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Review

Immune regulation and cytotoxic T cell activation of IL-10 agonists – Preclinical and clinical experience

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ABSTRACT

The expansion and activation of tumor antigen reactive CD8$^+$ T cells are primary goals of immunotherapies for cancer. IL-10 is an anti-inflammatory cytokine with an essential role in the development and proliferation of regulatory T cells, restricting myeloid and chronic inflammatory T cell responses. However, IL-10 is also essential for the expansion of antigen activated, tumor specific CD8$^+$ T cells, leading to spontaneous tumor development in IL-10 deficient patients and mice. IL-10 induces IFNγ and cytotoxic mediators in antigen activated T cells. In clinical trials, monotherapy with recombinant, pegylated IL-10 (Pegilodecakin) induced objective responses in cancer patients. Patients receiving pegilodecakin had a systemic increase of IFNγ and granzymes, proliferation and expansion of immune checkpoint positive CD8$^+$ T cells. Combination of pegilodecakin with anti-PD-1 appeared to improve on the efficacy of the single agents.

1. Introduction

Early successes in stimulating the immune system to recognize and eliminate tumor cells have been achieved with clinical use of cytokine therapies such as Interferon-α and interleukin-2 (IL-2). While associated with immune mediated toxicity, recombinant IL-2 has been being among the most clinically successful agents for the immune treatment inducing long lasting complete response, albeit only in a subset of patients with melanoma and renal cell cancer [1]. With the more recent success of immune checkpoint inhibitors, antibody therapies against cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed death-1 (PD-1) on T cells, immunotherapy as therapeutic avenue in cancer treatment has finally emerged into mainstream oncology. While anti–CTLA-4 antibodies were the first to be approved by the U.S. Food and Drug Administration for the treatment of melanoma, antibodies inhibiting the PD-1 receptor or its ligand transformed the treatment paradigms of many solid malignancies.

In the last twenty years, many studies demonstrated that infiltration of T cells is the single most important prognostic factor for the majority of human tumor types [2]. The inhibition of CTLA-4 and PD-1 alone or in combination, enhance CD8$^+$ T cell recognition of tumor antigens and achieve durable clinical responses in patients with melanoma [3–5]. T cells specific to tumor-associated antigens (TAA) are rare in the blood and tumors of patients with cancer. Anti–CTLA-4 but not anti–PD-1 treatment amplifies TAA-specific CD8$^+$ T-cells in responding patients with melanoma [6,7].The combination of T-cell checkpoint inhibitors, anti–PD-1 and anti–CTLA-4, revealed a stunning improvement over each therapy alone, with 53% of patients with melanoma receiving the combined regimen experiencing an objective responses [8]. Anti-PD-1 therapies are most efficacious in indications and tumors with a high number of somatic mutations or tumor mutational burden (TMB). High TMB increases the antigenic difference between the tumour and the immunological self (immunogenic tumors). This antigenic difference enables T cell recognition and may lead to a higher number of tumor infiltrating T cells (TIL) [9–11]. In the opposite scenario, immune therapies and immune checkpoint inhibition, tend to have poor response rates in indications or patients with lower mutational burden. Hence additional therapeutic approaches to increase the rare tumor antigen specific T cells in tumors with low TMB are needed.

IL-10 is the founding member of the IL-10 family of cytokines. It is a noncovalent homodimeric alpha helical cytokine with structural similarities to IFN-γ. The IL-10 receptor (IL10R) consists of two molecules of an IL-10–specific alpha chain IL10Ra and two molecules of IL10Rb that is shared with other cytokines. IL10R is expressed on the surface of most hematopoietic cells, including T cells, B cells, and macrophages. IL-10 was identified as a factor produced by Th2-polarized CD4$^+$ T cells, which suppresses the proliferation of CD4$^+$ T cells. In this setting IL-10 inhibits the secretion of cytokines in Th1 helper cells, in particular IL-12 and IFN-γ [12]. In vitro, IL-10 also reduces the antigen specific stimulation of CD4$^+$ T cell proliferation by monocytes through

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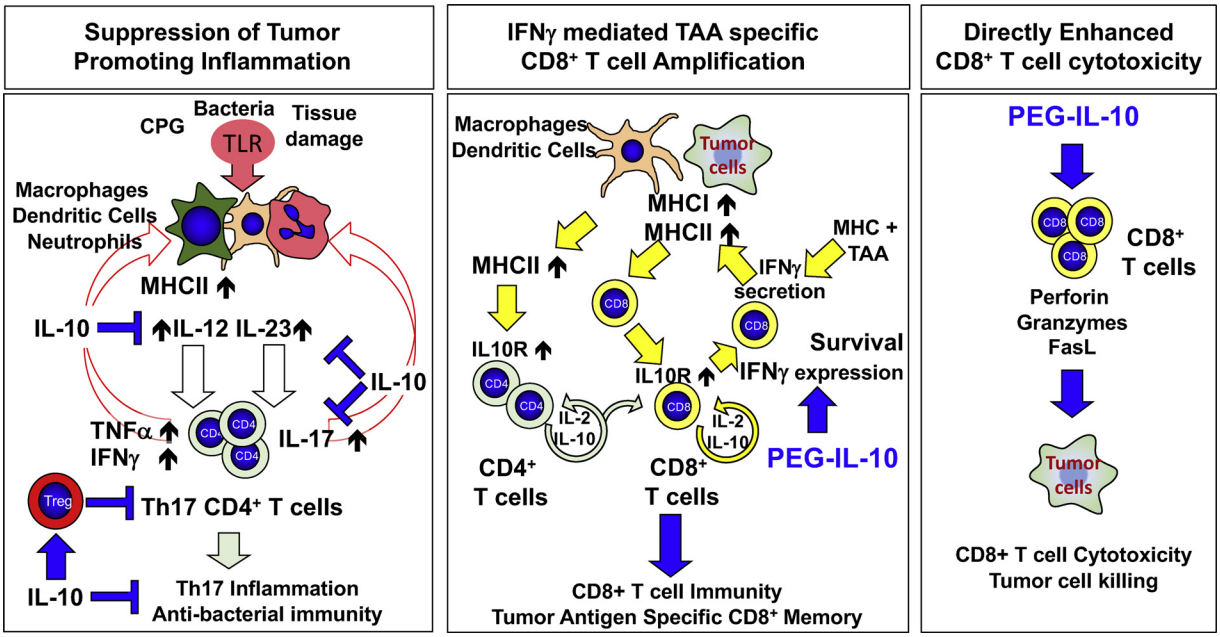

Fig. 1. IL-10 at the crossroad from inflammation to the stimulation of $CD8^+$ T cells. A). Toll like Receptors (TLR) or pattern recognition (PRR) mediated inflammatory responses induce the expression of IL-12 and IL-23 . IL-10 activates STAT3 which inhibits expression of the shared p40 subunit of IL-12 and IL-23. IL-10 also inhibits inflammatory Th17 Cells directly and indirectly through the stimulation of Tregs. Suppression of IL-17, IL-1 and TNFa inhibit neutrophil and macrophage activation in tumor associated inflammation and inflammatory diseases. B). Upon antigen stimulation, $CD8^+$ T cells upregulate the IL-10 receptor and IL-10. Autocrine and therapeutic IL-10 increases anti-apoptotic signals and IFNγ in those antigen experienced $CD8^+$ T cells. IFNγ is released once the $CD8^+$ T cell is recognizing the antigen (MHC + TAA) on tumor cells or dendritic cells (DC) in the tumor. This localized IFNγ release, leads to MHC I and MHC II upregulation in the tumor and enables tumor centric priming of $CD4^+$ and $CD8^+$ T cells. C). Pegilodecakin induces directly IFNγ, Granzyme and FasL expression in antigen activated $CD8^+$ T cells, facilitating the upregulation of MHC I on the tumor cell and induction of tumor cell killing.

downregulation of class II major histocompatibility complex (MHC-II) [13]. In contrast, IL-10 induced thymocyte proliferation by elevating the expression of CD3 and CD8 and IL-10 was shown to induce the cytotoxicity of $CD8^+$ T cells [14]. In mouse tumor models, IL-10 increased the expression of IFNγ in $CD8^+$ T cells, paradoxically leading to increased expression of MHC I and MHC II [15]. Deficiency in IL-10 or IL10R have - in human genetics and mouse models - clarified the nonredundant immunologic properties of the IL-10 pathway for both functions. In support of the anti-inflammatory and tumor-inhibitory function, IL-10–deficient mice and humans develop inflammatory bowel disease (IBD) and cancer [16–18].

In preclinical tumor models, IL-10 induces the rejection of tumors predominantly relying on the $CD8^+$ T cell activating property. The anti-inflammatory role of IL-10 has been well described mostly in engineered mouse models of IL-10 deficiency, whereas the well-documented IL-10–dependent stimulation of $CD8^+$ T-cell cytotoxicity in the immune response to cancer has received considerably less attention. This review will focus mostly on the use of the recombinant pegylated cytokine, its ability to stimulate antigen-specific $CD8^+$ T cell responses and its application in cancer immune therapy in mouse models of cancer and clinical trials in cancer patients.

2. IL-10 reduces TLR induced inflammatory responses

Understanding of the anti-inflammatory mechanism of IL-10 was early on revealed in a mouse model of endotoxemia. Single or repeated injections of bacterial lipopolysaccharides (LPS) induce an acute cytokine release syndrome (CRS), leading to a vascular shock and death. LPS activates Toll-like receptor 4 (TLR4), a pattern recognition receptor (PRR) expressed on many myeloid cells. PRRs alert the innate immune system of foreign molecules such as bacterial cell wall constituents or DNA, and of damaged host cells. The stimulation of PRRs is not antigen specific and leads to an inflammatory reaction, largely driven by the innate immune system. However, LPS also induces inflammatory cytokines IL-12/IL-23, which stimulate inflammatory T cells. IL-10 is induced by LPS as a negative feedback and inhibits the expression of the shared p40 subunit of IL-12/IL-23. This is achieved through a transcriptional cascade involving STAT3 and the transcriptional repressor nuclear factor, interleukin 3 regulated (NFIL3), which inhibits the transcription of p40 [19–21]. NFIL3 deficiency causes a microbiota dependent colitis [22]. Due to the absence of this feedback, IL-$10^{-/-}$ mice are sensitive to the LPS-induced shock, while IL-10 protects the mice from CRS [23,24]. IL-10–activated STAT3 also induces the suppressor of cytokine signaling 3 (SOCS3), which binds and inhibits the proinflammatory IL-6 and IL-12/IL-23 receptors [25]. IL-10 thereby intercepts the inflammatory responses at various levels.

At high local concentrations, the inflammatory response to PRR ligands injected into a tumor can result in sufficient local activation of the innate immune cells to reject a tumor. TLR7 agonist (imiquimod) is approved for the treatment of localized basal carcinoma of the skin [26]. Tumor rejection is driven by local activation of inflammatory T cells and macrophages. The local inflammatory reaction to imiquimod indeed serves as a prognostic indicator of treatment success [27]. In the absence of IL-10, the local inflammation is greatly exacerbated. Intratumoral injection of TLR ligands into experimental tumors have enhanced activity when applied in a IL-10-/- mouse or in combination with an IL-10 neutralizing antibody [28]. Systemic applications of TLR agonists, however lead to a life-threatening CRS. Consequently, and due to limiting toxicities, this approach has seen so far little or no success in systemic tumor therapy. Intratumoral injection of the PRR agonist in combination with IL-10 inhibition are pursued. Antibody mediated IL-10 abrogation (MK-1966) in combination with local TLR9 activation (SD-101) entered clinical trials in 2015 (NCT02521870) with an expected study completion in 2019.

