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Immunocytokines for cancer treatment: past, present and future

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Abstract

Immunocytokines are antibody-cytokine fusion proteins, with the potential to preferentially localize on tumor lesions and to activate anticancer immunity at the site of disease. Various tumor targets (e.g., cell membrane antigens and extracellular matrix components) and antibody formats (e.g., intact IgG and antibody fragments) have been considered for immunocytokine development and some products have advanced to clinical trials. In this review, we present relevant concepts and strategies for the design and use of anticancer immunocytokine products. In addition, we discuss emerging strategies for the pharmaceutical development and clinical application of this promising class of biopharmaceuticals.

From cytokines to immunocytokines

Cytokines are immunomodulatory proteins, which can activate or inhibit the activity of the immune system, depending on their properties and concentration, as well as the environment in which they operate. Some pro-inflammatory cytokines [e.g., interleukin-2 (IL2), tumor necrosis factor (TNF), interferon-alpha] have received marketing authorization for the treatment of certain types of malignancies. However, the systemic administration of these agents is often associated with dose-dependent side-effects (e.g., hypotension, flu-like symptoms, nausea, capillary leak), which prevent dose escalation to therapeutically active regimens. In principle, the fusion of cytokines to suitable antibody molecules, specific to tumor-associated antigens and capable of selective localization at the tumor site, should increase the therapeutic index of the bioactive payload.

Immunocytokine formats

The first experimental implementations of the immunocytokine concept relied on the use of antibodies in intact IgG format. At a later stage, antibody fragments (e.g., scFv fragments

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Conflicts of Interest

D.N. is co-founder, shareholder and board member of Philogen (www.philogen.com), a Swiss-Italian biotech company which develops novel immunocytokine products.

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and diabodies) were also considered for product development. While many cytokine payloads have been considered and have been tested at the preclinical level, most clinical activities are based on IL2, TNF and the heterodimeric interleukin-12 (IL12) cytokine. Both the antibody format and properties of the target antigen can have a profound impact on the performance and mechanism of action of immunocytokine products. Detailed reviews are available, which cover historical aspects related to development of anticancer immunocytokines [1–6].

Some of the most popular immunocytokine formats for IL2, IL12 and TNF are shown in Figure 1. Cytokine moieties can be genetically fused to various sites on IgG molecules (e.g., at the N- or at the C-terminus). If the immunoglobulin moiety of an immunocytokine has not been intentionaly altered (normally through specific mutations at judiciously chosen sites), it should retain its functional properties, such as binding to Fc gamma receptors, to the complement cascade-initiating factor C1q, the pH-dependent interaction with the neonatal FcRn receptor, as well as antigen binding activity. In addition, the cytokine component of the immunocytokine should also preserve an intact cytokine activity. Interestingly, the positioning of the cytokine moiety and the use of suitable linkers may facilitate the development of products, with adaptable immunostimulatory activity upon antigen binding [7].

Immunocytokines based on antibody fragments have a simpler functional activity, as they only consist of two moieties (i.e., the cytokine and the antigen binding portion of the antibody). In contrast to full IgG-based immunocytokines (~180 kDa), these smaller products (45-130 kDa) are rapidly cleared from circulation. However, favorable tumor:organ ratios have been reported for fragment-based immunocytokine products in various quantitative biodistribution studies performed in mouse models of cancer [e.g., 4, 8–10 and references therein]. Interestingly, certain payloads (e.g., IL2, IL4, IL6, IL9, IL10, IL12, interferon-alpha, TNF] can be efficiently delivered to the tumor by suitable antibodies, while other payloads (e.g., those with heavy glycosylation or with extreme isoelectric points) have been shown to abrogate the tumor targeting potential of the parental antibody [9–13]. Interestingly, payloads with abundant receptors distributed *in vivo* (e.g., interferon-gamma) can be adsorbed by these receptors when infused *in vivo* at low doses, thereby inhibiting targeting of the immunocytokine to the tumor. However, adequate tumor-targeting performance is recovered with higher dosing, once the cognate receptors have been saturated [14] or when interferon-gamma receptor knock-out mice are used [15].

