

Systemic Delivery of Targeted, siRNA-Containing Nanoparticles for the Treatment of Solid Tumors: Concept to Clinic

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ABSTRACT

Calando Pharmaceuticals is developing siRNA-containing nanoparticle formulations, and its leading formulation that will enter the clinic in 2008 contains the human transferrin protein as a targeting ligand and siRNA targeting the M2 subunit of ribonucleotide reductase (RRM2). Critical parameters in the formulation and function of this therapeutic system will be highlighted using data from pre-clinical investigations. Anti-tumor efficacy is enhanced when the targeted version of the nanoparticles is employed, and results from numerous safety and efficacy studies will be discussed.

Keywords: siRNA, RRM2, transferrin, polymer, cyclodextrin

1 BACKGROUND

Calando Pharmaceuticals has been advancing its RNAi/Oligonucleotide Nanoparticle Delivery (RONDELTM) technology toward clinical application. The system involves assembly of a nucleic acid, such as siRNA, with three synthetic components into nanoparticles that are suitable for intravenous administration *in vivo*. A summary of the RONDELTM technology is shown in Figure 1.

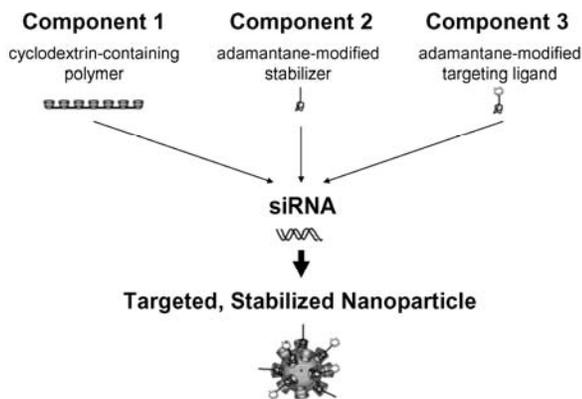


Figure 1. Schematic of RONDELTM technology.

The first synthetic component is a linear, cyclodextrin-containing polycation that interacts electrostatically with the polyanionic siRNA. The polymer and siRNA assemble into nanoparticles less than 100 nm in diameter that protect the siRNA from nuclease degradation, permitting the use of non-chemically-modified siRNA in these formulations. The cyclodextrin in the polymer enables the surface of the particles to be decorated by stabilizing agents and targeting ligands. These surface modifications are formed by proprietary methods involving cyclodextrin and inclusion complexes with adamantane within the surface-modifying agents, as discussed below.

The surface-modifying agents have terminal adamantane groups that form non-covalent inclusion complexes with the cyclodextrins within the polymer. These agents contain poly(ethylene glycol) (PEG) to endow the particles with properties that prevent aggregation, enhance stability and enable systemic administration. Ligands to cell surface receptors can be covalently attached to the adamantane-PEG modifier, enabling the siRNA-containing particles to be targeted to cell surface receptors of interest.

Ribonucleotide reductase (RR) catalyzes a rate-limiting step in the pathway for production of 2'-deoxynucleoside 5'-triphosphates that are necessary for DNA replication. Consequently, it has long been an important target for controlling pathologies that depend on DNA replication, such as cancer. Small molecule drugs designed against the M2 subunit of RR (RRM2) are not completely specific to the RRM2 protein, and nucleic acid-based approaches are currently under development. Calando has developed a potent siRNA sequence targeting RRM2 that significantly reduces cellular mRNA and protein levels and achieves an anti-proliferative effect in cancer cells of various types [1].

CALAA-01 incorporates this potent anti-RRM2 siRNA duplex into a RONDELTM-based nanoparticle formulation that includes the human transferrin protein as a targeting ligand.

2 PRE-CLINICAL EVALUATION OF CALAA-01

2.1 Efficacy

The efficacy of CALAA-01 has been examined in numerous murine cancer models. All experiments include a vehicle control (D5W, 5% dextrose in water) as well as an analogous formulation made using a non-targeting siRNA sequence (siCON).

A summary of results from three such experiments is shown in Figure 2. The effect of CALAA-01 on subcutaneous tumors of murine hepatoma (Hepa1-6), human colon adenocarcinoma (HT29), and human melanoma (HT144) cells was examined in mice. All data shown were obtained at 10 mg/kg/dose CALAA-01 (with respect to the siRNA component of the formulation) with intravenous administration on Days 1, 3, 8, and 10. Formulated siCON was administered at the same schedule (HT144) or on Days 1 and 8 (Hepa1-6, HT29). Median (n=5 animals per group) tumor volume is expressed as a percentage of the median tumor volume for D5W-treated animals (Days 1, 3, 8, and 10) in the same experiment. Statistically-significant ($P<0.05$) reduction in tumor volume was observed in all experiments.

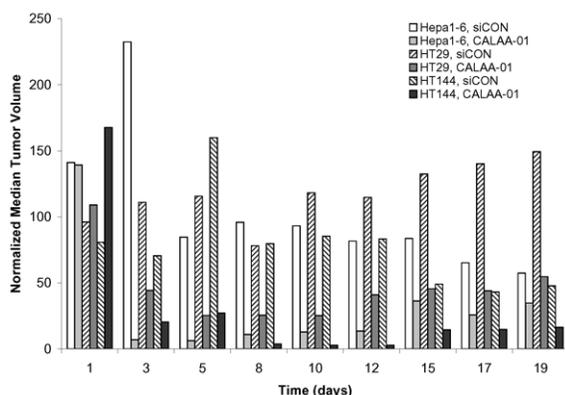


Figure 2. Summary of CALAA-01 efficacy results in murine cancer models.

2.2 Safety

Evaluations of safety of CALAA-01 have been performed in four different species. These include multi-dose, multi-cycle evaluations and assessment was made of numerous standard and relevant endpoints, including CBC, serum chemistry, coagulation, complement, toxicokinetics, and pathology. The results of an exploratory study of CALAA-01 in cynomolgus monkeys have been published [2], the first publication of multiple dosing of systemically-administered, targeted, formulated siRNA in primates.

The primary conclusions of this primate study have been confirmed in other species:

- Multidose, multicycle administration of CALAA-01 at doses at or above those that have been shown to be efficacious in mice (allometric scaling) are well-tolerated
- When doses are further escalated to supertherapeutic doses, evidence of renal impairment may be observed which is attributable to “free” (unbound) RONDEL™ components within the formulation
- An antibody response to the CALAA-01 formulation is observed and does not effect pharmacokinetics; it is specific to the human transferrin component of the formulation

3 SUMMARY AND FUTURE WORK

Calando has designed a Phase I protocol to evaluate the safety of CALAA-01 in patients having solid tumors that are refractory to standard-of-care treatment. The primary objectives of this open-label, dose-escalation trial are to evaluate the safety and tolerability of CALAA-01 in humans. Additional objectives include characterization of pharmacokinetics, evaluation of tumor response, and recommendation of a CALAA-01 dose level for future clinical studies.

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