1st ASEICA EDUCATIONAL SYMPOSIUM

Madrid, 14th and 15th November 2017

Clinically Relevant novel drivers in Glioblastoma

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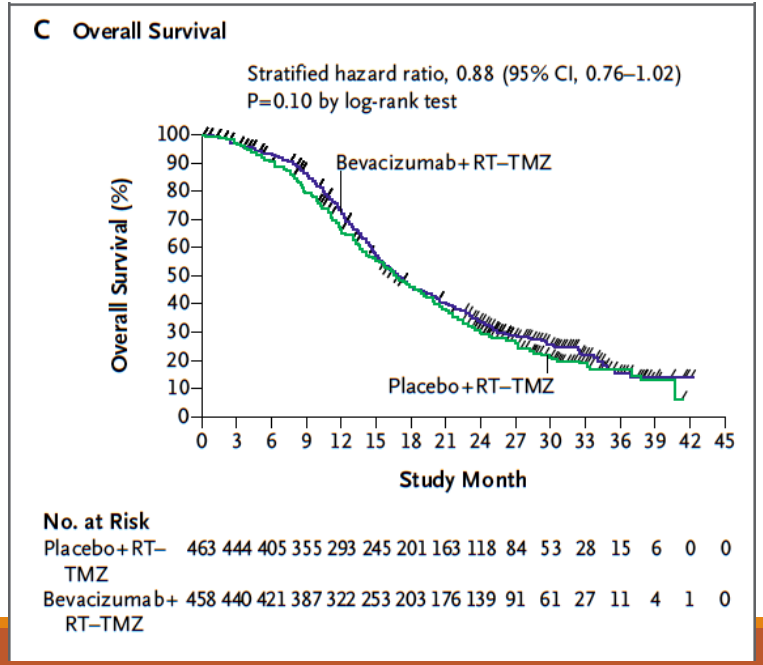
Unidad de Neurooncología
Hospital Universitario 12 de Octubre

aseica

Glioblastoma: Without progress since 2005



ESTUDIO AVAGLIO (NEJM 2014)

Stupp (Rt+TMZ) + Bevacizumab/placebo

TARGETED THERAPIES IN GBM: EGFR

Biology supports use of EGFR TKIs

- ~50% of GBM tumors are amplified for EGFR
- Predictor of poor prognosis
- Of EGFR amplified tumors, 50% express EGFRvIII

But, activity with EGFR TKIs has been modest to date in GBM:

First generation reversible EGFR inhibitors including **gefitinib and erlotinib** showed limited activity for several reasons but mainly because these agents were tested in an unselected population.

TARGETED THERAPIES?

Small Molecule Therapeutics

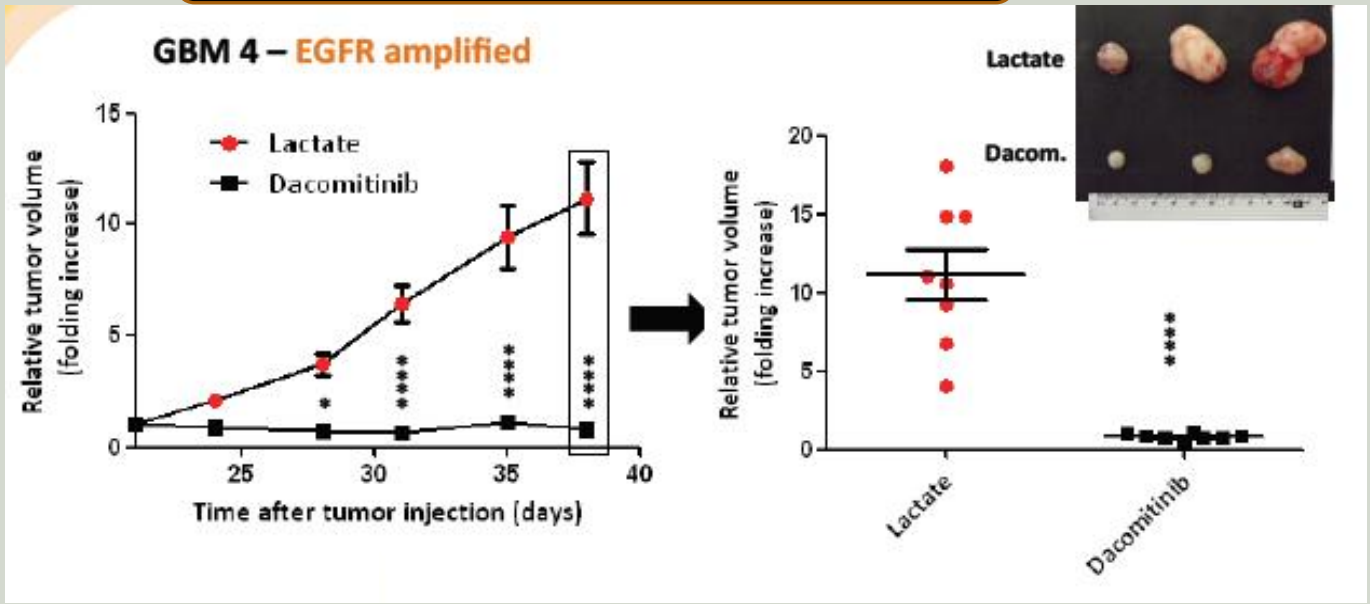
Molecular Cancer Therapeutics

Preclinical Test of Dacomitinib, an Irreversible EGFR Inhibitor, Confirms Its Effectiveness for Glioblastoma

Cristina Zahonero[1], Pilar Aguilera[1], Carmen Ramírez-Castillejo[1], Marta Pajares[1], Maria Victoria Bolós[2], Diana Cantero[3], Angel Perez-Nuñez[3], Aurelio Hernández-Laín[3], Pilar Sánchez-Gómez[1], and Juan Manuel Sepúlveda[3]

Dacomitinib in GBM. Preclinical Studies

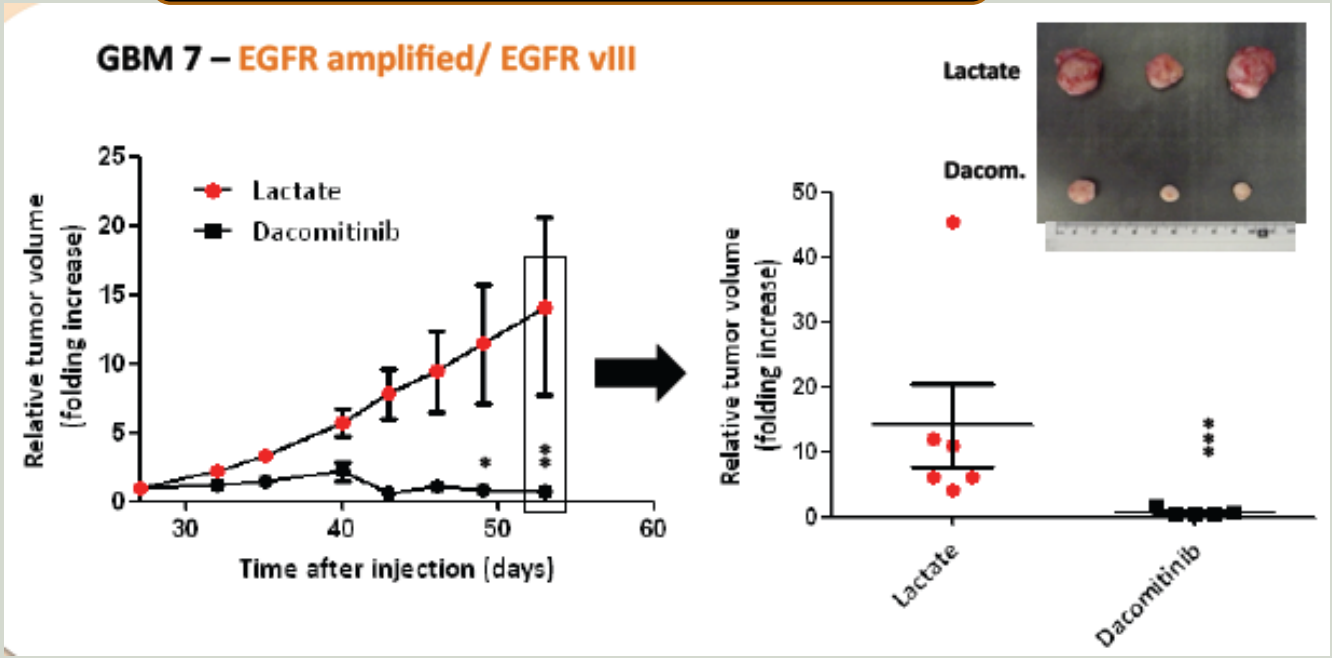
Dacomitinib inhibits growth in
EGFR amplified GBM xengrafts models



