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**Study of the radionuclide therapeutic potency of  
 $2\text{-}^{131}\text{I}\text{-D-Phenylalanine}$  in R1Mfluc tumour bearing mice.**

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# Introduction

- In earlier studies we have shown that  $^{123}\text{I}$ -2-D-Phenylalanine is typically transported into different types of human cancer cells by the over-expressed LAT1 system and that the uptake of this D-isomer in non target organs in NuNu mice and in humans is lower than of the L-isomer.
- The clearance from the body was fast and comparable in NuNu mice and humans.

**Therefore we decided to try  $^{131}\text{I}$ -2-D-Phenylalanine as a radionuclide therapeutic agent in NuNu mice inoculated with transfected R1M cells expressing the enzyme luciferase (R1Mfluc cells) allowing also follow up of the tumour growth by bioluminescence imaging.**

## Methods:

### Therapeutic and control formulations.

•Kit-labeling by the commonly known  $\text{Cu}^{1+}$  assisted nucleophilic isotopic exchange in presence of  $\text{SnSO}_4$ , gentisic acid and citrate (> 95%) and trapping of free  $^{131}\text{I}^-$  on a Ag-filter yielded a 1 mL solution containing

**C.A.  $^{131}\text{I}$ -2-I-D-Phenylalanine with a specific activity of ~1500 MBq/ mg**

- The “control Kit” contained  $\text{SnSO}_4$ , gentisic acid, citrate and 1 mg of non radioactive 2-I-D-Phenylalanine.
- The “control isotonic saline”: 0.9% NaCl solution
- The  $^{131}\text{I}^-$  solution: 1500 MBq  $^{131}\text{I}^-$  - NaI in 1 mL 0.9 % NaCl solution
- The solutions were sterilized by means of a 0.22  $\mu$  Millipore filter

## Methods:

### *In vitro* experiments

#### **Testing of LAT1 transport expression by transfected R1Mfluc cells**

The LAT1 ( Na<sup>+</sup> independent transport system) expression by the R1Mfluc cells was tested in vitro in 6-well-plates using at least three wells for each data-point. Influx and efflux were studied in HEPES- (where Choline Chloride replaces Na<sup>+</sup>)

#### **Sensitivity to <sup>131</sup>I radiation**

The cells were brought into 1 mL well plates with full MEM buffer (in which normal growth is assured ) and 50 µL of 37 KBq <sup>131</sup>I-2-I-D-Phe / Kit formulation were added.

The plates were incubated at 37°C for 24 hours in a 5% CO<sub>2</sub> atmosphere and the viability of the cells counted by means of trypan blue staining.

*The biomolecular aspects of the Generation of the luciferase-positive R1M rhabdomyosarcoma (R1Mfluc) cell line are described in detail by Keyaerts et al. (Eur. J Nucl Med Mol Imaging (2008) 35:999-1007)*

## Methods:

### *In vivo* experiments

The NuNu mice (weight ~30 g) were inoculated with  $10^5$  R1Mfluc cells and BLI measurements (constant amount of D-luciferine injected ) were performed daily up from day 2 after inoculation till day 17.

**100  $\mu$ L of the therapeutic dose (148 MBq  $^{131}\text{I}$ -2-I-Phe / Kit formulation) or the control solutions (Cold Kit and isotonic saline) were injected *as a bolus* in the tail vein on day 10.**

***At that moment the mean weight of the tumours was ~ 0.2 g and the amount of  $^{131}\text{I}$ -2-I-Phe activity in the tumours 30 min. p.i.v. injection 325 KBq (1.1% IA/g)***

