

TNFR2: Role in Cancer Immunology and Immunotherapy

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Abstract: Immune checkpoint inhibitors (ICIs), including anti-CTLA-4 (cytotoxic T lymphocyte antigen-4) and anti-PD-1/PD-L1 (programmed death-1/programmed death-ligand 1), represent a turning point in the cancer immunotherapy. However, only a minor fraction of patients could derive benefit from such therapy. Therefore, new strategies targeting additional immune regulatory mechanisms are urgently needed. CD4⁺Foxp3⁺ regulatory T cells (Tregs) represent a major cellular mechanism in cancer immune evasion. There is compelling evidence that tumor necrosis factor (TNF) receptor type II (TNFR2) plays a decisive role in the activation and expansion of Tregs and other types of immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs). Furthermore, TNFR2 is also expressed by some tumor cells. Emerging experimental evidence indicates that TNFR2 may be a therapeutic target to enhance naturally occurring or immunotherapeutic-triggered anti-tumor immune responses. In this article, we discuss recent advances in the understanding of the mechanistic basis underlying the Treg-boosting effect of TNFR2. The role of TNFR2-expressing highly suppressive Tregs in tumor immune evasion and their possible contribution to the non-responsiveness to checkpoint treatment are analyzed. Moreover, the role of TNFR2 expression on tumor cells and the impact of TNFR2 signaling on other types of cells that shape the immunological landscape in the tumor microenvironment, such as MDSCs, MSCs, ECs, EPCs, CD8⁺ CTLs, and NK cells, are also discussed. The reports revealing the effect of TNFR2-targeting pharmacological agents in the experimental cancer immunotherapy are summarized. We also discuss the potential opportunities and challenges for TNFR2-targeting immunotherapy.

Keywords: TNF, TNFR2, cancer immunology and immunotherapy, CD4⁺Foxp3⁺ regulatory T cells, tumor immune microenvironment

Introduction

Cancer immunotherapy, broadly defined as a treatment that can enhance anti-cancer immune responses by targeting certain immune cells or molecules, has achieved significant advances in the past decades and currently becomes a mainstream therapy in cancer treatment, alongside surgery, chemotherapy, and radiotherapy.¹ The successful clinical trials of immune checkpoint inhibitors (ICIs), with blockade antibodies such as ipilimumab and nivolumab/pembrolizumab against CTLA-4 or PD-1 respectively, have transformed the ICIs into major therapy in the advanced non-small cell lung cancer (NSCLC) and melanoma (FDA-approved ICIs are summarized in Table 1).^{2,3} However, only a minor fraction of patients can obtain a complete response after such treatment.⁴ Moreover, by stimulating the activation of immune cells, treatment with ICIs could result in inflammatory side effects

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Table 1 FDA-Approved Immune Checkpoint Inhibitors (ICIs) for Cancer Treatment

Drug (Trade Name and Company Name)	Target	Approval Date	Approved Indication	Immune-Related Adverse Events (irAEs)	Reference
Ipilimumab (Yervo [®] , Bristol-Myers Squibb Co.)	CTLA-4	28 March 2011	<ul style="list-style-type: none"> Advanced metastatic melanoma Renal cell carcinoma (RCC) Colorectal cancer Non-small cell lung cancer (NSCLC) Hepatocellular carcinoma Malignant Pleural Mesothelioma 	<ul style="list-style-type: none"> Hypophysitis Colitis Dermatitis (rash) Hepatitis Nephritis 	193,195
Pembrolizumab (Keytruda [®] , Merck & Co.)	PD-1	4 September 2014	<ul style="list-style-type: none"> Advanced melanoma NSCLC Small cell lung cancer (SCLC) Head and neck squamous cell cancer Classical Hodgkin lymphoma Primary mediastinal large B-Cell lymphoma Urothelial Carcinoma Microsatellite instability-high or mismatch repair deficient metastatic colorectal cancer Gastric cancer Cervical cancer Hepatocellular carcinoma Merkel cell carcinoma RCC Tumor mutational burden-high cancer Cutaneous squamous cell carcinoma Esophageal cancer Endometrial cancer 	<ul style="list-style-type: none"> Pneumonitis Colitis Hepatitis Rash Hypothyroidism Hyperthyroidism Type-1 diabetes (T1D) Hypophysitis Nephritis 	193,194,196
Nivolumab (Opdivo [®] , Bristol-Myers Squibb Co.)	PD-1	22 December 2014	<ul style="list-style-type: none"> Advanced melanoma Advanced SCLC and NSCLC Advanced RCC Classical Hodgkin's lymphoma Head and neck squamous cell carcinoma Urothelial cancer Colorectal cancer Hepatocellular cancer Malignant pleural mesothelioma Esophageal squamous cell carcinoma 	<ul style="list-style-type: none"> Pneumonitis Colitis Hepatitis Rash Hypothyroidism Hyperthyroidism T1D Hypophysitis Nephritis 	193,196

Atezolizumab (Tecentriq®, Roche)	PD-L1	18 May 2016	<ul style="list-style-type: none"> ● Locally advanced or metastatic urothelial carcinoma ● NSCLC ● SCLC ● Metastatic triple-negative breast cancer ● Hepatocellular cancer ● Advanced melanoma 	<ul style="list-style-type: none"> ● Pneumonitis ● Colitis ● Hepatitis ● Hypothyroidism ● Hyperthyroidism ● T1D ● Hypophysitis 	193,196
Avelumab (Bavencio®, Merck KGaA & Pfizer)	PD-L1	23 March 2017	<ul style="list-style-type: none"> ● Metastatic Merkel cell carcinoma ● Advanced or metastatic urothelial carcinoma ● Renal cell carcinoma 	<ul style="list-style-type: none"> ● Pneumonitis ● Colitis ● Hepatitis ● Hypothyroidism ● T1D ● Nephritis 	193,196
Durvalumab (Imfinzi®, AstraZeneca)	PD-L1	01 May 2017	<ul style="list-style-type: none"> ● Locally advanced or metastatic urothelial carcinoma ● Stage III NSCLC 	<ul style="list-style-type: none"> ● Pneumonitis ● Colitis ● Hepatitis ● Rash ● Hypothyroidism ● Hyperthyroidism ● T1D ● Hypophysitis ● Nephritis 	193,196
Cemiplimab (Libtayo®, Sanofi/Regeneron)	PD-1	28 September 2018	<ul style="list-style-type: none"> ● Metastatic or locally advanced cutaneous squamous cell carcinoma 	<ul style="list-style-type: none"> ● Rash ● Pneumonitis ● Hypothyroidism 	193,197

known as immune-related adverse events (irAEs).⁵ Treatment with these agents could also upregulate the expression of additional immune checkpoint molecules and consequently induce resistance to immunotherapy.⁶

The fact that TNF expression is elevated in the tumor environment and this cytokine can be further enhanced by immunotherapy has recently spurred the extensive discussion about the exact role of TNF in cancer immunology and cancer immunotherapy.^{7–10} TNF levels are increased in patients with irAEs, raising the possibility that TNF may play a major role in the inflammatory side effect of ICIs.¹¹ Indeed, it was reported that the treatment with anti-TNF antibody is effective in the control of irAEs in patients.¹² TNF is an extremely pleiotropic cytokine and its biological functions are mediated by one of its two surface receptors, TNFR1 (TNF receptor type I) and TNFR2. Unlike TNFR1, TNFR2 has no death domain, and its activation can lead to the activation of NF- κ B (Nuclear Factor Kappa B) and cell growth.^{13–16} TNFR1 is ubiquitously expressed, while the expression of TNFR2 is limited to a certain type of cells, including activated T lymphocytes especially CD4⁺Foxp3⁺ Tregs, endothelial cells, and neural cells.^{17–21} Our previous study indicated that TNF-TNFR2 interaction plays a decisive role in the activation, expansion, function, and phenotypical stability of Treg cells.^{22,23} As shown by studies based on various human and murine cancer, highly suppressive TNFR2-expressing Tregs represent a major cellular mechanism in cancer immune evasion.^{24–27}

We and others have proposed that targeting TNFR2 may be a novel strategy to enhance anti-tumor immune responses.²⁸ In this article, we review and analyze the recent progress in the study that has advanced the understanding of the molecular mechanism underlying the Treg-boosting effect of TNF-TNFR2 signaling. Latest studies on the roles of TNF and TNFR2 in tumor immunology are introduced. The emerging concept of TNFR2-targeting treatment as a novel strategy in cancer immunotherapy and as an adjuvant to enhance the efficacy of checkpoint inhibitors are discussed.

