Proceedings of the 6th International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/2026.38136

Combined GPR81 Blockade and STING Agonism Restore CD8⁺ T Cell–Mediated Tumor Control in Triple-Negative Breast Cancer

Sungjae Lee[1,†], Qingyu Che[2,*,†], Alex Pang[3,†], Kevin Yang[4,†], Rain Lin[5,†]

[1]Hangzhou International School, Hangzhou, China
[2]Institute of Epidemiology & Health Care, University College London, London, UK
[3]International School of Nanshan Shenzhen, Shenzhen, China
[4]Saint Anne's-Belfield School, Charlottesville, USA
[5]Jericho High School, Jericho, USA
**Corresponding Author. Email: freedom.che.25@ucl.ac.uk*
[†]These authors contributed equally to this work and should be considered as co-first authors

Abstract. Triple-negative breast cancer (TNBC) creates an immunosuppressive tumor microenvironment (TME) that limits the efficacy of checkpoint immunotherapy. Tumor-derived lactate activates GPR81 signaling on tumor-associated macrophages (TAMs), while the STING pathway, which normally drives innate immune activation, remains suppressed, together maintaining TAMs in an immunosuppressive state and limiting CD8⁺ T cell activity. This study aims to test whether combined GPR81 blockade and STING activation can synergistically repolarize TAMs and restore CD8⁺ T cell–mediated tumor control. Using a syngeneic 4T1 mouse model of TNBC, we will compare vehicle, single-agent, and combination treatment groups, alongside genetic GPR81 deletion and CD8⁺ T cell depletion to establish pathway specificity. We predict combination therapy will produce greater macrophage repolarization, CD8⁺ T cell infiltration, and tumor regression than either agent alone. These findings would support dual metabolic and innate immune targeting as a strategy to overcome immunotherapy resistance in breast cancer.

Keywords: Triple-negative breast cancer, GPR81, Lactate signaling, STING pathway, Tumor-associated macrophages

1. Introduction

Breast cancer is the most frequently diagnosed cancer and the second leading cause of cancer-related deaths among women in the United States. About 321,910 new cases and 42,140 deaths are expected there in 2026 [1]. It arises when some breast cells begin growing out of control and form masses called tumors, which can remain within the ducts or lobules or break through and enter nearby tissues. They may then travel to regional lymph nodes and even to distant sites [2]. Although precisely what causes this disease remains unclear, two well-known risk factors include advanced age as well as certain mutated versions of BRCA1 and BRCA2 inherited from parents [1]. In terms

© 2026 The Authors. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

DOI: 10.54254/2753-8818/2026.38136

of clinical classification according to ER, PR, and HER2 status, triple-negative breast cancer (TNBC) has none and hence tends to behave more aggressively while also having access to fewer targeted therapeutic alternatives [3].

There is standard treatment consisting of surgery, radiation, and systemic therapies adapted to each subtype: endocrine therapy if the cancer has hormone receptors and therapy targeting HER2 if HER2 is positive in the neoplastic cells. When metastasis is present, the treatment is mainly systemic. Cure is no longer possible, and the goal is instead to control the disease [2]. There is also a new treatment based on PD-1/PD-L1 inhibitors for some cases of triple-negative breast cancer, although responses are rare, and many patients develop resistance soon after starting this therapy. An important reason is probably that immune suppression caused by elements found in most types of TME makes it difficult for helpful antitumor immunity to work effectively in general [4].

There is one important contributor to this kind of immunosuppression: changes in tumor metabolism itself. By carrying out aerobic glycolysis, breast cancers generate large amounts of lactate, which they then release. Far from being just another metabolite, however, lactate acts as a signal. It attaches to GPR81 on immune cells, thereby inhibiting antigen presentation and decreasing the amount of proinflammatory cytokine produced by these cells [5]. Meanwhile, something else occurs: although the cGAS-STING pathway can usually stimulate innate immunity and thus produce very strong anticancer reactions if activated within the TME [6], it actually tends not to work well in most cases involving TAMs. Therefore, there will probably not be enough stimulation of innate immunity, leading instead to promotion of a pro-tumor state [7]. Consequently, too little priming occurs on the part of tumor-reactive $CD8^+$ T cells. Immunotherapy does not help much.

