Poster # 1961-LB

Targeted Removal of Pathogenic Proinsulin and Insulin Autoantibodies With TRAP Degraders for the Treatment of Type 1 Diabetes

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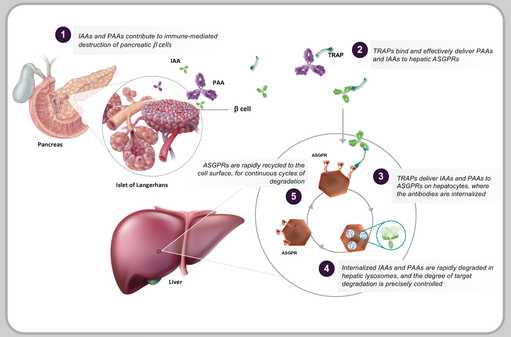
INTRODUCTION

- Approximately 8.4 million individuals worldwide live with type 1 diabetes (T1D), of whom nearly 18% (1.5 million) are under the age of 20[1]
- T1D is an organ-specific autoimmune disease characterized by immune-mediated destruction of insulin-producing β cells found in the pancreatic islets of Langerhans, leading to glycemic dysregulation[2,3]
- Proinsulin, a protein precursor of insulin that is secreted by islet β cells, has been identified as a pathogenic autoantigen in T1D[3,4]
- Insulin autoantibodies (IAAs) and proinsulin autoantibodies (PAAs), which bind and destroy islet β cells, are prevalent in the early stages of T1D and are often followed by formation of autoantibodies (AAbs) against other native β-cell proteins, such as glutamic acid decarboxylase 65 (GAD65), tyrosine phosphatase–related islet antigen 2 (IA-2), and zinc transporter 8 (ZnT8)[3,5-8]

Targeted Removal of Aberrant Protein in T1D

- TRAP™ degraders are bispecific molecules that leverage the novel MoDE™ platform to target aberrant proteins for rapid lysosomal degradation. TRAP 1 and TRAP 2 were specifically designed to mediate the formation of ternary complexes between IAAs/PAAs and asialoglycoprotein receptors (ASGPRs), which are expressed abundantly on hepatocytes in T1D. This binding event results in immediate endocytosis and subsequent efficient degradation of human IAAs and PAAs by lysosomal proteases (**Figure 1**)
- TRAP degrader technology can be used to eliminate antibody-induced inflammation of the pancreas through binding to IAAs and PAAs, potentially salvaging β-cell mass, restoring circulating levels of insulin and proinsulin in individuals with T1D, and promoting glucose homeostasis
- This series of studies explores the use of TRAP 1 and TRAP 2 for active removal of IAAs and PAAs, both in vitro using plasma samples from individuals with T1D and in vivo via a nonobese diabetic (NOD) mouse model

Figure 1. Proinsulin/Insulin TRAP Mechanism of Action



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METHODS & RESULTS

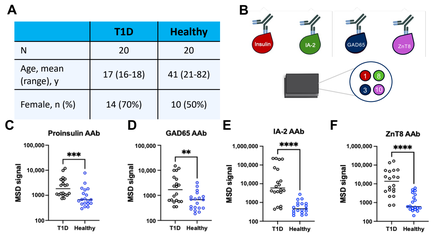
1. Islet Autoantibodies are Detected in Human Plasma Samples

- Plasma concentrations of islet AAbs were measured in a cohort of healthy controls and patients with T1D (**Figure 2A**) by capturing IgG with proinsulin, IA-2, GAD65, and ZnT8 using a multiplex Meso Scale Discovery (MSD) assay (**Figure 2B**)
- Proinsulin, GAD65, IA-2, and ZnT8 AAbs were significantly elevated in patients with T1D as compared with healthy controls (**Figure 2C-F**), consistent with reports in the literature

Figure 2. Detection of Islet Autoantibodies in Human Plasma Samples

A

	T1D	Healthy
N	20	20
Age, mean (range), y	17 (16-18)	41 (21-82)
Female, n (%)	14 (70%)	10 (50%)



$^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

2. TRAPs Selectively Bind IAAs and PAAs While Avoiding Interaction With Insulin Receptors

- First-generation TRAP (TRAP 1) and second-generation TRAP (TRAP 2) were developed to selectively target IAAs and PAAs in patients with T1D
- Binding of TRAPs to insulin receptors (insulin receptor isoform B [IR-B] and insulin-like growth factor 1 receptor [IGF-1R]) and to insulin and proinsulin antibodies was assessed by surface plasmon resonance. Compared to insulin, the TRAPs showed a 10- to 100-fold decrease in binding to anti-insulin receptors but maintained high affinity to both insulin and proinsulin antibodies (**Figure 3**)
- These data suggest that the TRAPs are capable of selectively binding IAAs and PAAs while avoiding interaction with insulin receptors and their downstream signaling

