* 2015; **141**: 769-784 [PMID: 25005786]

38 **Saenz del Burgo L**, Pedraz JL, Orive G. Advanced nanovehicles for cancer management. *Drug Discov Today* 2014; **19**: 1659-1670 [PMID: 24981660 DOI: 10.1016/j.drudis.2014.06.020]

39 **Sosnik A**, Carcaboso AM. Nanomedicines in the future of pediatric therapy. *Adv Drug Deliv Rev* 2014; **73**: 140-161 [PMID: 24819219 DOI: 10.1016/j.addr.2014.05.004]

40 **Federman N**, Denny CT. Targeting liposomes toward novel pediatric anticancer therapeutics. *Pediatr Res* 2010; **67**: 514-519 [PMID: 20118828 DOI: 10.1203/PDR.0b013e3181d601c5]

41 **Celgene Corporation**. To Find a Safe Dose and Show Early Clinical Activity of Weekly Nab-paclitaxel in Pediatric Patients with Recurrent/ Refractory Solid Tumors [updated 2014 Aug 11]. Available from: URL: http://clinicaltrials.gov/show/NCT01962103

42 **Callisto Pharmaceuticals, Inc**. Callisto Pharmaceuticals Opens L-Annamycin Phase I Clinical Trial in Pediatric Relapsed or Refractory Acute Leukemia. Available from: URL: http://www.prnewswire.co.uk/news-releases/callisto-pharmaceuticals-opens-l-annamycin-phase-i-clinical-trial-in-pediatric-relapsed-or-refractory-acute-leukemia-153539655.html

43 **Seif AE**, Reilly AF, Rheingold SR. Intrathecal liposomal cytarabine in relapsed or refractory infant and pediatric leukemias: the Children's Hospital of Philadelphia experience and review of the literature. *J Pediatr Hematol Oncol* 2010; **32**: e349-e352 [PMID: 20962675 DOI: 10.1097/MPH.0b013e3181ec0c25]

44 **Benesch M**, Siegler N, Hoff Kv, Lassay L, Kropshofer G, Müller H, Sommer C, Rutkowski S, Fleischhack G, Urban C. Safety and toxicity of intrathecal liposomal cytarabine (Depocyte) in children and adolescents with recurrent or refractory brain tumors: a multi-institutional retrospective study. *Anticancer Drugs* 2009; **20**: 794-799 [PMID: 19617818 DOI: 10.1097/CAD.0b013e32832f4abe]

45 **Spectrum Pharmaceuticals, Inc**. To Evaluate the Safety, Activity and Pharmacokinetics of Marqibo in Children and Adolescents with Refractory Cancer.. Available from: URL: http://clinicaltrials.gov/show/NCT01222780

46 **Children's Hospital Medical Center, Cincinnati**. Safety Study of CPX-351 in Children with Relapsed Leukemia or Lymphoma In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: http://clinicaltrials.gov/show/NCT01943682 NLM Identifier: NCT01943682

47 **Bricarello DA**, Smilowitz JT, Zivkovic AM, German JB, Parikh AN. Reconstituted lipoprotein: a versatile class of biologically-inspired nanostructures. *ACS Nano* 2011; **5**: 42-57 [PMID: 21182259 DOI: 10.1021/nn103098m]

48 **Basha R**, Sabnis N, Heym K, Bowman WP, Lacko AG. Targeted nanoparticles for pediatric leukemia therapy. *Front Oncol* 2014; **4**: 101 [PMID: 24860784 DOI: 10.3389/fonc.2014.00101]

49 **Johnson R**, Sabnis N, McConathy WJ, Lacko AG. The potential role of nanotechnology in therapeutic approaches for triple negative breast cancer. *Pharmaceutics* 2013; **5**: 353-370 [PMID: 24244833 DOI: 10.3390/pharmaceutics5020353]

50 **Zhang L**, Zhang S, Ruan SB, Zhang QY, He Q, Gao HL. Lapatinib-incorporated lipoprotein-like nanoparticles: preparation and a proposed breast cancer-targeting mechanism. *Acta Pharmacol Sin* 2014; **35**: 846-852 [PMID: 24902791 DOI: 10.1038/aps.2014.26]

51 **Mulkidjanian AY**, Galperin MY, Koonin EV. Co-evolution of primordial membranes and membrane proteins. *Trends Biochem Sci* 2009; **34**: 206-215 [PMID: 19303305 DOI: 10.1016/j.tibs.2009.01.005]

52 **Gotto AM**, Pownall HJ, Havel RJ. Introduction to the plasma lipoproteins. *Methods Enzymol* 1986; **128**: 3-41 [PMID: 3523141]

53 **Chapman MJ**. Animal lipoproteins: chemistry, structure, and comparative aspects. *J Lipid Res* 1980; **21**: 789-853 [PMID: 7003040]

54 **Hermanson G**. Bioconjugate Techniques. San Diego, CA: Academic Press; 1996

55 **Krieger M**, Smith LC, Anderson RG, Goldstein JL, Kao YJ, Pownall HJ, Gotto AM, Brown MS. Reconstituted low density lipoprotein: a vehicle for the delivery of hydrophobic fluorescent probes to cells. *J Supramol Struct* 1979; **10**: 467-478 [PMID: 229357]

56 **Hanahan D**, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646-674 [PMID: 21376230 DOI: 10.1016/j.cell.2011.02.013]

57 **Biswas S**, Lunec J, Bartlett K. Non-glucose metabolism in cancer cells--is it all in the fat? *Cancer Metastasis Rev* 2012; **31**: 689-698 [PMID: 22706846 DOI: 10.1007/s10555-012-9384-6]

58 **Warburg O**. [Origin of cancer cells]. *Oncologia* 1956; **9**: 75-83 [PMID: 13335077 DOI: 10.1126/science.123.3191.309]

59 **Sabnis N**, Nair M, Israel M, McConathy WJ, Lacko AG. Enhanced solubility and functionality of valrubicin (AD-32) against cancer cells upon encapsulation into biocompatible nanoparticles. *Int J Nanomedicine* 2012; **7**: 975-983 [PMID: 22393294 DOI: 10.2147/IJN.S28029]

60 **Vander Heiden MG**, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 2009; **324**: 1029-1033 [PMID: 19460998 DOI: 10.1126/science.1160809]

61 **Mooberry LK**, Nair M, Paranjape S, McConathy WJ, Lacko AG. Receptor mediated uptake of paclitaxel from a synthetic high density lipoprotein nanocarrier. *J Drug Target* 2010; **18**: 53-58 [PMID: 19637935 DOI: 10.3109/10611860903156419]

62 **McConathy WJ**, Paranjape S, Mooberry L, Lacko AG. Validation of the reconstituted high-density lipoprotein (rHDL) drug delivery platform using dilauryl fluorescein (DLF). *Drug Deliv Transl Res* 2011; **1**: 113–120

63 **McConathy WJ**, Nair MP, Paranjape S, Mooberry L, Lacko AG. Evaluation of synthetic/reconstituted high-density lipoproteins as delivery vehicles for paclitaxel. *Anticancer Drugs* 2008; **19**: 183-188 [PMID: 18176115 DOI: 10.1097/CAD.0b013e3282f1da86]

64 **Andalib S**, Varshosaz J, Hassanzadeh F, Sadeghi H. Optimization of LDL targeted nanostructured lipid carriers of 5-FU by a full factorial design. *Adv Biomed Res* 2012; **1**: 45 [PMID: 23326776 DOI: 10.4103/2277-9175.100147]

65 **Shao T**, Li X, Ge J. Target drug delivery system as a new scarring modulation after glaucoma filtration surgery. *Diagn Pathol* 2011; **6**: 64 [PMID: 21736763 DOI: 10.1186/1746-1596-6-64]

66 **Shahzad MM**, Mangala LS, Han HD, Lu C, Bottsford-Miller J, Nishimura M, Mora EM, Lee JW, Stone RL, Pecot CV, Thanapprapasr D, Roh JW, Gaur P, Nair MP, Park YY, Sabnis N, Deavers MT, Lee JS, Ellis LM, Lopez-Berestein G, McConathy WJ, Prokai L, Lacko AG, Sood AK. Targeted delivery of small interfering RNA using reconstituted high-density lipoprotein nanoparticles. *Neoplasia* 2011; **13**: 309-319 [PMID: 21472135]

67 **Sabnis N**, Pratap S, Akopova I, Bowman PW, Lacko AG. Pre-Clinical Evaluation of rHDL Encapsulated Retinoids for the Treatment of Neuroblastoma. *Front Pediatr* 2013; **1**: 6 [PMID: 24459664 DOI: 10.3389/fped.2013.00006]

68 **Liu M**, Li W, Larregieu CA, Cheng M, Yan B, Chu T, Li H, Mao SJ. Development of synthetic peptide-modified liposomes with LDL receptor targeting capacity and improved anticancer activity. *Mol Pharm* 2014; **11**: 2305-2312 [PMID: 24830852 DOI: 10.1021/mp400759d]

69 **Markakis KP**, Koropouli MK, Grammenou-Savvoglou S, van Winden EC, Dimitriou AA, Demopoulos CA, Tselepis AD, Kotsifaki EE. Implication of lipoprotein associated phospholipase A2 activity in oxLDL uptake by macrophages. *J Lipid Res* 2010; **51**: 2191-2201 [PMID: 20332422 DOI: 10.1194/jlr.M003558]

70 **Lacko AG**, Nair M, Paranjape S, Mooberry L, McConathy WJ. Trojan horse meets magic bullet to spawn a novel, highly effective drug delivery model. *Chemotherapy* 2006; **52**: 171-173 [PMID: 16691026]

71 **Zheng G**, Chen J, Li H, Glickson JD. Rerouting lipoprotein nanoparticles to selected alternate receptors for the targeted delivery of cancer diagnostic and therapeutic agents. *Proc Natl Acad Sci USA* 2005; **102**: 17757-17762 [PMID: 16306263]

72 **van Capelleveen JC**, Brewer HB, Kastelein JJ, Hovingh GK. Novel therapies focused on the high-density lipoprotein particle. *Circ Res* 2014; **114**: 193-204 [PMID: 24385512 DOI: 10.1161/CIRCRESAHA.114.301804]

73 **Zhang WL**, Gu X, Bai H, Yang RH, Dong CD, Liu JP. Nanostructured lipid carriers constituted from high-density lipoprotein components for delivery of a lipophilic cardiovascular drug. *Int J Pharm* 2010; **391**: 313-321 [PMID: 20214958 DOI: 10.1016/j.ijpharm.2010.03.011]

74 **Parker N**, Turk MJ, Westrick E, Lewis JD, Low PS, Leamon CP. Folate receptor expression in carcinomas and normal tissues determined by a quantitative radioligand binding assay. *Anal Biochem* 2005; **338**: 284-293 [PMID: 15745749]

75 **Yang M**, Chen J, Cao W, Ding L, Ng KK, Jin H, Zhang Z, Zheng G. Attenuation of nontargeted cell-kill using a high-density lipoprotein-mimicking peptide--phospholipid nanoscaffold. *Nanomedicine* (Lond) 2011; **6**: 631-641 [PMID: 21718175 DOI: 10.2217/nnm.11.10]

76 **Smith JD**. Apolipoprotein A-I and its mimetics for the treatment of atherosclerosis. *Curr Opin Investig Drugs* 2010; **11**: 989-996 [PMID: 20730693]

77 **Tuteja S**, Rader DJ. High-density lipoproteins in the prevention of cardiovascular disease: changing the paradigm. *Clin Pharmacol Ther* 2014; **96**: 48-56 [PMID: 24713591 DOI: 10.1038/clpt.2014.79]

78 **Tardif JC**. Emerging high-density lipoprotein infusion therapies: fulfilling the promise of epidemiology? *J Clin Lipidol* 2010; **4**: 399-404 [PMID: 21122683 DOI: 10.1016/j.jacl.2010.08.018]

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REVIEW

Progress in pancreatic cancer therapeutics: The potential to exploit molecular targets

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Abstract

Pancreatic ductal adenocarcinoma is an aggressive and devastating disease associated with poor survival outcomes. Even though significant advances have been made towards understanding the intricate pathology of this cancer, several important aspects remain unknown. Recently, key genetic mutations within the tumour have been identified, but the exact role they play in tumourigenesis has yet to be determined. For many years, the micro-tumour environment and stroma was thought to aid proliferation but there is now emerging research that suggests the contrary. Several novel targeted agents in pre-clinical and early clinical studies have been promising but it remains to be seen whether they will have a significant impact on patient outcomes. In this review we discuss the unique nature of pancreatic cancer biology, current treatment options and summarise the latest results from pre-clinical and clinical research. We also discuss the future strategies that are needed to improve outcomes for this disease.

Key words: Pancreatic cancer; Adenocarcinoma; Targeted therapy; Genomics; Stroma; KRAS; Chemotherapy

Core tip: Pancreatic ductal adenocarcinoma is a cancer with several significant genetic aberrations that have recently been identified by international research efforts. Despite these findings, standard therapy for advanced disease consists primarily of chemotherapy. In the last few years two new chemotherapy regimens, FOLFIRINOX and Gemcitabine/Nab-paclitaxel, have demonstrated survival benefits in large phase III trials resulting in a change to current practise. However, the advent of targeted treatments has not yet had a significant impact in this disease compared with other malignancies. Current research strategies include developing therapies directed towards the RAS-RAK-MEK pathway, PI3K-AKT-mTOR pathway, notch pathway and immunotherapies to name but a few, with several clinical trials underway. It is likely that the heterogeneous nature of pancreatic cancer necessitates a more personalised approach to management with targeted treatment guided by predictive biomarkers.

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is the 5th leading cause of cancer related mortality worldwide. Despite significant research efforts, 5-year survival for all stages remains stagnant at 3%-5%[1,2]. More than 80% of patients present with inoperable or advanced disease, so there remains an urgent need for more effective systemic treatments[3]. Although newer combination chemotherapy regimens offer improved survival outcomes, therapeutic options are limited and do not fully exploit the unique biology of the disease. To date no predictive or prognostic biomarker has been validated for use. This review will discuss current management and emerging therapeutic concepts.

THE MOLECULAR BIOLOGY OF PDAC

Pancreatic cancer is a genetic disease, the biology of which is both intricate and highly heterogeneous. Several extensive genomic studies have confirmed that the development of PDAC results from several complex genetic aberrations with mutations in both oncogenes and tumour suppressor genes[4]. The progression from the pre-malignant dysplastic cellular transformations to the final development of PDAC is associated with increasing mutational changes, confirming the importance of such genetic variations in cancer development. Nearly all pancreatic cancers harbour a KRAS mutation and the majority are also associated with inactivation of CDKN2a/INK4a, TP53 and DPC4/SMaD4[4-7]. Several groups have aimed to accurately depict the genomics of PDAC with each reporting numerous activating mutations. Importantly, there was heterogeneity amongst the different pancreatic tumours with several key pathways responsible for cancer progression differing between patient samples[7]. Clonal mutations found in metastatic lesions were identified in primary cancers but due to the unstable genetic nature of PDAC, these initial mutations continued to evolve resulting in heterogeneity amongst the different metastatic deposits in the same patient. This complex genetic landscape results in an aggressive pathology, often refractory to treatment resulting in poor survival outcomes.

A recent addition to the understanding of PDAC comes from recent studies that have identified a group of pancreatic cancer cells that display stem cell properties[4,8]. These cells appear to have the ability of self-renewal and asymmetric division. Preliminary data suggests that patients with tumours containing cancer stem cells (CSCs) are associated with poorer overall survival (OS). The identification of CSCs and the signalling pathways that they regulate, has led to newer therapeutic targets such as WnT, Hedgehog and Notch. Further research is needed to see whether these can be successfully exploited to produce meaningful clinical outcomes.

Despite promising pre-clinical studies, several chemotherapeutics and targeted agents have failed to reproduce positive results in patients (Table 1). One explanation for this relates to the complex micro-tumour environment that surrounds the cancer cells and the difficulties replicating this *in-vitro*. A significant bulk of the pancreatic tumour comprises not of malignant cells but of the encompassing dense fibrotic stromal matrix[9]. This micro-tumour environment results from the extensive desmoplastic reaction seen in PDAC and consists of an abundant extracellular matrix, pancreatic stellate cells, fibroblasts, immune cells, inflammatory cells and vasculature all of which were previously thought to aid proliferation, invasion and metastatic spread whilst also preventing adequate drug delivery leading to chemotherapy-resistance[10-14]. The success of nab-paclitaxel (as discussed later) appear to manipulate the distinct characteristics of the stroma for therapeutic benefit[15].

However recent emerging research using genetically modified mouse models suggest that depletion of the stroma (by genetic or pharmacological targeting of the Hedgehog[16] pathway) results in an unexpected increase in tumour vascularity and proliferation, thereby resulting in more aggressive tumours with reduced survival[17]. Furthermore, transgenic mice with the ability to delete αSMA$^+$ myofibroblasts in pancreatic cancer also demonstrated reduced survival[18]. Both studies suggest that rather than a promoter of cancer growth, the stroma (or at least part of it) may paradoxically act to suppress proliferation and angiogenesis thus targeting the stroma should be performed with caution. An intricate and crucial part of PDAC, further research into the stroma is needed in order to exploit its presence for therapeutic benefit.

CHEMOTHERAPY

Until recently the standard treatment for inoperable or metastatic disease was with the nucleoside analogue gemcitabine. This was based on the results of a phase Ⅲ trial in 1997 of 126 patients with advanced PDAC. Patients were randomised to receive gemcitabine (gemcitabine 1000 mg/m^2 weekly × 7 followed by 1 wk of rest, then weekly × 3 every 4 wk thereafter), or to fluorouracil (5-FU) (600 mg/m^2 once weekly)[19]. Both arms continued treatment until progression or unacceptable toxicities and the primary end point was clinical benefit, measured using a combined score of pain, performance status (PS) and weight loss. Clinical benefit response was experienced by 23.8% of gemcitabine-treated patients compared with 4.8% of 5-FU-treated patients (P = 0.0022). There was also a modest survival benefit with gemcitabine with a 12-mo

Table 1 Phase III trials with gemcitabine combination chemotherapy regimens

Date published	Target	Ref.	Sample size (*n*)	Treatment	OS (mo)	*P* value
2001	MMP	Bramhall *et al*[100]	414	Marimastat and Gem *vs* Gem	5.4 5.4	0.95
2004	FT	Van Cutsem *et al*[31]	688	Tipifarnib and Gem *vs* Gem + Placebo	5.9 6.3	0.75
2009	EGFR	Moore *et al*[61]	569	Erlotinib and Gem *vs* Gem	6.2 5.9	0.038
2008	EGFR/VEGF	Van Cutsem *et al*[88]	301	Gem, Erlotinib and Bevacizumab *vs* Gem, Erlotinib and Placebo	7.1 6.0	0.2087
2010	VEGF	Kindler *et al*[87]	535	Gem and Bevacizumab *vs* Gem and Placebo	5.8 5.9	0.95
2010	EGFR	Philip *et al*[75]	745	Gem *vs* Gem and cetuximab	5.9 6.3	0.23
2011	VEGF	Kindler *et al*[101]	630	Axitinib and Gem *vs* Gem	8.5 8.3	0.54
2012	VEGF, BRAF, PDGFR-B	Gonçalves *et al*[102]	104	Sorafenib and Gem *vs* Gem	8.0 9.2	0.23

Gem: Gemcitabine; OS: Overall survival; MMP: Matrix metalloproteinase; FT: Farnesyl transferase; EGFR: Epidermal growth factor receptor; VEGF: Vascular endothelial growth factor; PDGFR-B: Platelet derived growth factor receptor-B.

Table 2 Phase III trials with targeted treatment in metastatic pancreatic cancer

Date published	Regimen	Ref.	Sample size (*n*)	Median OS (mo)	*P* value
2001	Gem *vs* Gem + 5FU	Berlin *et al*[103]	322	5.4 6.7	0.09
2004	Gem *vs* Gem + Irinotecan	Rocha Lima *et al*[104]	360	6.6 6.3	0.789
2005	Gem *vs* GemOx	Louvet *et al*[[105]	326	7.1 9.0	0.13
2007	Gem *vs* Gem + cape	Herrmann *et al*[106]	319	7.2 8.4	0.234
2006	Gem *vs* Gem + Irinotecan	Stathopoulos *et al*[107]	145	6.4 6.5	0.970
2006	Gem *vs* Gem + Cisplatin	Heinemann *et al*[108]	195	6.0 7.5	0.15

Gem: Gemcitabine; 5FU: 5-Fluoruracil; GemOx: Gemcitabine + Oxaliplatin; cape: Capecitabine.

survival of 18% *vs* 2% ($P = 0.0025$) with a median OS of 5.6 m for patients treated with gemcitabine and 4.4 mo for those with 5-FU. Treatment was well tolerated and gemcitabine became standard treatment for inoperable and advanced disease.

Numerous clinical trials with various chemotherapy agents combined with gemcitabine ensued (Table 2) and following several disappointing outcomes, the majority of preclinical work focused on developing new targeted treatments. However, the most significant advances in PDAC management were with two new chemotherapy combinations that have recently demonstrated benefits over gemcitabine in large phase III trials and thus changed current practise. The combined treatment of oxaliplatin, irinotecan, leucovorin and fluorouracil (FOLFIRINOX) was associated with a median OS of 11.1 mo compared with 6.8 mo in patients treated with gemcitabine alone (HR for death 0.57, 95%CI: 0.45-0.73, $P \leqslant 0.001$)[20,21]. This phase III trial of 342 patients with PS 0 or 1 also demonstrated increases in median progression free survival (PFS) (6.4 m *vs* 3.3 m, $P \leqslant 0.001$) and objective response rate (ORR) (31.6 *vs* 9.4, $P \leqslant 0.001$)). Approved for use in first line metastatic disease, in practise this regimen is generally reserved for patients with an excellent performance status as unsurprisingly, toxicity was also significantly increased with this 3-drug combination. More recently a phase III trial compared combined nab-paclitaxel and gemcitabine with gemcitabine alone in patients with metastatic disease[22]. Median OS was 8.5 mo with the combination chemotherapy and 6.7 mo with gemcitabine (95%CI: 0.62-0.83, $P < 0.001$). ORR was significantly increased at 23% *vs* 7% ($P < 0.001$), leading to interest in the potential use as a means of down staging locally advanced disease. A further pre-specified sub-group analysis concluded that baseline Karnofsky score (KS), presence of liver metastases, age and number of metastatic sites were independent prognostic factors for OS and PFS[23]. Common adverse events of grade 3 or higher included neutropenia (38% in nab-paclitaxel and gemcitabine arm and 27% in the gemcitabine arm), fatigue (17% and 7%) and

neuropathy (17% and 1%). Nab-paclitaxel is a colloidal suspension of 130 nm particles homogenised in human serum albumin that is bound to paclitaxel. Pancreatic cancers are known to overexpress Secreted protein acidic and rich in cysteine (SPARC) and nab-paclitaxel improves efficacy *via* SPARC-albumin binding[24]. Pre-clinical models have confirmed that SPARC overexpression in the stroma promotes cell invasion and metastatic spread. Higher levels of SPARC appeared to correlate with improved survival in the original phase I/II trial of gemcitabine and nab-paclitaxel (mOS was 17.8 m in the high SPARC group compared with 8.1 m in the low SPARC group, P = 0.431). Further research is needed to confirm whether SPARC has the potential to be used as a predictive marker. The recently reported results from a prospective randomised adjuvant study have also suggested the prognostic significance of over-expressed SPARC in patients undergoing resection with curative intent. Disease free survival (DFS) was 7.4 mo in patients with higher levels of SPARC compared to 12.1 m in those with lower levels (P = 0.041) and OS was 14.1 and 25.6 m respectively (P = 0.011)[25,26]. Without a direct head- to -head trial of both combination chemotherapy regimens, it is difficult to ascertain whether FOLFIRINOX or gemcitabine and nab-paclitaxel is superior and both are now standard practise. However single agent gemcitabine remains treatment of choice for those patients that are not suitable for combination therapy.

RAS-RAF-MEK PATHWAY

Approximately 30% of all patients with solid malignancies have tumours that exhibit oncogenic Ras mutations[27]. In PDAC this figure is much higher as an excess of 95% have a small GTPase KRAS mutation resulting in a dominant activated form. These mutations cause the protein to be constitutively activated, which leads to aberrant down-stream signalling and increased proliferation[28]. Following the discovery of the Ras family, a concerted effort was made to develop agents that could block mutated Ras function with little success.

As KRAS requires binding to the plasma membrane *via* farnesylation or geranylgeranylation in order to become activated, several farnesyltransferase inhibitors (FFIs) have been developed but have proved ineffective in clinical trials. Two phase II trials using FFIs were negativeand a randomised doubled blind phase III trial of 688 patients comparing gemcitabine with or without the FFI tipifarnib, demonstrated no significant survival benefit in the combination arm compared with standard treatment[29-31]. A further study demonstrated that binding of mammalian PDEδ to KRAS using small molecule inhibitors can suppress oncogenic RAS signalling by virtue of selective binding to the prenyl-binding pocket of PDEδ and in PDAC cell lines resulted in reduced cell proliferation[32]. Other approaches include the development of small molecules that target son of sevenless (SOS) mediated nucleotide exchange and subsequently target KRAS[33] and recently KRASG12C inhibitors have demonstrated therapeutic potential by allosterically allowing KRAS to favour GDP over GTP[34]. Another recent approach to targeting KRAS is by the combined MEK/BCL-XL inhibition, a method developed after identification in a pooled shRNA screen[35]. This combination resulted in significant apoptosis in several KRAS mutated cell lines.

A recent pre-clinical study demonstrated a novel way of targeting KRAS in transgenic mouse models using an siRNA delivery system (Local drug EluteR or LODER)[36]. This model capitalises on the effects of siRNA and knockdown of KRAS, but *via* an innovative platform of a controlled and prolonged delivery for therapeutic benefit. The LODER against KRAS (siG12D LODER) decreased KRAS levels in pancreatic cancer cell lines resulting in reduced proliferation and epidermal-mesenchymal transition. Within *in-vivo* models, the growth of human pancreatic cancer cells was impaired and mouse survival was increased compared to controls. A phase 1 study in patients with locally advanced disease treated with siG12D LODER is on-going[37], and a further phase II study of siG12D LODER in combination with chemotherapy plans to open early next year[38]. Whilst these results are promising in the pursuit of an anti-KRAS therapy, it remains to be seen whether this can be translated in to an efficacious treatment in clinical trials.

With the limited success of inhibiting Ras, efforts have moved towards targeting downstream signalling activity. There are two main pathways that have been extensively interrogated, mitogen-activated protein kinases (MAPK) and phosphoinositide 3 kinase (PI3K) signalling (Figure 1). BRaf inhibitors, such as vemurafenib, work downstream from Ras and have had considerable success in Raf mutant tumours such as melanoma[39]. However, evidence now supports that there is a paradoxical up regulation of MAPK signalling when Raf is inhibited in KRAS mutated tumours[40]. In pancreatic cancer where Raf is wild type and Ras is nearly always mutated, Raf inhibitors create feedback activation of the MAPK signalling pathway therefore it is likely that targeting downstream by MEK inhibition will offer more promising results. Phase I/II clinical trials of various MEK inhibitors in combination with gemcitabine are currently underway following positive pre clinical work[41,42], Results from a phase IIa trial of 60 patients treated with gemcitabine in combination with the allosteric oral Mek1/2 inhibitor refametinib were presented at ASCO 2104. The best result was partial response in 35% of patients with median duration of response at 3.8 mo (117 d 95%CI: 83-265). Time to progression was 7.4 mo[43,44]. KRAS mutations were identified in 39 patients (65%) and the results suggested a trend towards improved survival outcomes in patients with KRAS wild type tumours. The OS for the KRAS mutant subgroup was 6.6 mo compared with 18.2

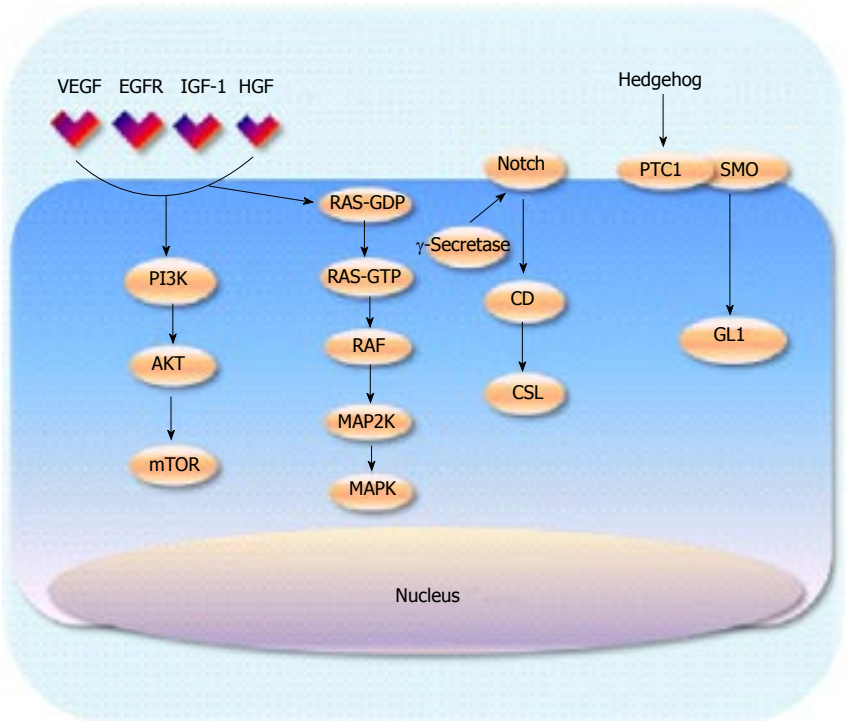

Figure 1 Simplified diagram of oncogenic targets in pancreatic cancer. The binding of ligands (including VEGF, EGF, IGF-1 and HGF) to receptors activates signalling pathways including the PI3K-Akt and the Ras pathways affecting downstream targets such as mTOR and MAPK. VEGF: Vascular endothelial growth factor; EGF: Epidermal growth factor; IGF-1: Insulin growth like factor-1; HGF: Hepatocyte growth factor; PI3K: Phosphatidylinositide 3-kinases; mTOR: Mammalian target of rapamycin; RAS: Rat activated sarcoma; GDP: Guanosine diphosphate; GTP: Guanosine triphosphate; MAP: Mitogen activated kinase; CD: Cytoplasmic domain; PTC1: Patched; SMO: Smooth muscle, GL1.

mo (HR = 0.27).

PI3K-MTOR PATHWAY

PI3K is an enzyme that lies downstream from RAS and is responsible for the activation of AKT, which in turn leads to activation of mammalian target of rapamycin (mTOR) (Figure 1). In normal tissue the PI3K-AKT pathway inhibits apoptosis and cell proliferation, thus deregulation of this pathway leads to unregulated cell[45]. Several PI3K inhibitors have been developed and are currently being investigated in a number of malignancies. Preliminary studies using transgenic mice have demonstrated reduced pancreatic tumour growth when PI3K is inhibited and therefore PI3K remains a valid therapeutic target that warrants further attention[46]. The dual PI3K/PDK inhibitor rigosertib, has demonstrated safety and some efficacy when combined with gemcitabine in pre-treated patients with advanced disease[47] and a phase Ⅲ trial in combination with chemotherapy is underway[48].

mTOR has been identified as a critical effector in cell signalling and the drug everolimus, an oral inhibitor of mTOR, has had success against solid tumours such as metastatic renal cell cancers and breast cancers, but also in pancreatic neuroendocrine tumours[49-51]. Preclinical studies showed that inhibition of the mTOR pathway suppressed proliferation in pancreatic cancer cell lines[52]. However in a phase Ⅱ study of 33 patients with gemcitabine refractory metastatic PDAC, there were no complete or partial responses and median PFS was 1.8 mo and OS was 4.5 mo[53]. A phase 1 trial combining gemcitabine with temsirolimus, another mTOR inhibitor, resulted in significant toxicity without any partial or complete responses. The commonly used anti-diabetic drug metformin is also known to inhibit mTOR and epidemiological studies have linked metformin use with reduced risk of developing malignancies[54,55]. Metformin is now the focus of a clinical trial and is being used in combination with chemotherapy[56]. Novel agents that comprise of both mTOR complex 1 and 2 inhibitors (mTORC1/2) have shown promising efficacy in cancer cells *in vitro.* One such agent, INK-128, led to pancreatic cancer cell apoptosis and necrosis *in vitro*. Furthermore INK-128 resulted in increased sensitivity of pancreatic cancer cells to gemcitabine suggesting potential benefit when used in combination with chemotherapy[57]. Other positive results have been reported with dual PI3K/mTOR kinase inhibitors *in vitro*[58]. Recently inhibitors against the p110δ isoform of PI3K demonstrated inactivation of regulatory T cells leading to $CD8^+$ cytotoxic cells and subsequent tumour regression in murine models[59]. Despite limited results in clinical trials thus far, recent pre-clinical efforts are more promising and the PI3K/AKT/mTOR signalling cascade remains an important pathway for future research.

EPIDERMAL GROWTH FACTOR RECEPTOR

The epidermal growth factor receptor (EGFR) has also emerged as an attractive therapeutic target for many malignancies. EGFR is a member of the erbB/human EGFR family of tyrosine kinases and when bound to a ligand, a conformational change is induced leading to dimerisation with other receptors[60]. This results in the activation of several cascades including the Ras/MAP kinase pathway and the PI3K/Akt/mTOR pathway.

Several small molecules have been developed that block EGFR with varying degrees of success. The only targeted drug to be approved for the management of advanced PDAC so far is the tyrosine kinase inhibitor (TKI) erlotinib when administered in combination with gemcitabine. A phase Ⅲ trial (PA.3) randomly assigned 569 patients with advanced disease to receive standard gemcitabine plus erlotinib (100 or 150 mg/d orally) or placebo[61]. The trial was double blinded with a primary end point of overall survival. The results showed a modest but significant survival benefit in the combination arm (6.24 m *vs* 5.91 m HR = 0.82, 95%CI: 0.69-0.99, $P \leqslant 0.038$), which led to FDA approval. Although the benefits appeared to be small, an unplanned retrospective subgroup analysis led the authors to hypothesise that patients who developed a skin rash on treatment experienced a higher disease control rate. Patients that were younger than 65 (P = 0.1) and those with a good PS (P = 0.03) were more likely to develop a rash. The median OS in patients with a grade 0, 1 or 2 rash were 5.3 m, 5.8 m and 10.5 m respectively with 1 year survival rates of 16%, 9% and 43% ($P < 0.001$). This was further assessed in a study correlating rash and survival outcomes, by analysing combined data from the PA.3 trial and a phase Ⅲ trial using erlotinib in advanced non-small cell lung cancer (BR.21)[62,63]. They found that the presence of grade 2 or higher rash correlated with improvements in PFS and disease control. These findings were echoed in a retrospective study of 174 patients that found that high-severity rash was associated with longer OS[64].

However, molecular studies have not been able to identify EGFR and KRAS mutations as predictive biomarkers of survival benefit and no association between KRAS mutation or EGFR gene copy number with rash has been identified. Erlotinib in combination with capecitabine has also been shown to have some activity in gemcitabine refractory patients as evidenced by a phase Ⅱ trial combining capecitabine and erlotinib in patients with advanced PDAC. The primary end point was response and this was found to be 10% of all 30 patients with a median OS of 6.5 mo[65]. A further phase Ⅲ trial comparing combined capecitabine and erlotinib followed by gemcitabine on progression compared with gemcitabine and erlotinib followed by capecitabine is on-going[66].

An alternative anti EGFR TKI, gefitinib, demonstrated anti-proliferative effects in the pre clinical setting and this has translated to positive survival benefit in patients with non small cell EGFR mutated lung cancer[67]. Gefitinib combined with gemcitabine has been assessed in a phase Ⅱ trial of 53 patients with locally advanced or metastatic PDAC. Patients were treated with gefitinib (250 mg) once daily and gemcitabine at the standard dose and schedule. 6 mo PFS was 30% with a median PFS of 4.1 mo. The 1-year survival rate was measured at 7.3 mo. Whilst these results were comparable to the PA 3 trial, there has yet to be a randomised trial of gefitinib to demonstrate significant benefit over single agent gemcitabine[68].

The anti-EGFR antibody cetuximab has shown significant clinical activity in both colorectal cancers and head and neck tumours in patients with wild type KRAS[69,70]. Despite the majority of patients with pancreatic cancer having KRAS mutations, preclinical activity suggested that it might be a useful therapy in advanced PDAC due to EGFR overexpression[71-73]. A phase Ⅱ trial evaluated gemcitabine and cetuximab in 41 treatment-naïve patients stratified according to EGFR expression using immunohistochemistry (4 patients were 1+, 20 patients were 2+ and 17 patient were 3+)[74]. Cetuximab was administered at a loading dose of 400 mg/m^2 followed by 250 mg/m^2 weekly and gemcitabine was administered 1000 mg/m^2 weekly for 7 wk and then 100 mg/m^2 every week for three weeks followed by a week's rest. Five patients achieved a partial response (12.5%) and 26 patients (63.4%) had disease stability. Median TTP was 3.8 mo and the median OS was 7.1 mo. Survival at 1 year was 31.7%. Toxicities were as previously reported with cetuximab chemotherapy combinations, most notably rash (87.7%), nausea (61.0%), weight loss (58.5%) and diarrhoea (53.7%). Despite the promising results from this phase Ⅱ trial, this was not reproduced in 2 phase Ⅲ trials. The S0205 trial conducted by the southwest oncology group (SWOG) reported that in 766 patients treated with either gemcitabine or gemcitabine plus cetuximab, there was no survival benefit seen in the combination arm[75]. A further trial combining gemcitabine and cisplatin with or without the addition of cetuximab, recruited 40 patients. Seven patients had a documented response in the antibody arm compared to 5 in the control arm but again no survival benefit was seen with cetuximab[76]. A further negative phase Ⅱ trial with gemcitabine and oxaliplatin with the addition of cetuximab recruited 64 patients. Patients received a combination of gemcitabine at 100 mg/m^2 on day 1 with oxaliplatin at 100 mg/m^2 on day 2, every 2 wk. Cetuximab was administered at a loading dose of 400 mg/m^2 followed by weekly dose of 250 mg/m^2. Although well tolerated, the findings (response rate 33%, median time to PFS 3.9 mo and OS 7.1 mo) were not superior to previously seen results using the chemotherapy combination alone[77]. The results of a phase Ⅱ trial presented at ASCO 2013, portrayed a significant survival benefit at 1 year with gemcitabine combined with the anti-EGFR antibody nimotuzumab compared to gemcitabine alone (34.4% *vs* 19.5%, P = 0.034, HR = 0.69) and the combination was well tolerated[78]. A phase Ⅱ study of nimotuzumab in pre-treated patients with advanced PDAC was also encouraging and a randomised placebo controlled phase Ⅱb/Ⅲa study comparing the combination of gemcitabine and nimotuzumab compared to gemcitabine and placebo has recently closed to recruitment and the results are awaited[78].

