

NOVEL APOLIPOPROTEIN-A1 (ApoA-I) MULTIMERS, CARGOMER[®], AS NEW TARGETED DELIVERY PLATFORM FOR THERAPEUTIC CANCER VACCINES WITH TUMOR NEO- AND SHARED ANTIGENS

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Background

Therapeutic vaccination is a highly promising tumor-specific approach in immuno-oncology but has shown insufficient clinical efficacy, so far. The development of new technologies to target the delivery of Tumor Antigens (TA) and immunostimulatory molecules into antigen-presenting cells (APC) both alone and in combination with immune checkpoint inhibitors opens up new opportunities for cancer treatments.

Cerenis' recombinant human (rh)ApoA-I pre- β discoidal HDL mimetic, CER-001 loaded with ⁸⁹Zr is currently evaluated for tumor imaging in a phase II clinical trial, TARGET, in esophageal cancer patients; preliminary data analysis shows ⁸⁹Zr 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.

Based on know-how gained with CER-001, we developed Cargomer[®], innovative multimeric rhApoA-I HDL mimetics, to enhance the targeted delivery of TA and an adjuvant to APC.

- HDLs are natural transporters in the blood as well as the lymph
- ApoA-1 is the major protein contained in HDL and binds to receptor SR-B1 for cholesterol transport
- SR-B1 is expressed on macrophages and dendritic cells and has been shown to be overexpressed in several cancer types^(2,3)
- CER-001 and Cargomer[®] are nanosize biocompatible particles, which consist of rhApoA-I and a controlled lipid composition

