ImmunoTargets and Therapy

Dovepress
Taylor & Francis Group

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REVIEW

# Cytokine Couture: Designer IL2 Molecules for the Treatment of Disease

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**Abstract:** Interleukin 2 (IL2) is a dual-acting cytokine, playing important roles in both immune activation and regulation. The role IL2 plays as a potent activator of CD8 T cells saw IL2 become one of the earliest immunotherapies, used for the treatment of cancer. In more recent years refined understanding of IL2, and the potent capacity it has for Treg stimulation, has seen low-dose IL2 therapy trialled for the treatment of auto-immune and inflammatory conditions. However, despite clinical successes, IL2 therapy is not without its caveats. The complicated receptor biology of IL2 gives rise to a narrow therapeutic window, made problematic by its short half-life. Armed with a better understanding of the structure of IL2 in complex with its receptors, many attempts have been made to create designer IL2 molecules which overcome these problems. A wide range of approaches have been used, resulting in >100 designer IL2 molecules. These include antibody complexes, fusion proteins, mutant IL2 molecules and PEGylation, each uniquely modifying the biological activity in an effort to enhance its therapeutic potential. Collectively, designer IL2 molecules form a blueprint outlining modification pathways available to other immunotherapeutics, paving the way for the next generation of immunotherapy.

**Keywords:** cytokine, protein engineering, T cell, treg, interleukin 2

## Introduction

Since the discovery of IL2 as T cell growth factor in 1978 by Kendal Smith and team,[1] IL2 has been an alluring target for therapeutic use. Based on the early understanding of IL2 in the promotion of effector T cell responses, initial drug development was based around stimulating anti-tumour responses. Indeed, the approval of Proleukin (Aldesleukin) for the treatment of renal carcinoma in 1992,[2] was arguably the first cancer immunotherapeutic. Although IL2 therapy provided promising results in cancer treatment, with 15–17% of patients experiencing an objective clinical regression in disease,[3] dosing was, and remains, difficult. Patients receive large bolus injections daily to reach therapeutic concentrations due to the short half-life of IL2.[4,5] In addition, off target effects of high dose IL2 can lead to serious side effects, such as vascular leak syndrome (VLS).[6]

Many of the early disappointments in IL2 therapeutics can, in retrospect, be attributed to the unappreciated role of IL2 as the key cytokine in regulatory T cell (Treg) homeostasis,[7,8] making the biology of this molecule more complicated than initially appreciated. The renaissance of IL2 as an anti-inflammatory biologic lead to reinvigorated therapeutic testing of IL2, with refined dosing to stimulate and promote Treg survival in a range of inflammatory diseases, such as GVHD and SLE.[9–13] Recently, with enhanced understanding of the structure of IL2 binding to its receptors,[14,15] the opportunity has arisen to modify this complicated cytokine to enhance the biological properties needed for therapeutic use in both inflammatory and anti-inflammatory contexts. Whilst designer IL2 molecules have been well reviewed in the context of cancer immunotherapy and inflammatory disease,[16,17] here we aim to create an extensive resource which brings together the <100 designer IL2 molecules in the clinic or in development as therapeutics and mouse analogues developed as key research tools, their design strategies, known biological features, and their potential to finally unlock the power of IL2 therapy.

Received: 25 January 2025
Accepted: 20 March 2025
Published: 4 April 2025

## A Hierarchy of Binding: IL2 and Its Receptors

The relationship between IL2 and its receptors lies at the heart of its dualistic behaviour, playing a critical role in both immune activation and regulation. Cellular selectivity of the IL2 molecule is driven through differential receptor expression on cellular targets which dictate the affinity and downstream signalling pathways. The IL2 receptor exists in three conformations, characterised by their relative affinity, each made up of a different assembly of the protein compartments IL2Rα (CD25), IL2Rβ (CD122) and IL2Rγ (CD132) (Figure 1). The highest affinity receptor is a trimer comprised of all three components.[18] At baseline, the high affinity receptor is expressed primarily on the surface of Tregs, with stimulation leading to increased Treg fitness and survival.[19,20] Upon IL2 engagement, the receptor complex activates downstream JAK-STAT signalling, particularly the STAT5 pathway, leading to enhanced Treg proliferation, survival, and suppressive function. This mechanism is crucial for maintaining immune tolerance and preventing autoimmunity. In contrast, the intermediate affinity receptor is a dimer comprised of an IL2Rβ and an IL2Rγ subunit which is primarily expressed on the surface of CD8 T and NK cells. This receptor configuration has an affinity ~100 fold lower than its trimeric counterpart.[18] Consequently, under normal physiological conditions, IL2 availability is limited for CD8+ T and NK cells, instead favouring Treg expansion. However, during immune activation and inflammatory responses, IL2 production can surge, leading to transient stimulation of effector cells through the intermediate-affinity receptor. This dynamic regulation allows IL2 to promote cytotoxic T cell and NK cell proliferation, differentiation, and effector function, which are essential for pathogen clearance and tumour immunity.[21] Additionally, IL2Rα (CD25) can function independently as a low-affinity receptor, binding IL2 without initiating downstream signalling. It can also be shed into the extracellular environment as a soluble form (sCD25), which has been proposed to act as a decoy receptor, sequestering excess IL2 and modulating its bioavailability.[22] While the exact physiological role of sCD25 remains incompletely understood, it is thought to contribute to immune regulation by limiting IL2-driven activation of effector cells. Elevated levels of sCD25 have been associated with various inflammatory and autoimmune diseases, suggesting its potential as a biomarker for immune dysregulation.[22–25] As IL2 binding to the IL2RA and IL2RB subunits is mediated through different interaction surfaces, the processes are largely, although not entirely, independent. This understanding has enabled the bioengineering of IL2 into potential therapeutics with altered affinity to one or both receptors. Alterations in this interface, or modifications to prolong the short half-life of the molecule, are the primary modifications present in the >100 altered IL2 molecules currently characterised.

## Anti-Inflammatory IL2Rα-Biased Agents

With the high therapeutic potential of harnessing the suppressive capacity of Tregs for treating inflammatory conditions, extensive work has gone into developing strategies to elevate the capacity of IL2 to work through the IL2Rα component.

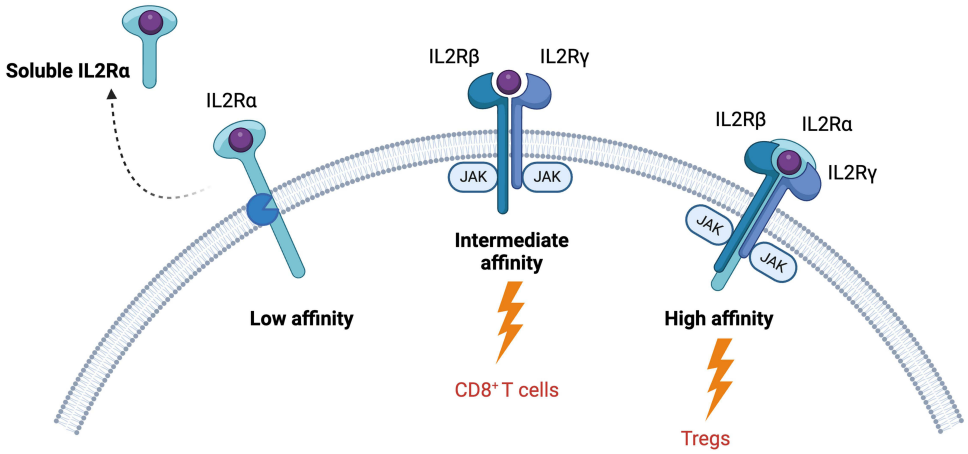


**Figure 1** IL2 receptor biology. IL2 is a pleiotropic cytokine; its dualistic behaviour arises from its relationship with its differentially expressed receptors. The low affinity receptor is comprised of only the IL2Rα subunit and can be cleaved to become a soluble cytokine receptor. It is thought to act as a decoy to quench excess IL2. The intermediate affinity receptor is primarily expressed by inflammatory cells such as CD8 T cells and in made up of the IL2Rβ and IL2Rγ subunits. Finally, the high affinity receptor is highly expressed on regulatory T cells and is comprised of all three subunits. Created in BioRender. Dashwood, A. (2025) https://BioRender.com/ h38f115.

