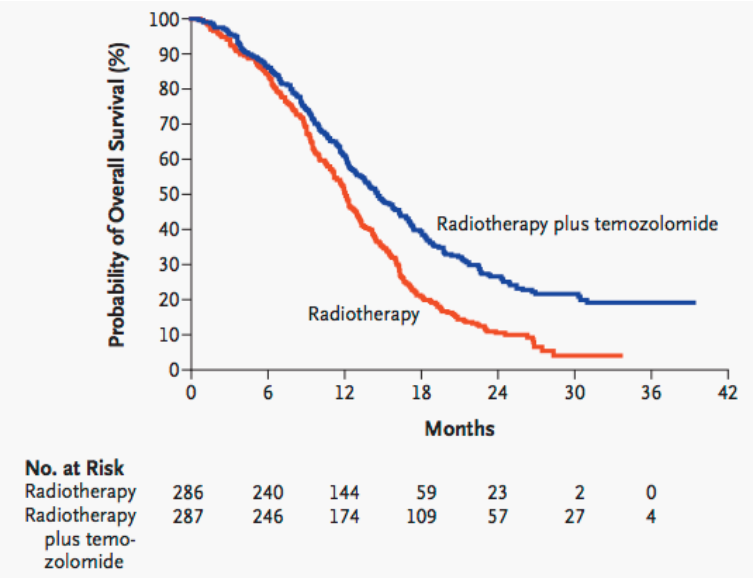
Targeting Notch pathway in glioblastoma prolongs survival in combination with standard of care

ICTR-2016

Jan Theys
www.maastrolab.com

Glioblastoma Multiforme (GBM)

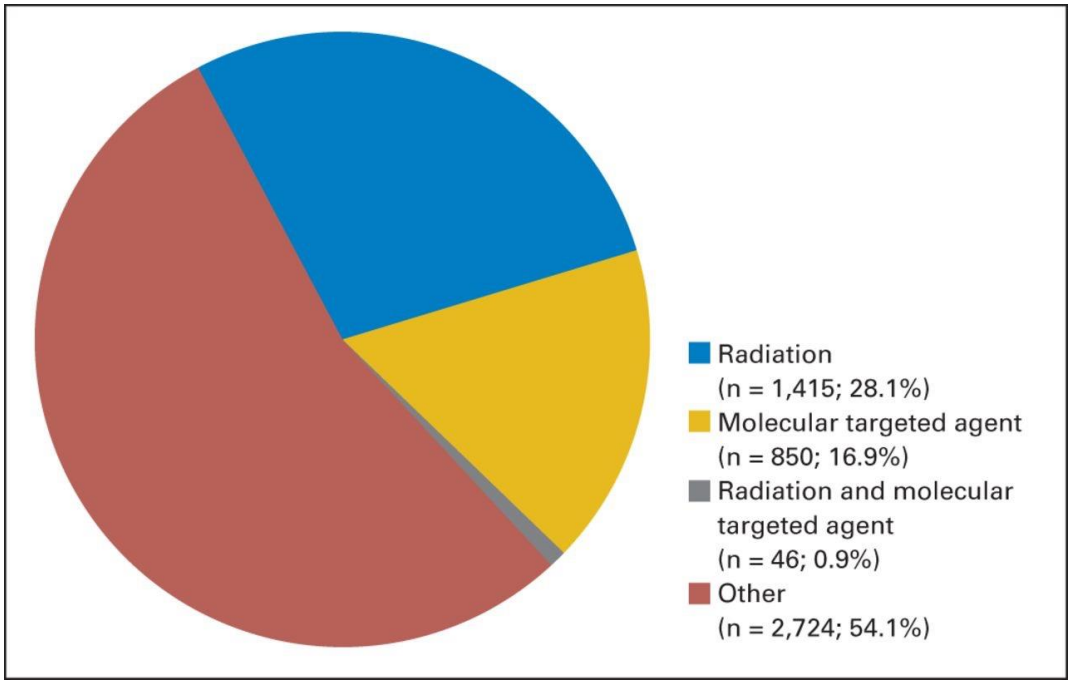


Most common and aggressive brain tumor (grade IV astrocytoma)
No curative treatment
First line treatment Surgery, Radiotherapy + Chemotherapy (temozolomide)
MGMT methylation predictive for temozolomide response
Median survival ~15 months after initial diagnosis

Need for new treatments/ targets

Stupp NEJM 2005

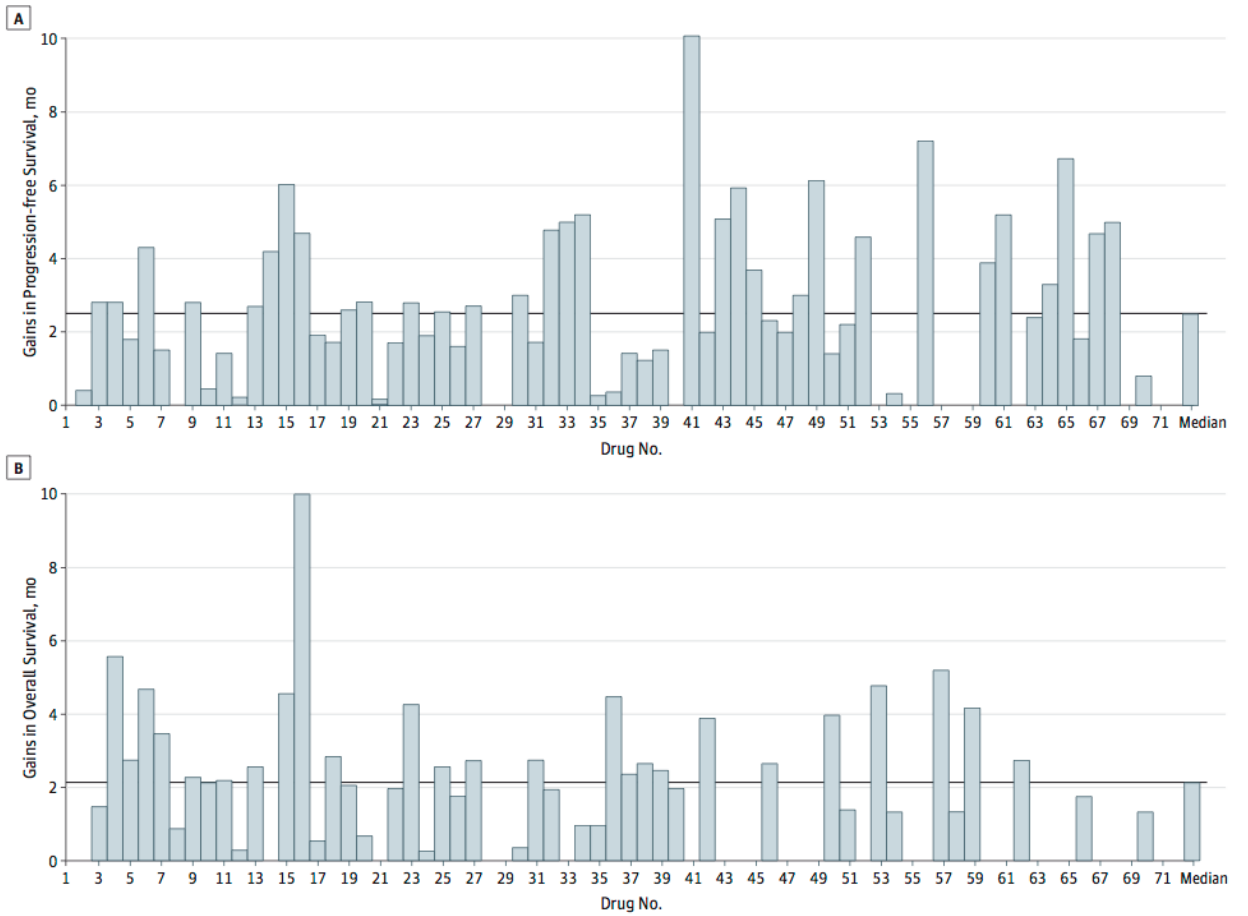
Distribution of current phase III clinical trials in oncology



