Stanford MEDICINE
School of Medicine

Engineering Endothelial Cells to Treat Pulmonary Arterial Hypertension

Heather Jackson-Pease, Anais Amaya
Matthew Porteus
CIRM, Stanford School of Medicine:Department of Pediatrics
Porteus Lab

Figures provided by BioRender

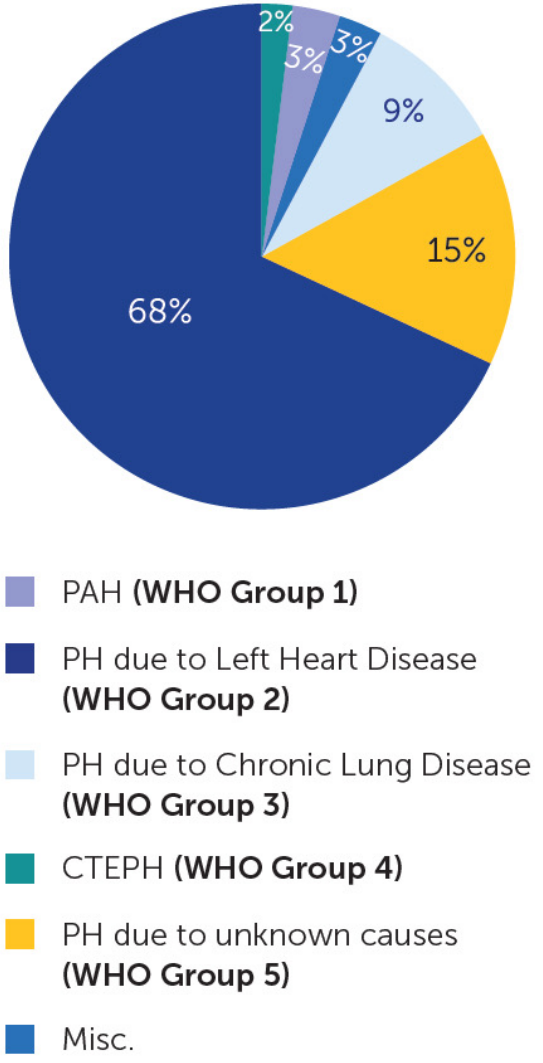
Introduction

Five Groups of Pulmonary Hypertension (PH)

Group 1 Associated : 51%	Group 2	Group 3	Group 4	Group 5
• Idiopathic **PAH** 44% • Hereditary PAH 4% • Drug induced PAH • Connective tissue disease associated PAH (e.g. scleroderma) 67% • Congenital heart disease associated PAH 12%	• Due to **left heart disease** • Risk factors for this type of PH include coronary artery disease, hypertension, diabetes, high cholesterol etc.)	• PH due to **lung disease or hypoxia** (low oxygen) • This can be caused by advanced lung disease including COPD, Interstitial lung diseases (e.g. IPF) or obstructive sleep apnoea	• **Chronic thromboembolic pulmonary hypertension** (CTEPH) is the most common cause of Group 4 PH.	• This is a '**miscellaneous**' group that includes causes of PH that are unclear or have multiple mechanisms such as sarcoidosis.

The National Pulmonary Hypertension Unit(Dublin, Ireland)

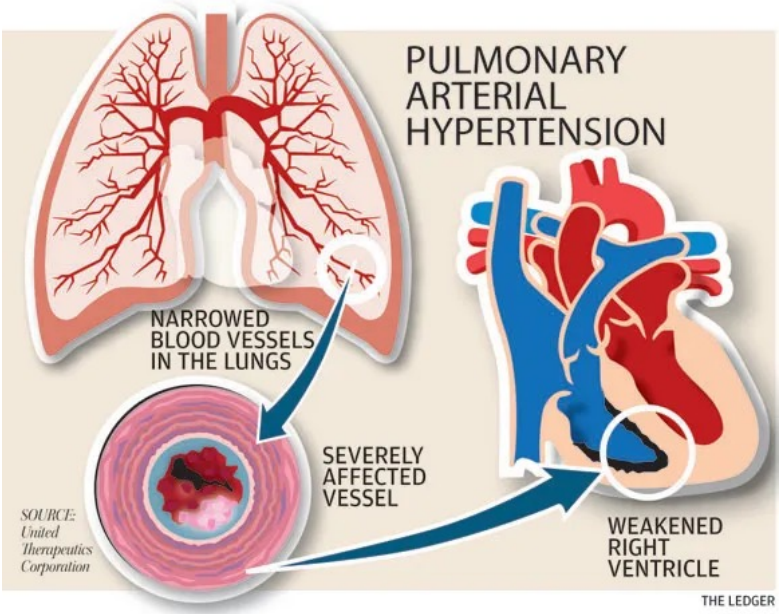
Hereditary: BMPR2 gene→ maturation of bone and cartilage



Strange G, et al. Heart. 2012;98(24):1805-11.

