


REVIEW

Roles of IL-2 in bridging adaptive and innate immunity, and as a tool for cellular immunotherapy

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Abstract

IL-2 was initially characterized as a T cell growth factor in the 1970s, and has been studied intensively ever since. Decades of research have revealed multiple and diverse roles for this potent cytokine, indicating a unique linking role between adaptive and innate arms of the immune system. Here, we review the literature showing that IL-2 is expressed in a plethora of cell types across the immune system, where it has indispensable functions in orchestrating cellular interactions and shaping the nature and magnitude of immune responses. Emerging from the basic research that has revealed the molecular mechanisms and the complexity of the biologic actions of IL-2, several immunotherapeutic approaches have now focused on manipulating the levels of this cytokine in patients. These strategies range from inhibition of IL-2 to achieve immunosuppression, to the application of IL-2 as a vaccine adjuvant and in cancer therapies. This review will systematically summarize the major findings in the field and identify key areas requiring further research in order to realize the potential of IL-2 in the treatment of human diseases.

KEYWORDS

myeloid cells, monocytes, cyclosporine A, Tacrolimus, calcineurin inhibitors

1 | INTRODUCTION

IL-2 was originally identified in the 1970's as the first T cell growth factor.^{1,2} As a result of this key property, it has been intensively researched ever since: thousands of papers now describe the details of IL-2's molecular and cellular biology (reviewed in Refs. 3,4 and others). What has emerged from these studies is a complex picture that extends well beyond the limited scope of a prototypical T cell growth factor; in fact, IL-2 occupies a central position in all immune responses and during homeostasis, being produced by, and acting upon, a plethora of cell types, with its effects determined by source, target, dose, and context.

Our understanding of IL-2's roles is underpinned by early studies that elucidated its molecular mechanism of expression in T cells, which led to the pivotal discovery of the NFAT family of transcrip-

tion factors.^{5,6} These studies showed that T cell receptor stimulation results in the opening of calcium channels in the cell membrane, and so increases levels of intracellular calcium, leading to activation of the calcium- and calmodulin-dependent serine/threonine protein phosphatase, calcineurin (CN). Activated CN then dephosphorylates NFAT in the cytoplasm, causing a change in its conformation that exposes a nuclear translocation sequence, leading to initiation of transcription of target genes, including of *IL2*.^{7,8} What we now know is that this process does not occur only in T cells, but also in cells of the innate immune system, such as dendritic cells (DC), monocytes and NK cells.⁹

NFAT family members are widely expressed across the immune system, but it is NFAT1 and NFAT2 that are required for IL-2 production.^{10,11} Despite this dependence, NFATs alone are unable to initiate gene expression, and require cooperation with other

Abbreviations: AML, acute myeloid leukemia; CAR, chimeric antigen receptor; CN, calcineurin; CsA, cyclosporine A; DC, dendritic cell; GVHD, graft-versus-host disease; TIL, tumor-infiltrating lymphocytes; Treg, T regulatory cells.

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transcription factors including NF- κ B,¹² AP-1,¹³ or T-bet¹² to promote IL-2 expression in different cell types. In contrast, in T regulatory cells (Treg), the binding of NFAT together with the FoxP3 transcription factor represses IL-2 expression,^{14,15} explaining the dependence of Treg on IL-2 from other cellular sources. Altogether these molecular interactions aim to assure balanced expression of IL-2 in innate and adaptive immune cells, which is essential for orchestrating an optimal immune response.

One reason that the discovery of NFAT as a molecular regulator of IL-2 signaling was so important is that it led to a means to target IL-2 expression and achieve clinical immunosuppression. It was first shown that inhibition of the phosphatase activity of CN using the fungal isolate cyclosporine A (CsA) or the synthetic inhibitor FK506 resulted in impaired NFAT signaling,^{8,16} leading to reduced expression of IL-2, and an efficient immunosuppressive effect.¹⁶ These immunosuppressive therapeutic approaches have since revolutionized the field of organ transplantation, reducing the rejection of solid grafts,¹⁷ and limiting graft-versus-host disease (GVHD) in hematopoietic stem cell transplant recipients.^{18,19} The success of CN inhibitors in the transplantation setting led to trials and eventual widespread use of CsA and FK506 in other pathologies, including psoriasis, eczema, rheumatoid arthritis, and Crohn's disease.²⁰ However, simultaneously, the recognition of the importance of IL-2 for maintaining immune-suppressive Treg has led to the somewhat counterintuitive strategy of treating some autoimmune conditions with low doses of exogenous IL-2 summarized in Table 1. Together, these opposing strategies demonstrate the complexity of IL-2's roles across the immune system, and the ongoing challenges of understanding this enigmatic cytokine.

