OncoImmunology 3, e29030; April 2014; © 2014 Landes Bioscience

Trial watch

Immunostimulatory cytokines in cancer therapy

Erika Vacchelli[1,2,3,4,†], Fernando Aranda[1,2,3,4,†], Florine Obrist[1,2,3,4], Alexander Eggermont[1], Jérôme Galon[2,5,6,7], Isabelle Cremer[2,6,8], Laurence Zitvogel[1,9], Guido Kroemer[2,3,5,10,11,‡], and Lorenzo Galluzzi[1,3,5,‡]

[1]Gustave Roussy; Villejuif, France; [2]INSERM, UMRS1138; Paris, France; [3]Equipe 11 labellisée par la Ligue Nationale contre le Cancer, Centre de Recherche des Cordeliers; Paris, France; [4]Université Paris-Sud/Paris XI; Le Kremlin-Bicêtre, France; [5]Université Paris Descartes/Paris V, Sorbonne Paris Cité; Paris, France; [6]Université Pierre et Marie Curie/Paris VI; Paris, France; [7]Laboratory of Integrative Cancer Immunology, Centre de Recherche des Cordeliers; Paris, France; [8]Equipe 13, Centre de Recherche des Cordeliers; Paris, France; [9]INSERM, U1015, CICBT507; Villejuif, France; [10]Pôle de Biologie, Hôpital Européen Georges Pompidou, AP-HP, Paris, France; [11]Metabolomics and Cell Biology Platforms, Gustave Roussy; Villejuif, France;

[†]These authors contributed equally contributed to this work.

[‡]These authors share senior co-authorship.

Keywords: chemokines, GM-CSF, IFN, IL-2, TGFβ1, TNFα

Abbreviations: AML, acute myeloid leukemia; CML, chronic myelogenous leukemia; DC, dendritic cell; FDA, Food and Drug Administration; FLT3L, fms-related tyrosine kinase 3 ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; mAb, monoclonal antibody; NSCLC, non-small cell lung carcinoma; NHL, non-Hodgkin lymphoma; PDGF, platelet-derived growth factor; RCC, renal cell carcinoma; SCLC, small cell lung carcinoma; SNP, single nucleotide polymorphism; TAA, tumor-associated antigen; TLR, Toll-like receptor; TNFα, tumor necrosis factor α; Treg, regulatory T cell

Tumor-targeting immune responses provide a significant contribution to (when they do not entirely account for) the clinical activity of diverse antineoplastic regimens, encompassing not only a large panel of immunotherapeutic strategies but also conventional cytotoxic molecules, targeted anticancer agents and irradiation. In line with this notion, several approaches have been devised to elicit novel or boost existing anticancer immune responses, including the administration of immunomodulatory cytokines. Such a relatively unspecific intervention suffices to mediate clinical effects in (at least a subset of) patients bearing particularly immunogenic tumors, like melanoma and renal cell carcinoma. More often, however, immunostimulatory cytokines are administered to boost the immunogenic potential of other agents, including (but not limited to) immune checkpoint-blocking antibodies, anticancer vaccines, oncolytic viruses and immunogenic chemotherapeutics. Here, we summarize the latest advances in the clinical development of recombinant cytokines as an immunomodulatory intervention for cancer therapy.

Introduction

The word 'cytokines' is commonly employed to refer to a large and heterogeneous group of small and for the most part soluble (glyco)proteins that regulate—in an autocrine, paracrine or endocrine manner—virtually all biological functions, including (but not limited to) proliferative responses, differentiation, chemotaxis, inflammatory reactions, innate and adaptive immunity, and cell death.[1-4] The cytokine family nowadays includes more than 140 distinct members, and this number is expected to grow as various cytokine-like molecules are discovered every year.[5-7] Several attempts have been made throughout the past 3 decades to classify cytokines based on structural and/or functional considerations, leading to the introduction of relatively unspecific terms like 'chemokines,' referring to small cytokines involved in the regulation of chemotaxis, 'interleukins,' referring to cytokines that regulate the crosstalk between leukocytes, and 'colony-stimulating factors,' referring to cytokines that control hematopoiesis.[8-10] Along with the realization of the astonishing pleiotropism of the cytokine system, however, such classifications turned out to be reductionist and relatively imprecise, and thus were abandoned.[5,6] This said, terms including interleukins, chemokines and colony-stimulating factors are still largely employed by the scientific community, mainly for historical reasons.

Cytokine signaling is highly pleiotropic, at least in part because (1) virtually all cell types throughout the body produce (one or several) cytokines;

*Correspondence to: Lorenzo Galluzzi, Email: deadoc@vodafone.it; Guido Groemer, Email: kroemer@orange.fr
Submitted: 04/25/2014; Accepted: 04/26/2014; Published Online: 06/03/2014
Citation: Aranda F, Vacchelli E, Obrist F, Eggermont A, Galon J, Cremer I, Zitvogel L, Kroemer G, Galluzzi L. Trial Watch: Immunostimulatory cytokines in cancer therapy. OncoImmunology 2014; 3:e29030; http://dx.doi.org/10.4161/onci.29030

(2) the same cytokine can signal via various receptors/receptor isoforms, which are generally characterized by differential binding affinity and expression patterns; (3) cytokines generally participate in signaling cascades that regulate the release of other biologically active molecules, including other cytokines; and (4) the activity of cytokines is heavily influenced by contextual parameters such as local concentration, cell type, receptor isoform and the presence of additional cytokines.[5,6,8,9]

Besides regulating homeostatic hematopoiesis[10] and participating in physiological angiogenesis[11,12] cytokines are released in response to a wide array of insults, such as traumatic events, infections, and cancer.[13-15] In these settings, cytokines are secreted in discrete waves to coordinate (1) the removal of the pathogenic stimulus, and (2) the re-establishment of tissue homeostasis.[16-18] When such an adaptive response fails and the initiating stimulus cannot be removed, however, the continuous secretion of specific cytokines may promote chronic inflammation. Of note, patients affected by a chronic inflammatory response, be it systemic or local, exhibit an increased propensity to develop some neoplasms, including colorectal carcinoma.[19-21] Most likely, this originates from the increased production of mutagenic reactive oxygen species at sites of inflammation, as well as from the local secretion of mitogenic cytokines. Altogether, these observations lend further support to the notion that the biological activity of several cytokines exhibits a high degree of context dependency.

As soon as it became clear that tumors do not go unnoticed by the immune system, several approaches have been developed to elicit novel (or reinstate pre-existent) tumor-targeting immune responses. These therapeutic strategies include highly specific interventions, such as dendritic cell (DC)-based, peptide-based and DNA-based anticancer vaccines,[22-24] as well as relatively non-specific maneuvers, such as the local or systemic administration of Toll-like receptor (TLR) agonists,[25,26] immunostimulatory antibodies,[27] or cytokines.[5,6] In spite of the fact that cytokines do not actively elicit a tumor-targeting immune response but rather boost the antineoplastic potential of natural, tumor-specific immune effectors, no less than three recombinant cytokines are currently approved by the US Food and Drug Administration (FDA) or equivalent regulatory agencies for use as standalone therapeutic interventions in adult cancer patients. First, interferon (IFN-)α2a (Roferon-A®) is used in subjects with hairy cell leukemia and chronic phase, Philadelphia chromosome-positive chronic myelogenous leukemia (CML), upon minimal pretreatment (within 1 y of diagnosis). Second, IFN-α2b (Intron A®) is employed for the therapy of hairy cell leukemia, AIDS-related Kaposi's sarcoma, follicular lymphoma, multiple myeloma, melanoma, condyloma acuminata and cervical intraepithelial neoplasms. Third, interleukin (IL)-2 (aldesleukin, Proleukin®) is approved for the treatment of metastatic forms of melanoma and renal cell carcinoma (RCC) (source http://www.fda.gov). The use of recombinant granulocyte colony-stimulating factor (G-CSF, also known as filgrastim, lenograstim or Neupogen®) and recombinant granulocyte monocyte colony-stimulating factor (GM-CSF, also known as molgramostim, sargramostim, Leukomax®, Mielogen® or Leukine®) in cancer patients has also been licensed by the US FDA. However, these cytokines are not (yet) harnessed for their ability to boost anticancer immune responses. Rather, they are employed as mitogenic factors, (1) to favor the reconstitution of the immune system in transplanted patients, who are generally subjected to lymphodepleting/lymphoablating regimens, as well as in patients treated with aggressive antimitotic chemotherapy, who are prone to develop febrile neutropenia;[28-30] (2) to recruit bone marrow precursors to the peripheral blood in the context of autologous stem cell transplantation;[31,32] (3) to prevent the neutropenia-inducing activity of specific chemotherapeutics;[33,34] and (4) to favor the replication of quiescent leukemic cells, thus exposing them to the antineoplastic activity of drugs that preferentially target actively proliferating cells.[35] Finally, recombinant tumor necrosis factor α (TNFα) is currently approved by multiple regulatory agencies including the European Medicine Agency (EMA), but not by the US FDA, for use in patients with limb-threatening soft tissue sarcoma and melanoma.[36-43] In this setting, TNFα is generally co-administered with melphalan (an alkylating agent) to isolated limbs under mild hyperthermic conditions, a safe and relatively simple procedure that has been associated with consistent rates of objective responses.[44-46]

Owing to their pleiotropic biological activity, cytokines can be associated with clinically relevant side effects, especially when administered systemically. There are 3 major concerns related to the use of cytokines in (cancer) patients: (1) the elicitation of an acute, sepsis-like, potentially lethal systemic reaction characterized by the massive release into the circulation of pyrogenic and cytotoxic cytokines;[47-51] (2) the exacerbation of chronic inflammatory foci that may initiate oncogenesis or accelerate tumor progression;[19-21] and (3) the activation of a mitogenic program in otherwise poorly proliferating cells, favoring the accumulation of genetic/epigenetic defects and hence increasing the likelihood of malignant transformation.[52-54] In fact, some cytokines including multiple members of the platelet-derived growth factor (PDGF) family cannot be employed as therapeutic interventions owing to their excessive mitogenic (and hence potentially oncogenic) potential.[5,6,55]

In previous issues of *OncoImmunology*, we discussed the scientific grounds supporting the use of cytokines as experimental immunostimulatory interventions in cancer patients as well as recent studies assessing the authentic clinical value of this regimen.[5,6] Here, we present the newest developments in this exciting area of investigation. Of note, studies assessing the clinical profile of cytokines as immunoreconstituting agents, studies involving FDA-approved immunostimulatory cytokines (*i.e.*, IFN-α2a, IFN-α2b and IL-2) employed as "on-label" interventions (see above), as well as studies investigating the antineoplastic activity of potentially oncotoxic cytokines, such as TNFα, will not be discussed here.

Literature Update

Since the submission of our latest Trial Watch dealing with topic (April 2013),[5] the results of at least 10 clinical studies evaluating the therapeutic profile of cytokines as off-label immunostimulatory interventions in cancer patients have been published in peer-reviewed scientific journals (source http://www.ncbi.nlm.nih.gov/pubmed).

