Orthotopic tumor models for glioma and NSCLC

ICTR Geneva 2016

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Valley of death
lab-clinic need better models that predict response

To evaluate (combination) treatments: need for 3D in vitro and orthotopic in vivo models

- tumors in own microenvironment
- high incidence of advanced disease
- limited number of tumors / site
- image guided (precision) RT
- longitudinal non-invasive imaging

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Lung cancer

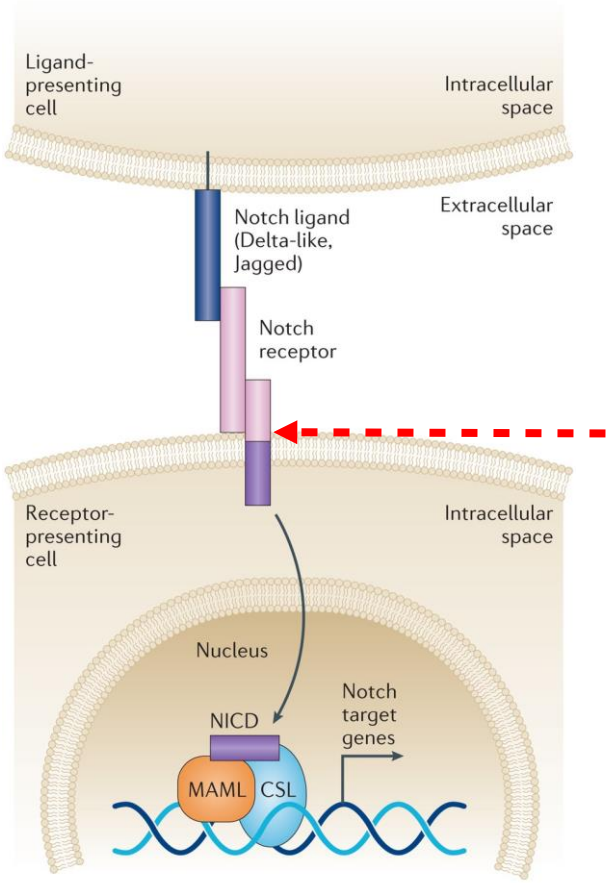
- **Types:**
  - Non-small cell lung cancer (85%)
  - Small cell lung cancer (15%)

- **Standard of care:** Chemo-radiation

- **Prognosis:** poor
  - 5-year survival:
    - 40-50% early stage
    - 1-5% advanced stage

Need for new treatments/ targets

NOTCH is a potential therapeutic target



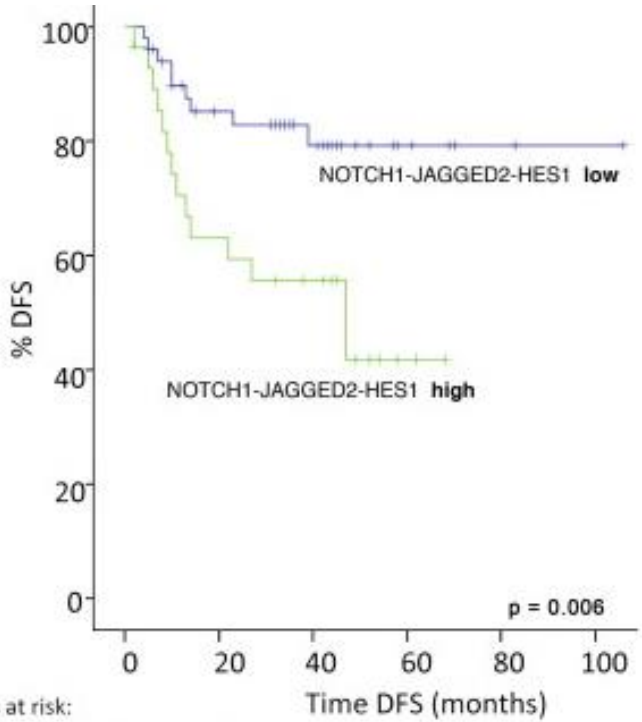
Nature Reviews | Drug Discovery

- 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 signaling is deregulated in NSCLC

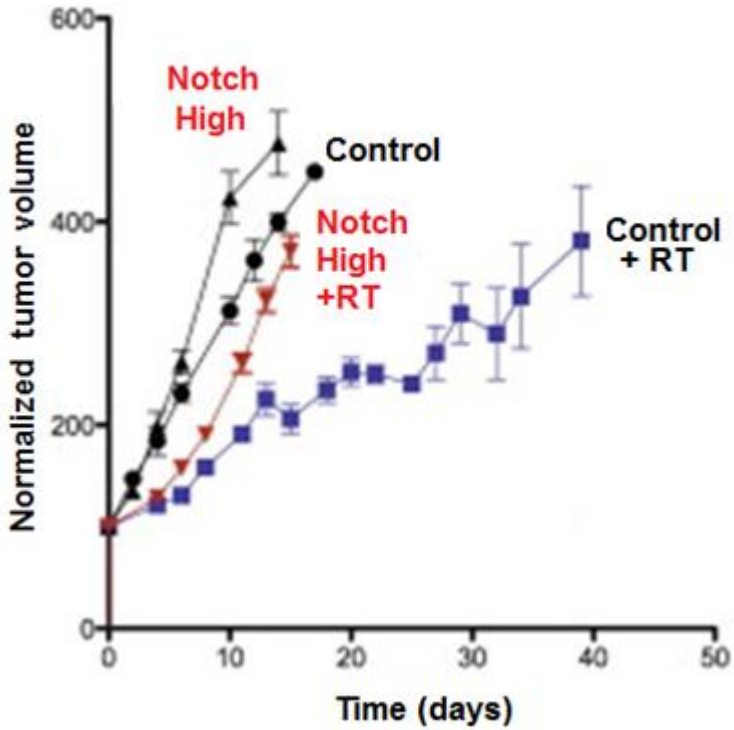
Patients



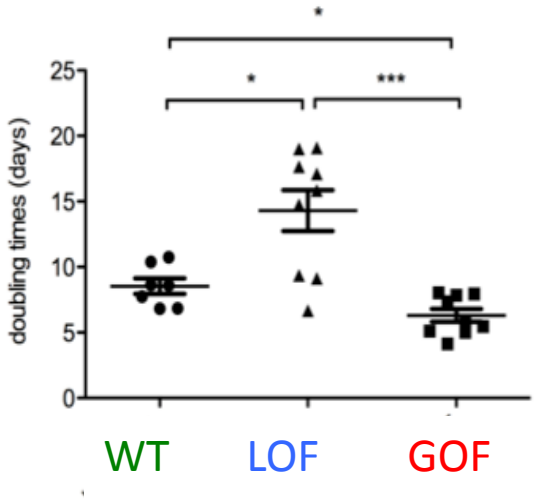
Patients at risk:

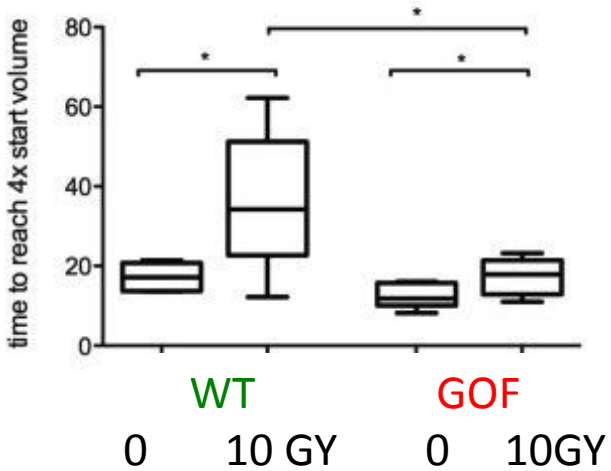
low	56	36	22	5	2	1
high	30	17	13	2	0	0

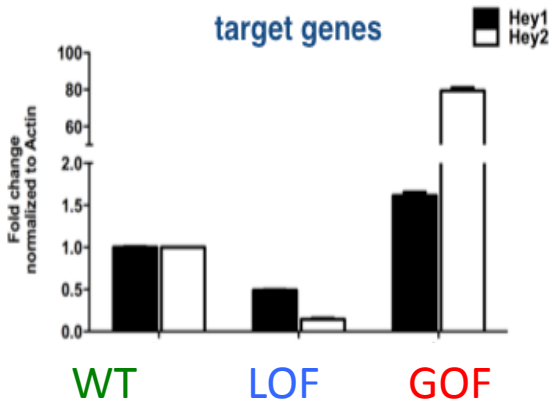
Mice xenograft

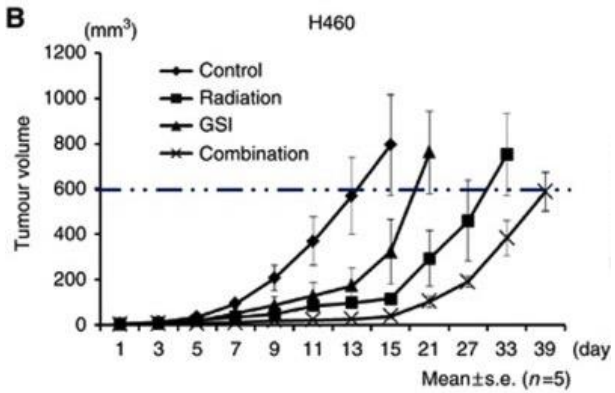


Gains and loss of NOTCH affect tumor growth and response to RT



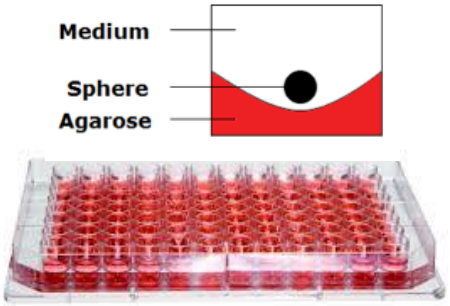






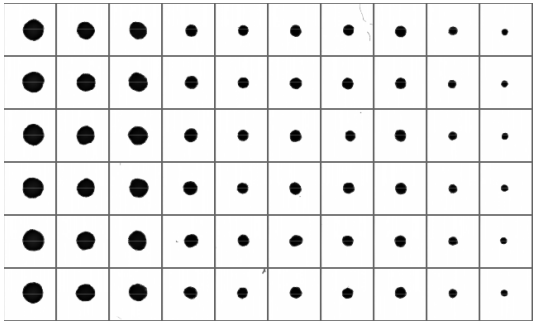
Theys et al., RO 2013 Mizugaki et al., BJC 2012
Sosa Iglesias et al., unpublished

Drug screening in 3D NSCLC spheroids



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

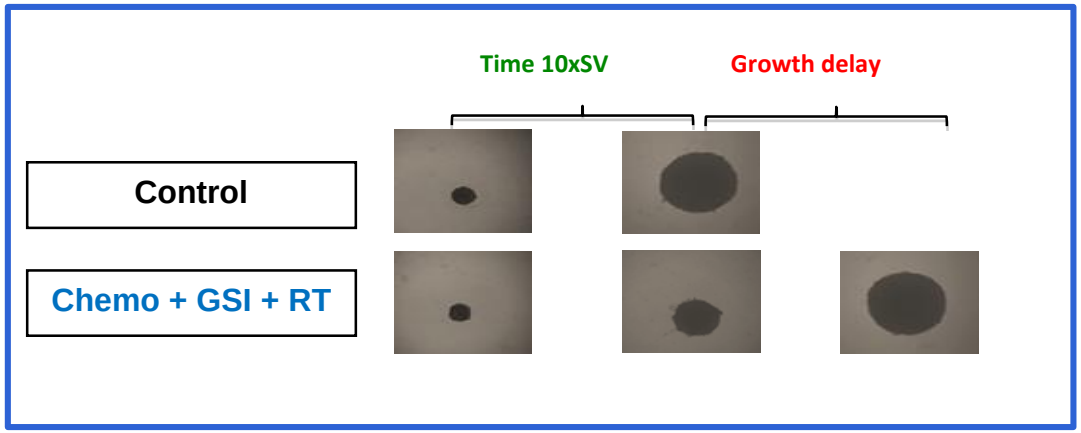
3. Make phase contrast images (3x/week, starting at d4 post-seeding)



