**Grant Impact Report**

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| Name | Eleni Ladikou |
| Job title | Haematology Specialty Registrar and Honorary Lecturer in Haematology |
| Organisation | University Hospitals Sussex NHS Foundation Trust |
| Co-investigators (if applicable) | NA |
| Grant awarded | Early-Stage Research start-up grant |
| 1. **Please summarise what the grant enabled you to achieve;** **what would not have been possible without the funding? (Up to 500 words)**   I am deeply grateful to the British Society of Haematology for the early start-up grant provided during my PhD. The funding enabled me to conduct essential experiments using patient samples, which were critical to the success of my research. I successfully established a unique artificial 2D bone marrow model, called the Bone Marrow Adhesion System (BMAS), which served as a drug-testing platform to try and identify novel anti-adhesion therapies for acute Myeloid Leukaemia (AML).  50% of AML patients relapse and exhibit poor long-term disease-free survival. This unmet clinical need is underpinned by the adherence of AML cells in the protective niche of the bone marrow micro-environment (BMME) where they are protected by cell mediated drug resistance (CAM-DR). The ability to disrupt this adhesion, and push AML cells into the peripheral blood (PB) where they are more susceptible to chemotherapy, could unlock new and exciting therapeutic strategies. While many *in-vitro* co-culture systems have previously been developed for AML, none had measured AML cell adhesion in a multi-cellular system that. Following extensive optimisation, using different bone marrow cell types with different AML cells (both cell lines and patient-derived cells) I successfully developed the BMAS model. BMAS replicates the adhesive and protective properties of the BMME using stromal cells (HS-5 cell line), endothelial cells (HUVEC cells), and osteoblasts (hFOB 1.19 cell line). The platform enabled me to test novel therapies aimed at detaching AML (Acute Myeloid Leukaemia) cells from the protective bone marrow niche and I was able to identify anti-CD44 as a therapy that can reduce adhesion and increase AML sensitivity to chemotherapy. Additionally, I was able to isolate BMAS persistently adherent AML cells and use RNA sequencing to transcriptionally characterise them. This led to the identification of a novel target, FAK, as a key contributor to AML cell adhesion in the bone marrow niche. Following this discovery, I was able to show that Defactinib, a FAK inhibitor, also prevented AML adhesion and that dual targeting of the FAK and CD44 pathways was the most effective approach for disrupting AML cell adhesion. Without the early start-up grant, none of this work in developing the BMAS would have been possible. Following on from the success of this work I was successful in securing a PhD studentship grant from the Sussex Cancer Fund which will enable continuation of this exciting project. The student started in October 2024, and I have been awarded an honorary contract as a Lecturer at Brighton and Sussex Medical school which will enable me to continue directing this research whilst I return to the clinical training programme. | |
| 1. **Briefly describe the aims and intended outcomes of this project. Please clearly indicate if there is any sensitive information in this report that should remain confidential for now. (Up to 300 words)**   **Aim 1: Develop a sophisticated multi-cellular co-culture system recapitulating the adhesive BMME (BMAS) and use this to identify the main components involved in AML cell adhesion.**  Initially, the BMAS will be developed. The effect of three major components of the BMME on AML adhesion will be systematically evaluated by co-culturing AML cell lines with HS-5 stromal cells, endothelial cells, and osteoblasts in a range of ratio-metric combinations. The optimally adhesive combination of these cell types will be then used to assess a range of endpoints comparing survival, cell cycle, oxidative stress and phenotype between adhered, non-adhered and monoculture AML cells.  **Aim 2: Identify the best agent for blocking adhesion of AML cells and combine that with the common DNA damage agent, Cytarabine, to test for reversal of cell adhesion mediated drug resistance (CAM-DR)**  Following the establishment of the optimal adhesive 2D system, known adhesion antagonists will be added to the system and adhesion levels assessed. The results from these experiments will help identify the most promising adhesion blocking agent(s) (monotherapy or in combination). In addition, conventional AML therapies, such as Cytarabine, will be added to compare their cytotoxic effect on both non-adhered and adhered AML cells and investigate whether this is increased by the addition of the most promising adhesion blocking agent(s).  **Aim 3: Utilise the model to isolate persistently adhered AML cells and perform paired transcriptomic analysis of adhered versus non-adhered cells to identify novel druggable targets**  Using the optimised model, AML cells will be treated with the best agent for blocking adhesion. Subsequently, persistently adhered, non-adhered and monoculture cells will be isolated, and their RNA sequenced in order to perform comparative transcriptomic analysis. The results from these experiments will help us identify novel targets from the persistently adhered AML cells in order to effectively increase adhesion blockade on our BMAS model. | |
