Agonistic SLAMF7 (CD319) signals mediate human CD8+ T-cell differentiation and responses against low affinity tumor associated antigens

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ABSTRACT

Immunotherapy, particularly immune checkpoint blockade (ICB), has made significant advances in cancer treatment. However, limited patient responses and immune-related adverse events often limit its efficacy. To enhance anti-tumour CD8+ T-cell responses, we identified the self-ligating receptor SLAMF7 as a promising target due to its immunoregulatory role on various immune cells.

In this study, we analysed the impact of SLAMF7 activation on CD8+ T cell differentiation, activation and effector function, as well as its potential to improve responses against the tumour antigen NY-ESO-1 as an alternative to ICB or in combination with ICB. We found that the frequency of SLAMF7+ CD8+ T cells increased upon co-stimulation and IL-12 exposure. Agonistic SLAMF7 signalling promoted T-cell clonal expansion and enhanced cytotoxic effector differentiation. Importantly, SLAMF7 activation enhanced immune responses against low affinity NY-ESO-1 and its combination with αPD-1/αPD-L1 blockade further enhanced CD8+ T-cell cytotoxicity.

These findings highlight SLAMF7 as a novel immunotherapeutic target for enhancing anti-tumour CD8+ T cell responses.

METHODS

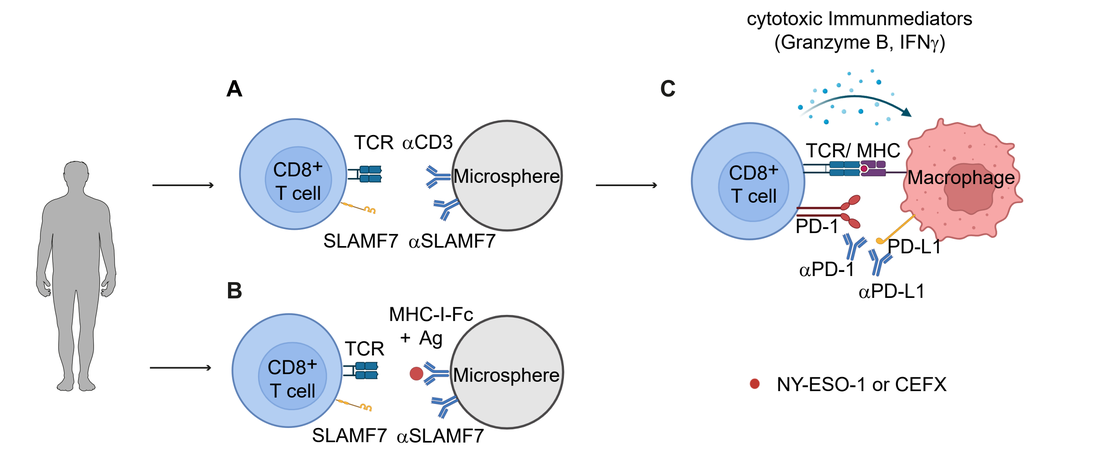

Fig. 1. Experimental setup. CD8+ T cells, isolated from peripheral blood, were activated via antibodies coupled on microspheres. An agonistic SLAMF7-antibody was used to investigate the effect of SLAMF7 crosslinking on CD8+ T cells during polyclonal activation (A) or antigen-specific stimulation (B). For antigen-specific stimulation a recombinant MHC-I molecule (HLA-A2:Ig) was loaded with either CEFX, a peptide mix of various infectious antigens, or the tumour antigen NY-ESO-1. To investigate the combinatory effect of agonistic SLAMF7 signals and ICB, CD8+ T cells were restimulated in an APC-co-culture were an Immune checkpoint blockade (αPD-1/αPD-L1) was established, APC´s were pulsed with NY-ESO-1. As read out flow cytometry and cytokine assays were used.

