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Significance of interleukin-13 receptor alpha 2–targeted glioblastoma therapy

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Glioblastoma multiforme (GBM) remains one of the most lethal primary brain tumors despite surgical and therapeutic advancements. Targeted therapies of neoplastic diseases, including GBM, have received a great deal of interest in recent years. A highly studied target of GBM is interleukin-13 receptor α chain variant 2 (IL13Rα2). Targeted therapies against IL13Rα2 in GBM include fusion chimera proteins of IL-13 and bacterial toxins, nanoparticles, and oncolytic viruses. In addition, immunotherapies have been developed using monoclonal antibodies and cell-based strategies such as IL13Rα2-pulsed dendritic cells and IL13Rα2-targeted chimeric antigen receptor–modified T cells. Advanced therapeutic development has led to the completion of phase I clinical trials for chimeric antigen receptor–modified T cells and phase III clinical trials for IL-13–conjugated bacterial toxin, with promising outcomes. Selective expression of IL13Rα2 on tumor cells, while absent in the surrounding normal brain tissue, has motivated continued study of IL13Rα2 as an important candidate for targeted glioma therapy. Here, we review the preclinical and clinical studies targeting IL13Rα2 in GBM and discuss new advances and promising applications.

Keywords: glioblastoma, glioma, immunotherapy, IL13Rα2, toxin.

Glioblastoma multiforme (GBM) is one of the most devastating primary brain tumors. Despite aggressive treatment, median survival in patients with GBM remains slightly longer than a year, with most deaths attributed to tumor recurrence.[1] Novel approaches are under development targeting glioma invasion, angiogenesis, proliferation, immune escape, and tumor recurrence.[2] Targeted therapy has emerged as a promising field of research in the treatment of malignancies.[3] Optimism on the use of targeted therapy has been even higher recently following impressive clinical data reporting complete remission in patients with B-cell malignancies after administration of genetically modified T lymphocytes targeting CD19.[4–6] The benefits of targeted therapy are not limited to hematological malignancies and have been borne out also in solid tumors. Highly invasive breast cancer that expresses human epidermal growth factor receptor 2 can be successfully targeted with antibody-drug conjugate agents, such as trastuzumab emtansine.[7] At the same time, targeting is not limited to tumor cells alone but can also be directed toward the tumor microenvironment and immune response. Cytotoxic T-lymphocyte antigen 4 blockade releases the inhibition of antitumor response in metastatic melanoma patients and increases their survival.[8] Hence, targeted therapy is the new emerging trend in oncology. This has increased the focus on discovering antigens present exclusively in glioma as targets for gene therapy and immunotherapy. A variety of tumor antigens have been used as targets in preclinical and clinical trials. One of the most extensively studied targets is the interleukin-13 receptor α chain variant 2 (IL13Rα2). Its selective expression on GBM, discovered almost 2 decades ago, led to its identification as a target.[9] Here, we will outline the modalities of pharmacologic therapies that target IL13Rα2 and review potential new therapeutic strategies.

IL13Rα2 Structure and Function

Interleukin-13 plays a major role in regulating immune responses and immune microenvironment not only during normal physiological conditions but also in cancer.[10–12] In most cells, IL-13 binds to an IL13Rα1 monomer with a low affinity and then joins IL4Rα to form a heterodimer complex. This complex can trigger Janus kinases and leads to downstream pathway activation of signal transducer and activator of transcription (STAT)6.[13] On the other hand, in some normal cells, like testis, and in cancer cells, IL-13 can bind to the high-affinity receptor IL13Rα2.[14,15] The RNA transcript for the IL13Rα2 gene that is located in Xq13.1-q28 encodes for a 380-amino-acid protein that includes a 26-amino-acid signaling sequence and a short 17-amino-acid

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intracellular domain.[16] While sparsely expressed in normal tissues, IL13Rα2 expresses up to 30 000 binding sites for IL-13 per cell in GBM.[17]

According to the current dogma, IL13Rα2 is a decoy receptor for IL-13. The IL13Rα2 binds available IL-13 with higher affinity than the ubiquitously expressed IL13Rα1.[13] This allows for sequestration of the ligand away from IL13Rα1. In nonmutated cells, IL-13 binding to IL13Rα1 activates STAT6, which translocates to the nucleus, where it exerts transcriptional control over genes containing the N6–growth arrest specific promoter, such as 15-lipooxygenase-1, which promotes apoptosis through increased caspase-3 activity.[18–20] IL-13 sequestration can be an apoptosis escape mechanism of tumor cells. Another mechanism described for the blockade of IL13Rα2 by IL13Rα1 is physical blocking of the docking of STAT6 to the receptor. The lack of STAT6 docking impedes downstream activation of apoptosis.[18] The selective expression of IL13Rα2 in glioma cells and the subsequent apoptosis inhibition in cells harboring the receptor are the bases for suggesting a possible role for IL13Rα2 as an oncogene.[19]

The IL-13 ligand binds at different cytokine receptor homology sites in IL13Rα1 compared with IL13Rα2; and furthermore, IL13Rα1 binding requires heterodimerization with IL4Rα for a high-affinity bond.[21,22] These 2 characteristics have allowed for generation of mutated IL-13 ligands with limited IL13Rα1/IL-13/IL4Rα binding while preserving affinity with IL13Rα2 (Fig. 1A). A specific sequence similarity between the glutamic acid at position 9 of IL-4 (IL4.E9) and that at position 13 of IL-13 (IL13.E13) is believed to be involved in the binding of IL-13 to IL4Rα and thus signaling through the IL-13/IL-4 pathway.[23] A mutation of glutamic acid to lysine (IL13.E13K) was effective in reducing the affinity of IL-13 binding to IL13Rα1[24] (Fig. 1B). Another IL-13 mutein, in which E13 was replaced with tyrosine (IL13.E13Y), was also shown to be effective in reducing IL-13 affinity to IL13Rα1.[25,26]

IL-13 binding to IL13Rα2, on the other hand, is dependent on the presence of Lys-105, Lys-106, and Arg-109 on IL13's α-helix D[27] (Fig. 1C). Mutations in these locations can decrease or increase the affinity to IL13Rα2. Screening among different mutations permitted the discovery of the high-affinity mutants IL13.K105R and IL13.R109K. Replacement of lysine with arginine at location 105 (IL13.K105R) and of arginine with lysine at 109 (IL13.R109K) increased affinity to the receptor 77- and 27-fold, respectively, compared with native IL-13[27] (Fig. 1D). At the same time, such mutations did not alter affinity to the ubiquitously expressed IL13Rα1. Therefore, the development of mutants with increased affinity to the target IL13Rα2 and low binding to IL13Rα1 is possible. This, in turn, may lead to increased efficacy and fewer adverse effects. Kong et al.[28] reported a double mutation of IL-13 (IL13.E13K.R109K), which reduced the affinity of IL-13 to IL13Rα1 while at the same time improving IL13's selectivity toward IL13Rα2.

