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Anticancer Cytokines: Biology and Clinical Effects of IFN-α2, IL-2, IL-15, IL-21, and IL-12

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Abstract

Efforts over nearly four decades have focused on ways to use cytokines to manipulate the host immune response towards cancer cell recognition and eradication. Significant advances were achieved with interleukin-2 (IL-2) and interferon-α (IFN-α), primarily in the treatment of patients with melanoma and renal cell carcinoma. However, the utility of other cytokines showing promise in the preclinical setting has not been established largely because of toxicity, the complex functionality of each cytokine and the difficulty mimicking in preclinical models the human environment. In this paper we will review the basic biology and the clinical experiences with IFN-α, IL-2, IL-15, IL-21 and IL-12. We will also review ongoing clinical trials and discuss future directions including potential use of cytokines in combination with other effective immunotherapy approaches which have come of age in recent years.

Keywords

cytokine; IL-2; IL-15; IL-21; IL-12; IFN-α

INTRODUCTION

Cytokine therapy has been established as one of the main pillars of human cancer immunotherapy, primarily in the management of patients with melanoma and renal cell carcinoma (RCC) where interleukin-2 (IL-2) and interferon alpha (IFN-α) were the mainstay of treatment for many years until recent advances in molecularly targeted therapies, anti-angiogenic therapies and immune checkpoint blockers. Since the 1980's, cytokines have been investigated in large-scale clinical trials for patients with breast cancer, RCC, glioblastoma, lymphoma, leukemia and melanoma[(1)]. Early studies with recombinant IFN-α demonstrated clinical activity in patients with melanoma, RCC, and hairy cell leukemia with

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objective tumor response rates of 10–20% observed[1]. IFN-α (Intron-A; Merck) was approved by the United States Food and Drug Administration (FDA) for therapy of patients with hairy cell leukemia in 1986, and in 1995, it became the first immunotherapy approved for adjuvant treatment of patients with high risk stage IIB/III melanoma[2, 3]. Subsequently, recombinant IL-2 demonstrated significant antitumor activity largely limited to patients with metastatic melanoma and RCC. IL-2 (Aldesleukin, Proleukin; Prometheus) was approved by the FDA in 1992 for the treatment of patients with metastatic RCC and in 1998 for the treatment of patients with metastatic melanoma. This review will discuss the role of multiple cytokines investigated as anticancer immunotherapy. It will focus specifically on our current understanding of the role of IFN-α, IL-2, IL-15, IL-21 and IL-12 in cancer management. This includes biomarker studies and a brief discussion of current combinations and opportunities for future studies.

INTERFERON-ALPHA

Interferon-α (IFN-α) belongs to the type I IFN family [4]. It has undergone extensive clinical evaluation. Clinically used recombinant formulations exist in three isoforms (α2a, α2b, α2c). IFN alpha is FDA approved as adjuvant treatment for patients with high-risk melanoma (both α2b, also in its pegylated form), as first line treatment for patients with metastatic RCC (α2a, α2b in combination with bevacizumab), AIDS-related Kaposi's Sarcoma (α2b), follicular lymphoma (α2b), hairy cell leukemia (α2a, α2b), chronic myelogenous leukemia (PH chromosome+, α2a), condyloma acuminata (α2b), and cervical intraepithelial neoplasms (α2b) [5]. IFN-α has significant immunomodulatory effects. It polarizes immune responses towards Th1, enhances cytotoxicity and survival of NK cells, induces the generation and survival of both CTL and memory CD8+ T cells, positively regulates antibody production, and promotes DC maturation, chemotaxis and CD8+ T cell priming against tumor antigens [6]. Furthermore, IFN-α exhibits direct antitumor activity by upregulation of MHC class I surface molecules, promotes caspase-dependent apoptosis in certain types of cancer, and has anti-angiogenic effects on tumor vasculature [7].

