

Antagonism of β -Catenin/BCL9 Interaction Suppresses Polymorphonuclear Myeloid-Derived Suppressor Cell Generation and Maintenance

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Poster #5562

Role of β -Catenin/BCL9 in MDSC Biology

- Mutations of the Wnt/ β -Catenin axis are foundational drivers of colorectal cancer (CRC), however their role in orchestrating the immunosuppressive tumor microenvironment (TME) remains a critical, less-understood barrier to effective therapy.
- Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of myeloid-lineage cells that contribute to the immunosuppressive TME¹. MDSCs may be classified as Polymorphonuclear MDSCs (PMN-MDSCs) or Monocytic MDSCs (M-MDSCs).
- PMN-MDSCs accumulate in tumors, peripheral blood and lymphoid organs of advanced CRC patients², yet little is known about their cell of origin or pathways required for maintenance.
- ST316 is a first-in-class clinical-stage peptide antagonist of the β -catenin/BCL9 protein complex that is currently being evaluated in a Phase 1/2 study (NCT05848739) in patients with advanced CRC.
- Here we characterize the impact of antagonism of the β -Catenin/BCL9 complex with ST316 on PMN-MDSC generation and maintenance in non-clinical and clinical studies.

Antagonism of β -Catenin/BCL9 inhibits PMN-MDSCs in CRC

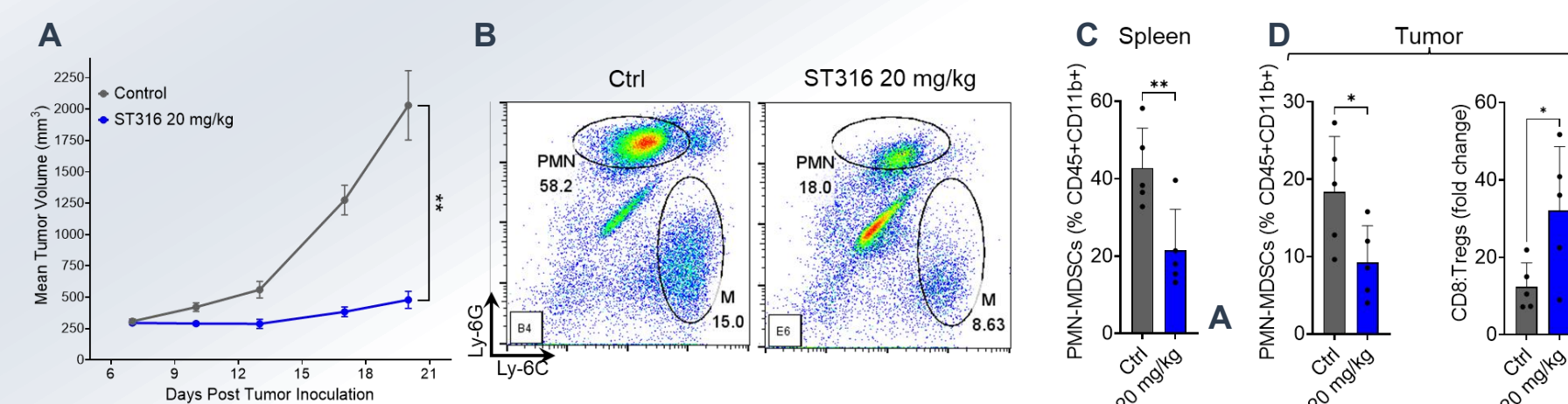


Figure 1. A) CT-26 tumor volumes following treatment with 20mg/kg ST316 1x/week or vehicle (Control). **B)** Flow cytometry plots of spleens from CT-26-bearing BALB/C mice treated with vehicle (Ctrl, left) or ST316 (right). Gates indicate PMN- and M-MDSCs. **C)** Frequency of PMN-MDSCs as fraction of Cd11b+CD45+ splenic myeloid cells. **D)** Frequency of PMN-MDSCs (left) and CD8:Tregs ratio (right) in the indicated conditions. Statistics, Student T-test; **p<0.01, *p<0.05, n=5/group. Error bars, standard deviations.