As Tregs have the highest basal expression of IL2Rα, these therapeutics shift the response towards Treg survival and fitness, creating a more anti-inflammatory environment. The strategies used to create these α-biased IL2 molecules are diverse, including antibody complexes, Fc fusions, point mutations, directed PEGylation, and de novo design (Table 1). Each strategy has had varying success with a several candidates making it into clinical trials.

**Table 1** IL2Rα-Biased (Treg-Promoting) IL2 Muteins

| ***Antibody Complexes*** | | | | | |
|---|---|---|---|---|---|
| **Name** | **Antibody** | **Species** | **Effect** | **Clinical Trials** | **References** |
| IL2/F5111 | Antibody complex with anti-IL2 F5111.2 | Human | Steric obstruction IL2Rβ binding site[26] | | [26,27] |
| IL2/JES6 | Antibody complex with anti-IL2 JES6 | Mouse | Sterically blocks IL2Rβγ interaction, allosterically lowers affinity to α favouring IL2Rα high cells[28] | | [29,30] |
| IL2/SD-01 | Antibody complex/fusion with anti-IL2 SD-01 | Human | Unable to bind IL2Rβγ[31] | | [31] |
| ***Point Muteins*** | | | | | |
| **Name** | **Mutation(s)** | **Species** | **Effect** | **Clinical Trials** | **References** |
| Fc.Mut24 | N103R, V106D | Mouse | Decreased IL2Rβ binding[32] | | [32–36] |
| N88D | N88D | Human | Decreased IL2Rβγ binding[37] | See MK-6194 | [37,38] |
| IL-2M | D34S, N103D, C140A, P51T | Mouse | D34S, N103D reduce IL2Rβ binding, C140A and P51T increase manufacturability[39] | | [39] |
| Fc.Mut27 | M33D, V106D | Mouse | IL2Rα-dependency[40] | | [40] |
| 2–4 IL-2 | N29S, Y31H, K35R, T37A, K48E, V69A, N71R, Q74P, N88D, I89V | Human | Increased IL2Rα binding affinity[41] | | [41,42] |
| V91R | V91R | Human | Disrupts IL2Rβ binding[42,43] | | [42,43] |
| Q126T | Q126T | Human | Disrupts IL2Rγ binding[42] | | [42] |
| WC9 | S4P, T10A, Q11R, V69A, Q74P, N88D, T133A | Human | Increased IL2Rα binding affinity[41] | | [41] |
| M6 | V69A, Q74P, I128T | Human | Increased IL2Rα binding affinity[41] | | [41,44] |
| WE3 | N30S, V69A, Q74P, I128T | Human | Increased IL2Rα binding affinity[41] | | [41] |
| 1b-8 | K8R, Q13R, N26D, N30T, K35R, T37R, V69A, Q74P, I92T | Human | Increased IL2Rα binding affinity[41] | | [41] |
| 1a-1 | N29S, Y31H, K35R, T37A, K48E, V69A, N71R, Q74P, N88D, I89V | Human | Increased IL2Rα binding affinity[41] | | [41] |
| M1 | Q74P, V69A | Human | Lower affinity to IL2Rβ | | [44] |
| IL2 REH | L18R, Q22E, Q126H | Human | Increased dependence on IL2Rα through weakened binding to IL2Rγ[45] | | [45] |
| BAY 50-4798/ AIC284 | N88R | Human | Defective binding to IL2Rβ[46] | HIV,[47] Melanoma and renal cancer.[48] | [46,49–53] |
| N90R, V91R, T131R | N90R, V91R, T131R | Human | V91R causes reduced binding to IL2Rβ without IL2Rα binding first. N90R and T131R boost production[43] | | [43] |
| V106R | V106R | Mouse | Reduced binding to IL2Rβ without IL2Rα binding first[43] | | [43] |

(*Continued*)


https://doi.org/10.2147/ITT.S500229

**Table 1** (Continued).

| ***Fusion Proteins/Antibody fusions*** | | | | |
|---|---|---|---|---|
| **Name** | **Fusion Protein/Antibody** | **Species** | **Clinical Trials** | **References** |
| HSA-IL2m | IL2 mutein fused with serum albumin | Human | Phase 1 healthy Individuals, given as mRNA-6231.[54] | [55] |
| CC-92252 (DEL 106) | IL2 fused with Fc domain | Human | Psoriasis[56] | |
| IL2-EHD2-sc-mTNF$_{R2}$ | IL2 fused with mutated TNF | Mouse | | [57] |
| Xmab564 (XmAb27564) | IL2 fused with Fc domain | Human | Healthy individuals,[58] Psoriasis or atopic dermatitis.[59] | |
| Amg 592 (Efavaleukin Alfa) | IL2 mutein (V91K, C145A) fused with Fc domain | Human | Healthy Individuals,[60] Lupus,[61,62] GVHD,[63] Arthritis[64] and Ulcerative colitis.[65,66] | [67–69] |
| MK-6194 (PT101) | IL2R$^{\alpha\text{-bias}}$ mutein (N88D) fused with IgG | Human | Healthy Individuals,[70] Ulcerative Colitis,[71] Atopic Dermatitis,[72] Vitiligo,[73] Lupus.[74] | [75,76] |
| IBI363 | IL2$^{\alpha\text{-bias}}$ mutein fused with anti-PD1 bispecific antibody | Human | Solid tumours or lymphoma,[77] Solid Malignancies or lymphoma,[78] Solid tumours,[79] Advanced melanoma,[80] Solid malignancies.[81] | [38] |
| Melredableukin alfa RO7049665 (RG-7835) | IL2$^{\alpha\text{-bias}}$ muteins (T3A, N88D, C125A) to IgG1K | Human | Healthy Individuals,[82] Autoimmune hepatitis,[83] Ulcerative colitis.[84] | [37] |
| Selectikine (NHS-IL2LT, EMD 521873) | IL2 muteins (D20T) fused with NHS76 (anti-DNA) antibody | Human | Solid Tumours[85], NSCLC.[86] | [87,88] |
| KY1043 | IL2R $^{\alpha\text{-bias}}$ mutein fused with anti-PDL1 antibody | Human | | [89] |
| ***De novo*** | | | | |
| **Name** | **Design** | **Species** | **Clinical Trials** | **References** |
| NEO-TRA1 | IL2 mimetic with targeting to ILRα via antibody fusion | De novo: human reactive | | [90] |
| ***PEGylated*** | | | | |
| **Name** | **PEGylation site(s)** | **Species** | **Clinical Trials** | **References** |
| KKC80 | desA1/C125S /1129oAzZLys_W-shaped 80 kDa PEG | Human | | [91] |
| Rezpegaldesleukin LY 3471851 (NKTR 358) | Pegylated at least one of the following amino acid residues 11, 12, 13, 15, 16, 18, 19, 20, 84, 87, 88, 91, 92, 108, 115, 119, 122, 123, and 130[92] | Human | Healthy Individuals,[93–95] SLE,[96,97] Psoriasis,[98] Eczema,[99] Ulcerative Colitis,[100] Alopecia Areata,[101] Atopic Dermatitis.[102] | [103–105] |
| Dual-31/51-20K | Dual 20 kDa PEG Tyr 31, Thr 51 | Human | | [106] |

## Antibody Complexes

The use of anti-IL2 antibodies to create immune complexes were the first inadvertent steps towards producing receptor-biased IL2 products. Initially generated to neutralise IL2, the conflicting effects of different IL2 antibodies were eventually identified to relate to the site of IL2 binding, with antibodies that bound the IL2Rα interface giving a relative boost to IL2Rβ signals, and vice versa.[107] An example of this being used to create IL2Rα bias is IL2/F5111, which is a complex between IL2 and the F5111 anti-IL2 antibody.[26,27] F5111 binds to IL2 in a manner that sterically blocks the binding site to the IL2Rβ receptor, preventing responses from cells that rely exclusively on this interface for signalling. Antibody complexes have the additional benefit that they

can extend protein half-life by increasing its molecular weight and altering its biophysical properties, therefore reducing clearance. This beneficial effect has potential issues when transferring to clinic, ie how long are these complexes stable for and can the complex dissociate within the body resulting in off target effects. For these reasons, it may be beneficial to covalently link the IL2 molecule, via the IL2Rβ-interaction face, to a fusion protein carrier, to achieve similar effects. Additionally, antibody complexes can bear further production costs over fusion proteins or muteins as two separate proteins need to be expressed and purified.

