Poster
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First-in-class, antigen-specific extracellular protein degrader BHV-1400, in development for treating IgA nephropathy, selectively targets galactose-deficient IgA for rapid and efficient endolysosomal degradation in the liver

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INTRODUCTION

- IgA nephropathy (IgAN) is the most prevalent form of glomerulonephritis, affecting nearly 3 of every 100,000 people worldwide[1]
- Individuals with IgAN present with a range of symptoms, including severe hypertension, hematuria, proteinuria, and nephrotic syndrome[2]
- It has been estimated that approximately 30-40% of individuals diagnosed with IgAN will develop end-stage kidney disease[2,3]
- Current treatment options for IgAN are symptomatic or broadly immunosuppressive. No currently approved treatments selectively target the pathogenic galactose-deficient IgA1 that triggers the disease

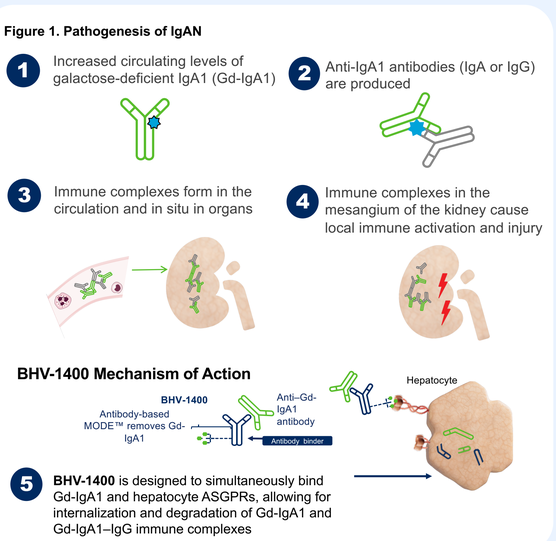
IgAN Pathophysiology

- IgAN is a heterogeneous autoimmune disorder characterized by the deposition of IgA1-containing immune complexes in the glomerular mesangium (**Figure 1**)[4]
- Overproduction of galactose-deficient IgA1 (Gd-IgA1) and the subsequent formation of Gd-IgA1–IgG immune complexes serve as key drivers of a proposed 4-hit pathogenic cascade[2]
- Lowering levels of circulatory Gd-IgA1 and associated immune complexes has potential to decrease mesangial deposition and improve kidney function
- Investigational immunomodulatory drugs have successfully reduced circulatory Gd-IgA1, with potential improvements in proteinuria and kidney function. With these therapies, however, concurrent reduction of other antibodies, such as IgM, occurs as well[5]

A Transformational MODE Drug Platform: Molecular Degraders of Extracellular Proteins (MODE)™ and BHV-1400

- The MODE platform develops bifunctional molecules that degrade extracellular protein targets, such as Gd-IgA1, via the asialoglycoprotein receptor (ASGPR)-mediated endosome/lysosome pathway
- Biohaven engineered BHV-1400—a novel, selective, anti-human bifunctional protein degrader—to specifically target extracellular Gd-IgA1[6]
- BHV-1400 has the potential to treat IgAN without causing broad immunosuppression

Figure 1. Pathogenesis of IgAN



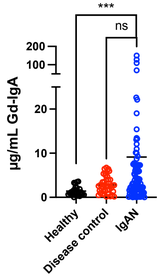
METHODS & RESULTS

1. Detection of Gd-IgA1 Levels in Human Plasma Samples

- Plasma concentrations of Gd-IgA1 antibody in patients with IgAN were measured using BHV-1400 lacking ASGPR-binding moiety (BH-5305) and SULFO-TAG anti-IgA antibody
- Gd-IgA1 mean levels in patient samples were ~7-fold higher than mean levels in healthy volunteers (**Figure 2**)
- Consistent with literature reports using diagnostic KM55 (a rat antibody raised against Gd-IgA hinge peptide), these findings confirm that the anti–Gd-IgA1 antibody recognizes significant levels of circulating human Gd-IgA1

Figure 2. Mean Gd-IgA levels in plasma samples

	Gd-IgA* (μg/mL)
Healthy (n = 26)	1.41 ± 1.03
Disease control** (n = 40)	2.81 ± 1.96
IgAN (n = 95)	9.12 ± 23.5



ns, not significant.
* Mean ± standard deviation.
** Disease control refers to a variety of kidney and autoimmune diseases.
*** $P < .001$.