Zachary S. Morris, and Paul M. Harari JCO 2014;32:2886-2893

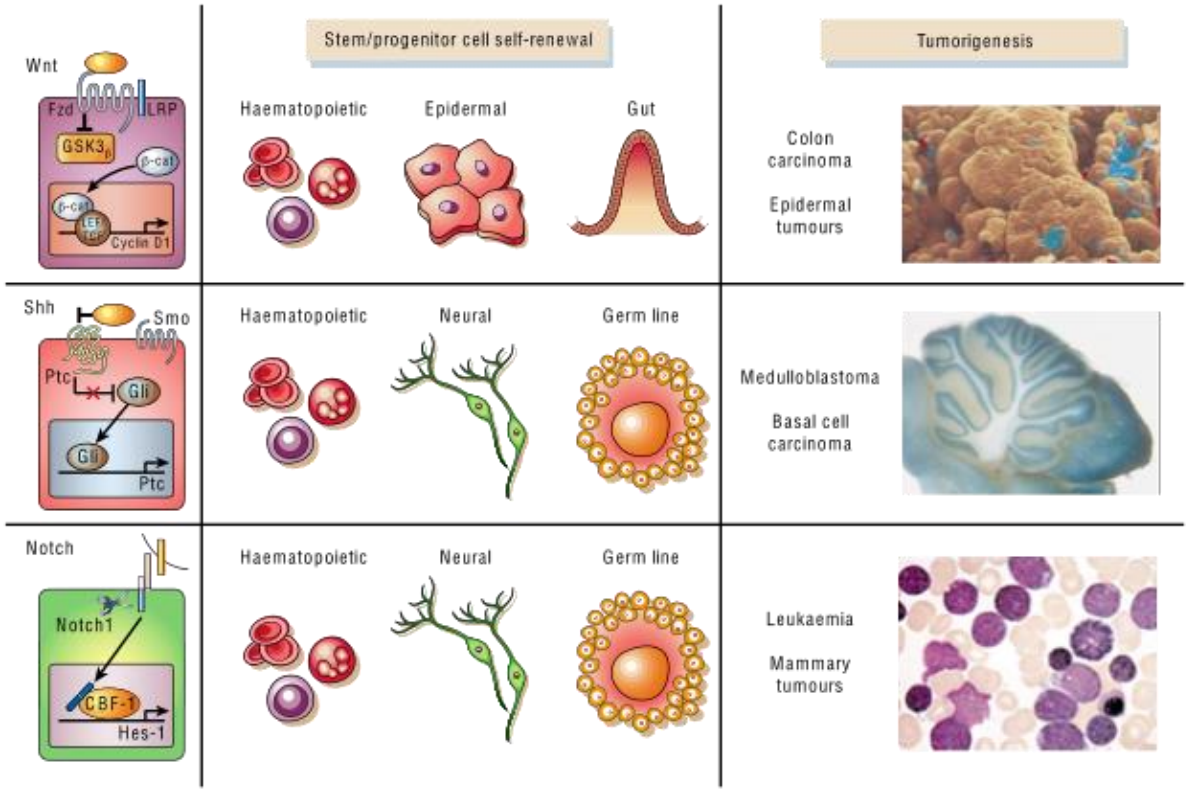
A need for NEW treatment approaches

Figure 1. Graphical Representation of the Results in Table 1: Gains in Progression-Free Survival (PFS) and Overall Survival (OS) for the 71 Drugs Approved by the FDA From 2002 to 2014 for Metastatic and/or Advanced and/or Refractory Solid Tumors



Fojo T et al JAMA Otolaryngol Head Neck Surg. 2014

Stem Cell Pathways are deregulated in Cancer



Cancer Stem Cells

- initiation,
- progression
- metastasis
- treatment resistance
- heterogeneity

Reya Nature 2001

NOTCH is an interesting therapeutic target

Tumor Cells

- Deregulated in many cancers, mutated in some
- promote self-renewal, block differentiation
- epithelial mesenchymal transition
- Response to hypoxia
- Cancer stem cells (heterogeneity)

Tumor microenvironment

- NOTCH ligands drives tumor angiogenesis
- Immune cells, Fibroblasts.. Other…

Role in treatment response

- chemotherapy, radiotherapy and targeted therapies

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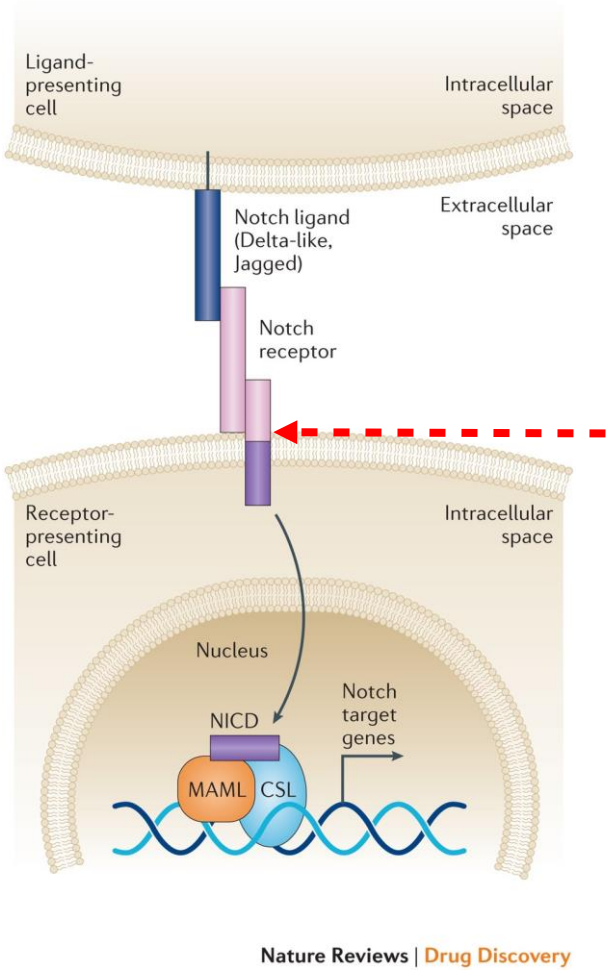
NOTCH receptor signaling



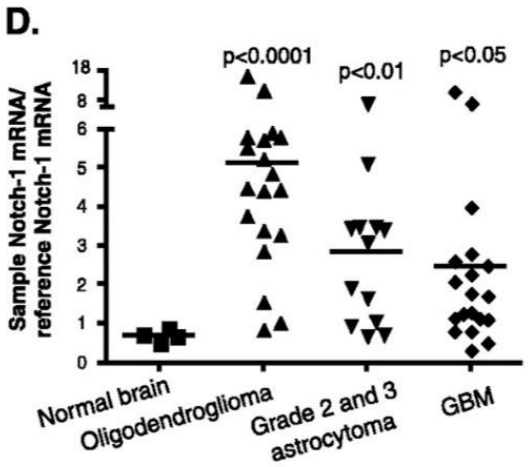
Nature Reviews | Drug Discovery

- Cell cell communication in development and adult tissues
- proliferation differentiation cell death and cell renewal

- ©-secretase inhibitors block Notch cleavage and activity
- > 40 clinical trials in leukemia's and solid cancers
- Only 2 clinical trials which include RT

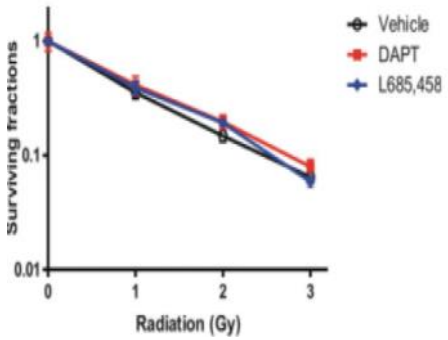
NOTCH pathway is active and a target in GBM

clinical

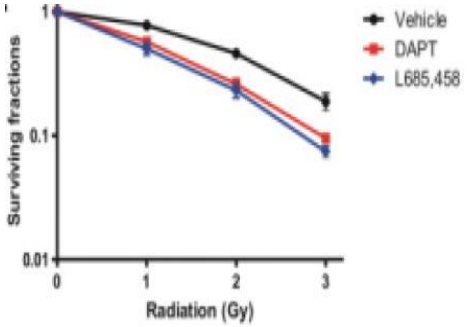


preclinical

Cd133- (bulk of tumor cells)



Cd133+ (tumor initiating cells)



but efficacy in orthotopic models in combination with standard treatment is lacking !

Purow B W et al. Cancer Res 2005

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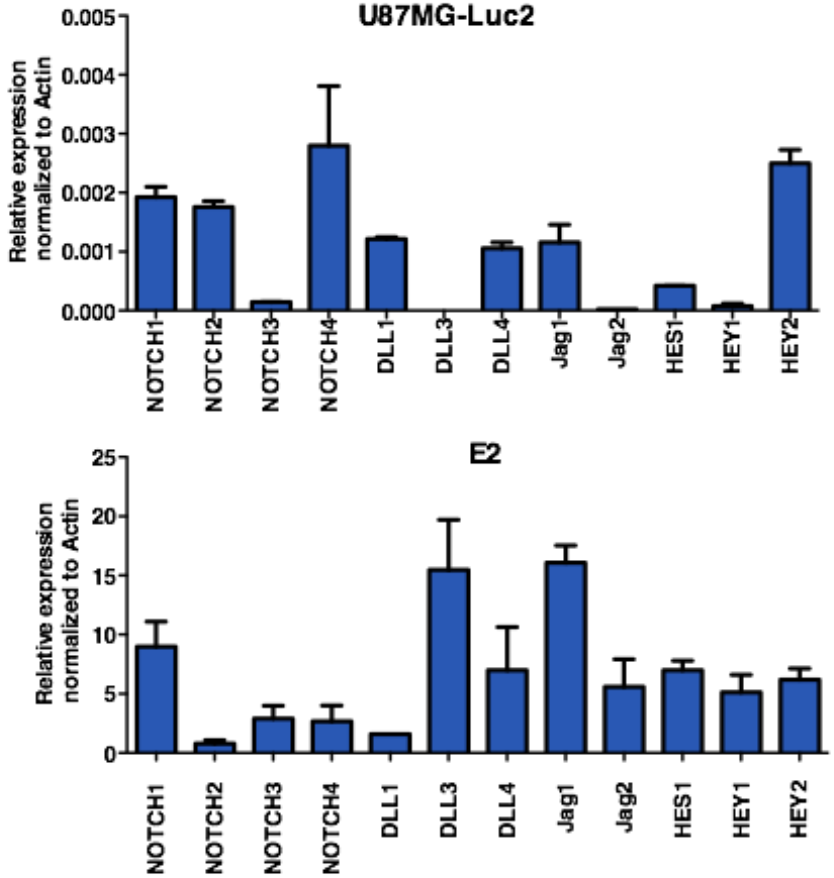
Study objective

To develop a preclinical platform for orthotopic glioblastoma
in which standard care treatment (RT+ TMZ) + Notch inhibitors can be studied mimicking clinical management of Glioblastoma in patients.

