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Dual Roles of IL-15 in Cancer Biology

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Abstract

IL-15 is an immune-enhancing cytokine belonging to the IL-2 family, which supports survival, proliferation and functional activities of NK, NK-T, T and B cells. Moreover, IL-15 may support the growth and survival of different lymphoid malignancies, suggesting that targeting of the IL-15/IL-15R system or its downstream signaling cascade may result in therapeutic effects, in these tumors. On the other hand, given its immune-enhancing activities IL-15 has been considered a good candidate for cancer immunotherapy. Indeed, IL-15 or IL-15 super agonists have shown anti-tumor activity in several animal tumor models either alone or combined with other immune-enhancing molecules. Therefore, clinical trials of IL-15 or IL-15 super-agonists are ongoing in different cancers. Here we will summarize the biological features of the IL-15/IL-15R system and discuss its duality in tumor biology and the potential applications of IL-15 agonists and antagonists in cancer.

Keywords: IL-15; IL-15Ra; Cancer immunotherapy; Lymphoid malignancy

Introduction

IL-15 belongs to a family of cytokines that bind to receptor (R) complexes sharing the common γ chain (γ_c or CD132), which is essential for signaling through the associated tyrosine kinase JAK3. Besides IL-15, this family also comprises IL-2, IL-4, IL-7, IL-9, and IL-21, which regulate the development and functions of lymphoid cells [1,2]. IL-4, IL-7, IL-9 and IL-21 receptor complexes consist of a cytokine-specific α chain and the γ_c, while IL-2 and IL-15, besides having specific α chains, also share the usage of a promiscuous IL-2/IL-15Rβ (CD122) chain [3,4]. Therefore, both IL-2 and IL-15 signal through JAK1/3 and STAT3/5 pathways and mediate the proliferation and differentiation of NK, NK-T and activated T and B cells, *in vitro*. The IL-2/IL-15Rβ/γ_c is constitutively expressed on resting NK cells and on T cell subsets, which can directly respond to IL-2 or IL-15 and acquire potent cytolytic activity against cancer cells [5]. This effect is related to the induction of granzyme B and perforin expression [6]. In addition, IL-15 cooperates with IL-12 to induce secretion of different cytokines such as IFN-γ, TNF-α, and MIP-1α in NK cells [7].

In spite of a functional overlap *in vitro*, IL-2 and IL-15 have specific functions *in vivo*, as demonstrated by the study of KO models. In particular, IL-15 or IL-15Rα are essential for the development and survival of NK, NK-T and specific T cell subsets [8,9]. Differently, IL-2 and IL-2Rα have a specific role in immune regulation and their deficiency results in lympho proliferation and autoimmunity [10,11]. These phenotypes may reflect immune-regulatory activities of IL-2, including the induction of the activation-induced cell death of T cells and the expansion and fitness of $CD4^+CD25$ high regulatory T cells. The specific functions of the two cytokines *in vivo* are related, at least in part, to their differential expression and regulation [12]. Indeed, IL-2 is specifically produced by activated T lymphocytes during the immune response, while IL-15 is expressed in different cell types, including monocytes, macrophages, DCs, stromal and some epithelial cells, in response to different signals [3,12]. Moreover, the IL-2Rα and IL-15Rα are also differentially regulated, are present in different cell types, and have different functional activities and affinities for their ligands [12,13].

