Interleukin 15 in Gene Therapy of Cancer

Maria C. Ochoa[1], Guillermo Mazzolini[2], Sandra Hervas-Stubbs[1], Miguel Fernandez de Sanmamed[1,3], Pedro Berraondo[1] and Ignacio Melero[1,3]

[1]Center for Applied Medical Research, University of Navarra. Avda Pio XII, 55; 31008 Pamplona, Spain; [2]Gene Therapy Laboratory, Austral University, Avda Presidente Peron, 1500; B1629ODT Buenos Aires, Argentina; [3]Department of Oncology, Clinica Universidad de Navarra, 31008 Pamplona, Spain

Abstract: Interleukin 15 (IL-15) exerts powerful stimulatory effects on lymphocyte subsets that result in antiviral and antitumoral activities. The functions of this cytokine are mainly mediated in a cell-to-cell contact fashion termed IL-15 trans-presentation. This function is mediated by a cell which tethers IL-15 to its plasmatic membrane complexed to IL-15 receptor alpha (IL-15Rα). Such surface complexes interact with interleukin 2 receptor beta and gamma on the adjacent cell to elicit signalling. Unlike interleukin 2, IL-15 protects from activation-induced cell death and does not promote regulatory cells. These features underlie its activity against transplanted tumors and its adjuvanticity in tumor and viral vaccines. The GMP-manufactured recombinant protein is undergoing clinical trials but its rapid renal clearance calls for biotechnological strategies to increase molecular weight and ensure IL-15Rα trans-presentation. Since early efforts with stable transfected tumor cells, IL-15 has been tested in a variety gene therapy approaches. Those mainly include transfer of expression cassettes to tumor cells, T cells, dendritic cells, vaccination sites and the liver as a biofactory organ. Detailed mechanistic knowledge of IL-15 biology is envisaged to make the most of a powerful immunotherapeutic tool ranked as one of the most promising for cancer immunotherapy.

Keywords: Cancer, gene therapy, interleukin 15, immunotherapy.

1. INTERLEUKIN 15

Interleukin 15 (IL-15) is four-helix bundle cytokine family member discovered in 1994 as a close relative to interleukin 2 (IL-2) cytokine that bound interleukin 2 receptor beta (IL-2Rβ) and common gamma chain (γc) but did not need IL-2Rα [1]. IL-2Rβ and γc form a heterotrimeric receptor with its own IL-15Rα subunit that binds the IL-2Rβ and γc through physical interactions different to those described for IL-2 and IL-2Rα [2].

Murine IL-15 (mIL-15) RNA is not expressed in T or NK cells, but it is found in monocytes, bone marrow stroma cells, fibroblasts, epithelial cells and different tissues such as placenta, skeletal muscle, spleen, liver, heart, lung and kidney [1, 3]. However, functional IL-15 is not expressed by all of these, due in part to various post-transcriptional control checkpoints. There are two described pre-IL-15 proteins that differ in the length of the signal peptide [4-7]. Both signal peptides act as a regulatory mechanism to constrain IL-15 secretion [8], however, the short signal peptide IL-15 isoform is not as bioactive as the long signal peptide IL-15 isoform because it forms less stable complexes with IL-15Rα [9]. As a result, the main cells that express functional IL-15 protein upon activation are macrophages, monocytes, dendritic cells, muscle cells, keratinocytes, renal epithelial cells, and endothelial cells [3, 10-12].

*Address correspondence to this author at the CIMA, Avda Pio XII, 55, 31008 Pamplona, Spain; Tel: +34 948 194700 4030; Fax: +34 948194716; E-mail: imelero@unav.es

IL-15 expression is upregulated by granulocyte-macrophage colony-stimulating factor [13] and toll-like receptor agonists such as poly I:C and LPS Fig. (**1A**). Type I IFNs induce IL-15 expression [12] in a wide variety of cells of the immune system. Indeed, IL-15 is essential for the development [14], survival [15, 16] and function of NK cells [12]. A hallmark of IL-15 activity is that it increases NK proliferation and cytotoxicity [17]. However, it has recently been shown that sustained stimulation with IL-15 during two weeks decreases some NK activities, such as IFNγ expression and cytotoxicity in comparison with a transient stimulation for two days [18]. However, sustained IL-15 stimulation with IL-15/IL-15Rα complexes enhances NK proliferation and it has been shown to protect NK cells from apoptosis upregulating Bcl-2 and Mcl-1 gene expression but downregulating Bim and Noxa [16, 19]. IFNγ-producing killer dendritic cells (IKDCs), a cell amidst NK and dendritic cell biology [20], also require IL-15 to proliferate and IKDC cytotoxic functions are increased by IL-15 [21].

Homeostatic proliferation of memory $CD8^+$ T cells [22-24] and epidermal γδ T cell survival [25] is mediated by IL-15. Homeostatic proliferation maintains the normal numbers of such lymphocytes as a result of competition for the cytokine. Following antigen exposure, this cytokine protects T cells from activation-induced cell death [26]. It has been shown [27] that IL-15 is necessary for the reversal of antitumor CD8 T cell tolerance following lymphopenia and tumoral irradiation [28]. With regard to naïve CD8 T lymphocytes, IL-15 can amplify their activation and proliferation

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upon TCR stimulation [29] and thereby increase its response to a cognate antigen.

Other effects involving IL-15 are stimulation of B-cell proliferation and antibody production [27, 30], production of secondary cytokines such as IL-5 and TNF-α by T cells [31, 32], IL-8 secretion by neutrophils [33] and IFN-γ by NK cells [11]. IL-15 plays a role in phagocytosis and morphological differentiation in human neutrophils [34]. Promotion of angiogenesis [35] and up-regulation of chemotaxis and adhesion of T lymphocytes are other functions reported for IL-15 [36, 37].

The intracellular signalling pathway of IL-15 includes tyrosine phosphorylation of JAK1 and JAK3 which mediate the phosphorylation and the activation of STAT5 and STAT3 [38, 39] Fig. (**1B**). STAT-5 phosphorylation is usually taken as a convenient functional biomarker of IL-15-mediated activation. IL-15 up-regulates the PI_3K/Akt, Shc and ERK1/2 signalling pathways [39, 40]. In addition, it has been reported that IL-15 induces LCK dephosphorylation and MAPK activation [41]. Interestingly, a form of IL-15 that is membrane-bound (probably forming IL-15/IL-15Rα complexes) in monocytes seems to carry out a reverse signalling function through diverse enzymes such as the Rho family GTPase Rac3 and the MAPK members, ERK1/2 and p38, which are in turn involved in adhesion and in cytokine production [42].

A study with serial administrations of human IL-15, as a soluble recombinant protein has been performed in rhesus macaques [43]. Animals received doses between 10 and 50 μg/kg i.v. daily for 12 days. At day 13 they showed a prominent expansion of NK and CD8 memory T cells in different tissues such as the spleen, lymph nodes and bone marrow, as well as in peripheral blood [43]. Treated animals presented a hypercellular bone marrow with smaller adipocytes and liver enlargement due to an inflammatory infiltrate composed of lymphocytes and neutrophils. The most severe adverse effect was a transient neutropenia. At day 48 from the beginning of treatment, all cellular subsets returned to homeostatic levels. Recently, another study in which rhesus macaques were administered IL-15 by continuous infusion and daily subcutaneous injections during 10 days has been published showing a remarkably enhanced lymphocyte accumulation over bolus discontinuous administration [44]. Indeed, continuous infusion of IL-15 achieved higher levels of $CD8^+$ cells than the subcutaneous injections (100 fold vs. 10 fold) at day 10. Both routes of administration increased circulating NK (10 fold and 5 fold), while the continuous infusion also showed an increase in monocytes. There were no changes in the amount of monocytes in the animals that received the subcutaneous injections.

