## Use of differentiated THP.1 cells to mimic nurse-like cells for its pro-survival effect on chronic lymphocytic leukaemia cells

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**Introduction**: Chronic lymphocytic leukaemia (CLL), the most common adult leukaemia in the West, is characterised by an accumulation of monoclonal CD5<sup>+</sup> mature B-cells in the peripheral blood and lymphoid tissues including lymph nodes and bone marrow. Despite the recent advance in treatment options, the disease is still incurable. The persistence of the leukemic cells is likely due to the presence of a protective microenvironment which provides pro-survival signals to the malignant cells. One of the major components of the CLL microenvironment is the monocyte-derived nurse-like cells (NLCs) which predominantly display an M2 macrophage phenotype and express CD68 and CD163.<sup>1</sup>

To date, studies of NLC-CLL interactions have relied on the use of fresh CLL samples from patients. Due to the variability in the number of monocytes between CLL samples, consistency in the development of NLCs *in vitro* remains a technical challenge. For example, the number of resultant NLCs varies significantly from case to case. Also, the experimental procedure to develop NLCs from fresh CLL samples is labour-intensive and time-consuming. Therefore, a reproducible and convenient model of NLCs is badly needed. Here we describe the use of differentiated THP.1 cells to mimic the pro-survival effect of NLCs on CLL cells.

**Methods**: To develop NLCs, PBMCs isolated from fresh blood samples of CLL patients were plated at a density of 10 x 10<sup>6</sup>/ml on multi-well plates and maintained in culture condition for up to 2 weeks. Morphological features of NLCs were observed via phase contrast microscopy and on light microscopy following May Grünwald Giemsa (MGG) staining. Phenotyping of NLCs was performed using immunofluorescence microscopy following staining with CD14, CD68 and CD163 antibodies. Co-culture experiments were performed using thawed autologous CLL cells that were cryopreserved in a -150°C freezer. Cell viability was measured using Annexin-V FITC/PI by flow cytometry.

Human monocytic leukemic THP.1 cell line was obtained from European Collection of Authenticated Cell Cultures (Catalogue No. 88081201, UK) and maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin and 100  $\mu$ g/mL streptomycin. THP.1 cells were induced to differentiate into macrophages using phorbol 12-myristate 13-acetate (PMA), as described. Co-culture experiments with primary CLL cells were performed similarly as described above and viability of co-cultured CLL cells monitored daily over the following 3 days. In addition, further polarising of differentiated THP.1 cells to M1 or M2 macrophages was performed using IL-4 (for M2) or IF $\gamma$  and LPS (for M1), respectively.

**Results**: We observed a time-dependent formation of NLCs from most of the CLL PBMC samples studied. The morphological features of NLCs observed by phase contrast microscopy and light microscopy following MGG staining (**Figure 1A-C**) were consistent with the published reports. Immunofluorescence microscopy also confirmed that NLCs expressed CD68 and CD163 (**Figure 1D and E**).

Co-culture of CLL cells with NLCs indeed enhanced viability of CLL cells over 5 days of observation when compared to CLL cells cultured alone (**Figure 2**).

Similarly, we co-cultured primary CLL cells with PMA-differentiated THP.1 cells. Interestingly, we also observed that CLL cells in such co-culture condition maintained better viability than CLL cells cultured alone (**Figure 3**). Using Student's t-test analysis (two tailed

and paired), we found that the difference between average viability of CLL cells cultured alone and those in co-culture was statistically significant with p values of 0.024 on Day 1, 0.026 on Day 2 and 0.020 on Day 3, respectively. The viability of CLL cells was 4.6%, 10.4% and 12.9% higher when cultured on THP.1 cells on Day 1, Day 2 and Day 3, respectively.

