A closed loop for normal and abnormal haematopoiesis: *in vivo -in vitro - in silico*

Maria Fuentes[a,b], Eirini Velliou[a,b], Ruth Misener[a,b], Eleni Pefani[a], Nicki Panoskaltsis[c], Athanasios Mantalaris[b], Michael C. Georgiadis[a], Efstratios N. Pistikopoulos[a]

[a] *Centre for Process Systems Engineering, Department of Chemical Engineering, Imperial College London, South Kensington Campus, London, SW7 2AZ, UK*
[b] *Biological Systems Engineering Laboratory, Department of Chemical Engineering, Imperial College London, South Kensington Campus, London, SW7 2AZ, UK*
[c] *Department of Haematology, Imperial College London, Northwick Park & St. Mark's Campus, London, HA1 3UJ, UK*

Keywords: chemotherapy optimization, cell cycle models, cyclin, 3D scaffold, AML, pharmacokinetics, pharmacodynamics, bioreactor optimization

Northwick Park Hospital, Leukaemia Research Fund · erc MOBILE · RICHARD THOMAS Leukaemia Fund · NHS National Institute for Health Research

INTRODUCTION

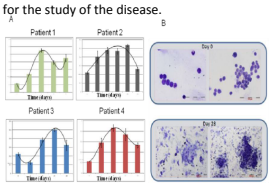
Acute Myeloid Leukaemia (AML) is one of the most common types of leukaemia in adults. Briefly, AML is a malignant disease of the bone marrow and blood. Immature white blood cells which are not able to develop into normal functioning blood cells are overproduced and build up in the bone marrow and blood. This inhibits the development of healthy blood and immune cells due to space restrictions and inhibitory and clonal factors specific to the disease. The most common treatment for AML is intensive chemotherapy. It is well known that this therapy can result in several life-threatening complications as only few patient-specific factors are taken into consideration in current protocols and choice of treatment often depends on the treating physician's experience.

Inter-patient and intra-leukaemia variability combined account for the added complexity in determining these treatment protocols.

In order to overcome these limitations, there is a need for personalised treatments that incorporate both the individual patient characteristics and features specific to the patient's leukaemia (different for every patient). Therefore, **the main focus of our research is to close the loop *in vitro-in silico-in vivo* for AML. For this purpose *in vitro* study of AML is taking place in our group, for collection of patient and disease specific data that will serve as an input for the development of an *in silico* model for personalised chemotherapy treatment.**

EX-VIVO CULTURE OF AML PATIENT CELLS

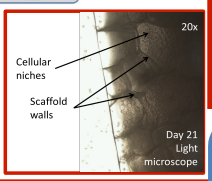
An adequate environment where normal and abnormal blood cells can be cultured was previously developed by our group. It consists of a 3-D polyurethane scaffold coated with collagen type I, which is naturally found in the bone marrow where blood cells are produced and is known to enhance cell adhesion. Cell growth is sustained for periods of over 2 months without the need for the addition of growth factors, which modify the inherent cellular growth kinetics. This ex-vivo mimicry recapitulates the environmental characteristics of the bone marrow, making it a suitable platform for the study of the disease.



(A) Growth Kinetics of AML primary cells from patients cultured in the 3D static biomimicry. The AML heterogeneity was evident.

(B) Wright-Giemsa staining of cultured cells highlights cell the maintenance of the leukaemic morphology and phenotype after 28 days.

Light microscopy image taken of a polyurethane collagen-coated scaffold containing patient cells in culture after 21 days. Cell growth occurs within the niches, where high cell density is observed.

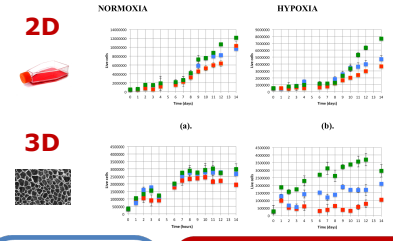


THE EFFECT OF ENVIRONMENTAL STRESS FACTORS ON LEUKEMIA

The absorption/ metabolism of a chemotherapy drug can highly vary within the same patient, depending on the condition of the patient during chemotherapy. Fluctuations of factors such as *temperature*, *oxygen* and *glucose* levels can highly vary within different body compartments at different time points as well as between different patients. Therefore, for the efficient prediction of the leukaemia evolution under chemotherapy it, essential to monitor the effect of these factors.

The leukaemia kinetics under *oxidative* and *starvation stress* at levels close to physiological (*in vitro* hypo- and hyper- glycemia) are monitored in our *in vitro* platform (3D *ex vivo* bone marrow mimicty) and compared to the traditional platform (2D suspension cultures).

The kinetic parameters derived from these studies will be incorporated in the developed predictive tool for a more accurate prediction of the cancer evolution under realistic environmental conditions.

