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PRE-β HDL DISCOIDAL MIMETIC, CER-001, AND NOVEL APOLIPOPROTEIN A-I (APOA-I) MULTIMERS, CARGOMER®, AS NEW TARGETED DELIVERY VEHICLES FOR THERAPEUTIC CANCER MEDICINES

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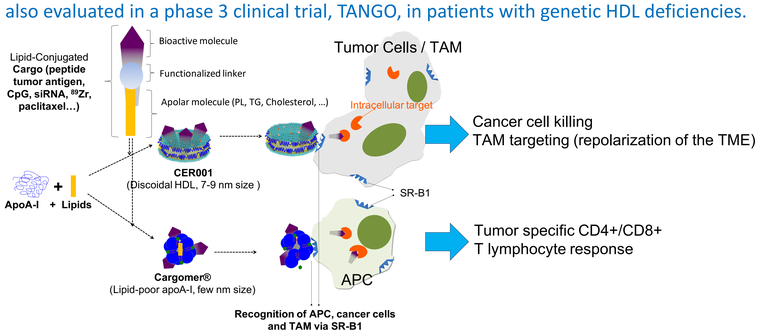
Background

Despite the huge potential of nanoparticles, the delivery efficiency of their payload to tumors has plateaued at 0.7% in the last decade (1). The ability to produce and manufacture recombinant human (rh)ApoA-I to design a new type of nanovehicles, CER-001 and Cargomer®, deploying the HDL natural features of targeted delivery system opens the way for improved means of drug delivery.

- HDLs are natural transporters in the blood as well as the lymph
- ApoA-1 is the major protein contained in HDL and binds to the scavenger receptor B1 (SR-B1) for cholesterol transport
- SR-B1 is particularly expressed on steroidogenic organs, in the liver, on macrophages including in tumor-associated macrophages and dendritic cells, and has been shown to be overexpressed in several cancer types (2)
- CER-001 and Cargomer® are negatively charged nanosize biocompatible particles, which consist of rhApoA-I and a controlled lipid composition

Cerenis' rhApoA-I pre-β discoidal HDL mimetic, CER-001, loaded with ^{89}Zr is currently evaluated for tumor imaging in a phase II clinical trial, TARGET, in esophageal cancer patients; preliminary data analysis shows ^{89}Zr accumulation in the tumor by PET/CT imaging(1). CER-001 is also evaluated in a phase 3 clinical trial, TANGO, in patients with genetic HDL deficiencies.



APC: Antigen-Presenting Cells, SR-B1: Scavenger receptor-B1, TMA: Tumor-Associated Macrophages, TME: Tumor MicroEnvironment

Figure 1: CER-001 and Cargomer® are HDL mimetics.
Those HDL nanoparticles are fully biocompatible and able to carry bioactive molecules to selectively bring them to tumor.

Objectives

- Assess the capacity of Cerenis tumor-targeted delivery HDL mimetic platform to accommodate multiple cargos as cytotoxic drugs and oligonucleotides as small interfering (si)RNA targeting oncogenes, or immuno-stimulating oligos targeting Toll-like receptor 9.
- Assess the delivery efficiency by the most advanced Cerenis' drug delivery technology, CER-001, of its cargo to tumor in vivo.

Design

- Production of HDL mimetic nanoparticle complexes consisting in CER-001 loaded with Paclitaxel (CER-001/Paclitaxel), a poorly soluble drug, or with modified cholesterol-conjugated siRNA (CER-001/Chol-siRNA) targeting the oncogenic KRAS mutant G12D (KRAS^{G12D}) through a proprietary manufacturing process.
- Production of an innovative multimeric rhApoA-1 lipid-poor particles, Cargomer®, loaded with modified cholesterol-conjugated CpG (Cargomer®/Chol-CpG) following a similar process.
- *In vivo* treatment with CER-001/Paclitaxel of tumor-bearing mice and measurement of delivery efficiency to tumor compared to positive control paclitaxel protein-bound nanoparticles (Abraxane®).

