

➤ Orthotopic tumor models

Implantation of tumor cells into the organ of origin (“orthotopically”) allows organotypical interaction between tumor cells and surrounding stroma. It has been shown that this interaction affects growth, differentiation, and drug sensitivity of tumor cells. Moreover, tumor cells can spread to metastatic sites in other organs, with specificities comparable to the human situation. However, it must be emphasized that in most orthotopically implanted *in vivo* models using typical immortalized cell lines metastasis occurs but is very heterogeneous and not detectable in all animals after implantation. Reaction Biology started working on more reliable *in vivo* models to address intentions aiming mainly at metastasis. Nevertheless, analysis of the primary tumors of orthotopically implanted cancer cells gives us a very prospective read out when testing a new compound.

➤ A172_Luc cells

A172 cells originate from the brain and represent a human glioblastoma cell line.

In order to detect the orthotopically implanted cells, a luciferase expressing cell pool was generated via transduction of a luciferase-neomycin construct and subsequent neomycin selection.

➤ Study outline

To initiate orthotopic growth, mice are anesthetized and placed in a stereotactic fixation device. Through a burr hole A172_Luc cells will be implanted intracranially into the cerebrum. Tumor cell growth is monitored via *in vivo* bioluminescence imaging (BLI). The animals are randomized into treatment groups according to the luminescence signal. During the study, tumor growth is monitored via BLI once per week, animal behavior is monitored daily and animal weights are measured three times per week. .

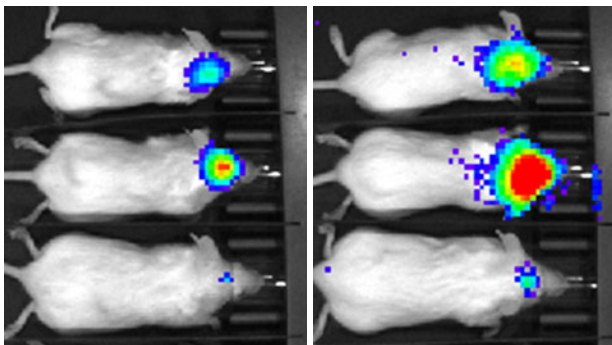


Figure 1: *In vivo* BLI of mice with A172_Luc cells injected intracranially were measured 40 days (left) or 82 days (right) after implantation.

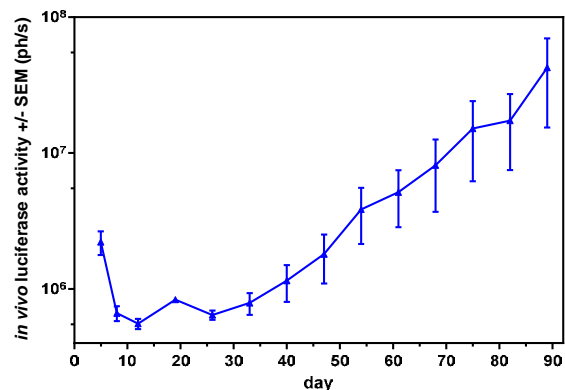


Figure 2: *In vivo* BLI of A172_Luc cells growing as glioblastoma tumors *in vivo*, luciferase activity, mean values +/- SEM

➤ Quality Assurance

- Routine authentication of tumor cell lines by STR profiling
- Mycoplasma testing of implanted tumor cells by PCR just prior to implantation
- Routine health monitoring of sentinel animals (according to FELASA guide lines)
- Animal work according to the 5R rules (reduce, refine, replace, responsible, remember)

Note: Graphs depicted are derived from study examples. Each study is a biological system of its own and subject to intrinsic variation.