

# World Journal of *Experimental Medicine*

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**MINIREVIEWS**

- 42 Recent clinical trials of cancer immunogene therapy in companion animals  
*Finocchiaro LME, Glikin GC*

**ORIGINAL ARTICLE**

**Basic Study**

- 49 Role of LIGHT in the pathogenesis of joint destruction in rheumatoid arthritis  
*Sabokbar A, Afrough S, Mahoney DJ, Uchiyama Y, Swales C, Athanasou NA*

## Contents

*World Journal of Experimental Medicine*  
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### ABOUT COVER

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## Recent clinical trials of cancer immunogene therapy in companion animals

Liliana ME Finocchiaro, Gerardo C Glikin

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### Abstract

This mini-review presents the results of veterinary clinical trials on immunogene therapy published from 2014 to 2016. A variety of tumors, among them melanoma (canine and equine), mastocytoma (canine), mammary

adenocarcinoma (canine) and fibrosarcoma (feline) were treated by using diverse strategies. Non-viral vectors were usually employed to transfer genes of cytokines, suicide enzymes and/or tumor associated antigens. In general terms, minor or no adverse collateral effects were related to these procedures, and treated patients frequently improved their conditions (better quality of life, delayed or suppressed recurrence or metastatic spread, increased survival). Some of these new methodologies have a promising future if applied as adjuvant treatments of standard approaches. The auspicious results, derived from immunogene therapy studies carried out in companion animals, warrant their imperative usage in veterinary clinical oncology. Besides, they provide a strong preclinical basis (safety assays and proofs of concept) for analogous human clinical trials.

**Key words:** Cancer; Gene therapy; Immunotherapy; Companion animals; Comparative oncology

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**Core tip:** Cancer immunogene therapy is a major growing area among human clinical trials. Until August 2016 there were about 2409 registered gene therapy trials, where 1554 were aimed to cancer, and among them 864 corresponded to immunotherapy. Working with veterinary cancer bearing patients can significantly speed up translational research and benefit both veterinary and human patients. New data demonstrated the safety and efficacy of different immunotherapy approaches. Following our previously published review on the subject covering from 1996 to 2014, this new mini-review is focused on veterinary cancer immunogene therapy covering published work in the field from 2014 to 2016.

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## INTRODUCTION

More than 20 years elapsed from the publication of the pioneering work of Quintin-Colonna *et al.*<sup>[1]</sup> in 1996 on *ex vivo* interleukin-2 (IL-2) immunogene therapy for canine melanoma and feline fibrosarcoma. As we had previously discussed in a previous review covering this subject from the early beginnings up to 2014<sup>[2]</sup>, nearly all veterinary cancer gene therapy clinical trials involved the stimulation of immune responses against tumor cells. In this new review we are covering the three years period from January 2014 to December 2016. As we did in the preceding review, we focused our interest only on reports of clinical data collected from client-owned patients with spontaneously arising tumors.

Why clinical trials of cancer immunogene therapy in companion animals do actually matter? Despite some progresses treating tumors in companion animals and human patients, there is still a need of new therapeutic approaches for diverse malignancies because of the lack of long lasting effective treatments and the unwanted side effects of most of them. In addition, spontaneous tumors in companion animals offer a very useful translational model because pets display relatively large sizes, diversity of genetic backgrounds and intact immune systems. They usually offer a strong support as proof of concept in a setting similar to the population of human patients, while directly providing data about toxicity and long-term efficacy. Nowadays there is a revival of the idea that researchers should turn to canine clinical trials to advance cancer therapies<sup>[3]</sup>.

Up to date we found 57 reports about veterinary immunogene therapy clinical trials: 45 canine, 7 feline and 5 equine. As seen in Table 1, the fourteen newly reported trials mainly involved non-viral gene transfer (13/14), sometimes enhanced by physical methods (10/13: 7 electrotransfer and 3 jet injection), while a viral vector was used in 1 trial (1/1: poxvirus). Reports about using genetically modified bacteria<sup>[4,5]</sup> or suicide gene expressing encapsulated mammalian cells<sup>[6]</sup> as vectors are not discussed in this mini-review.

New trials (7/14) were mainly reported from only three countries: 3 from United States (2 canine, 1 feline), 2 from Belgium (canine) and 2 from Italy (canine). The remaining five studies were: 1 from Argentina (canine), 1 from France (feline), 1 from Germany (equine), 1 from Slovenia (canine), 1 from United Kingdom (canine), 1 from South Africa (canine) and 1 multi-centric from Ukraine, Russia and Italy (canine).

Most of the current veterinary trials (6/11 canine, 1/2 feline and 1/1 equine) employed cytokine genes, mainly IL-2 and IL-12 as single (6/8) or combined genetic agents (2/8), followed by antigens (5/11 canine, 1/2 feline, 1/1 equine).

Immunogene therapy was applied alone (10/14),

or combined with chemotherapy (3/14) or suicide gene (1/14). Most trials were focused on feasibility and safety (8/14) with few treated animals and often carrying diverse kind of tumors. On the other hand, larger controlled trials (4/14) allowed the determination of clinical efficacy.

## CYTOKINE-BASED IMMUNOGENE THERAPY

Only five cytokine genes were assayed in veterinary clinical settings in the latest three years. Granulocyte-macrophage colony stimulating factor (GM-CSF) stimulates dendritic cells that then induce antitumor immune responses. The result is twofold: Direct destruction of the injected lesion and enhanced antigen presentation, leading to an immune response against metastatic melanoma. T-cells treated with GM-CSF have demonstrated increased antitumor responsiveness.

IL-2 is a naturally occurring glycoprotein secreted by T-cells to augment the immune response and was first used in clinical cancer studies in the early 1980s. This glycoprotein promotes T-lymphocyte proliferation and stimulates cytotoxic T-cells and natural killer cells. IL-2 has been used as immunotherapy for nearly 40 years. The treatment with the recombinant protein displays systemic cytotoxicity that deteriorates the patient's quality of life.

Interferon- $\beta$  (IFN- $\beta$ ) simultaneously displays anti-proliferative and anti-angiogenic effects as well as immunomodulatory activities. It was among the first cytokines approved for clinical use. Even though clinically effective, the completion of IFN- $\beta$  therapy is often impaired by its inherent systemic toxicity that negatively affects the patient's quality of life.

IL-12 and IL-18 exhibit different immune regulatory roles including activation of cytotoxic T-lymphocytes and natural killer cells, production of interferon- $\gamma$  (IFN- $\gamma$ ). In addition they have antiangiogenic properties and induce apoptosis in tumor cells. Although they produce from moderate to severe side effects, significant anti-tumor activity was demonstrated in clinical trials for recombinant IL-12 and IL-18 proteins.

### **Combination with suicide gene therapy and whole tumor cells extracts for canine melanoma**

Spontaneous canine melanoma is a highly aggressive tumor. It appears mainly in the oral cavity, footpads, digits and mucocutaneous junctions, displaying a clinical behavior analogous to aggressive human melanoma. Both diseases are resistant to chemotherapy, radiotherapy and treatments with modulators of biological responses. They share comparable location selectivity and metastatic phenotypes.

