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Use of enhanced interleukin-2 formulations for improved immunotherapy against cancer

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Abstract

The use of interleukin-2 (IL-2) for the stimulation of an effector immune response against metastatic cancer dates back to the early 1980s. Administration of unmodified IL-2, either alone or together with antigen-specific approaches, has resulted in remarkably long-term survival of some patients suffering from metastatic melanoma. However, such treatment is usually hampered by the appearance of toxic adverse effects, which has motivated the engineering of modified IL-2 formulations showing reduced toxicity while being more potent at stimulating anti-tumor effector immune cells. In this review we summarize and discuss the features and biological relevance of several enhanced IL-2 formulations, compare these to IL-15-based therapeutics, and try to foreshadow their potential in immunological research and immunotherapy.

Introduction

Interleukin-2 (IL-2) is a small 15.5 kDa four α-helical bundle cytokine, which plays crucial roles both during the resting and activated states of the immune system [1]. The main function of IL-2 during steady-state conditions appears to lie in the development and survival of $CD4^+$ forkhead box p3 $(Foxp3)^+$ regulatory T-cells (Tregs), thus governing peripheral immune tolerance [2]. Conversely, during an immune response, IL-2 acts as a 'growth factor' of effector immune cells by supporting the proliferation and expansion of effector and memory T-cells and natural killer (NK) cells, and also enhances effector functions of these cells [1-3].

IL-2 can bind to three different IL-2 receptors (IL-2R): IL-2Rα (CD25) alone, a heterodimer of IL-2Rβ (CD122) and IL-2Rγ (also referred to as common γ-chain receptor, γc), and a heterotrimer of CD25, CD122 and γc, which are called the low-, intermediate-, and high-affinity IL-2R, respectively (Figure 1A). CD122 and γc are crucial for signaling upon IL-2 binding, whereas CD25 does not appear to signal but increases receptor affinity [3,4].

While γc expression remains rather stable, CD25 is up-regulated on recently activated T-cells, along with a moderate elevation of CD122, thereby leading to increased sensitivity of these cells to autocrine and paracrine IL-2. On resting immune cells, CD25 is mainly confined to Tregs, which also express the other IL-2R subunits, thus showing high sensitivity to IL-2. Recently antigen-stimulated T-cells express CD25 mirroring their high dependency on IL-2 during the expansion phase [3,5]. Interestingly, during later stages of an immune response, antigen-experienced (memory) $CD8^+$ T-cells and NK cells express very high levels of CD122 (higher than on Tregs) along with γc and are thus able to compete with Tregs for IL-2 [6,7].

IL-2 is a secreted primarily by activated $CD4^+$ T-cells, but also $CD8^+$ T-cells, NK and NKT-cells, DCs, and mast cells are able to produce IL-2 following activation [1,2,8].

Owing to its potent T-cell growth-stimulating properties, IL-2 administration has been successfully used since the early 1980s as a cancer immunotherapy [9-11]. There, IL-2 proved beneficial in patients with end-stage metastatic melanoma or renal cell carcinoma (Figure 2), especially when given as high-dose (HD) IL-2 consisting of 600'000-720'000 international units per kg body weight per infusion, administered every 8 hours for a maximum of 14 doses [9,11]. However, the widespread use of IL-2 is hampered by dose-dependent adverse effects, such as hypotension, pulmonary edema, liver cell damage and renal failure. Pathophysiologically, these phenomena appear to result from increased vascular permeability leading to vascular leak syndrome (VLS) [12,13].

Another complicating factor is that IL-2 has a short half-life of 20-30 minutes in the blood *in vivo*, as it is rapidly cleared by the renal system upon intravenous (iv) injection [12,14,15]. Moreover, HD IL-2 expands Tregs, which can suppress tumor-specific effector $CD8^+$ T-cell responses [16,17].

