BHV-1400 TARGETS THE NON-IMMUNOSUPPRESSIVE, SELECTIVE DEPLETION OF CIRCULATING GALACTOSE-DEFICIENT IGA1 IN IGA NEPHROPATHY

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CONCLUSIONS

1. BHV-1400 is a novel, antibody-based, bifunctional conjugate designed to selectively bind and degrade pathogenic circulating Gd-IgA1 and Gd-IgA1-IgG immune complexes via ASGPR-mediated hepatocyte internalization
2. BHV-1400 has demonstrated compelling preclinical evidence for rapid and robust degradation of deglycosylated IgA and related protein aggregates in cellular and rodent experiments
3. BHV-1400 recognizes pathogenic Gd-IgA1 in human IgAN and kidney disease patient plasma samples
4. BHV-1400 holds therapeutic potential as a transformative, non-immunosuppressive, disease-modifying treatment for patients with IgAN

OBJECTIVE

- To share biophysical target engagement data, protein of interest cellular internalization data, and rodent pharmacokinetic (PK) and pharmacodynamic (PD) data related to the degradation of exogenous deglycosylated IgA (dg-IgA) by the preclinical development candidate BHV-1400

INTRODUCTION

- IgA nephropathy (IgAN) is the most prevalent primary glomerulonephritis, with an annual incidence of 2-10 cases per 100,000 person-years[1,2]
- Individuals with IgAN present with a range of symptoms, including hematuria, proteinuria, nephrotic syndrome, and severe hypertension[1]
- Approximately 30-40% of individuals diagnosed with IgAN will develop end-stage kidney disease[1,3]
- Currently, there are no approved disease-modifying, IgAN-specific therapies. Only symptomatic treatments are available[2]

IgAN Pathophysiology

- IgAN is a heterogeneous autoimmune disorder characterized by deposition of IgA1-containing immune complexes in the glomerular mesangium[2] (**Figure 1**)
- Overproduction of galactose-deficient IgA1 (Gd-IgA1) and the formation of Gd-IgA1-IgG immune complexes are key drivers of a proposed 4-hit pathogenic cascade[1]
- Lowering levels of circulatory Gd-IgA1 and associated immune complexes has potential to decrease mesangial deposition and improve kidney function
- Reduction of circulatory Gd-IgA1 has been achieved using investigational immunomodulatory drugs, with potential improvements in proteinuria and kidney function, but with concurrent reduction of other antibodies such as IgM[4]
- Selective protein degradation of circulatory Gd-IgA1 and its complexes with BHV-1400 has the potential to stabilize or reverse the progression of IgAN, without broad immunosuppression

Molecular Degraders of Extracellular Proteins (MoDE)™ and BHV-1400

- The MoDE platform discovers and develops bifunctional molecules that degrade extracellular protein targets, such as Gd-IgA1, via the asialoglycoprotein receptor (ASGPR)-mediated endosome/lysosome pathway
- Biohaven engineered a novel anti-human Gd-IgA1 chimeric antibody (BH5305) with human constant regions, using a human IgG1 Fc with reduced effector function
- The ASGPR-binding bifunctional conjugate BHV-1400 was assembled from BH5305 in 1 step, using proprietary FcIII-directed MATE™ technology[5]
  - Linker and ASGPR binder were attached via a stable amide connection
  - BHV-1400 was isolated with good yield and excellent homogeneity (binder-to-antibody ratio = 2)
  - Site-specific conjugation of dual Lys248 residues was confirmed by peptide mapping

