Combined diffuse light imaging tomographic (DLIT) and magnetic resonance imaging analysis of murine orthotopic and disseminated tumor models

Gehrig, Sebastian; Diana Groza; Fatih Alioglu; Petra Heffeter; Walter Berger; Ogris, Manfred

a) Laboratory of Macro Molecular Cancer Therapeutics (MMCT), University of Vienna, Department of Pharmaceutical Chemistry, Austria
b) Institute for cancer research Medical University of Vienna, Austria

Combined Diffuse Light Imaging and computed Tomography (DLIT/CT) as a technique for spatial reconstruction of BLI-signal helped to overcome limitations based on the 2D nature of classical BLI imaging by slightly decreased throughput and ease of use (Kuo et al., 2008). Limitations associated with DLIT/CT are mainly based on the limited soft tissue contrast provided by CT. Meanwhile combined DLIT/MRI has been demonstrated by a number of authors and can overcome limitations associated with lack of soft tissue contrast. (i.e Scott et al., 2016) Here we present an in house developed animal shuttle compatible with Aspect Imaging M3™ and PE Spectrum CT™, enabling for sequential combined DLIT/MRI maintaining ease of use and throughput of DLIT/CT which has recently been commercialized as the Aspect Imaging VivoFuse™ BLI Shuttle. Due to the easy to use, small footprint self shielded bench top MRI the solution is feasible for routine laboratory work behind hygienic barriers not requiring specialized imaging facilities.

VivoFuse™ workflow and fusion

Models used for proof of concept

Syngeneic Hepa 1c1c7 cells and Osteosarcoma CRL2836 were lentivirally transduced with Luciferase gene and sorted for Luc positive cells. 3 million Hepa 1c1c7 cells dissolved in 50 µl PBS where injected in lower part of the spleen of Black6 albino mice subsequent to surgical uncouaty. 0.1 Mio Osteo CRL2836 Cellsdissolved in 20µl PBS where injected in the proximal Tibia of Balb/C mice. Accurate placement of injection needle was verified by microCT (red arrows). Repeated combined MRI and DLIT/CT as described above was performed immediately after cell inoculation and then according to individual growth rate till animals needed to be humanely euthanized due to tumor load.

Accuracy of fusion

Accuracy of fusion was verified by checking DLIT – surface alignment with outer shape of animal as depicted by MRI. In all presented animals DLIT-surface accurately aligns with outer shape of animal.

DLIT/MRI vs DLIT/CT Hepa 1c1c7 Model

a. Immediately after cell inoculation. DLIT signal can be found associated with spleenic injection site and along portal vein and its collaterals (yellow arrows) in T2 weighted MRI. b. At day 21 p.i. a huge tumor adjacent to spleen parenchyma but within spleen capsule developed with DLIT signal mainly associated with cellular areas in T2 weighted MRI. CT doesn’t reveal these details. T2 weighted MRI also reveals areas of necrosis (yellow circles) and vessels (red arrows) within tumor. c. 2 DLIT signals associated with portal vein and a collateral can be found in liver parenchyma lacking a morphological correlate (yellow arrows). CT doesn’t reveal these details. Presented data is not quantitative. For quantification refer to d.

DLIT/MRI vs DLIT/CT Osteosarcoma Model

28 days after cell inoculation T2 weighted images show diffuse signal intense infiltration of lower hind limb. The distal part of the pathology is of lesser T2 signal intensity indicating the presence of necrosis. This is supported by the fact that DLIT signal colocalizes mainly with upper, cellular, part of the pathology. Apart from soft tissue swelling no further information can be obtained from DLIT/CT.

Summary, Conclusions and References

VivoFuse™ enables sequential combined DLIT/MRI. Information content compared to DLIT/CT increases dramatically by maintaining ease of use and throughput of DLIT/CT. Luc positive cells can be precisely allocated to anatomical structures as provided by MRI. Once tumors grow to a certain size BLI signal does not accurately reflect tumor load which can be compensated by additional MRI. Furthermore components of the tumor micro environment such as necrosis, cellularity and vascular structures can be distinguished.