**5-Nonyloxytryptamine Reveals Novel Approach for MHC-I Upregulation in Tumors**

**Background** Melanoma is categorized as an immunologically ‘cold’ tumor due to its unresponsiveness to immunotherapies. Efforts to enhance the immune response against tumor cells have focused on manipulating exhausted cytotoxic T lymphocyte (CTL) using monoclonal antibodies targeting PD1, LAG-3, and TIM3, as well as enhancing CTL priming through CD27 agonists. In the search of identifying novel enhancers of T cell-mediated anti-tumor responses, we conducted an ex vivo pharmacological screen that led to the discovery of 5-Nonyloxytryptamine (5-NL), a serotonin receptor agonist, that improves the ability of T cells to target tumor cells.

**Methods** To uncover new immune-modulating agents, we screened the NIH clinical collection library of small molecules using a co-culture setup involving melanoma B16F10 cells and T cells. The potential hit, 5-NL, was subsequently studied in the context of treating B16F10-inoculated wildtype C57BL/6J and immunocompromised NSG mice. Additionally, to uncover the mechanism of action of 5-NL through various techniques, such as immunoblots, histological staining, flow cytometry, and RNA sequencing analysis of tumor cells treated with 5-NL.

**Results** In vivo experiments demonstrated that 5-NL effectively slowed down tumor growth, and this effect was dependent on the host’s immune system. Notably, the difference in tumor growth was abolished when CD8+ T cells were depleted, highlighting the involvement of CD8+ T cells in the tumor growth inhibition mediated by 5-NL. Further investigations indicated that 5-NL’s pro-immune effects were not directly caused by its impact on T cells. Instead, 5-NL increased the transcriptional and protein levels of the antigen-presenting machinery in mice and human melanoma cells. This effect was recapitulated in other solid tumor types like colon cancer, but not in breast and lung cancer. Mechanistically, it was found that 5-NL increased the phosphorylation of cAMP response element-binding protein (CREB) without activating mitogenic pathways like MAPK. Interestingly, this action was independent of type-I interferon signaling and did not increased PD-L1. Additionally, pharmacological inhibition of phospho-CREB reversed the enhanced antigen presentation, validating the proposed mechanism of action. Furthermore, RNA sequencing pointed to the activation of the AMPK pathway, which was corroborated by phospho-AMPK immunoblotting. This activation ultimately resulted in inhibition of the PI3K/Akt/mTOR pathway and subsequent cell death in vitro (figure 1).

**Conclusions** This study introduces innovative prospects for enhancing immune responses in tumors with limited immunogenicity. The elevation of CREB phosphorylation induced by 5-NL offers a novel approach for augmenting antigen presentation, leading to the control of tumor growth in immunologically cold solid tumors.

**REFERENCES**


**Ethics Approval** Experiments were performed under the authorization of LANUV in accordance with German law for animal protection.