GB001 is a Potent, Insurmountable DP_2 Antagonist With Long Receptor Residence Time and Extended Pharmacodynamic Effects

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BACKGROUND

- GB001 is an oral antagonist of the prostaglandin D_2 receptor 2 (DP_2) in development for the treatment of moderate-severe asthma (NCT03683576) and chronic rhinosinusitis (NCT03956862)
- DP_2 antagonists block receptor activation and intracellular signaling induced by prostaglandin D_2 (PGD_2), which may inhibit recruitment of airway eosinophils and reduce airway inflammation[1]

OBJECTIVE

- Evaluate in vitro affinity, potency, and receptor residence time of GB001
- Assess the insurmountability of GB001, defined as the ability of the antagonist to block the receptor despite increasing agonist concentrations[2,3]

METHODS

- Competitive antagonism and biochemical kinetics of GB001 were assessed in ^{3}H-PGD_2 and ^{3}H-GB001 radioligand displacement assays in isolated DP_2 membranes prepared from CHO cells overexpressing human DP_2
- Cell-based potency for GB001 was measured using CHO cells overexpressing human DP_2 in assays measuring cAMP accumulation, β-arrestin recruitment, and calcium flux. Cells were treated with GB001 for 30-180 min followed by treatment with PGD_2.
- Insurmountability of GB001 was evaluated in a GTPγS assay using DP_2 membranes and calcium flux assay using CHO-DP_2 cells. Membranes were treated with GB001 for 180 min followed by treatment with PGD_2 for 15 or 90 min.
- Functional cellular residence time for GB001 was measured using CHO cells overexpressing human DP_2 in calcium flux assays. Cells were treated with GB001 for 180 min, washed, and incubated for 0-90 minutes, followed by treatment with PGD_2. Offset rate was measured using a method applying the Black/Leff operational model.[4]
- PGD_2-driven DP_2 internalization was assessed by flow cytometry in both human DP_2-overexpressing cell lines and human whole blood assays. Human whole blood was stimulated with PGD_2 antagonists for 30 minutes; cells were washed and immediately stimulated with PGD_2 for 1 hour.

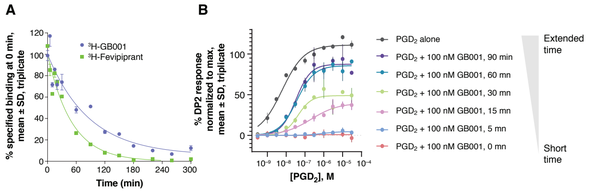
RESULTS

Table 1. GB001 in vitro potency

	nM
DP_2 ^{3}H-PGD_2 Ki	2[a]
DP_2 ^{3}H-GB001 Ki	12[a]
DP_2 GTPγS IC_{50}	4 ± 1[b]
DP_2-CHO cAMP IC_{50}	9 ± 4[b]
DP_2-CHO receptor internalization IC_{50}	16 ± 4[b]
DP_2-CHO calcium flux IC_{50}	39 ± 15[b]
DP_2-CHO b-arrestin IC_{50}	2 ± 1[b]

[a]n = 1; [b]data presented as mean ± SD (n = 2–5)