| 1. **Describe the key outcomes to date, including whether this grant has resulted in further research. Please summarise your conclusions**. **(Up to 600 words)**   Fifty percent of AML patients relapse and exhibit poor long-term disease-free survival. This unmet clinical need is underpinned by the adherence of AML cells in the protective niche of the BM micro-environment (BMME). Therefore, disrupting this adhesion and releasing AML cells into the peripheral blood, where they are more susceptible to chemotherapy, could unlock exciting new therapeutic strategies. During my 3 years at BSMS, I developed and optimised a robust, reproducible, in-vitro co-culture model of the AML-BMME; the BM adhesion system (BMAS). The BMAS comprises of stromal cells (HS-5), endothelial cells (HUVEC), and osteoblasts (hFOB 1.19) in equal proportions. I demonstrated that the BMAS exhibited many of the properties of the BMME and showed that adhered AML cells are more viable and metabolically active. I also demonstrated that the BMAS recapitulated the cell adhesion mediated drug resistance (CAM-DR) seen clinically by showing BMAS- adhered AML cells to be the most resistant to treatment with the common AML therapy Cytarabine. This verified the *in-vitro* BMAS system as a useful drug testing model to test whether blocking adhesion of AML cells would increase their susceptibility to chemotherapy. Initially I used the BMAS to establish differences in adhesion antigens between adhered and non-adhered AML cells and showed that adhered AML cells express higher levels of surface CD34 and CD44. Following the findings of these experiments, and a thorough literature search, I next tested several commercially based adhesion-blocking agents for their ability to reduce the number of adhered AML cells. Anti-CD44 treatment was established as the most effective in preventing adhesion and its combination with Cytarabine significantly increased AML cell apoptosis (death) compared to Cytarabine alone. However, even at the highest anti-CD44 dose, some AML cells remained persistently adhered. I therefore, isolated and characterised these transcriptionally (RNAseq) to identify novel druggable targets. Paired transcriptional analysis of anti-CD44 treated adhered versus non-adhered AML cells identified focal adhesion (FAK) as a novel potential target. The clinical grade FAK inhibitor, Defactinib prevented adhesion of the KG1a AML cell line, and its combination with anti-CD44 was additive. Strikingly, when the BMAS system was adapted to use primary AML cells and an autologous BMME (stromal cells derived from the patient), the combination of anti-CD44 and Defactinib was highly synergistic at preventing AML adhesion. Taken together, the unique BMAS has revealed novel ways to target persistently adherent AML cells within the BMME and provides a platform for testing combination therapies designed to improve AML chemotherapeutic susceptibility. It also has the potential to explore patient heterogeneity, develop personalised therapeutic strategies and be utilised to research other blood cancers. Importantly, anti-CD44 (available as a clinical grade drug RG7356) and Defactinib have been identified as a promising novel therapeutic strategy to overcome CAM-DR.  The findings from this project have been written up for publication and are currently with the co-authors for their input prior to submission. In addition, I have successfully secured grant funding from the Sussex Cancer Fund for a 3-year PhD studentship to continue this research. Following my maternity leave, I have now returned to clinical medicine in the department of haematology at Eastbourne District General Hospital, but I have an honorary contract with Brighton and Sussex Medical school which will enable me to continue my research and supervise this newly awarded PhD studentship (with the help of Professor Andrea Pepper). Furthermore, I will continue to collaborate with the Pepper and Mitchell teams at BSMS (www.pepper.science and www.mitchell.science) in other projects and will be helping them adapt the BMAS system for a collaborative project with Dr John Jones on Multiple Myeloma. I will also be contacting Professor Steve Knapper (Cardiff University) who has asked me to discuss joining the UK AML working party and participating in UK AML clinical trials. | |
| 1. **List published papers, oral and/or poster presentations as a result of this grant. Include manuscripts in preparation or in submission / under review, prefaced by an asterisk.**   **Publications**   * **\*Ladikou EE** et al. (2024) Modelling and targeting Acute Myeloid Leukaemia cells in the Bone Marrow protective niche. To be submitted/in preparation * Burley TA, Hesketh A, Bucca G, Kennedy E, **Ladikou EE**, Towler BP, Mitchell S, Smith CP, Fegan C, Johnston R, Pepper A, Pepper C. (2022) Elucidation of Focal Adhesion Kinase as a Modulator of Migration and Invasion and as a Potential Therapeutic Target in Chronic Lymphocytic Leukemia**.** Cancers * Burley TA, Kennedy E, Broad G, Boyd M, Li D, Woo T, West C, **Ladikou EE**, Ashworth I, Fegan C, Johnston R, Mitchell S, Mackay SP, Pepper AGS, Pepper C (2022) Targeting the Non-Canonical NF-κB Pathway in Chronic Lymphocytic Leukemia and Multiple Myeloma. Cancers * **Ladikou EE**, et al. (2019) Dissecting the role of the CXCL12/CXCR4 axis in Acute Myeloid Leukaemia. BJHaem * **Ladikou EE**, et al. (2019) AML in its niche - the Bone Marrow microenvironment in Acute Myeloid Leukemia. Current Oncology Reports.   **European Hematology association (EHA)**   * Poster presentation * Travel grant award covering conference registration   **European School of Haematology (ESH)**   * Poster presentation   **Leukaemic Stem Cell (LSC) Symposium London Stem Cell Network**   * Poster presentation   **Adam Weiler Doctoral Impact Award**   * Noted as one of three ‘highly commended’ candidates by the judging panel.   **University of Brighton 3MT competition**  **University of Brighton photo competition**   * 2nd place   **Bench to Bedside Cancer Symposium**   * Oral presentation | |