Role of IL13Rα2 in Oncogenesis

Increased expression of IL13Rα2 promotes tumor progression in glioma and other tumor models. Expression of IL13Rα2 increases with glioma malignancy grade and is a prognostic indicator for

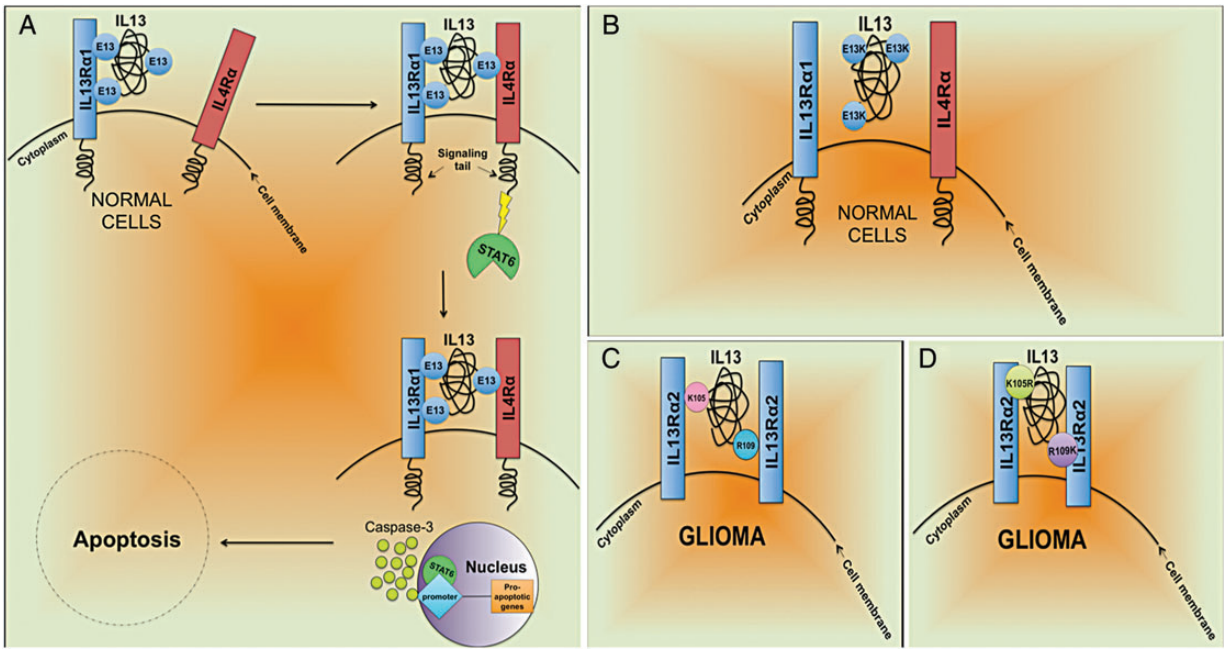

Fig. 1. IL-13 and its affinity with IL13Rα1 and IL13Rα2. (A) Description of IL-13's affinity with IL13Rα1 via E13 moiety, which also helps in binding of the IL13Rα1/IL-13 complex to IL4Rα to form the IL13Rα1/IL-13/IL4Rα complex on normal cells. This complex via the signaling tail activates STAT6, which then translocates to the nucleus and influences promoters of pro-apoptotic genes to secrete caspase-3. (B) Mutation of E13 moiety to E13K has been shown to reduce the binding affinity of IL-13 to both IL13Rα1 and IL4Rα, thus failing to form the signaling complex. (C) K105 and R109 moieties on IL-13 help in binding to the decoy receptor IL13Rα2, which lacks the signaling tail. (D) Mutations of K105 to K105R and of R109 to R109K have been shown to enhance the binding of IL-13 to IL13Rα2 on glioma cells.

poor patient survival.[29] IL13Rα2 not only blocks the normal apoptotic pathway induced by IL-13/IL-4 through STAT6 but also induces upregulation of STAT3 and B-cell lymphoma 2 in glioma cells.[30,31] In ovarian and pancreatic cancers, it promotes invasion and metastasis via the pathway of extracellular signal-regulated kinase/activator protein 1.[32,33] Expression of IL13Rα2 in immune cells, such as myeloid derived suppressor cells, also promotes tumor immune escape and progression via upregulation of transforming growth factor β.[34]

Targeting IL13Rα2 becomes even more important in light of such findings. Eradicating IL13Rα2-expressing cells would not only reduce tumor burden but also alter the tumor microenvironment.

Glioma Therapy Targeted at IL13Rα2

Since its discovery, IL13Rα2 has provided a well-defined target for novel therapies in gliomas, culminating in completion of a phase III clinical trial. Therefore, reviewing the promises and pitfalls of these approaches will aid in the design of better-targeted therapies in the future.

After it was proven that targeting a receptor with a fusion chimera protein (IL-13–*Pseudomonas* exotoxin A [PE]) was possible, more than 30 different biological agents have been engineered toward IL13Rα2. These targeting agents can be broadly characterized into 2 different groups: (i) IL-13–labeled therapeutic agents and (ii) IL13Rα2-targeted immunotherapy.

IL-13–labeled Therapeutic Agents

IL-13 Fusion Chimera Proteins

The therapeutic approach with ligand-toxin fusion chimera proteins did not originate with IL-13 targeting but, once revealed, gathered exceptional interest and produced research that could benefit future agents. The therapeutic effect of an IL-13–truncated PE fusion chimera protein (IL13PE38QQR) in glioma was recognized even before characterization of its receptor. IL13PE38QQR was serendipitously discovered to have a much higher potency than any previous ligand-associated toxin tested before, ~1000 times more than any other biological compound in vitro.[17] The increased efficacy was due to high expression of IL13Rα2 molecules on glioma cells.[35] When tested in xenograft animal models of human glioma, intratumoral injections of IL13PE38QQR cured 40% of the animals.[9] These promising animal studies allowed IL13PE38QQR to advance in clinical trials in GBM and other cancers with the commercial name cintredekin besudotox.[36]

IL13PE has not been the only toxin-mediated approach to brain tumors. The truncated diphtheria toxin (DT) fusion chimera protein to IL-13—DT-IL13QM—was shown to lyse a wide range of glioma cell lines in vitro.[37] To increase its specificity and potency toward glioma cells, different groups have relied on the expression of more than one ligand bound to the toxin. Truncated DT bound to a bipeptide from IL-13 and epidermal growth factor—DTEGF13—was more cytotoxic to glioma cells than were the single bound forms, DTEGF and DTIL13, both in vitro and in vivo.[38] On the other hand, a bispecific urokinase-type plasminogen activator and IL-13—DTAT13—was shown to be much less toxic to the kidneys and liver when injected into animals' brains than were its single-ligand bound counterparts.[39] DT ligand-targeted therapy remains a useful tool for targeted therapy that has yet to advance to clinical trials.

