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# Natural and modified IL-2 for the treatment of cancer and autoimmune diseases

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ABSTRACT

Interleukin-2 (IL-2) is a pleiotropic cytokine required for both effector lymphocyte proliferation/differentiation and regulatory T cell expansion/survival. Ability to receive IL-2 signals is defined by the affinity to distinct IL-2-receptor-complexes expressed on each subset of cells. While IL-2 targets anti-tumor cytotoxic lymphocytes (CTLs) for the treatment of patients with melanoma or renal cell carcinoma, IL-2 directed at regulatory T (Treg) cells could have potential therapeutic value in several immune-related diseases including chronic graft-versus-host disease (cGVHD), type 1 diabetes (T1D) and systemic lupus erythematosus (SLE). A variety of IL-2 alteration has been made to deliver IL-2 to the proper target, including mutant IL-2, IL-2-fusion proteins and anti-IL-2 antibodies. Experimental and clinical trials using IL-2 are expanding to diverse group of diseases including solid organ transplantation. Although the sustainability and efficiency of IL-2-responding cells in controlling disease activity are still not fully understood, the results of clinical trials will provide a basis of the most effective regimen for each disease.

## 1. Introduction

IL-2 was discovered more than 40 years ago as a soluble stimulator of leukocytes, produced by T cells. Identification and cloning of human IL-2 gene in 80's and the advent of recombinant DNA technology enabled over-physiological administration of recombinant IL-2. IL-2 was recognized as a growth factor of T cells and NK cells, and has shown anti-tumor effect promoting the activity of these cells. As a result, IL-2 was considered in cancer immunotherapy drug in 90's for renal cell carcinoma and melanoma. However, concept of IL-2 as an exclusive growth factor was questioned by the fact that IL-2/IL-2 receptor-deficient mice develop severe autoimmunity with uncontrolled lymphoproliferaion [1]. Identification of regulatory T cells (Tregs) and determination of indispensable roles of IL-2 for Tregs have resolved the contradiction. Tregs express IL-2 receptor complexes, IL-2R$\alpha\beta\gamma_c$ (CD25, CD122, CD132), which bind with high afinity IL-2. IL-2 is essential for differentiation, expansion and survival of Tregs say Treg cells not Tregs everywhere. On the other hand, $CD8^+$ T cells and NK cells express dimeric receptor IL-2R$\beta\gamma_c$ (CD122, CD132) with intermediate affinity to IL-2 [2]. Therefore, high amounts of IL-2 is required for the expansion of cytotoxic lymphocytes in vivo whereas Tregs require low doses of IL-2. Treg cells represent one potential bottleneck for cancer immunotherapy because they suppress the expansion and function of of cytotoxic lymphocytes. A variety of modified IL-2 and anti-IL-2 antibodies has been developed to overcome individual unfavorable effects. Numerous clinical trials have been done and are now ongoing using IL-2/modified IL-2 for the treatment of cancer, transplant rejection and autoimmune diseases. In this review, IL-2 based therapies in each disease area are discussed along with problems and concerns which have arisen.

## 2. Cancer immunotherapy and IL-2

### 2.1. High-dose IL-2 for cancer immunotherapy

The first patient with metastatic melanoma received IL-2 achieved a complete response and remained disease-free for 29 years [15]. In a phase II trial of IL-2 for the treatment of metastatic melanoma, 600,000–720,000 IU / kg of IL-2 (For example, 18MIU of aldesleukin = 1.1 mg protein) were administered every 8 h for up to 14 consecutive doses and partial or complete responders were around 17% [3]. IL-2 therapy of consecutive series of 509 patients with progressive metastatic melanoma or renal cell carcinoma attained an objective response of 22.6% for renal cancer and 16.3% for melanoma [4]. High-dose IL-2 therapy was approved by FDA for patients with melanoma or metastatic renal cell carcinoma. However, IL-2 is effective for a limited subset of patients and there is no appropriate marker that can identify those patients who will respond to IL-2. High-dose IL-2 therapy usually

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involves many adverse effects at totally more than 80% including diarrhea, fever/chills, arrhythmia, dyspnea, renal dysfunction [5]. These are mainly caused by vascular leak and fatal edema in multiple organs such as lung, heart, kidney and central nervous system. High-dose IL-2-associated mortality rate is reported up to 4% [6]. Therefore, completion of the treatment course is difficult because of induction of side effects. Other than the adverse effects, the limitation of this therapy involves Treg cell expansion, possibly leading to the suppression of anti-tumor immune responses. Moreover, repeated stimulation of lymphocyte induces unresponsiveness and activation-induced cell death (AICD) through the expression of Fas (CD95), which is one of the mechanisms of peripheral self-tolerance [7,8]. IL-2 promotes AICD of T cells when they are repeatedly stimulated with cognate antigen [9], resulting in reduction of the efficacy of IL-2 therapy. Therefore, injection of in-vitro expanded anti-tumor lymphocytes has been considered and tried to improve responses of IL-2 therapy.

