Einfluss der Antiepileptika : Phenytoin, Levetiracetam, Lamotrigin, Phenobarbital, Carbamazepin und Chemotherapeutika: Temozolomid, Lomustin auf die Akkumulation von Protoporphyrin IX in Zellen maligner Gliome.

Synthes Award 2007

10 Steps to a more radical Tumor Removal in GBM Patients

A in vitro study of interactions and apoptosis in the presence of 5ALA

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PIX Fluorescence in malignant Glioma surgery

- Diagnostic

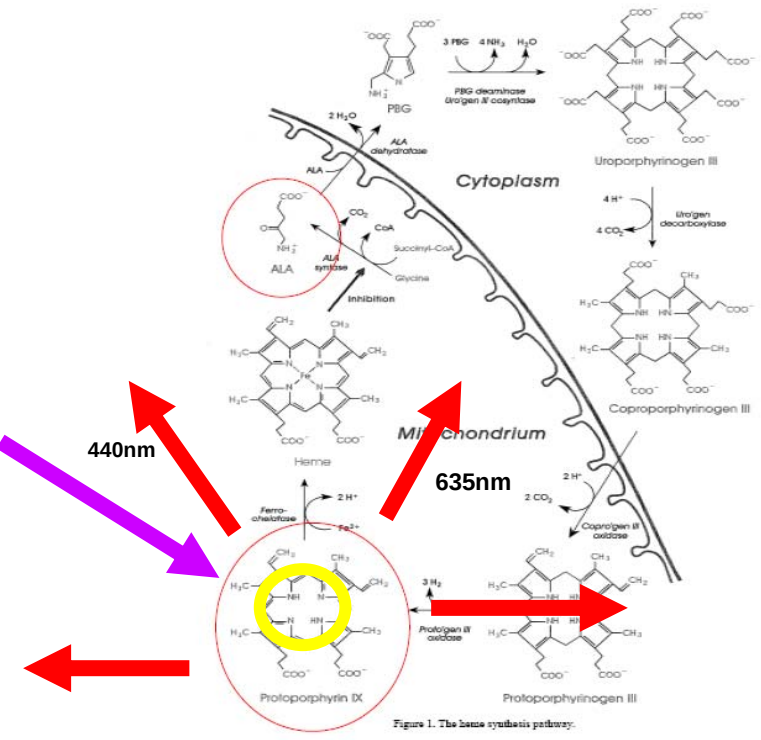
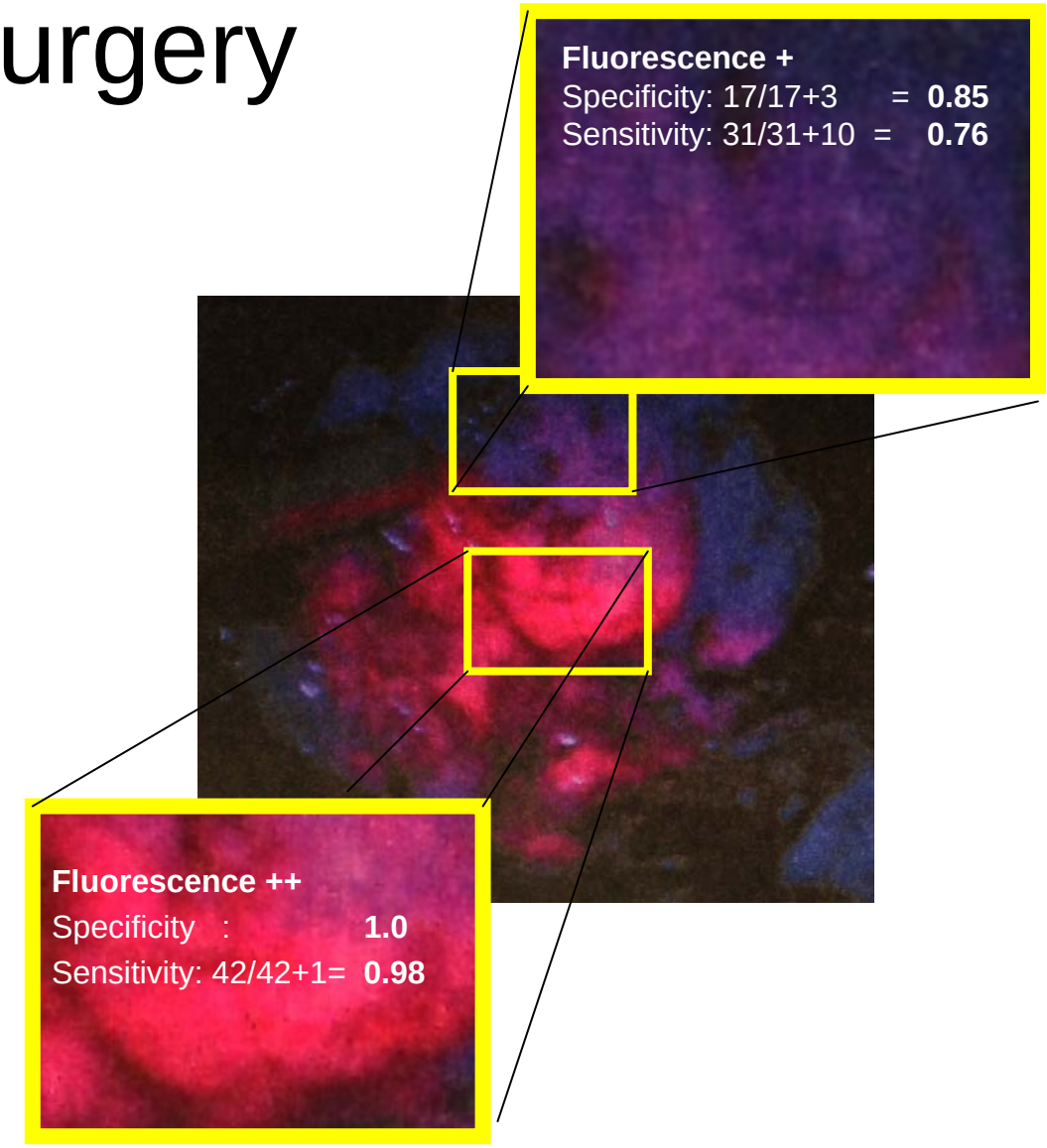


