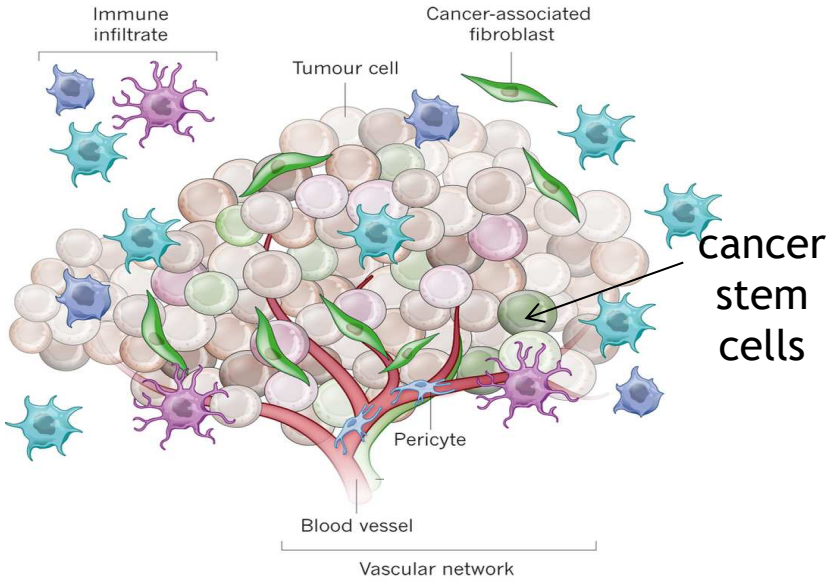
100 years nencki institute of experimental biology 1918-2018

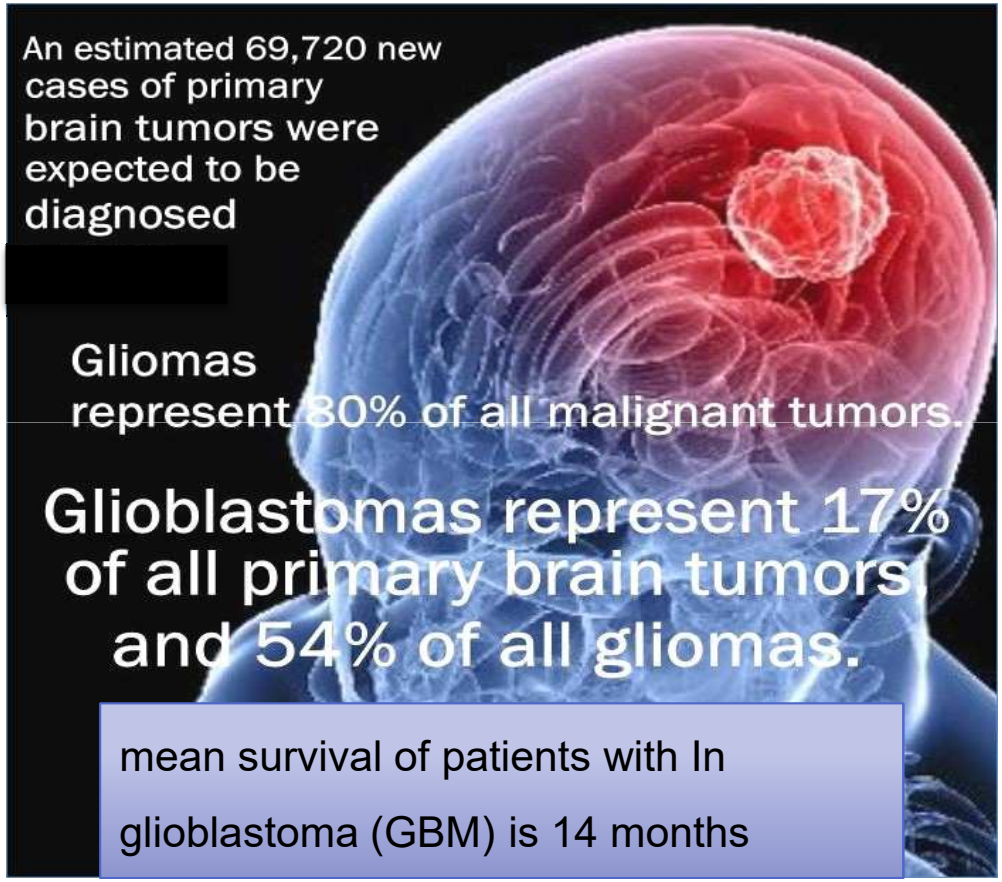
How to sensitize glioblastoma cells to chemotherapeutics?

Bozena Kaminska
Laboratory of Molecular Neurobiology
Neurobiology Center
www.kaminska-lab.pl



11th International Conference on Contemporary Oncology, Poznan 2019

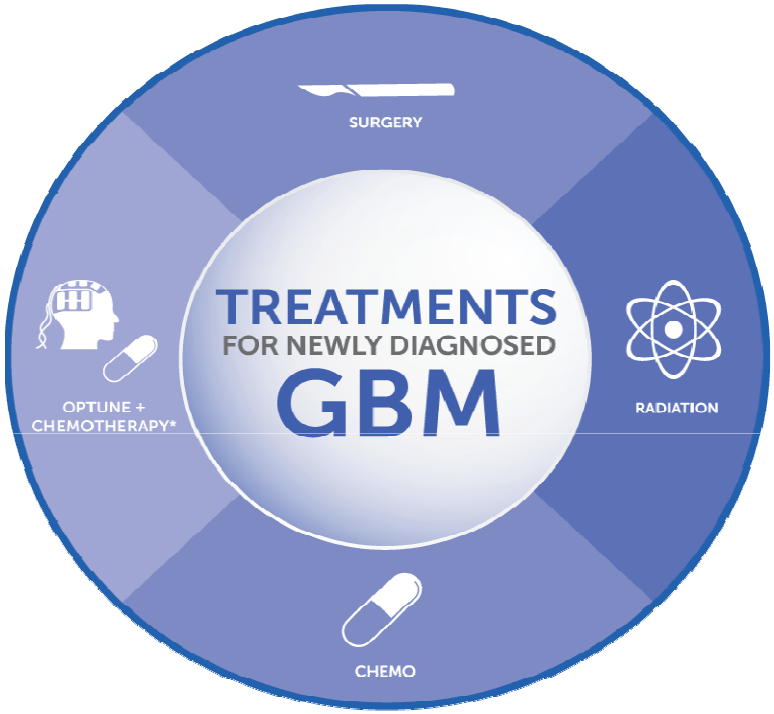
Gliomas represent are more freqeunt primary brain tumors

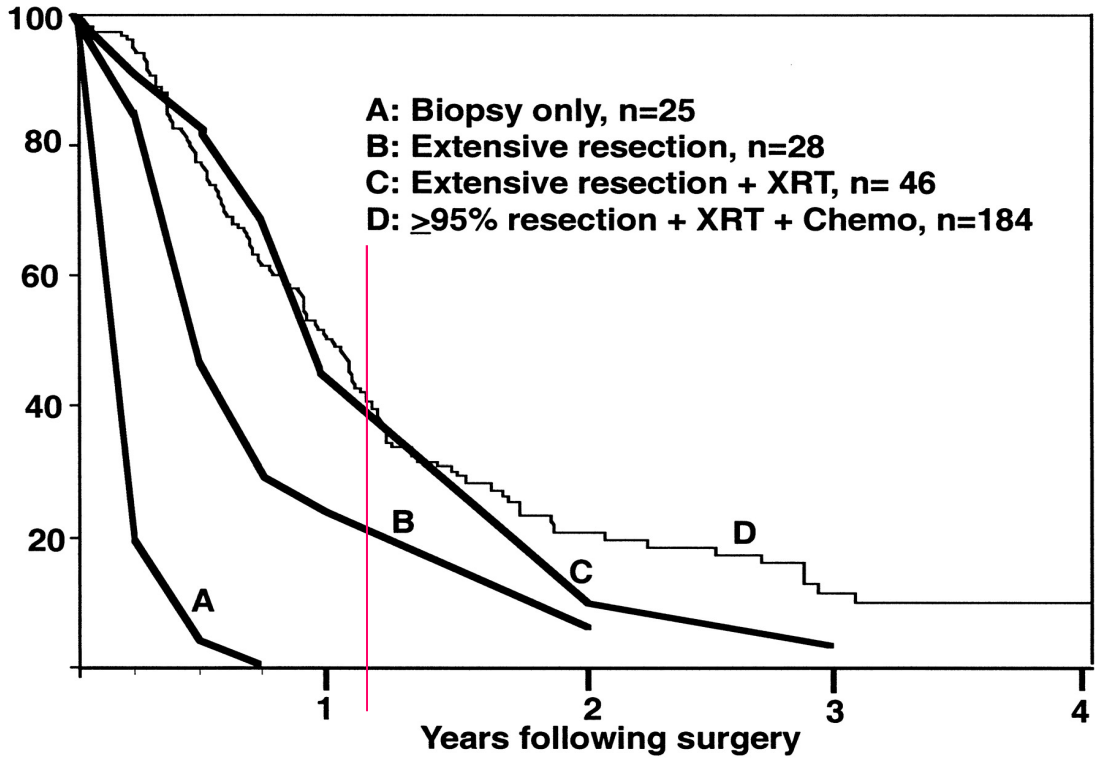


WHO divides gliomas into four malignancy grades based on histopathology: GI, GII, GIII, GIV

Glioblastoma (GBM, WHO grade GIV) is the most frequent and malignant primary brain tumor

Therapeutic options for glioblastoma (GBM)



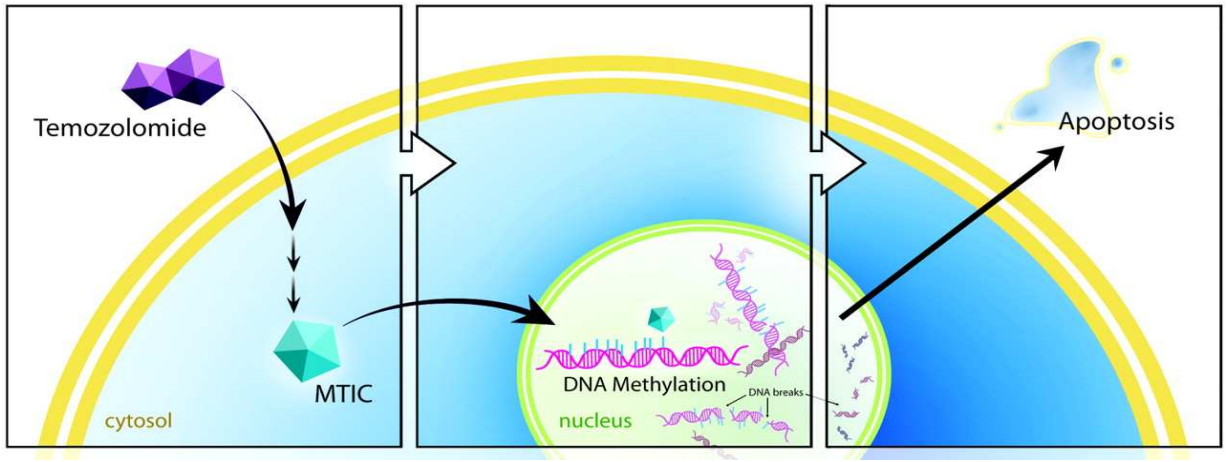


Stupp et al. NEJM 2005

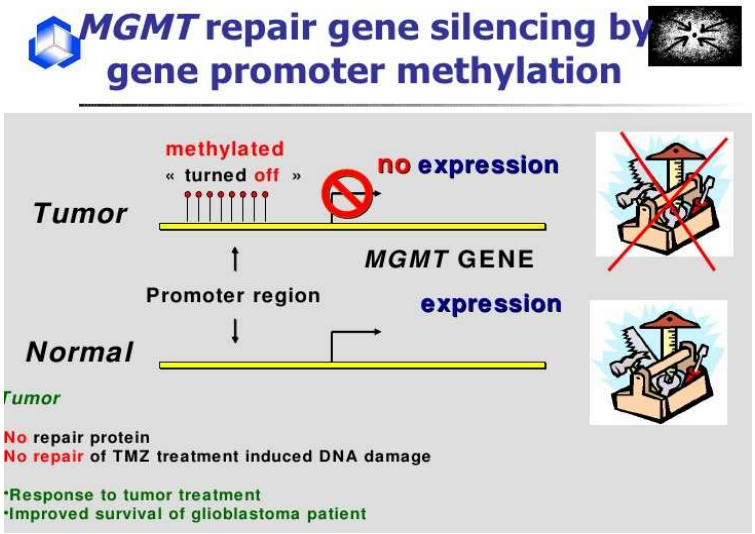
Median survival 14,6 months

GBM has almost a 100% relapse rate with a median time to recurrence of 7 months

Temozolomide has a limited anti-tumor activity

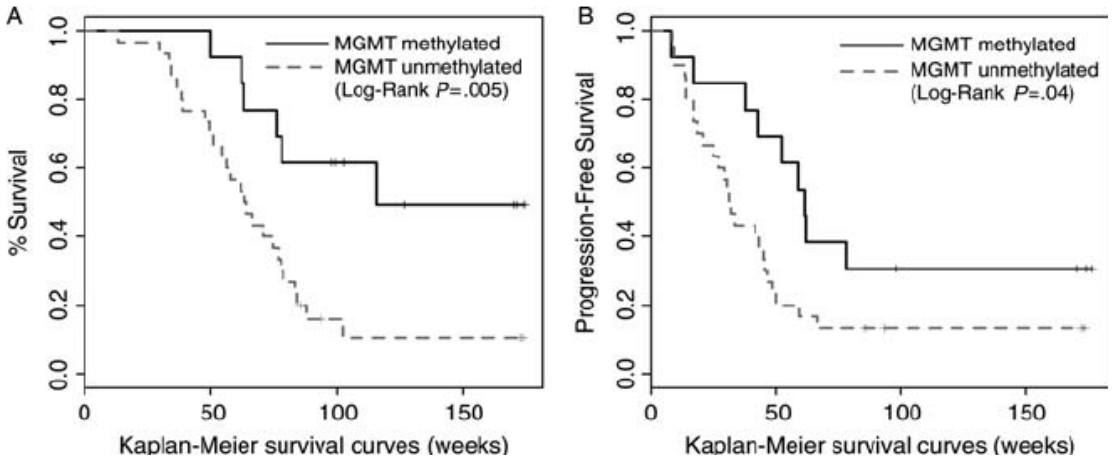


Temozolomide is an alkylating agent prodrug, delivering a methyl group to purine bases of DNA (O6-guanine; N7-guanine and N3-adenine). Cellular repair mechanisms cannot adjust, resulting in DNA nicks and ultimately apoptosis.

