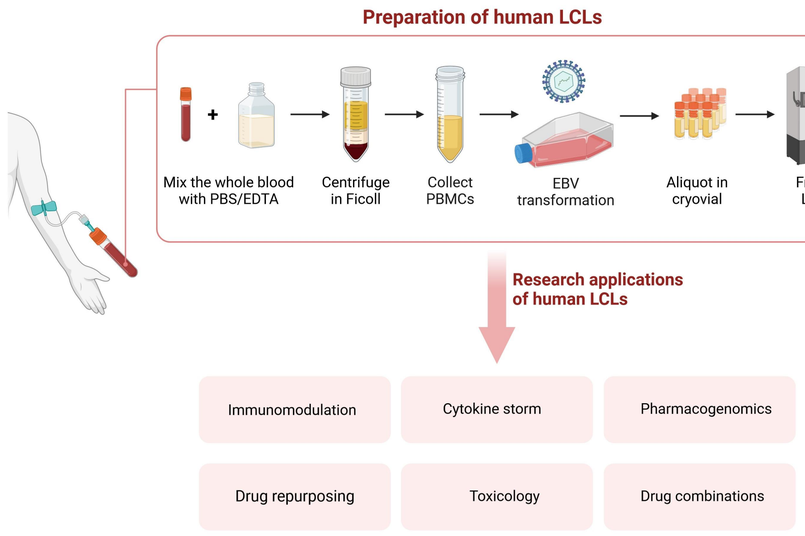
COVID-19 PATIENTS DERIVED LYMPHOBLASTOID CELL LINES AS AN ALTERNATIVE TO WHOLE BLOOD FOR COMPOUND SCREENING

Luka Hiti[1], Maša Kandušer[1], Tijana Markovič[1], Tilen Burnik[1], Mitja Lainščak[2,3], Emil Pal[2], Jerneja Farkaš Lainščak[2,4], Irena Mlinarič-Raščan[1]

[1]Faculty of Pharmacy, University of Ljubljana, Slovenia, [2]General Hospital Murska Sobota, Slovenia, [3]Faculty of Medicine, University of Ljubljana, Slovenia, [4]National Institute of Public Health, Slovenia

E-mail: luka.hiti@ffa.uni-lj.si

CYTOKINE STORM AND HUMAN LYMPHOBLASTOID CELL LINES FOR DRUG REPURPOSING



Cytokine storm, a COVID-19 complication, is a life-threatening systemic inflammatory syndrome involving the uncontrolled secretion of cytokines, which in severe cases leads to systemic organ failure and death (1).

We present here a novel *in vitro* cell model for evaluating the potential of compounds in decreasing the secretion of cytokines: immortalised B-lymphocytes, known as lymphoblastoid cell lines (LCLs).

LCLs are immortalised B-lymphocyte derived cell lines. They are produced by transfecting isolated donor B lymphocytes with Epstein-Barr virus (EBV) (2). Empirically, we found they secrete various cytokines, and can for that reason be used as an alternative to an established *in vitro* model of cytokine release, whole blood (3).

Figure 1. **Lymphoblastoid cell lines (LCLs) preparation through B lymphocyte Epstein-Barr virus (EBV) transfection and their applications in biomedical research.**
Adapted from "Harvest Peripheral Blood Mononuclear Cells (PBMCs) for Biomedical Applications", by BioRender.com. Retrieved from https://app.biorender.com/biorender-templates

