PIONYR
IMMUNOTHERAPEUTICS

Therapeutic Targeting of TREM2—Distinct Approaches for Different Diseases

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SUMMARY

Triggering receptor expressed on myeloid cells 2 (TREM2) has gained attention as a therapeutic target for diverse indications, including cancer, fibrosis, and neurodegenerative diseases; multiple companies are developing agents to target TREM2.

However, TREM2 is likely to have distinct roles in the pathogenesis of these disorders, so therapeutic agents in development employ different approaches to modify TREM2 activity.

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TREM2 AND THE ANTI-TUMOR IMMUNE RESPONSE

One immunotherapeutic strategy for cancer is to promote the anti-tumor activities of CD8+ T cells by use of immune checkpoint inhibitors (CPIs), such as antibodies against the T-cell inhibitory receptor PD-1 or its ligand PD-L1. These drugs can activate CD8+ T cells to kill cancer cells and have transformed treatment of some cancers, completely curing a subset of patients. However, there are many tumor types that do not respond to these drugs. A complementary approach under clinical investigation is to target myeloid cells within the tumor microenvironment (TME)—particularly tumor-associated macrophages (TAMs)—either alone or in combination with CPIs.

TAMs can have immune-suppressive and immune-promoting functions (1). Although an over-simplification, immune-stimulating macrophages are often called M1-like macrophages whereas immune-suppressive TAMs are called M2-like macrophages. Pionyr Immunotherapeutics (Pionyr) is developing a 'Myeloid Tuning'™ platform that focuses on shifting the balance of the TME from an immune-suppressive to an immune-activated environment, to promote anti-tumor immunity.

One subset of immunosuppressive TAMs has been characterized by the specific expression of TREM2 (2). TREM2 is a single-transmembrane protein with an extracellular immunoglobulin-like variable (IgV) domain that binds anionic ligands and has an intracellular domain that signals via interactions with the adaptor proteins DNAX activation protein 12 (DAP12) and DAP10, which in turn signal via spleen tyrosine kinase (Syk) and phosphatidylinositol 3-kinase (PI3K). This signaling pathway leads to downregulated transcription of genes that promote inflammation (*Tnf, Il1b, and Nos2*) (3). The TREM2 signaling pathway also leads to release of cytokines that prevent activation of anti-tumor CD8+ T cells (4). TREM2+ immunosuppressive TAMs correlate with the level of exhausted T cells in the human TME (5). A TREM2+ TAM-rich TME therefore appears to be immunosuppressive and might promote resistance to anti-PD1 therapies (5)

Although TREM2 expression is low in most normal tissues (4, 6), it is overexpressed in many human tumor types. TREM2+ macrophages were observed in 75% of the 126 samples in a multi-carcinoma tissue array, but not in peripheral tissues; the authors concluded that TREM2 is a marker of tumor-infiltrating macrophages (4). Cheng et al compared levels of *TREM2* mRNA among 33 cancer tissues and matched normal tissues using datasets from The Cancer Genome Atlas (TCGA), and found higher levels of expression in tumor compared to normal tissues in 18 cancer types, including head and neck squamous cell carcinoma, colon adenocarcinoma, and glioblastoma, as well as gynecologic, liver, gastric, kidney, breast, bladder, and esophageal cancers (6). They also found the level of *TREM2* mRNA to be positively associated with tumor mutation burden and microsatellite instability in 12 cancer types and associated with level of DNA methylation in 20 tumor types.

Cheng et al reported that in invasive breast carcinoma, cervical squamous cell carcinoma and endocervical adenocarcinoma, kidney renal clear cell carcinoma, lung squamous cell carcinoma, skin cutaneous melanoma, and stomach adenocarcinoma, there is a negative correlation between expression of TREM2 and tumor infiltration by immune cells (6). To extend this analysis, Binnewies et al (Pionyr) assessed *TREM2* RNA expression in 9736 tumor and 8587 normal tissue samples from the TCGA and the GTEx projects and observed a consistent increase in TREM2 levels in tumors (5). These findings support the tumor-enriched expression of TREM2 and the importance of therapeutically targeting it.

