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COMMENTARY

Preclinical platforms to study therapeutic efficacy of human γδT-cells for oncology indications

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Abstract

In this commentary, we discuss recent advances in the study of γδT cell-based immunotherapeutics. As an allo-compatible cell therapy chassis without clear functional homologs in mice, γδT cells represent a challenge and an opportunity for preclinical modelling. We discuss some of the techniques and approaches that can be used to demonstrate and characterise γδT cell behaviour in biomimetic systems.

γδT-cells are a subset of tumour-reactive human lymphocytes that recognise malignantly transformed cells through an interconnected web of innate stress-sensing and adaptive receptors. These include NK-like receptors such as NKG2D and DNAM-1, and subset-dependent T-cell receptor classes that range from restricted to highly polyclonal.[1] Only primates possess human-like γδT-cell repertoires and ligands, substantially complicating credible in-vivo testing. Robust methods for pre-clinical γδT-therapeutic / solid tumour-microenvironment interactome simulation remain an unmet need.

Recent work from Ou and colleagues describes the development of a 3D ex-vivo multicellular patient-derived melanoma model, in which they screened γδT-cell immunotherapeutic performance in the presence of suppressive immune bystanders, fibroblasts and stroma. Importantly, the platform enabled the identification of clinically-translatable adjuvant therapies, such as checkpoint inhibitors and epigenetic modifiers. We expect a proliferation of γδT-therapeutic platforms as translation into the clinic accelerates, and briefly discuss considerations for the design of pre-clinical models.

1 | SOLID TUMOUR MODELLING

Solid tumours represent a highly complex environment. In modelling the disease (Figure 1), the first consideration is the tumour substrate. Patient-derived immortalized or primary material is now widely available for pre-clinical screening from commercial and academic suppliers.[2] An important factor for innate immunotherapeutics especially is whether the unmet clinical need is for immunologically 'hot' or 'cold', mutationally high- or low-burden tumours.[3] Parameters such as sourcing from primary tumour or metastasis can further inform platform design, should the therapeutic intent be, for example,

