Trial Watch—Immunostimulation with cytokines in cancer therapy

Erika Vacchelli,a,b,c,d,e, Fernando Aranda,f,g, Norma Bloya,b,c,d,e, Aitziber Buqueta,b,c,d,e, Isabelle Cremera,b,c,d,e, Alexander Eggermonta,e, Wolf Hervé Fridmana,b,c,g, Jitka Fucikovah,i, Jérôme Galona,b,c,j, Radek Spisekh,i, Laurence Zitvogela,k, Guido Kroemer,a,b,c,d,l,m,n, and Lorenzo Galluzzi,a,b,c,d,e

Equally contributed to this work.

INSERM, U1138, Paris, France; Université Paris Descartes/Paris V, Sorbonne Paris Cité, Paris, France; Université Pierre et Marie Curie/Paris VI, Paris, France; Equipe 11 labellisée par la Ligue Nationale contre le Cancer, Center de Recherche des Cordeliers, Paris, France; Gustave Roussy Cancer Campus, Villejuif, France; Group of Immune receptors of the Innate and Adaptive System, Institut d’Investigacions Biomédiques August Pi i Sunyer (IDIBAPS); Equipe 13, Center de Recherche des Cordeliers, Paris, France; Sotio, Prague, Czech Republic; Dept. of Immunology, 2nd Faculty of Medicine and University Hospital Motol, Charles University, Prague, Czech Republic; Laboratory of Integrative Cancer Immunology, Center de Recherche des Cordeliers, Paris, France; INSERM, U1015, CICTS07, Villejuif, France; Pôle de Biologie, Hôpital Européen Georges Pompidou, AP-HP, Paris, France; Metabolomics and Cell Biology Platforms, Gustave Roussy Cancer Campus, Villejuif, France; Department of Women’s and Children’s Health, Karolinska University Hospital, Stockholm, Sweden

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 immune 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.

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

CONTACT Lorenzo Galluzzi deadoc@vodafone.it; Guido Kroemer kroemer@orange.fr

© 2016 Taylor & Francis Group, LLC

http://dx.doi.org/10.1080/2162402X.2015.1115942
death” (ICD). 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, like melanoma and renal cell carcinoma (RCC). 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) or oncotoxic (TNF) factors.  

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; (2) establish or perpetuate foci of chronic inflammation that may sustain oncogenesis or tumor progression; or (3) promote proliferation among otherwise non-proliferating cells, thereby promoting the fixation of potentially oncogenic genetic or epigenetic defects.

Owing to such non-negligible side effects as well as generally low-objective response rates, the interest of oncologists in using recombinant cytokines as systemic standalone anticaner 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. Rather, investigators and practitioners are focusing their efforts on the possibility to use recombinant cytokines (at low doses and locally) to boost the anticaner activity of other immunotherapeutic regimens, including checkpoint blockers, adoptive cell transfer, oncolytic virotherapy, DNA- and peptide-based vaccines, dendritic cell (DC)-based interventions, as well as other immunostimulatory agents like Toll-like receptor (TLR) agonists.

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 oncolgical indications (May 2014), 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, while only one trial tested the clinical profile of a hitherto experimental cytokine (i.e., IL-15).

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). 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 (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, or in combination with thalidomide, respectively; (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, and (4) 41 patients affected with advanced solid neoplasms, who were treated with GM-CSF s.c. in combination with local radiotherapy, with the specific aim to provoke systemic tumor-targeting immune responses (NCT02474186). 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.