High expression of TREM2 was associated with shorter survival times of patients with ovarian cancer (6), gastric cancer (5), lower-grade glioma, hepatocellular carcinoma, or renal clear cell carcinoma (6). In an analysis of the TGCA database, Molgora et al found an inverse correlation between TREM2 expression and overall survival and recurrence in patients with colorectal cancer and triple-negative breast cancer (4).

Pionyr used a TREM2 immunohistochemistry assay to profile TREM2 on patient samples from multiple solid tumors and reported at the 2021 AACR Annual Meeting (Abstract no. LB071) that TREM2 is most highly expressed in lung, gastric, kidney, breast, colon, and ovarian tumor tissues (**Figure 1**). In addition, TREM2 protein increased with higher grade in multiple solid tumors, including ovarian cancer, colon cancer, and gastric adenocarcinoma, and expression was associated with shorter survival times of patients with these tumor types (2019 EORTC Abstract no. C104).

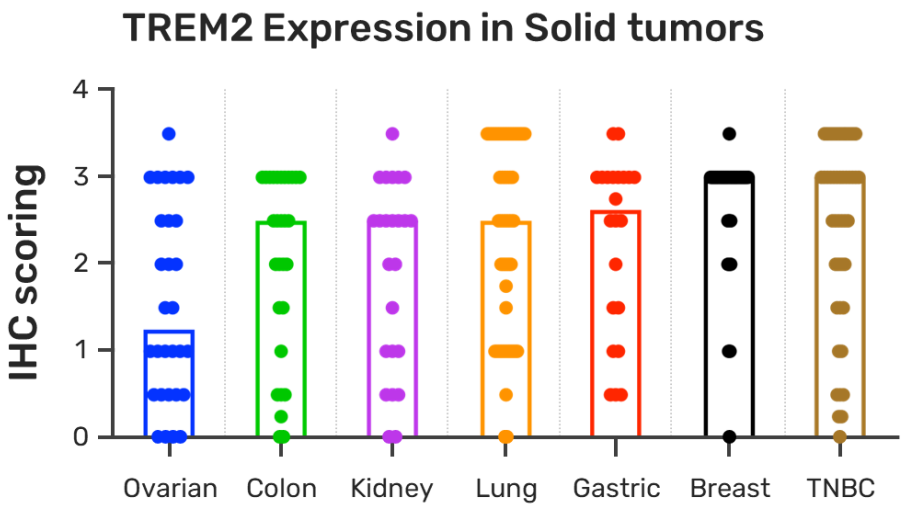


Figure 1. TREM2 Expression in Solid tumors

Median immunohistochemistry (IHC) scores (semi-quantitative manual scoring by a board-certified pathologist evaluating the percentage of TREM2-positive cells in the TME) for TREM2 staining in advanced tumor specimens. IHC scores ranging from 0 to 3.5 were given for each tumor tissue: 0 = <1% positive cells, 0.5 = 1%–10% positive cells, 1 = 10%–25% positive cells, 1.5 = 25%–50% positive cells, 2 = approximately 50% positive cells, 2.5 = 50%–75% positive cells, 3 = approximately 75% positive cells, and 3.5 = >75% positive cells over stroma. Each dot represents 1 tumor case, and the line indicates median score. TNBC, triple-negative breast cancer

At the 2021 Pathology Visions Conference (Abstract no. 10106), Pionyr reported the development and validation of the TREM2 immunohistochemical assay as a laboratory-developed test that can be used with clinical specimens to detect TREM2 in the TME. The assay detected TREM2+ TAMs with high levels of sensitivity and specificity, across 6 prioritized tumor indications. The study confirmed that TREM2+ TAMs are highly enriched whereas TREM2 expression is absent from most normal tissues.

Pionyr reported in Binnewies et al (5) and in an AACR abstract that, in human ovarian and other tumor specimens, expression of TREM2 protein and mRNA was restricted to TAMs, with minimal expression by most other types of immune cells. Ovarian cancer does not usually respond to CPI therapy and has a high density of TAMs (5).

