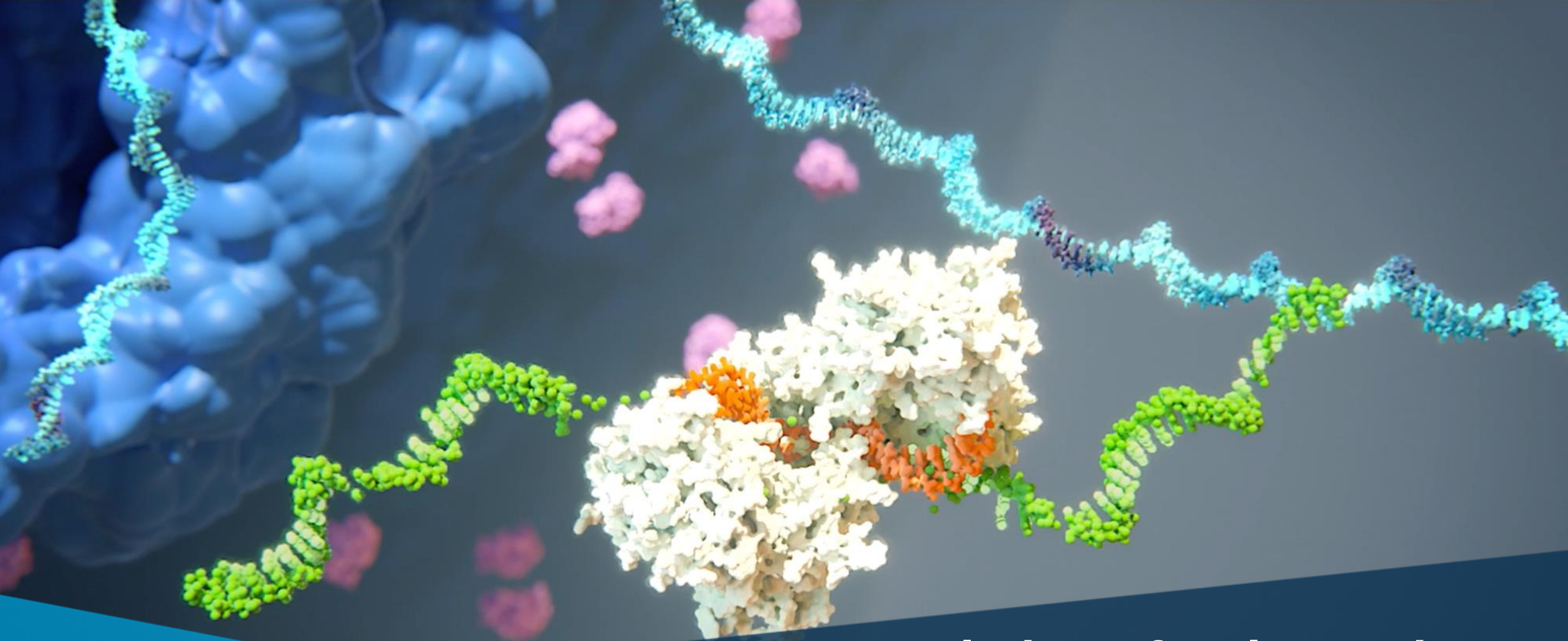


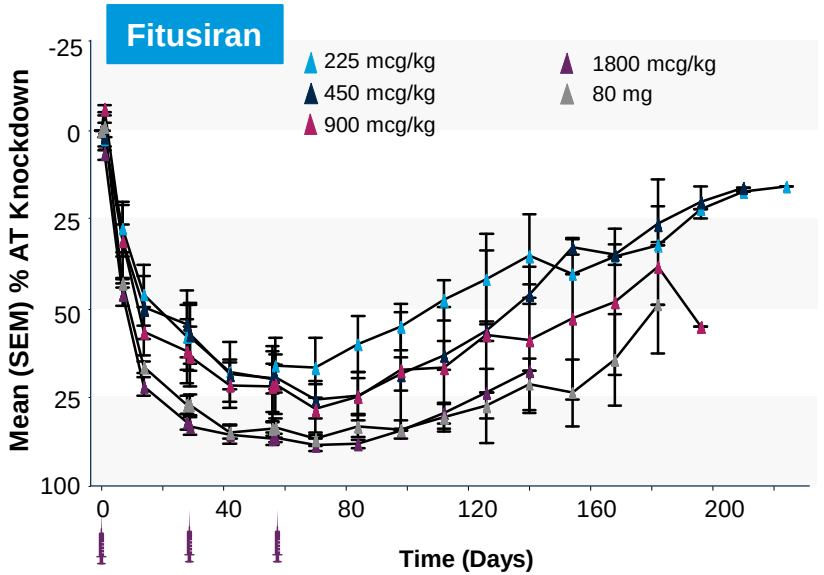
# Human Translation of GalNAc-siRNA Conjugates with Improved Specificity

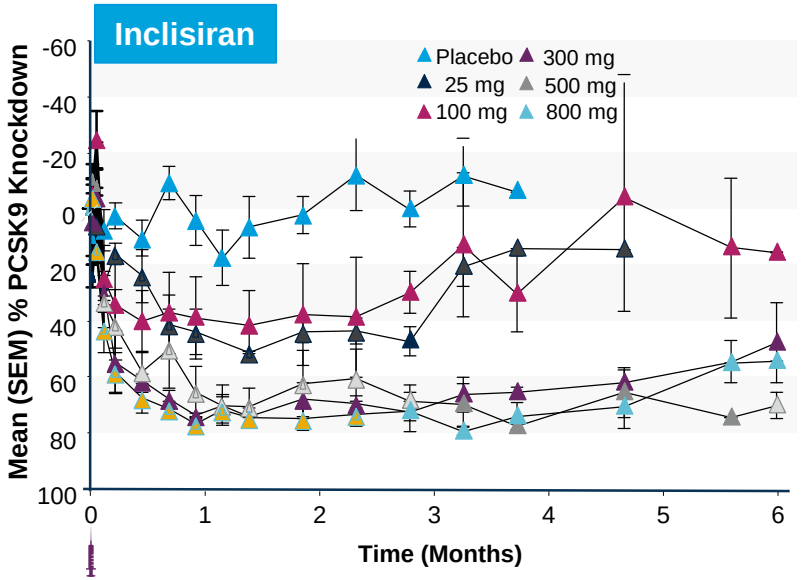
**Vasant Jadhav, PhD**
**Vice President, Research, Alnylam**