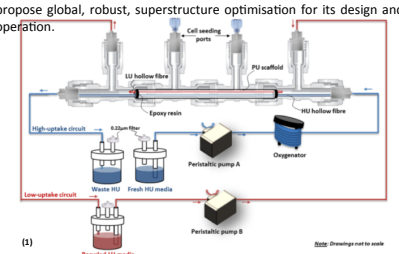


Growth Kinetics of AML cells in the 2D *in vitro* platform for (a). 20% O_2 and (b). 5% O_2 for (■) 4.3 g/L, (■) 1.2 g/L and (■) 0.6 g/L glucose.

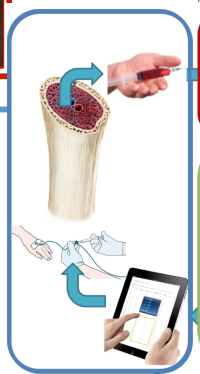
Growth Kinetics of AML cells in the 3D *in vitro* platform for (a). 20% O_2 and (b). 5% O_2 for (■) 4.3 g/L, (■) 1.2 g/L and (■) 0.6 g/L glucose.

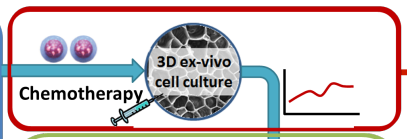
OPTIMISATION OF BIOREACTOR SUPERSTRUCTURE

Blood cell production requires delivering nutrients, oxygen, and growth factors to cells in a specialised 3D micro-environment in the bone marrow. Artificial ex vivo blood production, however, is typically performed in 2D liquid suspension; which is expensive due to the high levels of growth factors. Instead, the bioreactor we developed recapitulates the architectural and functional stimuli of blood formation reducing the need for expensive growth factors by over an order of magnitude while producing red blood cells of good quality. Use of this bioreactor is currently cost- and labour-intensive; we propose global, robust, superstructure optimisation for its design and operation.



1. Diagram of the bioreactor setup featuring a high uptake circuit (delivery of nutrients, disposal of waste) and a low uptake circuit (circulation of growth factors, removal of mature cells).
2. a Bioreactor cross section;
b,c Krogh Cylinder approximation

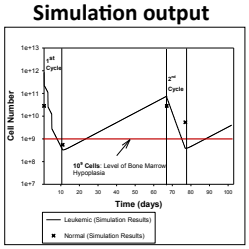




OPTIMISATION OF CHEMOTHERAPY DOSAGE

gPROMS

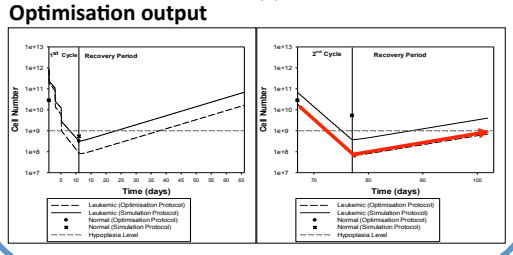
Simulation output

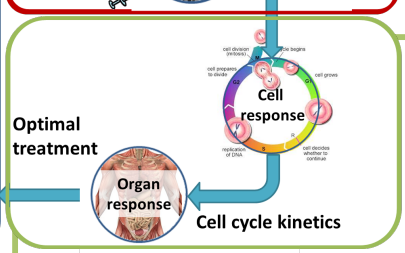


From the simulation output that took place taking as optimal drug dosages the ones suggested my the hospital, it can be seen that the leukaemic population did not eliminate after the 2nd chemotherapy cycle.

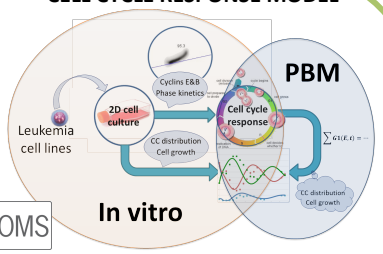
After optimisation of the chemotherapy dosage with *gOPT* of gPROMS elimination of the AML population was achieved.

Optimisation output



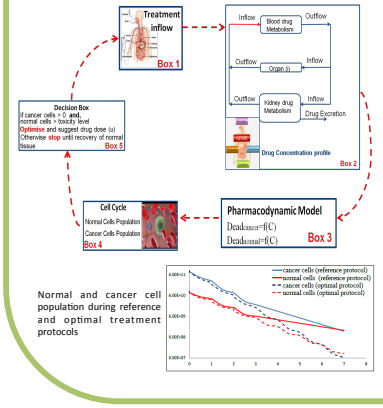


CELL CYCLE RESPONSE MODEL



ORGAN RESPONSE MODEL

gPROMS



Normal and cancer cell population during reference and optimal treatment protocols

CELL CYCLE PHASE