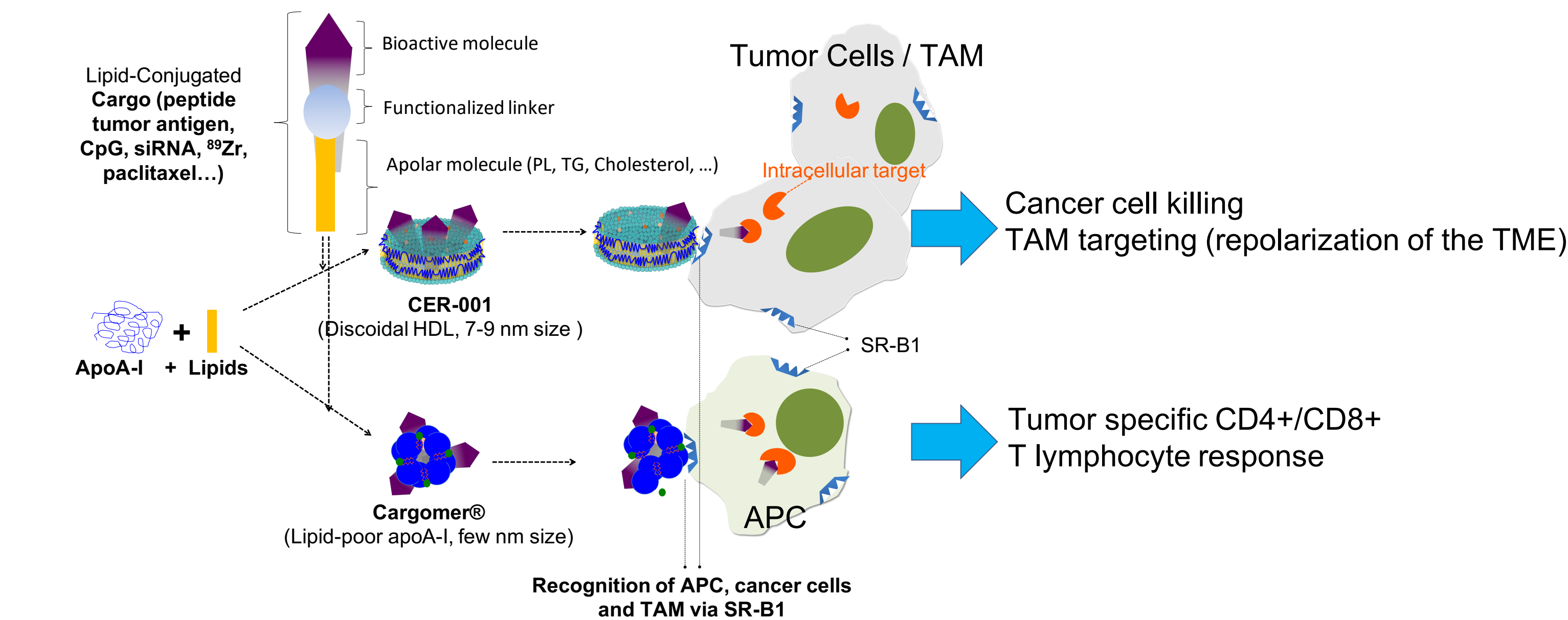


Figure 1: CER-001 and Cargomer[®] are HDL mimetics.

Those HDL nanoparticles are fully biocompatible and able to carry bioactive molecules to selectively bring them to APC and tumors

Objectives

- To develop an innovative multimeric rhApoA-I HDL mimetics, Cargomer[®], to enhance the targeted delivery of TA and immunostimulatory molecules to APC.
- To assess the immunotherapeutic potential of Cargomer[®] complexed with neopeptide peptides and a TLR9 agonist as a standalone treatment and in combination with immune checkpoint blockade (ICB) treatment in an aggressive mouse-tumor model.

Design

- Cargomer[®] have been complexed with three phospholipid-conjugated synthetic long peptides: M27 and M30, 2 neopeptides of the mouse melanoma B16F10 cells and Trp2 an overexpressed melanoma TA, as well as a cholesterol-conjugated CpG1826, a mouse TLR9 agonist.
- *In vitro* and *in vivo* experiments have been performed to assess the delivery capacity and induction of a therapeutic immunization in B16F10-tumor bearing mice.

In vitro Preparation and Activity of Immunotherapeutic Cargomer[®]