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

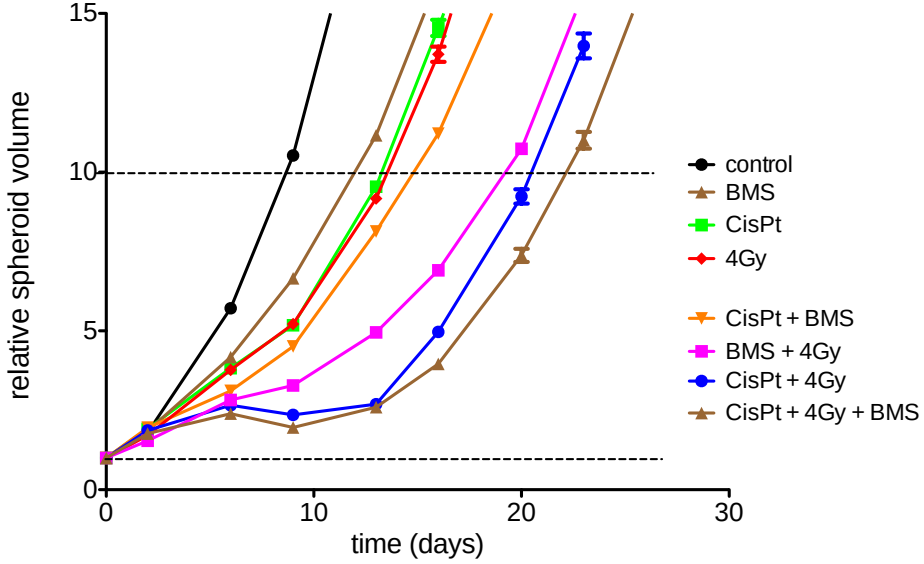
6. Automated spheroid analysis using MatLab-based software (active contour algorithm)

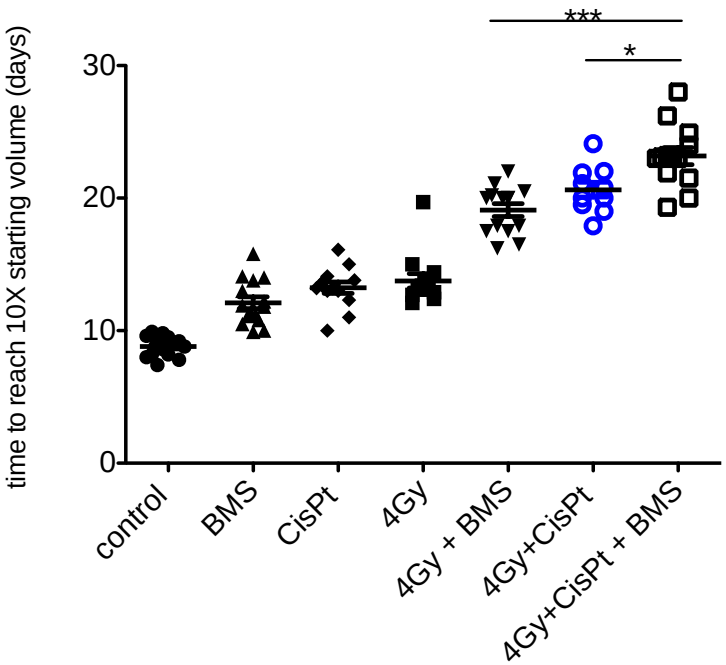
7. Use generated table with relevant morphometric information for data analysis



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

NSCLC spheroid growth delay chemotherapy+ RT+ GSI

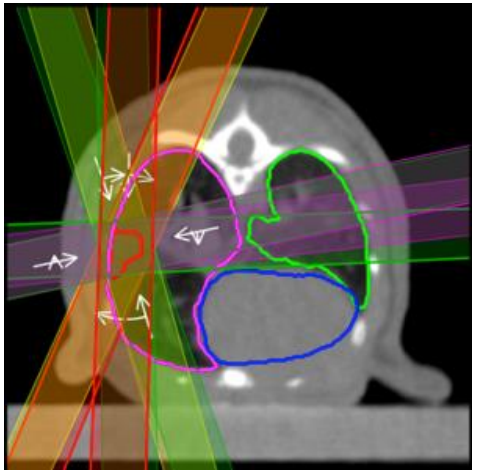




The addition of GSI in a chemoradiation standard of care treatment schedule enhances treatment efficacy.

Evaluation of combination treatments *in vivo*
A need to improve our models

Orthotopic NSCLC models in mice



Availability image-guided precision RT in lung

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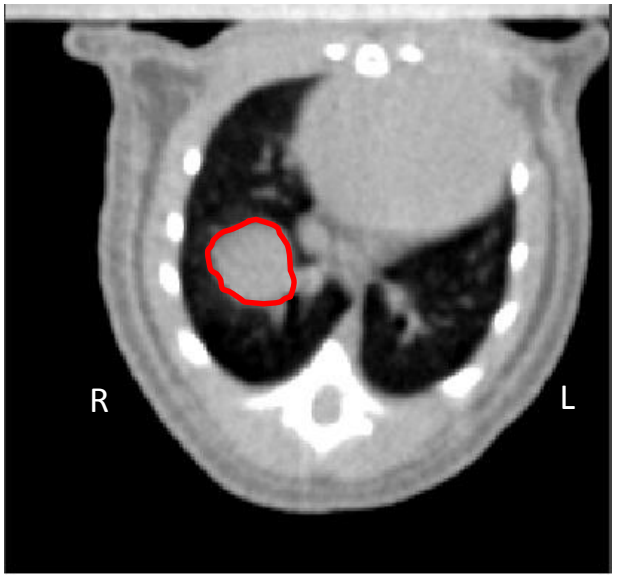
Modeling human lung cancer in mouse

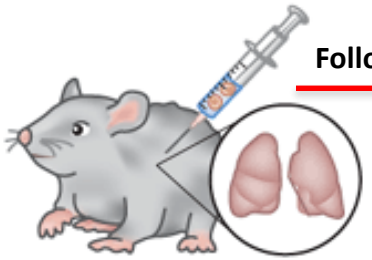
Human lung cancer

Mouse lung cancer

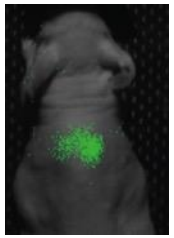


Orthotopic NSCLC model

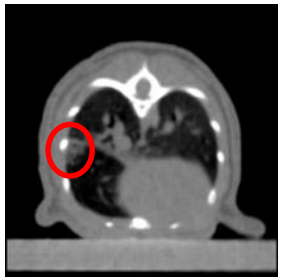
Inject NSCLC cells in lung



Follow-up tumor growth



Bioluminescence imaging (BLI) ***optimization ongoing***



Micro-CBCT

SmART

X-RAD 225Cx (Precision X-ray, North Branford, CT)

10 Gy

Treatment planning

Follow-up tumor growth 2-3x/week

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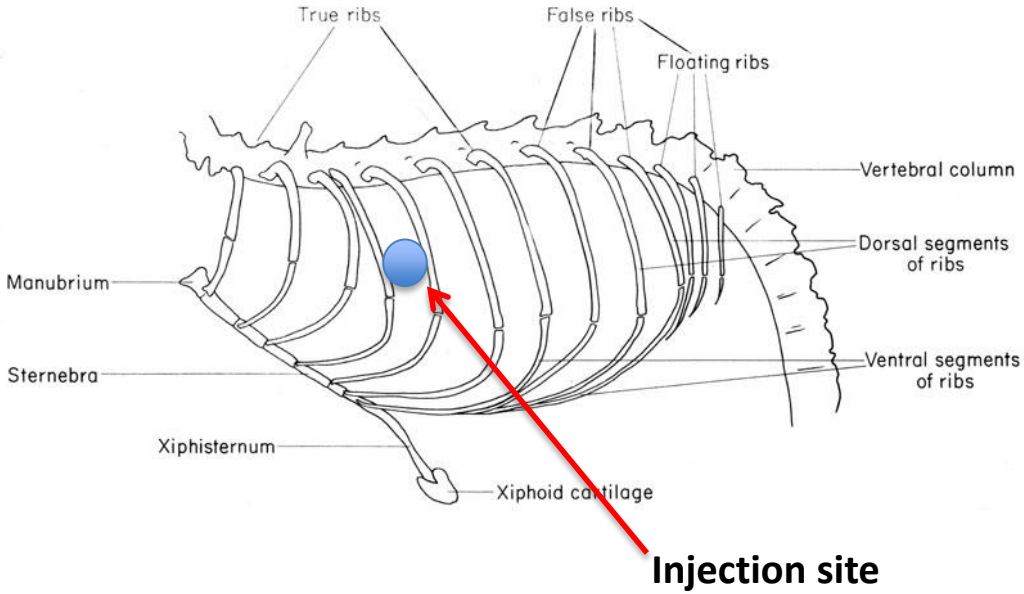
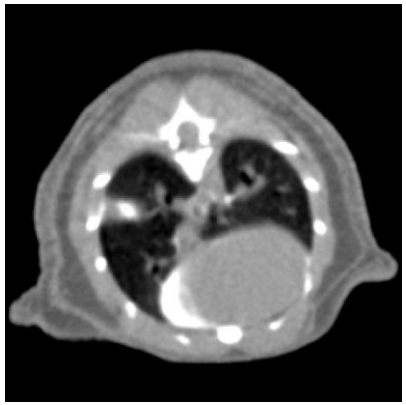
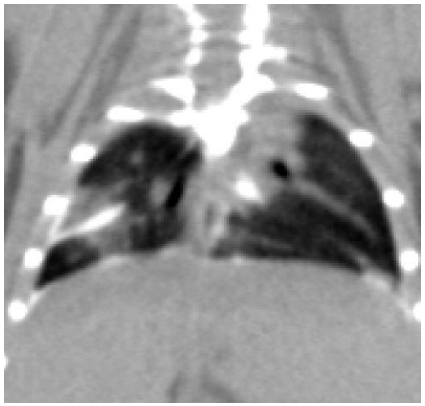
Orthotopic NSCLC model