3. Tumor associated inflammation

The regulation of proinflammatory and anti-inflammatory signals

and T cell populations is crucial for the maintenance of normal physiology as well as the suppression of tumor development. Locally induced inflammation can lead to the elimination of the injected tumor and sometimes abscopal lesions, but systemic inflammation appears to promote tumor incidence and progression. Chronic inflammation correlates with tumor incidence in experimental cancer models and in cancer epidemiology [29,30]. The development and progression of tumors in chronically inflamed tissue is thought to be the consequence of increased compensatory cell proliferation, a tumor-promoting microenvironment with increased angiogenesis and proteolytic activity, an increased mutation rate due to the release of oxygen radicals, and the conspicuous absence of tumor immunosurveillance by cytotoxic T cells.

IL-10 is an essential cytokine for the homeostasis of anti-inflammatory regulatory T cells (Tregs) and the suppression of proinflammatory IL-17–expressing T cells (Th17) (Fig. 1a). This anti-inflammatory role of IL-10 was earliest demonstrated in IL-10 knockout mice who spontaneously develop inflammatory bowel disease (IBD) [31]. Removal of commensal intestinal bacteria by antibiotic treatment protected IL-10 -/- mice from colitis [32], and the prevalence and severity of IBD in IL-10-/- mice is attenuated in SPF facilities. The relevance of this finding has been confirmed in patients carrying a somatic mutation in the IL-10 or the *IL10R* gene, who spontaneously develop severe, very early onset IBD [16]. Importantly, in the presence of this increased systemic inflammation, IL-10 knockout mice (IL-10-/-) spontaneously develop colon cancer [18], while patients deficient in IL-10 signaling develop lymphomas within the first decade of life [17]. It is not clear why mice do not develop lymphomas, or if IL-10R deficient patients would develop colon cancer with increased age. This difference in tumor type could arise from the difference in the duration of inflammation until tumor incidence, the anti-inflammatory medication in IL-10 deficient patients which may prevent the development of colon carcinomas in human, or a difference in the microbiota that modifies the organ specific cancer risk. In general, inflammation arising due to IL-10 deficiency may provide a fertile ground for tumor development and support the concept of tumor-promoting inflammation.

In autoinflammatory mouse models, IL-10 ablation leads to increased immune pathology mediated by proinflammatory Th17 cells expansion [33]. In this scenario, regulatory T cells function both as a rate limiting producer of IL-10 and an essential recipient of the cytokine, and deficiency of IL-10 or the IL10R in Treg causes colitis [34]. IL-10 induces STAT3 phosphorylation in Tregs, and STAT3 deficient Tregs fail to expand in the inflamed gut. In contrast, STAT3 is not required for Treg mediated suppression of CD4 T-cell proliferation *in vitro* [35]. This suggests that IL-10 regulates the inhibition of inflammation through the homeostasis of Tregs.

Mice with a mutation in the adenomatous polyposis coli gene ($APC^{\Delta 468}$) develop intestinal tumors, driven by focal inflammation to their microbial gut flora. Ablation of IL-10 in T cells increases the inflammation and increases the tumor burden in the colon [36]. In the small intestine of the same mice, overshooting severe inflammation prevented development of tumors at an early age, however progressive loss of $IFN\gamma^+$ T cells and cellular cytotoxicity led to cancer development [37]. Adoptive transfer of $CD25^{hi}$ T cells into $APC^{\Delta 468}$ mice with colonic tumors lead to an IL-10–dependent reduction of tumor burden [38]. Collectively, these data support a role of $CD4^+$ T cell derived IL-10 in the suppression of inflammation induced cancers.

Th17 cells are functionally dependent on the myeloid-derived cytokine IL-23. Genetic or therapeutic ablation of IL-23 in mice renders them resistant to experimentally induced autoinflammatory diseases [39]. IL-23 deficiency also severely restricts the development of experimentally induced tumors accompanied by a deficiency of inflammatory mediators such as IL-17, tumor-promoting inflammatory metalloproteases and inflammation driven angiogenesis [40,41]. Simultaneously, tumor-infiltrating $CD8^+$ T cells and their cytotoxic mediators and IFN-γ are highly prevalent [41]. The pro-inflammatory IL-23 also suppressed NK cell mediated tumor rejection [40]. The mutual exclusivity of “inflammatory” and “cytotoxic immunity” mediating cells is explained by the signature effector cytokines, IL-17 or IFN-γ. IL-17 attracts and activates granulocytes and myeloid cells promoting angiogenesis and wound repair. IFN-γ induces antigen presentation and the development of $CD8^+$ T cell immunity.

At the transcriptional level, this dichotomy is achieved by transcription factors such as RORγt, which defines the proinflammatory Th17 cells and proinflammatory Tregs [42,43]. T-cell–specific deletion of RORγt inhibits both inflammatory T-cell populations, suppresses tumor development in $APC^{\Delta 468}$ mice, and increases the expression of IL-10 [42]. In the absence of RORγt and inflammatory Th17 cells, cytotoxic granzymes and perforin-positive cells are increased in the gut, indicating the reciprocal regulation of the immune response. NFIL3, the transcriptional repressor mediating the IL-10–STAT3 induced inhibition of IL-12/23p40 expression [20], also mediates the repression of RORγt [44]. Intriguingly, STAT3 mediates both, inflammatory signals through IL-6 and IL-23 and anti-inflammatory signals by IL-10. STAT3 knockout mice were resistant to experimental uveitis due to deficient Th17 induction, and an increase of $IL\text{-}10^+$ $CD8^+$ T cells. However, upon herpesvirus infection, the STAT3 deficient $CD8^+$ T cells had less virus-specific $IFN\text{-}\gamma^+$ and $KLRG\text{-}1^+$ $CD8^+$ T cells demonstrating dependence on the IL-10 STAT3 stimulation of $CD8^+$ Teff cell survival (see also 4.1) [45].

The balance of the decision between cytotoxic and inflammatory cell fate of CD8 + T cells is also controlled by the T-box transcription factors, Eomesodermin and T-bet which are essential for NK cells and CD8 + T cell memory [46]. Intriguingly, in compound knockout mice lacking both, T-bet and Eomesodermin, CD8 + T cells are unable to mount a cytotoxic response after virus infection but instead develop a neutrophil mediated wasting syndrome, driven by IL-17 expressing CD8 + T cells (Tc17) [47]. These proinflammatory Tc17 are also devoid of IL-10 and IFNγ expression.

These data demonstrate the intricate antagonism of proinflammatory signaling and cellular cytotoxicity and the importance of IL-10 in the control of Th17 cells which are critically involved in the development of tumor-promoting inflammation. Intriguingly, increased $CD8^+$ T cell activity is observed when inflammatory responses are inhibited. While the rational for inflammation control or immune effector modification is supported by both preclinical mechanistic data and clinical correlations, it has not been explored clinically as aggressively as immune stimulatory therapies. The use of tocilizumab (anti-IL-6) to control immune mediated adverse events of immune checkpoint inhibitors, appears to be clinically safe and not inhibit anti-tumor immune function [48]. This supports the concept that inflammatory effector cytokines such as IL-6 are not essential in antigen specific tumor immunity. More specific combinations of activation of antigen specific T cells and anti-inflammatory therapies may provide means to a more tolerated and potentially more efficacious immune therapy regimen.

4. Regulation of $CD8^+$ T cells by IL-10

4.1. *In vitro* modulation of $CD8^+$ T cell activity by IL-10

IL-10 modifies the immune response to antigens and pattern recognition motives toward an antigen specific and $CD8^+$ T cell specific response. A stimulating function of IL-10 on $CD8^+$ T cells was first described as the B-cell–derived T-cell growth factor (B-TCGF), IL-10 induces the expression of CD3 and CD8 molecules on thymocytes and enhances the cytotoxic activity of $CD8^+$ T cells [14,49]. However IL-10 also inhibits CD8 + T cells in an allogenic reaction [50], potentially due to the nature and strength of the TCR signal in this setting. IL-10 enhanced the proliferation of $CD8^+$ T cells after direct stimulation of the T-cell receptor (TCR) signaling using anti-CD3 monoclonal antibodies (mAb). In addition, IL-10 induced the proliferation of $CD8^+$ T cells stimulated with anti-CD3 mAbs in the absence of exogenous IL-2 [50]. It is however feasible, that IL-10 in this setting merely enhanced the

responsiveness to endogenous, anti-CD3 induced IL-2 (see below).

$CD8^+$ T cell activation by TCR stimulation leads to an upregulation of the IL-10Ra and IL-10Rb [51,52]. This increases the sensitivity of TCR stimulated $CD8^+$ T cells to STAT3 induction by IL-10 and may selectively deliver anti-apoptotic signals to TCR stimulated $CD8^+$ T cells [52]. Simultaneously, activation of $CD8^+$ T cell leads to a temporary induction of immune checkpoint receptors such as PD-1 and Lag-3, and this activation signature is stabilized by the treatment with IL-10 [52]. IL-10 also directly induces GranzymeB expression in activated $CD8^+$ T cells, leading to a higher antigen specific cytotoxicity of those cells on target cells [15,52].

A more careful analysis of the autocrine and paracrine function of IL-10 on macrophages, dendritic cells and $CD8^+$ T cell priming showed that the TLR7/8 stimulation induced IL-10 in dendritic cells, but inhibited IL-10 production in monocytes. Moreover, autocrine IL-10, produced by dendritic cells did not inhibit DC maturation and priming to high affinity antigens, but lowered priming to low affinity antigens, potentially through reducing MHC I and CD86 expression. Reducing antigen stimulation by 50% may restrict the T cell response to a more mature immune response [53,54]. In addition, IL-10 enhanced the IL-15 induced TCR-independent proliferation of memory cytotoxic T cells. By selectively increasing the sensitivity of high-affinity $CD8^+$ T cells to IL-15, IL-10 may increase the prevalence of antigen activated CTL. Conversely, IL-10 inhibition may broaden the immune response by reducing antigen restriction. This could lead to a widened $CD8^+$ T cell repertoire, at the expense of TCR high affinity selection.

Antigen specific $CD8^+$ T cells, isolated from B cell lymphomas (DLBCL), had increased levels of PD-1 and IFNγ. Exogenous IL-10 improved the survival and activity of specific, $IFN\gamma^+$ but not of $IFN\gamma^-$ $CD8^+$ T cells. IL-10 reduced $CD8^+$ T cell apoptosis while inducing phosphorylation of STAT3 and the expression of the anti-apoptotic bcl2 [55].

In summary, IL-10 stabilizes the activity of antigen stimulated $CD8^+$ T cells and stimulates the expression of cytotoxic effector molecules (Fig. 1c).

4.2. IL-10 in the establishment of $CD8^+$ T cell responses and memory in vivo

4.2.1 Endogenous IL-10 in tumor and virus models A protective role of IL-10 against endogenous tumors was first observed when IL-10-/- mice spontaneously developed colon carcinoma [18]. Also, in mice with transgenic over-expression of IL-10 in antigen presenting cells (IL-10TG), syngeneic tumors establish normally, but are rejected within weeks without further treatment and dependent on $CD8^+$ T cell [56]. When IL-10-/- and IL-10TG mice were treated with chemical carcinogens to induce squamous skin tumors, IL-10-/- mice developed papillomas and carcinomas earlier and in larger numbers than control mice, while IL-10TG mice were protected from tumor development [15]. Skin carcinomas in IL-10-/- mice progressed and metastasized prematurely with all IL-10-/- mice succumbing prematurely to metastasis, while the immune system of cancer bearing control mice was at large capable to prevent metastasis, with most mice reaching normal age [15]. In early precancerous lesions, intratumoral $CD8^+$ T cells, IFNγ, MHC molecules, and granzymes were suppressed in IL-10-/- mice, but increased in IL-10TG mice.

