applications, particularly in terms of scalability and the ability to maintain cell quality during expansion. While the results are pending, the implications of this research could be significant, offering insights into the optimization of T-cell production processes and contributing to the advancement of therapeutic strategies.

P0775

Development of a novel iCasp9 kill switch for safer cell therapies

MS Wake[2] C Schindler[2] S Chin[2] J Sumner[2] L Vinall[2] R Dodd[2] A Sigurdardottir[2] T Malcolm[2] G Rees[2] C Graneli[2] N Tigue[2]

1: 2: AstraZeneca

Several Chimeric Antigen Receptor (CAR)-T cell therapies are approved for haematological tumours while many are in development and trials for solid tumours. However, due to the risk of lymphoproliferation, cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), it is important to engineer cell therapies with so-called suicide or kill switches that can be activated in such instances. Here, we describe SIM-iCasp9, a novel caspase-9-based kill switch activated via the orally available, clinically approved, small molecule simeprevir. This feature provides significant advantages over existing iCasp9 kill switches that are activated by small molecules that have not yet been approved. We have demonstrated that activation of the SIM-iCasp9 kill switch can provide effective killing of several cell types *in vitro* including cell lines, ES cells and immune cells, via rapid induction of apoptosis. Furthermore, when SIM-iCasp9-expressing tumour cells are implanted into mice, administration of a single dose of simeprevir led to complete tumour regression. Kill switches such as this are helping to drive these next generation cell therapies to the clinic by improving safety to the patients.

P0776

Augmenting expansion and tumor infiltration of chimeric antigen receptor T cells with a novel dendritic cell vaccine for solid tumors

ZR Wu[1] RD Hao[2] YT Li[2] MM Sun[2] B Zhai[1]

1: Renji Hospital, Shanghai Jiao Tong University School of Medicine 2: Suzhou Immunofoco Biotechnology Co., Ltd

Chimeric antigen receptor T (CAR-T) cell therapy faces challenges in treating solid tumors, particularly due to poor tumor infiltration and inadequate expansion. Here, we present T-booster, a novel dendritic cell (DC) vaccine co-engineered with tumor antigen receptor (TAA) and immunostimulatory cytokine and chemokine, which significantly augments the efficacy of CAR-T cell therapy against solid tumors. Firstly, Claudin18.2 (CLDN18.2) modified T-Booster and CLDN18.2 targeting CAR-T were used for proof of concept. In vitro assays demonstrated T-booster's ability to significantly increase CAR-T cell activation, proliferation, and cytotoxicity. In vivo studies utilizing both conventional and large-refractory gastric cancer xenograft models demonstrated that the combination of T-booster with CAR-T cell therapy resulted in complete tumor regression and prolonged survival, without incurring additional toxicity. Mechanistic studies elucidated that the modification of DC cells leading to their preferentially homing to the tumor site, thereby establishing a microenvironment enriched with these cytokines and chemokines, which are responsible for chemoattracting CAR-T. Additionally, CAR-T cells stimulated with T-booster exhibited reduced exhaustion markers and increased markers for T cell activation, memory formation, and apoptosis resistance. Furthermore, T-booster expressing EpCAM was shown to enhance the in vitro and in vivo efficacy of EpCAM-targeting CAR-T cells, underscoring its potential as a platform technology for various TAA. Collectively, our findings suggest that T-booster may represent a novel strategy to enhance anti-tumor efficacy of CAR-T cell therapy in solid tumors.

P0777

A programmable and automated microfluidic platform for massively parallel and sequential processing of single cell assay operations

LG Welch[1] RA Randle[1] J Estranero[1] P Tourlomousis[2] RC Wootton[1] V Radu[1] C Gonzalez-Fernandez [1] TJ Puchtler[1] CM Murzeau[1] NM Dieckmann[1] A Shibahara[1] BW Longbottom[1] C Bryant[2] EL Talbott[1]

1: Lightcast Discovery 2: Department of Veterinary Medicine, University of Cambridge

Recent advancements in cancer immunotherapy have revolutionised the treatment paradigm and significantly improved clinical outcomes for patients. Single cell profiling has been pivotal in the development of these immunotherapies. However, while traditional single cell technologies have yielded extensive 'omic datasets enabling users to infer biological function, they have not yet empowered the direct functional analysis at single cell level. We propose that such analysis is crucial for understanding the complex cellular interactions within the tissue microenvironment. To address this challenge, we introduce a novel platform that leverages droplet microfluidics and optical electrowetting-on-dielectric (oEWOD). This platform enables highly-controlled, sequential and multiplexed single cell assays in massively parallelised workflows. This capability enables functional profiling of single cells in a scalable screening format. Soluble reagents, cells, or assay beads are encapsulated into droplets in fluorous oil. These droplets are actively filtered based on size and content, ensuring only desirable droplets (e.g., single cell droplets) are retained for analysis, thereby overcoming the challenge of Poisson distribution, a limiting factor with many other single cell technologies. The droplets are arrayed on a temperature-controlled chip, and each droplet's history is tracked from filtering to workflow completion. On the chip, droplets undergo an automated suite of operations, including merging and multicolour fluorescent imaging. This enables precise execution of complex sequential assay workflows and multiparametric assay readouts providing functional insights that are not possible with many other single-cell approaches. To illustrate the platform's utility in immunotherapy research and development, we present single-cell functional workflows for antibody discovery and cell and gene therapy. We describe generation of droplets containing single immune effector cells, such as T-cells, and single target cells, which can be merged sequentially and repeatedly to test effector cell killing efficacy, specificity and exhaustion. In summary, our droplet-based approach empowers direct functional analysis at the single-cell level, advancing our understanding of cellular interactions and cellular function and accelerating progress in cancer immunotherapy research and development.