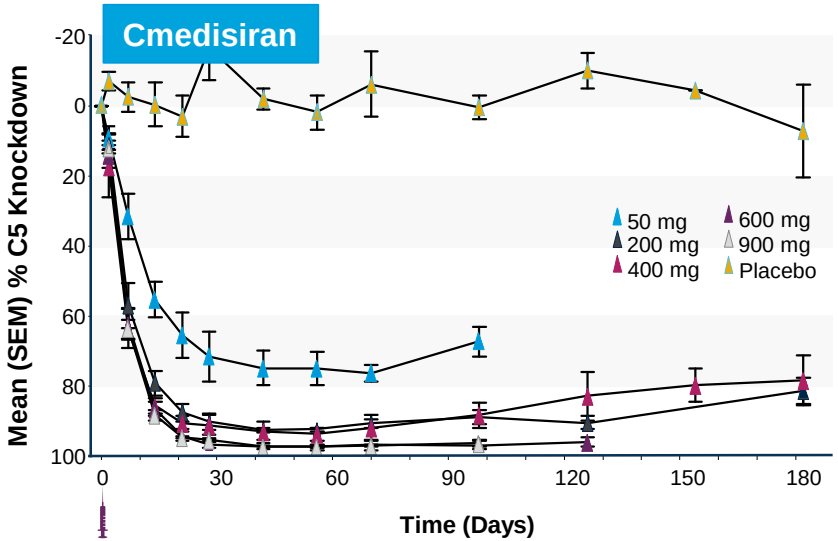
**OTS, September 26-29, 2020**

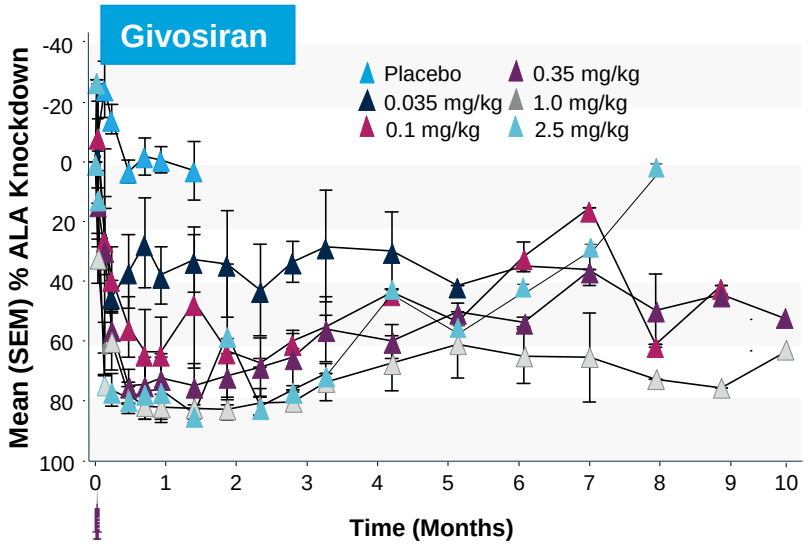
Alnylam® PHARMACEUTICALS

# Multiple Human POCs Demonstrate Reproducible and Modular Nature of ESC Conjugate Platform




1. Fitusiran
2. Inclisiran
3. Givosiran
4. Lumasiran
5. Vutrisiran
6. Cemdisiran
7. ALN-AAT
8. ALN-AAT02
9. ALN-AGT
10. ALN-HBV02 (VIR-2218)

# Inclisiran: Investigational RNAi Therapeutics Targeting PCSK9

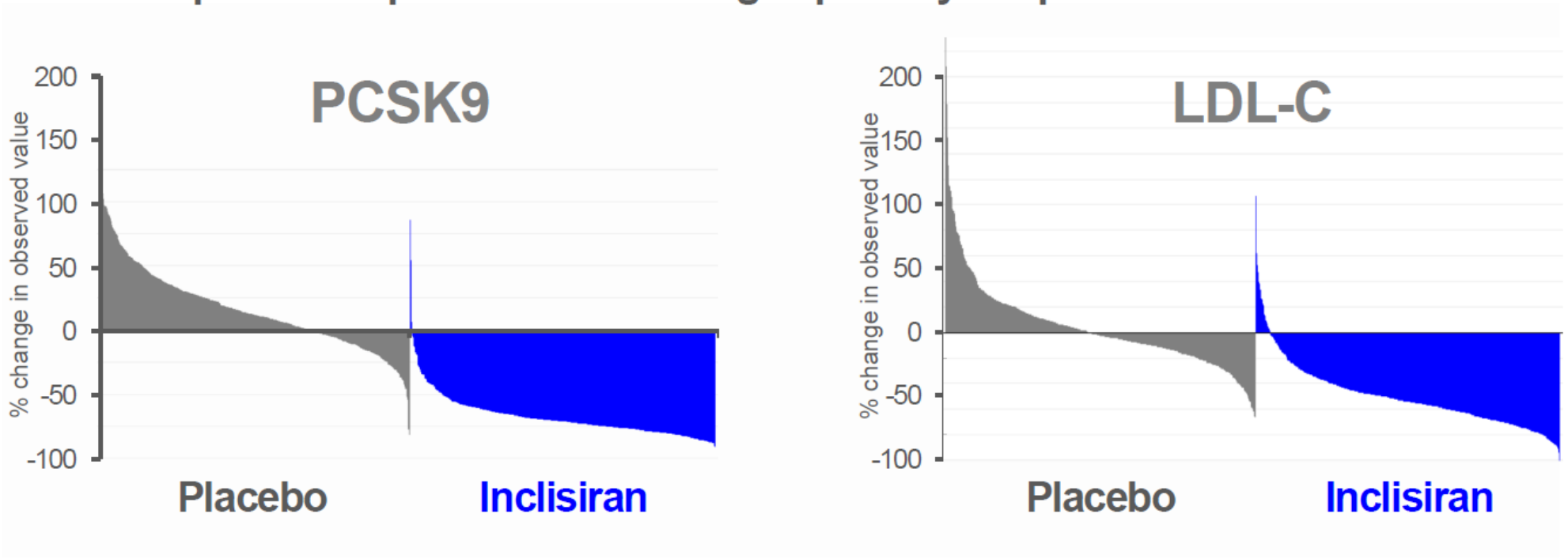
## Study Conducted by The Medicines Company; Acquired by Novartis International AG