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) along with the multitarget tyrosine kinase inhibitor sorafenib (UMIN000002466), along with the multitarget therapy was associated with severe side effects, resulting in the premature closure of the study.
enterotoxin A (naptumomab estafenato);100,105,108,113,129 (2) 313 patients with symptomatic indolent B-cell lymphoma, receiving IFN-α2a in combination with the CD20-targeting mAb rituximab130,131 (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 intraperitoneal 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 temsirolimus132,133 (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 tenasin C, TNG)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 (Vesalus 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) Marcais 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, 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 (NCT02499421) 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 (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) (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 capicitabine-based chemotherapy (NCT02218164). Finally, two clinical studies are assessing the safety and efficacy of solubilized form of crystalline recombinant IFN-α2b (known as rIFN-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 cytarbamine- 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 S-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 | NCT01045438 |
|         | Ovarian carcinoma | Terminated | n.a. | Combined with docetaxel and carboplatin | NCT02469016 |
|         | Prostate carcinoma | II    | Recruiting | n.a. | Combined with multimodal chemotherapy | NCT02494921 |
|         | Solid tumors | II    | Recruiting | s.c. | Formulated as Tbo-fligrastim | NCT02190721 |
| GM-CSF  | Brain tumors | 0     | Not yet recruiting | n.a. | Combined with a personalized peptide-based vaccine and polyICLC after temozolomide | NCT02510950 |
|         | Breast carcinoma | II    | Recruiting | s.c. | Combined with an ERBB2-targeting vaccine and/or trastuzumab | NCT02297698 |
|         | Colorectal carcinoma | II    | Recruiting | i.v. | In the context of the XELOX regimen | NCT02466906 |
|         | Glioblastoma | I     | Recruiting | s.c. | Combined with a personalized peptide-based vaccine and polyICLC after temozolomide | NCT01492255 |
|         | Neuroblastoma Osteosarcoma | II    | Recruiting | s.c. | Combined with autologous T cells armed with a GD2-targeting BiTE plus IL-2 | NCT01273093 |
|         | Soft tissue sarcoma | II    | Not yet recruiting | s.c. | Combined with dinutuximab, isotretinoin and IL-2 | NCT02169609 |
|         | Osteosarcoma | II    | Not yet recruiting | s.c. | Combined with a proteasomal inhibitor | NCT02424785 |
|         | Prostate carcinoma | I     | Recruiting | i.d. | Combined with a PD-L1-targeting mAb | NCT02387307 |
|         | Hematological malignancies | I     | Recruiting | s.c. | Combined with adoptively transferred NK cells and decitabine | NCT02173093 |
|         | Melanoma | I/II  | Recruiting | s.c. | Combined with triplet-based vaccination | NCT02240537 |
|         | Neuroblastoma | Not yet recruiting | n.a. | Combined with dinutuzumab, isoretinoin and IL-2 | NCT02185714 |
|         | Neuroblastoma Osteosarcoma | II    | Recruiting | s.c. | Combined with autologous T cells armed with a PD-1 target BiTE and IL-2 | NCT01492255 |
|         | Osteosarcoma | II    | Not yet recruiting | s.c. | Combined with a replicative adenovirus | NCT02197169 |
|         | Soft tissue sarcoma | II    | Not yet recruiting | s.c. | Combined with adoptively transferred NK cells and decitabine | NCT02169609 |
|         | Soft tissue sarcoma | II    | Enrolling by invitation | s.c. | Combined with a humanized anti-GD2 mAb | NCT02502786 |
|         | Soft tissue sarcoma | II    | Active not recruiting | i.d. | Combined with radiochemotherapy | NCT02464007 |
|         | Soft tissue sarcoma | II    | Active not recruiting | n.a. | Combined with adoptively transferred NK cells and decitabine | NCT02502786 |
