ELSEVIER

Immunocytokines: a novel class of potent armed antibodies

Nadine Pasche and Dario Neri

Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, Swiss Federal Institute of Technology (ETH Zürich), Wolfgang-Pauli-Strasse 10, CH-8093 Zürich, Switzerland

Several cytokines have been investigated in clinical trials, based on their potent therapeutic activity observed in animal models of cancer and other diseases. However, substantial toxicities are often reported at low doses, thus preventing escalation to therapeutically active regimens. The use of recombinant antibodies or antibody fragments as delivery vehicles promises to enhance greatly the therapeutic index of pro-inflammatory and anti-inflammatory cytokines. This review surveys preclinical and clinical data published in the field of antibody-cytokine fusions (immunocytokines). Molecular determinants (such as molecular format, valence, target antigen), which crucially contribute to immunocytokine performance *in vivo*, are discussed in the article, as well as recent trends for the combined use of this novel class of biopharmaceuticals with other therapeutic agents.

Many cytokines have shown potent antitumor activities in preclinical experiments and represent promising agents for cancer therapy. However, despite encouraging results in animal models, only a few cytokines {e.g. Proleukin® [interleukin 2 (IL2)], Roferon A® [interferon (IFN)α 2a], Intron A® (IFNα 2b), Beromun® [recombinant tumor necrosis factor (TNF)]} are approved as anticancer drugs. Current indications include metastatic renal cell cancer, malignant melanoma, hairy cell leukemia, chronic myeloid lymphoma, sarcoma and multiple myeloma, alone or in combination with chemotherapy. Additionally, certain cytokines are used in clinical practice for the treatment of viral and bacterial infections [1], whereas anti-inflammatory cytokines can confer a benefit to patients suffering from chronic inflammatory conditions.

Although in preclinical models of cancer certain cytokines can mediate complete tumor eradication, only a modest efficacy is often observed in the clinical setting. So, what are the reasons for such a striking difference between preclinical experiments and clinical trials?

In many cases, striking therapeutic results were obtained by intratumoral or peritumoral application of cytokines, intratumoral implantation of cytokine-producing cells or cytokine gene transfection of cancer cells before implantation [2–6]. These modalities are rarely applicable in the clinical setting and are typically not efficacious in the case of disseminated disease. By contrast, the systemic administration of cytokines rarely induces complete cure [4,7–9]. Considerable toxicities can be observed at low doses, which prevents escalation to therapeutically active regimens [10].

It is therefore well established that cytokines can achieve curative effects for cancer treatment, but only if high concentrations of localized drug in the tumor environment are administered. Because most cytokines do not preferentially localize at the tumor site after systemic administration [11], the targeted delivery of these immunostimulatory proteins could lead to an improved therapeutic index and to a more potent therapeutic benefit with acceptable side effects. A prominent example is represented by the antibody-based targeted delivery of IL12, which generates therapeutic effects comparable to the ones observed by the cytokine alone but at a 20-fold lower administered dose [12].

Immunocytokines represent a novel class of biopharmaceuticals that have great potential for the therapy of cancer and other serious diseases [13]. These products consist of a cytokine moiety fused to monoclonal antibodies or to an antibody fragment serving as the delivery vehicle for the selective localization of the immunostimulatory payload at sites of disease.

Corresponding author:. Neri, D. (neri@pharma.ethz.ch)

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2012.01.007

Immunocytokines specific to antigens expressed on tumor blood vessels (i.e. to markers of angiogenesis) are particularly attractive for the therapy of cancer and angiogenesis-dependent diseases, because new blood vessels are rarely found in the healthy human body but are a characteristic feature of tumors and of chronic inflammatory conditions. Markers expressed on pathological blood vessels are more easily reached *in vivo* by antibody-based therapeutic agents coming from the bloodstream. Several markers of angiogenesis have been reported so far. For some of them, antibodies have been generated with proven ability to localize selectively at the tumor site following intravenous administration. The extra domain A (EDA) and extra domain B (EDB) of fibronectin and the A1 domain of tenascin-C (TnC A1) possibly represent the most extensively studied markers of angiogenesis that have been drugged with immunocytokines based on the F8, L19 and F16 human monoclonal antibodies, respectively [14,15]. These alternatively spliced domains of extracellular matrix components exhibit a broad pattern of expression in many different types of solid cancer and lymphoma, whereas their expression in normal organs is mainly confined to the female reproductive system in the proliferative phase. To our knowledge, there are no reports in the literature about immunocytokines in clinical trials that are capable of selective targeting of antigens solely expressed on tumor endothelial cells. In principle, several vascular targets could be considered, including integrins, annexin A1, nucleolin, PSMA, vascular endothelial growth factor (VEGF)-A (and its receptors), endoglin (CD105) and phosphatidyl serine [14]. Advances in transcriptomic and proteomic technologies promise to improve our knowledge of the pathological neovasculature at the molecular level and to deliver novel targets for the generation of antibody-based immunomodulatory products [16,17].

Immunocytokine formats and biodistribution data

Several recombinant antibody formats can be considered for immunocytokine development (Fig. 1). They range from single-chain variable fragment (scFv) (MW ~28 kDa) to full immunoglobulin G (IgG) (MW ~150 kDa) and can differ in terms of their valence. The IgG format has been used for immunocytokine development, despite the fact that the Fc portion of the molecule could contribute to a long circulatory half-life and to the targeting of the cytokine moiety to cells bearing Fc receptors. Our group has preferred to focus on antibody fragments in the scFv format, which can form monomers or non-covalent homodimers ('diabodies' [18]) depending on the cytokine fusion modality and the length of the linker connecting VH and VL. Diabodies are particularly suited cytokine partners for immunocytokine construction, because their bivalent nature contributes to a high binding avidity and the resulting molecular weight is larger than the renal filtration threshold, thus mediating a rapid hepatobiliary clearance mechanism. Notably, tumor-targeting diabodies exhibit favorable tumor:organ ratios at early time points following intravenous administration, compared with other antibody formats [19,20].

Several cytokines have been used for the production of fusion proteins with disease-targeting antibodies. Figure 2 summarizes the immunocytokines that have been described in preclinical studies (i.e. *in vivo* biodistribution analysis and/or therapy), depicting the molecular format used for antibody-cytokine fusion. In most cases, antibodies have been genetically fused to cytokines and expressed in mammalian cells. In earlier works, however, chemical conjugates have been reported for TNF (ZME-TNF) [21] and IFN alpha (C2-2b-2b; 20-2b) [22,23]. The majority of preclinical studies with immunocytokines have been conducted in mouse models of cancer. However, some of them (L19-IL10, F8-IL10, L19-IL2, F8-IL2, L19-IL12, L19-TNF) have also been studied in mouse models of rheumatoid arthritis, psoriasis and endometriosis.

