

Central memory T-Cell phenotype is associated with response to anti-PD1 therapy and enhanced with ex vivo PI3K-delta inhibition in mice

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Background

- Despite exciting results of immunotherapy targeting PD-1/L1 for advanced head and neck squamous cell carcinoma, response rates are low and different responses are poorly understood.
- Central memory T-Cells (Tcm) within the tumor microenvironment have improved antitumor immunity compared to effector memory T-cells (Tem) in solid tumors
- Tcm phenotype in oral cavity cancer (OCC) is associated with favorable response to neoadjuvant PD-1 therapy.
- CAL-101, an FDA approved PI3K-delta inhibitor, has previously been used in *ex vivo* expansion to improve anti-tumor responses of adoptively transferred T cells in melanoma models but 10uM during expansion limits cell yield.

Materials and Methods

- C57BL/6 mice were implanted with non-PD1-responsive MOC2 or PD-1-responsive MOC22 tumors and treated with 3 doses of nivolumab. TIL were harvested, expanded and assessed for memory markers by flow cytometry
- C57BL/6 splenocytes were harvested, resuspended at 1×10^6 cell/mL, stimulated with a-CD3/a-CD28, then expanded in the presence 100IU/mL IL-2 and either:
 - Vehicle
 - 1uM CAL-101 x 7 days then 10uM CAL101 x 7 days (**aka RAMP**)
 - 10uM CAL-101 x 10 days

•T cells were then assessed for yield, markers of memory, and markers of exhaustion by flow cytometry

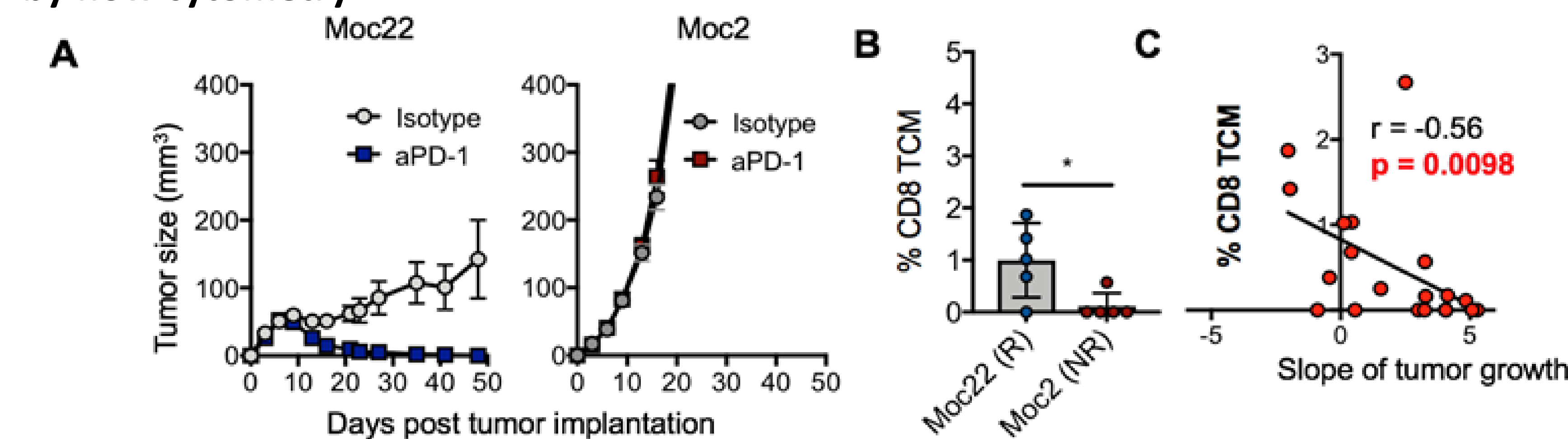


Figure 1. CD8 Tcm phenotype in mice OCC models is associated with response to PD-1 therapy. A) MOC22 or MOC2 tumors implanted on C57BL/6 mice, treated with aPD-1 on day 3, 6, and 9 (250ug/injection) vs isotype. $n = 7$ /group. B) Tumors harvested on day 12 for TIL analysis for CD8+CD44+CD62L+ (Tcm). $n = 5$ /group, Mann Whitney U test. C) Frequency of CD8+ Tcm correlated with slope of tumor growth. $n = 20$ mice, Spearman r correlation.

