ASGPR-Targeting Chimeras (ATACs): In Vitro and In Vivo Demonstration of the Degradation of Extracellular Proteins

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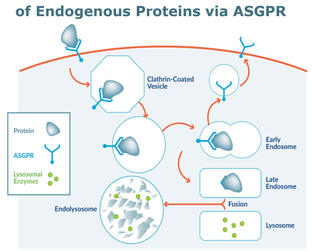
Abstract

A new targeted protein degradation approach has emerged that enables the endolysosomal degradation of extracellular proteins via the asialoglycoprotein receptor (ASGPR), an endocytic receptor expressed predominantly on the surface of hepatocytes. Here we describe the development of a new ASGPR targeting chimera (ATAC) platform using bifunctional molecules containing Avilar's novel, potent, small-molecule ASGPR-binding ligands. For proof-of-concept studies, ATACs were designed to target the extracellular protein IgG, a high plasma concentration and long half-life protein. In vitro characterization of the ATAC interactions with ASGPR and IgG revealed potent biochemical binding, ASGPR-mediated cellular uptake, and degradation of IgG via the endolysosomal pathway. A heterologous rat PK/PD model was used to test ATAC-mediated depletion of human IgG. Human IgG injected IV in rats was depleted from plasma within 4 hours after ATAC dose. Rat liver PK and histology revealed ATAC-dependent uptake of IgG into hepatocytes, subsequent trafficking of IgG to the endolysosome and degradation of IgG in vivo. ATAC-dependent depletion of endogenous IgG was also achieved in cynomolgus monkey, reaching 35% after a single dose and 85% after repeat dosing.

ASGPR Role in Body's Natural Cellular Degradation Machinery

- Cell surface receptor and part of natural cellular machinery for extracellular degradation[1,2] (like E3 ligases in intracellular degradation)
- Mediates the endocytosis and degradation of various endogenous glycoproteins in endolysosome[1,2]
- Highly expressed on hepatocytes (~1M receptors per cell in humans)[1,2]
- Endocytosed and recycled from endosome back to plasma membrane every ~15 minutes[1,2]

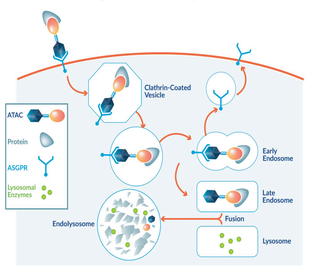
Natural Endocytosis and Degradation of Endogenous Proteins via ASGPR



ATACs Harness ASGPR Pathway to Degrade Extracellular Proteins

ATAC Construct

ATAC Mediated Protein Endocytosis and Degradation



- Bi-functional molecules comprising ASGPR binder, optimized 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

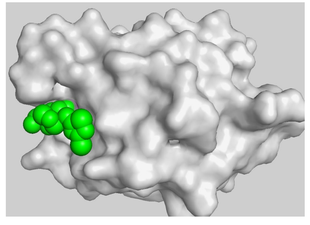
Linker (350–800 Da) — GalNAc ASGPR binder (3 × 200 Da)
Connecting components (3 × 250 Da)
Total MW ~1,700–2,150 Da (3–4x > Avilar)

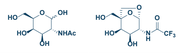
↑Affinity ↓Avidity ↓MW ↓Dose/Volume

Avilar Monodentate

Linker (~140–250 Da) — ASGPR binder[4] (~300 Da)
Total MW <550 Da

Structure-Guided ASGPR Ligand Design

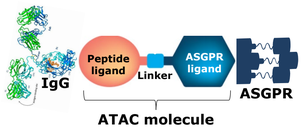




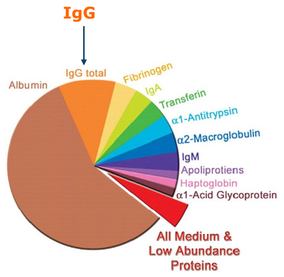
Compound ID	GalNAc	Pfizer[3]	AVI-1	AVI-2	AVI-3
ASGPR K_D (SPR) (nM)	52,800	1,650	720	210	24
Increase in Affinity (X Fold)	1	32	73	251	2200

