Personalized Medicine for Gliomas

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Abstract

Personalized medicine for cancer entails tailoring therapy for each patient based on unique features of the patient's tumor; physiologic, molecular, genetic and epigenetic. Our ability to molecularly characterize tumor cells has increased dramatically and shown that there are significant differences between samples from patients with the same tumor type. Given this extensive variability in mutations and pathways driving tumors in patients, seeking a single bullet is an unrealistic approach for achieving a cure. In glioblastoma multiforme (GBM), the most common adult brain tumor, this inter-tumoral heterogeneity is further complicated by intra-tumoral heterogeneity within the tumor. This suggests that for personalized therapy to work for GBMs, pharmacologic agents would not only be tailored to target the differences from patient to patient but also the clonal diversity within each patient's tumor. In this review, we provide a historical perspective on clinical trials for cancer. We also discuss the current state of molecular biology and immunology based strategies for personalized therapies for glioblastoma multiforme.

Key Words: Glioma, glioblastoma multiforme, personalized medicine, targeted therapy, tumor heterogeneity, tumor vaccines

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INTRODUCTION

Personalized medicine for cancer entails tailoring therapy for each patient based on genomic or epigenomic mutations unique to the tumor. High throughput analysis of hundreds of glioblastoma multiforme (GBM) patient samples show that gliomas may contain many types of mutations, including mutations to *TP53, INK4A/ARF, PTEN or NF-1*.[4,31] Some of these mutations are thought to contribute to the progression of the tumor.[16,31] Although the diagnostic criteria for GBMs are the same during pathological analysis, these tumors demonstrate significant histological diversity. It is speculated that combinations of distinct mutations contribute to this diversity and instil differential sensitivities to conventional therapy.[2,35,36] Therefore, proponents of personalized medicine believe that each tumor therapy be based on the patient's own unique set of molecular alterations.

The fight against cancer in the 1950s was driven by the philosophy that one did not need to completely understand the molecular and cellular biology to find a cure.[17] Paediatric pathologist, Sidney Farber began screening anti-microbial against different cancer types such as leukemias, lymphomas, sarcomas. He was ultimately successful with inducing remission in wilms tumor patients albeit the presence of devastating side effects. Others such as the National Cancer Institute's

Emil Freireich and Emil Frei reasoned that if one agent showed any efficacy for leukemia and two agents lead to remission, three or four agents would overcome resistance and cure the disease. Although these combinations induced remission and showed for the first time that inducing remission in cancer was a possibility, the cancer invariably recurred and the side effects during treatment were devastating. These findings re-ignited a focus on understanding the basic underpinning of cancer. A University of Chicago urological surgeon named Charles Huggins, who was concerned about the highly variable course of prostate cancer made an important physiologic discovery that changed our approach to cancer therapy and clinical trials. He observed that while some patients with prostate cancer lived with the disease indolently, others developed aggressive and metastatic lesions resulting in a painful disease course. Huggins recognized that there was something inherently different about the aggressive form of prostate cancer. He reasoned that since estrogen controlled the growth of breast tissue, male hormones by analogy could also control the growth of prostatic tissue. His studies demonstrated that injecting estrogen (Premarin) stopped the endogenous production of testosterone resulting in prostate cancer remission. Although patients eventually relapsed, it proved that we may not have to rely in indiscriminate use of DNA damage-inducing agents. It is possible to selectively target cancer sub-types based on unique clinical, physiologic, molecular observations using less toxic agents and without unwanted side effects.[17] Premarin use in metastatic prostate cancer represents one of the earliest use of personalized medicine for cancer based on clinical observation and physiology.

More recently, the promise of personalized medicine for cancer using molecular information was exemplified following the discovery of Imatinib (*Gleevac®*), a drug that inhibits a specific over-active tryosine kinase fusion protein BCR-Abl in chronic myelogenous leukemia (CML). Given that this translocation event represents the driver mutation in CML, *Gleevac* became one of the first cancer drugs to selectively target cancer cells based on a unique alteration resulting in clinically significant remission in patients. Following this discovery, identification of similar targets in other cancer types became and remains an intense area of research. Unfortunately, targeting single gene products or alterations may not be feasible in a majority of tumors especially GBMs given significant clonal diversity inherent to this tumor type. This represents one of the most frustrating aspects of research seeking to develop targeted therapy or personalized medicine for GBMs.

