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Original Research Article

Recent Advances on T Regulatory Cells

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ABSTRACT

Active suppression of tumour-reactive T cells can limit both immune surveillance and immunotherapy. Development of cancer results into the generation of immune suppressive network to inhibit anti-tumour activity and evade the host's immune response, which eventually facilitates tumour progression. Emerging evidence indicates that immunosuppressive cells; T regulatory cells (Tregs) may be largely responsible for inhibiting host T-cell activity against tumour-associated antigens and impair the effectiveness of anticancer immunotherapeutic approaches. Reducing the deleterious effects of Treg may increase the success of various immunotherapeutic modalities in cancer. However, an increase in our understanding of the local tumour microenvironment and the exact mechanisms of Treg induction and/or expansion in peripheral blood and tumour microenvironment of cancer patients should provide opportunities to test different treatments to target these immunosuppressive cells and alter the balance in favor of generating effective anti-tumour immune responses. The purpose of this study is to provide information about the phenotype, role and the function of Tregs and the mechanisms of their expansion in cancer.

1. Background

1.1. Introduction

Numerous approaches have been explored to harness the potency of the immune system to target cancer. To date, these have been essentially focused on enhancing the immunogenicity of the tumour or the induction and expansion of immune effectors to potentially target and eradicate the tumour. One plausible hypothesis is that the local tumour environment is strongly immune suppressive suggesting that there is a need to overcome immunosuppression mechanisms (Talmadge, 2007). It is hypothesised that as a tumour evolves during the early stages of neoplastic disease, there is an orchestrated generation of a pro-inflammatory microenvironment which provides mitogenic stimuli, supports neo-angiogenesis and circumvents T cell mediated antitumour responses (Dunn, Bruce, Ikeda, Old, & Schreiber, 2002). Evidence indicates that increase the level of immunosuppressive cells; Tregs and MDSCs may be responsible for the inhibition of host T cell activity against tumour-associated antigens (TAA). In addition, it is proposed that an increase in the numbers of these immune suppressive cells could negatively impair the effectiveness of anti-cancer immunotherapeutic approaches.

To clarify these roles and immunosuppression activity of Tregs, we have to understanding first of all; the interaction between immune cells (Tregs) and cancer cells. And secondly we need to know the functional activity of these immunosuppressive cells and reduce their effects, which may increase the success of design various and effective immunotherapeutic strategy in cancer patients. This review will focus on the immunosuppressive cells (Tregs) phenotype, functional activity and their roles in normal and cancer.

2. T regulatory cells (Tregs)

The importance of Tregs in both normal and pathological conditions (including tumour and microbial immunity, transplantation and allergy) is now better known (Yamaguchi, Wing, & Sakaguchi, 2011).

3. Definition of Tregs

Tregs cells were discovered by (Sakaguchi, Sakaguchi, Asano, Itoh, & Toda, 1995) group as double positive cells with CD4 and CD25 ($CD4^+CD25^+$) that main function was inhibiting the activity of T cells (Sakaguchi et al., 1995). Tregs cells are heterogeneous subset of cells composed of different phenotypes and functions (Mohr, Malhotra, Mayer, Gorochov, & Miyara, 2018). Moreover, there is also $CD8^+$ Tregs but still not well described yet (Holderried, Lang, Kim, & Cantor, 2013; H.-J. Kim, Verbinnen, Tang, Lu, & Cantor, 2010). In the presented study will be focused on $CD4^+$ Tregs and it is believed that there are two main subpopulations of $CD4^+$ Tregs with different origins; natural Tregs (nTregs) and induced Tregs (iTregs).

4. Phenotype of Tregs

Tregs express the canonical Treg markers such as, interleukin-2 (IL-2; CD25) receptor α-chain, FoxP3, glucocorticoid-induced TNF receptor (GITR) and cytotoxic T-lymphocyte associated protein 4 (CTLA4; CD152), the TNF receptor superfamily member 25 (TNFRSF25; DR3), CD73 and CD39 (Deaglio et al., 2007; B.-S. Kim et al., 2015; Lin et al., 2013; Madireddi et al., 2017; Zhao, Liao, & Kang, 2017). In addition, nTregs display higher levels of programmed cell death-1 (PD-1; CD279), neuropilin 1 (Nrp1), Helios, glycoprotein A repetitions predominant (GARP; LRRC32), lymphocyte activation antigen-3 (LAG-3; CD223) and latency-associated peptide (LAP) compared with iTregs (M.-L. Chen, Yan, Bando, Kuchroo, & Weiner, 2008; Huard, Prigent, Tournier, Bruniquel, & Triebel, 1995; Y. C. Kim et al., 2012; Lin et al., 2013; Sun, Jin, & Li, 2016; Tran, Andersson, Wang, et al., 2009; Weiss et al., 2012; Yadav et al., 2012).

5. Classification of Tregs

Treg cells are mainly subdivided into two population nTregs and iTregs as discussed in the following sections.

a. nTregs is also named as thymus-derived nTreg cells as it develops in the thymus and it is originally characterised as a subset of $CD4^+$ T cells constitutively expressing high levels of the interleukin-2 (IL-2) receptor α-chain (CD25) (Lin et al., 2013). They are antigen-specific, play a key role in maintaining self-tolerance, and comprise approximately 5-10% of total peripheral $CD4^+$ T cells. The main characteristic phenotype of these cells that they express the forkhead box P3 ($FoxP3^+$) proteins and also known as the winged helix transcription factor, this is crucial for the development and function of nTregs. and it was demonstrated that retroviral gene transfer of FoxP3 confers suppressor function on peripheral naïve $CD4^+CD25^-$ T cells (Fontenot, Gavin, & Rudensky, 2003; Hori, Nomura, & Sakaguchi, 2003; Rudensky, 2011). In addition to FoxP3, approximately 70% of CD4 Tregs express high level of the Ikaros family member Helios (Thornton et al., 2010). The co-expression of $Helios^+$ makes $CD4^+$ Tregs more effectively suppress cytokine production by T cells (Himmel, MacDonald, Garcia, Steiner, & Levings, 2013; Sugita et al., 2015) as well as stabilize $Helios^+$ $CD4^+$ Tregs under inflammatory conditions than $Helios^-$ $CD4^+$ Tregs (Dhuban et al., 2015; Dijke et al., 2016; Sharma et al., 2013). Studies demonstrated that deficient mice with Helios cells develop autoimmune with low suppressive activity of $CD4^+$ Tregs (H.-J. Kim et al., 2015; Sebastian et al., 2016).

