TNFR2 antagonist and agonist: a potential therapeutics in cancer immunotherapy

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Abstract
Tumour necrosis factor receptor 2 or TNFR2 is considered an appealing target protein due to its limited frequency to TREGs, which are highly immunosuppressive and present on human malignancies. Numerous studies have revealed that TNFR2 is primarily found on MDSCs (myeloid-derived suppressor cells) and CD + Foxp3 + regulatory T cells (TREGs). Therefore, it has great importance in the proliferation and functional activity of TREGs and MDSCs. TNFR2 suppression must be downregulated or upregulated as required to treat malignancies and diseases like autoimmune disorders. Therefore, at the molecular level, advances in the comprehension of TNFR2's complex structure and its binding to TNF have opened the door to structure-guided drug development. Two critical obstacles to cancer treatment are the dearth of TREG-specific inhibitors and the lack of widely applicable ways to target tumours via frequently expressed surface oncogenes directly. Many researchers have discovered potential antagonists and agonists of TNFR2, which were successful in inhibiting TREGs proliferation, reducing soluble TNFR2 secretion from normal cells, and expanding T effector cells. The data represented in the following review article elucidates the clinically administrated TNFR2 antagonist and agonist in treating cancers.

Keywords TREG · Antagonist · Agonist · MDSCs · CD + Foxp3 +

Introduction
Cancer treatment was utterly dependent on conventional therapies such as chemotherapy and radiation a few years ago. The traditional treatment of cancer was expensive to afford, but it also had severe adverse effects that were intolerable to patients. The invention of immunotherapy has signified a revolutionary change in cancer treatment. Applying gene therapy or immune checkpoint inhibitors has significantly advanced cancer treatment. However, their effectiveness and recurrence rates are unpredictable, and there is a chance that they can cause autoimmunity. Ipilimumab and nivolumab/pembrolizumab are immune checkpoint inhibitors which target CTLA-4 and PD-1, respectively. After successful clinical studies, this has become a critical therapeutic in advanced non-small cell lung cancer (NSCLC) and melanoma [1, 2].

TNF or tumour necrosis factor was initially termed after it was observed in a study that the introduction of bacterial endotoxin to mice led to the production of a serological protein with necrotic anti-tumour effect at high quantities. Tumour necrosis factor is a milestone in cancer immunotherapy [3]. TNF is a pleiotropic cytokine that acts as a principal part of the inflammatory and immune responses by two different receptors: TNFR1 and TNFR2. TNFR1 is also called p55 and TNFRSF1A, whereas TNFRII is also known as p75 and TNFRs1B. When TNFR1 is compared to TNFR2, TNFR1 is expressive primarily in all cells; it activates the nuclear factor kappa B (NF-kB) [4] and induces death in cells. At the same time, 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 expressed predominantly on the human and mouse CD4 + Foxp3 + regulatory T cells, also known as TREGs [6], which are generally immunosuppressive in mammals.
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-κB 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 tumour necrosis factor receptor 2 (TNFR2) is a well-known subtype. TREGs that express the TNFR2 gene in both mice and humans are potent immune suppressors that are excessively numerous in human and murine malignancies [11, 21–26].

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 tumour cells [27–32]. The signalling of nuclear factor B (NF-κB) is activated by TNFR2, resulting in constitutive downstream agonism and increased cell proliferation. It would be ideal if a single strategy could successfully suppress TREGs while directly inhibiting tumour development via the TNFR2 oncogene. T effector cells (Teffs) would ideally be able to grow and destroy the tumour using this way.

TNFR2-positive TREGs are common in the tumour microenvironment of several human and murine malignancies. In cancers, TNFR2 gene duplication and activating mutations have been discovered [28]. According to a study based on baboons, the known toxicity of high-dose TNF is entirely mediated by TNFR1, not TNFR2 [33, 34]. In naturally occurring TREGs in human blood, TNFR2 is found at a tenfold 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 [6, 34–44].

Several TNFR2-positive TREGs at the tumour site or in the circulation are signs of tumour 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 cancer cells [26, 28, 45, 46]. The total quantity of TNFR2-positive TREGs in the tumour 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 [26].

TNFR2 contains distinct characteristics. In suppressive T cell types, it acts as an immune system marker. It is also considered a master switch for TREG survival and fate and a newly invented highly expressed oncogene [47]. The most suppressive TREGs might express excessive quantities of TNFR2 (TNFR2hi TREGs) and exert significant immunosuppressive effects [26] in several cancers. In contrast, in patients with acute myeloid leukaemia [48] and lung cancer, lower TNFR2-positive TREGs correspond with better clinical outcomes [49]. TNFR2 can be expressed abnormally as a growth receptor oncogene on the tumour in colon cancer, multiple myeloma, Hodgkin’s lymphoma, ovarian cancer, and cutaneous T cell lymphomas [27–31, 50]. 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 [28]. TNFR2 is an attractive molecular target for TREG inactivation in the tumour microenvironment and direct tumour targeting as an aberrant surface oncogene. TNFRI expression does not differ between TREG and non-TREG cells; however, human TREG expresses higher amounts of TNFR2 than CD25+ T-conv cells. Furthermore, TNFR2+ TREG has the most suppressive ability [6, 11]. TNF’s impact on TREG suppressor function is still up for debate.

In mice and humans, sTNF retained or even boosted FOXP3 expression and TREG suppressive capability [12, 17, 47, 51]. 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 the in vitro experiment [52].

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 [52, 53]. In clinical trials, RA patients who received the
anti-TNF antibody adalimumab had more FOXP3 + cells, and their regulatory function was restored [54]. It is worth noting that the TNFR2 antibodies utilized in these investigations were most likely different (agonistic vs antagonistic) [47]. Recent research suggests that TNFR2 antagonisms and agonisms may affect TREG phenotype and suppressor activity in diverse ways [47].

