

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.

For more information visit www.intechopen.com



Interleukin 12: Stumbling Blocks and Stepping Stones to Effective Anti-Tumor Therapy

Hollie J. Pegram¹, Alena A. Chekmasova¹,
Gavin H. Imperato¹ and Renier J. Brentjens^{1,2,3,*}

¹Department of Medicine, Memorial Sloan-Kettering Cancer Center,

²Center for Cell Engineering, Memorial Sloan-Kettering Cancer Center,

³Molecular Pharmacology and Chemistry Program,

Memorial Sloan-Kettering Cancer Center,

USA

1. Introduction

Interleukin-12 (IL-12) is a heterodimeric pro-inflammatory cytokine long recognized to have properties capable of mediating immune effector functions in a manner compatible to enhancing endogenous anti-tumor immune responses. For this reason the cytokine has garnered significant interest from investigators in the field of immune mediated anti-cancer therapies. While the exact mechanisms whereby IL-12 mediates pro-inflammatory endogenous anti-tumor responses remains to be fully elucidated, pre-clinical murine tumor models demonstrate unequivocal anti-tumor benefit mediated by IL-12 (1-5). Preclinical studies demonstrate that the mechanisms of IL-12 mediated anti-tumor endogenous immune responses seen in these models are likely to be complex and multifactorial. Beyond the ability of IL-12 to induce an inflammatory Th1 CD4⁺ T cell response, studies have demonstrated the ability of IL-12 to enhance CD8⁺ T cell cytotoxicity. Additionally, preclinical studies have shown IL-12 to recruit and activate innate cytotoxic NK cells and modulate a pro-inflammatory macrophage phenotype. Further, studies have shown that T cell secretion of IFN γ mediated by IL-12 may reverse T cell anergy and confer effector T cell resistance to immune suppressive Tregs. The ability of IL-12 to activate the adaptive as well as the innate immune systems, but also further modulate the otherwise immune-hostile tumor microenvironment, suggests that the cytokine may serve as a potent immunotherapeutic agent. Significantly, pre-clinical murine tumor models have largely validated these predictions. These promising pre-clinical studies consequently spurred on a series of clinical trials treating patients with a variety of tumors with intravenous infusions of recombinant IL-12. Unfortunately, these studies have yielded only modest tumor responses in the context of associated severe and unforeseen toxicities. The IL-12 related toxicities seen in these early clinical trials served to markedly dampen enthusiasm for this cytokine as a potential anti-tumor therapeutic reagent in the clinical setting. However, subsequent clinical trials conducted utilizing direct infusion of IL-12 into accessible tumor sites has resulted in

* Corresponding Author

promising anti-tumor responses in the absence of toxicities induced by systemic infusion seen in earlier studies. Thus, based on these clinical trials, the potent anti-tumor effects of IL-12 can best be harnessed by restricting its administration directly into the tumor microenvironment. Therefore, optimal utilization of the anti-tumor efficacy of IL-12 may be realized utilizing novel approaches whereby the cytokine is delivered directly to the site of the tumor with limited systemic distribution to avoid previously observed toxicities. In this chapter, we review the biology of IL-12 and the predicted mechanisms whereby this cytokine may mediate anti-tumor endogenous immune responses. We further discuss pre-clinical studies to support the utilization of IL-12 in cancer therapy as well as clinical trial data, which, in part, have tempered enthusiasm for IL-12 as an effective anti-tumor reagent in the clinical setting due to associated toxicities. Finally, we present and discuss previously published approaches to overcome systemic toxicity through targeted delivery of IL-12 directly into the tumor microenvironment.

2. Basic biology and immune stimulatory effects of IL-12

IL-12 is biologically functional as a heterodimeric molecule consisting of an α and β chain, where covalently-linked p35 and p40 subunits together form the active molecule, IL-12p70 (6-8). The p35 subunit is expressed ubiquitously but only phagocytic cells produce the p40 subunit, therefore functional IL-12p70 is only produced by activated antigen-presenting cells (APCs), neutrophils and macrophages (9). The IL-12 receptor (IL-12R) is composed of two subunits, β 1 and β 2, and is expressed predominantly on dendritic cells (DCs), T cells and natural killer (NK) cells. The IL-12R mediates signal transduction through the Janus kinases (JAKs) but these pathways will not be discussed here (10).

Initially, IL-12 was described as “Natural killer-stimulating factor” and “cytotoxic lymphocyte maturation factor” and has since been reported to have important effects on the generation of an adaptive immune response (6, 11). It is a potent activator of NK cells, with IL-12 stimulation resulting in enhanced NK cell mediated cytotoxicity (9). The effects of IL-12 on T cells include enhanced cytotoxicity and CD4⁺ T cell differentiation into type-1 helper T cells (Th1) (12-14). It has also been demonstrated that IL-12 could provide “signal 3” for T cell activation, where signal 2 provides co-stimulation and signal 3 upregulates the expression of the lytic protein Granzyme B, leading to increased cytotoxic effector function and overcoming tolerance (15, 16). IL-12 also mediates significant effects on T cell proliferation. In a murine model of adoptive transfer, OT-1 T cells were inoculated into an irradiated syngeneic mouse and exhibited increased homeostatic proliferation when supported with injection of IL-12 (17). Further studies have demonstrated that T cell expansion was augmented with IL-12 as a result of decreased apoptosis. This was found to be due to decreased Fas expression, increased expression of anti-apoptotic FLIP proteins and inhibition of caspase activation (18). Additionally, it has been reported that IL-12 is important in the fate of CD8⁺ T cells where IL-12 promotes differentiation into functional effector cells and inhibits memory T cell formation (19).

An additional, but no less important outcome following IL-12 production is the induction of Interferon (IFN)- γ from B, T and NK cells (20-22). IL-12 has positive feedback loops whereby IL-12 stimulates DCs to produce more IL-12, thereby stimulating IFN γ production resulting in additional IL-12 produced by monocytes (23, 24). This IFN γ is able to further activate

innate and adaptive immune systems as well as influencing the tumor microenvironment, as discussed below. Many of these IL-12 mediated anti-tumor effects are abrogated upon neutralization of IFN γ thereby demonstrating the importance of this pro-inflammatory cytokine in IL-12 mediated immune stimulation (3, 4, 25).

3. IL-12 in the tumor microenvironment

The wide-ranging effects of IL-12 have profound impacts upon the tumor microenvironment; acting directly on tumor cells, influencing the surrounding tumor stroma/structure, and modulating infiltrating immune cells. These effects, detailed below, mediate the recruitment of lymphocytes, activation of tumor infiltrating lymphocytes, as well as direct effects on tumor cells to decrease angiogenesis which combine to result in tumor eradication or inhibition.

Direct effects of IL-12 on tumor cells may include the ability of IL-12 to up-regulate expression of molecules that induce immune recognition and death of tumor cells. It has been documented that adenoviral mediated expression of IL-12 in human osteosarcoma cells or chemo-resistant breast cancer cells increased expression of Fas, and subsequent apoptosis of tumor cells (26). This was postulated to be a function of the IL-12R β 1 chain activating NF- κ B, a signaling effect that is thought to be absent in lymphocytes ensuring that IL-12 stimulation of T and NK cells does not result in Fas up-regulation or apoptosis. In a mouse model of mammary adenocarcinoma, it was demonstrated that IL-12 induced IFN γ led to increased surface MHC expression on tumor cells (2), increasing the presentation of tumor-associated antigens to the immune system and resulting in increased endogenous anti-tumor immune responses.

IL-12 is widely reported to increase IFN γ expression, which is responsible for mediating effects directly on the tumor cells, including upregulation of inducible nitric oxide synthase (iNOS) and indolamine 2,3-dioxygenase (IDO) genes, as well as increased MHC expression. Utilizing a murine model of fibrosarcoma, systemic injection of IL-12 was found to induce IFN γ , which in turn mediated increased expression of IDO and iNOS mRNA by tumor cells (3). The products of these genes may slow the growth of tumor cells. IDO is thought to influence tumor growth through the deprivation of tryptophan (27). This was also demonstrated in a study employing a murine model of spontaneous breast cancer treated with systemic IL-12 injection (1).

An important tumor microenvironment modulatory effect by IL-12 is the inhibition of angiogenesis. Folkman and others have elegantly demonstrated the anti-angiogenic role of IL-12 against fibroblast growth factor-induced corneal neo-vascularization. This effect was abrogated upon neutralization of IFN- γ and a downstream effector, IP-10, implicating the latter as the mediator of this effect (28-31). IL-12 has further been shown to mediate down-regulation of pro-angiogenic gene vascular endothelial growth factor (VEGF)-C, as well as the pro-angiogenic proteins, VEGF and basic fibroblast growth factor (BFGF) on tumor cells and supporting fibroblast cells (32, 33). There is also evidence to suggest that NK cells and CD8⁺ T cells may contribute to the IL-12 mediated anti-angiogenic effect in some models (34, 35). Cytotoxic T and NK cells were shown to directly lyse epithelial cells, therefore contributing to inhibition of neo-vascularization. It is likely that both secreted factors, such as IP-10 from fibroblasts, as well as direct effects mediated by T and NK cells contribute to the overall anti-angiogenic effects of IL-12.