   On the other hand, coexpression of Helios and FoxP3 in murine $CD4^+$ Tregs increases the suppression activity of Tregs compared with Foxp3 alone (Fu et al., 2012). Additionally, it has been shown that Helios is also critical for $CD8^+$ Treg to mediate its function (H.-J. Kim et al., 2015).

b. iTregs, also known as adaptive or periphery Tregs (pTregs) which are generated in peripheral lymphoid tissue by the conversion of conventional naïve $CD4^+FoxP3^-$ T cells to $FoxP3^+$ Tregs (Li & Zheng, 2015). iTregs develop outside the thymus upon the activation of conventional T cells by polyclonal or antigen-specific activation in the presence of specific immune suppressive cytokines and exerting their function *in vivo* in a cytokine-dependent manner such as transforming growth factor beta (TGF-β) (Bluestone & Abbas, 2003; de Lafaille & Lafaille, 2009; Gallimore & Simon, 2008; Schallenberg, Tsai, Riewaldt, & Kretschmer, 2010). TGF-β is a multifunctional cytokine that has effect on wide range of cell growth and differentiation, it has been shown to induce the conversion of peripheral $CD4^+CD25^-$ naïve T cells to $CD4^+CD25^+FoxP3^+$ Tregs (W. Chen et al., 2003; Filippi et al., 2008). Three subsets of $CD4^+$ Tregs (Tr1, Th3 and iTr35) and $CD8^+$ Tregs ($CD8^+CD28^-$) as discussed in brief subsequently:

      I. Type 1 regulatory T cells (Tr1), it lacks the expression of $FoxP3^+$ but it secretes high levels of interleukin (IL-10), this is induced by antigen stimulation via an IL-10-dependent process both *in vitro* and *in vivo* (Grazia Roncarolo et al., 2006; Hawrylowicz & O'garra, 2005; Povoleri et al., 2013). Moreover, Tr1 can be induced and mediated by molecules such as cyclooxygenase 2 (COX-2) that expressed by tumour cells (Akasaki et al., 2004; Bergmann, Strauss, Zeidler, Lang, & Whiteside, 2007).

II. T helper cells (Th3), like Tr1, it also lacks FoxP3$^+$, but it secretes a high level of TGF-β, Th3 cells are induced as a result of antigen administration, in presence of TGF-β and IL-4 (Inobe et al., 1998; Povoleri et al., 2013; Weiner, 2001).

III. iTr35 cells do not express FoxP3 and they are induced and mediate their suppression activity via the inhibitory cytokine IL-35 (Collison et al., 2010; Povoleri et al., 2013). The exact role and function of iTr35 cells in immune response is still elusive and need further investigations.

IV. CD8$^+$ Tregs are recently described T cells that function to control and regulate various T cell responses, in particular, play critical role in cancer and autoimmunity (Chaput et al., 2009; Gupta & Agrawal, 2020). CD8$^+$ Tregs are induced following repeated activation of CD8$^+$ T cells with antigen and this activation leads to generation of suppressive T cells with phenotype CD8$^+$CD28$^-$ (Jiang et al., 1998).

Both nTregs and iTregs suppress a variety of physiological and pathological immune responses in a contact- and/or cytokine-dependent manners, with a wide potential immune suppressive molecules that processes FoxP3$^+$ Tregs in particular, reviewed largely (Shevach, 2009; Q. Tang & Bluestone, 2008).

6. Tregs expansion in cancer

In terms to the roles of Tregs cells in cancer, both nTregs and iTregs are thought to potentially contribute to its development by promoting tumour-specific T cell tolerance, inhibiting anti-tumour immune responses (Shevach, 2009). In addition to Treg cells, both CD4$^+$ and CD8$^+$ T suppressive cells have been described in a number of cancers (Andersen et al., 2009; Bailur, Gueckel, Derhovanessian, & Pawelec, 2015; Chaput et al., 2009; Preston et al., 2013; Tatsumi et al., 2003; Woo et al., 2001; Yamamoto et al., 2012; Zhou et al., 2020). Importantly, a large number of FoxP3$^+$ Treg infiltrate into tumours, and systemic removal of these cells enhances natural as well as vaccine-induced anti-tumour T cell responses, more details can be found in this review (Nishikawa & Sakaguchi, 2010). Studies demonstrated that Tregs are expanded in peripheral blood and tumour microenvironment in cancer patients and this usually correlates with poor prognosis and reduced survival (Chaudhary & Elkord, 2016; Filaci et al., 2007; Jacobs et al., 2010; Whiteside, 2015). It seems that tumours have diverse immunosuppressive mechanisms and networks to escape the antitumour immunity (Demaria, 2013). However, Dannull and colleagues have reported that vaccine efficacy (mediated antitumor immunity) was boosted in cancer patients following the depletion of Tregs (Dannull et al., 2005). The available evidence suggest that Tregs are implicated in the immunopathology of cancer and their depletion may enhance the immunotherapy efficacy.

Given the background on Tregs in cancers, however, it is still unknown whether systemic or local (tumour microenvironment) sources of Tregs are the key players. At least four potential sources for Tregs in the tumour microenvironment have been identified including 1) trafficking, 2) differentiation, 3) expansion by dysfunctional antigen-presenting cells (APCs) and 4) conversion of T cells into Tregs by TGF-β (Zou, 2006). The fourth mechanism, conversion of conventional T cells into Tregs has been supposed as a potential mechanism for Treg expansion in cancers and this was supported by *in vitro* and mouse model studies with no data from in vivo or human studies to support its role in enriching peripheral and tumor-infiltrating Tregs. Moreover, studies reported that some soluble mediators such as prostaglandin (E_2), vascular endothelia growth factor (VEGF) and TGF-β were detected at high levels in certain tumours and their roles may have negative effects on the APC and dendritic cells (DCs) maturation and functions (Gomella, Sargent, Linehan, & Kasid, 1989; Smyth et al., 2003; Wada et al., 2009) and more details can be found on the following review (Zhao et al., 2017).

Studies have been shown that reported that chemokine gradients in the tumour microenvironment can attract circulating Tregs. Expression of different chemokine receptors, such as CXCR3, CCR4, CCR6 and CCR9 were shown to promote nTreg subsets accumulation in non-lymphoid tissues, in order to exert tissue-specific immuneregulation (Hirahara et al., 2006; Kleinewietfeld et al., 2005; Santodomingo-Garzon, Han, Le, Yang, & Swain, 2009; Yamazaki et al., 2008). In addition to CCR6, it has been demonstrated to be specific for effector/memory nTregs, which associated with increased levels of IL-10 secretions (Guo et al., 2008; Kleinewietfeld et al., 2005).