## Decreased IL2Rβ Binding

With insights delivered on IL2 receptor binding by antibody neutralisation and structural studies, new mutated proteins, or muteins, could be designed that interfered with the capacity of IL2 to bind one of its receptors without the need for antibody blockade (Figure 2). In the case of Treg-biased muteins, the simplest approach is to mutate residues in IL2 which are important

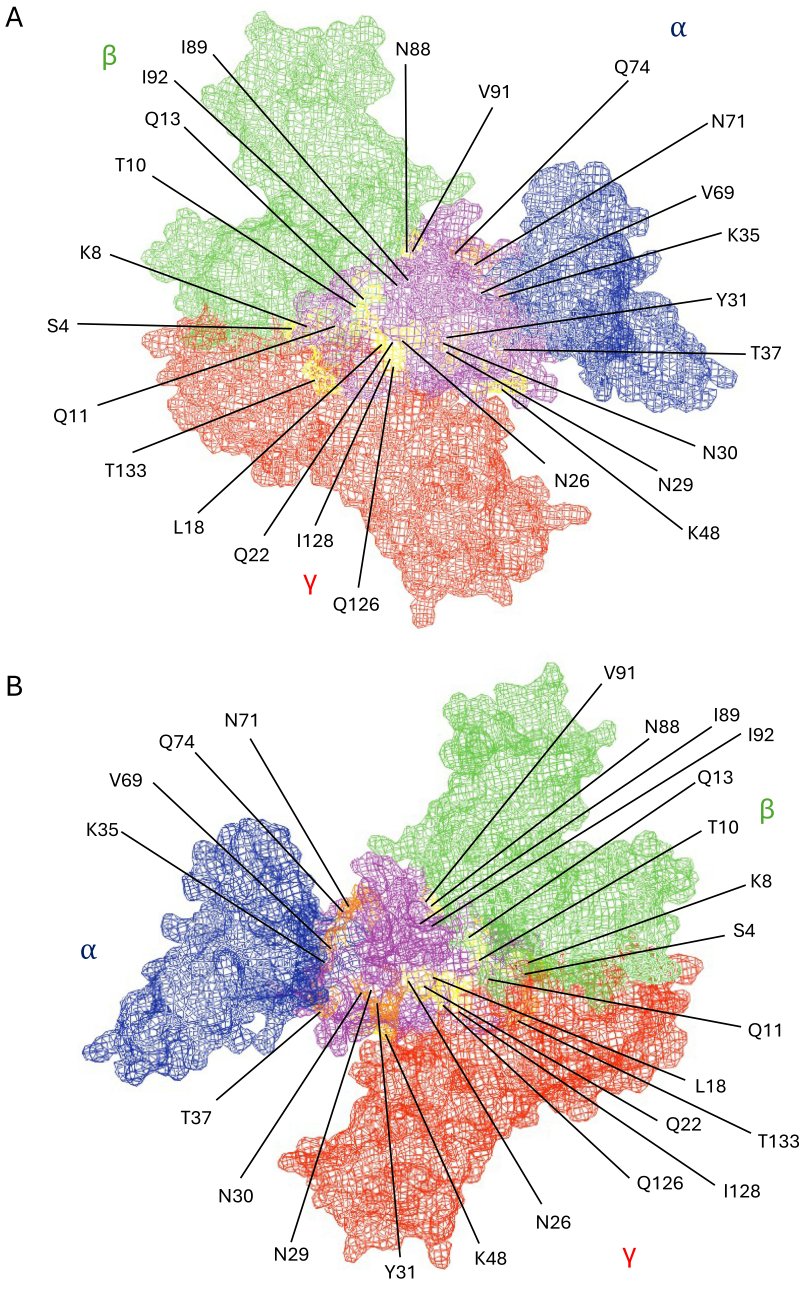


**Figure 2** IL2Rα-biased point muteins. IL2 (purple) in complex with receptor subunits IL2Rα (blue), IL2Rβ (green), and IL2Rγ (red), based on 2ERJ,[15] visualised from above the membrane (**A**) and from below the membrane (**B**). Residues of IL2 which have previously been targeted for mutation are highlighted in Orange (disrupt IL2Rα binding) and yellow (enhance IL2Rβγ binding). (Figure created in PyMol).


https://doi.org/10.2147/ITT.S500229

to binding the IL2Rβ interface, thereby preventing activation of the inflammatory cells that exclusively rely on IL2Rβ-IL2Rγ for signalling. An example of this approach is the IL2 mutant N88D, where mutating the asparagine at position 88 to an aspartic acid, decreased IL2 affinity to the IL2Rβ subunit.[37] The authors report a 30–80-fold reduced ability to activate receptors present on effector T cells and NK cells whilst having only a minimal reduction (6-fold) in the ability to activate the high affinity receptor on Tregs. This marginal decrease in overall bioactivity of IL2 is often seen in IL2Rβ-impeded muteins.[32,39,40,55] A probable explanation for this decreased bioactivity is the requirement to form the trimer to allow signalling, as the IL2Rα subunit cannot signal alone. However, despite this, N88D entered phase 1 clinical trials in the form of an Fc fusion protein (PT101), in ulcerative colitis.[71] Results of this clinical trial will help shed light on whether this decrease in bioactivity will affect overall performance in vivo.

### Increased IL2Rα Binding

An alternate way to mutate IL2 to enhance its anti-inflammatory effects is to increase affinity to the IL2Rα subunit. Rao et al performed this type of mutation with M1 and M6.[44] Both muteins possess point mutations, Q74P and V69A which lie at the interface with the IL2Rα subunit. M6 has one further mutation I128T which lies close to the predicted IL2Rβ interface. Both mutants were shown to have an increased affinity for the alpha subunit. However, when M1 and M6 were evaluated in proliferation assays using KIT-225 T cell line, M1 failed to perform better than WT IL2, suggesting that the increased binding to the IL2Rα subunit alone was not enough to confer increased biological potency. On the other hand, M6 performed better than WT IL2, increasing proliferation by 50–60%.[44] Neither mutein has yet progressed to pre-clinical or clinical studies.

## Pro-Inflammatory IL2Rβ/IL2Rγ-Biased Agents

For enhanced therapeutic use of IL2 in its original purpose, oncology, the reciprocal changes are required. IL2Rβ-biased IL2 modifications are expected to have enhanced or maintained expansion of CD8 T cell and NK cell anti-tumour responses, without inducing a suppressive Treg response through IL2Rα (Table 2). While the engineering strategies used closely parallel those of IL2Rα-biased agents, many more candidates have progressed to the clinic, likely due to the simpler structural problem of excluding a non-essential signalling component (IL2Rα).

### Antibody Complexes

Similar to the earliest IL2Rα-biased IL2 agents, antibody complexing was among the earliest approaches used to alter the biophysical properties and bioactivity of IL2 to bias it towards the dimeric receptor. These complexes result in pro-inflammatory responses. Several examples have made it into clinical trials in various types of cancer. Amongst these is IL2/TCB2, a complex formed of IL2 and an anti-IL2 which blocks binding to the α receptor subunit.[115,117] In preclinical studies the complex, which was prepared daily before IP injection, resulted in inhibition of tumour growth in three independent models. This effect was further enhanced when used in conjunction with anti-PD1, resulting in 100% tumour rejection of MC38 colon cancer cells.[115] IL2/TCB2 has since moved into phase 1/2 clinical trials, which are actively recruiting patients with solid tumours, as both a monotherapy and in combination with chemo-agent gemcitabine.[118]

### Decreased IL2Rα Binding

In the same way that designer IL2 muteins can be made with decreased IL2Rβ binding, to enhance Treg selectivity, muteins can be generated with decreased IL2Rα binding, to enhance inflammatory cell selectivity (Figure 3). One example of this is the no-alpha IL2 mutein where point mutations (R38A, F42A, Y45A, E62A, C125S) have been introduced at the IL2 interface with the IL2Rα subunit.[123] The resulting effect of this is a 1000-fold decrease in the $EC_{50}$ of the mutein on Tregs, whilst its ability to stimulate CD8 T cells is maintained. In preclinical models, treatment with this mutein translated to a reduction in tumour size which was later shown to be a direct effect of altering the CD8:Treg balance.[124] This provides a proof-of-concept for the utility of this class of agents in a clinical setting.

