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Review

Interleukin-2 therapy of cancer-clinical perspectives

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ABSTRACT

Interleukin (IL)-2 is a pleiotropic cytokine that displays opposing activities on immune system acting either in favor of or against cancer progression. Advanced/metastatic melanoma and renal cell carcinoma (RCC) are the two types of cancers that included most studies implemented for assessing the role of high-dose IL-2 therapy. The use of high-dose IL-2 therapy can, however, increase the rate of toxicities and interferes with the activity of endothelial cells (ECs) and effector T cells in tumor microenvironment (TME). This implies the need for adjusting strategies related to the cytokine therapy, such as suppressing signals that are interfering with the activity of this cytokine or the use of engineered IL-2 variants. The focus of this review is to discuss about pros and cons related to the IL-2 therapy and propose strategies to increase the efficacy of therapy. The outcomes of this literature will call for application of variants of IL-2 engineered to represent higher half-life and efficacy, and are more safe in the area of cancer immunotherapy.

1. Introduction

Cancer is an uncontrolled cellular growth condition that is considered as one of the most prevalent diseases and a leading cause of death worldwide [1–2]. Immunotherapy of cancer has received an intense focus in the recent years. Allison and Honjo started a ground-breaking work on cancer immunotherapy in the year 1992. The outcomes of this work was honored by Nobel Prize award in physiology and medicine at the year 2018 [3]. Their discovery for application of checkpoint inhibitors has been used for a number of cancers including tumors placed in the category of cold, and the outcomes were promising for some types of cancers in this category [4]. Cold cancers are low or irresponsive tumors that display low infiltration of $CD8^+$ T lymphocytes (CTLs) [5]. Despite advances, there are still challenges in the field of immunotherapy. The checkpoint inhibitor ipilimumab was the second drug that received indication for metastatic melanoma [6]. Low activity of solo immune checkpoint inhibitor (ICI) therapy and its unwanted effects on tumor microenvironment (TME) are issues that are still exist in the area [3–4]. TME is a heterogeneous and adaptive niche that add complexity and variability to the evolutionary tumorigenic processes [7].

The first immunotherapeutic approach approved in the USA for melanoma (stage IV) was high-dose interleukin (IL)-2 [8]. IL-2 is an immune-activating cytokine [9] and a key component of several cancer immunotherapeutic approaches [10]. IL-2 is discovered in the year 1976 and cloned in 1983 [9]. IL-2 acts for promoting expansion, function and survival of effector T cells. Besides the activation of immune system, it can also repress immune responses, thus rising challenges in regard with the use of this cytokine in cancer immunotherapy, concerning the toxicity and the adversarial impacts on other cells in TME [11]. IL-2 therapy pose a huge difference in functionality in different doses. The two types of cancers with the most focus in clinic for evaluation of the efficacy of IL-2 therapy are advanced or metastatic melanoma and renal cell carcinoma (RCC). Many of the clinical trials published so far in regard with the role of this cytokine were with the high-dose IL-2 therapy, but it can rise the rate of toxicity and exerting unwanted effects on TME. In this review, we aimed to discuss about both sides, and proposing strategies to increase the efficacy and to reduce the rate of negative impacts of IL-2 therapy, particularly in melanoma and RCC.

2. Interleukin-2

2.1. The structure of IL-2 receptor-ligand

IL-2 is a 4α-helix bundle member of cytokine family with 15.5 kDa which acts through binding to the IL-2 receptor (IL-2R) [12]. IL-2 receptor (IL-2R) is considered as a heterotrimeric protein that represents three subunits or chains: α (CD25), β (CD122) and γ (CD132). The different and separate expressions of these receptor chains on various

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immune cell types is associated with diverse functionality of IL-2 in TME. Assemblage of these receptor chains in different combinations will lead to the generation of IL-2Rs with three levels of affinities: low, intermediate and high. Low affinity occurs when α chain is involved in the interaction between receptor and ligand. The intermediate level of affinity occurs when β and δ chains are involved in the receptor/ligand interactions. Finally, IL-2R interacts through α, β and γ chains with high affinity, forming a stable complex. The first interaction occurs between IL and 2 with IL-2Rα. This interaction leads to a conformational alteration in IL-2, which allows for its efficient bondage with IL-2Rβ and IL-2Rγ [13]. ILs 2, 4, 7, 9, 15 and 21 share the γ receptor. IL-2 and IL-15 ligands represent the shared β and γ receptor chains [13]; this is indicative of the shared functionality between IL and 2 and IL-15, and can be a possible reason for the usage of IL-15 as an alternative to the IL-2 therapy [10], discussed further (Fig. 1).

2.2. IL-2 receptor/ligand activity in tumor microenvironment

IL-2 is generally considered as a critical growth factor for lymphocytes (T cells) [12]. CTLs are the key cells of adaptive immunity and the front-line defense against cancer that their retarded functionality is a key mechanism of immune escape by tumor [14–16]. Regulatory T cells (Tregs) are placed in another side in which their accelerated activity is related to the immunosuppression within TME [14]. Tregs are responsible for suppression of CTL activity, and promotion of tumor resistance, relapse and metastasis [17]. CD25 is expressed constitutively by Tregs. Tregs are thus representing as cell types highly responsive to the IL-2 content of TME [18–19]. Highly functional Tregs are responsible for curtailing anti-cancer responses, mediated via preventing T cell activation [20]. High rates of CD25 expression allows Tregs to be a winner in competition for IL-2 acquisition from TME; this will reduce the amount of IL-2 available for T cell induction, which can be partly due to the short half-life (about 7 min) of this cytokine in humans [18]. Endothelial cells (ECs) also express CD25, which can be a reason for IL-2-related toxic effects on blood vessels [18] and promoting a condition called vascular leak syndrome (VLS) [21]. The leaky vasculature is a common feature occurring in tumor vessels and is contributed to the restriction of drug accumulation in tumor area, thus promoting cancer resistance to therapy [15]. CD122/CD132 is generally expressed by naïve $CD8^+$ T cells; the expression of CD25 on CTLs is transient and occurs after stimulation of T cell receptor (TCR). Therefore, exploiting strategies to use long-lived IL-2 analogs that are able to preferentially target the intermediate affinity receptor (i.e. CD122/CD132) over the high affinity CD25/CD122/CD135 receptor will reduce the rate of toxicity and support the activity of effector T cells over Tregs, thereby potentiating the anti-tumor activity of IL-2 [18].

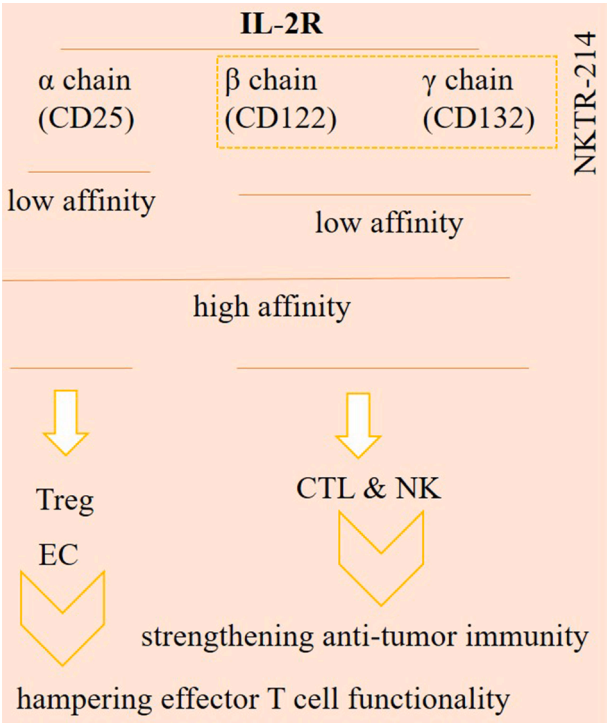

Fig. 1. Interleukin-2 (IL-2) receptor (IL-2R) and ligand activity. IL-2R has three subunits: α (CD25), β (CD122) and γ (CD132). Interaction between IL and 2 with IL-2R represents three levels of affinities: Low affinity occurs when α chain is involved in the interaction between receptor and ligand. The intermediate level of affinity occurs when β and δ chains are involved. Finally, IL-2R interacts through α, β and γ chains with high affinity, forming a stable complex. NKTR-214 (also called bempegaldesleukin) is an IL-2Rβγ agonist. CD25 is active in regulatory T cells (Tregs) and endothelial cells (ECs), and its upregulation is contributed to the T cell dysfunctionality and vascular toxicities, promoting a condition called vascular leak syndrome (VLS). By contrast, CD122 and CD132 are expressed on resting T cells ($CD4^+CD8^+$), natural killer (NK) cells, monocytes and macrophages, and are associated with T cell effector function against cancer, mediated via stimulating proliferation and activation of $CD8^+$ T cells (so called cytotoxic T lymphocytes [CTLs]) and NK cells. IL-2 release from NK cells can act in an autocrine way through increasing their infiltration into the tumor area and mediating their cytolysis activity.

Natural killer (NK) cells are known as the key cells of anti-tumor immunity [22–23]. NK cells are active for promoting the recruitment of dendritic cells (DCs), the activity of which is important for CTL priming [3]. DC formation is induced by IL-2 [24]. IL-2 promotes NK cell differentiation and T cell clonal expansion after antigen exposure [25]. IL-2 along with IL-15 are contributed to the expression of the activating receptor NKG2D on NK cell surface, and induce expression of CD137 from these cells [26]. CD137 directs cross-interactions between innate and adaptive immunity, thus allowing expansion of activated T cells [27]. Phosphoinositide 3-kinase (PI3K)/AKT, Janus kinase (JAK)-signal transducer and activator of transcription (STAT) and mitogen-activated protein kinase (MAPK) are the three main pathways downstream to the IL-2 signaling [12–13].