IL-12 may directly increase the expression of lymphocyte adhesion molecules within tumors, thereby increasing the infiltration of immune effectors into the tumor. For example, treatment of a poorly immunogenic mammary adenocarcinoma with IL-12 resulted in increased vascular cell adhesion molecule (VCAM)-1 expression within tumors (2). This result was also reported in a murine model of breast cancer (1). In addition, a recent report documented IL-12 mediated activation of lymphoid-tissue inducer (LTi) cells, which led to the up-regulation of adhesion molecules and increased leukocyte infiltration (36). Utilizing a murine model of melanoma, it was found that LTi cells expressing the NK cell receptor NKp46, were responsible for up-regulating both VCAM-1 and inter-cellular adhesion molecule (ICAM)-1 within the tumor microenvironment. Up-regulated expression of VCAM-1 has been demonstrated to increase the migration of lymphocytes, therefore allowing increased lymphocytic infiltration into IL-12 treated tumors (37).

A consequence of increased expression of adhesion molecules is the increased infiltration of tumors with immune effector cells, as reported in several studies wherein solid tumors were treated with IL-12. In murine models of mammary adenocarcinoma, lung alveolar carcinoma, fibrosarcoma and spontaneous breast cancer, tumor masses were infiltrated with lymphocytes following systemic IL-12 treatment (1-4, 25, 38). Infiltrating cells were NK cells, CD8⁺ T cells, CD4⁺ T cells, and macrophages, depending on the model utilized. Infiltration of lymphocytes is highly important for IL-12 mediated tumor regression, as depletion of CD4 or CD8 T cells prior to treatment abrogated the anti-tumor response in several murine models (2, 4, 5).

IL-12 has further been shown to mediate activation of tumor infiltrating lymphocytes (TILs). In the context of minimal residual disease following transplant in a murine model of lymphoma, IL-12 treatment was shown to activate splenocytes, as noted by up-regulation of CD25 (39). Subsequent studies have demonstrated that IL-12 therapy mediates substantial increase in Granzyme B expression, increased proportion of IFN γ secreting CD8⁺ T cells, and greater levels of IFN γ secretion in previously quiescent tumor infiltrating CD8⁺ T cells (38). A recent report has identified the specificity of endogenous T cells activated by IL-12 in a murine model of melanoma (40). This study demonstrated that IL-12 therapy stimulated a protective CD8⁺ T cell response, where T cells were specific for multiple tumor associated stromal antigens. Other cells activated in response to IL-12 include B cells, as demonstrated by an increase in tumor-reactive antibodies following IL-12 treatment (2). However, the clinical relevance of these tumor reactive antibodies currently remain unclear.

IL-12 may reverse the anergic state of T cells present within the tumor microenvironment. In elegant studies, primary human lung biopsy samples were transplanted into SCID mice and treated with intra-tumoral injection of IL-12 microbeads (41-43). IL-12 therapy was found to mediate regression of tumors, which was dependent on the reactivation of CD4⁺ T cells within the tumor. These cells were stimulated to proliferate, secrete IFN γ , and mediate complete eradication of the tumor. Additionally, anergy induced in murine CD4⁺ T cells by regulatory T cells can be overcome with the addition of IL-12 (44). The authors demonstrate that IL-12 mediates effects on the CD4⁺ effector T cell allowing proliferation and IFN γ secretion despite the presence of suppressive Tregs.

IL-12 has further been demonstrated to impact regulatory cell populations present in the tumor microenvironment. Using a murine model of lung carcinoma, it was demonstrated

that IL-12 microsphere therapy resulted in a reduction of CD4⁺ CD25⁺ suppressor T cells (38). This study reported an IFN γ dependent induction of apoptosis in the suppressive T cell subset. Relieving tumor resident T cells of suppressive factors may allow the generation of effective endogenous anti-tumor responses. Recent reports have demonstrated that IL-12 can also inhibit the expansion of regulatory T cells (45). IL-12 was found to inhibit the expansion of Tregs *in vitro* and *in vivo* in a murine model of lymphoma. Inhibition of Treg expansion was shown to occur in a IFN γ dependent fashion as IL-12 did not effect Treg expansion in IFN γ receptor deficient mice.

Tumor-associated macrophages (TAMs) play a major role in promoting tumor growth and metastasis and in suppressing the antitumor immune response. Such local immune dysfunction is recognized as one of the major barriers to cancer immunotherapy (46). Macrophages are also functionally plastic, meaning that they can convert between functional states (47). In particular, it has been reported that TAMs can be converted from a M2 suppressive phenotype to an M1 inflammatory phenotype following treatment of tumor-bearing mice with IL-12 containing microspheres (48, 49). Moreover, tumor lesions treated with tumor targeted T cells engineered to secrete IL-12 were infiltrated with activated M1 type of macrophages that were not found in tumors upon T cell therapy without IL-12. The accumulation of activated macrophages was critical to the antitumor immune response as depletion of these macrophages abolished the anti-tumor response (50).

4. Preclinical studies investigating the anti-tumor therapeutic potential of IL-12

Early studies investigating the utilization of IL-12 as an anti-cancer therapeutic agent provided encouraging results, conveying the anti-tumor potential of this powerful cytokine. Intra-tumoral and systemic administration of this cytokine demonstrated marked tumor regression in several murine models of cancer. These include a poorly immunogenic mammary adenocarcinoma, lung alveolar carcinoma, fibrosaroma, spontaneous breast cancer, ovarian carcinoma, lymphoma, renal cell carcinoma, as well as a model of pulmonary metastasis of melanoma (1-5, 38, 39, 51). IL-12 was shown to mediate disease eradication in primary tumors, as well as eradication of metastasis following surgical removal of primary tumors (2). Additionally, using a murine model of minimal residual lymphoma disease following transplant, IL-12 was shown to eradicate disease following transplant without affecting lympho-hematopoietic recovery (39). Significantly, IL-12 exerts anti-tumor effects and mediates tumor regression in models of early, intermediate and late stage disease, illustrating the efficacy of IL-12 anti-cancer therapy even in the setting of advanced disease (51). These studies provided rational for the use of this cytokine in cancer therapy and several clinical approaches to utilize IL-12 in cancer therapy have been developed as discussed below.

Other therapeutic strategies to deliver IL-12 to the tumor microenvironment include the sustained release of cytokine using nanoparticles. Anti-tumor efficacy has been demonstrated in a murine model of mammary carcinoma, wherein IL-12 microsphere treatment was found to result in NK cell mediated anti-tumor effects (52). Combination therapy involving IL-12 and TNF α receptor microspheres was found to lead to superior anti-tumor function with the recruitment of CD8⁺ T cells. Additional studies of IL-12 microsphere treatment utilized a combination of IL-12 and GM-CSF, which mediated

regression of lung alveolar carcinomas in a murine model (38, 53). This study reported IL-12 mediated effects including reactivation of tumor resident T cells as well as predicted apoptosis of regulatory T cells. The anti-tumor efficacy of polymer-mediated IL-12 delivery has also been tested in murine models of malignant glioma and disseminated ovarian cancer (54, 55). These studies validate the nanoparticle mediated delivery approach, demonstrating a sustained release and therapeutic efficacy, whilst contributing to the understanding of the mechanisms of IL-12 mediated anti-tumor efficacy.

As an alternative approach to systemic infusion of IL-12, specific delivery of IL-12 directly into the tumor site may be achieved through gene therapy strategies. One such approach utilizes adenoviral vector mediated delivery of the IL-12 gene with subsequent expression and secretion of IL-12 by the infected cell. In this manner, direct intra-tumoral injection of adenovirus encoding the IL-12 gene resulted in anti-tumor responses in murine models of melanoma, laryngeal squamous cell carcinoma, glioma, renal cell carcinoma and bladder cancer (40, 56-59). Other models utilizing combinations of adenovirus and other chemotherapies have also demonstrated encouraging results. It was shown that a combination of cyclophosphamide and intra-tumoral injection of adenoviral gene transfer of IL-12 resulted in tumor eradication in a murine model of colorectal cancer (60). Additional strategies to further improve the safety of this response involve the utilization of organ specific, drug inducible adenoviral vectors. One study reported the use of a liver-specific, mifepristone-inducible adenoviral vector encoding IL-12 for the treatment of colorectal cancer liver metastasis (61). This system allowed for controlled and long term expression of the vector following systemic infusion, and was enhanced by additional treatment with the chemotherapeutic agent oxaliplatin (62). Other strategies testing drug inducible IL-12 expression have yielded similar results, confirming the ability to tightly control IL-12 production *in vivo* (63).

Additional viral vector based strategies include the utilization of other oncolytic viruses, which preferentially infect and lyse tumor cells. Intra-tumoral injection of a vesicular stomatitis virus carrying an IL-12 transgene was demonstrated to reduce tumor volume in a murine model of squamous cell carcinoma (64). This reduction in tumor volume correlated to an increased survival of treated tumor bearing mice.

Other delivery strategies include electroporation, where a voltage is applied to a cell membrane, allowing entry of plasmid DNA into a cell. To achieve this *in vivo*, studies have injected plasmids encoding the IL-12 gene intra-tumorally, followed by *in vivo* electroporation to allow for the introduction and expression of the gene into and by the regional cells (65). Utilizing a murine model of melanoma, it was found that electroporation of the IL-12 gene into the tumor resulted in tumor eradication in 47% of mice (65). This study also demonstrated the ability of IL-12 to stimulate an endogenous anti-tumor immune response as surviving mice were resistant to tumor re-challenge. Therapy resulted in increased levels of IL-12 and IFN γ in the tumor, increased lymphocyte infiltration and reduction in vascularity. These findings are consistent with previously documented effects of IL-12. This approach has similarly been successfully applied to a murine model of fibrosarcoma (66).

