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ORAL ABSTRACTS

506.BONE MARROW MICROENVIRONMENT

Haemogenic Gastruloids Recapitulate Developmental Haematopoiesis and Provide an Ontogeny-Relevant Context to Dissect the Origins of Infant Leukemia

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Abstract Modelling of developmental hematopoiesis has historically been challenging due to the inability to produce hematopoietic stem cells (HSC) and recapitulate microenvironment interactions ex vivo. Gastruloids are 3D aggregates of embryonic stem (ES) cells which display developmentally-specific spatial and temporal organization that recapitulate gastrulation. We adapted the gastruloid protocol to introduce hematopoietic signalling cues, and generated an in vitro model of embryonic hematopoiesis that sequentially recapitulates the formation of hemogenic endothelium, hematopoietic progenitors, and pre-HSC, over a culture period of 216 hours. Flow cytometry analysis detected the presence of c-Kit+ endothelium at 120h, followed by emergence of CD41+ hematopoietic progenitors at 144h, and the appearance of CD45+ cells from 192h. CD45+ cells were observed in small clusters adjoining endothelium-lined structures, reminiscent of developmental hemogenic-to-endothelial transition and intra-aortic clusters. Single-cell RNA sequencing revealed specification of pre-definitive and definitive waves of embryonic hematopoiesis, aligning 144h-CD41+ cells with erythro-myeloid progenitors (EMP), and late CD45+ with lympho-myeloid progenitors and pre-HSC, altogether supporting the hemogenic gastruloid as a model that is temporally and topographically congruous with the embryo.

The close recapitulation of developmental ontogeny led us to explore hemogenic gastruloids to understand cell and stage-specific susceptibility to forms of Acute Myeloid Leukaemia exclusively observed in infants. The chromosomal translocation t(7;12)(q36;p13), characterized by the ectopic overexpression of the *MNX1* gene, is found in up to one third of infant AML cases, but has been challenging to model using conventional strategies, largely due to the inability of *MNX1* to transform adult hematopoietic cells. The age-selectivity of t(7;12) has been proposed to reflect a transient developmental window for a target cell of origin absent in adult life, but its nature is yet to be defined. In order to identify the context of *MNX1*-driven leukemogenesis, we produced hemogenic gastruloids using lentiviral-transduced mouse ES cells in which we overexpressed *MNX1* as a proxy of t(7;12). Although *MNX1* did not interfere with ES cell pluripotent cultures, it primed incipient hemogenic programmes and promoted hemogenic gastruloid formation. Critically, expression of *MNX1* resulted in transformation of gastruloid-derived hematopoietic cells, as assessed by serial colony-forming cell replating, with expansion of a phenotypic myeloid cell, a phenomenon not observed in adult tissues. Detailed analysis of the cellular composition of *MNX1*-overexpressing hemogenic gastruloids revealed a significant effect in the output of CD41+ and c-Kit+ populations at 144h, but no effect in CD45+ cells at 192-216h, suggesting that the target of *MNX1* lies within the EMP stage, an observation supported by single-cell RNA-seq analysis of *MNX1* vs control gastruloids. Systematic comparison of the temporal transcriptional profiles of hemogenic gastruloids, *MNX1*-overexpressing gastruloids, and t(7;12) patients, pinpoints the target cell of *MNX1* at the HE-to-EMP transition.

In summary, we propose a novel model of embryonic hematopoiesis capable of capturing developmentally-relevant cellularity and topography of the early hematopoietic microenvironment, with the ability to mechanistically elucidate developmental associations of infant leukemia.

Disclosures Martinez Arias: *Cambridge Enterprise Ltd:* Other: Patent application EP3853344A1. **Pina:** *Cambridge Enterprise Ltd:* Other: Patent application EP3853344A1.

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