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REVIEW

Trial Watch—Immunostimulation with cytokines in cancer therapy

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ABSTRACT

During the past decade, great efforts have been dedicated to the development of clinically relevant interventions that would trigger potent (and hence potentially curative) anticancer immune responses. Indeed, developing neoplasms normally establish local and systemic immunosuppressive networks that inhibit tumor-targeting immune effector cells, be them natural or elicited by (immuno)therapy. One possible approach to boost anticancer immunity consists in the (generally systemic) administration of recombinant immunostimulatory cytokines. In a limited number of oncological indications, immunostimulatory cytokines mediate clinical activity as standalone immunotherapeutic interventions. Most often, however, immunostimulatory cytokines are employed as immunological adjuvants, *i.e.*, to unleash the immunogenic potential of other immunotherapeutic agents, like tumor-targeting vaccines and checkpoint blockers. Here, we discuss recent preclinical and clinical advances in the use of some cytokines as immunostimulatory agents in oncological indications.

Abbreviations: 5-FU, 5-fluorouracil; AML, acute myeloid leukemia; CML, chronic myelogenous leukemia; DC, dendritic cell; ERBB2, erb-b2 receptor tyrosine kinase 2; FDA, Food and Drug Administration; FLT3LG, fms-related tyrosine kinase 3 ligand; ICD, immunogenic cell death; IFN, interferon; IL, interleukin; *i.v.*, *intra venam*; G-CSF, granulocyte colony-stimulating factor GM-CSF, granulocyte monocyte colony-stimulating factor; mAb, monoclonal antibody; MTOR, mechanistic target of rapamycin; NK, natural killer; NSCLC, non-small cell lung carcinoma; peg, pegylated; RCC, renal cell carcinoma *s.c.*, *sub cutem*; TLR, Toll-like receptor; TNF, tumor necrosis factor

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Introduction

Cytokines are a large and very heterogeneous family of small and generally soluble glycoproteins that regulate nearly all biological functions via autocrine, paracrine or endocrine circuitries.[1-4] At least in part, such a central position in the biology of mammalian organisms stems from the extreme pleiotropism of cytokine signaling.[5-8] Indeed, (1) virtually all mammalian cell types synthesize at least one cytokine; (2) one single cytokine can bind to, hence activating, distinct receptors or receptor isoforms, which normally exhibit differential binding and expression patterns; (3) cytokines often elicit the secretion of other mediators, including additional cytokines and (4) the biological outcome of cytokine signaling exhibit an elevated degree of context dependency, as it is profoundly influenced by contextual variables like local concentration, receptor type/isoform, cell type and differentiation state and presence of additional mediators.[5-8] The cytokine system is so pleiotropic that previous attempts to classify cytokines based on structural or functional aspects of their biology are now considered relatively reductionistic, imprecise and outdated.[5,6] Indeed, novel functions for members of the cytokine family as well as new cytokine-like proteins are discovered every year.[9-12]

Owing to their capacity to regulate various cellular responses including proliferation, differentiation, activation and regulated cell death, cytokines orchestrate complex organismal functions as different as hematopoiesis, angiogenesis, wound healing and inflammation.[13-19] Moreover, some cytokines play a critical role in the initiation, propagation and extinction of innate and adaptive immune responses, irrespective of whether such responses are initiated by microbial challenges or endogenous states of danger.[20-22] As a standalone example, Type I interferon (IFN) is not only a key determinant of antiviral immunity,[21,22] but also an important mediator of immune responses elicited by cancer cells undergoing so-called "immunogenic cell

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death" (ICD).[20,23-29] The immunostimulatory activity of some cytokines is so pronounced that (similar to other immunotherapeutics) they mediate clinical activity as standalone interventions in patients affected by particularly immunogenic neoplasms,[30-32] like melanoma and renal cell carcinoma (RCC).[33-35] In line with this notion, three cytokines are currently licensed by the US Food and Drug Administration (FDA) and equivalent regulatory agencies worldwide for use as immunostimulatory interventions in cancer patients, namely recombinant IFN-α2a (Roferon-A®), recombinant IFN-α2b (Intron A®) and recombinant interleukin (IL)-2 (aldesleukin, Proleukin®) (sources www.fda.gov and http://www.ema.europa.eu/ema/). Recombinant IFN-α2a is employed for the treatment of hairy cell leukemia and chronic phase, Philadelphia chromosome-positive chronic myelogenous leukemia (CML), upon minimal pretreatment (within 1 y of diagnosis); recombinant IFN-α2b is approved for use in patients with follicular lymphoma, multiple myeloma, hairy cell leukemia, AIDS-related Kaposi's sarcoma, melanoma, genital warts (*Condyloma acuminata*) and cervical intraepithelial neoplasms; and recombinant IL-2 is employed for the therapy of metastatic forms of melanoma and RCC (sources www.fda.gov and http://www.ema.europa.eu/ema/).

Of note, at least three other cytokines are approved by various regulatory agencies worldwide for use in cancer patients, namely recombinant granulocyte colony-stimulating factor (G-CSF, also known as Filgrastim, Lenograstim or Neupogen®), recombinant granulocyte monocyte colony-stimulating factor (GM-CSF, also known as Molgramostim, Sargramostim, Leukomax®, Mielogen® or Leukine®), and recombinant tumor necrosis factor (TNF) (sources www.fda.gov and http://www.ema.europa.eu/ema/). However, these molecules are not employed to boost tumor-targeting immune responses, but rather as immunoreconstituting (G-CSF, GM-CSF)[36-43] or oncotoxic (TNF) factors.[44-55]

Importantly, cytokines can cause relatively severe adverse effects (especially upon systemic administration), which *de facto* reflect their robust immunostimulatory activity and/or their biological pleiotropism. In particular, cytokines can (1) trigger an acute, potentially lethal systemic response that involves the release of pyrogenic and cytotoxic mediators (including additional cytokines) into the bloodstream;[56-60] (2) establish or perpetuate foci of chronic inflammation that may sustain oncogenesis or tumor progression;[61-63] or (3) promote proliferation among otherwise non-proliferating cells, thereby promoting the fixation of potentially oncogenic genetic or epigenetic defects.[64-67]

Owing to such non-negligible side effects as well to generally low-objective response rates, the interest of oncologists in using recombinant cytokines as systemic standalone anticancer agents had dropped. Relatively safe and efficient treatments are indeed available for all the indications mentioned above, perhaps with the single exception of Stage II–III melanoma, which is still treated with surgery followed by IFN-α2b-based immunotherapy.[35,68] Rather, investigators and practitioners are focusing their efforts on the possibility to use recombinant cytokines (at low doses and locally) to boost the anticancer activity of other immunotherapeutic regimens, including checkpoint blockers,[69-72] adoptive cell transfer,[73-76] oncolytic virotherapy,[77-81] DNA- and peptide-based vaccines,[82–87] dendritic cell (DC)-based interventions,[88-92] as well as other immunostimulatory agents like Toll-like receptor (TLR) agonists.[93-97] Here, we discuss recent preclinical and clinical advances in the development of recombinant cytokines for use as immunological adjuvants in cancer patients.

Update on the development of recombinant cytokines as immunostimulants for cancer therapy

Completed clinical studies

Since the submission of our latest Trial Watch dealing with the use of recombinant cytokines as immunological adjuvants in experimental or off-label oncological indications (May 2014),[6] the results of 17 clinical trials investigating this immunotherapeutic paradigm have been published in the peer-reviewed scientific literature (source http://www.ncbi.nlm.nih.gov/pubmed). Of note, 16 of these studies assessed the safety and efficacy of FDA-approved cytokines (*i.e.*, G-CSF, GM-CSF, IFN-α2a, IFN-α2b and IL-2) as off-label immunostimulatory interventions,[98-113] while only one trial tested the clinical profile of a hitherto experimental cytokine (*i.e.*, IL-15).[114]

The safety and efficacy of G-CSF have been assessed in 11 breast carcinoma patients, who were treated with subcutaneous G-CSF in combination with 5-fluorouracil (5-FU), epirubicin, cyclophosphamide and paclitaxel (NCT02225652).[98] The clinical profile of GM-CSF has been investigated in (1) 58 patients with newly diagnosed, treatment-naive CML, who received subcutaneous GM-CSF in combination with IFN-α,[101] (2) two cohorts of 125 and 11 subjects with castration-resistant prostate carcinoma, who were treated with GM-CSF *s.c.* as a maintenance therapy upon successful docetaxel- and prednisone-based chemotherapy,[115] or in combination with thalidomide,[116,117] respectively;[103,111] (3) 32 individuals with metastatic melanoma, receiving subcutaneous GM-CSF along with the FDA-approved, cytotoxic T lymphocyte-associated protein 4 (CTLA4)-blocking monoclonal antibody (mAb) ipilimumab;[109] and (4) 41 patients affected with advanced solid neoplasms, who were treated with GM-CSF *s.c.* in combination with local radiotherapy,[118-120] with the specific aim to provoke systemic tumor-targeting immune responses (NCT02474186).[110] Taken together, the results of these studies confirm that the subcutaneous administration of GM-CSF to cancer patients is safe and may boost the therapeutic activity of other treatments, at least in a proportion of individuals. Conversely, the combination of G-CSF with aggressive, multimodal chemotherapy was associated with severe side effects, resulting in the premature closure of the study.[98]