Other novel strategies to target IL-12 to the tumor site include anchoring IL-12 to a tumor-specific protein. One group utilized a single chain variable fragment (scFv) from an

antibody specific to erbB2 anchored to IL-12 to specifically deliver IL-12 to erbB2⁺ murine bladder cancers (67). This approach resulted in increased survival but failed to completely eradicate established disease. Similarly, investigators linked a IL-13R α 3 protein to IL-12 as a means of targeting murine melanoma (68). This group chose IL-13R α 3 as it can be a negative regulator of tumors that utilize IL-13 as a pro-tumorigenic factor. This therapy led to a significant NK T cell mediated inhibition of tumor growth *in vivo*.

Indeed, combinations of these treatment modalities have also yielded encouraging results in preclinical testing. Intra-tumoral injection of adenovirus encoding IL-12 and 4-1BBL combined with DC injection was found to mediate marked inhibition of tumor growth in a murine model of melanoma (69). Another study demonstrated the intra-tumoral injection of IL-12 encoding plasmid followed by DC vaccination led to the suppression of primary hepatocellular carcinoma and metastases (70). Significantly, utilization of this combined immune based therapy approach to augment surgical resection of primary tumor yielded superior results, leading to long term survival and resistance to tumor re-challenge in murine models of ovarian cancer, prostate cancer and hepatocellular carcinoma (51); (71); (72). Combination of IL-12 gene therapy with IL-27 gene therapy, or retinoic acid based therapies have also been described, with encouraging responses against systemic tumors (73, 74).

5. Clinical trials: Toxicity tempers the potential of IL-12 as an anti-cancer agent

Previous pre-clinical studies demonstrating the anti-tumor efficacy of IL-12 warranted the translation of this therapeutic agent to the clinical setting. A number of tumors were targeted in these trials, with modest, mixed responses. The first published trial of systemically administered IL-12 was a phase I dose escalation trial of intravenous (i.v.) administered recombinant human interleukin 12 (rhIL-12) (75). Cohorts of four to six patients with advanced solid tumor malignancies received escalating doses (3-1000 ng/kg/day) of rhIL-12 by bolus i.v. injection once and then, after a 2-week rest period, once daily for five days every 3 weeks. Forty patients were enrolled on this study including 20 with renal cell carcinomas, 12 with melanoma, and 5 with colon cancer. One melanoma patient experienced a complete regression of metastatic disease for a period of four weeks, while a second patient with renal cell carcinoma experienced a partial response that was ongoing at 22 months. Toxicities observed in this trial were fever, chills, fatigue, nausea, vomiting, and headache. Routine laboratory findings reported abnormalities including anemia, neutropenia, lymphopenia, hyperglycemia, thrombocytopenia, and hypoalbuminemia. Dose limiting toxicities included oral stomatitis and elevated transaminases. The maximum tolerated dose (MTD) (500 ng/kg) was associated with asymptomatic hepatic function test abnormalities in three patients and one on study death due to *Clostridia perfringens* septicemia. Lymphopenia was observed at all dose levels, with recovery occurring within several days of completing treatment without rebound lymphocytosis (75). These adverse events were hypothesized to be related to administration of recombinant human IL-12 and so the immune effects of this therapy were interrogated. Consistent with pre-clinical data, IL-12 was shown to up-regulate IFN γ , in a dose-dependent fashion. Additionally, a single 500 ng/kg dose of rhIL-12 was shown to increase NK cell

cytolytic activity and T cell proliferation, as determined by studies on peripheral blood samples collected pre and post treatment (76). In a subsequent Phase II study of 17 patients investigators observed unexpected toxicities related to the dosing schedule of IL-12 administration (77). On this study, 12 out of 17 patients required hospitalization and two patients died. Two patients deaths occurred during the phase II study were determined to be related to IL-12 administration. Postmortem examination of these two patients showed hemorrhagic ulceration in the large intestine (patient 1) and necrotizing aspiration pneumonia and diffuse hemorrhagic colitis (patient 2). The constitutional, cardiac, renal, hematopoietic, hepatic and neurologic toxicities observed in the phase II were similar to those dose-limiting toxicities observed on phase I studies with IL-12. These toxicities resulted in the suspension of IL-12 trials by the Food and Drug Administration (FDA). Significantly, investigators subsequently determined that a single IL-12 infused loading dose given two weeks prior to consecutive treatments (as done in the initial trial) abrogated these observed toxicities.

A subsequent study by Gollob and colleagues (78), the authors describe two patients with renal cell carcinoma treated with twice-weekly intravenous rhIL-12 during a phase I trial. A cycle of therapy lasted 6 weeks. The patients had grade 4 neutropenia and grade 3 hemolytic anemias. The severe neutropenia was associated with bone marrow agranulocytosis and a preponderance of large granular lymphocytes in the peripheral blood, whereas the hemolytic anemia was associated with splenomegaly. Both patients had stable disease 4 months after the IL-12 treatment was stopped with persisted agranulocytosis and hemolytic anemia.

Additionally, thirty-four patients with measurable metastatic, recurrent or inoperable cervical carcinoma were enrolled on phase I clinical trial to investigate the anti-tumor effect of i.v administrated IL-12 at 250 ng/kg daily up to 21 days. Over half of these patients had received prior cisplatin-based chemotherapy. The most common serious toxicities were hematologic or hepatic, and all were reversible. The median survival was 6.5 months. This was the first clinical trial to demonstrate induction of cell-mediated immune (CMI) responses to specific antigens (HPV16 E4, E6, and E7 peptides) following treatment with IL-12 in women with cervical cancer. However, this improvement in immune response was not associated with enhanced objective response or survival (79).

Pharmacokinetic advantages of intraperitoneal (i.p.) rhIL-12 infusion, tumor response to i.p. delivery of cytokines, as well as its potential anti-angiogenic effect provided the rationale for further evaluation of rhIL-12 in patients with refractory or relapsed ovarian or peritoneal carcinoma. In this study (80) rhIL-12 was administered to 29 previously treated patients with peritoneal carcinomatosis from Müllerian carcinomas, gastrointestinal tract carcinomas and peritoneal mesothelioma in a phase I trial. rhIL-12 doses were dose escalated between patients from 3 to 600 ng/kg weekly up to 6 months. Three or more patients at each level received weekly i.p. injections of rhIL-12. Dose-limiting toxicity (grade 3 elevated transaminase levels) occurred in 50% of treated patients at the 600 ng/kg dose. More frequent, but less severe, toxicities included fever, fatigue, abdominal pain, and nausea. Ten patients received 300 ng/kg with acceptable frequency and severity of side effects. Two patients (one with ovarian cancer and one with mesothelioma) had no remaining disease at laparoscopy. Eight patients had stable disease

and 19 patients had progressive disease. Cytokines including IL-1 α , IL-2, IL-10, TNF α , and IFN γ were determined in serum and peritoneal fluid samples during therapy. Immunobiological effects included peritoneal tumor cell apoptosis, decreased tumor cell expression of BFGF and VEGF, elevated IFN γ levels and IP-10 transcripts in peritoneal exudate, and increased proportions of peritoneal CD3⁺ T cells relative to CD14⁺ monocytes (80). In a subsequent phase II trial thirty-four patients with ovarian carcinoma or primary peritoneal carcinoma were treated i.p. with rIL-12 (300 ng/kg weekly) (81). 12 patients completed this second phase were evaluated for response. There were no treatment related deaths, peritonitis or significant catheter related complications. Toxicities included grade 4 neutropenia (1), grade 3 fatigue (4), headache (2), myalgia (2), non-neutropenic fever (1), drug fever (1), back pain (1), and dizziness (1). Two patients had stable disease (SD) and 9 had progressive disease (PD). The authors concluded that rIL-12 can safely be administered by i.p. scheduled to patients after first line chemotherapy for ovarian/peritoneal carcinoma. Future i.p. therapies with rhIL-12 will require better understanding and control of pleiotropic effects of IL-12 since proteins with potential for both anti-tumor (IFN γ , IP-10) and pro-tumor growth effects (VEGF, IL-8) were detected in this study (81).

To avoid toxicities associated with systemic infusion of rIL-12, others have investigated subcutaneous administration of IL-12. Rook and colleagues initiated a phase I dose escalation trial of rhIL-12 treating 10 patients with cutaneous T-cell lymphoma (CTCL) with dose escalating regimens of subcutaneous (s.c.) 50, 100, or 300 ng/kg rhIL-12 twice weekly or intralesional injections for up to 24 weeks (82). Histological analysis of regressing skin lesions revealed increased numbers of CD8⁺ T cells. In contrast to systemic rIL-12 infusion, sq or intralesional rIL-12 regimens were well tolerated with adverse effects limited to low-grade fevers and headaches.

Similarly, in another phase I trial, 28 patients with advanced renal cell carcinoma were treated s.c. with rhIL-12 that was administered on day 1 and followed on day 8 with repeated s.c. injections 3 times a week for 2 weeks. The MTD of the initial injection was evaluated at dose levels of 0.1, 0.5, and 1.0 μ g/kg. A dose limiting toxicity (DLT) was observed at 1.0 μ g/kg consisting of fever, perivascularitis of the skin, and leukopenia. Other notable toxicities were oral mucositis and transaminitis. These toxicities were more severe after the initial injection than after repeated injections at the same dose level. In this study, one patient had a partial response and seven patients had stable disease (83).