Although EGFR remains a critical receptor in pancreatic cell proliferation and metastatic spread, with

the exception of the modest benefits seen in the PA3 trial, there have not been any positive results with EGFR targeted therapy in large randomised trials. Whilst monoclonal antibodies that target EGFR have demonstrated efficacy in other solid tumours, its distribution within pancreatic cancer cells is not well known and may be an explanation for poor outcomes. It is also possible that the optimum doses and methods of drug delivery have not yet been elucidated. With regards to erlotinib and gefitinib, the excellent results that have been demonstrated in several large clinical trials in lung cancer have not been reproduced in PDAC and are likely due to the lack of activating mutations seen in these tumours. There is not enough evidence to suggest that even in those with an activating mutation, this can predict response to anti-EGFR therapy. Thus disappointingly expression or mutation of EGFR has not emerged so far as a predictive or prognostic biomarker[79-81]. Unlike lung and colon tumours KRAS mutation is not mutually exclusive with EGFR activation. Initiation of KRAS mutated PDAC appears to be dependent on EGF activation and a recent study reported that EGF inhibition has limited therapeutic benefit in tumours with p53 inactivation[82]. The study hypothesised that p53 loss might "reactivate" the PI3K/AKT and the STAT pathway independent from EGF activation suggesting EGFR inhibitors may only be of clinical benefit in patients with p53 wild type tumours.

ANGIOGENIC THERAPY

Angiogenesis describes the process by which a tumour initiates the formation of new vessels through remodelling of existing vasculature[83]. Once the "angiogenic switch" is initiated, the complex process of new vessel formation begins and subsequently plays a key role in tumour growth[84]. VEGF is vital to angiogenesis and is therefore a potential target in many tumour types with variable outcomes in clinical trials[85]. Anti-VEGF antibodies have been used without much success in pancreatic cancer. Bevacizumab, which offers improved outcomes in colorectal and ovarian cancer, is a monoclonal antibody that decreases the formation of new blood vessels *in vivo* and improves drug delivery to the cancer cell. A phase Ⅱ trial of bevacizumab and gemcitabine in patients with advanced PDAC, demonstrated that in 52 patients, 19% had a partial response and 48% had stable disease[86]. The median OS was 8.8 mo with a 6-mo survival of 77%. This led to 2 phase Ⅲ trials, both of which were disappointingly negative. The CALGB 80303 study treated patients with gemcitabine with or without bevacizumab[87,88]. 602 patients were enrolled and both overall response rate and 1 year survival outcomes failed to reach statistical benefit in the combination arm. The AViTA trial, comparing the combination of gemcitabine and erlotinib with the addition of bevacizumab was also negative[88]. Despite the changes in the vasculature seen in patients treated with these drugs, no benefit has been shown when targeting VEGF and the exact mechanism of failure remains unknown but is likely to be in part due to the hypovascularity of the surrounding stroma[11].

IMMUNOTHERAPY

Interest in immunotherapy has had a recent resurgence following the results of several positive clinical trials in solid malignancies including melanoma and prostate cancer[89,90]. Success has been more modest in pancreatic cancer although several newer agents remain under investigation. Based on the understanding that the innate immune system can distinguish between cancer cells and "normal self", exploitation of the immune system has been a topic of research for several decades. Not only do immune-deficient mice develop malignancies, evidence has also shown that patients with cancer develop B and T cells that can recognise antigens released by pancreatic tumour cells. The immune response created by the patient is invariably unsuccessful at eliminating malignancy but this reaction can be enhanced for therapeutic gain. Theoretically, immunotherapy should be active in pancreatic cancer, as the dense stroma is enriched with immune cells such as T cell and macrophages.

A recent positive trial presented at the Gastrointestinal Cancers Symposium in 2014 demonstrated significant survival benefit when combining two specific anti-cancer vaccines compared with monotherapy. GVAX is a vaccine made from 2 pancreatic cancer cell lines that have been irradiated to secrete granulocyte-macrophage colony-stimulating factor causing stimulation of the immune system[91]. Administered intra-dermally after low dose cyclophosphamide, it inhibits regulatory T cells. CRS-207 is made of live-attenuated Listeria monocytogenes engineered to stimulate an immune response against a protein called mesothelin that is expressed at high levels in pancreatic cancer cell lines. This phase Ⅱ trial compared the combination of CRS-207 and GVAX with GVAX alone with positive outcomes. Ninety patients with pre-treated PDAC were randomly assigned at a ratio of 2:1 to be treated with 2 dose of CY/GVAX followed by 4 doses of CRS-207 or 6 doses of CY/GVAX every three weeks. The primary end point was OS with safety, clinical response and immune response secondary. At the interim analysis median OS was 6.1 m with the combination treatment compared with 3.9 m for GVAX therapy. (HR = 0.59, two sided Log Rank P = 0.03). One-year survival was doubled with combination treatment (24% *vs* 12%). Following the encouraging results from the interim analysis, crossover was allowed. Toxicities included fevers, rigors and lymphopaenia, but were minimal and were not cumulative. Several other studies are due to open comparing combination CY/GVAX and CRS-207 with chemotherapy in the second line setting or in combination with immune checkpoint inhibitors of programmed death 1 (PD1) and its ligand PD-L1. PD1, which is a T-cell co-inhibitory receptor, and PD-L1 have shown considerable responses in certain

solid tumours including melanoma and lung cancers. An international phase 1 study using the intravenous anti-PD-L1 antibody treated 75 patients, 14 of whom had pancreatic cancer. Objective responses were seen in patients with non-small lung cancer, melanoma, renal and ovarian cancer but not in those with PDAC[92]. However there remains potential benefit with PD-1 in combination with other compounds. Recently the effects of PD-1 immunosuppression were enhanced when used in combination with chimeric antigen reception (CAR) T-cell therapy in Her2 transgenic mice. Further research with this combination is on going. A phase 1 study combining the agonist CD40 monoclonal antibody (CP-870,893) in combination with gemcitabine demonstrated tolerability and resulted in 4 out of 22 patients with advanced PDAC achieving a partial response suggesting that further clinical trials are warranted.

The results from a phase Ⅲ trial assessing the GV1001 vaccine, a promiscuous class Ⅱ epitope vaccine, recently reported no benefit when used in combination with gemcitabine and capecitabine compared to chemotherapy alone and was therefore terminated early[93]. Several other immunological treatments remain under review. The anti-CTLA4 antibody that has been approved for use in melanoma, also demonstrated no initial responders to therapy. However in this phase Ⅱ trial of 27 patients, 1 patient had a significant delayed response[94]. Single agent ipilimumab has not been taken forward to a phase Ⅲ trial but its safety when used in combination with gemcitabine is currently being assessed in an early phase trial[95]. As it has been suggested that immunotherapy is most successful in the absence of large disease burden, several clinical trials are assessing immunotherapy in the post-operative setting or as maintenance therapy following response to chemotherapy.

HEDGEHOG INHIBITORS

The hedgehog (Hh) pathway has been identified as another important signalling cascade in multiple cancers suggesting its potential as a therapeutic target. Two transmembrane proteins have been identified that activate the Hh signalling pathway, the tumour suppressor patched protein (PTCI) and smoothened (SMO) an oncogenic protein[96-98]. Pre-clinical studies have established that human pancreatic stellate cells (as seen in the stroma) express high levels of smoothened protein and low levels of Hh ligands unlike the pancreatic cancer cells, which demonstrate the converse expression pattern[11]. The majority of Hh inhibitors that have been developed target SMO. In transgenic Kras mutated mice the administration of a Hh inhibitor IPI-926 depleted the surrounding stroma enhancing the drug delivery of gemcitabine[11]. A phase Ib trial of IPI-926 in combination with gemcitabine demonstrated acceptable tolerability in 16 patients with untreated metastatic PDAC. Common AEs included fatigue, thrombocytopenia, anaemia, nausea, diarrhoea, vomiting and dose reductions of IPI-926 were required in 3 patients. DR of gemcitabine occurred in 11 patients. Five sixteenths (31%) had a radiological response while median PFS was more than 7 mo with 74% patients alive after 6 mo of entry in to the study. Whilst these results were promising, a phase Ⅱ trial was terminated early at the interim analysis as patients in the combination arm experienced worse outcomes than those on single agent gemcitabine[99]. These disappointing results may be partly explained by the results from recent pre-clinical studies suggesting the importance of the stroma (as discussed earlier) where depletion led to increased tumour growth[16,17].

However further research with Hh inhibitors are on-going. A single-arm study with the Hh inhibitor vismodegib combined with the chemotherapy regimen gemcitabine and nab-paclitaxel, presented an interim analysis at GI ASCO 2014[16]. Eighty percent of the 59 patients treated had stable disease or better. Median PFS was 5.5 mo and OS was 10 mo. Patient recruitment is on-going and based on the preliminary results, the final survival data is eagerly awaited.

CONCLUSION

The current prognosis for advanced PDAC remains poor, highlighting the urgent need for more effective systemic therapies. In order to develop targeted treatments and improve outcomes, research efforts needs to focus on three key areas; a greater understanding of the unique biology of PDAC and the key signalling pathways, comprehension of the unique desmoplastic reaction and micro-tumour environment, and the development of predictive and prognostic biomarkers. It may be that the future of pancreatic cancer treatment will see combining standard chemotherapy with targeted treatments to achieve better outcomes. It is likely that PDAC treatment will be dictated by the biology of the individual tumour rather than the "one shoe fits all" approach that is used today. The last few years have seen significant results towards this in the pre-clinical setting but it remains to be seen whether they can be translated into meaningful clinical outcomes.

REFERENCES

1 **WHO.int.** Cancer. International Childhood Cancer Day 2015. Available from: URL: http://www.who.int/cancer/en/

2 **Ryan DP**, Hong TS, Bardeesy N. Pancreatic adenocarcinoma. *N Engl J Med* 2014; **371**: 1039-1049 [PMID: 25207767 DOI: 10.1056/NEJMra1404198]

3 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013; **63**: 11-30 [PMID: 23335087 DOI: 10.3322/caac.21166]

4 **Hidalgo M**. New insights into pancreatic cancer biology. *Ann Oncol* 2012; **23** Suppl 10: x135-x138 [PMID: 22987949 DOI: 10.1093/annonc/mds313]

5 **Feldmann G**, Beaty R, Hruban RH, Maitra A. Molecular genetics of pancreatic intraepithelial neoplasia. *J Hepatobiliary Pancreat Surg* 2007; **14**: 224-232 [PMID: 17520196 DOI: 10.1007/

s00534-006-1166-5]
6 **Hezel AF**, Kimmelman AC, Stanger BZ, Bardeesy N, Depinho RA. Genetics and biology of pancreatic ductal adenocarcinoma. *Genes Dev* 2006; **20**: 1218-1249 [PMID: 16702400 DOI: 10.1101/gad.1415606]
7 **Jones S**, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 2008; **321**: 1801-1806 [PMID: 18772397 DOI: 10.1126/science.1164368]
8 **Hermann PC**, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, Bruns CJ, Heeschen C. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell* 2007; **1**: 313-323 [PMID: 18371365 DOI: 10.1016/j.stem.2007.06.002]
9 **Mahadevan D**, Von Hoff DD. Tumor-stroma interactions in pancreatic ductal adenocarcinoma. *Mol Cancer Ther* 2007; **6**: 1186-1197 [PMID: 17406031 DOI: 10.1158/1535-7163.MCT-06-0686]
10 **Liss AS**, Thayer SP. Therapeutic targeting of pancreatic stroma. Available from: URL: http://www.ncbi.nlm.nih.gov/books/NBK98931/
11 **Olive KP**, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, Madhu B, Goldgraben MA, Caldwell ME, Allard D, Frese KK, Denicola G, Feig C, Combs C, Winter SP, Ireland-Zecchini H, Reichelt S, Howat WJ, Chang A, Dhara M, Wang L, Rückert F, Grützmann R, Pilarsky C, Izeradjene K, Hingorani SR, Huang P, Davies SE, Plunkett W, Egorin M, Hruban RH, Whitebread N, McGovern K, Adams J, Iacobuzio-Donahue C, Griffiths J, Tuveson DA. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* 2009; **324**: 1457-1461 [PMID: 19460966 DOI: 10.1126/science.1171362]
12 **Michl P**, Gress TM. Improving drug delivery to pancreatic cancer: breaching the stromal fortress by targeting hyaluronic acid. *Gut* 2012; **61**: 1377-1379 [PMID: 22661496 DOI: 10.1136/gutjnl-2012-302604]
13 **Neesse A**, Krug S, Gress TM, Tuveson DA, Michl P. Emerging concepts in pancreatic cancer medicine: targeting the tumor stroma. *Onco Targets Ther* 2013; **7**: 33-43 [PMID: 24379681 DOI: 10.2147/OTT.S38111]
14 **Neesse A**, Frese KK, Chan DS, Bapiro TE, Howat WJ, Richards FM, Ellenrieder V, Jodrell DI, Tuveson DA. SPARC independent drug delivery and antitumour effects of nab-paclitaxel in genetically engineered mice. *Gut* 2014; **63**: 974-983 [PMID: 24067278 DOI: 10.1136/gutjnl-2013-305559]
15 **Minchinton AI**, Tannock IF. Drug penetration in solid tumours. *Nat Rev Cancer* 2006; **6**: 583-592 [PMID: 16862189 DOI: 10.1038/nrc1893]
16 **Jesus-Acosta D**. A phase II study of vimodegib, a hedgehog (Hh) pathway inhibitor, combined with gemciatbine and nab-paclitaxel (nab-P) in patients (pts) with untreated metastatic pancreatic ductal adenocarcinoma (PDA). *J Clinic Oncol* 2014; **32** suppl 3: abstr 257
17 **Rhim AD**, Oberstein PE, Thomas DH, Mirek ET, Palermo CF, Sastra SA, Dekleva EN, Saunders T, Becerra CP, Tattersall IW, Westphalen CB, Kitajewski J, Fernandez-Barrena MG, Fernandez-Zapico ME, Iacobuzio-Donahue C, Olive KP, Stanger BZ. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. *Cancer Cell* 2014; **25**: 735-747 [PMID: 24856585 DOI: 10.1016/j.ccr.2014.04.021]
18 **Özdemir BC**, Pentcheva-Hoang T, Carstens JL, Zheng X, Wu CC, Simpson TR, Laklai H, Sugimoto H, Kahlert C, Novitskiy SV, De Jesus-Acosta A, Sharma P, Heidari P, Mahmood U, Chin L, Moses HL, Weaver VM, Maitra A, Allison JP, LeBleu VS, Kalluri R. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. *Cancer Cell* 2014; **25**: 719-734 [PMID: 24856586 DOI: 10.1016/j.ccr.2014.04.005]
19 **Burris HA**, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD, Von Hoff DD. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* 1997; **15**: 2403-2413 [PMID: 9196156]
20 **Conroy T**, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardière C, Bennouna J, Bachet JB, Khemissa-Akouz F, Péré-Vergé D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011; **364**: 1817-1825 [PMID: 21561347 DOI: 10.1056/NEJMoa1011923]
21 **Papadatos-Pastos D**, Thillai K, Rabbie R, Ross P, Sarker D. FOLFIRINOX - a new paradigm in the treatment of pancreatic cancer. *Expert Rev Anticancer Ther* 2014; **14**: 1115-1125 [PMID: 25204327 DOI: 10.1586/14737140.2014.957188]
22 **Von Hoff DD**, Goldstein D, Renschler MF. Albumin-bound paclitaxel plus gemcitabine in pancreatic cancer. *N Engl J Med* 2014; **370**: 479-480 [PMID: 24476438 DOI: 10.1056/NEJMc1314761]
23 **Tabernero J**, Chiorean EG, Infante JR, Hingorani SR, Ganju V, Weekes C, Scheithauer W, Ramanathan RK, Goldstein D, Penenberg DN, Romano A, Ferrara S, Von Hoff DD. Prognostic factors of survival in a randomized phase III trial (MPACT) of weekly nab-paclitaxel plus gemcitabine versus gemcitabine alone in patients with metastatic pancreatic cancer. *Oncologist* 2015; **20**: 143-150 [PMID: 25582141 DOI: 10.1634/theoncologist.2014-0394]
24 **Arnold SA**, Rivera LB, Miller AF, Carbon JG, Dineen SP, Xie Y, Castrillon DH, Sage EH, Puolakkainen P, Bradshaw AD, Brekken RA. Lack of host SPARC enhances vascular function and tumor spread in an orthotopic murine model of pancreatic carcinoma. *Dis Model Mech* 2010; **3**: 57-72 [PMID: 20007485 DOI: 10.1242/dmm.003228]
25 **Sinn M**, Sinn BV, Striefler JK, Stieler J, Pelzer U, Prinzler J, Neuhaus P, Dietel M, Dörken B, Oettle H, Riess H, Denkert C. SPARC in pancreatic cancer; Results from the CONKO-001 study. ASCO annual meeting 4016. *J Clin Oncol* 2013; **31** suppl: abstr 4016
26 **Von Hoff DD**, Ramanathan RK, Borad MJ, Laheru DA, Smith LS, Wood TE, Korn RL, Desai N, Trieu V, Iglesias JL, Zhang H, Soon-Shiong P, Shi T, Rajeshkumar NV, Maitra A, Hidalgo M. Gemcitabine plus nab-paclitaxel is an active regimen in patients with advanced pancreatic cancer: a phase I/II trial. *J Clin Oncol* 2011; **29**: 4548-4554 [PMID: 21969517 DOI: 10.1200/JCO.2011.36.5742]
27 **Kanda M**, Matthaei H, Wu J, Hong SM, Yu J, Borges M, Hruban RH, Maitra A, Kinzler K, Vogelstein B, Goggins M. Presence of somatic mutations in most early-stage pancreatic intraepithelial neoplasia. *Gastroenterology* 2012; **142**: 730-733.e9 [PMID: 22226782 DOI: 10.1053/j.gastro.2011.12.042]
28 **Hruban RH**, van Mansfeld AD, Offerhaus GJ, van Weering DH, Allison DC, Goodman SN, Kensler TW, Bose KK, Cameron JL, Bos JL. K-ras oncogene activation in adenocarcinoma of the human pancreas. A study of 82 carcinomas using a combination of mutant-enriched polymerase chain reaction analysis and allele-specific oligonucleotide hybridization. *Am J Pathol* 1993; **143**: 545-554 [PMID: 8342602]
29 **Macdonald JS**, McCoy S, Whitehead RP, Iqbal S, Wade JL, Giguere JK, Abbruzzese JL. A phase II study of farnesyl transferase inhibitor R115777 in pancreatic cancer: a Southwest oncology group (SWOG 9924) study. *Invest New Drugs* 2005; **23**: 485-487 [PMID: 16133800]
30 **Cohen SJ**, Ho L, Ranganathan S, Abbruzzese JL, Alpaugh RK, Beard M, Lewis NL, McLaughlin S, Rogatko A, Perez-Ruixo JJ, Thistle AM, Verhaeghe T, Wang H, Weiner LM, Wright JJ, Hudes GR, Meropol NJ. Phase II and pharmacodynamic study of the farnesyltransferase inhibitor R115777 as initial therapy in patients

with metastatic pancreatic adenocarcinoma. *J Clin Oncol* 2003; **21**: 1301-1306 [PMID: 12663718 DOI: 10.1200/JCO.2003.08.040]

31 **Van Cutsem E**, van de Velde H, Karasek P, Oettle H, Vervenne WL, Szawlowski A, Schoffski P, Post S, Verslype C, Neumann H, Safran H, Humblet Y, Perez Ruixo J, Ma Y, Von Hoff D. Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. *J Clin Oncol* 2004; **22**: 1430-1438 [PMID: 15084616]

32 **Zimmermann G**, Papke B, Ismail S, Vartak N, Chandra A, Hoffmann M, Hahn SA, Triola G, Wittinghofer A, Bastiaens PI, Waldmann H. Small molecule inhibition of the KRAS-PDEδ interaction impairs oncogenic KRAS signalling. *Nature* 2013; **497**: 638-642 [PMID: 23698361]

33 **Maurer T**, Garrenton LS, Oh A, Pitts K, Anderson DJ, Skelton NJ, Fauber BP, Pan B, Malek S, Stokoe D, Ludlam MJ, Bowman KK, Wu J, Giannetti AM, Starovasnik MA, Mellman I, Jackson PK, Rudolph J, Wang W, Fang G. Small-molecule ligands bind to a distinct pocket in Ras and inhibit SOS-mediated nucleotide exchange activity. *Proc Natl Acad Sci USA* 2012; **109**: 5299-5304 [PMID: 22431598]

34 **Ostrem JM**, Peters U, Sos ML, Wells JA, Shokat KM. K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. *Nature* 2013; **503**: 548-551 [PMID: 24256730]

35 **Corcoran RB**, Cheng KA, Hata AN, Faber AC, Ebi H, Coffee EM, Greninger P, Brown RD, Godfrey JT, Cohoon TJ, Song Y, Lifshits E, Hung KE, Shioda T, Dias-Santagata D, Singh A, Settleman J, Benes CH, Mino-Kenudson M, Wong KK, Engelman JA. Synthetic lethal interaction of combined BCL-XL and MEK inhibition promotes tumor regressions in KRAS mutant cancer models. *Cancer Cell* 2013; **23**: 121-128 [PMID: 23245996]

36 **Zorde Khvalevsky E**, Gabai R, Rachmut IH, Horwitz E, Brunschwig Z, Orbach A, Shemi A, Golan T, Domb AJ, Yavin E, Giladi H, Rivkin L, Simerzin A, Eliakim R, Khalaileh A, Hubert A, Lahav M, Kopelman Y, Goldin E, Dancour A, Hants Y, Arbel-Alon S, Abramovitch R, Shemi A, Galun E. Mutant KRAS is a druggable target for pancreatic cancer. *Proc Natl Acad Sci USA* 2013; **110**: 20723-20728 [PMID: 24297898]

37 **Silenseed Ltd**. Phase 1 - Escalating dose study of siG12D LODER (Local Drug EluteR) in patients with advanced adenocarcinoma of the pancreas and a single dose study of siG12D LODER (Local drug EluteR) in patients with operable adenocarcinoma of the pancreas. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01188785 NLM Identifier: NCT01188785

38 **Silenseed Ltd**. A phase II study of siG12D LODER in combination with chemotherapy in paitents with unresectable locally advanced pancreatic cancer. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01676259 NLM Identifier: NCT01676259

39 **Chapman PB**, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, Hogg D, Lorigan P, Lebbe C, Jouary T, Schadendorf D, Ribas A, O'Day SJ, Sosman JA, Kirkwood JM, Eggermont AM, Dreno B, Nolop K, Li J, Nelson B, Hou J, Lee RJ, Flaherty KT, McArthur GA. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med* 2011; **364**: 2507-2516 [PMID: 21639808 DOI: 10.1056/NEJMoa1103782]

40 **Hatzivassiliou G**, Song K, Yen I, Brandhuber BJ, Anderson DJ, Alvarado R, Ludlam MJ, Stokoe D, Gloor SL, Vigers G, Morales T, Aliagas I, Liu B, Sideris S, Hoeflich KP, Jaiswal BS, Seshagiri S, Koeppen H, Belvin M, Friedman LS, Malek S. RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. *Nature* 2010; **464**: 431-435 [PMID: 20130576 DOI: 10.1038/nature08833]

41 **GlaxoSmithKline**. Study of GSK1120212 plus gemcitabine vs placebo plus gemcitabine in metastatic pancreatic cancer. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01231581 NLM Identifier: NCT01231581

42 **Merck KGaA**. Trial of gemcitabine with or without MSC1936369B in pancreatic cancer. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01016483 NLM Identifier: NCT01016483

43 **Bayer**. Combination with gemcitabine in advanced pancreatic cancer (BAGPAC). In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01251640 NLM Identifier: NCT01251640

44 **Riess H**, Van Laethem JL, Martens UM, Heinemann V, Michl P, Peeters M, Van Brummelen D, Weekes CD, Dueland S, Schmiegel WH, Giurescu M, Garosi VL, Schulz A, Seidel H, Childs BH, Teufel M. Phase II study of the MEK inhibitor refametinib (BAY 86-9766) in combination with gemcitabine in patietns with unresectable, locally advacned, or metastatic pancreatic cancer. 2014 ASCO annual meeting 4025. *J Clin Oncol* 2014; **32**: 5s (suppl; abstr 4129)

45 **Falasca M**, Selvaggi F, Buus R, Sulpizio S, Edling CE. Targeting phosphoinositide 3-kinase pathways in pancreatic cancer--from molecular signalling to clinical trials. *Anticancer Agents Med Chem* 2011; **11**: 455-463 [PMID: 21521159 DOI: 10.2174/187152011795677382]

46 **Eser S**, Reiff N, Messer M, Seidler B, Gottschalk K, Dobler M, Hieber M, Arbeiter A, Klein S, Kong B, Michalski CW, Schlitter AM, Esposito I, Kind AJ, Rad L, Schnieke AE, Baccarini M, Alessi DR, Rad R, Schmid RM, Schneider G, Saur D. Selective requirement of PI3K/PDK1 signaling for Kras oncogene-driven pancreatic cell plasticity and cancer. *Cancer Cell* 2013; **23**: 406-420 [PMID: 23453624 DOI: 10.1016/j.ccr.2013.01.023]

47 **Ma WW**, Messersmith WA, Dy GK, Weekes CD, Whitworth A, Ren C, Maniar M, Wilhelm F, Eckhardt SG, Adjei AA, Jimeno A. Phase I study of Rigosertib, an inhibitor of the phosphatidylinositol 3-kinase and Polo-like kinase 1 pathways, combined with gemcitabine in patients with solid tumors and pancreatic cancer. *Clin Cancer Res* 2012; **18**: 2048-2055 [PMID: 22338014 DOI: 10.1158/1078-0432.CCR-11-2813]

48 **Onconova Therapeutics, Inc**. Gemcitabine and ON 01910.Na in previously untreated metasatic pancreatic cancer (ONTRAC). In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01360853 NLM Identifier: NCT01360853

49 **Motzer RJ**, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, Grünwald V, Thompson JA, Figlin RA, Hollaender N, Urbanowitz G, Berg WJ, Kay A, Lebwohl D, Ravaud A. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. *Lancet* 2008; **372**: 449-456 [PMID: 18653228 DOI: 10.1016/S0140-6736(08)61039-9]

50 **André F**, O'Regan R, Ozguroglu M, Toi M, Xu B, Jerusalem G, Masuda N, Wilks S, Arena F, Isaacs C, Yap YS, Papai Z, Lang I, Armstrong A, Lerzo G, White M, Shen K, Litton J, Chen D, Zhang Y, Ali S, Taran T, Gianni L. Everolimus for women with trastuzumab-resistant, HER2-positive, advanced breast cancer (BOLERO-3): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet Oncol* 2014; **15**: 580-591 [PMID: 24742739 DOI: 10.1016/S1470-2045(14)70138-X]

51 **Yao JC**, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, Hobday TJ, Okusaka T, Capdevila J, de Vries EG, Tomassetti P, Pavel ME, Hoosen S, Haas T, Lincy J, Lebwohl D, Öberg K. Everolimus for advanced pancreatic neuroendocrine tumors. *N Engl J Med* 2011; **364**: 514-523 [PMID: 21306238 DOI: 10.1056/NEJMoa1009290]

52 **Matsubara S**, Ding Q, Miyazaki Y, Kuwahata T, Tsukasa K, Takao S. mTOR plays critical roles in pancreatic cancer stem cells through specific and stemness-related functions. *Sci Rep* 2013; **3**: 3230 [PMID: 24231729 DOI: 10.1038/srep03230]

53 **Wolpin BM**, Hezel AF, Abrams T, Blaszkowsky LS, Meyerhardt JA, Chan JA, Enzinger PC, Allen B, Clark JW, Ryan DP, Fuchs CS. Oral mTOR inhibitor everolimus in patients with gemcitabine-refractory metastatic pancreatic cancer. *J Clin Oncol* 2009; **27**:

193-198 [PMID: 19047305 DOI: 10.1200/JCO.2008.18.9514]
54 **Dowling RJ**, Zakikhani M, Fantus IG, Pollak M, Sonenberg N. Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells. *Cancer Res* 2007; **67**: 10804-10812 [PMID: 18006825]
55 **Evans JM**, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. *BMJ* 2005; **330**: 1304-1305 [PMID: 15849206 DOI: 10.1136/bmj.38415.708634.F7]
56 Academisch Medisch Centrum - Universiteit van Amsterdam (AMC-UvA). Metformin combined with chemotherapy for pancreatic cancer(GEM). In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01210911 NLM Identifier: NCT01210911
57 **Lou HZ**, Weng XC, Pan HM, Pan Q, Sun P, Liu LL, Chen B. The novel mTORC1/2 dual inhibitor INK-128 suppresses survival and proliferation of primary and transformed human pancreatic cancer cells. *Biochem Biophys Res Commun* 2014; **450**: 973-978 [PMID: 24971544 DOI: 10.1016/j.bbrc.2014.06.081]
58 **Tang JY**, Dai T, Zhang H, Xiong WJ, Xu MZ, Wang XJ, Tang QH, Chen B, Xu M. GDC-0980-induced apoptosis is enhanced by autophagy inhibition in human pancreatic cancer cells. *Biochem Biophys Res Commun* 2014; **453**: 533-538 [PMID: 25285629 DOI: 10.1016/j.bbrc.2014.09.115]
59 **Ali K**, Soond DR, Piñeiro R, Hagemann T, Pearce W, Lim EL, Bouabe H, Scudamore CL, Hancox T, Maecker H, Friedman L, Turner M, Okkenhaug K, Vanhaesebroeck B. Inactivation of PI(3)K p110δ breaks regulatory T-cell-mediated immune tolerance to cancer. *Nature* 2014; **510**: 407-411 [PMID: 24919154 DOI: 10.1038/nature13444]
60 **Zhang H**, Berezov A, Wang Q, Zhang G, Drebin J, Murali R, Greene MI. ErbB receptors: from oncogenes to targeted cancer therapies. *J Clin Invest* 2007; **117**: 2051-2058 [PMID: 17671639 DOI: 10.1172/JCI32278]
61 **Moore MJ**, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, Au HJ, Murawa P, Walde D, Wolff RA, Campos D, Lim R, Ding K, Clark G, Voskoglou-Nomikos T, Ptasynski M, Parulekar W. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 2007; **25**: 1960-1966 [PMID: 17452677 DOI: 10.1200/JCO.2006.07.9525]
62 **Wacker B**, Nagrani T, Weinberg J, Witt K, Clark G, Cagnoni PJ. Correlation between development of rash and efficacy in patients treated with the epidermal growth factor receptor tyrosine kinase inhibitor erlotinib in two large phase III studies. *Clin Cancer Res* 2007; **13**: 3913-3921 [PMID: 17606725 DOI: 10.1158/1078-0432.CCR-06-2610]
63 **Shepherd FA**, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, Campos D, Maoleekoonpiroj S, Smylie M, Martins R, van Kooten M, Dediu M, Findlay B, Tu D, Johnston D, Bezjak A, Clark G, Santabárbara P, Seymour L. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2005; **353**: 123-132 [PMID: 16014882 DOI: 10.1056/NEJMoa050753]
64 **Stepanski EJ**, Reyes C, Walker MS, Satram-Hoang S, Leon L, Wojtowicz-Praga S, Miller PJ, Houts AC, Schwartzberg LS. The association of rash severity with overall survival: findings from patients receiving erlotinib for pancreatic cancer in the community setting. *Pancreas* 2013; **42**: 32-36 [PMID: 22699203 DOI: 10.1097/MPA.0b013e318254f19a]
65 **Kulke MH**, Blaszkowsky LS, Ryan DP, Clark JW, Meyerhardt JA, Zhu AX, Enzinger PC, Kwak EL, Muzikansky A, Lawrence C, Fuchs CS. Capecitabine plus erlotinib in gemcitabine-refractory advanced pancreatic cancer. *J Clin Oncol* 2007; **25**: 4787-4792 [PMID: 17947726]
66 **Heinemann V.** Capecitabine/erlotinib followed of gemcitabine versus gemcitabine/erlotinib followed of capecitabine. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://www.clinicaltrials.gov/ct2/show/NCT00440167 NLM Identifier: NCT00440167
67 **Zhou X**, Zheng M, Chen F, Zhu Y, Yong W, Lin H, Sun Y, Han X. Gefitinib inhibits the proliferation of pancreatic cancer cells via cell cycle arrest. *Anat Rec* (Hoboken) 2009; **292**: 1122-1127 [PMID: 19645012 DOI: 10.1002/ar.20938]
68 **Fountzilas G**, Bobos M, Kalogera-Fountzila A, Xiros N, Murray S, Linardou H, Karayannopoulou G, Koutras AK, Bafaloukos D, Samantas E, Christodoulou C, Economopoulos T, Kalogeras KT, Kosmidis P. Gemcitabine combined with gefitinib in patients with inoperable or metastatic pancreatic cancer: a phase II Study of the Hellenic Cooperative Oncology Group with biomarker evaluation. *Cancer Invest* 2008; **26**: 784-793 [PMID: 18798073 DOI: 10.1080/07357900801918611]
69 **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 DOI: 10.1016/S1470-2045(09)70311-0]
70 **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]
71 **Bruns CJ**, Harbison MT, Davis DW, Portera CA, Tsan R, McConkey DJ, Evans DB, Abbruzzese JL, Hicklin DJ, Radinsky R. Epidermal growth factor receptor blockade with C225 plus gemcitabine results in regression of human pancreatic carcinoma growing orthotopically in nude mice by antiangiogenic mechanisms. *Clin Cancer Res* 2000; **6**: 1936-1948 [PMID: 10815919]
72 **Bloomston M**, Bhardwaj A, Ellison EC, Frankel WL. Epidermal growth factor receptor expression in pancreatic carcinoma using tissue microarray technique. *Dig Surg* 2006; **23**: 74-79 [PMID: 16717472 DOI: 10.1159/000093497]
73 **Uegaki K**, Nio Y, Inoue Y, Minari Y, Sato Y, Song MM, Dong M, Tamura K. Clinicopathological significance of epidermal growth factor and its receptor in human pancreatic cancer. *Anticancer Res* 1997; **17**: 3841-3847 [PMID: 9427790]
74 **Xiong HQ**, Rosenberg A, LoBuglio A, Schmidt W, Wolff RA, Deutsch J, Needle M, Abbruzzese JL. Cetuximab, a monoclonal antibody targeting the epidermal growth factor receptor, in combination with gemcitabine for advanced pancreatic cancer: a multicenter phase II Trial. *J Clin Oncol* 2004; **22**: 2610-2616 [PMID: 15226328 DOI: 10.1200/JCO.2004.12.040]
75 **Philip PA**, Benedetti J, Corless CL, Wong R, O'Reilly EM, Flynn PJ, Rowland KM, Atkins JN, Mirtsching BC, Rivkin SE, Khorana AA, Goldman B, Fenoglio-Preiser CM, Abbruzzese JL, Blanke CD. Phase III study comparing gemcitabine plus cetuximab versus gemcitabine in patients with advanced pancreatic adenocarcinoma: Southwest Oncology Group-directed intergroup trial S0205. *J Clin Oncol* 2010; **28**: 3605-3610 [PMID: 20606093 DOI: 10.1200/JCO.2009.25.7550]
76 **Cascinu S**, Berardi R, Labianca R, Siena S, Falcone A, Aitini E, Barni S, Di Costanzo F, Dapretto E, Tonini G, Pierantoni C, Artale S, Rota S, Floriani I, Scartozzi M, Zaniboni A. Cetuximab plus gemcitabine and cisplatin compared with gemcitabine and cisplatin alone in patients with advanced pancreatic cancer: a randomised, multicentre, phase II trial. *Lancet Oncol* 2008; **9**: 39-44 [PMID: 18077217 DOI: 10.1016/S1470-2045(07)70383-2]
77 **Kullmann F**, Hollerbach S, Dollinger MM, Harder J, Fuchs M, Messmann H, Trojan J, Gäbele E, Hinke A, Hollerbach C, Endlicher E. Cetuximab plus gemcitabine/oxaliplatin (GEMOXCET) in first-line metastatic pancreatic cancer: a multicentre phase II study. *Br J Cancer* 2009; **100**: 1032-1036 [PMID: 19293797 DOI: 10.1038/sj.bjc.6604983]
78 **Strumberg D**. Phase II, randomized, double-blind placebo-

controlled trial of nimotuzumab plus gemcitabine compared with gemcitabine alone in patients (pts) with advanced pancreatic cancer (PC). *J Clin Oncol* 2013; **31**: (suppl; abstr 4009)

79 **Immervoll H**, Hoem D, Kugarajh K, Steine SJ, Molven A. Molecular analysis of the EGFR-RAS-RAF pathway in pancreatic ductal adenocarcinomas: lack of mutations in the BRAF and EGFR genes. *Virchows Arch* 2006; **448**: 788-796 [PMID: 16598499 DOI: 10.1097/MPA.0b013e3181b8feb0]

80 **Kwak EL**, Jankowski J, Thayer SP, Lauwers GY, Brannigan BW, Harris PL, Okimoto RA, Haserlat SM, Driscoll DR, Ferry D, Muir B, Settleman J, Fuchs CS, Kulke MH, Ryan DP, Clark JW, Sgroi DC, Haber DA, Bell DW. Epidermal growth factor receptor kinase domain mutations in esophageal and pancreatic adenocarcinomas. *Clin Cancer Res* 2006; **12**: 4283-4287 [PMID: 16857803 DOI: 10.1158/1078-0432.CCR-06-0189]

81 **Faller BA**, Burtness B. Treatment of pancreatic cancer with epidermal growth factor receptor-targeted therapy. *Biologics* 2009; **3**: 419-428 [PMID: 19774209]

82 **Navas C**, Hernández-Porras I, Schuhmacher AJ, Sibilia M, Guerra C, Barbacid M. EGF receptor signaling is essential for k-ras oncogene-driven pancreatic ductal adenocarcinoma. *Cancer Cell* 2012; **22**: 318-330 [PMID: 22975375 DOI: 10.1016/j.ccr.2012.08.001]

83 **Hanahan D**, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996; **86**: 353-364 [PMID: 8756718]

84 **Bergers G**, Benjamin LE. Tumorigenesis and the angiogenic switch. *Nat Rev Cancer* 2003; **3**: 401-410 [PMID: 12778130 DOI: 10.1038/nrc1093]

85 **Jain RK**, Duda DG, Clark JW, Loeffler JS. Lessons from phase III clinical trials on anti-VEGF therapy for cancer. *Nat Clin Pract Oncol* 2006; **3**: 24-40 [PMID: 16407877 DOI: 10.1038/ncponc0403]

86 **Kindler HL**, Friberg G, Singh DA, Locker G, Nattam S, Kozloff M, Taber DA, Karrison T, Dachman A, Stadler WM, Vokes EE. Phase II trial of bevacizumab plus gemcitabine in patients with advanced pancreatic cancer. *J Clin Oncol* 2005; **23**: 8033-8040 [PMID: 16258101 DOI: 10.1200/JCO.2005.01.9661]

