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Interleukin-15 biology and its therapeutic implications in cancer

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Abstract

Cancer immunotherapy is designed to stimulate the immune system to reject and destroy tumors. Recently, interleukin-15 (IL-15), a member of the 4- α -helix bundle family of cytokines, has emerged as a candidate immunomodulator for the treatment of cancer. IL-15 acts through its specific receptor, IL-15R α , which is expressed on antigen-presenting dendritic cells, monocytes and macrophages. IL-15 exhibits broad activity and induces the differentiation and proliferation of T, B and natural killer (NK) cells. It also enhances cytolytic activity of CD8⁺ T cells and induces long-lasting antigen-experienced CD8⁺CD44^{hi} memory T cells. IL-15 stimulates differentiation and immunoglobulin synthesis by B cells and induces maturation of dendritic cells. It does not stimulate immunosuppressive T regulatory cells (Tregs). Thus, boosting IL-15 activity could enhance innate and specific immunity and fight tumors. Here we review aspects of IL-15 biology that make it a promising agent for anticancer therapy. We also discuss preclinical models in which IL-15 has demonstrated antitumor activity and highlight ongoing clinical trials of IL-15 in patients with cancer and HIV infection.

Interleukin-15

In the last decade immunotherapy has moved to the forefront of cancer research with promising results recently reported in a number of high profile clinical trials. This has led to the approval of a first-in-class vaccine for the treatment of castrate-resistant prostate cancer, and the approval of an immunostimulatory monoclonal antibody for the treatment of advanced melanoma [1, 2]. Importantly, patients receiving these novel agents, sipuleucel-T or ipilimumab, have for the first time demonstrated improved survival. In addition, several recombinant cytokines and hematopoietic growth factors are approved for use as supportive agents in cancer, whereas others such as interferon- α and interleukin-2 (IL-2) are used for the surgical adjuvant treatment of high-risk malignant melanoma, or to treat metastatic melanoma and renal cell cancer, respectively [3, 4].

Interleukin-15 (IL-15) was simultaneously co-discovered by two different laboratories in 1994 and characterized as a T cell growth factor [5, 6]. The heterotrimeric receptor of IL-15 shares the IL-2R/IL-15R β (CD122) and common gamma (γ c) chain (CD132) with the IL-2 receptor. In light of these common receptor components and a sharing of the JAK1/JAK3/

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STAT5 (Janus kinase/signal transducers and activators of transcription) signaling pathway, the two cytokines also share certain functions that include the stimulation of T cell proliferation, the generation of cytotoxic T lymphocytes, stimulation of immunoglobulin synthesis by B cells and the generation and persistence of NK cells [7]. However, in many adaptive immune responses, IL-2 and IL-15 also have distinct and often competing roles. Unlike IL-2, IL-15 is not required for the maintenance of T regulatory cells (Tregs) that can attenuate antitumor immune responses [8]. IL-2 in contrast to IL-15 inhibits T cell responses through activation-induced cell death (AICD) of CD8⁺ effector T cells [9]. However, IL-15 is required for the differentiation of NK, effector CD8⁺ and memory phenotype CD8⁺ T cells. In addition, based on pre-clinical studies, their toxicities appear to be different, with little vascular capillary leak observed with IL-15 in contrast to IL-2 [10]. These factors suggest that IL-15 may represent a more efficacious cytokine for cancer immunotherapy. Herein, we review the biology, immunology and current state of IL-15 clinical development.

Molecular biology

IL-15 is a 14–15 kDa glycoprotein encoded by a 34 kilobase (kb) region on chromosome 4q31 (Figure 1). The IL-15 gene is made up of nine exons and eight introns, 4 of which (exons 5 through 8) code for the mature protein [11]. There are 2 isoforms of IL-15 mRNA that differ in their signal peptides lengths. The originally identified isoform consisted of a 316 base pair (bp) 5' untranslated region (UTR), a 486 bp coding sequence, and a 400 bp 3'UTR that is translated into an IL-15 precursor protein with a long signal peptide of 48 amino acids (IL-15LSP). The long signal peptide is encoded within exons 3–5 [6]. The other isoform of IL-15 mRNA has a short signal peptide of 21 amino acids (IL-15SSP) encoded by exons 4A and 5. Both isoforms produce the same mature protein; however, the two differ in that they have distinct intracellular trafficking. IL-15LSP is targeted to the Golgi apparatus, early endosomes and the endoplasmic reticulum secretory pathway, whereas IL-15SSP is not secreted. IL-15SSP appears to be restricted to the cytoplasm and nucleus and may play a role in its transcriptional regulation [12].

IL-15 expression

IL-15 mRNA is expressed by a large number of tissues including fibroblasts, keratinocytes, epithelial cells of various tissues, nerve cells, monocytes, macrophages and dendritic cells [13]. Low levels of mRNA have also been detected in T-cells [7]. Although IL-15 mRNA expression is widespread, detection of IL-15 protein is largely limited to monocytes/macrophages and dendritic cells. This indicates that, although regulation of IL-15 protein production occurs at the transcription, translation and intracellular trafficking stages, the majority is post-transcriptional. Multiple AUG sequences in the 5'UTR, a long signal peptide and a negative regulatory element in the C-terminus of the coding sequence and mature protein, all act to limit translation [5]. The long or short signal peptides also influence the intracellular trafficking of IL-15 and its secretion [12]. Tight regulation of IL-15 expression is likely required because of its potency as an inflammatory cytokine. Indiscriminant expression, with the capacity of IL-15 to induce tumor necrosis factor alpha (TNF- α), IL-1 β , interferon- γ and other inflammatory cytokines could be associated with the induction of autoimmunity, indicating the reason for this tight regulation of IL-15 expression [14].