Alongside progress in understanding NFAT-mediated signaling leading to IL-2 production, a further layer of complexity has more recently become apparent from studies of IL-2's downstream signaling in target cells. Complex molecular mechanisms control cellular sensitivity to IL-2. One mechanism is through variable subunit composition of the heterotrimeric IL-2 receptor (IL-2R), whose affinity for the cytokine is determined by the combinations of the IL-2R α (CD25), IL-2R β (CD122), and the common gamma or γ_c (CD132) chains.^{3,54} The "low affinity" IL-2R is formed only by IL-2R α , whereas the "intermediate affinity IL-2R" consists of IL-2R β and γ_c , and the "high affinity IL-2R" is comprised of 1 of each of the 3 chains.⁵⁵ The expression of different versions of the IL-2R on responder cells (also shown in Fig. 1) has a profound impact on the effects of IL-2 on those cells (reviewed Ref. 56). Furthermore, the IL-2R β and γ_c chains are also able to bind IL-15 and IL-21,^{3,57} which are involved in the regulation of immunologic processes through overlapping actions or by competing with each other, and with IL-2, for receptor engagement.

Therefore, although the first role of IL-2 in T cells was described 50 years ago, advances in our understanding of the regulation of its production and signaling, and how it might be used effectively and precisely to modulate patients' immune systems, are still very much a topic of ongoing research. This review will examine recent findings in these areas, with particular emphasis on IL-2's emerging roles in the crosstalk between innate and adaptive immune cells, and its current and future uses in clinical immunotherapy.

2 | ROLES OF IL-2 IN ADAPTIVE AND INNATE IMMUNITY

2.1 | Roles of IL-2 in orchestrating adaptive immunity

IL-2 is a major modulator of the development, homeostasis and functions of various T cell subsets, and therefore has key role in orchestrating the balance of adaptive immune responsiveness. It has long been known that in the thymus IL-2 fuels the initial proliferation of naïve T cells⁵⁹ and is essential for maturation of Treg.⁶⁰ At the same time, IL-2 is also responsible for the expansion and cytotoxicity of effector T cells.⁵⁶ What remains debated is which cell types in the thymus are the key cellular sources of IL-2 for the different lymphocyte subtypes. For example, in the case of murine Treg, while 1 study showed that DC-derived IL-2 was important for their development in an ex vivo thymic slice model,⁶¹ another group using IL-15^{-/-} mice with *il-2* also deleted in T cells, B cells and DC, reported that only T cell-derived IL-2, and not IL-2 from B cells or DC, was essential for Treg development in the thymus in vivo.⁶²

In the periphery, IL-2 is a master regulator of T cell biology. Effector T cells are the main producers of IL-2 that they use for autocrine stimulation of their own proliferation, cytotoxicity, and the downstream development of memory T cells.⁶³ T cell homeostasis also relies on paracrine IL-2 signaling.⁶⁴ Interestingly, studies on human DC have revealed their ability to capture and present either DC- or T-cell produced IL-2 at the immunologic synapse in order to stimulate antigen-specific T cell proliferation.⁶⁵ These findings highlight a novel mechanism by which even extremely small amounts of IL-2 can be critical for the initiation of immune responses by acting, quite literally, as a molecular bridge/connection between the effector cells of the innate and adaptive arms of immunity.

Although the roles of IL-2 in stimulating immune responses are well known, early studies in mice lacking IL-2 or its α or β receptor chains also uncovered the role of IL-2 in preventing autoimmunity,⁶⁶⁻⁶⁸ which we now know relates to the dependence of Treg on this cytokine for their development and maintenance.⁶⁴ Similar to effector T cells in the periphery, studies in the mesenteric lymph nodes have revealed the importance of both T cell- and DC- derived IL-2 in Treg homeostasis. For example, in the gut mucosa, tolerance is largely maintained by Treg,⁶⁹⁻⁷¹ with IL-2 playing a key role via a range of mechanisms: mucosal Treg are maintained by the IL-2 from naïve CD4⁺ T cells⁷²; whereas in parallel, IL-2-driven Treg development inhibits the differentiation of naïve CD4⁺ T cells into Th17 cells,⁷³ though the cellular source of this IL-2 is unknown. It is an open question whether the same cellular sources are important for Treg maintenance across all lymphoid tissues, or whether the dominant cellular source of this cytokine varies by microenvironment. Alongside its importance for Treg functions, recent data suggest that mucosal-associated invariant T cells, which are innate T cells, necessary for gut immune system regulation, are also dependent on IL-2.⁷⁴ Taken together these studies show how IL-2 produced by innate immune DC and adaptive immune T cells, in the gut in particular, have distinct but complementary roles in managing the immune environment in the

