Evaluation of c-Jun N-terminal kinase (JNK) inhibitors in a vascularized microphysiological model of endometriosis

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Introduction/Background
Endometriosis is characterized by the growth of vascularized lesions rich with macrophages. C-Jun N-terminal kinase inhibitor (JNKi) bentamapimod has demonstrated lesion regression *in vivo* but lacks potency required for clinical translation. To evaluate novel JNKi, we have developed a microfluidic lesion model using a synthetic hydrogel.

Materials and Methods
Synthetic hydrogels are polyethylene glycol-based with a cell degradable crosslinker and adhesive peptides. Primary endometrial epithelial organoids (EEOs) and stromal cells (ESCs), uterine endothelial cells (HUTMVEC), and macrophages from CD14+ human monocyte were combined in a gel precursor and injected into a custom microfluidic device used in static or continuous culture and exposed to media containing JNKi. Vessel morphology and organoid growth assessed via imaging. JNKi impact on the macrophage secretome assessed via ELISA.

Results
We show formation of perfusable microvasculature with adenomyosis-relevant source cells, using HUTMVEC-ESC co culture and can incorporate EEOs to form lesion–like structures in microfluidic devices. Next, we established conditions to achieve microvascular network formation with macrophages at a ratio comparable to that found in endometriosis lesions *in vivo* and demonstrated macrophage functionality via IL-6 and MMP-9 secretion. Novel JNKi with over 100X the potency of bentamapimod enhanced the ability of ESCs to decidualize, quantified through prolactin secretion over 6 days. Moreover, in co-cultures with macrophages treated with inflammatory cytokine IL1B, bentamapimod *increased* TNFa detected in spent media while the novel JNKi dampened TNFa. We are currently translating these readouts to device cultures, where we can simultaneously evaluate vascularization of the lesion microenvironment.

Conclusion
Here we describe our efforts to systematically evaluate the effects of highly selective JNKi in a microphysiological hydrogel platform that recapitulates critical aspects of the endometriosis microenvironment. This microfluidic culture model has potential for impacts on a better scientific understanding of endometriosis and new drug development.

Key words
microphysiological system, inflammation, vascularization