2. THE IL-15/IL-15 RECEPTOR SYSTEM

IL-15 function is strictly dependent on its receptor system. The cytokine needs to bind IL-2Rβ and γc to exert its functions in T, TCRγδ T, NKT, NK and IKDC cells [10, 11, 21, 25, 27, 45]. IL-2Rβ and γc subunits were first identified as receptors for IL-2. IL-15 was described to form a heterotrimeric receptor complex with its own IL-15Rα subunit that associates IL-2Rβ and γc [2] Fig. (**1B**).

IL-15 binds IL-15Rα with high affinity (Kd ≈ 10^{-11}) due mainly to ionic forces operating at the interface between the cytokine and the receptor [46]. IL-15Rα is expressed on activated T lymphocytes, activated NK cells, neutrophils, macrophages, monocytes and DCs, and mRNA encoding IL-15Rα has a broad tissue distribution including the thymus, heart, spleen, lung, skeletal muscle, prostate, ovary, testis, colon, small intestine and it is especially abundant in the liver [10, 34, 47, 48]. IL-15Rα contains a single short consensus repeat domain (sushi domain) that is necessary for IL-15 binding and function [49], whereas the cytoplasmic domain of IL-15Rα plays a part in intra-cellular signalling [50] and is essential for the endosomal recycling of the IL-15/IL-15Rα complexes that has been described in monocytes [51]. Capture by this intracellular recycling system could be the reason for the low levels of IL-15 found in serum. It has been shown that IL-15/IL-15Rα complexes are formed intracellularly and then traffic to the membrane where they remain anchored [52, 53]. The intracellular co-expression of IL-15 and IL-15Rα provides stability to the molecules and decreases the rate of IL-15 elimination [9]. Moreover, IL-15Rα presents different isoforms generated by alternative splicing that modulate IL-15/IL-15Rα function [54].

IL-15 and IL-15Rα form complexes at the cell membrane that can induce activation and proliferation in neighbouring cells expressing the IL-2Rβ and γc upon cell-to-cell contact ("trans-presentation") Fig. (**1B**). In addition, IL-15 can bind to the high affinity trimeric receptor with the IL-15Rα, IL-2Rβ and γc subunits ("cis-presentation") acting on the same cell [51, 53, 55, 56] Fig. (**1C**). Experiments *in vitro* have shown that high artificial concentrations of IL-15 achieve signalling through IL-2Rβ and γc subunits in the absence of IL-15Rα [47, 57] Fig. (**1C**). However, IL-15Rα increases IL-15 bioactivity and in physiological conditions the trans-presentation phenomenon appears to be by far the most important. Indeed, the co-expression of IL-15 and IL-15Rα on the same DC is necessary for NK and CD8 T cell support as mediated by IL-15 [53, 58-62]. Moreover, IFNγ production by NK cells is dependent on IL-15 trans-presentation [63]. Recently, it has been shown that the requirement for trans-presentation in NK cell development is dose-dependent, in such a way that the level of IL-15Rα expression in the dendritic cells quantitatively determines the intensity of the response [64]. In accordance with this idea, proliferation of CD8 T cells and NK cell survival are not possible in IL-$15Rα^{-/-}$ mice even if the NK and T lymphocytes are from wild type donors [65, 66].

In some renal carcinoma cells, IL-15 also appears as a membrane-bound form not trans-presented by IL-15Rα, in spite of expressing IL-15Rα in the same cell [67] Fig. (**1C**). This IL-15 form can be shed upon cleavage by ADAM10 and ADAM17 metalloproteases thus becoming a soluble form of IL-15 that can interact with IL-15Rα forming soluble complexes [68], reported to be involved in epithelial-mesenchymal transition(EMT). Peripheral blood hematopoietic NK cell precursors also express this directly membrane-bound form IL-15 [69]. When such membrane-bound IL-15 interacts with soluble IL-15Rα, the ERK 1/2 and FAK signalling enzymes are activated leading to differentiation into an ill-defined type of regulatory NK cells [69]. The activation of the ERK 1/2 and FAK pathways in NK cells is

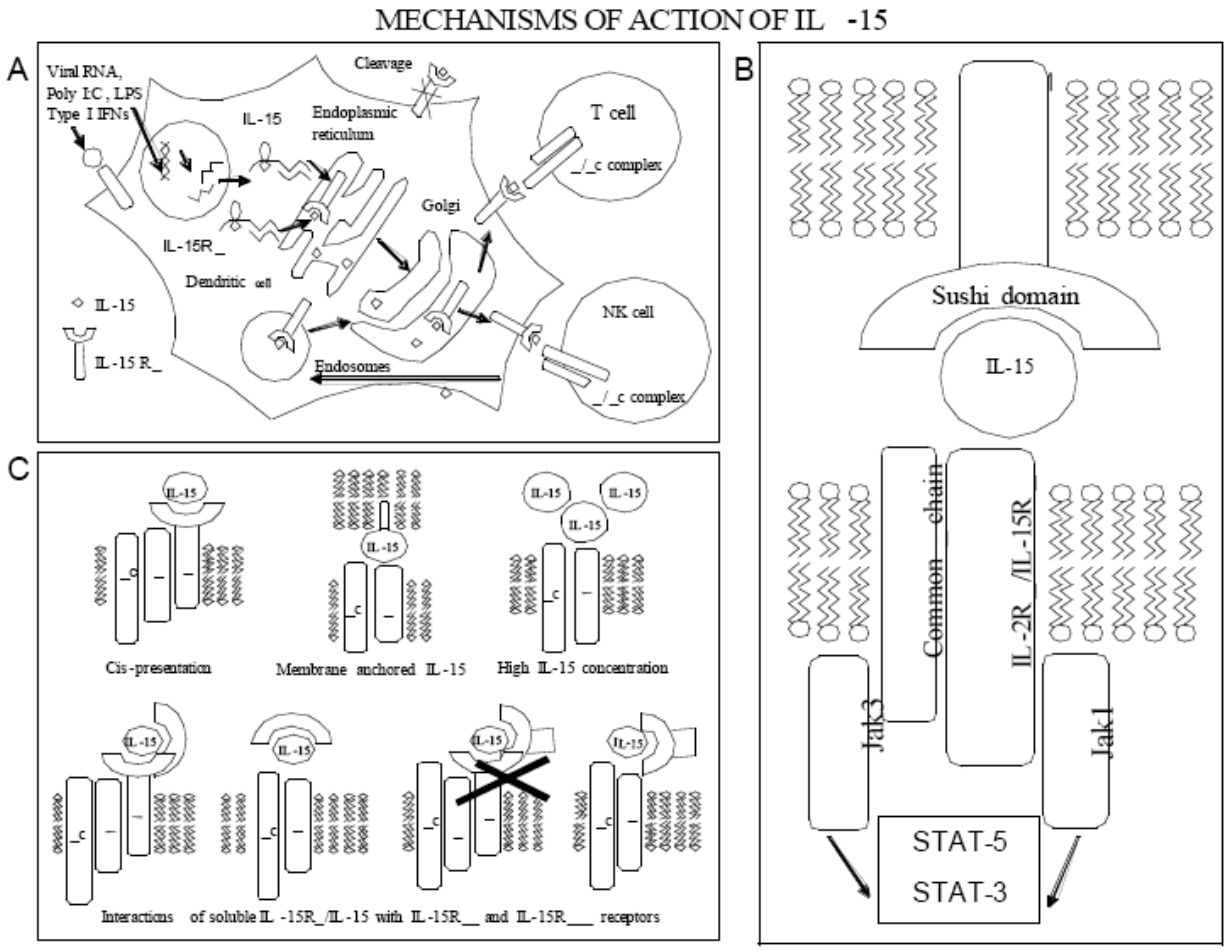

Fig. (1). Mechanisms of action of IL-15. (**A**) Model of a DC trans-presenting IL-15 tethered to IL-15Rα upon stimulation via pathogen-associated nucleic acids or type I IFN. The biosynthesis and surface trans-presentation of IL-15 are represented (**B**) Molecular players of classical IL-15 trans-presentation and the main elicited molecular signalling events. (**C**) Described modes of action of IL-15 on its receptors in addition to classical trans-presentation.

blocked by the membrane form of the IL-15Rα chain, so upon trans-presentation differentiation to regulatory NK cells would not take place.

