Trial watch

Immunostimulatory cytokines in cancer therapy

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

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

Introduction

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

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

Besides regulating homeostatic hematopoiesis50 and participating in physiological angiogenesis11,12 cytokines are released in response to a wide array of insults, such as traumatic events, infections, and cancer.13-15 In these settings, cytokines are secreted in discrete waves to coordinate (1) the removal of the pathogenic stimulus, and (2) the re-establishment of tissue homeostasis.16-18 When such an adaptive response fails and the initiating stimulus cannot be removed, however, the continuous secretion of specific cytokines may promote chronic inflammation. Of note, patients affected by a chronic inflammatory response, be it systemic or local, exhibit an increased propensity to develop some neoplasms, including colorectal carcinoma.19-21 Most likely, this originates from the increased production of mutagenic reactive oxygen species at sites of inflammation, as well as from the local secretion of mitogenic cytokines. Altogether, these observations lend further support to the notion that the biological activity of several cytokines exhibits a high degree of context dependency.

As soon as it became clear that tumors do not go unnoticed by the immune system, several approaches have been developed to elicit novel (or reinstate pre-existent) tumor-targeting immune responses. These therapeutic strategies include highly specific interventions, such as dendritic cell (DC)-based, peptide-based and DNA-based anticancer vaccines,22-24 as well as relatively non-specific maneuvers, such as the local or systemic administration of Toll-like receptor (TLR) agonists,25,26 immunostimulatory antibodies,27 or cytokines.28 In spite of the fact that cytokines do not actively elicit a tumor-targeting immune response but rather boost the antineoplastic potential of natural, tumor-specific immune effectors, no less than three recombinant cytokines are currently approved by the US Food and Drug Administration (FDA) or equivalent regulatory agencies for use as standalone therapeutic interventions in adult cancer patients. First, interferon (IFN)-α2a (Roferon-A®) is used in subjects with hairy cell leukemia and chronic phase, Philadelphia chromosome-positive chronic myelogenous leukemia (CML), upon minimal pretreatment (within 1 y of diagnosis). Second, IFN-α2b (Intron A®) is employed for the therapy of hairy cell leukemia, AIDS-related Kaposi’s sarcoma, follicular lymphoma, multiple myeloma, melanoma, condyloma acuminata and cervical intraepithelial neoplasms. Third, interleukin (IL)-2 (aldesleukin, Proleukin®) is approved for the treatment of metastatic forms of melanoma and renal cell carcinoma (RCC) (source http://www.fda.gov). The use of recombinant granulocyte colony-stimulating factor (G-CSF, also known as filgrastim, lenograstim or Neupogen®) and recombinant granulocyte monocyte colony-stimulating factor (GM-CSF, also known as molgramostim, sargramostim, Leukomax®, Mielogen® or Leukine®) in cancer patients has also been licensed by the US FDA. However, these cytokines are not (yet) harnessed for their ability to boost antitumor immune responses. Rather, they are employed as mitogenic factors, (1) to favor the reconstitution of the immune system in transplanted patients, who are generally subjected to lymphodepleting/lymphoablating regimens, as well as in patients treated with aggressive antimitotic chemotherapy, who are prone to develop febrile neutropenia;28-30 (2) to recruit bone marrow precursors to the peripheral blood in the context of autologous stem cell transplantation;31,32 (3) to prevent the neutropenia-inducing activity of specific chemotherapeutics;33,34 and (4) to favor the replication of quiescent leukemic cells, thus exposing them to the antineoplastic activity of drugs that preferentially target actively proliferating cells.35 Finally, recombinant tumor necrosis factor α (TNFα) is currently approved by multiple regulatory agencies including the European Medicine Agency (EMA), but not by the US FDA, for use in patients with limb-threatening soft tissue sarcoma and melanoma.36-41 In this setting, TNFα is generally co-administered with melphalan (an alkylating agent) to isolated limbs under mild hyperthermic conditions, a safe and relatively simple procedure that has been associated with consistent rates of objective responses.42-46

Owing to their pleiotropic biological activity, cytokines can be associated with clinically relevant side effects, especially when administered systemically. There are 3 major concerns related to the use of cytokines in (cancer) patients: (1) the elicitation of an acute, sepsis-like, potentially lethal systemic reaction characterized by the massive release into the circulation of pyrogenic and cytotoxic cytokines;47-50 (2) the exacerbation of chronic inflammatory foci that may initiate oncogenesis or accelerate tumor progression;51,52 and (3) the activation of a mitogenic program in otherwise poorly proliferating cells, favoring the accumulation of genetic/epigenetic defects and hence increasing the likelihood of malignant transformation.53-55 In fact, some cytokines including multiple members of the platelet-derived growth factor (PDGF) family cannot be employed as therapeutic interventions owing to their excessive mitogenic (and hence potentially oncogenic) potential.5,6,55

