Therapeutic drugs promote differentiation of Acute Myeloid Leukaemia (AML) blasts into a migratory and chemo-resistant phenotype facilitated by the bone marrow microenvironment.

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Introduction

The interaction of Acute Myeloid Leukaemia (AML) cells with the bone marrow (BM) microenvironment cells creates niches that offer cytoprotection to leukemic stem cells (LSC).
Using our in vitro co-culture experimental model of AML and BM cytoprotective cells we found that BM-mediated cytoprotection against daunorubicin and cytarabine correlated with an increased motile phenotype of AML surviving cells. We investigated the molecular mechanisms involved in this process.

Methodology

We have developed a fluorescence-based high throughput *in vitro* model that recreates BM-mediated cytoprotection of AML cells and allows for drug screening to overcome BM-mediated drug resistance. The platform consists of co-cultures of AML cell lines and the BM cytoprotective cell line HS5, expressing eGFP and mCherry, respectively. The level of expression of fluorescent proteins is proportional to the number of cells allowing for using fluorimetry as a read out for changes in cell proliferation. Fluorescent AML cells can also be used for live cell imaging and FACS sorting. In the current study, this platform was used for cytokine and phosphoproteomic essays and qPCR were performed as well as cell cycle analysis by flow cytometry.

Results

Our results showed that the interactions between AML and BM microenvironment cells play a crucial role in resistance to daunorubicin treatment (Figure 1). Daunorubicin resistant cells showed an abnormally differentiated phenotype (Figure 2) and migratory (Figure 3) AML phenotype correlating with expression of different epithelial-mesenchymal transition (EMT)-related genes (Figure 4).

Taking advantage of the high throughput capacity of our experimental platform, we performed phosphoproteomic and drug screening studies that converged in identifying JAK1/2 kinases as a critical mediators for BM microenvironment-mediated chemo-resistance to daunorubicin. The JAK1/2 inhibitors Ruxolitinib (RUX) and Baricitinib (BAR) sensitised AML cells to daunorubicin in the presence of BM stromal cells (Figure 5) and inhibited daunorubicin-induced increased migration (Figure 3).

Figure 1 **Cytoprotection of AML cells against daunorubicin (DNR) and cytarabine by BM stromal cells: cell cycle arrest in S phase**

Cell cycle progression of MOLM-14 cells cultured alone or in the presence of the BM stromal cell lines mCherry-HS5.

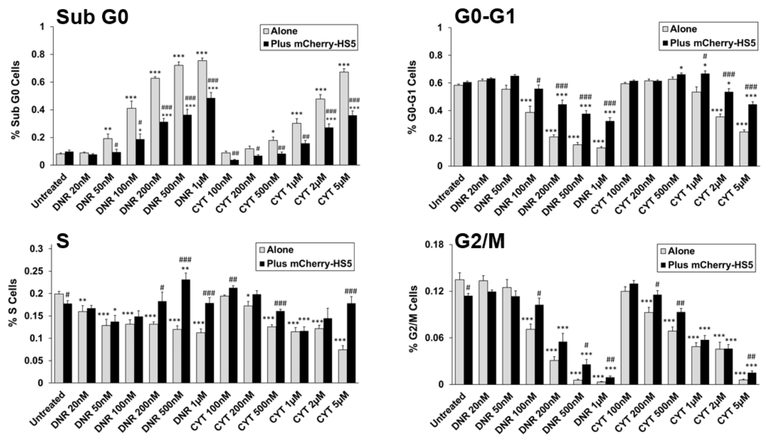
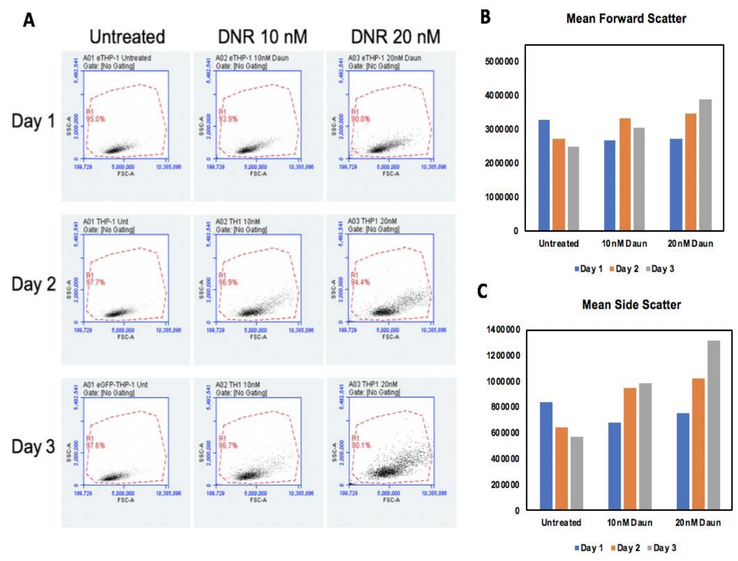