Interleukin-15 receptor

The heterotrimeric IL-15 receptor is composed of a beta subunit (IL-2R/15R β) that is shared with the IL-2 receptor, a common gamma subunit (γ c) shared with IL-2, IL-4, IL-7, IL-9 and IL-21, and a unique alpha subunit (IL-15R α) that confers receptor specificity to IL-15 [15]. The IL-2R/15R β subunit is a 525 amino acid receptor consisting of a 214 amino acid

extracellular segment, a 25 amino acid transmembrane region, and a 286 amino acid cytoplasmic domain [16]. The human γc consists of a 233 amino acid extracellular domain, a 28 amino acid transmembrane domain, and an 86 amino acid cytoplasmic region [17].

IL-15R α has a 173 amino acid extracellular domain, a single 21 amino acid membrane-spanning region, and a 37 amino acid cytoplasmic domain. It is structurally similar to IL-2R α with a conserved extracellular protein-binding Sushi domain. The IL-15R α gene also has a similar intron-exon organization to that of IL-2R α [17]. Despite these similarities, IL-15R α shares little sequence homology to IL-2R α .

Molecular biology of IL-15R α

The human IL-15R α gene maps to chromosome 10 and is made up of 7 exons, which, as a result of alternative splicing, can produce 8 different isoforms of IL-15R α [18]. In humans, there are three types of splicing events: (1) alternate usage of exon 7 or 7'; (2) deletion of exon 3 encoding the linker region and (3) deletion of exon 2 which encodes the Sushi domain resulting in an inability to bind IL-15 [18]. Exon 2 also has a putative nuclear localization signal, the deletion of which results in this isoform localizing only in the non-nuclear membrane compartments suggesting a role in post-translational routing of IL-15R α [18].

Interaction of Interleukin-15 with its receptor

IL-15R α is widely expressed in humans and mice independently of IL-2R/IL-15R β - γc [9]. It binds IL-15 with a high affinity ($K_d > 10^{11}M$) and retains IL-15 on the cell surface. IL-15R α *trans*-presents IL-15 to IL-2R/IL-15R β - γc on nearby effector NK and T cells by the formation of an immunological synapse (Figure 2) [19, 20]. This immunological synapse mechanism is believed to limit exposure to circulating IL-15, restricting aberrant immune stimulation and decreasing the risk of autoimmunity from uncontrolled IL-15 exposure. In lymphocytes, the presentation of membrane-bound IL-15 to IL-2R/IL-15R β - γc activates the JAK1/JAK3 and STAT3/STAT5 pathways, Syk kinase and phospholipase C_(PLC) γ , Lck kinase, and Shc resulting in the activation of PI3K/Akt and Ras/Raf/MAPK signaling cascades [21]. These pathways lead to the subsequent expression of *bcl-2*, *c-myc* and *c-fos/jun* and NF- κ B activation. IL-15 bound to IL-15R α can also recycle through endosomal vesicles for many days (endosomal recycling) resulting in the persistence of membrane-bound IL-15 [19].

Biological effects of interleukin-15

IL-15 was initially identified for its ability to stimulate T cell proliferation in an IL-2-like manner through common receptor components (IL-2R/IL-15R β - γc) and signaling through JAK1/JAK3 and STAT3/STAT5. Like IL-2, IL-15 has been shown to stimulate proliferation of activated CD4-CD8-, CD4+CD8+, CD4+ and CD8+ T cells as well as facilitate the induction of cytotoxic T-lymphocytes, and the generation, proliferation and activation of NK cells [7]. However, unlike IL-2 which is required to maintain forkhead box P3 (FOXP3)-expressing CD4+CD25+ Treg cells and for the retention of these cells in the periphery, IL-15 has little effect on Tregs [8]. This is important as FOXP3-expressing CD4+CD25+ Tregs inhibit effector T cells, thereby inhibiting immune responses including those directed against the tumor. IL-2 also has a crucial role in initiating activation induced cell death (AICD), a process that leads to the elimination of self-reactive T cells, whereas IL-15 is an anti-apoptotic factor for T cells [9]. IL-15 co-delivered with HIV peptide vaccines has been shown to overcome CD4+ T cell deficiency by promoting longevity of antigen-specific CD8+ T cells and blocking TRAIL-mediated apoptosis [22]. Furthermore, IL-15 promotes the long-term maintenance of CD8+CD44hi memory T cells [23].

The importance of IL-15 and IL-15R α to T and NK cell development is further highlighted by the phenotype of IL-15R $\alpha^{-/-}$ and IL-15 $^{-/-}$ mice. Knockout mice demonstrate decreased numbers of total CD8 $^{+}$ T cells, and are deficient in memory-phenotype CD8 $^{+}$ T cells, NK cells, NK/T cells and some subsets of intestinal intraepithelial lymphocytes, indicating that IL-15 provides essential positive homeostatic functions for these subsets of cells [24, 25]. The similarities in the phenotypes of these two strains of knockout mice suggest the importance of IL-15R α in maintaining physiologically relevant IL-15 signals.

