Poster
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Molecular Degraders of Extracellular Proteins (MoDEs™) Rapidly and Effectively Remove Interstitial IgG and Disease-Relevant Immune Complexes Through Endolysosomal Degradation in the Liver

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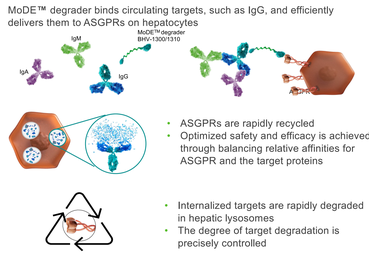
INTRODUCTION

- Novel extracellular IgG degraders (BHV-1300/1310) from the molecular degraders of extracellular proteins (MoDE) drug platform are being developed for the treatment of IgG-mediated autoimmune diseases
- IgG MoDEs have the potential to target IgG and IgG immune complexes, both in the circulation and in disease-relevant tissues
- Thus, this new modality offers a therapeutic strategy for targeting pathogenic autoantibodies and immune complexes

Molecular Degraders of Extracellular Proteins (MoDE)™ and BHV-1300/1310

- The MoDE platform discovers and develops bifunctional molecules that bind simultaneously to both IgG molecules and hepatic asialoglycoprotein receptors (ASGPRs), leading to internalization and endolysosomal degradation of IgG in the liver (**Figure 1**)
- Biohaven engineered BHV-1300/1310—novel, selective, anti-human bifunctional protein degraders—to specifically target and decrease serum IgG levels* in a rapid, robust, and selective manner
- This presentation shares data from in vivo and in vitro studies that evaluated the ability of IgG MoDEs to remove interstitial IgG and disease-relevant IgG immune complexes

Figure 1. Extracellular MoDE™ Degrader BHV-1300/1310 Mechanism of Action



*IgG1, IgG2, and IgG4.

CONCLUSIONS

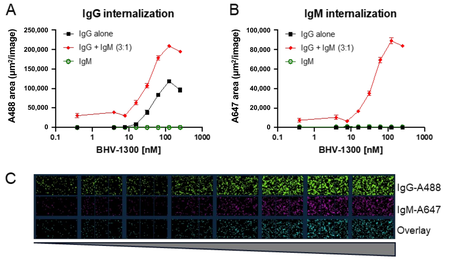
- In these in vitro and in vivo studies, extracellular IgG degraders (BHV-1300/1310) from the MoDE drug platform demonstrated the ability to remove interstitial IgG and disease-relevant immune complexes through endolysosomal degradation in the liver
- This new modality offers an attractive and differentiated therapeutic strategy for treating a spectrum of autoimmune conditions by targeting pathogenic autoantibodies and immune complexes that play a critical role at disease onset and during disease progression and organ damage

METHODS & RESULTS

1. BHV-1300 mediates internalization of large IgG and IgG-IgM immune complexes through ASGPR

- Dose-dependent, selective endocytosis of IgG and IgG-IgM immune complexes was observed with BHV-1300 in human embryonic kidney (HEK) cells transfected with human ASGPR1 but not in untransfected HEK cells (data not shown)
- BHV-1300 specifically internalized IgG and IgM complexed to IgG and spared free IgM (**Figure 2**)

Figure 2. BHV-1300 Internalizes IgG and IgG-IgM Complexes, Sparing IgM

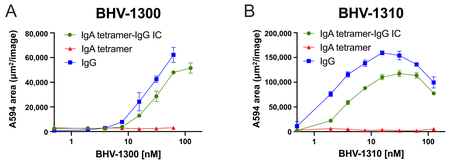


ASGPR1-expressing cells were incubated for 20 hours with human IgG conjugated to Alexa Fluor 488 (A488) and/or rabbit IgG anti-human IgM conjugated to Alexa Fluor 647 (A647), as well as a dose curve of BHV-1300. IgG internalization was monitored by measuring A488 area (A), and IgM internalization was monitored by measuring A647 area (B) in live-cell imaging, showing individual internalization of IgG and IgM, as well as the overlap with both signals(C). (A) IgG EC50 = 39 nM; IgG + IgM EC50 = 33 nM. (B) IgG + IgM EC50 = 41 nM. EC50, half-maximal effective concentration.