Results

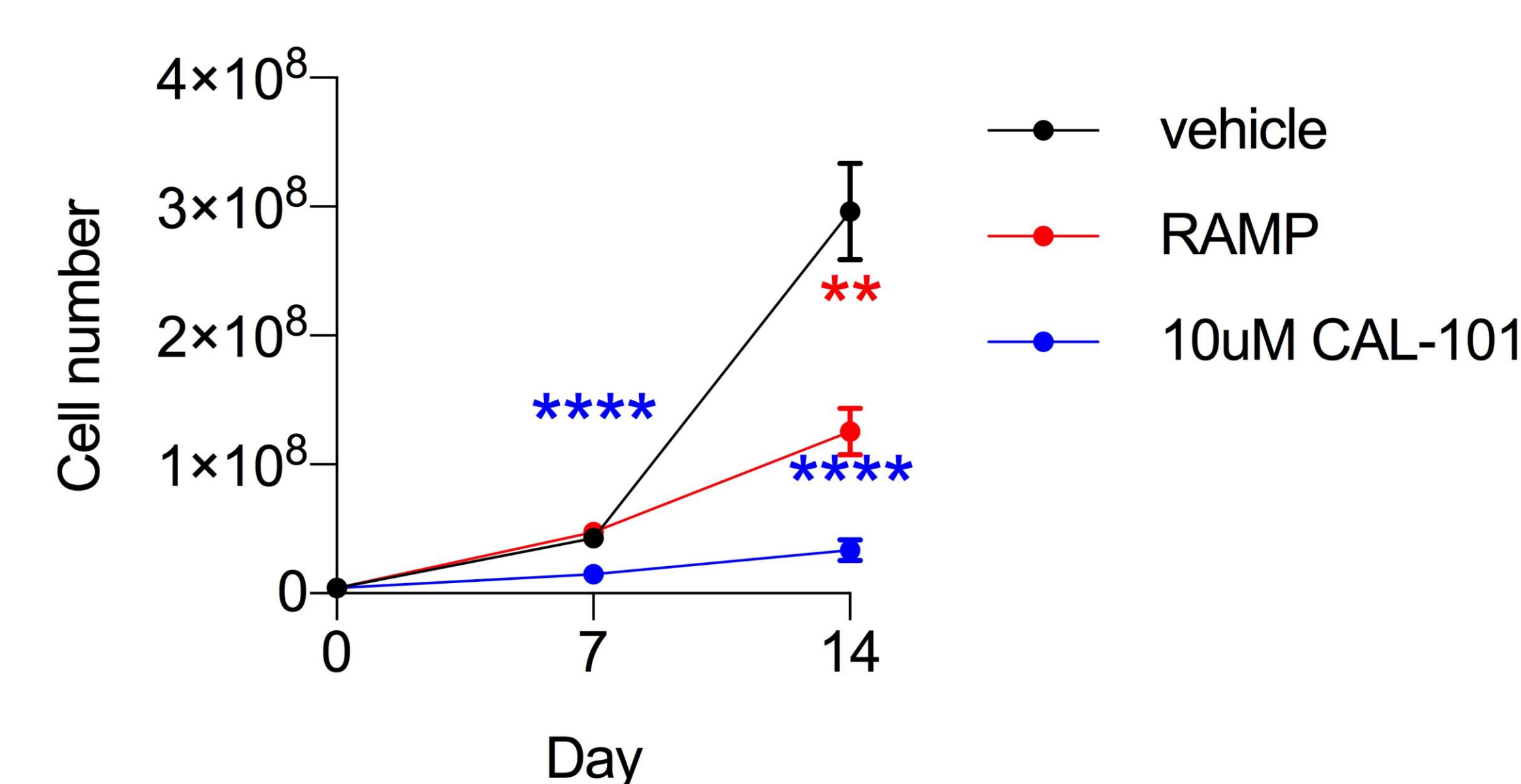


Figure 2. RAMP dosing improves T cell yield compared to traditional dosing. C57BL/6 mice splenocytes were harvested, resuspended at 1×10^6 cell/mL, stimulated with a-CD3/a-CD28, then expanded in the presence 100IU/mL IL-2 and either vehicle, RAMP dosing, or 10uM of CAL-101 and cells counted at day 7 and 14.

