IL-15 receptor alpha as the magic wand to boost the success of IL-15 antitumor therapies : the upswing of IL-15transpresentation

IL-15 receptor alpha as the magic wand to boost the success of IL-15 antitumor therapies: the upswing of IL-15 transpresentation

Authors: Johan MJ Van den Bergh*[1], Eva Lion[1,2], Viggo FI Van Tendeloo[1], Evelien LJM Smits[1,2,3]

Affiliations: [1] Laboratory of Experimental Hematology, Vaccine and Infectious Disease Institute (VAXINFECTIO), Faculty of Medicine & Health Sciences, University of Antwerp, Antwerp, Belgium; [2] Center for Cell Therapy and Regenerative Medicine, Antwerp University Hospital, Antwerp, Belgium; [3] Center for Oncological Research Antwerp, Faculty of Medicine & Health Sciences, University of Antwerp, Antwerp, Belgium

***Corresponding author:** Johan MJ Van den Bergh, Laboratory of Experimental Hematology, Antwerp University Hospital (UZA), Wilrijkstraat 10, B-2650 Edegem, Belgium.

Tel.: 003238214112; Fax.: 003238214456. E-mail address: johan.vandenbergh@uantwerpen.be

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Abstract

Interleukin (IL)-15 as a stand-alone therapy can activate the antitumor functions of immune effector cells resulting in significant tumor regression. Interestingly, combining IL-15 with the α-moiety of its receptor (IL-15Rα), also called IL-15 transpresentation, increases the *in vivo* half-life of IL-15 and enhances binding of IL-15 with cells expressing the IL-15Rβγ, such as NK cells and $CD8^+$ T cells. These features enlarge the signal transmission of IL-15, resulting in improved proliferation and antitumor activities of both NK cells and $CD8^+$ T cells, eventually leading to enhanced killing of tumor cells. In this review, we discuss the antitumor strategies in which this IL-15 transpresentation mechanism is implemented, that are currently under preclinical investigation. Furthermore, we give an overview of the studies in which the IL-15/IL-15Rα complexes are combined with other antitumor therapies. The promising results in these preclinical studies have incited several clinical trials to test the safety and efficacy of IL-15 transpresentation strategies to treat both hematological and advanced solid tumors.

Keywords

Interleukin-15, IL-15 receptor alpha, IL-15 transpresentation, antitumor immunotherapies, combination therapies

Abbreviations

ALT-803, IL-15 mutant (whereby the amino acid asparagine at position 72 is substituted by an aspartic acid) + sushi domain IL-15Rα (Figure 1); IL, interleukin; IL-15Rα, interleukin-15 receptor alpha; NK cells, natural killer cells; RLI, IL-15 coupled with a linker to the soluble IL-15Rα sushi domain (Figure 1)

Contents

1. Introduction: Discovery of IL-15 and IL-15Rα; a comparison with IL-2 and its receptor

More than two decades ago, interleukin (IL)-15 was discovered as a T cell growth factor with overlapping biological functions with IL-2 *in vitro* (Grabstein et al., 1994). Although their protein sequences show no similarities, IL-15 can be assigned to the IL-2 family based on the resembling three-dimensional structure, in this case the common four alpha-helical bundle (Grabstein et al., 1994). Due to their structural and biological similarities, the hypothesis that both cytokines bind to common receptor parts to transfer their signal was thoroughly investigated (Giri et al., 1994). It was found that both the beta and gamma chains of the IL-2 receptor (IL-2R) are required for IL-15 binding and signaling (Giri et al., 1994). However, several lines of evidence indicated the existence of an additional, IL-15-specific receptor compartment (Cosman et al., 1995; Giri et al., 1994). In this context, Cosman and colleagues discovered an IL-15 binding chain that, with co-transfection of IL-2Rβ, resulted in IL-15 stimulation of the transfected cells (Anderson et al., 1995; Cosman et al., 1995). Although this IL-15-specific receptor part, hereafter called IL-15Rα, showed to be structurally related to the IL-2 receptor alpha (IL-2Rα), both receptor parts have unique properties. First, IL-15Rα binds IL-15 with a 1000-fold higher affinity as compared to the binding of IL-2Rα and IL-2. Secondly, IL-15Rα is expressed by a wider variety of cells as compared to IL-2Rα, suggesting a broader range of cellular targets for IL-15 (Anderson et al., 1995). Altogether, the above mentioned discoveries about IL-15 and IL-15Rα underscored the need to scrutinize both molecules in order to unravel the signaling mechanisms and the corresponding functions. In this review, we will focus on the engagement of IL-15Rα in IL-15 antitumor therapies, on approaches that mimic IL-15 transpresentation to combat cancer cells and on treatments that combine IL-15/IL-15Rα complexes with other antitumor therapies.

