

Trial Watch

Immunostimulatory cytokines

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Abbreviations: BMP, bone morphogenic protein; CD40L, CD40 ligand; DC, dendritic cell; EGF, epidermal growth factor; EGFR, EGF receptor; EPO, erythropoietin; FASL, FAS ligand; FLT3L, FLT3 ligand; GIST, gastrointestinal stromal tumor; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte macrophage colony stimulating factor; IFN, interferon; IFNAR, IFN α receptor; IFNGR, IFN γ receptor; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; IL1R1, type I IL-1 receptor; IL1R2, type II interleukin-2 receptor; INH β , inhibin β ; LPS, lipopolysaccharide; LT, lymphotoxin; MAPK, mitogen-activated protein kinase; NK, natural killer; NRK, normal rat kidney; OX40L, OX40 ligand; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; RANKL, RANK ligand; rh, recombinant human; RCC, renal cell carcinoma; TGF β , transforming growth factor β ; Th17, IL-17-producing helper T; TNF, tumor necrosis factor; TNFR, TNF receptor; TPO, thrombopoietin; Tregs, FOXP3⁺ immunosuppressive T cells; TRAIL, TNF-related apoptosis-inducing ligand; TRAILR1, TRAIL receptor 1; VEGF, vascular endothelial growth factor.

During the last two decades, a number of approaches for the activation of the immune system against cancer has been developed. These include highly specific interventions, such as monoclonal antibodies, vaccines and cell-based therapies, as well as relatively unselective strategies, such as the systemic administration of adjuvants and immunomodulatory cytokines. Cytokines constitute a huge group of proteins that, taken together, regulate not only virtually all the aspects of innate and cognate immunity, but also several other cellular and organismal functions. Cytokines operate via specific transmembrane receptors that are expressed on the plasma membrane of target cells and, depending on multiple variables, can engage autocrine, paracrine or endocrine signaling pathways. Perhaps, the most appropriate term for defining the cytokine network is “pleiotropic”: cytokines are produced by—and operate on—multiple, often overlapping, cell types, triggering context-dependent biological outcomes as diverse as cell proliferation, chemotaxis, differentiation, inflammation, elimination of pathogens and cell death. Moreover, cytokines often induce the release of additional cytokines, thereby engaging self-amplificatory or self-inhibitory signaling cascades. In this Trial Watch, we will summarize the biological properties of cytokines and discuss the progress of ongoing clinical studies evaluating their safety and efficacy as immunomodulatory agents against cancer.

Introduction

The word “cytokine” (derived from a Greek term meaning “to set cells in motion”) is employed to describe a highly heterogeneous group of small signaling (glyco)proteins that nowadays includes more than 130 members.^{1,2} Cytokines are produced throughout the body by an incredible variety of distinct cells, encompassing virtually all cellular components of the immune system as well as epithelial, endothelial and stromal cells. Cytokine-delivered signals, which—depending on the context—can be autocrine, paracrine or endocrine, are transduced into biological outcomes thanks to specific receptors that are expressed on the surface of target cells.^{1,2} Perhaps, the most appropriate word to characterize the cytokine system is “pleiotropic:” taken together, cytokines exert highly diversified functions, yet in a partially overlapping and redundant fashion. Thus, cytokines can trigger biological outcomes as diverse as proliferation, chemotaxis, differentiation, inflammation, elimination of pathogens and cell death. Moreover, cytokines very often stimulate the production and release of additional cytokines, de facto functioning as part of finely regulated and highly intertwined signaling cascades. Such a pleiotropism reflects not only the heterogeneous identity of cytokines as a group, but also (1) the existence of multiple receptors that can bind the same cytokine with different affinity (which are frequently expressed on different target cells), and (2) the fact that the biological activity of one cytokine on a specific target cell is highly influenced by the concomitant presence of additional cytokines.^{1,2}

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A wide array of adverse conditions, encompassing inflammation, infection by pathogens and tumorigenesis, provokes the secretion of cytokines. In this context, cytokines underlie a host response that aims at minimizing the harmful effects of stress, favoring repair mechanisms and, eventually, restoring homeostasis. Indeed, cytokines are often released in subsequent waves, and the terminal molecules of the cascade normally function to extinguish the stress response, along with the reestablishment of homeostasis. One prominent example of this biological behavior is provided by the systemic response to the administration of lipopolysaccharide (LPS, mimicking widespread bacterial infection). In this model, a rapid secretion of tumor necrosis factor α (TNF α) precedes a wave of interleukin-1 β (IL-1 β), IL-6, IL-8, IL-17A, IL-18 and interferon γ (IFN γ) (all of which exert potent pro-inflammatory effects, at both local and system levels), followed by a relatively delayed secretion of anti-inflammatory IL-10.³⁻⁵ In some instances, however, repair mechanisms are inefficient and fail to resolve the cytokine-inducing stimulus, leading to persistent cytokine production and exacerbated tissue damage. This is particularly relevant for inflammation-driven carcinogenesis, as it implies that the sites of chronic inflammation are a source of potentially mutagenic chemicals (e.g., high levels of reactive oxygen species) as well as of cytokine cocktails that may promote survival, proliferation and angiogenesis.^{6,7} Taken together, these observations suggest that the administration of immunomodulatory cytokines for eliciting an antitumor immune response should always be carefully weighted not only against their acute toxicity (in some cases resembling a state of severe infection) but also against the possibility to exacerbate inflammation-associated oncogenesis.⁶ In addition, some cytokines are endowed with potent mitogenic functions, de facto precluding their use as anticancer agents (see below).

During the last three decades, there have been multiple attempts to classify cytokines based on structural and/or functional parameters. Thus, at some stage, terms including “lymphokines,” “interleukins” and “chemokines” have been introduced to indicate cytokines that are produced by lymphocytes, cytokines that mediate the communication between leucocytes, and cytokines that stimulate chemotaxis, respectively.^{1,2} Today, according to the Kyoto Encyclopedia of Genes and Genomes (www.genome.jp/kegg/), cytokines can be cataloged into nine main groups: (1) chemokines, small cytokines with chemotactic activities that can further be subdivided into C, CC, CXC and CX3C chemokines, depending on the number and arrangement of conserved cysteine residues; (2) hematopoietic growth factors (or hematopoietins), i.e., cytokines with a prominent role in hematopoiesis, which can be further grouped—based on their respective receptors—into gp130 (IL6ST) shared, IL13RA1 shared, IL12RB1 shared, IL3RB (CSF2RB) shared, ILRG shared and others; (3) interleukin-1 family members; (4) interleukin-10 family members; (5) interleukin-17 family members; (6) interferons (IFNs); (7) platelet-derived growth factor (PDGF) family members; (8) transforming growth factor β (TGF β) family members; and (9) tumor necrosis factors (Table 1).