During viral infection, murine hepatitis B virus specific $CD8^+$ T cells produce IL-10 upon antigen exposure and the autocrine IL-10 prevents antigen mediated apoptosis and increased IL-2 responsiveness [57]. During acute lymphocytic choriomeningitis virus (LCMV) infection, endogenous IL-10 suppresses $CD4^+$ T cells, but not $CD8^+$ T cells [58]. The *in vivo* maintenance of antiviral memory $CD8^+$ T cells requires STAT3 and IL-10 [59]. In viral liver infection and inflammation, TLR9 induces IL-10 producing $CD8^+$ T cell. These $IL\text{-}10^+$ $CD8^+$ T cells, did not resemble a regulatory T cell population, but produced IFNγ and contributed to liver inflammation [60]. During coronavirus infection, cytolyticly active $CD8^+$ T cells produce IL-10, which reduced $CD8^+$ T cells bystander cell activation, resulting in decreased tissue damage [61]. This is reminiscent to in vitro result, were IL-10 suppressed low affinity TCR activation [54] (4.1.1).

IL-10 expressing $CD8^+$ memory T cell are induced in syngeneic tumor models by treatment with recombinant IL-27. These self-renewing memory $CD8^+$ T cells were $Bcl\text{-}6^+$, $SOCS3^+$, $Sca\text{-}1^+$, and had increased phosphorylation of STAT3 in response to IL-10 [62]. IL-10 is also essential for $CD8^+$ T cell expansion in Trypanosoma infection, where IL-10 deficiency affects specifically the $CD8^+$ T cells, not $CD4^+$ T cell responses [63].

In summary, endogenous IL-10 is essential for the formation of a potent $CD8^+$ T cell memory to tumor and virus challenges, enabling high affinity TCR responses, while suppressing bystander T cell proliferation. The antiapoptotic function of STAT3 may protect $CD8^+$ T cells after antigen challenge. IL-10 increases expression of IFNγ, facilitating MHC expression and antigen recognition but potentially also mediating the suppression of T cell clones with weak TCR recognition [64].

4.2.1. Treatment with IL-10 agonist

When tumor cells engineered to express IL-10 were injected in immunocompetent hosts, tumors were rejected [65] and IL-10TG mice reject transplanted tumors [56]. Syngeneic mouse tumors treated with recombinant human IL-10 induced $CD8^+$ T-cell–dependent tumor rejection [66]. In a tumor vaccination, tumor challenge and treatment experiment, IL-10 application during the tumor vaccination did not enhance tumor rejection, while treatment with human IL-10 during the immune effector phase enhanced tumor rejection [67].

The single injection of systemic IL-10 or of a long-lived, pegylated version of IL-10 (PEG-IL-10) into tumor-bearing mice induced expression of IFN-γ and granzymes in tumor infiltrating $CD8^+$ T cells. Sustained treatment with PEG-IL-10 led to the $CD8^+$ T-cell–dependent tumor rejection in a variety of rather immune resistant mouse tumor models and eliminated lung metastasis in aggressive transplanted tumor models. Most strikingly PEG-IL-10 induces rejection of large endogenous breast cancers in Her2 transgenic mice. This is of particular interest, as tumors induced by a transgenic oncogenic driver mutations typically harbor a reduced frequency of somatic mutations and have therefore an increased resistance to immune therapies. Tumor rejection induced by PEG-IL-10 treatment led to a durable immune memory against the rejected tumors, with mice remaining resistant to tumor challenge, tested up to 8 months after the initial tumor rejection. IL-10 receptor ablation studies indicated that tumor immunity is mediated directly through activation of intratumoral $CD8^+$ T cells by IL-10, while IL-10R expression on other cells was dispensable for tumor control [51].

In intratumoral $CD8^+$ T cells, sustained IL-10 exposure induced phosphorylated STAT3 and STAT1 as well as IFN-γ. In contrast, $CD4^+$ T cells or $CD8^+$ T cells from lymphoid organs had only moderate STAT3 phosphorylation and failed to express IFN-γ in IL-10 treated mice [51]. STAT1 is particularly important in the induction of IFN-γ, because it induces T-bet, a transcription factor defining the Th1 and T_c1 lineages of IFN-γ–producing T cells [68]. T-bet and the TCR-activated transcription factor NFAT cooperatively induce IFN-γ and granzymes in cytotoxic T cells [69].

This may suggest that the observed tumor restricted induction of IFN-γ, granzymes, and perforin in $CD8^+$ T cells in PEG-IL-10 treatment only occurred in the presence of Tumor antigen mediated TCR stimulation. Also in the presence of intratumoral IFN-γ produced by $CD8^+$ T cells, both MHC class I and II molecules were induced, potentially allowing increased antigen presentation and recognition within the tumor [70] (Fig. 1b). Supporting this concept, analysis of antigen recognition and the expansion of tumor specific $CD8^+$ T cells in syngeneic mouse tumor models revealed that PEG-IL-10 increased the number of T cells which recognized the tumor cells from the tumors they were

isolated from, but not MHC matched control cells. Interestingly, this increase of tumor specific CD8$^+$ T cells was observed equally in T cells, isolated from the tumor and in the blood of PEG-IL-10 treated animals, suggesting that the IL-10 induced tumor reactive T cells circulate through the lymphatic and the blood [15]. Proliferation of tumor-directed T cells generally is thought to occur in the tumor-draining lymph node, with the post proliferative T cells returning to the tumor through the blood stream. However inhibition of lymphatic migration with an S1P inhibitor during the treatment with IL-10 did not reduce tumor efficacy or the increase of intratumoral CD8$^+$ T cells, suggesting that intratumoral T cell activation and expansion was sufficient to induce tumor rejection in response to IL-10 treatment [51]. IL-10 dependence of intratumoral T cell expansion was further tested by adoptive transfer of IL10R$^+$ and IL10R$^-$ T cells into tumor bearing T cell deficient (Rag-/-) mice. During IL-10 treatment, IL10R-expressing tumor-resident CD8$^+$ T cells rapidly expanded within the tumor tissue, at the expense of T cells not carrying the IL-10 receptor [51].

In summary, IL-10 induces the survival of antigen stimulated CD8$^+$ effector and effector memory T cells, increasing cytotoxic enzymes and IFNγ. CD8$^+$ T cell derived IFN-γ mediated upregulation of antigen presentation within the tumor (Fig. 1b). Due to the upregulation of IL-10R in response to antigen recognition, PEG-IL-10 treatment selectively expands tumor-resident, tumor-specific CD8$^+$ T cells (Fig. 2).

5. Clinical experience with IL-10 agents

5.1. Reduction of inflammation and induction of immune activation in patients treated with recombinant IL-10

IL-10 deficient animals develop inflammatory bowel disease [31] and inflammatory, Th17 mediated disease models are inhibited by IL-10 treatment [71]. Genome-wide association studies also confirmed the association of IL-10 with various inflammatory diseases such as Crohn's disease, ulcerative colitis, and Behçet disease [72] and humans with genetic deficiency in the IL-10 pathway develop very early onset inflammatory bowel disease [16]. Accordingly, clinical trials with recombinant IL-10 were initiated in the nineties with the intend to suppress inflammation in chronic inflammatory diseases. More than five thousand individuals, normal healthy volunteers and patients with inflammatory disease were treated predominantly by subcutaneous injections of recombinant IL-10 [73]. These clinical trials were conducted before a detailed understanding of the molecular and cellular mechanism of IL-10's anti-inflammatory function was established, biomarker analysis was in its infancy and it was therefore difficult to optimize the treatment. Moreover, the pharmacologic characteristics of the recombinant IL-10 limited the exposure to several hours after each subcutaneous injection. Disease-associated proinflammatory cytokines such as TNF-α, IL-1β, IL-12, and IL-17 were reduced significantly, indicating a pharmacodynamic activity of the treatment [74]. The suppression of IL-1 and TNF-α was detectable only during the short periods of elevated serum concentration of IL-10, returning to high values within 24 h after the IL-10 injection [75]. Nevertheless, encouraging

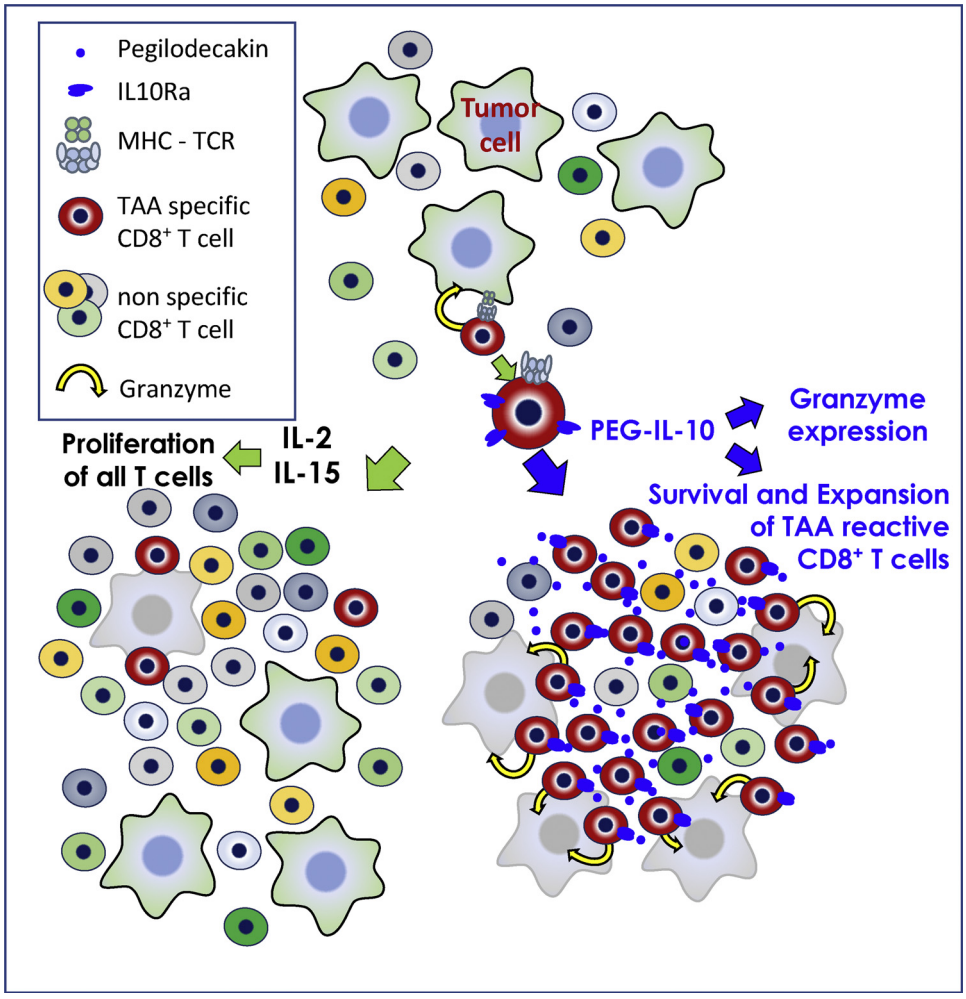

Fig. 2. Expansion of the most fitting – Pegilodecakin expands predominantly tumor recognizing CD8 + T cells. Antigen recognition upregulates IL-10Ra on CD8$^+$ T cells. Pegilodecakin selects preferentially CD8$^+$ T cells, which had a recent antigen encounter - by virtue of their IL-10Ra^{hi} status. IL-10 signaling leads to increased activation, survival and expansion of these TAA reactive CD8$^+$ T cells. In contrast, IL-2 or IL-15, induce proliferation and activation of CD4$^+$ and CD8$^+$ T cells independent of a TCR signal and NK cells. This leads to the activation and proliferation of a large proportion of all peripheral T cells, irrespective of antigen specificity.

efficacy of IL-10 was observed in psoriasis and Crohn disease [73]. In patients with interferon refractory chronic hepatitis C, IL-10 reduced liver inflammation and fibrosis [76]. However, in pivotal trials, frequently using reduced, three-times-weekly dosing regimen to avoid side effects, statistically significant disease modification has not been observed. Taken together, the variable concentration of IL-10 due to the short half-life of recombinant IL-10 in patients may have severely limited the therapeutic benefit.

