TNFR2 antagonist and agonist: A potential therapeutics in Cancer Immunotherapy

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Abstract:

Tumor necrosis factor receptor 2 or TNFR2 is considered as an appealing target protein due its limited frequency to Tregs which are highly immunosuppressive and its presence on human malignancies. Numerous studies have revealed that TNFR2 is primarily found on MDSCs (myeloid derived suppressor cells) and $CD^{+}Foxp3^{+}$ regulatory T cell (Tregs). It has a great importance in the proliferation and functional activity of Tregs and MDSCs. To treat malignancies and diseases like autoimmune disorder, the suppressor activity of TNFR2 must be eliminated by downregulation or upregulation. Therefore, at the molecular level, advances in comprehension of TNFR2's complex structure and its binding to TNF have opened the door to structure-guided drug development. Two key obstacles of cancer treatment are the dearth of Treg-specific inhibitors and the lack of widely applicable ways to directly target tumors via frequently expressed surface oncogenes. Many researchers have discovered potential antagonist and agonist of TNFR2 which were successful in the inhibition of Tregs proliferation, reduction of soluble TNFR2 secretion from normal cells and in the expansion of T effector cells. The representation of the data in the following review article elucidates the clinically administrated TNFR2 antagonist and agonist in the treatment of cancers.

Keywords: Treg, Antagonist, Agonist, MDSCs, CD+Foxp3+.

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Highlights:

1. Generally, the expression of TNFR2 is highly observed in the MDSCs and Tregs.
2. TNFR2 leads towards the functional activity of cell longevity and immune suppression. TNFR2 has been marked as a oncogene in around twenty five types of tumor cell.
3. Targeting TNFR2 with antagonist or agonist antibodies can be potential therapeutics against cancer.
4. Combined or Monotherapy of TNFR2 with immune checkpoint inhibitor is an appealing approach in cancer immunotherapy.

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Introduction:

A few years ago, treatment of cancer was solely depended on conventional therapies such as chemotherapy and radiation. The traditional treatment of cancer was not only expensive to afford but also it has severe adverse effects which were intolerable by patients. The invention of immunotherapy has signified a revolutionary change in cancer treatment. The application of gene therapy or immune checkpoint inhibitors have significantly advanced cancer treatment, but their effectiveness and recurrence rates are unpredictable and there are chance that they can cause autoimmunity. Ipilimumab and nivolumab/pembrolizumab are immune checkpoint inhibitors which target CTLA-4 and PD-1, respectively. This have become a key therapeutic in advanced non-small cell lung cancer (NSCLC) and melanoma after successful clinical studies [1,2].

TNF or tumor necrosis factor was initially termed after it was observed in a study that the introduction of bacterial endotoxin to mice led in the production of a serological protein with necrotic anti-tumor effect at high quantities. Tumor necrosis factor is hailed as a milestone is cancer immunotherapy [3]. TNF is known to be a pleiotropic cytokine which acts as a principal part in the inflammatory and immune responses by two different receptors: TNFRI and TNFR2. TNFR1 is also called as p55 and TNFRSF1A whereas TNFRII is also known as p75 and TNFRs1B. When TNFR1 is compared to TNFR2, TNFR1 is mostly expressive on all types of cells, it activates the nuclear factor kappa B (NF-kB) [4] and it also induces death in cells while TNFR2 is generally expressed in some cell types which includes endothelial cells [5], minor subsets of lymphocytes [6,7] and mesenchymal stem cells of human [8]. Furthermore, research has shown that TNFR2 was also found to be expressed predominately on the human and mouse $CD4^{+}Foxp3^{+}$ regulatory T cells or also known Tregs [6] which are generally immunosuppressive cells in mammals [9]. TNFR2 expression not only determines the most suppressive Treg subgroup but also plays a critical role in Treg proliferative growth, suppressive function, and phenotypic stability [10-17]. Studies has also shown that some cancer cells and tumor-

infiltrating immunosuppressive CD4+ FoxP3+ regulatory T cells(Tregs) express tumor necro sis factor receptor type II (TNFR2) . TNFR2 enhances cancer cell survival and tumor growth by stimulating the activation and multiplication of Tregs, a key checkpoint in antitumor immu ne responses. By blocking the expression of TNFRII through antibody antagonist, the proliferation of Tregs can be suppressed which may initially induce apoptosis to cancer cells. This following review article has briefly illustrated the clinically administrated anti TNFR2 antagonists and agonist which has been successfully targeted TNFR2 and marked as potential

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therapeutics against human cancers.

TNFR2: Expression and Effect on Treg

TNFR2 is a member of the TNF superfamily of receptors [18]. TNFR2 has a distinct signaling circuit than TNFR1. Instead of being related to a death domain, TNFR2 induces activation of NF-kB and cell proliferation. All lymphoid cells and parenchymal cells express most TNF superfamily receptors. TNFR2, on the other hand, has a limited immune system expression, is triggered by the ligands TNF and interleukin-2 (IL-2), and is restricted to minor lymphoid subpopulations such as potent Tregs, myeloid suppressor cells, endothelial cells, and select neurons during mammalian development [19,20]. Since of its limited expression, TNFR2 is a suitable therapeutic target because antibody-based therapies are less likely to induce systemic toxicity. TNFR2 is a compelling contender for a variety of reasons. A population of Tregs that expresses tumor necrosis factor receptor 2 (TNFR2) is a well-known subtype. Tregs that express the TNFR2 gene in both mice and humans are strong immune suppressors that are excessively numerous in human and murine malignancies [21-27].

Numerous cancer cells including colon cancer, multiple myeloma, renal cell carcinoma, Hodgkin's lymphoma and cutaneous non-lymphoma, Hodgkin's and ovarian cancer proliferate because of aberrant expression of TNFR2 on tumor cells [28-33]. The signaling of nuclear factor B (NF-kB) is activated by TNFR2 signaling, resulting in constitutive downstream agonism and increased cell proliferation. It would be ideal if there was a single strategy that could successfully suppress Tregs while also directly inhibiting tumor development via the TNFR2 oncogene. T effector cells (Teffs) would ideally be able to grow and destroy the tumor using this way.