ST316 Rescues T cell Activity in Presence of PMN-MDSCs suppression

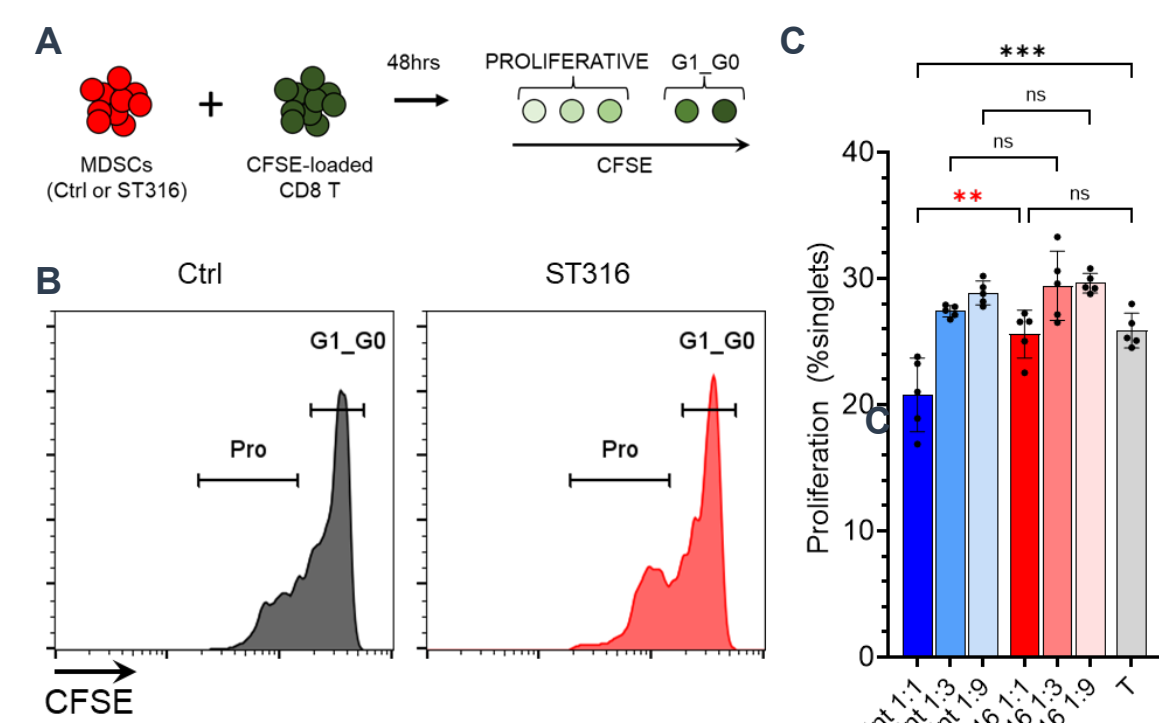


Figure 2. A) Outline of the CD8/PMN-MDSCs ex vivo suppressor assay. Syngeneic BALB/C, polyclonal expanded CD8 T cells were loaded with CFSE and incubated with PMN-MDSCs from spleens of Control or ST316-treated CT-26 bearing mice. **B)** Representative histogram plots for CD8 T proliferation following co-culture for 48hrs with Ctrl (grey) or ST316-treated PMN-MDSCs (red). G0_G1, Non proliferative fraction (undiluted CFSE). Proliferative fraction (Pro) was defined as CD8 cells with CFSE dilution. **C)** ST316-reprogrammed PMN-MDSCs lose immunosuppressive activity. CD8-PMN (1:1, 1:3, 1:9) ratios are indicated. T, T cell only culture. Statistics, 1-way Anova, T-test. **p<0.01; ***p<0.001; ns, not significant). Error bars represent sd.