Figure 1. The heme synthesis pathway.



PIX Fluorescence in malignant Glioma surgery

Therapy:

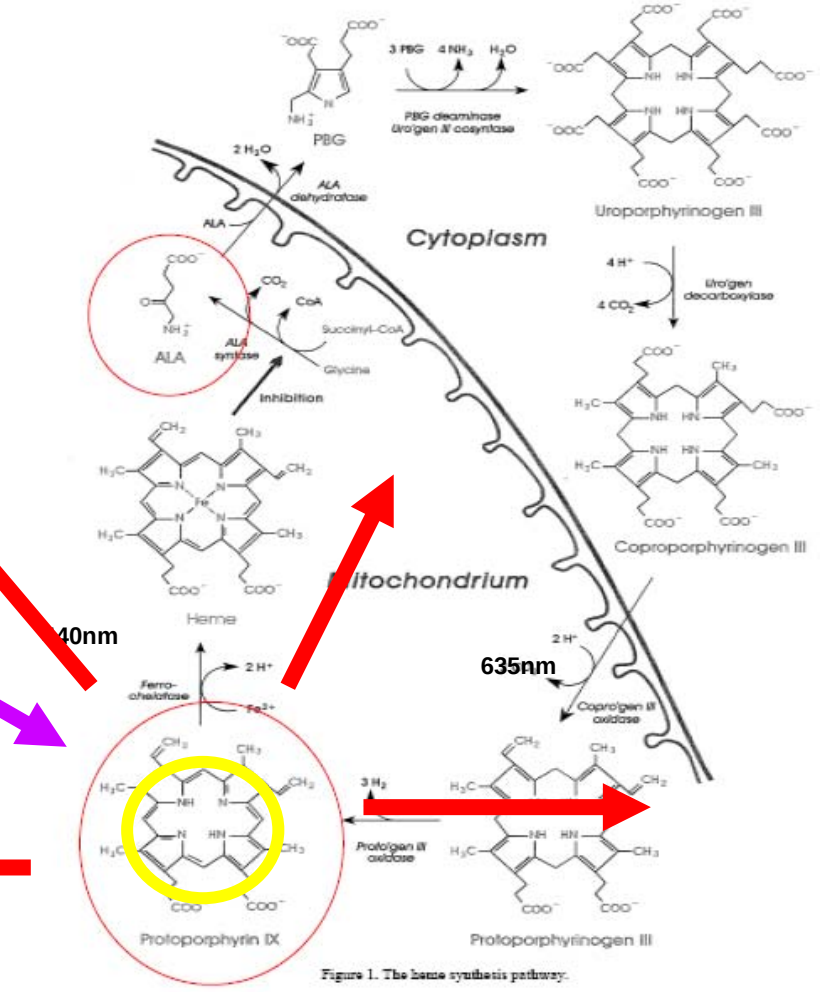
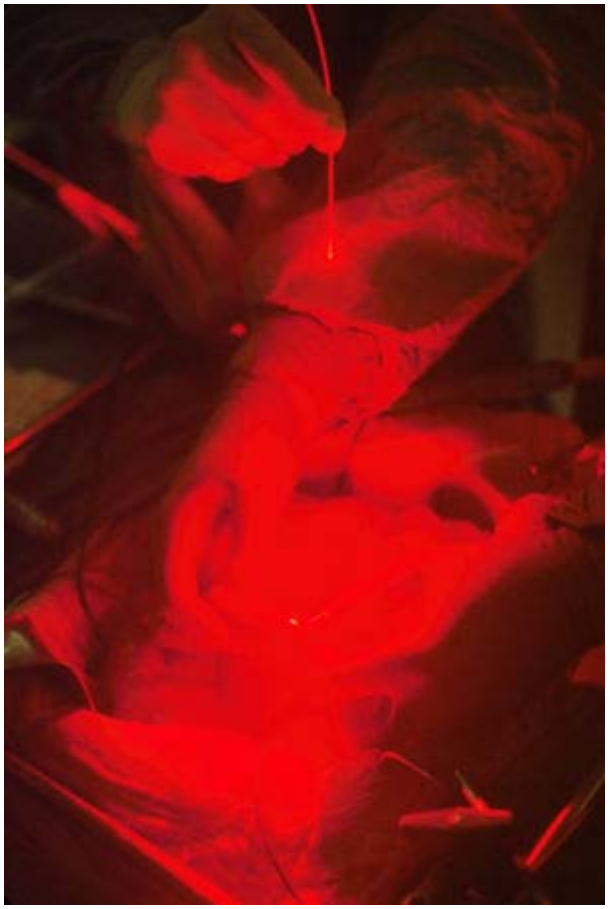