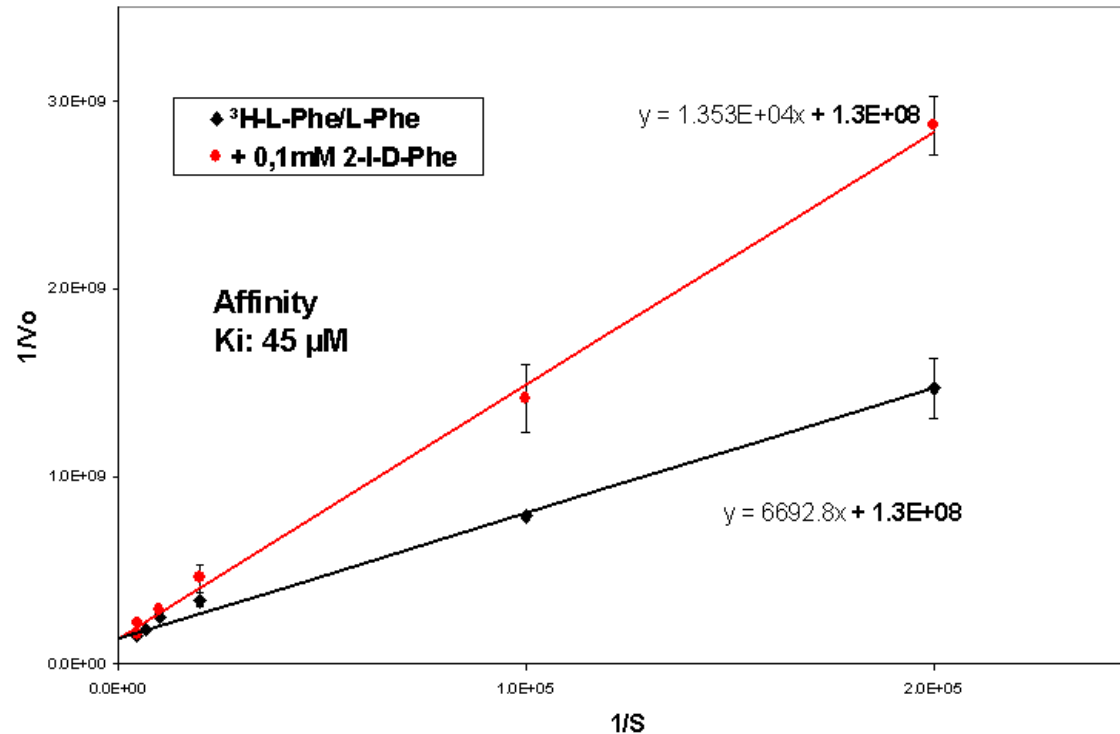
From day 10 -17 also calliper measurements were daily performed .

At day 17 the mice were sacrificed and the tumours dissected and weighed.

The weight of the mice was controlled each day before starting the BLI and caliper measurements.

## Results *In vitro*

### Control of expression of LAT1 transport system : Determination of affinity ( $K_i$ ) en competitiveness in R1Mfluc cellen