Whether lactate/GPR81 signaling and STING suppression converge on TAMs through a shared mechanism, or act through independent pathways that could be targeted together, remains untested. To address this gap, we propose a combination strategy that simultaneously targets metabolic and innate immune signaling: pharmacological blockade of the lactate receptor GPR81 together with activation of the STING pathway, with the aim of reprogramming TAMs from an immunosuppressive M2-like phenotype toward a pro-inflammatory M1-like state that reactivates $CD8^+$ T-cell priming and cytotoxic function.

In the following section, we summarize three primary research articles that our group selected as the foundation for this project, spanning lactate-mediated immune suppression, STING-dependent macrophage reprogramming, and combination immunotherapy targeting the lactate/GPR81 pathway.

2. Research articles the analysis is founded on

2.1. Lactate–GPR81 signaling suppresses antigen presentation in the tumor microenvironment

In fact, GPR81 had previously been shown to act directly upon tumour cells, so Brown et al [5].wanted to know if lactate derived from tumours also acts on immune cells present within the TME via such a paracrine GPR81 mechanism. Indeed, they discovered that lactate does activate GPR81 on dendritic cells, thereby decreasing their MHC-II cell-surface expression and reducing the amount of IL-6 and IL-12 released by these activated dendritic cells. As a result, fewer naive $CD4^+$ T cells become stimulated, and therefore more tumour escape occurs. Nevertheless, this work involved looking solely at dendritic cells and $CD4^+$ T cells, thus raising two further issues still needing clarification: first, whether GPR81 signalling also leads to macrophage polarization toward the M2-like state; secondly, whether inhibition of GPR81 activity will suffice by itself to restore normal anti-tumour immunity or else requires a second intervention consisting of administering

DOI: 10.54254/2753-8818/2026.38136

some kind of STING agonist so as to restore innate sensing capabilities and allow recovery of $CD8^+$ T-cell killing.

2.2. STING activation reprograms tumor-associated macrophages toward anti-tumor function

Then there was Miao et al. [7] , who investigated whether immune responses against gastric cancers might benefit from interference with the STING pathway present in TAMs, and if they did benefit from such interference, what the relevant mechanism might be. According to their findings, STING has indeed helped improve anti-tumor immunity when activated within those particular types of macrophages; STING-modified macrophages acquire pro-inflammatory traits and gain the ability to destroy gastric carcinoma cells on their own, while mice carrying macrophages targeted by STING display reduced tumor size. Here too, however, antitumor activity depends upon a specific signal-transduction route, namely the one involving IL6R–JAK–STAT–IL24. Many problems evidently still need solving, though. There remains the puzzle of why both stimulation and deletion of STING produce an anticancer effect. Another problem raised is whether what occurs only in rodents will also occur in humans, and concerns what substances may bind STING itself under these circumstances.

2.3. GPR81 blockade enhances $CD8^+$ T cell–mediated anti-tumor immunity

Chen et al. [4] investigated whether inhibiting GPR81 could reduce tumor-driven immune suppression and enhance $CD8^+$ T-cell-mediated anti-tumor responses. They found that GPR81 inhibition lowered immune tolerance, increased $CD8^+$ T-cell function, decreased tumor progression, and reduced lactate-associated metabolism, suggesting that combining GPR81 blockade with checkpoint inhibition could meaningfully improve anti-tumor immunity. The study left open the question of whether GPR81 blockade would be similarly effective in other cancer types.

