

Review

Immunocytokines

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Abstract: A number of cytokines have shown beneficial effects in preclinical animal models of cancer and chronic inflammatory diseases. However, cytokine treatment is often associated with severe side effects, which prevent the administration of clinically relevant doses in humans. Immunocytokines are a novel class of biopharmaceuticals, consisting of a cytokine moiety fused to monoclonal antibodies or to an antibody fragment, which selectively accumulate at the disease site and thereby enhance the therapeutic effects of cytokines. This review surveys the recent preclinical and clinical advances in the field, with a special focus on the impact of antibody formats, target antigen and cytokine moieties on the therapeutic performance *in vivo*. We also discuss emerging data about the possibility to combine immunocytokines with other pharmacological agents.

Keywords: immunotherapy; immunocytokines; cytokines; antibodies; antibody formats; cancer; chronic inflammatory disease

1. Introduction

A number of cytokines have shown beneficial effects in preclinical animal models of cancer and immune disorders and represent promising agents for therapy. However, despite encouraging results, only few cytokines are approved as drugs (e.g., interleukin 2 (IL2, Proleukin®), tumor necrosis factor (TNF, Beromun®), interferon alpha (IFN α , Roferon A® and Intron A®)). Current indications in cancer include metastatic renal cell cancer, malignant melanoma, hairy cell leukemia, chronic myeloid lymphoma, sarcoma and multiple myeloma, either as single agents or in combination with

chemotherapy. In addition, certain cytokines are used for the treatment of viral and bacterial infections in the clinic and are administered to patients suffering from chronic inflammatory conditions.

Unfortunately, considerable toxicities can be observed at low doses, which prevent escalation to therapeutically active regimens. For this reason the systemic administration of cytokines rarely induces complete cure, making the further clinical development of cytokines for therapeutic approaches difficult. Alternative delivery pathways have been successfully exploited, leading to complete tumor eradication in preclinical cancer models. The most promising results were obtained by intratumoral or peritumoral application of cytokines and gene therapy approaches, including intratumoral implantation of cytokine-producing cells or cytokine gene transfection of cancer cells before implantation [1–5]. However, these approaches are rarely applicable in the clinical setting and are typically not efficacious in the case of disseminated disease. Nonetheless, these findings do show that cytokines can promote disease eradication, if the drug is delivered to the disease environment at a sufficient concentration.

One way to achieve specific localization of cytokines at the disease site after systemic administration consists in the use of antibodies as delivery vehicles, leading to a new class of biopharmaceutical agents, termed “immunocytokines”. These products consist of a cytokine moiety fused to monoclonal antibodies or to an antibody fragment, capable of mediating a preferential localization of the immunostimulatory payload at the site of disease. Immunocytokines have been evaluated for applications in cancer and chronic inflammatory disease [6,7]. Their therapeutic potency depends on molecular format, target antigen and cytokine fusion, as well as their combinatory function with other pharmacological agents.

2. Molecular Formats

Monoclonal antibodies bind target proteins with high affinity and selectivity and therefore represent ideal mediators of targeted therapeutic approaches for applications in cancer and immune diseases.

