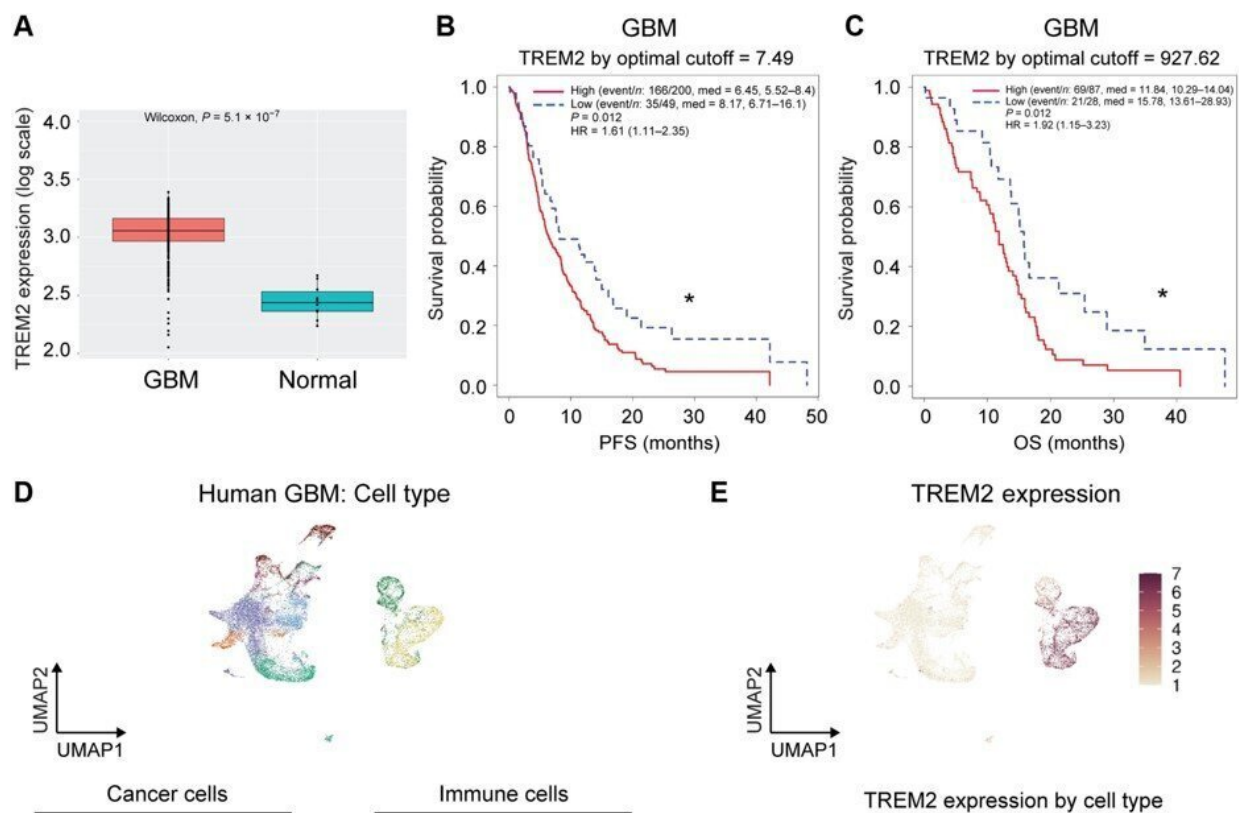


# Antitumor cell activity in glioblastoma regulated by inhibiting triggering receptor expressed on myeloid cells 2

May 29 2023, by Thamarasee Jeewandara



TREM2 expression is higher in GBM tumors than normal brain tissue and inversely correlates with patient outcome. (A) Box plots of TREM2 expression in IDH1/2–wild-type GBM tumors (n = 252) and normal brain specimens (n = 10). Wilcoxon test,  $P = 5.1 \times 10^{-7}$ . (B) Kaplan-Meier curves show a PFS of 249 patients with IDH1/2–wild-type GBM based on TREM2 expression. TREM2 expression was dichotomized into high/low according to the optimal cutoff. Log-

rank test,  $P = 0.012$ . (C) Kaplan-Meier curves show an OS of 115 patients with IDH1/2-wild-type GBM by high and low TREM2 expression. Patients were divided on the basis of the optimal cutoff of TREM2 expression. Log-rank test,  $P = 0.012$ . (D) UMAP plots of cell clusters identified in human GBM tumors. (E) UMAP and dot plots of TREM2 expression level in each cluster. AC, astrocyte; MES, mesenchymal; OPC, oligodendrocyte progenitor. \*P *Science Advances* (2023). DOI: 10.1126/sciadv.ade3559

