Human-Relevant Non-Animal Methodologies (NAMs)
Specialised Models Series: ***Cancer & Haematology***
Haematological/Blood & Stem Cell Models
Reference 1.2 in Full Scope Roadmap

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Legal & Scientific Imperative for Universities

Section 2A of the Animals (Scientific Procedures) Act 1986 imposes a statutory duty on the Secretary of State to ensure compliance with the principles of replacement, reduction and refinement. The principle of replacement requires that, wherever possible, a scientifically satisfactory method or testing strategy not entailing the use of protected animals must be used instead of a regulated procedure.

Traditional animal models in haematology—particularly murine bone marrow transplantation, systemic irradiation-induced immunosuppression, and xenograft models of human leukaemia—have well-documented limitations. These include profound species differences in haematopoietic niche architecture, cytokine cross-reactivity, immune surveillance, and stem cell homing mechanics. These limitations contribute to historically high attrition rates in haematological drug development and regenerative medicine, rendering animal models non-predictive of human clinical outcomes. At the cellular, microstructural, and microenvironmental levels, human and rodent haematopoietic systems exhibit critical disparities:

- **Haematopoietic Niche Architecture Mismatch:** The human haematopoietic niche relies on a highly complex, 3D architecture involving specific interactions between haematopoietic stem/progenitor cells (HSPCs), mesenchymal stromal cells (MSCs), and the endosteal/vascular niche. Rodent models frequently utilise "niche-less" systemic transplantation protocols that fail to recapitulate the human-specific mechanical and biochemical cues required for stem cell self-renewal and lineage-specific differentiation.

- **Cytokine and Receptor Specificity:** Key human cytokines and growth factors (e.g., IL-3, GM-CSF), often show limited or no cross-reactivity with rodent receptors. This necessitates the use of transgenic "humanised" mice which are costly, prone to significant variability, and still fail to replicate the full physiological repertoire of the human bone marrow microenvironment, leading to biased results in drug toxicity and myelosuppression assessments.

- **Irradiation and Conditioning Toxicity**: Achieving human-like engraftment phenotypes in rodents requires high-dose total-body irradiation or aggressive chemotherapy conditioning. This inflicts immense, high-severity suffering, resulting in systemic multi-organ damage, chronic inflammatory distress, and severe post-operative morbidity. This systemic trauma acts as a major confounding variable, masking the true efficacy and safety profile of novel haematopoietic therapies.

- **Systemic Distribution and Extravasation:** Studying the systemic trafficking of malignant cells or therapeutic CAR-T cells in rodents fails to account for the unique geometry of the human vascular network and the specific adhesion molecules governing human cell extravasation. Consequently, predictions regarding therapeutic biodistribution and off-target tissue toxicity remain poorly translatable to the human clinic.

Transitioning to human-relevant Non-Animal Methodologies (NAMs)—such as microfluidic marrow-on-a-chip platforms, 3D niche-mimetic organoids, and in silico computational modelling of clonal evolution—is a dual scientific and statutory mandate for UK academic research.

Landmark Regulatory & Scientific Momentum

This section details the transition from traditional animal-based haematological assays to high-fidelity, human-relevant NAMs. By leveraging organ-on-a-chip and 3D niche-mimetic platforms, institutions can replicate the intricate dynamics of human stem cell self-renewal, differentiation, and haematological disease pathology with unprecedented accuracy.

- **Haematopoietic niche-on-a-chip:** Microfluidic platforms that recreate the human bone marrow microenvironment, allowing for the study of stem cell mobilisation, homing, and leukaemic cell interactions under physiologically relevant flow conditions.
- **3D bone marrow organoids:** Self-assembled human tissues that mimic the architecture of the marrow niche, providing a stable platform for evaluating drug-induced myelotoxicity and bone marrow failure syndromes.
- **Microfluidic blood-brain barrier (BBB) & vascular models:** Integrated systems to study the extravasation of malignant haematological cells and the systemic distribution of novel blood-based therapies.
- **In silico haematopoietic modelling:** Computational frameworks that simulate clonal evolution and haematopoietic response to cytokine stimulation, reducing the need for longitudinal animal studies.