The NOTCH pathway is expressed in primary and established GBM cell lines



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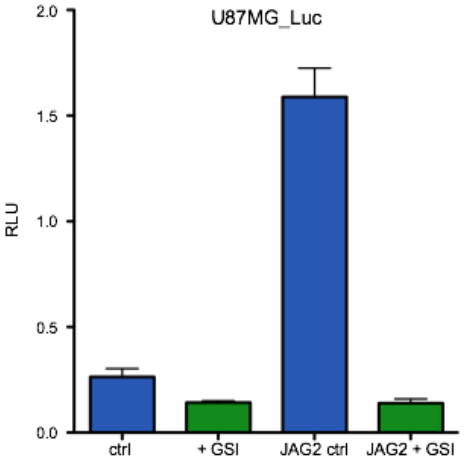
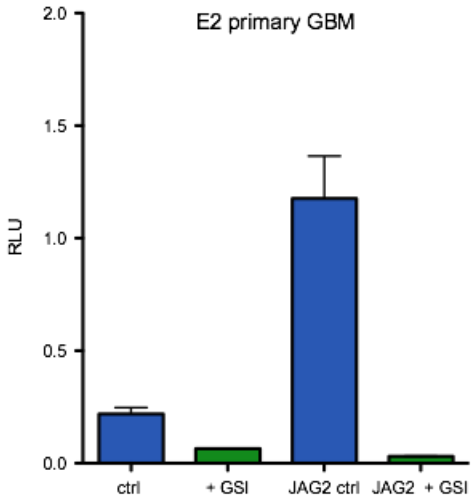
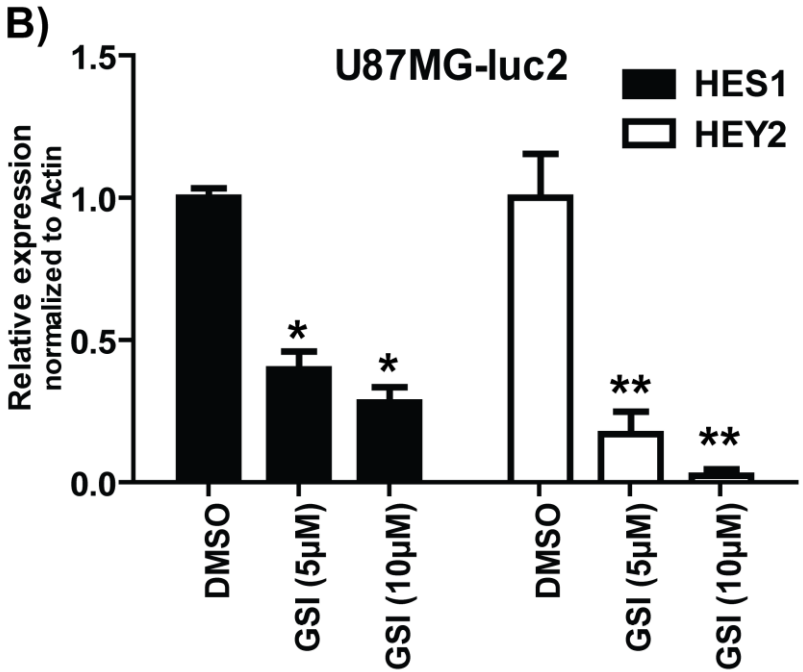
The NOTCH pathway is active in primary and established GBM cell lines







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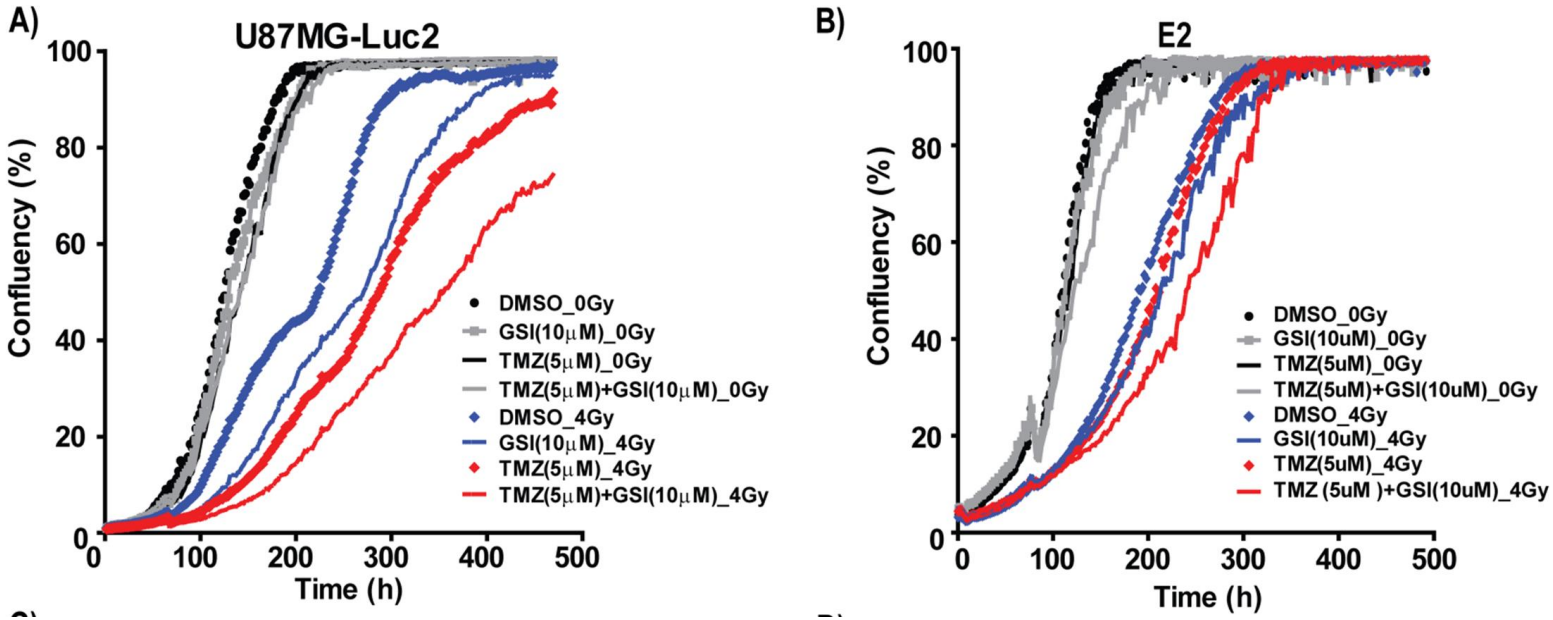
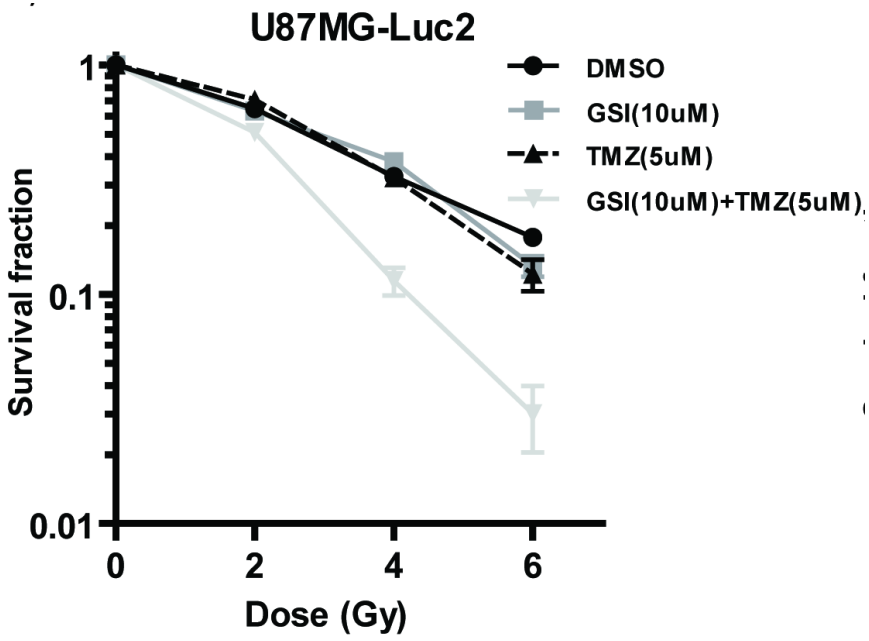
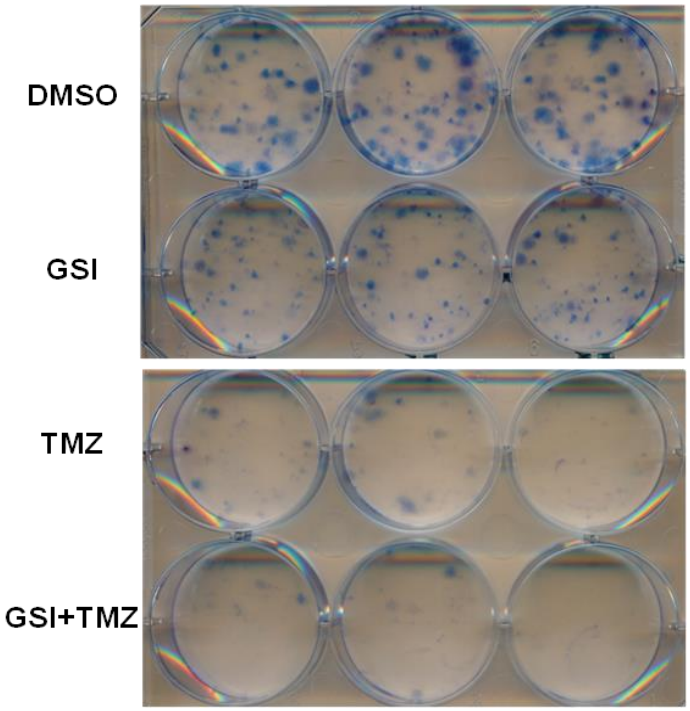
GSI+RT+TMZ affects proliferation (2D)