CYTOKINE RELEASE ASSAY: COMPARISON OF WHOLE BLOOD AND HUMAN LCLs

COMPOUND SELECTION

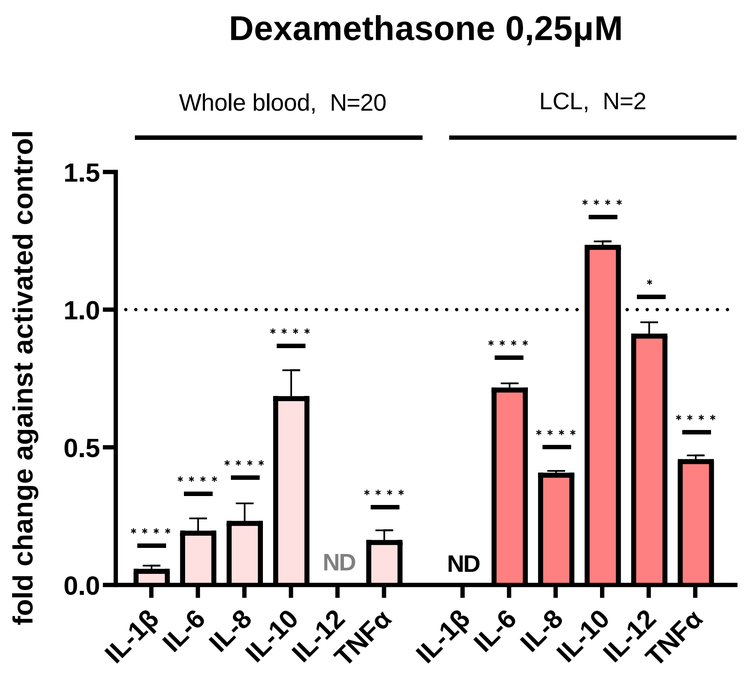
For drug screening we selected a set of compounds based on literature search or our previous research. Dexamethasone, a known immunomodulator, was used as a positive control.

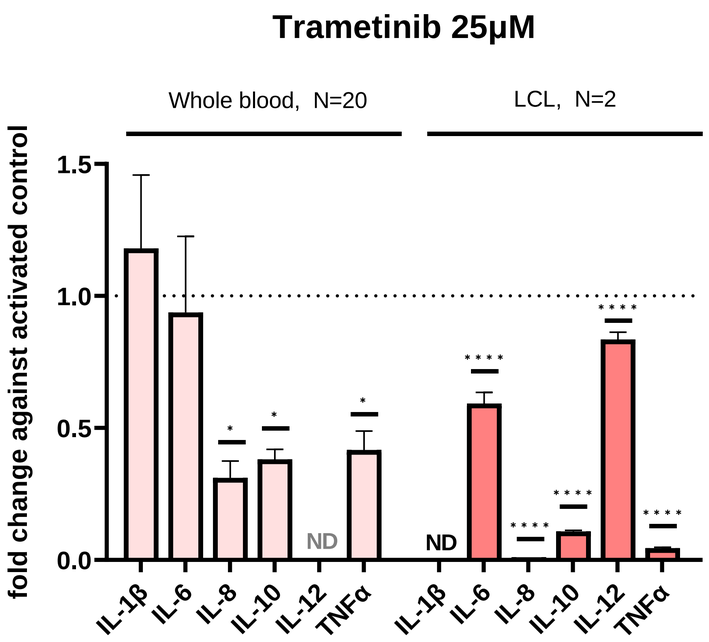
METHODS

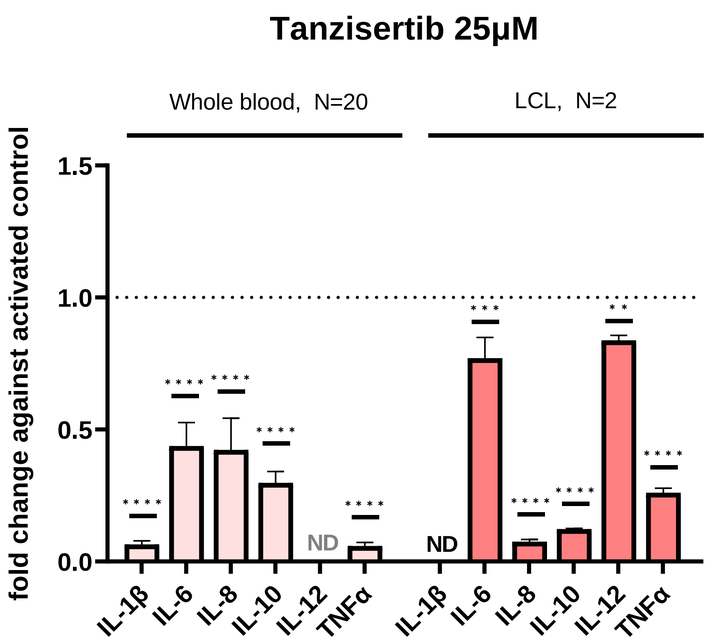
We compared LCL to an established *in vitro* cytokine release assay, whole blood. The production of cytokines was analysed as described previously (3,4). Briefly, cells were pre-treated for 1 h with the non-cytotoxic concentration of the compound of interest and activated by 100 ng/ml LPS (whole blood) or 0.5 mM ionomycin and 3.33 ng/ml PMA (LCLs) and incubated for 24 h. The resting, untreated cells and the LPS or ionomycin/PMA activated cells were used as controls. Cytokine production was assessed by BD Cytometric Bead Array (CBA) Human Inflammatory Cytokine kit (Contents: IL-1β, IL-6, IL-8, IL-10, IL-12, TNFα and) using AttuneNext flow cytometer.