TABLE 1 Overview of different approaches to the therapeutic use of IL-2

Group of disorders	Disease	References	IL-2 dose	Note	Major findings
Autoimmune diseases	Systemic lupus erythematosus	21-25	Low		IL-2 corrected Treg defects, promoted Treg/NK cell expansion → restoration of immune homeostasis
	HCV-induced vasculitis	26	Low		IL-2 led to Treg recovery without adverse effects
	Type 1 diabetes	27-30	Low		Determination of optimal doses of aldesleukin needed to expand Tregs. Partial desensitization of Treg to IL-2 on day 3 after treatment → improvement of dosing regimens for future trials. Selective Tregs responses to low IL-2 through IL-2-dependent transcriptional amplification mechanism.
	Alopecia areata	31	Low		IL-2 led to increased Treg count. No adverse event was reported.
	Rheumatoid arthritis	25	Low		IL-2 induced Treg expansion and activation without effector T cell activation.
	Crohn's disease, ulcerative colitis	25	Low		IL-2 induced Treg expansion and activation without effector T cell activation.
Transplantations	Graft-versus-host disease	32-35	Low		IL-2 administration was associated with preferential, sustained expansion of functional Tregs (while maintaining the immune response to infections) resulting in reduced chronic GVHD. IL-2 restores homeostasis of CD4 ⁺ T cell subsets through selective increase of Stat5 phosphorylation in Tregs and a decrease of phosphorylated Stat5 in conventional CD4 ⁺ T cells.
Inflammatory condition	Chronic kidney disease (CKD)	36	Low		Treg count is lower in CKD patients. IL-2 selectively expanded CD4 ⁺ CD25 ^{hi} and CD4 ⁺ CD25 ⁺ FoxP3 ⁺ Tregs and up-regulated the expression of FoxP3 mRNA.
	Cardiovascular diseases (preclinical model)	37	Low	IL-2/anti-IL-2 complex (IL-2C)	Tregs can suppress immunologic damage in myocardial ischemia/reperfusion injury (MIRI). IL-2C led to Treg expansion resulting in attenuated MIRI and improved myocardial recovery (mouse model).
Infection	Tuberculosis (TB)	38,39	High		IL-2 immunotherapy appears to promote the proliferation and conversion of CD4 ⁺ (Th1) and NK cells and decrease Th17 and Treg populations. The improved sputum culture and smear conversion of TB patients were reported.
	Persistent viral infections	40		IL-2C	IL-2C improved IL-2 signaling and enhanced the quality of the CD8 T cell response via up-regulation in granzyme B production increasing cytotoxicity and higher numbers of virus-specific CD8 T cells (mouse model).
	HIV	41,42		Intermittent administration of IL-2	IL-2 induced sustained increase of CD4 ⁺ T cells.
Cancer	Melanoma	43-46	High		IL-2 therapy displayed durable response and antitumor activity in some patient with metastatic melanoma. Selective inhibition of IL-2-mediated enhancement of T may be beneficial for IL-2 therapy.
	Renal cancer	45,46	High		IL-2 therapy displayed durable response in subset of patients. Selective inhibition of IL-2-mediated enhancement of T may be beneficial for IL-2 therapy.
	Acute myeloid leukemia	47-51	High, low, IL-2 diptheria toxin (IL2DT)		IL-2 used to expand the number of circulating NK cells before high-dose chemotherapy and autologous hematopoietic cell transplantation. Depletion Tregs with IL2DT improved efficacy of haploidentical NK cell therapy. HD IL-2 therapy. HD IL-2 therapy was related with adverse reactions.
	Lymphoma	52			IL-2-activated cells generate PBMCs with enhanced cytotoxicity against NK-resistant targets, and increase cytokine levels.
	Multiple myeloma	53			IL-2 and phosphostim stimulate in vitro expansion of $\gamma\delta$ T cells which are efficient in killing of human myeloma cells.

