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.

**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.

**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 APC 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 up to 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 naive 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 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 TH1/TH17 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 TH1/TH17 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
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. Two of the most relevant factors were soon identified as the IFN-γ–inducible genes IP-10 and Mig. Initial results obtained by Sgadari et al. 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. In addition, administration of neutralizing antibodies to IP-10 and Mig substantially reduced the antitumor effects of IL-12.

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. 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. Furthermore, IL-12–induced IFN-γ reduces activation of integrin αvβ3 on endothelial cells, leading to decreased endothelial cell adhesion and survival. The relevance of lymphocyte-endothelial cells cross-talks has been investigated by Strasly et al. They found that IL-12 activates an antiangiogenic program in lymphocytes that leads to the production of IP-10 and Mig. 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. 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 angiopeptin 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. 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.
Preclinical Models of IL-12 as Antitumor Agent

The antitumor and antimitotic 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 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-126-35 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.

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-126-35 and influenza matrix58-66 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>
<thead>
<tr>
<th>Tumors</th>
<th>Route of administration</th>
<th>Patients (n)</th>
<th>Objective response</th>
<th>Immune modulation</th>
<th>Angiogenesis-related effects</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Different solid tumors*</td>
<td>i.v.</td>
<td>40</td>
<td>5%</td>
<td>Dose-dependent ↑ sIFN-γ; peak at 24-48 h after IL-12; CD4+/CD8+ and CD16+ cells;</td>
<td>Urine bFGF in two of three patients with minor responses</td>
<td>(54, 55)</td>
</tr>
<tr>
<td>Melanoma*</td>
<td>s.c.</td>
<td>10</td>
<td>0% (three minor responses)</td>
<td>sIFN-γ within 24 h after the first IL-12 injection; IL-10 during the second cycle</td>
<td></td>
<td>(56, 57)</td>
</tr>
<tr>
<td>Renal cell carcinoma*</td>
<td>s.c.</td>
<td>51</td>
<td>2%</td>
<td>sIFN-γ with peak level at 24 h after the first maintenance dose</td>
<td></td>
<td>(58)</td>
</tr>
<tr>
<td>Cutaneous T cell lymphoma*</td>
<td>s.c. or intralesonally</td>
<td>10</td>
<td>56%</td>
<td>CD8+ and/or TIA-1+ T cells in skin biopsy</td>
<td></td>
<td>(59)</td>
</tr>
<tr>
<td>Melanoma, renal cell carcinoma*</td>
<td>i.v.</td>
<td>28</td>
<td>3%</td>
<td>Induction of IFN-γ, IL-15 and IL-18, maintained in patients with tumor regression or prolonged disease stabilization</td>
<td></td>
<td>(60)</td>
</tr>
<tr>
<td>Renal cell carcinoma †</td>
<td>s.c.</td>
<td>30</td>
<td>7%</td>
<td>sIFN-γ, IL-10 and neopterin, maintained in cycle 2</td>
<td></td>
<td>(61)</td>
</tr>
<tr>
<td>Abdominal tumors*</td>
<td>i.p.</td>
<td>29</td>
<td>7%</td>
<td>Peritoneal CD3+ and CD14+ cells</td>
<td>Urine bFGF and VEGF in tumor</td>
<td>(62)</td>
</tr>
<tr>
<td>Bladder cancer*</td>
<td>Intravesical</td>
<td>15</td>
<td>0%</td>
<td>No urine/serum</td>
<td>IFN-γ and IP-10 transcripts in peritoneal exudate cells</td>
<td>(63)</td>
</tr>
<tr>
<td>Renal cell carcinoma*</td>
<td>s.c.</td>
<td>26</td>
<td>NA</td>
<td>Dose-dependent ↑ sIFN-γ, TNF-α, IL-10, IL-6 and IL-8 at first injection Lymphopenia: further ↑ IL-10 during treatment</td>
<td></td>
<td>(64)</td>
</tr>
<tr>
<td>Cervical carcinoma †</td>
<td>i.v.</td>
<td>34</td>
<td>3%</td>
<td>Lymphoproliferative responses to HPV 16 E4, E6 and E7 peptides</td>
<td></td>
<td>(65)</td>
</tr>
<tr>
<td>Head-neck carcinoma*</td>
<td>Intratumoral</td>
<td>10</td>
<td>ND</td>
<td>CD56+ NK cells in the primary tumor</td>
<td></td>
<td>(66)</td>
</tr>
<tr>
<td>AIDS-related Kaposi's sarcoma*</td>
<td>s.c.</td>
<td>34</td>
<td>50% (71% at highest doses)</td>
<td>sIFN-γ after first dose, persisting after week 4</td>
<td>sIP-10 after the first dose, persisting after week 4</td>
<td>(67)</td>
</tr>
<tr>
<td>Mycosis fungoides †</td>
<td>s.c.</td>
<td>23</td>
<td>43%</td>
<td></td>
<td>sVEGF and sbFGF in 37% of patients</td>
<td>(68)</td>
</tr>
<tr>
<td>Non-Hodgkin's lymphoma and Hodgkin's lymphoma †</td>
<td>i.v. or s.c.</td>
<td>42</td>
<td>21%</td>
<td></td>
<td></td>
<td>(69)</td>
</tr>
</tbody>
</table>

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.
**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 the injected sites in four patients and at noninjected distant sites in one melanoma patient 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

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

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

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.
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 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


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 p2 subunit. Biochem Biophys Res Commun 2003;310:1188–93.


Interleukin-12: Biological Properties and Clinical Application

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


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