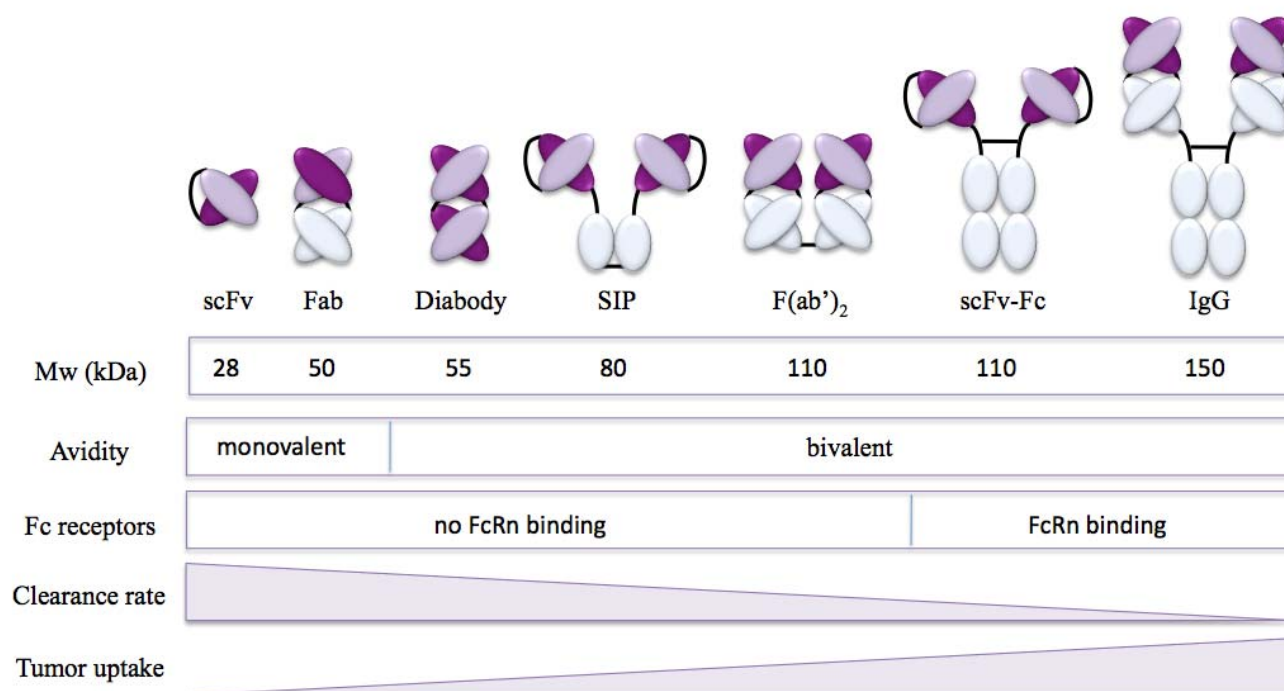
The full size IgG antibody is the most often used format for therapeutic development. However, protein engineering allows the generation of various antibody fragments with specific targeting properties defined by molecular size, blood clearance and tissue penetration and retention [8]. A number of these formats have been explored for the development of immunocytokines (Figure 1).

2.1. Targeting Properties of Antibody Formats

Full size IgG antibodies (Mw ~ 150 kDa) are Y-shaped multidomain proteins consisting of two heavy (H) chains and two light (L) chains, each of which contain a constant (C) and variable (V) region. Antigen binding is mediated in a bivalent fashion by V_H and V_L domains located on the two Fab ($V_H V_L C_H1 C_L$) arms of the antibody. The remaining constant domains ($C_H2 C_H3$) form the Fc region, which recruits effector functions. The bivalent nature of antibodies contributes to high affinity binding of the antigen and provides long retention times at the target site. IgGs typically display a long serum half-life ($t_{1/2}$) of several days, which is mainly mediated by interaction of the Fc region with neonatal Fc receptors (FcRn) [9,10]. Binding to FcRn allows the transport of IgGs within and across cells and salvages them from default lysosomal degradation [11]. The role of this interaction in mediating prolonged antibody half-life is underlined by the finding, that IgGs with lower binding affinity to FcRn are more rapidly cleared. A number of genetic modifications as well as isotype-specific Fc properties

have been explored to modify FcRn binding and serum half-life of IgG antibodies [12,13]. Tissue penetration of IgGs has been found to be slow and heterogeneous. In particular in solid tumors, which display elevated interstitial pressure, the large IgG molecule shows limited extravasation and diffusion capacity [14].

Figure 1. Antibody formats used for the development of immunocytokines and their targeting properties.