In addition to the effects on T and NK cells, IL-15 also has several effects on other components of the immune system. IL-15 protects neutrophils from apoptosis, modulates phagocytosis and stimulates the secretion of IL-8 and IL-1R antagonist. It functions through the activation of JAK2, p38 and ERK1/2 MAPK, Syk kinase and the NF- κ B transcriptional factor [26]. In mast cells, IL-15 can act as a growth factor and an inhibitor of apoptosis. In these cells IL-15 activates the JAK2/STAT5 pathway without the requirement of yc binding [27]. IL-15 also induces B lymphocyte proliferation and differentiation, and increases immunoglobulin secretion [28]. It also prevents Fas-mediated apoptosis and allows induction of antibody responses partially independent of CD4-help [29, 30]. Monocytes, macrophages and dendritic cells effectively transcribe and translate IL-15. They also respond to IL-15 stimulation. Macrophages respond by increasing phagocytosis, inducing IL-8, IL-12 and MCP-1 expression, and secreting IL-6, IL-8 and TNF α [21]. Dendritic cells incubated with IL-15 demonstrate maturation with increased CD83, CD86, CD40, and MHC class II expression, are also resistant to apoptosis, and show enhanced interferon- γ secretion [31].

IL-15 has also been shown to have effects on non-hematological cells including myocytes, adipocytes, endothelial and neural cells. IL-15 has an anabolic effect on muscle and may support muscle cell differentiation [32]. It stimulates myocytes and muscle fibers to accumulate contractile protein and is able to slow muscle wasting in rats with cancer-related cachexia [33]. IL-15 has also been shown to stimulate angiogenesis [34] and induce microglial growth and survival [35].

Interleukin-15 and cancer

As a potent proinflammatory cytokine, IL-15 plays an important and complex role in autoimmune disease and inflammation. There is increasing recognition of a link between inflammation and the development of cancer [36]. Because IL-15 stimulates proliferation and maintenance of NK cells, B and T lymphocytes, it is probable that IL-15 could play a role in certain hematological malignancies. In support of this, proliferation of the murine T cell lymphoma cell line LBC is enhanced by IL-15 [37] and spontaneous development of CD8 $^{+}$ T cell leukemia is observed in IL-15 transgenic mice [38, 39]. These mice demonstrate a rapid proliferation of NK and T cells, followed by spontaneous transformation to lethal leukemia with many features in common with aggressive variants of human NK and T cell large granular lymphocyte leukemia.

In vitro, IL-15 inhibits apoptosis of primary human NK leukemia cells and NK tumor cell lines [40], as well as promotes the growth of B cell chronic lymphocytic leukemia cells [41]. IL-15 mRNA expression has been detected in the peripheral blood mononuclear cells of patients with Sezary syndrome, the leukemic form of mycosis fungoides (a cutaneous T-cell lymphoma) and expression of IL-15 protein in the epidermal keratinocytes in the skin lesions of mycosis fungoides patients [42]. Increasing evidence indicates that an IL-15/IL-15R α autocrine growth stimulation loop plays a role in the transformation and progression of human T cell lymphotropic virus (HTLV-1)-associated adult T cell leukemia/lymphoma [43, 44]. The viral Tax protein activates expression of IL-15 and

IL-15R α . These cells constitutively express IL-15R α and proliferate in response to IL-15. In acute lymphocytic leukemia (ALL) cells, IL-15 expression was associated with mediastinal and lymph node involvement, and IL-15 expression in precursor B cell ALL was associated with an inferior 5-year relapse-free survival [45]. In children with ALL who did not have of the central nervous system (CNS) at diagnosis, IL-15 expression levels greater than the median were associated with a higher risk of CNS relapse [46].

In non-hematological malignancies, there is little evidence that IL-15 plays any direct role in the development of solid cancers, but rather it may play a role in immunosurveillance and protection from tumor formation. One study found no significant difference between serum IL-15 levels in 40 patients with various solid tumors, including 24 with metastatic disease, and serum IL-15 levels found in healthy individuals [47]. Indeed, IL-15 may offer protective effects against the development of cancer. The risk of cancer is known to increase with age. Studies in mice have demonstrated an age-related decline in serum IL-15 levels [48]. IL-15 may play a protective role against the development of tumors through enhancing immune surveillance that may be lost as part of the aging process.

Preclinical studies

The direct administration of IL-15 has been shown anti-tumor effects in several pre-clinical mouse tumor models (10, 49–51). In a recent study by Yu *et al.*, IL-15 was shown to prolong the survival of mice with metastatic CT26 colon cancer [51]. However, administration of IL-15 alone was not optimal; as it also activated immune system negative regulatory checkpoints that might also dampen the immune response. IL-15 induced the expression of the immunosuppressive receptor, programmed death-1 (PD-1) and increased the secretion of the immunosuppressive cytokine IL-10 by CD8 $^{+}$ T cells. Co-administration of anti-programmed cell death-1 ligand (PD-L1) and anti-cytotoxic lymphocyte antigen 4 (CTLA-4) monoclonal antibodies reduced PD-1 and IL-10 expression and resulted in greater anti-tumor responses than IL-15 alone [51]. Various other preclinical approaches have been examined to increase the efficacy of IL-15 immunotherapy, including co-administration of anti-CD40 to induce and enhance IL-15R α expression, and the covalent binding of IL-15 to soluble IL-15R α to mimic *trans*-presentation and increase the bio-stability of IL-15.[50, 53]. Both of these approaches produced greater anti-tumor responses than if IL-15 was used alone. Similarly, the co-administration of other cytokines such as IL-21 has augmented the anti-tumor efficiency of IL-15 in animal tumor models [52].