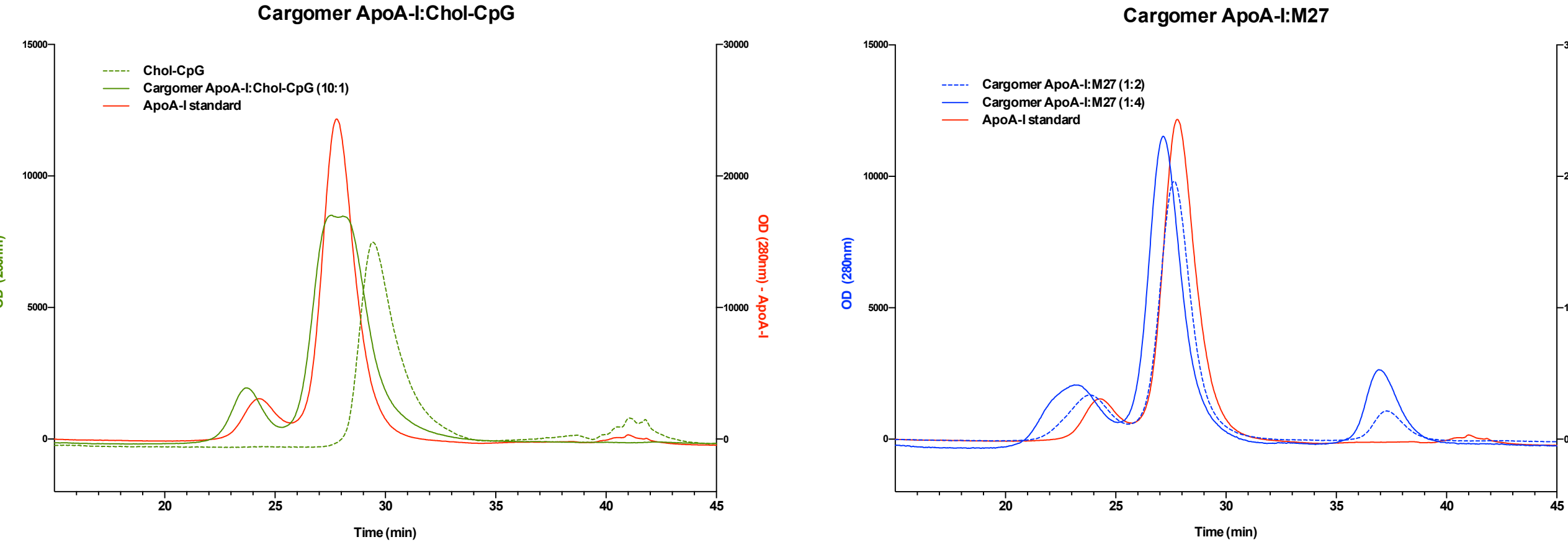


Figure 2: HPLC-SuperdexTM 200 analysis of Cargomer[®] loaded with peptides or CpG. Cargomer[®] have been loaded either with M27, M30, or TRP2 peptides chemically conjugated to phospholipids (PDP-PE) or with CpG1826 conjugated to Cholesterol (Chol-CpG) at 1:2 and 1:4 ApoA-I:peptide ratio, and 10:1 ApoA-I:Chol-CpG ratio. Chromatograms of Cargomer[®] ApoA-1:M27 at 1:2 and 1:4 ratio are shown as representative Cargomer[®] ApoA-1: peptide chromatograms,

⇒ **Multimeric ApoA-1 Cargomer[®] can be easily complexed with synthetic peptides and CpG oligo, both conjugated to lipids to form loaded HDL nanoparticles**

Groups	Formulation	OD
Neg .control	Buffer	0.5
Neg. control	ApoA-1 (empty Cargomer [®])	0.6
Pos. control	CpG/Lyovec TM	3.2
Test article	Chol-CpG	1.5
Test article	Cargomer [®] /Chol-CpG	2.1

Table 1: TLR9 activation by Cargomer[®]/Chol-CpG complexes in *in vitro* cell-based assay. Murine TLR9-expressing HEK 293 cells (HEK-BlueTM-TLR9 cells; InvivoGen) were incubated with Cargomer[®] ApoA-1:Chol-CpG (10:1 ratio) at same CpG concentration as CpG/LyovecTM positive control, or with negative controls at 37°C for 24h.

⇒ **Cargomer[®] efficiently deliver CpG to intracellular compartment to activate TLR9**

In Vivo Efficacy of Therapeutic Immunization in Tumor-Bearing mice

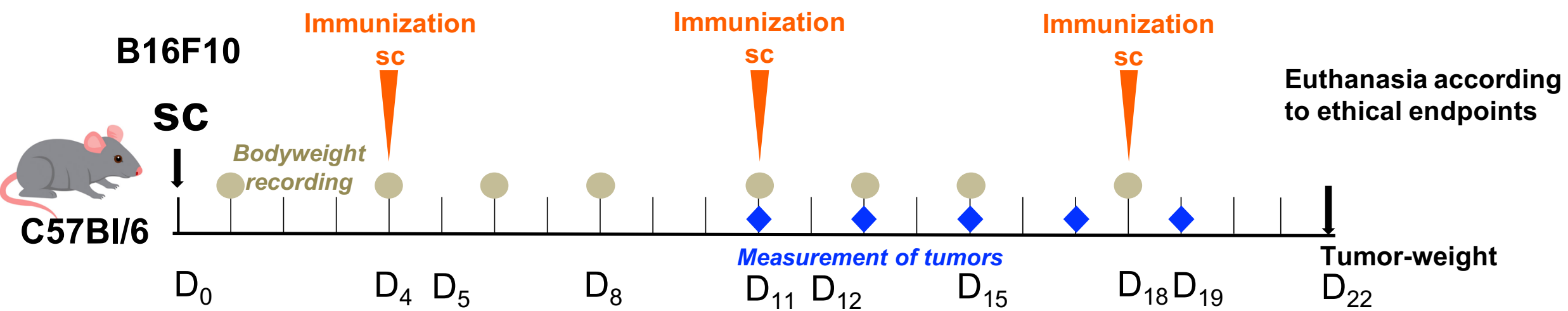


Figure 3: Immunization protocol of B16-F10-tumor bearing mice

- Group 1: PBS
- Group 2: free peptides + Chol-CpG
- Group 3: Cargomer[®] ApoA-1:peptide: (1:2) + Cargomer[®] ApoA-1:Chol-CpG (10:1)
- Group 4: Cargomer[®] ApoA-1:peptide: (1:4) + Cargomer[®] ApoA-1:Chol-CpG (10:1)

Equivalent doses of ApoA-1 (30nmol) complexed with peptides M27, M30 and Trp2 and Chol-CpG were administered per injection (8 mice per group). One representative experiment of 3.