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

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

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

Vitale and coworkers investigated the therapeutic profile of subcutaneous low-dose IL-2, combined with the somatostatin analog lanreotide, in 6 patients with symptomatic and advanced medullary thyroid carcinoma. The authors observed that a 6-mo regimen of lanreotide plus low-dose IL-2 was well tolerated by all patients, improved their quality of life and elicited some objective responses.75,76 Of note, the tumor rapidly became undetectable by magnetic resonance imaging and computer tomography, and the patient remained in complete remission for at least 6 y after the confirmed diagnosis of unresectable hepatocellular carcinoma.69

Robertson et al. performed a dose-escalation Phase I study to test the safety and therapeutic profile of recombinant human IL-18 in non-Hodgkin lymphoma (NHL) patients treated with the CD20-targeting mAb rituximab.70-73 Rituximab (375 mg/m²) was administered i.v. once weekly for a total of 4 wks, while escalating doses of IL-18 (1, 3, 10, 20, 30, and 100 µg/kg) were given as a 2 h intravenous infusion weekly for 12 consecutive wks. No dose-limiting toxicities were observed. Common side effects were chills, fever, headache and nausea, while abnormal laboratory findings included transient asymptomatic lymphopenia, hyperglycemia, anemia, hypoalbuminemia as well as temporary elevations in circulating bilirubin and hepatic enzymes. Of note, 5 out of 19 patients experienced objective clinical responses. Altogether, these findings suggest that recombinant human IL-18 is well tolerated at doses at which it may improve the therapeutic profile of rituximab in NHL patients.74

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

Cheung and coworkers investigated the ability of GM-CSF to improve the response of 79 patients with persistent osteomедullary neuroblastoma to 3F8, a mAb specific for GD2 ganglioside.78-80 Patients were treated with 3F8 plus GM-CSF for up to 24 mo, or until the development of neutralizing anti-3F8 antibodies. In the context of this Phase II clinical trial, toxicities were generally manageable and 38% of patients achieved an objective response as defined by metaiodobenzyl-guanidine scan. Moreover, the 5-y progression-free survival of patients receiving 3F8 plus subcutaneous GM-CSF was 24 ± 6%, which was significantly better than that of patients treated with 3F8 plus intravenous GM-CSF (11 ± 7%).81,85

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

Coker and colleagues performed a Phase 1 dose-escalation study of oral temozolomide, an alkylating agent, combined with subcutaneous pegylated IFN-α2b in 19 patients with refractory or advanced solid tumors. The authors identified the maximum tolerated dose of the combination in 100 mg/m² temozolomide on days 1–7 and 15–21 plus 1.5 µg/kg IFN-α2b per wk on 28-d cycles, and reported that the pharmacokinetics of pegylated IFN-α2b are not altered by the co-administration of temozolomide.85

Eto and collaborators prospectively investigated the predictive value of 11 single nucleotide polymorphisms (SNPs) affecting 8 distinct genes linked to immune responses among 203 RCC patients treated with 3 doses per wk of IFN-α (5 MIUs). The authors reported a response rate of 13.8% (9 complete responses, 19 partial responses), which was not influenced by any of the SNPs analyzed in this study. However, when
Table 1. Clinical trials recently launched to evaluate the safety and efficacy of immunostimulatory cytokines in cancer patients*