Experts in the field of cytokine research would probably criticize many aspects of this relatively inaccurate but simple

classification. Nevertheless, since our aim is not to dwell on the cytokine system in detail, we will use this scheme to provide a conceptual framework to our Trial Watch, in which we will briefly review the biological properties of cytokines and discuss the progress of ongoing (started after January 2008) clinical trials investigating their safety and efficacy as immunomodulatory agents against cancer. In line with this objective, our Trial Watch will deliberately disregard the use of cytokines for hematopoietic reconstitution upon chemotherapy or transplantation. Of note, only 2 cytokines out of more than 130 are nowadays approved by FDA for anticancer therapy: IFN α 2b and IL-2 (Table 2).

Chemokines

Chemokines are small (8–10 kDa) secreted proteins that share a highly conserved secondary structure and a distinguishing tetracysteine motif, constituting the basis of their systematic classification (see above). Chemokines have first been characterized for their ability to stimulate the migration of cell types as diverse as neutrophils, monocytes, lymphocytes, eosinophils, fibroblasts and keratinocytes. However, it is now clear that chemokines, as a family, contribute to a wide array of physiological and pathological processes, encompassing embryogenesis, immune system function, inflammation, oncogenesis and tumor progression. To date, 44 different human chemokines and 21 G protein-coupled chemokine receptors have been described.^{2,8}

During the last two decades, the inhibition of chemokines or their receptors has been extensively explored—in preclinical models—as a strategy against autoimmune and chronic inflammatory diseases, including rheumatoid arthritis, systemic lupus erythematosus and Type 2 diabetes.⁹ Nowadays, some of these approaches have been translated into clinical trials (www.clinicaltrials.gov). For instance, IL-8 receptor antagonists such as GSK1325756 and SB-656933,¹⁰ as well as anti-IL-8 monoclonal antibodies (ABX-IL8) are being tested in patients affected by chronic obstructive pulmonary disease or cystic fibrosis. MDX-1100 (a fully human anti-CXCL10 monoclonal antibody)¹¹ is currently under evaluation for the therapy of rheumatoid arthritis and ulcerative colitis. Moreover, strategies for the inhibition of the chemokine receptor CCR2, including the monoclonal antibody MLN1202¹² and the PEGylated antisense oligonucleotide NOX-E36¹³ are being explored in Phase I/II clinical trials to treat multiple sclerosis and Type 2 diabetes, respectively. Of note, maraviroc (an allosteric inhibitor of CCR5 also known as Selzentry) is currently approved by FDA for HIV treatment, as CCR5 is an essential co-receptor for the cellular entry of most HIV strains.¹⁴

Opposed to the large number of preclinical and clinical studies evaluating chemokine inhibition as a strategy for autoimmune disorders and chronic inflammation, only a few trials are evaluating the therapeutic potential of the modulation of the chemokine system in cancer patients (www.clinicaltrials.gov). Vaccines based on autologous dendritic cells (DCs) genetically modified to express CCL21 (a small member of the CC subfamily)¹⁵ are being investigated, either as single agents or in

Table 1. Operational classification of cytokines

Family	Subfamily	Members	Ref.
Chemokines	C subfamily	XCL1, XCL2	2,8
	CC subfamily	CCL1, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL13, CCL14, CCL15, CCL16, CCL17, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28	
	CXC subfamily	CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL16, PPBP	
	CX3C subfamily	CX3CL1	
Hematopoietins	IL6ST - shared	CLCF1, CNTF, CTF1, IL-11, IL-6, LIF, OSM	17,18
	IL13RA1 - shared	IL-4, IL-13	
	IL12RB1 - shared	IL12A, IL23A	
	IL3RB1 - shared	GM-CSF, IL-3, IL-5	
	ILRG - shared	IL-2, IL-4, IL-7, IL-9, IL-15, IL-21	
	Others	G-CSF, EPO, GH1, GH2, LEP, PRL, TSLP, TPO	
IL-1s		IL-1A, IL-1B, IL-18	43
IL-10s		IL-10, IL-19, IL-20, IL-22, IL-24, IL-28A, IL-28B, IL-29	61
IL-17s		IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, IL-17F	73
IFNs		IFN α 1, IFN α 2, IFN β 1, IFN ϵ 1, IFN γ , IFN κ , IFN ω	81–83
PDGFs		M-CSF, EGF, FLT3L, HGF, KITL, PLEKHQ1, PGDFA-D, VEGFA-D	89,90
TGF β s		AMH, BMP2, BMP7, GDF5, INHBA, INHBB, INHBC, INHBE, TGF β 1, TGF β 2, TGF β 3	103–106,108
TNFs		CD40L, EDA, FASL, LTA, LTB, RANKL, OX40L, TNF, TNFSF7, TNFSF8, TNFSF9, TNFSF12, TNFSF13, TNFSF13B, TNFSF14, TNFSF18, TRAIL	111–113

Abbreviations: AMH, anti-Mullerian hormone; BMP, bone morphogenetic protein; CD40L, CD40 ligand; CLC, cardiotrophin-like cytokine factor; CNTF, ciliary neurotrophic factor; CTF, cardiotrophin; EDA, ectodysplasin A; EGF, epidermal growth factor; EPO, erythropoietin; FASL, FAS ligand; FLT3L, FLT3 ligand; GDF, growth differentiation factor preproprotein; GH, growth hormone; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte macrophage colony stimulating factor; HGF, hepatocyte growth factor; IFN, interferon; IL, interleukin; INHB, inhibin β ; INHBE, activin β E; KITL, KIT ligand; LEP, leptin; LIF, leukemia inhibitory factor; LT, lymphotoxin; M-CSF, macrophage colony stimulating factor; OSM, oncostatin M, OX40L, OX40 ligand; PDGF, platelet-derived growth factor; PLEKHQ, PH domain-containing protein; PPBP, pro-platelet basic protein; PRL, prolactin; RANKL, RANK ligand; TGF β , transforming growth factor β , TNF, tumor necrosis factor; TPO, thrombopoietin; TSLP, thymic stromal lymphopoietin, VEGF, vascular endothelial growth factor.

Table 2. Currently approved cytokines for oncological indications*

Agent	Commercial names	Oncological indications
IFN α 2b	Intron A, Roferon-A	Cervical intraepithelial neoplasia, CML, FL, HCL, melanoma, MM
IL-2	Aldesleukin, Proleukin	Metastatic melanoma, metastatic renal cell carcinoma

Abbreviations: CML, chronic myeloid leukemia; FL, follicular lymphoma, HCL, hairy cell leukemia, IFN, interferon; IL, interleukin; MM, multiple myeloma. *by FDA or European Medicines Agency (EMA) at the day of submission.

combination with CD40L-expressing DCs, against melanoma and lung carcinoma. MLN1202 is currently under investigation as a single agent in unspecified metastatic neoplasms. Moreover, the safety and efficacy of a monoclonal antibody that specifically targets the CCR2 ligand (CNTO888) is being tested in metastatic prostate cancer patients (as monotherapy) as well as in patients affected by unspecified solid tumors (in combination with doxorubicin, gemcitabine, paclitaxel + carboplatin, or docetaxel). Reflecting the important role played by its ligand (CCL12) in normal and malignant hematopoiesis,¹⁶ an anti-CXCR4 monoclonal antibody (BMS-936564) is being administered to multiple myeloma patients, alone or in combination with lenalidomide, dexamethasone, or bortezomib, as well as to patients with multiple B-cell neoplasms. Along similar lines, the anti-CCL12 PEGylated antisense oligonucleotide NOX-A12 is under investigation as a single agent to treat multiple myeloma or lymphocytic leukemia patients.