Concomitantly, signs of IL-10–mediated stimulation of an immune response were observed. In healthy volunteers, HLA-DR$^+$ CD14$^+$ monocytes were elevated in the blood [77]. In patients with Crohn disease, treated daily with higher doses of IL-10, peripheral blood mononuclear cells (PBMC) secreted elevated levels of IFN-γ compared to controls [78]. A single injection of IL-10, given to healthy volunteers prior to or after a challenge with LPS, increased granzyme B and IFN-γ in the serum, but decreased IL-12 p40 [79]. On IL-10, IFNγ-producing CD4 T cells were increased in patients with psoriatic arthritis [80]. These results suggested that therapeutic doses of recombinant IL-10 induced in patients key elements reminiscent of a CD8$^+$ T-cell response to IL-10, including IFN-γ and granzymes. IL-10 induction of IFNγ appeared to be independent of the IL-12 since IFNγ induction occurred concomitantly to the suppression of IL-12 [79].

In summary, recombinant, IL-10 had limited efficacy as an anti-inflammatory treatment, due to its short half-life but also due to the stimulatory activity on CD8$^+$ T cells and IFNγ expression.

5.2. Clinical experience with recombinant pegylated IL-10

Supported by preclinical efficacy of pegylated murine IL-10 in advanced tumor models in mice, phase 1 studies with pegilodecakin were started in 2013 (NCT02009449). Preclinical results had indicated that continuous exposure to pegilodecakin would be required to achieve optimal anti-tumor efficacy [15,51]. Analysis of the cellular target population in mice suggested that tumor resident CD8$^+$ T cells would be the primary effector population necessary for the therapeutic effect of IL-10, indicating the tumor tissue as the relevant pharmacokinetic compartment. To achieve both, increased systemic exposure and sufficient intratumoral concentration of IL-10, daily subcutaneous injection of a pegylated IL-10 was chosen as the initial clinical strategy. The biologic effects of IL-10 on CD8$^+$ Teff cells are beneficial throughout tumor progression, patients were therefore allowed to continue therapy until disease progression [81].

5.2.1. Pegilodecakin monotherapy in dose escalation and early phase 1

The Phase 1 study enrolled patients with advanced solid tumors, which had failed all available prior therapies, including prior immune therapies with IL-2, anti-CTLA4 (ipilimumab) or anti-PD-1. Objective tumor responses were measured using immune related response criteria (irRC) [82]. Patients were allowed to interrupt dosing for brief intermissions for unrelated or related adverse events but could resume treatment until tumor progression or dose limiting toxicity (DLT). Pegilodecakin (AM0010) was well tolerated at escalating doses from 1μg/kg to 40μg/kg. This resulted in a sustained, meaningfully elevated serum concentration of IL-10 between 1 and 40 ng/ml [81], since the half maximal effective concentration (EC50) of pegilodecakin in cell-based in vitro assays was approximately 8 ng/mL and the serum concentration of IL-10 in cancer patients prior to dosing averaged at 3 pg/mL [52]. The DLT was not determined and adverse events (AE) included anemia, thrombocytopenia, fever and fatigue, while hypotension or durable auto-immune related adverse events were not recorded. Importantly, adverse events were transient, residing after short dose interruptions [81]. This allowed for patients with cytopenias and sustained tumor responses to continue treatment after short treatment interruption for several months and up to 2 years. The adverse event profile distinguishes pegilodecakin therapy from IL-2, were severe hypotension is dose and duration of treatment limiting despite the well-established clinical benefit of high-dose IL-2 treatment [1]. Confirming the anti-inflammatory function of IL-10, immune related, inflammatory adverse events (irAEs), such as colitis, pneumonitis or hepatitis were not observed. This differentiates IL-10 treatment also from immune checkpoint inhibitor therapies, which frequently induce irAEs but rarely cause transient cytopenias [83]. Pegilodecakin treated patients had systemic upregulation of immune stimulatory cytokines, such as IFNγ, IL-4 and IL-18, but reduced concentrations of the immune suppressive TGFβ, supporting the hypothesis that pharmacologically stabilized IL-10 may increase T cell activation in patients (see also chapter 5.5 for immune regulation in patients).

Objective responses on pegilodecakin monotherapy were observed in several patients in the dose escalation cohorts, including a renal cell cancer patient (RCC) and a patient with uveal melanoma. In an expansion cohort of 16 patients with pretreated RCC (1–7 prior lines of therapy), four had an objective response (objective response rate, ORR = 25%)[81]. The responses were durable with several of the patients remaining on therapy for more than 2 years. In RCC, Nivolumab (anti-PD-1) as monotherapy had a comparable responses rate of 20–22% in a phase 1 study [84]. In contrast, high dose IL-2 is reserved for previously not treated patients and is not expected to have responses in pretreated RCC patients. Similarly, a pegylated from of IL-2 (NKTR-214), did not have single agent objective tumor response in pretreated, advanced RCC patients [85] (established and emerging treatments for RCC are also discussed in [86]).

Taken together, pegilodecakin had very encouraging efficacy as monotherapy and in combination with chemotherapy (not discussed here) with an acceptable side effect profile. Phase 2 and phase 3 studies are currently in progress.

5.2.2. Pegilodecakin in combination with anti-PD-1

The introduction of immune checkpoint inhibitors progressively changed the landscape of cancer treatment in many indications. In recent years, anti-PD-1 antibodies were approved by the FDA as first line treatment alone or in various combinations for the treatment of melanoma, RCC, non-small cell lung cancer (NSCLC) and other indications [5,86–88]. Treatment of melanoma patients with pembrolizumab induced objective clinical responses in 38% in phase 1 [4]. Clinical responses were predominantly observed in patients whose tumors had prior to therapy a strong infiltration of CD8$^+$ T cells [9], suggesting that the pre-existing immunity is required for the clinical success of anti-PD-1. Intriguingly, the response to anti-PD-1 is correlated with a high burden of somatic mutations in NSCLC patients tumor [10] and the responsiveness of different tumor histologies correlates generally with the frequency of somatic mutations in the tumor type [11]. Preclinical data indicated that pegilodecakin increased the frequency and activity of tumor infiltrating CD8$^+$ T cells in poorly infiltrated tumors. CD8$^+$ T cell mediated tumor responses were also observed in endogenous tumors in a Her2 transgenic tumor model, which generally have a strongly reduced number of somatic mutations and accordingly a low infiltration by T cells [15,51]. Moreover, patients on Pegilodecakin monotherapy had an increase in the number of activated, tumor infiltrating CD8$^+$ T cells in tumors with low T cell infiltration prior to therapy [52].

T cell receptor stimulation simultaneously induces immune checkpoint molecules such PD-1 [89] and the IL-10 receptor but also expression of IL-10 in CD8$^+$ T cells [51]. Exhausted CD4$^+$ T cells in HIV patients have increased expression of both, PD-1 and IL-10Ra, suggesting that T cells exhausted during continuous antigen stimulation may be the primary target for both therapies. This raised the possibility that stimulation of the IL-10 pathway and inhibition of PD-1 may lead to improved clinical responses. In this scenario, pegilodecakin may increase the number of tumor infiltrating, antigen specific T cells and anti-PD-1 would restore TCR activity. To test this concept cohorts of patients with treatment refractory, advanced RCC or non-small cell lung cancer (NSCLC) were enrolled to receive a combination of

pegilodecakin plus one of two PD-1 inhibitory antibodies (pembrolizumab or nivolumab). In NSCLC patients the combination of pegilodecakin plus anti-PD-1 had an objective response rate of 43% and a progression free survival (PFS) of 9.4 months. The median overall survival (OS) of NSCLC patients on pegilodecakin plus anti-PD-1 was 24.1 months. In a similar population pembrolizumab monotherapy, had an ORR of 19.4%, PFS of 3.7 months and overall survival (OS) of 12 months [90]. The response of NSCLC to anti-PD-1 correlates with the expression of PD-L1 in tumor cells, with tumors expressing PD-L1 in more than half of the tumor cells (PD-L1^{hi}) responding to anti-PD-1 in 45% (ORR), while tumors with no PD-L1 expression show responses in less than 10% of cases [90]. PD-L1^{hi} NSCLCs had an ORR 83% and PD-L1 negative NSCLCs treated with pegilodecakin plus anti-PD-1 had objective responses in 33% and a PFS of 11 months. [91].

In RCC patients who had at least one prior therapy and no prior pegilodecakin, the ORR on pegilodecakin plus anti-PD-1 was 44%, 16.7 months PFS and a 1-year overall survival (1-year OS) of 93% [91]. A similar population of RCC patients receiving nivolumab after progression on first line sunitinib, had an ORR of 22%, a PFS of 4.2 months and an 1-year OS of less than 80% [84]. Treatment naïve RCC patients who received nivolumab and anti-CTLA4 (ipilimumab) as the first line treatment had a ORR of 42%, a PFS of 11.6 months and a 1-year OS of 80% [88].

In summary, combination treatment of lung and renal cancers with a combination of pegylated IL-10 and anti-PD-1, appeared to provide an increased tumor response rate but also increased progression free and overall survival, compared to either agent alone.

5.2.3. IL-10 treatment results in Th1 immunity and CD8$^+$ t cell activation

Patients treated with subcutaneous injection of recombinant IL-10 (2 μg/kg) had discontinuous exposure, with a peak concentration of more than 13 ng/mL and less than 1 ng/mL after 24 h [75,92]. Despite this, both anti-inflammatory activity and immune activation had been observed in clinical trials, including the suppression of IL-1 and TNFα and the enhancement of lipopolysaccharide induced IFNγ and Granzyme [79].

- Pegilodecakin Increases Th1 Cytokines and Cytotoxicity markers in the Blood

Cancer patients treated with pegilodecakin had sustained serum levels of IL-10 and an immune cytokine profile consistent with Th1 T cell polarization and CD8$^+$ T cell cytotoxicity dominated anti-inflammatory effects. IL-17, TGFβ and TNFα were consistently, but only partially suppressed. In contrast, IFNγ and T cell derived cytotoxic effector molecule, including FasL, LymphotoxinB were strongly induced [81]. IFNγ started to increase in the serum with a delay, increasing after 7 days of continuous pegilodecakin treatment. Thereafter, IFNγ is elevated throughout the pegilodecakin dosing period at least for several months, as data from cancer patients indicate [52]. However, the levels of IFNγ remained relatively low (below 10 pg/mL) and a cytokine release syndrome was not observed. In comparison, patients treated with i.v. bolus of recombinant IL-12 have a transient rise in serum IFNγ up to 1600 pg/mL [93] and patients on antibody-coupled NHS-IL-12 had spikes of IFNγ up to 20 ng/mL [94]. Pegilodecakin also induced sustained elevation of IL-18. IL-18 induction correlated directly with the tumor reduction in patients (irRC) [52]. IL-18 is both induced by IFNγ, and maintains IFNγ expression in T cells, potentially through the inhibition of activated induced cell death [95]. In support of an important function of IL-18 in T cell mediated tumor responses, CAR T cells expressing IL-18 in tandem with the chimeric antigen receptor (CAR) have strongly enhanced efficacy in mice [96]. Recombinant IL-18 had been studied in cancer patient at doses up to 1 mg/kg, with 2 partial responses observed 3–5 months after a 5 days treatment interval with IL-18 [97]. In a second study in 19 melanoma and RCC patients no responses have been observed [98]. It may be more plausible that IL-18 is not the only key factor mediating tumor responses but represent an important contributing element in the IL-10 mediated tumor response.