### Increased IL2Rβ Binding

IL2 can also be engineered to have an increased binding to the IL2Rβ subunit. This is the case for D10. D10 harbours a number of point mutations (Q74H, L80F, R81D, L85V, I86V, I92F) which increase the ability of IL2 to stimulate cells

**Table 2** IL2Rβ/IL2Rγ-Biased (CD8 T Cell-Promoting), Inflammatory Agents

| **Antibody Complexes** | | | | | |
|---|---|---|---|---|---|
| **Name** | **Antibody** | **Species** | **Effect** | **Clinical Trials** | **References** |
| AU-007 | Antibody complex with anti-IL2 (AI-designed antibody) | Human | Blocks IL2 interaction with IL2Rα while sparing its interaction with IL2Rβγ[108] | Locally advanced or metastatic cancer[109] | [108] |
| IL2/S4B6 | Antibody complex with anti-IL2 SB46 | Mouse | Sterically hinders interaction with IL2Rα whilst conformationally stabilizing IL2Rβγ interaction[28] | | [28,107,110–112] |
| IL2/MAB602 | Antibody complex with anti-IL2 MAB602 | Human | Blocks IL2Rα binding[110] | | [110,111,113,114] |
| IL2/NARA1 | Antibody complex with anti-IL2 NARA1 | Human | Binds near IL2Rα binding site, abolishing binding[115] | | [113,116] |
| IL2/TCB2 (SLC-3010) | Antibody complex with anti-IL2 TCB2 | Human | Blocks IL2Rα binding[115,117] | Advanced Solid Tumours[118] | [115,117,119] |
| ***Point Muteins*** | | | | | |
| **Name** | **Mutation(s)** | **Species** | **Effect** | **Clinical Trials** | **References** |
| SumIL2 | F42A, L80F, R81D, L85V, I86V, and I92F | Human | Decreased IL2Rα, increased IL2Rβ binding[120] | | [120,121] |
| No-alpha mutein | R38A, F42A, Y45A, E62A, C125S | Human | Reduced IL2Rα binding[122] | | [122–127] |
| FSD13 | P65L | Human | Low affinity to IL2Rα[128] | | [128] |
| MK-6 | K35A, R38A, K43A, Y45A, T3A, C125A | Human | Reduced IL2Rα binding[129] | | [129] |
| CGC-601 | Undisclosed | Human | IL2RBγ binding only[130] | | [130] |
| F42K | F42K | Human | Reduced IL2Rα binding[131] | | [131–133] |
| R38A | R38A | Human | Reduced IL2Rα binding[133] | | [132,133] |
| IL2v (STI-7349) | F42A, Y45A, L72G | Human | Abolished IL2Rα binding[134] | Solid tumours[135] | [38,134,136,137] |
| H9 (MDNA109) | L80F, R81D, L85V, I86V and I92F | Human | Enhanced IL2Rβ binding[138,139] | | [138,140–142] |
| MDNA109FEAA | L80F, R81D, L85V, I86V, I92F, F42A and E62A | Human | Enhanced IL2Rβ binding and no interaction with IL2Rα[143] | | [140,143–145] |
| HM16390 | Undisclosed | Human | Enhanced IL2Rβ binding[146] | Solid tumours[147] | [146,148] |
| H9-T | L80F, R81D, L85V, I86V and I92F, Q126T | Human | Enhanced IL2Rβ binding, reduced IL2Rγ binding – partial agonist[139] | | [139,141] |
| H9-RE | L18R, Q22E, L80F, R81D, L85V, I86V and I92F | Human | Enhanced IL2Rβ binding[139] | | [139] |
| H9-RET | L18R, Q22E, L80F, R81D, L85V, I86V and I92F, Q126T | Human | Enhanced IL2Rβ binding, reduced IL2Rγ binding – partial agonist[139] | | [139] |
| H9-RETR | L18R, Q22E, L80F, R81D, L85V, I86V and I92F, Q126T, S130R | Human | Enhanced IL2Rβ binding, reduced γ binding – partial agonist, antagonist of IL2 and IL15[139] | | [139] |
| M1 | F42A, P65A, L72A | Human | Reduced IL2Rαβ γ affinity[149] | | [149] |
| M2 | K35A, E61A, F42A | Human | Reduced IL2Rα affinity[149] | | [149] |
| D10 | Q74H, L80F, R81D, L85V, I86V, I92F | Human | Increased IL2Rβ affinity[142] | | [142,150] |
| D10_D81E | Q74H, L80F, R81E, L85V, I86V, I92F | Human | Increased IL2Rβ affinity β[150] | | [150] |

(*Continued*)


https://doi.org/10.2147/ITT.S500229

**Table 2** (Continued).

| | | | | | |
|---|---|---|---|---|---|
| D10_F92W | Q74H, L80F, R81D, L85V, I86V, I92W | Human | Increased IL2Rβ affinity[150] | | [150] |
| D10_N119E | Q74H, L80F, R81D, L85V, I86V, I92F, N119E | Human | Increased IL2Rβ affinity[150] | | [150] |
| Y45K | Y45K | Human | Decreased IL2Rα binding[43] | | [43] |
| Q30W | Q30W | Mouse | Enhanced IL2Rβ binding[43] | | [43] |
| Y59K | Y59K | Mouse | Decreased IL2Rα binding[43] | | [43] |
| N90R T131R E62K Y45K | N90R, T131R, E62K, Y45K | Human | E62K, Y45K disrupt binding to IL2Rα. N90R and T131R boost production[43] | | [43] |
| ***Fusion Proteins/Antibodies*** | | | | | |
| **Name** | **Fusion Protein/Antibody** | **Species** | **Clinical Trials** | | **References** |
| OMCPmutIL2 | mutIL2 (R38A, F42K, C125S) fused with OMCP, a ligand for NKGD. | Human | | | [151] |
| Pro IL2 | SumIL2-Fc masked by IL2RB linked by a MMP substrate | Human | | | [152] |
| Erb-sumIL2 | SumIL2 fused with anti-human EGFR | Human | | | [120] |
| Melittin-MhIL2 | Mutant IL2 (Arg88/Ala125) linked to melittin | Human | | | [153–156] |
| Simlukafusp alfa (FAP-IL2v, RO6874281/ RG7461) | IL2v fused with anti-FAP antibody | Human | Cancer[157–159] | | [38,134,136,160] |
| Cergutuzumab amunaleukin (CEA-IL2v, RG7813) | IL2v fused with anti-carcinoembryonic antigen (CEA)-specific antibody | Human | Solid tumours[161,162] | | [134,137] |
| Eciskafusp Alfa (PD1-IL2v, RO7284755/ RG6279) | IL2v fused with blocking anti-PD-1 antibody | Human | Combination therapy in solid tumours[163] | | [164–167] |
| AB248 | IL2 mutein fused with anti-CD8 antibody | Human | Combination therapy in solid tumours[168] | | [169–172] |
| $IL2^{3X}Fc$ | Fc Fusion with triple mutant IL2 triple mutant (R38D, E61R, K43E) fused with Fc domain | Human | | | [173] |
| AB359 | Affinity-attenuated IL2 mutein fused with anti-CD8 antibody | Human | | | [174] |
| INBRX-120 | IL2x fused to two anti-CD8α single domain antibodies | Human | | | [175] |
| Exenokine-2 | No-α-IL2 linked to anti-HAS single domain antibody | Human | | | [176] |
| Nemvaleukin alfa ALKS 4230 | Circularly-permuted IL2 fused with extracellular domain of IL2Rα | Human | Cancer[177–181] | | [182,183] |
| IL2K35C-moFA | IL2K35C conjugated to fatty acid moiety | Human | | | [184] |
| NARA1leukin | Human IL2 grafted unto light chain complementary determining region of NARA1 anti-IL2 antibody | Human | | | [116] |