Indeed, chemokine ligand (CCL22), which can be produced by tumour cells and tumour-associated macrophages, and mediates the specific recruitment chemokine receptor CCR4 Tregs into the tumour (Gobert et al., 2009; Klarquist et al., 2016). Moreover, it suggested that the reason for the relative increase in Treg levels in cancer patients with renal cell carcinoma (RCC) could be associated with the upregulation of antiapoptotic genes and downregulation of pro-apoptotic genes (Jeron et al., 2009). However, it is worth to mention that there is a better understanding of antigen recognition and interactions by CTL

or Th cells, of note, the requirement for antigen specificity for Tregs and their suppressive actions and mechanisms remains largely elusive. In addition, tumour-associated antigens (TAA) that expressed by tumour cells may have a critical role in the recruitment, maintenance and expansion of Tregs at the sites of tumour and this may indicate that Tregs to function as anti-tumour immune response (Curiel, 2008; Wang & Wang, 2007). Therefore, it is important to determine the TAA that recognised by Tregs, it has been shown in some mouse model studies that a superior suppressive activity of antigen-specific Tregs over non-antigen-specific Tregs (Samy, Parker, Sharp, & Tung, 2005). Previous studies showed that GARP, LAP and CD137 (4–1BB) were defined as Treg-cell activation markers following polyspecific Treg-cell activation (Schoenbrunn et al., 2012; Tran, Andersson, Hardwick, et al., 2009; Tran, Andersson, Wang, et al., 2009). In this regard, it has been demonstrated that GARP and LAP can be used as pure alloantigen-specific Treg cells. Same study demonstrated that naïve and activated Treg cells do not express CD40 ligand (CD154) (Noyan et al., 2014). Moreover, there are some ligands that expressed tumour infiltrating lymphocytes and recognized by $CD4^+$ Tregs of cancer patients. Such these ligands LAGE1 (a homolog of NY-ESO-1) and ARTC1 were previously identified as ligands for tumour-specific $CD4^+$ T cells that share the common characteristics of nTregs (Wang et al., 2004; Wang, Peng, Guo, Shevach, & Wang, 2005). It may indicate that generation of $CD4^+$ T-cell clones that have the same antigen specificity but with helper or suppressor functions.

Despite the growing understanding of how Tregs are increased in the cancer setting, the exact mechanism involved in elevating their levels in cancer patients remains unclear. Investigation of whether the increased numbers of Tregs are nTregs or iTregs would provide greater insights into potential mechanisms of cancer evasion. Additionally, exploring which of these mechanisms may have a role in a specific cancer setting is of particular interest as some of the processes involved could be amenable to therapeutic intervention.

7. Suppression mechanisms of Tregs

Tregs can inhibit various type of immune cells including B cells, natural killer cells (NK) cells, natural killer T (NKT) cells, monocytes, dendritic cells (DCs) as well as $CD4^+$, and $CD8^+$ T cells (A. Schmidt, Oberle, & Krammer, 2012). The exact suppression mechanism of Tregs is still incompletely understood, however, there are different mechanisms of Treg cells mediated its suppression activities that have been described; 1) suppression via co-inhibitory receptors (this review will only discuss this suppression mechanism in subsequent sections), 2) suppression via cytokines secretion including; TGF-β, IL-10 and IL-35, and there are other mechanisms that have been described in more details elsewhere (A. Schmidt et al., 2012; Zhao et al., 2017).

1. **Treg-mediated suppression via co-inhibitory receptors**
   a. CTLA-4

Tregs cells have the ability to regulate APCs via CTLA-4. CTLA-4 or CD152 is constitutively expressed on activated Tregs cells and function to bind with high affinity with B7 (CD80 and CD86) rather than CD28 resulting in reduction for co-stimulatory capacity of APCs (Wing, Yamaguchi, & Sakaguchi, 2011). Moreover, expression of CTLA-4 on Tregs can control their functions. A study demonstrated that the presence of CTLA-4 expression on Tregs lead to prevention of inflammatory tissue attack in arthritis (Klocke, Holmdahl, & Wing, 2017). It reported that deletion of CTLA-4 led to Tregs growth but with low suppressive activity (Peggs, Quezada, Chambers, Korman, & Allison, 2009; E. M. Schmidt et al., 2009). A study on mice model demonstrated that inhibition of CTLA-4 by using CTLA-4 specific blocking antibody rapidly induce Tregs proliferation (A. L. Tang et al., 2008). On the other hand, blocking B7 molecules on target cells using CTLA-4-Ig resulted reduction in level of memory Tregs (with robust suppressive function) (Holt, Punkosdy, Glass, & Shevach, 2017; Mohr et al., 2018). Taken together, deletion of CTLA-4 increases Tregs level whereas number of Tregs decrease as a result of loss of CD28. Moreover, monoclonal antibodies targeting the expression CTLA-4 have been shown a potent immunotherapy for some tumours (Eggermont et al., 2016; Robert et al., 2019; Robert et al., 2015).

   b. PD-1

Apart from CTLA-4, an inhibitory receptor PD-1 (CD279) is also expressed on activated B cells, CD4+ and CD8+ T cells in the periphery (Nishimura & Honjo, 2001). It shares similarity with CTLA-4 a member of CD28 family which provides a negative signal when it binds with its cognate ligands (PD-L1 and PD-L2) (Keir, Butte, Freeman, & Sharpe, 2008). A study demonstrated that positive Tregs for PD-1 displays strong suppression activity during viral infections (Ha et al., 2016). In addition, it has been shown that the inhibition of PD-1 contributed to enhancement of Tregs and production IL-10 (Woods, Ramakrishnan, Sodré, Berglund, & Weber, 2017). Combination of PD-1 with cytokine TGF-β led to the downregulation of TGF-β-mediated signals and thus induced the differentiation of naïve T cells to iTregs (Francisco et al., 2009). Nowadays, extensive research is going on on targeting PD-1 as a new immunotherapy besides CTLA-4 (Alsaab et al., 2017; Hodi et al., 2016; Postow et al.,

2015; Routy et al., 2018), however, a number of patients failed to respond to anti-PD-1 immunotherapy (Bu, Mahoney, & Freeman, 2016).

