Producing T cell competent blood progenitor cells from pluripotent stem cells in scalable dynamic suspension culture

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Summary

INTRODUCTION

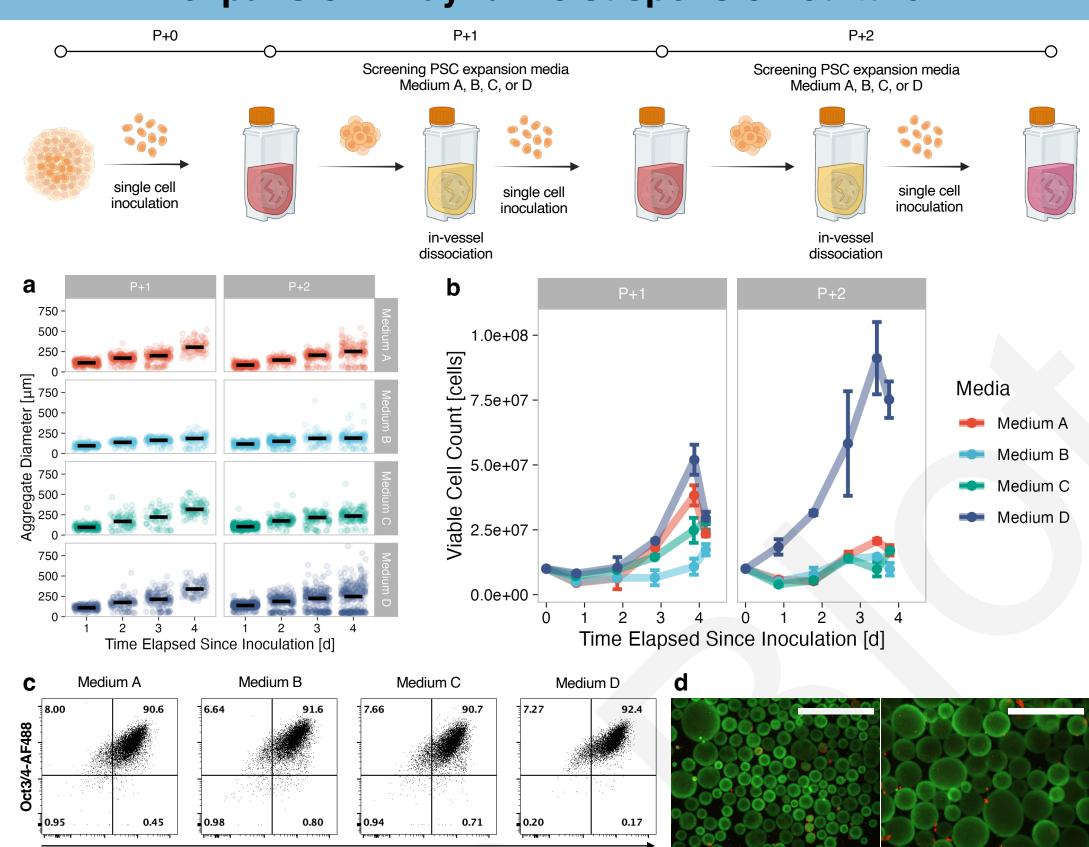
- Scaled-up bioprocesses will drive down the cost of pluripotent stem cell (PSC) derived T cell therapies
- ii. Current vein-to-vein model of T cell therapies are inaccessible, highly variable, and costly
- iii. Our capability to differentiate PSCs into blood progenitor cells with T lymphoid-competence provides an opportunity to leverage PSCs as an alternative source of T cell immunotherapies
- iv. A key challenge to enabling scalable cell manufacturing is the reliance on low-throughput microplate based adherent cultures