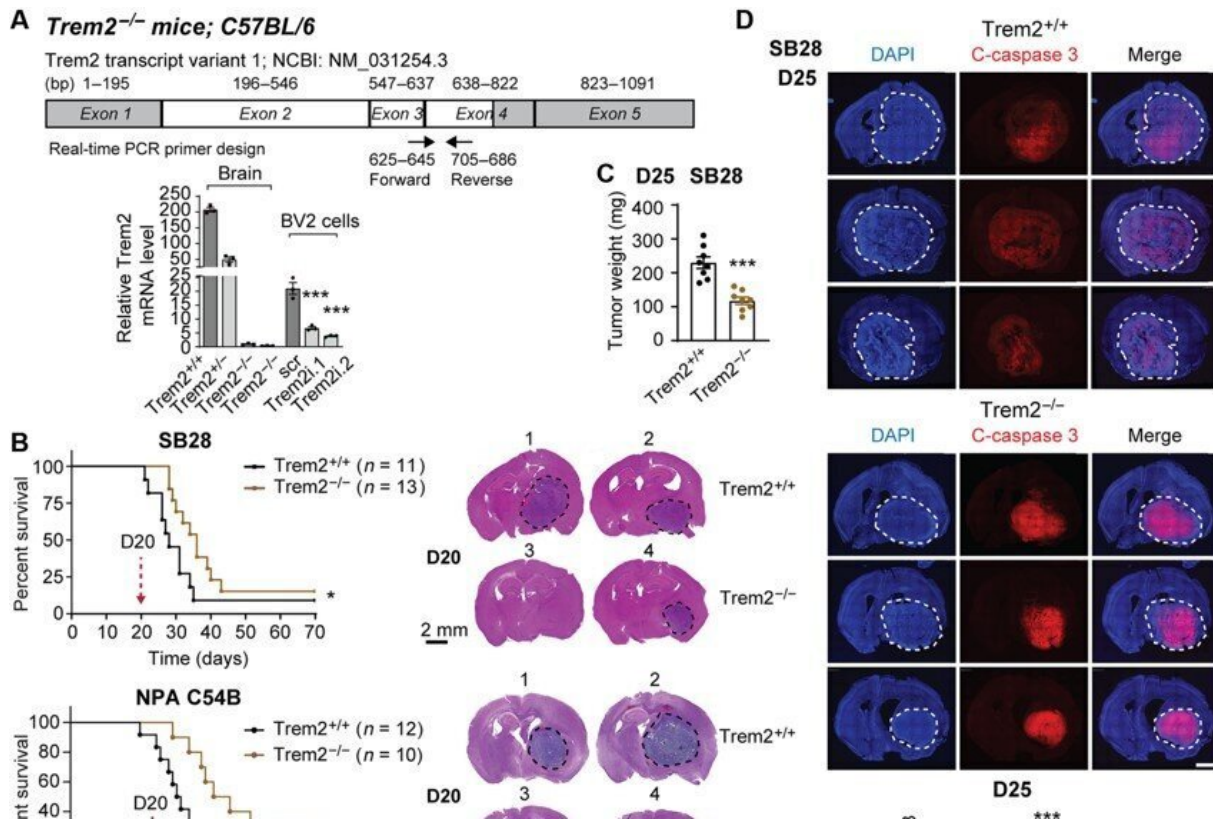
While the importance of the triggering receptor expressed on myeloid cells 2 (TREM2) is known in brain microglial function during neurodegenerative diseases, the function of the TREM2 cell receptor/protein is unknown in glioblastoma tumors. In a new report in *Science Advances*, Rui Sun, and a research team in the departments of neurosurgery, pathology, anesthesiology and developmental biology at the Washington University School of Medicine, in the U.S., found the cell receptor to be highly expressed in myeloid subsets including macrophages and microglia, by using examples of human and mouse glioblastoma tumors.