2. BHV-1300 and BHV-1310 mediate internalization of large IgG-IgA immune complexes in vitro and in vivo

- BHV-1300 and BHV-1310 promote efficient cellular internalization of IgG and IgG complexed to tetrameric IgA
- BHV-1300 and BHV-1310 mediate degradation of large immune complexes in a dose-dependent manner
- In vivo, BHV-1310 promotes internalization followed by subsequent degradation of large immune complexes via the lysosomal pathway in the liver
- BHV-1310 effectively prevents the deposition of IgA-IgG immune complexes in the kidneys (**Figures 3A-3D**)

Figures 3A and 3B. BHV-1300 and BHV-1310 Promote Cellular Uptake of IgG-IgA Immune Complexes

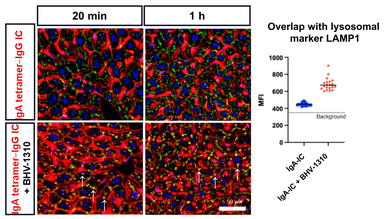


	EC50 [nM]
IgG	22.8
IgA tetramer	N/A
IgA tetramer–IgG IC	26.3

	EC50 [nM]
IgG	1.87
IgA tetramer	N/A
IgA tetramer–IgG IC	3.54

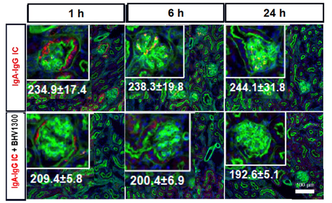
HEK293-ASGPR1 cells were incubated for 12 hours with human IgG anti-IgA, HPLC-purified IgA tetramer, or a combination of both, along with a dose curve of BHV-1300 (A) or BHV-1310 (B). IgA tetramer was pre-labeled with Alexa Fluor 594 (A594) for IgA tetramer and IgA tetramer–IgG IC readout, whereas IgG was labeled with A594 for IgG readout by live-cell imaging. EC50, half-maximal effective concentration; HPLC, high-performance liquid chromatography; IC, immune complex.

Figure 3C. BHV-1310 Promotes Degradation of Immune Complexes in the Liver



Animals were administered an IC consisting of tetrameric IgA (red fluorescence) Alexa Fluor 594 and anti-IgA IgG, followed by treatment with BHV-1310. Mouse liver sections were stained with anti-LAMP1 antibody (green fluorescence) to mark lysosomes. The ICs are shown in red in the figure. IC, immune complex; MFI, mean fluorescence intensity. †, co-localization of IgA–IgG IC and lysosomal LAMP1

Figure 3D. BHV-1310 Prevents Immune Complex Deposition in the Kidney

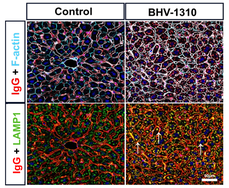


Animals were administered IgA-IgG immune complex (IC) pre-labeled with Alexa Fluor 594 (red) and anti-IgA IgG, followed by BHV-1310 treatment. Mouse kidneys were stained with phalloidin conjugated with Alexa Fluor 488 (green) to visualize tubules and glomeruli. The numbers below the insets represent the mean fluorescence intensity values with standard deviations of IgG signal measured in glomeruli (n = 12~18). The immune complexes are shown in red in the figures.

