

TARGETED THERAPY – A HOPE FOR MALIGNANT GLIOMA TREATMENT

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ABSTRACT

Malignant glioma therapy represent one of the greatest challenges in neuro-oncology. Despite technical advances in neurosurgery, radiotherapy and chemotherapy the prognosis of most patients remains very poor. Advances in understanding the molecular mechanisms involved in malignant glioma pathogenesis helped researchers to develop new agents that target the genetic and cellular alterations. This review presents the targeted agents used in research and clinical trials for malignant glioma treatment.

Key words: malignant glioma, therapy, targeted agents

INTRODUCTION

Primary CNS (central nervous tumors) account for 1.35% of all cancers and 2.2% of all cancer-related death. (1) Glial tumors represent 42% of all primary CNS cancers, and over three quarters are malignant. (2). Every year over 12,500 new cases are diagnosed in the United States (3).

Gliomas represent a group of cancers which have in common a presumed glial cell of origin, and these tumors include: astrocytomas, oligodendrogliomas, oligo-astrocytomas, ependymomas and choroids plexus tumors. World Health Organization (WHO) classifies gliomas based on their degree of differentiation and anaplasia in:

- pilocytic astrocytomas – grade I – relatively benign, localized, noninfiltrating tumors that generally occur in pediatric population
- low-grade or diffuse astrocytomas – grade II – tumors of well-differentiated cells that grow slowly but invade normal brain structures
- malignant or high-grade gliomas which include:
 - Anaplastic astrocytomas (AA) or oligodendrogliomas (AO) – grade III
 - Glioblastomas (GB) – grade IV

Which are characterized by the presence of mitoses, cellular and nuclear atypia. (4,5) They are among the most devastating neoplasms, and in spite of advances in therapy the mean survival time range from 9 to 12 month after initial diagnosis. Only 26.5% of recurrent glioblastoma multiforme (GBM) patients that receive optimal therapy survive 2 years (6).

Malignant gliomas occur most frequently between the ages of 40 and 60 and their clinical

presentation depends on the location of the tumor. GB is usually centered in the deep white matter of the cerebral hemispheres, most frequently in the frontal lobe. They manifest with mental and personality changes as early symptoms. Less than 50% of patients present the triad: headache, epilepsy and hemiparesis.

The management of glioma patients involves providing effective supportive care associated with definitive antitumor therapy (7). The definitive antitumor treatment usually consists of maximal surgical resection followed by radiation therapy which are considered the standard of care. Adjuvant chemotherapy appears to have some limited efficiency (8-11). Recent advances in understanding of molecular biology of gliomas have revealed genetic and cellular alterations that may lead to GB. The knowledge of these abnormalities have been translated into new therapies that target alterations in cell-signaling pathways and bring hope for patients with malignant brain tumors.

TARGETED THERAPY

Definitions

The US Food and Drug Administration (FDA) considered targeted therapy as a drug with an approved label in which there is a specific reference to a simultaneously or previously approved diagnostic test that must be performed before the patient can be considered eligible to receive the drug (12).

For oncologists, targeted therapy is defined as a drug with a focused mechanism that specifically acts on well-defined target or biologic pathway that, when inactivated, causes regression or destruction

of the malignant process. A monoclonal antibody is an additional type of targeted therapy.

Molecularly targeted therapy is represented by substances that kill cancer cells by targeting key molecules involved in cancer cell growth. The features of ideal anticancer target are:

- must be crucial to the malignant phenotype
- must not be significantly expressed in vital organs or tissues
- its biological relevance can be measured reproducibly in readily obtained clinical samples
- it is correlated with clinical outcome
- the clinical response must be obtained in a significant proportion of patients whose tumors express the target when target interrupted, interfered with or inhibited
- the minimal responses must be obtained in patients whose tumors do not express the target (13)

Targeted therapies for malignant gliomas

The agents that have been investigated for GB treatment include:

- Monoclonal antibodies
- Ligand conjugates
- Tyrosine kinases and other small molecule inhibitors
- Antisense oligonucleotides (14)

Monoclonal antibodies

Monoclonal antibodies (mAbs) recognize epitopes with high selectivity and affinity and they can initiate immune responses including complement-mediated or antibody-dependent cell-mediated cytotoxicity. They might also block protein-protein interactions such as: growth factor-receptor and integrin-ligand (15).

