GBR 830: AN OX40 ANTAGONIST ANTIBODY WITH A FAVORABLE TOXICITY PROFILE IN NON-HUMAN PRIMATES

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ABSTRACT

OX40 is a costimulatory receptor member of the NGFR/TNFR superfamily expressed predominantly on activated T cells. Ligation of OX40 by its ligand OX40L leads to enhanced T cell survival, proliferation, and effector functions. Blocking the OX40/OX40L pathway is therefore highly attractive to treat a broad range of T cell-mediated autoimmune diseases. While several OX40 agonist antibodies are under development in oncology, generating an OX40 antagonist devoid of agonist activity remains a challenge. GBR 830 is a humanized IgG1 targeting OX40 with a monovalent affinity for human OX40 (~90 nM as measured by surface plasmon resonance) and cross-reactivity to macaque OX40 albeit with a lower affinity. However, its apparent affinity drastically increases when GBR 830 binds bivalently. GBR 830 blocks OX40L binding and inhibits OX40L-mediated T cell proliferation at a low nM concentration. It also mediates low levels of antibody dependent cellular cytotoxicity and complement dependent cytotoxicity. Importantly, GBR 830 was evaluated for residual agonism by assessing its costimulatory effect on the proliferation of purified T cells from multiple donors. Compared to OX40L or anti-CD28 positive controls, GBR 830 did not stimulate T cells with or without addition of a crosslinking antibody. In a more sensitive experimental setup in which anti-OX40 antibodies were co-coated with an anti-CD3 antibody, no agonism was detected with GBR 830, whereas all other anti-OX40 antibodies tested showed agonism. When administered to cynomolgus monkeys in the repeat-dose intravenous/subcutaneous toxicity studies (6-weeks or 6-months duration), GBR 830 was well tolerated without any adverse findings. The no-observed-adverse-effect-level was 100 mg/kg/week. These data show that GBR 830 is able to block OX40L-induced proliferation without inducing receptor agonism, in contrast to other anti-OX40 antibodies.

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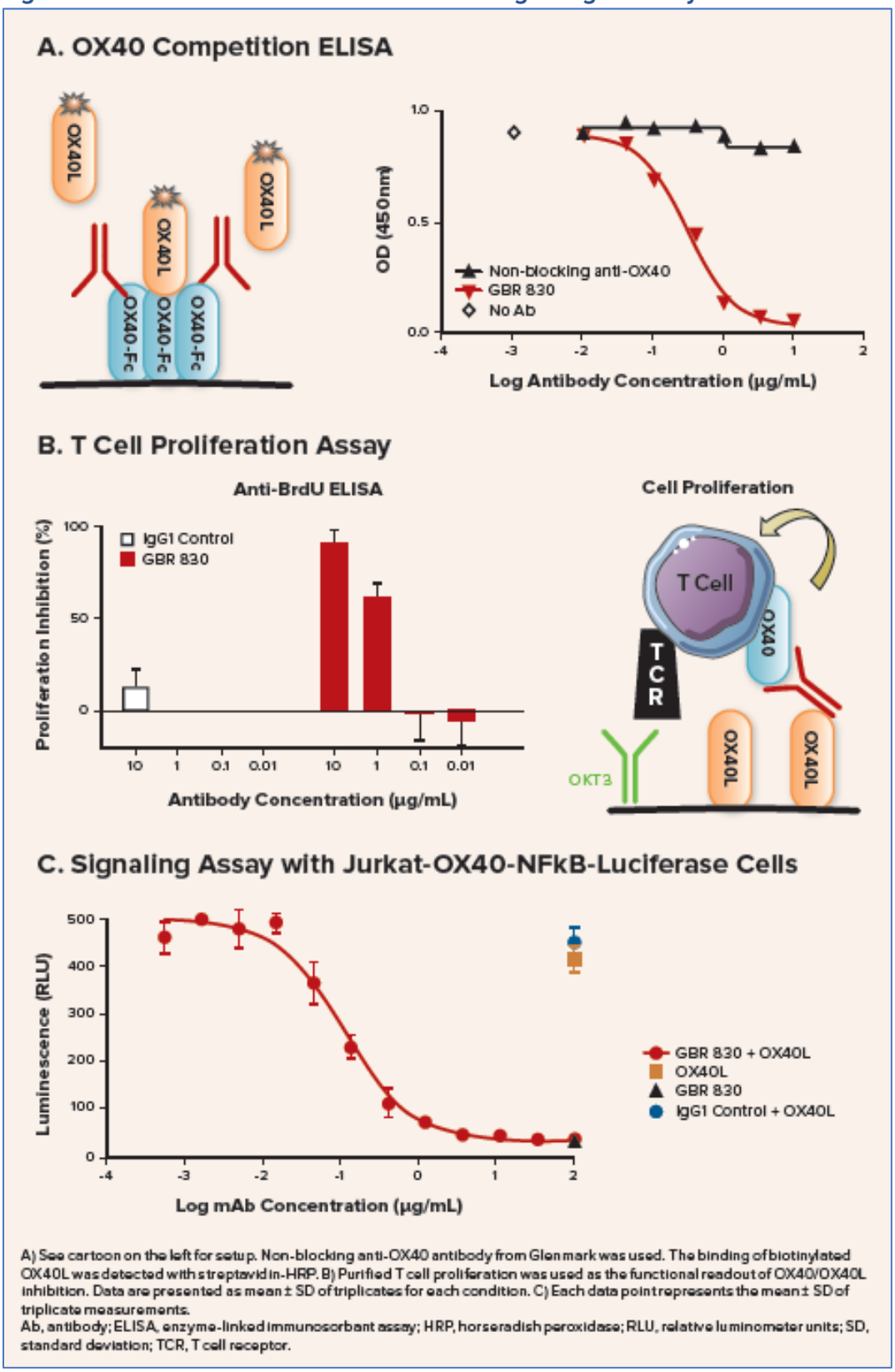
RESULTS

