The Allure of Immunotherapy for Glioblastoma (GBM)

Amy B. Heimberger, MD
The University of Texas
MD Anderson Cancer Center
Houston, Texas
Shortcomings of Conventional Therapy

Only 5% to 10% of the 15,000 patients diagnosed survive five years after diagnosis despite aggressive surgery, radiation and chemotherapy.

**NEJM: 1980**

Figure 1: Survival Curves for Each Treatment Group in the Total Randomized Population. The numbers of patients are shown in parentheses.

**Dose-dense temozolomide**

**NEJM: 2005**

Figure 3: Kaplan-Meier Estimates of Overall Survival According to Treatment Group. The hazard ratio for death among patients treated with radiotherapy plus temozolomide, as compared with those who received radiotherapy alone, was 0.63 (95% confidence interval, 0.52 to 0.75; P=0.001).

**VEGF-targeted therapy**

**EGFR-targeted therapy**

Dismantling the Notion of CNS “Immune Privilege”

- Thinned mouse skull window
- Bone
- BRAIN
- ICA
- Intravital Imaging
- Intra Carotid Artery
  Intra-cranial implantation
- Genetic CNS brain models
Image not available
What Is Needed for an Optimal Antitumor Immune Therapeutic Response?

- Immunologic target (ie, antigen) or a response that is not dependent on this (NK cells)
  - EGFRvIII, IDH1 mutant, IMA-950, CMV pp65, HSP, tumor homogenates

- Activate the T cell signal
  - 4-1BB antibodies/aptamers, OX40 antibodies, pro-inflammatory cytokines (GM-CSF, IFN-γ, IL-12, IL-15), KLH, TLR agonists (CpG, poly IC), dendritic cells, viral therapy, STING agonists

- Adequate trafficking to and infiltration of the tumor site
  - Chemokine (fractalkine/CX3CR1)

- Maintenance of T cell effector function
  - Inhibition of immune suppressive cytokines (TGF-β, IL-10), STAT3 inhibitors, checkpoint inhibitors

HSP, heat shock protein; KLH, keyhole limpet hemocyanin; NK, natural killer; TLR, toll-like receptor
Types of Cancer Immunotherapy

**PASSIVE**

- Components made outside the body (i.e., antibodies)
  - Ipilimumab (anti-CTLA-4), nivolumab (anti-PD-1), EGFR mAb-toxin constructs
- Patient’s immune system does not take an active role in fighting the cancer
- Directed toward single targets
- Mostly widely used form of cancer immunotherapy

**ACTIVE**

- Trigger the immune system to respond
  - Tumor cell vaccines, dendritic cells, peptides (EGFRvIII, Immatics), DNA vaccines, HSP
- Combined with others substances that boost the immune system (BCG, KLH, GM-CSF, IL-2)
- Cell based/adoptive (usually the patient’s own cells such as lymphocytes, NK) or vector based (engineered virus)

- Manufacturing challenges
- $$
- Poorly immunogeneic
- Autoreactivity
- Neutralizing antibodies
- Cells may not grow well
- Product can’t be made/release criterion
Chimeric Antigen Receptor T-Cell Therapies

Insert CAR into T cells

| scFv | Modified Hinge | IgG4 | CD28 Transmembrane | CD28 Cytoplasmic | CD3ζ Cytoplasmic |

B

- Untreated
- Cetux CAR
- Nimo CAR

Percent survival vs. Days
Immune Activation and Inhibition

- APC
  - MHC complex
  - glioma specific Ag
- TCR/CD3
- CD80/86
- CD28
- CTLA-4
- IL2Rα
- IL2Rβ
- IL2Rγ
- PD-1
- PD-L1
- TGFβR
- VEGFR
- STAT3
- IDO
- Arg-1
- Arg-2
- IL-2
- IL-10
- TGF-β
- VEGF

Glioma cell
- STAT3

Treg cell
- STAT3

Effector T cell
Berghoff Study: PD-L1 Staining

### Nduom Study: PD-L1 Is Only Expressed in a Subset of Patients With GBM

<table>
<thead>
<tr>
<th>% of PD-L1 Cells</th>
<th>% GBM Patients (n = 99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1</td>
<td>85.9</td>
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<td>13.0</td>
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<tr>
<td>&gt;25</td>
<td>1.1</td>
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</table>