The choice of the cytokine, the antigen recognized by the antibody and the molecular format used for immunocytokine production influence the ability of this class of biopharmaceuticals to localize selectively at sites of disease. Ideally, the disease targeting performance of an immunocytokine should be assessed by quantitative biodistribution studies with radiolabeled protein preparations in animal models and by nuclear medicine techniques in patients. Three main classes of immunocytokines can be identified based on published biodistribution data, they are:

(i) antibody-cytokine fusions that selectively localize at site of disease, with targeting performance that is largely independent of the dose used (in the mouse, this typically ranges between 1 and 100 μg). Prominent examples in this class include fusions based on IL2 or TNF [11,24].

(ii) immunocytokines where targeting performance varies as a function of the injected dose (usually exhibiting better results at higher concentrations). Prominent examples in

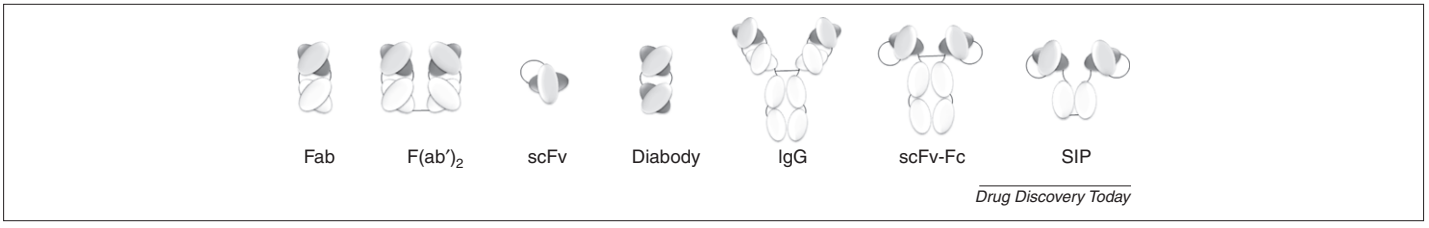

FIGURE 1

Schematic representation of seven different recombinant antibody formats, frequently used for immunocytokine development. The formats include Fab fragments (MW ~50 kDa) and the corresponding disulfide-linked homodimeric $F(ab')_2$ structure, which contains the hinge region (MW ~110 kDa). The single chain variable fragment (scFv; MW ~28 kDa) represents the smallest portion of the antibody molecule that retains the binding affinity (but not the avidity) of the parental antibody. When short peptide linkers are used between VH and VL domains, homodimeric diabody structures (MW ~55 kDa) can be generated. IgG (MW ~150 kDa). The scFv–Fc format consists of a scFv fragment fused to the Fc portion of an IgG molecule (MW ~110 kDa). A smaller version, named mini-antibody or small immune protein (SIP) uses the CH3 domain of an IgG or the εCH4 domain of an IgE antibody to mediate a stable homodimerization of scFv fragments (MW ~80 kDa).

this class include fusions based on granulocyte-macrophage colony-stimulating factor (GM-CSF) or IL7 [25,26].

(iii) fusion proteins in which the cytokine moiety abrogates the disease-targeting performance of the parental antibody. A prominent example is represented by IFNγ, which (upon fusion with the L19 antibody) targeted tumors in mice that were deficient in IFNγ receptor, but not in wild-type mice [27].

Selected preclinical therapy findings (single agent and combinations)

Figure 2 schematically summarizes key findings related to the tumor homing properties and therapeutic performance of immunocytokines in mouse models of cancer. When considering the anti-inflammatory products F8-IL10 and L19-IL10, preclinical data in mouse models of arthritis, psoriasis and endometriosis are also indicated [28–31]. In some cases (e.g. IL2) the human cytokine could be used for product development and preclinical evaluation, because the cytokine moiety cross-reacts with the murine receptor(s). In other cases (e.g. TNF and IL12) the murine cytokine should preferably be used, because of species barriers.

Anticancer activity is classified in various categories, according to the tumor growth retardation observed in animal models. Furthermore, Fig. 2 indicates whether a given fusion protein exhibited a superior therapeutic performance compared with closely related proteins used as the negative control (NC). In most cases, these comparisons were performed with the cytokine alone or with immunocytokines having the same molecular format but antibodies of irrelevant specificity in the mouse. In some cases, especially when IgGs were fused to cytokines, therapeutic performance was compared to the activity of the corresponding unconjugated naked immunoglobulin. In a few cases, tumor cells not expressing the antibody's antigen were used as the negative control. As a general trend, whenever a selective accumulation of an immunocytokine was observed at the tumor site as a result of the targeting antibody moiety used, a strong therapeutic benefit was observed compared with immunocytokines of irrelevant specificity used as negative controls. The best therapeutic results were reported for IL2, IL12, IL15 and TNF, in line with the positive tumor uptake data observed in biodistribution studies. By contrast, products based on the anti-inflammatory cytokine IL10 have so far scored best for the inhibition of arthritis and endometriosis, whereas the same products did not exhibit tumor growth retardation in the mouse.

Several studies have explored the combination of immunocytokines with other pharmacological agents, such as cytotoxic drugs [26,32–34] or intact immunoglobulins [35]. In general, immunocytokines appear to be ideal combination partners for established anticancer therapeutic agents, because they typically do not exhibit overlapping limiting toxicities with those associated with conventional chemotherapy (e.g. myelotoxicity, gastrointestinal toxicity, toxicity for kidney, liver or other organs). However, optimal combination partners must be judiciously identified based on preclinical studies that analyze the influence of dosage, schedule and administration sequence on therapeutic outcome. Most pro-inflammatory immunocytokines activate the endothelium, and the resulting vascular leakage of fluids and proteins, drop in blood pressure and fever-like symptoms are compatible with the simultaneous administration of chemotherapeutic agents. The vasoactive properties of certain cytokines (most notably IL2 and TNF) have been exploited to increase the uptake of other therapeutic agents at the tumor site [36,37].

A potentiation of antibody-dependent cell cytotoxicity (ADCC) has been observed in combination therapies with certain IgG therapeutics, because pro-inflammatory immunocytokines mediate the infiltration of many types of leukocytes into the tumor mass [34,38]. By contrast, to our knowledge, the combined use of immunocytokines with blocking antibodies (e.g. TNF or VEGF blockers) has not yet been reported.

In the recent past, the combined use of different immunocytokines began to be explored, with encouraging preclinical results. For example, the combined use of L19-IL2 and L19-TNF, or L19-IL12 and L19-TNF has led to the eradication of tumors in immunocompetent mouse models of the disease, which could not be cured by the individual immunocytokines used as single agents [24,36,39]. Moreover, the fusion of two cytokines onto the same targeting antibody (KS-IL12/IL2) could eradicate LCC-EpCAM tumors. The therapeutic activity of the novel double immunocytokine was comparable to that of the single immunocytokines (i.e. KS-IL12 and KS-IL2) injected simultaneously [40].