GBR 830 Binding and Downstream Effects

- GBR 830 blocks OX40/OX40L interaction (**Figure 1A**), inhibits T cell proliferation (**Figure 1B**), and inhibits OX40 signaling (**Figure 1C**)

Figure 1. GBR 830 Inhibits the OX40/OX40L Signaling Pathway

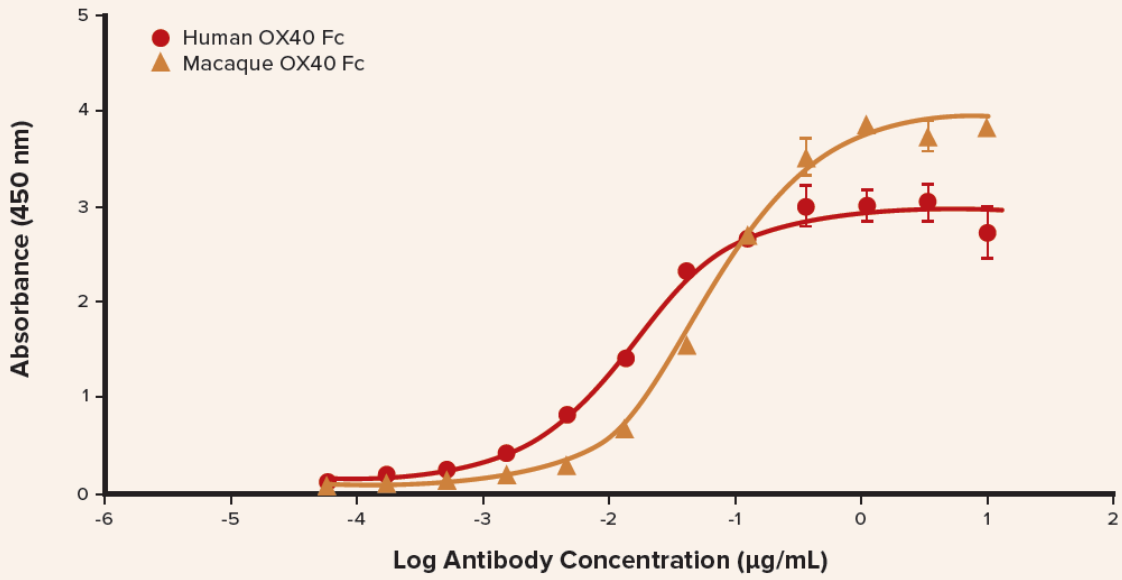


A) See cartoon on the left for setup. Non-blocking anti-OX40 antibody from Glenmark was used. The binding of biotinylated OX40L was detected with streptavidin-HRP. B) Purified T cell proliferation was used as the functional readout of OX40/OX40L inhibition. Data are presented as mean ± SD of triplicates for each condition. C) Each data point represents the mean ± SD of triplicate measurements.
