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Selected opportunities in Immunology

HLA-Class II Artificial Antigen Presenting Cells in CD4 + T Cell-Based Immunotherapy (BIO15322&BIO18370)

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HLA-Class II Artificial Antigen Presenting Cells in CD4 + T Cell-Based Immunotherapy (BIO15322&BIO18370)

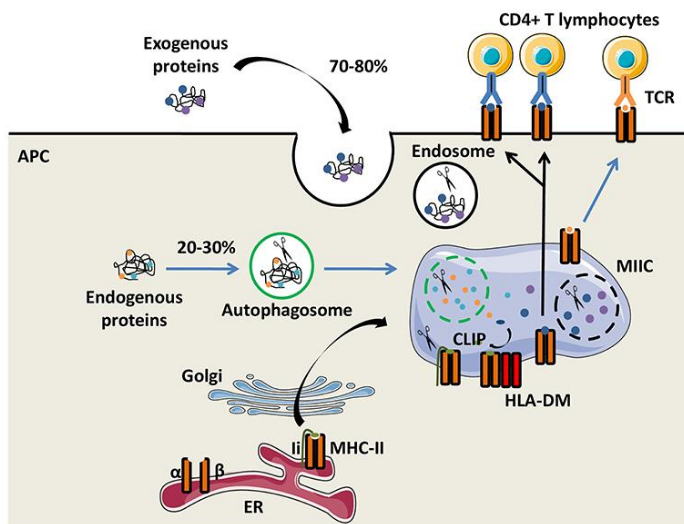
Product factsheet

stage

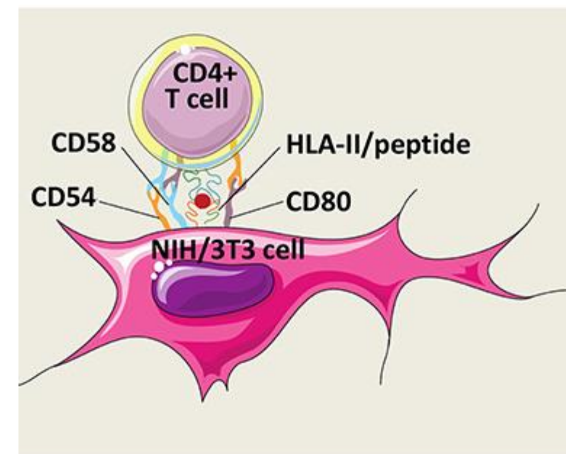
- **Product:**
  - HLA-Class II Artificial Antigen Presenting Cells
- **Application:**
  - CD4 + T Cell-Based Immunotherapy for, respectively, chronical viral infections and cancer, or severe autoimmune diseases and transplantation
- **Rational and POC:**
  - Stable artificial antigen presenting cells (AAPCs) derived from mouse fibroblasts were genetically modified to express HLA-DR molecules and the human accessory molecules B7.1, ICAM-1 and LFA-3.
  - AAPCs expressing HLA-DR1, HLA-DR15 or HLA-DR51 molecules exogenously (pulsing) or endogenously loaded with antigens derived from influenza hemagglutinin (HA), myelin basic protein or factor VIII were shown to activate specific CD4+ T cell clones more effectively than EBV-transformed B cells.
  - The inventors also showed that AAPCs were able to take up and process whole Ag proteins, and present epitopes to specific T cells.
  - Although AAPCs were less effective than autologous PBMCs to stimulate specific CD4+ T cells in primary culture, AAPCs were more potent to reactivate and expand memory Th1 cells with an effector and or transitional memory phenotype.
  - Finally specific memory regulatory T cells (Tregs) purified from circulating CD4+/CD25+ T cells (Thymic Tregs) and primed by Ag-loaded APCs in presence of rapamycin and IL-2 could be amplified by AAPCs in the same conditions.
- **Patent and publication:**
  - WO 2017/093464
  - WO 2020/120649
  - Couture A, Garnier A, Docagne F, Boyer O, Vivien D, Le-Mauff B, Latouche JB, Toutirais O. HLA-Class II Artificial Antigen Presenting Cells in CD4+ T Cell-Based Immunotherapy. Front Immunol. 2019 May 17;10:1081. doi: 10.3389/fimmu.2019.01081. PMID: 31156634; PMCID: PMC6533590.
  - Garnier A, Hamieh M, Drouet A, Leprince J, Vivien D, Frébourg T, Le Mauff B, Latouche JB, Toutirais O. Artificial antigen-presenting cells expressing HLA class II molecules as an effective tool for amplifying human specific memory CD4(+) T cells. Immunol Cell Biol. 2016 Aug;94(7):662-72. doi: 10.1038/icb.2016.25. Epub 2016 Feb 29. PMID: 26924643.

