The IL-4 and IL-13 pseudomonas exotoxins: new hope for brain tumor therapy

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✓ Targeting cell surface receptors with cytotoxins or immunotoxins provides a unique opportunity for brain tumor therapy. The authors have discovered that receptors for two cytokines, interleukin (IL)-4 and IL-13, are overexpressed on tumor biopsy samples and on cell lines derived from a variety of human tumors, including brain tumors. These investigators have demonstrated that the structure of these cytokine receptors on tumor cells is different from that found on normal immune cells. In human solid tumor cells, IL-4 binds to two chains (IL-4R α and IL-13R α 1), whereas IL-13 binds to three chains in many solid tumor cells, including glioma cells (to IL-4R α , IL-13R α 1, and IL-13R α 2). To target IL-4Rs and IL-13Rs, the authors generated two recombinant fusion cytotoxins composed of IL-4 or IL-13 and a mutated form of pseudomonas exotoxin (PE), which for simplicity are called IL4-PE and IL13-PE in this paper. These chimeric cytotoxins are highly toxic in vitro to human tumor cell lines and primary cell cultures, including glioma cells, and in vivo to animal models of human tumors, including gliomas. In contrast, normal cells, including immune, endothelial, and brain cells, are spared from their cytotoxic effects. Based on numerous preclinical studies, IL13-PE (also known as IL13-PE38QQR or cintredekin besudotox) has been tested in four Phase I/II clinical trials. The agent IL13-PE was administered intracranially by using convection-enhanced delivery (CED). The drug was delivered through catheters placed either directly into the tumor bed or in the peritumoral region after resection of the lesion. The CED of IL13-PE was fairly well tolerated, with a reasonable benefit/risk profile for treatment of patients with glioma. Based on Phase I/II clinical trials, the Phase III Randomized Evaluation of CED of IL13-PE Compared to Gliadel Wafer with Survival Endpoint Trial (also known as the PRECISE Trial) in patients with initial recurrence of glioblastoma multiforme has recently been completed. Patients are being monitored for safety of the agents, duration of overall survival, and quality of life.

KEY WORDS • glioblastoma multiforme • brain neoplasm • cintredekin besudotox • convection-enhanced delivery

MALIGNANT neoplasms of the central nervous system, in particular GBMs, are the most common type of brain tumors in adults. Approximately 13,000 cases are diagnosed in the US each year. These locally infiltrating, fatal tumors continue to challenge the effectiveness of current therapeutic interventions, including resection, chemotherapy, radiation therapy, and various combinations of these treatments. The prognosis of patients with GBM is dismal: a median survival duration of 1 year with available therapy at initial diagnosis and approximately 6 months following recurrence or progression. Recent progress achieved in understanding the fundamental biology of human tumors and GBM has facilitated

development of novel anticancer therapeutic approaches, including targeting of tumor-specific cell surface proteins by using a monoclonal antibody or a toxin.^{1,9} These targeted agents are composed of a toxin derived from bacteria, like PE or DT, coupled to either an antibody specific for a membrane antigen (immunotoxin) or a ligand specific for cell surface receptors (cytotoxin). Immunotoxins and cytotoxins exert specificity in their killing of tumor cells.

Cancer Therapy Targeting IL-4R

Discovery and Targeting of the Cytokine Receptor

Expression. Approximately 17 years ago, investigators in our laboratory serendipitously discovered receptors for an immunoregulatory cytokine, IL-4, on murine sarcoma and colon adenocarcinoma cells.²⁴ Later, we discovered that a variety of human tumor cell lines and patient tumor

Abbreviations used in this paper: CED = convection-enhanced delivery; DT = diphtheria toxin; GBM = glioblastoma multiforme; IL = interleukin; IL-2R, IL-4R, and IL-13R = IL-2, IL-4, and IL-13 receptors; PE = pseudomonas exotoxin.

samples, including glioma tumors, express high levels of IL-4R as determined by messenger RNA and protein expression.^{10,23} We determined the structure of IL-4R in tumor cells, and we identified immune cells on which IL-4 has prominent biological activities (Fig. 1). In our extensive studies we have demonstrated that IL-4 binds to IL-4R α chain, a primary IL-4 binding protein, and also to IL-13R α 1 chain, and that together they mediate signaling induced by IL-4 in tumor cells. In immune cells, IL-4R α chain forms a complex with IL-2R γ chain for IL-4 signaling.