Immunocytokines in clinical development

Despite the large number of immunocytokines in preclinical development, only a few of them have entered clinical trials (Fig. 3). IL2 fusion proteins account for the most advanced immunocytokines in clinical development, in line with the fact that the unconjugated cytokine is routinely used for the therapy of patients with metastatic renal cell carcinoma or melanoma. The typical adverse events observed with IL2-based immunocytokines resemble the ones of recombinant IL2, including hypotension, fever, rigor, neuropathic pain, hypoxia, pruritus, allergic reactions, hypophosphatemia, thrombocytopenia, leucopenia and neutropenia.

L19-IL2 was studied in patients with metastatic renal cell carcinoma. A recommended dose could be identified (i.e. 22.5 Mio IU/patient/day – three injections/week). Toxicities were manageable and reversible. Disease stabilization could be achieved in 83% of the metastatic renal cell carcinoma patients after the second cycle (i.e. six weeks). Median progression-free survival was eight months (1.5–30.5 months) [41].

Promising results have recently been reported for the combined use of L19-IL2 with dacarbazine in metastatic melanoma. After a dose escalation study in ten patients, 22 additional patients were enrolled. Toxicities were manageable and reversible. Efficacy could be evaluated in 29 out of the 32 patients and 28% of the patients had an objective response evaluable by response evaluation criteria in solid tumors (RECIST), including one patient who experienced a complete response and was still tumor free after 21 months. In fact, 61.5% of the patients treated at the recommended dose ($n = 26$) were still alive after 12 months, the median survival of pretreated melanoma patients is approximately six to nine months. A controlled Phase IIb study (L19-IL2 plus dacarbazine versus dacarbazine alone) with 90 metastatic melanoma patients is currently in progress [42].

Disease stabilization was also achieved in 58% of melanoma patients receiving ch14.18-IL2 after the first cycle (i.e. one week).

Name	Format	Antigen	Tumor model	Targeting *in vivo*	Efficacy	Clinic	Refs
TNF alpha							
FAP-TNF		FAP	HT1080-FAP^{+} s.c.[2]	n.a.	+, > NC	no	[55]
G250-TNF		CA IX	NU-12 s.c.[2]; SK-RC17/52 s.c.[2]	NU-12: (+) DD[a]	SK-RC 17/52: ++, > NC	no	[56]
scFvMEL-TNF		gp240	A375 s.c.[2]	(+)	+++, > NC	no	[57, 58]
L19-TNF		EDB	F9 s.c.[1]; WEHI 164 s.c.[1]; C51s.c.[1]	F9: ++	F9: +, > NC ; ++, > NC *(Melph)* WHEI 164, C51: +++, > NC *(Melph)*	p. II	[24, 39]
MFE23-TNF		CEA	LS174T s.c.[2]	++	n.a.	no	[59]
TNF-TNT3		DNA	LS174T s.c.[2]	+	n.a.	no	[60]
TNF-FuP		EGFR	BLM s.c.[3,4]	(+)[3]	+, ≈ NC[4]	no	[61]
TNF-B1		LeY	MCF-7 s.c.[2]	n.a.	+++, > NC	no	[62]
ZME/TNF		gp240	A375 s.c.[2]	(+)[b]	++, > NC	no	[21]
GM-CSF							
L19-GMCSF		EDB	F9 s.c.,i.v.[1]; C51 s.c.,i.v.[1]	F9 s.c.: (+)/+ DD	F9 s.c : +, > NC F9 i.v.; C51: +	no	[25]
Anti-HER2/*neu* IgG3-GMCSF		HER2/ *neu*	CT26 s.c.[1]; CT26 HER2^{+} s.c.[1]	HER2$^{+/-}$+[c]	HER2^{+}: +, > NC	no	[63]
CLL1-GMCSF		MHC II	ARH-77[2]	+[d]	n.a.	no	[64]
Interleukin 2							
L19-IL2		EDB	F9 s.c.[1,2]; C51 s.c.[2]; N52 s.c.[2]; Ramos[3] s.c., i.v.; A20 s.c.[1], DoHH-2 s.c.[3]	F9[2]: ++[a] Ramos s.c: + A20: +	F9[1,2]: C51, N52: ++, > NC Ramos s.c., i.v.; DoHH-2: +, > NC; +++ (*Ritux.*)	p. II	[11, 35]
F16-IL2		TnC A1	MDA-MB-231 s.c[2]; U87MG s.c., orthot,[2]	MDA: ++ U87 s.c.: ++	MDA: +, > NC; ++ (*Doxo/PTX*) U87: s.c. no effect; +++ (*TMZ*) orthot. +;++ (*TMZ*)	p. Ib/II	[33, 34]
F8-IL2		EDA	Caki-1[2]	+		no	[65]
KS-IL2		EpCAM	CT26-KSA i.s.,i.v.,s.c[1]; PC-3.MM2 i.v.[3]; 4T1 s.c., i.v.;LCC s.c.[1]	CT26-KSA i.v.: +[e]	CT26-KSA: i.v, i.s. +++, > NC; s.c. ++ (*PTX*) PC-3: +++, > NC 4T1: s.c.+++ (*CP*); i.v ++ (*CP*) LCC: ++ (*CP*)	p. I	[9, 32, 66]
ch14.18-IL2		GD2	M21 i.v.[3,4], i.s.[3], s.c.[2], B16-GD2 i.v.[1], i.s.[1]; SK-N-AS i.s.[4]; NX2S[1] i.v., s.c, spon. met.	M21 s.c.: + M21 i.v[3]/i.s: +[e] B16-GD2: (+)[f] NX2S s.c.: +	M21 i.v.[4]: +++, > NC B16-GD2: +++, > NC SK-N-AS: +++, > NC NX2S i.v: +++, > NC NX2S s.m: +++, > NC NX2S s.c.: +++ (*IL2*)	p. II	[7, 8, 67-71]
ch225-IL2		EGF	M24met i.s.[4]	n.a.	+++, > NC	no	[70]
antiCD20-IL2		CD20	Daudi i.v.[3,4]	n.a.	+++, > NC	no	[72]
Anti HER2/*neu* IgG3-IL2		HER2/ *neu*	CT26-HER2[1]	n.a.	+, > NC	no	[73]
CLL1-IL2		MHC II	ARH-77[2]	++[d]	n.a.	no	[64]
IL2-FuP		EGFR	BLM s.c.[3,4]	+[3]	+[4]	no	[61]
AntiCEA-IL2		CEA	MC-38, MC-38-CEA s.c.[7]	+	++, > NC	no	[74]
FUMK1-IL2		MK1 (=EpCAM)	MKN-74 s.c.[4]	n.a.	++, > NC	no	[75]
IL2-MOV19		αFR	CT26-α FR[1] s.c., i.v.	g	s.c.: ++, > NC	no	[76]
2aG4-IL2		PS	–	n.a.	Vaccine adjuvant!	no	[77]
NHS-IL2LT		DNA	NX2S i.v.[1], LCC i.v.[1]	n.a.	IL2 is in a low toxcity form! ++, > NC	p. I	[78]