3. Joint project direction and working hypothesis

Brown et al. [5] showed that lactate suppresses antigen presentation through GPR81; Miao et al. [7] showed that STING activation reprograms suppressive macrophages into pro-inflammatory effectors; and Chen et al. [4] showed that blocking GPR81 enhances checkpoint blockade. No study, however, has tested whether combining GPR81 blockade with STING activation produces a synergistic effect on TAM reprogramming. Although Miao et al. [7] observed anti-tumor effects with both STING activation and knockdown, we selected pharmacologic STING activation because the broader body of work on TAM reprogramming and the agents currently in clinical development center on agonism [6, 8]. Together, these findings suggest that simultaneously disrupting lactate-mediated immune suppression while activating STING-dependent macrophage reprogramming may restore $CD8^+$ T-cell-mediated tumor control. Based on these observations, we developed the following mechanistic working model.

We propose that lactate produced by highly glycolytic tumor cells continuously activates GPR81 signaling in TAMs, maintaining these cells in an immunosuppressive M2-like phenotype that limits antigen presentation and suppresses $CD8^+$ T-cell activation. Pharmacological inhibition of GPR81 is predicted to interrupt this suppressive signaling pathway, while simultaneous activation of the STING pathway provides a pro-inflammatory stimulus that promotes macrophage repolarization toward an M1-like phenotype. We hypothesize that these pathways act largely independently, so that removing the suppressive lactate signal while adding an activating STING signal will synergistically

DOI: 10.54254/2753-8818/2026.38136

restore innate immune activation, enhance CD8⁺ T-cell priming, and improve tumor control; whether the two pathways are in fact independent, rather than converging on a shared node, is directly tested in the first experiment below.

In fact, aerobically glycolytic (Warburg-effect) cancer cells release large amounts of lactate continually into the TME; this lactate then engages GPR81 receptors present on TAMs. Engagement of GPR81 stimulates the adoption of an immunosuppressive profile by the affected macrophages, which involves decreased MHC class II-dependent Ag presentation capacity, decreased expression of certain proinflammatory cytokines, and increased levels of some inhibitory molecules regulating adaptive immune responses. Thus, more muted forms of adaptive antitumor immunity will ensue.

There are thus two possible ways to interfere here: one can pharmacologically inhibit GPR81 itself through use of a selective antagonist, thereby preventing lactate-mediated repression by blocking reception of the signal in macrophages; or one can administer a STING agonist intratumorally to produce activation of intrinsic immune pathways responsible for stimulating a shift toward an "M1" phenotype. Either approach alone ought still to lead to some benefit in terms of helping such cells carry out their assigned tasks properly, but taken together they might act in concert: inhibiting inhibition on one hand, and provoking excitation on the other (see again Figure 1).

