

E-602 (Efgitasialase alfa) Enhances Memory B Cell Depletion by Rituximab and Reduces Profibrotic Macrophages via Desialylation in Autoimmune Disease



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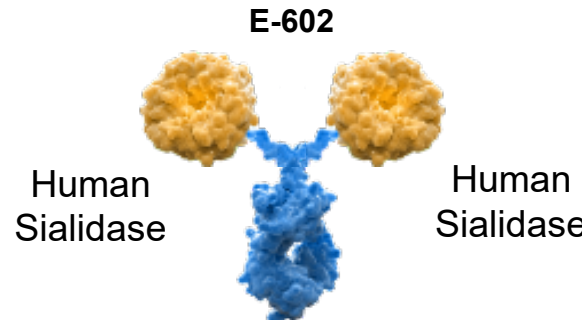
Introduction

Multiple clinical studies link higher levels of **CD27⁺ memory B cells** to suboptimal treatment outcomes in autoimmunity (1, 2), especially with B cell depleting agents. In addition, several studies link the persistence of CD163⁺ **M2 macrophages** to organ damage and fibrosis in autoimmunity (3). Therapeutic strategies that reduce these two key pathogenic immune cell subsets may have meaningful impact in treating autoimmune diseases.

Sialoglycans - cell surface glycans that terminate with sialic acid – contribute to regulating immune response and promoting cell survival. Sialoglycans play a role in the persistence of these two pathogenic immune cell populations.

