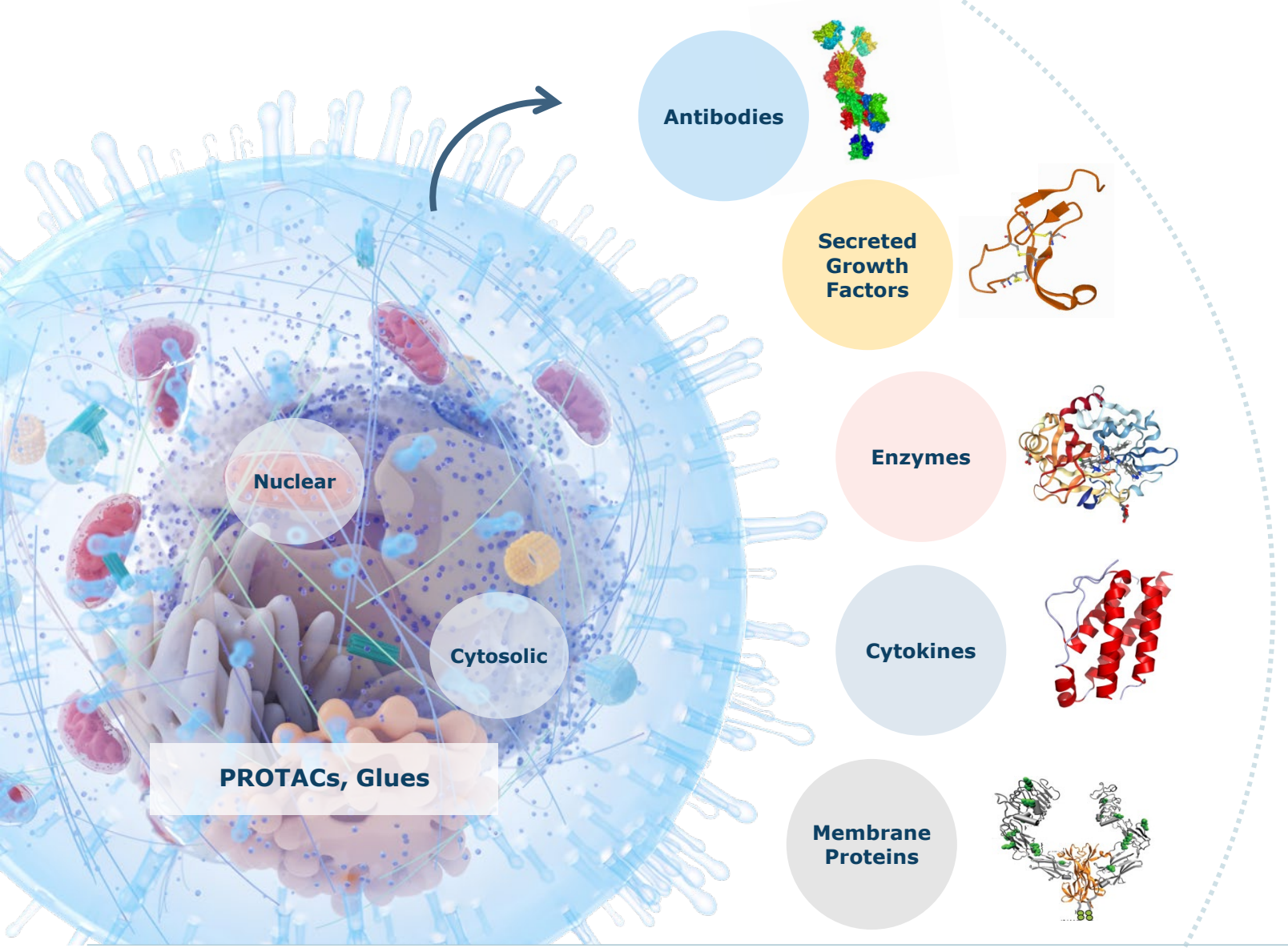
Targeted Degradation of Extracellular Proteins with ATACs (ASGPR Targeting Chimeras)

Discovery on Target Conference

Boston, MA
October 19, 2022

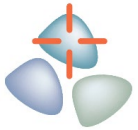
Universe of Extracellular and Membrane Proteins for Degradation



- First generation degraders target intracellular proteins
- Yet almost 40% of human proteins are extracellular (EC) 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 EC targets where ATACs have advantage

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

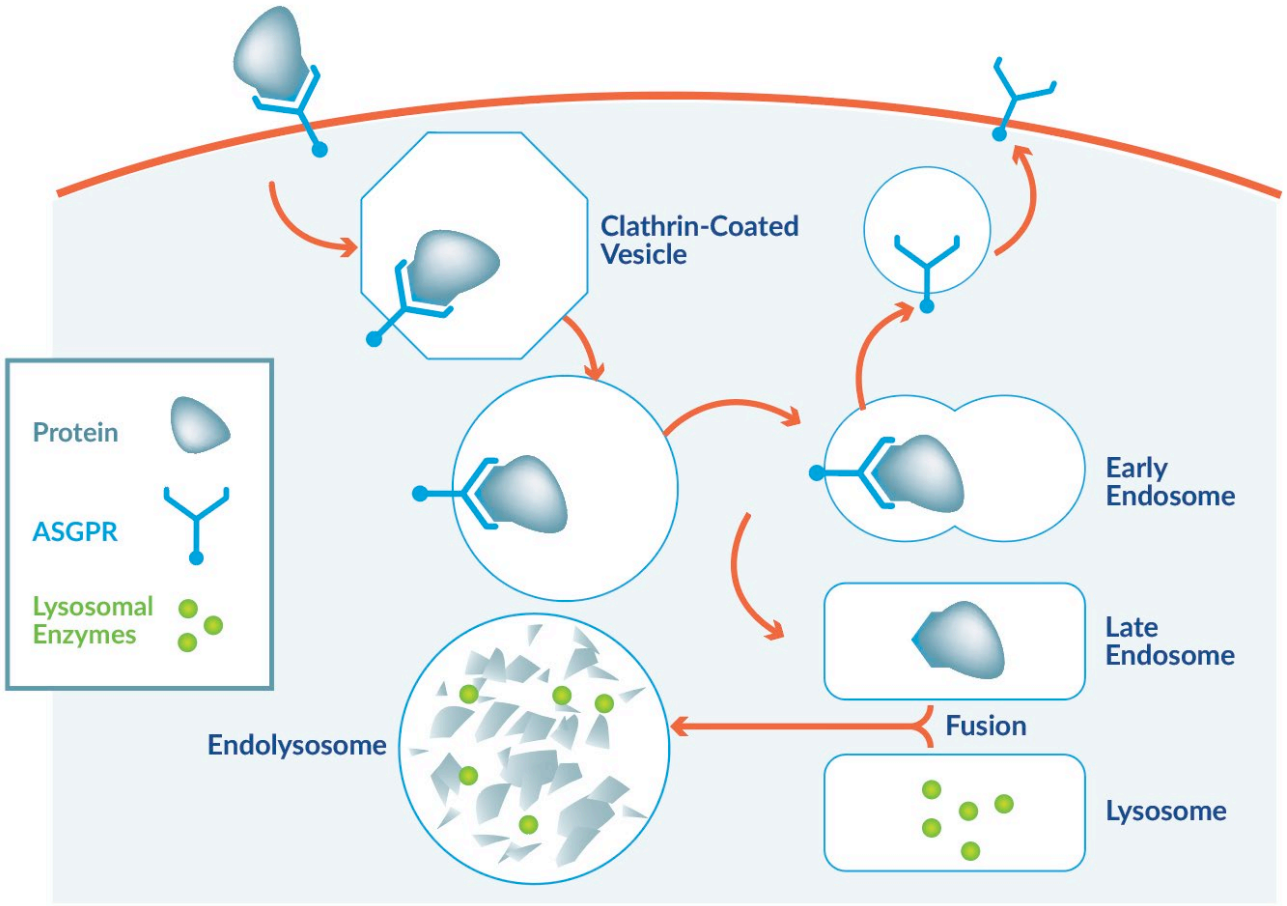
Novel Applications for ATAC Extracellular Protein Degraders