Biology of the IL-15/IL-15R system

The human IL15 gene consists of six coding exons and maps to chromosome 4q31. The study of an IL-15 reporter transgenic mouse showed that IL15 promoter activity is largely limited to myeloid lineages while lymphoid cells express very low IL15 promoter activity. Hematopoietic stem cells show high levels of IL-15 expression, which is down-regulated during T cell differentiation in a stepwise and Notch-dependent fashion [14]. Different transcription factors, including NF-kB, IRF-E, Myb, and INF2 mediate transcriptional activation of the IL-15 gene [15-17]. In addition to transcriptional control, the IL-15 expression is also regulated at the level of mRNA splicing and translation and protein intracellular trafficking [12,17]. Alternative splicing of the IL-15 transcript results in the generation of two mRNA isoforms encoding for IL-15 proteins bearing either a short (SSP) or a long signal peptide (LSP) [18]. The different hydrophobicity of the two signal peptides dictates differential intracellular trafficking, as the LSP-IL-15 enters the ER and can be exported outside the cell, while the SSP-IL-15 localizes to the cytoplasm and nucleus [19,20]. Finally, IL-15 mRNA translation is limited by the presence of multiple AUG codons in the 3'UTR, upstream the initiation codon [21]. These mechanisms greatly limit IL-15 secretion in cells expressing IL-15 mRNA, and IL-15 release or surface exposure occurs only in activated monocytes, macrophages, DCs and stromal cells.

The human Il15RA consists of seven exons, and alternative mRNA splicing may result in eight molecular IL-15Rα isoforms with different extra- or intracellular domains [13]. Full-length isoforms consist of an extracellular portion containing the Sushi (i.e. IL-15-binding) domain, a trans-membrane domain, and an intracellular tail. Different from IL-2Rα, the isolated IL-15α chain is a high-affinity receptor with a KD <10-11M. The high affinity is an important property of the IL-15Rα,

which can bind IL-15 at the surface of myeloid cells, in the absence of the IL-2/IL-15Rβ/γ_c dimers. In this way, activated DCs or macrophages can trans-present surface IL-15Rα/IL-15 complexes to IL-2/IL-15Rβ/γ_c $^+$ lymphocytes, through a "juxtacrine" mechanism involving cell-to-cell contacts [22-24] (Figure 1).

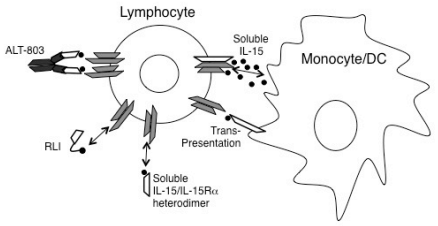

Figure 1: IL-15 transpresentation and IL-15 superagonists. IL-15 can act on target cells by several mechanisms: i) soluble IL-15 may act on neighbor cells expressing high affinity IL-15Rα/IL-2/IL-15Rα,β,γ_c complexes; ii) IL-15 bound to surface 15Rα chain is transpresented to lymphoid cells expressing IL-2Rβ/γ_c complex through cell-to-cell contact; iii) soluble IL-15/IL-15Ra complexes activate IL-2R β/γ_c complexes. Superagonists consist of IL-15 bound to IL-15R β/γ_c -sushi domain (RLI) or by a highly active IL-15 mutant (N72D) in complex with IL-15R-Sushi/IgFc (ALT-803).

Several pieces of evidence support the concept that IL-15 trans-presentation is the most important mechanism of action of IL-15. The IL-2/IL-15Rβ/γ_c dimer present on T or NK cells has a low/intermediate affinity for free IL-15, and its activation requires high IL-15 concentrations, which are not found *in vivo*. The study of KO models revealed an essential role of IL-15Rα in IL-15 biology. Both IL-15- and IL-15Rα-deficient mice displayed a defective development of NK, NK-T and intestinal intraepithelial CD8$^+$ T cells [8,9,25]. Other studies showed that IL-15 mediates the commitment of hematopoietic progenitors of the bone marrow, lymphoid organs and cord blood to the NK cell lineage [26-29], and IL-15 trans-presentation is essential for NK cell development *in vivo* [30]. T or NK cell responses require the presence of IL-15Rα on interacting activated myeloid cells co-expressing IL-15, whereas expression of IL-15Rα on T or NK cells is not necessary [25,30]. Trans-presentation of IL-15 also mediates NK cell survival, as NK cells from wild-type mice showed reduced survival when implanted into Il15ra-/- mice [9]. Indeed, IL-15 is an important survival factor for NK and T cells in peripheral tissues through Erk-1/2 and PI-3 kinase-mediated inhibition of the apoptosis-inducing molecule Bim, and up-regulation of the anti-apoptotic molecule Mcl-1 [31]. In addition, reduced CD8$^+$CD44 high memory T cells were reported in Il15ra-/- mice, indicating a role for IL-15Rα in the development of memory T cells. Again the presence of IL-15Rα on CD8$^+$ T cells is dispensable for T cell memory differentiation, indicating a prominent role for trans-presentation [32]. Membrane-bound IL-15 is an important mediator of the cross-talk between DCs and NK cells in secondary lymphoid organs [33]. DCs produce IL-15 in response to CD40 signalling and trans-present it to NK cells mediating their activation and proliferation [34]. On the other hand, IFN-γ produced by NK cells enhances surface IL-15 expression on the DCs [35]. Also, TLR agonists trigger DCs to produce IFN-type I, which induce IL-15 trans-presentation. Induction of IL-15 expression on DCs is relevant for adaptive immunity against pathogens [36], as it supports the proliferation of memory CD45RO$^+$ CD4$^+$ and CD8$^+$ T cells and naive CD45RO$^-$CD8$^+$ T cells.