IFN-α was the first recombinant cytokine to be investigated clinically in patients with stage IV melanoma. Initial phase I and II studies yielded overall response rates of 16% (about one third of them having complete response). Responses were observed as late as 6 months from therapy initiation and up to one third of them were durable [8]. IFN-α has been used either as monotherapy or as part of the biochemotherapy regimen [9–11]. Initial evidence of activity of IFN-α in patients with metastatic melanoma led to its testing in the adjuvant setting. The North Central Cancer Treatment group (NCCTG) trial [12] and the Eastern Cooperative Oncology Group (ECOG) trial E1684 [13] were the first 2 adjuvant randomized controlled trials. Both trials tested a high-dose IFN-α (HDI) regimen (>10 Million Units/dosage). In the ECOG E1684 trial, HDI was given at 20 million units (MU)/m^2 intravenously 5 days a week for 4 weeks and then at 10MU/m^2, 3 days a week for 48 weeks. At a median follow-up of 6.9 years, HDI demonstrated a statistically significant impact on RFS and OS as compared to observation. The estimated 5-year RFS in the treatment arm was 37% (95% confidence interval [CI], 30%–46%) versus 26% (95% CI, 19%–34%) in the control group. Median RFS was 1.72 versus 0.98 years (P = .0023), hazard ratio (HR) = 0.61 (P = .0013). The 5-year OS was 46% (95% CI, 39%–55%) versus 37% (95% CI, 30%–46%) in the treatment

and observation arms, respectively. Median OS was 3.82 versus 2.78 years (P = .0237); HR = 0.67 (P = .01) [14]. The outcomes of this trial led to US FDA approval 1995. The EORTC 18991 trial tested adjuvant therapy with pegylated-IFN alfa-2b versus observation for AJCC stage III melanoma, recruiting 1256 patients from 2000 to 2003 [15]. At a median follow-up of 7.6 years, the study showed an improvement in the primary endpoint of RFS (HR = 0.87, 95% CI, 0.76 –1.00, P = .05), but with no significant differences seen in OS or distant metastasis free survival (DMFS) between observation and treatment. Pegylated IFN-α was granted FDA approval in the US as adjuvant therapy for patients with high-risk resected melanoma with lymph node metastases.

IFN-α was also investigated in the neoadjuvant setting [16]. Patients with stage IIIB/C melanoma underwent an initial biopsy then received induction HDI (IV 20 MU/m^2 5 days a week for 4 weeks) followed by lymph node dissection and subsequent maintenance HDI (SC 10 MU/m^2 3 days a week for 48 weeks). Of the 20 patients enrolled, 3 had pathologic complete responses and 8 had partial responses documented at the time of node dissection for a total perioperative response rate of 55%. At a median follow-up of 18.5 months, 10 patients had no evidence of recurrent disease. In the context of this neoadjuvant study, HDI was found to up-regulate pSTAT1, whereas it down-regulated pSTAT3 and total STAT3 levels in both tumor cells and lymphocytes. Higher pSTAT1/pSTAT3 ratios in tumor cells pretreatment were associated with longer overall survival (P = 0.032). Clinical responders had significantly greater increases in intratumoral CD11c+ and CD3+ cells and significantly greater decreases in intratumoral CD83+ cells compared with non-responders [16]. Additional adjuvant trials testing IFN-alfa-2b in patients with high-risk melanoma and several meta-analyses are discussed at length in a prior paperl[17].

In contrast to melanoma, IFN-α has failed to demonstrate efficacy when administered in the adjuvant setting to patients with stage II-III RCC[18, 19]. However, it was broadly tested in patients with advanced RCC, alone or in combinations. Responses were reported, primarily in patients with good risk by MSKCC stratification and prior nephrectomy. The lower toxicity profile of IFN-α compared to HD IL-2 and its feasibility in a community setting made it the reference arm in many subsequent phase III RCC trials[20] testing VEGFR TKIs. IFN has generally performed poorly relative to the VEGFR TKIs greatly diminishing its use in patients with metastatic RCC. Although the combination of IFN-α with bevacizumab was shown to be superior to IFN-α alone in two phase III trials, leading to FDA approval for the combination, the added toxicity associated with IFN administration, lack of evidence that the combination was superior to bevacizumab alone and the availability of multiple oral VEGF pathway inhibitors has greatly limited the use of this combination in clinical practice[21, 22].