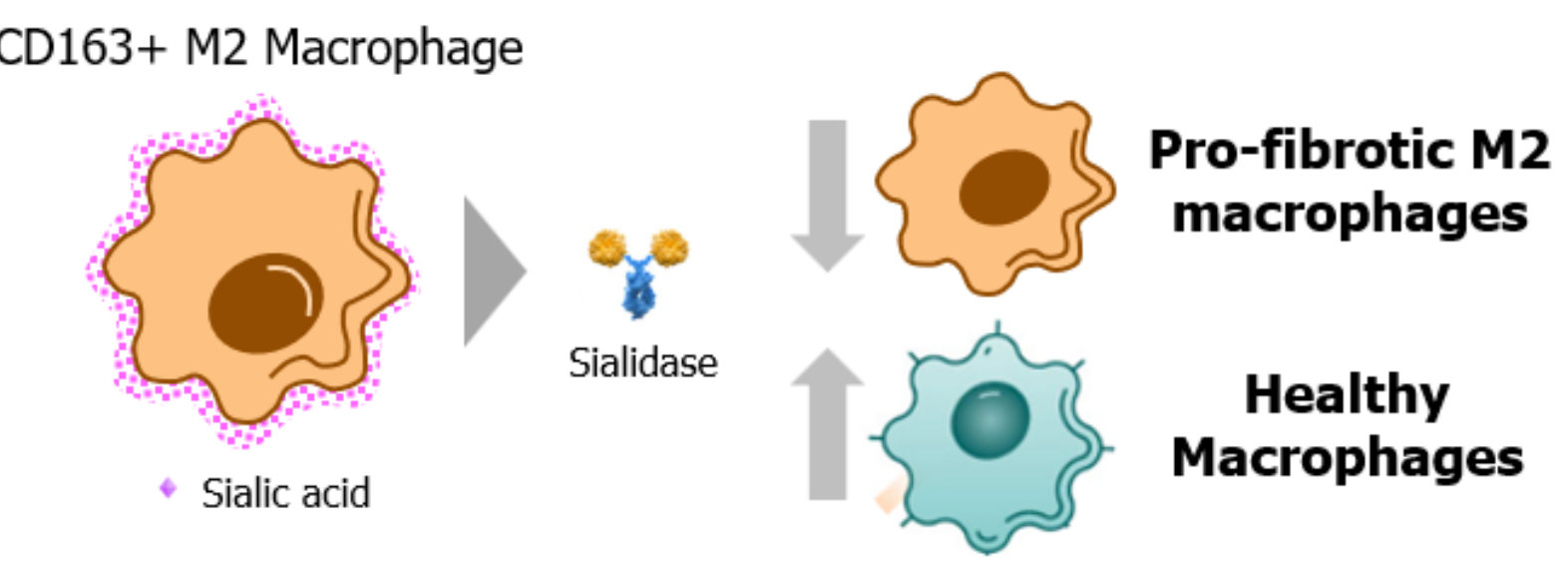
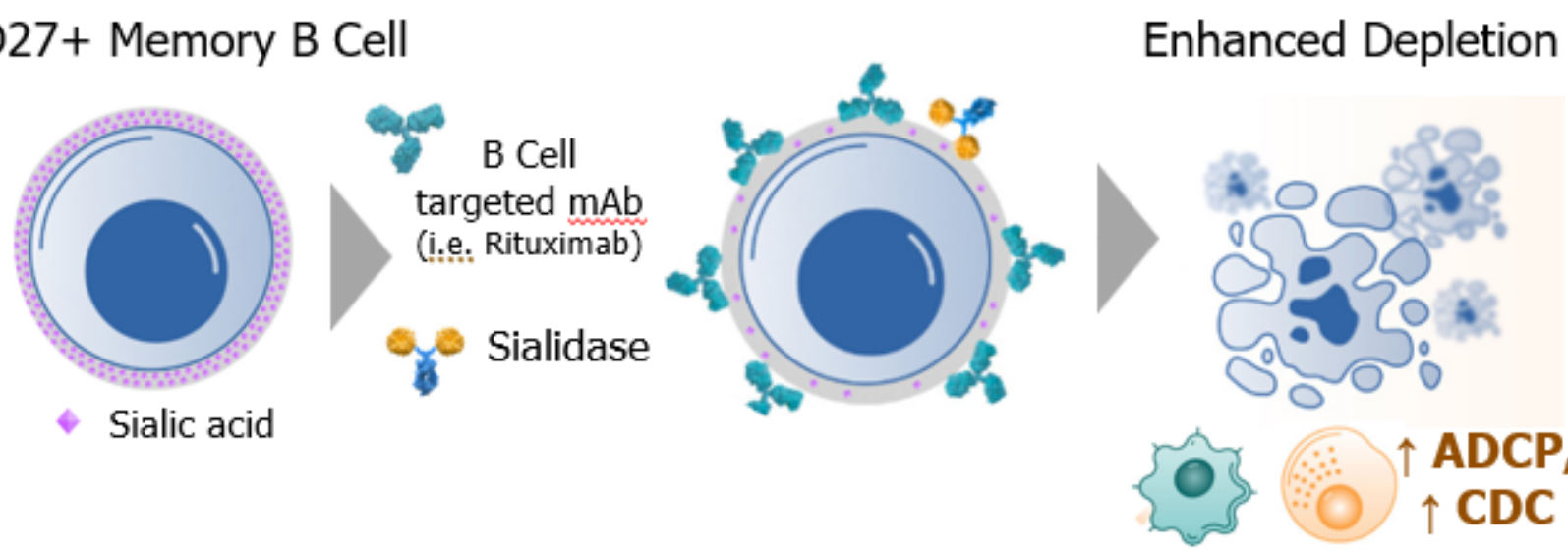
E-602 is a human sialidase-Fc dimer that removes sialic acid from cell surfaces with high sialoglycan levels. **Desialylation** with E-602 enhances the antibody-mediated **reduction of CD27⁺ memory B cells** and the direct clearance of **CD163⁺ M2 macrophages**.

E-602 thus offers a novel therapeutic strategy through enzymatic desialylation to enhance the clearance of these two critical pathogenic immune cell populations and restore immune balance in a wide range of patients with autoimmune diseases.