2. IL-15 transpresentation in cancer: gene therapy approaches

Although IL-15 in high concentrations does not require binding to IL-15Rα to transfer its signal towards IL-15Rβ/γ-expressing cells (Polansky et al., 2016), the main signaling mechanism remains IL-15 transpresentation (Stonier & Schluns, 2010). Hereby, IL-15 and IL-15Rα form a heterodimeric complex intracellularly, which is subsequently guided to the cell membrane, where it is bioactive (Bergamaschi et al., 2008; Dubois et al., 2002; Mortier et al., 2008). In this way, cell-cell contact between the IL-15-transpresenting cells and IL-15Rβ/γ-expressing cells can result in activation of the latter. Since both natural killer (NK) cells and $CD8^+$ T cells, as main killer cells of the immune system, express the βγ-moiety of the IL-15 receptor, IL-15 transpresentation has become a valuable signaling mechanism in antitumor immunotherapies. Therefore, researchers have focused on implementing both IL-15 and IL-15Rα DNA or mRNA into dendritic cells (Steel et al., 2010; Van den Bergh et al., 2015a) or tumor cells (Kobayashi et al., 2005; Morris et al., 2014; Rowley et al., 2008) to create a transcellular IL-15-presenting system with the intention to activate IL-15Rβ/γ-expressing cells (Table 1) (Figure 1A). According to these studies, transcellularly-presented IL-15 was able to increase the activation status of both NK cells (Kobayashi et al., 2005; Rowley et al., 2008; Van den Bergh et al., 2015a) and cytotoxic T lymphocytes (CTLs) (Kokaji et al., 2008; Morris et al., 2014; Rowley et al., 2008) in a superior way as compared to soluble IL-15. Importantly in an antitumor setting, the IL-15/IL-15Rα-activated NK cells demonstrated enhanced cytotoxic activity against leukemic cells (Van den Bergh et al., 2015a) and colon cancer cells *in vitro* (Kobayashi et al., 2005). In addition, *in vivo* mice experiments displayed increased percentages of tumor-infiltrating NK cells and $CD8^+$ T cells after treatment with IL-15/IL-15Rα- expressing cells. This increased tumor-infiltration resulted in the inhibition of tumor growth and led to improved survival of challenged

mice as demonstrated in a lung cancer mouse model (TC-1 cells) (Rowley et al., 2008), a breast cancer mouse model (TS/A cells) (Morris et al., 2014) and a prostate cancer mouse model (TRAMP-C2 cells) (Morris et al., 2014). Next to activation of NK cells and CTLs, treatment of tumor-bearing mice (BALB-neuT mice) with IL-15/IL-15Rα-expressing cells can also result in an antigen-specific antibody response, which is superior as compared to cells that only express IL-15. This antibody response is correlated with an increased antitumor effect in terms of decreased tumor development in a mammary carcinoma mouse model (BALB-neu T mice) (Steel et al., 2010). Overall, these results show that the IL-15 transpresentation mechanism is of great value in optimizing dendritic cell (Steel et al., 2010; Van den Bergh et al., 2015a) as well as tumor cell vaccination (Kobayashi et al., 2005; Morris et al., 2014; Rowley et al., 2008).

