







## Letter to the editor:

### AMPLIFYING CANCER IMMUNITY: AMPK ACTIVATORS AND $\gamma\delta$ T CELLS UNVEILED

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$\gamma\delta$ -T cells, which are part of an active immunity, have impressive anticancer capabilities and are able to broadly target tumors without requiring a patient-specific antigen or a specific type of leukocyte antigen on the cancer patient (Dai et al., 2024; Mensurado et al., 2023), making  $\gamma\delta$  T cells to sense the altered stress signals in prevalent in transformed cells (Sebestyen et al., 2020). Though we are still in juvenile phase to establish the precise mechanism governing and recognizing the stressed cells. Among human  $\gamma\delta$  T cells, V $\gamma$ 9V $\delta$ 2 T cells stand out as the most abundant subset. These cells identify a protein complex of butyrophilin 2A1 (BTN2A1) and BTN3A1 which are widely expressed on cell surface activated by phosphoantigens profusely produced by tumor cells (Mamedov et al., 2023), making them promising agents for the advancement of tumor immunotherapy. Innate immune response mediated by  $\gamma\delta$  T cell receptor, in cancer patients demonstrates broad reactivity but lacks the antigenic specificity seen in  $\alpha\beta$  TCR. Among  $\gamma\delta$  T cells, V $\gamma$ 9V $\delta$ 2 T cells, which are specific to the BTN2A1-BTN3A1-BTN3A2 complex, have shown significant potential in antitumor immunity. Elevated

expression of BTN3A1 in tumor cells regulated by the mevalonate pathway facilitates the recruitment of BTN2A1 thereby activating the V $\gamma$ 9V $\delta$ 2 T cells. This interaction enhances understanding of the complex signaling mechanism involved in  $\gamma\delta$  T cell-mediated tumor recognition and cytotoxicity. Many insight dynamics between cancer cells and V $\gamma$ 9V $\delta$ 2 T cells are yet to be deciphered due to poor understanding, making it more challenging for glioma scientists (Close et al., 2020; Thomas et al., 2024).

Mamedov et al. employed CRISPR technology to unveil the genes governing the cancer-killing capabilities of human V $\gamma$ 9V $\delta$ 2 T cells, on Daudi-Cas9 cell line expressing Cas9. Cells were treated with Zoledronic acid and were cultured with primary V $\gamma$ 9V $\delta$ 2 T cells from healthy donors to examine the live Daudi cells. Important regulators were identified in the interaction between cancer cells and V $\gamma$ 9V $\delta$ 2 T cells responsible for enhanced survival rates via genes related to the butyrophilin complex, mevalonate pathway enzymes, transporter genes, gene activators, and surface proteins (Rigau et al., 2020), providing significant insight for new strategies in cancer therapeutics. This breakthrough holds significant promise for advancing cancer treatment strategies. Metabolic pathways fundamental for survival during co-culture, with purine metabolism, oxidative phosphorylation and TCA cycle exhibited notable enrichment via gene set enrichment analysis (GSEA). A genome-wide CRISPR screen was conducted to identify key regulatory genes involved in modulating the expression and surface localization of BTN3A, aiming to elucidate the molecular pathways that govern its cell surface levels. The cytotoxic function of V $\gamma$ 9V $\delta$ 2 T cells on tumor cells is fundamentally associated with BTN3A levels on the cell surface. Significant expression FDR < 0.01 of BTN3A was observed in co-culture cells, deciphering the fundamental role of BTN3A in modulating the effectiveness of V $\gamma$ 9V $\delta$ 2 T cell-mediated killing (Yuan et al., 2024).

The V $\gamma$ 9V $\delta$ 2 TCR–BTN2A1/3A1 interaction is a function of phosphoantigen concentration, and is highly influenced by plays through mevalonate pathway (Yuan et al., 2023), however its surface expression in excess was yet to be deciphered. The study reveals that the knockout of the mevalonate pathway enzyme FDPS (farnesyl diphosphate synthase) along with enzymes involved in pathways such as *de novo* purine synthesis and Fe-S cluster synthesis results in increased BTN3A levels on the cell surface and improves the antitumor activity and efficiency. Furthermore, the adenosine monophosphate (AMP), regulates AMPK and ATP synthesis from *de novo* purine synthesis pathway, can further influence BTN3A and its impact on tumor cell destruction.

The result assay further indicates a reciprocal regulation by IRF1 and ZNF217, with interplay between oxidative phosphorylation and BTN3A expression. It was observed that no association between disturbance in the electron transport chain complex I contraindicate earlier studies that changes in ATP synthesis and the NADH:NAD<sup>+</sup> ratio influence BTN3A expression. Experiments have demonstrated that BTN3A expression relies on energy; heightened glucose levels and the inhibition of glycolysis or specific complexes raise surface BTN3A levels. They tested whether metformin, a secondary activator of AMPK, slightly increased the expression of BTN3A on the cell surface in WT Daudi-Cas9 cells, which was confirmed by direct activators, C991 and A-769662. Interestingly, breast and colon cancer organoids derived from patients treated with AMPK activators also demonstrated improved levels of BTN3A on the cell surface. It's worth noting that eliminating AMPK without exposing the cells to stress conditions did not lead to any alterations in BTN3A.