Figure 3. TRAPs and Surface Plasmon Resonance Binding Data

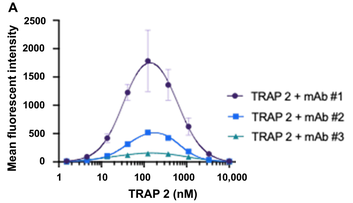
Sample	IR-B K_D (μM)	IGF-1R K_D (μM)	Insulin Ab K_D (μM)	Proinsulin Ab K_D (μM)
Insulin	0.34	1.24	0.11	—
TRAP 1	8.22	18	0.80	0.20
TRAP 2	43.5	No binding	1.26	0.28

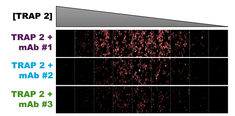
Ab, antibody; K_D, dissociation constant.

3. TRAP 2 Promotes Cellular Internalization and Degradation of Proinsulin Antibody in ASGPR-Expressing Cells

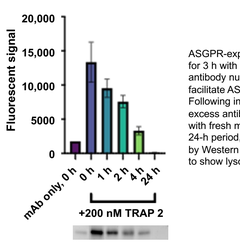
- TRAP 2 facilitates efficient cellular internalization of monoclonal proinsulin antibodies via membrane-bound ASGPR in a dose-dependent manner (EC50, 2-10 nM). At saturating concentrations of TRAP 2, an expected "hook effect" is observed due to increased TRAP-ASGPR and TRAP-antibody binary complex formation preventing ternary complex formation (**Figure 4A**)
- Internalized anti-proinsulin antibody is rapidly degraded in the lysosome, demonstrating depletion of target AAbs, as facilitated by TRAP 2 (**Figure 4B**)

Figure 4. TRAP 2–Mediated Cellular Internalization and Degradation of Proinsulin Antibodies





ASGPR-expressing HEK293 cells were incubated for 18 h with 50 nM fluorescently labeled monoclonal antibodies targeting distinct epitopes of proinsulin and a dose-curve of TRAP 2. Dose-dependent internalization of fluorescent antibody was captured using live-cell imaging.



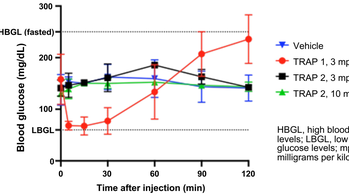
ASGPR-expressing HEK293 cells were incubated for 3 h with 100 nM of monoclonal anti-proinsulin antibody number 3 ± 200 nM of TRAP 2 to facilitate ASGPR-mediated internalization. Following internalization, cells were washed of excess antibody and drug and then incubated with fresh medium for up to 24 h. Throughout the 24-h period, lysates were collected and analyzed by Western blot with fluorescent anti-human IgG to show lysosomal degradation of anti-proinsulin.

mAb, monoclonal antibody.

4. TRAP 2 Maintains Glycemic Status More Effectively Than TRAP 1

- NOD mice spontaneously develop PAAs and IAAs and are an established model of T1D
- Administration of TRAP 2 in NOD mice demonstrated superior glycemic stability, compared to administration of TRAP 1 (**Figure 5**)
- In vivo tolerability studies conducted in NOD mice further supported the safety profile of TRAP 2, showing no observable toxic effects
- These findings suggest that TRAP 2 is more effective than TRAP 1 in maintaining glycemic stability and is well tolerated in a sensitive autoimmune-prone model

Figure 5. Blood Glucose Levels With TRAP 1 vs TRAP 2

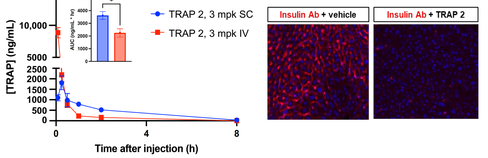


HBGL, high blood glucose levels; LBGL, low blood glucose levels; mpk, milligrams per kilogram.

