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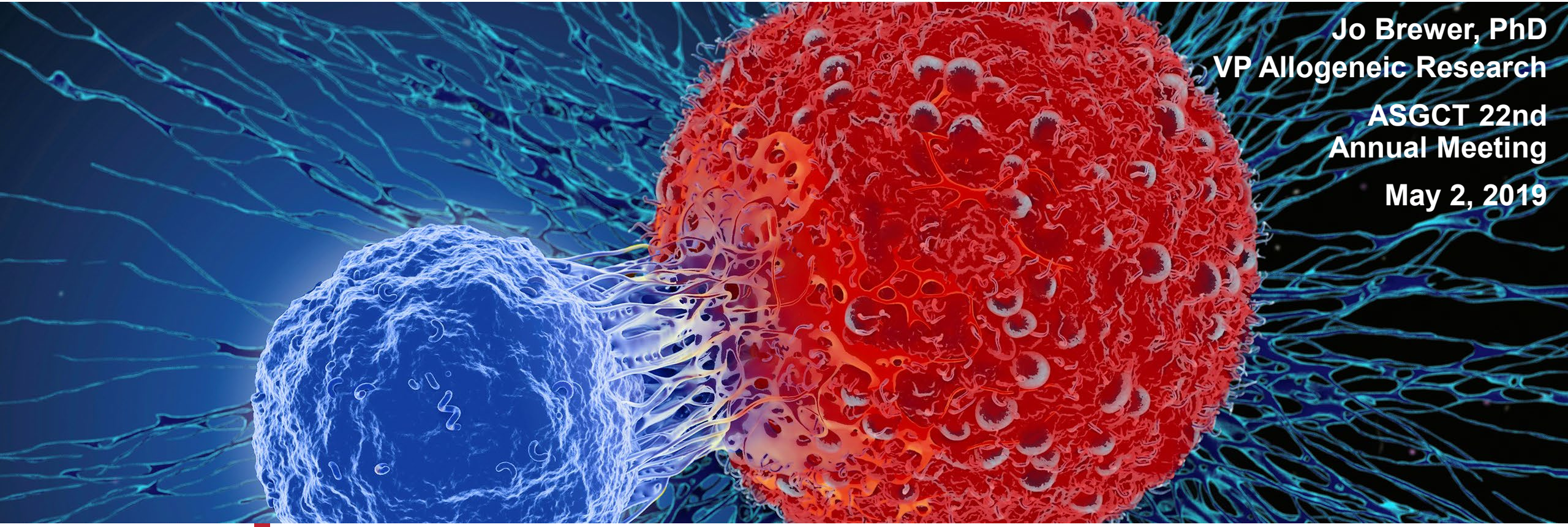
TRANSFORMING T CELL THERAPY



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VP Allogeneic Research

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# Differentiating T-Cells from hiPSCs to Create Off-the-Shelf SPEAR T-Cell Therapies

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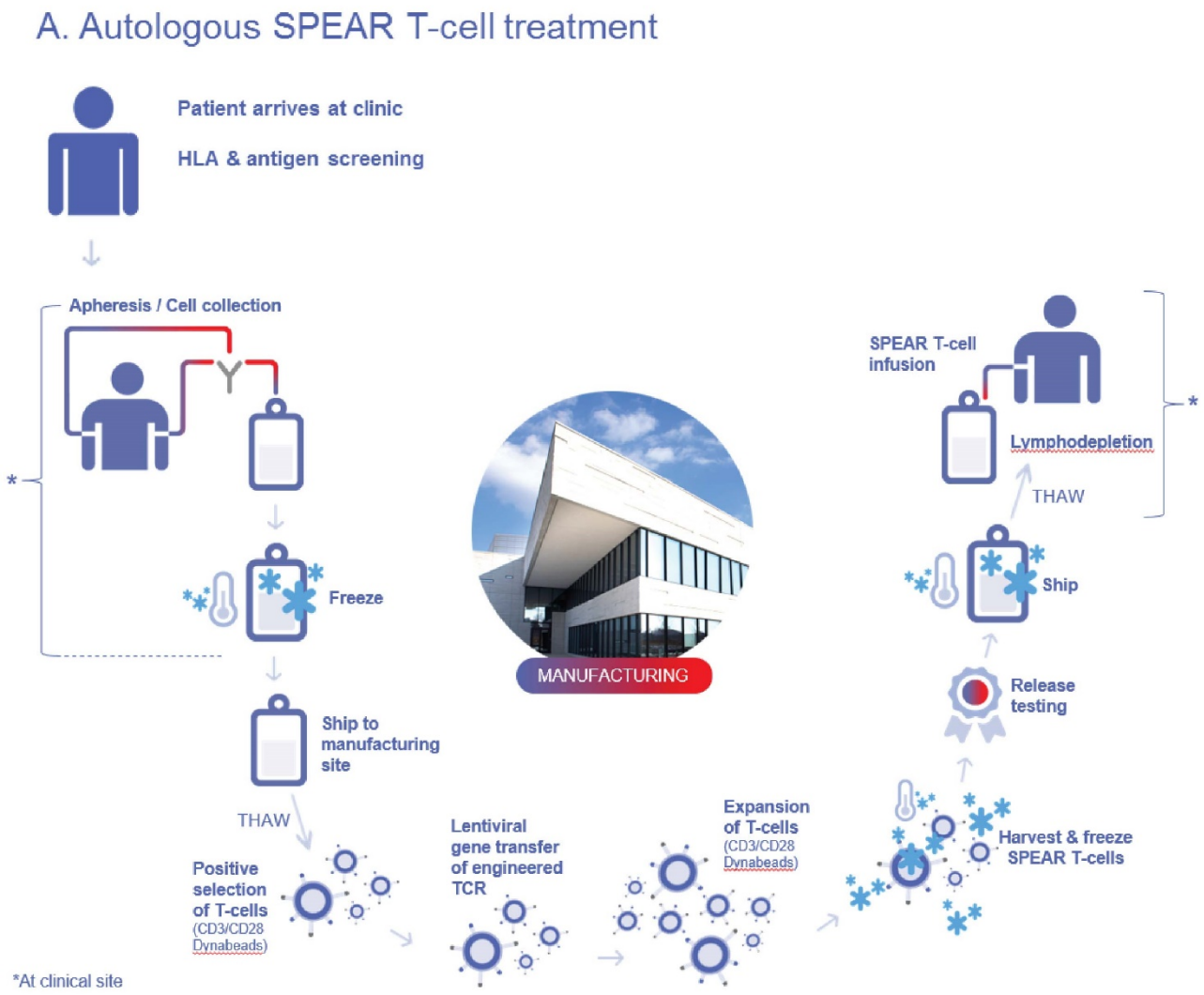
# Disclosures

