UniversityHospital Zurich

University of Zurich[UZH]

How to break immune tolerance in brain tumors

Roger Stupp, MD on behalf of
Michael Weller, MD
The Brain Tumor Center
University of Zurich / Switzerland

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?

Immunosuppression in glioblastoma: is it real?

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

Observation	Refs
Cutaneous anergy	1–3
Decreased antibody response to influenza virus and tetanus toxoid	4
Decreased percentage and absolute number of peripheral blood T cells	5,6,13
Decreased peripheral blood lymphocyte reactivity to mitogens and alloantigens	1,5–10

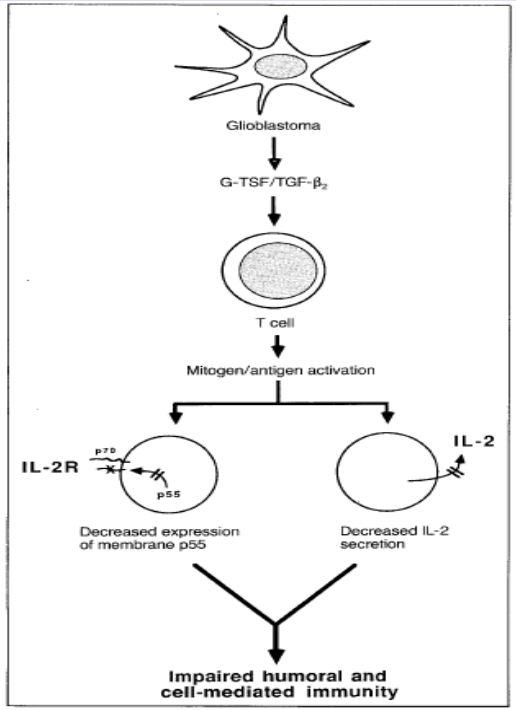
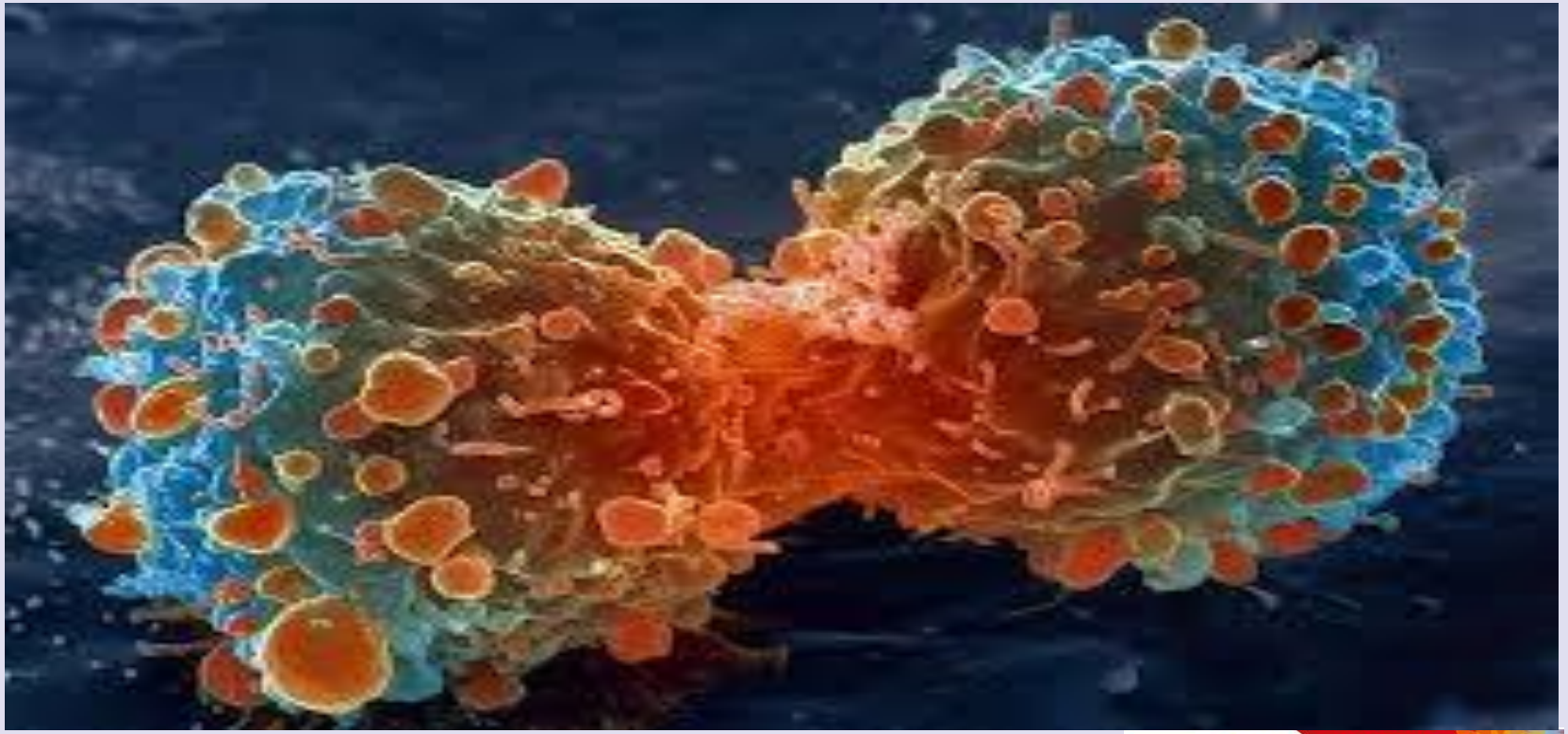


Fig. 1. *A possible scheme is shown that explains the broad suppression of humoral and cell-mediated immunity observed in patients with glioblastomas.*

Immunol Today. 1991;12:370-374

presented by R Stupp at

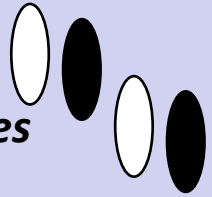
Modulating the Immunsystem



Immunosuppression in GBM: → focus on TGF-β

Cytokine synthesis ?
MHC class II ?
H_2O_2 and NO release ?