Proof of concept

Basic Principle



The MHC-II antigen presentation pathways. Major histocompatibility complex class II (MHC-II) α and β chains, expressed by antigen presenting cells (APCs), are synthetized in the endoplasmic reticulum (ER) where they form a heterotrimer with the invariant chain (Ii). After maturation in the Golgi apparatus, the heterotrimer (α/β/Ii) is delivered to the MHC class II compartment (MIIC) in which endocytosed and exogenous proteins but also Ii are degraded by proteases for generating peptides. Ii is progressively degraded into the Class II Invariant chain Peptide (CLIP) which binds to the MHC-II groove. The chaperone protein HLA-DM induces CLIP replacement by an antigenic peptide. Then, the peptide/MHC-II complexes move to the plasma membrane and are presented to T-cell receptors (TCRs) of CD4+ T lymphocytes.



Model of AAPCs derived from the mouse fibroblast NIH/3T3 cell line. NIH/3T3-AAPCs express CD54, CD58, CD80, and one HLA-II molecule.

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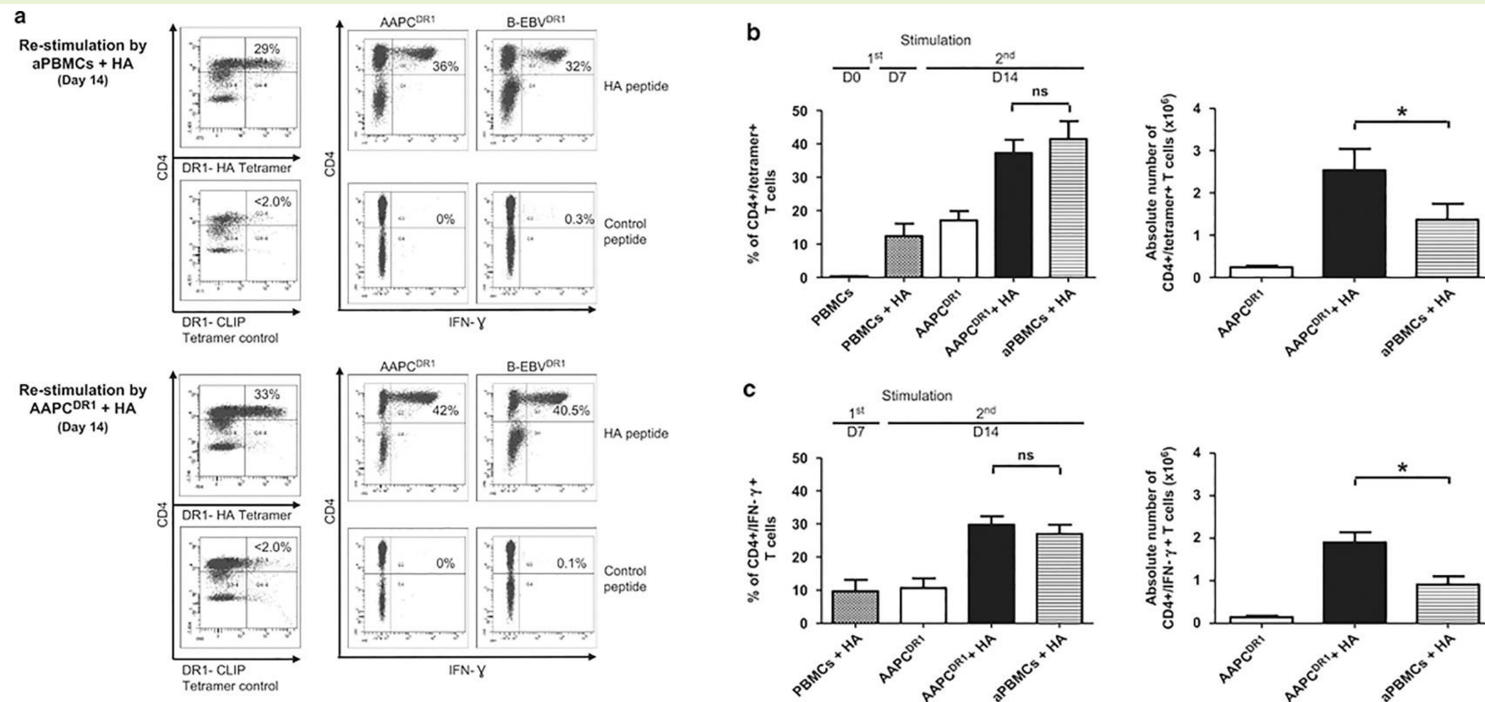
Proof of concept