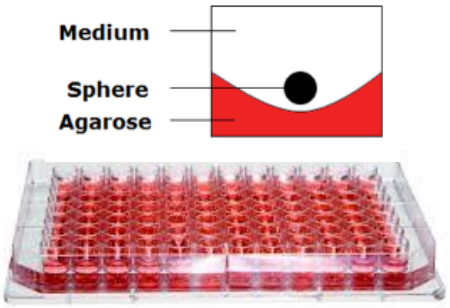
GSI+RT+TMZ affects long term clonogenic growth





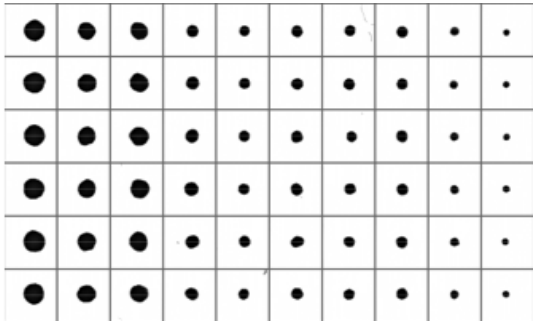
Yahyanejad et al., in revision

in vitro drug screening in 3D GBM spheroids



1. Prepare 96 well agarose-coated plates
2. Seed cells and grow spheroids in liquid overlay

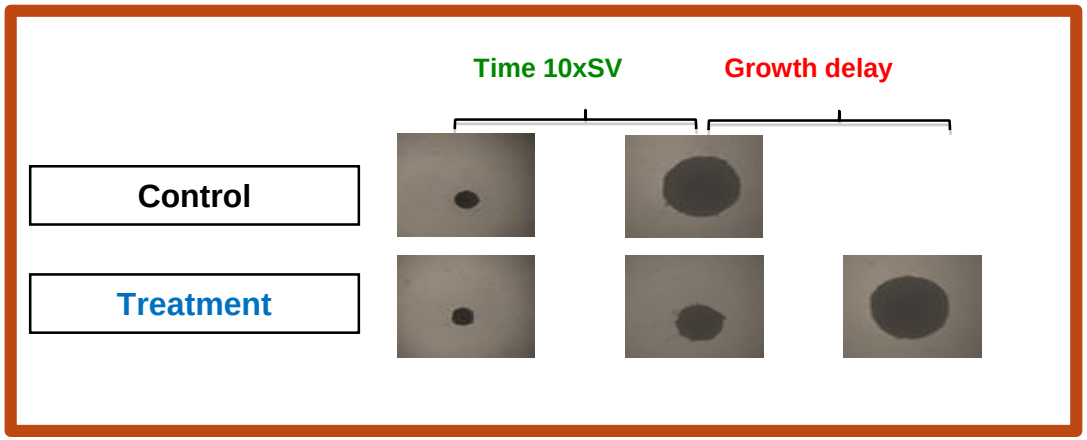
3. Make phase contrast images (3x/week, starting at d4 post-seeding)



4. Treat spheroids (n≥12 for each condition)
5. Re-fresh medium (3x/week)

6. Automated spheroid analysis using MatLab-based software (active contour algorithm)

7. Use generated table with relevant morphometric information for data analysis



Friedrich J et al. Nat Protoc. 2009;4(3):309-24
Chen W et al. J Vis Exp. 2014 Jul 8;(89)

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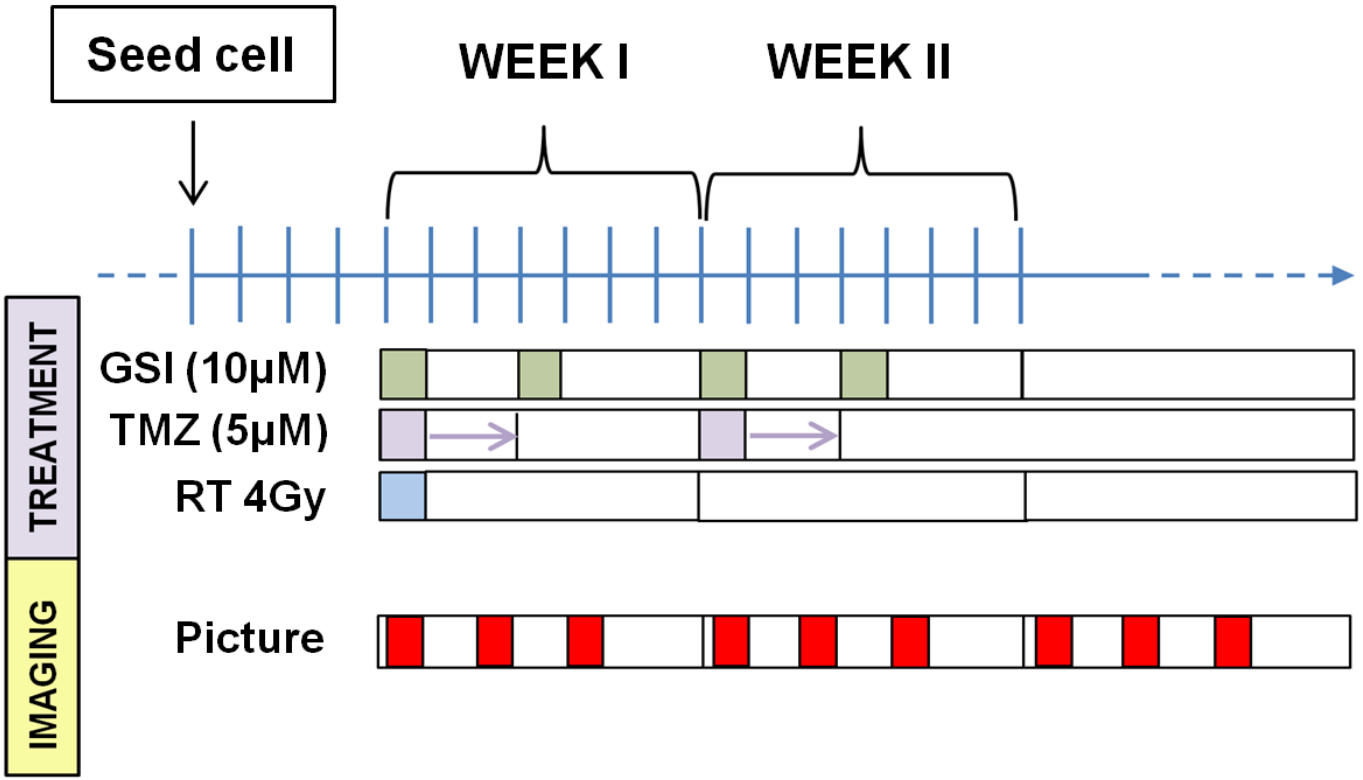
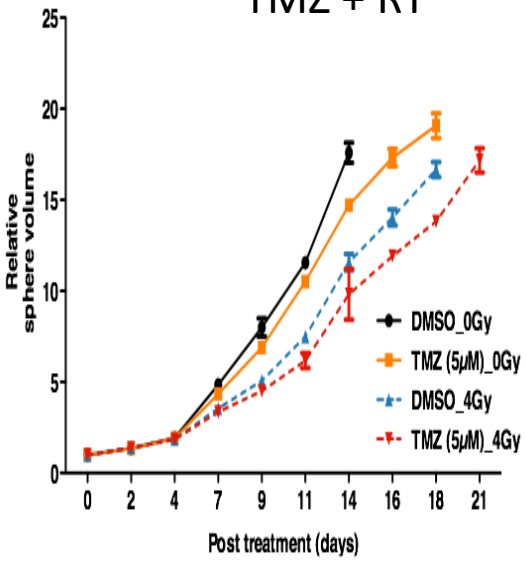
in vitro drug screening in 3D GBM spheroids