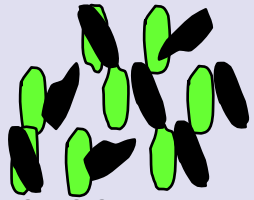
Microglia
Macrophages



Chemotaxis ?
DC maturation ?
Antigen presentation ?

T cells
NK cells

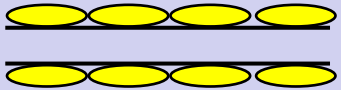
Proliferation ↓
Activity ↓
Apoptosis ↑
TGF-β synthesis ↑



Glioblastoma

Autocrine effects

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



Endothelial cells

Adhesion ↓

presented by R Stupp at
SINGAPORE 2015 ESMO ASIA 18-21 DECEMBER SINGAPORE

TGF-β and immunosuppression in glioblastoma: preclinical studies

[CANCER RESEARCH 64, 7596–7603, October 15, 2004]

RNA Interference Targeting Transforming Growth Factor-β Enhances NKG2D-Mediated Antiglioma Immune Response, Inhibits Glioma Cell Migration and Invasiveness, and Abrogates Tumorigenicity *In vivo*

Manuel A. Friese,[1] Jörg Wischhusen,[1] Wolfgang Wick,[1] Markus Weiler,[1] Günter Eisele,[1] Alexander Steinle,[2] and Michael Weller[1]

[CANCER RESEARCH 64, 7954–7961, November 1, 2004]

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,[1] Steffen Aulwurm,[1] Jörg Wischhusen,[1] Markus Weiler,[1] Jing Ying Ma,[2] Ramona Almirez,[2] Ruban Mangadu,[2] Yu-Wang Liu,[2] Michael Platten,[1] Ulrich Herrlinger,[1] Alison Murphy,[2] Darren H. Wong,[2] Wolfgang Wick,[1] Linda S. Higgins,[2] and Michael Weller[1]

Inhibiting TGF-β signaling restores immune surveillance in the SMA-560 glioma model

Thomas-Toan Tran, Martin Uhl, Jing Ying Ma, Lisa Janssen, Venkataraman Sriram, Steffen Aulwurm, Irene Kerr, Andrew Lam, Heather K. Webb, Ann M. Kapoun, Darin E. Kizer, Glenn McEnroe, Barry Hart, Jonathan Axon, Alison Murphy, Sarvajit Chakravarty, Sundeep Dugar, Andrew A. Protter, Linda S. Higgins, Wolfgang Wick, Michael Weller, and Darren H. Wong

Neuro-Oncology 9, 259–270, 2007

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

Mengxian Zhang[1,2,4], Susanne Kleber[5], Manuel Röhrich[2,4], Carmen Timke[2,4], Na Han[1], Jochen Tuettenberg[5], Ana Martin-Villalba[3], Juergen Debus[4], Peter Peschke[2], Ute Wirkner[2], Michael Lahn[6], and Peter E. Huber[2,4]

Cancer Res; 71(23); 7155–67.

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

Ryo Ueda,[1,5] Mitsugu Fujita,[1,5] Xinmei Zhu,[1,5] Kotaro Sasaki,[3,4] Edward R. Kastenhuber,[5] Gary Kohanbash,[1,5] Heather A. McDonald,[5] Jay Harper,[6] Scott Lonning,[6] and Hideho Okada[1,2,5]

Clin Cancer Res 2009;15(21) November 1, 2009

NEOPLASIA
www.neoplasia.com
Volume 13 Number 6 June 2011 pp. 537–549 537

Trimodal Glioblastoma Treatment Consisting of Concurrent Radiotherapy, Temozolomide, and the Novel TGF-β Receptor I Kinase Inhibitor LY2109761[1,2]

Mengxian Zhang*,†,‡,3, Tobias W. Herion†,3, Carmen Timke†, Na Han*, Kai Hauser†, Klaus J. Weber†, Peter Peschke†, Ute Wirkner†, Michael Lahn§ and Peter E. Huber†,‡

*Department of Oncology, Tongji Hospital, Tongji Medical College, Huazhong University of Science & Technology, Wuhan, China; †Department of Radiation Oncology, German Cancer Research Center, Heidelberg, Germany; ‡Department of Radiation Oncology, University Hospital Center, Heidelberg, Germany; §Oncology Early Clinical Investigation, Lilly Research Laboratories, Indianapolis, IN, USA

TGF-β and immunosuppression in glioblastoma: clinical studies

Phase I clinical trial of a TGF-β antisense-modified tumor cell vaccine in patients with advanced glioma

H Fakhrai[1], JC Mantil[2], L Liu[3], GL Nicholson[2], CS Murphy-Satter[2], J Ruppert[2] and DL Shawler[1]

Cancer Gene Therapy (2006) 13, 1052–1060

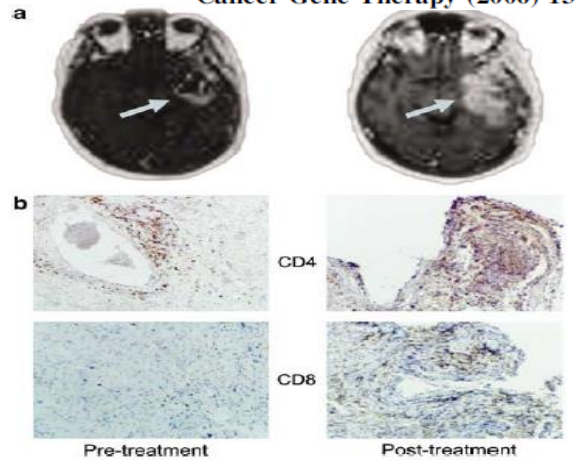


Figure 1 Pre- and post-treatment MRIs and immunohistology in Patient 1. (**a**) Brain MRIs taken before (left) and after therapy (right) show areas of increased contrast enhancement (arrows). The increased contrast enhancement in the post-therapy MRI is larger than the same area in the pre-therapy MRI. (**b**) CD4 and CD8 immunohistology shows that the increased contrast enhancement may have been caused by immune infiltration.