TNF priming causes TREG proliferation and activation both in vitro [17, 55] and in an acute mouse GvHD model via TNFR2 in clinical trials [15]. The activation of human TREG with a TNFR2-agonist antibody sustained a stable TREG phenotype and function following ex vivo growth [52]. The use of a TNFR2 agonist alone was enough to prevent the loss of FOXP3 expression. In contrast, 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 TNF knockout CD4 + T cells are reliant on the impairment of TNFR2-mediated NFkB activation [56].

Researchers hypothesize that a similar regulatory pathway exists in human TREG, in which TNFR2/NF-kB signalling 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 [55], a polycomb repressor complex with two components (PRC2). In addition, EZH2 is reported to bind to FOXP3, assisting FOXP3 in gene transcriptional repression regulation [57].

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 [47, 52, 58]. 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 all-HCT, as well as prolong longevity without compromising anti-leukaemia or anti-infective benefits [13]. These studies have revealed the therapeutic potential of new TNFR2-targeting drugs in the treatment of autoimmune and inflammatory illnesses. 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, and inflammatory bowel disease, and autoimmune liver disease is linked to TREG dysfunction [59]. 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 [60].

The accumulation of TNFR2-expressing TREGs in the tumour microenvironment is thought to be a fundamental biological mechanism of tumour immune evasion. Most tumour-infiltrating TREGs show extensive surface TNFR2 expression and are highly immunosuppressive in Lewis lung carcinoma mice and the 4T1 breast tumour model [6, 61]. The percentage of TNFR2 + TREGs in the peripheral blood or tumour-associated ascites is higher in lung and ovarian cancer patients [62, 63]. 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 [26]. Furthermore, it was observed that TNFR2 expression on TREGs is linked to increased lymphovascular invasion, a higher chance of malignancies, a higher clinical stage, and a worse response to therapy in individuals suffering from acute myeloid myeloma and lung cancer [61, 62, 64]. 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 [30]. In a mouse colon cancer model, our team discovered that using a TNFR2-blocking antibody significantly improved the efficiency of immunotherapy using CpG. [65]. This combination treatment reduced TNFR2 expression on tumour-infiltrating TREGs and increased tumour infiltration of interferon-gamma-producing CD8 + CTLs [66]. As a result, new TNFR2 antagonists might be used as cancer immunotherapy drugs.

The tumour microenvironment preferentially attracts TNFR2 + TREG cells, which have full immunosuppressive potential, making tumour immune escape easier. The absence of TNFR2 expressing TREG or the failure to establish systemic autoimmunity [44], 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 in Fig. 1:
TNFR2 antagonist in cancers

TNFR2 is an oncogene that has been found in at least 25 tumour 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 tumour itself [67]. Overexpression of the cytokine receptor TNFR2 leads to enhanced tumour cell proliferation and tumour 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 tumour cells and TREG is a hallmark of SS.

Gain-of-function mutations in TNFR2 increase non-canonical NF-κB activation [28], essential 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 tumour development. Such TNFR2-specific blocking agents would ideally limit TREG while allowing Tconv growth and activity, allowing antitumour immune responses to be restored and tumour regression. There are several TNFR2 recognizing antagonists and agonists that have shown positive outcomes against various cancers. In the following Table 1, the clinically administered antibody antagonists of TNFR2 are described below:

**APX601**

APX601 is recognized as a potential TNFR2 antagonist antibody which can reverse the immune suppression and cause tumour cell apoptosis by targeting TNFR2-expressing TREG and MDSC. In addition, research finding by Filbert et al. suggests 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 tumours [66]. Filbert and his colleagues have created antibodies in a broad spectrum for TNFR2 by using Apexigen’s proprietary rabbit monoclonal antibody technology or APXiMABTM.
APX601, a humanized IgG1 antibody, was chosen as the primary treatment candidate after thoroughly evaluating 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 tumour cells was examined both in vitro and in vivo [68].

The clinical experiments demonstrate 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 potent antagonist that inhibits the TNFR2-TNFα interaction (IC50 = 0.149 nM). It has two ways of restoring immune suppression: 1) 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 tumour cells via antibody-dependent cell cytotoxicity (EC50 = 1.14 nM) and ADCP (EC50 = 0.71 nM) effector functions [68].

TY101

TY101 is an anti-mouse TNFR2 antibody antagonist experimented in mice colorectal cancer cell lines CT26 and MC38. A study by Case et al. performed a pre-clinical trial on a murine model with colon cancer. They have the following three regimens in the experiment [69]. In the murine colon cancer CT26 and MC38 models, the researchers have tested the effectiveness of TY101 alone or with a combination of anti PD1 therapy. The research was mainly based on mono-therapy. The mice infected with cancer cells were treated with anti-PD-1 alone, anti TNFR2 alone, and the combination of PD1 and TNFR2.

The treatment with CT26 infected mice was started when the tumour was grown up to the threshold of the volume of 100-200mm3. Four different therapies with doses of 100 μg of sterile antibody were administrated for 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 had given effective results by lowering the tumour volume. Among
the therapies, anti TNFR2 upholds the best solo treatment, showing a cure rate of 10 among 18 (55%) mice by 20 to 21 days. On the other hand, the anti PD1 antibody has shown poor results by demonstrating a 25% cure rate. Finally, the combination therapy with anti PD1 and anti-TNFR2 antibodies has to outstand the other two solo therapy by showing a 62% cure rate [69].

On the other side, in the scenario of MC38 infected mice, the solo therapies showed 10% and 20% cure rates, respectively. The combined therapy with the anti PD1 and anti TNFR2 was highly efficacious by showing a 70% cure rate. Observing the FACS analysis explains that the mechanism of action of the in vitro and in vivo methods was to kill the immunosuppressive TREGs in the tumour 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 at a 70% cure rate, followed by the anti-TNFR2 antibody after anti PD1 with a 40% cure rate. The less effective one was anti PD1 after TNFR2 with a 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 helpful therapy for people who do not react to, or cannot tolerate, anti-PD-1, or other checkpoint inhibitors [69].