Ab, antibody; ELISA, enzyme-linked immunosorbant assay; HRP, horseradish peroxidase; RLU, relative luminometer units; SD, standard deviation; TCR, T cell receptor.

KEY FINDINGS

- GBR 830 cross-reacts with human OX40 and macaque OX40, albeit with reduced affinity (**Figure 2**)

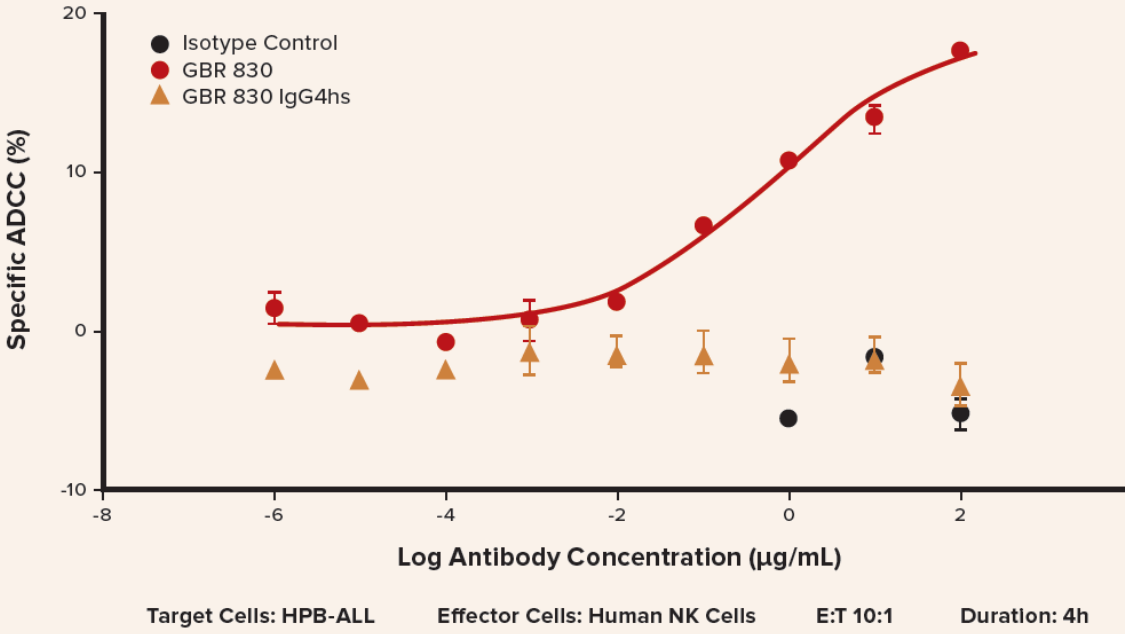
Figure 2. Cross-Reactivity ELISA



The binding of GBR 830 by ELISA on human or macaque OX40 is shown. Fc fusions of the ectodomain of OX40 from human and macaque were coated at 2 µg/mL on an ELISA plate and incubated with a concentration range of GBR 830 followed by detection with a peroxidase-conjugated anti-human IgG (Fab specific). The graph shows the mean ± SEM of duplicates for each condition.
ELISA, enzyme-linked immunosorbent assay; SEM, standard error of the mean.

