

## **CALAA-01 Results Show Delivery Technology to Meet Critical Need in RNAi Therapeutics, Putting Arrowhead in Position to Monetize Asset**

RNAi Therapeutics is widely considered to have the potential to be the next major drug development engine after small molecules and recombinant proteins. As a result, investment in RNAi Therapeutics has been widespread, including most of the major pharmaceutical companies. In order to unlock its full potential, however, systemic delivery technologies that allow the RNAi trigger ('siRNA') to reach its site of desired activity are critically needed. It is expected that technologies that can provide proof-of-concept for such gene knockdown in Man will be highly valued by the field.

The CALAA-01 study results that were just published in the highly prestigious journal Nature are evidence that Calando is leading the translation of RNAi into the clinic by demonstrating for the first time directly an RNAi mechanism of action in human subjects. For investors, the significance of the results extends well beyond the de-risking of CALAA-01 as a candidate for solid cancers as the underlying delivery technology ('RONDEL') can be applied to any target gene in cancer and other diseases for which it can reach target tissues. Moreover, the pioneering nature of the technology should allow it to command a leadership premium as early insights into clinical RNAi are sought after as they help speed up the development of the RNAi Therapeutics platform in general.

This report will first provide a brief introduction into RNAi Therapeutics and its potential to revolutionize drug development, then go on to highlight delivery as the critical unmet need and value creation opportunity, then explain the RONDEL delivery technology, and finally make the case why the study results should significantly increase the value of RONDEL in the RNAi Therapeutics marketplace.

### The Promise of RNAi Therapeutics

In the wake of sequencing the human genome, our understanding of the molecular basis of disease has been increasing exponentially. As a result, molecularly defined and targeted drug candidates are dominating current development pipelines. Unfortunately, traditional drug technologies can only address a limited number of the genetically defined potential drug targets, such as extracellular proteins and certain classes of enzymes. Even then, these strategies often suffer from poor target specificity and a translation gap between the genetic definition of the disease and non-genetic technologies.

RNA interference (RNAi) is ideally suited to close that gap by being able to address essentially any target and because it works at the genetic level. RNAi is a naturally occurring gene silencing pathway that operates in every cell of the human body. Following the recognition that RNAi is induced by double-stranded RNAs (dsRNAs), a discovery for which Fire and Mello were awarded the 2006 Nobel Prize in Physiology or Medicine, it has really revolutionized biomedical research when Tuschl reported in 2001 that short versions of dsRNAs, small interfering RNAs (siRNAs) could trigger the same mechanism in humans. This led to the immediate adoption of this technology in determining the function of genes, many of which had just been discovered,

and for target discovery and validation in drug development in virtually every pharmaceutical company. From this, it was not a stretch that RNAi would soon thereafter become recognized as having the potential as a therapeutic modality itself. Not only has it become conceivable to develop therapeutics for such genetically well defined diseases like Huntington's Disease, but since essentially every disease manifests itself through the inappropriate expression of genes, RNAi Therapeutics approaches are theoretically possible for the vast majority of diseases.

Another reason behind the attraction of RNAi Therapeutics as a drug modality is that once the basic technologies have been established, it should be a very efficient platform for drug development. This is because many of the development expenses for enabling technologies such as chemistry and delivery can be amortized across the platform, and also because it short-cuts the pre-clinical development stage by being able to directly go from target to drug candidate. Cancer is a good example of the amortization benefit, since cancers are really a heterogeneous group of diseases each with its peculiar genetics necessitating personalized approaches for best treatment outcomes. RNAi Therapeutics is ideally suited to address this need since all that has to be done in theory is to exchange the siRNA sequence of the drug while the disease tissue and associated delivery remains more or less the same. Therein also lies part of the significance of the present CALAA-01 results, as they should be translatable into many different types of oncology targets.