TNFR2-positive Tregs are common in the tumor microenvironment of several human and murine malignancies. In cancers, TNFR2 gene duplication and activating mutations have been discovered [29]. The known toxicity of high-dose TNF is entirely mediated by TNFR1, not TNFR2, according to a study based on baboons [34,35]. In naturally occurring Tregs in human blood, TNFR2 is found at a 10-fold greater density than TNFR1 [19]. The immune system of mice missing TNFR2 is better able to react to and destroy several cancer types without progressing to systemic autoimmunity [35-46].

Several TNFR2-positive Tregs at the tumor site or in the circulation are signs of tumor immunosuppression. Gene duplications or other mechanisms (as reported in cutaneous non-lymphoma Hodgkin's patients) might explain the higher cell surface abundance of TNFR2 on

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cancer cells [27,29,47,48]. The total quantity of TNFR2-positive Tregs in the tumor is higher than in the patient's peripheral blood in other malignancies, however the specific genetic foundation for their dysregulation has yet to be determined [27].

TNFR2 contains distinct characteristics. In suppressive T cell types, it acts as an immune system marker. It is also considered as a master switch for Treg survival and fate and a newly invented highly expressed oncogene [49]. The most suppressive Tregs might express excessive quantities of TNFR2 (TNFR2hi Tregs) and exert significant immunosuppressive effects [27] in several cancers, while in patients with acute myeloid leukemia [50] and lung cancer, lower levels of TNFR2-positive Tregs correspond with better clinical outcomes [51]. TNFR2 can be expressed abnormally as a growth receptor oncogene on the tumor itself in colon cancer, multiple myeloma, Hodgkin's lymphoma, ovarian cancer, and cutaneous T cell lymphomas [28-32,52]. The genetic foundation of aberrant TNFR2 expression in non-cutaneous T cell lymphomas is connected with constitutive overexpression of TNFR2 due to numerous gene duplications or cytoplasmic TNFR2 mutations that impart constitutive agonism [29]. As an aberrant surface oncogene, these characteristics make TNFR2 an attractive molecular target for Treg inactivation in the tumor microenvironment and direct tumor targeting. TNFR1 expression does not differ between Treg and non-Treg cells, however human Treg express higher amounts of TNFR2 than CD25- T-conv cells. Furthermore, TNFR2+ Treg have the most suppressive ability [6, 11]. TNF's impact on Treg suppressor function is still up for debate.

In both mice and humans, sTNF retained or even boosted FOXP3 expression and Treg suppressive capability [12, 17, 49,53]. In an inflammatory context, TNF-TNFR2 is required for maintaining FOXP3 expression and murine Treg stability [11]. A similar effect has been reported in human Treg in in-vitro experiment [54].

TNF has also been shown to have a deleterious impact on Treg function. TNF inhibits Treg function by lowering FOXP3 expression or increasing its dephosphorylation [54,55]. In clinical trials, RA patients who received the anti-TNF antibody adalimumab had a higher number of FOXP3+ cells and their regulatory function was restored [56]. It's worth noting that the TNFR2 antibodies utilized in these investigations were most likely different (agonistic vs. antagonistic) [49]. Recent research suggests that TNFR2 antagonisms and agonisms may affect Treg phenotype and suppressor activity in very diverse ways [49].

TNF priming causes Treg proliferation and activation both in vitro [17,57] and in an acute mouse GvHD model via TNFR2 in clinical trials [58]. The activation of human Treg with a

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TNFR2-agonist antibody sustained a stable Treg phenotype and function following ex vivo growth [54]. The use of a TNFR2 agonist alone was enough to prevent the loss of FOXP3 expression, whereas sustained hypomethylation of the TSDR (Treg-specific demethylated region) of the FOXP3 gene locus required both rapamycin and a TNFR2 agonist, implying that FOXP3 expression is stabilized by both the mTOR and the NF-kB signal pathways. In vitro restimulation of TNFR2 agonist-expanded Treg with rapamycin did not result in the loss of FOXP3 protein or an increase in IL-17A production, even under proinflammatory circumstances, showing Treg stability. Increased expression of RORyt and IL-17 production in TNFR2 knockout CD4+ T cells is reliant on the impairment of TNFR2-mediated NFkB activation [59]

Researchers hypothesize that a similar regulatory pathway exists in human Treg, in which TNFR2/NF-kB signaling acts as a double-edged sword, enhancing FOXP3 while inhibiting RORyt expression, hence contributing to Treg stability. Another possibility is that TNFR2 activation triggers an autocrine TNF-TNFR2 loop, which controls the production of histone methyltransferase EZH2 [57], a polycomb repressor complex 2 components (PRC2). EZH2 is reported to bind to FOXP3, assisting FOXP3 in gene transcriptional repression regulation [60].

TNFR2 agonist and antagonist on Tregs:

Several agonistic TNFR2-recognizing monoclonal antibodies have been produced to augment functional Treg populations in trials, with therapeutic efficacy in T1D and skin inflammation [49,54,61]. In a mouse model of GvHD, the STAR2 protein, a TNF-based TNFR2 agonist, has been demonstrated to enlarge host-type radiation-resistant Tregs and enhance the result after allo-HCT, as well as prolong longevity without compromising anti-leukemia or anti-infective benefits [13]. The therapeutic potential of new TNFR2-targeting drugs in the treatment of autoimmune and inflammatory illnesses has been revealed by these studies. However, a small molecule TNFR2 agonist has yet to be discovered. The development of autoimmune illnesses includes systemic lupus erythematosus, multiple sclerosis, type 1 diabetes (T1D), rheumatoid arthritis (RA), autoimmune thyroid disease, psoriasis, inflammatory bowel disease, and autoimmune liver disease is linked to Treg dysfunction [62]. As a result, for patients with autoimmune disorders and GvHD, restoring the function or raising the number of Tregs has become a therapeutic strategy and treatment aim [63].

