

Functional evaluation of CAR-T anti-CD123 from immune-mediated inflammatory diseases patients for novel pDCs-targeted immunotherapy in psoriasis

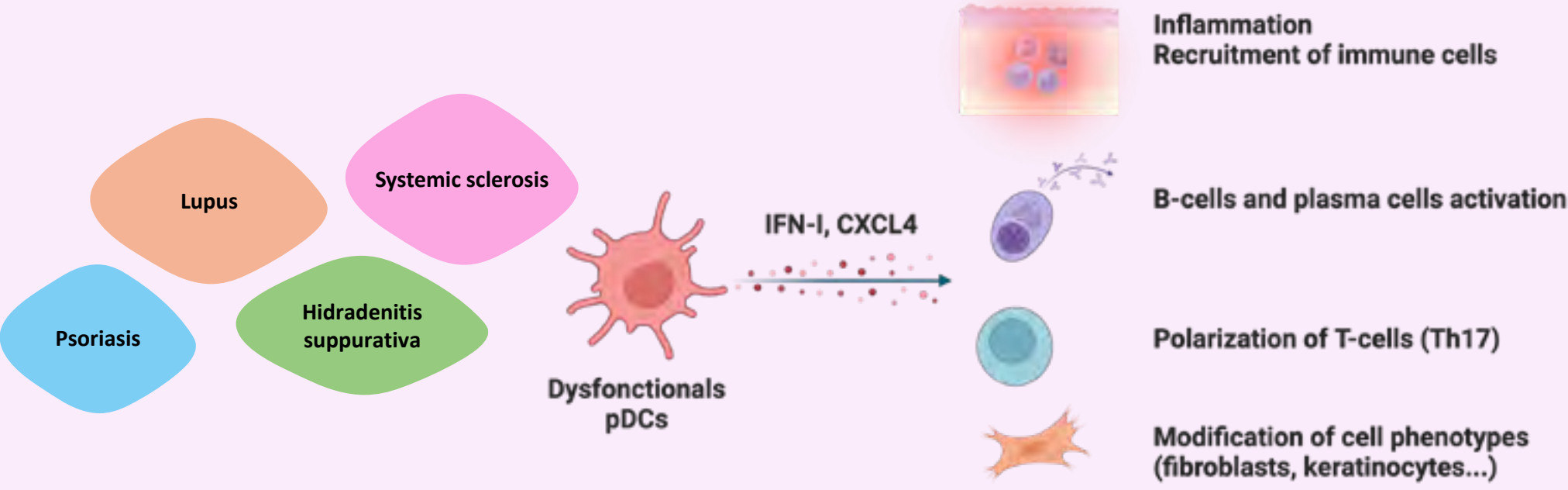
Blandine Caël^{1,2}, Elodie Bôle-Richard^{1,3}, Imane Belakri⁴, Ludivine Dal Zuffo¹, Jérémy Mazars⁵, Sébastien Humbert⁶, Fabiane Sônego⁷, Florent Creusat⁷, Kader Thiam⁷, Jeanne Galaine¹, Francois Aubin^{1,8}, Francine Garnache-Ottou^{1,9}

¹Univ. Marie et Louis Pasteur, INSERM, EFS BFC, UMR1098, RIGHT Interactions Greffon-Hôte-Tumeur/Ingénierie Cellulaire et Génique, Besançon, France; ²CHU Besançon, Autoimmunity and Allergy Laboratory, Besançon, France; ³FC Innov', Bionoveo, Besançon, France; ⁴EFS Bourgogne/Franche-Comté, Laboratoire de Contrôle Qualité des MTI, Besançon, France; ⁵Service Anatomopathologie CH Trévenans, France; ⁶Service de Médecine Interne, CH Louis Pasteur, Dole, France; ⁷GenOway, Lyon, France; ⁸Service Dermatologie, CHU Besançon, Besançon, France; ⁹CHU Besançon, Hematology and Cellular Immunology Laboratory, Besançon, France



INTRODUCTION

The success of targeted therapies using Chimeric Antigen Receptor-T (CAR T cells) in hematological malignancies has paved the way for other applications, such as immune-mediated inflammatory diseases (IMiDs). Since the landmark study by Mougiakakos *et al.*, which demonstrated complete remission of refractory systemic lupus erythematosus (SLE) following anti-CD19 CAR-T cell therapy, several clinical trials have confirmed the remarkable efficacy of this strategy across multiple IMiDs, with evidence of sustained remission (Mougiakakos *et al.*, NEJM 2021). Increasing evidence suggest that plasmacytoid dendritic cells (pDCs) are involved in the development of different pDC-driven IMiDs (Caël *et al.*, JACI 2025).