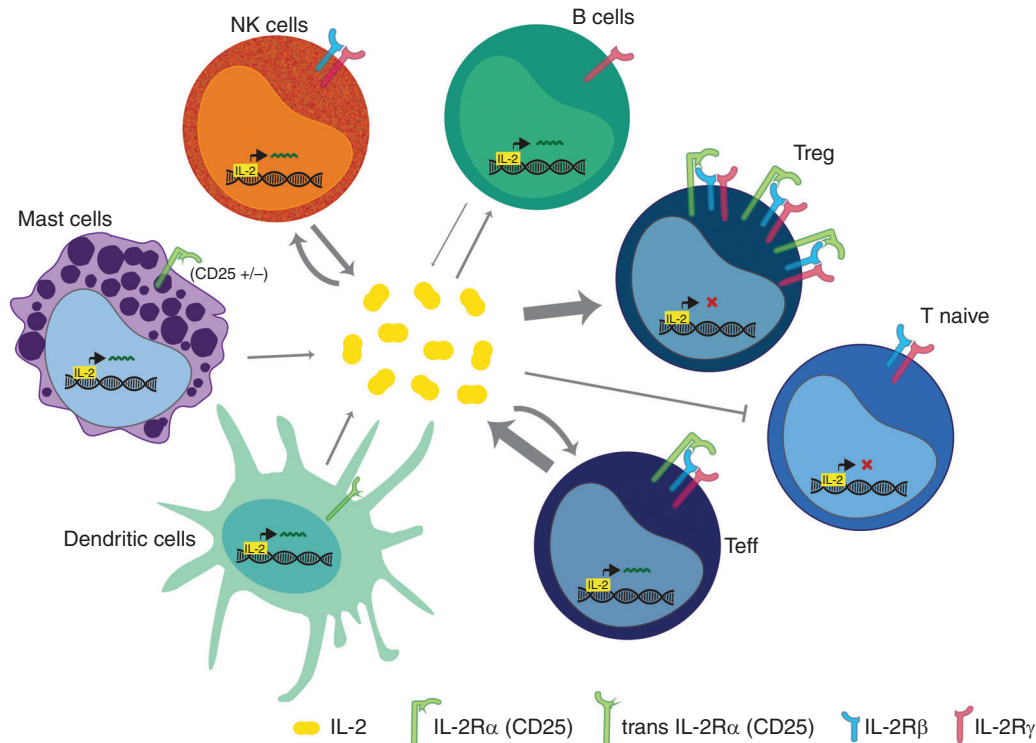


FIGURE 1 IL-2 as a bridge between adaptive and innate immunity. Both adaptive and innate immune cell subsets have been identified as a source of IL-2. Here, we summarized the main IL-2 producers as well as the capacity of these cells to bind IL-2 via 1 of the IL-2 receptors: low affinity IL-2R α (CD25) dimeric intermediate affinity IL-2R consisting of IL-2R β (CD122) and the common γ -chain (CD132) and high affinity IL-2R—heterotrimer of subunits α , β , and γ -chain. Activated T cell represents main producer of IL-2, although IL-2-driven regulation of B cell fate has been shown and to certain extent B cell are able to produce IL-2 as well. Naïve T cells are not responsive to IL-2 and they lack CD25. During early stage of immune response, after antigen activation, the expression of CD25 is induced leading to IL-2 expression, T cell proliferation, and differentiation. Unlike other T cell subsets, Treg cells constitutively express high levels of CD25, with intermediate levels of CD122 and γ chain and are dependent on exogenous source of IL-2. Within innate immune cells IL-2 expression was proved in NK cells, activated DC and mast cells. IL-2 is necessary for many NK cell functions. Although NK cells secrete IL-2, they are likely dependent on T cell derived IL-2, whereas intermediate affinity receptor helps to bind IL-15. Some subsets of mast cells express IL-2R α although likely independently of its IL-2-related functions.⁵⁸ DC are able to trans present IL-2 through CD25 and thus mediate T-cell activation

periphery. Whether IL-2's role was essential or was overlapping/redundant with that of other cytokines sharing the same beta and gamma receptor chain, such as IL-15, was for a long time controversial; nevertheless, experiments in mice with an IL-15^{-/-} background have now distinguished specific functions of IL-15 in the maintenance of CD8⁺ memory T cells, whereas IL-2 is indispensable for the maintenance of Treg.^{69,75}

Overall, it is now clear that IL-2 orchestrates T cell homeostasis through several different mechanisms ranging from paracrine signaling⁶⁴ to cross-presentation of T cell produced IL-2 by CD25-expressing DCs during the T-DCs interaction.⁶⁵ In summary IL-2 from both innate and adaptive immune cell sources plays the key role in T cell activation during the primary immune response and throughout reactivation of memory T cells; furthermore the other important role of IL-2 is to establish negative regulatory feedback loop around the T cell response by driving the expansion of Treg populations.³ The plasticity of T cell subsets' capacity to produce or sense IL-2 creates a complex regulatory environment controlling the process of adaptive immune responses on different levels. IL-2 is indispensable for the regulation of both immune activation and immunosuppressive responses

to foreign or self-antigens, and is the key to homeostatic maintenance of T cell populations.

