## Investigating the potential benefits of Vitamin D, C and zinc treatments on T cell-based cancer immunotherapy



By Evangelia Rakou (210474550), placement student (e.rakou2@ncl.ac.uk), Dr. Mark Levasseur, supervisor (mark.levasseur@ncl.ac.uk)

## Introduction

- Immunotherapy is a fast developing field of cancer research that works by using the innate ability of our immune system to fight cancer<sup>[1]</sup>
- The roles of Vitamin D, C and zinc in immune health and their positive responses to cancer treatment are well established<sup>[2]</sup>
- Granzyme B is secreted by activated T cells and plays pivotal role in tumour cell killing since it promotes a rapid onset of apoptosis

Aims

- Pre-treat T cells (HUT-78) with different concentrations of Vitamin D, C and zinc and investigate their activation and killing potential
- Determine the status of granzyme B (active/inactive) after the activation of the pretreated T cells





Figure 3. A. Merged images of activated HUT-78 cells pre-treated with vitamin D, C and zinc. Immunofluorescence performed using Alexa 594 antibody (green) for tubulin and Alexa 488 (red) for Granzyme B. B. Bar chart representing tubulin and granzyme B intensities for the samples in Figure 3A quantified using ImageJ-Fiji software.



fluorescent A localization cleavage protein domain t = 0

2 h after activation

Figure 4. A. Model of the DNA construct NES-VGPD-GFP. Using nuclear export signal (NES) as a localization domain, GFP is exported from the nucleus when granzyme B is inactive whereas VGPD cleavage site is cleaved when granzyme B is active and GFP diffuses inside the nucleus <sup>[3]</sup>. **B**. GFP distribution at t=0 and C. 2 h after PMA/ionomycin activation.

## Conclusions

- Vitamin D (1µM) caused the highest granzyme B secretion but on cellular level, vitamin C and zinc had a higher granzyme B intensity
- Combination treatments were collectively more effective than individual ones
- Granzyme B is inactive inside the HUT-78 cells which means that activation is linked with later stages of the activation pathway or the secretion itself

References 1. Böttger F, Vallés AV, Cahn L, Jimenez CR. High-dose intravenous vitamin C, a promising multi-targeting agent in Experimental and Clinical Cancer Research, 2021;40(1);343-, 2 Johnson CS, Deeb KK, Trump DL, Vitamin D signalling pathways in cancer; potential fo anticancer therapeutics. Nature reviews Cancer. 2007;7(9):684-700. 3. Liesche C, Sauer P, Prager I, Urlaub D, Claus M, Eils R, et al. Single-Fluorescent Protein Reporters Allow Parallel Quantification of Natural Killer Cell-Mediated Granzyme and Caspase Activities in Single Target Cells. Frontiers in immunology. 2018;9:1840-

## Results