Hematopoietic Growth Factors

The group of hematopoietic growth factors includes more than 25 cytokines that are required for the maintenance of hematopoietic stem cells, their proliferation, their differentiation into distinct hematopoietic lineages, as well as for the preservation of a stable equilibrium in the mature hematopoietic compartment. In addition to this homeostatic role, hematopoietins coordinate the organismal response to stimuli that require the expansion of specific hematopoietic cell lineages, (e.g., pathogen infection).¹⁷ The spectrum of cells targeted by different hematopoietins can be large, such in the case of the granulocyte macrophage colony stimulating factor (GM-CSF), or relatively restricted, such in the case of erythropoietin (EPO). In both cases, hematopoietins do not simply convey proliferative signals but also stimulate cell survival, differentiation, lineage commitment and the functional activation of mature cells.¹⁸

At present, several hematopoietic growth factors including EPO (Epopgen, Procrit), IL-2 (Aldesleukin, Proleukin), granulocyte colony stimulating factor (G-CSF, Filgrastim) and GM-CSF (Sargramostim, Molgramostim) are approved by FDA for the treatment of hematological deficiencies including anemia, neutropenia and thrombocytopenia.^{19,20} Although these conditions are highly relevant in oncological settings, as they often develop as dose-limiting side effects of chemotherapy, we will not discuss here clinical trials in which hematopoietins are employed (1) to prevent/ameliorate post-chemotherapy cytopenia; and (2) to facilitate immune reconstitution upon bone marrow transplantation. Rather, we will focus our discussion on the modulation of hematopoietins to obtain specific immunostimulatory or direct anticancer effects.

Besides being employed to boost the proliferation of hematopoietic cells, especially in protocols of adoptive cell transfer,²¹ IL-2 is currently approved by FDA to treat immunosensitive tumors such as melanoma and renal cell carcinoma (RCC) (Table 2). In line with this notion, IL-2 is also included in approximately 50 early (Phase I/II) clinical trials that evaluate various combination regimens for the therapy of melanoma and RCC. Furthermore, IL-2 is being extensively studied (19 Phase I/II clinical trials) as an off-label (co-)medication against multiple hematological and solid malignancies, including breast cancer (4 trials) and neuroblastoma (2 trials) (Table 3). In 11 of these trials, unmodified recombinant human IL-2 (rhIL-2) is used, while 7 others employ IL-2 fused to a tumor-targeting peptide (such as the recombinant antibody fragments F16 or L19).^{22,23} In a rather peculiar approach, the safety and efficacy of an attenuated strain of *Salmonella typhimurium* genetically engineered to express human IL-2 are being investigated in patients with unresectable hepatic metastases from a solid tumor. In a few cases, IL-2-based chimeras are tested as single agents. More often, IL-2 is co-administered with conventional chemotherapeutics or anticancer vaccines (www.clinicaltrials.gov).

G-CSF and GM-CSF stimulate the differentiation of bone marrow stem cells toward the granulocytic (both G-CSF and GM-CSF) and monocytic (GM-CSF only) lineages.²⁰ In addition, G-CSF promotes the mobilization of hematopoietic precursor cells from the bone marrow into the bloodstream.²⁴ Since their approval by FDA (in the early 1990s), G-CSF and GM-CSF have been used in millions of cancer patients to prevent/counterbalance chemotherapy-associated neutropenia.²⁰ Moreover, G-CSF has been tested for its ability to “prime” leukemic cells and hence render them more sensitive to conventional chemotherapy. However, results from a large randomized clinical trial indicate that G-CSF priming might extend disease-free (but not overall) survival, and only in patients with standard-risk acute myeloid leukemia.²⁵ Today, G-CSF and GM-CSF are employed in no less than 100 clinical trials, often, if not always, in combination with therapeutic strategies that are known (or expected) to provoke leucopenia. Multiple studies are testing G-CSF in combination with plerixafor (a derivative of bicyclam with immunostimulatory functions)²⁶ for mobilizing hematopoietic cell stem cells prior to autologous transplantation. Moreover, GM-CSF is being used extensively in the context of anticancer vaccine-based

clinical trials, either as a recombinant product (Sargramostim, Molgramostim) or upon genetic engineering of (re-)infused cells. Nevertheless, no clinical studies are currently testing whether G-CSF and GM-CSF might exert anticancer effects independent of their capacity to stimulate early hematopoiesis (www.clinicaltrials.gov).

IL-4 stimulates the differentiation of naive helper T cells into Th2 cells, in turn producing additional IL-4 as well as IL-10 (see below) and IL-13.²⁷ As it drives the local secretion of mitogenic cytokines, the Th2 response has been suggested to promote tumor growth.²⁸ In line with this notion, no trials are currently investigating the efficacy of IL-4 and IL-13 as anticancer agents. Nevertheless, as tumor cells often overexpress the IL-4 and/or the IL-13 receptor,²⁹ IL-4 and IL-13 are being tested as tumor-targeting partners for the enzymatically active portion of *Pseudomonas aeruginosa* exotoxin A (Table 3).

Besides participating in the acute phase response at the organismal level,^{3,4} IL-6 can function as a paracrine regulator of inflammation and immunity.³⁰ Moreover, some neoplasms (e.g., most variants of multiple myeloma) produce high levels of IL-6, and these are strictly required for tumor survival.³¹ Driven by promising preclinical observations,³² several monoclonal antibodies that specifically block IL-6 have been tested in cancer patients during the last decade.³¹ Nevertheless, the actual efficacy of these drugs for oncological indications remain unclear.^{33,34} One notable exception is represented by siltuximab (CNTO 328) for ovarian cancer patients experiencing paraneoplastic thrombocytosis.³⁵ The efficacy of siltuximab for oncological indications is currently being tested in no less than 15 Phase I/II clinical trials (www.clinicaltrials.gov).