Group 1: Pulmonary Arterial Hypertension(PAH)

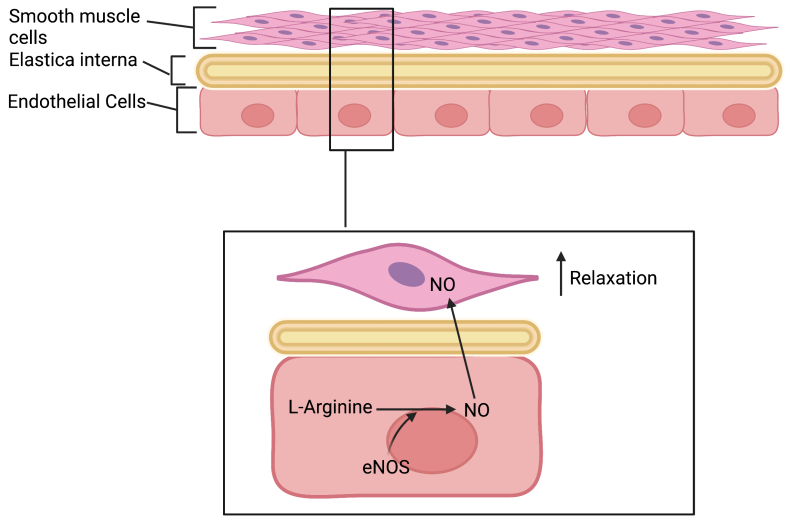
- About 500-1000 new cases of PAH diagnosed each year in U.S.(ALA)
- 50-70 million people WW (APS)
- Life expectancy about 2-3 years without treatment
- Predominately effects women (3:1) of color ages 30-60
  - Ages of patients are generally lower when drug/toxin induced
- Characterized by blood vessels in the pulmonary artery being narrowed or destroyed
  - Increased pressure in artery
  - Weakening of right side of the heart
- Believed to be the result of injury to the cells that line the blood vessels (endothelial cells) of the lungs

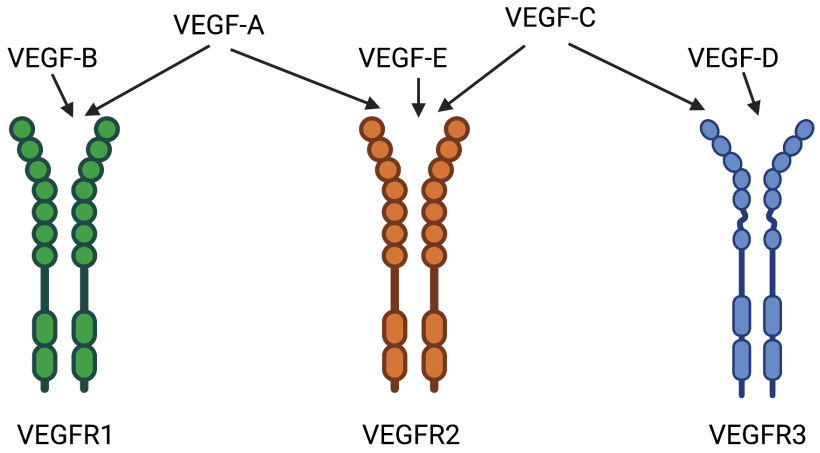


Endothelial Nitric Oxide Synthase leads to the production of Nitric Oxide(NO) & Vascular Endothelial Growth Factor promotes reendothelialization