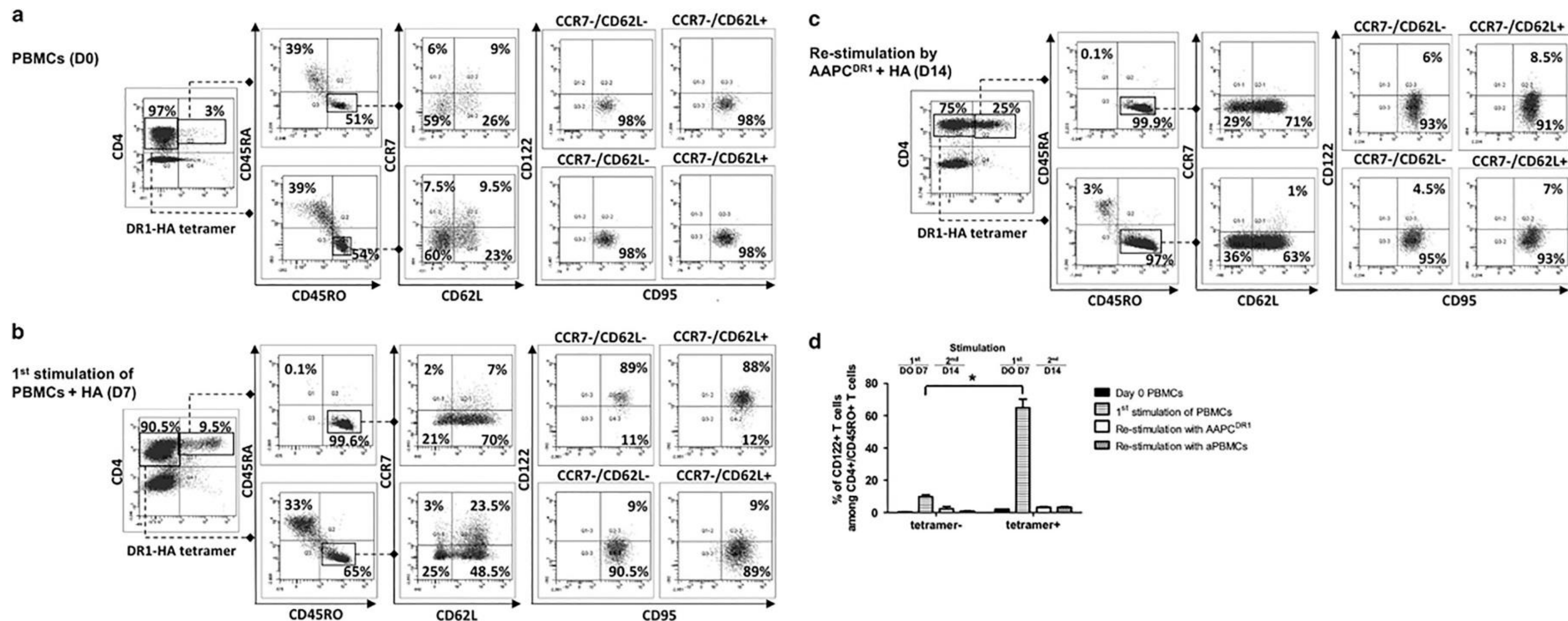
Artificial antigen-presenting cells expressing HLA class II molecules as an effective tool for amplifying human specific memory CD4+ T cells (exogenous)



Re-stimulation of Ag-specific memory CD4 T cells by AAPCs or autologous PBMCs. CD4+ T cells generated after primary culture of PBMCs with HA peptide for 7 days have been re-stimulated for 7 additional days with either AAPCDR1 or autologous PBMCs (aPBMCs) loaded or not with 10 µg ml–1 of HA peptide. At days 0, 7 and 14, the percentages and the absolute numbers of HA-specific T cells were evaluated by tetramer staining and intracellular cytokine staining (ICS). (a) A representative experiment of ICS performed after a 6h reactivation by AAPCs or B-EBV cell lines loaded with HA or control peptide. Data of tetramer staining (b) and ICS (c) are from five independent experiments with four donors. *P<0.05 (Student's paired t-test); NS, not significant.