- All authors are employed by and have stock or other ownership interests in Adaptimmune

# Autologous T-cell manufacturing




Factors that can affect the autologous product are:

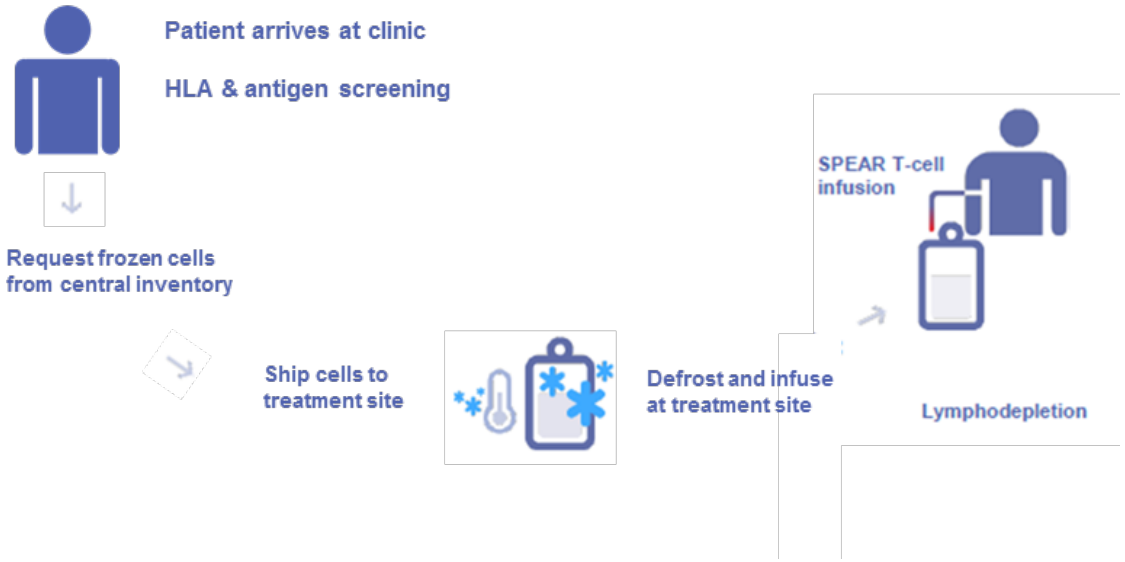
- Complex patient scheduling and supply chain
- Long lead time for individual products to be manufactured and released
- Patient leukapheresis is variable and hard to control
  - Cell composition
  - Age
  - Prior treatment regimens



SPEAR T-cell = specific peptide enhanced affinity receptor T-cell

# Advantages of manufacturing an off-the-shelf T-cell product

B. Off-the-shelf SPEAR T-cells derived from hiPSCs




- Routine patient scheduling and supply chain
- Products manufactured and released ahead of need, stored cryogenically (off-the-shelf)
- Product highly characterized
  - Defined cell composition
  - Demonstrated efficacy
  - Known safety profile
  - Reasonable cost of goods

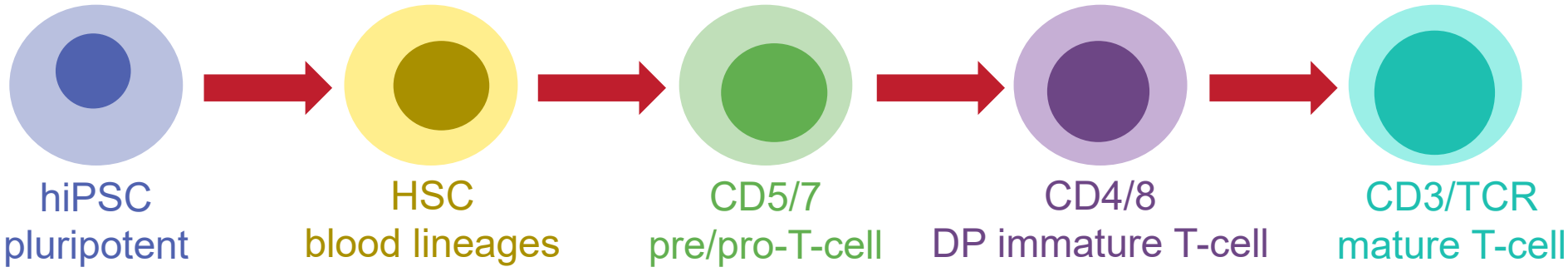


hiPSC = human induced pluripotent stem cell; SPEAR T-cell = specific peptide enhanced affinity receptor T-cell

# Our research aims to make off-the-shelf T-cell products a reality

- Key process features
  - hiPSC-directed differentiation without murine stroma
  - Enabling scale-up and GMP manufacture of edited off-the-shelf lines

