Improving CAR-T cell efficacy in a preclinical model of breast cancer through modified manufacturing methods and adjuvant therapy

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Introduction and Rationale

The efficacy of CAR-T products remains limited in solid malignancies. CellPryme™ is a small molecule that can be briefly incorporated into the **manufacturing (CellPryme-M)** process of CAR-T cells to confer a central memory phenotype for improvement of persistence. It can also be used as an **adjuvant (CellPryme-A)** to pre-condition solid tumours to enhance tumour penetrance and killing efficacy. Murine T cells were activated with anti-CD3/28 expanded in the presence of IL-2/7. T cells were then retrovirally transduced with a HER2-CD28z construct before pretreatment with CellPryme™ for the first 24 hours of the expansion phase

CellPryme™ enriches for central memory CD4+ and CD8+ T cells within 24 hours of pretreatment

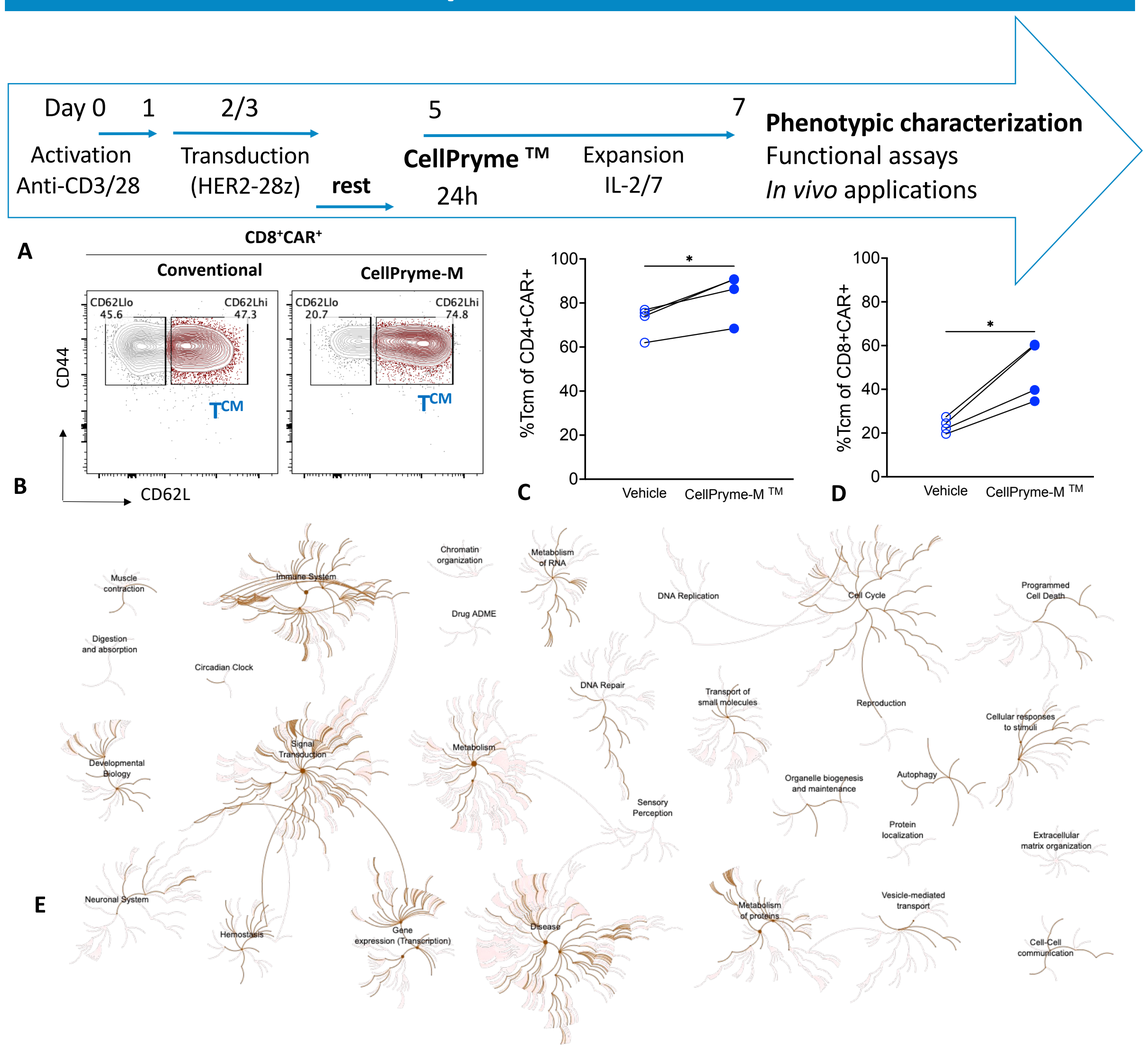

Figure 1. Utility of CellPryme™ as a manufacturing additive. Representative timeline of murine CAR-T cell production (A). Representative flow cytometric plots of central memory T cell (Tcm) phenotyping (B). CellPryme™ pretreatment increased Tcm