Current Oncology Reports (2021) 23: 83
https://doi.org/10.1007/s11912-021-01064-4

MELANOMA (RJ SULLIVAN, SECTION EDITOR)

Cytokines in the Treatment of Melanoma

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Accepted: 30 March 2021 / Published online: 18 May 2021

Abstract

Purpose of Review The use of cytokines in harnessing the immune system to eradicate cancer has been an important treatment modality. However, the dose-limiting toxicities of these cytokines limited their usage in clinic. Here, we review the basic biology of cytokines involved in the treatment of melanoma and discuss their therapeutic applications. Moreover, we describe several innovative technological approaches that have been developed to improve the pharmacokinetics, safety, and efficacy of these cytokines.

Recent Findings The safety and the anti-tumor activity of newly engineered cytokines including PEGylated IL-2 (NKTR-214), PEGylated IL-10 (AM0010), and IL-15 super agonist (ALT-803) have been evaluated in clinical trials with encouraging clinical activity and acceptable safety profile, both as single agents and in combination with immuno-oncology agents.

Summary A greater understanding of the mechanisms of action and effective dosing of these newly engineered cytokine together with determination of optimum combination therapy regimens may yield greater clinical benefits in the future.

Keywords Cytokines · Cancer · Melanoma · Therapy · Interferon · Interleukin 2 · Interleukin 10 · Interleukin15 · NKTR-214 · AM0010 · ALT-803

Introduction

Cytokines play an essential role in the regulation of the innate and adaptive immunity. They control the development, survival, and function of various immune cells. Cytokines can also directly inhibit the tumor cell growth by impairing their proliferation and promoting their apoptosis. In recent years, a number of cytokines, including interferon-α (IFN-α); interleukin-2 (IL-2), IL-10, IL-12, IL-15, and IL-21; and granulocyte macrophage colony-stimulating factor (GM-CSF) have been shown to mediate effective anti-tumor immunity in preclinical cancer models [1–8]. To date, only two cytokines have obtained FDA approval for melanoma treatment: high-dose IL-2 (HD-IL-2) for metastatic melanoma and IFN-α as adjuvant therapy for high-risk surgically resected melanoma (stage II B and stage III). However, these two cytokines were not consistently used in the clinic due to their short half-life and the need for high and frequent dosing, which often result in considerable toxicity. Several innovative approaches such as cytokine-antibody fusion molecules (immunocytokines), recombinant viral vectors to deliver cytokine genes, transgenic expression of cytokines in whole tumor cells, and chemical conjugation to polyethylene glycol (PEGylation) are being utilized to improve cytokine half-lives and their ability to target tumors more efficiently. In this review, we discuss the basic biology and therapeutic applications of the major cytokines involved in the treatment of melanoma. We also describe several innovative approaches that have been developed to improve the pharmacokinetics, safety, and efficacy of these cytokines.

This article is part of the Topical Collection on *Melanoma*

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IL-2

IL-2 is a potent cytokine for promoting the activation and proliferation of $CD8^+$ T cells and natural killer (NK) cells. The IL-2 receptor is composed of three subunits including IL-2Rα (CD25), IL-2β (CD122), and IL-2γ (CD132). At