There is a population of tumor-specific macrophages characterized by upregulation of TREM2 and its ligands, APOE and C1Q, validated by spatially resolved, quantitative multispectral immunofluorescence and single-cell RNA sequencing. The authors of this study concluded that tumor infiltration by TREM2+, APOE+, C1Q+ macrophage is a biomarker for recurrence of clear-cell renal carcinoma and that these cells are also therapeutic targets (5, 7). TREM2+ macrophages from human tumors also express CD68, CD163, CSF1R, and nuclear MAFB (4).

Pionyr Immunotherapeutics Inc (Pionyr) has developed an anti-TREM2 afucosylated IgG1 monoclonal antibody, called PY314, which is designed to selectively deplete the TREM2-positive, immunosuppressive TAMs via antibody-dependent cell mediated cytotoxicity (ADCC) and/or antibody-dependent cell mediated phagocytosis (ADCP) (5) (**Figure 2**). The absence of the core fucose on the Fc N-glycan (afucosylation) increases the effector function of IgG antibodies, leading to augmented IgG1 Fc binding affinity to the FcγRIIIa on immune effector cells, such as natural killer (NK) cells, and enhances ADCC (8). Studies have shown that afucosylated antibodies against TREM2 elicit higher levels of ADCC of TREM2-positive cells than non-glycoengineered antibodies against TREM2 (5).

Pionyr reported at the 2021 AACR conference (Abstract no. LB071) and at previous conferences (2019 EORTC Abstract no. C104, 2019 SITC abstract no. Abstract P800) that administration of an antibody against mouse TREM2 (PY314m) to mice bearing syngeneic tumors reduces tumor growth and alters the immune composition in the TME. Depletion of TREM2-positive cells in tumors by PY314m leads to recruitment of activated NK and CD8+ T cells and switches the TME to one in which the immune-activating (M1-like) macrophages predominate, thereby 'tuning' the myeloid infiltrate in the TME (**Figure 2**).

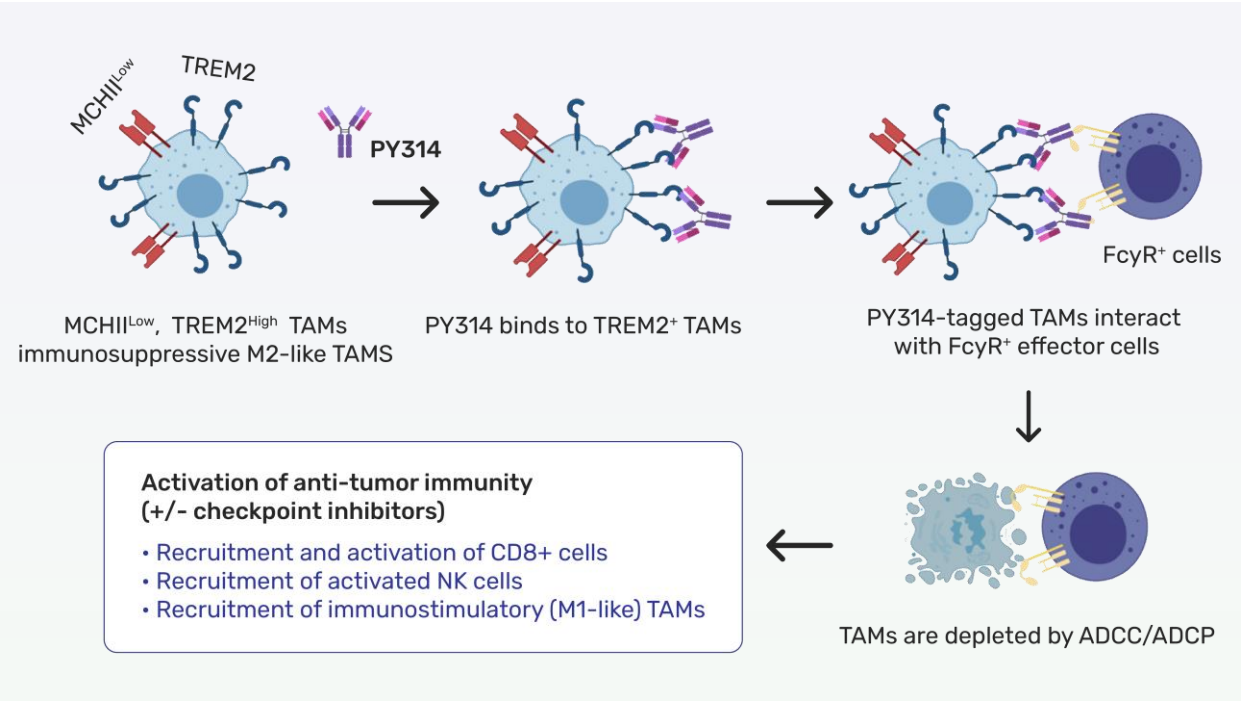


Figure 2. PY314 Depletion of Immune-suppressive TAMs (M2 Macrophages)

