

➤ 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.

➤ MiA-PaCa2 ELN cells

MiA-PaCa-2 is a poorly differentiated pancreatic ductal carcinoma cell line. When implanted orthotopically into nude mice, local metastasis was reported for this 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.

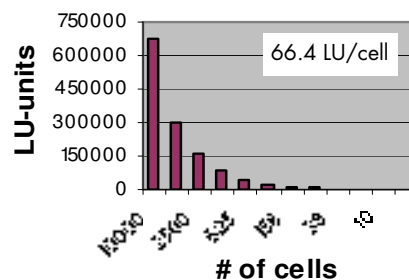


Figure 1: Luciferase assay. Serial dilutions of a cell lysate were tested for luciferase activity

➤ *In vivo* bioluminescence measurement

After surgery, the growth of the cells will be monitored via *in vivo* bioluminescence imaging (BLI). Using BLI, the animals are randomized into treatment groups according to apparent tumor sizes.

Moreover, once treatment is initiated, effects on the total *in vivo* bioluminescence signal, and thus primary tumor and potential metastatic loci may be monitored.

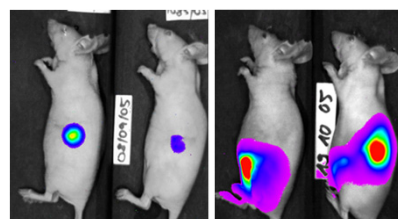


Figure 2: *In vivo* BLI. Mice with MIA-PaCa2 ELN cells orthotopically implanted into the pancreata were measured one week or 6 weeks (left panel) after surgery.

➤ Study example

Mice bearing orthotopically implanted MiA-PaCa2 tumors were treated with free or liposomal Gemcitabine.

Figure 3: Treatment.

Tumor growth was monitored using BLI (left panel). Tumor sizes were determined via *in vitro* BLI (right panel, dark) or caliper (light).