The accumulation of TNFR2-expressing Tregs in the tumor microenvironment is thought to be a fundamental biological mechanism of tumor immune evasion. The majority of tumor

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infiltrating Tregs show extensive surface TNFR2 expression and are highly immunosuppressive in the mice Lewis lung carcinoma and the 4T1 breast tumor model [6,64].The percentage of TNFR2+ Tregs in the peripheral blood or tumor-associated ascites is higher in lung cancer and ovarian cancer patients [65,66].When comparing Tregs to CD4+ effector T cells (Teffs) and CD8+ cytotoxic T lymphocytes (CTLs) in metastatic melanoma patients, single-cell RNA-Seq reveals that TNFR2 is one of the most significantly elevated genes expressed by Tregs, and its expression is linked to CD8+ CTL exhaustion [27].Furthermore, it was observed that TNFR2 expression on Tregs is linked to increased lympho-vascular invasion, a higher chance of malignancies, a higher clinical stage, and a worse response to therapy regarding individuals suffering from acute myeloid myeloma and lung cancer [64, 65,67]. TNFR2 is regarded as an oncogene, and antagonistic antibodies with TNFR2 have recently been investigated as a potential method in cancer immunotherapy.

An antagonistic antibody targeting TNFR2 has been shown to cause the death of both Tregs and OVCAR3 ovarian cancer cells, which exhibit high surface TNFR2 expression [68]. In a mouse colon cancer model, our team discovered that using a TNFR2-blocking antibody significantly improved the efficiency of immunotherapy using CpG. [69]. This combination treatment reduced TNFR2 expression on tumor-infiltrating Tregs and increased tumor infiltration of interferon-gamma-producing CD8+ CTLs as a result [70]. As a result, new TNFR2 antagonists might be used as cancer immunotherapy drugs.

The tumor microenvironment preferentially attracts TNFR2+ Treg cells, which have a strong immunosuppressive potential, making tumor immune escape easier. The absence of TNFR2 expressing Treg or the failure to establish systemic autoimmunity [46] or the lower numbers and poor function of MDSCs in TNFR2 knockout mice may explain their better immune responses to malignancies. Furthermore, elevated TNFR2 gene expression on Treg cells has been linked to CD8 cytotoxic T lymphocyte fatigue in individuals with metastatic melanoma. A mode of action of anti TNFR2 antagonist and agonist is illustrated below on Figure 1:

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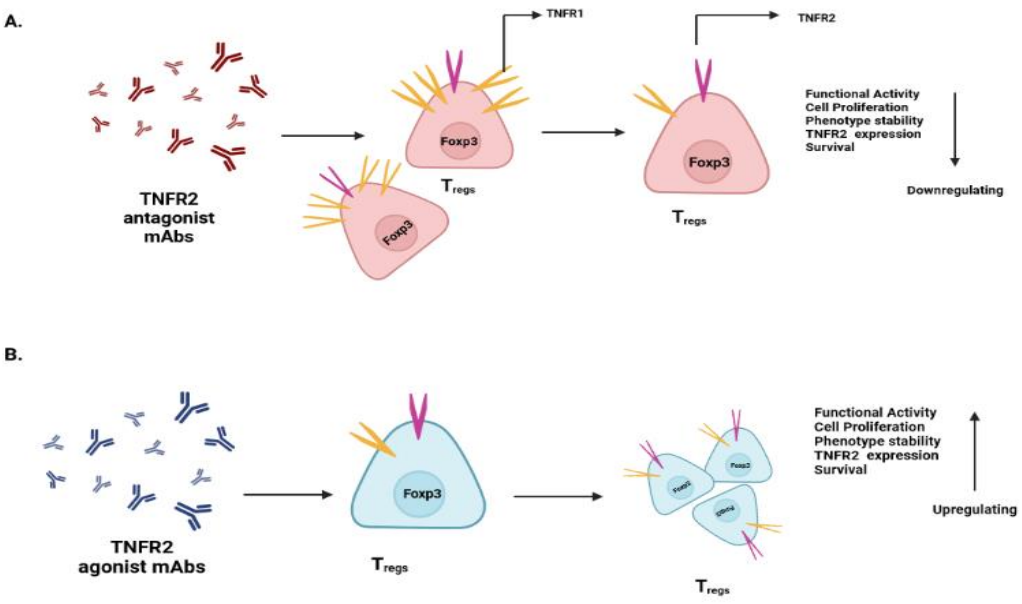

General Mode of Action of TNFR2 antagonist and agonistic mAbs. A. The TNFR2 antagonist antibodies has downregulated the functional activity, Treg proliferation, TNFR2 surface expression, Phenotype stability and Survival of Tregs. B. The TNFR2 agonist antibodies has stimulated TNFR2 after binding with it.

Figure 1: General mode of action of anti TNFR2 antagonist and agonist.

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TNFR2 antagonist in cancers:

TNFR2 is an oncogene that has been found in at least 25 tumor types, in addition to being a Treg expansion inducer. Human renal cell carcinoma, multiple myeloma, colon cancer, ovarian cancer, and cutaneous T-cell lymphomas (CTCL) have all been documented to have increased TNFR2 expression on the tumor itself [71]. Overexpression of the cytokine receptor TNFR2 leads to enhanced tumor cell proliferation and tumor development in general. Patients with Sezary syndrome (SS), an uncommon variant of CTCL that is generally resistant to therapy, have genetic mutations/genomic gains in the TNFRSF1B gene, which codes for the TNFR2 protein. The elevated expression of TNFR2 on tumor cells and Treg is a hallmark of SS.

Gain-of-function mutations in TNFR2 result in increased noncanonical NF-kB activation [72], which is important for cell proliferation and growth. By utilizing antagonistic compounds against TNFR2, it appears to be desired to use one technique that might successfully block powerful suppressive Treg while also directly preventing tumor development. Such TNFR2-specific blocking agents would ideally limit Treg while allowing Tconv growth and activity, allowing antitumor immune responses to be restored and tumor regression to occur. There are a number of TNFR2 recognizing antagonist and agonist which have shown positive outcomes against variety of cancers. In the following table 1, the clinically administrated antibody antagonists of TNFR2 are described below:

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Table 1: Anti TNFR2 antagonist antibodies in cancer treatment