The efficacy of s.c. rhIL-12 for the treatment of patients with early mycosis fungoides (MF; stage IA-IIA) has similarly been tested in a phase I clinical trial. In this study rhIL-12 was administered subcutaneously biweekly (100 ng/kg for 2 weeks; 300 ng/kg thereafter). Ten of 23 patients (43%) achieved partial responses (PR); 7 (30%) achieved minor responses; and 5 (22%) had stable disease. The duration of PRs ranged from 3 to more than 45 weeks. Twelve patients (52%) ultimately progressed with a mean time to progression of 57 days (range, 28-805). Seventeen patients had treatment-related adverse events that were generally mild to moderate in severity including asthenia, headache, chills, fever, injection site reaction, pain, myalgia, arthralgia, transaminitis, anorexia, and sweating. One patient in PR died of hemolytic anemia, possibly exacerbated by rhIL-12 treatment (84).

Little and colleagues conducted phase II clinical trial wherein 36 patients with AIDS-associated Kaposi sarcoma requiring chemotherapy were treated with six 3-week cycles of pegylated liposomal doxorubicin (20 mg/m²) plus interleukin-12 (300 ng/kg subcutaneously twice weekly), followed by 500 ng/kg subcutaneous IL-12 twice weekly for up to 3 years (85). Thirty patients had a major response, including 9 with a complete response, with an 83% overall response rate. Patients had elevated levels of IFN γ and IP-10 in their serum, indicative of an rIL-12 mediated immune response.

Finally, 42 previously treated patients (32 patients with relapsed or refractory non-Hodgkin's lymphoma (NHL) and 10 patients with relapsed Hodgkin's disease (HD)) were enrolled in a phase II clinical trial to evaluate the clinical activity and toxicity of rIL-12. Patients were treated with either intravenous (n = 11) or subcutaneous (n = 31) rIL-12. The patients had received a median of three prior treatment regimens, and 16 patients had undergone prior autologous stem cell transplantation. All patients were assessable for toxicity, and 39 of 42 (93%) patients were assessable for response. Six of 29 (21%) patients with NHL had a partial or complete response, whereas none of the 10 patients with HD responded to rIL-12 therapy. Furthermore, 15 patients had stable disease that lasted for up to 54 months. The most common toxicity was flu-like symptoms. Reversible grade 3 hepatic toxicity was observed in three patients requiring dose reduction (86). This study demonstrated increased numbers of peripheral blood CD8⁺ T cells as well as decreased VEGF and BFGF in 37% of the treated patients indicative of a rIL-12 mediated immune response.

Additional methods of delivering IL-12 to the tumor site have been investigated. Ten previously untreated patients with head and neck squamous cell carcinomas (HNSCC) received direct injection of rhIL-12 in the primary tumor weekly, at two dose levels of 100 or 300 ng/kg, as neoadjuvant therapy prior to surgical resection. In this trial the histologic and immunohistopathologic effects of intratumorally (i.t) infused rhIL-12 were evaluated in the primary tumors and regional lymph nodes. In the primary tumor, the number of CD56⁺ NK cells was increased in rhIL-12-treated patients compared with control non-rhIL-12 treated patients. After i.t. rhIL-12 treatment of HNSCC patients, significant effects were noted on B cells, with altered lymph node architecture in every IL-12-treated patient and excessive peritumoral infiltration of B cells in some patients (87, 88).

In a phase I/II clinical trial (89), plasmid DNA encoding human IL-12 was produced under good manufacturing practice (GMP) conditions and injected into lesions of nine patients with stage IV malignant melanoma previously treated with both standard and salvage chemotherapy regimens. Plasmid DNA was injected in cycles, three injections per cycle, for up to seven cycles. One cycle consisted of three injections at weekly interval, that is, on day 1, 8 and 15, followed by a resting period of about 8 days (89). Local injection site anti-tumor responses were seen in a majority of patients, with four patients exhibiting responses at distant metastases and a complete remission was achieved in one patient. Biopsies of lesions from responding patients demonstrated a predicted increase in IL-12, IFN γ and IP-10 expression analyzed by real-time polymerase chain reaction.

In a similar study, nine patients with metastatic melanoma were treated by intra-tumoral injection of a recombinant viral vector expressing human IL-12 derived from the canarypox virus (ALVAC-IL-12). Increases in IL-12 and IFN γ mRNA, were observed in ALVAC-IL-12-

injected tumors compared with saline-injected control tumors in four of the nine patients. ALVAC-IL-12-injected tumors were also characterized by increased T cell infiltration of the tumor (90). This therapy was well tolerated with no reported dose limiting toxicities. One patient achieved a complete response in the injected subcutaneous metastasis, but all patients developed neutralizing IgG antibodies to the viral vector, demonstrating a limitation to this viral delivery strategy.

Viral vectors, probably the most commonly used for gene delivery, often result in host immune response, systemic toxicity and integration into host genome. Plasmid DNA-based vectors avoid these problems but are lacking in efficient gene transfer efficiency. In vivo electroporation, which utilizes an electric charge to facilitate entry of macromolecules into the cell, can be a reproducible and highly efficient method to deliver plasmid DNA. A phase I dose escalation trial of plasmid IL-12 gene electroporation was studied in patients with metastatic melanoma. Patients received electroporation treatments on days 1, 5, and 8 during a single 39-day cycle, into metastatic melanoma lesions through a penetrating six-electrode array immediately after DNA injection. A sterile applicator containing six needle electrodes arranged in the circle was inserted into the tumor and six pulses at field strength of 1,300 Volts/cm and pulse duration of 100 μ s were applied using a Medpulsar DNA EPT System Generator. Twenty-four patients were treated at seven dose levels, with minimal systemic toxicity. Transient pain after electroporation was the primary adverse effect. Post-treatment biopsies showed plasmid dose proportional increases in IL-12 levels as well as marked tumor necrosis and increased lymphocytic infiltrate. Two of 19 patients with nonelectroporated distant lesions and no other systemic therapy showed complete regression of all metastases, whereas eight additional patients (42%) showed disease stabilization or partial responses (91).

A phase I trial to assess the safety and tolerability of i.p. injected human IL-12 plasmid (pIL-12) formulated with a synthetic lipopolymer, polyethyleneglycol-polyethyleneimine-cholesterol (PPC), was conducted in women with chemotherapy-resistant recurrent ovarian cancer. A total of 13 patients were enrolled in four dose-escalating cohorts and treated with 0.6, 3, 12 or 24 mg/m² of the formulated plasmid once every week for 4 weeks (92). This approach is attractive because of the ability of nanoparticles to transport larger amounts of genetic material than viral vectors, as well as the ability of this approach to bypass the induction of an endogenous immune response as is the case with viral vectors (93). However, nanoparticles lack the specificity required to home to sites of tumor (92). Intraperitoneal administration of this IL-12 gene bearing nanoparticle was well-tolerated, with mild to moderate fevers and abdominal pain reported for each patient. Treatment was associated with stable disease and decrease in serum cancer antigen (CA)-125 by 3% in one of the three patients in cohort-1; 36 and 86% in two of three patients in cohort-2; and 2, 11 and 16% in three of four patients in cohort-4 at the 5-week follow-up visit. There was an overall clinical response of 31% stable disease and 69% progressive disease at the 5 \pm 1 week post-treatment follow-up visits.

At present, several additional trials utilizing IL-12 as an anti-cancer therapy are currently enrolling patients. Avigan and colleagues are recruiting patients for a phase I/II trials to evaluate co-administration of a dendritic cell/tumor fusion vaccine with subcutaneously administered IL-12 to patients with stage IV breast cancer (Avigan D., Vaccination of patients with breast cancer with dendritic cell/tumor fusions and IL-12, NCT00622401).

Gajewski and colleagues are investigating the role of multi-peptide vaccination with or without an admixture of intradermally or subcutaneously delivered IL-12, with subsequent daclizumab therapy in patients with metastatic melanoma (Gajewski T.F., A randomized phase II study of multi-peptide vaccination with or without IL-12, then combined with regulatory T cell depletion using daclizumab in patients with metastatic melanoma, NCT01307618). A group at the National Cancer Institute is conducting a clinical trial using a novel IL-12 agent in patients with treatment-refractory solid tumors. This trial is designed to test the safety and effectiveness of experimental drug NHS-IL12 as a treatment for solid tumors that have not responded to standard treatments. The NHS-IL12 immunocytokine is composed of 2 IL-12 heterodimers, each fused to one of the V_H-chains of the NHS76 antibody, which has affinity for both single- and double-stranded DNA. Thus, NHS-IL12 targets delivery to regions of tumor necrosis where DNA has become exposed (NCT01417546). Other phase I/II study of metastatic melanoma will be conducted by Rosenberg group at NCI using lymphodepleting conditioning followed by infusion of tumor infiltrating lymphocytes genetically modified to express IL-12 (NCT01236573) (see below). These ongoing clinical trials convey the potential of this powerful immune stimulatory cytokine, while highlighting the necessity for careful dosing and more importantly, targeted delivery to reduce the risks of toxicity.

6. The promise of adoptive cell immunotherapy

Adoptive cell therapy involves the isolation, modification and expansion of endogenous immune cells, followed by the *ex vivo* expansion of and re-infusion of these cells into a tumor-bearing host. Indeed, the use of cells to deliver IL-12 to a tumor is attractive as natural immune cell features can be exploited whilst delivering IL-12 to the tumor microenvironment. One such example of this approach is the utilization of mesenchymal stem cells (MSCs) to deliver IL-12. MSCs have a widely reported ability to traffic to sites of tumor growth making them ideal delivery for IL-12 (94). Several groups have reported the use of MSCs to deliver IL-12 to tumors in murine models of glioma, renal cell carcinoma, breast cancer, melanoma, Ewing sarcoma and prostate cancer (95-99). These studies demonstrate that MSCs are successful delivery vehicles of IL-12 and the IL-12 delivered mediates anti-tumor responses in preclinical murine models involving increased IFN γ , increased infiltration of T cells and anti-angiogenic effects (95).