As a continuation of encouraging data obtained with a surgery adjuvant treatment that consisted of complete or cytoreductive surgery followed by a combination of suicide gene therapy with a subcutaneous vaccine<sup>[7]</sup>, a

Table 1 Veterinary cancer immunogene therapy trials 2014-2016

No.	Genes	Tumor	Vector	Mode	Results	Ref.
1	hIL-2 + hGM-CSF cIFN-β + HSV-tk	MEL	Plasmid lipofection: HSV-tk + IFN-β + GCV. Plasmid lipofection: hIL-2 and hGM-CSF	<i>In vivo</i> (SG) + (IFN-β) - i.t. <i>In vivo</i> (hIL-2 + hGM-CSF) + (TV) - s.c./+ SX	Combined treatment ( <i>n</i> = 301)/surgery controls ( <i>n</i> = 162). Complete surgery arms: Local disease-free patients: 83%/11%. Metastasis-free patients: 89%/44%. Median survival: > 2211 d/109 d. Metastasis-free median survival: > 2211 d/134 d ( <i>n</i> = 4) Dose escalation and safety. Two confirmed local and distant responses. No severe side effects	Finocchiaro <i>et al</i> <sup>[18]</sup>
2	ttIL12	SCC MEL	Plasmid/EGT	<i>In vivo</i> - i.t. EGT	( <i>n</i> = 13) 2 CR, 4 PR, 5 SD, 2 PD. No severe side effects	Cutreria <i>et al</i> <sup>[19]</sup>
3	cIL-12	AMB, PCY, SCC, STS	Naked plasmid + bleomycin/ gemcitabine EGT	<i>In vivo</i> - i.t./+ CHT	( <i>n</i> = 18) 13 CR, 2 PR, 1 SD, 2 PD. No severe side effects	Cutreria <i>et al</i> <sup>[12]</sup>
4	hIL12	MCT	Naked plasmid + cisplatin or bleomycin electrotransfer	<i>In vivo</i> - i.t./+ CHT	( <i>n</i> = 9) Safety studies and demonstration of immunogenic and anti-angiogenic effects. No significant clinical benefits	Cemazar <i>et al</i> <sup>[14]</sup>
5	hIL12	FSA, MAC, MCT, OSA, SCC, SCH	Plasmid/EGT	<i>In vivo</i> - i.t.	( <i>n</i> = 6) No significant unwanted side effects. Combined therapy slowed down tumor progression and improved QOL	Cicchelero <i>et al</i> <sup>[10]</sup>
6	hIL12	ADC, FSA, MEL, OSA, SCH	Plasmid/EGT + metronomic cyclophosphamide	<i>In vivo</i> - i.t. EGT/+ metronomic CHT	Combined treatment ( <i>n</i> = 25)/ Surgery + brachytherapy controls ( <i>n</i> = 23). Disease-free patients: 41%/72%. Median survival: > 730 d/287 d	Cicchelero <i>et al</i> <sup>[13]</sup>
7	fIL-2	FSA	Canary pox virus	<i>In vivo</i> - p.t.	( <i>n</i> = 27) No differences between eIL-12 + eIL-18 without ( <i>n</i> = 9) or with either hgp100 ( <i>n</i> = 9) or htyr ( <i>n</i> = 9). Tumor size reduction on day 120 approximately 20% ( <i>n</i> = 14) VAC/ ( <i>n</i> = 13) CTR	Jas <i>et al</i> <sup>[11]</sup>
8	eIL-12 + eIL-18 hgp100 htyr	MEL	Plasmid like	<i>In vivo</i> - i.m./p.t.	Median survival: VAC 532 d/ CTR 205 d	Mählmann <i>et al</i> <sup>[15]</sup>
9	CSPG4	MEL	Plasmid/EGT	<i>In vivo</i> - i.m./+ SX	( <i>n</i> = 7) 5PR (50%-75% reduction) 2 SD. Safety verified	Riccardo <i>et al</i> <sup>[23]</sup>
10	hp62	MAC	Plasmid	<i>In vivo</i> - i.m.	Retrospective study ( <i>n</i> = 38). Responding patients median survival: 870 d. Non-responding patients median survival: 240 d	Gabai <i>et al</i> <sup>[22]</sup>
11	htyr	MEL	Naked plasmid - Jet injection	<i>In vivo</i> - i.m./+ SX ± RX	Retrospective study ( <i>n</i> = 32). Median survival: 335 d. Progression-free median survival: 160 d	McLean <i>et al</i> <sup>[19]</sup>
12	htyr	MEL	Naked plasmid - Jet injection	<i>In vivo</i> - i.m./+ SX ± RX	( <i>n</i> = 23) VAC/ ( <i>n</i> = 19) CTR	Treggiari <i>et al</i> <sup>[20]</sup>
13	CSPG4	MEL	Plasmid/EGT	<i>In vivo</i> - i.m./+ SX	Median survival: VAC 684 d/ CTR 220 d	Piras <i>et al</i> <sup>[24]</sup>
14	htyr	MEL	Naked plasmid - Jet injection	<i>In vivo</i> - i.m./+ SX ± RX	Retrospective study ( <i>n</i> = 24). Eleven percent of manageable post-vaccination adverse effects. Safety verified. Forty-two percent died of unrelated causes or still alive	Sarbu <i>et al</i> <sup>[18]</sup>

The No. 1-8 genes are cytokines; the No. 9-14 genes are antigens; the tumors in No. 1-6 and 9-13 are canine; the tumors in No. 7 and 14 are feline; the tumors in No. 8 is equine. ADC: Adenocarcinoma; AMB: Acanthomatous ameloblastoma; FSA: Fibrosarcoma; MAC: Mammary adenocarcinoma; MCT: Mast cell tumor; MEL: Melanoma; PCY: Plasmocytoma; OSA: Osteosarcoma; SCC: Squamous cell carcinoma; SCH: Schwannoma; STS: Soft tissue sarcoma; CSPG4: Chondroitin sulfate proteoglycan-4; GM-CSF: Granulocyte macrophage colony-stimulating factor; gp100: Glycoprotein 100; HSV-tk: Herpes simplex thymidine kinase; IFN-β: Interferon-β; IL: Interleukin; p62: Protein 62; tyr: Tyrosinase; CR: Complete response; CTR: Control; GT: Gene therapy; PR: Partial response; SD: Stable disease; GCV: Ganciclovir; SG: Suicide gene; TV: Tumor vaccine; VAC: Vaccinated; i.m.: Intramuscular, i.t.: Intratumoral, p.t.: Peritumoral; s.c.: Subcutaneous; CHT: Chemotherapy; RX: Radiotherapy; SX: Surgical excision; c: Canine; e: Equine; f: Feline; h: Human; tt: Tumor targeted.

new local and vaccine formulation was assayed<sup>[8]</sup>. This new trial involved the injection (at the end of surgery)

of DMRIE/DOPE lipoplexes carrying two therapeutic genes: Canine IFN-β and herpes simplex virus thymidine

kinase. In parallel, a subcutaneous genetic vaccine made of lipoplexes carrying human GM-CSF and IL-2 genes and tumor formalized extracts was periodically administered. Taking surgery-only-treated (ST) as controls, it was found that the combined treatment (CT) that followed complete surgery (CS) significantly increased the portion of local distant metastases-free (M0) from 44% to 89% and disease-free canine patients from 11% to 83%. Even in the case of cytoreductive surgery (CR), CT had a better control of the systemic disease (M0: 82%) than ST (M0: 48%). Furthermore, taking the ST group as control, CT displayed a considerable > 17-fold (CS) and > 13-fold (CR) increase of metastasis-free survival (MFS), and 7-fold (CS) and 4-fold (CR) increase of overall survival. The remarkable rise of CS-recurrence-free survival (RFS) and CS-MFS (both > 2251 d) and CR-MFS (> 1321 d) revealed that CT was transforming a fast terminal disease into a chronic one. Finally as a surgery adjuvant, this CT was able to considerably postpone or preclude distant metastasis and postsurgical recurrence, while it extended disease-free and overall survival, and preserved the quality of life. The long-term safety and efficacy of this treatment was supported by the high number of canine patients involved and the wide follow-up (> 6 years) with negligible or null toxicity. Besides, this work confirmed that the most advantageous clinical situation is to implement this approach as a surgery adjuvant acting on the minimal residual disease.

#### **Single treatments for canine and feline solid tumors**

Electrotransfer was the preferred method for introducing IL-12 plasmids into cells. In a first report, the peptide-cytokine VNTANST-IL12 could successfully target expressed cell-surface vimentin while displaying the antitumor effects of cytokine IL-12. So, a tumor-targeted IL-12 plasmid DNA (ttIL12 pDNA) was safely delivered at clinical levels by electrotransfer to four tumor bearing canine patients, inducing antitumor immune responses in both treated and untreated tumors including metastatic lesions<sup>[9]</sup>.

