Oncolytic Virotherapy for the Treatment of Non-Hodgkin Lymphoma

[1]M. Holmes, [1]G. Scott, [2]D. Shafren, [1]A. Melcher, [1]F. Errington-Mais;
[1]Level 5 Wellcome Trust Brenner Building, Leeds Institute of Cancer and Pathology, University of Leeds
[2]Viralytics Limited, Sydney, New South Wales, Australia

Introduction

Non-Hodgkin Lymphoma (NHL) comprises a highly heterogeneous group of >60 indolent and aggressive cancers of the lymphatic system that are predominately B-Cell in origin. These include Follicular Lymphoma (FL), Burkitt's Lymphoma (BL), Diffuse Large B Cell Lymphoma- Germinal Centre B Cell (DLBCL-GCB) and DLBCL- Activated B Cell subset (DLBCL-ABC).

Some NHL subtypes respond well to chemotherapy, while others have poor prognoses due to the aggressiveness of the disease (DLBCL-ABC: 45% 3 year survival with current standard of care) or the presence of drug resistant cells residing in the bone marrow, warranting research into superior areas of therapeutic intervention.

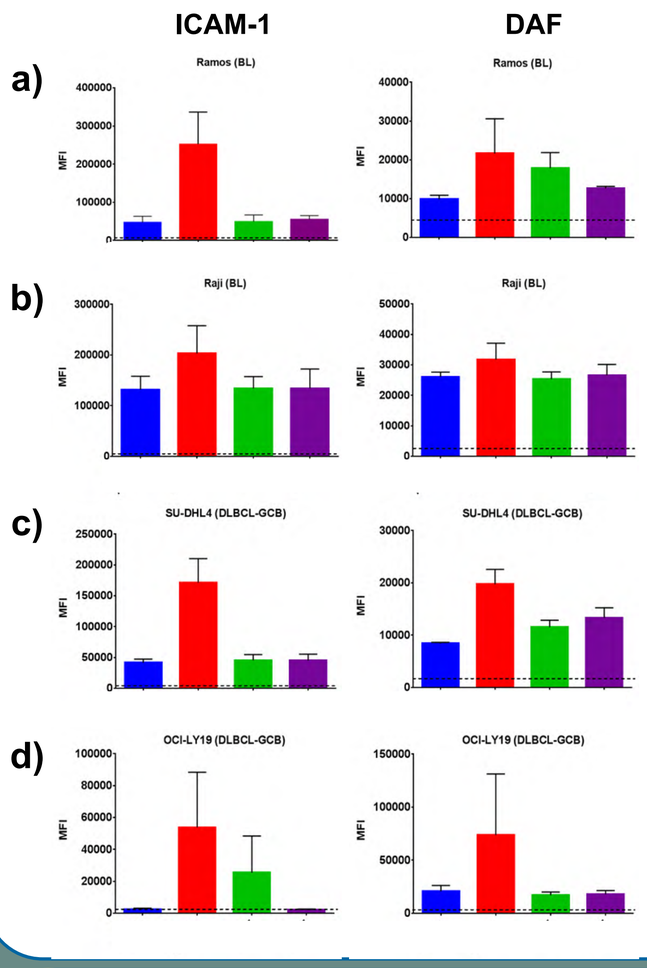
CAVATAK™, a non-manipulated oncolytic Coxsackievirus A21 currently in Phase I and II clinical trials in solid malignancies, was used to treat aggressive NHL cell lines that were co-cultured on lymph node (LN; $CD40L^+$ L929 mouse fibroblasts) and bone marrow (BM: HS5 and HS27 human bone marrow cells) feeder layers and analysed for the expression of the CAVATAK™ receptor (ICAM-1), the co-receptor (DAF) and cell viability, using Flow Cytometry.

Primary lymph node biopsy samples and normal B cells were analysed for ICAM-1 expression and viability following treatment with CAVATAK™.