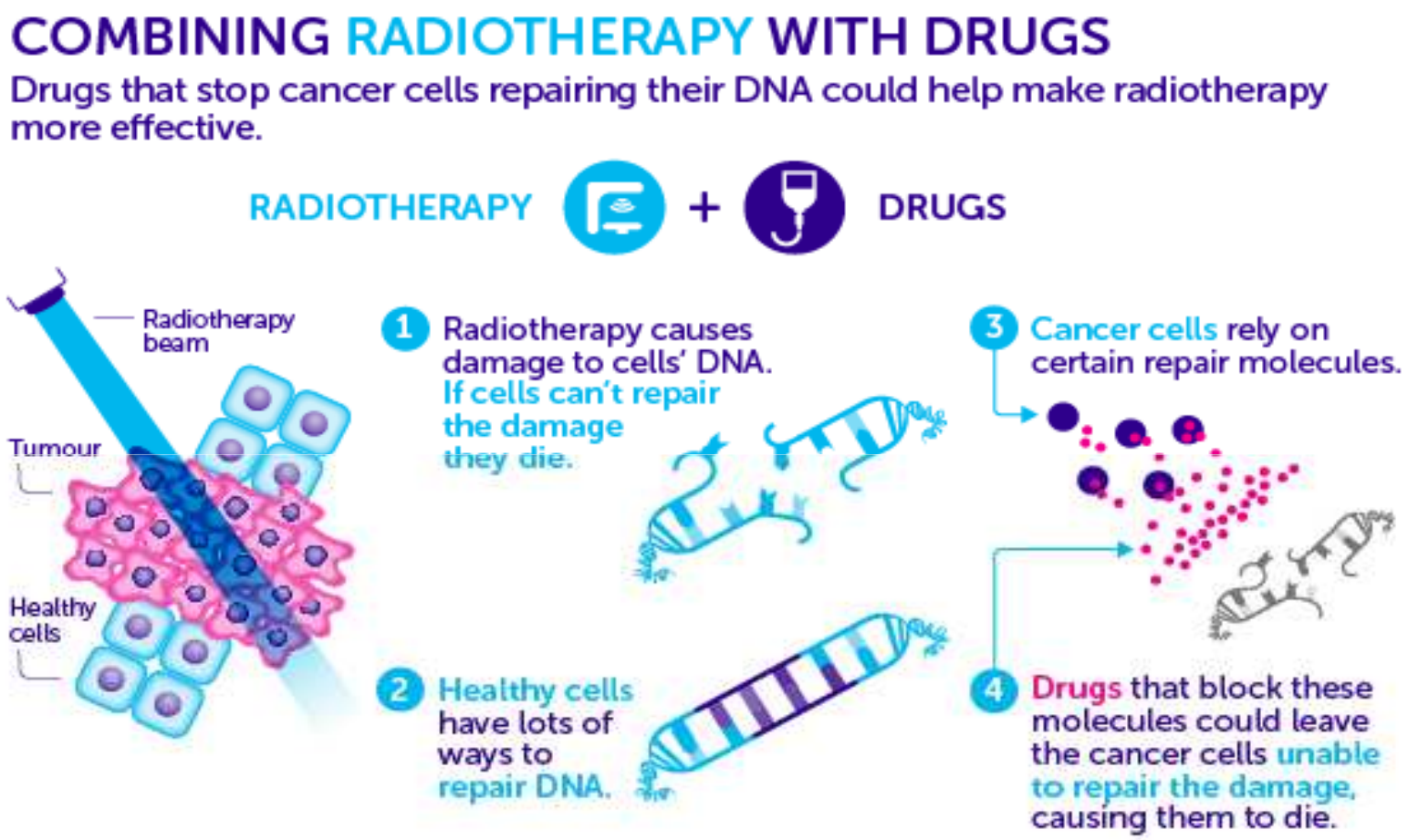


O6-methylguanine can be removed by methylguanine methyltransferase (**MGMT**) or tolerated in mismatch repair-deficient tumors

Butovsky et al. Neuro-Oncology 2011



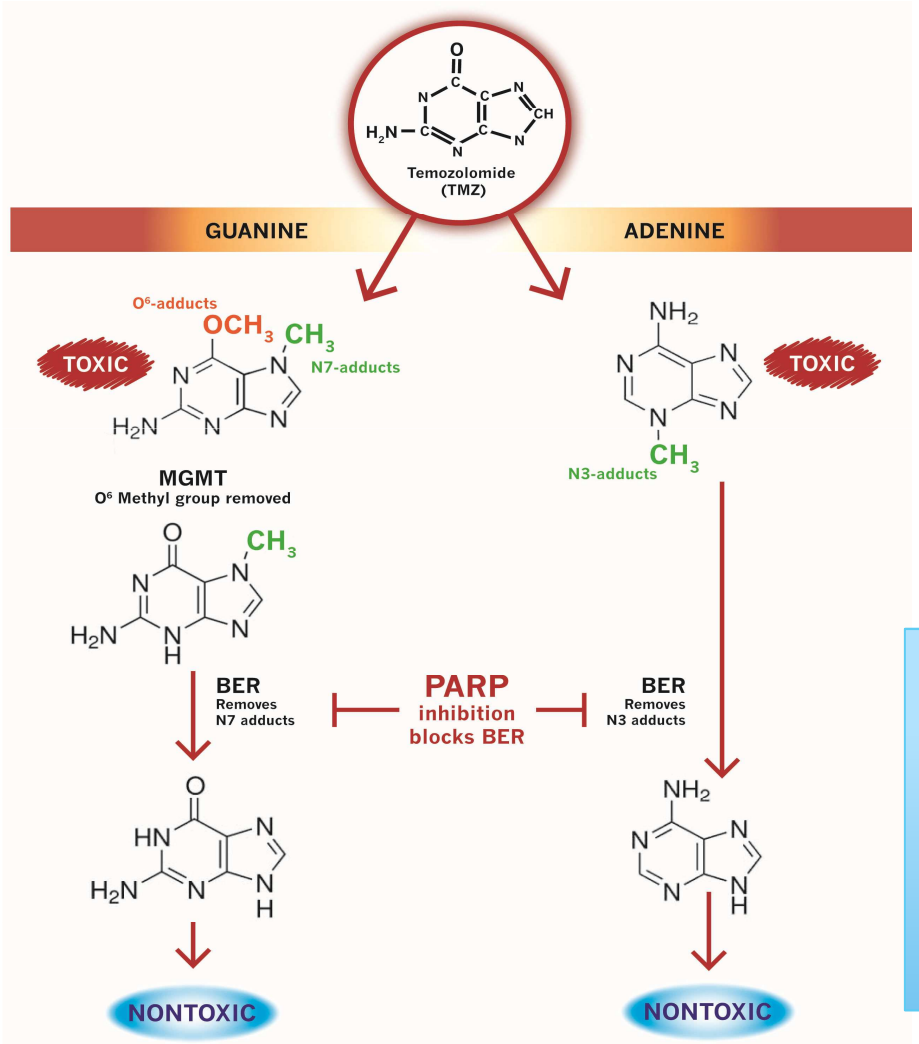
Therapeutic options for glioblastoma (GBM)

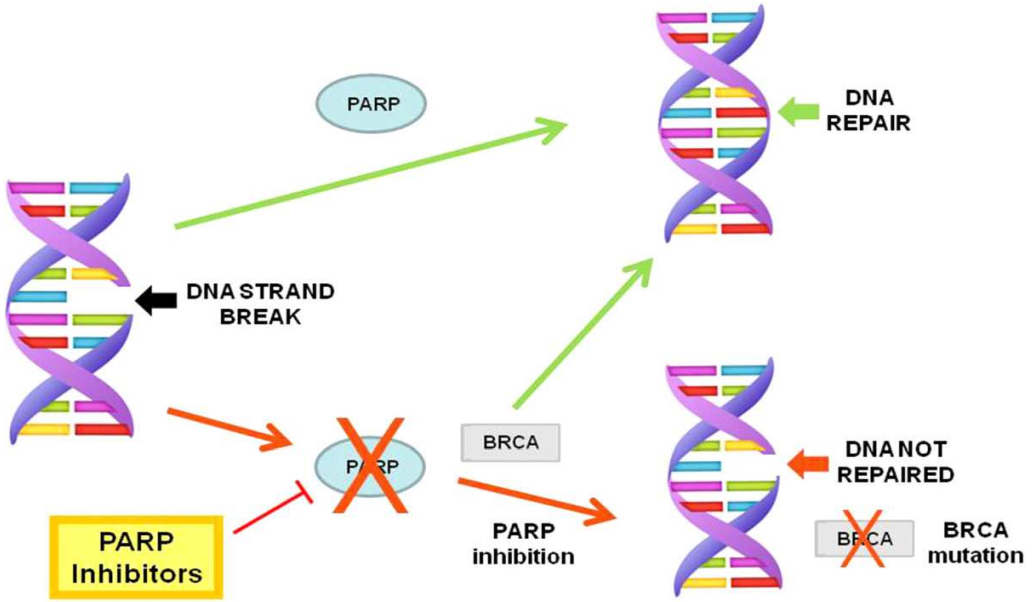


Intensive cell damage may boost an innate and adaptive immunity

CANCER RESEARCH UK

New therapeutic options for glioblastoma-blocking DNA repair mechanims



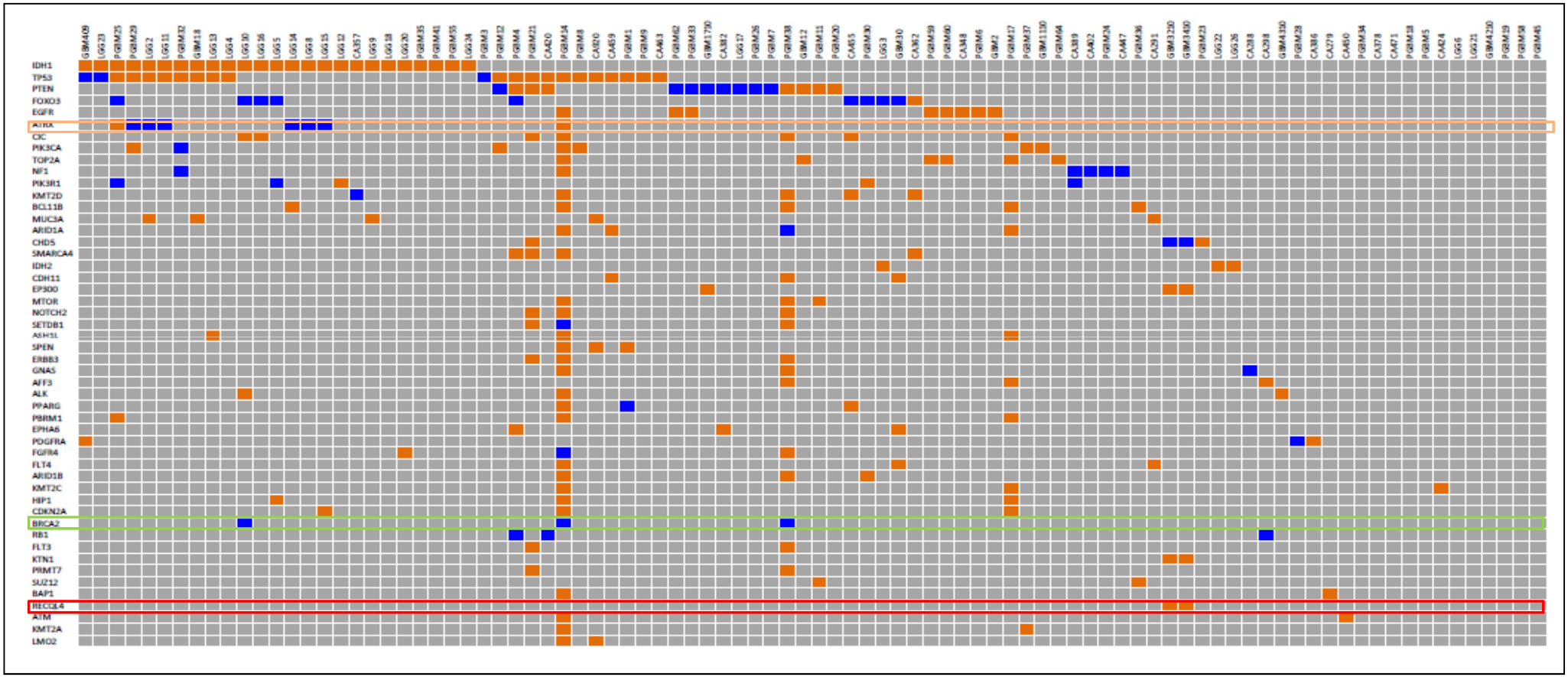


Tentori et al. BMC Cancer 2014 Mar 5;14:151. **Pharmacological inhibition of poly(ADP-ribose) polymerase-1 modulates resistance of human glioblastoma stem cells to temozolomide.**

Lesueur P et al. BMC Cancer 2019 Mar 4;19(1):198.
Phase I/IIa study of concomitant radiotherapy with olaparib and temozolomide in unresectable or partially resectable glioblastoma: OLA-TMZ-RTE-01 trial protocol.