When the bioengineers induced TREM2 loss-of-function experiments in human [macrophages](#) and mouse [myeloid cells](#) they increased [interferon- \$\gamma\$ -induced immunoactivation](#), proinflammatory polarization and tumoricidal capacity. The team used mouse models of glioblastoma to show how the chronic and acute TREM2 loss-of-function resulted in decreased tumor growth and improved survival. Inhibiting this cell receptor led to increased production of the [programmed cell death protein](#) and [CD8<sup>+</sup> T cells](#) in the tumor microenvironment. The deficiency of TREM2 therefore improved the effectiveness of anti-programmed cell death protein-1 (PD-1) treatment in preclinical experiments as a viable therapeutic strategy for intervention in patients with glioblastoma.

## Treatment strategies for glioblastoma tumors

Glioblastoma is a primary malignant brain tumor with short-term survival in patients, despite the availability of a range of aggressive, multimodal [therapeutic strategies](#). Treating the tumor microenvironment is challenging due to its "cold" nature, thereby preventing T-cell infiltration as well as pro-inflammatory responses to render the tumor resistant to immunotherapies such as [immune checkpoint blockade](#).

Myeloid cell populations including macrophages and microglia account for more than 50% [leukocyte](#) enriched in the tumor microenvironment, although they rarely exert tumoricidal functions—while maintaining an immunosuppressive environment instead. Oncologists and bioengineers therefore seek to develop new strategies to treat glioblastoma.



Trem2 inhibition in vivo inhibits tumor growth and improves animal survival. (A) Trem2<sup>-/-</sup> mice genotype verified by real-time quantitative polymerase chain reaction (RT-qPCR) (n = 3). Primers' loci in Trem2 mRNA were shown. Trem2 mRNA detected in brain tissue from Trem2<sup>+/+</sup>, Trem2<sup>+/-</sup>, and Trem2<sup>-/-</sup> (Trem2<sup>-/-</sup><sub>1</sub> and Trem2<sup>-/-</sup><sub>2</sub>) littermate mice. Control BV2 (scr) and Trem2 knockdown BV2 cells (Trem2i.1 or Trem2i.2) served the control. Comparisons performed as indicated. NCBI, National Center for Biotechnology Information. (B) Trem2<sup>-/-</sup> mice showed increased neurological deficit-free survival time than Trem2<sup>+/+</sup> mice in both SB28 and NPA C54B GBM models (left). Mice were euthanized at day 20 after tumor implantation. Representative histological images showing tumor size in Trem2<sup>+/+</sup> (1 and 2) and Trem2<sup>-/-</sup> (3 and 4) mice (right). Scale bars, 2 mm. (C) Tumor weight. SB28-bearing mice were euthanized at day 25. Whole-tumor mass was dissected and weighed (n = 8 per genotype). (D) Apoptotic cells in tumor tissue by cleaved caspase-3 (C-caspase 3) immunostaining (n = 3 per genotype). Quantified apoptosis shown as mean fluorescence intensity of tumor area (nuclei, DAPI) divided by C-caspase 3 intensity (red). Scale bar, 2 mm. (E) Acute Trem2 reduction by intracerebroventricular (ICV) injection of ASOs increased OS in GBM-bearing Trem2<sup>+/+</sup> mice. SB28, n = 10 per condition; NPA C54B, n = 6 per condition. Schematic showing experimental design (top). Kaplan-Meier curves demonstrated increased survival in animals injected with Trem2 ASO versus control ASO (inactive) (left). Mice were euthanized at day 20 after tumor implantation. Representative histological images showing tumor size (right). Scale bars, 2 mm. (F) Anti-Trem2 treatment increased animal survival in GBM-bearing mice (n = 5 per condition). Trem2<sup>+/+</sup> mice were injected intracerebrally (IC) with SB28 cells and treated intraperitoneally (i.p.) with anti-Trem2 or isotype antibody (200 μg per mouse). Experimental design (top). Survival curves (bottom). Data represent means ± SEM. \*P Science Advances (2023). DOI: 10.1126/sciadv.ade3559

## **Role of triggering receptor expressed on myeloid cells 2 (TREM2)**

Neuroscientists have extensively studied the triggering receptor

expressed on myeloid cells 2 (TREM2) for its function in microglia in neurodegenerative diseases such as [Alzheimer's disease](#). Recent studies have shown that specific solid tumors express a high level of TREM2 receptor/protein, although its role in cancer appears to depend on the tumor-type. While the TREM2 receptor/protein suppressed the progression of malignant colorectal cancer and afforded protection from [hepatocellular carcinoma](#), its increased expression in non-small cell lung and gastric tumors resulted in [poor prognosis](#).

