Scan the QR code to view more exemplar assay data here:

NEXUS BIOQUEST

3D tumour killing model

Screening different therapeutic modalities

Introduction

Tumour cell line spheroids co-cultured with or without immune cells allow the therapeutic assessment of novel checkpoint inhibitors, targeted monoclonal/ bi-specific antibodies, small molecule therapeutics or cell therapies in a system which more closely recapitulates solid *in vivo* tumours by including 3D structure. In this 3D spheroid model we test **antibody-drug conjugates, checkpoint inhibitor targeting antibodies** and **ADCC** modalities. The 3D spheroid model provides a robust model for lead candidate selection for *in vivo* immuno-oncology studies and can be performed with a bank of tumour line cell types and immune subsets.

Methodology

Fluorescently labelled tumour cells are seeded in 96 well plates and tumour spheroids generated. Immune cell subsets are added to the spheroids and immune mediated killing of tumour cells is measured by reduction of tumour spheroid area via live cell imaging on the CellCyte X. The ability of large molecules, small molecules or cell therapies at enhancing immune-mediated tumour killing is assessed, alone or in combination.

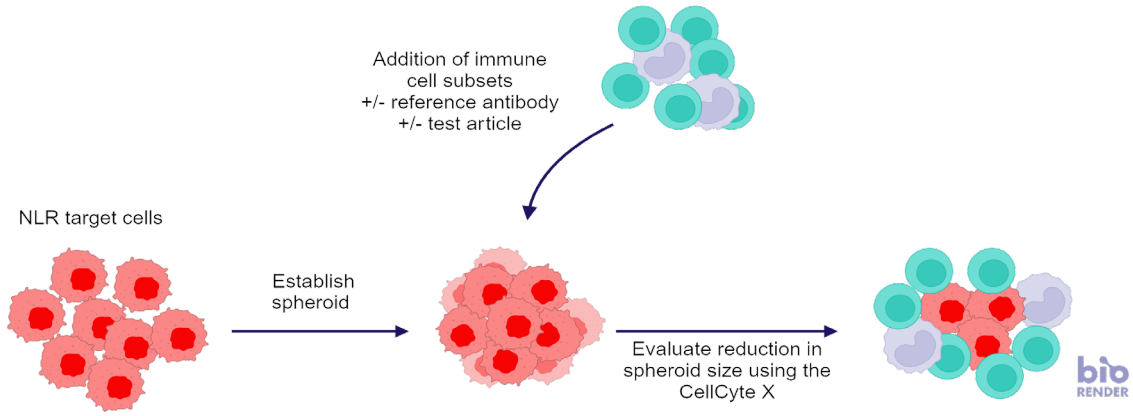


Figure 1: Antibody drug conjugates in a 3D model

These data show Trastuzumab deruxtecan-mediated killing as evidenced by a reduction in spheroid area in HER2-expressing SK-OV-3 and SK-BR-3 cancer cell lines. Trastuzumab deruxtecan did not induce killing in the HER2-negative BT-20 cancer cell line at any concentration. Percentage fold change in calculated spheroid area at 144 hours in Trastuzumab-deruxtecan compared to isotype control was determined **(A)**. Relative spheroid area normalized to T=0 was calculated for SK-OV-3 cells only **(B).**

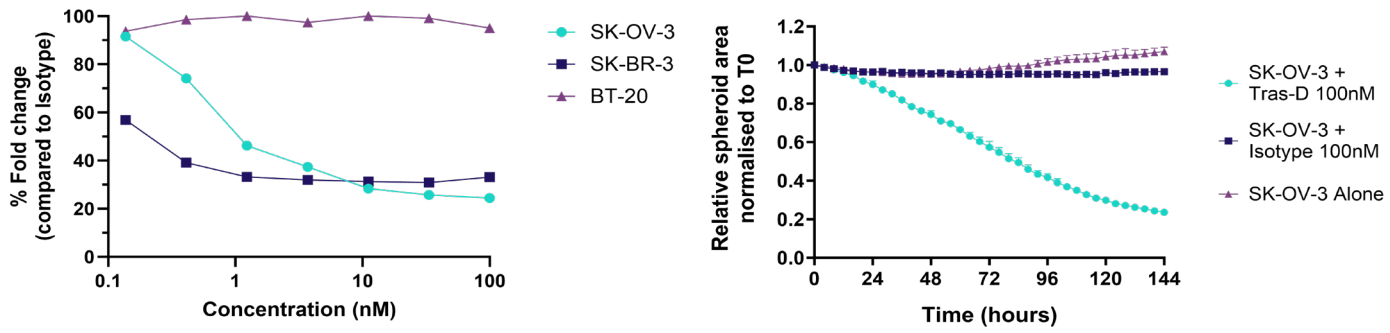


Figure 2: Checkpoint inhibitor sensitive 3D model

These data show the therapeutic window for checkpoint inhibitors (CPI) in a 3D immune mediated tumour killing assay. When PBMC +/- Pembrolizumab or IgG4 control are added to SK-OV-3 spheroids, tumour killing (spheroid reduction) is enhanced by CPI as compared to isotype control. Cells were imaged every 4 hours for a total of 144 hours using the CellCyte X to monitor tumour spheroid formation and area. Representative time course data (positive controls included) **(A).** Summary data in n=9 donors **(B).**