	**Drug Historically Undruggable Targets**	• Leverage ligands that bind but do not have – or need to have – functional activity to **degrade previously undruggable targets**
	**Degrade Very High Concentration Proteins**	• Degrade very high concentration proteins that would otherwise require **infeasibly or unattractively large doses of neutralizing mAb**
	**Selectively Target Relevant Proteins**	• Degrade **specific protein classes or subclasses** responsible for disease, while leaving other related proteins unaffected
	**Rapid Onset of Action**	• Rapidly degrade pathogenic protein to drive faster clinical benefit for **patients in crisis or in acute need**
	**Remove Pathogenic Complexes**	• Degrade **protein complexes** or necessary component elements of protein complexes causing diseases
	**Oral Degraders**	• Use small molecule ASGPR ligands + small molecule protein binders to create **oral ATACs** for proteins currently targeted by injectable biologics

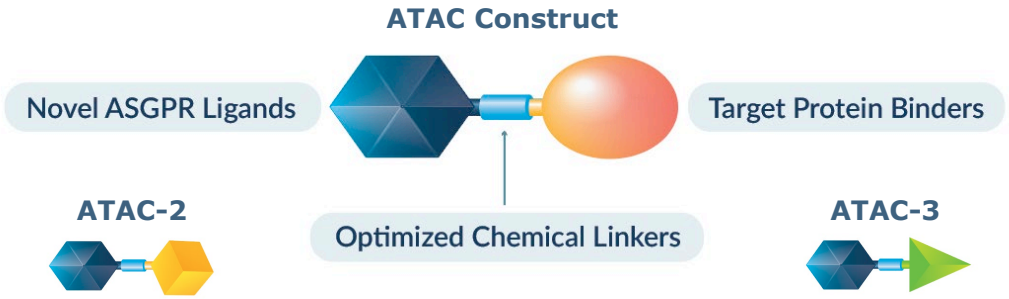
ASGPR Role in Body's Natural Cellular Degradation Machinery

- Cell surface receptor and part of natural cellular machinery for extracellular degradation (like E3 ligases in intracellular degradation)
- Mediates the endocytosis and degradation of various endogenous glycoproteins in endolysosome
- Highly expressed on hepatocytes (~1M receptors per cell in humans)
- Endocytosed and recycled from endosome back to plasma membrane every ~15 minutes

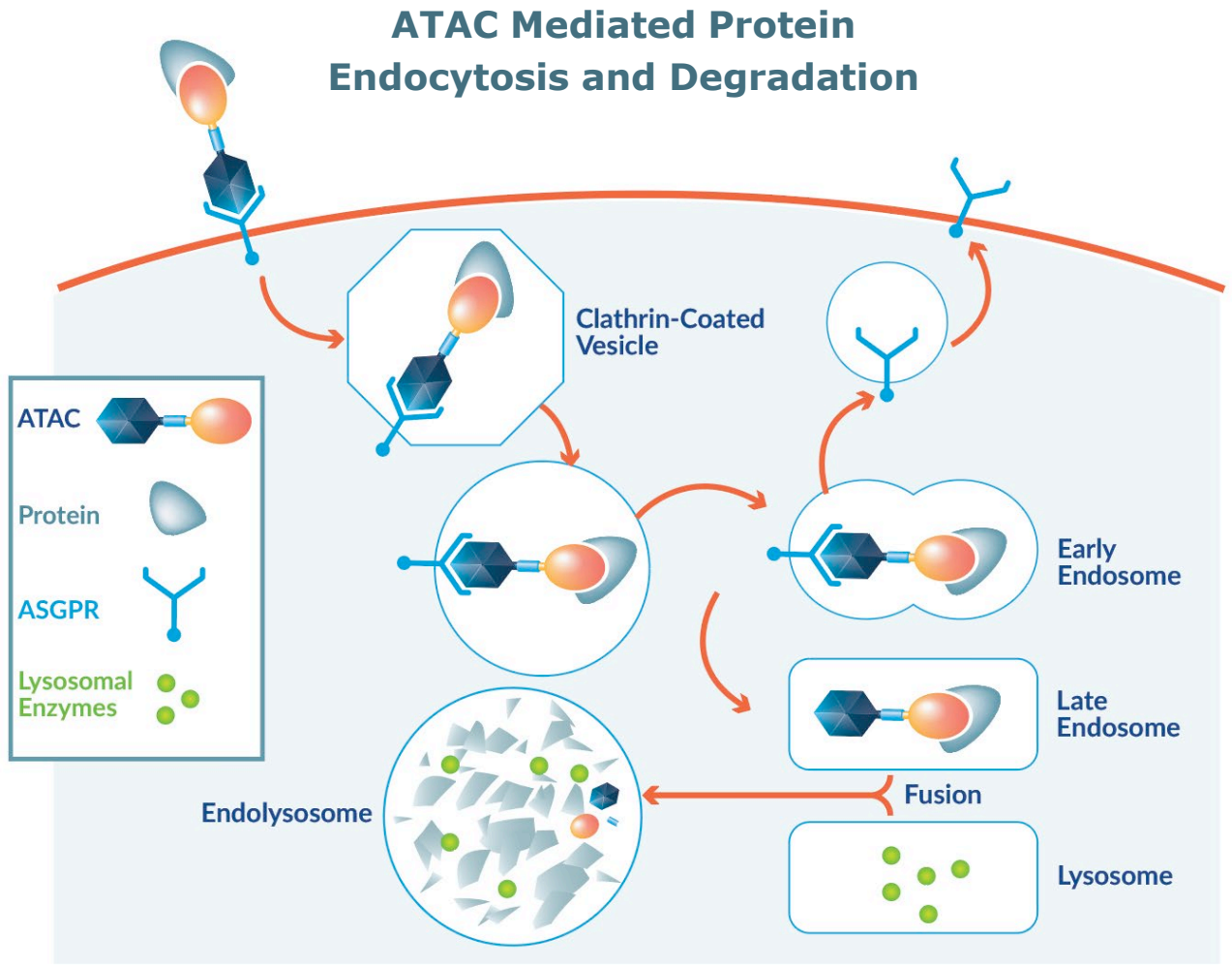
Natural Endocytosis and Degradation of Endogenous Proteins via ASGPR



