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QUANTITITATIVE, DYNAMIC AND LONG TERM *IN VIVO* IMAGING OF INTRAVASCULAR CIRCULATING TUMOR CELLS IN AWAKE ANIMALS, WITH A NOVEL MINIATURE FLUORESCENCE MICROSCOPE

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Introduction - Metastasis, the cause for 90% of cancer mortality[1], is a complex, poorly understood process. Circulating Tumor Cells (CTCs) provide a window into metastasis and are a biomarker bearing great potential prognostic value[2]. However, the rarity of these cells and a lack of reproducibility, specificity or sensitivity are rendering their interrogation by current techniques very challenging. Recent studies have shown that blood vessel invasion may happen at a very early stage, before the main tumor is detectable[3]. To provide an insight into this 'black box', we developed a novel intravital miniature microscopy setup capable of real-time long-term monitoring of CTCs in awake small animals. This system has the potential to uncover CTC dynamics over a tumor's lifetime - from primary tumor to metastasis.

Methods - As an alternative to conventional benchtop systems that are expensive, non-scalable and lack continuous imaging capabilities, we have engineered a miniature intravital microscopy system (IMM) for long term imaging in awake animals. The IMM can be mounted on the back of a mouse and is composed of a Dorsal Skinfold Window Chamber giving access to superficial vasculature, a custom-designed holder, and a novel lightweight Miniature Intravital Fluorescence Microscope with an excitation source at 488nm. Using a lentiviral construct encoding a fusion reporter gene, luciferase (Luc2) and enhanced Green-Flurescence Protein (eGFP), we have also developed an imageable orthotopic 4T1 model for breast cancer metastasis.

Results - In this model, following orthotopic implantation in mice in the mammary fat pad (n=20), primary tumor growth and metastatic burden can be monitored by whole-body bioluminescence imaging while CTCs can be followed continuously by IMM fluorescence imaging. We have demonstrated that we can observe lung metastasis as early as 6 days after primary tumor induction, with further metastatic sites in the liver and bone. This provides us with a window of 6 days where we propose to observe the continuous kinetics of CTCs using the IMM. We have monitored blood vessels of various sizes (40 - 90µm) for long periods of time (t=2h) in mice bearing a fluorescent model of metastatic breast cancer, based on systemic injection of labeled 4T1 cells (n=5). Using an in-house algorithm, we are able to detect, count and compute CTC trajectories, speed, and dynamics.

Conclusion - These data represent the first reported use of a miniature intravital microscopy setup for in vivo long-term imaging of CTCs in non-anesthetized animals and demonstrate its potential to uncover unexplored aspects of cancer metastasis.

Acknowledgements - This work has been supported by a Fulbright Science and Technology Fellowship Award, a Stanford Weiland Family Fellowship, a Graduate Student Fellowship from the Society for Nuclear Medicine and a Center for Cancer Nanotechnology Excellence grant.

References 1.Wittekind C, Cancer Invasion and Metastasis. Oncology. 2005.2.Paterlini-Brechot P, Circulating tumor cells (CTC) detection: clinical impact and future directions. Cancer Lett. 2007 3. Gray JW. Evidence emerges for early metastasis and parallel evolution of primary and metastatic tumors. Cancer Cell. 2003.