3. BHV-1310 induces endocytosis and lysosomal degradation of human IgG via ASGPR, facilitating hepatic absorption and IgG depletion across various tissues

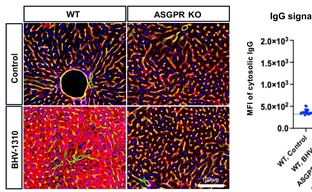
- BHV-1310 facilitates hepatic endocytosis and lysosomal degradation of human IgG, with ASGPR being essential for this process (**Figures 4 and 5**).
- BHV-1310 effectively promotes the reduction of circulating IgG through hepatic uptake in a mouse model (**Figure 6**)
- BHV-1310 promotes IgG depletion across various tissues, underscoring this agents' broad therapeutic potential for eliminating pathogenic autoantibodies and their immune complexes in a range of autoimmune diseases (**Figure 7**)

Figure 4. BHV-1310 Induces Hepatic Endocytosis and Lysosomal Degradation of Human IgG



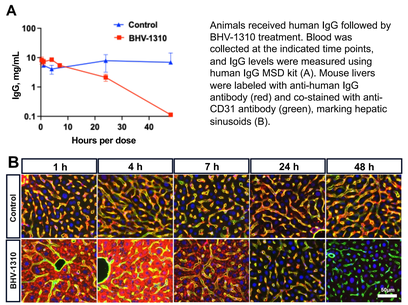
Animals were administered human IgG followed by BHV-1310 treatment. Mouse liver sections were stained with an anti-human IgG antibody (red) and phalloidin (cyan) to label the cell boundaries (F-actin), and anti-LAMP1 (green) to mark lysosomes. F-actin, actin filaments; MFI, mean fluorescence intensity. †, co-localization of IgA-IgG immune complex and lysosomal LAMP1

Figure 5. ASGPR Is Required for BHV-1310–Mediated Hepatic Endocytosis of IgG



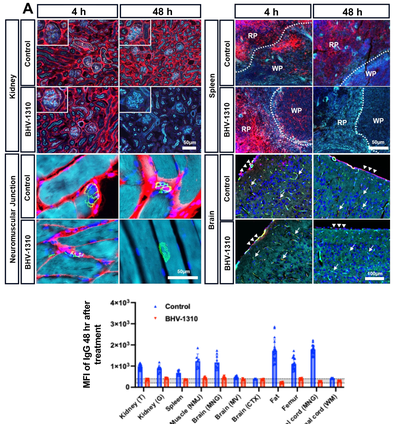
Animals were administered human IgG followed by BHV-1310 treatment. Mouse liver sections were stained with anti-human IgG antibody (red) and co-stained with anti-CD31 antibody (green) to mark hepatic sinusoids. KO, knockout; MFI, mean fluorescence intensity; WT, wild-type.

Figure 6. BHV-1310 Promotes the Reduction of Circulating IgG Through Hepatic Uptake in a Mouse Model



Animals received human IgG followed by BHV-1310 treatment. Blood was collected at the indicated time points, and IgG levels were measured using human IgG MSD kit (A). Mouse livers were labeled with anti-human IgG antibody (red) and co-stained with anti-CD31 antibody (green), marking hepatic sinusoids (B).

Figure 7. BHV-1310 Promotes IgG Depletion Across Various Tissues



Tissues were stained with anti-human IgG antibody (red) and co-stained with phalloidin (cyan) to mark IgG and tissue structure. Muscle and brain tissues were stained with α-bungarotoxin (green) to label neuromuscular junctions and anti-CD31 antibody (green) to label microvascular structures. Arrowheads in the brain images indicate meninges, and arrows mark microvascular structures. The shaded area indicates the ranges of background noise from the tissues. CTX, cortex; G, glomeruli; MFI, mean fluorescence intensity; MNG, meninges; MV, microvascular; NMJ, neuromuscular junction; RP, red pulp; T, tubule; WM, white matter; WP, white pulp.

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DISCLOSURES: SL, CS, KM, AE, HY, KC, BR, SDC, AMR, GD, DP, AB, VC, and BC are employed by and hold stock/stock options in Biohaven Pharmaceuticals. DS is a Scientific Advisor to Biohaven Pharmaceuticals.

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