In a parallel study, nine dogs with spontaneous cancer were subjected to three consecutive intratumoral IL-12 electrogene therapy (EGT) sessions to assess their clinical, anti-angiogenic and immunological effects<sup>[10]</sup>. Serum and tumor tissue displayed transitory increases of IFN- $\gamma$  and IL-12. Intratumoral IL-12 EGT did not produce clinically significant results, even though some anti-angiogenic and immunostimulatory activities appeared as well as transitory objective responses did. It was not possible to get a sustained tumor regression during the trial. Without severe side effects, safety was verified.

Fibrosarcoma is a lethal disease in cats that is frequently found at standard vaccine injection sites. Because of the high local recurrence rates after surgery alone, the addition of adjuvant treatments has been under investigation for the last few years. A randomized, controlled clinical study was performed to determine the safety and efficacy of a recombinant canarypox virus

carrying the feline IL-2 gene (ALVAC IL-2)<sup>[11]</sup>. Additionally to surgery and post-surgical brachytherapy and as adjuvant treatment, the vector was transferred to cats with a first occurrence of feline fibrosarcoma. The pet patients were distributed at random to a control group (23 cats), low dose injected group (25 cats) and high dose injected group (23 cats). The treatment consisted of six successive intratumoral injections of ALVAC IL-2. With limited mild local reactions, it was well tolerated. Injected animals displayed a significantly longer median time to relapse (> 730 d in the low dose injected group) than in the control group (287 d). The risk of relapse of treated cats displayed a considerable decrease: 56% at one year (injected groups compared to control group) and 65% at two years (low dose injected group compared to control group).

#### **Combination with electro-chemotherapy for canine solid tumors**

In a further report, a study demonstrated the efficacy and safety of recurring cycles of IL-12 gene therapy plus chemotherapy for long-term treatment of aggressive tumors<sup>[12]</sup>. In this case 13 canine patients were subjected to various rounds of electro-chemo-gene therapy (ECGT) with canine IL-12 plasmid DNA (pDNA) and either gemcitabine or bleomycin. This approach was versatile, being effective for many histotypes, and allowed fast eradication or debulk of large tumors (sarcoma excluded). It affected both treated tumors and non-treated metastatic tumors. Without severe adverse events reported, even after multiple treatment cycles, ECGT with IL-12 pDNA proved to be a very valuable approach for many types of spontaneous cancers including refractory, large and multiple tumor loads. Being this phase I trial designed for dose escalation/safety assessment, no data about long term quality of life and progression free survival could be obtained.

By combining with metronomic cyclophosphamide, the outcomes of three successive intratumoral IL-12 electrogene transfer treatments<sup>[13]</sup> were explored in six dogs with spontaneous tumors. This treatment induced erythema and swelling of the tumor, a transient increase in IL-12 levels, and a continuous increase in IFN- $\gamma$  and thrombospondin-1 concomitant with a continuous decrease in vascular endothelial growth factor. While the treatment improved the quality of life and weight, it slowed tumor progression in most of the patients (4/6).

Mast cell tumors (MCTs) are common cutaneous neoplasms in dogs, accounting for up to 21% of all canine skin tumors. Peritumoral IL-12 gene transfer was combined with electro-chemotherapy (cisplatin or bleomycin) targeting mast cell tumors in 18 canine patients<sup>[14]</sup>. Without emerging side effects, one month after the therapy a considerable local tumor control was evidenced. The complete responses rate increased up to 72% during the observation period. IL-12 gene electrotransfer produced detectable serum IL-12 and/or IFN- $\gamma$  levels in 78% of patients. This study showed a high antitumor efficacy of the combined treatment that also prevented recurrences or distant metastases.

In addition the safety and feasibility of this approach was demonstrated.

### **Combination with antigens for equine melanoma tumors**

Equine melanoma has a high incidence in grey horses. In a clinical trial<sup>[15]</sup>, 27 melanoma-bearing grey horses were allocated into 3 groups ( $n = 9$ ) and intramuscularly vaccinated on days 1, 22, and 78 with lipoplexes carrying DNA plasmids bearing equine *IL-18* and *IL-12* genes alone or combined with either human glycoprotein 100 or tyrosinase. In all groups, by day 120 the relative tumor volume significantly decreased about 20%. The combination of the two cytokines was safe, but the addition of DNA vectors encoding the human melanoma antigens did not potentiate their effects.

## **ANTIGEN-BASED IMMUNOGENE THERAPY**

As it happens in dogs, in cats malignant melanoma appears as locally aggressive and highly metastatic regardless of the primary site of origin. The standard modalities used for treatment are surgery and radiotherapy, with poor results in the long-term. Following the studies about the use of xenogeneic antigen vaccination for spontaneous canine malignant melanoma<sup>[16,17]</sup>, new data about intramuscular needle free jet injection of a plasmid containing human tyrosinase gene and its expression as a melanoma xen-antigen against feline and canine melanoma were reported.

In a retrospective study the safety of the canine melanoma DNA vaccine (Oncept<sup>®</sup>) following surgery in 24 feline patients was assessed<sup>[18]</sup>. The vaccine appeared to be well tolerated, with a low number of reported adverse events. Since most cats finally died from this disease, controlled prospective studies are necessary to determine the immunogenicity and efficacy of this vaccine originally designed for canine melanoma in cats with melanoma.

Furthermore, two additional independent retrospective analyses on the use of this DNA vaccine for canine melanoma<sup>[19,20]</sup> were in agreement on safety issues with previously published results<sup>[16,17]</sup>. In the first case 38 canine patients were evaluated: The median survival time of responding patients (42%) was 870 d, while it was 240 d in the case of non-responding ones (58%)<sup>[19]</sup>. Even though a relatively small number of patients was analyzed, melanotic oral melanoma appeared to give survival advantage as compared to its amelanotic counterpart. As expected, surgery with clean margins also improved the treatment outcome. In the second case, 32 oral melanoma patients received the DNA vaccine after surgery displaying a median survival time of 335 d<sup>[20]</sup>. No significant differences were found when compared to other adjuvant treatments. Because of the low statistical power due to small number and group heterogeneity, the authors proposed controlled studies to assess whether the addition of any adjuvant treatment to surgery, including immunogene therapy, is able to significantly prolong survival in cases of canine oral melanomas. A very recent

paper<sup>[21]</sup> analyzing the outcome of 69 canine melanoma patients after human tyrosinase DNA vaccination, reported a median survival time of 455 d, a value that is similar to those published earlier<sup>[19,20]</sup>. Since the authors observed responses in patients with macroscopic disease, they suggest that the vaccine could be considered as palliative treatment in dogs with remaining tumors or recurrence.

Mammary tumors are the most common tumors of unspayed female dogs with a prevalence of 40% of all tumors, and about half of them are malignant. The p62 protein (SQSTM1) is a major player in selective macroautophagy and acts as a signaling hub for many signal transduction pathways. It is noteworthy that p62 is expendable for normal tissues, but critical for development and survival of tumors. The effects of intramuscular p62 DNA vaccine on mammary tumors of seven dogs were tested<sup>[22]</sup>. Locally advanced lesions decreased (5/7) or stabilized (2/7) their growth while the overall toxic effects of p62 were absent. The observed antitumor activity was associated with lymphocyte infiltration and tumor encapsulation *via* fibrotic reaction.