Biomarkers for predicting efficacy of IFN-α treatment are not well established. One report from the Hellenic Oncology Group indicated that development of autoimmune disorders (vitiligo, thyroid dysfunction, enhanced serologic autoantibody titers) was strongly associated with favorable outcome in patients receiving adjuvant IFN treatment for melanoma[23] but later reports showed a weaker association [24, 25]. Serum S-100B blood levels were investigated in the E1694 trial high-risk patient population and found to correlate with increased recurrence and mortality risk[26]. Finally, a meta-analysis from two

European studies of adjuvant therapy for patients with high risk melanoma (EORTC 18952 and EORTC 18991) indicated tumor ulceration and lower stage disease (stage IIB/III-N1 vs. III-N2) as predictive factors[27] for freedom from IFN relapse and this notion is being prospectively investigated in the EORTC 18081 trial.

A phase II trial combining IFN-α with the anti-CTLA-4 antibody tremelimumab in patients with advanced melanoma yielded an overall response rate of 24% with long lasting remissions and evidence of downregulation of host immune suppressor mechanisms[28]. This study suggests that the activity of immunostimulatory agents like IFN-α can be augmented by inhibiting tumor related immune checkpoint mechanisms (such as CTLA4 and PD1). Clinical trials combining IFN-α with tyrosine kinase inhibitors, anti-PD-1/PDL-1/CTLA-4 antibodies, VEGF inhibition and DC vaccination strategies are ongoing.

INTERLEUKIN-2

Interleukin-2 (IL-2) is mostly produced by antigen-stimulated CD4+ T helper cells, and to a lesser extent by CD8+ T cells, NK - NKT cells and activated dendritic cells (DCs) [29]. The IL-2 receptor comprises three subunits including IL-2Rα (CD25), IL-2Rβ (CD122) and IL-2Rγ (CD132) known as the common cytokine receptor γ chain (γc). IL-2 induces the proliferation of NK cells augmenting their cytolytic capacity, drives proliferation and activation of CD8+ T cells and promotes the proliferation of B cells and antibody secretion [30]. IL-2 was originally seen as the key factor of augmenting an effector lymphocyte immune response. However, it also serves as a potent immune regulator by expanding immunosuppressive CD4+FOXP3+ T regulatory cells (Treg) [31] as well as promoting activation-induced death (AICD) of over-activated T cells[32]. IL-2 administration leads to an abundant release of pro-inflammatory cytokines (e.g. TNF-α, IL-1β, IL-6, IFN-γ), that likely contribute to the “flu-like” side effects of treatment, and increases angiopoietin 2 and nitric oxide levels that are felt to contribute to the capillary leak syndrome (CLS) and hypotension frequently observed in patients treated with high dose IL-2[33, 34].

Studies with high dose bolus (HDB) IL-2 used doses of 600,000–720,000 units/kg every 8 hours from days 1–5 (cycle 1) and 15–19 (cycle 2) with a maximum of 14 doses per cycle or 28 doses per course (1 course = 2 cycles). IL-2 was administered either as a single agent or in combination with immunologically active cells, so-called adoptive immunotherapy. The latter technique used 2 types of immune cells: lymphokine-activated killer (LAK) cells and tumor infiltrating lymphocytes (TIL). In RCC, results from seven phase II clinical trials including 255 patients receiving HD IL-2 demonstrated an overall response rate of 15%. The median duration of response for partial responders (PR) was 19 months while median response duration of complete responders (CR) had not been reached. In metastatic melanoma, a retrospective analysis of 8 trials using the HDB IL-2 regimen included 270 patients with follow-up through December 1998, and a subsequent update, demonstrated an objective response rate of 16%[3]. The median response duration was 8.9 months (range: 4–106+ months). Twenty-eight percent of responding patients, including 59% of those patients who achieved a complete response remained progression free at a median follow-up of 62 months. No patient with an ongoing response at 30 months has relapsed, with follow-up