(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

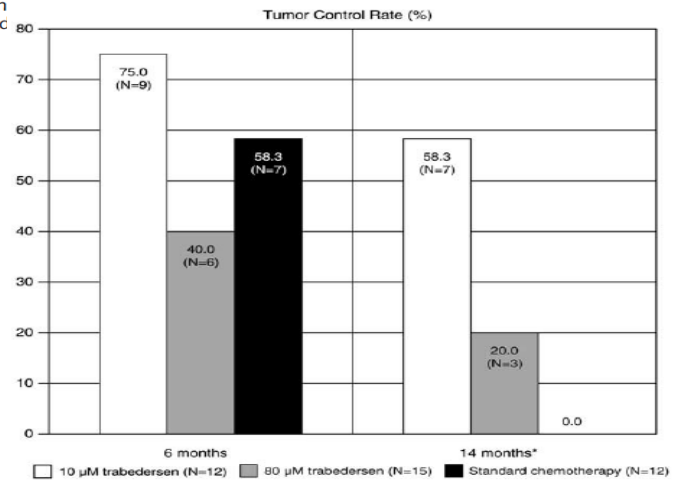
shows that the increased contrast enhancement may have been caused by immune infiltration.

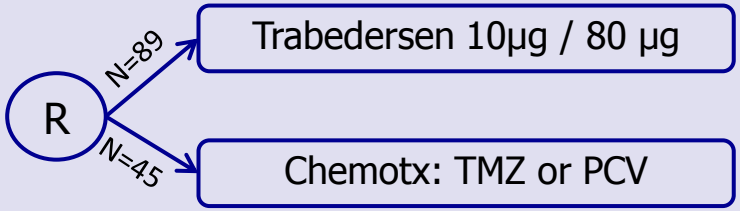
TGF-β and immunosuppression in glioblastoma: clinical studies

Neuro-Oncology 13(1):132–142, 2011.
doi:10.1093/neuonc/noq142
Advance Access publication October 27, 2010

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

U. Bogdahn, P. Hau, G. Stockhammer, N.K. Venkataramana, A.K. Mahapatra, A. Suri, A. Balasubramaniam, S. Nair, V. Oliushine, V. Parfenov, I. Poverennova, M. Zaaroor, P. Jach … id the Trabec





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

Trabedersen to target transforming growth factor-b: when the journey is not the reward

Wick & Weller. Letter to the editor: Neuro-Oncol 2011; 13:559-60

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 · Analía Azaro · Emiliano Calvo · Joan Seoane · Irene Braña · Elisabet Sicart · Ivelina Gueorguieva · Ann Cleverly · N. Sokalingum Pillay · Durisala Desaiah · Shawn T. Estrem · Luis Paz-Ares · Matthias Holdhoff · Jaishri Blakeley · Michael M. Lahn · Jose Baselga

Invest New Drugs (2015) 33:357–370

Summary *Purpose* Transforming growth factor-beta (TGF-β) signaling plays a key role in epithelial-mesenchymal 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 evaluations, the intermittent administration of galunisertib at 300 mg/day is safe for future clinical investigation.

Immunosuppression in GBM beyond TGF-beta:

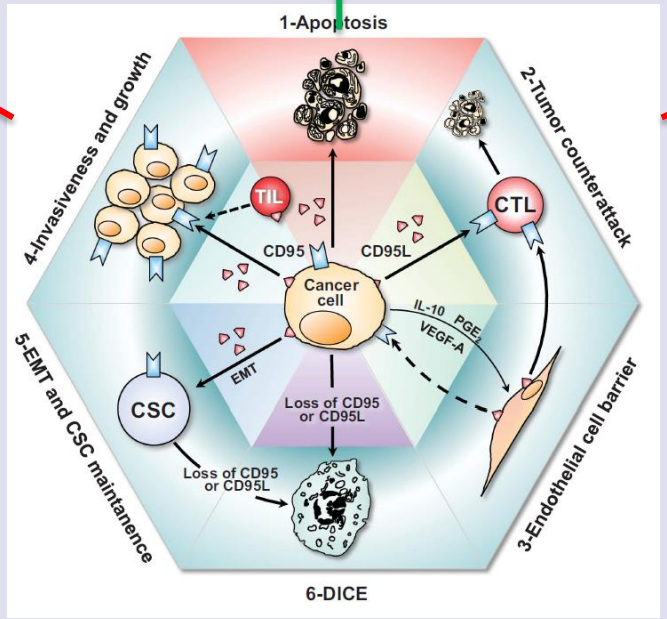
- prostaglandins
- IL-10
- galectin-1
- **CD95 Ligand**
- Placental mimikry
- **Checkpoint inhibition**

