REVIEW

Asunercept as an innovative therapeutic approach for recurrent glioblastoma and other malignancies

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Abstract: Glioblastoma is the most common and aggressive malignant tumor of the central nervous system. Despite the existing high unmet medical needs, the past few decades have seen no notable improvement in overall survival for glioblastoma patients. One active area of research to develop new therapeutic options for this disease is focusing on the CD95/Fas receptor and its ligand CD95L/FasL. It is now recognized that in addition to its role in programmed cell death, CD95/CD95L signaling is involved in a wide range of other apoptotic and non-apoptotic pathways directed toward T-effector cells and cells in the tumor microenvironment involved in tumor progression and invasiveness. Asunercept is a first-in-class recombinant glycosylated fusion protein, which has been designed to selectively bind to CD95L and therefore disrupt CD95/CD95L signaling. The current report provides a brief overview of the role of the CD95/CD95L signaling pathway in cancer pathogenesis and discusses how asunercept was designed to bind and neutralize CD95L and disrupt signaling thereby potentially improving outcomes in glioblastoma and other malignancies.

Keywords: CD95/CD95L, apoptosis, asunercept, APG101, glioblastoma, immuno-oncology

Short report

Glioblastoma (isocitrate dehydrogenase (IDH)-wildtype, IDH-mutant, and NOS glioblastoma according to the recent World Health Organization (WHO) classification of tumors of the central nervous system)[1] is the most common and aggressive malignant tumor of the central nervous system, with an age-adjusted incidence rate of 3.21 per 100,000.[2] Current guideline recommendations for patients with newly diagnosed glioblastoma consist of maximal surgical resection where safe and possible, and radiotherapy with concomitant and adjuvant temozolomide (TMZ) over several consecutive cycles.[3–6] However, as glioblastoma is characterized by diffuse infiltrative growth and frequently found in eloquent brain areas, extensive surgical resection is not generally feasible. Infiltrating cancer cells invariably remain, leading to recurrence and disease progression with a relative survival for the majority of patients of 12–15 months under available therapy.

Recurrent glioblastoma has no well-established standard of care. Treatment options may include repeat surgery, re-irradiation, TMZ, PCV (procarbazine, CCNU [lomustine], and vincristine) or single-agent CCNU, and supportive care where it is needed.[3,4,6] Access to ongoing investigational clinical trials is also considered a key therapeutic option, if available. However, many innovative therapeutics have failed to demonstrate clinical efficacy in newly diagnosed and recurrent glioblastoma, and in the last decade, only a few novel treatments have

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been approved.[5] In patients with recurrent glioblastoma, prognosis is poor, on average only about 1 year from diagnosis,[7,8] with 5-year survival rates less than 5.6%,[2] or even lower after disease progression.

Much research is ongoing to develop new therapeutic options for this disease. An approach targeting programmed cell death (apoptosis) may be one avenue to advance the treatment of recurrent glioblastoma, pending further confirmation in randomized, controlled trials. CD95/Fas and its ligand CD95L/FasL have been recognized for several decades for their role in apoptosis and as cancer hallmarks; however, only in recent years has clinical evidence emerged confirming CD95L as a promising novel target for the treatment of recurrent glioblastoma and potentially other malignancies. This short report describes the scientific rationale for CD95L inhibition in recurrent glioblastoma and presents evidence for asunercept, a selective CD95L inhibitor.