**TNFR2 antagonist antibodies against ovarian cancer and Sezary syndrome**

Two potent TNFR2 antagonistic antibodies with identical in vitro kinetics that inactivate human TREGs were discovered and produced by Torrey et al. and his team [65]. The antagonistic antibodies are even more effective than regular donors in inhibiting TNFR2 TREGs from ovarian cancer patients. Furthermore, these recently discovered TNFR2 antagonistic antibodies do not need Fc interaction, bind to the same area of the receptor, display dominance over TNF-mediated agonism, and inhibits intracellular NF-kB activation and phosphorylation, which is required for TNFR2 signaling-mediated cell proliferation [65].

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 forms a novel non-signaling complex from newly emerging surface TNFR2, which has therapeutic implications for TNF superfamily receptors, particularly TNFR2 [65].

Cancer TREGs, particularly those from tumour regions, are exceptionally effective immunosuppressors compared to TREGs from cancer patients' peripheral blood or even control participants [26]. Fresh ovarian cancer TREGs from ascites fluid were extracted and compared to normal TREGs separated from a regular blood donor to begin to comprehend the effectiveness of TNFR2 antagonistic antibody on a tumour residing TREG. The ovarian cancer TREGs were tenfold more susceptible to TNFR2 antagonism than the TREGs from a regular blood donor when it came to TNFR2 antagonism. With TNFR2 antagonist, increased tumour microenvironment TREG mortality trend was highly repeatable. In a pooled study, TREGs from ovarian cancer ascites were compared to TREGs from healthy donors. Even in short-term 48-h experiments, the killing of tumour 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 also be significant. 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 tumour microenvironmental TREGs [65].

The researchers wanted to know why the TNFR2 antagonistic antibodies were more selective and potent on TREGs' cancer site. 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 tumour 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. Such invention lends credence to the idea that tumour microenvironment specificity is partly influenced by TREG proliferation at the tumour site and antagonist capture of only freshly produced TNFR2 proteins [65].

Furthermore, TNFR2 + CD26 cells and TNFR2 + TREG cells in Sezary syndrome patients were diminished by antagonistic antibodies expanding Teff cells. Due to such characteristics, the TREG/Teff ratios were adjusted in the microenvironment of a tumour. The research group has also developed numerous new versions of human TNFR2-specific antagonistic antibodies that kill TNFR2-expressing tumour cells and TREGs with excellent TME specificity. In addition, 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 [70].
Moreover, the TNFR2 expression pattern explained the efficiency of antagonistic treatments. The tumour cell line with high TNFR2 expression has more effective TNFR2 antagonistic killing activity than the tumour cell line with low TNFR2 expression.

**M861**

M861 is a TNFR2 antagonist antibody which can limit the development of subcutaneously transplanted mice CT26 colon tumours by removing TNFR2+ TREG cells and boosting tumour infiltrating IFNγ+ CD8+ CTLs when a dosage of CpG oligodeoxynucleotide (CpG ODN) was paired sub-optimally [66]. Furthermore, in the mouse 4T1 breast cancer model, a combination of antagonist antibodies of anti-TNFR2 and anti-CD25 antibodies leads to an improved reduction of tumour development. Nie et al. and the 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) [66]. The cell surface abundance of TNFR2 on TREG cells was 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 h, the proportion of TNFR2+ TREG cells was significantly reduced by 64 per cent, and the abundance of TNFR2 on splenic TREG cells was significantly reduced by >56 per cent. 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 previous work [66].

They have treated female CT26 tumour-bearing Balb/c mice with M861 and CpG ODN or different controls to see if TNFR2 inhibition affected the effectiveness of tumour immunotherapy. When the tumour had grown to 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 [71].

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

**Clinically administrated anti-TNFR2 agonists**

The ex vivo TREG proliferative growth, phenotypic stability, and suppressive activity have been enhanced by TNFR2 agonists [52]. As a result, using a “TNFR2 agonist” to restore TREG functional activity or expand the number of TREGs justifies treating graft versus host disease (GvHD), neurodegenerative illness, and Type 1 diabetes (T1D), and other autoimmune disorders. [72–77]. In separate humanized mouse models, agonistic TNFR2 antibodies had substantial anti-tumour action [78–82]. Furthermore, in the WEHI-164 model, the Y9 therapy elicits more robust anti-tumour activity than anti-PD-1 treatment alone. On the contrary, the monotherapy of both (Y9 + anti-PD-1) elicited a higher survival rate in the CT26 and EMT6 syngeneic mice tumour models (Fig. 2) [78]. TNFR2 expression on tumour cells or TREG cell depletion did not seem to affect the action of Y9 [78, 79]. In cancer immunotherapy, anti-TNFR2 agonists have demonstrated successful outcomes in clinical trials (Table 2 and 3).

**MM-401**

It is a humanized agonistic anti-TNFR2 antibody that induces antibody-dependent cellular cytotoxicity in vitro to boost anti-tumour immunity (ADCC) [80, 82]. The quantity of TREG cells in human ovarian cancer ascites has decreased following treatment with MM-401. [80, 82]. Future research can reconcile these seemingly opposing effects because TNFR2 agonists appear to have anti-tumour and anti-inflammatory properties.

MM-401 was created via CDR grafting from a mouse hybridoma antibody and had other modifications that boosted affinity and physicochemical characteristics. As a result, MM-401 binds to a region of human TNFR2 that
matches the mouse TNFR2 epitope of our mouse 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) [82]. 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.

Table 2 Anti-TNFR2 agonist antibodies experimented in clinical trials

| Anti-TNFR2 agonist | 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) [82]. |
| Y9(Anti mouse TNFR2 agonist antibody) | Numerous syngeneic tumour models of mice | Decreased the surface expression TNFR2 on tumour infiltrating CD8+ and CD14+ T cells. TREG proliferation was not observed. Soluble TNFR2 was increased. Tumour 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 [78]. |
| TR75-54.7 and TR75-89 | Mouse colon cancer (CT26) | Bind to recombinant mouse FcYRII and FcYRIII. Improved anti-tumour activity. Survival rates of the mouse were increased. Two mice with anti-TNFR2 mAb TR75-54.7 showed complete tumour regression. Three mice with TR75-89 showed complete tumour regression [83]. |

![Fig. 2](image-url) A hypothetical overview of the effect of combination therapy (Y9 + anti PD-1) in WEHI-64, CT26, and EMT6 model mice.
samples. These findings imply that MM-401 may also boost anti-tumour immunity by directly mediating ADCC and directly co-stimulating T cell responses.