- GBR 830 mediates low levels of antibody-dependent cellular cytotoxicity (ADCC; **Figure 3**)

Figure 3. ADCC on HPB-ALL Cells



HPB-ALL (OX40+ T cell lymphoma cell line) cells were incubated with purified human natural killer cells (NK) for 4 hours. Specific killing was measured by lactate dehydrogenase release or by flow cytometry absolute counting.
ADCC, antibody-dependent cell-mediated cytotoxicity.

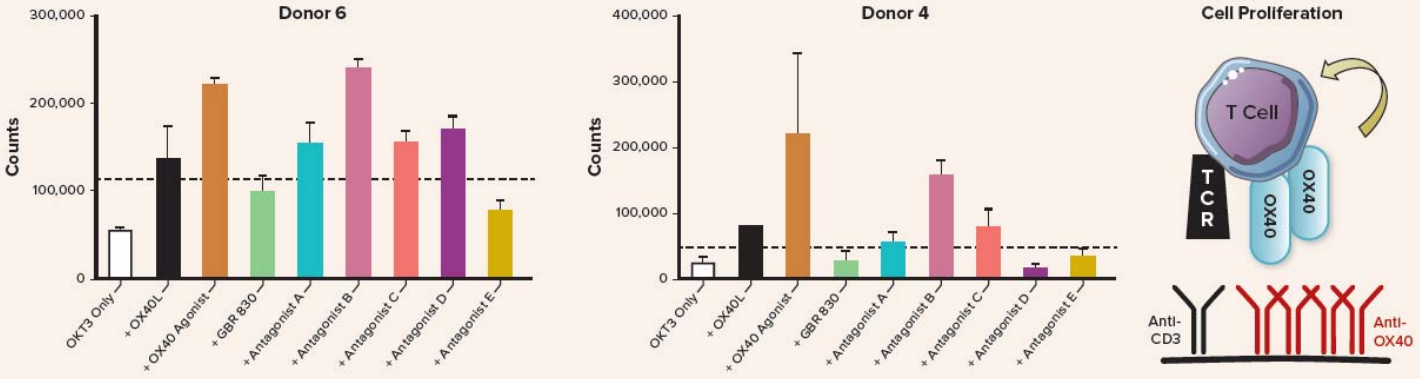
KEY FINDINGS

Benchmarking GBR 830 Against Competitor Monoclonal Antibodies for Agonism

- No agonism was detected with GBR 830 compared with competitor monoclonal antibodies (**Figure 4**)
  - All other anti-OX40 antibodies described as antagonistic show some agonism (**Figure 4B**)

Figure 4. GBR 830 Agonism Benchmarking

A. T Cell Proliferation Agonism Assay: Summary of 2 T Cell Donors



B. Proliferation Agonism Assay: Summary of All Donors Tested (Stimulation Index >2)

Controls			GBR 830	OX40 Antagonists				
OX40L (positive control)	OX40 Agonist (positive control)	IgG1 Control		Antagonist A	Antagonist B	Antagonist C	Antagonist D	Antagonist E
11/16	15/16	0/13	0/16	9/14	3/3	2/3	7/9	5/14

Purified human T cells from healthy donors were incubated with plate-bound anti-CD3 (OKT3)[a] indicated antibodies (10 µg/mL) or OX40L (5 µg/mL). The readout was ^{3}H thymidine incorporation after 96 hours. The proliferation index was calculated as: mean counts (sample)/mean counts (OKT3 only). A) The graphs show the mean ± SD of triplicates for each condition. B) A proliferation index >2 indicated a positive proliferative response. Red indicates agonism (positive proliferative response) with at least a proportion of donors. Green indicates no agonism detected with any donor. Some donors were excluded because they did not respond to OX40L and the OX40 agonist positive control or they showed agonism with the IgG control condition. Other anti-OX40 antibodies tested included an OX40 agonist control (positive control, Glenmark); Antagonist A: A10 (Genentech: WO2008106116); Antagonists B and C: Ab 131 and Ab 315[1]; Antagonist D: 112V8 (Kirin: WO2007062245); and Antagonist E: A26 (UCB Pharma: WO2010/096418).
Ab, antibody; SEM, standard error of the mean; TCR, T cell receptor.