Proof of concept

Artificial antigen-presenting cells expressing HLA class II molecules as an effective tool for amplifying human specific memory CD4+ T cells (exogenous)



Naive/memory phenotype of CD4+ T cells. PBMCs at day 0 (a), effector T cells collected after primary culture of PBMCs with HA peptide for 7 days (b) or after re-stimulation of primary effectors with AAPCDR1 loaded with HA peptide (day 14) (c) were stained with DR1-HA-tetramer and with anti-CD4, CD45RA, CD45RO, CCR7, CD62L, CD122 and CD95 mAbs. Frequencies of naive and memory subsets are represented on FACS dot plots. A representative experiment is shown from five independent experiments with four donors. (d) Frequencies of CD122+ memory cells among CD4+/CD45RO+ T cells gated in DR1-HA-tetramer negative or tetramer positive quadrant were analyzed before (day 0) and after the first PBMC stimulation or re-stimulation with AAPCDR1 or autologous PBMCs. Data are from five independent experiments with four donors. *P<0.005 (Student's paired t-test).

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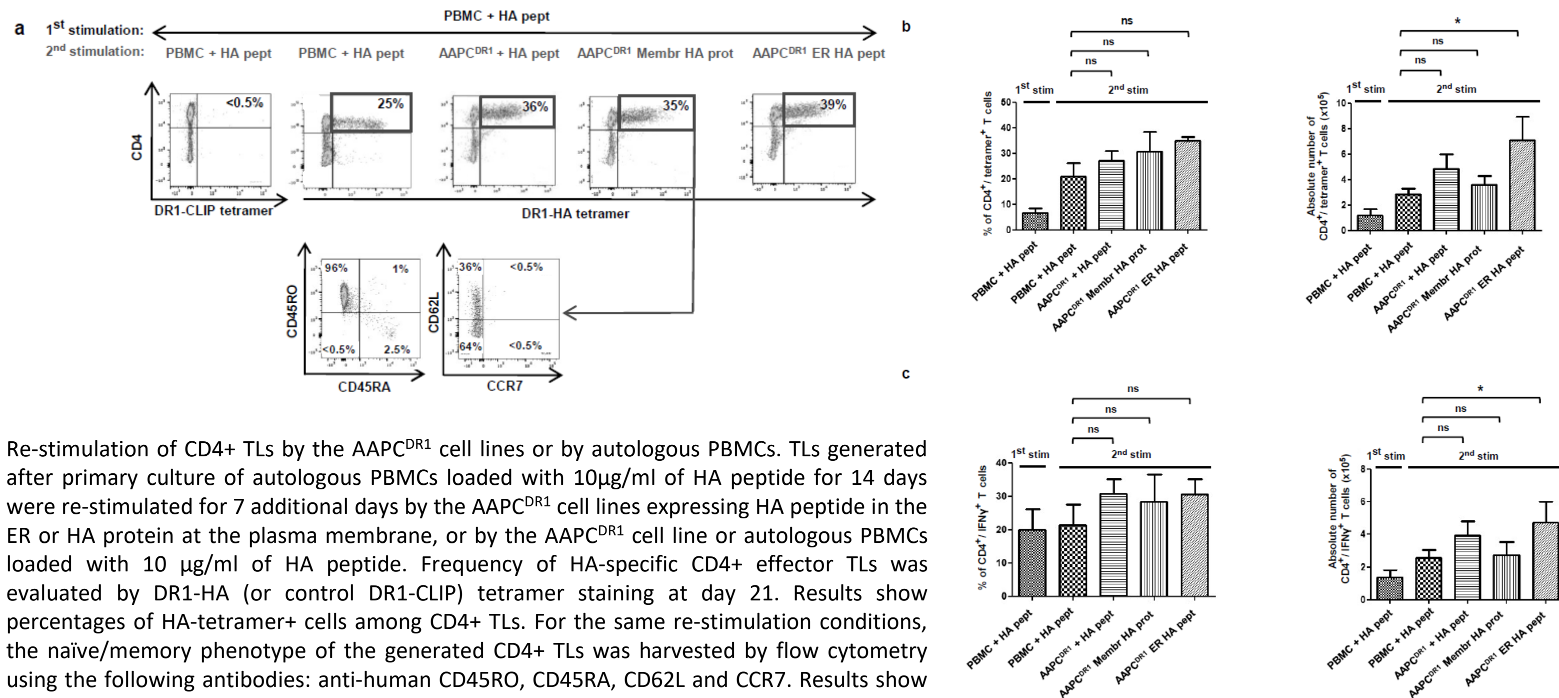

