Bioluminescent 3D Tumors with Immune Cell Co-culture for High Throughput Screening (HTS)

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ABSTRACT

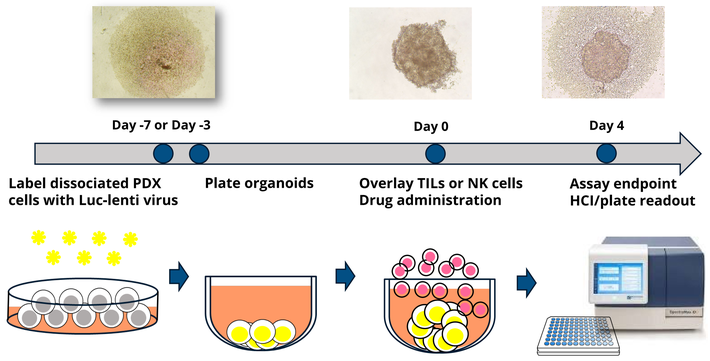
In the past decade, cancer immunotherapy has revolutionized treatment outcomes for patients with varied indications and thus offers an attractive option for drug discovery and development. Immunotherapies work by re-educating and boosting patient's immune systems to recognize and eliminate cancer cells. There is a growing need to recreate a relevant tumor immune microenvironment (TIME) that is easily traceable to quickly delineate the effect of fine tuning of the different compartments.

Recent advances in the Champions TumorGraft3D (CTG3D) platform has not only generated robust models for preclinical drug evaluation, but also elevates the translational relevance by efficiently mimicking the interactions between cancer organoids and immunocytes. Currently, available analytic approaches for coculture models are limited and are highly reliant on flow cytometry and high-resolution confocal imaging endpoints. These are often slow, cost-inefficient, and cloud computing heavy thereby limiting the bandwidth of studies and decision-making.

Easily traceable tags (e.g. bioluminescent and fluorescent) are attractive options that can aid the establishment of reliable effector (E) cells to the tumors (T) cell conditions, thus enabling the user to test multiple test agents cost-effectively and rapidly. At Champions Oncology, we have generated a reliable and stable Luc and GFP-tagged CTG3D-immune co-culture platform across various tumor indications (including colon, prostate, gastric, non-small cell lung cancer, and leukemia) with autologous TILs, allogenic TILs, and expanded NK cells. Herein, we describe a Luc-CTG3D-3631 colon adenocarcinoma (COAD) autologous TILs co-culture model, where the data suggests that naïve TILs when cocultured with organoids have limited killing, however the efficacy of killing increases in a dose response manner when E:T ratios are manipulated in a CD3/CD28 activated model system. Similarly, we achieved targeted killing in prostate adenocarcinoma (PRAD) activated coculture models by changing the E:T ratios. Additionally, a killing effect up to 95% was observed with expanded NK-organoid coculture model in PRAD showcasing the versatility of the platform.

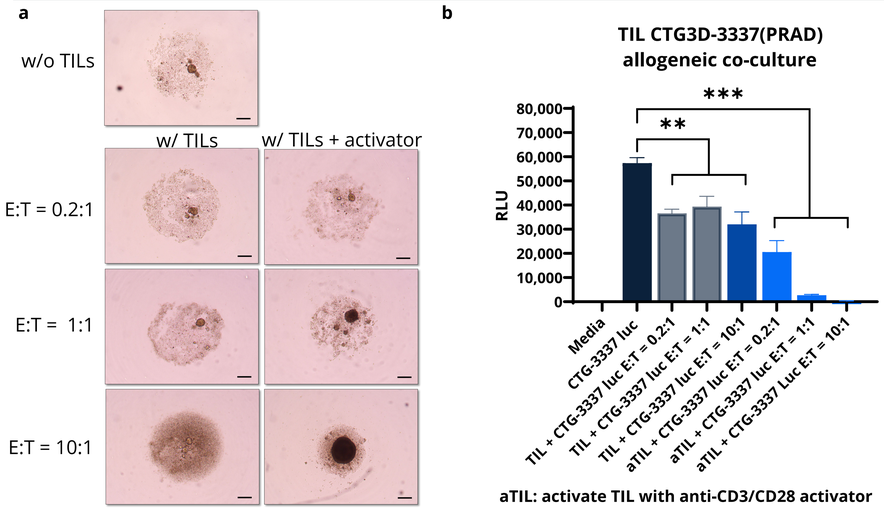
Furthermore, the original luc-labeled PDX cells can subcutaneously implant immune-compromised mice and maintain the bioluminescent signal for 12-16 weeks, thus allowing the end user to simultaneously validate conditions in vivo. Thus, this new luc-tagged CTG3D-immune coculture platform offers an exciting, fast, and cost-effective option to optimize the E: T ratios and monitor the IO drug performance both under in vitro and in vivo conditions.

