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Prognostic value of regulator T cells in patients with pancreatic cancer: a systematic review and meta-analysis

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Abstract. – OBJECTIVE: This study focused on investigating the relationship between the level of regulatory T cells (Tregs) infiltration and the prognosis of pancreatic cancer patients by using meta-analysis to identify new clinical diagnostic markers.

MATERIALS AND METHODS: We looked through PubMed, Embase, and the Cochrane Library for studies published between the database's inception and September 2021. We included studies that looked at the relationship between Tregs and pancreatic cancer prognosis. We exempted duplicate publications, studies without full text, insufficient information, or inability to conduct data extraction, animal experiments, reviews, and systematic reviews. The data were analyzed using STATA 15.1.

RESULTS: The pooled results show that high infiltrating Treg is highly correlated with lower OS of pancreatic cancer patients (HR=1.56, 95% CI: 1.23-1.990.000), but not with DFS of patients with pancreatic cancer (HR=1.29, 95% CI: 0.88-1.89, *p*=0.184). On the other hand, the results show that high infiltrating Treg is significantly associated with lower OS (HR=2.13, 95% CI: 1.70-2.67, *p*=0.000) and DFS (HR=1.79, 95% CI: 1.12-2.86, *p*=0.015) in patients from Asia, whereas it is not significantly associated with OS and DFS in patients from Europe and America.

CONCLUSIONS: This meta-analysis suggests that the assessment of Tregs may help to predict the prognosis in pancreatic cancer patients. Advanced histological techniques for obtaining more detailed information about Treg activity may assist in the identification of novel treatment strategies.

Key Words:
Prognostic value, Regulatory T cells, Pancreatic cancer, Systematic review and meta-analysis.

Introduction

Pancreatic cancer is one of the deadliest cancers, with less than 8% of the patients having a 5-year survival rate[1]. Pancreatic cancer incidence has risen in recent decades and is expected to rise further[2,3]. Therefore, there is a substantial opportunity to develop precision approaches to pancreatic cancer treatment. Pancreatic cancer genetic analysis reveals that there are currently few operable therapeutic targets[4-6]. The use of immunotherapy is a promising treatment that has produced long-term clinical benefits in the treatment of challenging cancers in the earlier days[7-9].

Immune cells from the adaptive and innate immune systems can infiltrate and fight cancer after recognizing a malignant tumor. CD8+T cells and regulatory T cells (Tregs) in tumor-infiltrating lymphocytes play an important role in tumor progression and immune escape[10]. Tregs have the ability to suppress the activation and formation of CD4+ T helper cells and CD8+ cytotoxic T cells, as well as shape the suppressive immune microenvironment, resulting in tumor immune escape[11]. In addition, Treg levels have been linked to clinical outcomes in a variety of cancers[12]. Besides, the existence of tumor-infiltrating lymphocytes and the proportions of the tumor microenvironment can be used to predict cancer prognosis and immunotherapy response[13,14]. Investigating the prognostic value of Tregs in pancreatic cancer can assist in improving prognosis and developing personalized treatment. The level of Tregs has been correlated with the clinical outcome in several malignancies[12]. Exploring the prognostic value of Tregs in pancreatic cancer can help improve the prognosis and formulate personalized treatment.

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There are currently no reliable clinical trials and sufficient basic studies that can clearly demonstrate the relationship between Tregs and pancreatic cancer prognosis. Even multiple papers have shown apparently opposite results. However, clarifying the relationship between pancreatic cancer prognosis and Tregs is particularly important for the exploration of precision therapy and immunotherapy for pancreatic cancer. Therefore, we conducted this analysis in order to obtain higher evidence-based medical evidence and to further explore new options for immunotherapy of pancreatic cancer.

This study conducted a meta-analysis by including articles reporting the prognostic value of Tregs in pancreatic cancer, with the goal of investigating the relationship between the level of Tregs infiltration and the prognosis of pancreatic cancer patients to identify new clinical diagnostic markers.

Materials and Methods

Literature Inclusion and Exclusion Criteria

The following were inclusion criteria: the study design was a cohort study, the study reported a correlation between Tregs and pancreatic cancer prognosis, and the language was limited to English and Chinese. Exclusion criteria included duplicate publication, research without full text, incomplete information, or inability to conduct data extraction, animal experiments, reviews, and systematic reviews.

Search Strategy

We searched PubMed, Embase, and the Cochrane Library from the database's inception to September 20, 2021, for this meta-analysis. The following search terms were used: "Treg", "regulatory T cell" and "pancreatic cancer", "pancreatic Neoplasm" and "prognosis" "prognostic" "indicator".

Literature Screening and Data Extraction

Two researchers worked independently on the literature search, screening, and information extraction. If there were any doubts or disagreements, the decision was made after consulting with a third party. The author, year, study area, research type, number of cases, and outcome indicators, such as overall survival (OS) and disease-free survival, were all extracted from the data.

Literature Quality Assessment

The Newcastle-Ottawa Scale (NOS) for cohort studies was used by two researchers independently to assess the quality of the literature[15]. NOS includes 4 items (4 points) for "Research Subject Selection", 1 item (2 points) for "Comparability between Groups" and 3 items (3 points) for "Result Measurement", for a total score of 9 points, with ≥7 points considered high-quality literature and <7 points classified as lower-quality literature. When the opinions are in disagreement, it is decided through discussion or consultation with a third party. The meta-analysis was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-analysis statement (PRISMA statement) and its related items[16].

Data Synthesis and Statistical Analysis

The data were analyzed using STATA version 15.1 (Stata Corporation, College Station, TX, USA). OS and DFS were evaluated using HR (95% CI). I^2 was used to evaluate the heterogeneity. If the heterogeneity test resulted $p \geq 0.1$ and $I^2 \leq 50\%$, this indicated that the studies were homogenous, and the fixed effects model was utilized for combined analysis; $p < 0.1$ and $I^2 > 50\%$ indicated that the study was heterogeneous, and sensitivity analysis was performed to determine the source of heterogeneity. If the heterogeneity remained high, the random effects model was utilized, or the combination of results was abandoned in favor of descriptive analysis. To investigate publication bias, funnel plots and Egger's tests were performed.

Results

Literature Search Results

497 studies were retrieved from the database for this study. After removing duplicate studies, 273 studies were included, and 191 studies were identified after reviewing the titles and abstracts. Finally, the meta-analysis included sixteen articles (Figure 1).