The safety and efficacy of IFN-α2a and IFN-α2b have been evaluated in (1) five cohorts of 791, 365, 53, 51 and 40 subjects with advanced RCC, who received IFN-α2a in combination with the FDA-approved tumor-targeting antibody bevacizumab (which neutralizes vascular endothelial growth factor A, VEGFA)[121-123] (NCT00719264), along with the multitarget tyrosine kinase inhibitor sorafenib (UMIN000002466),[124,125] in the context of a multimodal treatment involving potentially immunogenic chemotherapy plus bevacizumab *i.v.* and low dose IL-2 *s.c.*,[126-128]; or optionally combined with a trophoblast glycoprotein (TPBG)-redirected variant of staphylococcal

enterotoxin A (naptumomab estafenatox);[100,105,108,113,129] (2) 313 patients with symptomatic indolent B-cell lymphoma, receiving IFN-α2a in combination with the CD20-targeting mAb rituximab[130,131] (NCT01609010);[107] (3) 236 subjects with recurrent non-muscle-invasive bladder carcinoma, who were treated with intravesical IFN-α2b after intravesical mitomycin C-based chemotherapy;[106] (4) 23 individuals with recurrent epithelial ovarian carcinoma, receiving pegylated (peg)-IFN-α2b *s.c.* in the context of carboplatin- and doxorubicin-based chemotherapy plus a mAb targeting interleukin 6 receptor (IL6R) (NCT01637532);[112] and (5) 20 patients with advanced intrahepatic cholangiocarcinoma, who received subcutaneous peg-IFN-α2b along with hepatic arterial infusion 5-FU-based chemotherapy.[99] Cumulatively, these studies confirm that IFN-α2a and IFN-α2b can be safely administered to cancer patients as off-label indications. IFN-α2a improved the clinical efficacy of sorafenib-based chemotherapy for advanced RCC,[113] but failed to do so better than the mechanistic target of rapamycin (MTOR) inhibitors everolimus or temsirolimus[132,133] (although it was associated with a lower incidence of side effects) in patients with metastatic RCC co-treated with bevacizumab,[108,129] as it failed to ameliorate the therapeutic profile of rituximab in indolent B-cell lymphoma patients.[107] Moreover, the intravesical administration of IFN-α2b after mitomycin C-based chemotherapy was associated with an increased frequency of recurrence among patients with non-muscle-invasive bladder carcinoma as compared to the post-chemotherapy instillation of the Bacillus Calmette-Guérin (BCG).[106] These findings indicate that the use of IFN-α2a and IFN-α2b as immunological adjuvants may not be optimal for all oncological indications.

Finally, subcutaneous low-dose IL-2 has been tested as an adjuvant to autologous DCs in 10 patients with ovarian carcinoma;[102] the safety and efficacy of F16-IL2 (a variant of IL-2 retargeted to the extracellular domain A1 of tenascin C, TNC)[134] administered *i.v.* in combination with doxorubicin-based chemotherapy have been evaluated first in 10 subjects with advanced solid tumors (to identify a recommended dose) and subsequently in 19 breast carcinoma patients (at the recommended dose);[104] and the clinical profile of recombinant IL-15 given *i.v.* or *s.c.* has been assessed in 18 individuals with advanced melanoma or RCC.[114] These latter two studies identified doses of F16-IL2 and IL-15 that are suitable for further investigation.[104,114] In addition, IL-15 was found to markedly alter the homeostasis of various circulating lymphocyte subsets, notably natural killer (NK), γδ T and memory $CD8^+$ T cells.[114]

Preclinical and translational advances

Among recent preclinical and translational studies focusing on immunostimulation by cytokines, we found of particular interest the works of (1) Finisguerra and colleagues (Vesalius Research Center, Leuven, Belgium), who demonstrated that the proto-oncogene MET and its cognate cytokine hepatocyte growth factor (HGF) are required for the infiltration of neoplastic lesions by neutrophils with tumor-suppressive functions;[135] (2) Marçais and co-authors (International Center for Infectiology Research, Lyon, France), who found that MTOR is essential for the development and activation of NK cells in response to IL-15;[136] (3) O'Sullivan and collaborators (University of California San Diego, La Jolla, CA, US), who reported that the secretion of IL-17D by malignant cells stimulates the recruitment of NK cells to the tumor bed, and the consequent initiation of a tumor-targeting immune response that partially relies on M1 macrophages;[137] (4) Zhu *et al.* (Massachusetts Institute of Technology, Cambridge, MA, US), who found that the efficacy of a tumor-targeting mAb is significantly boosted by the co-administration of an IL-2 variant with extended serum half-life;[138] (5) Ruffell and colleagues (Oregon Health & Science University, Portland, OR, US), who characterized an IL-10-dependent mechanism whereby immunosuppressive macrophages block chemotherapy-driven anticancer immune responses at the level of IL-12 production by DCs;[139] (6) Hou and co-authors (Chinese Academy of Medical Sciences, Beijing, China), who demonstrated that the expression levels of DEAD (Asp-Glu-Ala-Asp) box polypeptide 58 (DDX58, best known as RIG-I) in malignant cells predict the response of hepatocellular carcinoma patients to IFN-α;[140] (7) Litvin and collaborators (Columbia University, New York, NY, US), who found that recombinant Type I IFN robustly enhances the efficacy of mitogen-activated protein kinase (MAPK) inhibitors in melanoma cells, but only when IFN signaling in baseline conditions is reduced;[141] (8) Escobar *et al.* (Vita-Salute San Raffaele University, Milan, Italy), who engineered the hematopoietic system of human hematochimeric mice so that IFN-α would be expressed selectively by differentiated monocytes, thereby endowing these animals with a superior ability to control the progression of experimental breast carcinomas;[142] and (9) Bald and colleagues (University of Bonn, Bonn, Germany), who demonstrated that melanoma lesions with limited immune infiltrate respond to the local administration of immunostimulatory RNA (which activates Type I IFN signaling) in combination with a mAb blocking programmed cell death 1 (PDCD1, best known as PD-1).[143] Moreover, our laboratories demonstrated that cancer cells succumbing to ICD inducers produce Type I IFN upon the activation of TLR3, driving an autocrine/paracrine signal transduction pathway that ultimately leads to the secretion of chemokine (C-X-C motif) ligand 10 (CXCL10).[144] Thus, Type I IFN stands out as a potent immunostimulatory cytokine in several distinct experimental settings. However, results from recent clinical studies indicate that IFN-α2a or IFN-α2b may not universally ameliorate the efficacy of other anticancer interventions (see above). Large, randomized clinical trials are urgently awaited to understand whether recombinant IFN-α2a or IFN-α2b might safely improve the therapeutic activity of specific FDA-approved chemo- and immune-therapeutics, including targeted anticancer agents like the BRAF inhibitor vemurafenib and checkpoint-blocking mAbs like ipilimumab, nivolumab and pembrolizumab.

Recently initiated clinical trials

During the past 17 mo, no less than 60 clinical trials have been launched to test recombinant cytokines as on-label immunoreconstituting or immunostimulatory interventions in cancer patients, but these studies will not be discussed here. In the same period, at least 59 additional trials have been initiated to test the safety and efficacy of recombinant cytokines as immunostimulatory agents in off-label oncological indications. Of these studies, eight involve G-CSF, 20 GM-CSF, 10 IFN-α, 13

IL-2, and the remaining eight other cytokines including fms-related tyrosine kinase 3 ligand (FLT3LG), IFNγ, IL-8, IL-12, IL-15 and IL-18 (Table 1) (source www.clinicaltrials.org).

The safety and efficacy of G-CSF are being assessed in (1) bladder carcinoma patients, who receive G-CSF *i.v.* in the context of dense multicomponent chemotherapy (NCT02177695); (2) breast carcinoma patients,[145] receiving subcutaneous G-CSF in combination with 5-FU, epirubicin, cyclophosphamide and paclitaxel (NCT02225652); (3) subjects with gastric adenocarcinoma, receiving G-CSF *i.v.* in the context of the so-called SIRINOX chemotherapeutic regimen (S-1 plus irinotecan plus oxaliplatin) (NCT02387138); (4) individuals with head and neck squamous cell carcinoma, who are treated with subcutaneous G-CSF together with mitomycin C-based chemotherapy (NCT02369458); (5) ovarian carcinoma patients, receiving G-CSF in combination with docetaxel and carboplatin (NCT02469116); (6) subjects with prostate carcinoma, who are treated with G-CSF in the context of chemotherapy alone (NCT02494921) or chemotherapy plus androgen ablation therapy (NCT02543255); and (7) patients with advanced solid malignancies without bone marrow involvement, who receive Tbo-filgrastim (a short-acting form of G-CSF) as standalone immunotherapeutic intervention (NCT02190721). All these studies are ongoing with the exception of NCT02225652, which has been completed (see above), and NCT02469116, which has been terminated owing to the withdrawal of financial support (source www.clinicaltrials.org).