high doses, IL-2 binds to heterodimeric IL2Rβγ causing expansion of $CD8^+$ T cells [9]. IL-2 also binds with higher affinity to its heterotrimeric receptor containing the subunit IL-2Rα (CD25) forming the IL-2αβγ complex, leading to expansion of $CD4^+$ T regulatory cells (Tregs) [10], which express high levels of IL-2Rα. HD-IL-2 was approved for the treatment of metastatic melanoma in 1998. HD-IL-2 induces objective clinical responses and durable complete response (CR) rates in 15–20% and 5–7% respectively in patients with advanced melanoma [11]. However, its therapeutic applications have multiple shortcomings such as the stimulation of Tregs and the need for high and frequent dosing, which increase the risks of severe adverse effects including capillary leak syndrome, gastrointestinal side effects, and constitutional symptoms such as fever and chills. Several approaches were used to increase the efficacy and improve the safety of IL-2. For instance, two novel IL-2 mutants (F42K and R38A) with different binding properties were created to expand $CD8^+$ T cells and NK without Treg expansion and minimal secretion of high levels of pro-inflammatory cytokine [12]. More recently, a novel IL-2 fusion protein, namely cergutuzumab amunaleukin (RG7813), has been developed using engineered mutated IL-2 variant with a reduced affinity to IL-2Rα. This mutant is fused to a human monoclonal antibody that targets carcinoembryonic antigen (CEA), a protein produced in very small amounts after birth but found at high levels on the surface of several types of tumor cells [13]. The safety and efficacy of RG7813 in combination with atezolizumab in patients with locally advanced and/or metastatic solid tumors including melanoma are being evaluated in a phase 1b clinical trial (NCT02350673). Another IL-2 fusion protein targeting fibroblast activation protein-α (FAP), namely RO6874281 (RG-7461), is being evaluated in phase 1a/1b study as a single agents in combination with trastuzumab or cetuximab in patients with advanced and/or metastatic solid tumors (NCT02627274). RG-7461 is further tested in phase 1b study in combination with pembrolizumab in patients with previously untreated advanced and/or metastatic melanoma (NCT03875079). ALKS 4230 is another engineered fusion protein composed of a circularly permuted interleukin-2 (IL-2) and IL-2 receptor (IL-2R) α designed to selectively activate the intermediate-affinity IL-2Rβγ. A phase 1/2 study is evaluating ALKS 4230 administered intravenously (IV) as monotherapy and in combination with pembrolizumab in subjects with advanced solid tumors (NCT02799095). Another phase 1/2 study is evaluating ALKS 4230 administered subcutaneously (SC) as monotherapy and in combination with pembrolizumab in subjects with advanced solid tumors (NCT03861793). PEGylation is another strategy to improve IL-2 efficacy. A novel IL-2 cytokine, namely bempegaldesleukin (NKTR-214), was designed to preferentially expand $CD8^+$ T cells and NK cells over Tregs. NKTR-214 is recombinant IL-2 molecule of aldesleukin conjugated with six releasable polyethylene glycol (PEG) chains. Upon IV administration, the PEG chains slowly release via hydrolysis to generate the active cytokine species (2-PEG-IL-2 and 1-PEG-IL-2) that have a peak plasma concentration 26–30 h after infusion. The slow generation of the 2-PEG-IL-2 and 1-PEG-IL-2 significantly mitigates the rapid-onset, systemic cytokine-related toxicities associated with high-dose IL-2. Furthermore, the prodrug design of NKTR-214 with its antibody-like dosing, and the long half-life, obviate the need for 5-day courses of thrice daily infusions, or daily subcutaneous injections. In addition, the polymer conjugation of NKTR-214 promotes biased signaling through the IL-2 receptor beta gamma (IL-2Rβγ). This unique feature preferentially increases the proliferation, $CD8^+$ T cells, and natural killer cells within the tumor microenvironment (TME) without expanding unwanted intra-tumoral Tregs that are activated through the IL-2 heterotrimeric receptor IL-2Rαβγ [14]. The phase 1 dose escalation trial of NKTR monotherapy assessing safety and tolerability enrolled 28 patients with locally recurrent or metastatic solid tumors including melanoma (NCT02869295). Patients received outpatient IV dosing over 15 min every 2 or 3 weeks. NKTR-214 had a favorable safety profile, with no immune-related AEs; grade 3 hypotension was observed in 3/25 patients and was rapidly reversible with fluids; notably, all three patients continued dosing after these events; no capillary leak syndrome was observed. Furthermore, the drug showed clinical activity including tumor shrinkage and durable disease stabilization in heavily pretreated patients [15•]. Based on the biological activity and tolerability of NKTR-214 as well as the non-overlapping toxicities with approved checkpoint inhibitors, NKTR-214 is being evaluated in ongoing phase 1/2 trial (PIVOT-02) combined with nivolumab in patients with select locally advanced or metastatic solid tumor malignancies (NCT02983045). Preliminary results from PIVOT2 study presented at the Society for Immunotherapy of Cancer (SITC) annual meeting in 2018 showed an acceptable toxicity profile and promising evidence of clinical activity (ORR, 53%; CR, 24%) in checkpoint inhibitor (CPI)–naïve metastatic melanoma patients [16]. NKTR-214 is further tested in combination with nivolumab plus NKTR-262, a small molecule agonist of Toll-like receptors (TLRs) 7/8 designed to be retained in the TME to activate antigen presenting cells (APCs) in patients with locally advanced or metastatic solid tumor malignancies (NCT03435640).