Dutcher and colleagues, in collaboration with the Eastern Cooperative Oncology Group, tested the ability of recombinant IL-1α to boost the antineoplastic activity of cyclophosphamide (an immunostimulatory alkylating agent)[56-60] in patients with advanced solid tumors. In this Phase I clinical study, 3 different IL-1α doses and administration schedules were evaluated. Common side effects included fever, chills, hypotension, nausea/emesis, and elevations in circulating hepatic enzymes. Moreover, the co-administration of IL-1α failed to rescue the neutropenic effects of cyclophosphamide, suggesting that other, comparatively more specific (and hence less toxic) cytokines may be best suitable to provide a hematopoietic support to chemotherapy.[61]

Vitale and coworkers investigated the therapeutic profile of subcutaneous low-dose IL-2, combined with the somatostatin analog lanreotide,[62-64] in 6 patients with symptomatic and advanced medullary thyroid carcinoma. The authors observed that a 6-mo regimen of lanreotide plus low-dose IL-2 was well tolerated by all patients, improved their quality of life and elicited a partial response or disease stabilization in 2 or 3 individuals, respectively.[64] Tomov and collaborators reported a case of hepatocellular carcinoma that recurred upon the surgical resection of a primary lesion of 60 mm initially misdiagnosed as hepatic adenoma. Seven new lesions developed upon surgery, and the patient received high-dose IL-2, bacillus Calmette-Guérin (an attenuated variant of *Mycobacterium bovis* that is currently employed for the treatment of transitional cell carcinomas of the bladder)[25,65] and melatonin (a pineal hormone that appears to mediate antineoplastic effects, especially in combination with IL-2).[66-68] Of note, the tumor rapidly became undetectable by magnetic resonance imaging and computer tomography, and the patient remained in complete remission for at least 6 y after the confirmed diagnosis of untreatable hepatocellular carcinoma.[69]

Robertson et al. performed a dose-escalation Phase I study to test the safety and therapeutic profile of recombinant human IL-18 in non-Hodgkin lymphoma (NHL) patients treated with the CD20-targeting mAb rituximab.[70-73] Rituximab (375 mg/m^2) was administered i.v. once weekly for a total of 4 wks, while escalating doses of IL-18 (1, 3, 10, 20, 30, and 100 mug/kg) were given as a 2 h intravenous infusion weekly for 12 consecutive wks. No dose-limiting toxicities were observed. Common side effects were chills, fever, headache and nausea, while abnormal laboratory findings included transient asymptomatic lymphopenia, hyperglycemia, anemia, hypoalbuminemia as well as temporary elevations in circulating bilirubin and hepatic enzymes. Of note, 5 out of 19 patients experienced objective clinical responses. Altogether, these findings suggest that recombinant human IL-18 is well tolerated at doses at which it may improve the therapeutic profile of rituximab in NHL patients.[74]

Gorin and colleagues tested the ability of G-CSF to boost the therapeutic profile of the anti-CD52 mAb alemtuzumab, which mostly originates from antibody-dependent cell-mediated cytotoxicity,[75,76] in 12 patients with relapsed or refractory acute lymphoblastic leukemia. In the context of this Phase II clinical study, patients received 5 mug/kg G-CSF per day along with 30 mg alemtuzumab 3 times per wk for a total of 12–18 infusions. Fever/chills, skin rash and bronchospasm were the most common side effects. Four patients achieved a complete response, defined as the disappearance of leukemic blasts from the bone marrow. Nonetheless, all patients progressed within a few months and all but one died. These results indicate that alemtuzumab plus G-CSF may induce robust but temporary clinical responses.[77]

Cheung and coworkers investigated the ability of GM-CSF to improve the response of 79 patients with persistent osteomedullary neuroblastoma to 3F8, a mAb specific for GD2 ganglioside.[78-80] Patients were treated with 3F8 plus GM-CSF for up to 24 mo, or until the development of neutralizing anti-3F8 antibodies. In the context of this Phase II clinical trial, toxicities were generally manageable and 38% of patients achieved an objective response as defined by metaiodobenzyl-guanidine scan. Moreover, the 5-y progression-free survival of patients receiving 3F8 plus subcutaneous GM-CSF was 24 ± 6%, which was significantly better than that of patients treated with 3F8 plus intravenous GM-CSF (11 ± 7%).[81-83]

Zarogoulidis et al. tested whether IFN-α and IFN-γ, administered alone (3 MIUs) or in combination (1.5 plus 1.5 MIUs) 3 times per wk, would improve the activity of carboplatin-, fosfamide- and etoposide-based chemotherapy in a cohort of 164 individuals with small cell lung carcinoma (SCLC). No differences in survival between groups were observed in the context of this Phase II clinical trial when all patients were included in the analysis. However, when only individuals with early disease were considered, IFN-α appeared to provide a survival benefit to SCLC patients treated with chemotherapy.[84]

Coker and colleagues performed a Phase I dose-escalation study of oral temozolomide, an alkylating agent, combined with subcutaneous pegylated IFN-α2b in 19 patients with refractory or advanced solid tumors. The authors identified the maximum tolerated dose of the combination in 100 mg/m^2 temozolomide on days 1–7 and 15–21 plus 1.5 μg/kg IFN-α2b per week on 28-d cycles, and reported that the pharmacokinetics of pegylated IFN-α2b are not altered by the co-administration of temozolomide.[85]

Eto and collaborators prospectively investigated the predictive value of 11 single nucleotide polymorphisms (SNPs) affecting 8 distinct genes linked to immune responses among 203 RCC patients treated with 3 doses per wk of IFN-α (5 MIUs). The authors reported a response rate of 13.8% (9 complete responses, 19 partial responses), which was not influenced by any of the SNPs analyzed in this study. However, when

Table 1. Clinical trials recently launched to evaluate the safety and efficacy of immunostimulatory cytokines in cancer patients*

Cytokine	Indication(s)	Status	Phase	Route	Notes	Ref.
FLT3L	Lymphoma	Recruiting	II	*i.t.*	Combined with radiotherapy and a TLR3 agonist	NCT01976585
GM-CSF	Breast carcinoma Ovarian carcinoma	Recruiting	I/II	*s.c.*	Combined with a FOLR1-targeting vaccine	NCT02019524
	Follicular B-cell lymphoma	Completed	II	*s.c.*	Combined with rituximab	NCT01939730
	GBM	Not yet recruiting	I/II	n.a.	Combined with multipeptide vaccine and imiquimod	NCT02078648
	GBM Gliosarcoma	Not yet recruiting	II	*s.c.*	Combined with a cell-based vaccine, bevacizumab and cyclophosphamide	NCT01903330
	Melanoma	Completed	III	*s.c.*	As single agent or combined with TYR-targeting vaccine	NCT01989572
		Recruiting	I/II	n.a.	Combined with ipilimumab	NCT02009397
	Mesothelioma	Recruiting	II	*s.c.*	Combined with a WT1-targeting vaccine	NCT01890980
	NSCLC	Recruiting	II	n.a.	Combined with an autophagosome-derived vaccine and imiquimod	NCT01909752
IFN-α IFN-α2b	AML	Recruiting	IV	*n.a.*	As single agent upon allogeneic stem cell transplantation	NCT02027064
	Gastrointestinal neuroendocrine tumors	Not yet recruiting	III	*s.c.*	As single agent	NCT01860742
	Anal intraepithelial neoplasia	Recruiting	I/II	*s.c.*	Combined with a HPV-16-targeting vaccine	NCT01923116
	Childhood craniopharyngioma	Not yet recruiting	II	*s.c.*	As single agent	NCT01964300
	CML	Not yet recruiting	II	n.a.	Combined with dasatinib	NCT01872442
		Not yet recruiting	II	*s.c.*	Combined with imatinib and nilotinib	NCT02001818
		Recruiting	I	*s.c.*	Combined with imatinib	NCT01933906
		Recruiting	II	*s.c.*	Combined with nilotinib	NCT01866553
	Melanoma RCC	Not yet recruiting	I/II	*s.c.*	Combined with anti-PDCD1 mAb	NCT02089685
IFNγ	Soft tissue sarcoma	Recruiting	n.a.	*s.c.*	As single agent	NCT01957709

Abbreviations: AML, acute myeloid leukemia; CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; CML, chronic myeloid leukemia; FLT3L, fms-related tyrosine kinase 3 ligand; FOLH1, folate hydrolase 1; FOLR1, folate receptor 1; GBM, glioblastoma multiforme; GM-CSF, granulocyte macrophage colony-stimulating factor; HPV-16, human papillomavirus Type 16; IFN, interferon; IL, interleukin; *i.t.*, *intra tumorem*; *i.v.*, *intra venam*; mAb, monoclonal antibody; MRD, minimal residual disease; n.a., not available; NHL, non-Hodgkin lymphoma; NK, natural killer; NSCLC, non-small cell lung carcinoma; PBL, peripheral blood lymphocyte; PDCD1, programmed cell death 1; RCC, renal cell carcinoma; SABR, stereotactic ablative body radiotherapy; *s.c.*, *sub cutem*; TNFα, tumor necrosis factor α; TYR, tyrosinase; WT1, Wilms tumor 1. *Between 2013, May 1st and the date of submission.

disease stabilization for >24 wks was included among clinically favorable outcomes, a SNP affecting signal transducer and activator of transcription 3 (*STAT3*) was statistically associated with clinical responses, confirming previous observations from the same group.[86,87]

Harmon and coworkers evaluated potential biomarkers of efficacy among 750 treatment-naïve metastatic RCC patients randomized to receive 50 mg/day sunitinib (a multi-targeted receptor tyrosine kinase inhibitor)[88-90] on a 4-wk on/2-wk off schedule or 9 MIUs subcutaneous IFN-α 3 times per wk. Circulating IL-8 and VEGF-A levels at baseline were associated with overall survival independent of treatment. However, no independent predictors of IFN-α efficacy were identified by multivariate analysis.[91]

Among recent translational studies focusing on immunostimulatory cytokines in general, we found of particular interest the works of (1) Guermonprez and colleagues, who discovered a signaling pathway triggered by Plasmodium infection that regulates DC homeostasis and adaptive immune response upon the release of fms-related tyrosine kinase 3 ligand (FLT3L);[92] (2) Sim and coworkers, who demonstrated that $CD4^+CD25^+FOXP3^+$ Tregs accumulating in melanoma patients treated with high-dose IL-2 express inducible T-cell co-stimulator (ICOS)

Table 1. Clinical trials recently launched to evaluate the safety and efficacy of immunostimulatory cytokines in cancer patients* (continued)