Natural compounds as AMPK activators include Metformin, Resveratrol, Salicylate. Small drug molecules like Thienopyridone, 991 (ex229), PF-06409577 (Compound 7), Compound-2/Compound-13, PT-1, MT 63-78 (Debio 0930), MT47-100, JJO1 (Guigas and Viollet, 2016) are already in use for various metabolic disorders. Their new mechanistic approach (Anagnostakis and Piperi, 2023) via empowering  $\gamma\delta$  T cells can open new avenues in retarding

the cases of glioma either by inhibiting glioma growth, inducing apoptosis, preventing angiogenesis or even changing the microenvironment that can inhibit the stress on neurons exploring the potential synergies of combining natural compounds with conventional glioma treatments with minimum adverse effects.

Drugs activating AMPK can lead to high expression of BTN immunoglobulin on cancer cells including gliomas with high chances of V $\gamma$ 9V $\delta$ 2 T cell receptor-mediated cytotoxicity suggesting new possibilities to boost  $\gamma\delta$  T cell anticancer efficacy. Transcription, post translation modifications and membrane alterations were already known to initiate  $\gamma\delta$  T cell responses, but small drug molecules that can be able to cross the blood brain barrier give new hope particularly related to ATP production via metabolic alteration by the drug molecules without putting stress on tumor cells.

The limitations surrounding above hypothesis are primarily due to the incomplete understanding of the precise mechanism by which  $\gamma\delta$  T cells recognize and attack stress tumor cells. While  $\gamma\delta$  T cells, particularly the V $\gamma$ 9V $\delta$ 2 subset, are known for their broad anticancer activity, current knowledge of how they sense altered stress signals and the complex interaction between the mevalonate pathway and other metabolic regulators remains underdeveloped. Despite the potential by AMPK activators as a new therapy targeting  $\gamma\delta$  T cell, further research is required to translate these findings into practical drug discovery strategies.

### **Consent of publication**

None.

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### **Conflict of interest**

The authors declare no conflict of interest, financial or otherwise.

## **REFERENCES**

- Anagnostakis F, Piperi C. Targeting options of tumor-associated macrophages (TAM) activity in gliomas. *Curr Neuropharmacol*. 2023;21:457-70.
- Close HJ, Stead LF, Nsengimana J, Reilly KA, Droop A, Wurdak H, et al. Expression profiling of single cells and patient cohorts identifies multiple immunosuppressive pathways and an altered NK cell phenotype in glioblastoma. *Clinical & Experimental Immunology*. 2020;200:33-44.
- Dai W, Li J, Liu J, Li H, Liu XF, Xia S, et al. BSI-093, a best-in-class anti-BTN3A monoclonal antibody for cancer immunotherapy by activation of V $\gamma$ 9V $\delta$ 2 T cells. *Cancer Res*. 2024;84(6, Suppl):5298.
- Guigas B, Viollet B. Targeting AMPK: from ancient drugs to new small-molecule activators. *AMP-activated Protein Kinase*. 2016:327-50.
- Mamedov MR, Vedova S, Freimer JW, Sahu AD, Ramesh A, Arce MM, et al. CRISPR screens decode cancer cell pathways that trigger  $\gamma\delta$  T cell detection. *Nature*. 2023;621:188-95.
- Mensurado S, Blanco-Domínguez R, Silva-Santos B. The emerging roles of  $\gamma\delta$  T cells in cancer immunotherapy. *Nat Rev Clin Oncol*. 2023;20:178-91.
- Rigau M, Ostrouska S, Fulford TS, Johnson DN, Woods K, Ruan Z, et al. Butyrophilin 2A1 is essential for phosphoantigen reactivity by  $\gamma\delta$  T cells. *Science*. 2020;367:eaay5516.
- Sebestyen Z, Prinz I, Déchanet-Merville J, Silva-Santos B, Kuball J. Translating gammadelta ( $\gamma\delta$ ) T cells and their receptors into cancer cell therapies. *Nat Rev Drug Discov*. 2020;19:169-84.
- Thomas P, Paris P, Pecqueur C. Arming V $\delta$ 2 T cells with chimeric antigen receptors to combat cancer. *Clin Cancer Res*. 2024;30:3105-16.
- Yuan L, Ma X, Yang Y, Qu Y, Li X, Zhu X, et al. Phosphoantigens glue butyrophilin 3A1 and 2A1 to activate V $\gamma$ 9V $\delta$ 2 T cells. *Nature*. 2023;621:840-8.
- Yuan M, Wang W, Hawes I, Han J, Yao Z, Bertaina A. Advancements in  $\gamma\delta$ T cell engineering: paving the way for enhanced cancer immunotherapy. *Front Immunol*. 2024;15:1360237.