The soluble form of IL-15Rα that is produced by proteolytic cleavage is able to avoid the interaction between membrane bound IL-15 and cell surface IL-15R receptor α inhibiting IL-15 function [70]. However, it has been reported that soluble form of IL-15Rα found on the serum of a head and neck cancer patient behaved as an agonist of IL-15 increasing proliferation and cytokine production in CD8 T lymphocytes [71]. This is because soluble IL-15Rα acts as an antagonist of IL-15 in cells that express the three subunits of IL-15R, but not in cells expressing only the Il-15Rβγ [72]. In line with this, exogenous soluble "sushi" domain of IL-15Rα has been demonstrated to act as an agonist of IL-15 in cells expressing IL-2Rβ and the γc chain [73] and it does not appear to be an antagonist on cells expressing the three subunits of the receptor [72] Fig. (**1C**). The reason for the lack of antagonistic activity of the sushi domain in cells expressing the α, β and γ subunits of IL-15 receptor is attributed to the 13 amino acid sequence that is located at the C-terminus of the "sushi" domain. This sequence seems responsible for the antagonist activity of soluble IL-15Rα [72].

A soluble form of the γc receptor has been described in mouse serum [74]. When T cells are activated, the concentration of soluble forms of γc increases while the membrane-attached expression of this receptor decreases. It has been proposed that the presence of soluble complexes of IL-2Rβ and γc in the milieu [75] could result in the trans-presentation of IL-15 in a reverse form.

Another form of IL-15/IL-15Rα has been observed in absence of IL-2Rβ in bone marrow-derived mast cells [76]. According to this report, IL-15 can induce JAK-2 and STAT-5 phosphorylation, probably through a different, as yet unidentified, receptor called IL-15R-X [77].

3. MICE DEFICIENT IN IL-15 OR IL-15Rα AND CONSEQUENCES OF IL-15 CHRONIC OVER-EXPRESSION

Knock-out mice for IL-15 and IL-15Rα were made in 2000 and 1998 respectively [78, 79]. Both of these develop as healthy adults and do not present any autoimmune disease. Indeed, they are slightly immunosuppressed since they are almost totally devoid of NK cells in the thymus, liver and spleen. IL-15$^{-/-}$ mice harbour only a very small population of immature NK cell in the spleen and bone marrow whose

development has been arrested at a defined stage [80]. Both strains also show a decreased population of CD8 memory T cells and the number of TCRγδ CD8αα intraepithelial lymphocytes is diminished, as are NKT cells and IKDC [81]. However, IL-15$^{-/-}$ mice have normal numbers of CD8α^{+} and CD8α^{-} DCs [82].

IL-15$^{-/-}$ and IL-15Rα$^{-/-}$ mice present a similar phenotype, indicating that both proteins interact in most of the functions of the cytokine. In conclusion, there are no indications of other receptors or cytokines that interact with IL-15 or IL-15Rα at least for their main functions on immune ontogeny.

Transgenic mice that over-express murine IL-15 under MHC class I promoter showed resistance to *Salmonella* infection and presented strong IFNγ production [6]. Their antiviral immune response was also increased whereas no signs of autoimmunity were ever observed. Indeed, studies with tumors derived from cell lines have shown that IL-15 transgenic mice presented antitumor NK activity against B16.44 MHC-I lacking tumors and that the growth of B16F10 tumors was retarded because of a CTL dependent mechanism [83].

Interestingly, transgenic mice engineered to overexpress IL-15, eliminating posttranscriptional checkpoints, develop leukemias with a T/NK cell phenotype when 12-30 weeks old [84]. These leukemias were similar to human large granular lymphocyte leukemias (LGLL). Half of the IL-15 transgenic mice developed leukemias of the NK phenotype and the other half displayed a T phenotype with expression of the NK antigens NK1.1 [85] and NKp46 [86].

There are also transgenic mice that express human IL-15 [87]. Those mice presented a very active immune system with a high density of NK cells in the liver and spleen together with an accumulation of splenic memory phenotype CD8 T cells. Moreover, in such transgenic mice, activation-induced cell death as mediated by IL-2 is blocked. These mice develop leukemia when older than 12 months, and it has been shown that expression of IL-15Rα in the leukemic cell is necessary to develop the malignancy [88].

4. THERAPY WITH IL-15 IN MOUSE TUMOR MODELS

Since its discovery IL-15 has been reported to mediate therapeutic effects in murine transplanted tumors [89-91]. IL-15 presents antitumoral effects mediated in part by the enhancement of NK cytotoxicity [92]. It has been proved that IL-15 increases NK-cell cytotoxicity in a NKG2D-dependent fashion [93] and this mechanism could be dependent on the presence of the NKG2D-ligand ULBP1 on the tumor cell [94]. There are additional ways in which NK cells are important for IL-15 antitumoral therapy. For instance, *in vitro* assays indicate that NK cells in presence of IL-15 become more efficacious mediators of ADCC against cultures of B-lymphoma cells in the presence of the anti-CD20 mAb rituximab [95].

CD8 T cells are also principal players in IL-15-induced antitumoral immunotherapy. IL-15 increases the number of specific CD8 T cells in adoptive T-cell therapy [96] and *in vitro* assays show that IL-15 can increase the proliferation and also IFNγ production of unresponsive CD8 T cells against Wilms´tumor antigen [97]. IL-15 systemic injection increases the number of CD8 cells when administered in combination with peptide pulsed DC [98] indicating that IL-15 could by a good adjuvant for dendritic-based vaccines.

In spite of the high expectations raised, IL-15 administration in animal tumor models has not achieved the expected results, probably due to the very short half-life of the cytokine in plasma (<1 h) [99] or the high capacity of self-regulation conferred by the receptor system. In this regard, some strategies have been developed to achieve better results with IL-15in cancer immunotherapy. Some strategies have focused on the trans-presentation system of IL-15 and researchers have devised treatments with IL-15 complexed to IL-15Rα. Other investigators have designed different combinations of IL-15 with other known antitumoral treatments. Different vectors to optimize IL-15 delivery have been constructed and in other experiments the IL-15 gene has been directly transferred to different cell types such as dendritic cells, tumoral cells and T lymphocytes. The ultimate aim is to attain a better antitumoral effect Fig. (**2**).

It must be considered that IL-15 can exert some protumoral effects in certain types of cancer that express IL-15 receptors. For example, IL-15 is an autocrine/paracrine growth factor for adult T-cell leukemia [100] probably because in cutaneous T-cell lymphoma cells IL-15 maintains *bcl-2* and *c-myb* gene expression, thereby protecting these malignant cells from apoptosis [101, 102]. Likewise, some human myeloma cells express IL-15Rα and this procures an anti-apoptotic effect *in vitro* in presence of IL-15 [103]. There is also a report in the sense that IL-15 is a factor that induces mucosal hyperplasia in colon cancer, potentially favouring the progression of the disease [104].