| Cytokine     | Indication(s)          | Status          | Phase | Route | Notes                                      | Ref.         |
|--------------|------------------------|-----------------|-------|-------|--------------------------------------------|--------------|
| FLT3L        | Lymphoma               | Recruiting      | II    | i.t.  | Combined with radiotherapy and a TLR3 agonist | NCT01976585 |
| GM-CSF       |                        |                 |       |       |                                            |              |
|              | Breast carcinoma       | Recruiting      | I/II  | s.c.  | Combined with a FOLR1-targeting vaccine   | NCT02019524 |
|              | Ovarian carcinoma      |                 |       |       |                                            |              |
|              | Follicular B-cell       | Completed       | II    | s.c.  | Combined with rituximab                    | NCT01939730 |
|              | lymphoma               |                 |       |       |                                            |              |
|              | GBM                    | Not yet         | I/II  | n.a.  | Combined with multipepptide vaccine and imiquimod | NCT02078648 |
|              |                        | recruiting      |       |       |                                            |              |
|              | GBM                    | Not yet         | II    | s.c.  | Combined with a cell-based vaccine, bevacuzumab and cyclophosphamide | NCT01903330 |
|              |                        | recruiting      |       |       |                                            |              |
|              | Melanoma               | Completed       | III   | s.c.  | As single agent or combined with TYR-targeting vaccine | NCT01989572 |
|              |                        | Recruiting      | I/II  | n.a.  | Combined with ipilimumab                   | NCT02009397 |
|              | Gastrointestinal       | Not yet         | III   | s.c.  | As single agent upon allogenic stem cell transplantation | NCT01860742 |
|              | neuroendocrine tumors  | recruiting      |       |       |                                            |              |
|              | Anal intraepithelial    | Recruiting      | I/II  | s.c.  | Combined with a HPV-16-targeting vaccine   | NCT01923116 |
|              | neoplasia              |                 |       |       |                                            |              |
|              | Childhood craniopharyngioma | Recruiting   | I/II  | s.c.  | Combined with a HPV-16-targeting vaccine   | NCT01964300 |
|              |                        |                 |       |       |                                            |              |
|              | CML                    | Not yet         | II    | n.a.  | Combined with dasatinib                    | NCT01872442 |
|              |                        | recruiting      |       |       |                                            |              |
|              | Melanoma               | Not yet         | II    | s.c.  | Combined with imatinib and nilotinib       | NCT02001818 |
|              | RCC                    | Recruiting      | I     | s.c.  | Combined with imatinib                     | NCT01933906 |
|              |                        | Recruiting      | II    | s.c.  | Combined with nilotinib                    | NCT01866553 |
|              |                        |                 |       |       |                                            |              |
|              | IFN-α                  | Recruiting      | IV    | n.a.  | As single agent                            | NCT02027064 |
|              | IFN-α2b                |                 |       |       |                                            |              |
|              | Gliosarcoma            | Not yet         | II    | s.c.  | Combined with anti-PD1 mAb                 | NCT02089685 |
|              |                        | recruiting      |       |       |                                            |              |
|              | Soft tissue sarcoma    | Recruiting      | n.a.  | s.c.  | As single agent                            | NCT01957709 |

Abbreviations: AML, acute myeloid leukemia; CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; CML, chronic myeloid leukemia; FLT3L, fms-related tyrosine kinase 3 ligand; FOLR1, folate hydrolyase 1; FOLR1, folate receptor 1; GBM, glioblastoma multiforme; GM-CSF, granulocyte macrophage colony-stimulating factor; HPV-16, human papillomavirus Type 16; IFN, interferon; IL, interleukin; i.t., intra tumorem; i.v., intra venam; mAb, monoclonal antibody; MRD, minimal residual disease; n.a., not available; NHL, non-Hodgkin lymphoma; NK, natural killer; NSCLC, non-small cell lung carcinoma; PBL, peripheral blood lymphocyte; PDCC1, programmed cell death 1; RCC, renal cell carcinoma; SABR, stereotactic ablative body radiotherapy; s.c., sub cutem; TNFα, tumor necrosis factor α; TYR, tyrosinase; WT1, Wilms tumor 1. *Between 2013, May 1st and the date of submission.

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

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

Among recent translational studies focusing on immunostimulatory cytokines in general, we found of particular interest the works of (1) Guermonprez and colleagues, who discovered a signaling pathway triggered by Plasmodium infection that regulates DC homeostasis and adaptive immune response upon the release of fms-related tyrosine kinase 3 ligand (FLT3L);92 (2) Sim and coworkers, who demonstrated that CD4+CD25+FOXP3+ Tregs accumulating in melanoma patients treated with high-dose IL-2 express inducible T-cell co-stimulator (ICOS).
and exhibit an activated phenotype, as indicated by elevated levels of CD39, CD73 and transforming growth factor β1 (TGFβ1). These latter findings confirm and extend previous results indicating that immune checkpoint-blocking agents such as the cytotoxic T lymphocyte-associated protein 4 (CTLA4)-targeting mAb ipilimumab may significantly ameliorate the therapeutic profile of immunostimulatory cytokines.96

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

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

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

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

| Cytokine | Indication(s) | Status | Phase | Route | Notes | Ref. |
|----------|---------------|--------|-------|-------|-------|------|
| | | | | | | |

