

CALANDO PHARMACEUTICALS INC. is using its proprietary technologies to design and create new, targeted siRNA therapeutics. Calando combines proprietary technologies in targeted polymeric delivery systems and siRNA design to create effective therapeutics. The company is pursuing this goal through both its internal R&D group and collaborations and partnerships with pharmaceutical and biotechnology companies.

CALANDO'S TECHNOLOGY

Calando's cyclodextrin-containing polymers form the foundation for its two-part siRNA delivery system. The first component is a linear, cyclodextrin-containing polycation that, when mixed with small interfering RNA (siRNA) binds to the anionic "backbone" of the siRNA. The polymer and siRNA self-assemble into nanoparticles of approximately 50 nm diameter that fully protect the siRNA from nuclease degradation in serum. 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 the cyclodextrins.

The surface-modifying agents have terminal adamantane groups that form inclusion complexes with the cyclodextrin and 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 tissues of interest.

The siRNA delivery system has been designed for intravenous injection. Upon delivery to the target cell, the targeting ligand binds to membrane receptors on the cell surface and the RNA-containing nanoparticle is taken into the cell by endocytosis. There, chemistry built into the polymer functions to unpackage the siRNA from the delivery vehicle.

PATENTS

Calando has filed or exclusively licensed a broad suite of US patents and patent applications for development and delivery of siRNA therapeutics, with counterparts filed in Europe, Japan and other key countries.

CALANDO'S siRNA ADVANTAGE

Calando has multiple proprietary technologies that encompass all the components necessary for effective siRNA-based therapeutics. Design of the siRNA and several delivery systems are included in Calando's portfolio of technologies and the company can provide:

Design of siRNA molecules – proven proprietary technology for sequence selection and construction of effective siRNA molecules

Generalized delivery systems – binds to and self-assembles with the siRNA to form uniform colloidal-sized particles. Analysis has shown that these particles are spherical and ca. 50 nm in diameter.

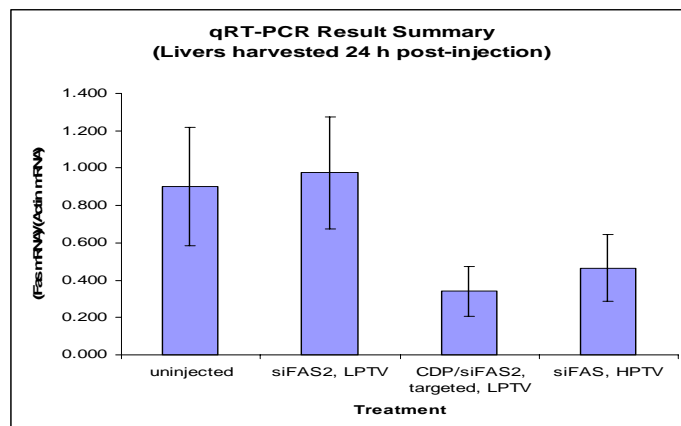
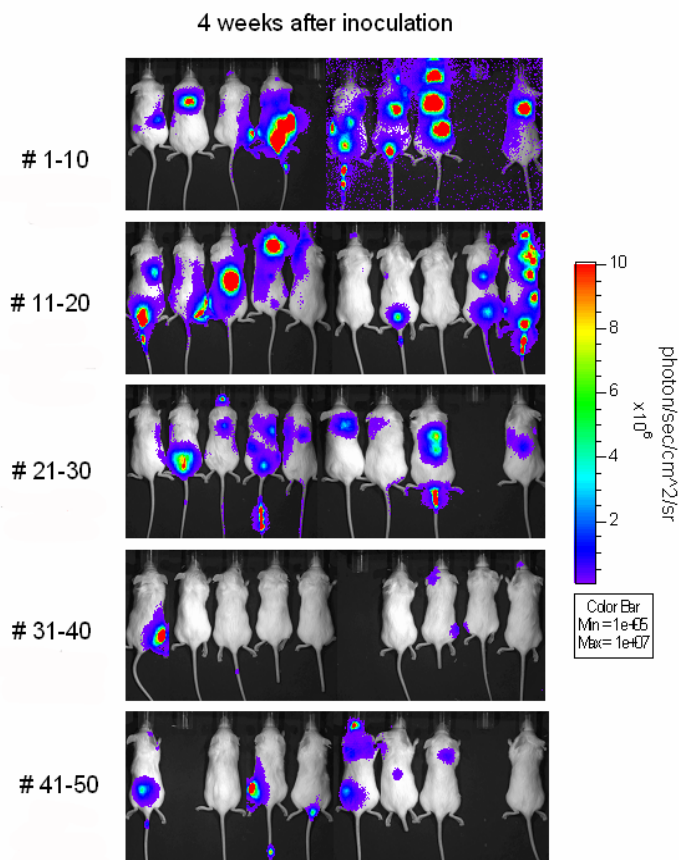
Stealthy to the immune system – The delivery vector allows for repeat dosing without the risk of immune reactions. **Unlike lipid delivery vehicles, the cyclodextrin-based delivery system does not cause an interferon response.**

Non-toxic – has been shown to be non-toxic in *in vitro* testing with human cell cultures, and the fully formulated polymer/siRNA particles produced no toxicity in mice even when repeated doses (up to 8 doses over a four week period) are used.

Stable under physiological conditions – particles have been shown to be stable under physiological conditions.

Efficient in targeted delivery – Calando and its partners have demonstrated successful delivery of functional siRNA therapeutics to tumor cells and to hepatocytes by systemic administration and confirmed sequence-specific gene inhibition.

Fully integrated system – Calando has made significant advances in the design and development of non-viral delivery systems that bypass the extra- and intra-cellular barriers to siRNA delivery. The company is the only one to integrate both siRNA technology with delivery technology to create an effective siRNA therapeutic.



EXEMPLARY RESULTS

Calando and its collaborators have generated preclinical data that demonstrate sequence-specific gene inhibition in tumors from the systemic administration of targeted formulations of siRNA. Workers at Caltech and Children’s Hospital-LA created a mouse model of Ewing’s sarcoma using luciferase-expressing, human Ewing’s sarcoma cells that allows for real time bioluminescence monitoring (see figure above, left). Mice 1-10 received sham injections, 11-20 naked anti-EWS-FLII siRNA, 21-30 fully formulated siRNA with an unrelated sequence to EWS-FLII, 31-40 fully formulated anti-EWS-FLII siRNA and 41-50 anti-EWS-FLII siRNA formulated with the delivery system that did not contain the targeting ligand. All injections were at 2.5mg/kg via the tail vein. The data in the figure show that only the targeted formulation provides any anti-tumor efficacy – control sequences and removal of the targeting ligand eliminates the anti-tumor effects. Additionally, no abnormalities in IL-12 and interferon-alpha, liver and kidney function tests, complete blood counts or pathology of major organs was observed. Of major significance is that the cyclodextrin-containing delivery system does not produce an interferon response like those obtained from lipid delivery of siRNA even when published immunostimulatory motifs are included in the siRNA.

Calando’s technology can be used with various targeting ligands. In addition to targeting tumors, the targeting of liver

has also been accomplished. Using anti-FAS siRNA, liver targeted delivery from tail vein injections in mice are able to provide FAS gene inhibition like that observed from a high pressure tail vein injection that is not clinically relevant. The data give in the figure above (right) show the reduction in FAS mRNA after a single injection (2.5 mg siRNA/kg).

These two examples demonstrate some of the technology available at Calando for the development of siRNA therapeutics.

Calando Pharmaceuticals Inc. is seeking additional collaborative arrangements for discovery and development of siRNA therapeutics.

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