Zahonero C, et al. Molecular Cancer Therapeutics 2015

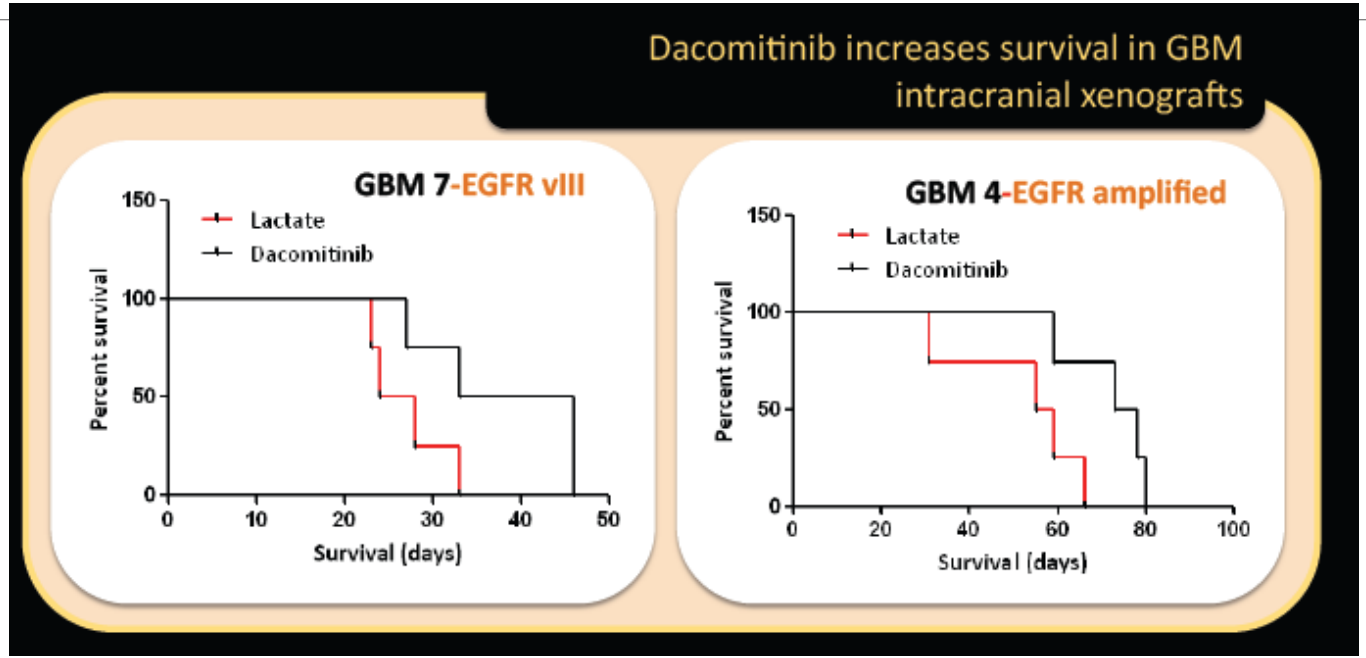
Dacomitinib in GBM. Preclinical Studies

Dacomitinib inhibits growth in
GBM EGFR amplified / EGFR vIII xenografts models



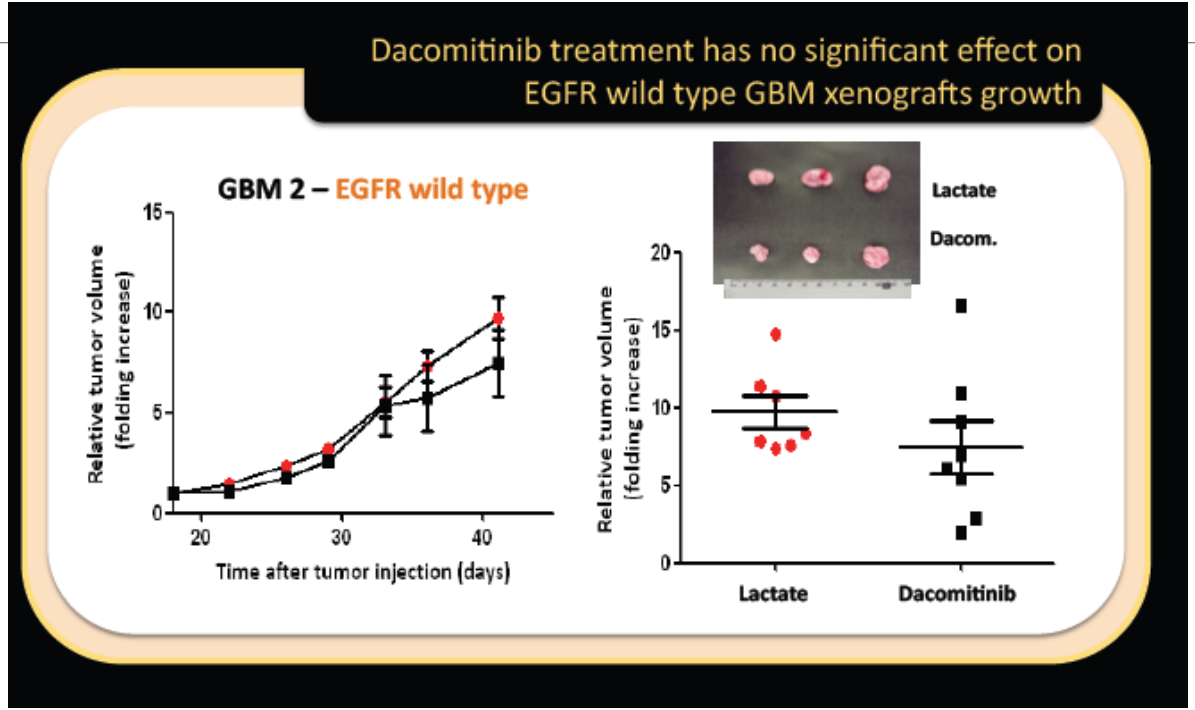
Zahonero C, et al. Molecular Cancer Therapeutics 2015

Dacomitinib in GBM. Preclinical Studies



Zahonero C, et al. Molecular Cancer Therapeutics 2015

Dacomitinib in GBM. Preclinical Studies



Zahonero C, et al. Molecular Cancer Therapeutics 2015

GEINO-11 TRIAL. FINAL RESULTS

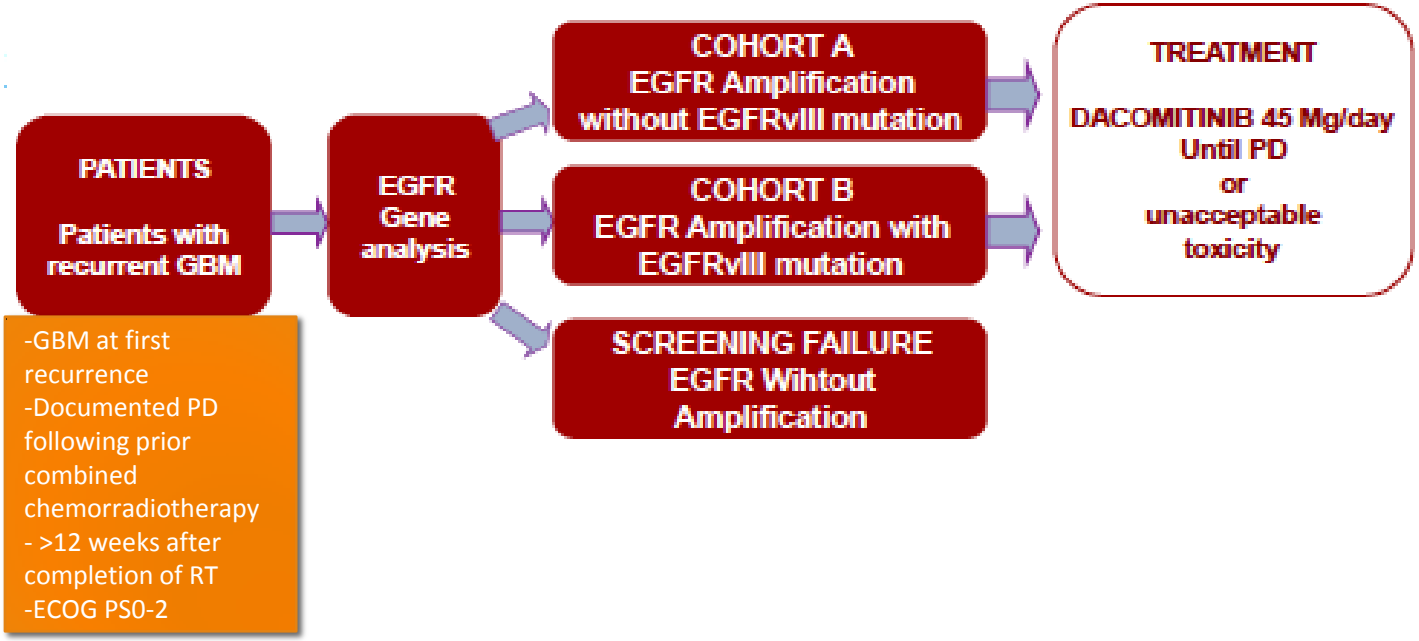
1522 Neuro-Oncology

19(11), 1522–1531, 2017 | doi:10.1093/neuonc/nox105 | Advance Access date 1 June 2017

Phase II trial of dacomitinib, a pan–human EGFR tyrosine kinase inhibitor, in recurrent glioblastoma patients with *EGFR* amplification

Juan Manuel Sepúlveda-Sánchez, María Ángeles Vaz, Carmen Balañá, Miguel Gil-Gil, Gaspar Reynés, Óscar Gallego, María Martínez-García, Elena Vicente, María Quindós, Raquel Luque, Ana Ramos, Yolanda Ruano, Pedro Pérez-Segura, Manuel Benavides, Pilar Sánchez-Gómez, and Aurelio Hernández-Laín

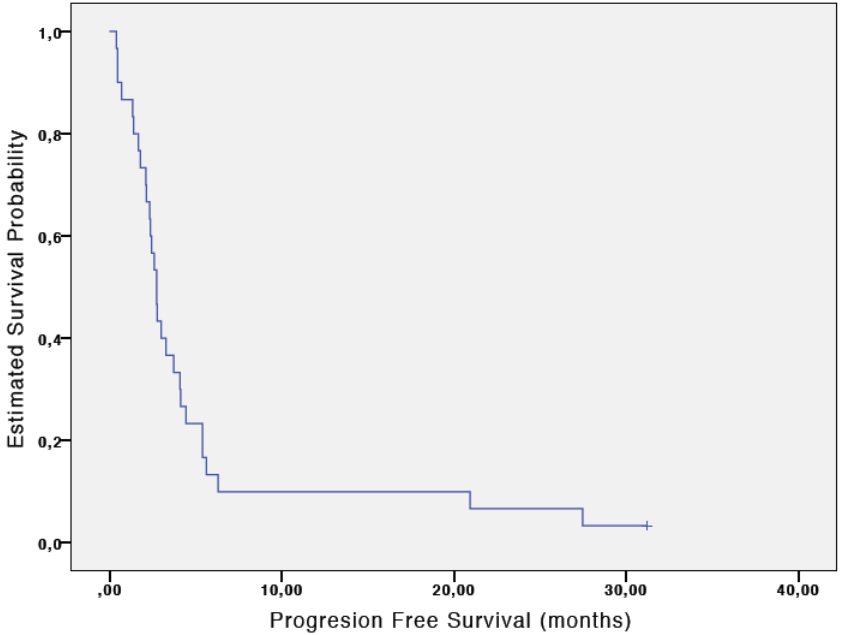
GEIMO 11. TRIAL DESIGN



RESULTS
Primary Endpoint: PFS6m

COHORT A. EGFRamp/EGFRvIII-

- 6 month PFS rate: 13.3% (95% CI: 0-26.5).
  - Patients without progression at 6 months: 3/30