IL-13 Ligand Expressing Viruses

IL-13 ligand targeting has proven effective and relatively safe and was thus adopted as a strategy to label gliomatropic viruses. Herpes simplex virus was modified to express IL-13 ligand (R5111) to transduce glioma cells in vitro with high selectivity based on IL13Rα2 expression.[40] Virus surface modification to express IL-13 ligand in adenovirus (LU-13) or lentivirus (MV $H_{c\Delta 18\text{-}AA}$–IL-13) resulted in >2-log increase in luciferase gene expression in glioma-bearing mice xenografts compared with their controls.[41,42] When viral vectors are administered in vivo, the main issue is their safety profile. Surface receptor modifications to express IL-13 ligand can make these constructs safer without altering their toxicity profile. Attenuated vaccine strains of measles virus were successful in preclinical models to prolong survival in glioma-bearing mice. Increased targeting potential via expression of IL-13 peptide on their surface has the potential to decrease dramatically their toxicity while preserving efficacy.[43] IL-13 retargeting of viral vectors can bring such therapeutic agents closer to clinical trials.

One of the major outcomes of a recent IL13Rα2-targeted phase III clinical trial using cintredekin besudotox was adverse neurotoxicity in almost 60% of the patients receiving the therapy.[44] This was possibly due to IL-13 binding to the physiological receptor IL13Rα1 that is expressed in normal brain.[30,45] Candolfi et al.[46] developed an adenoviral vector, Ad.mhIL4.TRE.mhIL13PE, to address this cross-reactive neurotoxicity and develop a sturdier gene therapy delivery mechanism.[46] The adenoviral vector expressed a modified human IL-13 (IL13.E13K or mhIL13) conjugated to PE (mhIL13PE), which helped increase the specificity of IL-13 toward IL13Rα2.[24,27] The vector also expressed a mutant human IL-4 (IL4.Y124D or mhIL4) that specifically bound to IL13R/IL4R, thus limiting any potential cross-binding of mhIL13PE. Mediated delivery of PE led to tumor reduction and long-term survival with minimal neurotoxicity in over 70% of xenografts as well as syngeneic graft models compared with those treated with cintredekin besudotox.[46]

IL-13–labeled Liposomes

Liposomes are nanoscale artificial vesicles that can be loaded with drugs and injected systemically. They are composed of a lipid bilayer whose surface can be modified to target specific receptors. Liposomes offer an advantage over other models of delivery because they can be injected systemically and target gliomas. Disadvantages of liposomes include possible uptake by the reticuloendothelial system and dumping, mostly due to faulty administration. The benefits of liposomes outweigh the disadvantages and therefore render them excellent candidates for a clinical trial program. IL-13–labeled liposomes were superior to nontargeted liposomes in achieving tumor size reduction and prolongation of survival in animals bearing glioma xenografts.[47,48] An interesting approach could entail the use of IL13Rα2-targeted liposomes as carriers for chemotherapeutic agents to deliver drugs to the tumor site, thereby potentially reducing the systemic toxicity of chemotherapy.

IL13Rα2-targeted Immunotherapy

Targeting IL13Rα2 With Antibodies

IL13Rα2 antibodies generated either by phage display libraries or by hybridoma technology have been successfully tested both in vitro and in vivo.[49,50] The advantage of increased specificity via antibody-based targeting has to be weighed against decreased potency compared with an IL-13 ligand approach. Kioi et al.[49] found that none of the IL13Rα2 antibody fragment variants conjugated to PE could match the potency of IL13PE in vitro or in vivo. This suggested that increasing affinity would yield better results. The antibodies generated by phage display technology tend to have low-affinity binding compared with hybridoma-generated antibodies. Using the latter technology, Balyasnikova et al.[50] showed the IL13Rα2 antibody to have high affinity to glioma cells either in vitro or ex vivo in tissue slides. Interestingly, this high-affinity antibody prolonged survival in mice coinjected intracranially with glioma cells. This is the only study showing that high-affinity binding to IL13Rα2 without any toxin increased animal survival. This was thought to result either from complement binding to the constant fragment of the IL13Rα2 antibody or from downstream signaling of IL13Rα2 inhibiting tumor formation via antibody binding. Another advantage that stems from the high-specificity targeting of this approach is the possibility of delivering antibodies systemically while minimizing side effects. Intraperitoneal or intravenous delivery of antibody fragment or peptide bound PE toxin may successfully home to glioma and reduce its growth in flank or orthotopic models.[49,51] The potency of this approach remains limited and understudied. Targeting very specific amino acid structures in highly mutated tumors carries the limitation of killing only a subgroup of cells. Generation of immune responses to a variety of specific glioma antigens may have a better outcome.

Titanium oxide microdiscs may be coated with anti-IL13Rα2 monoclonal antibody to target glioma cells. When an alternating magnetic field is applied, the microdisc vortices shift, creating an oscillation and transmitting a mechanical force to the tumor cells. A low-frequency field applied for only 10 min is sufficient to destroy 90% of cancer cells in vitro.[52,53]

Dendritic Cells Pulsed With Tumor-associated Antigens

Immunotherapy based on dendritic cells targets one of the main immune escape mechanisms discovered in glioma: poor tumor antigen presentation. Patients with high-grade gliomas have a poor systemic response to antigens.[54] More recent attempts to reboot antigen presentation have relied on ex vivo pulsation of dendritic cells with glioma antigens. To achieve maximum benefit, peripheral blood mononuclear cells are collected from patients; the cells of interest are sorted and then stimulated with glioma-associated antigens (GAAs) in the presence of immunostimulatory cytokines. Once loaded with immunostimulatory peptides, the antigen-presenting cells are injected back into the patient. Dendritic cells pulsed with GBM antigens are being tested in phase I/II clinical trials. The choice of antigen remains a critical element to this approach. Cell lysates offer an immense variety of different antigens that can be presented to effector cells in vivo. Instead, pulsation of dendritic cells with peptides from GAAs such as IL13Rα2, epidermal growth factor receptor variant III, and glycoprotein 100 can result in a more targeted immune response. Dendritic cells pulsed only with IL13Rα2 peptides induced robust immune responses in a subgroup of patients with the human leukocyte antigen–A*24/A*02 allele.[55,56] IL13Rα2 peptides have also been part of different mixtures of immunogenic molecules to provide more extensive coverage of the different cell populations.[57,58] Some of these antigen-cocktail pulsed dendritic cells are showing promising results in clinical trials. Recently, ICT-107, an intradermally administered autologous vaccine from dendritic cells pulsed with 6 different antigens, including IL13Rα2, showed a statistically significant prolongation of progression-free survival in a phase II multicenter study (NCT01280552). Median progression-free survival increased by 2 months overall and was even more evident in the group of patients who received at least 4 induction vaccinations.[59] Furthermore, the benefits of IL13Rα2-based vaccines are not limited to GBM. Recent data reported by Okada et al.[60] showed low toxicity and an even more potent immune response induction in low-grade glioma upon subcutaneous vaccinations with synthetic peptides for GAA epitopes that included IL13Rα2, EphA2, Wilms tumor 1, and Survivin.[60] These findings are promising but still far from the impressive potency of chimeric antigen receptor (CAR)–modified T cells in other malignancies.[4]

