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Nucleic Acids Research Breakthrough article describes insights into the key issue of duration of action for RNAi therapeutics

Research teams led by **Vasant Jadhav** at **Alnylam Pharmaceuticals** report the results of studies that help explain the exceptional duration of gene suppression by synthetic RNAi therapeutics, in "*Investigating the Pharmacodynamic Durability of GalNAc-siRNA Conjugates*".

Duplex RNA therapeutics that function through the RNA interference (RNAi) pathway are becoming an increasingly successful class of drugs, as illustrated by two recently approved drugs (ONPATTRO® and GIVLAARI®). Several other drug candidates have had impressive success during clinical trials. A critical aspect of compound design for therapeutics directed to gene targets primarily expressed in the liver is conjugation of the hepatocyte targeting ligand N-acetyl galactosamine (GalNAc) to one strand of the synthetic duplex small interfering RNA (siRNA). GalNAc binds the asialoglycoprotein receptor that is primarily expressed in hepatocytes, increasing the efficiency of targeting genes in the liver. Not only is potency increased but, remarkably, the duration of efficacy can be up to six months. This long duration greatly increases the potential for highly effective drugs in this class of RNAi therapeutics. Until this paper, however, there was little insight into how this durability of effect was achieved.

Christopher Brown et al. provide several lines of evidence that acidic intracellular compartments serve as a long-term depot for GalNAc-siRNA conjugates and are the major contributor to the extended duration of activity observed *in vivo*. Essentially, this serves as a living slow release mechanism that provides compound in the cytosol gradually, thereby enabling long term control of gene expression . This work explains the biological origins of the long duration of effect, and could improve strategies for drug development, clinical trial design, and long-term administration of siRNA drugs to patients.

The manuscript was nominated as a Breakthrough manuscript by our reviewers and editors. One reviewer noted that "*the prolonged duration of effect with siRNA in the clinic has certainly been one of the most interesting observations in nucleic acid therapeutics in recent years. I don't think anyone expected the duration to be so robust. The Alnylam group did a nice job to show that it is endosomal stability and slow release. Presumably, RISC loading is very efficient and small amounts of siRNA released in the cytosol can achieve efficient RISC loading*." NAR executive editor David Corey added "*Long duration effects have been the reason for much of the recent clinical progress with duplex RNA drugs. Everyone has been asking for an explanation for how a duplex RNA can be active for so long in the body. This paper provides an answer*."=

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Investigating the Pharmacodynamic Durability of GalNAc-siRNA Conjugates

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ABSTRACT

One hallmark of trivalent *N*-acetylgalactosamine (GalNAc)-conjugated siRNAs is the remarkable durability of silencing that can persist for months in preclinical species and humans. Here, we investigated the underlying biology supporting this extended duration of pharmacological activity. We found that siRNA accumulation and stability in acidic intracellular compartments is critical for long-term activity. We show that functional siRNA can be liberated from these compartments and loaded into newly generated Argonaute 2 protein complexes weeks after dosing, enabling continuous RNAi activity over time. Identical siRNAs delivered in lipid nanoparticles or as GalNAc conjugates were dose-adjusted to achieve similar knockdown, but only GalNAc-siRNAs supported an extended duration of activity, illustrating the importance of receptor-mediated siRNA trafficking in the process. Taken together, we provide several lines of evidence that acidic intracellular compartments serve as a long-term depot for GalNAc-siRNA conjugates and are the major contributor to the extended duration of activity observed *in vivo*.