Abstract

Mathematical Modelling and Sensitivity Analysis of Nurse Macrophage-Driven Erythropoiesis Disruption in Acute Myeloid Leukaemia

Acute myeloid leukaemia (AML) is an aggressive haematological malignancy characterised not only by uncontrolled expansion of leukaemic blasts, but also by profound disruption of normal haematopoiesis. Among its most clinically relevant consequences is anaemia, which is nearly universal in AML and substantially contributes to patient morbidity, transfusion dependence, and reduced quality of life. Although marrow infiltration by blasts contributes to haematopoietic suppression, increasing evidence indicates that AML-associated anaemia cannot be explained by space competition alone. Instead, AML actively remodels the bone marrow microenvironment, including erythropoietic niches known as erythroblastic islands (EBIs). These specialised structures consist of a central nurse macrophage surrounded by differentiating erythroblasts. Nurse macrophages support erythropoiesis by promoting erythroid proliferation and maturation, clearing extruded nuclei, recycling iron, and providing local cytokine- and adhesion-dependent signals. However, the mechanistic relationship between AML-driven niche remodelling, nurse macrophage dysfunction, EBI collapse, and erythropoietic failure remains insufficiently understood.

The aim of this thesis was to investigate how AML perturbs erythropoiesis through disruption of nurse macrophages and EBIs, and to develop an integrated experimental-computational framework capable of explaining this process quantitatively and identifying regulatory axes with therapeutic relevance. To address this, the study combined experimental observations from murine and human AML models with mechanistic mathematical modelling and sensitivity analysis.

The experimental component drew on syngeneic murine MLL-AF9 AML models and human AML xenograft systems. Flow cytometry and imaging flow cytometry were used to quantify haematopoietic progenitor populations, erythroid compartments, erythroblastic islands, and nurse macrophages across healthy and leukaemic conditions. Transcriptomic analysis of sorted nurse macrophages was used to explore molecular changes associated with niche dysfunction in AML. In addition, published and experimental evidence relating to M-CSF-mediated macrophage support was incorporated to interpret the role of macrophage survival signals in erythropoietic failure and potential rescue.

The computational component consisted of a mechanistic ordinary differential equation (ODE) model describing erythropoiesis across multiple developmental stages, including both bone marrow and stress-related splenic contributions. The model incorporated interactions between erythroid cells and nurse macrophages within EBIs, together with regulatory processes linked to cytokine support, macrophage turnover, erythroid proliferation, differentiation, and red blood cell output. Parameter values were informed by experimental measurements and the literature, and the calibrated model was fitted to fold-change data representing erythroid population dynamics across increasing AML burden. Model robustness and biological interpretation were further explored using one-at-a-time sensitivity analysis and broader global sensitivity analysis workflows. To support robust parameter prioritisation, several global sensitivity analysis methods, including

Sobol-type approaches, eFAST, polynomial chaos expansion (PCE), and variogram-based methods such as VARS, were benchmarked on test problems of different dimensionality and complexity before being applied to the erythropoiesis model.

The results support a niche-centred explanation of AML-associated erythropoietic failure. Experimentally and computationally, increasing AML burden was associated with progressive loss of nurse macrophages, destabilisation and collapse of EBIs, and impaired erythroid output, especially at later stages of differentiation. The calibrated model reproduced the stage-specific pattern observed in the data, in which late erythroid populations such as reticulocytes were more strongly reduced than earlier progenitors as AML infiltration increased. This behaviour supports the interpretation that AML disrupts terminal erythroid maturation primarily through damage to macrophage-supported niches rather than through simple replacement of erythroid cells within the marrow.

Analysis of fitted EBI-related parameters showed that EBIs contribute more strongly to erythroid differentiation than to proliferation across most developmental stages, with the notable exception of the proerythroblast stage. This finding suggests that, within the context of the model, erythroblastic islands function predominantly as maturation-supporting niches. LASSO-regularised fitting further indicated that all EBI-related regulatory terms contributed to model performance, reinforcing the view that macrophage-dependent support acts across multiple erythroid checkpoints rather than at a single isolated stage.