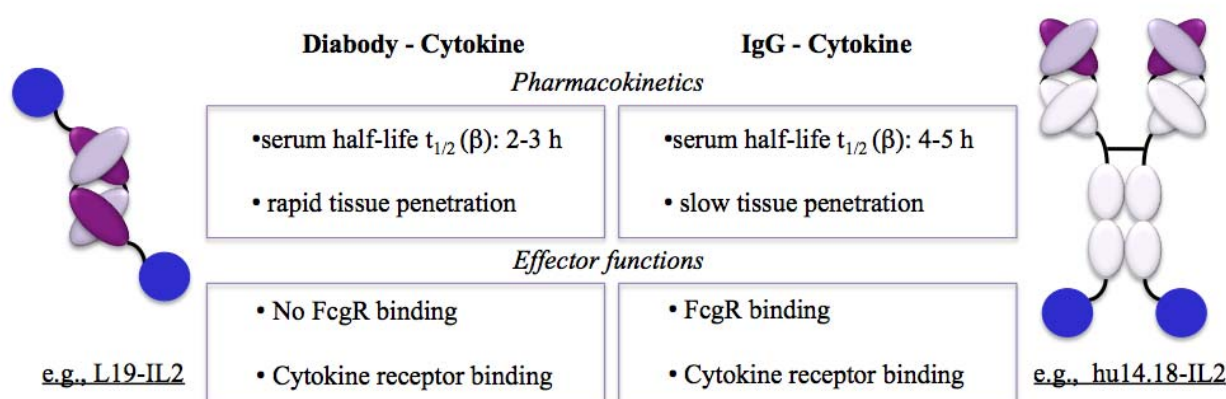


To the other extreme, single-chain Fvs (scFvs; Mw ~ 28 kDa) represent the smallest format, which retains the antigen-binding affinity, but not avidity, of the parental antibody. They consist of a variable V_H and a variable V_L domain fused by a flexible polypeptide linker. The low molecular weight of scFvs has shown to improve tissue penetration compared to IgGs [15,16]. However, rapid clearance of the small fragments combined with reduced retention property due to the monovalent nature of the antigen interaction leads to low absolute accumulation (8–10 fold lower than IgG) at the target site [17].

A number of multivalent antibody formats (*i.e.*, diabody, SIP, scFvFc) of intermediate size have been engineered to profit from longer target-site retention than scFvs, as well as deeper tissue penetration compared to full size IgGs [8,18]. For example, reducing the linker length of the scFv to 5 residues or less, promotes the formation of stable scFv homodimers, called diabodies (Mw ~ 55k Da) [19]. This bivalent format displays high tumor-to-blood ratios with an increased absolute tumor accumulation compared to scFv of the same antigen specificity.

2.2. Targeting Properties of Immunocytokines

The clinically most advanced immunocytokines are recombinant fusion proteins in which the antibody moiety is used in the full IgG or in diabody format (Figure 2).

Figure 2. Comparison of the antibody formats of clinical stage immunocytokines.

Immunocytokines have been reported to display a significantly reduced serum half-life compared to their corresponding naked antibody [20]. The IgG-IL2 fusion protein hu14.18-IL2, for example, displayed a serum half-life of ~4 h in melanoma and neuroblastoma patients [21]. In comparison, the serum half-life of the diabody L19 was 2–3 h when fused to IL2 [22]. In either case this represents a significant decrease of blood clearance of IL2 alone ($t_{1/2} \sim 30\text{--}45$ min) [20].

In some cases the cytokine moiety of immunocytokines can significantly interfere with targeting. One example is the immunocytokine L19-IFN γ , which targeted tumors in mice lacking IFN γ receptors, but not in wild-type mice [23].

Enrichment of cytokines at the target site is mediated by the antibody-antigen interaction. Recruiting effector functions is crucial for the therapeutic effect of immunocytokines and is mediated by binding of the cytokine moiety to the correspondent cytokine receptors on effector cells. For example, binding of the IL2 moiety of immunocytokines to the IL2 receptor stimulates the expansion and activation of immune cells such as cytotoxic T cells and natural killer (NK) cells. Newly generated antibody fusion proteins are validated for functional cytokine signaling.