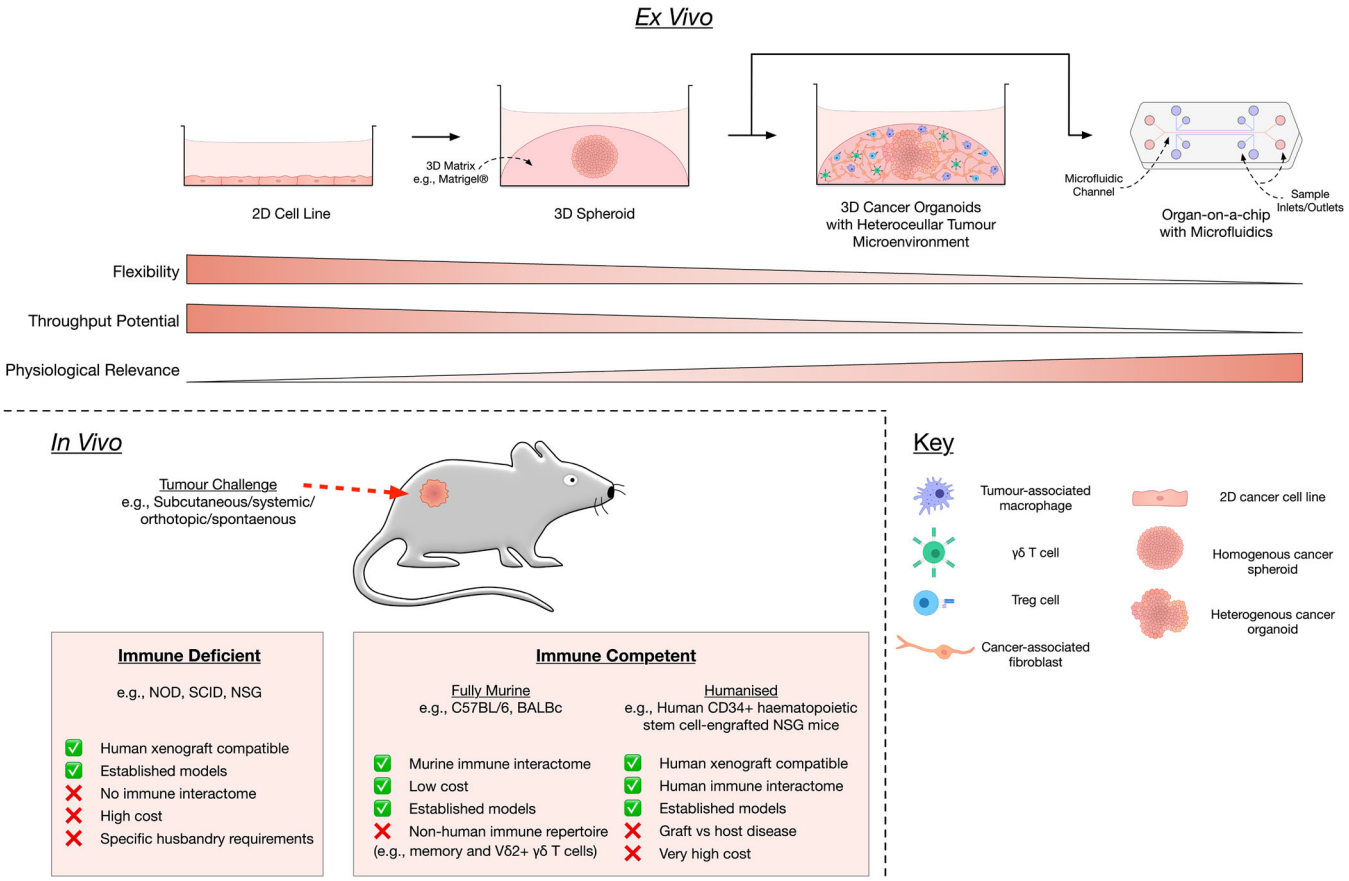

FIGURE 1 Modelling solid tumours across ex-vivo and in-vivo contexts

to treat dispersed metastases rather than large primary tumours.

1.1 | Ex-vivo

In the design of ex-vivo models, we next consider the model matrix. While often centring on generalizable, all-purpose materials such as Matrigel,[4] substantial recent advances have been made in studying the significance of the physical parameters of 3D cancer models, including stiffness and compartmentalisation.[5] Work from Bakkalci et al.,[6] for example, describes a model of bone cancer, ameloblastoma, which consisted of engineering active bone-forming stroma to probe the interaction of ameloblastoma with its native bony microenvironment. Such models are particularly attractive for studying phosphoantigen-reactive Vγ9Vδ2-T-cell immunotherapies that can be co-administered clinically with adjuvant aminobisphosphonate drugs of high hydroxyapatite affinity.

When considering bystander cells, Ou et al. identified the importance of suppressive immune infiltrate (e.g. tumour-associated myeloid cells), cancer-associated fibroblasts and stroma. Depending on the type of immunotherapy modelled, further data can be gleaned from including cell types that are activated upon immunotherapy administration, such as when dealing with secreted therapeutic modules like cytokines or opsonins.[7] While ideally autologous to the tumour, bystander cells allogeneic to the tumour can be used for modelling non-alloreactive therapeutics like $\gamma\delta$T-cells. Depending on availability and tolerance to various culture conditions, a mixture of primary and immortalized bystanders may be used, such as in colorectal carcinoma organoid modelling by Qin and colleagues.[4] When investigating the homing potential of cell-based therapies to a tumour and its microenvironment following intravenous infusion, 'organ-on-a-chip' microfluidic systems offer suitable advanced modelling strategies.