Search for genetic alterations in gliomas

We collected 300 brain tumor samples and up to now 180 were sequenced by targeted 700 gene panel **(FNP TEAM TECH CORE, EPTHERON projects)**



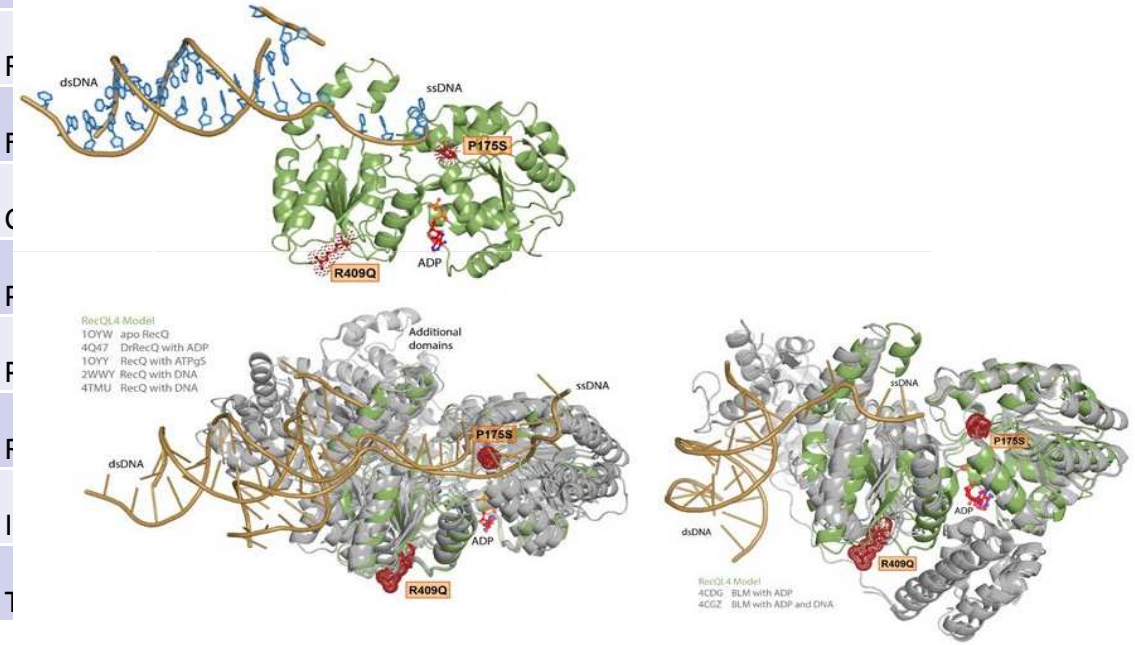
ATRX - as ATRX–DAXX-H3.3 complex maintains genomic stability and participates in replication independent DNA repair (15%, frequntly co-segregates with *IDH1* mutation)

RECQL4 – DNA helicase participates in DNA repair (4%)

BRCA2- participates in DNA repair and control the assembly and activity of complexes that monitor chromosome duplication, maintenance, and segregation across the cell cycle (4%)

Variants in the *RECQL4* gene in 12 patients with glioblastoma among 200 tested glioma samples

gene	chrom_position	frequency	grade	variant_type	amino_change	codon_change	variant_databases	
RECQL4	chr8:144511724	1	GBM	missense_variant	E/D	gaG/gaT	-	
RECQL4	chr8:144513628	1	GBM	missense_variant				
RECQL4	chr8:144514216	1	GBM	missense_variant				
RECQL4	chr8:144514518	1	GBM	missense_variant				
RECQL4	chr8:144514961	1	GBM	missense_variant				
RECQL4	chr8:144514962	1	GBM	missense_variant				
RECQL4	chr8:144513384	2	GBM	missense_variant				
RECQL4	chr8:144513623	2	GBM	missense_variant				
RECQL4	chr8:144513702	2	GBM	missense_variant				



Prepared by Dr. S. Glatt

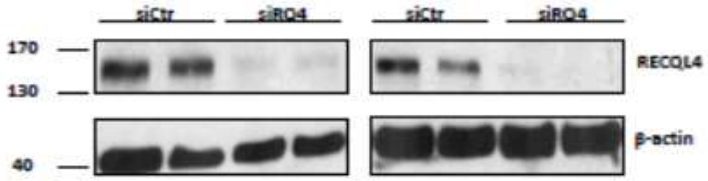
Patent filled with Selvita

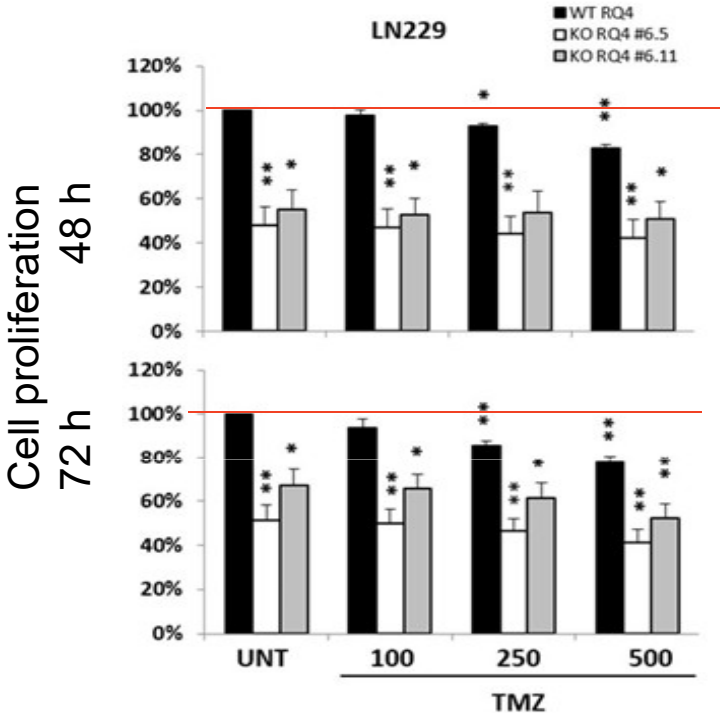
Kaczmarczyk, Krol, Wojtas….Kaminska#, in preaparation

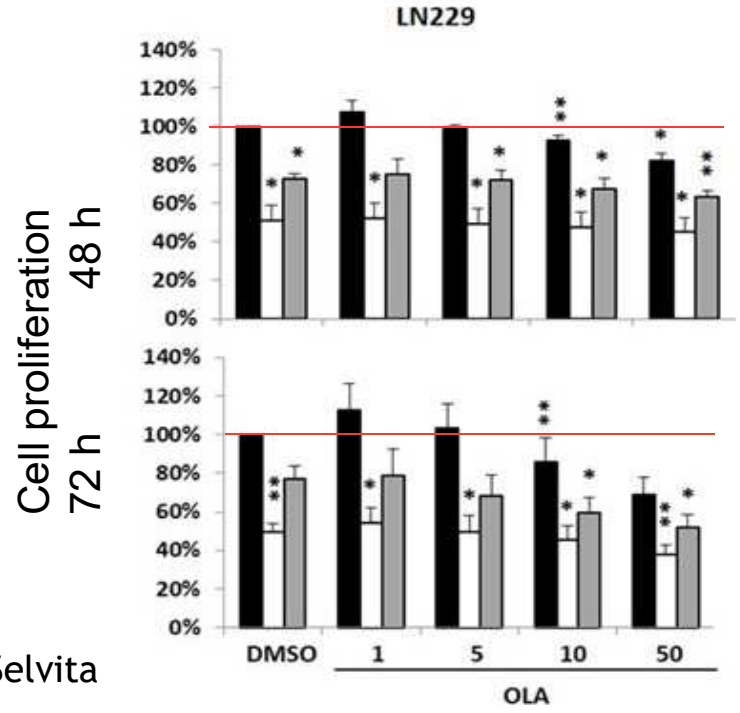
Knockdown of RECQL4 sensitizes human glioma cells to chemotherapeutics