RESULTS

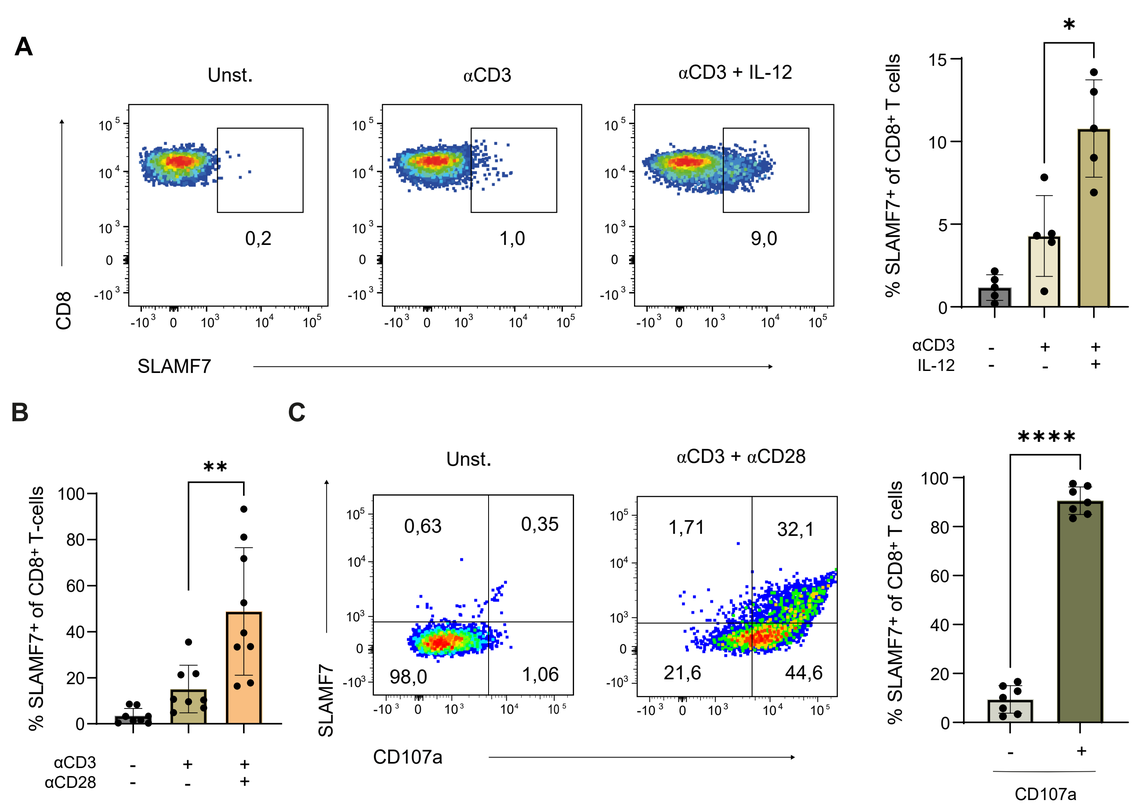

Fig. 2. Expression of SLAMF7 on CD8+ T cells. (A) IL-12 increases the frequency of SLAMF7 expressing CD8+ T cells. (B) The frequency of SLAMF7+ CD8+ T cells increases further by CD28-co-stimulation, in the presence of IL-12. (C) SLAMF7+ CD8+ T-cells, express CD107a, a marker for T-cell degranulation. The chart shows the frequency of CD107a- vs. CD107a+ CD8+ T cells within the SLAMF7+ CD8+ T-cell population. (one-way ANOVA with Tukey´s multiple comparison test (A+B) or paired t-test (C); * p < 0.05, ** p < 0.01, *** p < 0.001, and **** p < 0.0001)