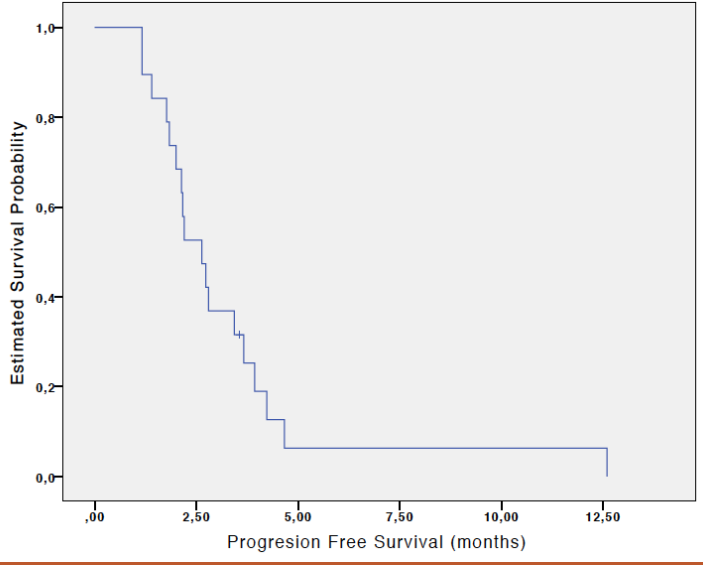


Median PFS: 2.7 months
(95% CI: 2.2-3.2)

RESULTS
Primary Endpoint

COHORT B. EGFRamp/EGFRvIII+

- Recruitment was closed after the planned interim analysis (First Stage). Only 1 patient did not progress after 6 months of treatment after the inclusion of the first 17 evaluable patients.
- 6 PFS rate: 6% (95% CI: 0-18)

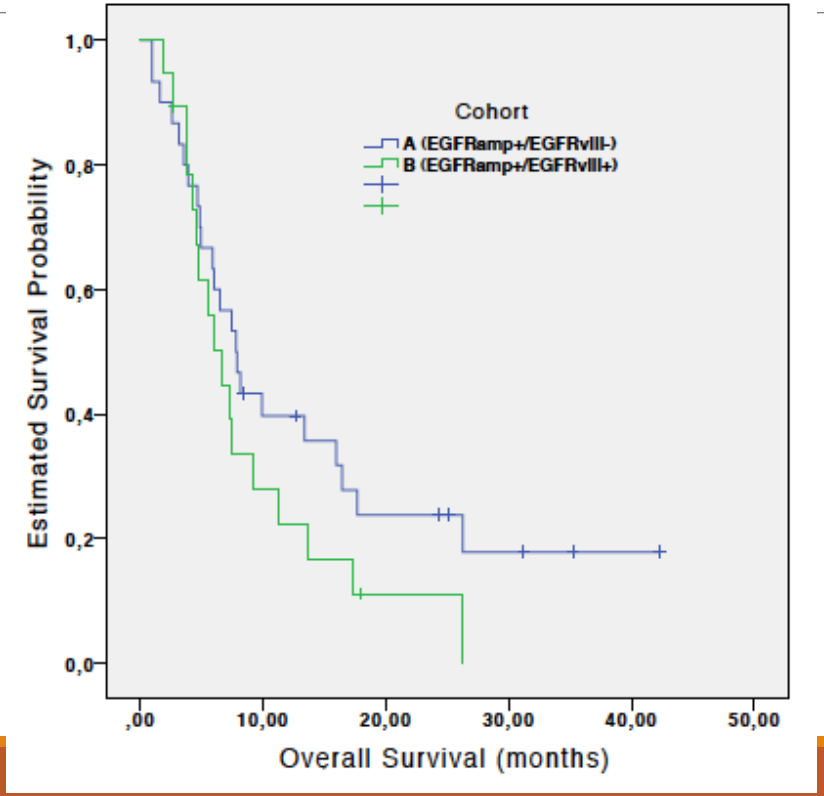


Median PFS: 2.6 months
CI 95%: 1.8-3.4

Best Overall Response

	COHORT		
	A (EGFRamp+/EGFRvIII-) N=30 n (%)	B (EGFRamp+/EGFRvIII+) N=19 n (%)	TOTAL N=49 N (%)
CR	1 (3.3)	0	1 (2)
PR	1 (3.3)	1 (5.3)	2 (4.1)
SD	8 (26.7)	4 (21)	12 (24)
PD	17 (56.7)	13 (68.4)	30 (61.2)
Not Evaluable	3 (10)	1 (5.3)	4 (8.2)

Overall Survival



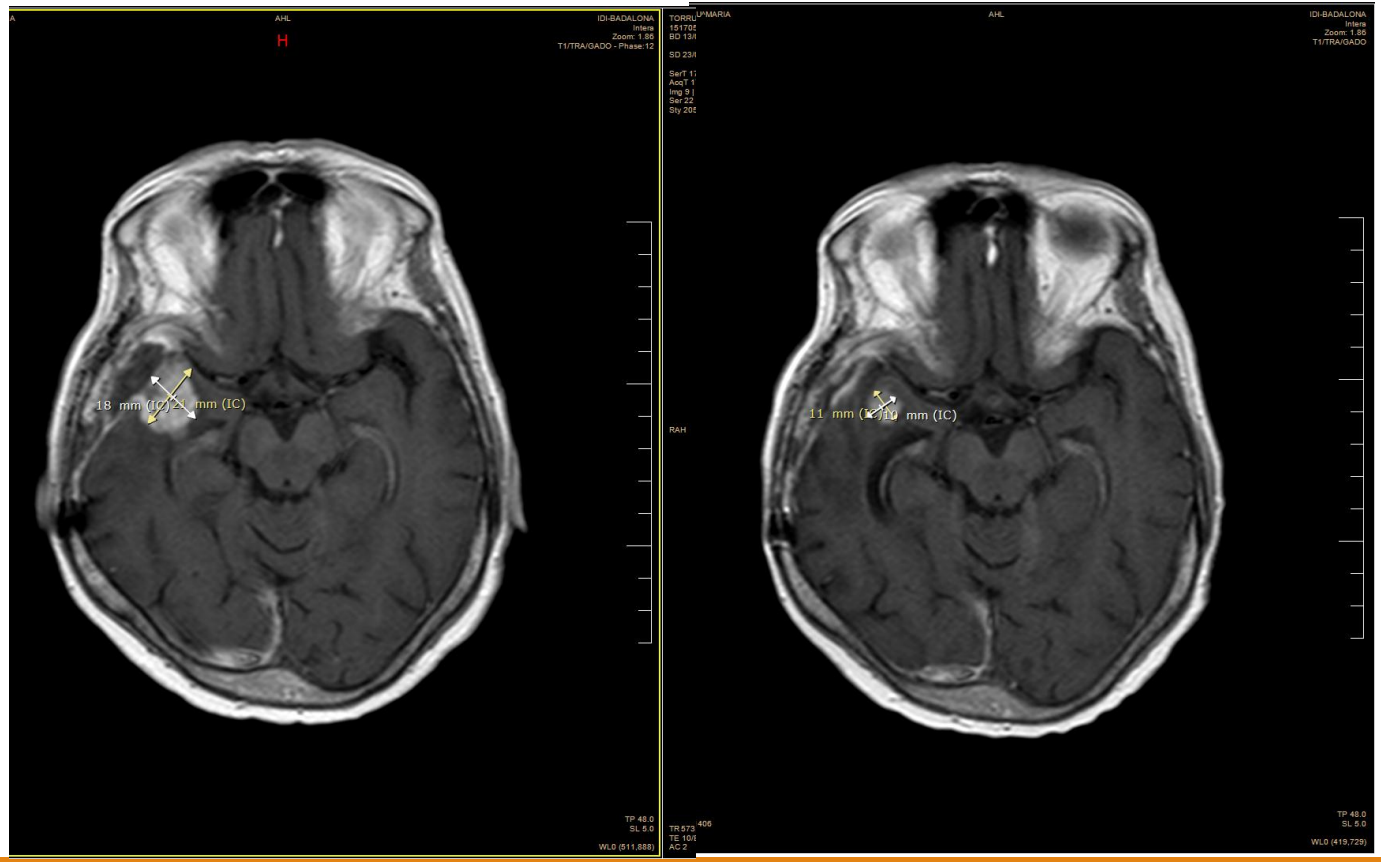
Cohort A

-Median OS: 7.8 months
(CI 95%: 5.6-10)