Our team has previously produced and characterized CD28/4-1BB CD123 CAR-T cells that can specifically target and kill CD123+ blasts cells. Given high CD123 expression on pDCs, we assessed the potential of CD123 CAR-T cells to eliminate pDCs across multiple IMiDs (Caël *et al.*, Br J Dermatol 2023). Our project aims to deeply characterize the phenotype and functional capacities of CD123 CAR-T using T cells from patients at different stages of disease and treated with various therapies. We evaluate the potential of a CD123 CAR-T cells to eliminate pDCs and improve skin inflammation in the context of psoriasis.

METHODS

Peripheral blood mononuclear cells (PBMC) from patients (CARDA21 study) and healthy donors (HD) were collected at day 0 (D0). A total of 44 patients were included in the study CARDA21 (24 psoriasis, 11 HS, 4 SSC and 5 SLE). PBMCs were activated and modified to express a 3rd generation CD123 CAR. CAR-T proliferation, transduction efficiency and phenotype were assessed before (day 0) , after CAR insertion and at the end of *ex-vivo* culture (day 10) (Figure 1).

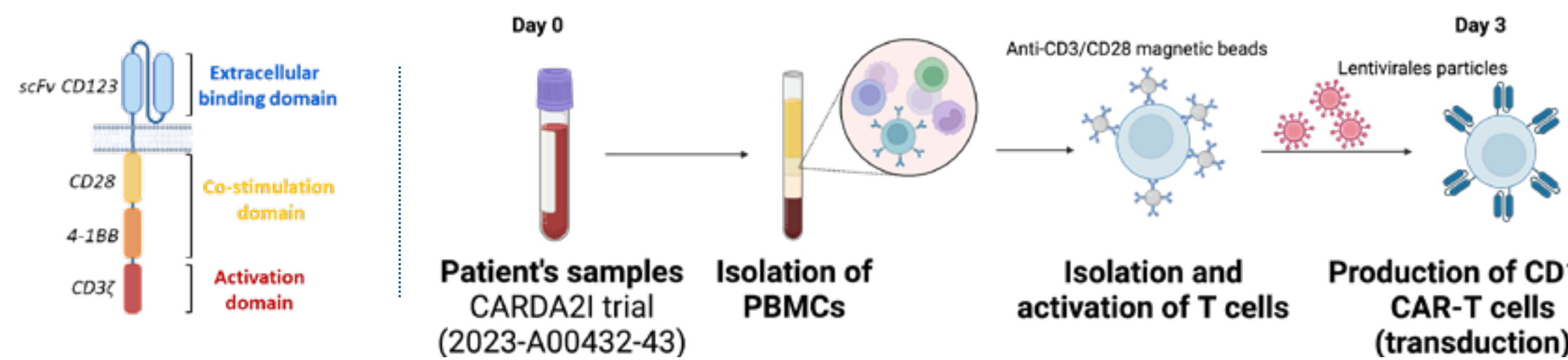


Fig 1: Full length third-generation CAR123 protein (left) and experimental setup to produced CD123 CAR-T cells from patient or healthy donors (right)

RESULTS

1) IMiDs patients T cells can be efficiently engineered to produce CAR-T cells with strong proliferative capacity and robust cytotoxicity

IMiDs patients T-cells can be efficiently engineered to produce CAR-T cells with a strong proliferative capacity (Figure 2). Transduction efficiency of the CAR123 was high in all patients (65.8% ± 19.9%, n=44) and healthy donors (HD) (78.9% ± 15.05%, n=8, ns) (Figure 2B).