5. STABILIZATION AND TRANS-PRESENTATION OF IL-15 FOR CANCER THERAPY

The trans-presentation system offers an opportunity to augment overall IL-15 bioactivity mainly because IL-15Rα enhances its efficiency to stimulate cells bearing the β and γ subunits of the receptor. Furthermore, complexes offer the opportunity to increase the molecular size over the renal filtration threshold to extend half-life (summarized data of this strategy are shown in Table **1**). In this sense, elegant studies have focused on the use of complexes of IL-15 and a fusion protein containing the extracellular domain of murine IL-15Rα chimerized with the Fc fragment of human IgG1 [105, 106]. *In vitro* assays have shown that such IL-15/IL-15Rα complexes enhance NK and CD8 T cell proliferation and cytotoxicity against tumor cells, showing higher efficiency than IL-15 alone [107]. Studies *in vivo* in animal models have corroborated these data [105, 108]. Stoklasek *et al.* showed therapeutic effects of treatment with IL-15/IL-15Rα complexes in B16-F1-derived metastatic lung nodules [108]. On day 21 after the treatment 90% of the IL-15/IL-15Rα-treated mice were free of metastases in comparison with 10% of the group that received IL-15 only. The immunologic effect of those complexes appears to be also dependent on the Fc receptors because weaker NK and CD8 T cell proliferation was observed when the IL-15/IL-15Rα complexes were administered to FcR$^{-/-}$ mice [105]. However, there is a report that shows that NK and peripheral CD8 T cells are not the

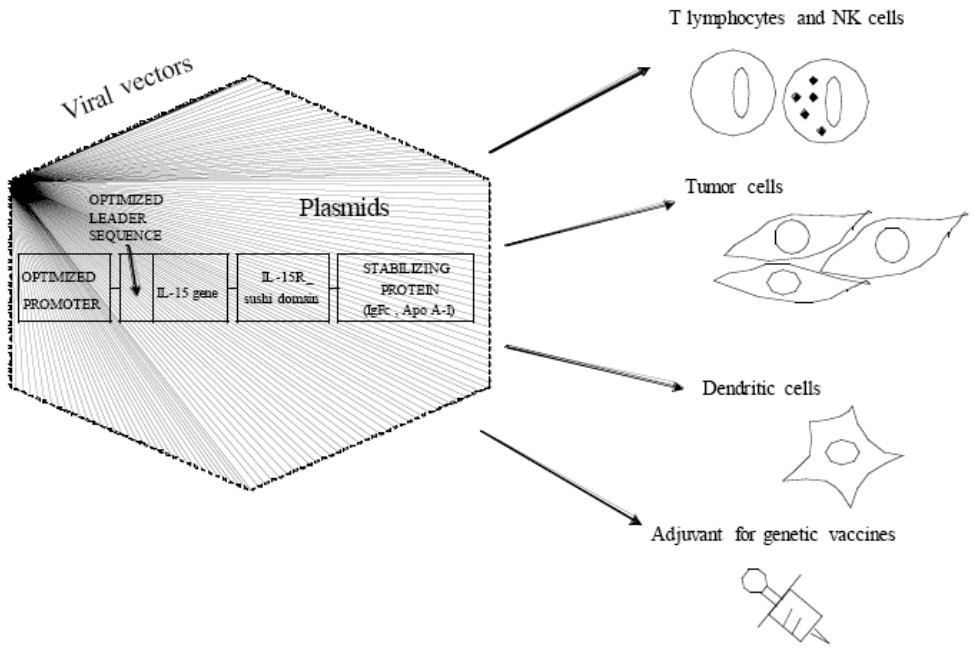

Fig. (2). Strategies of gene therapy with IL-15. Expression cassettes of IL-15 can be delivered by *ex-vivo* transfection or *in vivo* gene transfer with viral and non-viral vectors. IL-15 gene expression is optimized changing the leader peptide sequence and the promoters. Fusions with other proteins are made with the purpose of stabilization in plasma as a result of enhancing the molecular weight above the renal filtration threshold. Artificially engineering the trans-presentation of the cytokine to enhance bioactivity can be achieved by fusion partners as well. The main cellular targets for transgenic IL-15 are NK and T lymphocytes and antigen presenting DC.

Table 1. Antitumoral Strategies Based on Stabilized IL-15

Treatment	Model	Effect of IL-15	Reference
IL-15/IL-15Rα-Fc	B16-F1 melanoma cell line i.v.	Increased antitumoral effect. High NK and CD8 T cell proliferation and activation.	[108]
IL-15/IL-15Rα-Fc	B16 melanoma cell line i.v.	Antitumoral effect. High NK and CD8 T cell proliferation and activation. Effect dependent in part of FcR.	[105]
IL-15/IL-15Rα-Fc	B16F10 melanoma cell line injected i.v. and spontaneous pancreatic tumors of RIP1-Tag2 mice	Antitumoral effect by activating tumor resident CD8T cells	[109]
IL-15/L-15Rα fusion protein	HCT-116 human colorectal cancer cells derived tumors in the cecum of nude mice and B16F10 tumoral cells injected i.v. or intrasplenic	Increased antitumoral effect	[110]
Irradiation and specific transfer of CD8 T cells conjugated with IL-15/IL-15Rα-Fc – and IL-21–releasing nanoparticles	Established B16F10 derived melanomas	Induction of specific T cells. Strong antitumoral effect	[111]
IL-15 fused to the human antibody fragment scFv(L19), specific to the EDB domain of fibronectin	F9 and C51 derived carcinomas in subcutaneous and i.v. models	Increased antitumoral effect	[112]
IL-15 and IL-15Rα sushi domain fused to and antibody targeting the tumor stromal fibroblast activation protein	B16-FAP-derived melanomas injected i.v. in a lung metastasis model	Increased antitumoral effect	[113]

main players in the antitumoral effects of IL-15/IL-15Rα-Fc complexes against B16F10- derived lung metastases and insulinomas arising in RIP1-Tag2 transgenic mice. At least in those cases, resident CD8 T cells in the tumor (tumor infiltrating lymphocytes) appear to be the main antitumor players in those therapeutic settings [109].

Another group stabilized IL-15 and IL-15Rα designing a fusion protein encompassing both molecules [73, 110]. They found that the chimeric protein exerted therapeutic effects against HCT-116 human colorectal cancer implanted in the cecum of nude mice and against B16F10-derived lung and liver metastases. Conclusive experiments showed that the anti- B16F10 effect of the fusion protein was abrogated when NK cells were depleted with anti-asialo GM1. A very creative and efficacious combined therapeutic strategy consisted of irradiation and administration of CD8 specific T cells with nanoparticles attached onto the plasma membrane with the capacity to deliver both IL-15/IL-15Rα-Fc and IL-21 [111]. Such a therapy cured one-week established tumors derived from a syngenic melanoma cell line.

There is a study using a strategy for stabilization of IL-15 that does not include the IL-15Rα. It consisted of the delivery of IL-15 in the tumoral vasculature as a fusion of IL-15 to a specific antibody for a domain of fibronectin, a known angiogenesis marker targeting tumors [112]. This immunocytokine obtained a palliative reduction for F9 teratocarcinoma and subcutaneous growth of C51 tumors.Another study used IL-15 and IL-15Rα sushi domain fused to and antibody directed to the tumor stromal fibroblast activation protein and was found to exert antitumoral effects on a model of B16-FAP-derived melanomas injected i.v. to render a lung metastasis model [113].

