**Big Question**

Compare **DGAT1 vs. DGAT2** in **cancer (especially glioblastoma, GBM)** and how they influence the **immune response**.

**Approach**

1. **Manipulate DGAT1/2**
   * Use **KD/KO (knockdown/knockout)** with sgRNA (CRISPR) or shRNA.
   * Specifically target **DGAT1** and **DGAT2** separately.
2. **Cell Lines (GBM & others)**
   * Mouse GBM lines: **SB-28, CT-2A**.
   * Human GBM lines: **U305, G261, OV** (possibly ovarian, but here looks like GBM + others).
   * Also general cancer models.
3. **Readouts**
   * **Lipid storage**: LDs, triglycerides.
   * **Mitochondrial stress**: ROS, apoptosis.
   * **Tumor growth in vivo**: inject modified cells into **C57BL/6 mice**.
4. **Tumor vs. Immune**
   * Observe tumor growth (tumor shrinkage if DGAT1/2 are lost).
   * Track immune response: macrophages, microglia, T cells.
5. **Special Focus**
   * **Macrophages & microglia**: Does DGAT1/2 loss change their activation (M1 vs M2)?
   * **Cancer cells vs. immune cells**: Is DGAT1 more important for tumor survival? Is DGAT2 more relevant for immune cells?

**Expected Hypothesis Flow (based on notes + prior papers)**

* **DGAT1 loss in GBM cells** → fewer lipid droplets → more ROS → apoptosis → **smaller tumors**.
* **DGAT2 loss** → less clear (maybe compensates in some tissues; role in immune regulation less studied).
* **Immune cells (macrophages, microglia)**:
  + DGAT1 inhibition may lower inflammatory cytokines OR shift towards more Tregs (immunosuppression context-dependent).
* **In mice**: Compare tumor burden vs immune infiltration when DGAT1 or DGAT2 is knocked down.

**🗂️ How You Could Organize This Into a Workflow Diagram**

**Step 1: Gene Manipulation**

* sgRNA/shRNA → DGAT1↓ or DGAT2↓

**Step 2: Model Systems**

* In vitro: mouse/human GBM cell lines (SB-28, CT-2A, G261, U305, OV).
* In vivo: implant into C57BL/6 mice.

**Step 3: Measurements**

* Tumor growth.
* Lipid metabolism (LD formation, TGs).
* Stress & apoptosis (ROS, cytochrome c, caspases).
* Immune profiling (macrophages, microglia, T cells).

**Step 4: Compare DGAT1 vs DGAT2**

* Which one drives tumor growth more?
* Which one shapes immune cell behavior more?
* Are effects synergistic or distinct?

✅ This structure basically links **cancer metabolism (DGAT1/2) → tumor biology → immune response**.

**🧰 Master List: What You Might Need for DGAT1/2 Project**

**1.**

**Genetic Tools**

* **Lentiviral shRNA vectors**
  + pLKO.1-puro backbone (Sigma MISSION)
  + shDGAT1-1 / shDGAT1-2 (sequence in Supplement)
  + shDGAT2-1 / shDGAT2-2 (sequence in Supplement)
  + shNTC (non-target control)
* **Packaging plasmids**
  + psPAX2 (2nd gen packaging)
  + pMD2.G (VSV-G envelope)
* **Transfection reagents**
  + Polyethylenimine (PEI) or Lipofectamine 2000/3000
* **Concentration kit**
  + Lenti-X Concentrator (Takara)
* **Infection reagent**
  + Polybrene (8 µg/mL)

**2.**

**Pharmacological Compounds**

* **DGAT1 inhibitor (cell use):** A-922500 (20 µg/mL in vitro)
* **DGAT2 inhibitor:** PF-06424439 (20 µg/mL in vitro)
* **DGAT1 inhibitor (in vivo):** A-900225 (120 mg/kg/day PO; check vendor/availability)
* **Rescue controls**
  + N-acetyl-cysteine (NAC, 1 mM)
  + Etomoxir (CPT1 inhibitor, 6 µM)
  + Acylcarnitines (20 µM, mimic FA overload)

**3.**

**Western Blot Antibodies**

* **DGAT1** — Santa Cruz sc-32861
* **CPT1A** — Abcam ab128568
* **CPT1B** — Abcam ab104662
* **Cytochrome c** — BD 556433
* **Cleaved Caspase-3 (Asp175)** — CST 9661
* **Cleaved Caspase-9 (Asp330)** — CST 9501
* **PARP (46D11)** — CST 9532
* **BiP/GRP78 (C50B12)** — CST 3177
* **CHOP (L63F7)** — CST 2895
* **COX IV (3E11)** — CST 4850
* **PDIA1 (H-17)** — Santa Cruz sc-30932
* **β-Actin (loading control)** — Sigma A1978
* **TIP47 / Perilipin-3 (LD marker)** — Abcam ab47638