Name	Format	Antigen	Tumor model	Targeting *in vivo*	Efficacy	Clinic	Refs
Interleukin 12							
BC1-IL 12		EDB	PC3mm2[3] i.v., s.c.; A431 s.c.[3]; HT29 s.c.[3]	n.a.	PC3 i.v.: +++ PC3 s.c., HT29: +, > NC A431: +, ≈ NC	p. II	[79]
chTNT3-IL 12		DNA	LS147T s.c.[2]; DU145 s.c.[4]	LS147T: +	DU145: +, > NC	(NHS-IL12 p. I)	[80]
KS-IL12		EpCAM	DU145 i.v.[4]; CT26-EP21 i.v.[3]	n.a.	DU145: ++, >NC CT26: ++	no	[81]
KS-IL12/IL2			LCC-EpCAM s.c.[1]	n.a.	+++	no	[40]
mscIL-12 her2.IgG3		HER2/ *neu*	CT26-HER2 s.c.[1,5,6],i.v.[1]; CT26 s.c.[1]	n.a.	CT26HER2: s.c.[1],i.v[1] ++, ≈ NC; s.c[5,6] +, ≈ NC CT26: +, ≈ NC		[82, 83]
IL12-scFv(L19)		EDB	F9 s.c.[1]; C51[1] s.c., i.v.	F9: +	F9: ++, > NC; +++ (*L19-TNF*) C51: s.c. +, > NC; i.v. ++, > NC	no	[12, 36, 84]
IL12-SIP (L19)			F9 s.c.[1]	+	n.a.	no	[84]
L19p35/ p40L19			F9 s.c.[1]	++	++	no	[84]
F8p35/ p40F8		EDA	F9 s.c.[1]	++	n.a.	no	[85]
Interleukin 7							
F8-IL7		EDA	F9 s.c.[1]	–/(+) DD	+	no	[26]
F8-IL7-F8				+	+, ≈ NC	no	[26]
Interleukin 15							
L19-IL15		EDB	F9[1] s.c., i.v.; C51[1] s.c., i.v.	F9: +	F9: s.c. +, > NC; i.v. ++ C51; s.c +; i.v +	no	[25]
Interleukin 17							
F8-IL17		EDA	F9 s.c.[1]	+	no effect	no	[86]
Interleukin 18							
F8-IL18		EDA	F9 s.c.[1]	+/++DD	n.a.	no	[N.P., D.N.; unpub.]
Interleukin 10							
F8-IL10		EDA	F9 s.c.[1]	++	Rheumatoid arthritis, endometriosis	p. I	[28, 29]
F19-IL10		EDB	F9 s.c.[1]	++[h]	Rheumatoid arthritis, psoriasis	no	[30, 31]
IFN alpha							
F8-IFNα		EDA	F9 s.c.[1]; Cloudman S91 s.c.[1]	F9: ++ S91: –/+ DD	+, ≈ CN	no	[38]
CD20IgG3 IFNα		CD20	38C13-CD20 s.c.[1]; Daudi s.c.[2]	n.a.	38C13: +++, > NC Daudi: +++, > NC	no	[87]
AntiHER2/ *neu*-IFN		HER2/ *neu*	38C13-HER2+ s.c.[1]	n.a.	+++, > NC	no	[88]
C2-2b-2b		HLA-DR	Daudi i.v.[3]; CAG i.v.[3]	n.a.	Daudi: +++, > NC CAG: +++	no	[23]
20-2b		CD20	Daudi i.v.[3]; Raji i.v.[3].; NAMALWA i.v.[3]	n.a.	Daudi: +++, > NC Raji, NAMALWA: +(+), > NC	no	[22]
IFN gamma							
L19-IFNγ		EDA	F9 s.c. [1,2,8]; i.v.[1]; C51 s.c.,i.v[1]; CT26 s.c.[1]	F9 s.c.: [1,2], ++8	F9 s.c.: +, > NC F9 i.v.: ++ *(L19-IL2, Doxo)* C51; CT26 : –	no	[27]
TNT3-IFNγ		DNA	LS174T s.c.[2]; MAD109 s.c.[2]; RENCA i.v.[1]	LS174T: + MAD109: +	RENCA: ++, > NC	no	[60, 89]

Drug Discovery Today

FIGURE 2

Comprehensive summary of immunocytokines that have been tested in animal models (*in vivo* biodistribution studies and/or therapy experiments). Name, molecular format, cognate antigen and tumor model(s) used are indicated. Furthermore, targeting and therapy performance are schematically classified according to parameters indicated below. This figure also indicates if the product has been moved to clinical trials (Phase I or Phase II studies).
The animal models used in different publications (provided as References) were: 1. Immunocompetent mice; 2. Athymic mice; 3. SCID mice (lack of T and B cells); 4. SCID mice with human effector cells; 5. RAG2 KO mice; 6. SCID beige mice (lack of T, B and NK cells); 7. CEA transgenic mice; 8. IFNγ-R KO mice.

Compound	Generic name	Antibody format	Antigen	Indications	Highest phase	Organization
Interleukin 2						
L19 – IL2	Darleukin	Diabody	EDB of fibronectin	Melanoma	Phase II	Philogen
F16 – IL2	Teleukin	Diabody	A1 domain of Tenascin C	Breast cancer, lung cancer	Phase II	Philogen
KS – IL2	EMD 273066	IgG	Epithelial cell adhesion molecule	Ovarian cancer, colorectal cancer, NSCL carcinoma, prostate cancer	Phase I	Merck KGaA
Ch14.18 – IL2	EMD 273063	IgG	Ganglioside GD2	Melanoma, neuroblastoma	Phase I/II	Merck KGaA
NHS – IL2 LT	EMD 521873	IgG	DNA	Non-Hodgkin lymphoma, NSCL cancer	Phase I	Merck KGaA
Interleukin 12						
BC1 – IL12	AS1409	IgG	EDB of fibronectin	Melanoma	Phase I/II	Antisoma
NHS – IL12	MSB0010360	IgG	DNA/histone complexes	Epithelial and mesenchymal malignant tumors	Phase I	Merck KGaA
TNF alpha						
L19 –TNF	Fibromun	Trimeric scFv	EDB of fibronectin	Melanoma (isolated limb perfusion)	Phase I/II	Philogen
Interleukin 10						
F8 – IL10	Dekavil	Diabody	EDA of fibronectin	Arthritis	Phase I	Philogen

Drug Discovery Today

FIGURE 3

Immunocytokines in clinical development. Immunocytokines based on IL2, IL12, TNFα and IL10 are currently being investigated in clinical trials. Splice-isoforms of fibronectin and tenascin-C represent the most frequently used target antigens for cytokine delivery (i.e. five out of nine immunocytokines currently in clinical trials).