Aims

1) Examine the expression of the CAVATAK™ receptor ICAM-1 on NHL cell lines using *in vitro* models of clinically relevant anatomical sites: The LN and BM
2) Test the effects of these co-cultures on CAVATAK™ sensitivity
3) Analyse primary normal and NHL cells for CAVATAK™ receptor expression and susceptibility

Figure 1: NHL cell lines express CAVATAK™ receptors



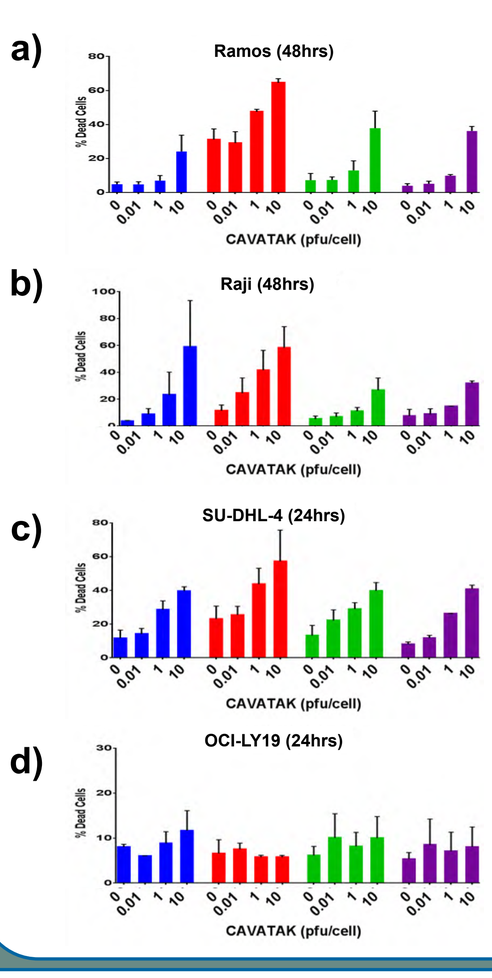
NHL cell lines were cultured alone or with feeder layers that represented the lymph node (LN) or bone marrow (BM) tumour microenvironments and analysed for the expression of the CAVATAK™ receptor, ICAM-1, and co-receptor, DAF.

The broken line represents the isotype threshold.

a) Ramos and **b) Raji** (Burkitt's Lymphoma) cells express ICAM-1 and this is increased in the LN environment.
c)SU-DHL-4 (DLBCL-GCB) cells express high levels of ICAM-1 which is up-regulated in the LN environment.
d) OCI-LY19 (DLBLC-GCB) cells up-regulate ICAM-1 on only a small subset of cells in the LN environment.

All cell lines expressed DAF which is necessary but not sufficient for CAVATAK™ infection.