Key points IL-2R contains α (CD25), β (CD122) and γ (CD132) chains. Potentiation of the activity of CD122/CD132 subunits can improve the anti-tumor potential of IL-2.

3. High-dose interleukin-2 therapy of cancer

IL-2 has pleiotropic effects, mediated via exerting both anti-tumor and pro-tumor activities. The 'dosage' of this cytokine is a possible contributor. IL-2 at low-dose activates specifically Tregs [19,28]. This is effective for patients with chronic inflammatory, autoimmune and certain metabolic diseases, and for patients receiving organ transplant [12,29]. Here, IL-2 can be engineered to bind selectively to the IL-2R with high affinity, which is for potentiating Treg functionality [30]. Low-dose IL-2 can be used for such purpose, namely expansion of Tregs to work against autoimmune diseases [12]. High-dose IL-2 is considered as one of the earliest approaches in immunotherapy of cancer [31]. High-dose IL-2 is reported to stimulate responses from NK cells and $CD4^+CD8^+$ T cells [29]. Clinical application of high-dose IL-2 stands for over 30 years [28]. Here, we will discuss responses from advanced/metastatic melanoma and RCC to the high-dose IL-2 therapy. The dose 600 000 IU/kg is used commonly in clinical trials as the high-dose treatment schedule for IL-2 therapy of metastatic melanoma and RCC [32–36]. In some papers the dose 720 000 IU/kg was used as a high-dose for IL-2 therapy of cancer [37–38].

3.1. Metastatic melanoma

High-dose IL-2 is the first immunotherapeutic agent approved by FDA for treatment of metastatic melanoma [38]. Its first approval for application in metastatic melanoma patients is dated back to the year

1998 [39]. In a study by Davar and colleagues the role of high-dose IL-2 (600,000 IU/kg) was evaluated in 237 patients with metastatic melanoma. The authors noticed the respective median progression-free survival (PFS) and overall survival (OS) of 2.8 months and 9.6 months [40]. The PFS is consistent with the result of study by Tarhini and colleagues (median PFS: 2.3 months) [36]. In the first study, 23% of patients showed stable disease (SD) [40], while the SD among patients in the second work was 48% [36]. A point important for consideration for interpreting the outcomes of the two studies is the big gap in the number of patients evaluated in each report (237 vs 29 cases in the second study), which may influence the outcomes. Objective response rate (ORR) was also evaluated in patients with metastatic melanoma treated with high-dose IL-2 and the ORR of 13% was resulted [41]. Taken together, it could be asserted that metastatic melanoma patients show moderate responses to the high-dose IL-2 therapy (Table 1 and Fig. 2).

3.2. Metastatic renal cell carcinoma

Metastatic renal cell carcinoma (RCC) is another choice for high-dose IL-2 therapy. The first approval for the use of this cytokine in treatment of RCC is dated back to the year 1992 [39]. Fishman and colleagues evaluated the efficacy of high-dose aldesleukin (IL-2) in patients with RCC. The authors noticed high median OS (64.5 months) and 2-year OS (73.8%) in patients with favorable risk, which were reduced in patients with intermediate and poor risk, in particular [37]. The efficacy of high-dose aldesleukin in patients with metastatic RCC was also evaluated by another group. Almost all patients (96%) were clear-cell subtype and had a previous history of nephrectomy (99%). Aldesleukin therapy showed durable remission, depicted by 3-year progression free of 11%, satisfactory responses (ORR: 25%) and prolonged survival (median OS: 42.8 months) [42]. Stenehjem and colleagues assessed the roles for high-dose IL-2 in patients mainly with intermediate risk metastatic RCC (the clear-cell subtype). The findings represented that about 50% of IL-treated patients showed meaningful survival benefit. Another finding from this work was the validity of assessment of SD in clinical setting for predicting responses to the high-dose IL-2 therapy [35]. Finally, Alva and colleagues investigated the efficacy of high-dose IL-2 therapy on metastatic melanoma and RCC patients. Evaluation of the median OS showed the respective 19.6 and 41 months in these patients, which is indicative of the higher efficacy of such therapy in patients with metastatic RCC. Collectively, 362 patients were assessed in this study, and there were no deaths related to the high-dose IL-2 therapy. This infers the safety of such therapy in melanoma and RCC cancer patients, but the general responses were higher in metastatic RCC patients [39] (Table 1 and Fig. 2).

Key points: High-dose IL-2 therapy show infrequent (modest) but durable responses in metastatic melanoma patients. High-dose IL-2 therapy is safe and possibly more effective in metastatic RCC patients compared to the melanoma. Overall survival is better improved in metastatic RCC patients with favorable risk

Table 1
High-dose interleukin (IL)-2 therapy of cancer.

Cancer type	Therapy phase, patients	Outcomes	Ref
metastatic melanoma	305 patients	ORR: 13%	[41]
metastatic melanoma	237 patients	SD: 23% DCR: 41% median OS: 9.6 months median PFS: 2.8 months	[40]
metastatic melanoma	phase 2, 29 patients	SD: 48% PFS: 2.3 months	[36]
metastatic RCC	81 poor risk patients	median OS: 14 months 2-year OS: 39.8%	[37]
metastatic RCC	120 patients	ORR: 25% median OS: 42.8 months	[42]
metastatic RCC	391 patients	ORR: 19%	[35]
metastatic RCC and melanoma	362 patients (RCC, 192; and melanoma, 170)	median OS: 41 vs. 19.6 months	[39]
metastatic RCC and melanoma	100 patients (RCC, 46; and melanoma, 54)	durable PFS (ranges from 5 to 30 years)	[28]

mAb, monoclonal antibody; SD, stable disease; DCR, disease control rate; PFS, progression-free survival; RCC, renal cell carcinoma; ORR, objective response rate; OS, overall survival.

4. IL-2 combination therapy

4.1. Chemo/targeted therapy

Histone deacetylases are enzymes responsible for removal of acetyl group from DNA histones in order to make DNA less reachable to the transcription factors. Hyperacetylation of histones causes structural chromatin remodeling and transcriptional gene activation. Entinostat is a synthetic benzamide and histone deacetylase inhibitor that possesses long half-life [43]. Pili and colleagues in a study evaluated the efficacy of high-dose IL-2 combination with entinostat in patients with metastatic clear-cell RCC. The authors noticed the depletion of Tregs after treatment with entinostat, and the combination therapy was resulted in the satisfactory clinical outcomes (median PFS of 13.8 months and median OS of 65.4 months). In fact, entinostat through suppression of Tregs will make the immune microenvironment less suppressive, which is beneficial for high-dose IL-2 in order to better exert the anti-tumor activity [34].

Sorafenib is a tyrosine kinase inhibitor (TKI) that acts for targeting vascular endothelial growth factor receptor (VEGFR), and is considered as an anti-angiogenic agent with vascular normalizing activities [44]. Maroto and coworkers in a phase 2 trial surveyed the effectiveness of immune cytokine therapy with IL-2 and interferon (IFN)-α followed by sorafenib in patients with metastatic RCC. The authors noticed satisfactory clinical responses to such therapy, rendering the ORR of 44%, the DCR of 94.4% and the median OS of 16.6 months [45]. However, the results are not meaningful when compared with the phase 3 trial of sorafenib therapy, reported by another group, which led to the median OS of 23.3 months [46], but the ORR is reasonable when comparing with the 19% [35] and 25% [42] responses to the high-dose IL-2 therapy for such cancer (Table 2).

4.2. Combination with other immunotherapeutic approaches

4.2.1. Immune checkpoint inhibitor therapy

4.2.1.1. Combination with PD-(L)1 blockade. ICI therapy is the current focus in cancer immunotherapy. ICI is a term used primarily for application of anti- programmed death-1 receptor (PD-1), anti- programmed death ligand 1 (PD-L1) and anti- CTL-associated antigen-4 (CTLA-4) drugs in advanced or metastatic cancers with the aim of reinvigorating the effector function of T cells against cancer [3–4,25,47]. PD-1/PD-L1 interactions send co-inhibitory signals to the effector T cells which subsequently cause self-tolerance, the continuity of such process finally causes T cell exhaustion and dysfunctionality [48].

Buchbinder and coworkers investigated the efficacy of high-dose IL-2 in patients with metastatic RCC and metastatic melanoma treated with anti-PD-1 or anti-PD-L1. The outcomes of this retrospective analysis showed that administration of high-dose IL-2 after ICI therapy was safe and led to the promising objective responses (ORR: 22.5% and 24% for melanoma and RCC, respectively) [31]. A point, however, need to be considered in the study by Buchbinder and colleagues is the large gap in the number of patients included for interpreting the outcomes of the two types of cancers (17 cases for metastatic RCC vs 40 patients for metastatic melanoma), which may interfere with the outcomes. This may

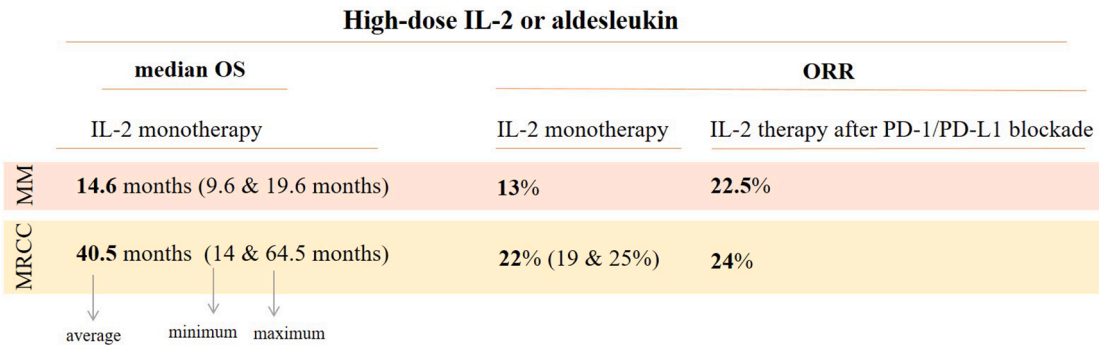

Fig. 2. Clinical responses to the high-dose interleukin (IL)-2 therapy. The efficacy of high-dose IL-2 are assessed as both monotherapy and in combination with immune checkpoint inhibitors (ICIs) in patients with advanced or metastatic melanoma (MM) and metastatic renal cell carcinoma (MRCC). Results of median overall survival (OS) and objective response rate (ORR) are indicative of the higher response from patients with MRCC to the IL-2 monotherapy. IL-2 combination with programmed death-1 receptor (PD-1)/programmed death ligand 1 (PD-L1) blockade and comparing the outcomes with that for IL-2 monotherapy are suggestive of the higher efficacy of such combination therapy in patients with MM.