RESULTS

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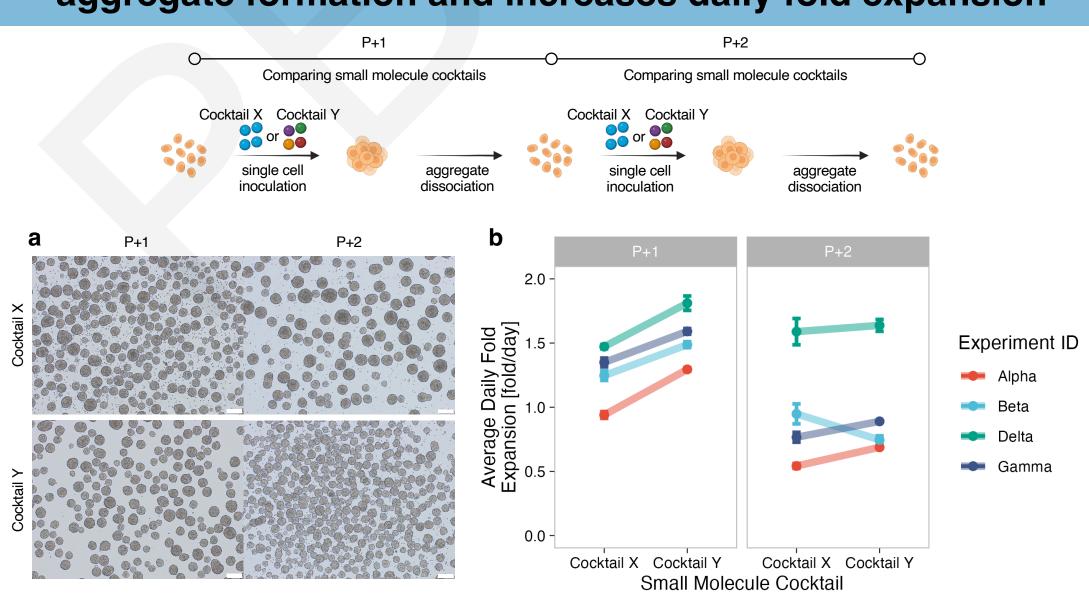
- 1. We identified commercially available PSC expansion media that is compatible with single-cell passaging and maintains stemness in dynamic suspension-based culture
- 2. We tested an alternative small-molecule cocktail to intensify PSC expansion and differentiation toward blood and immune cell lineages
- 3. We designed a scalable dynamic suspension culture-based bioprocess for blood progenitor cell production that eliminates ex situ CD34+ enrichment and exogenous notch ligands
- 4. We show that generated blood progenitor cells exhibit T lymphoid-competence

1. Screening commercially available media to maximize PSC expansion in dynamic suspension culture



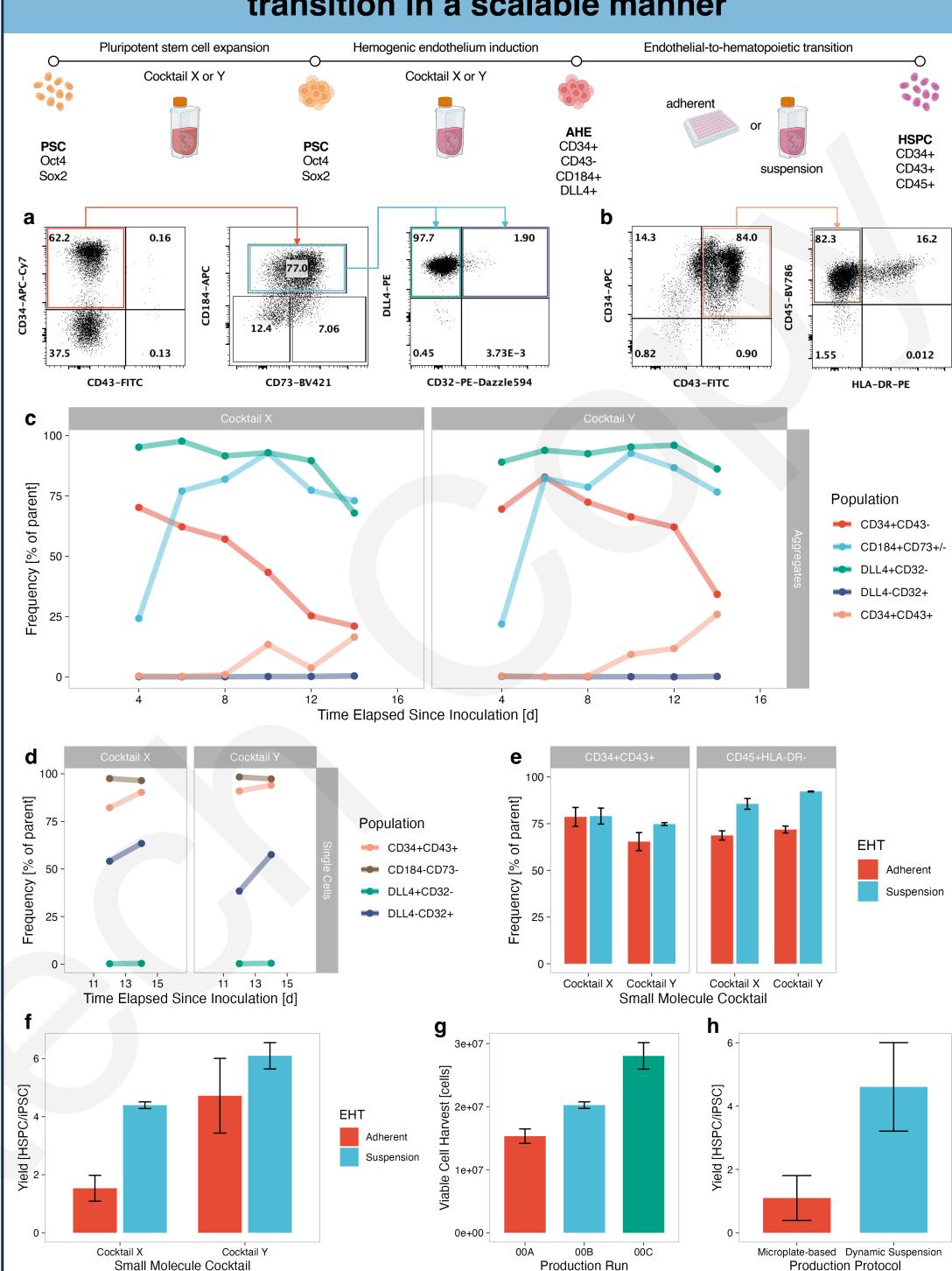
a,b. Aggregate diameter distribution and viable cell counts over two serial passages. c. Flow cytometry characterizing stemness marker expression (Oct-3/4 and Sox2) of harvested cells after two serial passages in dynamic suspension culture. d. Representative photomicrographs of aggregates stained with viability dyes (live/dead), scale bar = 1000 μ m.