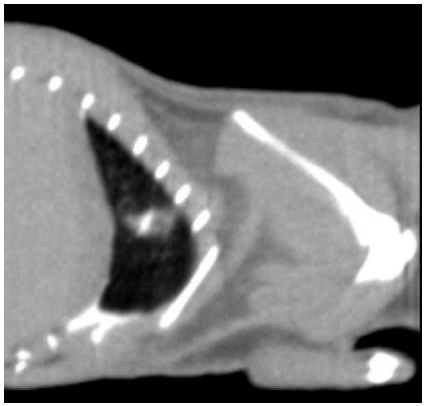




axial



coronal

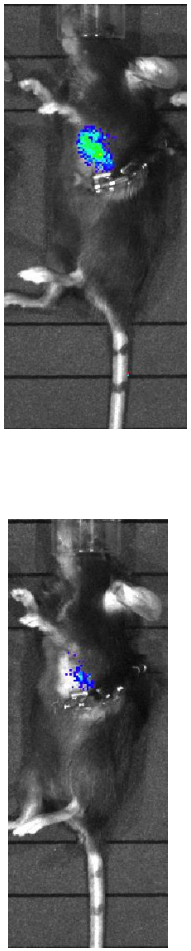


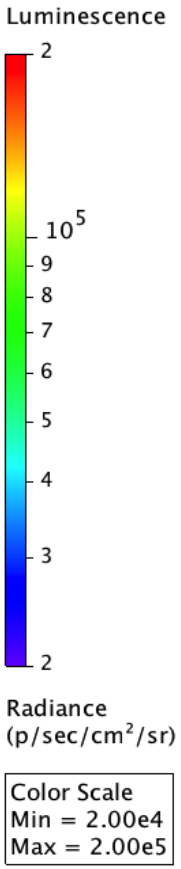
sagittal

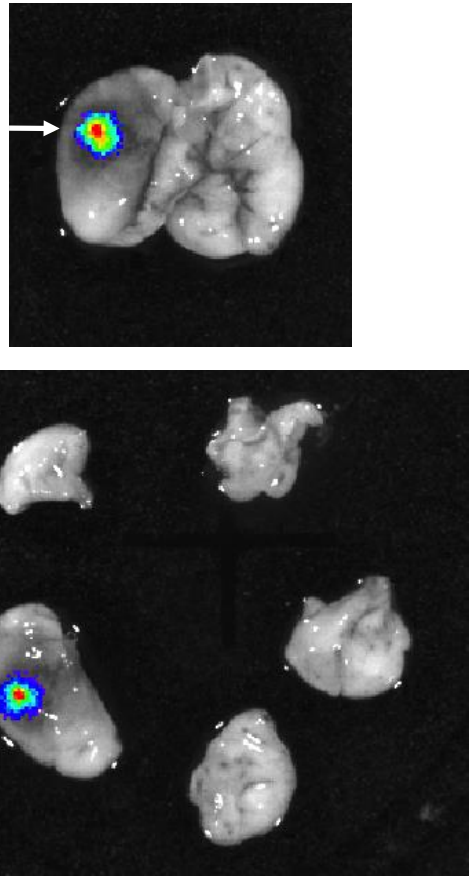
Orthotopic Lewis lung carcinoma

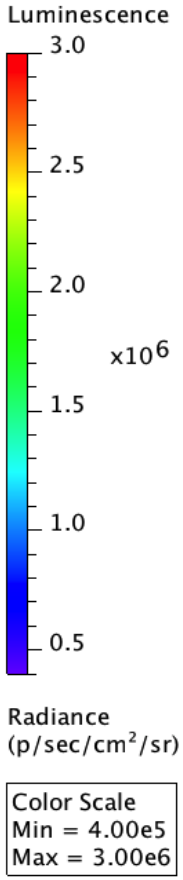
Day 0

Day 1



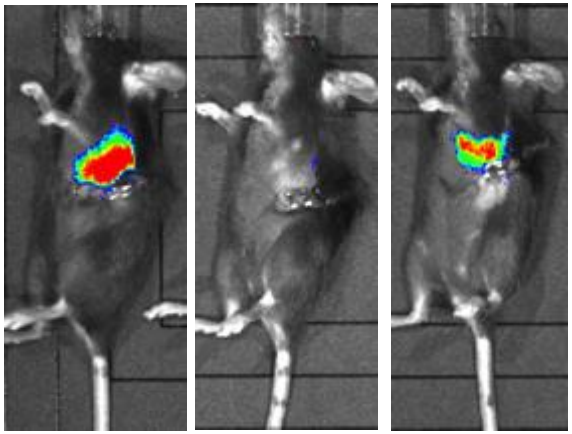




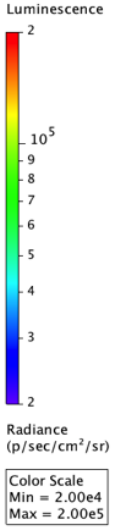


Days after tumor inoculation

1

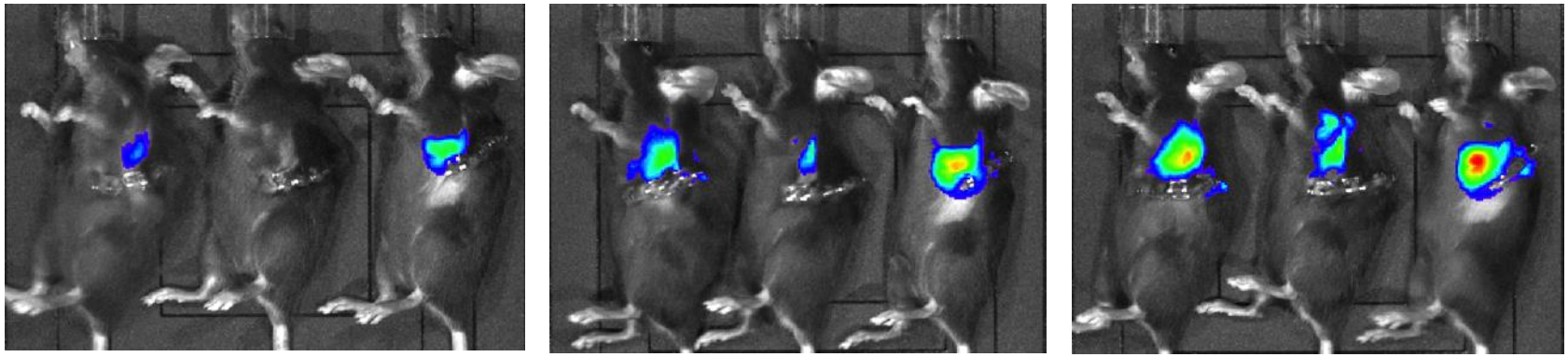