This review provides an overview of three unique and cutting edge strategies for personalized medicine for GBM. These include targeting inter-tumoral heterogeneity, inter-tumoral heterogeneity and tumor immunology. We also describe the state of research in each of these areas and speculate on diagnostic methods that may guide clinical decision making in the future.

Classification of gliomas

Gliomas are classified into four grades by the World Health Organization (WHO) based on pathologic features of such as cellularity, pleomorphism, endothelial proliferation/abnormal angiogenesis, mitotic figures and necrosis [Table 1].[15] Glioblastoma multiforme (GBM) represents the worst grade of gliomas (Grade IV) and is also the most common form of primary brain tumors in adults. Although the WHO grades have distinct median survival differences between grades (I: 8-10 years, II: 7-8 years, III: 2 years, IV:<1 year), it does not account for the variability in response to therapy within each grade that may be driven by heterogeneity at the molecular and cellular levels. Consequently, the goal of targeted therapy for glioma is to develop a clinically relevant algorithm that predicts response to specific therapy based on patient specific molecular/cellular features.

Targeted therapy for glioblastoma multiforme

There is currently no Food and Drug Administration (FDA) approved drugs designed for personalized therapy for patients with gliomas. There are signs, however, that this advance is in the near future. The DNA alkylating agent Temozolomide (TMZ) improves the survival of patients with GBM when used in combination with radiation therapy. Furthermore, GBMs with hypermethylation and suppression of O-6-Methylguanine DNA methyltransferase (*MGMT*) are more sensitive to the TMZ.[12] *MGMT* hypermethylation, however, represents a small minority of patients with GBM.[12] Given that TMZ plus adjuvant radiation improves survival of patient irrespective of *MGMT* methylation status and the lack of an alternative agent with clinical efficacy, designing prospective randomized clinical trials where one group of patients receive TMZ and others becomes problematic and unethical. So at this point, the sensitivity of *MGMT* hypermethylated tumors to TMZ only represents proof of concept that supports targeting a sub-set of GBM patients with specific molecular signatures. In the future, as more chemotherapeutic agents with similar efficacy are developed based on molecular alterations, it may be possible to design clinical trials assessing the differential sensitivities of patients with different molecular signatures and alterations to chemotherapy.

Inter-tumoral heterogeneity

Four GBM sub-types were recently reported based on gene expression profiling [Table 2].[34] These include classic, neural, pro-neural and mesenchymal sub-types. Each sub-type is driven by different molecular alterations, demonstrate differential responses to therapy and differ

Table 1: WHO classification of gliomas

Grade	Tumors	Median survival (years)
I	Pilocytic astrocytoma	8-10
II	Diffuse astrocytoma	7-8
III	Anaplastic astrocytoma	2-3
IV	Glioblastoma multiforme	<1

Table 2: Molecular classification of glioblastoma

Sub-classification	Frequently mutated genes
Proneural	TP53 (54), IDH1 (30), PIK3R (19), EGFR (16), PDGFRA (11)
Neural	EGFR (26), TP53 (21), PTEN (21), ERBB2 (16), NF-1 (16), PIK3R (11)
Classic	EGFR (32), PTEN (23), EGFRvIII (23)
Mesenchymal	NF-1 (37), TP53 (32), PTEN (32), RB1 (13)

in terms of survival.[6,34] While the mesenchymal sub-type demonstrated the worst prognosis, the pro-neural sub-type showed longer overall survival. Furthermore, there was a significant benefit from more intense chemo-radiation in the classic and mesenchymal sub-type. This effect was not seen in the pro-neural sub-type. In the next few sections we present ongoing translational and clinical efforts using available molecular information to create personalized treatments for patients with GBMs.