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

Update on Ongoing Clinical Trials

When this Trial Watch was being redacted (April 2014), official sources listed no less than 88 clinical trials launched after May 1st, 2013 that would evaluate the efficacy and safety of immunostimulatory cytokines in cancer patients (source http://www.clinicaltrials.gov). In 14 of these studies, IL-2 (14 trials), GM-CSF (4 trials), G-CSF (33 trials) and IFN-α (3 trials) were used as on-label interventions. These studies will not be discussed here. In addition, 34 clinical trials have been launched during the last 12 mo to investigate the immunostimulatory potential of various cytokines in off-label settings (Table 1).
T cells expressing a chimeric antigen receptor specific for folate hydrolase 1 (FOLH1, best known as PSMA),108,109 in prostate cancer patients subjected to non-myeloablative conditioning (NCT01929239); and (4) in combination with G-CSF, GM-CSF and a mAb specific for ganglioside GD2,110 for the treatment of minimal residual disease in children with advanced neuroblastoma treated with aggressive induction chemotherapy and stem cell transplantation (NCT01857934). Moreover, various studies have recently been launched to test the clinical profile of IL-2 variants retargeted to cancer cells by means of tumor-associated antigen (TAA)-specific antibodies or antibody fragments, i.e., IL-2-based immunocytokines.111-113 Thus, (1) the safety and therapeutic potential of a carcinoembryonic antigen (CEA)-directed IL-2 variant (RO6895882) of a carcinoembryonic antigen (CEA) are being assessed in patients with advanced and/or metastatic solid tumors (NCT02004106); (2) a fusion between IL-2 and the Fv fragment of a mAb specific for tenasin C (TNC) is being tested, in combination with the micotubular inhibitor paclitaxel, in Merkel cell carcinoma patients (NCT02054884); (3) the therapeutic value of a CD20-retargeted form of IL-2 is being investigated as a standalone intervention in subjects with NHL (NCT01874288); (4) the safety and efficacy of IL-2 fused to the Fv fragment of a mAb specific for the extracellular domain B of fibronectin (L19),114-117 administered i.t. in combination with L19-TNFα, are being evaluated in melanoma patients (NCT02076633); and (5) the therapeutic profile of L19-IL-2 administered as a standalone agent immediately after stereotactic ablative body radiotherapy18 is being investigated in subjects affected by metastatic non-small cell lung carcinoma (NSCLC) (NCT02086721). Moreover, (1) glycosylated recombinant human IL-7 is being tested as adjuvant to sipuleucel-T (an FDA-approved vaccine based on autologous peripheral blood mononuclear cells) in subjects with castration-resistant prostate cancer (NCT01881867); (2) pegylated recombinant human IL-10 administered s.c. is being assessed as a standalone therapeutic intervention in patients with advanced solid tumors (NCT02009449); and (3) the clinical profile of recombinant human IL-15 given i.v. in combination with autologous activated NK cells is under evaluation in children and young adults affected by solid neoplasms (NCT01875601).

The safety and clinical potential of IFN-α2b, invariably administered s.c., are being investigated in cohorts of (1) subjects with gastrointestinal neuroendocrine tumors that failed to respond to somatostatin analogs, who receive IFN-α2b as a standalone agent (NCT01860742); (2) pediatric patients with unresectable recurrent craniopharyngioma, who are treated with a pegylated variant of IFN-α2b (NCT01964300); (3) individuals with advanced melanoma or RCC, receiving pegylated IFN-α2b in combination with a mAb specific for programmed cell death 1 (PD1D1, best-known as PD-1) (NCT02089685); (4) patients with chronic myeloid leukemia, who are treated with normal or pegylated IFN-α2b plus tyrosine kinase inhibitors including imatinib124 (NCT01939306); dasatinib125 (NCT01872442) and nilotinib126 (NCT01866553; NCT02001818); and (5) adults affected by anal intraepithelial neoplasms, who are concurrently vaccinated with a human papillomavirus Type 16 (HPV-16)-targeting peptide-based vaccine24 (NCT0192316). In addition, a not-better defined variant of IFN-α is being tested as standalone intervention for molecular relapse in t(8; 21) AML patients who previously underwent allogeneic stem cell transplantation (NCT02027064); and recombinant IFN-γ is being assessed for its therapeutic efficacy in subjects with soft tissue sarcoma (NCT01957709).