Antigen presenting cells (APCs) can also be utilized in cell transfer therapy. Although APCs have the endogenous capacity to produce IL-12, the hostile tumor microenvironment often suppresses this immune stimulatory response. Adoptive transfer of APCs genetically modified to continually produce IL-12 is aimed at initiating an endogenous anti-tumor immune response. The most commonly utilized APCs for IL-12 delivery are DCs. Intratumoral injection of DCs modified to express IL-12 mediated complete regression of neuroblastoma tumor in a mouse model (100). This effect was shown to correlate with increased tumor-specific splenocyte cytotoxic capacity. Other groups have tested this approach in murine liver tumor models with similar encouraging responses (101). This latter study specifically demonstrated the generation of a protective immune response, which was dependent on T and NK cells. One alteration of this approach involves the pulsing of the IL-12 modified DCs with tumor lysates to increase the immune-stimulatory capacity of the injected cells. Using a model of colon cancer, mice were treated with tumor lysate pulsed,

IL-12 gene modified DCs which was found to dramatically inhibit tumor growth (102). This therapy resulted in an increase of endogenous immune tumor specific cytotoxicity and increased IFN γ levels, consistent with previously reported effects of IL-12. Injection of IL-12 gene modified macrophages was also found to inhibit tumor growth and spontaneous metastasis following prostatectomy in a murine model of prostate cancer (71). These studies support the utilization of APC mediated delivery of IL-12 to the tumor microenvironment as a method to initiate an effective endogenous anti-tumor response.

Other immune cells employed in adoptive transfer anti-cancer therapy include adaptive immune effector cells. Cytokine induced killer (CIK) cells are generated by the *ex vivo* activation and expansion of T cells, resulting in a cell population with both T and NK cell phenotypes (103). Using a preclinical, immunocompetent murine model of breast cancer, it was shown that augmentation of CIK therapy with IL-12 resulted in enhanced anti-tumor efficacy and complete remission in 75% of mice following therapy (104). This was found to be due to IL-12-mediated increased immune mediated cytotoxicity, improved homing and persistence, as well as *in vivo* proliferation of transferred CIK cells.

A similar strategy to deliver IL-12 specifically to tumor cells involves the use of tumor-specific T cells (50, 105). In an initial report, transgenic mouse T cells specific for the gp100 melanoma antigen were genetically modified to express an IL-12 transgene (105). These cells were then infused into irradiated mice bearing subcutaneous melanoma tumors. It was found that these IL-12 producing targeted T cells mediated rejection of tumor with dose responsive toxicity. When lower numbers of T cells were transferred into these mice, toxicity was absent and anti-tumor effects still eradicated advanced tumors, though effective therapy still required prior lymphodepletion. These anti-tumor effects were dependent on the IL-12 being present in the tumor microenvironment as T cells cultured *ex vivo* in IL-12 did not have similar anti-tumor activity. Consistent with previously published results, therapy was associated with CD8⁺ T and NK cell infiltration and a reduction of Foxp3 expression within the tumor. To further improve the safety of this approach, an additional study reported control of IL-12 expression by a promoter containing binding sites for nuclear factor of activated T cells (NFAT) resulting in IL-12 production only upon T cell activation within the targeted tumor microenvironment (106). An additional report describes the isolation of murine T cells, genetically engineered *ex vivo* to express the IL-12 transgene and a chimeric antigen receptor (CAR) targeted to the carcinoembryonic antigen expressed on colon cancers (50). This study demonstrated the translational applicability of this approach, and a novel potentially clinically applicable approach to tumor targeted delivery of the IL-12 cytokine. This approach is currently being investigated in a clinical trial, as described to above. Rosenberg and colleagues are currently enrolling patients in a trial utilizing *ex vivo* expanded tumor-infiltrating lymphocytes modified to produce IL-12 for the treatment of metastatic melanoma (NCT1236573).

7. Conclusion

IL-12 is a potent mediator of anti-tumor immunity. The exact mechanisms of IL-12 mediated anti-tumor effects continue to warrant further investigation. While translation to the clinical setting has been hampered by toxicity and modest anti-tumor efficacy, localized delivery of IL-12 directly into the tumor may prove to be a successful approach in limited numbers of accessible tumors. However, it is perhaps in the setting of adoptive T cell immunotherapy

utilizing IL-12 secreting, tumor specific T cells, where the full anti-tumor benefit of IL-12 therapy will be realized with tumor targeted, locally secreted cytokine. This approach will avert systemic toxicity while providing the requisite boost to the endogenous immune system to fully eradicate tumor. IL-12 remains a unique and promising cytokine with marked anti-tumor activity and warrants continued rigorous investigation in both the pre-clinical and clinical settings in order to realize the full anti-tumor potential of this reagent.

8. Competing interests statement

The authors have no competing professional, financial, or personal interests that may have affected the presentation of this manuscript.

9. References

- [1] Boggio K, Nicoletti G, Di Carlo E, Cavallo F, Landuzzi L, Melani C, Giovarelli M, Rossi I, Nanni P, De Giovanni C, Bouchard P, Wolf S, Modesti A, Musiani P, Lollini PL, Colombo MP, Forni G. 1998. Interleukin 12-mediated prevention of spontaneous mammary adenocarcinomas in two lines of Her-2/neu transgenic mice. *J Exp Med* 188: 589-96
- [2] Cavallo F, Di Carlo E, Butera M, Verrua R, Colombo MP, Musiani P, Forni G. 1999. Immune events associated with the cure of established tumors and spontaneous metastases by local and systemic interleukin 12. *Cancer Res* 59: 414-21
- [3] Yu WG, Yamamoto N, Takenaka H, Mu J, Tai XG, Zou JP, Ogawa M, Tsutsui T, Wijesuriya R, Yoshida R, Herrmann S, Fujiwara H, Hamaoka T. 1996. Molecular mechanisms underlying IFN-gamma-mediated tumor growth inhibition induced during tumor immunotherapy with rIL-12. *Int Immunol* 8: 855-65
- [4] Nastala CL, Edington HD, McKinney TG, Tahara H, Nalesnik MA, Brunda MJ, Gately MK, Wolf SF, Schreiber RD, Storkus WJ, et al. 1994. Recombinant IL-12 administration induces tumor regression in association with IFN-gamma production. *J Immunol* 153: 1697-706
- [5] Brunda MJ, Luistro L, Warriar RR, Wright RB, Hubbard BR, Murphy M, Wolf SF, Gately MK. 1993. Antitumor and antimetastatic activity of interleukin 12 against murine tumors. *J Exp Med* 178: 1223-30
- [6] Kobayashi M, Fitz L, Ryan M, Hewick RM, Clark SC, Chan S, Loudon R, Sherman F, Perussia B, Trinchieri G. 1989. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. *J Exp Med* 170: 827-45
- [7] Stern AS, Podlaski FJ, Hulmes JD, Pan YC, Quinn PM, Wolitzky AG, Familletti PC, Stremlo DL, Truitt T, Chizzonite R, et al. 1990. Purification to homogeneity and partial characterization of cytotoxic lymphocyte maturation factor from human B-lymphoblastoid cells. *Proc Natl Acad Sci U S A* 87: 6808-12
- [8] Carra G, Gerosa F, Trinchieri G. 2000. Biosynthesis and posttranslational regulation of human IL-12. *J Immunol* 164: 4752-61
- [9] Trinchieri G. 2003. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol* 3: 133-46
- [10] O'Shea JJ, Gadina M, Schreiber RD. 2002. Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. *Cell* 109 Suppl: S121-31

- [11] Wolf SF, Temple PA, Kobayashi M, Young D, Dicig M, Lowe L, Dzialo R, Fitz L, Ferenz C, Hewick RM, et al. 1991. Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and natural killer cells. *J Immunol* 146: 3074-81
- [12] Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. 1993. Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science* 260: 547-9
- [13] Del Vecchio M, Bajetta E, Canova S, Lotze MT, Wesa A, Parmiani G, Anichini A. 2007. Interleukin-12: biological properties and clinical application. *Clin Cancer Res* 13: 4677-85
- [14] Macgregor JN, Li Q, Chang AE, Braun TM, Hughes DP, McDonagh KT. 2006. Ex vivo culture with interleukin (IL)-12 improves CD8(+) T-cell adoptive immunotherapy for murine leukemia independent of IL-18 or IFN-gamma but requires perforin. *Cancer Res* 66: 4913-21
- [15] Curtsinger JM, Lins DC, Johnson CM, Mescher MF. 2005. Signal 3 tolerant CD8 T cells degranulate in response to antigen but lack granzyme B to mediate cytotoxicity. *J Immunol* 175: 4392-9
- [16] Curtsinger JM, Lins DC, Mescher MF. 2003. Signal 3 determines tolerance versus full activation of naive CD8 T cells: dissociating proliferation and development of effector function. *J Exp Med* 197: 1141-51
- [17] Kieper WC, Prlic M, Schmidt CS, Mescher MF, Jameson SC. 2001. IL-12 enhances CD8 T cell homeostatic expansion. *J Immunol* 166: 5515-21
- [18] Lee SW, Park Y, Yoo JK, Choi SY, Sung YC. 2003. Inhibition of TCR-induced CD8 T cell death by IL-12: regulation of Fas ligand and cellular FLIP expression and caspase activation by IL-12. *J Immunol* 170: 2456-60
- [19] Pearce EL, Shen H. 2007. Generation of CD8 T cell memory is regulated by IL-12. *J Immunol* 179: 2074-81
- [20] Micallef MJ, Ohtsuki T, Kohno K, Tanabe F, Ushio S, Namba M, Tanimoto T, Torigoe K, Fujii M, Ikeda M, Fukuda S, Kurimoto M. 1996. Interferon-gamma-inducing factor enhances T helper 1 cytokine production by stimulated human T cells: synergism with interleukin-12 for interferon-gamma production. *Eur J Immunol* 26: 1647-51
- [21] Lauwerys BR, Renauld JC, Houssiau FA. 1999. Synergistic proliferation and activation of natural killer cells by interleukin 12 and interleukin 18. *Cytokine* 11: 822-30
- [22] Yoshimoto T, Okamura H, Tagawa YI, Iwakura Y, Nakanishi K. 1997. Interleukin 18 together with interleukin 12 inhibits IgE production by induction of interferon-gamma production from activated B cells. *Proc Natl Acad Sci U S A* 94: 3948-53
- [23] Ma X, Chow JM, Gri G, Carra G, Gerosa F, Wolf SF, Dzialo R, Trinchieri G. 1996. The interleukin 12 p40 gene promoter is primed by interferon gamma in monocytic cells. *J Exp Med* 183: 147-57
- [24] Grohmann U, Belladonna ML, Bianchi R, Orabona C, Ayroldi E, Fioretti MC, Puccetti P. 1998. IL-12 acts directly on DC to promote nuclear localization of NF-kappaB and primes DC for IL-12 production. *Immunity* 9: 315-23
- [25] Zou JP, Yamamoto N, Fujii T, Takenaka H, Kobayashi M, Herrmann SH, Wolf SF, Fujiwara H, Hamaoka T. 1995. Systemic administration of rIL-12 induces complete tumor regression and protective immunity: response is correlated with a striking