Cytokine	Indication(s)	Status	Phase	Route	Notes	Ref.
IL-2	AML	Not yet recruiting	I	*s.c.*	Combined with adoptively transferred NK cells	NCT01898793
	Breast carcinoma Gastric carcinoma	Recruiting	I/II	*s.c.*	Combined with adoptively transferred NK cells and trastuzumab	NCT02030561
	Melanoma	Recruiting	II	*i.t.*	As an L19-fused immunocytokine combined with L19-TNFα	NCT02076633
	Merkel cell carcinoma	Recruiting	II	*i.v.*	As an F16-fused immunocytokine combined with paclitaxel	NCT02054884
	Multiple myeloma	Recruiting	II	n.a.	Combined with adoptively transferred NK cells	NCT01884688
	Neuroblastoma	Recruiting	II	*s.c.*	Coupled to an anti-GD2 mAb, G-CSF and GM-CSF for the treatment of MRD	NCT01857934
	NHL	Recruiting	I/II	*s.c.*	As a CD20-targeting immunocytokine	NCT01874288
	NSCLC	Not yet recruiting	I	*i.v.*	As an L19-fused immunocytokine after SABR	NCT02086721
	Prostate cancer	Recruiting	I/II	n.a.	Combined with FOLH1-specific CAR-expressing T cells	NCT01929239
	Solid tumors	Not yet recruiting	I	*s.c.*	Combined with NY-ESO-1-targeted PBLs and ipilimumab	NCT02070406
		Recruiting	I	*i.v.*	As a CEA-targeting immunocytokine	NCT02004106
			II	n.a.	Combined with NY-ESO-1-targeted PBLs	NCT01967823
IL-7	Prostate cancer	Not yet recruiting	II	*s.c.*	Combined with sipuleucel-T	NCT01881867
IL-10	Solid tumors	Recruiting	I	*s.c.*	As single agent	NCT02009449
IL-15	Solid tumors	Recruiting	I	*i.v.*	Combined with autologous activated NK cells	NCT01875601

Abbreviations: AML, acute myeloid leukemia; CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; CML, chronic myeloid leukemia; FLT3L, fms-related tyrosine kinase 3 ligand; FOLH1, folate hydrolase 1; FOLR1, folate receptor 1; GBM, glioblastoma multiforme; GM-CSF, granulocyte macrophage colony-stimulating factor; HPV-16, human papillomavirus Type 16; IFN, interferon; IL, interleukin; *i.t.*, *intra tumorem*; *i.v.*, *intra venam*; mAb, monoclonal antibody; MRD, minimal residual disease; n.a., not available; NHL, non-Hodgkin lymphoma; NK, natural killer; NSCLC, non-small cell lung carcinoma; PBL, peripheral blood lymphocyte; PDCD1, programmed cell death 1; RCC, renal cell carcinoma; SABR, stereotactic ablative body radiotherapy; *s.c.*, *sub cutem*; TNFα, tumor necrosis factor α; TYR, tyrosinase; WT1, Wilms tumor 1. *Between 2013, May 1st and the date of submission.

and exhibit an activated phenotype, as indicated by elevated levels of CD39, CD73 and transforming growth factor β1 (TGFβ1);[93] (3) Mortha and collaborators, who showed that GM-CSF is required for the establishment of Treg-dependent oral tolerance by intestinal macrophages;[94] and (4) West et al., who demonstrated that combining IL-2 with a mAb specific for CD274 mediates synergistic immunostimulatory effects.[95] These latter findings confirm and extend previous results indicating that immune checkpoint-blocking agents such as the cytotoxic T lymphocyte-associated protein 4 (CTLA4)-targeting mAb ipilimumab may significantly ameliorate the therapeutic profile of immunostimulatory cytokines.[96,97]

Update on Ongoing Clinical Trials

When this Trial Watch was being redacted (April 2014), official sources listed no less than 88 clinical trials launched after May 1st, 2013 that would evaluate the efficacy and safety of immunostimulatory cytokines in cancer patients (source http://www.clinicaltrials.gov). In 54 of these studies, IL-2 (14 trials), GM-CSF (4 trials), G-CSF (33 trials) and IFN-α (3 trials) were used as on-label interventions. These studies will not be discussed here. In addition, 34 clinical trials have been launched during the last 12 mo to investigate the immunostimulatory potential of various cytokines in off-label settings (**Table 1**).

Recombinant IL-2 is being tested (1) in combination with the adoptive transfer of natural killer (NK) cells,[55,98] in patients with relapsed or refractory acute myeloid leukemia (AML) (NCT01898793), multiple myeloma (MM) (NCT01884688), and *v-erb-b2* avian erythroblastic leukemia viral oncogene homolog 2 (ERBB2)$^+$ breast or gastric carcinoma (NCT02030561), in the latter setting coupled to the ERBB2-targeting monoclonal antibody (mAb) trastuzumab;[99-104] (2) in combination with autologous peripheral blood lymphocytes engineered to express a NY-ESO-1-specific T-cell receptor, alone or coupled to ipilimumab[105-107] in individuals affected by diverse solid tumors (NCT01967823; NCT02070406); (3) as an adjuvant to

T cells expressing a chimeric antigen receptor specific for folate hydrolase 1 (FOLH1, best known as PSMA),[108,109] in prostate cancer patients subjected to non-myeloablative conditioning (NCT01929239); and (4) in combination with G-CSF, GM-CSF and a mAb specific for ganglioside GD2,[110] for the treatment of minimal residual disease in children with advanced neuroblastoma treated with aggressive induction chemotherapy and stem cell transplantation (NCT01857934). Moreover, various studies have recently been launched to test the clinical profile of IL-2 variants retargeted to cancer cells by means of tumor-associated antigen (TAA)-specific antibodies or antibody fragments, i.e., IL-2-based immunocytokines.[111-113] Thus, (1) the safety and therapeutic potential of a carcinoembryonic antigen (CEA)-directed IL-2 variant (RO6895882) are being assessed in patients with advanced and/or metastatic solid tumors (NCT02004106); (2) a fusion between IL-2 and the Fv fragment of a mAb specific for tenascin C (TNC)[114] is being tested, in combination with the microtubular inhibitor paclitaxel, in Merkel cell carcinoma patients (NCT02054884); (3) the therapeutic value of a CD20-retargeted form of IL-2 is being investigated as a standalone intervention in subjects with NHL (NCT01874288); (4) the safety and efficacy of IL-2 fused to the Fv fragment of a mAb specific for the extradomain B of fibronectin (L19),[115-117] administered i.t. in combination with L19-TNFα, are being evaluated in melanoma patients (NCT02076633); and (5) the therapeutic profile of L19-IL-2 administered as a standalone agent immediately after stereotactic ablative body radiotherapy[118] is being investigated in subjects affected by metastatic non-small cell lung carcinoma (NSCLC) (NCT02086721). Moreover, (1) glycosylated recombinant human IL-7 is being tested as adjuvant to sipuleucel-T (an FDA-approved vaccine based on autologous peripheral blood mononuclear cells)[119] in subjects with castration-resistant prostate cancer (NCT01881867); (2) pegylated recombinant human IL-10 administered s.c. is being assessed as a standalone therapeutic intervention in patients with advanced solid tumors (NCT02009449); and (3) the clinical profile of recombinant human IL-15 given i.v. in combination with autologous activated NK cells is under evaluation in children and young adults affected by solid neoplasms (NCT01875601).

The safety and clinical potential of IFN-α2b, invariably administered s.c., are being investigated in cohorts of (1) subjects with gastrointestinal neuroendocrine tumors that failed to respond to somatostatin analogs, who receive IFN-α2b as a standalone agent (NCT01860742); (2) pediatric patients with unresectable of recurrent craniopharyngioma, who are treated with a pegylated variant of IFN-α2b (NCT01964300); (3) individuals with advanced melanoma or RCC, receiving pegylated IFN-α2b in combination with a mAb specific for programmed cell death 1 (PDCD1, best-known as PD-1)[120-123] (NCT02089685); (4) patients with chronic myeloid leukemia, who are treated with normal or pegylated IFN-α2b plus tyrosine kinase inhibitors including imatinib[124] (NCT01933906; NCT02001818), dasatinib[125] (NCT01872442) and nilotinib[126] (NCT01866553; NCT02001818); and (5) adults affected by anal intraepithelial neoplasms, who are concurrently vaccinated with a human papillomavirus Type 16 (HPV-16)-targeting peptide-based vaccine[24] (NCT01923116). In addition, a not-better defined variant of IFN-α is being tested as standalone intervention for molecular relapse in t(8; 21) AML patients who previously underwent allogeneic stem cell transplantation (NCT02027064); and recombinant IFN-γ is being assessed for its therapeutic efficacy in subjects with soft tissue sarcoma (NCT01957709).

GM-CSF is being intensively investigated for its ability to boost tumor-targeting immune responses elicited by wide panel of immunotherapeutic interventions. In particular, GM-CSF is being tested as an adjuvant to (1) ipilimumab,[27,70] in subjects with unresectable, Stage IIIC or IV melanoma (NCT02009397); (2) rituximab,[70,71] in individuals affected by follicular B-cell lymphoma (NCT01939730); (3) a peptide vaccine directed against folate receptor 1 (FOLR1, also known as FBP),[127,128] in patients with breast and ovarian carcinoma (NCT02019524); (4) SL-071, a multipeptide-based vaccine targeting several epitopes overexpressed by glioma cells,[129,130] administered to glioblastoma patients in combination with the TLR7 agonist imiquimod[131,132] (NCT02078648); (5) a peptide vaccine specific for Wilms tumor 1 (WT1),[133,134] in mesothelioma patients who have completed multimodal therapy (NCT01890980); (6) a tyrosinase (TYR)-targeting peptide vaccine,[135-137] in melanoma patients who underwent tumor resection (NCT01989572); (7) an autophagosome-based vaccine derived from allogeneic cancer cells,[138-140] administered to NSCLC patients in combination with imiquimod (NCT01909752); and (8) cyclophosphamide, the vascular endothelial growth factor (VEGF)-specific mAb bevacizumab[70,71,141,142] and a vaccine based on autologous cancer cells, in patients with glioblastoma multiforme or gliosarcoma (NCT01903330). Finally, the intratumoral administration of recombinant FLT3L[143-145] to B-cell lymphoma patients is being tested for its capacity to recruit DCs to neoplastic lesions and hence allow low-dose radiation therapy and a TLR3 agonist to induce clinically relevant anticancer immune responses (NCT01976585). This is a novel application for recombinant human FLT3L (also known as CDX-301), which is being developed as an alternative or a support to G-CSF for the mobilization of hematopoietic cell precursors in bone marrow donors, with promising results (source http://www.celldex.com/). Of note, official sources list NCT01989572 and NCT01939730 as "completed," yet no results are appear to be available at the moment.

As for the clinical trials listed in our previous Trial Watches dealing with this topic,[98,146] the following studies have changed status during the past 12 mo: NCT00589550, NCT00977145, NCT01099631, NCT01099631, NCT01131364, NCT01337544, which appear as 'terminated'; NCT00784524, NCT01236573 and NCT01490047, which are listed as 'suspended'; NCT00631371 and NCT00923351, which show as 'active, not recruiting' but are associated

with results; as well as NCT00601796, NCT00660270, NCT00719264 NCT00891475, NCT01021059, NCT01220648, NCT01404936, NCT01592045, NCT01637532, NCT01639885 and NCT01673217, which have been completed (source http://www.clinicaltrials.gov).