RECQL4 knockdown with CRISPRCas9

LN18 LN229

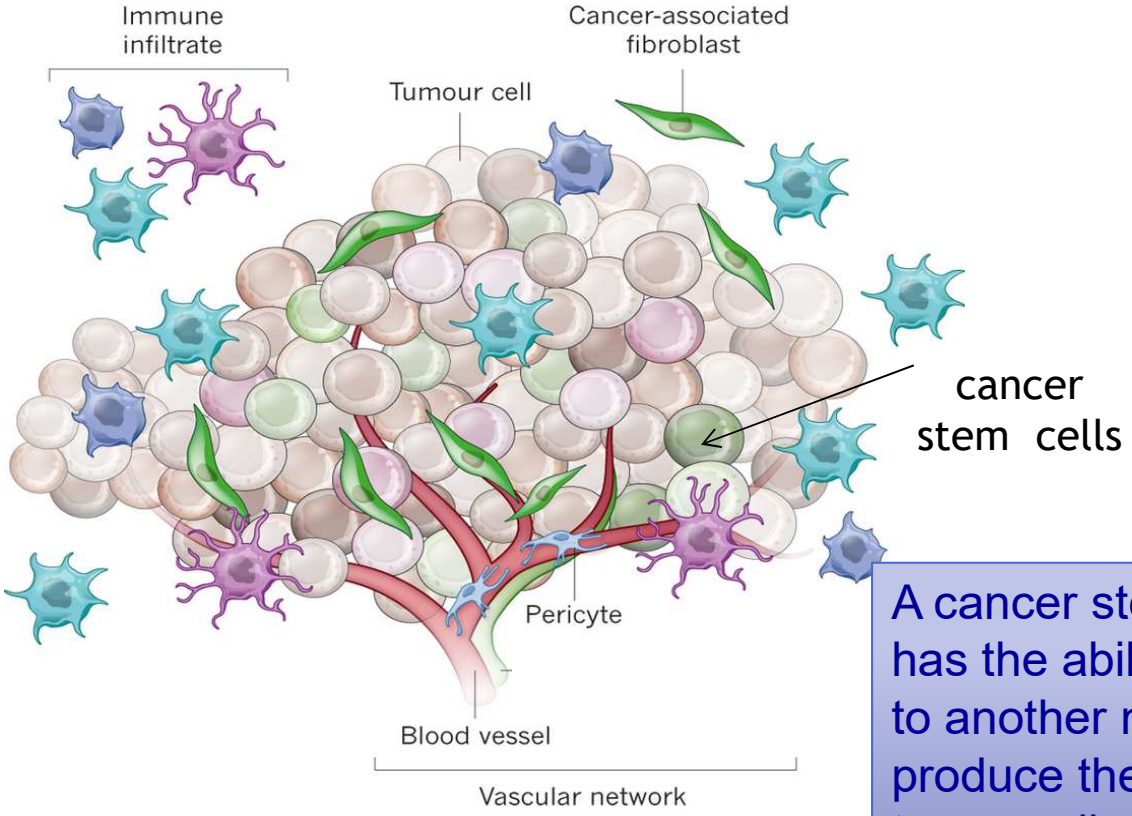






Patent filled with Selvita

Cancer stem cells, a rare but dangerous subpopulation of cancer



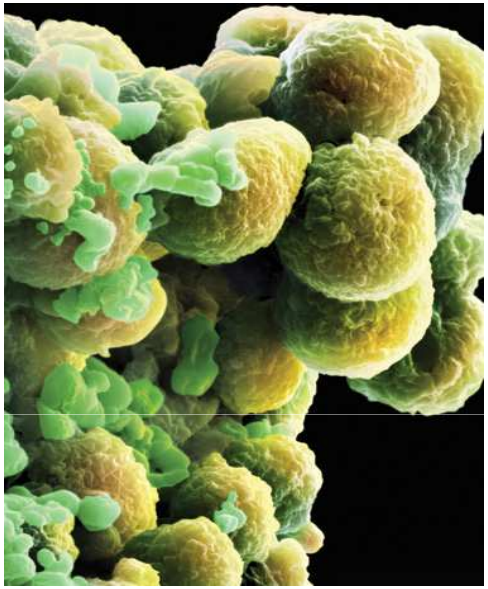
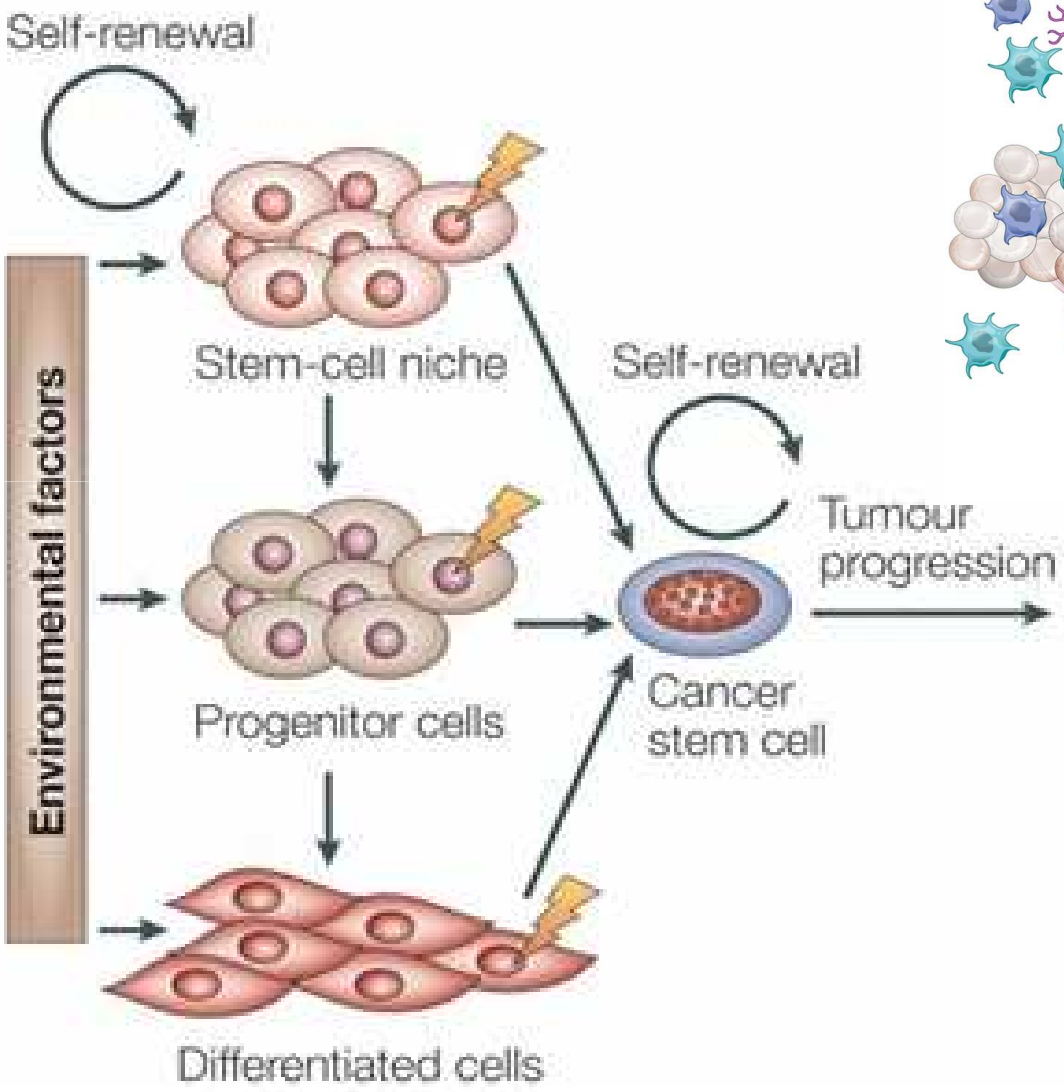
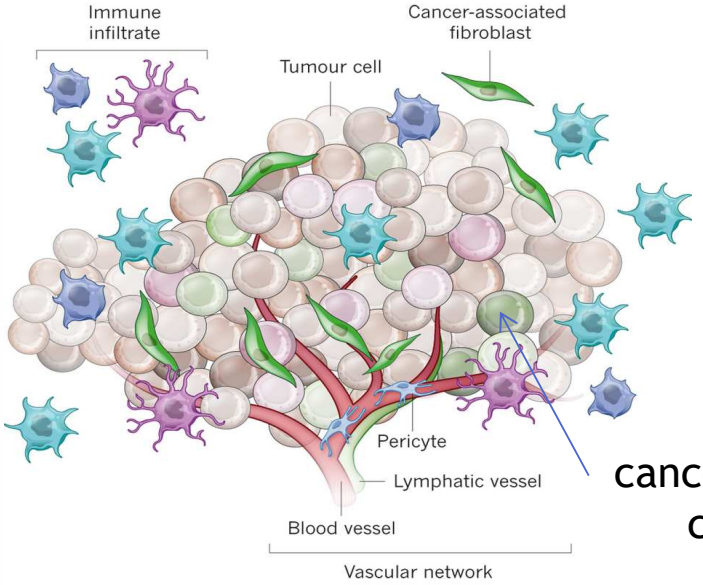


PHOTO RESEARCHERS, INC., DAVID MCCARTHY

A cancer stem cell (CSC) is defined as a cell that has the ability to self-renew, dividing to give rise to another malignant stem cell, as well as to produce the phenotypically diverse, differentiated tumor cells that form the bulk of the tumor.

Origin of cancer stem cells

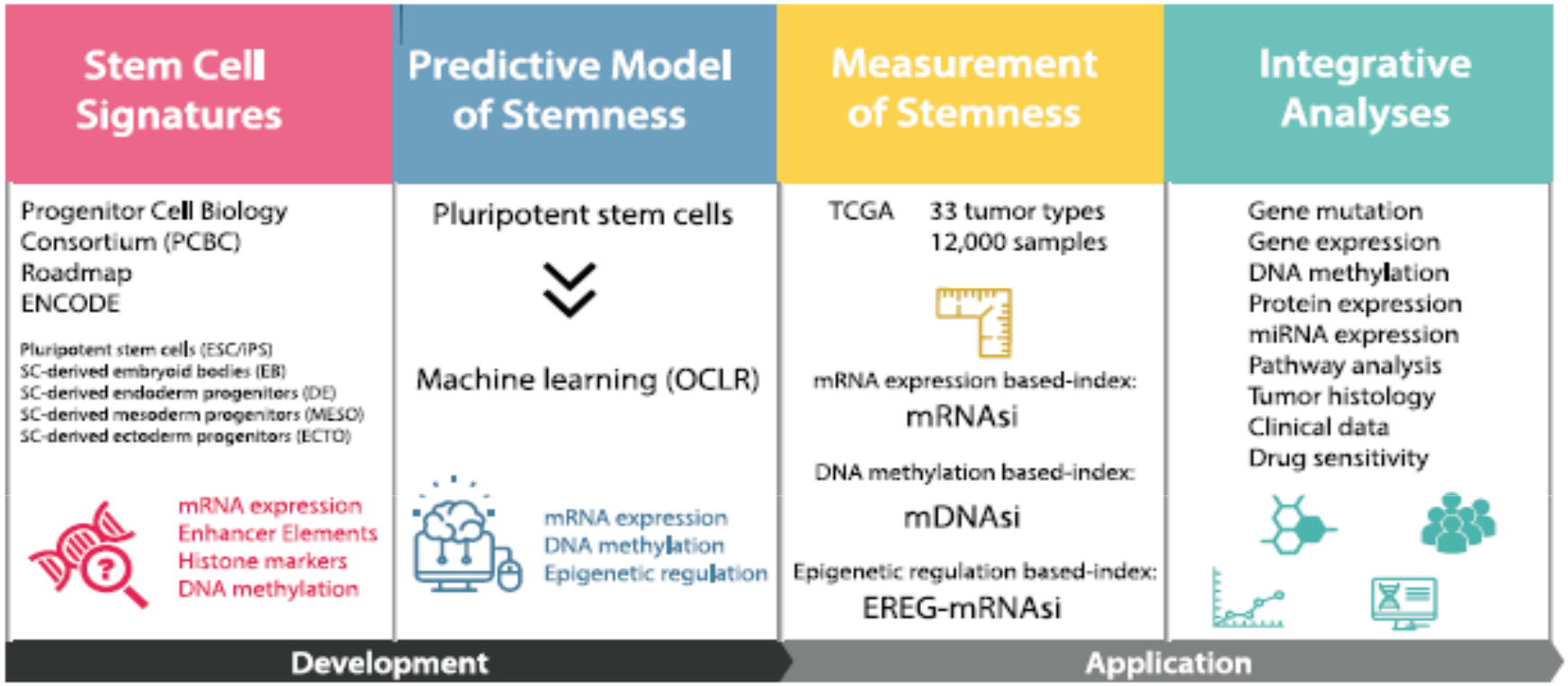




cancer stem cells

A cancer stem cell (CSC) is defined as a cell that has the ability to self-renew, dividing to give rise to another malignant stem cell, as well as to produce the phenotypically diverse, differentiated tumor cells that form the bulk of the tumor.

Tumor stemness project

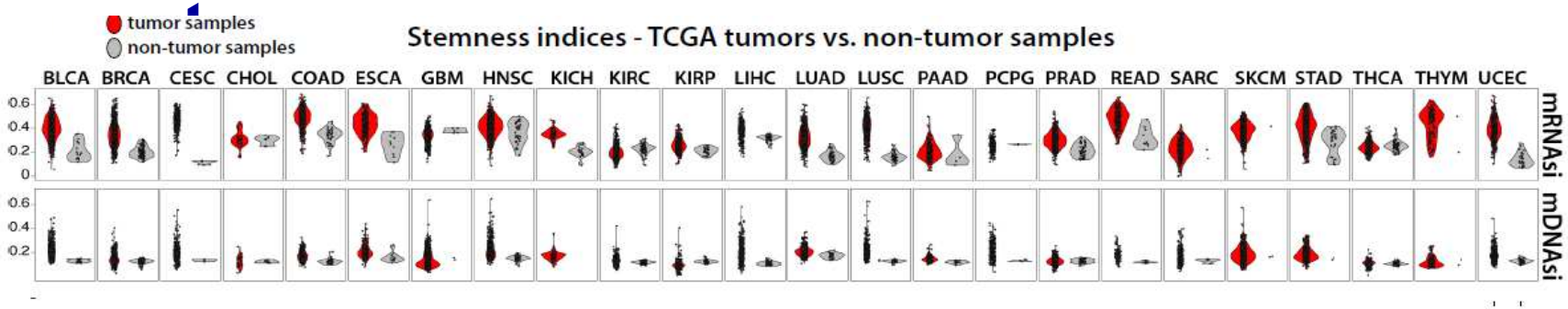


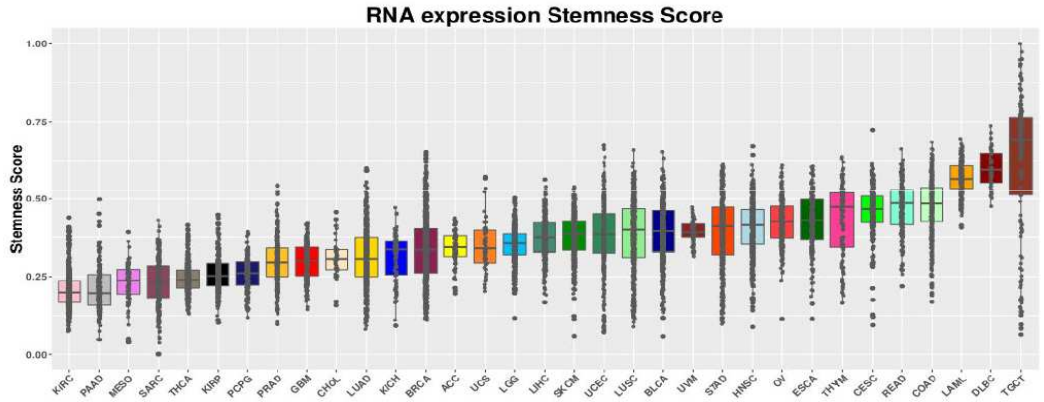
Cell

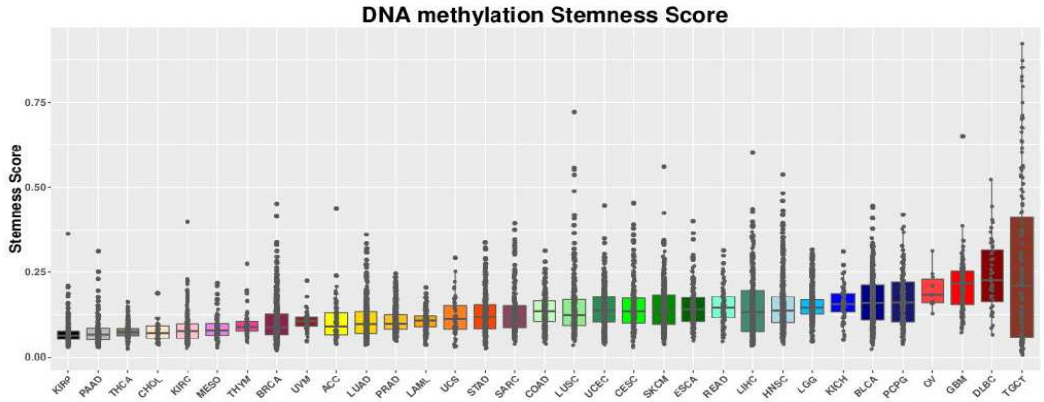
Machine Learning Identifies Stemness Features Associated with Oncogenic Dedifferentiation

Tathiane M. Malta,[1,2,23] Artem Sokolov,[3,23] Andrew J. Gentles,[4] Tomasz Burzykowski,[5] Laila Poisson,[1] John N. Weinstein,[6] Bożena Kamińska,[7] Joerg Huelsken,[8] Larsson Omberg,[9] Olivier Gevaert,[4] Antonio Colaprico,[10,11] Patrycja Czerwińska,[12] Sylwia Mazurek,[12,13] Lopa Mishra,[14] Holger Heyn,[15] Alex Krasnitz,[16] Andrew K. Godwin,[17] Alexander J. Lazar,[6] The Cancer Genome Atlas Research Network, Joshua M. Stuart,[18] Katherine A. Hoadley,[19] Peter W. Laird,[20] Houtan Noushmehr,[1,2,23,*] and Maciej Wiznerowicz[12,21,22,23,24,*]