Landmark Regulatory Milestone

In recent regulatory advancements, there is increasing acceptance of qualified human-relevant data for haematological toxicity and drug safety assessments. By moving away from restrictive gnotobiotic and radiation-induced murine models, this shift demonstrates that human-derived organ-on-a-chip systems can successfully support regulatory advancement in haematology and stem cell research, setting a clear precedent for replacement under Section 2A of the *Animals (Scientific Procedures) Act 1986*.

Core Specialised Cancer & Haematology Models & Enabling Platforms

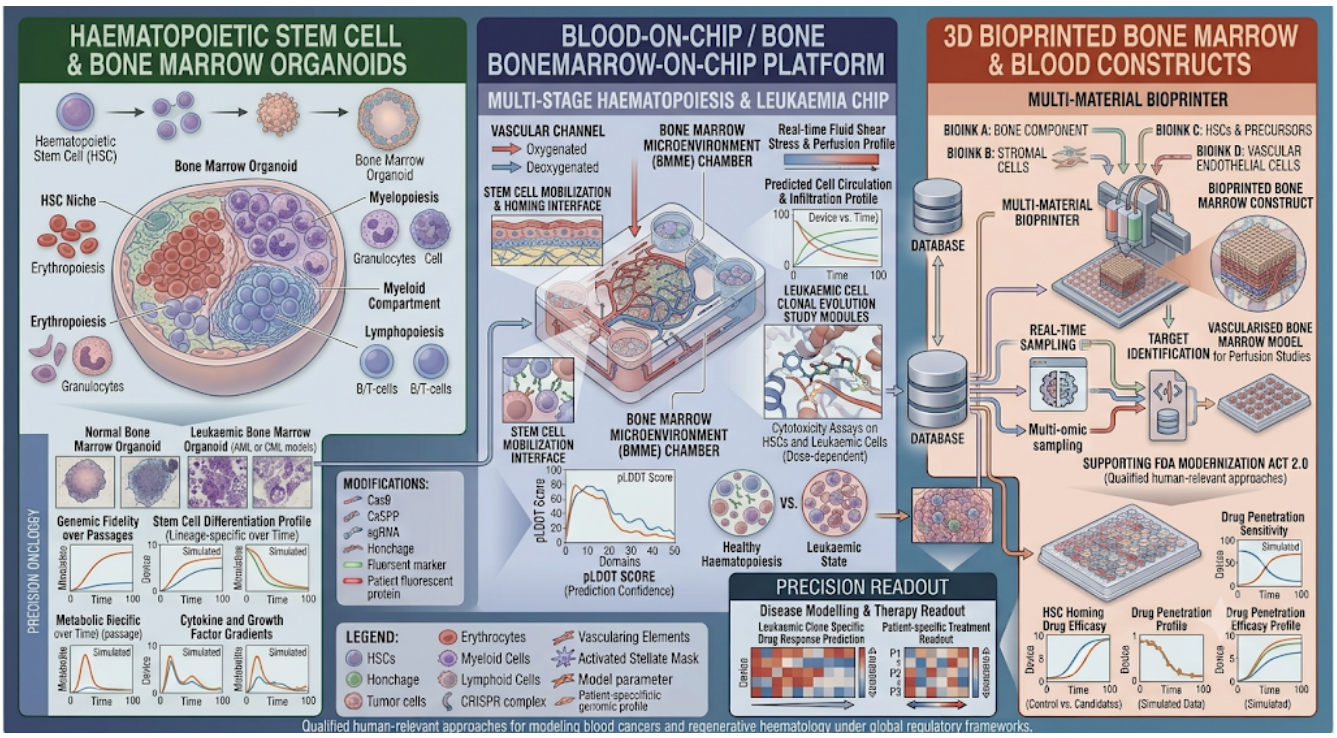

Figure 1: Core Specialised Haematological / Blood & Stem Cell Models — Blood / haematopoietic stem cell organoids or bone marrow organoids with erythroid, myeloid and lymphoid lineages (left), blood-on-chip or bone marrow-on-chip with vascular and immune components for haematopoiesis and leukaemia studies (centre), and 3D bioprinted blood or bone marrow constructs (right) for modelling blood cancers, genetic blood disorders, and regenerative haematology. These platforms support the FDA Modernization Act 2.0 and strengthen Replacement arguments under Section 2A of the Animals (Scientific Procedures) Act 1986.

