Interleukin-12: Biological Properties and Clinical Application

Michele Del Vecchio,[1] Emilio Bajetta,[1] Stefania Canova,[1] Michael T. Lotze,[4] Amy Wesa,[5] Giorgio Parmiani,[3] and Andrea Anichini[2]

Abstract Interleukin-12 (IL-12) is a heterodimeric protein, first recovered from EBV-transformed B cell lines. It is a multifunctional cytokine, the properties of which bridge innate and adaptive immunity, acting as a key regulator of cell-mediated immune responses through the induction of T helper 1 differentiation. By promoting IFN-γ production, proliferation, and cytolytic activity of natural killer and T cells, IL-12 induces cellular immunity. In addition, IL-12 induces an antiangiogenic program mediated by IFN-γ–inducible genes and by lymphocyte-endothelial cell cross-talk. The immunomodulating and antiangiogenic functions of IL-12 have provided the rationale for exploiting this cytokine as an anticancer agent. In contrast with the significant antitumor and antimetastatic activity of IL-12, documented in several preclinical studies, clinical trials with IL-12, used as a single agent, or as a vaccine adjuvant, have shown limited efficacy in most instances. More effective application of this cytokine, and of newly identified IL-12 family members (IL-23 and IL-27), should be evaluated as therapeutic agents with considerable potential in cancer patients.

Interleukin-12 (IL-12) is recognized as a master regulator of adaptive type 1, cell-mediated immunity, the critical pathway involved in protection against neoplasia and many viruses. This is supported by the analysis of numerous animal (1, 2) and human clinical studies that attribute improved clinical outcome (3) and mechanisms of IL-12–based therapy (4) to strong type 1 responses *in situ*. Since the initial preclinical and clinical studies of IL-12, done over a decade ago, basic and translational science studies have contributed to the greater understanding of IL-12 immunobiology. In addition to its noted effects in the priming of T helper 1 (TH1) cell responses and IFN-γ production by T and natural killer (NK) cells, more recent studies support its critical role as a third signal for $CD8^+$ T cell differentiation (5, 6), and its ability to serve as an important factor in the reactivation and survival of memory $CD4^+$ T cells (7). This is particularly relevant in the repolarization of $CD4^+$ T cells from dysfunctional antitumor TH2 into TH1 cells in the cancer setting (8). Here, we review the immunomodulatory and antiangiogenic functions of IL-12, as well as the results of preclinical and clinical studies in which IL-12 was used as an anticancer agent.

Authors' Affiliations: [1]Medical Oncology Unit 2 and [2]Human Tumor Immunobiology Unit, Department of Experimental Oncology, Fondazione IRCCS Istituto Nazionale per lo Studio e la Cura dei Tumori, [3]Unit of Immuno-Biotherapy of Solid Tumors, Istituto Scientifico S. Raffaele, Milan, Italy, [4]Translational Research, Molecular Medicine Institute, University of Pittsburgh Cancer Institute, and [5]Department of Dermatology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Received 4/6/07; revised 5/18/07; accepted 5/31/07.

Grant support: Associazione Italiana per la Ricerca sul Cancro, Milan (A. Anichini) and PO1 CA 101944-01 (M.T. Lotze).

Requests for reprints: Emilio Bajetta, Medical Oncology Unit 2, Fondazione IRCCS Istituto Nazionale per lo Studio e la Cura dei Tumori, Via Venezian 1, 20133 Milan, Italy. Phone: 39-02-23902500; Fax: 39-02-23902149; E-mail: emilio.bajetta@istitutotumori.mi.it.

doi:10.1158/1078-0432.CCR-07-0776

Bridging of Innate and Adaptive Immunity by IL-12

IL-12 was independently discovered by Trinchieri and colleagues (in 1989) and by Gately and colleagues (in 1990) as "natural killer–stimulating factor" and as "cytotoxic lymphocyte maturation factor", respectively (9, 10). It was identified as a heterodimeric cytokine composed of two covalently linked p35 and p40 subunits and was present in the supernatant of phorbol-ester–induced EBV-transformed B cell lines (9, 10). Initial characterization of its biological activities revealed that IL-12, when added to human peripheral blood lymphocytes, induced IFN-γ production, increased NK cell cytotoxicity as well as T cell proliferation in response to mitogenic lectins and phorbol diesters (see ref. 11 for review). Subsequent studies indicated that IL-12 could boost the generation of cytotoxic T cells by promoting the transcription of genes encoding cytolytic factors including perforin and granzymes (11). In 1993, Hsieh et al. (12) discovered that IL-12, produced by macrophages in response to microbial pathogens, was a key cytokine in TH1 T cell differentiation. This finding established the central role of IL-12 in a pathway in which innate immune cells drove the adaptive immune response, polarizing naïve $CD4^+$ cells towards the TH1 phenotype. The general model on the biological role of IL-12 predicts that this cytokine is required for resistance to bacterial and intracellular parasites, as well as for the establishment of organ-specific autoimmunity (11). According to such a model, IL-12, produced by activated hematopoietic phagocytic cells (monocytes, macrophages, and neutrophils), by dendritic cells (DC), and by the recently identified IFN-producing killer DC lineage (13), acts as a critical regulator of cell-mediated responses. The biological functions of IL-12 are mediated by the IL-12 receptor (14) composed of two chains (β1 and β2). Triggering of the receptor activates the JAK-STAT signaling pathway, with STAT4 being the predominant mediator of cellular responses activated by IL-12 (14).

Positive and Negative Control of IL-12 Expression

The production of the p35 and p40 subunits, as well as of the biologically active IL-12 heterodimer (i.e., IL-12p70) by phagocytic cells, require specific "priming signals" from bacterial products, and "amplification" signals dependent on cytokines produced by T cells or DC subtypes, and on cell-cell interactions. Production of the IL-12 p40 subunit by myeloid cells requires Toll-like receptor engagement by damage-associated molecular pattern molecules or pathogen-associated molecular pattern molecules, but T cell–derived cytokines such as IFN-γ are required for the optimal production of both p35 and p40 subunits (15). IL-12 production by conventional DCs in response to TLR9 agonists (CpG) requires IL-15 production leading to up-regulation of CD40 on such APCs (16). This in turn allows plasmacytoid DCs to promote IL-12 production in conventional DCs, through CD40L-CD40 interaction (16). At the T-DC interface, T cells contribute to amplify IL-12 production by DCs, in response to bacterial priming, through cell-cell interactions mediated by CD40L on activated T cells and CD40 on DCs (17). In addition, recent results indicate that IL-12 production by mature DCs requires a signaling event mediated by the tumor necrosis factor-α intracellular domain that is released upon intramembrane proteolysis by SPPL peptidases (18). In turn, the tumor necrosis factor-α intracellular domain can signal to the nucleus for IL-12 production (18). Furthermore, simultaneous stimulation of human monocytes with TLR4 and TLR8 agonists leads to IL-12p70 production, even in the absence of overt T cell help (19). Thus, DCs have a "combinatorial code" for optimal IL-12 production dependent on TLR3 and TLR4 acting in synergy with TLR7, TLR8, or TLR9 (20). Such "coding" may allow DCs to express an efficient TH1 polarizing program upon appropriate stimulation by pathogen- or damage-associated molecular pattern molecules.