Baseline Characteristics and Quality Assessment of the Included Studies

This meta-analysis included sixteen cohort studies[17-32] with patient sample sizes ranging from

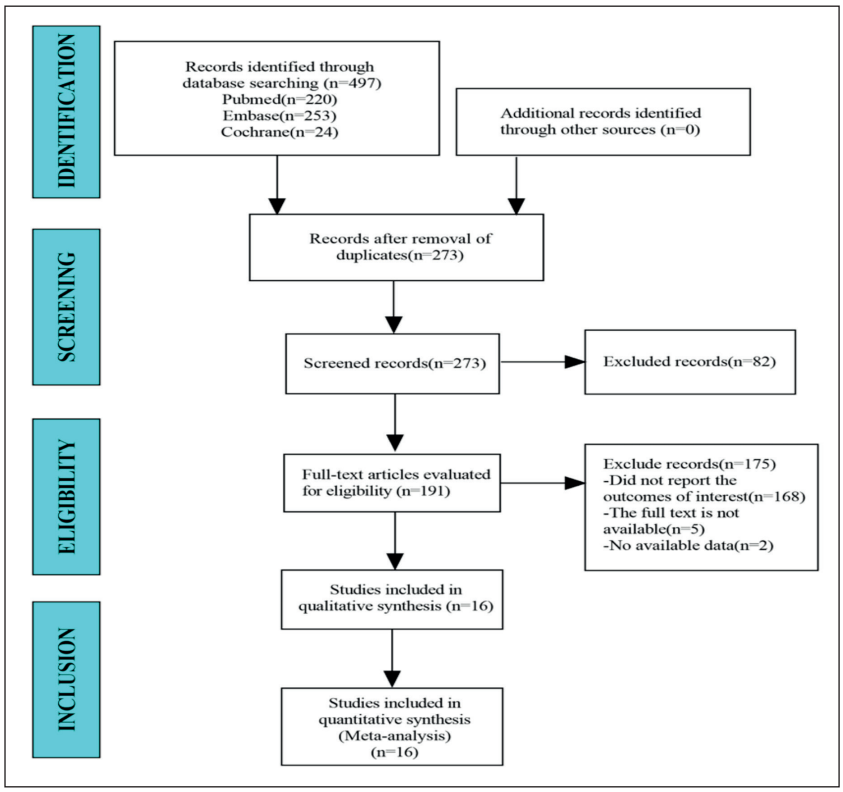

Figure 1. Flow diagram for selection of studies.

90 to 241, for a total of 2,345 patients. There were 1,261 Asian patients in eight of the studies[17,19,20,24,27-29,32], and 1,084 European and American patients in eight of the studies[18,21-23,25,26,30,31], with most of the patients being middle-aged and elderly. Our study aggregated OS and DFS to reflect the prognostic value of Treg in predicting pancreatic cancer patients. The NOS score used for quality assessment is all above 7 and meets the requirements (Table I).

Results

Overall Survival

There were sixteen studies[17-32] involving 2345 patients that report a connection between Treg levels and OS. Since there is significant heterogeneity (I^2=79.0%, p=0.000), a meta-analysis was conducted through a random effects model. The pooled results show that high infiltrating Treg levels are significantly associated with lower OS of pancreatic cancer patients (HR=1.56, 95% CI: 1.23-1.99, p=0.000) (Figure 2).

Disease-free survival

There are seven studies[18,20,26-28,31,32] with a total of 1,086 patients that report a link between Treg levels and DFS. Meta-analysis was performed using a random effects model, since the data are highly heterogeneous (I^2=84.2%, p=0.000). The pooled results show that the infiltrating level of Treg is not significantly correlated with DFS of patients with pancreatic cancer (HR=1.29, 95%CI: 0.88-1.89, p=0.184) (Figure 3).

Subgroup Analysis

Due to the heterogeneity of the research results, we performed a subgroup analysis for different regions. We started by looking at the differences in operating systems between Asia, Europe, and America. There are eight studies[17,19,20,24,27-29,32] with a total of 1,261 patients from Asia. Since there is significant heterogeneity (I^2=54.5%, p=0.030), a meta-analysis was conducted through a random effects model. The combined findings show that high infiltrating Treg levels are significantly related to lower OS in Asian patients (HR=2.13, 95% CI: 1.70-2.67, p=0.000). Whereas the infiltrating level of Treg is not significantly correlated with OS of patients from Europe and America (HR=1.11, 95% CI: 0.83-1.48, p=0.489; I^2=67.9%, p=0.003; enrolling 8 studies) (Figure 4).

Then, we examined the differences in DFS between Asia, Europe, and America. There are four studies[20,27,28,32], with 625 Asian patients. A

Table I. Baseline characteristics and quality assessment of the included studies.

Author	Year	Country	Study design	Sample size	Gender (male/female)	Age	Outcome	Follow-up duration (month)	NOS score
Pu et al[17]	2018	China	Cohort	90	56/34	/	OS	/	8
Karakhanova et al[18]	2015	Germany	Cohort	92	52/40	61.6 (33.0-77.0)	OS, DFS	/	7
Hiraoka et al[19]	2006	Japan	Cohort	198	114/84	63.0 (33.0-83.0)	OS	20	7
Liu et al[20]	2015	China	Cohort	92	68/24	61.0 (35.0-89.0)	OS, DFS	6	7
Carstens et al[21]	2017	USA	Cohort	132	75/57	64.4 (25.0-85.0)	OS	/	8
Hutcheson et al[22]	2016	USA	Cohort	223	121/102	65.0 (27.0-89.0)	OS	/	7
Knudsen et al[23]	2017	USA	Cohort	109	/	/	OS	/	7
Wang et al[24]	2017	China	Cohort	120	/	/	OS	/	7
Wartenberg et al[25]	2015	Switzerland	Cohort	117	64/53	64.0 (34.0-84.0)	OS	/	7
Diana et al[26]	2016	UK	Cohort	145	/	/	OS, DFS	20	7
Ino et al[27]	2019	Japan	Cohort	241	/	/	OS, DFS	21.2	8
Ino et al[28]	2013	Japan	Cohort	212	130/82	/	OS, DFS	18.8	8
Tang et al[29]	2014	China	Cohort	228	147/81	66.0 (33.0-78.0)	OS	/	7
Helm et al[30]	2014	Germany	Cohort	42	24/18	65.0 (46.0-85.0)	OS	/	7
Sideras et al[31]	2017	Netherlands	Cohort	224	132/92	67.2 (33.4-85.2)	OS, DFS	/	7
Zhao et al[32]	2018	China	Cohort	80	42/38	/	OS, DFS	33.2	8