ST316 inhibits Wnt-B-catenin pathway in PMN-MDSCs

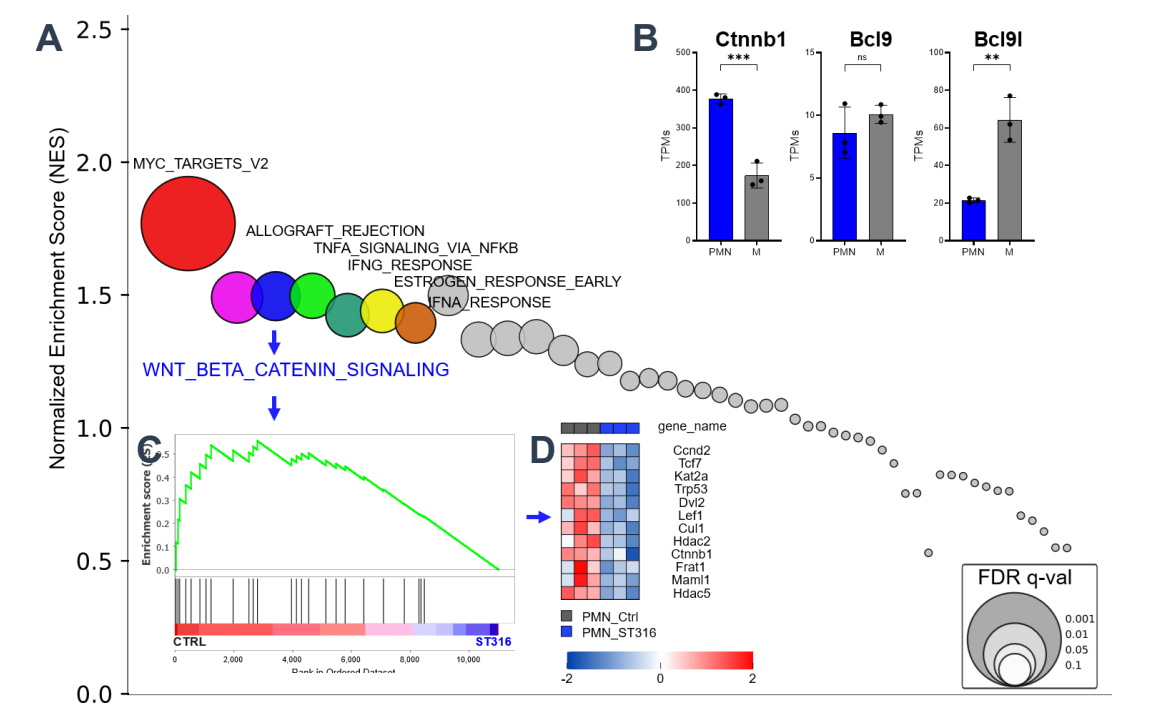


Figure 3. A) Bubble Plot for unbiased Hallmark pathway GSEA (n=50) for splenic PMN-MDSCs treated with vehicle (Ctrl) or ST316 20mg/kg for 1 week (2 total doses). Y-axis represent normalized enrichment score (ES); bubble size is inversely proportional to FDR q-val. **B)** Ctnnb1, Bcl9 and Bcl9l are robustly expressed in splenic MDSCs. Statistics, Student t-test (n=3/group). Gene Expression is in TPM units. **C-D)** GSEA plot (C) and heatmap for leading edge genes (D) for Hallmark Wnt- β -Catenin pathway.

β -Catenin/BCL9 Regulates Expression of Early Neutrophil Markers CD101 and Cd300c on PMN-MDSCs

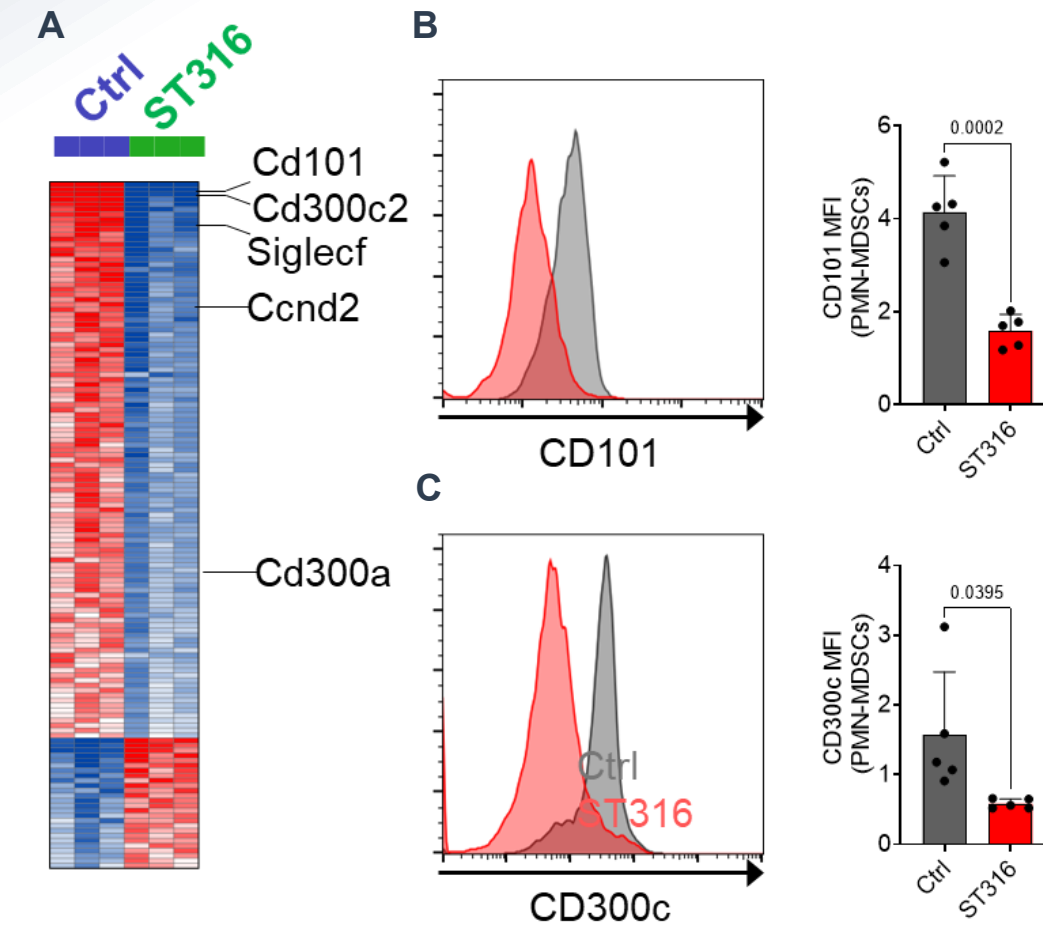


Figure 4. A) Supervised clustering of gene expression profiles of splenic PMN-MDSCs from mice treated with Vehicle (Ctrl) or ST316 as in Figure 1. Analysis shows 111 targets are suppressed by ST316 in PMN-MDSCs, including several myeloid receptors previously linked to immunosuppressive roles (Siglec-f, Cd101, Cd300c, Cd300a). Heatmap indicates genes with TPMs greater than 20 in at least two samples, fold change between Control and ST316-treated greater than 2 and a p-value less than 0.05 (BH correction). **B-C)** Flow cytometry analysis confirms reduction of CD101 (B) and CD300c (C) in splenic PMN-MDSCs in ST316 (red) treated mice vs. controls (grey). Left, Mode-normalized histogram; right, MFI statistics. P-values indicate Student T-test. Error bars represent sd (n=4).

Identification of a Ly-6G^{dim};Ly-6C^{int} Candidate Precursor Population for PMN-MDSCs (P-PMN-MDSCs)

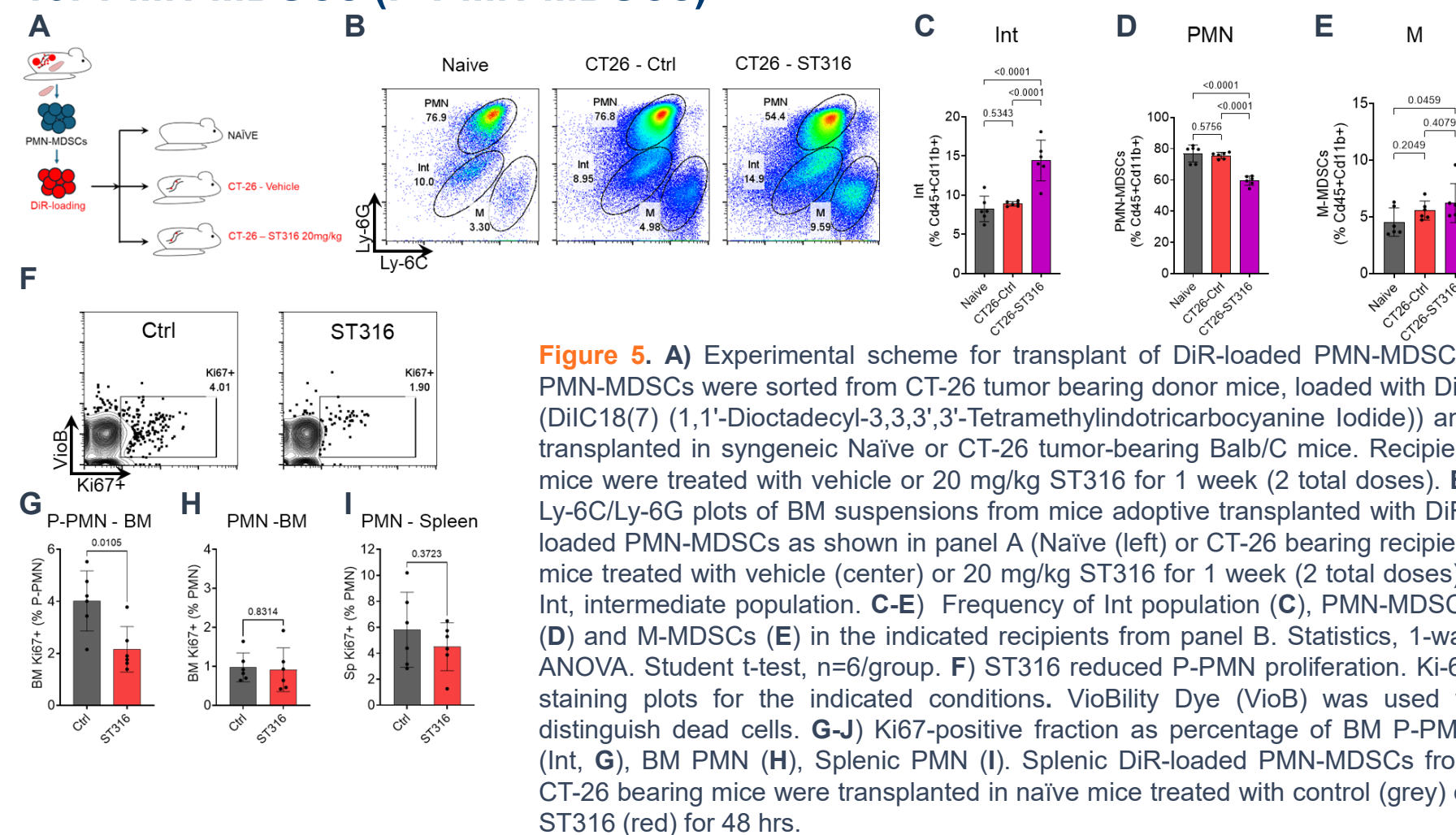


Figure 5. A) Experimental scheme for transplant of DiR-loaded PMN-MDSCs. PMN-MDSCs were sorted from CT-26 tumor bearing donor mice, loaded with DiR (DiC18(7) (1,1'-Diiododecyl-3,3',3'-Tetramethylindotricarbocyanine Iodide)) and transplanted in syngeneic Naive or CT-26 tumor-bearing Balb/C mice. Recipient mice were treated with vehicle or 20 mg/kg ST316 for 1 week (2 total doses). **B)** Ly-6C/Ly-6G plots of BM suspensions from mice adoptively transplanted with DiR-loaded PMN-MDSCs as shown in panel A (Naive (left) or CT-26 bearing recipient mice treated with vehicle (center) or 20 mg/kg ST316 for 1 week (2 total doses)). Int, intermediate population. **C-E)** Frequency of Int population (C), PMN-MDSCs (D) and M-MDSCs (E) in the indicated recipients from panel B. Statistics, 1-way ANOVA. Student t-test, n=6/group. **F)** ST316 reduced P-PMN proliferation. Ki-67 staining plots for the indicated conditions. Viobility Dye (Viob) was used to distinguish dead cells. **G-I)** Ki67-positive fraction as percentage of BM P-PMN (Int, G), BM PMN (H), Splenic PMN (I). Splenic DiR-loaded PMN-MDSCs from CT-26 bearing mice were transplanted in naive mice treated with control (grey) or ST316 (red) for 48 hrs.

ST316 Reduces Immunosuppressive Activity of PMN-MDSC and P-PMN-MDSC Populations

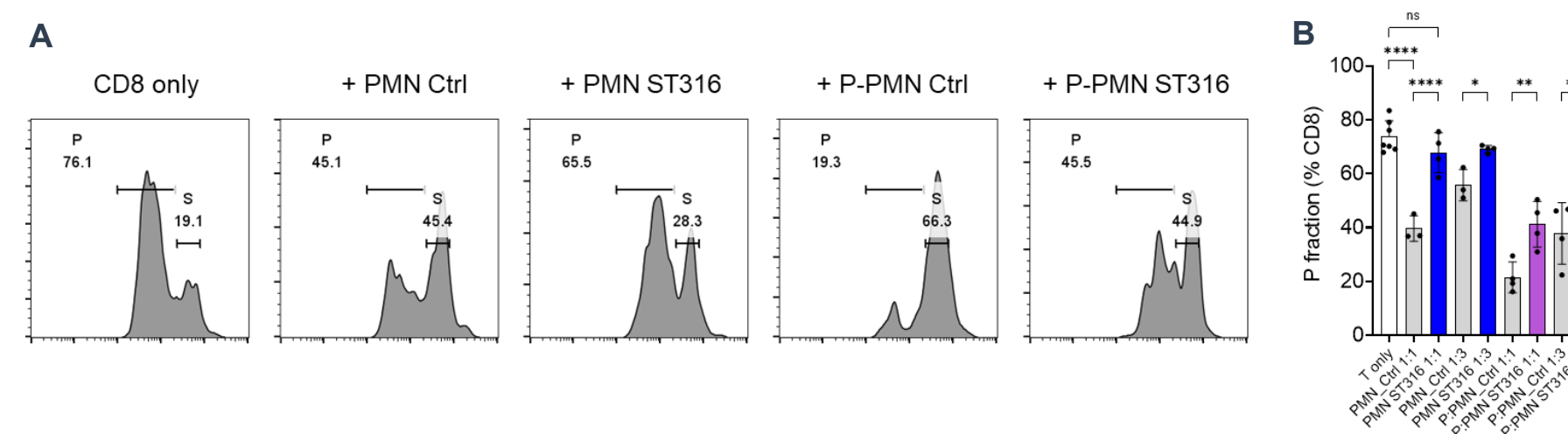


Figure 6. A) Representative histograms of CFSE-loaded CD8 T cells cultured for alone (T only) for 24 hrs or in the presence of PMN or P-PMN from bone marrow (BM) of vehicle-treated mice (PMN Ctrl or P-PMN Ctrl) or ST316-treated mice (PMN ST316 or P-PMN ST316). P, proliferative fraction; S, non-proliferative fraction. **B)** Fraction of proliferative CD8 T cells co-cultured with control PMN-MDSCs (grey), PMN-MDSC from ST316 treated mice (blue), or P-PMN-MDSCs from ST316 treated mice (violet). Statistics, 1-way ANOVA. Student t-test ***p<0.0001, **p<0.001, *p<0.05, **p<0.05, ns, not significant.