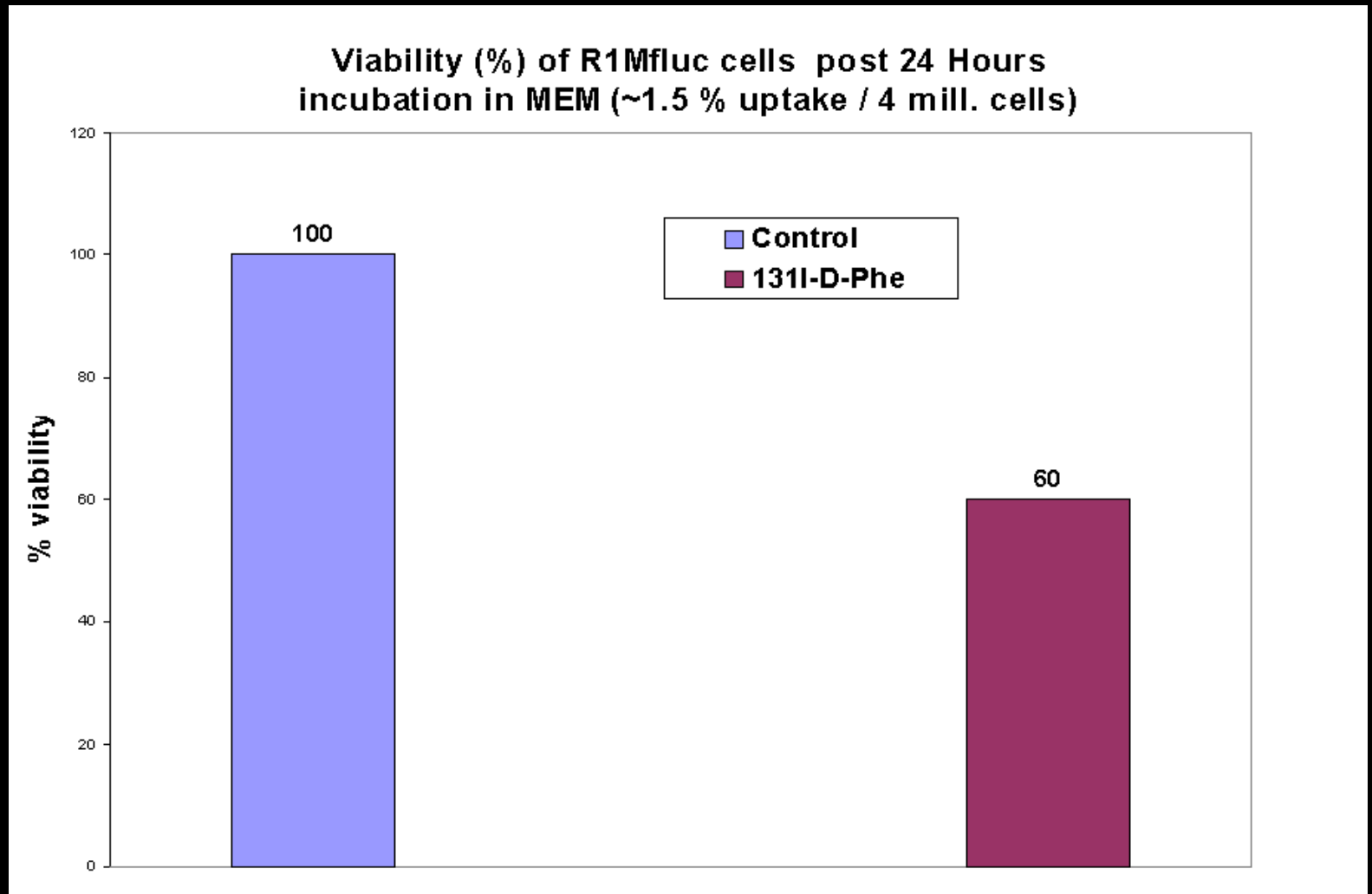


### R1Mfluc Cells

- $^3\text{H}$ -L-Phe and 2-I-D-Phe curve have the same intercept on the  $1/V_o$  axis  
→ competitive uptake sharing the same LAT1 transport system.
- The affinity for 2-I-D-Phe:  $K_i = 45 \mu\text{M}$ , comparable with R1Mwt cells.