Cytokines such as IL-10 and transforming growth factor-β1 can negatively regulate IL-12 production. Both IL-10 and transforming growth factor-β1 suppress transcription of the IL-12 p40 subunit, thus limiting the amount of biologically active p70 heterodimer (21, 22). In addition, the cytokine formed by homodimerization of the IL-12 p40 subunit [i.e., IL-12$(p40)_2$] acts as an antagonist of IL-12p70 biological activity by binding to the β1 subunit of the IL-12 receptor (23), although this mechanism may act primarily in mouse cells and not in human cells, due to the low affinity of the human p40 homodimer for the IL-12 receptor. Furthermore, recent evidence indicates that the IL-12$(p40)_2$ homodimer can have agonist activity, as suggested by its ability to promote DC migration to draining lymph nodes and naïve T cell activation in response to *Mycobacterium tuberculosis* infection (24).

IL-12 and TH1 Differentiation

Early models placing IL-12 at the beginning of the TH1 T cell differentiation pathway have been progressively revised, thanks to the emerging evidence supporting the role of T-bet transcription factor in TH1 commitment (25). In 2001, Mullen et al. (26) reported that, in the mouse, T-bet could specify TH1 effector fate without the involvement of IL-12. Such TH1 fate determination was achieved by the effects of T-bet on chromatin remodeling of IFN-γ alleles and by the induction of IL-12Rβ2 expression. This led to a revised TH1 differentiation model (27) placing T-bet upstream of IL-12. According to such a revised model, T-bet expression was induced in naïve T cells by signals derived from innate cells (i.e., NK cell–derived IFN-γ, signaling through STAT1), whereas IL-12 maintained a role as a survival signal acting on cells with a predetermined TH1 fate (27). Recent results further clarified that the contribution of T-bet to TH1 fate determination depends on its activity as a negative regulator of GATA-3, the main regulator of T cell commitment to the TH2 lineage, rather than as solely a positive regulator of the IFN-γ gene (28).

Newly Discovered IL-12 Family Members

The discovery of new IL-12 family members, IL-23 and IL-27 (29), has prompted a further revision of the role of IL-12 in the differentiation of TH lineages and in some T cell–dependent autoimmune and inflammatory diseases. IL-23 shares the p40 chain with IL-12, but such subunit associates with a p19 chain (29). IL-27 is a heterodimeric cytokine which is composed of the EBV-induced molecule 3 that associates with the IL-27 p28 chain (29). Similar to IL-12, IL-23 and IL-27 are produced predominantly by macrophages and dendritic cells and affect IFN-γ production by T and NK cells (29). IL-23 serves to promote TH4/TH_{17} cells producing the cytokines IL-6, IL-17, IL-22, and IL-25. All three cytokines (IL-12, IL-23, and IL-27) seem to play roles in the priming and re-activation of polarized T cell responses. Both IL-12p70 and IL-27 can exert effects in the priming of TH1 $CD4^+$ T cell responses (30, 31), with recent data suggesting a critical role for T regulatory cell survival. IL-12 and IL-23 enhance the responses of memory T cells (32), whereas IL-27 engagement with its receptor seems to limit inflammatory T cell responses (33). Furthermore, IL-27 suppresses the development of TH4/TH_{17} cells (34), whereas contributing to TH1 development by promoting the expression of T-bet and IL-12Rβ2 (29).

IL-12 as an Antiangiogenic Factor

Brunda et al. (35), as well as Tahara et al. (36) and Nastala et al. (37) showed that IL-12 has potent *in vivo* antitumor and antimetastatic activity against murine tumors. Interestingly, the efficacy of IL-12 was greatly reduced, but not abolished, in immune-incompetent mice. By building on these observations, in 1995, Folkman and colleagues discovered the potent antiangiogenic properties of IL-12 (38). They found that IL-12 treatment inhibited basic fibroblast growth factor–induced corneal neovascularization in both immunocompetent and immunodeficient mice (38). Suppression of angiogenesis by IL-12 was dependent on its ability to induce IFN-γ expression (see Fig. 1 for an outline of IL-12 effects on angiogenesis). Accordingly, administration of IFN-γ reproduced the antiangiogenic effects promoted by IL-12 (38). Trinchieri and colleagues (39) injected neoplastic cells engineered to secrete murine IL-12 or IL-18 in syngeneic mice and found significant protection against the growth of a concurrent tumor, not secreting cytokines, and injected at a distant site. This effect was shown to be mediated by inhibition of angiogenesis by IL-12 and IL-18 released by transfected neoplastic cells, rather than by the promotion of