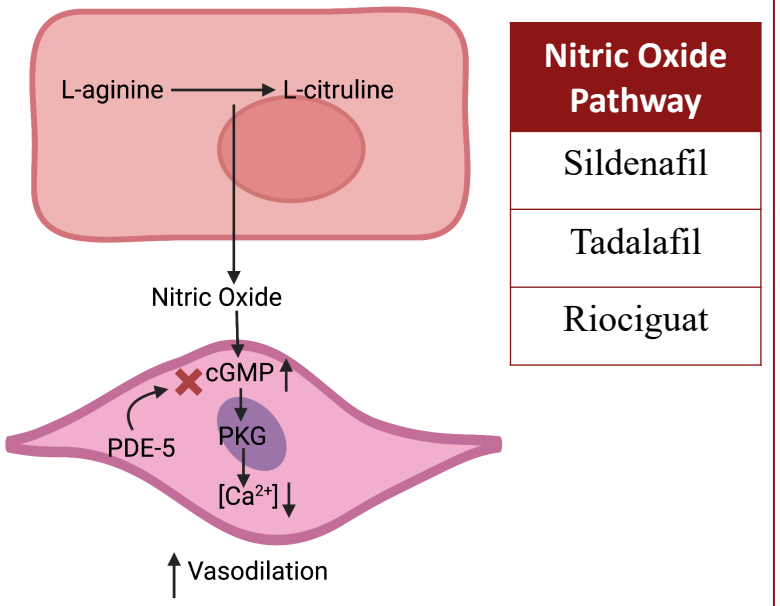
- NO diffuses into smooth muscle, activates guanylyl cyclase, and leads to vasodilation(Dr. Murad, 1977)
  - NO has a short half-life of 1-10 seconds
    - Quickly oxidizes to nitrite and then nitrate

- VEGF A, has shown to promote activation of angiogenic signaling pathways(Karanysheva, A.F., 2008) and reendothelialization (Chang, et al, 2018)
- The most abundant isoform of VEGF A, $VEGFA^{165a}$ stimulates proliferation and survival of ECs (Peiris-Pages, M., et al, 2012)

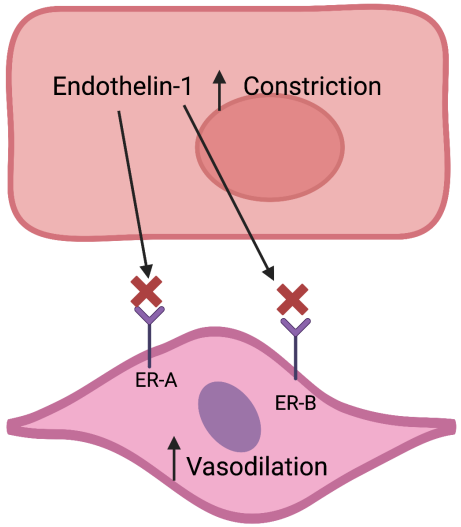




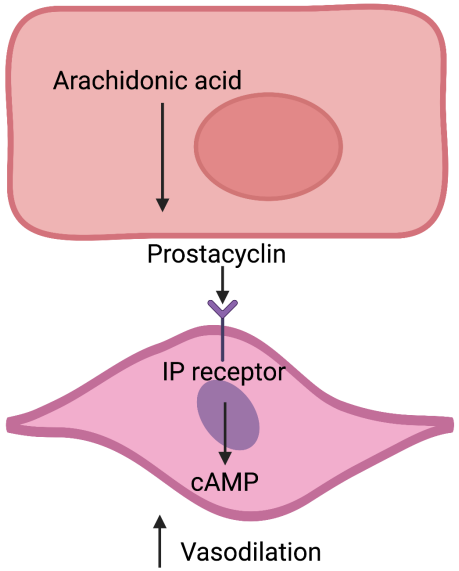
Current treatment for PAH target vasodilation pathways



Nitric Oxide Pathway
Sildenafil
Tadalafil
Riociguat



Endothelin Pathway
Macitentan
Ambrisentan

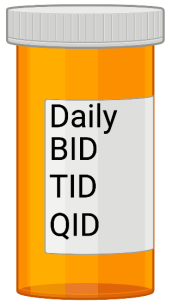


Prostacyclin Pathway
Oral selexipag
Inhaled iloprost
Subcutaneous treprostinil
Intravenous epoprostenol