Prognostic Impact of the PD-1 and PD-L1 Signaling Axis

TCGA Atlas Data Set

IHC Validating Data Set

* $P = .008$
Current State of the Art: Immune Checkpoints

THE MODEL


THE REALITY


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# Immune Suppression Is Enriched in GBM Subset

## Number of Cases; % mRNA Over Expression

<table>
<thead>
<tr>
<th>Immune Suppressor/Gene</th>
<th>Number of Cases</th>
<th>% mRNA Over Expression</th>
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<tbody>
<tr>
<td><strong>Immune suppressive cytokines and checkpoints</strong></td>
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<tr>
<td>Galectin-3/LGALS3</td>
<td>n = 141</td>
<td>2; 1</td>
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<tr>
<td>VEGF/VEGFA</td>
<td>n = 160</td>
<td>16; 11</td>
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<tr>
<td>IL-10/IL10</td>
<td>n = 147</td>
<td>4; 3</td>
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<tr>
<td>IL-23/IL23A</td>
<td>n = 147</td>
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<td>TGF-β/TGFB1</td>
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<td>PD-L1/PDL1</td>
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<td>CTLA-4/CTLA-4</td>
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<td><strong>Tumor-supportive macrophage chemotactic and skewing molecules</strong></td>
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<td>CSF-1/CSF</td>
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<td>CCL2/CCL2</td>
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<td>MIC-1/GDF15</td>
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<td>STAT3/STAT3</td>
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<td>SOCS3/SOCS3</td>
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<td>STAT5A/STAT5A</td>
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<td><strong>Markers of Tregs</strong></td>
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<tr>
<td>IDO/IDO1</td>
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<td>6; 4</td>
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</tbody>
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Monitoring the GBM-Infiltrating Immune Responses

Healthy Donor

GBM blood

GBM Infiltrating T Cells
Immune Checkpoint Clinical Trials in GBM

• Is there an increase in the frequency of the T cells?
• Is there modulation of checkpoint expression in tumor infiltrating immune cells?
• Are there increased functional T cell responses (IL-2R, IFN-γ, TNF-α, granzyme B, perforin)?

Activated CD4+ T cells are predominantly Tregs

CD8+ T cells are not activated

FoxP3
# Clinical Trials of Immune Checkpoint Inhibitors in Glioblastoma And Brain Metastases

<table>
<thead>
<tr>
<th>National Clinical Trial registration number</th>
<th>Mechanism of tested agent</th>
<th>Therapy and/or treatment groups</th>
<th>Tumour type</th>
<th>No. of patients</th>
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<td>NCT02017717 Anti-PD1, anti-CTLA4</td>
<td>Nivolumab (anti-PD1)</td>
<td>Recurrent glioblastoma</td>
<td>372</td>
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<td>Nivolumab + ipilimumab</td>
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<tr>
<td></td>
<td>(anti-CTLA4)</td>
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<td></td>
<td>Bevacizumab (control group)</td>
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<td>NCT01952769 Anti-PD1</td>
<td>Pidilizumab (two cohorts)</td>
<td>Relapsed glioblastoma, diffuse</td>
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<td>42</td>
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<td></td>
<td>TMZ + ipilimumab</td>
<td>or gliosarcoma</td>
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<td>TMZ + nivolumab + ipilimumab</td>
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<td>MEDI4736 + radiotherapy</td>
<td>glioblastoma</td>
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<td>MEDI4736 + bevacizumab</td>
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<td>NCT02115139 Anti-CTLA4</td>
<td>Ipilimumab + whole-brain</td>
<td>Melanoma BM</td>
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<td></td>
<td>radiotherapy</td>
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<td>followed by ipilimumab</td>
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<td></td>
<td>Stereotactic surgery</td>
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<td>NCT01703507 Anti-CTLA4</td>
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<td>Melanoma BM</td>
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<td>I</td>
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<td></td>
<td>Ipilimumab + stereotactic</td>
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<td>radiosurgery</td>
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<td>NCT01950195 Anti-CTLA4</td>
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<td>Melanoma BM</td>
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<td>radiosurgery</td>
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<tr>
<td>NCT02337491 Anti-PD1</td>
<td>Pembrolizumab monotherapy</td>
<td>Recurrent glioblastoma</td>
<td>79</td>
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<td>Pembrolizumab + bevacizumab</td>
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<td>NCT02085070 Anti-PD1</td>
<td>Pembrolizumab</td>
<td>Non-small cell lung cancer BM</td>
<td>64</td>
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<tr>
<td></td>
<td></td>
<td>or melanoma BM</td>
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Immune Suppression Mechanisms in GBM