Promiscuous CD95/CD95L signaling

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

Peter et al. CDD 2015;22:549–559

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



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

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

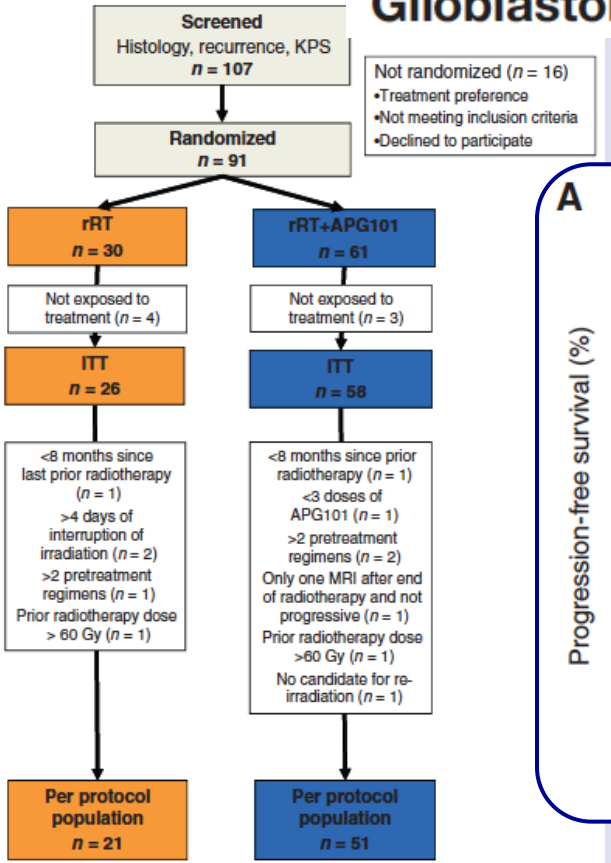
Tumor endothelium expresses CD95L and mediates apoptosis of effector T cells but not Tregs

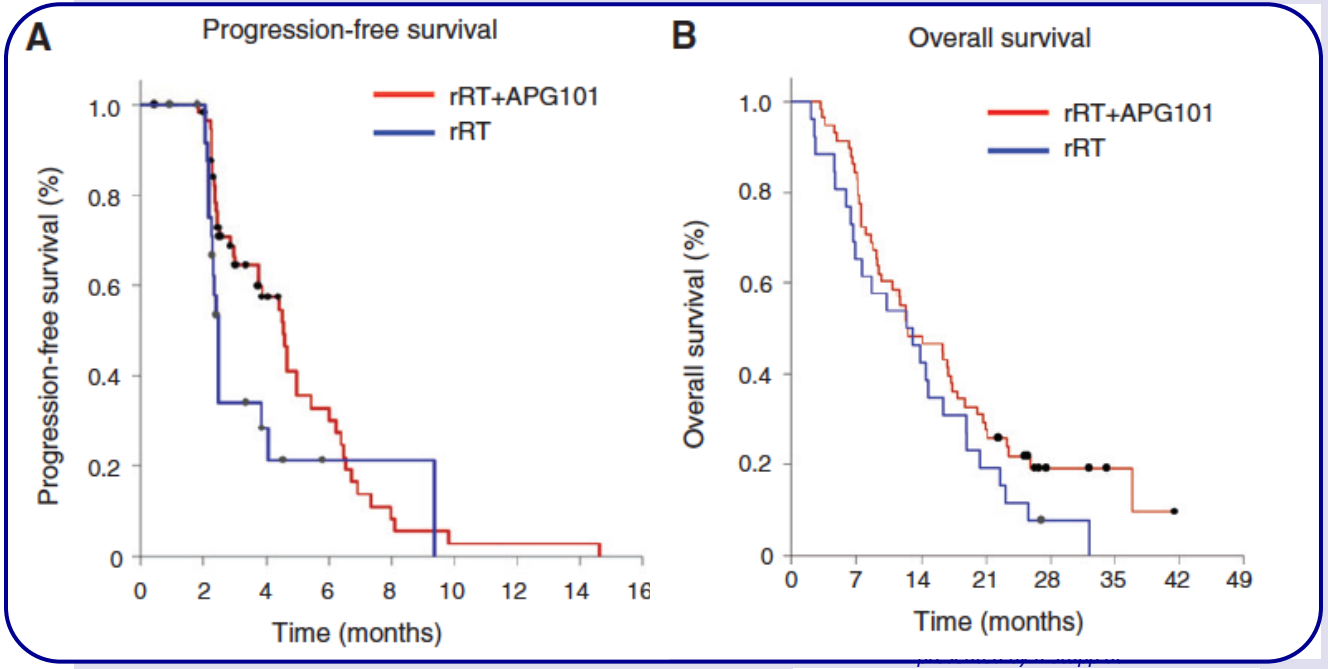
Death induced by CD95 or CD95 ligand elimination

A Phase II, Randomized, Study of Weekly APG101+Reirradiation versus Reirradiation in Progressive Glioblastoma

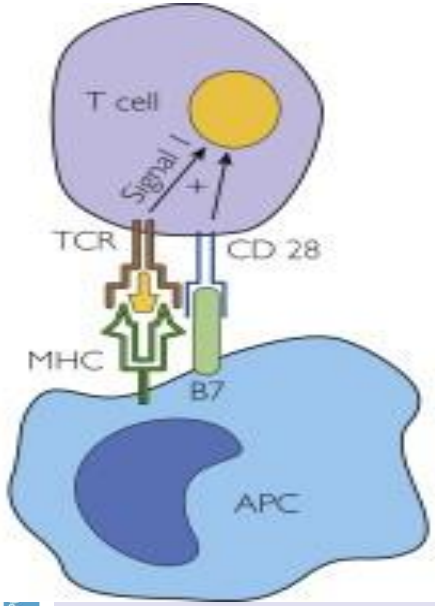
Wolfgang Wick[1,2], Harald Fricke[3], Klaus Junge[4], Grigory Kobyakov[5], Tobias Martens[6], Oliver Heese[6], Benedikt Wiestler[1,2], Maximilian G. Schliesser[2], Andreas von Deimling[7,8], Josef Pichler[9], Elena Vetlova[5], Inga Harting[10], Jürgen Debus[11], Christian Hartmann[7,8], Claudia Kunz[3], Michael Platten[1,12], Martin Bendszus[10], and Stephanie E. Combs[11]

