HepG2 via spleen injection
Orthotopic liver tumor model

IN VIVO PHARMACOLOGY

➢ 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.

➢ HepG2 cells

A human liver tumor cell line established from a hepatocellular carcinoma of a 15-year-old, white, male youth. The cell line exhibits epithelial-like morphology.

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

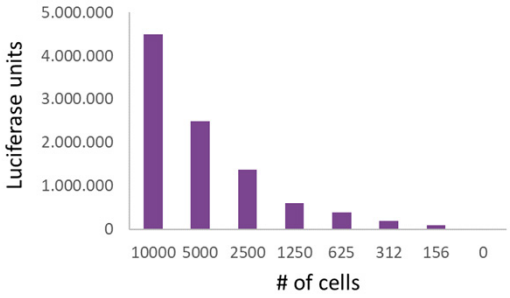

Figure 1: *Luciferase assay.*
Serial dilutions of a cell lysate were tested for luciferase activity.

➢ *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.

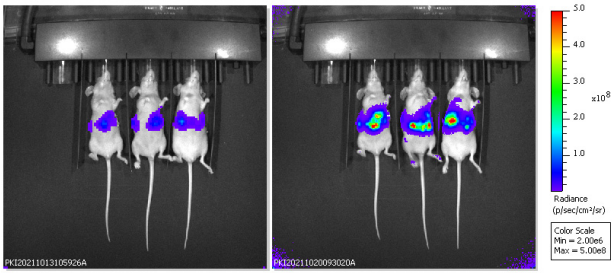

Figure 2: *In vivo bioluminescence.*
Mice with HepG2 tumors are shown after 2 days (left image), 23 days (right images) after surgery.

➢ Tumor growth

Luciferase-expressing HepG2 cells were injected in the spleen of immune compromised mice followed by splenectomy. Cells migrate via the vena lienalis into the liver initiating orthotopic tumor growth. The tumor growth was monitored in three mice in a feasibility study.

Figure 3: *Tumor growth. Growth of orthotopic HepG2 tumors was monitored in three mice via bioluminescence imaging using a IVIS Lumina III system within a feasibility study.*