

## News Release

### Title

Selective inhibition of low-affinity memory CD8<sup>+</sup> T cells by corticosteroids

### Key Points

- While immune checkpoint blockade (ICB) augments anti-tumor immune responses and improves overall survival in various types of cancer, immune-related adverse events (irAEs) are observed in some patients
- Management of irAEs often requires immunosuppressive drugs such as corticosteroids, but these medications have the potential to suppress anti-tumor immune responses elicited by ICB.
- Low--affinity memory T cells specific to self-antigens are dominantly suppressed by corticosteroids mainly by inhibition of fatty acid oxidation.

### Summary

Cancer immunotherapy in the form of immune checkpoint blockade (ICB) such as anti-cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and anti-programmed cell death 1 (PD-1) therapy reactivates cytotoxic T cells and facilitates killing of cancer cells, providing significant clinical efficacy across various types of cancer, even in patients with advanced disease. On the other hand, the patients treated with ICB sometimes experience immune-related adverse events (irAEs), requiring immuno-suppressive drugs such as corticosteroids, despite the possibility that immunosuppression could impair the anti-tumor effects of ICB. Here, we address the dilemma of using corticosteroids for the treatment of irAEs induced by ICB. ICB augmented neoantigen-specific CD8<sup>+</sup> T-cell responses, resulting in tumor regression. Simultaneous, but not late, administration of corticosteroids impaired anti-tumor responses with reduction of CD8<sup>+</sup> T-cell proliferation. Secondary challenge using tumors with/without the neoantigen showed selective progression in tumors lacking the neoantigen when corticosteroids were administered. Corticosteroids decreased low-, but not high-affinity memory T cells by suppressing fatty acid metabolism essential for memory T cells. In a small cohort of human melanoma patients, overall survival was shorter after treatment with CTLA-4 blockade in patients who received early corticosteroids or had low tumor mutation burden. Together, low-affinity memory T cells are dominantly suppressed by corticosteroids, necessitating careful and thoughtful corticosteroid use.

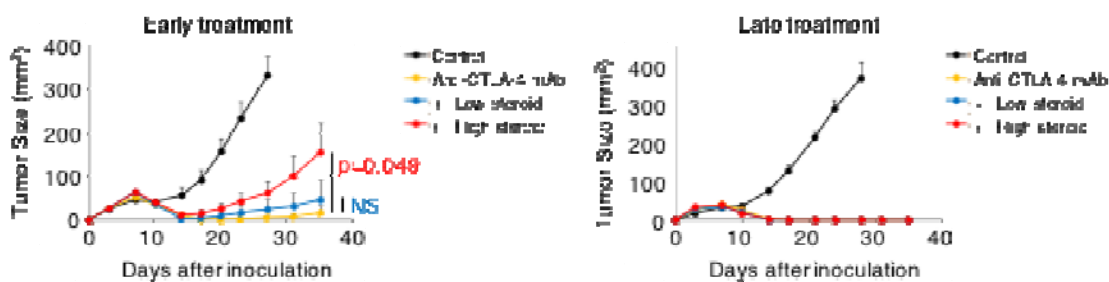
The study has been published online in The Journal of Experimental Medicine on Sep 19, 2019.

## Research Background

Patients treated with ICB sometimes experience irAEs and these patients receive immuno-suppressive drugs such as corticosteroids. However, these drugs have the possibility that immunosuppression could impair the anti-tumor effects of ICB. Here, we address the dilemma of using corticosteroids for the treatment of irAEs induced by ICB.