in CD4+ and CD8+ compartments (C-D); (* p<0.05, paired two-tailed t-test). Reactome pathway analysis showing pathways over-represented by mass spectrometry in T cells following 24 hours of CellPryme™ pretreatment. Only proteins significantly changed by ≥ 2 S.D. were included (E).

CellPryme™ pretreatment protects CAR-T cells against exhaustion and retains Tcm phenotype following chronic antigen challenge

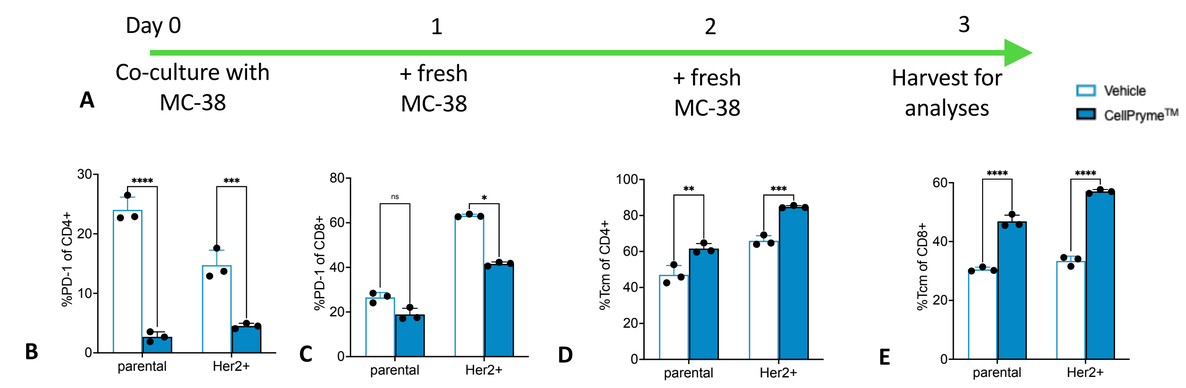

Figure 2. Functional testing of CellPryme™ pretreated CAR-T cells. When chronically challenged with parental or HER2+ MC-38 tumour cells (A), CellPryme-M™ pretreatment reduced percentage of PD-1+ CD4+ and CD8+ CAR-T cells (B-C) and maintained percentage of Tcm in CellPryme™ pretreated CD4+ and CD8+ CAR-T cells (D-E).

CellPryme™ pretreatment improves tumour killing and confers survival benefit *in vivo*

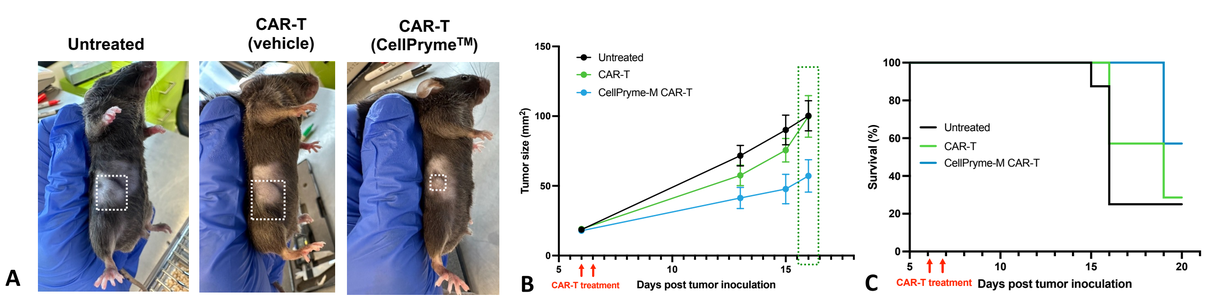

Figure 3. CellPryme™ pretreated CAR-T cells demonstrated improved tumour killing *in vivo* in an immunocompetent hHer2 breast cancer model. Representative images of tumours taken on day 16 (A). Pretreatment with CellPryme™ improved CAR-T cell killing of breast tumour E0771-Her2 (B). This coincided with improved survival (C).

