

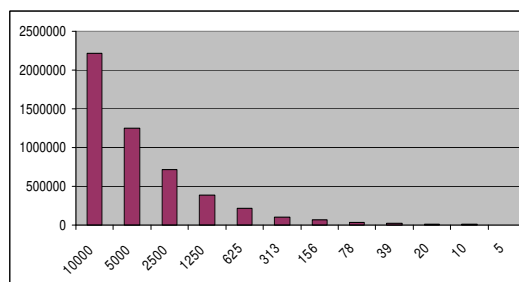
## ➤ Orthotopic tumor models

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.

## ➤ HCT-116 cells (CPQ-226)

HCT-116 cells originate from the colon and represent a human coloncarcinoma cell line.

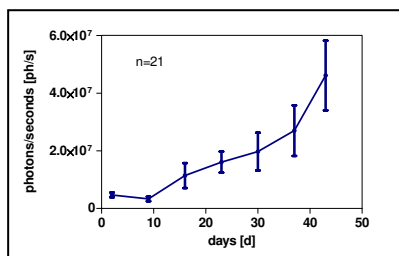
In order to detect orthotopic growth of implanted cells, a luciferase expressing cell pool was initially generated via transduction of a luciferase-neomycin construct and subsequent neomycin selection.



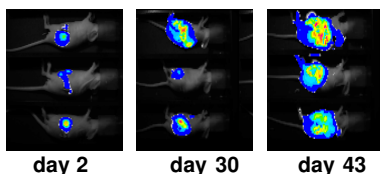
**Figure 1:** Luciferase assay. Serial dilutions of a cell lysate were tested for luciferase activity.

## ➤ In vivo bioluminescence measurement

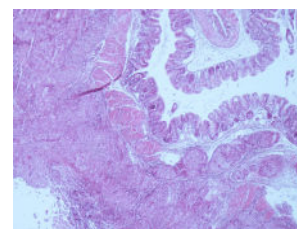
During surgery, tumors cells are implanted into the caecum, a part of the colon compartment. Thereafter tumor growth is monitored via in vivo bioluminescence imaging (BLI). Using BLI, animals are randomized into treatment groups and in vivo monitored during therapy.



**Figure 2:** In vivo BLI. In vivo tumor growth of HCT-116 was monitored once a week using BLI.



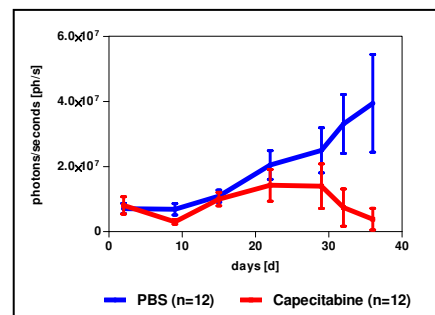
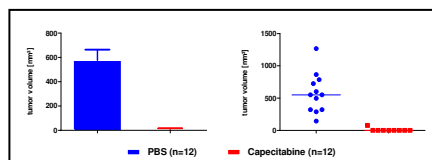
**Figure 3:** Pictures of in vivo BLI. Mice with orthotopically growing HCT-116 tumors measured on day 2, 30 and 43 after implantation



**Figure 4:** HE staining. HE staining of an orthotopically implanted HCT-116 tumor. Structures of colon mucosa are visible surrounded by tumor tissue.

## ➤ Study example

Mice bearing orthotopically implanted HCT-116 tumors were treated with Capecitabine.



**Figure 5:** HCT-116 tumors treated with Capecitabine. Tumor growth was monitored using BLI (right panel). Tumor volumes at necropsy are displayed as mean and as single values (above).