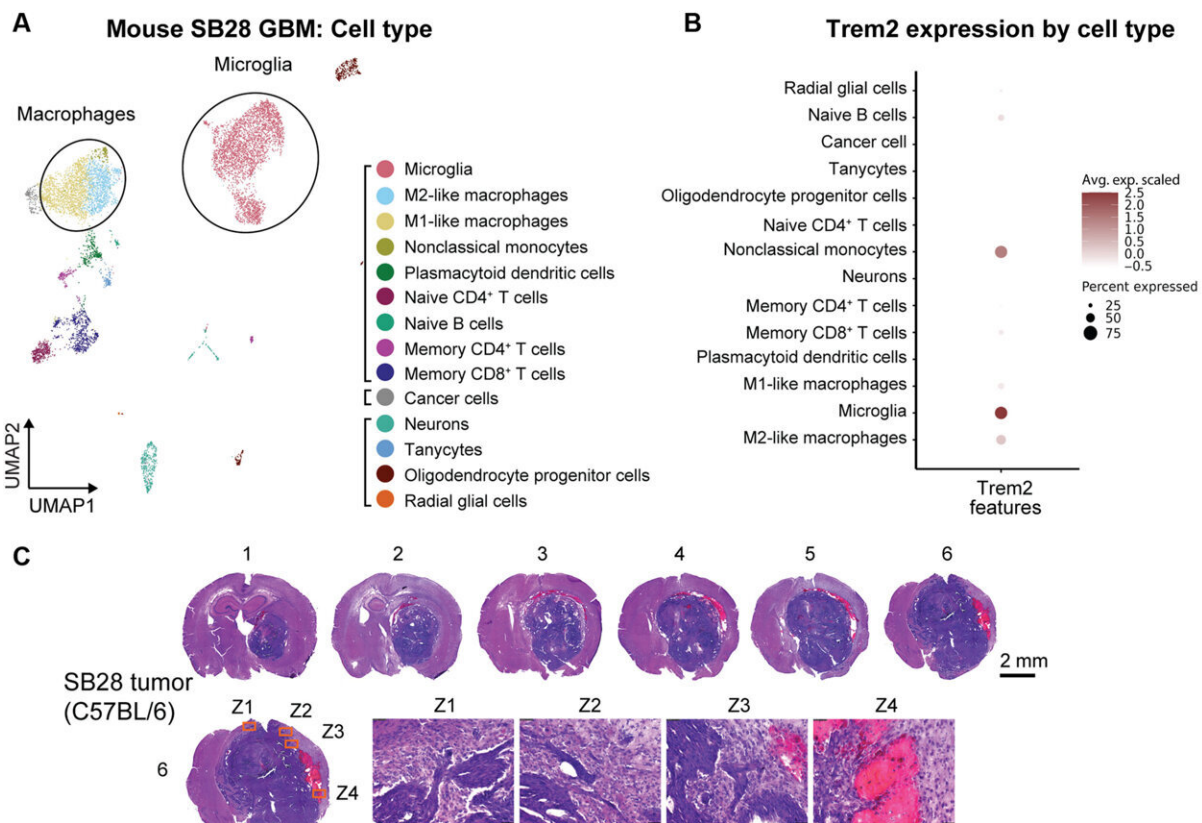
Research evidence has shown that the receptor of interest is widely expressed in tumor-associated macrophages, and its loss-of-function altered tumor macrophage behavior within solid tumors of the body for [improved immune checkpoint blockade](#). Nevertheless, much remains to be known of the role of TREM2 during glioblastoma progression including the mechanisms underlying its capacity to regulate [myeloid cell function](#).

## **TREM2 expression is higher in glioblastoma tumors when compared to normal brain tissue**

Sun and colleagues studied the clinical relevance of TREM2 in human glioblastoma by analyzing if its expression was altered in glioblastoma tumors; typically classified as [IDH1/2-wild type tumors](#) in the [cancer genome atlas](#). The mRNA levels of the TREM2 receptor were significantly higher in wild-type glioblastoma tumors, compared to normal brain tissue. In [gliomas](#) in general, the expression of the receptor correlated with the cancer grade, where the highest levels were found in grade 4 gliomas. The team studied if the TREM2 levels were associated with clinical outcomes by classifying the glioblastoma tumor type into high/low TREM2 expression categories.