5. TRAP 2 Depletes IAAs and PAAs in NOD Mice and Binds Autoantibodies of Humans Diagnosed With T1D

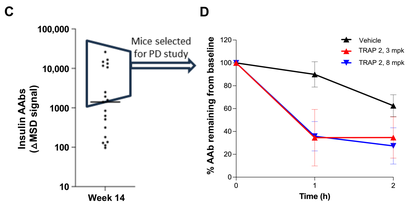
- Following single administrations of TRAP 2 in NOD mice, bioavailability was higher after subcutaneous (SC) vs intravenous (IV) administration (**Figure 6A**)
- TRAP 2 induced rapid clearance of human insulin antibodies in the liver of nude mice (**Figure 6B**)
- 14-week-old NOD mice containing high levels of PAAs and IAAs were pre-selected for a pharmacodynamic (PD) study (**Figure 6C**) and showed dose-dependent clearance of PAAs and IAAs by SC administration of TRAP 2 (**Figure 6D**)
- These findings suggest that TRAP 2 rapidly depletes endogenous PAAs and IAAs in NOD mice

Figure 6. TRAP Bioavailability, Antibody Binding, and Antibody Clearance



AUC = area under the curve; $^{*}P < 0.05$.

(A) NOD mice were injected with 3 mpk TRAP 2 by SC or IV injection in the pharmacokinetic assay. (B) Nude mice were injected with human insulin antibody conjugated with Alexa Fluor 594 and vehicle (left) or TRAP 2 (right). Livers were removed 30 min after injections. Presence of anti-insulin antibody (red) was observed on hepatic sinusoids.

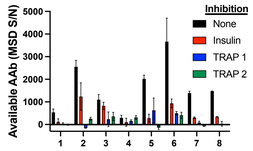


(C) NOD mice were screened for IAAs and PAAs by MSD assay for inclusion in the PD study. (D) In the PD study, the selected mice were injected with 3 mpk or 8 mpk TRAP 2 by SC injection.

6. TRAP 2 Effectively Binds and Neutralizes IAAs and PAAs of Patients With T1D

- TRAPs bound nearly all AAbs in T1D patient plasma containing detectable PAAs and inhibited the PAAs more than native insulin (**Figure 7**)

Figure 7. TRAP 1 and TRAP 2 Binding to AAbs From Patients With T1D



T1D patient plasma samples containing detectable levels of PAAs were pre-incubated with TRAP 1, TRAP 2, or insulin in a proinsulin MSD assay. S/N, signal-to-noise ratio.

CONCLUSIONS

- In vitro studies using plasma samples from patients with T1D showed robust binding of IAAs and PAAs to TRAP degraders
- In vivo studies with NOD mice demonstrated robust and targeted removal of IAAs and PAAs via TRAP degrader liver-mediated clearance while maintaining normal glycemic status and excellent tolerability
- TRAP degraders have the potential to address the underlying autoimmune etiology of T1D through efficient removal of AAbs directed against insulin and proinsulin

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DISCLOSURES: CS, BR, AE, HY, SDC, AB, BL, KP, RD, LM, DP, SI, CV, VC, and BC are employed by and hold stock/stock options in Biohaven Pharmaceuticals.

References: **1.** Gregory GA, Robinson TIG, Linklater SE, et al. Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040: a modelling study. *Lancet Diabetes Endocrinol.* 2022;10(10):741-760. doi:10.1016/S2213-8587(22)00218-2. **2.** Jeun R. Immunotherapies for prevention and treatment of type 1 diabetes. *Immunotherapy.* 2025;17(3):201-210. doi:10.1080/1750743X.2025.2473111 **3.** Narendran P, Mannering SI, Harrison LC. Proinsulin-a pathogenic autoantigen in type 1 diabetes. *Autoimmun Rev.* 2003;2(4):204-210. doi:10.1016/s1568-9972(03)00009-0 **4.** Chen W, Bergerot I, Elliott JF, et al. Evidence that a peptide spanning the B-C junction of proinsulin is an early autoantigen epitope in the pathogenesis of type 1 diabetes. *J Immunol.* 2001;167(9):4926-4935. doi:10.4049/jimmunol.167.9.4926 **5.** Böhmer K, Keilacker H, Kuglin B, et al. Proinsulin autoantibodies are more closely associated with type 1 (insulin-dependent) diabetes mellitus than insulin autoantibodies. *Diabetologia.* 1991 Nov;34(11):830-834. doi: 10.1007/BF00408359 **6.** Hazime R, Lamjaddi S, Guennouni M, et al. Autoantibodies in type 1 diabetes: prevalence and clinical profiles. *Diabet Epidemiol Manag.* 2025;17(6):100246. doi:10.1016/j.deman.2024.100246 **7.** DiMeglio LA, Evans-Molina C, Oram RA. Type 1 diabetes. *Lancet.* 2018;391(10138):2449-2462. doi:10.1016/S0140-6736(18)31320-5 **8.** Elding Larsson H, Vehik K, Gesualdo P, et al. Children followed in the TEDDY study are diagnosed with type 1 diabetes at an early stage of disease. *Pediatr Diabetes.* 2014;15(2):118-126. doi:10.1111/pedi.12066

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