87 **Kindler HL**, Niedzwiecki D, Hollis D, Sutherland S, Schrag D, Hurwitz H, Innocenti F, Mulcahy MF, O'Reilly E, Wozniak TF, Picus J, Bhargava P, Mayer RJ, Schilsky RL, Goldberg RM. Gemcitabine plus bevacizumab compared with gemcitabine plus placebo in patients with advanced pancreatic cancer: phase III trial of the Cancer and Leukemia Group B (CALGB 80303). *J Clin Oncol* 2010; **28**: 3617-3622 [PMID: 20606091 DOI: 10.1200/JCO.2010.28.1386]

88 **Van Cutsem E**, Vervenne WL, Bennouna J, Humblet Y, Gill S, Van Laethem JL, Verslype C, Scheithauer W, Shang A, Cosaert J, Moore MJ. Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. *J Clin Oncol* 2009; **27**: 2231-2237 [PMID: 19307500 DOI: 10.1200/JCO.2008.20.0238]

89 **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]

90 **May KF**, Gulley JL, Drake CG, Dranoff G, Kantoff PW. Prostate cancer immunotherapy. *Clin Cancer Res* 2011; **17**: 5233-5238 [PMID: 21700764 DOI: 10.1158/1078-0432.CCR-10-3402]

91 **Le DT**, Wang-Gillam A, Picozzi V, Greten TF, Crocenzi TS, Springett GM, Morse M, Zeh H, Cohen DJ, Fine RL, Onners B, Uram JN, Laheru D, Murphy A, Skoble J, Lemmens E, Grous JJ, Dubensky T, Brockstedt DG, Jaffee EM. A phase 2, randomized trial of GVAX pancreas and CRS-207 immunotherapy versus GVAZ alone in patients with metastatic pancreatic adenocarcinoma; Updated results. *J Clin Oncol* 2014; **32**: (suppl 3; abstr 177)

92 **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/NEJMoa1200690]

93 **Middleton G**, Silcocks P, Cox T, Valle J, Wadsley J, Propper D, Coxon F, Ross P, Madhusudan S, Roques T, Cunningham D, Falk S, Wadd N, Harrison M, Corrie P, Iveson T, Robinson A, McAdam K, Eatock M, Evans J, Archer C, Hickish T, Garcia-Alonso A, Nicolson M, Steward W, Anthoney A, Greenhalf W, Shaw V, Costello E, Naisbitt D, Rawcliffe C, Nanson G, Neoptolemos J. Gemcitabine and capecitabine with or without telomerase peptide vaccine GV1001 in patients with locally advanced or metastatic pancreatic cancer (TeloVac): an open-label, randomised, phase 3 trial. *Lancet Oncol* 2014; **15**: 829-840 [PMID: 24954781 DOI: 10.1016/S1470-2045(14)70236-0]

94 **Royal RE**, Levy C, Turner K, Mathur A, Hughes M, Kammula US, Sherry RM, Topalian SL, Yang JC, Lowy I, Rosenberg SA. Phase 2 trial of single agent Ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. *J Immunother* 2010; **33**: 828-833 [PMID: 20842054 DOI: 10.1097/CJI.0b013e3181eec14c]

95 **Northwestern University**. Ipililmumab and gemcitabine hydrochloride in treating patients with stage III-IV or recurrent pancreatic cancer that cannot be removed by surgery. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01473940 NLM Identifier: NCT01473940

96 **McMahon AP**, Ingham PW, Tabin CJ. Developmental roles and clinical significance of hedgehog signaling. *Curr Top Dev Biol* 2003; **53**: 1-114 [PMID: 12509125]

97 **Xie J**, Murone M, Luoh SM, Ryan A, Gu Q, Zhang C, Bonifas JM, Lam CW, Hynes M, Goddard A, Rosenthal A, Epstein EH, de Sauvage FJ. Activating Smoothened mutations in sporadic basal-cell carcinoma. *Nature* 1998; **391**: 90-92 [PMID: 9422511 DOI: 10.1038/34201]

98 **Amakye D**, Jagani Z, Dorsch M. Unraveling the therapeutic potential of the Hedgehog pathway in cancer. *Nat Med* 2013; **19**: 1410-1422 [PMID: 24202394 DOI: 10.1038/nm.3389]

99 **Infinity Pharmaceuticals, Inc.** A study evaluating IPI-926 in combination with gemcitabine in patients with metastatic pancreatic cancer. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01130142 NLM Identifier: NCT01130142

100 **Bramhall SR**, Rosemurgy A, Brown PD, Bowry C, Buckels JA. Marimastat as first-line therapy for patients with unresectable pancreatic cancer: a randomized trial. *J Clin Oncol* 2001; **19**: 3447-3455 [PMID: 11481349]

101 **Kindler HL**, Ioka T, Richel DJ, Bennouna J, Létourneau R, Okusaka T, Funakoshi A, Furuse J, Park YS, Ohkawa S, Springett GM, Wasan HS, Trask PC, Bycott P, Ricart AD, Kim S, Van Cutsem E. Axitinib plus gemcitabine versus placebo plus gemcitabine in patients with advanced pancreatic adenocarcinoma: a double-blind randomised phase 3 study. *Lancet Oncol* 2011; **12**: 256-262 [PMID: 21306953 DOI: 10.1016/S1470-2045(11)70004-3]

102 **Gonçalves A**, Gilabert M, François E, Dahan L, Perrier H, Lamy R, Re D, Largillier R, Gasmi M, Tchiknavorian X, Esterni B, Genre D, Moureau-Zabotto L, Giovannini M, Seitz JF, Delpero JR, Turrini O, Viens P, Raoul JL. BAYPAN study: a double-blind phase III randomized trial comparing gemcitabine plus sorafenib and gemcitabine plus placebo in patients with advanced pancreatic cancer. *Ann Oncol* 2012; **23**: 2799-2805 [PMID: 22771827 DOI: 10.1093/annonc/mds135]

103 **Berlin JD**, Catalano P, Thomas JP, Kugler JW, Haller DG, Benson AB. Phase III study of gemcitabine in combination with fluorouracil versus gemcitabine alone in patients with advanced pancreatic carcinoma: Eastern Cooperative Oncology Group Trial E2297. *J Clin Oncol* 2002; **20**: 3270-3275 [PMID: 12149301 DOI: 10.1200/JCO.2002.11.149]

104 **Rocha Lima CM**, Sherman CA, Brescia FJ, Brunson CY, Green

MR. Irinotecan/gemcitabine combination chemotherapy in pancreatic cancer. *Oncology* (Williston Park) 2001; **15**: 46-51 [PMID: 11301841]

105 **Louvet C**, Labianca R, Hammel P, Lledo G, Zampino MG, André T, Zaniboni A, Ducreux M, Aitini E, Taïeb J, Faroux R, Lepere C, de Gramont A. Gemcitabine in combination with oxaliplatin compared with gemcitabine alone in locally advanced or metastatic pancreatic cancer: results of a GERCOR and GISCAD phase III trial. *J Clin Oncol* 2005; **23**: 3509-3516 [PMID: 15908661 DOI: 10.1200/JCO.2005.06.023]

106 **Herrmann R**, Bodoky G, Ruhstaller T, Glimelius B, Bajetta E, Schüller J, Saletti P, Bauer J, Figer A, Pestalozzi B, Köhne CH, Mingrone W, Stemmer SM, Tàmas K, Kornek GV, Koeberle D, Cina S, Bernhard J, Dietrich D, Scheithauer W. Gemcitabine plus capecitabine compared with gemcitabine alone in advanced pancreatic cancer: a randomized, multicenter, phase III trial of the Swiss Group for Clinical Cancer Research and the Central European Cooperative Oncology Group. *J Clin Oncol* 2007; **25**: 2212-2217 [PMID: 17538165 DOI: 10.1200/JCO.2006.09.0886]

107 **Stathopoulos GP**, Syrigos K, Aravantinos G, Polyzos A, Papakotoulas P, Fountzilas G, Potamianou A, Ziras N, Boukovinas J, Varthalitis J, Androulakis N, Kotsakis A, Samonis G, Georgoulias V. A multicenter phase III trial comparing irinotecan-gemcitabine (IG) with gemcitabine (G) monotherapy as first-line treatment in patients with locally advanced or metastatic pancreatic cancer. *Br J Cancer* 2006; **95**: 587-592 [PMID: 16909140 DOI: 10.1038/sj.bjc.6603301]

108 **Heinemann V**, Quietzsch D, Gieseler F, Gonnermann M, Schönekäs H, Rost A, Neuhaus H, Haag C, Clemens M, Heinrich B, Vehling-Kaiser U, Fuchs M, Fleckenstein D, Gesierich W, Uthgenannt D, Einsele H, Holstege A, Hinke A, Schalhorn A, Wilkowski R. Randomized phase III trial of gemcitabine plus cisplatin compared with gemcitabine alone in advanced pancreatic cancer. *J Clin Oncol* 2006; **24**: 3946-3952 [PMID: 16921047 DOI: 10.1200/JCO.2005.05.1490]

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REVIEW

Appropriate prescribing in the elderly: Current perspectives

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Abstract

Advances in medical therapeutics have undoubtedly contributed to health gains and increases in life expectancy over the last century. However, there is growing evidence to suggest that therapeutic decisions in older patients are frequently suboptimal or potentially inappropriate and often result in negative outcomes such as adverse drug events, hospitalisation and increased healthcare resource utilisation. Several factors influence the appropriateness of medication selection in older patients including age-related changes in pharmacokinetics and pharmacodynamics, high numbers of concurrent medications, functional status and burden of co-morbid illness. With ever-increasing therapeutic options, escalating proportions of older patients worldwide, and varying degrees of prescriber education in geriatric pharmacotherapy, strategies to assist physicians in choosing appropriate pharmacotherapy for older patients may be helpful. In this paper, we describe important age-related pharmacological changes as well as the principal domains of prescribing appropriateness in older people. We highlight common examples of drug-drug and drug-disease interactions in older people. We present a clinical case in which the appropriateness of prescription medications is reviewed and corrective strategies suggested. We also discuss various approaches to optimising prescribing appropriateness in this population including the use of explicit and implicit prescribing appropriateness criteria, comprehensive geriatric assessment, clinical pharmacist review, prescriber education and computerized decision support tools.

Key words: Elderly; Inappropriate prescribing; Polypharmacy; Beers criteria; Screening Tool of Older Person's potentially inappropriate Prescriptions/Screening Tool to Alert to Right Treatment; Adverse drug reactions

Core tip: In this paper we discuss the challenges and complexities of prescribing for older people. We describe the important age-related changes in pharmacokinetics and pharmacodynamics that influence prescribing decisions and we highlight commonly encountered examples of drug-drug and drug-disease interactions. We present a detailed analysis of a complex clinical case in which several instances of potentially inappropriate prescribing exist and we suggest corrective actions. We explore a range of strategies aimed at optimizing prescribing appropriateness for older people including prescribing criteria, comprehensive geriatric assessment, clinical pharmacy interventions and computerized decision supports.

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INTRODUCTION

Over the last century, there have been dramatic increases in life expectancy owing largely to improvements in living standards and advances in diagnostics, pharmaceutical medicine and therapeutics. This is reflected in worldwide changes in population demographics, with ever-increasing numbers of older people. The United Nations define "older people" as being aged 60 years or older with the oldest old being 80 years or older. In 1990, 9.2% of the world's population was aged at least 60 years old. In 2013, this proportion was 11.3% and by 2050, it is estimated that 21.2% of the world's population will be aged 60 years and over[1]. The largest numbers of older adults currently reside in developed countries, however by 2050 it is estimated they will reside in developing countries. Presently the older population is predominantly female with an expected improvement in male mortality expected in the coming years[1].

Though increased longevity is to be celebrated, it is well established that increasing age brings with it an increase in the burden of co-morbidity and a corresponding increase in the consumption of medications. Appropriate selection and prescription of curative and preventative medicines is an essential element of high quality healthcare for older people, who are the greatest consumers of healthcare resources in most developed nations[2]. One in eight Americans is aged over 65 years, but this small proportion of the population consumes the greatest proportion of prescription medications[3]. Similarly, older Europeans consume over twice as many healthcare resources than their younger counterparts[4]. In the United Kingdom approximately one fifth of the population is aged over 65 years, but this group receives 45% of all dispensed drugs[5]. In Ireland, 11% of the population is over 65 years but account for up to 50% of medications dispensed through its reimbursement service[6]. In the United States approximately 30% of community-dwelling older adults are regularly prescribed five or more medications[7]. This number rises in hospitalized older patients and in nursing home residents, perhaps reflecting a greater disease burden.

It is estimated that older people consume approximately 40% of all over-the-counter (OTC) medications sold in the United States[8]. Concurrent use of OTC medication with regular prescription medications places patients at higher risk of adverse outcomes; one study reported that 46% of older patients were concurrently taking OTC medications with regular prescription medications and 1 in 25 of these patients were at risk of significant drug interactions[7]. In addition, there is emerging evidence that the consumption of complementary and alternative medicines amongst older adults is steadily increasing[9]. A recent study showed a significant rise in the use of herbal remedies in those aged $\geqslant$ 65 years from 13.2% in 2002 to 19.5% in 2007[10].

Prescribing for older patients with multiple chronic illnesses, especially frailer older patients with cognitive and functional impairments, presents many unique challenges, particularly with respect to the following variables: (1) polypharmacy; (2) altered pharmacokinetic and pharmacodynamic responses; (3) balancing the risk of harm *vs* long term therapeutic benefit; and (4) paucity of robust scientific evidence for use of commonly prescribed medications in older, frail patients with limited life expectancy.

Prescribers must be cognizant of important age-related anatomical, biochemical and physiological changes that affect drug pharmacokinetics, pharmacodynamics and homeostatic mechanisms. They must also be aware of the potential for interaction with concurrently prescribed drugs and co-existing disease states. Prescribers should have an appreciation of the potentially low therapeutic yield in very frail older patients with poor life expectancy where the risk of certain treatments can exceed the potential clinical benefit. These important tenets of appropriate prescribing for older patients are briefly summarised below.

Pharmacokinetics and ageing

The key pharmacokinetic changes commonly associated with ageing are summarized in Table 1. A more detailed description follows. Drug absorption is generally unaltered in healthy older people; however certain conditions may affect the rate of drug absorption. Drugs with anticholinergic effects may reduce saliva secretion, thus impeding the rate, but not necessarily the amount of drug absorbed through the oral mucosa, *e.g.,* buccal midazolam and sublingual nitrate. The rate of absorption of subcutaneous, intramuscular and transdermal medications can be affected by reduced tissue perfusion. Conversely, prokinetic agents such as domperidone or erythromycin can increase the rate of delivery of an oral drug to its absorption site. Reductions in small bowel active transport mechanisms can affect the extent of absorption of iron and vitamin B12. Intravenous absorption is generally not affected.

Plasma drug concentration is inversely related to its volume of distribution (Vd), which in turn, is dependent on the hydrophilic and lipophilic volumes in the body. As people age, there is a reduction in muscle mass and body water content with a proportionate increase in body fat[11]. Consequently, the Vd for hydrophilic drugs (*e.g.,* lithium) is reduced; this may result in toxicity if drugs are not dose-adjusted. Lipophilic drugs (*e.g.,* antipsychotic medications) have a higher Vd in older people, and therefore have an increased elimination

Table 1 Pharmacokinetics and ageing

Absorption	↓ amount of saliva
	↑ gastric pH
	↓ gastric acid secretion
	↑ gastric emptying time
	↓ gastric surface area
	↓ gastrointestinal motility
	↓ active transport mechanisms
Distribution	↓ cardiac output
	↑ peripheral vascular resistance
	↓ renal blood flow
	↓ hepatic blood flow
	↓ body water
	↑ body fat tissue
	↓ serum albumin levels
	↑ for lipid soluble and decrease for water soluble drugs
Metabolic	↓ microsomal hepatic oxidation
	↓ clearance
	↑ steady state levels
	↑ half lives
	↑ levels of active metabolites
	↓ first pass metabolism due to reduced ↓ blood flow
Excretion	↓ in renal perfusion
	↓ in renal size
	↓ in glomerular filtration rate
	↓ tubular secretion
	↓ in tubular reabsorption

↑: Increased; ↓: Reduced.

half-life, prolonged drug effect and accumulation with continued use thus increasing the potential for toxicity and adverse drug events (ADEs)[12].

Most drugs bind to protein (*e.g.,* albumin and α-1 glycoprotein) when circulating in plasma compartments, with only the unbound drug being pharmacologically active. In healthy older people, changes in serum albumin concentrations are minimal. In older people with chronic illnesses and malnutrition, serum albumin concentrations can be significantly reduced, leading to a reduction in bound drug concentrations and higher serum levels of free drug. This affects commonly prescribed drugs such as sodium valproate, warfarin and antipsychotics, thus increasing the potential for drug toxicity and adversity in patients with diminished circulating albumin. This is particularly relevant to frail, older hospitalised patients.

Hepatic mass and perfusion declines with age, thus reducing the liver's capacity for first pass metabolism[13]. Commonly prescribed drugs such as verapamil, amitriptyline and morphine may have higher bioavailability at standard doses in older people, thus leading to greater potential for adverse effects if not dose-adjusted. An example of this includes the risk of first dose hypotension with antihypertensive medications that have a high extraction ratio. This ratio would be reduced in older patients thus leading to greater bioavailability after hepatic extraction and thus greater potential for significant first-dose hypotension, so caution is needed when initiating antihypertensive treatment in an older patient with respect to dose and time of administration.

Table 2 Common cytochrome P450 isoenzyme inhibitors and inducers

Enzyme inhibitors	Enzyme inducers
Amiodarone	Carbamazepine
Allopurinol	Ethanol
Cimetidine	Isoniazid
Citalopram, sertraline	Phenytoin
Ciprofloxacin	Phenobarbital
Diltiazem, verapamil	Rifampcin
Fluxetine, paroxetine	St. Johns Wort
Erythromycin, clarithromycin	
Fluconazole, ketoconazole	
Omeprazole	
Sulphonamides	
Grapefruit Juice	

Another important consideration is the possibility of drugs interacting through inhibition and induction of cytochrome p450 isoenzymes. Commonly encountered enzyme inducers and inhibitors are detailed in Table 2. Enzyme induction may take several weeks to occur and may result in treatment failure in those taking multiple medications, *e.g.,* a patient may fail to respond to "drug A" because "drug B" has induced a cytochrome p450 isoenzyme which metabolizes "drug A".

With ageing, well-documented changes occur in renal size, perfusion and function (see Table 1)[14]. This is of particular relevance to older patients who are prescribed renally excreted drugs where reduced elimination can lead to increased and potentially toxic drug accumulation (Table 3). Glomerular filtration rate (GFR) should be estimated using readily available formulas such as the Cockcroft and Gault[15] and Modification of Diet in Renal Disease[16]. Prescribers should be aware that serum creatinine concentration alone is an unreliable marker of renal function in the elderly owing to reductions in muscle volume. Indeed, approximately 50% of those with normal creatinine levels have a reduced estimate GFR (eGFR)[17].

Pharmacodynamics and ageing

Older people often have significantly different pharmacodynamic responses than their younger counterparts to similar drug concentrations. Differences can be caused by a shift in receptor affinity, density, post-receptor events at the cellular level, or in adaptive homeostatic response mechanisms. Pathologic organ changes may also affect pharmacodynamic responses, particularly in frail older patients[17]. Prescribers should be aware of commonly encountered age-related pharmacodynamic differences as listed in Table 4. Some clinically relevant examples are present in Table 5. Generally, it is recommended to initiate medications at the smallest possible dose and titrate slowly according to response.

Polypharmacy

Polypharmacy is often defined by the number of prescribed medications, with ⩾ 6 drugs being a common

Table 3 Common used drug classes which require dose adjustment with chronic kidney disease

Drug class	Adjust dose in CKD stage 1-3	Avoid in CKD stages 4 and 5
ACE-inhibitors and Angiotensin 2 receptor blockers	All ACE inhibitors	Olmesartan
Diuretics	Potassium-sparing and thiazide diuretics	Potassium-sparing and thiazide diuretics
Beta-blockers	Acebutolol, atenolol, bisoprolol, nadolol, sotalol	Sotalol
Lipid lowering agents	Pravastatin, rosuvastatin, fibrates	Glyburide, metformin, exanitide
Hypoglycaemic agents	Gliclazide, acarbose, insulin, gliptins	
Analgesia (NSAIDs and opioids)	Codeine, tramadol, morphine, oxycodone,	All NSAIDs, pethidine
Psychotropic agents	Lithium, gabapentin, pregabalin, topiramate, vigabatrin, bupropion, duloxetine, paroxetine, venlafaxine	
Miscellaneous	Allopurinol, colchicine, digoxin	Dabigatran Rivaroxaban (CI stage 5, dose adjust in stage 4 CKD) Apixaban (CI stage 5, dose adjust in stage 4)

CKD: Chronic kidney disease; ACE-inhibitors: Angiotensin-converting-enzyme inhibitor; NSAIDs: Non-Steroidal anti-inflammatory drugs; CI: Contraindicated.

Table 4 Age-associated changes in pharmacodynamic response to commonly prescribed drugs

Drug type	Specific drug	Pharmacodynamic response in older people	Potential clinical consequence
Analgesia	Morphine	↑	Excessive sedation, confusion, constipation, respiratory depression
Anticoagulant	Warfarin	↑	Increased bleeding risk
	Dabigatran in those ⩾ 75 yr with a body weight of < 50 kg)		
Cardiovascular system drugs	Angiotensin II receptor blockers	↑	Hypotension
	Diltiazem	↑	
	Enalapril	↑	
	Verapamil	↑	
	Propranolol	↓	
Diuretics	Frusemide	↓	Reduced diuretic effect at standard doses
	Bumetanide	↑	
Psychoactive drugs	Diazepam	↑	Excessive sedation, confusion, postural sway, falls
	Midazolam	↑	
	Temazepam	↑	
	Haloperidol	↑	
	Traizolam	↑	
Others	Levodopamine	↑	Dyskinesia, confusion, hallucinations

↑: Increased pharmacodynamic response; ↓: Reduced pharmacodynamic response.

Table 5 Commonly used drugs - comparison of prescription between older and younger patients

Drug	Typical dose in younger patient (< 65 yr)	Typical dose in older patient (⩾ 65 yr)	Reason for different dose in the elderly
Anti-arrhythmics			
Digoxin	Loading dose is 1-1.5 mg in divided doses over 24 h Maintenance dose 125-250 mcg OD	Loading dose is 1 mg in divided doses over 24 h Maintenance dose 62.5-125 mcg OD	Water soluble contributing to increased plasma levels in the elderly
Anti-coagulants			
Warfarin	Standard initiation dose, *e.g.*, 10 mg daily for two days	Lower initiation dose, *e.g.*, 5 mg daily for two days	Increased sensitivity to anticoagulant effect
Dabigatran	150 mg BD	Patient > 80 yr 110 mg BD Patient 75-80 yr 150 mg BD in setting or normal eGFR	Increased sensitivity to anticoagulant effect
Anti-hypertensive			
Ramipril	Initiation dose 2.5 mg	Initiation dose 1.25 mg	Lower initial dose and gradual dose titration required (higher risk of ADE in the elderly)
Psychoactive drugs			
Diazepam	2 mg TDS	1 mg BD	Lipid soluble with higher volume of distribution in older people thus contributing to a prolonged duration of effect

OD: Once daily; BD: Twice daily; TDS: Three time daily.

Table 6 Important drug interactions in older patients

Drug	Drug	Interaction	Effect
Anti- hypertensive agents	NSAID	NSAID antagonizes hypotensive effect	↓ antihypertensive effect
Aspirin	NSAID, oral corticosteroids	↑ risk of peptic ulceration	Peptic ulceration
Calcium channel blockers	Enzyme inducers	↑ clearance of calcium channel blocker	↓ anti-hypertensive effect
Digoxin	Diuretics	Diuretic-induced hypokalaemia	↑ effect of digoxin (arrhythmia, toxicity)
Digoxin	Amiodarone, Ditiazem, Verapamil	↓ clearance of digoxin	↑ effect of digoxin (arrhythmia, toxicity)
TCA	Enzyme inhibitors	↓ clearance of TCA	Arrhythmia, confusion, orthostatic hypotension, falls
Phenytoin	Enzyme inhibitors	↓ clearance of phenytoin	↑ effect of phenytoin, toxicity
Thyroxine	Enzyme inducers	↑ clearance of thyroxine	↓ effect of thyroxine

NSAID: Non-steroidal anti-inflammatory drug; TCA: Tricyclic anti-depressants.

cut-off point[18]. Another definition of polypharmacy is the prescription of at least one drug without valid clinical indication[19]. Increasing numbers of medications is associated with a higher risk of ADEs with resultant increased frequency of hospitalisation, negative health outcomes and increased healthcare resource utilisation[20-25]. The risk of an adverse drug reaction (ADR) when taking two concurrent medications is 13%[26]. This risk rises to 38% in patients taking 4 medications and to 82% in those taking ⩾ 7 medications[26]. Polypharmacy can often be indicative of prescribing cascades, *i.e.,* where a new drug is used to treat a negative effect of an existing drug. Clearly, prescription of medications in such circumstances is inappropriate.

Prescription of multiple drugs impacts negatively on adherence and compliance. Clinicians are sometimes unaware of their patients complete prescription record perhaps because of multiple prescribers or under-reporting by patients at time of consultation. Frank *et al*[27] reported that almost 4 out of 10 patients were taking drugs unbeknownst to their doctors, and approximately 1 out of 20 patients were not taking medications listed on their prescription record. Prescribers should make every effort to obtain an accurate medication list. Pharmacy reconciliation protocols are useful for this purpose in hospital environments. Tools such as the Structured History of Medications can also be very useful in this regard, though they are time consuming to complete[28].

Drug interactions

One drug can interact with another drug through pharmacokinetic or pharmacodynamic mechanisms. Gurwitz *et al*[29] reported that drug interactions accounted for 13% of preventable prescribing errors. The risk increases with rising numbers of prescribed drugs and with multiple attending prescribers[30]. A study of over sixteen hundred older outpatients across six European countries found that 46% had at an important drug interaction with 1 in 10 having the potential for severe consequence[31]. Table 6 details some commonly encountered and potentially significant drug-drug interactions in older people.

Drugs can often worsen co-existing medical conditions. The risk of drug-disease interactions is higher in older adults who are on multiple medications to treat multiple conditions. Lindblad *et al*[32,33] reported that 15%-40% of hospitalized older adults were prescribed a drug that could potentially exacerbate a co-existing condition, *e.g.,* use of non-dihydropyridine calcium antagonists with heart failure. In the community-dwelling elderly, the prevalence of drug-disease interactions ranges from 6% to 30%[34-37]. Commonly encountered drug-disease interactions, which have the potential for clinically significant negative outcomes in older patients, are presented in Figure 1. Prescription of these medications in these clinical circumstances is potentially inappropriate, particularly if safer alternatives are available.

Appropriate prescribing

So far, we have described circumstances where prescribing decisions in older patients can be considered to be potentially inappropriate, *i.e.,* where the risk of a negative outcome exceeds the potential therapeutic gain. The term "appropriate prescribing" extends well beyond the aforementioned pharmacological principles to encompass a range of actions and attitudes that characterise the quality of prescribing that should be achieved in everyday practice[38] (summarised in Figure 2). This term encompasses several important domains including patient choice, therapeutic expectation, scientific and technical rationalisation and the general good for society[38]. A discussion of pharmacoeconomic rationalisation is beyond the scope of this paper, but it is becoming increasingly important that prescribers are economically just in their decisions so that the greatest number can receive the greatest benefit and that older individuals can be offered the least expensive available therapeutic options.

Inappropriate prescribing (IP) is a commonly used term. It pertains to use of medications that may cause more harm than good and perhaps, more importantly, the under-prescription of clinically indicated medications[38]. IP has been identified in 12%-40% of residents in long-term care facilities and in 14%-23% of community-dwelling older people[39,40]. The association between IP and negative outcomes such as ADRs has been shown in numerous studies in Europe[41,42], the

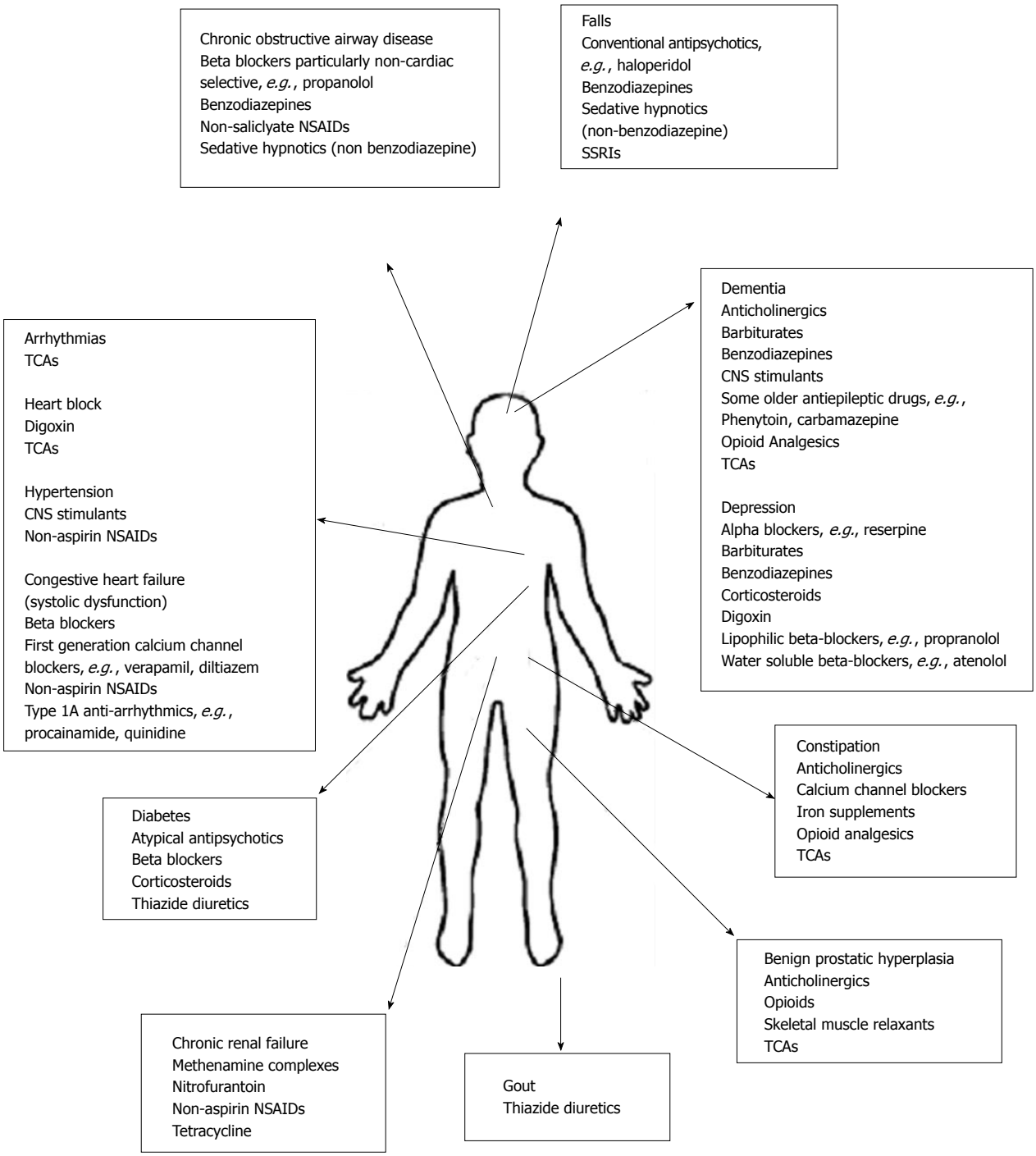

Figure 1 Common encountered clinically significant drug-disease interactions in older patients. The following conditions may be exacerbated by prescription of the *drug classes* listed below.TCA: Tricyclic anti-depressants; NSAID: Non-steroidal anti-inflammatory drug; SSRIs: Selective serotonin reuptake inhibitors.

United States[43-45] and Asia[46].

Clinical judgments of prescribing appropriateness with respect to therapeutic benefit are often difficult to make because of insufficient scientific evidence for the older population. Those with multiple co-morbidities and multiple medications are often poorly represented in clinical trials and physicians often have to extrapolate scientific evidence from the use of medications in younger, unrepresentative patient populations, with fewer illness and fewer concurrent medications. Only 2.1% of patients recruited to trials investigating the efficacy of non-steroidal anti-inflammatory drugs (NSAIDs) were aged 65 years and over, with less than 0.1% over 75 years[47]. Nonetheless, NSAIDs are commonly used to treat musculoskeletal disorders in older patients. It is well established that the risk of adverse events of NSAIDs such as peptic ulcer disease is much higher in older people. Indeed, inappropriate use of NSAIDs is a commonly encountered ADR in elderly inpatients[48], usually through incorrect dose, prolonged duration or failure to recognize impairment of renal function.

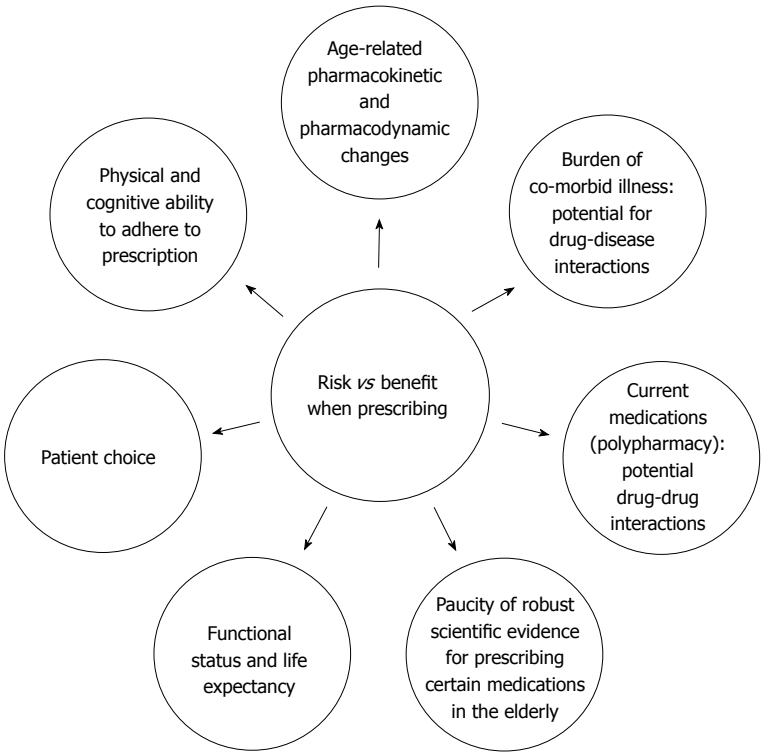

Figure 2 Important considerations when evaluating the quality of prescribing decisions in older people.

Under-prescribing of essential, often preventative medication is perhaps an even bigger concern than misuse of medications in older patients, particularly when the potential outcome of not treating the condition can be catastrophic[49]. The risk of cardio-embolic stroke in those with atrial fibrillation increases with age (1.2% to 2.5% annual risk in persons aged 60-69 years *vs* 7.3%-13.7% annual risk in persons aged 80 years and over)[50-52] but many do not receive evidence-based preventative anticoagulation[53]. The Irish Longitudinal Study on Ageing recently reported that 30% of patients had a potential prescribing omission (PPO), the most common PPO being appropriate anti-hypertensive therapy[54]. Prescribing omissions were twice as common as inappropriate prescriptions[55]. Even greater proportions of hospitalised older patients are reported to have potentially inappropriate prescribing omissions, with Barry *et al*[55] reporting 57% prevalence of prescribing omissions in one prospective study of over 600 hospitalised older patients in Ireland. The elderly have a higher burden of co-morbid illnesses, *e.g.,* a single patient may have hypertension, diabetes mellitus, chronic obstructive airways disease, dementia and recurrent falls. Every effort should be made to appropriately treat all illnesses bearing in mind the principles of appropriate prescribing as previously discussed.

Other considerations

Prescribing appropriateness must also take into account a patient's capacity to comply with the prescription as well as their physical ability to take the medication. In older adults post coronary artery bypass grafting it was found that in-hospital education was paramount in helping patients adhere to their medication regimens[56]. However, it must be acknowledged that almost 25% of patients aged ⩾ 80 years will have significant cognitive deficits and memory deficits can often contribute to improper medication use as patients can have difficulty understanding instructions[57]. Patients may fail to remember to take their medicines or may even take multiple doses concurrently thus placing them at an increased risk of adverse drug events[42]. Prescribers have a responsibility to ensure that medications can be taken safely and reliably. Sometimes this requires simple written instructions, the use of doset boxes or blister-packs, or direct supervision of administration by a carer or relative. Physical impairments such as hearing loss, visual loss and impaired manual dexterity can also impact on adherence to prescribed medications, thus resulting in poor therapeutic yield and consequent negative outcomes.