Table 2
Combination of interleukin (IL)-2 with chemo/targeted therapy.

Cancer type	Agent name	Patients and therapy phase	Outcomes	Ref
metastatic RCC	entinostat	47 patients, phase 1/2	median PFS: 13.8 months median OS: 65.3 months	[34]
metastatic RCC	sorafenib	41 patients, phase 2	median PFS: 7.4 months median OS: 16.6 months ORR: 44.4% DCR: 94.4%	[45]

RCC, renal cell carcinoma; PFS, progression-free survival; OS, overall survival; ORR, objective response rate; DCR, disease control rate.

suggest the need for further studies in order to make the justifications more accurate. Martini and colleagues in a quite recent retrospective review interpreted information from patients underwent second immunotherapy-based regimen. The authors noticed higher responses in ICI-naïve patients (than patients with prior experience of ICI) or in patients treated previously with IFN-γ or IL-2. This is indicative of the priming effect of IL-2 in cancer immunotherapy. Thus, IL-2 can be used synergistically with anti-PD-L1 therapy [25]. Comparing the outcomes with the ORR of 13% [41] and 25% [42], reported respectively for metastatic melanoma and metastatic RCC cancer patients treated with high-dose IL-2 monotherapy, it could be asserted that PD-(L)1 blockade followed by high-dose IL-2 is effective in patients with metastatic melanoma but not for metastatic RCC cases. This can also be understood from the outcomes of Fig. 2 and Table 3.

4.2.1.2. Combination with anti-CTLA-4. The CTLA-4 inhibitor ipilimumab is an effective drug for melanoma [49], being considered as the second drug approved for metastatic melanoma [6]. Intra-tumoral injection of both IL-2 and ipilimumab was assessed by Ray and colleagues in advanced melanoma. The results of this phase 1 trial showed an overall response of 40% and the clinical benefit in 50% of patients receiving combination therapy. Interestingly, local injection was led to an abscopal response in 89% of subjects, which is outstanding [50]. The abscopal response is referred to the effects distant from the localized response,such systemic and indirect effect can boost the activity of immune system against cancer and reduce the chance of metastasis [47,49]. Patel and coworkers evaluated the effect of sequential administration of high-dose IL-2 and ipilimumab in metastatic melanoma. The outcomes of this study showed the higher potency of the combination therapy over 3 mg/kg ipilimumab monotherapy for improving 1-year

Table 3
Combination of interleukin (IL)-2 with other immunotherapeutic approaches.

Cancer type	Agent name	Patients and therapy phase	Outcomes	Ref
Immune checkpoint inhibitors (ICIs)				
metastatic melanoma	ipilimumab: CTLA-4 inhibitor	29 patients	1-year OS: 77% (vs. 46% in ipilimumab alone)	[33]
advanced melanoma	ipilimumab	12 patients, phase 1	overall response: 40% clinical benefit: 50% abscopal response: 89%	[50]
metastatic melanoma	ipilimumab	15 patients, phase 2	SD: 3 patients DCR: 20%	[52]
metastatic RCC and melanoma	anti-PD-1/PD-L1	57 patients (17 RCC, and 40 melanoma)	ORR: 24% (RCC) and 22.5% (melanoma)	[31]
Cellular immunotherapy				
metastatic melanoma	TILs	phase 3, 26 patients	The 5-year tumor relapse was seen in 11 patients	[92]
Vaccination				
advanced melanoma	gp-100	phase 3, 185 patients	median OS: 17.8 months (vs. 11.1 months)PFS: 2.2 months (vs. 1.6 months) clinical response: 16% (vs. 6%)	[53]

RCC, renal cell carcinoma; CTLA-4, CTL-associated antigen-4 ; TIL, tumor-infiltrating lymphocyte; PFS, progression-free survival; OS, overall survival; SD, stable disease; DCR, disease control rate.

OS in treated patients (77% vs 46%). The number of patients involved in this study was 29, among them only 20 cases were met the criteria for evaluation, such small sample size indicates the need for more studies in the area [33]. Besides, the outcome of this study is inconsistent with the 4-year OS of 76.6% reported for melanoma patients (stage IIIC-IV) treated with 3 mg/kg ipilimumab [51]. The number of patients enrolled for these trials (29 vs 453 in the second study) and the period of time considered for the evaluation of the results can be possible contributors for such gap in the outcomes. Weide and colleagues in a phase 2 trial investigated the efficacy of intra-tumoral IL-2 (9 MIU) in combination with systemic ipilimumab for metastatic melanoma. The combination therapy resulted in the adverse events (AEs) similar to that seen for the respective monotherapy of either agent. In the 15 treated patients, 3 cases showed 12-week SD, rendering 20% disease control

rate (DCR) [52] (Table 3).

4.2.2. Cancer vaccination

The synthetic peptide gp-100 is used as a cancer vaccine. Smith and colleagues evaluated the efficacy of gp-100 vaccination in metastatic melanoma patients receiving high-dose IL-2 and found a considerable rise in the ORR (22%) compared with patients receiving IL-2 alone (ORR: 12%) [41]. Schwartzentruber and colleagues in a study involving 21 centers evaluated the efficacy of high-dose IL-2 combination with gp-100 in patients with advanced melanoma. In this phase 3 trial 185 patients were registered and the results showed the higher clinical response for the combination therapy (16%) compared to the IL-2 alone (6%) [53] (Table 3).

Key points: The efficacy of high-dose IL-2 therapy is improved when used in combination with immune activating agents, such as histone deacetylase inhibitors. Application of high-dose IL-2 after PD-(L)1 blockade therapy is safe and effective for metastatic melanoma, but it seems not to be effective for metastatic RCC. The long-term efficacy of high-dose IL-2 plus ipilimumab is seemed not to surpass the ipilimumab monotherapy. Combination with gp-100 improves responses from melanoma to the high-dose IL-2 therapy.

5. IL-2 in *ex vivo* cellular expansion for cellular immunotherapy

IL-2 is known as the T cell growth factor, so T cells expanded *ex vivo* under exposure to the IL-2 show enhanced tumor-killing effects [54]. Cytokine-induced killer (CIK) cells can be easily expanded *ex vivo*, and the protocol for their expansion is safe and inexpensive. Such benefits have raised growing interests for their application in cellular cancer immunotherapy. IL-2 drives the proliferation as well as the cytotoxic function of a number of cells including NK, lymphokine-activated killer (LAK), CIK and tumor-infiltrating lymphocyte (TIL) cells. CIK cells have virtues over the use of LAK cell,the top one among several advantages is that application of CIK cells does not need high-dose IL-2 administration. In fact, for CIK cell therapy IL-2 is used only *ex vivo*. By contrast, application of LAK cells required also the injection of high-dose IL-2, thus raising the rate of toxic effects [55]. Co-culture of CIK with DCs and the application of such combination cellular therapy is an interesting approach and the promising area in cancer immunotherapy. The idea behind exploiting such strategy is the ability of DCs to compensate tumor-related antigens specific for CIK cells [56–57]. DC-CIK combination therapy can be used safely and with efficient clinical outcomes for patients with various advanced solid cancers, such as gastrointestinal cancer [58–59], non-small cell lung cancer (NSCLC) [60], RCC [61–62] and breast cancer [63].

6. Factors affecting responses to the IL-2 therapy and managing strategies

6.1. Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) is a pro-angiogenic factor that has a modulatory role on tumor immunity (innate and adaptive). There is a positive relation between high tumor level of VEGF with the irresponsive IL-2 therapy. Tarhini and colleagues in a study compared the effect of VEGF inhibitor ziv-aflibercept plus high-dose IL-2 vs high-dose IL-2 in patients with metastatic melanoma. The authors noticed higher PFS in the combination group (6.9 vs 2.3 months), which is indicative of the VEGF inhibition as an appropriate combination with immunotherapeutic agents [36]. Clear-cell RCC is a subtype that displays excess production of VEGF. In a phase 3 trial, Donskov and colleagues evaluated the roles for cytokine therapy (IL-2 and IFN-alpha) in combination with bevacizumab (anti-VEGF mAb) in patients with metastatic RCC (clear-cell subtype). Evaluations were performed on favorable or intermediate risk cancer patients. The combination therapy did not add any new or unexpected toxicity, but it did not show efficacy beyond what observed for solo cytokine therapy [9]. An insignificant outcome with combination therapy is in contrast with the study by Tarhini and colleagues. The diversity in the type of VEGF inhibitor to goes with cytokine therapy along with the different types of cancers evaluated in the two studies may interfere with the outcomes. A 3-fold rise in the PFS for combination therapy (Tarhini and colleagues) is suggestive of additive effects of VEGF inhibition and IL-2 therapy. This, however, requires more research in order to grasp more knowledge in regard with the cross-talk between VEGF with IL-2 in patients receiving exogenous IL-2 therapy.