**The modification in the R1Mfluc cells has no apparent influence on LAT1 expression and properties.**

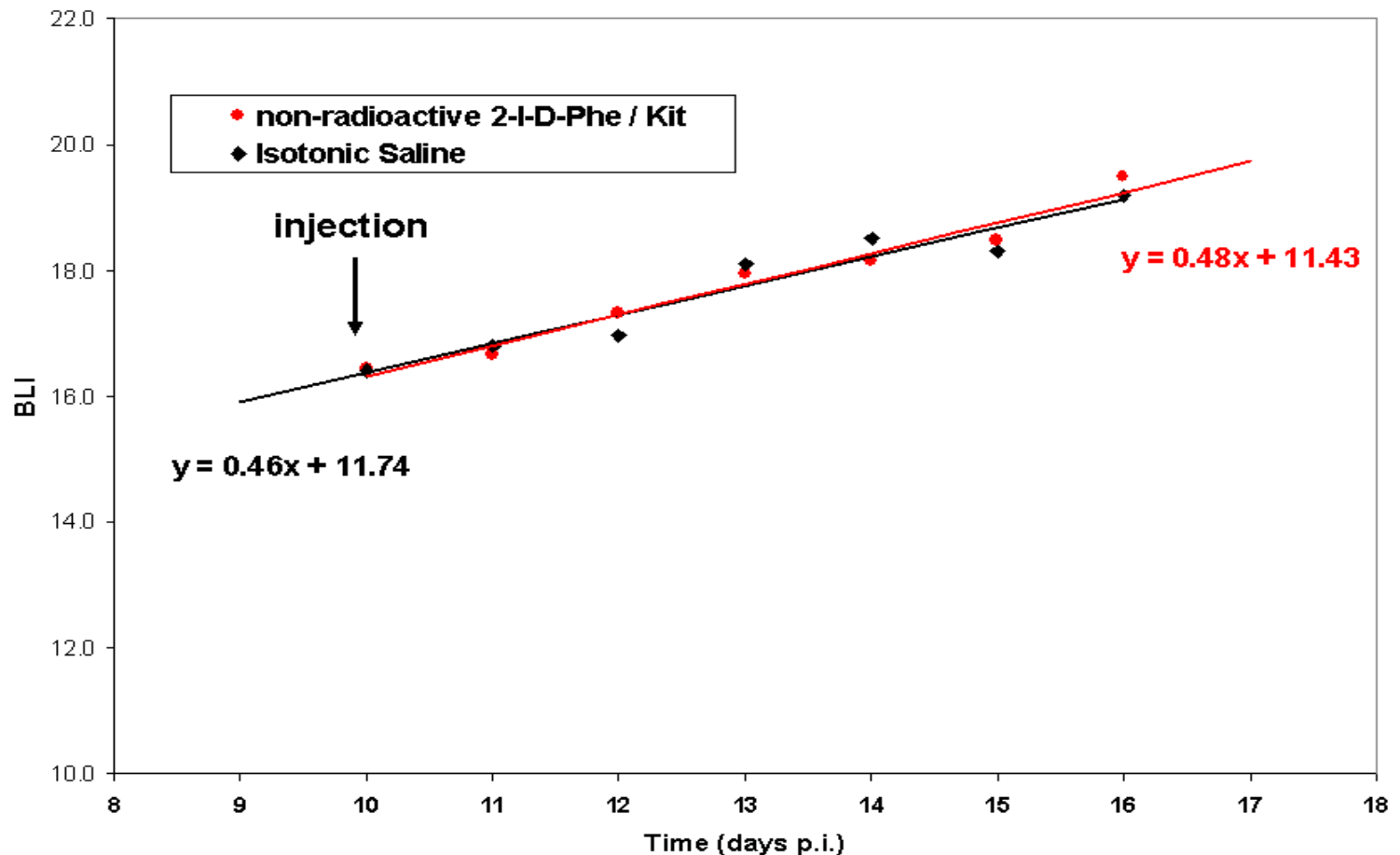
## Results *In vitro*



R1Mfluc cells are sensitive to  $^{131}\text{I}$  radiation present as  $^{131}\text{I}$ -2-I-D-Phe in the cells and in the surrounding buffer