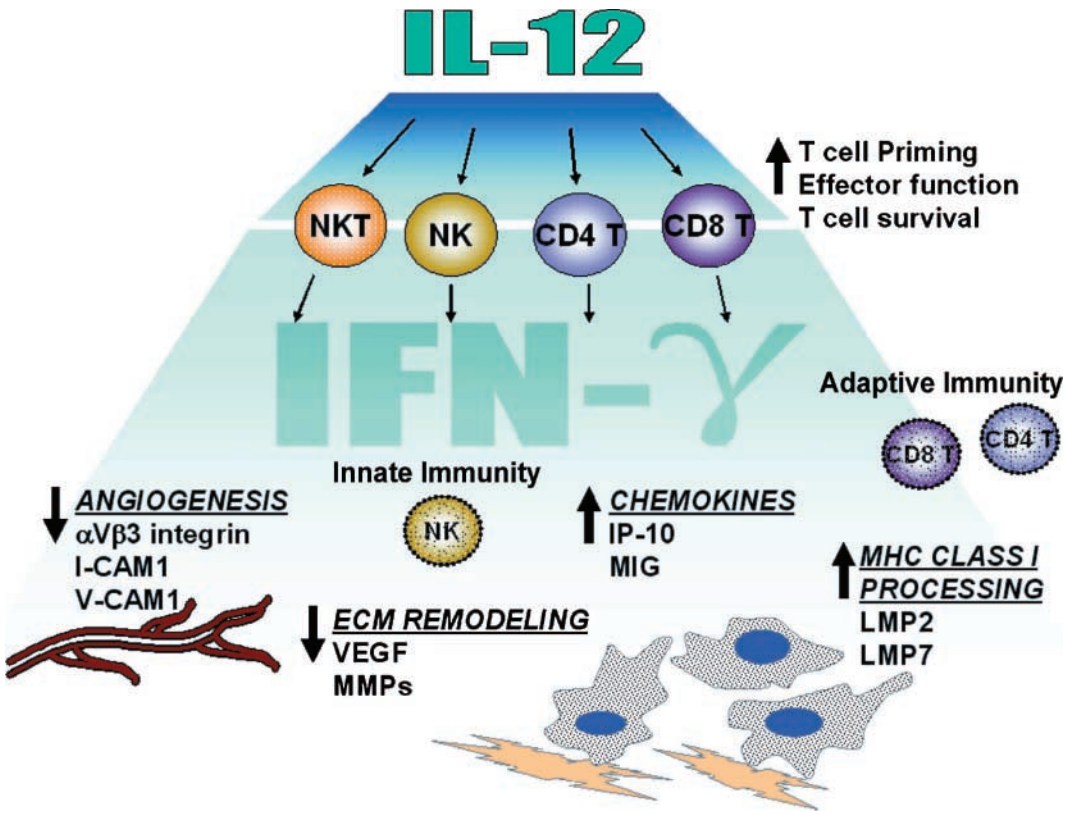

Fig. 1. A model of mechanisms involved in the antitumor effects of IL-12. IL-12 directly activates cells of the innate (NK and NK-T cells) and adaptive ($CD4^+$ and $CD8^+$ T cells) arms of immunity. In particular, IL-12 helps to prime T cells and increases their survival, enhances T cell, NK cell, and NK-T cell effector functions, and induces the secretion of IFN-γ, a nearly indispensable component of IL-12's antitumor effect. In turn, the production of IFN-γ by such antitumor effectors may act directly on the tumor cells, as well as stromal and endothelial components within the tumor microenvironment. Pathways targeted by IFN-γ signaling are activated, and result in (*a*) increased MHC class I processing and presentation (enhancing recognition of the tumor by T cells), (*b*) induction of chemokines IP-10 and MIG (that may recruit innate and adaptive immune effectors), which in turn lead to (*c*) alterations in extracellular matrix (*ECM*) remodeling, including inhibition of matrix metalloproteinase (*MMP*) expression, that reduces angiogenesis and tumor invasion, as well as (*d*) decreases in adhesion molecules expressed by endothelial cells that may further limit angiogenesis. These distinct pathways activated by IL-12 converge to obstruct tumor progression, and ultimately, facilitate the eradication of the tumor.

antitumor immunity (39). In a related article, the same group also showed that inhibition of tumor growth by IL-12 or IFN-γ required an intact signaling from IFN-γ receptors expressed in neoplastic cells. This indicated that IL-12 could inhibit tumor growth by inducing neoplastic cells to produce antiangiogenic factors (40). Two of the most relevant factors were soon identified as the IFN-γ–inducible genes *IP-10* and *Mig* (41, 42). Initial results obtained by Sgadari et al. (43) showed that intratumor delivery of Mig into Burkitt's tumors, growing subcutaneously in nude mice, led to tumor necrosis associated with vascular damage. Subsequently, these authors found that treatment *in vivo* with IL-12 led to the expression of *IP-10* and *Mig* genes in tumor cells (42). In addition, administration of neutralizing antibodies to IP-10 and Mig substantially reduced the antitumor effects of IL-12 (42).

Mechanisms of Angiogenesis Inhibition by IL-12

The available evidence puts IFN-γ and lymphocyte-endothelial cells cross-talk at the beginning of the process of inhibition of angiogenesis by this cytokine (Fig. 1). Dias et al. (44) found that IFN-γ, induced by IL-12, reduced tumor cell production of vascular endothelial growth factor. Moreover, IL-12 treatment reduced the production of metalloproteases playing a role in matrix remodeling, a process required during neoangiogenesis (45). Furthermore, IL-12–induced IFN-γ reduces activation of integrin αVβ3 on endothelial cells (46), leading to decreased endothelial cell adhesion and survival (46). The relevance of lymphocyte-endothelial cells cross-talks has been investigated by Strasly et al. (47). They found that IL-12 activates an antiangiogenic program in lymphocytes that leads to the production of IP-10 and Mig (Fig. 1). These factors in turn negatively modulate the cycle of endothelial cells, the production of matrix metalloproteinase-9, the ability of endothelial cells to adhere to vitronectin and to up-regulate intercellular adhesion molecule 1 and vascular cell adhesion molecule-1 expression (47). The antiangiogenic program activated in lymphocytes by IL-12 can also directly affect gene expression in neoplastic cells. In fact, up-regulation of STAT1, IRF-1, LMP2, LMP7, Mig, monocyte chemoattractant protein 1, and angiopoietin 2 genes, with down-modulation of vascular endothelial growth factor, has been documented in neoplastic cells exposed to soluble factors released by IL-12–stimulated lymphocytes (48). Further studies have also shown that NK cells are important effectors of the antiangiogenic activity of IL-12. In fact, NK cells accumulate at sites of IL-12–mediated inhibition of angiogenesis and are cytolytic for endothelial cells at such sites (49).

Preclinical Models of IL-12 as Antitumor Agent

The antitumor and antimetastatic activities of IL-12 have been extensively shown in murine models including melanomas, mammary carcinomas, colon carcinoma, renal carcinoma, and sarcomas (see ref. 50 for review). Some of these studies have addressed the issue of local IL-12 production versus systemic delivery (i.e., intraperitoneally). Production of IL-12 at the tumor site (by neoplastic cells engineered to release IL-12 by appropriate expression vectors) induces the rejection of neoplastic cells by $CD8^+$ T cells associated with macrophage infiltration, vessel damage, and necrosis (51). Interestingly, the cure rates of mice bearing established tumors were much higher following i.p. administration of rIL-12 when compared to vaccination with tumors releasing IL-12 (51). Improved antitumor effects have been shown when IL-12 was administered with other cytokines such as IL-2 and IL-18 (39) or with neoplastic cells expressing costimulatory molecules (52). Analysis of the immune mechanisms activated by IL-12 in these preclinical models has suggested the role of several subsets, including NK cells, $CD4^+$ and $CD8^+$ T cells, and $CD3^+$ $CD56^+$ NK-T cells expressing the Vα14 invariant T cell receptor (35, 53).

Clinical Studies with IL-12 as an Anticancer Agent

The activity of IL-12 has been investigated in patients with advanced solid tumors and hematologic malignancies (54–75), as either monotherapy (Table 1), or in combination with other therapies (Table 2). With the exception of the results obtained in cutaneous T cell lymphoma variants (59, 68), in AIDS-related Kaposi sarcoma (67) and non–Hodgkin's lymphoma (69), efficacy was minimal, with an objective response rate ranging between 0% and 11% (Tables 1 and 2). In the first published trial, Atkins and colleagues (54) enrolled 40 patients, including 20 with renal cancer and 12 with melanoma, in a phase I dose escalation study of i.v. administered recombinant human IL-12 (rHuIL-12). A transient complete response in a patient with melanoma and one partial response in a patient with renal cell cancer were documented. Bajetta and colleagues enrolled 10 pretreated patients with advanced melanoma in a pilot study (56). The patients received a fixed dose of rHuIL-12 (0.5 μg/kg) on days 1, 8, and 15 for two sequential 28-day cycles. No partial or complete responses were documented, but tumor shrinkage involving subcutaneous metastases, superficial adenopathy, and hepatic metastases was observed. Immune monitoring of these patients, by Mortarini et al. (57), indicated that IL-12 administration induced a striking burst, in the periphery, of HLA-restricted CTL precursors directed to autologous tumors and to an immunogenic tumor-associated antigen (Melan-A/Mart-1_{26-35} peptide). Interestingly, infiltration of neoplastic tissue by $CD8^+$ T cells with a memory and cytolytic phenotype was identified by immunohistochemistry in eight of eight posttreatment metastatic lesions, but not in five of five pretreatment metastatic lesions from three patients (57). These results provided the first evidence that rHuIL-12 can boost the frequency of circulating antitumor CTL precursors in tumor patients and promote infiltration of neoplastic lesions by $CD8^+$ memory T cells in a clinical setting.