### ORION-11: Efficacy

## Potent, consistent response to inclisiran

The Medicines Company

**Individual patient responses contributing to primary endpoint – 17 months**



# Inclisiran: Investigational RNAi Therapeutics Targeting PCSK9

## Study Conducted by The Medicines Company; Acquired by Novartis International AG

### ORION-11: Safety and tolerability

### No evidence of liver, kidney, muscle or platelet toxicity

| Laboratory tests<br>Safety population[1,2] | | Placebo<br>N = 804 | | Inclisiran<br>N = 811 | |
|---|---|---|---|---|---|
| Liver function | ALT >3x ULN | 4 | (0.5%) | 4 | (0.5%) |
| | AST >3x ULN | 4 | (0.5%) | 2 | (0.2%) |
| | ALP >2x ULN | 2 | (0.2%) | 1 | (0.1%) |
| | Bilirubin >2x ULN[3] | 8 | (1.0%) | 6 | (0.7%) |
| Kidney function | Creatinine >2 mg/dL | 11 | (1.4%) | 5 | (0.6%) |
| Muscle | CK >5x ULN | 9 | (1.1%) | 10 | (1.2%) |
| Hematology | Platelet count <75x$10^9$/L | 1 | (0.1%) | 0 | (0.0%) |

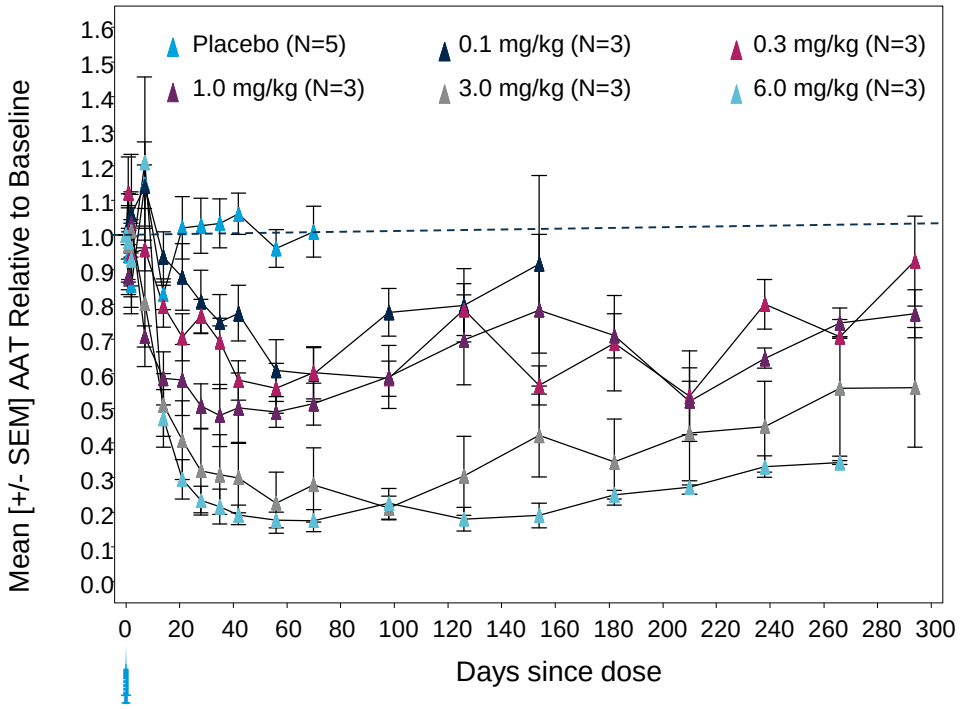
1. Safety population includes all patients who received at least 1 dose of study medication 2. Patients may be counted in more than one category 3. No cases met Hy's Law

- Inclisiran safety profile similar to placebo, with no adverse changes in laboratory markers
- Injection site events 4.2% - predominantly mild and none persistent
- Numerically fewer CV events reported for inclisiran than placebo (exploratory endpoint)

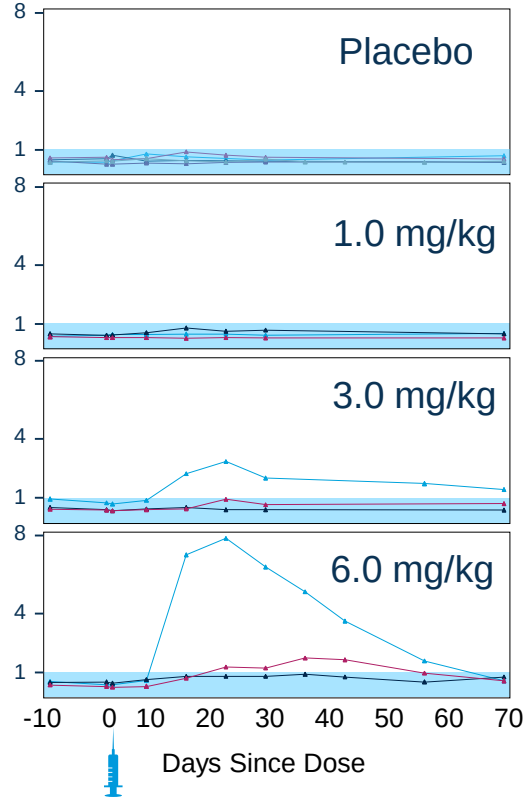
# Characteristics of the Human LFT Signal with Subset of ESC Conjugates: Does Not Occur Across All Programs, Suggesting Sequence Specificity

## ALN-AAT01 Phase 1/2 Interim Results

### PD: Serum AAT Protein Levels




### Safety: Serum ALT Levels

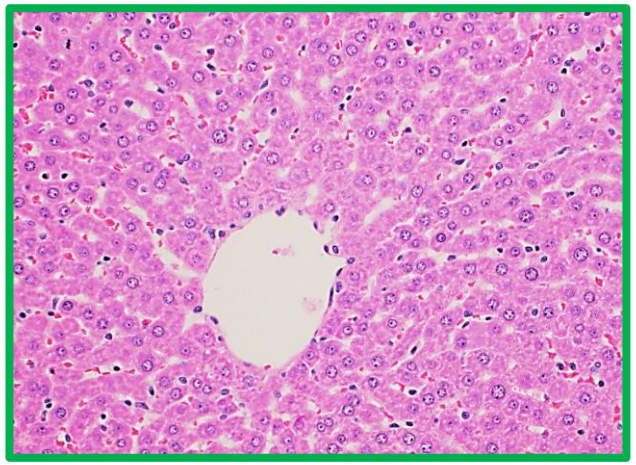



- Anecdotal evidence for RISC-mediated mechanism:
  - Onset of LFT elevations coincides with onset of maximum RNAi activity
  - High knockdown appears necessary (but not sufficient) for LFT elevations
- Similar profile seen in other programs with sporadic LFT elevations