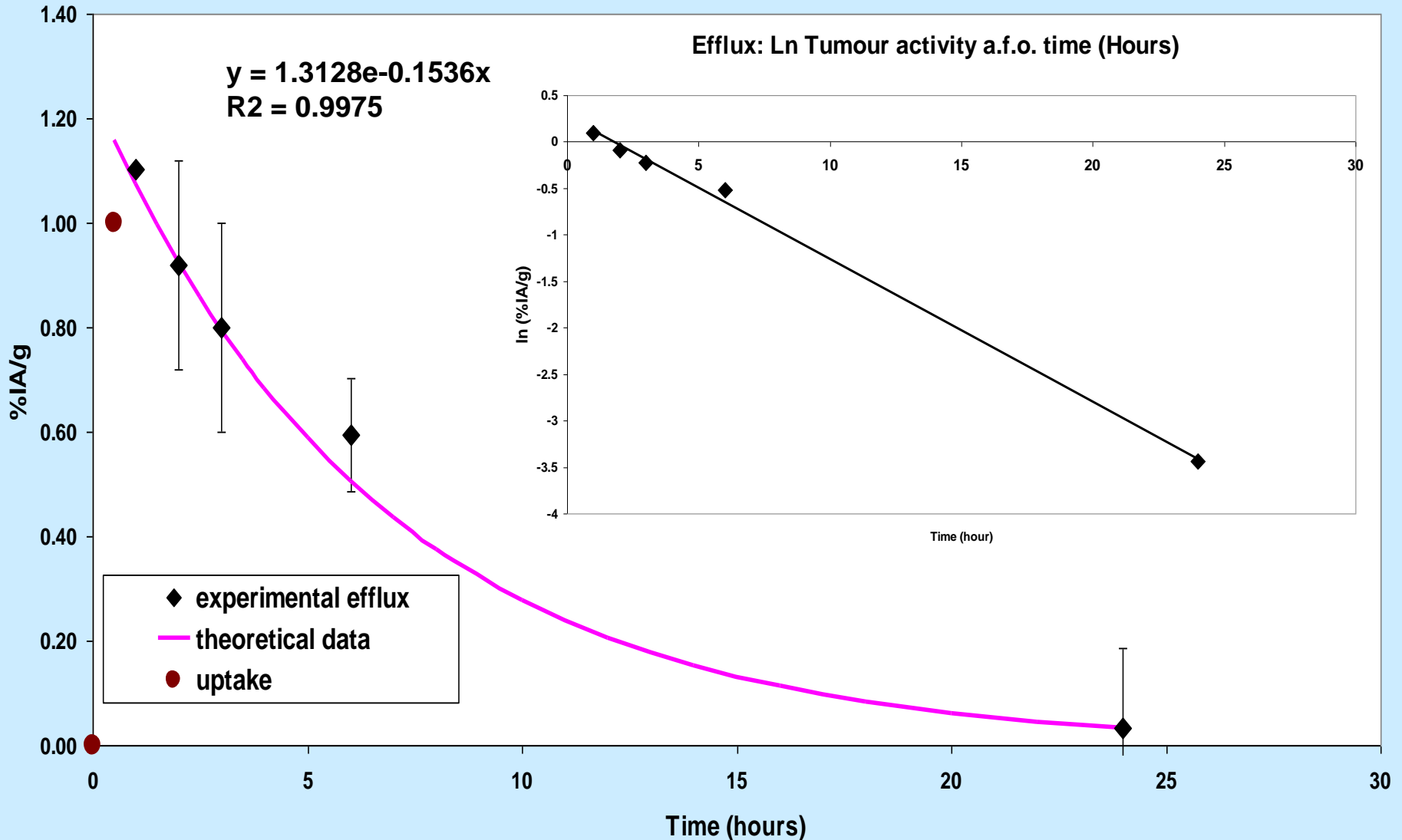
# Results *In vivo*

## Effect of Kit composition on tumour growth a.f.o. time compared to Isotonic Saline BLI measurements



- ❖ no effect of the injected “cold-kit” formulation as compared to isotonic saline
- *no influence on the weight and the behaviour of the mice.*

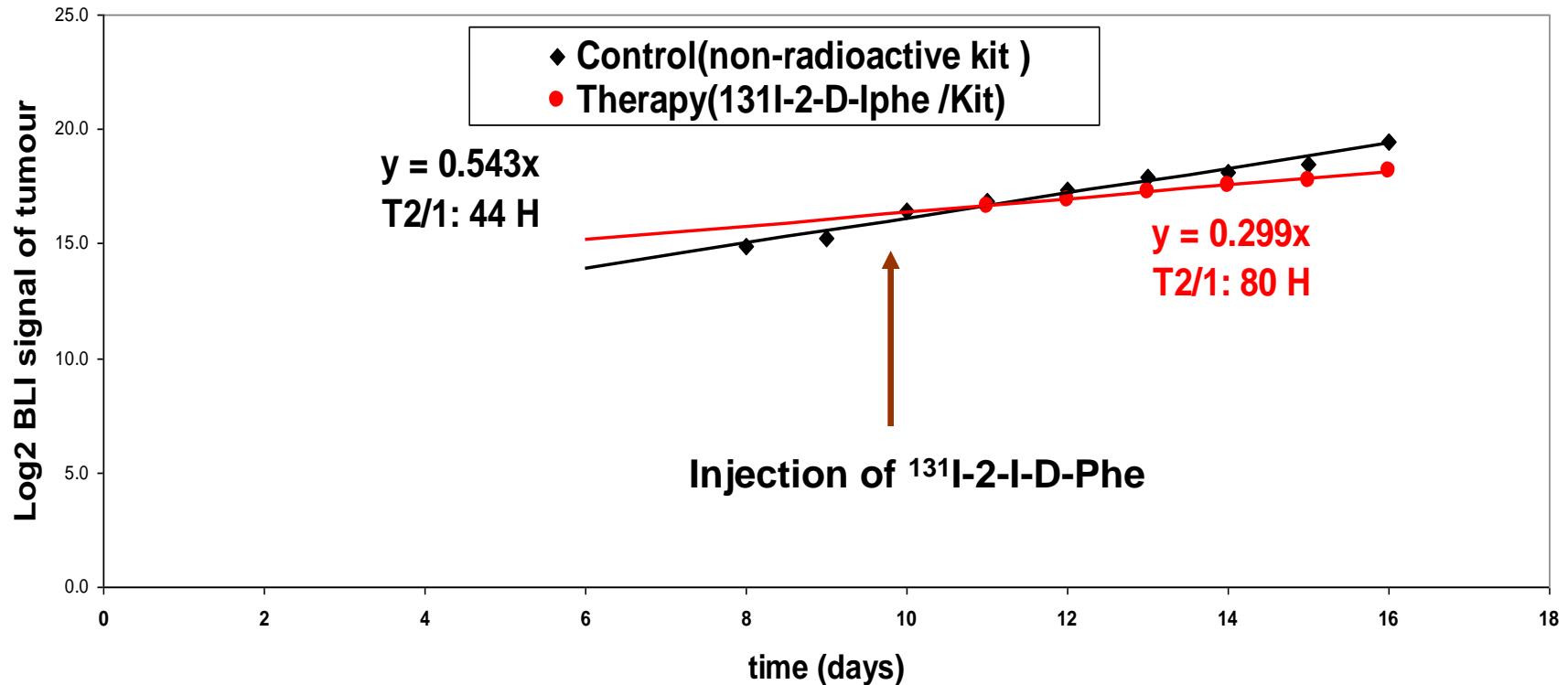
**NuNu mice model, day 10;  $^{131}\text{I}$ -2-I-D-Phe activity in R1Mfluc tumour  
 %IA/g a.f.o time  
 Resulting Dose of Tumour: 8.5 mGy / MBq (1.26 Gy / 148 MBq)**