Gollob et al. (60), using an administration schedule based on twice-weekly injections for 6 weeks, found raised blood levels of IFN-γ, IL-15, and IL-18 in treated patients. Interestingly, whereas IFN-γ and IL-15 induction was attenuated during the first cycle in patients with disease progression, patients with tumor regression or stable disease showed constant higher levels of IFN-γ, IL-15, and IL-18 (60). In 2001, Motzer et al. (61) reported the results of a randomized phase II trial on 46 patients comparing rHuIL-12 IFN-α in patients with previously untreated and advanced renal cell carcinoma. Only 2 of 30 patients treated with rHuIL-12 achieved a partial response.

In order to avoid the IL-12–related toxicity, observed after systemic administration, locoregional treatment with IL-12 was investigated in two small clinical trials. Lenzi et al. (62) administered IL-12 to 29 previously treated patients with peritoneal carcinomatosis from various abdominal cancers through an indwelling peritoneal catheter. Two patients (one with ovarian cancer and one with surgically pretreated mesothelioma and with small residual disease) had no remaining disease at laparoscopy, eight patients had stable disease and the remainder had progressive disease (62). In a phase I study, Weiss et al. (63) evaluated intravesical IL-12 administration in patients with recurrent superficial transitional cell carcinoma of the bladder that had failed at least one prior intravesical therapy, or had at least two recurrences of low-grade tumors. Each patient received intravesical IL-12 weekly for 6 weeks. The treatment was well-tolerated, but no objective responses were observed (63).

Clinical Studies Involving IL-12 as an Adjuvant to Vaccination

Lee et al. (70) reported a trial in which 48 patients with resected stage III or IV melanoma were immunized with peptides derived from tyrosinase and gp100, with or without s.c. administration of IL-12. IL-12 augmented peptide-specific delayed-type hypersensitivity reactivity to the gp100 antigen in 34 of 40 patients. Moreover, the treatment boosted the gp100-specific and tyrosinase-specific peripheral immune response, as measured by IFN-γ release in 37 of 42 patients. In another trial conducted by Cebon and colleagues (71), rHuIL-12 was administered s.c. or i.v. in two cohorts of stage III or IV melanoma patients expressing Melan-A/Mart-1 in their tumors. Melan-A/Mart-1_{26-35} and influenza matrix$_{58-66}$ peptides were administered intradermally. Clinical responses were mostly mixed, but one complete response and one stabilization of the disease were achieved in the i.v. arm, one partial response, and five stabilizations were attained in the s.c. arm. Cutaneous delayed-type hypersensitivity reactions were associated with $CD4^+$ and $CD8^+$ T lymphocyte infiltration capable of lysing a Melan-A/Mart-1 peptide-pulsed target *in vitro* (71). Peterson et al. (72) immunized 20 pretreated patients with advanced melanoma with PMBC loaded with Melan-A/Mart-1 peptide plus IL-12. Two patients achieved a complete response, five patients a minor or mixed response, and four patients had stable disease. The overall median survival was 12.25 months and seven patients remained alive at the time of data analysis, with all patients followed for more than 12 months (72).

Table 1. Clinical studies of systemic IL-12 alone

Tumors	Route of administration	Patients (*n*)	Objective response	Immune modulation	Angiogenesis-related effects	Refs.
Different solid tumors*	i.v.	40	5%	Dose-dependent ↑ sIFN-γ; peak at 24-48 h after IL-12 ↓ $CD4^+$/$CD8^+$ and $CD16^+$ cells; nadir at 24 h after IL-12 ↑ of NK cell adhesion molecules (CD2, LFA-1)	ND	(54, 55)
Melanoma*	s.c.	10	0% (three minor responses)	↑ sIFN-γ within 24 h after the first IL-12 injection ↑ IL-10 during the second cycle Lymphopenia and CD4/CD8 ratio inversion ↓ CD16+cells 24 h after the first injection ↑ Frequency of antitumor CTL precursors Tumor infiltration by CD8+ memory T cells	↓ Urine bFGF in two of three patients with minor responses	(56, 57)
Renal cell carcinoma*	s.c.	51	2%	↑ sIFN-γ with peak level at 24 h after the first maintenance dose	ND	(58)
Cutaneous T cell lymphoma*	s.c. or intralesionally	10	56%	↑ $CD8^+$ and/or $TIA\text{-}1^+$ T cells in skin biopsy from regressing lesions	ND	(59)
Melanoma, renal cell carcinoma*	i.v.	28	3%	Induction of IFN-γ, IL-15 and IL-18, maintained in patients with tumor regression or prolonged disease stabilization	ND	(60)
Renal cell carcinoma †	s.c.	30	7%	↑ sIFN-γ, IL-10 and neopterin, maintained in cycle 2	ND	(61)
Abdominal tumors*	i.p.	29	7%	↑ Peritoneal $CD3^+$ and ↓ $CD14^+$ cells	↓ bFGF and VEGF in tumor ↑ IFN-γ and IP-10 transcripts in peritoneal exudate cells	(62)
Bladder cancer*	Intravesical	15	0%	No urine/serum IFN-γ induction	ND	(63)
Renal cell carcinoma*	s.c.	26	NA	Dose-dependent ↑ sIFN-γ, TNF-α, IL-10, IL-6 and IL-8 at first injection Lymphopenia: further ↑ IL-10 during treatment	ND	(64)
Cervical carcinoma †	i.v.	34	3%	↑ Lymphoproliferative responses to HPV 16 E4, E6 and E7 peptides	ND	(65)
Head-neck carcinoma*	Intratumoral	10	ND	↑ $CD56^+$ NK cells in the primary tumor High IFN-γ mRNA expression at lymph node level	ND	(66)
AIDS-related Kaposi's sarcoma*	s.c.	34	50% (71% at highest doses)	↑ sIFN-γ after first dose, persisting after week 4	↑ sIP-10 after the first dose, persisting after week 4	(67)
Mycosis fungoides †	s.c.	23	43%	ND	ND	(68)
Non–Hodgkin's lymphoma and Hodgkin's lymphoma †	i.v. or s.c.	42	21% ‡	↑ Circulating $CD8^+$ T cells	↓ sVEGF and sbFGF in 37% of patients	(69)

Abbreviations: bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; TNF, tumor necrosis factor; NA, not available; ND, not done; s, serum; ↑, increase; ↓, decrease.
*Pilot/phase I trial.
† Phase II trial.
‡ Only in patients with non–Hodgkin's lymphoma.