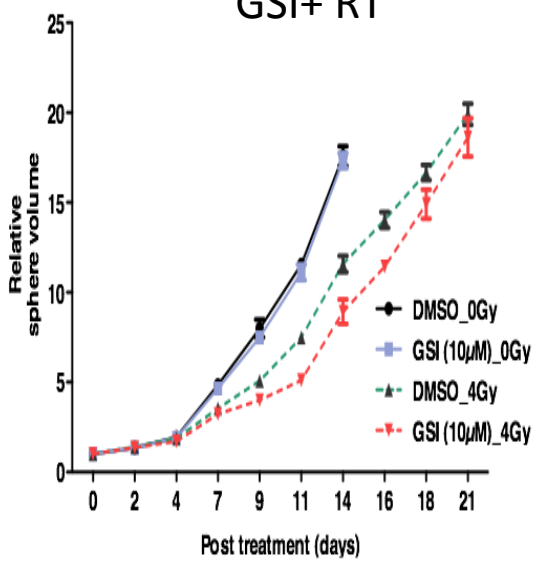


Effect of treatment on spheroid growth

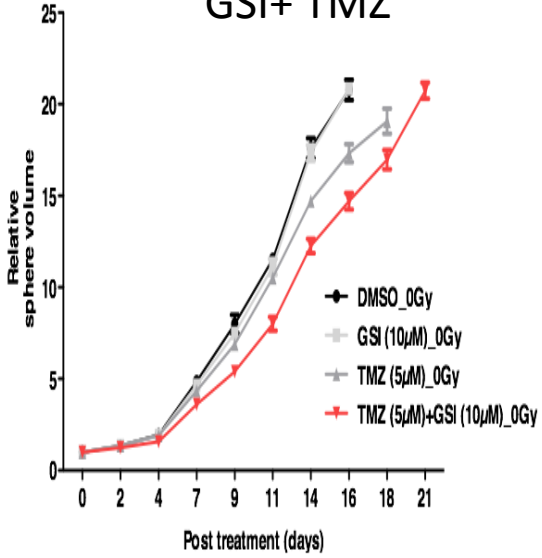
TMZ + RT



GSI+ RT



GSI+ TMZ



Yahyanejad et al., in revision

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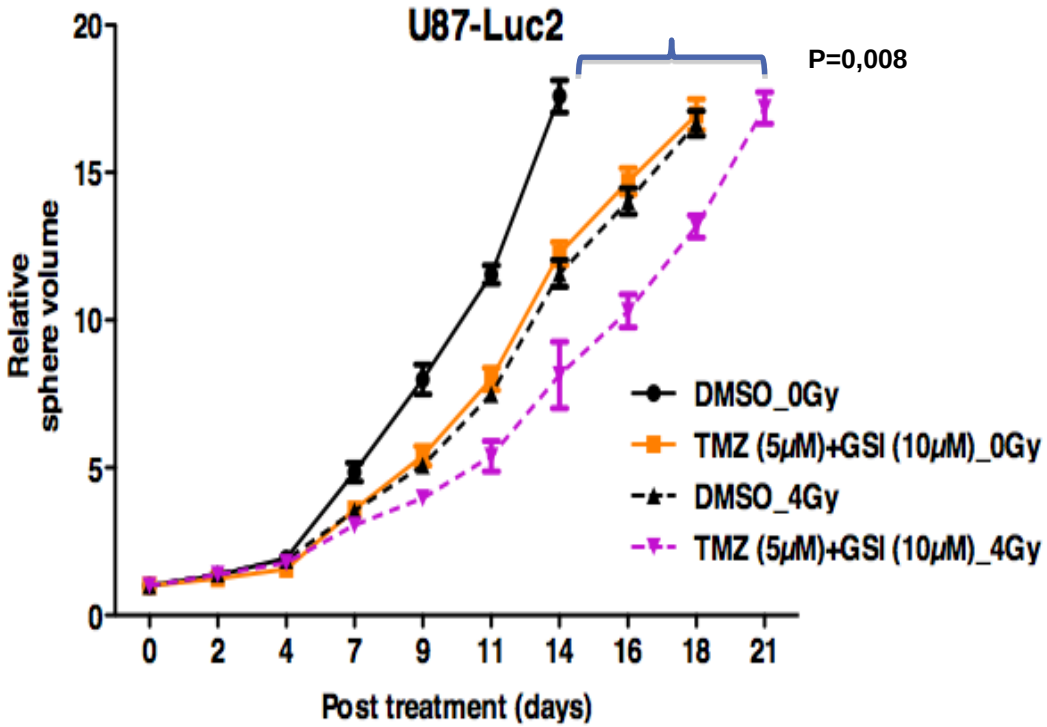
NOTCH inhibitors sensitize to TMZ and RT

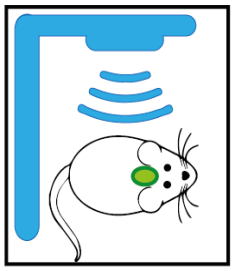


treatment	Td
Control	3.318
GSI	3,346
TMZ	3,440
TMZ+GSI	4,570
4Gy	4,629
4Gy +GSI	4,821
4Gy + TMZ	4,898
4Gy + TMZ + GSI	4,982

micro-IGRT for GBM “setting the right RT dose”

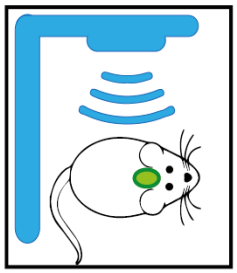
Implant GBM

uCT and BLI



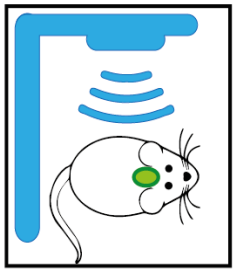
planning

RT



treatment

BLI

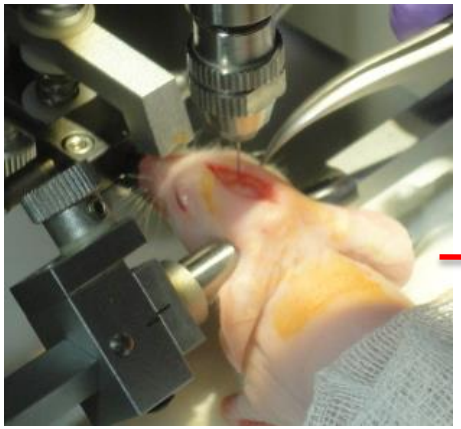


monitoring

BLI10 *or*
Humane endpoint

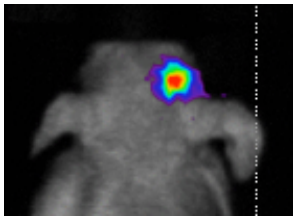
small animal **I**mage **G**uided **R**adio**T**herapy (SMART)

Implant U87-Luc2 cells



100K in PBS (5 ul)

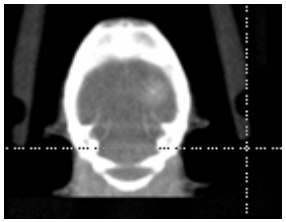
Bioluminescence imaging(BLI)



luciferase

Luciferin +ATP + O_2 ----------> OxiLuciferin + Light !

Contrast-enhanced micro-CT



iv omnipaque

X-rad PXi

Follow-up tumor growth
3x/week

+/- treatment

van Hoof S., Granton P. V., and Verhaegen F., Radiotherapy and Oncology, (2013)

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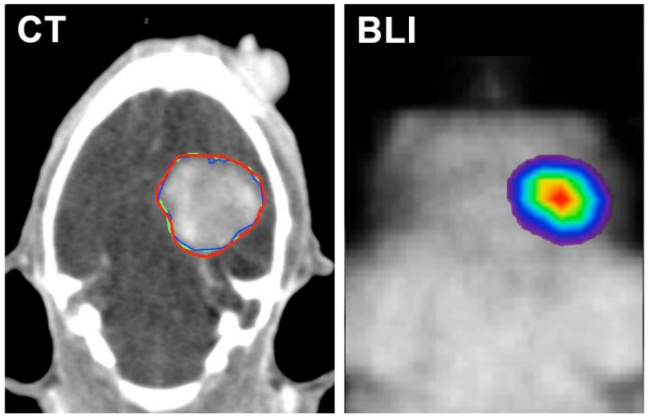
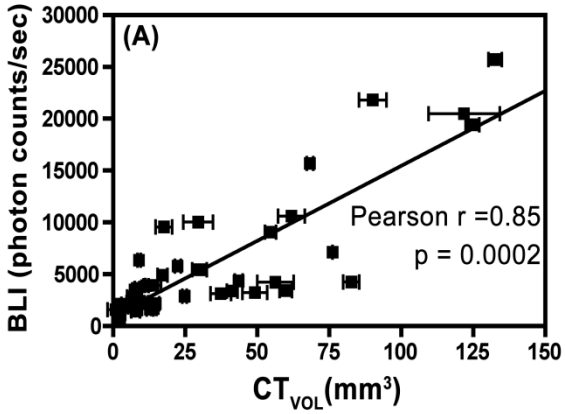
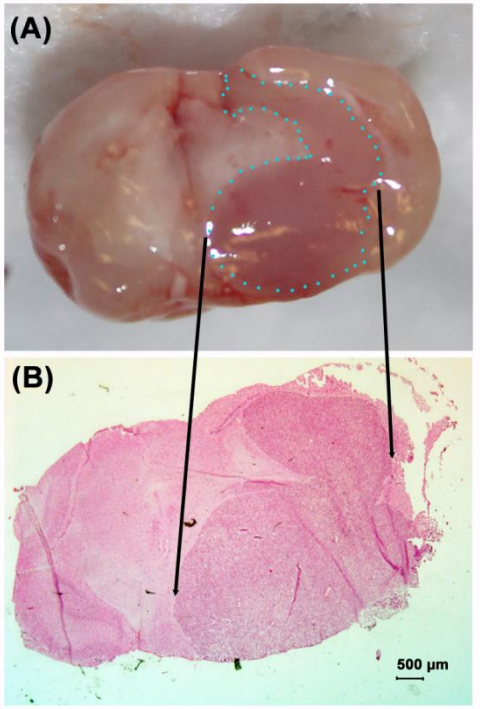
uIGRT platform for glioblastoma







Do a planning CT and follow growth by BLI

Yahyanejad et al., Mol Imaging 2014.