3.5e6 8.3e4 4.7e5

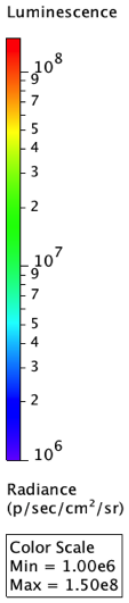


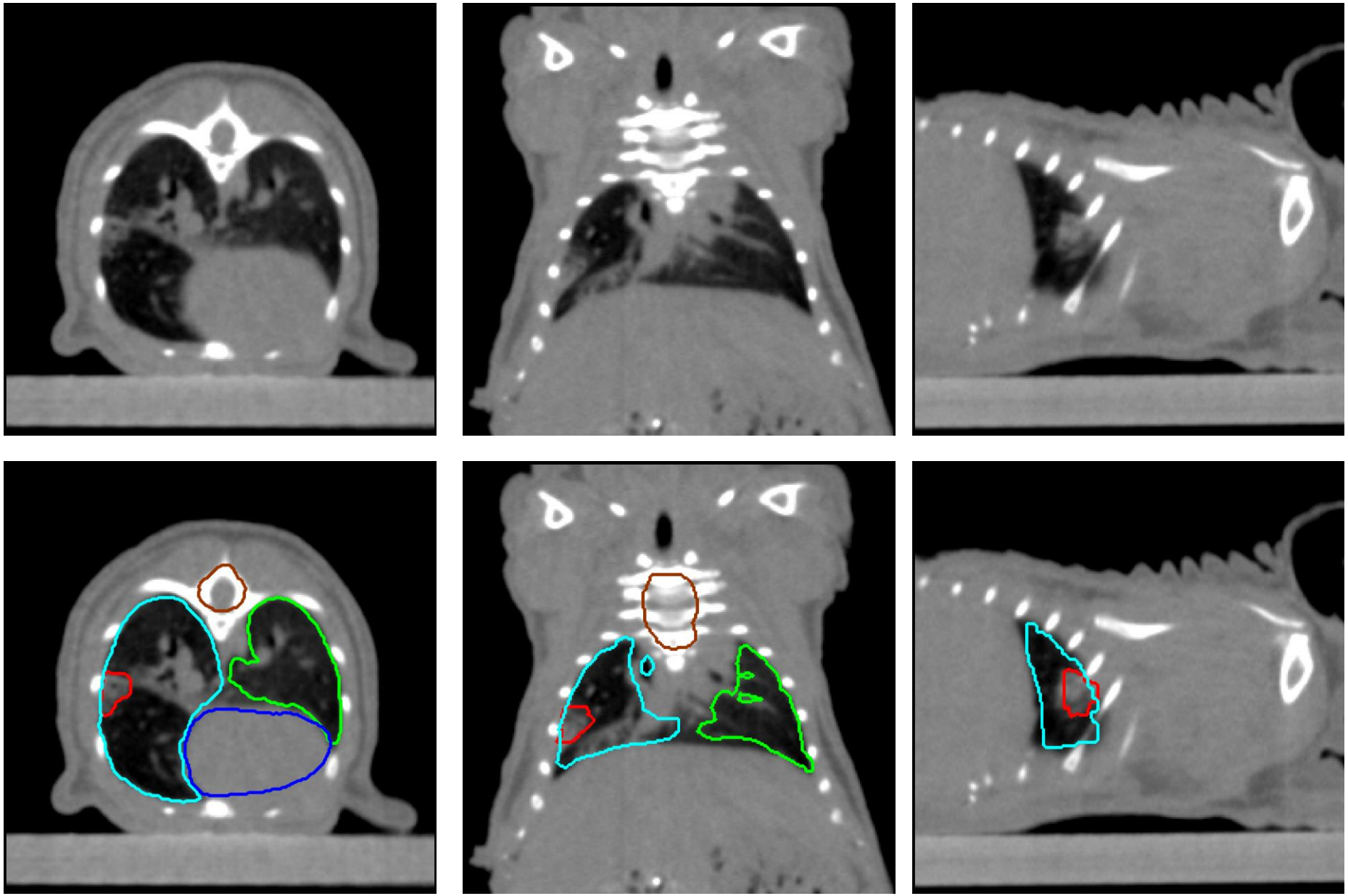
Days after tumor inoculation

6 9 14



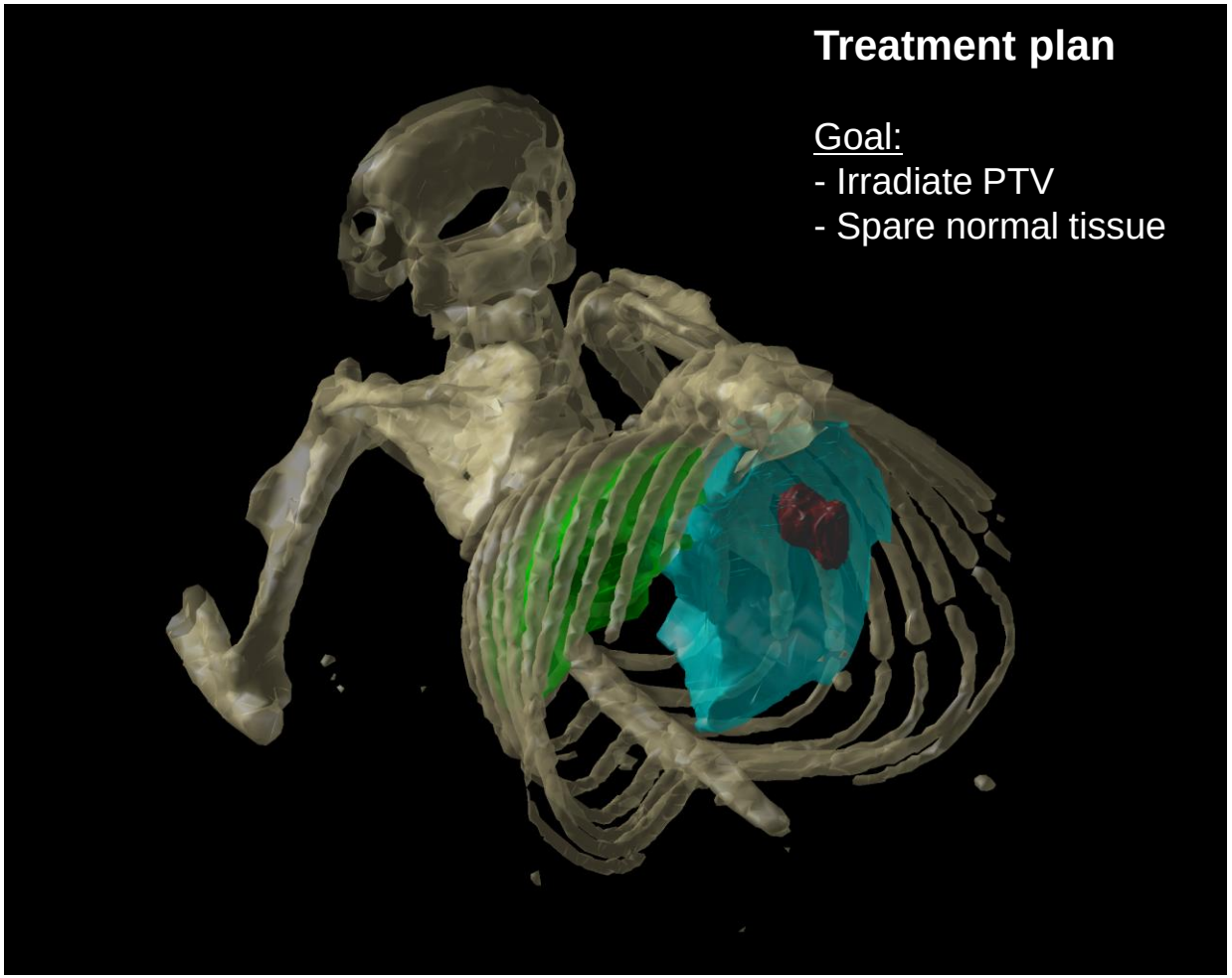
1.5e7 2.0e6 5.0e7 8.1e7 1.8e7 2.4e8 2.7e8 8.2e7 4.1e8

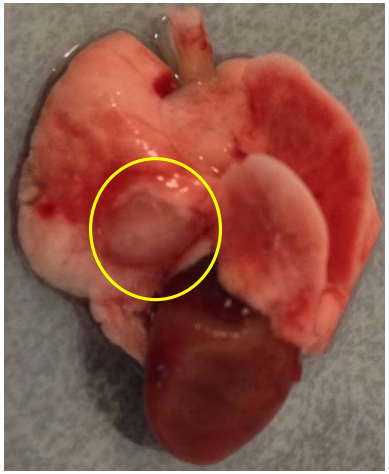




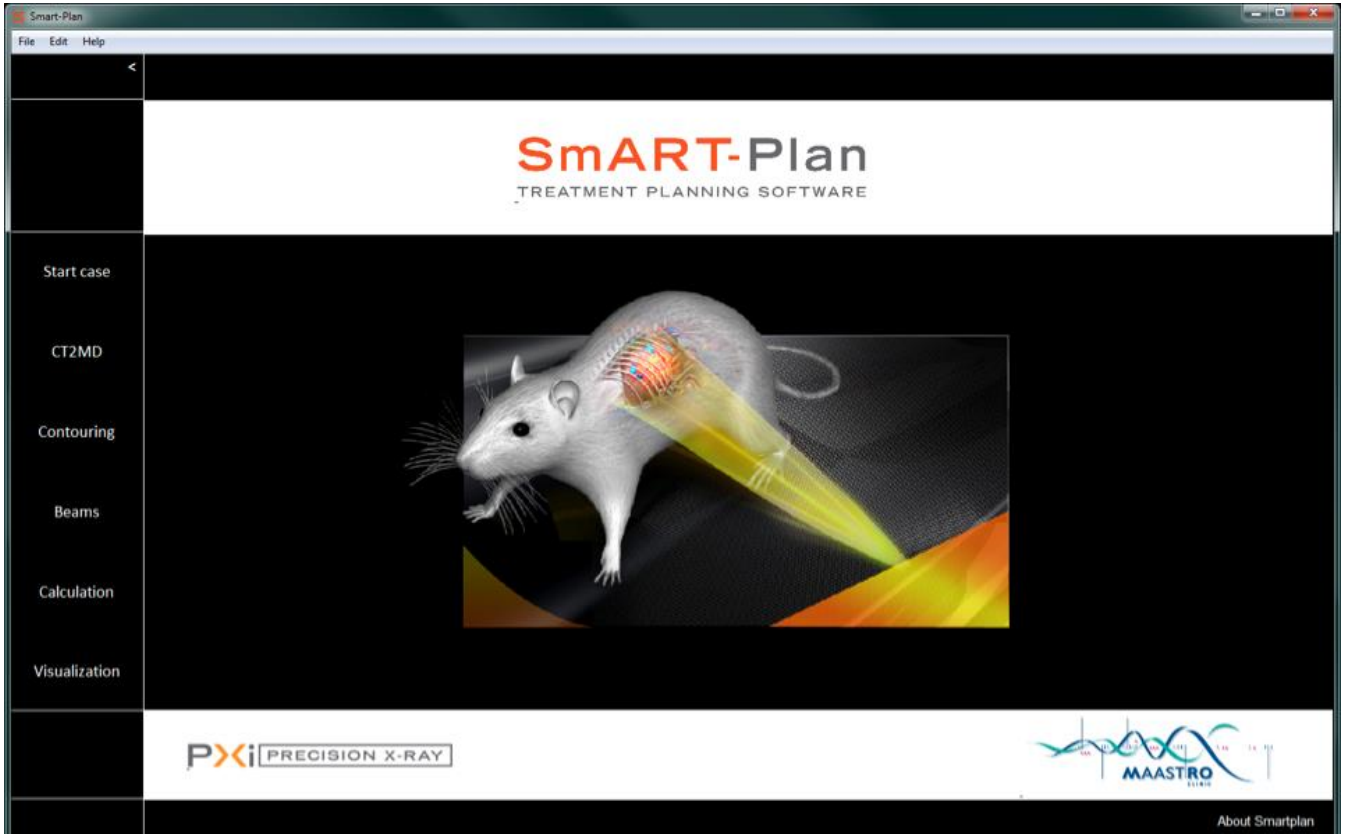
axial coronal sagittal

Orthotopic NSCLC 3D render

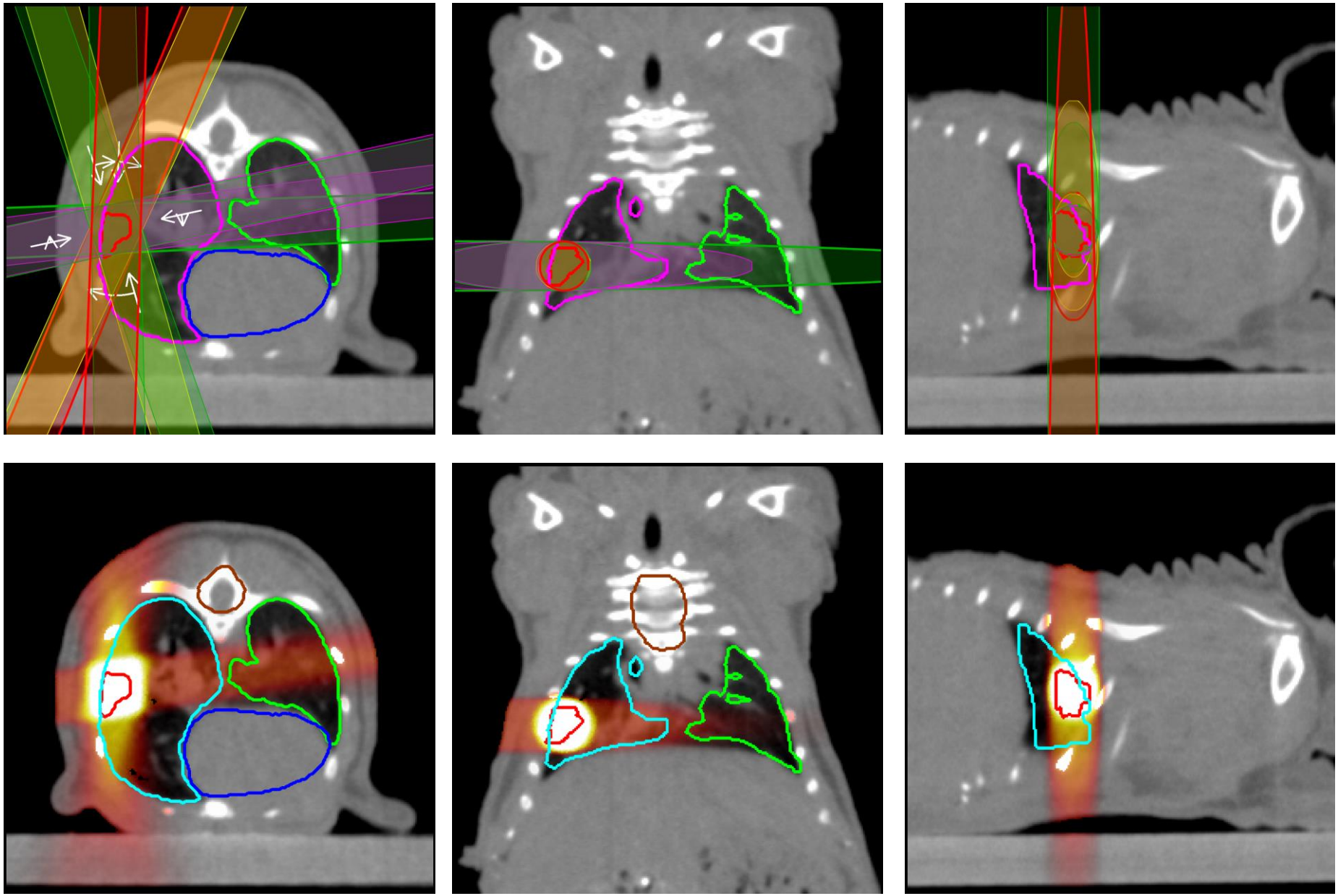




Small Animal Radiation Therapy
(SmART-Plan)