They studied the specific cell types expressing TREM2 in tumor

patients, by examining [single-cell RNA sequencing datasets](#) and using [unsupervised clustering by uniform manifold approximation and projection](#). They thereby identified immune cell markers and hypothesized the TREM2 expressing tumor-associated myeloid population to play a significant role during glioblastoma tumor progression.



Mouse GBM tumor shows abundant Trem2<sup>+</sup> myeloid cells in the TME. (A) UMAP plots of cell type clusters in mouse SB28 model of GBM in vivo. (B) Dot plots of TREM2 expression level in each cell cluster. (C) Representative histology of brain GBM (SB28). Cryosections of 30  $\mu$ m thickness (1 to 6) by hematoxylin and eosin staining (top). Scale bar, 2 mm. Histological images for invasive tumor front on section 6: Z1, Z2, Z3, and Z4. Scale bar, 50  $\mu$ m. (D) Representative immunostaining images. Whole-brain sections (left). Red, Iba1; green, SB28 tumor cells (tracking dye); blue, nucleus

[4',6-diamidino-2-phenylindole (DAPI)]. Scale bar, 2 mm. Iba1+ myeloid cells around and inside the tumor (right). Arrows indicate SB28 cells (yellow) and Iba1+ cells (white). Scale bars, 100  $\mu$ m. Credit: *Science Advances* (2023). DOI: 10.1126/sciadv.ade3559

## Regulating glioblastoma tumors with myeloid cells

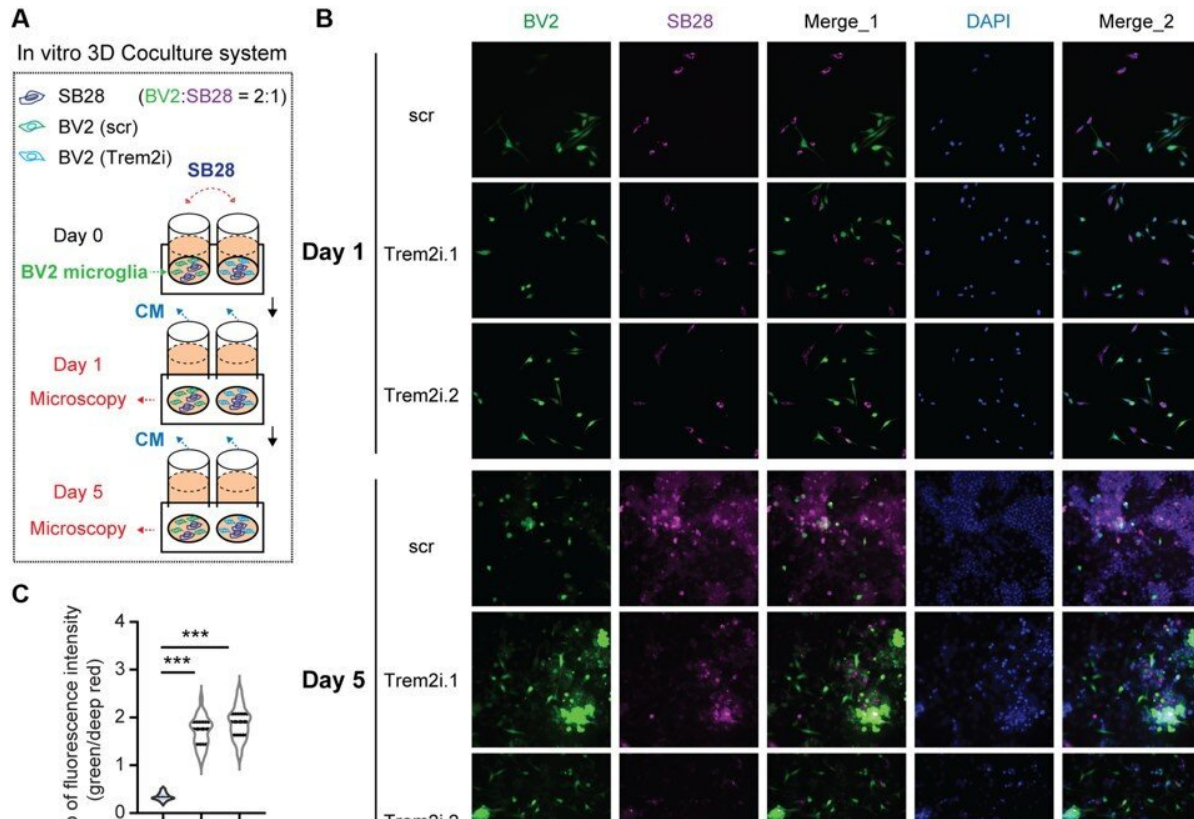
The scientists explored the role of TREM2 by using [glioblastoma mouse models](#) recapitulating characteristics of human glioblastoma, including resistance to [radiotherapy and chemotherapy](#), resistance to [immune checkpoint blockade](#), alongside a high fraction of myeloid cells in the tumor microenvironment. As with human tumors, single-cell RNA sequence results revealed predominant macrophage/monocyte lineages and microglial cells.

