Editorial

# Interfering with Helios-induced regulatory T cell stability as a strategy for cancer immunotherapy

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*Comment on:* Nakagawa H, Sido JM, Reyes EE, *et al.* Instability of Helios-deficient Tregs is associated with conversion to a T-effector phenotype and enhanced antitumor immunity. Proc Natl Acad Sci U S A 2016;113:6248-53.

Submitted Oct 24, 2016. Accepted for publication Oct 28, 2016.
doi: 10.21037/tcr.2016.11.23
**View this article at:** http://dx.doi.org/10.21037/tcr.2016.11.23

The molecular control of regulatory T cell (Treg) homeostasis and differentiation is not yet completely understood in particular during inflammation and tumorigenesis.

Since the identification of Tregs in 1995 (1) and Foxp3 as the Treg lineage-defining transcription factor in 2003 (2), significant advancements have been made in the knowledge of Treg biology and function. We have learned that Tregs differentiate in two different ways: either in the thymus, from $CD4^+$ thymocytes that strongly recognize self-antigens but bypass the negative selection and develop into anergic Tregs [natural Tregs (nTregs)]; or in the periphery, from $CD4^+Foxp3^-$ T cells under specific circumstances that promote conversion into Tregs [(induced Tregs (iTregs)]. Expression of the transcription factor Helios, a member of the Ikaros zinc-finger protein family, was initially reported as a distinctive marker of nTreg (3). Further studies, however, have revealed that Helios can be induced in $Foxp3^+$ Tregs independent of their origin (4,5), and indicated the involvement of Helios expression in the definition of stability and suppression function of Tregs (6,7).

In physiological conditions, the frequency of Tregs is kept constant within a specific range. In tumor bearing individuals, instead, Treg frequency increases in the periphery and to higher extents at the tumor site, indicating the failure of the homeostatic mechanisms that normally control the amount of Tregs that need to be produced. The molecular network underlying these changes has not been fully clarified yet. Given the unfavorable prognostic impact of intra-tumor Treg accumulation, understanding how to interfere with abnormal Treg development in this condition may help identify effective strategy for cancer immunotherapy.

The work by Nakagawa *et al*. explores the role of Helios-mediated Treg stability in anti-tumor immunity and investigates strategies to counteract Helios expression/function for cancer immunotherapy. Building upon their previous report demonstrating the requirement of Helios expression for the maintenance of functional Tregs in models of inflammation (7), here the authors study the relevance of targeting Helios to improve anti-tumor immunity. Remarkably, the growth of both highly immunogenic (MC38 colon adenocarcinoma) and aggressive non-immunogenic (B16F10 melanoma) mouse tumor models is affected in mice with Treg-selective Helios deficiency. Importantly, in these mice, Treg frequency at the tumor site does not increase and remains comparable to that found in the periphery. Increased production of Th1-type cytokines (TNFα and IFNγ) by tumor-infiltrating Helios-deficient Tregs points to a reverse conversion of Tregs into effector T cells (Teffs) promoted by Helios loss, or vice versa to a less efficient Teff to Treg conversion in the absence of Helios. In either case, these findings validate Helios as a critical transcription factor controlling development/maintenance of functional Tregs in the tumor microenvironment. In addition, they provide the rationale to exploit strategies able to affect Helios-mediated Treg stability for cancer immunotherapy. As an approach to substantiate this hypothesis, the authors use anti-GITR agonist antibodies, which they found to be effective in dampening Treg stability, as also previously reported by our group (8). GITR stimulation with the antibody