Clin Cancer Res. 2014 Dec 15;20(24):6304-13





Function of CTLA-4 and PD-1



Engager

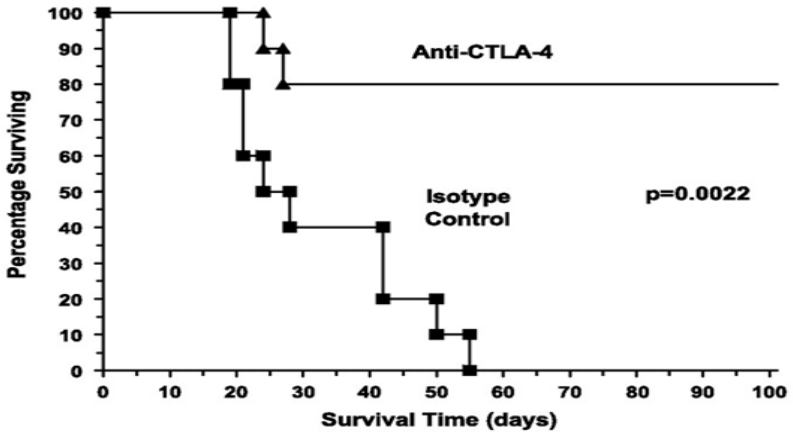
=> Inhibition of these «checkpoint» molecules may boost immune reponses against a tumor

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] Hidenobu Ochiai,[3] Duane A. Mitchell,[1] Peter M. Grossi,[1] Alison E. Sweeney,[1] Gary E. Archer,[1] Thomas Cummings,[2] James P. Allison,[4] Darell D. Bigner,[2] and John H. Sampson[1,2]

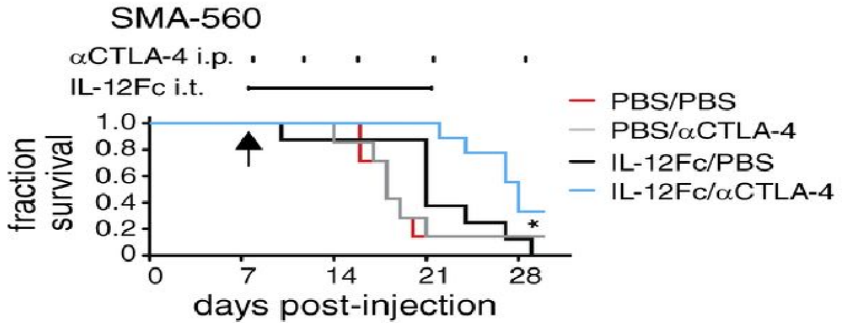
Clin Cancer Res 2007;13(7) April 1, 2007



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 Haimovici,[1] Paulina Kulig,[1] Anna Sledzinska,[1] Michael Weller,[2] and Burkhard Becher[1]

J. Exp. Med. 2013 Vol. 210 No. 13 2803-2811



Expression and functional role of PD-L1 in glioblastoma

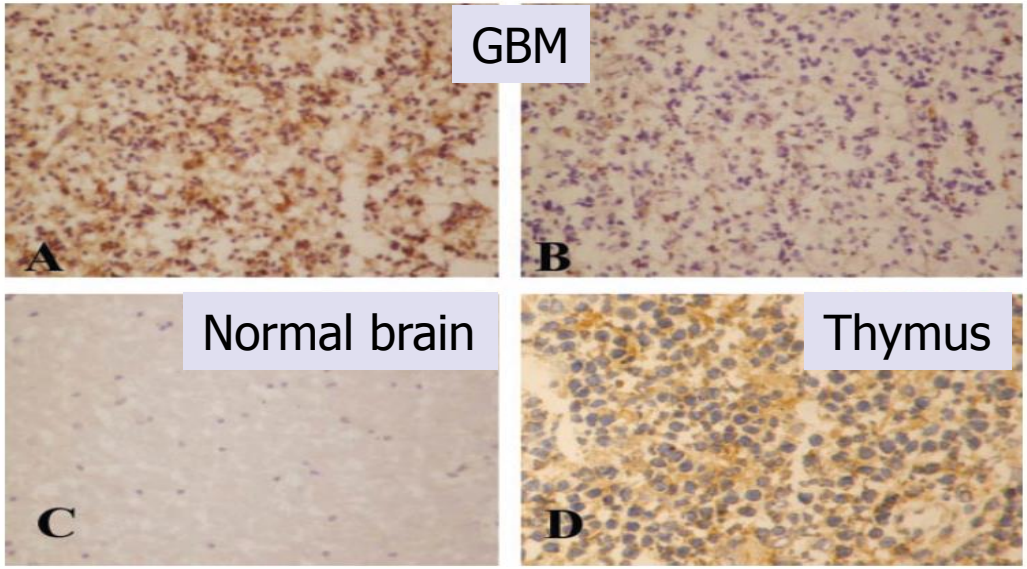
[CANCER RESEARCH 63, 7462–7467, November 1, 2003]

Expression of the B7-Related Molecule B7-H1 by Glioma Cells: A Potential Mechanism of Immune Paralysis[1]

Sabine Wintterle,[2] Bettina Schreiner,[2] Meike Mitsdoerffer, Dagmar Schneider, Lieping Chen, Richard Meyermann, Michael Weller, and Heinz Wiendl[3]

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.]