Transcriptomic profiling of AML-exposed nurse macrophages provided molecular context for these modelling results. Canonical EBI-associated genes remained detectable in the surviving macrophage population, but transcriptional patterns were compatible with reduced survival- and proliferation-associated programmes. These findings are consistent with the model-derived hypothesis that attenuation of extrinsic survival signals destabilises nurse macrophages and contributes to EBI collapse during AML progression.

Sensitivity analyses consistently highlighted macrophage-centred regulation as a dominant determinant of erythropoietic efficiency. Across methods, parameters linked to nurse-cell survival, macrophage support, M-CSF-dependent regulation, and erythroid differentiation ranked among the most influential in shaping system behaviour. These results support the conclusion that relatively small perturbations in macrophage-supportive pathways can have large downstream effects on erythroid output under leukaemic stress. In parallel, benchmarking of sensitivity analysis methods showed that PCE provided efficient and accurate performance for smooth low-interaction systems, while VARS offered a robust and computationally practical screening framework, particularly under constrained budgets and higher-dimensional settings. This methodological component strengthens confidence in the parameter-prioritisation results obtained in the biological model.

The therapeutic interpretation of the study focused especially on macrophage colony-stimulating factor (M-CSF), a key regulator of macrophage survival and homeostasis. Experimental observations and model interpretation together support a mechanism in which reduced M-CSF availability contributes to nurse macrophage attrition, EBI disruption, and impaired erythropoiesis in AML. Exogenous M-CSF treatment in leukaemic mice increased nurse macrophage abundance

and partially improved erythropoiesis, particularly in early and intermediate erythroid compartments, but did not produce measurable suppression of AML burden over the relevant experimental period. This suggests that M-CSF acts primarily as a niche-stabilising and erythropoiesis-supporting intervention rather than as a directly anti-leukaemic therapy. Simulations further suggested that combined modulation of macrophage support and inflammatory signalling, particularly through M-CSF restoration alongside anti-IL-6 treatment, could enhance erythroid rescue more effectively than single-pathway intervention alone. Additional simulations also indicated that enhancing basophilic erythroblast production could markedly attenuate AML-associated erythropoietic suppression, identifying another potentially relevant regulatory direction for future study.

Overall, this thesis provides an integrated experimental and computational framework linking AML-driven remodelling of the bone marrow microenvironment to erythropoietic failure. The work shows that disruption of nurse macrophages and erythroblastic islands is not a secondary consequence, but a central mechanism contributing to anaemia in AML. By combining quantitative data, mechanistic modelling, and sensitivity analysis, the study identifies macrophage-supportive pathways, particularly the nurse-cell/M-CSF axis, as tractable targets for improving erythroid output under leukaemic stress.

Several limitations should be acknowledged. Species-specific differences were observed between murine and patient-derived material, particularly in nurse macrophage phenotypes, which may reflect both biological divergence and technical challenges in preserving intact EBIs from clinical samples. In addition, the model simplifies several important regulatory layers, including explicit erythropoietin dynamics, systemic iron regulation, clonal heterogeneity, and the spatial complexity of the marrow microenvironment. Nevertheless, the framework provides a useful platform for hypothesis generation, mechanistic interpretation, and prioritisation of experimentally testable niche-based interventions. Future work should refine the model through richer multi-omics and single-cell data, incorporate additional regulatory loops such as EPO and iron handling, and validate predicted therapeutic strategies in patient-relevant AML systems.

In conclusion, this thesis supports a model in which AML-associated anaemia arises in large part from disruption of nurse macrophages and erythroblastic islands, leading to impaired terminal erythroid maturation and reduced red blood cell production. The study advances both biological understanding and computational methodology, and establishes a quantitative basis for future development of niche-stabilising strategies aimed at mitigating erythropoietic failure in AML.