2. Priming with cocktail Y in dynamic culture supports aggregate formation and increases daily fold expansion



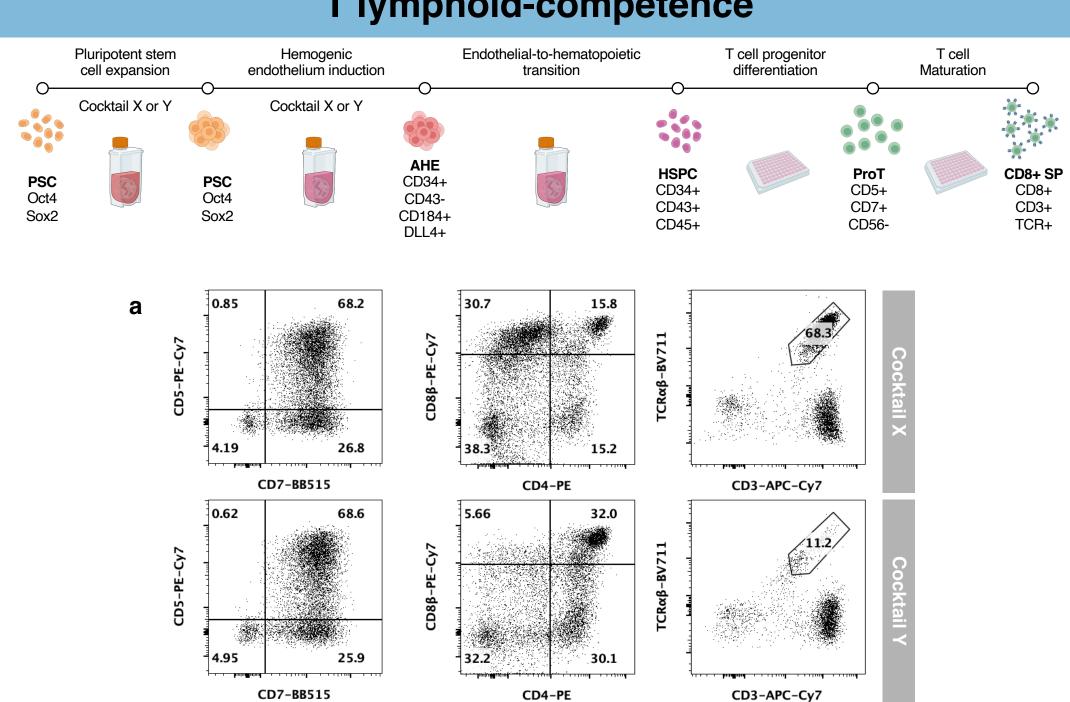
a. Aggregate formation in the presence of cocktail X or cocktail Y in dynamic suspension culture. Representative photomicrographs were taken 1 day post-inoculation, scale bar = 200 μ m. b. Quantification of the average daily fold expansion over two serial passages across different experiments.

3. Developed bioprocess allows endothelial-to-hematopoietic transition in a scalable manner



a,b. Representative flow cytometry plots of the aggregates pre-EHT (day 6) and the single cells post-EHT (day 16), respectively, in dynamic suspension culture. c,d. Phenotype tracking of blood progenitor cells in the aggregate and single cell phase, respectively, in dynamic suspension culture. e,f. Arterial hemogenic endothelial cells are subjected to EHT in adherent (CD34+ enrichment and transfer to DLL4/VCAM1 coated microwells) or suspension culture (no enrichment or exogenous DLL4/VCAM1 addition). Phenotypic characterization and yield, respectively, of the emergent blood progenitor cells in the free-floating single cell fraction post-EHT. g. Developed bioprocess produces up to 28 million viable blood progenitor cells per production run. h. Quantification of the yield comparing the developed bioprocess using dynamic suspension culture and the legacy protocol using microplate-based systems.

4. Generated blood progenitor cells exhibit T lymphoid-competence



a. Blood progenitor cells from the developed bioprocess have the ability to generate T cell progenitors and phenotypically mature αβT cells from pluripotent stem cells in our fully defined differentiation system.