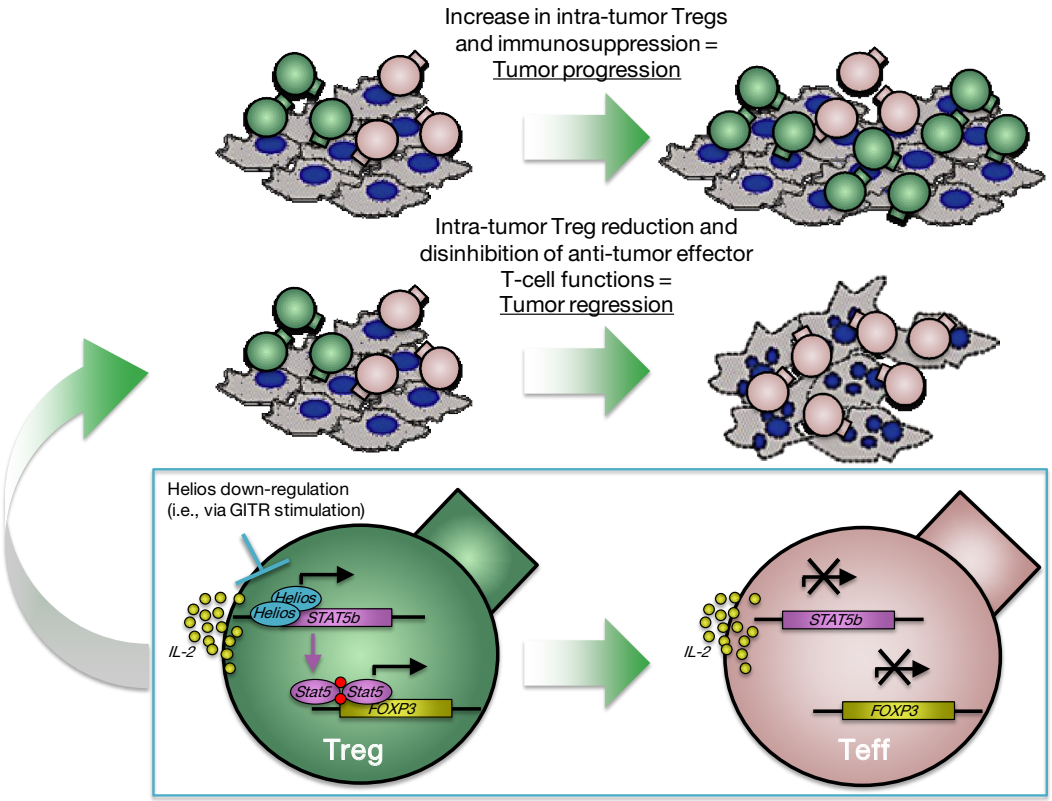


**Figure 1** Targeting Helios-induced Treg stability to promote therapeutic anti-tumor immune responses. As part of the mechanisms that promote tumor progression, Tregs accumulate in the tumor microenvironment and counteract the activity of tumor-specific Teffs, thus favoring tumor immune escape (top). Blocking this mechanism would enable Teffs to quantitatively predominate in the tumor microenvironment and exert anti-tumor functions with consequent tumor shrinkage (middle). The demonstration provided by Nakagawa *et al.* that Helios plays a key role in sustaining Foxp3 expression and phenotypic stability of tumor-infiltrating Tregs, probably by integrating signals in the IL2-STAT5 pathway as suggested in their previous work (7), opens the opportunity to target Helios for cancer immunotherapy (bottom). Antibody-dependent engagement of Treg surface receptors that trigger Helios down-regulation (i.e., GITR) can favor conversion of intra-tumor Tregs into Teffs thus enhancing anti-tumor immunity (bottom). Treg, regulatory T cell; Teff, effector T cell.

DTA-1 recapitulates the outcome achieved in mice with Treg-selective Helios deficiency, showing reduction in intra-tumor Treg frequency and stability coupled with delayed tumor growth. Even though it is still not completely clear if interfering with Helios affects a specific subset of Tregs (nTregs *vs.* iTregs), these results confirm the therapeutic impact of down-regulating Helios and the possibility to pharmacologically induce this effect (*Figure 1*).

As cancer immunotherapy with agonist anti-GITR antibodies is currently under clinical investigation, understanding the molecular interplay within the GITR-Helios-Foxp3 axis will be of paramount importance for the identification of relevant pharmacodynamic and predictive biomarkers of anti-GITR therapy. In their previous report, the authors demonstrate that Helios stabilizes Tregs and Foxp3 expression by promoting STAT5 transcription (*Figure 1*). Investigating the relative contribution of STAT5 modulation to the anti-tumor activity of anti-GITR therapy would thus represent the logical next step toward this goal.

## Acknowledgments

*Funding:* We would like to thank Swim Across America, Ludwig Institute for Cancer Research, Parker Institute for Cancer Immunotherapy, Center for Experimental Therapeutics at MSKCC (ETC), the Breast Cancer Research Foundation and the MSKCC Core Grant (P30 CA008748).

 

## Footnote

*Provenance and Peer Review:* This article was commissioned and reviewed by the Section Editor Chen Qian (Center for Inflammation & Epigenetics, Houston Methodist Hospital Research Institute, Houston, USA).

*Conflicts of Interest:* Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2016.11.23). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.



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**Cite this article as:** Zappasodi R, Merghoub T. Interfering with Helios-induced regulatory T cell stability as a strategy for cancer immunotherapy. Transl Cancer Res 2016;5(Suppl 6):S1116-S1118. doi: 10.21037/tcr.2016.11.23

 