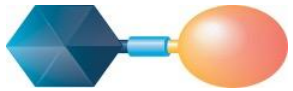
Heterobifunctional Molecules That Induce Targeted Degradation of Extracellular Proteins Through the Cell-Surface Asialoglycoprotein Receptor

- 6th Annual TPD Summit, Boston MA, Nov 2, 2023 -

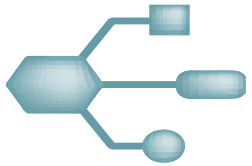
Protein Degradation at the Extracellular Frontier

Avilar Is Pioneering Extracellular Protein Degradation



ATACs (ASGPR TArgeting Chimeras) – New Class of Degraders

Designed to shuttle unwanted protein from circulation to endolysosome for degradation

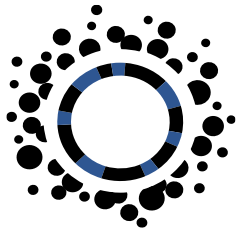


Proprietary discovery platform to design and build ATACs

Novel high-affinity ASGPR chemistry, extensive in vivo PoC, monovalent degraders

Advancing pipeline of first-in-class extracellular degraders

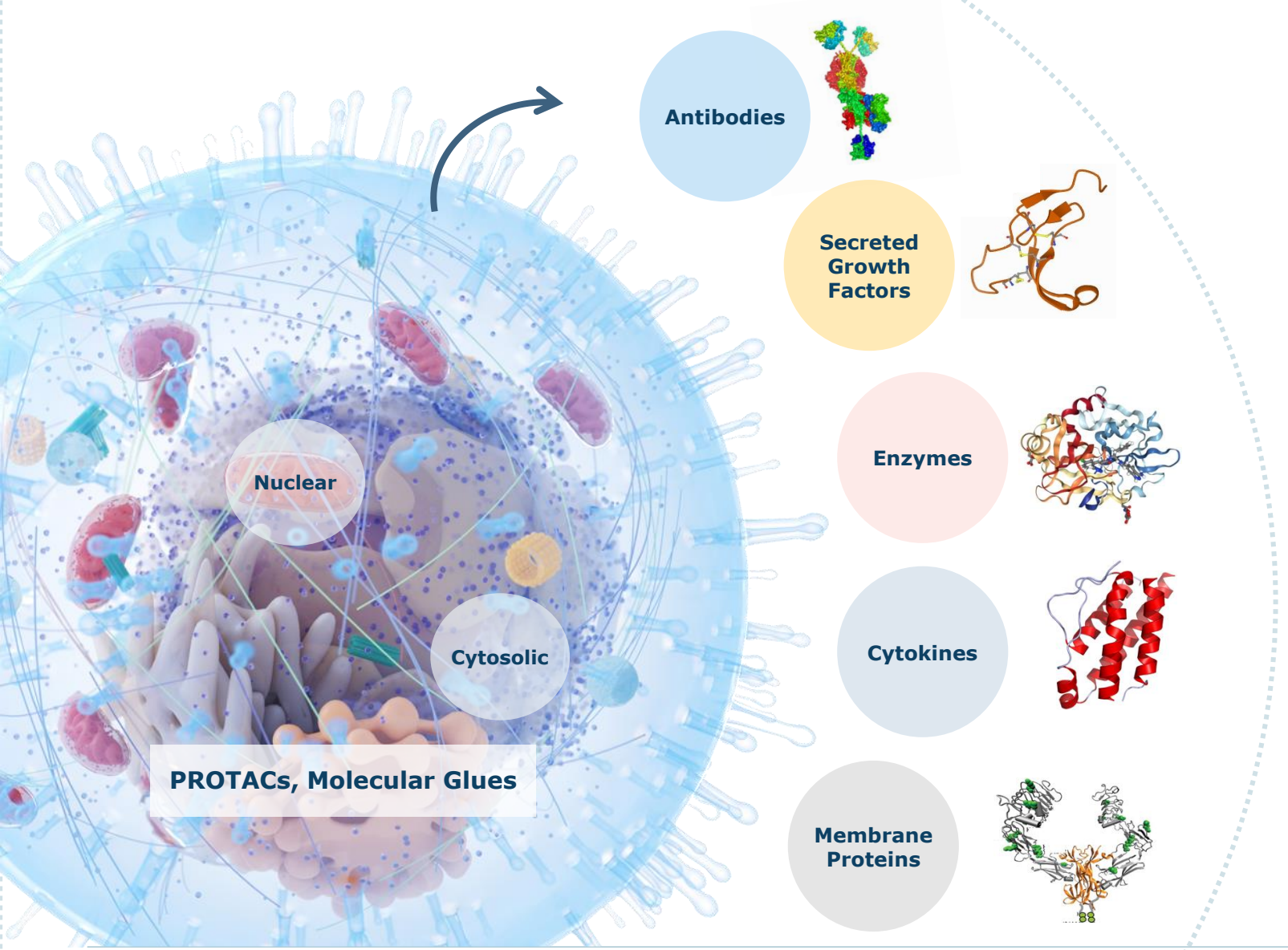
Opportunities for both internal pipeline and pharma collaborations



Leadership at the next frontier in protein degradation

Multi-product + multi-technology leadership in field of extracellular degradation

Universe of Extracellular and Membrane Proteins for Degradation



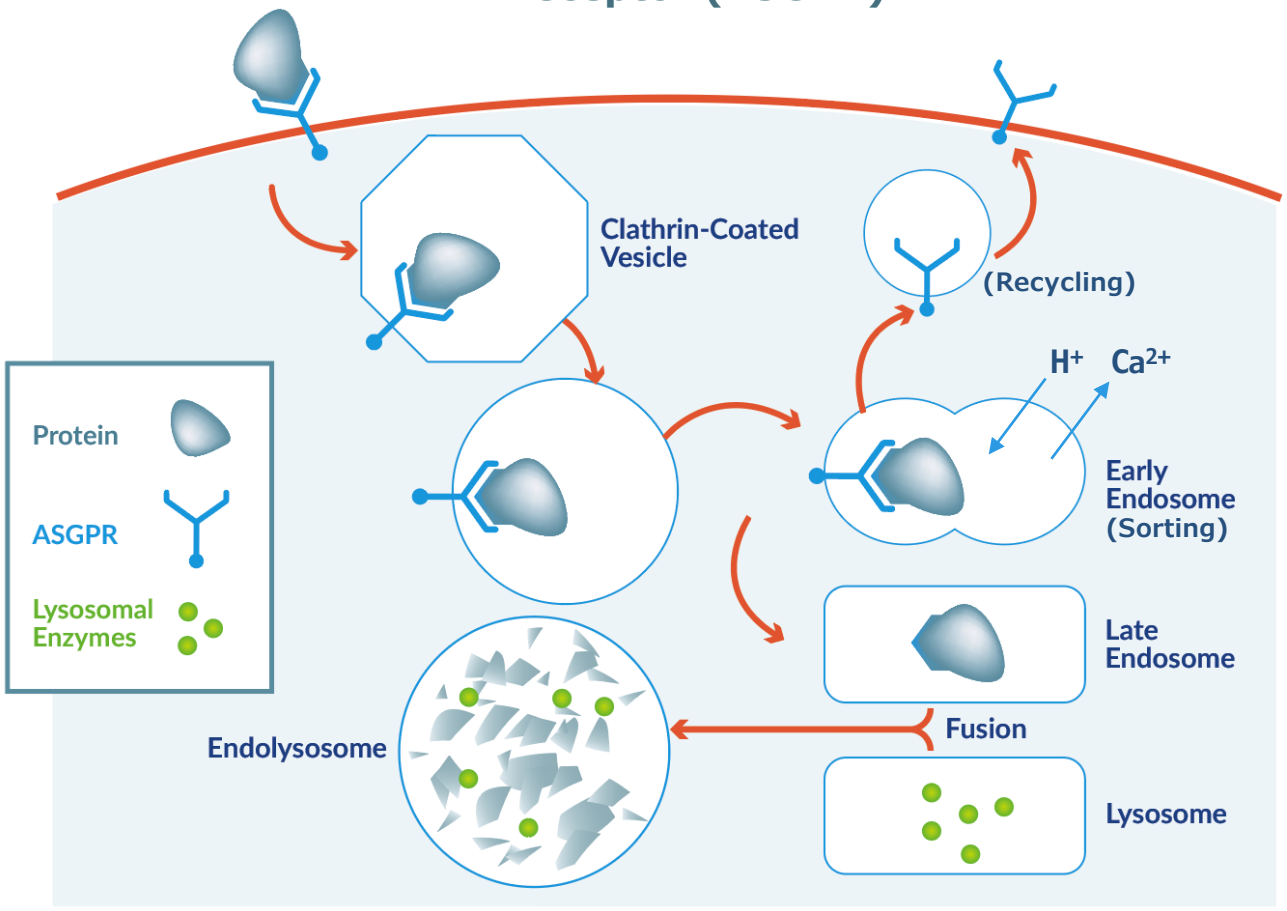
- First generation degraders target intracellular proteins
- Yet almost 40% of human proteins are extracellular or membrane-bound
- Multiple classes and hundreds with established role in pathogenesis of diseases
- Degradation of extracellular proteins would dramatically expand the “degradome”
- Avilar initial focus: validated yet poorly served targets where degrader has advantage

https://www.proteinatlas.org/humanproteome/tissue/secretome

ASGPR Role in Body's Natural Cellular Degradation Machinery

- Endocytotic C-type lectin receptor that recognizes galactose (Gal) and N-Ac-galactosamine (GalNAc) units
  - Ca^{2+}-dependent ligand binding
- Highly expressed on hepatocytes (~1M receptors per cell in humans)
- Mediates binding, internalization, and subsequent degradation of endogenous Gal/GalNAc-terminated (asialo) glycoproteins via endolysosome (asialofetuin, IgA, von Willebrand factor, etc)
- ASGPR–glycoprotein complex segregates in early endosome with increasing acidity and decreasing Ca^{2+} concentration
- ASGPR endocytosed and recycled from endosome back to plasma membrane every ~15 minutes
- Glycoprotein enters catabolic lysosomal pathway