Figure 2 **Daunorubicin (DNR) induces an unusual mixed M1/M2 differentiated migratory phenotype in AML cells that is sustained by the BM stroma**

DNR increases cells size and cytoplasmic complexity of AML cells



DNR increases expression of CD197 and CD163 (M1 and M2 macrophage markers) in AML cells

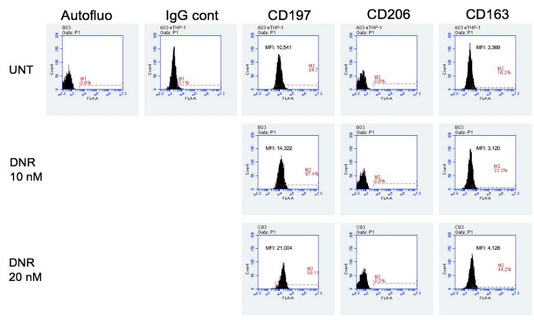


Figure 3 **The JAK1/2 inhibitors Ruxolitinib and Baricitinib inhibit the increase in the migratory capacity of AML cells induced by daunorubicin**

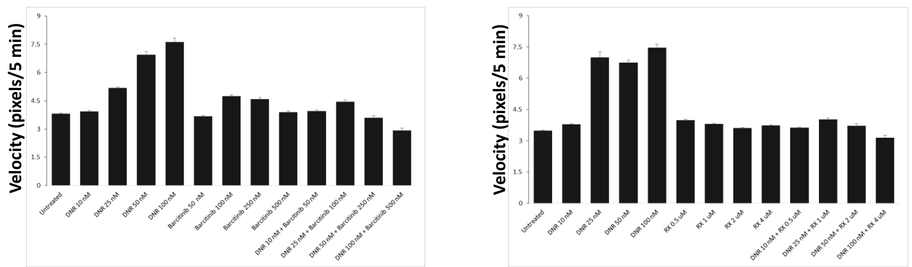
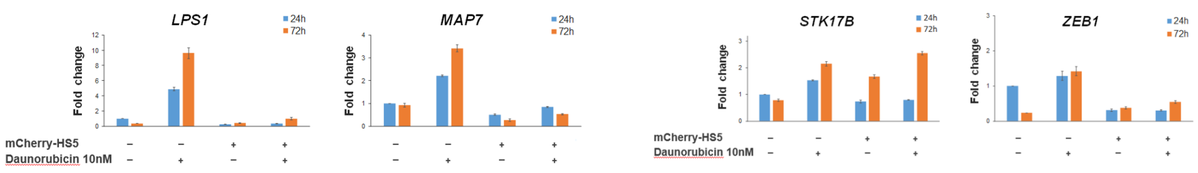


Figure 4 **Upregulation of EMT related genes in AML cells in response to daunorubicin**



Cytokine secretion in response to DNR treatment and the presence of cytoprotective BM stroma

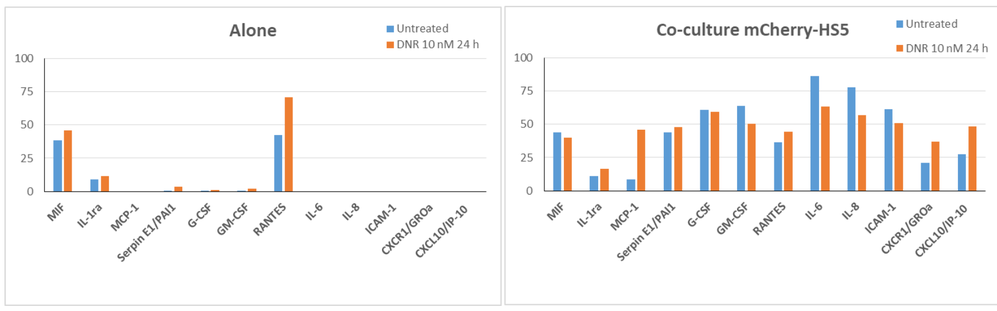


Figure 5 **The JAK1/2 inhibitors Ruxolitinib and Baricitinib increase the pro-apoptotic effect of daunorubicin in the presence of the cytoprotective BM stroma**