The immunostimulatory activity of GM-CSF is being tested in (1) patients with various malignancies of the central nervous system, who are treated with GM-CSF *s.c.* in combination with a personalized peptide-based vaccine and optionally a TLR3 agonist after temozolomide-based chemotherapy (NCT02149225; NCT02455557; NCT02510950), a DC-based vaccine after temozolomide-based chemotherapy (NCT02465268), or a replication competent oncolytic reovirus (NCT02444546); (2) women with breast carcinoma, who receive subcutaneous GM-CSF together with the erb-b2 receptor tyrosine kinase 2 (ERBB2)-specific mAb trastuzumab[146,147] and an ERBB2-targeting vaccine (NCT02297698); (3) subjects with colorectal cancer, receiving GM-CSF *s.c.* in the context of the so-called XELOX chemotherapeutic regimen (capecitabine plus oxaliplatin) (NCT02466906); (4) patients with gynecological malignancies, who are treated with subcutaneous GM-CSF and IL-2 as a support to autologous T cells armed with a bispecific T-cell engager (BiTE)[148-150] targeting ERBB2 (NCT02470559); (5) individuals with hematological neoplasms, who receive GM-CSF *s.c.* in support of a multipeptide-based vaccine (NCT02240537); (6) melanoma patients, who are treated with GM-CSF as a standalone immunotherapeutic intervention (NCT02451488) or in combination with ipilimumab and peptide-based vaccination targeting telomerase reverse transcriptase (TERT)[151,152] (NCT02275416); (7) subjects with neuroblastoma, osteosarcoma or soft tissue sarcoma, receiving subcutaneous GM-CSF and IL-2 along with autologous T cells armed with a BiTE targeting ganglioside GD2 (NCT02173093); (8) individuals with neuroblastoma, who are treated with subcutaneous GM-CSF plus IL-2 *i.v.* and dinutuximab (a chimeric ganglioside GD2-specific mAb)[153,154] (NCT02169609); (9) osteosarcoma patients, who receive GM-CSF *s.c.* plus dinutuximab or a humanized ganglioside GD2-specific mAb (NCT02484443; NCT02502786); (10) individuals with prostate carcinoma, who are treated with subcutaneous or intradermal GM-CSF in combination with cryotherapy[155] (NCT02250014) or a peptide-based vaccine (NCT02452307), respectively; (11) RCC patients, receiving intradermal GM-CSF as an adjuvant to peptide-based vaccination (NCT02429440); and (12) subjects with metastatic solid tumors, who receive GM-CSF in combination with radio- and chemo-therapy (NCT02474186) or with a ERBB2-targeting vaccine plus cyclophosphamide (NCT02276300). All these studies are ongoing (source www.clinicaltrials.org).

The clinical profile of IFN-α2a is being investigated in (1) acute myeloid leukemia (AML) patients, who receive peg-IFN-α2a in the context of hematopoietic stem cell transplantation (not as an immunoreconstituting agent) and methotrexate-based therapy (NCT02328755); (2) patients with advanced solid tumors, who are treated with IFN-α2a as standalone immunotherapeutic intervention (NCT02159482) or together with a mAb specific for the PD-1 ligand CD274 (best known as PD-L1)[156] (NCT02174172). Recombinant IFN-α2b is being tested in (1) subjects with astrocytomas or optic pathway gliomas, who receive peg-IFN-α2b *s.c.* as standalone immunotherapeutic agent (NCT02343224); (2) individuals with ovarian carcinoma or peritoneal surface malignancies, who are treated with intraperitoneal or intravenous IFN-α2b, respectively, as adjuvant to cisplatin plus DC-based vaccination (NCT02432378) or DC-based vaccination alone (NCT02151448); (3) RCC patients, receiving peg-IFN-α2b in combination with the proteasomal inhibitor ixazomib (NCT02447887); and (4) subjects with squamous cell carcinomas of the skin, who receive peg-IFN-α2b *s.c.* in together with capecitabine-based chemotherapy (NCT02218164). Finally, two clinical studies are assessing the safety and efficacy of solubilized form of crystalline recombinant IFN-α2b (known as rSIFN-co) administered *s.c.* as standalone immunotherapeutic agent to patients with advanced solid neoplasms (NCT02387307; NCT02464007). With the exception of NCT02159482, which has already been terminated owing to low accrual, all these studies are ongoing. Of 11 patients enrolled in NCT02159482, only 6 completed the vaccination protocol. Five of these individuals (83.33%) experienced moderate adverse effects, the most common of which were fatigue and fever. Moreover, fiveof these patients exhibited an increase in the magnitude of the tumor antigen-specific immune responses, as determined by ELISPOT analysis (source www.clinicaltrials.org).

The immunostimulatory activity of IL-2 is being assessed in (1) patients with hematological malignancies including AML, who are treated with IL-2 in combination adoptively transferred NK cells (NCT02123836), adoptively transferred NK cells plus cytarabine- or decitabine-based chemotherapy (NCT02229266; NCT02316964), or adoptively transferred T lymphocytes plus rituximab (NCT02315118) (2) individuals with gynecological malignancies, who receive subcutaneous GM-CSF and IL-2 as a support to autologous T cells armed with an ERBB2-targeting BiTE (NCT02470559); (3) subjects with non-Hodgkin lymphoma, receiving a de-immunized and humanized anti-CD20 mAb (Leu16) fused to IL-2 as

Table 1. Clinical trials recently started to investigate the safety and efficacy of immunostimulatory cytokines as off-label interventions for cancer therapy.

Molecule	Indication(s)	Phase	Status	Route	Notes	Ref.
FLT3LG	Melanoma	II	Recruiting	*s.c.*	Combined with a peptide-based vaccine targeted to DCs *in vivo*	NCT02129075
G-CSF	Bladder carcinoma	II	Recruiting	*i.v.*	In the context of dense multicomponent chemotherapy	NCT02177695
	Breast carcinoma	II	Completed	*s.c.*	Combined with 5-FU, epirubicin, cyclophosphamide and paclitaxel	NCT02225652
	Gastric adenocarcinoma	I	Recruiting	*i.v.*	In the context of the SIRINOX regimen	NCT02387138
	HNC	II	Recruiting	*s.c.*	Combined with mitomycin C-based chemotherapy	NCT02369458
	Ovarian carcinoma	II	Terminated	n.a.	Combined with docetaxel and carboplatin	NCT02469116
	Prostate carcinoma	I/II	Recruiting	n.a.	Combined with multimodal chemotherapy	NCT02494921
		II	Not yet recruiting	n.a.	Combined with multimodal chemotherapy and androgen ablation	NCT02543255
	Solid tumors	II	Recruiting	*s.c.*	Formulated as Tbo-filgrastim	NCT02190721
GM-CSF	Brain tumors	0	Not yet recruiting	n.a.	Combined with a personalized peptide-based vaccine and polyICLC after temozolomide	NCT02510950
		I	Recruiting	*s.c.*	Combined with replication competent reovirus	NCT02444546
		II	Not yet recruiting	*s.c.*	Combined with a DC-based vaccine after temozolomide-based chemotherapy	NCT02465268
	Breast carcinoma	II	Recruiting	*s.c.*	Combined with an ERBB2-targeting vaccine and/or trastuzumab	NCT02297698
	Colorectal carcinoma	II	Recruiting	*s.c.*	In the context of the XELOX regimen	NCT02466906
	Glioblastoma	I	Recruiting	*s.c.*	Combined with a personalized peptide-based vaccine and polyICLC after temozolomide	NCT02149225
		II	Recruiting	*s.c.*	Combined with a personalized peptide-based vaccine after temozolomide	NCT02455557
	Gynecological malignancies	I	Recruiting	*s.c.*	Combined with autologous T cells armed with a BiTE plus subcutaneous IL-2	NCT02470559
	Hematological malignancies	I	Recruiting	*s.c.*	Combined with multipeptide-based vaccination	NCT02240537
	Melanoma	I/II	Recruiting	*s.c.*	Combined with a TERT-targeting vaccine and ipilimumab	NCT02275416
		IV	Recruiting	n.a.	As standalone immunotherapeutic agent	NCT02451488
	Neuroblastoma	II	Not yet recruiting	n.a.	Combined with dinutuximab, isotretinoin and IL-2	NCT02169609
	Neuroblastoma Osteosarcoma Soft tissue sarcoma	I/II	Recruiting	*s.c.*	Combined with autologous T cells armed with a GD2-targeting BiTE plus IL-2	NCT02173093
	Osteosarcoma	II	Not yet recruiting	*s.c.*	Combined with dinutuximab	NCT02484443
			Recruiting	*s.c.*	Combined with a humanized anti-GD2 mAb	NCT02502786
	Prostate carcinoma	I	Not yet recruiting	*s.c.*	Combined with cryotherapy	NCT02250014
		I/II	Active not recruiting	*i.d.*	Combined with peptide-based vaccination	NCT02452307
	RCC	I/II	Active not recruiting	*i.d.*	Combined with peptide-based vaccination	NCT02429440
	Solid tumors	I	Recruiting	n.a.	Combined with an ERBB2-targeting vaccine	NCT02276300
		II	Active not recruiting	n.a.	Combined with radiochemotherapy	NCT02474186
IFNα-2a	AML	I/II	Recruiting	*s.c.*	In the context of HSCT and methotrexate-based chemotherapy	NCT02328755
	Solid tumors	I	Recruiting	*s.c.*	Combined with a PD-L1-targeing mAb	NCT02174172
		II	Terminated has results	*s.c.*	As standalone immunotherapeutic agent	NCT02159482
IFNα-2b	Astrocytomas Gliomas	II	Recruiting	*s.c.*	As standalone immunotherapeutic agent	NCT02343224
	Ovarian carcinoma	I/II	Not yet recruiting	*i.p.*	Combined with cisplatin plus DC-based vaccination	NCT02432378
	Peritoneal surface malignancies	I/II	Recruiting	*i.v.*	Combined with DC-based vaccination	NCT02151448
	RCC	I/II	Not yet recruiting	n.a.	Combined with a proteasomal inhibitor	NCT02447887
	Skin SCC	II	Recruiting	*s.c.*	Combined with capecitabine	NCT02218164
	Solid tumors	I	Not yet recruiting	*s.c.*	As single agent with specific formulation	NCT02464007
			Recruiting	*s.c.*	As single agent with specific formulation	NCT02387307
IFNγ	Glioblastoma Gliosarcoma	I	Recruiting	*i.t.*	Combined with a replicative adenovirus	NCT02197169
IL-2	AML	n.a.	Recruiting	*s.c.*	Combined with adoptively transferred NK cells and decitabine	NCT02316964
	Gynecological malignancies	I	Recruiting	*s.c.*	Combined with autologous T cells armed with a BiTE plus subcutaneous GM-CSF	NCT02470559
		II	Not yet recruiting	n.a.	Combined with adoptively transferred NK cells and cytarabine	NCT02229266
	Hematological malignancies	I	Recruiting	n.a.	Combined with adoptively transferred NK cells	NCT02123836
		I/II	Recruiting	n.a.	Combined with adoptively transferred T cells and rituximab	NCT02315118
	HNC	I/II	Recruiting	*s.c.*	Combined with adoptively transferred NK cells and cetuximab	NCT02507154
	MesotheliomaNSCLC	I/II	Recruiting	*s.c.*	Combined with cyclophosphamide and genetically modified T cells	NCT02408016
	Neuroblastoma	II	Not yet recruiting	n.a.	Combined with dinutuximab, isotretinoin and GM-CSF	NCT02169609
	NeuroblastomaOsteosarcomaSoft tissue sarcoma	I/II	Recruiting	*s.c.*	Combined with autologous T cells armed with a GD2-targeting BiTE plus GM-CSF	NCT02173093
	Non-Hodgkin's lymphoma	I/II	Enrolling by invitation	*s.c.*	As immunocytokine targeted to CD20	NCT02151903
	Ovarian carcinoma	I	Recruiting	*i.v.*	Combined with adoptively transferred TILs	NCT02482090
	Pleural mesothelioma	I/II	Not yet recruiting	*s.c.*	Combined with adoptively transferred TILs	NCT02414945
	Solid tumors Lymphoma	I	Recruiting	*s.c.*	Combined with adoptively transferred haploidentical NK cells	NCT02130869
IL-8	Solid tumors	I	Recruiting	*i.v.*	As standalone immunotherapeutic agent	NCT02536469
IL-12	AML	I	Not yet recruiting	*i.v.*	As standalone immunotherapeutic agent	NCT02483312
	CTCL	II	Not yet recruiting	*s.c.*	Combined with low-dose total skin electron beam therapy	NCT02542124
	DLBCL	II	Not yet recruiting	*s.c.*	As single agent (upon completion of chemotherapy)	NCT02544724
IL-15	AML	II	Recruiting	*s.c.*	Combined with adoptively transferred haploidentical NK cells	NCT02395822
IL-18	Gynecological malignancies	I	Completed	n.a.	Combined with adoptively transfer of vaccine-primed, CD3/CD28-costimulated T cells	NCT02277392