The Decisive Role of TNFR2 Signaling in the Biology of Tregs: New Mechanistic Insight

Tregs are a specialized subset of CD4⁺ T cells that play a fundamental role in the regulation of immune responses and the maintenance of immune homeostasis.^{29–31}

Impaired function or reduced number of Tregs leads to autoimmune inflammatory responses. In contrast, an increase in the number of highly suppressive Tregs promotes immune evasion of the tumor.^{32–35} Thus, modulation of Treg activity has important therapeutic implications in the control of physiological and pathological immune responses in clinical settings.³⁶ For example, there is ample evidence that the expansion of antigen-specific Tregs or strengthening their suppressive activity can be useful in the treatment of autoimmune inflammatory diseases and undesirable immune responses such as rejection of transplanted organs.^{29,35} In contrast, decreased Treg number or suppressive activity can evoke or enhance anti-tumor immune responses or enhance anti-microbial immunity in chronic infection.^{34,35} Transcription factor Foxp3 (Forkhead Box P3) is a master gene that controls the functionalities of Tregs.^{37,38} Immunosuppressive function of Treg cells is believed to be mediated by co-inhibitory receptors such as CTLA-4, PD-1, TIM3 (T cell Immunoglobulin and Mucin Domain-containing Protein 3), LAG3 (lymphocyte-Activation Gene 3), or TIGIT (T cell Immunoglobulin (Ig) and Immunoreceptor Tyrosine-based Inhibition Motif (ITIM) domain), or immunosuppressive cytokines such as IL-10 (Interleukin-10), TGF β (Transforming Growth Factor-beta), granzymes, and IL-35 (Interleukin-35).^{39–41} Nevertheless, they likely represent only part of effector molecules or functionality of Tregs, and the exact mechanism remains elusive.

We (Xin Chen and Joost J. Oppenheim) found and published the first clear evidence that TNF-TNFR2 signaling is fundamentally important for the biology of Tregs.²² We reported that TNF can enhance the expression of CD25 and Foxp3, promote the proliferation, and enhance the suppressive function of Tregs.²² Intriguingly, TNF selectively upregulates the expression of TNFR2 and other members of the TNF receptor superfamily (OX40, 4-1BB, and FAS) on Tregs.⁴² Tregs express markedly higher levels of TNFR2 than CD4⁺CD25⁻ effector T cells (Teffs).²² TNFR1 has a stronger affinity for soluble TNF (sTNF), while membrane-bound TNF (mTNF) preferentially binds to and activates TNFR2.^{43,44} There is compelling evidence that mTNF-TNFR2 signaling axis favors immune tolerance, which is presumably mainly based on the activation and expansion of Treg cells.^{45–47} It was reported that vitamin D3, adalimumab (a humanized anti-TNF antibody), and methotrexate up-regulate the expression of mTNF by dendritic cells (DCs) and monocytes. As a consequence, upregulation of mTNF by these

compounds promoted the activation and expansion of Tregs through TNFR2.^{45,46,48} Recently, we have reported that inhibition of two-pore channels with tetrandrine or siRNAs increases the number of CD4⁺Foxp3⁺ Tregs in an mTNF-TNFR2-dependent manner in mice. Using the mice colitis model, we showed that the expanded Tregs are attributable to the reduced colon inflammation after tetrandrine treatment.^{49–51} Furthermore, TNFR2 is closely associated with the immunosuppressive function of Treg cells, and the expression of TNFR2 can define the highly suppressive subset of Tregs.^{22,25,46,52} Moreover, the number of Tregs in TNFR2^{-/-} mice is markedly lower than in wild-type (WT) mice, and when stimulated with the septic challenge, they fail to expand.^{22,23} Therefore, TNFR2 plays a role in the homeostasis of Tregs in both steady state and inflammatory responses. This biological function of TNFR2 is at least partially transduced by I κ B kinase alpha (IKK α), as shown by the phenotype of mice with conditional knockout (KO) of IKK α in CD4⁺ T cells.⁵³

It was shown that in vitro treatment with antagonist leads to contraction of Tregs, while treatment with TNFR2 agonist can expand Tregs and promote the phenotypical stability and enhance the function of Tregs.^{54–56} Mechanistically, TNFR2 agonist promotes the stabilization of Foxp3 expression through mTOR (Mammalian Target of Rapamycin) and NF- κ B signal pathways.⁵⁷ Previously, it was shown that activation of TNFR2 in human CD4⁺ and CD8⁺ T cells results in the proliferative expansion through the IKK/NF- κ B pathway, including the activation of phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT), and these pathways of TNFR2 signaling are likely applicable to Tregs.^{54,58–62} Further, it was also reported that mitogen-activated protein kinase (MAPK) (Extracellular Signal-Regulated Protein Kinase (Erk1/2), p38, Jun N-Terminal Kinases (JNK)) signaling pathway attributable to the activation and proliferation of naturally occurring Treg cells (nTreg) by TNF-TNFR2 interaction.^{53,63–67} Moreover, a recent study indicates that the stimulatory effect of TNF on Tregs is partially through an epigenetic mechanism that demethylates the *foxp3* gene.⁶⁸ Ex vivo activation of Treg cells by the stimulation with anti-CD3/CD28 represses the mTOR pathway and disfavors glycolysis, which contrasts with conventional T (Tconvs) cells.^{69–71} The suppression of glycolysis in Treg cells promotes fatty acid oxidation-fueled oxidative phosphorylation and impairs the stability and function of Treg cells.^{72–74} Recently, it was shown that co-stimulation of human thymus-derived Treg (tTreg) cells via CD3/TNFR2 switches to PI3K-mTOR drives

glycolysis, and this helps to maintain the identity and suppressive function of tTreg.⁷⁵ In contrast to glycolytic Tconvs, Tregs use a diverse metabolic program downstream of glycolysis upon TNFR2 co-stimulation. Glycolytic tTreg cells produce lactate from glucose and participate in the complete glycolytic pathway upon TNFR2 co-stimulation, while the net lactate secretion remains unaltered.⁷⁵ Moreover, in contrast to glycolytic Tconvs, upon stimulation with CD3/TNFR2, the glycolytic tTreg cells markedly augment the levels of the labeled tricarboxylic acid cycle (TCA)-cycle intermediates.⁷⁵ This study also shows that human blood-derived TNFR2^{hi} effector tTreg cells exhibited high glycolytic activity.⁷⁵

The role of TNF-TNFR2 interaction in the activation of nTreg is well defined, while its role in TGF β -induced Treg cells (iTregs) remains controversial. It has been reported that TNFR2 contributes to the development of Treg cells in the thymus.⁷⁶ A recent study shows that the exogenous TNF boosts the differentiation and function of iTreg via TNFR2 signaling.⁷⁷ Further, this study shows that TNFR2 deficiency impairs the differentiation, proliferation, and function of iTreg cells in both in vitro and in vivo settings. In comparison, TNFR1 deficiency leads to the reduction of the differentiation of inflammatory T cells such as Th1 (T Helper Type 1) and Th17 (T Helper Type 17) cells, while the iTregs function remains intact.⁷⁷ However, an earlier in vivo study showed that the suppressive function of nTreg is dependent on TNF-TNFR2 signaling, while the activation of iTreg cells does not need the TNFR2 signal.⁷⁸ In the experimental mouse model of autoimmune encephalomyelitis (EAE), it was found that TNF-TNFR2 signaling impairs the function of iTregs through the activation of Akt-Smad3 (Protein Kinase B-Recombinant Human Mother Against Decapentaplegic Homolog) signaling pathways, which inhibits TGF β -induced Smad3 phosphorylation and reduces the transcription of *foxp3* genes.⁷⁹ Therefore, more definitive evidence is needed to clarify the exact role of the TNFR2 signal in iTregs.