Chondroitin sulfate proteoglycan-4 (CSPG4) is an early cell surface progression marker associated with tumor cell migration, invasion and proliferation. In a first trial, the immunogenicity, safety and therapeutic efficacy of a human CSPG4 DNA-based vaccine were evaluated<sup>[23]</sup>. Canine patients with stage II - III surgically excised CSPG4-positive oral malignant melanoma were treated by adjuvant intramuscular electrotransfer with human CSPG4-encoded plasmid from 6 to 20 mo. Overall (653 d vs 220 d) and disease-free (477 d vs 180 d) survival times were significantly longer in 14 vaccinated dogs as compared with 13 non vaccinated controls. No clinically relevant local or systemic side effects were found. This suggested that xenogeneic electrovaccination against CSPG4 can prevail over the host tolerance to the "self" antigen, being successful in treating canine malignant melanoma.

In a second trial, the same treatment was performed in 23 dogs with surgically excised CSPG4-positive oral canine melanoma. In parallel, 19 control dogs with CSPG4-positive tumors were subjected only to surgery<sup>[24]</sup>. Vaccinated dogs displayed a one year survival rate of 74%, with a median survival time of 684 d, and one year disease-free interval rate of 48%. Non-vaccinated dogs showed one year survival rate of 26%, with a median survival time of 200 d, and a one-year disease-free interval rate of 26%. The involvement of a fair amount of canine patients together with the relatively wide follow-up (2 years) with absent or minimal adverse side effects, added to the results of the previous report<sup>[23]</sup>, assure the significant efficacy and the long-term safety of this treatment.

## **CONCLUSION**

The suitability of comparative oncology using large animal naturally occurring cancer models to test new



cancer drugs, particularly gene medicines, was widely discussed<sup>[2,25]</sup>.

This approach can primarily benefit pets that do not have efficient treatments for some malignancies and ultimately human patients. The proof of concept and safety results obtained in companion animals can be readily used for designing clinical trials against the corresponding human malignancies.

Some immunogene therapy approaches similar to those presented here were also tested in human patients before 2014: Xenogeneic tyrosinase<sup>[26]</sup> and gp100<sup>[27]</sup>, IL-2<sup>[28]</sup>, GM-CSF<sup>[29]</sup> and IL-12<sup>[30]</sup>. On the other hand, encouraging results were recently reported about a clinical trial of *AdCD40L* gene therapy combined with cyclophosphamide chemotherapy for human melanoma<sup>[31]</sup> and p62 protein (SQSTM1) gene therapy for some human solid tumors<sup>[32]</sup>. These trials were respectively based on previous veterinary trials involving canine melanoma<sup>[33]</sup> and canine mammary adenocarcinoma<sup>[22]</sup> patients. In addition, our Unit is currently involved in a phase I clinical trial for human advanced melanoma based on the safety and efficacy of our previous long lasting veterinary clinical trials<sup>[7,8]</sup>.

Because of safety reasons and the cost of producing the suitable vectors, most of the veterinary gene therapy protocols were done with non viral vectors, being electrotransfer mainly used in the latest period (2014-2016).

Four controlled trials<sup>[8,11,23,24]</sup> showed statistically significant tumor control and survival time increase, while maintaining a good quality of life. These results strongly support further developments in immunogene therapy. Meanwhile, new immune-mediated gene therapy approaches are emerging for treating equine melanoma with a bacterial antigen<sup>[34]</sup> and canine lymphoma with a chimeric antigen receptor<sup>[35]</sup>. A different gene therapy approach is also under investigation for feline oral squamous cell carcinoma with RNAi oligonucleotides targeting feline CK2 $\alpha$  and CK2 $\alpha'$  (TBG-RNAi-fCK2 $\alpha\alpha'$ )<sup>[36]</sup>.

As we discussed earlier<sup>[2]</sup>, proper cancer models involving companion animals spontaneously induce the investigators to work in the duality between the veterinary perspective and the potential application to human medicine. However, these are not contradictory objectives, and more trials in humans based on the equivalent trials in pets will possibly come soon.

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## Basic Study

**Role of LIGHT in the pathogenesis of joint destruction in rheumatoid arthritis**

Afsie Sabokbar, Sara Afrough, David J Mahoney, Yoshinobu Uchihara, Catherine Swales, Nicholas A Athanasou

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**Author contributions:** All authors contributed equally to this work; Sabokbar A, Mahoney DJ and Uchihara Y performed the research; Swales C contributed clinical materials; Sabokbar A, Afrough S, Mahoney DJ, Uchihara Y and Athanasou NA analysed the data; Sabokbar A, Afrough S, Mahoney DJ and Athanasou NA wrote the paper and approved the final version of the article to be published.

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**Abstract****AIM**

To characterise the role of substitutes for receptor-activator nuclear factor kappa-B ligand (RANKL) in rheumatoid arthritis (RA) joint destruction.

**METHODS**

Synovial fluid (SF) macrophages isolated from the knee joint of RA patients were incubated with 25 ng/mL macrophage-colony stimulating factor (M-CSF) and 50 ng/mL LIGHT (lymphotoxin-like, exhibits inducible expression and competes with herpes simplex virus glycoprotein D for herpes virus entry mediator, a receptor expressed by T lymphocytes) in the presence and absence of 25 ng/mL RANKL and 100 ng/mL osteoprotegerin (OPG) on glass coverslips and dentine slices. Osteoclastogenesis was assessed by the formation of multinucleated cells (MNCs) expressing tartrate-resistant acid phosphatase (TRAP) on coverslips and the extent of lacunar resorption pit formation on dentine slices. The concentration of LIGHT in RA and osteoarthritis (OA) synovial fluid was measured

by an enzyme-linked immunosorbent assay (ELISA) and the expression of LIGHT in RA and OA synovium was determined by immunohistochemistry using an indirect immunoperoxidase technique.

## RESULTS

In cultures of RA SF macrophages treated with LIGHT and M-CSF, there was significant formation of TRAP + MNCs on coverslips and extensive lacunar resorption pit formation on dentine slices. SF-macrophage-osteoclast differentiation was not inhibited by the addition of OPG, a decoy receptor for RANKL. Resorption pits were smaller and less confluent than in RANKL-treated cultures but the overall percentage area of the dentine slice resorbed was comparable in LIGHT- and RANKL-treated cultures. LIGHT significantly stimulated RANKL-induced lacunar resorption compared with RA SF macrophages treated with either RANKL or LIGHT alone. LIGHT was strongly expressed by synovial lining cells, subintimal macrophages and endothelial cells in RA synovium and the concentration of LIGHT was much higher in RA compared with OA SF.

## CONCLUSION

LIGHT is highly expressed in RA synovium and SF, stimulates RANKL-independent/dependent osteoclastogenesis from SF macrophages and may contribute to marginal erosion formation.

**Key words:** Receptor-activator nuclear factor kappa-B ligand; Osteoclast; Rheumatoid arthritis; Resorption; LIGHT

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**Core tip:** Rheumatoid arthritis (RA) is an inflammatory joint condition characterised by the formation of marginal erosions due to the activity of bone resorbing osteoclasts. Osteoclasts can be formed from macrophages by both receptor-activator nuclear factor kappa-B ligand (RANKL)-dependent and RANKL-independent mechanisms. LIGHT is a potent RANKL substitute that induces significant osteoclast formation from cultures of RA synovial fluid macrophages; this results in comparable levels of resorption to that seen in RANKL-treated cultures. LIGHT also stimulated RANKL-mediated lacunar resorption. LIGHT is highly expressed in RA joints and synovial fluid and is likely to play a key role in the pathogenesis of marginal erosion formation in RA.