The efficacy of this therapy depends on several factors:

- the creation of non-immunogenic monoclonal antibodies
- the tissue dynamic of the tumor
- the integrity of targeted antigen
- the route of administration (16)

It is already known that the delivery of the substances into the brain is tightly regulated by the blood-brain barrier (BBB) permeability. In the progression of malignant brain tumors the hypoxic areas of tumor show alterations of normal BBB integrity (17,18). In consequence the large molecular weight of antibodies is likely to result in inefficient drug delivery into the brain. For this reason monoclonal antibody therapy is often delivered intratumorally.

Several mAbs have been studied *in vitro* and *in vivo*. Cetuximab is a chimeric human-mouse antibody against EGFRvIII, which binds to EGFR (epidermal growth factor receptor) and prevents ligand binding. Therefore the antibody blocks ligand induced tyrosine kinase activation and stimulates receptor internalization. It appears to induce apoptosis and inhibit angiogenesis *in vitro* (19-21). Cetuximab has not been clinically trialed in gliomas, because of disappointing results against lung cancer. Other mAbs that have gone under investigation including clinical trials are: mAbR3 which reached phase I clinical trial with encouraging results (22), mAb425 (EMD55,900). A phase I/II trial in 16 previously treated patients diagnosed with glioblastoma multiforme (GBM) or anaplastic astrocytoma (AA) found no therapeutic benefit. But mAb425 conjugated with I-125 have been conjugated to demonstrate a significant increase in median survival (23-25). Another antibody mAb528 have been shown to inhibit glioma proliferation *in vitro* and *in vivo* (26). The anti Δ EGFR antibody 806 inhibits growth factor xenografts in nude mice. The mAb recognizes the wild type of the receptor when it is highly expressed. This might give the antibody some efficacy against gliomas that overexpress EGFR as well as those that express Δ EGFR (27,28).

Another monoclonal antibody is 81C6 which is a I-131 tagged mAb against tenascin. Tenascin is an extracellular matrix glycoprotein that is highly expressed by gliomas, but is not expressed in normal brain. It's located in association with the perivascular matrix and may play a role in promoting angiogenesis as well as invasion (29-31). The phase II clinical trials with mAb81C6-I-131 injected directly into a surgically created resection cavity, followed by conventional external-beam radiation therapy and alkylator – based chemotherapy showed survival data exceeded that of historical controls treated with conventional radiation therapy and chemotherapy. Randomized phase III trials are underway (32,33).

Antibodies that undergo internalization have been armed with either radioisotopes or toxins to initiate cell killing. Radioisotopes have included: α -emitter At211, and β and λ -emitter I-131. They allow inside-out delivery of focal radiation to tumor bed. Toxins have included: Pseudomonas exotoxin and Diphtheria toxin both of which can kill a cell with a single internalized molecule (15).

Ligand – toxin conjugates

Glioma cells have some cell-surface receptors that show high-affinity to ligands. The ligands for

these receptors can be engineered to deliver a toxin at high selectivity and affinity. The delivery might be limited by the inherent binding propensity of growth factors. Currently a recombinant fusion protein (IL-13-PE38QQR) composed of IL-13 (interleukin 13) (34-36) and a mutated form of *Pseudomonas* exotoxin is being studied in brain tumor clinical trials. Other clinical trials have been initiated to evaluate the same idea but using other ligands such as: TGF- α (transforming factor alpha), IL-4 (interleukin 4), transferrin (37,38).