While NCT00977145, NCT00589550, NCT01099631 and NCT01392170 have been terminated owing to slow accrual, NCT01337544 has been halted because the parents of two patients enrolled who died presented a claim against the hospital. The reasons underlying the termination of NCT01131364 are not available. NCT00784524 has been suspended for interim analysis, whereas NCT01236573 has been temporarily halted for final data collection and primary outcome assessment. The reasons behind the suspension of NCT01490047 have not been reported. The results of NCT00660270,[147] NCT00719264, NCT00891475, NCT01021059, NCT01220648, NCT01592045, NCT01637532, NCT01639885 and NCT01673217 have not been disseminated yet. Conversely results are available for NCT00601796, NCT00631371, NCT00923351 and NCT01404936. In the context of NCT00601796, testing a GM-CSF-involving cell-based vaccine in combination with cyclophosphamide and all-*trans* retinoic acid in lung cancer patients, 5 immunological responses were observed among 14 evaluable patients, the median time to progression and median overall survival among 24 treated patients being 2.4 and 8 mo, respectively. Preliminary results from NCT00631371, which is still ongoing, revealed that bevacizumab plus IFN-α is not inferior to bevacizumab plus the mammalian target of rapamycin (mTOR) inhibitor temsirolimus for the treatment of advanced RCC patients, but associated with lower incidence of serious adverse effects (36.6% vs. 44.3%). NCT00923351 is investigating the ability of recombinant IL-7 to boost the therapeutic activity of a DC-based vaccine in patients with Ewing's Sarcoma, rhabdomyosarcoma or neuroblastoma. Preliminary results indicate that IL-7 may indeed promote the immunogenic potential of DCs loaded ex vivo with autologous cancer cell lysates but not increase the toxicity of the procedure. The number of patients enrolled and analyzed so far, however, appears to be excessively low for drawing robust conclusions from this study. NCT01404936 evaluated the combination of IFN-α2a with a multi-agent chemotherapeutic regimen in Hodgkin lymphoma patients. In this setting, 23 out of 30 patients achieved a complete response to treatment, while serious side effects affected only 10% of participants. Additional, randomized and comparatively larger clinical studies are required to validate these findings.

Concluding Remarks

The activation of novel or the reactivation of existing immune responses has been shown to underlie the clinical efficacy not only of an increasingly wide panel of immunotherapeutic interventions[148] but also of multiple radiotherapeutic and chemotherapeutic regimens.[56,57,149,150] Along with the realization that the immune system plays a fundamental role in the response of cancer patients to therapy, great interest has gathered around the possibility to harness the immunostimulatory potential of multiple cytokines to drive tumor-targeting immune responses. As discussed above, however, using cytokines as standalone immunostimulatory interventions does not suffice to elicit therapeutically relevant immune responses in a majority of cancer patients, exception made for individuals with melanoma and RCC, which are particularly immunogenic per se. Thus, current efforts focus on the use of immunostimulatory cytokines as adjuvants to other immunotherapeutic paradigms, especially immune checkpoint-blocking mAbs. IL-2 and GM-CSF are perhaps the molecules that have generated the greatest interest in this setting. However, recent preclinical and clinical data indicate that the immunological activity of both IL-2 and GM-CSF may exhibit a significant degree of context dependency. Indeed, high-dose IL-2 has been shown to promote the accumulation of immunosuppressive $CD4^+CD25^+FOXP3^+$ regulatory T cells (Tregs) in both cancer and HIV-1 patients,[93,151-156] while GM-CSF has been involved in the establishment of Treg-mediated oral tolerance by intestinal macrophages.[94] These data suggest that selectively targeting IL-2 or GM-CSF to specific immune effectors may further improve their immunostimulatory activity (and hence their clinical profile). So far, immunocytokines have mostly been designed to deliver immunostimulatory signals the tumor microenvironment in a relatively unspecific manner (i.e., they have been developed based on TAA-specific mAb). However, neoplastic lesions contain high amounts of Tregs, myeloid-derived suppressor cells and tumor-associated macrophages,[157] implying that such a strategy may promote the unwarranted expansion of immunosuppressive cells. Further studies are required to unveil whether targeting immunocytokines to specific populations of immune effector cells results in optimal immunostimulation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Authors are supported by the Ligue contre le Cancer (équipe labelisée); Agence National de la Recherche (ANR); Association pour la recherche sur le cancer (ARC); Cancéropôle Ile-de-France; AXA Chair for Longevity Research; Institut National du Cancer (INCa); Fondation Bettencourt-Schueller; Fondation de France; Fondation pour la Recherche Médicale (FRM); the European Commission (ArtForce); the European Research Council (ERC); the LabEx Immuno-Oncology; the SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE); the SIRIC Cancer Research and Personalized Medicine (CARPEM); and the Paris Alliance of Cancer Research Institutes (PACRI).

References

1. Tato CM, Cua DJ. SnapShot: Cytokines I. Cell 2008; 132:324. e1.
2. Tato CM, Cua DJ. SnapShot: cytokines II. Cell 2008; 132:500; PMID:18267079
3. Tato CM, Cua DJ. SnapShot: cytokines III. Cell 2008; 132:900; PMID:18329374