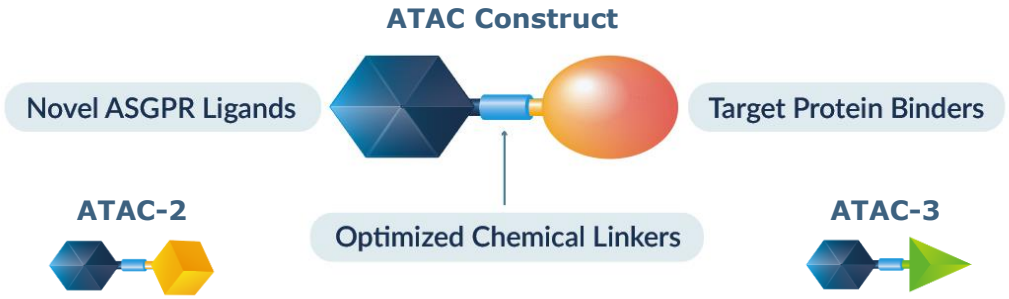
Natural Endocytosis and Degradation of Endogenous Proteins via Asialoglycoprotein Receptor (ASGPR)



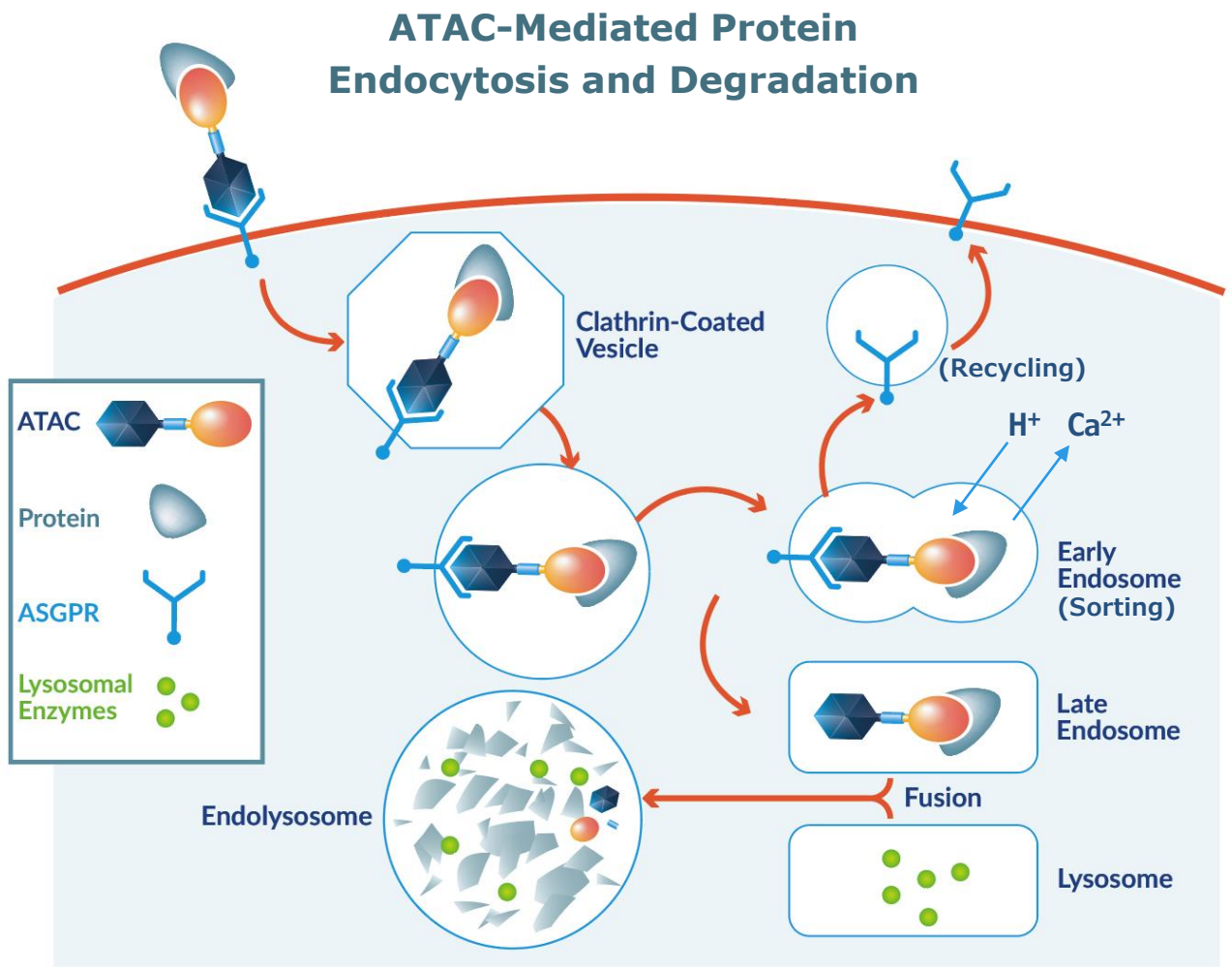
Andersen, C. B. F. et al. Trends Biochem. Sci. 2014; Onizuka, T. et al. FEBS J. 2012; Grewal, P. K. Methods Enzymol. 2010; Gerasimenko, J. V. et al. Curr. Biol. 1988

Avilar THERAPEUTICS

ATACs Harness ASGPR Pathway to Degrade Extracellular Proteins

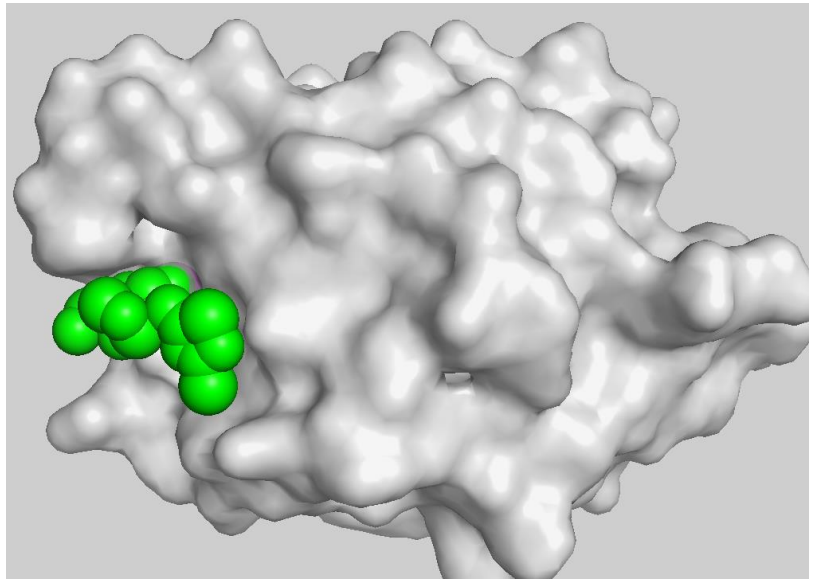


- Leverage natural cellular process to shuttle target protein from circulation to endolysosome for degradation
  - Key pH-/Ca^{2+}-dependent ATAC–ASGPR binding
- Employ novel bi-functional molecules comprising ASGPR ligand, selected linkers, and binder to target protein
- Modular: advantageous ASGPR ligands and linkers deployed in synthesis of ATACs with diverse protein targeting binders
- Established: ASGPR-mediated hepatocyte targeting safely delivers RNAi therapeutics to the liver (endosomal escape mechanism)
  - 1st approved drug: givosiran, 2019



Avilar Proprietary ASGPR Ligands With Dramatically Improved Affinity

Structure-Guided ASGPR Ligand Design



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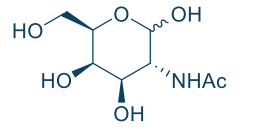
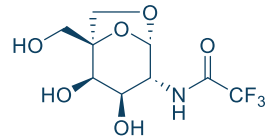
(54) Title: ASGPR-BINDING COMPOUNDS FOR THE DEGRADATION OF EXTRACELLULAR PROTEINS

- Multiple X-ray structures inform ligand library design
- Avilar's 1st patent application, US allowance Aug 2023

Compound ID	GalNAc	BC Ligand*	AVI-1	AVI-2	AVI-3	AVI-4
ASGPR K_D (SPR, pH 7.4, [Ca^{2+}] >1 mM)	53 μM	1.7 μM	0.72 μM	0.21 μM	0.10 μM	0.037 μM
Increase in Affinity	1x	~30x	~70x	~250x	~530x	~1,400