Anti TNFR2 antagonist antibodies	Types of condition	Outcomes of experiment
APX601	Solid Tumors	• APX601 binds specifically to human TNFR2 with high affinity (Kd = 47 pM) • Induces death in tumor cells • Inhibits the binding of TNFR2 to its ligand TNF-α • Depletion of TNFR2-expressing Tregs, MDSC and tumor cells through ADCC [73]
TY101	Mouse Colon cancer (CT26 & MC-38)	• In CT26 cell line • Anti TNFR2 upholds the best solo treatment (10/18 mice) • Anti PD-1 alone showed 25% of recovery • Combined therapy with anti PD-1 and anti TNFR2 has shown 62% cure rate. • In MC38, the recovery rate of solo therapies was 10% and 20%. • With the combined therapy, the recovery rate was 70% • In both cell lines, the death of tumor infiltrating

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		Tregs was induced and the IFNγ$^+$ CD8$^+$ CTLs were elevated [74].
TNFR2 antagonist antibody	Human Ovarian Cancer	• Induced death on tumor cells (in vitro experiment • Expansion of CD8$^+$ T effector cells • The functional activity of tumor associated CD4$^+$ Treg cells was suppressed • An inhibition of peripheral CD4$^+$ Treg cells or cells from healthy donors were seen (in small quantity) [66]
TNFR2 antagonist antibody	Sezary Syndrome	• Induced death of TNFR2$^+$ Sezary Syndrome of tumor cells • Proliferated the CD8$^+$ Teff cells. • Reinstated CD26$^-$ subpopulations. • Reset Treg/Teff ratio to normal. [69]
M861	Mouse Colon cancer (CT26)	• The mono or combined therapy with CpG ODN induced death on the tumor cell. • Boosts the tumor infiltrating IFNy+CD8+

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		CTLs production when combined with sub-optimal dosage of CpG ODN. • Decreased the quantity of TNFR2^{+} Treg cells [75]

APX601:

APX601 is recognized as a potential TNFR2 antagonist antibody which can reverse the immune suppression and cause tumor cell apoptosis by targeting TNFR2-expressing Treg and MDSC. Research finding by Filbert et al., suggest that APX60 is a promising immunotherapeutic antibody with numerous possible modes of action and can be developed further for the treatment of a range of solid tumors [70]. Filbert and his colleagues have created antibodies in a broad spectrum for TNFR2 by using Apexigen's proprietary rabbit monoclonal antibody technology or APXiMAB^{TM}.

APX601, a humanized IgG1 antibody, was chosen as the primary treatment candidate after a thorough evaluation of over 100 antibody candidates for TNFR2 binding, TNF-α blockade, and functional tests. In Treg and MDSC suppression experiments, the capacity of APX601 to reverse immune suppression was tested. Furthermore, utilizing the mouse Colo205 xenograft model, the potential of APX601 to deplete TNFR2-expressing Treg and tumor cells was examined both in vitro and in vivo [73].

The results of the clinical experiments demonstrates that APX601 recognizes a unique epitope in the CRD1 domain of human TNFR2 and binds to it with high affinity (Kd = 47 pM).In cell-based ligand binding studies, APX601 is a strong antagonist that inhibits the TNFR2-TNFα interaction (IC50 = 0.149 nM). It has two ways for restoring immune suppression: I. substantial inhibition of Treg and MDSC immunosuppressive capabilities by reducing TNFR2 binding to its ligand TNF-α, and 2) depletion of TNFR2-expressing Tregs, MDSC, and tumor cells via antibody-dependent cell cytotoxicity (EC50 = 1.14 nM) and ADCP (EC50 = 0.71 nM) effector functions [73].

TY101:

TY101 is an anti-mouse TNFR2 antibody antagonist which has been experimented in mice

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colorectal cancer cell lines CT26 and MC 38. A study by Case et al., performed a pre-clinical trial on murine model with colon cancer. They have followed three types of regimens in the experiment [74]. In the murine colon cancer CT26 and MC38 models, the researchers have tested the effectiveness of TY101 alone or with the combination of anti PD1 therapy. The research was mainly based on monotherapy. The mice who were infected with cancer cells was treated with anti PD-1 alone, anti TNFR2 alone and combination of anti PD1 and anti TNFR2. The treatment with CT26 infected mice were started when the tumor was grown up to threshold of volume of 100-200mm^3. Four different therapies with the doses of 100µg of sterile antibody were administrated up to 20 to 21 days. The therapies were placebo control, anti PD1 alone, anti TNFR2 alone, and the combination of PD1 and TNFR2. It was observed that the three active therapies have given effective results by lowering the volume of the tumor. Among the therapies, anti TNFR2 upholds the best solo treatment which have shown cure rate on 10 among 18 (55%) mice by 20 to 21 days. The anti PD1 antibody has shown poor result by demonstrating 25% of the cure rate. The combination therapy with anti PD1 and anti TNFR2 antibody has outstand the other two solo therapy by showing 62% cure rate [74].

On the other side, in the scenario of MC38 infected mice, the solo therapies shown 10% and 20 % cure rate respectively. The combined therapy with the anti PD1 and anti TNFR2 was seen to be highly efficacious by showing 70% cure rate. By observing the FACS analysis, it is explained that the mechanism of action of the in vitro and in vivo methods were to kill the immunosuppressive Tregs in the tumor micro-environment and increase the ratio of CD8+ T effectors (Teffs) to Tregs. The researchers have found out that there was an effect on the outcomes because of the sequence of antibody delivery method. Among the therapies, the most effective sequences were simultaneous delivery which was at 70% cure rate followed by the anti TNFR2 antibody after anti PD1 with 40% cure rate. The less effective one was by anti PD1 after anti TNFR2 with 10% cure rate. The research concluded that the simultaneous administration of anti-PD-1 and anti-TNFR2 improves anti-PD-1 efficacy, and anti-TNFR2 alone might be a useful therapy for people who do not react to, or cannot tolerate, anti-PD-1 or other check point inhibitors [74].

TNFR2 antagonist antibodies against ovarian cancer and Sezary syndrome:

Two strong TNFR2 antagonistic antibodies with identical in vitro kinetics that inactivate human Tregs were discovered and produced by Torrey et al., and his team [69]. The antagonistic antibodies are even more effective than normal donors at inhibiting TNFR2 Tregs from ovarian cancer patients. These recently discovered TNFR2 antagonistic antibodies do not need Fc interaction, bind to the same area of the receptor, display dominance over TNF-

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mediated agonism, inhibit intracellular NF-kB activation and phosphorylation, which is required for TNFR2 signaling–mediated cell proliferation [69].