3. Mimicking IL-15 transpresentation

In addition to transcellular IL-15 presentation, recent studies also focused on soluble IL-15/IL-15Rα complexes as antitumor therapy, because the cytoplasmatic part of IL-15Rα is not necessary for IL-15 transpresentation (Wu et al., 2008). To date, three main fusion proteins are being pursued in the pre-clinical treatment of cancer (Figure 1B, C and D) (Muller, 2012; Wu, 2013). The first is a fusion of IL-15 and the soluble IL-15Rα-Fc subunit, further called IL-15/IL-15Rα-Fc complex (Chertova et al., 2013; Rubinstein et al., 2006; Stoklasek et al., 2006). The second complex is known as RLI, which consists of IL-15 coupled with a linker to the soluble IL-15Rα sushi domain which was identified to have the most binding affinity for IL-15 (Mortier et al., 2006). Thirdly, pre-association of an IL-15 mutant (whereby the amino acid asparagine at position 72 is substituted by an aspartic acid) with the IL-15Rα sushi-Fc fusion complex is called ALT-803 (Zhu et al., 2009). All three IL-15 fusion proteins displayed improved

in vivo pharmacokinetics and a higher potency to activate immune cells as compared to soluble IL-15 (Chertova et al., 2013; Han et al., 2011; Mortier et al., 2006) (Table 2).

For the IL-15/IL-15Rα-Fc complex, enhanced proliferation and cytotoxicity of both NK cells and $CD8^+$ T cells *in vitro* and *in vivo* were observed as compared with the use of IL-15 alone (Chertova et al., 2013; Dubois et al., 2008; Sun & Liu, 2016; Wu & Xu, 2010). This enhanced cytotoxic profile of immune cells resulted in the reduction of tumor growth and increase in overall survival of melanoma- (Dubois et al., 2008), pancreatic cancer- (Epardaud et al., 2008) and Lewis lung cancer-bearing mice (Sun & Liu, 2016), which was seen after intraperitoneal (Chertova et al., 2013; Dubois et al., 2008), intravenous (Epardaud et al., 2008) and hydronamic tail vein (Sun & Liu, 2016) injection of the complex, respectively. Interestingly, despite a marked expansion of $CD8^+$ T cells in lymphoid organs and peripheral blood following treatment with IL-15/IL-15Rα-Fc complexes, the destruction of solid tumors was orchestrated by tumor-resident rather than newly infiltrating $CD8^+$ T cells (Epardaud et al., 2008).

As compared with the IL-15/IL-15Rα-Fc complexes that are described above, similar effects on proliferation of IL-15Rβ/γ-expressing cells were observed with RLI in *in vitro* (Mortier et al., 2006) and *in vivo* (Chang et al., 2010; Cheng et al., 2014) studies. In the *in vivo* studies, RLI treatment was shown to be an effective therapy against well-established metastatic and autochthonous liver cancers in mice. Although one study devoted the antitumor effects to increased levels of hepatic $CD8^+$ T cells rather than the presence of NK cells (Cheng et al., 2014), another study showed exactly the opposite (Chang et al., 2010). In the latter, it was demonstrated that depletion of NK cells abrogated the therapeutic effect, concluding that these effector cells also have their engagement in the eradication of the tumor cells.

The third fusion protein ALT-803, comparable to the other two fusion proteins in terms of function, was able to induce long-term survival and durable antitumor immune responses as evidenced in a multiple myeloma (Xu et al., 2013), glioblastoma (Mathios et al., 2016), melanoma (Rhode et al., 2016) and colon carcinoma mouse model (Kim et al., 2016; Rhode et al., 2016). Furthermore, ALT-803 promoted the conversion of memory $CD8^+$ T cells into innate-like effector cells with antitumor activity via an antigen-independent mechanism, which could be beneficial to combat tumor cells (Kim et al., 2016; Wong et al., 2013; Xu et al., 2013). Altogether, pre-clinical studies have proven that all three fusion proteins possess superior immunotherapeutic antitumor properties as compared to soluble IL-15, favoring clinical development of these molecules to treat various cancers. Although there is no evidence to suggest that one of the three IL-15 fusion proteins is better for cancer treatment, only ALT-803 is currently being investigated in clinical studies (vide infra).

