**Grant Impact Report**

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| Name | Dr Cristina Pina |
| Job title | Lecturer in Biomedical Sciences |
| Organisation | Brunel University London |
| Co-investigators (if applicable) | N/A |
| Grant awarded | £15,000.00 |
| Year awarded | 2019 |
| Date started | 1st March, 2020 |
| Date completed | 28th February, 2022 |
| Total amount expended (£) | £14,846.05 |

This is the BSH grant impact report form. Please enter the full grant details above, and fill out the form below. The form should be completed electronically and sent to [grants@b-s-h.org.uk](mailto:grants@b-s-h.org.uk). Please note that the report can only be accepted if all sections have been completed in full.

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| 1. **Please summarise what the grant enabled you to achieve;** **what would not have been possible without the funding? (Up to 500 words)**   The funding received was critical for my installation as a new Lecturer in Biomedical Sciences at Brunel University London, College of Health, Medicine and Life Sciences. It bridged my previous Fellowship work at the University of Cambridge into my first established Academic position, and allowed me to recruit a talented MPhil (Res) student, Ralph Samarista, to undertake the laboratory work while I got settled in the combined teaching, research, and administrative commitments of my new post. This student’s recruitment was particularly critical during the CoVID-19 Pandemic, which placed strong onus on teaching responsibilities with adaptation to remote, and later hybrid teaching, as well as extensive undergraduate student support. Furthermore, charity-based funding opportunities which have historically supported work in the Haematology and Leukaemia fields were significantly reduced and, in many cases suspended, during the last 2 years, and BSH funding was critical to sustain my research activity.  At an institutional level, my early establishment as an externally-funded researcher in the leukaemia field, supported my nomination as the Cancer Cluster Lead in the newly-established Centre for Genome Engineering and Maintenance. It prompted College-Level financial commitment towards the upgrading of the existing Novocyte flow cytometer analyser from 5 to 15 channels to enable my research. More recently, my sustained research activity proved critical to a successful College matched-funded application to the Wolfson Foundation for acquisition of a cell sorter.  Scientifically, the funding allowed me to analyse individual and combined contributions of epigenetic methylations and the unfolded protein response (UPR) in Acute Myeloid Leukaemia, and to explore the therapeutic potential of combined DNA demethylating agents and UPR modulators. I had previously identified these two pathways as the main candidate mediators of AML dependency on the MAT2A enzyme, which catalyses the synthesis of S-adenosyl-methionine, the main methyl donor in the cell. I have now shown that combined targeting of DNA methylation and activation of the pro-apoptotic arm of the UPR can cooperate in eliminating AML cells from different genetic backgrounds, both cultured AML cell lines and patient samples. I will now aim to extend these results *in vivo* to confirm their translational therapeutic potential. |
| 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)**   The project has 2 main aims:  1. To test additive and synergistic effects of DNA demethylation and loss of H3K36me3 in AML cells  2. To combine DNA demethylating agents with activation of unfolded protein response chaperones in eliminating AML cells  The intended outcomes are the development of new targeted combinatorial therapies to enhance survival of AML patients currently treated with DNA demethylating agents. These are typically elderly patients who cannot tolerate conventional chemotherapy ± bone marrow transplantation regimens, and who inevitably relapse with dismal survival.  We (Tzelepis et al *Cell Rep* 2016, 10.1016/j.celrep.2016.09.079) and others (Secker et al *Cancers* 2020, 10.3390/cancers12051342) have previously identified a vulnerability of AML cells to loss or inhibition of MAT2A (preliminary data for this proposal), the enzyme responsible for synthesis of S-adenosyl-methionine, the main methyl-group donor in the cell. We observed that MAT2A resulted in loss of DNA methylation, unique loss of Histone 3 K36 tri-methylation, and molecular activation of the unfolded protein response, and sought to explore combinatorial and/or synergistic effects of these pathways in AML.  The project is still on-going, and I would be grateful if the contents of this report could remain confidential until data publication. |
| 1. **Describe the key outcomes to date, including whether this grant has resulted in further research. Please summarise your conclusions**. **(Up to 600 words)**   The main outcomes to date concern Aim 2. **We demonstrated synergistic effects of p97 inhibition and DNA demethylation in eliminating AML cells, including candidate leukaemia stem/progenitor cells in cell lines and patient samples.**  In detail:  We have shown that inhibition of the p97 AAA ATPase in AML cell lines reflecting common genetic events in human disease, MOLM-13 (*KMT2A-MLLT3* and *FLT3-ITD*), and OCI-AML3 (mutant *NPM1* and *DNMT3A*) tested using 2 different inhibitors, NMS-873 and CB-5083 (Anderson et al, *Cancer Cell* 2015, 10.1016/j.ccell.2015.10.002), results in decreased cell growth and reduced cell viability. IC50 (viability) were calculated as CB-5083 400-450nM and NMS-873 1.8-2M in both cell lines, in line with observations in colon cancer (Anderson et al, 2015, above). At a cellular level, the results are mediated by increased apoptosis (p<0.01, NMS-873; p<0.001, CB-5083) and an accumulation of cells in the G0/G1 phase of the cell cycle (p<0.0001, both inhibitors), with no clear effects on cell differentiation. There is activation of genes in the apoptotic arm of the UPR, namely *CALR*, *DNAJC3*, *DNAJB9* and *DNAJB11*.  