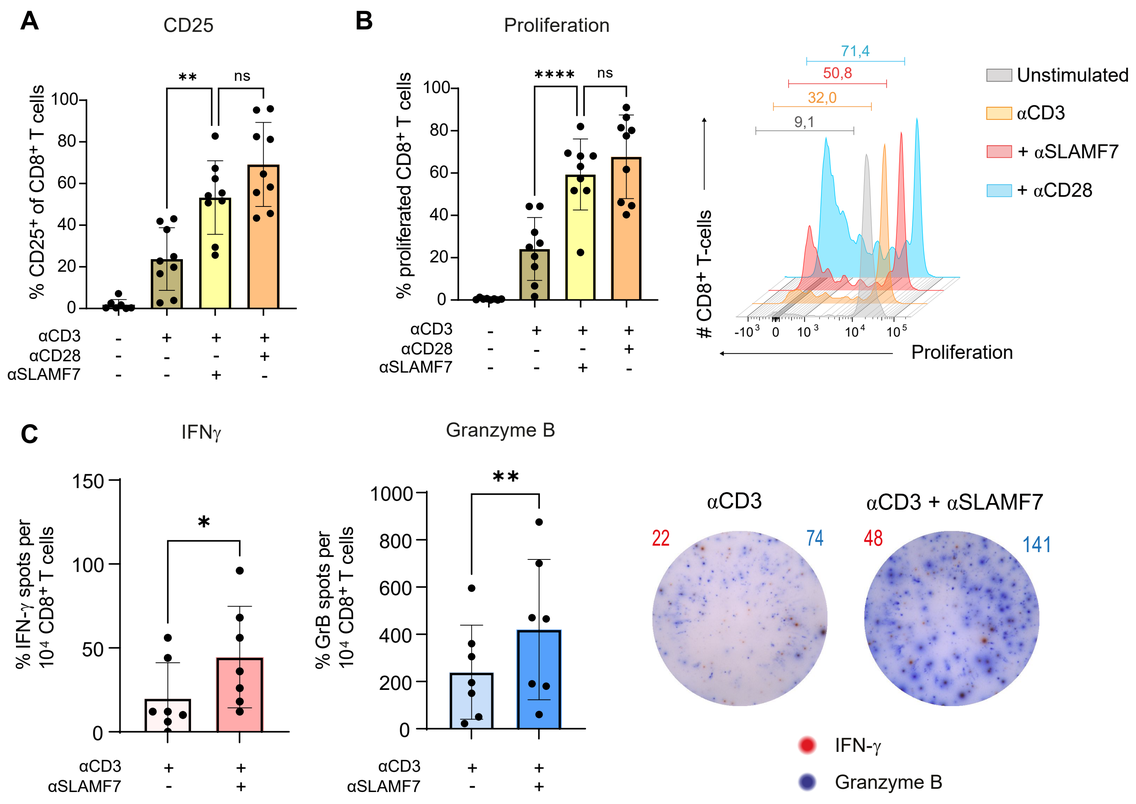

Fig. 3. Agonistic SLAMF7-signaling induces activation, proliferation & cytotoxic effector differentiation of CD8+ T cells. (A) Stimulation of SLAMF7 via agonistic αSLAMF7 coupled on microspheres enlarge the frequency of CD8+ T cells expressing CD25, a surrogate marker for T-cell activation and their proliferative capacity. (B) SLAMF7 activation induces proliferation of CD8+ T cells with significant difference compared to αCD3 stimulation alone. (C) Agonistic SLAMF7-signaling leads to increased number of cells releasing the effector molecules Interferon γ (red) and Granzyme B (blue). (one-way ANOVA with Tukey´s multiple comparison test (A+B), paired t-test (C (GrB)) or Wilcoxon-test (C (IFNγ)); * p < 0.05, ** p < 0.01, *** p < 0.001, and **** p < 0.0001).

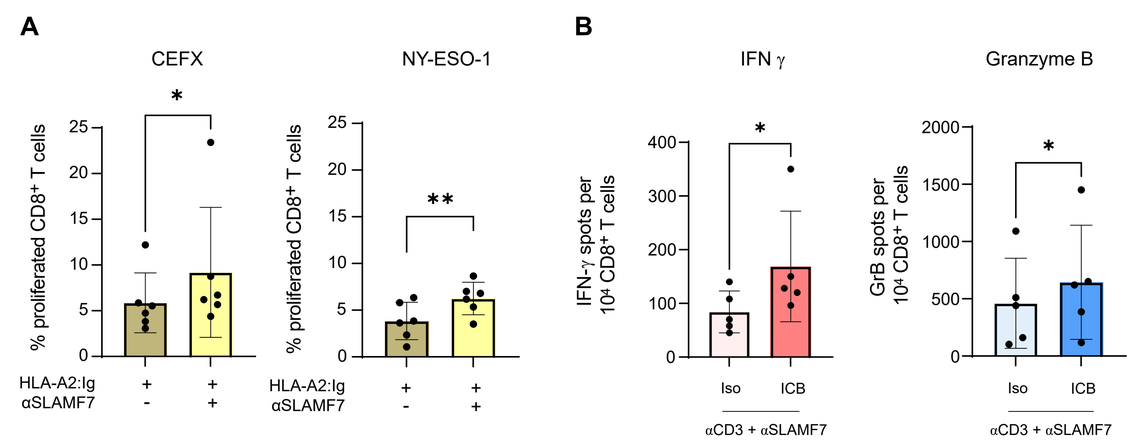

Fig. 4. SLAMF7 engagement during antigen-specific CD8+ T-cell activation and in combination with αPD-1/αPD-L1 blockade. (A) Agonistic SLAMF7 stimuli induce the expansion of antigen-specific CD8+ T cells, for infectious peptids as well as for the tumour antigen NY-ESO-1 (B) Immune checkpoint blockade (αPD-1/αPD-L1) in a co-culture of CD8+ T cells and NY-ESO-1 pulsed APCs enhances the SLAMF7-effect regarding the number of CD8+ T cells releasing Interferon γ and Granzyme B. (paired t-test (A (NY-ESO-1), B (GrB)) or Wilcoxon-test (A (CEFX), B (IFNγ)); * p < 0.05 and ** p < 0.01).

Summary & Conclusion

- Expression of SLAMF7 on CD8+ T cells is induced by proinflammatory cytokine IL-12 and CD28-co-stimulation.
- SLAMF7 is expressed on cytotoxic CD8+ T cells.
- Agonistic SLAMF7-signaling induces activation, proliferation & cytotoxic effector differentiation of CD8+ T cells.
- SLAMF7 engagment expands antigen-specific CD8+ T cells.
- SLAMF7 engagement & ICB enhance CD8+ T cell responses synergistically.
- SLAMF7 is a novel promising therapeutic target for cancer immunotherapy.

Conflict of interest declaration

The authors declare that they have no conflict of interest.

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