### 2.2. Adoptive cell therapy and IL-2

Cytotoxic lymphocytes including $CD8^+$ T cells and NK cells were originally called lymphokine activated killer (LAK) cells [10]. LAK were known to expand by IL-2 and exert tumor-lytic effect, therefore in vitro expanded LAK cells were transferred to patients with cancer in addition to IL-2 therapy and improved survival was observed [11]. However, a randomized trial of IL-2 treatment in 181 patients with melanoma and renal cancer with or without LAK cells did not showed significant improvement of survival [12]. Thereafter, in vitro expanded tumor-infiltrating lymphocytes (TILs), which were obtained from resected tumors or lymph nodes, were adoptively transferred to the patients with metastatic melanoma in combination with IL-2 therapy and the rate of response was greater in those patients who were treated with TILs from younger cultures, TILs with shorter doubling times, and TILs that exhibited higher lysis against autologous tumor targets [13,14]. To obtain more objective responders, improvement of treatment protocols has been achieved with IL-2 therapy, such as preparative lympho-depletion chemotherapy and total body irradiation. As a result, the highest response rate reached 72% [15,16]. Currently, several clinical trials of adoptive transfer of TILs with IL-2 are ongoing for the treatment of melanoma and mesothelioma (Table 1).

Based upon the concept that TILs include cells expressing tumor-antigen specific TCR, TCRs were cloned from isolated melanoma antigen MART-1- and gp100-reactive TILs, transduced into $CD8^+$ T cells from peripheral blood and injected back into patients [17]. This engineered lymphocyte therapy is currently modified to improve anti-tumor responses in vivo known as chimeric antigen receptor (CAR) containing T cell technology. A novel CAR-T cells, whose antigen-specific TCR containing IL-2Rβ cytoplasmic domain with CD3ζ and CD28 signaling domains, enabled enhanced proliferation and prevention of terminal differentiation [18]. These CAR-T cells demonstrated superior in vivo persistence and prolonged cytotoxicity against tumor in mice. Further, Sockolosky JT et al. recently engineered tumor-specific T cells that are selectively expanded by the interaction of a mutant IL-2 with altered IL-2Rβ system [19], minimizing off-target effect of IL-2.

### 2.3. Manipulation of IL-2 for anti-tumor activity

To improve the half-life and biological activity of IL-2, pegylated human recombinant IL-2 (PEG-IL-2) was generated and shown prolongation of plasma clearance and increased antitumor activity even at low doses [20].NKTR-214, a pegylated IL-2 displays favorable binding to IL-2β, produced by Nektar Therapeutics, is now in clinical trial in combination with anti-PD1 antibody, Nivolumab (NCT03635983 and more, see clinicaltrials.gov) [21,22]. To direct IL-2 signal toward cytotoxic lymphocytes, several mutant human IL-2 proteins that have reduced IL-2α binding have been designed [23,24]. For example, a mutant IL-2 protein 'superkine' that has increased affinity for IL-2Rβ with absent functional requirement for CD25, induced superior expansion of CTLs leading to improved antitumor responses [24]. Vazquez-Lombardi et al. generated human IL-2 with the Fc region of murine IgG2c ($IL\text{-}2^{WT}$-Fc) and abolished CD25-binding mutant IL-2 (R38D, K43E, E62R) with Fc proteins ($IL\text{-}2^{3\times}$-Fc) [25]. $IL\text{-}2^{3\times}$-Fc showed higher ability of selective expansion of CTLs than those of IL-2WT-Fc in vitro, but interestingly, anti-tumor activity was higher with $IL\text{-}2^{WT}$-Fc treatment than with $IL\text{-}2^{3\times}$-Fc, which was accompanied with Fc-mediated Treg cell depletion. Arenas-Ramirez et al. generated anti-human IL-2 antibody NARA1, which blocks CD25 binding site of IL-2. IL-2/NARA1 complex injection showed higher anti-tumor effects against melanoma than IL-2 alone (Fig. 1) [26].

