A multimodal animal cradle inlay system to facilitate optical, PET, MCT, and MRI co-registrations of a metastatic, colorectal cancer syngeneic mouse model

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Introduction

- Multimodal imaging can enhance studies of pre-clinical animal research models. Investigators in the fields of oncology and immuno-oncology have sought to add *in vivo* optical imaging data to complement PET/MCT/MRI analyses. In the proof-of-concept study presented here, we sensitively monitored and functionally characterized the progression of a lung metastatic colorectal cancer model, across the imaging modalities of optical (bioluminescence imaging, BLI), PET, MCT and MRI.
- A novel (experimental only) Cradle Inlay system allowed mouse anatomical positioning to be conserved across the range of imaging devices used. Furthermore, resulting image data was then driven to a centralized database via pmod 4.5 SDMS for data consolidation and multimodal data presentation, analysis and fusion—allowing the production of powerful, multiparametric evaluations of the disease model.

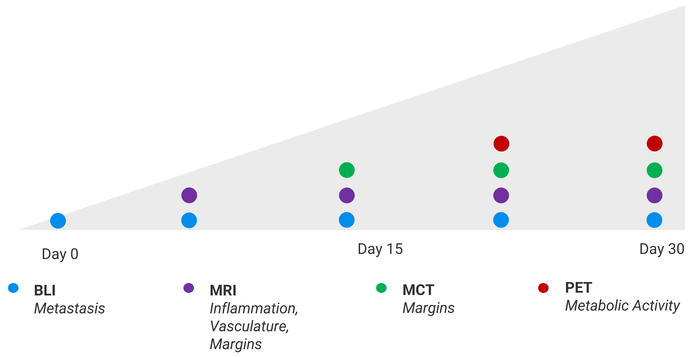

Figure 1. Multimodal monitoring of lung metastasis progression.

Methods

- **Mouse Model & Imaging Schedule.** A colorectal lung metastatic murine model was initiated by giving Balb/c female mice, 8 weeks of age (n=6), an IV tail vein inoculum of $5x10^5$ colorectal carcinoma cells (CT26.WT-Fluc-Neo, developed by Imanis Life Sciences, in turn, derived from ATCC CRL-2638), that expressed Red Firefly Luciferase. This murine model was set up by Hera Biolabs. Mice were imaged at several timepoints, across multiple modalities, over the span of 29 days (Figure 1).
- **Experimental Cradle Inlay System.** An experimental Cradle Inlay system was used to transfer mice between a set of imaging instruments (all from Bruker BioSpin) used to acquire images in the following modalities: bioluminescence (BLI) optical (Spectral Lago X), PET/MCT (PET/CT Si78) or MRI (BioSpec 7T). Critically, while being imaged across these device platforms, mouse anatomical positions were kept constant through a Cradle Inlay locking pin system designed to secure the inlay into each base cradle used (Figure 2).
- **Pmod v4.5 SDMS data consolidation.** All image DICOM files were directly pushed from individual imaging systems to a centralized database, located in a server workstation. This data organization allowed for multimodal image data presentation, analysis and fusion. The pmod image fusion tool (PFUS) has been used to apply calibrated, reproducible multimodal image fusions, e.g., for the fusion of BLI (Spectral Lago X) and MCT (PET/CT Si78) datasets.
- **Optical Scans.** All BLI optical scans were acquired in a Spectral Lago X, using Aura software version 4.5.1. Prior to BLI, mice were anesthetized by isoflurane vapor (in a Somni Scientific AMD-3+, EPS-3 system) and injected subcutaneously with freshly-prepared D-Luciferin substrate (GoldBio,150 mg/kg in 1XPBS). Mice were allowed to awaken briefly for 1 minute, re-anesthetized, and then imaged at 18 minutes post-substrate injection, a moment within the peak of the model's BL kinetic curve.
- **PET/MCT Scans.** Mice received 4-8 MBq 18F-FDG. Following 45 minutes uptake, mice were scanned (PET/CT Si78) for 20 minutes. PET data was reconstructed using MLEM-MAP, Voxel Size: 0.25 mm, 18 iterations. CT scans were performed at 200 µm, 0.8 mm angles, 360˚ projections, and 3 averages.
- **MRI Scans.** T2-TurboRARE MRI (BioSpec 70/20, 40mm volume coil) scans were made of the lung region using parameters TE/TR:24/830 ms, resolution 100X100 $µm^2$, slice thickness: 1 mm, scan time: > 3 min 26 secs, respiratory trigger per slice.