$n = 7$, ANOVA

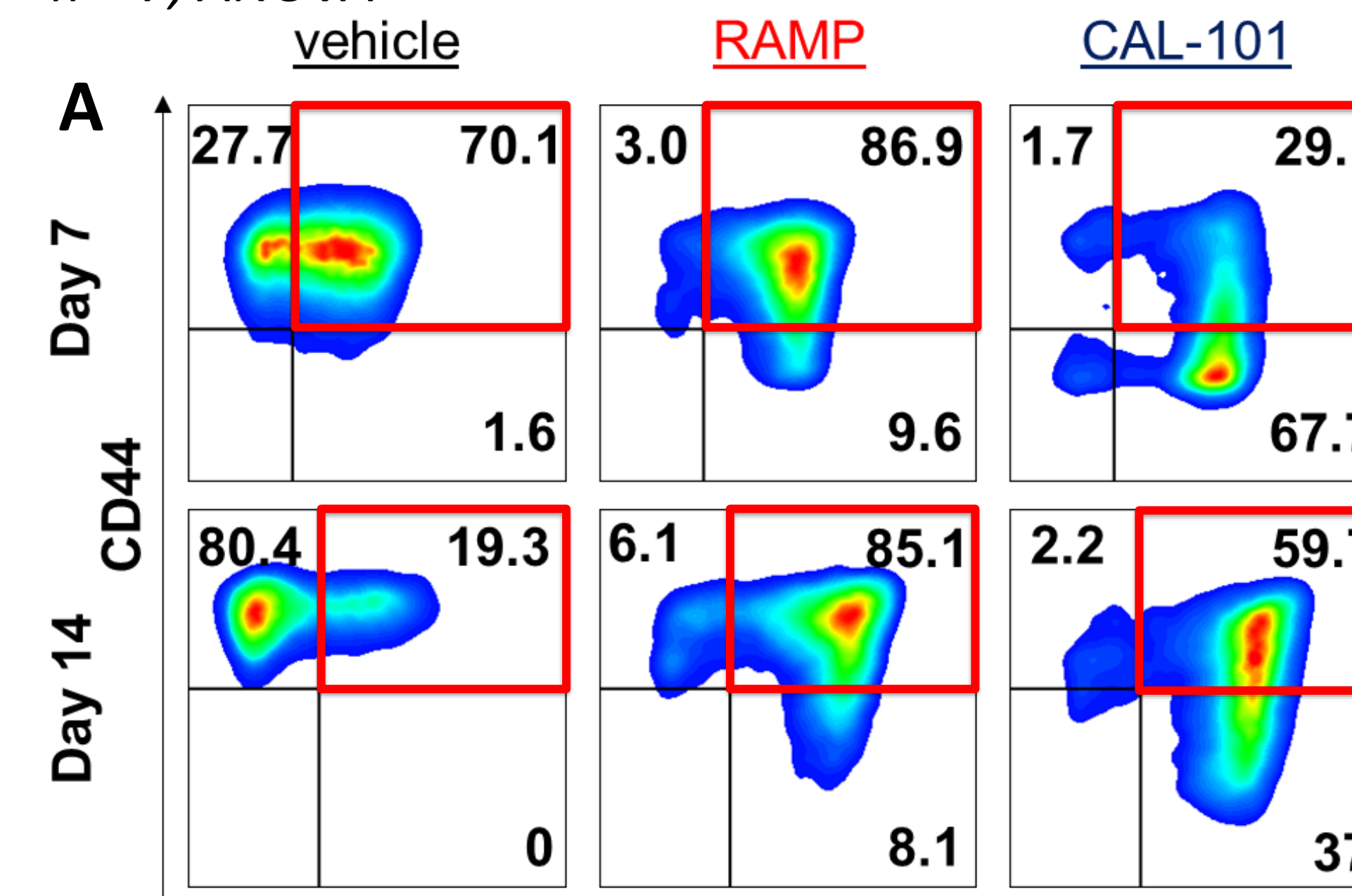