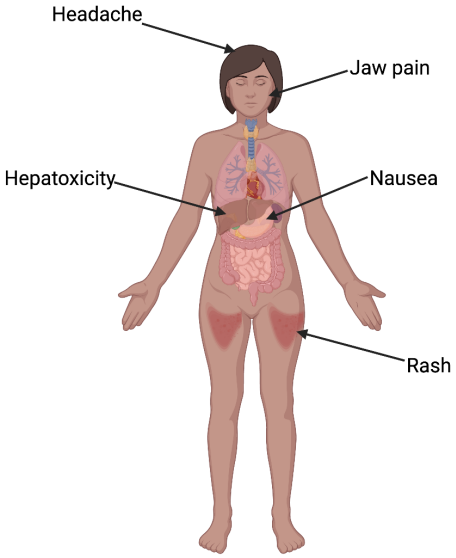
Adapted from: Humbert et al (2014)

Shortcomings of current treatments leaves room for improvement

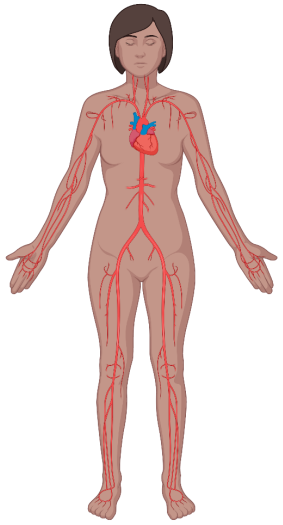
Frequent



Side effects



Not localized