GM-CSF is being intensively investigated for its ability to boost tumor-targeting immune responses elicited by wide panel of immunotherapeutic interventions. In particular, GM-CSF is being tested as an adjuvant to (1) ipilimumab,16,27,28 in subjects with unresectable, Stage IIIC or IV melanoma (NCT02009379); (2) rituximab,70,71 in individuals affected by follicular B-cell lymphoma (NCT01939730); (3) a peptide vaccine directed against folate receptor 1 (FOLR1, also known as FBP),127,128 in patients with breast and ovarian carcinoma (NCT02019524); (4) SL-071, a multipeptide-based vaccine targeting several epitopes overexpressed by glioma cells,129,130 administered to glioblastoma patients in combination with the TLR7 agonist imiquimod131,132 (NCT02078648); (5) a peptide vaccine specific for Wilms tumor 1 (WT1),133,134 in mesothelioma patients who have completed multimodal therapy (NCT01890980); (6) a tyrosinase (TYR)-targeting peptide vaccine,135,137 in melanoma patients who underwent tumor resection (NCT01989572); (7) an autophagosome-based vaccine derived from allogeneic cancer cells,138-140 administered to NSCLC patients in combination with imiquimod (NCT01909752); and (8) cyclophosphamide, the vascular endothelial growth factor (VEGF)-specific mAb bevacizumab70,71,141,142 and a vaccine based on autologous cancer cells, in patients with glioblastoma multiforme or gliosarcoma (NCT01903330). Finally, the intratumoral administration of recombinant FLT3L34-35 to B-cell lymphoma patients is being tested for its capacity to recruit DCs to neoplastic lesions and hence allow low-dose radiation therapy and a TLR3 agonist to induce clinically relevant antitumor immune responses (NCT01976585). This is a novel application for recombinant human FLT3L (also known as CDX-301), which is being developed as an alternative or a support to G-CSF for the mobilization of hematopoietic cell precursors in bone marrow donors, with promising results (source http://www.cellx.com/). Of note, official sources list NCT01989572 and NCT01939730 as “completed,” yet no results are expected to be available at the moment.

As for the clinical trials listed in our previous Trial Watches dealing with this topic,98,146 the following studies have changed status during the past 12 months: NCT00589550, NCT00977145, NCT01096631, NCT01099631, NCT01131364, NCT01337544, which appear as ‘terminated’; NCT00784524, NCT01236573 and NCT01490047, which are listed as ‘suspended’; NCT00631371 and NCT00923351, which show as ‘active, not recruiting’ but are associated...
with results; as well as NCT00601796, NCT00660270, NCT00719264, NCT00891475, NCT01021059, NCT01220648, NCT01404936, NCT01592045, NCT01637532, NCT01639885 and NCT01673217, which have been completed (source http://www.clinicaltrials.gov).

While NCT00977145, NCT00589550, NCT01099631 and NCT01392170 have been terminated owing to slow accrual, NCT01337544 has been halted because the parents of two patients enrolled who died presented a claim against the hospital. The reasons underlying the termination of NCT01131364 are not available. NCT00784524 has been suspended for interim analysis, whereas NCT01256573 has been temporarily halted for final data collection and primary outcome assessment. The reasons behind the suspension of NCT01490047 have not been reported. The results of NCT00660270, NCT00719264, NCT00891475, NCT01021059, NCT01220648, NCT01592045, NCT01637532, NCT01639885 and NCT01673217 have not been disseminated yet.

Conversely results are available for NCT00601796, NCT00631371, NCT00923351 and NCT01404936. In the context of NCT00601796, testing a GM-CSF-involving cell-based vaccine in combination with cyclophosphamide and all-trans retinoic acid in lung cancer patients, 5 immunological responses were observed among 14 evaluable patients, the median time to progression and median overall survival among 24 treated patients being 2.4 and 8 mo, respectively. Preliminary results from NCT00631371, which is still ongoing, revealed that bevazumab plus IFN-α is not inferior to bevacizumab plus the mammalian target of rapamycin (mTOR) inhibitor temsirolimus for the treatment of advanced RCC patients, but associated with lower incidence of serious adverse effects (36.6% vs. 44.3%). NCT00923351 is investigating the ability of recombinant IL-7 to boost the therapeutic activity of a DC-based vaccine in patients with Ewing’s Sarcoma, rhabdomyosarcoma or neuroblastoma. Preliminary results indicate that IL-7 may indeed promote the immunogenic potential of DCs loaded ex vivo with autologous cancer cell lysates but not increase the toxicity of the procedure. The number of patients enrolled and analyzed so far, however, appears to be excessively low for drawing robust conclusions from this study. NCT01404936 evaluated the combination of IFN-α2a with a multi-agent chemotherapeutic regimen in Hodgkin lymphoma patients. In this setting, 23 out of 30 patients achieved a complete response to treatment, while serious side effects affected only 10% of participants. Additional, randomized and comparatively larger clinical studies are required to validate these findings.

**Concluding Remarks**

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

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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