They established a model of dominant antagonism by these TNFR2 antibodies based on the binding and stabilization of a unique antiparallel dimeric conformation of surface TNFR2 that inhibits intracellular signaling, cannot bind TNF, cannot be cleaved to create soluble TNFR2, and has preferential potency against rapidly dividing cancer cells using linear and three-dimensional (3D) epitope mapping. TNFR2 antagonists, even at modest dosages, destroyed TNFR2-positive ovarian cancer cells in culture quickly. Dominant antagonism results in the formation of a novel nonsignaling complex from newly emerging surface TNFR2, which has therapeutic implications for TNF superfamily receptors, particularly TNFR2 [69].

Cancer Tregs, particularly those from tumor regions, are exceptionally effective immunosuppressors as compared to Tregs from cancer patients' peripheral blood or even control participants [27]. Fresh ovarian cancer Tregs from ascites fluid were extracted and compared to normal Tregs separated from a normal blood donor to begin to comprehend the effectiveness of TNFR2 antagonistic antibody on a tumor residing Treg. The ovarian cancer Tregs were tenfold more susceptible to TNFR2 antagonism than the Tregs from a normal blood donor when it came to TNFR2 antagonism. With TNFR2 antagonist, the trend of increased tumor microenvironment Treg mortality was highly repeatable. Tregs from ovarian cancer ascites were compared to Tregs from healthy donors in a pooled study. Even in short-term 48-hour experiments, killing of tumor-residing Tregs occurred at lower dosages and to a greater extent. In addition, if cancer Tregs are powerful and destroyed, the reciprocal Teff response should be significant as well. With TNFR2 antagonism, the Teffs of the cancer patient multiply more than the Teff of normal peripheral blood. They conclude that TNFR2 antagonists are selective for tumor microenvironmental Tregs [69].

In the study, the researchers wanted to know why the TNFR2 antagonistic antibodies were more selective and powerful on the cancer site Tregs. The structural biology studies imply that dominant TNFR2 antagonists bind to newly produced and membrane versions of TNFR2 on the cell surface, preventing increased Treg killing in the tumor microenvironment. There was either mitomycin C (50 g/ml) or no mitomycin C (50 g/ml) treatment of freshly isolated CD4 T cells before IL-2 with or without the TNFR2 agonist or antagonist antibodies. The studies suggest that mitomycin C inhibited cell division, which prevented TNFR2 antagonists from killing Treg cells. This lends credence to the idea that tumor microenvironment specificity is influenced in part by Treg proliferation at the tumor site and antagonist capture of only freshly produced TNFR2 proteins [69].

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Furthermore, TNFR2+CD26 cells and TNFR2+ Treg cells in Sezary syndrome patients was diminished by antagonistic antibodies while expanding Teff cells. Due to such characteristics, the Treg/Teff ratios was adjusted in the microenvironment of tumor. The research group has also developed numerous new versions of human TNFR2-specific antagonistic antibodies that kill TNFR2-expressing tumor cells and Tregs with excellent TME specificity. The hinge region (disulfide double mutations at C232S and C233S) was stabilized by the optimized anti-TNFR2 with IgG2 isoforms which shows higher TME specificity due to the wide separation of antibody arms [75].

Moreover, the TNFR2 expression pattern explained the efficiency of antagonistic treatments. The tumor cell line with high TNFR2 expression has more effective TNFR2 antagonistic killing activity than the tumor cell line with low TNFR2 expression.

M861:

M861 is an TNFR2 antagonist antibody which can limit the development of subcutaneously transplanted mice CT26 colon tumors by removing TNFR2+ Treg cells and boosting tumor infiltrating IFNy+CD8+ CTLs when a dosage of CpG oligodeoxynucleotide (CpG ODN) was paired sub-optimally [70]. Furthermore, in the mouse 4T1 breast cancer model, a combination of antagonist antibody of anti-TNFR2 and anti-CD25 antibody leads in an improved reduction of tumor development. Nie et al., and research team have performed research with CT26 cell lines where they have used M861 as a treatment and observed that it reduced TNF-induced proliferation and expansion of Treg cells in CD4+ T cells grown with interleukin-2 (IL-2) [70]. The cell surface abundance of TNFR2 on Treg cells were likewise inhibited by M861. Even though M861 did not decrease the quantity of Treg cells in the spleens and lymph nodes in LPS-challenged mice within 24 hours, the proportion of TNFR2+ Treg cells was significantly reduced by 64 percent, and the abundance of TNFR2 on splenic Treg cells was significantly reduced by >56 percent. Cell death was not the cause of the reduction in TNFR2+ Treg cells.M861 was not a Treg-depleting antibody, unlike two other antibodies that recognize human TNFR2 disclosed in a previous work [70].

They have treated female CT26 tumor-bearing Balb/c mice with M861 and CpG ODN or different controls to see if TNFR2 inhibition affected the effectiveness of tumor immunotherapy. When the tumor had grown to a diameter of 5 to 6 mm, treatment began (day 0). CpG ODNs were given as an intertumoral injection, which has previously been shown to have the best anticancer impact [76].

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To demonstrate the benefit of combination treatment, we combined M861 with a suboptimal dosage of CpG ODN, neither of which slowed tumor development much on its own .M861 and CpG ODN together effectively suppressed the development of primary CT26 tumors. Eighty percent of mice were tumor-free and lived to the conclusion of the trial, which was 60 days, whereas animals in other groups perished of tumor burden within 50 days after tumor injection. Individual tumor development curves differed; while a few animals showed delayed tumor growth when given PBS, CpG, or M861 alone, the anticancer impact of the M861 and CpG ODN combination was obvious. The surviving mice were reinoculated subcutaneously with CT26 tumor cells in their right flanks and 4T1 tumor cells in their left flanks to see if the tumor-free animals established long-term CT26 tumor-specific immunity. Both 4T1 tumor cells and CT26 tumor cells were implanted into naive mice in the same way as controls, and both tumors grew in all naive animals, as predicted. By day 26 following inoculation, all CT26 (intertumoral)–surviving animals had established quantifiable 4T1 tumors, but none of these mice had produced CT26 colon tumors. These findings suggest that a combination of M861 and CpG ODN therapy resulted in the formation of long-term tumor antigen-specific immunity. During analyzing the immune system, the scientist has also observed that the combination therapy elevated the production of IFN-*y* by CD8+ CTLs, and the proportion of IFN-*y* producing CD8+ T cells was greater than threefold as compared with CpG ODN treatment alone.