### 2.4. Treg cells in anti-tumor therapy

Expansion of Tregs is one of the limitations of IL-2 therapy in cancer patients. High dose IL-2 therapy promotes expansion of $CD4^+CD25^+Foxp3^+ICOS^+$ Tregs in melanoma patients and increase of Treg following the first cycle is associated with worse clinica outcome [27]. CTLA-4 blockade is a potential Treg cell targeted anti-tumor therapy. When patients with metastatic melanoma were treated with ipilimumab (anti-CTLA4) prior to high dose IL-2 administration, overall response rate was 21% as compared to 12% in the group that had not received ipilimumab [28]. PD-1 on Treg cells could also be a target. PD-1 is expressed highly on activated Treg and have an important role for immunosuppression [29]. However, some PD-1 positive Treg cells seem to be unstable phenotype. In patients with malignant glioma, high percentage of circulating PD-1 is observed and $PD\text{-}1^{high}$ Treg cells have less suppressive functions and produce IFNγ [30]. Moreover, anti-PD-1 in WT mice induces $IFN\gamma^+$ $Foxp3^+$ Treg that have reduced immunosuppressive function, indicating that PD-1 blockade drives Treg fragility[31,32]. When human Tregs cells were cultured in IL-2 plus anti-PD-1, PD-1 blockade enhances IL-2-induced temporal proliferation of Tregs but promotes apoptosis [33]. Collectively, combination therapy of IL-2 with immune checkpoint agents could affect Treg function as well as enhance CTLs. A number of trials with IL-2 in combination with anti-PD-1 and anti-PD-L1 is now ongoing (Table 1.) However, it is to be noted that Treg cells also play protective roles against IL-2-induced systemic toxicity. In humanized mice, low dose IL-2 administration in Treg-depleted mice induced vascular leak syndrome [34]. Therefore, systemic depletion of Treg cells is not favored and the most ideal immunological balance in cancer immunotherapy might be a condition of enhanced anti-tumor CTL activity without affecting systemic Treg cell function except tumor infiltrating Treg cells.

## 3. Autoimmune/immune-related diseases and IL-2

### 3.1. Development of low dose IL-2 therapy

While IL-2 can expand CTLs, IL-2 has pleiotropic function on $CD4^+$ T cells. IL-2 promotes expansion and differentiation of several subsets of $CD4^+$ T cells such as $T_H$ (T helper) 1, $T_H2$, $T_H9$ whereas constrains and inhibits $T_H17$ and follicular helper T ($T_{FH}$) cells [35]. Although IL-2 is critical for the cell fate decision to these subsets, it is dispensable for their generation. Treg cells express CD25-containing high affinity receptor complex for IL-2 despite its inability of IL-2 production, indicating that IL-2 is provided to Treg from IL-2-producing cells such as activated $CD4^+CD25^{low}$ T cells, NK and NKT cells [36]. Moreover, low IL-2R signaling is substantial for the production of effective population of Tregs while extensive IL-2R signaling is required for proliferation and function of conventional T cells [37]. Therefore, when IL-2 supplementation is low, Treg cells might be stimulated selectively without triggering effector T cell responses [38]. Low dose IL-2 administration was tried first as early as 1992 by Ritz et al. before identification of Treg for medically compromised patients underwent hematopoietic stem cell transplantation (HSCT) due to hematologic malignancy or solid tumor