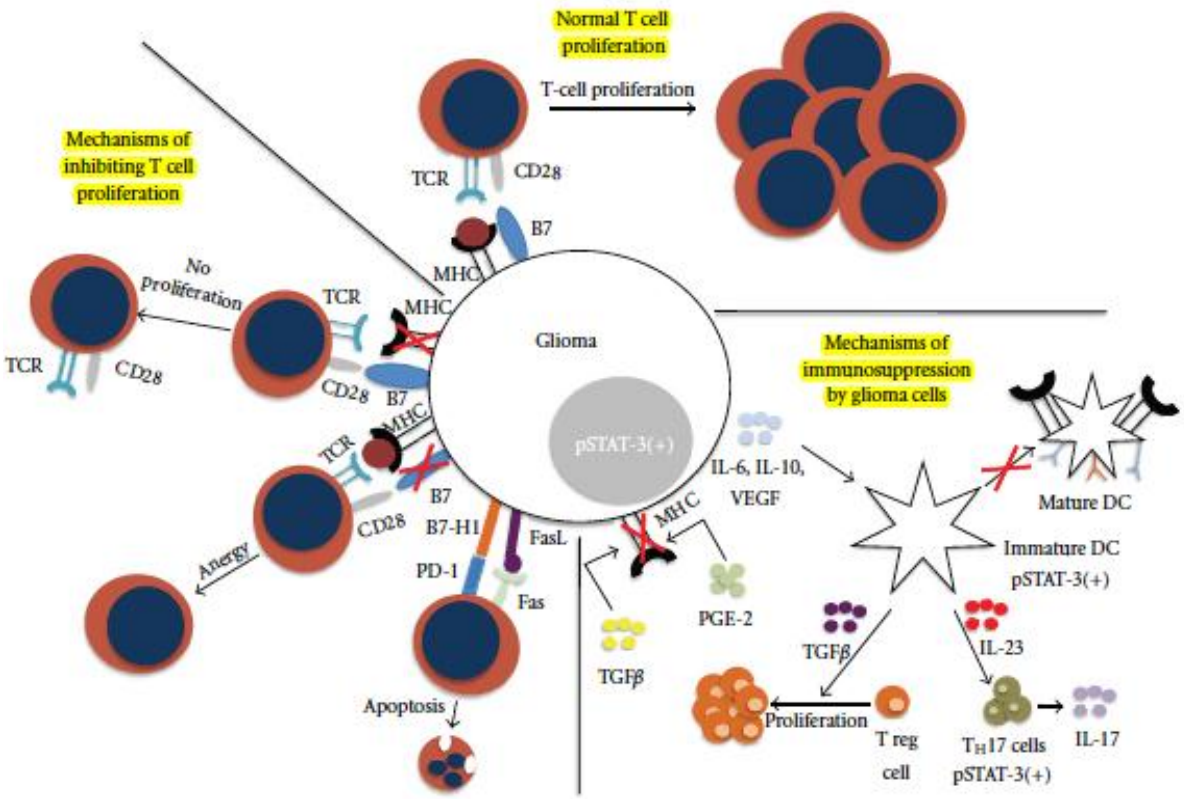
Cohort B

-Median OS: 6.7 months
(CI 95%: 4.4-8.9)

Partial Response after 6 months of treatment. EGFR amp without VIII mutation



GBM-related Inmunosupression



Immune Privilege in the Brain: Old Paradigm

Historical belief was that **"immune privilege"** in the brain was equivalent to **"immune isolation"**[1-3]

- Presence of BBB
- Absence of lymphatic drainage system
- Ability to tolerate foreign antigens without eliciting an inflammatory response
- Low baseline levels of MHC expression
- Altered expression of T-cell co-stimulatory molecules

BBB, blood-brain barrier; MHC, major histocompatibility complex.

1. Jackson CM et al. *Clin Cancer Res.* 2014;20(14):3651-3659.
2. Heimberger AB, Sampson JH. *Neuro Oncol.* 2011;13(1):3-13.
3. Carson MJ et al. *Immunol Rev.* 2006;213:48-65.

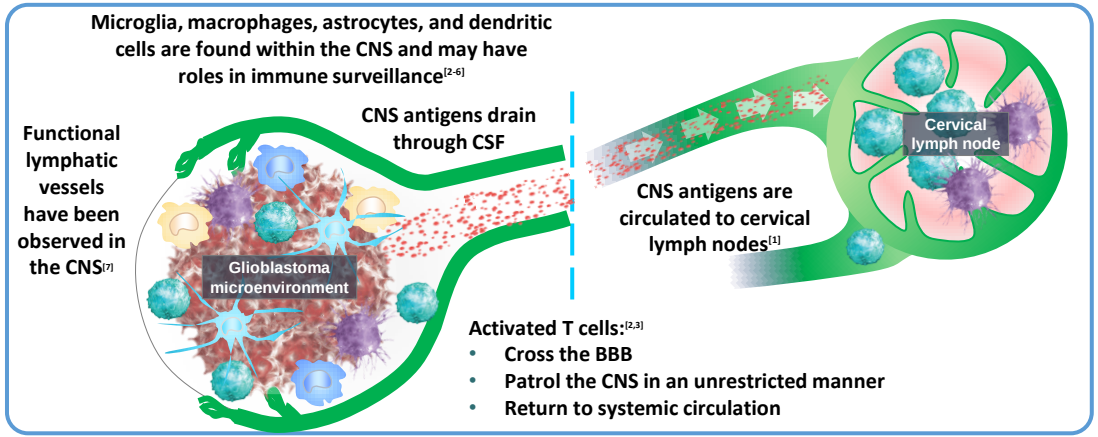
The BBB is Not an Absolute Barrier to Immune Response

Component	Effect / Impact on the BBB
Glioblastoma cells[1]	• Release factors that disrupt endothelial tight junctions
Glioblastoma-associated vasculature[1-4]	• Display abnormal structural features, including loss or abnormal morphology of tight junctions, as well as increased permeability
Activated T cells[5,6]	• High levels of Very Late Antigen-4 (VLA-4) and leukocyte-function-associated antigen (LFA-1) allow movement across BBB • Can patrol the CNS for antigens in an unrestricted manner
Peripheral immune system activation[6]	• BBB leakiness and movement of activated macrophages and dendritic cells across BBB

BBB, blood-brain barrier; CNS, central nervous system; LFA-1, leukocyte-function-associated antigen-1; VLA-4, very late antigen-4.

1. Schneider SW et al. *Acta Neuropathol.* 2004;107(3):272-276. 2. Rascher G et al. *Acta Neuropathol.* 2002;104(1):85-91. 3. Abbott NJ et al. *Neurobiol Dis*. 2010;37(1):13-25. 4. Wolburg H et al. *Acta Neuropathol.* 2003;105(6):586-592. 5. Ransohoff RM et al. *Nat Rev Immunol*. 2003;3(7):569-581. 6. Carson MJ et al. *Immunol Rev.* 2006;213:48-65.

“Immune privilege” is being redefined:
Communication between the CNS and immune system exists



BBB, blood-brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid.

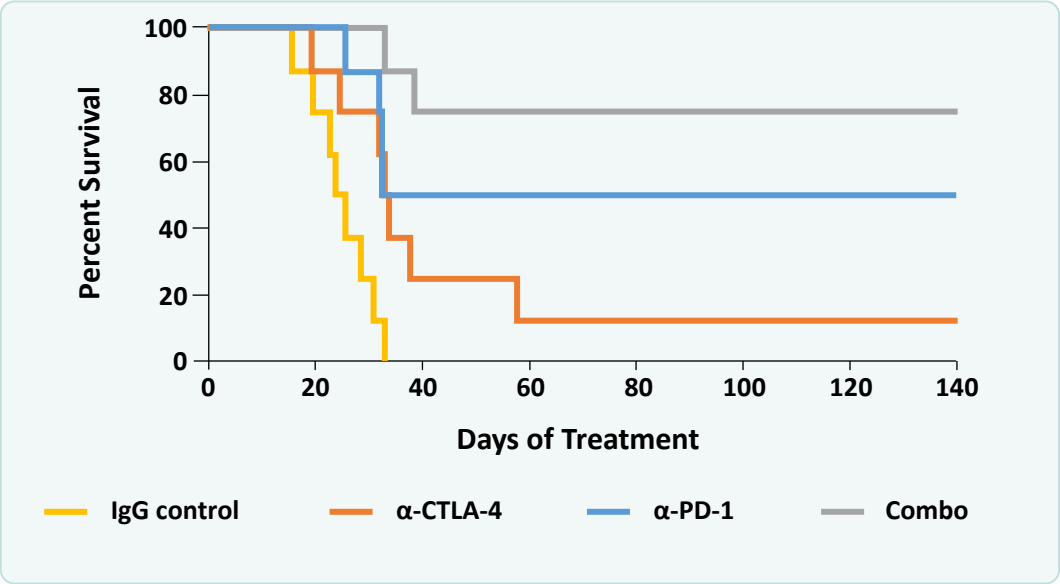
1. Jackson CM et al. *Clin Cancer Res*. 2014;20(14):3651-3659. 2. Heimberger AB, Sampson JH. *Neuro Oncol.* 2011;13(1):3-13. 3. Glass R, Synowitz M. *Acta Neuropathol*. 2014;128(3):347-362. 4. Carson MJ et al. *Immunol Rev.* 2006.213:48-65. 5. Ransohoff RM, Engelhardt B. *Nat Rev Immunol.* 2012;12(9):623-635. 6. Jackson C et al. *Clin Dev Immunol*. 2011:732413. doi: 10.1155/2011/732413. 7. Louveau A et al. *Nature*. 2015;523(7560):337-341. doi: 10.1038/nature14432.

Society for Neurooncology Nov 2015 Abstracts Related with Checkpoint inhibitors

Lead Author	Study	Title	Format
Sampson	CA209-143	Safety and Activity of Nivolumab Monotherapy and Nivolumab in Combination with Ipilimumab in Recurrent Glioblastoma: Updated Results from CHECKMATE-143	Oral
Sahebjam	CA209-143	Pseudoprogression and Immunotherapy in Glioblastoma: A Case Series from Cohort 1 of CHECKMATE-143 (NCT02017717)	Rapid Report Platform Presentation
Ramkissoon	CA209-143	Central Neuropathology Review to Assist in the Evaluation of Immune-Related Treatment Changes versus Disease Progression in CHECKMATE-143	Poster
Lucca	N/A	PD-1 Expression Identifies Exhausted Tumor-Infiltrating Regulatory T cells in Glioblastoma	Poster
Goods	N/A	Functional Differences Between PD-1+ and PD-1– CD4+ T effector Cells in Healthy Donors and Patients With Glioblastoma	Poster
Lowther	CA209-143	Immune biomarker results from a trial of nivolumab ± ipilimumab in patients with recurrent glioblastoma: CHECKMATE-143	Poster
Nayak	CA209-143	Description of clinical and patient reported outcomes assessments from a Phase 3, multicenter, randomized trial evaluating nivolumab monotherapy versus bevacizumab in recurrent glioblastoma: CHECKMATE-143	Poster
Signorovitch	HEOR	Patient relevant outcomes in recurrent glioblastoma: a systematic literature review	Poster

PD-1 and CTLA-4 Blockade Prolonged Survival in a Preclinical Mouse Model

GL261 murine glioma tumor-bearing mice were treated with anti-murine CTLA-4 and anti-murine PD-1 or anti-murine PD-L1 blocking antibodies:



CTLA-4, cytotoxic T-lymphocyte antigen-4; IgG, immunoglobulin G; PD-1, programmed death-1.

