TARGETING OX40 WITH GBR 830, AN OX40 ANTAGONIST, INHIBITS T CELL-MEDIATED PATHOLOGICAL RESPONSES

JULIE MACOIN[1]; RAMI LISSILAA[1]; PAVANKUMAR SANCHETI[2]; VENKATESHWAR REDDY[3]; JONATHAN BACK[1]; GERHARD WOLFF[3]

[1]GLENMARK PHARMACEUTICALS SA, SWITZERLAND; [2]GLENMARK PHARMACEUTICALS LTD., INDIA; [3]GLENMARK PHARMACEUTICALS INC., USA

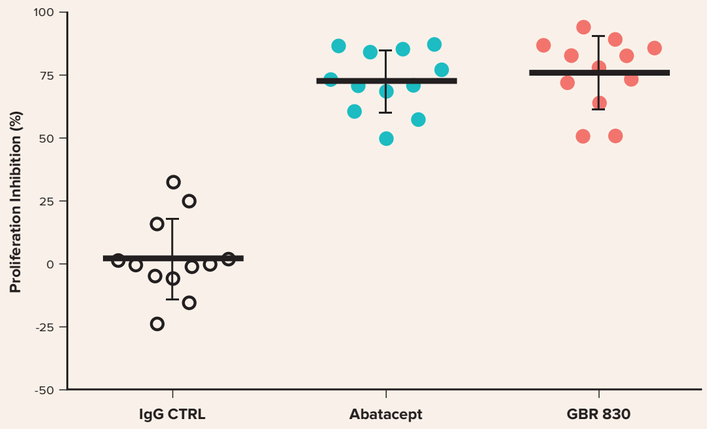
SYNOPSIS/OBJECTIVE

OX40 (TNFRSF4, CD134) is a costimulatory receptor member of the TNFR superfamily expressed predominantly on activated T cells. Binding of OX40 to its ligand OX40L (TNFSF4, CD252) leads to enhanced T cell survival, proliferation, and effector functions. Blocking the OX40/OX40L pathway is therefore a highly attractive target for a broad range of T cell-mediated autoimmune diseases. GBR 830, a humanized IgG1 monoclonal antibody targeting OX40 with proven antagonistic properties and no detectable agonistic activity, blocks OX40L binding and OX40L-mediated T cell proliferation in vitro. The studies presented herein characterize the mechanism of action and immunomodulatory capabilities of GBR 830.

METHODS AND RESULTS

- GBR 830 suppresses T cell-mediated allogeneic responses with a potency similar to positive controls abatacept (CD28 blocker; **Figure 1**) and efalizumab (LFA-1 blocker; data not shown)

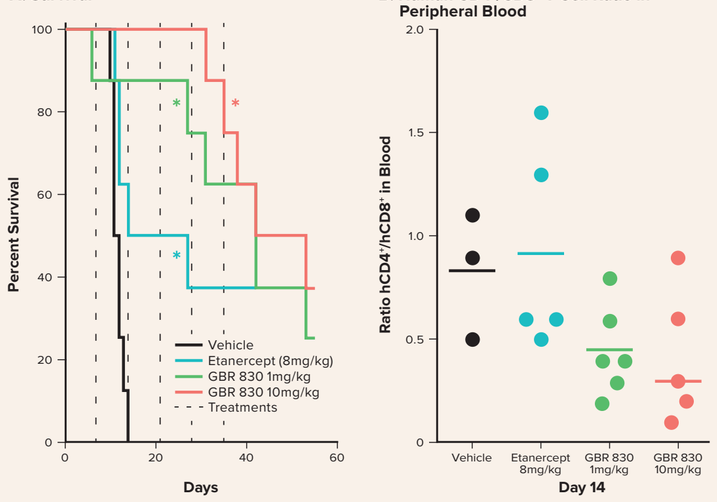
Figure 1. Human Allogenic Mixed Lymphocyte Reaction Assay



One-way mixed lymphocyte reaction measured by ^{3}H-thymidine incorporation. Multiple allogeneic combinations were performed, and each data point represents the maximum T cell proliferation inhibition observed for a given combination. Data are presented as mean ± SD.
CTRL, control.

- GBR 830 blocks a strong T helper-mediated response in a human xenogeneic graft versus host disease (GvHD) model (**Figure 2**)

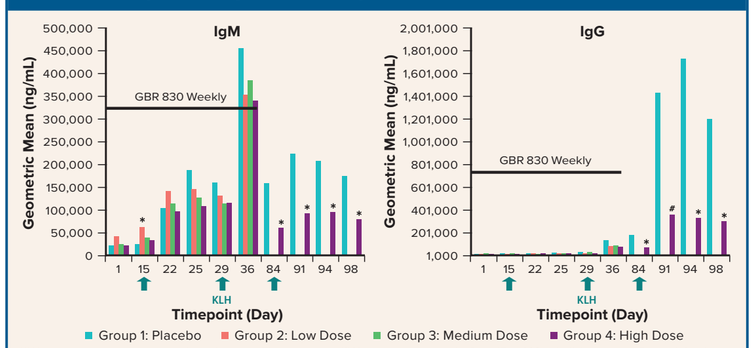
Figure 2. Xenogeneic Human Graft Versus Host Disease Model