The IL-15Rα/IL-15 complex may activate IL-2Rβ/$\gamma_c$$^+$ T or NK cells not only as membrane-bound form but also as soluble complex (Figure 1). An IL-15RαΔ3 soluble isoform generates functional complexes with IL-15, which exert potent biological activity on lymphoid cells [37]. Type I IFNs, viral infection, and CD40 stimulation induce the release of soluble IL-15Rα/IL-15 complexes, which may enhance immune responses, *in vivo* [38]. The demonstration that IL-15/IL-15Ra-Sushi domain complexes strongly stimulate lymphoid cells expressing IL-2/IL-15Rβ/γ_c provided the basis for the generation of new IL-15 superagonists [39].

IL-15Rα may also act as a signaling molecule in myeloid cells, as delivery of an IL15 gene in Rag2-/-γ_c-/- mice increased the number of myeloid cells in the spleen and IL-15 induced RANTES production through activation of JNK and NF-κB, in these cells [40].

Besides IL-15Rα-bound IL-15, alternative membrane forms of GPI-linked or trans-membrane IL-15 have been described on human monocytes [41]. The trans-membrane IL-15 may deliver signals by cell-to-cell contacts to lymphoid cells and could also mediate a reverse signal to monocytes through the Rho-GTPase Rac3 and the MAPK pathway, resulting in increased adhesion and IL-8 secretion [42].

Pro-tumor effects of IL-15

IL-15 stimulates growth and survival of normal T, B and NK cells, and may have similar effects on their neoplastic cellular counterparts. Indeed, several pieces of evidence indicate that IL-15 supports proliferation and survival of different types of neoplastic lymphoid cells, including those from Large Granular Lymphocyte Leukemia (LGLL) [43-45], B-Chronic Lymphocytic Leukemia (B-CLL) [46-48], Follicular Lymphoma (FL) [49] Hodgkin's Lymphoma (HL) [50] Cutaneous T cell Lymphoma (CTC) [51], Multiple Myeloma (MM) [52], Enteropathy-Associated T cell Lymphoma (EATL) [53,54], and Adult T cell Leukemia (ATL) [55].

An early study showed that IL-15 mediates the proliferation of T- or NK-type LGLL cells, which express IL-15Rα and IL-2/IL-15Rβ/γ_c suggesting that it may act as a growth factor in these lymphoproliferative disorders [44]. Also, the study of IL-15-transgenic mice showed that chronic hyper-expression of IL-15 *in vivo* may result in the development of LGLL, sharing similarities with the human disease [43]. Further studies confirmed that chronically high levels of IL-15 alone are sufficient to drive the neoplastic transformation of normal LGL in the mouse, through induction of JAK1/3 and STAT3/5 signalling [45]. The role of STAT3 in LGLL genesis was also indicated by the high frequency (30-40%) of STAT3 mutations, involving the SH2 domain in human T and NK-LGLL [56]. Chronic activation of the STAT3 pathway mediates high Myc expression resulting in: i) overexpression of Aurora kinase A and B, which mediate amplification of centrosomes and chromosome instability; ii) down regulation of mir-29b, which results in enhanced expression of DNA methyl transferases and epigenetic silencing of oncosuppressor genes [45].

