The value of micro-CT to monitor lung metastasis in an immune-competent mouse cancer model

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Aims
Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells and is a leading cause of death worldwide. Today, cancer accounts globally for one in every eight deaths. In 2012, there were an estimated 14.1 million cases of cancer diagnosed and 8.2 million deaths from the disease around the world. Moreover, the global cancer burden is growing at an alarming pace: in 2030 alone, about 21.7 million new cancer cases and 13.0 million cancer deaths are expected to occur, simply due to the growth and aging of the population [1].

With more patients dying from metastasis (i.e. the dissemination of the cancer) than from primary cancers, metastasis is a very important area in cancer research. Investigators thereby heavily rely on animal models of metastasis to common organs such as the lung in order to improve our insight into the pathogenesis and to research novel therapeutic approaches to combat the dissemination of cancer cells from primary tumors. In previous studies, mostly human-mouse xenograft models were used to study lung metastasis, in which human cancer cell lines are injected into immunocompromised mice. Disadvantages of these models include that tumors are a mixture of human cancer cells and mouse cells, which can influence metastasis. Moreover, as the mouse needs to be immunocompromised in order for human tumors to grow, the ability to examine the role of the immune system in cancer dissemination is lost in these models.

In this experimental context, novel tools that allow longitudinal monitoring of lung metastasis in individual animals are highly needed. We have therefore evaluated for the first time micro-computed tomography (μCT) as a very efficient means to non-invasively and repeatedly monitor metastasis to the lung in individual, free-breathing syngeneic mice, and compared it to bioluminescence imaging (BLI).

Method
Two individual clones of KLN205 cancer cells were intravenously injected in syngeneic DBA/2 mice and lung metastasis was weekly monitored during 3 weeks using μCT and compared to the current gold standard histology and the more recently introduced bioluminescence imaging (BLI). Retrospectively respiratory-gated μCT datasets of free-breathing, anesthetized mice (1.5-2% isoflurane in 100% oxygen) were acquired on a dedicated small animal μCT scanner (SkyScan 1076, software version 4.2, Bruker microCT, Kontich, Belgium) [2]. Briefly, images were acquired in list-mode with the following parameters: 50 kVp X-ray source voltage and 180 µA current combined with a composite X-ray filter of 0.5 mm aluminum, 120 ms exposure time per projection, 9 projections per view, acquiring projections with 0.7° increments over a total angle of 180°, 2 cm field of view covering the lungs, producing four reconstructed 3D datasets with 35 µm isotropic voxel size corresponding to four different phases of the breathing cycle (4D). Here we report data at end of expiration. Software provided by the
manufacturer (TSort, NRecon, DataViewer and CTan) was used to retrospectively gate, reconstruct, visualize and process μCT data as described [3]. Quantification of the mean lung density (CT number, expressed in grey values), lung tissue (including tumor) volume, aerated lung volume and total lung volume was done for a volume-of-interest covering the lung, comprised of regions-of-interest that were manually delineated on the coronal μCT images, thereby avoiding the heart and main blood vessels [4, 5]. The threshold used to distinguish aerated from lung tissue volume was manually set at 50 and kept constant for all datasets.

Figure 1: Representative BLI, μCT and ex vivo read outs. Lung BLI and μCT images at baseline, and 1, 2 and 3 weeks after intravenous injection of KLN205 cells for a representative mouse with low, medium and high lung metastasis load. A photograph and H&E staining of the corresponding dissected lungs are shown.
Results

μCT enabled us to visualize diffuse tumor morphology (Figure 1), but also to extract four different biomarkers that quantify not only tumor load, but also aerated space in the lung as a marker of vital lung capacity and potential compensatory mechanisms (Figure 2). Complementary to BLI, applying this novel μCT-based approach enabled us to sensitively and efficiently unravel differences in metastatic potential between two cellular clones.

Figure 2: Longitudinal in vivo lung μCT-derived biomarkers to monitor lung metastasis

Graphs present longitudinal changes of four lung biomarkers quantified from the μCT images. Graphs (A,C,E,G) show results for all individual mice (n=15-18) and graphs (B,D,F,H) for groups of mice injected with different KLN205 cell clones for the following biomarkers. Data represent mean (n = 6-9) ± standard error. * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001; ns = not significant; vx = voxels; a.u. = arbitrary units.
**Conclusion**

µCT and BLI offer biomarkers that describe different and complementary aspects of lung metastasis, underlining the importance of multi-modality follow-up. The added value of µCT-findings is important to better assess lung metastasis and host/lung response in preclinical studies, which will be valuable for translational applications.

**References**