Table 1: Core Specialised Haematological / Blood & Stem Cell Models

Platform / Mod	Description	Key Applications	Benefits for 3Rs
BM Organoids / HS Models	3D structures from human iP primary cells recapitulating th niche.	Modelling haematopoiesis, b disorders, and leukaemia.	Human-relevant alternativ rodent models.
Blood-on-Chip / Haematopoietic MF	Microfluidic platforms integra HSCs and niche cells under controlled flow.	Functional studies of blood c production and CAR-T devel	Enables dynamic, functio human haematopoietic st
3D Bioprinted Bon Marrow	Engineered constructs with controlled architecture and v channels.	Advanced niche modelling, leukaemia, and drug delivery	High experimental control reproducibility.
Patient-Derived iPS Disease Models	iPSC lines from patients with blood disorders/malignancies	Precision mechanistic studie gene therapy testing.	Enables mutation-specific human-centric research.
Multi-Organ Bone Marrow-Immune M	Interconnected chips linking with immune or liver compart	CAR-T toxicities, systemic ef and multi-organ drug toxicity.	Holistic study of systemic haematological processes
Ex Vivo Perfusion / Slice Models	Human bone marrow tissue maintained under perfusion c thin slices.	Short-term HSC behaviour a interaction studies.	Preserves native microenvironment; fast hu data.

