How to break immune tolerance in brain tumors

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Disclosures

Michael Weller

- Research grants: Acceleron, Actelion, Bayer, Isarna, MSD, Merck & Co, Novocure, PIQUR, Roche
- Honoraria for lectures or advisory board participation or consulting: Celldex, Immunocellular Therapeutics, Isarna, Magforce, MSD, Merck & Co, Northwest Biotherapeutics, Novocure, Pfizer, Roche, Teva

Roger Stupp

- Principal Investigator for clinical trials with temozolomide, cilengitide, tumor treating fields
- Honoraria to institution for advisory boards: Ipsen, Merck KGaA, MSD/Merck & Co, Novartis, Pfizer, Roche
- Spouse employee of Celgene
Challenging Popular Assumptions

- Brain is an immunoprivileged site: is there need for additional immunosuppression?
- Glioblastoma cells (may) lack tumor-specific antigens: why additional suppression of a „blinded“ immune system
- Is there evidence that immune surveillance accounts for the low incidence of systemic metastasis in glioblastoma?
- The increased incidence of glioblastoma in the elderly may relate to immune senescence, but why is there no increased risk with immunodeficiency states including AIDS?
REVIEW

Modulation of T-cell function by gliomas

Thomas Roszman, Lucinda Elliott and
William Brooks

T cells from glioma patients exhibit defects
• interleukin 2 secretion and in
• expression of the IL-2 receptor

Table 1. Some anomalies in the immune status of patients with glioblastomas

<table>
<thead>
<tr>
<th>Observation</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutaneous anergy</td>
<td>1–3</td>
</tr>
<tr>
<td>Decreased antibody response to influenza virus and tetanus toxoid</td>
<td>4</td>
</tr>
<tr>
<td>Decreased percentage and absolute number of peripheral blood T cells</td>
<td>5,6,13</td>
</tr>
<tr>
<td>Decreased peripheral blood lymphocyte reactivity to mitogens and alloantigens</td>
<td>1,5–10</td>
</tr>
</tbody>
</table>

Modulating the Immune System
Immunosuppression in GBM: focus on TGF-β

- Cytokine synthesis
- MHC class II
- H₂O₂ and NO release

Microglia
Macrophages

T cells
NK cells

- Proliferation ↓
- Activity ↓
- Apoptosis ↑
- TGF-β synthesis ↑

Glioblastoma

- Proliferation ↑↓
- MHC class II ↓
- MMP synthesis ↑
- Invasion/migration ↑
- Stem cell renewal ↑

Endothelial cells

- Adhesion ↓

Chemotaxis
DC maturation
Antigen presentation

Presented by R. Stupp at...
# TGF-β and Immunosuppression in Glioblastoma: Preclinical Studies

## Inhibiting TGF-β Signaling Restores Immune Surveillance in the SMA-560 Glioma Model


*Neuro-Oncology* 9, 259–270, 2007

## RNA Interference Targeting Transforming Growth Factor-β Enhances NKG2D-Mediated Antigloma Immune Response, Inhibits Glioma Cell Migration and Invasiveness, and Abrogates Tumorigenicity In Vivo

Manuel A. Friede, Jörg Wischhusen, Wolfgang Wick, Markus Weller, Günter Eisele, Alexander Steinle, and Michael Weller


## SD-208, a Novel Transforming Growth Factor β Receptor I Kinase Inhibitor, Inhibits Growth and Invasiveness and Enhances Immunogenicity of Murine and Human Glioma Cells In Vitro and In Vivo

Martin Uhl, Steffen Aubwurm, Jörg Wischhusen, Markus Weller, Jing Ying Ma, Ramona Almirante, Ruben Mangada, Yu-Wang Liu, Michael Platten, Ulrich Herrlinger, Alison Murphy, Darren H. Wong, Wolfgang Wick, Linda S. Higgins, and Michael Weller


## Blockade of TGF-β Signaling by the TGFβR-I Kinase Inhibitor LY2109761 Enhances Radiation Response and Prolongs Survival in Glioblastoma