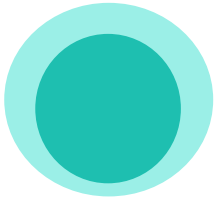
- hiPSCs are able to differentiate through multiple precursor stages toward a mature T-cell phenotype *in vitro*






DP = double positive; GMP = Good Manufacturing Practice; hiPSC = human induced pluripotent stem cell; HSC = hematopoietic stem cell; TCR = T-cell receptor

# A ready, reproducible supply of T-cells from a single, well-characterized source



CD3/TCR
mature T-cell

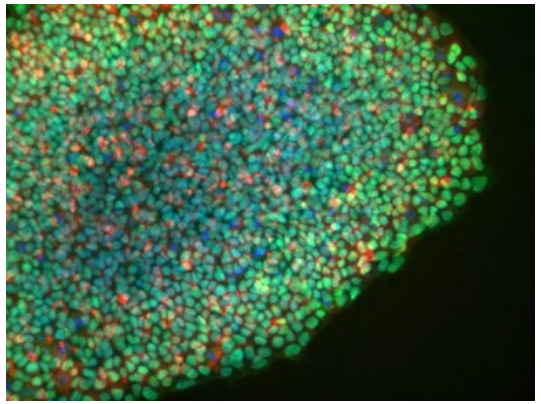
- hiPSC-derived T-cells display hallmark features of mature T-cells
  - Expression of lineage-specific genes (Biomark panel)
  - Surface TCRαβ or TCRγδ expression (flow cytometry)
  - Effector functions: cytokines, lytic granules, and CD69 upregulation



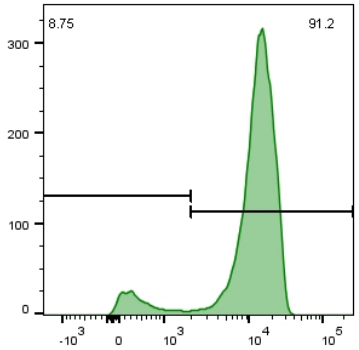
hiPSC = human induced pluripotent stem cell; TCR = T-cell receptor

# Starting with hiPSC before differentiation

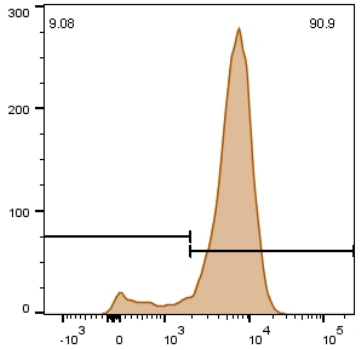
## Assessment of pluripotency



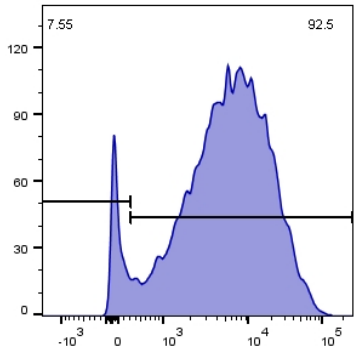
TRA-160 (red) Oct4 (green) nuclei (blue)