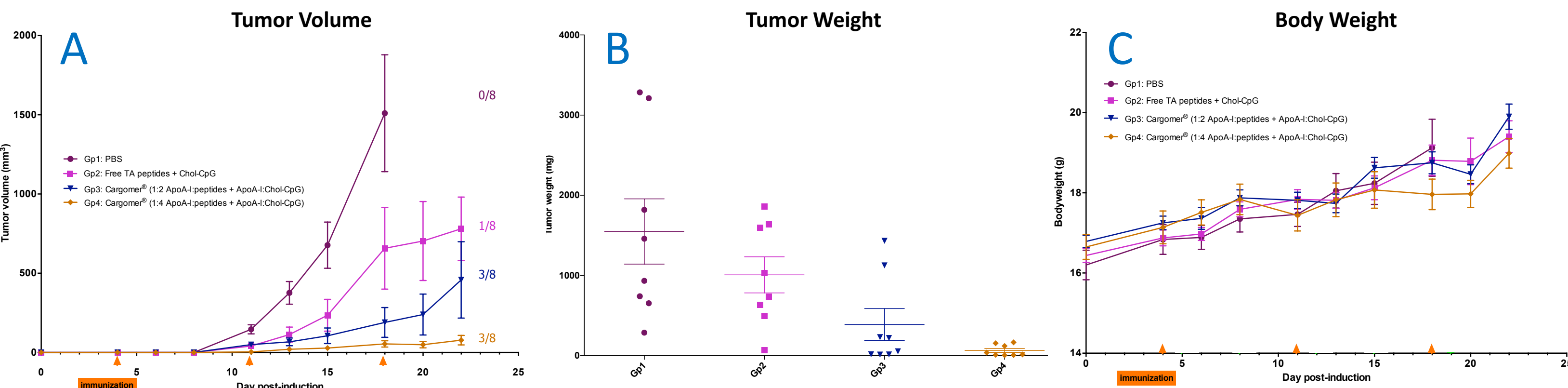


Figure 4: Tumor growth inhibition following Cargomer[®] therapeutic immunization.

A: tumor volume, B: tumor weight at euthanasia, C : body weight

⇒ **Cargomer[®] loaded with TA peptide cocktail and Chol-CpG induce a potent and well tolerated therapeutic anti-tumor response**