Mechanism of action

Immunocytokine products, which are specific to tumor-associated antigens on the cell membrane, have the potential to bridge tumor cells and certain leukocytes (e.g., T cells, or NK cells), in analogy to what could be achieved by using bispecific antibodies [16–19; Figure 2]. By contrast, immunocytokines which target tumor-associated extracellular matrix components (e.g., splice isoforms of fibronectin or of tenascin-C) [4], are believed to mainly display a biological activity which results from the high-density anchoring of the cytokine moiety at the site of disease. Analysis of tumor infiltrate following pro-inflammatory immunocytokine treatment in mouse models of cancer and in patients has clearly revealed an

increased density of leukocytes (including T cells and NK cells) in the neoplastic mass [20–24]. When created using an antibody that targets an antigen on the tumor cell membrane, bispecific antibodies and immunocytokine products could be considered as functionally similar tools for the selective activation of leukocytes at the tumor site. The type of leukocytes activated would be determined by the other antibody component of the bispecific antibody or the cytokine payload of the immunocytokine. For example both can potentially redirect the killing of tumor cells by cytotoxic T lymphocytes without MHC restriction. However, antigen loss by tumor cells may represent an escape strategy for the neoplasm from mAbs that recognize the surface antigen, and thus from bispecific antibodies or immunocytokines that use them. By contrast, immunocytokines that are created with antibodies that recognize tumor-related antigens that are not on the tumor cells themselves (such as extracellular matrix molecules or released necrotic nuclear components) are less affected by loss of antigen expression by individual tumor cells and can still simultaneously activate various types of leukocytes (e.g., T cells and NK cells).

Immunocytokines can activate various components of the immune system against neoplastic cells. The specific characteristics largely depend on the cytokine moiety. For example, IL2-based immunocytokines can potently activate both T cells and NK cells. It is widely assumed that $CD8^+$ T cells, directed against tumor-associated antigens or mutated peptides, may be present in cancer patients, sometimes with reduced activity due to a variety of suppressive mechanisms (e.g., action of regulatory T cells within the neoplastic mass). In addition, NK cells may recognize stress antigens (e.g., MIC-A) or other NK activating moieties on the surface of cancer cells, triggering the release of cytotoxic granuli. Changes in cytokine concentration at the tumor site may help boost these pre-existing components of the immune response against neoplasms. Interestingly, some cytokine payloads (e.g., TNF) have the ability to convert tumor masses into black scabs, which may eventually disappear without leaving a scar, revealing (both macroscopically and microscopically) a biological activity on the tumor neo-vasculature [25]. By contrast, other cytokine moieties may reduce tumor size without apparent macroscopic changes to the appearance of neoplastic lesions.

Combination strategies and clinical development programs

Many types of combination regimens have been evaluated for anti-tumor immunocytokines at the preclinical level. Combination partners included drugs [e.g., 25–27], intact antibodies [e.g., 28], radiation [e.g., 29,30] and other immunocytokine products [e.g., 23, 31–34]. The incorporation of two immunocytokine moieties into the same product has been proposed [35], but, depending upon configuration, may result in the abrogation of the tumor targeting properties of the parental immunocytokine products [31].

Table 1 reports a list of immunocytokine products, which have entered clinical trials (alone or in combination with other agents) for the treatment of patients with various types of malignancies. The products feature IL2, IL12 or TNF as pro-inflammatory moieties. Some (e.g., Hu14.18-IL2, NHS-IL2LT, anti-CEA-IL2v, BC1-IL12, DI-Leu16-IL2) are based on intact IgG formats, some (e.g., L19-IL2, F16-IL2, L19-TNF) on antibody fragments.

Preclinical data with non-mutated IL2 linked to intact IgG molecules have demonstrated far more potent antitumor efficacy in tumor-bearing mice than comparable amounts of the antibody and IL2 given simultaneously as separate molecules [36], even though these immunocytokines have a substantially shorter *in vivo* half-life (after intravenous injection) than the intact mAb. For example, DI-Leu16-IL2 (an anti-CD20 immunocytokine) was far more effective against human CD20+ lymphoma cells in immunodeficient mice than 25 fold greater doses of anti-CD20 mAb plus IL2 [37]. In the initial trial of DI-leu16-IL2, for B-cell lymphoma patients that relapsed following prior anti-CD20 therapy, 5 of 13 evaluable patients showed CR (3 patients) or PR (2 patients) [38]. Preclinical data with the hu14.18-IL2 (anti-GD2) immunocytokine showed that effects were most apparent against microscopic, rather than large tumors [39]. When tested in a phase-II study for children with relapsed/refractory neuroblastoma, no responses were seen in 13 children with bulky (radiologically evident) disease, but 5 children out of 23 with evaluable (but not bulky) disease (detected only by sensitive ^{123}I-MIBG nuclear scintigraphy, or by identification of tumor cells in bone marrow aspirates) showed CR [40], consistent with the preclinical data [39].