Human glioblastoma is a highly lethal tumor tl
immune inhibitory capabilities. B7-homologue 1 (B7-
tified homologue of B7.1/2 (CD80/86), has been descri
latory 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 alloreactive 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.



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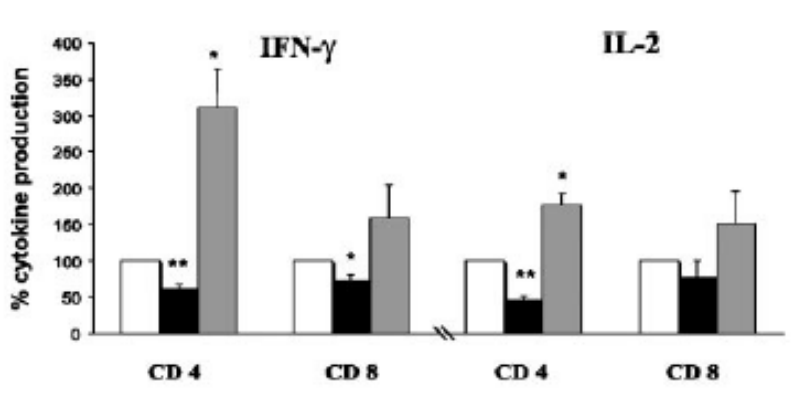
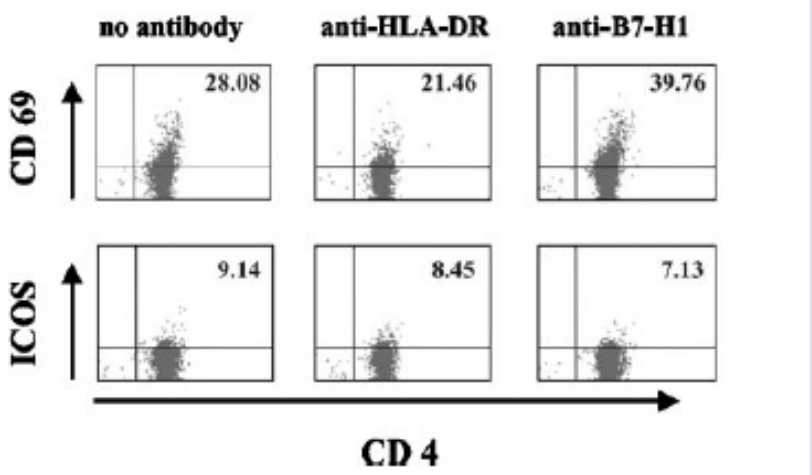
Department of Neurology [S. W., B. S., M. M., D. S., M. W., H. W.] and Institute of Brain Research [R. M.], University of Tübingen, Medical School, D-72076 Tübingen, Germany, and Department of Immunology, Mayo Clinic, Rochester, Minnesota 55905 [L. C.]