Role of CD95/CD95L signaling pathway in cancer

The CD95 (Fas or APO-1) receptor is a member of the tumor necrosis factor receptor superfamily and interaction with its natural ligand (CD95L) has been implicated in several pathways of cancer pathogenesis. CD95 is a transmembrane protein with an extracellular ligand-binding domain and an intracellular signaling domain. In the classical case, extracellular binding of CD95L to CD95 triggers intracellular formation of the death-inducing signaling complex and induction of cellular apoptosis, including that of cancer cells. However, apoptotic signaling is frequently disrupted in cancers, and a number of lines of evidence suggest that the CD95/CD95L pathway plays a crucial role in the escape of tumor cells from immune surveillance and in the induction of cancer resistance to radiotherapy and immunotherapy. Inhibition of CD95/CD95L interaction might, therefore, represent a potential therapeutic approach for cancer immunotherapy.[9–11]

Concomitantly, depending on the tissue and the conditions, CD95/CD95L signaling can also mediate apoptotic and non-apoptotic signaling pathways directed toward T-effector cells (CD8+), including their differentiation and infiltration into the tumor microenvironment (TME), suggesting it may serve a role as an immune checkpoint.[12] A number of other cells in the TME, including the tumor endothelium, and cancer-associated fibroblasts can also express CD95L and therefore induce T-cell apoptosis or suppress its function, for example when induced

by angiogenic growth factors.[10,11,13] Some evidence also suggests that the CD95/CD95L path might be connected to maintenance, and probably survival, of cancer stem cells (CSC), which can be associated with cancer cell persistence and relapse after radiation and/or chemotherapy, and lead to invasive growth.[10] CD95 is required for CSC survival and stimulation of CD95 on multiple tumor cells has been shown to result in conversion from non-CSC to CSC, independent of its apoptosis-inducing function, suggesting that CD95 expression could be a reliable surface marker for targeted therapy.[14]

Scientific rationale for CD95L inhibition as a therapeutic approach in recurrent glioblastoma

Increasing evidence shows that the non-apoptotic CD95/CD95L signaling pathways play an important role in glioblastoma invasiveness and progression, which is characterized by infiltrative and rapid growth of tumor cells into the surrounding intact brain tissue. The binding of CD95L to the CD95 receptor on glioblastoma cells stimulates tyrosine kinases including the Yes member of the Src family and the p85 subunit of phosphatidylinositol 3 kinase (PI-3K), which along with increased expression of matrix metalloproteinase (MMP) have been described as underlying mechanisms of tumor invasion.[15] When CD95 activity was neutralized, the invasive migration of glioblastoma cells was significantly reduced.[15]

It has been suggested that the recurrence-initiating cancer cells that emerge from the tumor cell population surviving initial therapy have stem cell-like properties because they can initiate a recurrent glioblastoma with a diversity of tumor cells.[16] In addition, such cells are more aggressive than primary tumor-derived glioblastoma stem cells,[17] which is consistent with the shorter survival of patients with recurrent glioblastoma; median overall survival after recurrence is 6.2 months despite treatment.[18]

Given the important role of CD95/CD95L signaling in tumor progression and recurrence, a number of lines of research are focusing on this pathway as a therapeutic target for recurrent glioblastoma. Asunercept is a first-in-class recombinant glycosylated fusion protein comprising the extracellular domain of human CD95 linked to the Fc domain of human IgG1. It has been designed to selectively bind to CD95L and therefore disrupt CD95/CD95L signaling. The scientific rationale for asunercept in recurrent glioblastoma is supported by a number of in vitro and in vivo non-clinical studies. CD95L is overexpressed in glioblastoma cells, which

are largely resistant to CD95-mediated apoptosis resulting in their growth and invasiveness. Stimulation of glioblastoma cell lines (both apoptosis-resistant and apoptosis-sensitive) with CD95L increased migration of cells in the apoptosis-resistant cell line, but not in the apoptosis-sensitive cell line, and could be prevented by blocking MMP activity.[15] In murine glioblastoma cell lines, knockdown of the CD95L gene had no effect on overall cell viability nor the ability of the cells to form tumor spheroids, but it reduced tumor cell invasiveness, which could be restored by addition of soluble recombinant CD95L and inhibited by asunercept.[19] Together these results suggest that CD95L from both autocrine and paracrine sources can contribute to the invasive nature of glioblastoma cells, and that asunercept can reduce this invasiveness by neutralizing CD95L.

