

# Preclinical Evaluation of $^{99m}\text{Tc}$ -Histidine-Folate for Folate Receptor-Positive Tumor Targeting

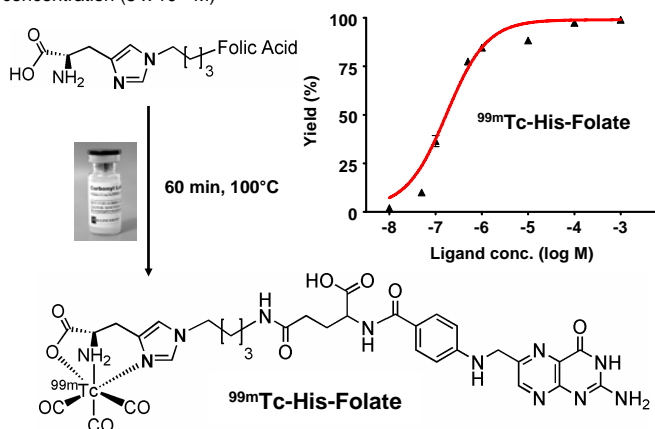
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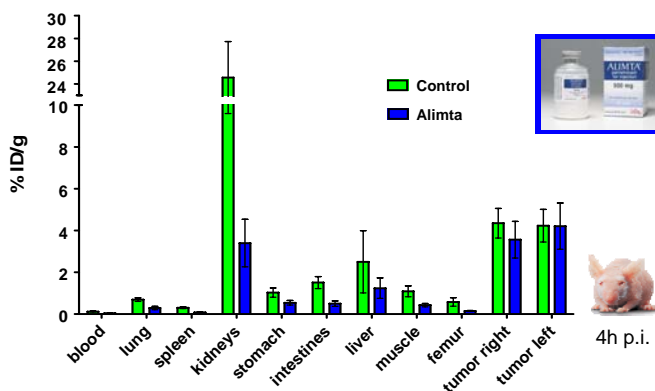
**Introduction.** The high affinity folate receptor (FR) is overexpressed in a number of tumors (e.g. ovarian, endometrial carcinomas). Thus, using the vitamin folic acid as a “Trojan Horse” is a promising strategy for specific delivery of diagnostic and therapeutic probes into FR-positive tumor cells [1,2]. This study describes the *in vitro* and *in vivo* evaluation of a novel folate derivative with a histidine chelator for tridentate coordination of the  $[\text{M}(\text{CO})_3]^+$ -core (M =  $^{99m}\text{Tc}$ ,  $\gamma$ -radiation for diagnosis;  $^{188}\text{Re}$ ;  $\beta$ -radiation for therapy) [3]. The  $^{99m}\text{Tc}$ -His-folate showed favorable *in vivo* characteristics compared to other organometallic radiofolates and the recently developed antifolate-protocol to block kidney retention was successfully applied [4].

## Radiolabeling of the Histidine-Folate

The  $^{99m}\text{Tc}$ -radiolabeling of the novel histidine-folate was carried out via the Isolink™ method. Labeling efficiency was high (> 90%), even at low ligand concentration ( $5 \times 10^{-6}$  M)



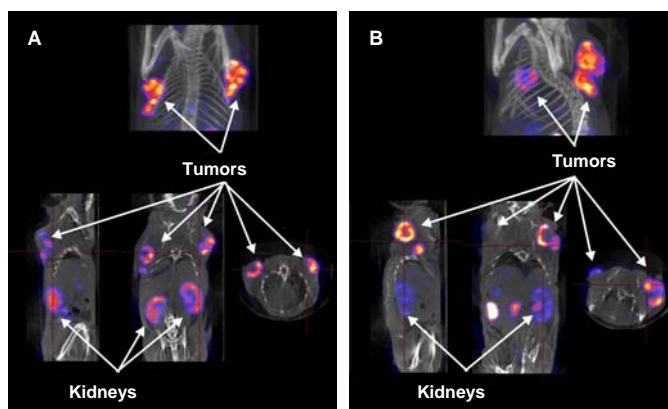
## Biodistribution Data of $^{99m}\text{Tc}$ -Histidine-Folate in Mice



Biodistribution studies were performed in male nude mice bearing human KB-tumor xenografts that overexpress the FR. Compared with controls (green), injection of Alimta® 1 h previous to the radiotracer reduced kidney retention of radioactivity significantly, whereas the tumor uptake was retained (blue).

## SPECT/CT-imaging of KB-Tumor Bearing Nude Mice

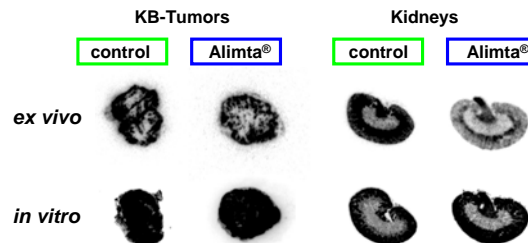
Scans were acquired with a dedicated small animal SPECT/CT camera (NanoSPECT™, Bioscan Inc.) 24 h p.i. of 300 MBq radiofolate.



**A:** In control mice a high tumor uptake was found along with high accumulation of radioactivity in FR-positive kidneys. **B:** Pre-injection of the mice with the antifolate Alimta® resulted in retained tumor uptake but significantly reduced radioactivity in the kidneys.

## Ex vivo and in vitro Autoradiography Studies

Frozen sections of KB-tumors and kidneys were used for *ex vivo* and *in vitro* autoradiography.



**Ex vivo AR:** Alimta pre-injection did not influence the tumor uptake of the radiofolate but in kidneys accumulated radioactivity was significantly reduced in the cortex where FRs are expressed. **In vitro AR:** FR-expression could be confirmed throughout the KB tumor tissue and in the cortex of the kidneys for tissues from control animals and animals previously injected with Alimta.

**Conclusion.** The novel organometallic  $^{99m}\text{Tc}$ -His-radiofolate showed favorable characteristics for its use as imaging agent of FR-positive tissue and organs. In combination with antifolates (e.g. Alimta®), undesired kidney retention could be significantly reduced while the tumor uptake was not affected. This led to a improved tumor-to-kidney ratio of radioactivity (> 1) and would potentially allow a therapeutic application with the same tracer when radiolabeled with the  $\beta$ -radiation emitting  $^{188}\text{Re}$ -radionuclide.