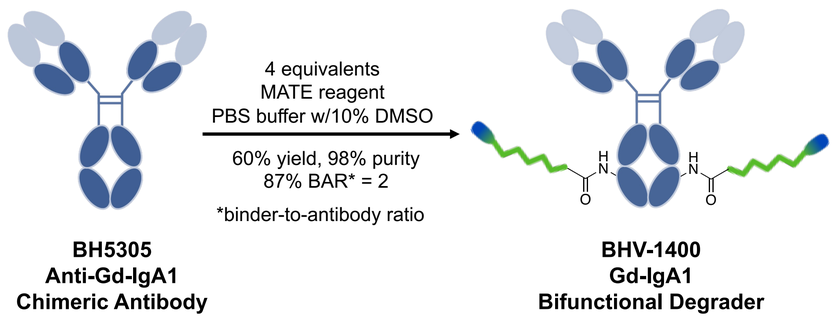
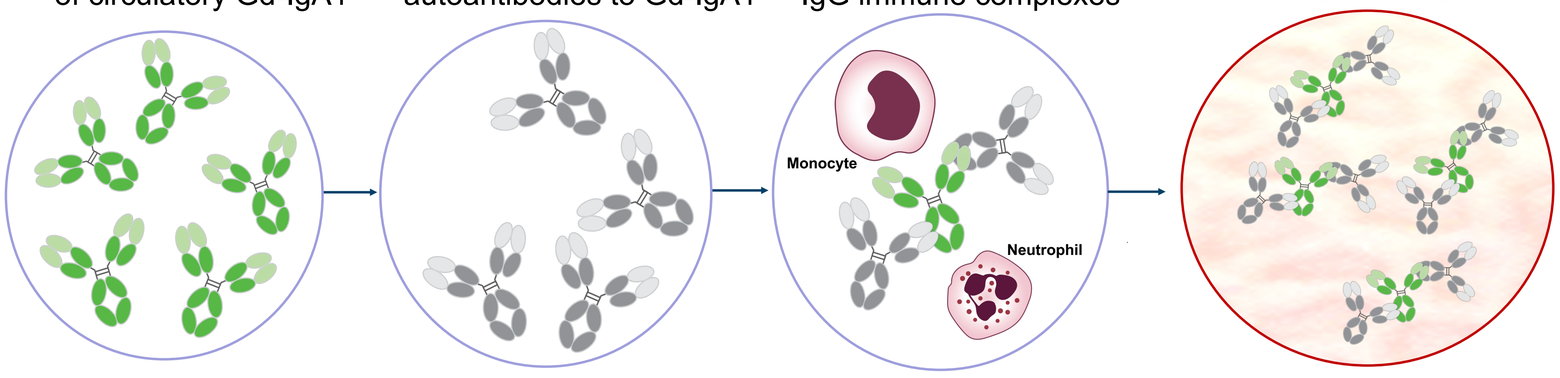
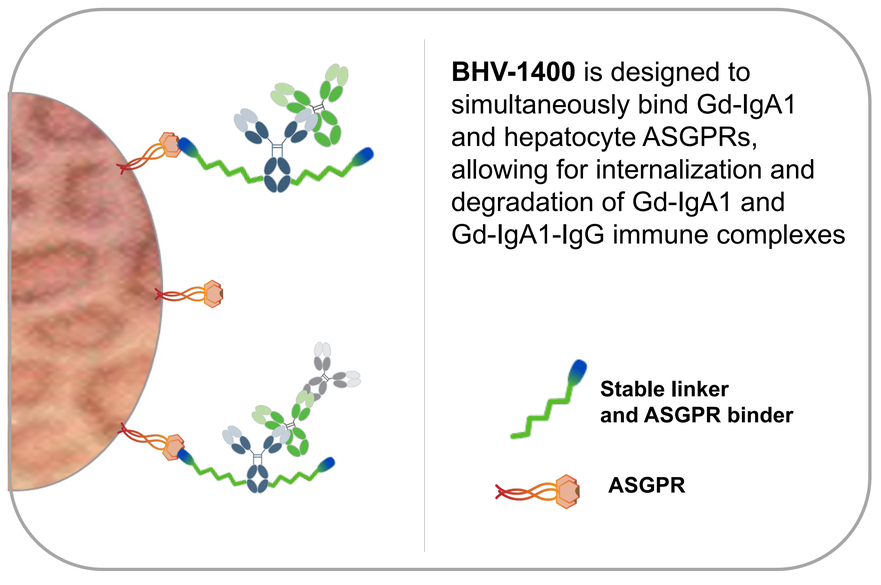


Figure 1. Pathogenesis of IgAN

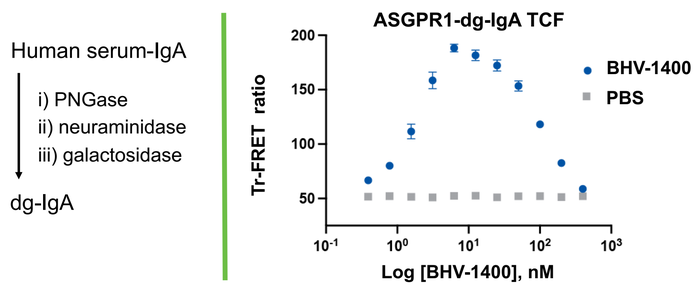




METHODS AND RESULTS

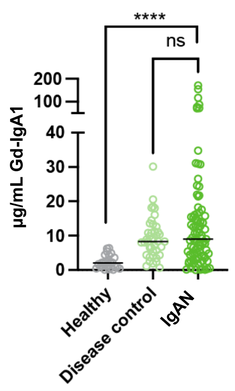
1) BHV-1400 Demonstrates Ability to Simultaneously Engage ASGPR and Protein of Interest (POI)