Table 2. Clinical studies of systemic IL-12 in combination with vaccines, other cytokines, or antitumor monoclonal antibodies

Tumors	Combined treatment	Patients (*n*)	Objective response	Immune modulation	Angiogenesis-related effects	Refs.
Melanoma*	gp100 and tyrosinase peptides	48	ND	Age-specific immune response against the peptide vaccine, as shown by ↑IFN-γ release in most patients	ND	(70)
	Melan-A/Mart-1 and influenza peptides	28	8%	↑ sIFN-γ within 24 h after the first IL-12 injection	ND	(71)
	Melan-A/Mart-1 peptide-pulsed PBMC †	20	10%	↑ IFN-γ – producing T cells directed to Melan-A/Mart-1 after vaccination	ND	(72)
Melanoma, renal cell carcinoma*	IL-2	28	11%	↑ IFN-γ production and expansion of NK cells	↑ IP-10 production	(73)
	IFN-α2b	26	11%	CD80 and IFN-γ induction in PBMCs of selected patients by RT-PCR	RT-PCR on PBMCs showed induction of IP-10 and IFN-γ in selected patients	(74)
$HER2^+$ tumors*	Trastuzumab	15	6%	↑ IFN-γ production by NK cells in responsive or stable patients; associated with IFN-γ gene polymorphism	↑ sMIP-1α, TNF-α and IP-10	(75)

Abbreviations: PBMC, peripheral blood mononuclear cells; TNF, tumor necrosis factor; NA, not available; ND, not done; s, serum; ↑, increase; ↓, decrease.
*Pilot/phase I trial.
† Phase II trial.

Clinical Studies of IL-12 Administered with Other Cytokines or with Antitumor Monoclonal Antibodies

In the study done by Gollob et al. (73), pretreated patients with metastatic renal cell cancer, melanoma, or transitional cell cancer were treated with 6-week cycles of twice-weekly i.v. rHuIL-12 plus IL-2 s.c. There was one partial response and two pathologic responses, all of which occurred in patients with melanoma. When administered at the maximum tolerated dose, IL-2 significantly augmented IFN-γ and IP-10 production by rHuIL-12 and led to a 3-fold expansion of NK cells (73). Alatrash et al. (74) treated 26 patients affected by metastatic melanoma or metastatic renal cell cancer with escalating doses of IL-12 and IFN-α2b. Three patients had a partial response and the median overall survival was 13.8 months (74). More recently, in a limited phase I clinical trial, IL-12 was administered with trastuzumab in patients with $Her2^+$ solid tumors (75), but the addition of the cytokine did not seem to enhance the efficacy of this antibody treatment. One patient had a complete response and two patients had stabilization of disease (75).

Clinical Studies of IL-12 Gene Therapy

Systemic administration of IL-12 in patients is limited by toxicity. Based on the promising results obtained in a large series of preclinical IL-12 gene therapy studies (see ref. 76 for review), clinical trials have been designed with the aim of achieving production of the cytokine at the tumor site, whereas maintaining low serum concentrations to reduce systemic toxicity. Kang et al. (77) enrolled seven patients with advanced malignancies accessible from the body surface in a phase I dose-escalation clinical study of peritumoral injection of IL-12–transduced autologous fibroblasts. Weekly injection consisted of sufficient fibroblasts to secrete an estimated 300 ng of IL-12 per 24 h. Transient reductions of tumor sizes were observed at the injected sites in four patients and at noninjected distant sites in one melanoma patient. Overall, five of seven patients showed clinical responses. No clinically significant toxicities were reported (77). In another study, patients with melanoma received s.c. injections at weekly intervals of autologous tumor cells transduced with two independent eukaryotic expression vectors coding for the p35 and p40 subunits of IL-12 (78). Two patients developed delayed-type hypersensitivity reaction against their autologous melanoma cells and one had a minor clinical response (78). Heinzerling et al. (79) did a study in which plasmid DNA encoding human IL-12 was injected into lesions of pretreated patients with advanced malignant melanoma. Intratumoral injection resulted in local responses at the injection site in all but one of the nine treated patients, and led to significant local tumor reduction in five patients. Overall, two instances of "stable disease" and one complete remission were achieved. Real-time quantitative PCR on biopsies taken 24 h after the last injection revealed a more pronounced increase in IL-12, IFN-γ, and IP-10 in responders than in nonresponders (79). Triozzi et al. examined the activity of the intratumoral injection in melanoma patients of two vectors, one encoding the costimulatory molecule B7.1 and the other encoding IL-12. Nine patients received only the B7.1-encoding vector and five received both vectors. No clinical responses were observed (80). Mazzolini et al. (81) described the intratumoral injection of dendritic cells engineered to secrete IL-12 in 17 patients with metastatic gastrointestinal tumors. A partial response was observed in a patient with

pancreatic carcinoma and infiltration of $CD8^+$ lymphocytes was documented in 3 of 11 biopsies analyzed.

New Perspectives

The reasons for the limited clinical efficacy of IL-12 as a biological response modifier in cancer patients remain incompletely understood. Early clinical studies (82) showed evidence of an "adaptive response" that down-regulated the pharmacodynamics of IL-12 following the first administration of cytokine. Subsequent studies (56, 57) confirmed that the effects of IL-12 on IFN-γ levels and the frequency of circulating tumor-specific T cells were greatly reduced after the first cytokine administration. These results suggested that the antitumor activity of IL-12 could be progressively inhibited upon continuing administration to patients. Thus, a strategy of "*crescendo ma non troppo*" (increasing but not too much), by increasing the dose of IL-12 to overcome the tachyphylactic response, was reasonable but never tested. In addition, the immune suppression–dominated microenvironment in advanced tumors (83) is likely a major factor contributing to limit the efficacy of IL-12–based therapies. Indeed, $CD4^+$ T cell depletion that presumably removes regulatory T cells has been reported to enhance the antitumor effectiveness of IL-12 in mice (84). However, IL-12 itself does not seem to directly modulate the function or frequencies of $CD4^+CD25^+FOXP3^+$ regulatory T cells *in vitro* (85), thus, combinational approaches designed to deplete preexisting regulatory immune cells, or to limit their function, could be used. A broad-based approach could include preconditioning immunodepletion such as low-dose cyclophosphamide (86), followed by an IL-12–based vaccine, or IL-12–based therapy in conjunction with antibodies targeting regulatory T cells (87), like the anti–CTLA-4 monoclonal antibody recently used in clinical studies. As IL-12 may itself contribute to immunoregulation through the compensatory induction of IL-10 (88), circumventing the immunosuppressive effects of IL-10 could also be envisioned to enhance the therapeutic index of IL-12. This could be achieved through blocking IL-10 binding to cellular receptors by coadministration of a soluble IL-10R or anti–IL-10 antibody, or preferably, by obstructing the STAT3 signaling pathway induced by IL-10 using a variety of inhibitors (89). As a corollary, negative feedback pathways mediated by SOCS-1 (90) or SOCS-3 (91) molecules induced by IL-12 signaling could also represent attractive targets for small molecule inhibitors or RNAi.