Chimeric Antigen Receptor–modified T Lymphocytes

CAR-modified T lymphocytes are artificially generated cytotoxic cells that recognize only one antigen. These T cells have the potential to kill target cells in the absence of costimulatory molecules or major histocompatibility class I, whose expression is altered in GBM. The first-generation CAR T cells were designed to have a specific single chain variable fragment of an antibody specific to the target or a targeting ligand (such as IL-13) connected with the intracellular signaling zeta-domain of CD3 (CD3ξ). The main issue with the first generation was their weak activation levels, which was resolved with the addition of a CD28 costimulatory domain in the second-generation CARs. The costimulatory domain not only increased cytotoxicity but also enhanced proliferation, cell survival, and memory formation.[61]

IL13Rα2 targeting has been feasible with both first- and second-generation CAR T cells. The CAR construct to target glioma cells contains a mutated IL-13 sequence at a single site (E13) that allows for increased affinity to IL13Rα2 and decreased binding to IL13Rα1.[26,28] Despite improved targeting of these T cells, the theoretical risk for adverse effect remains high and untested. All the studies to date have relied on local intracranial injections to show their efficacy.

The early successful preclinical studies on first-generation CAR T cells were initiated almost a decade ago. The IL13.E13Y zetakine receptor CD8+ T cells induced secretion of interferon γ, tumor necrosis factor α, and granulocyte-macrophage colony-stimulating factor only in presence on IL13Rα2-expressing cells. Also, when cultured in vitro together with glioma cells, they lysed only IL13Rα2-expressing U251 glioma.[26] In immunodeficient animal models, intratumoral injection of IL-13 zetakine CD8+ T cells cured mice with intracranial glioma. None of the animals had tumor recurrence, a finding that may be attributed to the lack of resistance to such therapy in glioma. Most other currently tested therapies suffer from tumor recurrence because a certain group of cells that are resistant become the dominant phenotype

or can act as a cell pool to originate tumor recurrence. Such cells are known as cancer stem cells because they are self-renewing, express stem cell markers, and can efficiently initiate tumor formation in vivo. Glioma stem cells have been shown to be responsible for resistance to current therapies and tumor recurrence. Glioma stem cells derived from IL13Rα2+ tumors express IL13Rα2 at levels similar to more differentiated cells and therefore are similarly sensitive in vitro to IL-13 zetakine T-cell therapy.[62] IL-13 zetakine T cells could block formation of tumor and induce its regression in a xenograft mouse model employing patient-derived glioma stem cells.[62] The T-cell presence in the glioma environment was limited to <15 days, a limitation shared by first-generation zetakine T cells. To address the limited potential of T cells to proliferate or survive after transplantation, costimulatory intracellular signals were introduced into them. The newly generated CAR zetakine T cells, containing CD28 and/or 41BB, expressed higher levels of Akt and mitogen-activated protein kinase upon contact with glioma cells, inducing a type 1 T helper cell cytotoxic phenotype in CD4+ cells. The increased activation via costimulatory domains allows for higher tumor suppressive activity and improves survival in animal models of glioma.[28,63]

Recently, a second-generation IL-13 "zetakine" CAR composed of a mutated IL-13 (IL13.E13K.R109K) extracellular domain linked to intracellular signaling elements of the CD28 costimulatory molecule and CD3ζ was reported by Kong et al.[28] IL13.E13K.R109K, a double-mutant IL-13, improved recognition of IL13Rα2+ tumors while reducing activity against cells expressing IL13Rα1+. The CAR-expressing T cells were efficient in killing IL13Rα2+ glioma cell targets with abundant secretion of cytokines IL-2 and interferon γ, and they displayed enhanced tumor-induced T cells in vitro. In an in vivo test with a human glioma xenograft model, single intracranial injections of CAR-expressing designer T cells into tumor sites resulted in marked increase in animal survival.

While intracellular signaling in CAR-expressing T cells has advanced dramatically in the last decade, not much has been accomplished in improving targeting of CAR T cells toward glioma. Further questions that need to be answered concern the survival of these therapeutic T cells in the presence of leucopenic chemotherapy drugs like temozolomide and the long-term host-immune effect of genetically modified T cells.

Clinical Trials Targeting IL13Rα2

Cintredekin Besudotox

Phase I/II clinical trials in gliomas with intracranial convection-enhanced delivery (CED) of IL13PE38QQR showed that the maximum tolerated intraparenchymal concentration was 0.5 μg/mL, which resulted in tumor necrosis.[44] No systemic toxicity was noted. Most common adverse effects were headaches, in up to 41% of patients, numbness, speech disorders, seizures, and occasional hemiparesis.[64,65] While most of these adverse effects were self-limited, hemiparesis was the most significant dose limiting toxicity, in 12% of patients. It correlated with suboptimal intratumoral diffusion and exposure of the normal brain parenchyma to twice the maximum tolerated intraparenchymal concentration, resulting in necrosis.[44] Another study, assessing the feasibility of IL13PE38QQR in newly diagnosed high-grade glioma, had a similar dose limiting toxicity profile, with 2 out of 22 patients suffering grade 3/4 adverse effects.[66] The other limited adverse effects related to IL13PE38QQR included fatigue (9%), gait disturbance (9%), nystagmus (9%), and confusion (9%); but no hemiparesis. Both studies highlighted the importance of catheter positioning and the need for real-time assessment of IL13PE38QQR diffusion to limit toxicities. The best timing to achieve maximum distribution of the reagent was when the injection regimen, 6 daily injections per protocol, was started 1 – 7 days postresection.[44,67] Timing of the injection was not the only predictor of success. The number of catheters placed also appeared to play an important role in the outcome. Survival of patients with only 1 catheter was significantly less than that of those patients who had ≥2 catheters placed properly via 3D MRI-based guidance.[44]

With these promising results, IL-13 targeting underwent testing in a phase III clinical trial in recurrent GBM in comparison with Gliadel wafer, the PRECISE study.[67] The study was designed to treat patients with proven histological diagnosis of GBM at their first disease recurrence. It included more than 50 centers across North America and Europe and recruited 296 patients during the 2004 to 2005 time interval. Placement of catheters, 2 to 4 per patient, was done based on post-op MRI findings, in the areas most suspicious for tumor infiltration or residual tumor, using either a stereotactic frame or frameless navigation. The study objectives were, first, to measure overall survival and, second, to assess the safety and toxicity of each treatment.

The PRECISE study showed IL13PE38QQR to be as efficacious in prolonging survival as the standard of care. Median survival ranged between 10 and 11 months in the group of patients who received at least 90% of the planned IL13PE38QQR dose.[67] There were little differences in the adverse effects between the 2 groups. Only vascular complications were statistically different, with the IL13PE38QQR group having more pulmonary embolism, likely due to more prolonged hospital stay.