Targeting. Although the significance of overexpression of IL-4R on tumor cells is not known, to target these receptors we generated fusion proteins composed of IL-4 and mutated forms of PE, which were found to be highly and specifically toxic to tumor cells.²³ To improve the binding affinity of IL-4 to IL-4Rs, we created a circularly permuted IL-4 and fused it to a mutated form of PE [the fusion protein was termed IL4(38-37)-PE38KDEL or cpIL4-PE] (Fig. 2).²² The cpIL4-PE is highly and specifically toxic to tumor cells, including brain tumor cells, in vitro, and has remarkable antitumor activity in animal xenograft models of a variety of human cancers.¹⁴ This molecule has demonstrated a prominent antitumor activity in animal models of brain tumors.²⁶

Animal and Clinical Trials

The safety of IL4-PE administration was tested in mice, rats, and monkeys.⁵ In addition, three Phase I clinical trials were undertaken. In two of the Phase I clinical trials, cpIL4-PE was delivered intratumorally by CED in patients with recurrent GBM.^{25,26,29,30} This was the preferred route of delivery of the agent due to limited trafficking of macromolecules to brain tumors because of the blood-brain barrier. The safety and tolerability of this cytotoxin was also demonstrated in an additional Phase I clinical trial for malignancies outside the central nervous system, in which cpIL4-PE was administered intravenously every other day for 3 days.⁵

Cytotoxicity of IL4-PE

The efficacy of cpIL4-PE was suggested by Rainov and Heidecke²⁵ and Rand, et al.,²⁶ who reported long-term survival of patients with recurrent malignant glioma following intratumoral infusion of IL4-PE. Additional clinical studies must be performed using cpIL4-PE either alone or in combination with other agents to realize the benefit of this promising targeted agent for cancer therapy in general and malignant glioma in particular, because CED can safely deliver a high local concentration of cpIL4-PE for cancer therapy.

Cancer Therapy Targeting IL-13R

Discovery and Targeting of the Cytokine Receptor

Expression. In the early to mid-1990s, as a new Th-2-derived cytokine called IL-13 was described, we were curious to know whether human tumor cells also express receptors for IL-13. Our laboratory was the first in which the overexpression of IL-13Rs was identified in human renal cell carcinomas.²⁰ Because the cpIL4-PE cytotoxin was already in development for malignant brain tumors

FIG. 1. Schematic model of IL-4R subunits. Type I IL-4R (left) consists of IL-4R α and IL-2R γ chains (γ c) and is expressed in T cells and natural killer (NK) cells. Tumor cells (renal cell carcinoma [RCC], colon carcinoma [colon ca], and brain tumors) express Type II IL-4R (right), which consists of IL-4R α and IL-13R α 1 chains. Both types of IL-4R complexes have been confirmed by reconstitution studies.

and CED can deliver cytotoxins effectively, we immediately tested whether malignant brain tumors also express IL-13R.^{3,12} We also reported that many human tumor cell lines and tissues originating from malignant glioma, acquired immunodeficiency syndrome-associated Kaposi sarcoma, squamous cell carcinoma of the head and neck, and medulloblastoma express large numbers of IL-13Rs.^{8,11,13} In contrast, human B cells, monocytes, T cells, and endothelial cells express undetectable or low numbers of these receptors.¹⁹ The IL-13 molecule mediates prominent biological activities in B cells and monocytes, and recently it has been shown to be a mediator or regulator of asthma, allergic and inflammatory diseases, and cancer.^{2,18,31}

The significance of overexpression of IL-13R in tumors is not clear. However, our extensive studies have demonstrated that IL-13 binds to two chains (IL-13R α 1 and IL-13R α 2 chains; Fig. 3). The IL-13R α 1 chain binds to IL-13 with a low affinity, whereas the IL-13R α 2 chain binds to IL-13 with a high affinity. In addition, IL-13 binds to the IL-4R α chain, and together with IL-13R α 1, IL-13 mediates signaling induced in tumor, endothelial, fibroblast, and immune cells. In immune cells, endothelial cells, and most solid tumor cell lines, the IL-13R α 2 chain is not present,¹⁹ but it is present at a high density in malignant gliomas and other solid tumors as listed earlier. The IL-13R α 2 chain neither forms a complex with IL-4R α nor mediates signaling through the Janus tyrosine kinase/signal transducer and activator of transcription (or JAK-STAT) pathway. Recent studies have shown that the extracellular domain of IL-13R α 2 may serve as a “decoy” receptor, and that IL-13R α 2 may be involved in signaling through the AP1 pathway.^{4,32}