In addition, immunocytokines can activate antigen dependent cellular cytotoxicity (ADCC). The Fc region of IgG-based fusion proteins mediates ADCC by binding Fc γ receptors (Fc γ R) on the surface of immune effector cells, such as NK cells. IL2 immunocytokines have shown that IL2 enhances the ADCC function of antibodies [7,24,25]. Recent findings describe the formation of activating immune synapses (AIS) between FcR-deficient NK cells and tumor cells mediated by IL2 immunocytokines. These functional AIS were formed by the interaction of the IL2 component of the immunocytokine and IL2 receptors on effector cells [26,27], demonstrating that IL2 immunocytokines can initiate direct target cell lysis even without Fc involvement.

3. Targets

Antibody-based delivery of cytokines to the site of disease requires the presence of accessible, abundant and stable target antigens, which allow a clear discrimination between diseased tissue and

healthy organs. Potential targets can be expressed either directly on the surface of cancer cells or in the disease environment. Both of these targeting strategies have been exploited for the development of immunocytokines. The targets of the most advanced immunocytokines are described in Table 1.

Table 1. Targets of the most advanced immunocytokines.

Antigen	Expression profile	Antibody
Disease cell surface		
EpCAM <i>Epithelial cell adhesion molecule</i>	Physiologically expressed in epithelia Over-expressed in breast, colorectal and pancreatic carcinomas.	KS
GD2 <i>Disialoganglioside</i>	Physiological expression restricted to cerebellum and peripheral nerves. Expressed in neuroectodermal tumors including melanoma, neuroblastoma.	Ch14.18
Disease environment		
EDA / EDB of fibronectin <i>Extra-domains EDA and EDB of fibronectin</i>	Physiological expression restricted to endometrium in the proliferating phase. Expressed around the neo-vasculature and in the stroma of aggressive tumours and in chronic inflammation.	F8 / L19 and BC1
A1 domain of Tenascin-C	Physiological expression restricted to endometrium in the proliferating phase. Expressed around the neo-vasculature and in the stroma of aggressive tumours and in chronic inflammation.	F16
DNA / Histone complexes	Normally not accessible. Exposed in necrotic tissue.	NHS

3.1. Targeting the Cell Surface

Epithelial cell adhesion molecule (EpCAM) is a well-described cell surface antigen for a broad range of carcinomas, such as breast, colorectal and pancreatic carcinomas. EpCAM overexpression is considered to be a marker of tumor initiating cells and is involved in cancer metastasis. However, the selectivity of EpCAM is limited by its expression in healthy epithelia [28,29].

GD2 disialoganglioside is expressed on the cell surface of neuroectodermal tumors, such as melanoma and neuroblastoma and soft tissue sarcomas [21,30,31]. In the healthy body, GD2 expression is restricted to neurons and peripheral pain fibers, and thereby displays a high degree of selectivity [32].

3.2. Targeting the Disease Environment

Progression of cancer or immune diseases involves the formation of a specific disease environment, which involves tissue remodeling, angiogenesis and the recruitment of stromal and immune cells [33,34]. Disease environments provide a number of well accessible target structures.

Newly formed blood vessels are particularly attractive targets for antibody-mediated delivery approaches. Vascular antigens are readily reached via the blood stream and angiogenesis is involved in the disease progression of most cancers as well as numerous inflammatory diseases [35]. Our group has extensively studied the *in vivo* performance of vascular targeting antibodies [36]. Splice isoforms of fibronectin and of tenascin-C represent some of the best-characterized markers of newly formed blood vessels [37]. Specifically, the alternatively-spliced extra-domains EDA and EDB of fibronectin, as well as the extra-domain A1 of tenascin-C, are virtually undetectable in normal adult tissues, but are strongly expressed at sites of physiological and pathogenic angiogenesis. The human monoclonal antibodies F8, L19 and F16 specifically recognize the EDA and EDB domains of fibronectin and the A1 domain of tenascin-C, respectively, and have shown selective accumulation at the disease site in a broad range of cancers and inflammatory disorders in animal models as well as in patients [38–41].