Antibodies blocking the IL-2Rβ/$\gamma_c$complex such as the Mikβ1 antibody inhibit the effects of IL-15 on LGL proliferation, *in vitro* [44]. However, clinical studies of murine or humanized Mikβ1antibody showed no significant clinical benefit in LGLL patients [57], possibly suggesting the involvement of other factors and/or the loss of IL-15-dependency during progression.

An initial report indicated that IL-15 supports B-CLL proliferation *in vitro* through the IL-2R [47]. Further studies showed that IL-15 triggers STAT3/5 and ERK1/2 activation, mediating proliferation and survival of B-CLL cells *in vitro* and that stimulation via CD40L increased sensitivity to IL-15 effects [46]. B-CLL cells express TLR9, which upon ligand engagement drives their apoptosis. However, a recent report showed that IL-15 inhibits TLR-9-induced apoptosis and that TLR9 and IL-15 rather co-stimulate B-CLL clonal expansion. B-CLL cells with chromosomal anomalies showed stronger proliferative responses, which correlated with reduced patient survival. In addition, the presence of IL-15-producing cells and apoptotic cells near B-CLL pseudofollicles in the spleen, suggest that DNA and IL-15 may co-stimulate B-CLL growth in secondary lymphoid organs [48]. Also, FL cells proliferate in response to IL-15 trans-presented by macrophages and CD40L signaling further increases this response [49]. Collectively, these studies indicate that IL-15 may cooperate with other stimuli such as TLR and CD40L to support neoplastic B cell growth in the microenvironment of lymphoid organs.

The IL-15/IL-15R system mediates mitogenic and anti-apoptotic signals in Hodgkin's and Reed Stenberg cells through the phosphorylation of ERK1/2 and STAT5, and enhanced the expression of inflammatory factors including IL-1α, IL-6, IL-9, IL-12β, and CCL3 [50].

In ATL, an autocrine IL-15 loop supports the proliferation of neoplastic cells. In this disease the human HTLV-1 transactivating protein TAX drives IL-15 and IL-15Rα over-expression, thus generating an autocrine IL-15 loop that may play a role in disease development and progression [12,16,17].

IL-15 has also been involved in CTC, where the skin shows overexpression of IL-15, which could mediate paracrine effects on CTC cells. Since IL-15 can act as a chemoattractant for T cells ( ), it is conceivable that it may be involved in the tropism of CTC cells for the skin. At advanced stages also CTC cells acquire the ability to produce autocrine IL-15, which can render cells less dependent on the support provided by the skin environment [51]. Similarly, autocrine IL-15 expression by MM cells supports their proliferation and survival, protecting them from spontaneous or activation-induced apoptosis and rendering them independent from the microenvironment [52].

IL-15, which is highly expressed in the gut of Celiac Disease (CD) patients, may also play a role in some complications of this disease, including Refractory CD and EATL. Indeed, in type II Refractory CD there is an accumulation of abnormal intraepithelial lymphocytes with a CD3- and CD8-negative phenotype and clonal rearrangements of the TCR, which are considered a low-grade intraepithelial lymphoma [53]. Instead, EATL is a rare but aggressive T cell lymphoma with inflammatory features. It is likely that chronic antigenic stimulation may act in concert with IL-15 to support the expansion of intraepithelial lymphocytes, a first step in the development of oligoclonal and monoclonal expansions and subsequent lymphoma development [53].

Collectively these data support the concept that IL-15 may play an important role as a paracrine growth factor in some lymphoid malignancies, where agents blocking the IL-15/IL-15Rα system or its downstream JAK/STAT3/5 pathway may provide potential therapeutic tools.