As a result of the potential of RNAi Therapeutics, investment in the area has been remarkable with investments approaching \$3 billion thus far, including the \$1.1B acquisition of Sirna Therapeutics by Merck (2006), ~\$700M in realized partnership funding for Alnylam's intellectual property, \$164M that Pfizer paid for remodeling oligonucleotide therapeutics company Coley Pharmaceuticals into its hub for RNAi Therapeutics development (2007), and \$125M paid by Roche for an early-stage delivery technology in 2008. Importantly, while most of the major pharmaceutical companies are evaluating RNAi Therapeutics, many of them are still expected to make investments to obtain necessary intellectual property and enabling technologies from external sources.

### The delivery challenge of RNAi Therapeutics

Despite the great potential of RNAi Therapeutics, there are also challenges in unlocking the full value of RNAi Therapeutics. These include siRNA chemical stability, the avoidance of off-targeting, RNAi triggers that do not stimulate immune responses and their delivery. While it is well documented that most of these challenges can be solved through nucleic acid chemistry, RNAi delivery has emerged as the one area for which solutions are still in high demand...and where there is need, there is opportunity as the \$125M acquisition of Mirus Bio by Roche highlights.

The delivery challenge arises because successful delivery is a multi-step problem involving first getting the RNAi trigger to the target tissue of interest, and once there facilitating the transport of the siRNA across the cell membrane into the cytoplasm where it can be recognized by the endogenous RNAi machinery as a substrate for gene silencing. As a result, many of the first-generation RNAi Therapeutics programs that have entered the clinic employed *local* delivery of siRNAs to the eye, brain, lung, skin, and kidney where the tissue delivery half of the equation is minimized. In these local delivery approaches, the siRNA is typically either directly injected

(eye, brain), inhaled (lung), applied (skin), or delivered intravenously as unformulated nucleic acid (kidney)<sup>1</sup>.

While there are many diseases that could be addressed by local delivery, the market is particularly interested in solutions for the systemic delivery of RNAi Therapeutics as this will be the required route of administration for most disease applications. For systemic delivery, before crossing the cell membrane, the challenge of localizing the RNAi trigger with the target tissue in the first place increases. Unformulated siRNAs do not do so on their own and will be rapidly removed from the body via the kidneys and urine. Consequently, it is important to formulate the siRNA in a manner that increases its circulation time so that it has the time to find its target tissue. Solutions can be broadly categorized into conjugate and nanoparticle ones. In conjugates, a moiety is directly added to the siRNA with the aim of increasing its circulation time either through increased size or association with components of the blood such as albumin or lipoproteins. In some cases, the conjugate is also intended to facilitate cellular uptake by recognizing certain cell surface receptors. Conjugate-siRNAs have been successful in delivering siRNAs throughout the body. Almost all of these approaches, however, suffer from the inability of these molecules to then cross the cellular membrane.

This is where nanoparticle approaches have an advantage as multiple functionalities, including cellular uptake, membrane crossing ability and triggered nanoparticle disassembly, can be engineered into them. Nanoparticle delivery of siRNA has been successfully applied in pre-clinical animal models to knock down various genes especially in the liver, tumors, and certain cells of the immune system. While the progress is very encouraging, there is still considerable uncertainty of how they will perform in humans. Liposomes for example have been widely adopted for systemic RNAi delivery, but there is still uncertainty about its safety profile in Man, such as liver toxicity and immune stimulatory potential. Moreover, most nanoparticle siRNA delivery systems are only pharmacokinetically targeted, although targeted delivery through the addition of cell surface receptor ligands is thought to have significant potential to enhance the efficacy and safety of delivery. Part of these challenges are simply the result of the chemical nature of the nanoparticles, partly the result of the difficulty of controlling the formulation of such complex particles. Consequently, there is tremendous interest in nanoparticle-siRNA delivery systems that show proof-of-concept RNAi activity following their systemic administration in Man.