extending beyond 20 years in some cases, suggesting that such patients are likely cured[3]. However, the major toxicities associated with this regimen, including CLS leading to hypotension, renal insufficiency and hypoxia, have precluded its widespread application. The use of HDB IL-2 is limited to specialized programs with experienced personnel, and it is generally offered only to patients with good performance and excellent organ function[35]. In addition, randomized studies have not shown superiority for IL-2 administered with LAK cells compared with therapy with HD IL-2 alone[36, 37]. Efforts continued in adoptive immunotherapy including the simplification and harvesting of TIL leading to significant advances [38]. Lymphodepletion induced by chemotherapy or total body radiotherapy prior to infusion of *ex* vivo expanded TIL with HDB IL-2 have conferred response rates of 50–72% in selected patients [38, 39] including many whose disease had failed to respond to HDB IL-2.

Biomarkers of Response

To date, the majority of studied markers associated with IL-2 response have been pretreatment clinical parameters (subcutaneous/cutaneous only metastasis) or post treatment variables such as the height of rebound lymphocytosis, treatment induced thrombocytopenia and the induction of autoimmune thyroiditis or vitiligo, or decreases in absolute numbers or frequencies of peripheral T-regulatory cells[40, 41]. In RCC, clear cell histology is a useful pretreatment predictor of IL-2 response. High levels of carbonic anhydrase IX expression by the tumor was also reported to be associated with response but prospective evaluation failed to validate this finding [42]. Proteomics analyses of the serum of patients reported high levels of VEGF and fibronectin as independent predictors of non-response to HDB IL-2, although these findings have not been confirmed by other groups[43]. Recent insights into tumor gene expression profiling have identified “immunogenic” tumors with high expression of immune-related genes that are associated with a higher likelihood of response in both patients with melanoma and RCC[44]. Further, NRAS mutations and normal serum LDH levels appear to correlate with better outcome in patients with melanoma [45]. These preliminary findings are currently being further investigated.

Combinations

Efforts to build upon the clinical activity of IL-2 monotherapy have included combinations with chemotherapy, vaccines, other cytokines, tyrosine kinase inhibitors, monoclonal antibodies, radiation and *ex vivo* activated immune cells. Overall, combining chemotherapy and IFN-α with IL-2 (labeled biochemotherapy) has produced inconsistent results[11, 46] with a prospective phase III trial ultimately showing no overall survival (OS) benefit for biochemotherapy relative to chemotherapy alone. Administration of a gp100 peptide vaccine yielded prolonged progression free survival (PFS) and OS relative to HDB IL-2 alone in one phase III trial[47] but the overall activity for the combination was not greater than what is typically seen with IL-2 alone, calling into question the true value of this result. A phase I/II study combined HDB IL-2 with ipilimumab, an anti-CTLA4 antibody, yielded a response rate of 22% with the majority of responses (75%) sustained for over a year[48]. Concurrent administration of IL-2 with anti-VEGF therapies (either sorafenib or bevacizumab) in metastatic RCC has been inconclusive [49, 50]. A phase 1 study of stereotactic body

radiotherapy (SBRT) and IL-2 demonstrated safety and supported further investigation of the combination and of CD4+ effector memory T cells as a predictor of response [51].

Ongoing trials are investigating HD IL-2 administration in combination with adoptive cell transfer, monoclonal antibodies targeting immune checkpoints, molecularly targeted therapies, other immunomodulating agents or radiation therapy. IL-2 conjugates with tumor specific antibodies are also appealing due to their theoretical targeted action and are currently being investigated in a broad spectrum of cancers.