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## INTRODUCTION

Rheumatoid arthritis (RA) is a common inflammatory

arthropathy which affects approximately 1% of the adult population<sup>[1]</sup>. RA is characterized by a heavy lymphocyte and plasma cell infiltrate in the synovium, synovial overgrowth; and extension of inflammatory tissue into bone; this leads to the formation of periarticular marginal erosions by osteoclasts, multinucleated cells which are specialised to carry out lacunar bone resorption. The osteoclast is a member of the mononuclear phagocyte system and is formed from CD14<sup>+</sup> macrophage precursors in the presence of macrophage-colony stimulating factor (M-CSF) and receptor activator for nuclear factor  $\kappa$ B ligand (RANKL), a tumour necrosis family (TNF) superfamily member<sup>[2-5]</sup>. RANKL is expressed by activated fibroblasts, osteoblasts and lymphocytes which also produce osteoprotegerin (OPG), a decoy receptor for RANKL that inhibits osteoclastogenesis<sup>[6,7]</sup>. The RANKL-OPG axis is key to controlling osteoclast formation and survival. A monoclonal antibody directed against RANKL, denosumab, has been shown to increase bone mineral density and to reduce pathological bone resorption in osteoporosis and RA<sup>[8-11]</sup>.

Several studies have shown that osteoclasts can be formed by a RANKL-independent pathway promoted by other TNF superfamily members<sup>[12-17]</sup>. LIGHT (lymphotoxin-like, exhibits inducible expression and competes with herpes simplex virus glycoprotein D for herpes virus entry mediator, a receptor expressed by T lymphocytes) is the most potent RANKL substitute identified to date<sup>[14,18,19]</sup>. LIGHT (TNFSF14) is a type 2 transmembrane glycoprotein which is expressed on activated T lymphocytes, monocytes and dendritic cells<sup>[20-22]</sup>. LIGHT binds to two membrane-bound members of the TNFR superfamily, herpes virus entry mediator (HVEM), which is expressed by many inflammatory cells including T cells, B cells, monocytes and dendritic cells, and lymphotoxin  $\beta$  receptor (LT $\beta$ R), which is expressed by many cell types such as fibroblasts, endothelial cells, stromal cells and monocytes but not lymphocytes<sup>[23-25]</sup>. LIGHT also binds to decoy receptor 3 (DcR3), a soluble non-signaling receptor which has been shown to modulate its function *in vitro* and *in vivo*<sup>[23,26]</sup>. LIGHT is expressed constitutively by dendritic cells and activated T cells and is mainly found in lymphoid tissues<sup>[20-22]</sup>. LIGHT has been shown to induce osteoclast formation by a RANKL-independent mechanism from monocytes<sup>[14,19]</sup>.

The role LIGHT plays in inducing pathological bone resorption in RA joints is uncertain. LIGHT levels are increased in the serum of patients with RA, and blocking the action of LIGHT has been shown to reduce the severity of murine collagen-induced arthritis<sup>[14,27]</sup>. Synovial fluid (SF) macrophages are known to differentiate into osteoclasts in the presence of RANKL and M-CSF<sup>[28]</sup>. In this study we have examined the role LIGHT plays in the pathogenesis of inflammatory joint destruction by determining whether LIGHT alone can stimulate osteoclastogenesis from SF macrophages isolated from RA joints. We have also examined whether the concentration of LIGHT is higher in RA than non-inflammatory osteoarthritis (OA) SF and compared the

expression of LIGHT in RA and OA synovium.

## MATERIALS AND METHODS

### *Patients samples and reagents*

SF samples were derived from six patients (four females, one male) undergoing therapeutic arthrocentesis for inflammatory joint disease. Non-inflammatory OA control SF was derived from four patients (two females, two males) undergoing unicompartmental knee replacement. All patients diagnosed with RA were seropositive for rheumatoid factor and met the ACR/EULAR diagnostic criteria for RA<sup>[29]</sup>. RA and OA synovial tissue was derived from biopsy specimens. All patients gave informed consent and the Oxford Clinical Research Ethics Committee approved the study.

For all tissue culture incubations,  $\alpha$ -minimum essential medium ( $\alpha$ -MEM) (Invitrogen, United Kingdom) was supplemented with 2 mmol/L (w/v) glutamine, 10  $\mu$ g/mL streptomycin, 100 IU/mL benzyl penicillin, 10% (v/v) heat-inactivated fetal bovine serum (FBS). Recombinant soluble human RANKL was purchased from Peprotech Europe (London, United Kingdom); all other cytokines were purchased from R and D Systems Europe (Abingdon, United Kingdom). Primary antibodies for immunohistochemistry were purchased from Abcam (Cambridge, United Kingdom).

### *Isolation and culture of SF macrophages*

SF macrophages were isolated from aspirates by centrifugation at 2500 rpm for 10 min at 4 °C; macrophages were subsequently used for osteoclastogenesis (see below) while the cell-free supernatant was frozen and stored at -80 °C for ELISA measurement of LIGHT levels. Following centrifugation of SF aspirates, the resultant cell pellet was re-suspended  $\alpha$ -MEM/FBS and then added at  $1 \times 10^6$  cells/well to 96 well tissue culture plates containing 5 mm dentine slices. After 2 h incubation, dentine slices were removed from the wells and washed vigorously in MEM/FBS to remove non-adherent cells; the cell suspension was subsequently transferred to a 24-well tissue culture plate containing 1 mL of MEM/FBS supplemented with various factors. As positive controls, SF macrophage cultures were maintained in the presence of 25 ng/mL M-CSF plus 50 ng/mL soluble RANKL. As negative controls, cultures were maintained with M-CSF alone. SF macrophages were also cultured in the presence of LIGHT (50 ng/mL)  $\pm$  OPG (100 ng/mL). All SF macrophage cultures were incubated for up to 14 d, during which time the entire culture medium containing all factors was replenished every 2-3 d.

### *Characterisation of osteoclast formation and activation*

**Tartrate-resistant acid phosphatase:** After 14 d in culture, cells were fixed in formalin and stained histochemically for tartrate-resistant acid phosphatase (TRAP), an osteoclast marker, using naphthol AS-BI as a substrate, in the presence of 1.0 mol/L tartrate.

**F-actin ring formation:** Multiple rows of podosomes

containing an F-actin core are often localised in the sealing zone of osteoclasts. To detect F-actin ring structure, dentine slices were fixed with 4% formaldehyde for 5 min and then permeabilised for 6 min in 0.5% Triton X-100 in phosphate buffered saline (PBS) and rinsed with PBS. The cells on dentine slices were then incubated with tetramethyl-rhodamine isothiocyanate-conjugated phalloidin (Sigma-Aldrich) for 30 min and observed using a fluorescence microscope (Olympus).

**Lacunar resorption:** Functional evidence of osteoclast formation was determined using a resorption assay system. Circular dentine slices (4 mm in diameter) were prepared from elephant tusk blocks, kindly supplied by the Customs and Excise, United Kingdom, and sterilised in absolute alcohol overnight. After 14-d incubation, dentine slices on which cells had been cultured, were removed from wells, rinsed in PBS, incubated in 1.0 M ammonium hydroxide for 24 h and sonicated in distilled water for 5-10 min. All cellular debris was thus removed from the dentine slice permitting examination of its surface for evidence of lacunar resorption. The slices were washed in distilled water, stained with 0.5% (w/v) toluidine blue in 1.0% (w/v) boric acid pH 5.0 and examined by light microscopy. To quantify the lacunar resorption, dentine slices were photographed and the resorbed areas highlighted and measured using Adobe Photoshop CS3 and Image J (NIH, Bethesda, MD, United States).

### *Quantification of LIGHT levels in synovial fluid of OA and RA patients*

Human LIGHT enzyme-linked immunosorbent assay (ELISA) kit (R and D Systems Europe) was used to determine the concentration of soluble LIGHT in the synovial fluid derived from RA ( $n = 5$ ) and OA ( $n = 4$ ) patients, as per manufacturer's instructions. The upper and lower detection limits of the ELISA were 31 pg/mL and 2 ng/mL, respectively.