c. CD39 and CD73

CD39 and CD73 (also named as ectonucleotidases) have been suggested as functional markers and highly expressed on Tregs cells. Both molecules play critical roles in conversion of ATP-induced proinflammatory to adenosine-induced anti-inflammatory state (Cekic & Linden, 2016). In addition, Tregs can suppress the proliferation of effector T cells via two mechanisms a) exosomes (containing both CD39 and CD73) and b) production of adenosine by CD39 and CD73 that expressed on Tregs cells in which bind with the adenosine receptors (A2A) on effector T cells, as a result, induce the intracellular cyclic AMP (cAMP) levels that inhibit effector T cells function (Gruenbacher et al., 2016). For more details, readers are advised to look at these references (Leonard et al., 2017; Zhao et al., 2017).

d. T cell Ig and ITIM domain (TIGIT)

TIGIT is defined as a new immune checkpoint, and expressed on natural killer cells (NK), nTregs and Tr1 (Anderson, Joller, & Kuchroo, 2016; Lozano, Dominguez-Villar, Kuchroo, & Hafler, 2012). TIGIT binds with cognate ligands CD155 and CD112 on the APCs and tumour cells (Joller et al., 2014; Kurtulus et al., 2015). Studies have demonstrated that TIGIT/CD226 has a superior immunosuppressive activity in vitro function to inhibit T cells (Joller et al., 2014; Manieri, Chiang, & Grogan, 2017).

8. Conclusion

Tregs are heterogenous cells in phenotype, classification, function and their suppression pathways in cancers. Large evidence indicates that Treg cells may be responsible for inhibiting host T-cell activity against tumour-associated antigens and decrease the efficacy of immunotherapy approaches. The current review attempt to enhance our knowledge of Tregs cells, definition, phenotype, classification and suppression functions based on recent studies. Understanding the exact mechanisms of Treg cells function in tumour microenvironment of cancer patients may support the future studies in developing different immunotherapeutic strategy for cancer patients.

References

[1] Akasaki, Y., Liu, G., Chung, N. H., Ehtesham, M., Black, K. L., & John, S. Y. (2004). Induction of a CD4+ T regulatory type 1 response by cyclooxygenase-2-overexpressing glioma. *The Journal of Immunology, 173*(7), 4352-4359.

[2] Alsaab, H. O., Sau, S., Alzhrani, R., Tatiparti, K., Bhise, K., Kashaw, S. K., & Iyer, A. K. (2017). PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: mechanism, combinations, and clinical outcome. *Frontiers in pharmacology, 8*, 561.

[3] Andersen, M. H., Sørensen, R. B., Brimnes, M. K., Svane, I. M., Becker, J. C., & thor Straten, P. (2009). Identification of heme oxygenase-1–specific regulatory CD8+ T cells in cancer patients. *The Journal of clinical investigation, 119*(8), 2245-2256.

[4] Anderson, A. C., Joller, N., & Kuchroo, V. K. (2016). Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. *Immunity, 44*(5), 989-1004.

[5] Bailur, J. K., Gueckel, B., Derhovanessian, E., & Pawelec, G. (2015). Presence of circulating Her2-reactive CD8+ T-cells is associated with lower frequencies of myeloid-derived suppressor cells and regulatory T cells, and better survival in older breast cancer patients. *Breast Cancer Research, 17*(1), 34.

[6] Bergmann, C., Strauss, L., Zeidler, R., Lang, S., & Whiteside, T. L. (2007). Expansion of human T regulatory type 1 cells in the microenvironment of cyclooxygenase 2 overexpressing head and neck squamous cell carcinoma. *Cancer research, 67*(18), 8865-8873.

[7] Bluestone, J. A., & Abbas, A. K. (2003). Natural versus adaptive regulatory T cells. *Nature Reviews Immunology, 3*(3), 253-257.

[8] Bu, X., Mahoney, K. M., & Freeman, G. J. (2016). Learning from PD-1 resistance: new combination strategies. *Trends in molecular medicine, 22*(6), 448-451.

[9] Cekic, C., & Linden, J. (2016). Purinergic regulation of the immune system. *Nature Reviews Immunology, 16*(3), 177.

[10] Chaput, N., Louafi, S., Bardier, A., Charlotte, F., Vaillant, J.-C., Ménégaux, F., . . . Taieb, J. (2009). Identification of CD8+ CD25+ Foxp3+ suppressive T cells in colorectal cancer tissue. *Gut, 58*(4), 520-529.

[11] Chaudhary, B., & Elkord, E. (2016). Regulatory T cells in the tumor microenvironment and cancer progression: role and therapeutic targeting. *Vaccines, 4*(3), 28.

[12] Chen, M.-L., Yan, B.-S., Bando, Y., Kuchroo, V. K., & Weiner, H. L. (2008). Latency-associated peptide identifies a novel CD4+ CD25+ regulatory T cell subset with TGFβ-mediated function and enhanced suppression of experimental autoimmune encephalomyelitis. *The Journal of Immunology, 180*(11), 7327-7337.

[13] Chen, W., Jin, W., Hardegen, N., Lei, K.-j., Li, L., Marinos, N., . . . Wahl, S. M. (2003). Conversion of peripheral CD4+ CD25− naive T cells to CD4+ CD25+ regulatory T cells by TGF-β induction of transcription factor Foxp3. *The Journal of experimental medicine, 198*(12), 1875-1886.

[14] Collison, L. W., Chaturvedi, V., Henderson, A. L., Giacomin, P. R., Guy, C., Bankoti, J., . . . Brown, S. A. (2010). IL-35-mediated induction of a potent regulatory T cell population. *Nature immunology, 11*(12), 1093.