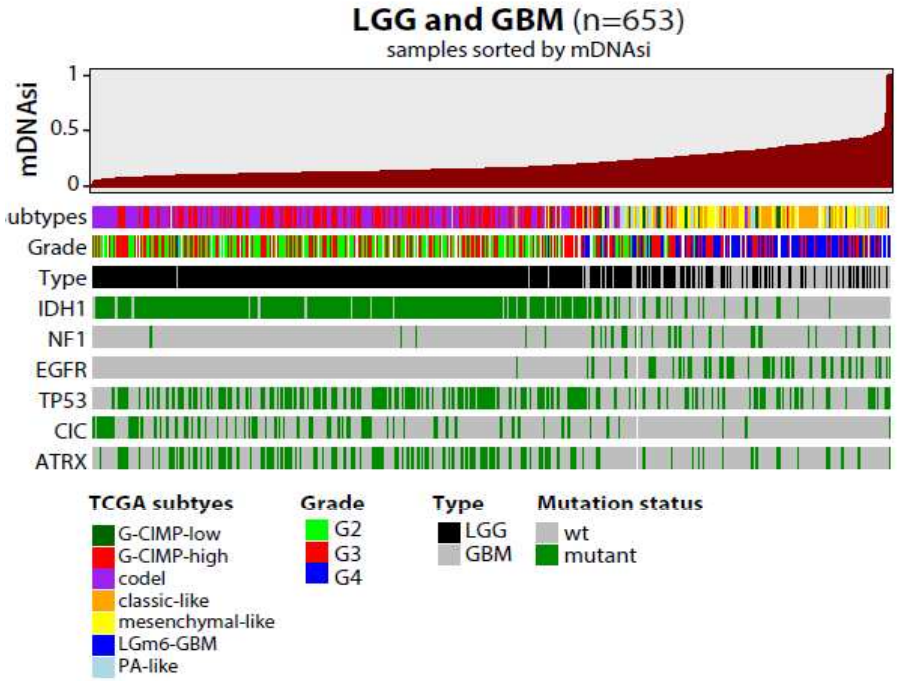
Stemness score across 33 cancer

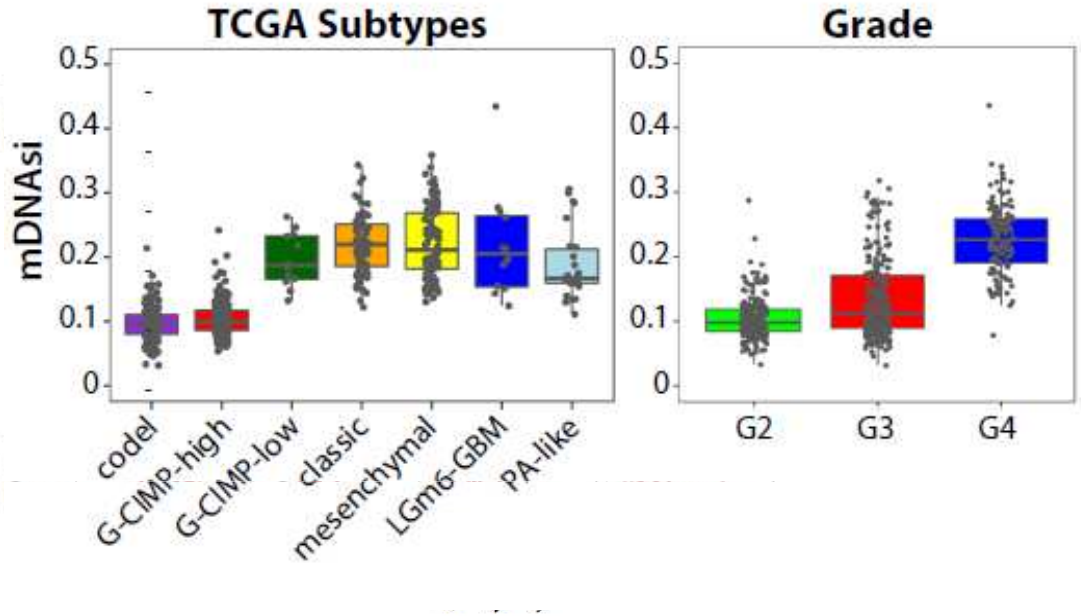


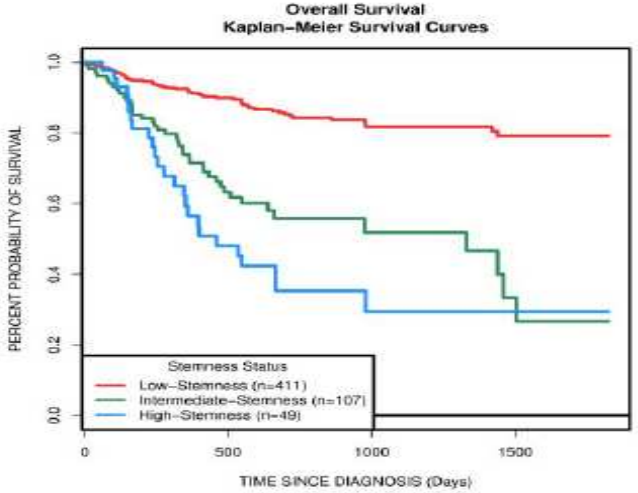


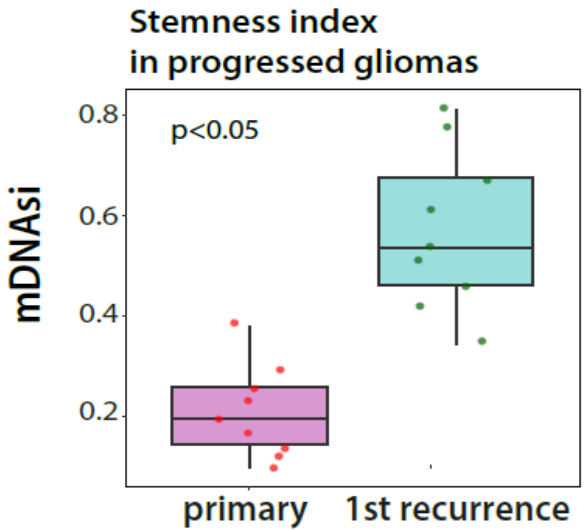


Stemness and patient survival in gliomas

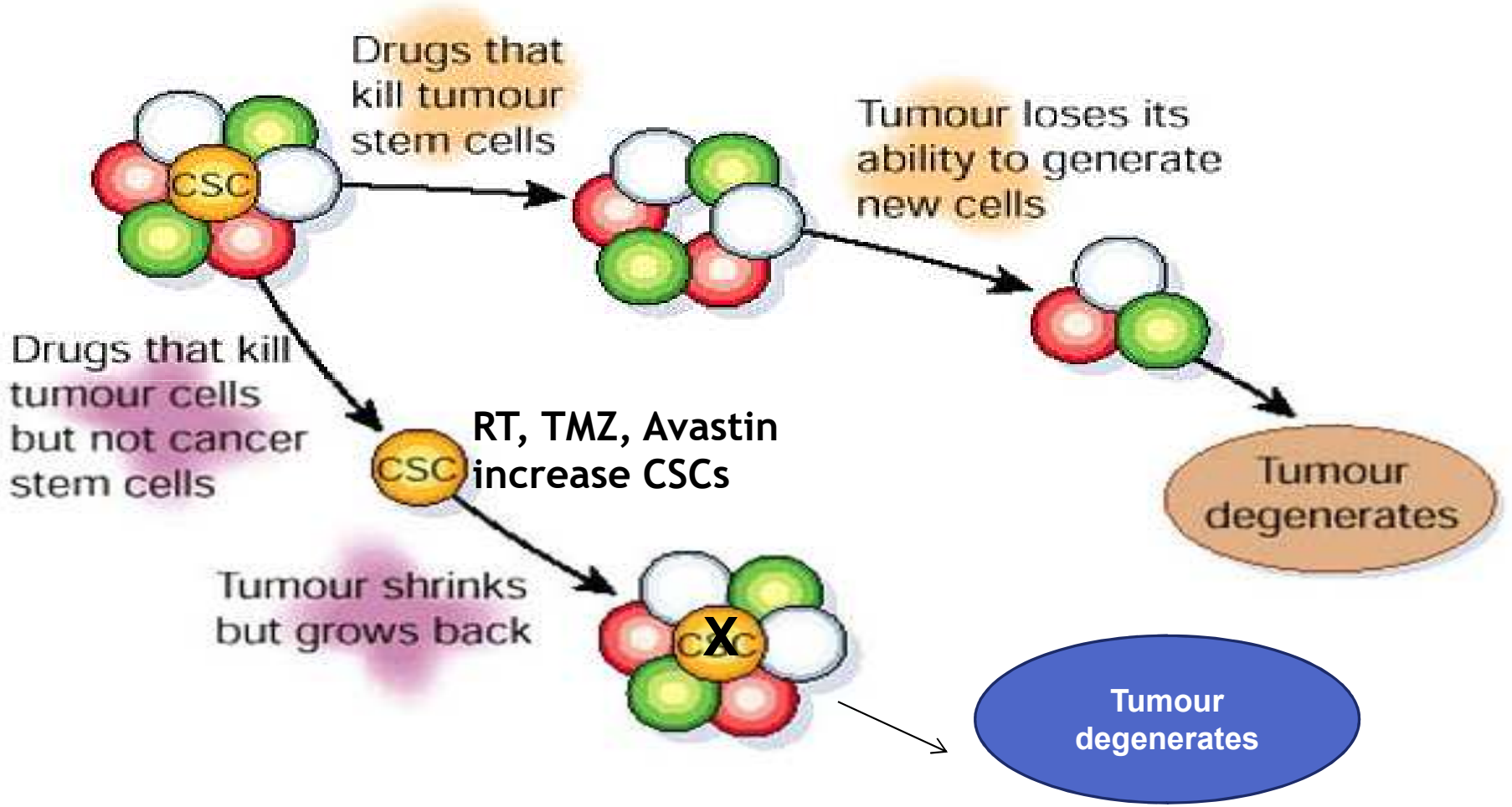




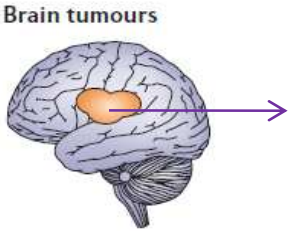


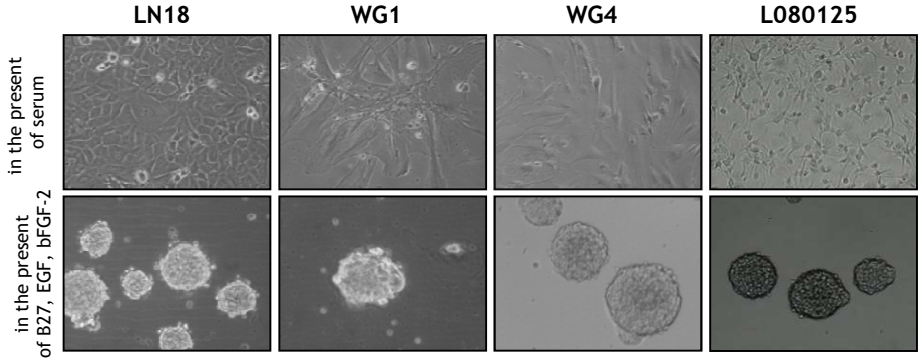


Cancer stem cells and therapy

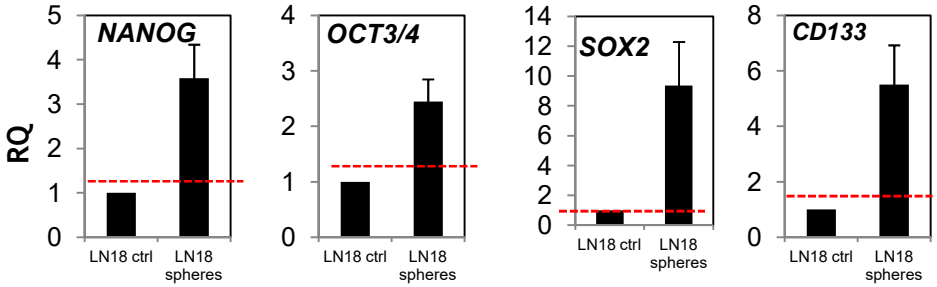


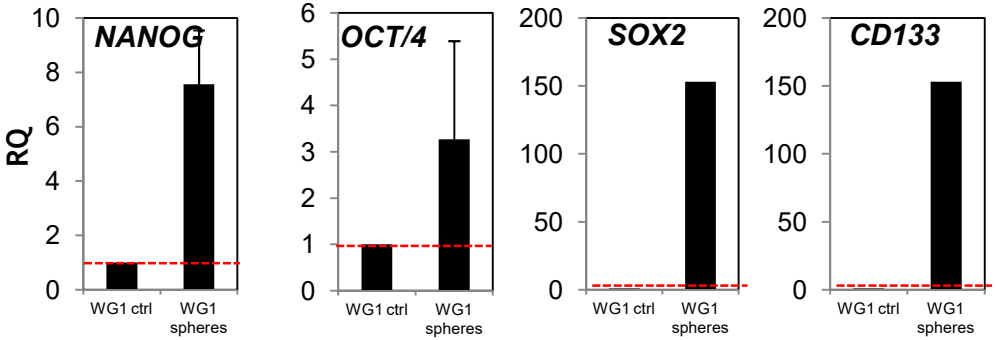
Glioblastoma spheres express stem cell markers