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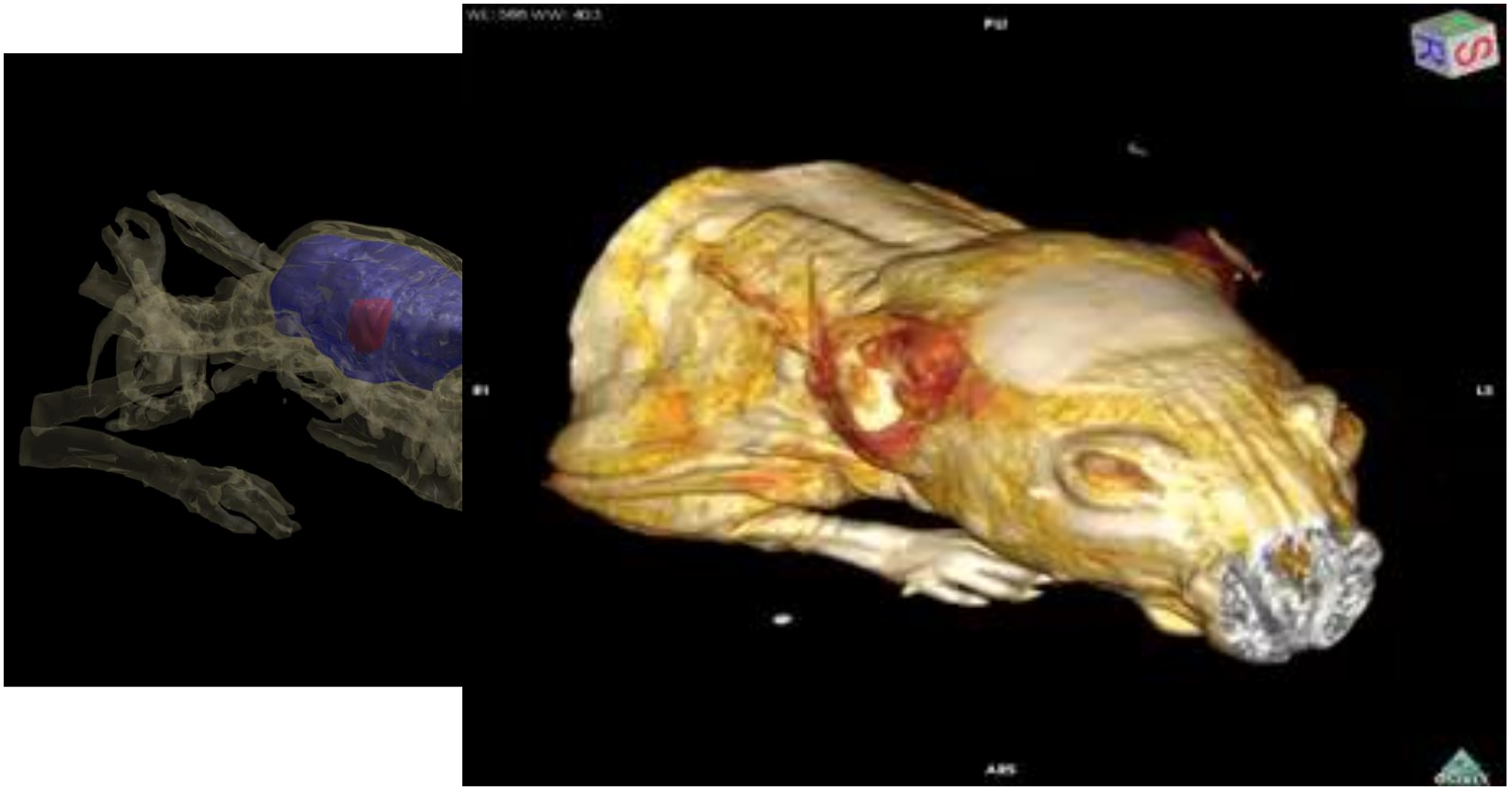
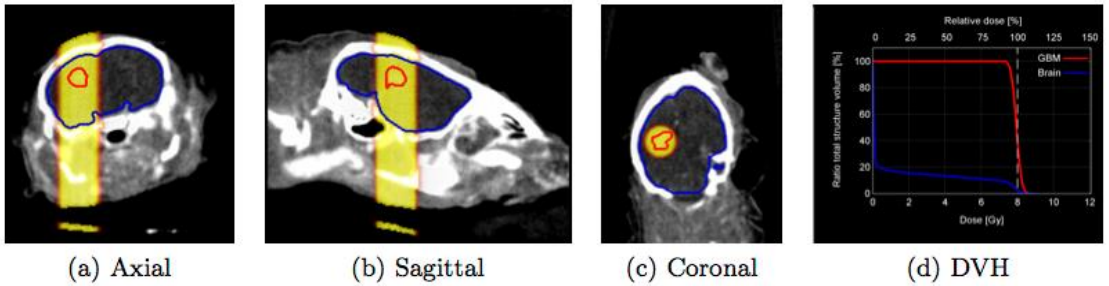
SmART micro-CT



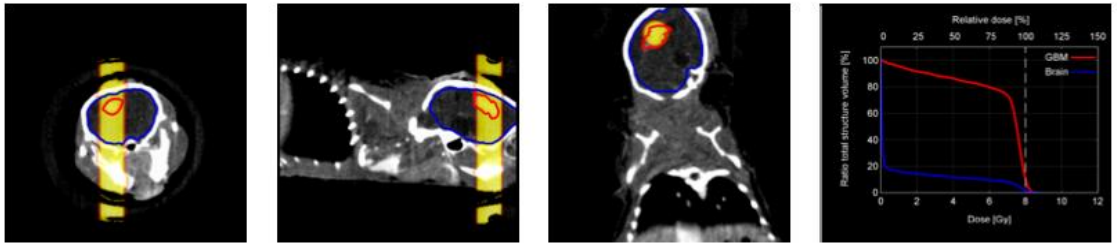
Yahyanejad et al., 2014 and Granton et al., J. Neuroncol 2015

Tumor delineation and treatment planning



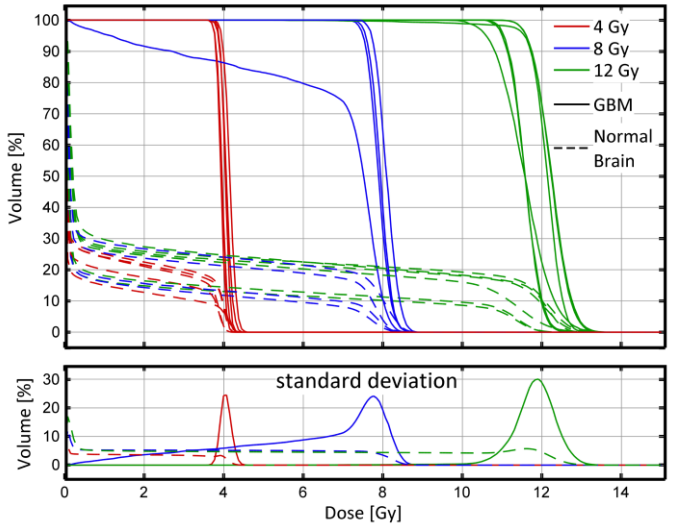
(a) Axial (b) Sagittal (c) Coronal (d) DVH

Figure 0.10: Mouse B4



(a) Axial (b) Sagittal (c) Coronal (d) DVH

Beam = 3 or 5 mm



Yahyanejad et al., Radiotherapy and Oncology 2015

micro-IGRT for GBM “setting the right RT dose”

Implant GBM

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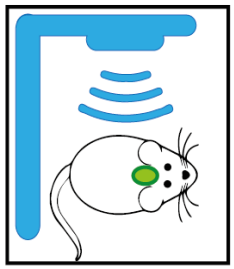
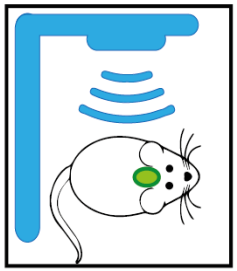


