Investigating the *in vitro* enhancement of differential immune cell responses by immunotherapies pembrolizumab, blinatumomab and trastuzumab

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ABSTRACT

Immunotherapies have revolutionised the treatment of several cancers by harnessing the power of the immune system to mediate a durable anti-tumour response. We have investigated three molecules with diverse mechanism of actions in a range of *in vitro* systems to characterise their impact with the aim of providing discovery platforms for the development of new and enhancing agents.

Pembrolizumab, a checkpoint inhibitor which blocks the PD-1/PDL-1 pathway, enhanced T cell IFNγ production, an effect especially pronounced in systems utilising T cells with an 'exhausted' phenotype. Blinatumomab, a CD19/CD3 bispecific T cell engager, induced potent killing of a Burkitt lymphoma cell line, and in a PBMC system, at a level that exceeded that of rituximab, an anti-CD20 monoclonal antibody. Finally, we tested the ability of trastuzumab to enhance T cell responses following tumour cell phagocytosis.

These assays highlight the diverse immunological mechanisms that can contribute to the anti-tumour response and are currently being applied to the development of new immunotherapeutic agents.

INTRODUCTION

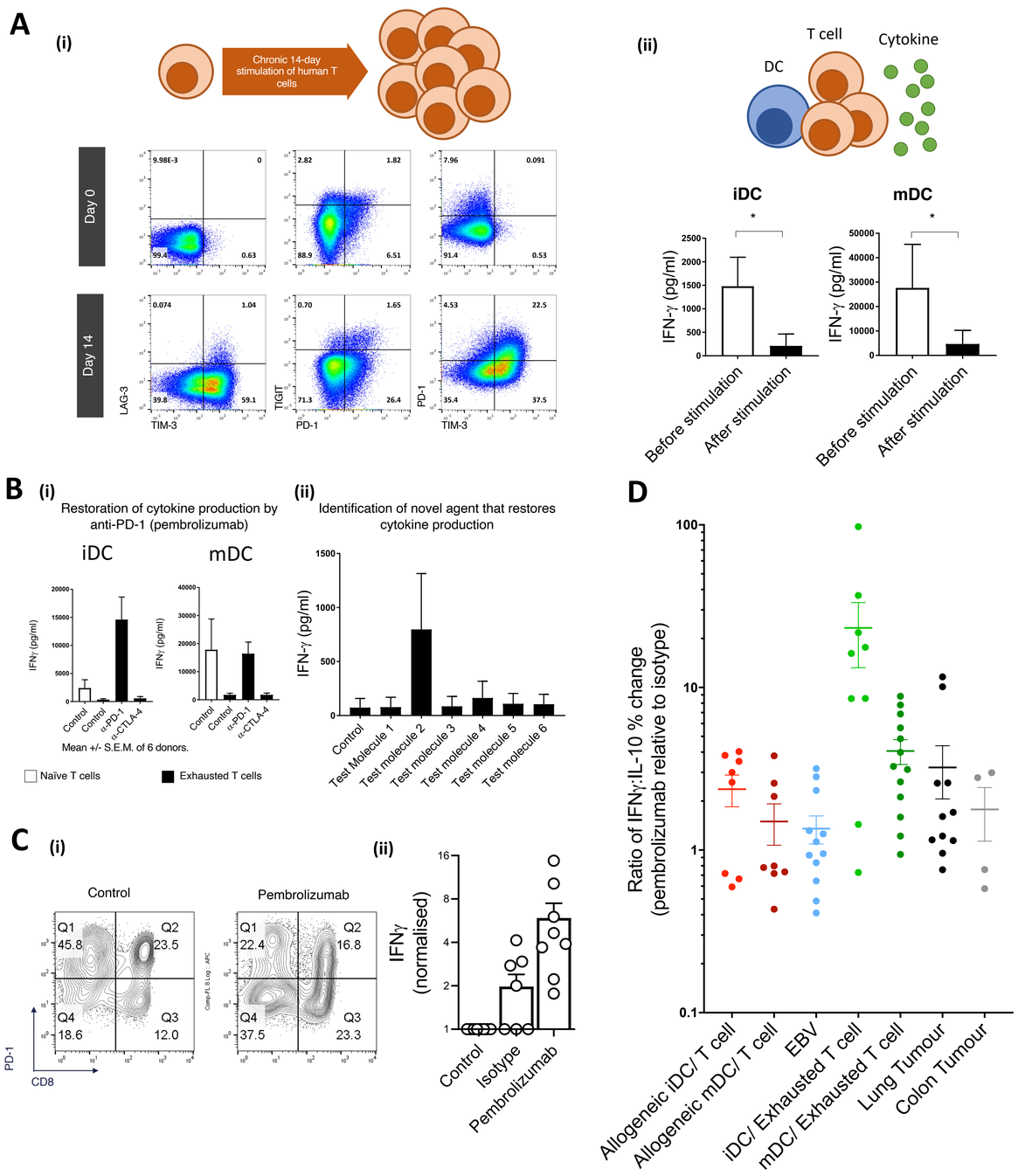
Immuno-oncology therapeutics have revolutionized cancer treatment by offering an often more targeting and less toxic approach than chemotherapy or radiotherapy, and often leading to durable responses and improved survival rates. These drugs stimulate the patient's own immune system to target and destroy tumour cells by directly engaging molecules on cancer cells, activating the immune system more generally, or both.