A further promising area of investigation, with potentially relevant clinical applications, stems from the emerging knowledge on newly discovered IL-12 family members. For example, in murine models, both IL-23 and IL-27 have been used to effectively treat tumors (92, 93). However, due to the role of IL-23 in inflammatory diseases, caution would be justified because such inflammation could conceivably contribute to tumor progression rather than tumor regression (94). One could envision combinatorial approaches using IL-12 and IL-27 to promote naïve T cell responses against tumor antigens, and/or IL-12 and IL-23 to promote memory antitumor responses. Carefully designed combinatorial IL-12 family regimens could potentially exploit a natural mechanism for regulating type 1 immune (re)activation, without triggering chronic inflammation. This could lead to a more durable and effective biological antitumor response than that obtained with IL-12 alone, and hence, greatly improve the therapeutic efficacy of IL-12–based treatments. Further clinical studies could be developed by exploiting the antiangiogenetic program promoted by IL-12 in lymphocytes and in neoplastic cells. To this end, IL-12–based therapy might be combined with other antiangiogenic molecules such as bevacizumab or raf-kinase inhibitors (95).

References

1. Hung K, Hayashi R, Lafond-Walker A, et al. The central role of CD4(+) T cells in the antitumor immune response. J Exp Med 1998;188:2357–68.
2. Tatsumi T, Huang J, Gooding WE, et al. Intratumoral delivery of dendritic cells engineered to secrete both interleukin (IL)-12 and IL-18 effectively treats local and distant disease in association with broadly reactive Tc1-type immunity. Cancer Res 2003;63:6378–86.
3. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313:1960–4.
4. van Herpen CM, Looman M, Zonneveld M, et al. Intratumoral administration of recombinant human interleukin 12 in head and neck squamous cell carcinoma patients elicits a T-helper 1 profile in the locoregional lymph nodes. Clin Cancer Res 2004;10:2626–35.
5. Curtsinger JM, Lins DC, Mescher MF. Signal 3 determines tolerance versus full activation of naive CD8 T cells: dissociating proliferation and development of effector function. J Exp Med 2003;197:1141–51.
6. Kalinski P, Hilkens CM, Wierenga EA, Kapsenberg ML. T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. Immunol Today 1999;20:561–7.
7. Yoo JK, Cho JH, Lee SW, Sung YC. IL-12 provides proliferation and survival signals to murine CD4+ T cells through phosphatidylinositol 3-kinase/Akt signaling pathway. J Immunol 2002;169:3637–43.
8. Wesa A, Kalinski P, Tatsumi T, et al. Polarized type-1 dendritic cells (DC1) producing high levels of IL-12 family members rescue patient Th1-type anti-melanoma CD4+ T cell responses *in vitro*. J Immunother 2007;30:75–82.
9. Kobayashi M, Fitz L, Ryan M, et al. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. J Exp Med 1989;170:827–45.
10. Stern AS, Podlaski FJ, Hulmes JD, et al. Purification to homogeneity and partial characterization of cytotoxic lymphocyte maturation factor from human B-lymphoblastoid cells. Proc Natl Acad Sci U S A 1990;87:6808–12.
11. Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat Rev Immunol 2003;3:133–46.
12. Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. Science 1993;260:547–9.
13. Chan CW, Crafton E, Fan HN, et al. Interferon-producing killer dendritic cells provide a link between innate and adaptive immunity. Nat Med 2006;12:207–13.
14. Trinchieri G, Pflanz S, Kastelein RA. The IL-12 family of heterodimeric cytokines: new players in the regulation of T cell responses. Immunity 2003;19:641–4.
15. Ma X, Chow JM, Gri G, et al. The interleukin 12 p40 gene promoter is primed by interferon γ in monocytic cells. J Exp Med 1996;183:147–57.
16. Kuwajima S, Sato T, Ishida K, Tada H, Tezuka H, Ohteki T. Interleukin-15-dependent crosstalk between conventional and plasmacytoid dendritic cells is essential for CpG-induced immune activation. Nat Immunol 2006;7:740–6.
17. Schulz O, Edwards AD, Schito M, et al. CD40 triggering of heterodimeric IL-12 p70 production by dendritic cells *in vivo* requires a microbial priming signal. Immunity 2000;13:453–62.
18. Friedmann E, Hauben E, Maylandt K, et al. SPPL2a and SPPL2b promote intramembrane proteolysis of TNFα in activated dendritic cells to trigger IL-12 production. Nat Cell Biol 2006;8:843–8.
19. Bekeredjian-Ding I, Roth SI, Gilles S, et al. T cell-independent, TLR-induced IL-12p70 production in primary human monocytes. J Immunol 2006;176:7438–46.
20. Napolitani G, Rinaldi A, Bertoni F, Sallusto F, Lanzavecchia A. Selected toll-like receptor agonist combinations synergistically trigger a T helper type-1-polarizing program in dendritic cells. Nat Immunol 2005;6:769–76.
21. D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, Trinchieri G. Interleukin 10 (IL-10) inhibits human lymphocyte interferon γ-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. J Exp Med 1993;178:1041–8.