The above results and study design have undergone extensive scrutiny. Patient selection was all-inclusive; every patient with GBM recurrence was eligible, despite tumor size or histological characteristics. The study considered all tumors as being uniformly positive for IL13Rα2, whereas multiple studies have now shown its expression to vary significantly. IL13Rα2 has been shown to be overexpressed in as low as 38% to as high as 70% of glioblastomas, based on immunohistochemistry, while its gene was overexpressed in 58% of patients.[29,68] No post-hoc analysis to evaluate efficacy in relation to IL13Rα2 expression was possible because IL13Rα2 status has remained unknown for those samples. Also, data regarding IL13Rα2 expression were collected in gliomas that had not undergone chemo/radiotherapy. Instead, IL13Rα2 expression in recurrent glioma remains to be studied.[69] With better knowledge of GBM pathobiology, future studies may not be all-inclusive. Different molecularly characterized GBM subtypes may respond better to certain therapies and henceforth would require separate study.[70,71] Indeed, recent studies have shown a correlation between IL13Rα2 expression and the mesenchymal signature gene expression.[29]

Convection-enhanced delivery of IL13PE38QQR remains experimental. Although DT-transferrin was the first immunotoxin administered via CED, IL13PE38QQR as a therapeutic agent to the human brain, along with Cotara and TransMID, was one of the first to be extensively studied for the use of CED.[72] The study protocol included a detailed algorithm by which the

injection would be targeted. The study was conducted in 50 medical centers across North America and Europe with different technological capabilities, as evidenced by the fact that some centers relied on frameless technology for catheter placement and some did not. The role of catheter positioning in patient outcome was analyzed retrospectively.[73,74] Only 51% of the catheters were accurately placed based on the protocol recommendations. The overall differences in catheter placement did affect progression-free survival, but not overall survival. Also, a statistical difference in overall survival was noted between experienced centers (>2 patients treated) and inexperienced centers.[74] Moving forward, it may be more beneficial if such complex delivery protocol trials are restricted to high-volume centers. The pharmacokinetics of CED in brain tumors need further studies for better understanding of volumes of distribution and the importance of catheter positioning for future trials.

IL13Rα2-targeting Chimeric Antigen Receptor T Cells

Initial clinical experience at City of Hope Hospital in 2 phase I clinical trials with intracranial administration of first-generation IL-13–zetakine CAR+ CD8+ T-cell clones in patients with high-grade glioma has demonstrated the potential of targeting IL13Rα2-expressing brain tumors. In the first pilot phase I clinical trial, 3 research participants with recurrent/refractory glioblastoma were treated with autologous first-generation IL13(E13Y)-zetakine+ CD8+ T-cell clones in cycles of escalating cell dose infusions up to 10^8. The CAR T-cell product also expressed a hygromycin resistance gene/herpes simplex virus 1 thymidine kinase fusion (HyTk) to serve as a selection/suicide marker, and a PET reporter gene.[26] A case study on one of the research participants has been reported with respect to the noninvasive detection of the autologous IL13(E13Y)-zetakine/HyTK+ CD8+ T cells using 9-[4-[18F]fluoro-3-(hydroxymethyl)butyl]guanine PET after adoptive transfer.[75] This study provides evidence for detection of the CAR T cells at the site of injection, as well as at a secondary site of recurrence near the corpus callosum, thus suggesting the potential of the CAR T cells to traffic to distant sites of infiltrative disease. In the second phase I clinical trial, an allogeneic CAR CD8+ T-cell product, termed GRm13Z40-2, generated from a healthy donor, was modified to express the first-generation IL-13–zetakine/HyTK CAR as described above (NCT01082926; http://www.clinicaltrials.gov/ct2/show/NCT01082926). Additionally, these T cells were deleted for the glucocorticoid receptor to render the allogeneic T-cell product resistant to steroids following adoptive transfer. In this trial, 6 research participants with nonresectable recurrent/refractory glioblastoma were treated in conjunction with IL-2 with repetitive doses of 10^8 CAR T cells. For all 9 research participants in both trials, clinical experience demonstrated the feasibility of this approach and the absence of serious therapy-related side effects and provided evidence for transient antiglioma responses for patients with IL13Rα2-expressing tumors. Second-generation IL13Rα2-CAR T cells are anticipated to demonstrate superior antitumor efficacy and improved T-cell persistence compared with the first-generation IL-13–zetakine CD8+ T-cell clones.[76,77] A phase I clinical trial evaluating the feasibility and safety of intratumoral/intracavitary administration of autologous second-generation CAR T cells for the treatment of recurrent IL13Rα2-positive glioblastoma is scheduled to initiate at City of Hope in 2014.

Conclusion

IL13Rα2 is expressed in ~58% of adult and 83% of pediatric brain tumors.[29,78] IL13Rα2 is also expressed on glioma-initiating cells,[62] thus making it an important target for glioma therapy. Despite recent developments, heterogeneity of IL13Rα2 expression in GBM has been attributed to the mixed success in IL13Rα2-targeted therapy. A wealth of reports have confirmed abundant IL13Rα2 overexpression in GBM.[9,29,62,78–80] Only one study, however, reported expression of IL13Rα2 in <50% of GBM cases.[68] In a recent study, it was demonstrated in vitro that escapees from IL13Rα2-targeted therapy of GBM primary cell lines were significantly less tumorigenic and were vulnerable to radiation and chemotherapy compared with the parent cells.[81] This implies that while the higher IL13Rα2-expressing tumors may be eliminated by targeted therapies, the tumors with lower expression, which often escape the therapy, may be less malignant in nature. Nevertheless, targeting IL13Rα2 has motivated the development of highly effective therapies and novel administration strategies (Table 1), which can be used as building blocks for future clinical trials for GBM.

Early clinical trials targeting IL13Rα2, via intratumoral CED of IL-13–conjugated PE, showed a promising safety and efficacy profile that can be improved with better catheter positioning.[44,66] Use of intraoperative MRI, labeled reagents, and computerized simulated models, such as iPlan Flow software by Brain-Lab, can increase efficacy significantly.[82] At the same time, better targeting of IL13Rα2 could be achieved by mutating sequences on IL-13 that reduce binding to normal tissues while allowing for enhanced binding to glioma cells.[25,83,84] These factors have been the limitation not only of IL13Rα2 but also of other glioma targets, such as transferrin receptor. A diphtheria toxin bound to transferrin (Tf-CRM107) administered to glioma via high-flow CED