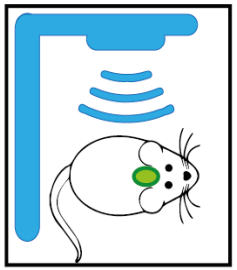
planning

RT



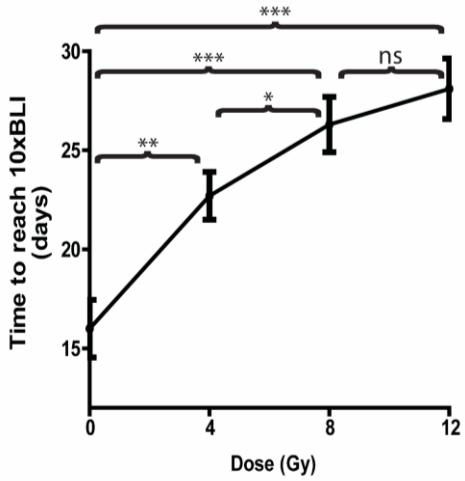
treatment

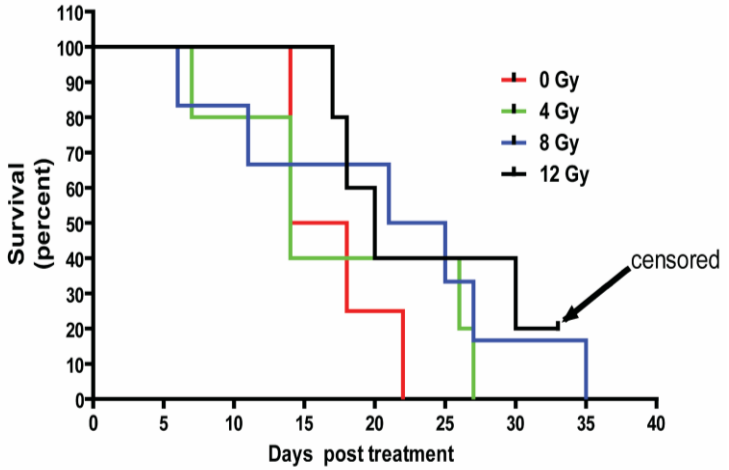
BLI



monitoring

BLI10 *or*
Humane endpoint





Yahyanejad et al., Radiotherapy and Oncology 2015

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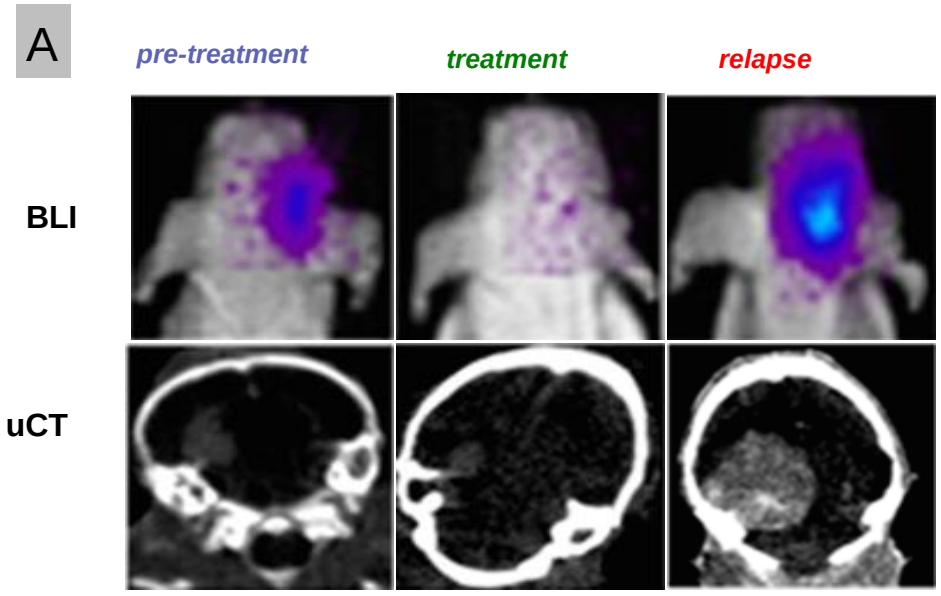
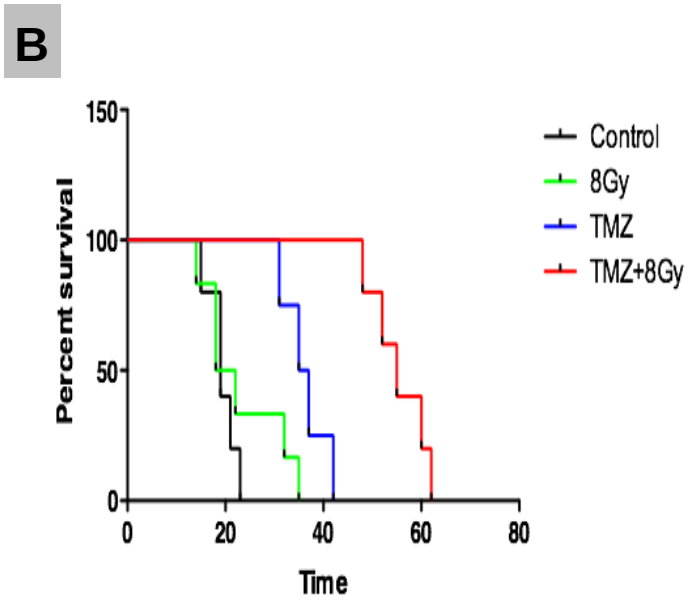
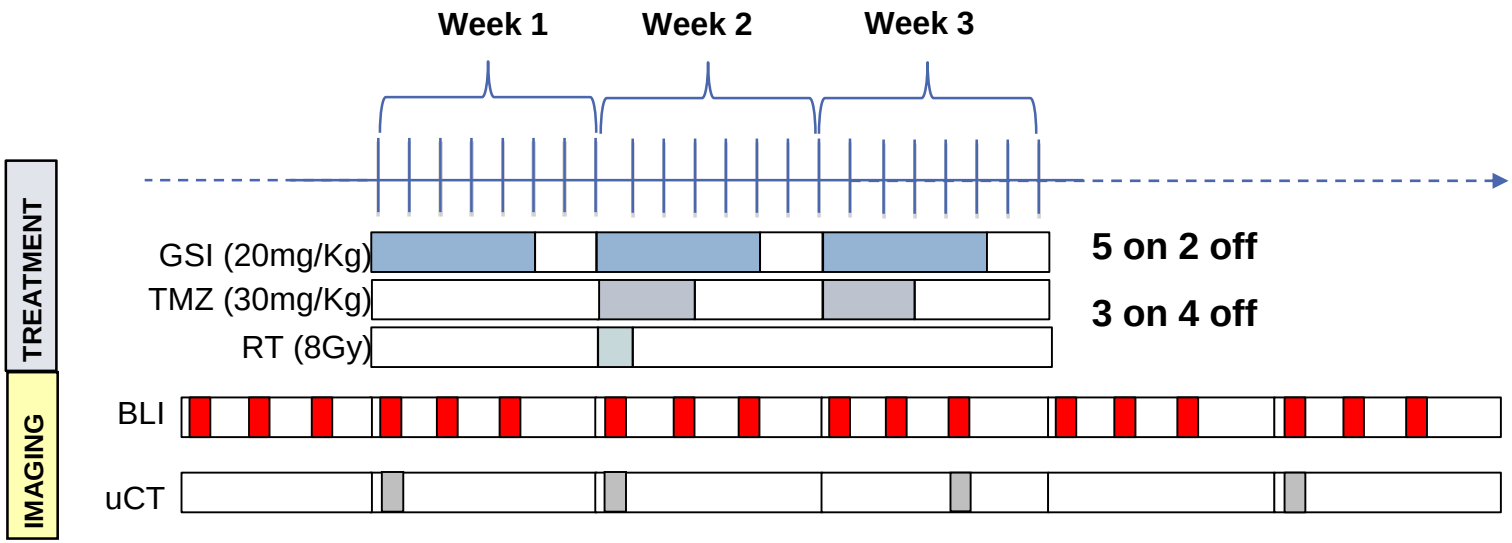
Response and relapse to standard of care





Yahyanejad et al RO 2015 and in revision

Study set up: therapy scheduling

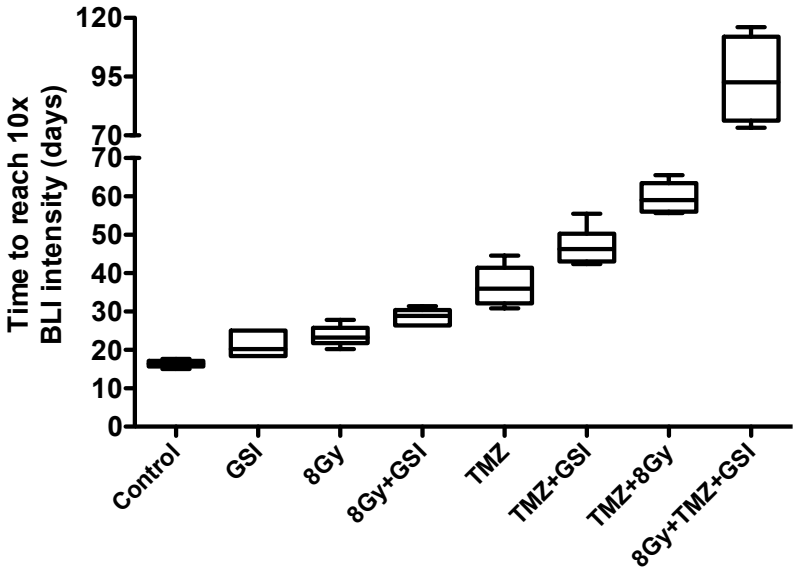


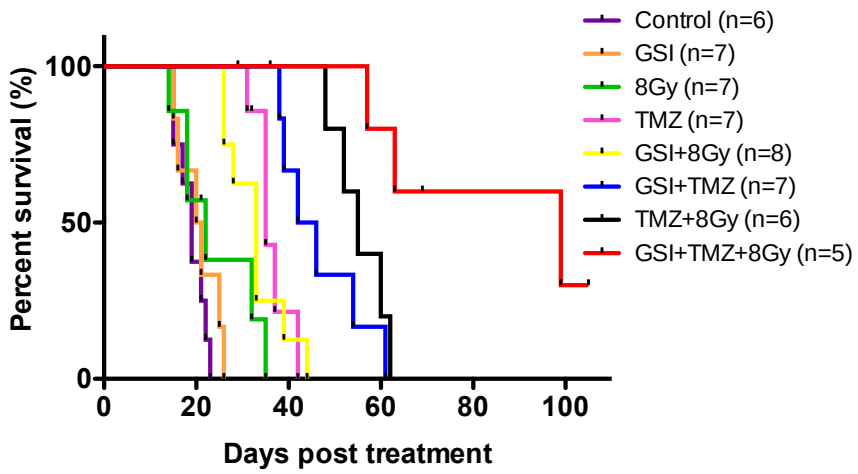
Start treatment after 2 consecutive doublings by BLI (30 mm^3)