TAMs that express low levels of MHC class II (MHCII^{low}) and high levels of TREM2 are considered immunosuppressive, or M2-like macrophages. Binding of the therapeutic antibody PY314 to TREM2 on these cells increases interactions with FCγR+ effector cells, which targets the immunosuppressive TAMs for depletion by ADCC or ADCP. Depletion of these TAMs from the TME results in activation of an anti-tumor immune response, via recruitment and activation of CD8+ T cells, NK cells, and immunostimulatory (M1-like) TAMs. Addition of CPIs might also increase activation of CD8+ T cells and increase overall anti-tumor immunity (box at bottom of figure).

There is synergy between anti-PD1 and anti-TREM2. This synergy increases the abundance of intra-tumoral CD8+ T cells and inflammatory cytokines (IFNG and TNF) (**Figure 3**). Administration of mPY314 to tumor-bearing mice depletes TAMs and promotes anti-tumor immunity—as a single agent and in combination with anti–PD1 (5).

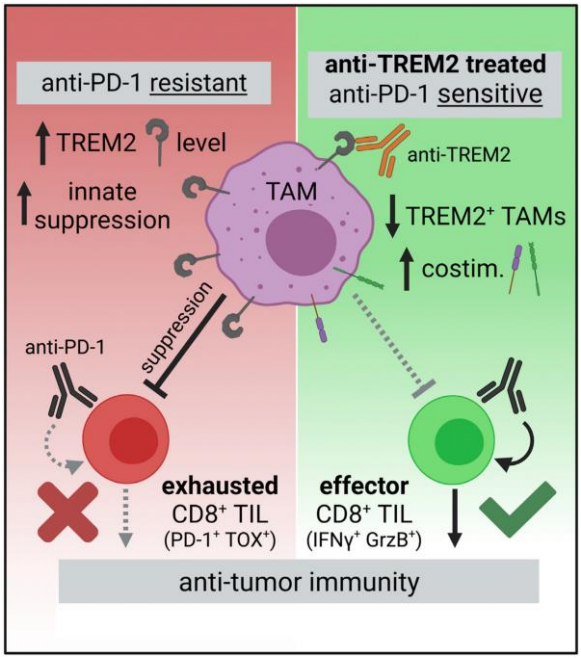


Figure 3. Anti-tumor Effects of TREM2 and PD1 Inhibition

Tumors that are resistant to CPI-based therapies (such as anti-PD1) have increased expression of TREM2 on innate immune cells such as TAMs. These TAMs suppress cytotoxic CD8+ T cells (tumor-infiltrating lymphocytes, TILs), and inhibit the effects of anti-PD1. PY314 depletes the TREM2+ TAMs via ADCC and ADCP. This allows CD8+ T cells to become activated and shift from an exhausted to an effector state—especially in the presence of anti-PD1—and produce cytokines that activate an anti-tumor immune response.

BEYOND ONCOLOGY–TREM2 AS A THERAPEUTIC TARGET FOR NEURODEGENERATIVE DISEASES

In the brain, TREM2 is expressed on microglia (9). Binding of apolipoproteins, such as ApoE, to TREM2 promotes phagocytosis of apoptotic neurons or the uptake of amyloid beta by microglia (10). Additionally, TREM2 interacts with pathological beta-amyloid oligomers (11), anionic and zwitterionic lipids (12), and lipo- and apolipoproteins (APOE and CLU/APOJ) (13), which, together with amyloid-beta, form plaques characteristic of Alzheimer's disease. Variants of TREM2 that encode proteins with reduced affinity for ligands have been associated with Alzheimer's disease (14).