Because of the diverse mechanisms of action of immuno-therapeutic agents, screening assays for their development need to encompass a range of mechanisms. Here, using three immunotherapeutic agents, pembrolizumab, blinatumomab and trastuzumab as examples, we show how they modulate anti-tumour responses in diverse *in vitro* cell-based systems.

PD-1/PDL-1 BLOCKADE BY PEMBROLIZUMAB ON EXHAUSTED T CELLS

Pembrolizumab is a checkpoint inhibitor and acts by blocking the interaction of the inhibitory PD-1 receptor expressed on T cells with its ligand PDL-1. This leads to enhanced T cell activation leading to an anti-tumour response. Resting naïve human T cells generally do not express high levels of PD-1 and this is true of several immune checkpoints associated with T cell exhaustion. We have therefore investigated a range of *in vitro* assay systems that enable the study of immune checkpoint blockade, and compared the responses in these systems to those of dissociated tumour cells. Our results highlight how an 'exhausted' T cell system demonstrated the greatest assay window when measuring cytokine production following PD-1 blockade with pembrolizumab.

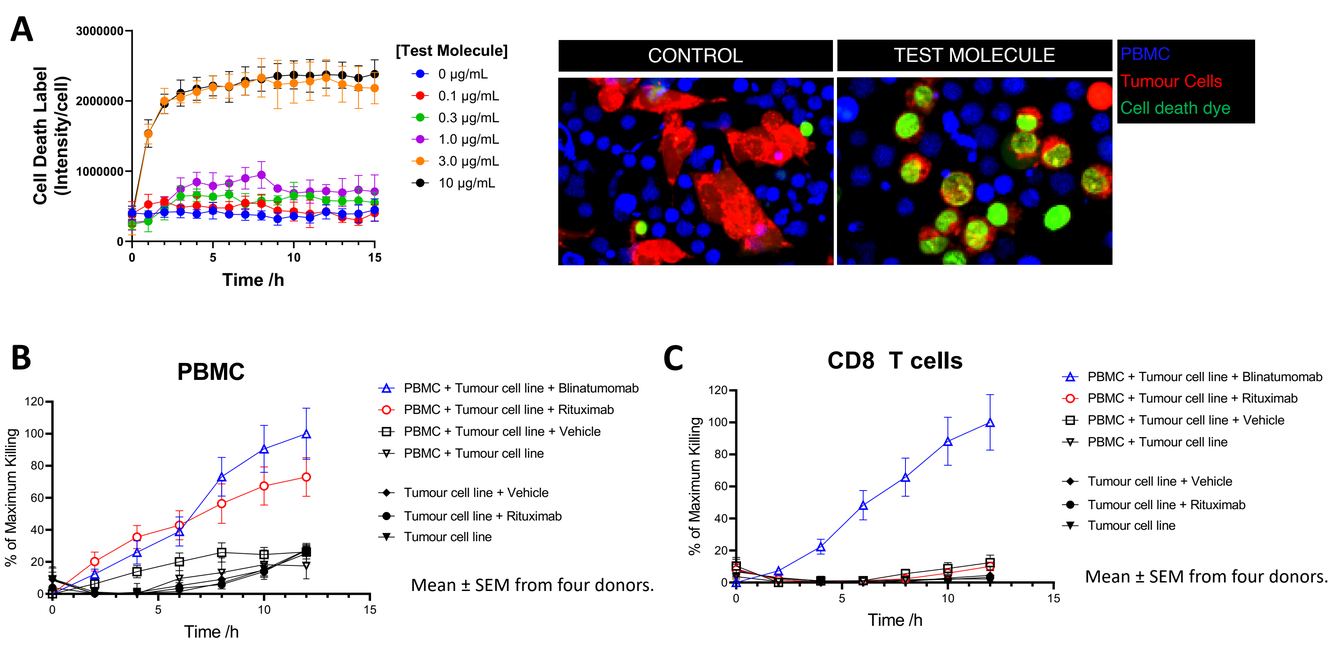
A Chronic stimulation of human CD4 T cells leads to upregulation of exhaustion markers (i) and impaired cytokine production on restimulation (ii). **B** 'Exhausted' phenotype is sensitive to reversal by pembrolizumab (i) and system can be used for the identification of novel agents that restore cytokine production (ii). **C** Comparison of response with human dissociated tumour cells. (i) Reduction of PD-1 labelling on tumour infiltrating T cells following pembrolizumab addition. (ii) Enhancement of IFNγ production in dissociated tumour cell preparations from certain patients. **E** Comparison of stimulatory (IFNγ) vs inhibitory (IL-10) cytokine ratio across assay systems shows greatest PD-1 evoked change in an exhausted T cell model.