4. Tato CM, Cua DJ. SnapShot: Cytokines IV. Cell 2008; 132:e1-2; PMID:18358817
5. Vacchelli E, Eggermont A, Fridman WH, Galon J, Zitvogel L, Kroemer G, Galluzzi L. Trial Watch: Immunostimulatory cytokines. Oncoimmunology 2013; 2:e24850; PMID:24073369; http://dx.doi.org/10.4161/onci.24850
6. Vacchelli E, Galluzzi L, Eggermont A, Galon J, Tartour E, Zitvogel L, Kroemer G. Trial Watch: Immunostimulatory cytokines. Oncoimmunology 2012; 1:493-506; PMID:22754768; http://dx.doi.org/10.4161/onci.20459
7. Papatriantafyllou M. Cytokines: true to their family name. Nat Rev Immunol 2013; 13:544-5; PMID:23827957; http://dx.doi.org/10.1038/nri3496
8. Borish LC, Steinke JW. 2. Cytokines and chemokines. J Allergy Clin Immunol 2003; 111(Suppl):S460-75; PMID:12592293; http://dx.doi.org/10.1067/mai.2003.108
9. Steinke JW, Borish L. 3. Cytokines and chemokines. J Allergy Clin Immunol 2006; 117(Suppl Mini-Primer):S441-5; PMID:16455343; http://dx.doi.org/10.1016/j.jaci.2005.07.001
10. Metcalf D. The colony-stimulating factors and cancer. Nat Rev Cancer 2010; 10:425-34; PMID:20495576; http://dx.doi.org/10.1038/nrc2843
11. Neufeld G, Kessler O. Pro-angiogenic cytokines and their role in tumor angiogenesis. Cancer Metastasis Rev 2006; 25:373-85; PMID:17006765; http://dx.doi.org/10.1007/s10555-006-9011-5
12. Benelli R, Lorusso G, Albini A, Noonan DM. Cytokines and chemokines as regulators of angiogenesis in health and disease. Curr Pharm Des 2006; 12:3101-15; PMID:16918437; http://dx.doi.org/10.2174/138161206777947461
13. Ohlsson K, Björk P, Bergenfeldt M, Hageman R, Thompson RC. Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. Nature 1990; 348:550-2; PMID:2147233; http://dx.doi.org/10.1038/348550a0
14. Tracey KJ, Fong Y, Hesse DG, Manogue KR, Lee AT, Kuo GC, Lowry SF, Cerami A. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature 1987; 330:662-4; PMID:3317066; http://dx.doi.org/10.1038/330662a0
15. Andrews DM, Chow MT, Ma Y, Cotterell CL, Watt SV, Anthony DA, Akira S, Iwakura Y, Trapani JA, Zitvogel L, et al. Homeostatic defects in interleukin 18-deficient mice contribute to protection against the lethal effects of endotoxin. Immunol Cell Biol 2011; 89:739-46; PMID:21263463; http://dx.doi.org/10.1038/icb.2010.168
16. Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. Nat Immunol 2005; 6:1191-7; PMID:16369558; http://dx.doi.org/10.1038/ni1276
17. Brenner C, Galluzzi L, Kepp O, Kroemer G. Decoding cell death signals in liver inflammation. J Hepatol 2013; 59:583-94; PMID:23567086; http://dx.doi.org/10.1016/j.jhep.2013.03.033
18. Senovilla L, Galluzzi L, Zitvogel L, Kroemer G. Immunosurveillance as a regulator of tissue homeostasis. Trends Immunol 2013; 34:471-81; PMID:23891238; http://dx.doi.org/10.1016/j.it.2013.06.005
19. Dranoff G. Cytokines in cancer pathogenesis and cancer therapy. Nat Rev Cancer 2004; 4:11-22; PMID:14708024; http://dx.doi.org/10.1038/nrc1252
20. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002; 420:860-7; PMID:12490959; http://dx.doi.org/10.1038/nature01322
21. Coussens LM, Zitvogel L, Palucka AK. Neutralizing tumor-promoting chronic inflammation: a magic bullet? Science 2013; 339:286-91; PMID:23329041; http://dx.doi.org/10.1126/science.1232227
22. Vacchelli E, Vitale I, Eggermont A, Fridman WH, Fučíková J, Cremer I, Galon J, Tartour E, Zitvogel L, Kroemer G, et al. Trial watch: Dendritic cell-based interventions for cancer therapy. Oncoimmunology 2013; 2:e25771; PMID:24286020; http://dx.doi.org/10.4161/onci.25771
23. Pol J, Bloy N, Obrist F, Eggermont A, Galon J, Hervé Fridman W, Cremer I, Zitvogel L, Kroemer G, Galluzzi L. Trial Watch: DNA vaccines for cancer therapy. Oncoimmunology 2014; 3:e28185; PMID:24800178; http://dx.doi.org/10.4161/onci.28185
24. Aranda F, Vacchelli E, Eggermont A, Galon J, Sautès-Fridman C, Tartour E, Zitvogel L, Kroemer G, Galluzzi L. Trial Watch: Peptide vaccines in cancer therapy. Oncoimmunology 2013; 2:e26621; PMID:24498550; http://dx.doi.org/10.4161/onci.26621
25. Vacchelli E, Eggermont A, Sautès-Fridman C, Galon J, Zitvogel L, Kroemer G, Galluzzi L. Trial Watch: Toll-like receptor agonists for cancer therapy. Oncoimmunology 2013; 2:e25238; PMID:24083080; http://dx.doi.org/10.4161/onci.25238
26. Yamazaki T, Hannani D, Poirier-Colame V, Ladoire S, Locher C, Sistigu A, Prada N, Adjemian S, Catani JP, Freudenberg M, et al. Defective immunogenic cell death of HMGB1-deficient tumors: compensatory therapy with TLR4 agonists. Cell Death Differ 2014; 21:69-78; PMID:23811849; http://dx.doi.org/10.1038/cdd.2013.72
27. Aranda F, Vacchelli E, Eggermont A, Galon J, Fridman WH, Zitvogel L, Kroemer G, Galluzzi L. Trial Watch: Immunostimulatory monoclonal antibodies in cancer therapy. Oncoimmunology 2014; 3:e27297; PMID:24701370; http://dx.doi.org/10.4161/onci.27297
28. Arellano M, Lonial S. Clinical uses of GM-CSF, a critical appraisal and update. Biologics 2008; 2:13-27; PMID:19707424; http://dx.doi.org/10.2147/BTT.S1355
29. Khoury HJ, Loberiza FR Jr., Ringdén O, Barrett AJ, Bolwell BJ, Cahn JY, Champlin RE, Gale RP, Hale GA, Urbano-Ispizua A, et al. Impact of posttransplantation G-CSF on outcomes of allogeneic hematopoietic stem cell transplantation. Blood 2006; 107:1712-6; PMID:16239431; http://dx.doi.org/10.1182/blood-2005-07-2661
30. Sebban C, Lefranc A, Perrier L, Moreau P, Espinouse D, Schmidt A, Kammoun L, Ghesquieres H, Ferlay C, Bay JO, et al. A randomised phase II study of the efficacy, safety and cost-effectiveness of pegfilgrastim and filgrastim after autologous stem cell transplant for lymphoma and myeloma (PALM study). Eur J Cancer 2012; 48:713-20; PMID:22248711; http://dx.doi.org/10.1016/j.ejca.2011.12.016
31. Hosing C. Hematopoietic stem cell mobilization with G-CSF. Methods Mol Biol 2012; 904:37-47; PMID:22890920
32. Demirer T, Ayli M, Ozcan M, Gunel N, Haznedar R, Dagli M, Fen T, Genc Y, Dincer S, Arslan O, et al. Mobilization of peripheral blood stem cells with chemotherapy and recombinant human granulocyte colony-stimulating factor (rhG-CSF): a randomized evaluation of different doses of rhG-CSF. Br J Haematol 2002; 116:468-74; PMID:11841454; http://dx.doi.org/10.1046/j.1365-2141.2002.03264.x
33. Naeim A, Henk HJ, Becker L, Chia V, Badre S, Li X, Deeter R. Pegfilgrastim prophylaxis is associated with a lower risk of hospitalization of cancer patients than filgrastim prophylaxis: a retrospective United States claims analysis of granulocyte colony-stimulating factors (G-CSF). BMC Cancer 2013; 13:11; PMID:23298389; http://dx.doi.org/10.1186/1471-2407-13-11
34. Chan KK, Siu E, Krahn MD, Imrie K, Alibhai SM. Cost-utility analysis of primary prophylaxis versus secondary prophylaxis with granulocyte colony-stimulating factor in elderly patients with diffuse aggressive lymphoma receiving curative-intent chemotherapy. J Clin Oncol 2012; 30:1064-71; PMID:22393098; http://dx.doi.org/10.1200/JCO.2011.36.8647
35. Pabst T, Vellenga E, van Putten W, Schouten HC, Graux C, Vekemans MC, Biemond B, Sonneveld P, Passweg J, Verdonck L, et al.; Dutch-Belgian Hemato-Oncology Cooperative Group (HOVON); German AML Study Group (AMLSG); Swiss Collaborative Group for Clinical Cancer Research (SAKK). Favorable effect of priming with granulocyte colony-stimulating factor in remission induction of acute myeloid leukemia restricted to dose escalation of cytarabine. Blood 2012; 119:5367-73; PMID:22422824; http://dx.doi.org/10.1182/blood-2011-11-389841
36. Deroose JP, Eggermont AM, van Geel AN, Burger JW, den Bakker MA, de Wilt JH, Verhoef C. Long-term results of tumor necrosis factor alpha- and melphalan-based isolated limb perfusion in locally advanced extremity soft tissue sarcomas. J Clin Oncol 2011; 29:4036-44; PMID:21931039; http://dx.doi.org/10.1200/JCO.2011.35.6618
37. Deroose JP, Eggermont AM, van Geel AN, de Wilt JH, Burger JW, Verhoef C. 20 years experience of TNF-based isolated limb perfusion for in-transit melanoma metastases: TNF dose matters. Ann Surg Oncol 2012; 19:627-35; PMID:21879272; http://dx.doi.org/10.1245/s10434-011-2030-7
38. Deroose JP, Grünhagen DJ, van Geel AN, de Wilt JH, Eggermont AM, Verhoef C. Long-term outcome of isolated limb perfusion with tumour necrosis factor-α for patients with melanoma in-transit metastases. Br J Surg 2011; 98:1573-80; PMID:21739427; http://dx.doi.org/10.1002/bjs.7621
39. Eggermont AM. The success of TNF alpha in isolated limb perfusion for irresectable extremity soft tissue sarcomas, melanoma and carcinomas: observations in patients and preclinical perfusion models. Gan To Kagaku Ryoho 1996; 23:1357-70; PMID:8854755
40. Eggermont AM, Schraffordt Koops H, Klausner JM, Kroon BB, Schlag PM, Liénard D, van Geel AN, Hoekstra HJ, Meller I, Nieweg OE, et al. Isolated limb perfusion with tumor necrosis factor and melphalan for limb salvage in 186 patients with locally advanced soft tissue extremity sarcomas. The cumulative multicenter European experience. Ann Surg 1996; 224:756-64, discussion 764-5; PMID:8968230; http://dx.doi.org/10.1097/00000658-199612000-00011
41. Eggermont AM, Schraffordt Koops H, Klausner JM, Schlag PM, Kroon BB, Ben-Ari G, Lejeune FJ. Isolated limb perfusion with high-dose tumor necrosis factor-alpha for locally advanced extremity soft tissue sarcomas. Cancer Treat Res 1997; 91:189-203; PMID:9286497; http://dx.doi.org/10.1007/978-1-4615-6121-7_13
42. Eggermont AM, Schraffordt Koops H, Liénard D, Kroon BB, van Geel AN, Hoekstra HJ, Lejeune FJ. Isolated limb perfusion with high-dose tumor necrosis factor-alpha in combination with interferon-gamma and melphalan for nonresectable extremity soft tissue sarcomas: a multicenter trial. J Clin Oncol 1996; 14:2653-65; PMID:8874324
43. Eggermont AM, Suciu S, Testori A, Santinami M, Kruit WH, Marsden J, Punt CJ, Salès F, Dummer R, Robert C, et al. Long-term results of the randomized phase III trial EORTC 18991 of adjuvant therapy with pegylated interferon alfa-2b versus observation in resected stage III melanoma. J Clin Oncol 2012; 30:3810-8; PMID:23008300; http://dx.doi.org/10.1200/JCO.2011.41.3799