2.2 | Roles of IL-2 in orchestrating innate immunity

2.2.1 | Myeloid cells and IL-2

Although IL-2 was long-considered purely a T cell cytokine, there is clear evidence that functional calcium-NFAT signaling also occurs within some myeloid cell subsets, as reviewed.^{20,76,77} Activation of the CN-NFAT pathway was first described in DC in response to whole bacteria or LPS,^{78,79} and since, also in response to stimulation with the fungal components zymosan,⁸⁰ or curdlan.⁸¹ These findings led others to investigate the activation of the CN-NFAT pathway in macrophages, which was found to be stimulated upon phagocytosis of fungal conidia,^{82,83} and in human macrophages by exposure to *Aspergillus fumigatus*.⁸⁴

Intensive research followed these initial findings, aiming to establish the molecular mechanisms of NFAT activation in myeloid cells. Together, they revealed multiple pathways leading to CN-NFAT

signaling: in murine macrophages and DC, Dectin-1 ligation by yeast or zymosan particles resulted in NFAT activation⁸⁵; whereas murine DC exposed to LPS or whole bacteria showed CN-NFAT activation followed by IL-2 expression that relied on TLR4 ligation.^{78,79} Later studies refined this work by showing that CD14 was capable of mediating bacterial ligand-induced CN-NFAT activation alone in these cells.⁸⁶ Furthermore, the interaction of TLR-9 and Dectin-1 with β -glucans within the fungal cell wall leads to activation of Bortom's tyrosine kinase (BTK)-CN-NFAT in murine macrophages during experimental pulmonary aspergillosis.⁸² Last, paralleling aspects of T cell biology, the ligation of Dectin-1 and other C-type lectin receptors on murine macrophages initiates the activation of the ITAM, resulting in NFAT activation through Syk phosphorylation.⁸⁵

Perhaps due to the pervading dogma of IL-2 as a T cell cytokine, few studies that reported CN-NFAT activation in myeloid cells initially looked for IL-2 expression. Therefore, the ability of NFAT to initiate IL-2 transcription in myeloid cells, and the possible biologic relevance of this, remained an open question for many years. Granucci et al.^{78,79} first identified the expression of IL-2 in murine DC, but it was more than a decade before Yu et al.⁸⁷ successfully used a human DC cell line to perform genome-wide mapping and found the target sites of NFAT1 binding, confirming that NFAT1 was able to modulate expression of IL-2 in human DC.

At around the same time, evidence began to build for an important biologic role of innate immune cell-derived IL-2 in modulating systemic adaptive immune responses. Mice lacking IL-2 expression in CD11c⁺ cells were found to express higher levels of IL-17 in their lungs, resulting in increased susceptibility to infection with *A. fumigatus* as a result of a pathologic Th17 response.⁸⁸ Parallel clinical observations were made in human patients, where there is some evidence that treatment with CN inhibitors increases patients' susceptibility to fungal infections, but the overall picture remains somewhat unclear.^{20,89} A similar approach has also been used to investigate the role of myeloid cell-derived IL-2 in gut homeostasis. Mencarelli et al.⁹⁰ compared mice lacking either CN or IL-2 in either CD11c⁺ or LysM⁺ cells, and showed that impairment of CN or of IL-2 in myeloid cells leads to spontaneous intestinal inflammation and increased susceptibility to experimental colitis. Interestingly, they also found that distinct DC subsets in the gut produce different levels of IL-2, with CD103-expressing DC the most, although the biologic significance of this difference is not yet known. Interestingly, this study also confirmed earlier findings by Han et al.⁹¹ revealing a CN-NFAT signaling-independent mechanism of IL-2 production in murine DC, instead utilizing the TLR-TRAF6-NF κ B cascade. In the Han study, mice lacking TRAF6 specifically in DC exhibited spontaneous enteritis that was associated with unrestrained Th2 responses and decreased Treg numbers; importantly, DC in these mice expressed significantly lower levels of IL-2, and the aberrant immunophenotype could be rescued by the addition of exogenous IL-2,⁹¹ implicating DC-derived IL-2 in the maintenance of adaptive immune tolerance in the murine gut. Intriguingly, the same study also identified the microbiota as an important component of the TRAF6-deficient DC immunophenotype, but the mechanisms underlying this phenomenon and the relationship between intestinal microbiota,

DC-derived IL-2 and T cell homeostasis in the gut were elusive. Recent findings may, however, provide a further piece of the jigsaw: Zhou et al.⁹² found that innate lymphoid cells are also important producers of IL-2 in the murine intestine, dependent on macrophages being stimulated by the microbiota to produce IL-1 β . Importantly, this study also showed that innate lymphoid cells from patients with Crohn's disease produced significantly less IL-2, which was associated with lower frequencies of Treg in their intestine.