COMPARISON OF KILLING OF A BURKITT LYMPHOMA CELL LINE BY BLINATUMOMAB AND RITUXIMAB

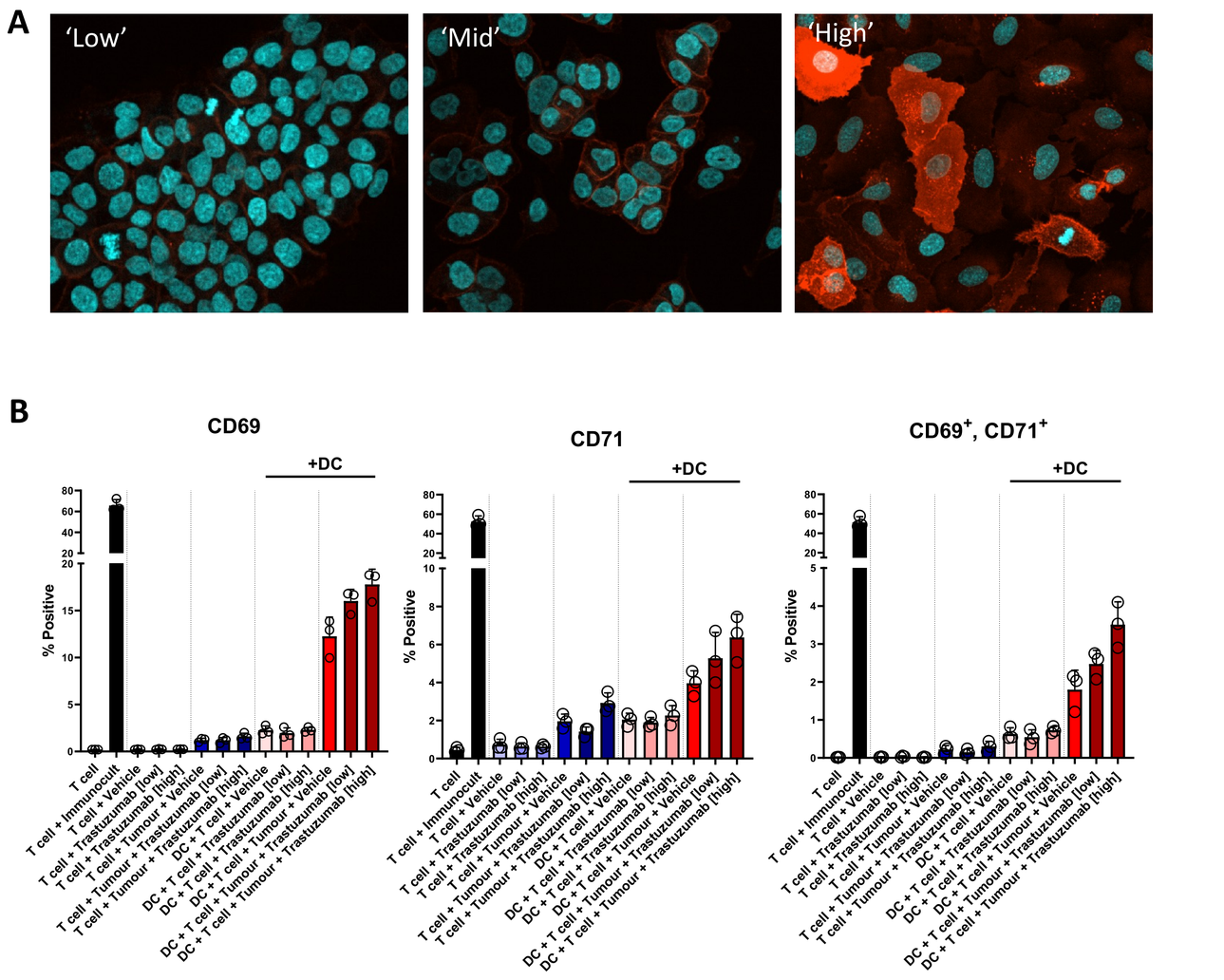
Monoclonal antibodies against tumour-expressed targets may engage Fc-receptors on cells such as NK cells, macrophages and neutrophils to mediate antibody-dependent cellular cytotoxicity. More recently, bispecific T cell engagers which link a tumour antigen with a T cell surface molecule have been developed. We have made a comparison of rituximab, a monoclonal antibody that binds to CD20 and used as a treatment for certain types of lymphoma or leukaemia, and blinatumomab, which engages CD19 on the target cell and CD3 on the T cell.

Initially, we generated a model to monitor tumour cell killing using labelled PBMCs, a labelled cancer cell line, and a cell death dye. Cells were visualised over time by high-throughput confocal microscopy and killing in response to a test molecule quantified by automated analysis (**A**). We then used this system to characterise the killing of a Burkitt lymphoma cell line by either PBMCs (**B**) or purified CD8 T cells (**C**), in the absence or presence of rituximab or blinatumomab. While tumour cell line killing by PBMCs was evident with both rituximab and blinatumomab, killing by CD8 T cells was only mediated by blinatumomab and not rituximab.



ENHANCEMENT OF T CELL ACTIVATION BY TRASTUZUMAB

Tumour cells exhibit multiple suppressive mechanisms that may inhibit the activation of immune cells. We have therefore investigated T cell activation in the presence of dendritic cells and tumour cells, in the absence or presence of trastuzumab. We first tested labelling of trastuzumab across tumour cell lines with low-, mid- and high Her2 expression (**A**). The 'high' expressing cells were chosen for subsequent co-culture with dendritic cells and T cells in the absence or presence of trastuzumab (**B**)**.** A trastuzumab-dependent enhancement of T cell activation (assessed by flow cytometry using the activation markers CD69 and CD71) was evident when dendritic cells, tumour cells and T cells were co-cultured indicating trastuzumab enhancement of T cell activation can be modelled *in vitro*.



FUTURE WORK

The assays described on this poster are currently being applied to the development of new immuno-oncology agents.

ACKNOWLEDGEMENTS

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