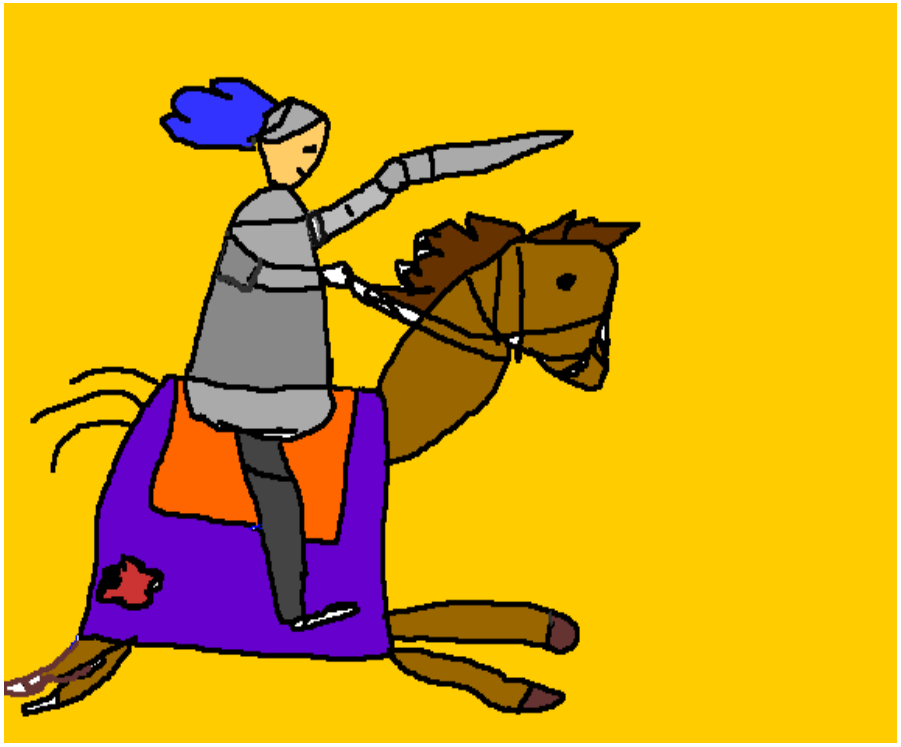
Figure 1. The heme synthesis pathway.



The Quest

- Do commonly adminstered antiepileptic and chemotherapeutic drugs interact with PIX accumulation of in GBM?

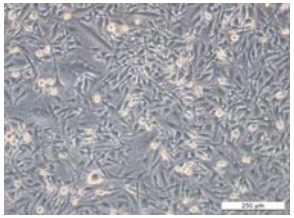
- What is the optimal light source an exposure time for a selective therapy in PDT with GBM?