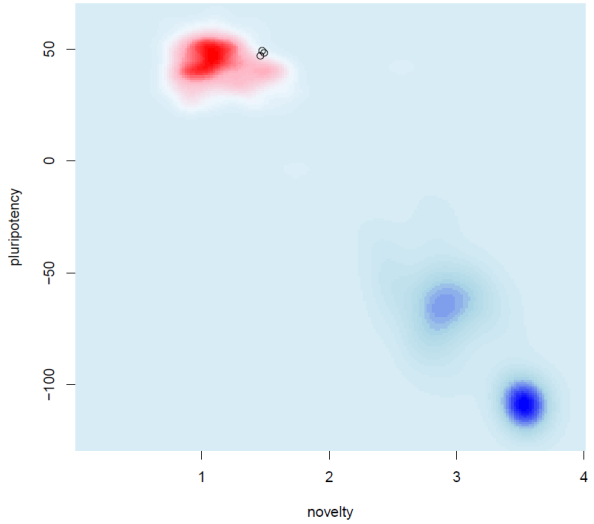

Oct4

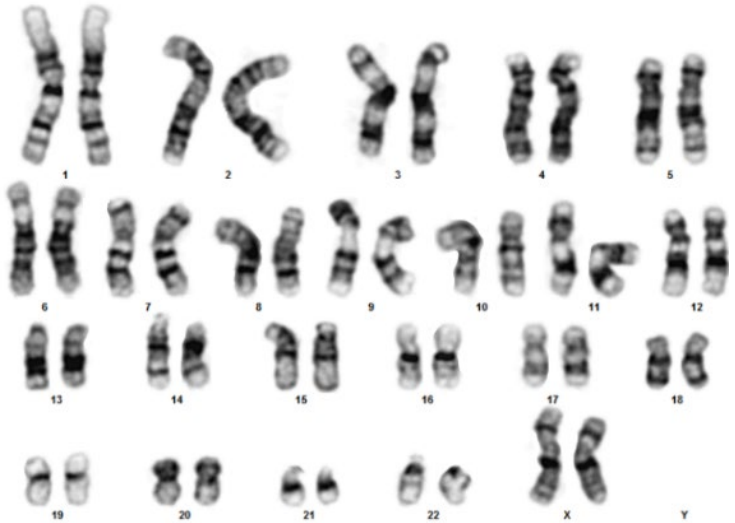


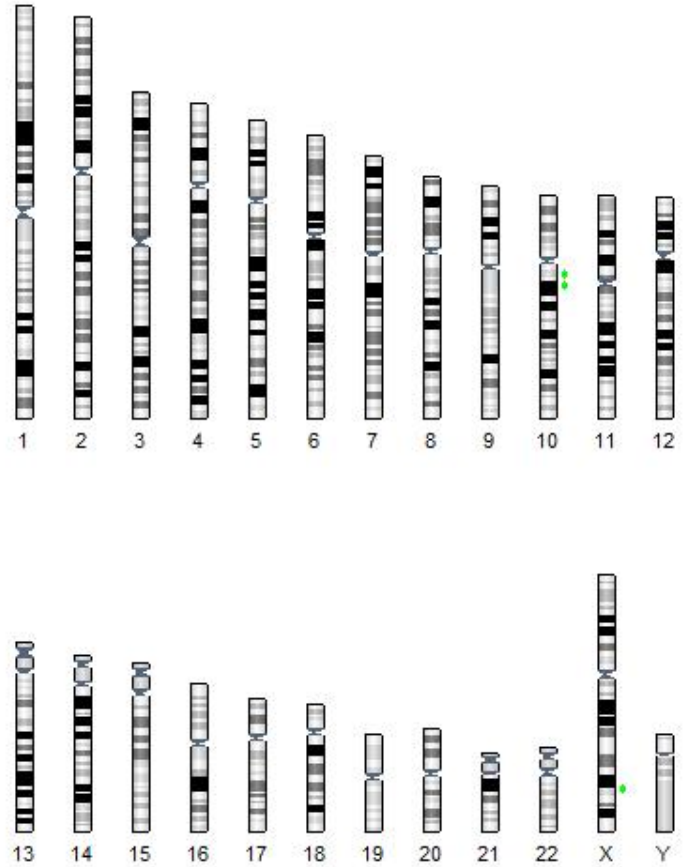

Nanog




TRA-160








hiPSC = human induced pluripotent stem cell

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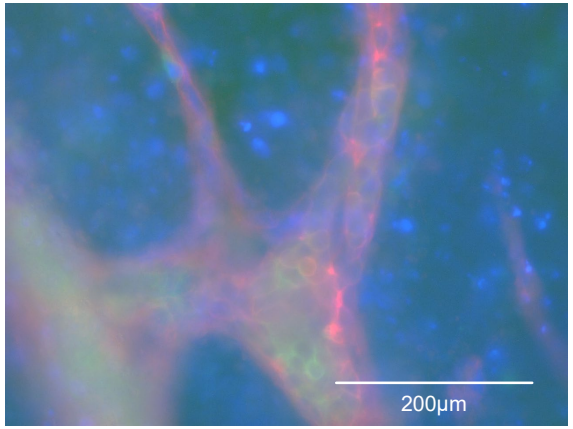
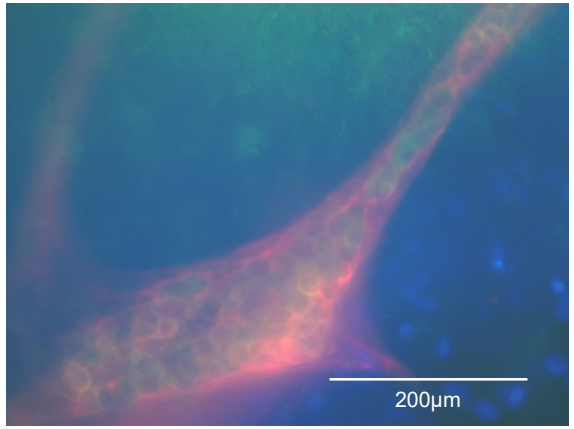
# Generating $CD34^{+}CD45^{+}$ hematopoietic progenitors and early pro-T-cells…

## Intermediate developmental stages

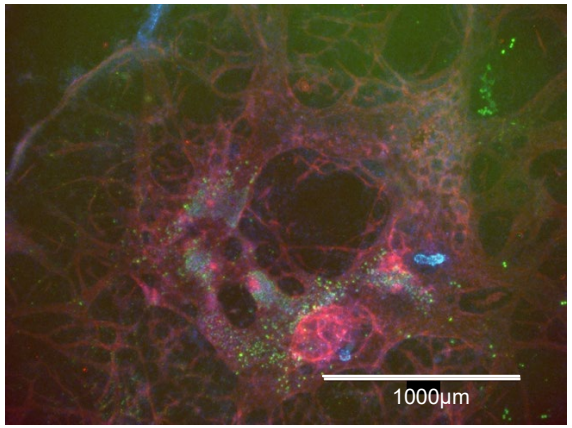
HSC
blood lineages




CD34 (red) CD45 (green) nuclei (blue)

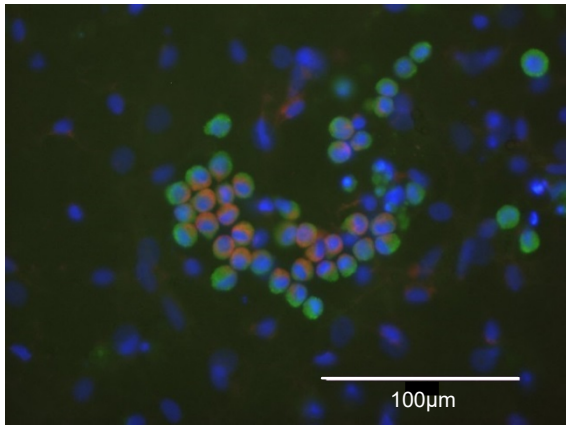



CD34 (red) CD45 (green) nuclei (blue)