IL-15 has been shown to be effective when administered as a vaccine adjuvant in pre-clinical models of cancer and infectious diseases. This is highlighted in a recent study by Steel and colleagues in which IL-15 was genetically expressed in a dendritic cell (DC) vaccine targeting a *neu*-positive mouse breast cancer model [30]. Animals vaccinated with DC expressing IL-15 and truncated *neu* gene remained tumor-free significantly longer than those vaccinated with *neu* alone. This effect was further increased by modifying the DC vaccine to express IL-15R α along with the IL-15. This study also showed that IL-15 could overcome defects in CD4-help, as well as enhance antitumor antibody responses. IL-15 has also been shown to overcome defects in CD4-help to allow CD8 $^{+}$ immune responses by inhibiting TRAIL-mediated apoptosis [22]

IL-15 is being evaluated as an ancillary agent to stimulate cells *ex vivo*. Preclinical studies have shown that NK, CD8 $^{+}$ T cells, CD8 $^{+}$ memory T cells and dendritic cells cultured in the presence of IL-15 exhibit increased function when adoptively transferred into animals (54–56).

Interleukin-15 toxicology

IL-15 plays an important role in autoimmune diseases and inflammation. Diseases that manifest elevated IL-15 levels, disordered expression, or abnormal signaling by IL-15 include pemphigus vulgaris, rheumatoid arthritis, systemic lupus erythematosus, sarcoidosis, multiple sclerosis, celiac disease, as well as inflammatory bowel disease [57]. IL-15 has been hypothesized to sit at the apex of a pyramid of proinflammatory cytokines that includes TNF α , IL-1 β , IL-6, IL-8, granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage inflammatory protein-1 alpha (MIP-1 α), and MIP-1 β [22]. This suggests that autoimmunity might result from treatment with IL-15. In addition, CD8+ T cell leukemia has been reported in IL-15 transgenic mice [38, 39]. This is thought to be a minimal risk in the therapeutic setting owing to the transient nature of the exposure to IL-15.

The safety of IL-15 has been evaluated in mice and in non-human primates. IL-15 appears to lack the vascular leak syndrome (VLS) associated with IL-2. VLS is characterized by the extravasation of fluids and proteins into the tissues due to an abnormal increase in vascular permeability. This may result in fluid retention, peripheral edema, pleural and pericardial effusions, ascites, anasarca and in the most serious forms, lead to pulmonary edema and cardiovascular failure. Munger *et al.* administered 30, 60, or 180 μ g (approximately 1200–7200 μ g/kg) of recombinant simian IL-15 by intraperitoneal injection to C57Bl/6 mice three times per day for nine doses [10]. The mice were examined for pulmonary VLS using radiolabeled bovine serum albumin and compared to mice receiving the same doses of IL-2. At 30 μ g, IL-2 induced significant pulmonary VLS while the mice receiving IL-15 180 μ g demonstrated minimal VLS suggesting that VLS would not be dose limiting in human trials of IL-15.

Non-human primate studies have further evaluated the potential adverse events associated with pharmacological dosing of IL-15. Mueller *et al.* subcutaneously injected SIV infected macaques with recombinant rhesus macaque IL-15 (rMamu IL-15) at doses of 10 μ g/kg or 100 μ g/kg twice weekly for 4 weeks [58]. Animals were monitored for toxicity through various methods, including serial measurements of serum chemistries, liver function tests, leukocytes, red blood cells, hemoglobin, hematocrit, platelets, white blood count and differential, and for clinical signs of toxicity and weight loss. IL-15 induced a nearly three-fold increase in peripheral CD8+CD3- NK cells. CD8+ T cells increased more than two-fold mainly, due to an increase in effector memory CD8+ T cells. All clinical laboratory results remained within normal limits with the exception of a non-significant increase in platelet counts in all groups including the untreated control animals. No changes in weight or other abnormalities were observed. Berger and colleagues administered human recombinant IL-15 at doses of 2.5 to 15 μ g/kg by daily subcutaneous injection for up to 14 days to macaques [8]. A second set of macaques received IL-15 every 3 days at doses of 2.5 μ g/kg, 5 μ g/kg, or 10 μ g/kg for 24 days. The animals were monitored for clinical toxicity, as well as by complete blood count, platelets, white blood count and differential, serum chemistries, peak and trough IL-15 levels, and bone marrow aspirate and biopsy. The daily administration schedule resulted in persistently elevated plasma IL-15 levels. Intermittent IL-15 administration allowed clearance of IL-15 between doses and was found to be safe. Daily administration of IL-15 for up to 14 days caused reversible severe neutropenia, a massive expansion of T cells, anemia, weight loss, and generalized skin rash. The bone marrow of a neutropenic animal was found to be hypocellular, but rapidly recovered when the IL-15 was discontinued.