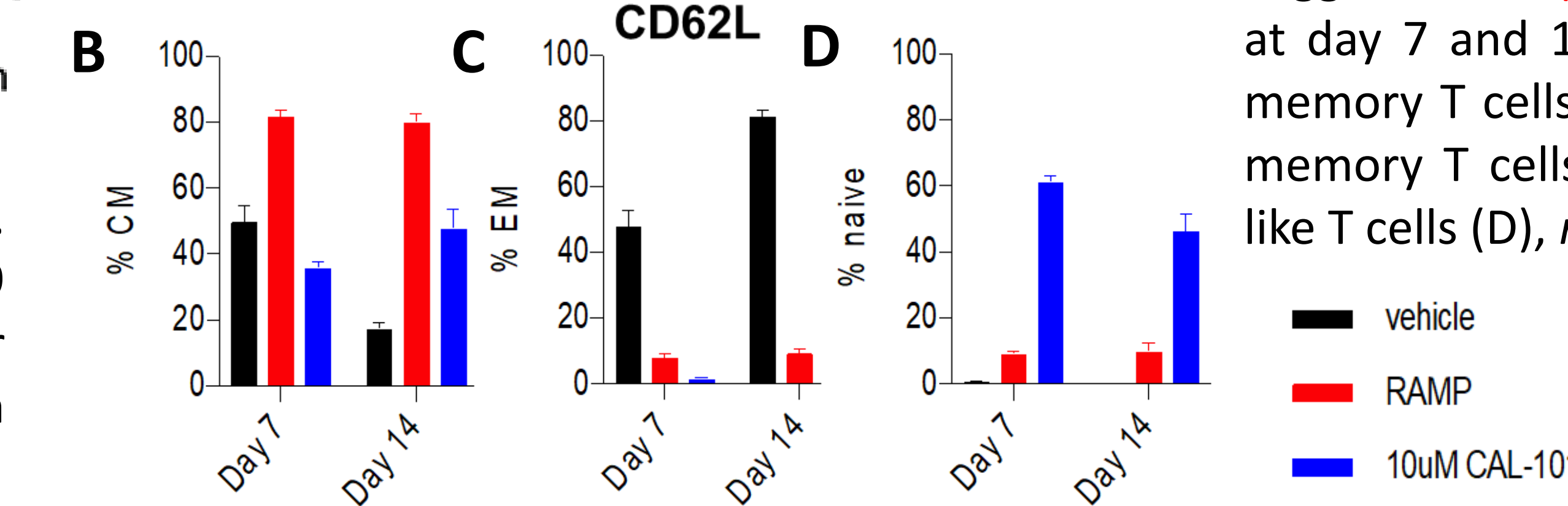