GalNAc



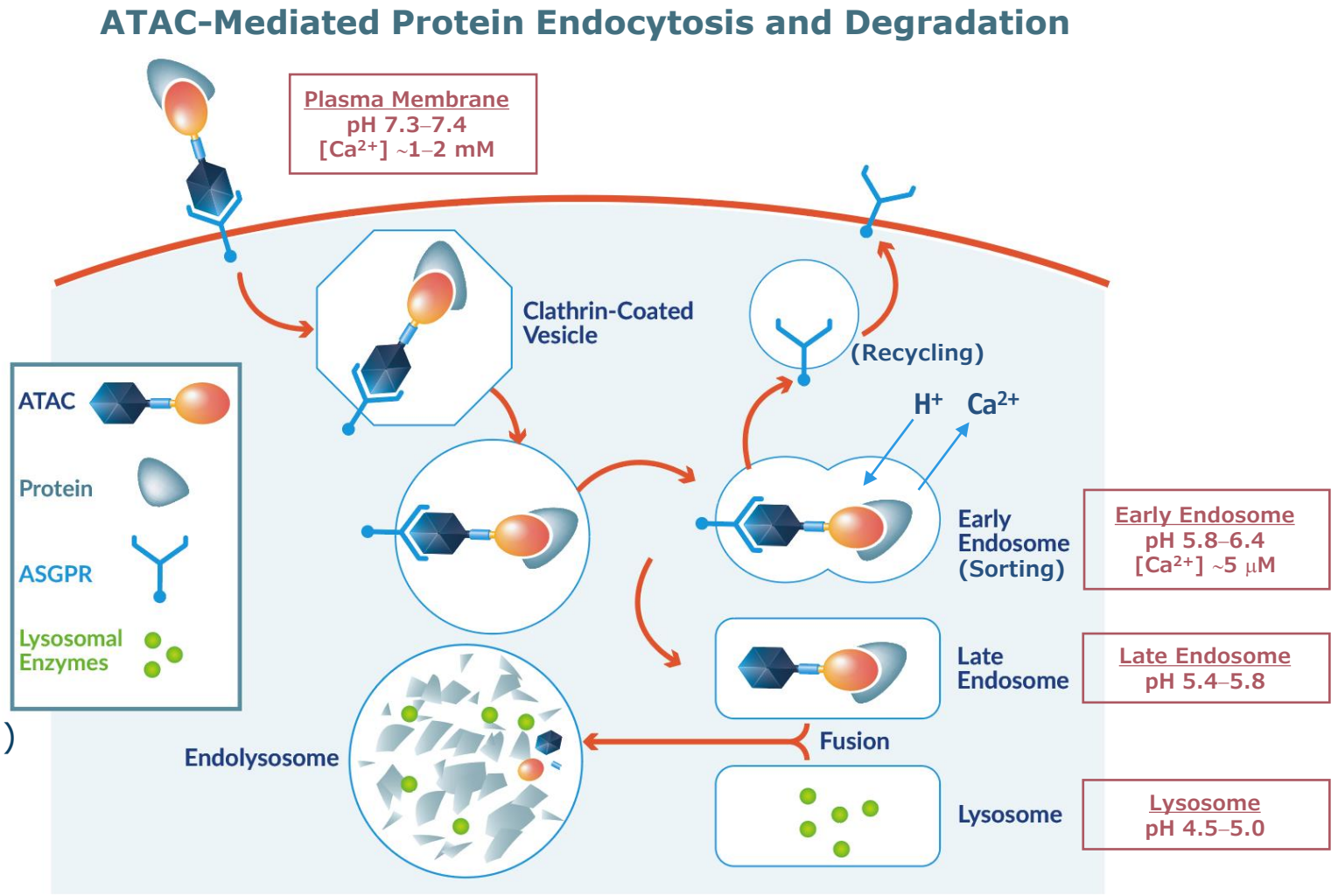
Bicyclic (BC) Ligand

- pH and Ca^{2+} levels mimic physiological conditions on plasma membrane for ligand–ASGPR engagement

*Liras, S. et al. U.S. Patent 9,340,553 (Pfizer, 2014)

Proprietary Ligands Mimic Natural Glycoprotein Endocytotic Processing

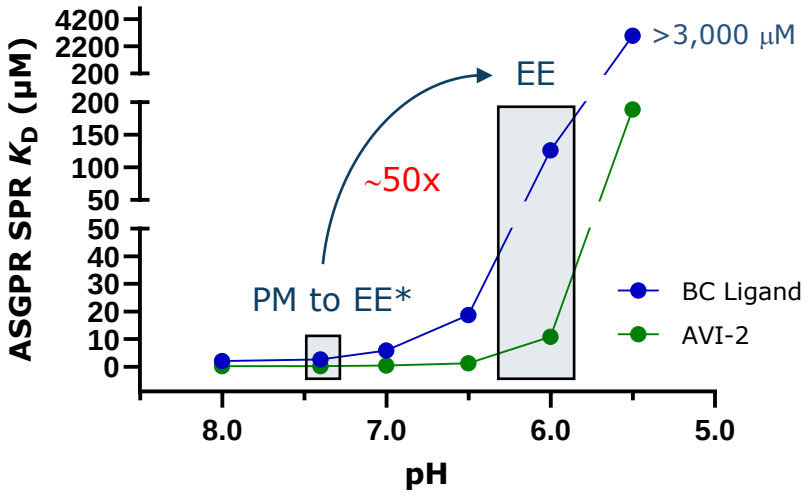
- ATAC–protein complexes taken up by cells experience an increasingly acidic environment as they progress through the endolysosomal pathway
- Rapid acidification driven by V-ATPase H^+ pump in early endosome
  - Low pH required later for activity of hydrolases in lysosome
- Most Ca^{2+} taken up through endocytosis is quickly lost during initial course of endosomal acidification
- Weak acidification of early endosome and decrease in Ca^{2+} concentration are key for ligand–receptor segregation (affinity switch)
  - Receptor recycled to plasma membrane
  - Ligand continues to endolysosome for degradation



Gasnier, B. et al. Ion and Molecule transport in Lysosomes 2020; Garrity, A. G. et al. eLife 2016; Jaworska, A. et al. Analyst 2015; Andersen, C. B. F. et al. Trends Biochem. Sci. 2014; Morgan, A. J. et al. Biochem. J. 2011; Casey, J. R. et al. Nat. Rev. Mol. Cell Biol. 2010; Gerasimenko, J. V. et al. Curr. Biol. 1988

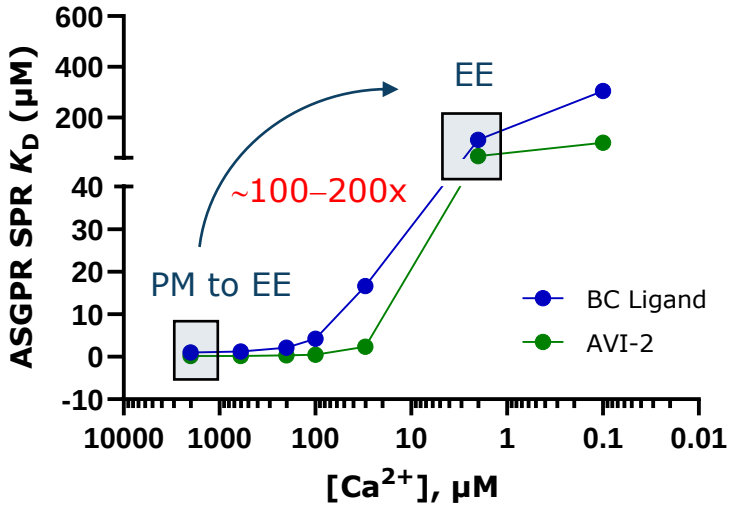
Avilar ASGPR Ligands Retain Key pH/Ca^{2+}-Dependent Binding

pH-Dependent hASGPR binding



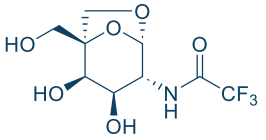
Affinity decreases as pH decreases
(All measurements at [Ca^{2+}] = 2 mM)

Ca^{2+}-Dependent hASGPR Binding



Affinity decreases as Ca^{2+} level decreases
(All measurements at pH 7.4)

AVI-2
(Proprietary Ligand)



Bicyclic (BC) Ligand
(Non-proprietary Control)

	ASGPR SPR K_D (μM)		
	2 mM Ca^{2+}	5 μM Ca^{2+}	
	pH 7.4	pH 6.5	pH 6.0
AVI-2	0.21	>3,000	>3,000
Bicyclic	1.7	>3,000	>3,000

PM conditions
(High affinity, binds)

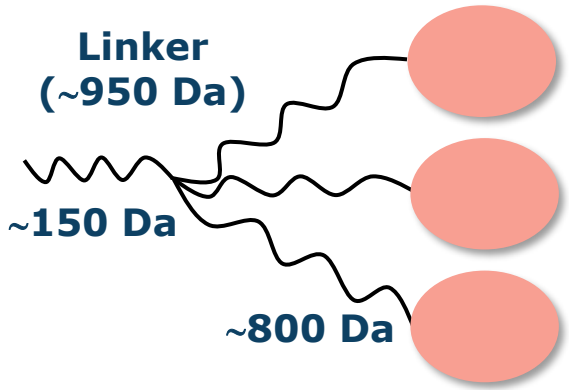
EE conditions
(Low affinity, releases)

- Both pH and Ca^{2+} data consistent with ligand release mechanism in early endosome, enabling ASGPR recycling and degradation of ATAC–protein complex
  - Results agree between lower affinity non-proprietary ligand and higher affinity proprietary ligand
  - Also agree with previously published NMR study of GalNAc release from ASGPR**

*PM, Plasma Membrane; EE, Early Endosome **Onizuka, T. et al. FEBS J. 2012

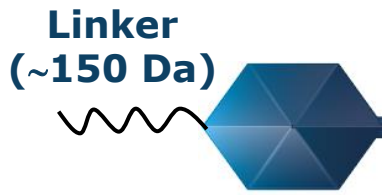
Avilar ASGPR Ligands Enable Design of Low MW Monovalent ATACs

Conventional Tridentate GalNAc
Total MW = ~1600 Da (~3.5x > Avilar)



GalNAc
ASGPR ligands
(3 x ~220 = ~660 Da)

Avilar Monodentate ASGPR Ligand
Total MW = ~450 Da



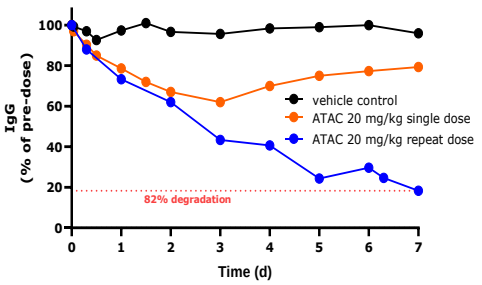
Avilar
ASGPR ligand
(~300 Da)

- Traditional low-affinity ligands (e.g., GalNAc, K_D ~50 μM) require multivalency (avidity) to achieve nM affinity
- Novel high-affinity ASGPR ligands enable monodentate presentation with lower MW and improved drug-like properties
  - Low MW ASGPR ligands enable low dose/volume SC ATACs
  - Low MW ASGPR ligands combined with SM binders to POI enable oral dosing

ATACs Demonstrated Degradation of Diverse Extracellular Proteins

- **IgG In Vivo PoC**
- **POI Ligand: Peptide**
- MW: 150 kDa
- Conc: 80 µM

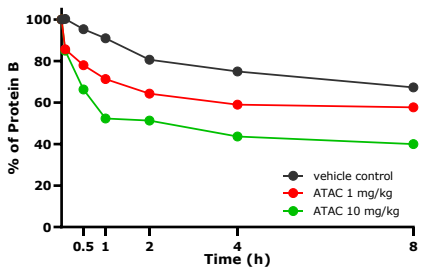
IgG Degradation in NHP



20 mg/kg of ATAC dosed IV once (red circles) or every 2 days for 4 cycles (blue circles). Plasma collected out to 14 days for exposure and IgG analyses. Graph represents mean of n=3 NHPs per group.