Clearly, prescribing for older patients is complex and sometimes time-consuming particularly when all of the aforementioned variables are considered. In addition, older patients are a heterogeneous group, with wide variation in physical, cognitive and functional status. The most important clinical question when deciding on prescribing appropriateness is whether or not there is a clear clinical indication for the treatment. This requires a clear diagnosis and a clear expectation of the therapeutic goal. Evaluation of the therapeutic goal must take into account the scientific rational of using a drug as well as the potential benefit to improving the

Table 7 Key considerations when prescribing for older patients

Use non-pharmacological treatment whenever possible
Include the patient (and carer where appropriate) in prescribing decisions
Ensure each medication has an appropriate indication and a clear therapeutic goal (this involves careful clinical assessment and appreciation of time to obtain treatment effect and life expectancy)
Start at the smallest dose and titrate slowly according to response and efficacy
Use the simplest dosing regimen (*e.g.*, once a day preferable to three times per day) and most appropriate formulation
Provide verbal and written instructions on indication, time and route of administration and potential adverse effects of each medication
Regularly review prescriptions in the context of co-exiting disease states, concurrent medications, functional and cognitive status and therapeutic expectation
Be aware that new presenting symptoms may be due to an existing medication, drug-drug interaction or drug-disease interaction (avoid prescribing cascade)
When stopping a medication check that it can be stopped abruptly or whether it needs to be tapered, *e.g.*, long-term steroids, benzodiazepines

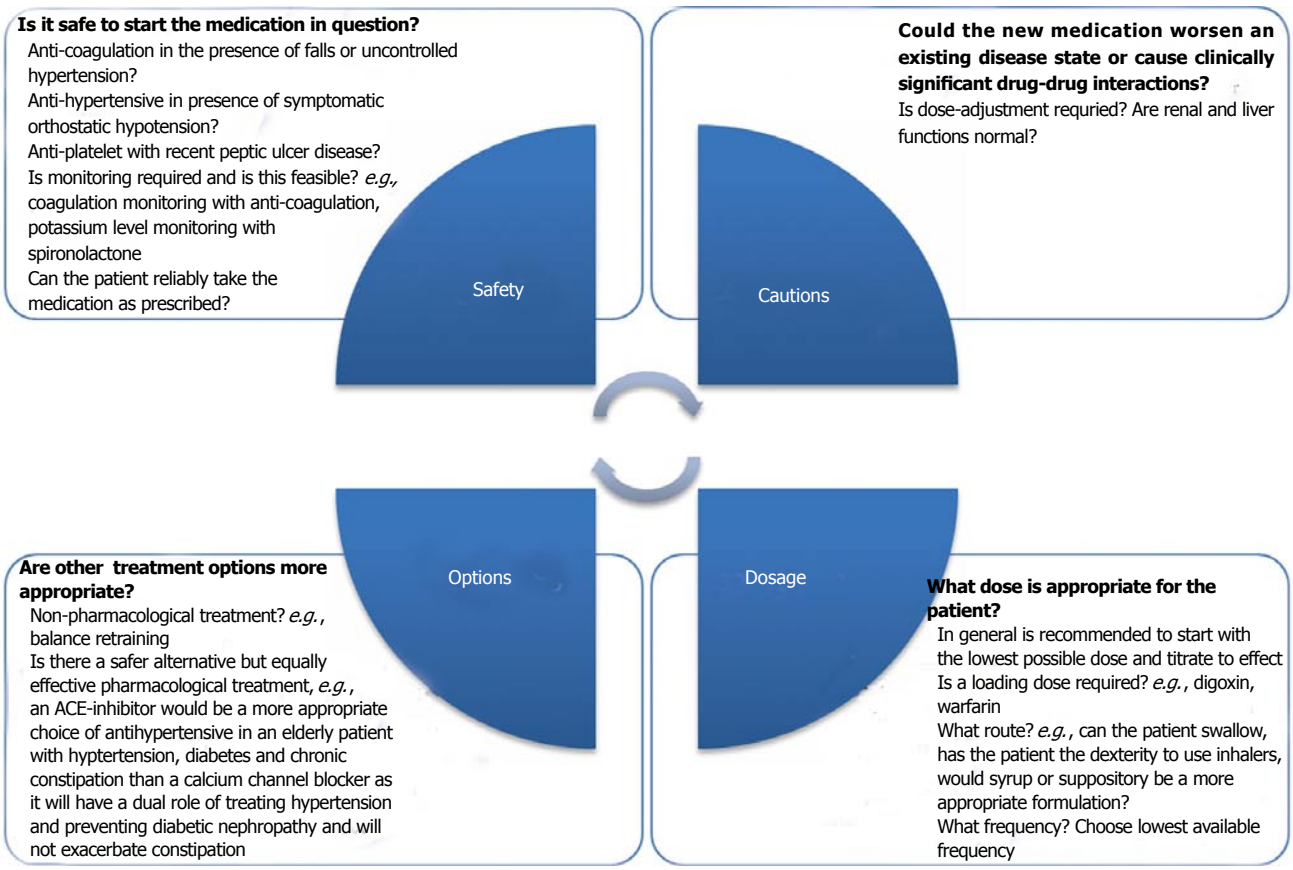

Figure 3 Influential factors when prescribing for the elderly with some examples.

condition. Prescribers must ensure that people take the appropriate medicine at the correct dose; thereby minimizing risks of adversity (see Table 7).

A case history, displayed in Table 8, illustrates the complexities of making appropriate prescribing decisions in older people and also some of the negative clinical consequences of IP decisions. Other examples of important considerations with respect to prescribing safety, cautions, dosage and therapeutic options are presented in Figure 3.

ADVERSE DRUG EVENTS AND ADRS

An adverse drug event (ADE) is defined as "any injury resulting from the use of a drug"[58]. This broad definition encompasses any harm caused directly by the medication and any event that occurs during its use (including dose reductions and harm from discontinuation of the drug). An ADR is defined as a "response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of disease or for the modification of physiologic function"[58,59].

Unsurprisingly, ADEs are highly prevalent in the elderly. Those with multiple co-morbidities and who are prescribed multiple medications are at the highest risk. It is widely accepted that the crude prevalence rate of ADEs in community-dwelling older people is approximately 30%[60]. One study reported that ADEs accounted for 1 in 10 of all emergency

Table 8 Clinical example

An 80-year-old lady is referred with a four day history of general malaise, nausea, vomiting and recurrent falls. Her past medical history includes paroxysmal atrial fibrillation, non-obstructive coronary artery disease, hypertension, recurrent episodes of acute gout, dependent lower limb edema and "vertigo/dizziness". Prior to this episode she was functionally independent and had normal cognition

Her medications were as follows: Simvastatin 40 mg daily; Verapamil 240 mg daily; Quinine Sulphate 300 mg daily, Perindopril 5 mg/Indapamide 1.5 mg daily; Digoxin 250 mcg daily; Diclofenac 75 mg twice daily; Frusemide 40 mg daily; Betahistine 16 g three times per day; Paracetamol 1 g as required; Warfarin as per INR (target INR 2-3); Flurazepam 30 mg nocte. She was not taking OTC medications

On assessment she was pale and tired. Supine blood pressure was 122/70 mmHg; erect blood pressure after one minute was 92/62 mmHg

Pulse was 52 beats per minute. She had no clinical signs of congestive cardiac failure. She scored 9/10 on a short mental test score

Investigations showed a eGFR of 38 mL/min, serum potassium 2.8 mmol/L (low) and serum sodium 126 mmol/L (low). Haemoglobin was 10.2 g/dL with MCV 72fl (hypochromic microcytic anemia)

When evaluating the appropriateness of an older person's prescription medications it is important to consider the following two questions:

1 Is there a clinical indication for the drug?

2 Could the drug be contributing to the presenting symptoms?

Using this approach each medication should be evaluated in turn and corrective action implemented

Medication	Clinical indication?	Contributing to presenting symptoms?	Action taken?
Simvastatin 40 mg	Yes (hyperlipidaemia, high cardiovascular risk)	Could cause muscle cramps and myopathy which could lead to falls (note patient prescribed quinine)	Check fasting lipid profile and creatine phosphokinase. Revise dose according to target lipid levels
Verapamil 240 mg	Yes (hypertension, arrhythmia)	Could cause hypotension and bradycardia. Increased risk of myopathy when prescribed with simvastatin	Consider discontinuation. Beta-blocker may be more appropriate choice as rate controlling agent
Quinine 300 mg	No clear indication	No	Muscle cramps may be due to statin. Review choice of statin. Discontinue Quinine
Perindopril 5 mg	Yes (hypertension)	Could contribute to postural hypotension and acute renal injury	Consider temporary withdrawal while investigating cause of renal dysfunction
Indapamide 1.5 mg	Yes (hypertension)	Could contribute to postural hypotension, acute renal injury, hyponatraemia and hypokalaemia. Can precipitate digoxin toxicity, hyperuricaemia and recurrent episodes of gout	Discontinue
Digoxin 250 mcg	Yes (atrial fibrillation)	Symptoms of digoxin toxicity. Dose too high given level of renal dysfunction	Discontinue. Beta-blocker may be more appropriate choice of rate controlling agent
Diclofenac 75 mg	Yes (acute gout)	Yes. Diclofenac may be causing renal impairment. Gastritis/peptic ulcer disease should also be considered because of nausea, vomiting and microcytic anemia. NSAIDs should not be prescribed with warfarin because of significantly increased risk of bleeding	Discontinue. Consider addition of allopurinol for gout prophylaxis
Frusemide 40 mg	Yes (hypertension)	Yes (hypotension, hyponatraemia, hypokalaemia, renal impairment)	Frusemide is not required as an anti-hypertensive in this patient. It has been prescribed to treat dependent lower limb edema. Leg elevation and compression stockings would be more appropriate
Betahistine 16 mg	No (prescribed for dizziness which is actually related to orthostatic hypotension)	No	Discontinue. No indication
Paracetamol 1 g	Yes (pain)	No	Continue
Warfarin	Yes (atrial fibrillation embolic prophylaxis)	May be contributing to anemia. Should not be co-prescribed with diclofenac as there is an increased risk of bleeding	Investigate cause of anemia. Consider future suitability for anticoagulation if high falls risk persists
Flurazepam 30 mg	No	Yes (falls, malaise)	Contact GP and pharmacy for prescription history. Do not suddenly discontinue because of risk of benzodiazepine withdrawal

INR: International normalized ratio; OTC: Over-the-counter; eGFR: Estimated glomerular filtration rate; MCV: Mean corpuscular volume.

department attendances in those aged ⩾ 65 years[61]. Approximately one third of those with an ADE had a potential drug interaction. The most common offending medications were NSAIDs, antibiotics, anticoagulants, diuretics, hypoglycemic agents, β-blockers, calcium-channel blockers, and chemotherapeutic agents[61]. ADEs are common in hospitalized older patients, with prevalence rates of up to 25% being reported in some studies[44,62-64]. Most ADEs are predictable with 27% of ADEs in community-dwelling older patients[30] and 42% of ADEs in nursing home facilities thought to be avoidable[63].

One large study of over 18000 hospital admissions found that ADRs were responsible for 1 in 16 hospitalisations (6.5%), 4% of hospital bed capacity and 0.15% of deaths[65]. In the United States, it has been

reported that ADRs are amongst the leading causes of death[59]. The majority of ADRs (> 80%) in older people are predictable in that they are related to the known pharmacological effect of the drug and often escalate with increasing dose[66].

PRESCRIBING APPROPRIATENESS CRITERIA

With changing demographics and ever-increasing availability of therapeutic agents, the frequency of IP in older patients is not abating. Various strategies to identify, measure and reduce potentially inappropriate prescribing have been the focus of worldwide research endeavors over the last thirty years. A detailed analysis of all such endeavors is beyond the scope of this paper. Instead we will focus on the principal prescribing appropriateness criteria, their relationship to adverse healthcare outcomes and the evidence to support their role in optimising prescribing appropriateness.

Explicit criteria for appropriate prescribing comprise lists of medications that are known to cause harm in older adults; either through predictable pharmacological or predictable physiological mechanisms. In general, they have been developed from expert consensus techniques[67]. Explicit criteria can often be utilised in the absence of detailed clinical data[68]. However, this may also be a limitation, particularly in older patients, where clinical detail is an essential requirement for any treatment decision, particularly in relation to burden of co-morbidity[69], patient preference and consideration of previously unsuccessful treatment approaches. Furthermore, explicit criteria need regular updating so as to incorporate emerging evidence.

Beers criteria focus principally on over-prescribing and mis-prescribing. They comprise a list drugs that are inappropriate to prescribe for the elderly under any circumstances and a list of drugs that should be avoided with particular clinical illnesses and syndromes[70-73]. Screening Tool of Older Person's potentially inappropriate Prescriptions (STOPP)/Screening Tool to Alert to Right Treatment (START) Criteria are organised according to physiological system and include criteria that highlight when medications should be considered in older people, with certain conditions, where no contraindication exists, *e.g.,* anticoagulation in patients with atrial fibrillation and calcium and vitamin D supplementation in patients with osteoporosis[74,75]. Table 9 summarises the principal explicit prescribing criteria, their advantages and disadvantages.

Implicit criteria focus on several domains of prescribing appropriateness. The medication appropriateness index (MAI) is the most widely cited implicit tool which measures prescribing appropriateness according to 10 criteria including indication, effectiveness, dose, administration, drug-drug and drug-disease interactions and cost[76,77]. Clinical expertise and detailed clinical and pharmaceutical information is required to apply some of the criteria, thus making this tool time consuming to use in everyday clinical practice. The MAI does not address prescribing omissions. Three of the MAI criteria (indication, effectiveness and duplication) can be combined as a measure of unnecessary polypharmacy, one study of 384 frail older patients at the point of hospital discharge showing that 44% were prescribed at least one unnecessary drug, the most common drug classes being gastrointestinal, central nervous system and therapeutic nutrients or minerals[78]. Another study of 397 frail elderly inpatients showed that 365 patients (92%) met at least one MAI criterion, the most common problems being use of the most expensive drugs (70%), impractical directions (55%), and incorrect dosages (51%)[79]. One advantage of the MAI is that it encompasses elements for drug prescribing that are applicable to any medication and to any clinical condition in any clinical setting. The Assessment of Underutilisation (AOU) of Medications tool is based on an instrument reported by Lipton *et al*[80] and simply requires the user to match the patient's active illnesses to his/her prescription drugs thus establishing if a condition is under-treated by omission of an indicated medication. One study showed that 64% of older patients had evidence of under prescribing according to the AOU instrument[81]. The labeling of a prescription as "potentially inappropriate" implies that the prescription in question should be predictive of an adverse outcome. Ideally, the drugs highlighted by explicit IP criteria should be associated with preventable ADEs. Prospective use of IP screening criteria should, theoretically curtail the occurrence of ADEs.

The reported prevalence rates of potentially inappropriate prescribing according to various explicit criteria range from 24% to 44% depending on the populations and proportions of criteria applied[82]. The reported associations between IP and adverse outcomes also vary. Pasina *et al*[83] showed the prevalence of at least one PIM was 20.1% and 20.3% according to the 2003 and 2012 iterations of Beers' criteria respectively. However an association between IP and health outcomes was not demonstrated. Conversely, medications listed in STOPP[48] criteria have been associated with a higher proportion of patients requiring admission to hospital because of IP-related adverse events than those listed in Beers' criteria (11.5% *vs* 6%, respectively). A recently published randomised controlled trial of 400 older hospitalized patients showed that unnecessary polypharmacy, incorrect dosing, and potential drug-drug and drug-disease interactions were significantly lower at time of discharge and for up to 6 mo post discharge when patients were screened with STOPP/START criteria within 72 h of hospitalization (absolute risk reduction 37.5%, number needed to screen to yield improvement in MAI = 2.8)[75]. Reduction of underutilisation of clinically indicated medications was also observed (absolute risk reduction 21.2% with a number need to yield reduction of 4.7). However, a recent systematic review of the application

Table 9 Explicit criteria for potentially inappropriate prescribing in older patients

Explicit criteria	Advantages	Disadvantages
Beers criteria[70]	Assesses prescribing quality Useful for education	Several drugs unavailable outside United States Does not include underuse of drugs, drug-drug interactions or duplicate drugs No under-prescribing indicators
Beers criteria[71]	Concise explanation of inappropriateness Severity ratings of adverse outcomes Assesses prescribing quality Useful for education	Several drugs unavailable outside United States Does not include underuse of drugs, drug-drug interactions or duplicate drugs No under-prescribing indicators
Beers criteria[72]	Concise explanation of inappropriateness Severity ratings of adverse outcomes Can be used by computerized clinical information systems	Several drugs unavailable outside the United States Controversy over some drugs labeled as inappropriate No drug to drug interaction No drug disease interactions No under prescribing
Beers criteria[73]	Concise explanation of inappropriateness Structured according to therapeutic classes and organ systems Drug disease interactions	Several drugs unavailable outside United States No drug-drug interaction No under prescribing
STOPP/START[74]	Organised by physiological system Concise list on inappropriate medications Includes drug and disease interactions, therapeutic duplications and prescribing omissions	Does not suggest safer alternatives Does not address certain domains of prescribing, *e.g.*, indication
McLeod criteria[113]	Concise list of inappropriate medications with safer alternatives suggested Useful for education	Obsolete indicators, *e.g.*, beta blockers in heart failure No under-prescribing indicators Several drugs unavailable outside United States
IPET 2000 (Improved prescribing in the elderly tool)[114]	Concise Useful for education	Not comprehensive Predominantly cardiovascular and psychotropic drugs No under-prescribing indicators
Zhans criteria[115]	Less restrictive than previous criteria	Several drugs unavailable outside United States No drug to drug interaction No drug disease interactions No under-prescribing indicators
French Consensus Panel List[116]	Concise explanation of inappropriateness Includes drug duplications Safer alternatives suggested	No clinical studies to date No under prescribing
Rancourt[117]	26 Drug drug interactions 10 drug duplications	Large number of criteria to get through in clinical practice Data only on long term care setting
Australian Prescribing Indicators Tool[118]	Includes drug duplication Includes under-prescribing	Not validated and time consuming Derived from Australian data sources limiting international applicability
Norwegian General Practice (NORGEP) Criteria[119]	Can be applied to medication list with no clinical information	No drug prescribing No drug-disease interactions No studies to date outside Norway
Priscus List[120]	Provides therapeutic alternatives Recommendations on dose adjusting and monitoring	No studies to date published outside Germany
Thailand Criteria[121]	Drug interactions Drug disease interactions	No studies to date outside country of origin

of STOPP/START criteria concluded that there was limited evidence found in relation to the clinical and economic impact of the STOPP/START criteria. This is the subject of ongoing research endeavors as described below.

All prescribing appropriateness criteria are designed to assist decision-making and not to substitute good clinical decision-making. However, for prescribing appropriateness criteria to continue to facilitate decision-making they will need to remain clinically valid *via* regular updates in tandem with evolving clinical evidence and new medications. No criteria exist specifically for guidance of prescribing in frail older long term care residents with reduced life expectancy and indeed this cohort is likely to increase with changing demographics and prolonged survival[66].

OTHER APPROACHES TO OPTIMIZING PRESCRIBING APPROPRIATENESS IN OLDER PATIENTS

Comprehensive geriatric assessment

Geriatric medicine multidisciplinary teams comprise doctors, nurses, pharmacists and other allied health professionals who offer detailed assessment of older patients' physical, cognitive and functional abilities as well as optimization of medications. Several trials have shown improvements in all domains of prescribing appropriateness following comprehensive geriatric assessment (CGA). Schmader *et al*[84] demonstrated a significant reduction in the prevalence of potentially inappropriate prescribing, including under-prescribing,

in older inpatients that were randomised to receive CGA when compared to routine inpatient care. In the same study, outpatients who received CGA were shown to have a 35% reduction in the risk of a serious ADEs and prescribing omissions when compared with standard care[84].

Saltvedt *et al*[85] reported a lower prevalence of anticholinergic drug use and potential drug interactions at hospital discharge in acutely ill elderly patients who were randomized to receive inpatient CGA compared with standard hospital care. In addition, antipsychotic drugs were more likely to be withdrawn in the intervention cohort. An Australian study of 154 long term care residents with challenging behavior showed that an intervention comprising two case conferences between a care of the elderly physician, general practitioner, pharmacist and nursing home staff resulted in significant improvements in the prevalence of IP, particularly with respect to the use of benzodiazepines[86]. A Finnish study of 400 patients with cardiovascular disease showed a significant improvement in the use of evidence-based cardiovascular medications following geriatrician review with subsequent improvement in risk factor profile, but no improvement in three year cardiovascular morbidity or mortality[87].

CGA affords a complete overview of an older patient's health status and functional abilities and enables the prescriber to make informed prescribing decisions in the context of such variables. However, comprehensive geriatric assessment is time-consuming and resource intensive and is, in reality, only applicable to patients attending hospital, either as an inpatient or as an outpatient. It is not feasible in most health services for all older patients to undergo comprehensive geriatric assessment, thereby limiting the value of this approach at the population level.

Clinical pharmacy intervention

Clinical pharmacists perform systematic assessments of a patients' medication regimen and generate pharmaceutical care plans with the aim of optimizing the clinical impact of treatment, minimizing adverse effects of treatment and reducing waste[88]. An intervention comprising detailed review of medications by a clinical pharmacist with subsequent recommendations for the attending physician including patient counseling showed significant improvement in MAI scores over a twelve month period when compared to usual outpatient care[89]. However, there were no improvements in other outcomes including ADEs and healthcare use. Similarly, Crotty *et al*[90] reported improvements in MAI scores and a lower hospital re-admission rate in older patients whose medications were reviewed and discussed in detail by doctors and pharmacists. However, significant reductions in ADEs and other adverse outcomes were not identified. In Belgium, one hospital-based study has shown that a combined pharmacy and geriatrician intervention improves prescribing appropriateness[91].

Similar to CGA, specialist pharmacy input is resource intensive and is, in reality, confined to patients attending the hospital. Not all pharmacists have specialist training in geriatric pharmacotherapy and the success of this intervention depends upon the availability of the medical record to the pharmacist as well as the acceptance of the pharmaceutical care plan by both the patient and the prescribing physician. Therefore, clinical pharmacists need to work in close liaison with prescribers. The impact of the community pharmacist with no specialist training in geriatric pharmacotherapy on prescribing appropriateness has not been studied.

Prescriber education, audit and feedback

Several studies have shown that most physicians receive inadequate training in geriatric pharmacotherapy at an undergraduate and postgraduate level[92-94]. Therefore, educational strategies targeted specifically at those who prescribe for older patients would appear to be highly relevant. Numerous studies have investigated the impact of different educational approaches on the quality of prescribing in older patients, with mixed results. In general, interactive approaches with direct feedback that target multiple disciplines[53,95,96] are more effective than passive approaches involving didactic lectures and written dissemination of educational and feedback material[97,98]. However, most of these studies pertain to specific drugs or drug classes, *e.g.*, antibiotics[99], psychotropic drugs[100,101] analgesics[101] or avoidance of potentially inappropriate anticholinergic drugs[95]. The effect of educational interventions on broader measures of prescribing appropriateness and on health-related outcomes remains to be seen.

A recent systematic review investigated whether education interventions improved prescribing by undergraduate students and postgraduate junior physicians. No definitive answer was found. The trials included were small and flawed in their methodology. The better quality studies used the World Health Organization guide that directs students through a six-step problem-solving process when prescribing. Improvement in prescribing skills has been demonstrated in simulated environments. However, further research is required into the long-term benefits of such educational interventions[102].

Electronic prescribing and computerized alerts

Electronic prescribing systems provide user-guidance in relation to medication selection, dosage, price, potential interactions and need for monitoring[103,104]. They have the added potential of reducing prescribing errors of transcription when transferring between places of care, *e.g.,* from hospital to community, or from community to nursing home thereby improving communication[105]. Though challenging and costly to install, these tools can be applied at the point of medication initiation with great potential to minimize ADEs[106].

Existing electronic prescribing systems have been developed for the general adult population and are not specifically refined for elderly patients with complex co-morbidities and altered pharmacokinetics

and pharmacodynamics. Therefore, existing tools may not be suitable for use in older patients. Furthermore, physicians often over-ride the therapeutic flags generated by computerised systems[107] perhaps because many of them are perceived as being falsely positive or clinically unimportant, *e.g.*, a sodium level only marginally below the laboratory reference range may be acceptable in clinical practice. If physicians are overloaded with computerised alerts, they are unlikely to respond to true high-risk safety situations. A disadvantage of computerised prescribing systems is that they are dependent on the quality of the computer programming. There have been reports that computerized decision support systems have themselves resulted in medication errors and related adverse drug events[107-109]. Therefore, computerized decision support systems should be used to enhance a prescribing decision or to flag a potentially inappropriate prescription but can never substitute a comprehensive clinical assessment.

Several exiting research projects are currently underway in Europe[110,111] and the United States[112], the aim being to develop software engines to optimize prescribing appropriateness and to investigate the clinical and economic impacts of their utilisation. A new Software ENgine for the Assessment and optimization of drug and non-drug Therapy in Older persons (SENATOR) trial is presently recruiting throughout seven European centres (http://www.senator-project.eu/). It will assess and optimise drug and non-drug therapy in older persons with multimorbidity and provide recommendations to the attending clinician. The software engine aims to simultaneously reduce inappropriate prescribing, ADRs, and costs alongside optimising medications.

CONCLUSION

Prescribing for older patients presents many unique challenges. Prescribers must be aware of the key pharmacological differences in older people and the principal domains of prescribing appropriateness as described in this paper. Criteria are available to assist prescribers in appropriate decision making, but cannot replace good clinical judgment and cannot be applied in a "one size fits all" manner. Data are limited as to the health-outcome and economic effects of prescribing appropriateness criteria, but important research is ongoing into these areas. Continuous prescriber education at undergraduate and postgraduate level and regular audit of prescribing practice is very important. CGA and clinical pharmacist input are clearly of benefit in optimizing prescribing appropriateness, particularly in hospitalised older patients. However, these interventions are resource intensive. Exciting research into computerized prescribing supports for older people is ongoing. Finally, more older patients with complex co-morbidities should participate in clinical trials to ensure that evidence-based practice and guideline development is based on the testing and use of drugs in representative populations.

REFERENCES

1 **United Nations**. World Population Ageing 2013. Available from: URL: http://www.un.org/en/development/desa/population/publications/pdf/ageing/WorldPopulationAgeing2013.pdf

2 **Chrischilles EA**, Foley DJ, Wallace RB, Lemke JH, Semla TP, Hanlon JT, Glynn RJ, Ostfeld AM, Guralnik JM. Use of medications by persons 65 and over: data from the established populations for epidemiologic studies of the elderly. *J Gerontol* 1992; **47**: M137-M144 [PMID: 1512428 DOI: 10.1093/geronj/47.5.M137]

3 **National Centre for Health Statistics.** Health, United States, 2013: With Special Feature on Prescription Drugs. Hyattsville (MD): National Center for Health Statistics (US), 2014 [PMID: 24967476]

4 **O'Connor K**, O'Mahony D. Drugs and Ageing. In: Liston R, Mulkerrin EC, editors. Medicine for older patients: cases and practice. Dublin, Ireland: Eireann Healthcare Publications, 2003: 205

5 **Wynne HA**, Blagburn J. Drug treatment in an ageing population: practical implications. *Maturitas* 2010; **66**: 246-250 [PMID: 20399044 DOI: 10.1016/j.maturitas.2010.03.004]

6 **Richardson K**, Moore P, Pekdar J, Galvin R, Bennett K, Kenny RA. The Irish Longitudinal Study on Ageing. Polypharmacy in adults over 50 in Ireland: Opportunities for cost saving and improved healthcare [accessed 2012 Dec]. Available from: URL: http://tilda.tcd.ie/assets/pdf/PolypharmacyReport.pdf

7 **Qato DM**, Alexander GC, Conti RM, Johnson M, Schumm P, Lindau ST. Use of prescription and over-the-counter medications and dietary supplements among older adults in the United States. *JAMA* 2008; **300**: 2867-2878 [PMID: 19109115 DOI: 10.1001/jama.2008.892]

8 **Hanlon JT**, Fillenbaum GG, Ruby CM, Gray S, Bohannon A. Epidemiology of over-the-counter drug use in community dwelling elderly: United States perspective. *Drugs Aging* 2001; **18**: 123-131 [PMID: 11346126 DOI: 10.2165/00002512-200118020-00005]

9 **Cheung CK**, Wyman JF, Halcon LL. Use of complementary and alternative therapies in community-dwelling older adults. *J Altern Complement Med* 2007; **13**: 997-1006 [PMID: 18047447 DOI: 10.1089/acm.2007.0527]

10 **Wu CH**, Wang CC, Kennedy J. Changes in herb and dietary supplement use in the U.S. adult population: a comparison of the 2002 and 2007 National Health Interview Surveys. *Clin Ther* 2011; **33**: 1749-1758 [PMID: 22030445 DOI: 10.1016/j.clinthera.2011.09.024]

11 **Meyer BR**. Clinical pharmacology and ageing. In: Evans JG, Williams TF, Beattie BL, Michael JP, Wilcock G, editors. Oxford textbook of geriatric medicine. 2nd ed. Oxford: Oxford University Press, 2003: 127-136

12 **Resnick NM**. Geriatric medicine. In: Fouci AS, Braunwald E, Isselbacher KJ, editors. Harrison's principles of internal medicine, vol 1. 14th ed. New York: McGraw-Hill Companies Inc, 1998: 37-46

13 **Woodhouse KW**, Wynne HA. Age-related changes in liver size and hepatic blood flow. The influence on drug metabolism in the elderly. *Clin Pharmacokinet* 1988; **15**: 287-294 [PMID: 3203484 DOI: 10.2165/00003088-198815050-00002]

14 **Mangoni AA**, Jackson SH. Age-related changes in pharmacokinetics and pharmacodynamics: basic principles and practical applications. *Br J Clin Pharmacol* 2004; **57**: 6-14 [PMID: 14678335 DOI: 10.1046/j.1365-2125.2003.02007.x]

15 **Cockcroft DW**, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; **16**: 31-41 [PMID: 1244564 DOI: 10.1159/000180580]

16 **Levey AS**, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999; **130**: 461-470

[PMID: 10075613 DOI: 10.7326/0003-4819-130-6-199903160-00002]

17 **Corsonello A**, Pedone C, Corica F, Mussi C, Carbonin P, Antonelli Incalzi R. Concealed renal insufficiency and adverse drug reactions in elderly hospitalized patients. *Arch Intern Med* 2005; **165**: 790-795 [PMID: 15824299 DOI: 10.1001/archinte.165.7.790]

18 **Bushardt RL**, Massey EB, Simpson TW, Ariail JC, Simpson KN. Polypharmacy: misleading, but manageable. *Clin Interv Aging* 2008; **3**: 383-389 [PMID: 18686760 DOI: 10.2147/CIA.S2468]

19 **Hanlon JT**, Schmader KE, Ruby CM, Weinberger M. Suboptimal prescribing in older inpatients and outpatients. *J Am Geriatr Soc* 2001; **49**: 200-209 [PMID: 11207875 DOI: 10.1046/j.1532-5415.2001.49042.x]

20 **Holbrook AM**, Pereira JA, Labiris R, McDonald H, Douketis JD, Crowther M, Wells PS. Systematic overview of warfarin and its drug and food interactions. *Arch Intern Med* 2005; **165**: 1095-1106 [PMID: 15911722 DOI: 10.1001/archinte.165.10.1095]

21 **Juurlink DN**, Mamdani MM, Kopp A, Rochon PA, Shulman KI, Redelmeier DA. Drug-induced lithium toxicity in the elderly: a population-based study. *J Am Geriatr Soc* 2004; **52**: 794-798 [PMID: 15086664 DOI: 10.1111/j.1532-5415.2004.52221.x]

22 **Shorr RI**, Ray WA, Daugherty JR, Griffin MR. Concurrent use of nonsteroidal anti-inflammatory drugs and oral anticoagulants places elderly persons at high risk for hemorrhagic peptic ulcer disease. *Arch Intern Med* 1993; **153**: 1665-1670 [PMID: 8333804 DOI: 10.1001/archinte.1993.00410140047006]

23 **Battistella M**, Mamdami MM, Juurlink DN, Rabeneck L, Laupacis A. Risk of upper gastrointestinal hemorrhage in warfarin users treated with nonselective NSAIDs or COX-2 inhibitors. *Arch Intern Med* 2005; **165**: 189-192 [PMID: 15668365 DOI: 10.1001/archinte.165.2.189]

24 **Juurlink DN**, Mamdani M, Kopp A, Laupacis A, Redelmeier DA. Drug-drug interactions among elderly patients hospitalized for drug toxicity. *JAMA* 2003; **289**: 1652-1658 [PMID: 12672733 DOI: 10.1001/jama.289.13.1652]

25 **Onder G**, Pedone C, Landi F, Cesari M, Della Vedova C, Bernabei R, Gambassi G. Adverse drug reactions as cause of hospital admissions: results from the Italian Group of Pharmacoepidemiology in the Elderly (GIFA). *J Am Geriatr Soc* 2002; **50**: 1962-1968 [PMID: 12473007 DOI: 10.1046/j.1532-5415.2002.50607.x]

26 **Goldberg RM**, Mabee J, Chan L, Wong S. Drug-drug and drug-disease interactions in the ED: analysis of a high-risk population. *Am J Emerg Med* 1996; **14**: 447-450 [PMID: 8765105 DOI: 10.1016/S0735-6757(96)90147-3]

27 **Frank C**, Godwin M, Verma S, Kelly A, Birenbaum A, Seguin R, Anderson J. What drugs are our frail elderly patients taking? Do drugs they take or fail to take put them at increased risk of interactions and inappropriate medication use? *Can Fam Physician* 2001; **47**: 1198-1204 [PMID: 11421047]

28 **Prins MC**, Drenth-van Maanen AC, Kok RM, Jansen PA. Use of a structured medication history to establish medication use at admission to an old age psychiatric clinic: a prospective observational study. *CNS Drugs* 2013; **27**: 963-969 [PMID: 23959814 DOI: 10.1007/s40263-013-0103-9]

29 **Gurwitz JH**, Field TS, Harrold LR, Rothschild J, Debellis K, Seger AC, Cadoret C, Fish LS, Garber L, Kelleher M, Bates DW. Incidence and preventability of adverse drug events among older persons in the ambulatory setting. *JAMA* 2003; **289**: 1107-1116 [PMID: 12622580 DOI: 10.1001/jama.289.9.1107]

30 **Tamblyn RM**, McLeod PJ, Abrahamowicz M, Laprise R. Do too many cooks spoil the broth? Multiple physician involvement in medical management of elderly patients and potentially inappropriate drug combinations. *CMAJ* 1996; **154**: 1177-1184 [PMID: 8612253]

31 **Beard K**. Adverse reactions as a cause of hospital admission in the aged. *Drugs Aging* 1992; **2**: 356-367 [PMID: 1504448 DOI: 10.2165/00002512-199202040-00008]

32 **Lindblad CI**, Hanlon JT, Gross CR, Sloane RJ, Pieper CF, Hajjar ER, Ruby CM, Schmader KE. Clinically important drug-disease interactions and their prevalence in older adults. *Clin Ther* 2006; **28**: 1133-1143 [PMID: 16982290 DOI: 10.1016/j.clinthera.2006.08.006]

33 **Lindblad CI**, Artz MB, Pieper CF, Sloane RJ, Hajjar ER, Ruby CM, Schmader KE, Hanlon JT. Potential drug-disease interactions in frail, hospitalized elderly veterans. *Ann Pharmacother* 2005; **39**: 412-417 [PMID: 15687479 DOI: 10.1345.aph.1E467]

34 **Chin MH**, Wang LC, Jin L, Mulliken R, Walter J, Hayley DC, Karrison TG, Nerney MP, Miller A, Friedmann PD. Appropriateness of medication selection for older persons in an urban academic emergency department. *Acad Emerg Med* 1999; **6**: 1232-1242 [PMID: 10609925]

35 **Giron MS**, Wang HX, Bernsten C, Thorslund M, Winblad B, Fastbom J. The appropriateness of drug use in an older nondemented and demented population. *J Am Geriatr Soc* 2001; **49**: 277-283 [PMID: 11300238 DOI: 10.1046/j.1532-5415-2001.4930277.x]

36 **Gosney M**, Tallis R. Prescription of contraindicated and interacting drugs in elderly patients admitted to hospital. *Lancet* 1984; **2**: 564-567 [PMID: 6147611 DOI: 10.1016/S0140-6736(84)90775-X]

37 **Hanlon JT**, Schmader KE, Boult C, Artz MB, Gross CR, Fillenbaum GG, Ruby CM, Garrard J. Use of inappropriate prescription drugs by older people. *J Am Geriatr Soc* 2002; **50**: 26-34 [PMID: 12028243 DOI: 10.1046/j.1532-5415.2002.50004.x]

38 **Spinewine A**, Schmader KE, Barber N, Hughes C, Lapane KL, Swine C, Hanlon JT. Appropriate prescribing in elderly people: how well can it be measured and optimised? *Lancet* 2007; **370**: 173-184 [PMID: 17630041 DOI: 10.1016/S0140-6736(07)61091-5]

39 **Willcox SM**, Himmelstein DU, Woolhandler S. Inappropriate drug prescribing for the community-dwelling elderly. *JAMA* 1994; **272**: 292-296 [PMID: 8028142 DOI: 10.1001/jama.272.4.292]

40 **Ennis KJ**, Reichard RA. Maximizing drug compliance in the elderly. Tips for staying on top of your patients' medication use. *Postgrad Med* 1997; **102**: 211-213, 218, 223-224 [PMID: 9300029 DOI: 10.3810/pgm.1997.09.323]

41 **Hamilton H**, Gallagher P, Ryan C, Byrne S, O'Mahony D. Potentially inappropriate medications defined by STOPP criteria and the risk of adverse drug events in older hospitalized patients. *Arch Intern Med* 2011; **171**: 1013-1019 [PMID: 21670370 DOI: 10.1001/archinternmed.2011.215]

42 **Lindley CM**, Tully MP, Paramsothy V, Tallis RC. Inappropriate medication is a major cause of adverse drug reactions in elderly patients. *Age Ageing* 1992; **21**: 294-300 [PMID: 1514459 DOI: 10.1093/ageing/21.4.294]

43 **Lund BC**, Carnahan RM, Egge JA, Chrischilles EA, Kaboli PJ. Inappropriate prescribing predicts adverse drug events in older adults. *Ann Pharmacother* 2010; **44**: 957-963 [PMID: 20460558 DOI: 10.1345/aph.1M657]

44 **Page RL**, Ruscin JM. The risk of adverse drug events and hospital-related morbidity and mortality among older adults with potentially inappropriate medication use. *Am J Geriatr Pharmacother* 2006; **4**: 297-305 [PMID: 17296535 DOI: 10.1016/j.amjopharm.2006.12.008]

45 **Hanlon JT**, Schmader KE. What types of inappropriate prescribing predict adverse drug reactions in older adults? *Ann Pharmacother* 2010; **44**: 1110-1111 [PMID: 20460555 DOI: 10.1345/aph.1P182]

46 **Liu CL**, Peng LN, Chen YT, Lin MH, Liu LK, Chen LK. Potentially inappropriate prescribing (IP) for elderly medical inpatients in Taiwan: a hospital-based study. *Arch Gerontol Geriatr* 2012; **55**: 148-151 [PMID: 21820189 DOI: 10.1016/j.archger.2011.07.001]

47 **Rochon PA**, Berger PB, Gordon M. The evolution of clinical trials: inclusion and representation. *CMAJ* 1998; **159**: 1373-1374 [PMID: 9861206]

48 **Gallagher P**, O'Mahony D. STOPP (Screening Tool of Older Persons' potentially inappropriate Prescriptions): application to acutely ill elderly patients and comparison with Beers' criteria. *Age Ageing* 2008; **37**: 673-679 [PMID: 18829684 DOI: 10.1093/ageing/afn197]

49 **Rochon PA**, Gurwitz JH. Prescribing for seniors: neither too much nor too little. *JAMA* 1999; **282**: 113-115 [PMID: 10411177 DOI: 10.1001/jama.282.2.113]

50 **Wolf PA**, Abbott RD, Kannel WB. Atrial fibrillation as an independent risk factor for stroke: the Framingham Study. *Stroke* 1991; **22**: 983-988 [PMID: 1866765 DOI: 10.1161/01.STR.22.8.983]

51 **Lake FR**, Cullen KJ, de Klerk NH, McCall MG, Rosman DL. Atrial fibrillation and mortality in an elderly population. *Aust N Z J Med* 1989; **19**: 321-326 [PMID: 2789508 DOI: 10.1111/j.1445-5994.1989.tb00271.x]

52 **Furberg CD**, Psaty BM, Manolio TA, Gardin JM, Smith VE, Rautaharju PM. Prevalence of atrial fibrillation in elderly subjects (the Cardiovascular Health Study). *Am J Cardiol* 1994; **74**: 236-241 [PMID: 8037127 DOI: 10.1016/0002-9149(94)90363-8]

53 **Elliott RA**, Woodward MC, Oborne CA. Antithrombotic prescribing in atrial fibrillation: application of a prescribing indicator and multidisciplinary feedback to improve prescribing. *Age Ageing* 2002; **31**: 391-396 [PMID: 12242203 DOI: 10.1093/ageing/31.5.391]