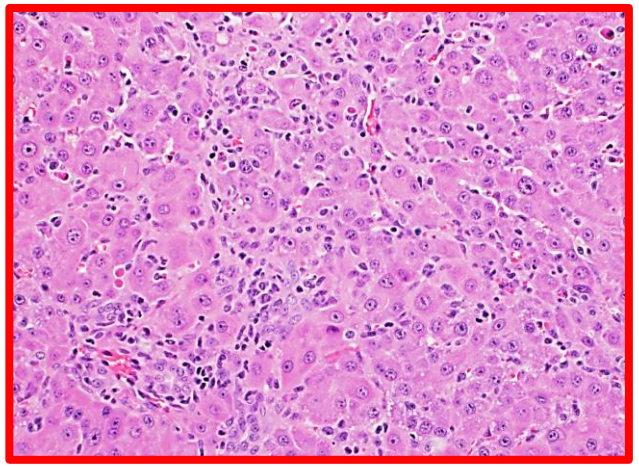
# Subset of ESC Conjugates Show Rat Hepatotoxicity at Exaggerated Doses

*In silico* prediction & *In vitro* efficacy → *In vitro* screen for predicted off-targets → Rodent Knockdown → Rat Tox @ >100x PD dose → NHP Knockdown → DC

**No hepatotoxicity (60%)**



**Show hepatotoxicity (40%)**

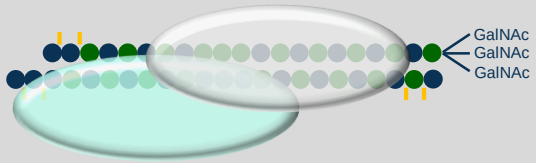


*These compounds drop out of DC selection process*

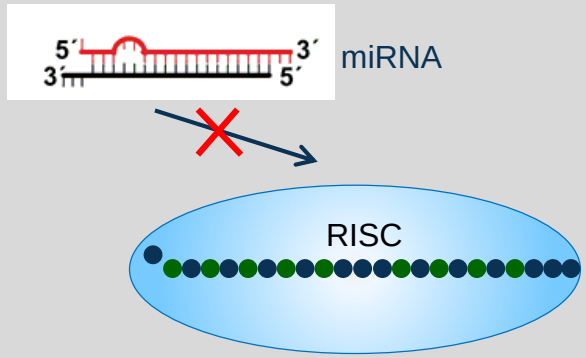
Single cell necrosis and/or hepatocellular degeneration with ↑LFT 2x upper limit of normal

# Seed-Based Off-Target Effects Are Important Drivers of Rodent Hepatotoxicity for Subset of ESC Conjugates

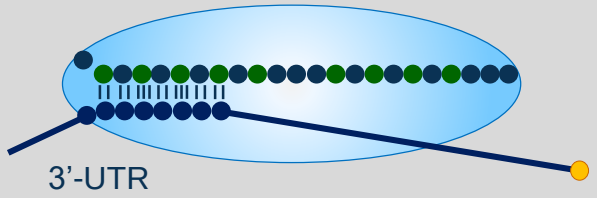
1. Non-RNAi effects
   e.g. siRNA chemistry, metabolites, protein binding, drug accumulation




2. Competition for RISC loading with miRNAs




3. Undesired seed-based off-target activity




Off-target binding
Partial sequence match

nature COMMUNICATIONS

Janas, Schlegel et al. *Nat Commun*. **2018**

ARTICLE

DOI: 10.1038/s41467-018-02989-4 OPEN

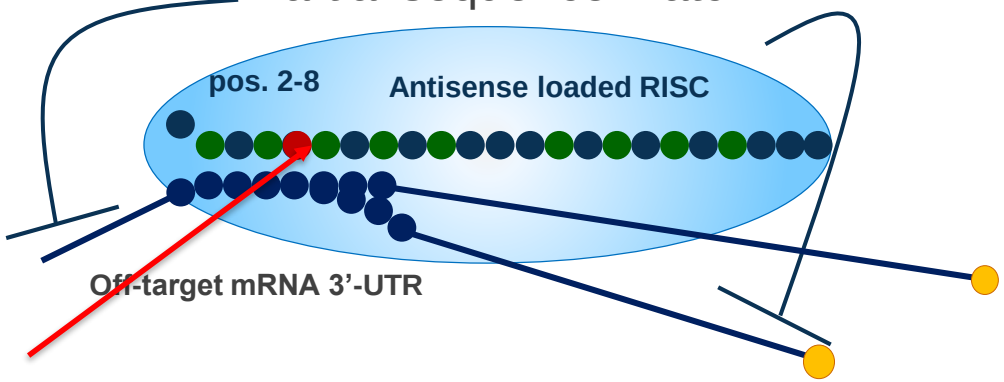
Selection of GalNAc-conjugated siRNAs with limited off-target-driven rat hepatotoxicity

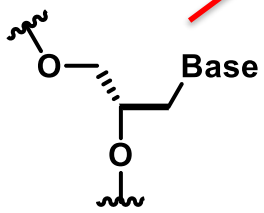
Maja M. Janas[1], Mark K. Schlegel[1], Carole E. Harbison[1], Vedat O. Yilmaz[1], Yongfeng Jiang[1], Rubina Parmar[1], Ivan Zlatev[1], Adam Castoreno[1], Huilei Xu[1], Svetlana Shulga-Morskaya[1], Kallanthottathil G. Rajeev[1], Muthiah Manoharan[1], Natalie D. Keirstead[1], Martin A. Maier[1] & Vasant Jadhav[1]

