Dectin-2 agonist antibodies reprogram tumor-associated macrophages to drive anti-tumor immunity

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ABSTRACT 2883

INTRODUCTION

Tumor-associated macrophages (TAMs) are an abundant immune cell population in most cancers that support tumor progression through their immunosuppressive effects. We discovered that TAMs express the pattern recognition receptor Dectin-2 (*CLEC6A*), an activating C-type lectin receptor (CLR) that binds to high-mannose glycans on fungi and other microbes and induces protective immune responses against infectious disease. Dectin-2 is selectively expressed by myeloid cells, and upon ligation, mediates enhanced phagocytosis, antigen processing and presentation, and pro-inflammatory cytokine production. Given these properties, we evaluated the therapeutic potential of targeting Dectin-2 using naturally derived ligands. We then generated a human Dectin-2-targeted agonistic antibody, BDC-3042, capable of robustly activating immunosuppressive "M2" or TAM-like macrophages. Our studies show that primary TAMs from a range of tumor types express Dectin-2 and respond to treatment with the agonist antibody ex vivo. We further found that BDC-3042 treatment suppressed tumor growth and was well tolerated in mice with humanized immune systems.

Dectin-2 agonism activates TAMs and elicits anti-tumor immune response

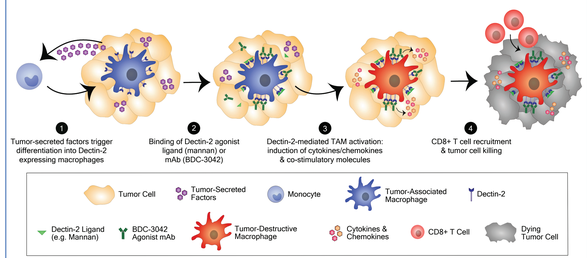

Figure 1: Schematic of proposed mechanism of action driving Dectin-2-mediated anti-tumor activity.

RESULTS

Dectin-2 gene expression is elevated across tumor types

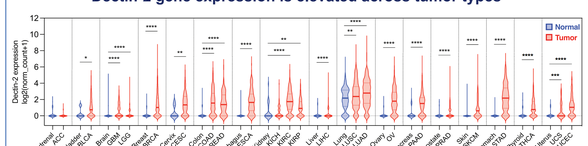

Figure 2: Dectin-2 gene expression is elevated in tumors but low in most normal tissues. Dectin-2/*CLEC6A* mRNA expression in human tissue samples from the TCGA (tumor) and GTEX (normal) datasets. TCGA study abbreviations are shown for the tumor subtypes. TCGA and GTEX data were processed using a uniform bioinformatic pipeline and obtained from UCSC Xena (xena.ucsc.edu). Median and interquartile range are shown on the violin plots. Statistics were calculated by Mann-Whitney U test; *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

RESULTS

Dectin-2 agonist activity is CD8 T cell dependent and elicits immunological memory

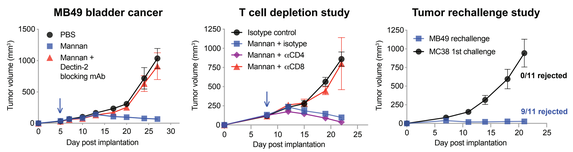

Figure 3: Dectin-2 agonist activity is Dectin-2 and CD8 T cell dependent and elicits immunological memory. (Left) MB49 tumor-bearing mice were treated systemically every 2 days with the naturally derived Dectin-2 ligand, *S. cerevisiae* mannan (12.5 mg/kg i.v.), with or without co-administration of Dectin-2 blocking antibody (5 mg/kg i.p.). (Middle) MB49 tumor-bearing mice were treated systemically with mannan, and 250 ug of isotype IgG, anti-CD4, or anti-CD8 depleting antibody every 3 days (n=4-5 mice, day of treatment initiation marked with a blue arrow). (Right) Mice that experienced complete regression of MB49 tumors following mannan treatment were rechallenged with MB49 cells as well as an unrelated tumor cell line (MC38) >70 days after the last treatment (n=11 mice). Data are shown as mean with SEM.