**Table 1**
Ongoing clinical trials cancer immunotherapy using IL-2 and modified IL-2.

| | Cells/combination | IL-2 dose | Disease | Phase | NCT no |
|---|---|---|---|---|---|
| **rIL-2** | TILs | n.d. | Recurrent ovarian cancer, fallopian tube cancer, primary peritoneal cancer | I | 01883297 |
| | TILs + cyclophosphamide + fludarabine | 0.72 MIU/kg iv every 8 h × 15doses | Metastatic Melanoma | ? | 00604136 |
| | TILs + cyclophosphamide + fludarabine | n.d. | Pleural mesothelioma | I/II | 02414945 |
| | TILs + cyclophosphamide + fludarabine + Dndritic cell vaccine | 0.1 MIU/kg iv every 8 h × 14doses | Melanoma | I | 01946373 |
| | Cord blood derived and culture expanded NK cells (PNK007) | n.d. | Multiple myeloma | I | 02955550 |
| | 4SCAR19/22T cells | 0.25 MIU/$m^2$ every other day | B cell lymphoma, leukemia | I | 03098355 |
| | Radiotherapy | 18 MIU/$m^2$ iv 2 days every 3 weeks × 4 cycles | Renal cell carcinoma, metastatic melanoma | II | 01884961 |
| | Stereotactic body radiation | 0.6 MIU/kg every 8 h 14 doses × 14 cycles | Metastatic renal cancer | II | 02306954 |
| | recMAGE5-A3 + AS15 (tumor-antigen immunization) | n.d. | Melanoma | II | 01266603 |
| | Pembrolizumab | 0.6 MIU/kg every 8 h 14 doses × 2 cycles | Metastatic melanoma | pilot | 03476174 |
| | Pembrolizumab | n.d. | Stage III-IV melanoma | Ib/II | 02748564 |
| | Pembrolizumab | n.d. | Metastatic renal cancer | I | 03260504 |
| | Nivolumab | 0.6 MIU/kg iv every 8 h two 5 day cycles | Metastatic clear cell renal cell cancer | Ib/II | 02989714 |
| | Entinostat (HDAC inhibitor) | 0.6 MIU/kg iv every 8 h two 5 day cycles | Renal cell carcinoma | II | 03501381 |
| **NKTR-214** | –<br>Nivolumab + ipilimumab | n.d.<br>0.003–0.009 mg/kg every 3 weeks | unspecified solid tumor<br>Melanoma, Renal cell carcinoma, Non small cell lung cancer, urothelial carcinoma, triple negative breast cancer | I/II<br>I/II | 02869295<br>02983045 |

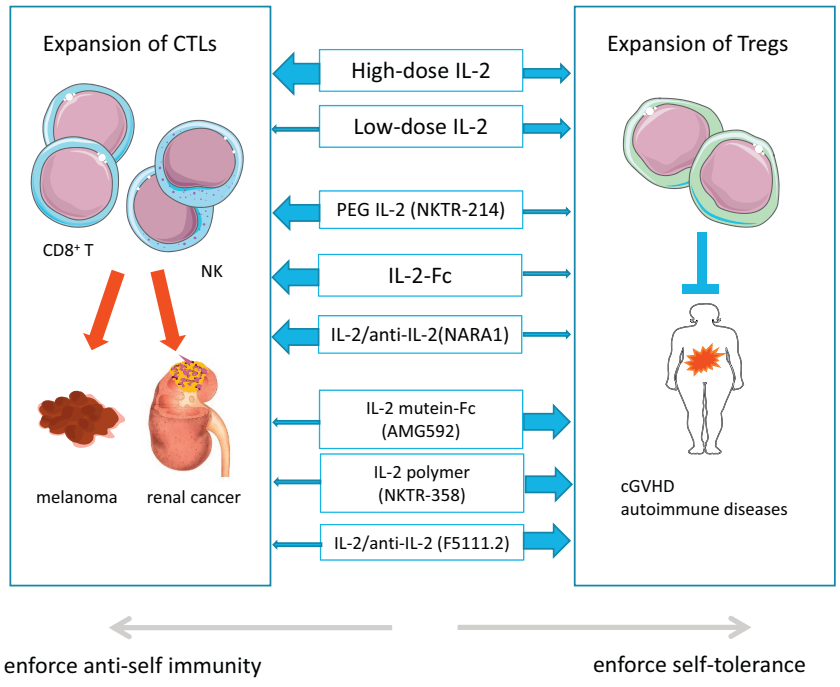


**Fig. 1.** Current and future clinical usage of IL-2 and modified IL-2. Various modified IL-2 agents were designed and investigated to have maximum effects on one side of cells and minimal effects on the other side. From immune-tolerance viewpoint, IL-2 can act on antithetical disease conditions, anti-self immunity and self-tolerance.