Two IL2-based immunocytokines (L19-IL2 and F16-IL2), featuring antibody fragments in non-covalent diabody formats, have been tested in Phase I and Phase II clinical trials. The L19 antibody is specific to the alternatively-spliced EDB domain of fibronectin, a marker of tumor angiogenesis, while F16 recognizes the A1 domain of tenascin-C. L19-IL2 has been used as monotherapeutic agent for the treatment of metastatic renal cell carcinoma [41] and in combination with dacarbazine for the treatment of metastatic melanoma [42]. The product is currently being studied in combination with rituximab for the last-line treatment of patients with diffuse large B-cell lymphoma [28]. F16-IL2 has been used in combination with doxorubicin or paclitaxel for the treatment of various types of malignancies [43], or in combination with low-dose cytarabine for the treatment of acute myeloid leukemia [27; 44].

L19-TNF is a non-covalent homotrimeric immunocytokine, resulting from the fusion of human TNF with the L19 antibody in scFv format. The product has been administered systemically to patients with various types of malignancies [45], as well as in isolated limb perfusion procedures to patients with in-transit melanoma metastases [46]. Following the observation of cancer cures and the induction of protective immunity in immunocompetent mouse models of soft-tissue sarcoma (STS), L19-TNF is currently being investigated in combination with doxorubicin for the treatment of STS.

Interestingly, the IL2 and TNF moieties have been found to potently synergize, when the corresponding immunocytokines were administered systemically [47] or intralesionally [34] to tumor-bearing mice. L19-IL2 has been given intralesionally to patients with Stage III melanoma, with encouraging results [48]. The therapeutic activity was potentiated by the combination with L19-TNF [49] and a neo-adjuvant Phase III clinical trial has recently started.

Preclinically, the intratumoral injection of the hu14.18-IL2 anti-GD2 immunocytokine (versus intravenous administration), induces greater NK and T cell infiltration into tumors, has a more potent anti-tumor effect and depends on the activity of both NK and T cells [50].

When combined with local measures of tumor destruction [either radiofrequency ablation (RFA) or external beam radiation therapy] to the same tumor receiving intratumoral immunocytokine, the local action against the treated tumor is more potent; a tumor-specific T cell response is induced that shows epitope spread to antigens other than GD2 on the tumor and antitumor activity is seen against distant disease [51,52]. In this setting the intratumoral immunocytokine combined with the RFA or radiotherapy is enabling the injected tumor to function as an effective *in situ* vaccine (treating locally in order to act systemically).

Outlook

While immunocytokine products are advancing in clinical trials, future research activities will be needed in order to more precisely characterize their mechanism of action. Progress in HLA-peptidome analysis [53] and in the generation of multiplex tetramer reagents [54] has made it possible, for the first time, to characterize the binding specificity of individual T-cells before and after immunotherapy. These investigations should provide a better understanding of the nature and role of tumor rejection antigens, as well as information on the dynamics and breadth of T cell specificities in response to therapy [55]. In contrast to the study of T cells, for which analytical tools are available, the mechanistic investigation of NK cells is more difficult. However, since this cell type potently contributes to therapeutic action of immunocytokines, as demonstrated by depletion experiments in preclinical models and by Killer Immunoglobulin-like Receptor (KIR) genotype analyses of clinical data [56], a more detailed understanding of the key molecular events responsible for the anti-tumoral activity of activated NK cells would be desirable.

Conclusions

In summary, immunocytokines represent a promising class of activators of the immune system, with the potential to be used alone or in combination with other therapeutic modalities. Proinflammatory immunocytokines are not myelotoxic and can be potentially combined with a number of therapeutic modalities, including other immunotherapies as well as more conventional treatments. More research is needed, in order to identify which cytokine payloads should be preferred for individual cancer indications and which pharmaceutical agents would benefit most from immunocytokine combinations. Finally, while this article focuses exclusively on oncology applications, it should be mentioned that certain immunocytokine products have shown promising therapeutic activity for the treatment of chronic inflammatory conditions [e.g., 57–60] and of endometriosis [61,62] in immunocompetent preclinical models.

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* = of special interest

** = of outstanding interest

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Highlights

- Certain immunocytokines can modulate the activity of leukocytes at the tumor site
- They mediate the influx of T cells and NK cells into the tumor mass
- Various anticancer immunocytokines are being studied in clinical trials
- Intratumoral administration is being considered for neo-adjuvant purposes