- reversal of suppressed IFN-gamma production by anti-tumor T cells. *Int Immunol* 7: 1135-45
- [26] Lafleur EA, Jia SF, Worth LL, Zhou Z, Owen-Schaub LB, Kleinerman ES. 2001. Interleukin (IL)-12 and IL-12 gene transfer up-regulate Fas expression in human osteosarcoma and breast cancer cells. *Cancer Res* 61: 4066-71
- [27] Burke F, Knowles RG, East N, Balkwill FR. 1995. The role of indoleamine 2,3-dioxygenase in the anti-tumour activity of human interferon-gamma in vivo. *Int J Cancer* 60: 115-22
- [28] Voest EE, Kenyon BM, O'Reilly MS, Truitt G, D'Amato RJ, Folkman J. 1995. Inhibition of angiogenesis in vivo by interleukin 12. *J Natl Cancer Inst* 87: 581-6
- [29] Angiolillo AL, Sgadari C, Taub DD, Liao F, Farber JM, Maheshwari S, Kleinman HK, Reaman GH, Tosato G. 1995. Human interferon-inducible protein 10 is a potent inhibitor of angiogenesis in vivo. *J Exp Med* 182: 155-62
- [30] Angiolillo AL, Sgadari C, Tosato G. 1996. A role for the interferon-inducible protein 10 in inhibition of angiogenesis by interleukin-12. *Ann N Y Acad Sci* 795: 158-67
- [31] Sgadari C, Angiolillo AL, Tosato G. 1996. Inhibition of angiogenesis by interleukin-12 is mediated by the interferon-inducible protein 10. *Blood* 87: 3877-82
- [32] Ferretti E, Di Carlo E, Cocco C, Ribatti D, Sorrentino C, Ognio E, Montagna D, Pistoia V, Airolidi I. 2010. Direct inhibition of human acute myeloid leukemia cell growth by IL-12. *Immunol Lett* 133: 99-105
- [33] Duda DG, Sunamura M, Lozonschi L, Kodama T, Egawa S, Matsumoto G, Shimamura H, Shibuya K, Takeda K, Matsuno S. 2000. Direct in vitro evidence and in vivo analysis of the antiangiogenesis effects of interleukin 12. *Cancer Res* 60: 1111-6
- [34] Yao L, Sgadari C, Furuke K, Bloom ET, Teruya-Feldstein J, Tosato G. 1999. Contribution of natural killer cells to inhibition of angiogenesis by interleukin-12. *Blood* 93: 1612-21
- [35] Wigginton JM, Gruys E, Geiselhart L, Subleski J, Komschlies KL, Park JW, Wilttrout TA, Nagashima K, Back TC, Wilttrout RH. 2001. IFN-gamma and Fas/FasL are required for the antitumor and antiangiogenic effects of IL-12/pulse IL-2 therapy. *J Clin Invest* 108: 51-62
- [36] Eisenring M, vom Berg J, Kristiansen G, Saller E, Becher B. 2010. IL-12 initiates tumor rejection via lymphoid tissue-inducer cells bearing the natural cytotoxicity receptor Nkp46. *Nat Immunol* 11: 1030-8
- [37] Ogawa M, Tsutsui T, Zou JP, Mu J, Wijesuriya R, Yu WG, Herrmann S, Kubo T, Fujiwara H, Hamaoka T. 1997. Enhanced induction of very late antigen 4/lymphocyte function-associated antigen 1-dependent T-cell migration to tumor sites following administration of interleukin 12. *Cancer Res* 57: 2216-22
- [38] Kilinc MO, Aulakh KS, Nair RE, Jones SA, Alard P, Kosiewicz MM, Egilmez NK. 2006. Reversing tumor immune suppression with intratumoral IL-12: activation of tumor-associated T effector/memory cells, induction of T suppressor apoptosis, and infiltration of CD8+ T effectors. *J Immunol* 177: 6962-73
- [39] Verbik DJ, Stinson WW, Brunda MJ, Kessinger A, Joshi SS. 1996. In vivo therapeutic effects of interleukin-12 against highly metastatic residual lymphoma. *Clin Exp Metastasis* 14: 219-29
- [40] Zhao X, Bose A, Komita H, Taylor JL, Kawabe M, Chi N, Spokas L, Lowe DB, Goldbach C, Alber S, Watkins SC, Butterfield LH, Kalinski P, Kirkwood JM, Storkus WJ. 2011.

- Intratumoral IL-12 gene therapy results in the crosspriming of Tc1 cells reactive against tumor-associated stromal antigens. *Mol Ther* 19: 805-14
- [41] Broderick L, Yokota SJ, Reineke J, Mathiowitz E, Stewart CC, Barcos M, Kelleher RJ, Jr., Bankert RB. 2005. Human CD4⁺ effector memory T cells persisting in the microenvironment of lung cancer xenografts are activated by local delivery of IL-12 to proliferate, produce IFN- γ , and eradicate tumor cells. *J Immunol* 174: 898-906
- [42] Hess SD, Egilmez NK, Bailey N, Anderson TM, Mathiowitz E, Bernstein SH, Bankert RB. 2003. Human CD4⁺ T cells present within the microenvironment of human lung tumors are mobilized by the local and sustained release of IL-12 to kill tumors in situ by indirect effects of IFN- γ . *J Immunol* 170: 400-12
- [43] Broderick L, Brooks SP, Takita H, Baer AN, Bernstein JM, Bankert RB. 2006. IL-12 reverses anergy to T cell receptor triggering in human lung tumor-associated memory T cells. *Clin Immunol* 118: 159-69
- [44] King IL, Segal BM. 2005. Cutting edge: IL-12 induces CD4⁺CD25⁻ T cell activation in the presence of T regulatory cells. *J Immunol* 175: 641-5
- [45] Cao X, Leonard K, Collins LI, Cai SF, Mayer JC, Payton JE, Walter MJ, Piwnica-Worms D, Schreiber RD, Ley TJ. 2009. Interleukin 12 stimulates IFN- γ -mediated inhibition of tumor-induced regulatory T-cell proliferation and enhances tumor clearance. *Cancer Res* 69: 8700-9
- [46] Sica A, Schioppa T, Mantovani A, Allavena P. 2006. Tumour-associated macrophages are a distinct M2 polarised population promoting tumour progression: potential targets of anti-cancer therapy. *Eur J Cancer* 42: 717-27
- [47] Gordon S. 2003. Alternative activation of macrophages. *Nature Reviews Immunology* 3: 23-35
- [48] Stout RD, Watkins SK, Suttles J. 2009. Functional plasticity of macrophages: in situ reprogramming of tumor-associated macrophages. *J Leukoc Biol* 86: 1105-9
- [49] Watkins SK, Egilmez NK, Suttles J, Stout RD. 2007. IL-12 rapidly alters the functional profile of tumor-associated and tumor-infiltrating macrophages in vitro and in vivo. *J Immunol* 178: 1357-62
- [50] Chmielewski M, Kopecky C, Hombach AA, Abken H. 2011. IL-12 Release by Engineered T Cells Expressing Chimeric Antigen Receptors Can Effectively Muster an Antigen-Independent Macrophage Response on Tumor Cells That Have Shut Down Tumor Antigen Expression. *Cancer Res* 71: 5697-706
- [51] Mu J, Zou JP, Yamamoto N, Tsutsui T, Tai XG, Kobayashi M, Herrmann S, Fujiwara H, Hamaoka T. 1995. Administration of recombinant interleukin 12 prevents outgrowth of tumor cells metastasizing spontaneously to lung and lymph nodes. *Cancer Res* 55: 4404-8
- [52] Sabel MS, Su G, Griffith KA, Chang AE. 2010. Intratumoral delivery of encapsulated IL-12, IL-18 and TNF- α in a model of metastatic breast cancer. *Breast Cancer Res Treat* 122: 325-36
- [53] Hill HC, Conway TF, Jr., Sabel MS, Jong YS, Mathiowitz E, Bankert RB, Egilmez NK. 2002. Cancer immunotherapy with interleukin 12 and granulocyte-macrophage colony-stimulating factor-encapsulated microspheres: coinduction of innate and adaptive antitumor immunity and cure of disseminated disease. *Cancer Res* 62: 7254-63