**Table 2**
Ongoing clinical trials of autoimmune or immune-related diseases with IL-2.

| | IL-2 dose | Disease | Additional Tx | Phase | NCT No |
|---|---|---|---|---|---|
| **rIL-2** | Self-sc 8 weeks | Steroid-refractory cGVHD | Treg infusion | I | 01937468 |
| | 0.3–3 MIU/m$^2$ sc 8 weeks | GVHD | | I | 00529035 |
| | Low dose IL-2 sc 8 weeks | cGVHD | Photopheresis | II | 02340676 |
| | Low dose IL-2 sc 12 weeks | cGVHD | Photopheresis | II | 03007238 |
| | 0.4 MIU/m$^2$ 3 times a week 90 days | Acute GVHD | | II | 02659657 |
| | 0.3 MIU sc 4 weeks | Liver transplantation | | I | 02739412 |
| | n.d. | Liver transplantation | | I | 02949492 |
| | 1 MIU 5 days every 2 weeks for up to 180 days | Autoimmune and inflammatory diseases (Rheumatoid Arthritis, Ankylosing spondylitis, SLE, Psoriasis, Behcet's disease, GPA, Takayasu's disease, Crohn's disease, Ulcerative colitis, Autoimmune hepatitis, Sclerosing cholangitis, Sjogren syndrome, Idiopathic thrombocytopenic purpura, Systemic sclerosis) | | II | 01988506 |
| | 1.5 MIU 5 day every 3 weeks 4 cycles | SLE | | II | 03312335 |
| | 1 MIU 5 days | Type 1 diabetes | polyclonal Treg infusion | I | 02772679 |
| | 1.5–3.0 MIU sc 5 day 4 cycles | Autoimmune hemolytic anemia | | I/II | 02389231 |
| | 0.3–1.5 MIU sc 8 weeks | Ulcerative colitis | | I | 02200445 |
| **AMG592** | n.d. | SLE | | I/II | 03451422 |
| | n.d. | Rheumatoid arthritis | | I/II | 03410056 |
| | Weekly or biweekly up to 52 weeks | cGVHD | | I/II | 03422627 |
| **NKTR-358** | | SLE | | I | 03556007 |

(0.2 MIU/m$^2$/day intravenously, 3 months) for aiming to prevention of GVHD [39]. Prolonged infusion of IL-2 at low doses was found to be safe and to increase NK cell number. They confirmed later with the patient samples that Treg cells were significantly expanded and FOXP3 expression was increased more than 9-fold [40]. Afterwards, they tried low dose IL-2 (0.3 MIU-3.0 MIU daily subcutaneous for 8 weeks) to treat chronic GVHD and 12 out of 23 patients showed objective clinical responses with substantial increase of Treg in peripheral blood [41]. Phase 1-phase 2a clinical study of low dose IL-2 administration (1.5 MIU-3.0 MIU 5 day for 4 cycles) in patients with HCV-induced vasculitis also revealed that clinical signs of disease were ameliorated without adverse effects [42]. IL-2-based therapy is currently tried to several autoimmune diseases including type 1 diabetes, rheumatoid arthritis and systemic lupus erythematosus (Table 2).

### 3.2. Impaired IL-2/IL-2R signaling in type 1 diabetes

Type 1 diabetes (T1D) is an autoimmune disease against pancreatic beta cells associated with autoantibodies such as antibodies to insulin (IAA), glutamic acid decarboxylase (GAD), protein tyrosine phosphatase (IA2) and zinc transporter 8 (ZnT8). Function of beta cells could be preserved by controlling autoimmunity with cyclosporin, however, the rate of remission is limited. In humans, genetic risk loci of T1D other than HLA complex have been identified more than 50 sites. Among them, non-HLA genetic polymorphisms with stronger association have been mapped to CTLA-4, insulin (INS), PTPN22 and CD25 (IL2RA) [43]. Fifty-four SNPs were identified near the regulatory regions or exons of IL2RA [44]. Individuals with the IL2RA T1D susceptible SNPs have reduced CD25 expression on Tregs and memory T cells, and reduced IL-2 receptor signaling phospho-STAT5 [45]. The important role of IL-2 in protecting from T1D has also been confirmed in non-obese diabetic (NOD) mice. IL2 gene is encoded among the insulin dependent diabetes (Idd) locus, a major contributor of diabetes susceptibility. NOD mice showed lower IL-2 level and anti-IL-2 antibody administration accelerates the onset of diabetes [36,46]. By contrast, congenic NOD mice with Idd loci from diabetes-free C57BL/6 mice are protected from diabetes with increased IL-2 production [47]. Accordingly, depletion of Treg by genetic ablation of Foxp3 in NOD mice rapidly induce diabetes and adoptive transfer of islet β cell-specific Treg cells completely protect NOD mice from disease development [46]. Taken together, IL-2 could be a proper therapeutic target for the treatment of T1D.