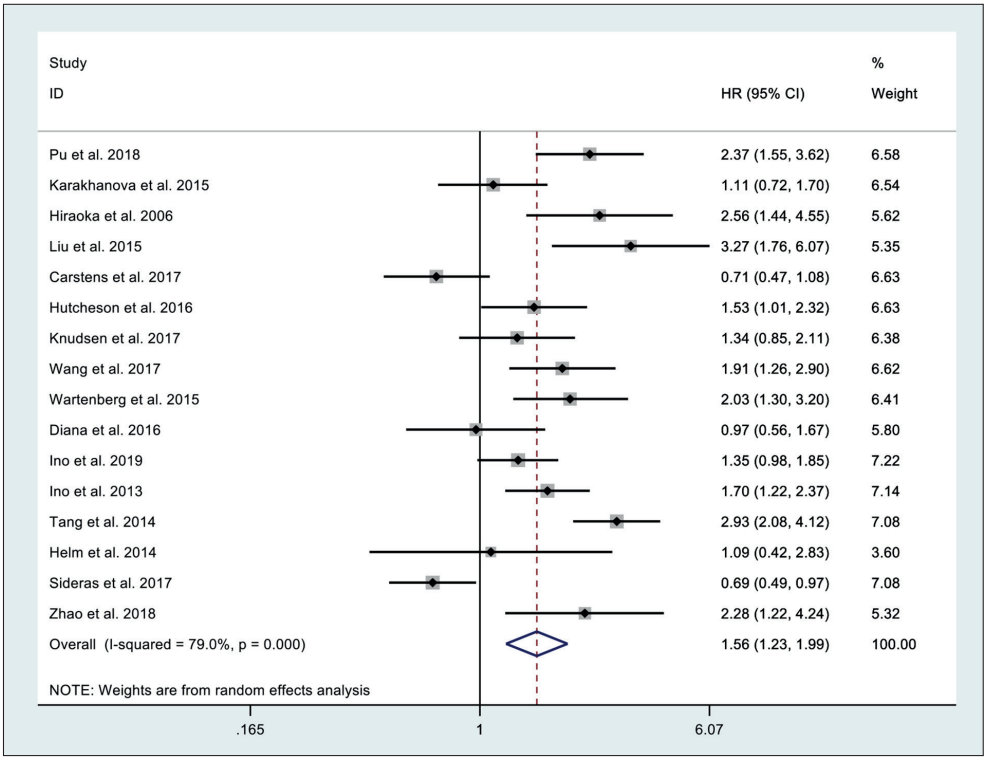

Figure 2. Forest plots on the level of Treg and overall survival (OS).

random effects model was used for the meta-analysis because the results are having a significant heterogeneity (I^2=81.5%, p=0.001). The merged results of the study show that a high infiltrating Treg level is significantly correlated with lower DFS in Asian patients (HR=1.79, 95% CI: 1.12-2.86, p=0.015), but not in the European and American patients (HR=0.82, 95% CI: 0.60-1.10, p=0.183; I^2=36.7%, p=0.206 enrolling 3 studies) (Figure 5).

Sensitivity Analysis

We performed sensitivity analysis by removing each included study one at a time and per-

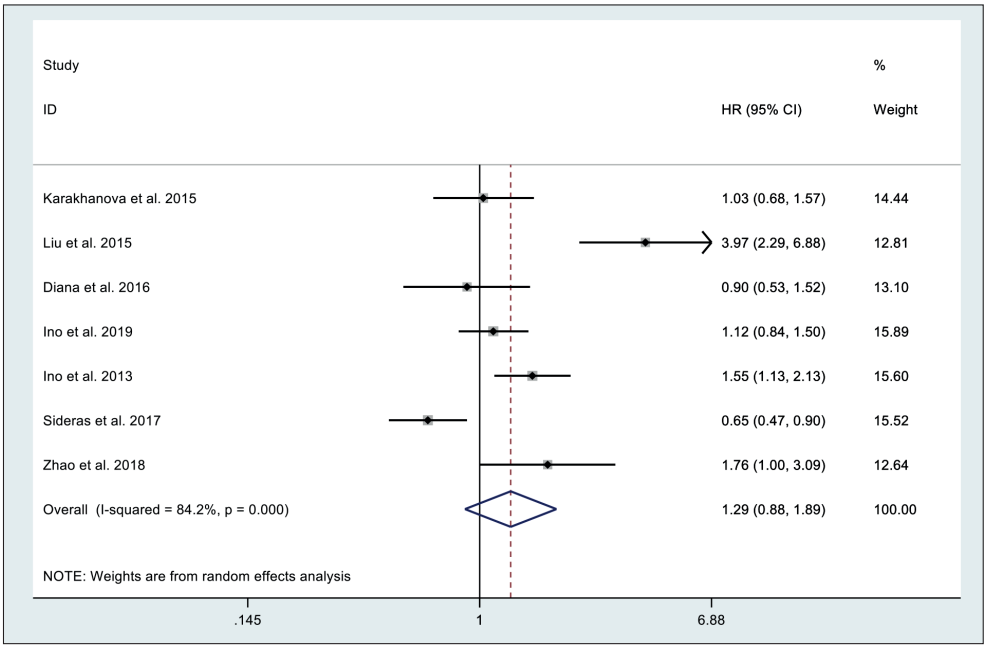

Figure 3. Forest plots on the level of Treg and disease-free survival (DFS).