54 **Galvin R**, Moriarty F, Cousins G, Cahir C, Motterlini N, Bradley M, Hughes CM, Bennett K, Smith SM, Fahey T, Kenny RA. Prevalence of potentially inappropriate prescribing and prescribing omissions in older Irish adults: findings from The Irish LongituDinal Study on Ageing study (TILDA). *Eur J Clin Pharmacol* 2014; **70**: 599-606 [PMID: 24493365 DOI: 10.1007/s00228-014-1651-8]

55 **Barry PJ**, Gallagher P, Ryan C, O'mahony D. START (screening tool to alert doctors to the right treatment)--an evidence-based screening tool to detect prescribing omissions in elderly patients. *Age Ageing* 2007; **36**: 632-638 [PMID: 17881418 DOI: 10.1093/ageing/afm118]

56 **Sengstock D**, Vaitkevicius P, Salama A, Mentzer RM. Underprescribing and non-adherence to medications after coronary bypass surgery in older adults: strategies to improve adherence. *Drugs Aging* 2012; **29**: 93-103 [PMID: 22239673 DOI: 10.2165/11598500-000000000-00000]

57 **Murray MD**, Morrow DG, Weiner M, Clark DO, Tu W, Deer MM, Brater DC, Weinberger M. A conceptual framework to study medication adherence in older adults. *Am J Geriatr Pharmacother* 2004; **2**: 36-43 [PMID: 15555477 DOI: 10.1016/S1543-5946(04)90005-0]

58 Medicines and Healthcare products Regulatory Agency. Available from: URL: http://www.gov.uk/government/organisations/medicines-and-healthcare-products-regulatory-agency

59 Adverse Drug Events, Adverse Drug Reactions and Medication Errors. VA center for Medication Safety and VHA Pharmacy Benefits Management Strategic Healthcare Group and the Medical Advisory Panel Nov 2006. Available from: URL: http://www.va.gov/ms/professionals/medications/adverse_drug_reaction_faq.pdf

60 **Hanlon JT**, Schmader KE, Koronkowski MJ, Weinberger M, Landsman PB, Samsa GP, Lewis IK. Adverse drug events in high risk older outpatients. *J Am Geriatr Soc* 1997; **45**: 945-948 [PMID: 9256846]

61 **Hohl CM**, Dankoff J, Colacone A, Afilalo M. Polypharmacy, adverse drug-related events, and potential adverse drug interactions in elderly patients presenting to an emergency department. *Ann Emerg Med* 2001; **38**: 666-671 [PMID: 11719747 DOI: 10.1067/mem.2001.119456]

62 **O'Connor MN**, Gallagher P, Byrne S, O'Mahony D. Adverse drug reactions in older patients during hospitalisation: are they predictable? *Age Ageing* 2012; **41**: 771-776 [PMID: 22456465 DOI: 10.1093/ageing/afs046]

63 **Gurwitz JH**, Field TS, Avorn J, McCormick D, Jain S, Eckler M, Benser M, Edmondson AC, Bates DW. Incidence and preventability of adverse drug events in nursing homes. *Am J Med* 2000; **109**: 87-94 [PMID: 10967148 DOI: 10.1016/S0002-9343(00)00451-4]

64 **Tangiisuran B**, Wright J, Van der Cammen T, Rajkumar C. Adverse drug reactions in elderly: challenges in identification and improving preventative strategies. *Age Ageing* 2009; **38**: 358-359 [PMID: 19420141 DOI: 10.1093/ageing/afp050]

65 **Pirmohamed M**, James S, Meakin S, Green C, Scott AK, Walley TJ, Farrar K, Park BK, Breckenridge AM. Adverse drug reactions as cause of admission to hospital: prospective analysis of 18 820 patients. *BMJ* 2004; **329**: 15-19 [PMID: 15231615 DOI: 10.1136/bmj.329.7456.15]

66 **Rawlins MD**, Thompson JP. Pathogenesis of adverse drug reactions. In: Davies DM, editor. Textbook of adverse drug reactions. Oxford: Oxford University Press, 1977: 44 [DOI: 10.1017/S0033291700016500]

67 **Campbell SM**, Cantrill JA. Consensus methods in prescribing research. *J Clin Pharm Ther* 2001; **26**: 5-14 [PMID: 11286603 DOI: 10.1046/j.1365-2710.2001.00331.x]

68 **Anderson GM**, Beers MH, Kerluke K. Auditing prescription practice using explicit criteria and computerized drug benefit claims data. *J Eval Clin Pract* 1997; **3**: 283-294 [PMID: 9456428 DOI: 10.1046/j.1365-2753.1997.t01-1-00005.x]

69 **Boyd CM**, Darer J, Boult C, Fried LP, Boult L, Wu AW. Clinical practice guidelines and quality of care for older patients with multiple comorbid diseases: implications for pay for performance. *JAMA* 2005; **294**: 716-724 [PMID: 16091574 DOI: 10.1001/jama.294.6.716]

70 **Beers MH**, Ouslander JG, Rollingher I, Reuben DB, Brooks J, Beck JC. Explicit criteria for determining inappropriate medication use in nursing home residents. UCLA Division of Geriatric Medicine. *Arch Intern Med* 1991; **151**: 1825-1832 [PMID: 1888249 DOI: 10.1001/archinte.1991.00400090107019]

71 **Beers MH**. Explicit criteria for determining potentially inappropriate medication use by the elderly. An update. *Arch Intern Med* 1997; **157**: 1531-1536 [PMID: 9236554 DOI: 10.1001/archinte.1997.00440350031003]

72 **Fick DM**, Cooper JW, Wade WE, Waller JL, Maclean JR, Beers MH. Updating the Beers criteria for potentially inappropriate medication use in older adults: results of a US consensus panel of experts. *Arch Intern Med* 2003; **163**: 2716-2724 [PMID: 14662625 DOI: 10.1001/archinte.163.22.2716]

73 **American Geriatrics Society 2012 Beers Criteria Update Expert Panel.** American Geriatrics Society updated Beers Criteria for potentially inappropriate medication use in older adults. *J Am Geriatr Soc* 2012; **60**: 616-631 [PMID: 22376048 DOI: 10.1111/j.1532-5415.2012.03923.x]

74 **Gallagher P**, Ryan C, Byrne S, Kennedy J, O'Mahony D. STOPP (Screening Tool of Older Person's Prescriptions) and START (Screening Tool to Alert doctors to Right Treatment). Consensus validation. *Int J Clin Pharmacol Ther* 2008; **46**: 72-83 [PMID: 18218287 DOI: 10.5414/CPP46072]

75 **Gallagher PF**, O'Connor MN, O'Mahony D. Prevention of potentially inappropriate prescribing for elderly patients: a randomized controlled trial using STOPP/START criteria. *Clin Pharmacol Ther* 2011; **89**: 845-854 [PMID: 21508941 DOI: 10.1038/clpt.2011.44]

76 **Hanlon JT**, Schmader KE, Samsa GP, Weinberger M, Uttech KM, Lewis IK, Cohen HJ, Feussner JR. A method for assessing drug therapy appropriateness. *J Clin Epidemiol* 1992; **45**: 1045-1051 [PMID: 1474400 DOI: 10.1016/0895-4356(92)90144-C]

77 **Samsa GP**, Hanlon JT, Schmader KE, Weinberger M, Clipp EC, Uttech KM, Lewis IK, Landsman PB, Cohen HJ. A summated score for the medication appropriateness index: development and assessment of clinimetric properties including content validity. *J Clin Epidemiol* 1994; **47**: 891-896 [PMID: 7730892 DOI: 10.1016/0895-4356(94)90192-9]

78 **Hajjar ER**, Hanlon JT, Sloane RJ, Lindblad CI, Pieper CF, Ruby CM, Branch LC, Schmader KE. Unnecessary drug use in frail older people at hospital discharge. *J Am Geriatr Soc* 2005; **53**: 1518-1523 [PMID: 16137281 DOI: 10.1111/j.1532-5415.2005.53523.x]

79 **Hanlon JT**, Artz MB, Pieper CF, Lindblad CI, Sloane RJ, Ruby CM, Schmader KE. Inappropriate medication use among frail elderly inpatients. *Ann Pharmacother* 2004; **38**: 9-14 [PMID: 14742785 DOI: 10.1345/aph.1D313]

80 **Lipton HL**, Bero LA, Bird JA, McPhee SJ. The impact of clinical pharmacists' consultations on physicians' geriatric drug prescribing. A randomized controlled trial. *Med Care* 1992; **30**: 646-658 [PMID: 1614233 DOI: 10.1097/00005650-199207000-00006]

81 **Steinman MA**, Landefeld CS, Rosenthal GE, Berthenthal D, Sen S, Kaboli PJ. Polypharmacy and prescribing quality in older people.

J Am Geriatr Soc 2006; **54**: 1516-1523 [PMID: 17038068 DOI: 10.1111/j.1532-5415.2006.00889.x]

82 **Blanco-Reina E**, Ariza-Zafra G, Ocafia-Riola R, Leon-Ortiz M. 2012 American Geriatrics Society Beers criteria: enhanced applicability for detecting potentially inappropriate medications in European older adults? A comparison with the Screening Tool of Older Person's Potentially inappropriate Prescriptions. *J Am Geriatr Soc* 2014; **62**: 1217-1223 [PMID: 24917083 DOI: 10.111/jps.12891]

83 **Pasina L**, Djade CD, Tettamanti M, Franchi C, Salerno F, Corrao S, Marengoni A, Marcucci M, Mannucci PM, Nobili A. Prevalence of potentially inappropriate medications and risk of adverse clinical outcome in a cohort of hospitalized elderly patients: results from the REPOSI Study. *J Clin Pharm Ther* 2014; **39**: 511-515 [PMID: 24845066 DOI: 10.1111/jcpt.12178]

84 **Schmader KE**, Hanlon JT, Pieper CF, Sloane R, Ruby CM, Twersky J, Francis SD, Branch LG, Lindblad CI, Artz M, Weinberger M, Feussner JR, Cohen HJ. Effects of geriatric evaluation and management on adverse drug reactions and suboptimal prescribing in the frail elderly. *Am J Med* 2004; **116**: 394-401 [PMID: 15006588 DOI: 10.1016/j.amjmed.2003.10.031]

85 **Saltvedt I**, Spigset O, Ruths S, Fayers P, Kaasa S, Sletvold O. Patterns of drug prescription in a geriatric evaluation and management unit as compared with the general medical wards: a randomised study. *Eur J Clin Pharmacol* 2005; **61**: 921-928 [PMID: 16307267 DOI: 10.1007/s00228-005-0046-2]

86 **Crotty M**, Halbert J, Rowett D, Giles L, Birks R, Williams H, Whitehead C. An outreach geriatric medication advisory service in residential aged care: a randomised controlled trial of case conferencing. *Age Ageing* 2004; **33**: 612-617 [PMID: 15385274 DOI: 10.1093/ageing/afh213]

87 **Strandberg TE**, Pitkala KH, Berglind S, Nieminen MS, Tilvis RS. Multifactorial intervention to prevent recurrent cardiovascular events in patients 75 years or older: the Drugs and Evidence-Based Medicine in the Elderly (DEBATE) study: a randomized, controlled trial. *Am Heart J* 2006; **152**: 585-592 [PMID: 16923435 DOI: 10.1016/j.ahj.2006.02.006]

88 **Holland R**, Smith R, Harvey I. Where now for pharmacist led medication review? *J Epidemiol Community Health* 2006; **60**: 92-93 [PMID: 16415254 DOI: 10.1136/jech.2005.035188]

89 **Hanlon JT**, Weinberger M, Samsa GP, Schmader KE, Uttech KM, Lewis IK, Cowper PA, Landsman PB, Cohen HJ, Feussner JR. A randomized, controlled trial of a clinical pharmacist intervention to improve inappropriate prescribing in elderly outpatients with polypharmacy. *Am J Med* 1996; **100**: 428-437 [PMID: 8610730 DOI: 10.1016/S0002-9343(97)89519-8]

90 **Crotty M**, Rowett D, Spurling L, Giles LC, Phillips PA. Does the addition of a pharmacist transition coordinator improve evidence-based medication management and health outcomes in older adults moving from the hospital to a long-term care facility? Results of a randomized, controlled trial. *Am J Geriatr Pharmacother* 2004; **2**: 257-264 [PMID: 15903284 DOI: 10.1016/j.amjopharm.2005.01.001]

91 **Spinewine A**, Swine C, Dhillon S, Lambert P, Nachega JB, Wilmotte L, Tulkens PM. Effect of a collaborative approach on the quality of prescribing for geriatric inpatients: a randomized, controlled trial. *J Am Geriatr Soc* 2007; **55**: 658-665 [PMID: 17493184 DOI: 10.1111/j.1532-5415.2007.01132.x]

92 **Eleazer GP**, Doshi R, Wieland D, Boland R, Hirth VA. Geriatric content in medical school curricula: results of a national survey. *J Am Geriatr Soc* 2005; **53**: 136-140 [PMID: 15667390 DOI: 10.1111/j.1532-5415.2005.53023.x]

93 **Bragg EJ**, Warshaw GA. ACGME requirements for geriatrics medicine curricula in medical specialties: progress made and progress needed. *Acad Med* 2005; **80**: 279-285 [PMID: 15734811 DOI: 10.1097/00001888-200503000-00014]

94 **Warshaw GA**, Bragg EJ. The training of geriatricians in the United States: three decades of progress. *J Am Geriatr Soc* 2003; **51**: S338-S345 [PMID: 12823665 DOI: 10.1046/j.1365-2389.2003.51345.x]

95 **van Eijk ME**, Avorn J, Porsius AJ, de Boer A. Reducing prescribing of highly anticholinergic antidepressants for elderly people: randomised trial of group versus individual academic detailing. *BMJ* 2001; **322**: 654-657 [PMID: 11250852 DOI: 10.1136/bmj.322.7287.654]

96 **Stein CM**, Griffin MR, Taylor JA, Pichert JW, Brandt KD, Ray WA. Educational program for nursing home physicians and staff to reduce use of non-steroidal anti-inflammatory drugs among nursing home residents: a randomized controlled trial. *Med Care* 2001; **39**: 436-445 [PMID: 11317092 DOI: 10.1097/00005650-200105000-00004]

97 **Pimlott NJ**, Hux JE, Wilson LM, Kahan M, Li C, Rosser WW. Educating physicians to reduce benzodiazepine use by elderly patients: a randomized controlled trial. *CMAJ* 2003; **168**: 835-839 [PMID: 12668540]

98 **Fick DM**, Maclean JR, Rodriguez NA, Short L, Heuvel RV, Waller JL, Rogers RL. A randomized study to decrease the use of potentially inappropriate medications among community-dwelling older adults in a southeastern managed care organization. *Am J Manag Care* 2004; **10**: 761-768 [PMID: 15623266]

99 **Lutters M**, Harbarth S, Janssens JP, Freudiger H, Herrmann F, Michel JP, Vogt N. Effect of a comprehensive, multidisciplinary, educational program on the use of antibiotics in a geriatric university hospital. *J Am Geriatr Soc* 2004; **52**: 112-116 [PMID: 14687324 DOI: 10.1111/j.1532-5415.2004.52019.x]

100 **Crotty M**, Whitehead C, Rowett D, Halbert J, Weller D, Finucane P, Esterman A. An outreach intervention to implement evidence based practice in residential care: a randomized controlled trial [ISRCTN67855475]. *BMC Health Serv Res* 2004; **4**: 6 [PMID: 15066200 DOI: 10.1186/1472-6963-4-6]

101 **Rahme E**, Choquette D, Beaulieu M, Bessette L, Joseph L, Toubouti Y, LeLorier J. Impact of a general practitioner educational intervention on osteoarthritis treatment in an elderly population. *Am J Med* 2005; **118**: 1262-1270 [PMID: 16271911 DOI: 10.1016/j.amjmed.2005.03.026]

102 **Ross S**, Loke YK. Do educational interventions improve prescribing by medical students and junior doctors? A systematic review. *Br J Clin Pharmacol* 2009; **67**: 662-670 [PMID: 19594535 DOI: 10.1111/j.1365-2125.2009.03395.x]

103 **Bates DW**, Gawande AA. Improving safety with information technology. *N Engl J Med* 2003; **348**: 2526-2534 [PMID: 12815139 DOI: 10.1056/NEJMsa020847]

104 **Garg AX**, Adhikari NK, McDonald H, Rosas-Arellano MP, Devereaux PJ, Beyene J, Sam J, Haynes RB. Effects of computerized clinical decision support systems on practitioner performance and patient outcomes: a systematic review. *JAMA* 2005; **293**: 1223-1238 [PMID: 15755945 DOI: 10.1001/jama.293.10.1223]

105 **Weiner M**, Callahan CM, Tierney WM, Overhage JM, Mamlin B, Dexter PR, McDonald CJ. Using information technology to improve the health care of older adults. *Ann Intern Med* 2003; **139**: 430-436 [PMID: 12965971 DOI: 10.7326/0003-4819-139-5_Part_2-200309021-00010]

106 **Tamblyn R**, Huang A, Perreault R, Jacques A, Roy D, Hanley J, McLeod P, Laprise R. The medical office of the 21st century (MOXXI): effectiveness of computerized decision-making support in reducing inappropriate prescribing in primary care. *CMAJ* 2003; **169**: 549-556 [PMID: 12975221]

107 **Koppel R**, Metlay JP, Cohen A, Abaluck B, Localio AR, Kimmel SE, Strom BL. Role of computerized physician order entry systems in facilitating medication errors. *JAMA* 2005; **293**: 1197-1203 [PMID: 15755942 DOI: 10.1001/jama.293.10.1197]

108 **Zhan C**, Hicks RW, Blanchette CM, Keyes MA, Cousins DD. Potential benefits and problems with computerized prescriber order entry: analysis of a voluntary medication error-reporting database. *Am J Health Syst Pharm* 2006; **63**: 353-358 [PMID: 16452521 DOI: 10.2146/ajhp050379]

109 **Horsky J**, Kuperman GJ, Patel VL. Comprehensive analysis of a medication dosing error related to CPOE. *J Am Med Inform Assoc* 2005; **12**: 377-382 [PMID: 15802485 DOI: 10.1197/jamia.M1740]

110 Development and clinical trials of a new Software ENgine for the Assessment & Optimization of drug and non-drug Therapy in Older

peRsons. Available from: URL: http://www.senator-project.eu/
111 **University College Cork**. Clinical Trial of a New Software ENgine for the Assessment & Optimization of Drug and Non-drug Therapy in Older peRsons (SENATOR). In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). [updated 2014 Mar 28]. Available from: URL: https://clinicaltrials.gov/ct2/show/NCT02097654 NLM Identifier: NCT02097654
112 **Baystate Medical Center**. Using Clinical Alerts to Decrease Inappropriate Medication Prescribing. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). [updated 2014 Mar 7]. Available from: URL: http://clinicaltrials.gov/ct2/show/NCT01034761 NLM Identifier: NCT01034761
113 **McLeod PJ**, Huang AR, Tamblyn RM, Gayton DC. Defining inappropriate practices in prescribing for elderly people: a national consensus panel. *CMAJ* 1997; **156**: 385-391 [PMID: 9033421]
114 **Naugler CT**, Brymer C, Stolee P, Arcese ZA. Development and validation of an improving prescribing in the elderly tool. *Can J Clin Pharmacol* 2000; **7**: 103-107 [PMID: 10958706]
115 **Zhan C**, Sangl J, Bierman AS, Miller MR, Friedman B, Wickizer SW, Meyer GS. Potentially inappropriate medication use in the community-dwelling elderly: findings from the 1996 Medical Expenditure Panel Survey. *JAMA* 2001; **286**: 2823-2829 [PMID: 11735757 DOI: 10.1001/jama.286.22.2823]
116 **Laroche ML**, Charmes JP, Merle L. Potentially inappropriate medications in the elderly: a French consensus panel list. *Eur J Clin Pharmacol* 2007; **63**: 725-731 [PMID: 17554532 DOI: 10.1007/s00228-007-0324-2]
117 **Rancourt C**, Moisan J, Baillargeon L, Verreault R, Laurin D, Grégoire JP. Potentially inappropriate prescriptions for older patients in long-term care. *BMC Geriatr* 2004; **4**: 9 [PMID: 15488143 DOI: 10.1186/1471-2318-4-9]
118 **Basger BJ**, Chen TF, Moles RJ. Inappropriate medication use and prescribing indicators in elderly Australians: development of a prescribing indicators tool. *Drugs Aging* 2008; **25**: 777-793 [PMID: 18729548 DOI: 10.2165/00002512-200825090-00004]
119 **Rognstad S**, Brekke M, Fetveit A, Spigset O, Wyller TB, Straand J. The Norwegian General Practice (NORGEP) criteria for assessing potentially inappropriate prescriptions to elderly patients. A modified Delphi study. *Scand J Prim Health Care* 2009; **27**: 153-159 [PMID: 19462339 DOI: 10.1080/02813430902992215]
120 **Holt S**, Schmiedl S, Thürmann PA. Potentially inappropriate medications in the elderly: the PRISCUS list. *Dtsch Arztebl Int* 2010; **107**: 543-551 [PMID: 20827352 DOI: 10.3238/arztebl.2010.0543]
121 **Winit-Watjana W**, Sakulrat P, Kespichayawattana J. Criteria for high-risk medication use in Thai older patients. *Arch Gerontol Geriatr* 2008; **47**: 35-51 [PMID: 17675177 DOI: 10.1016/j.archger.2007.06.006]

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Therapeutic options and vaccine development in the treatment of leishmaniasis

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Abstract

Early treatment of leishmaniasis is critical to achieve cure, prevent psychological and social distress, and prevent transmission of disease. Untreated Leishmaniasis- cutaneous leishmaniasis, mucocutaneous leishmaniasis and visceral leishmaniasis - results in disfiguring scars and high rates of morbidity and mortality in highly endemic regions of the world. However, cure rates with available therapeutics are limited due to cost, therapeutic toxicity and the growing rate of resistance. New therapeutic targets for medications and vaccine development are under investigation to provide improved healing and efficacy for the treatment of *Leishmania spp*.

Key words: Leishmania; Visceral; Cutaneous; Mucocutaneous; Amphoterecin; Vaccine

Core tip: Leishmaniasis is an old disease, hard to diagnose and even harder to treat. Limited treatment is available. Early treatment of leishmaniasis is critical to achieve cure, prevent psychological and social distress, and prevent transmission of disease. Untreated Leishmaniasis - cutaneous leishmaniasis, mucocutaneous leishmaniasis and visceral leishmaniasis - results in disfiguring scars and high rates of morbidity and mortality in highly endemic regions of the world. Cure rates with available therapeutics are limited due to cost, therapeutic toxicity and the growing rate of resistance. There is an emergent need for development of new therapeutic options with improved tolerability, improved healing process minimizing scarring, and improved efficacy amongst all *Leishmania spp*.

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INTRODUCTION

Leishmania spp. are intracellular protozoa transmitted between mammals by the bite of a female sandfly, genus *Phlebotmus* in the Old World (the Middle East, Asia, Eastern Europe, Western Europe, and Africa) and genus *Lutzomyia* in the New World (Central and South America)[1,2]. A variety of animals, including humans, can be infected with *Leishmania spp.* and many animals serve as natural reservoirs[1]. Leishmaniasis is endemic in 98 countries with an estimated prevalence of 12 million people infected and 350 million people at risk of infection[1-4]. There are more than 20 known *Leishmania spp.* that cause human disease[1,5,6]. *Leishmania spp.* cause four main human syndromes: Cutaneous leishmaniasis (CL), diffuse cutaneous leishmaniasis (DCL), mucocutaneous leishmaniasis (MCL) and visceral leishmaniasis (VL). The clinical syndrome varies based on the *Leishmania spp.*, the geographic location and the host immune system[1,6,7]. However, all forms of leishmaniasis are severely debilitating and affect the livelihood of those living in endemic areas of the world. An estimated loss of 2357000 disability-adjusted life years (DALYs) is attributed to leishmaniasis alone[7,8].

CL accounts for approximately 1.2 million new cases of Leishmaniasis per year reported in 83 countries[4]. The majority of CL cases occur in Afghanistan, Algeria, Brazil, Colombia, Iran, Peru, Ethiopia, Costa Rica, North Sudan, Saudi Arabia and Syria[3,6,8,9]. CL is typically caused by *Leishmania major* (*L. major*), *Leishmania tropica* (*L. tropica*), *Leishmania infantum* (*L. infantum*) and *Leishmania donovani* (*L. donovani* in the old world and *L. mexicana*, *L. amazonensis*), *Leishmania guyanensis* (*L. guyanensis*), *Leishmania panamensis* (*L. panamensis*) and *Leishmania braziliensis* (*L. braziliensis*) in the new world[1,8]. It may present as a single ulcerative or nodular lesion near the site of the sandfly bite on uncovered areas of the body[1]. In some cases, however, individuals may have a more severe diffuse infection called DCL, with nodular lesions of variable size in various locations (DCL)[1,10]. Lesions evolve over weeks to months and may resolve spontaneously over months to years. Treatment of primary CL depends on the *Leishmania spp*., the geographic region, and the clinical presentation[9]. For many species of leishmaniasis, cutaneous disease is self-limiting and will be cured over time. In Old World leishmaniasis, *L. major* spontaneously heals in 40%-70% of cases at 3 mo and close to 100% of cases by 12 mo, whereas *L. tropica* spontaneously resolves in less than 1% of cases at 3 mo and close to 100% by 3 years[9]. In New World leishmaniasis, *L. mexicana* may resolve spontaneously within 3-4 mo but *L. braziliensis*, *L. panamensis, L. guyanensis* and *L. peruviana* may take more than 6 mo to self-resolve[9]. After resolution patients may be left with disfiguring cutaneous scars[1]. Scarring caused by CL has a distinctive appearance particularly when involving sensitive areas such as the face. Scars often have a central depressed surface that is covered by rounded hyper-pigmented skin[11]. Years after spontaneous resolution, CL lesions have the potential to relapse, a condition known as leishmaniasis recidivans[1]. Despite the possibility a lesion will self-heal, initiation of treatment, either systemic or local therapy, may hasten resolution of disease and may prevent further transformation to MCL[1].

MCL occurs most commonly due to progression of CL caused by *L. braziliensis*. Metastasis of the parasite into the mucosal tissue causes significant tissue destruction and disfigurement[1,9]. Almost 90% of MCL occurs in Bolivia, Brazil, and Peru; up to 30% of *L. braziliensis* cases progress to mucocutaneous disease[6,9]. MCL typically involves the nose, palate, pharynx, and larynx and occurs months to years after resolution of the primary lesions[1]. Ulcerated lesions of the nasal septum, which may lead to perforation and deformities of the nasal pyramid, larynx, and pharynx, can cause significant morbidity and social rejection[12]. Mucocutaneous disease always requires treatment for cure; however, it may be refractory to current available therapeutic chemotherapy. With continued destruction of mucosal membranes, patients are at risk for secondary super-infections and severe malnutrition[1]. Because of the risk of secondary morbidity and mortality, systemic treatment is preferred[9].

VL, also known as Kala azar, is caused by *L. donovani* in India, Pakistan, China and several countries in Africa and by *L. infantum* in the Mediterranean region and in the New World[1,8]. VL occurs secondary to proliferation of parasites in macrophages in the liver, spleen and bone marrow which causes hepatosplenomegaly and bone marrow suppression with subsequent pancytopenia and immunosuppression[1]. There are an estimated 200000-400000 new cases of VL each year with a case fatality rate of more than 10%[3,4,6]. Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan report over 90% of all VL cases worldwide[4,6]. Without treatment, VL is almost universally fatal[1,8]. Systemic therapy is the current standard of care.

An appropriate cellular immune response is essential for the control and eradication of leishmania in the human host. With exposure to leishmania, the host T cells produce cytokines, specifically interferon gamma (INF-γ) and tumor necrosis factor-alpha (TNF-α), that activate host macrophages[12]. The activation of host macrophages produces nitric oxide (NO), peri-nitrate and oxygen derivates that are directly involved with leishmania killing and eradication[7,12]. Patients with immunocompromised conditions such as human immunodeficiency virus (HIV) are at increased risk of progressive, debilitating disease states. Interestingly, patients who recover from leishmaniasis often have a spectrum of resistance to re-infection or acquired anti-leishmanial immunity. Host resistance is mediated by both innate and adaptive immune responses including activation of macrophages, dendritic cells, and antigen specific CD4 and CD8 T cells[7]. These host responses allow for immunity against re-infection and

highlight possible new avenues for therapeutic drug development.

CURRENT TREATMENT

Leishmania spp. vary in their sensitivity to available drugs[8]. The choice of treatment is based on the region where the infection was acquired, local experience with treatment, and known species resistance patterns[8]. Currently the gold standard therapy for most forms of leishmaniasis remains pentavalent antimony (Sb^v), meglumine antimoniate, or sodium stibogluconate[1]. The mechanism of action against *Leishmania spp*. is still poorly understood and thought to act on the parasite indirectly through augmentation of the host's macrophage parasiticidal activity[1]. Sb^v can be administered by intravenous (typical dose of 20 mg/kg), intramuscular or intra-lesion route and typically requires at least 20-28 d course of treatment[1,8,9,13]. Despite its common use, Sb^v have different cure rates between species, ranging from 60%-80%[8,9,11]. Furthermore, recent studies involving the use of Sb^v in children showed significantly lower cure rates and significantly higher metabolic elimination of the drug compared with adults[14]. Adverse effects are also common with Sb^v and include cardiotoxicity such as arrhythmias, QTc prolongation, and sudden cardiac death; elevated aminotransaminases; elevated pancreatic enzymes; pancytopenia; and electrolyte abnormalities[1]. Because of these adverse effects, administration of Sb^v is highly restricted in pregnant and lactating women, infants, and patient with drug sensitivities[15]. Intralesional injections of Sb^v are the most established local therapy available for the treatment of CL and do not cause the same systemic adverse effects as intravenous and intramuscular formulations. However, there is a lack of standardization of dosing and treatment regimens with varying cure rates among geographic regions due to the development of resistance[16]. While intralesional injections do not cause significant systemic adverse effects, local therapy can cause itching, erythema, pain, and hyperpigmentation of the lesion, and put the patient at increased risk of bacterial super infection[9]. Sb^v chemotherapeutic agents currently available for the treatment of Leishmaniasis are toxic, costly, and not readily available in every community, and require a long duration of therapy as well as daily systemic administration with medical monitoring. These limitations promote poor treatment adherence within a community[1,17]. Due to the wider geographic distribution of leishmaniasis, the toxic chemoprophylaxis treatment available and the emergence of drug resistant *Leishmania* strains, new antimicrobial therapies and strategies are being developed to address the growing problem[1,3,7].

SYSTEMIC TREATMENT

Systemic therapies are recommend in complex CL, MCL and VL[9] (Table 1). However, current alternative systemic agents to Sb^v are limited.

Table 1 Common therapeutic options for treatment of leishmaniasis

Medication	Disease	Dosing	Adverse effects	Ref.
Antimony Sodium stibogluconate	CL, VL	IM, IV, IL	IM/IV: Cardiotoxicity, elevated aminotransaminases, elevated pancreatic enzymes, pancytopenia, electrolyte abnormalities IL: pain, hyperpigmentation, risk of bacterial infection	[1,13,15,89]
Amphotericin	CL, MCL, VL	IV	Renal insufficiency, electrolyte abnormalities	[1,13,18,20]
Pentamidine	CL, VL	IM, IV	Hypoglycemia, elevated amiontransaminases, nausea, vomiting, bone marrow toxicity, nephrotoxicity, cardiotoxicity	[1,13,22,23]
Miltefosine	VL, CL, MCL	PO	Vomiting, nausea, diarrhea, teratogen	[1,13,15,18]
Paromomycin	CL, VL	IM, PO, topically	Ototoxicity, vestibular instability, nephrotoxicity	[1,22]
Pentoxyfylline	CL, MCL	PO	Nausea, vomiting, headache, dizziness	[27]
Azoles	CL, VL	PO	Hepatic toxicity	[1,17]
Imiquimod	CL	Topical	Irritation at site of application	[1,11]
Thermotherapy	CL	Topical	Pain, post-inflammatory hyperpigmentation	[16,17,30]
Cryotherapy	CL	Topical	Local blistering, secondary bacterial infection	[8,16]
Phototherapy	CL	Topical	Pain	[30]

CL: Cutaneous leishmaniasis; VL: Visceral leishmaniasis; MCL: Mucocutaneous leishmaniasis; IM: Intramuscular; IV: Intravenous; IL: Intralesional; PO: Oral.

Amphotericin B

More recent clinical trials and clinical experience have highlighted the use of polyenes such as Amphotericin B in the treatment of leishmaniasis. Both liposomal and deoxycholate formulations have been found to have high affinity to the ergosterol membrane of *Leishmania spp.* and create membrane instability[1,18]. In areas of India, Bangladesh, Bhutan and Nepal where high resistance of Sb^v exists, Amphotericin B is the therapeutic drug of choice for VL. In these studies, using high doses of Amphotericin 10 mg/kg and 15 mg/kg demonstrated cure rates of 96% and 100% after a single dose[8,18]. In other areas where resistance to Sb^v is not as high but drug toxicity is a concern, such as in patients co-infected with *Leishmania spp.*

and HIV, and in travelers returning from regions where VL is endemic, Liposomal Amphotericin B again is the recommended drug of choice[8]. Treatment of CL and MCL caused by *L. braziliensis* requires systemic therapy. Studies comparing Liposomal Amphotericin B to Sb^v have shown superior results when treated with Liposomal Amphotericin B[19,20]. There is no standard dosing regimen for treatment of VL with Amphotericin B; however, lower dosing using Liposomal Amphotericin B at 3 mg/kg per day administered intravenously for days 1-5, 14 and 21 (total 21 mg/kg) has been used to treat CL, although further studies to evaluate optimal dose and duration are still needed[13,20,21]. Despite the promising results of Amphotericin formulations there remain many limitations including the need for intravenous administration, the significant cost of the medication, the limited availability of the medication, the emergence of *Leishmania spp*. resistance, and the significant adverse effect profile including renal insufficiency and electrolyte abnormalities[1,18]. Despite its high cost, cost analysis studies have shown the expense for total treatment with a shorter duration of therapy with Liposomal Amphotericin is less than with full treatment with Sb^v[19].

Pentamidine

Pentamidine isethionate is an intravenous or intramuscular formulation used to treat cutaneous Leishmaniasis caused by *L. amazonensis, L. guyanensis, L. panamensis* and *L. peruviana*. Pentamidine also serves as an alternative agent for patients with recurrence of cutaneous *L. braziliensis* or an alternative agent for recurrent VL after treatment failure with Sb^v or amphotericin[9,11]. The mechanism of action remains unknown but studies suggest the drug may target protozoa mitochondria and interfere with biosynthesis of macromolecules[1,9,22]. The optimal dosing of pentamidine is currently unknown with proposed dosing of 2-4 mg/kg per day *im* or *iv* for 21 d[13]. Adverse effects of Pentamidine include hypoglycemia, worsening of diabetes, elevated aminotransaminases, musculoskeletal pain, anorexia, nausea, vomiting, headaches, bone marrow toxicity, nephrotoxicity, and cardiotoxicity with arrhythmias, heart failure and hypotension[1,22,23]. The extensive side effect profile limits the use of pentamidine.

Paromomycin

An alternative systemic agent against leishmaniasis is Paromomycin, an aminoglycoside antibiotic that blocks protein synthesis through binding of 16S ribosomal RNA. Paromomycin can be administered intramuscularly, orally, or topically[1,22]. Paromomycin has been shown to be effective against CL and VL in areas with susceptible protozoa, although cure rates vary greatly amongst geographic locations[16]. It can be used alone or in combination with Sb^v or amphotericin B and has been associated with increasing time to resolution of lesions caused specifically by *L. major*[1]. A Phase 3 clinical study evaluating the efficacy of topical combination therapy with 15% Paromomycin and 0.5% Gentamicin applied to each lesion once a day for 20 d to treat CL has shown promise in advancing cure rates with reduced systemic absorption[24,25]. Phase 2 trials using topical paromomycin plus gentamicin formulation showed a 6 mo cure rate of 87% compared to paromomycin alone at 60%[26]. Despite these advancements, paromomycin has limiting adverse effects of systemic formulations, which include ototoxicity, vestibular instability and nephrotoxicity, as well as with the topical formulations, which include erythema, pain, edema as well as ototoxicity[1,22].

Miltefosine

Miltefosine, an alkylphosphocholine, is a promising oral agent, recently approved by the United States Food and Drug Administration (FDA) to treat VL, complicated CL and MCL cases that do not respond to first line therapeutics[1,23-25,27]. In adults, the treatment regimen consists of one 50-mg oral capsule twice a day for 28 consecutive days[13]. The oral formulation of miltefosine alleviates the risk, cost, and time demands of daily intramuscular or intravenous injections[14,18]. The mechanism of action is associated with interruption of phospholipid biosynthesis and alkyl-lipid metabolism in specific *Leishmania spp*[22]. As with other therapeutics to treat leishmania, the efficacy of Miltefosine is variable based on species and geographic location[14]. Studies in children specifically showed promising results comparing Miltefosine to the standard of care, Sb^v, for treatment of CL[14]. Miltefosine has been shown to be effective against CL by *L. major* but may also be effective in new world CL with *L. panamensis*[1]. Additional studies have shown improved cure rates in treating VL in India particularly when used in combination with paromomycin[1,15]. However, other New World studies have shown inferiority of miltefosine to Sb^v in the treatment of CL in Colombia. The finding of inferiority in this particular study was thought to be species specific. Treatment of CL with Miltefosine in Colombia demonstrated a cure rate of only 69.8%, which fell to 49% when administered to patient with lesions caused by *L. braziliensis*[15]. Miltefosine tends to be well tolerated with minimal non-specific adverse effects, including vomiting, nausea, diarrhea, and headache. However, miltefosine is a teratogen and an abortifacient and must be used with caution in females of reproductive age[1,18]. Females of reproduction age who are taking Miltefosine should be provided with contraception during the course of treatment as well as for 5 mo post-therapy[13,15]. Miltefosine also remains costly and requires prolonged therapy presenting additional barriers to therapeutic adherence[15].