## Research Results

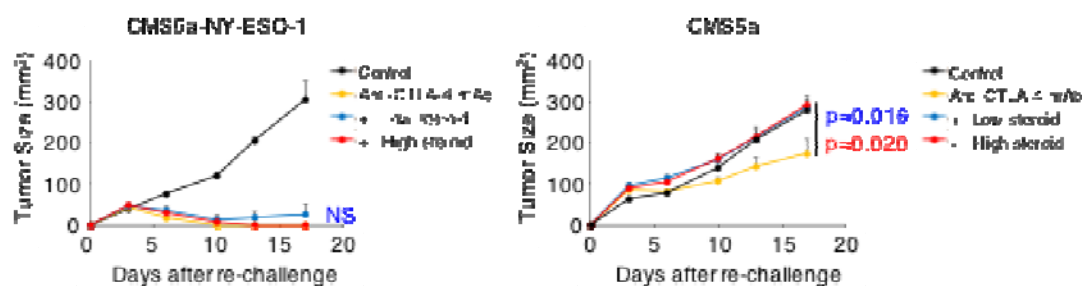
We first investigated the impact of corticosteroids on anti-tumor immune responses elicited by CTLA-4 blockade using animal models. BALB/c mice were inoculated with CMS5a with stable expression of a model tumor neoantigen NY-ESO-1 (CMS5a-NY-ESO-1) and treated with anti-CTLA-4 mAb. Methylprednisolone was administered beginning on the same day or after the tumor-regressing day as anti-CTLA-4 mAb treatment. Dosing of corticosteroids was selected based on human equivalent dose; low-dose corticosteroids was 20 µg per mouse and high-dose corticosteroids was 2000 µg per mouse. Early administration of corticosteroids was not controlled in 40% of mice treated with corticosteroids, whereas neither low- nor high-dose corticosteroid administration reduced anti-tumor activity by anti-CTLA-4 mAb in the late timing. (Fig. 1).



**Fig 1. Early corticosteroid treatment reduces anti-tumor activity by anti-CTLA-4 mAb in a dose-dependent fashion**

BALB/c mice were inoculated with CMS5a-NY-ESO-1 and injected with anti-CTLA-4 mAb on day 3, 6 and 9 after tumor inoculation. Corticosteroid administration was started on the same day (left) or after the tumor-regressing day (right) with anti-CTLA-4 mAb.

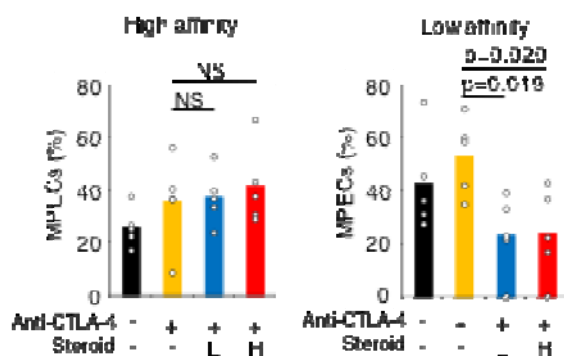
Some mice treated with early corticosteroids experienced re-growth of the tumor after initial tumor regression. This observation suggests that early corticosteroids may inhibit memory CD8<sup>+</sup> T cells that are involved in durable anti-tumor responses. We then asked whether memory T-cell differentiation was influenced by corticosteroids. Mice that had completely eradicated the initial tumors after anti-CTLA-4 mAb with or without early corticosteroid treatment were secondarily challenged with CMS5a-NY-ESO-1 cells (right hind flank) and parental CMS5a cells (left hind flank). Almost all mice rejected the CMS5a-NY-ESO-1 cells regardless of corticosteroid treatment. The growth of parental CMS5a was delayed in anti-CTLA-4 mAb-treated mice, but this tumor growth inhibition was abrogated in mice treated with both low- and high-dose corticosteroids (Fig 2).



**Fig 2. Early corticosteroid treatment reduces anti-tumor activity against parental cells by anti-CTLA-4 mAb**

Mice that had completely rejected the initial tumors were collected and secondarily inoculated with CMS5a-NY-ESO-1 (left) and parental CMS5a (right).

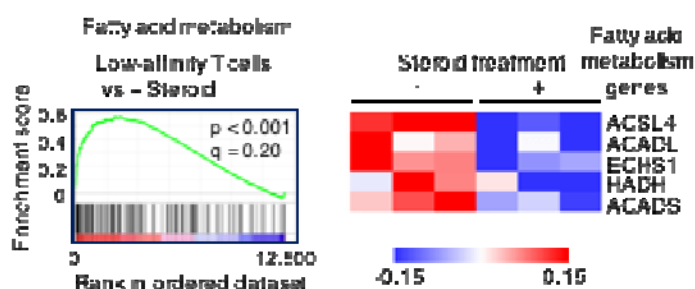
Since memory precursor effector cells (MPECs) generate long-lived  $CD8^+$  memory T cells, we analyzed the proportion of MPECs in tumor tissues. These cells reportedly express high levels of CD127 and low levels of KLRG1. When anti-CTLA-4 mAb and corticosteroids were administered, the proportion of MPECs in low-affinity  $CD8^+$  T cells was significantly decreased whereas MPECs in high-affinity  $CD8^+$  T cells in CMS5a-NY-ESO-1 tumors were not affected by corticosteroids (Fig 3).