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-tumour immunity modulator for cancer therapy.

### TR75-54.7 and TR75-89

Another research by Williams et al. has experimented with TR75-4.7 and TR75-89 (two anti-mouse TNFR2 agonists) on the 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 [84]. Using bio-layer interferometry and a cell-based NF-kB reporter system, TR75-54.7 was a TNF-α competitive TNFR2 agonist, showing that this mAb is a viable surrogate for the TNFR2-binding agonist DARPin. Another anti-TNFR2 mAb (clone TR75-89), a TNFR2 agonist that does not compete with TNF-α, was studied. Because of cross-linking by FcyR-expressing cells, monoclonal antibodies targeting TNFRSF members can now be used as agonists in pre-clinical trials [85–87]. TR75-54.7 and TR75-89 were shown to bind to recombinant mouse FcγRII and FcγRIII. However, 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 tumours in immunocompetent mouse models when contrasted with 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 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 the initial dosage, relying on the serum half-lives for two days [80]. As a result, the suppression length of tumour 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 those treated with TR75-89 showed complete tumour remission. There was no tumour development when the TR75-54.7 and TR75-89 treated mice were re-challenged with CT26 cells line is thirty days following tumour regression. The untreated control mice were transplanted with CT26 cells previously thought they normally developed later.

### Table 3 Anti-TNFR2 blockade agents and their characteristics

| Biological agents | Types of cancer or condition | Features |
|-------------------|-----------------------------|----------|
| Infliximab (Anti-TNFR2 Blockade) | Melanoma with severe colitis treated with immune checkpoint inhibitors | Cured severe colitis without melanoma prognosis [92] |
| 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 [93, 94] |
| Cyclophosphamide (DNA alkylating agent) | Mouse Tumour Model | It is shown that cyclophosphamide treatment depletes TNFR2+ TREG via inducing the death of replicating TREG that co-express TNFR2 and KI-67 [95] |
| Thalidomide and fludarabine | Chronic lymphocytic leukemia (CLL) | Decreased the number of CLL and TREG cells simultaneously [90] |
| 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. [48, 93, 94] |
| 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 tumour in melanoma bearing mice [96, 97] |

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Thus it suggests that TNFR2 mAbs caused CT26 tumour cells to develop long-term immunological memory.

**Y9(Anti-mouse TNFR2 agonist antibody)**

Tam et al. and their colleagues have discovered five novel anti-TNFR2 antibodies named Y9, M3, Y7, Y10, and H5L10. Y9 has shown higher binding affinity among the five novel antibodies, which made it stand out among others. To evaluate the efficiency of Y9, researchers have applied it to eight synergistic tumour models. All the mouse tumour models were treated with 300 mg. They found positive responses in five out of eight tumour models and four mice models showing complete responses. However, the tumour sizes were reduced to 60mm3, and they continued to regress till the end of the treatment [78].

Later on, the researchers experimented with the combined therapy with Y9 and anti-PD-1. First, the researchers developed the hamster anti-mouse PD-1 antibody J43 into a murine form [88]. Anti-PD-1-sensitive (Sal/N) and anti–PD-1–resistant (MBT-2) syngeneic mice models evaluated both antibodies. Y9 therapy alone resulted in CR in all treated animals in both scenarios. Next, 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-1 had equivalent antitumour activity in the WEHI-164 model, and the combination did not increase the antitumour activity. In the CT26 and EMT6 models, however, the combination of Y9 and anti–PD-1 therapy resulted in a higher survival rate.

They also created a mouse version of the programmed death-ligand 1 (PD-L1) antibody MPDL3280a [89] and found that the results were comparable in all three animals. As a result, anti-TNFR2 has an anticancer activity comparable to, in some cases, better than anti–PD-1. Therefore, may be used with anti–PD-1 or anti–PD-L1 for improved antitumour activity in less immunogenic tumours.

The other mouse models, B16-F10, A20, LLC1, and NR: 4T1, did not show significant changes like CT26, EMT6, and WEHI-164. Anti TNFR2 agonist Y9 has expanded and improved the tumour antigen-specific IFNγ + CD8 + CTLs during the research and also can reduce the expression of TNFR2 on the surface of tumour-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 [90, 91]. After that, they conducted 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 six 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). In the study’s conclusion, splenomegaly was only found in mice treated with anti–CTLA-4. 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. Significantly, Y9 did not affect 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 tumour 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 suggest that the anti-TNFR2 antibody Y9 does not cause spontaneous immune cell activation in healthy and tumour-bearing mice.

**TNFR2 blocking agents other than potential antagonist and agonists**

We have already known that several antagonist and agonist has been developing against TNFR2. Other than the anti-TNFR2 antagonist and agonist, some pharmaceutical agents can regulate the expression of TNF. In the following table, the biological agents are listed:
Conclusion

The role of TNFR2 in promoting cell survival and proliferation is well recognized. Such property of TNFR2 is likely appropriate to tumour cells that express it [4, 98–100]. It gives a compelling reason to conduct more research into TNFR2 antagonists as a cancer therapy. TNFR2 is expressed at greater levels in a wide variety of tumour cells than in normal tissues. Furthermore, 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 tumours, the amounts of TNFR2 expressed by tumours should be addressed in future clinical trials. Moreover, the elevated levels of TNFR2 expression in tumour cells might be used as a biomarker for TNFR2-targeting treatment, which should be investigated further in future research.

TNFR2 is 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 influential TREG subsets but is also a feature of tumour 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 more significant than in healthy people. Small-molecule agonists or antagonists to target TNFR2 is a promising but more significant than in normal tissues. If the TNFR2-targeting therapy is meant to act promptly on tumours, the amounts of TNFR2 expressed by tumours should be addressed in future clinical trials. Moreover, the elevated levels of TNFR2 expression in tumour cells might be used as a biomarker for TNFR2-targeting treatment, which should be investigated further in future research.