# ESC+ Seed Pairing Destabilization Strategy Improved Specificity and Therapeutic Index in Rats

## Off-target binding

Partial sequence match



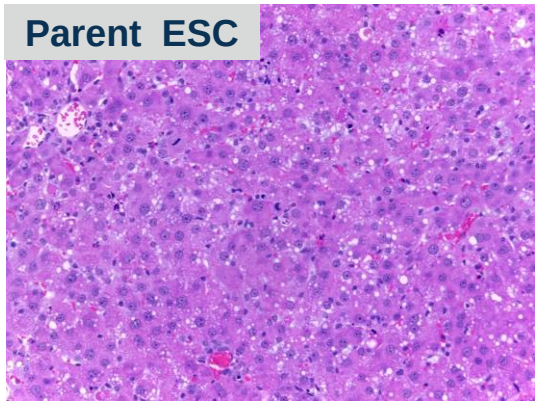




(*S*)-GNA

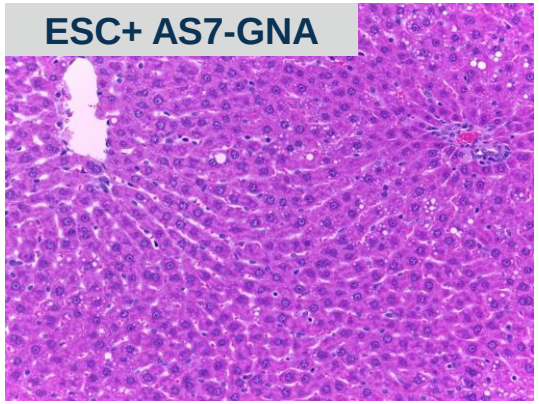
Unde[illegible]d off-target activity

**Rat Liver Histopath for 30 mg/kg dose**

Parent ESC



ESC+ AS7-GNA



## Important Considerations

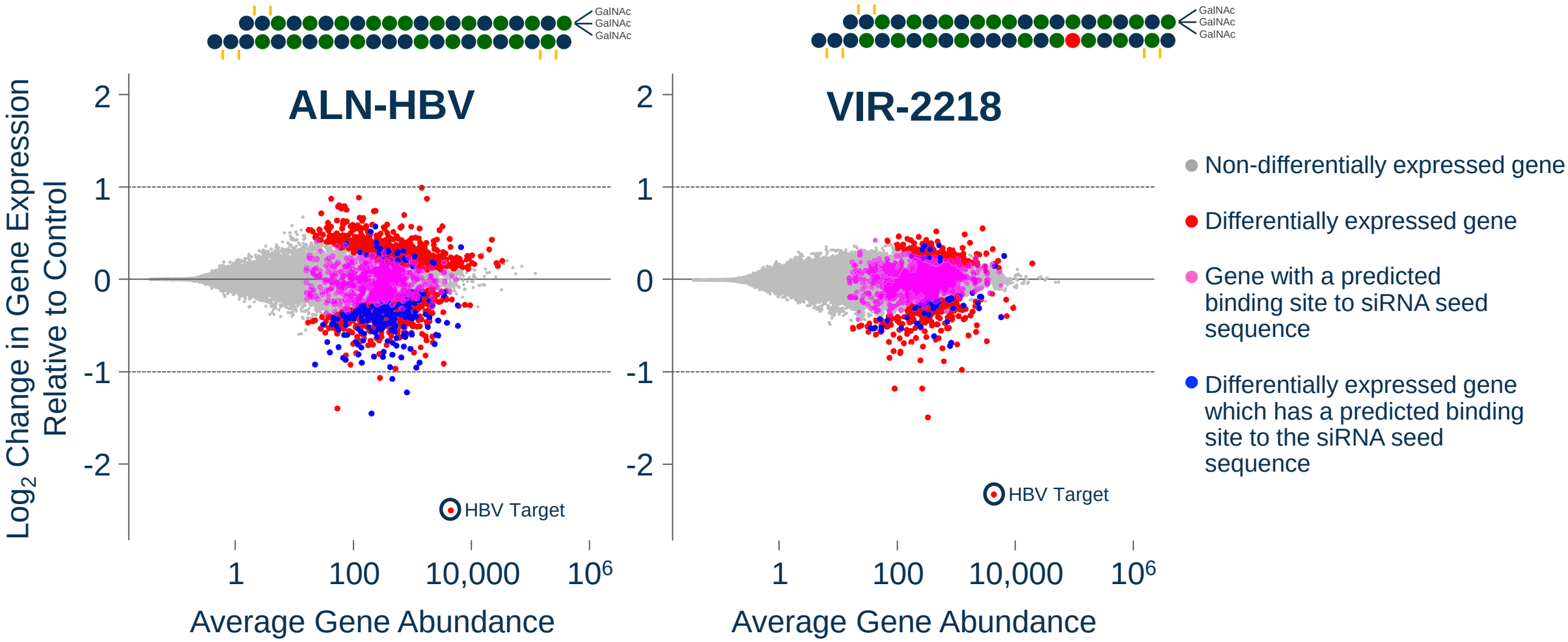
1. On-target potency must be maintained *in vivo*
2. Off-target activity should be minimized

**How would ESC+ design translate in humans?**

- Evaluated ESC+ versions of ALN-HBV and ALN-AAT01

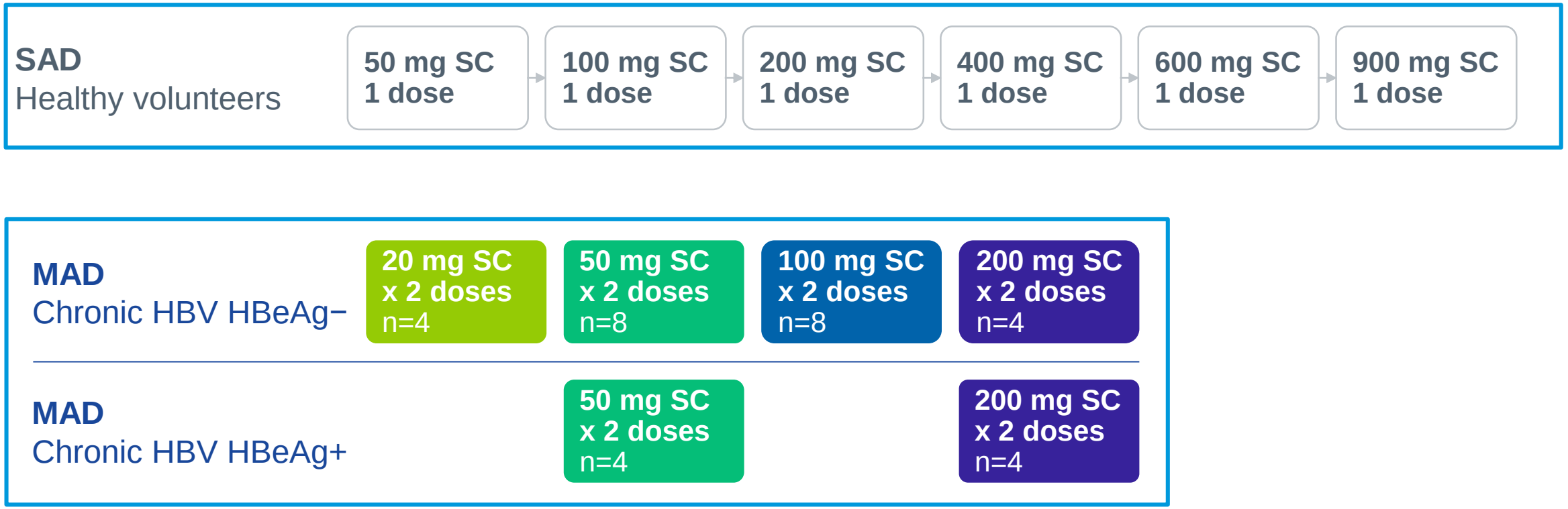

Bramsen et. al. *Nucleic Acids Res*. **2010**
Vaish et. al. *Nucleic Acids Res.* **2011**
Lee et. al. *Nat. Comm.* **2015**
Janas, Schlegel et al. *Nat. Comm.* **2018**