ATAC PoC Studies Demonstrating Degradation of IgG

- Immunoglobulin G (IgG) is the most common antibody; 2nd most abundant plasma protein
  - High plasma concentration[5]: 1.06 g/kg total body IgG or 74.2 g in 70 kg human
  - Long half life: 21 days in humans[5], 5 days in cyno
  - Resynthesis rate[5]: 32 mg/kg/day; ~3% of total IgG/day
- ATACs synthesized using a peptide ligand that binds both human and cynomolgus monkey IgG



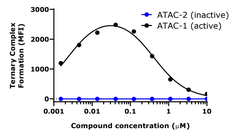
Major Human Plasma Proteins[6]



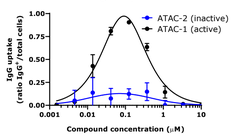
IgG-ATAC Function is Dependent on ASGPR Binding

ASGPR-inactive control IgG-ATAC does not engage with ASGPR

Ternary Complex Formation

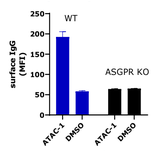


IgG uptake



IgG-ATAC does not engage with HepG2 cells lacking ASGPR

Ternary Complex Formation



IgG uptake

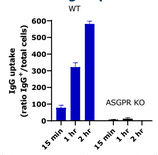


Figure legend. Ternary Complex Formation assays: WT HepG2 cells (top) or WT HepG2 and ASGPR-KO cells (bottom) were incubated with fluorescently labeled human IgG and ATAC-1, ATAC-2 or DMSO on ice for 1 h. Cells were washed and surface IgG was measured by flow cytometry (MFI). **Uptake assays**: WT HepG2 cells (top) or WT HepG2 and ASGPR-KO cells (bottom) were incubated with fluorescently labeled human IgG and ATAC-1, ATAC-2 or DMSO at 37°C for 6 h (top) or 15 min to 2 h (bottom). Cells were washed and surface+internal IgG was measured by fluorescence microscopy (ratio of IgG+ cells/total cells).

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

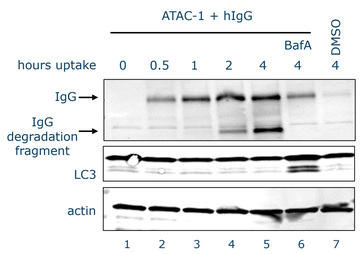
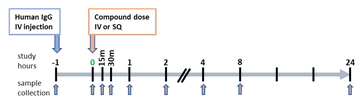
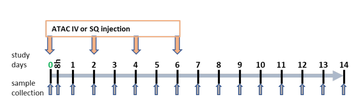


Fig legend: ATAC-1 and hIgG are incubated with HepG2 cells for 1 h +/- bafilomycin A. Cells are washed to remove excess IgG and further incubated for 0-4 h. Cells are then lysed, proteins are separated by SDS-PAGE and detected by Western blot with anti-IgG, anti-LC3 or anti-actin antibodies. Data is representative of 3 separate experiments in HepG2 cells.

Models to Evaluate In Vivo ATAC-Mediated Plasma IgG Depletion





Rat Model

IgG-ATACs do not bind rat IgG, enabling control of exogenous target level

Study design
- Human IgG, introduced IV (~ 15 μM)
- IgG-ATAC, dosed once IV or SQ

Data acquired
- PK: plasma [ATAC] & liver [ATAC] by mass spectroscopy
- PD: plasma [hIgG] by ELISA
  liver [hIgG] and localization by IHC

Cynomolgus Monkey Model

IgG-ATACs bind monkey IgG, enabling degradation of endogenous IgG

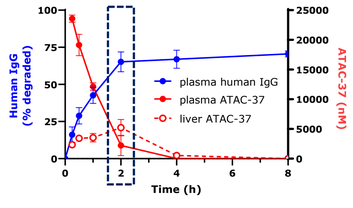
Study design
- Cyno IgG, native levels (~ 35-60 μM)
- IgG-ATAC, dosed once or 4 times IV or SQ

Data acquired
- PK: plasma [ATAC] by mass spectroscopy
- PD: plasma [cyno IgG] by ELISA

ATAC-37 Mediates IgG Trafficking and Degradation In Vivo

ATAC-37 PK and human IgG PD in Rat

- ATAC-37 dosed 6 mg/kg IV
- 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