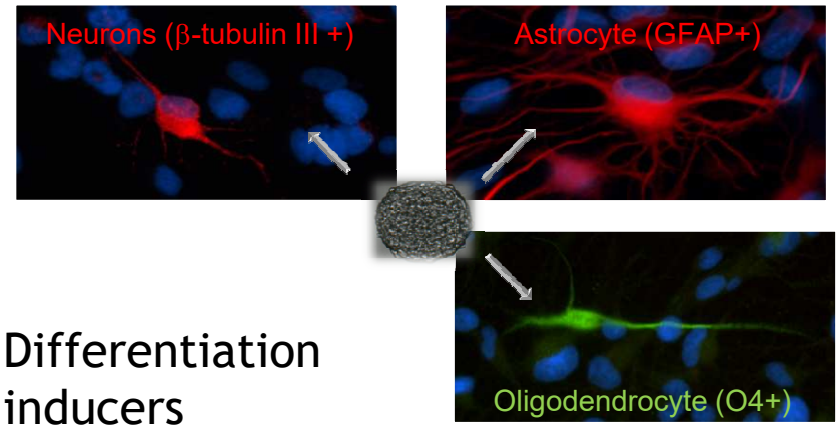




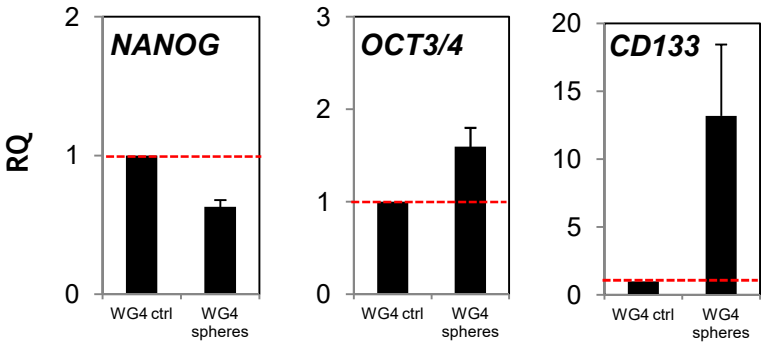
LN18, an established cell line
WG1, WG4 patient derived spheres
L080125 glioma spheres (Dr. R. Galli)





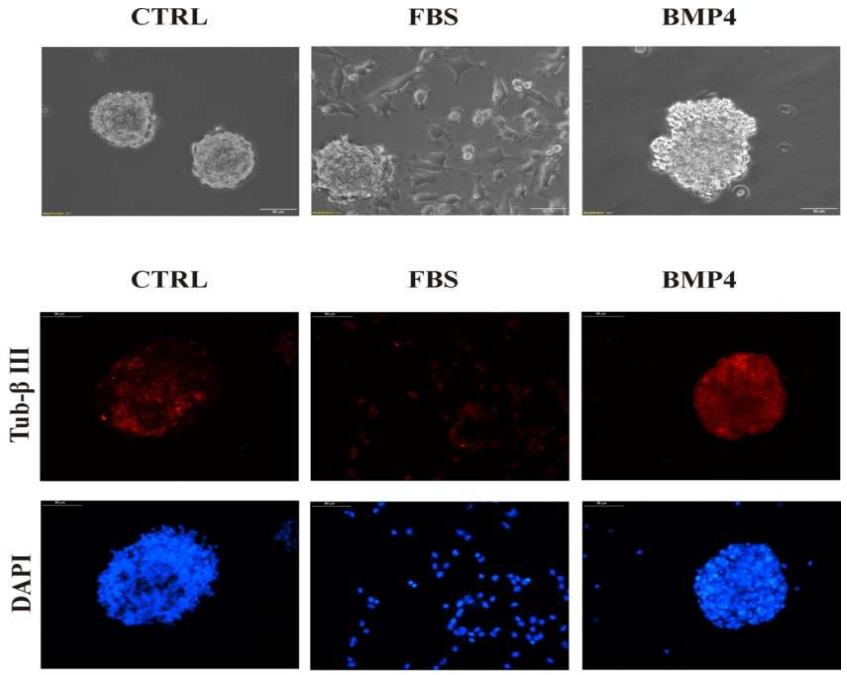


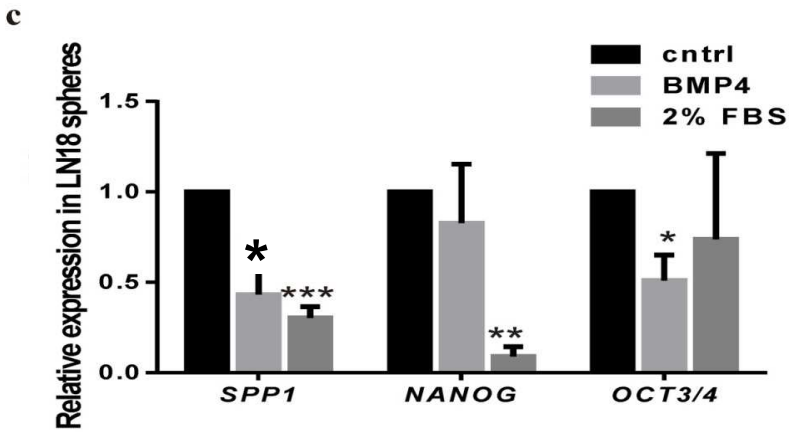
Differentiation inducers



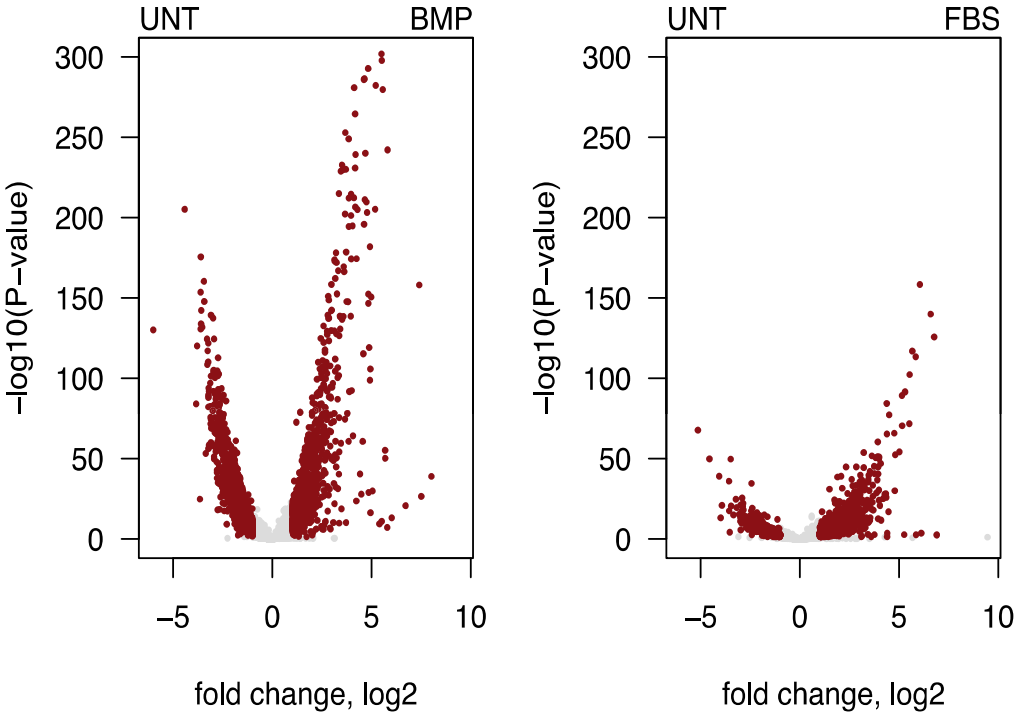
I.Ciechomska

Forced differentiation of cancer stem cells





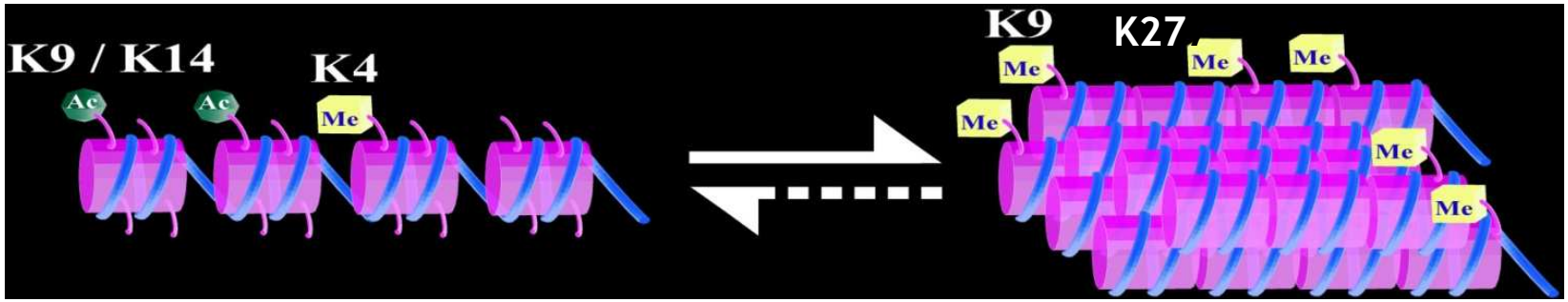
Kijewska et al. Oncotarget 2016



Jakub Mleczkowski

How to remove cancer stem cells from bulk tumor? Does such treatment would increase a vulnerablity of glioma cells to TMZ?

Inhibitors of epigenetic enzyme affect histone modifications and regulate chromatin openness

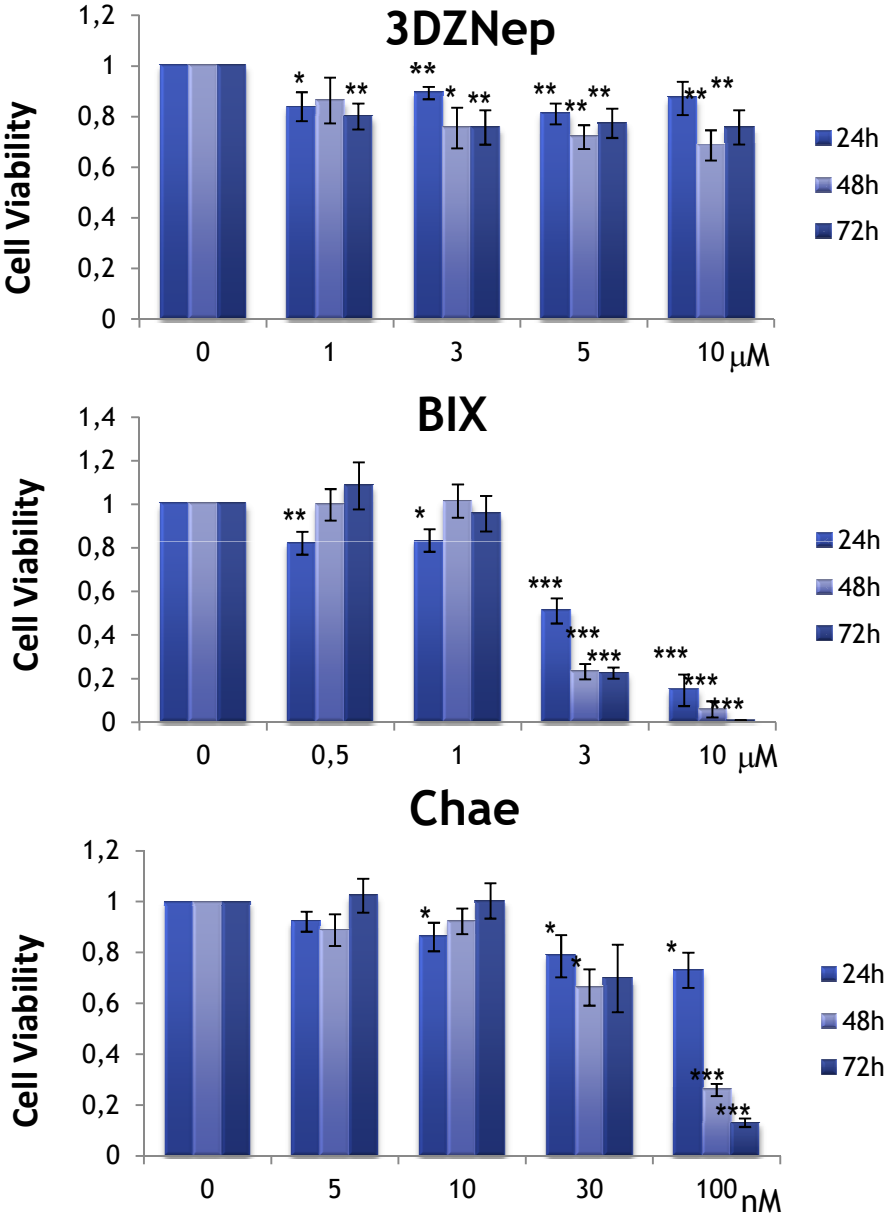


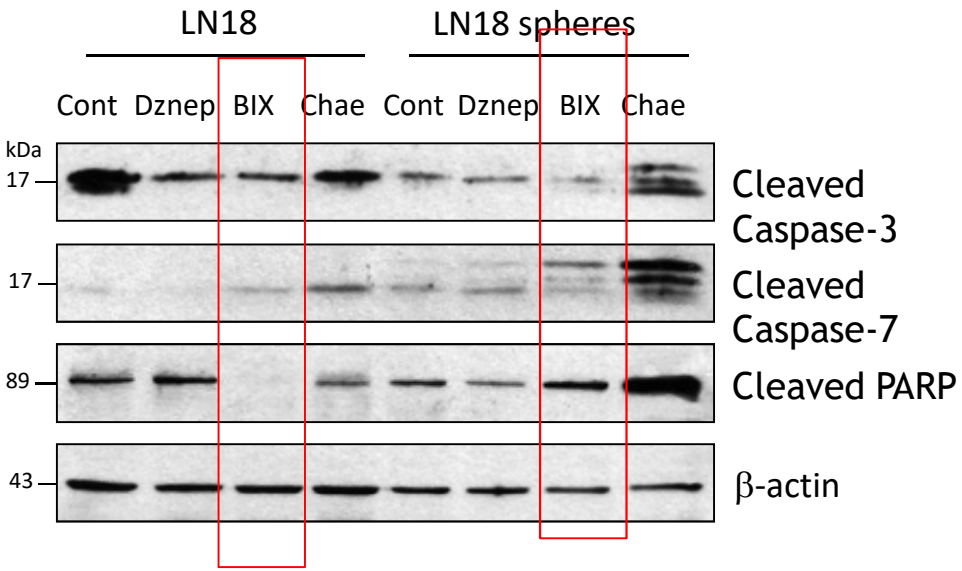
‘active’ or ‘open’ chromatin

‘silent’ chromatin

3DZNep	Ezh2	H3K27me3 ↓
BIX01294	G9a	H3K9me2, H3K27me3 ↓
Chaetocin	Suv39	H3K9me3 ↓