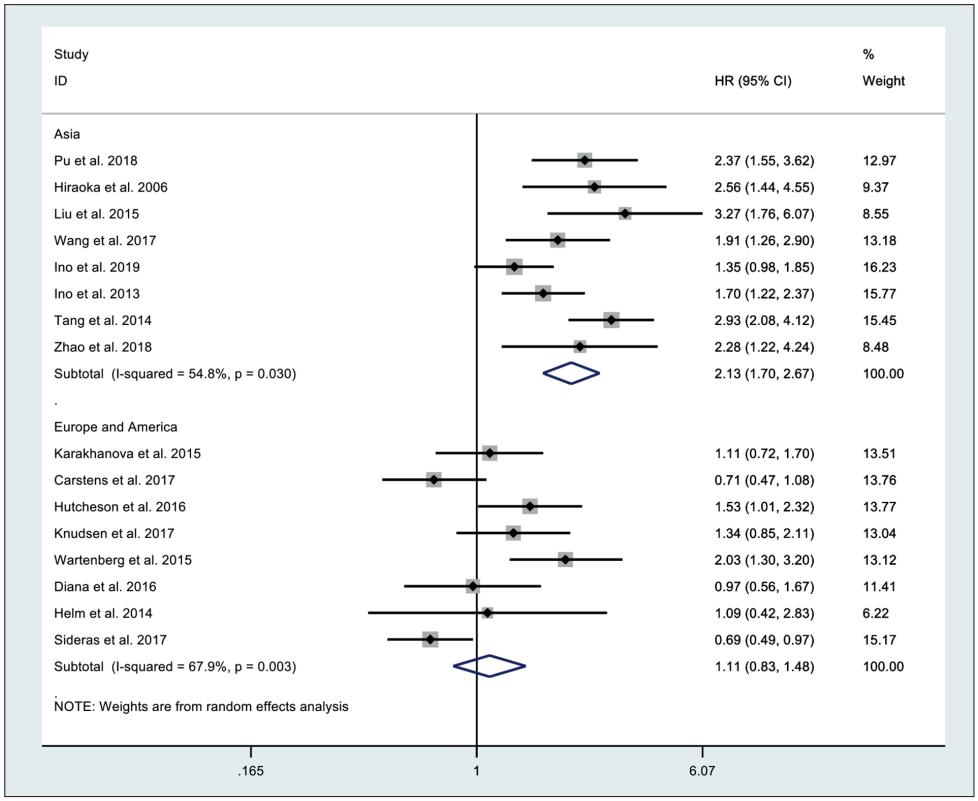

Figure 4. Subgroup analysis of the correlation between Treg and OS in patients in different regions.

forming a summary analysis on the remaining studies. Our research results revealed that none of the studies had a significant impact on the meta-analysis results, implying that the research results of this meta-analysis are stable and reliable (Figures 6 and 7).

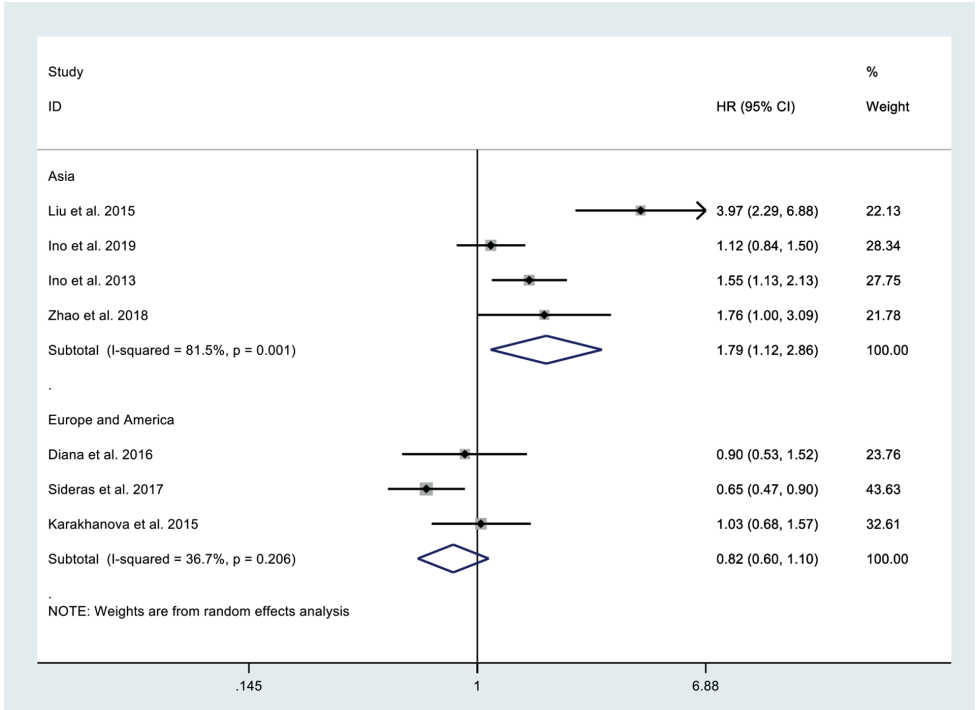

Figure 5. Subgroup analysis of the correlation between Treg and DFS in patients in different regions.

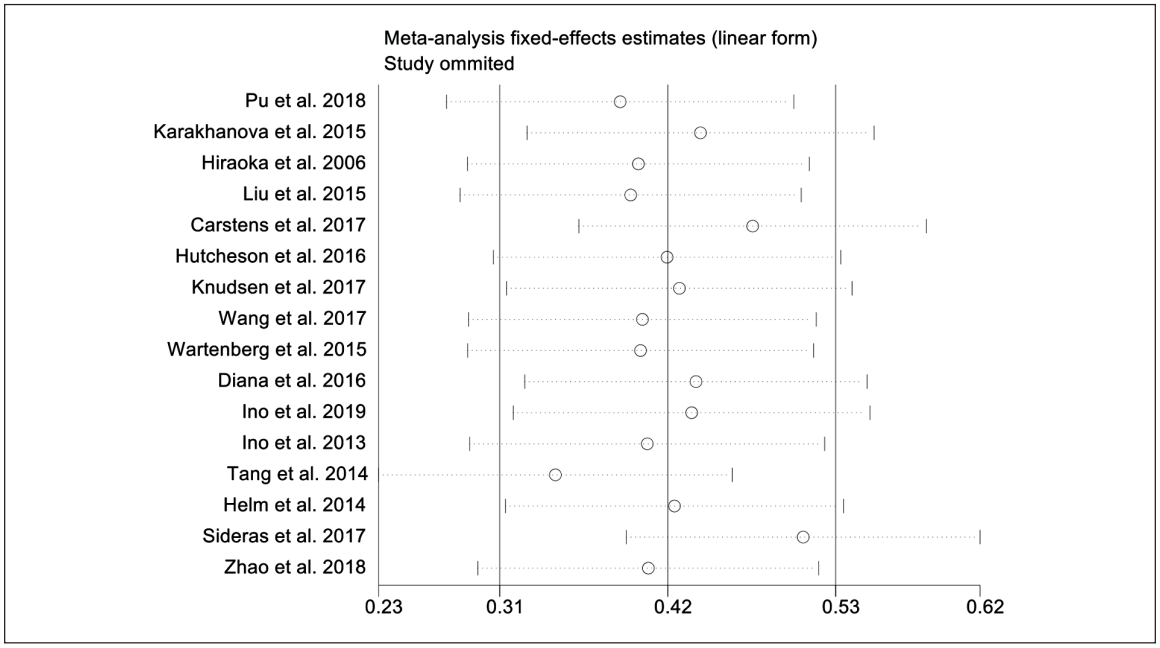

Figure 6. Sensitivity on the level of Treg and OS.

Publication Bias

Figure 8 depicts the funnel plot for this research. It can be observed that the funnel plot shows an asymmetric result, and the *p*-value of Egger's test is 0.583, suggesting that this study appears to contain a publication bias. One possible explanation for this publication bias is that some negative results were not published.