Enhanced CD27⁺ Memory B Cell Depletion
High sialoglycan levels on CD27⁺ memory B cell surfaces play a critical role in promoting their resistance to depleting antibody-mediated clearance (4,5). Desialylation enhances mAb-targeted depletion of **CD27⁺ memory B cells**

Reduced CD163⁺ M2 Macrophages
High sialoglycans also enable the persistence of CD163⁺ M2 macrophages in disease tissues, which contributes to fibrosis and tissue damage. Desialylation increases the clearance of **CD163⁺ M2 macrophages**



Results

E-602 enhanced ADCP and CDC function of anti-CD20 mAbs

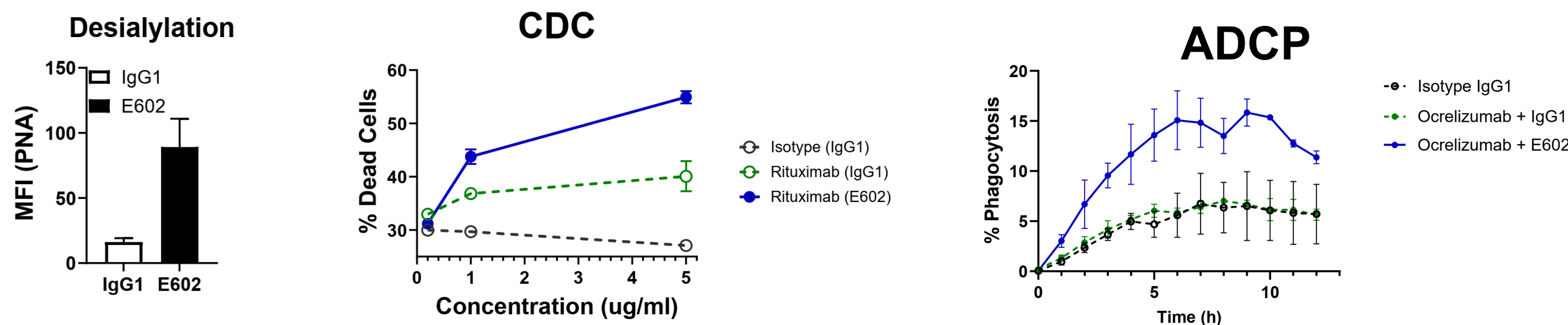


Figure 1. (left) Desialylation of primary human B cells. **(middle)** E-602 enhanced rituximab-mediated CDC of primary human B cells. **(right)** Time course of phagocytosis using live cell imaging demonstrates E-602 mediates early and sustained increase in ocrelizumab-mediated ADCP of Raji cells

E-602 durably desialylates B cells *in vivo*, in cynomolgus monkeys and in human subjects