Mengxian Zhang, Susanna Kiefer, Manuel Röhrich, Carmen Timke, Na Han, Jochen Tuettenberg, Ana Martin-Villalba, Jürgen Dedus, Peter Peschke, Ute Wirkner, Michael Lahm, and Peter E. Huber


## Systemic Inhibition of Transforming Growth Factor-β in Glioma-Bearing Mice Improves the Therapeutic Efficacy of Glioma-Associated Antigen Peptide Vaccines

Ryo Ueda, Mitsuqi Fujita, Xinmei Zhu, Kotaro Sasaki, Edward R. Kastenhuber, Gary Kohanbash, Heather A. McDonald, Jay Harper, Scott Lonning, and Hideko Okada


## TGF-β and Immunosuppression in Glioblastoma: Preclinical Studies

Mengxian Zhang, Tobias W. Herton, Carmen Timke, Na Han, Ute Wirkner, Klaus J. Weber, Peter Peschke, Michael Lahm, and Peter E. Huber

(2009) *Neoplasia*, 11(6), 537–549

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**Zurich University Cancer Center**
TGF-β and immunosuppression in glioblastoma: clinical studies

(a) Brain MRIs taken before (left) MRI after treatment (right) increased contrast enhancement is larger than in the pre-therapy MRI.

(b) CD4 and CD8 immunohistology shows that the increased contrast enhancement may have been caused by immune infiltration.
TGF-β and immunosuppression in glioblastoma: clinical studies

Targeted therapy for high-grade glioma with the TGF-β2 inhibitor trabedersen: results of a randomized and controlled phase IIb study


Six-month tumor control rates (1° endpoint) were not different

Median survival
10 mM trabedersen 39.1 months
80 mM trabedersen 35.2 months
chemotherapy 21.7 months

Trabedersen 10µg / 80 µg
Chemotx: TMZ or PCV

R
N=89
N=45

Six-month tumor control rates (1° endpoint) were not different

Median survival
10 mM trabedersen 39.1 months
80 mM trabedersen 35.2 months
chemotherapy 21.7 months

not significant
Despite the disappointing outcome of this trial, we remain confident that TGF-b is a relevant target in glioblastoma. Going forward, [...] to investigate the extent to which the oligonucleotide inhibits its target in vivo. It will be important to incorporate biological endpoints into future immunotherapy trials in glioblastoma.

[...] Trabedersen neutralizes only TGF-b2, whereas TGF-b1 or TGF-b3 released by glioma or glioma-infiltrating (e.g., microglial) cells will at least not directly be affected. Despite persistent safety concerns, the future of anti-TGF-b agents may be brighter for small molecule antagonists of the TGF-b receptor, which have shown truly promising activity in relevant rodent glioma models.
PHASE I STUDIES

Pharmacokinetic, pharmacodynamic and biomarker evaluation of transforming growth factor-β receptor I kinase inhibitor, galunisertib, in phase 1 study in patients with advanced cancer

Jordi Rodón · Michael Carducci · Juan M. Sepulveda-Sánchez · Analia Azaro · Emiliano Calvo · Joan Seoane · Irene Braña · Elisabet Sicart · Ivelina Gueorguieva · Ann Cleverly · N. Sokalingum Pillay · Durisala Desaih · Shawn T. Estrem · Luis Paz-Ares · Matthias Holdhoff · Jaishri Blakeley · Michael M. Lahn · Jose Baselga