There is significant progress towards developing a robust pre-clinical model for testing the susceptibility of the various GBM sub-types to anti-cancer agents. To achieve this, the predominant molecular alteration within each GBM sub-group is over-expressed or deleted within specific clone of cells using cell type specific factors unique to the clone as drivers for recombinase enzymes (for instance, *Cre*-loxP recombinase system® for genetic deletion). Interestingly GBMs have arisen from inducing recombination in mouse neural stem cells, oligodenrocyte precursor cells or astrocytes.[14,16] Although, this approach is slow and tedious given that mutations/deletions to single genes rarely cause malignant transformation, it has led to the development of some robust models for each GBM sub-type.

The pro-neural sub-group of GBM is dominated by platelet derived growth factor receptor (PDGFR) signalling.[13,34] Forced expression of *Pdgf-A/B* in mice brains leads to glioma formation that mimics low grade and high grade gliomas with good penetrance. The grade of these tumors is further determined by other mutations including *p53* or *Ink4a/Arf*. Interestingly, expression profiling of these mouse tumors are similar to the pro-neural sub-type in humans. Therefore, there are ongoing pre-clinical studies evaluating the susceptibility of this sub-type to various chemotherapeutic agents including PDGFR-A inhibitors and anti-Vascular endothelial growth factor (VEGF) therapy.[13] The classic sub-type is characterized by an

activated Epidermal growth factor receptor (EGFR).[34] Recent studies in mice show that forced expression of a constitutively active variant *Egfr version III (vIII)* alone, in some cases, results in GBMs that are similar to classic human GBMs.[13] Ongoing studies are also underway to evaluate the utility of this model. The mesenchymal subtype of GBM is driven by *NF-1* and *TP53* mutations. Clinically, this sub-type also shows the worst prognosis of all GBM sub-types.[6,31] The tumors derived from these mice however show variable similarities to both mesenchymal sub-types and pro-neural sub-type. Finally, no specific drivers have been deciphered for the neural sub-group, so there are no mouse models that mimic this sub-type of GBMs. Ultimately, we suspect that these models will hopefully provide clues about the differential susceptibility of each GBM sub-type to various chemotherapeutic agents.

Following pre-clinical studies in mouse models using candidate chemotherapeutic agents, clinical trials that assess the effectiveness of these agents in clinic would be warranted. Currently, there are no prospective randomized double blind clinical trials designed using these GBM sub-types as enrolling criteria. In the foreseeable future however, retrospective analysis of prior trials may help determine if these sub-types have clinical relevance for therapy. Results from such analysis would inform the design of prospective randomized clinical trials using molecular information derived from large scale studies such as the cancer genome atlas (TCGA). Given our limited understanding of tumor heterogeneity, however, these studies remain premature and may not yield any useful results. Further complicating issues is the possibility that these GBM sub types potentially interconvert during the evolution of glioma formation. Ultimately, if the mutations that define the subgroups occur late in the evolution of GBM development they may not be very good therapeutic targets.

In tens of diagnostics, research into non-invasive means of detecting the GBM sub-types is ongoing.[18] Several studies have shown that MRI combined with NMR spectroscopy could be usefull for detecting specific glioma subtypes such as the mesenchymal sub-type[18] or Isocitrate dehydrogenase 1 (*IDH1*) mutant tumors without invasive tests.[5,8] In the future, there may be serum or CSF biomarkers that can also be used to detect each sub-type and pre-emptively assign patients to appropriate treatment arms.

Intra-tumoral heterogeneity

The discovery of cells with different driver mutations existing side by side within tumors suggests that targeting a single mutation may be an ineffective strategy in GBMs. Recent evidence suggests that cells with mutations to Epidermal growth factor receptor (*EGFR*), Platelet derived growth factor receptor (*PDGFR*) and

Receptor tyrosine kinase (*RTK*) co-exist within the same GBM.[29,30] Therefore, a single agent targeting the EGFR mutation may not show efficacy in these tumor types.[9] This has led to the search for a common progenitor cell type within GBM such as the cancer stem cell (CSC) or tumor initiating cell (TIC). It is hypothesized that during tumor progression, multi-lineage differentiation potential of CSCs allows for molecular divergence in response to micro-environment induced stress. Targeting these putative mediators of intra-tumoral heterogeneity, however, remains a difficult task *in vitro* and *in vivo*.