However, only 24% of patients did not have any progression after the second cycle (i.e. six weeks). Phase II studies revealed that only two out of nine melanoma patients receiving ch14.18-IL2 showed disease stabilization. No objective responses were observed [43,44].

In children with neuroblastoma treated with ch14.18-IL2 a recommended dose could be identified (i.e. 110 Mio IU/m^2 given over three days). Stable disease was achieved in 54% of the patients after two or more cycles (i.e. at least six weeks). In a subsequent Phase II trial it was found that 22% of the patients having a disease evaluable only by metaiodobenzylguanidine (MIBG) scintigraphy and/or bone marrow histology (i.e. low tumor load) enjoyed a complete response lasting at least nine months up to more than 35 months. Unfortunately, ch14.18-IL2 did not have any impact on bulky disease [45,46]. Additionally, a second immunocytokine (KS-IL2; Fig. 2) has been studied in Phase I clinical trials [47].

In the case of non-targeted IL2, a variety of regimens have been used in the clinic, ranging from low-dose regimens at 75 Mio IU/week [48] to the intense regimens at 900 Mio IU/week (in young patients and in intensive care units) [49]. By contrast, IL2-based immunocytokines have so far been administered in the 67.5–110 Mio IU IL2 equivalents/week range [41,46], because companies have preferred to avoid the intensive care unit and develop a product broadly available to cancer patients. L19-IL2 and ch14.18-IL2 are administered as long infusions (1 h, 4 h) and have similar pharmacokinetics, with a terminal half-life of about three hours [41,44]; whereas KS-IL2 appears to have a prolonged half-life (4–6.7 h) [47]. When Proleukin® is administered as a short infusion (5 min), it displays a shorter half-life ($t_{1/2}$ distribution = 13 - min, elimination = 85 min). However, when the same product is administered by subcutaneous injection, its terminal half-life is approximately five hours [50].

Two IL12 fusion proteins are also currently being evaluated in clinical trials. Although NHS-IL12 is still in Phase I, results for a Phase I study with BC1-IL12 in melanoma and renal cell carcinoma patients have recently been reported. A recommended dose could be identified and two out of 13 patients experienced a partial response lasting seven and 17 months before disease progression (i.e. 15 μg/kg weekly) [51].

Because therapeutic performance appears to correlate with tumor localization efficiency, it is desirable to use nuclear medicine techniques [e.g. positron emission tomography (PET)] for the tomographic assessment of the tumor targeting properties of immunocytokine products in individual patients. Immuno-PET methodologies can be facilitated by the recent availability of

Targeting performance *in vivo* was classified on the basis of tumor:organ ratios observed for the majority of tested organs at 24 h: − [<1]; (+) [between 1 and 2]; + [between 2 and 10]; ++ [>10]. Whenever experimental data did not enable this classification, results were presented on the basis of: **(a)** tumor:blood ratio at 24 h; **(b)** tumor:blood ratio at 72 h; **(c)** $HER2^+$ tumor:$HER2^-$ tumor ratio at 12 h, tumor:blood ratio at 12 h; **(d)** tumor:organs ratio at 72 h; **(e)** metastatic organs:healthy organs ratio at 24 h; **(f)** metastatic organs:healthy organs ratio at 12 h; **(g)** accumulation in lung CT26-αFR positive tumor; **(h)** targeting performance assessed in psoriatic mice [+] and arthritic mice (based on autoradiographic signals). DD indicates dose dependence.
Therapy efficacy was defined as growth retardation (GR) compared with the saline group: − [no GR]; + [GR <50%]; ++ [GR >50%]; +++ [cure]. Performance of the immunocytokine compared with negative control [NC, untargeted cytokine (fused to an irrelevant antibody or alone), naked antibody, tumors not expressing the antigen] was also assessed. >NC (superior performance of immunocytokine), ≈NC (comparable performance of immunocytokine and NC).
Abbreviations: IL17, interleukin 17; IL18, interleukin 18; FAP, fibroblast-activating protein; CA IX, carbonic anhydrase IX; CEA, carcinoembryonic antigen; EGFR, epidermal growth factor receptor; HER2/neu, human epidermal growth factor receptor 2; MHC II, major histocompatibility complex II; EpCAM, epithelial cell adhesion molecule; EGF, epidermal growth factor; αFR, alpha folate receptor; PS, phosphatidylserine; HLA-DR, human leukocyte antigen DR; i.v., intravenous; s.c., subcutaneous; i.s., intrasplenic; DD, dose dependence; n.a., not assessed.

clinical-grade iodine-124, a PET radionuclide with a half-life of four days, which matches the antibody residence at the tumor site [52]. Furthermore, microscopic analysis of biopsy samples can facilitate the assessment of targeting microheterogeneity and of leukocyte infiltration into the tumor mass. From a logistical point of view, however, it is still not easy to incorporate immuno-PET procedures and analysis of biopsies into the execution of clinical trials.

The individual response to treatment might depend not only on immunocytokine localization at the tumor site but also on the immunological status of the patient. Most studies have so far reported the effect of the therapeutic immunocytokine on peripheral blood lymphocytes [41]. However, the recent discovery that cancer cells release peptides bound to soluble HLA-I molecules and that hundreds of these peptides can be identified by affinity capture and mass spectrometric analysis [53] indicates avenues for the non-invasive assessment of tumor-antigens that are displayed at the tumor site.

Challenges in the translation of preclinical data into clinical results

The use of preclinical models in cancer research has certain important limitations. The use of genetically identical cancer cell lines and of inbred mice reduces the variation that is usually observed in human patients. Immunotherapeutic strategies based on cytokines should ideally be investigated in immunocompetent mice. In many publications, however, xenografted tumors are implanted in athymic mice or SCID mice injected with human effector cells. Finally, tumors often grow in mice more rapidly than in humans, and the function of cytokines in mice might be different from the function of the homologous cytokine in human.

Immunogenicity issues could also complicate the preclinical analysis of immunocytokines. Most antibody-based biopharmaceuticals used are based on human antibodies, which frequently generate an immunogenic reaction in animals. This response could give rise to hypersensitivity reactions, neutralize the immunocytokine or alter its pharmacokinetic behavior.

