Development of a novel high content, high throughput platform for patient specific immunotherapy-based combinatorial drug screening

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Background

- **Diffuse large B-cell lymphoma (DLBCL):** Aggressive non-Hodgkin's lymphoma
- **R-CHOP:** First line, immunochemotherapeutic regimen
- **40%** of DLBCL patients eventually relapse or are non-responsive
- **New treatment options needed**
- **VAY736**: Novel, anti-BAFF-R, monoclonal antibody
  - Main mechanism of action: **Antibody-dependent cellular cytotoxicity (ADCC)**

Objectives

- **Main aim:** To pinpoint optimal drug combinations for VAY736 as therapeutic options
- **Research gap:** Lack of appropriate drug screening modalities that assess the effects of immunomodulatory drugs
- **Solution:** To develop a high-throughput drug screening method that includes immune components and measures ADCC killing in the target-specific manner

Methods

A. Standard calcein release assay

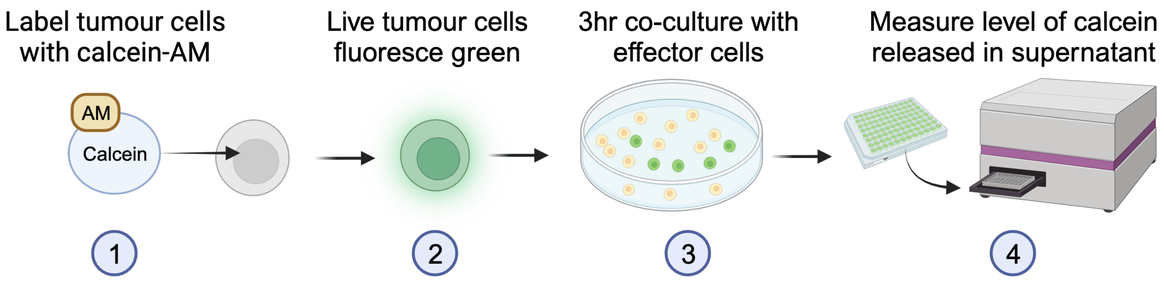

Figure 1. Workflow of calcein release assay. Live tumour (target) cells were labelled with calcein-acemethoxy (AM), where intracellular esterases hydrolyses the AM moiety, causing labelled target cells to fluoresce green. The extent of tumour cell death was assessed by quantifying the amount of calcein released by lysed target cells with a microplate reader.

B. CFSE-DAPI ADCC assay

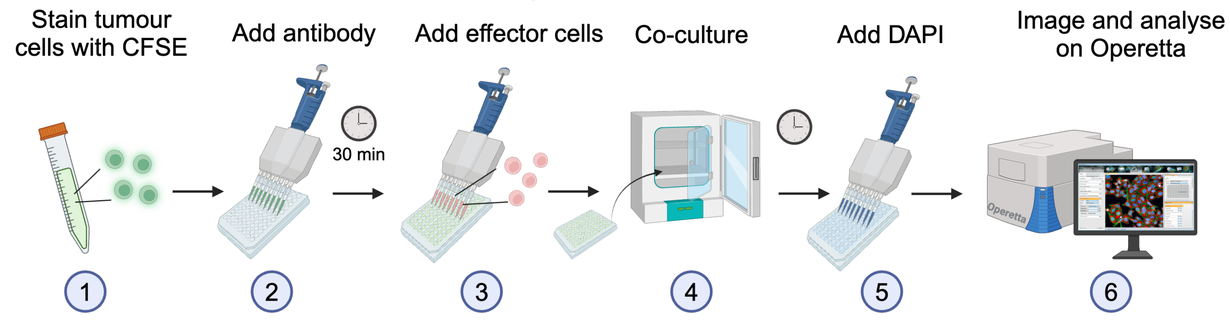

Figure 2. CFSE-DAPI ADCC assay workflow. Tumour (target) cells were labelled with carboxyfluoroscein succinimidyl ester (CFSE). CFSE+ cells were opsonized with antibodies for 30 minutes and co-cultured with effector cells (e.g. peripheral blood mononuclear cells (PBMCs) or natural killer (NK) cells) for the indicated duration. Dead cells were labelled with DAPI and the percentage of dead target cells (CFSE+ DAPI+) was calculated using the Operetta®.