- **Protein B In Vivo PoC**
- **POI Ligand: Undisclosed**
- MW: Undisclosed
- Conc: Undisclosed

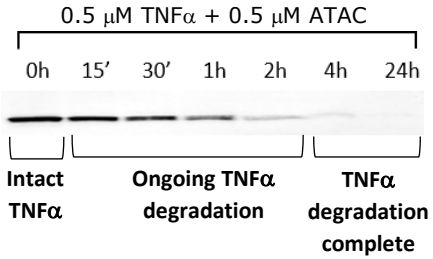
Protein B Degradation in Rodents



Animals injected with human protein B 1h prior to IV ATAC injection. Plasma collected at various timepoints up to 24h for exposure and protein B analyses. Graph represents mean of n=3 animals per group.

- **TNFα In Vitro PoC**
- **POI Ligand: SM**
- MW: 17 kDa
- Conc: 2 pM

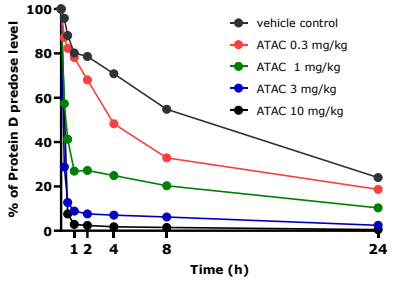
TNFα Degradation in HepG2 Cells



ATAC and human TNFα pre-incubated to allow binary complexes formation. ATAC / TNFα complexes or TNFα alone then incubated for 2h with HepG2 cells. TNFα detected with Western blot analysis.

- **Protein D In Vivo PoC**
- **POI Ligand Undisclosed**
- MW: Undisclosed
- Conc: Undisclosed

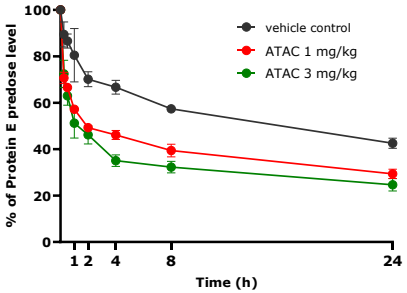
Protein D Degradation in Rodents



Animals injected with human protein D 1h prior to IV ATAC injection. Plasma collected at various timepoints up to 24h for exposure and protein D analyses. Graph represents mean of n=3 animals per group.

- **Protein E In Vivo PoC**
- **POI Ligand Undisclosed**
- MW: Undisclosed
- Conc: Undisclosed

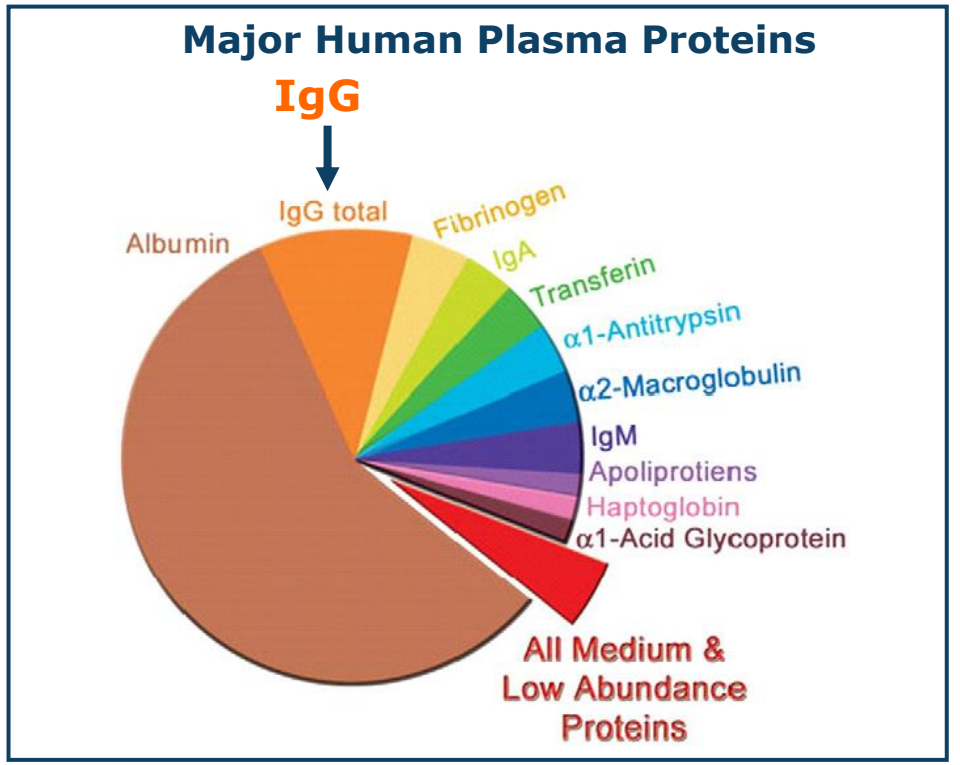
Protein E Degradation in Rodents

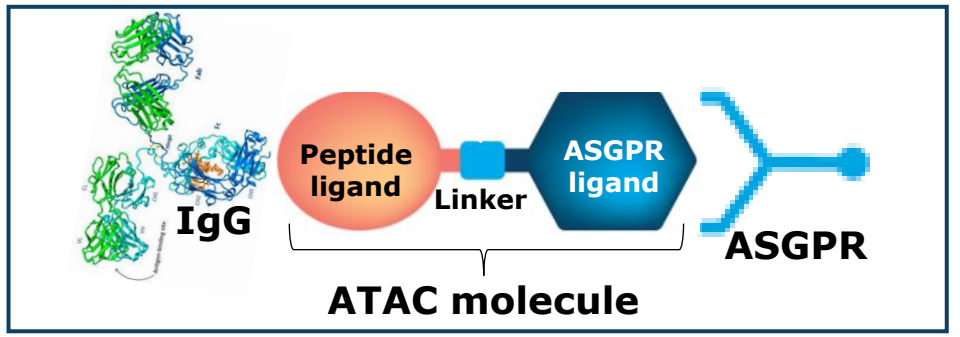


Animals injected with human protein E 1h prior to IV ATAC injection. Plasma collected at various timepoints up to 24h for exposure and protein E analyses. Graph represents mean of n=3 animals per group.

Proof-of-Concept Studies Demonstrating Degradation of IgG

- 2nd most abundant plasma protein
  - High plasma concentration: 1.06 g/kg total body IgG or 74.2 g in 70 kg human (plasma ~80 μM)
  - Resynthesis rate: 32 mg/kg/day; ~3% of total IgG/day
- Long half-life: ~21 days (human), ~5 days (cyno)
- ATACs synthesized using peptide ligand for IgG
- Multiple studies completed, including:
  - Monodentate and bidentate ATACs
  - IV bolus and SC administration
  - Single and repeat dose in vivo studies
  - MOA elucidation studies
  - PK/PD modeling simulations

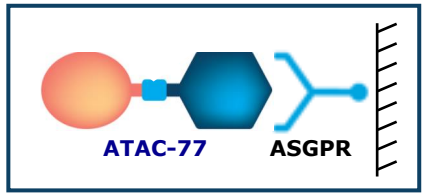




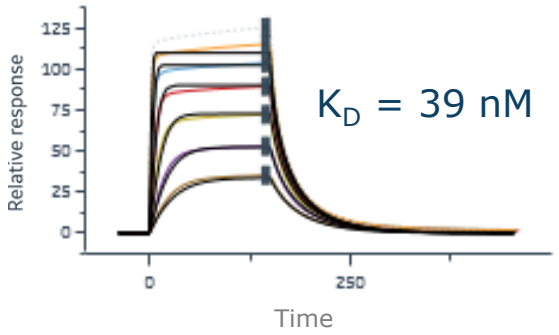
Nezlin, R., The Immunoglobulins 1998; Waldmann et al., Prog. Allergy 1969; Kratz et al., J. Control. Release 2012

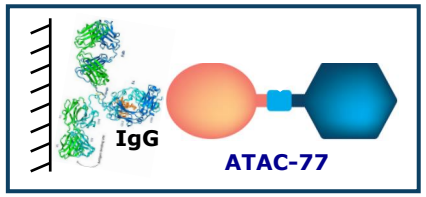
Monodentate ATAC-77 Binds Human ASGPR and IgG In Vitro

- Binary complexes: ATAC-77 binds to human ASGPR and IgG as shown by SPR
  - IgG selectivity: human IgA and IgE K_Ds >100 μM

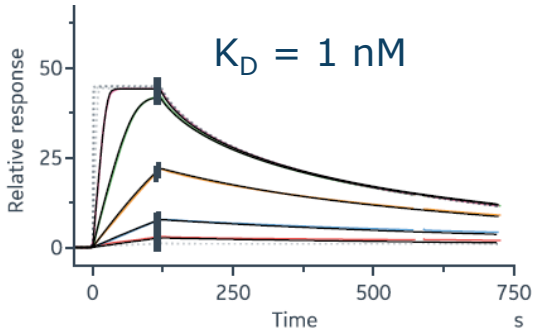


Binding to Human ASGPR

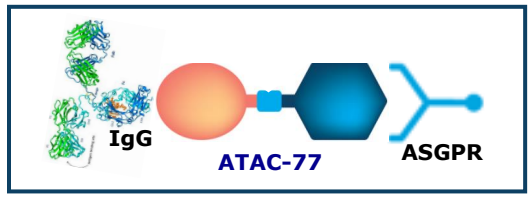




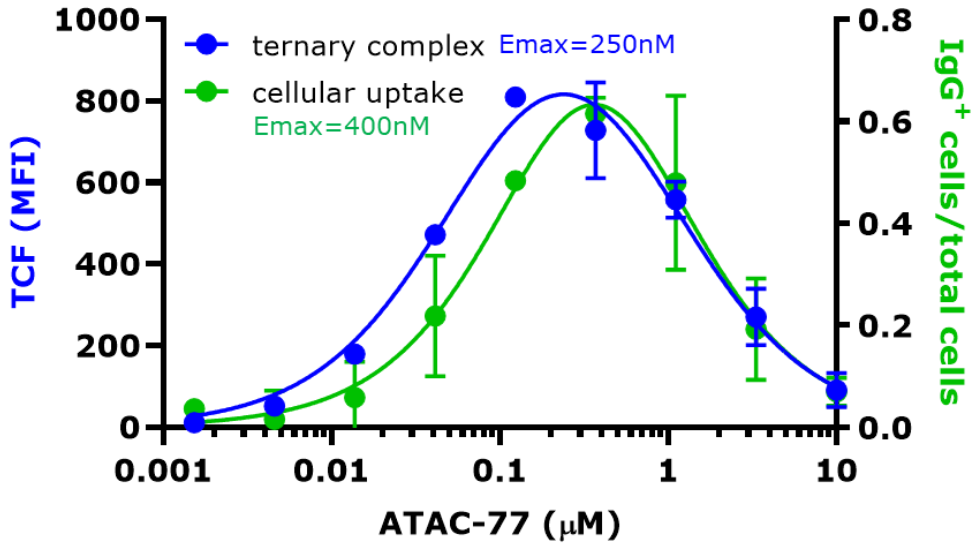
Binding to Human IgG



- ATAC-77 mediates ternary complex formation and cellular uptake of human IgG in HepG2 cells