SCID mice were sublethally irradiated and reconstituted (intraperitoneal) with 30 million human PBMCs (8 mice/group with PBMCs from 2 different donors) on Day 0. Mouse NK cells were depleted by twice weekly injections of TMbeta1 antibody. GBR 830, etanercept, or vehicle were given weekly (intravenous) for six consecutive doses with first doses administered two days before PBMC injection. **A)** Percent survival (including ethical sacrifice). **B)** Ratio of human CD4$^+$/CD8$^+$ T cells in peripheral blood measured by flow cytometry at Day 14. *p <0.05, Log-Rank test between indicated group and vehicle group.
h, human; NK, natural killer; PBMC, peripheral blood mononuclear cell.

- GBR 830 significantly reduced memory antibody response to keyhole limpet hemocyanin (KLH) in cynomolgus monkeys from Day 84 onward, with no effect on primary antibody response to KLH (**Figure 3**)

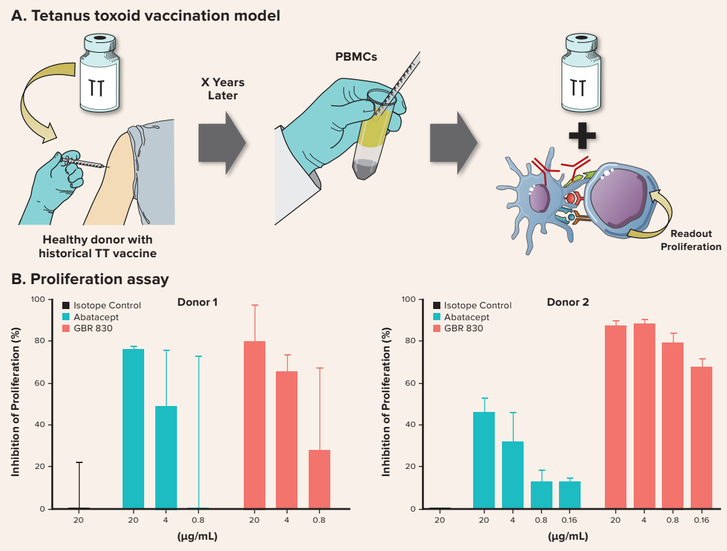
Figure 3. T Cell-dependent Antibody Response to KLH in Cynomolgus Monkeys



KLH immunization timepoints are indicated in green. The primary anti-KLH response was tested at Days 15 and 29 with all dose groups: 5 animals/sex/group for Group 1 (placebo) and Group 4 (GBR 830 high dose); 3 animals/sex/group for Group 2 (GBR 830 low dose) and Group 3 (GBR 830 medium dose). The memory response was tested at Day 84 onward only with Group 1 and Group 4 recovery animals (2 animals/sex/group). The geometric mean values are calculated from the pooled data from males and females for each dose group.
#p<0.05, *p<0.01 versus placebo.
KLH, keyhole limpet hemocyanin.

- Out of 6 healthy donors, GBR 830 demonstrated equal efficacy (n=3; representative Donor 1) or greater efficacy (n=3; representative Donor 2) versus abatacept in suppressing memory reactivation to tetanus toxoid (**Figure 4**)

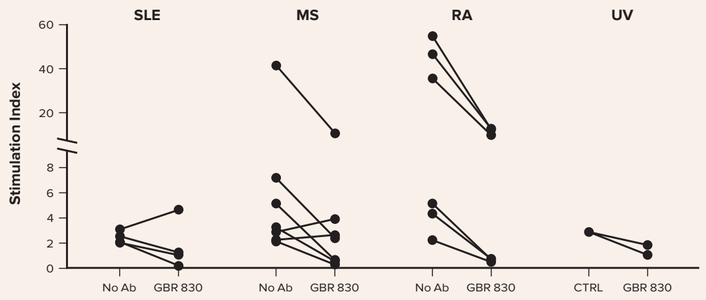
Figure 4. Memory Reactivation with Tetanus Toxoid



A) PBMCs were collected from healthy donors previously vaccinated with TT and incubated for 5 days with purified TT and isotype control, abatacept, or GBR 830. **B)** Proliferation was quantified by ^{3}H-thymidine incorporation. Percent inhibition of proliferation was determined relative to control with PBMC and TT only.
PBMC, peripheral blood mononuclear cell; TT, tetanus toxoid.

