TARGETING NOVEL PATHWAYS IN CHRONICALLY ACTIVATED T CELLS PREVENTS FUNCTIONAL EXHAUSTION


Background In cancer, persistent antigenic challenge leads to T cells acquiring a hyporesponsive cell state, also referred to as T cell exhaustion. We have previously demonstrated that human CD3+ T cells repeatedly stimulated in vitro, via their TCR, develop phenotypical characteristics of exhausted T cell found in vivo, including increased expression of inhibitory receptors PD-1, TIM-3 and LAG-3 and diminished responsiveness to dendritic cell activation and cancer cell cytotoxicity. We showed that PD-1 blockade with nivolumab and treatment with an IKZF3 inhibitor, lenalidomide, reinvigorated the exhausted T cells. We next wished to evaluate if blocking of IKZF3 during the development of T cell exhaustion could protect them from acquiring the exhausted phenotype.

Methods Human CD3+ T cells, isolated from healthy PBMC donors, were repeatedly stimulated with anti-CD3/anti-CD28 coated beads to mimic chronic antigenic challenge. The stimulations were conducted in the presence or absence of lenalidomide. T cells, both CD4+ and CD8+, were assessed for changes in expression of inhibitory receptors and cytokine release was quantified. Following the final round of TCR stimulation, T cells were co-cultured with allogeneic dendritic cells to determine if their functionality has been altered by lenalidomide treatment. T cell proliferation and IFN-g release were assessed as well as changes in inhibitory receptor expression at the end of the co-culture.

Results We demonstrated that lenalidomide had no impact on T cell viability or CD4+/CD8+ ratio in repeatedly stimulated cultures. Lenalidomide did however affect inhibitory receptor expression and impacted cytokine secretion from chronically stimulated T cells. Lenalidomide led to increased PD-1 expression on CD8+ T cells and LAG-3 expression on CD4+ T cells whereas TIM-3 expression was downregulated on both T cells subsets in the presence of compound. Lenalidomide enhanced production of cytokines in repeatedly stimulated T cells. To assess if lenalidomide-driven changes had any impact on T cell functionality, we cultured lenalidomide-treated and untreated repeatedly activated T cells with allogeneic dendritic cells. Whilst repeatedly stimulated T cells not exposed to lenalidomide showed diminished ability to proliferate and secrete IFN-g, consistent with their exhausted cell state, lenalidomide treated T cells displayed increased IFN-g secretion suggesting that lenalidomide protected repeatedly stimulated T cells from acquiring an exhausted phenotype.

Conclusions The in vitro assays described herein offer the opportunity to investigate the effect of candidate therapeutics, including combination therapies, on exhausted T cell development and function.