In microglia, TREM2 regulates clearance of neuronal debris (3). TREM2 deficiency and haploinsufficiency promote accumulation of amyloid-beta due to dysfunctional responses of microglia, which become apoptotic and fail to cluster around amyloid-beta plaques (12). In mice, defective TREM2 function affects microglial responses to amyloid-beta plaques, exacerbating tissue damage, whereas TREM2 overexpression reduces pathology (15). This study also found that an antibody agonist of TREM2 activates microglia and reduces amyloid beta plaques in mice. So, in the central nervous system, TREM2 deficiency results in impaired clearance of apoptotic neurons and inflammation, which might contribute to brain degeneration (3). Patients with Alzheimer's disease might therefore benefit from TREM2 activation to clear debris that promotes neurodegeneration (15).

TREM2 agonists for treatment of these disorders

Given the links between loss of TREM2 function and neurodegenerative diseases, several companies are evaluating TREM2 agonists for treatment of neurodegenerative diseases (see **Table 1**).

TABLE 1. AGENTS IN DEVELOPMENT FOR THERAPEUTIC TARGETING OF TREM2

Agent name	Molecule Type	Effects	Indication	Company	Testing phase and results
Oncology					
PY314	Glycoengineered antibody (antagonist)	Bind and deplete TAMs via ADCC and/or ADCP	Oncology (solid tumors)	Pionyr	Phase 1b dose-expansion study https://www.pionyrtx.com/pipeline/
AMV800	Designer proteins (inhibitors)	Bind and deplete TAMs	Oncology (solid tumors)	Amphivena	Discovery phase: https://amphivena.com/our-programs/#pipeline
Neurodegenerative Disorders					
DNL-919	Antibody with Fc engineered to bind transferrin receptor (agonist)	Normalize microglial function	Alzheimer's disease	Denali	IND-enabling, clinical hold* https://www.denalitherapeutics.com/pipeline
VGL101	Antibody (agonist)	Promote microglia proliferation and migration to sites of brain injury or neuron death	ALSP, Alzheimer's disease, cALD	Vigil	Phase 1 https://www.vigilneuro.com/trem2
SM	Small molecule (agonist)		Alzheimer's disease	Vigil	IND enabling https://www.vigilneuro.com/trem2
AL002	Antibody (agonist)	Activate TREM2 in microglia	Alzheimer's disease	Alector	Phase 2 https://alector.com/pipeline/
Not disclosed	Peptide agonists with binders to transferrin receptor	Activate TREM2	Dementia	Bicycle Therapeutics; Dementia Discovery Fund; Alzheimer's Research UK Oxford Drug Discovery Institute	Discovery https://investors.bicycletherapeutics.com/news-releases/news-release-details/bicycle-therapeutics-announces-significant-progress-across

ALSP, adult-onset leukoencephalopathy with axonal spheroids and pigmented glia; cALD, childhood cerebral adrenoleukodystrophy

*As of February 2022

However, there are key differences between agents that target TREM2 for treatment of neurodegenerative diseases and Pionyr's PY314 for treatment of cancer. Whereas PY314 selectively depletes TREM2-expressing cells to activate an anti-tumor response, agents in development for neurodegenerative disorders, such as AL002, an anti-TREM2 monoclonal antibody developed by Alector Therapeutics (Alector), are agonists that stimulate TREM2 in microglia to activate metabolism and proliferation (see **Figure 4**).