Taken together, there is now clear evidence for an important role of innate cell-derived IL-2 in mediating adaptive immune homeostasis and responses to microorganisms (whether pathogenic or commensal) in the lung and gut. Future studies are needed to resolve which cell types are important in different locations/situations within these tissues, and also in different tissues. Pressing research questions remain particularly in the area of tolerance in the gut, driven by innate cell-derived IL-2 production, and linked to interactions with the microbiota. Given the observations in human Crohn's patients made by Han et al., further studies in this area could be highly productive in understanding immune-related gut pathologies and in developing improved immune-targeted treatments.

2.2.2 | NK cells and IL-2

Immune surveillance provided by NK cells is a key mechanism to eliminate infected or cancerous cells. NK cell expansion, maturation, activity, and cytotoxicity are strongly dependent on levels of IL-2.^{54,93,94} Although NK cells are able to express their own IL-2 upon activation of the CN-NFAT pathway,⁹⁵ other reports have described their dependence on IL-2 produced from T cells,⁹⁶ and, more recently, on DC-derived IL-2 for activation of IFN- γ production.^{97,98}

IL-2 also sits at the crossroads of innate NK cell regulation and the regulation of Treg from the adaptive arm of the immune system. In NK cells, the absence of various chains of the IL-2 receptor, or the lack of IL-2, results in impaired NK cell homeostasis.⁵⁶ In particular, CD127⁺ immature NK cells expand in an IL-2-dependent manner, which is strongly inhibited by presence of Treg due to competition for IL-2; accordingly, depletion of Treg results in expansion of NK cell numbers.⁹⁹ Mechanistically, CD127⁺ NK cells are thought to compete for IL-2 binding with Treg via expression of the high affinity IL-2R α , whereas the IL-2R γ_c chain, which recognizes IL-2, IL-15, and IL-21, mediates the multiple facets of NK cell activation, maturation, and proliferation that are modulated by these closely-related cytokines.^{100,101}

3 | IL-2 IN IMMUNOTHERAPY

The prominent role of IL-2 in T cell stimulation led to it being the first human cytokine employed therapeutically. Almost 4 decades ago, IL-2 was used with some success to treat cancer,¹⁰² and today, researchers continue to dissect its importance in this disease, with a recent study showing that single nucleotide polymorphisms in the IL-2 gene are associated with colorectal cancer prognosis.¹⁰³ However, the range of conditions that IL-2 is used to help treat stretches beyond cancer to

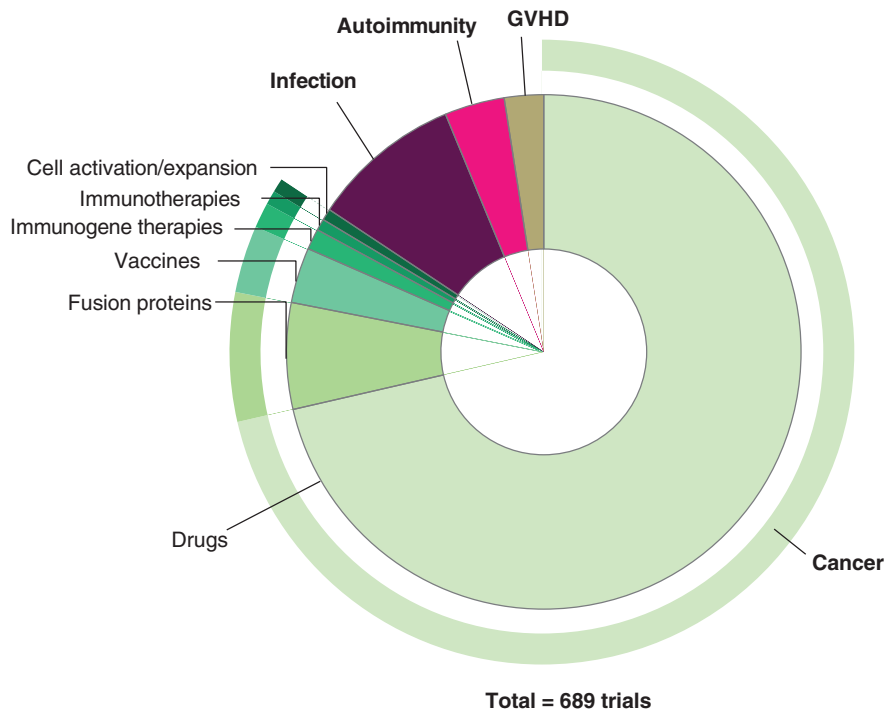


FIGURE 2 Overview of recent clinical studies using IL-2 alone or in combination with other drugs in the therapy against cancer, autoimmune disorders, graft-versus-host disease, or infection