Several strategies have been employed to optimize the efficacy of IL-2 for tumor immunotherapy. These include the use of particular anti-IL-2 monoclonal antibodies (mAb), which bind recombinant or endogenous IL-2 and form so-called IL-2/anti-IL-2 mAb complexes (IL-2-cx), thereby preferentially directing IL-2 to immune cells expressing high levels of CD122. Other strategies encompass the introduction of specific mutations to IL-2 (IL-2 muteins) to favor binding to either CD25 or CD122, i.e. dimeric or trimeric IL-2Rs, thus preferentially stimulating certain immune cell subsets. Alternatively, IL-2 has been linked to mAbs specific for tumor bed or tumor vasculature thereby generating so-called IL-2 fusion proteins (IL-2-FP) (Table 1).

In this review, we discuss recent developments in IL-2 tumor immunotherapy by focusing on IL-2-based compounds with improved *in vivo* properties, and compare these strategies to alternative therapeutics targeting IL-2R subunits, such as IL-15-based approaches.

IL-2/anti-IL-2 mAb complexes

IL-2-cx are generated by the association of murine or human IL-2 with specific mAbs (such as clone S4B6 for murine IL-2), whereby the mAb directs IL-2 to cells expressing high levels of CD122 (in addition to γc), including memory $CD8^+$ T-cells and NK cells (Figure 1B) [6]. Use of murine or human IL-2-cx in one or more treatment cycles consisting of 3-7 daily injections induced robust expansion of $CD8^+$ T-cells by 20-100 fold and of NK cells by 20-30 fold *in vivo* [6,18], which translated into significant anti-tumor immune responses and inhibition of tumor growth in several mouse models [15,18,19]. IL-2-cx immunotherapy show several advantages over HD IL-2, such as prolonged *in vivo* half-life, preferential stimulation of effector T-cells over Tregs, and reduction of IL-2-related toxic adverse effects (Table 1) [6,18,20]. While the process of complexing IL-2 with a specific anti-IL-2 mAb is rather simple, the complete dissociation of IL-2 from the mAb can be avoided by introducing a flexible linker between IL-2 and the mAb [21].

With the production of humanized or fully human anti-human IL-2 mAbs and the generation of single-molecule IL-2-cx, this technology might soon become available for testing in the clinic.

IL-2 muteins

Other strategies have focused on mutated forms of IL-2 (IL-2 muteins). While some IL-2 muteins were designed to target dimeric IL-2Rs, hence leading to the preferential stimulation of memory $CD8^+$ T-cells and NK cells, other IL-2 muteins favor cells expressing high levels of CD25, such as recently-activated T-cells, but also Tregs, the latter of which was less obvious when these molecules were engineered (Table 1).

The mutein 'IL-2 superkine' was generated by several amino acid substitutions between positions 80 and 92 of IL-2 [19]. Notably, a change of leucine for valine at position 85 (L85V) resulted in a 5.7-fold increase of affinity for CD122, whereas a set of four additional mutations (L80F, R81D, I86V and I92F) led to an additional 35-fold increase, resulting in an overall increase of affinity for CD122 of about 200-fold over wild-type IL-2. IL-2 superkine was shown to bind IL-2Rs with high affinity in a CD25-independent manner. Compared to wild-type IL-2, IL-2 superkine exerted superior anti-tumor properties in different murine tumor models, being comparable to CD122-targeting IL-2-cx when using a brief treatment course of five daily injections. However, compared to IL-2-cx, the anti-tumor efficacy of IL-2 superkine was weaker and could not be significantly improved by repetitive treatment cycles or formulation of an IL-2 superkine-Fc construct with longer *in vivo* activity (unpublished data). As for toxic adverse effects, IL-2 superkine showed, similar to IL-2-cx, reduced IL-2-related pulmonary edema and liver cell damage.

