

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^[1]
- The roles of Vitamin D, C and zinc in immune health and their positive responses to cancer treatment are well established^[2]
- 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

Results

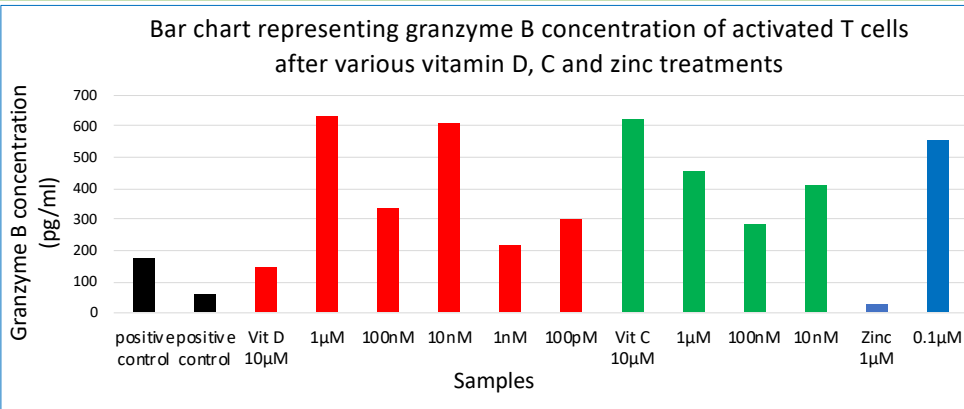


Figure 1. Granzyme B concentration levels secreted by HUT-78 cells pretreated with Vitamin D (red), C (green) and zinc (blue) for 72 h. Samples were activated with PMA/ionomycin for 4 h, granzyme B Sandwich ELISA assay was used and the absorbance was measured using Polarstar Omega plate reader.

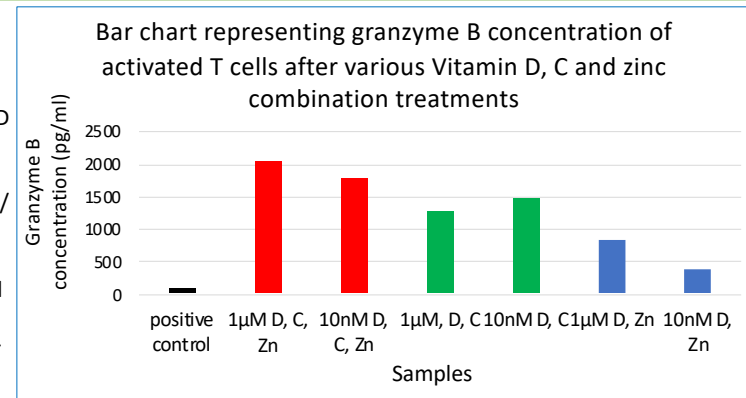


Figure 2. Granzyme B concentration levels secreted by HUT-78 cells pretreated with Vitamin D (1µM or 10nM), Vitamin C (10µM) and zinc (0.1µM). Sample activation, ELISA and absorbance measurements as per Figure 1.

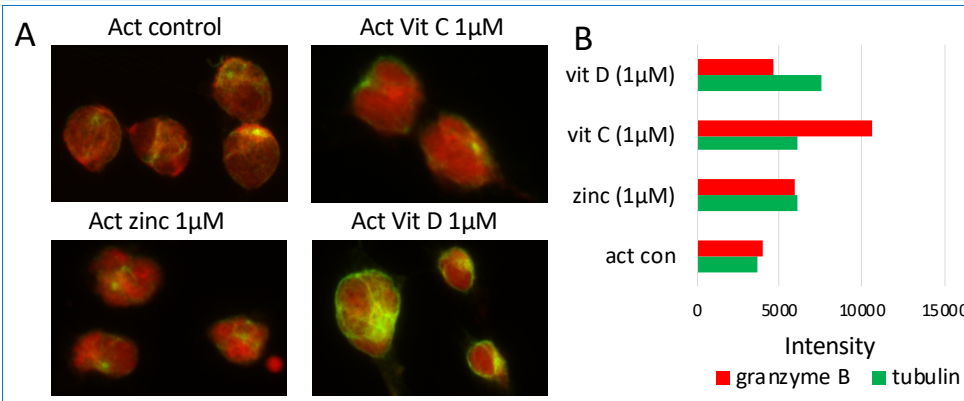
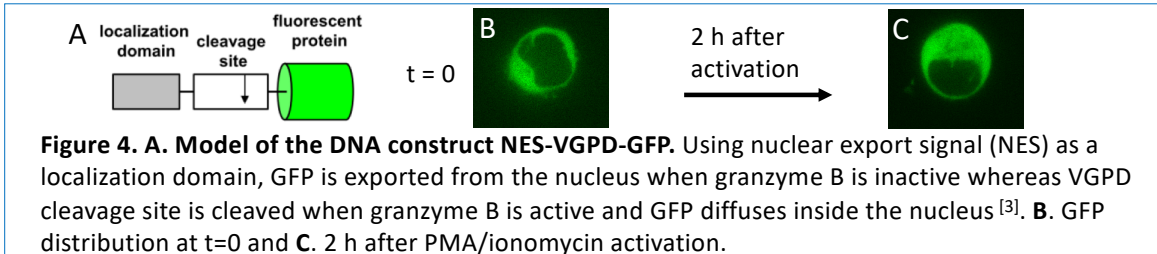


Figure 3. A. Merged images of activated HUT-78 cells pre-treated with vitamin D, C and zinc. Immunofluorescence performed using Alexa 594 antibody (green) for tubulin and Alexa 488 (red) for Granzyme B. **B.** Bar chart representing tubulin and granzyme B intensities for the samples in Figure 3A quantified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



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