For instance, till date, no mouse model demonstrates the significant intra-tumoral heterogeneity seen in human GBM. Some have speculated that developing a mouse model that is constantly evolving may result in a heterogenous population.[13] Mouse cell types, however, may not possess the ability to evolve or not be under the same pressure from the micro-environment to evolve or not have enough time to evolve in the context of a pre-clinical study.[19-21] To overcome this, induction of stress via hypoxia may be needed to force tumor evolution and promote heterogeneity. Another approach, may involve the development of mouse models from CSCs or TICs that have the inherent ability/plasticity to undergo multi-lineage differentiation during tumor initiation and progression in mice.

Clinical trials targeting intra-tumoral heterogeneity are not in the foreseeable future because there is limited understanding of drivers of heterogeneity-micro-environment, unstable genome, tumor evolution and terminally blocked CSC differentiation. Furthermore, the functional relevance of various clones within GBM remains unclear. Do these clones show a hierarchal or stochastic co-existence? If hierarchal, then the goal clinically would be to target the founder cell or the cancer stem cell potentially with single agents.[9,10] If, however, the organization of GBM cells is stochastic, a multi-agent approach will be necessary to eradicate all tumor forming clones within the GBM.

Clinically, determining the extent of tumor heterogeneity would require a tumor biopsy. Following this, some have used fluorescent *in situ* hybridization (FISH) to detect the identity of multiple clones within GBM.[29,31] Theoretically, these data could then be quantified to determine the predominance of specific clones. Such information would ultimately guide clinical decision making. It may be feasible to quantify the predominance of each of these mutations in a given GBM sample and use this information to guide further therapies. Unfortunately, drugs targeting these 'predominant clones' may only provide temporary remission as other clones re-emerge to re-populate the tumor. If a drug, however, were to target the source of these different clones of cells within cancer such as the hypothesized TIC or CSC, then intra-tumoral heterogeneity and consequently tumor recurrence could be blunted by administering anti-CSC agents in addition to conventional therapy.

Tumor immunology
The differential expression of antigens also provides an avenue for personalized medicine for GBMs. Tumor vaccines are being developed from exposure of immune cells to patient tumor antigens.[1,11,34] Administration of these vaccines to patients will theoretically allow for selective targeting of patient tumors. This aspect of personalized medicine could be used to selectively target a clone of GBM cells such as the cancer stem cell that hypothetically re-constitutes the tumor following treatment. Each patient may have a different antigen expressing GBM cancer stem cell therefore; this treatment provides a personalized intervention targeting an important sub-population within cancer.[24] In the future, flow cytometry may allow for sorting of specific tumor clones based not only on surface markers but also on functional characteristics such as self-renewal and differentiation potential. Following sorting, tumor vaccines could then be established against these cells and administered to the patient to hypothetically prevent recurrence.

Mouse studies over the last several decades have utilized patient derived tumor xenografts (PDX) to perform pre-clinical tests on hundreds of putative anti-cancer agents.[28] Although some of these trials have led to major advances in select cancer types, these pre-clinical studies have not yielded any promising agents for GBMs. Moreover, this approach may be problematic for evaluating classic immunologic based strategies that utilize immune cells such as dendritic cells. The most obvious reason is that most PDX are derived from immunocompromised mice strains such as nude mice (athymic; deficient mature T-cells) or severe combined immunodeficiency (*scid*) mice strains like NOD *scid* gamma (NSG; Deficient T and B cell, natural killer cells, complement and dendritic cell function) mice and BALB *scid* (T and B-cell deficient).[27]

Given these constraints, some have taken a unique approach to immunologic studies for GBM in genetically engineered mouse models (GEMMs) with intact immune systems. It was observed that GBM associated macrophages promote tumor progression. A recent study using an RCAS-*hPDGFB* driven GEMM showed that inhibition of macrophage colony stimulating factor 1R (CSF-1R) and hence GBM associated macrophages did not kill the macrophages but coerced them to eliminate glioblastoma cells.[25] These results demonstrate that the immune system is an integral part of tumor biology. It also demonstrates that in patients with GBMs that elicit a significant macrophage response, CSF-1R inhibition may provide one avenue for slowing tumor progression. Clearly, in addition to vaccine development, some are targeting other aspects of tumor immunology to curb tumor progression.