ATACs Harness ASGPR Pathway to Degrade Extracellular Proteins

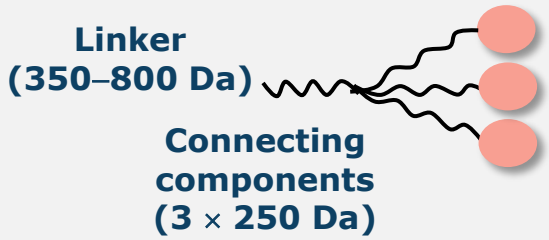


- Bi-functional molecules comprising ASGPR binder, specialized linker, and binder to a target protein
- Shuttle target protein from circulation to endolysosome for degradation
- Modular: proprietary ASGPR binders and linkers deployed in synthesis of ATACs with diverse protein targeting binders



Proprietary ASGPR Ligands with Significantly Improved Affinity

Conventional GalNAc Tridentate



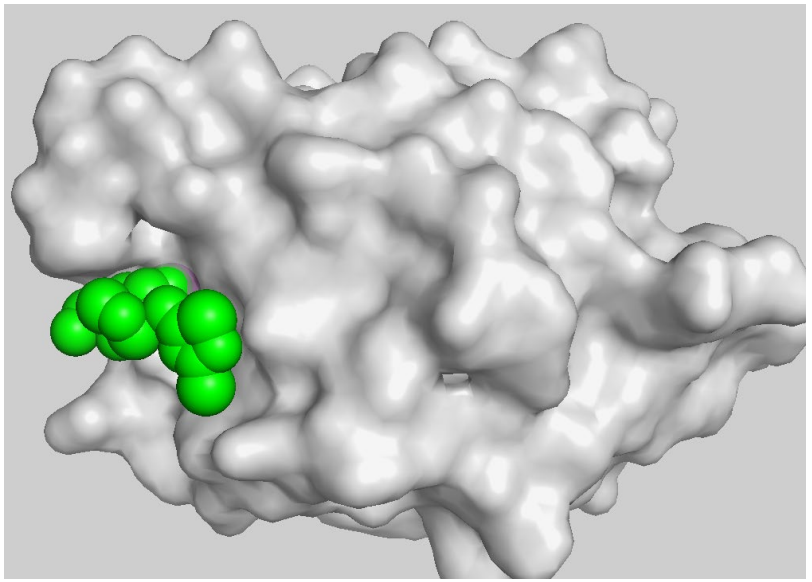
Total MW ~1,700–2,150 Da (3–4x > Avilar)

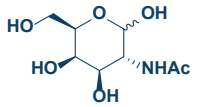
↑Affinity ↓Avidity ↓MW ↓Dose/Volume

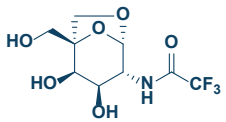
Avilar Monodentate

Total MW <550 Da

Structure-Guided ASGPR Ligand Design



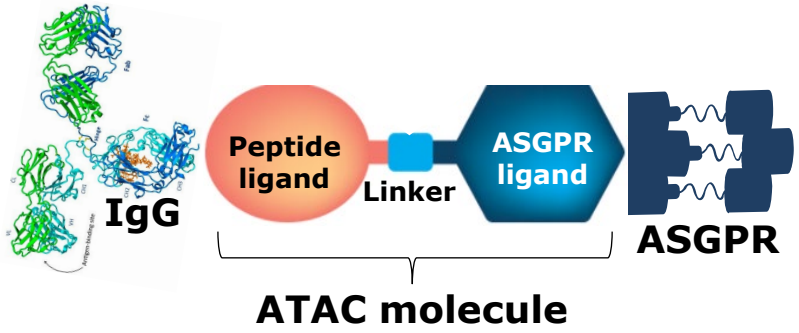




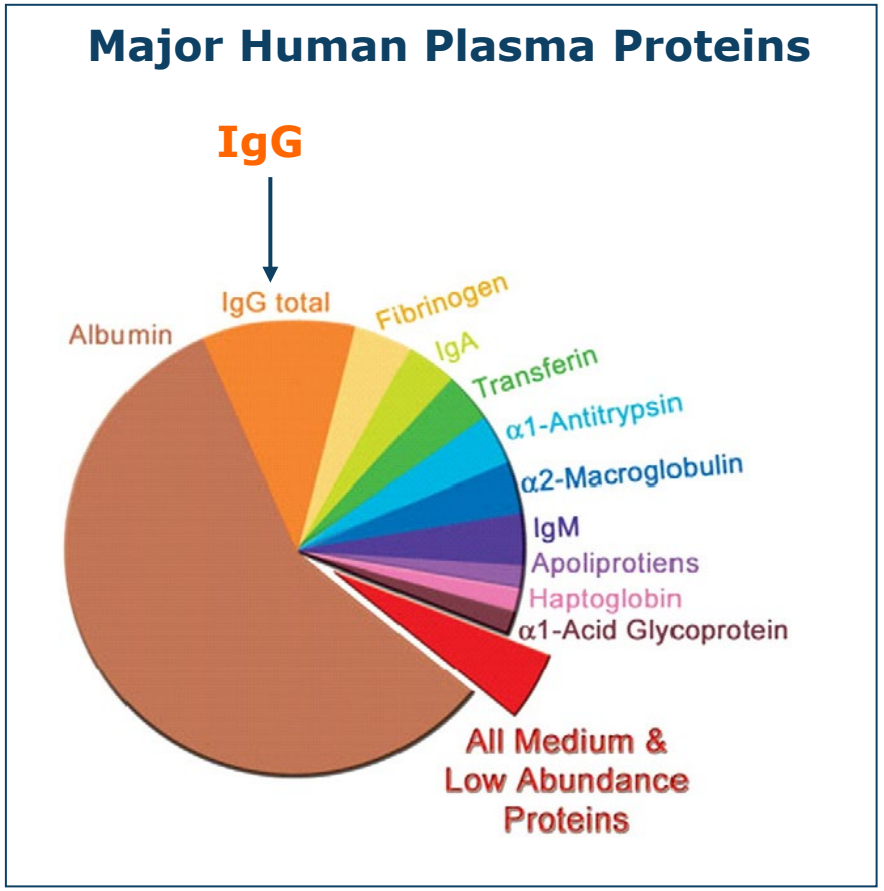
Compound ID	GalNAc	Pfizer	AVI-1	AVI-2	AVI-3
ASGPR K_D (SPR) (nM)	52,800	1,650	720	210	24
Increase in Affinity (X Fold)	1	32	73	251	2200