GBM cell lines

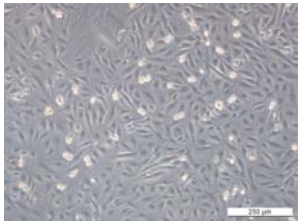
- U373 MG

  well known specifications



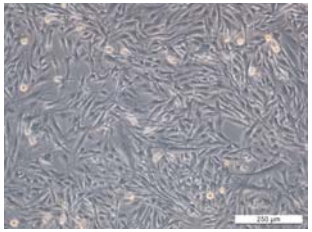
- U251 MG

  well known specifications



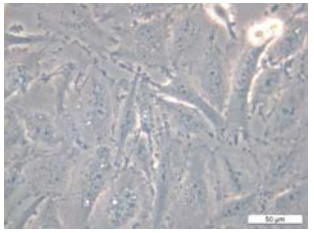
- SNB 19

  laminin slows growth and motility of cells



- U87 MG

  wild p53 less prone to apoptosis



Drugs

- Phenytoin Phenhydan ®
- Levetiracetam Keppra ®
- Lamotrigin Lamictal ®
- Phenobarbital Luminal ®
- Carbamazepin Tegretol ®
- Temozolomid Temodal ®
- Lomustin Cecenu ®

Preliminary study
Step 1-5

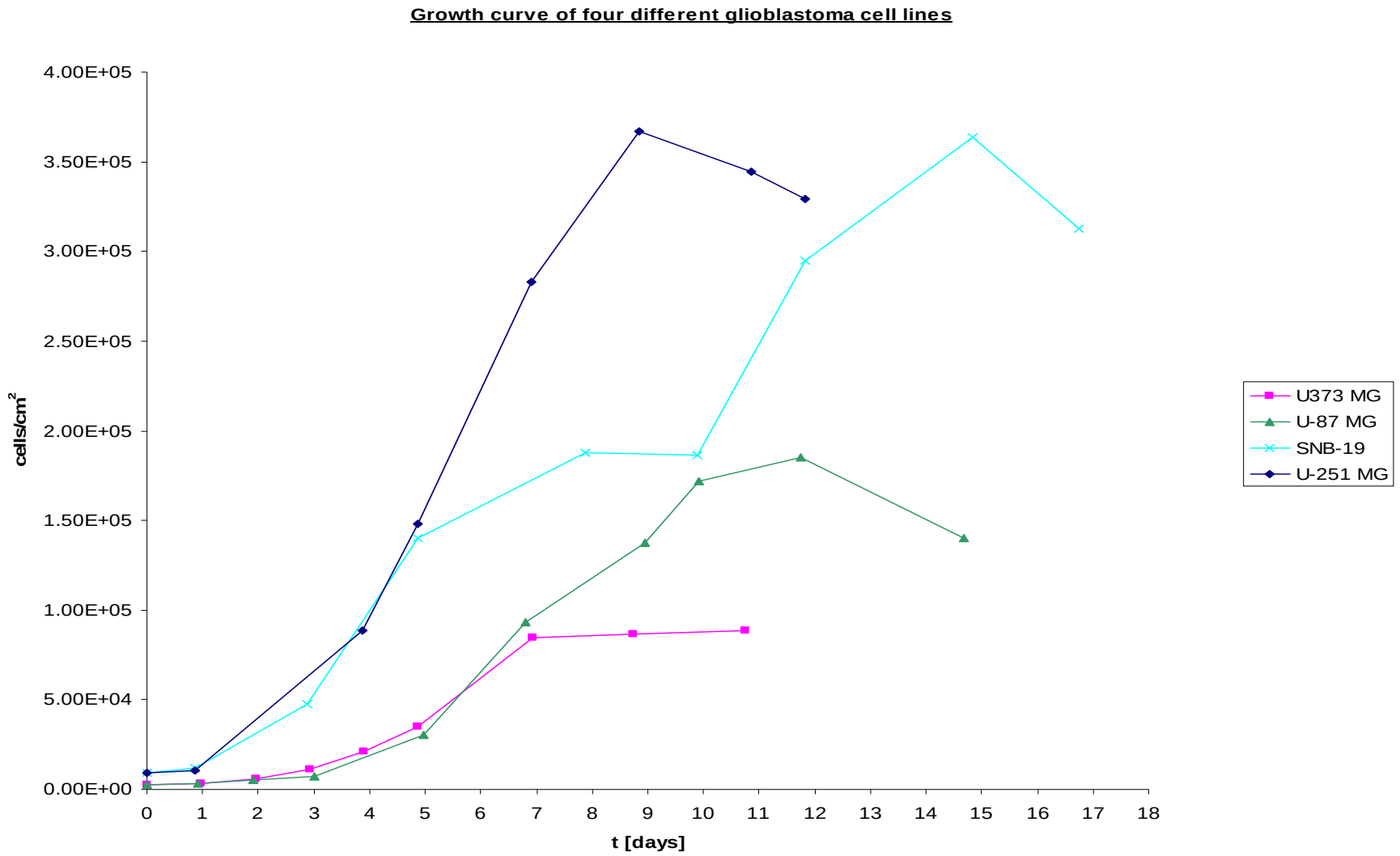
1. Do all GBM cell lines accumulate PIX in the presence of 5ALA?