|         | Soft tissue sarcoma | II    | Terminated | n.a. | Combined with a peptide-based vaccine after temozolomide | NCT02455557 |
|         | Gynecological malignancies | I     | Recruiting | s.c. | Combined with autologous T cells armed with a BiTE plus GM-CSF | NCT02470539 |
|         | Hematological malignancies | I     | Recruiting | s.c. | Combined with adoptively transferred IL2 | NCT02240537 |
|         | Melanoma | I/II  | Recruiting | s.c. | Combined with a TERT-targeting vaccine and ipilimumab | NCT02275416 |
|         | Neuroblastoma | Not yet recruiting | n.a. | Combined with dinutuzumab, isoretinoin and IL-2 | NCT02470539 |
|         | Neuroblastoma Osteosarcoma | II    | Recruiting | s.c. | Combined with autologous T cells armed with a GD2-targeting BiTE plus IL-2 | NCT01273093 |
|         | Soft tissue sarcoma | II    | Not yet recruiting | s.c. | Combined with dinutuzumab | NCT02484443 |
|         | Osteosarcoma | II    | Recruiting | s.c. | Combined with a proteasomal inhibitor | NCT02424785 |
|         | Prostate carcinoma | I     | Recruiting | i.d. | Combined with a TERT-targeting vaccine | NCT02267300 |
|         | Hematological malignancies | I     | Recruiting | s.c. | Combined with a calcineurin inhibitor | NCT01741886 |
|         | Melanoma | I/II  | Recruiting | i.d. | Combined with a replicative adenovirus | NCT02387307 |
|         | Neuroblastoma | Not yet recruiting | n.a. | Combined with a calcineurin inhibitor | NCT02464007 |
|         | Neuroblastoma Osteosarcoma | II    | Recruiting | s.c. | Combined with a replicative adenovirus | NCT02157421 |
|         | Soft tissue sarcoma | II    | Not yet recruiting | s.c. | Combined with a replicative adenovirus | NCT02173093 |
|         | Gynecological malignancies | I     | Recruiting | i.t. | Combined with adoptively transferred NK cells and decitabine | NCT02470539 |
|         | Hematological malignancies | I     | Recruiting | s.c. | Combined with adoptively transferred NK cells and decitabine | NCT02292966 |
|         | Melanoma | I/II  | Recruiting | i.p. | Combined with a calcineurin inhibitor | NCT02423278 |
|         | Neuroblastoma | Not yet recruiting | i.v. | Combined with a calcineurin inhibitor | NCT02421548 |
|         | Neuroblastoma Osteosarcoma | II    | Recruiting | s.c. | Combined with a calcineurin inhibitor | NCT02421548 |
|         | Soft tissue sarcoma | II    | Not yet recruiting | n.a. | Combined with a calcineurin inhibitor | NCT02421548 |
|         | Gynecological malignancies | I     | Recruiting | i.d. | Combined with a calcineurin inhibitor | NCT02421548 |
|         | Hematological malignancies | I     | Recruiting | s.c. | Combined with adoptively transferred NK cells and decitabine | NCT02292966 |
|         | Melanoma | I/II  | Recruiting | i.p. | Combined with a calcineurin inhibitor | NCT02423278 |
|         | Neuroblastoma | Not yet recruiting | i.v. | Combined with a calcineurin inhibitor | NCT02421548 |
|         | Neuroblastoma Osteosarcoma | II    | Recruiting | s.c. | Combined with a calcineurin inhibitor | NCT02421548 |
|         | Soft tissue sarcoma | II    | Not yet recruiting | n.a. | Combined with a calcineurin inhibitor | NCT02421548 |
|         | Ovarian carcinoma | II    | Recruiting | i.v. | Combined with adoptively transferred NK cells and decitabine | NCT02292966 |
|         | Peritoneal surface malignancies | II    | Recruiting | i.v. | Combined with a calcineurin inhibitor | NCT02421548 |
|         | RCC | II    | Not yet recruiting | n.a. | Combined with a calcineurin inhibitor | NCT02421548 |
|         | Skin SCC | II    | Recruiting | s.c. | Combined with a calcineurin inhibitor | NCT02421548 |
|         | Solid tumors | II    | Not yet recruiting | s.c. | Combined with a calcineurin inhibitor | NCT02421548 |
|         | Gynecological malignancies | I     | Recruiting | i.d. | Combined with adoptively transferred NK cells and decitabine | NCT02470539 |