4. Combinatorial approaches with IL-15/IL-15Rα complexes

Although IL-15/IL-15Rα complexes have proven to have superior antitumor activity as compared to IL-15 alone, combinatorial approaches together with these IL-15 superagonists could even enhance the antitumor effect (Van den Bergh et al., 2015b). In this context, combinations that could (i) eradicate tumor cells directly, (ii) further enhance the antitumor properties of immune cells, (iii) deliver IL-15/IL-15Rα-complexes via linked antibodies directly to the tumor site or (iv) block inhibitory checkpoints, in addition to the already described effects of IL-15/IL-15Rα complexes (vide supra), have been examined (Table 3).

In the first category, combining IL-15/IL-15Rα complexes with either chemotherapy (Sun & Liu, 2016), stereotactic radiosurgery (Mathios et al., 2016) or oncolytic viruses (Gaston et al., 2013;

Tosic et al., 2014) resulted in increased tumor cell killing as compared to one of the treatments alone, shown in both *in vitro* and *in vivo* cancer models, such as brain tumors (Gaston et al., 2013; Mathios et al., 2016), melanoma (Tosic et al., 2014) and lung cancer (Sun & Liu, 2016).

In the second category, multiple treatments that can enhance the antitumor properties of NK cells and/or $CD8^+$ T cells to treat various tumors have been combined with the IL-15/IL-15Rα therapy. One of these treatment combinations is the use of a fusion protein achieved by fusing IL-15, IL-15Rα and 4-1BBL to treat metastatic lung tumors (Kermer et al., 2014). The trifunctional antibody fusion protein resulted in enhanced interferon (IFN)-γ production of $CD8^+$ T cells, $CD8^+$ T cell proliferation and reduction in metastases. Other studies in this category combine the use of IL-15/IL-15Rα complexes with anti-CD20 antibodies to treat indolent B-cell non-Hodgkin lymphoma (Rosario et al., 2016) or an intravesicular Bacillus Calmette-Guérin treatment to cure bladder cancer (Gomes-Giacoia et al., 2014). In both studies, the combination regimen led to tumor reduction as a result of enhanced cytotoxic activation of NK cells (Rosario et al., 2016) and $CD8^+$ T cells (Gomes-Giacoia et al., 2014).

The third category contains the linkage of IL-15/IL-15Rα complexes to molecules that can target tumors to increase the antitumor effects of IL-15 transpresentation at the tumor site (Stone et al., 2012). In this perspective, a fusion of IL-15/IL-15Rα and apolipoprotein A-I (Apo A-I) to treat metastatic liver cancer (Ochoa et al., 2013) was constructed. The combination treatment resulted in the delivery of the fusion protein to the liver, followed by the infiltration of both cytotoxic T cells and NK cells, resulting in eradication of metastatic tumors. In another study, IL-15/IL-15Rα complexes were fused with an anti-GD2 ganglioside antibody (Vincent et al., 2013a; Vincent et al., 2013b). The anti-GD2-IL-15/IL-15Rα fusion retained the cytokine potential of IL-15/IL-15Rα and the antibody effector functions, which are antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity. Interestingly, the fusion protein displayed

higher tumor cell killing and increased survival of tumor-bearing mice as shown in both a T cell lymphoma and neuroblastoma mouse model, as compared with the treatments alone or even in a non-fused combination.