An alternative approach, termed target necrosis therapy (TNT), targets necrotic tissue, which is present primarily in the hypoxic environment of solid tumors and is absent in the healthy body. The monoclonal antibody NHS76 recognizes DNA / histone complexes, which have been demonstrated to be exposed by necrotic cells in the tumor core as well as metastases [42,43].

3.3. Target Identification

The identification of novel disease markers is of great importance for the development of targeted therapies. This process is driven mainly by comparative transcriptomic [44] and proteomic technologies, which have rapidly advanced in the past years [44–46]. For example, *in vivo* labeling followed by proteomic analysis has led to the identification a number of vascular accessible antigens [47,48], some of which are expressed in a broad range of diseases [39,49], while others are more restricted to specific malignancies [50].

4. Fusion Partners

Cytokines are a diverse group of immune-modulators, which can have pro-inflammatory or anti-inflammatory activity, depending on the microenvironment. Under normal conditions pro- and anti-inflammatory stimuli are carefully balanced to ensure an adequate immune response. This balance is disturbed in pathological settings, such as cancer progression and inflammatory disorders [51,52]. Understanding the functions of cytokines as well as the immunological environment of pathologies is important when choosing antibody fusion partners for the generation of immunocytokines as therapeutic agents. A number of cytokines have been tested for the generation of immunocytokines. A conclusive list of preclinical findings can be found in [6]. In this review we will focus on the clinical stage antibody–cytokine fusions, which are listed in Table 2.

4.1. Cytokines in Cancer

Tumor formation and progression is a complex process involving a multitude of changes within both cancer cells and the body's defense mechanisms. The tumor microenvironment harbors cytokines and other inflammatory mediators, which influence immunosurveillance, the growth of cancer cells, tissue remodeling and angiogenesis [33]. Anti-inflammatory factors, mainly IL10 and TGFβ, interfere

with the cell-mediated immune response against cancer cells and promote tumor development. However, pro-inflammatory signals and chronic inflammation also play a crucial role in tumor growth and progression [51,53].

The development of immunocytokines for cancer therapies has so far focused on the delivery of pro-inflammatory cytokines, which mediate the infiltration of leukocytes into the tumor mass and promote an antitumor immune response. The most promising results were reported for fusion proteins with interleukin 2 (IL2), interleukin 12 (IL12) and tumor necrosis factor (TNF).

Table 2. Immunocytokines in clinical development.

Compound	Antibody format	Target antigen	Indications	Clinical phase	Company
Pro-inflammatory					
F16-IL2	Diabody	A1 domain of tenascin-C	Breast cancer and lung cancer	Phase II	Philogen
L19-IL2	Diabody	EDB of fibronectin	Melanoma	Phase II	Philogen
hu14.18-IL2	IgG	GD2	Melanoma and neuroblastoma	Phase I/II	Merck KGaA
KS-IL2	IgG	EpCAM	Ovarian cancer, colorectal cancer, prostate cancer and NSCL carcinoma	Phase I	Merck KGaA
NHS-IL2LT	IgG	DNA/ histone	Non-Hodgkin lymphoma and NSCL carcinoma	Phase I	Merck KGaA
NHS-IL12	IgG	DNA/ histone	Epithelial and mesenchymal malignant tumors	Phase I	Merck KGaA
BC1-IL12	IgG	EDB of fibronectin	Melanoma	Phase I/II	Antisoma
L19-TNF	Trimeric scFv	EDB of fibronectin	Melanoma (isolated limb perfusion)	Phase I/II	Philogen
Anti-inflammatory					
F8-IL10	Diabody	EDA of fibronectin	Rheumatoid arthritis	Phase I	Philogen

4.1.1. IL2-Based Immunocytokines

Recombinant IL2 (rIL2, Proleukin®) is commonly used in the clinic for the treatment of patients with metastatic renal cell carcinoma and melanoma [54,55]. For this reason, IL2 fusion proteins account for the most advanced immunocytokines in clinical development. There are currently five IL2 based immunocytokines in clinical trials.

