CERTIS™ ONCOLOGY INTELLIGENCE

Development of Bioluminescent PDX Models to Study Metastasis and Evaluate Therapeutic Interventions

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ABSTRACT

Metastatic disease continues to be a significant cause of mortality among cancer patients. Despite advancements in patient-derived xenografts (PDXs), translational models of metastasis are lacking. To overcome this challenge, we have developed bioluminescent patient-derived and PDX-derived cultures and utilized optical imaging to create robust translational *in vivo* models of metastasis. Here, we show that these models can be implanted orthotopically into common metastatic sites or injected intravenously or intracardially to study spontaneous metastasis. Furthermore, we apply these models in humanized NOG-EXL (hGM-CSF/hIL-3 NOG) mice to demonstrate immunophenotypic differences among tumors in different anatomical sites and evaluate the pharmacology of checkpoint inhibitors.

METHODS

Three-dimensional spheroid cultures were generated directly from patient biopsies or from patient-derived xenograft models. To generate luciferase expressing cells for implantation, single cell suspensions were transduced with the Luciferase, firefly (CMV, Puro) virus (Gentarget: LVP325) and maintained under antibiotic selection with 0.5ug/ml puromycin dihydrochloride. Luciferase expression was validated using the Bio-Glo™ Luciferase Assay System (Promega: G794) prior to implantation.

For intracardiac injection, $1.0x10^6$ cells were resuspended in 100uL of PBS and injected into the left ventricle of animals. For intrapulmonary injections, $1.0x10^6$ cells were resuspended in 100uL of PBS and injected into the superior lobes of the left lungs. For intracranial injections, $0.3x10^6$ cells were resuspended in 4uL of PBS and injected into the striatum using a Hamilton syringe and Pump 11 Elite Nanomite from Harvard Apparatus.

Animals were monitored weekly for bioluminescence (BLI) using the Vilber NEWTON 7.0. Histology on formalin-fixed paraffin embedded tumors samples was performed using anti-human nucleoli (Abcam: ab190710), anti-CD8 (Fisher Scientific™: MS457SO), and Foxp3 (Cell Signaling: 98377S). Spectral flow cytometry was performed using the from Cytek™ Biosciences Aurora 3.

CONCLUSIONS

The development of bioluminescent patient-derived and PDX-derived models enabled the study of late-stage and spontaneous metastasis, through orthotopic implantation or intravenous/intracardial injection, respectively. We demonstrate the application of this model to humanized NOG-EXL mice and recapitulation of checkpoint inhibitor activity. This platform will enable additional insights into the molecular underpinnings of metastasis and facilitate the preclinical study of experimental immunotherapies.

CITATIONS & ACKNOWLEDGEMENTS

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All animals were provided or sponsored by TACONIC Models For Life.

RESULTS

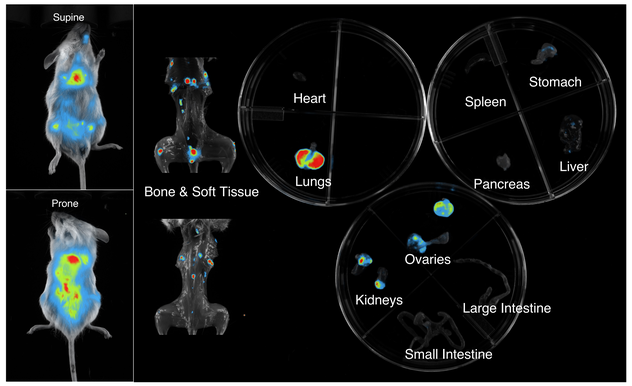


Figure 1. Intracardiac injection of CRT_295luc. Animals were injected with a lung adenocarcinoma line derived from a metastatic lesion on the iliac crest. Representative whole-body imaging of a mouse in supine and prone positions shows BLI signal throughout the body. *Ex vivo* imaging reveals signal in the spine and bones, lungs, ovaries, kidneys, pancreas, stomach and liver.