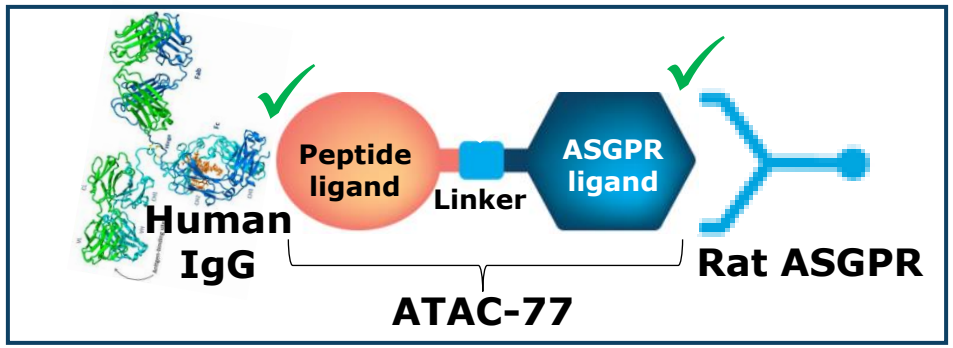
Ternary Complex Formation and Cellular Uptake

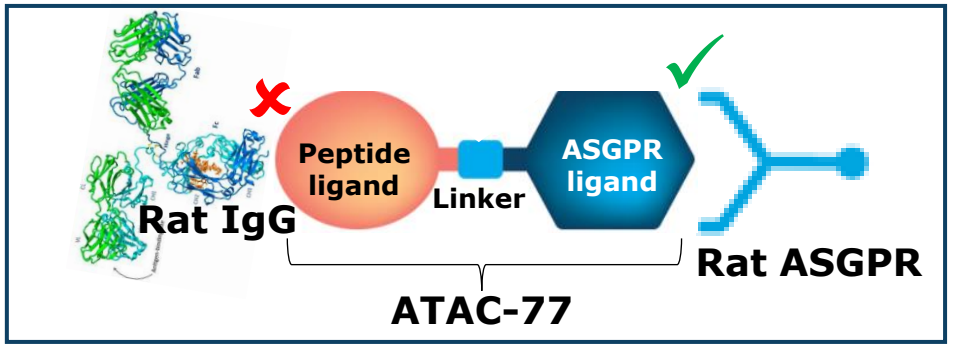


Study Designs: SPR studies: ATAC-77 was added to recombinant human IgG1-Fc or recombinant human ASGPR. TCF studies: ATAC-77 and fluorescently-labeled human IgG were added to HepG2 cells on ice. Cell-associated fluorescence was measured by flow cytometry (MFI). Uptake studies: ATAC-77 and fluorescently-labeled human IgG were added to HepG2 cells at 37 °C. Cell-associated fluorescence was measured by fluorescence microscopy and presented as ratio of IgG+ cells/total cells.

ATAC-77 Does Not Bind Rat IgG

- Key amino acids near peptide ligand binding site differ in rat vs. human IgG, resulting in drastic loss in affinity
  - Human and cyno IgG K_D ~1 nM, whereas rat IgG K_D >10 μM (no binding detected at C_{max})



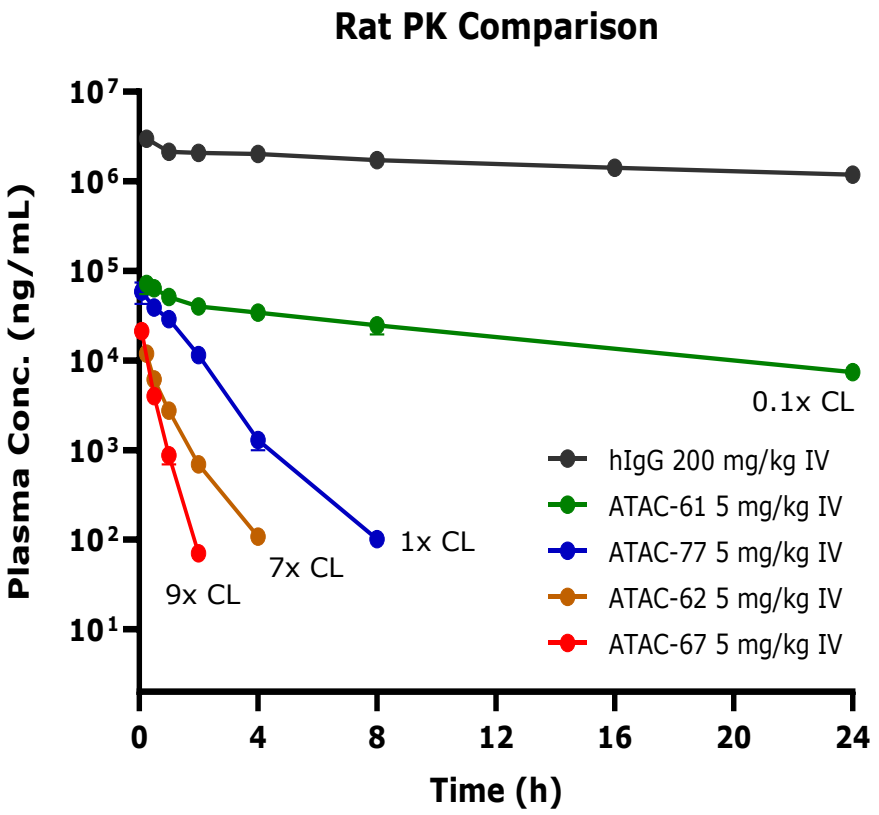


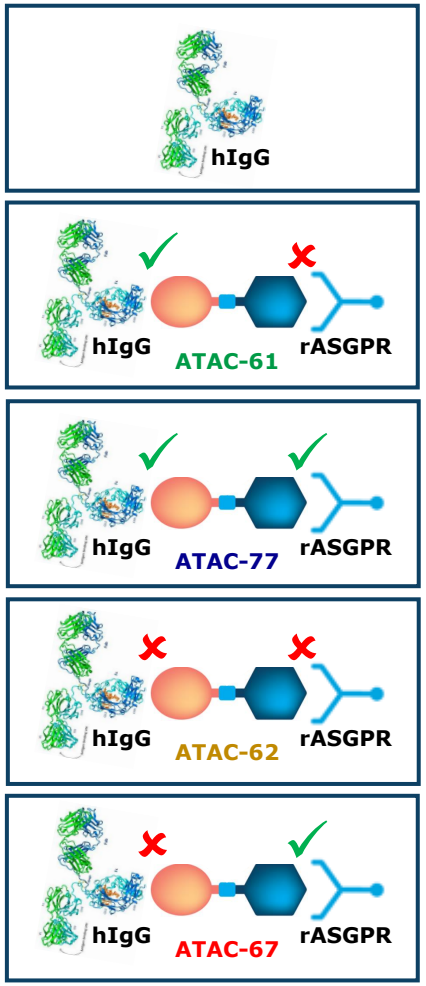
- PK/PD studies of ATAC-77 in rat require co-administration of human IgG
  - Mimic endogenous rat IgG plasma level (~10 μM*) with 200 mg/kg dose of human IgG

*Chang, H.–Y. et al. mAbs 2018

ATAC-77 Rat PK Highly Influenced by hIgG and rASGPR Binding

- PK study design
  - Administered 200 mg/kg IV bolus of hIgG followed by ATAC at 1 h postdose
- Elimination of ATAC-77 from plasma is rapid with estimated $t_{1/2}$ <1 h
- Binding to IgG leads to lower clearance, while binding to ASGPR leads to higher clearance
  - PK of ATAC-61 similar to target protein human IgG (high-affinity PPB)
  - PK of ATAC-67 similar to givosiran, first approved triGalNAc-conjugated RNAi therapeutic (liver-targeting delivery)
  - PK of ATAC-62 resembles ATAC-67, though elimination slower without ASGPR activity