- [54] Fewell JG, Matar MM, Rice JS, Brunhoeber E, Slobodkin G, Pence C, Worker M, Lewis DH, Anwer K. 2009. Treatment of disseminated ovarian cancer using nonviral interleukin-12 gene therapy delivered intraperitoneally. *J Gene Med* 11: 718-28
- [55] Sonabend AM, Velicu S, Ulasov IV, Han Y, Tyler B, Brem H, Matar MM, Fewell JG, Anwer K, Lesniak MS. 2008. A safety and efficacy study of local delivery of interleukin-12 transgene by PPC polymer in a model of experimental glioma. *Anticancer Drugs* 19: 133-42
- [56] Tian L, Chen X, Sun Y, Liu M, Zhu D, Ren J. 2010. Growth suppression of human laryngeal squamous cell carcinoma by adenoviral-mediated interleukin-12. *J Int Med Res* 38: 994-1004
- [57] Chiu TL, Lin SZ, Hsieh WH, Peng CW. 2009. AAV2-mediated interleukin-12 in the treatment of malignant brain tumors through activation of NK cells. *Int J Oncol* 35: 1361-7
- [58] Chen L, Chen D, Block E, O'Donnell M, Kufe DW, Clinton SK. 1997. Eradication of murine bladder carcinoma by intratumor injection of a bicistronic adenoviral vector carrying cDNAs for the IL-12 heterodimer and its inhibition by the IL-12 p40 subunit homodimer. *J Immunol* 159: 351-9
- [59] Siders WM, Wright PW, Hixon JA, Alvord WG, Back TC, Wiltrout RH, Fenton RG. 1998. T cell- and NK cell-independent inhibition of hepatic metastases by systemic administration of an IL-12-expressing recombinant adenovirus. *J Immunol* 160: 5465-74
- [60] Malvicini M, Rizzo M, Alaniz L, Pinero F, Garcia M, Atorrasagasti C, Aquino JB, Rozados V, Scharovsky OG, Matar P, Mazzolini G. 2009. A novel synergistic combination of cyclophosphamide and gene transfer of interleukin-12 eradicates colorectal carcinoma in mice. *Clin Cancer Res* 15: 7256-65
- [61] Gonzalez-Aparicio M, Alzuguren P, Mauleon I, Medina-Echeverez J, Hervas-Stubbs S, Mancheno U, Berraondo P, Crettaz J, Gonzalez-Asequinolaza G, Prieto J, Hernandez-Alcoceba R. 2011. Oxaliplatin in combination with liver-specific expression of interleukin 12 reduces the immunosuppressive microenvironment of tumours and eradicates metastatic colorectal cancer in mice. *Gut* 60: 341-9
- [62] Wang L, Hernandez-Alcoceba R, Shankar V, Zabala M, Kochanek S, Sangro B, Kramer MG, Prieto J, Qian C. 2004. Prolonged and inducible transgene expression in the liver using gutless adenovirus: a potential therapy for liver cancer. *Gastroenterology* 126: 278-89
- [63] Komita H, Zhao X, Katakam AK, Kumar P, Kawabe M, Okada H, Braughler JM, Storkus WJ. 2009. Conditional interleukin-12 gene therapy promotes safe and effective antitumor immunity. *Cancer Gene Ther* 16: 883-91
- [64] Shin EJ, Wanna GB, Choi B, Aguila D, 3rd, Ebert O, Genden EM, Woo SL. 2007. Interleukin-12 expression enhances vesicular stomatitis virus oncolytic therapy in murine squamous cell carcinoma. *Laryngoscope* 117: 210-4
- [65] Lucas ML, Heller L, Coppola D, Heller R. 2002. IL-12 plasmid delivery by in vivo electroporation for the successful treatment of established subcutaneous B16.F10 melanoma. *Mol Ther* 5: 668-75
- [66] Pavlin D, Cemazar M, Kamensek U, Tozon N, Pogacnik A, Sersa G. 2009. Local and systemic antitumor effect of intratumoral and peritumoral IL-12 electrogene therapy on murine sarcoma. *Cancer Biol Ther* 8: 2114-22

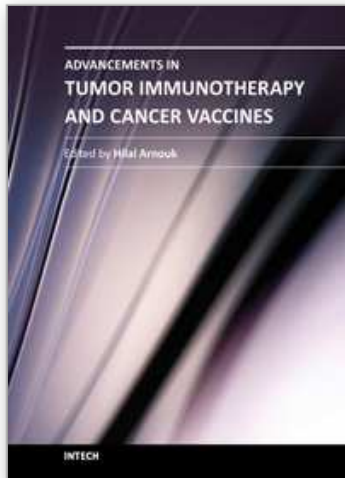
- [67] Tsai YS, Shiau AL, Chen YF, Tsai HT, Lee HL, Tzai TS, Wu CL. 2009. Enhancement of antitumor immune response by targeted interleukin-12 electrogene transfer through antiHER2 single-chain antibody in a murine bladder tumor model. *Vaccine* 27: 5383-92
- [68] Hebelers-Barbosa F, Rodrigues EG, Puccia R, Caires AC, Travassos LR. 2008. Gene Therapy against Murine Melanoma B16F10-Nex2 Using IL-13Ralpha2-Fc Chimera and Interleukin 12 in Association with a Cyclopalladated Drug. *Transl Oncol* 1: 110-20
- [69] Huang JH, Zhang SN, Choi KJ, Choi IK, Kim JH, Lee MG, Kim H, Yun CO. 2010. Therapeutic and tumor-specific immunity induced by combination of dendritic cells and oncolytic adenovirus expressing IL-12 and 4-1BBL. *Mol Ther* 18: 264-74
- [70] Kayashima H, Toshima T, Okano S, Taketomi A, Harada N, Yamashita Y, Tomita Y, Shirabe K, Maehara Y. 2010. Intratumoral neoadjuvant immunotherapy using IL-12 and dendritic cells is an effective strategy to control recurrence of murine hepatocellular carcinoma in immunosuppressed mice. *J Immunol* 185: 698-708
- [71] Tabata K, Watanabe M, Naruishi K, Edamura K, Satoh T, Yang G, Abdel Fattah E, Wang J, Goltsov A, Floryk D, Soni SD, Kadmon D, Thompson TC. 2009. Therapeutic effects of gelatin matrix-embedded IL-12 gene-modified macrophages in a mouse model of residual prostate cancer. *Prostate Cancer Prostatic Dis* 12: 301-9
- [72] Kayashima H, Toshima T, Okano S, Taketomi A, Harada N, Yamashita Y, Tomita Y, Shirabe K, Maehara Y. 2010. Intratumoral Neoadjuvant Immunotherapy Using IL-12 and Dendritic Cells Is an Effective Strategy To Control Recurrence of Murine Hepatocellular Carcinoma in Immunosuppressed Mice. *Journal of Immunology* 185: 698-708
- [73] Zhu S, Lee DA, Li S. 2010. IL-12 and IL-27 sequential gene therapy via intramuscular electroporation delivery for eliminating distal aggressive tumors. *J Immunol* 184: 2348-54
- [74] Charoensit P, Kawakami S, Higuchi Y, Yamashita F, Hashida M. 2010. Enhanced growth inhibition of metastatic lung tumors by intravenous injection of ATRAcationic liposome/IL-12 pDNA complexes in mice. *Cancer Gene Ther* 17: 512-22
- [75] Atkins MB, Robertson MJ, Gordon M, Lotze MT, DeCoste M, DuBois JS, Ritz J, Sandler AB, Edington HD, Garzone PD, Mier JW, Canning CM, Battiato L, Tahara H, Sherman ML. 1997. Phase I evaluation of intravenous recombinant human interleukin 12 in patients with advanced malignancies. *Clin Cancer Res* 3: 409-17
- [76] Robertson MJ, Cameron C, Atkins MB, Gordon MS, Lotze MT, Sherman ML, Ritz J. 1999. Immunological effects of interleukin 12 administered by bolus intravenous injection to patients with cancer. *Clin Cancer Res* 5: 9-16
- [77] Leonard JP, Sherman ML, Fisher GL, Buchanan LJ, Larsen G, Atkins MB, Sosman JA, Dutcher JP, Vogelzang NJ, Ryan JL. 1997. Effects of single-dose interleukin-12 exposure on interleukin-12-associated toxicity and interferon-gamma production. *Blood* 90: 2541-8
- [78] Gollob JA, Veenstra KG, Mier JW, Atkins MB. 2001. Agranulocytosis and hemolytic anemia in patients with renal cell cancer treated with interleukin-12. *Journal of Immunotherapy* 24: 91-8
- [79] Wadler S, Levy D, Frederickson HL, Falkson CI, Wang YX, Weller E, Burk R, Ho G, Kadish AS. 2004. A phase II trial of interleukin-12 in patients with advanced