Tyrosine kinase inhibitors

Tyrosine kinase inhibitors (RTKs) are a new class of small-molecule drugs. These drugs interfere with intrinsic tyrosine kinase activity blocking receptor autophosphorylation and activation (39).

There are several classes of small molecule RTK-inhibitors such as:

- compounds that compete at the ATP binding site of RTKs (quinazolines, pyridopyrimidines, phenylaminopyrimidines)
- molecules that act in a non-competitive fashion against ATP or peptide substrates (indoles, oxindoles)
- thyrphostins which are competitive inhibitors of RTKs at either or both ATP or substrate binding sites (40)

Several receptor tyrosine kinases inhibitors have been tested in brain tumor clinical trials.

EGFR INHIBITORS

The majority of malignant gliomas have aberrant EGFR activity through EGFR overexpression, amplification or mutation (41). The EGFR gene is amplified in more than 40% of glioblastomas and forty percent of tumors with EGFR amplification also have a constitutively active mutant (EGFRIII) (42,43). Several small molecule inhibitors of the EGFR such as: Gefitinib (Iressa, ZD1839) and Erlotinib (Tarceva, OSI-774) have been evaluated in malignant gliomas. The agents are generally well tolerated but responses tend to be limited (44,45).

PDGFR INHIBITORS

Overexpression of platelet-derived growth factor (PDGF) and PDGF receptors (PDGFR) is thought to occur early in gliomagenesis (46). PDGF tyrosine kinase inhibitors like Imatinib mesylate (Gleevec, STI-571) have also been tested in clinical trials. The North American Brain Tumor Consortium (NABTC) and the European Organization for

Research and Treatment of Cancer (EORTC) conducted phase II trials of Gleevec in patients with malignant gliomas and observed only minimal activity (47,48).

VEGFR INHIBITORS

Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) play a key role in stimulating angiogenesis and their expression is associated with clinical outcome in high grade gliomas (49). Small molecule inhibitors against VEGF and VEGFR such as semaxanib (SLK5416) are currently in clinical trial stage for recurrent malignant glioma (50).

INHIBITION OF PI3K/AKT PATHWAY

Malignant gliomas often display dysregulation and overactivation of the phosphatidylinositol 3-kinase pathway (PI3K/PTEN/AKT pathway) (51). Several agents that inhibit the PI3K pathway are being studied. LY294002 is an inhibitor that has shown efficacy in cultured glioma cells (52). Also mTOR has emerged as an interesting therapeutic target. mTOR inhibitors such as temsirolimus (CCI-779) have shown modest activity as monotherapy for gliomas (53,54).

INHIBITION OF THE Ras /MAPK PATHWAY

In malignant glioma cell lines exists an increased expression of Ras (55). Farnesyltransferase-inhibitors such as: tipifarnib (R111577) and Iorafarnib (SCH66336) have shown results in preclinical studies and are being tested in clinical trials for malignant gliomas (56,57).

Antisense Oligonucleotides

It is a strategy used to induce growth inhibition in cultured glioma cell lines and *in vivo* studies but has not been tested in a clinical trial. Antisense oligonucleotides effects are cytostatic and time and dose dependent. Their delivery to tumors has also been problematic. Various molecules implicated in gliomagenesis have been targeted experimentally by antisense oligomers such as: fibroblast growth factor, telomerase, p21waf1, matrix metalloproteinase-9, EGFR, MDM2 (58-63).

CONCLUSIONS

Recent advances in molecular biology helped us to understand the genetic and cellular alterations that may lead to malignant gliomas. This knowledge

is very important to developing new targeted therapies for GB treatment. These new drugs have as target: membrane receptors, signaling pathways and proteins or factors which are important in cell cycle

regulation or angiogenesis. They may also be more selective, less toxic to normal cells and may prevent tumor growth. Therefore the targeted therapy brings hope for malignant glioma diagnosed patients.

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