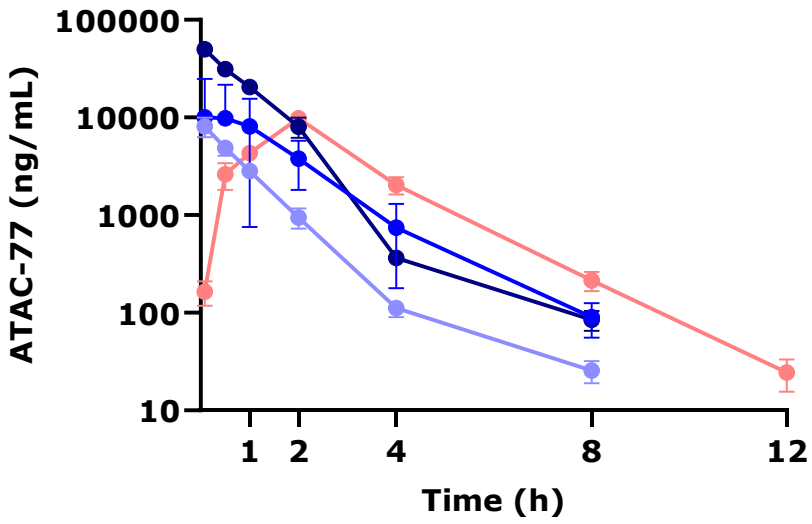


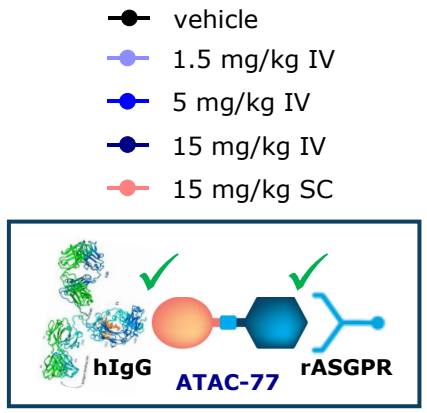


Givosiran: Li, J. et al. Drug Metab. Dispos. 2021

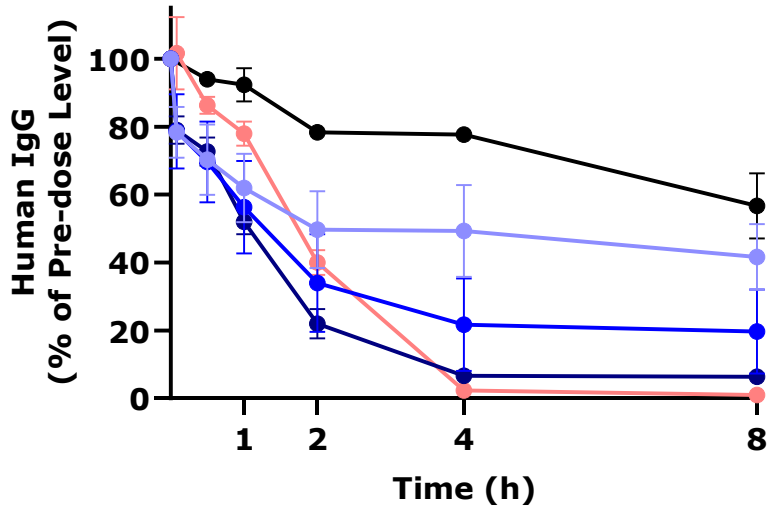
ATAC-77 Dosed IV/SC Degrades Human IgG in Rat PK/PD Model

ATAC-77 Plasma Exposure





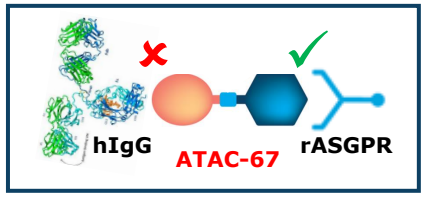
ATAC-77 Mediated Degradation of hIgG



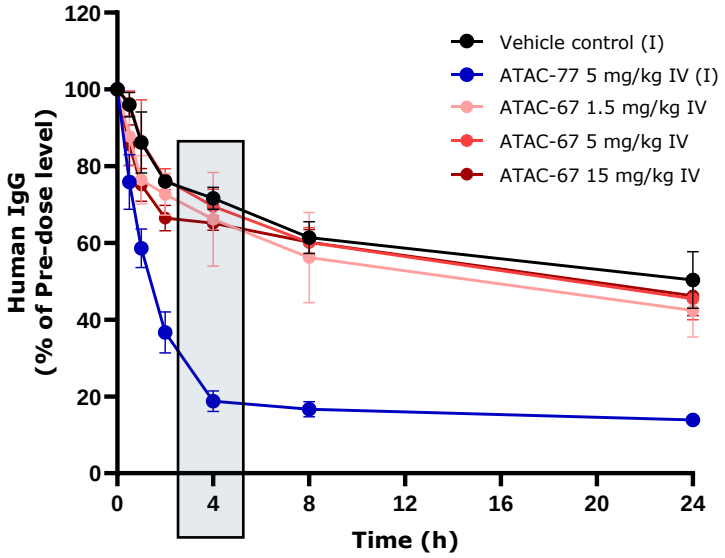
- Human IgG administered via single IV bolus injection at t = –1 h followed by ATAC-77 at t = 0 h (ATAC-77 does not bind rat IgG)
- ATAC-77 effectively degrades human IgG from rat plasma in a dose-dependent manner
- SC dose (15 mg/kg) results in 75% depletion of human IgG (vs. vehicle) in 4 h despite ~2.3x lower plasma AUC than IV dose (71% IgG depletion at 15 mg/kg)
  - SC liver exposure likely higher than IV liver exposure, as previously demonstrated for triGalNAc-conjugated RNAi therapeutic givosiran
  - More efficient SC liver uptake due to gradual increase in plasma concentration, potentially limiting saturation of ASGPR-mediated hepatic uptake

Study Design: All animals were injected IV with hIgG. Single IV or SC administration of monodentate ATAC-77 1h post hIgG injection (except vehicle group). Plasma collected over 12 h for PK and PD analyses.
PK: plasma ATAC concentration by mass spectrometry. PD: plasma human IgG concentration by ELISA. Graphs represent the mean +/-SD of n=3 rats per group

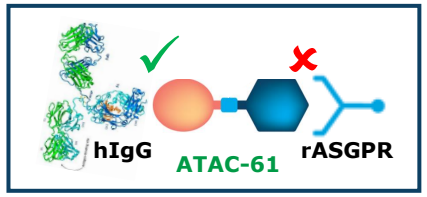
No Degradation Observed With Inactive IgG and/or ASGPR Ligands



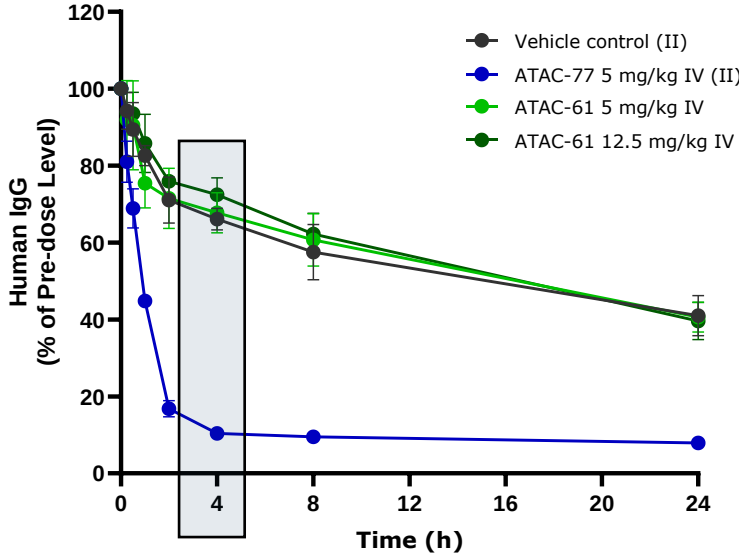
No hIgG Degradation by ATAC-67



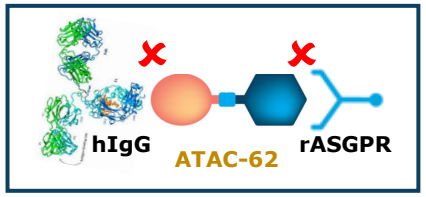
IgG alone	~28% depletion
With ATAC-67	~33% depletion
With ATAC-77	~81% depletion