6.2. The activity of Fcγ receptors on immune cells

Fcγ receptors (FcγRs) are expressed on cells of immune system that upon binding to the Fc region of IgG antibodies the interaction initiates immune activation against cancer. NK cells express FcγR2C and FcγR3A, while DCs, monocytes and macrophages express FcγR2A [64]. NK cells, for instance, express CD16 (also called FcγR3), the engagement of which with the Fc region of antibody mediates antibody-dependent cell-mediated cytotoxicity (ADCC), this is different from antibody-independent cytotoxic activity of NK cells [65–66]. Erbe and colleagues reported a link between FcγRs genotypes with the anti-tumor outcomes of high-dose-IL-2. Here, the results were confirmed in metastatic RCC. In fact, immune cells through FcγRs are engaged with the endogenous anti-tumor antibodies, the activity of which is enhanced by high-dose IL-2 and the outcome is tumor destruction and prolonged survival. High-dose IL-2 also augmented antibody-enhanced antigen presentation by tumor, mediated through activation of immune responses (both innate and adaptive immunity) [64].

6.3. Melanoma-associated antigen 3

Melanoma-associated antigen 3 (MAGE-A3) is considered as a tumor-specific antigen that is expressed in most of the melanoma cancer patients, while it lacks in normal tissues except for placenta and testes. Combination of MAGE-A3 targeted immunotherapy with high-dose IL-2 or ICI can be used for treatment of advanced cancer patients. Melanoma patients show low but durable responses to such therapy [38].

Key points High activity of VEGF renders IL-2 irresponsiveness. The anti-tumor activity of high-dose IL-2 is due partly to the activity of Fcγ receptors expressed on cells of immune cells.

7. Pro-tumor activity of interleukin-2

IL-2 exerts diverse effects on T cells, which is depended on the 'dose' of this cytokine. As stated above, the diverse functionality is mediated by immunosuppressive role at 'low' doses but immune activation at 'high' doses [67–68]. IL-2 is known as a marker of T cell expansion and effector function that its progressive loss is contributed to the T cell exhaustion [67]. However, there are reports indicated that high-dose IL-2 therapy can secondary cause Treg expansion [18] and promotes immunosuppression in the TME. Jiang and colleagues in the recent study reported a link between IL and 2 with induction of T cell exhaustion. They found that high and constituent presence of IL-2 in the TME resulted in the consistent activation of signal transducers and activators of transcription 5 (STAT5) in the $CD8^+$ T cells and the subsequent increased activity of tryptophan hydroxylase-1, which led finally to the stimulation of inhibitory receptors and suppressed production of cytokines and effector molecules from $CD8^+$ T cells, rendering a dysfunctional state in the cells [69]. Such exhausted cells are failed to make a control over tumor growth, the result of which is tumor progression and relapse [67]. Exhausted $CD8^+$ T cells display high levels of expression for IL-2Rβ. High activity of this receptor is contributed to the expression of PD-1, a known T cell exhaustion marker. Presence of such exhaustion phenotype is abrogated when IL-2Rβ is removed or downregulated [70] (Fig. 3).

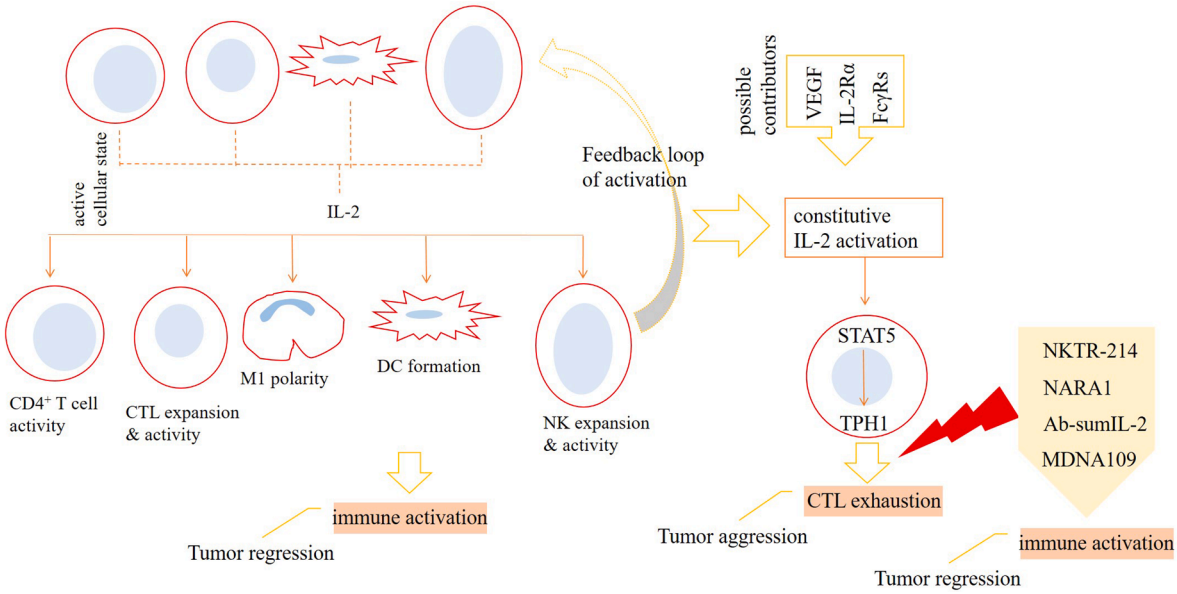

Fig. 3. The suppressive effect of interleukin (IL-2) on T cell effector function. Natural killer (NK) cells, dendritic cells (DCs), CD4$^+$ T cells and CD8$^+$ T cells upon activation release IL-2 into the tumor microenvironment (TME), which acts for strengthening immune activation against cancer. Polarization of macrophages toward anti-tumor type-1 phenotype (M1 cells), formation of DCs, and higher expansion and activity of cytotoxic T lymphocytes (CTLs) and NK cells are the consequences of IL-2 activity. Such activation can thus promote a feedback loop of activation in the anti-tumor immune cells of TME, which can direct a diverse route. Here, constitutive activation of IL-2 can cause hyperactive signal transducers and activators of transcription 5 (STAT5) signaling which further leads to the increased activity of tryptophan hydroxylase-1 (TPH1) and finally promoting an exhaustion state in CTLs. The activity of vascular endothelial growth factor (VEGF), IL-2Rα and melanoma-associated antigen 3 (MAGE-A3) may influence such unwanted activity for IL-2. To avoid this, a preferred strategy is to use IL-2 variants engineered to act selectively on CD132 and in particular CD122. Examples of these agents which are under current clinical evaluations are NKTR-214, NARA1, Ab-sumIL-2 and MDNA109.

7.1. Suppressive role of interleukin-2 on T follicular helper cell activity

T follicular helper (Tfh) cells are known as a subset of CD4$^+$ T helper cells that are located in the B cell zone of secondary lymphoid organs including spleen, tonsil and lymph nodes. The B cell zone of these organs has germinal centers, within which Tfh cells interact with B cells. Infiltration of Tfh cells into the tumor area is believed to increase patient survival. Tfh cells help formation and responses from germinal centers and are contributed to the durable antibody responses [71]. Tfh cells are, in fact, transducing signals of differentiation and survival which act for maintaining the development of germinal centers [72]. Tfh cells support B cell responses. Cross-talking between B and Tfh cells promote survival and differentiation of both cell types. In infection conditions, IL-2 is reported to act for limiting the differentiation of Tfh cells and further suppression of germinal centers [71]. This is mediated via the activity of STAT5 on repressing the expression of B-cell lymphoma 6 protein (Bcl-6) [72] (Fig. 3).

Key notes Controversies exist in regard with the use of high-dose IL-2 in cancer immunotherapy. It seems that continuous presence of high levels of IL-2 in the TME may pose a negative impact on the activity of effector T cells in the TME. Besides, it may increase the rate of toxicity. Retarded activity of Tfh cells is also expected in response to the IL-2 signaling.

8. IL-15: Is it a good substitute for IL-2?

IL-15 is a cytokine produced mainly from antigen-presenting cells (APCs), such as DCs and monocytes [73]. IL-15 is responsible for development, activation and expansion of NK cells [74–75]. Transformation of NK cells from CD56^{bright} into the strong cytotoxic CD56^{dim} subset under L-15 priming is a clear example of the role of IL-15 in the potentiation of NK cell functionality [76]. Monocytes, γδ T cells and memory CD8$^+$ T cells are other cells that are activated under exposure to the IL-15 [75,77].

IL-15 is beginning to have a use in oncology. A question rose in regard with the use of ILs in cancer therapy is that whether IL-15 is a substitute to the IL-2 therapy or not. IL-2 and IL-15 are members of 4α-helix cytokine family. As mentioned, both cytokines display shared IL-2Rβγ, and that IL-15, unlike IL-2, dose not bind with IL-2Rα, a receptor that interacts with Tregs and is contributed to their expansion [10]. Instead, it expresses IL-15Rα (CD215) that acts for IL-15 recycling and its *trans*-presentation to the IL-15Rβγ, being important for promoting survival of NK, natural killer T (NKT) and CD8$^+$ memory T cells [78]. This implies that IL-15 shows lower tendency to interact with Tregs, thus the expansion of such cells is presumably less common in patients receiving IL-15. This virtue may infer that patients receiving IL-15 therapy possibly have more chance to recover immune functionality, and that the rate of toxicity is presumably less common with such therapy. However, a point important for consideration is the 'sustainability' of IL-2 cytokine therapy. It seems that responses to the IL-2 therapy are more durable when the free cytokine is removed in the area [10]. It is proven in animal model that after transferring of adoptive activated CD8$^+$ T cells, this is the IL-2 (but not IL-15) that mediates anti-tumor immunity [10]. Recombinant human IL-15 (rhLI-15) is administered via an intravenous route for solid cancer patients. Conlon and colleagues in this study reported a considerable expansion in the fraction of CD8$^+$ T effector cells and NK. The high rate of NK cell fraction (a 38-fold rise in the total number of NK cells) is justifiable. However, a question arises from this work is a dramatic increase in the number of CD56^{bright} NK cells (by 358-fold rise), which is surprising [79] due to that CD56^{bright} NK cells are active in the cytokine production rather than cytotoxicity [80].