(*Continued*)

**Table 2** (Continued).

| | | | | |
|---|---|---|---|---|
| ANV419 | Human IL2 grafted unto light chain complementary determining region of NARA1 anti-IL2 antibody | Human | Solid tumours[185] and Melanoma[186] | [187] |
| BNT151 | Liposome encapsulated mRNA transcript of mutated human IL2R$^{\beta\text{-bias}}$ fused with albumin | Human | Solid tumours[188] | [189] |
| MDNA11 | MDNA109FEAA genetically linked to human albumin | Human | Tumours[190] | [140,143,191] |
| CUE-101 | IL2$^{\beta\gamma\text{-bias}}$ variant fused to effector attenuated IgG1 Fc HLA-A*0201. HPV-16 E7 (amino acids 11–20) are loaded unto the HLA | Human | HNSCC[192] and HPV+ OPSCC[193] | [194] |
| GI-101 | Fusion protein consisting of CD80, IgG4 Fc, and IL2R$^{\beta\gamma\text{-bias}}$ variant | Human | Solid tumours[195] | [196] |
| GI-102 | CD80-IgG4 Fc-IL2v Bispecific Fusion Protein | Human | Solid Tumours[197] | |
| XTX202 | IL2$^{\beta\gamma\text{-bias}}$ variant inactivated until cleavage by matrix metalloproteases | Human | Solid Tumours[198] | [199,200] |
| ***De novo*** | | | | |
| **Name** | **Design** | **Species** | **Clinical Trials** | **References** |
| Neoleukin-2/15 (Neo-2/15, NL-201) | Computationally designed alpha-independent agonist of the IL2 and IL-15 receptors | De novo: human reactive | Cancer[201,202] | [201,203,204] |
| ***PEGylation*** | | | | |
| **Name** | **PEGylation site(s)** | **Species** | **Clinical Trials** | **References** |
| Pegenzileukin THOR-707 (SAR-444245) | PEG moiety irreversibly bound to a novel amino acid via click chemistry at P65 | Human | Solid tumours: In combination therapy with pembrolizumab,[205] Skin Cancer: In combination with cemiplimab,[206] HNSCC: combination therapy,[207] GI Cancer: combination therapy,[208] B cell lymphoma,[209] Lung cancer,[210] Solid Tumours: monotherapy[211] | [205,212] |
| Bempegaldesleukin (NKTR-214) | Six releasable PEG chains located at interface between IL2 and IL2Rα | Human | Cancer,[213–232] Covid 19[233] | [234–237] |
| TransCon IL2 β/γ | Methoxy polyethylene glycol (mPEG) moiety in the IL2Rα binding site | Human | Cancer[238,239] | [238] |
| STK-012 | PEGylated, IL2R$^{\alpha\beta\text{-bias}}$ mutein (L18R, Q22E, Q126K) | Human | Solid tumours[240] | [241,242] |
| SHR-1916 | PEGylated IL2R$^{\beta\gamma\text{-bias}}$ variant | Human | Locally advanced or Metastatic Solid Tumours[243] | [244] |
| NL-201 | Pegylated Neolukin-2/15 | Human | Advanced solid tumours[245] | [201,246] |

expressing the intermediate affinity receptor by strengthening the binding to IL2Rβ and signalling through the IL2Rβ-IL2Rγ dimer.[150] In particular, the mutation at position 74 (Q74H) caused a conformational change which allows D10 to create stronger hydrophobic interactions in the receptor pocket whilst mutations R81D, I92F contributed to a higher binding energy.[150] These gain-of-function mutations provide a theoretical biotechnological benefit compared to the loss-of-function strategies, as the predicted protein production required to achieve functional thresholds should be reduced.

## Ortho IL2 – Designer IL2 to Match Designer T Cells

Another exciting concept in the IL2 mutein space are Ortho-IL2 cytokines. Ortho IL2 cytokines are IL2 replacement molecules designed to bind in pairs with a replacement (orthogonal IL2) extracellular domain fused to the IL2 receptor.


https://doi.org/10.2147/ITT.S500229

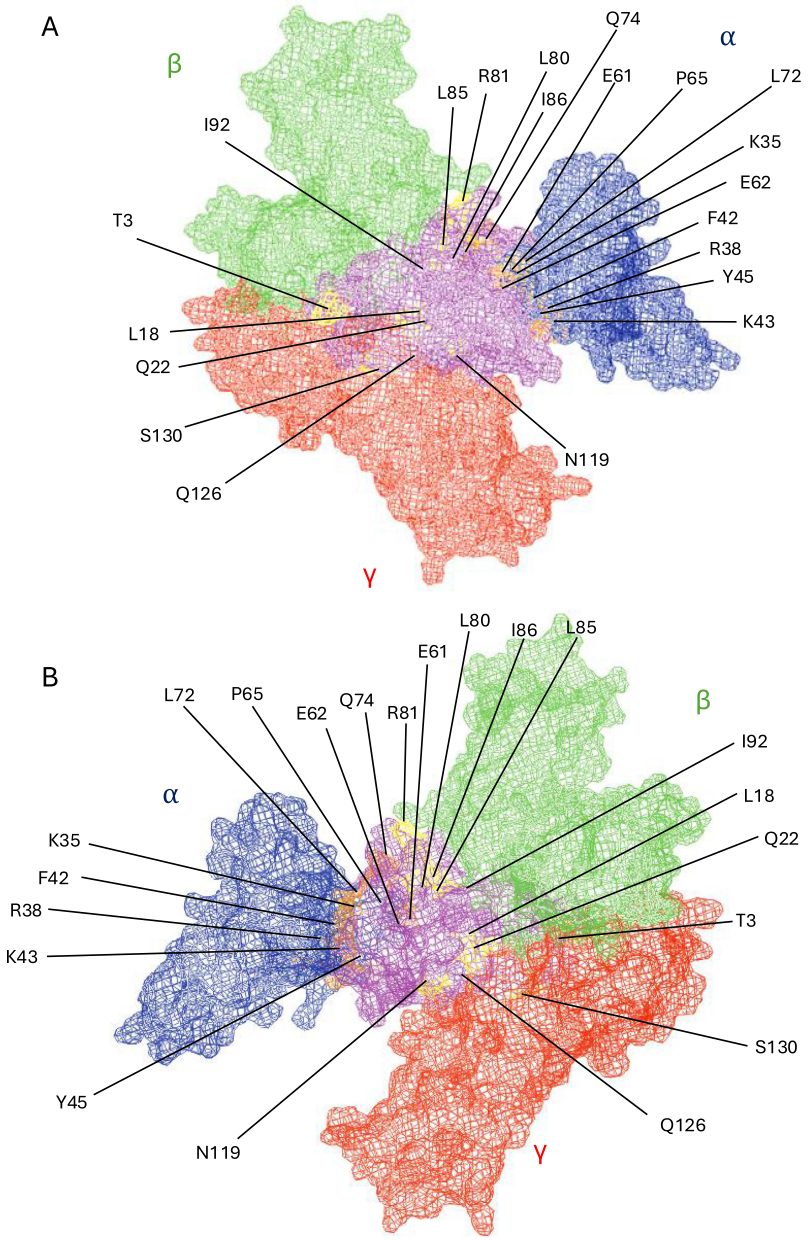


**Figure 3** IL2Rβγ-biased point muteins. IL2 (purple) in complex with receptor subunits IL2Rα (blue), IL2Rβ (green), and IL2Rγ (red), based on 2ERJ,[15] visualised from above the membrane (**A**) and from below the membrane (**B**). Residues of IL2 which have previously been targeted for mutation are highlighted in Orange (enhance IL2Rα binding) and yellow (disrupt IL2Rβγ binding). (Figure created in PyMol).