With the aim of reducing the affinity of IL-2 to CD25, another IL-2 mutein (termed 'no-α mutein') was generated by introducing an alanine at positions R38, F42, Y45, and E62, resulting in decreased affinity for CD25 while maintaining normal binding with IL-2R$\beta\gamma$ [22]. No-α mutein inhibited, in an NK-cell dependent manner, the metastasis of the B16 melanoma-variant MB16F0 and of 3LL-D122 Lewis lung

carcinoma in mice, while exerting lower toxic adverse effects compared to wild-type IL-2. The latter aspect was also confirmed by us using the F42A mutant of IL-2 that displays lower affinity to CD25, which was better tolerated in terms of pulmonary edema than wild-type IL-2 ([19], and unpublished data).

Another research group formulated various IL-2 muteins displaying higher affinity to CD25. *In vitro*, these IL-2 muteins showed superior capacity to stimulate CD25-expressing KIT-225 cells [23]. *In vivo* toxicity of these IL-2 muteins was not assessed, however, based on the dependency of IL-2-mediated adverse effects on CD25 [18], we expect to observe similar, or maybe increased, toxicity with CD25-directed IL-2 muteins compared to IL-2. Moreover, administration of CD25-directed IL-2 muteins will likely result in significant expansion of Tregs, as is the case with other CD25-directed IL-2 formulations in mice [6,18] and with wild-type IL-2 in humans [24]. Notably, BAY 50-4798 is a CD25-directed human IL-2 mutein, containing the amino acid substitution N88R, thereby disfavoring binding to CD122 by about 225,000-fold [25]. The rationale for generating BAY 50-4798 was to reduce IL-2 binding to NK cells, thereby decreasing NK-cell-derived pro-inflammatory cytokines contributing to VLS [26]. However, the clinical response rate following BAY 50-4798 was low: only two out of 45 patients (4%; one renal cell carcinoma and one metastatic melanoma patient) receiving BAY 50-4798 experienced a partial response. Administration of BAY 50-4798 to patients increased counts of $CD4^+CD25^+$ T-cells, including Tregs, while $CD8^+$ T-cell and NK-cell counts changed only minimally [27].

These results show that administration of CD25-directed IL-2 formulations leads to preferential stimulation of $CD25^+$ Tregs. However, CD25-directed IL-2 formulations might be suitable when combined with antigen-specific cancer vaccines by boosting the expansion of recently-primed - and thus $CD25^+$ [3,5] - tumor antigen-

specific T-cells [15]. Yet, this approach might program T-cells to become short-lived effectors rather than memory cells, the latter of which receive lower intensity IL-2 signals during their expansion phase and depend on CD122-mediated survival signals during memory [1].

Comparing IL-2 muteins to IL-2-cx and wild-type IL-2, IL-2 muteins show similar pharmacokinetic properties as wild-type IL-2, such as a short *in vivo* half-life [14], and will thus require frequent administration to maintain therapeutic levels (Table 2). IL-2-cx do not possess this disadvantage because of their prolonged half-life [20]. Moreover, IL-2 muteins contain a potentially immunogenic neoepitope, targeting of which might lead to abrogation of its biological activity. In line with this, 27% of patients developed BAY 50-4798-specific antibodies already after one round of treatment [27]. Conversely, conventional IL-2-cx consist of two components able to fully dissociate from each other, unless they are associated by a covalent linker [21].

IL-2 fusion proteins

Various IL-2-FPs (also termed IL-2 immunocytokines) have been generated in the past twenty years (reviewed in [28]). IL-2-FPs were devised to deliver the cytokine to the tumor microenvironment thereby increasing the local dose of IL-2 to levels sufficient to stimulate tumor-specific immune cells, while keeping systemic IL-2 levels and toxic adverse effects low. To this end, IL-2 has been fused to different antibodies or antibody fragments targeting tumor-associated antigens [28].