These agents include antibodies blocking the IL-15R complex, such as the Mikb1 antibody. This antibody showed no effects in LGLL [57], but two studies in advanced CD (NCT01893775) and HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis (NCT00076843) are recruiting patients. However, in some situations IL-15 may not be the only cytokine driving lymphoma pathogenesis. In this case, the use of small molecule inhibitors targeting common downstream signalling pathways of tumor supportive cytokines, such as the JAK inhibitors ruxolitinib or tofacitinib, may represent more powerful strategies. Indeed, CP-690,550 (tofacitinib) treatment prolonged the survival of mice bearing a CD8+ JAK3-mutant T-ALL [58] or a CD8+ T cell IL-15-transgenic leukemia [59]. In addition, a clinical study of ruxolitinib in ATL is currently recruiting patients (NCT01712659) and a very recent pilot study on nine patients with rheumatoid arthritis-associated LGLL suggested clinical benefit [60].

Anti-tumor activities of IL-15

IL-2 has been a milestone in cancer immunotherapy, but it has a remarkable toxicity and is active only in a limited proportion of melanoma or renal cancer patients. The immune-enhancing activities and data from pre-clinical studies of IL-15 suggested that it could represent an alternative candidate [12,17,61]. Indeed, several studies in pre-clinical models of cancer supported this hypothesis. An early study showed that recombinant simian IL-15 has low toxicity in mice, and inhibits the growth of lung metastases in a syngeneic sarcoma model [62]. Also, mammary adenocarcinoma cells, genetically modified to secrete human IL-15, showed reduced growth rates when implanted in syngeneic mice. Vaccination with irradiated IL-15-secreting tumor cells inhibited the growth of lung metastases of parental adenocarcinoma. These effects were mediated by the induction of T and NK-cell responses and by IFN-γ [63]. Another strategy is based on the expression of IL-15 induced by an oncolytic virus, harboring the IL15 gene, which mediates T cell responses and survival of mice bearing colon carcinoma implants [64].

The IL-15 anti-tumor activity can be increased by co-administration of other immune-enhancing molecules or immune checkpoint inhibitors. For example, combined IL-12 and IL-15 gene transfer in human small cell lung cancer cells resulted in complete loss of their tumorigenic potential upon implant in SCID or nude mice, while each cytokine alone had limited activity [65]. This cooperative effect was mediated by the activation of cytotoxic macrophages. The combined use of IL-12 gene-modified tumor cells and IL-15 administration showed synergistic effects also in a melanoma model through stimulation of CTLs and IFN-γ [66]. Differently, the combined use of IL-12 and IL-15 gene transfer in breast adenocarcinoma cells induced anti-tumor immunity through the induction of CD8+ T cells and TNF-α, in syngeneic IFN-γ-deficient mice [67]. These data indicate that IL-15 combined with IL-12 mediates the activation of different anti-tumor mechanisms, in pre-clinical models.

IL-15 anti-tumor activity can also be enhanced by combination with agonistic anti-CD40 mAbs, which enhance the expression of IL-15Rα on DC and their ability to trans-present IL-15. Treatment of syngeneic mice bearing colon cancer with IL-15 combined with anti-CD40 mAb resulted in increased mice survival, compared to each treatment alone [68].

Other studies combined IL-15 with immune checkpoint blockers. Indeed, IL-15 has immune stimulatory effects, but it also induces the expression of the PD-1 inhibitory receptor on CTLs, suggesting that IL-15 anti-tumor activity may be enhanced by simultaneous checkpoint blockade. The co-treatment of colon carcinoma-bearing mice with IL-15, anti-CTLA-4 and anti-PD-L1 mAbs produced much stronger therapeutic effects than each treatment alone [69]. This combined treatment showed enhanced efficacy also in a transgenic mouse prostate cancer model [70].