This creates classical IL2 signals, through the interaction of entire new ligand-receptor pairs. In practice, this requires engineering T cells to express the orthogonal IL2 receptor, transplanting them into the patient and then exposing to Ortho-IL2. As Ortho-IL2 has no affinity for the native IL2 receptor, it will specifically activate the ortho-IL2 receptor on the engineered (transplanted) T cells. There are many different orthogonal pairs which have been developed, which are now being tested in different pre-clinical models, for example, in cancer,[247,248] in transplantation models[249] and in GVHD.[250] This elegant method bypasses issues relating to the IL2 molecule itself, and overcomes the issue that even with strong receptor biasing, off-target effects may still occur due to receptor co-expression, for example, expression of IL2Rα on activated CD8 T cells. However, Ortho-IL2 is only suitable for approaches that utilise transplanted and engineerable T cells, limiting its utility and making it a costly therapeutic approach.

## IL2 Engineering Designed to Increase Half-Life

Distinct from the problem of biasing the specificity of IL2 towards pro- or anti-inflammatory functions, additional engineering has been performed to increase the half-life (Table 3). The serum half-life of IL2 is only ~12 minutes,[251] making it more difficult to use as a therapeutic without using large bolus doses to sustain therapeutic concentrations. Unfortunately, the bolus dose approach influences the hierarchy of reactivity, by supporting higher affinity reactions, and can trigger adverse events through triggering atypical IL2 pathways.[252,253] This narrowing of the therapeutic window limits utility, making it desirable to create IL2 molecules with a longer half-life.

While the function of antibody complexes described above focused on altering the specificity of IL2 by shielding one of its two receptor interfaces, antibody binding also substantially lengthens the half-life of IL2, allowing lower doses to be used with greater spacing. The effect of antibody complexing on half-life is likely brought about by both slower degradation and decreased consumption by off-target cells. For example, Boyman and team showed IL2/$mAb_{CD122}$ complexes, using SB46 (mouse) and MAB602 (human), relied on increased serum half-life brought about by FcRn to enhance IL2s biological function.[110] In addition, they showed IL2/$mAb_{CD25}$ complexes using JES6-1 had a further increased half-life, suggesting differing consumption rates may also play a factor.[110] Another interesting case was reported by Ward et al who, when designing CD8-biased fusion proteins, instead, discovered that an IL2-sCD25 fusion protein forms inactive head-to-tail dimers. These dimers which slowly dissociate in vivo, act as a slow-release system for IL2, boosting Treg responses.[263] A similar effect on half-life-extension can be generated through engineering a fusion protein combining IL2 with a protein with improved pharmacodynamics. These fusion proteins can even be based off IL2 muteins, to incorporate multiple improvements in the same molecule. For example, HSA-IL2m is a fusion protein made up of a mutated IL2 (for IL2Rα-biased specificity) and serum albumin,[55] to extend half-life.[265] The HSA-IL2 mutein even has an extended half-life over the already augmented HSA-IL2 (wildtype) molecule, potentially due to restricted consumption from IL2Rβ binding.[55]

Half-life extension can also be generated through fusion to non-protein carriers, chiefly polyethylene glycol (PEG). This was first explored in the context of IL2 as early as 1989 by Zimmerman et al, who showed PEG-IL2 to prolong the half-life of IL2 in mouse[261] and again, shown by Yang et al in 1991, who reported PEG-IL2 to have a half-life 25 times longer than WT.[262] Pegylated-IL2 progressed into clinical trials, with historical trials in cancer and HIV having limited

**Table 3** IL2 Agents Designed to Have an Increased Half-Life

| ***Increased half-life*** | | | | | |
|---|---|---|---|---|---|
| **Name** | **Modification(s)** | **Species** | **Effect** | **Clinical Trials** | **References** |
| B6 | IL2 Bioconjugate Modified with Fatty Acids by Sortase A | Human | Prolonged half-life | | [254] |
| IL2-poly(HPMA) | IL2 covalently conjugated to synthetic semitelechelic polymeric carrier | Human | Prolonged half-life | | [255] |
| C1 | I128T | Human | No change in binding, effect thought to be due to increased recycling[44] | | [44] |
| 2D1 | L18M, L19S | Human | Increased recycling instead of lysosomal degradation following receptor engagement[256] | | [256] |
| PEG-IL-2 | PEGylated human WT IL2 | Human | Prolonged circulating half-life[257] | Cancer,[257] HIV[258–260] | [261,262] |
| IL-2/CD25 | Fusion protein of mouse IL2 and mouse IL2Rα | Mouse | Forms inactive head-to-tail dimers that slowly dissociate into active monomer[263] | | [263] |
| Dextran MS IL2 | Dextran microspheres designed as a slow-release system for IL2 | Human | Slow release | | [264] |


https://doi.org/10.2147/ITT.S500229

success.[258,259,262] Like antibody binding, PEGylation of a molecule can serve as dual purpose, as targeted PEGylation can be used to interfere with a protein-receptor binding interface through the addition of a bulky group to key binding residues. This approach was used by Zhang et al, where they used targeted PEGylation to add 20kDa PEG molecules to tyrosine 31 and threonine 51 to create dual-31/51-20K.[106] These amino acid residues are close to the binding interface with the IL2Rβ subunit of the receptor. As a result, the selectivity of dual-31/51-20K, shown as EC50 ratio of CD8 T cell/Treg cells in pSTAT5 assays, was increased almost 3-fold, creating a Treg bias response,[106] albeit at the cost of a ~30-fold decrease in total bioreactivity.[106] The reverse bias can also be introduced through PEGylation, for IL2 molecules moving into the clinical space for the treatment of cancer. Many of them use PEGylation at the IL2Rα binding interface to change binding affinity. THOR-707 is the most progressed mutein, reporting success in multiple clinical trials.[205,266] THOR-707 utilises click chemistry to create a site-specific PEGylated IL2 at P65, resulting in a 10-fold decrease in $EC_{50}$ on Tregs whilst maintaining CD8 responses.[212] Clinical trials in a range of cancer subtypes have revealed THOR-707 to have a large clinical safety window, whilst being successful in producing minor-partial responses.

Finally, there are a few other non-protein carriers that have been used to extend IL2 half-life, without introducing a receptor usage bias. B6 is a bioconjugate of IL2 modified with fatty acid molecules using sortase A.[254] The addition of fatty-acid moieties helps increase half-life by non-covalent bonding to serum albumin. This technique has been used in a number of FDA-approved drugs including long-acting insulin Levemir where fatty acid groups have been added to lysine.[267] Due to the presence of many Lysine residues in IL2, the technique was altered using sortase A to create a single modification site. Clearance of B6 from the serum is 15-fold slower than WT IL2.[254] In addition to the beneficial increase in half life, fatty acid molecules also increased the bioavailability of IL2 by increasing the hydrophilicity, giving an added advantage over antibody fusions or pegylated IL2 molecules.[254] Using these techniques can make IL2 more favourable for therapeutic use, decreasing the number of injections needed whilst also generating more stable pharmacodynamics.

