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REVIEW SERIES: TREGS IN CANCER: WHERE ARE WE NOW?
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Regulatory T cells in cancer: where are we now?

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Summary

There have been substantial strides forward in our understanding of the contribution of regulatory T (Treg) cells to cancer immunosuppression. In this issue, we present a series of papers highlighting emerging themes on this topic relevant not only to our understanding of the fundamental biology of tumour immunosuppression but also to the design of new immunotherapeutic approaches. The substantially shared biology of $CD4^+$ conventional T (Tconv) and Treg cells necessitates a detailed understanding of the potentially opposing functional consequences that immunotherapies will have on Treg and Tconv cells, a prominent example being the potential for Treg-mediated hyperprogressive disease following anti-PD-1 therapy. Such understanding will aid patient stratification and the rational design of combination therapies. It is also becoming clear, however, that Treg cells within tumours exhibit distinct biological features to both Tconv cells and Treg cells in other tissues. These distinct features provide the opportunity for development of targeted immunotherapies with greater efficacy and reduced potential for inducing systemic toxicity.

T cells have an ability to recognize and kill cancer cells but their function is often suppressed within tumours. Whereas $CD4^+$ and $CD8^+$ conventional T (Tconv) cells promote immune activation, $CD4^+$ regulatory T (Treg) cells, dependent upon the transcription factor Foxp3, suppress Tconv cell responses and are required for immune homeostasis in both mice and humans.[1,2] Beyond this beneficial function, Treg cells can cause profound suppression of immune function within tumours.[3,4] In a variety of murine tumour models, ablation of Treg cells results in activation of $CD4^+$ or $CD8^+$ Tconv cells and rejection of solid tumours.[5–8] Moreover, high Treg ratios relative to total T cells or $CD8^+$ Tconv cells are associated with poorer survival in breast cancer,[9] non-small-cell lung carcinoma,[10] hepatocellular carcinoma,[11] ovarian cancer,[12,13] renal cell carcinoma,[14] pancreatic cancer,[15] colorectal carcinoma,[16] gastric cancer[17] and cervical cancer.[18] An understanding of a powerful role of Treg cells in tumour immunosuppression is emerging with extensive evidence from experimental mouse models complemented by a growing body of evidence in human cancer. In this Review series, we consider the progress made in our understanding of the mechanisms that lead to the accumulation and suppressive function of Treg cells within tumours, the unique properties of tumour-infiltrating Treg cells, and our means to selectively target them in cancer.

Although their immunosuppressive function make Treg cells in themselves an attractive target for specifically directed therapy, it is also important to consider the effects upon Treg cells of conventional immunotherapies thought to primarily target Tconv cells. Despite striking efficacy in some cases, therapies targeting programmed death 1 (PD-1)/ programmed death ligand 1 (PD-L1) signalling are ineffective at inducing durable responses in a majority of patients and can induce rapidly progressive disease referred to as 'hyperprogression' in a minority of patients.[19,20] A recent study suggests that hyperprogression is in part attributable to blockade of PD-1 signalling on Treg cells which, in susceptible individuals, results in enhanced Treg suppressive function.[21] It remains to be determined whether a similar phenomenon underlies

poor clinical responses to PD-1 therapy in subsets of patients but the findings highlight the need to consider the opposing effects that immunotherapies may have on the Tconv and Treg compartments. Indeed, such consideration may provide a basis for patient stratification or the rational design of combination immunotherapy. Evidence from both mouse models[22] and human cancer patients[23] indicate that the activity of anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) therapy is in part attributable to antibody-dependent cellular cytotoxicity-mediated depletion of intratumoural Treg cells, which express high levels of CTLA-4. Indeed, in patients with advanced melanoma, favourable response to treatment with the anti-CTLA4 monoclonal antibody ipilimumab was associated, among patients with inflamed tumours, with the presence of a coding polymorphism within CD16a/FcγRIIIa that results in its higher affinity for Fc, suggesting that FcγRIIIa-dependent antibody-dependent cellular cytotoxicity is involved in the efficacy of ipilimumab therapy in humans.[23] As Lim and Okkenhaug point out, Treg and Tconv cells also have substantially shared intracellular signalling pathways and the balance to which distinct immunomodulatory agents affect Treg suppression versus Teff cell-mediated anti-tumour immunity determines their net effect upon tumour progression as is exemplified by the net immunostimulatory effect of genetic or pharmacological disruption of Phosphoinositide 3-kinase (PI3K)-δ activity.[24] Moreover, it is likely that shared expression of CCR4 on tumour-infiltrating Treg cells and activated $CD4^+$ and $CD8^+$ Tconv cells may have contributed to the lack of robust clinical efficacy of antibody reagents targeting these molecules.[25] Finally, as discussed by Yano *et al.*,[26] checkpoint immunotherapy may result in reactive recruitment of Treg cells to tumours in response to increased inflammation. Hence, a theme emerging from a number of reviews in this series is the substantially shared biology of Treg and Tconv cells and the need to consider the effects of therapy on both Treg and Tconv compartments.