Abbreviations: 5-FU, 5-fluorouracil; AML, acute myeloid leukemia; BiTE, bispecific T-cell engager; CTCL, cutaneous T cell lymphoma; DC, dendritic cell; DLBCL, diffuse large B-cell lymphoma; FLT3LG, fms-related tyrosine kinase 3 ligand; G-CSF; granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; HNC, head and neck cancer; HSCT, hematopoietic stem cell transplantation; *i.d.*, *intra dermam*; IFN, interferon; IL, interleukin; *i.p.*, *intra peritoneum*; *i.t.*, *intra tumorem*; *i.v.*, *intra venam*; mAb, monoclonal antibody; n.a., not available; NK, natural killer; NSCLC, non-small cell lung carcinoma; poly-ICLC, polyinosinic:polycytidylic acid; RCC, renal cell carcinoma; *s.c.*, sub cutem; SCC, squamous cell carcinoma; SIRINOX, S-1 + irinotecan + oxaliplatin; TIL, tumor infiltrating lymphocyte; XELOX, capecitabine + oxaliplatin.

* initiated between 2014, May 1st and 2015, Sept 30th.

standalone immunotherapeutic intervention (NCT02151903); (4) patients with head and neck squamous cell carcinoma or nasopharyngeal carcinoma, who are treated with IL-2 *s.c.* as adjuvant to the infusion of autologous NK cells and the FDA-approved epidermal growth factor (EGFR)-targeting mAb cetuximab[157] (NCT02507154); (5) individuals with mesothelioma or non-small cell lung carcinoma (NSCLC), receiving subcutaneous IL-2 along with cyclophosphamide-based chemotherapy and T lymphocytes genetically redirected against the tumor-associated antigen Wilms tumor 1 (WT1) (NCT02408016); (6) subjects with neuroblastoma, osteosarcoma or soft tissue sarcoma, who receive subcutaneous GM-CSF and IL-2 along with autologous T cells armed with a BiTE targeting ganglioside GD2 (NCT02173093), or along with dinutuximab-based therapy (NCT02169609); (7) individuals with ovarian carcinoma or pleural mesothelioma patients, who are treated with intravenous or subcutaneous IL-2, respectively combined with the adoptive transfer of autologous tumor-infiltrating lymphocytes (NCT02482090; NCT02414945); and (8) children with high-risk solid tumors or lymphomas, receiving IL-2 *s.c.* as adjuvant to the adoptive transfer of haploidentical NK cells (NCT02130869). All the studies are ongoing (source www.clinicaltrials.org).

Moreover, (1) intravenous IL-8 is being tested as a standalone immunotherapeutic agent in patients affected by advanced solid tumors (NCT02536469); (2) the safety and efficacy of IL-12 are being investigated in AML patients, who receive IL-12 *i.v.* as a single immunotherapeutic intervention (NCT02483312), cutaneous T-cell lymphoma, who are treated with subcutaneous IL-12 in combination with low-dose total skin electron beam therapy (NCT02542124), and diffuse large B-cell lymphoma, receiving IL-12 *s.c.* after standard chemotherapy (NCT02544724); (3) IL-15 is being tested as a standalone immunological adjuvant to haploidentical NK-cell transfer in AML patients (NCT02395822); (4) the clinical profile of IL-18 is being evaluated in subjects with gynecological neoplasms, who are treated with IL-18 in support of vaccine-primed, CD3/CD28-stimulated autologous T cells (NCT02277392); (5) the safety and immunostimulatory activity of FLT3LG are being tested in melanoma patients, who receive FLT3LG *s.c.* together with a TLR3 agonist as a support to a peptide-based vaccine targeted to DCs *in vivo* (NCT02129075); and (6) IFNγ is being tested in combination with a replicative oncolytic adenovirus in glioblastoma and gliosarcoma patients (NCT02197169). These clinical trials are ongoing, with the sole exception of NCT02277392, which has already been completed (but for which results are not yet available) (source www.clinicaltrials.org).

Of the clinical studies discussed in our previous Trial Watches dealing with immunostimulatory cytokines in off-label oncological indications,[5,6] the following have changed status during the past 17 mo: NCT01579188, which currently appears as "Not yet recruiting;" NCT01580696, NCT01671774, NCT01784913, NCT01872442, NCT01929239, NCT01989325, NCT02019524, NCT02089685, and NCT02092922, which are nowadays listed as "Active not recruiting;" NCT01727076, NCT01881867, NCT01898793, NCT01903330, NCT01964300, NCT02001818, NCT02070406, and NCT02078648, which are now "Recruiting" participants; NCT01701479, and NCT01806272, whose status is now "Unknown;" NCT01940601, which has been "Withdrawn;" NCT00923351, NCT01767194, and NCT01572493, which have been "Suspended;" NCT00589550, and NCT00784524, which have been "Terminated;" and NCT00631371, NCT00719264, NCT01592045, NCT01609010, NCT01629758, NCT01658813, NCT01673217, NCT01875601, NCT01989572, NCT02076633, and NCT02087176, which have been "Completed" (source www.clinicaltrials.org).

NCT01940601 (a Phase II study testing the pharmacodynamics of human serum albumin-bound G-CSF in pediatric patients with solid tumors) has been withdrawn prior to enrollment for undisclosed reasons. NCT00923351 (a Phase I/II trial testing IL-17 as immunological adjuvant to the adoptive transfer of autologous T cells alone or given in combination with a DC-based vaccine in subjects with neuroectodermal tumors) has been suspended owing to the availability of IL-7, but preliminary results indicate that IL-7 exerts robust immunostimulatory effects in the absence of added toxicity (as compared to adoptive cell transfer alone). NCT01572493 (a Phase I study assessing the safety and efficacy of IL-15 as standalone immunotherapeutic intervention in adults with advanced malignancies) has been suspended for undisclosed reasons, whereas NCT01767194 (a Phase II trial evaluating the clinical profile of subcutaneous or intravenous GM-CSF in neuroblastoma patients receiving chemotherapy plus everolimus or dinutuximab) has been suspended for assessment. Both NCT00784524 (a Phase II study assessing the immunostimulatory activity of subcutaneous IL-2 given as adjuvant to a peptide-based vaccine in breast carcinoma patients) and NCT00589550 (a Phase I trial investigating the clinical profile of subcutaneous peg-IFN-α2b plus sorafenib-based chemotherapy in advanced RCC patients) have been terminated owing to slow accrual (source www.clinicaltrials.org).

We discussed the results of NCT0063137, NCT01609010 and NCT00719264 above. Preliminary results from NCT01592045 (a Phase I/II study comparing the activity of two distinct mAbs targeting ganglioside GD2 adjuvanted with G-CSF and IL-2 in neuroblastoma patients) indicate that this approach is not associated with an increased incidence or a different panel of side effects as compared to the administration of anti-GD2 mAbs alone (based on historical cohorts). Results from NCT02087176 (a large, randomized Phase III trial testing whether subcutaneous GM-CSF increases the clinical efficacy a multipeptide-based vaccine in melanoma patients) suggest that the administration of GM-CSF *s.c.* does not improve progression-free and overall survival in subjects with melanoma receiving a multipeptide-based vaccine, nor does it change the incidence of side effects. It remains to be clarified whether these negative results reflect the inability of vaccination to initiate tumor-targeting immune responses (irrespective of GM-SCF administration) in this setting. To the best of our knowledge, the results of NCT01629758 (a Phase I trial investigating the safety and efficacy of recombinant IL-21 plus nivolumab in subjects with advanced solid tumors), NCT01658813 (a Phase II study assessing the clinical profile of 5-FU followed by IFN-α2b in patients with metastatic gastrointestinal, kidney or lung cancers), NCT01673217 (a Phase I trial evaluating the safety and efficacy of a peptide-based vaccine adjuvanted with

subcutaneous GM-CSF in combination with conventional chemotherapeutics in women with gynecological tumors), NCT01875601 (a Phase I study testing autologous activated NK cells plus intravenous IL-15 in children and young adults with advanced solid tumors), NCT02076633 (a Phase II trial investigating the therapeutic profile of tumor-redirected IL-2 plus tumor-redirected TNF in melanoma patients), and NCT02087176 (a Phase II study testing the efficacy of a targeted anticancer agent given in combination with peg-GM-CSF and placebo or docetaxel in NSCLC patients) have not yet been disseminated (source www.clinicaltrials.org).