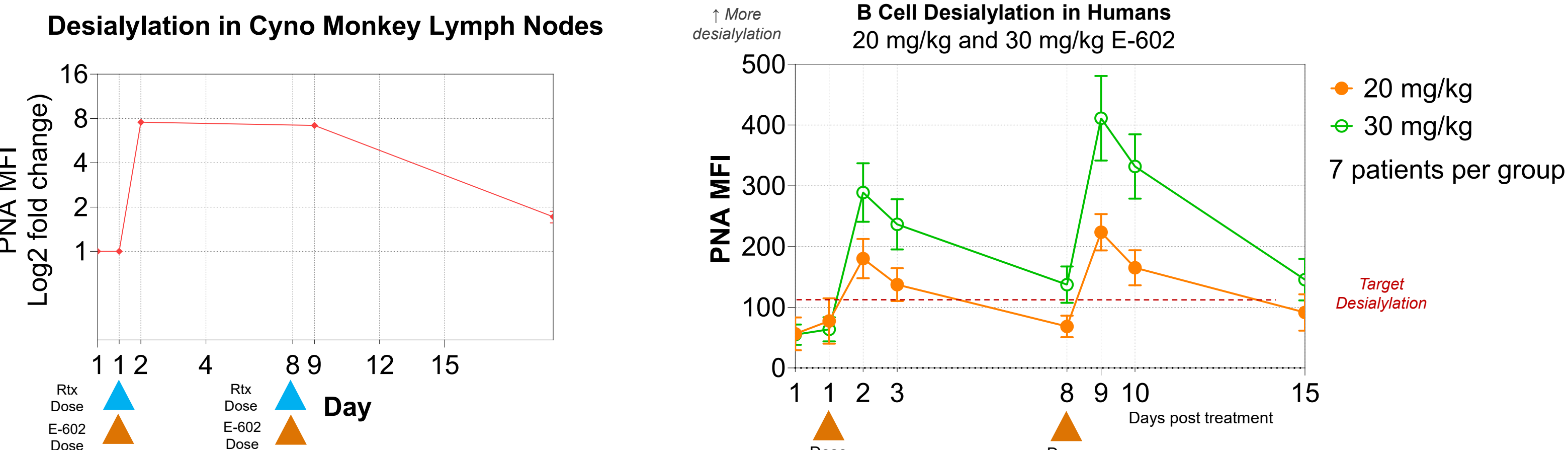


Figure 2. (left) E-602 desialylation of lymph nodes of cynomolgus monkeys, as measured by PNA staining on CD3⁺ cells (since B cells have been depleted). N = 4 animals/group, all treatment was given i.v, rituximab dosed at 20mg/kg, E-602 dosed at 90mg/kg. **(right)** Desialylation of B cells as measured by PNA, in peripheral blood of human subjects dosed with 20 mg/kg or 30 mg/kg of E-602 in the GLIMMER-01 study. MFI: Mean Fluorescence Intensity.

E-602 enhanced Rituximab-mediated B cell depletion *in vivo*

Lymph Node B Cell Depletion Non-Human Primate

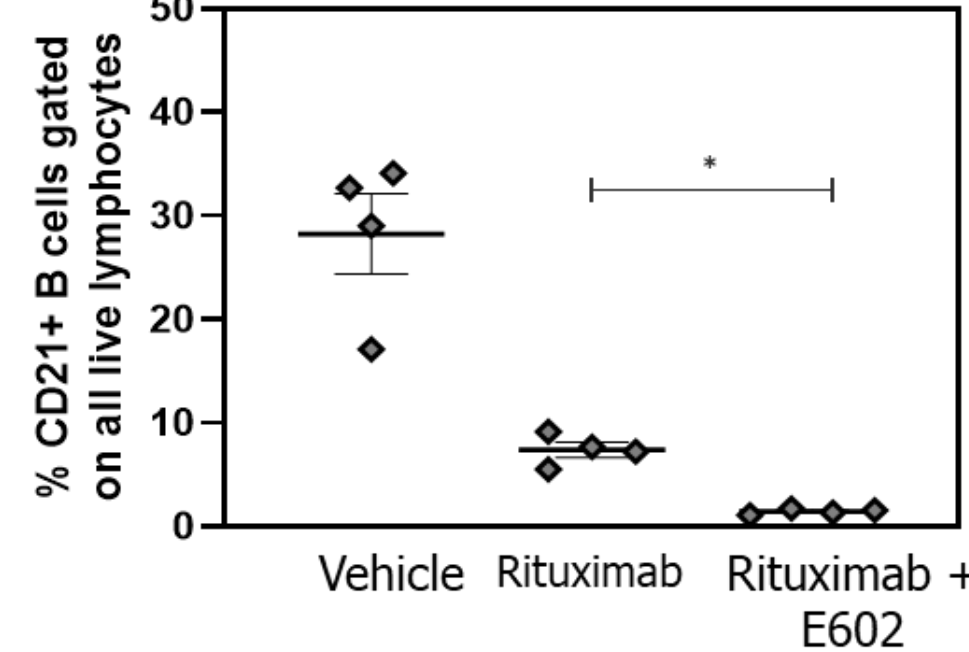


Figure 3. E-602 desialylation enhanced rituximab-mediated depletion of B cells in lymph nodes of cynomolgus monkeys *in vivo*. N = 4 animals/group, all treatment groups were given i.v, rituximab dosed at 20mg/kg, E-602 dosed at 90mg/kg. Lymph nodes were biopsied at 2 days post dosing. (* P < 0.05, ANOVA)

E-602 enhanced rituximab-mediated memory B cell depletion in PBMCs from SLE patients