Interferon-α

IFN-α is a potent cytokine with anti-proliferative, anti-tumor, and immunomodulatory properties [17, 18]. IFN-α can directly inhibit the growth of melanoma cells and promote their apoptosis [19–22]. In addition, IFN-α induces the

upregulation of major histocompatibility complex (MHC) class I molecules on tumor cells [23, 24] and promote dendritic cell (DC) maturation. IFN-α promotes also NK cell-mediated cytotoxicity and induces the activation and proliferation of memory $CD8^+$ cytotoxic T lymphocytes (CTLs) [25, 26]. High-dose interferon-α (HD-IFN-α) was approved in 1996 as adjuvant therapy for the treatment of resected stage IIB/III melanoma based on the results of Eastern Cooperative Oncology Group (ECOG) E1684 trial ($n = 287$). In this study, HD-IFN-α regimen was administrated to patients with stages IIB, III, or IV received as an induction phase with IFN-α given at a dose of 20 MU/m^2/day IV for 5 days per week for 4 weeks, followed by a maintenance phase of subcutaneous IFN-α at a dose of 10 MU/m^2/day every other day three times each week for an additional 48 weeks [27]. Clinical data from this trial showed an improvement in the relapse-free survival (RFS) and overall survival (OS). Other clinical trials, ECOG 1690 ($n = 642$) and ECOG 1694 ($n = 880$) [28, 29], showed a significant treatment improvement in RFS of HD-IFN versus low-dose IFN (LD-IFN) and HD-IFN versus GMK vaccine. The toxicity profile of IFN-α is significant, but tolerable in the majority of patients with dose interruptions and reductions. Flu-like symptoms including fever, fatigue, headaches, gastrointestinal symptoms, and myalgias occurred in most patients [30–32]. IFN-α also increases hepatic enzymes in some patients. Thrombocytopenia, leukopenia, and neutropenia have also been reported in most patients. More serious side effects are the neuropsychiatric issues, including depression, confusion, mania, and some cases of suicide [33]. HD-IFN-α toxicity is usually dose-related. In addition, HD-IFN-α inconvenient three to five times weekly maintenance dosing schedule resulted in poor patient compliance with therapy. A PEGylated formulation of IFN-α (PEG-IFN) was developed to improve the half-life of the drug, optimizing its pharmacokinetics profile and allowing administration in a once weekly regimen. PEG-IFN was approved in 2011 as an adjuvant therapy for stage III melanoma based on the results of EORTC 1899 trial [34]. In this study, 1256 patients received prolonged weekly administration of PEG-IFN treatment for up to 5 years. PEG-IFN regimen was administrated first as an induction phase of 6 μg/kg once weekly for 8 weeks, followed by maintenance dose of 3 μg/kg once weekly. PEG-IFN therapy was generally well tolerated and provides a significant improvement in RFS [34]. Although HD-IFN-α was approved as adjuvant therapy for the treatment of resected stage IIB/III melanoma, the drug was not consistently used mainly because of considerable toxicity. Moreover, IFN-α was largely replaced by PD-1 inhibitors [35, 36]. It has been shown in preclinical mouse model that IFN-α induce the upregulation of PD1 in T cells, and its combination with PD1 inhibitors was synergetic [37]. Recently, the safety of PEG-IFN in combination with pembrolizumab in patients with metastatic stage IV melanoma was evaluated in a phase 1b/2 study [38•]. Overall, this combination showed an acceptable toxicity profile with promising evidence of clinical activity in PD-1-naïve metastatic melanoma patients.