- GBR 830 blocks memory reactivation to autoimmune antigens from various autoimmune diseases compared with no antibody or IgG1 isotype control treatment (**Figure 5**)

Figure 5. Memory Reactivation to Autoimmune Antigens



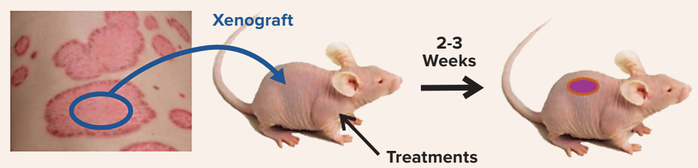
	SLE	MS	RA	UV
Proportion of Antigen Responders Showing an Effect of GBR 830	4/5	5/7	4/4	3/3

PBMCs were incubated with or without relevant antigens in presence or absence of GBR 830 or control antibodies. The response to antigen was measured by ^{3}H-thymidine incorporation. The graph shows the stimulation index for all antigens in the control condition (no antibody or IgG1 control) and GBR 830-treated condition. For RA, some samples responded to more than one antigen. The table shows the proportion of responders for which GBR 830 produced an inhibition of proliferation. Each condition was performed in at least triplicates. The following antigens were selected for each disease: MS, myelin basic protein purified from human brain; RA, citrulinated peptides from aggrecan, vimentin, and fibrinogen proteins[1]; SLE, Sm$D_{183-119}$ peptide[2]; UV, human soluble antigen.[3] If the stimulation index (ratio between the condition with the antigen and without antigen) was ≥2, the donor was considered a responder to that antigen. The effect of GBR 830 was assessed on all responders (SLE, n=5; MS, n=7; RA, n=4; UV, n=3).
Ab, antibody; CTRL, control; MS, multiple sclerosis; PBMC, peripheral blood mononuclear cell; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus. UV, uveitis.

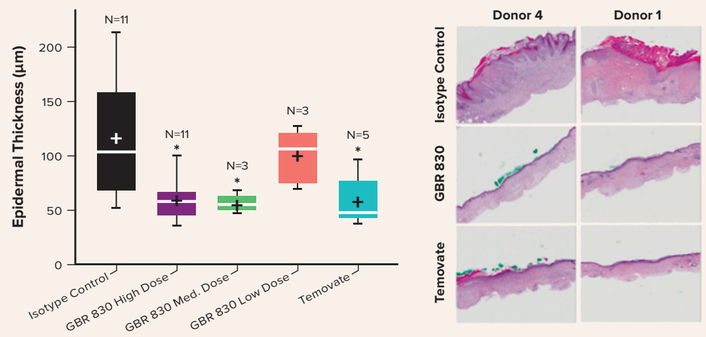
- GBR 830 was equally effective as clobetasol propionate (Temovate®) compared with isotype control in ameliorating the psoriasis phenotype in a human psoriatic skin transplant model (**Figure 6**)
- A reduction in CD3$^+$ T cell number was observed in the GBR 830 treatment group but was not statistically significant from the isotype control group

Figure 6. Human Psoriatic Skin Transplant in SCID Mice

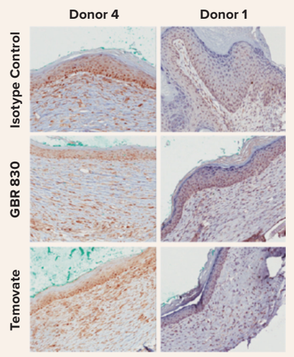
A. Human psoriatic skin transplant model



B. Epidermal thickness



C. T cell infiltration



CD3$^+$/Total	Average	Standard Deviation
Isotype Control	0.237	0.31
GBR 830	0.142	0.15
Temovate	0.136	0.162

A) Full-thickness, 6-mm lesional skin punch biopsies (epidermis + dermis) from adult volunteers with psoriasis were grafted onto the dorsal area of SCID mice. Three to 5 days after graft transplantation, mice were treated with isotype control, GBR 830 (both intraperitoneal), or Temovate® topical at 2x/week for 2 weeks. **B)** Left panel: mean epidermal area and length of tissue were measured using Aperio slide scanning software. Mean epidermal thickness was calculated as area/length. *p< 0.05 (Mann-Whitney two-tailed or student two-tailed unpaired t-test). N indicates the number of donors and + indicates mean. Right panel: Representative images of transplanted tissue cross-sections stained with hematoxylin and eosin. **C)** Left panel: immunohistochemistry of CD3$^+$ T cells (dark brown) in transplanted tissue. Right panel: T cell density based on CD3 immunohistochemistry. No statistical significance was observed due to inter-donor variability. Donors 1 and 4 were treated with the high dose of GBR 830. Histology of transplanted tissue was analyzed using a Scan scope at 10x magnification.
SCID, severe combined immunodeficiency.

CONCLUSIONS

- These data suggest that GBR 830 has immunomodulatory capabilities in memory/chronic T helper cell-mediated pathological responses without pan immunosuppression (no impact on primary antibody responses)
- Strong immune suppression focused on memory and chronic T cell responses but spared naïve T cell function
- Blockade of OX40 by GBR 830 is expected to be a relevant therapeutic target in a broad range of autoimmune diseases

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DISCLOSURES

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