### *Immunohistochemistry of RA and non-inflammatory OA synovial tissue*

Formalin-fixed, paraffin-embedded synovial biopsies of RA and OA synovium were cut (3  $\mu$ m), deparaffinized with xylene and rehydrated through a series of graded alcohols. After blocking endogenous peroxidase with 0.2% (v/v) hydrogen peroxide in 80% alcohol for 30 min, antigen retrieval was performed in 500 mL 10 mmol/L Tris + 1 mol/L EDTA (BDH, United Kingdom) buffer (pH 8.5) using a microwave for 20 min. Immunohistochemistry was performed using an indirect immunoperoxidase technique with 3,3-diaminobenzidine chromogen (EnVision™ + Dual Link System-HRP, Liquid DAB + Substrate Chromogen System, Dako, United Kingdom). Sections were incubated with an anti-LIGHT antibody (anti-hLIGHT/HVEM-L, R and D systems, United States) 1:5 overnight at room temperature followed by 30 min incubation with labeled polymer and 10 min in 3,3-diaminobenzidine. Slides were counterstained using Mayer's haematoxylin for 3 min, blued in 2% hydrogen sodium carbonate, dehydrated,



cleared in xylene and mounted using DePeX (Surgipath, United Kingdom). All sections were examined by light microscopy.

### **Ethical consideration**

All tissue specimens and blood samples from RA and OA patients were taken after informed consent and ethical permission was obtained for participation in the study.

### **Statistical analysis**

The statistical review of the study was performed prior and after the study was conducted and with consultation with a biomedical statistician. Data is represented as mean  $\pm$  SEM. For assessment of osteoclast resorption, the area of lacunar resorption was normalized and expressed as a percentage of RANKL-induced lacunar resorption (positive control). Statistical significance was determined by Mann-Whitney test or one-way ANOVA, using GraphPad Prism (Version 6.03). *P* value  $< 0.05$  were considered as statistically significant.

## **RESULTS**

### **LIGHT induces RANKL-independent osteoclastogenesis from SF macrophages**

In 14-d cultures of SF macrophages incubated with M-CSF and RANKL, numerous TRAP<sup>+</sup> and F-actin ring<sup>+</sup> multinucleated cells were generated (Figure 1); these cells were capable of lacunar resorption when cultured on dentine slices (Figure 1). Cultures of SF macrophages with LIGHT and M-CSF under similar conditions also resulted in the formation of comparable numbers of TRAP<sup>+</sup> and F-actin ring<sup>+</sup> multinucleated cells (Figure 1A) and a similar level of lacunar resorption on dentine slices (Figures 1B). The addition of excess molar concentrations of OPG (100 ng/mL) did not result in a decrease in lacunar resorption pit formation compared with cultures treated with LIGHT alone (Figures 1B and C), confirming that LIGHT-induced osteoclast formation did not occur *via* the RANKL pathway. Resorption pits formed in LIGHT-treated SF macrophage cultures were smaller and more often single than in RANKL-treated cultures where most resorption pits were compound or confluent with many long resorption tracks being produced (Figure 1B). The overall percentage area of the dentine slice resorbed in LIGHT-treated cultures was comparable to that seen in RANKL-treated cultures (Figure 1C).

### **LIGHT augments RANKL-induced osteoclastogenesis from SF macrophages**

In 14-d cultures of RA SF macrophages on dentine slices incubated with M-CSF and RANKL, a significant increase in lacunar resorption was seen compared with RA SF macrophage cultures treated with RANKL alone (Figure 2).

### **LIGHT in RA and OA synovial fluid**

The concentration of LIGHT was significantly elevated in the synovial fluid derived from inflammatory RA compared with non-inflammatory OA joints (Figure 3). The mean level of LIGHT detected in the synovial fluid of RA patients was  $452.5 \pm 70.5$  pg/mL. This was significantly higher (*P*  $< 0.05$ ) compared with that found in non-inflammatory OA patients ( $45.43 \pm 19.8$  pg/mL).

### **Expression of LIGHT in RA and OA synovium**

Immunohistochemistry showed that there was little or no expression of LIGHT in OA synovium, but in RA synovium LIGHT was strongly expressed by synovial lining cells and subintimal macrophages (Figure 4). Endothelial cells were also weakly stained, but lymphoid cells and fibroblasts were negative.

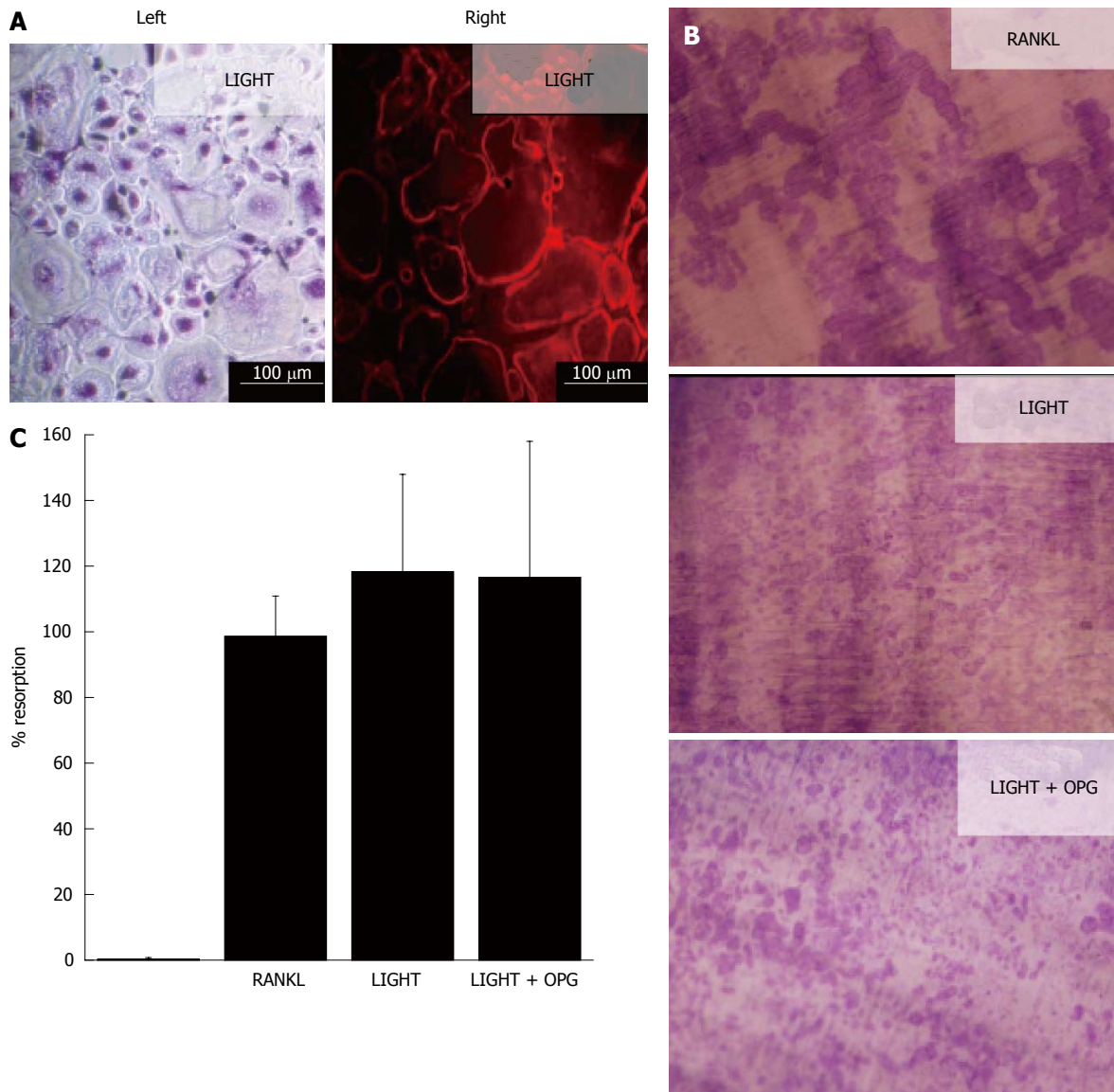
## **DISCUSSION**

The canonical RANKL/OPG axis is the main pathway of osteoclastogenesis and is believed to play a major role in pathological bone resorption in RA<sup>[30,31]</sup>. RANKL expression is upregulated by inflammatory cytokines found in RA joints such as IL-1, IL-6, IL-17 and tumor necrosis factors (TNF)- $\alpha$ <sup>[30-32]</sup>. RANKL is a member of the TNF superfamily and it has been shown that other TNF superfamily ligands can induce RANKL-independent osteoclastogenesis. In this study we show that one of these factors, LIGHT, can substitute for RANKL and induce osteoclast formation from SF macrophages derived from RA joints. LIGHT-induced osteoclast formation was not inhibited by OPG and resulted in comparable levels of lacunar resorption to that seen in RANKL-treated cultures. LIGHT also augmented RANKL-mediated lacunar resorption and was found to be highly expressed in RA synovium and SF.