Taken together, recent studies have greatly advanced our understanding of signaling pathways required for TNFR2 in the activation of Tregs and its mechanism, as well as the molecular basis underlying the immunosuppressive function of Tregs. However, the key signaling events of TNFR2 in Tregs and transcriptional regulation of Foxp3 expression by TNFR2 signals remain elusive. These unanswered questions merit further investigation. Since TNFR2 expression identifies the highly suppressive subset of Tregs and the expression of TNFR2 by Tregs can

be preferentially upregulated by TNF stimulation, this system represents an ideal model for understanding the molecular basis and definitive suppressor mechanism of Tregs.

TNFR2-Expressing Tregs: A Major Cellular Mechanism in Tumor Immune Evasion

A large number of Treg cells accumulate in the tumor microenvironment, presumably resulting from the conversion of iTregs from naïve CD4⁺ T cells, or metabolic adaptation, or stabilized Foxp3 expression, or response to neoantigen and self-antigen.³⁴ The prevalence of Treg cells promotes immune evasion of cancer by suppressing tumor-reactive Teff cells.⁸⁰ In tumor microenvironment (TME), Treg cells are reprogrammed and consequently acquire an activated phenotype and enhanced suppressor function.⁸¹ Elimination of Tregs activity can enhance anti-tumor immune responses by targeting several cell surface proteins, including TNFR2.^{22,28} TNFR2 is expressed preferentially by highly suppressive Treg cells including those in TME, and expression of TNFR2 is considered as a master control switch for the activation and expansion of Treg population.^{82,83} There is some experimental evidence that the TNF-TNFR2 interaction is responsible for the activation and expansion of Tregs in TME, and TNFR2-expressing Tregs represents a major cellular mechanism in immune evasion of the tumor.^{25,80} Therefore, we (Xin Chen and Joost J. Oppenheim) and Dr. Faustman proposed that TNFR2 may be harnessed as a druggable target to enhance anti-tumor immune responses.^{28,61,82,84–89} This idea is supported by a number of studies, as summarized in Table 2.

We for the first time reported that highly suppressive TNFR2⁺ Treg cells accumulate in the TME of mouse Lewis lung carcinoma (LLC) and 4T1 breast carcinoma.^{25,90} Similar observation was also made in mouse hepatocellular carcinoma and CT26 colon cancer.⁹¹ In TNFR2-deficient mice, hepatic metastases of the colon (MC-38) and lung (H-59) carcinoma are markedly reduced, accompanied by the decrease in the number of intrahepatic MDSCs as well as Tregs in sites of metastases.⁹² Using a luciferase-expressing variant of the syngeneic B16-F10 melanoma mouse model of lung metastasis, Chopra and colleagues reported that exogenous TNF could enhance metastasis by promoting the expansion of Treg cells through TNFR2, and deficiency of either TNF or TNFR2 on immune cells results in the

reduction of lung metastasis and decrease in the numbers of Treg cells in the lungs.⁹³

Certain human tumor cells can aberrantly express TNFR2, and tumor infiltrates are dominated by highly suppressive TNFR2⁺ Tregs.^{94,95} It was reported that Tregs in the peripheral blood of lung cancer patients and ascites in ovarian cancer patients express high levels of TNFR2, and the levels of TNFR2 expression are positively related to a more advanced clinical stage, immune invasion, and progressive metastasis.^{24,27,96} Tumor-infiltrating Treg cells express higher levels of TNFR2 as compared with Teff cells in surgical resection samples from three lung cancer patients.⁹⁷ In patients with cervical cancer and cervical intraepithelial neoplasia, TNFR2⁺ Tregs are increased in both peripheral blood and tumor environment.⁹⁸ Sézary syndrome (SS), a rare form of cutaneous T-cell lymphomas, is also characterized by high expression of TNFR2 on both tumor cells and Tregs.^{95,99} A recent study shows that the proportion of TNFR2⁺ cells was markedly higher in CD4⁺ T cells than CD19⁺ B cells or CD8⁺ T cells in the tumor-draining lymph nodes derived from breast cancer patients.¹⁰⁰ Further, it was reported that TNFR2 expression is much higher on Tregs than Tconv isolated from the peripheral blood of gastric cancer patients and healthy individuals.¹⁰¹ Moreover, the proportion of total Foxp3⁺ Tregs in the CD4⁺ T cells and the percentage of TNFR2⁺ Tregs increased with tumor progression and lymphatic metastasis.¹⁰¹

Taken together, high expression of TNFR2 is a characteristic of tumor-infiltrating Tregs that may represent a major cellular mechanism of cancer immune evasion. Since levels of TNFR2 expression on Tregs are related to the clinical pathology and metastasis status of patients with cancer, TNFR2 expression could be a promising molecular marker for the prognosis of cancer.

Expression of TNFR2 on Tumor Cells

It was reported that TNFR2 is expressed by at least 25 types of tumors, including human renal cell carcinoma, multiple myeloma, colon cancer, ovarian cancer, and cutaneous T-cell lymphomas.⁸² A recent study of 788 commercially available human cancer cell lines from diverse cancer tissues indicates that various levels of TNFR2 are expressed, while hematopoietic and lymphoid cell lines express the highest levels of TNFR2.⁸⁶ An immunohistochemistry (IHC) study of 431 tissue specimens from esophageal squamous cell carcinoma (ESCC) patients found that TNFR2 expression is higher in

Table 2 Effect of TNFR2-Targeting Treatment on Experimental Tumor Models

Therapeutics Agents	Types of Tumor Model	Studies Outcomes	Reference
Anti-mouse TNFR2 antagonistic antibody (TY101) (Mouse IgG1)	Mice colorectal cancer (CT26 & MC-38)	<ul style="list-style-type: none"> ● Monotherapy, or in combination with anti-PD-1, inhibited tumor growth.^a ● Induced death of tumor-infiltrating Tregs, increased IFNγ⁺ CD8⁺ CTLs. ● Concurrent or a prior dose of TY101 with anti-PD-1 showed maximum benefits. ● The prior dose of anti-PD-1 with TY101 should be avoided. 	84
Anti-mouse TNFR2 agonist antibody (Y9) (Mouse IgG1)	Various syngeneic mice tumor model	<ul style="list-style-type: none"> ● Expanded and improved tumor antigen-specific IFNγ⁺ CD8⁺ CTLs. ● Reduced surface TNFR2 expression on tumor-infiltrating CD8⁺ T cells and CD4⁺ T cells. ● No change in Treg population, but soluble TNFR2 increased. ● Response rate varied (CR: CT26, EMT6, WEHI-164, MC-38, Sa I/N, and MBT2; Without CR: A20; NR: 4T1, B16-F10, and LLC1).^b ● Combination with anti-PD-1 or anti-PD-L1 improved survival rate and anti-tumor response (WEHI-164, CT26, and EMT6). ● The toxicity profile was better than anti-CTLA-4. 	159
Ab1 (chimeric antibody) and Ab2 (Humanized form) (Human IgG1)	Humanized mouse models (HT-29, MDA-MB-231, and LG1306)	<ul style="list-style-type: none"> ● Increased activity and proliferation of CD8⁺ and CD4⁺ T cells. ● Monotherapy, or in combination with anti-PD-1, inhibited tumor growth. ● Combination treatments were more efficacious than anti-PD-1 alone. 	
TNFR2 antagonistic antibody	Sézary syndrome (SS)	<ul style="list-style-type: none"> ● Killed TNFR2⁺ SS tumor cells and TNFR2⁺ Treg cells of SS subjects. ● Expanded the CD8⁺ Teff cells. ● Restored CD26⁻ subpopulations. ● Reset Treg/Teff ratio to normal. 	85
TNFR2 blocking antibody (M861) (Mouse IgG)	Mice colorectal cancer (CT26)	<ul style="list-style-type: none"> ● Combination with CpG ODN inhibited the tumor growth. ● Reduced the proportion of TNFR2⁺ Treg cells and enhanced the IFNγ⁺ CD8⁺ CTLs in the tumor-infiltrating lymphocytes. 	88
TNFR2 antagonistic antibody (IgG)	Human ovarian cancer (OVCAR3)	<ul style="list-style-type: none"> ● In vitro killing of the tumor. ● Suppressed the activity of tumor-associated CD4⁺ Treg cells. ● Little inhibitory effects on peripheral CD4⁺ Treg cells or cells from healthy donors. ● Enhanced proliferation of CD8⁺ Teff cells. 	99
Anti-mouse TNFR2 agonists (TR75-54.7 and TR75-89) (Hamster IgG)	Mice colorectal cancer (CT26)	<ul style="list-style-type: none"> ● Enhanced anti-tumor activity. ● Improved median survival time.^c ● Increased IFNγ⁺ CD8⁺ CTLs. ● Reset Treg/Teff ratio to normal. 	97

(Continued)

Table 2 (Continued).