MATERIALS & METHODS



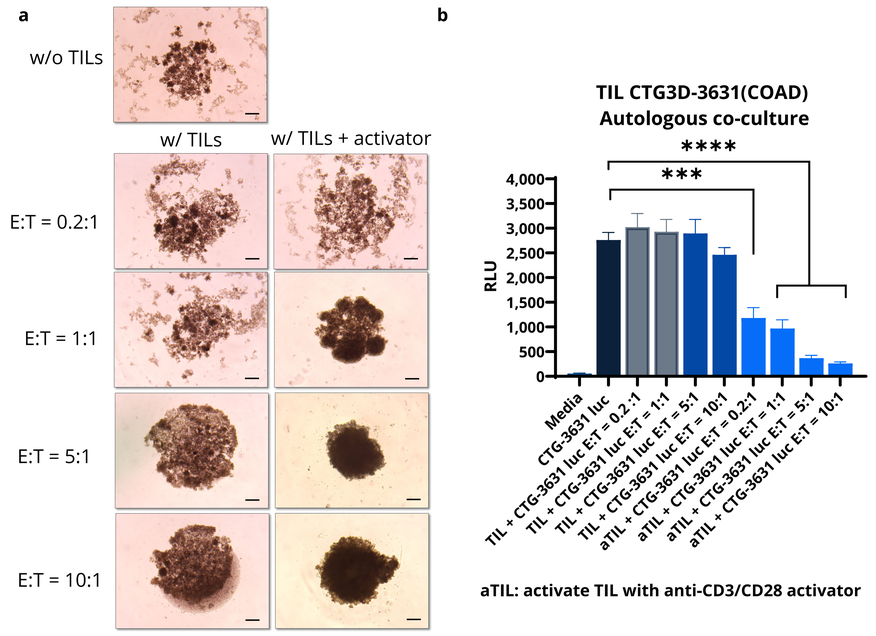
Surgical resection from patients were used to generate patient-derived xenografts (PDX) and tumor infiltrating lymphocytes in parallel (alternative options allogenic TILs or expanded NK cells). PDX were resected and digested to establish PDX-derived organoids. PDX tumor cells were transduced with firefly luciferase lentivirus and cultured for 3-7 days to form the 3D organoid structure. TILs and organoids were further coculture together with test agents for another 96 hours and endpoints are evaluated with bioluminescent readout.

RESULTS



aTIL: activate TIL with anti-CD3/CD28 activator

Figure 1. Allogenic TILs and Luc-labeled Organoids Co-culture. Luc-labeled prostate adenocarcinoma (PRAD) organoids were seeded in 96 well plates and were overlayed with allogenic TILs in ratio = 0.2:1, 1:1, and 10:1. Allogenic TILs and tumor organoids co-culture were subjected with and without anti-CD3/CD28 activator in quadruplicates. Tumor killing was analyzed after 96 hours of incubation. (a) Representative 4x bright field microscopy images, scale bar = 100 µm (b) Quantification of tumor killing effect in PRAD allogenic immune organoids co-culture. (multiple-comparison with Bonferroni test: ** p<0.01, *** p< 0.001).



aTIL: activate TIL with anti-CD3/CD28 activator

Figure. 2 Autologous TILs and Luc-labeled Organoids Co-culture. Luc-labeled colon adenocarcinoma (COAD) organoids were seeded in 96 well plates and were overlayed with autologous TILs in ratio = 0.2 :1, 1:1, 5:1, and 10:1. Autologous TILs and tumor organoids co-culture were subjected with and without anti-CD3/CD28 activator in quadruplicates. Tumor killing effect was analyzed after 96 hours of incubation. (a) Representative 4x bright field microscopy images, , scale bar = 100 µm (b) Quantification of tumor killing effect in PRAD allogenic immune organoids co-culture. (multiple-comparison with Bonferroni test: ** p<0.01, *** p< 0.001).