Table 1. IL13Rα2-targeted therapeutic approaches

Therapies	References
(A) IL-13 bound therapeutic agents	
1. IL-13 toxin fusion chimera proteins	
a. Truncated *Pseudomonas* exotoxin A*	17,40
b. Mutated *Pseudomonas* exotoxin A	83
c. Truncated diphtheria toxin	37,38,86
d. Polypeptide targeted diphtheria toxin	39
2. IL-13 expressing viruses	
a. Herpes simplex virus (R5111)	40
b. Adenovirus (LU-13) (mhIL13)	41,46
c. Lentivirus (MV $H_{c\Delta 18\text{-}AA}$–IL-13)	87
d. Measles virus (MV-GFP-H(AA)–IL-13)	43
3. IL-13–labeled liposomes	47,48
(B) IL13Rα2 directed immunotherapy	
1. Cell-based immunotherapy	
a. Pulsed dendritic cells	56,58
b. CAR-expressing cytotoxic T cells*	26,28,62
2. Antibodies targeting IL13Rα2	
a. Phage display or hybridoma based technology	49,50
b. IL13Rα2-antibody bound microdisc	53

*Have progressed to clinical trials.

had a similar safety/efficacy profile in phase I/II studies that later proved inferior to the best chemotherapeutic regimen.[85] These trials had similar delivery issues. However, their design may have been their major limitation: the trials were designed to compete with current standards of care instead of being adjunct measures. Future efficacy trials targeting IL13Rα2 should be designed as adjuncts to current therapies and should not be limited to recurrent disease. A phase I trial in newly diagnosed high-grade glioma of IL13PE38QQR via CED in combination with the current standard of care (temozolomide and radiotherapy) showed similar toxicity profiles, with about 10% of patients developing grade 3/4 adverse effects.[44,66] It may prove that targeting more than one antigen is necessary and that benefits could be limited to a subgroup of patients.

The field of targeted therapies in glioma holds a lot of promise. IL13Rα2, with its 2 decades of preclinical and clinical studies, is in an optimal position to materialize those promises.

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References

1. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol*. 2009;10(5):459–466.
2. Van Meir EG, Hadjipanayis CG, Norden AD, et al. Exciting new advances in neuro-oncology: the avenue to a cure for malignant glioma. *CA Cancer J Clin*. 2010;60(3):166–193.
3. Wainwright DA, Nigam P, Thaci B, et al. Recent developments on immunotherapy for brain cancer. *Expert Opin Emerg Drugs*. 2012;17(2):181–202.
4. Porter DL, Levine BL, Kalos M, et al. Chimeric antigen receptor–modified T cells in chronic lymphoid leukemia. *N Engl J Med*. 2011;365(8):725–733.
5. Kochenderfer JN, Dudley ME, Feldman SA, et al. B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. *Blood*. 2012;119(12):2709–2720.
6. Brentjens RJ, Riviere I, Park JH, et al. Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. *Blood*. 2011;118(18):4817–4828.
7. Verma S, Miles D, Gianni L, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. *N Engl J Med*. 2012;367(19):1783–1791.
8. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010;363(8):711–723.
9. Debinski W, Gibo DM, Hulet SW, et al. Receptor for interleukin 13 is a marker and therapeutic target for human high-grade gliomas. *Clin Cancer Res*. 1999;5(5):985–990.
10. Minty A, Chalon P, Derocq JM, et al. Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses. *Nature*. 1993;362(6417):248–250.
11. Terabe M, Matsui S, Noben-Trauth N, et al. NKT cell-mediated repression of tumor immunosurveillance by IL-13 and the IL-4R-STAT6 pathway. *Nat Immunol*. 2000;1(6):515–520.
12. Fichtner-Feigl S, Strober W, Kawakami K, et al. IL-13 signaling through the IL-13alpha2 receptor is involved in induction of TGF-beta1 production and fibrosis. *Nat Med*. 2006;12(1):99–106.
13. Arima K, Sato K, Tanaka G, et al. Characterization of the interaction between interleukin-13 and interleukin-13 receptors. *J Biol Chem*. 2005;280(26):24915–24922.
14. Debinski W, Gibo DM. Molecular expression analysis of restrictive receptor for interleukin 13, a brain tumor-associated cancer/testis antigen. *Mol Med*. 2000;6(5):440–449.
15. Andrews AL, Nasir T, Bucchieri F, et al. IL-13 receptor alpha 2: a regulator of IL-13 and IL-4 signal transduction in primary human fibroblasts. *J Allergy Clin Immunol*. 2006;118(4):858–865.
16. Tabata Y, Khurana Hershey GK. IL-13 receptor isoforms: breaking through the complexity. *Curr Allergy Asthma Rep*. 2007;7(5):338–345.
17. Debinski W, Obiri NI, Powers SK, et al. Human glioma cells overexpress receptors for interleukin 13 and are extremely sensitive to a novel chimeric protein composed of interleukin 13 and pseudomonas exotoxin. *Clin Cancer Res*. 1995;1(11):1253–1258.
18. Rahaman SO, Sharma P, Harbor PC, et al. IL-13R(alpha)2, a decoy receptor for IL-13 acts as an inhibitor of IL-4-dependent signal transduction in glioblastoma cells. *Cancer Res*. 2002;62(4):1103–1109.
19. Hsi LC, Kundu S, Palomo J, et al. Silencing IL-13Ralpha2 promotes glioblastoma cell death via endogenous signaling. *Mol Cancer Ther*. 2011;10(7):1149–1160.
20. Viita H, Pacholska A, Ahmad F, et al. 15-Lipoxygenase-1 induces lipid peroxidation and apoptosis, and improves survival in rat malignant glioma. *In Vivo*. 2012;26(1):1–8.
21. Hilton DJ, Zhang JG, Metcalf D, et al. Cloning and characterization of a binding subunit of the interleukin 13 receptor that is also a component of the interleukin 4 receptor. *Proc Natl Acad Sci U S A*. 1996;93(1):497–501.
22. Aman MJ, Tayebi N, Obiri NI, et al. cDNA cloning and characterization of the human interleukin 13 receptor alpha chain. *J Biol Chem*. 1996;271(46):29265–29270.
23. Zurawski SM, Vega F Jr., Huyghe B, et al. Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction. *EMBO J*. 1993;12(7):2663–2670.
24. Debinski W, Gibo DM, Obiri NI, et al. Novel anti-brain tumor cytotoxins specific for cancer cells. *Nat Biotechnol*. 1998;16(5):449–453.
25. Debinski W, Thompson JP. Retargeting interleukin 13 for radioimmunodetection and radioimmunotherapy of human high-grade gliomas. *Clin Cancer Res*. 1999;5(10 Suppl):3143s–3147s.
26. Kahlon KS, Brown C, Cooper LJ, et al. Specific recognition and killing of glioblastoma multiforme by interleukin 13-zetakine redirected cytolytic T cells. *Cancer Res*. 2004;64(24):9160–9166.
27. Madhankumar AB, Mintz A, Debinski W. Interleukin 13 mutants of enhanced avidity toward the glioma-associated receptor, IL13Ralpha2. *Neoplasia*. 2004;6(1):15–22.
28. Kong S, Sengupta S, Tyler B, et al. Suppression of human glioma xenografts with second-generation IL13R-specific chimeric antigen receptor-modified T cells. *Clin Cancer Res*. 2012;18(21):5949–5960.
29. Brown CE, Warden CD, Starr R, et al. Glioma IL13Ralpha2 is associated with mesenchymal signature gene expression and poor patient prognosis. *PLoS One*. 2013;8(10):e77769.