6. IMMUNOTHERAPY WITH TUMORAL CELLS ENGINEERED TO EXPRESS IL-15

A strategy to exploit the IL-15 as an antitumoral agent has been to use the transference of tumoral cells with an expression plasmid coding for a secretable form of IL-15 (summarized data of this strategy are shown in Table **2**). For example, the murine adenocarcinoma cell line TS/A was engineered to express and secrete active IL-15 [114]. This cell line showed a reduced growth rate when implanted in syngenic mice and vaccination with irradiated IL-15–engineered TS/A cells displayed therapeutic activity against TS/A experimental lung metastases. The antitumoral effect of the vaccination of mice with TS/A cells expressing IL-15 was CD8+ T cell-dependent and immunohistochemical analyses of shrinking tumors revealed the presence of an inflammatory infiltrate with NKs, macrophages, and granulocytes as predominant components. Interestingly, this IL-15 transfected cell line grew normally in IFNγ knock out mice, whereas the tumors were rejected in these mice when the cells were engineered to express both IL-15 and IL-12 [115]. It was concluded that the IFNγ-dependent antitumoral effect of IL-12 and IL-15 appears to be mainly mediated by CD8 T cells. The cell line Meth-A has also been transferred with a IL-15 expression cassette and a clear antitumoral effect was observed as mediated by T cells in syngenic mice [116]. In experimental neuroblastoma it has been shown that a two-step treatment firstly with cells expressing IL-12 and followed 10 days later the same cells engineered to express IL-15 cured 41% of the tumor-bearing mice [117]. These studies suggest the potential of combined cytokine gene expression by tumor cells as a common theme to enhance therapeutic efficacy.

Murine IL-15 delivered by a recombinant adenovirus (Ad.mIL-15) has also been successfully used against an orthotopic Lewis lung carcinoma model [118]. Survival of LLC cells infected *in vitro* by Ad.mIL-15 vector significantly increased in comparison to LLC cells transduced by a control adenovirus expressing α-galactosidase. Cured animals were re-challenged with LLC cells but tumor growth was blunted. These effects were abolished by NK-cell-depleting anti-asialo GM1 antibodies. The human cancer cell line N592 also was transfected with IL-15 and this transfectant grew in nude mice more slowly than the untransfected control cell line [119]. Similar observations were made with human pancreatic cancer cells (AsPC-1) in which secretion of IL-15 produced an antitumoral effect dependent on host NK cells [120]. However, when N592 cells were engineered to express simultaneously IL-15 and IL-12 there was an antitumoral effect unexpectedly found to be independent of NK and CD8 cells [121]. Further investigations have shown that this effect could be mediated by antitumor specific antibodies, suggesting that the antitumoral effect of the transfection of IL-12 and IL-15 in syngenic N592 cells is due mainly to B cells and the cross-talk between innate immunity and B cells [122]. However, when this strategy was implemented in a syngenic murine model, such as the CT26 tumor cell line, such an effect was not observable [123].

The human prostate carcinoma cell line PC-3 was transferred with a vector expression hIL-15 consisting of the hIL-2 signal peptide, the hIL-15 mature peptide-coding sequences, and an out-of-frame human growth hormone gene. Transfection resulted in a decreased ability of those human prostate cancer cells to graft and grow subcutaneously in nude mice (7 out of 11 mice rejected tumors) [124]. When animals were treated with anti-asialo GM1 mAb the antitumoral effects were abolished.

The amount of IL-15 expressed by the transfected tumoral cells must be an important parameter for the antitumoral effect. When the fibrosarcoma cell line Meth A was transduced with a vector constructed with IL-15 cDNA including an alternative exon 5 that results in a higher expression of IL-15, a stronger antitumoral effect was observed than when the cells were transferred with natural IL-15 [125]. However, the optimal concentration/dose of the cytokine in the tumor microenvironment or at draining lymph nodes remains to be established.

All these experiments with tumoral cells transferred with IL-15 involve an interplay between IL-15 and CD8 or NK cells acting in *cis* or mediated by a third-party transpresenting cell. To study the possible effects of transpresentation between tumoral cells and CD8 or NK cells, Rowley *et al.* transferred TC-1 cells with a retrovirus encoding IL-15 linked to IL-15Rα. The tumoral cells expressed IL-15/IL-15Rα complexes at the plasma membrane and induced an inhibition in tumor growth as well as a dramatic increase in the number and activity of NK and CD8 T cells in tumor infiltrates [126]. The colon carcinoma cell line MC38 was

Table 2. Studies Performed with IL-15 Gene Therapy

Vector/Strategy	Animal Model	Mechanism of Antitumor Effect	Ref.
DC engineered to express OVAhsp70 and IL-15	B16 melanoma model in C57BL/B6 mice	Tumor protection; Th1 and CTL responses	[136]
DC engineered by adenoviruses to express a truncated NEU antigen, IL-I5, and IL-15Rα	Spontaneous mammary tumors in BALB-neuT mice	Improved tumor-free survival; specific anti-NEU antibody induction	[137]
DC engineered to express IL-15 by an rSV40-IL-15	I.t. injection of rSV40IL-15, DC-rSV40IL-15 or *in vitro* infection of tumor cells; CT26 colorectal carcinoma, s.c. in BALB/c mice	Only DC infected with rSV40IL-15 showed antitumoral effect; CTL induction; efficacy was mediated mainly by CD8+ T lymphocytes and NK cells	[123]
GD2 ganglioside mimotope vaccine (47-LDA) + plasmids encoding IL-15 and IL-21 genes	NXS2 neuroblastoma cell line, s.c. in A/J mice	NK-cell as well CD4+ and CD8+-T lymphocyte depletion abrogates protection against tumor challenge	[138]
Hydrodynamic injection with plasmids encoding IL-15 and IL-21	Liver metastatic RLmale1 lymphoma model	Protective and therapeutic antimetastatic activity; increased NK activity	[139]
rAAV2 expressing hIL-15 (intramusculary)	JC breast cancer cells s.c. in BALB/c mice; preventive model	Increased LAK cytotoxicity; tumor cell apoptosis	[141]
rAAV2 expressing hIL-15 (intramusculary)	Cervical cancer cells (HeLa cells) s.c in nude mice	Tumor growth inhibition in pretreated mice.	[142]
Recombinant fowlpox (rF) containing B7-1, ICAM-1, and LFA-3 genes+rF expressing GM-CSF or IL-15	I.t. injections in orthotopic RCC#15 (renal cell carcinoma cells) model, in BALB/c mice	Increased antitumoral effect	[144]
Sindbis vector delivering mIL-15	Human ES-2 ovarian cancer cells i.p. in SCID	Higher antitumoral effect than Sindbis control	[145]
AAV8 delivering IL-15/IL-15Rα Sushi domain	Metastatic hepatocellular carcinoma model (BNLh1 cells) in BALB/c mice	Increased antitumoral effect by increasing NK cytotoxicity	[147]
Heparin-polyethylenimine-plasmid encoding IL-15 (HPEI-pIL-15)	Colorectal (CT26 cells) and melanoma (B16-F10 cells) lung metastatic model in mice; HPEI-pIL-15 i.v. injected	Anti-metastatic effect; increased NK activity and systemic TNF-α and IFN-γ levels	[148]
Plasmid expressing hIL-15	I. t. injection followed by electroporation in s.c. B16F10 melanoma in mice	Increased survival compared with phIL-15 alone	[149]
Oncolytic vesicular stomatatis virus expressing hIL-15	I.v. injection in CT26 colorectal carcinoma, s.c. and lung metastatic model, in BALB/c mice	Antitumoral effect based on specific CD8+ T cells	[156]
Bicistronic plasmid encoding Her-2/Neu and IL-15, IL-18 or GM-CSF	CT26 colorectal cancer cells expressing Her-2/Neu, s.c. in BALB/c mice	Protective and therapeutic antitumor activity	[137]
Mimovirus composed of a cell-penetrating peptide, a CTL epitope peptide survivin and a plasmid containing mIL-15	CT26-derived tumor model, s.c. in BALB/c mice	Increased antitumoral effect and animal survival	[150]
Intravesical liposomal plasmid encoding IL-15	Orthotopic bladder cancer (MBT-2 cells) model in C3H/HeN mice	CD8+ T cell infiltrate; CTL induction; Increased antitumoral effect	[151]
T lymphocytes expressing a CAR targeting CD19+IL15+inducible caspase-9-based suicide gene	Daudi and Raji (Burkitt lymphoma cell lines) and HDLM-2 (Hodgkin lymphoma cell line), i.p.and s.c. in SCID mice	Reduced PD-1 receptor on T cells; antitumoral activity	[157]
Epstein-Barr specific CTLs engineered to produce IL-15	LCL cells in matrigel plug in SCID mice	Higher antitumoral effect than untransfected CTLs	[158]
T lymphocytes expressing a chimeric antigen receptor targeting the CD19 antigen and IL-15	Raji-GL tumor cells in NOD/SCID/γ null mice	Increased antitumoral effect by increasing the number and effector functions of T cells transferred	[159]