**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; i.p., intra peritoneum; 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 cetuximab152 (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 (NCT02482890; NCT02419495); 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) IFN-y 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 (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, NCT01690910, 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 II/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, NCT01690910 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-CSF 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-directed IL-2 plus tumor-directed 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.

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.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

Authors are supported by the Ligue contre le Cancer (équipe labelisée); Agence Nationale 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 PARIS Alliance of Cancer Research Institutes (PACRI).

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; 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/10.1067/mai.jaci.2005.07.001
9. Gruenbacher G, Nussbaumer O, Gander H, Steiner B, Leonti C, Thurnheer A, Thurnheer S, Thurnheer F, Thurnheer T, Thurnheer M, Stress-related and homeostatic cytokines regulate Vγ9Vδ2 T-cell surveillance of malonaldehyde metabolism. Oncoimmunology 2014; 3:e953410; PMID:25960933; http://dx.doi.org/10.4161/21624011.2014.953410
10. Papapantakos K, Mervent M, Cytokines: true to their family name. Nat Rev Immunol 2013; 13:544-5; PMID:23827975; http://dx.doi.org/10.1038/nri3496
11. Hombach AA, Abken H. Targeting two co-operating cytokines efficiently shapes immune responses. Oncoimmunology 2013; 2:e23025; PMID:23802072; http://dx.doi.org/10.4161/onci.23025
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/10.1016/0074-7696(97)90162-7; PMID:18358817; http://dx.doi.org/10.4161/onci.24850
13. Metalf 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:17006755; 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/10.2174/1381612067797497461
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/10.1038/34850a0
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, Cotterrell CL, Watt SV, Anthony DA, Akira S, Iwakura Y, Trapani JA, Zitvogel L et al. Homeostatic defects in interleukin 18-deficient mice contribute to protective resistance 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, Mader 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; 15:405-47; PMID:26027717; http://dx.doi.org/10.1038/nri3845
37. Khoury HJ, Loberiza FR, Jr., Wack A, O’Garra A. Type I interferons in infectious disease. Nat Rev Immunol 2015; 15:23-42; PMID:25790790; http://dx.doi.org/10.1038/nri3806

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 transplantation for lymphoma and myeloma (PALM study). Eur J Cancer 2012; 48:713-20; PMID:22484711; http://dx.doi.org/10.1016/j.ejca.2011.12.016

39. Haining C. Hematopoietic stem cell mobilization with G-CSF. Methods Mol Biol 2012; 904:347-77; 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, Teng, Geng 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.1365-2411.2002.03264.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, Veke- mans MC, Biemond B, Sonneveld P, Passweg J, Verdonck L et al. Favorable effect of priming with granulocyte colony-stimulating fac- tor in remission induction of acute myeloid leukemia restricted to dose escalation of cytarabine. Blood 2012; 119:5367-73; PMID:22428224; http://dx.doi.org/10.1182/blood-2011-11-389841

44. Derose JP, Eggermont AM, van Geel AN, Burger JW, den Bakker MC, Biemond B, Sonneveld P, Passweg J, Verdonck L et al. Favorable effect of priming with granulocyte colony-stimulating fac- tor in remission induction of acute myeloid leukemia restricted to dose escalation of cytarabine. Blood 2012; 119:5367-73; PMID:22428224; http://dx.doi.org/10.1182/blood-2011-11-389841

45. Deroose JP, Eggermont AM, van Geel AN, Burger JW, den Bakker MC, Biemond B, Sonneveld P, Passweg J, Verdonck L et al. Favorable effect of priming with granulocyte colony-stimulating fac- tor in remission induction of acute myeloid leukemia restricted to dose escalation of cytarabine. Blood 2012; 119:5367-73; PMID:22428224; http://dx.doi.org/10.1182/blood-2011-11-389841

46. Derose JP, Eggermont AM, van Geel AN, Burger JW, den Bakker MC, Biemond B, Sonneveld P, Passweg J, Verdonck L et al. Favorable effect of priming with granulocyte colony-stimulating fac- tor in remission induction of acute myeloid leukemia restricted to dose escalation of cytarabine. Blood 2012; 119:5367-73; PMID:22428224; http://dx.doi.org/10.1182/blood-2011-11-389841