Cross-Cutting & Enabling Platforms for Haematological Research

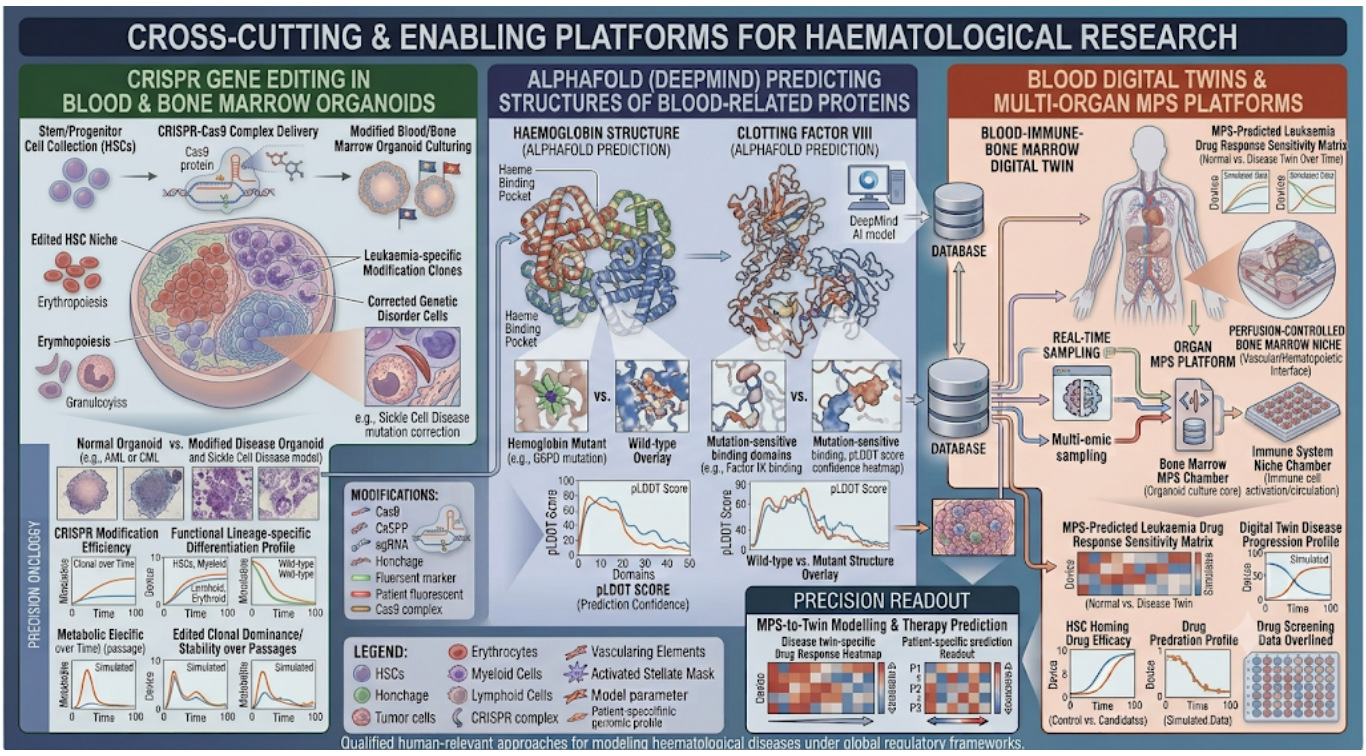

Figure 2: Cross-Cutting & Enabling Platforms for Haematological Research *— CRISPR gene editing in blood or bone marrow organoids for leukaemia or genetic blood disorders (left), AlphaFold (DeepMind) predicting structures of blood-related proteins such as haemoglobin and clotting factors (center), and blood digital twins or multi-organ blood–immune / blood–bone marrow MPS platforms (right) for disease modelling and drug response prediction. These enabling technologies support qualified human-relevant approaches under the FDA Modernization Act 2.0Table 2: Cross-Cutting & Enabling Platforms in Cancer & Haematology Research*

Table 2: Cross Cutting & Enabling Platforms in Haematological Research

Platform / Model	Description	Key Applications	Benefits for Universities & 3Rs
CRISPR-Based Functional Genomics in Blood & Bone Marrow Models	Gene editing applied in blood/bone marrow organoids and iPSC lines to model leukaemia drivers, genetic blood disorders, or study stem cell function.	Mechanistic studies of leukaemia and genetic blood disorders, gene therapy research, synthetic lethality screening, target validation.	Directly addresses animal model limitations in genetic modelling. Powerful for mechanistic haematology, gene therapy, and personalised blood disorder research.
AlphaFold & Structural Biology for Blood-Related Proteins	Use of AlphaFold and related tools to predict structures of blood-related proteins (e.g. haemoglobin, clotting factors, surface receptors) for drug design and mutation impact analysis.	Structure-based drug design for blood disorders, mapping disease-causing mutations, accelerating discovery of targeted therapies for leukaemia and genetic blood diseases.	Accelerates target discovery and drug design while minimising animal use. Ideal for computational + experimental haematology workflows.
Blood Digital Twins & Multi-Organ Blood–Immune / Blood–Bone Marrow MPS	Computational digital twins of the blood system combined with interconnected chips linking blood/bone marrow with immune or other compartments.	Systemic effects of blood cancers, personalised blood disorder modelling, drug response prediction, complex multi-organ haematological research.	Enables holistic, human-relevant modelling of blood–systemic interactions impossible in conventional animal models. Strong for advanced regulatory science and personalised medicine.

Regulatory Software & Strategy Frameworks

Regulatory acceptance of NAMs—referred to as "fit-for-purpose qualification"—is not a one-size-fits-all process. Researchers should utilise the following frameworks to ensure their computational and biological strategies align with the *Animals (Scientific Procedures) Act 1986* and industry best practices:

- **Context of Use (COU) Documentation:** Before deploying models, clearly define the specific scientific question the model is intended to address. This is critical for demonstrating that the NAM is reliable and suitable for its intended regulatory purpose.
- **Technical Characterisation:** Maintain comprehensive Standard Operating Procedures (SOPs) and study plans for both the biological NAM and any software used to analyse its output. This transparency is vital for successful project licence applications.
- **Early Regulatory Engagement:** Leverage institutional or external regulatory support to track "fit-for-purpose" milestones. Early engagement with the Home Office/ASRU is strongly encouraged to test the acceptability of a computational or NAM-based approach before full project submission.
- **Weight-of-Evidence (WoE) Frameworks:** Use integrated digital dashboards to aggregate data from both *in vitro* (NAM) and *in silico* (computational) sources. Building a strong "weight-of evidence" argument is the most effective way to strengthen Replacement justifications when moving away from traditional animal models.

Skills • Training, and Capacity Building

Successful adoption of high-maturity Non-Animal Methodologies (NAMs) requires a strategic shift in workforce development. To ensure the long-term success of the platforms outlined in this series, universities should prioritise the following training initiatives to build institutional capacity:

- **Integrated Oncology & Data Science:** Training programmes should bridge the gap between bench-top laboratory biology and computational analysis. Researchers require dual proficiency in 3D culture techniques and the digital pipelines used to interpret complex imaging and high-throughput drug-response data.
- **Regulatory Competency:** Staff should receive formal training on "Fit-for-Purpose" qualification and the specific regulatory expectations of the Home Office/ASRU. This includes understanding how to document evidence for the 3Rs within formal project licence applications to ensure they meet legal standards.
- **Technological Literacy:** Skills in **haematopoietic stem cell (HSC) expansion cultures, flow cytometry-based immunophenotyping, haematopoietic colony-forming assays (CFAs), and image-enabled cell sorting** are increasingly critical for modern haematology research. Universities should facilitate internal workshops or establish external partnerships to provide PhD students and postdoctoral fellows with hands-on experience in these specialised tools.
- **Ethics & Translation:** Education should focus on the scientific imperative of human-relevant research, emphasising why NAMs provide more predictive insights for clinical outcomes than traditional rodent models. This fosters a culture of excellence and ethical research design that is fully aligned with modern international standards.