Figure 3. RAMP dosing enhances Tcm phenotype but less naïve-like CD8+ cells compared to 10uM CAL-101. A) C57BL/6 mice splenocytes were harvested, resuspended at 1×10^6 cell/mL, stimulated with a-CD3/a-CD28, then expanded in the presence 100IU/mL IL-2 and either vehicle, RAMP dosing, or 10uM of CAL-101. T-cells were then assessed for marker of activation (CD44+) and marker of memory (CD62L). CD44+CD62L+ cell population suggests Tcm (red boxes) Cell proportion at day 7 and 14 of CD44+CD62L+ central memory T cells (B), CD44+CD62L- effector memory T cells, and CD44-CD62L+ naïve-like T cells (D), $n = 7$.



Results

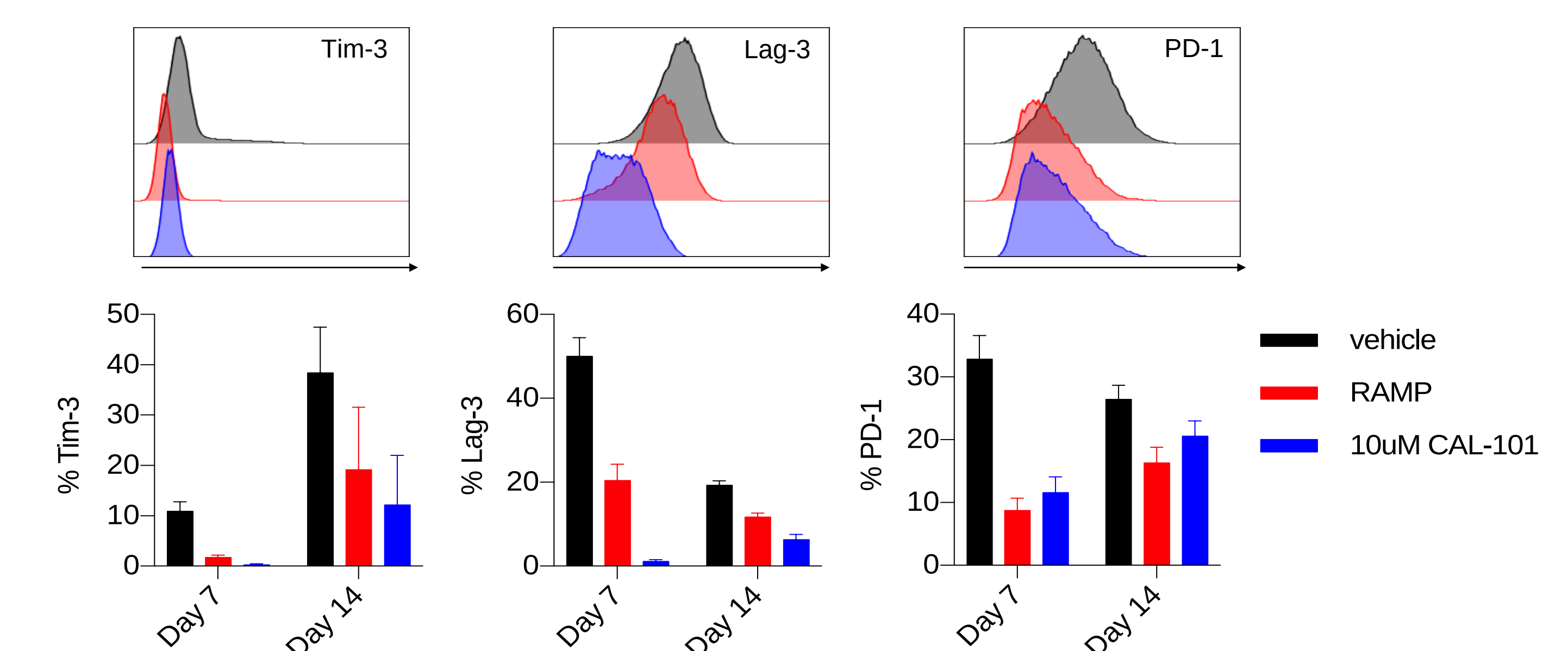


Figure 4. RAMP and 10uM CAL-101 limit proportion of T cells expressing markers of exhaustion. C57BL/6 mice splenocytes were harvested, resuspended at 1×10^6 cell/mL, stimulated with a-CD3/a-CD28, then expanded in the presence 100IU/mL IL-2 and either vehicle, RAMP dosing, or 10uM of CAL-101. T-cells were then assessed for markers of exhaustion including Tim-3, Lag-3, and PD-1 at day 7 and day 14 using flow cytometry, $n = 7$.

Discussion

- Tcm phenotype in the mouse oral cavity model MOC2 and MOC22 is associated with response to PD-1 therapy.
- A “ramped” up dosing regimen of CAL-101 improves T-cell yield compared to traditional expansion dosing while preserving Tcm phenotype
- RAMP dosing enhances Tcm phenotype in mice splenocytes, but less naïve-like CD8+ cells compared to traditional expansion dosing of CAL-101
- The markers of T-cell exhaustion Tim-3, Lag-3, and PD-1 were decreased when expanded in the presence of CAL-101

Conclusion

- Nivolumab responsive OCC tumors are enriched for markers of Tcm
- Expansion of T cells in the presence of the FDA approved PI3K-delta inhibitor CAL-101 enhances Tcm phenotype.
- These findings are important for potentially inducing a favorable tumor microenvironment for anti-PD1 therapy non-responders or for future emerging therapies for Head and Neck cancer, such as adoptive T-cell therapy.