In vitro Preparation of CER-001 and Cargomer® complexes with Paclitaxel or oligonucleotides

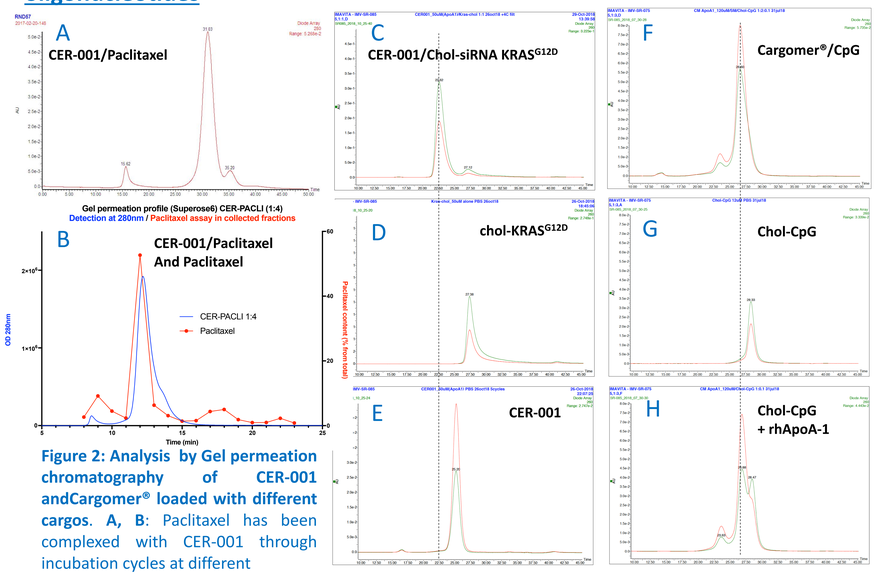


Figure 2: Analysis by Gel permeation chromatography of CER-001 andCargomer® loaded with different cargos. **A, B**: Paclitaxel has been complexed with CER-001 through incubation cycles at different temperatures to form CER-001/Paclitaxel HDLparticle mimetics composed of ApoA-1/DPPG/ Sphingomyelin/Paclitaxel at the ratio 1/2.7/97/3. C: CER-001 particles have been loaded with modified cholesterol-conjugated siRNA targeting oncogenic KRASG12D at ratio 1:1 ApoA1:Chol-siRNA KRASG12D. D, E: chol-KRAS^{G12D} alone and CER-001 alone, respectively. F: Cargomer®, containing sphingomyelin, loaded with modified cholesterol-conjugated CpG. G, H: Chol-CpG alone, Chol-CpG mixed with rhApoA-1 without sphingomyelin, respectively. Sphingomyelin is a key component of Cargomer® to allow the formation of nanoparticle complexes.

⇒ **rhApoA-1 CER-001, a discoidal HDL mimetic can be easily complexed with apolar molecules such as paclitaxel, a cancer cytotoxic drug, or cholesterol-conjugated siRNA.**

⇒ **Multimeric ApoA-1, Cargomer®, can be easily complexed with cholesterol-conjugated oligonucleotides such as CpG.**

In Vivo efficacy of CER-001/Paclitaxel nanoparticles in an orthotopic human breast cancer Tumor model

Gps	N b of animals	Treatment group	Dose of Paclitaxel (mg/kg)	Route / Frequency
1	10	Vehicle	0	IV / Daily for 5 consecutive days, then, twice weekly
2	10	Abraxane®	10	
3	10	CER-001/Paclitaxel	10	

Table 1: Study design.
Female NOD-SCID mice were xenografted in the mammary fat pad with 2x106 MDA-MB-231 cells, a human breast cancer cell line. Treatment was initiated at day 25 post tumor cell engraftment until euthanasia at d57, 24H after last treatment administration. Tumor growth and body weight were monitored during the full course of the experiment. Tumors were harvested at euthanasia. Placlitaxel content in tumors was measured by HPLC/UV.