Since only 30–40% of peripheral TREGs in normal mice are TNFR2-expressing cells, most tumour-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, human TREGs in the periphery express TNFR2 more extensively than mouse TREGs. Therefore, the following research should explore the danger of triggering autoimmune inflammatory responses by TNFR2-targeting medication in human patients. 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 [4, 60]. As a result, using TNFR2 agonistic antibodies in cancer treatment may increase TREG activity and lead to more excellent immune suppression. In the future, this possibility should also be carefully considered.

Given these problems to solve, existing preclinical evidence supports the notion that targeting TNFR2 with antagonists or agonists with antibodies or TNF eliminating agents might be a new and creative method in cancer immunotherapy.

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Declarations

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References

1. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, Gottfried M, Peled N, Tafreshi A, Cuffe S, O’Brien M, Rao S, Hotta K, Leiby MA, Lubinecki GM, Shentu Y, Rangwala R, Brahmer JR. KEYNOTE-024 Investigators. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med. 2016;375(19):1823–33. https://doi.org/10.1056/NEJMoa1600774.

2. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, Schadendorf D, Dummer R, Smylie M, Rutkowski P, Ferrucci PF, Hill A, Wagstaff J, Carlino MS, Haenen JB, Maio M, Marquez-Rodas I, McArthur GA, Ascierto PA, Long GV, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med. 2015;373(1):23–34. https://doi.org/10.1056/NEJMoa1504030.

3. Baeyens KJ, De Bondt HL, Raeymaekers A, Fiers W, De Ranter CJ. The structure of mouse tumour-necrosis factor at 2.2 Å resolution: towards modulation of its selectivity and trimerization. Acta Crystallogr D Biol Crystallogr. 1999;55(Pt 4):772–8. https://doi.org/10.1107/s0907444998018435.

4. Naudé PJ, den Boer JA, Luiten PG, Eisel UL. Tumor necrosis factor receptor cross-talk. FEBS J. 2011;278(6):888–98. https://doi.org/10.1111/j.1742-4658.2011.08017.x.

5. Luo D, Luo Y, He Y, Zhang H, Zhang R, Li X, Dobrucki WL, Sinusas AJ, Sessa WC, Min W. Differential functions of tumor necrosis factor receptor 1 and 2 signaling in ischemia-mediated arteriogenesis and angiogenesis. Am J Pathol. 2006;169(5):1886–98. https://doi.org/10.2353/ajpath.2006.060603.

6. Chen X, Subleski JJ, Kopf H, Howard OM, Männel DN, Oppenheim JJ. Cutting edge: expression of TNFR2 defines a maximally suppressive subset of mouse CD4+CD25+FoxP3+ T regulatory cells: applicability to tumor-infiltrating T regulatory cells. J Immunol (Baltimore, Md). 2008;180(10):6467–71. https://doi.org/10.4049/jimmunol.180.10.6467.
7. Zhao X, Rong L, Zhao X, Li X, Liu X, Deng J, Wu H, Xu X, Erben U, Wu P, Sybre U, Sieper J, Qin Z. TNF signaling drives myeloid-derived suppressor cell accumulation. J Clin Investig. 2012;122(11):4094–104. https://doi.org/10.1172/JCI64115.

8. Böcker W, Docheva D, Prall WC, Egea V, Pappou E, Roßmann O, et al. IKK-2 is required for TNF-α-induced invasion and proliferation of human mesenchymal stem cells. J Mol Med. 2008;86(10):1183–92.

9. Josewoicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol. 2012;30:531–64. https://doi.org/10.1146/annurev.immunol.25.022206.141623.

10. Grinberg-Bleyer Y, Saadoun D, Baeyens A, Billiard F, Goldstein JD, Grégoire S, Martin GH, Elbage R, Derian N, Carpenter J, et al. IKK-2 is required for TNF-α-induced invasion and proliferation of human mesenchymal stem cells. J Mol Med. 2008;86(10):1183–92.

11. Chen X, Wu X, Zhou Q, Howard OM, Netea MG, Oppenheim JJ. TNF receptor type 2 promotes expansion and function of regulatory CD4+FoxP3+ T cells. Blood. 2016;128(6):866–71. https://doi.org/10.1182/blood-2016-04-711275.

12. Zaragoza B, Chen X, Oppenheim JJ, Baeyens A, Gregoire S, Chader D, Gorovoch G, Miyara M, Salomon BL. Proliferative activity of human regulatory T cells is maintained in the presence of TNF. Nat Med. 2016;22(1):16–7. https://doi.org/10.1038/nm.4019.

13. Quazi S. Elucidation of CRISPR-Cas9 application in novel cellular immunotherapy. Preprints. 2021. https://doi.org/10.20944/preprints202108.0387.v1.

14. Chopra M, Biel M, Steinfatt T, Brandl A, Kums J, Amich J, Simons JG, Ding S, Van Landeghem L, Erben U, Wu P, Syrbe U, Sieper J, Qin Z. TNF signaling drives myeloid-derived suppressor cell accumulation. J Exp Med. 2012;213(9):1881–900. https://doi.org/10.1084/jem.20115156.

15. Leclerc M, Naserian S, Pilon C, Thiolat A, Martin GH, Pouchy C, Dominique C, Belkacemi Y, Charlotte F, Maury S, Salomon BL, Cohen JL. Control of GVDH by regulatory T cells depends on TNF produced by T cells and TNFR2 expressed by regulatory T cells. Blood. 2016;128(12):1651–9. https://doi.org/10.1182/blood-2016-02-700849.

16. Pierini A, Stroher W, Moffett C, Baker J, Nishikii H, Alvarez M, Pan Y, Schneidawind D, Meyer E, Negrin RS. TNF-α priming enhances CD4+FoxP3+ regulatory T-cell suppressive function in murine GVDH prevention and treatment. Blood. 2016;128(6):866–71. https://doi.org/10.1182/blood-2016-04-711275.