[15] Curiel, T. J. (2008). Regulatory T cells and treatment of cancer. *Current opinion in immunology, 20*(2), 241-246.
[16] Dannull, J., Su, Z., Rizzieri, D., Yang, B. K., Coleman, D., Yancey, D., . . . Gilboa, E. (2005). Enhancement of vaccine-mediated antitumor immunity in cancer patients after depletion of regulatory T cells. *The Journal of clinical investigation, 115*(12), 3623-3633.
[17] de Lafaille, M. A. C., & Lafaille, J. J. (2009). Natural and adaptive foxp3+ regulatory T cells: more of the same or a division of labor? *Immunity, 30*(5), 626-635.
[18] Deaglio, S., Dwyer, K. M., Gao, W., Friedman, D., Usheva, A., Erat, A., . . . Oukka, M. (2007). Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. *The Journal of experimental medicine, 204*(6), 1257-1265.
[19] Demaria, S. (2013). Immune escape: immunosuppressive networks *Cancer Immunotherapy* (pp. 149-164): Elsevier.
[20] Dhuban, K. B., d'Hennezel, E., Nashi, E., Bar-Or, A., Rieder, S., Shevach, E. M., . . . Piccirillo, C. A. (2015). Coexpression of TIGIT and FCRL3 identifies Helios+ human memory regulatory T cells. *The Journal of Immunology, 194*(8), 3687-3696.
[21] Dijke, I., Hoeppli, R., Ellis, T., Pearcey, J., Huang, Q., McMurchy, A., . . . Larsen, I. (2016). Discarded human thymus is a novel source of stable and long-lived therapeutic regulatory T cells. *American Journal of Transplantation, 16*(1), 58-71.
[22] Dunn, G. P., Bruce, A. T., Ikeda, H., Old, L. J., & Schreiber, R. D. (2002). Cancer immunoediting: from immunosurveillance to tumor escape. *Nature immunology, 3*(11), 991-998.
[23] Eggermont, A. M., Chiarion-Sileni, V., Grob, J.-J., Dummer, R., Wolchok, J. D., Schmidt, H., . . . Richards, J. M. (2016). Prolonged survival in stage III melanoma with ipilimumab adjuvant therapy. *New England Journal of Medicine, 375*(19), 1845-1855.
[24] Filaci, G., Fenoglio, D., Fravega, M., Ansaldo, G., Borgonovo, G., Traverso, P., . . . Rizzi, M. (2007). CD8+ CD28− T regulatory lymphocytes inhibiting T cell proliferative and cytotoxic functions infiltrate human cancers. *The Journal of Immunology, 179*(7), 4323-4334.
[25] Filippi, C. M., Juedes, A. E., Oldham, J. E., Ling, E., Togher, L., Peng, Y., . . . von Herrath, M. G. (2008). Transforming growth factor-β suppresses the activation of CD8+ T-cells when naive but promotes their survival and function once antigen experienced: a two-faced impact on autoimmunity. *Diabetes, 57*(10), 2684-2692.
[26] Fontenot, J. D., Gavin, M. A., & Rudensky, A. Y. (2003). Foxp3 programs the development and function of CD4+ CD25+ regulatory T cells. *Nature immunology, 4*(4), 330-336.
[27] Francisco, L. M., Salinas, V. H., Brown, K. E., Vanguri, V. K., Freeman, G. J., Kuchroo, V. K., & Sharpe, A. H. (2009). PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *Journal of Experimental Medicine, 206*(13), 3015-3029.
[28] Fu, W., Ergun, A., Lu, T., Hill, J. A., Haxhinasto, S., Fassett, M. S., . . . Chan, S. (2012). A multiply redundant genetic switch'locks in'the transcriptional signature of regulatory T cells. *Nature immunology, 13*(10), 972.
[29] Gallimore, A. M., & Simon, A. (2008). Positive and negative influences of regulatory T cells on tumour immunity. *Oncogene, 27*(45), 5886-5893.
[30] Gobert, M., Treilleux, I., Bendriss-Vermare, N., Bachelot, T., Goddard-Leon, S., Arfi, V., . . . Olive, D. (2009). Regulatory T cells recruited through CCL22/CCR4 are selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome. *Cancer research, 69*(5), 2000-2009.
[31] Gomella, L. G., Sargent, E. R., Linehan, W. M., & Kasid, A. (1989). Transforming growth factor-beta inhibits the growth of renal cell carcinoma in vitro. *The Journal of urology, 141*(5), 1240-1244.
[32] Grazia Roncarolo, M., Gregori, S., Battaglia, M., Bacchetta, R., Fleischhauer, K., & Levings, M. K. (2006). Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. *Immunological reviews, 212*(1), 28-50.
[33] Gruenbacher, G., Gander, H., Rahm, A., Idzko, M., Nussbaumer, O., & Thurnher, M. (2016). Ecto-ATPase CD39 inactivates isoprenoid-derived Vγ9Vδ2 T cell phosphoantigens. *Cell reports, 16*(2), 444-456.
[34] Guo, Z., Jang, M. H., Otani, K., Bai, Z., Umemoto, E., Matsumoto, M., . . . Matsushima, K. (2008). CD4+ CD25+ regulatory T cells in the small intestinal lamina propria show an effector/memory phenotype. *International immunology, 20*(3), 307-315.
[35] Gupta, S., & Agrawal, S. (2020). In vitro Effects of CD8+ Regulatory T Cells on Human B Cell Subpopulations. *International Archives of Allergy and Immunology*, 1-5.
[36] Ha, S.-J., Park, H. J., Park, J. S., Jeong, Y. H., Son, J., Ban, Y. H., . . . Chung, D. H. (2016). Role of PD-1 in regulatory T cells during chronic virus infection: Am Assoc Immnol.
[37] Hawrylowicz, C., & O'garra, A. (2005). Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. *Nature Reviews Immunology, 5*(4), 271-283.
[38] Himmel, M. E., MacDonald, K. G., Garcia, R. V., Steiner, T. S., & Levings, M. K. (2013). Helios+ and Helios− cells coexist within the natural FOXP3+ T regulatory cell subset in humans. *The Journal of Immunology, 190*(5), 2001-2008.
[39] Hirahara, K., Liu, L., Clark, R. A., Yamanaka, K.-i., Fuhlbrigge, R. C., & Kupper, T. S. (2006). The majority of human peripheral blood CD4+ CD25highFoxp3+ regulatory T cells bear functional skin-homing receptors. *The Journal of Immunology, 177*(7), 4488-4494.
[40] Hodi, F. S., Chesney, J., Pavlick, A. C., Robert, C., Grossmann, K. F., McDermott, D. F., . . . Agarwala, S. S. (2016). Combined nivolumab and ipilimumab versus ipilimumab alone in patients with advanced melanoma: 2-year overall survival outcomes in a multicentre, randomised, controlled, phase 2 trial. *The Lancet Oncology, 17*(11), 1558-1568.
[41] Holderried, T. A., Lang, P. A., Kim, H.-J., & Cantor, H. (2013). Genetic disruption of CD8+ Treg activity enhances the immune response to viral infection. *Proceedings of the National Academy of Sciences, 110*(52), 21089-21094.
[42] Holt, M. P., Punkosdy, G. A., Glass, D. D., & Shevach, E. M. (2017). TCR signaling and CD28/CTLA-4 signaling cooperatively modulate T regulatory cell homeostasis. *The Journal of Immunology, 198*(4), 1503-1511.
[43] Hori, S., Nomura, T., & Sakaguchi, S. (2003). Control of regulatory T cell development by the transcription factor Foxp3. *Science, 299*(5609), 1057-1061.