Results

1. Calcein-AM labelling has high spontaneous release and is cytotoxic

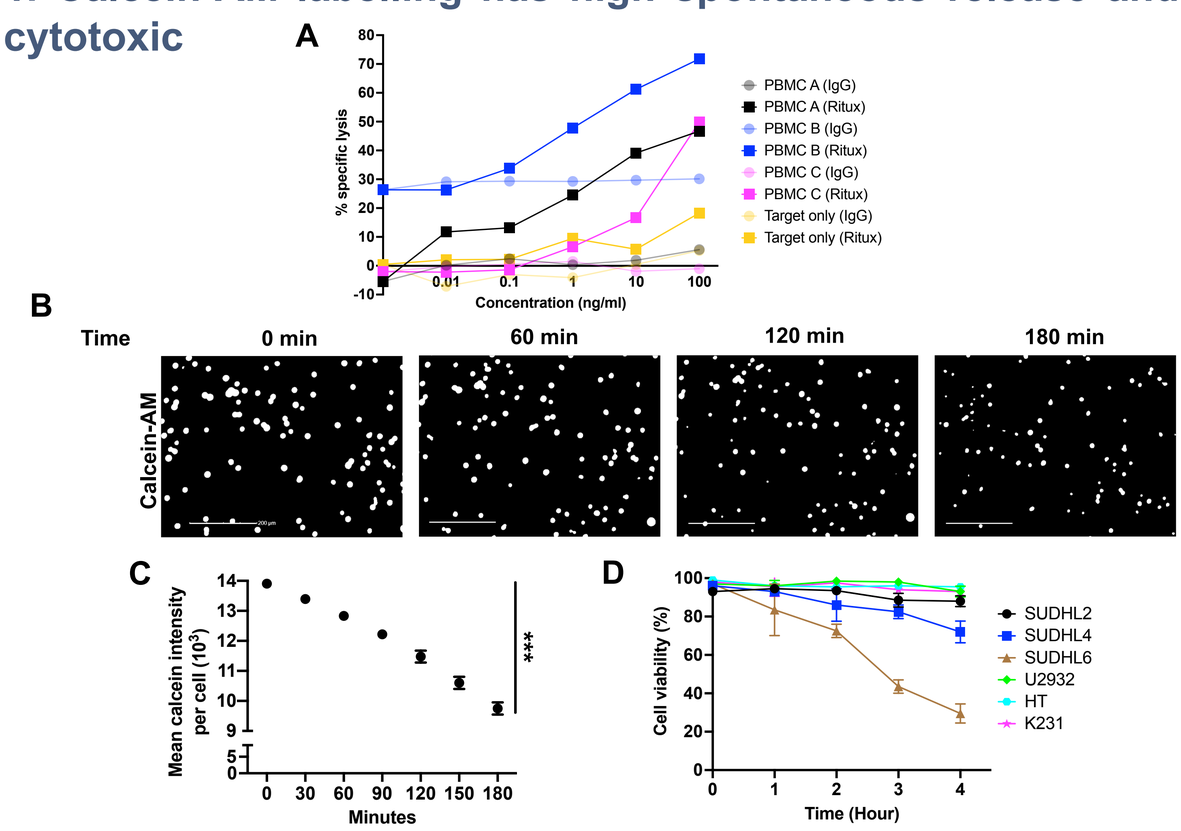

Figure 3. Evaluation of calcein release assay as a drug screen readout. (**A**) Standard calcein release assay using PBMCs from three healthy donors as effector (E) cells and SUDHL4 as target (T) cells. Cells were co-cultured for three hours of rituximab or IgG1κ at E:T = 10:1. (**B**) Time lapse imaging of calcein-labelled SUDHL4 only. Timelapse images were captured using the Operetta® for three hours. Scale bar = 200μm. (**C**) Mean calcein intensity per labelled SUDHL4 (***p <0.001). (**D**) Cell viabilities of six calcein-AM labelled DLBCL cell lines were tracked with trypan blue over four hours. N=2 for each cell line, error bars represent standard deviation (SD).