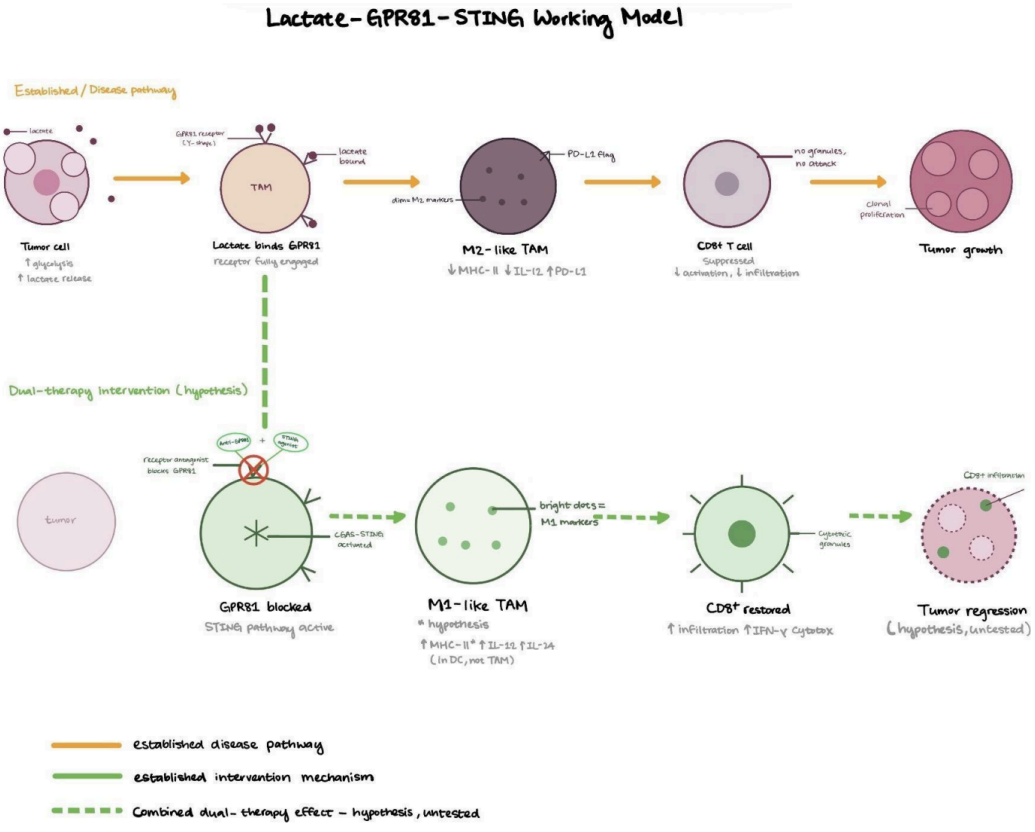


Figure 1. Working model of lactate–GPR81–STING signaling in tumor-associated macrophages and its consequences for CD8⁺ T cell–mediated tumor control

And, since expression of the antigen-presenting apparatus and production of proinflammatory cytokines increase when TAMs change from an M2-like to an M1-like state, more antigen presentation ought to lead to better stimulation and attraction of tumor-specific CD8⁺ T cells, while more inflammation helps these cells multiply and become cytotoxic. Thus, there should then be

DOI: 10.54254/2753-8818/2026.38136

more killing of tumor cells themselves, less tumor growth, and even long-term disappearance of tumors altogether. Based on this reasoning, we think that blocking GPR81 and activating STING together will work better than using just one therapy alone.

Lactate produced by tumors binds to GPR81 present on TAMs; this promotes development of an M2-like immunosuppressive phenotype characterized by reduced expression of MHC class II molecules and other components required for efficient antigen processing and presentation and increased PD-L1 levels, thus inhibiting $CD8^+$ T cells and allowing tumors to grow unchecked. Blocking GPR81 together with stimulation of STING switches TAMs to adopt a pro-inflammatory profile; consequently, more antigen is presented and T-cell responses are strengthened. This results in destruction of neoplastic cells and disappearance of tumors.

4. Proposed experimental approach

4.1. Experimental outline

It is meant to see if lactate/GPR81 signaling and STING signaling regulate TAMs by a mechanism separate from that controlled by STING itself, thereby making such pathways combinable therapeutic targets. The central question is whether they operate independently enough that removing one kind of "off" signal while turning on another yields more benefit than either option alone. Before asking this about therapeutics, however, we need first to find out whether lactate signaling actually changes what STING does inside TAMs. Thus, we want to learn whether there is less activation of canonical STING outputs (phospho-TBK1, phospho-IRF3, interferon-stimulated genes) in macrophage cultures treated with conditioned medium from tumors when lactate has been removed from the medium beforehand or blocked from entering cells via its own receptor. In this manner, we hope to discover whether lactate inhibits STING altogether in certain circumstances or whether their activities occur separately.

All in vivo work will involve use of the 4T1 syngeneic orthotopic model of TNBC implanted into the mammary fat pad of immunocompetent BALB/c mice [9]. This model retains both intact macrophages and $CD8^+$ T-cell responses, along with preservation of the entire mammary fat-pad milieu. We will directly measure intratumoral lactate concentrations to confirm that a physiologic gradient is present. Animals will be randomized when their tumours reach a certain defined size; the numbers per treatment arm will reflect what was calculated beforehand according to statistical principles, and assessment of results will be performed blind [10].