[44] Huard, B., Prigent, P., Tournier, M., Bruniquel, D., & Triebel, F. (1995). CD4/major histocompatibility complex class II interaction analyzed with CD4-and lymphocyte activation gene-3 (LAG-3)-Ig fusion proteins. *European journal of immunology, 25*(9), 2718-2721.
[45] Inobe, J. i., Slavin, A. J., Komagata, Y., Chen, Y., Liu, L., & Weiner, H. L. (1998). IL-4 is a differentiation factor for transforming growth factor-β secreting Th3 cells and oral administration of IL-4 enhances oral tolerance in experimental allergic encephalomyelitis. *European journal of immunology, 28*(9), 2780-2790.
[46] Jacobs, J. F., Idema, A. J., Bol, K. F., Grotenhuis, J. A., de Vries, I. J. M., Wesseling, P., & Adema, G. J. (2010). Prognostic significance and mechanism of Treg infiltration in human brain tumors. *Journal of neuroimmunology, 225*(1-2), 195-199.
[47] Jeron, A., Pfoertner, S., Bruder, D., Geffers, R., Hammerer, P., Hofmann, R., . . . Schrader, A. J. (2009). Frequency and gene expression profile of regulatory T cells in renal cell carcinoma. *Tumor Biology, 30*(3), 160-170.
[48] Jiang, S., Tugulea, S., Pennesi, G., Liu, Z., Mulder, A., Lederman, S., . . . Suciu-Foca, N. (1998). Induction of MHC-class I restricted human suppressor T cells by peptide priming in vitro. *Human immunology, 59*(11), 690-699.
[49] Joller, N., Lozano, E., Burkett, P. R., Patel, B., Xiao, S., Zhu, C., . . . Yajnik, V. (2014). Treg cells expressing the coinhibitory molecule TIGIT selectively inhibit proinflammatory Th1 and Th17 cell responses. *Immunity, 40*(4), 569-581.
[50] Keir, M. E., Butte, M. J., Freeman, G. J., & Sharpe, A. H. (2008). PD-1 and its ligands in tolerance and immunity. *Annu. Rev. Immunol., 26*, 677-704.
[51] Kim, B.-S., Nishikii, H., Baker, J., Pierini, A., Schneidawind, D., Pan, Y., . . . Negrin, R. S. (2015). Treatment with agonistic DR3 antibody results in expansion of donor Tregs and reduced graft-versus-host disease. *Blood, The Journal of the American Society of Hematology, 126*(4), 546-557.
[52] Kim, H.-J., Barnitz, R. A., Kreslavsky, T., Brown, F. D., Moffett, H., Lemieux, M. E., . . . Chan, S. (2015). Stable inhibitory activity of regulatory T cells requires the transcription factor Helios. *Science, 350*(6258), 334-339.
[53] Kim, H.-J., Verbinnen, B., Tang, X., Lu, L., & Cantor, H. (2010). Inhibition of follicular T-helper cells by CD8+ regulatory T cells is essential for self tolerance. *Nature, 467*(7313), 328-332.
[54] Kim, Y. C., Bhairavabhotla, R., Yoon, J., Golding, A., Thornton, A. M., Tran, D. Q., & Shevach, E. M. (2012). Oligodeoxynucleotides stabilize Helios-expressing Foxp3+ human T regulatory cells during in vitro expansion. *Blood, The Journal of the American Society of Hematology, 119*(12), 2810-2818.
[55] Klarquist, J., Tobin, K., Oskuei, P. F., Henning, S. W., Fernandez, M. F., Dellacecca, E. R., . . . Mehrotra, S. (2016). Ccl22 diverts T regulatory cells and controls the growth of melanoma. *Cancer research, 76*(21), 6230-6240.
[56] Kleinewietfeld, M., Puentes, F., Borsellino, G., Battistini, L., Rötzschke, O., & Falk, K. (2005). CCR6 expression defines regulatory effector/memory-like cells within the CD25+ CD4+ T-cell subset. *Blood, 105*(7), 2877-2886.
[57] Klocke, K., Holmdahl, R., & Wing, K. (2017). CTLA-4 expressed by FOXP3+ regulatory T cells prevents inflammatory tissue attack and not T-cell priming in arthritis. *Immunology, 152*(1), 125-137.
[58] Kurtulus, S., Sakuishi, K., Ngiow, S.-F., Joller, N., Tan, D. J., Teng, M. W., . . . Anderson, A. C. (2015). TIGIT predominantly regulates the immune response via regulatory T cells. *The Journal of clinical investigation, 125*(11), 4053-4062.
[59] Leonard, J. D., Gilmore, D. C., Dileepan, T., Nawrocka, W. I., Chao, J. L., Schoenbach, M. H., . . . Savage, P. A. (2017). Identification of natural regulatory T cell epitopes reveals convergence on a dominant autoantigen. *Immunity, 47*(1), 107-117. e108.
[60] Li, X., & Zheng, Y. (2015). Regulatory T cell identity: formation and maintenance. *Trends in immunology, 36*(6), 344-353.
[61] Lin, X., Chen, M., Liu, Y., Guo, Z., He, X., Brand, D., & Zheng, S. G. (2013). Advances in distinguishing natural from induced Foxp3+ regulatory T cells. *International journal of clinical and experimental pathology, 6*(2), 116.
[62] Lozano, E., Dominguez-Villar, M., Kuchroo, V., & Hafler, D. A. (2012). The TIGIT/CD226 axis regulates human T cell function. *The Journal of Immunology, 188*(8), 3869-3875.
[63] Madireddi, S., Eun, S.-Y., Mehta, A. K., Birta, A., Zajonc, D. M., Niki, T., . . . Croft, M. (2017). Regulatory T Cell–Mediated Suppression of Inflammation Induced by DR3 Signaling Is Dependent on Galectin-9. *The Journal of Immunology, 199*(8), 2721-2728.
[64] Manieri, N. A., Chiang, E. Y., & Grogan, J. L. (2017). TIGIT: a key inhibitor of the cancer immunity cycle. *Trends in immunology, 38*(1), 20-28.
[65] Mohr, A., Malhotra, R., Mayer, G., Gorochov, G., & Miyara, M. (2018). Human FOXP 3+ T regulatory cell heterogeneity. *Clinical & Translational Immunology, 7*(1), e1005.
[66] Nishikawa, H., & Sakaguchi, S. (2010). Regulatory T cells in tumor immunity. *International journal of cancer, 127*(4), 759-767.
[67] Nishimura, H., & Honjo, T. (2001). PD-1: an inhibitory immunoreceptor involved in peripheral tolerance. *Trends in immunology, 22*(5), 265-268.
[68] Noyan, F., Lee, Y. S., Zimmermann, K., Hardtke-Wolenski, M., Taubert, R., Warnecke, G., . . . Manns, M. P. (2014). Isolation of human antigen-specific regulatory T cells with high suppressive function. *European journal of immunology, 44*(9), 2592-2602.
[69] Peggs, K. S., Quezada, S. A., Chambers, C. A., Korman, A. J., & Allison, J. P. (2009). Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti–CTLA-4 antibodies. *Journal of Experimental Medicine, 206*(8), 1717-1725.
[70] Postow, M. A., Chesney, J., Pavlick, A. C., Robert, C., Grossmann, K., McDermott, D., . . . Agarwala, S. S. (2015). Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *New England Journal of Medicine, 372*(21), 2006-2017.
[71] Povoleri, G. A. M., Scottà, C., Nova-Lamperti, E. A., John, S., Lombardi, G., & Afzali, B. (2013). Thymic versus induced regulatory T cells–who regulates the regulators? *Frontiers in immunology, 4*, 169.