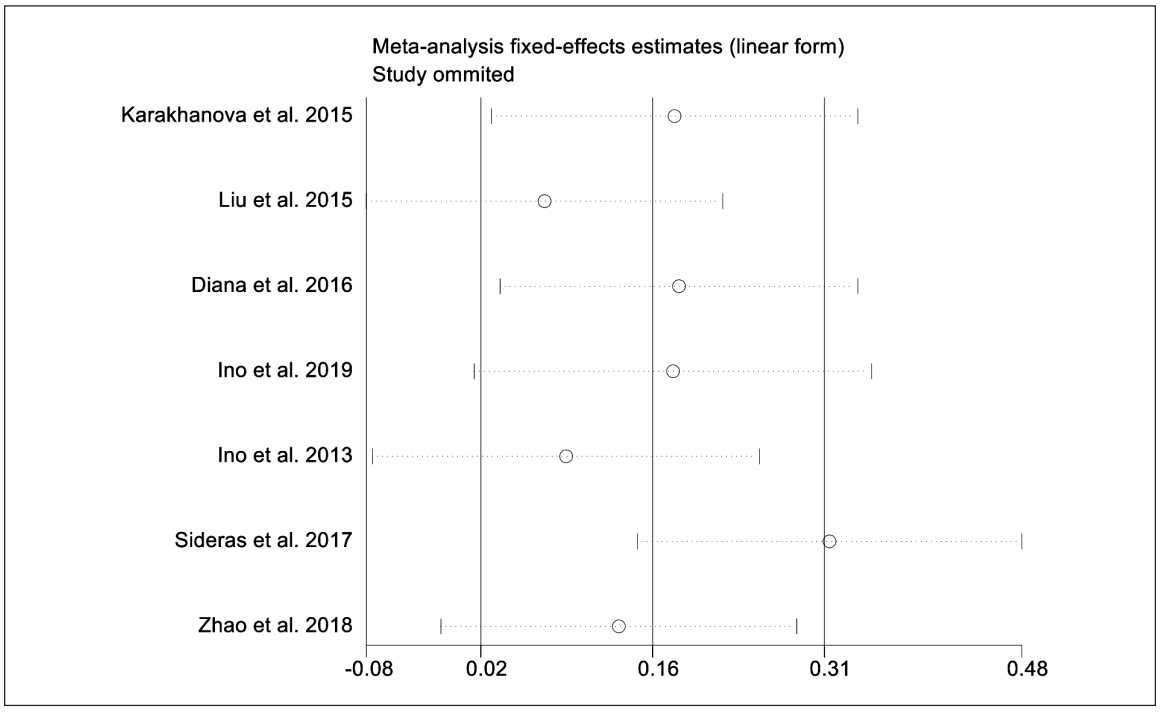

Figure 7. Sensitivity on the level of Treg and DFS.

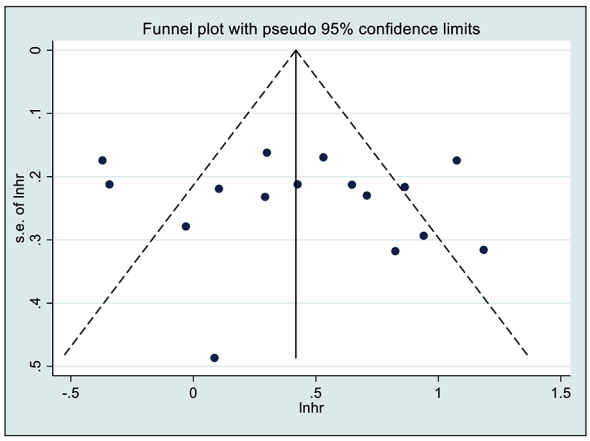

Figure 8. Funnel plot for evaluating the publication bias of this meta-analysis

Discussion

The presence of immune cell infiltrating in the microenvironment of various cancers does not always result in favorable treatment outcomes. In particular, Tregs have been demonstrated to have anti-inflammatory functions, as well as to inhibit anti-tumor immunity, thereby promoting tumor cell survival[33,34].

The primary function of Tregs in the body is to maintain homeostasis of the immune system and to prevent over-immune responses to self-antigens. Tregs are involved in the immunosuppressive population of CD4+ T cells that constitute the transcription factor FOXP3, while FOXP3 is essential for the development and function of Tregs[35,36]. Tregs also have a pivotal role in tumorigenesis and progression, mainly in the suppression of antitumor immunity. Intratumoral Tregs levels have been shown to be much higher in many cancers than in peripheral blood or healthy tissue. Also, increased amounts of FOXP3+ Tregs in the tumor microenvironment are often associated with adverse effects[37,38].

Since 1995, after Sakaguchi et al[39] linked the composition and high expression of CD25 to Tregs, research in the field of Treg cell function was revitalized and thus provided a new reference for Treg-based cell therapy[39]. Among many studies, Sakaguchi et al[39] identified FOXP3 as the master gene regulator of Treg cells and that FOXP3 is involved in regulating various cellular functions. This view was generally accepted[40-42]. The suppressive nature of Treg is not limited to T cells but extends to other immune cell such as NK cells and neutrophils and group II innate lymphocytes (ILC2)[43]. As an important player in the tumor microenvironment, Tregs directly or indirectly inhibit the anti-tumor capacity. In the tumor microenvironment, Tregs interfere with the anti-tumor capacity by working with some specific chemokines and their receptors[44,45]. In breast cancer, Tregs have a significant prognostic value in different subtypes of breast cancer[46]. The role Tregs played in the treatment of pancreatic cancer piques our interest. Tregs are abundant in human Pancreatic Intraepithelial Neoplasia (PanIN) and pancreatic cancer, according to a recent study published in 2020 by Zhang et al[47] Treg depletion accelerates pancreatic cancer and alters fibroblast populations in PanIN lesions. After Treg depletion, multiple CCR1 ligands are upregulated in epithelial cells and fibroblasts. CCR1 inhibition prevents PanIN progression induced by Treg depletion. The specific mechanisms are summarized comprehensively in a new review. Treg cells secrete TGFβ and promote differentiation of fibroblasts into tumor-restraining smooth muscle actin-expressing cancer-associated fibroblasts (myCAF). Furthermore, Treg cell depletion leads to lower TGF levels and an increase in tumor-promoting fibroblasts, which attract pro-tumorigenic myeloid cells *via* CCR1. Depletion of Treg cells also produces a pathologic Th2 response, which co-orchestrates pancreatic carcinogenesis[48]. Investigating the prognostic value of Tregs in pancreatic cancer can assist in improving prognosis and developing personalized treatment.