In the tumor tissue, treatment with pegilodecakin led to increased GranzymeB$^+$, phospho-STAT-3$^+$ CD8$^+$ T cells and upregulation of MHC-I on tumor and tumor infiltrating cells, similar to previously reported data in mouse models [15]. The expression of MHC molecules in human cancers is correlated with improved prognosis for the patients [99]. In summary, pegilodecakin induced a systemic signature consistent with Th1 polarization and CD8$^+$ T cell activation.

- Induction of CD8$^+$ T cell Invigoration

In patients, Pegilodecakin did not induce significant changes in the overall lymphocyte counts and the number of regulatory T cells or widespread NK and T cell proliferation [52]. This is in contrast to therapies using IL-2 [100] or pegylated IL-2 [85], which induce transient lymphopenia followed proliferation and expansion of CD4$^+$ T cells and Tregs, CD8$^+$ T cells and NK cells. Rather, on pegilodecakin, the percentage of proliferating PD-1$^+$Lag-3$^+$ CD8$^+$ T cells increased in the peripheral blood. PD-1$^+$ and Lag-3$^+$ CD8$^+$ T cells were also increased in tumor biopsies of treated patients. Antigen recognition upregulates the expression of immune checkpoints, thereby indicating an activated state of the T cell. However, the increase of multiple immune inhibitory receptors, in particular the upregulation of Tim-3 on non-proliferating T cells indicates functional exhaustion [101]. Therapy induced proliferation of such immune checkpoint positive T cells is reminiscent of the reinvigoration of exhausted - immune checkpoint expressing CD8$^+$ T cells on anti-PD-1 and immune checkpoint inhibitors [102]. Transient proliferation and expansion of PD-1$^+$ CD8$^+$ T cells has been observed in melanoma patients on anti-PD-1 and the ratio of proliferation to tumor burden correlated with anti-tumor response [103]. However, the anti-PD-1 induced invigoration was transient in patients, with anti-PD-1 induced proliferation occurring only within the first month of treatment [104]. On pegilodakin treatment, PD-1$^+$Lag-3$^+$ CD8$^+$ T cells continued to expand in the blood of patients with continued treatment and the expansion correlated directly with the improved tumor response [52]. Also, the more exhausted PD-1$^+$ Lag-3$^+$ Tim-3$^+$ CD8$^+$ T cell population was not increased on pegilodecakin [52]. TCR stimulation simultaneously upregulates immune checkpoints and the expression of IL-10 receptors, providing a plausible explanation for the selective rescue of this population in pegilodecakin treated patients.

- Pegilodecakin Induced Clonal T cell Expansion

The increase of immune checkpoint positive CD8$^+$ T cells could reflect on an increase of recently activated CD8$^+$ T cells, potentially as a consequence of increased antigen stimulation due to IFNγ induced MHC expression or the rescue of previously exhausted CD8$^+$ T cells. T cell receptor (TCR) sequence analysis on the blood of patients on pegilodecakin showed a clonal expansion of CD8$^+$ T cell clones which were rare or undetectable prior to treatment. Expanding clones continued the expansion throughout the analyzed treatment period. While all patients on pegilodecakin had more expanding than contracting T cell clones, patients who had an objective tumor response had further increased numbers of expanding T cell clones in the blood. The number of expanding T cells per patient also correlated directly with the tumor shrinkage [52]. The expansion of newly discoverable T cell clones and the expansion of PD-1$^+$ Lag-3$^+$ T CD8$^+$ T cells in the blood of patients is reminiscent to the expansion of tumor specific CD8 + T cells in the blood of PEG-IL-10 treated tumor models. It remains to be seen if pegilodecakin indeed expanded a tumor specific T cell population in patients.

Combination of pegilodakin with anti-PD-1 (pembrolizumab) appeared to have an increased objective tumor response rate. However, expansion and proliferation of PD-1$^+$ Lag-3$^+$ CD8$^+$ T cells and the

expansion of novel T cell clones in the blood was similar to pegilodecakin alone [52]. It is plausible, that in the combination therapy, anti-PD-1 primarily enhances antigen specific recognition and elimination of tumor cells within the tissue.

In summary, the analysis of patients treated with pegilodecakin suggests that IL-10 induces a Th1 polarization of the immune system and $CD8^+$ T cell invigoration. Further combination studies with anti-IL-10 are in progress.

6. Conclusions

Since the cloning of IL-10, the anti-inflammatory properties and the cytotoxicity stimulatory function on $CD8^+$ T cell have been studied in vitro, in mouse models and in patients. While the inhibition of immune mediated inflammation showed great promise in a variety of inflammatory diseases, clinical success has been ultimately limited, most likely due to pharmacological properties of recombinant IL-10 used in clinical studies.

In cancer patients, both element of the immune regulation encapsulated in IL-10 may be beneficiary. Tumor promoting, Th17 polarized chronic inflammation induces the recruitment of macrophages and neutrophils to the tumor and enables inflammation induced angiogenesis to fuel tumor growth and metastasis. On the other hand, the reinvigoration of exhausted and anergic T cells, and the inhibition of activation induced cell death of tumor antigen specific $CD8^+$ T cells may enable rejection of large tumors. IL-10 sits at the crossroads of both immune processes.

Pegylated IL-10 increased antigen specific $CD8^+$ T cell cytotoxicity and anti-tumor immune responses and supported an immune stimulatory role of IL-10 at higher and sustained concentrations. However, IL-10 mediated reduction of the inflammatory Th17 pathway was also detectable in cancer patients. IL-10 induced stimulation of T cell derived IFNγ appeared to overcome a suppressive effect of IL-10 on MHC expression, both in mice and in humans. In contrast to other therapeutic modalities, IL-10 appears to induce the key immunostimulatory cytokine IFN-γ only in $CD8^+$ T cells, strictly dependent upon antigen-stimulated TCR signaling. IL-10 may also skew the T cells toward clones recognizing high affinity antigens, excluding bystander clones. The specific expansion of $PD\text{-}1^+$ $Lag\text{-}3^+$ $CD8^+$ T cells reflecting recent antigen exposure and selected T cell clones in patients may support these properties of IL-10. Exploration of the antigen specificity of the pegilodecakin induced T cell repertoire in cancer patients would be warranted.

In contrast, the therapeutic stimulation with inflammatory cytokines such as IL-2 and IL-12 induces broad T cell stimulation, the systemic release of high levels of IFN-γ, leading to systemic immune toxicity (Fig. 2). Inhibition of immune checkpoints bypasses the restrictions of the TCR signal on T cells imposed by negative regulatory pathways, thereby allowing T cells to be activated by lower affinity tumor antigens. Enlisting an increased repertoire of T cells in the antitumor response leads to enhanced tumor immunity but may potentially also explain autoimmune-related side effects of checkpoint inhibitors. The combination of IL-10 activation with immune checkpoint inhibitors may overcome two essential limitations of immune suppression in the tumor microenvironment and represent a promising novel therapeutic avenue for the treatment of patients with cancer

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References

[1] M.B. Atkins, M.T. Lotze, J.P. Dutcher, R.I. Fisher, G. Weiss, K. Margolin, J. Abrams, M. Sznol, D. Parkinson, M. Hawkins, C. Paradise, L. Kunkel, S.A. Rosenberg, High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993, J. Clin. Oncol. 17 (7) (1999) 2105-16.

[2] W.H. Fridman, F. Pages, C. Sautes-Fridman, J. Galon, The immune contexture in human tumours: impact on clinical outcome, Nature reviews, Cancer 12 (4) (2012) 298–306.

[3] J.R. Brahmer, C.G. Drake, I. Wollner, J.D. Powderly, J. Picus, W.H. Sharfman, E. Stankevich, A. Pons, T.M. Salay, T.L. McMiller, M.M. Gilson, C. Wang, M. Selby, J.M. Taube, R. Anders, L. Chen, A.J. Korman, D.M. Pardoll, I. Lowy, S.L. Topalian, Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates, J. Clin. Oncol. 28 (19) (2010) 3167-75.

[4] O. Hamid, C. Robert, A. Daud, F.S. Hodi, W.J. Hwu, R. Kefford, J.D. Wolchok, P. Hersey, R.W. Joseph, J.S. Weber, R. Dronca, T.C. Gangadhar, A. Patnaik, H. Zarour, A.M. Joshua, K. Gergich, J. Elassaiss-Schaap, A. Algazi, C. Mateus, P. Boasberg, P.C. Tumeh, B. Chmielowski, S.W. Ebbinghaus, X.N. Li, S.P. Kang, A. Ribas, Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma, N. Engl. J. Med. 369 (2) (2013) 134-44.

[5] J. Larkin, V. Chiarion-Sileni, R. Gonzalez, J.J. Grob, C.L. Cowey, C.D. Lao, D. Schadendorf, R. Dummer, M. Smylie, P. Rutkowski, P.F. Ferrucci, A. Hill, J. Wagstaff, M.S. Carlino, J.B. Haanen, M. Maio, I. Marquez-Rodas, G.A. McArthur, P.A. Ascierto, G.V. Long, M.K. Callahan, M.A. Postow, K. Grossmann, M. Sznol, B. Dreno, L. Bastholt, A. Yang, L.M. Rollin, C. Horak, F.S. Hodi, J.D. Wolchok, Combined nivolumab and ipilimumab or monotherapy in untreated melanoma, N. Engl. J. Med. 373 (1) (2015) 23–34.

[6] J. Yuan, M. Adamow, B.A. Ginsberg, T.S. Rasalan, E. Ritter, H.F. Gallardo, Y. Xu, E. Pogoriler, S.L. Terzulli, D. Kuk, K.S. Panageas, G. Ritter, M. Sznol, R. Halaban, A.A. Jungbluth, J.P. Allison, L.J. Old, J.D. Wolchok, S. Gnjatic, Integrated NY-ESO-1 antibody and CD8+ T-cell responses correlate with clinical benefit in advanced melanoma patients treated with ipilimumab, Proc. Natl. Acad. Sci. U.S.A. 108 (40) (2011) 16723-8.

[7] J.S. Weber, R.R. Kudchadkar, B. Yu, D. Gallenstein, C.E. Horak, H.D. Inzunza, X. Zhao, A.J. Martinez, W. Wang, G. Gibney, J. Kroeger, C. Eysmans, A.A. Sarnaik, Y.A. Chen, Safety, efficacy, and biomarkers of nivolumab with vaccine in ipilimumab-refractory or -naive melanoma, J. Clin. Oncol. 31 (34) (2013) 4311-8.

[8] J.D. Wolchok, H. Kluger, M.K. Callahan, M.A. Postow, N.A. Rizvi, A.M. Lesokhin, N.H. Segal, C.E. Ariyan, R.A. Gordon, K. Reed, M.M. Burke, A. Caldwell, S.A. Kronenberg, B.U. Agunwamba, X. Zhang, I. Lowy, H.D. Inzunza, W. Feely, C.E. Horak, Q. Hong, A.J. Korman, J.M. Wigginton, A. Gupta, M. Sznol, Nivolumab plus ipilimumab in advanced melanoma, N. Engl. J. Med. 369 (2) (2013) 122-33.