[72] Preston, C. C., Maurer, M. J., Oberg, A. L., Visscher, D. W., Kalli, K. R., Hartmann, L. C., . . . Knutson, K. L. (2013). The ratios of CD8+ T cells to CD4+ CD25+ FOXP3+ and FOXP3-T cells correlate with poor clinical outcome in human serous ovarian cancer. *PloS one, 8*(11).
[73] Robert, C., Ribas, A., Schachter, J., Arance, A., Grob, J.-J., Mortier, L., . . . Lotem, M. (2019). Pembrolizumab versus ipilimumab in advanced melanoma (KEYNOTE-006): post-hoc 5-year results from an open-label, multicentre, randomised, controlled, phase 3 study. *The Lancet Oncology, 20*(9), 1239-1251.
[74] Robert, C., Schachter, J., Long, G. V., Arance, A., Grob, J. J., Mortier, L., . . . Lotem, M. (2015). Pembrolizumab versus ipilimumab in advanced melanoma. *New England Journal of Medicine, 372*(26), 2521-2532.
[75] Routy, B., Le Chatelier, E., Derosa, L., Duong, C. P., Alou, M. T., Daillère, R., . . . Roberti, M. P. (2018). Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. *Science, 359*(6371), 91-97.
[76] Rudensky, A. Y. (2011). Regulatory T cells and Foxp3. *Immunological reviews, 241*(1), 260-268.
[77] Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., & Toda, M. (1995). Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *The Journal of Immunology, 155*(3), 1151-1164.
[78] Samy, E. T., Parker, L. A., Sharp, C. P., & Tung, K. S. (2005). Continuous control of autoimmune disease by antigen-dependent polyclonal CD4+ CD25+ regulatory T cells in the regional lymph node. *The Journal of experimental medicine, 202*(6), 771-781.
[79] Santodomingo-Garzon, T., Han, J., Le, T., Yang, Y., & Swain, M. G. (2009). Natural killer T cells regulate the homing of chemokine CXC receptor 3-positive regulatory T cells to the liver in mice. *Hepatology, 49*(4), 1267-1276.
[80] Schallenberg, S., Tsai, P.-Y., Riewaldt, J., & Kretschmer, K. (2010). Identification of an immediate Foxp3− precursor to Foxp3+ regulatory T cells in peripheral lymphoid organs of nonmanipulated mice. *Journal of Experimental Medicine, 207*(7), 1393-1407.
[81] Schmidt, A., Oberle, N., & Krammer, P. H. (2012). Molecular mechanisms of treg-mediated T cell suppression. *Frontiers in immunology, 3*, 51.
[82] Schmidt, E. M., Wang, C. J., Ryan, G. A., Clough, L. E., Qureshi, O. S., Goodall, M., . . . Walker, L. S. (2009). Ctla-4 controls regulatory T cell peripheral homeostasis and is required for suppression of pancreatic islet autoimmunity. *The Journal of Immunology, 182*(1), 274-282.
[83] Schoenbrunn, A., Frentsch, M., Kohler, S., Keye, J., Dooms, H., Moewes, B., . . . Wu, P. (2012). A converse 4-1BB and CD40 ligand expression pattern delineates activated regulatory T cells (Treg) and conventional T cells enabling direct isolation of alloantigen-reactive natural Foxp3+ Treg. *The Journal of Immunology, 189*(12), 5985-5994.
[84] Sebastian, M., Lopez-Ocasio, M., Metidji, A., Rieder, S. A., Shevach, E. M., & Thornton, A. M. (2016). Helios controls a limited subset of regulatory T cell functions. *The Journal of Immunology, 196*(1), 144-155.
[85] Sharma, M. D., Huang, L., Choi, J.-H., Lee, E.-J., Wilson, J. M., Lemos, H., . . . Mellor, A. L. (2013). An inherently bifunctional subset of Foxp3+ T helper cells is controlled by the transcription factor eos. *Immunity, 38*(5), 998-1012.
[86] Shevach, E. M. (2009). Mechanisms of foxp3+ T regulatory cell-mediated suppression. *Immunity, 30*(5), 636-645.
[87] Smyth, G. P., Stapleton, P. P., Barden, C. B., Mestre, J. R., Freeman, T. A., Duff, M. D., . . . Daly, J. M. (2003). Renal cell carcinoma induces prostaglandin E 2 and T-helper type 2 cytokine production in peripheral blood mononuclear cells. *Annals of surgical oncology, 10*(4), 455-462.
[88] Sugita, K., Hanakawa, S., Honda, T., Kondoh, G., Miyachi, Y., Kabashima, K., & Nomura, T. (2015). Generation of Helios reporter mice and an evaluation of the suppressive capacity of Helios+ regulatory T cells in vitro. *Experimental dermatology, 24*(7), 554-556.
[89] Sun, L., Jin, H., & Li, H. (2016). GARP: a surface molecule of regulatory T cells that is involved in the regulatory function and TGF-β releasing. *Oncotarget, 7*(27), 42826.
[90] Talmadge, J. E. (2007). Pathways mediating the expansion and immunosuppressive activity of myeloid-derived suppressor cells and their relevance to cancer therapy. *Clinical Cancer Research, 13*(18), 5243-5248.
[91] Tang, A. L., Teijaro, J. R., Njau, M. N., Chandran, S. S., Azimzadeh, A., Nadler, S. G., . . . Farber, D. L. (2008). CTLA4 expression is an indicator and regulator of steady-state CD4+ FoxP3+ T cell homeostasis. *The Journal of Immunology, 181*(3), 1806-1813.
[92] Tang, Q., & Bluestone, J. A. (2008). The Foxp3+ regulatory T cell: a jack of all trades, master of regulation. *Nature immunology, 9*(3), 239-244.
[93] Tatsumi, T., Herrem, C. J., Olson, W. C., Finke, J. H., Bukowski, R. M., Kinch, M. S., . . . Storkus, W. J. (2003). Disease stage variation in CD4+ and CD8+ T-cell reactivity to the receptor tyrosine kinase EphA2 in patients with renal cell carcinoma. *Cancer research, 63*(15), 4481-4489.
[94] Thornton, A. M., Korty, P. E., Tran, D. Q., Wohlfert, E. A., Murray, P. E., Belkaid, Y., & Shevach, E. M. (2010). Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. *The Journal of Immunology, 184*(7), 3433-3441.
[95] Tran, D. Q., Andersson, J., Hardwick, D., Bebris, L., Illei, G. G., & Shevach, E. M. (2009). Selective expression of latency-associated peptide (LAP) and IL-1 receptor type I/II (CD121a/CD121b) on activated human FOXP3+ regulatory T cells allows for their purification from expansion cultures. *Blood, The Journal of the American Society of Hematology, 113*(21), 5125-5133.
[96] Tran, D. Q., Andersson, J., Wang, R., Ramsey, H., Unutmaz, D., & Shevach, E. M. (2009). GARP (LRRC32) is essential for the surface expression of latent TGF-β on platelets and activated FOXP3+ regulatory T cells. *Proceedings of the National Academy of Sciences, 106*(32), 13445-13450.
[97] Wada, J., Suzuki, H., Fuchino, R., Yamasaki, A., Nagai, S., Yanai, K., . . . Morisaki, T. (2009). The contribution of vascular endothelial growth factor to the induction of regulatory T-cells in malignant effusions. *Anticancer research, 29*(3), 881-888.