### 3.3. Low dose IL-2 therapy in type 1 diabetes

IL-2 therapy has been considered and tried to treat T1D in mice and human. In mice, administration of low IL-2 with anti-IL-2 antibody complexes into NOD mice significantly increased Tregs in the pancreas and periphery and prevented the development of disease [48]. Intramuscular vaccination of adeno-associated virus encoding IL-2 prevented diabetes in NOD mice with increased Treg cells in islets and pancreatic draining lymph nodes [49]. A single center phase 1/2 study of low dose IL-2 in patients with T1D was completed. Twenty-four adult patients (18–55 years of age) with established T1D were randomly assigned to placebo or IL-2 at 0.33 MIU, 1 MIU, 3 MIU per day administration for 5-day course. IL-2 was well tolerated at all doses with no serious adverse events [50]. Currently a numbers of clinical trials of IL-2 therapy for T1D has been completed and other are ongoing. It should be noted that a subset of T1D patients have anti-IL-2 autoantibodies [51].

### 3.4. IL-2 deficiency in SLE

IL-2 production by conventional T cells (Tconv) is impaired in SLE [52]. Cyclic AMP response element modulator alpha (CREMα) is overexpressed by SLE Tconv cells and it silences the expression of the *IL-2* gene. Enhanced activity of calcium calmodulin kinase IV (CaMK4) [53] and decreased expression of serine/arginine-rich splicing factor 1 (SRSF1) [54] are also involved in the transcriptional repression of IL-2 [55]. IFNγ-producing $T_H1$ cells and IL-17-producing $T_H17$ cells are increased in organs such as the skin and the kidney [56] probably because the absence of IL-2 favors differentiation and expansion of these populations [57]. Recent study confirmed that IL-2 production is impaired significantly in naïve $CD4^+$ T cells, and more importantly, this abnormality is associated with a defect in IL-2 signaling [58]. Although Treg cell numbers decrease in lupus-prone mice as progress of age and disease [59], several studies in human analyzed the frequency of Tregs in SLE reported conflicted results [60]. A report showed that significant increase in the frequency of $CD4^+CD25^{++}Foxp3^+$ Tregs was observed in active SLE [61], but others described that CD25 expression levels on the surface of Tregs was decreased in SLE patients [62]. IL-2 receptor-dependent activation of transcription factor STAT5 is essential for the suppressive function of Tregs [63], however, it is not known whether the function of Tregs in SLE patients is affected even under condition of IL-2 deficiency.

### 3.5. IL-2 therapy in lupus-prone mice

The first report of IL-2 treatment for lupus-prone mice appeared in 1990 prior to the discovery of Tregs [64]. An IL-2-encoding vaccinia virus was used to deliver IL-2 in vivo in MRL*lpr* mice. Treated mice survived longer and had reduced lymphadenopathy and kidney pathology. $TCR\alpha\beta^+CD4^-CD8^-$ (double negative, DN) T cells, which are the most likely culprit of lymphoadenopathy in MRL*lpr* mice, were consistently decreased after treatment with IL-2 and these findings have been confirmed [65–67]. Despite its unclear origin, DN T cells from lupus-prone mice and patients with SLE produce IL-17 [67,68], indicating their involvement in the pathogenesis of SLE. By IL-2 supplementation, Treg numbers increased substantially in lymphoid organs and the periphery in NZB/NZW F1 mice and MRL/*lpr* mice [59], however, Treg-specific expansion following the administration of IL-2/anti-IL-2 antibody complexes did not lead to the reduction of DN T cells [67], suggesting that an effect of IL-2 on non-Treg population might contribute to the inhibition of DN T cell expansion.