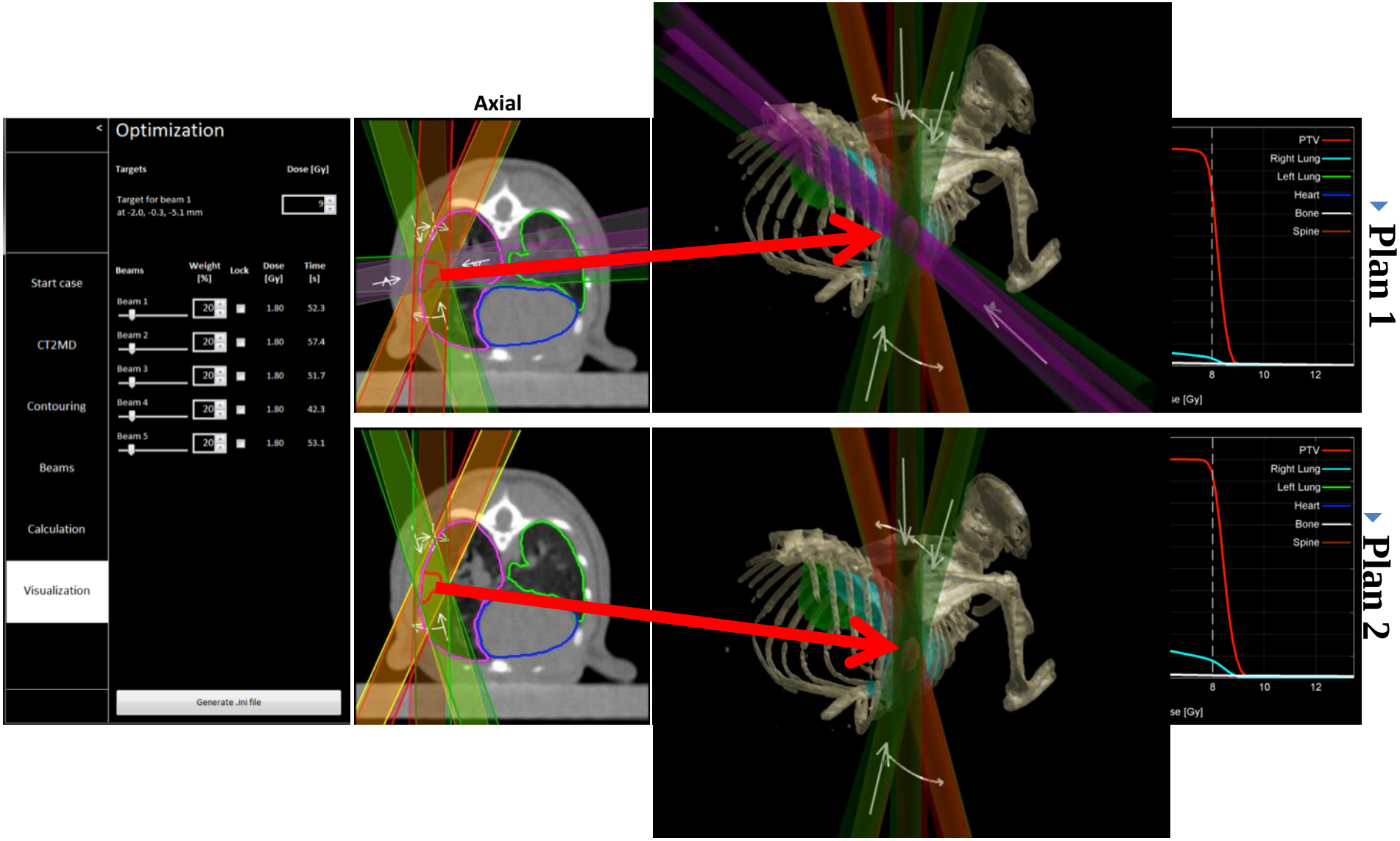


van Hoof SJ et al. Radiother Oncol 2013;109(3):361-6

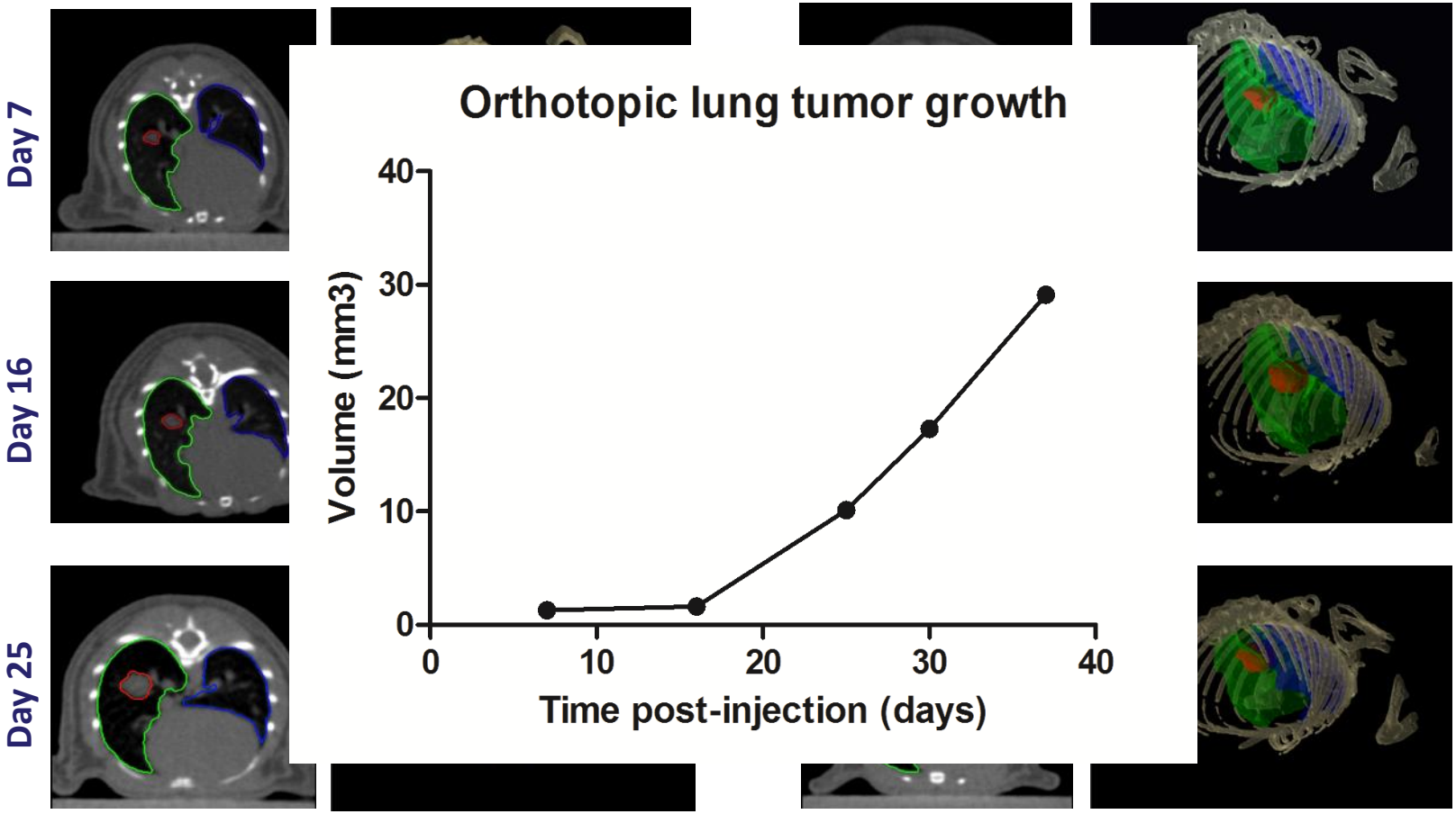


axial coronal sagittal

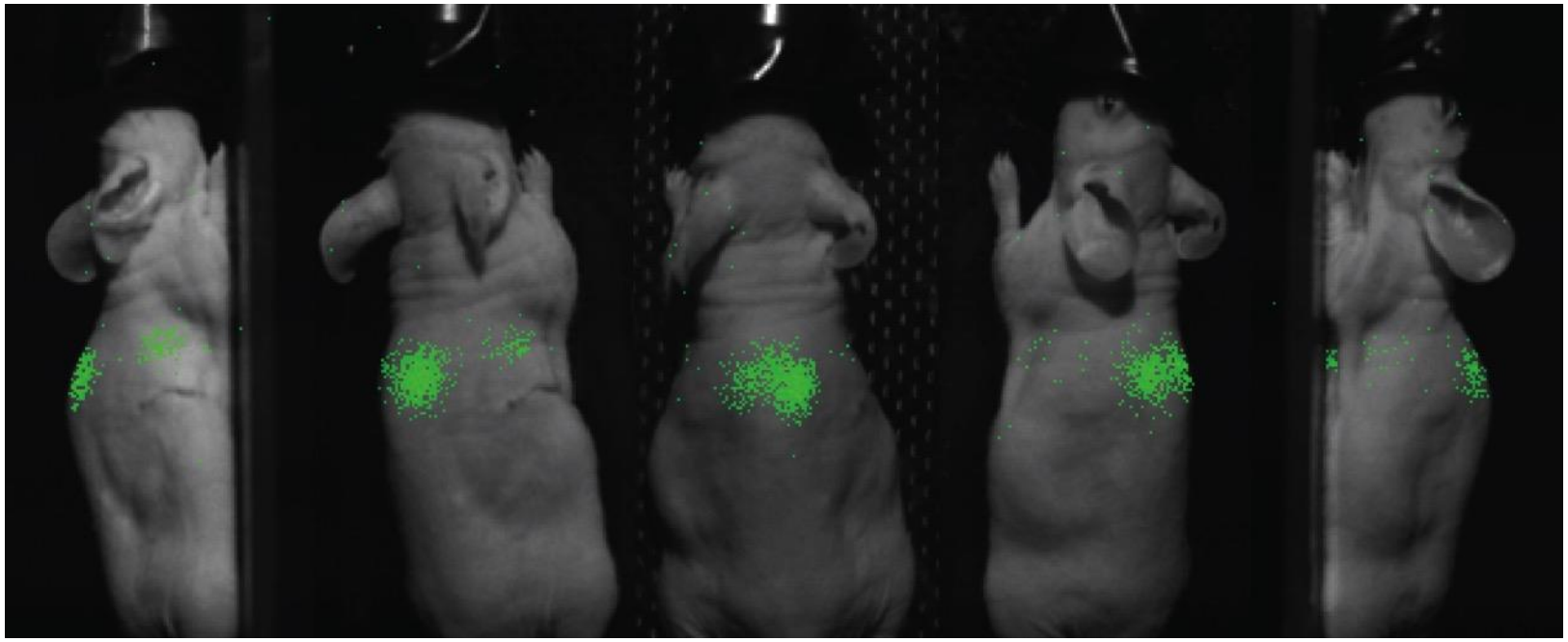
Individualized treatment planning



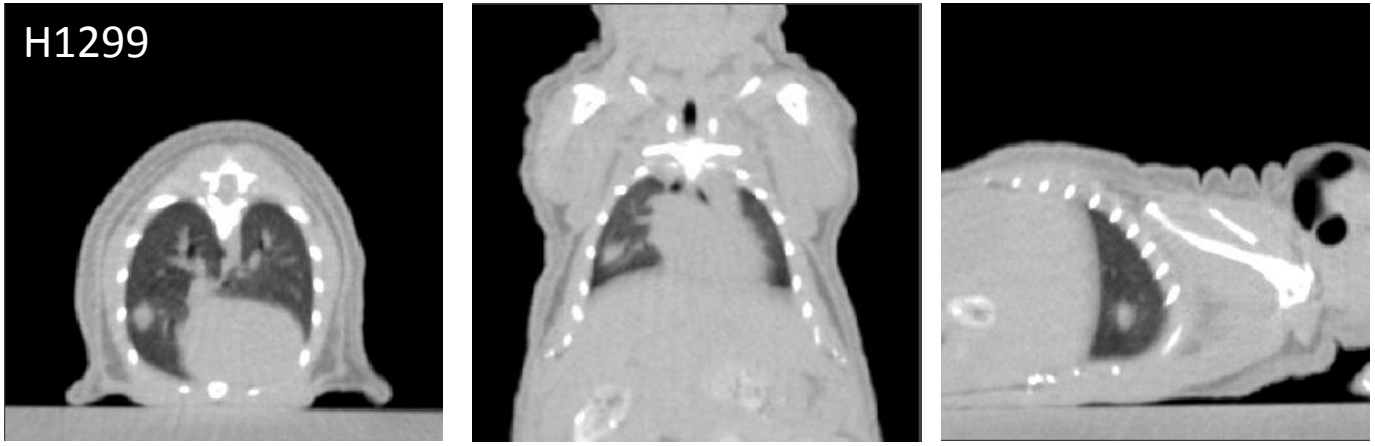
Tumor growth follow-up with micro-CBCT (SmART)