## Engineering of Targeted IL2 Delivery

An additional class of IL2 engineering covers those modifications which seek to overcome off target systemic effects of IL2 therapy by directing IL2 localisation to target tissues, fine-tuning the dose and reducing off-target detrimental effects (Table 4). The classical approach to targeting IL2 to a particular tissue is through antibody fusion, where the antibody provides the localisation signal. For example, Simlukafusp alfa is a fusion between a IL2Rβ-selective IL2v and anti-fibroblast activation protein (FAP) antibody.[136] Coupling the IL2v to anti-FAP gives a double pronged approach. The IL2v ensures CD8 selective stimulation whilst the anti-FAP targets this to the tumour specifically. In principle, this should reduce effects in off-target tissues and reduce the amount of agent needed by increasing the local concentration in the tumour only. Simlukafusp has achieved some success in a clinical trial in metastatic cervical cancer patients. When treated in combination with atezolizumab (anti-PD-L1) 44 out of 47 patients had an observed response.[160]

An alternative way to target IL2 to tumours is to create fusion proteins which are activated only within the tumour. IL2FP is a fusion protein between mouse IL2 and IL2Rα linked via an MMP2/9 cleavage site. The cleavage site is designed to exploit the dysregulated protease activity in the tumour microenvironment, releasing IL2 from its bound receptor only in the tumour.[298] In mouse tumour models, IL2FP was able to be cleaved within the tumour site, resulting in a change in immune composition in the tumour, inducing IFNγ secretion and leading to a reduction in tumour burden and an increase in survival.[298]

Directed delivery can also take advantage of microenvironmental changes. OMN-400 utilises pH-activated nanoparticles, with the metabolic acidosis of the tumour microenvironment triggering delivery of the IL2 cargo to the tumour.[268] At normal physiological pH, the IL2 is held inactive, encapsulated within the nanoparticle. However, in the acidic environment inside the tumour, the nano-particle is denatured, and the IL2 is released. Encapsulation resulted in reduced renal clearance and increased tumour retention of IL2, resulting in a decrease in tumour burden when compared to un-encapsulated IL2.[268] In a similar vein, IL2 itself can be engineered to activate in an acidic environment. The Switch-2 IL2 mutein is engineered to have enhanced potency under acidic conditions, aiding tumour rejection with fewer effects in off-target tissues.[353]

## IL2 Muteins with Increased Production

Finally, moving away from modifications, which alter specificity and efficacy of IL2, are muteins which boost production (Table 5). Biological therapeutics are more expensive to produce than their small molecule competitors, making them less

**Table 4** Delivery Systems for Optimised IL2 Dosing

| ***Delivery*** | | | | | |
|---|---|---|---|---|---|
| **Name** | **Modification(s)** | **Species** | | **Clinical Trials** | **References** |
| OMN-400 | pH-activated nanoparticle encapsulating IL2 | Human | Native/WT IL2 | | [268] |
| BNT153 | Liposome encapsulated mRNA transcript of native Human IL2 | Human | Native/WT IL2 | Cancer[269] | [270,271] |
| AVB-001 | IL2-expressing human retinal pigmented epithelial (RPE) cells encapsulated in alginate-based microparticles | Human | Native/WT IL2 | High grade serous adenocarcinoma of the ovary, peritoneum, fallopian tube[272] | [273,274] |
| TG4010 | Modified vaccinia Ankara virus engineered to express the Mucin 1 tumour antigen and IL2 | Human | Native /WT IL2 | NSCLC,[275–278] Prostate cancer,[279] Advanced cancer.[280] | [281–284] |
| SJNB-JF–IL2 | IL2- and Lymphotactin- expressing neuroblastoma cells implanted into tumour site | Human | Native/WT IL2 | Neuroblastoma[285,286] | [287] |
| AML Cell Vaccine | AML Cell Vaccine expressing CD80 and IL2 to boost cancer rejection | Human | Native/WT IL2 | High Risk MDS RAEB-2 and Acute Myeloid Leukaemia[288] | [289] |
| Saltikva | Attenuated strain of Salmonella typhimurium engineered to produce IL2 | Human | Native/WT IL2 | Pancreatic Cancer[290] | [291–293] |
| IL-2-expressing Salmonella | Attenuated strain of Salmonella typhimurium engineered to produce IL2 | Human | Native/WT IL2 | Unresectable hepatic metastases from a solid tumour cancer[294] | [295] |
| Prskavec | Cancer vaccine composed of Prostate-specific antigen (PSA) with IL2 and granulocyte-macrophage colony-stimulating factor (GMCSF) as adjuvants | Human | Native/WT IL2 | Prostate cancer[296] | [297] |
| IL2FP | IL2/IL2Ra fusion with an MMP2/9-specific cleavage site | Mouse | Native/WT IL2 | [298] | [298] |
| ALT-801 | WT IL2 fused with anti-p53 (amino acids 262–272) single-chain TCR | Human | Native/WT IL2 | Metastatic malignancies,[299] Melanoma,[300] Leukaemia,[301] Myeloma,[302] Bladder cancer,[303] Urothelial cancer.[304] | [305,306] |
| Hu14.18-IL2 | WT IL2 fused with anti-ganglioside GD2 antibody | Human | Native / WT IL2 | Neuroblastoma[307–311] and Melanoma.[312–314] | [315–317] |
| DI-Leu16–IL2 | WT IL2 fused with Anti-CD20 (Leu16) antibody | Human | Native / WT IL2 | Non-Hodgkin lymphoma[318–320] | [321,322] |
| Darleukin | WT IL2 fused with anti-Fibronectin (L19) single-chain fragment variable | Human | Native / WT IL2 | Advanced Solid Tumours,[323–325] Melanoma,[326] Lymphoma,[327] NSCLC[328,329] | [330–333] |
| F16-IL2 | WT IL2 fused with anti-Tenascin C (L19) single-chain fragment variable | Human | Native / WT IL2 | Solid tumours,[334,335] Merkel cell carcinoma.[336] | [337–339] |
| TILT-123 | Human 5/3 chimeric adenovirus modified to secrete IL2 and TNF alpha | Human | Native / WT IL2 | Melanoma,[340] Solid Tumours,[341,342] Ovarian Cancer,[343] NSCLC.[344] | [345–348] |
| WTX-124 | An IL2 molecule linked to a half-life extension domain and inactivation domain, that is cleaved in the tumour microenvironment | Human | Native / WT IL2 | Solid Tumours[349] | [350] |
| NNC0361-0041 | Plasmid encoding IL2, transforming growth factorβ1, IL10, and pre-proinsulin. Intended to induce antigen-specific Treg responses in the pancreas. | Human and mouse? | Native / WT IL2 | Type 1 diabetes[351] | [352] |
| Switch-2 | Acid-tolerant IL2 mutein T37H, R38L, T41S, F42Y, K43G | Human | Binds IL2Ra with higher affinity at acidic pH[353] | | [353] |
| PHP.*GFAP*-IL-2 | AAV vector encoding IL2 under GFAP promoter with minocycline on/off system. | Human | Native/ WT IL2 | | [354] |


https://doi.org/10.2147/ITT.S500229

**Table 5** IL2 Muteins with Increased Production

| *Increased Production* | | | | | |
|---|---|---|---|---|---|
| **Name** | **Modification(s)** | **Species** | **Effect** | **Clinical Trials** | **References** |
| K35E | K35E | Human | Enhanced secretion of IL2 human Fc Fusion proteins. | | [355,356] |
| N90R T131R | N90R T131R | Human | Rescues production of E62K Y45K with neutral effect on bioactivity.[43] | | [43] |

accessible to the wider patient population. This is due in part to the expensive processes used in their production, with biological limits on the amount of protein able to be produced in reactors. These limitations arise due to folding constraints that do not exist under the physiological protein concentrations at which these proteins have evolved to be produced—concentrations much lower than those achieved in bioreactors. Often proteins are maladapted to increased production, quickly reaching concentrations where solubility is affected and aggregates form. It is possible to alter production efficiency by mutating IL2. This was first described by Rojas et al, who screened a phage display library of IL2 muteins for those with increased display levels.[355] They found a single mutation, K35E, resulted in increased secretion of IL2-containing fusion proteins up to 20-fold.[355] Taking a more directed approach, we have recently used SoluBIS, a computational algorithm-based method to predict aggregation-prone linear segments of IL2.[43] Human IL2 was found to possess two aggregation-prone regions. Mutations N90R and T131R were predicted to disrupt these regions and help boost production.[43] Whilst alone, this was not significant, these mutations did provide rescue to a poorly produced CD8 bias mutein E62K Y45K. By increasing production efficiency using these muteins, the cost of protein production will be proportionally reduced.

## Clinical Status

Despite credible advances in the field, no designer IL2 molecules have yet been approved by regulatory authorities for routine clinical use. Several designer molecules have, however, reached clinical trials, largely in the oncology space with IL2Rβγ biased-molecules. Bempegaldesleukin (NKTR-214), a pegylated IL2 variant designed to stimulate inflammatory cells, initially appeared promising, advancing to melanoma and renal carcinoma Phase 3 trials in combination with the anti-PD-1 antibody pembrolizumab. However, after failing to meet primary endpoints for response rates and survival, its development was suspended.[357] This may reflect the tumour's ability to suppress immune responses rather than an inherent failure of the molecule itself, highlighting the broader challenge of improving immune checkpoint inhibitor therapies. Similarly, development of BAY 50–4798, an IL2 variant with an N88R mutation, appears to have been discontinued after Phase 1 trials revealed a short half-life (2 hours) and limited advantages over wild-type IL2.[48] The need for frequent dosing and lack of improvements in bioactivity and pharmacokinetics made further development unviable.