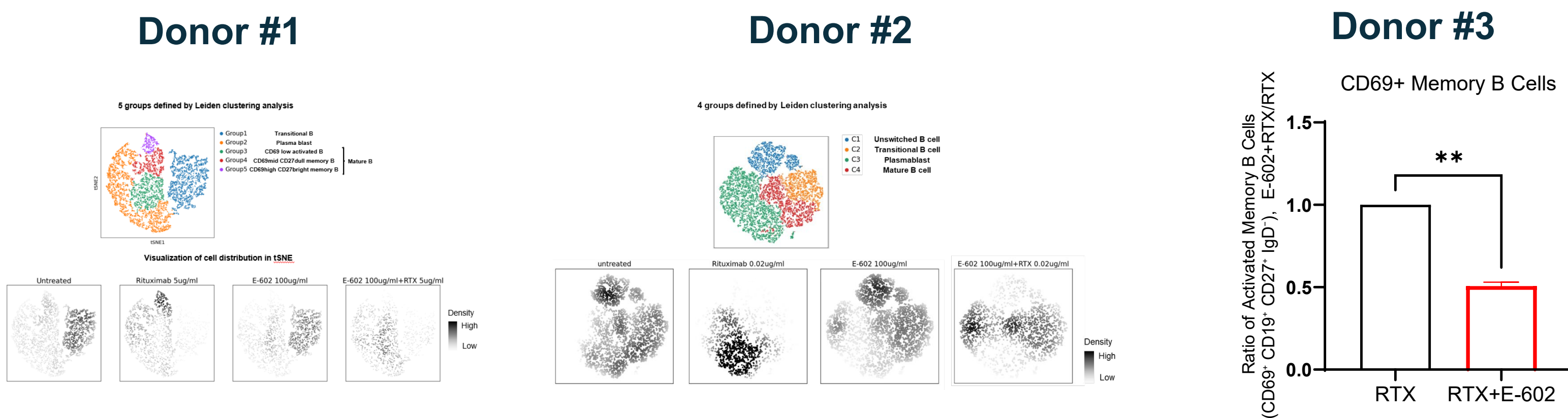


Figure 4. (left) t-SNE diagram of B cell subsets of PBMC of SLE donor #1. Treatment of E-602 alone did not affect the proportion of each B cell subset. Rituximab treatment alone significantly depleted subset 1, and most remaining B cells were from subset 5 (CD69high mature memory B cells). This type of cell was reported to be resistant to antibody-mediated B cell depletion (6). After E-602 treatment, the depletion of subset 5 by rituximab was significantly enhanced. **(middle)** Rituximab mainly depleted transitional B cells or unswitched B cells, which are in the early stages of B cell differentiation. With E-602 pre-treatment to remove sialic acid on the surface of B cells, the depleting function of rituximab on late stage or mature memory B cells or plasmablasts was significantly enhanced. **(right)** E-602 enhanced the depletion of CD69+ memory B cells by rituximab in human SLE PBMCs.

E-602 enhanced anti-CD20 mediated B cell depletion, and improved disease scores in MRL/lpr SLE mouse model