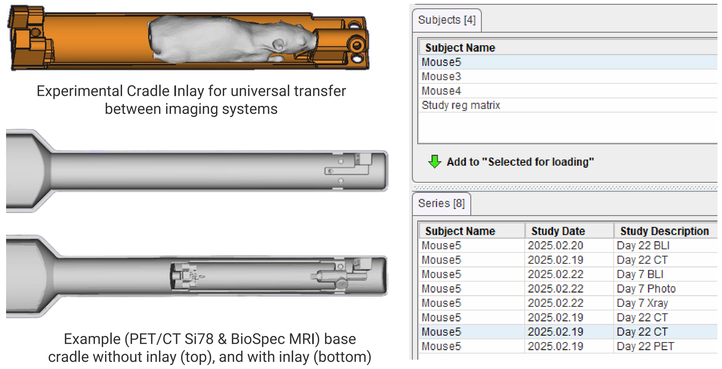

Figure 2. Cradle Inlay System (experimental only) and pmod 4.5 SDMS study consolidation.

Results

- **BLI:** BLI screening determined that all 6 CT-26 inoculated mice developed pulmonary tumor engraftments. BLI detected these CT-26 cell engraftments as early as Day 1 post-inoculation (Figure 3A), the first imaging time point performed. A persistent increase in viable CT-26 cell burden over the study time course was suggested by a continued increase of *in vivo* BL signal levels over time (Figure 3B). BLI monitoring also detected the occurrence of secondary, abdominal metastases in 1 of the 6 engrafted mice (an example is shown in Figure 3A). Abdominal metastases were later confirmed by PET and MRI (Figure 4B and 6A). **Note:** BLI detected pulmonary CT-26 cells at Day 1 post-inoculation, while other modalities (Figures 4, 5, and 6) detected tumors and associated pathologies starting at approximately Day 7 (MRI), Day 14 (MCT), and Day 22 (PET).

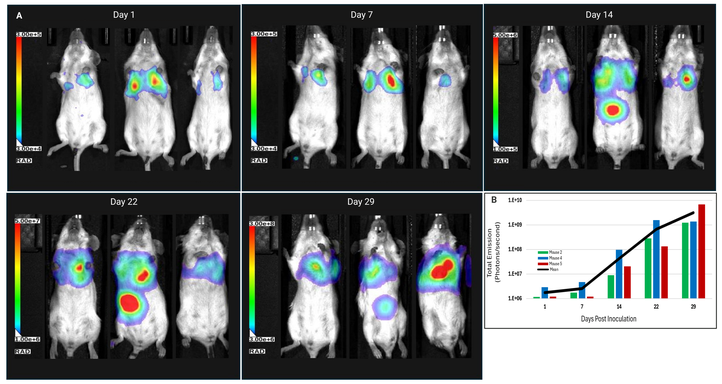

Figure 3. BLI *in vivo* monitoring of CT-26 tumor progression over time.