Frequency of Treg cells in the tumour immune infiltrate often far exceeds that in normal tissues, suggesting that co-option of Treg cells by tumours is an important feature of cancer development and a requisite for cancer progression in a number of tumour types. Stockis *et al.*[27] consider the mechanisms that drive Treg cell accumulation within tumours, reviewing our understanding of the molecular basis for recruitment and maintenance of Treg cells within tumours, and proposing that selective recruitment of thymic Treg cells rather than *de novo* induction of induced Treg cells is the dominant mechanism by which Treg cells accumulate in cancer. While experimental observations supportive of this conclusion are presented, the relative functional contribution of thymic Treg and induced Treg cells to tumour immunosuppression has yet to be formally established. Treg cells within tumours express high levels of specific chemokine receptors, such as CCR2, CCR4, CCR8 and CCR10, and it is plausible, though again not clearly established, that expression of these receptors drives the recruitment of Treg cells into tumours. In addition, the association of tumours with tertiary lymphoid structures contributes to recruitment of Treg cells into tumours.[28] The tumour environment provides an environment supportive of Treg cell proliferation, and Stockis *et al.* also review the role of co-stimulatory and co-inhibitory receptor and cytokine signalling on Treg cell maintenance and activation in tumours. It is clear, however, that more work is needed to better dissect the distinct functions of chemokine, cytokine and co-stimulatory/co-inhibitory receptors on Treg and Tconv cell migration and function, respectively.

Given the shared involvement of Treg cells in immunological tolerance and tumour immunosuppression, selective targeting of Treg cells in tumours is desirable but requires an understanding of their specific biological characteristics. Yano *et al.*[26] consider the specific molecular and functional characteristics of Treg cells in tumours, observing that a number of molecular, cellular and metabolic characteristics distinguish them from Treg cells in other tissues. Joshi *et al.* describe attempts made to target the immunosuppressive function of Treg cells in preclinical mouse tumour models and in the clinic.[28] Such attempts include systemic administration of P300/HAT, EZH2 and BET inhibitors whose consequence upon Treg cell suppression results in augmented tumour immunity. Green *et al.* review the role of tissue-resident Treg cells in promoting both non-immune processes and immune processes associated with wound healing. In part, this activity is mediated by release of the epidermal growth factor-like growth factor amphiregulin by Treg cells, whose activity extends beyond its canonical function in wound repair to promoting the release of bioactive transforming growth factor-β through inside-out activation of integrins. The extent to which the function of Treg-derived amphiregulin in promoting tumour immunosuppression involves canonical and non-canonical amphiregulin functions has yet to be determined.[29]

In summary, there have been substantial strides in our understanding of the contribution of Treg cells to tumour immunosuppression. A detailed understanding of the often opposing effects of immunotherapies on both the Tconv and Treg compartments will aid the design of new immunotherapy approaches and the interpretation of their outcomes. In addition, there is a growing awareness of the involvement of Treg cells in influencing the outcome of conventional checkpoint inhibitor therapy responses, with potential functional contributions as profound and deleterious as anti-PD-1-induced hyperprogression. In this context, the shared biology of Treg and Tconv cells presents both an obstacle and an opportunity, especially for patient stratification and rational design of

combination immunotherapies. The observation that Treg cells in tumours harbour distinct molecular profiles that contribute to their selective migration and function and that distinguish them from Treg cells in other tissues provides extremely important opportunities for the selective targeting of Treg cells in cancer.

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References

1 Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell* 2008; **133**:775–87.

2 Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. *Annu Rev Immunol* 2012; **30**:531–64.

3 Roychoudhuri R, Eil RL, Restifo NP. The interplay of effector and regulatory T cells in cancer. *Curr Opin Immunol* 2015; **33**:101–11.

4 Quezada SA, Peggs KS, Simpson TR, Allison JP. Shifting the equilibrium in cancer immunoediting: from tumor tolerance to eradication. *Immunol Rev* 2011; **241**:104–18.

5 Klages K, Mayer CT, Lahl K, Loddenkemper C, Teng MW, Ngiow SF *et al.* Selective depletion of Foxp3$^+$ regulatory T cells improves effective therapeutic vaccination against established melanoma. *Can Res* 2010; **70**:7788–99.

6 Teng MW, Swann JB, von Scheidt B, Sharkey J, Zerafa N, McLaughlin N *et al.* Multiple antitumor mechanisms downstream of prophylactic regulatory T-cell depletion. *Can Res* 2010; **70**:2665–74.

7 Bos PD, Plitas G, Rudra D, Lee SY, Rudensky AY. Transient regulatory T cell ablation deters oncogene-driven breast cancer and enhances radiotherapy. *J Exp Med* 2013; **210**:2435–66.

8 Teng MW, Ngiow SF, von Scheidt B, McLaughlin N, Sparwasser T, Smyth MJ. Conditional regulatory T-cell depletion releases adaptive immunity preventing carcinogenesis and suppressing established tumor growth. *Can Res* 2010; **70**:7800–9.