1.2 | In-vivo

While not always suitable, some pre-clinical questions can only be meaningfully probed using animals, which in practical effect often means murine models. Classically, this entails immunocompromised animals xenografted with human tumours and human T-cells. Such models have been important for assessing optimal

immunotherapy administration routes[8] and the potential for homing-enhancing co-treatments.[9] Conceivably, more sophisticated models could be developed with the knock-in of γδT-cell ligands and other interactome-relevant components, such as human *BTN* and *BTNL*.[1]

2 | EXTRACTING USEFUL INFORMATION

A major limitation for high-throughput pre-clinical γδT-cell modelling ex-vivo or in-vivo is the complexity of data acquisition and subsequent analysis arising from multi-component models. Historically, other than basic tumour luminescence, volume measurements or animal survival, γδT-cell / tumour architecture has been probed with immunohistochemistry or immunofluorescent microscopy, which is more recently aided by tumour and effector cell transduction with fluorescent proteins.[4] Substantial advances have been made in this area to improve throughput, parameter count and data processing with analyzers such as the PhenoCycler-Fusion (or CODEX) system that enables up to 50 simultaneous parameter analyses, organisation of cells into functional 'neighbourhoods' and visualisation of tissue architecture.

Disaggregated tissue analysis has also advanced from basic Western Blotting and low-parameter fluorescence-based cytometry to high-parameter spectral flow cytometry and cytometry by time of flight. Transcriptomic analysis too has advanced from bulk to single-cell to spatial single-cell RNA-sequencing, enabling the generation of ultra-high-resolution datasets.

As data generation methodologies continue to evolve, however, we note that data analysis techniques do not always keep up to speed. The bioinformatics required for meaningful high-parameter data analysis remains complex and is often hindered by a lack of access to relevant bioinformatics infrastructure and skills. Substantial improvement in the transparency of and access to algorithmic and statistical analysis tools is required to meaningfully interpret the multitude of data emerging from increasingly complex pre-clinical γδT-cell tumour models. Moreover, few widely used sorting algorithms currently provide resolution as far as γδT-cells are concerned, with γδT-cells often being sorted incorrectly based on the transcriptional signature, for example, into more widely recognised 'NK-cell' or 'cytotoxic $CD8^+$-αβT-cell' categories.[10]

γδT-cell-specific reagents, such as media or sequencing probes, remain few and far between. With the increasing clinical presence of γδT-cell immunotherapies, we expect this to change in the coming years.

3 | CONCLUSION

The greatest enhancement of γδT-cell immunotherapy design will arise from improvements based on data from well-designed and scientifically-probing clinical trials. As has become common practice for high-quality CAR-αβT studies, γδT-cell clinical trials need to expand their research component to include tracking of product transcriptome, persistence, homing to and infiltration into the tumour. Tumour-infiltrating γδT-cell phenotype needs to be characterised, correlated to clinical efficacy and compared across different malignancies and product manufacturing methods. Pairing high-resolution and research-oriented clinical trials with advanced γδT-specific pre-clinical modelling will aid the field in reaching its full potential. The work of Ou and colleagues demonstrates some of the potentials for using such modelling to inform the next generation of clinical studies.

When designing experiments that utilise a solid tumour model there are a variety of different options available. Traditionally, 2D cancer cell lines have been used which offer great flexibility and throughput potential, albeit at a cost in terms of physiological relevance in the context of a solid tumour and its microenvironment. More recent strategies involving cancer cell lines embedded in 3D matrices have increased in popularity, with spheroids being a common example. Aside from the 3D nature of this model, the matrices themselves also exert stiffness onto the cells which mimic the forces experienced in vivo. More sophisticated 3D models involving cancer organoids are increasing in popularity and availability. These heterocellular and self-organising systems can be derived from a variety of tissue sources (e.g. tumour resections and biopsies) and can be cultured alongside various immune and stromal cells, including anti-cancer cell-based therapies such as γδ T cells; these models aim to better recapitulate the complex tumour microenvironment surrounding a patient's tumour but result in a much more expensive and low throughput system. For experiments investigating the ability of cells to traffic and home across or between tissues, including extravasation from the vasculature, models involving microfluidic channels between varying cellular compartments are available such as organ-on-a-chip systems. When a solid tumour in vivo experiments are necessitated, there are a variety of murine models available depending on the tumour engraftment or immune system requirements. Typically, immune-deficient mice are used in the context of human xenograft models and there are a variety of well-established strains and cell lines used. Some strains come at a higher cost and do not contain a native murine immune interactome. For

those requiring a competent murine immune system, well established fully murine strains and models exist and are often lower cost. However, if experiments require a human immune interactome, humanised mice are required which comes with a very high cost.

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