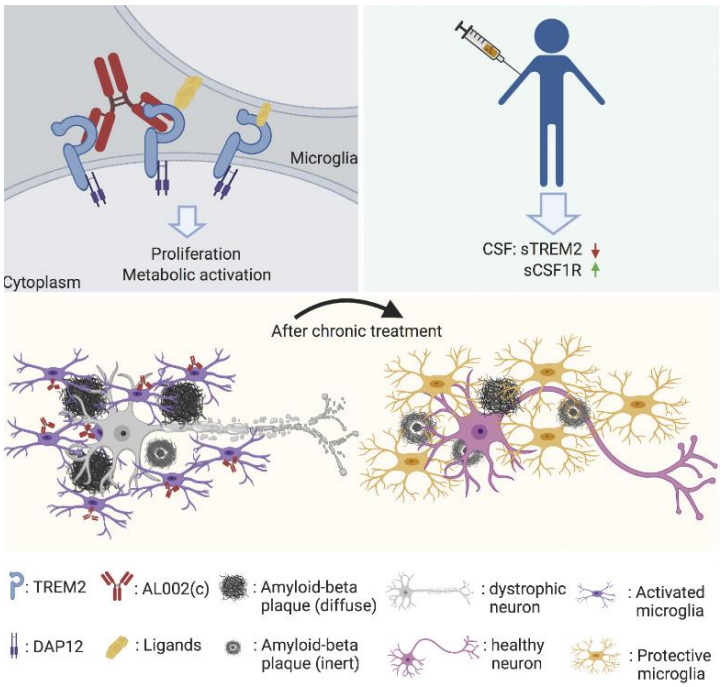


Figure 4. Mechanism of TREM2 Agonists for Treatment of Alzheimer's Disease

In contrast to Pionyr's TREM2 antagonist antibody PY314, antibodies in development for treatment of Alzheimer's disease and dementia are agonists that bind TREM2 to activate metabolism and proliferation of microglia. Alector's anti-TREM2 agonist antibody AL002c reduces plaques and activates microglia in mouse models. A phase 1 trial identified reduced soluble TREM2 (sTREM2) and increased soluble CSF1R (sCSF1R) as biomarkers of TREM2 engagement (15).

TREM2 agonists must cross the blood–brain barrier to stimulate microglial TREM2. Alector found that a small percentage (0.11% that of circulating plasma) of their systemically delivered antibody (AL002) was able to cross the blood–brain barrier in mice (15). To counteract the poor blood–brain barrier penetrance, these TREM2 agents for neurodegenerative diseases are often delivered at high concentrations (up to 60 mg/kg) or include transporter domains that facilitate transport across the blood–brain barrier.

CLINICAL TRIALS

A phase 1a/b trial is underway to characterize the safety and tolerability of PY314 as a single agent, and in combination with pembrolizumab (anti–PD1), in subjects with advanced refractory solid tumors, including those refractory to checkpoint inhibitors if approved for that indication (NCT04691375). The phase 1a portion of the study has evaluated PY314 (at 1, 3, 10, and 20 mg/kg) alone and in combination with 200 mg pembrolizumab in subjects with advanced solid tumors using a 3+3 dose escalation design, with subjects given intravenous doses once every 3 weeks.

Pionyr reported ASCO 2022 (Abstract no. 2648 and poster) that PY314 has been well tolerated, with an excellent safety profile as a single agent and in combination with pembrolizumab (16). A dose of 10 mg/kg has been selected as the recommended dose for an expansion study (phase 1b) in 6 tumor types.