**Fig 3. Low-affinity  $CD8^+$  T cells was significantly decreased by corticosteroid treatment.**

MPECs population in high-affinity and low-affinity  $CD8^+$  T cells in CMS5a-NY-ESO-1 were examined after tumor inoculation. Percentages of MPECs in high- (left) and low-affinity (right)  $CD8^+$  T cells.

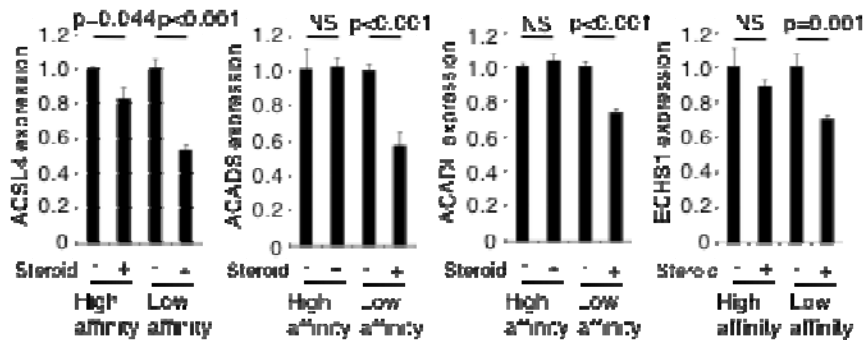
To gain insight into the mechanism(s) underlying selective inhibition of low-affinity T cells by corticosteroids, we examined the target genes for corticosteroids by investigating gene expression with or without corticosteroids. Gene-set enrichment analysis (GSEA) revealed significant enrichment of genes involved in fatty-acid oxidation (FAO) metabolism in corticosteroid-treated and -untreated low-affinity T cells (Fig 4)



**Fig 4. Corticosteroids compromise fatty acid oxidation in low-affinity T cells.**

(Left) GSEA of fatty acid metabolism-related genes in low-affinity T cells treated with corticosteroids. (Right) Heat map of expression of FAO-related genes.

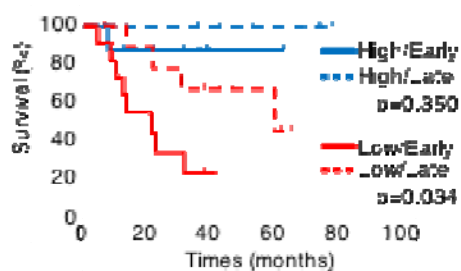
We further examined whether corticosteroid administration suppressed FAO-related gene expression in low-affinity T cells in vivo. In accordance with the in vitro data, low-affinity, but not high-affinity T cells in CMS5a-NY-ESO-1 tumors treated with corticosteroids also showed markedly less FAO-related gene expression and decreased mitochondrial function associated with FAO (Fig 5)



**Fig 5. Corticosteroids reduce FAO-related genes in low-affinity T cells.**

FAO-related mRNA expression in high- or low-affinity CD8<sup>+</sup> T cells in CMS5a-NY-ESO-1 tumors.

Finally, to examine the influence of corticosteroids on the clinical activity of ICB in cancer patients, we analyzed patients with malignant melanoma treated with the anti-CTLA-4 mAb ipilimumab. While no clinical impact of corticosteroid administration was observed in patients with high-mutation burden, patients with a low-mutation burden who were treated with corticosteroids at an early timepoint had a significantly worse prognosis than patients who received corticosteroids later (Fig 6).



**Fig 6. Low-mutation burden and early corticosteroid administration in patients treated with anti-CTLA-4 mAb are associated with poor prognosis.** Kaplan–Meier curve for OS of malignant melanoma patients treated with anti-CTLA-4 mAb. Early / late corticosteroid administration to patients with high-/low-mutation burdens.

## Research Summary and Future Perspective

In conclusion, we provide evidence that corticosteroids inhibit anti-tumor efficacy of ICB in a dose- and timing-dependent manner. Additionally, low- but not high-affinity memory T cells specific to self-antigens are dominantly suppressed by corticosteroids mainly by inhibition of FAO. Furthermore, well-timed usage of corticosteroids could potentially selectively control autoimmunity without negatively impacting anti-tumor immunity. Additional clinical studies with large cohorts are warranted to determine the appropriate timing and dosage of corticosteroid treatment as well as other interventions used for treatment of irAEs (e.g. TNF blockade, mycophenolate mofetil).

## Publication

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