ALT-803 is novel recombinant and super-agonist of IL-15 that is introduced as an immune-oncology drug for enhancing the activity of immune system against cancer [75]. ALT-803 is in fact an agent that targets the shared IL-2/IL-15Rβγ pathway [76]. Margolin and colleagues in a study evaluated the efficacy of ALT-803 in phase 1 trial of advanced solid cancers. Subcutaneous injection of ALT-803 was well-tolerated,

rendering minimal cytokine-related toxicities and causing considerable rise in the number of NK cells. The authors suggested the application of ALT-803 in combination with other anti-cancer agents [75]. In a phase 1b trial, Wrangle and colleagues investigated safety and efficacy of subcutaneous ALT-803 in combination with intravenous nivolumab for NSCLC patients. The authors noticed no dose-limiting toxicities related to the use of ALT-803 and the clinical efficacy of combination therapy was promising. Such combination regimen can of the highest importance for cases experiencing failure of therapy with PD-1 blockade drugs [81]. From what discussed above, it is fair to assert that both cytokines have their own pros and cons, and the area of therapy will determine selection of either one as a suitable regimen for cancer therapy. For example, in adoptive T cell therapy, IL-2 is a preferred choice. In regard with safety and the power of activity, IL-15 can possibly be an appropriate choice. Therefore, is seems that IL-15 has a place in the future of cancer immunotherapy. Due to limited data in regard with actual functions of IL-15 in human solid cancers we could not strictly advice the use of IL-15 over IL-2, which calls for more work in the area. What it is understood so far is the promising safety and clinical activity of targeting the shared IL-2/IL-15Rβγ pathway using appropriate agents, such as ALT-803 in immunotherapy of cancer.

9. IL-2/IL-2R selective engineering

9.1. Ab-sumIL-2

One of the advances in the area of cytokine therapy is the use of synthetic IL-2/receptor pairs. Such ligand/receptor pairs can be engineered to transmit IL-2-related signals without interacting with the endogenous IL-2/IL-2R signaling. This can be an appropriate strategy for mitigating toxic effects related to the cytokine therapy [11], so it can possibly be an appropriate choice to go with T cell engineering in adoptive cell therapy. Recombinant immunocytokines can be constructed to have an antibody to target tumor along with a super mutant (called Ab-sumIL-2). This is for reducing bondage to the CD25, instead enhancing binding capacity to CD122. The virtues of this recombinant immunocytokine are reduced toxicity and increased anti-tumor potential, mediated via specific bondage to CTLs. Administration of Ab-sumIL-2 also overcomes therapy resistance, and it can be an appropriate adjuvant to goes with ICIs [82].

9.2. Nara1

NARA1 is a monoclonal antibody (mAb) developed to human IL-2, which can be used as a strategy for improving IL-2-based cancer immunotherapy. NARA1 is, in fact, acting as CD25 mimicry agent that displays higher affinity (by 10-fold) for IL-2 than that for CD25, thus precluding IL-2/CD25 interaction [21]. The outcomes of such preferential interaction (IL-2/NARA1 complex) are the lowering toxicity and improved immune activation against cancer.

9.3. Nktr-214

Pegylation is a way for improving solubility of a drug and its pharmacokinetics. Pegylation in specific sites of a protein ligand will lead to an alteration in the binding activity of its specific domains. In regard with IL-2, pegylation can be a way to make a balance in TME favoring effector T cell activity [83]. Bempegaldesleukin (also called NKTR-214) is an IL-2 agonist engineered to enhance the anti-tumor activity of this cytokine, as well as for improving half-life and the tolerability [18,83]. NKTR-214 contains six molecules of polyethylene glycol (PEG) that are released to attach subunit α of IL-R. Such attachment preferentially reduces the binding activity of IL-2 to the α chain, instead the tendency for binding to the CD122/CD132 will be increased. This will lead to the reduced toxicity and enhanced anti-tumor activity of immune system [18]. NKTR-214 is thus considered as an IL-2Rβγ-based agonist of IL-2 pathway that acts for stimulation of the proliferation and activity of CTL and NK cells and revoking the undesired expansion of Tregs [84]. In a study, NKTR-214 was administered to 28 patients with advanced solid cancers, and the outcomes showed no objective responses. However, 35% of cases showed a rate of reduced tumor volume, ranged from 2 to 30% [85]. Bentebibel and colleagues in a study evaluated the effects of NKTR-214 on advanced or metastatic solid cancer patients, most of them had melanoma and RCC. In this phase 1 trial, 28 cases were participated and the heavily pre-treated cases showed tumor shrinkage along with the long-lasting disease stabilizing effects. Repeated administration of this IL-2R agonist has found to increase effector immune cell fraction and activity. The authors declared that NKTR-214 can be an optimal immunotherapy approach and an appropriate adjuvant to be used with ICIs [84]. To proceed with this hypothesis, the same group evaluated the efficacy of NKTR-214 plus the PD-1 inhibitor nivolumab in advanced cancer patients. In this dose-escalating trial 38 patients were registered, and the ORR was satisfactory (59.5% including 7 patients with complete responses) [86]. Such promising ORR is suggestive of the efficacy of IL-2Rβγ agonists in combination with ICI possibly in patients with other advanced cancers. However, more works are needed to power the idea.

9.4. Mdna109

MDNA109 is a variant of IL-2 cytokine engineered to bind selectively to the CD122. MDNA109 is known as an IL-2 superagonist that represents an affinity much higher than that for proleukin (native IL-2), representing about 1000 times higher binding ability; the result of such extensive affinity is the preferential expansion and responses of NK and $CD8^+$ T cells over Tregs. Besides, the toxicity related to the MDNA109 is by far lower [87]. Both MDNA109 [87] and NKTR-214 [85,88] preferentially activate $CD8^+$ T cells over Tregs. Due to the higher affinity of MDNA109 for CD122, its efficacy on this preferential activity is by far higher than that for NKTR-214.

Key notes Engineering IL-2 variants with selective activity on preferential receptors (CD122 in particular) is under way and is the current focus in cancer immunocytokine therapy. NKTR-214 is an IL-2Rβγ agonist with a stimulatory effect on effector immune cell functionality, which is considered as appropriate combination with ICIs. MDNA109 represents selective binding activity for CD122 and the preferential role on expanding effector population of T cells over Tregs.

10. Toxicities related to the high-dose IL-2

10.1. Normal organ toxicity of IL-2

Tachycardia, hypotension and augmented vascular permeability are hallmark of IL-2 cytokine storm. Hypotension is the most common toxicity related to the high-dose IL-2 therapy and is largely unavoidable [89]. VLS is a serious systemic side effect of IL-2 therapy [90], which will cause fluid infusion, edema and weight gain [89]. Toxicities related to the high-dose IL-2 can be life-threatening indeed. Patients may even go to the coma, so supervision by an oncologist is suggested [68]. In a study, high-dose IL-2 was used, and about 87% and 73% of patients (from 391 cases) showed hypotension and capillary leak or edema, respectively [35]. By contrast, safety is higher in patients receiving NKTR-214. Based on the outcomes of one study, patients heavily treated with this IL-2Rβγ agonist were well-tolerated the therapy and most of the AEs were grade 1/2. Grade 3 hypotension was also rapidly managed in NKTR-214-treated cases, so the drug is represented to be safe for advanced or metastatic cancer patients [84].

After administration of IL-2, release of other cytokines into the blood stream occurs; this will augment permeability of capillary beds and reducing vascular resistance, a result of which is the fluid shift from blood into the extracellular spaces [91]. High-dose IL-2 therapy is thus not suggested for patients with fluid collections, such as plural effusion or abdominal ascites. Expansions of fluid contents can increase the risk

of infections, so patients must be assessed carefully for any infections prior to the start of high-dose IL-2 [89]. Hypovolemia related to the VLS can cause multi-organ issues, marked by reduced blood flow to the heart, kidney, gut and brain causing ischemia, oliguria and confusion [91]. VLS in the capillary bed of lungs can also cause pulmonary congestion and dyspnea, and the severity of such condition will be increased in patients receiving continuous IL-2 therapy [32].

High-dose IL-2 therapy can increase total serum bilirubin and liver transaminases. Hypothyroidism may also occur with such therapy. Common neurologic side effects of high-dose IL-2 are depression, anxiety, dizziness, delirium and somnolence [32]. Cytokine storm occurring due to the exogenous IL-2 therapy can also cause symptoms mimicking of what seen in flu, such as chills, fever, arthralgias and myalgias [91].

10.2. Management of IL-2-related toxicity

A point here is that toxicities related to the IL-2 therapy are reversible and are generally disappear within 2–3 days of treatment completion. Toxicity of IV bolus administration of high-dose IL-2 is higher than subcutaneous or IV bolus administration of low-dose IL-2. In addition, the rate of toxicity is higher with continuous IV injection of high-dose, compared to the bolus prescription of the same dose. Thus, management of the dose, route and the frequency of administration are possible strategies. It is suggested to reassess patients for eligibility prior to the injection of each dose. Warm blanket and meperidine are requested for patients experiencing chills. Fever can be managed by acetaminophen. Patients who develop infection are suggested to withheld IL-2 therapy until recovery. Routine check-up for blood pressure is suggested [91]. It is requested to monitor patients constantly for pulse oxygen due to the possibility of dyspnea [32].

11. Conclusion and future perspectives

From what discussed in this literature it could be asserted that high-dose IL-2 in the native form can be problematic in patients with advanced melanoma and RCC. It represents low half-life which demands for it continuous administration, a result of which is its higher rate of severity. As discussed, high-dose IL-2 therapy can secondary cause Treg expansion that are act for hampering the activity of effector T cells [18]. However, due to the importance of this cytokine in modulating the activity of cells within TME it is suggested to use this cytokine in the native form in *ex vivo* culturing of cells for cellular immunotherapy. Modulation of factors implicated in the harnessing IL-2 responses can be a way for improving responses to the high-dose IL-2 therapy and reducing its AEs. Nowadays, a key focus is for engineering IL-2 variants that represent higher half-life, lower toxicity and higher efficacy compared with the conventional high-dose IL-2 therapy. Fortunately, progresses are made in the field, and some agents, such as MDNA109 are introduced and can have a place in the future of cancer immunotherapy.