Re-stimulation of CD4+ TLs by the AAPC^{DR1} cell lines or by autologous PBMCs. TLs generated after primary culture of autologous PBMCs loaded with 10µg/ml of HA peptide for 14 days were re-stimulated for 7 additional days by the AAPC^{DR1} cell lines expressing HA peptide in the ER or HA protein at the plasma membrane, or by the AAPC^{DR1} cell line or autologous PBMCs loaded with 10 µg/ml of HA peptide. Frequency of HA-specific CD4+ effector TLs was evaluated by DR1-HA (or control DR1-CLIP) tetramer staining at day 21. Results show percentages of HA-tetramer+ cells among CD4+ TLs. For the same re-stimulation conditions, the naïve/memory phenotype of the generated CD4+ TLs was harvested by flow cytometry using the following antibodies: anti-human CD45RO, CD45RA, CD62L and CCR7. Results show percentages of cells subsets among CD4+ HA-tetramer+ TLs **(a)**. At days 14 and 21, the percentages and absolute numbers of HA-specific TLs were evaluated by tetramer staining. A representative experiment is shown from four independent experiments with four donors **(b)**. They were also evaluated by intracellular IFN-γ staining. A representative experiment is shown from six independent experiments with six donors **(c)**.*$P<0.05$ (repeated measures of one-way ANOVA analysis followed by Dunnett's post test); ns, not significant.

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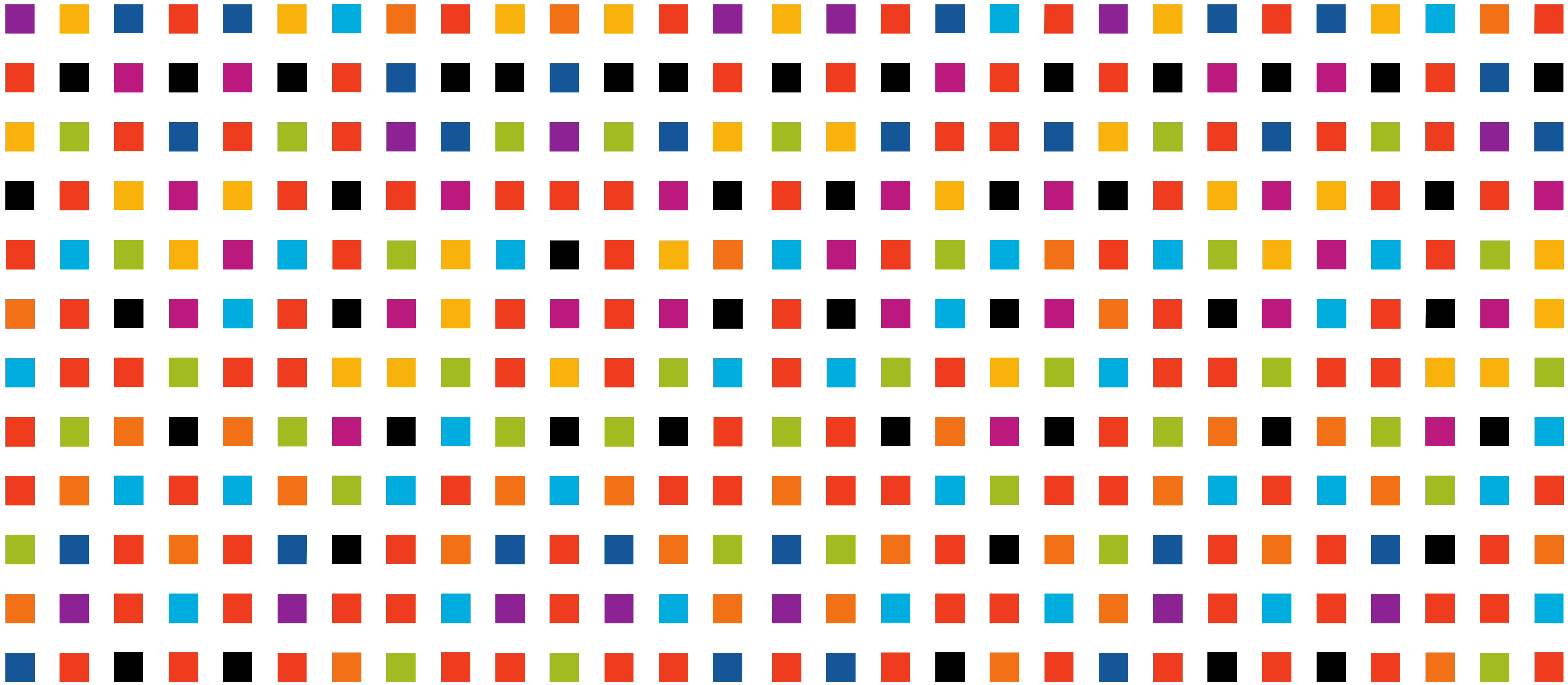

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