Long Term Immunotherapeutic Efficacy and Combination with ICB

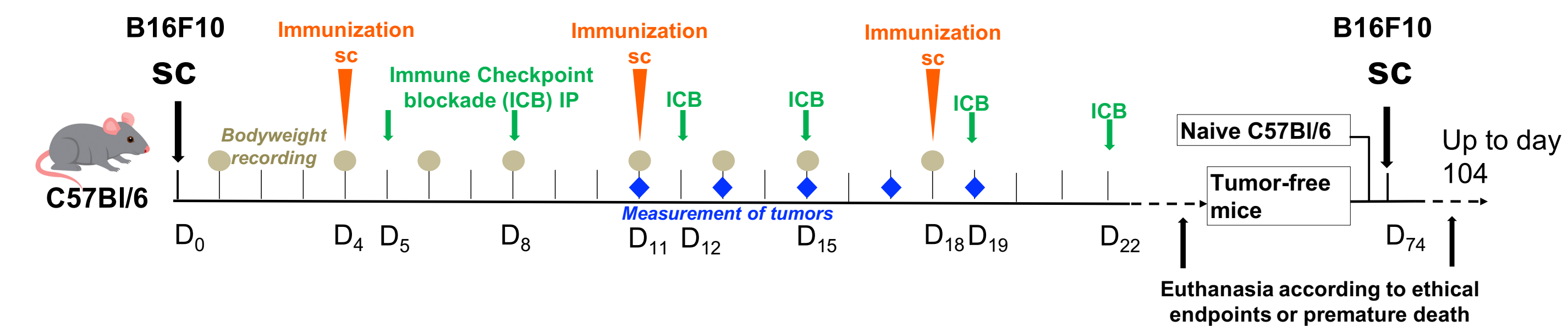


Figure 5: Treatment protocol for long term follow up and tumor rechallenge. Same immunization protocol as described in figure 3. In addition, groups 6, 7, and 8 received ICB treatment, i.e. anti-CTLA-4 and anti-PD-1 mouse antibodies. See groups in Table 2: 8 naive mice per group at day 0 and 6 naive and tumor-free mice from treated groups. 4, 5, 6, 7 were rechallenged with B16F10 tumor cells

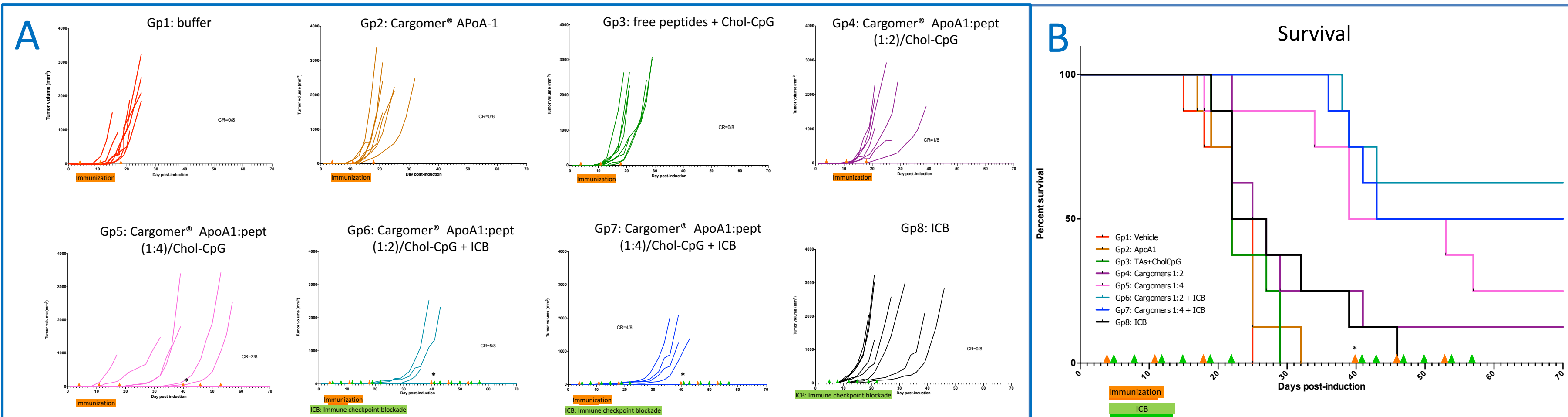


Figure 6: Effect of Cargomer[®] immunization on tumor growth and survival in B16F10-bearing mice A: Individual tumor growth curve per group; B: Kaplan-Meier plot per treatment group.

*Some animals presenting late tumor development from day 40 to 57 post-induction received additional treatments before day 70

Gp	Treatments	Med. Surv. (days)	Tumor-Free Mice (d70)
1	Buffer	23.5	0/8
2	rhApoA-I (empty Cargomer [®])	22	0/8
3	TA peptides + Chol-CpG	22	0/8
4	Cargomer [®] 1:2 ApoA-1:peptide/Chol-CpG	26	1/8
5	Cargomer [®] 1:4 ApoA-1:peptide/Chol-CpG	46	2/8
6	Cargomer [®] 1:2 ApoA-1:peptide/Chol-CpG + ICB	>70	5/8
7	Cargomer [®] 1:4 ApoA-1:peptide/Chol-CpG + ICB	56.5	4/8
8	ICB	24.5	0/8

Table 2: Median survival time and number of tumor-free mice per group at day 70. Tumor-free mice were rechallenged with B16F10 (see fig. 7).