### The RONDEL delivery system

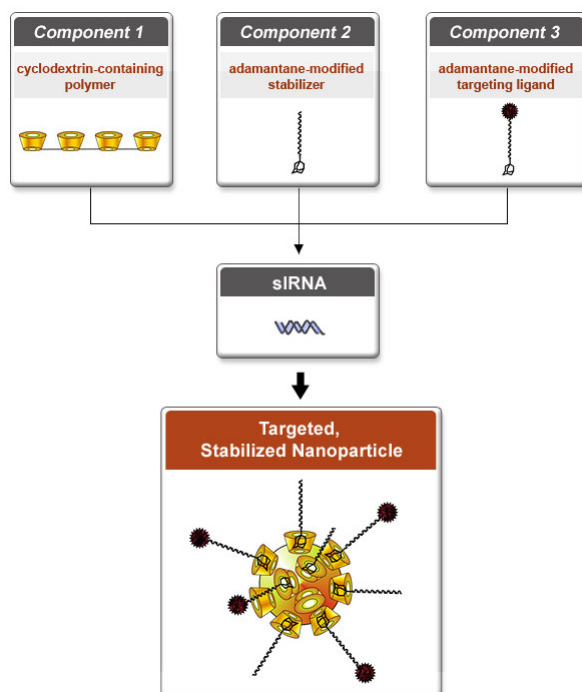
The RONDEL delivery system that underlies CALAA-01 has a long history of leading the development of nanoparticle-siRNA delivery systems. This is because it is able to draw on extensive experience with the technology based on efforts starting back in the mid 90's where it was originally conceived as a system for the delivery of plasmid DNA, a larger form of nucleic acid compared to siRNAs but in many respects essentially identical in terms of pharmacological properties. The reason, however, why siRNAs are a more promising payload for the system is because for it to be pharmacodynamically active, it only has to reach the cytoplasm, whereas

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<sup>1</sup> while delivery to the kidney involves systemic administration, pharmacologically it is a local RNAi delivery problem

plasmids have to overcome the additional hurdle of having to enter the nucleus. Milestones that illustrate the leading role that RONDEL delivery has played in RNAi Therapeutics delivery include a publication of RONDEL-siRNA in early 2007 that showed it to be safe and well tolerated in non-human primates, the initiation of the first clinical trial for a truly systemically administered (and moreover targeted) siRNA-nanoparticle, and now the first direct demonstration of RNAi activity following systemic siRNA administration in a human disease target tissue.

RONDEL is a smart, modular platform that comprises a core of a cyclodextrin-containing copolymer that can both bind to and protect the siRNA payload and is responsive to changes in the environment that facilitates its triggered release into the cytoplasm. In order to increase its stability in the blood circulation, it furthermore contains a hydrophilic shell of PEG (polyethyleneglycol). Some of these PEG chains further contain a ligand, in the case of CALAA-01 transferrin, in order to facilitate enhanced and targeted delivery of the siRNA through interaction with cell surface receptors. It is important to note that this is a highly versatile system in which the pharmacological properties can be tuned for a particular application by simply changing the nature of the individual components. This includes tailoring particle circulation times by increasing/decreasing the stability of the particle, altering the siRNA binding capacity and triggered release properties by slightly altering the co-polymer core, or exchanging the ligand to reach new cell types. Most importantly, the system is indifferent to the siRNA target sequence. All this is made possible by the self-assembly of the nanoparticle by mixing the individual components. As a result, while the initial efforts were focused on the already significant opportunity in solid cancer, it is expected that the system has further applications for other areas such as inflammation.



*Schematic of RonDel Delivery System*

RONDEL-siRNA delivery has also played a pioneering role in the field in understanding the various functionalities of such nanoparticles. It was thus an important insight that the utility in the (transferrin) ligand does not reside so much in enriching the nanoparticle in the target tissue, an assumption that many other nanoparticle systems have been built on, but once there, to significantly enhance cellular uptake. Such insights will speed up the development of next-generation RONDEL-siRNA systems, for example by taking advantage of cell surface receptors that best combine nanoparticle uptake with cell selectivity, thus adding to the attractiveness of accessing the RONDEL-siRNA system.

### CALAA-01 Phase I Results

CALAA-01 is Calando's lead RNAi Therapeutics program based on RONDEL delivery. The active RNAi ingredient is an siRNA targeting ribonucleotide reductase M2, an enzyme involved in nucleotide metabolism and required for DNA replication. RRM2 is considered a promising target for cell proliferative diseases, especially cancers.