- Vehicle treated rats: human IgG resides in the sinusoidal space and non-hepatocyte liver cells
- ATAC-treated rats: human IgG co-localizes with endolysosomal marker LAMP2 in hepatocytes

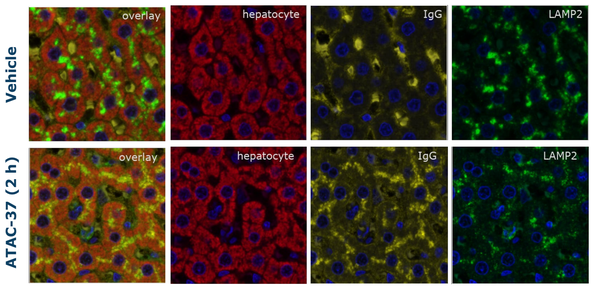


Figure legend. Rat liver ATAC-37 PK and hIgG PD. **Left:** Plot showing ATAC-37 concentration (right axis, nM) in rat plasma (solid red circles) or rat liver homogenate (open red circles) and human IgG amount in plasma (solid blue circle; left axis, % of total degraded) over time. **Right.** Rat liver sections from vehicle and 6 mg/kg ATAC-37 treated groups (at 2hrs post dose) were stained with Dapi (nuclei, blue) and with antibodies to hepatocyte plasma membrane marker (red; identifies hepatocytes only), human IgG (yellow) and LAMP2 (green; marker of endolysosomal/lysosomal compartments).

References

1. Spiess, Biochemistry (1990)
2. Stockert, J. Physiol. Rev. (1995)
3. Spiros et. al., U.S. Patent 9,340,553 (2016)
4. Saulnier et. al., WO2021155317 (2021)
5. Waldmann et.al., Prog. Allergy (1969)
6. Kratz et. al, J. Control Release (2012)

Acknowledgements

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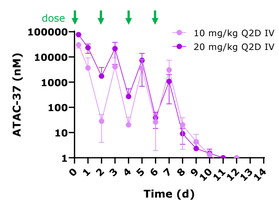
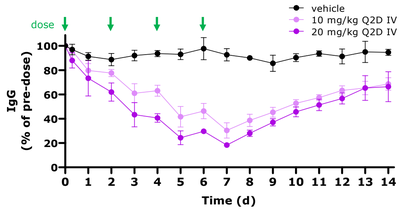
Repeat Dose IV of Bidentate ATAC-37 Degrades > 80% IgG in NHP

ATAC-37 Plasma PK



Cyno % IgG Degradation

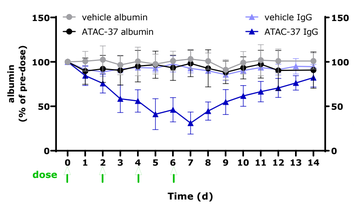


- Q2D dosing to accommodate short 5 day cyno IgG half-life
- Dose-dependent ATAC-37 exposure and IgG degradation after repeat dose
- 20 mg/kg: 27% (10 μM) at 24 h and max 82% (32 μM) at 7 d

Figure legend. PK and PD profiles of ATAC-37 in cyno PK/PD model. Vehicle or ATAC-37 was dosed at 10 or 20 mg/kg IV every 2 days for 4 cycles in cynomolgus monkeys. Blood samples were taken at various time points and plasma was processed for PK and PD measurements. **Left.** ATAC-37 concentration (nM) by LC/MS/MS. **Right.** Cyno IgG levels by ELISA expressed as a percent of the predose time point. Green arrows depict dosing days.

Repeat Dosing of ATAC-37 Does Not Affect Albumin, LDL levels

ATAC-37 does not decrease albumin levels while decreasing IgG levels by 70%



ATAC-37 does not increase LDL levels

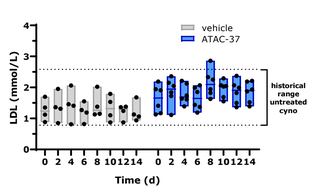
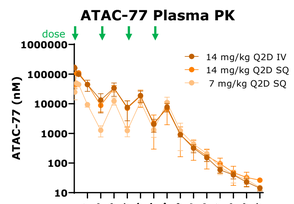