Despite limited success of the few designer molecules to complete clinical trials, new IL2 candidates continue to advance into clinical trials. XTX202, an IL-2Rβγ-biased variant, remains inactive until cleaved by matrix metalloproteases in the tumour microenvironment, allowing for localized activation.[358] In preclinical non-human primate studies, XTX202 was well tolerated at doses >42-fold higher than IL2 surrogates, suggesting a significantly improved therapeutic index due to its targeted activation.[358] As of January 2024, XTX202 is actively progressing through clinical trials, with initial results showing a favourable safety profile and localized tumour activation, making it a strong candidate for combination therapy.[359] By integrating localized activation and IL2βγ biasing, XTX202 offers a superior safety profile compared to high-dose IL2 while potentially enhancing anti-tumour immune responses. A plethora of IL2Rα-biased molecules are also available for clinical progression although with a different balance of safety profiles and regulatory barriers compared to the development of IL2Rβγ-based molecules in oncology. While the development of designer IL2 molecules has been a complex and challenging process with several setbacks for first-generation molecules, new multi-strategy approaches and more sophisticated design features offer renewed hope for success.

## Future Directions

While classical point mutations and protein complexes have allowed for the discovery of many interesting designer IL2 molecules, new technologies and design strategies continue to build on this to create more sophisticated candidates. A novel approach to creating receptor bias in IL2 is to start from scratch and build de novo synthetic mimetics, using the receptor binding sites as a starting point. NEO-TRA1 is a synthetic molecule developed by Neoleukin Therapeutics, a company which specializes in de novo protein development using computational, artificial intelligence (AI) methods. NEO-TRA1 is designed as an IL2 signalling mimetic, without intrinsic receptor binding capacity, instead being coupled to an anti-IL2Rα antibody for high selectivity for Tregs cells.[90] Such de novo design allows for fine tuning of the protein, adding and removing custom binding sites. However, as the protein is non-native, the risk of immunogenicity is increased. NEO-TRA1 may prove an interesting test-case of this trade-off. Taking this approach a step further, Neoleukin has developed a de novo mimetic with enhanced specificity to IL2Rβ. Neoleukin-2/15 is a computationally designed IL2Rα-independent agonist of the IL2 and IL-15 receptors.[203] It has a binding site for the IL2Rβ-IL2Rγ heterodimer but not for the IL2Rα subunit. It has four helices – three which are involved in binding and a fourth which holds the first three in place. In vitro, Neoleukin-2/15 stimulates $IL2R\alpha^{-}$ cells more potently that WT IL2, whilst some potency for $IL2R\alpha^{+}$ cells is lost but not completely abolished.[203] In vivo, treatment with Neoleukin-2/15 results in an increase in the CD8:Treg ratio, driving a decrease in tumour burden and increase in survival in mouse models of melanoma.[203] Importantly, in these preclinical models, there are no signs of immunogenicity, despite the non-native structure, and while the clinical development has been suspended, a lack of immunogenicity was also observed in the Phase 1 clinical trial,[360] suggesting the use of de novo proteins may be safer than initially thought. As AI technology improves, it is likely that in silico design of cytokine mimetics will become more common with increased success rates.

Beyond protein engineering, significant advancements are also being made in cytokine delivery systems, including viral vectors and lipid encapsulation. One example of improving the therapeutic properties of IL2 through delivery developed is the use of AAV delivery systems. IL2 can be encoded within an AAV vector, with expression determined by tissue trophism of the capsid. Depending on the capsid and route of delivery, this can cause enrichment within particular tissues, allowing a tissue-biased expansion of Tregs.[361,362] The specificity of delivery can be further enhanced by the use of a tissue-specific promoter. We previously utilised this technique to expand Tregs, specifically in the brain. In this example, IL2 is delivered to the mouse brain using the PHB.B capsid and an astrocyte-specific promoter, GFAP.[354] An additional benefit of these AAV-delivery approaches is the sustained production of the cytokine, allowing steady levels of IL2 to be achieved without a bolus injection or multiple dosing as well as the inclusion of a druggable switch. As yet, these systems have not reached the clinic. An alternative delivery approach is to administer mRNA encoding proteins rather than the protein itself. BNT151 is a lipid encapsulated mRNA which encodes mutated human $IL2R^{\beta\text{-bias}}$ fused with albumin being developed by BioNTech.[189] It is currently in phase 1/2a clinical trials in solid tumours, however no outcomes have yet been published. This method provides several advantages over recombinant protein delivery, including controlled cytokine expression, localized activation, and the potential for combination with cancer vaccines and immunotherapies. Additionally, the ability to produce cytokines in vivo rather than relying on large-scale recombinant protein manufacturing offers significant cost and scalability benefits.

The future of IL2-based immunotherapy is likely to be shaped by continued advancements in AI-driven cytokine engineering and next-generation delivery systems, both of which aim to enhance therapeutic efficacy while minimizing toxicity. As novel de novo cytokines and innovative gene delivery technologies continue to emerge, these approaches may overcome many of the limitations associated with traditional cytokine therapies, paving the way for more precise and effective immunomodulatory treatments.

## Conclusion

IL2 has proven to be fertile territory for biomodifications. Over a hundred altered molecules, mimics or fusions have been made, with five major design classes targeting different aspects of biology: IL2Rα-selective, IL2Rβ-selective, half-life extenders, tissue-specific delivery agents, and super secreters. Many approaches have been tried in both mouse and human, with mouse analogues being crucial for use as research tools and to explore in vivo effects in cases where human

agents do not elicit equivalent cross-reactivity in pre-clinical studies. To achieve these respective changes in biology a multitude of different protein engineering approaches have been applied, including antibody complexing, fusion proteins, point mutations, PEGylation and novel delivery systems, with varying success.

Protein engineering of IL2 has proven to be tricky balancing act, with the native IL2 still the gold standard to beat for clinical efficacy. As seen with many of the aforementioned examples, engineered alterations in specificity often come at a cost of reduced overall bioactivity. As a result, larger quantities of designer IL2 may be needed to reach therapeutic range, increasing cost and the likelihood of off-target effects, mitigating the advantages gained by the enhanced specificity. In addition, the more sophisticated and synthetic approaches such as de novo mimetics may end up enhancing immunogenicity and thus increasing the potential for harmful anti-drug reactions. As more designer IL2 molecules progress further into the clinic, it will be informative to see which molecules provoke such problems.

Currently, although there are many candidates in clinical trials, none of the above designer IL2 molecules have been approved for wide-scale clinical use. The reasons for this are likely to be multifactorial, with a low therapeutic window, improved utility of the native IL2 shifting the goal-posts, and new technologies and approaches rendering first-generation molecules obsolete before they can complete the clinical pipeline. The next generation of IL2 engineering is likely to incorporate multiple design features in a single molecule, improving selectivity and specificity while prolonging half-life and targeting the delivery to the site of action. Such multi-dimensional approaches, harnessing the progress made by different groups, are likely needed to tackle issues of tissue specificity, cellular selectivity, dose titration, production costs, and bio-availability. However, despite the slow progress at the clinical level, the many lessons learned from the incredible protein engineering of IL2 provide a rationale path forward for the modification of other cytokines and immunotherapies.

## Disclosure

Dr Amy Dashwood and Professor Adrian Liston report that The University of Cambridge is joint owner of a patent for AAV-based delivery of IL2 and a pending patent application for IL2 muteins, with the authors being potential financial beneficiaries of commercialization. AL and JD are founders of Aila Biotech Ltd. Amy Dashwood reports a patent GB2412771.4 pending to Cambridge Enterprise Limited; Katholieke Universiteit Leuven; VIB VZW; Babraham Institute. The authors report no other conflicts in this work.

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