# Improved Specificity of RNAi Activity by VIR-2218




RNA-Seq analysis in HepG2.2.15 cells showed fewer differentially expressed genes and a lower magnitude of gene dysregulation, supporting reduced off-target effects with VIR-2218 compared with ALN-HBV

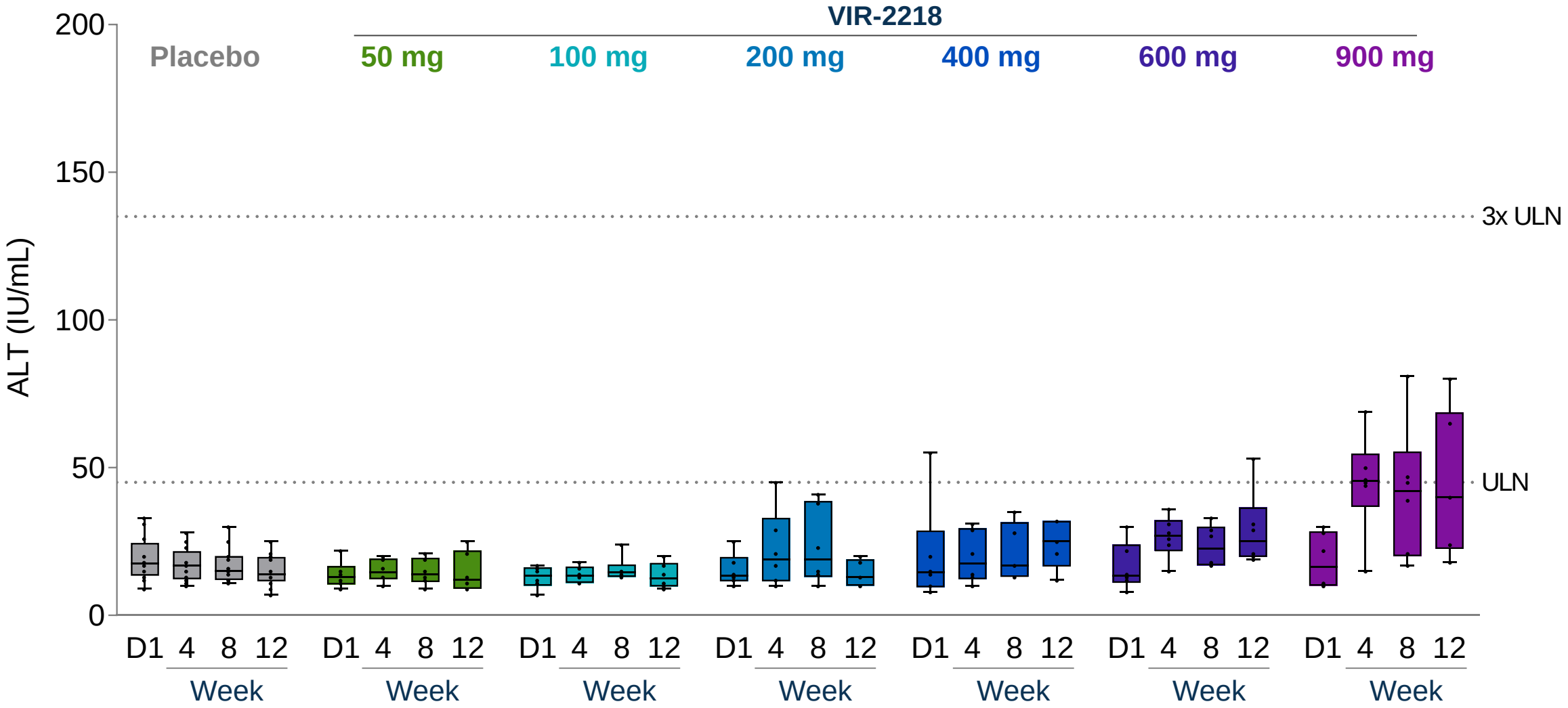
# VIR-2218-1001: Phase 1/2 study design



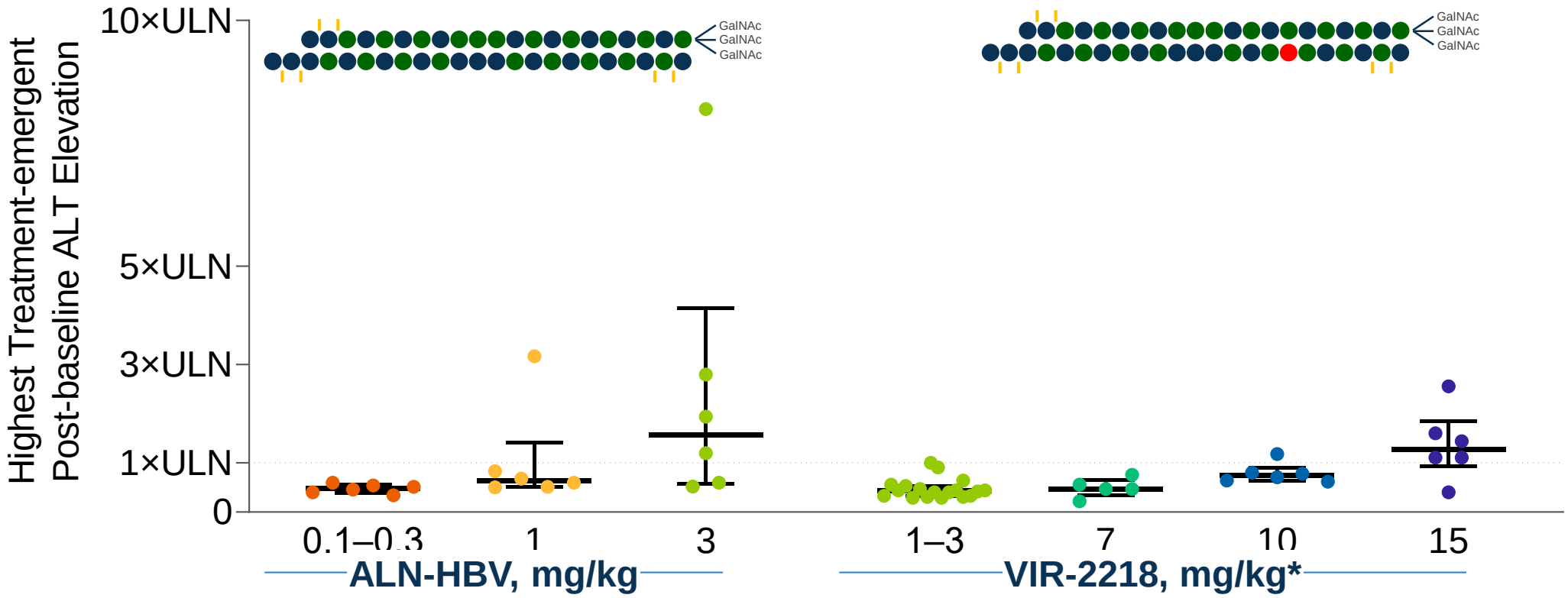

Double-blind, randomized, placebo-controlled, MAD study in patients with chronic HBV infection
At each dose level, 4 or 8 patients randomized 3 active:1 placebo