ATAC PoC Studies Demonstrating Degradation of IgG

- IgG is the most common antibody; 2nd most abundant plasma protein
  - High plasma concentration: 1.06 g/kg total body IgG or 74.2 g in 70 kg human
  - Long half life: 21 days in humans
  - Resynthesis rate: 32 mg/kg/day; ~3% of total IgG/day
- ATACs synthesized using a peptide ligand for IgG

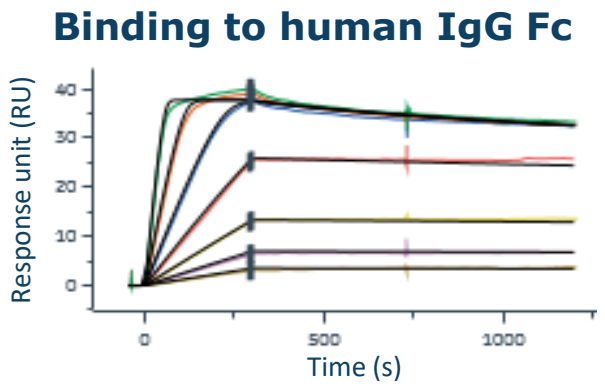


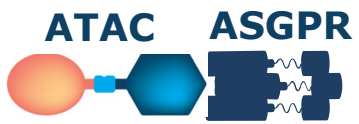
- Studies completed with ATACs targeting IgG:
  - Monodentate and bidentate ATACs, dosed IV and SQ
  - Single and repeat dose in vivo studies
  - MOA elucidation studies

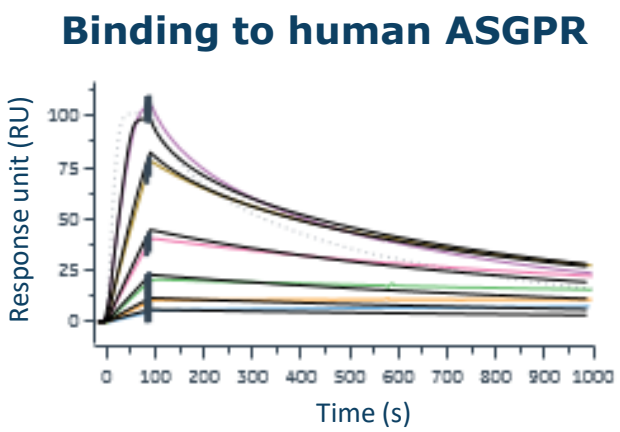


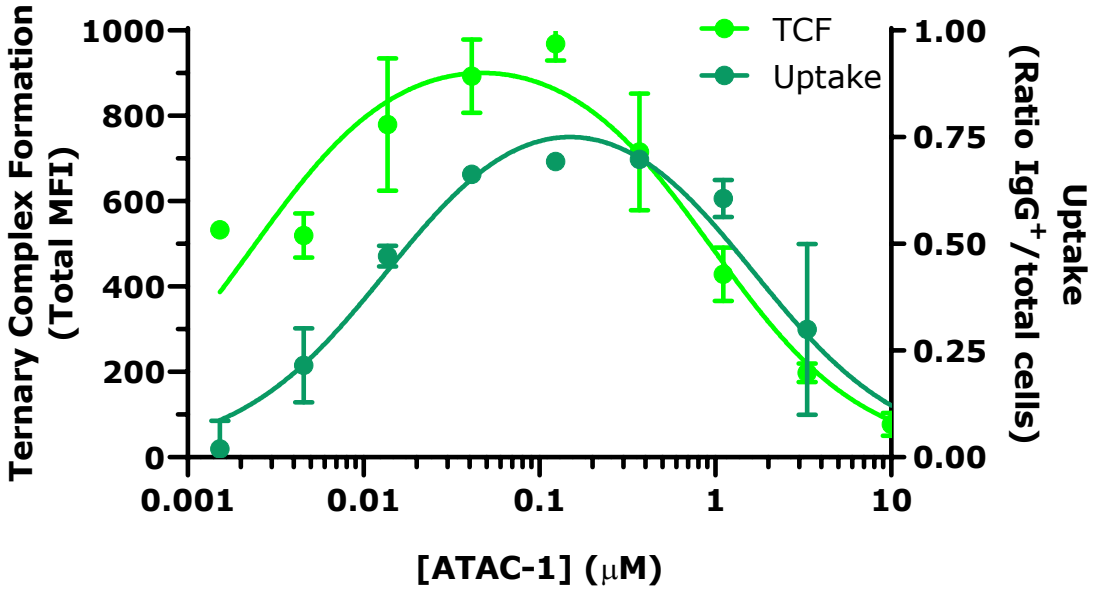
ATAC Binds In Vitro and Forms Ternary Complex

- Binary complexes: ATAC-1 binding to human IgG and ASGPR measured by SPR
- Ternary complex formation (TCF) and cellular uptake into HepG2 cells measured by flow