LIGHT is a potent RANKL-independent osteoclastogenic factor that has been shown in several studies to play a role in RA<sup>[13]</sup>. An increase in LIGHT levels has been noted in the serum of RA patients<sup>[14]</sup>. LIGHT is upregulated on B-lymphocytes and monocytes in RA and it has been shown to induce the expression of pro-inflammatory cytokines and metalloproteinases in macrophages and synoviocytes<sup>[33,34]</sup>. RA synovial fibroblasts express the LIGHT receptors HVEM and LT $\beta$ R; LIGHT activates synovial fibroblasts, resulting in an increase in cytokines and growth factors that promote inflammation and resorption; LIGHT also induces the proliferation of RA synovial fibroblasts through LT $\beta$ R<sup>[35,36]</sup>. It has also been shown that CD14<sup>+</sup> monocytes interact with stromal cells in RA synovium to induce the formation of TRAP<sup>+</sup> MNCs and that LIGHT enhances the generation of MNCs that release metalloproteinases including MMP9 and MMP12, both of which are found at sites of joint erosion in RA<sup>[37]</sup>.

Our data indicates that LIGHT is likely to play a



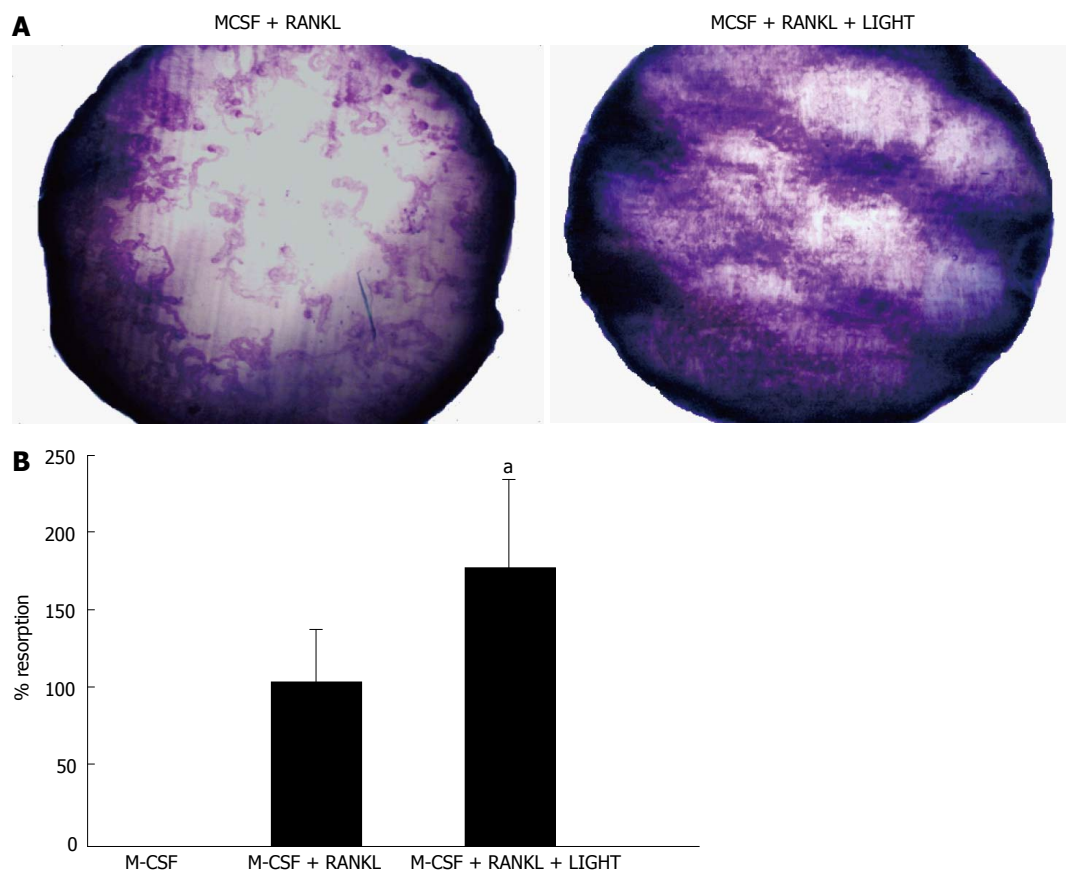


**Figure 1** LIGHT induces receptor-activator nuclear factor kappa-B ligand-independent osteoclastogenesis from rheumatoid arthritis synovial fluid macrophages. A: Osteoclast differentiation in 14-d cultures of RA SF macrophages incubated with M-CSF and LIGHT showing (left) TRAP<sup>+</sup> multinucleated osteoclasts and (right) F-actin-ring formation; B: Dentine slices stained with Toluidine blue showing lacunar resorption in 14-d RA SF macrophage cultures treated with M-CSF and sRANKL, LIGHT or LIGHT  $\pm$  OPG; C: Percentage surface area lacunar resorption on dentine slices in LIGHT- ( $\pm$  OPG) treated SF macrophage cultures relative to sRANKL - treated controls; data is expressed as mean  $\pm$  SEM of three independent experiments where each condition was carried out in triplicate. RANKL: Receptor-activator nuclear factor kappa-B ligand; RA: Rheumatoid arthritis; SF: Synovial fluid; M-CSF: Macrophage-colony stimulating factor; OPG: Osteoprotegerin.

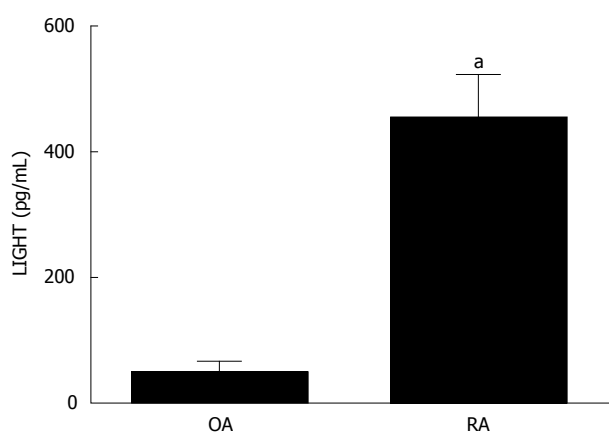
significant role in pathological bone resorption in RA. SF macrophages, like monocytes, are CD14<sup>+</sup> cells that are capable of differentiating into osteoclasts<sup>[28]</sup>. These cells are present in increased numbers in RA compared with OA joints and joint fluid, as are other inflammatory cells that are known to express LIGHT. This accords with our finding that the concentration of LIGHT is increased in RA compared with non-inflammatory OA joint fluid. There was little expression of LIGHT in OA synovium whereas it was strongly expressed in RA synovium mainly in synoviocytes and subintimal macrophages. The combination of an increased number of CD14<sup>+</sup> mononuclear phagocyte osteoclast precursors and high levels of LIGHT in the SF provides the conditions for LIGHT-induced osteoclastogenesis to exist in inflamed

RA joints.