Therapeutics Agents	Types of Tumor Model	Studies Outcomes	Reference
Azacitidine + panobinostat/azacitidine + lenalidomide	Acute myeloid leukemia	<ul style="list-style-type: none"> ● Decreased TNFR2⁺ Treg cells in the peripheral blood and bone marrow of LAML patients. ● No change in TNFR2⁺ Treg cells. ● Increased in cytokines [IL-2 and IFNγ] production by effector T cells. ● Panobinostat and lenalidomide repressed the expression of TNFR2 on Treg cells. 	178,198

Notes: ^aCure Rate (complete tumor regression and elimination): In CT26-tumor implanted animals, TY101 alone: 55%, Anti-PD-1: 25%, and TY101+Anti-PD-1: 62%. In MC38-tumor implanted animals, TY101 alone: 20%, Anti-PD-1: 10%, and TY101+Anti-PD-1: 70%. ^bComplete Response (CR): Tumor below 60 mm³ and continued to regress until the end of the study. NR: No Response. ^cMedian survival: Control: 22 days; Hamster IgG control mAbs: 25 days; TR75-89: 30.5 days; TR75-54: 7.36 days.

malignant tissue than non-tumor esophageal tissues, and the expression of TNFR2 is positively correlated with invasion depth, advanced clinical stage, low differentiation degree, and poor overall survival.¹⁰² Moreover, TNFR2 expression is strongly correlated with the prognosis of middle thoracic ESCC patients than of lower thoracic ESCC patients.¹⁰² Another IHC study from 71 primary human non-small cell lung carcinoma (NSCLC) patients and 25 normal tissue samples found that tumor tissues express markedly higher TNFR2 than normal tissues, and the rate of TNFR2^{hi} expression in NSCLC tissue is 35%.¹⁰³ Moreover, human NSCLC cell lines (A549, H1299, and H1975) also express markedly higher TNFR2 in both mRNA and protein levels than those in the normal human lung epithelial cell line (BEAS-2B).¹⁰³ We analyzed TCGA (The Cancer Genome Atlas) and GTEx (Genotype-Tissue Expression) database with web-based tool Gene Expression Profiling Interactive Analysis (GEPIA, <http://gepia2.cancer-pku.cn/>), and found that the overall abundance of TNFR2 gene expression is markedly higher than that of PD-L1 and CTLA-4 in the most of tumor tissues (Figure 1).¹⁹⁹ More specifically, TNFR2 gene expression is markedly higher in six human cancer types including glioblastoma multiforme, kidney renal clear cell carcinoma, brain lower grade glioma, pancreatic adenocarcinoma, stomach adenocarcinoma, and testicular germ cell tumors (Figure 2, p<0.01). In contrast, CTLA-4 gene expression is upregulated in four types of human cancer (lymphoid neoplasm diffuse large B-cell lymphoma, head and neck squamous cell carcinoma, pancreatic adenocarcinoma, and skin cutaneous melanoma), and PD-L1 gene expression is upregulated in only two cancers (lymphoid neoplasm diffuse large B-cell lymphoma and thymoma, Figure 2. P<0.01). Nevertheless, TNFR2 gene expression is lower in DLBC (lymphoid neoplasm diffuse large B-cell lymphoma) and THYM (thymoma) than in the normal control tissue (p<0.01). High expression of TNFR2 by tumor tissues is a premise to explore the possibility of tumor treatment with TNFR2-targeting agents. Future studies may need to focus on those types of human cancers with high TNFR2 gene expression.

The functional consequence of TNFR2 expression on cancer cells has also been studied. In colorectal cancer tissue from human patients, the expression of TNFR2 is positively correlated with Ki-67 expression.¹⁰⁴ Further, Ki-67 expression is up-regulated by overexpression of TNFR2 in SW1116 cells and inhibited by TNFR2 silence in HT29 cells.¹⁰⁴ Importantly, TNFR2 expression is found to promote the growth and clone formation of these two lines via the

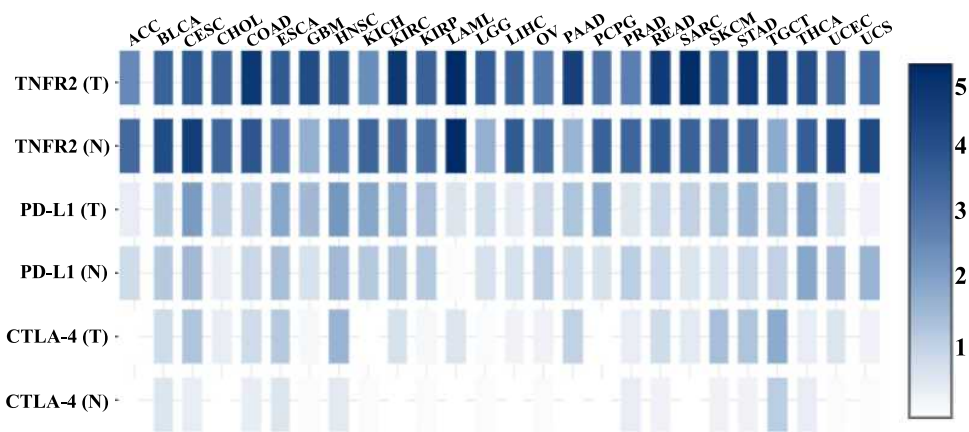


Figure 1 TNFR2, PD-L1, and CTLA-4 gene expression profiles across diverse human cancer and normal tissues. The transcriptomic analyses of indicated gene expression by human cancers and paired normal tissues were performed with GEPIA (Gene Expression Profiling Interactive Analysis) online database (<http://gepia2.cancer-pku.cn/>); **Figure 1** is drawn according to specific data in the GEPIA database.¹⁹⁹ Log-scale was set to log₂ (TPM+1) in the analysis.

Abbreviations: T, tumor; N, normal; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, hepatocellular carcinoma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma.

