Development of a “tumour-educated” T cell killing assay for predictive in vitro assessment of anti-PD-L1 antibodies

Kymab Ltd, The Bennett Building, Babraham Research Campus, Cambridge CB22 3AT, U.K. (email: morgane.lecointre@kymab.com)

Introduction

T cells within the tumour microenvironment (TME) differentiate into an exhausted phenotype, characterized by the expression of inhibitory receptors, decreased proinflammatory cytokines and reduced cytotoxic activity.1,2 PD-1/PD-L1 interaction has been shown to inhibit specific TCR-mediated lysis by cytotoxic T cells in vitro. This contributes to immune evasion, and consequently enhances tumorgenesis in vivo.1,2 Modulating the function of exhausted T cells with antibodies blocking immune checkpoints such as PD-1/PD-L1 represents an immunotherapeutic strategy with proven clinical activity.2 It is therefore essential to have a model of tumour-specific exhausted T cells that mimics the physiological state and expression profile of T cells within the TME to aid the screening of functional antibodies.

Material and methods

HLA-A2-matched CD4+ and CD8+ T cells were purified from human PBMCs isolated from 7 healthy donors and co-cultured with HLA-A2* (HLA-DR*) A375 melanoma cells for 20 days. (A) to generate T cells expressing markers associated with activation and exhaustion3 (B). Refer to poster P077 for more details.

These ‘tumour-educated’ T cells were then co-cultured for 4 days with red fluorescent A375 cells in the presence of a Kymab fully human anti-PD-L1 antibody, called KY1003.

Tumour growth was monitored overnight in the IncuCyte® by imaging and quantifying target cells. The number of cells undergoing caspase 3/7-mediated apoptosis was also quantified. PD-1-L1 HT1080 cells were used as control for non-specific killing.

Assay supernatants were collected at the end of the co-culture and analysed by MSD to assay secretion of effector cytokines.

“Tumour-educated” T cell killing is specific

CD8+/CD4+ T cell mediated killing of A375 but not HT1080 was observed. Addition of antigen+ T cells enhanced specific T cell mediated response.

Efficacy observed in 2D translates to 3D model

A375 spheroids were used to assess the ability of KY1003 to enhance T cell mediated killing in a 3D model that better mimics solid tumour growth, and is therefore more predictive of in vivo efficacy.

KY1003 has strong in vivo anti-tumour efficacy

An A375/D23 humanised mouse xenograft model was set up using in-vitro generated “tumour-educated” T cells, allowing for testing of non cross-reactive anti-PD-L1 antibodies such as KY1003. Strong anti-tumour efficacy was obtained with KY1003 (no tumour growth) (D).

Conclusions & perspectives

• A375 “tumour-educated” T cells expressing markers associated with activation and exhaustion were generated in vitro.

• T cell exhaustion was reversed in the presence of KY1003, which resulted in increased A375 tumour specific killing (2D and 3D cultures).

• Effect was seen in multiple donors and was statistically significant.

• Enhanced T cell mediated killing associated with the presence of KY1003 was correlated with increased production of proinflammatory cytokines, such as IFNγ and IL-2, in a dose dependent manner.

• In vitro data generated with KY1003 translates into strong anti-tumour efficacy in vivo, in a human melanoma xenograft model.

• This assay is therefore a biologically relevant approach for validation of antibodies targeting T cell immune-modulatory molecules.

• Biological relevance can be further improved by using other immunosuppressive effector cells to better model the TME complexity, allowing for a more predictive and meaningful assessment of monotherapies, combination treatments and bispecific molecules.