# Results In vivo

## Therapeutic effect of C.A. $^{131}\text{I}$ -2-I-D-Phenylalanine

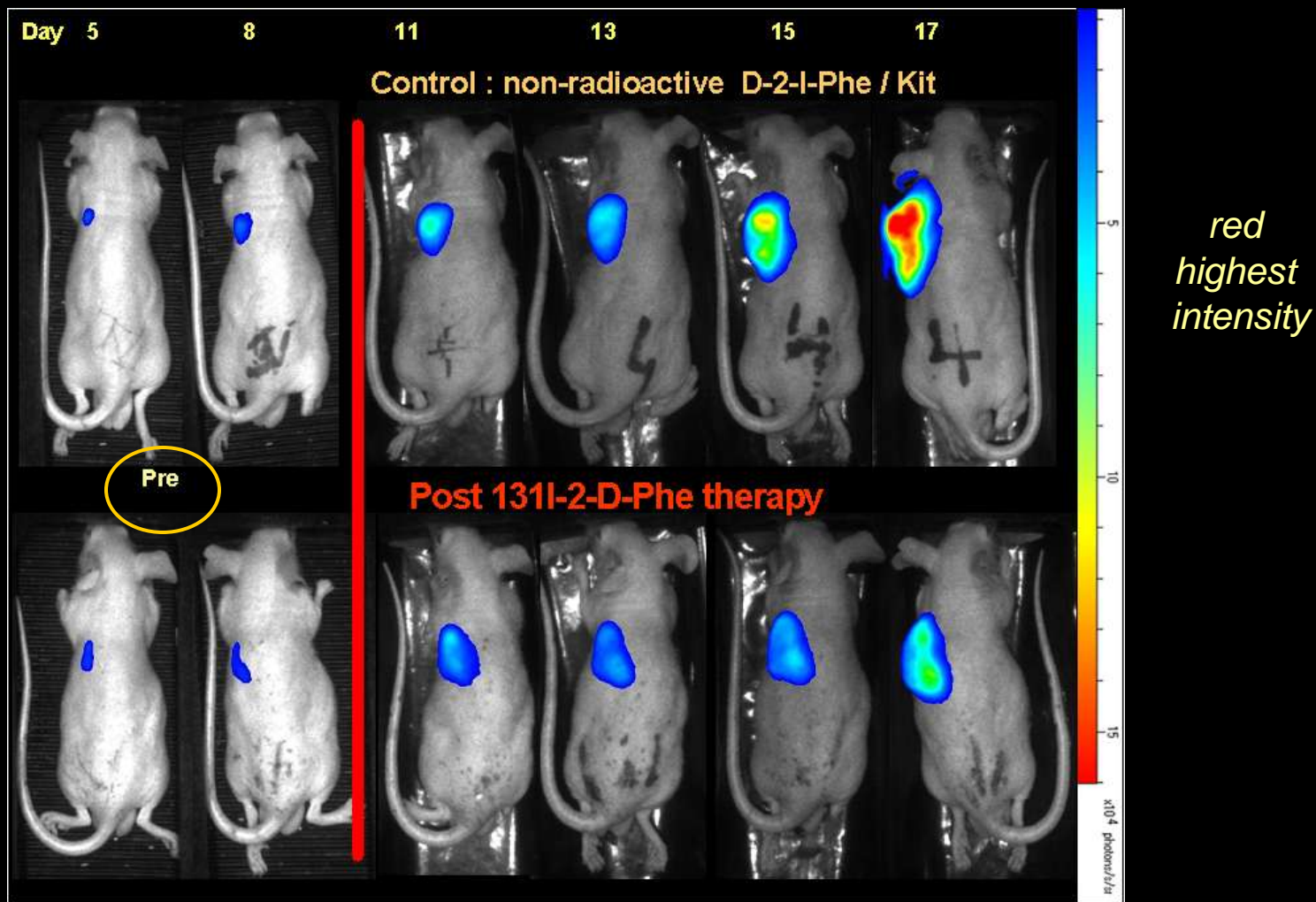
Influence of  $^{131}\text{I}$ -2-I-D-Phe on BLI R1M tumour growth signal a.f.o. time  
Cell doubling time  $T_{2/1} = 1/\text{Slope}$



- After injection of the therapeutic dose the slope decreased from 0.54 to 0.30 →  $T_{2/1}$  (doubling time) values almost doubled
- This means that the induced radiotoxicity has a more drastic effect than “once-only killing” a part of the tumour cells that should yield a parallel curve