1. Reardon DA et al. Poster presentation at ASCO 2014. 2084.

Randomized Phase 3 Study Evaluating the Efficacy and Safety of Nivolumab vs Bevacizumab in Patients With Recurrent Glioblastoma: CheckMate 143

David A. Reardon,[1,a] Antonio Omuro,[2,a] Alba A. Brandes,[3] Johannes Rieger,[4,5] Antje Wick,[6] Juan Manuel Sepulveda,[7] Surasak Phuphanich,[8] Paul de Souza,[9] Manmeet S. Ahluwalia,[10] Michael Lim,[11] Gordana Vlahovic,[12,b] John Sampson[12,b]

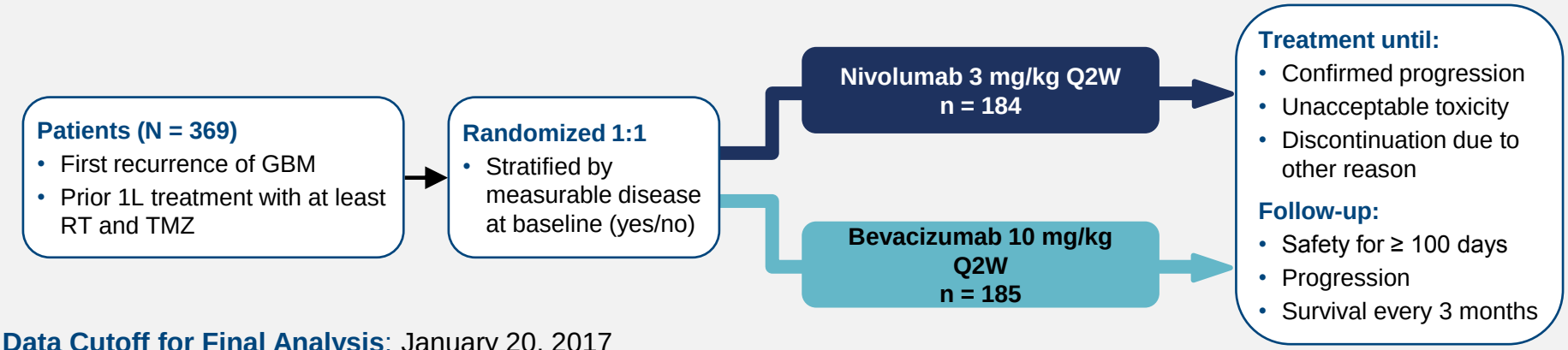
[1]Dana-Farber Cancer Institute and Harvard University School of Medicine, Boston, MA; [2]Memorial Sloan Kettering Cancer Center, New York, NY; [3]AUSL-IRCCS Institute of Neurological Sciences, Bologna, Italy; [4]Klinikum der Goethe-Universität, Frankfurt, Germany; [5]University of Tübingen, Tübingen, Germany; [6]Neurology Clinic, University of Heidelberg, National Center for Tumor Diseases, Heidelberg, Germany; [7]Hospital Universitario 12 De Octubre, Madrid, Spain; [8]Cedars-Sinai Medical Center, Los Angeles, CA; [9]University of Western Sydney School of Medicine, Liverpool, Australia; [10]Cleveland Clinic, Cleveland, OH; [11]The Johns Hopkins Hospital, Baltimore, MD; [12]Duke University Medical Center, Durham, NC

5th Quadrennial Meeting of the World Federation of Neuro-Oncology Societies
May 4-7, 2017; Zurich, Switzerland

[a] Co-first authors.
[b] Co-senior authors.

CheckMate 143 Cohort 2 Study Design

Nivolumab vs Bevacizumab in Recurrent GBM



Data Cutoff for Final Analysis: January 20, 2017

Median (range) Follow-Up: nivolumab, 9.8 (1.3–26.3) months; bevacizumab, 9.4 (0–26.8) months

Endpoints:

- **Primary**: OS in all randomized patients
- **Secondary**: investigator-assessed ORR and PFS (RANO); 12-month OS rate
- **Other key endpoints**: safety; biomarkers

Assessments:

- **Tumor**: contrast-enhanced MRI Q6W until week 13, then Q8W (RANO)
- **Safety**: CTCAE v4.0

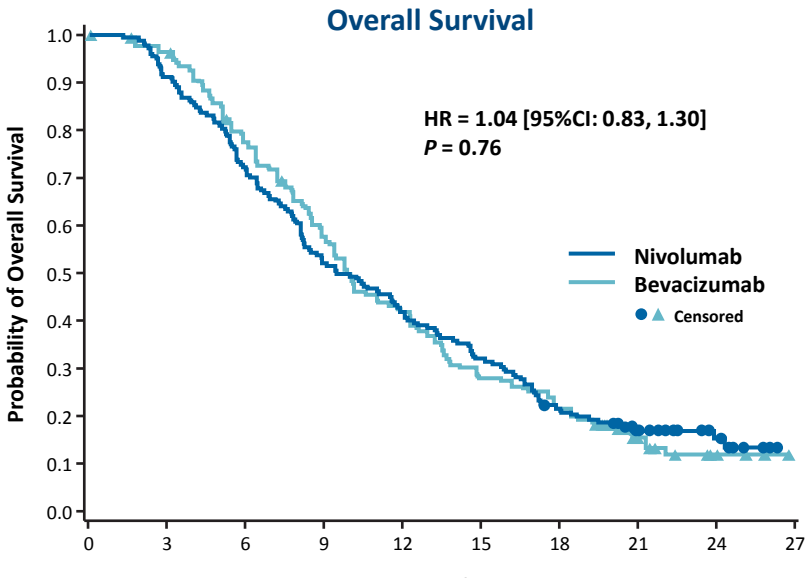
1L, first line; CTCAE, Common Terminology Criteria for Adverse Events; MRI, magnetic resonance imaging; ORR; objective response rate; PFS, progression-free survival; Q2W, every 2 weeks; Q6W, every 6 weeks; Q8W, every 8 weeks; RANO, Radiologic Assessment in Neuro-Oncology criteria.

Overall Survival and Progression-Free Survival

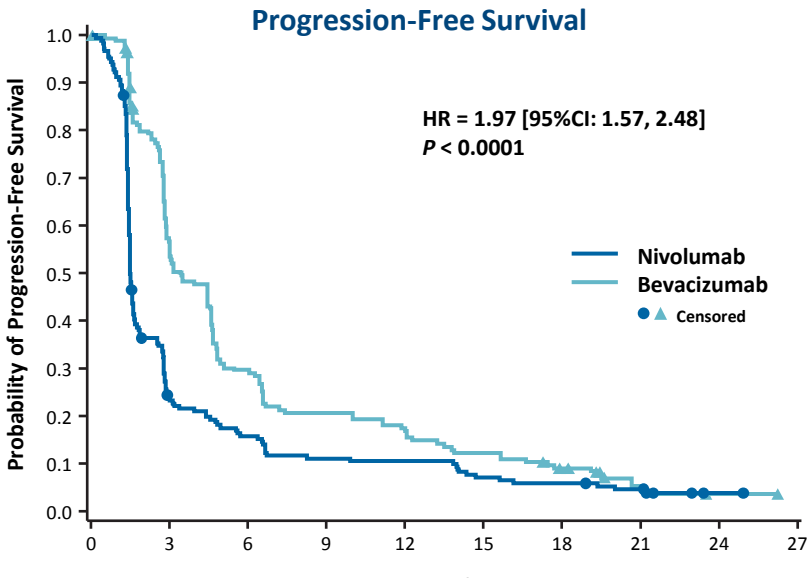
Nivolumab vs Bevacizumab in Recurrent GBM

	Events, n	Median OS [95% CI], months	12-Month OS Rate [95% CI], months
Nivolumab	154	9.8 [8.2, 11.8]	41.8 [34.7, 48.8]
Bevacizumab	147	10.0 [9.0, 11.8]	42.0 [34.6, 49.3]

	Events, n	Median PFS [95% CI], months	12-Month PFS Rate [95% CI], months
Nivolumab	171	1.5 [1.5, 1.6]	10.5 [6.5, 15.5]
Bevacizumab	146	3.5 [2.9, 4.6]	17.4 [11.9, 23.7]



No. at Risk										
Nivolumab	184	168	133	96	77	59	39	24	9	0
Bevacizumab	185	169	135	99	72	48	37	14	5	0

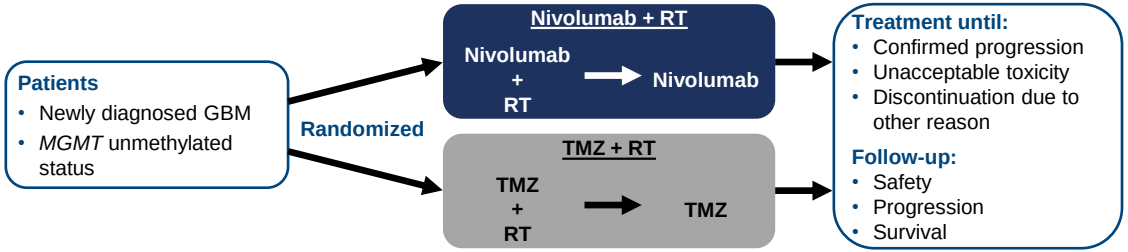


No. at Risk										
Nivolumab	184	41	27	19	18	12	10	7	1	0
Bevacizumab	185	88	46	32	27	19	12	3	1	0

HR, hazard ratio.