IL-7 is a potent hematopoietic growth factor, stimulating the differentiation of hematopoietic stem cells into lymphoid progenitors as well as the proliferation of mature cells of the lymphoid lineage.³⁶ In spite of early concerns on the possibility that IL-7 might per se sustain hematopoietic tumorigenesis,³⁷ IL-7 is nowadays being investigated in the clinic, in both non-oncological (e.g., in HIV patients in combination with conventional antiretroviral drug) and oncological settings (Table 3). In particular, IL-7 is being tested either as a single agent in metastatic breast cancer patients (mainly to contain lymphopenia and divpenia, i.e., a severe restriction in the TCR repertoire, but also to stimulate an anticancer immune response)³⁸ or in combination with vaccines against RCC, various types of sarcoma or neuroblastoma (www.clinicaltrials.gov).

Besides being involved in the differentiation of naive T cells, IL-12 potently stimulates the functions of T and natural killer (NK) cells. In particular, it promotes the secretion of TNF α and IFN γ and inhibits the generation of IL-4, de facto stimulating a cytotoxic Th1 response.³⁹ In line with this notion, multiple distinct approaches for employing IL-12 in anticancer therapy are being evaluated (Table 3). Thus, in 4 Phase I/II clinical trials, rhIL-12 is used in combination with cetuximab (a monoclonal antibody targeting the epidermal growth factor receptor, EGFR) or other cytokines (IL-2 + GM-CSF, delivered with microspheres) in head and neck cancer patients, as well as to boost vaccine-driven anticancer responses in melanoma or breast

cancer patients. As an alternative, the IL-12-encoding gene is administered either via plasmid electroporation (to melanoma and Merkel cell carcinoma patients), either with a replication-incompetent adenoviral strain (to melanoma and metastatic breast cancer patients), either within a non viral vector, alone or combined with chemotherapeutics (to colorectal, ovarian and primary peritoneal carcinoma patients), or in the context of adoptive cell transfer immunotherapy with genetically-engineered tumor-infiltrating lymphocytes (www.clinicaltrials.gov).

Similar to IL-12, both IL-15 and IL-21 regulate the proliferation and activation of NK and T cells.^{40,41} Moreover, IL-15 has been shown to promote the survival of a specific subset of

memory CD8⁺ T cells.⁴² Besides a few studies in which rhIL-15 is being tested for its ability to stimulate the immune system in HIV⁺ patients, there are 8 Phase I/II clinical trials in which the safety and efficacy of IL-15 and IL-21 is tested in oncological indications (Table 3). In six studies, rhIL-15 and rhIL-21 are given, either as standalone medications or combined with targeted anticancer agents, to patients with immunosensitive neoplasms like melanoma and RCC. Alternatively, IL-15 is administered to boost the immune function of freshly infused NK cells, in an adoptive cell transfer approach, or used to engineer DCs for the generation of an efficient vaccine against melanoma (www.clinicaltrials.gov).

Table 3. Clinical trials* on hematopoietins in cancer therapy (main trends)

Hematopoietin	Agent	Tumor type	Trials*	Phase	Notes	Refs.
IL-2	ALT801	Metastatic urothelial cancer	1	I-II	Combined with cisplatin and gemcitabine	NCT01326871
	CD40L/IL-2-expressing tumor cell vaccine	B-CLL	1	I	As single agent	NCT00609076
	DI-Leu16-IL2 immunocytokine	Hematological neoplasms	1	I	Combined with rituximab	NCT00720135
	F16IL2 immunocytokine	Breast cancer	1	I-II	Combined with paclitaxel	NCT01134250
		Breast cancer Solid tumors	1	I-II	Combined with doxorubicin	NCT01131364
	hu14.18-IL2 immunocytokine	NB	1	II	Combined with GM-CSF and isotretinoin	NCT01334515
	rhIL-2	AML	1	II	Combined with famotone	NCT01289678
		Breast cancer	1	II	Combined with anastrozol, aromasin, femar, pulsed DCs and thymosin 1α	NCT00935558
		MDS	1	I-II	Combined with azacitidine and ceplene	NCT01324960
		Melanoma	1	II	Combined with anticancer vaccine	NCT00784524
		Metastatic breast cancer	1	II	Combined with anticancer vaccine	NCT00784524
		NB	1	I	Combined with zoledronic acid	NCT01404702
		NHL	1	II	Combined with rituximab	NCT00994643
		Plasma cell neoplasms	1	II	Combined with anticancer vaccine	NCT00616720
	IL-2-expressing bacteria	Liver cancer	1	I	As single agent	NCT01099631
L19IL2 immunocytokine		Advanced solid tumors	1	I-II	As single agent	NCT01058538
		Pancreatic cancer	1	I-II	Combined with gemcitabine	NCT01198522
Microsphere delivery		HNC	1	n.a.	Combined with GM-CSF and IL-12	NCT00899821
IL-4		IL-4PE	Glioblastoma	1	II	As single agent
IL-7	rhIL-7	NB Sarcoma	1	I-II	Combined with anticancer vaccine	NCT00923351
	MGN1601	Metastatic breast cancer	1	II	As single agent	NCT01368107
		RCC	1	I-II	Genetically modified anticancer vaccine	NCT01265368

Abbreviations: AML, acute myeloid leukemia; B-CLL, B-cell chronic lymphocytic leukemia; CD40L, CD40 ligand; DC, dendritic cell; FOLFIRI, folinic acid, 5-fluorouracil, irinotecan; FOLFOX, folinic acid, 5-fluorouracil, oxaliplatin; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; HNC, head and neck cancer; IL-, interleukin; MDS, myelodysplastic syndrome; RCC, renal cell carcinoma; NB, neuroblastoma; NHL, non-Hodgkin lymphoma; p, plasmid-encoded; NK, natural killer; rh, human recombinant; TIL, tumor infiltrating lymphocyte. *Started after January 1, 2008 and not terminated at the day of submission.

Table 3. Clinical trials* on hematopoietins in cancer therapy (main trends) (continued)

Hematopoietin	Agent	Tumor type	Trials*	Phase	Notes	Refs.
IL-12	Ad-IL-12	Metastatic breast cancer	1	I	As single agent	NCT00849459
	Ad-RTS-hIL-12	Melanoma	1	I	Combined with activator ligand	NCT01397708
	EGEN-001	CRC	1	I-II	Alone or combined with FOLFIRI or FOLFOX	NCT01300858
		Reproductive tract cancer	1	I	Combined with doxorubicin	NCT01489371
	rhIL-12	Breast cancer	1	II	As single agent	NCT01118052
			1	I-II	Combined with DC/tumor cell fusion vaccine	NCT00622401
			1	II	Combined with daclizumab and anticancer vaccine	NCT01307618
	IL-12-expressing TILs	Metastatic melanoma	1	I-II	Combined with cetuximab	NCT01468896
			1	I-II	Combined with cyclophosphamide, fludarabine and G-CSF	NCT01236573
	Microsphere delivery	HNC	1	n.a.	Combined with GM-CSF and IL-2	NCT00899821
	NHS-IL12	Epithelial and mesenchymal malignancies	1	I	As single agent	NCT01417546
	pIL-12	Melanoma	1	II	As single agent	NCT01502293
Merkel cell cancer		1	II	As single agent	NCT01440816	
IL-13	IL13-PE38QQR	Brain tumors	1	I	As single agent	NCT00880061
IL-15	IL-15-expressing DCs	Melanoma	1	I-II	Autologous DC-based vaccine	NCT01189383
	rhIL-15	Metastatic cancer	1	I	As single agent	NCT01572493
		Metastatic melanoma Metastatic RCC	1	I		NCT01021059
IL-15-activated NK cells	Pediatric refractory solid tumors	1	I-II	Combined with haploidentical stem cell transplantation	NCT01337544	
IL-21	rhIL-21	Melanoma	2	I	Combined with ipilimumab	NCT01489059
		1	II	NCT01152788		
		Metastatic melanoma RCC	1	II	As single agent	NCT00601861
			1	I-II		NCT00617253