Pentoxifylline

Pentoxyfylline, a xanthine derivative, is an orally administered immunomodulator that is an attractive therapeutic alternative for CL and MCL. *In vitro* there is

no evidence that pentoxifylline directly kills *Leishmania spp*. but the major contribution of pentoxifylline is reduction of the TNF-α mediated tissue damage caused by *Leishmania spp*[28]. Pentoxyfylline blocks the transcription of TNF-α mRNA from macrophages leading to reduction in TNF-α synthesis, decreases leukocyte migration, and decreases leukocyte adhesion[28]. Pentoxifylline also causes significant vasodilation and increase in red blood cell flexibility for improved circulation and migration of host defense cells to the damaged tissue[12,28]. While Pentoxyfylline has been demonstrated to reduce the concentration of TNF-α in damaged tissue caused by *Leishmania spp*. in CL and MCL, monotherapy has not been associated with cure[28]. Pentoxyfylline is more commonly used as an adjuvant immunomodulating therapeutic agent[28]. In combination therapy regimens, pentoxifylline allows for reduction in the inflammatory response and promotes improved defense against protozoa by Sb^v[12]. Recent studies have shown higher cure rates and reduction of time to cure using combination of Sb^v and pentoxifylline. The reduction in time to cure has allowed for shorter Sb^v dosing regimens reducing the risk of adverse effects, the total cost of therapy, and the total hospital stay associated with prolonged Sb^v[8,12,28]. Along with the improved efficacy, pentoxifylline is associated with minimal adverse effects even with chronic use[12]. Adverse effects including nausea, vomiting, dizziness and headache occur in less than 2.2% of patients[28]. Additionally there are reports of safe use in children although large clinical trials are currently not available[12].

Azoles

Azoles, *e.g.,* posaconazole, itraconazole, fluconazole and ketoconazole, are oral therapeutic alternatives for treatment of Leishmaniasis. Azoles inhibit ergosterol synthesis through alteration of sterol demethylation causing the accumulation of sterols[1,17,27,29]. Decreased production of ergosterol, which composes the cell wall, inhibits leishmania growth and causes structural instability of the protozoa[27,29]. *In vitro* murine studies suggest azoles have anti-parasitic activity against certain *Leishmania spp.* causing VL such as *L. infantum* but are less active against *L. donovani*[27]. Ketoconazole and fluconazole have also been shown to hasten healing of CL caused by *L. mexicana, L. panamensis* and *L major*[1,29]. While several *in vitro* studies demonstrate effective anti-parasitic activity, clinical studies have not been as promising[1]. One clinical study did show comparable outcomes of Ketoconazole to standard Sb^v in the treatment of *L. panamensis* CL, although more recent studies have shown clinical benefit is achieved only with high dosing[29]. Azoles given at high doses expose patients to significant hepatic toxicity[1,17]. In order to reduce the high dosing, further studies evaluating azoles in combination with other therapeutic options may provide increased efficacy at lower dosing[17,27]. Topical imiquimod in combination with itraconazole has been shown to have better cure rates when either of the therapeutics were used alone[17].

LOCAL TREATMENTS

Local treatments can be used to treat CL when the *Leishmania spp.* has low potential to advance to MCL; there are a limited number of lesions (less than four); the lesions are small (< 4-5 cm); the lesions are not localized on delicate areas of the body; and the host is not immunosuppressed[9,23]. The use of local agents is favorable in these circumstances as they tend to have less systemic toxicity and allow for outpatient treatment regimens[9]. Local therapies are currently considered first line treatment in most cases of CL[9]. Despite these advantages, there is a need for standardization and highly scrutinized efficacy studies for the use of local therapies[23].

Imiquimod

Imiquimod, a topical imidazole quinolone cream, is a potent immune-modulator and Toll-like receptor 7 agonist that induces macrophage activation through production of pro-inflammatory cytokines interleukin-2, INF-γ and TNF-α[1,11]. Direct activation of macrophages mediates intracellular killing of *Leishmania spp.*[11]. Topical imiquimod can be used as monotherapy; however, the rate of treatment failure is currently unknown. When used alone imiquimod has demonstrated rapid initial healing but failed to maintain response after treatment was stopped. As a result, when imiquimod is used as monotherapy, patients may need a prolonged treatment course to ensure therapeutic cure[11]. More commonly imiquimod is added in combination with Sb^v[1,11]. Addition of imiquimod cream to a Sb^v based regimen to treat Sb^v-resistant CL showed increased rate of cure and higher sustained treatment response compared with persons treated with Sb^v alone[11,23]. Combination therapy also had increased rates of healing and an improved overall cosmetic effect with reduced scarring and reduced hyperpigmentation of the wounds compared to Sb^v therapy alone[11,23]. Imiquimod has been associated with lower treatment cost and fewer adverse effects compared to standard of care due to reduced need for prolonged Sb^v[11]. Imiquimod is generally well tolerated with the main adverse effect being irritation at the site of application[1].

Cryotherapy

Cryotherapy uses liquid nitrogen applied directly to CL lesions and has been proven effective in Old World CL including *L. tropica*, *L aethiopica* and *L infantum*, as well as New World CL that has low potential to progress to MCL such as *L. mexicana, L. panamensis and L. amazonensis*[1,8]. Application of liquid nitrogen is completed 2-3 times each session and repeated every 1-4 wk until complete healing of the lesion is achieved[9]. When used as monotherapy, cryotherapy has shown cure rates superior to spontaneous healing

and comparable to intralesional Sb^v[16]. However, superior results are observed with Cryotherapy in combination with intralesional Sb^v, with a cure rate of 89% compared to cryotherapy alone (75%) or intralesional Sb^v alone (67.8%)[1,9,16,17]. Cryotherapy, while safe and effective, can be painful and cause post-inflammatory hyperpigmentation[16,17,30]. The availability of cryotherapy in endemic regions of the world as well as unknown relapse rates further limit its consistent use as a therapeutic option for CL[16,17].

Thermotherapy

Thermotherapy, *i.e.,* heating the CL lesion to 50 degree Celsius for 30 s once weekly for 4 wk, has been used for the treatment of New world CL caused by *Leishmania spp.* with low likelihood of progression to MCL, such as *L. mexicana, L. panamensis, L. amazonensis*[1,8,9]. Through application of heat radiofrequency, the protozoa are directly killed[9]. Compared to intralesional or parenteral Sb^v, the duration of therapy and the adverse effects were reduced when using thermotherapy monotherapy[16]. Thermotherapy may put patients at risk for local blistering and secondary bacterial infection during the healing period[16]. CO2 Laser is a type of thermotherapy which operates through thermolysis on damaged tissues without causing damage to the surrounding healthy tissue. The CO2 laser is used in one single session and has been shown to be more effective than combined therapy of cryotherapy plus intralesional Sb^v[9]. With disfiguring facial lesions or lesions at sites at risk of significant scaring, CO2 thermotherapy may be an alternative therapeutic option[8]. Despite the positive effects of thermotherapy on healing of wounds, cure rates remain variable from 48%-83% amongst different *Leishmania spp*[16]. While shown to be effective in certain species, thermotherapy requires costly advanced technology equipment and adjuvant medications including local anesthetic and prophylactic antibiotics that are not readily available in endemic areas[8,16].

Phototherapy

Photodynamic therapy is an additional new treatment modality that uses light-mediated cytolysis of protozoa. The photodynamic therapy is applied once weekly for a total of 4 wk and does not induce drug resistance even after repeated applications[9,30]. Conventionally, photodynamic therapy requires activation of a topical photosensitizer, usually aminolevulinic acid (ALA) or methyl aminolevulinate, followed by irradiation by a visible light source[30]. Activation of the photosensitizer in the presence of oxygen results in the generation of reactive oxygen species, activation of host macrophages and subsequent destruction of the infected tissue[30]. This process can be time consuming and expensive and requires specialized technology[30]. New technology is emerging that uses daylight activation of the topical photosensitizers, abolishing the need for specialized light sources[30]. It has proven to be effective in the treatment of CL caused by both *L. major* and *L. tropica*, with an overall cure rate of 88.9%; however, efficacy is dependent on weather conditions in geographic locations[30]. Adverse effects associated with phototherapy include pain caused by the sudden activation of the photosensitizer[30].

Approach to chemotherapeutics selection

Choosing the appropriate initial therapy for a patient with leishmaniasis is dependent on the disease (CL, MCL, DCL or VL), the geographic location, the *Leishmania spp.*, and the state of the host immune response.

Currently the WHO recommends pentavalent antimonial, sodium stibogluonate 20 mg/kg per day for 21 d, and IV, as first line therapy for CL and VL[10,21]. However, Liposomal amphotericin B has been found to be as effective in treatment of VL, and superior in treatment for MCL, and better tolerated compared to Sb^v. As a result the US FDA has approved amphotericin B as first line therapy for VL caused by *L .infantum* and *L. donovani*[21]. Patients with CL, DCL and MCL caused by *L. braziliensis*, patients in the New World with leishmaniasis of unknown species, and patients with complicated CL including lesions on the face or lesions over the joints should also be treated with Liposomal amphotericin B 3 mg/kg on Days 1-5, 14, 21[20,21,30]. All patients with VL, CL, DCL or MCL who are immunocompromised should be treated with systemic therapy, either antimony or amphotericin B, as treatment failure and disease progression is more common in this group[21]. Due to the reduced side effects and reduced duration of therapy, Liposomal Amphotericin B should be the first line therapy in immunocompromised patients if available. Miltefosine is an appropriate alternative to Amphotericin B in DCL, MCL and VL caused by *L. donovani* and *L. infantum*[10,21].

For cutaneous disease that has low potential to advance to MCL; is caused by species other than *L. braziliensis*; where the patient has a limited number of lesions (less than four); where the lesions are small (< 4-5 cm); where the lesions are not localized on delicate areas; and where the host is not immunosuppressed; topical therapies such as intra-lesional chemotherapeutics, thermotherapy, phototherapy or cryotherapy or combination therapies should be used as first line therapy are to minimize adverse effects[20,31].

Vaccines

Preventative and therapeutic vaccines are recognized as the most efficacious and most cost-effective protection against leishmaniasis. Currently there is no licensed vaccine against human leishmaniasis; however, several vaccine candidates have been tried and several others are currently under further investigation. Vaccine development has been challenging due to the complexity of the protozoa pathogenesis and the interaction with the host cell-mediated immune response[2,7]. Despite the complexity of vaccine development, the cost-

effectiveness of leishmania vaccines makes further investigation, production and clinical development an attractive endeavor. Cost-analysis studies have shown that a vaccine even with a relatively short duration of protection will affect cost savings and prevent cases of leishmaniasis. The study found that a vaccine with 10 years protection used in endemic areas such as Brazil, Bolivia, Colombia, Ecuador, Peru and Venezuela that have a country-wide incidence of at least 0.03% in a total population of approximately 308 million people could prevent 41000-144784 CL cases at a cost less than the cost of chemotherapy[10]. This held true for vaccines with 5 years of protection as well[10]. Leishmania vaccines currently receiving attention include a live leishmania vaccine, whole killed or fractions of leishmania, live attenuated and DNA vaccines.

Live parasites were first tried for vaccine development by isolating *L. major* promastigotes from free culture and injecting into the patient. While promising results from live parasite exposure were identified, the standardization and quality control were lacking and concerns about possibility of transmission remain valid[7]. While live vaccines may prevent future infection, they are not currently reasonable options for vaccine development.

First generation vaccines consisting of whole killed leishmania or fractions of the protozoa have also been explored. Killed isolated *L. amazonensis* has been used as a therapeutic vaccine in combination with chemotherapy and has been shown to reduce the required dose of Sb^{v} to achieve cure[7]. Furthermore, in Venezuela autoclaved killed *L. mexicana* has been used to treat patients with Sb^{v} non-responsive CL[7]. Killed vaccines are valuable due to their safety in administration[32]. Despite the potential therapeutic value and minimal safety profile of killed leishmania vaccines, preventive vaccines have not shown significant protection[32]. In studies of autoclaved *L. major* vaccine, the host did not mount a robust immunogenic response. However, with better adjuvants that are able to maintain effector memory cell activation to achieve protection, the vaccine potency increases[32]. Addition of different adjuvants including alum, saponin, cationic liposomes and MPL-A have all been studied and are associated with significant cell mediated immune response, humoral immune response and reduced parasite load[32]. If an adequate adjuvant is used to produce improved immunogenicity with standardized preparation, it is possible that killed leishmaniasis vaccines may be candidates for further vaccine discovery as they are safe, low cost, stable, and composed of the complete protozoa spectrum of antigens[7,32,33].

Live attenuated, recombinant proteins and DNA vaccines are new vaccine strategies under consideration[7]. While some target proteins are conserved proteins across species, others are species and life cycle stage specific, making them limited in use[7]. Important recombinant protein candidate vaccines to date include surface expressed glycoprotein leishmaniolysin (gp63); Leishmania activated C kinase (LACK); parasite surface antigen (PSA); Leishmania-derived recombinant polyprotein (Leish-111f); serine proteases; LEISH-F1; and LEISH-F2[7,33]. LEISH-F1, three recombinant proteins conserved in *L. donovani*, *L. chagasi* and *L. braziliensis*, respectively, and LEISH-F2 re-designed recombinant protein have undergone phase 1 and phase 2 clinical trials with significant success against CL and VL in multiple target locations[33]. Both LEISH-F1 and LEISH-F2 have proven to be immunogenic, safe and well tolerated[33]. The next generation LEISH-F3, another recombinant protein vaccine, is currently under investigation in phase 1 clinical trials for VL[32-34]. Mucosal vaccination through oral and intranasal vaccine, using Leishmanial antigen, has shown promise in mice with *L. amazonensis* in protection against developing CL[35]. Additional mechanisms of combining recombinant parasite-derived nucleoside hydrolase with antigens from the sand fly genus *Lutzomyia* for *L. mexicana* CL have also been under investigation with initial successful results[10,33]. Naked DNA vaccines are another new approach that have shown promise in animal models[7,33]. Cloned genes encoding the target proteins are expressed in mammalian plasmids and injected intra-dermally or intramuscularly[7]. Replication within the host leads to expression of the recombinant proteins for longer periods of time in order to sustain a more robust immunologic response[7]. As no pathogenic organisms are used, the potential for infection is non-existent. It is possible that these DNA vaccines may be used therapeutically for CL cure as well[7]. Studies of live-attenuated leishmaniasis and naked DNA vaccines are limited, as vaccine development is still in its early stages. However great strides have been achieved recently in the development of safe, immunogenic vaccines[7].

Lastly, to achieve control of Leishmaniasis, control of animal reservoirs must also be addressed. *L infantum* is a primarily zoonotic disease, affecting millions of dogs around the world, and remains a source of leishmania transmission. To break the cycle of transmission new canine vaccine candidates are also under further investigation[7].

CONCLUSION

Early treatment of leishmaniasis is critical to achieve cure, prevent psychological and social distress, and prevent transmission of disease[17]. Untreated Leishmaniasis - CL, MCL and VL - result in disfiguring scars and high rates of morbidity and mortality in highly endemic regions of the world[11]. Cure rates with available therapeutics are limited due to cost, therapeutic toxicity and the growing rate of resistance[11]. The growing rate of drug resistance amongst all therapeutic options is of particular concern as little is known about the mechanism of resistance[22]. There is an emergent need for development of new therapeutic

options with improved tolerability, improved healing process minimizing scarring, and improved efficacy amongst all *Leishmania spp*[11]. Despite this need, the challenges associated with therapeutic development are vast due to parasite diversity across continents, the complexity of the host response, and the lack of full understanding of protozoa pathogenesis[23]. Gaining greater understanding on the pathogenesis of the disease and the interaction with host immune response might unveil new therapeutic targets, particularly for vaccine development.

REFERENCES

1 **McGwire BS**, Satoskar AR. Leishmaniasis: clinical syndromes and treatment. *QJM* 2014; **107**: 7-14 [PMID: 23744570 DOI: 10.1093/qjmed/hct116]

2 **Stockdale L**, Newton R. A review of preventative methods against human leishmaniasis infection. *PLoS Negl Trop Dis* 2013; **7**: e2278 [PMID: 23818997 DOI: 10.1371/journal.pntd.0002278]

3 **Desjeux P**. The increase in risk factors for leishmaniasis worldwide. *Trans R Soc Trop Med Hyg* 2001; **95**: 239-243 [PMID: 11490989 DOI: 10.1016/S0035-9203(01)90223-8]

4 **Alvar J**, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, den Boer M. Leishmaniasis worldwide and global estimates of its incidence. *PLoS One* 2012; **7**: e35671 [PMID: 22693548 DOI: 10.1371/journal.pone.0035671]

5 **Kumar R**, Engwerda C. Vaccines to prevent leishmaniasis. *Clin Transl Immunology* 2014; **3**: e13 [PMID: 25505961 DOI: 10.1038/cti.2014.4]

6 **World Health Organization**. Leishmaniasis. [accessed 2015 Jan 23]. Available from: URL: http://www.who.int/leishmaniasis/en/

7 **Mutiso JM**, Macharia JC, Kiio MN, Ichagichu JM, Rikoi H, Gicheru MM. Development of Leishmania vaccines: predicting the future from past and present experience. *J Biomed Res* 2013; **27**: 85-102 [PMID: 23554800 DOI: 10.7555/JBR.27.20120064]

8 **Hodiamont CJ**, Kager PA, Bart A, de Vries HJ, van Thiel PP, Leenstra T, de Vries PJ, van Vugt M, Grobusch MP, van Gool T. Species-directed therapy for leishmaniasis in returning travellers: a comprehensive guide. *PLoS Negl Trop Dis* 2014; **8**: e2832 [PMID: 24787001 DOI: 10.1371/journal.pntd.0002832]

9 **Monge-Maillo B**, López-Vélez R. Therapeutic options for old world cutaneous leishmaniasis and new world cutaneous and mucocutaneous leishmaniasis. *Drugs* 2013; **73**: 1889-1920 [PMID: 24170665 DOI: 10.1007/s40265-013-0132-1]

10 **Bacon KM**, Hotez PJ, Kruchten SD, Kamhawi S, Bottazzi ME, Valenzuela JG, Lee BY. The potential economic value of a cutaneous leishmaniasis vaccine in seven endemic countries in the Americas. *Vaccine* 2013; **31**: 480-486 [PMID: 23176979 DOI: 10.1016/j.vaccine.2012.11.032]

11 **Arevalo I**, Tulliano G, Quispe A, Spaeth G, Matlashewski G, Llanos-Cuentas A, Pollack H. Role of imiquimod and parenteral meglumine antimoniate in the initial treatment of cutaneous leishmaniasis. *Clin Infect Dis* 2007; **44**: 1549-1554 [PMID: 17516397 DOI: 10.1086/518172]

12 **Ribeiro de Jesus A**, Luna T, Pacheco de Almeida R, Machado PR, Carvalho EM. Pentoxifylline down modulate in vitro T cell responses and attenuate pathology in Leishmania and HTLV-I infections. *Int Immunopharmacol* 2008; **8**: 1344-1353 [PMID: 18687297 DOI: 10.1016/j.intimp.2008.03.020]

13 **Centers for Disease Control and Prevention**. Leishmaniasis. [accessed 2015 Jan 23]. Available from: URL: http://www.cdc.gov/parasites/leishmaniasis

14 **Rubiano LC**, Miranda MC, Muvdi Arenas S, Montero LM, Rodríguez-Barraquer I, Garcerant D, Prager M, Osorio L, Rojas MX, Pérez M, Nicholls RS, Gore Saravia N. Noninferiority of miltefosine versus meglumine antimoniate for cutaneous leishmaniasis in children. *J Infect Dis* 2012; **205**: 684-692 [PMID: 22238470 DOI: 10.1093/infdis/jir816]

15 **Vélez I**, López L, Sánchez X, Mestra L, Rojas C, Rodríguez E. Efficacy of miltefosine for the treatment of American cutaneous leishmaniasis. *Am J Trop Med Hyg* 2010; **83**: 351-356 [PMID: 20682881 DOI: 10.4269/ajtmh.2010.10-0060]

16 **Bailey MS**. Editorial commentary: local treatments for cutaneous leishmaniasis. *Clin Infect Dis* 2013; **57**: 381-383 [PMID: 23633110 DOI: 10.1093/cid/cit275]

17 **Al-Mutairi N**, Alshiltawy M, El Khalawany M, Joshi A, Eassa BI, Manchanda Y, Gomaa S, Darwish I, Rijhwani M. Tropical medicine rounds: Treatment of Old World cutaneous leishmaniasis with dapsone, itraconazole, cryotherapy, and imiquimod, alone and in combination. *Int J Dermatol* 2009; **48**: 862-869 [PMID: 19673049 DOI: 10.1111/j.1365-4632.2008.04010.x]

18 **Singh N**, Kumar M, Singh RK. Leishmaniasis: current status of available drugs and new potential drug targets. *Asian Pac J Trop Med* 2012; **5**: 485-497 [PMID: 22575984 DOI: 10.1016/S1995-7645(12)60084-4]

19 **Solomon M**, Baum S, Barzilai A, Scope A, Trau H, Schwartz E. Liposomal amphotericin B in comparison to sodium stibogluconate for cutaneous infection due to Leishmania braziliensis. *J Am Acad Dermatol* 2007; **56**: 612-616 [PMID: 17276541 DOI: 10.1016/j.jaad.2006.06.044]

20 **Wortmann G**, Zapor M, Ressner R, Fraser S, Hartzell J, Pierson J, Weintrob A, Magill A. Lipsosomal amphotericin B for treatment of cutaneous leishmaniasis. *Am J Trop Med Hyg* 2010; **83**: 1028-1033 [PMID: 21036832 DOI: 10.4269/ajtmh.2010.10-0171]

21 **Ramanathan R**, Talaat KR, Fedorko DP, Mahanty S, Nash TE. A species-specific approach to the use of non-antimony treatments for cutaneous leishmaniasis. *Am J Trop Med Hyg* 2011; **84**: 109-117 [PMID: 21212212 DOI: 10.4269/ajtmh.2011.10-0437]

22 **Kaur G**, Rajput B. Comparative analysis of the omics technologies used to study antimonial, amphotericin B, and pentamidine resistance in leishmania. *J Parasitol Res* 2014; **2014**: 726328 [PMID: 24900912 DOI: 10.1155/2014/726328]

23 **Reveiz L**, Maia-Elkhoury AN, Nicholls RS, Romero GA, Yadon ZE. Interventions for American cutaneous and mucocutaneous leishmaniasis: a systematic review update. *PLoS One* 2013; **8**: e61843 [PMID: 23637917 DOI: 10.1371/journal.pone.0061843]

24 **Ravis WR**, Llanos-Cuentas A, Sosa N, Kreishman-Deitrick M, Kopydlowski KM, Nielsen C, Smith KS, Smith PL, Ransom JH, Lin YJ, Grogl M. Pharmacokinetics and absorption of paromomycin and gentamicin from topical creams used to treat cutaneous leishmaniasis. *Antimicrob Agents Chemother* 2013; **57**: 4809-4815 [PMID: 23877689 DOI: 10.1128/AAC.00628-13]

25 **Clinical trials.gov.** Phase 3 Study to Evaluate WR 279,396 vs. Paromomycin Alone to Treat Cutaneous Leishmaniasis (in Tunisia) [accessed 2015 Jan 23]. Available from: URL: https://clinicaltrials.gov/ct2/show/NCT00606580

26 **Sosa N**, Capitán Z, Nieto J, Nieto M, Calzada J, Paz H, Spadafora C, Kreishman-Deitrick M, Kopydlowski K, Ullman D, McCarthy WF, Ransom J, Berman J, Scott C, Grogl M. Randomized, double-blinded, phase 2 trial of WR 279,396 (paromomycin and gentamicin) for cutaneous leishmaniasis in Panama. *Am J Trop Med Hyg* 2013; **89**: 557-563 [PMID: 23857024 DOI: 10.4269/ajtmh.12-0736]

27 **de Macedo-Silva ST**, Urbina JA, de Souza W, Rodrigues JC. In vitro activity of the antifungal azoles itraconazole and posaconazole against Leishmania amazonensis. *PLoS One* 2013; **8**: e83247 [PMID: 24376670 DOI: 10.1371/journal.pone.0083247]

28 **Lessa HA**, Machado P, Lima F, Cruz AA, Bacellar O, Guerreiro J, Carvalho EM. Successful treatment of refractory mucosal leishmaniasis with pentoxifylline plus antimony. *Am J Trop Med Hyg* 2001; **65**: 87-89 [PMID: 11508396]

29 **Saenz RE**, Paz H, Berman JD. Efficacy of ketoconazole against Leishmania braziliensis panamensis cutaneous leishmaniasis. *Am J Med* 1990; **89**: 147-155 [PMID: 2166429 DOI: 10.1016/0002-9343(90)90292-L]

30 **Enk CD**, Nasereddin A, Alper R, Dan-Goor M, Jaffe CL, Wulf

HC. Cutaneous leishmaniasis responds to daylight-activated photodynamic therapy: proof of concept for a novel self-administered therapeutic modality. *Br J Dermatol* 2015; **172**: 1364-1370 [PMID: 25363817 DOI: 10.1111/bjd.13490]

31 **Brown M**, Noursadeghi M, Boyle J, Davidson RN. Successful liposomal amphotericin B treatment of Leishmania braziliensis cutaneous leishmaniasis. *Br J Dermatol* 2005; **153**: 203-205 [PMID: 16029352 DOI: 10.1111/j.1365-2133.2005.06670.x]

32 **Thakur A**, Kaur H, Kaur S. Evaluation of the immunoprophylactic potential of a killed vaccine candidate in combination with different adjuvants against murine visceral leishmaniasis. *Parasitol Int* 2015; **64**: 70-78 [PMID: 25316605 DOI: 10.1016/j.parint.2014.10.003]

33 **Beaumier CM**, Gillespie PM, Hotez PJ, Bottazzi ME. New vaccines for neglected parasitic diseases and dengue. *Transl Res* 2013; **162**: 144-155 [PMID: 23578479 DOI: 10.1016/j.trsl.2013.03.006]

34 **Clinical trials.gov**. Phase 1 LEISH-F3 SLA-SE Vaccine Trial in Healthy Adult Volunteers [accessed 2015 Jan 23]. Available from: URL: https://clinicaltrials.gov/ct2/show/NCT02071758.

35 **de Matos Guedes HL**, da Silva Costa BL, Chaves SP, de Oliveira Gomes DC, Nosanchuk JD, De Simone SG, Rossi-Bergmann B. Intranasal vaccination with extracellular serine proteases of Leishmania amazonensis confers protective immunity to BALB/c mice against infection. *Parasit Vectors* 2014; **7**: 448 [PMID: 25239157 DOI: 10.1186/1756-3305-7-448]

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Challenges and opportunities in the treatment of ulcerative colitis

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Abstract

Ulcerative colitis (UC) is an inflammatory destructive disease of the large intestine occurred usually in the rectum and lower part of the colon as well as the entire colon. Drug therapy is not the only choice for UC treatment and medical management should be as a comprehensive whole. Many synthetic drugs are available for the treatment of UC like 5-aminosalicylic acid, oral or systemic corticosteroids, immunomodulator, *etc*. but these drugs are associated with many serious side effects after long term use or have certain disadvantage or not suitable for the use in some patients. In short synthetic drugs have many disadvantages and for this reason effective and safe alternative drug treatment for the UC is the challenge. Herbal drugs are found to be very promising results of the treatment of UC and enzymatic level. Researchers explored many herbal drugs for the treatment and even many more may found effective in the treatment of UC. At this point we feel herbal medicine is the better alternative for the treatment of UC.

Key words: Ulcerative colitis; Herbal drugs; Synthetic drugs

Core tip: Ulcerative colitis (UC) is one of the diseases of gastro intestinal tract having many serious complications. Many synthetic drugs are available for the treatment of UC but they have many serious side effects after long term use. This review presents potential of traditional/

herbal drugs in the treatment of ulcerative colitis. Herbal drugs have great potential with safety which could be better alternative to synthetic drugs.

Nirmal SA, Gangurde SS, Dumbre PS, Pal SC, Mandal SC. Challenges and opportunities in the treatment of ulcerative colitis. *World J Pharmacol* 2015; 4(2): 219-226 Available from: URL: http://www.wjgnet.com/2220-3192/full/v4/i2/219.htm DOI: http://dx.doi.org/10.5497/wjp.v4.i2.219

INTRODUCTION

Ulcerative colitis (UC) is chronic idiopathic inflammatory bowel disorders (IBD) in which patients require lifelong treatment[1]. Corticosteroids, immunosuppressants and antitumour necrosis factor (TNF) antibodies are used in conventional treatment of IBD but due to long term use they are associated with very serious side effects like malignancy[2,3] and this is the reason nowdays patients prefer complementary and alternative medicine (CAM) as it is more safe compared to synthetic drugs. This is proved by a survey conducted to observe patients preference for CAM drugs[4]. CAM treatment involves use of herbal medicine, dietary supplementation like probiotics and prebiotics and acupuncture and moxibustion treatments in IBD patients[5].

SOME FACTS ABOUT UC

Incidence of UC depends on gender, age, and geography[6,7] but men and women have an equal risk for UC. IBD is more prevalent in young people (10-19 years age), but it can occur at any age. Caucasians have more risk than Africans for the incidence of IBD while Asian and South American have lower incidences. Smokers have lower than incidences of UC than other patients. Study showed that smoking protects against UC. Breast feeding reduces risk of UC. Left-handed people have a significantly higher risk for IBDs than others. Persons with history of depression or anxiety have higher risk of UC because depression alters immunity and makes person more susceptible to UC[6,8].

SYMPTOMS OF UC

Ulcerative colitis can be diagnosed by symptoms like fever, fever with chills, loss of appetite, weight loss, impaired growth in children, tenesmus, ulcers and fistulae, recurrent diarrhea[7,9,10], presence of blood is observed in stools, constipation, an ache around the top of the hipbone, or cramps in the middle of the abdomen, bloating, nausea, vomiting, anemia, toxic megacolon, abdominal pain and distention, dehydration, and malnutrition[6,8,11], colon cancer, arthritis, red eyes, vision impairment and diseases of the liver and bile ducts.

SIDE EFFECTS OF SYNTHETIC DRUGS

Both medications and surgery have been used for the treatment of UC[9]. Generally surgery is done for life-threatening complications. As there is no perfect medication for UC, the goals of treatment with synthetic drug is to prevent remissions of UC and to improve the quality of patients life[12,13]. Table 1 summarizes various drugs used for the treatment of UC with their side effects.

INFLAMMATORY MEDIATORS

Humoral immunity is increased in IBD patients that can be confirmed by increased levels of immune globulins (IgG1)[21] and autoantibodies[22,23]. Same thing is evidenced when mucosal plasma cells from UC patients showed increased levels of IgG1[21]. Anticolon and antineutrophil antibodies are observed in UC patients[22,23]. Exaggerated Th2 response - elevated interleukin-5 (IL-5) profile is observed in UC patients[24]. Levels of various cytokines such as IL-1, IL-6, IL-8 and tumor necrosis factor-alpha (TNF-α) are found elevated in IBD[25,26].

OXIDATIVE STRESS IN ULCERATIVE COLITIS

It is observed that level of oxidative stress is increased in the intestinal mucosa of ulcerative colitis patients. Oxidative DNA damage may occur in IDB patients, which can be evidenced by production of 8-hydroxy-deoxyguanosine (8-OHdG). Levels of vitamins A and E are found to reduce in UC patients as compared to normal persons. Mucosal biopsies of UC patients showed increased reactive oxygen intermediates, DNA oxidation products (8-OHdG), and iron in inflamed tissue compared to normal at the same time levels of copper and zinc is found reduced which acts as cofactors for the endogenous antioxidant superoxide dismutase[27]. Reactive oxygen species (ROS) activate nuclear factor-kappa B (NF-κB) which increases production of TNF-α and again TNF-α cause production of ROS. In this way a cycle in induced by increased oxidative stress.

ROLE OF GLYCOSAMINOGLYCANS

Glycosaminoglycans (GAGs) is a important substance that affect permeability and immune/inflammatory reactions of the colon. Altered GAG content in the colon of IBD patients is observed. UC patients showed abnormal distribution of GAGs, with significantly greater amounts of total glycosaminoglycans, heparan sulfate, and hyaluronic acid than normal person[28]. It is hypothesize that change in negatively charged sulfated compounds can affect the passage of substances through the colonic mucosa, leading to leakage of proteins and fluids, thrombosis, and extensive remo-

Table 1 Allopatic drugs used for the treatment of ulcerative colitis and their side effects

Drug	Use	Dosage form	Disadvantages	Ref.
5-aminosalicylic acid compounds	Treatment of tissue inflammation	Enema or orally	Enema solution cannot reach high enough to treat inflammation in the upper colon and if taken orally, however, the stomach and upper small intestine absorb most of the drug before it reaches the colon	[13]
Sulfasalazine	Decreasing intestinal inflammation and relieving symptoms of abdominal pain, diarrhea, and rectal bleeding	Orally	Nausea, heartburn, headache, anemia, skin rashes, and in rare instances, hepatitis and kidney inflammation. In men, sulfasalazine can reduce the sperm count	[14]
Olsalazine (consists of two 5-ASA molecules linked together)	Decreasing intestinal inflammation and relieving symptoms of abdominal pain, diarrhea, and rectal bleeding	Orally	Secretory diarrhea	[15]
Corticosteroids (prednisone, prednisolone, hydrocortisone, *etc.*)	Corticosteroids do not require direct contact with inflamed intestinal tissues to be effective. These are potent anti-inflammatory agents	Orally, intravenous	Some patients become corticosteroid dependent and consistently develop symptoms of colitis Whenever the corticosteroid dose is below a certain level. Common side effects include rounding of the face (moon face), acne, increased body hair, diabetes, weight gain, high blood pressure, cataracts, glaucoma, increased susceptibility to infections, muscle weakness, depression, insomnia, mood swings, personality changes, irritability, and thinning of bones (osteoporosis) with an accompanying increased risk of compression fractures of the spine. Children on corticosteroids can experience stunted growth	[16]
Immunomodulator medications	Immunomodulators decrease tissue inflammation by reducing the population of immune cells and/or by interfering with their production of proteins that promote immune activation and inflammation	Orally	Risks of infection due to weakened immunity	[17]
Azathoprine and 6-Mercaptopurine	These are used mainly in the following situations, UC and CD patients with severe diseases not responding to corticosteroids, patients experiencing undesirable corticosteroid-related side effects, patients dependent on corticosteroids and unable to discontinue them without developing relapses		Increased vulnerability to infections, inflammation of the liver (hepatitis) and pancreas (pancreatitis), and bone marrow toxicity (interfering with the formation of cells that circulate in the blood). 6-MP can reduce the sperm count in men When the partners of male patients on 6-MP conceive, there is a higher incidence of miscarriages and vaginal bleeding. There also are respiratory difficulties in the newborn	[18]
Methotrexate	Helpful in treating patients with moderate to severe CD who neither respond to 6-MP and azathioprine nor tolerate these two medications	Orally or intramuscular	Development of liver cirrhosis when the medication is given over a prolonged period of time (years). Low white blood cell counts and inflammation of the lungs. Methotrexate should not be used in pregnancy	[19]
Cyclosporine	Treatment of severe UC. It is useful in fulminant UC and severely ill patients who do not respond to systemic corticosteroids	Orally as well as intravenously	High blood pressure, renal function impairment, tingling sensations in the extremities, anaphylactic shock and seizures	[20]

5-ASA: 5-aminosalicylic acid; UC: Ulcerative colitis; CD: Crohn's disease.

deling observed in UC and IBD[29]. These changes leads to the inflammatory process as hyaluronic acid can interact directly with lymphocytes, inhibit macrophage response to cytokines, and increase phagocytosis. GAG content has been associated with alteration in the distribution of macrophages reactive to TNF-α[30].