30. Liu H, Jacobs BS, Liu J, et al. Interleukin-13 sensitivity and receptor phenotypes of human glial cell lines: non-neoplastic glia and low-grade astrocytoma differ from malignant glioma. *Cancer Immunol Immunother*. 2000;49(6):319–324.
31. Rahaman SO, Vogelbaum MA, Haque SJ. Aberrant Stat3 signaling by interleukin-4 in malignant glioma cells: involvement of IL-13Ralpha2. *Cancer Res*. 2005;65(7):2956–2963.
32. Fujisawa T, Joshi B, Nakajima A, et al. A novel role of interleukin-13 receptor alpha2 in pancreatic cancer invasion and metastasis. *Cancer Res*. 2009;69(22):8678–8685.
33. Fujisawa T, Joshi BH, Puri RK. IL-13 regulates cancer invasion and metastasis through IL-13Ralpha2 via ERK/AP-1 pathway in mouse model of human ovarian cancer. *Int J Cancer*. 2012;131(2):344–356.
34. Fichtner-Feigl S, Terabe M, Kitani A, et al. Restoration of tumor immunosurveillance via targeting of interleukin-13 receptor-alpha 2. *Cancer Res*. 2008;68(9):3467–3475.
35. Mintz A, Gibo DM, Slagle-Webb B, et al. IL-13Ralpha2 is a glioma-restricted receptor for interleukin-13. *Neoplasia*. 2002;4(5):388–399.
36. Barth S. hIL-13-PE38QQR. NeoPharm. *Curr Opin Investig Drugs*. 2001;2(9):1309–1313.
37. Liu TF, Cai J, Gibo DM, et al. Reoxygenation of hypoxic glioblastoma multiforme cells potentiates the killing effect of an interleukin-13-based cytotoxin. *Clin Cancer Res*. 2009;15(1):160–168.
38. Stish BJ, Oh S, Vallera DA. Anti-glioblastoma effect of a recombinant bispecific cytotoxin cotargeting human IL-13 and EGF receptors in a mouse xenograft model. *J Neurooncol*. 2008;87(1):51–61.
39. Rustamzadeh E, Vallera DA, Todhunter DA, et al. Immunotoxin pharmacokinetics: a comparison of the anti-glioblastoma bi-specific fusion protein (DTAT13) to DTAT and DTIL13. *J Neurooncol*. 2006;77(3):257–266.
40. Zhou G, Ye GJ, Debinski W, et al. Engineered herpes simplex virus 1 is dependent on IL13Ralpha 2 receptor for cell entry and independent of glycoprotein D receptor interaction. *Proc Natl Acad Sci U S A*. 2002;99(23):15124–15129.
41. Ulasov IV, Tyler MA, Han Y, et al. Novel recombinant adenoviral vector that targets the interleukin-13 receptor alpha2 chain permits effective gene transfer to malignant glioma. *Hum Gene Ther*. 2007;18(2):118–129.
42. Ou W, Marino MP, Suzuki A, et al. Specific targeting of human IL-13 receptor alpha2-positive cells with lentiviral vectors displaying IL-13 [published online ahead of print]. *Hum Gene Ther Methods*. 2012.
43. Allen C, Paraskevakou G, Iankov I, et al. Interleukin-13 displaying retargeted oncolytic measles virus strains have significant activity against gliomas with improved specificity. *Mol Ther*. 2008;16(9):1556–1564.
44. Kunwar S, Prados MD, Chang SM, et al. Direct intracerebral delivery of cintredekin besudotox (IL13-PE38QQR) in recurrent malignant glioma: a report by the Cintredekin Besudotox Intraparenchymal Study Group. *J Clin Oncol*. 2007;25(7):837–844.
45. Liu H, Prayson RA, Estes ML, et al. In vivo expression of the interleukin 4 receptor alpha by astrocytes in epilepsy cerebral cortex. *Cytokine*. 2000;12(11):1656–1661.
46. Candolfi M, Xiong W, Yagiz K, et al. Gene therapy-mediated delivery of targeted cytotoxins for glioma therapeutics. *Proc Natl Acad Sci U S A*. 2010;107(46):20021–20026.
47. Madhankumar AB, Slagle-Webb B, Mintz A, et al. Interleukin-13 receptor-targeted nanovesicles are a potential therapy for glioblastoma multiforme. *Mol Cancer Ther*. 2006;5(12):3162–3169.
48. Madhankumar AB, Slagle-Webb B, Wang X, et al. Efficacy of interleukin-13 receptor-targeted liposomal doxorubicin in the intracranial brain tumor model. *Mol Cancer Ther*. 2009;8(3):648–654.
49. Kioi M, Seetharam S, Puri RK. Targeting IL-13Ralpha2-positive cancer with a novel recombinant immunotoxin composed of a single-chain antibody and mutated Pseudomonas exotoxin. *Mol Cancer Ther*. 2008;7(6):1579–1587.
50. Balyasnikova IV, Wainwright DA, Solomaha E, et al. Characterization and immunotherapeutic implications for a novel antibody targeting interleukin (IL)-13 receptor alpha2. *J Biol Chem*. 2012;287(36):30215–30227.
51. Pandya H, Gibo DM, Garg S, et al. An interleukin 13 receptor alpha 2-specific peptide homes to human Glioblastoma multiforme xenografts. *Neuro Oncol*. 2012;14(1):6–18.
52. Rozhkova EA, Ulasov I, Lai B, et al. A high-performance nanobio photocatalyst for targeted brain cancer therapy. *Nano Lett*. 2009;9(9):3337–3342.
53. Kim DH, Rozhkova EA, Ulasov IV, et al. Biofunctionalized magnetic-vortex microdiscs for targeted cancer-cell destruction. *Nat Mater*. 2010;9(2):165–171.
54. Fadul CE, Fisher JL, Hampton TH, et al. Immune response in patients with newly diagnosed glioblastoma multiforme treated with intranodal autologous tumor lysate-dendritic cell vaccination after radiation chemotherapy. *J Immunother*. 2011;34(4):382–389.
55. Shimato S, Natsume A, Wakabayashi T, et al. Identification of a human leukocyte antigen-A24-restricted T-cell epitope derived from interleukin-13 receptor alpha2 chain, a glioma-associated antigen. *J Neurosurg*. 2008;109(1):117–122.
56. Iwami K, Shimato S, Ohno M, et al. Peptide-pulsed dendritic cell vaccination targeting interleukin-13 receptor alpha2 chain in recurrent malignant glioma patients with HLA-A*24/A*02 allele. *Cytotherapy*. 2012;14(6):733–742.
57. Saikali S, Avril T, Collet B, et al. Expression of nine tumour antigens in a series of human glioblastoma multiforme: interest of EGFRvIII, IL-13Ralpha2, gp100 and TRP-2 for immunotherapy. *J Neurooncol*. 2007;81(2):139–148.
58. Okada H, Kalinski P, Ueda R, et al. Induction of CD8+ T-cell responses against novel glioma-associated antigen peptides and clinical activity by vaccinations with {alpha}-type 1 polarized dendritic cells and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in patients with recurrent malignant glioma. *J Clin Oncol*. 2011;29(3):330–336.
59. A Study of ICT-107 Immunotherapy in Glioblastoma Multiforme (GBM). Available at: http://clinicaltrials.gov/show/NCT01280552. Accessed January 23, 2014.
60. Okada H, Butterfield L, Hamilton R, et al. Robust inductions of type-1 CD8+ T-cell responses in WHO grade II low-grade glioma patients receiving peptide-based vaccines in combination with poly-ICLC [abstract]. *Neuro Oncol*. 2013;15:iii71.
61. Emtage PC, Lo AS, Gomes EM, et al. Second-generation anti-carcinoembryonic antigen designer T cells resist activation-induced cell death, proliferate on tumor contact, secrete cytokines, and exhibit superior antitumor activity in vivo: a preclinical evaluation. *Clin Cancer Res*. 2008;14(24):8112–8122.
62. Brown CE, Starr R, Aguilar B, et al. Stem-like tumor-initiating cells isolated from IL13Ralpha2 expressing gliomas are targeted and killed by IL13-zetakine-redirected T cells. *Clin Cancer Res*. 2012;18(8):2199–2209.