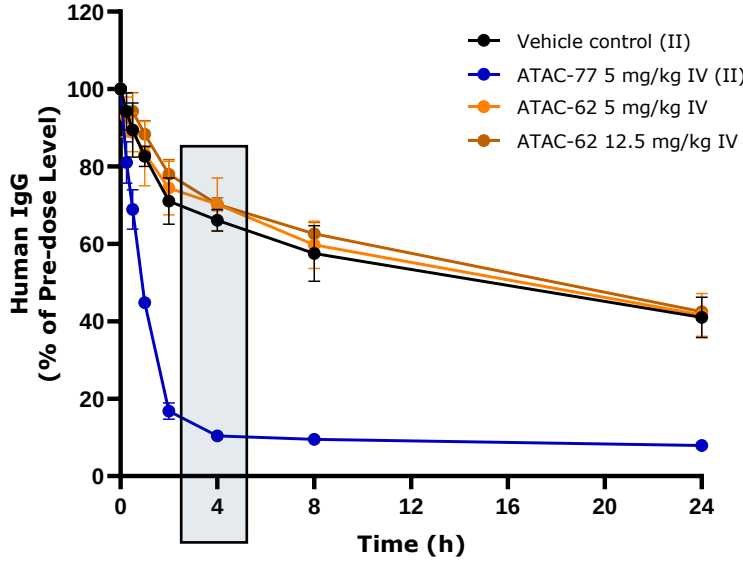
No hIgG Degradation by ATAC-61



IgG alone	~34% depletion
With ATAC-61	~30% depletion
With ATAC-77	~90% depletion



No hIgG Degradation by ATAC-62

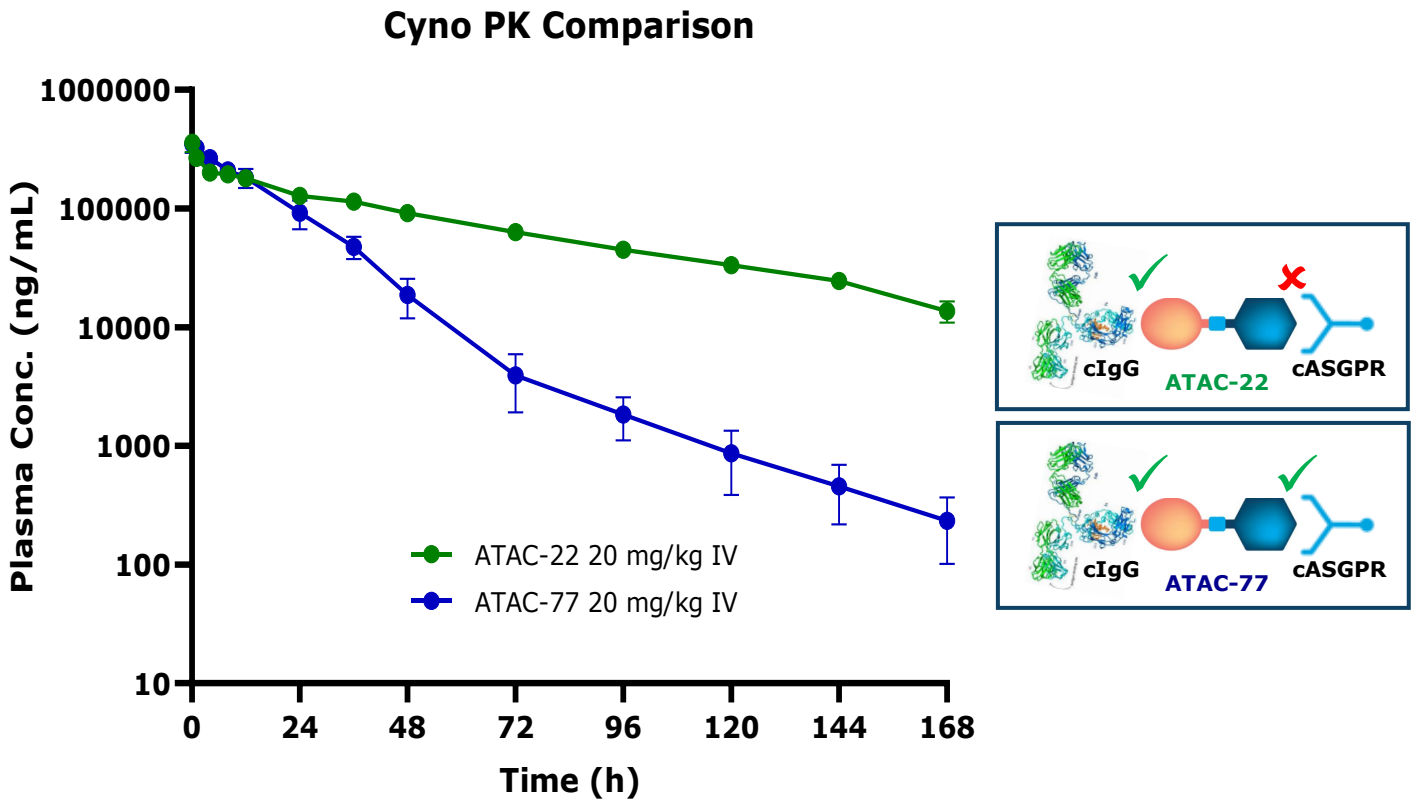


IgG alone	~34% depletion
With ATAC-62	~30% depletion
With ATAC-77	~90% depletion

- Unlike active ATAC-77, no apparent human IgG degradation in rats dosed with inactive ATAC-67, ATAC-61, or ATAC-62

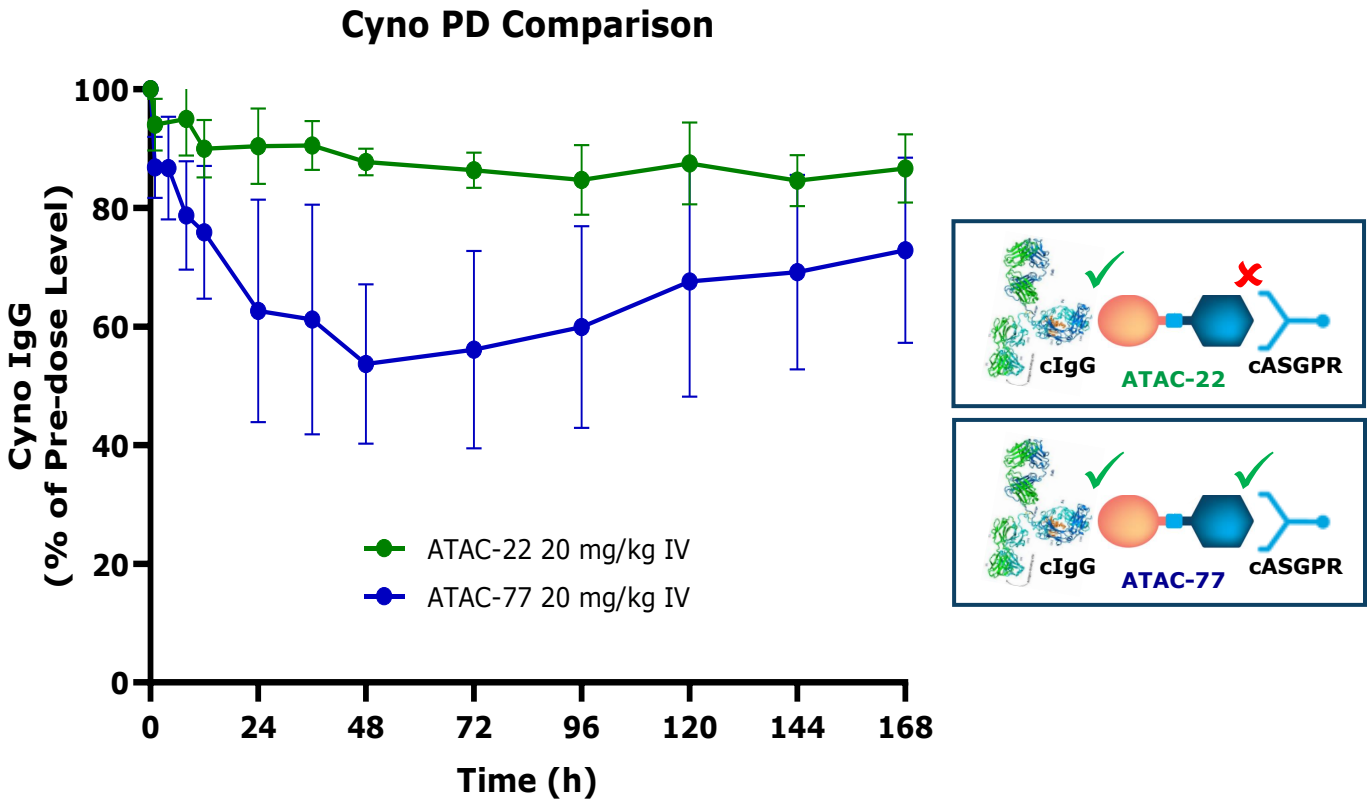
Monodentate ATAC-77 Cyno PK Highly Influenced by cASGPR Binding

- Design of single-dose PK studies
  - Administered single IV bolus doses of ATACs
- ATAC-77 exhibited higher in vivo clearance than related ATAC-22 with inactive ASGPR ligand
  - Indicates ASGPR-mediated hepatic uptake plays a major role in cyno PK behavior
- No human IgG pre-dosing required because IgG ligand is cross reactive with cyno IgG
  - Can now evaluate degradation of endogenous protein with repeat dose studies



Single Dose Monodentate ATAC-77 (IV Bolus) Degrades Cyno IgG

- Design of single-dose PD studies
  - Administered single exploratory IV bolus doses of ATACs (dose/formulation not optimized)
- Single IV bolus dose of 20 mg/kg ATAC-77 leads to 46% IgG degradation at 48 h
- By contrast, no degradation with ASGPR-inactive ATAC-22
- High variability in % degraded related to high variability of baseline (30–60 μM) observed in cyno
- Single dose data inform repeat dose study design



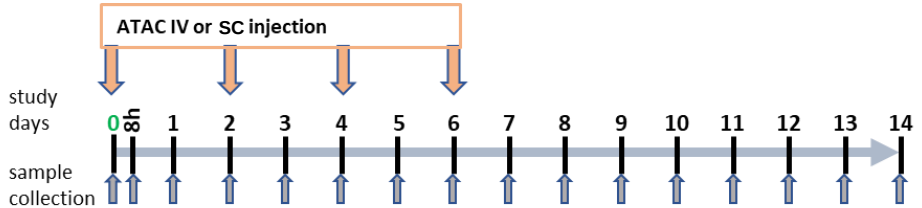
Study Design: IV bolus administration of single dose of monodentate ATAC-77 at 20 mg/kg. Plasma collected for 7 days for PK analyses. PD: plasma cyno IgG concentration by ELISA. Graphs represent the mean +/-SD of n=3 NHPs per group

Repeat Dose PK/PD Studies with Monodentate ATAC-77 in Cyno

- Goal to explore various clinically relevant dosing and route of administration regimens
  - Repeat dose bolus IV and SC
- Key elements of repeat dose design informed by single dose data
  - Assess Q2D dosing to match time of IgG concentration nadir in single dose study (48 h)
  - IgG baseline prescreen and randomization performed due to high variability (30–60 μM) observed in cyno

Study design

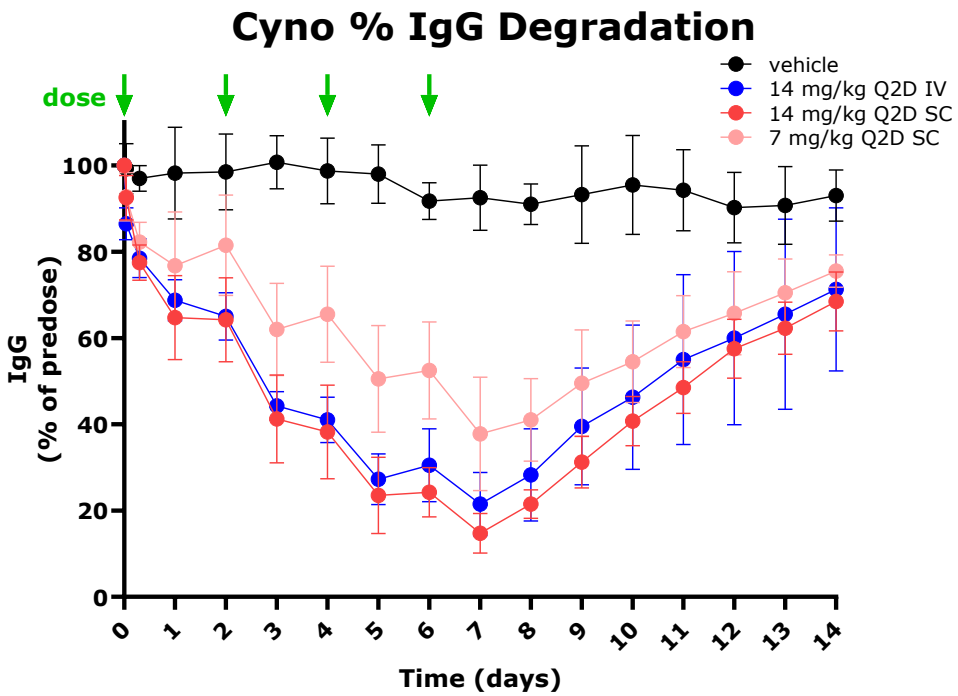
- Cyno IgG, native levels (~50 μM)
- IgG-ATAC, dosed 4 times IV bolus or SC

