Abstract Submission

03. Acute myeloid leukemia - Biology & Translational Research

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MODELLING AND TARGETING ACUTE MYELOID LEUKAEMIA CELLS IN THE BONE MARROW PROTECTIVE NICHE

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Background: AML is a therapeutic challenge due to its aggressiveness and biological heterogeneity. Although 80% of patients initially achieve a complete remission, the long-term disease-free survival is poor. One issue contributing to disease relapse, is persistence of disease in the protective niche of the Bone Marrow microenvironment (BMME). Here, AML cells are surrounded by other cell types that promote their survival, which enables them to evade therapeutic destruction and promotes the emergence of drug resistance. Clinical trials of single agent mobilising drugs, like Plerixafor, have yielded promising results but they are not curative. So, the development of combinatorial therapies that simultaneously target different components of AML cell adhesion may release more AML cells into the circulation, where they can be targeted by standard therapies.

Aims: a) To develop a multi-cellular, co-culture system to recapitulate the adhesive BMME, b) To test rational drug combinations and identify targets with the potential to mobilise anchored AML cells and c) To perform paired transcriptomic and phenotypic analysis of persistently adhered versus mobilised AML cells to detect novel targets.

Methods: We have developed a novel 96-well plate multi-cellular, co-culture system using stromal cells (HS5), endothelial cells (HUVEC), osteoblasts (hFOB 1.19) and AML cells (OCI-AML3 and KG1a). Multiple mobilisation drugs, alone and in combination, were tested and AML cell release was quantified by flow cytometry. Drugs tested to date include: Plerixafor (CXCR4 inhibitor), Natalizumab (anti-α4-integrin antibody), ONO-7161 (novel CXCR4 inhibitor), purified anti-CD44 and anti-E-Selectin. Phenotypic and transcriptional comparisons were made between the persistently adherent and mobilised AML cells.

Results: The most adhesive physiological “BMME mix” was identified as HS5, HUVEC and hFOB 1.19 in equal 1/3 proportions (p=0.0001). Co-culture with AML cells resulted in 74% and 68% adherence of KG1a and OCI-AML3, respectively. Phenotypic analysis of adhered vs non-adhered AML cells identified CD44, CXCR4, CD49d and CD38 as important markers of optimal BMME adhesion. Subsequently, our novel co-culture system was used as a drug testing platform to assess clinically available agents. Plerixafor and Natalizumab increased detachment by 1.3-fold (p=0.0132) and 1.2-fold (p=0.015) respectively. In contrast, ONO7161 and anti-E-selectin had little effect. The most promising candidate, anti-CD44, resulted in a 2-fold and 1.6-fold detachment of KG1a (p=0.004) and OCI-AML3 (p=0.027), respectively. However, even in the presence of the maximum dose of anti-CD44 (5μg/mL)), 46% of KG1a and 48% of OCI-AML3 cells remained persistently adhered. Synergy experiments with anti-CD44 in combination with either Plerixafor or Natalizumab yielded no better release than anti-CD44 alone.

Summary/Conclusion: We have developed a novel, multi-cellular co-culture system that recapitulates the adhesive BMME. We are using this as a drug testing platform for AML to identify the most potent release agents and test the effects of cytotoxic drugs on those most persistently adhered. To-date, anti-CD44 is the most effective release agent in our model, but it still failed to release all AML cells. Transcriptomic analysis of the persistently adherent AML cells should enable us to delineate the critical biological interactions required for BMME adhesion. This will enable us to identify rational new targets to effectively remove chemo-resistant AML cells from the protective BM niche.

Keywords: Acute myeloid leukemia, Adhesion, Bone Marrow, Microenvironment