Clinical Trials of Nivolumab in Newly Diagnosed GBM

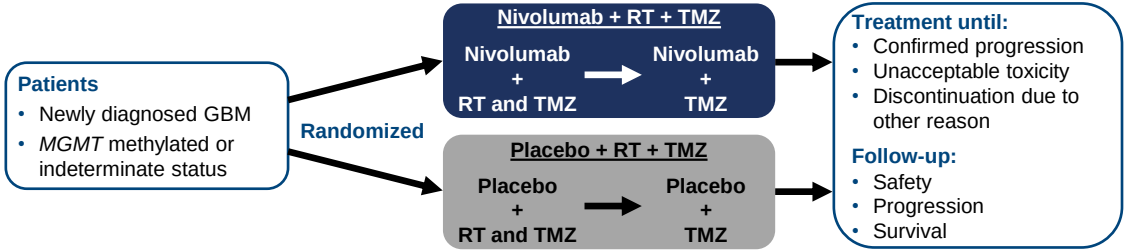
CheckMate 498 (NCT02617589): Nivolumab or TMZ in combination with RT in newly diagnosed patients with *MGMT*-unmethylated GBM



Endpoints:

- **Primary**: OS
- **Secondary**: PFS and 12-month OS rate

CheckMate 548 (NCT02667587): Nivolumab or placebo in combination with RT + TMZ in newly diagnosed patients with *MGMT*-methylated or indeterminate GBM

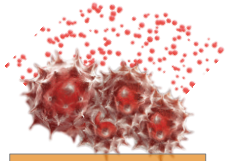


Endpoints:

- **Primary**: OS
- **Secondary**: PFS

Trial Design for ACT IV (Rindopepimut: EGFRvIII)

ACT IV: Ph III study investigating the addition of rindopepimut to standard of care in newly diagnosed GBM (N=700)



EGFRvIII vaccine

Key Eligibility Criteria

- Newly diagnosed, EGFRvIII–positive GBM
- Attempted surgical resection followed by conventional chemoradiation
- No PD after initial therapy

Randomized:

- **Rindopepimut (+ GM-CSF) + TMZ**
- **KLH (control) + TMZ**

Start Date:
November 2011

Primary Endpoint:
OS

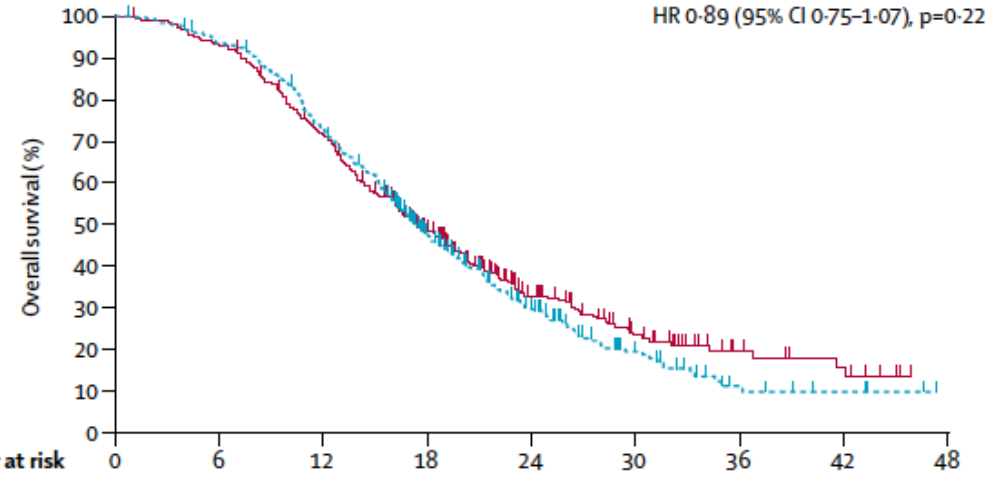
Secondary Endpoints:
PFS, safety and tolerability

EGFRvIII, epidermal growth factor receptor variant III; GBM, glioblastoma; GM-CSF, granulocyte macrophage colony-stimulating factor; KLH, keyhole limpet hemocyanin; OS, overall survival; PD, progressive disease; PFS, progression-free survival; TMZ, temozolomide.

1. Clinicaltrials.gov. NCT01480479.

ACT IV Trial (Rindopepimut) Results

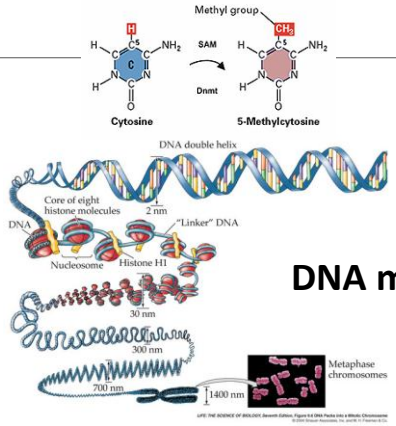
OS. Intention to treat population



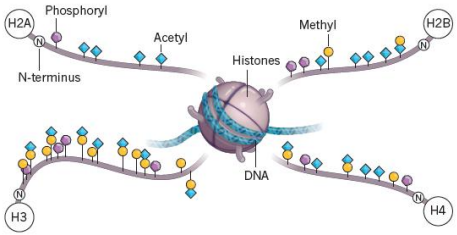
Number at risk (number censored)	0	6	12	18	24	30	36	42	48
Rindopepimut + temozolomide	371 (0)	345 (1)	261 (5)	159 (25)	72 (68)	32 (92)	12 (108)	7 (111)	0 (117)
Control + temozolomide	374 (0)	347 (3)	268 (7)	149 (35)	73 (61)	25 (90)	8 (98)	4 (101)	0 (105)

The Epigenetic Code is Written Over the Genome and Controls Its Expression

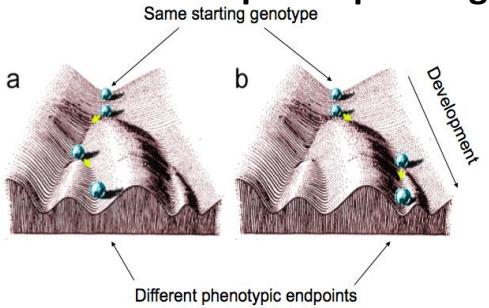
DNA methylation



Histone tail modifications

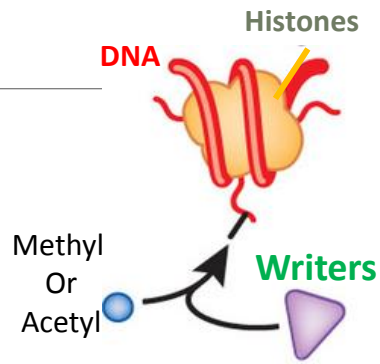


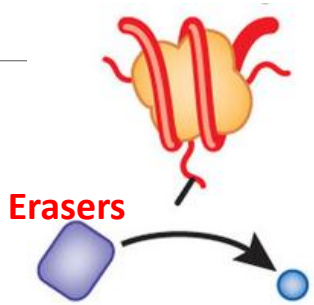
DNA methylation and Histone modifications can activate or repress specific genes

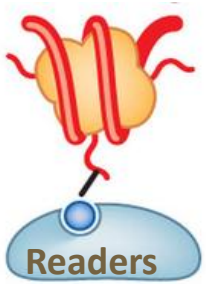


The distribution of DNA methylation and histone modifications across the genome of a cell describes its epigenetic "landscape"

The Epigenetic Code is Dynamically Written, Erased and Read by Families of Enzymes







Add methyl groups to
- DNA (DNMT)
- Histones (HMT)

Add acetyl groups to Histones (HAT)

Remove methyl groups from
- DNA (TET)
- Histones (HDM)

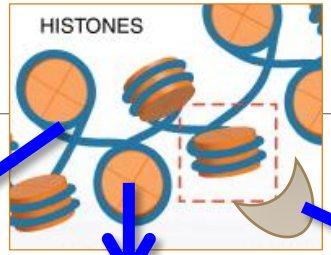
Remove acetyl groups from Histones (HDAC)

Recognize and bind to methyl or acetyl groups on
- DNA (MBD)
- Histones (BET (incl BRD2, 3, 4))

Control accessibility of genes to transcriptional machinery

Recruit transcriptional machinery to specific genes

Epigenetics Pathway Regulation



DNA Modifiers

- DNA methyl transferases (DNMTs)
  For ex: MGMT

Histone Modifiers

- Histone acetyl transferases (HATs)
- Histone deacetylases (HDACs)
- Histone methyl transferases (HMTs)
- Histone demethylases
  For ex: LSD1

Readers

- BET Family
  (BRD2, 3, 4)