ROLE OF HERBAL MEDICINES IN THE TREATMENT OF ULCERATIVE COLITIS

Herbal medicine means use of folk and/or traditional medicinal practice by using plants and/or plant extracts for the treatment of various diseases or disorders. Eighty percent population from developing country depends on herbal medicines for the treatment of their diseases. Recent studies showed that about 20%-26% patients use TCM therapies for the treatment of GI symptoms and another observation is that generally patients having chronic GI disorders use or prefer TCM therapies[31,32]. This use of TCM for the treatment of IBD is spread to Western world and in many Asian countries[33]. In ancient times Chinese people's are using herbals for the treatment of UC and this is evidenced by Chinese literature. The main intention of using herbals for the treatment of UC was the belief that natural drugs are safe and around 30% peoples think that herbal preparations don't cause any harm or side effects. Herbal drugs contains numerous chemicals and that is the reason its effect is unpredictable[34]. Today, TCM is most popular way of treatment for UC in Eastern Asian

Table 2 Medicinal plants used for the treatment of ulcerative colitis with their mechanism of action

Sr. No.	Plant	Mechanism	Ref.
1	*Acacia ferruginea*	Acacia ferruginea extract significantly resist UC *via* modulation of oxidant/anti-oxidant balance and inhibition of inflammatory mediators. Extract inhibited the activation and translocation of transcription factors (NF-κB subunits (p65/p50)	[37]
2	*Passiflora edulis*	Passiflora edulis peel can significantly resist 2,4,6-trinitrobenzenesulphonic acid-induced ulcerative colitis by modulating microbiota and could be used as a source of fiber and polyphenols in the prevention of oxidative stress through the improvement of serum and tissue antioxidant status	[38]
3	*Arnebia euchroma*	Effectiveness of a hydroxynaphthoquinone fraction from Arnebia euchroma was evaluated with TNBS-induced UC. The underlying mechanism may be associated with TNF-α inhibition in colonic tissue with the dose of 10 mg/kg	[39]
4	*Cannabis*	Cannabis had been used successfully to relieve the symptoms associated with IBD by inhalational route	[40]
5	*Rhizophora apiculata*	R. apiculata significantly resist acetic acid induced colitis in experimental mouse model by increasing anti-oxidant enzymes such as SOD and GSH and reducing LPO, NO and inflammatory mediators such as MPO, LDH, iNOS, COX-2 and TNF-α expressions. And also inhibits the translocation of NF-κB p65 and p50 subunits	[41]
6	*Hymenaea stigonocarpa*	Total flavonoid of stem bark extract and fruit pulp flour of Hymenaea stigonocarpa significantly resist TNBS-induced colonic damage in rats. Its mechanism may be related to inhibition of MPO and AP activities, reduction in colon MDA content, and counteraction of GSH depletion induced by inflammatory process	[42]
7	*Helicteres angustifolia*	Aqueous extract of Helicteres angustifolia significantly resist UC induced by TNBS by keeping balance of inflammatory factors in blood and also by improving increasing expression of IL-10 and decreasing the expressions of IL-6 and TNF-α	[43]
8	*Ilex kudingcha*	Methanol extracts of Ilex kudingcha increased glutathione and reduced colonic myeloperoxidase and malondialdehyde levels in the colon tissue and prevented edema, mucosal damage and loss of crypts. It also decreased anti-inflammatory effects by decreasing the levels of TNF-α, IL-1β and IL-6 in the colon tissues on 3% DSS-induced UC in mice	[44]
9	*Peony*	TGP showed positive effect in 2,4,6-trinitrobenzene sulfonic acid TNBS/ethanol-induced colitis by significant improvements of DAI, CMDI, HPS, and MPO activity. Moreover, administration of TGP (50 or 100 mg/kg per day) decreased the up-regulated levels of serum TNF-α and IL-1β, and expression of TNF-α and IL-1β mRNA and protein in colonic tissues, and increased the serum IL-10 and colonic IL-10 mRNA and protein level	[45]
10	*Olea europaea*	Total phenolic secoiridoid of oleuropein, the major phenolic secoiridoid in Olea europaea significantly resisted dextran sodium sulfate-induced chronic colitis in mice. It decreased inflammatory cell and released the inflammatory cytokines IL-1β and IL-6 with increased IL-10 levels in colon tissue. The anti-inflammatory mechanism of oleuropein was associated with the suppression of the phosphorylation of p38 mitogen-activated protein kinase and might be mediated by up-regulation of annexin A1	[46]
11	*Plumbagin plants*	Naphthoquinone constituent of Plumbagin plants significantly resist ulcerative colitis in mice by reducing the levels of proinflammatory cytokines (TNF-α, IFN-γ, and IL-17) but cytokine levels remained unaffected with restoration of goblet cells in colon of mice	[47]
12	*Euphorbia granuleta*	Alcohol extracts of Euphorbia granuleta (Euphorpiaceae) significantly resist UC by reducing the pro-inflammatory cytokine TNF-α and colonic MDA contents	[48]
13	*Amaranthus roxburghianus*	Combination of hydroalcoholic extract of roots of A. roxburghianus and piperine showed minimal ulceration, hemorrhage, necrosis and leucocyte infiltration and significantly reduced malondialdehyde and myeloperoxidase levels and increased glutathione levels in blood and tissue	[49]
14	*Curcumin*	Oral curcumin extract decreases colon injury with decreased inflammatory reactions, lipid peroxidation, apoptotic cell death, and modulating p38- and JNK-MAPK pathways in acetic acid-induced colitis	[50]
15	*Bauhinia tomentosa*	Extract of Bauhinia tomentosa significantly inhibit colitis by regulating the antioxidant and inflammatory mediators with decreasing myeloperoxidase, tumor necrosis factor and inducible nitric oxide synthase expression on acetic acid induced ulcerative colitis	[51]
16	*Cistanche tubulosa*	Echinacoside enriched extract of Cistanche tubulosa significantly resist DSS-induced colitis by protecting intestinal epithelium from inflammatory injury and by upregulating TGF-β1 which enhanced *in vitro* wound healing activity as well as with an increase in the number of Ki67(+) proliferating cells in diseased colons	[52]
17	*Garcinia kola*	A natural biflavonoid, kolaviron from the seeds of Garcinia kola significantly increase the inflammatory mediators, IL-1β and tumour necrosis factor alpha, in the colon of DSS-induced ulcerative colitis in rats The anti-colitis effect of kolaviron is related to its intrinsic anti-inflammatory and anti-oxidative properties	[53]
18	*Marine mangrove Avicennia marina*	Marine mangrove Avicennia marina extract significantly decreased the colonic lipid peroxides, glutathione peroxidase, serum nitric oxide and significantly increased the colonic and erythrocyte superoxide dismutase and glutathione levels against acetic acid-induced colitis due to the presence of higher levels of decanoic acid, DEHA, pentanoic acid, pyrrolidine, 4-chlorophenyl, thiazolidinones, and arabinopyranoside (flavonoid)	[54]
19	*Soy extract*	FSG with standardized isoflavone and stable BBI content exert cumulative or synergistic protection based on protease inhibition and ER-ligand activity in colitic rats. It improved the severity of colitis, by decreasing the TNBS-induced rise in gut permeability, visceral sensitivity, faecal proteolytic activity and PAR-2 expression at all post-TNBS points	[55]

20	*Aegle marmelos*	Effect of Aegle marmelos unripe fruit extract was studied on acetic acid induced ulcerative colitis and indomethacin-induced enterocolitis in Wistar albino rats. Its mechanism is related with protection in mast cell degranulation by significantly decreasing the MDA levels and increased SOD activity. Extract produced anti-inflammatory, antioxidant, and mast cell stabilizing effects demonstrating protective effect in inflammatory bowel disease	[56]
21	*Andrographis paniculata*	A. panculata extract at a dose of 1800 mg daily were found to be effective in the treatment of ulcerative colitis	[57]
22	*Terminalia chebula*	Fruit pulp of Terminalia chebula TCE (600 mg/kg) shows healing effect against acetic acid induced colitis in rats. TCE showed antibacterial activity and both TCE and SS enhanced the antioxidants, but decreased free radicals and myeloperoxidase activities affected in acetic acid-induced colitis	[58]
23	*American ginseng*	HAG cause apoptosis and suppress mouse colitis through a p53-mediated mechanism. HAG might be very effective in targeting the inflammatory cells and cancer cells since it induces apoptosis of inflammatory cells and cell cycle arrest in both p53-/- and WT p53 colon cancer cells	[59]
24	*Vaccinium myrtillus*	Anthocyanins from Vaccinium myrtillus were shown to have antioxidative and anti-inflammatory effects are useful in the treatment of UC	[60]
25	*Moringa oleifera and Citrus sinensis*	Results show that a combination of Moringa oleifera root extracts with Citrus sinensis fruit rind extract is effective in the treatment of UC and results are comparable with the standard drug prednisolone. The combination reduced the levels of MPO and MDA in blood and tissue	[61]
26	*Lavandula intermedia*	Lavandula intermedia protected acute colitis in a mouse model of caused by Citrobacter rodentium. It reduced intestinal tissue damage, and decreased infiltration of neutrophils and macrophages, with reduced levels of TNF-α, IFN-γ, IL-22, macrophage inflammatory protein-2α, and inducible nitric oxide synthase expression	[62]
27	*Chelidonium majus*	The CA, a constituent of Chelidonium majus L., has protective effects against DSS-induced UC. CA was found to regulate levels of IL-6 and tumor necrosis factor-α in serum. In colonic tissues, prostaglandin E(2) production levels and COX-2 and HIF-1α expression levels were increased by DSS, but CA attenuated increases in COX-2 and HIF-1α levels	[63]
28	*Boswellia serrata*	Extracts of the plant Boswellia serrata showed significant reduction in lipid peroxidation and SOD level and GPx glutathione level increased significantly. The activity might be due to presence of antioxidant substances	[64]
29	*Apple polyphenol extract*	Apple extract treatment reduced the severity of colitis. Apple polyphenols reduced the degradation of tissue transglutaminase protein occurring through calpain action. The efficacy of apple extract is mediated by its effects on COX-2 and TNF-α. The unbalance between calpain and tissue transglutaminase may play a role in colonic damage and future therapeutic interventions in ulcerative colitis can target this mechanisms	[65]
30	*Cordia dichotoma*	The bark of Cordia dichotoma f. (Boraginaceae) was used in the treatment of ulcerative colitis. Apigenin (5 mg/kg, *p.o.*) isolated from methanol extract of C. dichotoma bark showed significant healing and reduction in inflammatory enzymes when screened for UC	[66,67]
31	*Vitex negundo*	Ethanol extract of Vitex negundo Linn. (Verbenaceae) root is effective in the treatment of UC. Extract reduced MPO and MDA levels in blood and tissue	[68]

UC: Ulcerative colitis; TNBS: 2,4,6-trinitrobenzene sulfonic acid; IBD: Inflammatory bowel disorders; SOD: Superoxide dismutase; GSH: Glutathione; LPO: Lipid peroxides; NO: Nitric oxide; MPO: Myeloperoxidase; LDH: Lactate dehydrogenase; iNOS: Nitric oxide synthase; COX-2: Cyclooxygenase-2; TNF-α: Tumor necrosis factor-α; AP: Alkaline phosphatase; MDA: Malondialdehyde; DSS: Dextran sulfate sodium; TGP: Total glucosides of peony; CMDI: Colon macroscopic damage index; DAI: Disease activity index; HPS: Histopathological score; IL: Interleukin; TGF: Transforming growth factor; DEHA: Diethylhydroxylamine; FSG: Fermented soy germ extract; BBI: Bowman-birk bnhibitors; ER: Estrogen receptor; HAG: Hexane fraction of American ginseng; CA: Chelidonic acid; HIF-1α: Hypoxia induced factor-1α; GPx: Glutathione peroxidase.

countries. Slippery elm, fenugreek, devil's claw, Mexican yam, tormentil, and Wei tong ning (a TCM) are some of the herbal remedies for the treatment of IBD[34,35]. Chen *et al*[36] compared TCM and synthetic drugs for the management of UC and result showed that 118 cases of UC patients were treated with integration of TCM and 86 cases of UC were treated with prednisone as controls (Table 2).

CONCLUSION

Ulcerative colitis is a chronic disease condition in which patients need to be treated with medicines throughout their lives to either to prevent relapse or to reduce other threats. This is the way by which quality of life of patients suffering from UC can be improved. Many synthetic drugs prescribed for the treatment of UC are associated with large side effects. Large number of herbal medicines is available with promising results for the treatment of UC. Now it is proved that herbal medicines and TCM can treat conditions of ulcerative colitis. We feel it is the duty of physicians to guide UC patients to inform them regarding availability of TCM treatment which is more effective and safe. These herbal medicines have opened new avenues for the treatment of UC. Thus we feel that herbal medicines are better option for the treatment of UC.

REFERENCES

1 **Kane SV**, Robinson A. Review article: understanding adherence to medication in ulcerative colitis - innovative thinking and evolving concepts. *Aliment Pharmacol Ther* 2010; **32**: 1051-1058 [PMID: 20815833 DOI: 10.1111/j.1365-2036.2010.04445]

2 **Toruner M**, Loftus EV, Harmsen WS, Zinsmeister AR, Orenstein R, Sandborn WJ, Colombel JF, Egan LJ. Risk factors for opportunistic infections in patients with inflammatory bowel disease.

Gastroenterology 2008; **134**: 929-936 [PMID: 18294633 DOI: 10.1053/j.gastro.2008.01.012]
3 **Lichtenstein GR**, Rutgeerts P, Sandborn WJ, Sands BE, Diamond RH, Blank M, Montello J, Tang L, Cornillie F, Colombel JF. A pooled analysis of infections, malignancy, and mortality in infliximab- and immunomodulator-treated adult patients with inflammatory bowel disease. *Am J Gastroenterol* 2012; **107**: 1051-1063 [PMID: 22613901 DOI: 10.1038/ajg.2012.89]
4 **Fernández A**, Barreiro-de Acosta M, Vallejo N, Iglesias M, Carmona A, González-Portela C, Lorenzo A, Domínguez-Muñoz JE. Complementary and alternative medicine in inflammatory bowel disease patients: frequency and risk factors. *Dig Liver Dis* 2012; **44**: 904-908 [PMID: 22795615 DOI: 10.1016/j.dld.2012.06.008]
5 **Hilsden RJ**, Verhoef MJ, Rasmussen H, Porcino A, DeBruyn JC. Use of complementary and alternative medicine by patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2011; **17**: 655-662 [PMID: 20848543 DOI: 10.1002/ibd.21360]
6 **van Staa TP**, Cooper C, Brusse LS, Leufkens H, Javaid MK, Arden NK. Inflammatory bowel disease and the risk of fracture. *Gastroenterology* 2003; **125**: 1591-1597 [PMID: 14724810 DOI: 10.1053/j.gastro.2003.09.027]
7 **Thuraisingam A**, Leiper K. Medical management of ulcerative colitis. *Hosp Med* 2003; **64**: 703-707 [PMID: 14702780 DOI: 10.12968/hosp.2003.64.12.2360]
8 **Diculescu M**, Ciocîrlan M, Ciocîrlan M, Pițigoi D, Becheanu G, Croitoru A, Spanache S. Folic acid and sulfasalazine for colorectal carcinoma chemoprevention in patients with ulcerative colitis: the old and new evidence. *Rom J Gastroenterol* 2003; **12**: 283-286 [PMID: 14726972]
9 **Raychaudhuri SP**, Raychaudhuri SK. Role of NGF and neurogenic inflammation in the pathogenesis of psoriasis. *Prog Brain Res* 2004; **146**: 433-437 [PMID: 14699978 DOI: 10.1016/S0079-6123(03]
10 **Lichtenstein GR**. Evaluation of bone mineral density in inflammatory bowel disease: current safety focus. *Am J Gastroenterol* 2003; **98**: S24-S30 [PMID: 14697915 DOI: 10.1016/j.amjgastroenterol.2003.11.003]
11 **Winther KV**, Jess T, Langholz E, Munkholm P, Binder V. Survival and cause-specific mortality in ulcerative colitis: follow-up of a population-based cohort in Copenhagen County. *Gastroenterology* 2003; **125**: 1576-1582 [PMID: 14724807 DOI: 10.1053/j.gastro.2003.09.036]
12 **Solem CA**, Loftus EV, Tremaine WJ, Sandborn WJ. Venous thromboembolism in inflammatory bowel disease. *Am J Gastroenterol* 2004; **99**: 97-101 [PMID: 14687149 DOI: 10.1046/j.1572-0241.2003.04026.x]
13 **Russinko PJ**, Agarwal S, Choi MJ, Kelty PJ. Obstructive nephropathy secondary to sulfasalazine calculi. *Urology* 2003; **62**: 748 [PMID: 14550462 DOI: 10.1016/S0090-4295(03]
14 **Loftus EV**, Kane SV, Bjorkman D. Systematic review: short-term adverse effects of 5-aminosalicylic acid agents in the treatment of ulcerative colitis. *Aliment Pharmacol Ther* 2004; **19**: 179-189 [PMID: 14723609 DOI: 10.1111/j.0269-2813.2004.01827.x]
15 **Edmond LM**, Hopkins MJ, Magee EA, Cummings JH. The effect of 5-aminosalicylic acid-containing drugs on sulfide production by sulfate-reducing and amino acid-fermenting bacteria. *Inflamm Bowel Dis* 2003; **9**: 10-17 [PMID: 12656132 DOI: 10.1097/00054725-200301000-00002]
16 **Card T**, West J, Hubbard R, Logan RF. Hip fractures in patients with inflammatory bowel disease and their relationship to corticosteroid use: a population based cohort study. *Gut* 2004; **53**: 251-255 [PMID: 14724159 DOI: 10.1136/gut.2003.026799]
17 **Corominas H**, Baiget M. Clinical utility of thiopurine S-methyltransferase genotyping. *Am J Pharmacogenomics* 2004; **4**: 1-8 [PMID: 14987117 DOI: 10.2165/00129785-200404010-00001]
18 **Menachem Y**, Gotsman I. Clinical manifestations of pyoderma gangrenosum associated with inflammatory bowel disease. *Isr Med Assoc J* 2004; **6**: 88-90 [PMID: 14986464]
19 **Feagan BG**. Maintenance therapy for inflammatory bowel disease. *Am J Gastroenterol* 2003; **98**: S6-S17 [PMID: 14697913 DOI: 10.1016/j.amjgastroenterol.2003.11.002]
20 **Keven K**, Sahin M, Kutlay S, Sengul S, Erturk S, Ersoz S, Erbay B. Immunoglobulin deficiency in kidney allograft recipients: comparative effects of mycophenolate mofetil and azathioprine. *Transpl Infect Dis* 2003; **5**: 181-186 [PMID: 14987202 DOI: 10.1111/j.1399-3062.2003.00035.x]
21 **Kett K**, Rognum TO, Brandtzaeg P. Mucosal subclass distribution of immunoglobulin G-producing cells is different in ulcerative colitis and Crohn's disease of the colon. *Gastroenterology* 1987; **93**: 919-924 [PMID: 3308623]
22 **Das KM**, Dasgupta A, Mandal A, Geng X. Autoimmunity to cytoskeletal protein tropomyosin. A clue to the pathogenetic mechanism for ulcerative colitis. *J Immunol* 1993; **150**: 2487-2493 [PMID: 8450225]
23 **Duerr RH**, Targan SR, Landers CJ, Sutherland LR, Shanahan F. Anti-neutrophil cytoplasmic antibodies in ulcerative colitis. Comparison with other colitides/diarrheal illnesses. *Gastroenterology* 1991; **100**: 1590-1596 [PMID: 2019365]
24 **Blumberg RS**, Strober W. Prospects for research in inflammatory bowel disease. *JAMA* 2001; **285**: 643-647 [PMID: 11176874 DOI: 10.1001/jama.285.5.643]
25 **Mahida YR**, Ceska M, Effenberger F, Kurlak L, Lindley I, Hawkey CJ. Enhanced synthesis of neutrophil-activating peptide-1/interleukin-8 in active ulcerative colitis. *Clin Sci* (Lond) 1992; **82**: 273-275 [PMID: 1312411]
26 **MacDonald TT**, Murch SH. Aetiology and pathogenesis of chronic inflammatory bowel disease. *Baillieres Clin Gastroenterol* 1994; **8**: 1-34 [PMID: 8003737 DOI: 10.1016/S0950-3528(06]
27 **Lih-Brody L**, Powell SR, Collier KP, Reddy GM, Cerchia R, Kahn E, Weissman GS, Katz S, Floyd RA, McKinley MJ, Fisher SE, Mullin GE. Increased oxidative stress and decreased antioxidant defenses in mucosa of inflammatory bowel disease. *Dig Dis Sci* 1996; **41**: 2078-2086 [PMID: 8888724 DOI: 10.1007/BF02093613]
28 **Symonds DA**. The glycosaminoglycans of the human colon in inflammatory and neoplastic conditions. *Arch Pathol Lab Med* 1978; **102**: 146-149 [PMID: 580362]
29 **Murch SH**, MacDonald TT, Walker-Smith JA, Levin M, Lionetti P, Klein NJ. Disruption of sulphated glycosaminoglycans in intestinal inflammation. *Lancet* 1993; **341**: 711-714 [PMID: 8095623 DOI: 10.1016/0140-6736(93]
30 **Vantrappen G**, Geboes K. Glycosaminoglycans and the gut. *Lancet* 1993; **341**: 730-731 [PMID: 8095632]
31 **Comar KM**, Kirby DF. Herbal remedies in gastroenterology. *J Clin Gastroenterol* 2005; **39**: 457-468 [PMID: 15942431 DOI: 10.1097/01.mcg.0000165650.09500.3a]
32 **Tillisch K**. Complementary and alternative medicine for gastrointestinal disorders. *Clin Med* 2007; **7**: 224-227 [PMID: 17633940 DOI: 10.7861/clinmedicine.7-3-224]
33 **D'Inca R**, Garribba AT, Vettorato MG, Martin A, Martines D, Di Leo V, Buda A, Sturniolo GC. Use of alternative and complementary therapies by inflammatory bowel disease patients in an Italian tertiary referral centre. *Dig Liver Dis* 2007; **39**: 524-529 [PMID: 17433794 DOI: 10.1016/j.dld.2007.03.001]
34 **Langmead L**, Rampton DS. Review article: complementary and alternative therapies for inflammatory bowel disease. *Aliment Pharmacol Ther* 2006; **23**: 341-349 [PMID: 16422993 DOI: 10.1111/j.1365-2036.2006.02761.x]
35 **Langmead L**, Makins RJ, Rampton DS. Anti-inflammatory effects of aloe vera gel in human colorectal mucosa in vitro. *Aliment Pharmacol Ther* 2004; **19**: 521-527 [PMID: 14987320 DOI: 10.1111/j.1365-2036.2004.01874.x]
36 **Chen Q**, Zhang H. Clinical study on 118 cases of ulcerative colitis treated by integration of traditional Chinese and Western medicine. *J Tradit Chin Med* 1999; **19**: 163-165 [PMID: 10921142]
37 **Sakthivel KM**, Guruvayoorappan C. Protective effect of Acacia ferruginea against ulcerative colitis via modulating inflammatory mediators, cytokine profile and NF-κB signal transduction pathways. *J Environ Pathol Toxicol Oncol* 2014; **33**: 83-98 [PMID: 24941292 DOI: 10.1615/JEnvironPatholToxicolOncol.2014008425]

38 **Cazarin CB**, da Silva JK, Colomeu TC, Batista AG, Vilella CA, Ferreira AL, Junior SB, Fukuda K, Augusto F, de Meirelles LR, Zollner Rde L, Junior MR. Passiflora edulis peel intake and ulcerative colitis: approaches for prevention and treatment. *Exp Biol Med* (Maywood) 2014; **239**: 542-551 [PMID: 24623393 DOI: 10.1177/1535370214525306]

39 **Fan HY**, Zhang ZL, Liu K, Yang MY, Lv WH, Che X, Xu H, Song WW. Effectiveness of a hydroxynaphthoquinone fraction from Arnebia euchroma in rats with experimental colitis. *World J Gastroenterol* 2013; **19**: 9318-9327 [PMID: 24409058 DOI: 10.3748/wjg.v19.i48.9318]

40 **Storr M**, Devlin S, Kaplan GG, Panaccione R, Andrews CN. Cannabis use provides symptom relief in patients with inflammatory bowel disease but is associated with worse disease prognosis in patients with Crohn's disease. *Inflamm Bowel Dis* 2014; **20**: 472-480 [PMID: 24407485 DOI: 10.1097/01.MIB.0000440982.79036.d6]

41 **V VP**, C G. Protective effect of marine mangrove Rhizophora apiculata on acetic acid induced experimental colitis by regulating anti-oxidant enzymes, inflammatory mediators and nuclear factor-kappa B subunits. *Int Immunopharmacol* 2014; **18**: 124-134 [PMID: 24269623 DOI: 10.1016/j.intimp.2013.11.007]

42 **Orsi PR**, Seito LN, Di Stasi LC. Hymenaea stigonocarpa Mart. ex Hayne: A tropical medicinal plant with intestinal anti-inflammatory activity in TNBS model of intestinal inflammation in rats. *J Ethnopharmacol* 2014; **151**: 380-385 [PMID: 24211392 DOI: 10.1016/j.jep.2013.10.056]

43 **Gao YQ**, Su D, Hu Y, Lin H, Zhang WX, Dai WB. Effect of Helicteres angustifolia on rats with ulcerative colitis. *Zhongyaocai* 2013; **36**: 597-600 [PMID: 24134009]

44 **Song JL**, Qian Y, Li GJ, Zhao X. Anti-inflammatory effects of kudingcha methanol extract (Ilex kudingcha C.J. Tseng) in dextran sulfate sodium-induced ulcerative colitis. *Mol Med Rep* 2013; **8**: 1256-1262 [PMID: 23969782 DOI: 10.3892/mmr.2013.1635]

45 **Zhang Y**, Zhou R, Zhou F, Cheng H, Xia B. Total glucosides of peony attenuates 2,4,6-trinitrobenzene sulfonic acid/ethanol-induced colitis in rats through adjustment of TH1/TH2 cytokines polarization. *Cell Biochem Biophys* 2014; **68**: 83-95 [PMID: 23771723 DOI: 10.1007/s12013-013-9696-3]

46 **Giner E**, Recio MC, Ríos JL, Giner RM. Oleuropein protects against dextran sodium sulfate-induced chronic colitis in mice. *J Nat Prod* 2013; **76**: 1113-1120 [PMID: 23758110 DOI: 10.1021/np400175b]

47 **Pile JE**, Navalta JW, Davis CD, Sharma NC. Interventional effects of plumbagin on experimental ulcerative colitis in mice. *J Nat Prod* 2013; **76**: 1001-1006 [PMID: 23742275 DOI: 10.1021/np3008792]

48 **Awaad AS**, El-Meligy RM, Al-Jaber NA, Al-Muteeri HS, Zain ME, Alqasoumi SI, Alafeefy AM, Donia Ael R. Anti-ulcerative colitis activity of compounds from Euphorbia granuleta Forssk. *Phytother Res* 2013; **27**: 1729-1734 [PMID: 23580316 DOI: 10.1002/ptr.4985]

49 **Nirmal SA**, Ingale JM, Pattan SR, Bhawar SB. Amaranthus roxburghianus root extract in combination with piperine as a potential treatment of ulcerative colitis in mice. *J Integr Med* 2013; **11**: 206-212 [PMID: 23570686 DOI: 10.3736/jintegrmed2013022]

50 **Topcu-Tarladacalisir Y**, Akpolat M, Uz YH, Kizilay G, Sapmaz-Metin M, Cerkezkayabekir A, Omurlu IK. Effects of curcumin on apoptosis and oxidoinflammatory regulation in a rat model of acetic acid-induced colitis: the roles of c-Jun N-terminal kinase and p38 mitogen-activated protein kinase. *J Med Food* 2013; **16**: 296-305 [PMID: 23566056 DOI: 10.1089/jmf.2012.2550]

51 **Kannan N**, Guruvayoorappan C. Protective effect of Bauhinia tomentosa on acetic acid induced ulcerative colitis by regulating antioxidant and inflammatory mediators. *Int Immunopharmacol* 2013; **16**: 57-66 [PMID: 23538025 DOI: 10.1016/j.intimp.2013.03.008]

52 **Jia Y**, Guan Q, Jiang Y, Salh B, Guo Y, Tu P, Du C. Amelioration of dextran sulphate sodium-induced colitis in mice by echinacoside-enriched extract of Cistanche tubulosa. *Phytother Res* 2014; **28**: 110-119 [PMID: 23512684 DOI: 10.1002/ptr.4967]

53 **Farombi EO**, Adedara IA, Ajayi BO, Ayepola OR, Egbeme EE. Kolaviron, a natural antioxidant and anti-inflammatory phytochemical prevents dextran sulphate sodium-induced colitis in rats. *Basic Clin Pharmacol Toxicol* 2013; **113**: 49-55 [PMID: 23336970 DOI: 10.1111/bcpt.12050]

54 **Rise CL**, Prabhu VV, Guruvayoorappan C. Effect of marine mangrove Avicennia marina (Forssk.) Vierh against acetic acid-induced ulcerative colitis in experimental mice. *J Environ Pathol Toxicol Oncol* 2012; **31**: 179-192 [PMID: 23216642 DOI: 10.1615/JEnvironPatholToxicolOncol.v31.i2.90]

55 **Moussa L**, Bézirard V, Salvador-Cartier C, Bacquié V, Lencina C, Lévêque M, Braniste V, Ménard S, Théodorou V, Houdeau E. A low dose of fermented soy germ alleviates gut barrier injury, hyperalgesia and faecal protease activity in a rat model of inflammatory bowel disease. *PLoS One* 2012; **7**: e49547 [PMID: 23166707 DOI: 10.1371/journal.pone.0049547]

56 **Behera JP**, Mohanty B, Ramani YR, Rath B, Pradhan S. Effect of aqueous extract of Aegle marmelos unripe fruit on inflammatory bowel disease. *Indian J Pharmacol* 2012; **44**: 614-618 [PMID: 23112424 DOI: 10.4103/0253-7613.100389]

57 **Sandborn WJ**, Targan SR, Byers VS, Rutty DA, Mu H, Zhang X, Tang T. Andrographis paniculata extract (HMPL-004) for active ulcerative colitis. *Am J Gastroenterol* 2013; **108**: 90-98 [PMID: 23044768 DOI: 10.1038/ajg.2012.340]

58 **Gautam MK**, Goel S, Ghatule RR, Singh A, Nath G, Goel RK. Curative effect of Terminalia chebula extract on acetic acid-induced experimental colitis: role of antioxidants, free radicals and acute inflammatory marker. *Inflammopharmacology* 2013; **21**: 377-383 [PMID: 22956243 DOI: 10.1007/s10787-012-0147-3]

59 **Poudyal D**, Cui X, Mai Le P, Davis T, Hofseth AB, Jin Y, Chumanevich AA, Wargovich MJ, Nagarkatti M, Nagarkatti PS, Windust A, Hofseth LJ. A limited role of p53 on the ability of a Hexane fraction of American ginseng to suppress mouse colitis. *J Biomed Biotechnol* 2012; **2012**: 785739 [PMID: 22899889 DOI: 10.1155/2012/785739]

60 **Biedermann L**, Mwinyi J, Scharl M, Frei P, Zeitz J, Kullak-Ublick GA, Vavricka SR, Fried M, Weber A, Humpf HU, Peschke S, Jetter A, Krammer G, Rogler G. Bilberry ingestion improves disease activity in mild to moderate ulcerative colitis - an open pilot study. *J Crohns Colitis* 2013; **7**: 271-279 [PMID: 22883440 DOI: 10.1016/j.crohns.2012.07.010]

61 **Gholap PA**, Nirmal SA, Pattan SR, Pal SC, Mandal SC. Potential of Moringa oleifera root and Citrus sinensis fruit rind extracts in the treatment of ulcerative colitis in mice. *Pharm Biol* 2012; **50**: 1297-1302 [PMID: 22849565 DOI: 10.3109/13880209.2012.674142]

62 **Baker J**, Brown K, Rajendiran E, Yip A, DeCoffe D, Dai C, Molcan E, Chittick SA, Ghosh S, Mahmoud S, Gibson DL. Medicinal lavender modulates the enteric microbiota to protect against Citrobacter rodentium-induced colitis. *Am J Physiol Gastrointest Liver Physiol* 2012; **303**: G825-G836 [PMID: 22821949 DOI: 10.1152/ajpgi.00327]

63 **Kim DS**, Kim SJ, Kim MC, Jeon YD, Um JY, Hong SH. The therapeutic effect of chelidonic acid on ulcerative colitis. *Biol Pharm Bull* 2012; **35**: 666-671 [PMID: 22687399 DOI: 10.1248/bpb.35.666]

64 **Hartmann RM**, Morgan Martins MI, Tieppo J, Fillmann HS, Marroni NP. Effect of Boswellia serrata on antioxidant status in an experimental model of colitis rats induced by acetic acid. *Dig Dis Sci* 2012; **57**: 2038-2044 [PMID: 22451119 DOI: 10.1007/s10620-012-2134-3]

65 **D'Argenio G**, Mazzone G, Tuccillo C, Ribecco MT, Graziani G, Gravina AG, Caserta S, Guido S, Fogliano V, Caporaso N, Romano M. Apple polyphenols extract (APE) improves colon damage in a rat model of colitis. *Dig Liver Dis* 2012; **44**: 555-562 [PMID: 22381211 DOI: 10.1016/j.dld.2012.01.009]

66 **Ganjare AB**, Nirmal SA, Patil AN. Use of apigenin from Cordia dichotoma in the treatment of colitis. *Fitoterapia* 2011; **82**: 1052-1056 [PMID: 21745550 DOI: 10.1016/j.fitote.2011.06.008]

67 **Ganjare AB**, Nirmal SA, Rub RA, Patil AN, Pattan SR. Use of Cordia dichotoma bark in the treatment of ulcerative colitis. *Pharm*

Biol 2011; **49**: 850-855 [PMID: 21696332 DOI: 10.3109/13880209.2010.551539]

68 **Zaware BB**, Nirmal SA, Baheti DG, Patil AN, Mandal SC. Potential of Vitex negundo roots in the treatment of ulcerative colitis in mice. *Pharm Biol* 2011; **49**: 874-878 [PMID: 21591873 DOI: 10.3109/13880209.2010.551778]

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ORIGINAL ARTICLE

Basic Study

Protective effect of fu-qi granule on carbon tetrachloride-induced liver fibrosis in rats

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Institutional animal care and use committee: In the study entitled "Protective Effect of Fu-qi Granule on CCl_4-induced Liver Fibrosis in Rat", all procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Human and Animal Subjects in Teaching and Research (HASC) of the 302 Military Hospital of China. (IACUC protocol number: DWFL-2013-D018). Sixty Sprague-Dawley male rats (180-200 g) were obtained from Experimental Animal Center of Medical Sciences of Chinese People's Liberation Army of Beijing, China. Animal certificate was SCXK-(Army) 2012-0004.
Conflict-of-interest: The authors declared that they have no competing interests.
Data sharing: Technical appendix, statistical code, and dataset are stored and available at China Military Institute of Chinese Medicine, 302 Military Hospital of China. E-mail: zhaoyl2855@126.com. Participants gave informed consent for data sharing.

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Abstract

AIM: To investigate the efficacy of fu-qi granule (FQG) on carbon tetrachloride (CCl_4) induced liver fibrosis in rats and the underlying mechanisms.

METHODS: Sixty rats were randomly divided into six

groups: normal control group, CCl_4 induced liver fibrosis group, AnluoHuaxianWan group and three treatment groups of FQG. Treatment of rats with intraperitoneal injection of carbon tetrachloride solution at 0.3 mL per 100 g body weigh twice a week for 8 wk. The normal control group the rats were given the media (olive oil) at the same time. In the first 2 wk, rats were raised with feedstuff (80% corn meal, 20% lard, 0.5% cholesterol). Serum samples were collected for alanine transaminase, aspartate aminotransferase, alkaline phosphatase, albumin, total protein assay and typical histopathological changes was observed in Hematoxylin-eosin staining sections. Smooth muscle alpha actin (α-SMA) was analyzed with immunohistochemistry. Mammalian target of rapamycin (mTOR) and hypoxia-inducible factor-1 (HIF-1α) expressions were detected by Western blotting. Tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) and matrix metalloproteinases-9 (MMP-9) were measured with semi-quantitative reverse transcriptase-polymerase chain reaction.

RESULTS: FQG significantly reduced the serum levels of alanine transaminase, aspartate aminotransferase, alkaline phosphatase and increased the serum contents of albumin, total protein in rats with liver fibrosis. Moreover, FQG promoted extracellular matrix degradation by increasing MMP-9 and inhibiting TIMP-1 and α-SMA. mTOR and HIF-1α expression in liver significantly decreased in the rats treated with FQG.

CONCLUSION: The results indicated that FQG significantly reverse fibrosis induced by CCl_4, which should be developed as a new and promising preparation for the prevention of liver fibrosis.

Key words: Protective effect; Fu-qi granule; Carbon tetrachloride; Mammalian target of rapamycin/p70S6K signal pathway; Liver fibrosis

Core tip: Fu-qi granule (FQG) is traditional Chinese medicine preparation to prove the antifibrotic properties of traditional Chinese drug composed of six medicinal herbs in rats treated with carbon tetrachloride. we checked activities of liver enzymes, histopathological changes within the liver as well as the expression of mammalian target of rapamycin (mTOR) and hypoxia-inducible factor-1 and tissue inhibitor of matrix metalloproteinases-1 and matrix metalloproteinases-9. FQG can attenuate liver fibrosis induced by carbon tetrachloride *via* mTOR/p70S6K signal transduction pathway.

Zhong L, Sun YL, Shi WL, Ma X, Chen Z, Wang JB, Li RS, Song XA, Liu HH, Zhao YL, Xiao XH. Protective effect of fu-qi granule on carbon tetrachloride-induced liver fibrosis in rats. *World J Pharmacol* 2015; 4(2): 227-235 Available from: URL: http://www.wjgnet.com/2220-3192/full/v4/i2/227.htm DOI: http://dx.doi.org/10.5497/wjp.v4.i2.227

INTRODUCTION

As one of the common response to different kinds of liver injuries, such as autoimmune diseases, metabolic diseases, alcohol abuse, cholestatic liver disease[1], together with hepatitis. Liver fibrosis usually results in hepatic microstructure distortion and liver dysfunction with characterization of extracellular matrix (ECM) overproduction and irregular deposition in liver tissues[2], which seriously threatens human health. Therefore, it could be given a hint that the prevention of the progression from liver injury to liver cirrhosis may due to interruption and reversion of hepatic fibrosis.

Although there are many pathways and targets involving in liver fibrogenesis, however, by now, the mammalian targets of rapamycin (mTOR)/p70S6 kinase (p70S6K) signal pathways are receiving more and more attentions. It is reported that mTOR which were an regarded as an internal evolutionarily hidebound kinase, are taking advantage of controling serine threonine synthesis *via* phosphorylation of its downstream targets[3]. Being a mitogen-activated protein kinase, the p70S6K is playing an extremely major role in cell life cycle survival, proliferation or regulation[4]. Additionally, mTOR/p70S6K signaling pathways inhibited hepatic stellate cell (HSC) proliferation, which could be as an effective anti-fibrotic strategy[5].

Currently, several agents have showed promising anti-fibrogenesis effect in liver[6]. However, there still seems a long way to apply the agents in the clinical application[7]. Having been used for thousands of years in China, Traditional Chinese medicines (TCM) has been playing a special role in liver diseases treatment from a unique perspective[8]. With good therapeutic effects on liver fibrosis, traditional Chinese medicine has attracted more and more attentions and people are trying to explore new preparation of TCM and investigating the potential mechanisms[9]. Since TCM has specific characteristics with multi-constituents, multi-ways and less side effects, studies on traditional Chinese medicine with anti-fibrosis effects have been shown more important in today[10].

Fu-qi granule (FQG) is a new type with traditional Chinese medicine preparation. It was prepared by the astragalus membranaceus (Fisch.), broussonetia papyrifera (L.), poria cocos (Schw.) and angelica sinensis (Oliv.) *via* water extraction. Then the extraction was filtrated and the filtration liquid was enriched and dried to powder. The powder was mixed with Equus asinus L and Fermentative cordycepic fungal powder and the mixture was added dextrin to the preparation of FQG. These plants are chosen for the anti-fibrosis agent is because of their pharmacological properties and clinical curative effect is better against liver fibrosis in 302 Military Hospital of China.

Based on the theory of TCM, liver fibrosis is characterized by humidity, fever, blood-stasis, poison, and both gas and yin asthenia[11,12]. Therefore, FQG is used to treat liver fibrosis by clearing heart and damp,

removing stasis and toxin in the liver. In modern pharmacy, Astragalus membranaceus and Poria cocos were also widely investigated in liver desease. Astragalosides was a potent chemical ingredient and it can protect acute liver injury and fibrogensis[13]. In addition, in order to compare the anti-fibrotic effecacy of FQG, AnluoHuaxianWan group (ALHXW) was also used as a positive-control drug in the experiment. According to the basic theories of TCM and results of modern pharmacology, the aim of this research is looking into the function of FQG regarding anti-liver fibrosis. Meanwhile, its underlying mechanisms on FQG for liver fibrosis were also investigated.

MATERIALS AND METHODS

Materials

The composition of FQG included Astragalus membranaceus (Fisch.), Angelica sinensis (Oliv.), Poria cocos (Schw.), Broussonetia papyrifera (L.), Equus asinus L. and Fermentative cordycepic fungal powder. It was prepared by the Astragalus membranaceus (Fisch.), Angelica sinensis (Oliv.), Poria cocos (Schw.) and Broussonetia papyrifera (L.) *via* water extraction. Then the extraction was filtrated and the filtration liquid was enriched and dried to powder. The powder were mixed with Equus asinus L and Fermentative cordycepic fungal powder and the mixture was added dextrin to the preparation of FQG.

Animals and treatments

Sixty Sprague-Dawley male rats (180-200 g) were obtained from Experimental Animal Center of Medical Sciences of Chinese People's Liberation Army (Beijing, China). Animal certificate was SCXK-(Army) 2012-0004.

Rats were housed 5 per cage with food and water ad libitum. All procedures involving animals and their care were following the regulations of the Committee on use of Human and Animal Subjects in Teaching and Research of the 302 Military Hospital of China. A total of 60 rats were randomly divided into six groups: normal control group, CCl_4 induced liver fibrosis group, ALHXW and three treatment groups of FQG (n = 10, respectively). Except for the normal control group, all the rats were administered with carbon tetrachloride solution (CCl_4 dissolved in olive oil, 5:5, v/v) at 0.3 mL/100 g body weight for the first time by intraperitoneal injection, and then with carbon tetrachloride solution (CCl_4 dissolved in olive oil, 3:7, v/v) at 0.3 mL per 100 g body weigh twice a week for 8 wk[14,15]. The normal control group the rats were given the media (olive oil) at the same time. In the first 2 wk, rats were raised with feedstuff (80% corn meal, 20% lard, 0.5% cholesterol)[16].