CellPryme™ pretreated CAR-T cells retain central memory phenotype *in vivo* and upregulate chemokine receptors

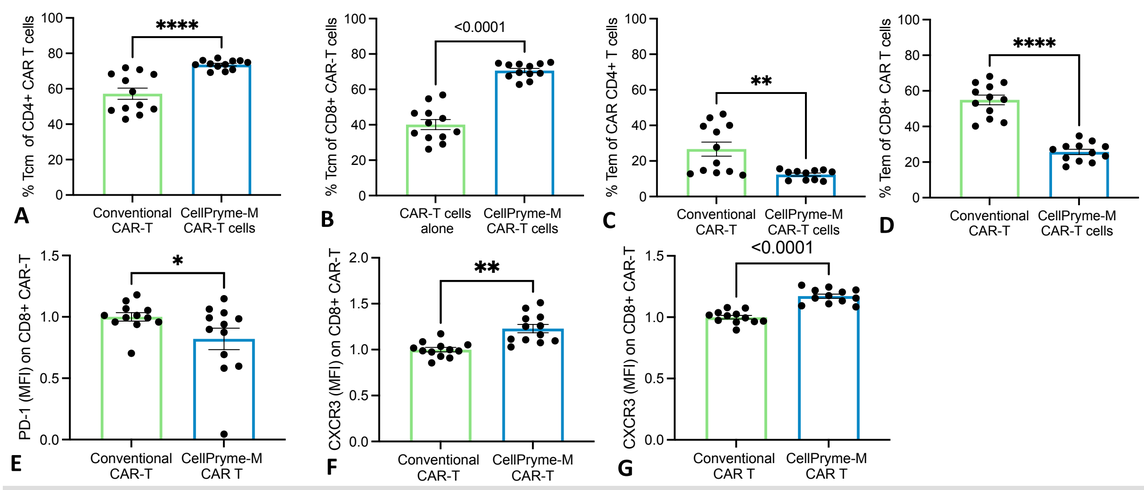

Figure 4. CellPryme™ pretreated CAR-T cells retain their phenotype *in vivo*. Following transfer into tumour bearing immunocompetent mice, the percentage of Tcm remained higher in CD4+ and CD8+ CAR-T cells (A-B) while the percentage of Tem was lower (C-D). CellPryme™ pretreatment also reduced PD-1, and increased CXCR3 and CXCR5 expression on CD8+ CAR-T cells a week after *in vivo* transfer (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, one-tailed t-test).

When used as an adjuvant, CellPryme™ confers survival and works synergistically with pretreated CAR-T

