**Abstract:** PF1145

**Title:** FORMATION OF TUNNELING NANOTUBES IN CHRONIC LYMPHOCYTIC LEUKEMIA AND ASSOCIATED MITOCHONDRIAL TRANSFER TRIGGER (CAR)-T CELL EXHAUSTION

**Author(s):** **Narendra Varma Gottumukkala,** **Jakub Pospisil,** **Michal Smida**

**Type:** Poster Presentation

**Session title:** Gene therapy, cellular immunotherapy and vaccination - Biology & translational research

**Background:**
CAR-T cell therapy has achieved remarkable responses in acute lymphoblastic leukemia and some B-cell lymphomas, nevertheless, it largely fails in other hematological malignancies like chronic lymphocytic leukemia (CLL). The mechanisms of failure are multiple, but the predominant feature is the low quality of isolated T cells, which display exhausted phenotype. Recently, it has been shown that various malignant cells are able to form cellular channels with T cells called tunneling nanotubes. Tumor cells use these nanotubes to hijack T-cell mitochondria, thereby weakening them and dampening T-cell function.

**Aims:**
The aim of this study was to explore whether also CLL cells are able to form such nanotubes and whether they are in this way stealing the mitochondria from T cells, as this can trigger T-cell exhaustion.

**Methods:**
Using confocal and super-resolution microscopy, we were observing the nanotubes formed among different B cell lines or primary CLL cells as well as between malignant B cells and T cells in the co-culture experiments. Flow cytometry was used to detect the expression of exhaustion markers and to assess the extent of mitochondrial exchange between CLL and T cells or CAR-T cells.

**Results:**
We have observed increased expression of different exhaustion markers on the surface of T cells isolated from CLL patients as compared to healthy donors. We have then discovered that CLL cells are forming nanotubes among themselves, which was confirmed in both CLL cell lines and in primary CLL patient cells of diverse genetic backgrounds. We have observed the exchange of mitochondria through such nanotubes. More importantly, CLL cells were able to form the nanotubes with T cells as well as with CAR-T cells in the co-culture experiments. Importantly, we again detected that mitochondria were transferred via these nanotubes and the transfer was predominantly towards leukemic B cells, thereby draining the T cells. Finally, when we analysed total mitochondrial mass present in T cells and B cells in the healthy samples and in CLL patients, we observed skewed ratio of T/B mitochondrial content, where CLL patients exhibited reduced mitochondria presence in T cells and on contrary higher mitochondrial mass in their B cells.

**Summary/Conclusion:**
We have for the first time detected the formation of tunneling nanotubes and the mitochondria exchange in the CLL setting. We propose that this results in the weakening of T cells and their exhaustion, thereby diminishing their activity within the CAR-T cell therapy.

*This work was supported by a grant from the Czech Science Foundation (project no. 2235273S), by the project MUNI/A/1685/2024 and project NICR (EU program EXCELES, No. LX22NPO5102).*