9 Bates GJ, Fox SB, Han C, Leek RD, Garcia JF, Harris AL *et al.* Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse. *J Clin Oncol* 2006; **24**:5373–80.

10 Petersen RP, Campa MJ, Sperlazza J, Conlon D, Joshi MB, Harpole DH Jr *et al.* Tumor infiltrating Foxp3$^+$ regulatory T-cells are associated with recurrence in pathologic stage I NSCLC patients. *Cancer* 2006; **107**:2866–72.

11 Gao Q, Qiu SJ, Fan J, Zhou J, Wang XY, Xiao YS *et al.* Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis of hepatocellular carcinoma after resection. *J Clin Oncol* 2007; **25**:2586–93.

12 Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F *et al.* Intraepithelial CD8$^+$ tumor-infiltrating lymphocytes and a high CD8$^+$/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. *Proc Natl Acad Sci USA* 2005; **102**:18538–43.

13 Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P *et al.* Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* 2004; **10**:942–9.

14 Griffiths RW, Elkord E, Gilham DE, Ramani V, Clarke N, Stern PL *et al.* Frequency of regulatory T cells in renal cell carcinoma patients and investigation of correlation with survival. *Cancer Immunol Immunother* 2007; **56**:1743–53.

15 Hiraoka N, Onozato K, Kosuge T, Hirohashi S. Prevalence of FOXP3$^+$ regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its premalignant lesions. *Clin Cancer Res* 2006; **12**:5423–34.

16 Sinicrope FA, Rego RL, Ansell SM, Knutson KL, Foster NR, Sargent DJ. Intraepithelial effector (CD3$^+$)/regulatory (FoxP3$^+$) T-cell ratio predicts a clinical outcome of human colon carcinoma. *Gastroenterology* 2009; **137**:1270–9.

17 Perrone G, Ruffini PA, Catalano V, Spino C, Santini D, Muretto P *et al.* Intratumoural FOXP3-positive regulatory T cells are associated with adverse prognosis in radically resected gastric cancer. *Eur J Cancer* 2008; **44**:1875–82.

18 Jordanova ES, Gorter A, Ayachi O, Prins F, Durrant LG, Kenter GG *et al.* Human leukocyte antigen class I, MHC class I chain-related molecule A, and CD8$^+$/regulatory T-cell ratio: which variable determines survival of cervical cancer patients? *Clin Cancer Res* 2008; **14**:2028–35.

19 Ferrara R, Mezquita L, Texier M, Lahmar J, Audigier-Valette C, Tessonnier L *et al.* Hyperprogressive disease in patients with advanced non-small cell lung cancer treated with PD-1/PD-L1 inhibitors or with single-agent chemotherapy. *JAMA Oncol* 2018; **4**:1543–52.

20 Kim CG, Kim KH, Pyo KH, Xin CF, Hong MH, Ahn BC *et al.* Hyperprogressive disease during PD-1/PD-L1 blockade in patients with non-small-cell lung cancer. *Ann Oncol* 2019.

21 Kamada T, Togashi Y, Tay C, Ha D, Sasaki A, Nakamura Y *et al.* PD-1$^+$ regulatory T cells amplified by PD-1 blockade promote hyperprogression of cancer. *Proc Natl Acad Sci USA* 2019; **116**:9999–10008.

22 Simpson TR, Li F, Montalvo-Ortiz W, Sepulveda MA, Bergerhoff K, Arce F *et al.* Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. *J Exp Med* 2013; **210**:1695–710.

23 Arce Vargas F, Furness AJS, Litchfield K, Joshi K, Rosenthal R, Ghorani E *et al.* Fc effector function contributes to the activity of human anti-CTLA-4 antibodies. *Cancer Cell* 2018; **33**:649–663 e644.

24 Lim EL, Okkenhaug K. PI3Kδ is a Treg target in cancer immunotherapy. *Immunology* 2019; **157**:210–8.

25 Kurose K, Ohue Y, Wada H, Iida S, Ishida T, Kojima T *et al.* Phase Ia study of FoxP3$^+$ CD4 Treg depletion by infusion of a humanized anti-CCR25 antibody, KW-0761, in cancer patients. *Clin Cancer Res* 2015; **21**:4327–36.

26 Yano H, Andrews LP, Workman CJ, Vignali DAA. Intratumoral regulatory T cells: markers, subsets and their impact on anti-tumor immunity. *Immunology* 2019; **157**:232–47.

27 Stockis J, Roychoudhuri R, Halim TYF. Regulation of regulatory T cells in cancer. *Immunology* 2019; **157**:219–31.

28 Joshi NSM, Akama-Garren EH, Lu Y, Lee DY, Chang GP, Li A *et al.* Regulatory T cells in tumor-associated tertiary lymphoid structures suppress anti-tumor T cell responses. *Immunity* 2015; **43**:579–90.

29 Green JA, Arpaia N, Schizas M, Dobrin A, Rudensky AY. A nonimmune function of T cells in promoting lung tumor progression. *J Exp Med* 2017; **214**:3565–75.