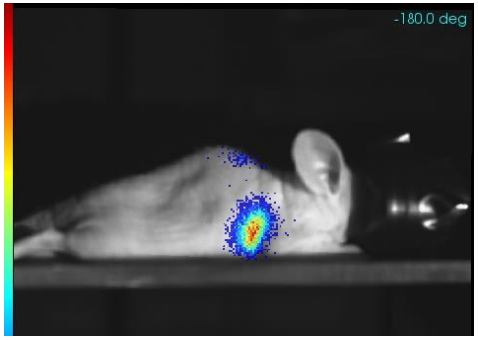
Tumor growth follow-up with bioluminescence (SmART)

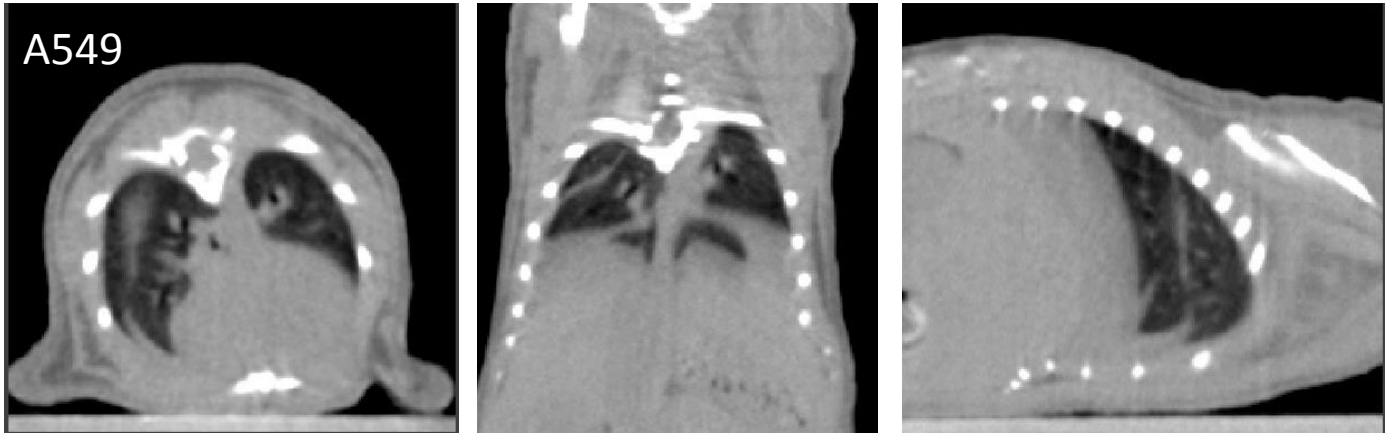


Tumor growth follow-up with bioluminescence + micro-CBCT (SmART)

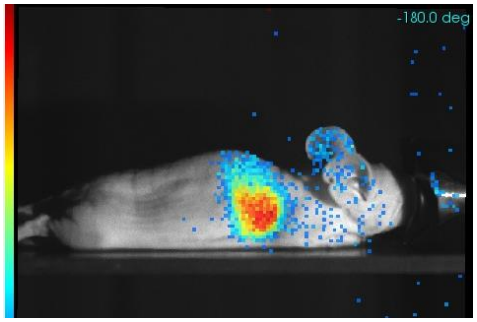


50 days p.i.

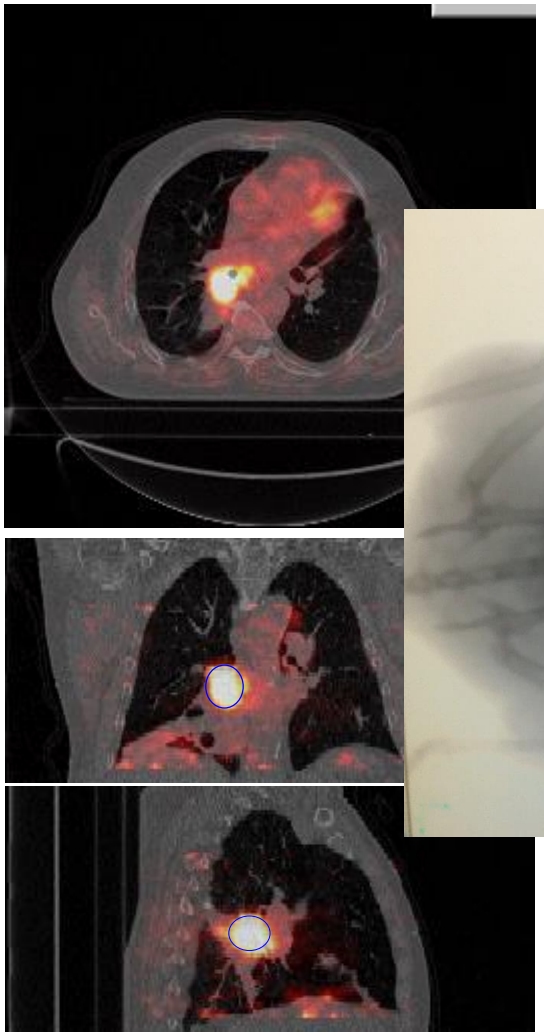




40 days p.i.



Orthotopic NSCLC
breathing/bowel movement



4D CT imaging

eathing and bowel movement
eates underdosed tumor areas
ormal tissue areas.

on of the margin (5 mm)
leads to a 30 % reduction in volume!!

Verellen D et al. Nature Rev Cancer 2007

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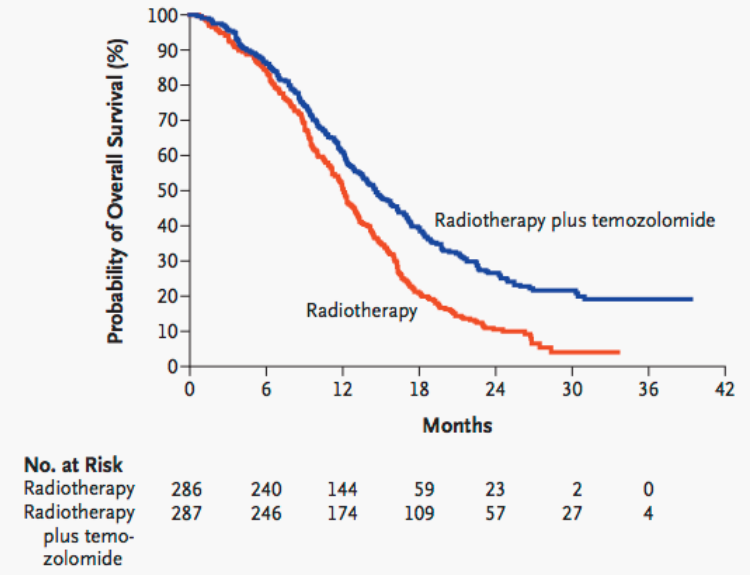
Glioblastoma Multiforme (GBM)

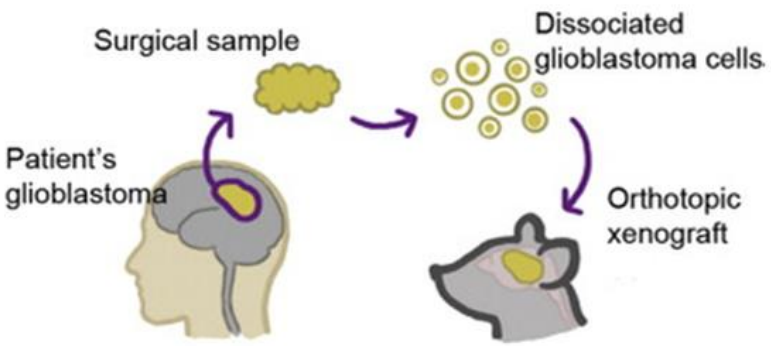


Most common and aggressive brain tumor (grade IV astrocytoma)
No curative treatment
First line treatment Surgery, Radiotherapy + Chemotherapy (temozolomide)
MGMT methylation predictive for temozolomide response
Median survival ~15 months after initial diagnosis

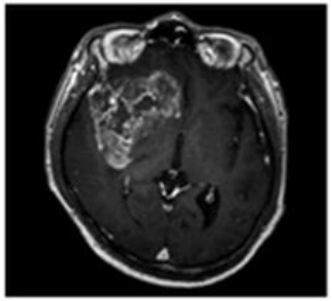
Need for new treatments/ targets

Stupp NEJM 2005

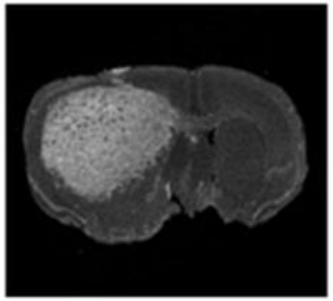
Orthotopic glioblastoma model



Patient's glioblastoma

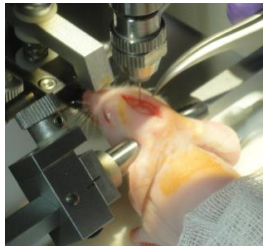


Mouse glioblastoma



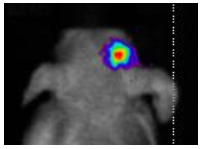
Orthotopic glioblastoma model: workflow

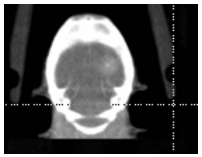
Implant
Glioblastoma cells



Follow-up tumor growth

Bioluminescence imaging(BLI)