[9] P.C. Tumeh, C.L. Harview, J.H. Yearley, I.P. Shintaku, E.J. Taylor, L. Robert, B. Chmielowski, M. Spasic, G. Henry, V. Ciobanu, A.N. West, M. Carmona, C. Kivork, E. Seja, G. Cherry, A.J. Gutierrez, T.R. Grogan, C. Mateus, G. Tomasic, J.A. Glaspy, R.O. Emerson, H. Robins, R.H. Pierce, D.A. Elashoff, C. Robert, A. Ribas, PD-1 blockade induces responses by inhibiting adaptive immune resistance, Nature 515 (7528) (2014) 568-71.

[10] N.A. Rizvi, M.D. Hellmann, A. Snyder, P. Kvistborg, V. Makarov, J.J. Havel, W. Lee, J. Yuan, P. Wong, T.S. Ho, M.L. Miller, N. Rekhtman, A.L. Moreira, F. Ibrahim, C. Bruggeman, B. Gasmi, R. Zappasodi, Y. Maeda, C. Sander, E.B. Garon, T. Merghoub, J.D. Wolchok, T.N. Schumacher, T.A. Chan, Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer, Science 348 (6230) (2015) 124-8.

[11] M. Yarchoan, B.A. Johnson 3rd, E.R. Lutz, D.A. Laheru, E.M. Jaffee, Targeting neoantigens to augment antitumour immunity, nature reviews, Cancer 17 (4) (2017) 209–222.

[12] D.F. Fiorentino, M.W. Bond, T.R. Mosmann, Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones, J. Exp. Med. 170 (6) (1989) 2081-95.

[13] R. de Waal Malefyt, J. Haanen, H. Spits, M.G. Roncarolo, A. te Velde, C. Figdor, K. Johnson, R. Kastelein, H. Yssel, J.E. de Vries, Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression, J. Exp. Med. 174 (4) (1991) 915-24.

[14] I.A. MacNeil, T. Suda, K.W. Moore, T.R. Mosmann, A. Zlotnik, IL-10, a novel growth cofactor for mature and immature T cells, J. Immunol. 145 (12) (1990) 4167-73.

[15] J.B. Mumm, J. Emmerich, X. Zhang, I. Chan, L. Wu, S. Mauze, S. Blaisdell, B. Basham, J. Dai, J. Grein, C. Sheppard, K. Hong, C. Cutler, S. Turner, D. Laface, M. Kleinschek, M. Judo, G. Ayanoglu, J. Langowski, D. Gu, B. Paporello, E. Murphy, V. Sriram, S. Naravula, B. Desai, S. Medicherla, W. Seghezzi, T. McClanahan, S. Cannon-Carlson, A.M. Beebe, M. Oft, IL-10 elicits IFNgamma-dependent tumor immune surveillance, Cancer Cell 20 (6) (2011) 781-96.

[16] E.O. Glocker, D. Kotlarz, K. Boztug, E.M. Gertz, A.A. Schaffer, F. Noyan, M. Perro, J. Diestelhorst, A. Allroth, D. Murugan, N. Hatscher, D. Pfeifer, K.W. Sykora, M. Sauer, H. Kreipe, M. Lacher, R. Nustede, C. Woellner, U. Baumann, U. Salzer, S. Koletzko, N. Shah, A.W. Segal, A. Sauerbrey, S. Buderus, S.B. Snapper, B. Grimbacher, C. Klein, Inflammatory bowel disease and mutations affecting the interleukin-10 receptor, N. Engl. J. Med. 361 (21) (2009) 2033-45.

[17] B. Neven, E. Mamessier, J. Bruneau, S. Kaltenbach, D. Kotlarz, F. Suarez, J. Masliah-Planchon, K. Billot, D. Canioni, P. Frange, I. Radford-Weiss, V. Asnafi, D. Murugan, C. Bole, P. Nitschke, O. Goulet, J.L. Casanova, S. Blanche, C. Picard, O. Hermine, F. Rieux-Laucat, N. Brousse, F. Davi, V. Baud, C. Klein, B. Nadel, F. Ruemmele, A. Fischer, A Mendelian predisposition to B-cell lymphoma caused by IL-10R deficiency, Blood 122 (23) (2013) 3713-22.

[18] D.J. Berg, N. Davidson, R. Kuhn, W. Muller, S. Menon, G. Holland, L. Thompson-Snipes, M.W. Leach, D. Rennick, Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4(+) TH1-