**HBeAg, hepatitis B e antigen; MAD, multiple ascending dose; SAD, single ascending dose.**

# VIR-2218-1001: Phase 1 SAD (Part A, healthy volunteers): ALT



# Human: Treatment-emergent post-baseline ALT elevations in healthy volunteers with normal ALT at baseline

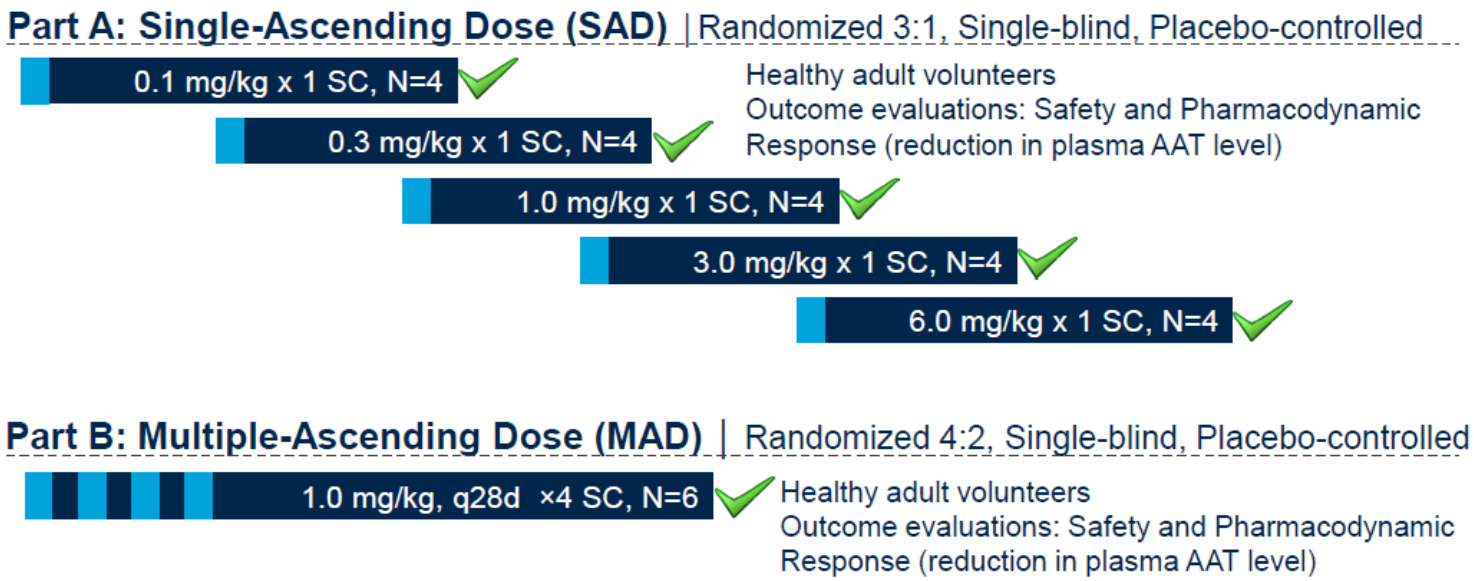



- No post-baseline ALT elevations to >ULN in the VIR-2218 or ALN-HBV cohorts were associated with increases in bilirubin >ULN
- No changes in functional status of the liver (eg, albumin, coagulation parameters) or clinical signs/symptoms of hepatic dysfunction were observed in any ALN-HBV- or VIR-2218-treated patient



***Approximate mg/kg dose based on an average adult weight of 60 kg; fixed doses ranged from 50–900 mg. ULN, upper limit of normal.**

# ALN-AAT01 Phase 1/2 Study Design



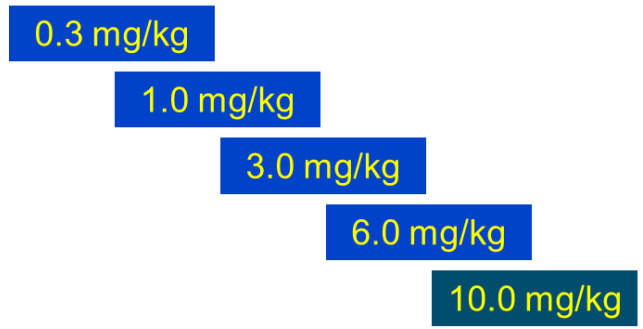

# ALN-AAT02 Phase 1/2 Study Design

**SAD-only Phase 1 in males and females**

**Part A:**

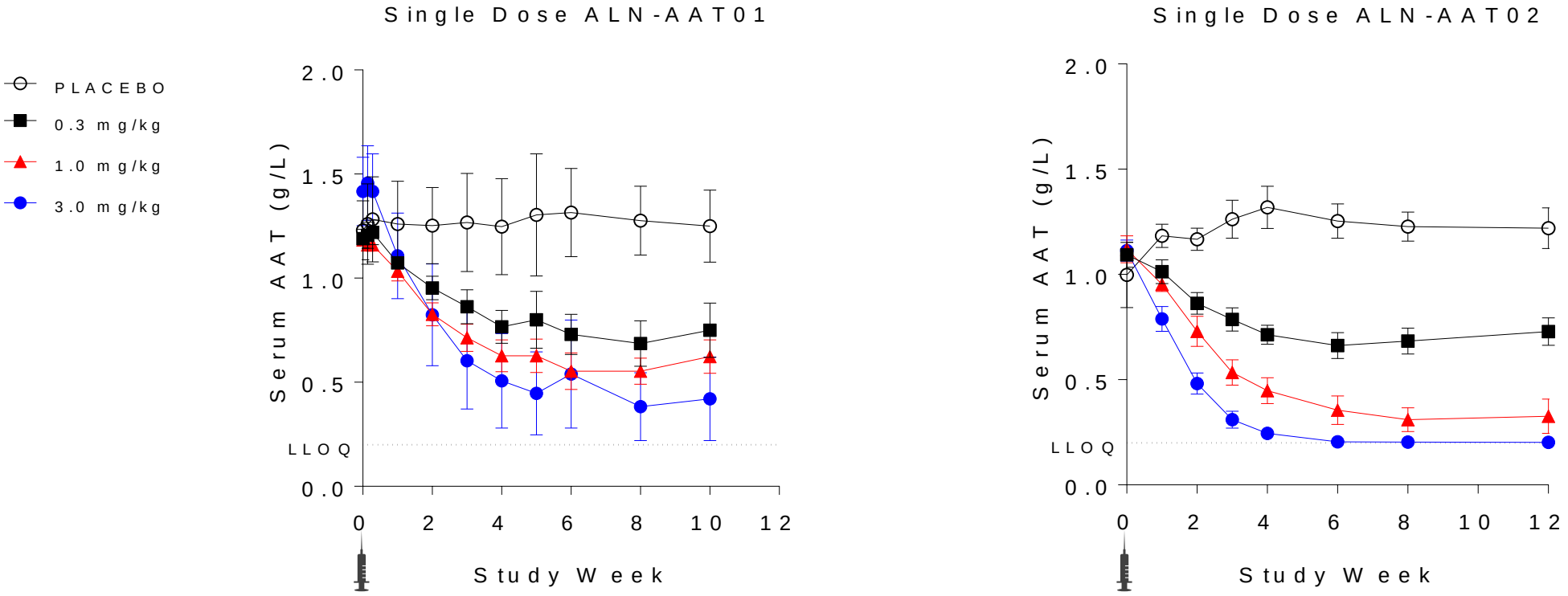
**N= 8 per cohort, double blind, randomized 3:1 active:placebo**