22. Du C, Sriram S. Mechanism of inhibition of LPS-induced IL-12p40 production by IL-10 and TGF-β in ANA-1 cells. J Leukoc Biol 1998;64:92–7.
23. Ling P, Gately MK, Gubler U, et al. Human IL-12 p40 homodimer binds to the IL-12 receptor but does not mediate biologic activity. J Immunol 1995;154: 116–27.
24. Khader SA, Partida-Sanchez S, Bell G, et al. Interleukin 12p40 is required for dendritic cell migration and T cell priming after *Mycobacterium tuberculosis* infection. J Exp Med 2006;203:1805–15.
25. Peng SL. The T-box transcription factor T-bet in immunity and autoimmunity. Cell Mol Immunol 2006; 3:87–95.
26. Mullen AC, High FA, Hutchins AS, et al. Role of T-bet in commitment of TH1 cells before IL-12-dependent selection. Science 2001;292:1907–10.
27. Murphy KM, Reiner SL. The lineage decisions of helper T cells. Nat Rev Immunol 2002;2:933–44.
28. Usui T, Preiss JC, Kanno Y, et al. T-bet regulates TH1 responses through essential effects on GATA-3 function rather than on IFNG gene acetylation and transcription. J Exp Med 2006;203:755–66.
29. Hunter CA. New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. Nat Rev Immunol 2005;5:521–31.
30. Pflanz S, Timans JC, Cheung J, et al. IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4(+) T cells. Immunity 2002;16:779–90.
31. Takeda A, Hamano S, Yamanaka A, et al. Cutting edge: role of IL-27/WSX-1 signaling for induction of T-bet through activation of STAT1 during initial Th1 commitment. J Immunol 2003;170:4886–90.
32. Oppmann B, Lesley R, Blom B, et al. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity 2000;13:715–25.
33. Villarino A, Hibbert L, Lieberman L, et al. The IL-27R (WSX-1) is required to suppress T cell hyperactivity during infection. Immunity 2003;19:645–55.
34. Batten M, Li J, Yi S, et al. Interleukin 27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17-producing T cells. Nat Immunol 2006;7:929–36.
35. Brunda MJ, Luistro L, Warrier RR, et al. Antitumor and antimetastatic activity of interleukin 12 against murine tumors. J Exp Med 1993;178:1223–30.
36. Tahara H, Ze H, Storkus WJ, et al. Fibroblasts genetically engineered to secrete interleukin-12 can suppress tumor growth *in vivo* and induce antitumor immunity to a murine melanoma. Cancer Res 1994; 54:182–9.
37. Nastala CL, Edington HD, McKinney TG, et al. Recombinant IL-12 administration induces tumor regression in association with IFN-γ production. J Immunol 1994;153:1697–706.
38. Voest EE, Kenyon BM, O'Reilly MS, Truitt G, D'Amato RJ, Folkman J. Inhibition of angiogenesis *in vivo* by interleukin 12. J Natl Cancer Inst 1995;87:581–6.
39. Coughlin CM, Salhany KE, Wysocka M, et al. Interleukin-12 and interleukin-18 synergistically induce murine tumor regression which involves inhibition of angiogenesis. J Clin Invest 1998;101:1441–52.
40. Coughlin CM, Salhany KE, Gee MS, et al. Tumor cell responses to IFNγ affect tumorigenicity and response to IL-12 therapy and antiangiogenesis. Immunity 1998; 9:25–34.
41. Sgadari C, Angiolillo AL, Tosato G. Inhibition of angiogenesis by interleukin-12 is mediated by the interferon-inducible protein 10. Blood 1996;87:3877–82.
42. Kanegane C, Sgadari C, Kanegane H, et al. Contribution of the CXC chemokines IP-10 and Mig to the antitumor effects of IL-12. J Leukoc Biol 1998;64: 384–92.
43. Sgadari C, Farber JM, Angiolillo AL, et al. Mig, the monokine induced by interferon-γ, promotes tumor necrosis *in vivo*. Blood 1997;89:2635–43.
44. Dias S, Boyd R, Balkwill F. IL-12 regulates VEGF and MMPs in a murine breast cancer model. Int J Cancer 1998;78:361–5.
45. Mitola S, Strasly M, Prato M, et al. IL-12 regulates an endothelial cell-lymphocyte network: effect on metalloproteinase-9 production. J Immunol 2003;171: 3725–33.
46. Ruegg C, Yilmaz A, Bieler G, et al. Evidence for the involvement of endothelial cell integrin αVβ3 in the disruption of the tumor vasculature induced by TNF and IFN-γ. Nat Med 1998;4:408–14.
47. Strasly M, Cavallo F, Geuna M, et al. IL-12 inhibition of endothelial cell functions and angiogenesis depends on lymphocyte-endothelial cell cross-talk. J Immunol 2001;166:3890–9.
48. Cavallo F, Quaglino E, Cifaldi L, et al. Interleukin 12-activated lymphocytes influence tumor genetic programs. Cancer Res 2001;61:3518–23.
49. Yao L, Sgadari C, Furuke K, et al. Contribution of natural killer cells to inhibition of angiogenesis by interleukin-12. Blood 1999;93:1612–21.
50. Colombo MP, Trinchieri G. Interleukin-12 in anti-tumor immunity and immunotherapy. Cytokine Growth Factor Rev 2002;13:155–68.
51. Cavallo F, Signorelli P, Giovarelli M, et al. Antitumor efficacy of adenocarcinoma cells engineered to produce interleukin 12 (IL-12) or other cytokines compared with exogenous IL-12. J Natl Cancer Inst 1997; 89:1049–58.
52. Zitvogel L, Robbins PD, Storkus WJ, et al. B7.1 costimulation markedly enhances IL 12-mediated antitumor immunity *in vivo*. Eur J Immunol 1996;26: 1335–41.
53. Cui J, Shin T, Kawano T, et al. Requirement for Vα14 NKT cells in IL-12-mediated rejection of tumors. Science 1997;278:1623–6.
54. Atkins MB, Robertson MJ, Gordon M, et al. Phase I evaluation of intravenous recombinant human interleukin 12 in patients with advanced malignancies. Clin Cancer Res 1997;3:409–17.
55. Robertson MJ, Cameron C, Atkins MB, et al. Immunological effects of interleukin 12 administered by bolus intravenous injection to patients with cancer. Clin Cancer Res 1999;5:9–16.
56. Bajetta E, Del Vecchio M, Mortarini R, et al. Pilot study of subcutaneous recombinant human interleukin 12 in metastatic melanoma. Clin Cancer Res 1998; 4:75–85.
57. Mortarini R, Borri A, Tragni G, et al. Peripheral burst of tumor-specific cytotoxic T lymphocytes and infiltration of metastatic lesions by memory CD8+ T cells in melanoma patients receiving interleukin 12. Cancer Res 2000;60:3559–68.
58. Motzer RJ, Rakhit A, Schwartz LH, et al. Phase I trial of subcutaneous recombinant human interleukin-12 in patients with advanced renal cell carcinoma. Clin Cancer Res 1998;4:1183–91.
59. Rook AH, Wood GS, Yoo EK, et al. Interleukin-12 therapy of cutaneous T-cell lymphoma induces lesion regression and cytotoxic T-cell responses. Blood 1999;94:902–8.
60. Gollob JA, Mier JW, Veenstra K, et al. Phase I trial of twice-weekly intravenous interleukin 12 in patients with metastatic renal cell cancer or malignant melanoma: ability to maintain IFN-γ induction is associated with clinical response. Clin Cancer Res 2000;6: 1678–92.
61. Motzer RJ, Rakhit A, Thompson JA, et al. Randomized multicenter phase II trial of subcutaneous recombinant human interleukin-12 versus interferon-α 2a for patients with advanced renal cell carcinoma. J Interferon Cytokine Res 2001;21:257–63.
62. Lenzi R, Rosenblum M, Verschraegen C, et al. Phase I study of intraperitoneal recombinant human interleukin 12 in patients with Mullerian carcinoma, gastrointestinal primary malignancies, and mesothelioma. Clin Cancer Res 2002;8:3686–95.
63. Weiss GR, O'Donnell MA, Loughlin K, et al. Phase 1 study of the intravesical administration of recombinant human interleukin-12 in patients with recurrent superficial transitional cell carcinoma of the bladder. J Immunother 2003;26:343–8.
64. Portielje JE, Lamers CH, Kruit WH, et al. Repeated administrations of interleukin (IL)-12 are associated with persistently elevated plasma levels of IL-10 and declining IFN-γ, tumor necrosis factor-α, IL-6, and IL-8 responses. Clin Cancer Res 2003;9:76–83.
65. Wadler S, Levy D, Frederickson HL, et al. A phase II trial of interleukin-12 in patients with advanced cervical cancer: clinical and immunologic correlates. Eastern Cooperative Oncology Group study E1E96. Gynecol Oncol 2004;92:957–64.
66. van Herpen CM, van der Laak JA, de Vries IJ, et al. Intratumoral recombinant human interleukin-12 administration in head and neck squamous cell carcinoma patients modifies locoregional lymph node architecture and induces natural killer cell infiltration in the primary tumor. Clin Cancer Res 2005;11: 1899–909.
67. Little RF, Pluda JM, Wyvill KM, et al. Activity of subcutaneous interleukin-12 in AIDS-related Kaposi sarcoma. Blood 2006;107:4650–7.
68. Duvic M, Sherman ML, Wood GS, et al. A phase II open-label study of recombinant human interleukin-12 in patients with stage IA, IB, or IIA mycosis fungoides. J Am Acad Dermatol 2006;55:807–13.
69. Younes A, Pro B, Robertson MJ, et al. Phase II clinical trial of interleukin-12 in patients with relapsed and refractory non-Hodgkin's lymphoma and Hodgkin's disease. Clin Cancer Res 2004;10:5432–8.
70. Lee P, Wang F, Kuniyoshi J, et al. Effects of interleukin-12 on the immune response to a multipeptide vaccine for resected metastatic melanoma. J Clin Oncol 2001;19:3836–47.
71. Cebon J, Jäger E, Shackleton MJ, et al. Two phase I studies of low dose recombinant human IL-12 with Melan-A and influenza peptides in subjects with advanced malignant melanoma. Cancer Immun 2003;3:7.
72. Peterson AC, Harlin H, Gajewski TF. Immunization with Melan-A peptide-pulsed peripheral blood mononuclear cells plus recombinant human interleukin-12 induces clinical activity and T-cell responses in advanced melanoma. J Clin Oncol 2003;21: 2342–8.
73. Gollob JA, Veenstra KG, Parker RA. Phase I trial of concurrent twice-weekly recombinant human interleukin-12 plus low-dose IL-2 in patients with melanoma or renal cell carcinoma. J Clin Oncol 2003;21: 2564–73.
74. Alatrash G, Hutson TE, Molto L, et al. Clinical and immunologic effects of subcutaneously administered interleukin-12 and interferon alfa-2b: phase I trial of patients with metastatic renal cell carcinoma or malignant melanoma. J Clin Oncol 2004;22:2891–900.
75. Parihar R, Nadella P, Lewis A, et al. A phase I study of interleukin 12 with trastuzumab in patients with human epidermal growth factor receptor-2-overexpressing malignancies: analysis of sustained interferon γ production in a subset of patients. Clin Cancer Res 2004;10:5027–37.
76. Sangro B, Melero I, Qian C, Prieto J. Gene therapy of cancer based on interleukin 12. Curr Gene Ther 2005;5:573–81.
77. Kang WK, Park C, Yoon HL, et al. Interleukin 12 gene therapy of cancer by peritumoral injection of transduced autologous fibroblasts: outcome of a phase I study. Hum Gene Ther 2001;12:671–84.
78. Sun Y, Jurgovsky K, Moller P. Vaccination with IL-12 gene-modified autologous melanoma cells: preclinical results and a first clinical phase I study. Gene Ther 1998;5:481–90.
79. Heinzerling L, Burg G, Dummer R, et al. Intratumoral injection of DNA encoding human interleukin-12 into patients with metastatic melanoma: clinical efficacy. Hum Gene Ther 2005;16:35–48.
80. Triozzi PI, Strong TV, Bucy RP, et al. Intratumoral administration of a recombinant Canarypox virus expressing interleukin 12 in patients with metastatic melanoma. Hum Gene Ther 2005;16:91–100.
81. Mazzolini G, Alfaro C, Sangro B, et al. Intratumoral injection of dendritic cells engineered to secrete interleukin-12 by recombinant adenovirus in patients with metastatic gastrointestinal carcinomas. J Clin Oncol 2005;23:999–1010.

