



We have produced novel radioimmunoconjugates for detection and therapy of head & neck cancer. This was done by combining the radiometals  $^{111}\text{In}$  and  $^{177}\text{Lu}$  with the chimeric monoclonal antibody U36.

The labelled conjugates were evaluated *in vitro*, demonstrating no adverse effects from labelling. The  $^{111}\text{In}$ -labelled conjugate was then evaluated *in vivo* in tumour bearing mice. Studies demonstrated a favourable biodistribution, and tumours as small as 60 mg were clearly visualised in gamma camera studies.

## Introduction:

Radiometals are promising radionuclides for imaging and therapy of head and neck squamous cell carcinoma (HNSCC) due to their suitable physical properties, availability, and favourable intracellular retention.

In this study, we have combined the anti-CD44v6 chimeric monoclonal antibody (CMab) U36 with  $^{111}\text{In}$  and  $^{177}\text{Lu}$ . An  $^{111}\text{In}$ -labelled antibody is suitable for imaging in HNSCC using planar gamma camera and SPECT. The low-energy beta emitter  $^{177}\text{Lu}$  labelled to an antibody is a suitable conjugate for radioimmunotherapy in small metastases in HNSCC.

## Materials & methods:

CMab U36 was radiolabelled with  $^{111}\text{In}$  and  $^{177}\text{Lu}$  using the linker CHX-A''-DTPA. The humanised antibody (HuMab) A33 was labelled the same way and used as a negative control.

Specificity, uptake and retention studies of the conjugates were evaluated *in vitro* using LigandTracer® yellow. Immunoreactivity and affinity evaluations were performed in cultured HNSCC (SCC9) cells.

Biodistribution and imaging studies of  $^{111}\text{In}$ -CMab U36 were performed *in vivo* using SCC9-tumour bearing nu/nu mice.

## Results:

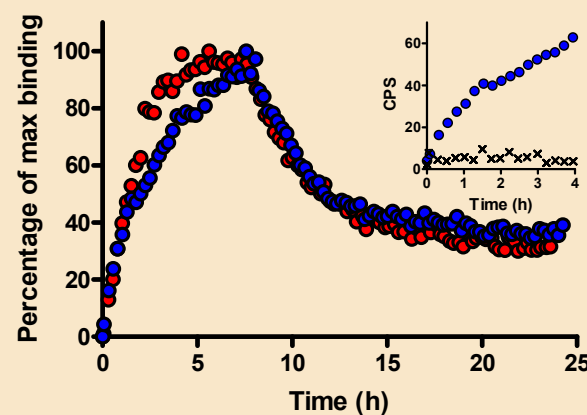
*In vitro* results demonstrated high immunoreactivity and affinity (Table 1). LigandTracer® analyses revealed a specific uptake of both conjugates in HNSCC cells, with a cellular retention of approximately 30% after 16 hours (Figure 1).

*In vivo* studies of  $^{111}\text{In}$ -CMab U36 demonstrated a favourable biodistribution with a tumour specific uptake (Figure 2), and a high and increasing tumour to blood ratio (Figure 2: inset).

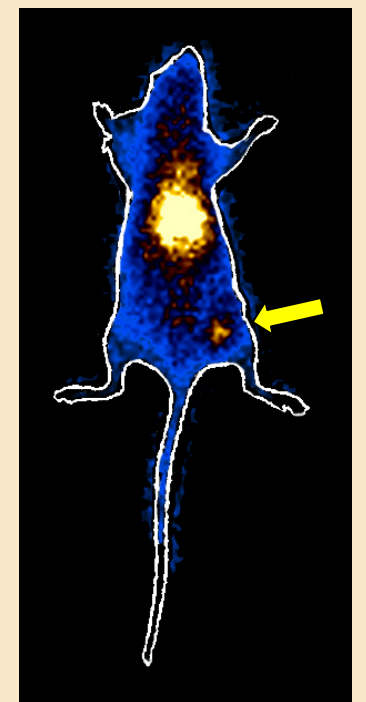
Tumours, ranging from 60 mg to 260 mg, were clearly visualised in gamma camera imaging studies 72h p.i. (Figure 3).

**Table 1.** Immunoreactivity and affinity of  $^{111}\text{In}$ -CMab U36 and  $^{177}\text{Lu}$ -CMab U36

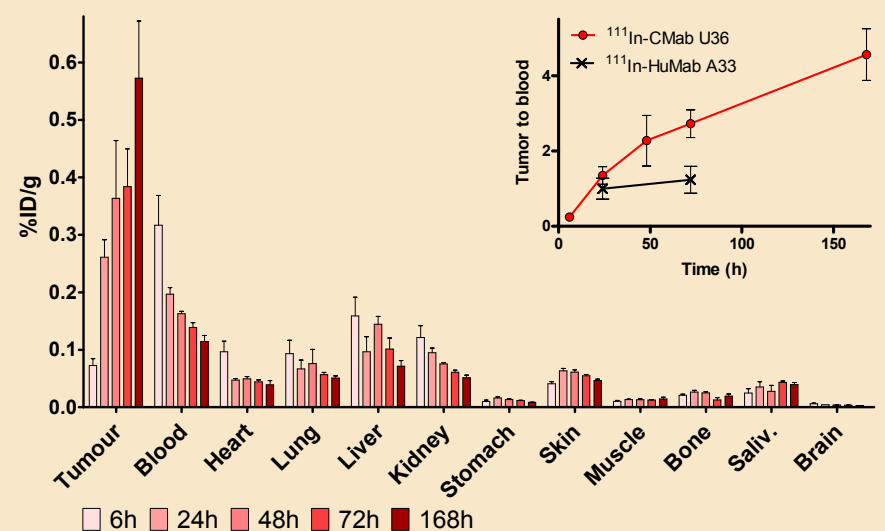
Conjugate	Immuno-reactivity	Affinity
$^{111}\text{In}$ -CMab U36	95%	$5.1 \pm 1.1$ nM
$^{177}\text{Lu}$ -CMab U36	96%	$4.7 \pm 2.2$ nM



**Figure 1.** LigandTracer® binding traces (uptake 8h, retention 16h) for CMAb U36 labelled with  $^{177}\text{Lu}$  (blue) and  $^{111}\text{In}$  (red) in the cell line SCC9. *Inset:* Uptake of  $^{177}\text{Lu}$ -CMAb U36 (blue) vs. the negative control  $^{177}\text{Lu}$ -HuMab A33 (X).



**Figure 3.** Gamma camera image 72h p.i. of  $^{111}\text{In}$ -CMAb U36 in a mouse bearing a 60 mg HNSCC tumour. Tumour is indicated by yellow arrow.



**Figure 2.** Biodistribution of  $^{111}\text{In}$ -CMAb U36 in SCC9 tumour bearing mice. *Inset:* Tumour to blood ratio over time for  $^{111}\text{In}$ -CMAb U36 (red) vs. the negative control  $^{111}\text{In}$ -HuMab A33 (X).

## Conclusions:

This study demonstrates the potential of radiometals for radioimmunotargeting. By combining a tumour targeting agent with  $^{111}\text{In}$  or  $^{177}\text{Lu}$ , two novel conjugates for imaging and therapy of HNSCC were created. Labelling was efficient and well tolerated, demonstrating high affinity, retained specificity and high cellular retention of the conjugates. The specific, high, and accumulating tumour to blood ratio of  $^{111}\text{In}$ -CMAb U36 indicated a favourable retention of the conjugate, and tumours were clearly visible in gamma camera studies.