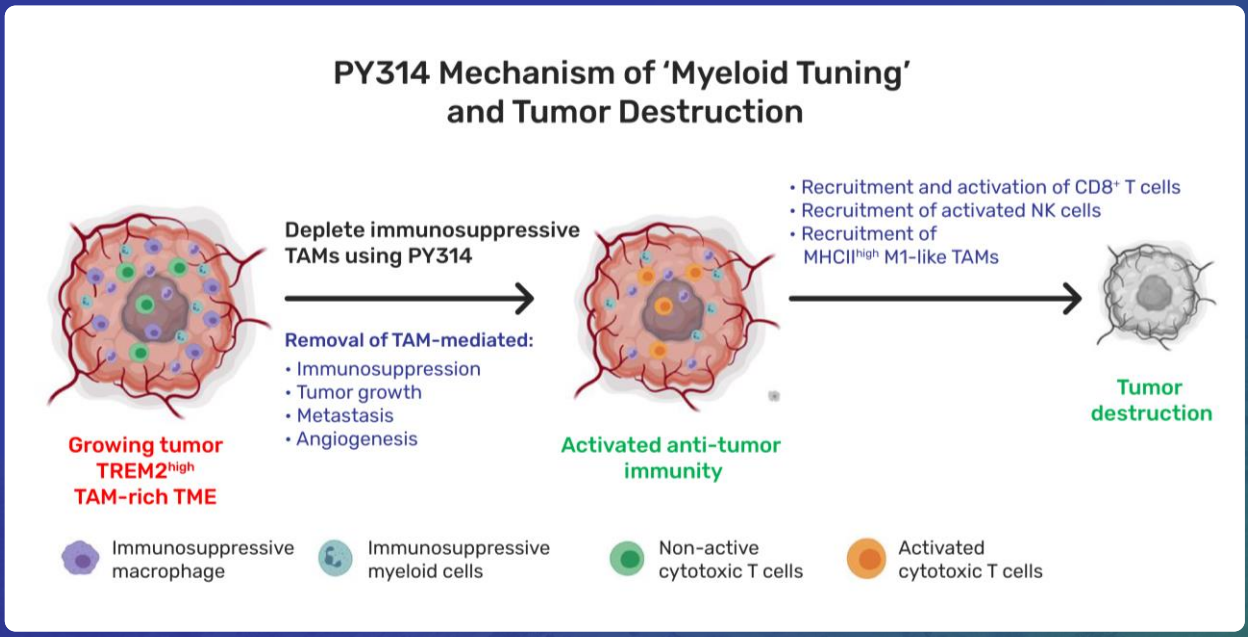
Several TREM2 agonist antibodies are, or will soon be, in clinical trials for neurodegenerative diseases. AL002 (Alector) was tested in a phase 1 trial of healthy adults and patients with mild–moderate Alzheimer's disease (NCT03635047), at doses up to 60 mg/kg. The researchers observed dose-dependent decreases in soluble TREM2 and CSF1R in cerebrospinal fluid, suggesting TREM2 engagement (15), and they observed increased levels of IL1RN and SPP1 in the cerebrospinal fluid, indicating microglial activation (AAIC poster, 2021). More recently, Alector announced in a press release that a small number of serious adverse events occurred among subjects with 2 alleles of a specific APOE mutation in their ongoing Phase 2 trial (NCT04592874). Alector is amending their phase-2 trial protocol to exclude subjects with these mutations.

Vigil's phase 1 trial of VGL101, in development for neurodegenerative diseases (**Table 1**), is being tested in healthy volunteers. Having completed a 20 mg/kg dose cohort in their single ascending-dose study, Vigil initiated a 20 mg/kg multiple ascending-dose cohort. However, Vigil recently announced that the FDA had placed a partial clinical hold on doses of its TREM2 agonist antibody above 20 mg/kg for its phase 2 trial in ALSP.

Denali's agent, DNL-919, in development for Alzheimer's disease (**Table 1**), uses a transporter vehicle to facilitate delivery of its agonist antibody across the blood–brain barrier. The phase 1 studies of this agent have been placed on clinical hold as Denali works to address the FDA's concerns about findings from preclinical toxicology studies (press release).

PY314, the afucosylated TREM2 antibody in development by Pionyr for cancer treatment, has a distinct mechanism and different dosing regimens than TREM2 agonists in development for treatment of neurodegenerative diseases. Pionyr reports at ASCO 2022 that PY314 has been well tolerated with an excellent safety profile as a single agent and in combination with pembrolizumab.

CONCLUSION



Several programs that target TREM2 are in development. However, it is important to recognize the distinct mechanisms of the therapeutic agents for cancer vs neurodegenerative diseases. Pionyr is developing an antibody against TREM2 (PY314), designed to deplete inhibitory TAMs and thereby stimulate an anti-tumor immune response in patients with cancer. For neurodegenerative diseases, other companies are developing antibodies, peptides, and small molecules that activate TREM2 in microglia to promote clearance of cell debris in the central nervous system and prevent neurodegeneration (15).

Pionyr is currently the only company in clinical development with a TREM2-depleting monoclonal antibody for oncology. To date, PY314 appears to be well tolerated as a single agent and in combination with pembrolizumab (ASCO 2022 abstract 2648) (16). Although there is at least one other company (Amphivena) with a TREM2 program for cancer therapy, it is still in preclinical investigation stages. Through myeloid tuning, PY314 has the potential to become a potent contributor to immunotherapies for multiple cancer types, including those resistant to checkpoint inhibitors.

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