- dg-IgA is a semi-synthetic Gd-IgA1 surrogate prepared from pooled human serum IgA in 3 enzymatic steps
- BHV-1400 demonstrated strong affinity for dg-IgA (KD = 4 nM) and the carbohydrate recognition domain of ASGPR1(148-291) (KD= 9 nM)
- Evidence for the formation of a viable ternary complex with hook effect was provided by a proximity-based time-resolved fluorescence resonance energy transfer assay



2) Detection of Gd-IgA1 Levels in Human Plasma Samples

- Gd-IgA1 antibody concentrations in patient plasma samples were measured using Meso Scale Discovery with immobilized BHV-1400 and SULFO-TAG anti-IgA antibody
- Gd-IgA1 median levels in samples from patients with kidney disease and IgAN were ~4-fold higher than median levels in healthy volunteers
- Results were consistent with the literature reports using KM55 diagnostic and confirm that BHV-1400 recognizes significant levels of circulating human Gd-IgA1 and corresponding immune complexes

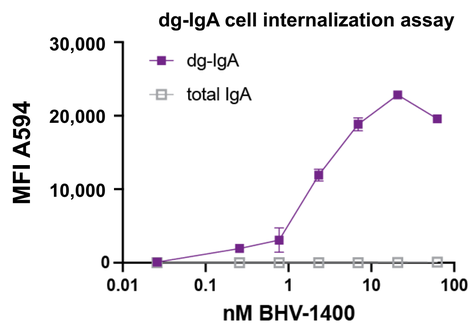


	Healthy participants (n = 26)	Participants with kidney disease (n = 40)	Participants with IgAN (n = 95)
Gd-IgA1 (µg/mL)*	2 ± 2	8 ± 6	9 ± 30

*median levels reported

3) In Vitro Cellular Internalization of dg-IgA with BHV-1400

- Dose-dependent, selective endocytosis of dg-IgA vs IgA was observed with BHV-1400 in human embryonic kidney (HEK) cells transfected with human ASGPR1 (hASGPR1)
- Low-nanomolar half-maximal effective concentration and robust mean fluorescence were observed at 12 hours for internalization of dg-IgA conjugated to Alexa Fluor 594
- BHV-1400 internalized POI for lysosomal degradation but spared normal IgA

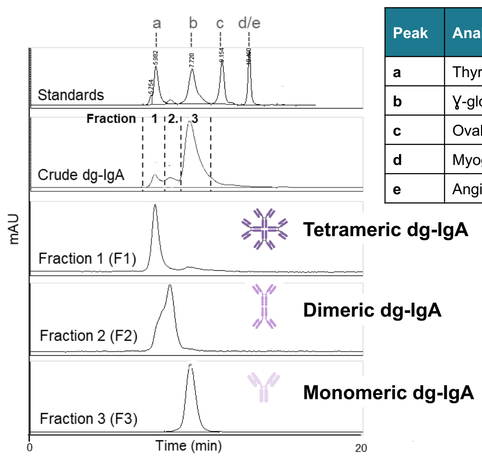


	dg-IgA	Total IgA
Max MFI	22,824	91.4
EC_{50} (nM)	1.75	27.5
S/N	201	101
Z prime	0.96	0.65

HEK293 cells transfected with ASGPR1 were used to measure endocytosis of 1 µg/mL dg-IgA and total IgA conjugated to Alexa Fluor 594. MFI = mean fluorescence intensity, S/N = signal-to-noise ratio.