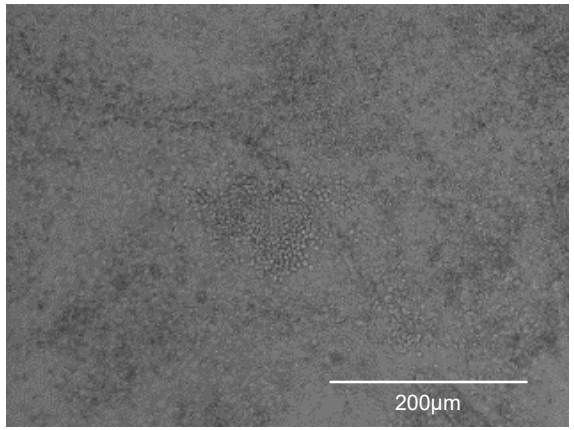



CD34 (red) CD3 (green) nuclei (blue)

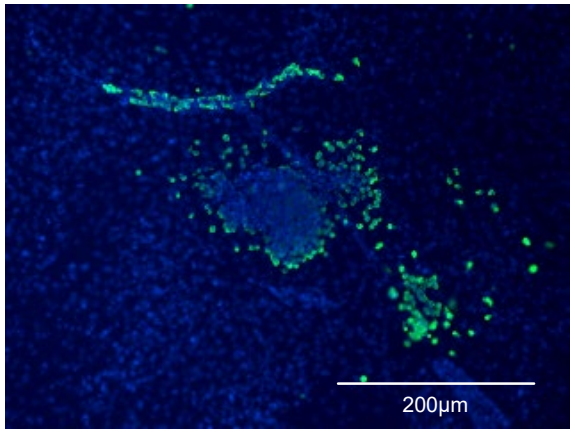
CD4/8
DP immature T-cell




CD8 (red) CD4 (green) nuclei (blue)




Brightfield




CD3 (green) nuclei (blue)

CD3/TCR
mature T-cell

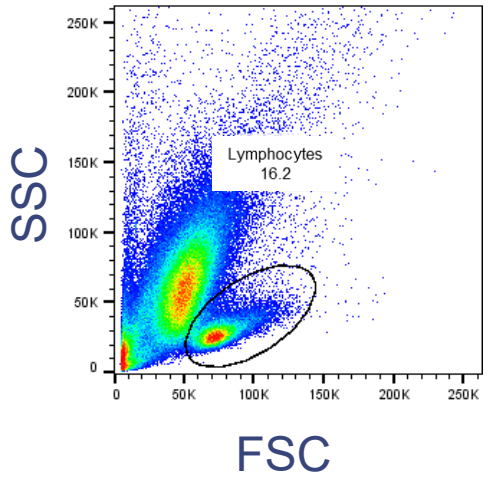


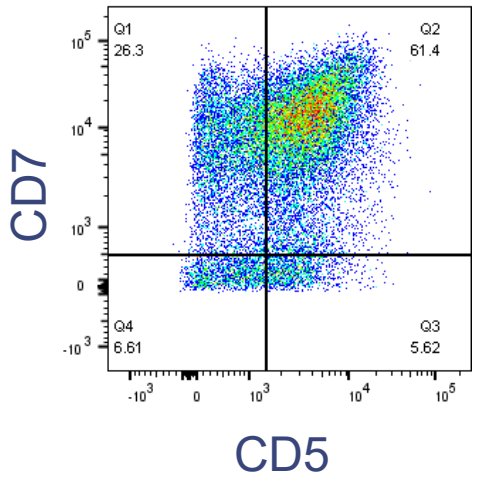
DP = double positive; HSC = hematopoietic stem cell; TCR = T-cell receptor