17. Chen X, Bäumel M, Männel DN, Howard OM, Oppenheim JJ. Interaction of TNF with TNF receptor type 2 promotes expansion and function of mouse CD4+CD25+ T regulatory cells. J Immunol (Baltimore, Md). 2007;179(1):154–61. https://doi.org/10.4049/jimmunol.179.1.154.

18. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol. 2003;3(9):745–56.

19. Heinrich M, Burger D, Wang L, Tahhan G, Reinhold P, Zhao M, et al. TNFR1 and TNFR2 expression and induction in human TREG cells from type 1 diabetic subjects. Antibodies. 2015;4(1):34–47.

20. Pol J, Remke A, Weber S, Schmidt D, Weber-Steifens D, Pierryga-Krieger A, Müller N, Ritter U, Mostböck S, Männel DN. Myeloid suppressor cells require membrane TNFFR2 expression for suppressive activity. Immunity Inflammt Disease. 2014;2(2):121–30. https://doi.org/10.1002/iid3.19.
baboon. J Exp Med. 1994;179(4):1185–91. https://doi.org/10.1084/jem.179.4.1185.

35. Chopra M, Riedel SS, Biehl M, Krieger S, von Krosigk V, Bäuerlein CA, Brede C, Jordan Garrote AL, Kraus S, Schäfer V, Ritz M, Mattenheimer K, Degla A, Mottak A, Einsele H, Wajant H, Beilhack A. Tumor necrosis factor receptor 2-dependent homeostasis of regulatory T cells as a player in TNF-induced metastasis. Carcinogenesis. 2013;34(6):1296–303. https://doi.org/10.1093/carcin/bgt038.

36. Sasi SP, Bae S, Song J, Perepletchikov A, Schneider D, Carrozza J, Yan X, Kishore R, Enderling H, Roukass DA. Therapeutic non-toxic doses of TNF induce significant regression in TNFR2-p75 knockdown Lewis lung carcinoma tumor implants. PLOS ONE. 2014;9:e92373.

37. Yu M, Zhou X, Niu L, Lin G, Huang J, Zhou W, Gan H, Wang J, Jiang X, Yin B, Li Z. Targeting transmembrane TNF-α suppresses breast cancer growth. Can Res. 2013;73(13):4061–74. https://doi.org/10.1158/0008-5472.CAN-12-3946.

38. Ham B, Wang N, D’Costa Z, Fernandez MC, Bourdeau F, Auguste P, Illemann M, Eefsen RL, Høyer-Hansen G, Vainer IS, Lin CY. Blockade of TNF-α signaling benefits cancer patients. Cancer Immunol Immunother. 2015;64(11):1475–85. https://doi.org/10.1007/s00262-015-1751-z.

39. Nakayama S, Iida K, Tsuzuki T, Iwashita T, Murakami H, Asai N, Iwata Y, Ichihara M, Ito S, Kawai K, Asai M, Kurokawa K, Takahashi M. Implication of expression of GDNF/Ret signaling components in differentiation of bone marrow haemopoietic cells. Br J Haematol. 1999;105(5):50–7.

40. Nie H, Zheng Y, Li R, Guo TB, He D, Fang L, Liu X, Xiao L, Chen X, Wan B, Chin YE, Zhang JZ. Phosphorylation of FOXP3 controls regulatory T cell function and is inhibited by TNF-α in rheumatoid arthritis. Nat Med. 2013;19(3):322–8. https://doi.org/10.1038/nm.3085.

41. Jain SS, Bird RP. Elevated expression of tumor necrosis factor-alpha signaling molecules in colonic tumors of Zucker obese (fa/fa) rats. Int J Cancer. 2010;127(9):2042–50. https://doi.org/10.1002/ijc.25232.

42. Quazi S. Artificial intelligence and machine learning in precision and genomic medicine. Preprints. 2021. https://doi.org/10.20944/preprints202111.0011.v1.

43. Chang LY, Lin YC, Chiang JM, Mahalingam J, Su SH, Huang CT, Chen WT, Huang CH, Jeng WJ, Chen YC, Lin SM, Sheen XM, Fu L. Overexpression of TNF-alpha and TNFRII in invasive micropapillary carcinoma of the breast: clinicopathological correlations. Histopathology. 2008;53(4):381–8. https://doi.org/10.1111/j.1365-2559.2008.03128.x.

44. Okubo Y, Mera T, Wang L, Faustman DL. Homogeneous expansion of human T-regulatory cells via tumor necrosis factor receptor 2. Sci Rep. 2013;3:3153. https://doi.org/10.1038/srep03153.

45. Govindaraj C, Tan P, Walker P, Wei A, Spencer A, Plebanski M. Reducing TNF receptor 2+ regulatory T cells via the combined action of azacitidine and the HDAC inhibitor, panobinostat for clinical benefit in acute myeloid leukemia patients. Clin Cancer Res. 2014;20(3):724–35. https://doi.org/10.1158/1078-0432.CCR-13-1576.

46. D’Costa Z, Fernandez MC, Bourdeau F, Auguste P, Illemann M, Eefsen RL, Høyer-Hansen G, Vainer IS, Lin CY. Blockade of TNF-α signaling benefits cancer patients. Cancer Immunol Immunother. 2015;64(11):1475–85. https://doi.org/10.1007/s00262-015-1751-z.

47. Okubo Y, Torrey H, Butterworth J, Zheng H, Faustman DL. TREG activation defect in type 1 diabetes: correction with TREG2 agonism. Clin Transl Immunol. 2016;5(1):e56. https://doi.org/10.1038/cti.2015.43.

48. Chen X, Yang Y, Zhou Q, Weiss JM, Howard OZ, McPherson JM, et al. Effective chemoimmunotherapy with anti-TGFβ antibody and cyclophosphamide in a mouse model of breast cancer. PLoS ONE. 2014;9(1):e85398.

49. Fang Y, Du R, Wei F, Zhao H, Yu J, Wang C, Zhan Z, Ding T, Ren X, Chen X, Li H. Expression of TNFR2 by regulatory T cells in peripheral blood is correlated with clinical pathology of lung cancer patients. Cancer Immunol Immunother. 2015;64(11):1475–85. https://doi.org/10.1007/s00262-015-1751-z.

