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Establishment of cancer immunotherapy in vitro model

Jana Veprkova[1*], Andrej Besse[1,2], Lenka Sedlarikova[1], Lenka Besse[1,2]

[1]*Department of Biology, Faculty of Medicine, Masaryk University Brno, Czechia*
[2]*Department of Oncology and Hematology Kantonsspital St. Gallen St. Gallen, Switzerland*

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***Correspondence** Jana Veprkova. Shurin. Department of Biology,Faculty of Medicine, Masaryk University Brno, Czechia.
E-mail: jana.veprkova@med.muni.cz
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Abstract

T cells play a central role in cancer immunotherapy, yet the lack of standardized *in vitro* models limits the reproducibility and translational potential of immune-cancer cell interaction studies. Existing models often lack reproducibility due to variability in experimental conditions, immune cell sources, and functional assays.

To address this gap, we established a robust and reproducible *in vitro* co-culture model, which serves to evaluate efficacy of the immune cell-based cytotoxicity using peripheral blood mononuclear cells (PBMCs) and multiple cancer cell lines. Healthy donor PBMCs supplemented with IL-2 are co-cultured with (educated) or without (naïve) cancer cell lines for two weeks. The model cell lines are equipped with a luciferase reporter, enabling real-time quantification of immune-mediated killing through assessment of luminescence intensity.

Single-cell RNA sequencing analysis of educated vs. naïve immune cells revealed favorable expansion and differentiation of $CD8^+$ cytotoxic T cells while compromised expansion of $CD4^+$ helper T cells. Moreover, the strong upregulation of Granzyme and Perforin expression in $CD8^+$ T cells is indicative of cytolytic function.

Initially validated using the pancreatic cancer cell line PANC-1, the model was further applied to lung cancer (A549) and multiple myeloma (AMO-1), demonstrating its versatility across malignancies. Despite the MHC non-restricted nature of PBMC cytotoxicity—due to lack of HLA compatibility—PBMCs exhibited a 40-80% increase in selective killing of the cancer cell line to which they were educated, compared to non-educated conditions.

While the use of healthy donor PBMCs poses a potential limitation in clinical settings due to the risk of graft-versus-host disease (GVHD), this model provides a highly adaptable and reproducible *in vitro* platform for studying T-cell mediated anti-cancer responses and optimizing immunotherapeutic approaches.