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References

[1] K. Mortezaee, M. Najafi, B. Farhood, A. Ahmadi, D. Shabeeb, A.E. Musa, NF-κB targeting for overcoming tumor resistance and normal tissues toxicity, J. Cell. Physiol. 234 (10) (2019) 17187–17204.
[2] K. Mortezaee, A. Narmani, M. Salehi, H. Bagheri, B. Farhood, H. Haghi-Aminjan, M. Najafi, Synergic effects of nanoparticles-mediated hyperthermia in radiotherapy/chemotherapy of cancer, Life Sci. 119020 (2021).
[3] K. Mortezaee, Immune escape: a critical hallmark in solid tumors, Life Sci. 118110 (2020).
[4] J. Majidpoor, K. Mortezaee, The efficacy of PD-1/PD-L1 blockade in cold cancers and future perspectives, Clin. Immunol. 108707 (2021).
[5] K. Mortezaee, Enriched cancer stem cells, dense stroma, and cold immunity: Interrelated events in pancreatic cancer, J. Biochem. Mol. Toxicol. 35 (4) (2021), e22708.
[6] P.A. Prieto, J.C. Yang, R.M. Sherry, M.S. Hughes, U.S. Kammula, D.E. White, C. L. Levy, S.A. Rosenberg, G.Q. Phan, CTLA-4 blockade with ipilimumab: long-term follow-up of 177 patients with metastatic melanoma, Clin. Cancer Res. 18 (7) (2012) 2039–2047.
[7] M. Najafi, K. Mortezaee, R. Ahadi, Cancer stem cell (a) symmetry & plasticity: tumorigenesis and therapy relevance, Life Sci. 231 (2019), 116520.
[8] D. Schadendorf, F.S. Hodi, C. Robert, J.S. Weber, K. Margolin, O. Hamid, D. Patt, T.-T. Chen, D.M. Berman, J.D. Wolchok, Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma, J clinical oncology 33 (17) (2015) 1889.
[9] F. Donskov, N.V. Jensen, T. Smidt-Hansen, L. Brøndum, P. Geertsen, A randomized phase II trial of interleukin-2 and interferon-α plus bevacizumab versus interleukin-2 and interferon-α in metastatic renal-cell carcinoma (mRCC): results from the Danish Renal Cancer Group (DaRenCa) study-1, Acta Oncol. 57 (5) (2018) 589–594.
[10] E.W. Su, C.J. Moore, S. Suriano, C.B. Johnson, N. Songalia, A. Patterson, D. J. Neitzke, K. Andrijauskaite, E. Garrett-Mayer, S. Mehrotra, IL-2Rα mediates temporal regulation of IL-2 signaling and enhances immunotherapy, Sci translational med 7 (311) (2015), 311ra170-311ra170.
[11] J.T. Sockolosky, E. Trotta, G. Parisi, L. Picton, L.L. Su, A.C. Le, A. Chhabra, S. L. Silveria, B.M. George, I.C. King, Selective targeting of engineered T cells using orthogonal IL-2 cytokine-receptor complexes, Science 359 (6379) (2018) 1037–1042.
[12] U. Karakus, D. Sahin, P.R. Mittl, P. Mooij, G. Koopman, O. Boyman, Receptor-gated IL-2 delivery by an anti-human IL-2 antibody activates regulatory T cells in three different species, Sci. Transl. Med. 12 (574) (2020).
[13] T. Wang, Y. Hu, E. Wangkahart, F. Liu, A. Wang, E. Zahran, K.R. Maisey, M. Liu, Q. Xu, M. Imarai, Interleukin (IL)-2 is a key regulator of T helper 1 and T helper 2 cytokine expression in fish: functional characterization of two divergent IL2 paralogs in salmonids, Front. Immunol. 9 (2018) 1683.
[14] M. Najafi, A. Ahmadi, K. Mortezaee, Extracellular-signal-regulated kinase/mitogen-activated protein kinase signaling as a target for cancer therapy: an updated review, Cell Biol. Int. 43 (11) (2019) 1206–1222.
[15] K. Mortezaee, Hypoxia induces core-to-edge transition of progressive tumoral cells: A critical review on differential yet corroborative roles for HIF-1α and HIF-2α, Life Sci. 242 (2020), 117145.
[16] K. Mortezaee, Redox tolerance and metabolic reprogramming in solid tumors, Cell Biology Interl 45 (2) (2021) 273–286.
[17] M. Najafi, K. Mortezaee, J. Majidpoor, Stromal reprogramming: a target for tumor therapy, Life Sci. 239 (2019), 117049.
[18] M. Sharma, H. Khong, F. Fa'ak, S.-E. Bentebibel, L.M. Janssen, B.C. Chesson, C.A. Creasy, M.-A. Forget, L.M.S. Kahn, B. Pazdrak, Bempegaldesleukin selectively depletes intratumoral Tregs and potentiates T cell-mediated cancer therapy, Nat. Commun. 11 (1) (2020) 1–11.
[19] C. Camisaschi, P. Filipazzi, M. Tazzari, C. Casati, V. Beretta, L. Pilla, R. Patuzzo, A. Maurichi, A. Cova, M. Maio, Effects of cyclophosphamide and IL-2 on regulatory CD4+ T cell frequency and function in melanoma patients vaccinated with HLA-class I peptides: impact on the antigen-specific T cell response, Cancer Immunol. Immunother. 62 (5) (2013) 897–908.
[20] D. Sahin, N. Arenas-Ramirez, M. Rath, U. Karakus, M. Hümbelin, M. van Gogh, L. Borsig, O. Boyman, An IL-2-grafted antibody immunotherapy with potent efficacy against metastatic cancer, Nat. Commun. 11 (1) (2020) 1–12.
[21] N. Arenas-Ramirez, C. Zou, S. Popp, D. Zingg, B. Brannetti, E. Wirth, T. Calzascia, J. Kovarik, L. Sommer, G. Zenke, Improved cancer immunotherapy by a CD25-mimobody conferring selectivity to human interleukin-2, Science translational medicine 8 (367) (2016), 367ra166-367ra166.
[22] J. Majidpoor, K. Mortezaee, Steps in metastasis: an updated review, Med. Oncol. 38 (1) (2021) 1–17.
[23] K. Mortezaee, Organ tropism in solid tumor metastasis: an updated review, Future Oncology (0) (2021).
[24] M.E. Raeber, R.A. Rosalia, D. Schmid, U. Karakus, O. Boyman, Interleukin-2 signals converge in a lymphoid–dendritic cell pathway that promotes anticancer immunity, Sci. Transl. Med. 12 (561) (2020).
[25] D.J. Martini, Y. Liu, J.M. Shabto, C. Lewis, M.R. Kline, H. Collins, M. Akce, H. T. Kissick, B.C. Carthon, W.L. Shaib, Clinical outcomes of advanced stage cancer patients treated with sequential immunotherapy in phase 1 clinical trials, Invest. New Drugs 37 (6) (2019) 1198–1206.
[26] M. Kim, T.-J. Kim, H.M. Kim, J. Doh, K.-M. Lee, Multi-cellular natural killer (NK) cell clusters enhance NK cell activation through localizing IL-2 within the cluster, Sci. Rep. 7 (1) (2017) 1–8.

[27] R.A. Wilcox, K. Tamada, S.E. Strome, L. Chen, Signaling through NK cell-associated CD137 promotes both helper function for CD8+ cytolytic T cells and responsiveness to IL-2 but not cytolytic activity, J Immunology 169 (8) (2002) 4230–4236.

[28] J.I. Clark, B. Curti, E.J. Davis, H. Kaufman, A. Amin, A. Alva, T.F. Logan, R. Hauke, G.P. Miletello, U. Vaishampayan, Long-term progression-free survival of patients with metastatic melanoma or renal cell carcinoma following high-dose interleukin-2, J Investigative Med (2021).

[29] G. Churlaud, C. Abbara, P.-A. Vinot, G. Fourcade, P.-G. Ritvo, R. Lorenzon, M. Rosenzwajg, B. Diquet, D. Klatzmann, Pharmacodynamics of regulatory T cells in mice and humans treated with low-dose IL-2, Journal of Allergy and Clinical Immunology 142(4) (2018) 1344-1346. e3.

[30] L. Khoryati, M.N. Pham, M. Sherve, S. Kumari, K. Cook, J. Pearson, M. Bogdani, D. J. Campbell, M.A. Gavin, An IL-2 mutein engineered to promote expansion of regulatory T cells arrests ongoing autoimmunity in mice, Sci. Immunol. 5 (50) (2020).

[31] E.I. Buchbinder, J.P. Dutcher, G.A. Daniels, B.D. Curti, S.P. Patel, S.G. Holtan, G. P. Miletello, M.N. Fishman, R. Gonzalez, J.I. Clark, Therapy with high-dose Interleukin-2 (HD IL-2) in metastatic melanoma and renal cell carcinoma following PD1 or PDL1 inhibition, J immunotherapy of cancer 7 (1) (2019) 1–7.

[32] J.C. Poust, J.E. Woolery, M.R. Green, Management of toxicities associated with high-dose interleukin-2 and biochemotherapy, Anticancer Drugs 24 (1) (2013) 1–13.

[33] S.P. Patel, D. Milton, M.M. Milhem, L.E. Flaherty, S. Hallmeyer, L.G. Feun, R. J. Hauke, L.D. Cranmer, G.A. Daniels, G.C. Doolittle, Sequential administration of high-dose interleukin-2 and ipilimumab in patients with metastatic melanoma, American Society of, Clinical Oncology (2016).