- cervical cancer: clinical and immunologic correlates Eastern Cooperative Oncology Group study E1E96. *Gynecologic Oncology* 92: 957-64
- [80] Freedman RS, Lenzi R, Rosenblum M, Verschraegen C, Kudelka AP, Kavanagh JJ, Hicks ME, Lang EA, Nash MA, Levy LB, Garcia ME, Platsoucas CD, Abbruzzese JL. 2002. Phase I study of intraperitoneal recombinant human interleukin 12 in patients with Mullerian carcinoma, gastrointestinal primary malignancies, and mesothelioma. *Clinical Cancer Research* 8: 3686-95
- [81] Lenzi R, Edwards R, June C, Seiden MV, Garcia ME, Rosenblum M, Freedman RS. 2007. Phase II study of intraperitoneal recombinant interleukin-12 (rhIL-12) in patients with peritoneal carcinomatosis (residual disease < 1 cm) associated with ovarian cancer or primary peritoneal carcinoma. *Journal of Translational Medicine* 5
- [82] Rook AH, Wood GS, Yoo EK, Elenitsas R, Kao DM, Sherman ML, Witmer WK, Rockwell KA, Shane RB, Lessin SR, Vonderheid EC. 1999. Interleukin-12 therapy of cutaneous T-cell lymphoma induces lesion regression and cytotoxic T-cell responses. *Blood* 94: 902-8
- [83] Portielje JEA, Kruit WHJ, Schuler M, Beck J, Lamers CHJ, Stoter G, Huber C, de Boer-Dennert M, Rakhit A, Bolhuis RLH, Waiter E. 1999. Phase I study of subcutaneously administered recombinant human interleukin 12 in patients with advanced renal cell cancer. *Clinical Cancer Research* 5: 3983-9
- [84] Duvic M, Sherman ML, Wood GS, Kuzel TM, Olsen E, Foss F, Laliberte RJ, Ryan JL, Zonno K, Rook AH. 2006. 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* 55: 807-13
- [85] Little RF, Aleman K, Kumar P, Wyvill KM, Pluda JM, Read-Connole E, Wang V, Pittaluga S, Catanzaro AT, Steinberg SM, Yarchoan R. 2007. Phase 2 study of pegylated liposomal doxorubicin in combination with interleukin-12 for AIDS-related Kaposi sarcoma. *Blood* 110: 4165-71
- [86] Younes A, Pro B, Robertson MJ, Flinn IW, Romaguera JE, Hagemester F, Dang NH, Fiumara P, Loyer EM, Cabanillas FF, McLaughlin PW, Rodriguez MA, Samaniego F. 2004. Phase II clinical trial of interleukin-12 in patients with relapsed and refractory non-Hodgkin's lymphoma and Hodgkin's disease. *Clin Cancer Res* 10: 5432-8
- [87] van Herpen CML, van der Laak JAW, de Vries IJM, van Krieken JH, de Wilde PC, Balvers MGJ, Adema GJ, De Mulder PHM. 2005. 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. *Clinical Cancer Research* 11: 1899-909
- [88] van Herpen CML, van der Voort R, van der Laak JAWM, Klasen IS, de Graaf AO, van Kempen LCL, de Vries IJM, Duiveman-de Boer T, Dolstra H, Torensma R, van Krieken JH, Adema GJ, De Mulder PHM. 2008. Intratumoral rhIL-12 administration in head and neck squamous cell carcinoma patients induces B cell activation. *International Journal of Cancer* 123: 2354-61
- [89] Heinzerling L, Burg G, Dummer R, Maier T, Oberholzer PA, Schultz J, Elzaouk L, Pavlovic J, Moelling K. 2005. Intratumoral injection of DNA encoding human interleukin 12 into patients with metastatic melanoma: clinical efficacy. *Hum Gene Ther* 16: 35-48

- [90] Triozzi PL, Strong TV, Bucy RP, Allen KO, Carlisle RR, Moore SE, Lobuglio AF, Conry RM. 2005. Intratumoral administration of a recombinant canarypox virus expressing interleukin 12 in patients with metastatic melanoma. *Hum Gene Ther* 16: 91-100
- [91] Daud AI, DeConti RC, Andrews S, Urbas P, Riker AI, Sondak VK, Munster PN, Sullivan DM, Ugen KE, Messina JL, Heller R. 2008. Phase I Trial of Interleukin-12 Plasmid Electroporation in Patients With Metastatic Melanoma. *Journal of Clinical Oncology* 26: 5896-903
- [92] Anwer K, Barnes MN, Fewell J, Lewis DH, Alvarez RD. 2010. Phase-I clinical trial of IL-12 plasmid/lipopolymer complexes for the treatment of recurrent ovarian cancer. *Gene Ther* 17: 360-9
- [93] Hallaj-Nezhadi S, Lotfipour F, Dass C. 2010. Nanoparticle-mediated interleukin-12 cancer gene therapy. *J Pharm Pharm Sci* 13: 472-85
- [94] Feng B, Chen L. 2009. Review of mesenchymal stem cells and tumors: executioner or coconspirator? *Cancer Biother Radiopharm* 24: 717-21
- [95] Ryu CH, Park SH, Park SA, Kim SM, Lim JY, Jeong CH, Yoon WS, Oh WI, Sung YC, Jeun SS. 2011. Gene therapy of intracranial glioma using interleukin 12-secreting human umbilical cord blood-derived mesenchymal stem cells. *Hum Gene Ther* 22: 733-43
- [96] Gao P, Ding Q, Wu Z, Jiang H, Fang Z. 2010. Therapeutic potential of human mesenchymal stem cells producing IL-12 in a mouse xenograft model of renal cell carcinoma. *Cancer Lett* 290: 157-66
- [97] Eliopoulos N, Francois M, Boivin MN, Martineau D, Galipeau J. 2008. Neo-organoid of marrow mesenchymal stromal cells secreting interleukin-12 for breast cancer therapy. *Cancer Res* 68: 4810-8
- [98] Wang H, Yang G, Timme TL, Fujita T, Naruishi K, Frolov A, Brenner MK, Kadmon D, Thompson TC. 2007. IL-12 gene-modified bone marrow cell therapy suppresses the development of experimental metastatic prostate cancer. *Cancer Gene Ther* 14: 819-27
- [99] Duan X, Guan H, Cao Y, Kleinerman ES. 2009. Murine bone marrow-derived mesenchymal stem cells as vehicles for interleukin-12 gene delivery into Ewing sarcoma tumors. *Cancer* 115: 13-22
- [100] Shimizu T, Berhanu A, Redlinger RE, Jr., Watkins S, Lotze MT, Barksdale EM, Jr. 2001. Interleukin-12 transduced dendritic cells induce regression of established murine neuroblastoma. *J Pediatr Surg* 36: 1285-92
- [101] Tatsumi T, Takehara T, Yamaguchi S, Sasakawa A, Miyagi T, Jinushi M, Sakamori R, Kohga K, Uemura A, Ohkawa K, Storkus WJ, Hayashi N. 2007. Injection of IL-12 gene-transduced dendritic cells into mouse liver tumor lesions activates both innate and acquired immunity. *Gene Ther* 14: 863-71
- [102] He XZ, Wang L, Zhang YY. 2008. An effective vaccine against colon cancer in mice: use of recombinant adenovirus interleukin-12 transduced dendritic cells. *World J Gastroenterol* 14: 532-40
- [103] Schmidt-Wolf IG, Lefterova P, Mehta BA, Fernandez LP, Huhn D, Blume KG, Weissman IL, Negrin RS. 1993. Phenotypic characterization and identification of effector cells involved in tumor cell recognition of cytokine-induced killer cells. *Exp Hematol* 21: 1673-9

- [104] Helms MW, Prescher JA, Cao YA, Schaffert S, Contag CH. 2010. IL-12 enhances efficacy and shortens enrichment time in cytokine-induced killer cell immunotherapy. *Cancer Immunol Immunother* 59: 1325-34
- [105] Kerkar SP, Muranski P, Kaiser A, Boni A, Sanchez-Perez L, Yu Z, Palmer DC, Reger RN, Borman ZA, Zhang L, Morgan RA, Gattinoni L, Rosenberg SA, Trinchieri G, Restifo NP. 2010. Tumor-specific CD8⁺ T cells expressing interleukin-12 eradicate established cancers in lymphodepleted hosts. *Cancer Res* 70: 6725-34
- [106] Zhang L, Kerkar SP, Yu Z, Zheng Z, Yang S, Restifo NP, Rosenberg SA, Morgan RA. 2011. Improving adoptive T cell therapy by targeting and controlling IL-12 expression to the tumor environment. *Mol Ther* 19: 751-9

IntechOpen



Advancements in Tumor Immunotherapy and Cancer Vaccines

Edited by Dr. Hilal Arnouk

ISBN 978-953-307-998-1

Hard cover, 218 pages

Publisher InTech

Published online 03, February, 2012

Published in print edition February, 2012

Harnessing the potential of the human body's own immune system to attack malignant tumor cells has been the goal of many scientific investigators in recent years, with advances in cancer biology and immunology enabling cancer immunotherapy to become a reality. World-class bench and clinical researchers have joined forces to collaborate and review current developments and trends in cancer immunology for the purposes of this book, and the result is a promising review of contemporary clinical treatments. In each chapter the authors present the scientific basis behind such therapeutic approaches, including cancer vaccines with special focus on prostate cancer, melanoma and novel approaches utilizing both innate and adaptive immune responses.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Hollie J. Pegram, Alena A. Chekmasova, Gavin H. Imperato and Renier J. Brentjens (2012). Interleukin 12: Stumbling Blocks and Stepping Stones to Effective Anti-Tumor Therapy, *Advancements in Tumor Immunotherapy and Cancer Vaccines*, Dr. Hilal Arnouk (Ed.), ISBN: 978-953-307-998-1, InTech, Available from: <http://www.intechopen.com/books/advancements-in-tumor-immunotherapy-and-cancer-vaccines/interleukin-12-stumbling-blocks-and-stepping-stones-to-effective-anti-tumor-therapy>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen