PYX-106, a highly differentiated anti-Siglec-15 monoclonal antibody, reverses Siglec-15 mediated immune suppression and enhances cytotoxic activity in human lymphocytes

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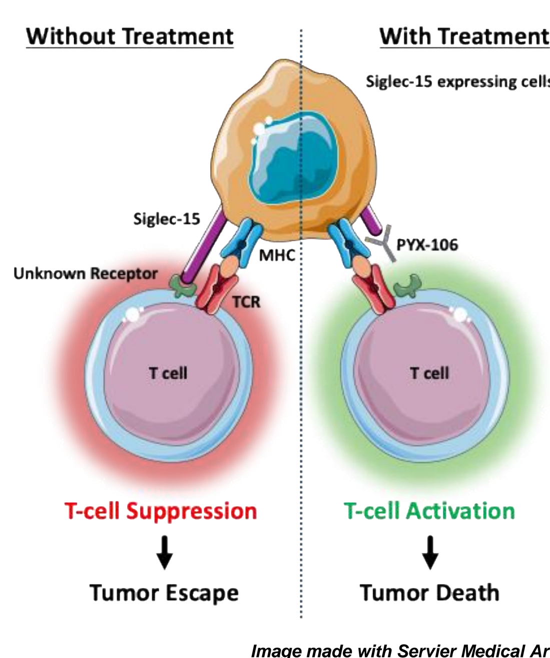
Abstract #: 567

Background

Sialic acid-binding immunoglobulin-like lectin 15 (Siglec-15) is a single-pass type I membrane glycoprotein that functions as an anti-tumor immune suppressor. Siglec-15 expression is upregulated across many cancer indications [1]. Pre-clinical studies have demonstrated that Siglec-15 promotes tumor outgrowth by inhibiting T cell functionality [1], suggesting that blockade of this molecule could serve as a novel cancer immunotherapy. Our data show that PYX-106, a fully human IgG1 monoclonal antibody, binds to human Siglec-15 at a unique epitope site with high specificity and affinity. Importantly, our data demonstrates that PYX-106 is a potent antagonistic antibody that reverses Siglec-15 mediated immune suppression and enhances T cell functionality.

[1] Wang J, Sun J, Liu LN, Flies DB, Nie X, Toki M, et al. Siglec-15 as an immune suppressor and potential target for normalization cancer immunotherapy. Nat Med 2019;25:656–66.

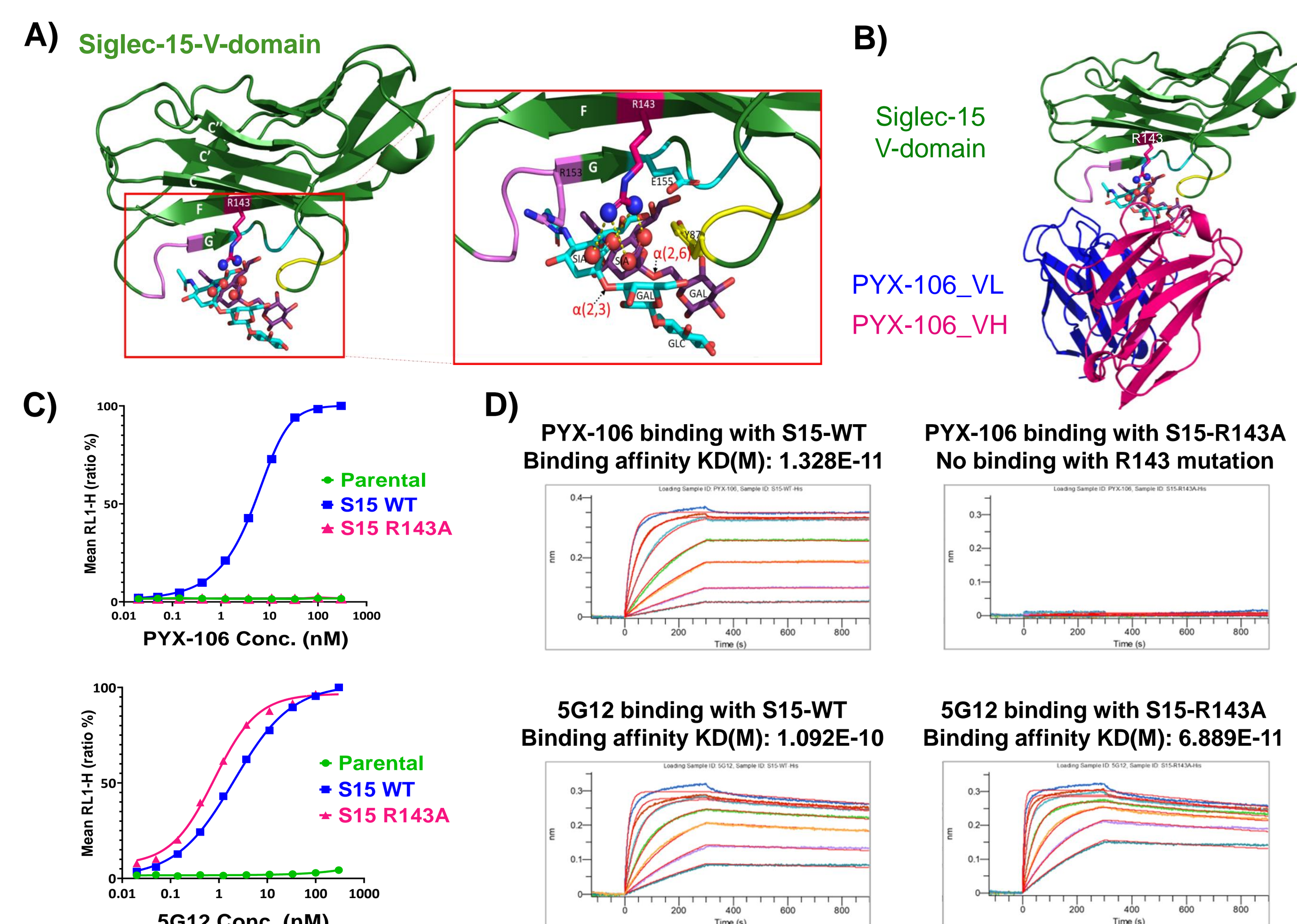


Methods

The binding and biophysical properties of PYX-106 and benchmark anti-Siglec-15 antibody (Clone 5G12) were determined and compared by plate and cell-based assays. Structural modeling and site-directed mutagenesis were used to identify the key residue that PYX-106 interacts with on Siglec-15. The functional activity of PYX-106 and 5G12 were also tested by *in vitro* assays involving stimulated peripheral blood mononuclear cells (PBMC) from healthy donors and cancer patients.

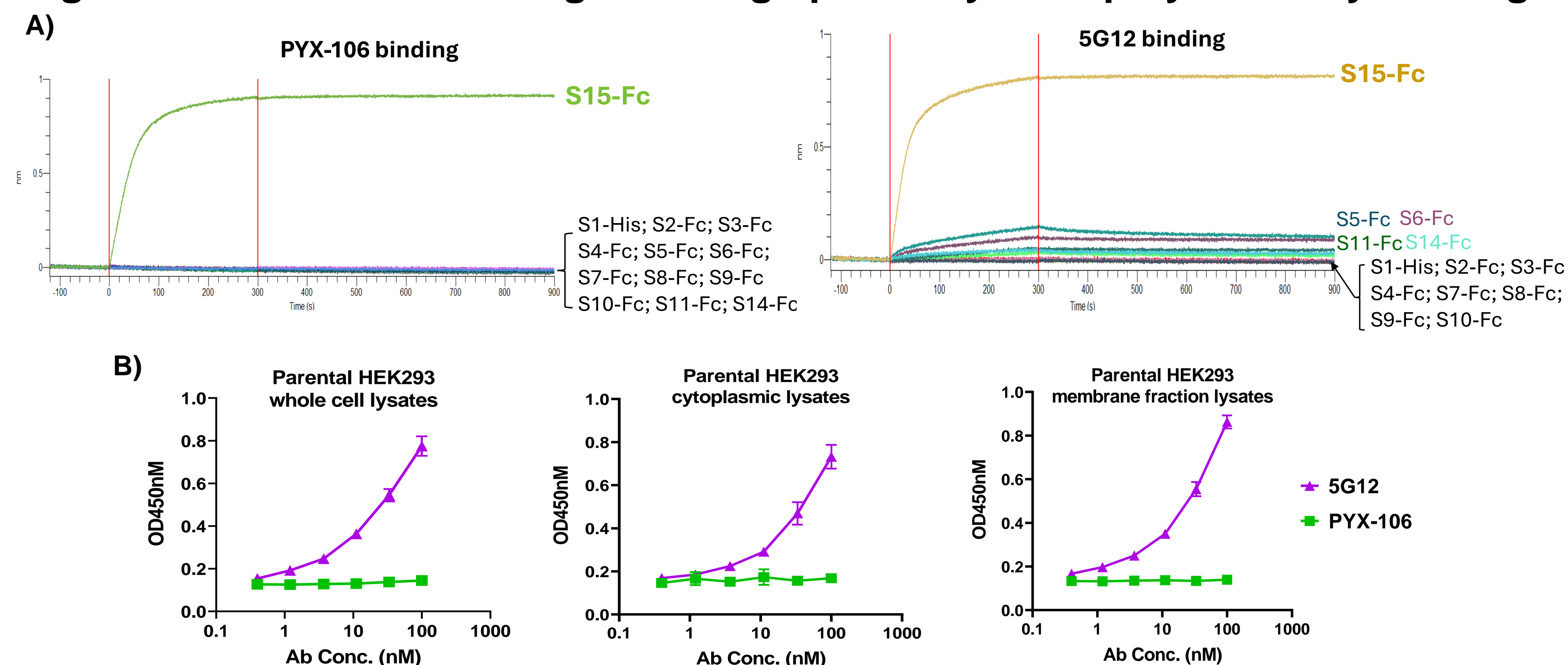
Results

Figure 1: PYX-106 uniquely targets Siglec-15 sialic acid recognizing residue R143



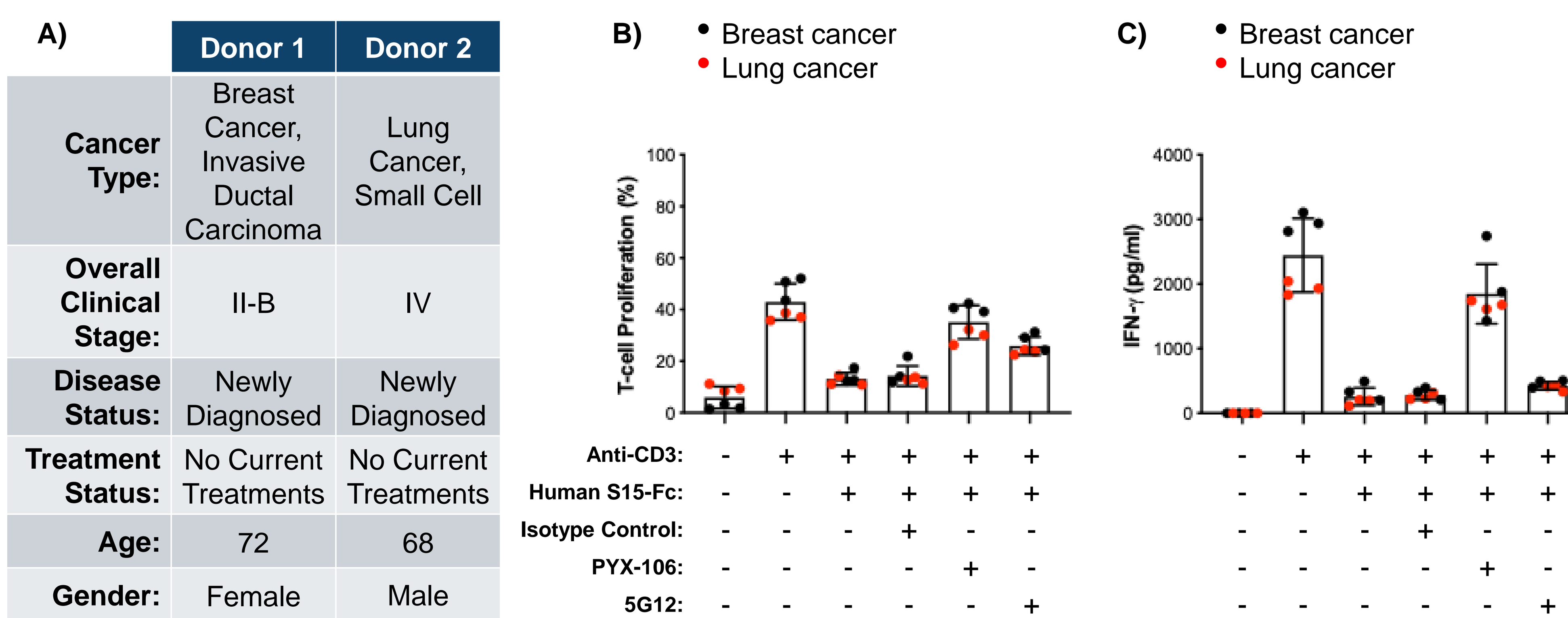
Unique epitope of PYX-106. A) PyMOL illustrates that a bidentate salt bridge formed between the guanidinium group (NH2) of Siglec-15 (S15) R143 residue, an "essential" conserved arginine for recognizing sialic acid, and the carboxylate group (COOH) of sialic acid. **B)** PyMOL modeling predicts that PYX-106 directly competes with sialic sugar motif binding to R143. **C)** PYX-106 binds onto HEK293 OE human S15-WT cells, but not human S15-R143A cells. In contrast, 5G12 binds onto both human S15-WT and human S15-R143A cells, indicating that R143 is essential for PYX-106, not for 5G12, binding to S15. **D)** Octet BLI sensorgrams for PYX-106 and 5G12 binding to S15 WT and R143A mutant proteins. R143A mutation abrogates the binding between PYX-106 and S15, further demonstrating that PYX-106 directly interacts with R143 residue to block the sialoglycan ligand binding motif of S15.

Figure 2: PYX-106 shows high binding specificity & no polyreactivity binding



Biophysical characterization of anti-S15 mAbs. A) The binding specificity against 13 Siglec family members was measured by Octet. 7.5 μg/mL PYX-106 and 5G12 were captured onto BLI biosensors, respectively; binding affinity with 50 nM recombinant Siglecs were measured individually. PYX-106 specifically binds to S15-Fc with high affinity, and no detectable binding to other Siglecs. In contrast, 5G12 showed the high binding affinity with S15-Fc, as well as the non-specific binding to multiple other Siglec family proteins. **B)** ELISA-based polyspecificity reactivity (PSR) assays (n=3) were performed using parental HEK293 whole cell lysate, cytoplasmic fraction and membrane fraction cell lysates, respectively, to assess antibody polyreactivity. In contrast to 5G12, PYX-106 demonstrated non-detectable PSR in all analyzed cell lysates.

Figure 3: PYX-106 effectively blocks Siglec-15 mediated suppression and enhances the functionality of T cells from cancer patients

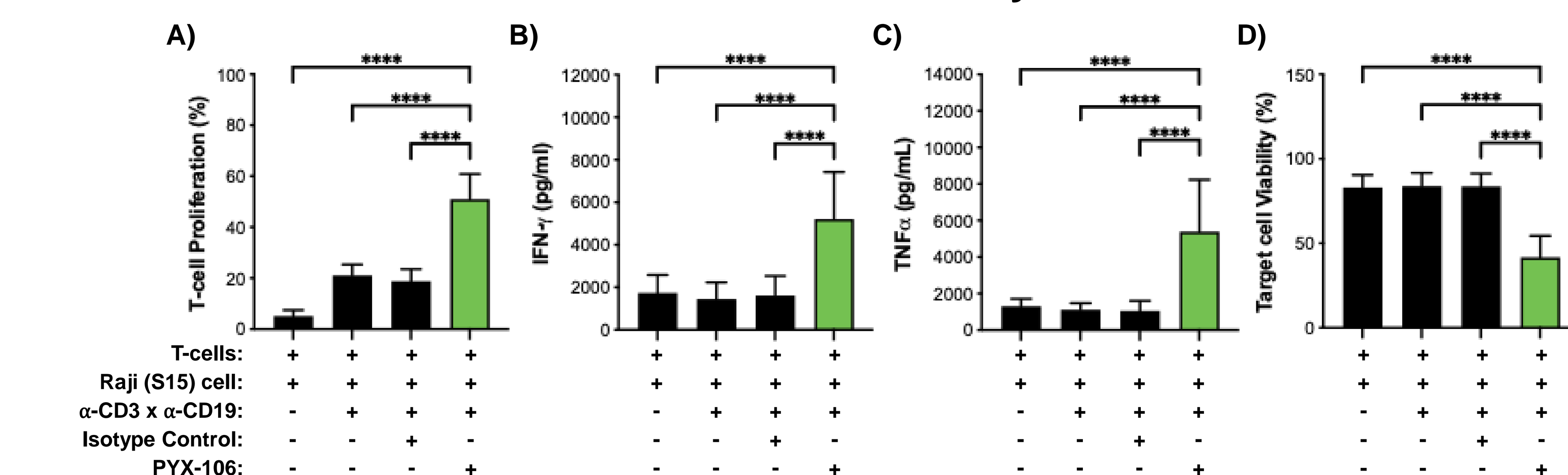


A)

	Donor 1	Donor 2
Cancer Type:	Breast Cancer, Invasive Ductal Carcinoma	Lung Cancer, Small Cell
Overall Clinical Stage:	II-B	IV
Disease Status:	Newly Diagnosed	Newly Diagnosed
Treatment Status:	No Current Treatments	No Current Treatments
Age:	72	68
Gender:	Female	Male

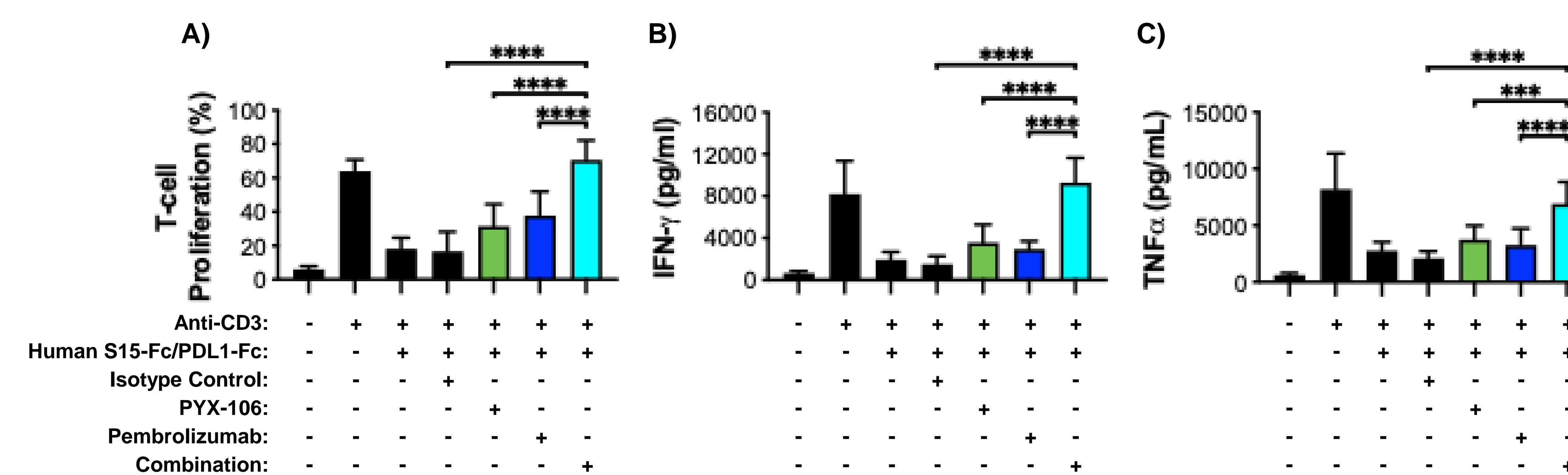
***In vitro* functionality of anti-S15 mAbs. A)** Bulk PBMC from indicated cancer patients (Discovery Life Science) were incubated with 10 μg/mL (90 nM) of soluble recombinant human Siglec-15 Fc-protein (S15-Fc) and sub-optimal concentrations of anti-CD3 (OKT3) mAb for three days in absence (-) or presence (+) of isotype control (huIgG1), PYX-106, or 5G12 at a fixed concentration of 30 μg/mL (200 nM). **B)** T cell proliferation was measured by flow cytometry using the CellTrace CFSE Cell Proliferation kit. **C)** Supernatants from test wells were collected at the end of the incubation period and IFNɣ concentrations were determined by ELISA. Data is from a single experiment involving two separate cancer indications.

Figure 4: PYX-106 significantly enhances T cell functionality and killing capacity in cell-based assay



Effectiveness of PYX-106 in cell-based assay. Bulk PBMC from healthy donors were co-cultured with Raji cells overexpressing human Siglec-15 and sub-optimal concentrations of anti-CD3 and anti-CD19 bi-specific T cell engager for three days in absence (-) or presence (+) of isotype control (huIgG1) or PYX-106 at a fixed concentration of 30 μg/mL (200 nM). **A)** T cell proliferation was measured by flow cytometry using the CellTrace CFSE Cell Proliferation kit. **B-C)** Supernatants from test wells were collected at the end of the incubation period and IFNɣ and TNFα concentrations were determined by ELISA. **D)** Viability of target Raji cells overexpressing human Siglec-15 was measured by flow cytometry using DAPI counter-stain. Data is from three independent experiments involving six healthy donors. P values were calculated by a one-way ANOVA analysis followed by a Tukey post-test. *P≤0.05; **P≤0.01; ***P≤0.001; ****P≤0.0001.

Figure 5: PYX-106 combined with Pembrolizumab significantly enhances T cell functionality than monotherapy treatment



***In vitro* combination approaches.** Bulk PBMC from healthy donors were incubated with 10 μg/mL (90 nM) of soluble recombinant human S15-Fc, 5 μg/mL (50 nM) plate-bound recombinant human PDL1-Fc and sub-optimal concentrations of anti-CD3 (OKT3) mAb for three days in absence (-) or presence (+) of isotype control (huIgG1), PYX-106, and Pembrolizumab at a fixed concertation of 30 μg/mL (200 nM). **A)** T cell proliferation was measured by flow cytometry using the CellTrace CFSE Cell Proliferation kit. **B-C)** Supernatants from test wells were collected at the end of the incubation period and IFNɣ and TNFα concentrations were determined by ELISA. Data is from two independent experiments involving four healthy donors. P values were calculated by a one-way ANOVA analysis followed by a Tukey post-test. *P≤0.05; **P≤0.01; ***P≤0.001; ****P≤0.0001.

Conclusions

These results support that PYX-106 could serve as a potent therapeutic antibody in many cancer indications, including those non-responsive to conventional immunotherapies. PYX-106 is a promising immunotherapy which is currently under investigation in a Phase I clinical trial (NCT05718557).

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