The first objective tests if lactate produced by tumors themselves induces this change in TAMs. Therefore, we will isolate TAMs by flow cytometric sorting and investigate their phenotype and function. On the one hand, surface levels of MHC-I, MHC-II, and PD-L1 will be determined by flow cytometry, and secretion of IL-12 will be measured by ELISA in cell culture supernatants of TAMs. Since priming of $CD8^+$ T cells require MHC-I–restricted (cross-)presentation, MHC-I counts most here as the measure of antigen presentation. In addition, MHC-II and IL-12 provide information about general pro-inflammatory activation. Thus, switching to more MHC-I/MHC-II, more IL-12, and less PD-L1 shows repolarization toward an antigen-presenting and pro-inflammatory cell type, whereas finding the opposite supports retention of the immunosuppressive status instead. Then there has to be some antigen-presenting ability. Therefore, they will carry out TAM–$CD8^+$ T-cell assays using TAMs presenting their own endogenous 4T1 tumor antigen AH1 (gp70, H-$2L^d$–restricted) to AH1-specific $CD8^+$ T cells. They cannot use the OT-I system here because this is on an H-2^d BALB/c background (not H-$2K^b$). In these experiments, they will determine whether TAMs can activate $CD8^+$ T cells by carrying out ex vivo co-culture assays

DOI: 10.54254/2753-8818/2026.38136

measuring both T-cell proliferation (via CFSE dilution) and IFN-γ production. More proliferation or more IFN-γ output means that TAMs have regained the ability to initiate priming of effector $CD8^+$ T cells. And then there is receptor specificity. Thus, we will include both pharmacologic inhibition and genetic deletion of GPR81 in myeloid cells. There is considerable doubt about the specificity of many commonly used GPR81 antagonists [11]. Therefore, genetic validation is necessary to attribute effects to this receptor.

The second aim determines whether STING activation reverses lactate-driven suppression in TAMs. We will assess whether STING agonism restores interferon signaling, antigen presentation, and T-cell priming capacity in macrophages exposed to high lactate. We will also evaluate whether STING activation directly affects tumor cells to separate macrophage-mediated effects from tumor-intrinsic responses.

There are three main goals. In the third, they carry out their combined treatment in vivo, according to a two-by-two factorial design: vehicle control, GPR81 blockade alone, STING activation alone, and both together (Table 1). Only thus can one obtain an estimate of interaction, which is necessary to claim synergy. Endpoints include tumor-growth curves, macrophage phenotype, $CD8^+$ T-cell infiltration, and $CD8^+$ effector function. For immune-dependence controls, there will be $CD8^+$ depletion arms for all conditions; if they want to determine what macrophages contribute, they will repeat the experiments in animals bearing myeloid-specific deletions of GPR81.

4.2. What may be expected, caveats, and alternative approaches

There has been some question about whether certain so-called selective agents such as 3-OBA truly block their supposed targets; many originally described as selective have since proved unable to inhibit the receptor reproducibly [11]. Thus, there are two ways here of stopping what the inhibitor stops: through drugs and by removing the relevant gene specifically from myeloid cells. Nor does triggering STING always help fight cancer; most attempts thus far to bring STING stimulators into patients have not worked well, while triggering STING everywhere produces damage and, sometimes, promotes tumors instead of helping the body eliminate them [8]. Limiting its stimulation to one site largely avoids these hazards. However, we will monitor whether T-cells become activated. If the two maneuvers together turn out not to work better than either alone, then they probably converge further along onto the same pathway rather than operating separately; this is what the interaction term in our factorial design should show, if anything does.