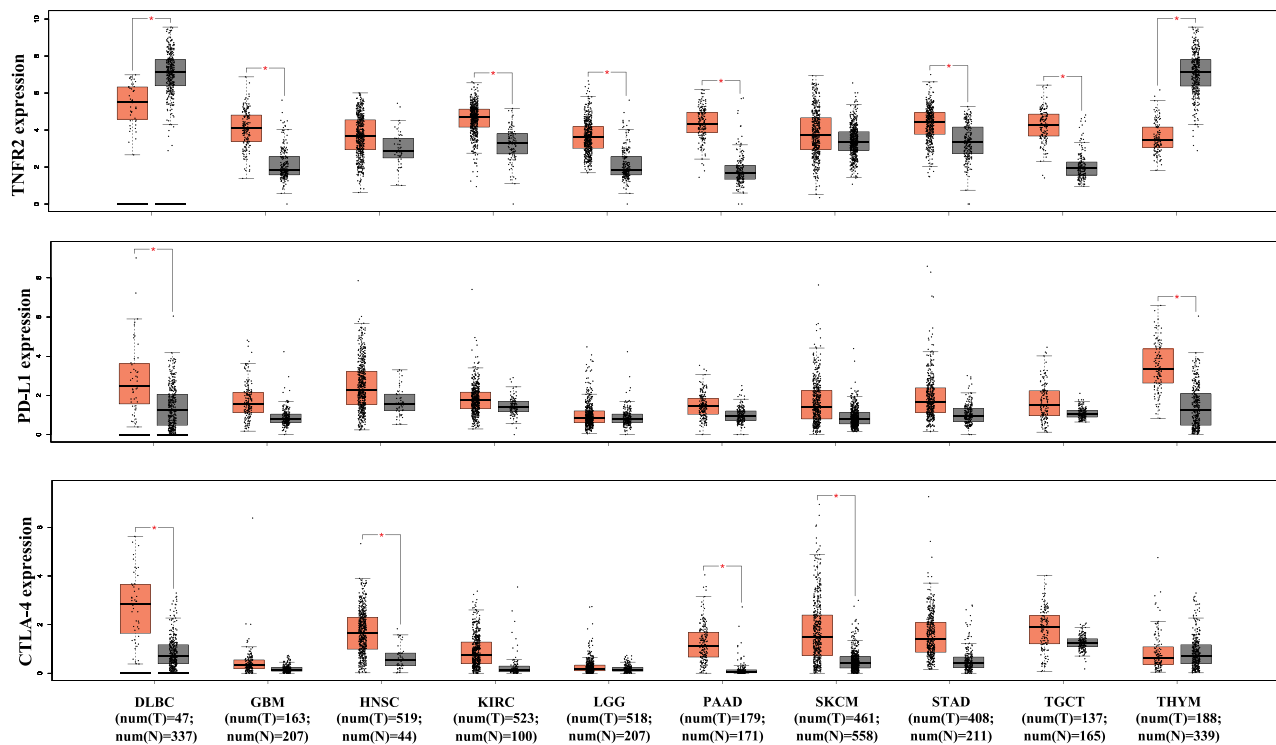


Figure 2 Comparison of TNFR2, PD-L1, CTLA-4 gene expression levels by human cancers and paired normal tissues. The transcriptomic analyses of indicated gene expression by human cancers and paired normal tissues were performed with GEPIA (Gene Expression Profiling Interactive Analysis) online database (<http://gepia2.cancer-pku.cn/>); **Figure 2** is drawn according to specific data in the GEPIA database.¹⁹⁹ Log-scale was set to log₂ (TPM+1) in the analysis. The transcriptomic analyses were performed as described in Y-axis: transcript per million. X-axis: tumor (T, red) and paired normal tissues (N, grey). The number (num) of samples is indicated. The solid black line represents medium value. The box is the upper and lower quartiles and the two lines outside the box stand for the highest and lowest expression levels. Comparison between tumor and paired normal tissue: *p<0.01 (analyzed by one-way ANOVA).

Abbreviations: DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KIRC, kidney renal clear cell carcinoma; LGG, brain lower grade glioma; N, normal; PAAD, pancreatic adenocarcinoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THYM, thymoma; T, tumor.

PI3K/AKT signaling pathway.¹⁰⁴ TNFR2 expression levels are also associated with drug-resistant to adriamycin in MDA-MB-231 and MCF-7 breast cancer cells by regulating the PARP (Poly (ADP-ribose) polymerase) via the AKT signaling pathway.¹⁰⁵ It was reported that allelic polymorphisms of TNFR2 (196 M/R (methionine/arginine)-TNFR2 variation) are associated with the development of breast carcinoma: 196 M allelic variant is associated with late-onset of breast cancer in post-menopausal patients, while 196R-TNFR2 in patients with breast carcinoma is associated with increased overall disease-free survival as compared with those absences of 196R allele.¹⁰⁶ Furthermore, a recent study found that the loss of one TNFR2 allele enhances the incidence of breast cancer in MMTV (mouse mammary tumor virus)-Wnt1 mouse model and results in a more aggressive phenotype and metastasis by activating the canonical NF- κ B signaling pathway and autocrine production of TNF.¹⁰⁷ The exact role of allelic polymorphisms of TNFR2 in tumorigenesis and progression should be further studied.

The levels of soluble TNFR2 (sTNFR2) in the malignant ovarian neoplasms are markedly higher than that in the benign ovarian neoplasms, and high levels of sTNFR2 were associated with tumor grades differentiation.¹⁰⁸ It would be interesting to ask which types of cells are the source of sTNFR2: is it mainly released by tumor cells or other TNFR2-expressing cells including Tregs?

Other Types of TNFR2-Expressing Cells in the Tumor Environment

MDSCs

Myeloid-derived suppressor cells (MDSCs) are another type of major immune suppressors that promote immune evasion of cancer by cooperating with Tregs in TME.^{109–111} Interestingly, MDSCs also express TNFR2, and the expression of TNFR2 promotes survival and suppressive activity of MDSCs.^{18,92,112–116} In tumor-bearing TNFR2^{-/-} mice, the development of MDSCs is impaired, and this contributes to the growth inhibition of tumor in mice deficient in TNFR2.¹¹⁴ It was reported that TNFR2 mediates the stimulation of mTNF in the upregulation of CXCR4 (C-X-C chemokine receptor type 4) expression on MDSCs, that is required for the infiltration of MDSCs to the tumor tissue.¹¹² Deficiency of TNFR2 in MDSCs fails to accumulate in pre-metastatic lesions and results in the down-regulation of arginase-1 expression and reduces liver metastasis of lung cancer in mice.⁹² In mouse 4T1 breast cancer model, the immunosuppressive function and accumulation of MDSCs in TME as

well as up-regulation of arginase-1 expression by MDSCs are dependent on TNFR2, through the activation of NF- κ B and phosphorylation of p38.¹¹³

MSCs

Mesenchymal stem cells (MSCs) are another type of immunosuppressive cells that represent an important component in TME with the capacity to promote tumor growth by suppressing the activation of tumor reactive immune cells.^{117,118} For example, several *in vivo* and *in vitro* studies have shown that MSCs are attributable to the immunosuppressive environment in TME by inhibiting the activation and maturation of DCs, reducing the killing ability of natural killer (NK) cells, promoting Tregs expansion, and suppressing functions of Teff cells.^{119–121} TNF can promote the expression of immunosuppressive proteins on MSCs.^{122–124} MSCs-boosting effect of TNF appears to be mediated by TNFR2, since it was reported that signal of TNFR2 plays a key role in the immunosuppressive function of MSCs, including inhibition of T cell activation and pro-inflammatory cytokine production, induction of more suppressive Tregs.¹²⁵ A recent study showed that the expression of TNFR2 by T cells is crucial for the induction of Tregs by MSCs.¹²⁶ Moreover, TNFR2-KO MSCs also reduce their proliferation capacity, major characteristics markers (Sca1, CD90, CD105, CD44, and CD73), and functional properties (tissue/cell regeneration functions and endothelial pro-angiogenic support).¹²⁷

ECs and EPCs

Endothelial cells (ECs) and their precursor endothelial progenitor cells (EPCs) play an important role in cancer-associated angiogenesis and neo-vascularization, thus promoting tumor progression and metastasis.^{128,129} Both ECs and EPCs express TNFR2 with a dominance of TNFR1 expression.^{130,131} It has been reported that TNF could increase the expression of pro-angiogenic mediators such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor, and IL-8 expression by ECs.¹³² There is *in vivo* evidence that the survival, mobilization, differentiation, and function of endothelial cells also depend on the TNF-TNFR2 signaling pathway.^{133,134} Intriguingly, a recent study has shown that priming EPCs with TNF markedly up-regulates TNFR2 expression on EPCs and the interaction of TNF with TNFR2 enhances the immunosuppressive function of EPCs.^{135,136} Blockade of TNFR2 with anti-TNFR2 monoclonal antibodies (mAbs) leads to the