Concluding remarks

This review surveys the preclinical and clinical progress made over the past decade in the design, cloning, expression and characterization of therapeutic immunocytokines. These products might be able to localize at sites of disease, thus increasing the therapeutic index of the corresponding cytokine. Although pro-inflammatory cytokines are typically used for cancer therapy applications, the antibody-based delivery of anti-inflammatory cytokines represents a promising strategy for the treatment of chronic diseases such as rheumatoid arthritis and endometriosis. When used alone, immunocytokines rarely exhibit complete cures for cancer in animal models and in patients. However, combination therapies have resulted in complete and long-lasting tumor eradications that cannot be achieved by conventional chemotherapy. It is still largely unknown why some patients respond to therapy and other patients do not. Similarly, different therapeutic outcomes are sometimes observed in different mouse models of cancer. Interestingly, the contribution of different classes of leukocytes can vary as a function of the tumor model used. For example, for some tumors the efficacy of IL2-based therapeutics is largely based on natural killer (NK) cells and is independent of the contribution from T cells [11,54], whereas for other cancer types a crucial role of T cells could be demonstrated [7]. Interestingly, the combined use of certain chemotherapeutic regimens with pro-inflammatory cytokines might lead to complete cancer eradication and to the protection against future challenges with homologous and heterologous tumors, pointing at a tumor vaccination effect [39].

We anticipate that immunocytokines will find an increasing use in the clinical setting, not only for the therapy of cancer but also to fight chronic inflammatory processes and, potentially, other disease types (e.g. infectious disease and cardiovascular conditions). Progress in this field will crucially rely on the identification of accessible good-quality markers of pathology, on the engineering of suitable protein formats and on the judicious choice of suitable cytokines and combination partners.

Conflict of interest

DN is founder and shareholder of Philogen, a biotech company that owns the F8, L19 and F16 antibodies.

Acknowledgements

Financial contributions from the Swiss National Science Foundation, the ETH Zürich, the European Union (ADAMANT Project), the Swiss Cancer League, the Swiss-Bridge Foundation and the Stammbach Foundation are gratefully acknowledged.

References

1 Walsh, G. (2010) Biopharmaceutical benchmarks 2010. *Nat. Biotechnol.* 28, 917–924
2 Aoki, T. *et al.* (1992) Expression of murine interleukin 7 in a murine glioma cell line results in reduced tumorigenicity *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.* 89, 3850–3854
3 Barker, S.E. *et al.* (2007) Immunotherapy for neuroblastoma using syngeneic fibroblasts transfected with IL-2 and IL-12. *Br. J. Cancer* 97, 210–217
4 Jackaman, C. *et al.* (2003) IL-2 intratumoral immunotherapy enhances CD8+ T cells that mediate destruction of tumor cells and tumor-associated vasculature: a novel mechanism for IL-2. *J. Immunol.* 171, 5051–5063
5 Koshita, Y. *et al.* (1995) Efficacy of TNF-alpha gene-transduced tumor cells in treatment of established *in vivo* tumor. *Int. J. Cancer* 63, 130–135
6 Miller, P.W. *et al.* (2000) Intratumoral administration of adenoviral interleukin 7 gene-modified dendritic cells augments specific antitumor immunity and achieves tumor eradication. *Hum. Gene Ther.* 11, 53–65
7 Becker, J.C. *et al.* (1996) T cell-mediated eradication of murine metastatic melanoma induced by targeted interleukin 2 therapy. *J. Exp. Med.* 183, 2361–2366
8 Becker, J.C. *et al.* (1996) Eradication of human hepatic and pulmonary melanoma metastases in SCID mice by antibody-interleukin 2 fusion proteins. *Proc. Natl. Acad. Sci. U.S.A.* 93, 2702–2707
9 Xiang, R. *et al.* (1997) Elimination of established murine colon carcinoma metastases by antibody-interleukin 2 fusion protein therapy. *Cancer Res.* 57, 4948–4955
10 Leonard, J.P. *et al.* (1997) Effects of single-dose interleukin-12 exposure on interleukin-12-associated toxicity and interferon-gamma production. *Blood* 90, 2541–2548
11 Carnemolla, B. *et al.* (2002) Enhancement of the antitumor properties of interleukin-2 by its targeted delivery to the tumor blood vessel extracellular matrix. *Blood* 99, 1659–1665
12 Halin, C. *et al.* (2002) Enhancement of the antitumor activity of interleukin-12 by targeted delivery to neovasculature. *Nat. Biotechnol.* 20, 264–269
13 Schrama, D. *et al.* (2006) Antibody targeted drugs as cancer therapeutics. *Nat. Rev. Drug Discov.* 5, 147–159