like responses, J. Clin. Invest. 98 (4) (1996) 1010-20.
[19] K.W. Moore, R. de Waal Malefyt, R.L. Coffman, A. O'Garra, Interleukin-10 and the interleukin-10 receptor, Annu. Rev. Immunol. 19 (2001) 683–765.
[20] T. Kobayashi, K. Matsuoka, S.Z. Sheikh, H.Z. Elloumi, N. Kamada, T. Hisamatsu, J.J. Hansen, K.R. Doty, S.D. Pope, S.T. Smale, T. Hibi, P.B. Rothman, M. Kashiwada, S.E. Plevy, NFIL3 is a regulator of IL-12 p40 in macrophages and mucosal immunity, J. Immunol. 186 (8) (2011) 4649-55.
[21] A.M. Smith, J.E. Qualls, K. O'Brien, L. Balouzian, P.F. Johnson, S. Schultz-Cherry, S.T. Smale, P.J. Murray, A distal enhancer in Il12b is the target of transcriptional repression by the STAT3 pathway and requires the basic leucine zipper (B-ZIP) protein NFIL3, J. Biol. Chem. 286 (26) (2011) 23582-90.
[22] T. Kobayashi, E.C. Steinbach, S.M. Russo, K. Matsuoka, T. Nochi, N. Maharshak, L.B. Borst, B. Hostager, J.V. Garcia-Martinez, P.B. Rothman, M. Kashiwada, S.Z. Sheikh, P.J. Murray, S.E. Plevy, NFIL3-deficient mice develop microbiota-dependent, IL-12/23-driven spontaneous colitis, J. Immunol. 192 (4) (2014) 1918-27.
[23] D.J. Berg, R. Kuhn, K. Rajewsky, W. Muller, S. Menon, N. Davidson, G. Grunig, D. Rennick, Interleukin-10 is a central regulator of the response to LPS in murine models of endotoxic shock and the Shwartzman reaction but not endotoxin tolerance, J. Clin. Invest. 96 (5) (1995) 2339-47.
[24] M. Howard, T. Muchamuel, S. Andrade, S. Menon, Interleukin 10 protects mice from lethal endotoxemia, J. Exp. Med. 177 (4) (1993) 1205-8.
[25] A. Yoshimura, T. Naka, M. Kubo, SOCS proteins, cytokine signalling and immune regulation, nature reviews, Immunology 7 (6) (2007) 454-65.
[26] H.J. Schulze, B. Cribier, L. Requena, J. Reifenberger, C. Ferrandiz, A. Garcia Diez, V. Tebbs, S. McRae, Imiquimod 5% cream for the treatment of superficial basal cell carcinoma: results from a randomized vehicle-controlled phase III study in Europe, Br. J. Dermatol. 152 (5) (2005) 939-47.
[27] A.M. Powell, A.M. Robson, R. Russell-Jones, R.J. Barlow, Imiquimod and lentigo maligna: a search for prognostic features in a clinicopathological study with long-term follow-up, Br. J. Dermatol. 160 (5) (2009) 994-8.
[28] A. O'Garra, F.J. Barrat, A.G. Castro, A. Vicari, C. Hawrylowicz, Strategies for use of IL-10 or its antagonists in human disease, Immunol. Rev. 223 (2008) 114-31.
[29] F. Balkwill, K.A. Charles, A. Mantovani, Smoldering and polarized inflammation in the initiation and promotion of malignant disease, Cancer Cell 7 (3) (2005) 211-7.
[30] B. Ritter, F.R. Greten, Modulating inflammation for cancer therapy, J. Exp. Med. 216 (6) (2019) 1234–1243.
[31] R. Kuhn, J. Lohler, D. Rennick, K. Rajewsky, W. Muller, Interleukin-10-deficient mice develop chronic enterocolitis, Cell 75 (2) (1993) 263-74.
[32] R.K. Sellon, S. Tonkonogy, M. Schultz, L.A. Dieleman, W. Grenther, E. Balish, D.M. Rennick, R.B. Sartor, Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice, Infect. Immun. 66 (11) (1998) 5224-31.
[33] M.J. McGeachy, K.S. Bak-Jensen, Y. Chen, C.M. Tato, W. Blumenschein, T. McClanahan, D.J. Cua, TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology, Nat. Immunol. 8 (12) (2007) 1390-7.
[34] A. Chaudhry, A.Y. Rudensky, Control of inflammation by integration of environmental cues by regulatory T cells, J. Clin. Invest. 123 (3) (2013) 939-44.
[35] A. Chaudhry, R.M. Samstein, P. Treuting, Y. Liang, M.C. Pils, J.M. Heinrich, R.S. Jack, F.T. Wunderlich, J.C. Bruning, W. Muller, A.Y. Rudensky, Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation, Immunity 34 (4) (2011) 566-78.
[36] K.L. Dennis, Y. Wang, N.R. Blatner, S. Wang, A. Saadalla, E. Trudeau, A. Roers, C.T. Weaver, J.J. Lee, J.A. Gilbert, E.B. Chang, K. Khazaie, Adenomatous polyps are driven by microbe-instigated focal inflammation and are controlled by IL-10-producing T cells, Cancer Res. 73 (19) (2013) 5905-13.
[37] K.L. Dennis, A. Saadalla, N.R. Blatner, S. Wang, V. Venkateswaran, F. Gounari, H. Cheroutre, C.T. Weaver, A. Roers, N.K. Egilmez, K. Khazaie, T-cell Expression of IL10 Is Essential for Tumor Immune Surveillance in the Small Intestine, Cancer Immunol. Res. 3 (7) (2015) 806-14.
[38] S.E. Erdman, J.J. Sohn, V.P. Rao, P.R. Nambiar, Z. Ge, J.G. Fox, D.B. Schauer, CD4+CD25+ regulatory lymphocytes induce regression of intestinal tumors in ApcMin/+ mice, Cancer Res. 65 (10) (2005) 3998–4004.
[39] R.A. Kastelein, C.A. Hunter, D.J. Cua, Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation, Annu. Rev. Immunol. 25 (2007) 221-42.
[40] M.W. Teng, D.M. Andrews, N. McLaughlin, B. von Scheidt, S.F. Ngiow, A. Moller, G.R. Hill, Y. Iwakura, M. Oft, M.J. Smyth, IL-23 suppresses innate immune response independently of IL-17A during carcinogenesis and metastasis, Proc. Natl. Acad. Sci. U.S.A. 107 (18) (2010) 8328-33.
[41] J.L. Langowski, X. Zhang, L. Wu, J.D. Mattson, T. Chen, K. Smith, B. Basham, T. McClanahan, R.A. Kastelein, M. Oft, IL-23 promotes tumour incidence and growth, Nature 442 (7101) (2006) 461-5.
[42] N.R. Blatner, M.F. Mulcahy, K.L. Dennis, D. Scholtens, D.J. Bentrem, J.D. Phillips, S. Ham, B.P. Sandall, M.W. Khan, D.M. Mahvi, A.L. Halverson, S.J. Stryker, A.M. Boller, A. Singal, R.K. Sneed, B. Sarraj, M.J. Ansari, M. Oft, Y. Iwakura, L. Zhou, A. Bonertz, P. Beckhove, F. Gounari, K. Khazaie, Expression of RORgammat marks a pathogenic regulatory T cell subset in human colon cancer, Sci. Transl. Med. 4 (164) (2012) 164ra159.
[43] L. Zhou, I.I. Ivanov, R. Spolski, R. Min, K. Shenderov, T. Egawa, D.E. Levy, W.J. Leonard, D.R. Littman, IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways, Nat. Immunol. 8 (9) (2007) 967-74.
[44] X. Yu, D. Rollins, K.A. Ruhn, J.J. Stubblefield, C.B. Green, M. Kashiwada, P.B. Rothman, J.S. Takahashi, L.V. Hooper, TH17 cell differentiation is regulated by the circadian clock, Science 342 (6159) (2013) 727-30.
[45] C.R. Yu, I.M. Dambuza, Y.J. Lee, G.M. Frank, C.E. Egwuagu, STAT3 regulates proliferation and survival of CD8+ T cells: enhances effector responses to HSV-1 infection, and inhibits IL-10+ regulatory CD8+ T cells in autoimmune uveitis, Mediators Inflamm. 2013 (2013) 359674.
[46] A.M. Intlekofer, N. Takemoto, E.J. Wherry, S.A. Longworth, J.T. Northrup, V.R. Palanivel, A.C. Mullen, C.R. Gasink, S.M. Kaech, J.D. Miller, L. Gapin, K. Ryan, A.P. Russ, T. Lindsten, J.S. Orange, A.W. Goldrath, R. Ahmed, S.L. Reiner, Effector and memory CD8+ T cell fate coupled by T-bet and eomesodermin, Nat. Immunol. 6 (12) (2005) 1236-44.
[47] A.M. Intlekofer, A. Banerjee, N. Takemoto, S.M. Gordon, C.S. Dejong, H. Shin, C.A. Hunter, E.J. Wherry, T. Lindsten, S.L. Reiner, Anomalous type 17 response to viral infection by CD8+ T cells lacking T-bet and eomesodermin, Science 321 (5887) (2008) 408-11.
[48] C.R. Stroud, A. Hegde, C. Cherry, A.R. Naqash, N. Sharma, S. Addepalli, S. Cherukuri, T. Parent, J. Hardin, P. Walker, Tocilizumab for the management of immune mediated adverse events secondary to PD-1 blockade, J. Oncol. Pharm. Pract. 25 (3) (2019) 551–557.
[49] W.F. Chen, A. Zlotnik, IL-10: a novel cytotoxic T cell differentiation factor, J. Immunol. 147 (2) (1991) 528-34.
[50] H. Groux, M. Bigler, J.E. de Vries, M.G. Roncarolo, Inhibitory and stimulatory effects of IL-10 on human CD8+ T cells, J. Immunol. 160 (7) (1998) 3188-93.
[51] J. Emmerich, J.B. Mumm, I.H. Chan, D. LaFace, H. Truong, T. McClanahan, D.M. Gorman, M. Oft, IL-10 directly activates and expands tumor-resident CD8(+) T cells without de novo infiltration from secondary lymphoid organs, Cancer Res. 72 (14) (2012) 3570-81.
[52] A. Naing, J.R. Infante, K.P. Papadopoulos, I.H. Chan, C. Shen, N.P. Ratti, B. Rojo, K.A. Autio, D.J. Wong, M.R. Patel, P.A. Ott, G.S. Falchook, S. Pant, A. Hung, K.L. Pekarek, V. Wu, M. Adamow, S. McCauley, J.B. Mumm, P. Wong, P. Van Vlasselaer, J. Leveque, N.M. Tannir, M. Oft, PEGylated IL-10 (Pegilodecakin) induces systemic immune activation, CD8(+) t cell invigoration and polyclonal t cell expansion in Cancer patients, Cancer Cell 34 (5) (2018) 775–791 e3.
[53] J. Geginat, P. Larghi, M. Paroni, G. Nizzoli, A. Penatti, M. Pagani, N. Gagliani, P. Meroni, S. Abrignani, R.A. Flavell, The light and the dark sides of Interleukin-10 in immune-mediated diseases and cancer, Cytokine Growth Factor Rev. (2016).
[54] G. Nizzoli, P. Larghi, M. Paroni, M.C. Crosti, M. Moro, P. Neddermann, F. Caprioli, M. Pagani, R. De Francesco, S. Abrignani, J. Geginat, IL-10 promotes homeostatic proliferation of human CD8(+) memory T cells and, when produced by CD1c(+) DCs, shapes naive CD8(+) T-cell priming, Eur. J. Immunol. 46 (7) (2016) 1622-32.
[55] H. Qiu, X. Hu, L. Gao, L. Chen, J. Chen, J. Yuan, C. Huang, X. Xu, J. Yang, Interleukin 10 enhanced CD8+ T cell activity and reduced CD8(+) T cell apoptosis in patients with diffuse large B cell lymphoma, Exp. Cell Res. 360 (2) (2017) 146–152.
[56] H. Groux, F. Cottrez, M. Rouleau, S. Mauze, S. Antonenko, S. Hurst, T. McNeil, M. Bigler, M.G. Roncarolo, R.L. Coffman, A transgenic model to analyze the immunoregulatory role of IL-10 secreted by antigen-presenting cells, J. Immunol. 162 (3) (1999) 1723-9.
[57] J. Fioravanti, P. Di Lucia, D. Magini, F. Moalli, C. Boni, A.P. Benechet, V. Fumagalli, D. Inverso, A. Vecchi, A. Fiocchi, S. Wieland, R. Purcell, C. Ferrari, F.V. Chisari, L.G. Guidotti, M. Iannacone, Effector CD8(+) T cell-derived interleukin-10 enhances acute liver immunopathology, J. Hepatol. 67 (3) (2017) 543–548.
[58] D.G. Brooks, K.B. Walsh, H. Elsaesser, M.B. Oldstone, IL-10 directly suppresses CD4 but not CD8 T cell effector and memory responses following acute viral infection, Proc. Natl. Acad. Sci. U.S.A. 107 (7) (2010) 3018-23.
[59] W. Cui, Y. Liu, J.S. Weinstein, J. Craft, S.M. Kaech, An interleukin-21-interleukin-10-STAT3 pathway is critical for functional maturation of memory CD8+ T cells, Immunity 35 (5) (2011) 792–805.
[60] J.E. Rood, S.W. Canna, L.K. Weaver, J.W. Tobias, E.M. Behrens, IL-10 distinguishes a unique population of activated, effector-like CD8(+) T cells in murine acute liver inflammation, J. Leukoc. Biol. 101 (4) (2017) 1037–1044.
[61] K. Trandem, J. Zhao, E. Fleming, S. Perlman, Highly activated cytotoxic CD8 T cells express protective IL-10 at the peak of coronavirus-induced encephalitis, J. Immunol. 186 (6) (2011) 3642-52.
[62] Z. Liu, J.Q. Liu, F. Talebian, L.C. Wu, S. Li, X.F. Bai, IL-27 enhances the survival of tumor antigen-specific CD8+ T cells and programs them into IL-10-producing, memory precursor-like effector cells, Eur. J. Immunol. 43 (2) (2013) 468-79.
[63] A.M. Pino-Martinez, C.G. Miranda, E.I. Batalla, S.M. Gonzalez-Cappa, C.D. Alba Soto, IL-10 participates in the expansion and functional activation of CD8(+) T cells during acute infection with Trypanosoma cruzi, J. Leukoc. Biol. 105 (1) (2019) 163–175.
[64] O. Sercan, D. Stoycheva, G.J. Hammerling, B. Arnold, T. Schuler, IFN-gamma receptor signaling regulates memory CD8+ T cell differentiation, J. Immunol. 184 (6) (2010) 2855-62.
[65] C.M. Gerard, C. Bruyns, A. Delvaux, N. Baudson, J.L. Dargent, M. Goldman, T. Velu, Loss of tumorigenicity and increased immunogenicity induced by interleukin-10 gene transfer in B16 melanoma cells, Hum. Gene Ther. 7 (1) (1996) 23–31.
[66] R.M. Berman, T. Suzuki, H. Tahara, P.D. Robbins, S.K. Narula, M.T. Lotze, Systemic administration of cellular IL-10 induces an effective, specific, and long-lived immune response against established tumors in mice, J. Immunol. 157 (1) (1996) 231-.
[67] S. Fujii, K. Shimizu, T. Shimizu, M.T. Lotze, Interleukin-10 promotes the maintenance of antitumor CD8(+) T-cell effector function in situ, Blood 98 (7) (2001) 2143-51.