Several clinical trials are underway assessing the efficacy of targeting tumors via the immune system. Some expose immune cells such as dendritic cells to either single or multiple antigens.[24] One of such co-operative Phase II trials called ACTIVATE, used a peptide vaccine that induces the immune system to target the constitutively active EGFR *viii* receptor.[7] The results demonstrated a median survival of 26 months for GBM patients (Compared to 14 months with standard therapy). Another trial (ACT II) incorporated Temozolomide, the standard of care in a multi-center study to see if there is an improvement in survival for patients with this devastating disease. These results have led to the initiation of a Phase III trial using the same tumor vaccine. These results demonstrate that as more unique antigens are characterized, this will remain a promising area of personalized medicine for GBM.

Non-vaccine mediated immunologic strategies are also demonstrating efficacy in human clinical trials for other cancers. It was shown that antibody mediated inhibition of the T-cell receptor programed death 1 (PD-1) or its ligand (PD-L1) induced objective responses in human patients with advanced melanoma, non-small cell lung cancer, renal cell carcinoma.[3,32] This suggests that some cancers may evade the immune system by activating receptors such as PD-1 to blunt any anti-tumor T-cell response.[26] Although there are no published data for brain tumors, this approach is a promising area given the success of the CSF-1R inhibition in GBM associated macrophages.

As for diagnostics, a literature search showed no validated non-invasive methods for detecting tumor antigens. There are however ongoing clinical trials assessing whether tumor antigens can be detected in peripheral blood draws. Although these trials have not published data, such case controlled trials will hopefully provide insights about the hypothetical GBM circulating tumor cells and their utility for both vaccine and non-vaccine mediated immunological therapy.

Challenges and strategies for the future

'Personalized medicine' or 'target specific' therapy entails the use of agents that preferentially kill cancer cells based on unique molecular features or protein expression. This endeavour poses a challenge to both basic and clinical researchers given issues such as intra-tumoral heterogeneity discussed above. There is, however, significant progress in our understanding of the molecular biology of gliomas especially GBMs. This advance is driven by the invention of high-throughput assays that detect alterations at the clonal or cellular level across hundreds of patient samples. The cancer genome atlas (TCGA) project begun by the National Institutes of Health (NIH) in collaboration with multiple institutes across the country is providing useful information on not just GBMs but also other cancers. For GBMs, it led to the discovery that apart from the classic oncogenes and tumor suppressor genes such as TP53 and PTEN, there are other previously unrecognized mediators of gliomagenesis with high prevalence within GBM. Some of these mutations including NF-1 or PI3K were not previously recognized to be involved in gliomagenesis. Furthermore, the TCGA has also demonstrated that epigenetics may also represent an area of research that could provide an avenue for targeted therapy. For instance, it is reported that hypermethylation phenotypes may be associated with better prognosis in patients with GBM.[23] In such patients, it is conceivable that DNA or histone demethylation agents may play a more significant role in curbing tumor progression. These large scale studies are showing that there are more targets within GBM that could be used for selectively targeting these malignant cells. Therefore, we are now in an era where these discoveries need to be translated into clinically relevant therapies. For diseases such as GBMs with extensive inter- and intra-tumoral heterogeneity, these clinically relevant therapies have to be designed with specific patient sub-populations in mind. To this end, clinical trials testing new agents against each molecular sub-type will have to assign patients to treatment arms not based on age, tumor grade or stage but based on distinct molecular profiles. Unfortunately, apart from being cost-prohibitive and high risk, these clinical trials are difficult to design because new agents cannot be evaluated as stand-alone therapies given that standard therapy improves survival.