INTERLEUKIN-15

Interleukin-15 (IL-15) shares common features with IL-2 in receptor binding, signaling and biologic activity, but also has significant differences in mediating innate and adaptive immune responses [52]. Although IL-15 mRNA is abundantly found in diverse tissues, its production occurs mainly from monocytes, macrophages and dendritic cells as a result of complex translational checkpoints and intracellular protein trafficking control[53]. IL-15 receptor consists of the high affinity IL-15Rα (CD215) chain along with IL-2R/IL-15Rβ chain and the common γ cytokine chain[54]. IL-15 expression is stimulated by type I Interferons and lipopolysaccharide (LPS) or other toll-like receptor agonists in dendritic cells [55]. IL-15 mainly promotes proliferation and differentiation of CD8+ T cells[56] and NK cells and serves as a homing factor in recruiting target immune cells to sites of production[57]. In comparison to IL-2, IL-15 appears to promote a more durable CD8+ memory T cell phenotype, induce more enhanced cell proliferation[58] and increase cell survival by upregulation of anti-apoptotic genes such as bcl-2 or downregulation of proapoptotic genes such as BIM and NOXA, even protecting them from IL-2 induced AICD[59, 60].

The strong immunostimulating capabilities of IL-15 have driven research to explore its role as an anticancer treatment. Numerous preclinical studies have demonstrated antitumor efficacy. *In vitro* and animal studies show enhanced NK cytolytic potency upon IL-15 activation via an NKG2D dependent fashion and also increased ADCC function. Enhanced cytotoxic lymphocytic (CTL) function as well as more efficient *ex vivo* expansion of TIL suitable for adoptive cell transfer have also been reported [61]. Since IL-15 signaling occurs mainly via trans presentation, IL-15/IL-15Rα complexes appear to be more potent immunostimulators than IL-15 alone [62]. Thus, modified IL-15/sIL-15Rα complexes have been developed and have demonstrated superior preclinical activity compared to rIL-15 in several preclinical models [63].

The results of a completed phase I study were recently presented [64]. Bolus I.V. administration in 18 patients with metastatic melanoma or RCC was associated with dose dependent toxicity. DLTs were grade 3 hypotension, thrombocytopenia, and elevations of ALT and AST. Strong immunostimulation for both NK and memory $CD8^+$ T cells was observed but preliminary results on antitumor activity yielded stable disease as a best response.

A number of clinical trials testing rIL-15 are ongoing. These involve administration of rIL-15 alone (NCT01727076, NCT01572493) or in conjunction with NK transfer

(NCT01385423, NCT018756010) or DC vaccination (NCT01189383). ALT-803, a mutant IL-15N72D/IL-15Rα sushi domain complex[65] is also being evaluated in Phase I/II studies in hematologic and solid malignancies (NCT01885897, NCT01946789) as well as intravesical administration with BCG in patients with non-muscle invasive bladder cancer (NCT 02138734).

INTERLEUKIN-21

Interleukin-21 (IL-21) is a pleiotropic cytokine that has emerged as a strong immune modulator functionally linking innate and adaptive immunity. It has been studied as a therapeutic target for the treatment of autoimmune diseases and as immune modulator for the treatment of patients with cancer. Its primary source is activated CD4+ T cells [65] and it has autocrine, as well as diverse functions on target cells. IL-21 binds to a specific IL-21R and signals through the common cytokine γc receptor as do IL-2 and IL-15[66]. A distinct feature of IL-21 is preferential transduction mainly through STAT1 and STAT3 and to a lesser degree through STAT5A/B, the dominant pathway for IL-2/IL-15 [67]. IL-21 can also signal through PI3K and MAPK pathways [67]. This means that it can promote both apoptosis (STAT1) and cellular proliferation (STAT3)[68]. Interestingly, patients with autoimmune diseases demonstrate IL-21R overexpression in cells of affected tissues [69]. IL-21 alone is not essential for lymphocytic development but rather acts as a strong co-stimulant of activated lymphocytes and IL-21R deficient patients display normal lymphocyte numbers but impaired B cell activity [70].

IL-21 alone has minimal effects on $CD8^+$ resting T cells. However, after cell activation, IL-21 promotes CD8+ T cell expansion and antitumor capacity, driving differentiation into a CTL or memory like phenotype[71]. It also supports sustained survival of human NK cells, induces proliferation and drives NK maturation finally leading to NK apoptosis [72]. B cells express high levels of IL-21R and IL-21 is a key cytokine for inducing differentiation of B and plasma cells establishing memory phenotype and long term humoral responses[73]. However, as an analog to IL-2's AICD function, IL-21 can also promote apoptosis possibly eliminating inappropriately activated B or plasma cells [74].