[68] M. Afkarian, J.R. Sedy, J. Yang, N.G. Jacobson, N. Cereb, S.Y. Yang, T.L. Murphy, K.M. Murphy, T-bet is a STAT1-induced regulator of IL-12R expression in naive CD4+ T cells, Nat. Immunol. 3 (6) (2002) 549-57.
[69] L.H. Glimcher, M.J. Townsend, B.M. Sullivan, G.M. Lord, Recent developments in the transcriptional regulation of cytolytic effector cells, Nature reviews, Immunology 4 (11) (2004) 900-11.
[70] M. Oft, IL-10: master switch from tumor-promoting inflammation to antitumor immunity, Cancer Immunol. Res. 2 (3) (2014) 194-9.
[71] D.J. Cua, H. Groux, D.R. Hinton, S.A. Stohlman, R.L. Coffman, Transgenic interleukin 10 prevents induction of experimental autoimmune encephalomyelitis, J. Exp. Med. 189 (6) (1999) 1005-10.
[72] A. Franke, T. Balschun, T.H. Karlsen, J. Sventoraityte, S. Nikolaus, G. Mayr, F.S. Domingues, M. Albrecht, M. Nothnagel, D. Ellinghaus, C. Sina, C.M. Onnie, R.K. Weersma, P.C. Stokkers, C. Wijmenga, M. Gazouli, D. Strachan, W.L. McArdle, S. Vermeire, P. Rutgeerts, P. Rosenstiel, M. Krawczak, M.H. Vatn, I.S. group, C.G. Mathew, S. Schreiber, Sequence variants in IL10, ARPC2 and multiple other loci contribute to ulcerative colitis susceptibility, Nat. Genet. 40 (11) (2008) 1319-23.
[73] K. Asadullah, W. Sterry, H.D. Volk, Interleukin-10 therapy–review of a new approach, Pharmacol. Rev. 55 (2) (2003) 241-69.
[74] K. Reich, C. Garbe, V. Blaschke, C. Maurer, P. Middel, G. Westphal, U. Lippert, C. Neumann, Response of psoriasis to interleukin-10 is associated with suppression of cutaneous type 1 inflammation, downregulation of the epidermal interleukin-8/CXCR2 pathway and normalization of keratinocyte maturation, J. Invest. Dermatol. 116 (2) (2001) 319-29.
[75] R.D. Huhn, E. Radwanski, J. Gallo, M.B. Affrime, R. Sabo, G. Gonyo, A. Monge, D.L. Cutler, Pharmacodynamics of subcutaneous recombinant human interleukin-10 in healthy volunteers, Clin. Pharmacol. Ther. 62 (2) (1997) 171-80.
[76] D.R. Nelson, G.Y. Lauwers, J.Y. Lau, G.L. Davis, Interleukin 10 treatment reduces fibrosis in patients with chronic hepatitis C: a pilot trial of interferon nonresponders, Gastroenterology 118 (4) (2000) 655-60.
[77] R.D. Huhn, E. Radwanski, S.M. O'Connell, M.G. Sturgill, L. Clarke, R.P. Cody, M.B. Affrime, D.L. Cutler, Pharmacokinetics and immunomodulatory properties of intravenously administered recombinant human interleukin-10 in healthy volunteers, Blood 87 (2) (1996) 699–705.
[78] H. Tilg, C. van Montfrans, A. van den Ende, A. Kaser, S.J. van Deventer, S. Schreiber, M. Gregor, O. Ludwiczek, P. Rutgeerts, C. Gasche, J.C. Koningsberger, L. Abreu, I. Kuhn, M. Cohard, A. LeBeaut, P. Grint, G. Weiss, Treatment of Crohn's disease with recombinant human interleukin 10 induces the proinflammatory cytokine interferon gamma, Gut 50 (2) (2002) 191-5.
[79] F.N. Lauw, D. Pajkrt, C.E. Hack, M. Kurimoto, S.J. van Deventer, T. van der Poll, Proinflammatory effects of IL-10 during human endotoxemia, J. Immunol. 165 (5) (2000) 2783-9.
[80] I.B. McInnes, G.G. Illei, C.L. Danning, C.H. Yarboro, M. Crane, T. Kuroiwa, R. Schlimgen, E. Lee, B. Foster, D. Flemming, C. Prussin, T.A. Fleisher, D.T. Boumpas, IL-10 improves skin disease and modulates endothelial activation and leukocyte effector function in patients with psoriatic arthritis, J. Immunol. 167 (7) (2001) 4075-82.
[81] A. Naing, K.P. Papadopoulos, K.A. Autio, P.A. Ott, M.R. Patel, D.J. Wong, G.S. Falchook, S. Pant, M. Whiteside, D.R. Rasco, J.B. Mumm, I.H. Chan, J.C. Bendell, T.M. Bauer, R.R. Colen, D.S. Hong, P. Van Vlasselaer, N.M. Tannir, M. Oft, J.R. Infante, Safety, Antitumor Activity, and Immune Activation of Pegylated Recombinant Human Interleukin-10 (AM0010) in Patients With Advanced Solid Tumors, J. Clin. Oncol. (2016).
[82] J.D. Wolchok, A. Hoos, S. O'Day, J.S. Weber, O. Hamid, C. Lebbe, M. Maio, M. Binder, O. Bohnsack, G. Nichol, R. Humphrey, F.S. Hodi, Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria, Clin. Cancer Res. 15 (23) (2009) 7412-20.
[83] J.S. Weber, F.S. Hodi, J.D. Wolchok, S.L. Topalian, D. Schadendorf, J. Larkin, M. Sznol, G.V. Long, H. Li, I.M. Waxman, J. Jiang, C. Robert, Safety profile of nivolumab monotherapy: a pooled analysis of patients with advanced melanoma, J. Clin. Oncol. 35 (7) (2017) 785–792.
[84] R.J. Motzer, B.I. Rini, D.F. McDermott, B.G. Redman, T.M. Kuzel, M.R. Harrison, U.N. Vaishampayan, H.A. Drabkin, S. George, T.F. Logan, K.A. Margolin, E.R. Plimack, A.M. Lambert, I.M. Waxman, H.J. Hammers, Nivolumab for metastatic renal cell carcinoma: results of a randomized phase II trial, Journal of clinical oncology: official journal of the American Society of Clinical Oncology 33 (13) (2015) 1430-7.
[85] S.E. Bentebibel, M.E. Hurwitz, C. Bernatchez, C. Haymaker, C.W. Hudgens, H.M. Kluger, M.T. Tetzlaff, M.A. Tagliaferri, J. Zalevsky, U. Hoch, C. Fanton, S. Aung, P. Hwu, B.D. Curti, N.M. Tannir, M. Sznol, A. Diab, A first-in-Human study and biomarker analysis of NKTR-214, a novel IL2Rbetagamma-Biased cytokine, in patients with advanced or metastatic solid tumors, Cancer Discov. 9 (6) (2019) 711–721.
[86] M.B. Atkins, N.M. Tannir, Current and emerging therapies for first-line treatment of metastatic clear cell renal cell carcinoma, Cancer Treat. Rev. 70 (2018) 127–137.
[87] C.J. Langer, S.M. Gadgeel, H. Borghaei, V.A. Papadimitrakopoulou, A. Patnaik, S.F. Powell, R.D. Gentzler, R.G. Martins, J.P. Stevenson, S.I. Jalal, A. Panwalkar, J.C. Yang, M. Gubens, L.V. Sequist, M.M. Awad, J. Fiore, Y. Ge, H. Raftopoulos, L. Gandhi, K.-. investigators, Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study, Lancet Oncol. 17 (11) (2016) 1497–1508.
[88] R.J. Motzer, N.M. Tannir, D.F. McDermott, O. Aren Frontera, B. Melichar, T.K. Choueiri, E.R. Plimack, P. Barthelemy, C. Porta, S. George, T. Powles, F. Donskov, V. Neiman, C.K. Kollmannsberger, P. Salman, H. Gurney, R. Hawkins, A. Ravaud, M.O. Grimm, S. Bracarda, C.H. Barrios, Y. Tomita, D. Castellano, B.I. Rini, A.C. Chen, S. Mekan, M.B. McHenry, M. Wind-Rotolo, J. Doan, P. Sharma, H.J. Hammers, B. Escudier, I. CheckMate, Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma, N. Engl. J. Med. 378 (14) (2018) 1277–1290.
[89] Y. Agata, A. Kawasaki, H. Nishimura, Y. Ishida, T. Tsubata, H. Yagita, T. Honjo, Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes, Int. Immunol. 8 (5) (1996) 765-72.
[90] E.B. Garon, N.A. Rizvi, R. Hui, N. Leighl, A.S. Balmanoukian, J.P. Eder, A. Patnaik, C. Aggarwal, M. Gubens, L. Horn, E. Carcereny, M.J. Ahn, E. Felip, J.S. Lee, M.D. Hellmann, O. Hamid, J.W. Goldman, J.C. Soria, M. Dolled-Filhart, R.Z. Rutledge, J. Zhang, J.K. Lunceford, R. Rangwala, G.M. Lubiniecki, C. Roach, K. Emancipator, L. Gandhi, K.- Investigators, Pembrolizumab for the treatment of non-small-cell lung cancer, N. Engl. J. Med. 372 (21) (2015) 2018-28.
[91] A. Naing, P. Wong, J.R. Infante, W.M. Korn, R. Aljumaily, K.P. Papadopoulos, K.A. Autio, S. Pant, R.M. Bauer, A. Drakaki, N. Daver, A. Hung, N.P. Ratti, S. McCauley, P. Van Vlasselaer, R. Verma, D. Ferry, M. Oft, A. Diab, E.B. Garon, N.M. Tannir, Pegilodecakin combined with pembrolizumab or nivolumab for patients with advanced solid tumours (IVY): a multicentre, open-label, phase 1b trial, Lancet Oncol. (2019) in press.
[92] E. Radwanski, A. Chakraborty, S. Van Wart, R.D. Huhn, D.L. Cutler, M.B. Affrime, W.J. Jusko, Pharmacokinetics and leukocyte responses of recombinant human interleukin-10, Pharm. Res. 15 (12) (1998) 1895-901.
[93] J.A. Gollob, K.G. Veenstra, H. Jyonouchi, A.M. Kelly, P. Ferrieri, D.J. Panka, F. Altare, C. Fieschi, J.L. Casanova, D.A. Frank, J.W. Mier, Impairment of STAT activation by IL-12 in a patient with atypical mycobacterial and staphylococcal infections, J. Immunol. 165 (7) (2000) 4120-6.
[94] J. Strauss, C.R. Heery, J.W. Kim, C. Jochems, R.N. Donahue, A.S. Montgomery, S. McMahon, E. Lamping, J.L. Marte, R.A. Madan, M. Bilusic, M.R. Silver, E. Bertotti, J. Schlom, J.L. Gulley, First-in-Human phase I trial of a tumor-targeted cytokine (NHS-IL12) in subjects with metastatic solid tumors, Clin. Cancer Res. 25 (1) (2019) 99–109.
[95] W. Li, S. Kashiwamura, H. Ueda, A. Sekiyama, H. Okamura, Protection of CD8+ T cells from activation-induced cell death by IL-18, J. Leukoc. Biol. 82 (1) (2007) 142-51.
[96] B. Hu, J. Ren, Y. Luo, B. Keith, R.M. Young, J. Scholler, Y. Zhao, C.H. June, Augmentation of antitumor immunity by human and mouse CAR t cells secreting IL-18, Cell Rep. 20 (13) (2017) 3025–3033.
[97] S.E. Robertson, J.D. Young, S. Kitson, A. Pitt, J. Evans, J. Roes, D. Karaoglu, L. Santora, T. Ghayur, F.Y. Liew, J.A. Gracie, I.B. McInnes, Expression and alternative processing of IL-18 in human neutrophils, Eur. J. Immunol. 36 (3) (2006) 722-31.
[98] M.J. Robertson, J.M. Kirkwood, T.F. Logan, K.M. Koch, S. Kathman, L.C. Kirby, W.N. Bell, L.M. Thurmond, J. Weisenbach, M.M. Dar, A dose-escalation study of recombinant human interleukin-18 using two different schedules of administration in patients with cancer, Clin. Cancer Res. 14 (11) (2008) 3462-9.
[99] M.D. Walsh, O.F. Dent, J.P. Young, C.M. Wright, M.A. Barker, B.A. Leggett, L. Bokey, P.H. Chapuis, J.R. Jass, G.A. Macdonald, HLA-DR expression is associated with better prognosis in sporadic Australian clinicopathological Stage C colorectal cancers, International journal of cancer, Int. J. Cancer Suppl. 125 (5) (2009) 1231-7.
[100] M.B. Atkins, Cytokine-based therapy and biochemotherapy for advanced melanoma, Clinical cancer research : an official journal of the American Association for Cancer Research 12(7 Pt 2) (2006) 2353s–2358s.
[101] E.J. Wherry, M. Kurachi, Molecular and cellular insights into T cell exhaustion, Nature reviews, Immunology 15 (8) (2015) 486-99.
[102] S.J. Im, M. Hashimoto, M.Y. Gerner, J. Lee, H.T. Kissick, M.C. Burger, Q. Shan, J.S. Hale, J. Lee, T.H. Nasti, A.H. Sharpe, G.J. Freeman, R.N. Germain, H.I. Nakaya, H.H. Xue, R. Ahmed, Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy, Nature 537 (7620) (2016) 417–421.
[103] A.C. Huang, M.A. Postow, R.J. Orlowski, R. Mick, B. Bengsch, S. Manne, W. Xu, S. Harmon, J.R. Giles, B. Wenz, M. Adamow, D. Kuk, K.S. Panageas, C. Carrera, P. Wong, F. Quagliarello, B. Wubbenhorst, K. D'Andrea, K.E. Pauken, R.S. Herati, R.P. Staupe, J.M. Schenkel, S. McGettigan, S. Kothari, S.M. George, R.H. Vonderheide, R.K. Amaravadi, G.C. Karakousis, L.M. Schuchter, X. Xu, K.L. Nathanson, J.D. Wolchok, T.C. Gangadhar, E.J. Wherry, T-cell invigoration to tumour burden ratio associated with anti-PD-1 response, Nature 545 (7652) (2017) 60–65.
[104] K.E. Pauken, M.A. Sammons, P.M. Odorizzi, S. Manne, J. Godec, O. Khan, A.M. Drake, Z. Chen, D.R. Sen, M. Kurachi, R.A. Barnitz, C. Bartman, B. Bengsch, A.C. Huang, J.M. Schenkel, G. Vahedi, W.N. Haining, S.L. Berger, E.J. Wherry, Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade, Science 354 (6316) (2016) 1160–1165.