- MRI: MRI detected CT-26 tumor-associated inflammation as early as Day 7 post-inoculation (white arrows, Figure 4A), while tumor margins (orange arrows, Figures 4A, 4B) and tumor-associated vasculature (red arrows, Figure 6D) were observed at later timepoints (Day 15 and 22) post-inoculation. MRI tumor margin resolution was improved by "Smart Noise Reduction" analyses (Figure 4A, lower panel series).
- MCT: MCT clearly detected pulmonary loci of CT-26 tumors (Figure 5A), and segmental analyses of normal mouse lung volumes, starting on Day 7, were seen to decline over time (Figure 5B).
- BLI/MCT/PET/MRI: Multimodal imaging analyses of mouse CT-26 tumor engraftments collectively provided a multiparametric evaluation that included: tumor distributions (BLI/MCT/PET/MRI, Figure 6A), tumor volume (MCT, Figure 6B), tumor metabolic activity (PET, Figure 6B), tumor segmentation (MCT, Figure 6C), tumor margins (MRI, orange arrow, Figure 6D), and tumor-associated inflammation and vasculature (MRI, red arrow, Figure 6D).

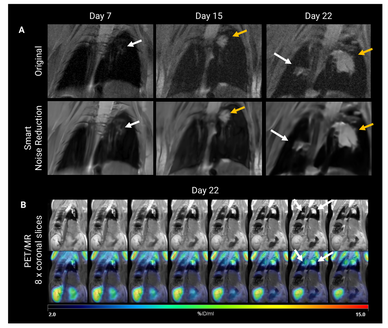

Figure 4. MRI *in vivo* detection of CT-26 tumor margin and tumor-associated inflammation (A). PET/MRI fusion of lung tumor inflammation and tumor tissue metabolic activity (B).

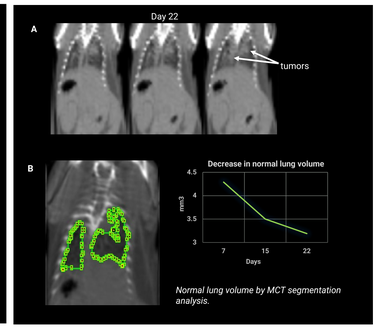

Figure 5. MCT *in vivo* detection of pulmonary CT-26 tumors (A). MCT segmental analysis of normal lung volume (B)

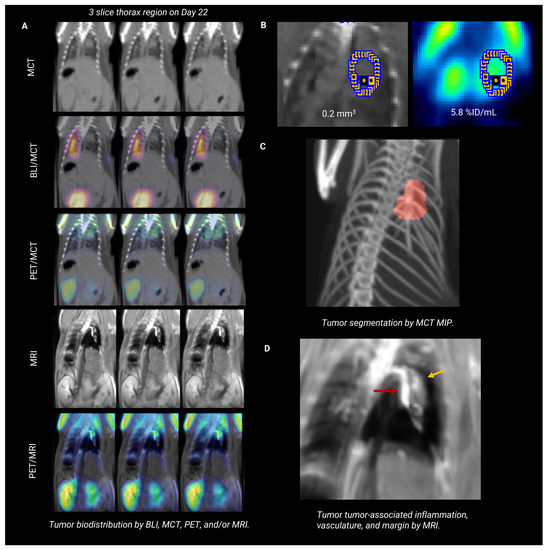

Figure 6. Multimodal acquisitions and multiparametric analyses made possible through the combined use of a new (experimental only) Cradle Inlay system, along with pmod 4.5 SDMS for data consolidation.

Summary

- An experimental Cradle Inlay system enabled easy, multimodal *in vivo* imaging of a pulmonary oncology mouse model across BLI, PET, MCT, and MRI platforms.
- Pmod 4.5 with SDMS was used: (1) to centralize and make available all DICOM files of resulting datasets in a single workstation, and then (2) to enable a full range of analyses, including image segmentation, statistics, and fusion.
- A multiparametric model evaluation was achieved by a combined use of an experimental Cradle Inlay system and pmod 4.5. Tumor-associated parameters analyzed included: distribution (BLI), 3D tomography (PET, MCT, MRI), metabolic activity (PET), inflammation (MRI), margins (MRI), and vascularization (MRI).

Acknowledgment

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