50. Chua J, Oh J, Moon J, Park S, Lee J, Kim Y, et al. Improved survival of AML patients with high-risk cytogenetics treated with azacitidine and the HDAC inhibitor, panobinostat. Leukemia. 2016;30(5):1073–40. https://doi.org/10.1038/leu.2016.96.
peripheral blood is correlated with clinical pathology of lung cancer. Cancer Immunol Immunother. 2015;64(11):1475–85. https://doi.org/10.1007/s00262-015-1751-z.

64. Tirosh I, Izar B, Prakadan SM, Wadsworth MH 2nd, Treacy D, Trombetta JJ, Rotem A, Rodman C, Lian C, Murphy G, Fallahi-Sichani M, Dutton-Regester K, Lin JR, Cohen O, Shah P, Lu D, Genthaas A, Hughes TK, Ziegler CG, Kazer SW, et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. Science (New York, NY). 2016;352(6282):189–96. https://doi.org/10.1126/science.aad0501.

65. Torrey H, Butterworth J, Mera T, Okubo Y, Wang L, Baum D, Defusco A, Plager S, Warden S, Huang D, Vanamee E, Foster R, Faustman DL. Targeting TNFR2 with antagonistic antibodies inhibits proliferation of ovarian cancer cells and tumor-associated TREGs. Sci signal. 2017;10(462):eaaf8608. https://doi.org/10.1126/scisignal.aaf8608.

66. Nie Y, He J, Shirota H, Trivett AL, Yang D, Klinman DM, Oppenheim JJ, Chen X. Blockade of TNFR2 signaling enhances the immunotherapeutic effect of CpG ODN in a mouse model of colon cancer. Sci signal. 2018;11(511):eaan0790. https://doi.org/10.1126/scisignal.aan0790.

67. Vanamee ES, Faustman DL, TNFR2: a novel target for cancer immunotherapy. Trends Mol Med. 2017;23(11):1037–46.

68. Filbert E, Krishnan S, Alvarado R, Huang G, Bahjat F, Yang X. 693 APX601, a Novel TNFR2 antagonist antibody for cancer immunotherapy. J Immunother Cancer. 2020;8(Suppl 3):A417–A417.

69. Case K, Tran L, Yang M, Zheng H, Kuhtreiber WM, Faustman DL. Targeting TNFR2 with antagonistic antibodies inhibits proliferation of ovarian cancer cells and tumor-associated TREGs. Sci signal. 2017;10(462):eaaf8608. https://doi.org/10.1126/scisignal.aaf8608.

70. Torrey H, Khodadoust M, Tran L, Baum D, Defusco A, Kim YH, Faustman DL. Targeted killing of TNFR2-expressing tumor cells and TREGs by TNFR2 antagonistic antibodies in advanced Sézary syndrome. Leukemia. 2019;33(5):1206–18. https://doi.org/10.1038/s41375-018-0292-9.

71. Shirota Y, Shirota H, Klinman DM. Intratumoral injection of CpG oligonucleotides induces the differentiation and reduces the immunosuppressive activity of myeloid-derived suppressor cells. J Immunol. 2012;188(4):1592–9. https://doi.org/10.4049/jimmunol.1101304.

72. Torrey H, Kuhtreiber WM, Okubo Y, Tran L, Case K, Zheng H, Vanamee E, Faustman DL. A novel TNFR2 agonist antibody expands highly potent regulatory T cells. Sci signal. 2020;13(661):caba9600. https://doi.org/10.1126/scisignal.caba9600.

73. Zou H, Li R, Hu H, Hu Y, Chen X. Modulation of regulatory T Cell activity by TNF receptor type II-targeting pharmacological agents. Front Immunol. 2018;9:594. https://doi.org/10.3389/fimmu.2018.00594.

74. Atrekhany KSN, Mufazalov IA, Dunst J, Kuchmiy A, Zou H, Li R, Hu H, Hu Y, Chen X. Promotion of colon cancer. Sci signal. 2018;11(462):eaaf8608. https://doi.org/10.1126/scisignal.aaf8608.

75. Faustman DL. TNF, TNF inducers, and TNFR2 agonists: a new translational medicine. 2019. https://doi.org/10.1126/scitranslmed.aax0720.

76. Fulton RB, Camblin A, Sampson JF, Richards J, Wong C, Koshkaryev A, et al. Mechanism of action of a novel agonist TNFR2 antibody that induces co-stimulation of T cells and promotes robust anti-tumor immunity. Cancer Res. 2019;79(13 Supplement):3270.

77. Sampson JF, Fulton RB, Kurella VB, Richards JM, Camblin AJ, Wong CS, et al. A novel TNFR2 antibody induces T cell co-stimulation and promotes durable anti-tumor immunity. Sci Transl Med. 2019. https://doi.org/10.1126/scitranslmed.aax0720.

78. Tam EM, Fulton RB, Sampson JF, Muda M, Camblin A, Richards J, et al. Antibody-mediated targeting of TNFR2 activates CD8+ T cells in mice and promotes antitumor immunity. Science translational medicine. 2019. https://doi.org/10.1126/scitranslmed.eda00720.

79. Fulton RB, Camblin A, Sampson JF, Richards J, Wong C, Koshkaryev A, et al. Mechanism of action of a novel agonist TNFR2 antibody that induces co-stimulation of T cells and promotes robust anti-tumor immunity. Cancer Res. 2019;79(13 Supplement):3270.

80. Sampson JF, Fulton RB, Kurella VB, Richards JM, Camblin AJ, Wong CS, et al. A novel TNFR2 antibody induces T cell co-stimulation, robust anti-tumor activity and immune memory. Cancer Res. 2019;79(13 Supplement):555.

81. Richards J, Wong C, Koshkaryev A, Fulton R, Camblin A, Sampson J, et al. MM-401, a novel anti-TNF-α antibody that induces T cell co-stimulation, robust anti-tumor activity and immune memory. Cancer Res. 2019;79(13 Supplement):555.