Selectikine is a fully humanized IL-2-FP developed for the treatment of solid tumors and B-cell non-Hodgkin lymphoma [29-31]. Selectikine comprises mAb NHS76 recognizing single or double-stranded DNA (often released from dying tumor cells) and a CD25-directed IL-2 mutein with a D20T mutation aimed at disrupting a three-amino-acid-sequence motif in IL-2, which appears to be responsible for IL-2-

binding to endothelial cells, thereby causing endothelial cell damage and VLS [32]. Compared to IL-2, Selectikine induced only mild (grade 1) hypotension and VLS suggesting improved tolerability [31]. Selectikine monotherapy stabilized disease for more than 6 weeks in 23% (9/39) and combined with low-dose cyclophosphamide in 33% (3/9) of patients with metastatic or local advanced tumors (mostly carcinomas) refractory to standard treatments [30]. Despite the transient increase of total leukocyte, lymphocyte and monocyte counts after each infusion, treatment with Selectikine failed to expand tumor-specific $CD8^+$ T-cells or promote their effector functions. Notably, circulating Tregs were massively expanded upon Selectikine, however, no correlation existed between blood Treg numbers and overall survival, although intratumoral Tregs were not assessed.

Two other IL-2-FPs (termed GA504 and GA501) consist of an IL-2 mutein with abolished binding to CD25 fused to humanized mAbs targeting carcinoembryonic antigen (CEA). GAS504, given three times weekly, has been reported to strongly expand and activate NK cells, $CD8^+$ T-cells and $\gamma\delta$ T-cells and lead to favorable ratios of $CD8^+$ to $CD4^+$ T-cells in blood, lymphoid organs and the tumor site. GA504 and GA501 were more efficient in controlling tumor growth of the syngeneic MC38-CEA and PancO2-CEA murine tumors compared to non-targeted IL-2-FP or wild-type IL-2 [33,34].

Another IL-2-FP undergoing clinical testing is hu14.18–IL-2. This immunocytokine is made of wild-type human IL-2 linked to each IgG heavy chain of the hu14.18 mAb, which recognizes disialoganglioside on tumors of neuroectodermal origin, such as neuroblastoma and melanoma [35]. In a phase II clinical trial, fourteen patients received at least two treatment cycles of hu14.18–IL-2, resulting in one partial response (7.1%) [36]. Subsequently, intratumoral administration of hu14.18–IL-2, instead of iv, was tested in the murine NXS2 neuroblastoma model, leading to

increased intratumoral infiltration of NK cells and $CD8^+$ T-cells and improved anti-tumor effects [37]. However, intratumoral administration might limit the application of hu14.18–IL-2 to patients with solid tumors accessible for injection.

Interestingly, intratumoral administration was shown to induce also systemic immune responses as demonstrated recently using L19–IL2, an IL-2-FP binding the alternatively spliced extra-domain B of fibronectin [38]. Twenty-five patients received once weekly ten million IL-2 international-unit equivalents of L19–IL2 with all lesions visible at screening being injected. Treatment resulted in an objective responses rate of 53.9% with 6 patients (25%) experiencing a complete disappearance of all treated lesions. Administration of L19–IL2 led to a transient increase of peripheral Tregs and NK cells, while lowering the numbers of myeloid-derived suppressor cells in blood. Toxic adverse effects were comparable to wild-type IL-2.

Another example of the potent therapeutic effects induced by targeting IL-2 to blood vessels was shown by administration of F16-IL2 and cytarabine to a patient with disseminated extramedullary acute myeloid leukemia [39].

IL-15-based immunotherapeutics

IL-15 shares many similarities to IL-2 as it also binds to CD122 and γc. However, instead of CD25, IL-15 interacts with high affinity ($Kd \approx 10^{-11}$ M) with its private α-chain, termed IL-15Rα or CD215. IL-15 signaling is facilitated via *cis* or *trans*-presentation of IL-15 by IL-15Rα [40]. Unlike IL-2, IL-15 minimally stimulates $CD25^+$ Tregs and causes less CD25-mediated toxicity, while efficiently activating $CD8^+$ T-cells and NK cells [7]. We assessed IL-15 and several IL-15-based formulations with extended *in vivo* half-lives in comparison to IL-2 and IL-2-cx. IL-15-based formulations included complexes of IL-15 with recombinant IL-15Rα linked to Fc (IL-