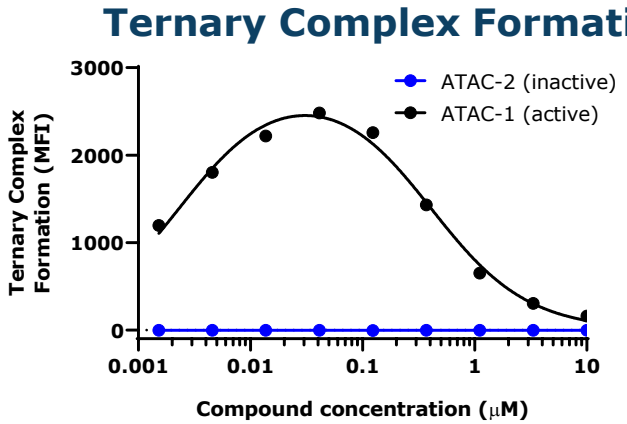


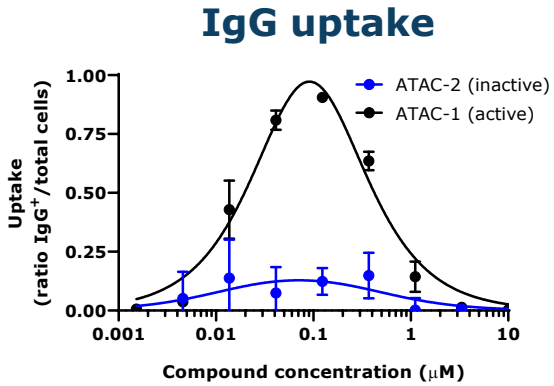




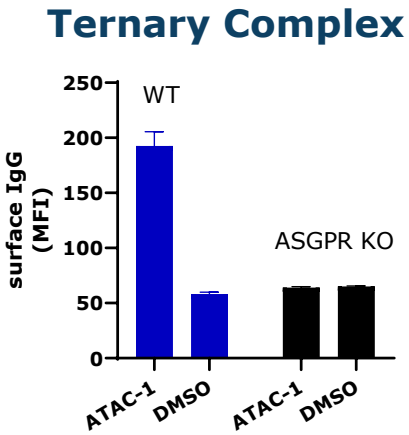


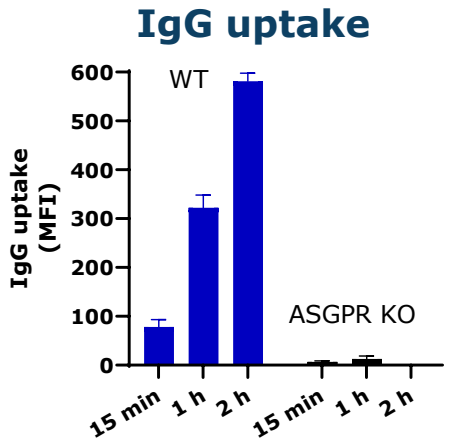
IgG-ATAC Cellular Function is Dependent on ASGPR Binding





- ASGPR-inactive control IgG-ATAC (ATAC-2) does not engage with ASGPR (absence of TCF and uptake)

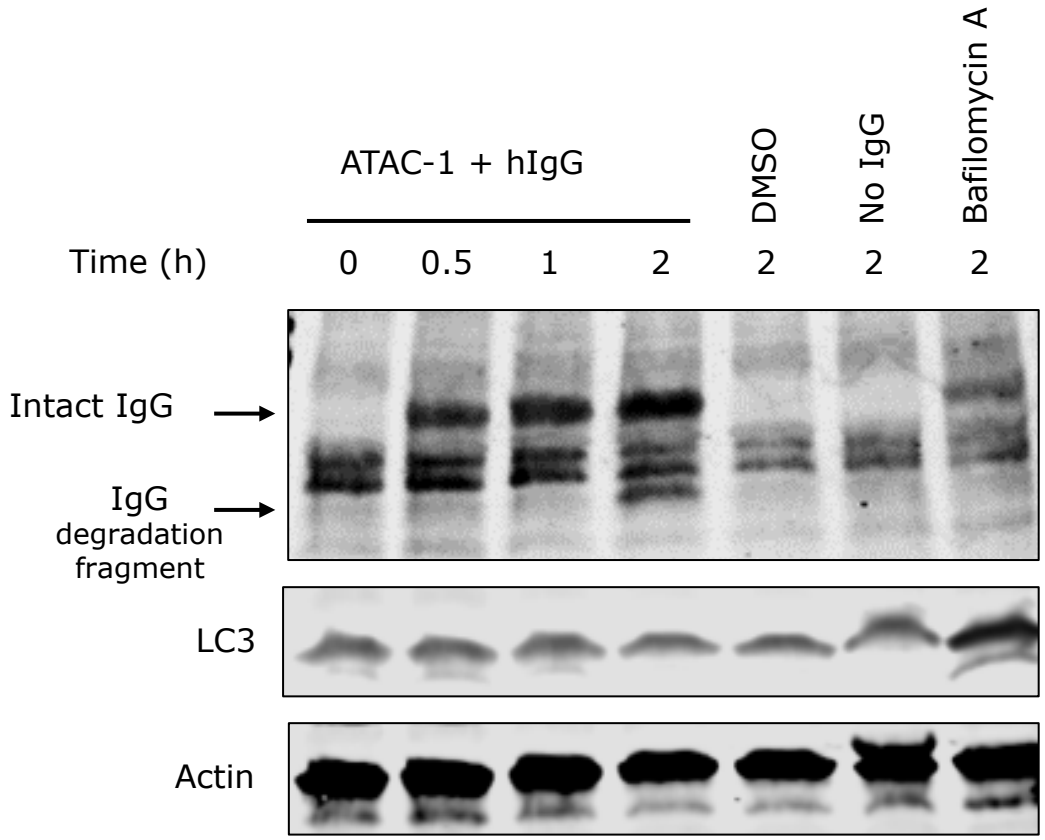




- IgG-ATAC (ATAC-1) does not engage with HepG2 cells lacking ASGPR (absence of TCF and uptake)

IgG Degradation by ATAC Requires Lysosomal Function

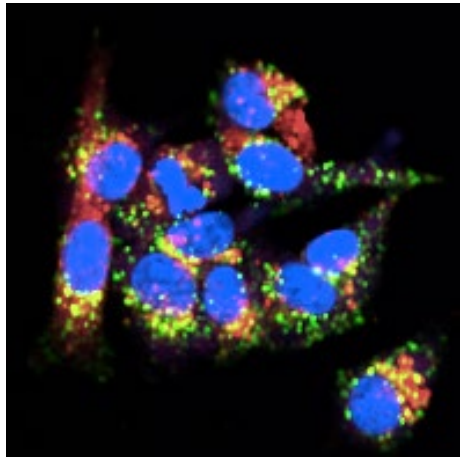
- Uptake of human IgG requires presence of IgG-ATAC
- IgG degradation after uptake is dependent on endolysosomal function
- IgG degradation kinetics consistent in HepG2 cells and primary rat hepatocytes