2. CFSE-DAPI ADCC assay stably and specifically tracks VAY736-mediated ADCC

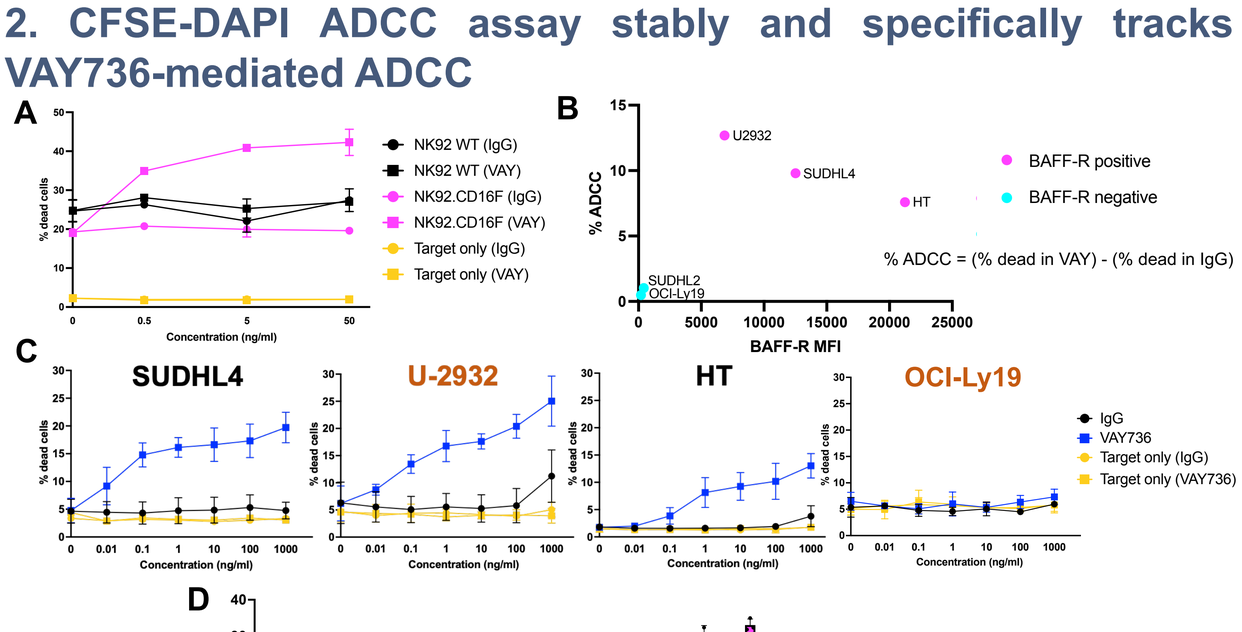

Figure 4. Specificity and stability of CFSE-DAPI ADCC assay. (**A**) Co-culture of SUDHL4, WT or CD16F.NK92 cells (E:T = 1:1) with increasing concentrations of IgG or VAY736. (**B**) Correlation of VAY736-specific % ADCC and mean fluorescence intensity (MFI) of BAFF-R (target of VAY736). (**C**) Dose response curves of BAFF-R-positive (SUDHL4, U2932, HT) and negative (OCI-Ly19) DLBCL cell lines to VAY736. DLBCL cell were co-cultured with NK92.CD16F for 48 hours, E:T=1:5. N = 3 (**D**) Variability of VAY736-specific ADCC across 12 healthy donors. PBMCs were co-cultured with SUDHL4 cells at E:T = 10:1 for five hours. Error bars represent SD.