Table 3: Skills • Training and Capacity Building

Capability Domain	Focus Area	Impact on 3Rs/Replacement
Technical	3D stem cell culture & niche engineering	Replaces invasive animal bone marrow transplants
Bioinformatics	Single-cell RNA-seq & lineage tracing	Bypasses animal-based flow cytometry studies
Regulatory	ASPA 1986 Compliance & AWERB	Facilitates faster ASRU approvals
Computational	Haematopoietic feedback modelling	Eliminates high-severity irradiation models

Strategic Note: This investment in human expertise is a key component of a robust, future-proof institutional roadmap, demonstrating to regulators that the institution is committed to the safe and effective operation of high-maturity NAMs.

Clinical Translation Case Studies

Case Study 1: Bone Marrow Organoids in Leukaemia Research and Drug Development

Patient-derived bone marrow organoids are being used by academic centres and pharmaceutical companies to model acute myeloid leukaemia (AML) and other blood cancers. These models have successfully recapitulated leukaemia stem cell behaviour and drug resistance mechanisms that were poorly modelled in conventional xenograft systems, leading to improved target identification and predictive preclinical testing.

Case Study 2: Blood-on-Chip Platforms for Gene Therapy and CAR-T Research

Blood-on-chip and bone marrow-on-chip platforms with vascular and immune components are being adopted to study CAR-T cell trafficking, engraftment, and toxicity in a human-relevant microenvironment. These systems have provided mechanistic insights into cytokine release syndrome and neurotoxicity that were difficult to study in animal models, supporting safer translation of gene and cell therapies.

Case Study 3: iPSC-Derived Blood Disorder Models in Personalised Medicine

iPSC-derived blood and bone marrow organoids from patients with genetic blood disorders (e.g. sickle cell disease, thalassaemia, inherited bone marrow failure syndromes) are being used for personalised drug screening and gene therapy development. Several programmes have advanced these models into translational pipelines, demonstrating strong potential for clinical application in precision haematology.

Executive Summary & Compliance Dashboard

Executive Summary

Traditional animal models of haematopoiesis often fail to reflect the human-specific immunological and niche-based regulation of stem cells. This roadmap transitions research toward human-relevant NAMs, including marrow-on-a-chip and 3D organoid systems. By adopting these high-fidelity models, the institution enhances scientific reproducibility, minimizes systemic animal suffering, and fulfills the ASPA 1986 mandate to replace regulated procedures with scientifically superior, human-centric methodologies.

Compliance Dashboard

Focus Area	Primary Animal Model	NAM Replacement Technology	ASPA 1986 Status
Haematopoietic Niche	Murine Transplant/ Radiation	Marrow-on-a-Chip & Organoids	High

Maturity Matrix

Phase 1 (Exploratory): Audit animal use in haematological disease and drug toxicity studies.
Phase 2 (Pilot): Integrate human-derived marrow organoids into toxicity protocols.
Phase 3 (Integration): Implement multi-organ vascular-haematopoietic chips.
Phase 4 (Standardisation): Replace 100% of high-severity radiation and transplant protocols.

Final Compliance Statement

All proposed models satisfy the Section 2A statutory duty of ASPA 1986. By adopting these methodologies, the institution positions itself at the forefront of the global transition toward human-centric, cruelty-free medical science.

Integration Context: Roadmap Conclusion

The modules detailed in this roadmap—from fundamental oncology (1.1) and haematology (1.2) models through the systemic complexities explored in our broader research series—demonstrate that replacement is an integrated institutional strategy. By mapping these specialised NAMs into a unified framework, the institution moves toward a standardised, human-centric research paradigm. This systemic integration is the essential prerequisite for fulfilling the ASPA 1986 statutory duty, ensuring all research is scientifically accurate, ethically aligned, and regulatory compliant.