A different approach to enhance IL-15 activity is based on the generation of super-agonists consisting of IL-15 bound to part of the extracellular domain of IL-15Rα, to mimic IL-15 trans-presentation in a soluble form. A fusion protein, termed RLI, was constructed by binding IL-15 to the Sushi domain of IL-15Rα via an amino acid linker [39]. RLI was a more potent stimulator of NK and T cells than IL-15, on a molar basis and showed prolonged half-life and stronger anti-tumor activity than IL-15 or IL-2 in metastatic B16F10 melanoma models [71]. Moreover, RLI reduced tumor growth and metastasis of human colon carcinoma cells in an orthotopic nude mouse model.

A further enhancement of IL-15 anti-tumor properties was achieved by the generation of fusion proteins consisting of RLI linked to antibodies targeting tumor-associated antigens, such as the ganglioside GD2 [72], the CD20 B-cell lymphoma antigen [73], or the Fibroblast Activation Protein (FAP) of the tumor stroma [74]. These reagents showed that antibody-targeted delivery of an IL-15-transpresenting moiety at the tumor site is suitable to enhance IL-15 activity for tumor therapy.

Finally, a complex of a mutant IL-15 superagonist and a dimeric Sushi domain/Fc fusion protein termed ALT-803 showed much more potent biologic activity on NK and T cells than IL-15, *in vivo* [75]. A single dose of ALT-803 prolonged survival of syngeneic mice bearing 5T33P and MOPC-315P myeloma while IL-15 was ineffective [75]. ALT-803 promoted rapid expansion of CD8+CD44high memory T cells *in vivo*, resulting in CTL- and IFN-γ-dependent immunity to re-challenge with the same tumor cells. Similarly, ALT-803 in combination with stereotactic surgery or anti-PD-1 antibody induced potent antitumor immune responses resulting in prolonged survival and complete remissions in a syngeneic glioblastoma model. These effects required both $CD4^+$ and $CD8^+$ T cells and IFN-γ production [76]. Moreover, ALT-803 augmented ADCC activity and IFN-γ secretion by human NK cells targeted by anti-CD20 mAbs against B-cell lymphoma cells. The combination of ALT-803 and anti-CD20 mAb significantly reduced mouse B cell lymphoma growth and increased survival [77].

Another report showed that IL-15 may directly act on a peculiar population of human $CD105^+$ renal cancer stem cells (CSCs) *in vitro*. These are cell populations resistant to conventional therapy, capable of self-renewal and driving tumorigenesis and relapses. IL-15 mediated epithelial differentiation of $CD105^+$ renal CSCs, which lose their stem cell characteristics, and acquire epithelial markers and the capability to self-produce IL-15 [78].

All these studies exploited the anti-tumor activity of IL-15 in various tumor models. Differently a recent report addressed the role of endogenous IL-15 in inflammation-induced colon cancer [79]. Il15-/- but not Il15rα-/- mice showed higher tumor incidence and increased colon weight than wild-type mice. Gene expression analysis showed up-regulation of pro-inflammatory cytokines involved in progression, such as IL-1β, IL-22, IL-23, Cxcl5, and Spp1 in tissues from Il15-/- mice [79]. Altogether, these findings suggest that IL-15 signaling via low-affinity IL-2/IL-15Rβ/γ_c suppresses colon carcinogenesis through induction of antitumor immune-surveillance and modulation of the tumor-associated inflammation.

A TAX-LUC mouse model of ATL allows to study lymphomagenesis by transgenic expression of HTLV-1 Tax, which drives the development of luciferase expressing lymphomas. As IL-15 is an autocrine factor in HTLV-1 adult T cell leukemia [16], the role of IL-15 in lymphomagenesis was studied in IL-15 TAX-LUC mice. Unexpectedly, the study of this model showed increased lymphomagenesis and mortality, indicating that IL-15 is not strictly required for the development of Tax-mediated lymphomas, whereas IL-15 seems involved in anti-lymphoma immune surveillance. Lymphomas developing in the absence of IL-15 showed a significant increase in IL-1α and IL-1α-regulated cytokine expression, suggestive of a lymphoma-promoting role of these cytokines, in the absence of IL-15 [80].