### 3.6. IL-2 therapy for patients with SLE

Since the decreased production of IL-2 in patients with SLE contributes to various immune defects, it is conceivable that low dose IL-2 treatment can restore these pathogenic processes [69]. Humrich and colleagues first reported a patient with SLE who experienced clinical improvement following treatment with low dose IL-2. Specifically, subcutaneous injection of 1.5 to 3 MIU IL-2 on 5 consecutive days resulted in disappearance of skin lesions, myositis and serum anti-dsDNA antibody. The SLE Disease Activity Index (SLEDAI) score decreased significantly from 14 to 4 and glucocorticoids were reduced from 30 mg/day to 10 mg/day. The percentage of $CD4^+CD25^+Foxp3^+CD127^{lo}$ Treg among $CD4^+$ T cells was transiently upregulated by 40% [70]. Subsequently, they started a combined phase I/IIa clinical trial to address the safety, tolerability, efficacy and immune response of low dose IL-2 therapy in patients with active and refractory SLE (PRO-IMMUN, EudraCT-number: 2013-001599-40, Germany) [71]. In this study Tregs from SLE patients displayed lower CD25 levels and co-culture of $CD4^+$ T cells with peripheral blood mononuclear cells showed deficient ex vivo IL-2 production by immune cells from patients with SLE. After five patients were treated with daily subcutaneous injection of IL-2 at 1.5MIU for 5 days, they confirmed that low dose IL-2 therapy induced substantial increases of the numbers of Tregs without major side effects. As the primary endpoint (immune response rate) has been completed, phase II trial is now ongoing. The latest clinical trial of low dose IL-2 in 38 SLE patients was conducted in China (NCT02084238) demonstrated that IL-2 treatment significantly decreased SLEDAI after 12 weeks [72]. One MIU IL-2 was administered alternate-day for 7 times at 3 cycles. More than 80% of patients achieved SLE response index (SRI) with 4-point drop in SLEDAI, with increased numbers of Tregs, decreased $T_H17$, Tfh and DN T cells. Unfortunately, the study was not controlled and various observations including the rapid disappearance of DNA antibodies remain unexplained. A phase I/II clinical study involving the induction of Tregs by low-dose IL-2 in SLE and other autoimmune and inflammatory diseases (NCT01988506, NCT03312335) is now in progress [73](Table 2). Since all studies are non-controlled ones, controlled prospective study is necessary in the future. Taken together, low-dose IL-2 treatment in SLE patients could mitigate severity of disease by altering the balance of T-cell subsets.

### 3.7. Efficacy, safety and durability of low-dose IL-2 therapy

In phage 2 study of cGVHD, efficacy and durability of clinical responses in low dose IL-2 therapy was accessed [74]. Patients received daily subcutaneous IL-2 (1 MIU/$m^2$) for 12 weeks. Among 35 enrolled patients, 5 patients (14%) required IL-2 dose reduction for constitutional adverse events such as flu-like symptoms, fatigue, malaise, arthralgia/myalgia. Except for 2 patients who withdrew early, 20 of 33 patients (61%) had objective partial responses (PR) by week 12. There is no patient with complete response (CR) and 3 patients (9%) progressed the disease. Twenty-three patients continued daily injection for over 1 years and 15 patients for more than 2 years among them. This extended low dose IL-2 therapy was well tolerated and 50% of steroid reduction was achieved, but 2 patients had hematologic relapse. The early predictors of IL-2 clinical response was identified; (1) earlier IL-2 beginning after HSCT, (2) Baseline and week 1 Treg:Tcon ratios of $\geqq$ 0.07 and $\geqq$ 0.2, respectively. Further analysis using mass cytometry of low-dose IL-2 treatment in cGVHD patients revealed that $CD4^+CD25^+Foxp3^+Helios^+$ Tregs and $CD56^{bright}CD16^-$ NK cells were selectively expanded [75]. $Helios^+$ Tregs were shown to be fully demethylated at the Treg-specific demethylated region (TSDR) and was recognized as a subset with enhanced suppressive potential [76]. Ki67 expression was increased one week after starting IL-2 but declined to baseline after 12 weeks. It is notable that even 48 weeks after daily treatment with low dose IL-2, phosphorylation of STAT5 and increased expression of Foxp3, CTLA-4, CD25 and Bcl-2 were sustained [75]. Function of Treg might be sustained at least during IL-2 treatment.

### 3.8. Modification of IL-2 for targeting Treg cells

Even though low-dose IL-2 can expand Tregs, frequent injection is required for the induction of effective Tregs because IL-2 has a very short half-life in human serum (5–7 min). Bell et al. recently developed monovalent or bivalent IL-2-fused with non-FcR binding human IgG1 molecules which had a prolonged half-life in vivo and caused prolonged activation and proliferation of Tregs after a single ultra-low dose [77]. Amgen is launching a phase I/II clinical trial to evaluate the efficiency of Amg592, an investigational IL-2 mutein-Fc fusion protein designed for greater Treg selectivity and longer half-life than recombinant IL-2, for the treatment of active SLE (NCT03451422) and cGVHD (NCT03422627). NKTR-358, Aldesleukin-based IL-2-polymer conjunction protein that has high affinity to IL-2αβγ but low to IL-2βγ were developed at Nektar Therapeutics. This new molecule enables selective Treg expansion more efficiently than IL-2, and prolonged half-life to inject only once or twice a month subcutaneously. The phase 1 clinical trial for SLE has just started (NCR03556007).