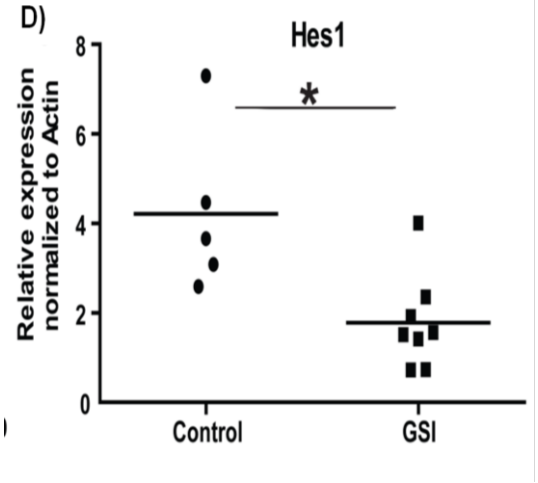
End treatment 10 x BLI / humane endpoint

Yahyanejad et al., in revision

Combined effect of GSI, TMZ and RT in vivo

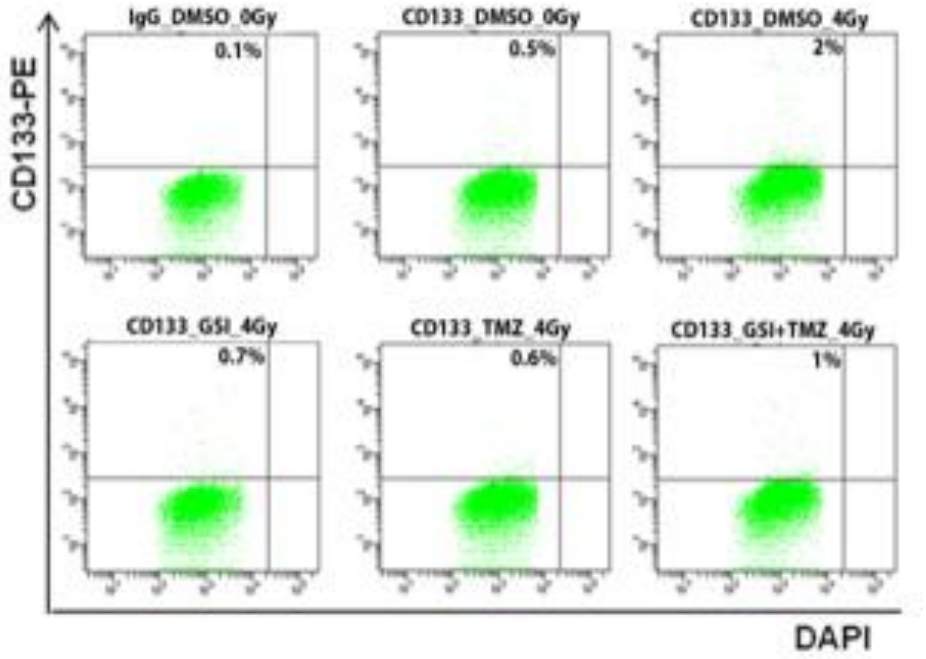


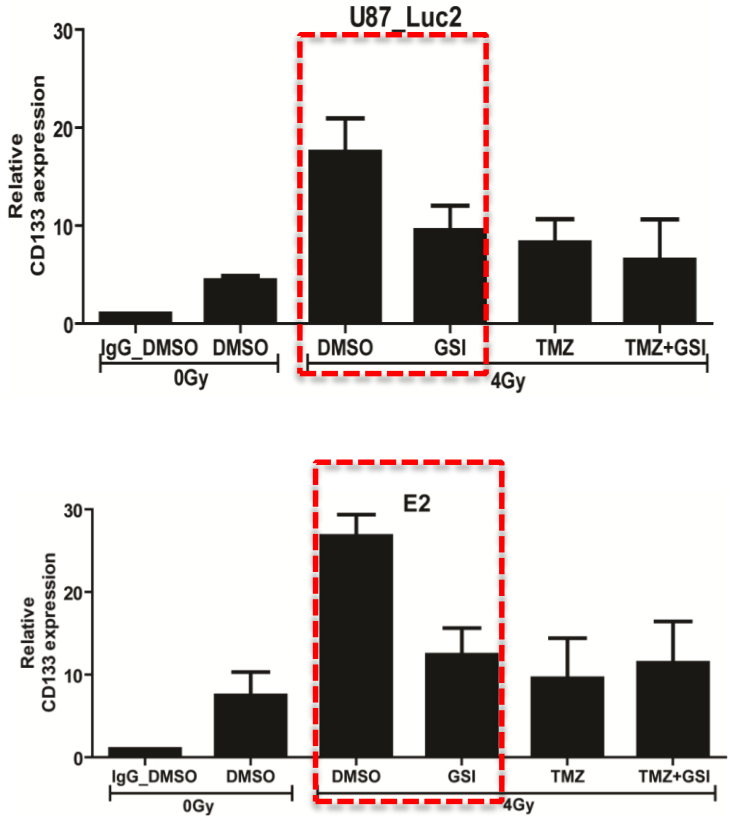




Yahyanejad et al., under revision

NOTCH inhibition blocks radiation induced increase of CD133 glioma stem cell marker (Neurospheres)





Yahyanejad et al., in revision

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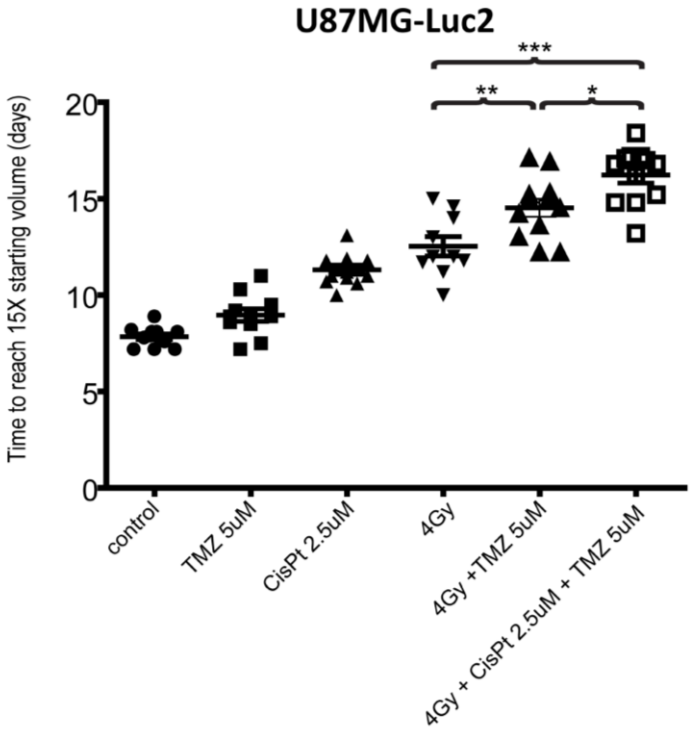
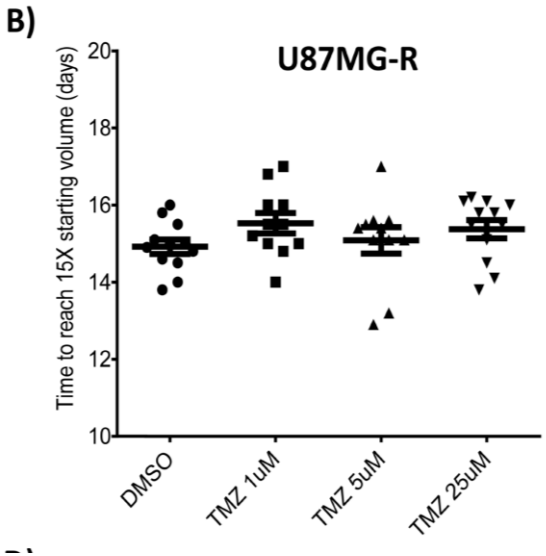
novel combination treatments for glioma

Enhancing other chemotherapeutics



Overcoming Temozolomide resistance



Yahyanejad, Van Hoof, Theys et al., Radiotherapy and Oncology 2015

Summary

- Image guided radiation therapy (SMART) allows accurate treament planning and irradiation followed by treatment monitoring in intracranial glioblastoma
- Notch inhibition combined with standard of care prolongs survival in GBM model
- NOTCH inhibition affect radiation sensitivity / clonogenic growth through regulation of CD133 glioma stem cell marker.
- Spheroid screening assays may be useful to discover novel treatment interactions.
- Can NOTCH inhibitors sensitize Temozolomide resistance (ongoing)

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