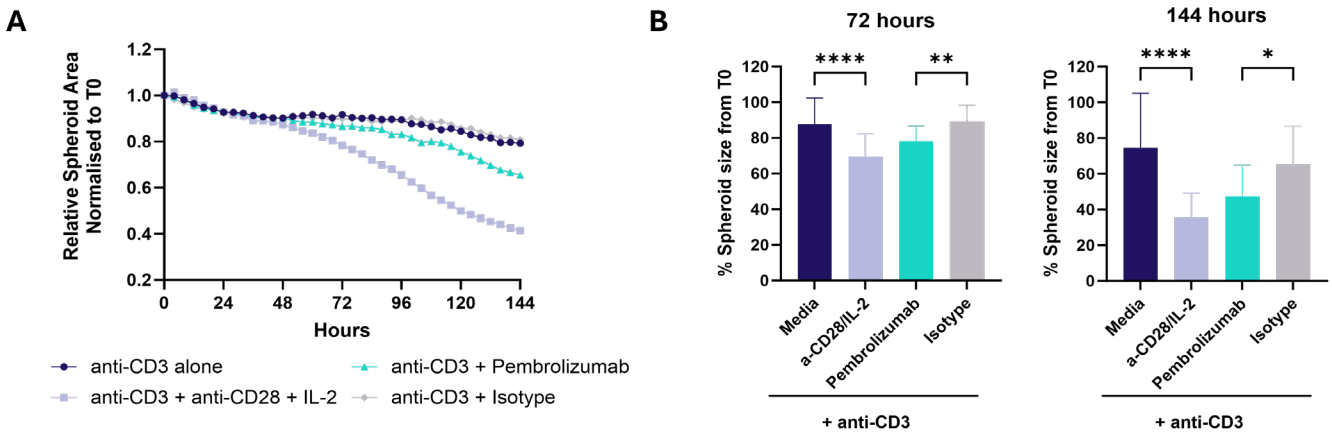
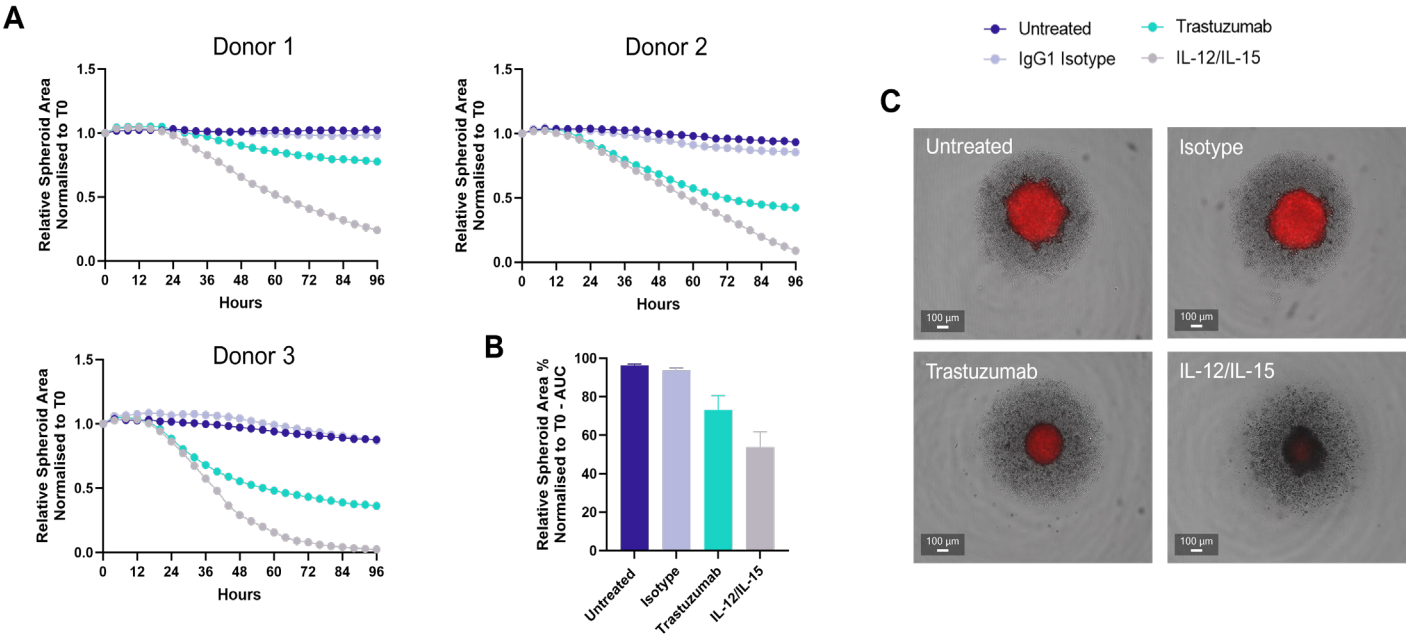


Figure 3: Antibody dependent cell cytotoxicity (ADCC) 3D model

These data demonstrate the therapeutic window for targeted monoclonal antibodies (ADCC) in a 3D immune mediated tumour killing assay (n=3 donors). When purified NK cells +/- Trastuzumab are added to SK-OV-3 tumour spheroids, tumour killing (spheroid reduction) is enhanced by targeted HER2 antibodies as compared to isotype control. Relative spheroid area (%) was measured **(A)** and the AUC statistics calculated using GraphPad Prism v9.5.0 **(B)** and live cell imaging representative images **(C).** Bar graph shows mean of n=3 donor tumour killing +/- SEM.



Conclusion

- At Nexus BioQuest, we offer a selection of clinically relevant NUCLight Red labelled tumour cells, from tissues such as lung, breast, colorectal, prostate, ovarian and melanoma.
- Our 3D spheroids can also be customised by incorporating additional stromal components or modifying immune cells to recreate the tumour microenvironment.

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