# Improving IL-15 antitumor efficacy:
## fusion protein strategy

- **Apolipoprotein A-I (Apo-AI)** is the major protein constituent of the HDL particles.
- **Apo-AI fusion proteins** have an optimized pharmacokinetic/pharmacodynamic profile as they show the following properties:
  - Apo-AI increases the plasmatic **half-life** and the **liver** retention of the fused proteins.
  - **Blood-brain barrier active transport**, mediated by a saturable transporter that limits the amount of the fusion protein in the central nervous system.
  - **Modulation of activity**: Apo-AI activates MAPKs through ABCA1 and SR-BI and, therefore, modulates the therapeutic effect of fused proteins.
- **pSushi-IL-15-Apo** is a new **proprietary triple fusion protein** developed combining apolipoprotein A-I, IL-15 and IL-15Rα's sushi, with:
  - Superior antitumor effect than IL-15 as a single agent.
  - Higher capacity than hIL15 to enhance antibody dependent cell cytotoxicity (ADCC) when combined with monoclonal antibodies.
  - Increased stability in circulation.
  - Facilitated trans-presentation.
- **Primary Indication**: adjuvant of tumor specific antigen antibodies in lymphoma, breast cancer and colorectal cancer treatment.

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| **Medical need** | Enhancement of the immune response against the tumors with emphasis in **synergistic approaches** with the current standard of care. |
| **Proof of Concept** | · Immunotherapeutic effects against metastatic disease in *in vivo* tumor models, (B16OVA lung metastasis of melanoma & MC38 colon cancer liver metastasis).<br>· *In vitro* assays testing the ADCC capacity of Sushi-IL15-Apo in combination with cetuximab against colorectal cancer-derived cell lines. |
| **Safety** | · Toxicity is an on-target side effect; a therapeutic window has been found.<br>· Repeated doses are feasible without desensitization or cumulative toxicity. |

**Product Profile**

- A plasmid encoding a triple fusion protein combining apolipoprotein A-I, IL-15 and the Sushi domain.
- Apo A-I acts as a natural vehicle that facilitates the IL-15/IL-15Ra sushi domain anchorage for trans-presentation of the cytokine.
- pSushi-IL-15-Apo is highly active and plasmid doses within the therapeutic range have been identified.
- These plasmid doses were tolerated and promoted the proliferation and accumulation of NK and memory CD8+ T cell in the spleen and in the liver (Fig. 1 and Fig. 2).
- In a classical cromium release assays it has been shown that human Sushi-IL15-Apo protein increases ADCC capacity of cetuximab against a tumoral cell line (Fig. 3).

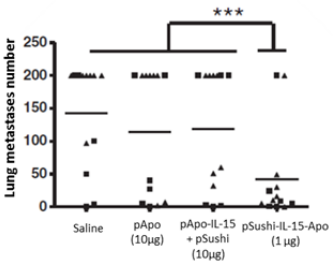


Fig.1. pSushi-IL-15-Apo gene transfer to the liver ameliorates melanoma lung metastasis of B16OVA.

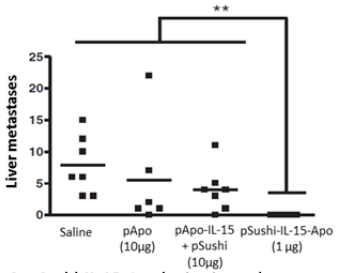


Fig.2. pSushi-IL-15-Apo hydrodynamic gene transfer is active against liver metastases of MC38 colon carcinoma.

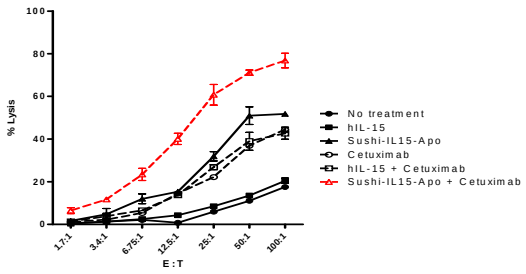


Fig.3. Human Sushi-IL-15-Apo increases ADCC capacity of cetuximab in chromium$^{51}$ release assays against HT-29 colon-carcinoma cells.

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| **Apo Platform: other fusion proteins** | · InterApo: ApoA-I fused to IFNα.<br>· Apo-Linker-144: Apo-AI fused to P144, an inhibitor peptide of TGFβ.<br>· Apo-CT1: Apo-AI fused to cardiotrophin 1.<br>· Other fusion proteins are under development. |
| **Intellectual Property** | PCT/ES2009/070224. Conjugates for the administration of biologically active compounds (licensed to Digna Biotech).<br>PCT/ES2010/070818. Novel conjugates and compositions for immunotherapy and antineoplastic treatment (licensed to Digna Biotech). |

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