47. Derose JP, Eggermont AM, van Geel AN, Burger JW, den Bakker MC, Biemond B, Sonneveld P, Passweg J, Verdonck L et al. Favorable effect of priming with granulocyte colony-stimulating fac- tor in remission induction of acute myeloid leukemia restricted to dose escalation of cytarabine. Blood 2012; 119:5367-73; PMID:22428224; http://dx.doi.org/10.1182/blood-2011-11-389841

48. Derose JP, Eggermont AM, van Geel AN, Burger JW, den Bakker MC, Biemond B, Sonneveld P, Passweg J, Verdonck L et al. Favorable effect of priming with granulocyte colony-stimulating fac- factor in remission induction of acute myeloid leukemia restricted to dose escalation of cytarabine. Blood 2012; 119:5367-73; PMID:22428224; http://dx.doi.org/10.1182/blood-2011-11-389841
ONCOIMMUNOLOGY
transient alpha-2b for advanced intrahepatic cholangiocarcinoma. Ann Surg Oncol 2014; 21:3638-45; PMID:24817369; http://dx.doi.org/10.1043/014-0766-7

100. Donini M, Buti S, Lazzarelli S, Bazzetti R, Rovinelli 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). Targt Oncol 2015; 10:277-86; PMID:25230695; http://dx.doi.org/10.1007/s11523-014-0337-6

101. Zeidner NF, Gladstone DE, Zahrak 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, Gonzalez-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:24652352; http://dx.doi.org/10.4161/19336918.2014.937875

105. Ellkord 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 napatumomab estafenatox plus IFN-alpha versus IFN-alpha. Oncotarg 2015; 6:4428-39; PMID:25669986; http://dx.doi.org/10.18632/oncotarget.2922

106. Jarvinen R, Murtilla T, Kassinen E, Rintala E, Aaltomaa S, Kallio L, Liukkonen T, Puolakkia VM, Seppainen M, Tuukanen K et al. Long-term outcome of patients with frequently recurrent non-muscle-invasive bladder carcinoma treated with one periperalve 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:25478117; 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 fixation with or without interferon-alpha2b: final results from a randomized phase III study in symptomatic indolent B-cell lymphomas. Leuk Lymphoma 2015; 56:5298-607; PMID:25686644; http://dx.doi.org/10.3109/10428194.2015.1014363

108. Ravaud A, Barrios CH, Alesbee B, Tay MH, Agarwala SS, Yalcin S, Zitvogel L, Kroemer G et al. Pilot study of flt3 ligand support followed by weekly paclitaxel in women with primary breast cancer. Oncologist 2015; 20:239-40; PMID:25637379; http://dx.doi.org/10.1016/j.theconco.2014-0326

109. 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
125. Kharrazia P, De Raeve H, Fristedt C, Li Q, Gruber A, Johnsson P, Kokarakis 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. Zitzvogel 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, Zitzvogel 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, Harathan 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:24573579; 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.1671

135. Finiguerra V, Di Conza G, Di Matteo M, Serneels J, Costa S, Thompson AA, Wauters E, Walmsley S, Prehen 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. Marciai A, Cherfils-Vicini J, Viant C, Degouve S, Viol 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; 7:1744-52; PMID:24794411; 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
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

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

Litvin O, Schwartz S, Wan Z, Schild T, 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

Escobar G, Moi D, Ranghetti A, Ozkal-Baydin P, Squadrito ML, Kajaste-Rudnitski A, Bondanza A, Gentner B, De Palma M, Mazzi 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

Bald T, Landsberg J, Lopez-Ramos D, Ruggiero S, Andre F, Zitvogel L. Natural killer cells engender 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

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/10.1586/14760584.7.10.881

Nair SK, Heiser A, Boczkowski D, Majumdar A, Naeo 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

Dinutuximab approved for high-risk neuroblastoma. Cancer Discov 2015; 5:OF5; PMID:25851859; http://dx.doi.org/10.1158/2159-8290.CD-15-0144

Reichert JM. Antibodies to watch in 2015. MAbs 2015; 7:1-8; PMID:25484055; http://dx.doi.org/10.4161/19420862.2015.989944

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

Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, Tang Y, Zhang K, 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

Ceresi 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

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