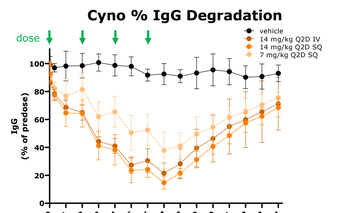
Figure Legend. ATAC-37 was dosed IV at 20 mg/kg 4 times Q2D in cynos. **Left panel:** IgG and albumin levels measured by ELISA. Graph represents the mean +/-SD of n=4 vehicle or n=6 treated cynos per group. **Right panel:** LDL measured in cyno plasma using clinical chemistry panel. Boxplot shows min to max values with lines at the median and all individual data points for n=4 vehicle and n=6 ATAC-37 treated cynos. Dotted lines show historical normal range of LDL levels in untreated cyno.

Repeat Dose IV/SQ of Monodentate ATAC-77 Degrades IgG in NHP

ATAC-77 Plasma PK



Cyno % IgG Degradation



- Small monodentate ATAC-77 (low mw) dosed SQ has high bioavailability
- Dose-dependent plasma exposure and cyno IgG depletion
- Repeat SQ dosing of ATAC-77 enables 85% reduction of cyno IgG by day 7 (43 μM cyno IgG)

Figure legend. PK and PD profiles of ATAC-77 in cyno PK/PD model. Vehicle or ATAC-77 was dosed at 7 or 14 mg/kg SQ or 14 mg/kg IV every 2 days for 4 cycles in cynomolgus monkeys. Blood samples were taken at various time points and plasma was processed for PK and PD measurements. **Left.** ATAC-77 concentration (nM) by LC/MS/MS. **Right.** Cyno IgG levels by ELISA expressed as a percent of the predose time point. Green arrows depict dosing days.

Monodentate & Bidentate ATACs Efficient at IgG Degradation in Cyno

- Equimolar dose comparison after repeat dosing in cyno
- Monodentate ATAC-77 (14 mg/kg dose) and bidentate ATAC-37 (20 mg/kg dose) IgG-ATACs are both highly efficacious in vivo

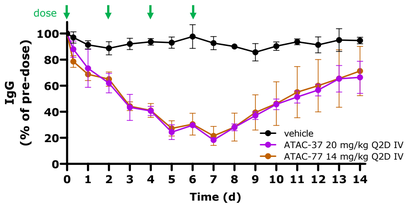
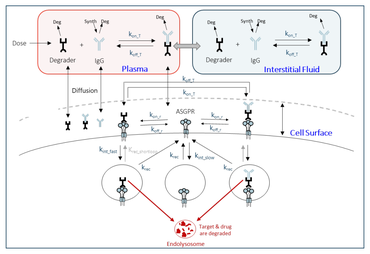
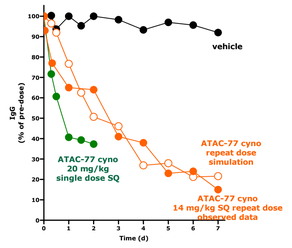


Figure legend. PD profiles of monodentate ATAC-77 and bidentate ATAC-37 from repeat dose studies in above panels overlayed on the same graph. Cyno IgG levels expressed as a percent of the predose time point. Green arrows depict dosing days.

Single Dose Modeling Simulation Predicts Repeat Dose NHP Data



- Multiple parameters (target protein level, half-life, ASGPR level, ASGPR recycling rate, etc.) are used to model IgG degradation across model systems



- Data from repeat dose study in cynos matches the simulated profile

Summary

- Avilar has created a unique proprietary platform for developing ATACs as novel extracellular protein degraders
- Demonstrated in vitro ASGPR-dependent ternary complex formation and cellular uptake, and endolysosomal-dependent degradation of cargo with ATACs
- Confirmed ATAC-mediated human IgG trafficking to hepatocyte endolysosome and degradation in rats
- Showed ATACs targeting IgG have dose-dependent exposure and degradation activity in NHP
- Achieved 85% IgG degradation efficacy using monodentate ATAC-77 in NHP
- Avilar's proprietary extracellular degradation modeling simulation technology, customized for ASGPR-mediated uptake and degradation, predicted the dose, dosing regimen, and PK/PD profile of ATACs in vivo