We tested the effects of CB-5083-mediated p97 inhibition in colony-forming cell (CFC) assays, a surrogate for the presence of culture-initiating leukaemia progenitor activity. We could not observe a reduction in CFC with CB-5083 alone. However, combinatorial use of CB-5083 (450nM) and azacytidine (AZA, 7M) resulted in reduced CFC activity, which exceeded with activity of AZA alone (p<0.01, 2-fold reduction compared with AZA). The same effect was observed in liquid cultures of both MOLM-13 and OCI-AML3 cells, with a combination of CB-5083 and AZA further decreasing growth and viability relative to AZA alone (p<0.05). Interesting, the enhanced effect could also be observed with half-dose of AZA (p<0.01, MOLM-13) or half-dose of both compounds (p<0.05, OCI-AML3), suggesting synergistic activity. The possibility of using lower doses of both compounds may have therapeutic implications for reduced toxicity. Preliminary investigation of the effects of CB-5083 ± AZA in patient samples (n=4 analysed, distinct mutational profiles), resulted in reduced %CD34+ cells in 24-hour liquid culture with SCF, Flt3L and TPO (40-71% of AZA, 14-34% of DMSO), indicating clinical relevance of the findings.  At a molecular level, RNA-sequencing analysis of MOLM-13 cells treated with CB-5083, AZA or CB-5083+AZA, revealed that the additional effects of CB-5083 could be attributed to induction of cell senescence (GSEA, NES=2.88, p-val=0, FDR q-val=0). Interestingly, AZA itself results in UPR activation (GSEA, NES=1.33, p-val=0, FDR q-val-0.11), suggesting convergent rather than independent effects.  Aim 1 is currently being pursued. The goal is to knockdown *SETD2* expression to ablate H3K36 tri-methylation activity in AML cells, and investigate possible synergism with DNA demethylating agents. To this end, we cloned and sequence-validated 3 independent knockdown-validated SETD2 shRNA from the MISSION siRNA database into our usual pLL3.7 lentiviral backbone (Arede et al, *Blood Adv* 2022, 10.1182/bloodadvances.2020002842). In contrast with the non-targeting shRNA control-transduced cells, we failed to observe growth of SETD2sh-transduced MOLM-13 cells, suggesting a strong anti-leukaemia effect, in line with recent reports (Skucha et al, *Nat Commun* 2018, 10.1038/s41467-018-04329-y). However, we faced technical difficulties in recovering cells for demonstration of gene expression knockdown and loss of H3K36me3. We are currently pursuing an inducible knockdown approach and extending the panel of shRNA to obtain a more gradual ablation in which synergistic effects of histone and DNA methylation can be explored. This work is still on-going. |
| 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.**  * \*(in preparation): Zeka K, Taylor AV, Samarista RC, Suen C-W, Forte D, Ragusa D, Byrne C, Meduri E, Huntly BJ, Curti A, Pina C, MAT2A contributes to AML maintenance through synergistic effects on DNA methylation and activation of the unfolded protein response. |
| 1. **Did any patent applications arise from this work? (If yes, please detail. Up to 200 words)**   No patent applications. |
| 1. **Were you successful in any further grant applications as a result of this work? (If yes, please detail. Up to 200 words)**   I plan to submit grant applications for analysis of the combined effects of DNA demethylating agents and p97 inhibition in patient samples from the last quarter of 2022, upon submission of the manuscript currently in preparation and its deposition in *biorXiv* as a pre-print.  As detailed, BSH funding enabled me to maintain research activity throughout the CoVID-19 Pandemic despite prolonged lab closures (March-September 2020), reduced lab occupancy (September 2020 – September 2021), and periods of shortage in lab supplies (September 2020 – June 2021). Continued results were critical in the leadership of a successful funding application to the Wolfson Foundation (£155,000, granted 01/2022) for the establishment of the Phenotyping and Cell Sorting Laboratory, specifically the acquisition of a cell sorter currently going into tender. |
| 1. **Did new collaborations arise from this work? (If yes, please detail. Up to 400 words)**   I established a collaboration with Dr Annabelle Lewis, a new Lecturer in Biomedical Sciences also at Brunel University London, who has a background in fundamental analysis of DNA methylation in development and cancer. We are analysing DNA methylation effects of DNA demethylating agents and p97 inhibition on the promoters of UPR genes, using targeted bisufite sequencing. Specifically, we are optimising a PCR approach that can be used in patient samples and may be tested to predict synergistic effects of the compounds.  I am currently in the process of establishing collaborations with Clinicians involved with the newly-established Brunel Medical School for access to patient samples, and future clinical studies. |
| 1. **What was the funding amount you received and how was it actually spent? (detail item/activity and amount spent in pounds)**   Awarded Amount - £15,000.00  Amount Received - £0.00  Amount Spent - £14,846.05  Laboratory Supplies / Consumables / Chemicals and Gases - £10,439.05  Studentship Tuition Fees - £4,407.00  Please see attached Excel Spreadsheet for detailed analysis of spend. |
| 1. **What are the future research priorities in this area?**   **The priorities are to extend the analysis of combinatorial effects of p97 inhibition and DNA demethylating agents to AML patient samples, and potentially to MDS samples, to support the clinical relevance of our observations. Ultimately, the results can be translated to the clinic is clinical trials.** |