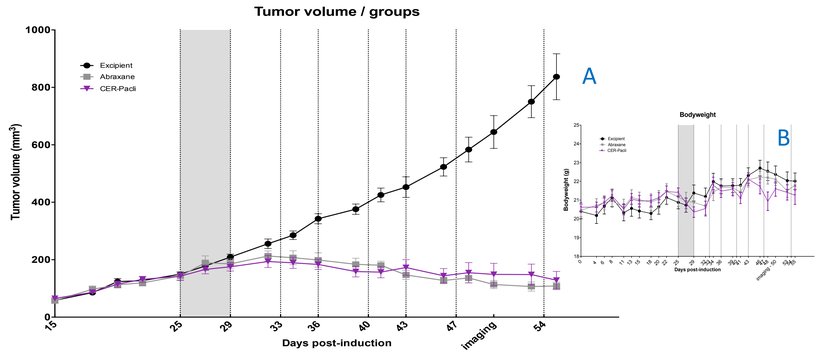


Figure 3: Tumor growth inhibition following CER-001/Paclitaxel treatment. Tumors were palpable and measurable in all animals at day 15 and reached a volume of 50-100 mm3 around day 20. Treatment started at day 25. **A**: tumor growth; **B** insert: body weight.

⇒ **CER-001 with paclitaxel at the dose of 10mg/kg and a ratio of 1:3 ApoA-1:Paclitaxel shows a therapeutic activity as potent as Abraxane® with tumor regression observed in all mice. Body weight evolution was similar in all groups.**

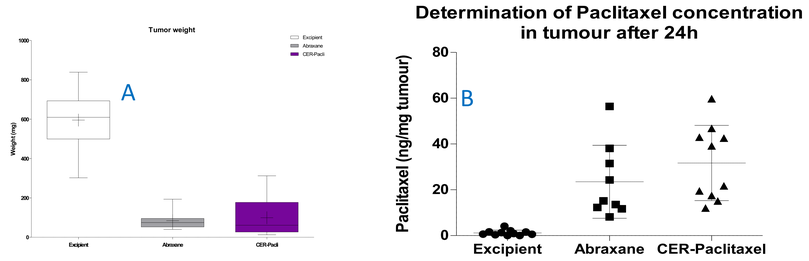


Figure 4. A: tumor weight at day 57, B: Paclitaxel contained in tumor.

Day	D25	D57	D57
Groups	**Tumor vol. (mm3)**		**paclitaxel : tumor weight ratio**
Vehicle	150	864	1.2
Abraxane®	143	105	23.5
CER-001/Paclitaxel	143	141	31.7

Table 2: Delivery efficiency. The dosage of Paclitaxel in tumor tissue was performed 24h post last treatment dose.

⇒ **Delivery efficiency of paclitaxel by CER-001/Paclitaxel nanoparticles in tumor *in vivo* is at least as potent as with Abraxane® treatment.**

Conclusion

⇒ **The CER-001 and Cargomer® HDL mimetics are flexible targeting nanoparticles that can accommodate multiple cargos including cytotoxic cancer drugs as Paclitaxel or oligonucleotides targeting oncogenic and immunogenic pathways as siRNA or antisense DNA and CpG, respectively.**

⇒ **CER-001-Paclitaxel is at least as efficacious as Abraxane® nanoparticles to deliver and treat tumor-bearing mice.**

(1) Wilhelm et al, Nat. Rev._Materials, 2016, Analysis of nanoparticle delivery to tumours; (2) Moobery et al, Front Pharmacol, 2016, Targeting the SR-B1 Receptor as a Gateway for Cancer Therapy and Imaging ; (3) First results of TARGET PHASE II study demonstrate the ability of CER-001, an HDL mimetic, to target tumor in patients with esophageal cancer - Cerenis Therapeutics Press release 25/06/2018