Concluding remarks

Preclinical and clinical data accumulating over the past two decades demonstrate that some cytokines, including (but presumably not limited to) G-CSF, GM-CSF, IFN-α2a, IFN-α2b, IL-2, IL-12 and IL-15 mediate robust immunostimulatory effects that may be harnessed to boost natural or therapy-elicited anticancer immune responses in patients. However, using these molecules as standalone immunostimulatory agents is associated with (a relatively low proportion of) objective responses in a limited number of oncological indications (*e.g.*, melanoma, RCC). Recent efforts have therefore been redirected to use immunostimulatory cytokines as adjuvants to other chemo-, radio- or immuno-therapeutic regimens. Moreover, it turned out that—besides being associated with non-negligible side effects—the systemic delivery of some cytokines (like IL-2) actually provides a preferential benefit to immunosuppressive, rather than effector, cells of the immune system.[158] As a consequence, various strategies have been devised to target recombinant cytokines to specific anatomical locations or selected cell populations, including variations in dosage, schedule and administration route as well as genetic/molecular engineering. A majority of recently launched clinical trials involves cytokines that are already licensed by regulatory agencies for use in humans, *i.e.,* G-CSF, GM-CSF, IFN-α2a, IFN-α2b and IL-2. In the era of checkpoint blockade, however, changes in the approval status of these molecules do not seem to stand next door.

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References

1. Tato CM, Cua DJ. SnapShot: Cytokines I. Cell 2008; 132:324.e1; PMID:18243106
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; 132:1062.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, Aranda F, 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; PMID:25083328; http://dx.doi.org/10.4161/onci.29030
7. Borish LC, Steinke JW. 2. Cytokines and chemokines. J Allergy Clin Immunol 2003; 111:S460-75; PMID:12592293; http://dx.doi.org/10.1067/mai.2003.108
8. Steinke JW, Borish L. 3. Cytokines and chemokines. J Allergy Clin Immunol 2006; 117:S441-5; PMID:16455343; http://dx.doi.org/10.1016/j.jaci.2005.07.001
9. Gruenbacher G, Nussbaumer O, Gander H, Steiner B, Leonhartsberger N, Thurnher M. Stress-related and homeostatic cytokines regulate Vgamma9Vdelta2 T-cell surveillance of mevalonate metabolism. Oncoimmunology 2014; 3:e953410; PMID:25960933; http://dx.doi.org/10.4161/21624011.2014.953410
10. Papatriantafyllou M. Cytokines: true to their family name. Nat Rev Immunol 2013; 13:544-5; PMID:23827957; http://dx.doi.org/10.1038/nri3496
11. Hombach AA, Abken H. Targeting two co-operating cytokines efficiently shapes immune responses. Oncoimmunology 2013; 2:e23205; PMID:23802072; http://dx.doi.org/10.4161/onci.23205
12. Sada A, Tumbar T. New insights into mechanisms of stem cell daughter fate determination in regenerative tissues. Int Rev Cell Mol Biol 2013; 300:1-50; PMID:23273858; http://dx.doi.org/10.1016/B978-0-12-405210-9.00001-1
13. Metcalf D. The colony-stimulating factors and cancer. Nat Rev Cancer 2010; 10:425-34; PMID:20495576; http://dx.doi.org/10.1038/nrc2843
14. 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
15. 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
16. Ohlsson K, Bjork 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
17. 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
18. 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
19. Seifert AW, Maden M. New insights into vertebrate skin regeneration. Int Rev Cell Mol Biol 2014; 310:129-69; PMID:24725426; http://dx.doi.org/10.1016/B978-0-12-800180-6.00004-9
20. Zitvogel L, Galluzzi L, Kepp O, Smyth MJ, Kroemer G. Type I interferons in anticancer immunity. Nat Rev Immunol 2015; 15:405-14; PMID:26027717; http://dx.doi.org/10.1038/nri3845

21. Crouse J, Kalinke U, Oxenius A. Regulation of antiviral T cell responses by type I interferons. Nat Rev Immunol 2015; 15:231-42; PMID:25790790; http://dx.doi.org/10.1038/nri3806
22. McNab F, Mayer-Barber K, Sher A, Wack A, O'Garra A. Type I interferons in infectious disease. Nat Rev Immunol 2015; 15:87-103; PMID:25614319; http://dx.doi.org/10.1038/nri3787
23. Dudek AM, Garg AD, Krysko DV, De Ruysscher D, Agostinis P. Inducers of immunogenic cancer cell death. Cytokine Growth Factor Rev 2013; 24:319-33; PMID:23391812; http://dx.doi.org/10.1016/j.cytogfr.2013.01.005
24. Garg AD, Martin S, Golab J, Agostinis P. Danger signalling during cancer cell death: origins, plasticity and regulation. Cell Death Differ 2014; 21:26-38; PMID:23686135; http://dx.doi.org/10.1038/cdd.2013.48
25. Golden EB, Frances D, Pellicciotta I, Demaria S, Helen Barcellos-Hoff M, Formenti SC. Radiation fosters dose-dependent and chemotherapy-induced immunogenic cell death. Oncoimmunology 2014; 3:e28518; PMID:25071979; http://dx.doi.org/10.4161/onci.28518
26. Kepp O, Senovilla L, Kroemer G. Immunogenic cell death inducers as anticancer agents. Oncotarget 2014; 5:5190-1; PMID:25114034; http://dx.doi.org/10.18632/oncotarget.2266
27. Krysko DV, Garg AD, Kaczmarek A, Krysko O, Agostinis P, Vandenabeele P. Immunogenic cell death and DAMPs in cancer therapy. Nat Rev Cancer 2012; 12:860-75; PMID:23151605; http://dx.doi.org/10.1038/nrc3380
28. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. Annu Rev Immunol 2013; 31:51-72; PMID:23157435; http://dx.doi.org/10.1146/annurev-immunol-032712-100008
29. Kepp O, Senovilla L, Vitale I, Vacchelli E, Adjemian S, Agostinis P, Apetoh L, Aranda F, Barnaba V, Bloy N et al. Consensus guidelines for the detection of immunogenic cell death. Oncoimmunology 2014; 3:e955691; PMID:25941621; http://dx.doi.org/10.4161/21624011.2014.955691
30. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, Lee W, Yuan J, Wong P, Ho TS et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015; 348:124-8; PMID:25765070; http://dx.doi.org/10.1126/science.aaa1348
31. Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, Ivanova Y, Hundal J, Arthur CD, Krebber WJ et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature 2014; 515:577-81; PMID:25428507; http://dx.doi.org/10.1038/nature13988
32. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, Walsh LA, Postow MA, Wong P, Ho TS et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med 2014; 371:2189-99; PMID:25409260; http://dx.doi.org/10.1056/NEJMoa1406498
33. Negrier S, Escudier B, Lasset C, Douillard JY, Savary J, Chevreau C, Ravaud A, Mercatello A, Peny J, Mousseau M et al. Recombinant human interleukin-2, recombinant human interferon alfa-2a, or both in metastatic renal-cell carcinoma. Groupe Francais d'Immunotherapie. N Engl J Med 1998; 338:1272-8; PMID:9562581; http://dx.doi.org/10.1056/NEJM199804303381805
34. Tsao H, Atkins MB, Sober AJ. Management of cutaneous melanoma. N Engl J Med 2004; 351:998-1012; PMID:15342808; http://dx.doi.org/10.1056/NEJMra041245
35. Eggermont AM, Spatz A, Robert C. Cutaneous melanoma. Lancet 2014; 383:816-27; PMID:24054424; http://dx.doi.org/10.1016/S0140-6736(13)60802-8
36. 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
37. Khoury HJ, Loberiza FR, Jr., Ringden 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
38. 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
39. Hosing C. Hematopoietic stem cell mobilization with G-CSF. Methods Mol Biol 2012; 904:37-47; PMID:22890920; http://dx.doi.org/10.1007/978-1-61779-943-3_3
40. 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
41. 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
42. 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
43. Pabst T, Vellenga E, van Putten W, Schouten HC, Graux C, Vekemans MC, Biemond B, Sonneveld P, Passweg J, Verdonck L et al. 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
44. 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
45. 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
46. Deroose JP, Grunhagen DJ, van Geel AN, de Wilt JH, Eggermont AM, Verhoef C. Long-term outcome of isolated limb perfusion with tumour necrosis factor-alpha for patients with melanoma in-transit metastases. Br J Surg 2011; 98:1573-80; PMID:21739427; http://dx.doi.org/10.1002/bjs.7621
47. 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
48. Eggermont AM, Schraffordt Koops H, Klausner JM, Kroon BB, Schlag PM, Lienard 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 64-5; http://dx.doi.org/10.1097/00000658-199612000-00011; PMID:8968230
49. 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
50. Eggermont AM, Schraffordt Koops H, Lienard 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