In a toxicity study carried out at the USA National Cancer Institute (NCI), adult rhesus macaques received intravenous recombinant human IL-15 at doses of 10, 20 or 50 μ g/kg daily for 12 days [59, 60]. The animals were evaluated at predetermined time points by

hematology, serum chemistries, autoimmune markers, lymphocyte subpopulations by flow cytometry and coagulation studies, as well as for recombinant human IL-15 (rhIL-15) pharmacokinetics, and a complete necropsy with gross and histopathological examination. Transient grade 3/4 neutropenia was observed in 3 of 6 macaques receiving 20 mcg/kg and 3 of 6 receiving 50 mcg/kg; however, no infections were documented. Bone marrow examination showed a hypercellular marrow containing all elements of the neutrophil series. Neutrophils were also observed in the sinusoids of enlarged livers and spleens. This suggested that the neutropenia observed in these animals was as a result of a redistribution of neutrophils out of the circulation and into the tissues [60]. Neutrophil counts were restored within 72 hours of discontinuation of the IL-15. No animal developed positive autoimmune markers. No VLS, hemodynamic instability or renal failure was observed in either of these studies. Neutropenia associated with IL-15 appears to be dose and schedule dependent, and fully reversible. Further characterization of associated adverse effects must await the completion of human phase I trials.

Clinical trials of interleukin-15

To date there have been 7 clinical trials initiated using IL-15 (see Table 1). These trials are either ongoing or recently completed and have yet to be published. Of the 7 trials, 4 targeted cancer with the remaining 3 targeting HIV infections. Two of the cancer trials use rhIL-15 protein administered either alone (www.clinicaltrials.gov: NCT01021059), or combined with the administration of patient-derived tumor infiltrating lymphocytes (TIL) (www.clinicaltrials.gov: NCT01369888). The primary objective of these trials are to determine the safety, toxicity profile, dose-limiting toxicity and maximum tolerated dose of intravenous rhIL-15 administered as a daily intravenous bolus infusion for 12 consecutive days alone, or 10 consecutive days following TIL administration, respectively. Secondary objectives include determination of rhIL-15 pharmacokinetics and the immunogenicity of rhIL-15, determination of the biological effects of rhIL-15 on various peripheral blood mononuclear cell populations, and to estimate the antitumor activity of rhIL-15. The three HIV trials utilize IL-15 as a genetic adjuvant for the HIV vaccine (www.clinicaltrials.gov: NCT00775424, [NCT00115960](http://www.clinicaltrials.gov: NCT00115960), and [NCT00528489](http://www.clinicaltrials.gov: NCT00528489)). In these trials the vaccine is composed of DNA plasmids expressing HIV antigens and rhIL-15. The primary objective of these trials was to examine if IL-15 could increase the immunogenicity of the HIV vaccine that has limited immunogenicity when used alone. In the remaining two trials, IL-15 was used as an *ex vivo* ancillary agent to enhance dendritic cell (www.clinicaltrials.gov: NCT01189383) or NK cell (www.clinicaltrials.gov: NCT01337544) expansion and function when used for anticancer vaccination or immunotherapy.

Concluding remarks

The broad immunological activity coupled with an apparent lack of toxicity seen in preclinical studies makes IL-15 an exciting candidate for cancer therapy. The similarity it shares with IL-2 has led to the speculation that it might be effective in cancers in which IL-2 has been effective, i.e. renal cancer and melanoma, and to date these are the cancers being examined in the first clinical trials of IL-15. Improved safety while retaining or improving efficacy, when compared to IL-2, would make IL-15 attractive for treating these cancers, so the results of these clinical trials are eagerly awaited.

The next step in the IL-15 story may lie in the boosting of its effectiveness through the use of IL-15R α resulting in an increase biological stability and activity and the promotion of *trans*-presentation. Whether the best approach for this, is stimulating native IL-15R α expression using of agents such as anti-CD40, genetically altering cells to overexpress IL-15R α or by pre-associating IL-15 with soluble IL-15R α -IgG1-Fc is still under debate

with all showing promising preclinical results. Whether the increase in effectiveness using these approaches will also leads to increased toxicity or auto-immunity in humans will need to be examined.

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Abbreviations

AICD	activation-induced cell death
bp	base pair
FoxP3	forkhead box P3
GM-CSF	granulocyte-macrophage colony stimulating factor
IL	interleukin
IL-15Rα	interleukin-15 receptor-alpha
IFN	interferon
HIV	human immunodeficiency virus
JAK	janus kinase
LSP	long signal peptide
MCP-1	monocyte chemotactic protein-1
MIP-1	macrophage inflammatory protein-1
NK	natural killer
SSP	short signal peptide
STAT	signal transducer and activator of transcription
TNF-α	tumor necrosis factor-alpha
Tregs	T regulatory cells
VLS	vascular leak syndrome
UTR	untranslated region

References

1. Kantoff PW, et al. Sipuleucel-T Immunotherapy for Castration-Resistant Prostate Cancer. *N. Engl. J. Med.* 2010; 363(5):411–422. [PubMed: 20818862]
2. Hodi FS, et al. Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. *N. Engl. J. Med.* 2010; 363(8):711–723. [PubMed: 20525992]
3. Mocellin S, et al. Interferon alpha adjuvant therapy in patients with high-risk melanoma: a systematic review and meta-analysis. *J. Natl. Cancer Inst.* 2010; 102(7):493–501. [PubMed: 20179267]
4. Tarhini AA, Agarwala SS. Interleukin-2 for the treatment of melanoma. *Curr. Opin. Investig. Drugs.* 2005; 6(12):1234–1239.