2. What ist the $[PIX]_{max}$ per individual GBM cell

3. What is the $[PIX]_{max}$ corresponding fluorescence intensity per cell?

4. Cell lines with no visibly descernable fluorescence (eg. Medulloblastoma), what is their $[PIX]_{max}$ and corresponding fluorescence intensity?

5. GBM and Medulloblastoma in co culture, how accurate can we define the borderline by fluorescence



Lugano 27-10-2007

Main Study
Step 6 and 7
drug related interaction

- Step 6: Dose response analysis, Culture vs. Drug; MTT Assay

1. Drug related mitochondrial inactivity

- Step 7: Dose response analysis, Culture, Drug, 5ALA; MTT and FL

1. 5ALA/ drug MTT – drug MTT
2. 5ALA related mitochondrial inactivity
3. Drug related fluorescence intensity

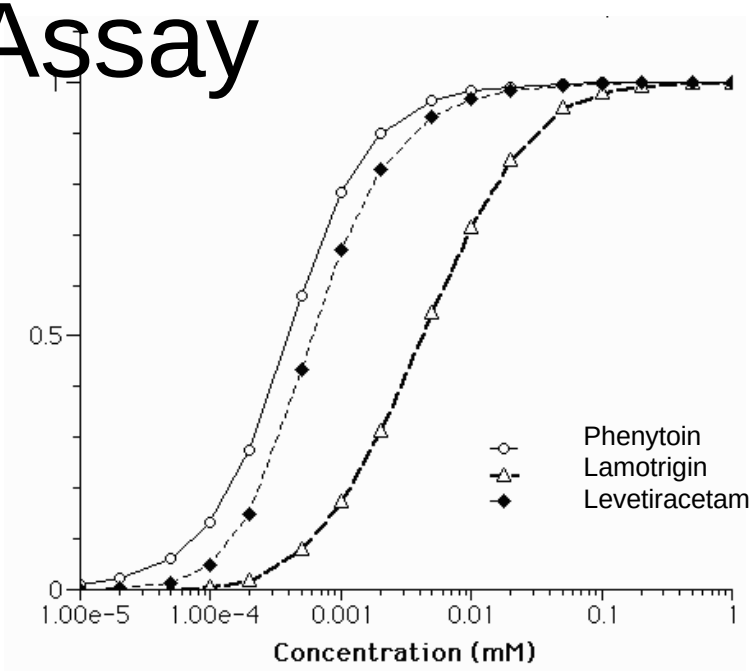
Dose Response MTT Assay

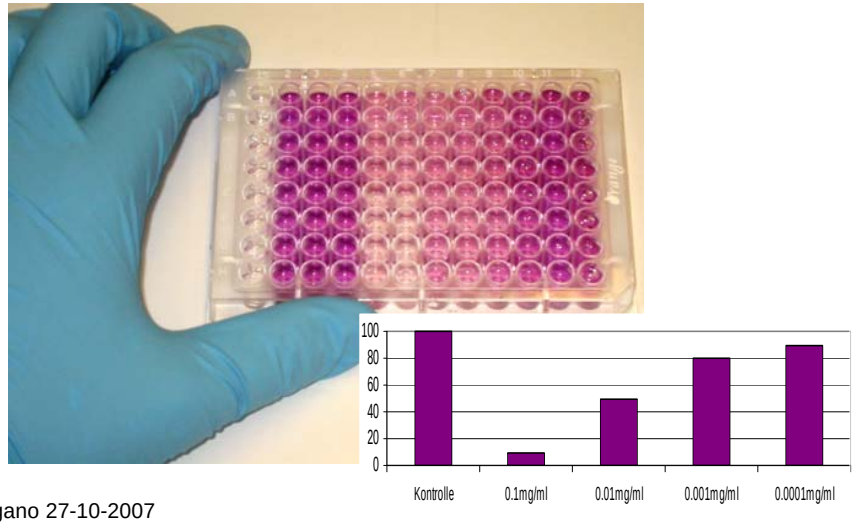
- U373 MG
- U251MG
- SNB19
- U87 MG

to

- Phenytoin, Levetiracetam, Lamotrigin, Phenobarbital, Carbamazepin and Temozolomide, Lomustin

