The STRIKE Platform Enables Kidney-Selective Gene Silencing via Megalin-Mediated Uptake

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Proximal tubular epithelial cells (PTECs) play a pivotal role in solute reabsorption and systemic homeostasis, making them an attractive target for therapeutic intervention in both systemic and renal diseases. Despite this potential, oligonucleotide-based therapeutics such as siRNAs have shown limited efficacy in this compartment due to inefficient delivery. Judo Bio's STRIKE (Selectively Targeting RNA Into KidnEy) platform addresses this challenge by enabling receptor-mediated uptake of ligand-conjugated siRNAs into specific renal cell types. We target megalin-a highly expressed, rapidly internalized, and recycling receptor on PTECs-to achieve durable, cell-specific gene silencing in the kidney.

Megalin-targeting ligand-siRNA conjugates ("STRIKERs") were rationally designed and evaluated in both *in vitro* and *in vivo* models to assess delivery and efficacy. Tissue distribution and drug exposure were analyzed using fluorescence imaging, mass spectrometry, and stem-loop qPCR. Gene silencing was quantified using gene-specific methods such as qPCR, and global approaches including RNA-seq. Rodents and non-human primates (NHPs) received subcutaneous administration following various dosing regimens. Safety and tolerability were evaluated in both species.

In mice, a single administration of Megalin-STRIKERs produced kidney-specific, dose-dependent gene knockdown exceeding 70%, with sustained duration. Biodistribution analyses confirmed selective localization to the kidney, particularly in PTECs. Megalin-STRIKERs translated effectively from rodents to NHPs, with no adverse events observed in either species.

The STRIKE platform enables potent, megalin-mediated delivery of siRNAs to PTECs, resulting in robust and selective gene silencing. These results demonstrate cross-species translatability and establish a foundation for targeted RNA-based interventions in renal and systemic diseases. STRIKE represents a promising modality for precisely modulating solute carrier proteins for therapeutic gain.

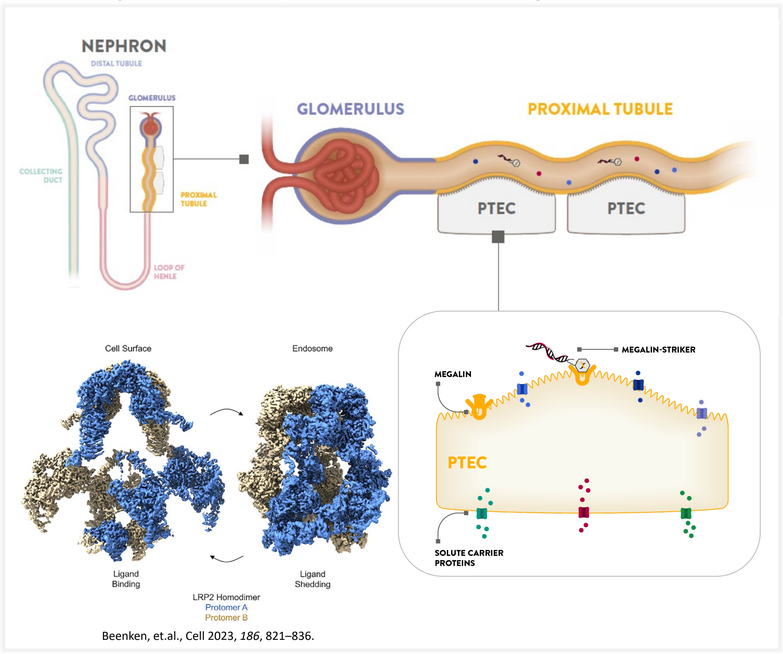

Figure 1: Each kidney contains about one million nephrons, the functional unit of the kidney, that together filter approximately 50 gallons of blood per day. Blood flows through each nephron, beginning at the glomerulus and continuing through the proximal tubule before exiting via the collecting duct. Megalin is a cell-surface receptor that is highly expressed on proximal tubular epithelial cells (PTECs). It is rapidly internalized, slowly degraded, and has a high recycling capacity, making it an ideal entry point for the intracellular delivery of ligand-siRNA conjugates (Megalin structures refer to PDB code 8EM4 and 8EM7).

STRIKE Platform enables targeting of specific receptors and delivery of siRNA payload in a modular fashion

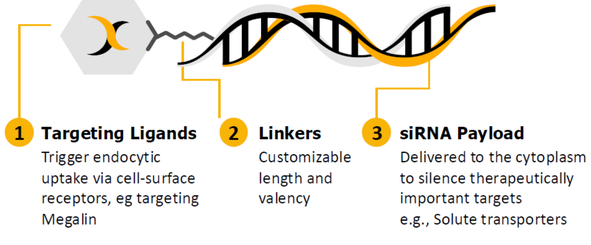

Figure 2: The Judo ligand was modified to achieve an optimal combination of cellular uptake and tissue specificity and was conjugated to siRNA via a linker optimized for multiple chemical properties, including length and valency.