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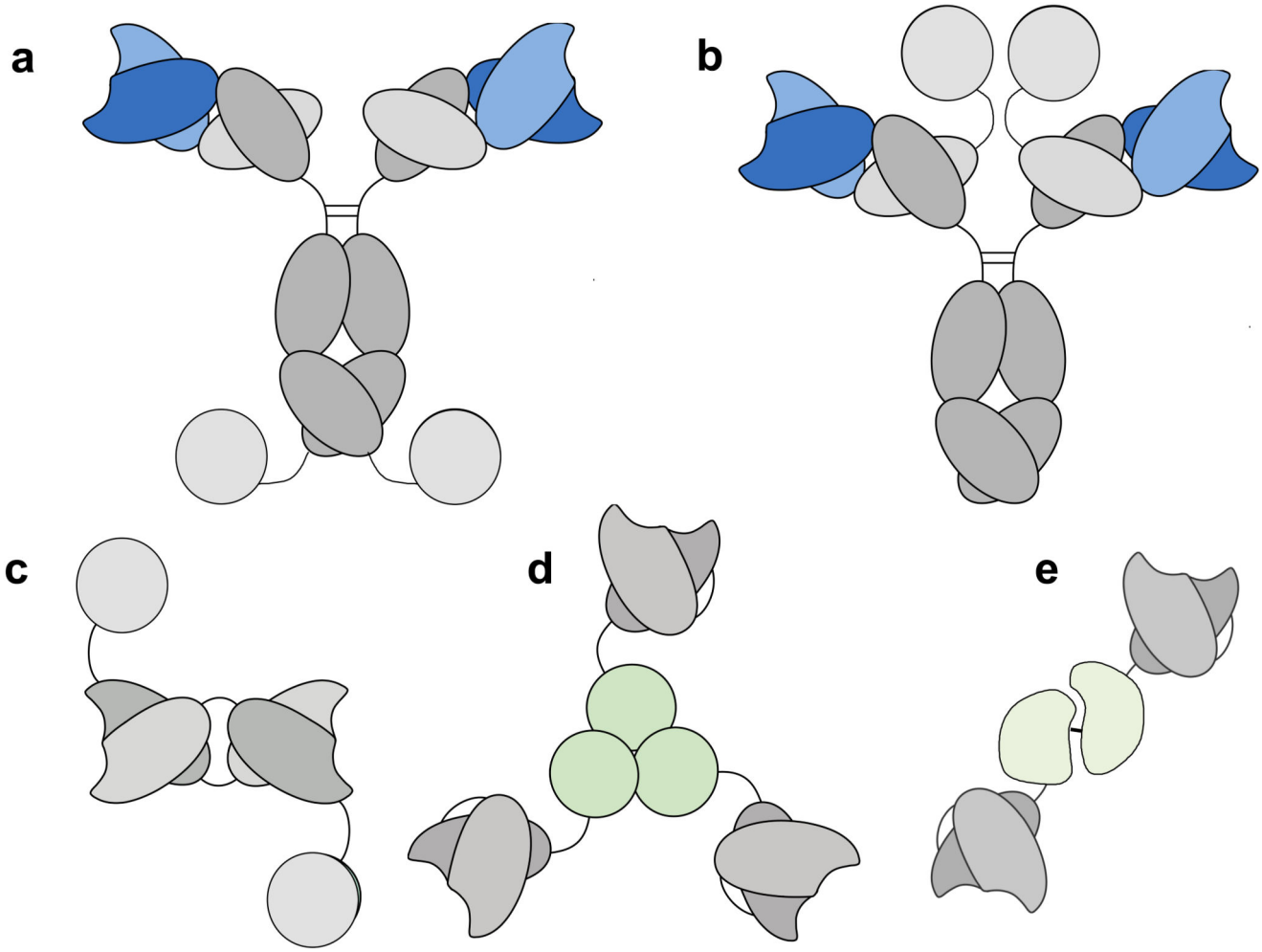

Figure 1.

Schematic representation of various antibody formats, used for the production of immunocytokines based on IL2, IL12 or TNF. (a and b): IgG- based immunocytokines with the IL2 moiety at the C-terminal end of heavy and light chain, respectively; (c) diabody-IL2 fusion protein format; (d) homotrimeric scFv-TNF fusion proteins; (e) heterodimeric immunocytokines, in which a scFv moiety is fused to both p40 and 35 subunits of IL12.