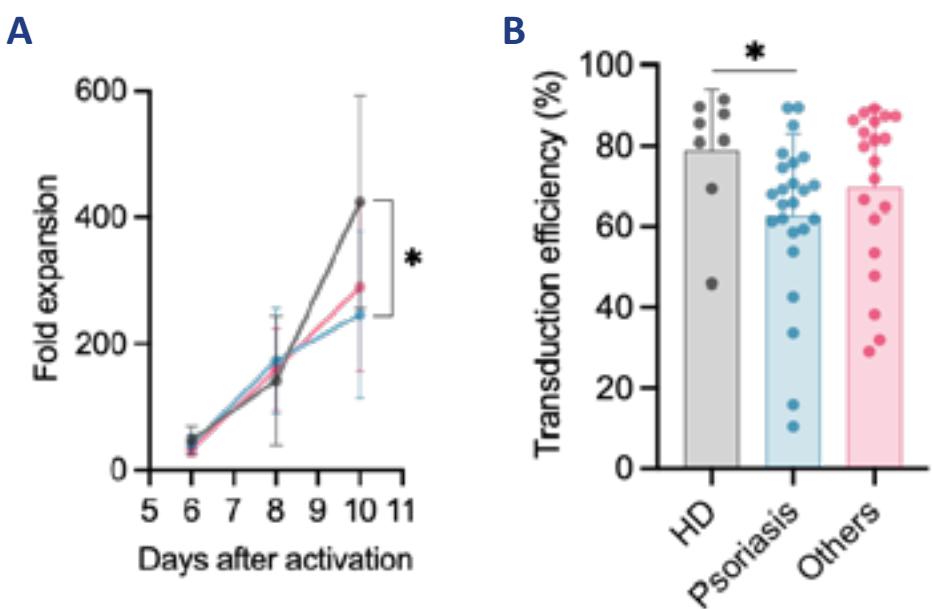


Fig 2: IMiDs patient-derived T cells can be used to generate CD123 CAR-T cells. A/ Fold expansion of CAR-T cells between day 6 and day 10 following activation, measured by T cell counting; B/ Transduction efficiency assessed by flow cytometry on day 10.

After co-culture, CAR-T cells from patients and HD displayed comparable and robust cytotoxicity against pDCs and monocytes (Figure 3A,B). As expected, no cytotoxicity was observed toward CD123 negative B cells (Figure 3B). The cytokine secretion profile revealed comparable levels of inflammatory mediators between CAR-T cells from HD and those from patients (Figure 3C).

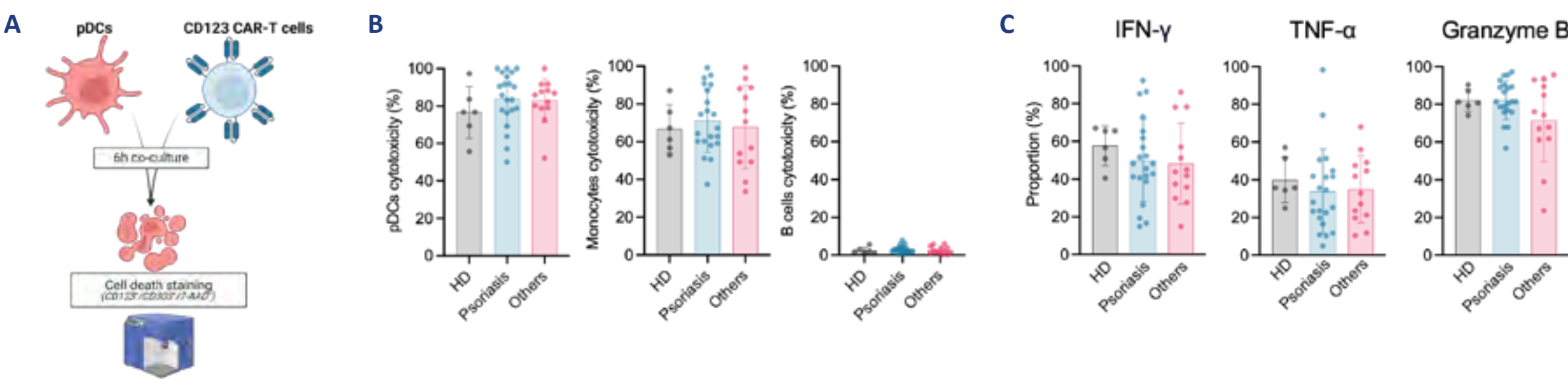


Fig 3: Cytotoxicity in co-culture with autologous pDC, monocytes and B-cells. A/ Experimental design of the co-culture to assess CAR-T cells functionality. B/Cytotoxic activity of CAR-T cells against autologous pDCs, monocytes and B cells assessed by flow cytometry. C/Capacity of cytokine secretion by CAR-T cells after co-culture evaluated by flow cytometry.