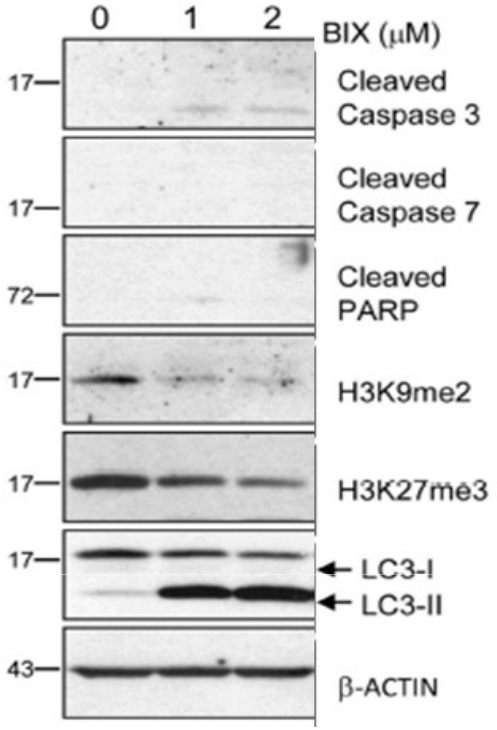
Effects of histone methyltransferases on viability of LN18 cells and spheres

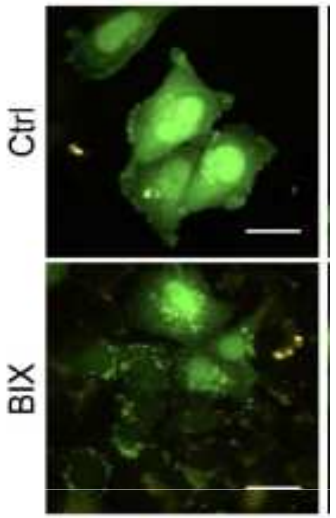


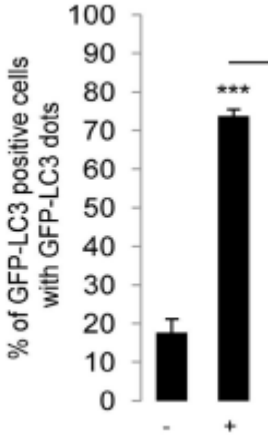


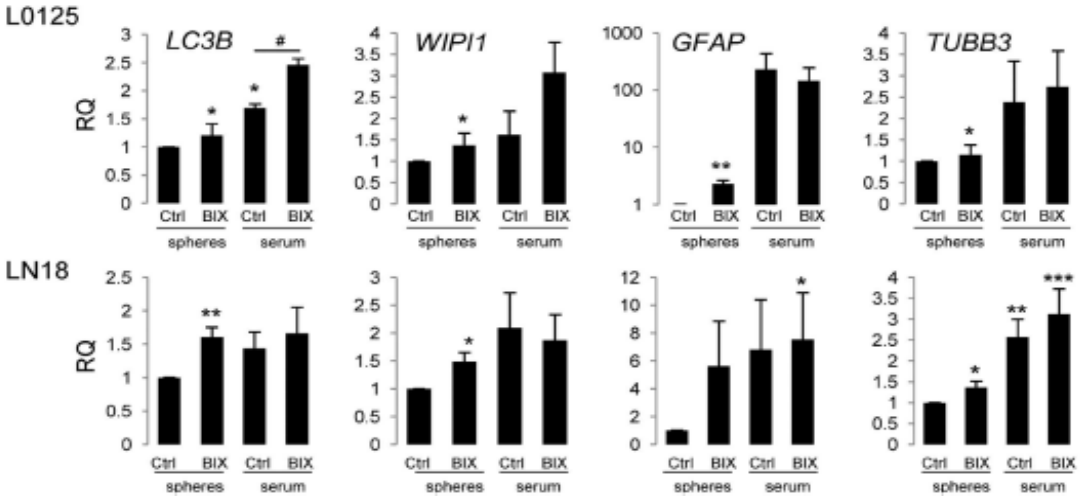
Ciechomska et al. Scientific Rep 2016

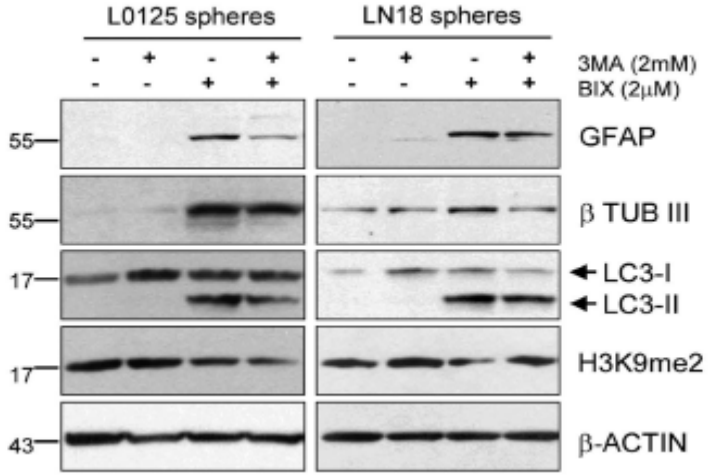
BIX 01294 induces autophagy and differentiation of glioma spheres



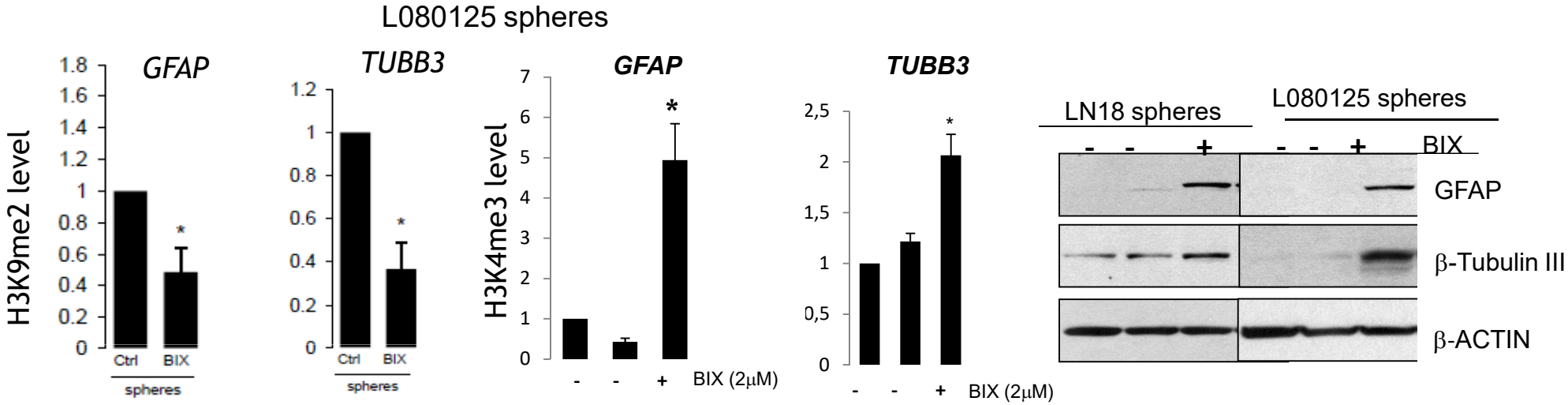






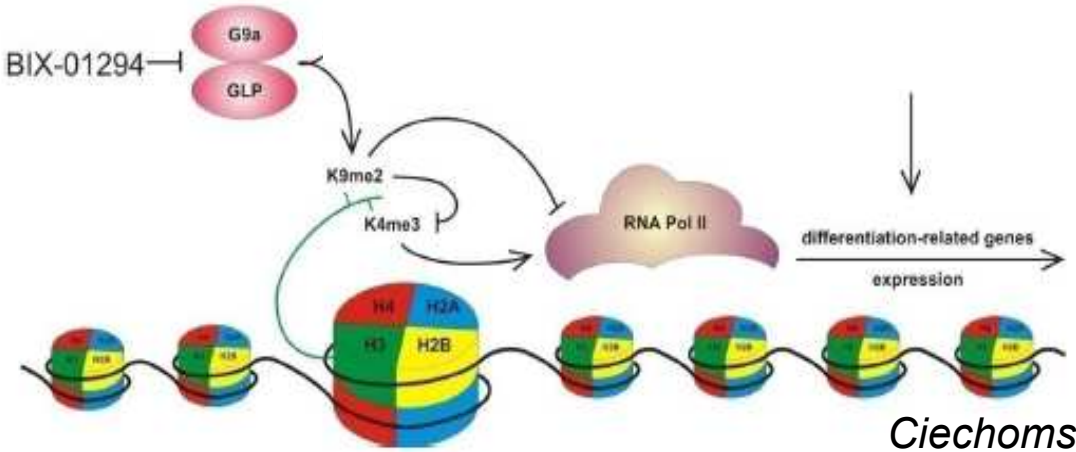


BIX 01294 – the G9a histone methyltransferase inhibitor - induces differentiation of glioma spheres