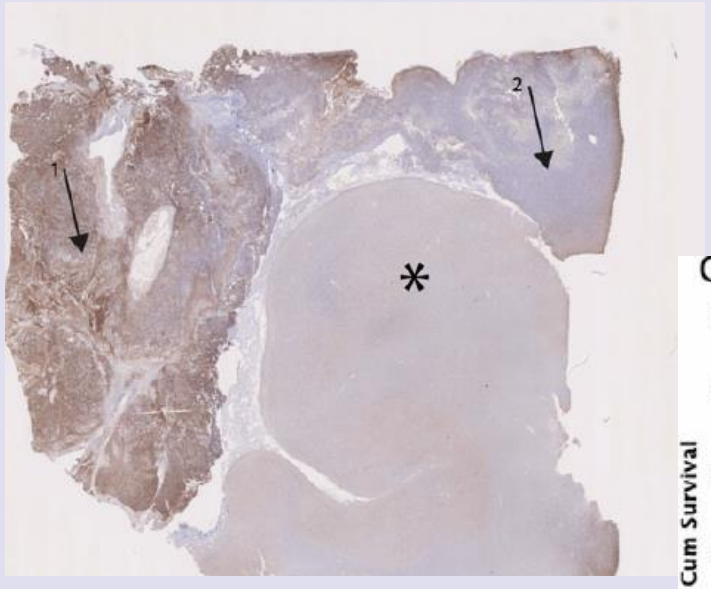


- ☐ No antibody
- ■ Anti-HLA-DR
- ■ Anti-B7

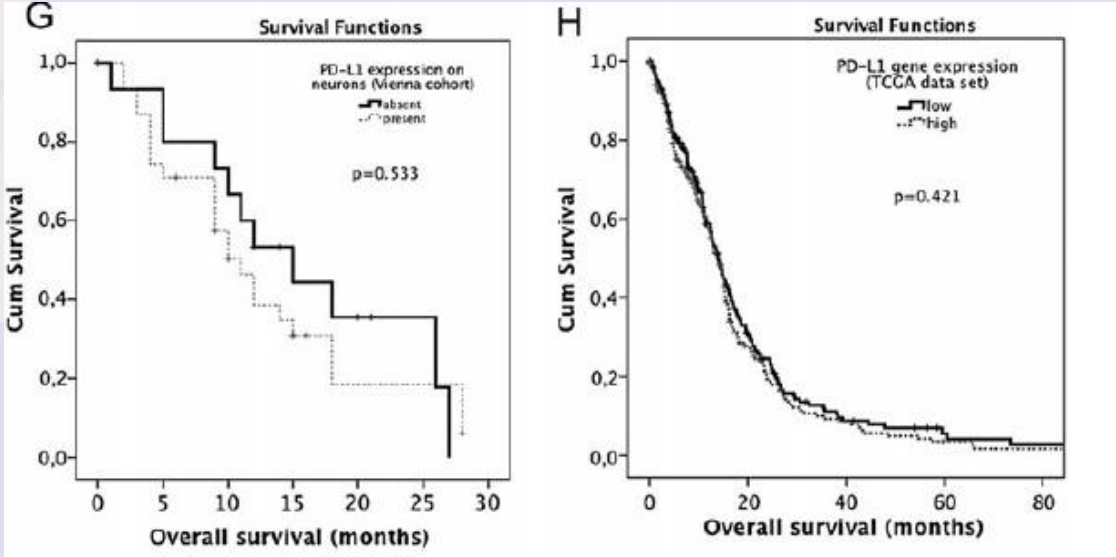
presented by R Stupp at

SINGAPORE 2015 ESMO ASIA 18-21 DECEMBER SINGAPORE

PDL1 expression in GBM



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

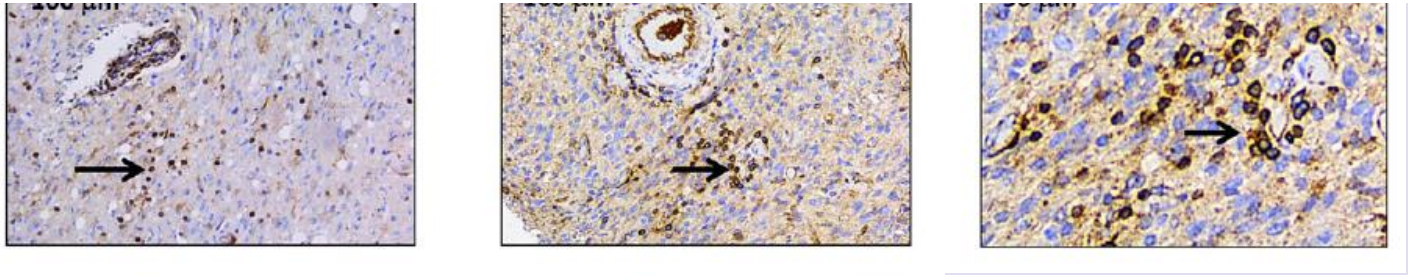


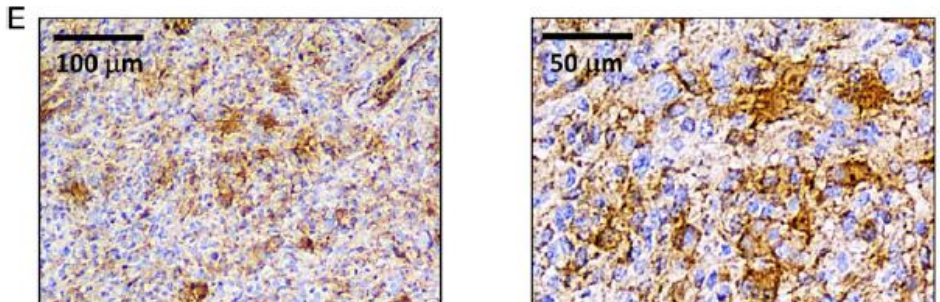
Berghoff et al.
Neuro-Oncology 17(8), 1064–1075, 2015

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

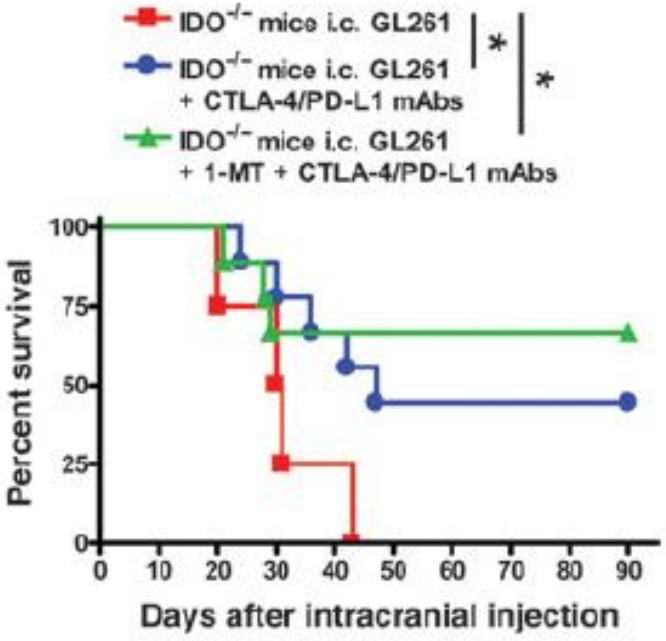
Clin Cancer Res 2014; 20: 5290–301

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.