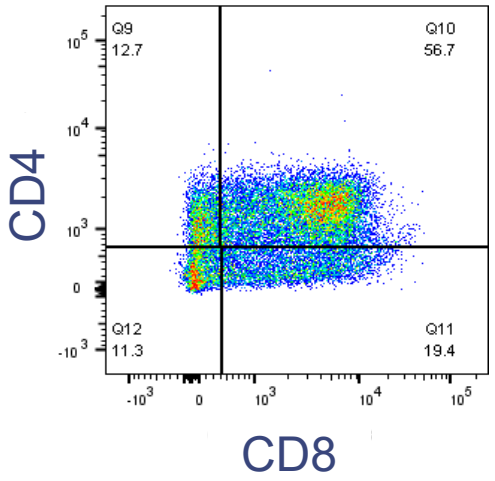
# …and finally to T-cells

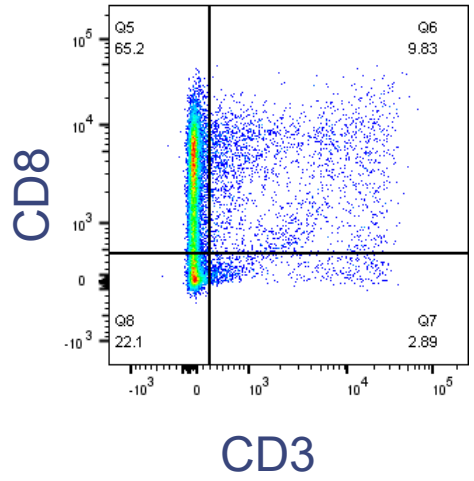
Intermediate-stage T-cell progenitors

CD4/8
DP immature T-cell



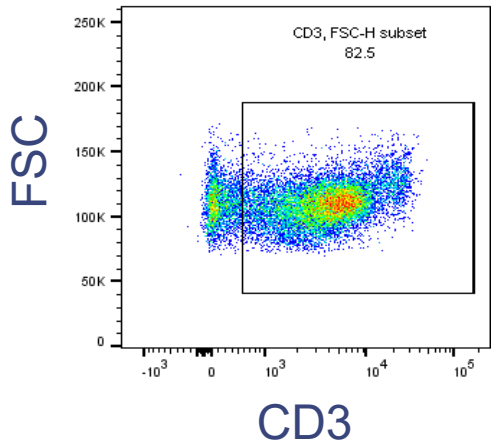



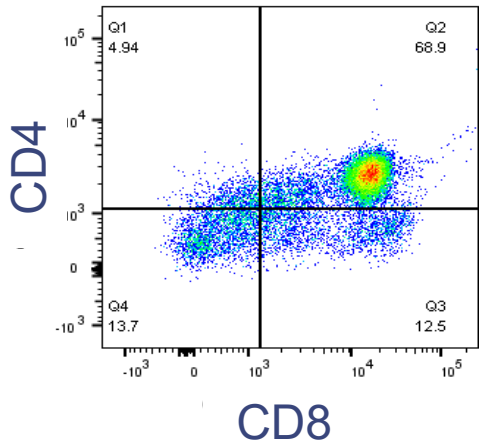



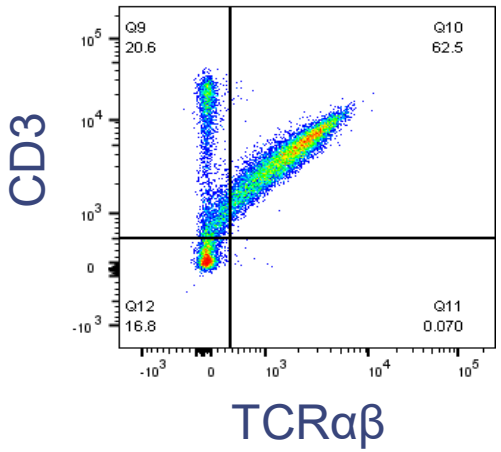



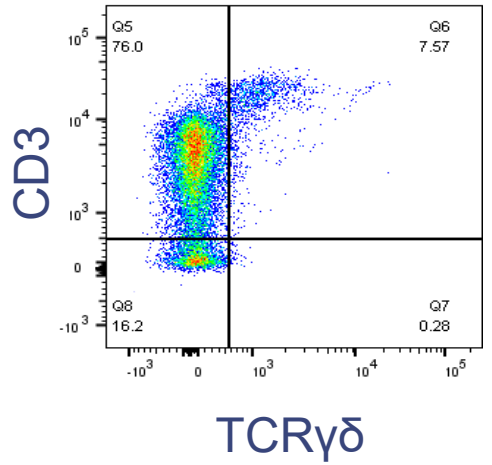

End-of-process differentiated T-cells

CD3/TCR
mature T-cell






DP = double positive; FSC = forward scatter; hiPSC = human induced pluripotent stem cell; SSC = side scatter; TCR = T-cell receptor