Lastly, a couple of studies used the combination of ALT-803 with immune checkpoint inhibitors, such as a blocking antibodies against programmed death protein (PD)-1 or its ligand PD-L1 (Kim et al., 2016; Mathios et al., 2016) and cytotoxic T-lymphocyte antigen 4 (CTLA-4) (Kim et al., 2016). The combination treatment displayed an additive survival effect of glioblastoma- or colon carcinoma-bearing mice, respectively, as compared with the treatments alone. In summary, IL-15/IL-15Rα combination treatments have proven to be superior as compared to one of the treatments alone in preclinical antitumor studies, so it would be very interesting to further explore these combinations in a clinical setting as well. The preferred combination might be highly dependent on the tumor type and associated tumor microenvironment, which stresses the importance of further research.

5. Clinical developments

The promising preclinical results of IL-15/IL-15Rα complexes to increase the antitumor immune system, have led to the implementation of this therapy in several registered clinical studies. At the moment, all eight clinical studies implemented the use of ALT-803 alone or in combination with other antitumor therapies to treat a range of both hematological and solid tumors, such as melanoma, renal cell cancer, pancreatic cancer, lung cancer, bladder cancer and indolent B cell non-Hodgkin lymphoma (Table 4). The primary goal of these studies is to evaluate the safety and determine both the maximum tolerated dose and the minimum effective dose. As secondary outcome measures, the immunogenicity and pharmacokinetic profile of ALT-803 will be characterized in treated patients, as well as progression-free survival and duration of the response.

Since none of the current clinical studies where IL-15/IL-15Rα complexes are used as biological agents is completed at this moment, no results on the safety and efficacy of this treatment have been published so far. In the meanwhile, we envisage that more antitumor therapies will be tested in a clinical setting and, therefore, more combinations with IL-15/IL-15Rα complexes will be subject to investigate their antitumor effect in all kind of tumors.

6. Discussion

Although IL-15 has become one of the most promising molecules for antitumor immunotherapy (Cheever, 2008), the cytokine as monotherapy may not be optimal to treat cancer patients due to several reasons, such as a short half-life *in vivo* and a low binding affinity with the IL-15 βγ-receptor to transfer its signal. However, combining IL-15 with IL-15Rα or methods that can increase the IL-15Rα-expression on cells (Zhang et al., 2012), can dramatically increase the *in vivo* bioactivity of IL-15 and subsequently results in superior expansion and antitumor activity of both NK cells and $CD8^+$ T cells as compared to IL-15 alone (Chertova et al., 2013; Huntington et al., 2009; Mortier et al., 2008). For these reasons, researchers have investigated several ways to present IL-15 to IL-15Rβγ-expressing cells to enhance the therapeutic effect of this antitumor immunotherapy. In this review, we focused on IL-15 transpresentation, whereby IL-15 is presented to neighboring cells in contrast to cis-presentation of this cytokine, whereby it is presented to the same cell that produces the IL-15. Although transpresentation is the main mechanism of IL-15 signaling (Stonier & Schluns, 2010), it is noteworthy to mention that IL-15 cis-presentation is also capable of increasing the proliferation and cytotoxic capacity of $CD8^+$ T cells, but not NK cells, *in vivo* (Delconte et al., 2016; Ota et al., 2010; Rowley et al., 2009).

In the context of IL-15 transpresentation, two different subgroups can be made, which are the soluble and the membrane-bound IL-15/IL-15Rα complexes. Both groups differ in the mechanism that is used to pass the IL-15 signal, resulting in different modes of actions. Soluble complexes activate only those cells expressing low-affinity IL-15Rβγ, while membrane-bound complexes activate high-affinity IL-15Rβγ-expressing cells (Giron-Michel et al., 2005). This can be an explanation why membrane-bound IL-15/IL-15Rα complexes are able to activate NK cells, and soluble complexes are not (Mortier et al., 2008). To circumvent this problem, Fc-molecules were linked to the soluble IL-15/IL-15Rα complexes, making them suitable to attach on Fc-receptors on the membrane of cells. Next to the membrane-bound state of IL-15/IL-15Rα complexes, the configuration (which is the mode of cytokine presentation) of these complexes on the cell surface is of crucial importance to induce optimal immune responses (Hong et al., 2016). In this context, Hong and colleagues describe that transpresentation of IL-15/IL-15Rα in a multivalent fashion on the surface of nanoparticle-treated dendritic cells stimulates antigen-specific $CD8^+$ T cells, resulting in significantly delayed tumor progression as compared to monovalent IL-15/IL-15Rα complexes (Hong et al., 2016). As shown by these reports, the design of the IL-15/IL-15Rα strategy is of crucial importance to obtain the most optimal antitumor immune responses.