2. In Vitro Cellular Internalization of dg-IgA with BHV-1400

- Deglycosylated IgA (dg-IgA) is a semi-synthetic Gd-IgA1 surrogate prepared from pooled human serum IgA in 3 enzymatic steps (**Figure 3A**)
- BHV-1400 causes dose-dependent, selective endocytosis of dg-IgA vs IgA in human embryonic kidney (HEK) cells transfected with human ASGPR1 (hASGPR1) (**Figure 3B**)
- Low-nanomolar half-maximal effective concentration and robust mean fluorescence were observed at 12 hours for internalization of dg-IgA conjugated to a fluorescent reporter (Alexa Fluor 594)
- BHV-1400 internalized dg-IgA for lysosomal degradation but spared normal IgA

Figure 3A. Production of deglycosylated IgA (surrogate Gd-IgA1)

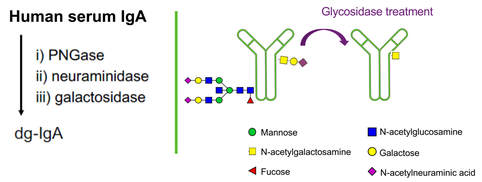
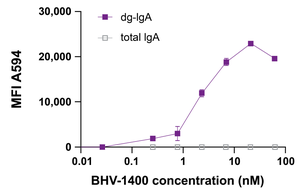


Figure 3B. dg-IgA cell internalization assay

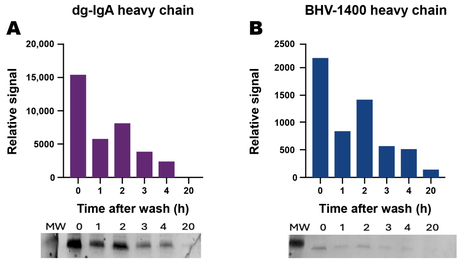


HEK293 cells transfected with ASGPR1 were used to measure endocytosis of 1 μg/mL dg-IgA and total IgA conjugated to Alexa Fluor 594. A594, Alexa Fluor 594; MFI, mean fluorescence intensity.

3. Cells Efficiently Degrade dg-IgA and BHV-1400

- ASGPR1-expressing cells were incubated with dg-IgA and BHV-1400, washed to remove exogenous ligand and compound, added to fresh media, and harvested for intracellular protein analysis at multiple time points
- The presence of dg-IgA (**Figure 4A**) and BHV-1400 (**Figure 4B**) were assessed via Western blot analysis, which showed a decrease in signal intensity over time, suggesting a gradual degradation of both antibody and compound every hour and almost complete degradation after 20 hours
- These data confirm that dg-IgA and BHV-1400 do not aggregate within cells but are degraded to almost completion within 20 hours

Figure 4. Endocytosed dg-IgA and BHV-1400 degrade intracellularly

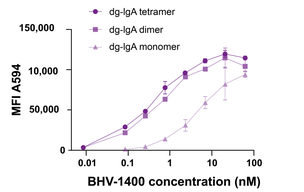


*MW, molecular weight marker.

4. Cellular Internalization of Surrogate Gd-IgA1 Antibody Complexes

- Size exclusion chromatography was used to isolate tetramers, dimers, and monomers of dg-IgA (**Figure 5**)
- At 12 hours, BHV-1400 demonstrated efficient internalization of both dimeric and tetrameric dg-IgA complexes in HEK (hASGPR1) cells
- These data confirm that BHV-1400 can target and mediate internalization of large antibody complexes