BDC-3042 binds to Dectin-2 and activates human macrophages

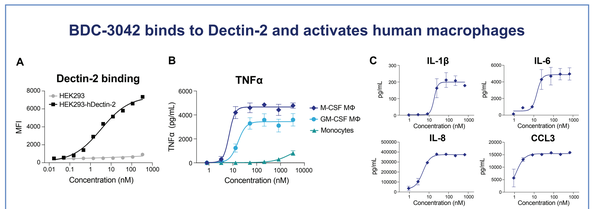

Figure 4: BDC-3042 binds to Dectin-2 and activates in vitro-generated macrophages. (A) BDC-3042 binds to cells expressing Dectin-2 with single digit nM EC_{50}s, while minimal binding is detected with HEK293T cells lacking Dectin-2 expression. (B) Fresh human monocytes (n=12 donors) or monocyte-derived macrophages generated with M-CSF (n=5) or GM-CSF (n=12) were stimulated overnight with the Dectin-2 agonist mAb, followed by cytokine analysis by ELISA. (C) Human M-CSF macrophages (n=3) were stimulated overnight with the Dectin-2 mAb, followed by cytokine and chemokine analysis using MSD kits.

BDC-3042 activates primary human TAMs ex vivo

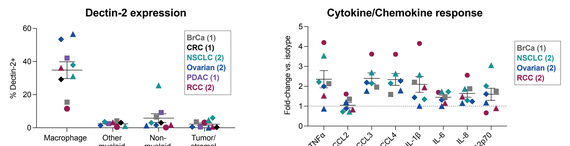

Figure 5: BDC-3042 activates primary human TAMs ex vivo. (Left) Human tumors were processed into single cell suspensions and analyzed by flow cytometry. TAMs were defined as viable CD45+CD11b+CD14+HLA-DR+ cells. (Right) Primary human tumor samples were processed into single cell suspensions and cultured overnight with BDC-3042 or a non-binding isotype control antibody. Data are shown with mean and SEM; Breast Cancer (BrCa, n=1), Colorectal Cancer (CRC, n=1), Non-Small Cell Lung Cancer (NSCLC, n=2), Ovarian Cancer (n=2), Pancreatic Ductal Adenocarcinoma (PDAC, n=1), Renal Cell Carcinoma (RCC, n=2).

BDC-3042 elicits dose-dependent activation of TAMs in renal cell carcinoma

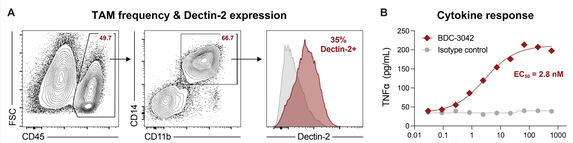

Figure 6: BDC-3042 elicits dose-dependent activation of TAMs in renal cell carcinoma (RCC). (A) Human RCC tumor sample was processed into single cell suspension and analyzed by flow cytometry. TAMs were defined as viable CD45+CD11b+CD14+ cells. (B) Cell suspension was incubated for 24 hours with BDC-3042 or isotype control mAb. TNFα secretion was measured by ELISA.

Dectin-2 expression is elevated in basal/triple-negative breast (TNBC) tumors

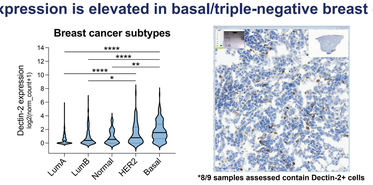

Figure 7: Dectin-2 expression is elevated in basal-like/triple-negative breast (TNBC) tumors. (Left) Dectin-2 mRNA expression in human breast tumor samples from TCGA data downloaded from cBioPortal.org. Median with interquartile range shown in violin plots. (Right) Dectin-2 staining in a frozen tissue section from human TNBC sample. Data are representative, where 8 of 9 samples assessed contained Dectin-2 positive cells.