In most studies where the IL-15 transpresentation mechanism is tested for its antitumor effects, both NK cells and $CD8^+$ T cells are described as important mediators of the therapeutic effect of the IL-15/IL-15Rα complex antitumor therapy. Nevertheless, some studies show that mainly NK cells (Chang et al., 2010; Gomes-Giacoia et al., 2014) or $CD8^+$ T cells (Mathios et al., 2016; Xu et al., 2013) are involved in the eradication of tumor cells and not the other subset by depleting one of these two immune cell subsets. A possible explanation for this phenomenon can be that

chronic exposure of IL-15/IL-15Rα complexes can lead to accumulation of NK cells with an exhausted phenotype and impaired effector functions (Elpek et al., 2010). In this case, NK cells can still infiltrate the tumor site, but are unable to kill the tumor cells anymore. These studies show us the importance of the dose and administration scheme of IL-15 transpresentation strategies to activate both NK cells and $CD8^+$ T cells in order to kill tumor cells. In addition, different tumor models and tumor types were used to test the antitumor effect of IL-15/IL-15Rα in the aforementioned studies. The different biology of each tumor, the various interactions of immune cells with tumor cells and the tumor (suppressor) microenvironment of each tumor type and tumor model might explain why some immune cells are more dominantly involved in some studies as compared to others, even when the same IL-15/IL-15Rα therapy is used (Gomes-Giacoia et al., 2014; Mathios et al., 2016; Xu et al., 2013).

In addition to selective expansion of NK cells and $CD8^+$ T cells, the use of IL-15 in combination with IL-15Rα can result in some other adverse events, such as hypothermia, weight loss, liver injury and mortality (Guo et al., 2015). These adverse consequences are mainly mediated by hyperproliferation of NK cells and the production of the pro-inflammatory cytokine IFN-γ. Once again, since these effects are dose- and time-dependent, it will be very important to administer the correct dose of IL-15/IL-15Rα complexes to cancer patients and optimize the time-point of administration(s) to gain optimal antitumor efficacy of the complexes with a minimum of adverse events.

Next to the antitumor properties and adverse consequences of IL-15/IL-15Rα complexes, two studies describe the pro-tumoral role of soluble IL-15Rα in cancer (Badoual et al., 2008; Khawam et al., 2009). In the first study, increased serum soluble IL-15Rα concentrations in head-

and-neck cancer patients were correlated with poor clinical outcome. Here, soluble IL-15Rα was mainly produced by tumor cells via proteolytic cleavage of IL-15Rα and functioned as an enhancer of IL-15-induced pro-inflammatory cytokines that may promote tumor progression (Badoual et al., 2008). In a second human *ex vivo* study, as seen in renal cancer, stimulation of membrane-bound IL-15 by soluble IL-15Rα results in epithelial-to-mesenchymal transition, a crucial process in tumor progression (Khawam et al., 2009). These studies show us that IL-15/IL-15Rα can also be involved in tumor evasion mechanisms, pointing to a potential dual face of these complexes in cancer.