5. Burton JD, et al. A lymphokine, provisionally designated interleukin T and produced by a human adult T-cell leukemia line, stimulates T-cell proliferation and the induction of lymphokine-activated killer cells. *Proc. Natl. Acad. Sci. USA.* 1994; 91(11):4935–4939. [PubMed: 8197160]
6. Grabstein KH, et al. Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor. *Science.* 1994; 264(5161):965–968. [PubMed: 8178155]
7. Waldmann TA, Tagaya Y. The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens. *Annu. Rev. Immunol.* 1999; 17:19–49. [PubMed: 10358752]
8. Berger C, et al. Safety and immunologic effects of IL-15 administration in nonhuman primates. *Blood.* 2009; 114(12):2417–2426. [PubMed: 19605850]
9. Marks-Konczalik J, et al. IL-2-induced activation-induced cell death is inhibited in IL-15 transgenic mice. *Proc. Natl. Acad. Sci. USA.* 2000; 97:11445–11450. [PubMed: 11016962]
10. Munger W, et al. Studies evaluating the antitumor activity and toxicity of interleukin-15, a new T cell growth factor: comparison with interleukin-2. *Cell. Immunol.* 1995; 165:289–293.
11. Krause H, et al. Genomic structure and chromosomal localization of the human interleukin 15 gene (IL-15). *Cytokine.* 1996; 8:667–674. [PubMed: 8932977]
12. Tagaya Y, et al. Generation of secretable and nonsecretable interleukin 15 isoforms through alternate usage of signal peptides. *Proc. Natl. Acad. Sci. USA.* 1997; 94:14444–14449. [PubMed: 9405632]
13. Anderson DM, et al. Functional characterization of human interleukin 15 receptor α chain and close linkage of IL-15RA and IL-2RA genes. *J. Biol. Chem.* 1995; 270:29862–29869. [PubMed: 8530383]
14. Waldmann TA. Targeting the interleukin-15/interleukin-15 receptor system in inflammatory autoimmune diseases. *Arthritis Res. Ther.* 2004; 6(4):174–177. [PubMed: 15225362]
15. Giri JG, et al. Identification and cloning of a novel IL-15 binding protein that is structurally related to the alpha chain of the IL-2 receptor. *EMBO J.* 1995; 14(15):3654–3663. [PubMed: 7641685]
16. Hatakeyama M, et al. Interleukin-2 receptor β chain gene: generation of three receptor forms by cloned human α and β chain cDNAs. *Science.* 1989; 244:551–556. [PubMed: 2785715]
17. Takeshita T, et al. Cloning of the γ chain of the human IL-2 receptor. *Science.* 1992; 257:379–382. [PubMed: 1631559]
18. Dubois S, et al. Natural splicing of exon 2 of human interleukin-15 receptor alpha-chain mRNA results in a shortened form with a distinct pattern of expression. *J. Biol. Chem.* 1999; 274:26978–26984. [PubMed: 10480910]
19. Dubois S, et al. IL-15R α recycles and presents IL-15 in trans to neighboring cells. *Immunity.* 2002; 17(5):537–547. [PubMed: 12433361]
20. Kobayashi H, et al. Role of trans-cellular IL-15 presentation in the activation of NK cell-mediated killing, which leads to enhanced tumor immunosurveillance. *Blood.* 2005; 105(2):721–727. [PubMed: 15367431]
21. Budagian V, et al. IL-15/IL-15 receptor biology: a guided tour through an expanding universe. *Cytokine Growth Factor Rev.* 2006; 17(4):259–280. [PubMed: 16815076]
22. Oh S, et al. IL-15 as a mediator of CD4⁺ help for CD8⁺ T cell longevity and avoidance of TRAIL-mediated apoptosis. *Proc. Natl. Acad. Sci. USA.* 2008; 105(13):5201–5206. [PubMed: 18362335]
23. Kanegane H, Tosato G. Activation of naive and memory T cells by interleukin-15. *Blood.* 1996; 88(1):230–235. [PubMed: 8704178]
24. Lodolce JP, et al. IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity.* 1998; 9:669–676. [PubMed: 9846488]
25. Kennedy MK, et al. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J. Exp. Med.* 2000; 191:771–780. [PubMed: 10704459]
26. Pelletier M, et al. Mechanisms involved in interleukin-15-induced suppression of human neutrophil apoptosis: role of the anti-apoptotic Mcl-1 protein and several kinases including Janus kinase-2, p38 mitogen-activated protein kinase and extracellular signal-regulated kinases-1/2. *FEBS Lett.* 2002; 532(12):164–170. [PubMed: 12459483]

