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506.BONE MARROW MICROENVIRONMENT

Establishment of Human Bone Marrow-on-Chip As a Preclinical Model to Evaluate Drug-Induced Toxicities and Myelofibrosis Patient-Specific Pathophysiology

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Hematopoiesis takes place in the bone marrow (BM) and is supported by a complex cellular and molecular network in the BM microenvironment. The inaccessibility of living human BM hampers the study of its pathophysiology under stress induced by drugs, radiation, or genetic mutations. Commonly used models of the human BM microenvironment rely on mouse models and 2D or 3D static tissue cultures. Although existing *in vitro* hematopoiesis models have led to advances in the field, they fail to recapitulate the biology of cellular interactions, cell extrinsic factors, and complex functions of living human BM hematopoiesis. This has also limited our understanding of human BM biology and the influence of BM niche cells in BM defects in diseases such as myeloproliferative neoplasms (MPNs) and leukemias. Consequently, there is an unmet need to develop a preclinical human BM hematopoiesis model that supports growth and differentiation of multilineage hematopoietic stem cells to enable evaluation of investigational compounds for myeloerythroid toxicity and efficacy. Microphysiologic systems (MPS), such as organ-on-chips, emulate the complex interplay between intrinsic and extrinsic cell factors in the BM microenvironment, the 3D tissue architecture, and are more clinically relevant compared with static co-culture approaches *(PMID: 31988457; PMID: 37359774)*.

Here we present the development of a human BM-on-chip MPS model to recapitulate both healthy and MPN disease and evaluate the alterations in the BM niche environment. We utilized a commercially available organ-on-chip microfluidic platform (Emulate, Boston, MA) to develop a living human BM with a functional hematopoietic niche *in vitro* by perfusion with culture medium. This fluidic-controlled modular chip comprises an osteogenic apical channel separated from a vascular basal channel by a semiporous membrane, which allows for media perfusion, cellular crosstalk, and intercellular signaling. The osteogenic apical channel is filled with a fibrin gel that allows the co-culture of all critical cells of the BM niche, including osteoblasts, endothelial cells, mesenchymal stem cells, hematopoietic stem and progenitor cells. The parallel basal channel is lined by human vascular endothelium and is perfused with culture medium optimized to allow survival and development of functional hematopoietic lineage niche cell populations. This unidirectional fluidic MPS model allows for collection of cell effluents for evaluation of cell-extrinsic factors such as cytokines, extracellular vesicles, and metabolites. The modular and accessible nature of this system provides an advantage to investigate the effects of the microenvironment and drug candidates individually on the osteogenic and vascular compartments.

This human BM-on-chip has been tested with cells from several healthy and myelofibrosis donors. Utilizing imaging, flow cytometry, cytokine profiling, and transcriptomics, we demonstrate that the vascularized human BM-on-chip recapitulates the living human hematopoietic niche, with BM relevant cytokine levels, retains hematopoietic stem and progenitor cells in *in vivo*-like proportions for over 3 weeks in culture, allows for the differentiation of BM niche lineage cell populations similar to human BM aspirate, and captures human donor variabilities. We also show that BM-on-chips derived from MPN patients recapitulate the hematopoietic defects to understand the biology and disease response in myelofibrosis.

Our robust experimental workflow and bioinformatics platform will be utilized to evaluate candidate compounds for BM myelotoxicities, drug mechanisms of action, and biomarkers in a multifactorial manner to support preclinical programs. This biomimetic platform offers a new approach for analysis of drug responses and toxicities in BM as well as for study of hematopoiesis and hematologic diseases such as myelofibrosis.

Disclosures Guha: *Incyte Corporation:* Current Employment, Current equity holder in publicly-traded company. **Dove:** *Incyte Corporation:* Current Employment, Current equity holder in publicly-traded company. **Gebeyehu:** *Incyte Corporation:* Cur-

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