15/IL-15Rα-Fc), IL-15/anti-IL-15 mAb complexes, and IL-15 coupled covalently via a linker to IL-15Rα (termed RLI, [41]). IL-2-cx conferred slightly better tumor control against subcutaneous B16F10 melanoma than the IL-15-based formulations, and both groups of formulations largely surpassed IL-2 and IL-15 for anti-tumor efficacy. This effect was paralleled by $CD8^+$ T-cell expansion. Interestingly, modest Treg expansion occurred following IL-2-cx but also IL-15 formulations, with the latter also appearing to affect myeloid-derived cell subsets (unpublished data). In line with our observations, the potential for clinical application of IL-15 formulations for cancer immunotherapy has been shown in B16F10 melanoma and pancreatic cancer in RIP1-Tag2 mice [42,43]. Significant reduction in tumor burden and increased survival of mice treated with IL-15–IL-15Rα-FP was observed, while treatment with IL-15 showed a negligible effect in these settings.

Final comments and conclusions

IL-2 cancer immunotherapy is experiencing a revival thanks to the advent of several IL-2 formulations, especially CD122-directed and tumor-targeting compounds, with superior immune stimulatory properties and robust anti-tumor efficacy in pre-clinical and early clinical situations compared to wild-type IL-2. The majority of these novel IL-2 formulations display significantly reduced toxic adverse effects in comparison to IL-2. While improved IL-2 formulations might be useful as monotherapies, their combination with other anti-cancer immunotherapies, such as adoptive T-cell transfer regimens, antigen-specific vaccination, and blockade of inhibitory molecules, e.g. cytotoxic T-lymphocyte antigen-4 and programmed cell death-1, to expand and maintain efficacious tumor-specific T-cell responses, might hold the promise of controlling metastatic cancer.

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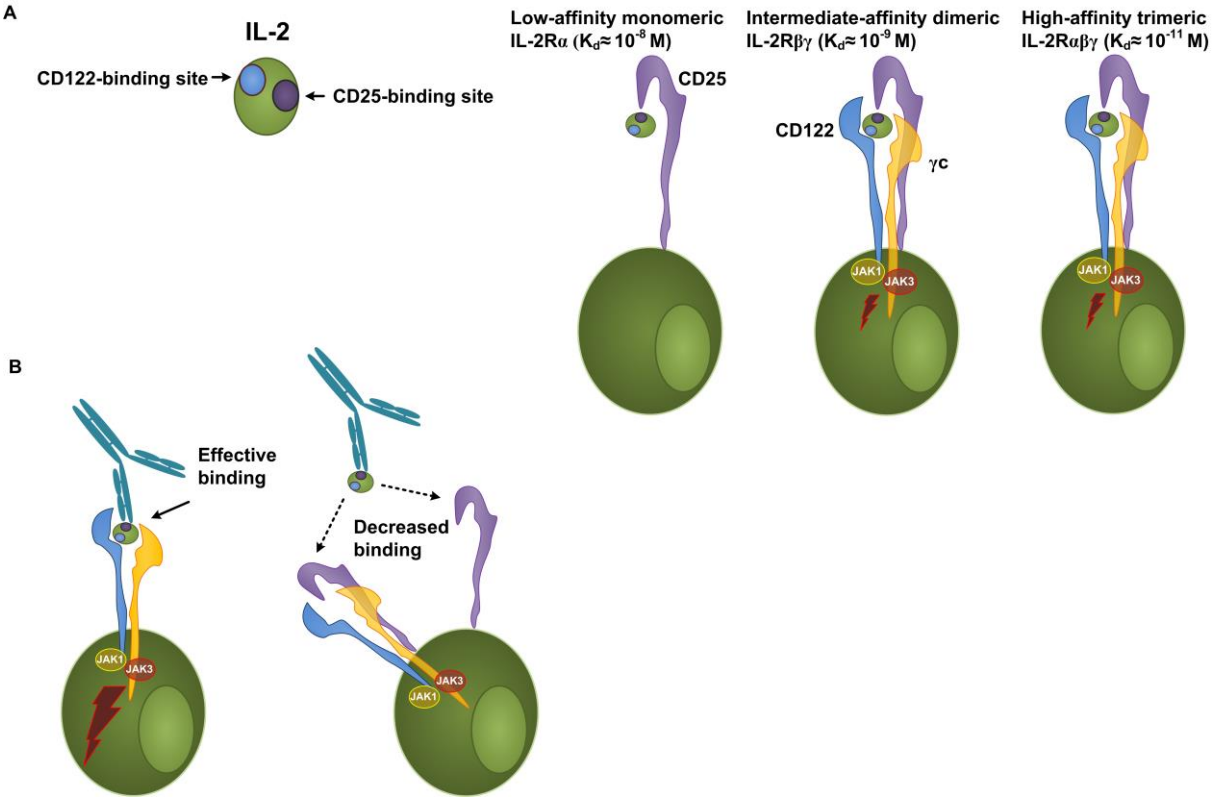