Megalin-STRIKERs distribute specifically to PTECs in kidneys

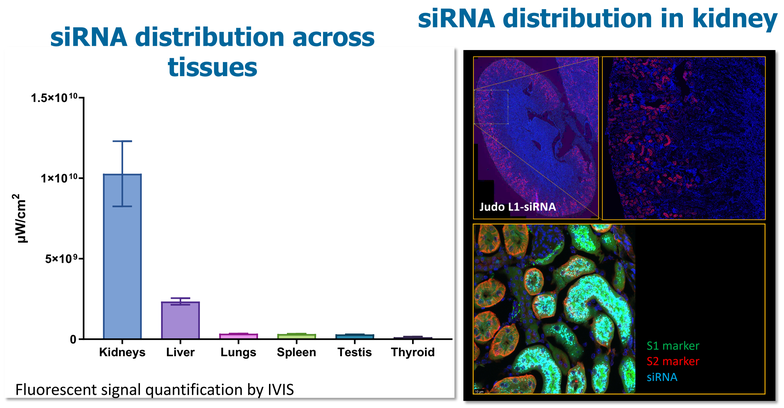

Figure 3: Mice were treated with Megalin-STRIKERs attached to fluorescent dye at a dose of 5 mg/kg and sacrificed 4 hours post-administration. Fluorescence associated with different organs was quantified and is shown in the graph. Tissues were sectioned for fluorescent imaging. Judo Ligand-conjugated siRNA demonstrated strong uptake in the kidney, specifically within PTECs.

Megalin-STRIKERs platform is modular

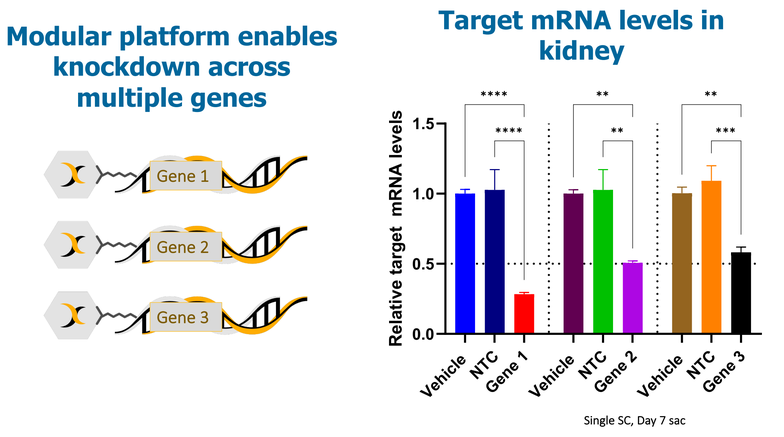

Figure 4: The same ligand-linker was conjugated to siRNA targeting three different genes. Mice (n=4 per group) were dosed subcutaneously at 10ul/g and necropsied on Day 7. Across all targets, knockdown of approximately 40~70% was achieved in the kidneys. The percentage expression in PTECs varies across these genes: gene 1 > gene 2 > gene 3. These results highlight the modularity of the STRIKE platform, allowing the the same ligand-linker design to be applied across different siRNA targets. Statistical analysis by one-way ANOVA, ****: P<0.0001

Dose-dependent knockdown with Megalin-STRIKERs

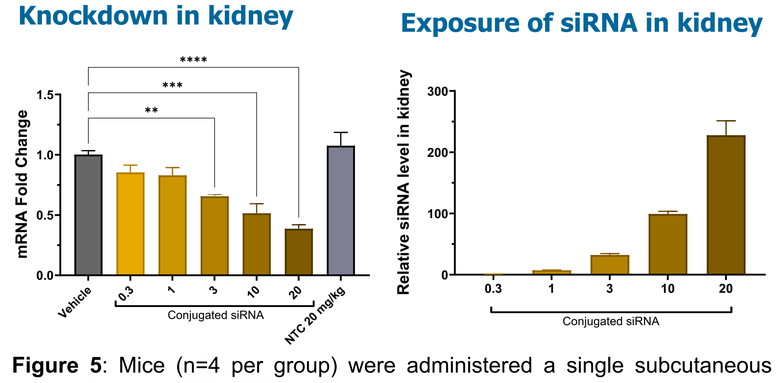

Figure 5: Mice (n=4 per group) were administered a single subcutaneous dose of Megalin-STRIKERs at varying dose levels (mg/kg) and necropsied on Day 14. Dose-dependent knockdown of the target gene was observed in the kidney, which corresponded with a dose proportional increase in siRNA levels, as measured by SL-PCR. Statistical analysis by one-way ANOVA, **: P<0.0021, ***: P<0.0002, ****: P<0.0001

