S100-A9 Inhibition Enhances CAR-T Cell Function and Overcomes Immune Suppression in CLL

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Introduction

Adoptive cell therapy, particularly CAR-T, has transformed treatment for B-cell neoplasms like ALL and NHL. However, in CLL, CAR-T efficacy is hindered by severe T-cell exhaustion and an immunosuppressive tumor microenvironment (TME). Targeting the alarmin protein S100-A9 may enhance CAR-T effectiveness, as S100-A9 drives immune suppression by recruiting myeloid-derived suppressor cells (MDSCs) in the TME. Our recent findings showed that S100-A9 activates inflammatory pathways in B-CLL cells and targeting this protein slow down CLL progression . We hypothesize that S100-A9 inhibition could improve T cell fitness and reduce exhaustion, offering a novel strategy to potentiate CAR-T therapy in CLL.

Methods

- Flow cytometry assessed S100-A9 expression in B-CLL cells in the PB and BM of 20 CLL patients.
- Nurse-like cells (NLCs) were obtained using PBMCs from individuals with CLL.
- MDSCs were differentiated from the BM of Eμ-TCL1 mice and treated in vitro with an S100-A9 inhibitor.
- To evaluate the *in vivo* immunomodulatory effect of targeting S100-A9, C57BL/6 were injected with splenocytes from Eμ-TCL1 mice and treated with the oral S100-A9 inhibitor, tasquinimod (TasQ).
- CART manufacturing: T cells were isolated from the spleen of TasQ-treated and control mice, activated with CD28/CD3 beads, transduced with the CAR construct, and then injected into Eμ-TCL1 mice with established disease.

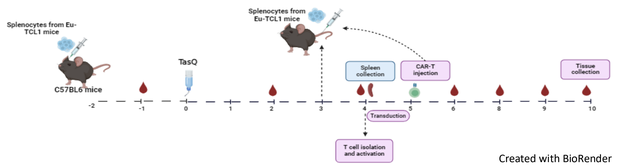


Fig. 2 Inhibition of S100-A9 Decreases Suppressive Myeloid Cells in CLL

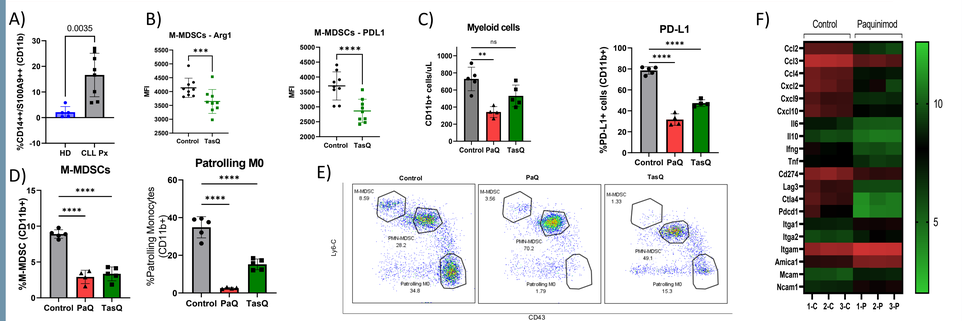


Fig. 2 A) A higher percentage of monocytes expressing S100-A9 was found in the PB of CLL patients compared to HD. B) In vitro treatment with TasQ decreased Arg1 and PDL-1 in M-MDSCs differentiated from the BM of Eμ-TCL1 mice. C-E) Eμ-TCL1 AT mice were treated with PaQ, TasQ or vehicle for 4 weeks. Spleens were collected for flow cytometry and Nanostring analysis. C) Absolute count of total myeloid cells D) PD-L1 expression was downregulated in myeloid cells from mice treated with PaQ and TasQ. E) The fractions of M-MDSCs and patrolling monocytes were significantly reduced after treatment with S100-A9 inhibitors. F) CD11b+ cells were isolated from control and PaQ treated mice, then the mRNA was used for Nanostring analysis. Heatmap showing the most differential expressed genes among the two groups. ****p < 0.0001; ***p < 0.001; ns p ≥ 0.05