⇒ **Cargomer[®] immunotherapy induces durable anti-tumor response and synergizes with ICB *In vivo***

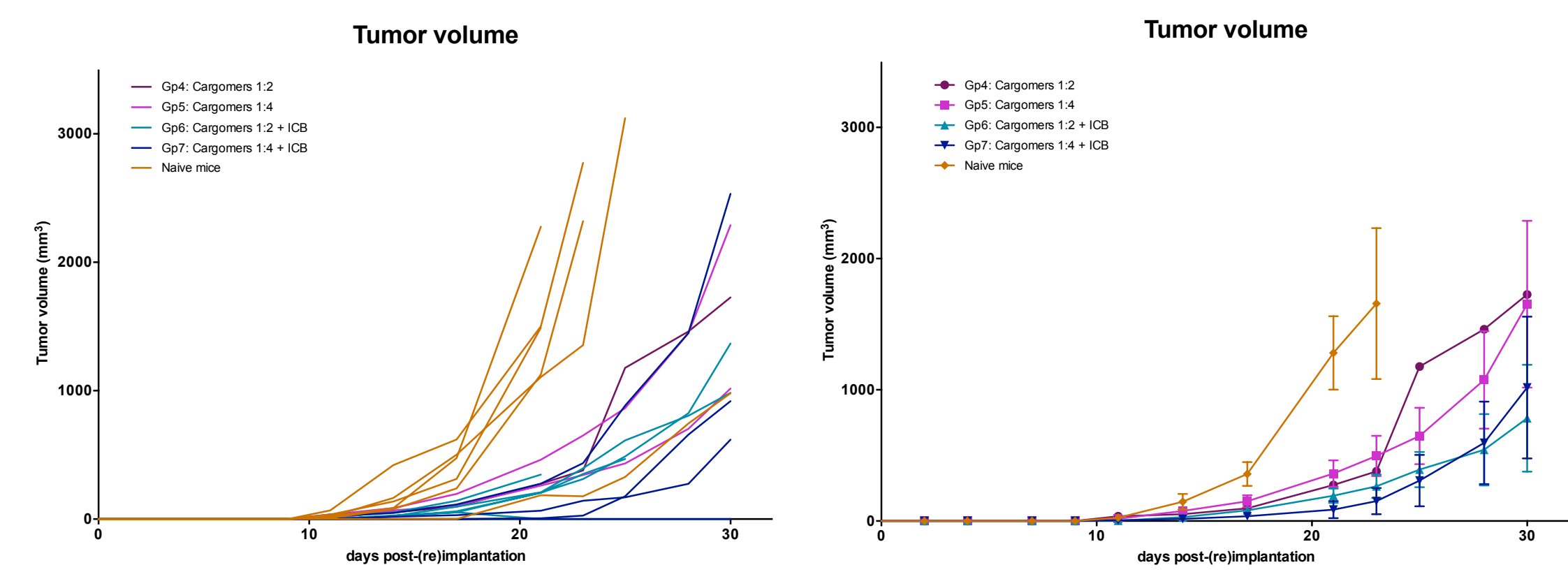


Figure 7: late tumor rechallenge in tumor-free survivors: mouse survivors following treatments and naive mice were challenged SC with 10⁵ B16F10 cells at day 74.

⇒ **Cargomer[®] immunotherapy induced immune memory**

Conclusion

⇒ **Immunization with Cargomer[®] loaded with peptides and CpG conjugated to lipids induces a potent and long lasting anti-tumor immunotherapeutic response *in vivo***

⇒ **Cargomer[®] is a flexible and promising targeted delivery platform for immunotherapy and use in personalized neoantigen therapeutic vaccine protocols**

⁽¹⁾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; ⁽²⁾Barth et al, J. Virol, 2008, Scavenger Receptor Class B Is Required for Hepatitis C Virus Uptake and Cross-Presentation by Human Dendritic Cells; Scavenger Receptor Class B Is Required for Hepatitis C Virus Uptake and Cross-Presentation by Human Dendritic Cells; ⁽³⁾ Mooberry et al, Front Pharmacol, 2016, Targeting the SR-B1 Receptor as a Gateway for Cancer Therapy and Imaging