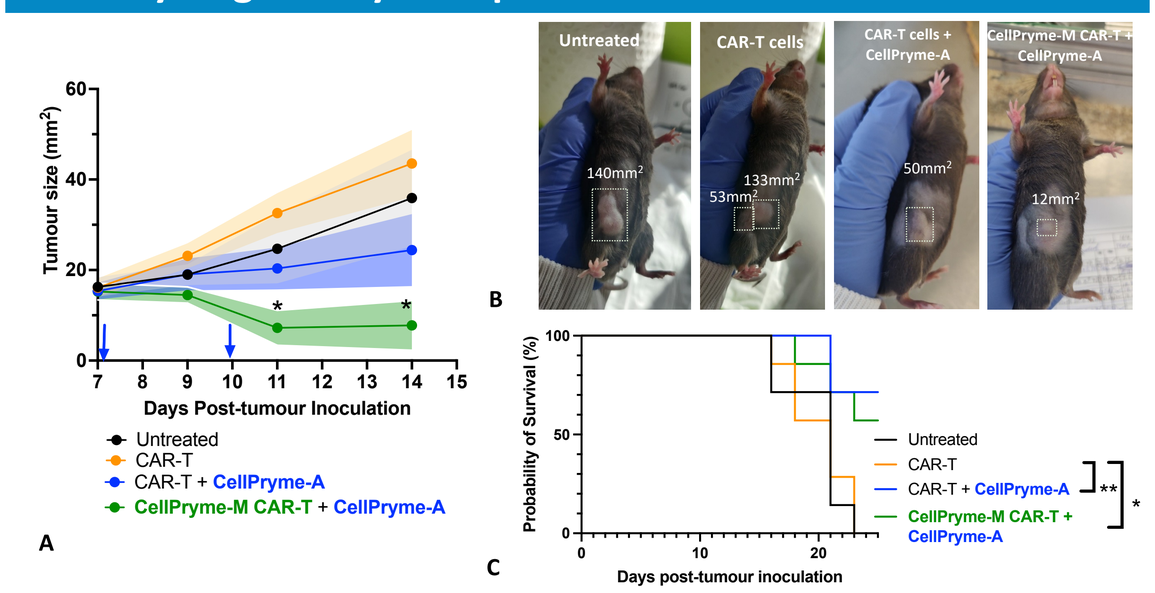

Figure 5. The utility of CellPryme™ as an adjuvant (CellPryme-A) for cellular immunotherapy in an immunocompetent model of breast cancer. CellPryme™ was administered intraperitoneally at 25mg/kg coinciding with CAR-T transfer and 3 days later, in combination with either conventionally produced CAR-T cells or CellPryme™ pretreated CAR-T cells. This this model, conventionally produced CAR-T cells confer no therapeutic benefit unless combined with CellPryme-A. The greatest impact on tumour killing was observed when CellPryme-A was used in combination with pretreated CAR-T cells (A). Representative images of tumours (B). The impact of CellPryme-A and pretreatment on survival benefit is significant (C). *p<0.05, **p<0.01, Mantel-Cox test.

CellPryme™ reduces intra-tumoral Tregs and enables *in vivo* CAR-T expansion when administered as an adjuvant

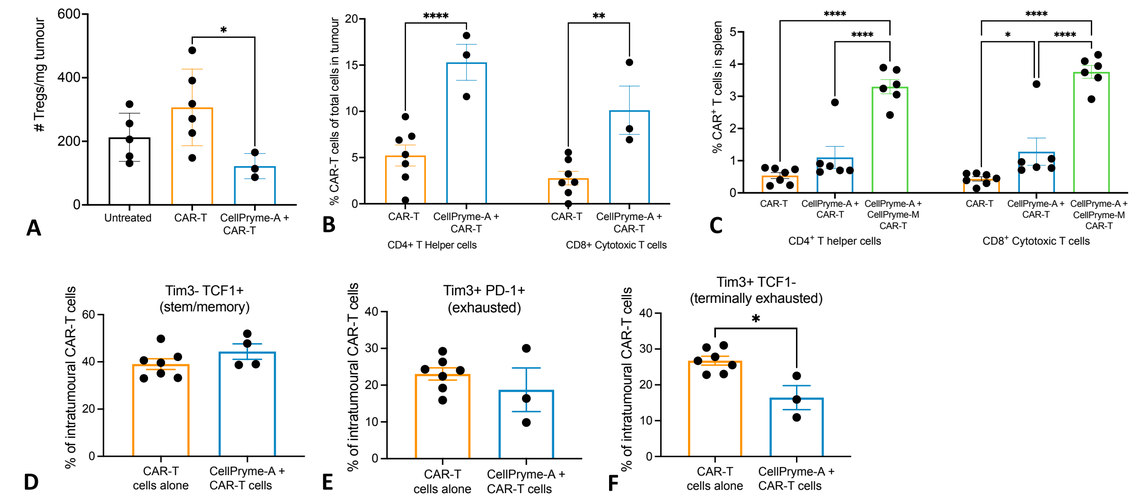

Figure 6. CellPryme™ addresses the immunosuppressive tumour microenvironment when used as an adjuvant (CellPryme-A) for cellular immunotherapy in an immunocompetent model of breast cancer. As an adjuvant, CellPryme™ reduced the numbers of regulatory T cells (Tregs) in solid tumours (A) and increased the percentage of CD4+ and CD8+ CAR-T cells (B). Adjuvant use of CellPryme™ enabled *in vivo* expansion of CD8+ CAR-T cells, and this was most profound and observed in both CD4+ and CD8+ compartments when the CAR-T cells were first pretreated with CellPryme™ (C). Notably, when administered as an adjuvant CellPryme™ modifies the tumour microenvironment but has no effect on the stem/memory (D, Tim3-TCF1+) or exhaustion (E, Tim3+PD-1+) phenotypes of CAR-T cells. However, the reduction of intra-tumoural Tregs may contribute to the reduced percentages of terminally exhausted (Tim3+ TCF-1-) intratumoural CAR-T cells (F). *p<0.05, **p<0.01,****p<0.0001. One-tailed Mann-Whitney t-test, one- or two-way ANOVA performed where appropriate.

Conclusions

CellPryme™ is a small molecule compound that can improve CAR-T efficacy through enrichment of Tcm during the manufacturing process. As an adjuvant, it addresses the immunosuppressive tumour microenvironment for cellular immunotherapy. Samples can be made available for testing under a Materials Transfer Agreement.