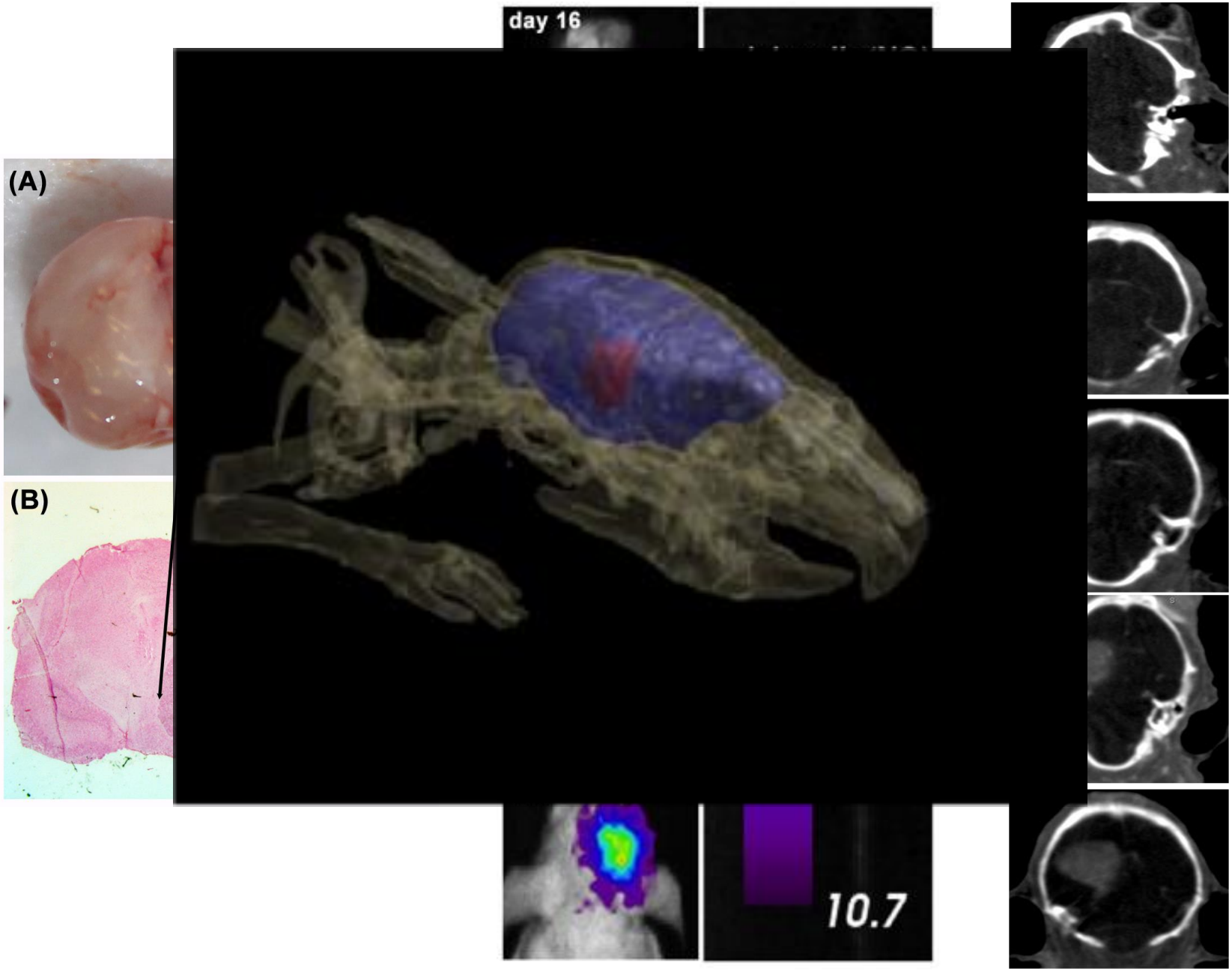




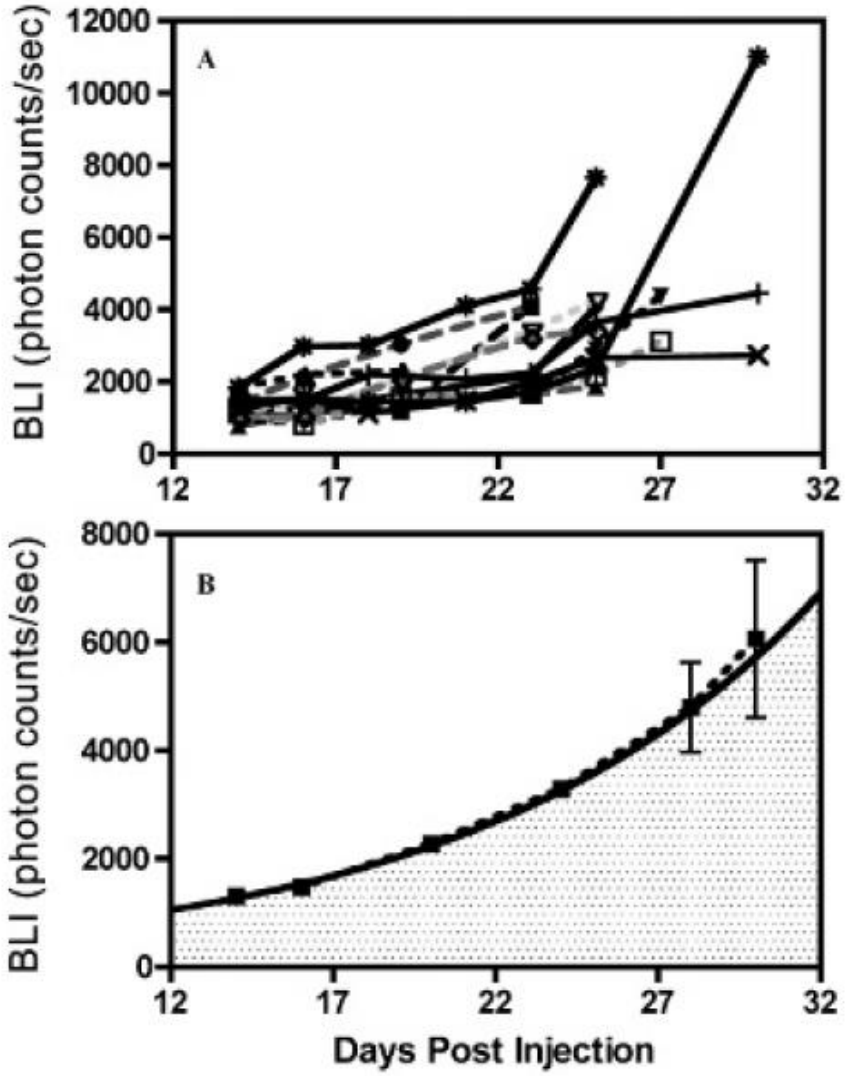
Contrast-enhanced micro-CT

Treatment
planning

Follow-up tumor growth
3x/week

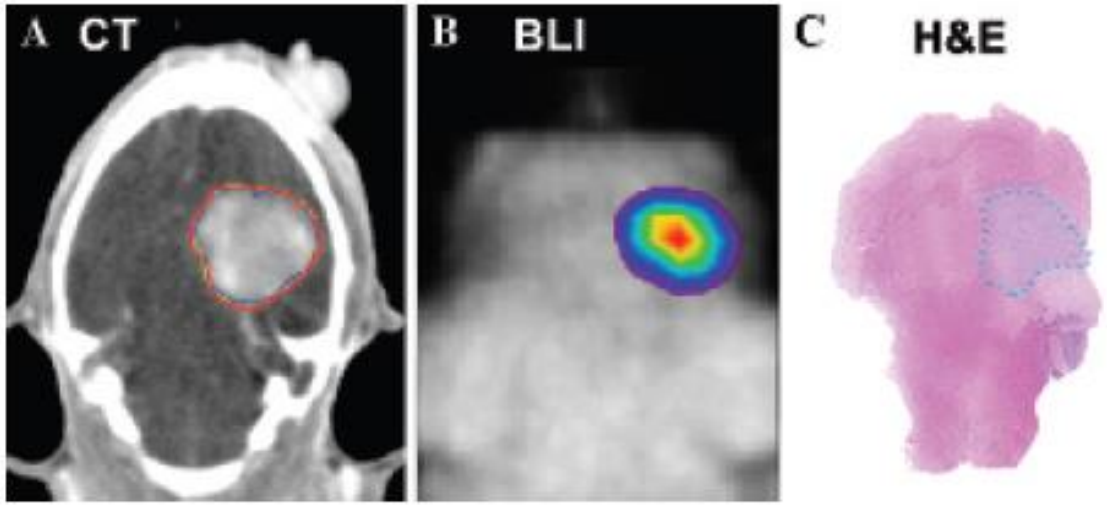

(A)
(B)
day 16
10.7

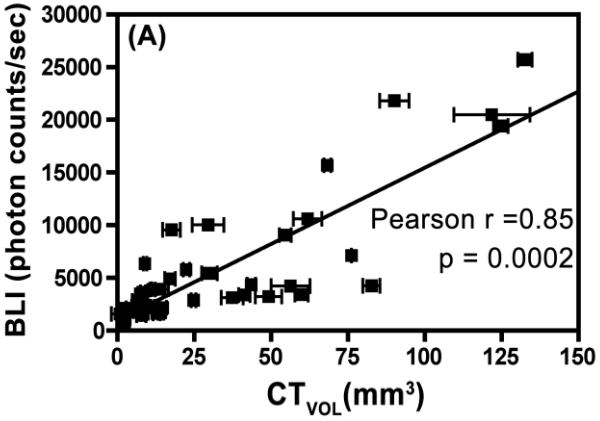
Follow-up intracranial tumor growth:
Bioluminescence quantification

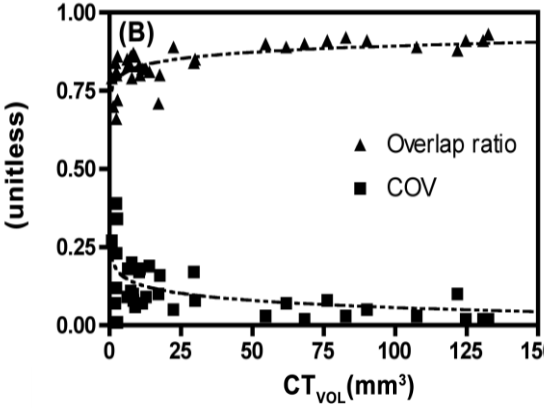


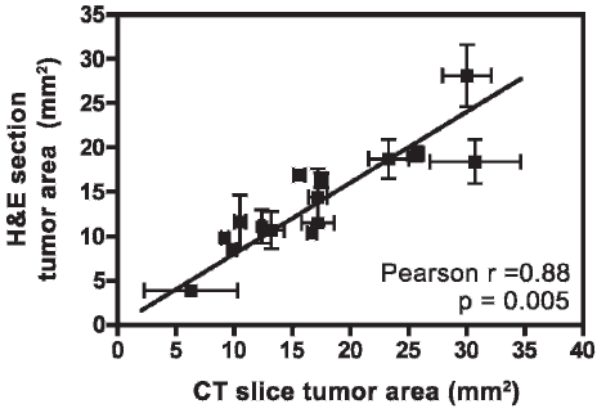
Yahyanejad et al., Mol Imaging 2014.