14 Neri, D. and Bicknell, R. (2005) Tumour vascular targeting. *Nat. Rev. Cancer* 5, 436–446
15 Neri, D. and Supuran, C.T. (2011) Interfering with pH regulation in tumours as a therapeutic strategy. *Nat. Rev. Drug Discov.* 10, 767–777
16 Borgia, B. *et al.* (2010) A proteomic approach for the identification of vascular markers of liver metastasis. *Cancer Res.* 70, 309–318
17 Schliemann, C. *et al.* (2010) In vivo biotinylation of the vasculature in B-cell lymphoma identifies BST-2 as a target for antibody-based therapy. *Blood* 115, 736–744
18 Holliger, P. *et al.* (1993) 'Diabodies': small bivalent and bispecific antibody fragments. *Proc. Natl. Acad. Sci. U.S.A.* 90, 6444–6448
19 Borsi, L. *et al.* (2002) Selective targeting of tumoral vasculature: comparison of different formats of an antibody (L19) to the ED-B domain of fibronectin. *Int. J. Cancer* 102, 75–85
20 Wu, A.M. *et al.* (1996) Tumor localization of anti-CEA single-chain Fvs: improved targeting by non-covalent dimers. *Immunotechnology* 2, 21–36
21 Rosenblum, M.G. *et al.* (1995) An antimelanoma immunotoxin containing recombinant human tumor necrosis factor: tissue disposition, pharmacokinetic, and therapeutic studies in xenograft models. *Cancer Immunol. Immunother.* 40, 322–328
22 Rossi, E.A. *et al.* (2009) CD20-targeted tetrameric interferon-alpha, a novel and potent immunocytokine for the therapy of B-cell lymphomas. *Blood* 114, 3864–3871
23 Rossi, E.A. *et al.* (2011) Preclinical studies on targeted delivery of multiple IFNalpha2b to HLA-DR in diverse hematologic cancers. *Blood* 118, 1877–1884
24 Borsi, L. *et al.* (2003) Selective targeted delivery of TNFalpha to tumor blood vessels. *Blood* 102, 4384–4392
25 Kaspar, M. *et al.* (2007) The antibody-mediated targeted delivery of interleukin-15 and GM-CSF to the tumor neovasculature inhibits tumor growth and metastasis. *Cancer Res.* 67, 4940–4948
26 Pasche, N. *et al.* (2011) Cloning and characterization of novel tumor-targeting immunocytokines based on murine IL7. *J. Biotechnol.* 154, 84–92
27 Ebbinghaus, C. *et al.* (2005) Engineered vascular-targeting antibody-interferon-gamma fusion protein for cancer therapy. *Int. J. Cancer* 116, 304–313
28 Schwager, K. *et al.* (2011) The antibody-mediated targeted delivery of interleukin-10 inhibits endometriosis in a syngeneic mouse model. *Hum. Reprod.* 26, 2344–2352
29 Schwager, K. *et al.* (2009) Preclinical characterization of DEKAVIL (F8-IL10), a novel clinical-stage immunocytokine which inhibits the progression of collagen-induced arthritis. *Arthritis Res. Ther.* 11, R142
30 Trachsel, E. *et al.* (2007) Antibody-mediated delivery of IL-10 inhibits the progression of established collagen-induced arthritis. *Arthritis Res. Ther.* 9, R9
31 Trachsel, E. *et al.* (2007) A human mAb specific to oncofetal fibronectin selectively targets chronic skin inflammation *in vivo*. *J. Invest. Dermatol.* 127, 881–886
32 Holden, S.A. *et al.* (2001) Augmentation of antitumor activity of an antibody-interleukin 2 immunocytokine with chemotherapeutic agents. *Clin. Cancer Res.* 7, 2862–2869
33 Marlind, J. *et al.* (2008) Antibody-mediated delivery of interleukin-2 to the stroma of breast cancer strongly enhances the potency of chemotherapy. *Clin. Cancer Res.* 14, 6515–6524
34 Pedretti, M. *et al.* (2010) Combination of temozolomide with immunocytokine F16-IL2 for the treatment of glioblastoma. *Br. J. Cancer* 103, 827–836
35 Schliemann, C. *et al.* (2009) Complete eradication of human B-cell lymphoma xenografts using rituximab in combination with the immunocytokine L19-IL2. *Blood* 113, 2275–2283
36 Halin, C. *et al.* (2003) Synergistic therapeutic effects of a tumor targeting antibody fragment, fused to interleukin 12 and to tumor necrosis factor alpha. *Cancer Res.* 63, 3202–3210
37 Hornick, J.L. *et al.* (1999) Pretreatment with a monoclonal antibody/interleukin-2 fusion protein directed against DNA enhances the delivery of therapeutic molecules to solid tumors. *Clin. Cancer Res.* 5, 51–60
38 Frey, K. *et al.* (2011) Antibody-based targeting of interferon-alpha to the tumor neovasculature: a critical evaluation. *Integr. Biol. (Camb)* 3, 468–478
39 Balza, E. *et al.* (2006) Targeted delivery of tumor necrosis factor-alpha to tumor vessels induces a therapeutic T cell-mediated immune response that protects the host against syngeneic tumors of different histologic origin. *Clin. Cancer Res.* 12, 2575–2582
40 Gillies, S.D. *et al.* (2002) Bi-functional cytokine fusion proteins for gene therapy and antibody-targeted treatment of cancer. *Cancer Immunol. Immunother.* 51, 449–460
41 Johannsen, M. *et al.* (2010) The tumour-targeting human L19-IL2 immunocytokine: preclinical safety studies, phase I clinical trial in patients with solid tumours and expansion into patients with advanced renal cell carcinoma. *Eur. J. Cancer.* 46, 2926–2935
42 Eigentler, T.K. *et al.* (2011) A dose-escalation and signal-generating study of the immunocytokine L19-IL2 in combination with dacarbazine for the therapy of patients with metastatic melanoma. *Clin. Cancer Res.* 17, 7732–7742
43 King, D.M. *et al.* (2004) Phase I clinical trial of the immunocytokine EMD 273063 in melanoma patients. *J. Clin. Oncol.* 22, 4463–4473
44 Ribas, A. *et al.* (2009) Phase I/II open-label study of the biologic effects of the interleukin-2 immunocytokine EMD 273063 (hu14.18-IL2) in patients with metastatic malignant melanoma. *J. Transl. Med.* 7, 68
45 Osenga, K.L. *et al.* (2006) A phase I clinical trial of the hu14.18-IL2 (EMD 273063) as a treatment for children with refractory or recurrent neuroblastoma and melanoma: a study of the Children's Oncology Group. *Clin. Cancer Res.* 12, 1750–1759
46 Shusterman, S. *et al.* (2010) Antitumor activity of hu14.18-IL2 in patients with relapsed/refractory neuroblastoma: a Children's Oncology Group (COG) phase II study. *J. Clin. Oncol.* 28, 4969–4975
47 Ko, Y.J. *et al.* (2004) Safety, pharmacokinetics, and biological pharmacodynamics of the immunocytokine EMD 273066 (huKS-IL2): results of a phase I trial in patients with prostate cancer. *J. Immunother.* 27, 232–239
48 Yang, J.C. *et al.* (2003) Randomized study of high-dose and low-dose interleukin-2 in patients with metastatic renal cancer. *J. Clin. Oncol.* 21, 3127–3132
49 Rosenberg, S.A. *et al.* (1998) Durability of complete responses in patients with metastatic cancer treated with high-dose interleukin-2: identification of the antigens mediating response. *Ann. Surg.* 228, 307–319
50 Kirchner, G.I. *et al.* (1998) Pharmacokinetics of recombinant human interleukin-2 in advanced renal cell carcinoma patients following subcutaneous application. *Br. J. Clin. Pharmacol.* 46, 5–10
51 Rudman, S.M. *et al.* (2011) A phase 1 study of AS1409, a novel antibody-cytokine fusion protein, in patients with malignant melanoma or renal cell carcinoma. *Clin. Cancer Res.* 17, 1998–2005
52 Tijink, B.M. *et al.* (2009) (124)I-L19-SIP for immuno-PET imaging of tumour vasculature and guidance of (131)I-L19-SIP radioimmunotherapy. *Eur. J. Nucl. Med. Mol. Imaging* 36, 1235–1244
53 Bassani-Sternberg, M. *et al.* (2010) Soluble plasma HLA peptidome as a potential source for cancer biomarkers. *Proc. Natl. Acad. Sci. U.S.A.* 107, 18769–18776
54 Lode, H.N. *et al.* (1998) Natural killer cell-mediated eradication of neuroblastoma metastases to bone marrow by targeted interleukin-2 therapy. *Blood* 91, 1706–1715
55 Bauer, S. *et al.* (2004) Targeted bioactivity of membrane-anchored TNF by an antibody-derived TNF fusion protein. *J. Immunol.* 172, 3930–3939
56 Bauer, S. *et al.* (2009) Targeted therapy of renal cell carcinoma: synergistic activity of cG250-TNF and IFNg. *Int. J. Cancer* 125, 115–123
57 Liu, Y. *et al.* (2004) Recombinant single-chain antibody fusion construct targeting human melanoma cells and containing tumor necrosis factor. *Int. J. Cancer* 108, 549–557
58 Liu, Y. *et al.* (2006) The antimelanoma immunocytokine scFvMEL/TNF shows reduced toxicity and potent antitumor activity against human tumor xenografts. *Neoplasia* 8, 384–393
59 Cooke, S.P. *et al.* (2002) In vivo tumor delivery of a recombinant single chain Fv::tumor necrosis factor-alpha fusion [correction of factor: a fusion] protein. *Bioconjug. Chem.* 13, 7–15
60 Sharifi, J. *et al.* (2002) Generation of human interferon gamma and tumor necrosis factor alpha chimeric TNT-3 fusion proteins. *Hybrid Hybridomics* 21, 421–432
61 Christ, O. *et al.* (2001) Efficacy of local versus systemic application of antibody-cytokine fusion proteins in tumor therapy. *Clin. Cancer Res.* 7, 985–998
62 Scherf, U. *et al.* (1996) Cytotoxic and antitumor activity of a recombinant tumor necrosis factor-B1(Fv) fusion protein on LeY antigen-expressing human cancer cells. *Clin. Cancer Res.* 2, 1523–1531
63 Dela Cruz, J.S. *et al.* (2000) Recombinant anti-human HER2/neu IgG3-(GM-CSF) fusion protein retains antigen specificity and cytokine function and demonstrates antitumor activity. *J. Immunol.* 165, 5112–5121
64 Hornick, J.L. *et al.* (1997) Chimeric CLL-1 antibody fusion proteins containing granulocyte-macrophage colony-stimulating factor or interleukin-2 with specificity for B-cell malignancies exhibit enhanced effector functions while retaining tumor targeting properties. *Blood* 89, 4437–4447
65 Frey, K. *et al.* (2010) The immunocytokine F8-IL2 improves the therapeutic performance of sunitinib in a mouse model of renal cell carcinoma. *J. Urol.* 184, 2540–2548
66 Dolman, C.S. *et al.* (1998) Suppression of human prostate carcinoma metastases in severe combined immunodeficient mice by interleukin 2 immunocytokine therapy. *Clin. Cancer Res.* 4, 2551–2557
67 Lode, H.N. *et al.* (1997) Targeted interleukin-2 therapy for spontaneous neuroblastoma metastases to bone marrow. *J. Natl. Cancer Inst.* 89, 1586–1594
68 Sabzevari, H. *et al.* (1994) A recombinant antibody-interleukin 2 fusion protein suppresses growth of hepatic human neuroblastoma metastases in severe combined immunodeficiency mice. *Proc. Natl. Acad. Sci. U.S.A.* 91, 9626–9630