| 1. **Did any patent applications arise from this work? (If yes, please detail. Up to 200 words)**   no | |
| 1. **Were you successful in any further grant applications as a result of this work? (If yes, please detail. Up to 200 words)**   I have successfully secured further funding from Sussex Cancer Fund (with matched funding from BSMS) for a PhD studentship. The new PhD student will work on a follow-on project from my PhD. They will utilise the novel co-culture system BMAS, in order to characterise chemo-resistant AML cells transcriptionally, phenotypically and functionally. This will help to identify novel targeted therapeutic approaches to prevent AML relapse. More specifically, they will optimise the isolation and subsequent analysis of chemo-resistant cells from BMAS using the KG1a AML cell line. Subsequently, these experiments will be repeated using therapy naive primary de novo AML cells. This will be achieved using the already established an AML biobank with bone marrow (BM) and peripheral blood (PB) donated by AML patients at the Royal Sussex County Hospital and Eastbourne General Hospital, consisting of 10 PB and 6 BM samples, four of which are paired. We are currently actively collecting more chemotherapy naïve and post chemotherapy samples. Using primary samples is a better model to investigate chemo-resistant AML cells compared to an AML cell line, as it allows us to capture the clinical heterogeneity that characterises AML. This project will be led by myself as the principal investigator and helped by the two co-investigators Prof. Andrea Pepper and Dr Fabio Simoes. | |
| 1. **Did new collaborations arise from this work? (If yes, please detail. Up to 400 words)**   Using both cell lines and primary AML cells, my project findings indicated that the combination of Defactinib and anti-CD44 is an exciting anti-adhesion therapeutic strategy to explore further. This is particularly interesting given the ongoing solid tumour clinical trials of drugs targeting FAK (Defactinib) and CD44 (RG7356) independently. For AML, my findings strongly suggest that they would be a synergistic combination therapeutic approach.  To test this in another model, I collaborated with Dr Giles Best at Flinders University, Australia. Dr Best further optimised my BMAS using primary AML cells and autologous BM cells (i.e.. all of the cells are from the patient). He subsequently treated with Defactinib and anti-CD44 alone or in combination and confirmed my findings; a significantly higher number of non-adhered cells were present in the samples treated with either anti-CD44 or Defactinib alone. Like me, he found that the combination therapy led to a substantial augmentation in the number of non-adherent cells across all patient samples, surpassing the outcomes observed with either drug alone. Importantly, a highly synergistic drug combination was identified which was much greater than the summative effect of the two drugs alone. | |
| 1. **What was the funding amount you received and how was it actually spent? (detail item/activity and amount spent in pounds)**   16,500 GBP  Please see attached excel sheet | |
| 1. **What are the future research priorities in this area?**   Future research priorities in Acute Myeloid Leukaemia (AML) and the Bone Marrow Microenvironment (BMME) focus on improving our understanding of the complex interactions between leukaemic cells and their surrounding niche, developing novel therapeutic strategies, and addressing challenges related to resistance and relapse. Key areas of priority include:   * Understanding how AML cells interact with the BMME to develop resistance and how to disrupt these protective mechanisms. Developing therapies that target the BMME to prevent it from nurturing AML cells and making them resistant to treatment. * Identifying and eradicating minimal residual disease, which often leads to relapse. Advanced methods for MRD detection using next-generation sequencing and flow cytometry will enable earlier and more precise interventions such as targeting resistant clones in MRD, potentially by modulating the immune response or targeting specific mutations. * Understanding the Leukemic Stem Cell (LSC) Niche and how these cells remain quiescent and resistant to therapy. Efforts to disrupt the interaction between LSCs and the BMME could enhance the effectiveness of treatments and prevent disease relapse. * Combining therapies that target both AML cells and their microenvironment (e.g., FAK and CD44 inhibitors) alongside traditional chemotherapy and novel agents will likely be a key strategy for improving outcomes. * Understanding the evolution of AML, especially in the context of therapy-induced selective pressures, can help in developing strategies to prevent relapse. Research will focus on identifying what drives disease relapse.   By advancing knowledge in these areas, future research hopes to improve survival rates, reduce relapse, and ultimately lead to more effective, targeted treatments for AML patients. | |