Correlation BLI and CT: towards multimodal imaging



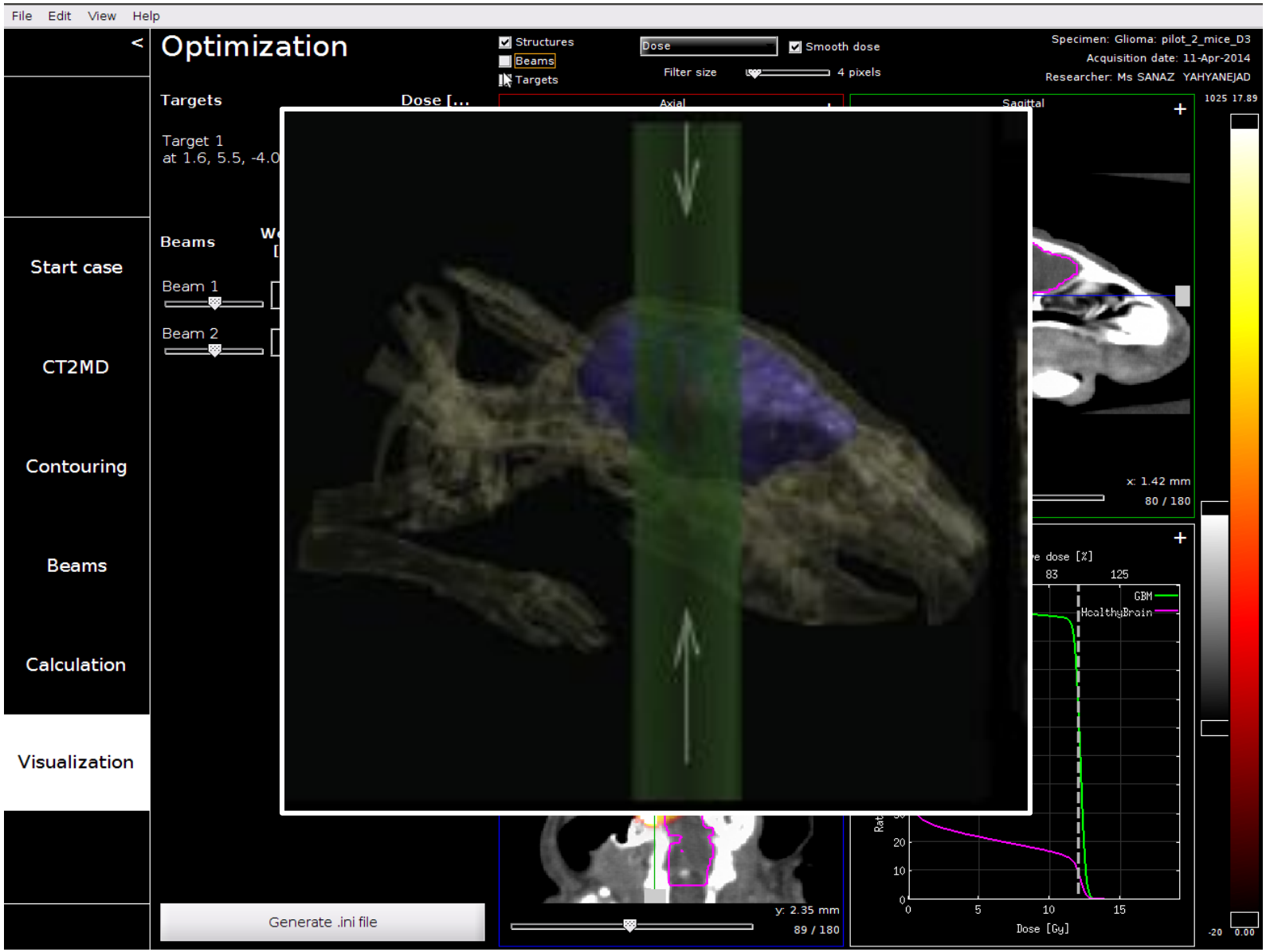




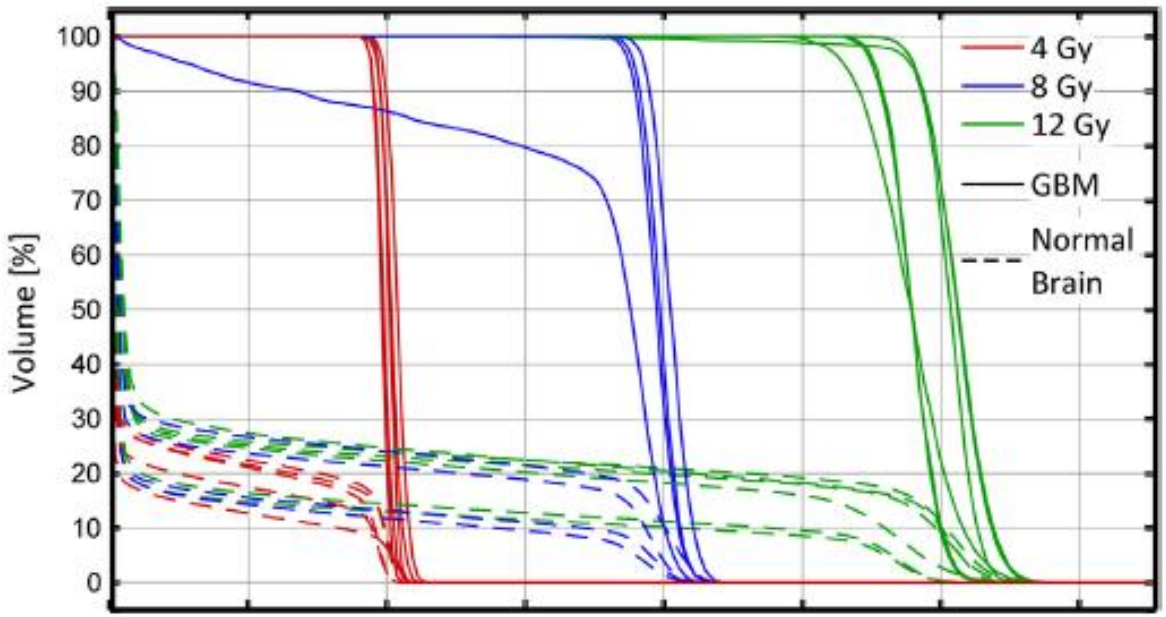


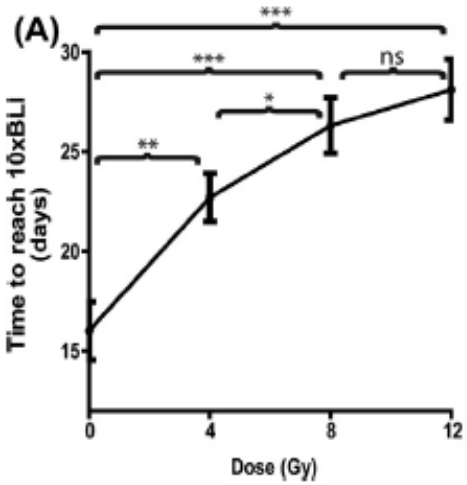
Yahyanejad et al., Mol Imaging 2014.

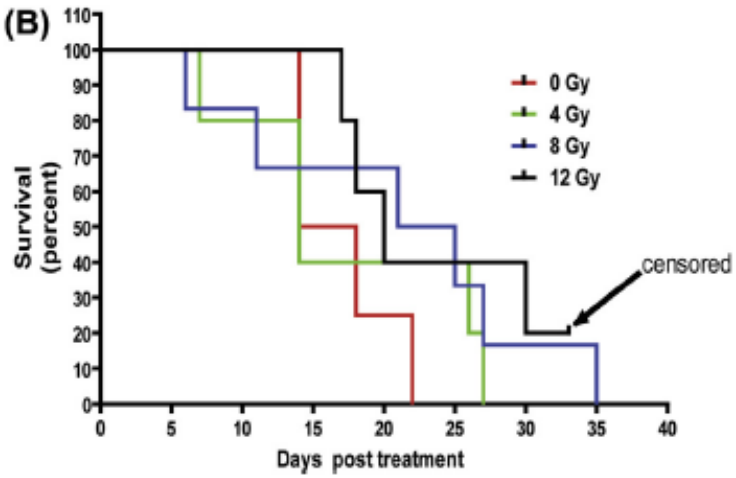
Radiation treatment set-up: SmART Plan



Radiation treatment: DVH and tumor response

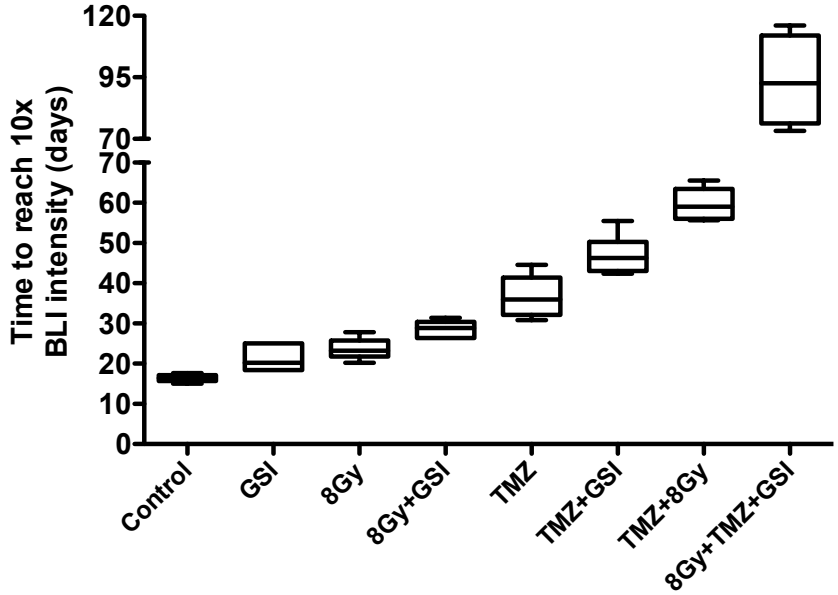


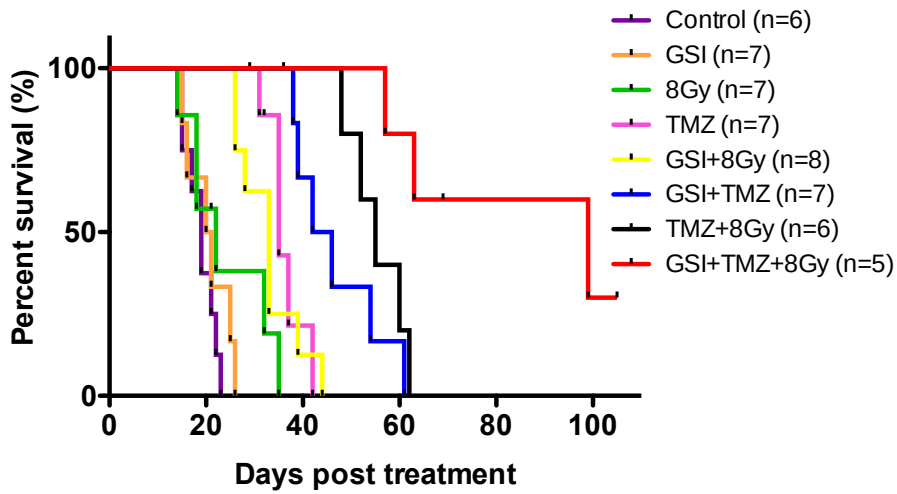




Yahyanejad, Van Hoof, Theys et al., R&O, 2015.

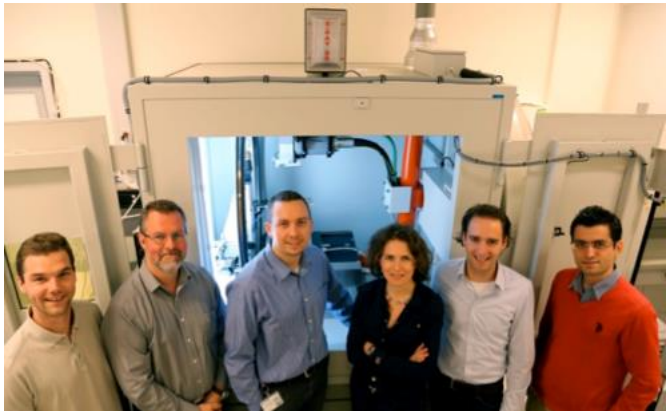
Combination treatment: RT, TMZ and GSI





Yahyanejad et al., in revision.

Acknowledgements



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