Quantify T Cell Response in 3D Tumor Spheroids Using Advanced Flow Cytometry and Live-Cell Analysis

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Summary & Impact

- Antigen recognition by T cells induces a cascade of downstream events which are critical in the immune system's fight against cancer.
- Robust in vitro assays are required to evaluate the potential for novel therapeutics, for example CAR-T cells and bispecific antibodies, to enhance T cell response against cancer.
- Conventional assays rely on the use of suspension cells or 2D cell monolayers, but these lack many of the complex cell-cell and cell-extracellular matrix interactions found in vivo.
- Here we provide two 3D tumor spheroid-based solutions to study immune cell-tumor interactions: immune cell killing (ICK) and tumor infiltrating lymphocytes (TILs).
- Therapeutics can improve T cell function in ICK by several mechanisms, including enhancing actuation and killing, reducing exhaustion and promoting memory T cell formation.
- High numbers of TILs are linked to increased response to neoadjuvant chemotherapy and improved pathological complete response rates.
- These data demonstrate the ability to study complex 3D tumor models using advanced flow cytometry and live-cell analysis-based workflows as a translational approach to in vitro characterisation of immunotherapeutics.

Activation status of TILs is higher than non-infiltrated T cells

- BT474 (breast cancer) spheroids were co-cultured with Incucyte®-labeled green labeled FRMCs (E:T, 1:1) and activated in well with CD3/CD28 Dynabeads for 40 h.
- T cell activation in non-infiltrated CD3+ cells was similar to external stimuli, with CD69 expression increasing from 2.3% to 66.5% at 4.7% with increasing Dynabead density.
- CD3+ TILs maintained high CD69 expression (average of 67.1 ± 3.9%) regardless of the number of Dynabeads present.
- Non-infiltrated TILs required 40-fold greater quantities of Dynabeads to elicit comparable activation marker expression.
- Control plots identify a clear increase in CD3+CD69+ TILs compared to non-infiltrated cells.

T cell activation and exhaustion during spheroid killing

- Incucyte®-labeled green spheroids were co-cultured with Dynabeads for 8 days.
- Incucyte® images quantified for spheroid fluorescence intensity on day 8 showed a 96.2% reduction in the presence of 50K Dynabeads compared to the non-activated control, indicating increased CD3+ cell death (representative images shown).
- Subsets analysis using FlowJo® cell characterization kits showed peak CD3+ cell death (early activation marker) expression on day 1 (68 ± 1% 30X Dynabeads), PD-1 peaked on day 4 then declined by day 8 (suggesting recovery from exhaustion after antigen clearance).
- Supersaturate samples were analyzed for cytokine concentrations using Qux biochip®. IFNγ and Granzyme B (not shown) both increased over time and with Dynabead density.

CD3/CD28 promotes transition to T memory cells during ICK

- BT474 spheroids were formed and incubated with FRMCs (E:T, 1:1) and Dynabeads for 8 days.
- After 8 h, spheroids were dissociated and cell subsets were analyzed using the Quix® Human T Cell Memory Kit.
- Dot plots show gating of Tcm (stem cell memory) and T effector (effectors) populations using the template provided by the kit.
- Kaplan-Meier survival curves from Qux biochip® show the shift from the early stage Tcm phenotype towards the later stage T memory phenotype with increasing Dynabead concentration. This is accompanied by a loss in stem renewal potential.

Spheroids containing fibroblasts have reduced numbers of TILs

- BT474 (breast cancer) spheroids were formed for 3 days before pre-activated Incucyte®-labeled green labeled FRMCs were added for 24 h.
- Spheroids were washed three times with PBS as per the iQue® Incucyte® assay protocol.
- Images taken using the Incucyte® after each wash step were used to validate the washing protocol.
- These images showed a clear reduction in the number of non-infiltrated immune cells surrounding the spheroid with each successive wash step, with negligible numbers remaining after the 3rd wash.
- Quantification of spheroid green intensity after the final wash indicated increased numbers of green labeled TILs with increasing concentrations of Dynabeads.

Targeted activation and killing by anti-HER2 CAR-T cells

- Spheroids formed from either a high HER2 expressing cell line, AUS5 cells (MFI, 1,600±100), or a HER2-negative cell line, MDA-MB-468 cells (MFI, 4,200±70) were incubated with anti-HER2 CAR-T cells (T:1) or non-transduced control T cells (CTL). 
- iQue® analysis at all HER2 targets was observed, but not of the HER2 negative targets or with the CTL.
- Specific killing was associated with increased activation marker expression and release of cytotoxic proteins in Granzyme B.