63. Chang L, Chang WC, McNamara G, et al. Transgene-enforced co-stimulation of CD4+ T cells leads to enhanced and sustained anti-tumor effector functioning. *Cytotherapy*. 2007;9(8):771–784.
64. Kunwar S. Convection enhanced delivery of IL13-PE38QQR for treatment of recurrent malignant glioma: presentation of interim findings from ongoing phase 1 studies. *Acta Neurochir Suppl*. 2003; 88:105–111.
65. Kunwar S, Chang SM, Prados MD, et al. Safety of intraparenchymal convection-enhanced delivery of cintredekin besudotox in early-phase studies. *Neurosurg Focus*. 2006;20(4):E15.
66. Vogelbaum MA, Sampson JH, Kunwar S, et al. Convection-enhanced delivery of cintredekin besudotox (interleukin-13-PE38QQR) followed by radiation therapy with and without temozolomide in newly diagnosed malignant gliomas: phase 1 study of final safety results. *Neurosurgery*. 2007;61(5):1031–1037; discussion 1037–1038.
67. Kunwar S, Chang S, Westphal M, et al. Phase III randomized trial of CED of IL13-PE38QQR vs Gliadel wafers for recurrent glioblastoma. *Neuro Oncol*. 2010;12(8):871–881.
68. Jarboe JS, Johnson KR, Choi Y, et al. Expression of interleukin-13 receptor alpha2 in glioblastoma multiforme: implications for targeted therapies. *Cancer Res*. 2007;67(17):7983–7986.
69. Bozinov O, Kalk JM, Krayenbuhl N, et al. Decreasing expression of the interleukin-13 receptor IL-13Ralpha2 in treated recurrent malignant gliomas. *Neurol Med Chir (Tokyo)*. 2010;50(8):617–621.
70. Verhaak RG, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell*. 2010;17(1):98–110.
71. Engler JR, Robinson AE, Smirnov I, et al. Increased microglia/macrophage gene expression in a subset of adult and pediatric astrocytomas. *PLoS One*. 2012;7(8):e43339.
72. Vandergrift WA, Patel SJ, Nicholas JS, et al. Convection-enhanced delivery of immunotoxins and radioisotopes for treatment of malignant gliomas. *Neurosurg Focus*. 2006;20(4):E13.
73. Mueller S, Polley MY, Lee B, et al. Effect of imaging and catheter characteristics on clinical outcome for patients in the PRECISE study. *J Neurooncol*. 2011;101(2):267–277.
74. Sampson JH, Archer G, Pedain C, et al. Poor drug distribution as a possible explanation for the results of the PRECISE trial. *J Neurosurg*. 2010;113(2):301–309.
75. Yaghoubi SS, Jensen MC, Satyamurthy N, et al. Noninvasive detection of therapeutic cytolytic T cells with 18F-FHBG PET in a patient with glioma. *Nat Clin Pract Oncol*. 2009;6(1):53–58.
76. Berger C, Jensen MC, Lansdorp PM, et al. Adoptive transfer of effector CD8+ T cells derived from central memory cells establishes persistent T cell memory in primates. *J Clin Invest*. 2008;118(1):294–305.
77. Wang X, Berger C, Wong CW, et al. Engraftment of human central memory-derived effector CD8+ T cells in immunodeficient mice. *Blood*. 2011;117(6):1888–1898.
78. Kawakami M, Kawakami K, Takahashi S, et al. Analysis of interleukin-13 receptor alpha2 expression in human pediatric brain tumors. *Cancer*. 2004;101(5):1036–1042.
79. Debinski W, Gibo DM, Slagle B, et al. Receptor for interleukin 13 is abundantly and specifically over-expressed in patients with glioblastoma multiforme. *Int J Oncol*. 1999;15(3):481–486.
80. Joshi BH, Plautz GE, Puri RK. Interleukin-13 receptor alpha chain: a novel tumor-associated transmembrane protein in primary explants of human malignant gliomas. *Cancer Res*. 2000;60(5):1168–1172.
81. Nguyen V, Conyers JM, Zhu D, et al. IL-13Ralpha2-targeted therapy escapees: biologic and therapeutic implications. *Transl Oncol*. 2011; 4(6):390–400.
82. Buonerba C, Di Lorenzo G, Marinelli A, et al. A comprehensive outlook on intracerebral therapy of malignant gliomas. *Crit Rev Oncol Hematol*. 2011;80(1):54–68.
83. Nash KT, Thompson JP, Debinski W. Molecular targeting of malignant gliomas with novel multiply-mutated interleukin 13-based cytotoxins. *Crit Rev Oncol Hematol*. 2001;39(1–2):87–98.
84. Kioi M, Kawakami K, Puri RK. Analysis of antitumor activity of an interleukin-13 (IL-13) receptor-targeted cytotoxin composed of IL-13 antagonist and Pseudomonas exotoxin. *Clin Cancer Res*. 2004;10(18 Pt 1):6231–6238.
85. Li YM, Vallera DA, Hall WA. Diphtheria toxin-based targeted toxin therapy for brain tumors. *J Neurooncol*. 2013;114(2):155–164.
86. Rustamzadeh E, Hall WA, Todhunter DA, et al. Intracranial therapy of glioblastoma with the fusion protein DTIL13 in immunodeficient mice. *Int J Cancer*. 2006;118(10):2594–2601.
87. Ou W, Marino MP, Suzuki A, et al. Specific targeting of human interleukin (IL)-13 receptor alpha2-positive cells with lentiviral vectors displaying IL-13. *Hum Gene Ther Methods*. 2012;23(2): 137–147.