

## Rationale

### Luciferase mediated detection of tumor cells in mice

- Luciferase expression is widely used to detect cells and tissues in transgenic animals and implanted cells.
- The present study was an evaluation of the system
  - to test novel anti-cancer drugs in orthotopic models
  - to investigate and modulate the metastatic pattern of tumor cell lines
- Two tumor models were tested, RENCA, and A375.

### The RENCA model

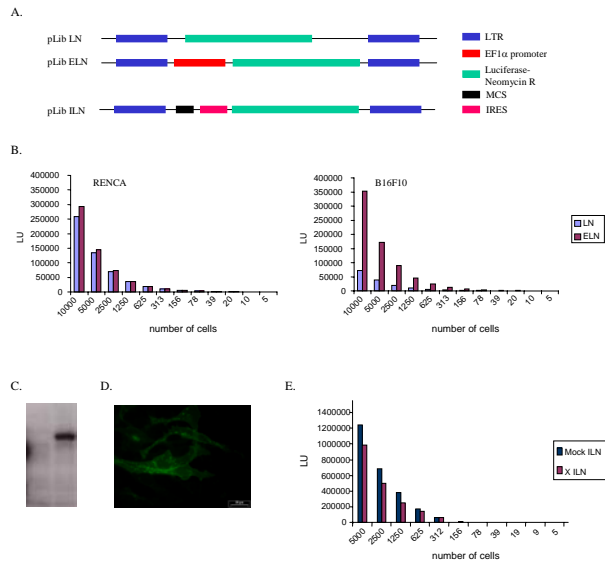
- Cells derived from a primary kidney tumor are inoculated by sub capsular renal injection.
- The cells form a primary tumor in the kidney, and metastasize mainly to the lung.
- Due to difficulties measuring primary tumor and metastases in the live animal, these parameters are usually determined as necropsy findings.
- An imaging system that would allow the detection of primary tumor and metastases in the live animal, and allow a quantitative determination of the metastatic burden would greatly increase the value of this model.

### A375 melanoma cell line metastasis dissemination model

- A375 is a human melanoma cell line that was investigated for its metastatic pattern using intra-cardiac injection technique.
- To allow the detection of metastases in the live animal, and to check the distribution of the tumor cells in the organs, a sensitive detection method is required.
- To study the influence of gene of interest on the metastatic dissemination, a vector that allows co-expression of a given gene with a marker gene should be generated.
- Since A375 lack endogenous expression of gene X, this was used to test a co-expression model.

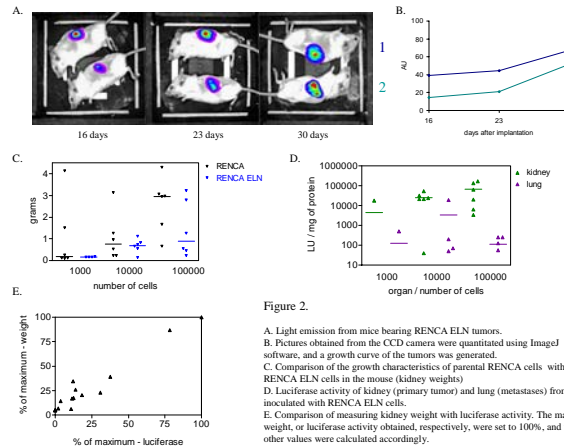
## Generation of cell lines expressing luciferase

- Transduction of a given cell line with the adequate retroviral construct (see Fig. 1A)
- Selection with 1-3 mg/ml G418 / Neomycin
- In vitro luciferase assay



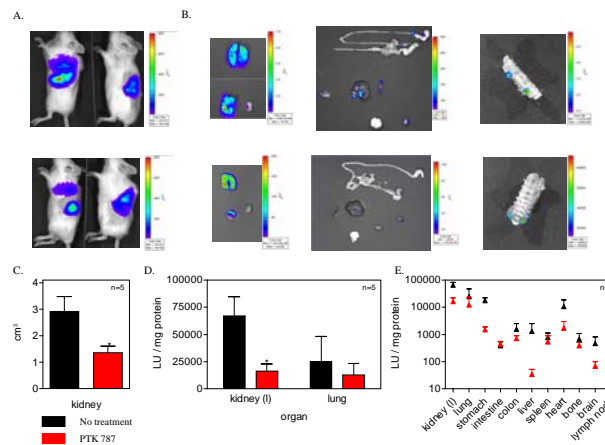
## Comparison of parental RENCA with RENCA ELN cells, and generation of a tumor growth curve based on luciferase activity in live animals