27. Tagaya Y, et al. Identification of a novel receptor/signal transduction pathway for IL-15/T in mast cells. *EMBO J.* 1996; 15(18):4928–4939. [PubMed: 8890166]
28. Armitage RG, et al. IL-15 has stimulatory activity for the induction of B cell proliferation and differentiation. *J. Immunol.* 1995; 154:483–490. [PubMed: 7814861]
29. Demirci G, Li XC. IL-2 and IL-15 exhibit opposing effects on Fas mediated apoptosis. *Cell Mol. Immunol.* 2004; 1(2):123–128. [PubMed: 16212899]
30. Steel JC, et al. Interleukin-15 and its receptor augment dendritic cell vaccination against the neu oncogene through the induction of antibodies partially independent of CD4 help. *Cancer Res.* 2010; 70(3):1072–1081. [PubMed: 20086176]
31. Anguille S, et al. Short-term cultured, interleukin-15 differentiated dendritic cells have potent immunostimulatory properties. *J. Transl. Med.* 2009; 7:109. [PubMed: 20021667]
32. Quinn LS, et al. Interleukin-15: a novel anabolic cytokine for skeletal muscle. *Endocrinology.* 1995; 136:3669–3672. [PubMed: 7628408]
33. Figueras M, et al. Interleukin-15 is able to suppress the increased DNA fragmentation associated with muscle wasting in tumour-bearing rats. *FEBS Lett.* 2004; 569(13):201–206. [PubMed: 15225634]
34. Angiolillo AL, et al. Interleukin-15 promotes angiogenesis in vivo. *Biochem. Biophys. Res. Commun.* 1997; 233:231–237. [PubMed: 9144429]
35. Hanisch UK, et al. Mouse brain microglia express interleukin-15 and its multimeric receptor complex functionally coupled to Janus kinase activity. *J. Biol. Chem.* 1997; 272:28853–28860. [PubMed: 9360952]
36. Malamut G, et al. IL-15 triggers an antiapoptotic pathway in human intraepithelial lymphocytes that is a potential new target in celiac disease-associated inflammation and lymphomagenesis. *J. Clin. Invest.* 2010; 120(6):2131–2143. [PubMed: 20440074]
37. Gravisaco MJ, et al. IL-2, IL-10, IL-15 and TNF are key regulators of murine T-cell lymphoma growth. *Int. J. Mol. Med.* 2003; 12(4):627–632. [PubMed: 12964046]
38. Fehniger TA, et al. Fatal leukemia in interleukin 15 transgenic mice follows early expansions in natural killer and memory phenotype CD8+ T cells. *J. Exp. Med.* 2001; 193(2):219–231. [PubMed: 11208862]
39. Sato N, et al. Development of an IL-15-autocrine CD8 T-cell leukemia in IL-15-transgenic mice requires the cis expression of IL-15R α . *Blood.* 2011; 117(15):4032–4040. [PubMed: 21304101]
40. Yamasaki S, et al. Growth and apoptosis of human natural killer cell neoplasms: role of interleukin-2/15 signaling. *Leuk. Res.* 2004; 28(10):1023–1031. [PubMed: 15289013]
41. Trentin L, et al. Interleukin-15 promotes the growth of leukemic cells of patients with B-cell chronic lymphoproliferative disorders. *Blood.* 1996; 87(8):3327–3335. [PubMed: 8605349]
42. Leroy S, et al. Interleukin-15 expression in cutaneous T-cell lymphoma (mycosis fungoides and Sézary syndrome). *Br. J. Dermatol.* 2001; 144(5):1016–1023. [PubMed: 11359391]
43. Azimi N, et al. Human T cell lymphotropic virus type I Tax protein trans-activates interleukin 15 gene transcription through an NF-kappaB site. *Proc. Natl. Acad. Sci. USA.* 1998; 95(5):2452–2457. [PubMed: 9482906]
44. Kukita T, et al. Autocrine and/or paracrine growth of adult T-cell leukaemia tumour cells by interleukin-15. *Br. J. Haemato.* 2002; 119(2):467–474.
45. Wu S, et al. Expression of interleukin 15 in primary adult acute lymphoblastic leukemia. *Cancer.* 2010; 116(2):387–392. [PubMed: 19924795]
46. Cario G, et al. High interleukin-15 expression characterizes childhood acute lymphoblastic leukemia with involvement of the CNS. *J. Clin. Oncol.* 2007; 25(30):4813–4820. [PubMed: 17947730]
47. Lissoni P, et al. Blood concentrations of interleukin-15 in cancer patients and their variations during interleukin-2 immunotherapy: preliminary considerations. *Int J. Biol. Markers.* 1998; 13(3): 169–171. [PubMed: 10079393]
48. Quinn LS, et al. Serum and muscle interleukin-15 levels decrease in aging mice: correlation with declines in soluble interleukin-15 receptor alpha expression. *Exp. Gerontol.* 2010; 45(2):106–112. [PubMed: 19854259]

49. Oh S, et al. Coadministration of HIV vaccine vectors with vaccinia viruses expressing IL-15 but not IL-2 induces long-lasting cellular immunity. *Proc. Natl. Acad. Sci. USA*. 2003; 100(6):3392–3397. [PubMed: 12626740]
50. Zhang M, et al. Interleukin-15 combined with an anti-CD40 antibody provides enhanced therapeutic efficacy for murine models of colon cancer. *Proc. Natl. Acad. Sci. USA*. 2009; 106(18):7513–7518. [PubMed: 19383782]
51. Yu P, et al. Simultaneous blockade of multiple immune system inhibitory checkpoints enhances antitumor activity mediated by interleukin-15 in a murine metastatic colon carcinoma model. *Clin. Cancer Res*. 2010; 16(24):6019–6028. [PubMed: 20924130]
52. Zeng R, et al. Synergy of IL-21 and IL-15 in regulating CD8+ T cell expansion and function. *J. Exp. Me*. 2005; 201(1):139–148.
53. Dubois S, et al. Preassociation of IL-15 with IL-15R alpha-IgG1-Fc enhances its activity on proliferation of NK and CD8+/CD44high T cells and its antitumor action. *J. Immunol*. 2008; 180(4):2099–2106. [PubMed: 18250415]
54. Salagianni M, et al. NK cell adoptive transfer combined with Ontak-mediated regulatory T cell elimination induces effective adaptive antitumor immune responses. *J. Immunol*. 2011; 186(6):3327–35. [PubMed: 21317394]
55. Klebanoff CA, et al. IL-15 enhances the in vivo antitumor activity of tumor-reactive CD8+ T cells. *Proc. Natl. Acad. Sci. USA*. 2004; 101(7):1969–1974. [PubMed: 14762166]
56. Anguille S, et al. Short-term cultured, interleukin-15 differentiated dendritic cells have potent immunostimulatory properties. *J. Transl. Med*. 2009; 7:109. [PubMed: 20021667]
57. Di, Sabatino A., et al. Role of IL-15 in immune-mediated and infectious diseases. *Cytokine Growth Factor Rev*. 2011; 22(1):19–33. [PubMed: 21074481]
58. Mueller YM, et al. Interleukin-15 increases effector memory CD8+ T cells and NK cells in simian immunodeficiency virus-related macaques. *J. Virol*. 2005; 79(8):4877–4885. [PubMed: 15795273]
59. Lugli E, et al. Transient and persistent effects of IL-15 on lymphocyte homeostasis in nonhuman primates. *Blood*. 2010; 116(17):3238–3248. [PubMed: 20631381]
60. Waldmann TA, et al. Safety (toxicity), pharmacokinetics, immunogenicity, and impact on elements of the normal immune system of recombinant human IL-15 in rhesus macaques. *Blood*. 2011; 117(18):4787–4795. [PubMed: 21385847]