Abbreviations: AML, acute myeloid leukemia; B-CLL, B-cell chronic lymphocytic leukemia; CD40L, CD40 ligand; DC, dendritic cell; FOLFIRI, folinic acid, 5-fluorouracil, irinotecan; FOLFOX, folinic acid, 5-fluorouracil, oxaliplatin; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; HNC, head and neck cancer; IL-, interleukin; MDS, myelodysplastic syndrome; RCC, renal cell carcinoma; NB, neuroblastoma; NHL, non-Hodgkin lymphoma; p, plasmid-encoded; NK, natural killer; rh, human recombinant; TIL, tumor infiltrating lymphocyte. *Started after January 1, 2008 and not terminated at the day of submission.

Interleukin-1 Family Members

The IL-1 family of cytokines includes three members: IL-1 α , IL-1 β and IL-18. IL-1-like proteins are mainly produced by activated macrophages, and play a prominent role in local and systemic inflammation.⁴³ IL-1 family members are synthesized as inactive precursors and are cleaved by intracellular proteases (pro-IL-1 α mainly by calpains, pro-IL-1 β and pro-IL-18 mainly by the caspase-1-containing complex called inflammasome) before secretion.⁴⁴⁻⁴⁶ So far, two distinct types of monomeric IL-1 receptors and one heteromeric IL-18 receptor have been identified, all belonging to the immunoglobulin superfamily.^{47,48} Of note, while the type I IL-1 receptor (IL1R1) is biologically active and transduces IL-1 signals, the type II receptor (IL1R2) often acts as a soluble decoy receptor.^{49,50} Moreover, IL-1 α and IL-1 β are functionally antagonized by the IL-1 receptor antagonist (IL-1RA), a soluble factor that binds non-productively to IL-1 receptors.⁵¹

Blockade of IL-1 β signaling with specific monoclonal antibodies or with a recombinant derivative of IL-1RA (anakinra)

compromises the therapeutic effects of anthracyclines and oxaliplatin in immunocompetent, but not immunodeficient, mouse models.^{52,53} This suggests that IL-1 β plays a key role in chemotherapeutic responses by virtue of its immunostimulatory functions. Probably due to such a potent pro-inflammatory profile, correlating with a high risk for severe side effects, the administration of recombinant IL-1 to cancer patients has not yet been tested. Conversely, the inhibition of IL-1 signaling with anakinra is being extensively tested, with encouraging results, in patients affected by Type 2 diabetes and many other autoimmune disorders (www.clinicaltrials.gov).⁵⁴⁻⁵⁶

Results from a recent Phase II trial evaluating the safety and efficacy of rhIL-18 (SB-485232) in metastatic melanoma patients suggest that SB-485232 is safe but has limited anticancer activity as a single agent.⁵⁷ Nowadays, SB-485232 is being tested, alone or in combination with doxorubicin, in patients affected by epithelial ovarian cancers (Phase I, NCT00659178) (www.clinicaltrials.gov), but the results of these trials have not yet been published. Intriguingly, in spite of its potent pro-inflammatory

profile, IL-18 has recently been shown to suppress metastasis surveillance by NK cells.⁵⁸ In line with this notion, elevated levels of circulating IL-18 have been associated with poor disease outcome in pancreatic and lung cancer patients.^{59,60} Taken together, these observations suggest that the administration of SB-485232 may be beneficial to patients affected by specific neoplasms (such as melanoma) but detrimental in others.

Interleukin-10 Family Members

According to current classifications, nine cytokines belong to the IL-10 family: IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28A, IL-28B and IL-29.⁶¹ With the notable exception of IL-24,⁶² these proteins operate through heteromeric receptors that share the IL-10 receptor β subunit.^{63,64} Nevertheless, the biological outcomes of IL-10 family members are extremely heterogeneous, encompassing consistent immunosuppression (such as that triggered by IL-10),⁶⁵ the induction of an antiviral state (by IL-28A, IL-28B and IL-29, which are also known as Type III IFNs),⁶⁶ as well as (often STAT3-dependent) mitogenic and pro-survival effects (by IL-19, IL-20, IL-22 and IL-26).⁶⁷ Of note, *Il10*^{-/-} mice develop a chronic and fatal enterocolitis, which can be stopped by administration of IL-10.⁶⁸ In line with the potent immunosuppressive functions of IL-10, early clinical trials were launched to study the safety and efficacy of rhIL-10 in patients affected by psoriasis and psoriatic arthritis.⁶⁹ Following the encouraging results of these studies, rhIL-10 (nowadays produced under the name of prevascar or ilodecakin) is being tested in other clinical settings featuring excessive/chronic inflammation, including wound healing and ulcerative colitis (in the latter scenario, IL-10 is produced in situ by genetically engineered *Lactococcus lactis*). In addition, a humanized anti-IL-10 antibody (SCH708980) is under investigation as an anti-immunosuppressive agent during visceral Leishmaniasis. Of note, there are no ongoing clinical trials that evaluate the therapeutic potential of cytokines from the IL-10 family (or their inhibitors) in cancer patients (www.clinicaltrials.gov). This said, there is a vast literature that correlate elevated serum levels of IL-10 with poor prognosis in multiple hematological and solid malignancies,⁷⁰⁻⁷² suggesting that—at least in some patients—the inhibition of IL-10 may turn out to be therapeutically beneficial.

