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TriApex's Considerations for Detecting GalNAc-siRNA Concentrations in Liver and Kidney: Insights from Nonclinical Studies

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Background

- GalNAc-siRNA, a conjugate of N-acetylgalactosamine (GalNAc) and small interfering RNA (siRNA), binds to the asialoglycoprotein receptor (ASGPR) on hepatocytes, enabling liver-targeted pharmacodynamic effects. However, GalNAc-siRNA also distributes to other vascularized tissues (e.g. kidney) and undergoes renal excretion. Prolonged tissue half-lives may lead to histopathological changes (e.g., histiocyte vacuolation, basophilic granules, and single-cell necrosis). Consequently, rigorous monitoring of parent compounds and metabolites in liver and kidney is essential to address potential toxicity risks.
- Following absorption, GalNAc-siRNA circulates in the bloodstream as a double-stranded form. Antisense (AS) and sense strands (SS) are generated through its mechanism of action. Single strand concentration could reflect the intact drug levels, which is why AS and SS detection is incorporated in nearly all related studies. Notably, AS strands mediate pharmacodynamic activity in vivo, making their quantification particularly critical in target tissues such as liver and kidney.

Objective

This study aims to share TriApex's strategic framework for determining GalNAc-siRNA concentrations in liver and kidney during nonclinical evaluations.

Methods

1. Study Design

- Analyzed 10 projects conducted in TriApex involving tissue concentration analysis in liver and kidney.
- Integrated nonclinical data from FDA-approved GalNAc-siRNA therapies to refine detection protocols.
- Applied case-by-case approaches based on regulatory requirements and compound specific characteristics.

2. Detection Workflow

- Tissue collection: Primary method via necropsy; in vivo biopsy as an alternative.
- Analytes: Parent GalNAc-siRNA including AS and SS (if necessary), and key metabolites (e.g., AS (N-1)3', AS(N-2)3').
- Species selection: NHPs as the primary model, supplemented by rodent data.

3. Key Metrics

- Conducted dose range-finding (DRF) studies with extensive tissue sampling to inform GLP-compliant protocols.
- Aligned metabolite profiling with FDA guidelines for oligonucleotide therapeutics.

Results

Comprehensive analyte detection was prioritized in dose range-finding (DRF) studies over GLP-compliant toxicity studies to optimize resource allocation (Fig. 1). The metabolite profiling identified AS (N-1)3' and AS(N-2)3' as the primary analytes characterized across these studies (Fig. 2).

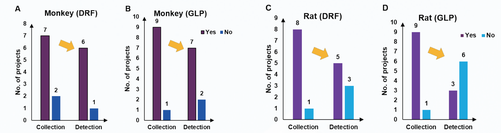

Fig 1. Tissue collection and detection statistics in DRF/GLP studies

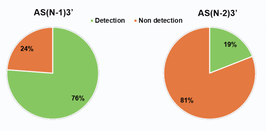

Fig 2. Metabolites types detected in TriApex

1. Tissue Distribution

Liver vs. Plasma: Drug concentrations in liver remained significantly higher than in plasma at 8-week recovery (D87) in both sexes (Fig. 3).
Liver vs. Kidney: Liver concentration was higher than kidney in NHP, consistent with GalNAc-mediated hepatocyte targeting (Fig. 4). While kidney concentration was higher in selected rat studies, suggesting species-specific toxicity risks (Fig. 5).

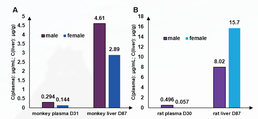

Fig 3. Comparative concentrations in plasma and liver

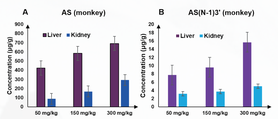

Fig 4. Concentrations of parent GalNAc-siRNA and metabolite were higher in liver

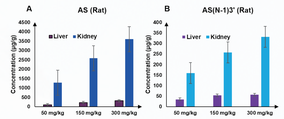

Fig 5. Concentrations of parent GalNAc-siRNA and metabolite were higher in kidney

2. Metabolite Profiles

AS strands dominated tissue activity, correlating with pharmacodynamic effects.
Metabolite detection in ≥1 study met regulatory safety evaluation criteria.
More complicated metabolite profiles might be found in liver and kidney (Tab 1).
Metabolites detected in TriApex were consistent with the report of FDA-approved GalNAc-siRNA therapies.

Tab 1. Major metabolites across siRNA candidates

siRNA	Main metabolites of AS
Patisiran	Nucleotide chains of different lengths
Givosiran	AS(N-1)3'
Inclisiran	AS(N-1)3'
Lumasiran	AS(N-1)3', 3 '-end deamination metabolites appeared in monkey liver
Vutrisiran	AS(N-2)3', AS(N-5)3'
Candidates in TriApex	**AS(N-1)3'; AS(N-2)3'**

Conclusion

TriApex recommends comprehensive concentration analysis of GalNAc-siRNA candidates (including metabolites) in liver and kidney during nonclinical pilot toxicity studies. This approach supports robust toxicity interpretation and provides critical data for clinical metabolite safety assessments. In practice, we also believe that study design should be considered on a case-by-case basis depending on regulatory application needs and the actual situation.

TriApex

With extensive experience in evaluating GalNAc-siRNA therapeutics (Fig. 6), TriApex has conducted over 30 nonclinical siRNA studies across diverse therapeutic areas (ophthalmology, CNS, metabolic diseases). Leveraging advanced NHP disease models and expertise in oligonucleotide pharmacokinetics/safety assessment, we accelerate translational research and regulatory success for siRNA-based therapies.

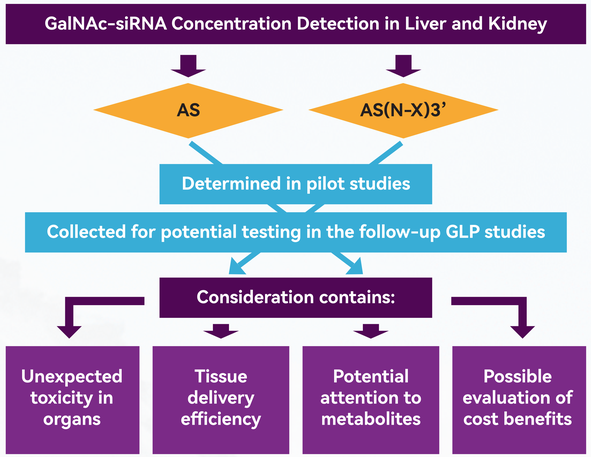

Fig 6. Detection Flowchart of GalNAc-siRNA concentrations in liver and kidney

Ref: A. Paul, A. Muralidharan, A. Biswas, et al, siRNA therapeutics and its challenges: recent advances in effective delivery for cancer therapy. OpenNano, 7 (2022)
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