44. Grünhagen DJ, Brunstein F, ten Hagen TL, van Geel AN, de Wilt JH, Eggermont AM. TNF-based isolated limb perfusion: a decade of experience with antivascular therapy in the management of locally advanced extremity soft tissue sarcomas. Cancer Treat Res 2004; 120:65-79; PMID:15217218; http://dx.doi.org/10.1007/1-4020-7856-0_4
45. Trabulsi NH, Patakfalvi L, Nassif MO, Turcotte RE, Nichols A, Meguerditchian AN. Hyperthermic isolated limb perfusion for extremity soft tissue sarcomas: systematic review of clinical efficacy and quality assessment of reported trials. J Surg Oncol 2012; 106:921-8; PMID:22806575; http://dx.doi.org/10.1002/jso.23200
46. Grünhagen DJ, de Wilt JH, ten Hagen TL, Eggermont AM. Technology insight: Utility of TNF-alpha-based isolated limb perfusion to avoid amputation of irresectable tumors of the extremities. Nat Clin Pract Oncol 2006; 3:94-103; PMID:16462850; http://dx.doi.org/10.1038/ncponc0426
47. Atkins MB, Lotze MT, Dutcher JP, Fisher RI, Weiss G, Margolin K, Abrams J, Sznol M, Parkinson D, Hawkins M, et al. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. J Clin Oncol 1999; 17:2105-16; PMID:10561265
48. Fyfe GA, Fisher RI, Rosenberg SA, Sznol M, Parkinson DR, Louie AC. Long-term response data for 255 patients with metastatic renal cell carcinoma treated with high-dose recombinant interleukin-2 therapy. J Clin Oncol 1996; 14:2410-1; PMID:8708739
49. Fyfe G, Fisher RI, Rosenberg SA, Sznol M, Parkinson DR, Louie AC. Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy. J Clin Oncol 1995; 13:688-96; PMID:7884429
50. Hauschild A. Adjuvant interferon alfa for melanoma: new evidence-based treatment recommendations? Curr Oncol 2009; 16:3-6; PMID:19526078; http://dx.doi.org/10.3747/co.v16i3.447
51. Anger B, Porzsolt F, Leichtle R, Heinze B, Bartram C, Heimpel H. A phase I/II study of recombinant interferon alpha 2a and hydroxyurea for chronic myelocytic leukemia. Blut 1989; 58:275-8; PMID:2736308; http://dx.doi.org/10.1007/BF00320165
52. Hershman D, Neugut AI, Jacobson JS, Wang J, Tsai WY, McBride R, Bennett CL, Grann VR. Acute myeloid leukemia or myelodysplastic syndrome following use of granulocyte colony-stimulating factors during breast cancer adjuvant chemotherapy. J Natl Cancer Inst 2007; 99:196-205; PMID:17284714; http://dx.doi.org/10.1093/jnci/djk028
53. Di Cosimo S, Ferretti G, Papaldo P, Carlini P, Fabi A, Ruggeri EM, Alimonti A, Nardoni C, Cognetti F. Does the concurrent use of anthracycline and granulocyte colony-stimulating factor influence the risk of secondary leukaemia in breast cancer women? Ann Oncol 2005; 16:1209-10; PMID:15857847; http://dx.doi.org/10.1093/annonc/mdi201
54. Relling MV, Boyett JM, Blanco JG, Raimondi S, Behm FG, Sandlund JT, Rivera GK, Kun LE, Evans WE, Pui CH. Granulocyte colony-stimulating factor and the risk of secondary myeloid malignancy after etoposide treatment. Blood 2003; 101:3862-7; PMID:12531808; http://dx.doi.org/10.1182/blood-2002-08-2405
55. Aranda F, Vacchelli E, Obrist F, Eggermont A, Fridman WH, Galon J, et al. Trial Watch: Adoptive cell transfer for anticancer immunotherapy. OncoImmunology 2013; 2:e24238; http://dx.doi.org/10.4161/onci.24238
56. Galluzzi L, Senovilla L, Zitvogel L, Kroemer G. The secret al.y: immunostimulation by anticancer drugs. Nat Rev Drug Discov 2012; 11:215-33; PMID:22301798; http://dx.doi.org/10.1038/nrd3626
57. Zitvogel L, Galluzzi L, Smyth MJ, Kroemer G. Mechanism of action of conventional and targeted anticancer therapies: reinstating immunosurveillance. Immunity 2013; 39:74-88; PMID:23890065; http://dx.doi.org/10.1016/j.immuni.2013.06.014
58. Viaud S, Saccheri F, Mignot G, Yamazaki T, Daillère R, Hannani D, Enot DP, Pfirschke C, Engblom C, Pittet MJ, et al. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science 2013; 342:971-6; PMID:24264990; http://dx.doi.org/10.1126/science.1240537
59. Becker JC, Schrama D. The dark side of cyclophosphamide: cyclophosphamide-mediated ablation of regulatory T cells. J Invest Dermatol 2013; 133:1462-5; PMID:23673502; http://dx.doi.org/10.1038/jid.2013.67
60. Connor JP, Cristea MC, Lewis NL, Lewis LD, Komarnitsky PB, Mattiacci MR, Felder M, Stewart S, Harter J, Henslee-Downey J, et al. A phase 1b study of humanized KS-interleukin-2 (huKS-IL2) immunocytokine with cyclophosphamide in patients with EpCAM-positive advanced solid tumors. BMC Cancer 2013; 13:20; PMID:23320927; http://dx.doi.org/10.1186/1471-2407-13-20
61. Dutcher JP, Neuberg D, Atkins MB, Tester WJ, Wadler S, Stewart JA, Chachoua A, Schuchter LM. Report of a Phase I Evaluation of Dose and Schedule of Interleukin-1 Alpha and Cyclophosphamide in Patients with Advanced Tumors: An Eastern Cooperative Oncology Group Study (PX990) and Review of IL-1-Based Studies of Hematopoietic Reconstitution. J Interferon Cytokine Res 2014; 34:376-84; PMID:24433038; http://dx.doi.org/10.1089/jir.2013.0010
62. Susini C, Buscail L. Rationale for the use of somatostatin analogs as antitumor agents. Ann Oncol 2006; 17:1733-42; PMID:16801334; http://dx.doi.org/10.1093/annonc/mdl105
63. Reubi JC, Schonbrunn A. Illuminating somatostatin analog action at neuroendocrine tumor receptors. Trends Pharmacol Sci 2013; 34:676-88; PMID:24183675; http://dx.doi.org/10.1016/j.tips.2013.10.001
64. Vitale G, Lupoli G, Guarrasi R, Colao A, Dicitore A, Gaudenzi G, Misso G, Castellano M, Addeo R, Facchini G, et al. Interleukin-2 and lanreotide in the treatment of medullary thyroid cancer: in vitro and in vivo studies. J Clin Endocrinol Metab 2013; 98:E1567-74; PMID:23884781; http://dx.doi.org/10.1210/jc.2013-1443
65. Vacchelli E, Galluzzi L, Eggermont A, Fridman WH, Galon J, Sautès-Fridman C, Tartour E, Zitvogel L, Kroemer G. Trial watch: FDA-approved Toll-like receptor agonists for cancer therapy. Oncoimmunology 2012; 1:894-907; PMID:23162757; http://dx.doi.org/10.4161/onci.20931
66. Cutando A, López-Valverde A, Arias-Santiago S, DE Vicente J, DE Diego RG. Role of melatonin in cancer treatment. Anticancer Res 2012; 32:2747-53; PMID:22753734
67. Vishwas DK, Mukherjee A, Haldar C. Melatonin improves humoral and cell-mediated immune responses of male golden hamster following stress induced by dexamethasone. J Neuroimmunol 2013; 259:17-25; PMID:23582490; http://dx.doi.org/10.1016/j.jneuroim.2013.03.002
68. Srinivasan V, Pandi-Perumal SR, Brzezinski A, Bhatnagar KP, Cardinali DP. Melatonin, immune function and cancer. Recent Pat Endocr Metab Immune Drug Discov 2011; 5:109-23; PMID:22074586; http://dx.doi.org/10.2174/187221411799015408
69. Tomov B, Popov D, Tomova R, Vladov N, Den Otter W, Krastev Z. Therapeutic response of untreatable hepatocellular carcinoma after application of the immune modulators IL-2, BCG and melatonin. Anticancer Res 2013; 33:4531-5; PMID:24123026
70. Vacchelli E, Eggermont A, Galon J, Sautès-Fridman C, Zitvogel L, Kroemer G, Galluzzi L. Trial watch: Monoclonal antibodies in cancer therapy. Oncoimmunology 2013; 2:e22789; PMID:23482847; http://dx.doi.org/10.4161/onci.22789
71. Vacchelli E, Aranda F, Eggermont A, Galon J, Sautès-Fridman C, Zitvogel L, Kroemer G, Galluzzi L. Trial Watch: Tumor-targeting monoclonal antibodies in cancer therapy. Oncoimmunology 2014; 3:e27048; PMID:24605265; http://dx.doi.org/10.4161/onci.27048
72. Akhtar S, Maghfoor I. Rituximab plus CHOP for diffuse large-B-cell lymphoma. N Engl J Med 2002; 346:1830-1, author reply 1830-1; PMID:12050349; http://dx.doi.org/10.1056/NEJM200206063462317
73. Coiffier B, Lepage E, Briere J, Herbrecht R, Tilly H, Bouabdallah R, Morel P, Van Den Neste E, Salles G, Gaulard P, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 2002; 346:235-42; PMID:11807147; http://dx.doi.org/10.1056/NEJMoa011795
74. Robertson MJ, Kline J, Struemper H, Koch KM, Bauman JW, Gardner OS, Murray SC, Germaschewski F, Weisenbach J, Jonak Z, et al. A dose-escalation study of recombinant human interleukin-18 in combination with rituximab in patients with non-Hodgkin lymphoma. J Immunother 2013; 36:331-41; PMID:23799412; http://dx.doi.org/10.1097/CJI.0b013e31829d7e2e
75. Galluzzi L, Vacchelli E, Fridman WH, Galon J, Sautès-Fridman C, Tartour E, Zucman-Rossi J, Zitvogel L, Kroemer G. Trial Watch: Monoclonal antibodies in cancer therapy. Oncoimmunology 2012; 1:28-37; PMID:22720209; http://dx.doi.org/10.4161/onci.1.1.17938
76. Hale G, Bright S, Chumbley G, Hoang T, Metcalf D, Munro AJ, Waldmann H. Removal of T cells from bone marrow for transplantation: a monoclonal antilymphocyte antibody that fixes human complement. Blood 1983; 62:873-82; PMID:6349718
77. Gorin NC, Isnard F, Garderet L, Ikhlef S, Corm S, Quesnel B, Legrand O, Cachanado M, Rousseau A, Laporte JP. Administration of alemtuzumab and G-CSF to adults with relapsed or refractory acute lymphoblastic leukemia: results of a phase II study. Eur J Haematol 2013; 91:315-21; PMID:23738686
78. Kushner BH, Cheung NK. GM-CSF enhances 3F8 monoclonal antibody-dependent cellular cytotoxicity against human melanoma and neuroblastoma. Blood 1989; 73:1936-41; PMID:2653466
79. Heiner JP, Miraldi F, Kallick S, Makley J, Neely J, Smith-Mensah WH, Cheung NK. Localization of GD2-specific monoclonal antibody 3F8 in human osteosarcoma. Cancer Res 1987; 47:5377-81; PMID:3115567
80. Cheung NK, Lazarus H, Miraldi FD, Abramowsky CR, Kallick S, Saarinen UM, Spitzer T, Strandjord SE, Coccia PF, Berger NA. Ganglioside GD2 specific monoclonal antibody 3F8: a phase I study in patients with neuroblastoma and malignant melanoma. J Clin Oncol 1987; 5:1430-40; PMID:3625258
81. Cheung IY, Hsu K, Cheung NK. Activation of peripheral-blood granulocytes is strongly correlated with patient outcome after immunotherapy with anti-GD2 monoclonal antibody and granulocyte-macrophage colony-stimulating factor. J Clin Oncol 2012; 30:426-32; PMID:22203761; http://dx.doi.org/10.1200/JCO.2011.37.6236