**Results**  
*In vivo*

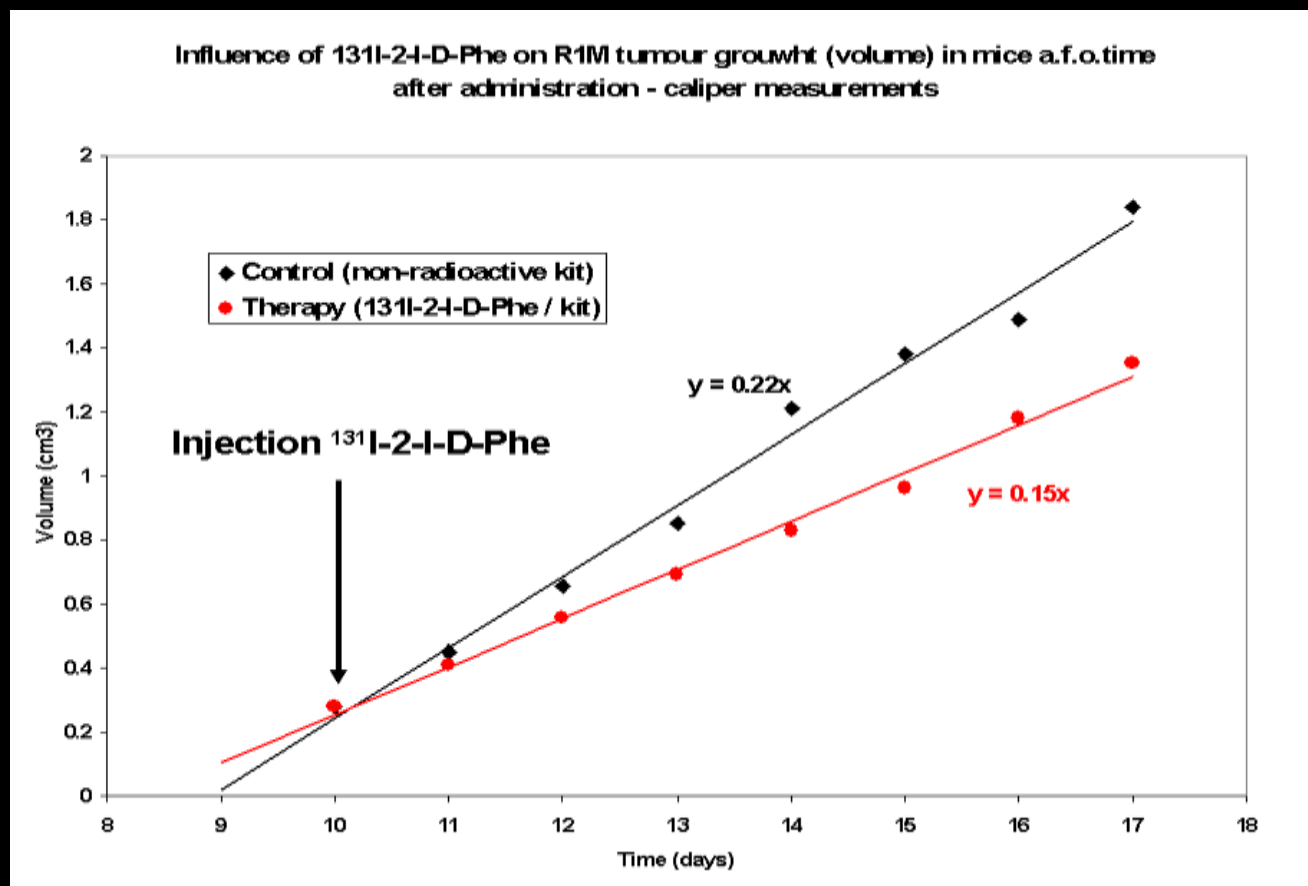
**BLI images: Therapy versus Kit-Control**



The BLI images over the period after therapy illustrate former results a significant lower light intensity for the Therapy series (row under) than for the control-Kit series (upper row)

## Therapy: Calliper measurements

### Results In vivo



□ Increase in tumour volume with time 30 % less for the Therapy series .



□ Mean weight of dissected tumours at day 17 was respectively 1.2 g for “control” and 0.9 g for the “therapy” series (loss of ~ 25 %).