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

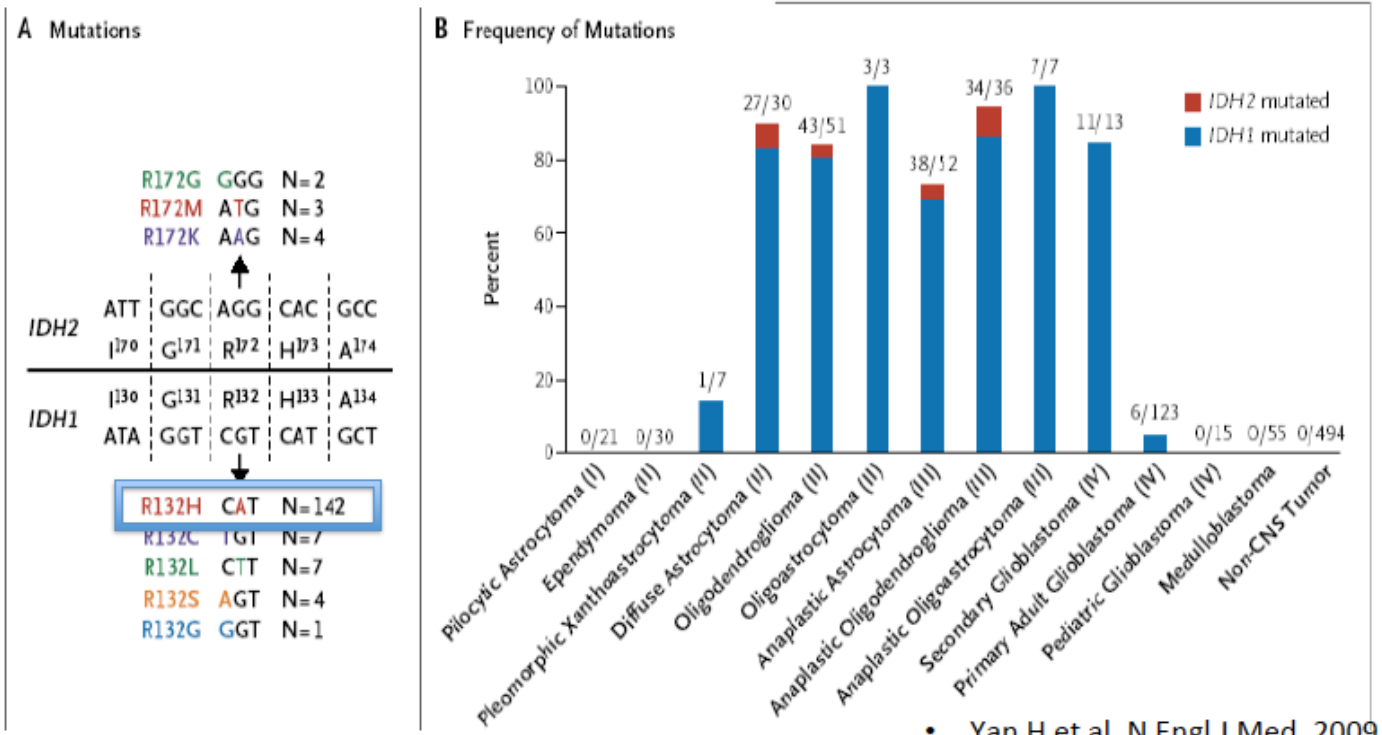
IDH

Secuencian 445 tumores SNC y 494 no-SNC

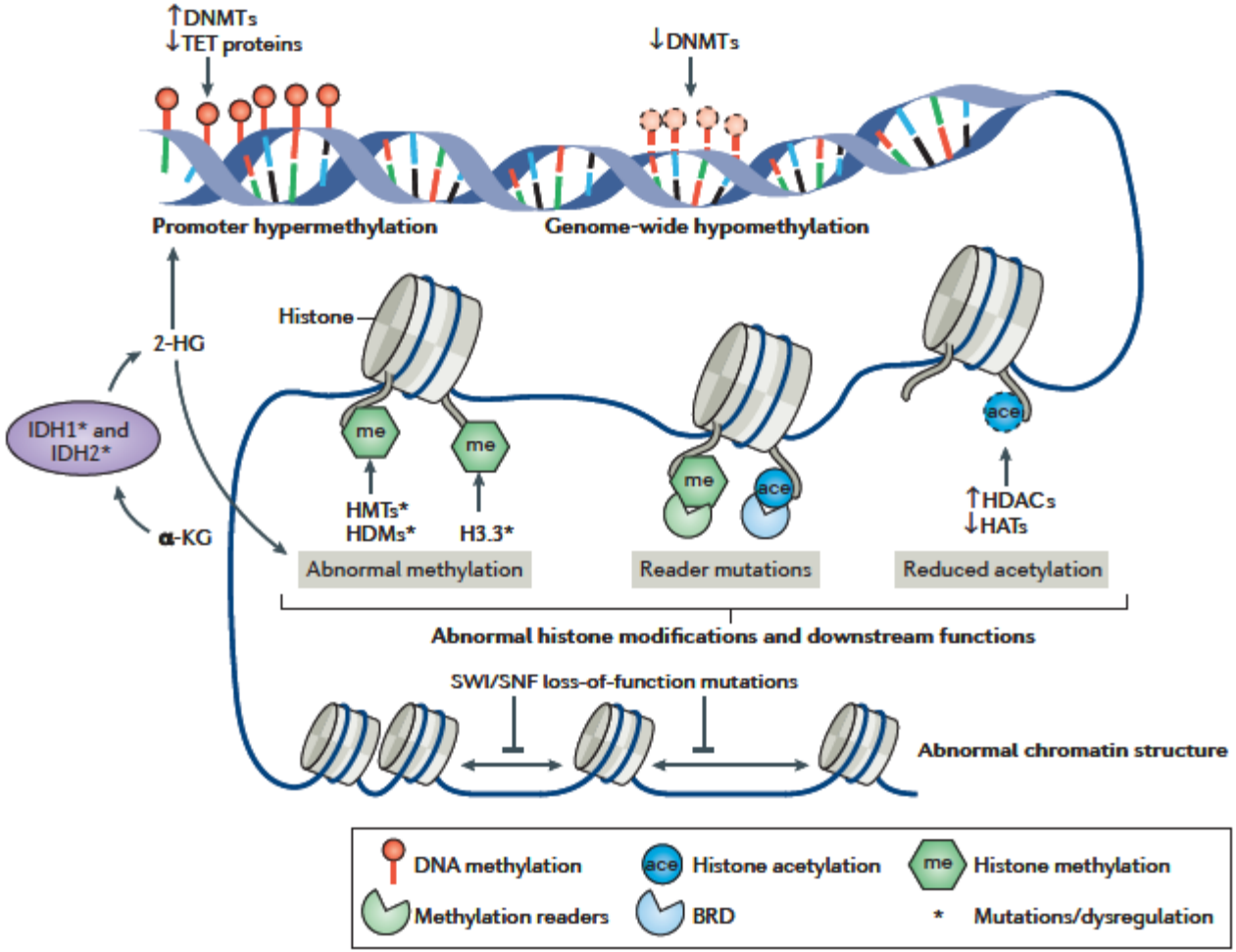
IDH1 and *IDH2* Mutations in Gliomas

Hai Yan, M.D., Ph.D., D. Williams Parsons, M.D., Ph.D., Genglin Jin, Ph.D., Roger McLendon, M.D., B. Ahmed Rasheed, Ph.D., Weishi Yuan, Ph.D., Ivan Kos, Ph.D., Ines Batinic-Haberle, Ph.D., Siân Jones, Ph.D., Gregory J. Riggins, M.D., Ph.D., Henry Friedman, M.D., Allan Friedman, M.D., David Reardon, M.D., James Herndon, Ph.D., Kenneth W. Kinzler, Ph.D., Victor E. Velculescu, M.D., Ph.D., Bert Vogelstein, M.D., and Darell D. Bigner, M.D., Ph.D.

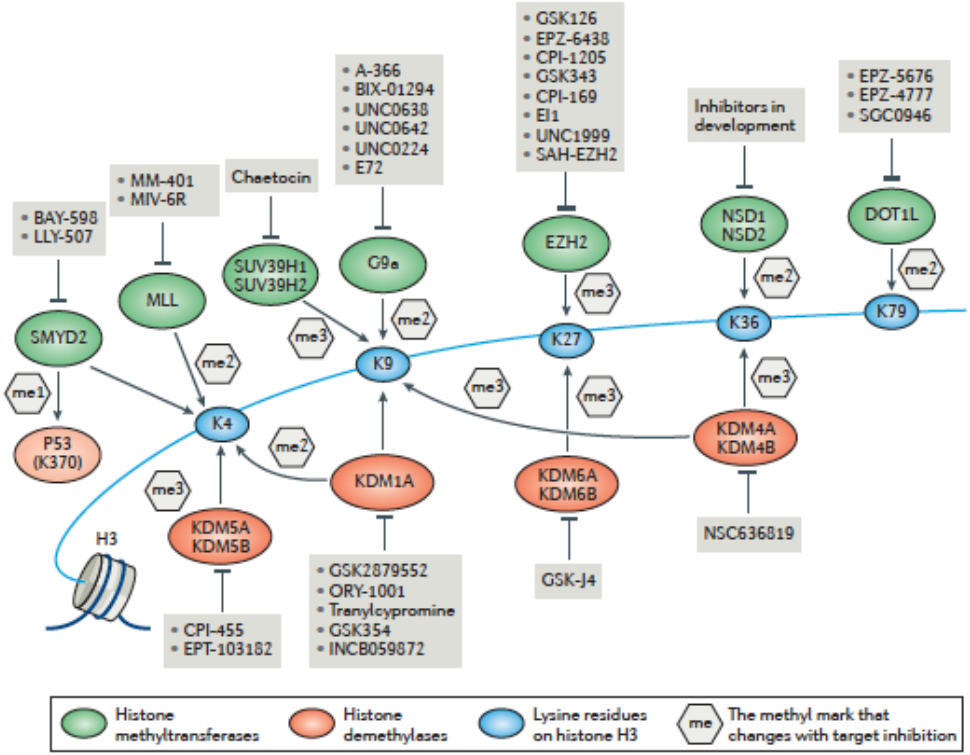
Mutaciones en 85% grado II, III y 2ºGBM



- Yan H et al. N Engl J Med. 2009


↑DNMTs
↓TET proteins
↓DNMTs
Promoter hypermethylation
Genome-wide hypomethylation
Histone
2-HG
IDH1* and IDH2*
α-KG
me
me
HMTs*
HDMs*
H3.3*
me
ace
ace
↑HDACs
↓HATs
Abnormal methylation
Reader mutations
Reduced acetylation
Abnormal histone modifications and downstream functions
SWI/SNF loss-of-function mutations
Abnormal chromatin structure
DNA methylation
Histone acetylation
Histone methylation
Methylation readers
BRD
* Mutations/dysregulation

Targeting gain-of-function mutations and overerexpression of HMTs and HDMs



Epigenetic regulation and Immune evasion in Glioma

-Antigen presentation may be compromised by DNA Hypermethylation or recruitment of HDAC