Targeting. To target IL-13Rs for cancer therapy, we developed a recombinant fusion protein composed of IL-13 as a tumor-targeting molecule and a mutated form of PE as a cytotoxic moiety (IL13-PE38QQR [also known as cintredekin besudotox or IL13-PE]). The PE molecule is

FIG. 2. Schematic model of IL4(38-37)-PE38KDEL. The circularly permuted IL-4 ligand contains the peptide GGNGG, joining the native COOH terminus to the native NH₂ terminus of IL-4. Another peptide connector of the sequence ASGGPE joins the COOH terminus of the ligand with toxin. The protein PE38KDEL consists of PE amino acids 253–364 and 381–608, followed by the KDEL sequence.

one of the pathogenic proteins secreted by *P. aeruginosa* and is composed of three domains. The NH₂-terminal domain Ia is a cell-binding domain that binds ubiquitously to receptors presented on eukaryotic cells. To generate IL-13 cytotoxin, domain Ia was removed to avoid non-specific binding, and the DNA sequence encoding IL-13 was inserted upstream of PE. Three amino acids in domain III (K590, K606, and K613) were replaced by Q, Q, and R, respectively (Fig 4). The IL13-PE was expressed in inclusion bodies of *Escherichia coli* that were transformed with expression vector plasmid. Highly purified chimeric protein was obtained by ion-exchange and gel-filtration chromatography.

Cytotoxicity of IL13-PE

The IL13-PE was found to be highly selective and potent in its capacity to kill human tumor cells in vitro, including GBM cells, while sparing normal cells (including immune cells, endothelial cells, and normal human astrocyte).⁹ An extremely low concentration of IL13-PE often achieved a concentration of cytotoxin at which 50% inhibition of protein synthesis occurs at less than 1 ng/ml. We have demonstrated that IL-13R α 2 chain is primarily responsible for IL13-PE-mediated cytotoxicity of tumor cells, including glioma cells.^{9,13}

Cell Culture Experiments and Animal Models

The IL-13R α 2 gene interference in GBM cells showed decreased ligand binding, and consequently IL13-PE exhibited less cytotoxicity to both U251MG and U373MG GBM cell lines. Consistent with its in vitro cytotoxic activity, IL13-PE elicited remarkable antitumor activity in human glioma xenograft models.^{7,9} Intravenous and intraperitoneal administration of IL13-PE in nude strains of experimental animals harboring human glioma tumors showed significant antitumor activity. Because GBM is a local/regional disease, intratumoral injection of IL13-PE is an ideal approach to bypass the blood–brain barrier. Intratumoral delivery of IL13-PE to tumor-bearing hosts mediated remarkable antitumor effects. We demonstrated that a significant level of IL13-PE can be achieved at the tumor site for a longer period when the agent is injected directly into tumors without flowing into the blood circulation. We also determined the safety and efficacy of IL13-PE against intracranial xenografts of tumors in mice and toxicity in rat brain. It was reported that treatment of established intracranial gliomas resulted in a highly sig-

FIG. 3. Schematic model of IL-13R subunits. In Type I IL-13R, the IL-13 binds to IL-13R α 1, IL-13R α 2, and IL-4R α subunits, and the IL-13R α 1 chain also binds to IL-4. In Type II IL-13R, the IL-13R α 2 subunit is absent. Both IL-13 and IL-4 bind to IL-13R α 1 and IL-4R α , thus cross-competing each other's binding. A431, COLO201, HT29 = cancer cell lines; SCCHN = squamous cell carcinoma of the head and neck.

nificant prolongation of the survival of mice after intratumoral injection of IL13-PE.

Delivery Methods for IL13-PE

Because sustained availability of drug is of paramount importance for achieving better efficacy for cancer therapy, we administered IL13-PE by continuous infusion, using micropumps in glioma tumor models. These results indicate that IL13-PE is better tolerated when administered by continuous infusion compared with bolus administration. The safety and toxicity of IL13-PE was determined in mice, rats, and monkeys.⁹ Intracerebral administration of IL13-PE in mouse and rat brain indicated that a 100 μ g/ml dose was well tolerated, with minimal histological changes in normal brain.

Clinical Trials of IL13-PE for Recurrent GBM: Phase I/II Studies

Encouraging preclinical results have led our group to initiate several Phase I/II clinical trials in patients with recurrent GBM.²¹ As described earlier, due to the high molecular weight of IL13-PE, it was necessary to develop a unique drug delivery system based on CED, one that uses positive-pressure infusion to achieve local/regional delivery of therapeutic agents through intracerebral catheters.¹⁶ Given the infiltrative nature of malignant glioma, a new set of approaches was applied in our clinical trials.