Summary Purpose Transforming growth factor-beta (TGF-β) signaling plays a key role in epithelialmesenchymal transition (EMT) of tumors, including malignant glioma. Small molecule inhibitors (SMI) blocking TGF-β signaling reverse EMT and arrest tumor progression. Several SMIs were developed, but currently only LY2157299 monohydrate (galunisertib) was advanced to clinical investigation. Design The first-in-human dose study had three parts (Part A, dose escalation, n=39; Part B, safety combination with lomustine, n=26; Part C, relative bioavailability study, n=14). Results A preclinical pharmacokinetic/pharmacodynamic (PK/PD) model predicted a therapeutic window up to 300 mg/day and was confirmed in Part A after continuous PK/PD. PK was not affected by co-medications such as enzyme-inducing anti-epileptic drugs or proton pump inhibitors. Changes in pSMAD2 levels in peripheral blood mononuclear cells were associated with exposure indicating target-related pharmacological activity of galunisertib. Twelve (12/79; 15 %) patients with refractory/relapsed malignant glioma had durable stable disease (SD) for 6 or more cycles, partial responses (PR), or complete responses (CR). These patients with clinical benefit had high plasma baseline levels of MDC/CCL22 and low protein expression of pSMAD2 in their tumors. Of the 5 patients with IDH1/2 mutation, 4 patients had a clinical benefit as defined by CR/PR and SD ≥6 cycles. Galunisertib had a favorable toxicity profile and no cardiac adverse events. Conclusion Based on the PK, PD, and biomarker adverse events, the intermittent administration of galunisertib at 300 mg/day is safe for future clinical investigation.

Immunosuppression in GBM beyond TGF-beta:

- prostaglandins
- IL-10
- galectin-1
- CD95 Ligand
- Placental mimikry
- Checkpoint inhibition
Promiscuous CD95/CD95L signaling

CD95/CD95L is a major system used by CTL and CD4+ effector cells to kill tumor cells

CD95-mediated non-apoptotic functions involve NF-kB and MAPK stimulation of cell invasiveness and angiogenesis

CD95 stimulation maintains the cancer stem cell population, converts non-CSC to CSC and induces EMT through β-catenin

CD95L on tumor cells enables a powerful counterattack against antitumor effector cells

Tumor endothelium expresses CD95L and mediates apoptosis of effector T cells but not Tregs

Death induced by CD95 or CD95 ligand elimination

Peter et al. CDD 2015;22:549–559
A Phase II, Randomized, Study of Weekly APG101+Reirradiation versus Reirradiation in Progressive Glioblastoma

Wolfgang Wick1,2, Harald Fricke3, Klaus Junge4, Grigory Kobyakov5, Tobias Martens6, Oliver Heese6, Benedikt Wiestler1,2, Maximilian G. Schlesser2, Andreas von Deimling7,8, Josef Pichler6, Elena Vetlouva6, Inga Harting10, Jürgen Debus11, Christian Hartmann7,8, Claudia Kunz2, Michael Platten1,2, Martin Bendszus10, and Stephanie E. Combs11


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**A. Progression-free survival**

- **Progression-free survival (%)**
  - rRT+APG101
  - rRT

**B. Overall survival**

- **Overall survival (%)**
  - rRT+APG101
  - rRT

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*Images and figures are not fully transcribed due to the nature of the task.*
Function of CTLA-4 and PD-1

Engager

=> Inhibition of these «checkpoint» molecules may boost immune responses against a tumor
Immunosuppression in glioblastoma: focus on checkpoint inhibition – CTLA-4

Systemic CTLA-4 Blockade Ameliorates Glioma-Induced Changes to the CD4+ T Cell Compartment without Affecting Regulatory T-Cell Function

Peter E. Fecci,1,2 Hidetoshi Ochiai,3 Duane A. Mitchell,1 Peter M. Grossi,1 Alison E. Sweeney,2 Gary R. Archer,3 Thomas Cummings,4 James P. Allison,6 Darel D. Bigner,6 and John H. Sampson1,2

Clin Cancer Res 2007;13(7) April 1, 2007

Intratumoral IL-12 combined with CTLA-4 blockade elicits T cell-mediated glioma rejection

Johannes vom Berg,1 Melissa Vrohlings,1 Sergio Haller,1 Aladin Haimovic,1 Paulina Kulig,1 Anna Sledzinska,1 Michael Weller,2 and Burkhard Becher1