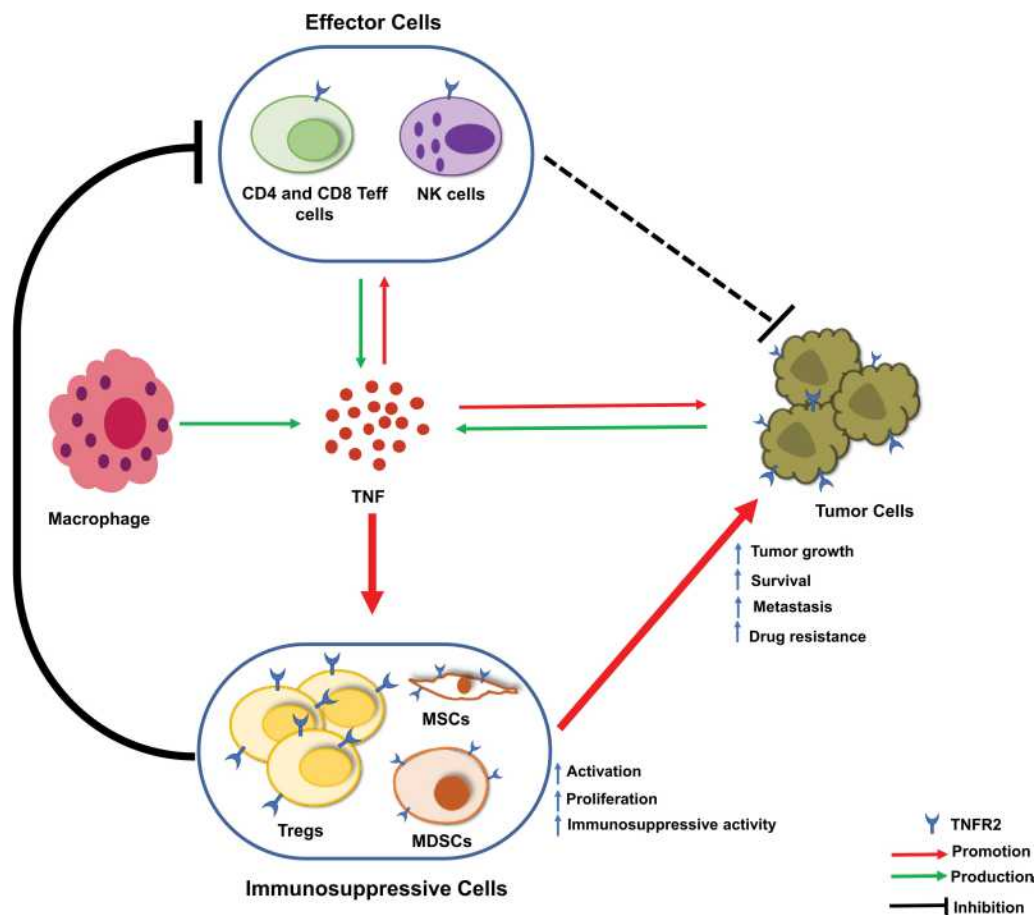


Figure 3 Current understanding of the role of TNF-TNFR2 signaling in the tumor microenvironment. In the tumor microenvironment (TME), tumor-associated macrophages, effector cells ($CD4^+$ and $CD8^+$ T effector (Teff) cells and natural killer (NK) cells), and tumor cells are the major source of TNF. In response to TNF stimulation, the number of $CD4^+Foxp3^+TNFR2^+$ Treg cells are increased. These expanded Treg cells in TME are more stable in phenotype and more immunosuppressive. Moreover, TNF activates $TNFR2^+$ myeloid-derived suppressor cells (MDSCs) and $TNFR2^+$ mesenchymal stem cells (MSCs). Tregs, MDSCs, and MSCs likely operate collaboratively in the inhibition of the anti-tumor immune response and the promotion of tumor evasion. Further, $TNFR2$ signaling also promotes the survival, metastasis, and growth of the tumor.

polarization of EPCs towards pro-inflammatory and immunogenic phenotype via TNF-TNFR1 pathway.¹³⁵ Since the expression of TNFR2 is higher in human liver cancer ECs than human normal liver sinusoidal ECs,¹³⁷ suggesting that TNFR2 signaling may play an important role in the pro-angiogenic and immunosuppressive function of EPCs or ECs in the TME. This possibility should be further studied.

CD8⁺ Tregs

Similar to $CD4^+$ Tregs, Foxp3-expressing $CD8^+$ T cells with immunosuppressive activity are considered as $CD8^+$ regulatory T cells ($CD8^+$ Tregs).^{138,139} Phenotypically, $CD8^+CD122^+$ cells, $CD8^+CD28^-$ cells, and $CD8^+CD103^+$ cells were reported to be $CD8^+$ Tregs in humans or mice.^{140–142} It has been reported that $CD8^+Foxp3^+$ Tregs can also express TNFR2 and

TNF can induce the activation, proliferation, and suppressive function of $CD8^+Foxp3^+$ Tregs in a TNF-TNFR2 dependent manner.^{143,144} A number of studies indicate that $CD8^+$ Tregs could be found in the tumor microenvironment and contribute to tumor immune escape. For example, $CD8^+CD28^-$ Tregs are detected in tumor tissues of patients with various cancers.¹⁴⁵ They potently inhibit the proliferation and cytotoxicity of Teff cells in IL-10 dependent manner.¹⁴⁵ In patients with colorectal cancer, $CD8^+CD25^+Foxp3^+$ Tregs directly isolated from the tumor can potently inhibit the proliferation of $CD8^+CD25^-$ Teff cells from tumor tissue.¹⁴⁶ It is possible that TNF-TNFR2 interaction also plays a role in the activation of tumor-infiltrating $CD8^+$ Tregs, while the exact role of TNFR2 signal in cancer immune evasion mediated by $CD8^+$ Tregs remains to be further clarified.^{139,145–148}

Bregs

IL-10-producing B cells with immunosuppressive function found in the experimental models of autoimmunity, parasitic infection, cancer, and transplantation indicates that regulatory B cells (Bregs) are also a player in the maintenance of immune tolerance.^{149–152} It was reported that TNFR2 is expressed on Bregs as well, and the activation of TNFR2 with its agonist TNC-scTNF (143N/145R) increases their production of IL-10.¹⁵³ It was shown that splenic IL-10-producing CD19⁺CD21⁺ Bregs promote the development of papilloma and growth of cancer in skin carcinogenesis induced by 7, 12-dimethylbenzanthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA).¹⁵⁴ Interestingly, TNF secreted by tumor cells inhibits anti-tumor immune responses and promotes tumor growth by stimulating the differentiation of Breg cells in the tumor microenvironment.¹⁵⁴ The requirement of TNFR2 signal in this process should be further studied in the future.

CD8⁺ CTLs

In addition, similar to immunosuppressive cells (MSCs, MDSCs, and MSCs); TNFR2 can also be expressed by tumor responsive Teff cells. For example, several studies reported that TNFR2 expression plays a crucial role in the activation, proliferation, and function of CD8⁺ T cells.^{60,155–157} The proportion of tumor-specific CD8⁺ T cells are markedly reduced in tumor-draining lymph nodes of TNFR2^{-/-} mice, suggesting that the sustainability of early proliferation of CD8⁺ T cells depends on the activation of TNFR2.¹⁵⁸ Furthermore, the production of IL-2 and IFN γ (interferon-gamma) by tumor-specific CD8⁺ T cells are reduced in TNFR2^{-/-} mice, indicating that TNFR2 plays a role in the optimal production of effector cytokines crucial for mount anti-tumor immune responses.¹⁵⁸ In sharp contrast, it was shown that the treatment with the antagonist of TNFR2 in tumor-bearing mice results in the activation of CD8⁺ T cells and long-term tumor-free survival.^{88,159} This may be explained by the increased survival of CD8⁺ CTLs, as TNF-TNFR2 signaling in CD8⁺CTLs can result in activation-induced cell death (AICD).^{157,160,161} Therefore, the TNFR2 signal in tumor responsive CD8⁺ CTLs is very complicated and should be further investigated.

