Establishment of Orthotopic Syngeneic Models using Bioluminescent Imaging to Recapitulate the Tumor Microenvironment and Evaluate Immunotherapies

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INTRODUCTION

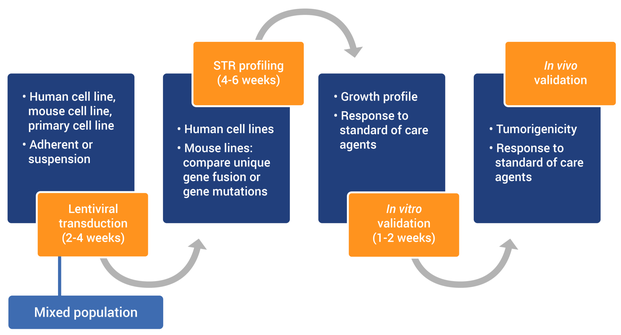
The emergence of immuno-oncology (I/O) therapies has shown promising benefits in various cancer types. The full potential of these therapies are still being realized, with the use of combinatorial treatments and overcoming resistance being areas of significant interest. Subcutaneous syngeneic models have been widely used to evaluate the efficacy, and to understand the mechanisms of action, of I/O therapies. One main limitation of subcutaneous models is the lack of a clinically relevant tumor microenvironment (TME), which is known to play a pivotal role in the success of I/O therapies. More clinically relevant TME can be established with orthotopic models where tumor cells are inoculated in a relevant organ-specific location to recapitulate the immune and stromal component interactions with the tumor, which can also facilitate metastatic spread. Using bioluminescent imaging (BLI), tumor development and progression, together with response to therapy, can be monitored in real-time.

We have generated a panel of bioluminescent syngeneic cell lines that are used to evaluate various therapies in both orthotopic and metastatic settings.

METHODS

To facilitate measurement of tumor development in immune competent murine models, a series of bioluminescent variants of syngeneic cell lines were generated in house by transducing the cell lines with a lentiviral vector carrying the firefly luciferase gene. This panel represents a diverse range of cancer types including liver (Hepa 1-6 and H22), breast (4T1), colon (CT26.WT), brain (GL261), lung (LL/2), and pancreas (Pan02). These bioluminescent cells were orthotopically implanted into immune competent mice, where primary tumor growth was measured by imaging using the IVIS® SpectrumCT *In Vivo* Imaging System.

Fig 1. Generation of bioluminescent cell models



RESULTS

Fig 2. Establishment of baseline response to standard of care and checkpoint inhibitors in the subcutaneous and orthotopic setting. H22 or Pan02 cells were subcutaneously or orthotopically implanted into mouse liver or pancreas. Following tumor establishment, mice were treated with vehicle, sorafenib, and/or anti-mCTLA-4. Tumor development was monitored by caliper **(A)** or imaged using bioluminescence **(B and C)**, the latter were used to quantitate orthotopic tumor development and data are shown as total flux (photons/second). Both sorafenib and anti-mCTLA-4 in the H22 subcutaneous setting were found to significantly inhibit tumor development ($p<0.001$, two-way ANOVA) but no effect was observed in the orthotopic setting. Anti-mCTLA-4 led to more significant inhibition of Pan02 tumor growth in the orthotopic than in the subcutaneous setting ($p=0.0133$ vs $p=0.248$, two-way ANOVA)

