ANTI-CLEC2D-TLR9 AGONIST CONJUGATE BINDS TO AND INTERNALIZES CLEC2D ON MYELOID CELLS, PLASMACYTOID DCS AND B CELLS LEADING TO ROBUST TLR PATHWAY ACTIVATION AND INFLAMMATORY CYTOKINE PRODUCTION


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Background

Current immunotherapies fail to provide benefit to tumors poorly infiltrated with T and NK cells. However, activation of myeloid cells and B cells can lead to recruitment of functional T and NK cells turning the ‘cold’ tumor microenvironment (TME) into ‘hot’. CLEC2D is broadly expressed on germinal center B cells, activated primary plasmacytoid DCs (pDC) and tumor associated macrophages (TAMs). Upon internalization, CLEC2D acts as a vehicle to deliver histone/CpG complexes to endosomal TLR9, stimulating an inflammatory response. Furthermore, CLEC2D is the ligand for CD161 which is an immune checkpoint expressed on both T and NK cells, making it a target for additional immunotherapeutic intervention.

Methods

We have developed a fully human anti-CLEC2D monoclonal antibody that binds and triggers receptor internalization on CLEC2D+ cells. Next, we conjugated this antibody to a CpG oligonucleotide to produce an anti-CLEC2D-TLR9-ISAC (Immune Stimulating Antibody Complex) molecule for systemic delivery of TLR9 agonist to CLEC2D+ cells. We have utilized multiple assays to get functional proof of concept (POC) of triggering TLR9 activation in myeloid cells, B cells and pDCs by this ISAC molecule.

Results

First, we have used THP1-TLR9 reporter cell lines to monitor successful activation of TLR9 pathway. We have shown that anti-CLEC2D-TLR9-ISAC treatment will induce both IRF and NFkB reporter activation over CpG alone. Next, treatment of in vitro generated human pDCs with this ISAC molecule dramatically increased production of IFN-a, a critical cytokine for induction of anti-tumor T cells. To test functionality on primary B cells, we have developed an in vitro assay with CLEC2D expressing primed B cells. Treatment of these B cells with the ISAC molecule results in sustained B cell proliferation and upregulation of co-stimulatory molecules such as CD80, CD86 and CD40 enabling stronger induction of T cell immune responses. Many tumors are highly infiltrated by immunosuppressive macrophages. We have shown that there is CLEC2D expression on TAMs within tumors, as well as on in vitro generated TAM like macrophages. Treatment of TAMs with the ISAC reversed TAM mediated suppression of T cell proliferation and activation, indicating that TLR9 agonism can reprogram TAMs towards inflammatory state. Finally, we show that treatment of normal human PBMCs with anti-CLEC2D-ISAC molecules did not trigger release of inflammatory cytokines providing preliminary safety data.

Conclusions

Overall, anti-CLEC2D antibody is a novel molecule that can effectively deliver CpG to endosomal TLR9. This results in activation of both myeloid and B cells enabling induction of sustained T cell immunity.

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