NK Cells

Natural Killer (NK) cells, an important component of the innate system, play a pivotal role in TME due to their natural

ability of cytotoxicity.^{162,163} It has been reported that TNFR2 is expressed on NK cells and the TNFR2 signal can induce the differentiation of NK cells.^{97,164} It was reported that, in response to TNF-TNFR2 stimulation, NK cells promote metastatic dissemination of B16-F10 melanoma in the mouse model.¹⁶⁵ In contrast, it was shown that the crosstalk between DCs and NK cells in mice required TNF-TNFR2 interaction: TNFR2 expressed on the surface of NK cells is required for DC-mediated NK-cells proliferation and amplification of NK cell's cytotoxic activity.¹⁶⁶ Moreover, there is compelling in vitro and in vivo evidence that TNF increases NK cell-mediated IFN γ production via TNFR2 dependent manner.¹⁶⁷

Taken together, in TME, TNFR2 is expressed on certain tumor cells and immune cells including CD4⁺ Treg cells, CD8⁺ Treg cells, CD8⁺ CTLs, Breg cells, NK cells, MDSCs, and other types of cells such as ECs, EPCs, and MSCs. As a consequence, these cells play important roles in tumor immune evasion, tumor growth, and tumor progression (Figure 3). However, how TNF-TNFR2 signaling regulates the biological function of these cells in TME and key signaling events with mechanistic details remain elusive and should be further studied.

TNFR2-Targeting Agents in Cancer Immunotherapy

Anti-TNF Biologics

Melanoma is a prominent type of cancer that benefits most from the treatment with ICIs.⁸ However, approximately 1% of patients with advanced melanoma treated with ICIs develop severe colitis.⁸ Anti-TNF therapy is widely used to treat autoimmune diseases, such as rheumatoid arthritis (RA), Crohn's disease, and ulcerative colitis¹⁶⁸ and thus has been used in the treatment of irAEs. For example, infusion of infliximab, the first-generation chimeric TNF blocking monoclonal antibody, was found to effectively cure colitis induced by ICIs in most patients without affecting melanoma prognosis.¹⁶⁹ Interestingly, TNF blockade can inhibit the AICD and consequently increase CD8⁺ tumor-infiltrating lymphocytes (TILs) and decrease PD-L1 and TIM-3 expression.^{170,171} Recently, it was reported that anti-TNF therapy through inhibition of AICD of CD8⁺ TILs could enhance the efficacy of combination therapy with anti-PD-1 and anti-CTLA-4 in mouse tumor models.¹⁷² To date, the exact role of TNF in cancer progression and TNF inhibitors in cancer treatment is still a matter of debate.⁷ The observed benefit of anti-TNF antibodies on the efficacy of ICIs in treating

tumors may be at least partially attributable to the blockade of TNFR2 and this possibility should be experimentally addressed in future studies.

Thalidomide Analogs

Thalidomide and its analog lenalidomide and pomalidomide are small molecule glutamic acid derivatives and are now classified as immunomodulatory drugs (IMiDs) to treat several inflammatory diseases and tumors.^{173,174} Thalidomide and its analogs all belong to “class I” IMiDs, characterized as broad inhibitors of LPS-induced inflammatory cytokines like TNF, IL-6, and IL-12.¹⁷⁵ Thalidomide and its analogs are effective in the treatment of hematological and solid malignant diseases.¹⁷⁶ It was reported that the combination therapy with thalidomide and fludarabine is effective in the treatment of patients with chronic lymphocytic leukemia (CLL). This treatment can reduce the number of CLL and Treg cells simultaneously.¹⁷⁷ Moreover, in acute myeloid leukemia patients, the treatment with lenalidomide reduces the number of TNFR2⁺ Tregs, and it is likely attributable to the reduction of TNFR2 expression on Tregs by lenalidomide.¹⁷⁸

TNFR2 Antagonistic Antibody

Antagonistic antibodies specific to TNFR2 may be the most straightforward approach to inactivate TNFR2-positive Tregs.⁹⁹ It was shown that human TNFR2-specific antagonistic antibodies that can inhibit the proliferation of Tregs while promoting the expansion of effector T cells (Teffs). The capacity of these TNFR2 antibodies in the killing of Tregs isolated from ovarian cancer ascites is stronger than that from healthy donor samples, indicating that these antibodies may preferentially act on tumor-infiltrating Tregs, which characteristically express high levels of TNFR2.⁹⁹ Moreover, antagonistic antibodies can also reduce TNFR2⁺CD26⁻ cells and TNFR2⁺ Treg cells, and expand the Teff cells in Sézary syndrome patients to corrected Treg/Teff ratios in the tumor microenvironment.⁸⁵ Recently, this team also designed several new variants of human TNFR2-specific antagonistic antibody to achieve high TME specificity by killing TNFR2-expressing tumor cells and Tregs. The optimized version of anti-TNFR2 with IgG2 isoforms stabilize hinge region (disulfide double mutations at C232S and C233S) and the wide separation of antibody arms have demonstrated better TME specificity.⁸⁶ Besides, they also reported that the TNFR2 expression pattern provided the rationale for antagonistic treatment effectiveness. The TNFR2 antagonistic killing activity is more potent in the cancer cell line with high TNFR2 expression than the cancer cell line with

low TNFR2 expression.⁸⁶ Furthermore, the combination treatment with murine-directed anti-TNFR2 antibody (TY101) and anti-PD-1 has a greater rate of tumor regression and elimination over single treatment with either anti-TNFR2 or anti-PD-1. Even anti-TNFR2 antibody alone possesses a better anti-tumor effect than anti-PD-1 alone in the murine model of CT26 and MC38. Mechanistically, the treatment restores the Treg/Teff ratio by inducing the death of immunosuppressive Treg cells in the TME.⁸⁴ We found that TNFR2 blocking antibody M861 combined with a sub-optimal dose of CpG oligodeoxynucleotide (CpG ODN) can synergistically inhibit the growth of subcutaneously transplanted mouse CT26 colon tumor by eliminating TNFR2⁺ Treg cells and increasing tumor-infiltrating IFN γ ⁺CD8⁺ CTLs. Moreover, the combination treatment with anti-TNFR2 blocking antibody and anti-CD25 antibody also results in an enhanced inhibition of tumor growth in mouse 4T1 breast cancer model.⁸⁸

TNFR2 Agonistic Antibody

“TNFR2 agonist” has been repeatedly shown to enhance the *in vivo* proliferative expansion of Tregs, maintain the stability of the Treg cell phenotype, and preserve their suppressive function.⁵⁵ Therefore, the restoration of the function or increase in the number of Tregs via “TNFR2 agonist” provided a rationale as a therapeutic strategy for the treatment of Type 1 diabetes (T1D), graft versus host disease (GvHD), neurodegenerative disease, and other autoimmune diseases.^{56,89,179–182} However, agonistic TNFR2 antibodies (Y9, and MM-401) were potent anti-tumor activity in different humanized mouse models.^{159,183–186} Furthermore, Y9 treatment alone elicits more potent anti-tumor activity than the treatment with anti-PD-1 alone in the WEHI-164 model, while a combination of both (Y9+anti-PD-1) showed greater survival in CT26 and EMT6 syngeneic mice tumor models.¹⁵⁹ Interestingly, the activity of Y9 did not depend on TNFR2 expression on tumor cells or the depletion of Treg cells.^{159,183} MM-401, a humanized agonistic anti-TNFR2 antibody that promoted anti-tumor immunity *in vitro* via induction antibody-dependent cellular cytotoxicity (ADCC).^{184–186} It is reported that after the treatment with MM-401, the number of Treg cells in human ovarian cancer ascites is reduced.^{184,186} As TNFR2 agonists appear to have both anti-tumor and anti-inflammatory effects, future studies can reconcile these seemingly opposite effects.