Figure 2: Increased ICAM-1 expression enhances sensitivity to CAVATAK™

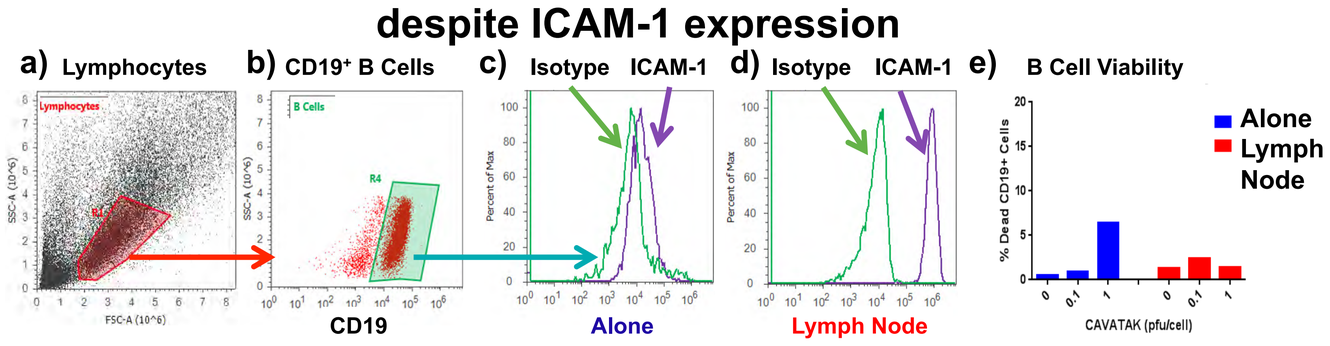


NHL cell lines were cultured with or without the LN or BM feeder layers for 24 hours. They were harvested, re-seeded and treated with CAVATAK™. Cell viability was then measured.

a) Ramos (Burkitt's Lymphoma) cells were mildly sensitive to CAVATAK™ at high doses. LN co-culture enhanced sensitivity at lower doses by 48hrs.
b) Raji cells were also sensitive to CAVATAK™ treatment. LN co-culture enhanced sensitivity at 48hrs at lower doses only.
c) SU-DHL-4 (DLBCL-GCB) cells showed sensitivity to CAVATAK™ within 24 hours. LN co-culture very slightly enhanced this sensitivity at higher doses only.
d) OCI-LY19 (DLBLC-GCB) express low levels of ICAM-1, and upregulate this on only a small subset of cells after the LN simulation, remain resistant to CAVATAK™ .

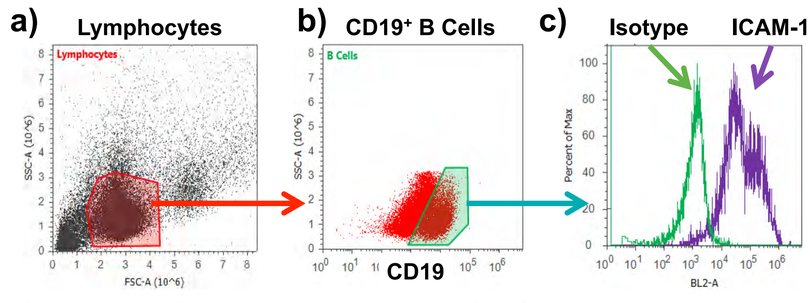
These data show that the upregulation of ICAM-1 enhanced the susceptibility of some cell lines to CAVATAK™. BM stimulated remain sensitive to CAVATAK™.

Figure 3: Normal B cells are CAVATAK™-resistant despite ICAM-1 expression



3) B Cells from healthy donors were treated with CAVATAK™ for 72hrs and analysed for CAVATAK™ receptor (ICAM-1) expression and viability
a) Forward Scatter/Side Scatter plot of isolated B Cells. b) Dot plot to identify the $CD19^+$ cells.
c) ICAM-1 is expressed on normal B cells, and **d)** is upregulated in the LN environment
e) Healthy B Cells, alone or after LN stimulation, are largely resistant to CAVATAK™

Figure 4: FL B Cells express ICAM-1



4) B Cells from a fresh FL sample were analysed for ICAM-1 expression.
a) Forward Scatter/Side Scatter plot of PBMCs within lymphocyte gate
b) CD19 identification gate highlighting the $CD19^+$ B cell population
c) ICAM-1 can be detected, above isotype levels, on $CD19^+$ B cells from a FL sample.
Future viability experiments on these cells will elucidate whether or not they are susceptible to CAVATAK™.

Conclusions

1. **3/4 NHL cell lines express the CAVATAK™ receptor ICAM-1, which is upregulated in the LN environment**
2. **Upregulation of ICAM-1 enhanced the susceptibility of NHL cell lines to CAVATAK™ at lower doses**
3. **BM-stimulated NHL cells remain sensitive to CAVATAK™**
4. **Normal B cells express ICAM-1 which is upregulated on the CD40L layer, but are resistant to CAVATAK™**
5. **FL B cells express ICAM-1, hopefully making them sensitive to CAVATAK™**

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