Table 1. Expected results from the seven-arm in vivo experiment described above

Group	Mouse Genotype / Background	Treatment	Expected TAM Phenotype	Expected $CD8^+$ T Cell State	Expected Tumor Growth
1 (Control)	Wild-Type (BALB/c)	Vehicle	M2-like	Exhausted / Suppressed	Rapid Progression
2	Wild-Type (BALB/c)	GPR81 Antagonist	Partial Repolarization	Moderate Activation	Delayed Growth
3	Wild-Type (BALB/c)	STING Agonist	Partial Repolarization	Moderate Activation	Delayed Growth

DOI: 10.54254/2753-8818/2026.38136

Table 1. (continued)

4 (Combo)	Wild-Type (BALB/c)	GPR81 Antagonist + STING Agonist	M1-like	Highly Active	Synergistic Regression
5 (Genetic Control)	Myeloid-specific GPR81 KO	Vehicle	Partial Repolarization	Moderate Activation	Delayed Growth
6 (Genetic Combo)	Myeloid-specific GPR81 KO	STING Agonist [a]	M1-like	Highly Active	Synergistic Regression
7 (Causal Control)	Wild-Type (BALB/c) with $CD8^+$ Depletion	GPR81 Antagonist + STING Agonist	M1-like	Absent	Progression (reduced control)

Wild-type and myeloid-specific GPR81 knockout BALB/c mice carrying established 4T1 tumors will receive either vehicle alone, a selective GPR81 antagonist, an STING pathway agonist, or both. Depletion of $CD8^+$ T cells will also occur in certain cases to demonstrate immune dependence. Accordingly, the table indicates what should happen regarding TAM phenotype, $CD8^+$ T-cell activation state, and the course of neoplastic disease in each case. Note that although STING-stimulated macrophages could still exert some degree of killing by themselves (according to Miao et al.), removal of their T-helper counterpart (arm 7) should therefore reduce but not abolish such cooperation altogether, thus providing another means of confirming that long-term suppression requires help from $CD8^+$ lymphocytes if this turns out to be true.

[a]Group 6 performs genetic ablation of GPR81 instead of pharmacologic blockade and therefore adds only the STING agonist, so there will not be two agents inhibiting GPR81.

Note that although STING-stimulated macrophages could still exert some degree of killing by themselves [7], removal of their T-helper counterpart (arm 7) should therefore reduce but not abolish such cooperation altogether, thus providing another means of confirming that long-term suppression requires help from $CD8^+$ lymphocytes if this turns out to be true.

There is then more work and expense involved compared with single-agent methods: there are two separately controlled drugs instead of just one; there is therefore more room to vary their relative timing and dosage. If there is indeed some helpful interaction found concerning 4T1 tumors, then this effect will require re-optimization when transferred to another situation later on. It takes additional months to obtain enough myeloid-specific GPR81 knockouts for a seven-group experiment in addition to what is needed for simple pairwise comparison. But such extra expenditures make sense as part of this investigation, since otherwise no interactions can appear without full-factorial testing.

And beyond what was mentioned above, there are several more things worth considering if one wants to interpret these results well. One needs to follow lactate concentration or pH inside the actual tumor over time, instead of looking only at baseline values, in order to determine whether blocking GPR81 changes the amount of lactate found within the cancer itself or affects only its receptors. One also needs some way of following blood vessels inside each treated animal's tumor throughout the entire study period, because otherwise no one will know afterward whether any

DOI: 10.54254/2753-8818/2026.38136

tumor control seen derives mainly from immunity or instead stems mostly from STING-dependent normalization, as occurs in certain cases. Finally, someone has to measure cytokines systemically rather than just locally within each animal's body before anyone can predict anything about toxicity when moving further along this path.

Assuming the combination therapy acts according to prediction, then logically its next phase will involve testing how far it generalizes outside the 4T1 TNBC model. Upregulation of GPR81 also occurs in colon, liver, lung, pancreatic, and cervical cancers in addition to breast cancer, so there seems good reason to assume that the existence of a lactate-GPR81 suppressive axis is true not just of breast tumors but of other types of solid neoplasm [12]. One could therefore extend the investigation of combination therapy efficacy to include syngeneic models of those kinds of malignancy, thereby determining whether the relation between GPR81 and STING shown here is found wherever there is much lactate present, or else depends on particular features of the TME of triple-negative disease.