51. Beug ST, LaCasse EC, Korneluk RG. Smac mimetics combined with innate immune stimuli create the perfect cytokine storm to kill tumor cells. Oncoimmunology 2014; 3:e28541; PMID:25050221; http://dx.doi.org/10.4161/onci.28541
52. 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
53. Grunhagen 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
54. 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
55. Grunhagen 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
56. 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
57. 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
58. 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
59. 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
60. 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
61. 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
62. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002; 420:860-7; PMID:12490959; http://dx.doi.org/10.1038/nature01322
63. 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
64. 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
65. 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
66. 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
67. Hinchcliffe EH. Centrosomes and the art of mitotic spindle maintenance. Int Rev Cell Mol Biol 2014; 313:179-217; PMID:25376493; http://dx.doi.org/10.1016/B978-0-12-800177-6.00006-2
68. Eggermont AM, Suciu S, Santinami M, Testori A, Kruit WH, Marsden J, Punt CJ, Salès F, Gore M, Mackie R et al. Adjuvant therapy with pegylated interferon alfa-2b versus observation alone in resected stage III melanoma: final results of EORTC 18991, a randomised phase III trial. Lancet 2008; 372:117-26; PMID:18620949; http://dx.doi.org/10.1016/S0140-6736(08)61033-8
69. 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
70. Vacchelli E, Eggermont A, Galon J, Sautes-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. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, Hassel JC, Rutkowski P, McNeil C, Kalinka-Warzocha E et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med 2015; 372:320-30; PMID:25399552; http://dx.doi.org/10.1056/NEJMoa1412082
72. Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, 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
73. Aranda F, Vacchelli E, Obrist F, Eggermont A, Galon J, Herve Fridman W, Cremer I, Tartour E, Zitvogel L, Kroemer G et al. Trial Watch: Adoptive cell transfer for anticancer immunotherapy. Oncoimmunology 2014; 3:e28344; PMID:25050207; http://dx.doi.org/10.4161/onci.28344
74. 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
75. Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: harnessing the T cell response. Nat Rev Immunol 2012; 12:269-81; PMID:22437939; http://dx.doi.org/10.1038/nri3191
76. Willimsky G, Protzer U, Knolle P, Heikenwalder M. Adoptive T-cell therapy to treat liver cancer: is the liver microenvironment key? Oncotarget 2013; 4:1117-8; PMID:23907631; http://dx.doi.org/10.18632/oncotarget.1179
77. Pol J, Bloy N, Obrist F, Eggermont A, Galon J, Cremer I, Erbs P, Limacher JM, Preville X, Zitvogel L et al. Trial Watch: Oncolytic viruses for cancer therapy. Oncoimmunology 2014; 3:e28694; PMID:25097804; http://dx.doi.org/10.4161/onci.28694
78. Vacchelli E, Eggermont A, Sautes-Fridman C, Galon J, Zitvogel L, Kroemer G, Galluzzi L. Trial watch: Oncolytic viruses for cancer therapy. Oncoimmunology 2013; 2:e24612; PMID:23894720; http://dx.doi.org/10.4161/onci.24612
79. Donnelly O, Ilett E, Kottke T, Thompson J, Vile R, Melcher A. Cytokine-enhanced intravenous oncolytic virotherapy. Lancet 2014; 383:S4; http://dx.doi.org/10.1016/S0140-6736(14)60305-6
80. Lichty BD, Breitbach CJ, Stojdl DF, Bell JC. Going viral with cancer immunotherapy. Nat Rev Cancer 2014; 14:559-67; PMID:24990523; http://dx.doi.org/10.1038/nrc3770
81. Tong Y, You L, Liu H, Li L, Meng H, Qian Q, Qian W. Potent antitumor activity of oncolytic adenovirus expressing Beclin-1 via induction of autophagic cell death in leukemia. Oncotarget 2013; 4:860-74; PMID:23765161; http://dx.doi.org/10.18632/oncotarget.1018
82. Pol J, Bloy N, Obrist F, Eggermont A, Galon J, Herve 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
83. Hamilton DH, Litzinger MT, Jales A, Huang B, Fernando RI, Hodge JW, Ardiani A, Apelian D, Schlom J, Palena C. Immunological targeting of tumor cells undergoing an epithelial-mesenchymal

transition via a recombinant brachyury-yeast vaccine. Oncotarget 2013; 4:1777-90; PMID:24125763; http://dx.doi.org/10.18632/oncotarget.1295

84. Senovilla L, Vacchelli E, Garcia P, Eggermont A, Fridman WH, Galon J, Zitvogel L, Kroemer G, Galluzzi L. Trial watch: DNA vaccines for cancer therapy. Oncoimmunology 2013; 2:e23803; PMID:23734328; http://dx.doi.org/10.4161/onci.23803
85. Aranda F, Vacchelli E, Eggermont A, Galon J, Sautes-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
86. Gabai V, Venanzi FM, Bagashova E, Rud O, Mariotti F, Vullo C, Catone G, Sherman MY, Concetti A, Chursov A et al. Pilot study of p62 DNA vaccine in dogs with mammary tumors. Oncotarget 2014; 5:12803-10; PMID:25296974; http://dx.doi.org/10.18632/oncotarget.2516
87. Blankenstein T, Coulie PG, Gilboa E, Jaffee EM. The determinants of tumour immunogenicity. Nat Rev Cancer 2012; 12:307-13; PMID:22378190; http://dx.doi.org/10.1038/nrc3246
88. Palucka K, Banchereau J. Dendritic-cell-based therapeutic cancer vaccines. Immunity 2013; 39:38-48; PMID:23890062; http://dx.doi.org/10.1016/j.immuni.2013.07.004
89. Bloy N, Pol J, Aranda F, Eggermont A, Cremer I, Fridman WH, Fučíková J, Galon J, Tartour E, Spisek R et al. Trial Watch: Dendritic cell-based anticancer therapy. Oncoimmunology 2014; 3:e963424; PMID:25941593; http://dx.doi.org/10.4161/21624011.2014.963424
90. Vacchelli E, Vitale I, Eggermont A, Fridman WH, Fucikova 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
91. Tacken PJ, de Vries IJ, Torensma R, Figdor CG. Dendritic-cell immunotherapy: from ex vivo loading to in vivo targeting. Nat Rev Immunol 2007; 7:790-802; PMID:17853902; http://dx.doi.org/10.1038/nri2173
92. Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. Nat Rev Cancer 2012; 12:265-77; PMID:22437871; http://dx.doi.org/10.1038/nrc3258
93. Wang J, Lin D, Peng H, Shao J, Gu J. Cancer-derived immunoglobulin G promotes LPS-induced proinflammatory cytokine production via binding to TLR4 in cervical cancer cells. Oncotarget 2014; 5:9727-43; PMID:25179302; http://dx.doi.org/10.18632/oncotarget.2359
94. Huang SW, Kao JK, Wu CY, Wang ST, Lee HC, Liang SM, Chen YJ, Shieh JJ. Targeting aerobic glycolysis and HIF-1alpha expression enhance imiquimod-induced apoptosis in cancer cells. Oncotarget 2014; 5:1363-81; PMID:24658058; http://dx.doi.org/10.18632/oncotarget.1734
95. Vacchelli E, Eggermont A, Sautes-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
96. Aranda F, Vacchelli E, Obrist F, Eggermont A, Galon J, Sautes-Fridman C, Cremer I, Henrik Ter Meulen J, Zitvogel L, Kroemer G et al. Trial Watch: Toll-like receptor agonists in oncological indications. Oncoimmunology 2014; 3:e29179; PMID:25083332; http://dx.doi.org/10.4161/onci.29179
97. Rakoff-Nahoum S, Medzhitov R. Toll-like receptors and cancer. Nat Rev Cancer 2009; 9:57-63; PMID:19052556; http://dx.doi.org/10.1038/nrc2541
98. Pietri E, Andreis D, Fabbri F, Menna C, Schirone A, Kopf B, Rocca A, Amadori D, De Giorgi U. A phase II study of a dose-density regimen with fluorouracil, epirubicin, and cyclophosphamide on days 1 and 4 every 14 days with filgrastim support followed by weekly paclitaxel in women with primary breast cancer. Oncologist 2015; 20:239-40; PMID:25637379; http://dx.doi.org/10.1634/theoncologist.2014-0326
99. Kasai K, Kooka Y, Suzuki Y, Suzuki A, Oikawa T, Ushio A, Kasai Y, Sawara K, Miyamoto Y, Oikawa K et al. Efficacy of hepatic arterial infusion chemotherapy using 5-fluorouracil and systemic pegylated interferon alpha-2b for advanced intrahepatic cholangiocarcinoma. Ann Surg Oncol 2014; 21:3638-45; PMID:24817369; http://dx.doi.org/10.1245/s10434-014-3766-7
100. Donini M, Buti S, Lazzarelli S, Bozzetti R, Rivoltini L, Camisaschi C, Castelli C, Bearz A, Simonelli C, Lo Re G et al. Dose-finding/phase II trial: bevacizumab, immunotherapy, and chemotherapy (BIC) in metastatic renal cell cancer (mRCC). Antitumor effects and variations of circulating T regulatory cells (Treg). Target Oncol 2015; 10:277-86; PMID:25230695; http://dx.doi.org/10.1007/s11523-014-0337-6
101. Zeidner JF, Gladstone DE, Zahurak M, Matsui WH, Gocke C, Jones RJ, Smith BD. Granulocyte-macrophage colony stimulating factor (GM-CSF) enhances the clinical responses to interferon-alpha (IFN) in newly diagnosed chronic myeloid leukemia (CML). Leuk Res 2014; 38:886-90; PMID:25012565; http://dx.doi.org/10.1016/j.leukres.2014.05.012
102. Baek S, Kim YM, Kim SB, Kim CS, Kwon SW, Kim Y, Kim H, Lee H. Therapeutic DC vaccination with IL-2 as a consolidation therapy for ovarian cancer patients: a phase I/II trial. Cell Mol Immunol 2015; 12:87-95; PMID:24976269; http://dx.doi.org/10.1038/cmi.2014.40
103. Aggarwal RR, Beer TM, Weinberg VK, Higano C, Taplin ME, Ryan CJ, Lin AM, Alumkal J, Graff JN, Nordquist LT et al. Intermittent chemotherapy as a platform for testing novel agents in patients with metastatic castration-resistant prostate cancer: A department of defense prostate cancer clinical trials consortium randomized phase II trial of intermittent docetaxel with prednisone with or without maintenance GM-CSF. Clin Genitourin Cancer 2015; 13:e191-8; PMID:25557266; http://dx.doi.org/10.1016/j.clgc.2014.12.004
104. Catania C, Maur M, Berardi R, Rocca A, Giacomo AM, Spitaleri G, Masini C, Pierantoni C, González-Iglesias R, Zigon G et al. The tumor-targeting immunocytokine F16-IL2 in combination with doxorubicin: dose escalation in patients with advanced solid tumors and expansion into patients with metastatic breast cancer. Cell Adh Migr 2015; 9:14-21; PMID:25562532; http://dx.doi.org/10.4161/19336918.2014.983785
105. Elkord E, Burt DJ, Sundstedt A, Nordle O, Hedlund G, Hawkins RE. Immunological response and overall survival in a subset of advanced renal cell carcinoma patients from a randomized phase 2/3 study of naptumomab estafenatox plus IFN-alpha versus IFN-alpha. Oncotarget 2015; 6:4428-39; PMID:25669986; http://dx.doi.org/10.18632/oncotarget.2922
106. Jarvinen R, Marttila T, Kaasinen E, Rintala E, Aaltomaa S, Kallio J, Liukkonen T, Puolakka VM, Seppänen M, Tuhkanen K et al. Long-term outcome of patients with frequently recurrent non-muscle-invasive bladder carcinoma treated with one perioperative plus four weekly instillations of mitomycin C followed by monthly bacillus calmette-guerin (BCG) or alternating BCG and interferon-alpha2b instillations: Prospective randomised finnBladder-4 study. Eur Urol 2015; 68:611-7; PMID:25748117; http://dx.doi.org/10.1016/j.eururo.2015.02.022
107. Kimby E, Ostenstad B, Brown P, Hagberg H, Erlanson M, Holte H, Linden O, Johansson AS, Ahlgren T, Wader K et al. Two courses of four weekly infusions of rituximab with or without interferon-alpha2a: final results from a randomized phase III study in symptomatic indolent B-cell lymphomas. Leuk Lymphoma 2015; 56:2598-607; PMID:25686644; http://dx.doi.org/10.3109/10428194.2015.1014363
108. Ravaud A, Barrios CH, Alekseev B, Tay MH, Agarwala SS, Yalcin S, Lin CC, Roman L, Shkolnik M, Anak O et al. RECORD-2: phase II randomized study of everolimus and bevacizumab versus interferon alpha-2a and bevacizumab as first-line therapy in patients with metastatic renal cell carcinoma. Ann Oncol 2015; 26:1378-84; PMID:25851632; http://dx.doi.org/10.1093/annonc/mdv149
109. Luke JJ, Donahue H, Nishino M, Giobbie-Hurder A, Davis M, Bailey N, Ott PA, Hodi FS. Single Institution Experience of Ipilimumab 3 mg/kg with Sargramostim (GM-CSF) in Metastatic Melanoma. Cancer Immunol Res 2015; 3:986-91; PMID:25943535; http://dx.doi.org/10.1158/2326-6066.CIR-15-0066
110. Golden EB, Chhabra A, Chachoua A, Adams S, Donach M, Fenton-Kerimian M, Friedman K, Ponzo F, Babb JS, Goldberg J et al. Local radiotherapy and granulocyte-macrophage colony-stimulating factor