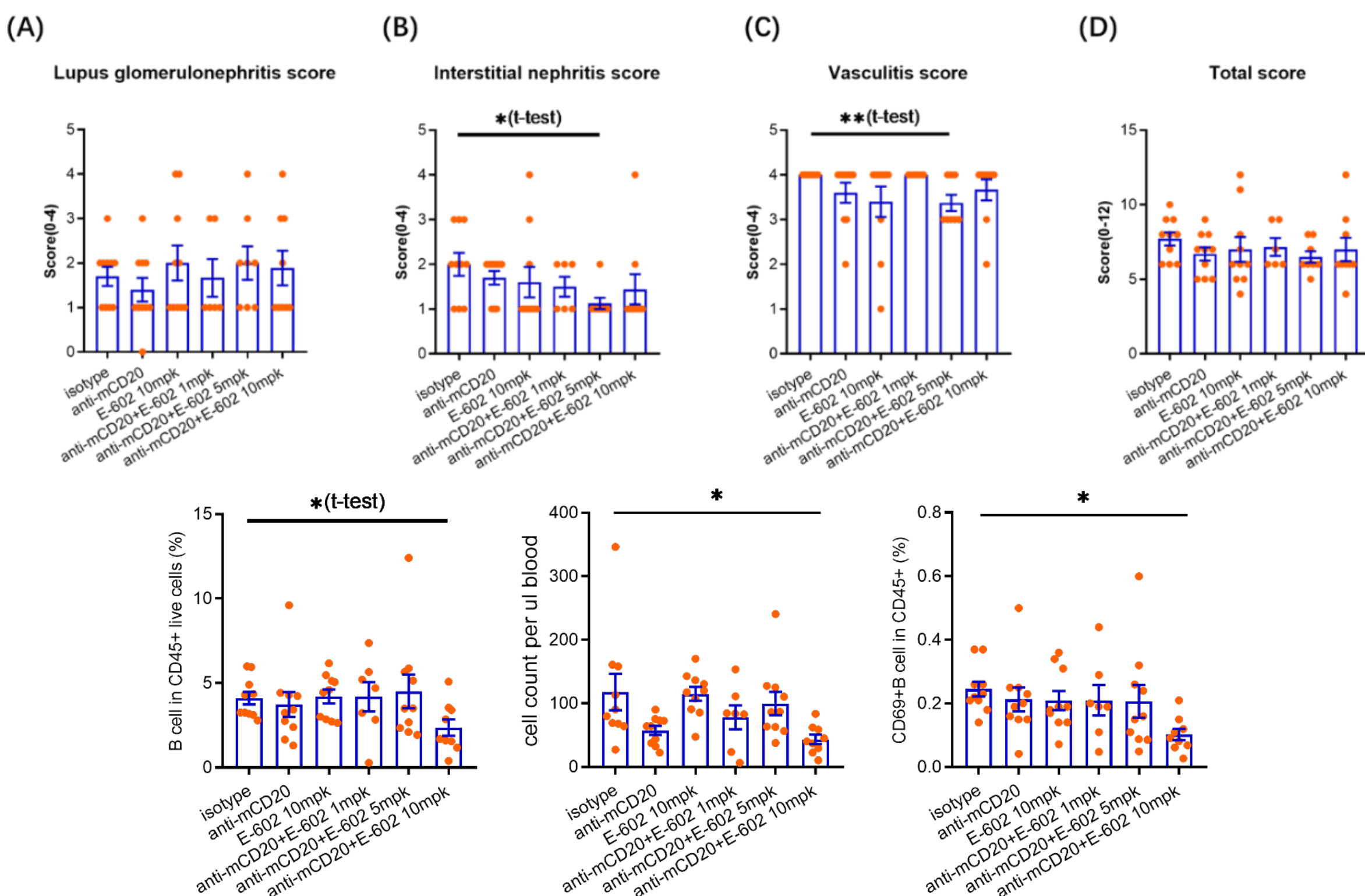


Figure 5. Combination of E-602 and anti-CD20 enhanced B cell and CD69⁺ activated B cell depletion in blood. Changes in peripheral blood B cells in 16-week MRL/lpr mice, *p<0.05 (Mean ±SEM, except for the data marked with t-test, others were tested using one-way ANOVA).

Figure 6. Combination of E-602 and anti-CD20 improved interstitial nephritis and vasculitis scores in kidney of MRL/lpr mice. Mouse renal pathological scores, lupus glomerulonephritis (A), interstitial nephritis (B), vasculitis (C) and total score (D), *p<0.05, **p<0.01 (Mean ±SEM, t-test).