Fig. 3 Targeting S100-A9 Mitigates T Cell Exhaustion in CLL Mice

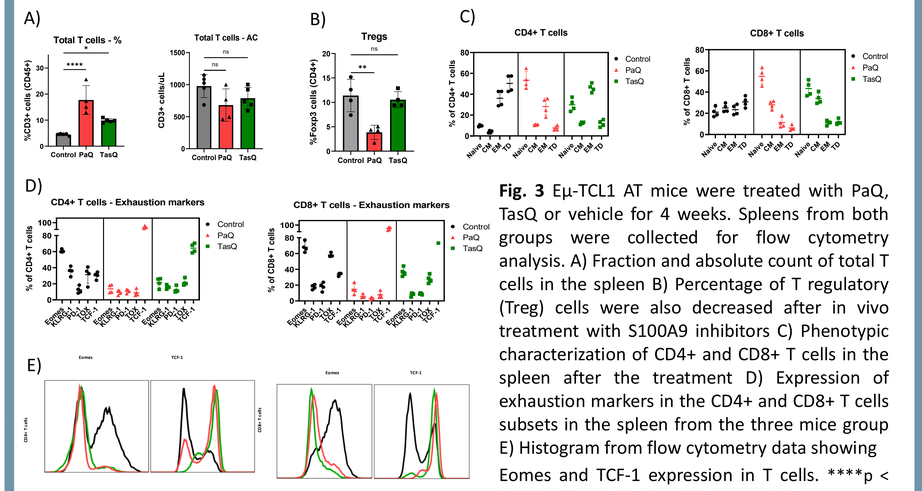


Fig. 3 Eμ-TCL1 AT mice were treated with PaQ, TasQ or vehicle for 4 weeks. Spleens from both groups were collected for flow cytometry analysis. A) Fraction and absolute count of total T cells in the spleen B) Percentage of T regulatory (Treg) cells were also decreased after in vivo treatment with S100A9 inhibitors C) Phenotypic characterization of CD4+ and CD8+ T cells in the spleen after the treatment D) Expression of exhaustion markers in the CD4+ and CD8+ T cells subsets in the spleen from the three mice group E) Histogram from flow cytometry data showing Eomes and TCF-1 expression in T cells. ****p < 0.0001; ***p < 0.01; *p< 0.05*; ns p ≥ 0.05

Fig. 4 Tasquinimod Enhances the Efficacy of CAR-T cells in a Preclinical CLL Model

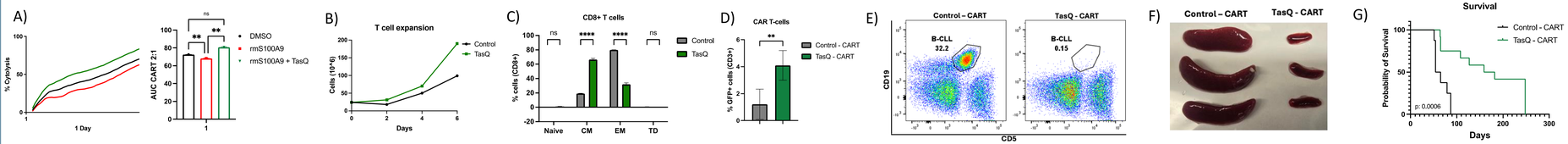


Fig. 4 A) CAR-T cells engineered from T cells isolated from the spleen of Eμ-TCL1 mice were exposed in vitro to rmS100A9 with and without TasQ, CAR-T cell cytotoxicity was measured using xCelligence RTCA. B-G) Eμ-TCL1 AT mice were treated with TasQ or vehicle for 4 weeks. Then, T cells were isolated from the spleens of both groups, activated with CD28/CD3 beads, transduced with the CAR construct, and then injected into Eμ-TCL1 mice with >20% of disease. B) CAR-T cells were counted at different time points during the manufacturing process, the results shows that CAR-T from TasQ-treated mice expanded faster *in vitro* C) Phenotype of CD8+ CAR-T cells D) Percentage of GFP+ CAR-T cells in PB of AT Eμ-TCL1 mice one week after injection E) Representative dot plot of one CAR-T control and one CAR-T TasQ mouse after 4 weeks of injection F) Representative spleens from both groups at 4 weeks after injection of CAR-T cells G) Survival curve of both CAR-T group. ****p < 0.0001; ***p < 0.01;* ns p ≥ 0.05

Fig. 1 TME Signaling Increases S100-A9 Expression in Primary B-CLL Cells

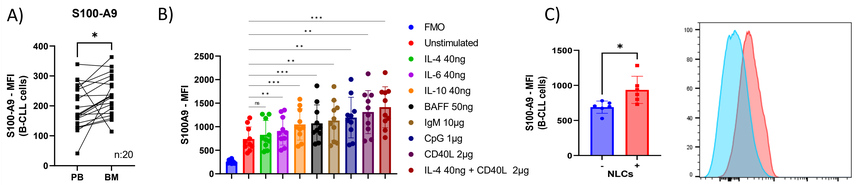
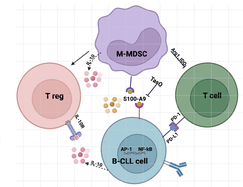


Fig. 1 A) Primary B-CLL cells from the BM have higher expression of S100-A9 compared to B-CLL cells isolated from the PB of the same patient. B) Several stimulation factors mimicking the CLL TME upregulate S100-A9 expression in B-CLL lymphocytes. C) S100-A9 MFI increases in leukemic CLL cells when PBMCs are co culture with NLCs. ***p < 0.001; ***p < 0.01; *p< 0.05*

Conclusions

1. B-CLL cells isolated from the BM of CLL patients have higher S100-A9 expression than their counterpart in PB.
2. NLCs as well as stimuli mimicking the TME promotes S100-A9 expression in primary B-CLL cells .
3. Targeting S100-A9 reprogram myeloid cells toward a less suppressive/inflammatory phenotype.
4. Mouse treated with S100-A9 inhibitors show less accumulation of exhausted T cells.
5. CAR-T cells engineered from Eμ-TCL1 mice exhibit reduced cytotoxic activity when exposed to S100A9 in vitro, and this effect is rescued by TasQ.
6. CAR-T cells generated from TasQ-treated donor murine cells prolonged survival in CLL-bearing mice compared with controls.



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