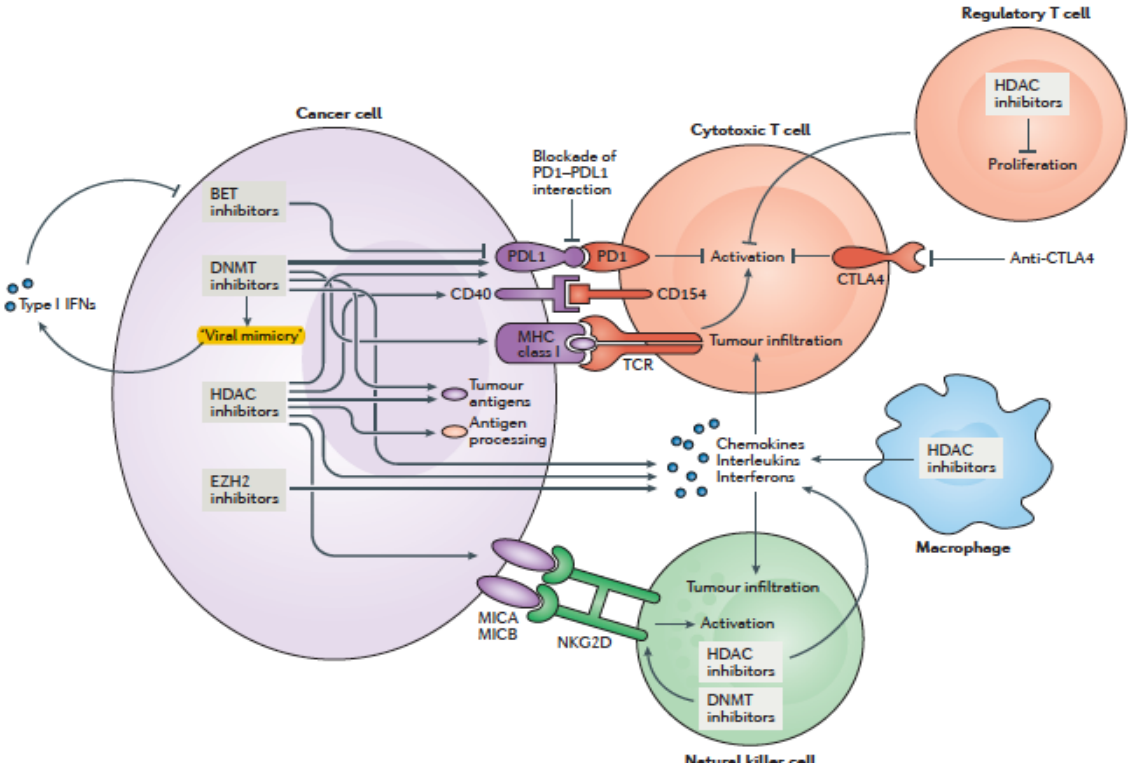
- -Mutated genes are not expressed (neantigens)

-MHC genes are hypermethylated in GBM

-*TAP1* transcription is blocked by incorrect histone acetylation in its promoter

- *TAP1* encodes Antigen Pepetide transporter

Epigenetic inhibitors in immunotherapy



Mutant IDH1 regulates the tumor-associated immune system in gliomas

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Gliomas harboring mutations in isocitrate dehydrogenase 1/2 (IDH1/2) have the CpG island methylator phenotype (CIMP) and significantly longer patient survival time than wild-type IDH1/2 (wtIDH1/2) tumors. Although there are many factors underlying the differences in survival between these two tumor types, immune-related differences in cell content are potentially important contributors. In order to investigate the role of IDH mutations in immune response, we created a syngeneic pair mouse model for mutant IDH1 (muIDH1) and wtIDH1 gliomas and demonstrated that muIDH1 mice showed many molecular and clinical similarities to muIDH1 human gliomas, including a 100-fold higher concentration of 2-hydroxygluratate (2-HG), longer survival time, and higher CpG methylation compared with wtIDH1. Also, we showed that IDH1 mutations caused down-regulation of leukocyte chemotaxis, resulting in repression of the tumor-associated immune system. Given that significant infiltration of immune cells such as macrophages, microglia, monocytes, and neutrophils is linked to poor prognosis in many cancer types, these reduced immune infiltrates in muIDH1 glioma tumors may contribute in part to the differences in aggressiveness of the two glioma types.

[*Keywords*: IDH mutation; immuno-oncology; glioma]

Supplemental material is available for this article.

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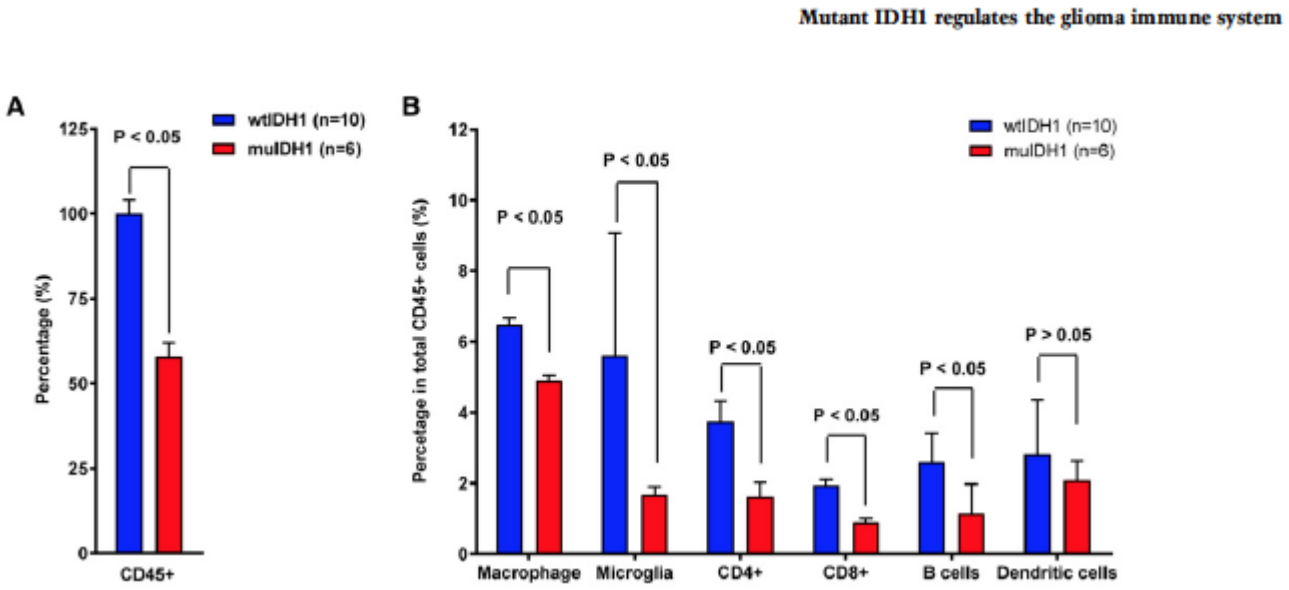
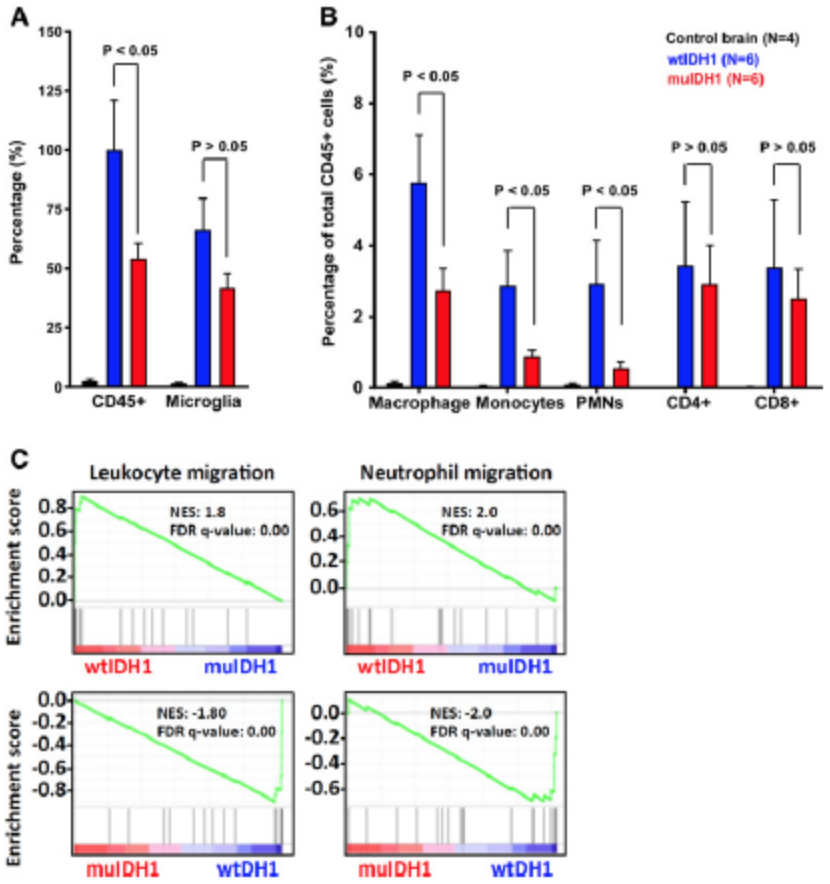
Mutant IDH1 has different immune infiltration




A
Percentage (%)
150
125
100
75
50
25
0
P < 0.05
P > 0.05
CD45+
Microglia
B
Percentage of total CD45+ cells (%)
10
8
6
4
2
0
Control brain (N=4)
wtIDH1 (N=6)
muIDH1 (N=6)
P < 0.05
P < 0.05
P < 0.05
P > 0.05
P > 0.05
Macrophage
Monocytes
PMNs
CD4+
CD8+
C
Leukocyte migration
Neutrophil migration
Enrichment score
NES: 1.8
FDR q-value: 0.00
NES: 2.0
FDR q-value: 0.00
wtIDH1
muIDH1
NES: -1.80
FDR q-value: 0.00
NES: -2.0
FDR q-value: 0.00
muIDH1
wtDH1

CONCLUSIONS

-GBMs are very aggressive neoplasms without effective treatments.

-Despite the high rate of EGFR mutations, EGFR inhibitors have not been effective in this tumours

-Checkpoint inhibitors are not active in recurrent GBM but clinical trials in first line are enrolling patients testing nivolumab associated with RT and TMZ

-Gliomas are tumors with significant epigenetic alterations and drugs such us BET inhibitos or HDAC inhibitors are under development.

-Epigenetic modulators could improve the response to immunotherapy through more efficient antigen presentation

Thanks for your attention