IL-15

IL-15 is a potent immune stimulatory cytokine mainly produced by activated myeloid cells as a membrane-bound heterodimer associated with IL-15Rα, a transmembrane protein that facilitate the trafficking and trans-presentation of IL-15 to NK and T cells. IL-15 signals through IL-2/IL-15Rβ/γc, a shared receptor with IL-2 [39, 40]. IL-15 shares functional similarities with IL-2; both cytokines promote the activation and proliferation of $CD8^+$ T cells and NK cells. In contrast to IL-2, IL-15 lacks the capacity to stimulate Tregs and does not cause a substantial toxicity necessitating intense supportive care. However, the use of recombinant hIL-15 (rhIL-15) in the clinic is not optimal due to the low expression of IL-15Rα, which plays an essential role in stabilizing and increasing the biological activity of IL-15 and the need for high doses to achieve biological responses in vivo [41, 42]. Preclinical studies in mice showed that IL-15 had a more favorable safety profile than IL-2, with robust efficacy including induction of anti-tumor immunity [43–45]. The safety and efficacy of rhIL-15 as a single agent were evaluated in a phase 1 dose escalation trial. In this study, rhIL15 was administrated as a daily intravenous bolus infusion for 12 consecutive days to five patients in patients with metastatic malignant melanoma or metastatic renal cell cancer (NCT01021059). Treatment with rhIL-15 was shown to induce biological activity on NK cells and $CD8^+$ memory T cells. However, this treatment proved to be difficult due to clinical toxicities produced by intense cytokine secretion that occurred in the first 2 h after treatment [46]. To reduce the toxicity, an alternative dosing strategy was initiated in phase 1 dose escalation trial of subcutaneous (SC) rhIL15 in refractory solid tumor cancer patients. Treatment consisted of daily SC injections of rhIL15 for two consecutive weeks (10 total doses/cycle) (NCT01727076). Overall, the treatment was well tolerated and was shown to induce biological activity on NK cells and $CD8^+$ memory T cells. Despite the absence of objective response, the drug showed clinical activity including disease stabilization in several patients including a renal cell carcinoma patient who continued protocol treatment for 2 years [47]. As described earlier, the biological activity of IL-15 is limited by the availability of IL-15Rα and could be further augmented by pre-association with its soluble receptor (IL-15Rα) [48–51]. Recently, a novel IL-15 super agonist complex named ALT-803 has been developed to improve pharmacokinetic properties of native IL-15 and enhances its anti-tumor activity. ALT-803 consists of an IL-15 mutant (IL-15N72D) bound to an IL-15 receptor α/IgG1 Fc fusion protein. ALT-803 showed

Table 1 Summary of selected clinical trials investigating new engineered cytokines as single agents and in combination with immuno-oncology agents including checkpoint inhibitor

Cytokine	Main mechanism of action	Name of drug	Treatment	Clinical trial	Clinical stage
IL-2	Clonal expansion of NK and T cells	Cergutuzumab amunaleukin (RG7813)	RG7813 + atezolizumab	NCT02350673	1b
		RO6874281 (RG-7461)	RG-7461 + atezolizumab	NCT03386721	2
			RG-7461 + pembrolizumab	NCT03875079	1b
			RG-7461	NCT02627274	1a/1b
			RG-7461 + trastuzumab or cetuximab	NCT02627274	1a/1b
		ALKS 4230	ALKS 4230	NCT02799095	1/2
			ALKS 4230 + pembrolizumab	NCT02799095	1/2
			ALKS 4230	NCT03861793	1/2
			ALKS 4230 + pembrolizumab	NCT03861793	1/2
		Bempegaldesleukin (NKTR-214)	NKTR-214	NCT02869295	1
			NKTR-214 + nivolumab	NCT02983045	1/2
			NKTR-214 + NKTR-264+ nivolumab	NCT03435640	1/2
IL-10	Promotion of cytotoxic $CD8^+$ T cells	Pegilodecakin (AM0010)	AM0010	NCT02009449N	1/1b
			AM0010 + chemotherapy	CT02009449	1/1b
			AM0010 + PD-1 inhibitors	NCT02009449	1/1b
IL-15	Clonal expansion of NK and T cells	ALT-803	ALT-803	NCT01946789	1
			ALT-803 + nivolumab	NCT03228667	2b