4) Isolation of dg-IgA Aggregates by Size Exclusion Chromatography

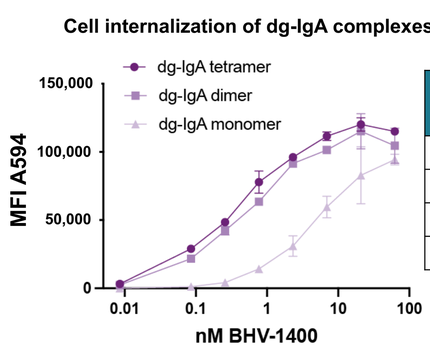
- dg-IgA conjugated to Alexa Fluor 594 was separated into 3 fractions corresponding to tetrameric, dimeric, and monomeric forms
- All 3 forms of dg-IgA were advanced into the cell internalization assay



Peak	Analyte	MW (daltons)
a	Thyroglobulin	670,000
b	Ɣ-globulin	150,000
c	Ovalbumin	45,000
d	Myoglobin	17,000
e	Angiotensin II	1000

5) Cellular Internalization of Surrogate Gd-IgA1 Antibody Complexes

- BHV-1400 demonstrated efficient internalization of dimeric and tetrameric dg-IgA complexes in HEK (hASGPR1) cells at 12 hours
- BHV-1400 can internalize and degrade large IgA antibody complexes

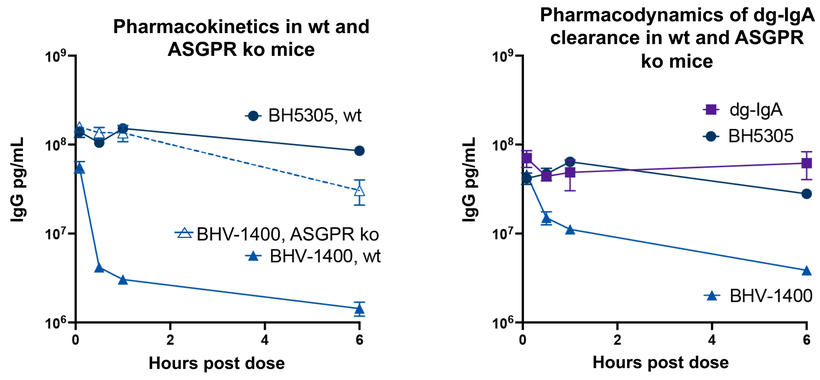


	Tetrameric dg-IgA	Dimeric dg-IgA	Monomeric dg-IgA
Max MFI	120,138	115,102	94,422
EC_{50} (nM)	0.37	0.48	3.93
S/N	57.9	139.9	678.9
Z prime	0.87	0.66	0.87

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.

6) BHV-1400 Achieves Robust Degradation of Exogenous dg-IgA in Mice

- BHV-1400 demonstrated ASGPR-dependent clearance in wild-type (wt) mice vs ASGPR1 knockout (ko) mice after intravenous (IV) administration. Results were consistent with BHV-1400 mechanism of action
- BHV-1400 depleted exogenous dg-IgA in wt mice to 58% area under the curve of control at 2:1 (drug:target) ratio after sequential IV administration
- Parent control antibody, BH5305, had no effect on degradation of exogenous dg-IgA in wt mice, as anticipated
- BHV-1400 rapidly and robustly degraded dg-IgA administered to mice



BHV-5305 IV, 200 µg/mouse; BHV-1400 IV, 200 µg/mouse = 8 mpk; dg-IgA IV 100 µg = 4 mpk (5× higher than patient's median Gd-IgA1 level); dg-IgA was administered 5 min prior to drug treatment in the pharmacodynamic study.

References: 1. Lai KN, Tang SC, Schena FP, et al. IgA nephropathy. *Nat Rev Dis Primers*. 2016;2:16001. **2.** Rajasekaran A, Julian BA, Rizk DV. IgA nephropathy: an interesting autoimmune kidney disease. *Am J Med Sci.* 2021;361(2):176-194. **3.** Knoppova B, Reily C, King RG, Julian BA, Novak J, Green TJ. Pathogenesis of IgA nephropathy: current understanding and implications for development of disease-specific treatment. *J Clin Med.* 2021;10(19):4501. **4.** Barratt J, Tumlin J, Suzuki Y, et al. Randomized phase II JANUS study of atacicept in patients with IgA nephropathy and persistent proteinuria. *Kidney Int Rep.* 2022;7(8):1831-1841. **5.** Rastelli L, Spiegel DA, Welsch ME, et al, inventors; Kleo Pharmaceuticals, Inc., assignee. Directed conjugation technologies. International application PCT/US2020/061127. May 27, 2021. **6.** Data on file.

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