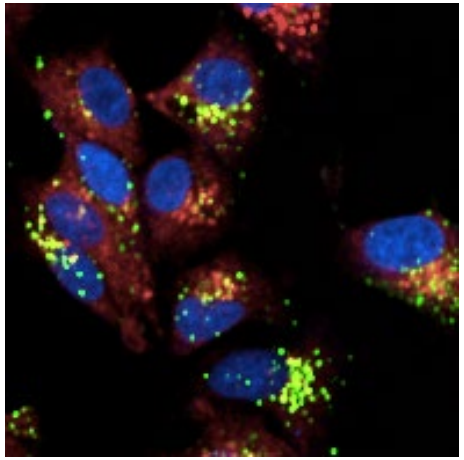
IgG Intracellular Localization Following ATAC-Mediated Uptake

IgG + ATAC-1

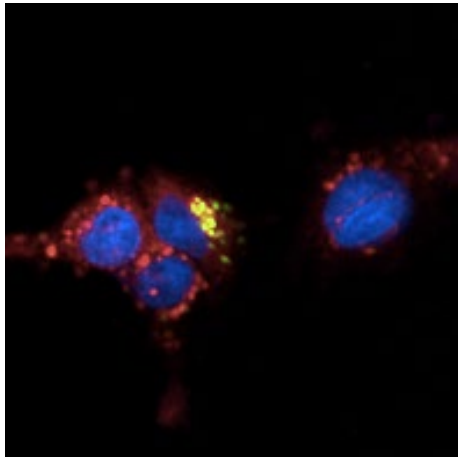
IgG + ATAC-1, T = 0 h



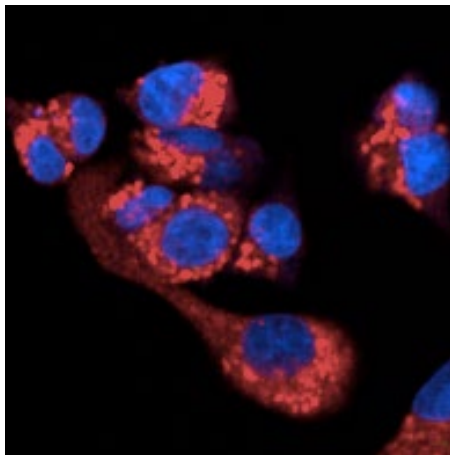
T = 1 h



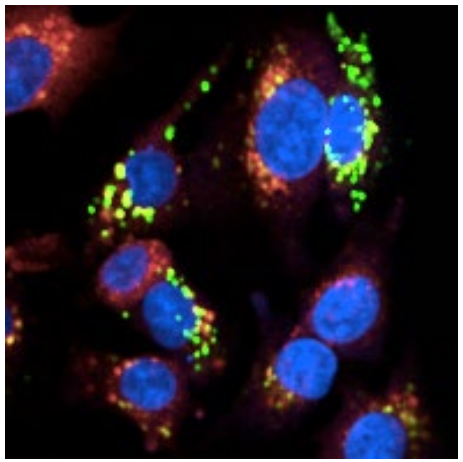
T = 2 h



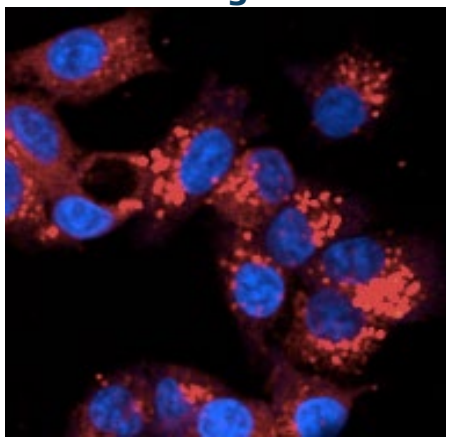
T = 4 h



BafA pre-treat, T = 4 h



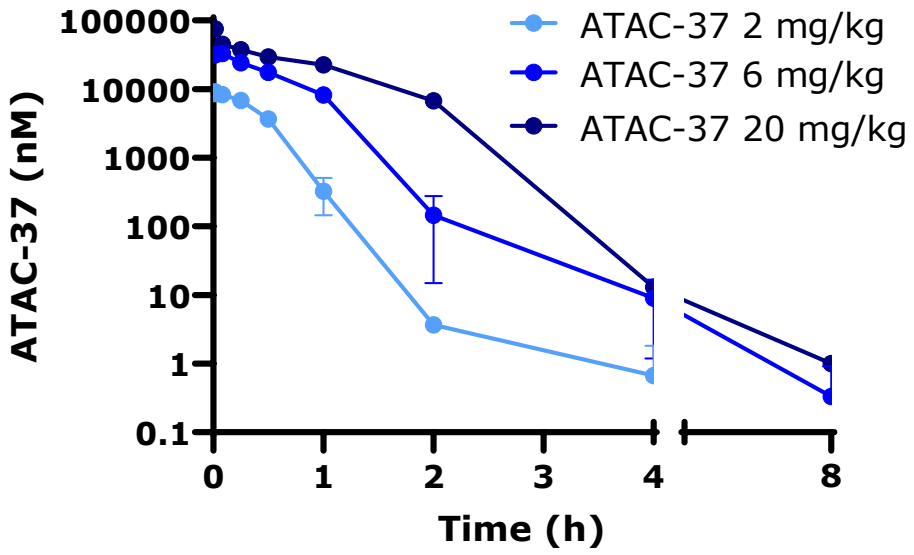
Control: IgG + DMSO



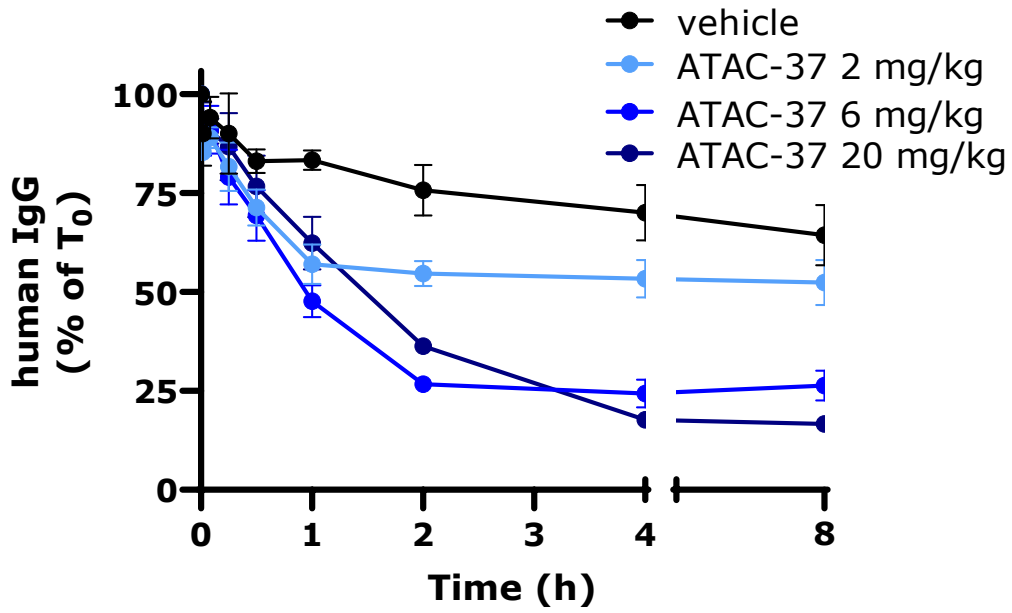