ST316 Reprograms PMN-MDSCs Ontogenesis

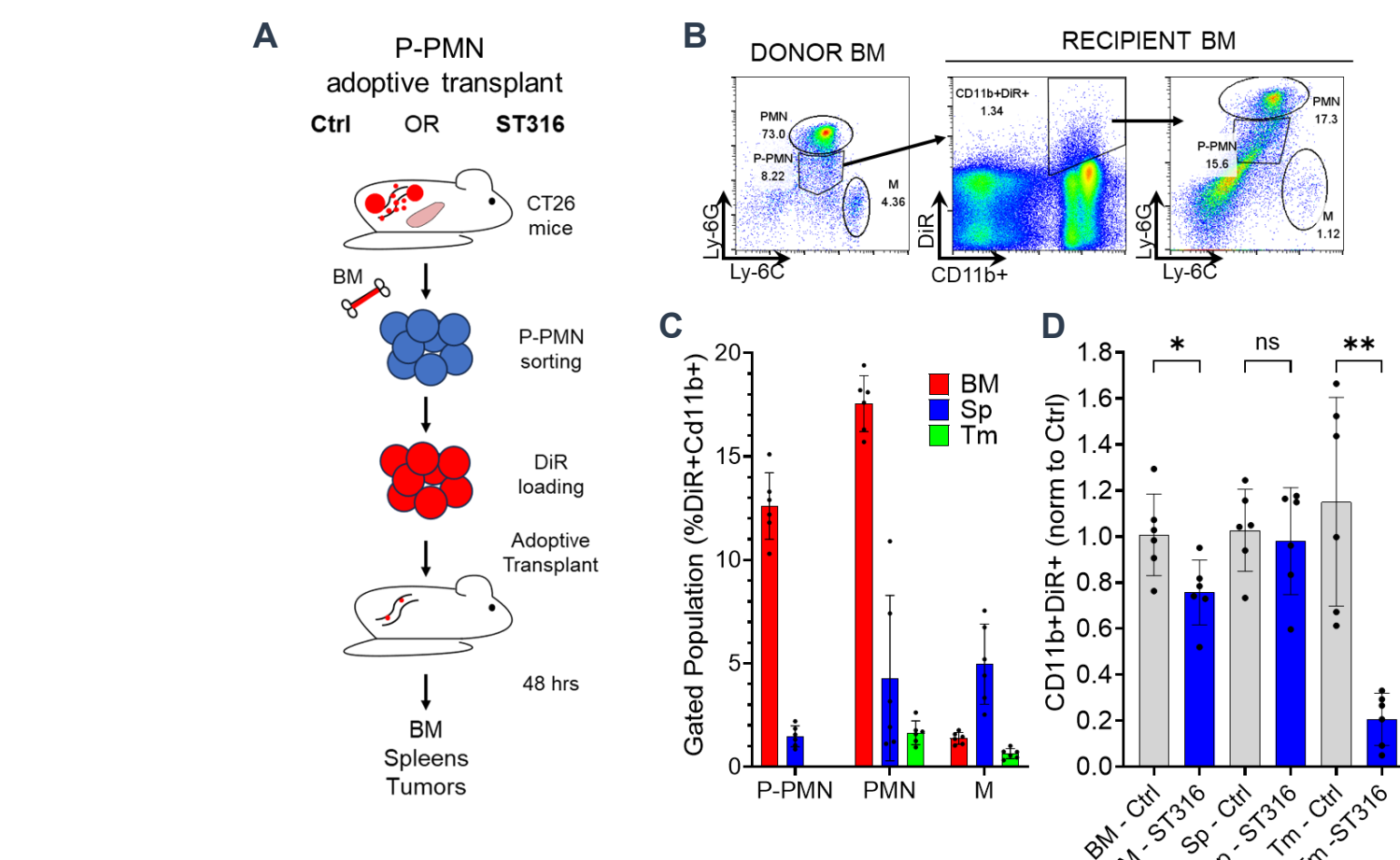


Figure 7. A) BM from CT-26 bearing mice treated with vehicle (Ctrl) or ST316 (20mg/kg 2x/week for 1 week) were collected, P-PMN fraction was sorted, loaded with DiR and transplanted in recipient mice bearing CT-26 tumors. BM, Spleen (Sp) and Tumors (Tm) were harvested and analyzed for P-PMN, PMN and M DiR+ content. **B)** Gating for donor P-PMN-MDSCs sorting (left) and recipient analysis. DiR+ Cd11b+ cells were gated in recipient mice BM (center), Sp and Tm fraction and P-PMN, PMN and M-MDSCs were sequentially gated (right). **C)** Distribution of DiR+ positive cells from control mice in BM, Sp and Tm in Control mice. Transplanted P-PMN predominantly home to the BM where they reconstitute the PMN pool. In spleens, P-PMN DiR+ cells show ~5% reconstitution for both PMN and M populations. In tumors, DiR+ cells show a marked preference for PMN generation over M-MDSCs. **D)** ST316-reprogrammed P-PMN-MDSCs display impaired short-term ability to generate PMN in BM and significantly reduced tumor PMN colonization. For each compartment, percentages are normalized to the control mean. (Statistics, 1-way ANOVA, *p<0.05, **p<0.01).