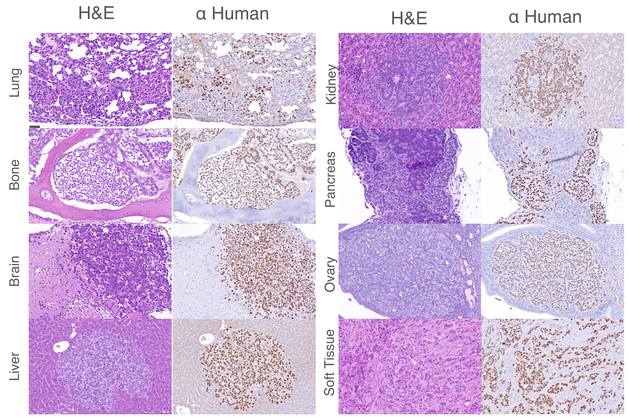
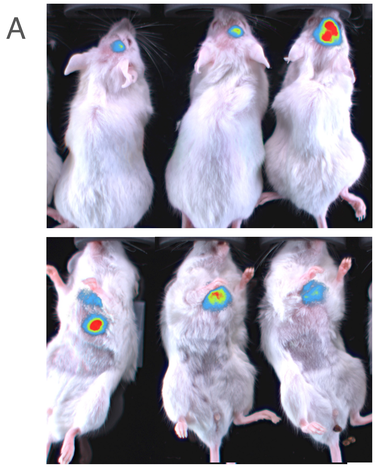


Figure 2. Histology of and immunohistochemistry of distant organs from intracardiac injection of CRT_295luc. Representative images of hematoxylin and eosin, and anti-human nucleoli IHC confirm infiltration of tumor cells into distant organs. Scale bar: 40 uM.



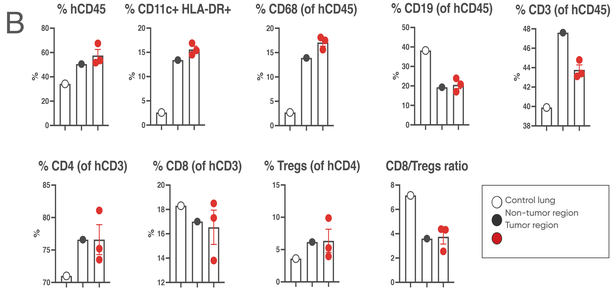
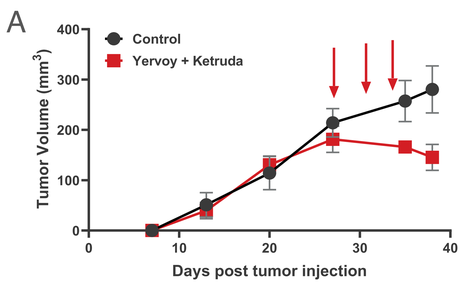


Figure 3. Orthotopic implantation of luc-lines in CD34+ humanized NOG-EXL animals. **A.** Bioluminescence Imaging of intracranial and intrapulmonary injections of CRT_295. **B.** TIL analysis of the lungs from control animals, and the injected and contralateral lungs of implanted animals.



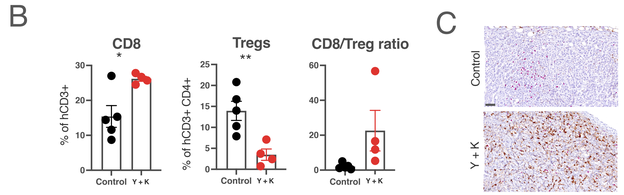


Figure 4. *In vivo* pharmacology of subcutaneous CRT_295 in CD34+ humanized NOG-EXL animals using checkpoint inhibitors. A. Three administrations of Ipilimumab and Pembrolizumab results in tumor growth inhibition. **B.** Treatment induced a significant increase in CD8 cells and significant decrease in Tregs in tumors. **C.** Histological confirmation showing differences in CD8 (brown) and Foxp3 (red) in treated and control tumors. Scale bar: 50 uM.

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