- ATAC-mediated uptake of IgG and its cellular localization are followed over time through the endocytotic pathway
- IgG is degraded, and its degradation is dependent on lysosomal function

IgG ATAC Shows Dose-Dependent Exposure and Activity in Rats

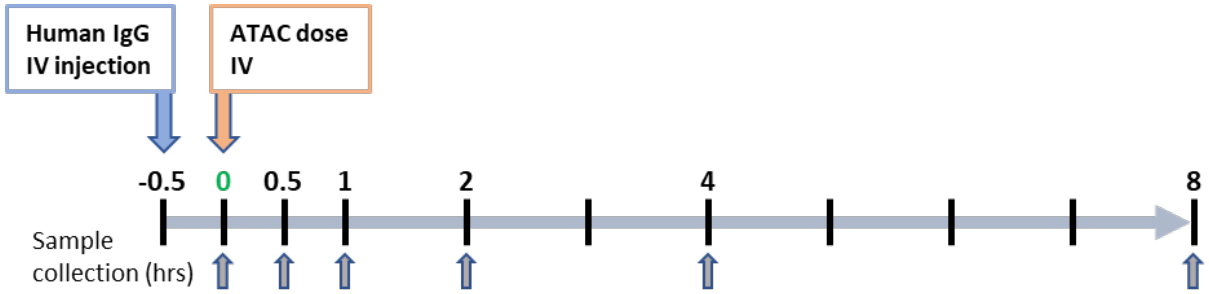
Dose-Dependent Exposure of ATAC



Dose-Dependent Degradation of Human IgG

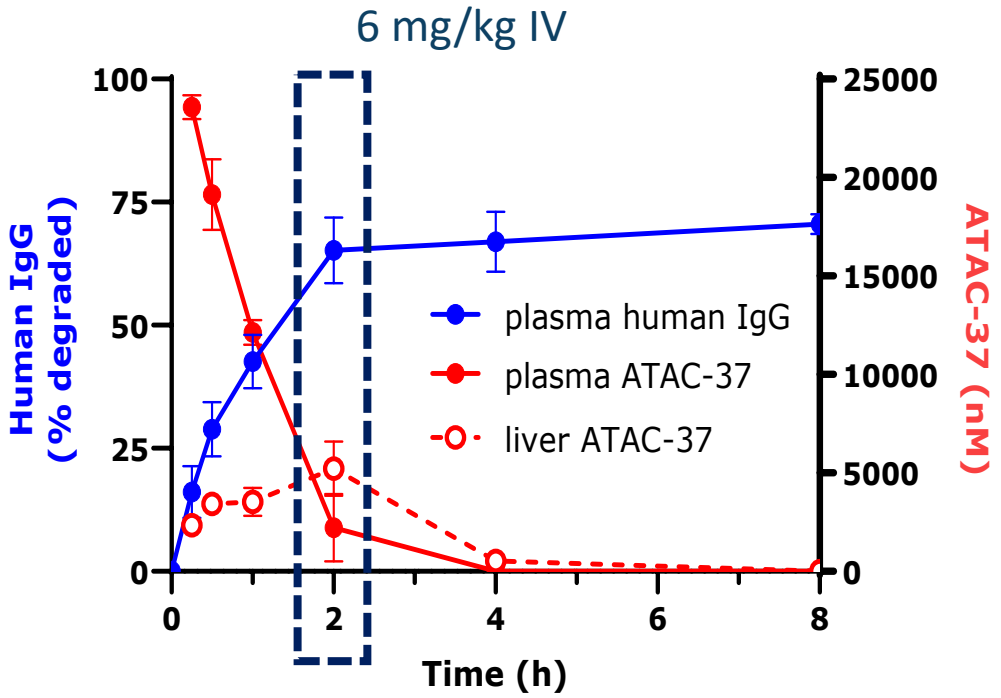


Experimental Protocol



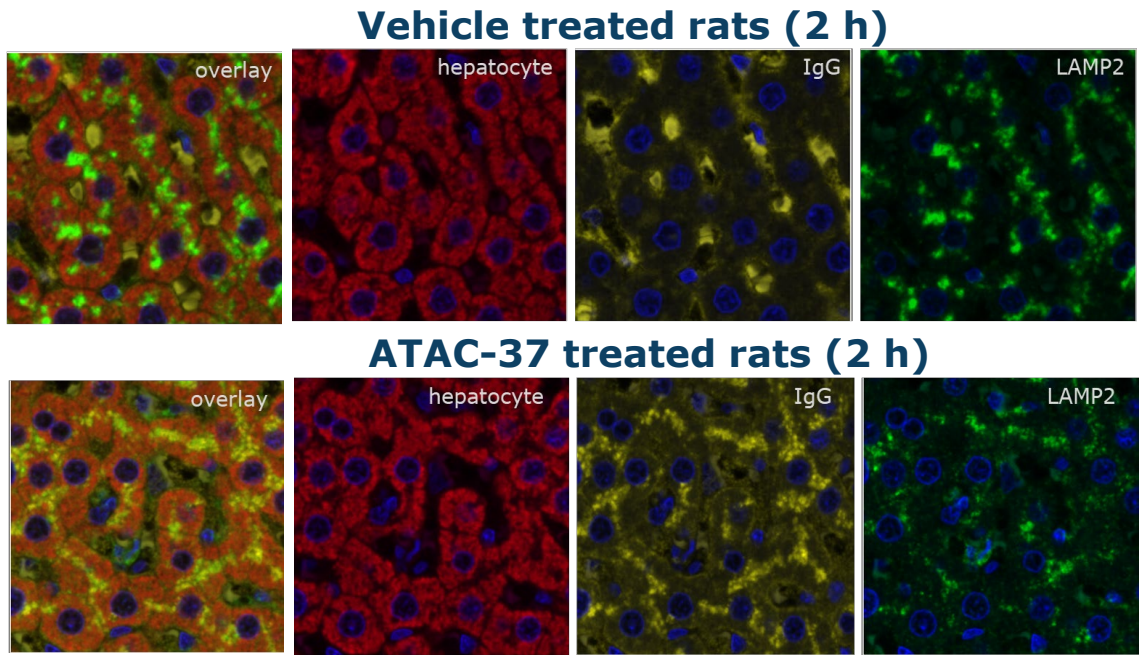
ATAC Mediates IgG Trafficking and Degradation In Vivo