The antitumor activity of IL-21 has been shown in several preclinical studies. High dose IL-21 achieved via encoding plasmid injection in mice, inhibited tumor growth of poorly immunogenic tumors like B16F1 melanoma, MethA fibrosarcoma, colon or mammary adenocarcinoma, through NK and/or CD8+T cell mediated killing, with sustained memory protection against tumor rechallenge[75, 76]. Adoptive cell transfer (ACT) of *in vitro* IL-21-primed TIL has also been tested in melanoma bearing mice showing augmented antitumor activity and longevity *in vivo* as compared to IL-2 or IL-15 TIL priming[71].

Clinical trials have been conducted since 2004 testing rIL-21 alone, in combination with targeted therapies, monoclonal antibodies, chemotherapy or for ACT immune cell expansion. Overall, rIL-21 administration was relatively safe and adverse events more commonly included grade 1–2 headache, fatigue, pyrexia, nausea, myalgia/arthralgia, rash, diarrhea and injection site reaction. Severe adverse events requiring rIL-21 dose reduction or discontinuation were rare and mostly grade 3–4 hematological (granulocytopenia) and/or

hepatic (ALT/AST elevations). While an initial single arm phase II study reported a promising response rate in patients with metastatic melanoma, a subsequent randomized phase II study versus dacarbazine reported minimal clinical activity[77, 78]. Efforts with rIL-21 in patients with RCC yielded modest results[79] or were terminated early due to toxicity[80], whereas a phase I study of the combination with rituximab in patients with low grade lymphomas was reported as promising[81]. While these earlier results have led to a relative halt in the investigation of rIL-21 as anti-cancer therapy, a number of studies are ongoing including the investigation of rIL-21 in combinations with anti-CTLA4 in patients with metastatic melanoma (NCT01489059), anti-PD1 in patients with advanced solid tumors (NCT01629758) or alone in patients with AML (NCT01787474).

INTERLEUKIN-12

Interleukin 12 (IL-12), a pro-inflammatory heterodimeric cytokine, is the first member of the IL-12 cytokine family, also including IL-23, IL-27 and IL-35. IL-12 is mainly secreted from antigen presenting cells (phagocytes, dendritic cells) in response to pathogens, promoting CD4+ cell differentiation into Th1 cells[82]. It has a positive synergistic proliferative effect on pre-activated NK and T cells, enhances independently and/or synergistically the cytolytic ability of both NK and CD8+ T cells by upregulation of genes that encode cytotoxic cell granule-associated proteins[82]. It also increases ADCC in antibody coated tumors at considerably lower concentrations than IL-2 [83]. Effector cells stimulated by IL-12 produce several cytokines such as GMCSF and TNF-α but primarily IFN-γ. IL-12 consists of a p35 and a p40 subunit, the latter also shared by IL-23 [84]. The IL-12 receptor (IL-12R) consists of two chains, IL-12Rβ1 and IL-12Rβ213 and signals predominately through STAT4 [85]. The IL-12R is expressed mainly by activated NK and T cells; it is barely detectable in resting T cells but is expressed at a low level in NK, probably explaining their rapid response to IL-12[84]. Nevertheless, TCR activation and co-stimuli like B7, IFN-α, IFN-γ and IL-12 itself upregulate IL-12R expression (particularly IL-12Rβ2)[86].