**Secondary antibodies**

* Anti-mouse HRP — CST 7076
* Anti-rabbit HRP — CST 7074
* Alexa Fluor secondaries (488, 568) — Thermo A-11034, A-11036, A-11004

**4.**

**qPCR Setup**

* **Housekeeping gene:** 36B4 (RPLP0)
* **Target primers** (from Table S1 of paper — order exact sequences):
  + DGAT1
  + DGAT2
  + CPT1A
  + CHOP
  + BiP
  + (plus any cytokines you want to test: IL-6, TNFα, IFNβ, etc.)
* **Reagents**
  + TRIzol (RNA extraction)
  + iScript cDNA synthesis kit
  + SYBR Green PCR Master Mix (ABI)
* **Instrument:** ABI 7900HT

**5.**

**Dyes, Stains, Kits**

* **BODIPY493/503** — lipid droplet staining (IF, microscopy)
* **MitoTracker Red** — mitochondrial morphology
* **CellROX Deep Red** — ROS detection
* **ECL reagent** — for WB detection
* **Seahorse XF reagents** — Oligomycin, FCCP, Rotenone/Antimycin A (for OCR assays)

**6.**

**Cell Models**

* **Human GBM lines**: U251, U87/EGFRvIII
* **Patient-derived GBM**: GBM30, GBM30-luc (for intracranial BLI)
* **Other lines mentioned in PI’s notes**: SB-28, CT-2A, G261, M005 (mouse glioma lines)

**7.**

**Animal Models**

* **C57BL/6 mice** — for syngeneic models (SB-28, CT-2A)
* **Nude or NOD-SCID mice** — for human xenografts (U87, GBM30)
* **Intracranial implantation** setup — stereotactic injector + luciferase imaging system (if using luc-tagged cells)

**The Story of DGAT1 in Immunology**

Think of **DGAT1** as a **storage manager** in immune cells. Its main job is to **pack fatty acids into triglycerides and store them safely in lipid droplets (LDs)** — like putting extra food into a fridge so it doesn’t spoil.

This storage affects how immune cells behave in inflammation, infection, and disease.

**🦠 1.**

**Macrophages: The Big Eaters**

* Normally, macrophages don’t store many fats.
* But when they sense **danger (LPS, IFN-γ)**, they **build lipid droplets** to handle stress.
* **DGAT1 is needed** for these triglyceride stores.
* If you block DGAT1:
  + Less TG and LDs form.
  + Less **pro-inflammatory cytokine** production (weaker “alarm”).
  + Also less **PGE2**, a lipid messenger.
* In a mouse sepsis model, blocking DGAT1 **reduced inflammation**.

👉 **Takeaway**: DGAT1 helps macrophages build up energy and signals for a strong inflammatory response. Blocking it calms inflammation.

**🌬️ 2.**

**Innate Lymphoid Cells (ILCs): The Barrier Guards**

* ILC2s, found in skin, lung, and gut, need fatty acids to survive stress.
* During infection or allergen exposure (e.g., fungus *Alternaria*), ILC2s **store fats in lipid droplets**.
* This storage depends on DGAT1.
* If DGAT1 is blocked, ILC2s can’t store fats → they die from **lipotoxicity**.

👉 **Takeaway**: DGAT1 protects ILC2s in inflamed tissues; without it, they are more vulnerable.

**🧑‍🤝‍🧑 3.**

**T Cells: The Decision-Makers**

* T cells need fatty acids for different roles:
  + Effector T cells (Th1, Th2, Th17) = **fat builders**.
  + Memory T cells & Tregs = **fat burners**.
* DGAT1 influences **retinoid metabolism**:
  + Normally DGAT1 stores vitamin A as **retinyl esters**.
  + Without DGAT1, more **retinoic acid (RA)** forms.
* RA tilts the balance toward **more Tregs** (regulatory T cells).
* In a mouse MS model (EAE):
  + DGAT1 KO or inhibitor treatment = fewer symptoms, more Tregs.
* But results are mixed:
  + Some studies show blocking DGAT1 increases Tregs.
  + Others show it decreases FoxP3+ Treg numbers.
  + Th17 response (IL-17 secretion) is also variably affected.

👉 **Takeaway**: DGAT1 affects how T cells differentiate by controlling retinoid levels, which shifts the immune balance.