82. Cheung NK, Cheung IY, Kramer K, Modak S, Kuk D, Pandit-Taskar N, Chamberlain E, Ostrovnaya I, Kushner BH. Key role for myeloid cells: Phase II results of anti-GD2 antibody 3F8 plus granulocyte-macrophage colony-stimulating factor for chemoresistant osteomedullary neuroblastoma. Int J Cancer 2014; (Forthcoming); PMID:24644014; http://dx.doi.org/10.1002/ijc.28851
83. Cheung NK, Guo H, Hu J, Tassev DV, Cheung IY. Humanizing murine IgG3 anti-GD2 antibody m3F8 substantially improves antibody-dependent cell-mediated cytotoxicity while retaining targeting in vivo. Oncoimmunology 2012; 1:477-86; PMID:22754766; http://dx.doi.org/10.4161/onci.19864
84. Zarogoulidis K, Ziogas E, Boutsikou E, Zarogoulidis P, Darwiche K, Kontakiotis T, Tsakiridis K, Porpodis K, Latsios D, Chatzizisi O, et al. Immunomodifiers in combination with conventional chemotherapy in small cell lung cancer: a phase II, randomized study. Drug Des Devel Ther 2013; 7:611-7; PMID:23901264; http://dx.doi.org/10.2147/DDDT.S43184
85. Coker SA, Dandamudi UB, Beelen AP, Crosby NA, Fisher JL, Obrocea M, Ernstoff MS, Lewis LD. A phase I, dose-escalation study of cyclical weekly oral temozolomide and weekly PEG-interferon alpha-2b in patients with refractory or advanced solid tumours. J Chemother 2013; 25:362-8; PMID:24093213; http://dx.doi.org/10.1179/1973947813Y.0000000102
86. Eto M, Kamba T, Miyake H, Fujisawa M, Kamai T, Uemura H, Tsukamoto T, Azuma H, Matsubara A, Nishimura K, et al.; Japan Immunotherapy SNPs-Study Group for Kidney Cancer. STAT3 polymorphism can predict the response to interferon-α therapy in patients with metastatic renal cell carcinoma. Eur Urol 2013; 63:745-52; PMID:23063454; http://dx.doi.org/10.1016/j.eururo.2012.09.052
87. Ito N, Eto M, Nakamura E, Takahashi A, Tsukamoto T, Toma H, Nakazawa H, Hirao Y, Uemura H, Kagawa S, et al. STAT3 polymorphism predicts interferon-alfa response in patients with metastatic renal cell carcinoma. J Clin Oncol 2007; 25:2785-91; PMID:17602083; http://dx.doi.org/10.1200/JCO.2006.09.8897
88. Faivre S, Demetri G, Sargent W, Raymond E. Molecular basis for sunitinib efficacy and future clinical development. Nat Rev Drug Discov 2007; 6:734-45; PMID:17690708; http://dx.doi.org/10.1038/nrd2380
89. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, Oudard S, Negrier S, Szczylik C, Kim ST, et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 2007; 356:115-24; PMID:17215529; http://dx.doi.org/10.1056/NEJMoa065044
90. Nicholaou T, Wong R, Davis ID. Tumour lysis syndrome in a patient with renal-cell carcinoma treated with sunitinib malate. Lancet 2007; 369:1923-4; PMID:17560435; http://dx.doi.org/10.1016/S0140-6736(07)60903-9
91. Harmon CS, DePrimo SE, Figlin RA, Hudes GR, Hutson TE, Michaelson MD, Négrier S, Kim ST, Huang X, Williams JA, et al. Circulating proteins as potential biomarkers of sunitinib and interferon-α efficacy in treatment-naïve patients with metastatic renal cell carcinoma. Cancer Chemother Pharmacol 2014; 73:151-61; PMID:24220935; http://dx.doi.org/10.1007/s00280-013-2333-4
92. Guermonprez P, Helft J, Claser C, Deroubaix S, Karanje H, Gazumyan A, Darasse-Jèze G, Telerman SB, Breton G, Schreiber HA, et al. Inflammatory Flt3l is essential to mobilize dendritic cells and for T cell responses during Plasmodium infection. Nat Med 2013; 19:730-8; PMID:23685841; http://dx.doi.org/10.1038/nm.3197
93. Sim GC, Martin-Orozco N, Jin L, Yang Y, Wu S, Washington E, Sanders D, Lacey C, Wang Y, Vence L, et al. IL-2 therapy promotes suppressive ICOS+ Treg expansion in melanoma patients. J Clin Invest 2014; 124:99-110; PMID:24292706; http://dx.doi.org/10.1172/JCI46266
94. Mortha A, Chudnovskiy A, Hashimoto D, Bogunovic M, Spencer SP, Belkaid Y, Merad M. Microbiota-dependent crosstalk between macrophages and ILC3 promotes intestinal homeostasis. Science 2014; 343:1249288; PMID:24625929; http://dx.doi.org/10.1126/science.1249288
95. West EE, Jin HT, Rasheed AU, Penaloza-Macmaster P, Ha SJ, Tan WG, Youngblood B, Freeman GJ, Smith KA, Ahmed R. PD-L1 blockade synergizes with IL-2 therapy in reinvigorating exhausted T cells. J Clin Invest 2013; 123:2604-15; PMID:23676462; http://dx.doi.org/10.1172/JCI67008
96. Omori R, Eguchi J, Hiroishi K, Ishii S, Hiraide A, Sakaki M, Doi H, Kajiwara A, Ito T, Kogo M, et al. Effects of interferon-α-transduced tumor cell vaccines and blockade of programmed cell death-1 on the growth of established tumors. Cancer Gene Ther 2012; 19:637-43; PMID:22790963; http://dx.doi.org/10.1038/cgt.2012.42
97. Prieto PA, Yang JC, Sherry RM, Hughes MS, Kammula US, White DE, Levy CL, Rosenberg SA, Phan GQ. CTLA-4 blockade with ipilimumab: long-term follow-up of 177 patients with metastatic melanoma. Clin Cancer Res 2012; 18:2039-47; PMID:22271879; http://dx.doi.org/10.1158/1078-0432.CCR-11-1823
98. Vacchelli E, Eggermont A, Fridman WH, Galon J, Tartour E, Zitvogel L, Kroemer G, Galluzzi L. Trial Watch: Adoptive cell transfer for anticancer immunotherapy. Oncoimmunology 2013; 2:e24238; PMID:23762803; http://dx.doi.org/10.4161/onci.24238
99. Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE Jr., Davidson NE, Tan-Chiu E, Martino S, Paik S, Kaufman PA, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 2005; 353:1673-84; PMID:16236738; http://dx.doi.org/10.1056/NEJMoa052122
100. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, Goldhirsch A, Untch M, Smith I, Gianni L, Baselga J, Bell R, Jackisch C, et al.; Herceptin Adjuvant (HERA) Trial Study Team. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 2005; 353:1659-72; PMID:16236737; http://dx.doi.org/10.1056/NEJMoa052306
101. Mavilio D, Galluzzi L, Lugli E. Novel multifunctional antibody approved for the treatment of breast cancer. Oncoimmunology 2013; 2:e24567; PMID:23802090; http://dx.doi.org/10.4161/onci.24567
102. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, et al.; ToGA Trial Investigators. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 2010; 376:687-97; PMID:20728210; http://dx.doi.org/10.1016/S0140-6736(10)61121-X
103. Gianni L, Eiermann W, Semiglazov V, Manikhas A, Lluch A, Tjulandin S, Zambetti M, Vazquez F, Byakhow M, Lichinitser M, et al. Neoadjuvant chemotherapy with trastuzumab followed by adjuvant trastuzumab versus neoadjuvant chemotherapy alone, in patients with HER2-positive locally advanced breast cancer (the NOAH trial): a randomised controlled superiority trial with a parallel HER2-negative cohort. Lancet 2010; 375:377-84; PMID:20113825; http://dx.doi.org/10.1016/S0140-6736(09)61964-4
104. Smith I, Procter M, Gelber RD, Guillaume S, Feyereislova A, Dowsett M, Goldhirsch A, Untch M, Mariani G, Baselga J, et al.; HERA study team. 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial. Lancet 2007; 369:29-36; PMID:17208639; http://dx.doi.org/10.1016/S0140-6736(07)60028-2
105. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010; 363:711-23; PMID:20525992; http://dx.doi.org/10.1056/NEJMoa1003466
106. Mavilio D, Lugli E. Inhibiting the inhibitors: Checkpoints blockade in solid tumors. Oncoimmunology 2013; 2:e26535; PMID:24244910; http://dx.doi.org/10.4161/onci.26535
107. Robert C, Thomas L, Bondarenko I, O'Day S, M D JW, Garbe C, Lebbe C, Baurain JF, Testori A, Grob JJ, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 2011; 364:2517-26; PMID:21639810; http://dx.doi.org/10.1056/NEJMoa1104621
108. Kloss CC, Condomines M, Cartellieri M, Bachmann M, Sadelain M. Combinatorial antigen recognition with balanced signaling promotes selective tumor eradication by engineered T cells. Nat Biotechnol 2013; 31:71-5; PMID:23242161; http://dx.doi.org/10.1038/nbt.2459
109. Schroten C, Kraaij R, Veldhoven JL, Berrevoets CA, den Bakker MA, Ma Q, Sadelain M, Bangma CH, Willemsen RA, Debets R. T cell activation upon exposure to patient-derived tumor tissue: a functional assay to select patients for adoptive T cell therapy. J Immunol Methods 2010; 359:11-20; PMID:20460126; http://dx.doi.org/10.1016/j.jim.2010.04.006
110. Modak S, Kushner BH, Kramer K, Vickers A, Cheung IY, Cheung NK. Anti-GD2 antibody 3F8 and barley-derived (1 → 3),(1 → 4)-β-D-glucan: A Phase I study in patients with chemoresistant neuroblastoma. Oncoimmunology 2013; 2:e23402; PMID:23802080; http://dx.doi.org/10.4161/onci.23402
111. Govindan SV, Goldenberg DM. Designing immunoconjugates for cancer therapy. Expert Opin Biol Ther 2012; 12:873-90; PMID:22679911; http://dx.doi.org/10.1517/14712598.2012.685153
112. Albertini MR, Hank JA, Gadbaw B, Kostlevy J, Haldeman J, Schalch H, Gan J, Kim K, Eickhoff J, Gillies SD, et al. Phase II trial of hu14.18-IL2 for patients with metastatic melanoma. Cancer Immunol Immunother 2012; 61:2261-71; PMID:22678096; http://dx.doi.org/10.1007/s00262-012-1286-5
113. Pasche N, Neri D. Immunocytokines: a novel class of potent armed antibodies. Drug Discov Today 2012; 17:583-90; PMID:22289353; http://dx.doi.org/10.1016/j.drudis.2012.01.007
114. Brack SS, Silacci M, Birchler M, Neri D. Tumor-targeting properties of novel antibodies specific to the large isoform of tenascin-C. Clin Cancer Res 2006; 12:3200-8; PMID:16707621; http://dx.doi.org/10.1158/1078-0432.CCR-05-2804
115. Gutbrodt KL, Schliemann C, Giovannoni L, Frey K, Pabst T, Klapper W, Berdel WE, Neri D. Antibody-based delivery of interleukin-2 to neovasculature has potent activity against acute myeloid leukemia. Sci Transl Med 2013; 5:ra118; PMID:24005158; http://dx.doi.org/10.1126/scitranslmed.3006221