[34] R. Pili, D.I. Quinn, H.J. Hammers, P. Monk, S. George, T.B. Dorff, T. Olencki, L. Shen, A. Orillion, D. Lamonica, Immunomodulation by entinostat in renal cell carcinoma patients receiving high-dose interleukin 2: a multicenter, single-arm, phase I/II trial (NCI-CTEP# 7870), Clin. Cancer Res. 23 (23) (2017) 7199–7208.

[35] D.D. Stenehjem, M. Toole, J. Merriman, K. Parikh, S. Daignault, S. Scarlett, P. Esper, K. Skinner, A. Udager, S.K. Tantravahi, Extension of overall survival beyond objective responses in patients with metastatic renal cell carcinoma treated with high-dose interleukin-2, Cancer Immunol. Immunother. 65 (8) (2016) 941–949.

[36] A.A. Tarhini, P. Frankel, C. Ruel, M.S. Ernstoff, T.M. Kuzel, T.F. Logan, N. I. Khushalani, H.A. Tawbi, K.A. Margolin, S. Awasthi, NCI 8628: A randomized phase 2 study of ziv-aflibercept and high-dose interleukin 2 or high-dose interleukin 2 alone for inoperable stage III or IV melanoma, Cancer 124 (22) (2018) 4332–4341.

[37] M. Fishman, J. Dutcher, J. Clark, A. Alva, G. Miletello, B. Curti, N. Agarwal, R. Hauke, K. Mahoney, H. Moon, Overall survival by clinical risk category for high dose interleukin-2 (HD IL-2) treated patients with metastatic renal cell cancer (mRCC): data from the PROCLAIM SM registry, J immunotherapy of cancer 7 (1) (2019) 1–7.

[38] J.L. McQuade, J. Homsi, C.A. Torres-Cabala, R. Bassett, R.M. Popuri, M.L. James, L. M. Vence, W.-J. Hwu, A phase II trial of recombinant MAGE-A3 protein with immunostimulant AS15 in combination with high-dose Interleukin-2 (HDIL2) induction therapy in metastatic melanoma, BMC cancer 18 (1) (2018) 1–9.

[39] A. Alva, G.A. Daniels, M.K. Wong, H.L. Kaufman, M.A. Morse, D.F. McDermott, J. I. Clark, S.S. Agarwala, G. Miletello, T.F. Logan, Contemporary experience with high-dose interleukin-2 therapy and impact on survival in patients with metastatic melanoma and metastatic renal cell carcinoma, Cancer Immunol. Immunother. 65 (12) (2016) 1533–1544.

[40] D. Davar, F. Ding, M. Saul, C. Sander, A.A. Tarhini, J.M. Kirkwood, H.A. Tawbi, High-dose interleukin-2 (HD IL-2) for advanced melanoma: a single center experience from the University of Pittsburgh Cancer Institute, J immunotherapy of cancer 5 (1) (2017) 1–10.

[41] F.O. Smith, S.G. Downey, J.A. Klapper, J.C. Yang, R.M. Sherry, R.E. Royal, U. S. Kammula, M.S. Hughes, N.P. Restifo, C.L. Levy, Treatment of metastatic melanoma using interleukin-2 alone or in conjunction with vaccines, Clin. Cancer Res. 14 (17) (2008) 5610–5618.

[42] D.F. McDermott, S.-C. Cheng, S. Signoretti, K.A. Margolin, J.I. Clark, J.A. Sosman, J.P. Dutcher, T.F. Logan, B.D. Curti, M.S. Ernstoff, The high-dose aldesleukin "select" trial: a trial to prospectively validate predictive models of response to treatment in patients with metastatic renal cell carcinoma, Clin. Cancer Res. 21 (3) (2015) 561–568.

[43] S.L.H. Yeruva, F. Zhao, K.D. Miller, A.J. Tevaarwerk, L.I. Wagner, R.J. Gray, J. A. Sparano, R.M. Connolly, E2112: randomized phase iii trial of endocrine therapy plus entinostat/placebo in patients with hormone receptor-positive advanced breast cancer, npj Breast Cancer 4 (1) (2018) 1–5.

[44] J. Majidpoor, K. Mortezaee, Angiogenesis as a hallmark of solid tumors-clinical perspectives, Cellular Oncology (2021) 1–23.

[45] J. Maroto, X. Del Muro, B. Mellado, J. Perez-Gracia, R. Andrés, J. Cruz, E. Gallardo, M. Domenech, J. Arranz, J.A. Meana, Phase II trial of sequential subcutaneous interleukin-2 plus interferon alpha followed by sorafenib in renal cell carcinoma (RCC), Clin. Transl. Oncol. 15 (9) (2013) 698–704.

[46] T.E. Hutson, S. Al-Shukri, V.P. Stus, O.N. Lipatov, Y. Shparyk, A.H. Bair, B. Rosbrook, G.I. Andrews, N.J. Vogelzang, Axitinib versus sorafenib in first-line metastatic renal cell carcinoma: overall survival from a randomized phase III trial, Clinical genitourinary cancer 15 (1) (2017) 72–76.

[47] K. Mortezaee, M. Najafi, Immune system in cancer radiotherapy: Resistance mechanisms and therapy perspectives, Critical Reviews in Oncology/Hematology 103180 (2020).

[48] K. Mortezaee, Myeloid-derived suppressor cells in cancer immunotherapy-clinical perspectives, Life Sci. 277 (2021), 119627.

[49] J.W. Welsh, C. Tang, P. De Groot, A. Naing, K.R. Hess, J.V. Heymach, V. A. Papadimitrakopoulou, T.R. Cushman, V. Subbiah, J.Y. Chang, Phase II trial of ipilimumab with stereotactic radiation therapy for metastatic disease: outcomes, toxicities, and low-dose radiation-related Abscopal responses, Cancer immunology research 7 (12) (2019) 1903.

[50] A. Ray, M.A. Williams, S.M. Meek, R.C. Bowen, K.F. Grossmann, R.H. Andtbacka, T.L. Bowles, J.R. Hyngstrom, S.A. Leachman, D. Grossman, A phase I study of intratumoral ipilimumab and interleukin-2 in patients with advanced melanoma, Oncotarget 7 (39) (2016) 64390.

[51] P.A. Ascierto, M. Del Vecchio, M. Mandalá, H. Gogas, A.M. Arance, S. Dalle, C. L. Cowey, M. Schenker, J.-J. Grob, V. Chiarion-Sileni, Adjuvant nivolumab versus ipilimumab in resected stage IIIB–C and stage IV melanoma (CheckMate 238): 4-year results from a multicentre, double-blind, randomised, controlled, phase 3 trial, Lancet Oncol. 21 (11) (2020) 1465–1477.

[52] B. Weide, A. Martens, K. Wistuba-Hamprecht, H. Zelba, L. Maier, H.-P. Lipp, B. D. Klumpp, D. Soffel, T.K. Eigentler, C. Garbe, Combined treatment with ipilimumab and intratumoral interleukin-2 in pretreated patients with stage IV melanoma—safety and efficacy in a phase II study, Cancer Immunol. Immunother. 66 (4) (2017) 441–449.

[53] D.J. Schwartzentruber, D.H. Lawson, J.M. Richards, R.M. Conry, D.M. Miller, J. Treisman, F. Gailani, L. Riley, K. Conlon, B. Pockaj, gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma, New England J Med 364 (22) (2011) 2119–2127.

[54] S.A. Rosenberg, N.P. Restifo, Adoptive cell transfer as personalized immunotherapy for human cancer, Science 348 (6230) (2015) 62–68.

[55] E. Cappuzzello, R. Sommaggio, P. Zanovello, A. Rosato, Cytokines for the induction of antitumor effectors: The paradigm of Cytokine-Induced Killer (CIK) cells, Cytokine Growth Factor Rev. 36 (2017) 99–105.

[56] A. Märten, C. Ziske, B. Schöttker, S. Renoth, S. Weineck, P. Buttgereit, F. Schakowski, A. von Rücker, T. Sauerbruch, I.G. Schmidt-Wolf, Interactions between dendritic cells and cytokine-induced killer cells lead to an activation of both populations, J. Immunother. 24 (6) (2001) 502–510.

[57] Y. Zhang, J. Ellinger, M. Ritter, I.G. Schmidt-Wolf, Clinical Studies Applying Cytokine-Induced Killer Cells for the Treatment of Renal Cell Carcinoma, Cancers 12 (9) (2020) 2471.

[58] H. Du, J. Yang, Y. Zhang, Cytokine-induced killer cell/dendritic cell combined with cytokine-induced killer cell immunotherapy for treating advanced gastrointestinal cancer, BMC cancer 20 (2020) 1–11.

[59] Y. Mu, C.-H. Zhou, S.-F. Chen, J. Ding, Y.-X. Zhang, Y.-P. Yang, W.-H. Wang, Effectiveness and safety of chemotherapy combined with cytokine-induced killer cell/dendritic cell–cytokine-induced killer cell therapy for treatment of gastric cancer in China: A systematic review and meta-analysis, Cytotherapy 18 (9) (2016) 1162–1177.

[60] Y. Zhao, G. Qiao, X. Wang, Y. Song, X. Zhou, N. Jiang, L. Zhou, H. Huang, J. Zhao, M. Morse, Combination of DC/CIK adoptive T cell immunotherapy with chemotherapy in advanced non-small-cell lung cancer (NSCLC) patients: A prospective patients' preference-based study (PPPS), Clin. Transl. Oncol. 21 (6) (2019) 721–728.

[61] D. Wang, B. Zhang, H. Gao, G. Ding, Q. Wu, J. Zhang, L. Liao, H. Chen, Clinical research of genetically modified dendritic cells in combination with cytokine-induced killer cell treatment in advanced renal cancer, BMC cancer 14 (1) (2014) 1–7.