82. Rakhit A, Yeon MM, Ferrante J, et al. Down-regulation of the pharmacokinetic-pharmacodynamic response to interleukin-12 during long-term administration to patients with renal cell carcinoma and evaluation of the mechanism of this "adaptive response" in mice. Clin Pharmacol Ther 1999;65:615–29.
83. Beyer M, Schultze JL. Regulatory T cells in cancer. Blood 2006;108:804–11.
84. Nagai H, Hara I, Horikawa T, et al. Elimination of CD4(+) T cells enhances anti-tumor effect of locally secreted interleukin-12 on B16 mouse melanoma and induces vitiligo-like coat color alteration. J Invest Dermatol 2000;115:1059–64.
85. Pasare C, Medzhitov R. Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells. Science 2003;299:1033–6.
86. Lutsiak ME, Semnani RT, De Pascalis R, et al. Inhibition of CD4(+)25(+) T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide. Blood 2005;105:2862–8.
87. Dannull J, Su Z, Rizzieri D, et al. Enhancement of vaccine-mediated antitumor immunity in cancer patients after depletion of regulatory T cells. J Clin Invest 2005;115:3623–33.
88. Meyaard L, Hovenkamp E, Otto SA, Miedema F. IL-12-induced IL-10 production by human T cells as a negative feedback for IL-12-induced immune responses. J Immunol 1996;156:2776–82.
89. Jing N, Tweardy DJ. Targeting STAT3 in cancer therapy. Anticancer Drugs 2005;16:601–7.
90. Eyles JL, Metcalf D, Grusby MJ, et al. Negative regulation of interleukin-12 signaling by suppressor of cytokine signaling-1. J Biol Chem 2002;277:43735–40.
91. Yamamoto K, Yamaguchi M, Miyasaka N, Miura O. SOCS-3 inhibits IL-12-induced STAT4 activation by binding through its SH2 domain to the STAT4 docking site in the IL-12 receptor β2 subunit. Biochem Biophys Res Commun 2003;310:1188–93.
92. Hisada M, Kamiya S, Fujita K, et al. Potent antitumor activity of interleukin-27. Cancer Res 2004;64:1152–6.
93. Overwijk WW, de Visser KE, Tirion FH, et al. Immunological and antitumor effects of IL-23 as a cancer vaccine adjuvant. J Immunol 2006;176:5213–22.
94. Langowski JL, Zhang X, Wu L, et al. IL-23 promotes tumour incidence and growth. Nature 2006;442:461–5.
95. Motzer RJ, Bukowski RM. Targeted therapy for metastatic renal cell carcinoma. J Clin Oncol 2006;24:5601–8.

Clinical Cancer Research

Interleukin-12: Biological Properties and Clinical Application

Michele Del Vecchio, Emilio Bajetta, Stefania Canova, et al.

Clin Cancer Res 2007;13:4677-4685.