L19-IL2 successfully targeted the neo-vasculature of several tumors in preclinical mouse models, as determined by quantitative biodistribution studies and therapy experiments [56,57]. Clinical trials in patients with metastatic renal carcinoma resulted in disease stabilization in 83% of the patients after the two cycles of the recommended dose (*i.e.*, 22.5 Mio IU/patient/day—3 injections/week), promoting a median progression-free survival of 8 months (1.5–30.5 months) [22].

F16-IL2, a second vascular targeting antibody-IL2 fusion protein, is currently being tested in a phase II trial in patients with breast or lung cancer [58].

Ch14.18-IL2, which recognizes GD2, was clinically tested for applications in melanoma and neuroblastoma. Disease stabilization was achieved in 58% of melanoma patients after the first cycle (*i.e.*, one week) of ch14.18-IL2 administration in a phase I trial. However, after the second cycle (*i.e.*, six weeks) only 24% of the patients still displayed stable disease. The maximal tolerated dose was determined to be 7.5 mg/m²/d. In line with the results from the phase I trial, only 2 out of 9 patients reached disease stabilization after treatment with 4–6 cycles (at 4 mg/m²/d) of ch14.18-IL2 in phase II studies. No objective responses were observed [59,60]. In children with neuroblastoma, the recommended dose of ch14.18-IL2 was determined (110 Mio IU/m² over 3 days) and its administration led to disease stabilization in 54% of the patients in a phase I trial. In a subsequent phase II trial 21.7% of patients with low tumor load (*i.e.*, disease evaluable only by (¹²³I)metaiodobenzylguanidine scintigraphy or bone marrow histology) experienced a complete response, whereas patients with bulky disease unfortunately did not respond [61,62].

Two additional immunocytokines, the EpCAM targeting KS-IL2 and the DNA targeting NHS-IL2LT, have been tested in phase I clinical trials [63,64].

In general, the adverse side effects caused by IL2 immunocytokines are comparable to the ones reported for IL2 alone, including hypotension, fever, rigor, neuropathic pain, hypoxia, pruritus, allergic reactions, hypophosphatemia, thrombocytopenia, leucopenia and neutropenia. IL2 based immunocytokines have so far been administered at 67.5–110 Mio IU IL2 equivalents/m² per week for 4–6 treatment cycles (one cycle: 21–28 days) [22,62]. In comparison, rIL2 is applied from 100 Mio IU/m² per week for at least 6 cycles as a low dose regimen to 900 Mio IU/ week as an intense regimen [65,66]. The reduction in dose, which can be achieved by selective delivery of IL2 to the site of disease helps reduce the incidence and severity of side effects.

4.1.2. IL12- and TNF-Based Immunocytokines

IL12 plays an important role in the activities of natural killer cells and T lymphocytes. The antitumor activity of recombinant IL12 (rIL12) has been demonstrated in several animal models and further evaluated in a number of clinical trials in humans. However, the systemic administration of

1–1.5 µg/week of rIL12 was associated with significant toxicities, which prevented further clinical development of rIL12 as a single agent for cancer therapy [67].

Preclinical findings show that the antibody-based targeted delivery of IL12 mediated the infiltration of macrophages, lymphocyte-activated killer (LAK) cells, NK cells, and T lymphocytes into the tumor mass and resulted in an increase of anti-angiogenic stimuli. IL12-based immunocytokines can greatly improve the effects of IL12, allowing therapeutic benefits at (up to 20-fold) lower administered doses [68].

Two IL12 based immunocytokines have progressed to clinical trials.