RESULTS

Our results on a selected set of compounds indicate that the trend of cytokine secretion suppression is comparable between whole blood and human LCLs. The most promising candidates for drug repurposing were identified as MAPK inhibitors (trametinib, a registered drug; and tanzisertib and SB203580, two research compounds,).







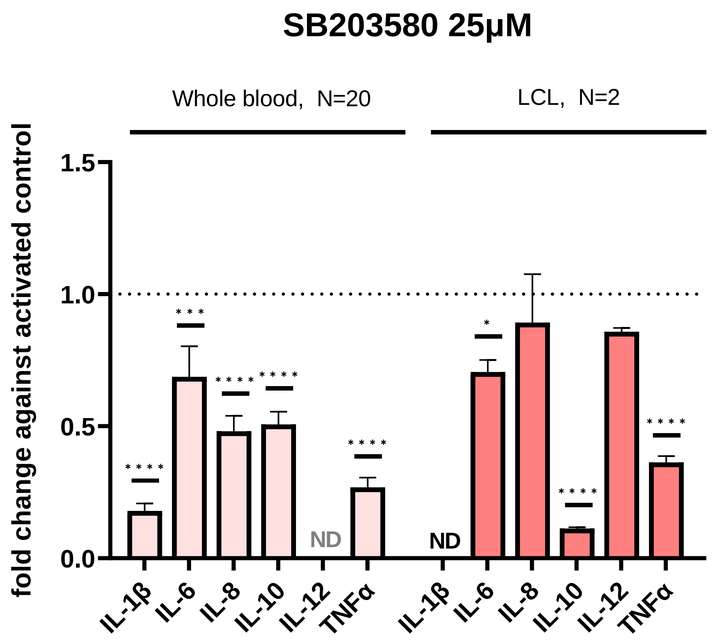


Figure 2. Cytokine profiles for compounds tested in fold change against untreated activated cells*: dexamethasone (0,25 µM), trametinib (25 µM), tanzisertib (25 µM), SB203580 (25 µM). Fold change in cytokine (IL-1β, IL-6, IL-8, IL-10, IL-12, and TNFα) concentration compared to activated cells after 24 h; 100 ng/ml LPS for whole blood, and 0,5 µM ionomycin and 3,33 ng/ml PMA for LCL, respectively. Cytokine production was assessed on twenty whole blood and two LCL samples. Bars above columns represent SEM, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, using Dunnett's test. ND – not detected.*

COVID-19 PATIENTS DERIVED HUMAN LCLs BIOBANK

Our results on a selected set of compounds indicate that the trend of cytokine secretion suppression is comparable between whole blood and human LCLs. This supports the use of human LCLs as a suitable *in vitro* cell model and as a good personalised medicine platform for cytokine-release related compound screening, since our LCL COVID-19 patients derived biobank accounts for interindividual differences.

To cover different phenotypes, we have prepared a biobank of human LCLs derived from 71 reconvalescent COVID-19 donors with differing severity of disease. Human LCLs were generated from fresh lymphocytes isolated from blood samples donated for this purpose by consenting healthy adults within the scope of the clinical study (no. 0120-496/2022/6) approved by Medical Ethics Committee of the Republic of Slovenia.

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