Moving toward clinical trials would require testing in models that better approximate human immune-tumor interactions than syngeneic mice. Humanized patient-derived xenograft (PDX) models, in which immunodeficient mice are engrafted with both a human tumor and a reconstituted human immune system, would allow assessment of whether human TAMs and $CD8^+$ T cells respond to GPR81 blockade and STING agonism the way their murine counterparts do, and would surface species-specific differences in receptor pharmacology before human trials begin [13]. Because the 4T1 model is strongly immunosuppressive but its suppression is dominated by murine myeloid populations, such as MDSCs, that differ from the immune composition of many human TNBC tumors, confirming that the mechanism holds in a model with human-level immune cell diversity and a more heterogeneous TME would be a necessary step before this strategy could be considered for early-phase clinical testing.

5. Conclusions

TNBC is still difficult to treat because the tumor microenvironment suppresses the antigen presentation and T-cell priming that checkpoint inhibitors depend on. This proposal identifies lactate/GPR81 signaling and STING suppression as two mechanistically distinct contributors to that suppression and tests whether removing one while activating the other reprograms tumor-associated macrophages more effectively than either intervention alone. The factorial design, combined with pharmacologic and genetic controls, isolates the contribution of each pathway and determines whether their effects are additive or synergistic. Confirming synergy would identify a specific point of convergence between metabolic and innate immune signaling in TAMs and support combination dosing as a strategy for extending checkpoint inhibitor efficacy to TNBC patients whose tumors currently resist treatment.

References

[1] American Cancer Society. (2026). *Cancer facts & figures 2026*. American Cancer Society, Atlanta, GA.
[2] National Cancer Institute. What is breast cancer? https://www.cancer.gov/types/breast/what-is-breast-cancer, accessed on June 22nd, 2026.
[3] Bianchini, G. et al. (2022). Treatment landscape of triple-negative breast cancer — expanded options, evolving needs. *Nature Reviews Clinical Oncology, 19*, 91–113.
[4] Chen, S. et al. (2021). Dual blockade of lactate/GPR81 and PD-1/PD-L1 pathways enhances the anti-tumor effects of metformin. *Biomolecules, 11*(9), 1373.

DOI: 10.54254/2753-8818/2026.38136

[5] Brown, T. P. et al. (2020). The lactate receptor GPR81 promotes breast cancer growth via a paracrine mechanism involving antigen-presenting cells in the tumor microenvironment. *Oncogene, 39*(21), 3292–3304.
[6] Corrales, L., et al. (2015). Direct activation of STING in the tumor microenvironment leads to potent and systemic tumor regression and immunity. *Cell Reports, 11*(7), 1018–1030.
[7] Miao, L., et al. (2020). Targeting the STING pathway in tumor-associated macrophages regulates innate immune sensing of gastric cancer cells. *Theranostics, 10*(2), 498–515.
[8] Shi, J., et al. (2025). Precision targeting of STING: Challenges, innovations, and clinical outlook for cancer therapy. *The Innovation, 6*, 101074.
[9] Pulaski, B. A., & Ostrand-Rosenberg, S. (2000). Mouse 4T1 breast tumor model. *Current Protocols in Immunology, 39*(1), Unit 20.2.
[10] Percie du Sert, N., et al. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLOS Biology, 18*(7), e3000410.
[11] Mohammad Nezhady, M. A., & Chemtob, S. (2021). 3-OBA is not an antagonist of GPR81. *Frontiers in Pharmacology, 12*, 803907.
[12] Brown, T. P., & Ganapathy, V. (2020). Lactate/GPR81 signaling and proton motive force in cancer: Role in angiogenesis, immune escape, nutrition, and Warburg phenomenon. *Pharmacology & Therapeutics, 206*, 107451.
[13] Park, C.-K., et al. (2025). Humanized mouse models for immuno-oncology research: A review and implications in lung cancer research. *JTO Clinical and Research Reports, 6*(3), 100781.