Shedding smart light on the effectiveness of chemotherapy; using Raman spectroscopy and machine learning to differentiate the effects of cytarabine toxicity and crosstalk of leukaemic and bone marrow stromal cells.

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Mesenchymal stromal cells (MSC) protect leukaemic cells from drug-induced toxicity within the bone marrow niche, with increasing evidence of leukaemic impact on supportive stroma. The nucleoside analogue, cytarabine (ara-C), is a front-line agent for acute myeloid leukaemia (AML); yet over a third of patients do not show continued response to ara-C-based regimens. DNA damage by agents such as ara-C can persist in bone marrow-MSC, which remain of host-origin post-allogeneic stem cell transplant, affecting functionality and compounding poor clinical outcomes, including bone marrow failure and secondary malignancies. There is a clinical need for rapid evaluation of AML cell chemosensitivity, in order to avoid unnecessary toxicity from patient exposure to ineffective agents, with current methods for testing genotoxicity or chemosensitivity proving time-consuming and costly. Raman spectroscopy enables probing for chemical changes within cells, correlated to cell health, and may provide an alternative rapid approach to assess treated cell toxicity. This study aimed to develop a novel method, combining AML-MSC co-culture, Raman spectroscopy and machine learning for the differentiation of cell types and drug handling responses, to evaluate toxicity and better understand chemoresistance mechanisms.

AML cells (HL-60/K562) and MSC (HS-5) were mono-cultured or co-cultured in a developed model, allowing bidirectional crosstalk, prior to treatment with physiological dose ara-C (25 µM, equivalent to 100-200 mg/m^2) for 1 or 48 h. Genotoxicity modulation by AML-MSC co-culture was assessed in ara-C treated cells by micronucleus incidence, with cytotoxicity modulation assessed by CellTiter-Glo ATP assay. Fixed or live cells were analysed by confocal Raman microscopy imaging, followed by analysis through supervised and unsupervised machine learning and principle component analysis.

Genotoxicity was significantly decreased in HL-60 ($P = 0.0007$) and K562 ($P = 0.003$) following co-culture with HS-5, while significantly increased in HS-5 following co-culture with HL-60 ($P = 0.0214$) and K562 ($P = 0.0013$). HS-5 were additionally sensitised to ara-C-induced cytotoxicity by leukaemic cell impact, with significant decreases in ATP production following co-culture with HL-60 ($P = 0.0144$) and K562 ($P = 0.0002$). Mono-cultured cells were successfully identified by Raman spectroscopy using a leave-one-out fivefold cross-validation paradigm and a radial basis function support vector machine with moderate accuracy of 0.77 (+/− 0.34). HL-60 were reliably characterised (1.0), while HS-5 (0.8) and K562 (0.5) had lower identification accuracy. Identification of the difference between untreated and ara-C-treated K562 cells showed moderate accuracy of 0.72 (+/− 0.08). Principle component analysis showed some clustering, however, with variance between different cell types.

This study shows the potential for use of confocal Raman microscopy as a method for delineation of chemotherapeutic and crosstalk effects in AML cells and MSC within the

context of the tumour microenvironment. Rapid identification of chemosensitive and chemoresistant patients may aid clinicians in selecting appropriate treatment strategies. Interpretation of Raman spectra may also provide mechanistic information linked to cellular chemical changes occurring in co-culture and following drug exposure, elucidating chemoresistance mechanisms in the leukaemic microenvironment.