This meta-analysis included 16 articles and 2,345 patients to investigate the relationship between the level of Treg infiltration and the OS and DFS of pancreatic cancer patients. The pooled results show that high infiltrating Treg is significantly correlated with lower OS in pancreatic cancer patients (HR=1.56, 95% CI: 1.23-1.99, p=0.000), but not with DFS in pancreatic cancer patients (HR=1.29, 95% CI: 0.88-1.89, p=0.184). This result first shows that the level of Treg invasion is significantly related to the survival of patients with pancreatic cancer, and the higher the level of Treg invasion, the worse the survival of patients. Single-cell RNA-seq reveals Treg infiltration levels increase significantly throughout the pancreatic cancer development[49]. Immunotherapy, including PD-1/PD-L1 agonists, was found to have limited efficacy in pancreatic cancer, according to the current study[50]. As we all know, the combination of PDL1 on the surface of tumor cells and PD1 on

$CD8^+$T cells can promote the $CD8^+$T apoptosis, allowing tumor immune escape. The role of PD1/PDL1 inhibitors is to block this process. However, the latest research has found that the expression of PD1 on Treg can enhance the inhibitory ability of Treg and promote the proliferation of Treg[51]. This indicates that the high infiltration of Treg may be the reason for the limited efficacy of PD1/PDL1 inhibitors in cancer, and also the possible reason for the short survival time of patients with pancreatic cancer. However, we note that the pooled results indicate that there is no correlation between Treg and the degree of disease progression in patients with pancreatic cancer. One of the possible reasons is that Treg is not directly involved in the evolution of the disease. Additionally, considering that there is a large heterogeneity in the research, it may lead to unobjective results. Therefore, we conducted a subgroup analysis for different regions.

The pooled results show that high infiltrating Treg is significantly associated with lower OS and DFS of patients from Asia, while the infiltrating level of Treg is not significantly correlated with OS and DFS of patients from Europe and America. After conducting subgroup analysis, we found that the heterogeneity had decreased to a certain extent, implying that the source of the patient may be the cause of the heterogeneity. This finding explains, to some extent, why there is no significant correlation between the degree of Treg infiltration and the DFS of patients with pancreatic cancer, as mentioned in the previous studies[18,20,26-28,31,32]. In addition, our study found that the level of Treg invasion cannot predict the prognosis of pancreatic cancer patients from Europe and America. This situation may be caused by genetic differences between different races. It has been well documented that there are large differences in the immune systems of European and African populations, which may have biological, geographic and social causes[52,53]. Although there is no clear literature analyzing the immune system disparities between Asian and European populations, we believe that there must be some immune system disparities between the two populations based on the origin of the two races and social geography. However, the detailed mechanism is still unclear, and we need to further explore it.

Our study also has certain limitations: although our subgroup analysis of patient source reduced heterogeneity to some extent, it did not completely rule out heterogeneity, indicating that other possible causes exist. However, since the included articles did not specifically describe other basic patient information, we were unable to carry out other subgroup analyses.

Conclusions

The observations of this meta-analysis suggest that assessing Tregs may help predicting prognosis for pancreatic cancer patients. Advanced histological methods for obtaining more detailed information about Treg activity may facilitate the identification of novel treatment strategies.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

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References

1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018; 68: 7-30.
2) Saad AM, Turk T, Al-Husseini MJ, Abdel-Rahman O. Trends in pancreatic adenocarcinoma incidence and mortality in the United States in the last four decades; a SEER-based study. BMC Cancer 2018; 18: 688.
3) Jemal A, Ward EM, Johnson CJ, Cronin KA, Ma J, Ryerson B, Mariotto A, Lake AJ, Wilson R, Sherman RL, Anderson RN, Henley SJ, Kohler BA, Penberthy L, Feuer EJ, Weir HK. Annual Report to the Nation on the Status of Cancer, 1975-2014, Featuring Survival. J Natl Cancer Inst 2017; 109.
4) Witkiewicz AK, McMillan EA, Balaji U, Baek G, Lin WC, Mansour J, Mollaee M, Wagner KU, Koduru P, Yopp A, Choti MA, Yeo CJ, McCue P, White MA, Knudsen ES. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. Nat Commun 2015; 6: 6744.
5) Bailey P, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, Miller DK, Christ AN, Bruxner TJ, Quinn MC, Nourse C, Murtaugh LC, Harliwong I, Idrisoglu S, Manning S, Nourbakhsh E, Wani S, Fink L, Holmes O, Chin V, Anderson MJ, Kazakoff S, Leonard C, Newell F, Waddell N, Wood S, Xu Q, Wilson PJ, Cloonan N, Kassahn KS, Taylor D, Quek K, Robertson A, Pantano L, Mincarelli L, Sanchez LN, Evers L, Wu J, Pinese

M, Cowley MJ, Jones MD, Colvin EK, Nagrial AM, Humphrey ES, Chantrill LA, Mawson A, Humphris J, Chou A, Pajic M, Scarlett CJ, Pinho AV, Giry-Laterriere M, Rooman I, Samra JS, Kench JG, Lovell JA, Merrett ND, Toon CW, Epari K, Nguyen NQ, Barbour A, Zeps N, Moran-Jones K, Jamieson NB, Graham JS, Duthie F, Oien K, Hair J, Grutzmann R, Maitra A, Iacobuzio-Donahue CA, Wolfgang CL, Morgan RA, Lawlor RT, Corbo V, Bassi C, Rusev B, Capelli P, Salvia R, Tortora G, Mukhopadhyay D, Petersen GM, Australian Pancreatic Cancer Genome I, Munzy DM, Fisher WE, Karim SA, Eshleman JR, Hruban RH, Pilarsky C, Morton JP, Sansom OJ, Scarpa A, Musgrove EA, Bailey UM, Hofmann O, Sutherland RL, Wheeler DA, Gill AJ, Gibbs RA, Pearson JV, Waddell N, Biankin AV, Grimmond SM. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature 2016; 531: 47-52.