- 5 x 1000, 10000, or 100000 RENCA or RENCA ELN cells were implanted into the left kidney of Balb/c mice.
- 16, 23, and 30 days after implantation, two mice from each group that received the RENCA ELN cells were anesthetized, injected with luciferin, and analysed using a CCD camera.
- After 30 days, the mice were sacrificed, and the weight of the left kidney was determined. Lungs and kidneys from mice that had received the RENCA ELN cells were subsequently homogenized, and the luciferase activity was measured per mg of protein.



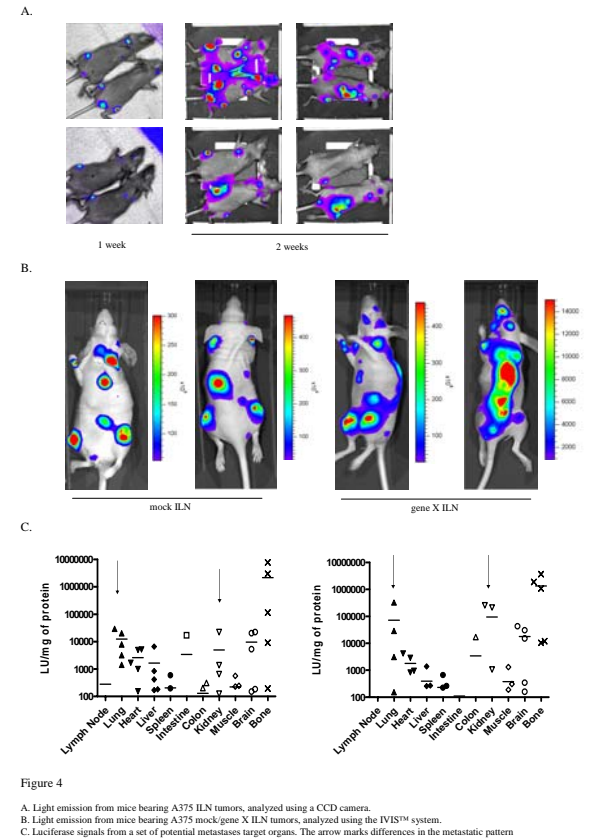
## Detection of RENCA tumors and metastases in vivo / ex vivo / in vitro

- 400000 RENCA ELN cells were orthotopically implanted into the left kidney of Balb/c mice.
- Half of the animals were treated with 100 mg PTK787 daily p.o.
- 18 days after implantation, the mice were anesthetized, injected with luciferin, and analysed using the IVIS™ system.
- The mice were then sacrificed, and the left kidneys bearing the primary tumor, were weighed.
- Kidneys, lungs, and a set of organs were homogenized, and the luciferase activity was measured per mg of protein.



## Impact of a gene, X, on the metastatic pattern of A375 cells

- A375 melanoma cells expressing either the IRES-Luciferase-Neomycin (ILN) cassette (mock), or gene X along with the ILN cassette, were injected in the left ventricle of the heart in nude mice.
- To follow the development of metastases, luciferase activity was measured 2 and 3 weeks after injection. Luciferin was injected intra-peritoneally and the mice were placed under a CCD camera.
- After 3 weeks, the mice were sacrificed and a set of organs were harvested, homogenized, and the luciferase activity was assayed.



## Conclusions

- Cell lines stably expressing luciferase as a selectable neomycin fusion protein were generated, and could be detected in the live animal.
- RENCA ELN cells show similar growth characteristics as their parental cell line, forming tumors in the kidney, and metastasizing to the lung, although the growth of the primary tumor may be slightly delayed.
- The primary tumor, as well as lung metastases, are detectable in the live animal.
- Smaller metastases are detectable in isolated organs, and if organs are homogenized.
- Luciferase based detection of tumor cells is a powerful tool to follow tumor progression, and test the efficacy of drugs in oncology.
- The metastatic distribution of A375 expressing luciferase only, or along with a gene potentially involved in the metastatic process could be followed in the live animal, and quantitated from tissue homogenates.

Luciferase marked tumor cell lines are excellent tools for tumor biology and drug development !