RESULTS

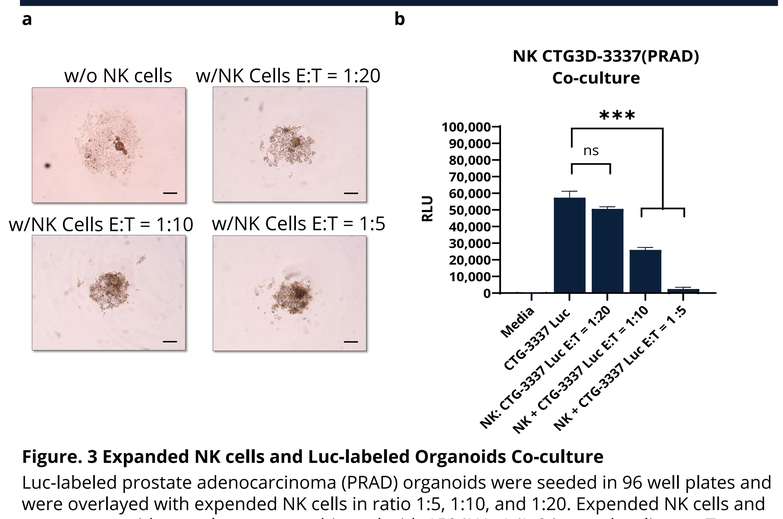


Figure. 3 Expanded NK cells and Luc-labeled Organoids Co-culture

Luc-labeled prostate adenocarcinoma (PRAD) organoids were seeded in 96 well plates and were overlayed with expended NK cells in ratio 1:5, 1:10, and 1:20. Expended NK cells and tumor organoids co-culture were subjected with 150 IU/mL IL-2 in quadruplicates. Tumor killing effect was analyzed after 96 hours of incubation. (a) Representative 4x bright field microscopy images, scale bar = 100 µm (b) Quantification of tumor killing effect in PRAD immune organoids co-culture. (multiple-comparison with Bonferroni test: ** p<0.01, *** p< 0.001).

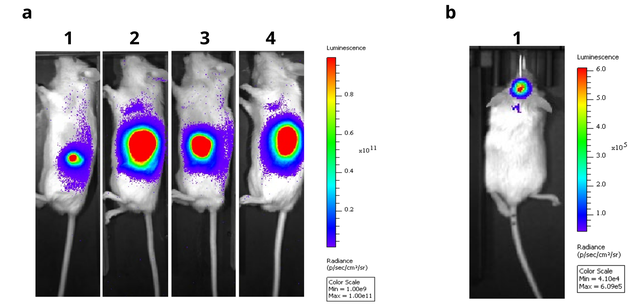


Figure. 4 Application of Luc-PDX Cells for in vivo Studies

(a) Luc-labeled Glioblastoma multiforme (GBM) PDX cells were subcutaneously (SC) injected in NSG mice to expand the materials for the next step in vivo studies. (b) Luc-labeled GBM-PDX were implanted in NGS mice via intracranial route for evaluating the therapeutic efficacy of test agent.

SUMMARY

- The established bioluminescent 3-dimensional immune organoids co-culture offer a quicker way to screen for novel test agents.
- The new bioluminescent labeling technique allow user to select the PDX and PDXO models with various genetic background from the bank to test drug's performance in tumor specific immune microenvironment.
- Bioluminescent CTG3D-immune coculture platform provides users a faster and cost-effective option to optimize the E: T ratio and evaluated the IO drug performance for high content or advanced endpoints.
- Bioluminescent PDX and PDXO cells provide user new options to evaluate therapeutic efficacy and monitor tumor metastasis via in vivo image system.

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