Clinically administrated anti TNFR2 agonists:

The ex vivo Treg proliferative growth, phenotypic stability, and suppressive activity have all been found to be enhanced by TNFR2 agonists [54]. As a result, using a "TNFR2 agonist" to restore Treg functional activity or expand the number of Tregs provides a justification for treating graft versus host disease (GvHD), neurodegenerative illness, Type 1 diabetes (T1D), and other autoimmune disorders. [77,78-82]. In separate humanized mouse models, Moreover, agonistic TNFR2 antibodies had substantial anti-tumor action [83-87]. Furthermore, in the WEHI-164 model, the Y9 therapy alone elicits more robust anti-tumor activity than anti-PD-1 treatment alone. On the contrary, the monotherapy of both (Y9+anti-PD-1) elicited higher survival rate in the CT26 and EMT6 syngeneic mice tumor models (Figure 2) [83]. TNFR2 expression on tumor cells or Treg cell depletion did not seem to affect the action of Y9 [83,84]. In cancer immunotherapy, anti TNFR2 agonists has demonstrated successful outcomes in clinical trials (Table 2).

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Table 2: Anti TNFR2 agonist antibodies experimented in clinical trials.

Anti TNFR2 agonists	Types of Condition	Outcomes of the experiment
MM-401	Ovarian Cancer ascites	• Stimulates TNFR2 signaling • When incubated with CD4+ and CD8+ T cells from healthy human blood, the production of activation markers and cytokines were elevated. • Enhances ADCC and reduce Tregs (in NF-kB in vitro experiment [87]
Y9(Anti mouse TNFR2 agonist antibody)	Numerous syngeneic tumor models of mice	• Decreased the surface expression TNFR2 on tumor infiltrating CD^{+8} and CD^{+4} T cells • Treg proliferation was not observed • Soluble TNFR2 was increased • tumor antigen specific IFNγ+ CD8+ CTLs was improved after drug administration, and it was also expanded • WEHI-164, MC-38, EMT6, CT26, Sa1/N, and MBT-2 showed complete response to Y9 • Three models such as WEHI-164, CT26, and EMT6 showed improved results by combined therapy with anti PD-1. • Improved toxicity profile than anti CTLA-4 [83]
TR75-54.7 and TR75-89	Mouse colon cancer (CT26)	• Bind to recombinant mouse FcγRII and FcγRIII • Improved anti-tumor activity • Survival rates of the mouse were increased • Two mice with anti-TNFR2 mAb TR75-54.7 showed complete tumor regression • Three mice with TR75-89 showed complete tumor regression [88]

MM-401:

It is a humanized agonistic anti-TNFR2 antibody that induced antibody-dependent cellular cytotoxicity in vitro to boost anti-tumor immunity (ADCC) [85-57]. The quantity of Treg cells in human ovarian cancer ascites has been observed to decrease following treatment with MM-401. [85,87]. Future research can reconcile these seemingly opposing effects because TNFR2 agonists appear to have both anti-tumor and anti-inflammatory properties.

MM-401 was created via CDR grafting from a mouse hybridoma antibody and has other modifications that boost affinity and physicochemical characteristics. As a result, MM-401 binds to a region of human TNFR2 that matches to the mouse TNFR2 epitope of our mouse

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surrogate antibody with low nanomolar affinity. Even though the antibody competes with TNF for receptor binding, MM-401 possesses agonistic action and stimulates TNFR2 signaling, as demonstrated by an NF-kB reporter cell experiment. A study by Sampson et al, observed elevation of activation markers and cytokine production when MM-401 was incubated with CD4+ and CD8+ T cells from healthy human blood, like utomilumab (anti-4-1BB), MEDI6469 (anti-OX40), and TRX518 (anti-GITR) [87]. In an NK cell-mediated in vitro experiment, MM-401 enhances antibody-dependent cellular cytotoxicity (ADCC), as well as a reduction in the number of regulatory T (Treg) cells in ovarian cancer ascites samples. These findings imply that MM-401 may also boost anti-tumor immunity by mediating ADCC and co-stimulating T cell responses directly.

MM-401 is now being tested in humanized mice created utilizing NSG-SGM3 mice with cord blood CD34+ hematopoietic stem cells in patient derived xenograft (PDX) models. These findings support the further development of MM-401 as an anti-tumor immunity modulator for cancer therapy.

TR75-54.7 and TR75-89:

Another research by Williams et al., has experimented TR75-4.7 and TR75-89 (two anti-mouse TNFR2 agonist) on CT26 cell line (mice colorectal cancer). When cross-linked in vitro, clone TR75-54.7 hamster anti-mouse TNFR2 mAb was found to compete with TNF-α and operate as a TNFR2 agonist, as measured by CT26 T cell proliferation [89]. Using bio-layer interferometry and a cell-based NF-kB reporter system, TR75-54.7 was found to be a TNF-α competitive TNFR2 agonist, showing that this mAb is a viable surrogate for the TNFR2-binding agonist DARPins. Another anti-TNFR2 mAb (clone TR75-89), which is likewise a TNFR2 agonist but does not compete with TNF-α, was studied. Because of cross-linking by FcγR-expressing cells, monoclonal antibodies targeting TNFRSF members can now be used as agonists in pre-clinical trials [90-92]. TR75-54.7 and TR75-89 were shown to bind to recombinant mouse FcγRII and FcγRIII, but no interaction with FcγRI or FcγRIV was identified, indicating that these mAbs can be cross-linked by a subset of mice FcγRs but should not be anticipated to promote antibody-dependent cellular phagocytosis (ADCP).