At the end of the 8th week, the ALHXW was oral administered with ALHXW (2.16 g/kg per day), which used for a positive-control drug. The treatment group was treated with high, medium and low doses of FQG (5.4, 2.7 and 1.35 g/kg per day, respectively) by oral administration. The control group and CCl_4 induced liver fibrosis group were given equivalent saline every day for 6 wk. All animals were anesthetized after the last administration. Blood was taken from the inferior vena cava, centrifuged at 3000 r/min, 4 ℃, for 10 min, and serum was kept at -20 ℃ for assay. Liver samples were taken and washed immediately with ice cold saline. Subsequently, the liver was divided into two parts. One was immediately stored at -80 ℃ for future experiment, and the other one was fixed in 4% formalin solution for histopathologic examination.

Serum biochemical analysis

Serum levels of alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin (ALB) and total protein (TP) were measured by commercially available kits (Mindray bio-medical electronics co, LTD. Shenzhen, China) according to the manufacturer's instructions.

Histopathological examination

Left lobe liver tissues were fixed in 4% buffered paraformaldehyde and dehydrated with different graded alcohol series. After paraffin embedded, and cut into 5 μm sections, they were stained with hematoxylin and eosin (HE) for histopathological examination.

Immunohistochemical staining

The same part of liver in each group was fixed with 4% paraformaldehyde, dehydrated by ethanol gradients, paraffin embedded, sectioned into thickness of 5 microns, and underwent regular dewaxing. Endogenous peroxidase activity was blocked with 3% H_2O_2. After microwave treatment with 0.1 mol/L citrate buffer and blockage of non-specific antigen with horse serum, rabbit polyclonal antibody against rat Smooth muscle alpha actin (α-SMA) (Calbiochem Biotechnology, San Diego, CA, United States) (1:150 diluted in blocking buffer) was added, followed by overnight incubation at 4 ℃. The membrane was washed three times with 0.1% Tween-PBS. Antibody-antigen complexes were detected with DAB as the substrate. An interstitial brown stellate structure was regarded as positive for α-SMA.

Western blot analysis for mTOR and hypoxia-inducible factor-1

The liver tissues were washed with PBS and total cell lysates were prepared by adding cell lysis buffer (50 mmol/L Tris·HCl, pH = 8.0, 150 mmol/L solid acid sodium, 1% TritonX-100). The proteins were separated by electrophoresis on 10% SDS-PAGE gel with Bio-Rad electrophoresis system[17], (Bio-Rad Laboratories, Hercules, CA, United States). The membrane was blocked and incubated with primary antibodies overnight at 4 ℃. The primary antibodies, phospho-mTOR (serine 2448) and hypoxia-inducible factor-1α (HIF-1α) (Cell signaling TECHNOLOGY), were used for detection of

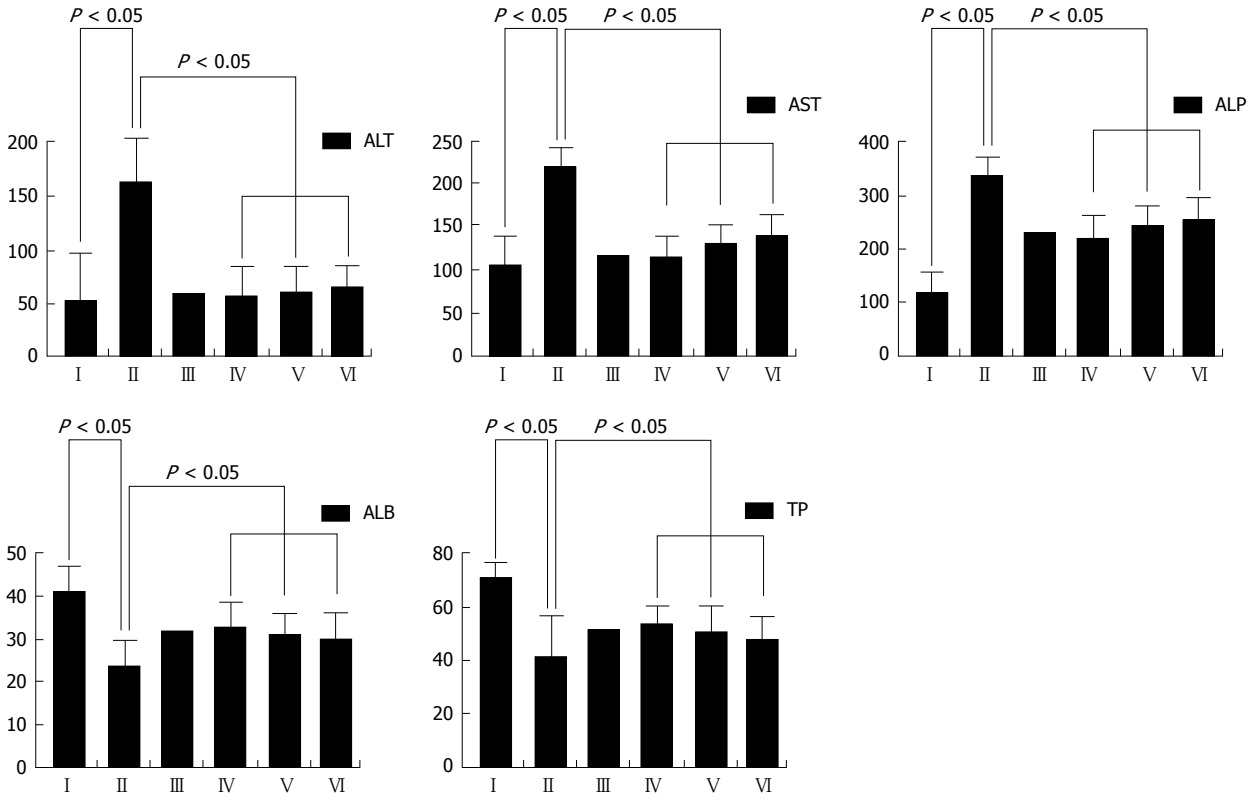

Figure 1 Serum alanine transaminase, aspartate aminotransferase, alkaline phosphatase, albumin and total protein levels. Ⅰ: Control group; Ⅱ: Model group; Ⅲ: ALHXW group; Ⅳ: High-dose of FQG groups; Ⅴ: Medium-dose of FQG; Ⅵ: Low-dosage of FQG group. ALT: Alanine transaminase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; ALB: Albumin; TP: Total protein; FQG: Fu-qi granule.

mTOR and HIF-1α, respectively. GAPDH protein was used as the internal control.

Reverse transcription polymerase chain reaction analysis of tissue inhibitor of matrix metalloproteinases-1 and matrix metalloproteinases-9

Total RNA was extracted from liver tissues of each group with Trizol reagent (Takara Biotechnology Dalian CO., LTD.) according to the manufacturer's protocol. The isolated RNA was dissolved in RNase-free water stored at -80 ℃ immediately. RNA was quantified by optical density measurement at 260 nm on a spectrophotometer.

Reverse transcription reaction was performed with 2 μg of total RNA, which was used for polymerase chain reaction (PCR) amplification of cDNA products. The PCR of β-actin cDNA, which was used as an internal control, was carried out in the same tubes as for the genes. The products of PCR amplification were analyzed by electrophoresis on 1.5% agarose gel. The PCR product signal intensities were measured by scanning the gels. Tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) and matrix metalloproteinases-9 (MMP-9) densitometric measurement was normalized with the internal control β-actin. The primers used were as follows: TIMP-1, Forward GACCACCTTATACCAGCGTT and Reverse TCGAGACCCCAAGGTATTG; MMP-9, Forward CTGTATGGCTTCTGTCCTA and Reverse GGCTTCCTCCGTGATT.

Statistical analysis

Results were expressed as mean ± SD. Test data were analyzed with one-way variance (SPSS 20.0). Deviations with $P < 0.05$ were considered the presence of statistically significant.

RESULTS

FQG rescues CCl_4-induced live functional damage

The blood serum AST, ALT, ALB, ALP and TP activity in various experiment groups is seen in Figure 1. Activity of ALT, AST, ALP have increased markedly, both serum ALB and TP activity were created a dramatic decreased in CCl_4-duplicate liver fibrosis group ($P < 0.05$). Whereas three treatment groups of FQG showed the opposite response, which serum ALT, AST and ALP activities in rats were significantly improvement and the levels of ALB, TP were increased respectively higher than model group.

FQG protect liver damage induced by CCl_4

In order to evaluate the pathological changes in liver tissue, HE stain methods were employed in examination of liver tissue. Liver tissues of the group with normal saline have inerratic lobular composition with central veios and hepatic cords (Figure 2A). In the CCl_4 group, serious injuries such as fibrous tissues hyperplasia, formed complete septa and pseudo lobule was observed in liver morphology ($P < 0.05$) (Figure

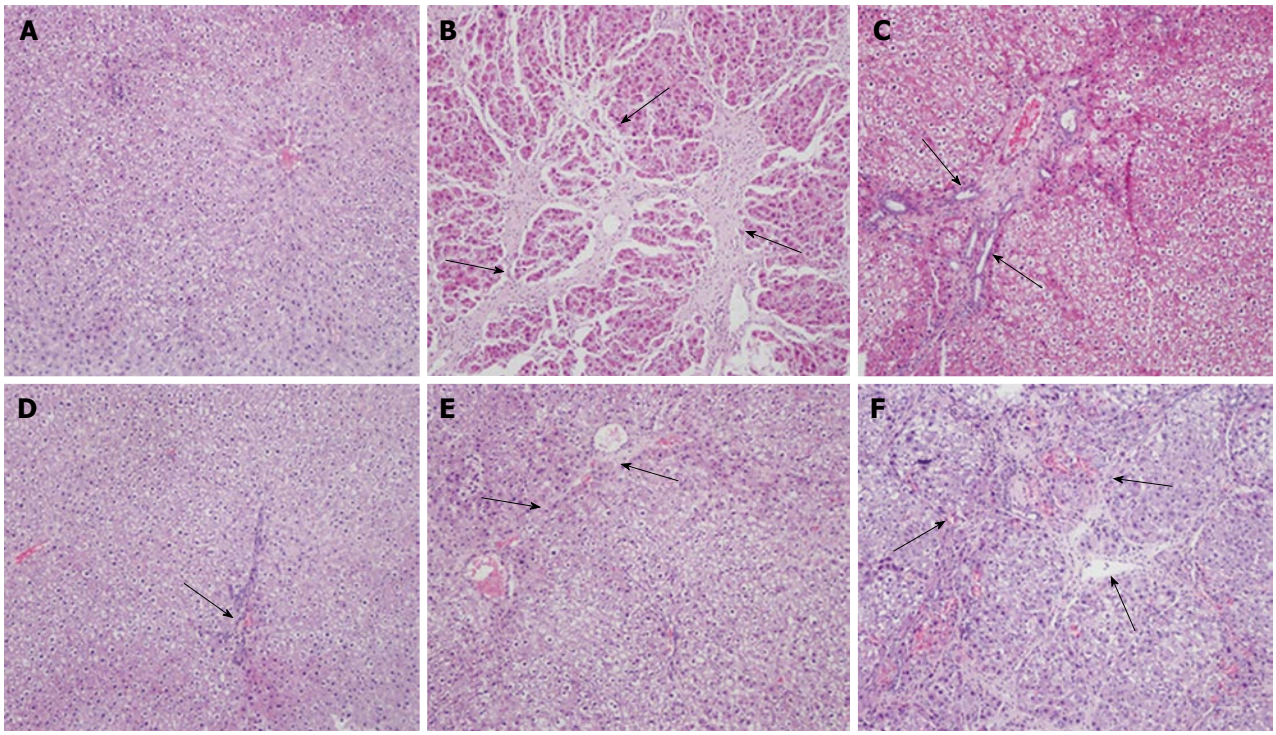

Figure 2 Effect of fu-qi granule on histopathological changes of liver (hematoxylin and eosin × 10). A: Control group; B: Model group; C: ALHXW group; D: High-dose of FQG-treated rats; E: Medium-dosage of FQG-treated rats; F: Low-dose of FQG-treated rats. Black arrow represents the pathological section. FQG: Fu-qi Granule.

2B). In the groups treated with ALHXW, high dosages and middle dosages with FQG groups correspondingly appear alleviate tissue destroy compared with model group (Figure 2C-E). However, low-dose of FQG groups had no obvious effect ($P < 0.05$, Figure 2F).

FQG inhibits α-SMA expression in CCl_4 treated rats

Compared with control group, α-SMA expression was increased significantly in the model group by method of immunohistochemical assay (Figure 3A and B). In the FQG treated rats groups with high-dose and meddle-dose, α-SMA expression of the liver tissues was noticeable reduction compared with CCl_4 induced model groups (Figure 3D and E). Besides, expression of α-SMA was drastically diminished in rats does by high-dose of FQG compare with ALHXW and low-dose of FQG (Figure 3C and F).

HIF-1α and mTOR protein expression

The mTOR and HIF-1α expression was detected undergo Western blotting. Studies have discovered that mTOR and HIF-1α expressions with hepatic tissue in models group were markedly increased, however, it was to observe the expression of mTOR and HIF-1α was significantly lowered in experimental rats with FQG intragastric administration group (Figure 4).

Detection of MMP-9 and TIMP-9 with reverse transcription-PCR

The results of semiquantitative reverse transcription-PCR (RT-PCR) expressions of MMP-9 and TIMP-1 in the hepatic tissues of different groups are exhibited in Figure 5. Experimental results showed that TIMP-1 expression dramatically stronger in treated with carbon tetrachloride than normal group, and its expression declined sharply in rats treated with high dosage of FQG. In contrast, the expression of MMP-9 was inferior in rats injected with CCl_4 relatively physiological saline, which down-regulation could obviously inhibit by treated with FQG.

DISCUSSION

Liver fibrosis induced by CCl_4 works as a kind of classic model in anti-fibrosis agents exploring and evaluation[18]. In the course of CCl_4 being transformed to free radicals, the cytochrome P450 plays a most important role. Furtherly, lipid oxidation reactions were initiated by free radicals and leaded to liver cell damnification, retrograde, and even death. This kind of stimulation again and again can definitely form liver fibrosis[19]. It will further worsening or permanence of cirrhosis. Thus, the prevention and reversal of fibrosis is an important means to prevent the formation of liver cirrhosis[20]. Some studies evidence suggests that liver is likely to recover from fibrosis[21]. Recently research on treatment of liver fibrosis by TCM preparation has made some progress, such as ALHXW was typically used to cure this disease in this area, and marketed in china (National Drug permit Registry Z20010098). Chinese medicinal preparations showed the influences on liver fibrosis mainly embodied itself in composition with abundant

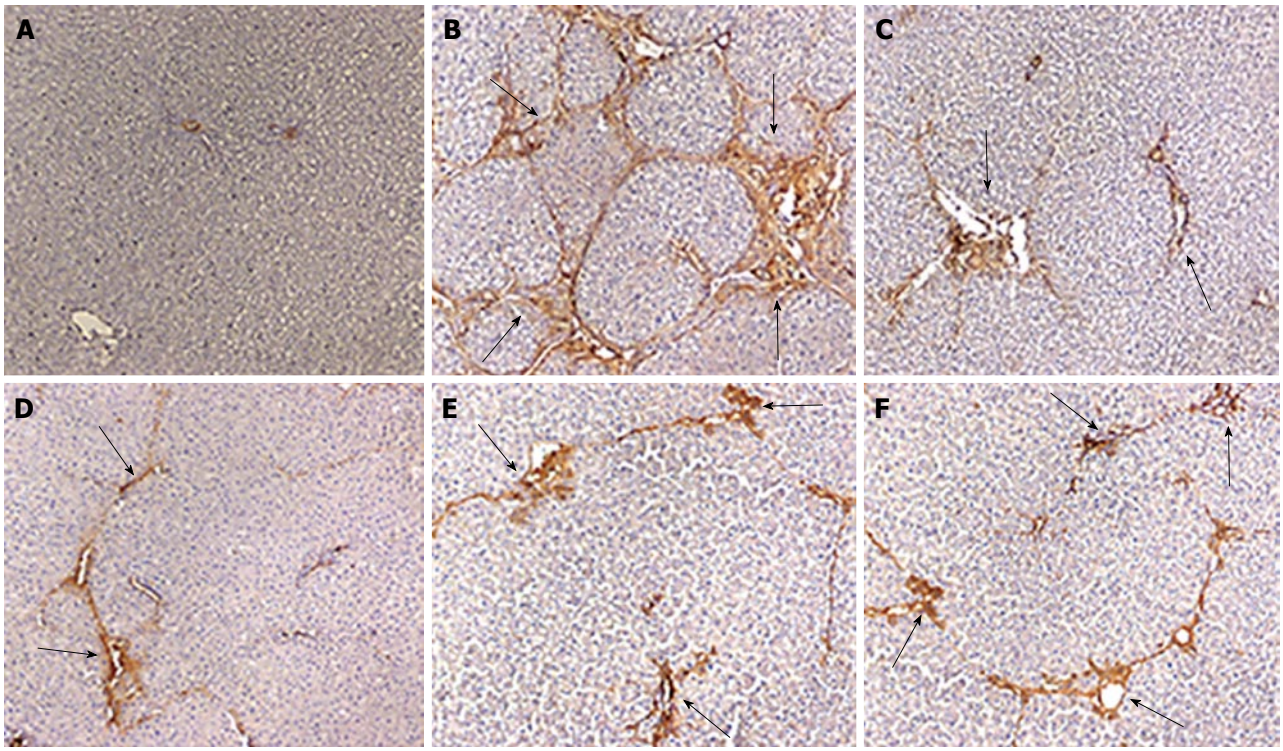

Figure 3 Immunohistochemical analysis of α-smooth muscle actin in liver from rats with liver fibrosis (× 10). A: Control group; B: Model group; C: ALHXW group; D: High-dosage of FQG-treated rats; E: Medium-dosage of FQG-treated rats; F: Low-dosage of FQG-treated rats. Black arrow represents the pathological section. FQG: Fu-qi granule.

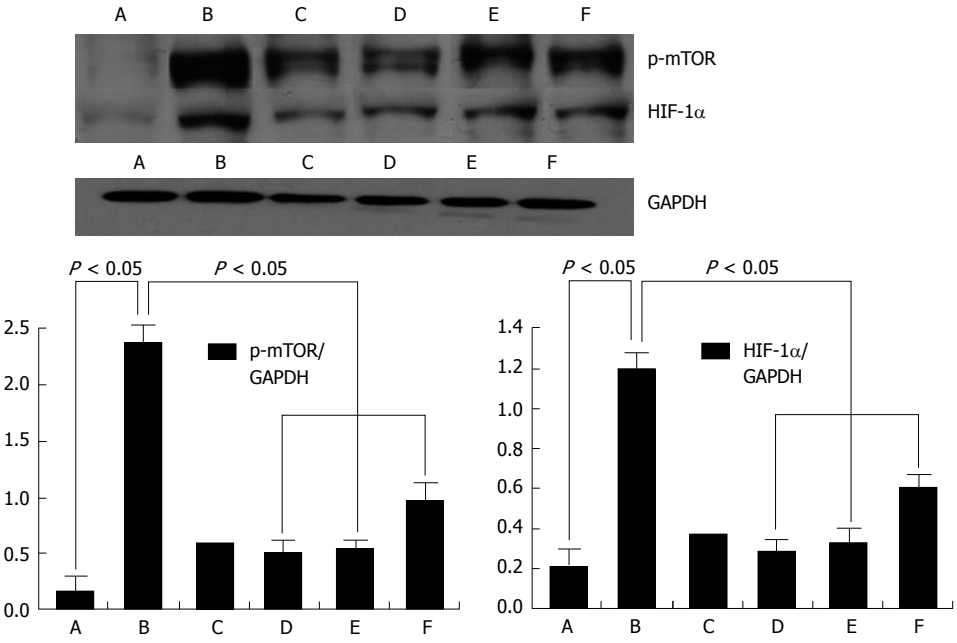

Figure 4 Western blot analysis of mammalian target of rapamycin and hypoxia-inducible factor-1α expression. A: Control group; B: Model group; C: ALHXW group; D: High-dosage of FQG-treated rats; E: Medium-dosage of FQG-treated rats; F: Low-dosage of FQG-treated rats. mTOR: Mammalian target of rapamycin; HIF-1α: Hypoxia-inducible factor-1 α; FQG: Fu-qi granule.

and complex[22,23].

The research adopt FQG to examine the therapeutic effects on hepatic fibrosis. ALT and AST is an enzyme, Increasing of which were considered due to damage of liver cell. ALP is an ectoenzyme of plasma membrane, its ascension is partly a reflection of hepatocyte plasma membrane was damaged. The other, ALB, TP were depressed. In this study, compared with model rats *via* CCl_4 treatment, effect of FQG makes serum ALT, AST and ALP levels significantly lowered, in the same time,

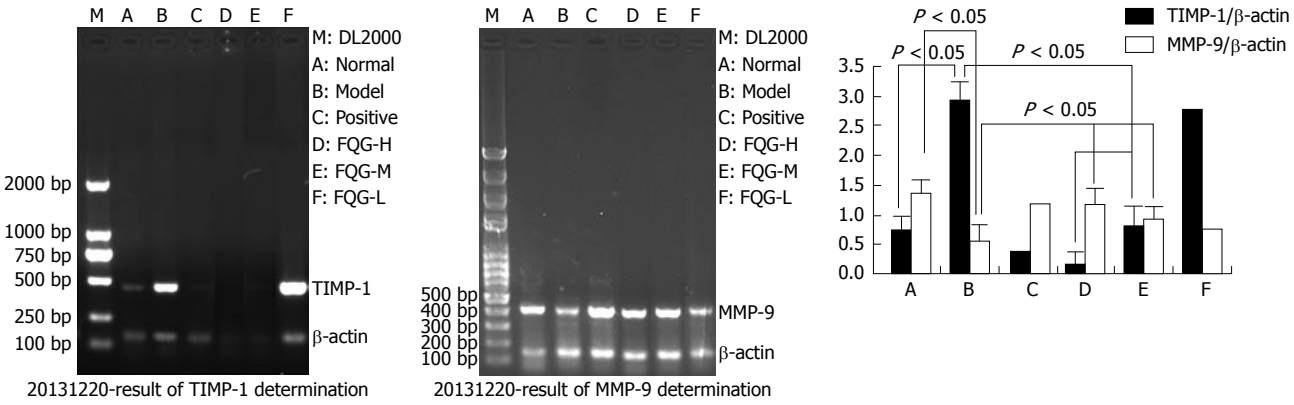

Figure 5 Effect of Fu-qi granule on expression of tissue inhibitor of matrix metalloproteinases-1 and matrix metalloproteinases-9 in carbon tetrachloride induced fibrotic liver of rats. A: Normal control; B: Model control; C: ALHXW group; D: High-dosage of FQG-treated rats; E: Medium-dosage of FQG-treated rats; F: Low-dosage of FQG-treated rats; M: Marker; TIMP-1: 455 bp; MMP-9: 679 bp; TIMP-1: Tissue inhibitor of matrix metalloproteinases-1; MMP-9: Matrix metalloproteinases-9; FQG: Fu-qi granule.

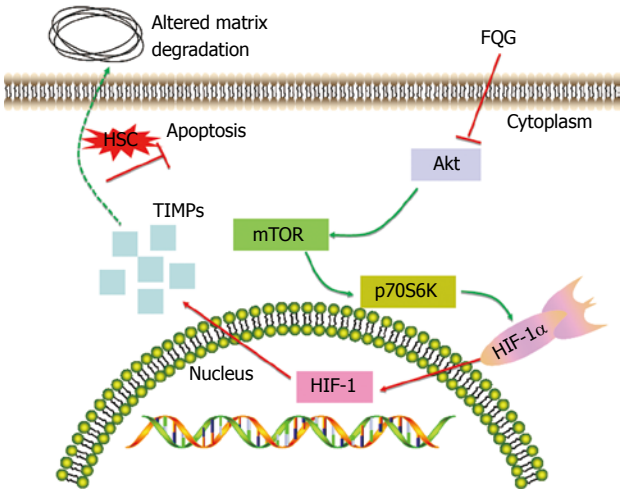

Figure 6 Mammalian target of rapamycin/P70S6 kinase signaling pathway activation maybe participate in the process of liver fibrosis. FQG is the major negative-regulation target of mTOR. FQG paradoxically down-regulates the expression of mTOR and HIF-1α by Western blot. Then subsequently inhibit the activation of mTOR/p70S6K pathway. mTOR: Mammalian target of rapamycin; p70S6K: P70S6 kinase; FQG: Fu-qi granule; TIMP: Tissue inhibitor of matrix metalloproteinases; HIF-1α: Hypoxia-inducible factor-1 α.

it also elevated activity of serum ALB and TP in rats with treated FQG. These results exhibited an obvious therapeutic effect for liver fibrosis.

From a cellular perspective, in general, HSCs activation is the most important characteristic of liver fibrosis. During the process of HSC activation, α-SMA has started to overexpression, which is mostly found in smooth muscle cell. Increasing of α-SMA activated HSCs resulting in collagen fibers protein were secretion, ultimately leads to fibrosis. In this research, immunohistochenical suggested that α-SMA expression was obviously increased in rats with CCl_4 stimulate. However, its expression was markedly improvement in rats with treated FQG. This study showed that FQG could inhibit the activity of HSC, thus emerging anti-fibrosis effect.

TIMPs was served as a vital factor in the process of liver fibrosis. It was secreted through activated HSC and can produce a variety of cytokines that are significantly increased in liver fibrosis[24]. MMPs are able to degrade ECM, and play a critical role in preventing inflammation and tumor progression[25]. Under physiological conditions, the expressions of TIMPs and MMP-9 are in dynamic equilibrium to maintain the stability of ECM in liver[26]. RT-PCR analysis of TIMP-1 and MMP-9 showed that FQGs could effectively inhibit TIMP-1 protein expressions, meanwhile, MMP-9 was enhanced during hepatic fibrosis in rats. This result also indicated that the regulation of TIMP-1 and MMP-9 levels can promote degradation of ECM.

Activation of HSC is the core of liver fibrosis, clinical treatment of hepatic fibrosis is sticks chiefly to intervene activation process[27]. mTOR is one of the phosphoinositide 3-kinase related kinases family members and plays a vital role in cell proliferation regulation[28]. Previous research have showed that liver fibrosis could alleviated by mTOR inhibitor[29]. p70S6K is directly concerned to the matrix with mTOR, while the mTOR/p70S6K pathways is related to regulate of cell proliferation[30]. HIF-1 was used to grasp the expression of hypoxia inducible genes and further to decrease oxidation ability in cells[31]. Some studies have proved that increasing expression of HIF-1α though mTOR signaling can significantly result in pulmonary fibrosis, renal fibrosis or peritoneal angiogenesis, whereas mTOR inhibitor such as FQG is able to effectively alleviate liver fibrosis[32,33]. In fact, the expression intensity of mTOR and HIF-1α in experiment rats with CCl_4 injected was up-regulated by western blot analysis. Moreover, anoxia is likely what lead HIF-1α to up-regulation in hepatic tissues. Nevertheless, it was down-regulated for FQG treat group, which perhaps raising matrix to degrade and promoting HSC to apoptosis, consequently inhibiting deterioration of hepatic fibrosis. From what has been discussed above, we speculate that the FQG effect might be due to inhibition of CCl_4-induced p70S6K activation (Figure 6).

COMMENTS

Background

The incidence rate was high in patients with liver fibrosis in world, accordingly, if the issue was appropriately to regard liver fibrosis during this stage. It will prevent the development of cirrhosis and relieve the pain of the patients. However, there still lack of satisfactory treat medical for liver fibrosis at present. Based on the theories of Traditional Chinese medicines, humid, blocked lifeblood circulation, imbalance of yin and yang will give rise to liver fibrosis. Fu-qi granule (FQG) can activate blood and remove stasis, therefore, the current situation is to explore the effect and its underlying mechanisms of FQG on liver fibrosis duplicated by carbon tetrachloride (CCl_4) in rats.

Research frontiers

Recent research showed liver fibrosis can be relieved by regulating collagen metabolism, inhibiting hepatic stellate cell (HSC) activation. Moreover, amelioration of hepatic fibrosis was regulated by mammalian target of rapamycin (mTOR) inhibitors. mTOR/p70S6K pathway is blocked will lead to decrease of HSCs proliferation.

Innovations and breakthroughs

This study has confirmed that FQG can improve liver function, alleviate liver fibrosis, which is probably associated with its regulating mTOR/p70S6K signal transduction pathway.

Applications

The FQG can prevent liver fibrosis, which implies that it will be a good medicine and promising preparation for patients with liver fibrosis, this study can provide some scientific data for its application and development.

Terminology

Liver fibrosis is a chronically ill, which was caused by excessive cumulation of extracellar stroma proteins. HSCs become a crucial role in liver fibrosis and cirrhosis with portal hypertension incidence of pathological basis.

Peer-review

This paper reinforced my conviction that there is protective effect of FQG on liver fibrosis rats with CCl_4-stimulated. The study is interesting and the analysed parameters are well matched to the mechanism of hepatic fibrosis. Data are clear and convincing.

REFERENCES

1 **Lee TY**, Chang HH, Chen JH, Hsueh ML, Kuo JJ. Herb medicine Yin-Chen-Hao-Tang ameliorates hepatic fibrosis in bile duct ligation rats. *J Ethnopharmacol* 2007; **109**: 318-324 [PMID: 16989967 DOI: 10.1016/j.jep.2006.07.042]

2 **Friedman SL**. Liver fibrosis -- from bench to bedside. *J Hepatol* 2003; **38** Suppl 1: S38-S53 [PMID: 12591185 DOI: 10.1016/S0168-8278(02)00429-4]

3 **Osaka E**, Suzuki T, Osaka S, Yoshida Y, Sugita H, Asami S, Tabata K, Hemmi A, Sugitani M, Nemoto N, Ryu J. Survivin as a prognostic factor for osteosarcoma patients. *Acta Histochem Cytochem* 2006; **39**: 95-100 [PMID: 17327929 DOI: 10.1267/ahc.06005]

4 **Zhou Q**, Deng Z, Zhu Y, Long H, Zhang S, Zhao J. mTOR/p70S6K signal transduction pathway contributes to osteosarcoma progression and patients' prognosis. *Med Oncol* 2010; **27**: 1239-1245 [PMID: 19936974]

5 **Gäbele E**, Reif S, Tsukada S, Bataller R, Yata Y, Morris T, Schrum LW, Brenner DA, Rippe RA. The role of p70S6K in hepatic stellate cell collagen gene expression and cell proliferation. *J Biol Chem* 2005; **280**: 13374-13382 [PMID: 15677443 DOI: 10.1074/jbc.M409444200]

6 **Friedman SL**. Reversibility of hepatic fibrosis and cirrhosis--is it all hype? *Nat Clin Pract Gastroenterol Hepatol* 2007; **4**: 236-237 [PMID: 17476207 DOI: 10.1038/ncpgasthep0813]

7 **Bataller R**, Brenner DA. Hepatic stellate cells as a target for the treatment of liver fibrosis. *Semin Liver Dis* 2001; **21**: 437-451 [PMID: 11586471 DOI: 10.1055/s-2001-17558]

8 **Li WL**, Zheng HC, Bukuru J, De Kimpe N. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. *J Ethnopharmacol* 2004; **92**: 1-21 [PMID: 15099842 DOI: 10.1016/j.jep.2003.12.031]

9 **Zou YH**, Yang Y, Li J, Wu Q, Li WP, Lu JT, Roberts MS. Potential therapeutic effects of a traditional Chinese formulation, BJ-JN, on liver fibrosis induced by carbon tetrachloride in rats. *J Ethnopharmacol* 2008; **120**: 452-457 [PMID: 18951966 DOI: 10.1016/j.jep.2008.09.023]

10 **Lin X**, Zhang S, Huang Q, Wei L, Zheng L, Chen Z, Jiao Y, Huang J, Fu S, Huang R. Protective effect of Fufang-Liu-Yue-Qing, a traditional Chinese herbal formula, on CCl4 induced liver fibrosis in rats. *J Ethnopharmacol* 2012; **142**: 548-556 [PMID: 22658988]

11 **Liu P**, Hu YY, Ni LQ. [On establishing comparative reference system for syndrome classification study from the thinking characteristics of syndrome differentiation dependent therapy]. *Zhongguo Zhongxiyi Jiehe Zazhi* 2006; **26**: 451-454 [PMID: 16883917]

12 **Arias M**, Lahme B, Van de Leur E, Gressner AM, Weiskirchen R. Adenoviral delivery of an antisense RNA complementary to the 3' coding sequence of transforming growth factor-beta1 inhibits fibrogenic activities of hepatic stellate cells. *Cell Growth Differ* 2002; **13**: 265-273 [PMID: 12114216]

13 **Zhang YD**, Shen JP, Zhu SH, Huang DK, Ding Y, Zhang XL. [Effects of astragalus (ASI, SK) on experimental liver injury]. *Yaoxue Xuebao* 1992; **27**: 401-406 [PMID: 1442065]

14 **Nakamura T**, Akiyoshi H, Saito I, Sato K. Adenovirus-mediated gene expression in the septal cells of cirrhotic rat livers. *J Hepatol* 1999; **30**: 101-106 [PMID: 9927156 DOI: 10.1016/S0168-8278(99)80013-0]

15 **Yang FR**, Fang BW, Lou JS. Effects of Haobie Yangyin Ruanjian decoction on hepatic fibrosis induced by carbon tetrachloride in rats. *World J Gastroenterol* 2010; **16**: 1458-1464 [PMID: 20333785 DOI: 10.3748/wjg.v16.i12.1458]

16 **Mullen KD**, McCullough AJ. Problems with animal models of chronic liver disease: suggestions for improvement in standardization. *Hepatology* 1989; **9**: 500-503 [PMID: 2921000 DOI: 10.1002/hep.1840090326]

17 **Yen CC**, Lai YW, Chen HL, Lai CW, Lin CY, Chen W, Kuan YP, Hsu WH, Chen CM. Aerosolized human extracellular superoxide dismutase prevents hyperoxia-induced lung injury. *PLoS One* 2011; **6**: e26870 [PMID: 22046389 DOI: 10.1371/journal.Pone.0026870]

18 **Brautbar N**, Williams J. Industrial solvents and liver toxicity: risk assessment, risk factors and mechanisms. *Int J Hyg Environ Health* 2002; **205**: 479-491 [PMID: 12455270 DOI: 10.1078/1438-4639-00175]

19 **Berger ML**, Bhatt H, Combes B, Estabrook RW. CCl4-induced toxicity in isolated hepatocytes: the importance of direct solvent injury. *Hepatology* 1986; **6**: 36-45 [PMID: 3943788 DOI: 10.1002/hep.1840060108]

20 **Ueberham E**, Löw R, Ueberham U, Schönig K, Bujard H, Gebhardt R. Conditional tetracycline-regulated expression of TGF-beta1 in liver of transgenic mice leads to reversible intermediary fibrosis. *Hepatology* 2003; **37**: 1067-1078 [PMID: 12717387 DOI: 10.1053/jhep.2003.50196]

21 **Lou JL**, Jiang MN, Li C, Zhou Q, He X, Lei HY, Li J, Jia YJ. Herb medicine Gan-fu-kang attenuates liver injury in a rat fibrotic model. *J Ethnopharmacol* 2010; **128**: 131-138 [PMID: 20056141 DOI: 10.1016/j.jep.2009.12.D38]

22 **Zimmermann GR**, Lehár J, Keith CT. Multi-target therapeutics: when the whole is greater than the sum of the parts. *Drug Discov Today* 2007; **12**: 34-42 [PMID: 17198971 DOI: 10.1016/j.drudis.2006.11.008]

23 **Gu P**, Chen H, Yu T. Ontology-oriented diagnostic system for traditional Chinese medicine based on relation refinement. *Comput Math Methods Med* 2013; **2013**: 317803 [PMID: 23533534]

24 **Cheung KF**, Ye DW, Yang ZF, Lu L, Liu CH, Wang XL, Poon RT, Tong Y, Liu P, Chen YC, Lau GK. Therapeutic efficacy of Traditional Chinese Medicine 319 recipe on hepatic fibrosis induced by carbon tetrachloride in rats. *J Ethnopharmacol* 2009; **124**: 142-150 [PMID: 19501992 DOI: 10.1016/j.jep.2009.03.005]

25 **Jackson BC**, Nebert DW, Vasiliou V. Update of human and mouse matrix metalloproteinase families. *Hum Genomics* 2010; **4**: 194-201 [PMID: 20368140]

26 **Zhou X**, Hovell CJ, Pawley S, Hutchings MI, Arthur MJ, Iredale JP, Benyon RC. Expression of matrix metalloproteinase-2 and -14 persists during early resolution of experimental liver fibrosis and might contribute to fibrolysis. *Liver Int* 2004; **24**: 492-501 [PMID: 15482348 DOI: 10.1111/j.1478-3231.2004.0946.x]

27 **Ueberham E**, Löw R, Ueberham U, Schönig K, Bujard H, Gebhardt R. Conditional tetracycline-regulated expression of TGF-beta1 in liver of transgenic mice leads to reversible intermediary fibrosis. *Hepatology* 2003; **37**: 1067-1078 [PMID: 12717387 DOI: 10.1053/jhep.2003.50196]

28 **Fingar DC**, Blenis J. Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. *Oncogene* 2004; **23**: 3151-3171 [PMID: 15094765 DOI: 10.1038/sj.onc.1207542]

29 **Patsenker E**, Schneider V, Ledermann M, Saegesser H, Dorn C, Hellerbrand C, Stickel F. Potent antifibrotic activity of mTOR inhibitors sirolimus and everolimus but not of cyclosporine A and tacrolimus in experimental liver fibrosis. *J Hepatol* 2011; **55**: 388-398 [PMID: 21168455 DOI: 10.1016/j.jhep.2010.10.044]

30 **Shamji AF**, Nghiem P, Schreiber SL. Integration of growth factor and nutrient signaling: implications for cancer biology. *Mol Cell* 2003; **12**: 271-280 [PMID: 14536067 DOI: 10.1016/j.molcel.2003.08.016]

31 **Sun S**, Gao YQ, Gao WX, Fan M. Hypoxia-inducible factor-1 and PI3K/Akt/mTOR signaling pathway. *Chinese Bulletin of Life Sciences* 2005; **17**: 311-314

32 **Mehrad B**, Burdick MD, Strieter RM. Fibrocyte CXCR4 regulation as a therapeutic target in pulmonary fibrosis. *Int J Biochem Cell Biol* 2009; **41**: 1708-1718 [PMID: 19433312 DOI: 10.1016/j.biocel.2009.02.020]

33 **Rozen-Zvi B**, Hayashida T, Hubchak SC, Hanna C, Platanias LC, Schnaper HW. TGF-β/Smad3 activates mammalian target of rapamycin complex-1 to promote collagen production by increasing HIF-1α expression. *Am J Physiol Renal Physiol* 2013; **305**: F485-F494 [PMID: 23761672 DOI: 10.1152/ajprenal.00215.2013]

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