Antitumor activity of systemic or local administration of IL-12 has been established in preclinical studies against various tumor cell lines [82]. Dose and model dependent response as well as a memory antitumor effect were observed. Other studies suggest that IL-12 is more efficacious against early stage or microscopic tumors than advanced disease. The T cell response is responsible for its antitumor activity and NK/NKT cell activation seems to prevent metastasis [87]. In addition to effector T cell (Teff) mediated tumor control, IL-12 inhibits tumor derived Treg either through suppression of T cell IL-2 production, induction of apoptosis or by IFN-γ mediated cell arrest, thus enhancing the Teff/Treg ratio both *in vitro* and *in vivo*[88, 89]. IL-12 was also reported to mediate reprogramming of intratumoral myeloid derived suppressor cells (MDSC) to enhance CTL activity in a B16 melanoma model, reversing their suppressive role *in vivo*. IL-12Rβ2 has also emerged as a potential tumor suppressor gene, particularly in hematologic malignancies where epigenetic IL-12Rβ2 gene silencing by promoter hypermethylation or gene downregulation were observed in multiple B-cell myeloma specimens and IL-12 significantly reduced tumor load upon receptor restoration[90]. Consistently, IL-12Rβ2−/− mice develop spontaneous malignancies

and lymphoproliferative diseases [91]. IL-12 also has "direct" antitumor activity in IL-12Rβ1 expressing tumors through IFN-γ mediated upregulation of MHC Class I molecules [92].

Initial clinical trials were temporarily halted after unexpected toxicity related deaths were observed in phase II trial in patients with metastatic RCC and melanoma, although the precedent phase I trial had established a safe MTD[93, 94]. This was attributed to a slight, though biologically significant alteration in the treatment schedule involving elimination of a test dose prior to a 5 day treatment course. Evaluation suggested that the test dose attenuated the effect of subsequent IL-12 on IFN-γ production through as yet unknown mechanism. Various IL-12 regimens were subsequently tested but with modest clinical results in patients with solid tumors and more promising results in hematologic malignancies [95]. Since IL-12 is a key mediator of the Th1 response, efforts focused on using IL-12 as an adjunct to vaccine therapy against tumor associated antigens, both for resectable or metastatic disease. In general, IL-12 has improved immune responses but this did not translate into clinically significant anti-tumor effects. The toxicity was also significant [96, 97].

The demonstration in preclinical models that locally delivered IL-12 was efficacious without the detriments of systemic administration prompted research into development of ways to express IL-12 intratumorally (IT)[98]. A study of IT injection of IL-12 into the primary tumor of patients with head and neck cancer revealed induced B cell activation that correlated with increased survival[99]. Rendering tumor cells able to produce IL-12 has been of interest and a number of small studies using viral vectors or plasmid DNA tested this approach with limited results[100, 101]. Antibody formation against viral vectors and undocumented efficiency of gene delivery were limiting factors. A new way to administer IL-12 locally is by the injection of plasmid DNA coding for IL-12 and electroporation of superficial tumors to facilitate cell entry. Results of a phase I study demonstrated antitumor activity, both at local injection sites and systemic uninjected sites [102]. Two trials in patients with gynecological malignancies assessed a PEG-PEI-cholesterol lipopolymer formulated IL-12 plasmid delivered by intraperitoneal administration but clinical benefit was modest [103, 104]. Current studies testing IL-12 are focused mainly on gene therapy including gene electroporation mediated plasmid transfer, adenoviral vector combined with orally administered activator ligand or mesenchymal engineered cells for IL-12 expression. NHS-IL-12 is a novel immunokine consisting of two IL-12 molecules fused to a tumor necrosis-targeting human IgG1 with longer half-life, improved efficacy and toxicity profile in preclinical models. It has now entered phase I clinical trial testing[105].

CONCLUSIONS

Multiple anticancer cytokines have demonstrated significant antitumor activity in preclinical studies. However, only IL-2 and IFN-α have made it into the clinic as monotherapy both with limited success. IL-12, IL-15 and IL-21 continue to be investigated through multiple novel approaches that appear promising especially in the context of adoptive cell transfer. Tremendous advances in cancer immunotherapy have been achieved during the last decade utilizing inhibitors and modulators of immune checkpoints (e.g. anti-CTLA4 and anti-PD1/PDL1 monoclonal antibodies). Combinations of cytokines with various checkpoint

inhibitors may provide a means of improving the outcome of checkpoint blockade and a restored foothold for cytokines in cancer immunotherapy. Early combination studies of CLTA4 and PD1/PDL1 blockade with IL-2 and IFN-α have either been completed with some promising results or are ongoing [10, 48].

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