Figure 1. Interleukin-15 genomic organization and gene expression

IL-15 is encoded by a 34 kb region on human chromosome 4q31. The *IL-15* gene is made up of nine (1–8 and 4A) exons and eight introns of which 4 exons (5 through 8) code for the mature protein. There are 2 isoforms of IL-15 mRNA that differ in the length of their signal peptides. The originally identified form consisted of a 316 bp 5'-untranslated region (UTR), a 486 bp coding sequence, and a 400 bp 3'UTR that is translated into an IL-15 precursor protein with a long signal peptide (IL-15LSP) of 48 amino acids. The long signal peptide is encoded within exons 3–5. The other isoform has a short signal peptide of 21 amino acids (IL-15SSP) encoded by exons 4A and 5. Both isoforms produce the same mature protein; however, the two differ in that they have distinct intracellular trafficking. IL-15LSP is targeted to the Golgi apparatus, early endosomes and the endoplasmic reticulum secretory pathway where it is complex with IL15R α . IL-15SSP is not secreted and appears to be restricted to the cytoplasm and nucleus.

Figure 2. Interaction of interleukin-2 (IL-2) with its receptor (IL-2R) contrasted to that of IL-15 and its receptor (IL-15R)

The high affinity IL-2R is a heterotrimeric complex composed of the common cytokine receptor gamma subunit (γc), a beta subunit (IL-2R/IL-15R β) that is also shared with the IL-15 receptor (IL-15R), and a private alpha subunit (IL-2R α). All three subunits are expressed on activated NK, T and other immune cells. Receptor specificity is conferred by the alpha subunit of each cytokine (IL-2R α or IL-15R α) and signal transduction is mediated through IL-2R/IL-15R β and γc . For IL-2, all three subunits (IL-2R α , IL-2R/IL-15R β , and γc) are expressed by the same cell and as a complex directly bind soluble IL-2. In contrast, the interaction of IL-15 with its receptor is more complex. Little IL-15 appears to be expressed in a free soluble state. Rather, IL-15 is presented in *trans* pre-associated with IL-15R α by antigen-presenting cells to the IL-2R/IL-15R β and γc receptor subunits expressed on NK and T cells. IL-15 is expressed by monocytes/macrophages and dendritic cells and is stabilized by being associated with IL-15R α in the endosomal-Golgi apparatus. The IL-15/IL-15R α complex is secreted and expressed on the surface of monocytes and dendritic cells to present IL-15 to the IL-2R/IL-15R β and γc subunits expressed on NK and CD8⁺ T cells.

Table 1

Human clinical trials using IL-15.

Clinical trial	Trial type	Agents	Disease
A Phase I Study of Intravenous Recombinant Human IL-15 (rhIL-15) in Adults With Refractory Metastatic Malignant Melanoma and Metastatic Renal Cell Cancer.	Cancer Immunotherapy	IL-15	Melanoma or Renal cell cancer
Use of IL-15 After Chemotherapy and Lymphocyte Transfer in Metastatic Melanoma.	Cancer Immunotherapy (Adjuvant)	Tumor-infiltrating lymphocytes, IL-15	Melanoma
IL-15 Dendritic Cell Vaccine for Patients With Resected Stage IIIc and Stage IV Melanoma.	Cancer Vaccine	Dendritic cells grown in culture with IL-15 (Ancillary agent)	Melanoma
Haploidentical Stem Cell Transplantation and IL-15 NK Cell Infusion for Paediatric Refractory Solid Tumours.	Cancer Immunotherapy	NK-cells grown in culture with IL-15 (Ancillary agent)	Pediatric cancers
PENNVAX-B With or Without IL-12 or IL-15 as a DNA Vaccine for HIV Infection.	HIV Vaccine	PENNVAX-B, IL-12 plasmid DNA, or IL-15 plasmid DNA	HIV/AIDS
Safety of and Immune Response to an HIV Preventive Vaccine (HIV-1 Gag DNA Alone or With IL-15 DNA) Given With or Without 2 Different Booster Vaccinations in HIV Uninfected Adults.	HIV Vaccine	HIV vaccine, IL-15 plasmid DNA	HIV/AIDS
Safety and Effectiveness of PENNVAX-B Vaccine Alone, With IL-12, or IL-15 in Healthy Adults.	HIV Vaccine	PENNVAX-B, IL-12 plasmid DNA, IL-15 plasmid DNA	HIV/AIDS