Interleukin-17 Family Members

So far, six different IL-17 variants have been described (IL-17A-F).⁷³ As a family, IL-17 (which is most often secreted by helper T cells in response to IL-23) exerts potent pro-inflammatory functions, stimulating the release of many other cytokines (such as IL-1 β , IL-6, GM-CSF, TGF β and TNF α), chemokines (e.g., MCP-1), and prostaglandins (e.g., PGE2).⁷⁴ IL-17 family members operate by binding to a heterodimeric receptor, which can be assembled by 2 out of 5 different subunits (IL17RA-E).⁷⁵ The exact role of IL-17 and IL-17-producing helper T (Th17) cells in tumor progression and response to therapy is controversial.⁷⁶ On one hand, IL-17-secreting $\gamma\delta$ T cells that arise in response to IL-1 β + IL-23 appear to be required for optimal therapeutic

responses, presumably due to the fact that they are prone to secrete GM-CSF and IFN γ .^{52,53} On the other hand, Th17 cells that differentiate in the presence of TGF β and IL-6 produce IL-17 and IL-10, de facto exerting immunosuppressive effects.⁷⁷ Hence, there are no ongoing clinical trials that evaluate the therapeutic potential of this cytokine in oncological settings (www.clinicaltrials.gov). Conversely, monoclonal antibodies targeting IL-17A (ixekizumab and secukinumab) or IL17RA (brodalumab) have given encouraging results for the treatment of diseases with an auto-immune component such as uveitis, rheumatoid arthritis and psoriasis.⁷⁸⁻⁸⁰ Ixekizumab (also known as LY2439821) is currently being tested in a few clinical trials to optimize dosage and administration schedules (www.clinicaltrials.gov).

Interferons

Interferons (IFNs) have first been characterized for their ability to “interfere” with viral replication, and indeed one of their major functions is the establishment of a robust antiviral state in response to infection.⁸¹ In addition, IFNs activate immune cells and facilitate the recognition of tumor cells by the immune system as they stimulate antigen presentation to T lymphocytes.⁸² IFNs are usually subdivided into three classes: Type I (including IFN α , IFN β and IFN ω), Type II (in humans: IFN γ), and Type III IFNs (including IL-28A, IL-28B and IL-29, see above). Type I IFNs signal through a receptor complex known as the IFN α receptor (IFNAR), consisting of IFNAR1 and IFNAR2 chains. Along similar lines, IFN γ functions by binding to a heterodimeric IFN γ receptor (IFNGR), which consists of IFNGR1 and IFNGR2 chains.⁸³ Recent data indicate that both IFNAR and IFNGR are required for optimal responses to immunogenic chemotherapy, at least in preclinical settings.⁸⁴⁻⁸⁶

During the last two decades, great efforts have been spent at optimizing strategies to modulate the IFN system. On one hand, humanized monoclonal antibodies specific for IFN γ (fontolizumab) have been developed and tested in clinical settings. Thus, fontolizumab has been shown to be safe and clinically efficient in patients with chronic inflammatory pathologies such as rheumatoid arthritis or moderate to severe Crohn disease.^{87,88} On the other hand, recombinant IFN α 2b has been approved by FDA for a wide range of indications, including chronic hepatitis B and C, hairy cell leukemia, chronic myeloid leukemia, multiple myeloma, follicular lymphoma and malignant melanoma (Table 2). Nowadays, the efficacy of IFN α 2 against cancer is being tested in 58 clinical trials, of which 22 evaluate IFN α 2b in FDA-approved clinical settings (most often in melanoma patients). Of the remaining 36 studies, 12 investigate the therapeutic potential of IFN α 2 in RCC patients, while the others span multiple solid and hematopoietic neoplasms, including bladder cancer, hepatocellular carcinoma, colorectal carcinoma, mesothelioma, pancreatic cancer and Hodgkin lymphoma (Table 4). Frequently (11/58 trials), IFN α 2 is tested as monotherapy. As an alternative, combination regimens with bevacizumab (7/58 trials), dacarbazine (3/58 trials), cisplatin (3/58 trials), cyclophosphamide (2/58 trials), sorafenib (2/58 trials) or decitabine (2/58 trials) are being investigated. Of note, a relatively high proportion

Table 4. Clinical trials* on IFNs as anticancer agents (main trends)

Chemokine	Tumor type	Trials*	Phase	Notes	Ref.
Early clinical trials (Phase I-II)					
IFN α	Adult T-cell leukemia/lymphoma	1	n.a.	Combined with valproic acid and zidovudine	NCT00854581
	Advanced solid tumors	1	I	Combined with decitabine	NCT00701298
		1	I	Combined with cyclophosphamide and vinorelbine	NCT00908869
		1	I	Combined with sodium stibogluconate	NCT00629200
		1	II		NCT01479309
	Bladder cancer	1	II	As single agent	NCT01162785
	Cervical cancer	1	II	Combined with radiotherapy and RA	NCT01276730
				Combined with nilotinib	NCT01294618
	CML	3	II	As single agent	NCT01392170
				Combined with nilotinib	NCT01220648
	CRC	1	I-II	Combined with celecoxib and poly-ICLC	NCT01545141
	Cutaneous T-cell lymphoma	1	n.a.	Combined with UV light	NCT00724061
	HCC	1	I	As single agent	NCT00838968
	Hodgkin lymphoma	1	II	Combined with ABVD	NCT01404936
	Kidney cancer	4	II	Combined with radioablation	NCT00891475
				Combined with pazaponib	NCT01513187
				Combined with sorafenib	NCT00589550
				Combined with bevacizumab	NCT00619268 NCT00719264 NCT00796757 NCT00873236
				Combined with celecoxib	NCT01158534
				Combined with GM-CSF and IL-2	NCT01176552
				Combined with bevacizumab and IL-2	NCT01274273
	Malignant pleural mesothelioma	2	I	Combined with cisplatin and pemetrexed	NCT01119664
				As single agent	NCT01212367
	NHL	1	II	In alkylating agent- or anthracycline-based regimens plus rituximab	NCT00842114
	Neurofibroma	1	II	As single agent	NCT00678951
	Pancreatic cancer	2	I	Combined with 5-FU, cisplatin and gemcitabine	NCT00660270
				Combined with 5-FU, docetaxel, gemcitabine and oxaliplatin	NCT00761241
	Various solid tumors	1	II	Combined with DC- and TIL-based immunotherapy plus cyclophosphamide and TNF α	NCT00610389
IFN γ	CRC	1	II	Combined with bevacizumab, 5-FU and folinic acid	NCT00786643
	Melanoma	1	n.a.	Combined with peptide-based vaccine	NCT00977145
		1	I-II	Combined with TIL and IL-2	NCT01082887
	Plasma cell neoplasms	1	II	Combined with antitumor vaccine	NCT00616720
	Soft tissue sarcoma	1	II	Combined with autologous NY-ESO-1-specific CD8 $^+$ T cells, cyclophosphamide and IL-2	NCT01477021
Advanced clinical trials (Phase III-IV)					
IFN α	Bladder cancer	1	III	Combined with BCG, epirubicin and mitomycin C	NCT01094964
	CRC	1	III	Combined with 5-FU and folinic acid	NCT01060501
	HCV-associated HCC	1	IV	Combined with ribavirin	NCT00834860
	Kidney cancer	1	III	Combined with bevacizumab	NCT00738530
	Urogenital cancer	1	III		NCT00631371