each

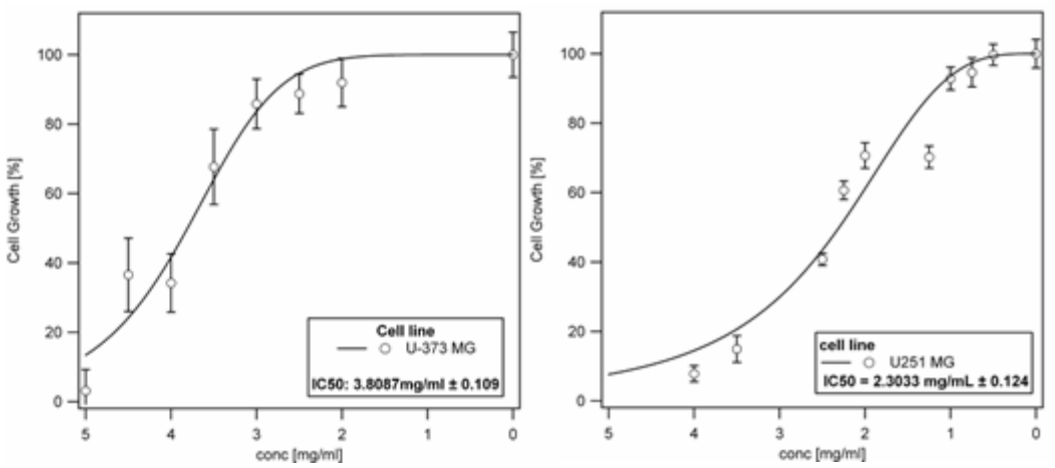




Dose Response
MTT and FL Assay

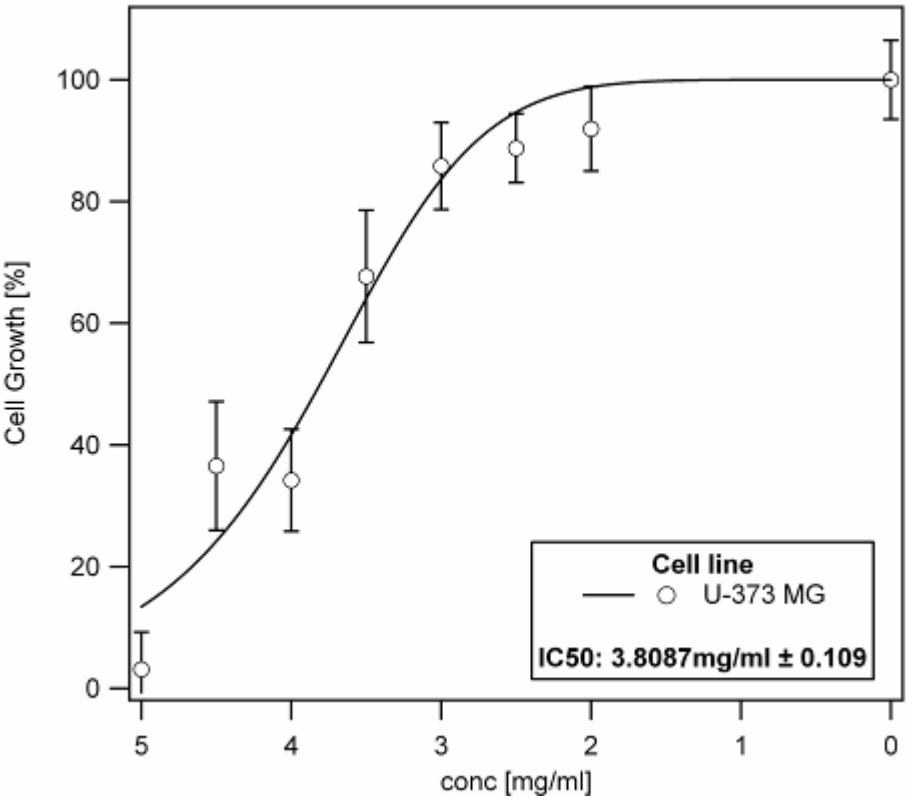
- U373 MG
- U251MG
- SNB19
- U87 MG
- to
- Phenytoin, Levetiracetam, Lamotrigin, Phenobarbital, Carbamazepin and Temozolomide, Lomustin
- each and
- 5 ALA