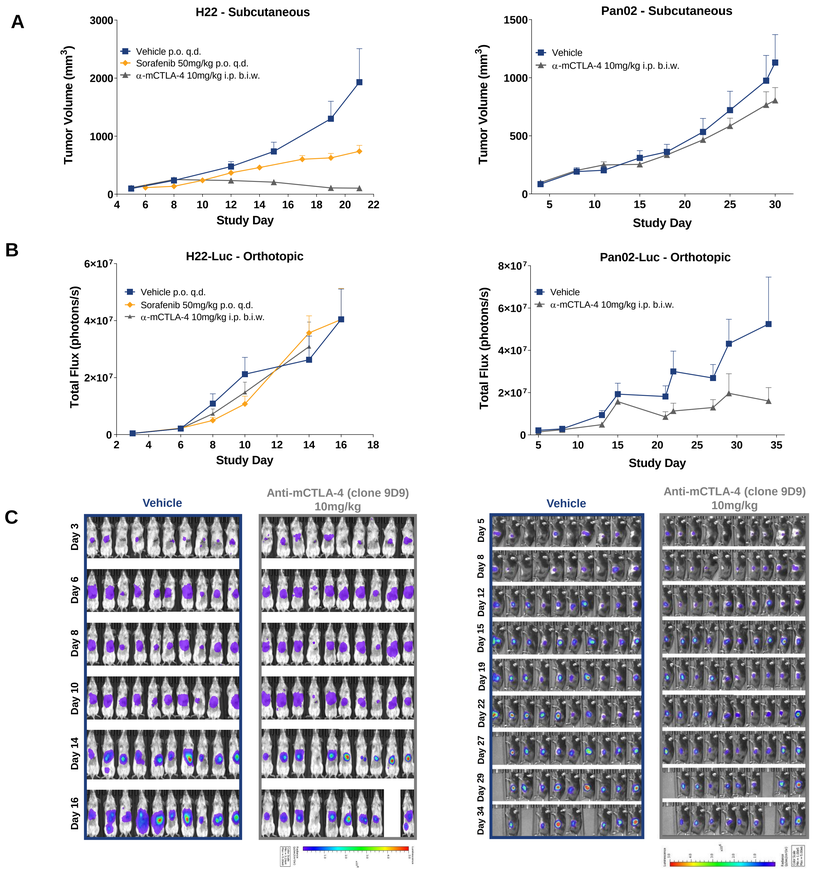


Fig 3. Bioluminescent imaging of CT26.WT-Luc tumor development. (A) CT26.WT-Luc cells were orthotopically implanted into mouse colon; **(B)** Tumor development was quantitated using bioluminescence data, with data shown as total flux (photons/second)

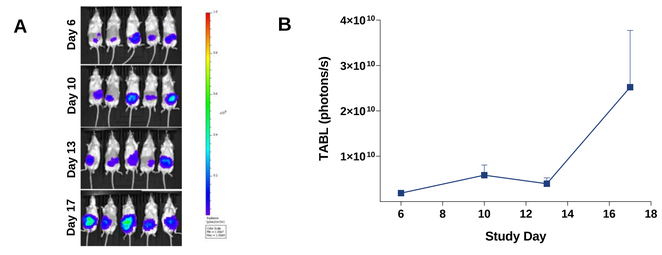
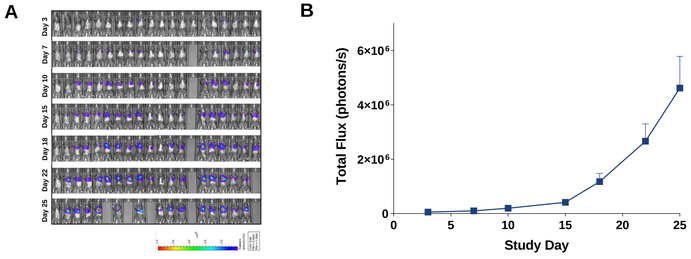


Fig 4. Bioluminescent imaging of LL/2-Luc tumor development. (A) LL/2-Luc cells were orthotopically implanted into mouse lung; **(B)** Tumor development was quantitated using bioluminescence data, with data being shown as total flux (photons/second)



SUMMARY

- Syngeneic orthotopic mouse models offer a valuable platform for testing I/O therapies with a more clinically relevant TME compared to a subcutaneous site
- The establishment of a range of models covering diverse tumor types allows for interrogation of the therapeutic potential of novel anticancer agents across various cancers
- Various responses were observed when compared with subcutaneous models. These models were first evaluated using various checkpoint inhibitors to establish baseline response. For example, Hepa 1-6 showed very good response to anti-PD-1 antibody in both the subcutaneous and orthotopic setting (data not shown). Interestingly, another liver cancer model, H22, responded to anti-mCTLA-4 antibody as a subcutaneous model but was refractory to the same dosing scheme in the orthotopic model. The Pan02 model showed greater response to the α-mCTLA-4 in the orthotopic setting than subcutaneous setting