In keeping with previous studies<sup>[14]</sup>, we found that LIGHT-induced osteoclast differentiation from RA SF macrophages occurred in the absence of RANKL. LIGHT-induced osteoclastogenesis was not inhibited by the RANKL inhibitor OPG and was comparable to that seen in RANKL-treated SF macrophage cultures in terms of formation of TRAP + MNC and lacunar resorption. We also found that LIGHT significantly augmented osteoclast formation in the presence of RANKL, in keeping with the findings of Ishida *et al.*<sup>[37]</sup> who noted that LIGHT promotes RANKL-induced formation of TRAP + MNCs from CD14<sup>+</sup> precursors. Our findings indicate that LIGHT is therefore likely to play a role in both RANKL-dependent and RANKL-independent osteoclast formation and pathological bone



**Figure 2** LIGHT augments receptor-activator nuclear factor kappa-B ligand-induced osteoclastogenesis from rheumatoid arthritis synovial fluid macrophages. A: Dentine slice stained with Toluidine blue showing lacunar resorption pits in 14-d RA SF macrophage cultures incubated with M-CSF and sRANKL  $\pm$  LIGHT; B: Percentage surface area lacunar resorption on dentine slices in 14-d RA SF macrophage cultures incubated with M-CSF and sRANKL  $\pm$  LIGHT. Data is expressed as mean  $\pm$  SEM of three independent experiments where each condition was carried out in triplicate; <sup>a</sup> $P < 0.05$ . RANKL: Receptor-activator nuclear factor kappa-B ligand; RA: Rheumatoid arthritis; SF: Synovial fluid; M-CSF: Macrophage-colony stimulating factor.

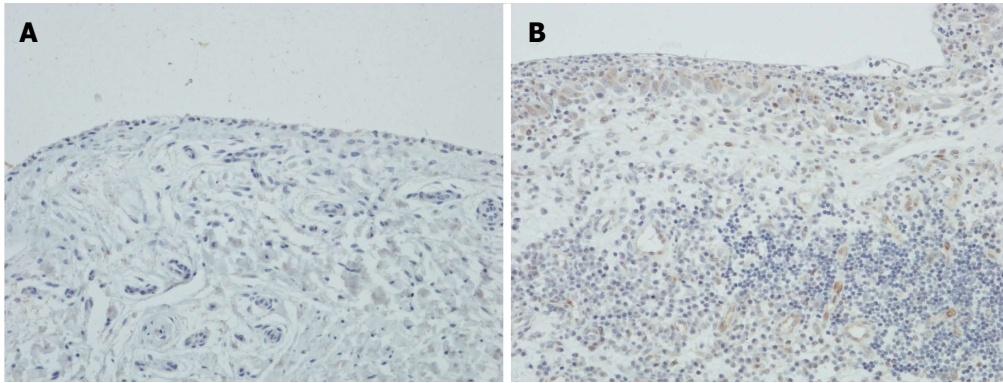


**Figure 3** LIGHT levels are significantly higher in rheumatoid arthritis than osteoarthritis synovial fluid. LIGHT concentration is significantly increased in SF of RA patients compared with OA joints. Data is expressed as mean  $\pm$  SEM of three independent experiments where each condition was carried out in triplicate; <sup>a</sup> $P < 0.05$ . RA: Rheumatoid arthritis; SF: Synovial fluid; OA: Osteoarthritis.

resorption in RA. In keeping with this conclusion, Fava *et al.*<sup>[27]</sup> showed that prophylactic treatment with the LIGHT pathway inhibitor protein LT $\beta$ R-Ig blocks induction of collagen-induced arthritis in mice and adjuvant arthritis in Lewis rats. There is likely to be a complex interplay

between RANKL-independent and RANKL-dependent mechanisms in inflamed RA joints. The role that T cells and synovial fibroblasts play in RANKL/LIGHT-induced osteoclast formation is likely to be key given that these cells are known to express both RANKL and LIGHT. It has been shown the LIGHT contributes to the survival and activation of synovial fibroblasts in RA, resulting in pannus formation which promotes the generation of metalloproteinases, inflammatory cytokines and adhesion molecules<sup>[33-36]</sup>.

RANKL-independent mechanisms of osteoclast formation induced by TNF superfamily members have been shown to play a role in the osteolysis associated with several neoplastic and non-neoplastic diseases of bone and joint, including giant cell tumour of bone, Ewing sarcoma, metastatic breast carcinoma, melanoma and myeloma<sup>[13,38]</sup>. LIGHT is the most potent TNF superfamily member identified to date that induces RA NKL-independent osteoclast formation. LIGHT is likely to represent a potentially useful therapeutic target in RA as inhibiting its action would not only reduce synovial inflammation but also LIGHT- and RANKL-mediated lacunar resorption, resulting in more complete healing of marginal erosions and preservation of periarticular bone in RA.



**Figure 4 Expression of LIGHT in rheumatoid arthritis and osteoarthritis synovium.** Immunohistochemical staining of (A) RA and (B) OA synovium, showing expression of LIGHT on synovial lining cells and subintimal macrophages in RA synovium with no staining for LIGHT in OA synovium. Magnification  $\times 200$ . RA: Rheumatoid arthritis; OA: Osteoarthritis.

A number of studies have shown that Denosumab, a fully humanised antibody that specifically binds RANKL can be used to treat joint erosions and periarticular bone loss in RA<sup>[39-43]</sup>. Denosumab, however, unlike LIGHT, has no effect on joint inflammation<sup>[8,9,39]</sup>. It is well recognized that in the treatment of giant cell tumour of bone, withdrawal of Denosumab results in the re-emergence of osteoclasts with consequent osteolysis and regrowth of the tumour<sup>[44,45]</sup>. The use of Denosumab to treat bone loss in RA is also likely to encounter this problem as fibroblastic stromal cells that express RANKL would persist in the inflammatory environment. Following this therapy targeting LIGHT would therefore be useful in this context as it would not only inhibit the proliferation and activation of RANKL-expressing synovial fibroblasts but also reduce LIGHT- and RANKL-mediated bone resorption.

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## COMMENTS

### Background

Rheumatoid arthritis (RA) is characterized by a heavy lymphocyte and plasma cell infiltrate in the synovium, which leads to the formation of periarticular marginal erosions by specialized multinucleated cells, osteoclasts, which carry out lacunar bone resorption. Osteoclast formation and activation is controlled by a receptor-activator nuclear factor kappa-B ligand (RANKL)-OPG axis, in RA as well as RANKL-independent pathway. One of the RANKL-independent mediators of osteoclast activation is an activated T-cell product, known as LIGHT which is also known to be expressed by dendritic cells.

### Research frontiers

Serum levels of LIGHT are increased in RA patients and blocking the action of LIGHT has been shown to reduce the severity of murine collagen-induced arthritis. It is uncertain whether LIGHT is involved in activation of RA synovial fluid macrophages to osteoclasts.

### Innovations and breakthroughs

This is the first study evaluating the role of LIGHT in osteoclastogenesis of RA

patients as well as demonstrating the increased LIGHT levels in synovial fluid of RA as compared to osteoarthritic patients.

### Applications

The elevated levels of LIGHT in synovial fluid of RA patients and the ability of LIGHT to induce RANKL-mediated osteoclast activation indicate that this protein plays a significant role in pathogenesis of RA, which had not been previously reported.

### Terminology

LIGHT is an abbreviation for lymphotoxin-like, exhibits inducible expression and competes with herpes simplex virus glycoprotein D for herpes virus entry mediator, a receptor expressed by T lymphocytes) is the most potent RANKL (receptor activator for nuclear factor  $\kappa$ B ligand) substitute identified to date, both of which are tumour necrosis family superfamily members.

### Peer-review

The authors demonstrated the possible role of LIGHT in stimulating osteoclast resorption in synovial fluid macrophages. This type of activity was enhanced in combination of RANKL and macrophage-colony stimulating factor. Overall, the paper is logical and well-organized.

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