[62] X. Zhao, Z. Zhang, H. Li, J. Huang, S. Yang, T. Xie, L. Huang, D. Yue, L. Xu, L. Wang, Cytokine induced killer cell-based immunotherapies in patients with different stages of renal cell carcinoma, Cancer Lett. 362 (2) (2015) 192–198.

[63] J. Hu, J. Hu, X. Liu, C. Hu, M. Li, W. Han, Effect and safety of cytokine-induced killer (CIK) cell immunotherapy in patients with breast cancer: A meta-analysis, Medicine 96 (42) (2017).

[64] A.K. Erbe, W. Wang, J. Goldberg, M. Gallenberger, K. Kim, L. Carmichael, D. Hess, E.A. Mendonca, Y. Song, J.A. Hank, FCGR polymorphisms influence response to IL2 in metastatic renal cell carcinoma, Clin. Cancer Res. 23 (9) (2017) 2159–2168.

[65] T. Ishikawa, T. Okayama, N. Sakamoto, M. Ideno, K. Oka, T. Enoki, J. Mineno, N. Yoshida, K. Katada, K. Kamada, Phase I clinical trial of adoptive transfer of expanded natural killer cells in combination with I g G 1 antibody in patients with gastric or colorectal cancer, Int. J. Cancer 142 (12) (2018) 2599–2609.

[66] M. Brillantes, A.M. Beaulieu, Memory and memory-like NK cell responses to microbial pathogens, Front. Cell. Infect. Microbiol. 10 (2020) 102.

[67] W. Jiang, Y. He, W. He, G. Wu, X. Zhou, Q. Sheng, W. Zhong, Y. Lu, Y. Ding, Q. Lu, Exhausted CD8+ T Cells in the Tumor Immune Microenvironment: New Pathways to Therapy, Front. Immunol. 11 (2021) 3739.

[68] L. Mortara, E. Balza, A. Bruno, A. Poggi, P. Orecchia, B. Carnemolla, Anti-cancer therapies employing IL-2 cytokine tumor targeting: Contribution of innate, adaptive and immunosuppressive cells in the anti-tumor efficacy, Front. Immunol. 9 (2018) 2905.

[69] Y. Liu, N. Zhou, L. Zhou, J. Wang, Y. Zhou, T. Zhang, Y. Fang, J. Deng, Y. Gao, X. Liang, IL-2 regulates tumor-reactive CD8+ T cell exhaustion by activating the aryl hydrocarbon receptor, Nat. Immunol. 22 (3) (2021) 358–369.

[70] R. Spolski, P. Li, W.J. Leonard, Biology and regulation of IL-2: from molecular mechanisms to human therapy, Nat. Rev. Immunol. 18 (10) (2018) 648–659.

[71] A. Ballesteros-Tato, B. León, B.A. Graf, A. Moquin, P.S. Adams, F.E. Lund, T. D. Randall, Interleukin-2 inhibits germinal center formation by limiting T follicular helper cell differentiation, Immunity 36 (5) (2012) 847–856.

[72] A. Papillion, M.D. Powell, D.A. Chisolm, H. Bachus, M.J. Fuller, A.S. Weinmann, A. Villarino, J.J. O'Shea, B. León, K.J. Oestreich, Inhibition of IL-2 responsiveness by IL-6 is required for the generation of GC-TFH cells, Sci. Immunol. 4 (39) (2019).
[73] R.M.S. Carrero, F. Beceren-Braun, S.C. Rivas, S.M. Hegde, A. Gangadharan, D. Plote, G. Pham, S.M. Anthony, K.S. Schluns, IL-15 is a component of the inflammatory milieu in the tumor microenvironment promoting antitumor responses, Proceedings National Acad Sci 116 (2) (2019) 599–608.
[74] L. Fisher, M. Zinter, S. Stanfield-Oakley, L.N. Carpp, R.W. Edwards, T. Denny, Z. Moodie, F. Laher, L.-G. Bekker, M.J. McElrath, Vaccine-induced antibodies mediate higher antibody-dependent cellular cytotoxicity after interleukin-15 pretreatment of natural killer effector cells, Front. Immunol. 10 (2019) 2741.
[75] K. Margolin, C. Morishima, V. Velcheti, J.S. Miller, S.M. Lee, A.W. Silk, S.G. Holtan, A.M. Lacroix, S.P. Fling, J.C. Kaiser, Phase I trial of ALT-803, a novel recombinant IL15 complex, in patients with advanced solid tumors, Clin. Cancer Res. 24 (22) (2018) 5552–5561.
[76] R. Romee, S. Cooley, M.M. Berrien-Elliott, P. Westervelt, M.R. Verneris, J. E. Wagner, D.J. Weisdorf, B.R. Blazar, C. Ustun, T.E. DeFor, First-in-human phase 1 clinical study of the IL-15 superagonist complex ALT-803 to treat relapse after transplantation, Blood 131 (23) (2018) 2515–2527.
[77] K.C. Conlon, E. Lugli, H.C. Welles, S.A. Rosenberg, A.T. Fojo, J.C. Morris, T. A. Fleisher, S.P. Dubois, L.P. Perera, D.M. Stewart, Redistribution, hyperproliferation, activation of natural killer cells and CD8 T cells, and cytokine production during first-in-human clinical trial of recombinant human interleukin-15 in patients with cancer, J clinical oncology 33 (1) (2015) 74.
[78] X.-L. Chen, D. Bobbala, Y.C. Donates, M. Mayhue, S. Ilangumaran, S. Ramanathan, IL-15 trans-presentation regulates homeostasis of CD4+ T lymphocytes, Cell. Mol. Immunol. 11 (4) (2014) 387–395.
[79] K.C. Conlon, E.L. Potter, S. Pittaluga, C.-C.R. Lee, M.D. Miljkovic, T.A. Fleisher, S. Dubois, B.R. Bryant, M. Petrus, L.P. Perera, IL15 by continuous intravenous infusion to adult patients with solid tumors in a phase I trial induced dramatic NK-cell subset expansion, Clin. Cancer Res. 25 (16) (2019) 4945–4954.
[80] A. Silva, D.M. Andrews, A.G. Brooks, M.J. Smyth, Y. Hayakawa, Application of CD27 as a marker for distinguishing human NK cell subsets, Int. Immunol. 20 (4) (2008) 625–630.
[81] J.M. Wrangle, V. Velcheti, M.R. Patel, E. Garrett-Mayer, E.G. Hill, J.G. Ravenel, J. S. Miller, M. Farhad, K. Anderton, K. Lindsey, ALT-803, an IL-15 superagonist, in combination with nivolumab in patients with metastatic non-small cell lung cancer: a non-randomised, open-label, phase 1b trial, Lancet Oncol. 19 (5) (2018) 694–704.
[82] Z. Sun, Z. Ren, K. Yang, Z. Liu, S. Cao, S. Deng, L. Xu, Y. Liang, J. Guo, Y. Bian, A next-generation tumor-targeting IL-2 preferentially promotes tumor-infiltrating CD8+ T-cell response and effective tumor control, Nat. Commun. 10 (1) (2019) 1–12.
[83] W.W. Overwijk, M.A. Tagliaferri, J. Zalevsky, Engineering IL-2 to Give New Life to T Cell Immunotherapy, Annual Review of Med 72 (2020).
[84] 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, A First-in-Human Study and Biomarker Analysis of NKTR-214, a Novel IL2Raf-Biased Cytokine, in Patients with Advanced or Metastatic Solid Tumors, (2019).
[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, A first-in-human study and biomarker analysis of NKTR-214, a novel IL2Rβγ-biased cytokine, in patients with advanced or metastatic solid tumors, Cancer discovery 9 (6) (2019) 711–721.
[86] A. Diab, N.M. Tannir, S.-E. Bentebibel, P. Hwu, V. Papadimitrakopoulou, C. Haymaker, H.M. Kluger, S.N. Gettinger, M. Sznol, S.S. Tykodi, Bempegaldesleukin (NKTR-214) plus nivolumab in patients with advanced solid tumors: phase I dose-escalation study of safety, efficacy, and immune activation (PIVOT-02), Cancer discovery 10 (8) (2020) 1158–1173.
[87] M. Rafei, S. Fidai, R. Merchant, F. Merchant, MDNA109: Effect of an interleukin-2 superkine on CD8 T-cell properties in the tumor microenvironment, American Society of, Clinical Oncology (2019).
[88] G. Parisi, J.D. Saco, F.B. Salazar, J. Tsoi, P. Krystofinski, C. Puig-Saus, R. Zhang, J. Zhou, G.C. Cheung-Lau, A.J. Garcia, Persistence of adoptively transferred T cells with a kinetically engineered IL-2 receptor agonist, Nat. Commun. 11 (1) (2020) 1–12.
[89] J.P. Dutcher, D.J. Schwartzentruber, H.L. Kaufman, S.S. Agarwala, A.A. Tarhini, J. N. Lowder, M.B. Atkins, High dose interleukin-2 (Aldesleukin)-expert consensus on best management practices-2014, J immunotherapy of cancer 2 (1) (2014) 1–23.
[90] P. Hu, M. Mizokami, G. Ruoff, L.A. Khawli, A.L. Epstein, Generation of low-toxicity interleukin-2 fusion proteins devoid of vasopermeability activity, Blood 101 (12) (2003) 4853–4861.
[91] R.N. Schwartz, L. Stover, J.P. Dutcher, Managing toxicities of high-dose interleukin-2, Oncology (Williston Park, NY) 16 (11 Suppl 13) (2002) 11–20.
[92] A. Khammari, J.-M. Nguyen, M.-T. Leccia, B. Guillot, S. Saiagh, M.-C. Pandolfino, A.-C. Knol, G. Quéreux, A. Chiffolettau, N. Labarrière, Tumor infiltrating lymphocytes as adjuvant treatment in stage III melanoma patients with only one invaded lymph node after complete resection: results from a multicentre, randomized clinical phase III trial, Cancer Immunol. Immunother. 69 (8) (2020) 1663–1672.