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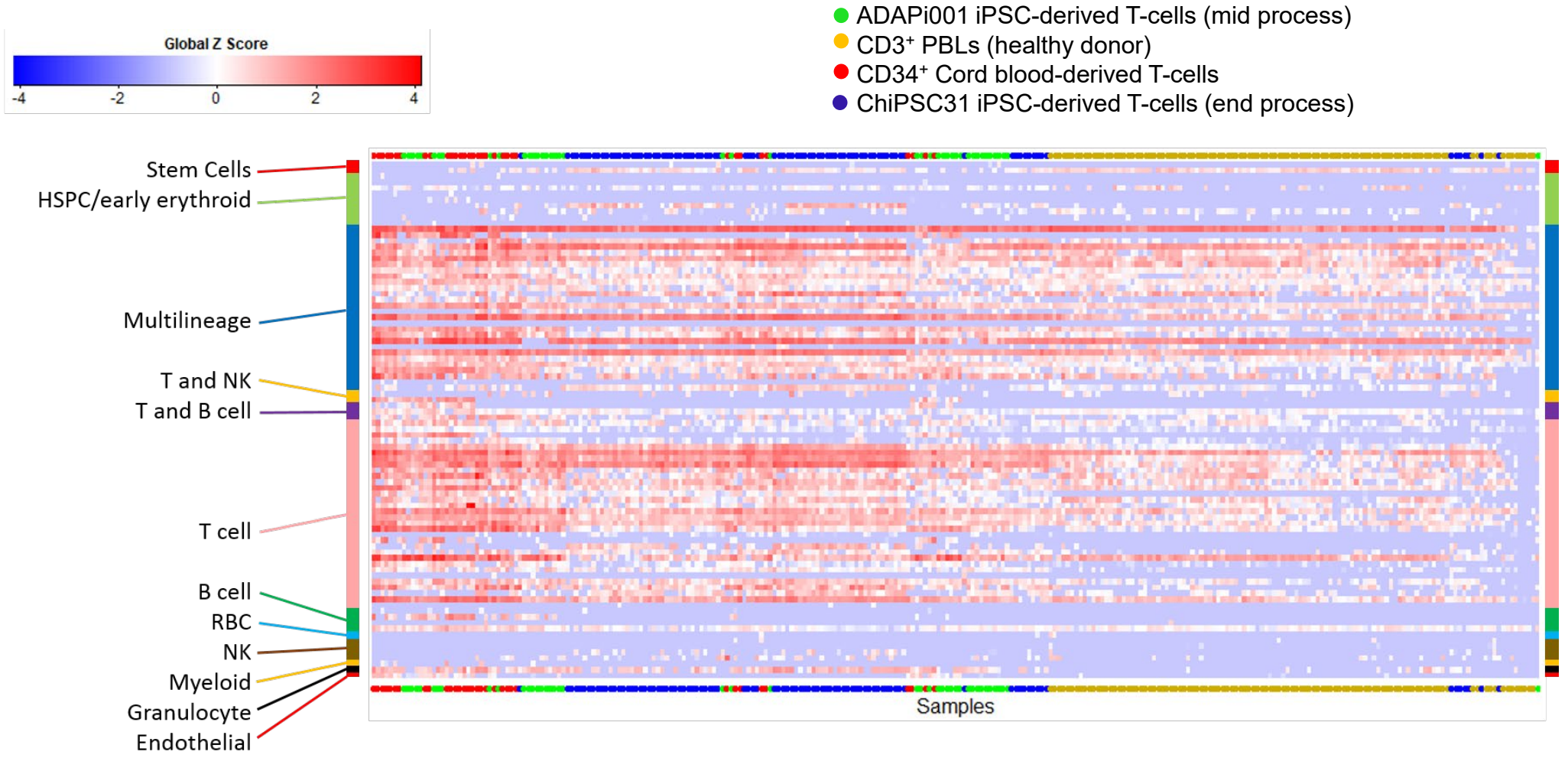
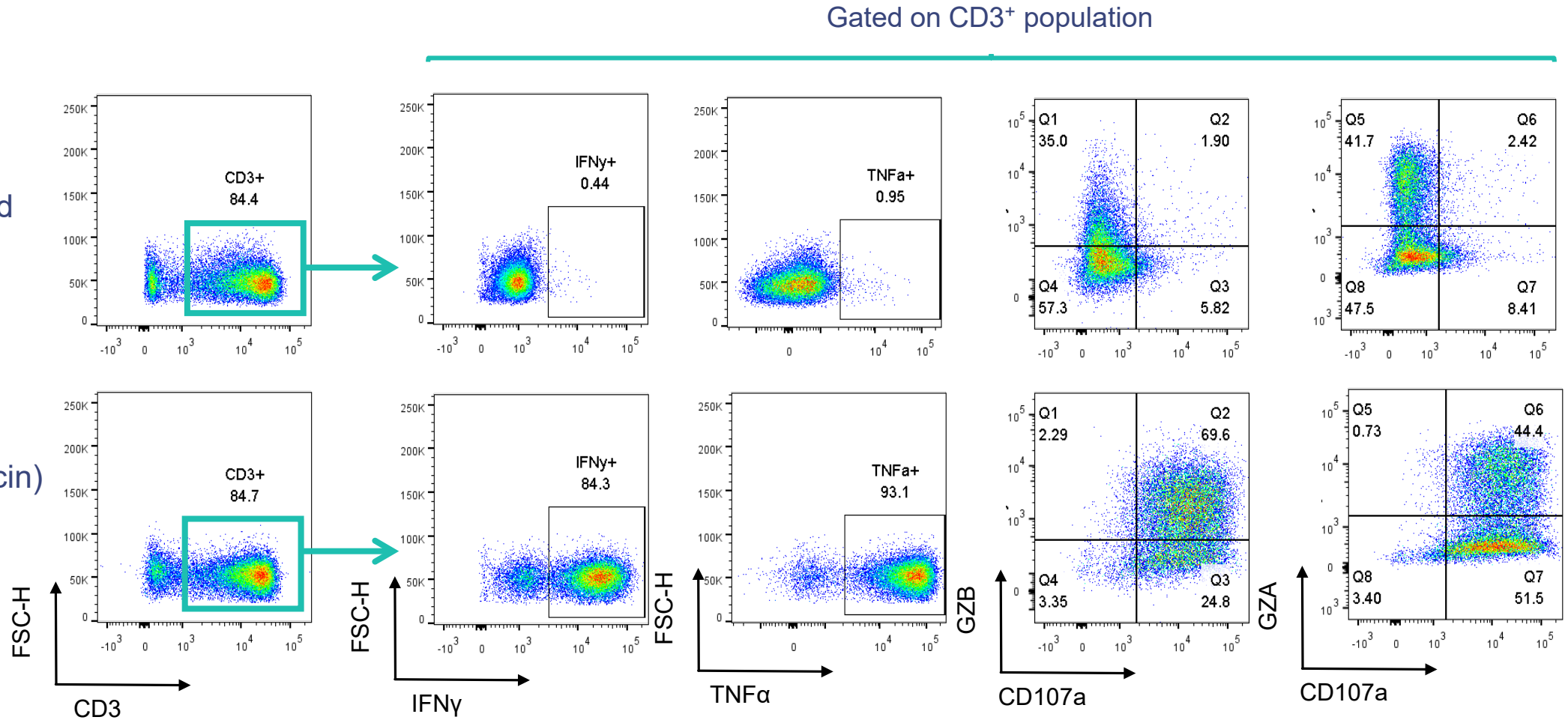
# Transcriptional profiling of hiPSC- and cord-derived CD3⁺ T-cells together with peripheral T-cells reveals similar gene signatures






hiPSC = human induced pluripotent stem cell; HSPC = hematopoietic stem and progenitor cell; iPSC = induced pluripotent stem cell; NK = natural killer (cell); PBL = peripheral blood lymphocyte; RBC = red blood cell