2) Expanded IMiDs patients CAR-T cells express similar immunophenotypic profile to Healthy Donors (HD)

Following transduction and expansion, patients-derived CAR-T cells displayed an immunophenotype comparable to HD derived CAR-T (Figure 4A), with increased activation marker expression (Figure 4B) and reduced senescence (Figure 4C).

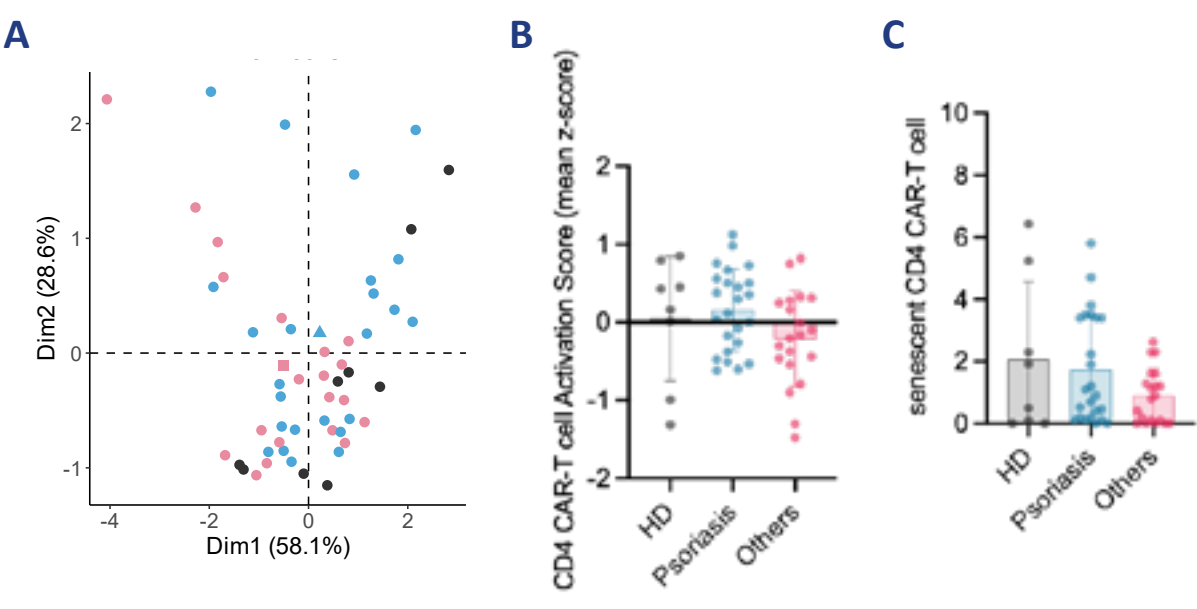


Fig 4: Absence of phenotypic alterations of IMiDs T cells at the end of the production process on CD4 T-cells. A/ PCA performed on several phenotypic markers evaluated by FC related to T cell activation, senescence, exhaustion, differentiation profile and cytokine expression B/ Composite activation scores calculated from the combined expression of activation markers and evaluated by FC. C/ CD4+ T cell senescence markers evaluated by FC. Similar results have been obtained on CD8

3) CD123 CAR-T cells induce selective depletion of pDCs and improvement of skin lesions in imiquimod-induced psoriasis model in a humanized mice

Using Flt3L-treated genO-BRGSF-HIS mouse model, CD123 CAR-T cells were generated from human T cells isolated from spleens of humanized mice (Figure 5A). These CAR-T cells were efficiently transduced (63.9% ± 12.6, n=3) (Figure 5B), and robustly expanded (Figure 5C). *In vitro* co-culture assays confirmed their cytotoxicity against CD123+ CAL-1 target cells (Figure 5D).