Desialylation reduced tumor associated M2-like macrophages in mouse models

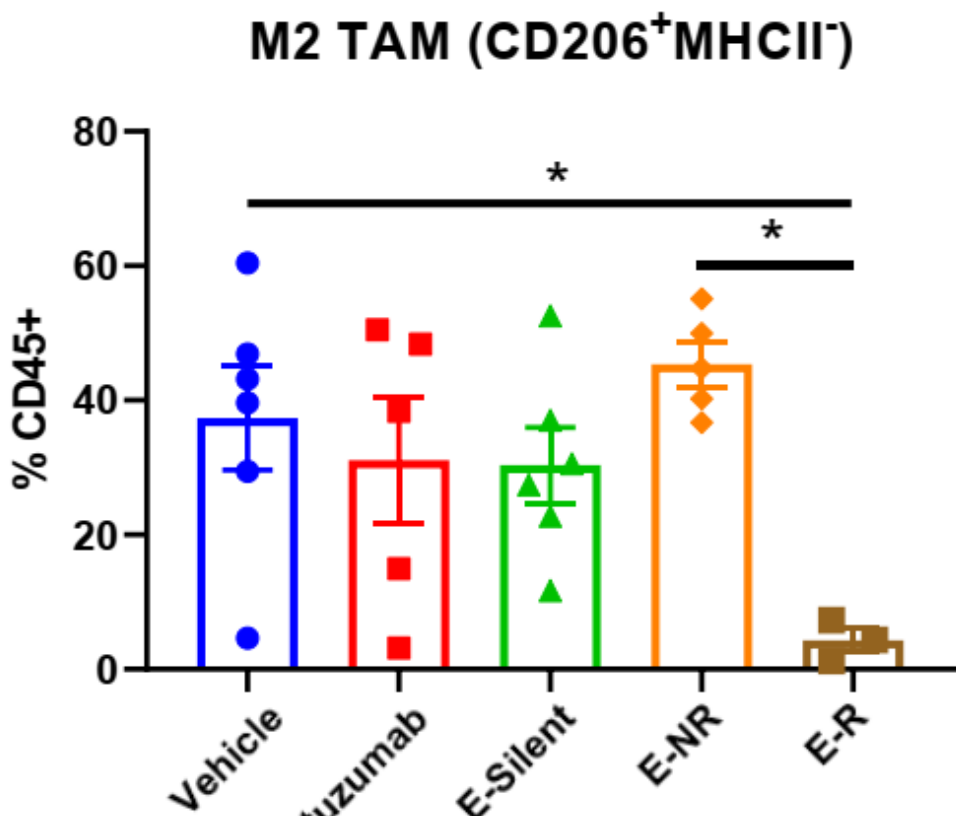


Figure 7. Desialylation by a HER2-targeted engineered human sialidase in syngeneic EMT6-hHER2 mouse tumor model. NR, R designated non-responder and responder, respectively based on tumor growth inhibition at time of tumor collection. E-Silent was a loss of function mutant sialidase. Treatment was given intraperitoneally to animals, twice per week for 5 total doses. After the last dose, tumors were harvested for analysis by flow cytometry. (*, p < 0.05, ANOVA)