ST316 Depletes PMN-MDSCs From Peripheral Blood of Phase 1 Patients

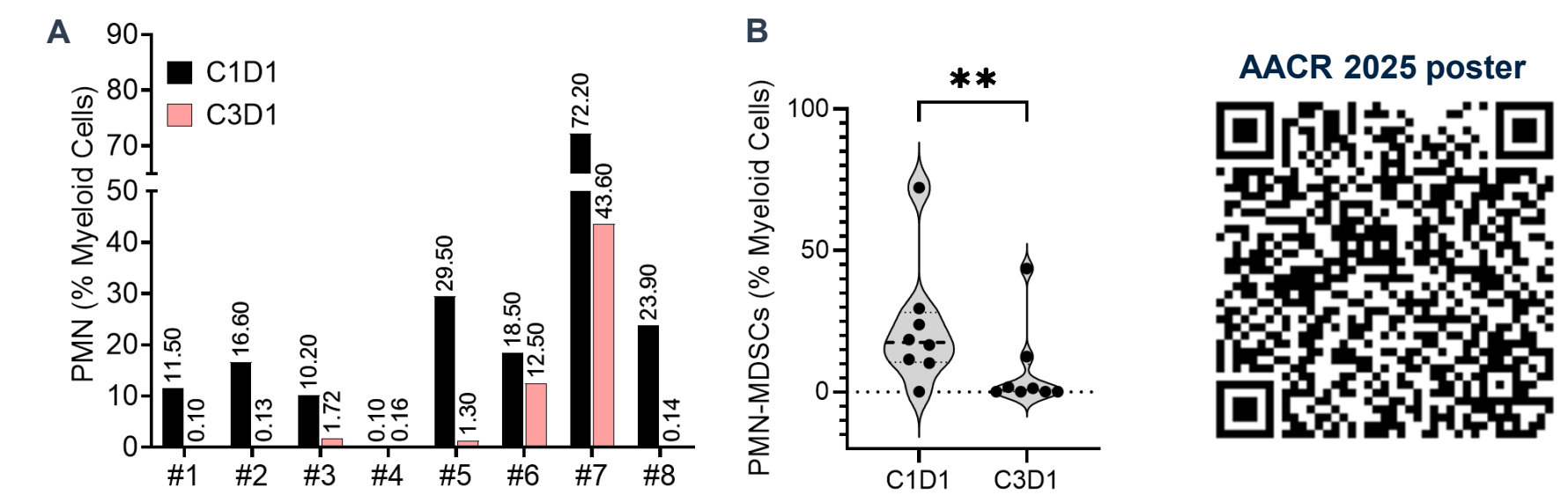


Figure 8. A) Peripheral blood (PB) fractions were analyzed pre-dosing (C1D1) or at Cycle 3 (C3D1) in 8 patients in the phase 1 ST316 trial. PMN-MDSCs fractions are reported as percentages of total myeloid cells. **B)** Violin plot for aggregate statistics of PMN-MDSCs in pretreatment and at Cycle 3. Statistics, Paired Student's-t test, n=8. **p<0.01. Scan the QR code for the AACR 2025 poster with extended data on clinical PMN-MDSC as ST316 pharmacodynamic biomarker.

Conclusions

- ST316 antagonism of the β -Catenin/BCL9 complex contracts PMN-MDSCs in secondary lymphoid organs and tumors in immunocompetent CT-26 CRC models. Similar data for APC^{min} model not shown.
- ST316 modulates the Wnt/ β -catenin program in PMN-MDSCs to suppress expression of myeloid immune checkpoints CD101 and CD300c. Similar data for CD170 not shown.
- ST316 reduces the proliferation and immunosuppressive potential of a Ly-6G^{dim};Ly-6C^{int} BM resident population distinct from PMN-MDSCs, identified here as PMN precursors (P-PMN-MDSCs).
- The β -Catenin/BCL9 complex is crucial for P-PMN-MDSCs proliferation, maturation into PMN-MDSCs in BM and homing to tumors.
- PMN-MDSCs were elevated in the PB of most evaluable patients in ST316 Phase 1 study (7/8). ST316 exposure resulted in MDSCs reduction in the PB of all patients who displayed baseline elevations (7/7).
- This study identifies a necessary role for β -Catenin/BCL9 in PMN-MDSC maturation from Ly-6G^{dim};Ly-6C^{int} BM precursor cells and immunosuppressive activity.
- PMN-MDSC modulation is a putative pharmacodynamic biomarker for ST316 activity in advanced CRC.

¹ Veglia et al, J Exp Med 2018; ² Toor et al, Front Immunol 2016