In vivo models involving intracranial injection of cells from a murine glioblastoma into a syngeneic host may better reflect the TME and have illustrated the requirement for tumor/host interaction. Two weeks after intracranial injection there was significantly increased expression of both CD95 and CD95L in cells from the implanted tumor compared with levels expressed in cultured cells.[15] Furthermore, when cells extracted from the implanted tumors were incubated with a CD95L neutralizing antibody, the invasive potential of the tumor cells was reduced by approximately 50%. Using a similar syngeneic mouse model of glioblastoma, asunercept was associated with a dose-dependent inhibition of tumor growth when assessed by MRI volumetry.[20]

Non-clinical models also demonstrate an increase in invasive growth of glioblastoma cells following radiotherapy, similar to that which is observed in patients. However, asunercept treatment was able to counteract this radiotherapy-triggered invasive potential of glioma-derived malignant cells and was associated with a survival advantage of up to 18 days over the control-arm ($p<0.05$).[20] Interestingly, in the same research, asunercept labeled with ^{123}I crossed the blood-brain barrier and enriched glioblastoma cells, suggesting a synergistic effect in combination with radiotherapy or chemotherapy.[20]

Asunercept clinical evidence in recurrent glioblastoma patients

The rationale for disruption of the CD95/CD95L pathway with asunercept and its clinical implications have been confirmed in a proof-of-concept Phase II study which evaluated the combination of asunercept and radiotherapy vs radiotherapy alone in patients with recurrent glioblastoma.[21]

Eligible patients were those experiencing recurrence of a histologically confirmed glioblastoma that was either not resectable or in whom there remained residual tumor after resection. Rates of progression-free survival (PFS) at 6 months were 3.8% (95% CI 0.1–19.6) for radiotherapy alone and 20.7% (95% CI 11.2–33.4) for the asunercept/radiotherapy combination (p=0.048). Median PFS was 2.5 months (95% CI 2.3–3.8 months) and 4.5 months (95% CI 3.7–5.4 months), respectively, with a hazard ratio of 0.49 (p=0.0162). In this trial, the combination of asunercept and radiotherapy was well tolerated with no serious adverse events and no impairment of radiotherapy tolerability. A recent update on overall survival of patients has revealed that 7% of asunercept/radiotherapy-treated recurrent glioblastoma patients were alive after 5 years compared to 0% of the patients treated with radiotherapy alone.[22] The combination was associated with a significant improvement in time to first deterioration and maintained quality of life compared with radiotherapy treatment alone.[23]

In an attempt to identify biomarkers that predict the treatment response of asunercept, archived primary tumor tissues from patients with glioblastoma at first or second progression were analyzed for expression levels of CD95L and DNA methylation status of the CD95L promoter.[21,24] These studies suggested that patients displaying a low level of methylation responded best to asunercept therapy (hazard ratio 0.19, 95% CI 0.06–0.58). There was a significant survival benefit for patients with low methylation when treated with a combination of asunercept and radiotherapy vs radiotherapy alone (HR 0.34, p=0.024), suggesting differential CD95L promoter methylation may represent a potential biomarker to predict response to asunercept treatment. Preliminary results suggest that CD95L promoter methylation correlates with the respective transcriptional activity and might, therefore, be responsible for regulating CD95L protein levels. Further research is warranted to determine whether this potential biomarker can also predict which patients would respond best to a combination of asunercept with radiotherapy or with chemotherapy.

Asunercept clinical evidence in myelodysplastic syndrome (MDS) patients

The CD95/CD95L signaling pathway and asunercept, as a selective CD95L inhibitor, offer broad potential applicability to a range of malignancies with aberrant CD95/CD95L signaling, beyond recurrent glioblastoma patients. In MDS, a decrease in physiological erythropoiesis is associated with