3. CFSE-DAPI ADCC combinatorial drug screen identified various drug-induced perturbations of VAY736-mediated ADCC

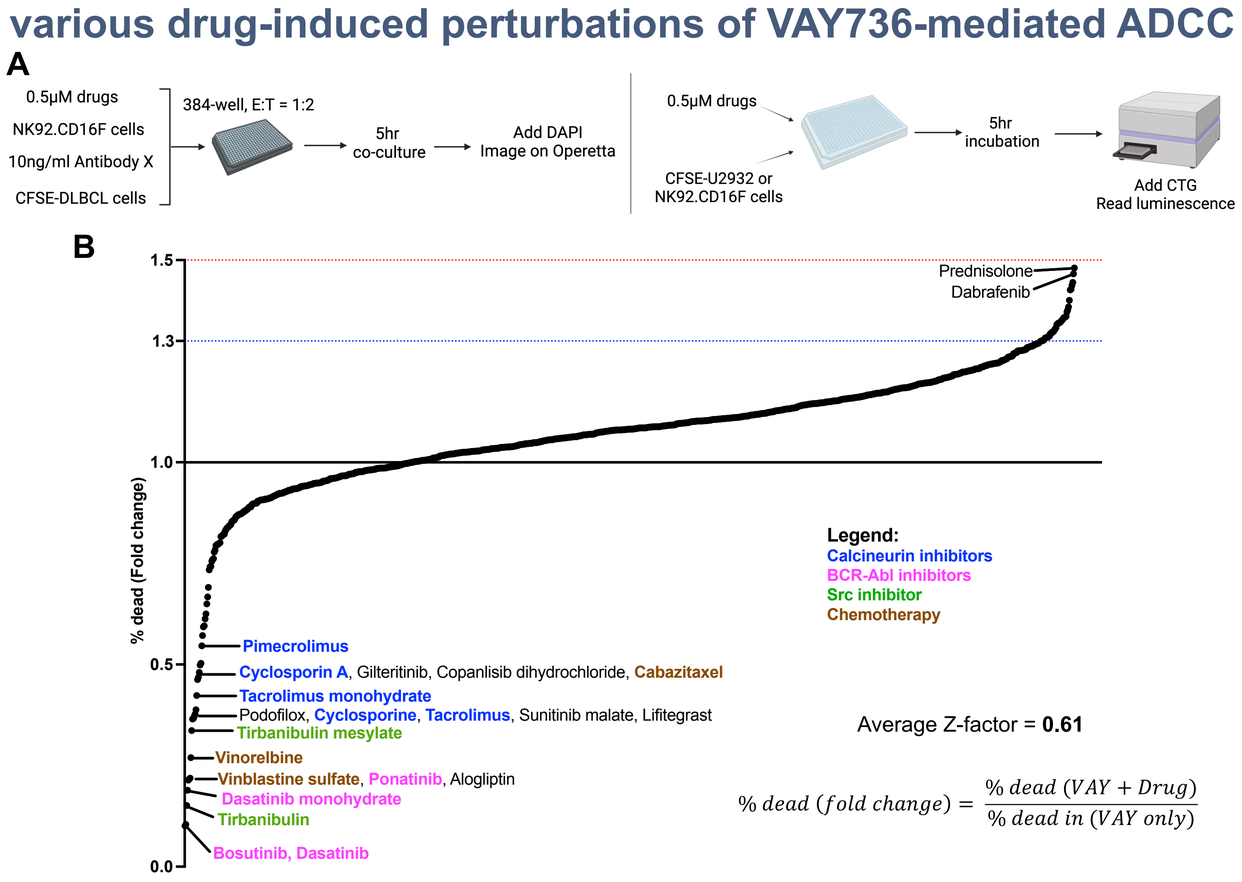

Figure 5. VAY736 ADCC drug screen using the CFSE-DAPI ADCC assay and FDA-approved drugs. (**A**) Workflow of the ADCC combinatorial drug screen where tumour and NK92.CD16 cells were co-cultured (**left**) or individually cultured (**right**) with 0.5μM FDA-approved drugs for five hours. Cell viabilities were determined with DAPI or CTG, respectively. **(C)** Dot plot indicating the fold change of VAY736-mediated U2932 cell death by each FDA approved drug compared to VAY736 (no drug) only.