GBR 830 Toxicity Studies in Cynomolgus Monkeys

- GBR 830 was safe without adverse findings after repeat dosing up to 6 months in cynomolgus monkeys (**Tables 1 & 2**)

Table 1. Summary of GBR 830 Repeat Dose Toxicity Studies in Cynomolgus Monkeys

Study	6-week study in young cynomolgus monkeys with 8-week recovery	6-month study in sexually mature cynomolgus monkeys
Age of monkeys	2-4 years	4-8 years
Number of animals	Treatment: 3/sex/group Recovery: 2/sex for control and high dose groups	Treatment: 4/sex/group
Dose level	IV: 10, 30, and 100 mg/kg/week	IV: 10, 30, and 100 mg/kg/week SC: 100 mg/kg/week
Route	IV infusion (15 minutes)	IV infusion (15 minutes)/SC injection (bolus)
Parameters	Standard toxicology parameters plus flow cytometry, cytokines, TDAR, safety pharmacology parameters (ECG, BP, respiratory rate, body temperature, neurological examination)	Standard toxicology parameters plus flow cytometry, cytokines, ECG, fertility end points (menstrual cycle, sperm evaluation, testicular size, and standard histopathology of reproductive organs)
Results	-No treatment-related adverse effects noted in any of the evaluated parameters -No adverse effects noted at the site of IV injection -No immunogenicity noted -NOAEL (IV) = 100 mg/kg/week	-No treatment-related adverse effects noted in any of the evaluated parameters -No adverse effects noted at the site of IV and SC injections -No immunogenicity noted -NOAEL (IV and SC) = 100 mg/kg/week

BP, blood pressure; ECG, electrocardiogram; IV, Intravenous; NOAEL, no-observed-adverse-effect-level; SC, subcutaneous; TDAR, T cell dependent antibody response.

KEY FINDINGS

Table 2. Summary of Serum Toxicokinetic Parameters Following Weekly IV Infusion or SC Injection in Cynomolgus Monkeys (6-Week and 6-Month Studies)

Dosing week	Dose (mg/kg/week)	Gender	6-week study IV		6-month study			
					IV		SC	
			C_{max} (µg/mL)	AUC_{0-168} (µg-h/mL)	C_{max} (µg/mL)	AUC_{0-168} (µg-h/mL)	C_{max} (µg/mL)	AUC_{0-168} (µg-h/mL)
Week 1	10	M	307	23900	341	23300	-	-
		F	305	22500	340	23600	-	-
	30	M	844	52900	853	63500	-	-
		F	895	36500	760	64500	-	-
	100	M	2440	188000	2830	232000	775	105000
		F	2860	208000	2960	225000	1050	138000
Week 6	10	M	607	48200	-	-	-	-
		F	449	45500	-	-	-	-
	30	M	1210	118000	-	-	-	-
		F	929	102000	-	-	-	-
	100	M	3540	343000	-	-	-	-
		F	3930	367000	-	-	-	-
Week 26	10	M	-	-	926	96200	-	-
		F	-	-	796	78200	-	-
	30	M	-	-	2070	224000	-	-
		F	-	-	1990	222000	-	-
	100	M	-	-	7590	808000	3100	453000
		F	-	-	6320	647000	2610	389000

Summary of toxicokinetics:
-Broadly dose-proportional increase in systemic exposures
-Approximately 2-4–fold accumulation after repeated dosing
-No gender differences
-SC bioavailability at 100 mg/kg/week = Approximately 45%-64%

AUC_{0-168}, area under the serum concentration-time curve over 168 hours post-dose; C_{max}, maximum concentration; F, female; IV, Intravenous; M, male; SC, subcutaneous.

CONCLUSIONS

- These data show that GBR 830 can block OX40L-induced proliferation without inducing receptor agonism, in contrast to other anti-OX40 antibodies
- GBR 830 was also safe and well-tolerated in non-human primates without any adverse effects after repeated administration up to 6 months