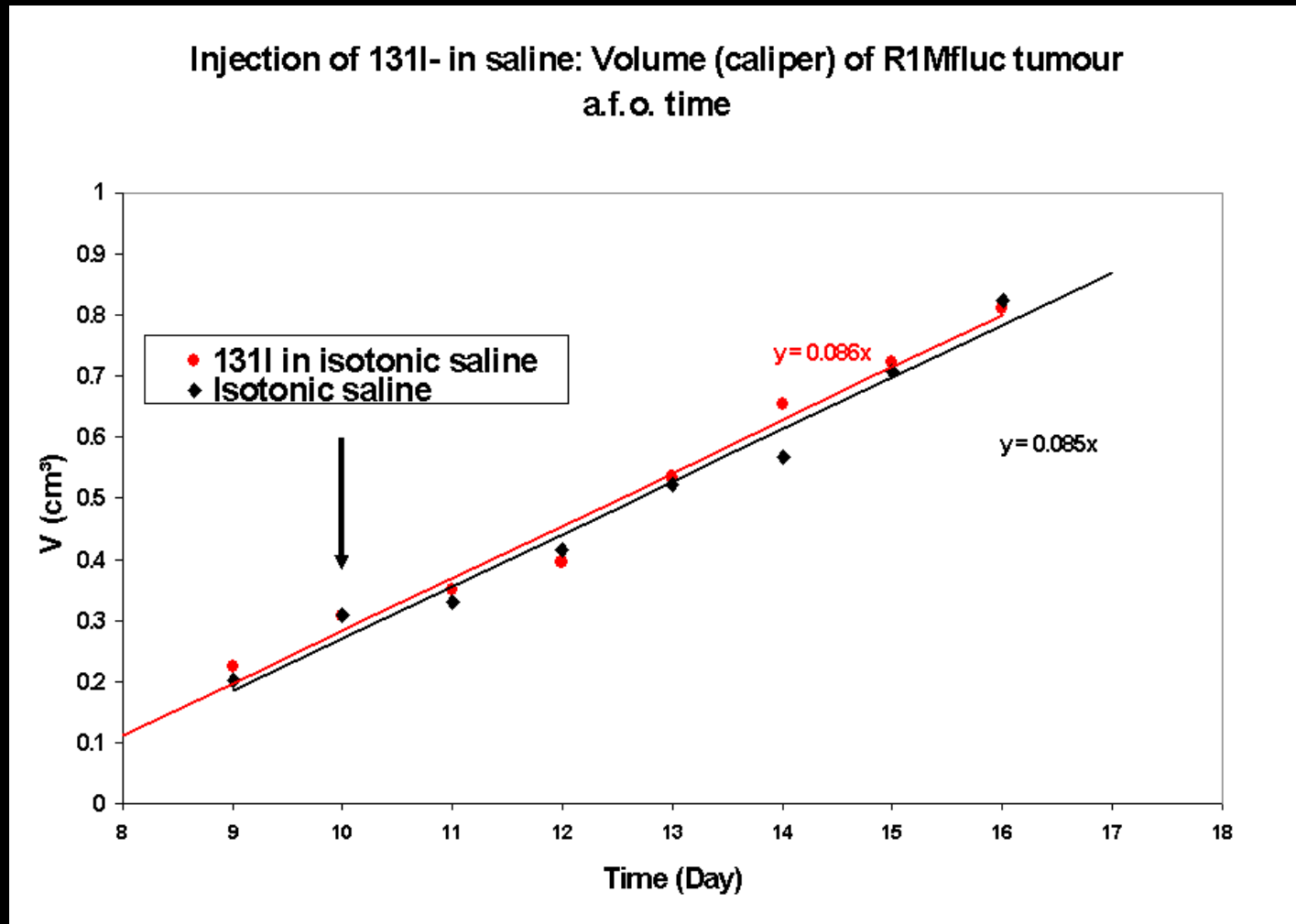
*In the weight values necrotic tissue is included*

*The therapy animals showed a small but significant weight loss of 1%*

# Results

## In vivo

### Potential influence of extra-cellar $^{131}\text{I}^-$



There was **no** influence of the injected  $^{131}\text{I}^-$ -NaI solution on tumour growth measured by calliper

# Conclusion

The results of the BLI measurements and the calliper measurements revealed a **significant therapeutic effect** of the injection of 148 MBq of the  $^{131}\text{I}$ -2-I-Phe / Kit formulation within 7 days past administration appearing as

- a decreased cell proliferation velocity and
  - a decreased tumour volume
- vis à vis the “ control mice” series.

A reduction of 25 % of the tumour weight after dissection confirmed these results

As the injection of  $^{131}\text{I}$ - NaI in isotonic saline did not show any effect on evolution of the tumour volume it may be assumed that the **therapeutic effect was  $^{131}\text{I}$ -2-I-Phe – specific**

# Thank you for your attention