**🧪 4.**

**Neutrophils: The First Responders**

* Neutrophils express the **highest DGAT1 levels** among immune cells.
* They normally store triglycerides and form lipid droplets when activated.
* In psoriasis models:
  + DGAT1 deficiency did **not stop lipid droplet accumulation**, but…
  + It reduced **neutrophil migration** to inflamed skin.
* Why? Because without DGAT1, more RA builds up → RA acts like a **signal jammer** and **slows neutrophil chemotaxis**.

👉 **Takeaway**: DGAT1 doesn’t just store fat — it controls RA levels, which decides how actively neutrophils move to inflammation sites.

**🎯 Overall Message**

DGAT1 is more than a fat-storage enzyme — it’s an **immunomodulator**.

* In **macrophages**, it fuels pro-inflammatory cytokines.
* In **ILC2s**, it protects against lipotoxicity.
* In **T cells**, it tilts the balance toward Tregs or effector cells, depending on RA levels.
* In **neutrophils**, it regulates migration to inflamed tissues.

👉 Whether DGAT1’s role is **good or bad** depends on the context:

* Blocking it can **reduce harmful inflammation** (sepsis, autoimmunity).
* But it may also **weaken protective responses** (tumor killing, pathogen clearance).

**1.**

**DGAT1 is up in cancers**

* Many tumors (glioblastoma, ovarian, breast, gastric, pancreatic, etc.) show **higher DGAT1 expression**.
* More DGAT1 = more triglycerides and lipid droplets (LDs).
* Patients with **high DGAT1** generally have a **worse prognosis** (poorer survival).

👉 Analogy: DGAT1 acts like a “storage manager” that lets tumors safely stash away fat. The more storage space they have, the more aggressively they grow.

**2.**

**Why tumors need DGAT1**

* Cancer cells take in and make lots of fatty acids (fuel + building blocks).
* Without DGAT1, these free fatty acids would overwhelm cells → toxic.
* With DGAT1, tumors **pack them into droplets**, avoiding lipotoxicity and oxidative stress.
* This **protects tumor mitochondria**, keeping the cancer alive and resistant to stress/therapy.

**3.**

**DGAT1 and immune suppression**

Here’s where it gets interesting — lipid droplets aren’t just helping tumor cells, they’re also **messing with immune cells**:

* **Tumor microenvironment = fatty, stressful**
  + Excess fatty acids spill over into immune cells infiltrating the tumor.
  + This overload **impairs immune attack** (CD8+ T cells, NK cells).
  + At the same time, it **boosts suppressor cells** (Tregs, TAMs, MDSCs).
* **Specific immune cells affected**:
  + **TAMs (tumor-associated macrophages, CD206+)**:
    - Load up on LDs.
    - Become more suppressive (anti-inflammatory M2-like).
    - Promote tumor growth & metastasis.
  + **Dendritic cells**:
    - Accumulated lipids block peptide–MHC class I presentation.
    - Means **CD8+ T cells don’t get activated** → weak anti-tumor immunity.
  + **MDSCs (myeloid-derived suppressor cells)**:
    - High DGAT1 expression → more lipid droplets → stronger suppression of T cell activity.

👉 Analogy: Tumor lipids are like “sugar-coated bullets” — they disarm the soldiers (CD8+ T cells, NK cells) and instead feed the “peacekeepers” (TAMs, Tregs, MDSCs) who calm the fight.

**4.**

**What happens if you block DGAT1 (A922500 inhibitor)?**

* In tumor cells:
  + LD formation blocked → fatty acids pile into mitochondria → ROS burst → apoptosis → smaller tumors.
* In immune cells:
  + **TAMs**: fewer LDs → less mitochondrial respiration → less suppressive → can’t protect tumor.
  + **CD8+ T cells**: infiltration ↑ and activity ↑ (better killing).
  + **MDSCs**: neutral lipid load ↓ → weaker immunosuppressive activity.

👉 Blocking DGAT1 both **kills cancer cells** *and* **frees immune cells** from suppression.

**5.**

**Big picture**

* DGAT1 is a **double enabler**:
  + Helps tumor cells survive fatty environments.
  + Turns infiltrating immune cells into **suppressors instead of fighters**.
* Inhibition of DGAT1:
  + **Weakens tumors directly** (lipotoxicity).
  + **Strengthens immune response indirectly** (fewer suppressive TAMs/MDSCs, more active CD8+ T cells).

👉 That’s why DGAT1 inhibitors are being considered not just as “metabolic drugs” but also as **immunotherapy helpers**.

Oleszycka, E., Kwiecień, K., Grygier, B., Cichy, J., & Kwiecińska, P. (2025). The many faces of DGAT1. *Life Sciences*, *362*, 123322. <https://doi.org/10.1016/j.lfs.2024.123322>