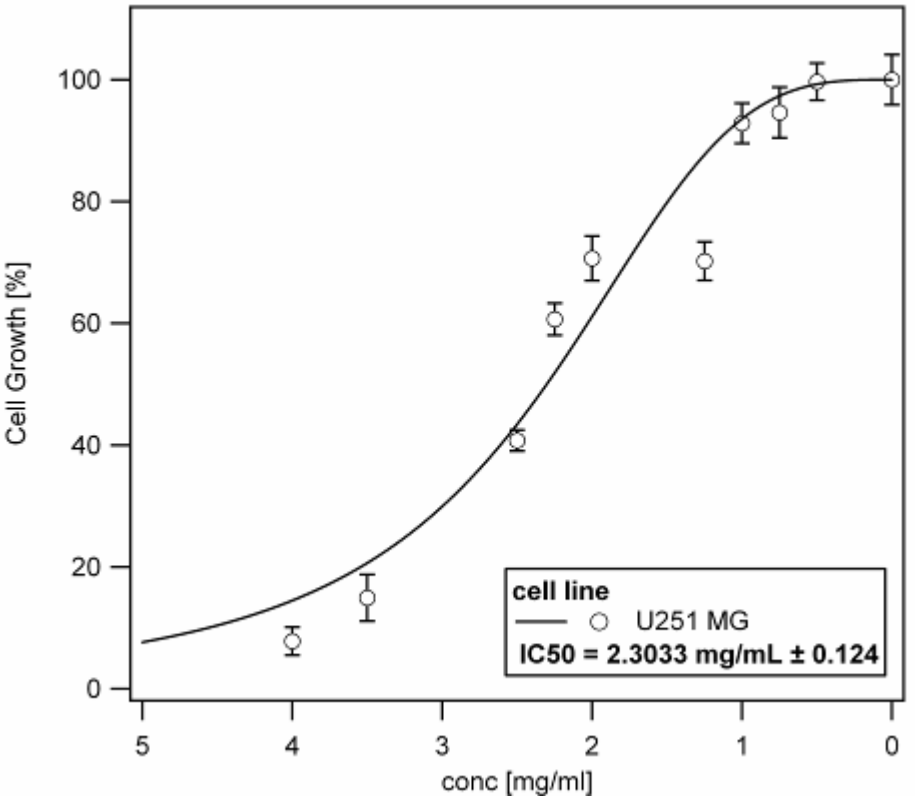
Dose-Response curves: Determination of IC50 after having incubated the glioblastoma cell lines U-373MG and U251 MG with ALA for 48 hours



Response: MTT and PIX

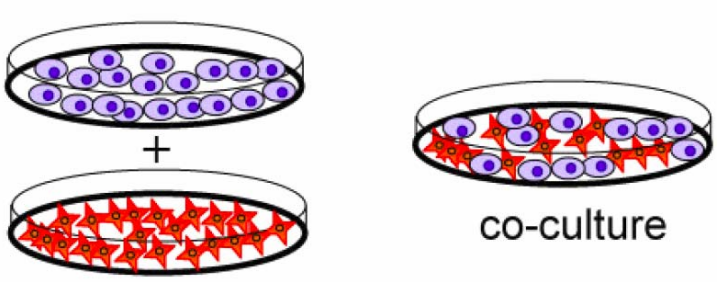
Dose-Response curves: Determination of IC_{50} after having incubated the glioblastoma cell lines U-373MG and U251 MG with ALA for 48 hours