116. Spitaleri G, Berardi R, Pierantoni C, De Pas T, Noberasco C, Libbra C, González-Iglesias R, Giovannoni L, Tasciotti A, Neri D, et al. Phase I/II study of the tumour-targeting human monoclonal antibody-cytokine fusion protein L19-TNF in patients with advanced solid tumours. J Cancer Res Clin Oncol 2013; 139:447-55; PMID:23160853; http://dx.doi.org/10.1007/s00432-012-1327-7
117. Johannsen M, Spitaleri G, Curigliano G, Roigas J, Weikert S, Kempkensteffen C, Roemer A, Kloeters C, Rogalla P, Pecher G, et al. The tumour-targeting human L19-IL2 immunocytokine: preclinical safety studies, phase I clinical trial in patients with solid tumours and expansion into patients with advanced renal cell carcinoma. Eur J Cancer 2010; 46:2926-35; PMID:20797845; http://dx.doi.org/10.1016/j.ejca.2010.07.033
118. Vacchelli E, Vitale I, Tartour E, Eggermont A, Sautès-Fridman C, Galon J, Zitvogel L, Kroemer G, Galluzzi L. Trial Watch: Anticancer radioimmunotherapy. Oncoimmunology 2013; 2:e25595; PMID:24319634; http://dx.doi.org/10.4161/onci.25595
119. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, et al.; IMPACT Study Investigators. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010; 363:411-22; PMID:20818862; http://dx.doi.org/10.1056/NEJMoa1001294
120. PD-1 inhibitors raise survival in NSCLC. Cancer Discov 2014; 4:6.
121. Weintraub K. Drug development: Releasing the brakes. Nature 2013; 504:S6-8; PMID:24352363; http://dx.doi.org/10.1038/504S6a
122. Dranoff G. Immunotherapy at large: Balancing tumor immunity and inflammatory pathology. Nat Med 2013; 19:1100-1; PMID:24013749; http://dx.doi.org/10.1038/nm.3335
123. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, Wolchok JD, Hersey P, Joseph RW, Weber JS, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med 2013; 369:134-44; PMID:23724846; http://dx.doi.org/10.1056/NEJMoa1305133
124. Chen LL, Gouw L, Sabripour M, Hwu WJ, Benjamin RS. Combining targeted therapy with immunotherapy (interferon-α): Rational, efficacy in gastrointestinal stromal tumor model and implications in other malignancies. Oncoimmunology 2012; 1:773-6; PMID:22934279; http://dx.doi.org/10.4161/onci.19729
125. Yang Y, Lizée G, Hwu P. Strong emerging rationale for combining oncogene-targeted agents with immunotherapy. Oncoimmunology 2013; 2:e22730; PMID:23524978; http://dx.doi.org/10.4161/onci.22730
126. Feldhahn N, Arutyunyan A, Stoddart S, Zhang B, Schmidhuber S, Yi SJ, Kim YM, Groffen J, Heisterkamp N. Environment-mediated drug resistance in Bcr/Abl-positive acute lymphoblastic leukemia. Oncoimmunology 2012; 1:618-29; PMID:22934254; http://dx.doi.org/10.4161/onci.20249
127. Kim DK, Lee TV, Castilleja A, Anderson BW, Peoples GE, Kudelka AP, Murray JL, Sittisomwong T, Wharton JT, Kim JW, et al. Folate binding protein peptide 191-199 presented on dendritic cells can stimulate CTL from ovarian and breast cancer patients. Anticancer Res 1999; 19(4B):2907-16; PMID:10652572
128. Peoples GE, Anderson BW, Lee TV, Murray JL, Kudelka AP, Wharton JT, Ioannides CG. Vaccine implications of folate binding protein, a novel cytotoxic T lymphocyte-recognized antigen system in epithelial cancers. Clin Cancer Res 1999; 5:4214-23; PMID:10632363
129. Okada H, Kalinski P, Ueda R, Hoji A, Kohanbash G, Donegan TE, Mintz AH, Engh JA, Bartlett DL, Brown CK, et al. Induction of CD8+ T-cell responses against novel glioma-associated antigen peptides and clinical activity by vaccinations with alpha-type 1 polarized dendritic cells and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in patients with recurrent malignant glioma. J Clin Oncol 2011; 29:330-6; PMID:21149657; http://dx.doi.org/10.1200/JCO.2010.30.7744
130. Okada H, Lieberman FS, Walter KA, Lunsford LD, Kondziolka DS, Bejjani GK, Hamilton RL, Torres-Trejo A, Kalinski P, Cai Q, et al. Autologous glioma cell vaccine admixed with interleukin-4 gene transfected fibroblasts in the treatment of patients with malignant gliomas. J Transl Med 2007; 5:67; PMID:18093335; http://dx.doi.org/10.1186/1479-5876-5-67
131. Holcmann M, Drobits B, Sibilia M. How imiquimod licenses plasmacytoid dendritic cells to kill tumors. Oncoimmunology 2012; 1:1661-3; PMID:23264929; http://dx.doi.org/10.4161/onci.22033
132. Galluzzi L, Vacchelli E, Eggermont A, Fridman WH, Galon J, Sautès-Fridman C, Tartour E, Zitvogel L, Kroemer G. Trial Watch: Experimental Toll-like receptor agonists for cancer therapy. Oncoimmunology 2012; 1:699-716; PMID:22934262; http://dx.doi.org/10.4161/onci.20696
133. Zauderer MG, Krug LM. Novel therapies in phase II and III trials for malignant pleural mesothelioma. J Natl Compr Canc Netw 2012; 10:42-7; PMID:22223868
134. Krug LM, Dao T, Brown AB, Maslak P, Travis W, Bekele S, Korontsvit T, Zakhaleva V, Wolchok J, Yuan J, et al. WT1 peptide vaccinations induce CD4 and CD8 T cell immune responses in patients with mesothelioma and non-small cell lung cancer. Cancer Immunol Immunother 2010; 59:1467-79; PMID:20532500; http://dx.doi.org/10.1007/s00262-010-0871-8
135. Tarhini AA, Leng S, Moschos SJ, Yin Y, Sander C, Lin Y, Gooding WE, Kirkwood JM. Safety and immunogenicity of vaccination with MART-1 (26-35, 27L), gp100 (209-217, 210M), and tyrosinase (368-376, 370D) in adjuvant with PF-3512676 and GM-CSF in metastatic melanoma. J Immunother 2012; 35:359-66; PMID:22495394; http://dx.doi.org/10.1097/CJI.0b013e31825481fe
136. Aarntzen EH, De Vries IJ, Lesterhuis WJ, Schuurhuis D, Jacobs JF, Bol K, Schreibelt G, Mus R, De Wilt JH, Haanen JB, et al. Targeting CD4(+) T-helper cells improves the induction of antitumor responses in dendritic cell-based vaccination. Cancer Res 2013; 73:19-29; PMID:23087058; http://dx.doi.org/10.1158/0008-5472.CAN-12-1127
137. Aarntzen EH, Bol K, Schreibelt G, Jacobs JF, Lesterhuis WJ, Van Rossum MM, Adema GJ, Figdor CG, Punt CJ, De Vries IJ. Skin-test infiltrating lymphocytes early predict clinical outcome of dendritic cell-based vaccination in metastatic melanoma. Cancer Res 2012; 72:6102-10; PMID:23010076; http://dx.doi.org/10.1158/0008-5472.CAN-12-2479
138. Su S, Zhou H, Xue M, Liu JY, Ding L, Cao M, Zhou ZX, Hu HM, Wang LX. Anti-tumor efficacy of a hepatocellular carcinoma vaccine based on dendritic cells combined with tumor-derived autophagosomes in murine models. Asian Pac J Cancer Prev 2013; 14:3109-16; PMID:23803088; http://dx.doi.org/10.7314/APJCP.2013.14.5.3109
139. Li Y, Wang LX, Pang P, Cui Z, Aung S, Haley D, Fox BA, Urba WJ, Hu HM. Tumor-derived autophagosome vaccine: mechanism of cross-presentation and therapeutic efficacy. Clin Cancer Res 2011; 17:7047-57; PMID:22068657; http://dx.doi.org/10.1158/1078-0432.CCR-11-0951
140. Twitty CG, Jensen SM, Hu HM, Fox BA. Tumor-derived autophagosome vaccine: induction of cross-protective immune responses against short-lived proteins through a p62-dependent mechanism. Clin Cancer Res 2011; 17:6467-81; PMID:21810919; http://dx.doi.org/10.1158/1078-0432.CCR-11-0812
141. Muhsin M, Graham J, Kirkpatrick P. Bevacizumab. Nat Rev Drug Discov 2004; 3:995-6; PMID:15645606; http://dx.doi.org/10.1038/nrd1601
142. Ferrara N, Hillan KJ, Gerber HP, Novotny W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. Nat Rev Drug Discov 2004; 3:391-400; PMID:15136787; http://dx.doi.org/10.1038/nrd1381
143. Lim SN, Kuhn S, Hyde E, Ronchese F. Combined TLR stimulation with Pam3Cys and Poly I: C enhances Flt3-ligand dendritic cell activation for tumor immunotherapy. J Immunother 2012; 35:670-9; PMID:23090076; http://dx.doi.org/10.1097/CJI.0b013e318270e135
144. Hennies CM, Reboulet RA, Garcia Z, Nierkens S, Wolkers MC, Janssen EM. Selective expansion of merocytic dendritic cells and CD8DCs confers anti-tumour effect of Fms-like tyrosine kinase 3-ligand treatment in vivo. Clin Exp Immunol 2011; 163:381-91; PMID:21235535; http://dx.doi.org/10.1111/j.1365-2249.2010.04305.x
145. Curran MA, Allison JP. Tumor vaccines expressing flt3 ligand synergize with ctla-4 blockade to reject preimplanted tumors. Cancer Res 2009; 69:7747-55; PMID:19738077; http://dx.doi.org/10.1158/0008-5472.CAN-08-3289
146. Galluzzi L, Vacchelli E, Eggermont A, Fridman WH, Galon J, Sautès-Fridman C, Tartour E, Zitvogel L, Kroemer G. Trial Watch: Adoptive cell transfer immunotherapy. Oncoimmunology 2012; 1:306-15; PMID:22737606; http://dx.doi.org/10.4161/onci.19549
147. Linehan DC, Tan MC, Strasberg SM, Drebin JA, Hawkins WG, Picus J, Myerson RJ, Malyapa RS, Hull M, Trinkaus K, et al. Adjuvant interferon-based chemoradiation followed by gemcitabine for resected pancreatic adenocarcinoma: a single-institution phase II study. Ann Surg 2008; 248:145-51; PMID:18650621; http://dx.doi.org/10.1097/SLA.0b013e318181e4e9
148. Couzin-Frankel J. Breakthrough of the year 2013. Cancer immunotherapy. Science 2013; 342:1432-3; PMID:24357284; http://dx.doi.org/10.1126/science.342.6165.1432
149. Kroemer G, Zitvogel L. Abscopal but desirable: The contribution of immune responses to the efficacy of radiotherapy. Oncoimmunology 2012; 1:407-8; PMID:22754758; http://dx.doi.org/10.4161/onci.20074
150. Galluzzi L, Kepp O, Kroemer G. Immunogenic cell death in radiation therapy. Oncoimmunology 2013; 2:e26536; PMID:24404424; http://dx.doi.org/10.4161/onci.26536
151. Ahmadzadeh M, Rosenberg SA. IL-2 administration increases CD4+ CD25(hi) Foxp3+ regulatory T cells in cancer patients. Blood 2006; 107:2409-14; PMID:16304057; http://dx.doi.org/10.1182/blood-2005-06-2399
152. Cesana GC, DeRaffele G, Cohen S, Moroziewicz D, Mitcham J, Stoutenburg J, Cheung K, Hesdorffer C, Kim-Schulze S, Kaufman HL. Characterization of CD4+CD25+ regulatory T cells in patients treated with high-dose interleukin-2 for metastatic melanoma or renal cell carcinoma. J Clin Oncol 2006; 24:1169-77; PMID:16505437; http://dx.doi.org/10.1200/JCO.2005.03.6830

153. Wei S, Kryczek I, Edwards RP, Zou L, Szeliga W, Banerjee M, Cost M, Cheng P, Chang A, Redman B, et al. Interleukin-2 administration alters the CD4+FOXP3+ T-cell pool and tumor trafficking in patients with ovarian carcinoma. Cancer Res 2007; 67:7487-94; PMID:17671219; http://dx.doi.org/10.1158/0008-5472.CAN-07-0565
154. Brandenburg S, Takahashi T, de la Rosa M, Janke M, Karsten G, Muzzulini T, Orinska Z, Bulfone-Paus S, Scheffold A. IL-2 induces in vivo suppression by CD4(+)CD25(+)Foxp3(+) regulatory T cells. Eur J Immunol 2008; 38:1643-53; PMID:18493984; http://dx.doi.org/10.1002/eji.200737791
155. Camisaschi C, Filipazzi P, Tazzari M, Casati C, Beretta V, Pilla L, Patuzzo R, Maurichi A, Cova A, Maio M, et al. Effects of cyclophosphamide and IL-2 on regulatory CD4+ T cell frequency and function in melanoma patients vaccinated with HLA-class I peptides: impact on the antigen-specific T cell response. Cancer Immunol Immunother 2013; 62:897-908; PMID:23589107; http://dx.doi.org/10.1007/s00262-013-1397-7
156. Weiss L, Letimier FA, Carriere M, Maiella S, Donkova-Petrini V, Targat B, Benecke A, Rogge L, Levy Y. In vivo expansion of naive and activated CD4+CD25+FOXP3+ regulatory T cell populations in interleukin-2-treated HIV patients. Proc Natl Acad Sci U S A 2010; 107:10632-7; PMID:20498045; http://dx.doi.org/10.1073/pnas.1000027107
157. Senovilla L, Vacchelli E, Galon J, Adjemian S, Eggermont A, Fridman WH, Sautès-Fridman C, Ma Y, Tartour E, Zitvogel L, et al. Trial watch: Prognostic and predictive value of the immune infiltrate in cancer. Oncoimmunology 2012; 1:1323-43; PMID:23243596; http://dx.doi.org/10.4161/onci.22009