6) Waddell N, Pajic M, Patch AM, Chang DK, Kassahn KS, Bailey P, Johns AL, Miller D, Nones K, Quek K, Quinn MC, Robertson AJ, Fadlullah MZ, Bruxner TJ, Christ AN, Harliwong I, Idrisoglu S, Manning S, Nourse C, Nourbakhsh E, Wani S, Wilson PJ, Markham E, Cloonan N, Anderson MJ, Fink JL, Holmes O, Kazakoff SH, Leonard C, Newell F, Poudel B, Song S, Taylor D, Waddell N, Wood S, Xu Q, Wu J, Pinese M, Cowley MJ, Lee HC, Jones MD, Nagrial AM, Humphris J, Chantrill LA, Chin V, Steinmann AM, Mawson A, Humphrey ES, Colvin EK, Chou A, Scarlett CJ, Pinho AV, Giry-Laterriere M, Rooman I, Samra JS, Kench JG, Pettitt JA, Merrett ND, Toon C, Epari K, Nguyen NQ, Barbour A, Zeps N, Jamieson NB, Graham JS, Niclou SP, Bjerkvig R, Grutzmann R, Aust D, Hruban RH, Maitra A, Iacobuzio-Donahue CA, Wolfgang CL, Morgan RA, Lawlor RT, Corbo V, Bassi C, Falconi M, Zamboni G, Tortora G, Tempero MA, Australian Pancreatic Cancer Genome I, Gill AJ, Eshleman JR, Pilarsky C, Scarpa A, Musgrove EA, Pearson JV, Biankin AV, Grimmond SM. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature 2015; 518: 495-501.

7) Sharma P, Allison JP. The future of immune checkpoint therapy. Science 2015; 348: 56-61.

8) Page DB, Postow MA, Callahan MK, Allison JP, Wolchok JD. Immune modulation in cancer with antibodies. Annu Rev Med 2014; 65: 185-202.

9) Peggs KS, Quezada SA, Korman AJ, Allison JP. Principles and use of anti-CTLA4 antibody in human cancer immunotherapy. Curr Opin Immunol 2006; 18: 206-213.

10) Zheng C, Zheng L, Yoo JK, Guo H, Zhang Y, Guo X, Kang B, Hu R, Huang JY, Zhang Q, Liu Z, Dong M, Hu X, Ouyang W, Peng J, Zhang Z. Landscape of Infiltrating T Cells in Liver Cancer Revealed by Single-Cell Sequencing. Cell 2017; 169: 1342-1356 e16.

11) Huang M, Peng X, Yang L, Yang S, Li X, Tang S, Li B, Jin H, Wu B, Liu J, Li H. Non-coding RNA derived from extracellular vesicles in cancer immune escape: Biological functions and potential clinical applications. Cancer Lett 2021; 501: 234-246.

12) Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 2012; 12: 298-306.

13) Galon J, Bruni D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. Nat Rev Drug Discov 2019; 18: 197-218.

14) Galon J, Bruni D. Tumor Immunology and Tumor Evolution: Intertwined Histories. Immunity 2020; 52: 55-81.

15) Cook DA, Reed DA. Appraising the quality of medical education research methods: the Medical Education Research Study Quality Instrument and the Newcastle-Ottawa Scale-Education. Acad Med 2015; 90: 1067-1076.

16) Bernardo WM. PRISMA statement and PROSPERO. Int Braz J Urol 2017; 43: 383-384.

17) Pu N, Zhao G, Yin H, Li JA, Nuerxiati A, Wang D, Xu X, Kuang T, Jin D, Lou W, Wu W. CD25 and TGF-beta blockade based on predictive integrated immune ratio inhibits tumor growth in pancreatic cancer. J Transl Med 2018; 16: 294.

18) Karakhanova S, Ryschich E, Mosl B, Harig S, Jager D, Schmidt J, Hartwig W, Werner J, Bazhin AV. Prognostic and predictive value of immunological parameters for chemoradioimmunotherapy in patients with pancreatic adenocarcinoma. Br J Cancer 2015; 112: 1027-1036.

19) 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: 5423-5434.

20) Liu L, Zhao G, Wu W, Rong Y, Jin D, Wang D, Lou W, Qin X. Low intratumoral regulatory T cells and high peritumoral CD8(+) T cells relate to long-term survival in patients with pancreatic ductal adenocarcinoma after pancreatectomy. Cancer Immunol Immunother 2016; 65: 73-82.

21) Carstens JL, Correa de Sampaio P, Yang D, Barua S, Wang H, Rao A, Allison JP, LeBleu VS, Kalluri R. Spatial computation of intratumoral T cells correlates with survival of patients with pancreatic cancer. Nat Commun 2017; 8: 15095.

22) Hutcheson J, Balaji U, Porembka MR, Wachsmann MB, McCue PA, Knudsen ES, Witkiewicz AK. Immunologic and Metabolic Features of Pancreatic Ductal Adenocarcinoma Define Prognostic Subtypes of Disease. Clin Cancer Res 2016; 22: 3606-3617.

23) Knudsen ES, Vail P, Balaji U, Ngo H, Botros IW, Makarov V, Riaz N, Balachandran V, Leach S, Thompson DM, Chan TA, Witkiewicz AK. Stratification of Pancreatic Ductal Adenocarcinoma: Combinatorial Genetic, Stromal, and Immunologic Markers. Clin Cancer Res 2017; 23: 4429-4440.

24) Wang X, Lang M, Zhao T, Feng X, Zheng C, Huang C, Hao J, Dong J, Luo L, Li X, Lan C, Yu W, Yu M, Yang S, Ren H. Cancer-FOXP3 directly activated CCL5 to recruit FOXP3(+)Treg cells in pancreatic ductal adenocarcinoma. Oncogene 2017; 36: 3048-3058.

25) Wartenberg M, Zlobec I, Perren A, Koelzer VH, Gloor B, Lugli A, Karamitopoulou E. Accumulation of FOXP3+T-cells in the tumor microenvironment is associated with an epithelial-mesenchymal-transition-type tumor budding phenotype and is an independent prognostic factor in surgically resected pancreatic ductal adenocarcinoma. Oncotarget 2015; 6: 4190-4201.

26) Diana A, Wang LM, D'Costa Z, Allen P, Azad A, Silva MA, Soonawalla Z, Liu S, McKenna WG, Muschel RJ, Fokas E. Prognostic value, localization and correlation of PD-1/PD-L1, CD8 and FOXP3 with the desmoplastic stroma in pancreatic ductal adenocarcinoma. Oncotarget 2016; 7: 40992-41004.

27) Ino Y, Oguro S, Yamazaki-Itoh R, Hori S, Shimada K, Hiraoka N. Reliable evaluation of tumor-infiltrating lymphocytes in pancreatic cancer tissue biopsies. Oncotarget 2019; 10: 1149-1159.

28) Ino Y, Yamazaki-Itoh R, Shimada K, Iwasaki M, Kosuge T, Kanai Y, Hiraoka N. Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer. Br J Cancer 2013; 108: 914-923.

29) Tang Y, Xu X, Guo S, Zhang C, Tang Y, Tian Y, Ni B, Lu B, Wang H. An increased abundance of tumor-infiltrating regulatory T cells is correlated with the progression and prognosis of pancreatic ductal adenocarcinoma. PLoS One 2014; 9: e91551.