Humanized mice for in vivo assessment of BDC-3042 anti-tumor activity

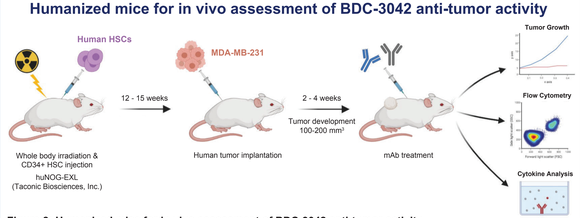

Figure 8: Humanized mice for in vivo assessment of BDC-3042 anti-tumor activity.

Immune landscape & Dectin-2 expression in tumor-bearing humanized mice

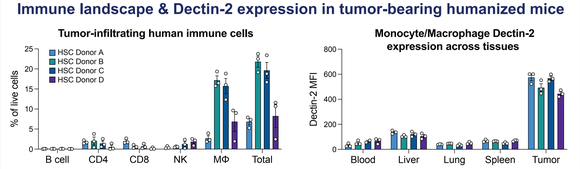

Figure 9: Immune landscape and Dectin-2 expression in tumor-bearing humanized mice. (Left) The tumor immune compartment was assessed in huNOG-EXL mice from four unique HSC donors (n=3 mice/donor). Quantification of human immune cell subsets is shown as the percentage of total live cells, and total human immune cells (CD45+) are marked as "Total". (Right) Dectin-2 expression was measured on human monocytes and macrophages recovered from the indicated tissues in tumor-bearing huNOG-EXL mice.

BDC-3042 activates TAMs from MDA-MB-231 tumors in humanized mice

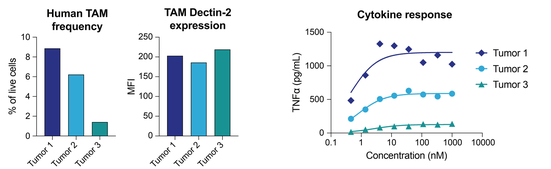

Figure 10: BDC-3042 activates TAMs from MDA-MB-231 tumors in humanized mice. MDA-MB-231 tumors were harvested from 3 huNOG-EXL mice (denoted as tumors 1-3) and digested into single cell suspensions (DTCs). The percentage of human TAMs within the tumor was assessed by flow cytometry and is shown as the percentage of total live cells. Dectin-2 expression was assessed with a commercial antibody. MDA-MB-231 DTCs were incubated for 18 hours with BDC-3042 or isotype control (data not shown). Human TNFα secretion was measured by ELISA.

BDC-3042 mediates anti-tumor activity in MDA-MB-231 tumor-bearing humanized mice

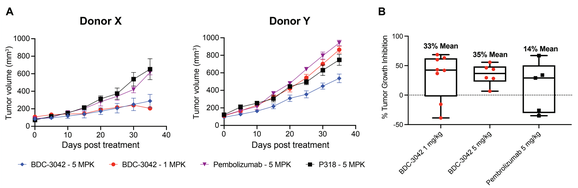

Figure 11: BDC-3042 mediates anti-tumor activity in MDA-MB-231 tumor-bearing humanized mice. huNOG-EXL mice from distinct human CD34+ HSC donors were implanted with bilateral MDA-MB-231 tumors. (A) Representative tumor growth curves following treatment with the indicated test article via IP administration Q5D x 6-7. P318 is the Fc-matched isotype control antibody. (B) Tumor growth inhibition (TGI) was calculated at Day 35 for all but one cohort of mice which was calculated at Day 30 as compared to an isotype control antibody.

CONCLUSIONS

- Dectin-2 is a novel immuno-oncology target expressed by TAMs across a range of tumor types
- BDC-3042 is an agonist antibody targeting Dectin-2 that has the potential to reprogram tumor-supportive macrophages into tumor-destructive macrophages as a novel anti-tumor immunotherapy
- BDC-3042 activates in vitro-generated macrophages and primary human TAMs, eliciting secretion of an array of pro-inflammatory cytokines and chemokines
- BDC-3042 mediates anti-tumor activity and is well tolerated in MDA-MB-231 tumor-bearing humanized mice
- Preclinical data support clinical evaluation of therapeutic potential of BDC-3042, with an IND filing planned for early 2023