~70% durable target gene knockdown attained in mouse kidneys with Megalin-STRIKERs

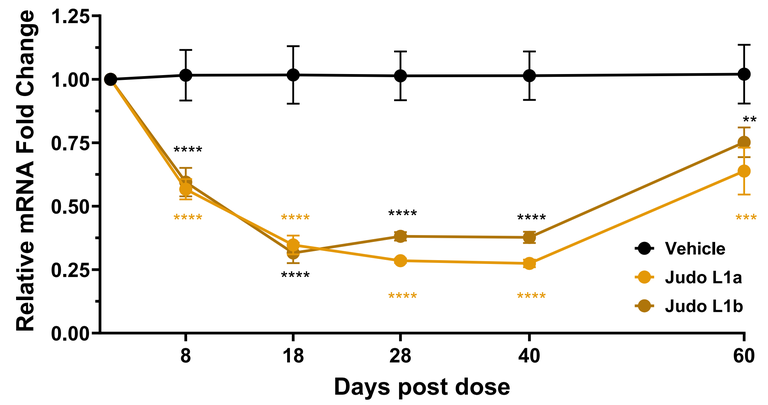

Figure 6: Mice (n=4 per group) were administered a single 10 mg/kg subcutaneous dose of two variants of Judo Ligand-1 (Judo L1a and L1b). Significant knockdown of the target gene was observed in the kidney at day 7, with greater knockdown of variant L1a.

A single subcutaneous dose administered on day 0 resulted in sustained knockdown lasting up to 2 months, the final time point tested. Both L1a- and L1b-conjugated siRNAs demonstrated durable gene silencing in the kidney. Statistical analysis by two-way ANOVA, **: P<0.0021, ***: P<0.0002, ****: P<0.0001

~70% durable target gene knockdown attained in Proximal tubule of NHP kidneys with Megalin-STRIKERs

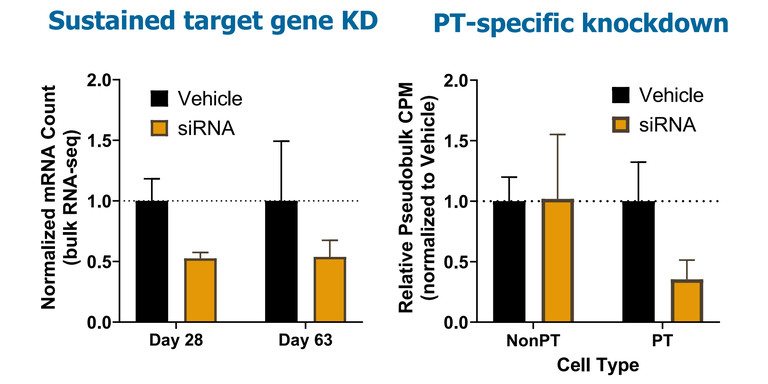

Figure 7: Non-human primates (NHPs) were administered a single subcutaneous dose of Megalin-STRIKERs targeting a tool gene. A significant knockdown of the target gene was observed in the kidney by day 28, with sustained suppression lasting up to two months post-dose, as measured by RNA-seq in whole kidney samples.

To understand the cell-type specificity of gene silencing, Week 9 samples were further processed. The results demonstrated selective knockdown of the target gene in proximal tubular epithelial cells, with no detectable knockdown in non-proximal tubule populations, highlighting the specificity and durability of the STRIKE platform in NHPs.

Summary

The STRIKE (Selectively Targeting RNA Into KidnEy) platform enables selective delivery of siRNAs to renal cells. Megalin-STRIKERs specifically target proximal tubular epithelial cells (PTECs) by engaging the megalin receptor. These conjugates are enriched in PTECs within kidney, with minimal distribution to other tissues. This modular platform enables dose-dependent, durable knockdown across rodents and non-human primates (NHPs).

These results highlight the utility of our approach in optimizing gene silencing for therapeutic intervention in systemic and renal diseases. Future research will focus on linking molecular knockdown to functional outcomes and disease modification in both rodent and NHP models, establishing a clear path toward therapeutic applications in systemic diseases.