In view of the anti-tumor effects of IL-15 in preclinical models, recombinant human IL-15 was further developed at clinical grade and tested for toxicity in rhesus macaques using different schedules [81,82]. IL-15 was biologically active particularly in the i.v. settings, as it increased circulating NK cells and central and effector memory $CD8^+$ T cells. An initial phase I clinical trial of IL-15 was performed in refractory metastatic renal cancer and/or melanoma, which are sensitive to IL-2-based immunotherapy. In principle, the use of IL-15 may better support effector memory T cell survival and functions than IL-2 and avoid the induction of activation-induced cell death and the stimulatory activity on Treg cells functions and fitness, which are typical of IL-2 [83]. In this clinical study IL-15 showed toxicity, including grade 3 fever, hypotension, thrombocytopenia, and increased transaminase levels, at 3 or 1 μg/kg/day for 12 days dose levels. The maximal tolerated dose was established at 0.3 μg/kg/day. After an initial rapid efflux of NK and memory CD8 T cells from the blood within minutes of IL-15 administration, influx and hyperproliferation resulted in 10-fold expansions of NK cells. Serum levels of multiple inflammatory cytokines, including IFN-g and IL-6 increased up to 50-fold, and may be involved in some toxic effects. No objective responses were observed and disease stabilization was recorded as best response [84]. A phase I/II study of i.v. IL-15 administration following a non-myeloablative lymphocyte depleting chemotherapy and autologous tumor-infiltrating lymphocytes transfer in metastatic melanoma was recently terminated due to autoimmune toxicity (NCT01369888). Other trials of IL-15 using different schedules are ongoing, e.g. a phase I/II study of s.c. rIL-15 in adults with advanced cancers (NCT01727076). In addition, other trials will address the use of IL-15 super agonists in cancer patients. A clinical study of s.c. hetIL-15, are combinant heterodimer of IL15/sIL-15Rα, in adults with metastatic cancers (NCT02452268) is recruiting patients. Two other studies of the IL-15 super agonist ALT-803 have been initiated in patients with advanced solid tumors [NCT01946789] and in relapses of hematologic malignancy after allogeneic stem-cell transplantation [NCT01885897]. It is hoped that ongoing studies will unravel the potential and the best formulation and schedules of IL-15-based immunotherapies in order to achieve clinical efficacy in cancer patients.

Conclusion

IL-15 is a pleiotropic cytokine, which is essential for NK cell development and promotes proliferation, differentiation and functions of T, B and NK cells. These effects are not limited to normal lymphoid

cells, as IL-15 can promote the growth of several types of malignant lymphoid cells, *in vitro*. Also, IL-15-transgenic mice develop spontaneous T or NK-type LGL leukaemias, supporting an *in vivo* role of IL-15 in leukaemia genesis. Therefore, the use of IL-15 for the treatment of lymphoid tumors should be avoided and, instead, the use of antibodies blocking the IL-15/IL-15R system or inhibitors targeting its downstream JAK/STAT signalling pathway is currently investigated. In this context, preliminary findings suggest that the JAK inhibitor tofacitinib has clinical activity in rheumatoid arthritis-associated LGLL.

On the other hand, the ability of IL-15 to stimulate both NK and T cell responses, and its well-documented anti-tumor activity in preclinical models, support the development of clinical studies of IL-15 in cancer. In addition, IL-15 showed acceptable toxicity profiles in mouse and primate models. Therefore, clinical studies of IL-15 or IL-15 superagonists, consisting of IL-15 linked to IL-15Rα portions, have been initiated. Also, studies combining IL-15 with adoptive transfer of T or NK cells are ongoing in cancer patients. However, a study combining IL-15 and TILs was recently terminated due to autoimmune toxicity, suggesting that IL-15's immune enhancing activity is powerful and may result in exaggerated reactions. The ongoing and future clinical studies will elucidate the potential of IL-15 or of its superagonists in immunotherapy and provide indications on the best treatment schedules. Finally, preclinical models have shown that the combination of IL-15 with other immune enhancing cytokines, CD40-agonists, or immune checkpoint blockers may result in cooperative anti-tumor effects, supporting the development of combinational therapies in clinical settings.

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