69 Neal, Z.C. *et al.* (2004) Enhanced activity of hu14.18-IL2 immunocytokine against murine NXS2 neuroblastoma when combined with interleukin 2 therapy. *Clin. Cancer Res.* 10, 4839–4847

70 Becker, J.C. *et al.* (1996) Long-lived and transferable tumor immunity in mice after targeted interleukin-2 therapy. *J. Clin. Invest.* 98, 2801–2804

71 Pancook, J.D. *et al.* (1996) Eradication of established hepatic human neuroblastoma metastases in mice with severe combined immunodeficiency by antibody-targeted interleukin-2. *Cancer Immunol. Immunother.* 42, 88–92

72 Gillies, S.D. *et al.* (2005) An anti-CD20-IL-2 immunocytokine is highly efficacious in a SCID mouse model of established human B lymphoma. *Blood* 105, 3972–3978

73 Penichet, M.L. *et al.* (2001) A recombinant IgG3-(IL-2) fusion protein for the treatment of human HER2/neu expressing tumors. *Hum. Antibodies* 10, 43–49

74 Xu, X. *et al.* (2000) Targeting and therapy of carcinoembryonic antigen-expressing tumors in transgenic mice with an antibody-interleukin 2 fusion protein. *Cancer Res.* 60, 4475–4484

75 Matsumoto, H. *et al.* (2002) Targeting of interleukin-2 to human MK-1-expressing carcinoma by fusion with a single-chain Fv of anti-MK-1 antibody. *Anticancer Res.* 22, 2001–2007

76 Melani, C. *et al.* (1998) Targeting of interleukin 2 to human ovarian carcinoma by fusion with a single-chain Fv of antifolate receptor antibody. *Cancer Res.* 58, 4146–4154

77 Huang, X. *et al.* (2011) Enhancing the potency of a whole-cell breast cancer vaccine in mice with an antibody-IL-2 immunocytokine that targets exposed phosphatidylserine. *Vaccine* 29, 4785–4793

78 Gillies, S.D. *et al.* (2011) A low-toxicity IL-2-based immunocytokine retains antitumor activity despite its high degree of IL-2 receptor selectivity. *Clin. Cancer Res.* 17, 3673–3685

79 Lo, K.M. *et al.* (2007) huBC1-IL12, an immunocytokine which targets EDB-containing oncofetal fibronectin in tumors and tumor vasculature, shows potent anti-tumor activity in human tumor models. *Cancer Immunol. Immunother.* 56, 447–457

80 Li, J. *et al.* (2004) chTNT-3/hu IL-12 fusion protein for the immunotherapy of experimental solid tumors. *Hybrid Hybridomics* 23, 1–10

81 Gillies, S.D. *et al.* (1998) Antibody-IL-12 fusion proteins are effective in SCID mouse models of prostate and colon carcinoma metastases. *J. Immunol.* 160, 6195–6203

82 Peng, L.S. *et al.* (1999) A single-chain IL-12 IgG3 antibody fusion protein retains antibody specificity and IL-12 bioactivity and demonstrates antitumor activity. *J. Immunol.* 163, 250–258

83 Peng, L.S. *et al.* (2001) Mechanism of antitumor activity of a single-chain interleukin-12 IgG3 antibody fusion protein (mscIL-12.her2.IgG3). *J. Interferon Cytokine Res.* 21, 709–720

84 Gafner, V. *et al.* (2006) An engineered antibody-interleukin-12 fusion protein with enhanced tumor vascular targeting properties. *Int. J. Cancer* 119, 2205–2212

85 Sommavilla, R. *et al.* (2010) Expression, engineering and characterization of the tumor-targeting heterodimeric immunocytokine F8-IL12. *Protein Eng. Des. Sel.* 23, 653–661

86 Pasche, N. *et al.* (2011) The targeted delivery of IL17 to the mouse neo-vasculature enhances angiogenesis but does not reduce tumor growth rate. *Angiogenesis* (Epub ahead of print)

87 Xuan, C. *et al.* (2010) Targeted delivery of interferon-alpha via fusion to anti-CD20 results in potent antitumor activity against B-cell lymphoma. *Blood* 115, 2864–2871

88 Huang, T.H. *et al.* (2007) Targeting IFN-alpha to B cell lymphoma by a tumor-specific antibody elicits potent antitumor activities. *J. Immunol.* 179, 6881–6888

89 Mizokami, M.M. *et al.* (2003) Chimeric TNT-3 antibody/murine interferon-gamma fusion protein for the immunotherapy of solid malignancies. *Hybrid Hybridomics* 22, 197–207