Figure 1. Properties of different IL-2 receptors and CD122-directed IL-2-cx. (A) IL-2 can be bound by CD25 (also known as IL-2 receptor α, IL-2Rα) alone, a heterodimer of CD122 (IL-2Rβ) and γc (common γ-chain receptor or alternatively known as IL-2Rγ), or a heterotrimer of CD25, CD122 and γc. CD122 and γc are able to signal, whereas CD25 increases IL-2R affinity [1,2,4,8]. (B) Preferential stimulation of cells expressing high levels of CD122 is achieved by complexing IL-2 with a particular anti-IL-2 mAb (such as clone S4B6 for murine IL-2), which binds IL-2 at a site within or neighboring the CD25-binding site thus impairing binding to CD25 and trimeric IL-2Rs [1,6,20].

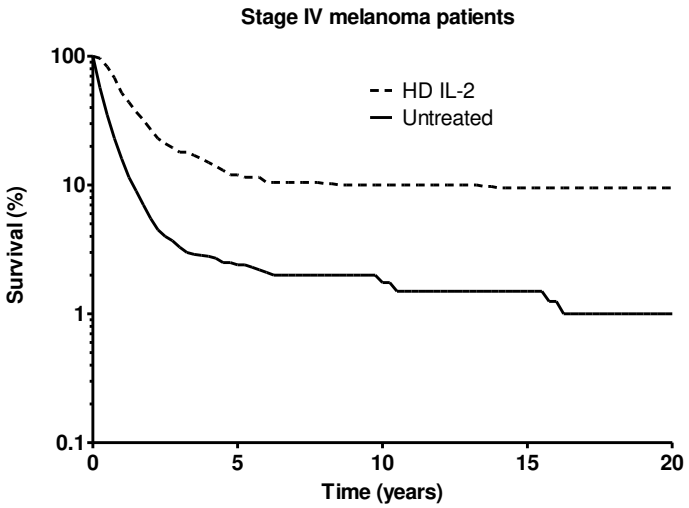

Figure 2. Overall survival of stage IV melanoma patients with or without high-dose IL-2 treatment. Shown are survival data of untreated patients with stage IV melanoma and of stage IV melanoma patients following high-dose (HD) IL-2 immunotherapy, adapted from [44] and [10], respectively.

Table 1. Biochemical, pharmacological and immunological properties of IL-2 formulations compared to IL-15 compounds.