**5th (10mg/kg) cohort optional**



# Positive ESC+ Human POC

## ALN-AAT02 Clinical Activity and Safety




| | ALN- AAT01 | ALN-AAT02 |
|---|---|---|
| **Structure*** |   |   |
| **Efficacy** | Up to **89% KD** | Up to **89% KD** |
| **Liver Safety (ALT >3x ULN)** | **1/15** (up to 6 mg/kg dose) | **0/18** (up to 6 mg/kg dose) |

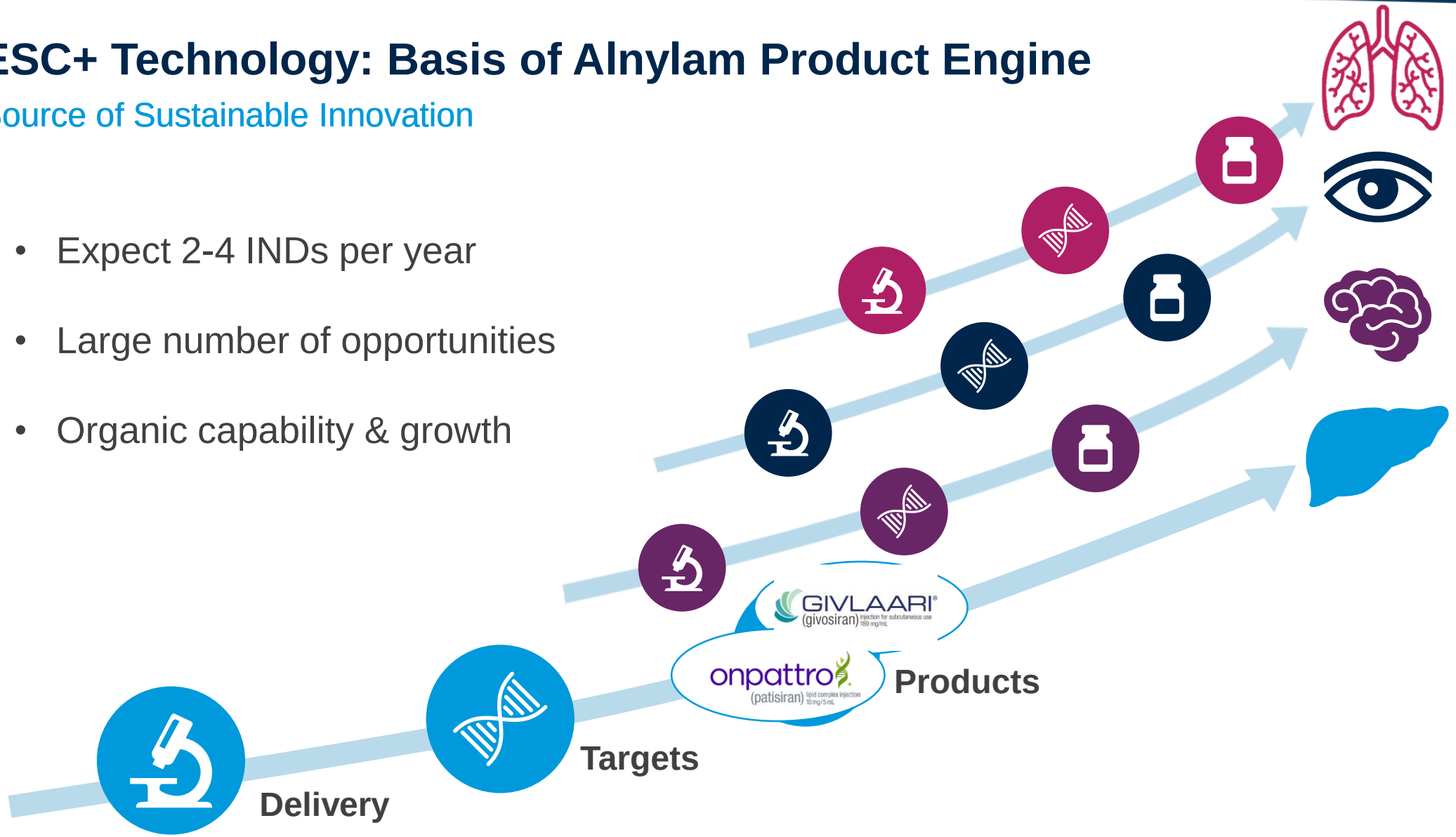


* Images are representative

# ESC+ Technology: Basis of Alnylam Product Engine

## Source of Sustainable Innovation

- Expect 2-4 INDs per year
- Large number of opportunities
- Organic capability & growth



# Summary

- ESC+ strategy mitigates seed-mediated off-target effects, improves specificity and further expands therapeutic window of siRNA conjugates in preclinical species
- Achieved encouraging translation of ESC+ design in humans
  - Directly assessed the impact of the new ESC+ design with follow-on compounds in two separate programs (same sequence but new ESC+ design)
- Multiple additional ESC+ conjugates have advanced into clinical development

# Acknowledgements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Christy Hebner

Phil Pang
Xiao Ding
Cara Pilowa
Richie Phan


**Ed Gane**
Professor of Medicine
New Zealand Liver Transplant Unit

To those who say "impossible, impractical, unrealistic," we say:

**CHALLENGE ACCEPTED**