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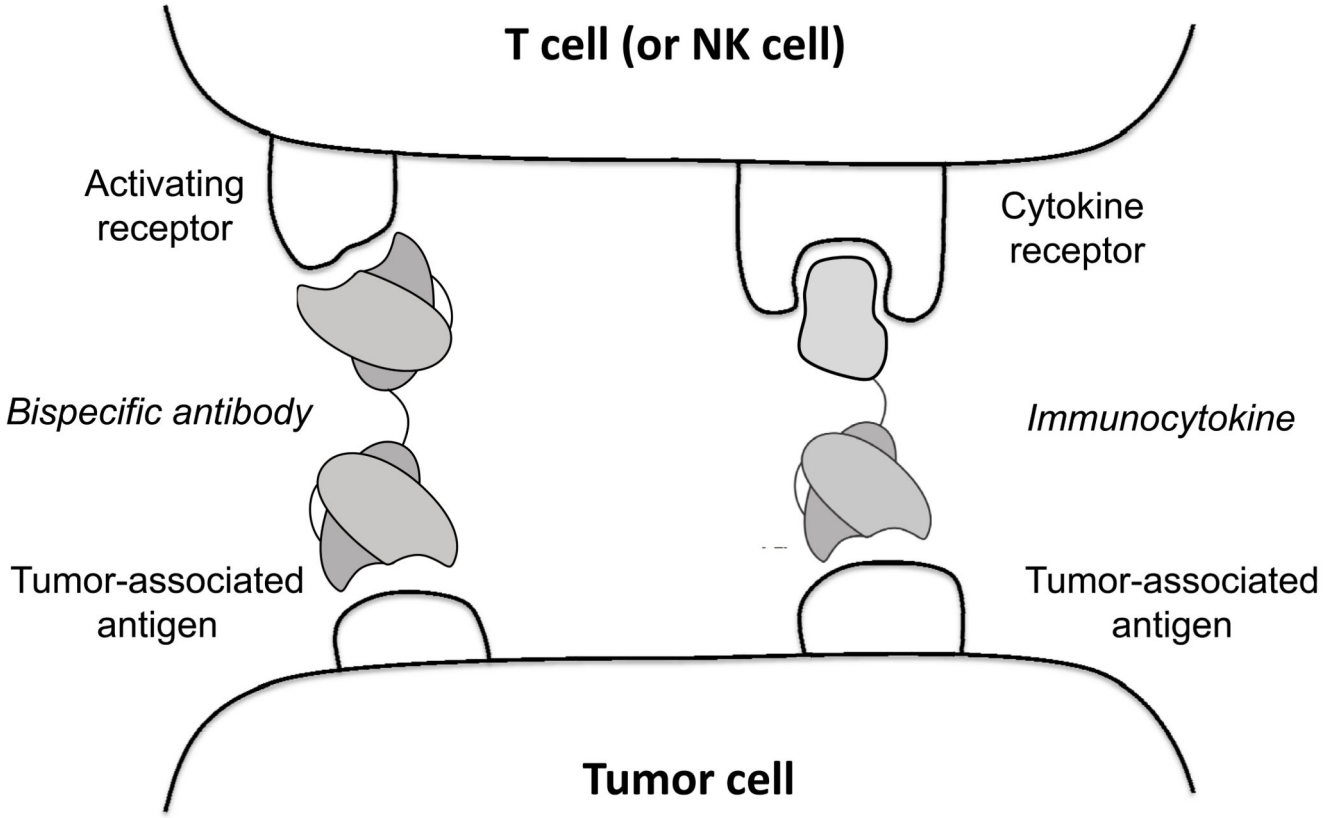

Figure 2.

Schematic representation of a comparative analysis, between the simultaneous engagement of tumor cells and T cells (or NK cells) by bispecific antibodies and by IL2-based immunocytokines.

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Table 1
List of anticancer immunocytokine products in clinical trials

List of antibody-cytokine fusion proteins, which have been investigated in clinical trials in oncology. The cytokine moiety and the target antigen are indicated. Nuclear antigen = histone recognized by the hTNT3 antibody; GD2 = disialoganglioside; CEA = carcinoembryonic antigen. Domain 7 of FN = epitope on domain 7 of fibronectin, which is cryptic in the presence of the alternatively-spliced EDB domain. EDB of FN = EDB domain of fibronectin. EpCAM = Epithelial Cell Adhesion Molecule. A1 of Tn-C = alternatively-spliced A1 domain of tenascin-C.

Product Name	Cytokine	Target Antigen	Format	Reference
Hu14.18-IL2	IL2	GD2	IgG	40
NHS-IL2LT	IL2	Nuclear antigen	IgG	29
anti-CEA-IL2v	IL2	CEA	IgG	
DI-Leu16-IL2	IL2	CD20	IgG	38
HuKS-IL2	IL2	EpCAM	IgG	64
NHS-IL12	IL12	Nuclear antigen	IgG	
BC1-IL12	IL12	Domain 7 of FN	IgG	63
L19-IL2	IL2	EDB of FN	diabody	41,42,48,49
F16-IL2	IL2	A1 of Tn-C	diabody	27,43,44
L19-TNF	TNF	EDB of FN	scFv	45,46,49