autoimmunity and chronic infections. Therapeutic approaches involving IL-2 can be broadly divided into those using the cytokine directly in vivo either alone or together with adoptive cell transfer, and those employing IL-2 for the pretreatment or expansion of therapeutic cells in vitro.

3.1 | Direct uses of IL-2 in vivo or in combination with adoptive cell transfer protocols

Intense research and numerous clinical trials culminated, in 1992, in the approval of IL-2 infusion as the first licensed immunotherapy for the treatment of renal cancer.¹⁰² Since then many clinical trials testing IL-2's ability, either alone or in combination with other therapeutic approaches, to treat a range of conditions have been conducted or are ongoing (Fig. 2). Although high-dose IL-2 remains a good treatment option for a subgroup of metastatic renal cancer and melanoma patients, its more widespread use has been limited by its toxicity, relatively short half-life, and variability in patient responses.¹⁰⁴ Despite this, therapy related mortality remains very low, and IL-2 treatment can offer significant improvements to survival, particularly in patients with metastatic melanoma.⁴³

Although high-dose IL-2 therapy has mostly been used to target late-stage cancers, low-dose infusion therapies were developed to promote Treg expansion and thereby treat autoimmunity and disorders of inflammation. Following promising data from experimental models, the first clinical studies in 2011 confirmed that low-doses of IL-2 were effective in treating GVHD and hepatitis virus C-induced vasculitis via their proliferative effects on Treg.^{32,26}

This proven potential leads to ongoing research into ways to optimize IL-2 therapy, by manipulating, for example, dosage or route of

administration, or via the administration of engineered IL-2 derivatives (see trails listed in Table 1 and strategies illustrated in Fig. 2). An important development has been the generation of protocols for the production of clinical grade recombinant fusion proteins. This allowed the first progress towards extending IL-2's half-life, by using an engineered IL-2 analog with improved binding and activator function.¹⁰⁵ The same technology also permitted the investigation of strategies targeting IL-2 to specific cells at the site of action. For example, a fusion molecule of a fragment of diphtheria toxin conjugated to IL-2 (Ontak[®]) or its improved equivalent E7777 has been investigated for its effectiveness in Treg depletion in clinical trials.^{106,107} In this case, when the fusion protein is bound and internalized by cells expressing IL2-R α , the diphtheria toxin is released from acidic vesicles into the cytoplasm where it inhibits protein synthesis, leading to subsequent cell death. Nevertheless, the safety, efficacy as well as exact effect of this agent on immune cells remain to be elucidated.

Other novel approaches include administration of IL-2 in immune complexes. These have been used to activate the immune system during chronic viral infections, such as HIV or herpes, where the IL-2 therapy resulted in increased numbers of Th cells.⁴⁰⁻⁴² Recent reports also describe the successful combination of IL-2 treatment with cell cycle checkpoint inhibitors, which was able to overcome previous resistance to these drugs in patients with advanced melanoma,¹⁰⁸ paving the way for further studies on the adjuvant use of IL-2 to improve responses to existing chemotherapeutic agents.

In summary, IL-2 continues to be used and explored as part of the therapeutic protocol for a large number of diseases (Table 1); however, its success has until now been limited due to a short in vivo half-life, its toxicity, and the cytokine's ability to amplify Treg. More recent studies have focused on how to overcome these drawbacks, and have

identified the use of engineered forms of IL-2,^{109,110} and its combination with traditional chemotherapeutic drugs¹⁰⁸ as highly promising strategies. A pressing area in need of further work is how to understand which patients will benefit most from IL-2 immunotherapies, and the mechanisms underlying variable responses to these treatments.