PEG-IL-2, already has undergone phase I/II clinical trials in cancer

patients [78], was recently revisited and tried in mice with asthma [79]. Nanoscale liposomal polymeric gels (nanolipogels) are biologically compatible and slowly biodegradable agents. Fahmy and collegues recently developed nanolipogels with encapsulated recombinant IL-2 and a TGFβ and anti-CD4-labeled nanolipogels with IL-2 and TGFβ successfully expand Tregs in vitro and in vivo [80]. Use of IL-2-nanoparticles tagged with an antibody recognizing specific tissues will result in more specific delivery and lower toxicity than systemic administration. Clinical trials of chemical agent-containing nanoparticles targeted tumor-specific antigen are ongoing in several cancer treatment. IL-2/anti-IL-2 complexes can also prolong the half-life of IL-2. In mice, IL-2/anti-IL-2 complexes have been well analyzed: IL-2/JES6-1A12 specifically binds to CD25 and IL-2/S4B6 to CD122. IL-2/JES6-1A12 and IL-2/S4B6 induce specific expansion of Tregs and cytotoxic lymphocytes respectively. IL-2/JES6-1A12 administration expands efficiently both peripheral and tissue Tregs [81,82]. Recently, Bluestone et al. developed anti-human IL-2 antibody that exhibit Treg expansion when complexed with human IL-2. They screened human antibodies against human IL-2 from a single-chain variable fragment phage display library. Relevant candidates were developed into full human IgGs, then IgGs were further screened to identify antibodies that inhibit IL-2 binding to IL-2Rβ and reduce binding to IL-2Rα. An anti-IL-2 clone F5111.2, when complexed with human IL-2, induced remission of T1D in NOD mice, reduced disease severity in a mouse model of autoimmune encephalomyelitis (EAE) and protected against xenogeneic GVHD in NOD scid gamma mice. When F5111.2 antibody is injected, it is expected that the antibody binds endogenous IL-2 to make conformational change for facilitating Treg expansion. They will be useful for the specific expansion of target cells and will probably require less frequent injections (Figure) [83].

### 3.9. Function and stability of IL-2-expanded Tregs

Although Treg-targeted IL-2 therapy is effective on autoimmune diseases and cGVHD, it still remains to be elucidated whether the broken immune tolerance is re-constituted and restored by IL-2 treatment. Analysis of IL-2-treated patients blood samples of cGVHD described that Helios$^+$ Tregs were expanded and Foxp3 was rapidly upregulated, indicating that expanded Tregs had activated and enhanced suppressive potential [75]. However, it is to be noted that these Tregs also increase the expression of Fas [75], indicating high susceptibility to apoptosis. IL-2 is reported to inhibit Fas-mediated apoptosis of Tregs, however, it is still not fully understood whether activated Tregs keep alive and functions continuously in vivo. It should also be considered that Tregs expand only for the 1st four weeks and no further growth or rather, decrease in number was seen at least in peripheral blood during IL-2 injection [74]. This might be due to the limited replicative capacity of activated Treg resulting from poor telomerase inducibility and extensive telomere erosion, and they might be cleared rapidly in vivo [84]. Therefore, it should be elucidated whether disease remission could be maintained after stopping long-term IL-2 treatment in cGVHD and autoimmune diseases. Also, additional strategies could be considered to enforce and stabilize Tregs such as retinoic acid and vitamin D, reported to enhance induced Treg stability [85]. Further investigation is required to clarify how to keep expanded Tregs stable in vivo.

## 4. Conclusion and future perspectives

Application of IL-2 are expanding to other immune disease animal models, including solid organ transplantation and allergic diseases. It is intriguing that low-dose recombinant IL-2 administration could protect mice from food allergy and the immune tolerance was sustained for more than 7 months after the last treatment of IL-2 [86]. For solid organ transplantation, clinical trial of subcutaneous low dose IL-2 administration in liver transplant recipients is now ongoing (NCT02739412). In solid organ transplant rejection, allogeneic memory CD8$^+$ T cells might play a key role for costimulation-independent recall response [87]. Low dose IL-2 treatment to non-human primates with accepted renal allografts for 1–10 years via mixed chimerism, abolished tolerance and trigger allograft rejection [88]. This result indicates the existence of effector cells highly-sensitive to IL-2 in certain immunological condition. From the results of clinical and experimental trials with IL-2 from the multiple research fields of cancer immunology, transplant immunology and autoimmunity, integrating and understanding the effects of IL-2 will help for the improvement and establishment of the most effective therapeutic strategy.

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