also engineered to express IL-15Rα and mice bearing these tumors survived longer than those challenged with the untransfected wild type form. The mechanisms underlying this antitumoral effect were attributed to NK cells [127]. A recent study from the group of Hans Schreiber has shown that neutralization of IL-15 allows the IL-15-transfected transplanted tumor cells to grow, but once released from inhibition, IL-15 induces NK cell mediated rejections of large tumor masses [128].

By contrast, Tasaki *et al.* found an antitumoral effect transfecting Colon26 murine colon carcinoma cells with IL-15, but in this case the investigators did not observe differences between NK depleted nude mice and undepleted nude mice [129]. In humans, a pioneering study reported that IL-15 in conjunction with CpG oligonucleotides enhances circulating NK activity in human patients suffering from cutaneous T-cell lymphoma [130].

7. CANCER THERAPY WITH IL-15 AND DENDRITIC CELLS BASED VACCINES

Addition of IL-15 to the culture of DC derived from monocytes increases the capacity of DC to prime specific CD8 T cells [131, 132] and it has been previously demonstrated that an IL-15 expression cassette increases the efficacy of DNA vaccines (summarized data of this strategy are shown in Table **2**) [133]. However, an antitumoral *in vivo* assay with C6VL tumor lysate-pulsed dendritic cell DC in combination with IL-15 failed to show any IL-15 antitumoral effect, in spite of the observation that IL-15 caused an increase in the number of memory CD8 T cells and NK cells [134].

DC can be efficaciously transfected with different viral and non viral methods [135]. Some authors have obtained good therapeutic results gene-transferring DCs to express IL-15. Considering that DC-derived IL-15 may facilitate DC activation, Tian and colleagues hypothesized that restricted expression and secretion of IL-15 *in vivo* by DC can increase the efficacy of DNA-based vaccines. Employing a gene gun strategy on the skin, these authors immunized mice with a CMV-OVAhsp70-CD11c-IL-15 construct and were able to elicit long-lasting Ag-specific Th1 and CTL responses against B16 melanoma, achieving tumor protection that was not seen with the corresponding controls [136]. As IL-15Rα has been shown to stabilize IL-15 and increase the half-life of the cytokine, Steel *et al.* explored a vaccination strategy using DC engineered to express the oncoprotein HER2/neu (overexpressed in human carcinomas with a poor prognosis) together with IL-15 and IL-15Rα to treat a spontaneous mammary carcinoma model [137]. As a result of such a DC vaccination strategy, the onset of mammary tumors was significantly delayed in female mice (including 10% of mice which were free of tumor for the duration of the experiment). Importantly, this strategy was able to induce potent anti-NEU antibodies and the serum from vaccinated mice inhibited tumor growth in third party mice. In this model, $CD4^+$ T cells were not required to induce antitumoral responses by DC transfected with Ad.Neu+Ad.m-IL15+Ad.mIL-15Rα. This finding may be important given the immune status of patients with advanced cancer. In contrast with this study, Chang *et al.* demonstrated that vaccination with a plasmid encoding truncated Her-2/neu elicited immunity against Her-2/neu-expressing cancer cells (human breast carcinoma SK-BR-3 cell line)[138]. This effect was increased by the use of bicistronic plasmids coexpressing Her-2/neu with IL-18 or GM-CSF cytokines. However, in this case the use of pIL-15 did not improve the efficacy of the vaccination scheme.

We have compared the therapeutic effects of IL-12 and IL-15 genes transferred to tumor cells or to DCs, which were subsequently injected into the tumor nodules of mice carrying established colorectal carcinoma [123]. For these experiments, we used viral vectors based on simian virus 40 (rSV40) and observed that infection of CT-26 tumor cells with rSV40 expressing IL-15 (rSVIL-15) failed to inhibit tumor growth. However, the intratumoral administration of syngeneic DCs transduced with rSVIL-15 was associated with a strong antitumor response. This antitumor effect was associated with the *in vivo* priming of tumor-specific CD8+ T lymphocytes and depletion studies showed that rSVIL-15-mediated antitumor efficacy was mainly mediated by CD8+ T lymphocytes and NK cells.

8. GENE THERAPY WITH IL-15 IN EXPERIMENTAL TUMOR MODELS

The IL-15 gene has been successfully used by Kowalczyk *et al.* to construct a mimotope vaccine for a weakly immunogenic antigen in combination with plasmid-derived IL-15 and IL-21 cytokines that was tested for neuroblastoma treatment [139]. Vaccination of mice with s.c. NXS2-derived neuroblastoma tumors using the combined strategy administered 24 h after tumor cell challenge resulted in a potent antitumoral effect (8 out of 8 tumors failed to graft). When a plasmid encoding IL-15 alone plus another plasmid encoding only IL-21 (without the mimotope antigen) were injected under similar experimental conditions, no antitumoral activity was observed. Importantly, the combined strategy has the ability to induce a specific CTL response and protection against NXS2 tumor rechallenge by stimulation of both innate and adaptive cell-mediated immunity. Using a similar combination of plasmids, Kishida and colleagues showed that when these cytokine encoding plasmids were hydrodinamically transduced to the liver, a potent antitumoral effect occurred against established metastatic lymphoma. Indeed, both cytokines exerted a synergistic stimulation of NK cell activity [140].

Among the viral-based vectors employed in gene therapy, rAAV is one of the most promising and has many attractive features including demonstrated safety and efficacy in humans [141]. An AAV vector carrying hIL15 gene was used by Yu and colleagues to maintain long-term gene expression by a single intramuscular injection in mice with experimental breast cancer in a prophylactic model [142]. Animals pretreated with rAAVhIL-15 showed delayed tumor appearance and increased overall survival as a result of tumor cell apoptosis and a potent cytotoxic activity exerted by lymphokine-activated killer cells. No signs of overt toxicity were observed in treated animals. A similar rAAV vector was used by Yiang *et al.* to transfer hIL-15 into mice prior to inoculation with human cervical cancer cells (HeLa cells) [143]. As a result, gene transfer before tumor cell inoculation reduced

xenografts, but not when rAAV-IL-15 injection was performed 24 h after tumor cell challenge.