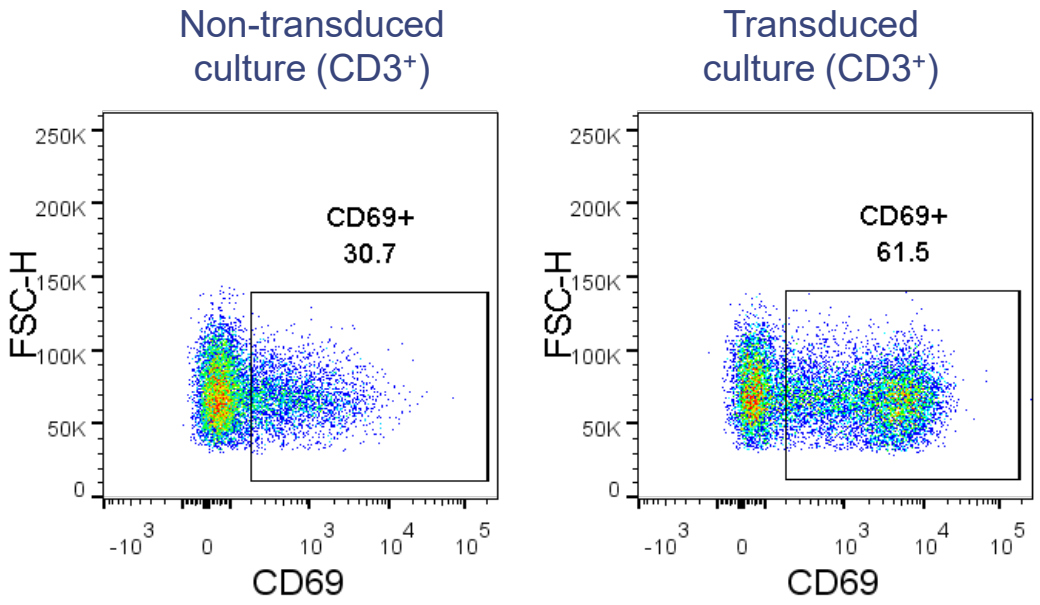
# Functionality of stem cell–derived T-cells following stimulation with PMA/ionomycin

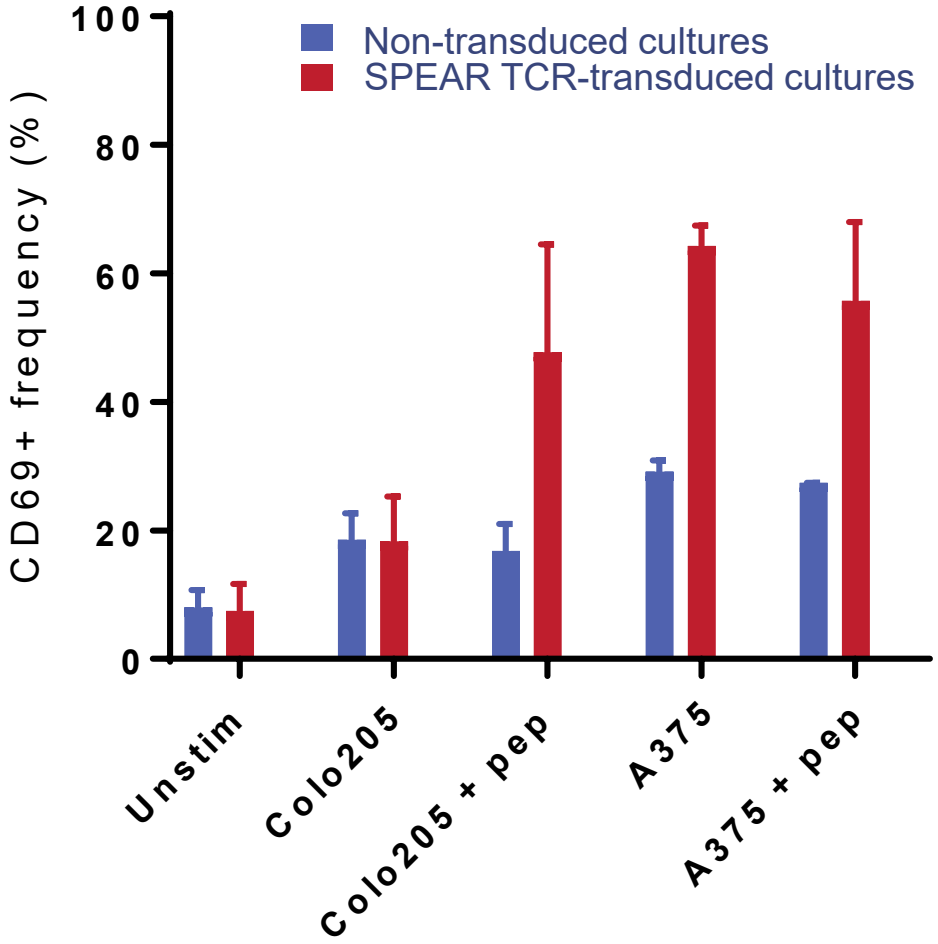





FSC-H = forward scatter-height; GZA = Granzyme A; GZB = Granzyme B; IFN = interferon; TNF = tumor necrosis factor

# TCR-transduced hiPSC-derived T-cells are activated in an antigen-dependent manner






FSC-H = forward scatter-height; hiPSC = human induced pluripotent stem cell; SPEAR = specific peptide enhanced affinity receptor; TCR = T-cell receptor

# Conclusions

- These hiPSC-derived T-cells show many characteristics that make them ideal for allogeneic, off-the-shelf T-cell therapies for oncology
  - Typical T-cell marker expression mimicking mature peripheral blood T-cells
  - Cytokine production after stimulation
  - Lytic granule production and release after stimulation
  - Tumor antigen-specific activation of TCR signaling pathway when engineered to express SPEAR TCR
  - Consistent batches can be manufactured to treat multiple patients

- This source of T-cells, when combined with genetic engineering to enhance tumor recognition with SPEAR TCR and prevent GVHD by preventing endogenous TCR expression, could accelerate a new generation of off-the-shelf T-cell therapies



hiPSC = human induced pluripotent stem cell; GVHD = graft versus host disease; SPEAR = specific peptide enhanced affinity receptor; TCR = T-cell receptor

# Acknowledgments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  - Jaqueline Viera
  - Darius Pillsbury
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  - Chris Barnes
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