The microglia showed markedly high levels of Trem2 expression among a range of anti-inflammatory cell lines. They noted M1-like interferon- $\gamma$  induced cells to maintain relatively low-levels of TREM2 expression. The team used transgenic mice deficient to the [cell receptor](#) (Trem2<sup>-/-</sup>) and noticed more apoptotic cells in such tumors, indicating that its deficiency inhibited tumor growth by increasing tumor cell death.

## Additional TREM2 loss-of-function experiments in mouse and human glioblastoma models

The scientists conducted a series of experiments to dissect the role of [myeloid cells](#) during glioblastoma progression by using bone-marrow derived macrophages from transgenic mice deficient to TREM2 expression. The results showed increased phagocytic function and cytotoxicity towards the glioblastoma cell line. When they tested TREM2 knockdown in human macrophages, they noted an inclination

for enhanced tumor cell death in patient-derived glioblastoma stem cells. The researchers further tested and validated the human model system of patient-derived glioblastoma stem cells in the lab.



Trem2 loss-of-function myeloid cells show antitumor effects both in vitro and in vivo. (A to C) Trem2 knockdown in BV2 microglia by Trem2-targeted shRNA lentivirus transduction (Trem2i.1 and Trem2i.2). Nonsilencing shRNA used as control (scr). BV2 and SB28 cells were stained with green and deep-red tracking dye, respectively, and cocultured in vitro. (A) Timeline schematic for treatment strategy. (B) Representative images. Green, BV2; purple, SB28; blue, nucleus (DAPI). Scale bar, 100  $\mu$ m. (C) Violin plot indicating the ratio of mean fluorescent intensity (green/purple) at day 5 (quantified for 30 fields per condition). (D) GBM model of intracranial (IC) coimplantation of BV2 microglia and SB28 cells. Neurological deficit-free survival curves (right; n = 5 per group). WT, wild-type. (E) GBM model of BMDMs and SB28 coinjection. Neurological deficit-free survival curves (right; n = 4 per group). Data represent



means  $\pm$  SEM. \*P Science Advances (2023). DOI: 10.1126/sciadv.ade3559

When they used [microglia](#), i.e., brain resident macrophages distinct from those arising from the blood lineage, they noted TREM2 deficiency increased phagocytic function, tumoricidal activity and a pro-inflammatory phenotype in mouse models. The myeloid cells with loss-of-function showed anti-tumor effects both in vitro and in vivo, while reprogramming tumor-associated myeloid populations towards a pro-inflammatory phenotype. Additional observations showed TREM2 deficiency to increase T cells in the [tumor microenvironment](#), while combining the programmed cell death protein for therapeutic strategies and improved animal survival. The deficiency of TREM2 further improved the effectiveness of anti-programmed cell death protein-1 (PD-1) treatment in preclinical experiments.

## Outlook

In this way, Rui Sun and colleagues noted higher expression of TREM2 in [glioblastoma](#) tumors associated with patients experiencing worse survival rates. They noted increased TREM2 expression in [myeloid cells](#) populations within [tumor](#) microenvironments, including microglia and macrophage subsets.

The team carried out extensive experiments in vitro and in vivo in human and mouse model systems to show effects of TREM2 inhibition on [tumor growth](#) reduction, with the promise to formulate additional therapeutic opportunities in the future.

**More information:** Rui Sun et al, TREM2 inhibition triggers antitumor cell activity of myeloid cells in glioblastoma, *Science Advances* (2023). [DOI: 10.1126/sciadv.ade3559](https://doi.org/10.1126/sciadv.ade3559)

Cyril Neftel et al, An Integrative Model of Cellular States, Plasticity, and Genetics for Glioblastoma, *Cell* (2019). [DOI: 10.1016/j.cell.2019.06.024](https://doi.org/10.1016/j.cell.2019.06.024)

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