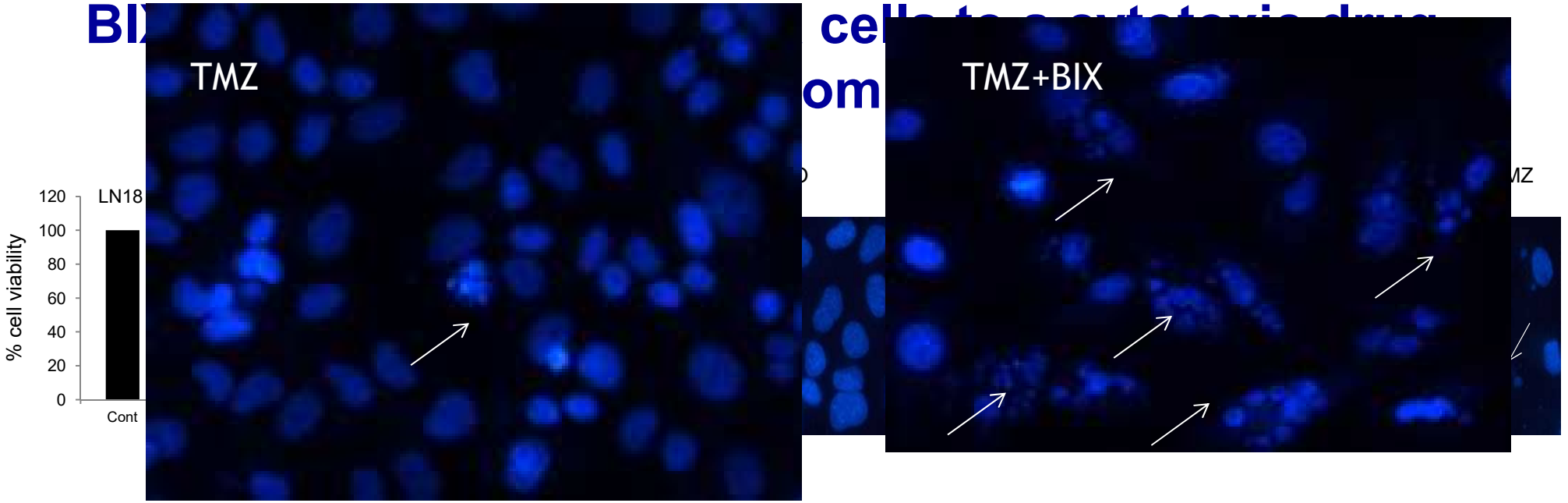
a repressive mark

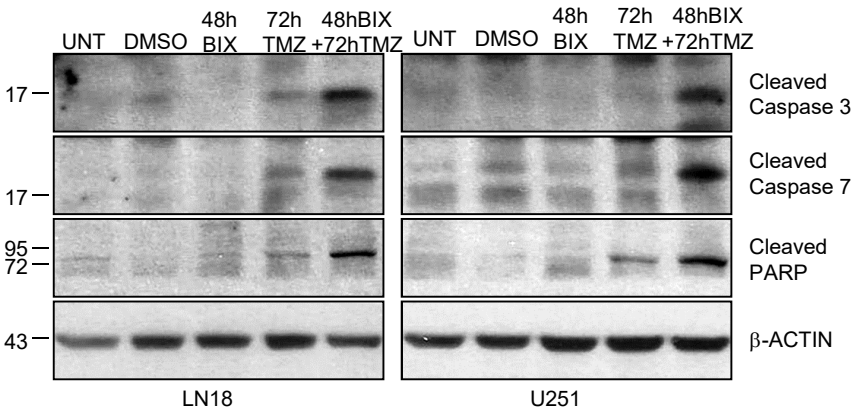
an activating mark

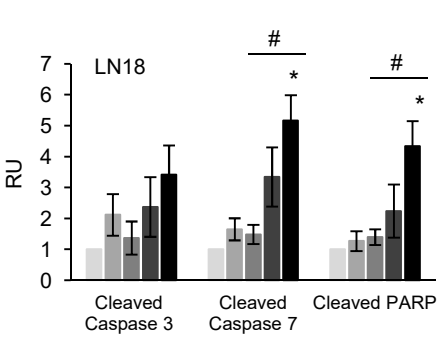


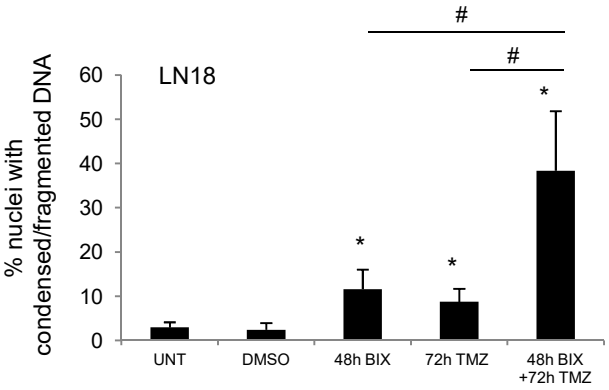
Ciechomska et al. Scientific Rep 2016

BI… cell… to a cytotoxic drug… om…









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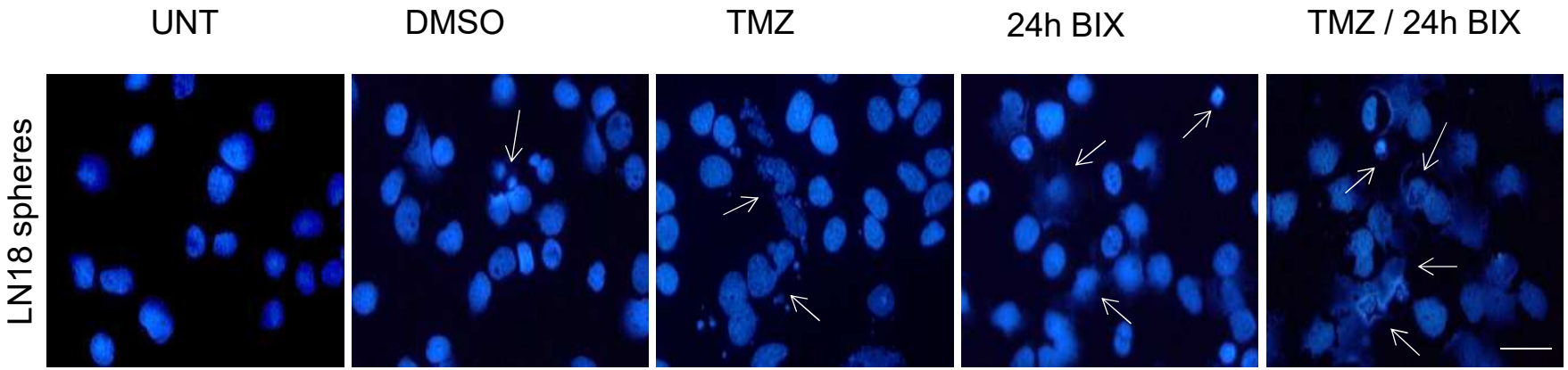
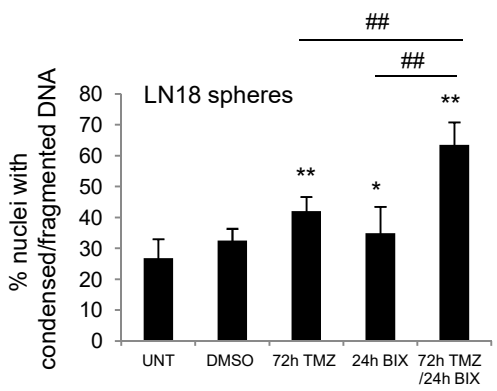
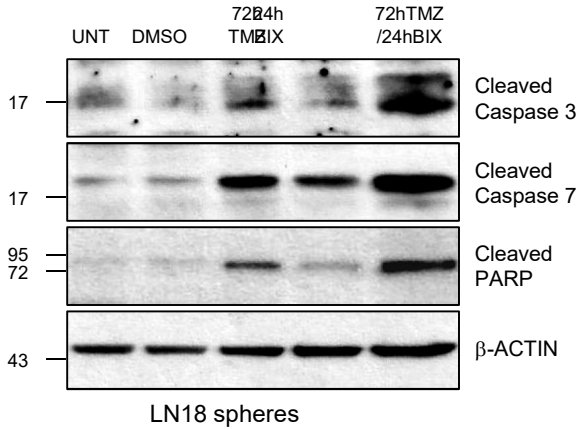
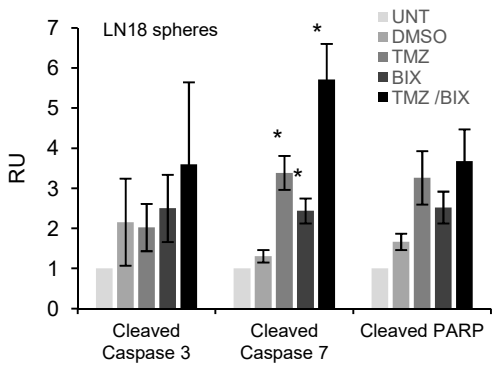
BIX 01294 sensitizes glioma cells to temozolomide



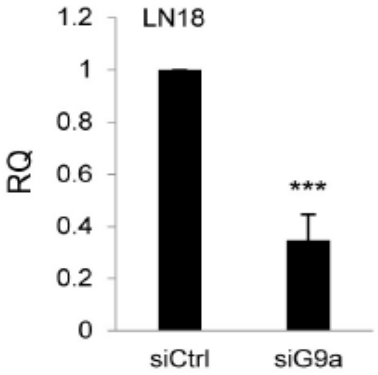


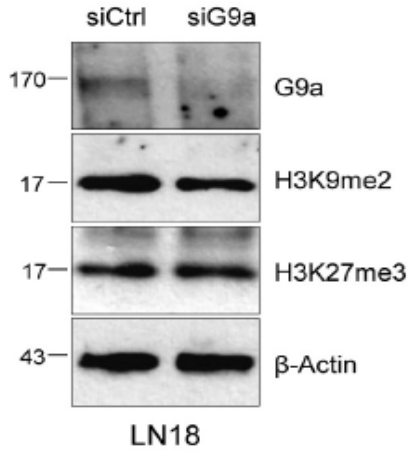


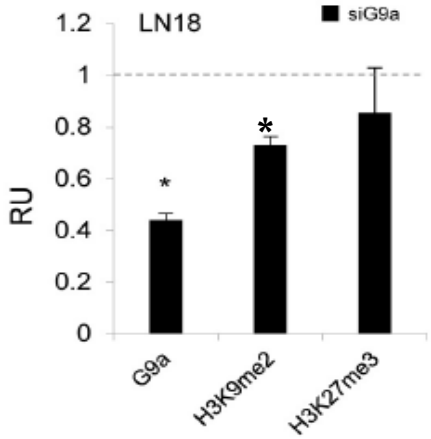


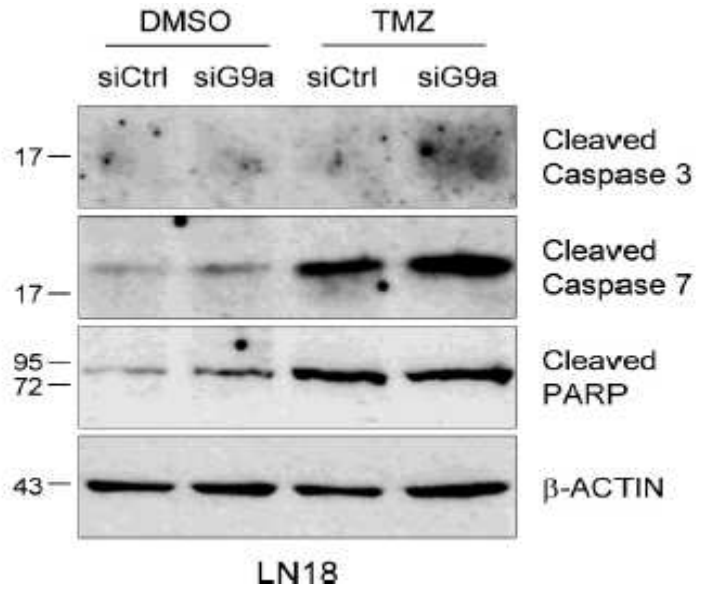
Ciechomska et al. Front Pharm., 2018

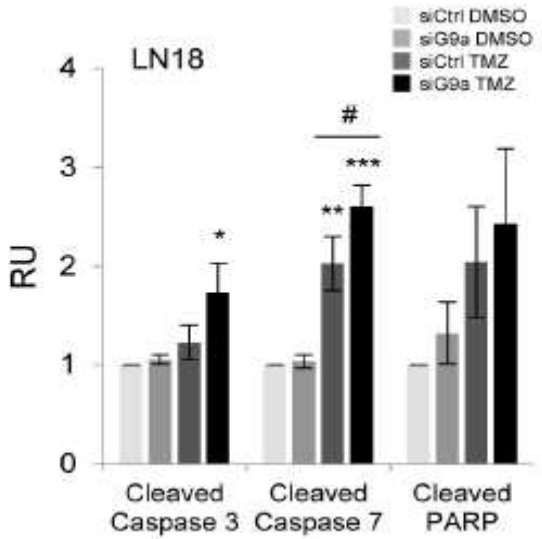
G9a konckdown sensitizes glioma cells to temozolomide











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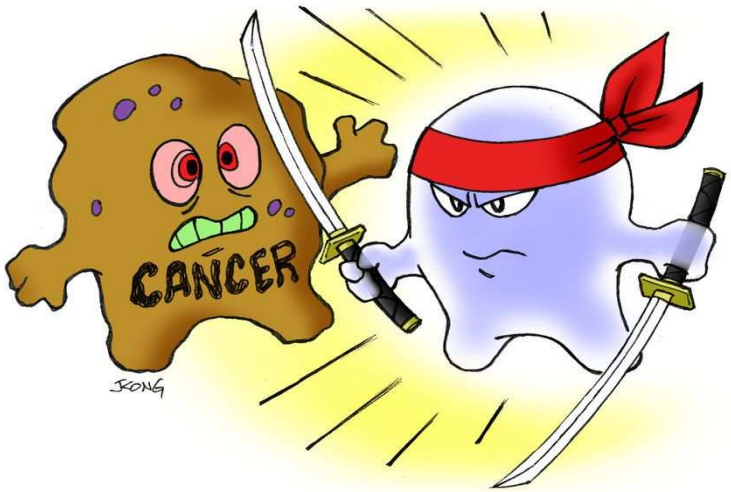

Summary

FIRST PART

- **We found a group of GBM patients with genomic alterations sensitizng them to TMZ**
- **Combining TMZ with PARP inhibitors may be a new option**

SECOND PART

- **Glioma CSCs are sensitive to treatment with epigenetic enzyme inhibitors.**
- **Inhibitors of a histone methyltransferase G9a sensitize glioma cells to TMZ.**
- **Interestingly, both pretreatment or posttreatment with a G9a inhibitor sensitizes glioma cells to TMZ.**

Kaminska's group

nencki institute of experimental biology

Dr. Iwona Ciechomska
Dr. Jakub Mieczkowski
Dr. Bartosz Wojtas
Dr. Marta Maleszewska
Dr. Ola Ellert-Miklaszewska
Dr. Rafał Guzik
Dr. Wenson D. Rajan,
Dr. Agnieszka Kaczmarczyk

Sylwia K. Król,
Karolina Stepniak,
Bartłomiej Gielniewski,
Katarzyna Poleszak,
Maria Pasierbińska,
Natalia Ochocka,
Kacper Walentynowicz,
Beata Kaza,
Paulina Pilanc,
Paulina Wiechecka, Paulina Szadkowska, Bartosz Czapski,
Kamil Wojnicki,
Pawel Segit, Adria Roura-Canalda

Rosella Galli, UnIversity of Milan, Italy

Clinicians:

Andrzej Marchel, Tomasz Czernicki, Andrzej Koziarski, Grzegorz Zielinski, Andrzej Styk, Maciej Kawecki, Cezary Szczylik, Ryszard Czepko, Maciej Banach, Wojciech Kaspera, Wojciech Szopa, Wiesława Grajkowska, Katarzyna Kotulska-Jozwiak, Marcin Roszkowski

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