promising immunostimulatory activity in B16 melanoma mouse model with relatively longer half-life than IL-15 and retention in lymph nodes [52]. In addition, the pharmacokinetics, safety, and efficacy of ALT-803 were evaluated in nonhuman primates. Results from this study showed that ALT-803 exhibit longer half-life estimated at 7–8 h. More importantly, the drug induced expansion of NK and T cells in both blood and TME without triggering a cytokine storm. Based on these compiling findings, several clinical trials are currently open to evaluate the safety and to determine both the maximum tolerated dose and the minimum effective dose of ALT-803 as a monotherapy in patients with advanced solid tumors including melanoma patients (NCT01946789). In this study, ALT-803 was administered as a weekly IV infusion or SC injection for 4 consecutive weeks, every 6 weeks until toxicity or progression. Overall, the therapy was well tolerated with minimal cytokine toxicities. The therapy induced expansion and activation of both NK and $CD8^+$ T cells. Clinical activity was not observed [53]. ALT-803 is being evaluated in combination with PD-1/PD-L1 checkpoint inhibitors in patients with advanced cancer who have progressed following an initial response to treatment with PD-1/PD-L1 checkpoint inhibitor therapy (NCT03228667). The phase 1b clinical trial assessing the safety and efficacy of ALT-803 in combination with nivolumab enrolled 23 patients with previously treated stage IIIB or stage IV NSCLC with escalating doses of ALT-803 [54••]. In this study, ALT-803 was administrated as a weekly SC injection to 21 patients on weeks 1–5 of four 6-week cycles for 6 months. The treatment was well tolerated; no dose-limiting toxicities were observed. The median progression-free survival was 9.4 months, and the median overall survival was 17.4 months and 29% of patients achieved an objective response.

IL-10

Interleukin-10 (IL-10) is an anti-inflammatory cytokine mainly produced by activated T cells and APCs. IL-10 plays crucial role in preventing inflammatory and autoimmune pathologies. Recombinant IL-10 has been studied in psoriasis, inflammatory disease, and liver fibrosis clinical trials as an anti-inflammatory molecule [55]. IL-10 has been considered an immunosuppressive cytokine at lower concentrations. However, there is growing evidence supporting an anti-tumor activity of IL-10. At higher doses, IL-10 was shown to induce activation and proliferation of $CD8^+$ tumor-infiltrating T cells (TIL) [56–58]. A PEGylated formulation of recombinant IL-10 named pegilodecakin (AM0010) was developed to improve the half-life of the drug, optimizing its pharmacokinetics profile and allowing sustained exposure. It has been shown in mouse tumor models that this form of IL-

10 elicits potent IFN-γ and $CD8^+$ T cell–dependent anti-tumor effects [59]. Given these compelling findings, a phase 1/1b clinical trial evaluating the safety and efficacy of AM0010 as a monotherapy or in combination with chemotherapy or PD-1 inhibitors in patients with advanced treatment-refractory solid tumors including melanoma (NCT02009449). AM0010 was self-administrated subcutaneously daily at a dose of 1 to 40 μg/kg. Results from the monotherapy dose escalation portion on 33 heavily pretreated patients demonstrated that AM0010 has acceptable toxicity profile and a potent capacity to stimulate the expansion and cytotoxicity of $CD8^+$ TIL [60•, 61•]. The most common AEs were anemia, fatigue, thrombocytopenia, injection site reactions, and fever. Grade 3 to 4 non-hematopoietic treatment-related AEs were observed in 15% of patients. Treatment-related grade 3 to 4 anemia or thrombocytopenia was observed in 18% of patients. AM0010 is now being evaluated in combination with pembrolizumab in advanced treatment-refractory solid tumors including melanoma (NCT02009449).

Conclusions

Cytokine-based therapy can elicit potent and durable anti-tumor responses in several cancers. High-dose IL-2 and IFN-α have been approved in the treatment of melanoma. However, the pleiotropic effects and dose-limiting toxicities of these cytokines limited their usage in clinic. In recent years, several technological approaches such as immunocytokines, and PEGylation, are being developed to improve the safety and anti-tumor activity of cytokines. The promising results of newly engineered cytokine in promoting effective anti-tumor immunity in preclinical studies have led to the implementation of several clinical trials (Table 1). A greater understanding of the mechanisms of action and effective dosing of these newly engineered cytokine together with determination of optimum combination therapy regimens may yield greater clinical benefits in the future.

Declarations

Conflict of Interest Salah-Eddine Bentebibel declares that he has no conflict of interest. Adi Diab has received a commercial research grant from Nektar Therapeutics and declares he is a consultant/advisory board member for Nektar Therapeutics.

Human and Animal Rights of Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

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- • Of importance
- •• Of major importance

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