Abbreviations: 5-FU, 5-fluorouracil; ABVD, bleomycin, dacarbazine, doxorubicin, vinblastine; BCG, bacillus Calmette-Guérin; CML, chronic myeloid leukemia; CRC, colorectal carcinoma; DC, dendritic cell; GM-CSF, granulocyte macrophage colony stimulating factor; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; n.a., not available; NHL, Non-Hodgkin's lymphoma; RA, retinoic acid; RCC, renal cell carcinoma; TIL, tumor infiltrating lymphocyte; TNF, tumor necrosis factor; UV, UV. *Started after January 1, 2008 and not terminated at the day of submission.

of IFN α 2-based clinical trials (around 20%) consists of advanced studies (Phase III/IV), presumably reflecting the limited safety issues associated with the use of a FDA-approved molecule. At present, rhIFN γ (which is approved by FDA for the therapy of granulomatous disease and severe osteopetrosis) is being tested against a wide range of cancer-unrelated pathologies, including infections and inflammatory diseases. Moreover, there are five ongoing clinical studies that evaluate the safety and efficacy of IFN γ in oncological indications. In one case, IFN γ is used alone or in combination with bevacizumab, 5-fluorouracil and folinic acid against colorectal cancer. In the others, IFN γ is employed to boost anticancer immune responses driven by the administration of autologous T cells or peptide vaccines (in patients affected by soft tissue sarcoma, melanoma or plasma cell neoplasms) (www.clinicaltrials.gov).

PDGF Family Members

The PDGF family of cytokines includes four PDGF isoforms (PDGFA-D), four isoforms of the vascular endothelial growth factor (VEGFA-D) and other six rather heterogeneous proteins. PDGFs, which activate cellular responses via two distinct tyrosine kinase receptors (PDGFR α and β), can be released by activated platelets, as well as smooth muscle cells, activated macrophages, and endothelial cells.⁸⁹ PDGFs exert potent mitogenic functions over a plethora of distinct cell types and play an important role in development and angiogenesis.⁹⁰ In line with this notion, PDGFRs are often hyperactivated in cancer, often due to point mutations in PDGFRA or PDGFRB, such as in the case of gastrointestinal stromal tumors (GISTs).⁹¹ The same holds true for other PDGF-like protein receptors including KIT (which delivers potent mitogenic signals and is mutated in more than 70% GIST patients),⁹² EGFR (which is overexpressed or mutated in colorectal and lung cancer)⁹³ and FLT3 (which is frequently hyperactivated in acute myeloid leukemia patients).⁹⁴ Along similar lines, tumor cells often overexpress VEGF, stimulating the so-called “angiogenic switch,” i.e., the transition of a tumor mass from a non-vascularized to a vascularized state.⁹⁵ The FLT3 ligand (FLT3L) has been shown to expand DCs *in vivo* (in mice), resulting in the elicitation of NK-mediated antitumor effects.^{96,97} Nevertheless, given their potent mitogenic (and hence potentially tumorigenic) properties, it is not surprising that PDGF family members are not employed as immunostimulatory agents against cancer, neither alone nor in combination with other drugs. Rather, chemicals that selectively inhibit the tyrosine kinase activity of PDGFR, KIT and FLT3 (including imatinib, lapatinib and sorafenib), as well as monoclonal antibodies that specifically block EGFR (i.e., cetuximab, panitumumab) or VEGFA (i.e., bevacizumab), are currently approved by FDA for an ever-increasing number of oncological indications and are associated with consistent rates of remissions.⁹⁸ These agents are the subject of an intense wave of clinical trials, aimed at finding new indications as well as at optimizing dosage and administration schedules (www.clinicaltrials.gov). The detailed discussion of such studies largely exceeds the scope of the present Trial Watch and can be found elsewhere.⁹⁹ This said, it is worth noting that several compounds that were

developed to specifically target the receptors for PDGF family members, including imatinib and many others, are less specific than expected, and *de facto* exert potent off-target immunostimulatory effects.^{84,100,101} This property has raised great expectations and—as many of these agents have already been approved by FDA for anticancer therapy—will presumably be tested in the short future in appropriate clinical trials.

TGF β Family Members

TGF β 1 has first been characterized for its ability to trigger the growth of normal rat kidney (NRK) fibroblast colonies in soft-agar assays (though only if small amounts of EGF were present).¹⁰² Since then, three distinct subtypes of human TGF β have been identified (TGF β 1-3), together with at least additional eight proteins that are now considered part of the TGF β family, including polypeptides of the activin-inhibin hormonal system and at least two bone morphogenic proteins (BMP2 and BMP7).¹⁰³ TGF β s signals are transduced intracellularly by homo- or heterodimeric serine/threonine kinase receptors, which can be assembled starting from three distinct subunits (TGFBR1-3).¹⁰⁴ In normal cells, TGF β s, and in particular TGF β 1, exert antiproliferative effects by upregulating the expression of cell cycle inhibitors such as p21^{CIP1} and p15^{INK4B} or trigger cell death by activating SMAD- or DAXX-dependent apoptosis.¹⁰⁵ Conversely, cancer cells often become refractory to the inhibitory effects of TGF β and, at least in some cases, also overexpress it.¹⁰⁶ In this setting, TGF β stimulates angiogenesis and facilitates the conversion of effector T lymphocytes into FOXP3⁺ immunosuppressive T cells (Tregs) or Th17 cells, *de facto* exerting pro-tumor functions.^{106,107} Of note, BMP2 and BMP7 also activate the SMAD signaling pathway, yet mainly function as osteogenic mediators.¹⁰⁸ Over the two last decades, two distinct monoclonal antibodies against TGF β have been developed: metelimumab (CAT-192, which specifically targets TGF β 1) and fresolimumab (GC-1008, which can block TGF β 1-3).¹⁰⁹ In people affected by systemic sclerosis, metelimumab was found to be safe but ineffective,¹¹⁰ leading the proprietary pharmaceutical company to focus on fresolimumab. Today, fresolimumab is being tested, alone or combined with other interventions, in pulmonary fibrosis, myelofibrosis and focal segmental glomerulosclerosis patients, as well as in patients affected by glioma, mesothelioma, melanoma and metastatic breast cancer (www.clinicaltrials.gov). To our knowledge, any other clinical trial is currently investigating the modulation of TGF β signaling in oncological indications.