Concluding Remarks

Recent pre-clinical studies indicate that targeting TNFR2 with either antagonistic or agonistic antibodies results in tumor inhibition by mobilizing anti-tumor immune responses,

eliminating Treg activity, or stimulating the activation of CD8⁺ CTLs, respectively.^{85,99,159} A number of cell types in the tumor microenvironment can express TNFR2, such as Tregs, MDSCs, MSCs, ECs, EPCs, CD8⁺ CTLs, NK cells, and tumor cells themselves. The responses of these cells to TNF-TNFR2 interaction are different and could even result in the opposite effect on immune responses to the tumor (Figure 3). Thus, the cellular target and mechanism of action of TNFR2-targeting agents could be very complicated. Moreover, the *in vivo* effect of antibodies could be different from that observed in the *in vitro* experiment. For example, the biological activity of IL-2 and IL-15 can be enhanced by *in vivo* treatment with anti-IL-2 mAbs and IL-15R α -Fc chimera, respectively.¹⁸⁷ The unpredictable *in vivo* effect of antibodies could add an additional layer of complicity to analyze the exact action and underlying mechanism of TNFR2 specific antibodies in cancer treatment. A more carefully designed study may help clarify this issue.

TNFR2 is well known for its function in the promotion of the survival and proliferation of cells and this feature of TNFR2 is likely applicable to tumor cells that express TNFR2.^{188,189} This provides a strong rationale to pursue further study on TNFR2 antagonists in the treatment of tumors. Indeed, a wide spectrum of tumor cells express higher levels of TNFR2 (as discussed in “Expression of TNFR2 on tumor cells”) than paired normal tissues (Figure 1). Nevertheless, some tumors express even lower levels of TNFR2 than normal tissues (Figure 1). Thus, the levels of TNFR2 expressed by tumors should be considered in the design of the future clinical study if the TNFR2-targeting agent is designed to act directly on tumors. Furthermore, high expression levels of TNFR2 on tumor tissues may be a biomarker for TNFR2-targeting therapy, and this possibility should be assessed in future studies.

Preferentially elimination of TNFR2^{hi} Tregs in TME is a scientific premise for the development of TNFR2-targeting agents. This is mainly based on the fact that only 30~40% of peripheral Tregs are TNFR2-expressing cells in normal mice, while the majority of mouse tumor-infiltrating Tregs are TNFR2^{hi} cells; thus, it is reasonable to assume that inactivation or even depletion of TNFR2-expressing Tregs would not compromise the peripheral tolerance to self-antigen.²⁵ However, TNFR2 is more broadly expressed by human Tregs in the periphery than mouse Tregs,⁵² therefore, the risk to trigger autoimmune inflammatory responses by TNFR2-targeting treatment in human patients should be considered in future study. In contrast, several recent studies clearly show that the treatment with TNFR2 agonistic antibodies can inhibit

autoimmune inflammatory responses by promoting the activation and expansion of Tregs.^{181,190–192} Therefore, application of TNFR2 agonistic antibodies in cancer treatment may boost Treg activity and result in more profound immune suppression. This possibility should also be carefully assessed in the future.

Despite these unanswered questions, the current pre-clinical data encourage and support the idea that targeting TNFR2 may represent a new avenue and novel strategy in cancer immunotherapy.

Abbreviations

CTLA-4, Cytotoxic T Lymphocyte Antigen-4; PD-1, Programmed Death-1; PD-L1, Programmed Death-Ligand 1; TNF, Tumor Necrosis Factor; TNFR1, Tumor Necrosis Factor Receptor Type I; TNFR2, Tumor Necrosis Factor Receptor Type II; Foxp3, Forkhead Box P3; Tregs, Regulatory T cells; Teffs, Effector T cells; MDSCs, Myeloid-Derived Suppressor Cells; MSCs, Mesenchymal Stem Cells; ECs, Endothelial Cells; EPCs, Endothelial Progenitor Cells; NK cells, Natural Killer Cells; Bregs, Regulatory B cells; CTL, Cytotoxic T Cells; TIL, Tumor-Infiltrating Lymphocytes; ICIs, Immune Checkpoint Inhibitors; FDA, The United States Food and Drug Administration; irAEs, Immune-Related Adverse Events; TIM3, T cell Immunoglobulin and Mucin Domain-containing Protein 3; LAG3, Lymphocyte-Activation Gene 3; TIGIT, T cell Immunoglobulin (Ig) and Immunoreceptor Tyrosine-based Inhibition Motif (ITIM) domain; IL-10, Interleukin-10; IL-35, Interleukin-35; TGF β , Transforming Growth Factor-beta; NF- κ B, Nuclear Factor Kappa B; sTNF, Soluble TNF; mTNF, Transmembrane TNF; DCs, Dendritic Cells; WT, Wild-Type; KO, Knock-Out; IKK α , I κ B Kinase alpha; Akt, Protein Kinase B; Smad3, Mothers Against Decapentaplegic Homolog 3; Th1, T Helper Type 1; Th17, T Helper Type 17; mTOR, Mammalian Target of Rapamycin; PI3K, Phosphatidylinositol 3-Kinase; Erk1/2, Extracellular Signal-Regulated Protein Kinase 1/2; MAPK, Mitogen-Activated Protein Kinase; JNK, Jun N-Terminal Kinases; Tconvs, Conventional T cells; tTregs, Thymus-derived Treg cells; TCA, Tricarboxylic Acid Cycle; nTregs, Naturally-occurring Treg cells; iTregs, Induced Treg cells; EAE, Experimental Autoimmune Encephalomyelitis; TME, Tumor Microenvironment; LLC, Lewis Lung Carcinoma; TCGA, The Cancer Genome Atlas; GTEX, Genotype-Tissue Expression; GEPIA, Gene Expression Profiling Interactive Analysis; SS, Sézary Syndrome; IHC, Immunohistochemistry; NSCLC, Non-Small Cell Lung

Carcinoma; ESCC, Esophageal Squamous Cell Carcinoma; DLBC, Lymphoid Neoplasm Diffuse Large B-Cell Lymphoma; THYM, Thymoma; M/R, Methionine or Arginine; PARP, Poly (ADP-ribose) Polymerase; sTNFR2, Soluble TNFR2; CXCR4, C-X-C Chemokine Receptor Type 4; VEGF, Vascular Endothelial Growth Factor; IL-8, Interleukin-8; mAbs, Monoclonal Antibodies; AICD, Activation-Induced Cell Death; IFN γ , Interferon Gamma; IL-2, Interleukin-2; RA, Rheumatoid Arthritis; IMiDs, Immunomodulatory Drugs; IL-6, Interleukin-6; IL-12, Interleukin-12; CLL, Chronic Lymphocytic Leukemia; CpG ODN, CpG Oligodeoxynucleotide; T1D, Type 1 Diabetes; GvHD, Graft Versus Host Disease; ADCC, Antibody-Dependent Cellular Cytotoxicity; IL-15, Interleukin-15; IL-15R α , Interleukin-15 Receptor alpha; T, Tumor; N, Normal; ACC, Adrenocortical Carcinoma; BLCA, Bladder Urothelial Carcinoma; CESC, Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma; CHOL, Cholangiocarcinoma; COAD, Colon Adenocarcinoma; ESCA, Esophageal Carcinoma; GBM, Glioblastoma Multiforme; HNSC, Head and Neck Squamous Cell Carcinoma; KICH, Kidney Chromophobe; KIRC, Kidney Renal Clear Cell Carcinoma; KIRP, Kidney Renal Papillary Cell Carcinoma; LAML, Acute Myeloid Leukemia; LGG, Brain Lower Grade Glioma; LIHC, Hepatocellular Carcinoma; OV, Ovarian Serous Cystadenocarcinoma; PAAD, Pancreatic Adenocarcinoma; PCPG, Pheochromocytoma and Paraganglioma; PRAD, Prostate Adenocarcinoma; READ, Rectum Adenocarcinoma; SARC, Sarcoma; SKCM, Skin Cutaneous Melanoma; STAD, Stomach Adenocarcinoma; TGCT, Testicular Germ Cell Tumors; THCA, Thyroid Carcinoma; UCEC, Uterine Corpus Endometrial Carcinoma; UCS, Uterine Carcinosarcoma; RCC, Renal Cell Carcinoma; SCLC, Small Cell Lung Cancer; IgG, Immunoglobulin G; Sca1, Stem Cell Antigen-1; DMBA, 7, 12-dimethylbenzanthracene; TPA, 12-O-tetradecanoylphorbol-13-acetate.

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Disclosure

The authors report no conflicts of interest in this work.

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