4. BCR-Abl inhibitors differentially affect VAY736-mediated ADCC, possibly via inhibition of Src family kinases

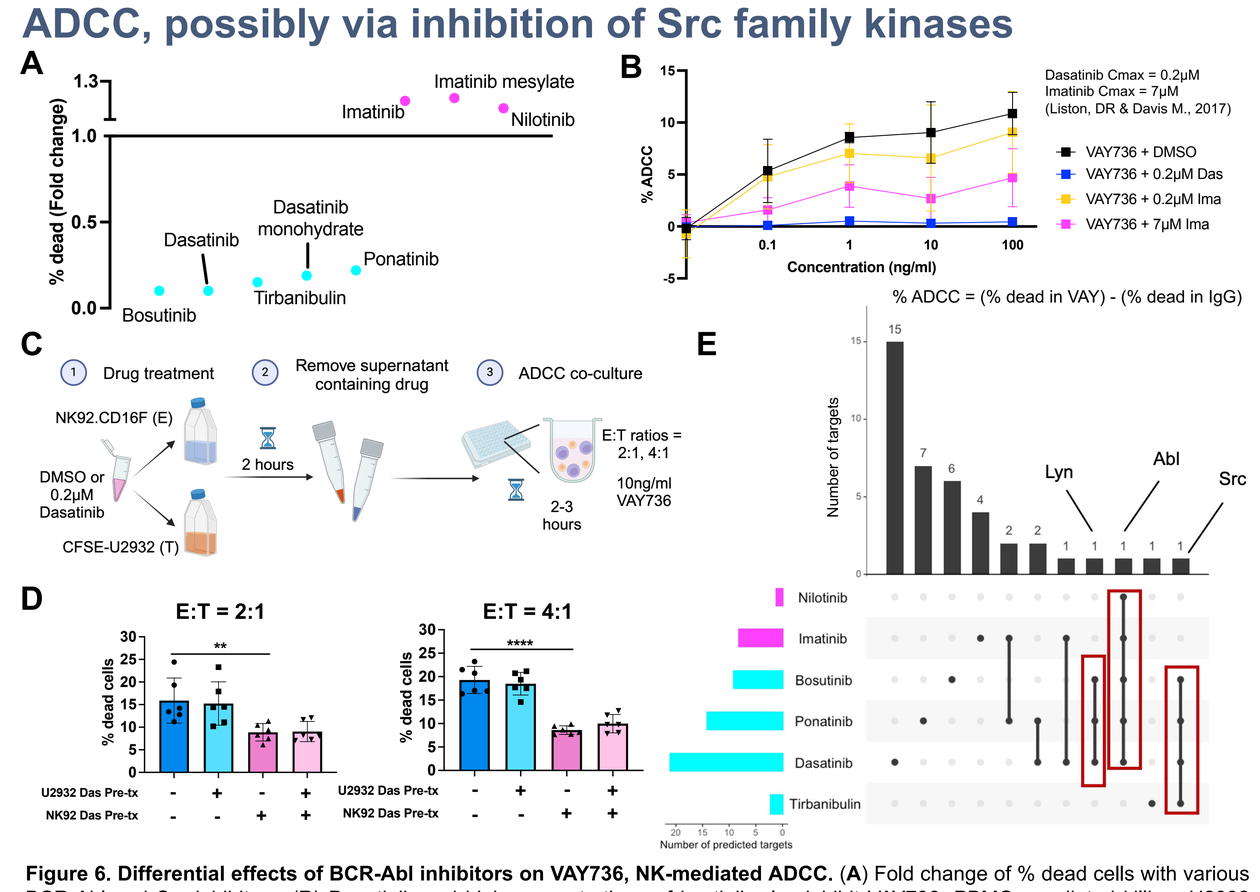

Figure 6. Differential effects of BCR-Abl inhibitors on VAY736, NK-mediated ADCC. (**A**) Fold change of % dead cells with various BCR-Abl and Src inhibitors. (**B**) Dasatinib and high concentrations of imatinib also inhibit VAY736, PBMCs-mediated killing. U2932 cells were co-cultured with PBMCs and respective drugs at E:T = 10:1 for 24 hours. N = 3 (**C**) Schematic of dasatinib pre-treatment with NK92.CD16F or CFSE-labelled U2932. (**D**) Dasatinib pre-treated NK92.CD16F, not U2932, diminished ADCC. N = 3, error bars represent SD (**E**) UpSet plot comparing predicted targets of each drug (go.drugbank.com) and common targets of ADCC inhibitors.

Conclusion and Future Directions

The CFSE-DAPI ADCC assay is:

- a feasible drug combination screening tool
- a useful tool for ADCC mechanism studies
- amenable to the incorporation of patient-derived materials
- adaptable for eventual patient-specific drug combinations screens to pinpoint optimal, personalised immunochemo-combination regimens

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