30) Helm O, Mennrich R, Petrick D, Goebel L, Freitag-Wolf S, Roder C, Kalthoff H, Rocken C, Sipos B, Kabelitz D, Schafer H, Oberg HH, Wesch D, Sebens S. Comparative characterization of stroma cells and ductal epithelium in chronic pancreatitis and pancreatic ductal adenocarcinoma. PLoS One 2014; 9: e94357.

31) Sideras K, Biermann K, Yap K, Mancham S, Boor PPC, Hansen BE, Stoop HJA, Peppelenbosch MP, van Eijck CH, Sleijfer S, Kwekkeboom J, Bruno MJ. Tumor cell expression of immune inhibitory molecules and tumor-infiltrating lymphocyte count predict cancer-specific survival in pancreatic and ampullary cancer. Int J Cancer 2017; 141: 572-582.

32) Zhao KL, Liu J, Jiang WN, Hao JH. [Prognostic value of tumor infiltration immune cells in pancreatic cancer]. Zhonghua Wai Ke Za Zhi 2018; 56: 464-470.

33) Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008; 454: 436-444.

34) Mantovani A, Germano G, Marchesi F, Locatelli M, Biswas SK. Cancer-promoting tumor-associated macrophages: new vistas and open questions. Eur J Immunol 2011; 41: 2522-2525.

35) Ohkura N, Hamaguchi M, Morikawa H, Sugimura K, Tanaka A, Ito Y, Osaki M, Tanaka Y, Yamashita R, Nakano N, Huehn J, Fehling HJ, Sparwasser T, Nakai K, Sakaguchi S. T cell receptor stimulation-induced epigenetic changes and Foxp3 expression are independent and complementary events required for Treg cell development. Immunity 2012; 37: 785-799.

36) Ono M. Control of regulatory T-cell differentiation and function by T-cell receptor signalling and Foxp3 transcription factor complexes. Immunology 2020; 160: 24-37.

37) Togashi Y, Shitara K, Nishikawa H. Regulatory T cells in cancer immunosuppression - implications for anticancer therapy. Nat Rev Clin Oncol 2019; 16: 356-371.

38) Bruni D, Angell HK, Galon J. The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy. Nat Rev Cancer 2020; 20: 662-680.

39) Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. 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. J Immunol 1995; 155: 1151-1164.

40) Fontenot JD, Gavin MA, Rudensky AY. Pillars Article: Foxp3 Programs the Development and Function of CD4+CD25+ Regulatory T Cells. Nat. Immunol. 2003. 4: 330-336. J Immunol 2017; 198: 986-992.

41) Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003; 299: 1057-1061.

42) Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in CD4+CD25+ T regulatory cells. Nat Immunol 2003; 4: 337-342.

43) Rigas D, Lewis G, Aron JL, Wang B, Banie H, Sankaranarayanan I, Galle-Treger L, Maazi H, Lo R, Freeman GJ, Sharpe AH, Soroosh P, Akbari O. Type 2 innate lymphoid cell suppression by regulatory T cells attenuates airway hyperreactivity and requires inducible T-cell costimulator-inducible T-cell costimulator ligand interaction. J Allergy Clin Immunol 2017; 139: 1468-1477. e2.

44) Stamoula E, Ainatzoglou A, Stamatellos VP, Dardalas I, Siafis S, Matsas A, Stamoulas K, Papazisis G. Atypical antipsychotics in multiple sclerosis: A review of their in vivo immunomodulatory effects. Mult Scler Relat Disord 2022; 58: 103522.

45) Ashrafizadeh M, Farhood B, Eleojo Musa A, Taeb S, Najafi M. The interactions and communications in tumor resistance to radiotherapy: Therapy perspectives. Int Immunopharmacol 2020; 87: 106807.

46) Liu J, Wang X, Deng Y, Yu X, Wang H, Li Z. Research Progress on the Role of Regulatory T Cell in Tumor Microenvironment in the Treatment of Breast Cancer. Front Oncol 2021; 11: 766248.

47) Zhang Y, Lazarus J, Steele NG, Yan W, Lee HJ, Nwosu ZC, Halbrook CJ, Menjivar RE, Kemp SB,

Sirihorachai VR, Velez-Delgado A, Donahue K, Carpenter ES, Brown KL, Irizarry-Negron V, Nevison AC, Vinta A, Anderson MA, Crawford HC, Lyssiotis CA, Frankel TL, Bednar F, Pasca di Magliano M. Regulatory T-cell Depletion Alters the Tumor Microenvironment and Accelerates Pancreatic Carcinogenesis. Cancer Discov 2020; 10: 422-439.

48) Aykut B, Chen R, Miller G. Regulatory T Cells Keep Pancreatic Cancer at Bay. Cancer Discov 2020; 10: 345-347.

49) Chen K, Wang Q, Li M, Guo H, Liu W, Wang F, Tian X, Yang Y. Single-cell RNA-seq reveals dynamic change in tumor microenvironment during pancreatic ductal adenocarcinoma malignant progression. EBioMedicine 2021; 66: 103315.

50) Karamitopoulou E, Andreou A, Pahud de Mortanges A, Tinguely M, Gloor B, Perren A. PD-1/PD-L1-associated immunoarchitectural patterns stratify pancreatic cancer patients into prognostic/predictive subgroups. Cancer Immunol Res 2021; 9: 1439-1450.

51) Zhao Y, Zhang Z, Lei W, Wei Y, Ma R, Wen Y, Wei F, Fan J, Xu Y, Chen L, Lyu K, Lin H, Wen W, Sun W. IL-21 Is an Accomplice of PD-L1 in the Induction of PD-1-Dependent Treg Generation in Head and Neck Cancer. Front Oncol 2021; 11: 648293.

52) Nedelec Y, Sanz J, Baharian G, Szpiech ZA, Pacis A, Dumaine A, Grenier JC, Freiman A, Sams AJ, Hebert S, Page Sabourin A, Luca F, Blekhman R, Hernandez RD, Pique-Regi R, Tung J, Yotova V, Barreiro LB. Genetic Ancestry and Natural Selection Drive Population Differences in Immune Responses to Pathogens. Cell 2016; 167: 657-669. e21.

53) Wallace TA, Prueitt RL, Yi M, Howe TM, Gillespie JW, Yfantis HG, Stephens RM, Caporaso NE, Loffredo CA, Ambs S. Tumor immunobiological differences in prostate cancer between African-American and European-American men. Cancer Res 2008; 68: 927-936.