- Cytokines – IL-10, TGF, PGE2
- Lack of functional antigen presenting cells, i.e., immunosuppressive microglia/macrophages (microglia, paucity of myeloid dendritic cells)
- Induction of T-cell apoptosis (FasL; Galectin-3)
- Treg recruitment to the tumor
- Increased expression of immune checkpoints
- Loss of antigen
- Decreased B_2_ microglobulin and/or HLA
- Induction of inappropriate T-helper function (skewing to Th2)
- Cancer stem cells/initiating cells
- Tumor hypoxia/HIF-1α

Is there a common pathway?
STAT3: The Global Regulator of Tumor-Mediated Immune Suppression


• Induces the expression of immune suppressive cytokines

• STAT3 activity turns off antigen presenting cells like dendritic cells

• STAT3 suppresses macrophage/microglia activation and function; induces M2 macrophages

• STAT3 is a transcriptional regulator of FoxP3 in Tregs

• Ablating STAT3 in only the immune cells results in marked antitumor activity (Kortylewski M, et al. Nat Med. 2005;11(12):1314-1321)

• STAT3 blockade in the immune cells from tumor patients can restore T-cell proliferation and responses

• STAT3 is up regulated in the peripheral blood of cancer patients
STAT3: A Key Driver of Cancer

- Activation is observed in majority of many malignancies
- Can be induced by a variety of factors produced in by surrounding cells (EGF, PDGF, IL-6, IFN, CMV, TLR-9)
- Upon phosphorylation of tyrosine\(^{705}\) (p-STAT3), dimerization occurs and subsequent nuclear translocation
- The p-STAT3 is a potent transcriptional factor that regulates key factors that mediate tumor proliferation and survival, migration and invasion, and angiogenesis
- Is a negative prognostic factor for survival
- Shown to mediate the low-grade to high-grade transition
- Maintains cancer stem cells which give rise to recurrence and treatment resistance
WP1066

*In vivo models with documented activity*

- AML (Ferrajoli, Cancer Research, 2007)
- Squamous Cell (Kupferman, J Exp Ther Oncol., 2009; Zhou Oncol Rep, 2014)
- Renal Cell (Horiguchi, Br J Cancer, 2010)
- Non-small cell **lung carcinoma** (Chiu, Biochem, Pharmcol, 2011)
- Gastric (Judd, PloS One, 2014)
- **Breast** Cancer (Lee, Oncotarget, 2015)
A Phase I Trial of WP1066: NCT01904123

Key Features of the Trial:

- Enrolling both primary brain tumors and metastatic disease to the brain
- Dose escalation proceeds according to an accelerated titration design
- Biomarker endpoints include pharmacokinetic bioavailability of WP1066, p-STAT3 levels, and immune monitoring
- Measurement of effect includes radiographic responses and advanced imaging techniques
- The primary objective is to determine maximum tolerated dose (MTD), safety, and tolerability
- Total number of patients: 21
Combinatorial Strategies

Challenges/Opportunities for Immunotherapy

- Does the immune system really prevent the development of gliomas? If so, what is the trigger?
- Uncharacterized immune suppressive mechanisms (exosome)
- Immune suppressive features of low grade glioma
- Cost and production ease of GMP cellular products
- Target only membrane expressed targets
- Redundancy of immune suppression
- Assumption of generalized immune assays for monitoring responses (harmonization)
- Polyvalent immune response
- Immunotoxicity
- Synergy with conventional chemotherapeutics/radiation/steroids
- Radiographic monitoring of immune responses
IMMUNOTHERAPY IN GLIOBLASTOMA MULTIFORME: EMERGING OPTIONS IN PRECISION MEDICINE