[98] Wang, H. Y., Lee, D. A., Peng, G., Guo, Z., Li, Y., Kiniwa, Y., . . . Wang, R.-F. (2004). Tumor-specific human CD4+ regulatory T cells and their ligands: implications for immunotherapy. *Immunity, 20*(1), 107-118.

[99] Wang, H. Y., Peng, G., Guo, Z., Shevach, E. M., & Wang, R.-F. (2005). Recognition of a new ARTC1 peptide ligand uniquely expressed in tumor cells by antigen-specific CD4+ regulatory T cells. *The Journal of Immunology, 174*(5), 2661-2670.

[100] Wang, H. Y., & Wang, R.-F. (2007). Regulatory T cells and cancer. *Current opinion in immunology, 19*(2), 217-223.

[101] Weiner, H. L. (2001). Induction and mechanism of action of transforming growth factor-β-secreting Th3 regulatory cells. *Immunological reviews, 182*(1), 207-214.

[102] Weiss, J. M., Bilate, A. M., Gobert, M., Ding, Y., Curotto de Lafaille, M. A., Parkhurst, C. N., . . . Ruocco, M. G. (2012). Neuropilin 1 is expressed on thymus-derived natural regulatory T cells, but not mucosa-generated induced Foxp3+ T reg cells. *Journal of Experimental Medicine, 209*(10), 1723-1742.

[103] Whiteside, T. L. (2015). The role of regulatory T cells in cancer immunology. *ImmunoTargets and therapy, 4*, 159.

[104] Wing, K., Yamaguchi, T., & Sakaguchi, S. (2011). Cell-autonomous and-non-autonomous roles of CTLA-4 in immune regulation. *Trends in immunology, 32*(9), 428-433.

[105] Woo, E. Y., Chu, C. S., Goletz, T. J., Schlienger, K., Yeh, H., Coukos, G., . . . June, C. H. (2001). Regulatory CD4+ CD25+ T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. *Cancer research, 61*(12), 4766-4772.

[106] Woods, D. M., Ramakrishnan, R., Sodré, A. L., Berglund, A., & Weber, J. (2017). PD-1 blockade induces phosphorylated STAT3 and results in an increase of Tregs with reduced suppressive function: Am Assoc Immnol.

[107] Yadav, M., Louvet, C., Davini, D., Gardner, J. M., Martinez-Llordella, M., Bailey-Bucktrout, S., . . . Kuster, D. J. (2012). Neuropilin-1 distinguishes natural and inducible regulatory T cells among regulatory T cell subsets in vivo. *Journal of Experimental Medicine, 209*(10), 1713-1722.

[108] Yamaguchi, T., Wing, J. B., & Sakaguchi, S. (2011). *Two modes of immune suppression by Foxp3+ regulatory T cells under inflammatory or non-inflammatory conditions.* Paper presented at the Seminars in immunology.

[109] Yamamoto, T., Yanagimoto, H., Satoi, S., Toyokawa, H., Hirooka, S., Yamaki, S., . . . Kwon, A.-H. (2012). Circulating CD4+ CD25+ regulatory T cells in patients with pancreatic cancer. *Pancreas, 41*(3), 409-415.

[110] Yamazaki, T., Yang, X. O., Chung, Y., Fukunaga, A., Nurieva, R., Pappu, B., . . . Panopoulos, A. D. (2008). CCR6 regulates the migration of inflammatory and regulatory T cells. *The Journal of Immunology, 181*(12), 8391-8401.

[111] Zhao, H., Liao, X., & Kang, Y. (2017). Tregs: where we are and what comes next? *Frontiers in immunology, 8*, 1578.

[112] Zhou, W., Deng, J., Chen, Q., Li, R., Xu, X., Guan, Y., . . . Li, J. (2020). Expression of CD4+ CD25+ CD127Low regulatory T cells and cytokines in peripheral blood of patients with primary liver carcinoma. *International Journal of Medical Sciences, 17*(6), 712.