Tumor Necrosis Factors

The TNF family of cytokines includes more than 15 distinct proteins, all of which can, at least in some settings, trigger cell death upon binding to specific transmembrane receptors.¹¹¹⁻¹¹³ The best known member of the family, TNF α , has first been identified in 1975, thanks to the work of Lloyd Old at the Memorial Sloan-Kettering Cancer Center (New York).¹¹⁴ Another TNF, lymphotoxin (LT, also known as TNF β), had been discovered a few years earlier, in 1968,^{115,116} but it was not until the cloning

of TNF α and LT cDNAs, in 1985, that the homology between these two proteins became evident.¹¹⁷ TNF α can bind two differentially expressed transmembrane receptors: while Type I TNFR (TNFR1) is found at the surface of virtually all cells, type II TNFR (TNFR2) is expressed only by cells of the immune system.¹¹³ TNFR1 ligation can induce biological outcomes as diverse as the activation of the NF κ B system (which normally delivers pro-survival signals), the initiation of mitogen-activated protein kinase (MAPK)-transduced signaling cascades (which can regulate proliferation, differentiation and cell death), and the induction of cell death (via apoptosis or regulated necrosis).^{118,119} The TNF family also includes FAS and CD40 ligands (FASL and CD40L, both exerting a major role in the development, homeostasis and function of the immune system),^{120,121} RANK ligand (RANKL, which has been involved in the differentiation of osteoclasts, in the maturation of DCs and in hormone-driven breast carcinogenesis),¹²²⁻¹²⁴ as well as TNF-related apoptosis-inducing ligand (TRAIL).¹²⁵

TNF α has been shown to play a crucial role in the pathogenesis of multiple inflammatory diseases, including (though presumably not limited to) rheumatoid arthritis, Crohn disease, psoriasis, psoriatic arthritis, ankylosing spondylitis and juvenile idiopathic arthritis.¹²⁶ During the past 20 y, a large battery of TNF inhibitors has been developed and approved by FDA for the treatment of these conditions. Such agents include anti-TNF α monoclonal antibodies like infliximab (Remicade), adalimumab (Humira), certolizumab pegol (Cimzia), and golimumab (Simponi), as well as the artificial fusion protein etanercept (Enbrel), consisting in two soluble human TNFR moieties linked to the Fc portion of an IgG₁.^{99,126} Denosumab (a fully human anti-RANKL antibody), is currently approved by FDA for the treatment of post-menopausal osteoporosis and for the prevention of skeletal-related events in patients with bone metastases from solid tumors (Table 2).^{127,128} Furthermore, a monoclonal antibody that neutralizes the OX40

ligand (OX40L, an important co-stimulatory molecule during immune responses) is currently being tested for the prevention of allergen-induced airway obstruction in adults with mild allergic asthma (www.clinicaltrials.gov).

In addition to these applications and studies (which do not directly relate to anticancer therapy), TNF α has been extensively employed in combination with melphalan (an alkylating agent) for isolated limb perfusion in metastatic melanoma and sarcoma patients,¹²⁹ an approach associated with high rates of limb salvage but limited impact on overall survival.^{130,131} Moreover, there are 22 ongoing clinical trials that evaluate whether the modulation of TNF family members is beneficial or not to cancer patients (www.clinicaltrials.gov). Three of these 22 studies (all of which are in Phase I/II) investigate whether the administration of rhTNF α (as such or fused to a tumor-targeting antibody fragment)¹³² is efficient (alone or in combination with doxorubicin or melphalan) in patients affected by lymphoma, melanoma or other advanced solid tumors (Table 5). Four clinical trials are testing whether monoclonal antibodies that can activate the TRAIL receptor 1 (TRAILR1), de facto mimicking TRAIL cytotoxicity, exert therapeutic effects, if combined with other chemotherapeutics, against hematological and solid tumors including cervical and colorectal carcinoma (Table 5). The safety and efficacy of strategies based on CD40L are being investigated in seven clinical trials. In this setting, CD40L is either introduced directly into the tumor by local injection of adenoviral vectors, or transfected into autologous DCs or cancer cells that are used to generate vaccines. In addition, CP-870,893 (a monoclonal antibody that activates CD40, de facto mimicking CD40L) is tested, in combination with carboplatin and paclitaxel, in patients bearing advanced solid tumors (Table 5). Finally, denosumab is currently under investigation (in nine Phase II/III clinical trials) for its capacity to prevent/treat bone metastase in patients affected by multiple myeloma and solid tumors including breast and prostate cancer (www.clinicaltrials.gov).

Table 5. Clinical trials* on TNFs and TNF-mimicking agents in anticancer therapy (main trends)

Chemokine	Agent	Tumor type	Trials*	Phase	Notes	Refs.
CD40L	CP-870,893	Metastatic solid tumors	1	I	Combined with carboplatin and paclitaxel	NCT00607048
		B-CLL	1	I	CD40L-expressing autologous tumor cell-based vaccine	NCT00609076
	rhCD40L	Bladder cancer	1	I-II	Adenoviral gene therapy	NCT00891748
		Lung cancer	2	II	CD40L-expressing allogeneic tumor cell-based vaccine	NCT00601796
		MDS	1	I	CD40L-expressing cell-based vaccines	NCT01433172
TNF α	L19TNF α	Melanoma	1	I	Adenoviral gene therapy	NCT01455259
		Advanced solid tumors	1	I-II	As single agent	NCT01253837
	rhTNF α	Melanoma	1	I	Combined with melphalan	NCT01213732
		Advanced solid tumors	1	I	Combined with doxorubicin	NCT01490047
TRAIL	Conatumumab	Lymphoma	1	I	Combined with doxorubicin	NCT01490047
		Advanced hematological and solid tumors	2	II	Alone or combined with bevacizumab, FOLFOX and ganitumab	NCT01327612
	Dulanermin	Advanced solid tumors	1	I-II	Combined with AMG479	NCT00819169
		CRC	1	I	Combined with bevacizumab	NCT00873756
Mapatumumab	Advanced cervical cancer	1	I-II	Combined with cisplatin and radiotherapy	NCT01088347	

Abbreviations: Ad, adenovirus; B-CLL, B-chronic lymphocytic leukemia; CRC, colorectal cancer; CD40L, CD40 ligand; DC, dendritic cell; FOLFOX, folinic acid, fluorouracil, oxaliplatin; MDS, myelodysplastic syndrome; MM, multiple myeloma, rh, recombinant human; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand. *Started after January 1, 2008 and not terminated at the day of submission.

Concluding Remarks

Cytokines constitute a highly heterogeneous group of proteins that, taken together, regulate virtually all aspects of the cell biology. Some of them, such as GM-CSF, potently stimulate the replication and survival of hematopoietic cell precursors, and are mainly employed in tumor patients to help them reconstituting the hematopoietic system upon chemotherapy or transplantation. Others, including multiple members of the PDGF family, exert potent mitogenic functions and hence cannot be employed in cancer therapy, as they would stimulate, rather than prevent, oncogenesis and tumor progression. Finally, there are cytokines that have been approved by FDA (or are being clinically tested) for oncological indications, owing to their ability to stimulate anti cancer immune responses. For instance, IL-2 has been associated with consistent rates of tumor regression in melanoma and RCC patients,^{133,134}

probably because these malignancies are able to elicit per se elevated levels of antitumor lymphocytes. Although the elevated pleiotropism of the cytokine system constitutes an obstacle for the development of highly targeted therapeutics (implying that these proteins may be intrinsically prone to elicit side effects), we surmise and hope that ongoing and/or future clinical trials will lead to the approval of additional cytokines for use in humans against cancer.

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