Expression and functional role of PD-L1 in glioblastoma

Human glioblastoma is a highly lethal tumor with immune inhibitory capabilities. B7-homologue 1 (B7-H1), a homologue of B7.1/2 (CD80/86), has been described to mediate and immune regulatory functions. We investigated the expression and the functional activity of B7-H1 in human glioma cells in vitro and in vivo. Although lacking B7.1/2 (CD80/86), all 12 glioma cell lines constitutively expressed B7-H1 mRNA and protein. Exposure to IFN-γ strongly enhanced B7-H1 expression. Immunohistochemical analysis of malignant glioma specimens revealed strong B7-H1 expression in all 10 samples examined, whereas no B7-H1 expression could be detected on normal brain tissues. To elucidate the functional significance of glioma cell-related B7-H1 expression, we performed coculture experiments of glioma cells with allogeneic CD4+ and CD8+ T cells. Glioma-related B7-H1 was identified as a strong inhibitor of CD4+ as well as CD8+ T-cell activation as assessed by increased cytokine production (IFN-γ, interleukin-2, and interleukin-10) and expression levels of the T-cell activation marker (CD69) in the presence of a neutralizing antibody against B7-H1 (mAb 5H1). B7-H1 expression may thus significantly influence the outcome of T-cell tumor cell interactions and represents a novel mechanism by which glioma cells evade immune recognition and destruction.
Expression and functional role of PD-L1 in glioblastoma

Expression of the B7-Related Molecule B7-H1 by Glioma Cells: A Potential Mechanism of Immune Paralysis

Sabine Wintterle, Bettina Schreiner, Meike Mitsdoerffer, Dagmar Schneider, Lieping Chen, Richard Meyermann, Michael Weller, and Heinz Wiendl

Department of Neurology [S.W., B.S., M.M., D.S., M.W., H.W.] and Institute of Brain Research [R.M.], University of Tübingen, Medical School, D-72076 Tübingen, Germany, and Department of Immunology, Mayo Clinic, Rochester, Minnesota 55905 [L.C.]

Expression and functional role of PD-L1 in glioblastoma

- No antibody
- Anti-HLA-DR
- Anti-B7
PDL1 expression in GBM

- Patchy in tumor tissue (→)
- Absent in normal brain (*)

Berghoff et al.
Neuro-Oncology 17(8), 1064–1075, 2015
PD-L1 expression on tumor-infiltrating lymphocytes and on GMB cells (patchy, focal)

Results. The median percentage of PD-L1-expressing cells in GBM by cell surface staining is 2.77% (range: 0%–86.6%; n = 92), which is similar to the percentage found by ex vivo flow cytometry. The majority of GBM patients (61%) had tumors with at least 1% or more PD-L1-positive cells, and 38% had at least 5% or greater PD-L1 expression. PD-L1 is commonly expressed on the GBM-infiltrating T cells. Expression of both PD-L1 and PD-1 are negative prognosticators for GBM outcome.

Conclusions. The incidence of PD-L1 expression in GBM patients is frequent but is confined to a minority subpopulation, similar to other malignancies that have been profiled for PD-L1 expression. Higher expression of PD-L1 is correlated with worse outcome.

Nduom ... & Heimberger. Neuro Oncol. 2015 Aug 30. [Epub]

Presented by R Stupp at
**Durable Therapeutic Efficacy Utilizing Combinatorial Blockade against IDO, CTLA-4, and PD-L1 in Mice with Brain Tumors**

Derek A. Wainwright, Alan L. Chang, Mahua Dey, Irina V. Balyasnikova, Chung Kwon Kim, Alex Tobias, Yu Cheng, Julius W. Kim, Jian Qiao, Lingjiao Zhang, Yu Han, and Maciej S. Lesniak

**Purpose:** Glioblastoma (GBM) is the most common form of malignant glioma in adults. Although protected by both the blood-brain and blood-tumor barriers, GBMs are actively infiltrated by T cells. Previous work has shown that IDO, CTLA-4, and PD-L1 are dominant molecular participants in the suppression of GBM immunity. This includes IDO-mediated regulatory T-cell (Treg; CD4+CD25+FoxP3+) accumulation, the interaction of T-cell-expressed, CTLA-4, with dendritic cell-expressed, CD80, as well as the interaction of tumor- and/or macrophage-expressed, PD-L1, with T-cell-expressed, PD-1. The individual inhibition of each pathway has been shown to increase survival in the context of experimental GBM. However, the impact of simultaneously targeting all three pathways in brain tumors has been left unanswered.