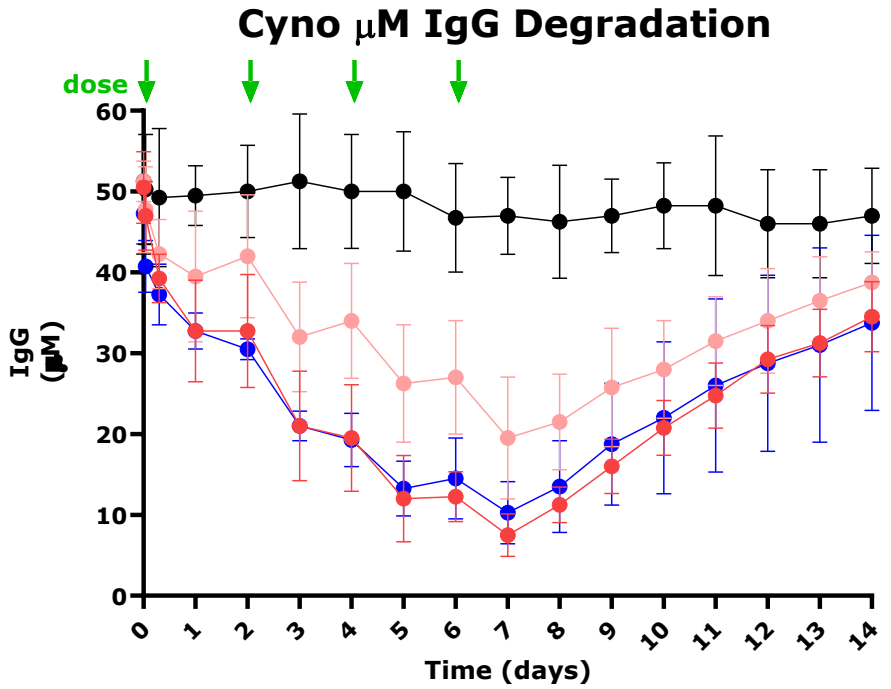


Data acquired for 14 days

- PK: plasma [ATAC] by mass spectrometry
- PD: plasma [cyno IgG] by ELISA

Repeat Dose IV/SC of Monodentate ATAC-77 Degrades IgG in Cyno

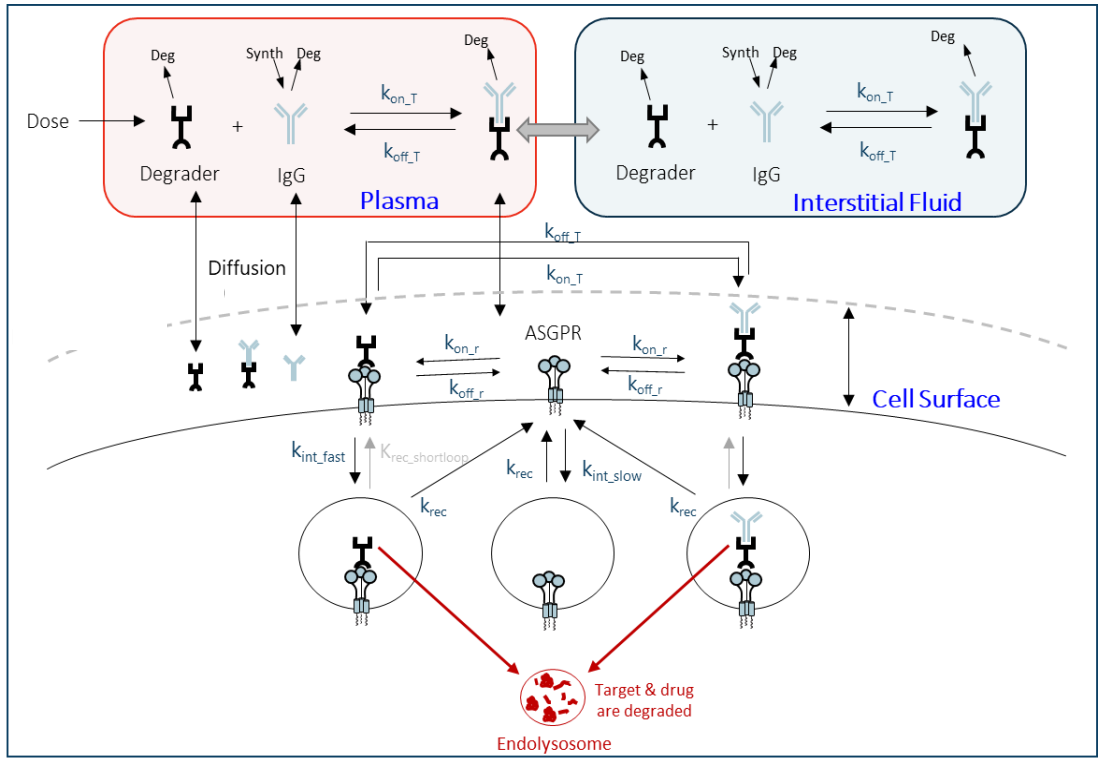


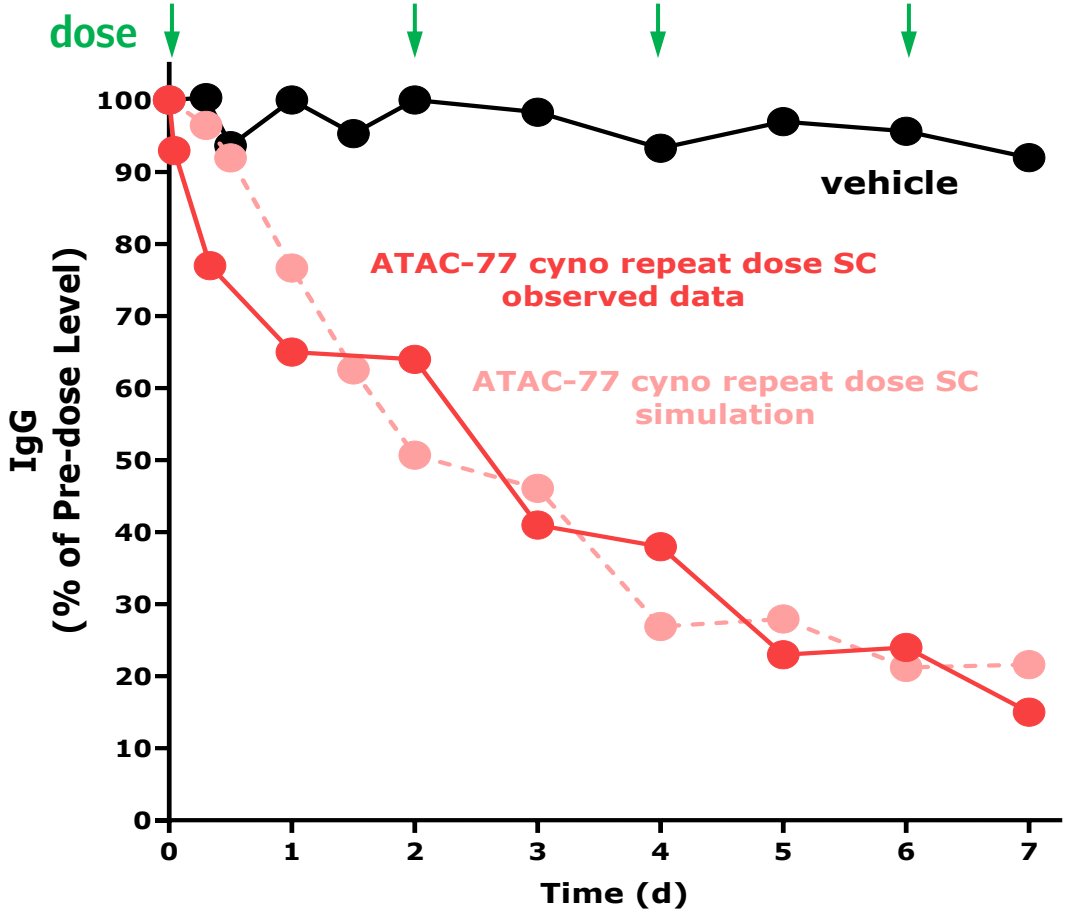


- Dose-dependent degradation observed after repeat dosing with ATAC-77
  - 7 mg/kg SC: 24% (12 μM) at 24 hours and max 62% (32 μM) at 7 days
  - 14 mg/kg SC: 35% (18 μM) at 24 hours and max 85% (43 μM) at 7 days
  - 14 mg/kg IV: 31% (15 μM) at 24 hours and max 79% (37 μM) at 7 days

Study Design: IV or SC administration of 4 doses of monodentate ATAC-77 at 7 or 14 mg/kg every 2 days. Plasma collected for 14 days for PK and PD analyses. PK: plasma ATAC concentration by mass spectrometry. PD: plasma cyno IgG concentration by ELISA. Graphs represent the mean +/-SD of n=4 NHPs per group

Proprietary Protein Degradation Modeling & PK/PD Simulations





- Proprietary modeling integrates key parameters (target protein level and half-life, ASGPR level and recycling rate, etc.) to drive PK/PD simulations

- Observed data from repeat dose study in cyno matches simulated profile based on single dose cyno study
- Similar agreement between simulated and observed for multiple ATACs (data not shown)

Summary

- Created ATAC platform to harness ASGPR pathway for uptake and endolysosomal degradation of extracellular proteins
- Platform centered around proprietary small molecule ASGPR ligands with dramatically improved affinity vs. previous ligands, including natural GalNAc
- Improved affinity of proprietary ASGPR ligands enables low MW monovalent ATAC design (vs previous trivalent approaches)
  - Lower MW enables lower dose/lower volume SC products
  - Lower MW enables oral delivery when paired with SM binder to protein
- ATACs confirmed to mimic natural ASGPR processing of endogenous proteins
  - Retain key pH- and Ca^{2+}-dependent binding properties to permit normal ASGPR-mediated endocytosis, followed by ASGPR release/recycling after ATAC internalization in early endosome
- Demonstrated proof of concept for ATAC-mediated IgG degradation
  - In vitro ternary complex formation and cellular uptake
  - In vivo degradation of endogenous and exogenous IgG in two preclinical species

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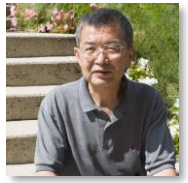

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