Figure 1. GB001 has prolonged biochemical and functional residence times

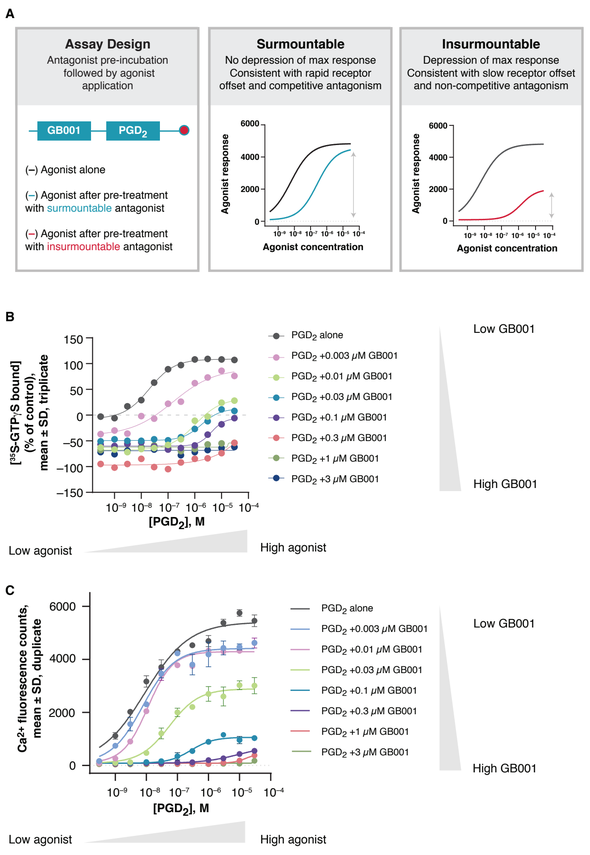


- The residence time of GB001 was assessed in CHO-DP_2 membranes using radiolabeled ^{3}H-GB001 (**Figure 1A**) and using a calcium flux assay after a washout to measure the recovery of response (**Figure 1B**)

Table 2. Receptor Residence Time Across Different DP_2 Antagonists

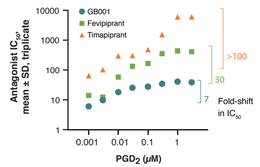
	Binding ([^{3}H]GB001 or [^{3}H]Fevi)	Functional (Ca^{2+} assay)
Data – Residence Time		
GB001	95 min	4.5 hr
Fevipiprant	50 min	1.2 hr
Timapiprant	Not tested	11 min
Assay considerations		
Physiological relevance	Low	Moderate
Biological material	Isolated membranes	Whole cells

Figure 2. GB001 is an insurmountable antagonist in GTPγS and calcium flux assays



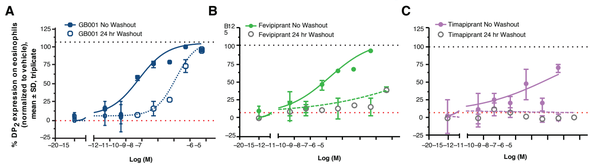
- Insurmountability refers to receptor blockade by the antagonist despite increasing agonist concentrations (**Figure 2A**). Insurmountability was assessed using GTPγS binding (**Figure 2B**) and calcium flux (**Figure 2C**) assays. All membranes or cells were treated with a dose-response of PGD_2 alone or after treatment with a range of GB001 concentrations.

Figure 3. GB001 inhibition potency is less sensitive to increasing PGD_2 concentrations



- GB001 and DP_2 inhibitor potencies were evaluated across a wide range of' PGD_2 concentrations in a calcium flux assay. GB001 IC_{50} shifts by under 10-fold, whereas other DP_2 antagonists change by greater amounts.

Figure 4. GB001 maintains inhibition of PGD_2-induced DP_2 internalization in a human whole blood assay after washout



- All compounds were potent antagonists of PGD_2-induced receptor internalization (GB001 IC_{50} 2 nM) (**Figure 4**)
- Following washout, GB001 was still able to inhibit PGD_2-induced internalization (dotted blue line, IC_{50} 127 nM) (**Figure 4A**); these data support an extended PD effect of GB001 in vitro

CONCLUSIONS

- GB001 demonstrated high potency, insurmountability, and slow disassociation from the DP_2 receptor
- These features endow GB001 with prolonged biologic effects and may be key properties to effectively control chronic inflammation.
- Clinical data are needed to confirm if these in vitro features are associated with a differentiated clinical response

REFERENCES

1. Asano K, Sagara H, Ichinose M, et al. *J Allergy Clin Immunol Pract* 2020; 8(4):1275-1283e1.
2. Vauquelin G, Van Liefde I, Vanderheyden P. *Trends Pharmacol Sci* 2002; 23(11):514-518.
3. Kenakin T, Jenkinson S, Watson C. *J Pharmacol Exp Ther* 2006; 319(2):710-723.
4. Black JW, Leff, P. *Proc R Soc Lond B Biol Sci* 1983; 220:141-162.

DISCLOSURES

KTM, SM, TSC, SS, GJO, LC, HO, and LSC are employed by Gossamer Bio, Inc. TK and SH are consultants to Gossamer Bio, Inc.

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