increased apoptosis of CD34+ hematopoietic progenitor cells. CD95 is overexpressed on CD34+ progenitors and erythrocytes in around two thirds of patients with lower risk MDS, and its activation is thought to negatively regulate erythrocyte production in the bone marrow.[25] Hypothesizing that asunercept inhibition of CD95L signaling would protect the CD34+ erythrocyte precursors from CD95L-induced apoptosis, a proof-of-concept study was conducted in 20 transfusion-dependent low- and intermediate-risk MDS patients resistant to treatment with erythropoiesis-stimulating agents. Asunercept administration was associated with a decrease in transfusion need and frequency and was well tolerated.[26] Analysis of bone marrow biopsies from these patients revealed that the decreased transfusion need during the study was associated with an increase in burst-forming unit-erythroid progenitor activity compared to baseline levels.[26] These results confirm that direct inhibition of CD95/CD95L signaling plays an important role in rescuing erythropoiesis with potential clinical implications for hematological malignancies such as MDS.

Asunercept safety and tolerability

The safety and tolerability of asunercept have been evaluated in healthy volunteers as well as in proof-of-concept clinical studies in patients with recurrent glioblastoma and transfusion-dependent patients with low- and intermediate-risk MDS.[21,26,27] The proportions of subjects reporting any adverse event after administration of asunercept or placebo were similar at 60.0% and 57.1%, respectively.[27] Only one adverse event in an asunercept-treated subject, one episode of mild headache, was considered related to the investigational medicine. In patients with recurrent glioblastoma randomized to asunercept 400 mg/week plus radiotherapy or radiotherapy alone, the combination was well tolerated and not associated with any serious adverse events.[21] Mild and moderate nervous system disorders, mostly headache, were more common in the combination group (20.7% and 53.4%, respectively) than in patients receiving radiotherapy alone (15.4% and 30.8%, respectively). Asunercept was tested in a clinical study with low- and intermediate-risk MDS patients who received doses of 100 mg or 400 mg/week. The relationship of adverse events to investigational medication was suspected with a possible or probable categories in 9 (45%) patients.[26] Overall two patients experienced serious adverse events that were suspected to be drug-related, one patient experienced a hypersensitivity reaction and one patient with pre-existing neutropenia had septic complications.

Potential implications in other tumors

As research with the selective CD95L inhibitor asunercept advances, opportunities might be directed toward other tumor types with high levels of CD95L expression on cancer- and TME cells. Non-clinical studies conducted using the TiRP mouse melanoma model, which mimics resistance signature and TME observed in human melanomas, have shown that these tumors are enriched in myeloid-derived suppressor cells which express high levels of CD95L and subsequently induce apoptosis of tumor-infiltrating lymphocytes.[9] Importantly, this could be prevented by inhibiting CD95L, providing a rationale for the use of asunercept beyond recurrent glioblastoma, for example in cutaneous melanoma. It is reasonable to consider that the use of asunercept may be particularly effective in combination with other immune checkpoint therapies antagonizing T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1).[28] Analysis of human cancer tissue samples has also found high levels of CD95L expression associated with certain types of breast, colon, renal, bladder, prostate, head and neck, pancreatic, and ovarian adenocarcinomas,[11] suggesting that targeting the CD95/CD95L pathway with asunercept may also offer a promising innovative treatment approach and should be considered for further evaluation and confirmation in clinical studies.

Conclusion

Selective inhibition of the CD95/CD95L pathway plays a critical role in reducing the invasive growth that characterizes recurrent glioblastoma and potentially other tumors with high levels of CD95L expression, and in parallel serves to improve the ability of cytotoxic T cells to infiltrate tumors. Proof-of-concept studies have demonstrated that asunercept, a fully human selective CD95L inhibitor, might provide clinical efficacy in patients with recurrent glioblastoma and low to intermediate-risk MDS with a good tolerability profile at intended therapeutic dose. Targeting the CD95/CD95L pathway with a tailored therapy such as asunercept may, therefore, offer a promising innovative treatment alternative and is currently under further clinical evaluation.

Disclosure

AK and CG are full time employees of Apogenix AG. AK was a former Hexal AG employee within the last 36

months. The authors report no other conflicts of interest in this work.

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