In the key Phase I study, patients underwent an initial tumor biopsy procedure, followed by the placement of one

FIG. 4. Schematic model of IL13-PE38QQR. Three amino acids in domain III, lysine residues at positions 590, 606, and 613 (K590, K606, and K613), were replaced by glutamine residues and arginine residue (Q, Q, and R), respectively.

intratumoral catheter, and then IL13-PE was administered by CED over a period of 48 hours (dose escalation 0.25–2 $\mu\text{g/ml}$, 400 $\mu\text{l/hour}$). After resection of the tumor, two or three catheters were inserted into the region adjacent to the tumor resection cavity (peritumoral infusion). Postresection CED of IL13-PE (0.25 $\mu\text{g/ml}$, 750 $\mu\text{l/hour}$) was then administered for 96 hours in an effort to kill residual tumor cells and reduce the risk for recurrence of the lesion. When a histologically effective concentration of IL13-PE was achieved, intratumoral administration was omitted, and escalation of the IL13-PE concentration during postresection peritumoral infusion continued until the maximum tolerated dose, 0.5 $\mu\text{g/ml}$, was identified.

Adverse events noted across all Phase I studies included headache (27%), hemiparesis (16%), aphasia (9%), seizures (9%), and fatigue (9%). An encouraging overall median survival duration was observed in all Phase I studies of IL13-PE in patients receiving peritumoral infusion (0.25 and 0.5 $\mu\text{g/ml}$, 42.7 weeks, 95% confidence interval 35.4–59.3, 42 patients). The outcomes were even better when two or more catheters were adequately positioned and the drug was consequently optimally distributed (0.25 and 0.5 $\mu\text{g/ml}$, 57.4 weeks, 95% confidence interval 35.6–75.3, 24 patients).

These data indicate that peritumoral infusion of IL13-PE by using CED is an innovative and promising strategy for the treatment of patients with GBM who undergo resection. A target accrual of 294 patients (276 with confirmed recurrent GBM) was achieved in December 2005 for our “Phase III Randomized Evaluation of CED of IL13-PE Compared to Gliadel Wafer with Survival Endpoint” Trial (which we also call the PRECISE Trial), which was conducted in 50 centers worldwide. Patients enrolled in the study will continue to be monitored until we perform the interim efficacy analysis at 160 deaths and a final efficacy analysis at 215 deaths. Analysis of the clinical data at these points will serve to elaborate further the safety and efficacy of IL13-PE as a treatment for GBM. A Phase I clinical trial of IL13-PE for the treatment of malignant glioma at initial diagnosis is ongoing and nearing completion. Similarly, a Phase I/II trial involving intracerebral injection of IL13-PE for treatment of pediatric patients with supratentorial recurrent malignant glioma is also underway.

Future Investigations

In addition to IL-4R and IL-13R, several growth factor receptors that have been identified on brain tumors may potentially serve as targets for tumor-directed therapy. Targeted toxins include DT fused to ligands specific to epidermal growth factor (called DAB₃₈₉-EGF), transferrin (named Tf-CRM107), or urokinase-type plasminogen activator (called DTAT); PE fused to ligands specific to transforming growth factor- α (such as TP-38) or to high-molecular-weight melanoma-associated antigen are also used. These fused constructs have demonstrated antitumor activity both in vitro and in an animal model of GBM, and some of them are in various stages of clinical trials, indicating the level of enthusiasm for immunotoxins and cytotoxins for use in GBM therapy.^{6,15,17,27,28}

Conclusions

The IL13-PE and IL4-PE molecules appear to be promising novel therapeutic agents for the treatment of patients with GBM. Clinical trials to assess the safety and efficacy of these agents are ongoing, with Phase III clinical trial data on IL13-PE for recurrent GBM becoming available in the near future. Because GBM represents a heterogeneous tumor expressing several different types of cell surface receptors, we will continue to explore additional therapeutic approaches such as combination therapy in which immunotoxins and cytotoxins targeting different receptors will be used to overcome this deadly disease. Because IL-4 and IL-13 are directed at two different targets, it will be of interest to test whether using both cytotoxins mediates a synergistic effect on gliomas.

Disclaimer

The views presented in this article do not necessarily reflect those of the US Food and Drug Administration.

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