**Experimental Design and Results:** In this report, we demonstrate that, when dually challenged, IDO-deficient tumors provide a selectively competitive survival advantage against IDO-competent tumors. Next, we provide novel observations regarding tryptophan catabolic enzyme expression, before showing that the therapeutic inhibition of IDO, CTLA-4, and PD-L1 in a mouse model of well-established glioma maximally decreases tumor-infiltrating Tregs, coincident with a significant increase in T-cell-mediated long-term survival. In fact, 100% of mice bearing intracranial tumors were long-term survivors following triple combination therapy. The expression and/or frequency of T cell expressed CD44, CTLA-4, PD-1, and IFN-γ depended on timing after immunotherapeutic administration.

**Conclusions:** Collectively, these data provide strong preclinical evidence that combinatorially targeting immunosuppression in malignant glioma is a strategy that has high potential value for future clinical trials in patients with GBM. *Clin Cancer Res* 20(20); 5290–301. ©2014 AACR.
NCT02017717: Randomized Study of Nivolumab vs Bevacizumab and a Safety Study of Nivolumab Combined With Ipilimumab in Adult Subjects With Recurrent Glioblastoma (GBM) (CheckMate 143)

1° Endpoint: Safety and tolerability (cohort 1 and 1b), OS (cohort 2)
Key 2° Endpoints: OS at 12 months, PFS, ORR

Recurrent GBM (first recurrence) N=260

Randomized

Cohort 1
Nivolumab 1mg/kg + ipilimumab 3 mg/kg q3w for four doses, then nivolumab 3 mg/kg q2w

Cohort 1b
Nivolumab 3 mg/kg + ipilimumab 1 mg/kg q3w for four doses, then nivolumab 3 mg/kg q2w

Cohort 2
Nivolumab 3 mg/kg q2w

Cohort 2
Bevacizumab 10 mg/kg q2w

Progression or toxicity

n=20

n=20

n=220

Study Start Date: January 2014
Estimated Primary Completion Date: January 2017 (Final data collection date for primary outcome measure)

GBM, glioblastoma multiforme; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

Checkmate Studies (BMS): Nivolumab in newly diagnosed GBM

**Diagnosis**
- Step 1: central *MGMT* methylation status assay
- MGMT unmethyl.
  - + Nivolumab
  - Radiotherapy
- Concomitant Phase
- Adjuvant (maintenance) Phase

**Registration**
- Randomization
- MGMT methyl.
  - + Nivolumab
  - Radiotherapy
- Concomitant Phase
- Adjuvant (maintenance) Phase

**Step 2: Randomization**
- Versus Control
- TMZ
- Radiotherapy

Presented by R Stupp at Checkmate Studies (BMS):
- Nivolumab in newly diagnosed GBM
- MGMT unmethyl.
- MGMT methyl.
- Radiotherapy
- Concomitant Phase
- Adjuvant (maintenance) Phase
Current status of PD-L-1 in glioblastoma

- PD-L1 is expressed in glioblastoma
- TCGA / other public data bases do not define a major prognostic role for PD-L1 expression
- The relevance of PD-L1 expression as a biomarker of tumoral and/or non-tumoral tissue remains to be determined
Immunosuppression in glioblastoma

**take home messages**

- Glioblastomas are a rich source of immunosuppressive molecules, including soluble mediators and cell surface proteins.
- This provides a rationale for maximum safe surgery which will relieve immunosuppression.
5th Quadrennial Meeting of the World Federation of Neuro-Oncology
Zurich / Switzerland, May 3 - 7, 2017
How to break the immune tolerance in brain tumours!

Michael Weller

presented by R Stupp at