The immunocytokine NHS-IL12, which targets DNA / histone complexes in necrotic regions of tumors, is currently being evaluated in a phase I trial [69].

The antibody BC1-IL12 recognizes the neo-vascular antigen EDB of fibronectin and was tested in a phase I trial in patients with malignant melanoma or renal cell carcinoma. The recommended dose was set at 15 µg/kg weekly (corresponds to a 3–5 fold molar increase compared to rIL12) and treatment promoted disease stabilization in 2 out of 13 patients [70].

TNF is one of the most potent antitumor cytokines. However, its substantial toxic side effects prevent systemic administration at therapeutically effective doses. The clinical use of TNF has therefore been limited to regional applications, such as Isolated Limb Perfusion for the treatment of nonresectable sarcoma and melanoma [71]. The immunocytokine L19-TNF is being evaluated in a phase I/II clinical trial [72,73].

4.2. Cytokines in Chronic Inflammatory Diseases

Chronic inflammatory diseases, such as rheumatoid arthritis, psoriasis, endometriosis, atherosclerosis or inflammatory bowel disease, are characterized by persistent inflammation mediated by complex interactions between immune cells and the disease microenvironment. These commonly both activate the immune system and interfere with pathways involved in the resolution of inflammation [34,52].

Immunocytokines based on the anti-inflammatory cytokine interleukin 10 (IL10) have been tested for applications in rheumatoid arthritis, psoriasis and endometriosis.

4.2.1. IL10 Immunocytokines

Recombinant IL10 (rIL10, TenovilTM) demonstrated promising *in vitro* immunomodulatory activity as well as therapeutic benefit in a number of animal models of chronic inflammation. And clinical evaluation in patients with inflammatory disorders showed an excellent tolerability profile at doses up to 25 µg/kg. However, the clinical development of rIL10 was discontinued due to lack of efficacy [74,75]. IL10-based immunocytokines targeting the neo-vasculature (F8-IL10 and L19-IL10) were tested in preclinical models of rheumatoid arthritis, psoriasis and endometriosis with encouraging outcomes. F8-IL10 has progressed to the clinic and is currently being tested in a phase I trial in patients with rheumatoid arthritis [76–78].

5. Combination Studies

5.1. Selected Preclinical Combination Therapies

A number of preclinical studies have been performed to explore the combinatorial effect of immunocytokines with other therapeutic agents, such as chemotherapeutics, monoclonal antibodies and other biologicals.

Most chemotherapeutics that are used for cancer therapy are traditionally considered to be immunosuppressive by killing or inhibiting the progression of dividing cells, including lymphocytes. But accumulating evidence indicates that cytotoxic drugs and radiotherapy also indirectly and directly effect the immune system to contribute to tumor regression [79].

For example, preclinical combination experiments with F8-IL2 [80] and KS-IL2 [81] showed that paclitaxel, when administered prior to the immunocytokine, enhances the antibody-targeted delivery of IL2 to the disease site and promotes a synergistic anti-cancer effect in different cancer models. Encouraging results were also observed for the combination of F16-IL2 and temozolomide in an orthotopic glioblastoma mouse model, demonstrating the effective delivery of cytokines to a pathological disorder in the brain [82]. In this case simultaneous administration of the immunocytokine and the chemotherapeutic agent led to complete eradication of lymphoma xenografts [57].

Radiofrequency ablation (RFA) followed by KS-IL2 treatment enhanced the antitumor effect and survival, compared to mice treated with RFA or KS-IL2 alone, in a murine colon adenocarcinoma model [83].

Some antibody–cytokine fusions (in particular IL2- and TNF-based immunocytokines) display vasoactive properties and can therefore also enhance the uptake of therapeutic agents at disease site, if administered as a pretreatment [84]. In general, immunocytokines and conventional chemotherapeutic agents are promising combination partners because they typically do not exhibit overlapping limiting toxicities. But the ideal combination partners, dose regimen and also administration sequence have to be elucidated for different disease settings.