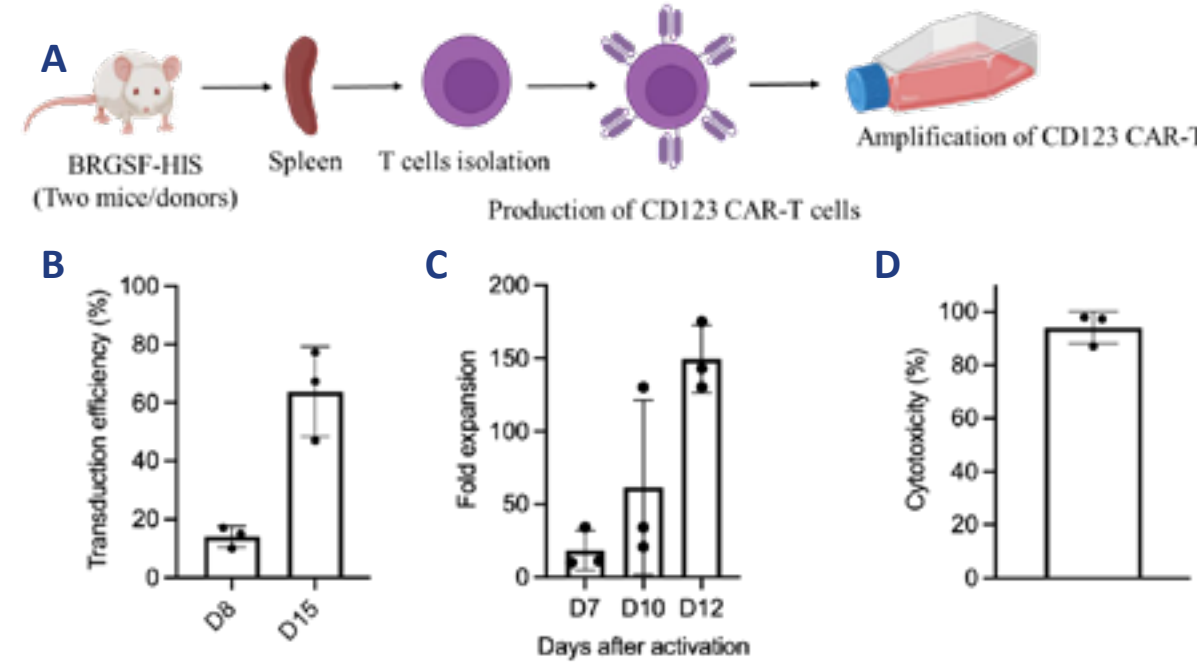


Fig 5: Production, characterization, and *in vitro* activity of autologous CAR-T cells derived from human T cells in a BRGSF-HIS mouse model. A/ Experimental schematic of autologous CAR-T cell production. B/ Transduction efficiency (hCD3+/hCD19+) measured by FC at the end of CAR-T cell production. C/ Expansion of CAR-T cells during *in vitro* production. D/ Cytotoxic activity of CAR-T cells in co-culture with CAL-1 cells at a 1:5 effector-to-target ratio.

Flt3L-treated genO-BRGSF-HIS humanized mice were then treated with topical imiquimod to reproduce psoriasis (Figure 6A). In addition, one group of mice received 10x10⁶ autologous CD123 CAR-T cells. Dexamethasone was used as positive control (Figure 6A). After sacrifice at day 15, CD123 CAR-T mice demonstrated a significant decrease of circulating pDCs (Figures 6B) and into the skin (Figures 6C) compared with all other groups. CD123 CAR-T were detected among both circulating total human T cells (Figure 6D) and into the skin (Figure 6E). Skin analysis skin analysis showed that imiquimod induced cutaneous infiltration of human CD45+ leucocytes in all groups including CD123 CAR-T and dexamethasone-treated mice (Figure 6F). Histologically, imiquimod-induced epidermal alterations (acanthosis, orthokeratosis, parakeratosis) were attenuated in CD123 CAR-T mice (Figures 6G).