Compound	Binding to CD25	Binding to CD122	Stimulation of $CD4^+CD25^+$ Tregs	Stimulation of $CD8^+$ T-cells	Stimulation of NK cells	Half-life in blood (hours)	Ref.
Wild-type IL-2							
HD IL-2	++	+	+++	++	++	+ (0.5-1 h)	[9,45]
LD IL-2	++	+	++	+	+	+ (0.5-1 h)	[45]
IL-2-cx	–	+	++	++++	+++	+++ (>24 h)	[15,18]
IL-2 muteins							
CD25-directed IL-2 muteins	+++	+	+++ (*in vitro*) °	ND	ND	+	[23]
BAY 50-4798	++	–	+++	+	+	+	[25,27]
IL-2-superkine	++	+++	++	+++	+++	+	[19]
No-α mutein	+	+	+	+	+	+	[22]
IL-2-FPs							
hu14.18–IL-2	++	+	ND	ND	ND	++ (2-4 h)	[46]
F8-IL2 / F16-IL2	++	+	ND	ND	ND	+++ #	[39]
L19-IL2	++	+	++	++	++	+++	[47,48]
IL-2-mutein-FPs							
Selectikine	++	+	+++	ND	+	++ (5 h)	[30,31]
CEA-IL2v (GA504 & GA501)	–	+	+/++	+++	+++	+++ #	[33,34]
IL-15 formulations							
IL-15/mAb-cx	–	+	+/++	+++	+++	+++ #	UP
IL-15/IL-15Rα-cx	–	+++	+/++	+++	+++	+++ #	[42]
RLI	–	+++	+/++	+++	+++	++ (3 h)	[41]

ND = not determined, UP = unpublished data.
– no effect, + weak, ++ mild, +++ strong, ++++ very strong, compared to control.
° The human $CD25^+$ KIT-225 T-cell line was used to assess potency of IL-2Rα-targeting muteins [23].
Estimated.

Table 2. Therapeutic anti-tumor properties of IL-2 formulations compared to IL-15 compounds.

Compound	Anti-tumor efficacy				Adverse effects		Ref.
	Pre-clinical [a]	Clinical objective response (%)			Pre-clinical [a]	Clinical (grade 3 and 4) [b]	
		0 – 0.5 years	0.5 – 5 years	> 5 years			
Wild-type IL-2							
HD IL-2	++[18]	40-48%	10-36%	8-9.3%	+++	36 - 45% ˣ	[9,45]
LD IL-2	+	ND	ND	ND	+	2.9% ˣ	[45]
IL-2-cx	+++	ND	ND	ND	+	ND	[15,18]
IL-2 muteins							
CD25-directed IL-2 muteins	ND	ND	ND	ND	ND	ND	[23]
BAY 50-4798	+++	0% (0/48)	ND	ND	+	70-80%	[25,27]
IL-2-superkine	++/+++	ND	ND	ND	+	ND	[19]
No-α mutein	+++	ND	ND	ND	+	ND	[22]
IL-2-FPs							
hu14.18–IL-2	++/+++	0% (0/9) □	ND	ND	+	41%	[46]
F8-IL2 / F16-IL2	+++	1/1 (case study)	ND	ND	+	ND	[39]
L19-IL2	+++	53.9%	ND	ND	ND	10-20%	[47,48]
IL-2-mutein-FPs							
Selectikine	++	7-14%	ND	ND	+	10%	[30,31]
CEA-IL2v (GA504 & GA501)	+++	ND	ND	ND	ND	ND	[33,34]
IL-15 formulations							
IL-15/mAb-cx	++	ND	ND	ND	+	ND	UP
IL-15/IL-15Rα-cx	++/+++	ND	ND	ND	+	ND	[42]
RLI	++/+++	ND	ND	ND	+	ND	[41]

ND = not determined, UP = unpublished data; objective responses = % of complete plus partial response of treated patients, excluding stable disease.

Clinical adverse effects of IL-2 are shown only for grade 3 and 4 reactions, whereby (ˣ) indicates hypotension.

□ A 98% humanized version of the hu14.18-IL-2 containing a single point mutation (*K322A*), termed hu14.18K322A showed better objective responses (6/31, 20%) in patients with recurrent neuroblastoma [49].

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Highlights

- IL-2 immunotherapy can induce durable tumor regression of metastatic cancer.
- IL-2 can lead to toxic adverse effects and stimulate regulatory $CD4^+$ T cells.
- Compared to IL-2, CD122-directed IL-2 formulations show a better therapeutic profile.
- CD122-directed formulations include IL-2/anti-IL-2 mAb complexes and IL-2 muteins.