Figure 5. Cell internalization of dg-IgA complexes



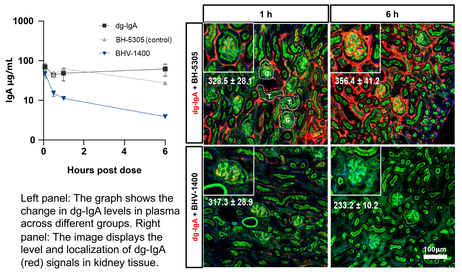
	Tetrameric dg-IgA	Dimeric dg-IgA	Monomeric dg-IgA
Max MFI	120,138	115,102	94,422
EC_{50} (nM)	0.37	0.48	3.93
S/N	57.9	140	679
Z prime	0.87	0.66	0.87

HEK293 cells transfected with ASGPR1 were used to measure endocytosis of 1 μg/mL monomeric, dimeric, and tetrameric dg-IgA conjugated to Alexa Fluor 594. EC_{50}, half-maximal effective concentration; Max MFI, maximum mean fluorescence intensity; S/N, signal-to-noise ratio.

5. BHV-1400 Achieves Robust Degradation of Exogenous dg-IgA in Mice

- In mice, BHV-1400 depleted exogenous dg-IgA to 58% area under the curve of control at a 2:1 (drug:target) ratio after sequential intravenous (IV) administration
- The parent control antibody lacking ASGPR-binding moiety, BH-5305, had no effect on degradation of exogenous dg-IgA in mice, as anticipated
- BHV-1400 rapidly and robustly degraded dg-IgA administered to mice and prevented the deposition of dg-IgA in kidney tissue (**Figure 6**)

Figure 6. dg-IgA clearance in the blood circulation and kidney



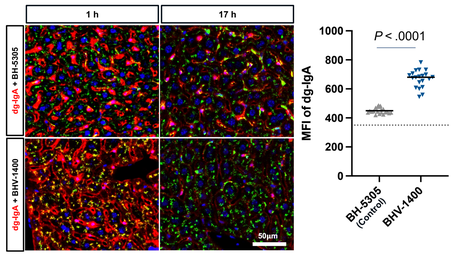
Left panel: The graph shows the change in dg-IgA levels in plasma across different groups. Right panel: The image displays the level and localization of dg-IgA (red) signals in kidney tissue.

Smaller inset images showcase glomeruli. The numbers below the insets represent the mean fluorescence intensity (MFI) values with standard deviation of dg-IgA signal measured in glomeruli (n = 22–25). G, glomerulus; T, tubule.

6. BHV-1400 Depletes Exogenous Tetrameric dg-IgA in Mouse Hepatocytes

- BHV-1400 induced endocytosis and lysosomal degradation of tetrameric dg-IgA within 1 hour in mouse liver tissues, with depletion observed 17 hours after treatment
- The parent control antibody, BH-5305, had minimal impact on the endocytosis and degradation of exogenous tetrameric dg-IgA, as expected
- These results suggest that in vivo, BHV-1400 has the potential to remove larger immune complexes composed of multimeric dg-IgA (**Figure 7**)

Figure 7. Cellular internalization of tetrameric dg-IgA complexes



Left panel: Images show tetrameric dg-IgA signal (red) in the liver tissue. The mouse liver sections were stained with the lysosomal marker lysosomal-associated membrane protein 1 (LAMP-1) (green) and DAPI (blue) for nuclei.
Right panel: The graph shows the measured pixel intensity of the dg-IgA signal in lysosomes at the 1-hour time point.

CONCLUSIONS

- BHV-1400 is a novel, antibody-based, bifunctional conjugate designed to selectively bind and degrade pathogenic circulating Gd-IgA1 and Gd-IgA1 immune complexes via ASGPR-mediated hepatocyte internalization
- BHV-1400 binds to pathogenic Gd-IgA1 in human IgAN and in plasma samples from patients with kidney disease
- BHV-1400 demonstrates compelling preclinical evidence for rapid and robust degradation of dg-IgA and dg-IgA–containing immune complexes in cellular and rodent experiments
- BHV-1400 holds therapeutic potential as a transformative, non-immunosuppressive, disease-modifying treatment for patients with IgAN

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DISCLOSURES: SL, CS, AE, KM, HY, AMKR, BR, SC, GD, DP, BC, LM, AB, and VC are employed by and hold stock/stock options in Biohaven Pharmaceuticals.

* SL and CS contributed equally to poster development.

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