In conclusion, IL-15/IL-15Rα complexes have shown to be superior in activating the antitumor functions of both NK cells and $CD8^+$ T cells as compared to IL-15 alone. IL-15/IL-15Rα strategies have proven their efficacy in *in vivo* mice experiments and now various immunotherapy schemes and doses are being investigated in clinical trials to test the safety and efficacy of these complexes. To further improve their antitumor effects, IL-15/IL-15Rα complexes can be combined with other antitumor therapies.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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7. Figure and figure legend

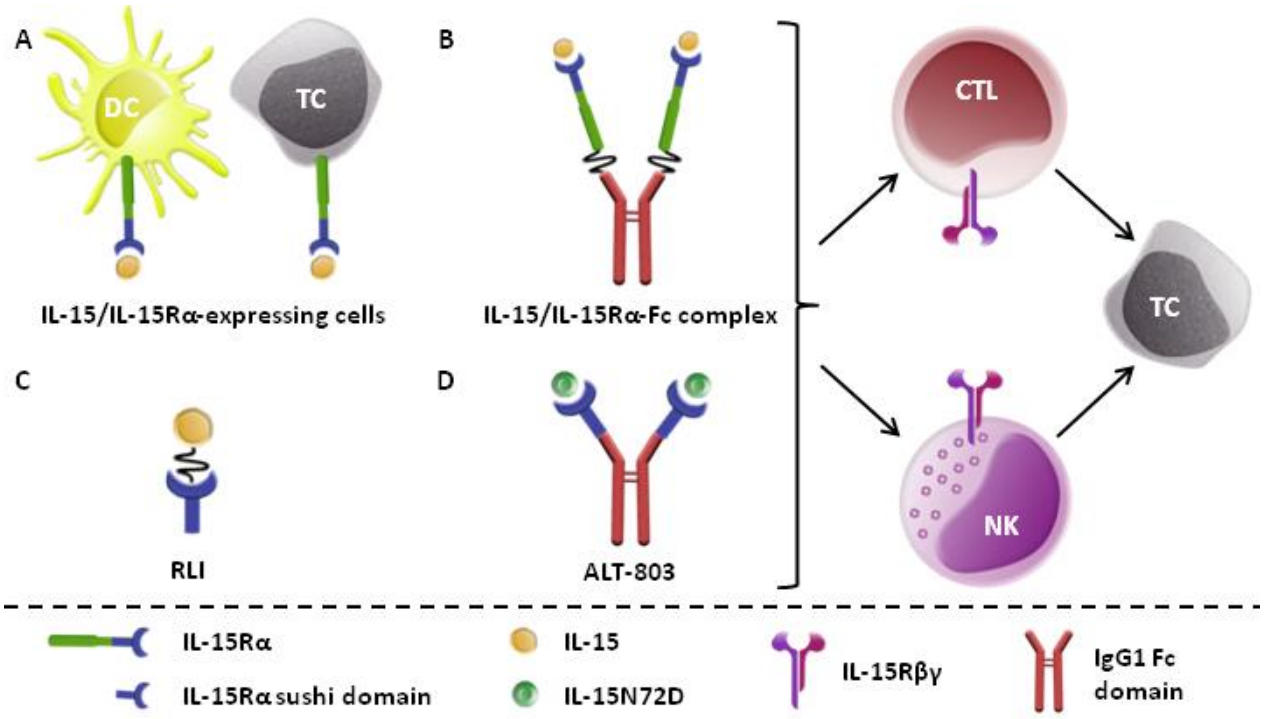

Figure 1. IL-15 transpresentation mechanisms used in antitumor immunotherapies. IL-15-transpresenting dendritic cells or tumor cells (**A**), IL-15/IL-15Rα-Fc complex (**B**), RLI (**C**) and ALT-803 (**D**) are used in both *in vitro* and *in vivo* studies to enhance the amount and antitumor activity of IL-15Rβγ-expressing cells, such as NK cells and CTLs, to eradicate tumor cells. **Abbreviations:** CTL, Cytotoxic T Lymphocyte; DC, Dendritic Cell; Fc, Fragment crystallizable region; IL, Interleukin; IL-15R, interleukin-15 Receptor; NK, Natural Killer cell; TC, Tumor Cell.