Another viral vector that has raised expectations for its application against cancer is the replication-defective fowlpox virus (called rfTRICOM because of co-expressing B7-1, ICAM-1, and LFA-3). This vaccine vector has been previously employed in phase I and II clinical trials in patients with carcinoembryonic antigen-expressing carcinomas and prostate cancer [144]. Kudo-Saito *et al.* used rfTRICOM in combination with a replication-defective fowlpox virus expressing IL-15 (rfIL-15) and this resulted in a potent antitumor activity acting in synergy with rfTRICOM to reduce tumor growth in mice with syngenic renal cell carcinomas [145].

Epithelial ovarian cancer remains a disease with a poor prognosis at the stage of peritoneal carcinomatosis. To test the efficacy of Sindbis vectors in the advanced disease, Tseng *et al.* used replication-defective Sindbis vectors including one encoding mIL-15 gene (obtained from the pORF-IL-15 plasmid) in an SCID xenograft model after i.p. inoculation of ES-2 ovarian cancer cells [146]. The virus was injected systemically and it was documented that no Sindbis vector infection of other organs occurs in mice, while cancer cells showed intense transgene expression. Importantly, mice treated with the Sindbis vector expressing mIL-15 showed a clear delay of disease progression.

Hepatocellular carcinoma is one of the most common cancers worldwide (3rd cause of cancer-related deaths) [147] and there is an urgent need for new therapeutic options for advanced disease. For example, Chang *et al.* used a rAAV vector serotype 8 to deliver an IL-15 superagonist (Il-15/IL-15RαS), consisting of IL-15 covalently linked to the N-terminal sushi domain of the IL-15Rα chain. This vector expanded the number of hepatic NK cells and induced potent antitumor activity in a liver metastatic murine HCC model (BNL cells); importantly, NK-cell depletion abrogated the therapeutic effect [148].

Gene transfer to the lungs is still a tough challenge in the field of gene therapy of cancer. One of the most interesting non-viral vectors is based on the cationic polymer polyethylenimine (PEI) given via the airway. Such an approach was shown to be effective in protecting DNA from undesirable degradation during the transduction process. However, PEI has toxicity limitations when its molecular weight increases. To solve this, Zhou *et al.* [149] used heparin to modify PEI, generating nanogels with a plasmid expressing a modified hIL-15 (containing an IL-2 signal peptide inserted upstream of IL-15 gene). This therapeutic tool was tested in lung metastatic models using two different cell lines. A strong inhibition of B16-F10 and CT26 lung metastasis was observed accompanied by increased apoptosis of tumor cells, tumor infiltration by NK cells, significant cytotoxic activity of splenocytes and systemic production of TNF-α and IFN-γ. Also, in a model of CT-26-derived tumor model, a mimovirus composed of a cell-penetrating peptide, a CTL epitope peptide and a plasmid coding for IL-15 showed increased antitumoral effect [150], and orthotopic bladder cancer with MBT-2 cells presented a higher CD8 T cells infiltrate and an increased antitumoral effect if treated with an intravesical liposomal plasmid [151].

Ugen *et al.* used intratumoral injection of an optimized plasmid expressing hIL-15. The optimization meant replacement of the existing Kozak sequence with a stronger Kozak sequence as well as removing upstream inhibitory AUGs. These constructs were used in established s.c. B16F10 melanoma tumors followed by in-vivo electroporation to increase the expression of the transgene, as previously performed by Heller`s group for IL-12 genes [152, 153]. Electroporation of *in vivo* transfected tumors significantly increased antitumoral response to pIL-15 (complete tumor regressions: 63%) in comparison to non-electroporated mice (19%) regressions). Despite these promising results, it should be borne in mind that the usefulness of this technique is limited to accessible superficial tumor nodules.

Oncolytic viruses are a promising tool for gene therapy of cancer because of the extensive preclinical and clinical experience in their use in addition to their safety profile [154]. Although there are important advances in the clinic, especially with conditional replication adenoviruses [155], there is still room for improvement in the field. Vesicular stomatitis virus (VSV) is one of the oncolytic vectors that demonstrated efficacy in experimental tumor models [156]. Stephenson *et al.* showed that a i.v. injection of VSV vector expressing a highly secreted form of optimized hIL-15 (VSV-hIL-15) displayed a potent antitumoral effect in mice with established pulmonary metastases of colorectal carcinoma and almost 50% of mice were cured in comparison to none in control groups [157]. At least in part, the antitumoral effect was based on specific $CD8^+$ T lymphocytes induced after treatment with the VSV-hIL-15 vector. In our laboratory we have constructed a chimera encompassing apolipoprotein A-I-IL-15 and the IL-15Rα sushi domain. The transgenic construct, when hydrodynamically given to the liver, complexes with circulating high-density lipoproteins. Longer pharmacokinetics and sushi-mediated trans-presentation result in strong *in vivo* bioactivity, thereby dramatically increasing NK and CD8 memory T cells (MC Ochoa *et al.* submitted).

9. ANTITUMORAL STRATEGIES BASED IN IL-15 BASED-MODIFIED T CELLS

It has been demonstrated that adoptive tumor immunotherapy using T cells can produce potent anticancer immune responses, including dramatic remissions in patients with metastatic melanoma [158]. The effectiveness of this strategy has been strengthened by the application of re-directed antitumor T cells with chimeric antigen receptors (CARS) [159]. In accordance with this, T cells engineered to express a chimeric receptor targeting the CD19 antigen of B-cell lymphomas have been shown to persist longer in circulation when they co-express IL-15 [160]. These double engineered T cells showed reduced surface expression of the programmed death 1 (PD-1) receptor upon antigen stimulation and exerted increased antitumor effects *in vivo* in a lymphoma animal model. Importantly, these T cells transduced both with a chimeric TCR and the IL15 gene were successfully eliminated upon pharmacological activation of a caspase-9 suicide gene cotransfected for safety reasons. The same group had previously demonstrated in a less complex model that transgenic expression of IL-2 or IL-15 increased the expansion of EBV-CTLs, using a SCID mouse model in

which enhanced antitumor activity against EBV-transformed lymphoblastoid cell lines (LCLs) intravenously administered was observed [161]. Markey *et al.* used CD19-specific human primary T cells transferred to express different γc cytokines and their antitumoral performance against a Raji-GL cell derived tumor in NOD/SCID/$\gamma c^{-/-}$ mice and a clear improvement was substantiated. Seven out of 23 mice that received T lymphocytes expressing IL-15 were cured, in contrast to 3 out of 23 which received cells transferred with a control plasmid [162].

10. COMBINATION THERAPIES INCLUDING IL-15

One of the first combination strategies for tumor therapy encompassing IL-15 was the addition of IL-2. However, it was shown that the addition of IL-2 to splenocyte cultures with target cells in the presence of IL-15 does not increase the cytotoxicity of these cells [163]. This could be due to the loss of one of the advantages of treatment with IL-15, namely that it does not increase Treg and MDSC in the tumor microenvironment, while IL-2 does [164].