TNFR2 mAbs decreased the development of CT26 tumors in immunocompetent mouse models when contrasted to control animals that undergone saline or hamster IgG control mAbs. Mice receiving TR75-54.7 or TR75-89 had a median survival of 36 and 30.5 days after implantation, respectively, compared to animals who was receiving saline or hamster IgG control mAbs for 22 to 25 days. About 90% of overall anti-TNFR2 mAbs exposure occurred within ten days of

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the initial dosage relying on the serum half-lives for two days [85]. As a result, the suppression length of tumor growth and improved survival were comparable to TNFR2 agonist exposure. Two of the thirty-four mice treated with TR75-54.7 (anti-TNFR2 mAb) and three of the thirty-four mice treated with TR75-89 showed complete tumor remission. There was no development of tumor when the TR75-54.7 and TR75-89 treated mice were re-challenged with CT26 cells line's thirty days following tumor regression. The untreated control mice were transplanted with CT26 cells previously though they developed normally later. This suggests that TNFR2 mAbs caused CT26 tumor cells to develop long-term immunological memory.

Y9(Anti-mouse TNFR2 agonist antibody):

Tam et al., and his colleagues have discovered five novel anti TNFR2 antibodies named Y9, M3, Y7, Y10, and H5L10. Among the five novel antibodies Y9 has shown higher binding affinity which made it to out-stand other antibodies. To evaluate the efficiency of Y9 , researchers has applied it to eight synergistic tumor model. All the mouse tumor model were treated with 300 mg. They found out positive responses in five out of eight tumor models, along with four mice models showing complete responses. The tumor sizes were reduced to $60mm^3$ and it continued to regress till the end of the treatment [83].

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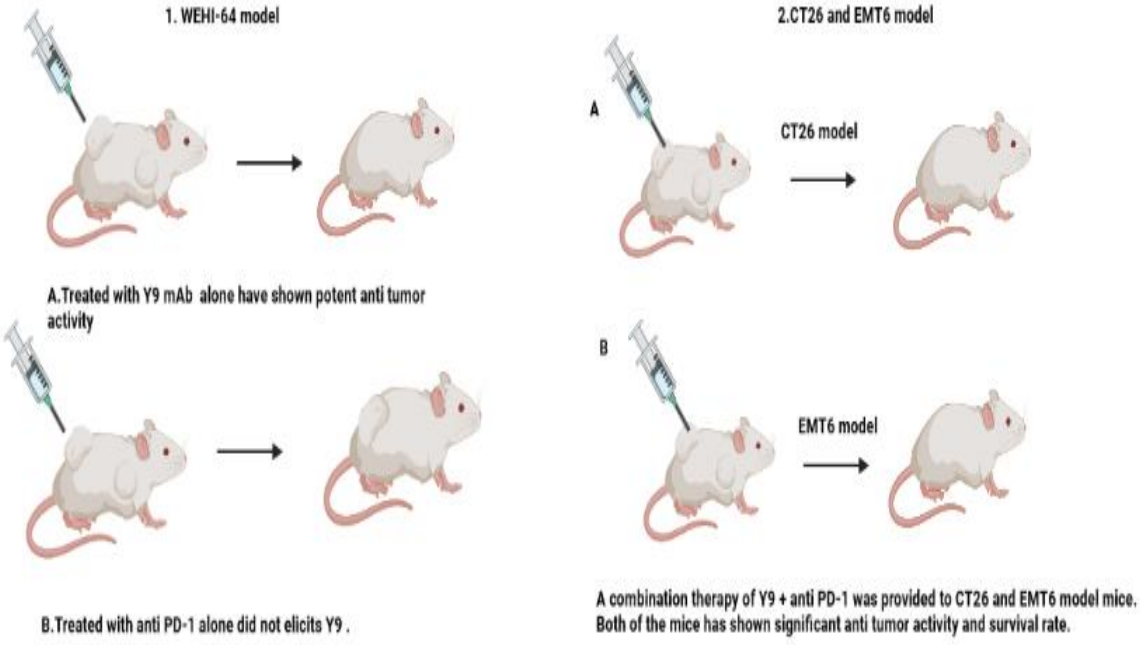

Figure 2: A hypothetical overview of the effect of combination therapy (Y9+anti PD-1) in WEHI-64, CT26 and EMT6 model mice.

Later on the researchers experimented the combined therapy with Y9 and anti PD-1.The hamster anti-mouse PD-1 antibody J43 was developed by the researchers into a murine form [93].Anti-PD-1–sensitive (Sa1/N) and anti–PD-1–resistant (MBT-2) syngeneic mice models were used to evaluate both antibodies.Y9 therapy alone resulted in CR in all treated animals in both scenarios. They investigated the therapy combination for activity in three syngeneic mice models to see if treatment with Y9 may improve the response to anti–PD-1. The alone treatment of Y9 anti–PD-1had equivalent anti-cancer activity in the WEHI-164 model, and the combination did not increase the antitumor activity. In the CT26 and EMT6 models, however, the combination of Y9 and anti–PD-1 therapy resulted in a higher rate of survival.

They also created a mouse version of the programmed death-ligand 1 (PD-L1) antibody MPDL3280a [94] and found that the results were comparable in all three animals. As a result,

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anti-TNFR2 has anticancer activity that is comparable to, and in some cases better than, anti–PD-1 or anti–PD-L1, and it may be used in conjunction with anti–PD-1 or anti–PD-L1 for improved antitumor activity in tumors that are less immunogenic.

The other mouse models B16-F10, A20, LLC1, and NR: 4T1 did not show any significant changes like CT26, EMT6 and WEHI-164. Anti TNFR2 agonist Y9 has expanded and improved the tumor antigen-specific IFNγ^+ CD8$^+$ CTLs during the research and also has the ability to reduce the expression of TNFR2 on the surface of tumor-infiltrating CD8$^+$ T cells and CD4$^+$ T cells.

Tam and his colleagues have also compared the toxicity of anti CTLA-1 with Y9.Due to documented immune-related adverse effects in the clinic, they used an anti–CTLA-4 antibody with a mIgG2a-Fc as a positive control and comparator [95,96]. After that, they conducted a long-term exposure research in healthy 6- to 8-week-old BALB/c and C57BL/6 female mice, comparing weekly injections (1 mg) for both antibodies. For the first 6 weeks of therapy, there was no change in weight between groups in BALB/c mice; however, following the seventh dosage, substantial weight loss in the anti–CTLA-4 group was seen ($P< 0.001$). Splenomegaly was only found in mice treated with anti–CTLA-4 at the study's conclusion. Only the anti–CTLA-4 group showed significant increases in blood alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ($PALT< 0.001$; $PAST< 0.01$;), while all other groups were within normal limits.