3.2 | Uses of IL-2 in cellular immunotherapy expansion protocols

Together with the success of IL-2 direct infusion therapies, this cytokine is also an indispensable tool for the *in vitro* expansion and activation of T cells including chimeric antigen receptor (CAR) T cells, $\gamma\delta$ T cells or NK cells prior to adoptive transfer. The first clinical studies in this area, over 3 decades ago, used IL-2-expanded autologous tumor-infiltrating lymphocytes (TIL) to treat metastatic melanoma.¹¹¹ However, the application of this approach to other cancers was limited by low expansion of TIL from some patients, the lack of available TIL from other tumor types, and tumor evasive mechanisms. To harness the power of IL-2 while overcoming the issues associated with TIL transfers, researchers turned to NK cells.

NK cells can exert powerful antitumor effects, which led to interest in their use for cancer immunotherapy.^{112,113} Initial studies used the patients' own purified NK cells that had been cultured *in vitro* with IL-2, and transferred back into the patient following lymphodepletion. In patients with advanced renal cancer or melanoma, the cultured NK cells were able to lyse tumor cells *in vitro* but did not induce clinical responses *in vivo*.¹¹⁴ Investigations in mice suggested that combining IL-2 with the closely related cytokine IL-15, and IL-18, could generate NK cells with antitumor actions that were retained *in vivo*.¹¹⁵ Since then, improvements in expansion protocols coupled with the elimination of normal lymphocytes through nonmyeloablative conditioning has increased the success of clinical studies in this area (reviewed in Ref. 102).

Related work has focused on the application of IL-2 during haplo-identical NK cell infusion treatment for hematologic malignancy. Initial studies reported complete hematologic remission in 30% of poor-prognosis acute myeloid leukemia (AML) patients treated with subcutaneous IL-2 after NK cell infusion⁵⁰; however, the success of this strategy was limited by the rapid expansion of Treg cells following IL-2 administration.^{51,116} To overcome this, following promising data in mice,^{92,117} an engineered IL-2 diphtheria toxin construct was used to deplete Treg in AML patients prior to NK cell infusion; this resulted in increased donor NK cell expansion post-transplant and significant improvements in remission and survival rates.⁵¹

Alternative strategies have looked at the use of an NK cell line, NK-92 genetically engineered to express own IL-2, in cellular immunotherapies for pathologies that are difficult to target with CAR T cells, such as AML.^{118,119} Preclinical testing resulted in promising findings as NK-92 improved survival in an AML allograft model.¹²⁰ This approach is paving the way for the use of genetically-modified NK cells in the clinic.

As well as NK cells, $\gamma\delta$ T cells also have the potential to express strong antitumor activities.^{121,122} When $\gamma\delta$ T cells are expanded *in vitro* in the presence of IL-2 and a synthetic agonist,⁵³ or IL-15¹²³⁻¹²⁵ they

exhibit powerful killing of human cancer cells and cell lines. These promising data led to trials in human cancer patients. When daily IL-2 treatment was used as part of an autologous $\gamma\delta$ T cells transfer protocol in a small group of people with advanced hematologic malignancy, 3 out of 4 achieved complete remissions lasting between 2 and 8 months.¹²⁶

Here, we have summarized the main strategies used to expand cells for adoptive transfer therapies. Of note, the majority of the reported studies have shown success using these immunotherapies in patients with late stage and/or refractory disease, some of whom had already been treated unsuccessfully with IL-2 alone or by hematopoietic stem cell transplant. These therapies are likely to be even more successful in patients with earlier stage disease, where higher functioning immune cells would be expected and tumor immune-suppressive and evasive mechanisms would be less established, and/or in combination with established chemotherapeutic protocols. Alongside, however, it is important to keep in mind that IL-2's central position at the intersection of adaptive and innate immunity means that strategies manipulating its levels either locally or systemically will directly affect the broader immune landscape. At this time there are few data on how the administration of IL-2 affects other nontargeted immune subsets, and such findings might prove important for avoiding treatment-associated side effects or realizing the full efficacy of IL-2 as a therapeutic agent.

4 | CONCLUSIONS AND PERSPECTIVES

It is a testament to IL-2's importance and enduring intrigue that it has been so actively researched for the past 50 years, and we are still making new discoveries even now. Here, we have highlighted the roles of IL-2 in adaptive and innate immunity, and at their intersection, confirming IL-2 as a key factor in the maintenance of immune homeostasis across multiple cell types (Fig. 1). Furthermore, many of these findings have now been successfully translated into effective immunotherapies. The overview of currently running clinical studies assures that IL-2 use in immunotherapy will be further expanding in the future, most probably broadening amount of clinical protocols and well as targeted disorders.

AUTHORSHIP

KB wrote manuscript and prepared the figures, JF conceptualized and wrote manuscript.

DISCLOSURES

The authors declare no conflicts of interest.

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