to generate abscopal responses in patients with metastatic solid tumours: a proof-of-principle trial. Lancet Oncol 2015; 16:795-803; PMID:26095785; http://dx.doi.org/10.1016/S1470-2045(15)00054-6

111. Song L, Zhou X, Li X. Phase II trial of granulocyte-macrophage colony-stimulating factor plus thalidomide in older patients with castration-resistant prostate cancer. Mol Clin Oncol 2015; 3:865-8; PMID:26171197; http://dx.doi.org/10.3892/mco.2015.571
112. Dijkgraaf EM, Santegoets SJ, Reyners AK, Goedemans R, Wouters MC, Kenter GG, van Erkel AR, van Poelgeest MI, Nijman HW, van der Hoeven JJ et al. A phase I trial combining carboplatin/doxorubicin with tocilizumab, an anti-IL-6R monoclonal antibody, and interferon-alpha2b in patients with recurrent epithelial ovarian cancer. Ann Oncol 2015; 26:2141-9; PMID:26216383; http://dx.doi.org/10.1093/annonc/mdv309
113. Eto M, Kawano Y, Hirao Y, Mita K, Arai Y, Tsukamoto T, Hashine K, Matsubara A, Fujioka T, Kimura G et al. Phase II clinical trial of sorafenib plus interferon-alpha treatment for patients with metastatic renal cell carcinoma in Japan. BMC Cancer 2015; 15:667; PMID:26452347; http://dx.doi.org/10.1186/s12885-015-1675-1
114. Conlon KC, Lugli E, Welles HC, Rosenberg SA, Fojo AT, Morris JC, Fleisher TA, Dubois SP, Perera LP, Stewart DM et al. Redistribution, hyperproliferation, activation of natural killer cells and CD8 T cells, and cytokine production during first-in-human clinical trial of recombinant human interleukin-15 in patients with cancer. J Clin Oncol 2015; 33:74-82; PMID:25403209; http://dx.doi.org/10.1200/JCO.2014.57.3329
115. Galluzzi L. New immunotherapeutic paradigms for castration-resistant prostate cancer. Oncoimmunology 2013; 2:e26084; http://dx.doi.org/10.4161/onci.26084
116. Semeraro M, Galluzzi L. Novel insights into the mechanism of action of lenalidomide. Oncoimmunology 2014; 3:e28386; PMID:25340011; http://dx.doi.org/10.4161/onci.28386
117. Semeraro M, Vacchelli E, Eggermont A, Galon J, Zitvogel L, Kroemer G, Galluzzi L. Trial Watch: Lenalidomide-based immunochemotherapy. Oncoimmunology 2013; 2:e26494; PMID:24482747; http://dx.doi.org/10.4161/onci.26494
118. Vacchelli E, Vitale I, Tartour E, Eggermont A, Sautes-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. Gameiro SR, Jammeh ML, Wattenberg MM, Tsang KY, Ferrone S, Hodge JW. Radiation-induced immunogenic modulation of tumor enhances antigen processing and calreticulin exposure, resulting in enhanced T-cell killing. Oncotarget 2014; 5:403-16; PMID:24480782; http://dx.doi.org/10.18632/oncotarget.1719
120. Bloy N, Pol J, Manic G, Vitale I, Eggermont A, Galon J, Tartour E, Zitvogel L, Kroemer G, Galluzzi L. Trial Watch: Radioimmunotherapy for oncological indications. Oncoimmunology 2014; 3:e954929; PMID:25941606; http://dx.doi.org/10.4161/21624011.2014.954929
121. Mesange P, Poindessous V, Sabbah M, Escargueil AE, de Gramont A, Larsen AK. Intrinsic bevacizumab resistance is associated with prolonged activation of autocrine VEGF signaling and hypoxia tolerance in colorectal cancer cells and can be overcome by nintedanib, a small molecule angiokinase inhibitor. Oncotarget 2014; 5:4709-21; PMID:25015210; http://dx.doi.org/10.18632/oncotarget.1671
122. Vacchelli E, Aranda F, Eggermont A, Galon J, Sautes-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
123. Chen P, Bonaldo P. Role of macrophage polarization in tumor angiogenesis and vessel normalization: implications for new anticancer therapies. Int Rev Cell Mol Biol 2013; 301:1-35; PMID:23317816; http://dx.doi.org/10.1016/B978-0-12-407704-1.00001-4
124. Giovannini C, Baglioni M, Baron Toaldo M, Ventrucci C, D'Adamo S, Cipone M, Chieco P, Gramantieri L, Bolondi L. Notch3 inhibition enhances sorafenib cytotoxic efficacy by promoting GSK3b phosphorylation and p21 down-regulation in hepatocellular carcinoma. Oncotarget 2013; 4:1618-31; PMID:24113128; http://dx.doi.org/10.18632/oncotarget.1221
125. Kharaziha P, De Raeve H, Fristedt C, Li Q, Gruber A, Johnsson P, Kokaraki G, Panzar M, Laane E, Osterborg A et al. Sorafenib has potent antitumor activity against multiple myeloma in vitro, ex vivo, and in vivo in the 5T33MM mouse model. Cancer Res 2012; 72:5348-62; PMID:22952216; http://dx.doi.org/10.1158/0008-5472.CAN-12-0658
126. 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
127. Vacchelli E, Aranda F, Eggermont A, Galon J, Sautes-Fridman C, Cremer I, Zitvogel L, Kroemer G, Galluzzi L. Trial Watch: Chemotherapy with immunogenic cell death inducers. Oncoimmunology 2014; 3:e27878; PMID:24800173; http://dx.doi.org/10.4161/onci.27878
128. Vacchelli E, Senovilla L, Eggermont A, Fridman WH, Galon J, Zitvogel L, Kroemer G, Galluzzi L. Trial watch: Chemotherapy with immunogenic cell death inducers. Oncoimmunology 2013; 2:e23510; PMID:23687621; http://dx.doi.org/10.4161/onci.23510
129. Rini BI, Bellmunt J, Clancy J, Wang K, Niethammer AG, Hariharan S, Escudier B. Randomized phase III trial of temsirolimus and bevacizumab versus interferon alfa and bevacizumab in metastatic renal cell carcinoma: INTORACT trial. J Clin Oncol 2014; 32:752-9; PMID:24297945; http://dx.doi.org/10.1200/JCO.2013.50.5305
130. Cai Q, Chen Y, Zou D, Zhang L, Badillo M, Zhou S, Lopez E, Jiang W, Huang H, Lin T et al. Clinical outcomes of a novel combination of lenalidomide and rituximab followed by stem cell transplantation for relapsed/refractory aggressive B-cell non-hodgkin lymphoma. Oncotarget 2014; 5:7368-80; PMID:25228589; http://dx.doi.org/10.18632/oncotarget.2255
131. Wagner JY, Schwarz K, Schreiber S, Schmidt B, Wester HJ, Schwaiger M, Peschel C, von Schilling C, Scheidhauer K, Keller U. Myeloablative anti-CD20 radioimmunotherapy +/- high-dose chemotherapy followed by autologous stem cell support for relapsed/refractory B-cell lymphoma results in excellent long-term survival. Oncotarget 2013; 4:899-910; PMID:23765188; http://dx.doi.org/10.18632/oncotarget.1037
132. Park MS, Kim BR, Dong SM, Lee SH, Kim DY, Rho SB. The antihypertension drug doxazosin inhibits tumor growth and angiogenesis by decreasing VEGFR-2/Akt/mTOR signaling and VEGF and HIF-1alpha expression. Oncotarget 2014; 5:4935-44; PMID:24952732; http://dx.doi.org/10.18632/oncotarget.2064
133. Blagosklonny MV. Immunosuppressants in cancer prevention and therapy. Oncoimmunology 2013; 2:e26961; PMID:24575379; http://dx.doi.org/10.4161/onci.26961
134. Pezzolo A, Marimpietri D, Raffaghello L, Cocco C, Pistorio A, Gambini C, Cilli M, Horenstein A, Malavasi F, Pistoia V. Failure of anti tumor-derived endothelial cell immunotherapy depends on augmentation of tumor hypoxia. Oncotarget 2014; 5:10368-81; PMID:25362644; http://dx.doi.org/10.18632/oncotarget.2015
135. Finisguerra V, Di Conza G, Di Matteo M, Serneels J, Costa S, Thompson AA, Wauters E, Walmsley S, Prenen H, Granot Z et al. MET is required for the recruitment of anti-tumoural neutrophils. Nature 2015; 522:349-53; PMID:25985180; http://dx.doi.org/10.1038/nature14407
136. Marcais A, Cherfils-Vicini J, Viant C, Degouve S, Viel S, Fenis A, Rabilloud J, Mayol K, Tavares A, Bienvenu J et al. The metabolic checkpoint kinase mTOR is essential for IL-15 signaling during the development and activation of NK cells. Nat Immunol 2014; 15:749-57; PMID:24973821; http://dx.doi.org/10.1038/ni.2936
137. O'Sullivan T, Saddawi-Konefka R, Gross E, Tran M, Mayfield SP, Ikeda H, Bui JD. Interleukin-17D mediates tumor rejection through recruitment of natural killer cells. Cell Rep 2014; 7:989-98; PMID:24794441; http://dx.doi.org/10.1016/j.celrep.2014.03.073
138. Zhu EF, Gai SA, Opel CF, Kwan BH, Surana R, Mihm MC, Kauke MJ, Moynihan KD, Angelini A, Williams RT et al. Synergistic innate and adaptive immune response to combination immunotherapy with anti-tumor antigen antibodies and extended serum half-life IL-2. Cancer Cell 2015; 27:489-501; PMID:25873172; http://dx.doi.org/10.1016/j.ccell.2015.03.004