IL-15 has also been used as an adjuvant cytokine in vaccination strategies. Lasek *et al.* designed a vaccine against B78-HI melanoma with irradiated tumoral cells engineered to express IL-12 and in addition they administered IL-15 (2μg per dose daily during 6 days) [165]. They obtained an additive therapeutic effect with an impressive result, 100% of tumor-free mice. With this combinatorial treatment they also found higher numbers of CD4 and CD8 T cells in draining lymph nodes accompanied by higher cytotoxicity and IFNγ production. This work was continued by Basak *et al.* [166] and it was shown that the combination of IL-15 with irradiated melanoma-derived cells transfected to produce TNF-α, GM-CSF or IL-6/sIL-6R was synergistically or at least additively effective.

IL-15 has been found to be effective in combination with monoclonal antibodies for cancer therapy. IL-15 enhances PD-1 expression in CD8 T cells and also increases IL-10 production, but when IL-15 is administered with antibodies that block PD-1 and CTLA-4, IL-10 production and PD-1 expression decreases [167]. With such a treatment combination, a longer survival of lung metastases-bearing mice was readily observed. In another setting, IL-15 has been proven to enhance the antibody- mediated cytotoxicity of rituximab against chronic lymphocytic leukemia cells *in vitro* [168]. An agonistic anti-CD40 antibody has also been shown to be more effective in combination with IL-15. In this case, mice with CT-26 and MC38-derived lung metastasis treated with anti-CD40 antibodies and IL-15 survived longer than mice receiving each treatment separately. Moreover, the NK cells of those mice had a higher granzyme B expression and displayed a more robust cytotoxicity [169].

IL-15 antitumoral activity is generally enhanced when administered in combination with chemotherapy. Administration of a dose of 200mg/kg of cyclophosphamide followed by IL-15 daily during 20 days increased survival of mice harbouring lung metastases from 76-9 rhabdomyosar coma [170]. Treated mice had higher amounts of CD8 and NK cells when compared to mice that only received cyclophosphamide. Another possible clinical use of IL-15 could be in combination with 5-fluorouracil. This strategy was explored in a model using rats transplanted with the chemically induced Ward colorectal carcinoma, administration of IL-15 with 5-fluorouracil and leucovorin resulted in a lower tumor growth rate and in milder toxicity [90].

There are also studies focusing on the adjuvant effect of IL-15 in T-cell adoptive transfer immuno-therapy. One of these used a J558Neo cells derived plasmacytoma model that expresses the P1A antigen. In this work, mice were adoptively transferred with CD8 T lymphocytes specific for this antigen. A group of animals then received a daily dose of IL-15 during 180 days resulting in a decreased tumor growth rate and extended animal survival , while 50% of IL-15 treated mice were alive at the end of the experiment all the mice that did not receive IL-15 died before 60 days [171]. IL-15 was shown to maintain tumor-specific T cells for a longer period of time.

As mentioned above, a relatively simple and effective strategy to potentiate IL-15 antitumor profile is its combination with different cytokines. In the first study using this strategy in a malignant pleursy model with syngeneic Meth A fibrobrosarcoma [172], the intrapleural administration of recombinant IL-12 and IL-15 showed a synergistic effect with long term surviving mice. Moreover, T cells in the pleural effusion showed higher IFNγ production capacity. Interestingly, IFNγ blockade with a specific antibody abrogated the observed antitumoral effect. Another immunotherapeutic combinatory approach using IL-15 as a partner cytokine was taken by Habibi *et al.* [173]. In this work, radiofrequency ablation was combined with intratumoral administration of IL-15 and IL-7. The strategy was tested in a 4T1-derived mammary carcinoma model and in another breast cancer model using cells derived from tumors of FVBN202 transgenic spontaneous breast tumors mice. It was reported that the combination of the three agents reduced tumor volume and the density of intratumoral myeloid-derived suppressor cells. There are two studies combining IL-6 with IL-15 published by the group of Chu *et al.* In both the authors used a canine transmissible venereal tumor model that is a kind of tumor that produces a notable output of TGF-β. Treatment consisted of intramuscular electroporation of both cytokines 7 days after the tumoral cell inoculation. In the first study [174], they transferred the tumor to SCID mice and observed a higher antitumoral effect in the group treated with both cytokines than in the groups that received each expression plasmid separately. This antitumoral effect in xenografted mice was again dependent on NK cells. The second study was performed in beagle dogs and showed similar results [175]. Summarized data of combinatorial treatments with IL-15 are shown in (Table **3**).

CONCLUDING REMARKS

As a recombinant protein, IL-15 given systemically is undergoing dose-finding and safety clinical trials (NCT 01369888, NCT01385423, NCT01021059, NCT 01572493). However, strategies based on gene therapy should not be disregarded [176].

Drawbacks of recombinant protein include that bolus administration of IL-15 might result in multiple `peaks and valleys´ pharmacokinetics. This could easily result in toxicity and suboptimal efficacy. Furthermore, in our view,

Table 3. Summary of Main Anti-Tumoral Studies Performed in Combination with IL-15

Combination	Model	Effect of IL-15	References
IL-15 and irradiated B78-HI melanoma cells transfected to elicit cytokines	B78-HI melanoma cell line in footpad	Increased antitumoral effect. Infiltrates with higher percentage of granulocytes, CD3+, CD4+ and CD8+ cells	[162, 163]
IL-15 and vaccines of C6VL tumor lysate-pulsed dendritic cells	C6VL tumors	No anti-tumoral effect	[134]
IL-15 with anti-PD-L1 and anti-CTLA-4 antibodies	CT26 colon carcinoma cell line i.v.	Increased antitumoral effect.	[164]
IL-15 and an agonistic anti-CD40 antibody	CD26 and MC38 i.v.	Increased antitumoral effect by increasing NK cytotoxicity	[166]
Cyclophosphamide and IL-15	Metastatic model of 76-9 murine rhabdo-myosarcoma derived cell line	Antitumoral effect, depending on NK, αβ and γδ T cells and impaired by B cells	[167]
5-Fluorouracil, leucovorin and IL-15	Ward colorectal carcinoma cell line	Increased antitumoral effect and decreased toxicity	[90]
Specific CD8 T cells for P1A antigen and IL-15	Plasmacytoma J558Neo cells expressing the P1A antigen	Increased antitumoral effect.	[168]
IL-15 and IL-12 intrapleural	Intrapleural administered MethA fibrosar-coma	Increased antitumoral effect depending on CD8+ T and NK cells. Higher production of IFNγ in the tumor	[169]
Radiofrequency thermal ablation, IL-7 and IL-15	Breast carcinoma derived from FVBN202 transgenic mouse and 4T1 tumors	Increased antitumoral effect. Higher production of IFNγ in the tumor. Lower percentage of splenic MDSC	[170]
Intramuscular electroporation of pIL-6 and pIL-15	CTVT cell line s.c. in SCID mice	Increased antitumoral effect by inhibition of TGF-β and increased NK cytotoxicity	[171]
Intratumoral electroporation with pIL-6 and pIL-15	Canine transmissible venereal tumor (CTVT) in beagles	Antitumoral effect higher proportion of CD8+ T cells infiltrating the tumor and higher tumor-specific cytotoxicity	[175]

strategies to exploit the trans-presentation biology system may be complex to achieve with recombinant protein products.

The advantages of gene therapy include locoregional production, ability to generate fusion constructs and versatility for combination strategies. The main disadvantages would be unreliable delivery and the intrinsic immunogenicity of the viral vectors.

All in all, the considerable experience gained with gene therapy in rodent models contrasts with the complete absence of clinical trials. Optimized forms of IL-15 immunotherapy ought to be excellent partners in combined strategies for cancer treatment. Gene therapy certainly offers interesting features to exploit IL-15 as a vaccine adjuvant and to sustain NK and T cell-dependent antitumor immunity.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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