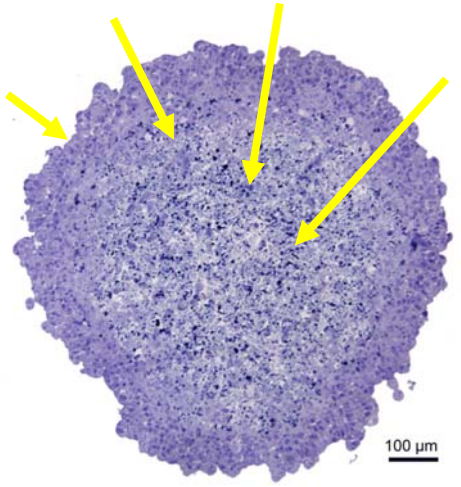


Main Study
Step 8, 9 and 10
Photodynamic Therapy

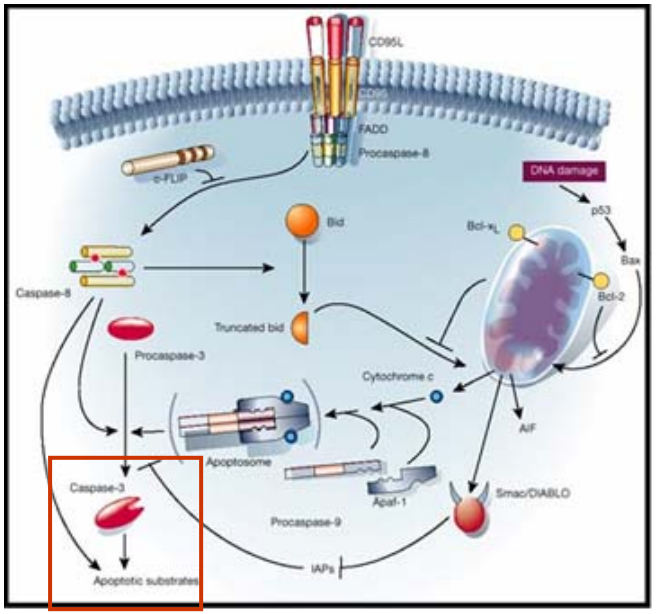
- Step 8: Dose response analysis, Culture and Co Culture, 5ALA, exposure time, MTT Assay

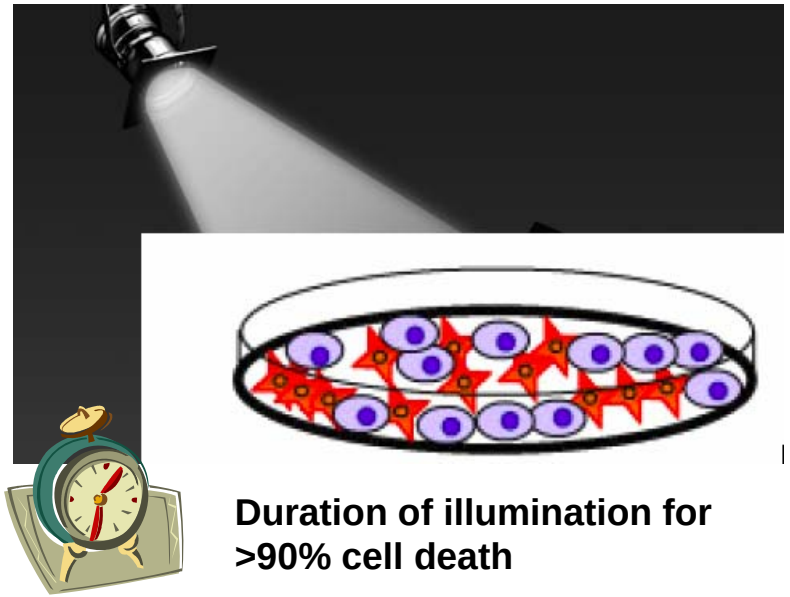


- Step 9: PCR and immunostaining for the detection of activated aptoposis genes as proof of succesfull PDT

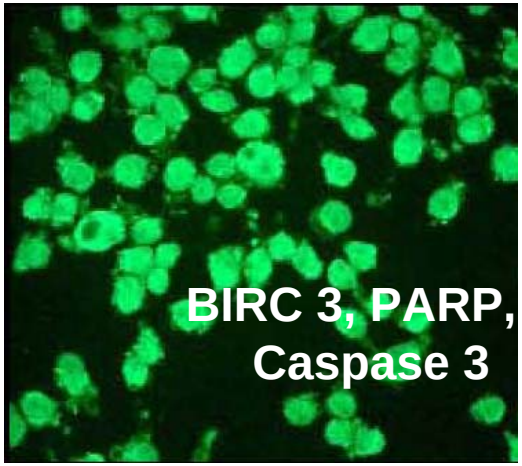


- Step 10: PDT in 3D GBM culture, evidence of BIRC 3, PARP, Caspase 3 activity to define penetration depth





Duration of illumination for >90% cell death



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What we learn

- Influence of common antiepileptic and chemotherapeutic medication on PIX Fluorescence intensity in GBM

- Optimal light source and exposure time for PIX PDT in GBM for max cell death and specificity and exposure depth in malignant Gliomas

Perspectives

Ina Albert Christine Galiagousis Tea D'Angelo