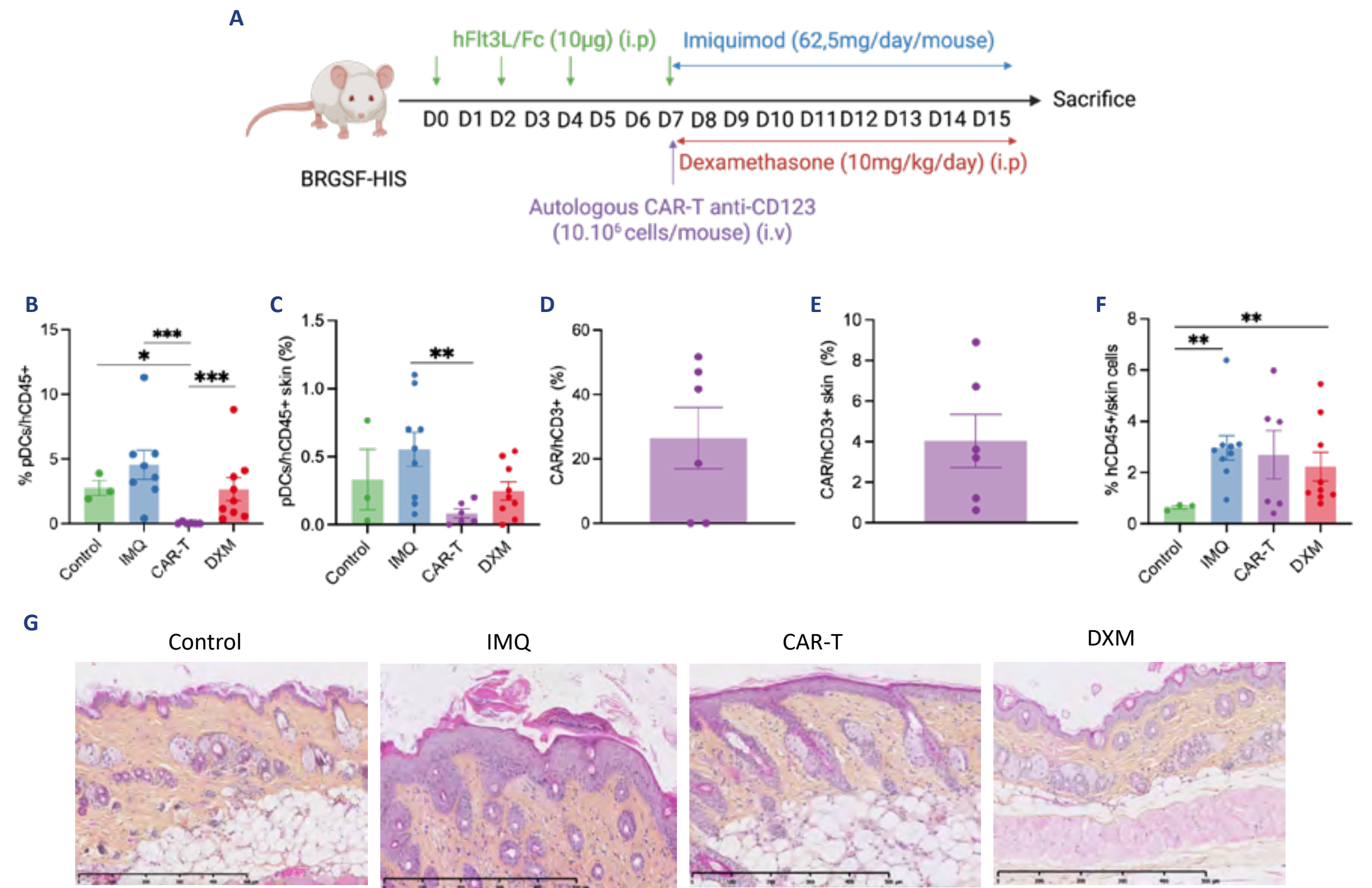


Fig 6: Injection of CD123 CAR-T cells into BRGSF-HIS mice depletes circulating pDCs and prevents their skin infiltration, thereby reducing epidermal damage. A/ Schematic representation of the in vivo experimental protocol. B/ Human pDCs frequency in blood at the end of the experiment, assessed by FC. C/ Proportion of CAR-T cells among human CD3+ T cells at the end of the experiment, assessed by FC. D/ Percentage of human cells CD45+ in mouse skin by FC. E/ Percentage of CAR-T cells among human CD3+ T cells in mouse skin assessed by FC. F/ Percentage of human cells pDCs in mouse skin evaluated by FC. G/ Representative Hematoxylin-Eosin-Safran stained images of mouse skin sections (x200). *p < 0.05; **p< 0.01; ***p < 0.001.

CONCLUSION

In conclusion, we showed that T cells from IMiDs patients can be used to generate functional CAR-T cells despite a more senescent and exhausted phenotype at baseline. In addition, **autologous T lymphocytes from patients with IMiDs can generate functional CD123 CAR-T cells capable of effectively eliminating pDCs and improving psoriasis-related skin disorders.** Altogether, our results confirm the **potential of CD123 CAR-T cells as a novel therapy in pDC-induced IMiDs.**