ATAC PK and human IgG PD in Rat



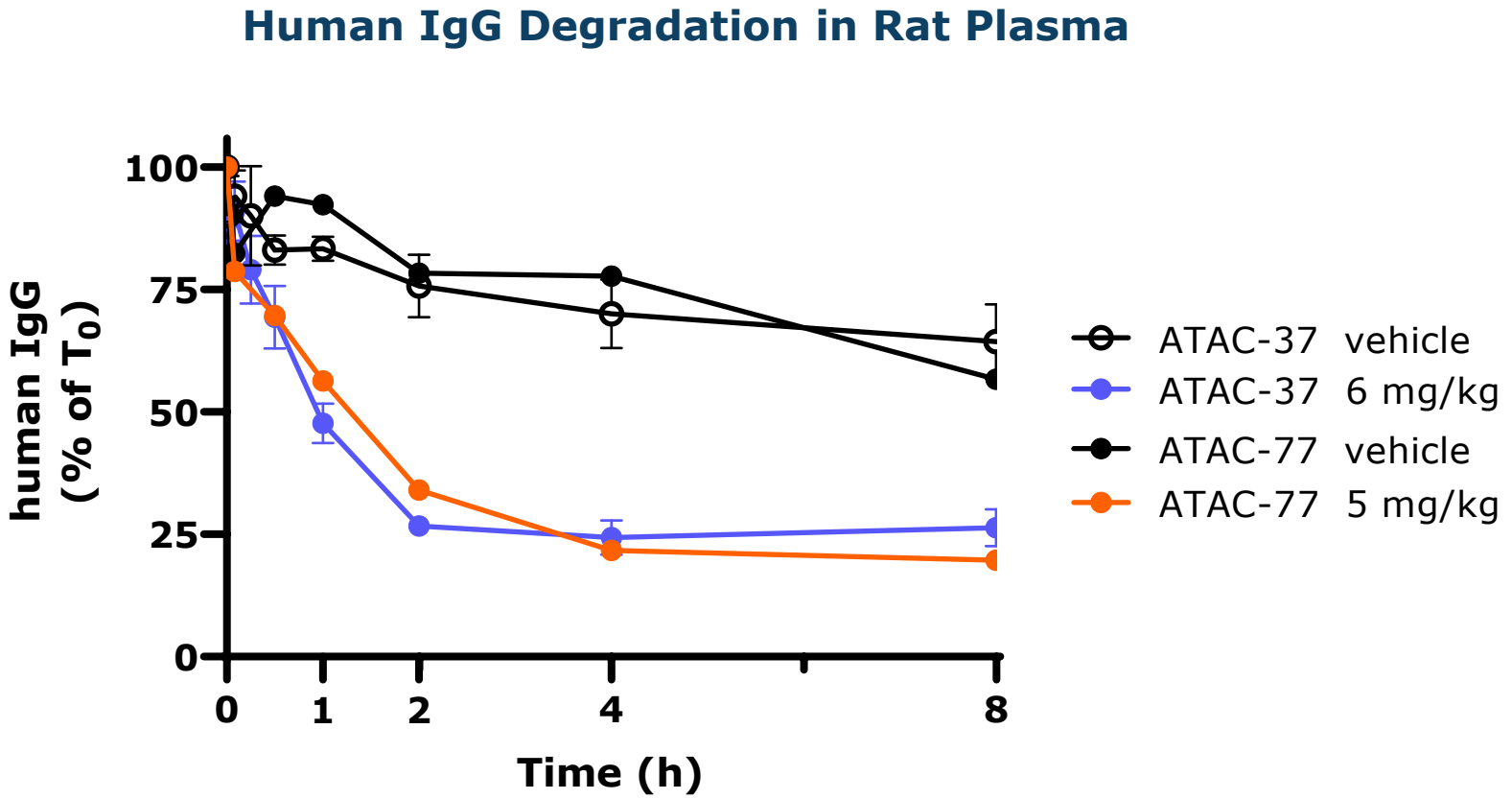
- Peak ATAC concentration in liver at 2 h coincides with high human IgG degradation in rat plasma

ATAC-induced IgG Localization and Degradation in Rat Liver

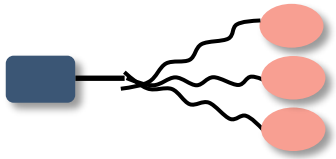


- In vehicle treated rats, human IgG resides in the sinusoidal space and non-hepatocyte liver cells
- In ATAC-treated rats at 2 h, human IgG co-localizes with endolysosomal marker LAMP2 in hepatocytes

Monodentate ATAC is Highly Efficacious to Degrade Protein in Rat



Previous Protein Degraders Display Three GalNAc Monosaccharide ASGPR Motifs



Avilar Bidentate ATAC-37

Avilar Monodentate ATAC-77

Avilar Therapeutics – Summary

- Created a unique proprietary platform for creating and developing ATACs as novel extracellular protein degraders leveraging ASGPR
- Demonstrated in vitro ligand binding, ternary complex formation, cellular uptake, and degradation of IgG with ATACs, establishing platform PoC
- Showed ATACs targeting IgG have dose-dependent exposure and degradation activity in vivo
- Confirmed ATAC-mediated IgG trafficking to hepatocyte endolysosome and degradation in vivo
- Created monodentate ATACs that are highly efficacious to degrade protein in vivo

Expert Team of Biopharma Executives and R&D Leaders

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