**Conclusions**: Altogether, our study confirmed that NLCs developed from the CLL PBMCs protected CLL cells from spontaneous apoptosis when cultured together. Moreover, we have shown that PMA-differentiated THP.1 cells also exhibited the cytoprotective effects on primary CLL cells, thus resembling at least in part the action of NLCs. Our work thus shows that the chemically-differentiated THP.1 cells could be a useful model to mimic the action of NLCs for the study of CLL microenvironment. Current ongoing work is to determine whether the cytoprotective effects are provided by the M1 or M2 macrophages from the differentiated THP.1 cells.

## References:

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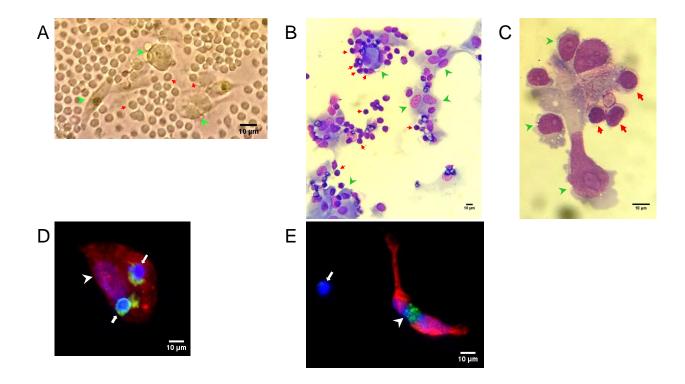


Figure. 1 Morphological Characterization and Phenotyping of NLCs. A. Phase contrast image of CLL cells (Red arrow) and NLCs (Green arrow head). B. Light microscope at lower magnification of MGG stained CLL cells (Red arrow) and NLCs (Green arrow head). C. Light microscope at higher magnification of MGG stained CLL cells (Red arrow) and NLCs (Green arrow head). D. Immunofluorescence microscopy of CLL cells (Arrow) and NLCs (Arrow head) stained with CD163 (Red) and CD19 (Green) antibodies, and counterstained with DAPI (Blue). E. Immunofluorescence microscopy of CLL cell (Arrow) and NLC (Arrow head) stained with CD163 (Red) and CD68 (Green) antibodies, and counterstained with DAPI (Blue).

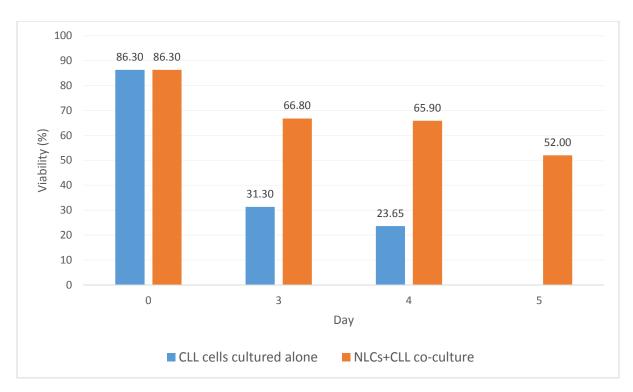


Figure 2. Viability of CLL cells cultured alone vs In Co-culture with NLCs (n=2).

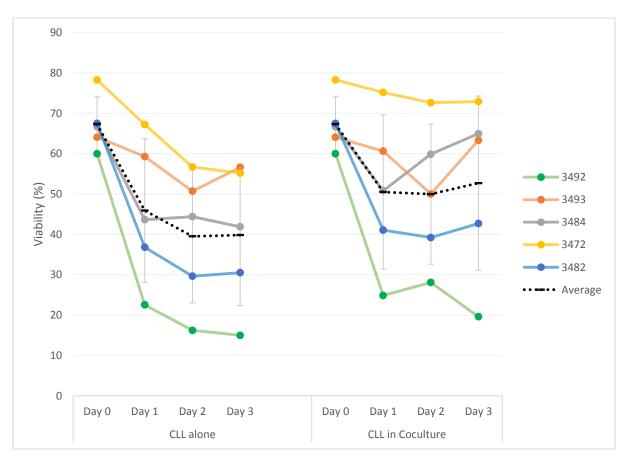


Figure 3. Viability of CLL cells cultured alone vs. In Co-culture with Differentiated THP.1 Cells.