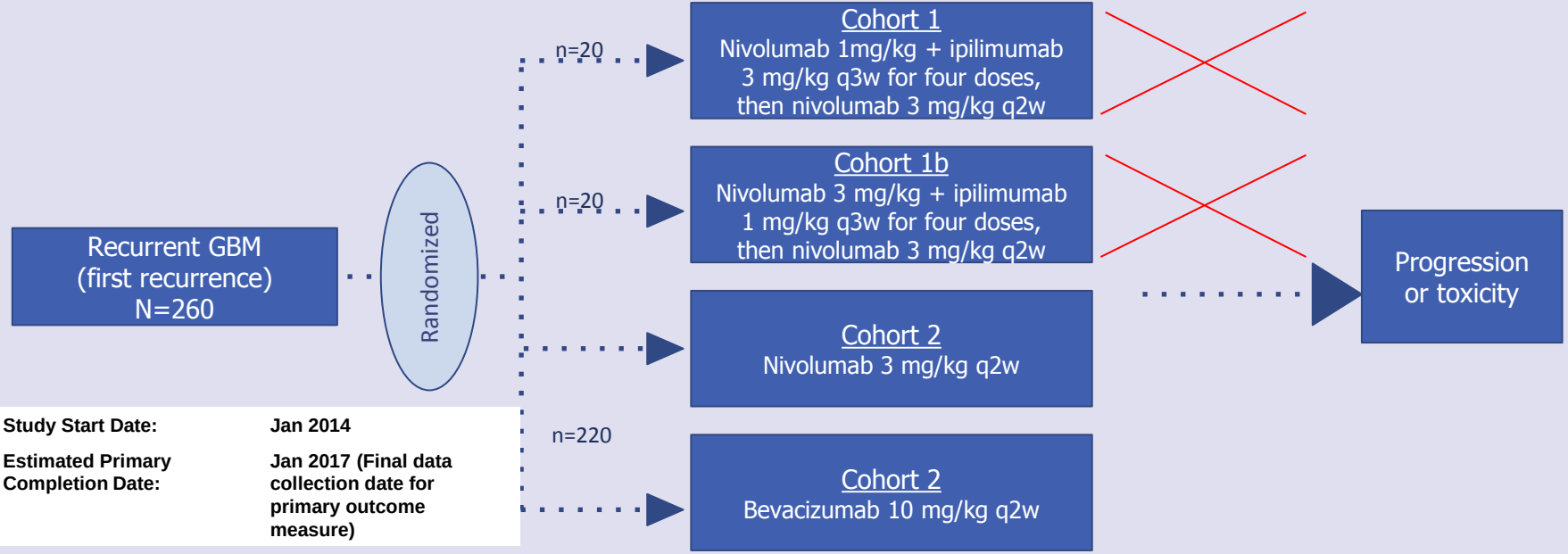
Experimental Design and Results: In this report, we demonstrate that, when dually challenged, IDO-deficient tumors provide a selectively competitive survival advantage against IDO-competent tumors. Next, we provide novel observations regarding tryptophan catabolic enzyme expression, before showing that 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



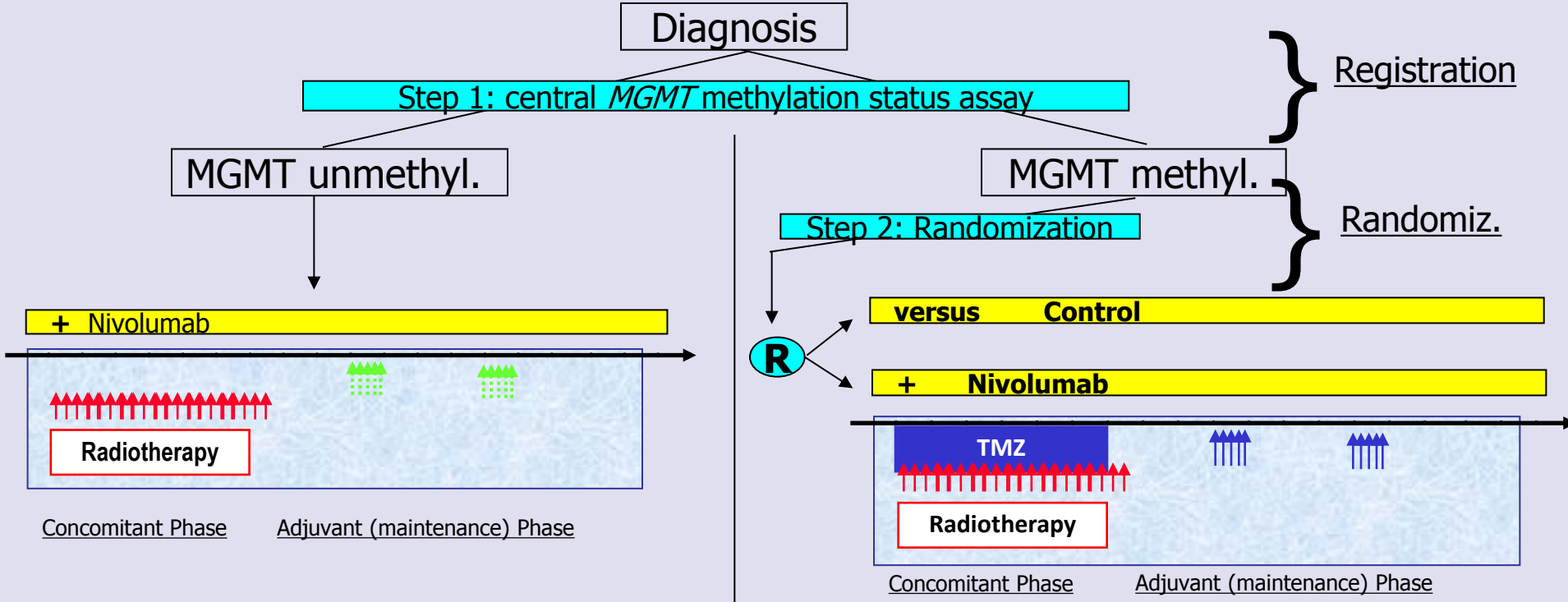
Study Start Date:	**Jan 2014**
Estimated Primary Completion Date:	**Jan 2017 (Final data collection date for primary outcome measure)**

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

1. ClinicalTrials.gov. U.S. National Institutes of Health. NCT02017717. http://clinicaltrials.gov/show/ NCT02017717. Accessed November 5, 2014.

presented by R Stupp at
SINGAPORE 2015 ESMO ASIA 18-21 DECEMBER SINGAPORE

Checkmate Studies (BMS): Nivolumab in newly diagnosed GBM



presented by R Stupp at

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