82. Sampson JF, Kurella VB, Paragas V, Kumar S, Lulo JE, Qiu JA, et al. A novel human TNFR2 antibody (MM-401) modulates T cell responses in anti-cancer immunity. Cancer Res. 2019;79(13 Supplement):555.

83. Williams GS, Mistry B, Guillard S, Ulrichsen JC, Sandercock AM, Wang J, et al. Phenotypic screening reveals TNFR2 as a promising target for cancer immunotherapy. Oncotarget. 2016;7(42):68278.

84. Sheehan KC, Pinckard JK, Arthur CD, Dehner LP, Goeddel DV, Schreiber RD. Monoclonal antibodies specific for murine p55 and p75 tumor necrosis factor receptors: identification of a novel in vivo role for p75. J Exp Med. 1995;181(2):607–17.

85. Li F, Ravetch JV. A general requirement for FcRRIIB co-engagement of agonistic anti-TNF antibodies. Cell Cycle. 2012;11(18):3343–4.

86. Quazi S, Jangi R. Artificial intelligence and machine learning in medicinal chemistry and validation of emerging drug targets. Preprints. 2021. https://doi.org/10.20944/preprints202105.0567.v1.

87. Stewart R, Hammond SA, Oberst M, Wilkinson RW. The role of Fc gamma receptors in the activity of immunomodulatory antibodies for cancer. J Immunother Cancer. 2014;2(1):1–10.

88. Agata Y, Kawasaki A, Nishimura H, Ishida Y, Tsubat T, Yagita H, Horio T. Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. Int Immunol. 1996;8(5):765–72.

89. Powles T, Eder JP, Fine GD, Braiteh FS, Loriot Y, Cruz C, Bellmunt J, Burris HA, Petrylak DP, Teng SL, Shen X, Boyd Z, Hegde PS, Chen DS, Vogelzang NJ. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature. 2014;515(7528):558–62. https://doi.org/10.1038/nature13904.

90. Paz-Ares L, Luft A, Vicente D, Tafreshi A, Güümüş M, Mazieres J, Hermes B, Çay Senler F, Csösz T, Fülöp A, Rodríguez-Cid J, Wilson J, Sugawara S, Kato T, Lee KH, Cheng Y, Novello S, Halmos B, Li X, Lubinecki GM, KEYNOTE-407 Investigators. Pembrolizumab plus chemotherapy for squamous non-small-cell lung cancer. N Engl J Med. 2018;379(21):2040–51. https://doi.org/10.1056/NEJMoa1810865.

91. Weber JS, Postow M, Lao CD, Schadendorf D. Management of adverse events following treatment with anti-programmed cell death-1 agents. Oncologist. 2016;21(10):1230–40. https://doi.org/10.1634/theoncologist.2016-0055.

92. Incidence and Clinical Impact of Anti-TNFα Treatment of Severe Immune Checkpoint Inhibitor-induced Colitis in Advanced Melanoma: The Mecolit Survey.

93. Lesage C, Longvert C, Prey S, Maanouei S, Dréno B, Machet L, Zehou O, Kramkimel N, Jeudy G, Skowron F, Aubin F, Visseaux
L. Mansard S, Dereure O, Lesage FX, Guillot B. French Group of Onco-Dermatology. J Immunother. 2019;42(5):175–9.

94. Govindaraj C, Madondo M, Kong YY, Tan P, Wei A, Plebanski M. Lenalidomide-based maintenance therapy reduces TNF receptor 2 on CD4 T cells and enhances immune effector function in acute myeloid leukemia patients. Am J Hematol. 2014;89(8):795–802. https://doi.org/10.1002/ajh.23746.

95. van der Most RG, Currie AJ, Mahendran S, Prosser A, Darabi A, Robinson BW, Nowak AK, Lake RA. Tumor eradication after cyclophosphamide depends on concurrent depletion of regulatory T cells: a role for cycling TNFR2-expressing effector-suppressor T cells in limiting effective chemotherapy. Cancer Immunol Immunother. 2009;58(8):1219–28. https://doi.org/10.1007/s00262-008-0628-9.

96. Giannopoulos K, Dmoszynska A, Kowal M, WasiK-Szczeniak E, Bojarska-Junak A, Roinski J, Döhner H, Stilgenbauer S, Bullinger L. Thalidomide exerts distinct molecular antileukemic effects and combined thalidomide/fludarabine therapy is clinically effective in high-risk chronic lymphocytic leukemia. Leukemia. 2009;23(10):1771–8. https://doi.org/10.1038/leu.2009.98.

97. Wei X, Gong J, Zhu J, Wang P, Li N, Zhu W, Li J. The suppressive effect of triptolide on chronic colitis and TNF-alpha/TNFRIII signal pathway in interleukin-10 deficient mice. Clinical Immunol. 2008;129(2):211–8. https://doi.org/10.1016/j.clim.2008.07.018.

98. Liu B, Zhang H, Li J, Lu C, Chen G, Zhang G, Lu A, He X. Triptolide downregulates TREG cells and the level of IL-10, TGF-β, and VEGF in melanoma-bearing mice. Planta Med. 2013;79(15):1401–7. https://doi.org/10.1055/s-0033-1350708.

99. Grell M, Becke FM, Wajant H, Männel DN, Scheurich P. Tumor necrosis factor (TNF) receptor type 2 mediates thymocyte proliferation independently of TNF receptor type 1. Eur J Immunol. 1998;28(1):257–63. https://doi.org/10.1002/(SICI)1521-4141(199801)28:01<257::AID-IJIM2573.0.CO;2-G.

100. Lamontain V, Schmid T, Weber-Steffens D, Zeller D, Jenei-Lanzl Z, Wajant H, Straub RH, Männel DN. Stimulation of TNF receptor type 2 expands regulatory T cells and ameliorates established collagen-induced arthritis in mice. Cell Mol Immunol. 2019;16(1):65–74. https://doi.org/10.1038/cmi.2017.138.

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