E-602 reduced CD163⁺ macrophages in patients in GLIMMER-01 trial

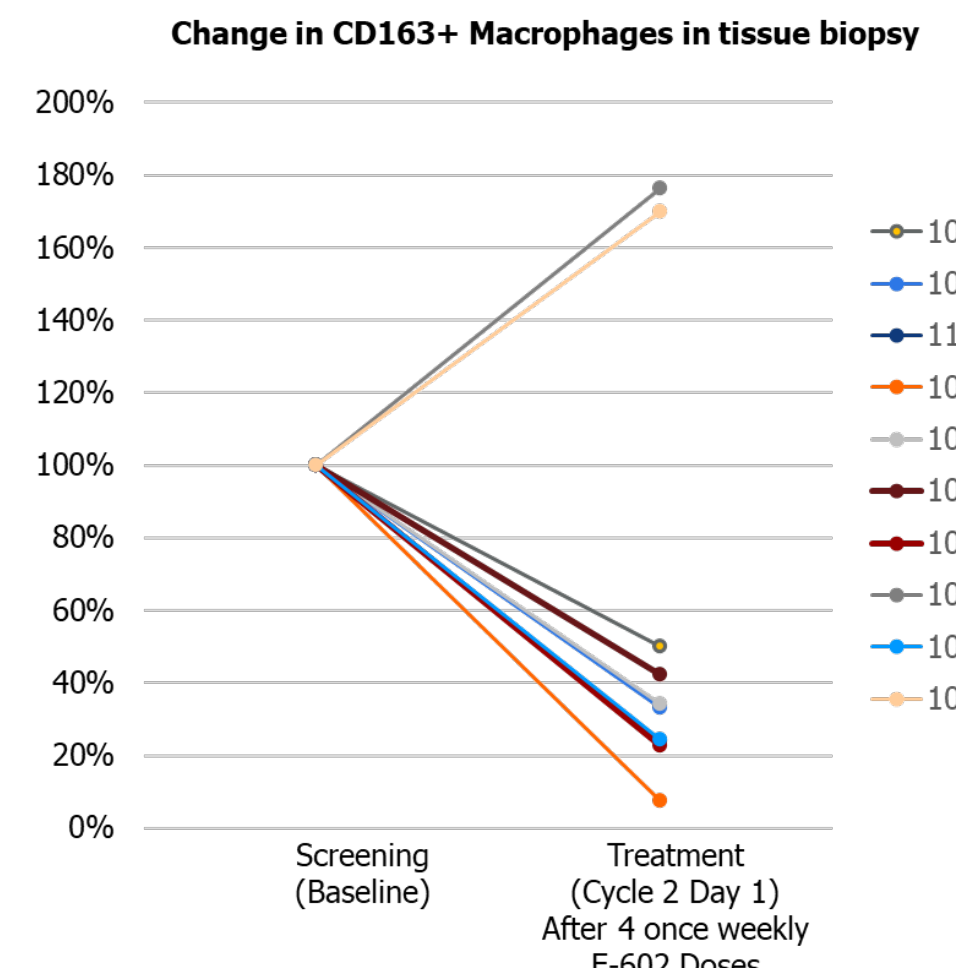


Figure 8. Change in CD163⁺ macrophages in biopsy from patients treated with E-602 in GLIMMER-01 trial.

Evolving data shows strong association of decreases in M2 macrophages with outcomes and is used as a biomarker of end organ response in renal autoimmune diseases (6)

- CD163⁺ M2 macrophages are the major subset driving tissue fibrosis in autoimmunity
- E-602 has potential to reduce fibrosis

Conclusions

	CD27 ⁺ Memory B Cell	CD163 ⁺ M2 Macrophage
Role in Healthy Immunity	Immune Memory Long-lived cells that secrete antibodies to previously encountered antigens	Wound Healing Play important role in wound healing and scar formation
Dysfunction in Autoimmunity	Driver of Active Inflammatory Injury: Produce autoantibodies, present antigens to T cells, and secrete inflammatory cytokines	Driver of Fibrosis CD163 ⁺ M2 macrophages cause excess collagen deposition, fibrosis, and loss of organ function in autoimmunity
Validation as a Target	B cell depletion is clinically validated strategy; memory B cell reconstitution is associated with relapse	Macrophage reduction is a clinically validated strategy in autoimmunity (CSF-1R inhibition in cGVHD)
Role of Sialoglycans	Memory B cells are naturally hypersialylated making them more resistant to antibody-mediated depletion	High sialoglycans allow dysfunctional CD163 ⁺ M2 macrophages to persist in inflamed tissue
Impact of Desialylation	Desialylation improves mAb-mediated depletion of memory B Cells <i>Improved Reconstitution Favoring Naive over Memory B Cells</i> <i>In combination with B Cell mAb</i>	Desialylation reduces pro-fibrotic M2 macrophages, reducing fibrosis <i>Enhanced Clearance of Pro-fibrotic CD163⁺ M2 Macrophages</i> <i>Single Agent</i>

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Next Steps: Ongoing Phase 2

E-602 offers a novel therapeutic strategy through enzymatic desialylation of pathogenic immune cells to enhance their depletion and restore immune balance. E-602 has completed a first-in-human trial in oncology (GLIMMER-01), demonstrating excellent safety and tolerability. A total of sixty-nine (69) subjects were treated in the study, the median number of doses of E-602 administered was 8 (range 2 – 43 doses), and the highest dose administrated was 30mg/kg, weekly. There were no SAEs that were considered related to E-602 administration, and the reported SAEs were consistent with those reported for advanced cancer populations. E-602 is currently in a Phase 2 study in combination with a rituximab biosimilar for glomerulonephritis (Membranous Nephropathy and Lupus Nephritis, ClinicalTrials.gov identifier NCT07038382). The Phase 2, part A is a multi-ascending dose (MAD) study with 3 doses of E-602 + 375mg/ m2 rituximab x 4 . Each arm will compare 6 pts to 2 pts on rituximab only. The phase 2, part B is a randomized double-blind, placebo-controlled study (N = 150 subjects), testing E-602 at 2 dose levels in combination with rituximab, and in the single arm controls. Endpoints include safety, clinical efficacy (PR and CR) based on eGFR and key biomarkers (autoantibodies levels including PLA2R), B cell repertoire, and urinary CD163 level.