139. Ruffell B, Chang-Strachan D, Chan V, Rosenbusch A, Ho CM, Pryer N, Daniel D, Hwang ES, Rugo HS, Coussens LM. Macrophage IL-10 blocks CD8+ T cell-dependent responses to chemotherapy by suppressing IL-12 expression in intratumoral dendritic cells. Cancer Cell 2014; 26:623-37; PMID:25446896; http://dx.doi.org/10.1016/j.ccell.2014.09.006
140. Hou J, Zhou Y, Zheng Y, Fan J, Zhou W, Ng IO, Sun H, Qin L, Qiu S, Lee JM et al. Hepatic RIG-I predicts survival and interferon-alpha therapeutic response in hepatocellular carcinoma. Cancer Cell 2014; 25:49-63; PMID:24360797; http://dx.doi.org/10.1016/j.ccr.2013.11.011
141. Litvin O, Schwartz S, Wan Z, Schild T, Rocco M, Oh NL, Chen BJ, Goddard N, Pratilas C, Pe'er D. Interferon alpha/beta enhances the cytotoxic response of MEK inhibition in melanoma. Mol Cell 2015; 57:784-96; PMID:25684207; http://dx.doi.org/10.1016/j.molcel.2014.12.030
142. Escobar G, Moi D, Ranghetti A, Ozkal-Baydin P, Squadrito ML, Kajaste-Rudnitski A, Bondanza A, Gentner B, De Palma M, Mazzieri R et al. Genetic engineering of hematopoiesis for targeted IFN-alpha delivery inhibits breast cancer progression. Sci Transl Med 2014; 6:217ra3; PMID:24382895; http://dx.doi.org/10.1126/scitranslmed.3006353
143. Bald T, Landsberg J, Lopez-Ramos D, Renn M, Glodde N, Jansen P, Gaffal E, Steitz J, Tolba R, Kalinke U et al. Immune cell-poor melanomas benefit from PD-1 blockade after targeted type I IFN activation. Cancer Discov 2014; 4:674-87; PMID:24589924; http://dx.doi.org/10.1158/2159-8290.CD-13-0458
144. Sistigu A, Yamazaki T, Vacchelli E, Chaba K, Enot DP, Adam J, Vitale I, Goubar A, Baracco EE, Remédios C et al. Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. Nat Med 2014; 20:1301-9; PMID:25344738; http://dx.doi.org/10.1038/nm.3708
145. Kroemer G, Senovilla L, Galluzzi L, Andre F, Zitvogel L. Natural and therapy-induced immunosurveillance in breast cancer. Nat Med 2015; 21:1128-38; PMID:26444637; http://dx.doi.org/10.1038/nm.3944
146. Duffy MJ, Crown J, Mullooly M. ADAM10 and ADAM17: New players in trastuzumab tesistance. Oncotarget 2014; 5:10963-4; PMID:25460503; http://dx.doi.org/10.18632/oncotarget.2794
147. Vici P, Sperati F, Maugeri-Sacca M, Melucci E, Di Benedetto A, Di Lauro L, Pizzuti L, Sergi D, Terrenato I, Esposito L et al. p53 status as effect modifier of the association between pre-treatment fasting glucose and breast cancer outcomes in non diabetic, HER2 positive patients treated with trastuzumab. Oncotarget 2014; 5:10382-92; PMID:25071015; http://dx.doi.org/10.18632/oncotarget.2060
148. Choi BD, Pastan I, Bigner DD, Sampson JH. A novel bispecific antibody recruits T cells to eradicate tumors in the "immunologically privileged" central nervous system. Oncoimmunology 2013; 2:e23639; PMID:23734318; http://dx.doi.org/10.4161/onci.23639
149. Walter RB. Biting back: BiTE antibodies as a promising therapy for acute myeloid leukemia. Expert Rev Hematol 2014; 7:317-9; PMID:24617311; http://dx.doi.org/10.1586/17474086.2014.896190
150. Turini M, Chames P, Bruhns P, Baty D, Kerfelec B. A FcgammaRIII-engaging bispecific antibody expands the range of HER2-expressing breast tumors eligible to antibody therapy. Oncotarget 2014; 5:5304-19; PMID:24979648; http://dx.doi.org/10.18632/oncotarget.2093
151. Beatty GL, Vonderheide RH. Telomerase as a universal tumor antigen for cancer vaccines. Expert Rev Vaccines 2008; 7:881-7; PMID:18767939; http://dx.doi.org/10.1586/14760584.7.7.881
152. Nair SK, Heiser A, Boczkowski D, Majumdar A, Naoe M, Lebkowski JS, Vieweg J, Gilboa E. Induction of cytotoxic T cell responses and tumor immunity against unrelated tumors using telomerase reverse transcriptase RNA transfected dendritic cells. Nat Med 2000; 6:1011-7; PMID:10973321; http://dx.doi.org/10.1038/79519
153. Dinutuximab approved for high-risk neuroblastoma. Cancer Discov 2015; 5:OF5; PMID:25851859; http://dx.doi.org/10.1158/2159-8290.CD-NB2015-044
154. Reichert JM. Antibodies to watch in 2015. MAbs 2015; 7:1-8; PMID:25484055; http://dx.doi.org/10.4161/19420862.2015.988944
155. Zamarin D, Postow MA. Immune checkpoint modulation: rational design of combination strategies. Pharmacol Ther 2015; 150:23-32; PMID:25583297; http://dx.doi.org/10.1016/j.pharmthera.2015.01.003
156. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, Tang Y, Zhang Y, Kang S, Zhou T et al. EBV-driven LMP1 and IFN-gamma up-regulate PD-L1 in nasopharyngeal carcinoma: Implications for oncotargeted therapy. Oncotarget 2014; 5:12189-202; PMID:25361008; http://dx.doi.org/10.18632/oncotarget.2608
157. Ceresa BP, Peterson JL. Cell and molecular biology of epidermal growth factor receptor. Int Rev Cell Mol Biol 2014; 313:145-78; PMID:25376492; http://dx.doi.org/10.1016/B978-0-12-800177-6.00005-0
158. 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