Thus far, in the patients that have participated in the trial, CALAA-01 has been well tolerated and no serious adverse effects have been observed. Three with melanoma volunteered to have biopsies taken allowing for more detailed molecular analyses of the effects of CALAA-01. Through this, it was possible to confirm the dose-dependent accumulation of CALAA-01 in the melanoma cancer target tissues. This has not been established before for any RNAi Therapeutics clinical candidate. Most importantly, however, and in yet another first for RNAi Therapeutics, it was possible to isolate RNA nucleic acid material and by this unambiguously confirm through a modified form of PCR, the so called 5' RACE assay, that the siRNA not only accumulated in the target tissue, but was also successful in directing mRNA targets for degradation through an RNAi mechanism of action. While the 5' RACE assay is non-quantitative, it still requires a robust level of cleaved mRNA in order to be successfully applied. Accordingly, RRM2 mRNA knockdown was established by quantitative realtime PCR. Moreover, the knockdown was long-lasting and could still be detected after more than a month. In aggregate, the data provide first-ever direct proof of target mRNA knockdown through an RNAi mechanism of action in Man. With more patients being enrolled and analyzed, a more quantitative picture should emerge. It is very encouraging, that these data could be observed without having reached dose-limiting toxicity.

### Financial implications of the CALAA-01 study results

The CALAA-01 interim results establish that RONDEL-siRNA delivery is able to knock down genes in Man without signs of show-stopping toxicities. This is a major de-risking event for RONDEL as a clinically relevant platform technology and puts it in a leading position among RNAi delivery technologies. No matter how promising the pre-clinical results, first-in-Man studies have the potential for surprises that could put an end to the further clinical development. Because of the modularity of the system, the results also lay a sound foundation for the further optimization of the technology and its development into broader applications.

Potential partners will recognize that the RONDEL technology and Calando's related regulatory know-how will enable them to obtain early human clinical data so as to lead in the development

of the RNAi Therapeutics platform. Since solid cancers is an expressed priority of many of the major pharmaceutical companies, it will be obvious to them that RONDEL can also deliver siRNAs for their targets of choice and therefore be of immediate value to them. More generally, the financial flexibility of a larger entity will allow it to more fully realize the value of the technology. Part of the value of RONDEL technology also derives from the fact that it is a technology that differentiates itself in a field that has become quite reliant on lipid-based solutions. Companies with a broad interest in RNAi Therapeutics may therefore view RONDEL as an opportunity to diversify their delivery technology risks.

## Outlook

In summary, the CALAA-01 results not only increase the value of CALAA-01 as a promising candidate for solid cancer in its own right, but the RONDEL delivery technology as a whole. By this, RONDEL compares favorably for example to the 'dynamic polyconjugate' siRNA delivery technology for which Roche paid \$125M in 2008. Moreover, because of the unmet need in RNAi delivery, it is to be expected that the financial terms for such technologies, especially those that have shown clinical proof of concept, are about to increase considerably. Ultimately, the Nature studies should capture wide attention and put Calando in an excellent position to monetize the asset either through an out-licensing strategy or outright sale.

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*This report was written by Dirk Haussecker, Ph.D., an independent biotech consultant with an expertise in RNAi therapeutics and interest in RNAi-related business and investment. Dr. Haussecker recently completed a post doctoral fellowship at Stanford University and is the author of several articles on the field of RNAi therapeutics. Dr. Haussecker blogs about RNAi Therapeutics at <http://rnaitherapeutics.blogspot.com>. Although the report was underwritten by Arrowhead Research Corporation, the views expressed herein are solely those of the author and should not be attributed to Calando Pharmaceuticals, Inc. or Arrowhead Research Corporation. The report is provided for informational purposes only and should not be relied on as the basis for an investment decision. For more information about Arrowhead Research Corporation and Calando Pharmaceuticals, Inc. and the risks associated with an investment therein, please refer to Arrowhead's Annual Report on Form 10-K, Quarterly Reports on Form 10-Q, Current Reports on Form 8-K and other filings with the Securities and Exchange Commission, available at the SEC's website at [www.sec.gov](http://www.sec.gov).*

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