They also studied peripheral blood T cells and dendritic cells (DCs) from skin-draining lymph nodes to see how therapy affected immune cell phenotype. The frequency of acutely activated (PD-1+) and proliferating (Ki-67+) CD4 + and CD8+ T cells increased in mice treated with anti–CTLA-4 compared to isotype controls. Anti-TNFR2 did not produce spontaneous activation and proliferation of peripheral T cells, unlike anti–CTLA-4. Animals treated with Y9 exhibited no increase in T cell proliferation or acute activation markers. Furthermore, anti–CTLA-4 increased the expression of the costimulatory ligand CD86 on DCs, but Y9 did not. Importantly, Y9 had no effect on lymphoid or non-lymphoid tissues histologically, whereas anti–CTLA-4 produced broad immune cell infiltration. Anti–CTLA-4 induced a chronic rise in blood IFN-g, TNF, IL-6, IL-5, and IL-10, whereas Y9 generated a mild and transitory increase in serum TNF and interleukin-6 (IL-6). In the EMT6 tumor model, the anti–CTLA-4 antibody had similar side effects, but mice treated with Y9 showed no symptoms of toxicity. The scientists also found that Y9 in conjunction with anti–PD-1 did not cause spontaneous T cell activation, but anti–PD-1 in combination with anti–CTLA-4 did. Overall, their findings

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suggest that in healthy and tumor-bearing mice, the anti-TNFR2 antibody Y9 does not cause spontaneous immune cell activation.

TNFR2 blocking agents other than potential antagonist and agonists:

We have already known that a number of antagonist and agonist has been develop against TNFR2. Other than anti TNFR2 antagonist and agonist there has some pharmaceutical agents which are able to regulate the expression of TNF. In the following table, the biological agents are listed:

Table 3: Anti TNFR2 blockade agents and their characteristics.

Biological Agents	Types of Cancer or Condition	Features
1.Infliximab (Anti TNFR2 Blockade)	Melanoma with severe colitis treated with immune checkpoint inhibitors.	Cured severe colitis without melanoma prognosis [97].
2.Azacitidine and lenalidomide (thalidomide derivatives)	Acute myeloid leukemia	Reduce TNFR2 expression on T cells as well as TNFR2+ Treg in vivo, leading to enhanced effector immune function [98]
3.Cyclophosphamide (DNA alkylating agent)	Mouse Tumor Model	It is shown that cyclophosphamide treatment depletes TNFR2+ Treg via inducing the death of replicating Treg that co-express TNFR2 and KI-67 [100]
4.Thalidomide and fludarabine	Chronic lymphocytic leukemia (CLL).	Decreased the number of CLL and Treg cells simultaneously [95].
5.Azacitidine + Panobinostat	Acute myeloid leukemia	It can decrease $TNFR2^{+}$ Treg cells in the peripheral blood and bone marrow of LAML patients. There is no change in $TNFR2^{-}$ Treg cells. The combined therapy increases in cytokines [IL-2 and IFNγ] production by effector T cells. Panobinostat repressed the expression of TNFR2 on Treg cells. [50,98] .
6.Triptolide	Melanoma, Colitis	It decreased the TNF and TNF2 expression in the colon of colitis mice. It has been also seen to reduce Tregs proliferation and deduct the growth of tumor in melanoma bearing mice [101,102].

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Conclusion:

The role of TNFR2 in promoting cell survival and proliferation is well recognized. Such property of TNFR2 is likely appropriate to tumor cells that express it [103-106]. This gives a compelling reason to conduct more research into TNFR2 antagonists as a cancer therapy. TNFR2 is expressed at greater levels in a broad variety of tumor cells than in comparable normal tissues. TNFR2 is regulated at significantly lower amounts in certain cancers than in normal tissues. If the TNFR2-targeting therapy is meant to act promptly on tumors, the amounts of TNFR2 expressed by tumors should be addressed in the strategy of future clinical trials. Moreover, the elevated levels of TNFR2 expression in tumor cells might be used as a biomarker for TNFR2-targeting treatment, which should be investigated further in future research.

TNFR2 being the most abundant in the TME among other Treg surface receptors. TNFR2 is an efficient receptor on T regulatory cells. Its expression on the cell surface not only identifies powerful Treg subsets, but it is also a feature of tumor infiltrating Treg. On some cancer infiltrating Treg, TNFR2 expression is 100 times greater than on circulating Treg in control people. In various cancers, the number of TNFR2+ Treg in the peripheral blood is greater than in healthy people. The use of small molecule agonists or antagonists to target TNFR2 is a promising but difficult endeavor. Given Treg's suppressive properties and decreased activities in many immune-pathologies, innovative (tumor-specific) antagonists targeting TNFR2 are unquestionably promise for cancer immunotherapy. Combining TNFR2 inhibition with immune checkpoint inhibitors appears to be an appealing method in redefining contemporary cancer immunotherapy from a therapeutic standpoint. A scientific underpinning for the development of TNFR2-targeting drugs is the preferential removal of TNFR2hi Tregs in TME. Since only 30-40% of peripheral Tregs in normal mice are TNFR2-expressing cells, whereas the majority of tumor-infiltrating Tregs in mice are TNFR2hi cells; thus, inactivation or even depletion of TNFR2-expressing Tregs would not compromise peripheral tolerance to self-antigen. However, because human Tregs in the periphery express TNFR2 more extensively than mouse Tregs, the danger of triggering autoimmune inflammatory responses by TNFR2-targeting medication in human patients should be explored in the following research. TNFR2 agonistic antibodies, on the other hand, have been shown in multiple recent investigations to decrease autoimmune inflammatory responses by boosting the activation and expansion of Tregs [63,105]. As a result, using TNFR2 agonistic antibodies in cancer treatment may increase Treg activity and lead to greater immune suppression. In the future, this possibility should also be carefully considered.

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Given these problems to solve, existing pre-clinical evidence encourages and supports the notion that targeting TNFR2 with antagonist or agonist with antibodies or TNF eliminating agents might be a new route and creative method in cancer immunotherapy.

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