The combined use of therapeutic antibodies and cytokines has been shown to potentiate the ADCC, due to an increase of effector cells [85,86]. And in fact, a synergistic therapeutic effect was observed for F8-IL2 and the monoclonal antibody sunitinib in mouse models of renal cell carcinomas [87]. A comparable effect was described for the combination of L19-IL2 and rituximab in a mouse model of B-cell lymphoma [57].

In addition, the combination of different immunocytokines, such as L19-IL12 and L19-TNF, demonstrated enhanced therapeutic benefit compared to the administration of each immunocytokine alone [88]. In accordance with these findings, bifunctional antibody cytokine fusion proteins (KS-IL2/IL12) displayed synergistic effects *in vivo* [89].

5.2. Combination Therapies in Clinical Development

Some clinical results of combination therapies with immunocytokines and chemotherapeutic agents have recently been reported. The combination of KS-IL2 with cyclophosphamide and the combination of F16-IL2 with doxorubicin or with paclitaxel are being studied in phase Ib clinical trials in patients with solid tumors [90,91]. And the combined use of L19-IL2 (at the same dose applied as

monotherapy) with dacarbazine showed an excellent tolerability profile and led to objective responses in 8/29 patients and a complete response in one patient in a phase Ib trial in patients with metastatic melanoma. This combination therapy is currently being evaluated in a randomized phase II trial [92].

6. Concluding Remarks

This review surveys the recent preclinical and clinical advances in the field of immunocytokines, with a special focus on the impact of molecular formats, target antigen, cytokine moieties and combination approaches on therapeutic performance.

Several antibody formats and their specific targeting properties have been explored during the development of immunocytokines. The clinically most advanced immunocytokines are recombinant fusion proteins in which the antibody moiety is in the full IgG or in the diabody format.

The identification of selective antigens is a prerequisite for the antibody-based delivery of cytokines to the site of disease. A number of potential targets can be considered, including those located on the surface of diseased cells (e.g., cancer cells) or in the modified extracellular matrix. The targeting of newly formed pathological blood vessels is particularly attractive and has found application in a wide range of malignancies, but also for the treatment of chronic inflammatory diseases.

Preclinical and clinical experiments with antibody–cytokine fusion proteins have demonstrated that increased accumulation at the disease site enhances the therapeutic index of the corresponding cytokine [6]. The development of immunocytokines for cancer therapies has focused on the delivery of pro-inflammatory cytokines (IL2, IL12 and TNF), whereas anti-inflammatory cytokines (IL10) are used for the treatment of chronic diseases, such as rheumatoid arthritis.

The translation of preclinical findings to clinical settings, however, remains one of the main challenges for the future.

When used as single agent, immunocytokines are rarely curative. For this reason, a number of combination therapies with other pharmacological agents have been tested. The combined use of immunocytokines and chemotherapeutics for cancer therapy has been extensively studied and is currently being investigated in clinical trials.

The large diversity in disease characteristics as well as immunological state of patients may have a significant impact on the function of immunocytokines. To address this issue imaging techniques (e.g., nuclear medicine techniques like positron emission tomography) could in principle be used to screen patients for antigen expression and selective targeting of the antibody prior to treatment [93]. In addition, the recent discovery that cancer cells release peptides bound to soluble HLA-I molecules, which can be identified and quantified by mass spectrometry, indicates potential avenues for non-invasive assessment of tumor-antigens that are displayed at the tumor site [94].

Taken together, progress in antibody engineering, identification of accessible and selective markers of disease, use of different cytokine moieties and the combination with other therapeutics has driven the development of immunocytokine therapies. We anticipate that immunocytokines will find an increasing use in clinical settings, in particular for the treatment of cancers and chronic inflammatory diseases.

Conflict of Interest

Dario Neri is a co-founder and shareholder of Philogen (www.philogen.com), the company that owns the F8, L19 and F16 antibodies.

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