This stagnation of translational research drove the National Institutes of Health to establish its newest institute called the National Center for Advancing Translational Science (NCATS).[12] The goal of NCATS is to "accelerate drug discovery through predictive toxicology" and rational drug design. It will 'emphasize biomarker research, predictive toxicology, target validation and de-risk the therapeutic pipeline'. One mechanism it may use to achieve this goal is to form a pioneering relationship between government and industry to help bring life-saving therapy to patients. For patients with GBM, this is welcoming news because such an approach has yielded results for other types of cancers such as *Gleevac* and needs Registeraed trademark (CML; BCL-Abl inhibitor) and *Zelboraf* and needs registered trademark symbol (Melanoma; B-Raf/MEK inhibitor). Although these discoveries may represent an exception in cancer since they impinge on single mutations driving progression, it is proof that personalized medicine through activation of endogenous immune surveillance (CSF-1R, PD1, PD1-L) or targeting tumor initiating clone-specific pathways is feasible when a multi-disciplinary research team includes input from industry.
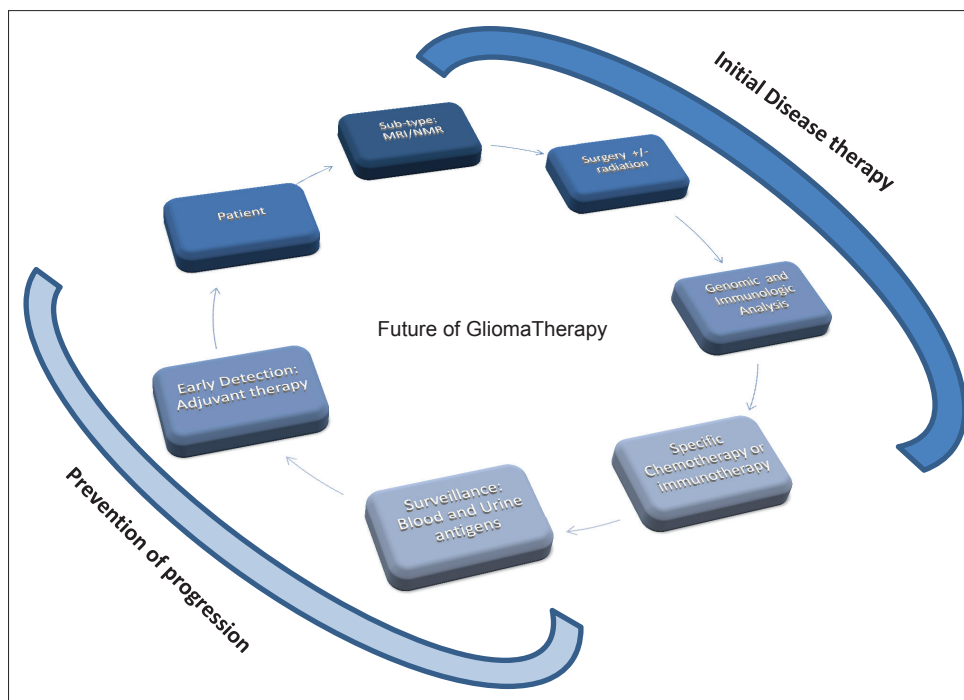
Figure 1: Personalized Medicine for Glioma (Scheme): Proposed model for the future of personalized medicine for gliomas. It involves a multi-modality approach to treatment of the disease including molecular biology, surgery and radiation therapy

CONCLUSION

The lack of progress in therapies for GBM is a complex topic beyond the scope of this review. It is important to state, however, that a lack of progress in personalized therapy for this disease is most likely a limited understanding of the clonal heterogeneity within a single GBM sample that has been brought to light by technology not previously available.[9,13,19-22,29,31] Targeting intra-tumoral heterogeneity using a multi-agent approach may help prevent recurrence in GBMs by selectively eliminating patient specific tumor initiating clones. This is reminiscent of the Frei/Freiriech 3-4 agent approach in the 1950s but will be more selective for patient specific glioblastoma dependent pathways at the clonal level and with less toxicity associated with treatment- *Personalized medicine* [Figure 1; Scheme].

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