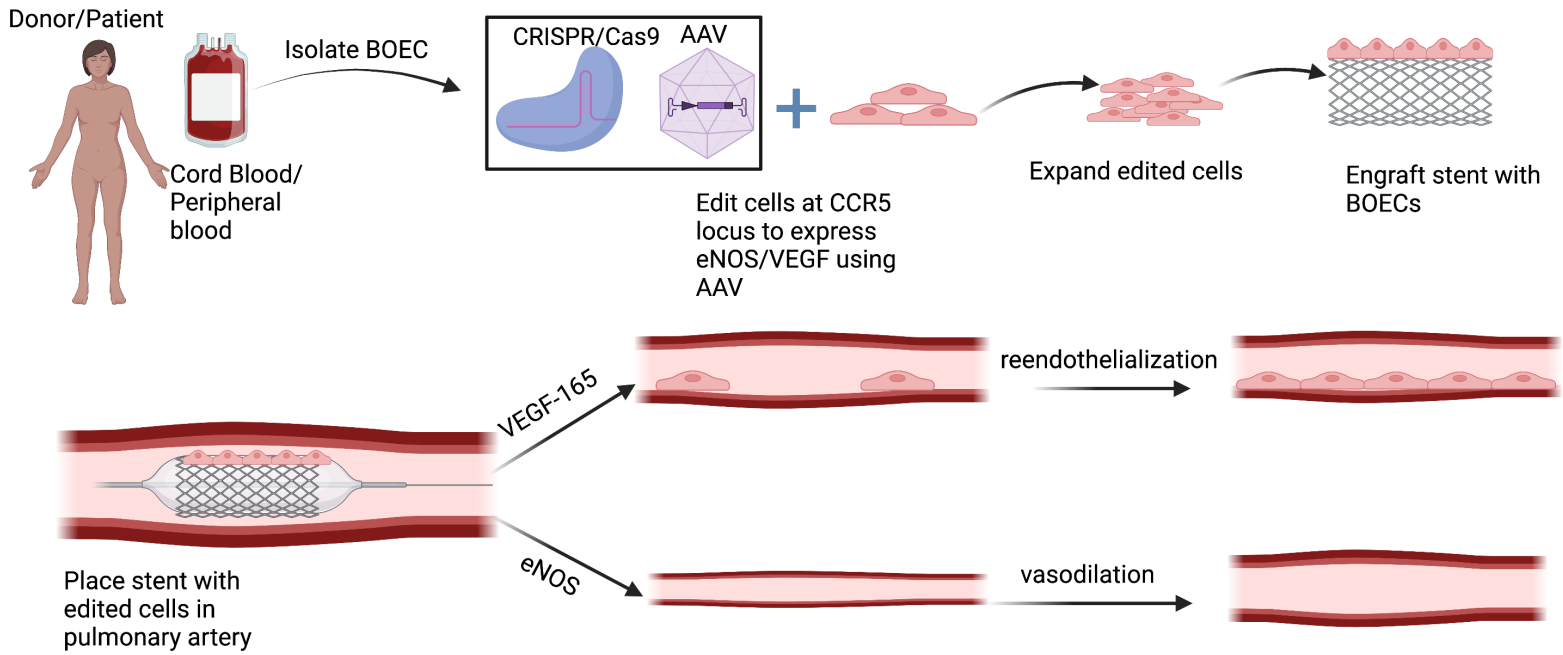


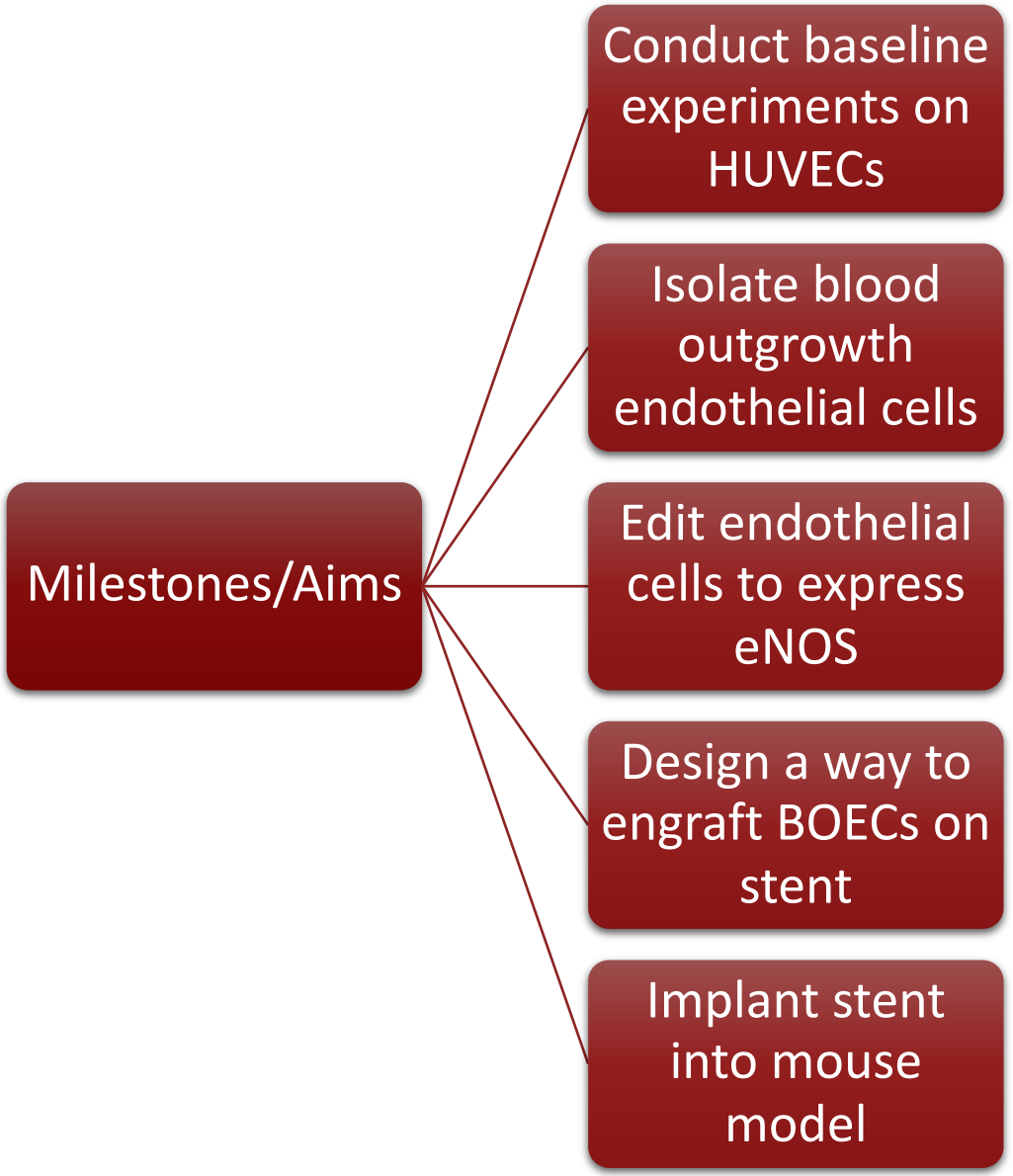
Not suitable for pregnant people

Improved Mortality

~6-10 years

The plan: Engraft edited ECs on stent, implant stent in PA

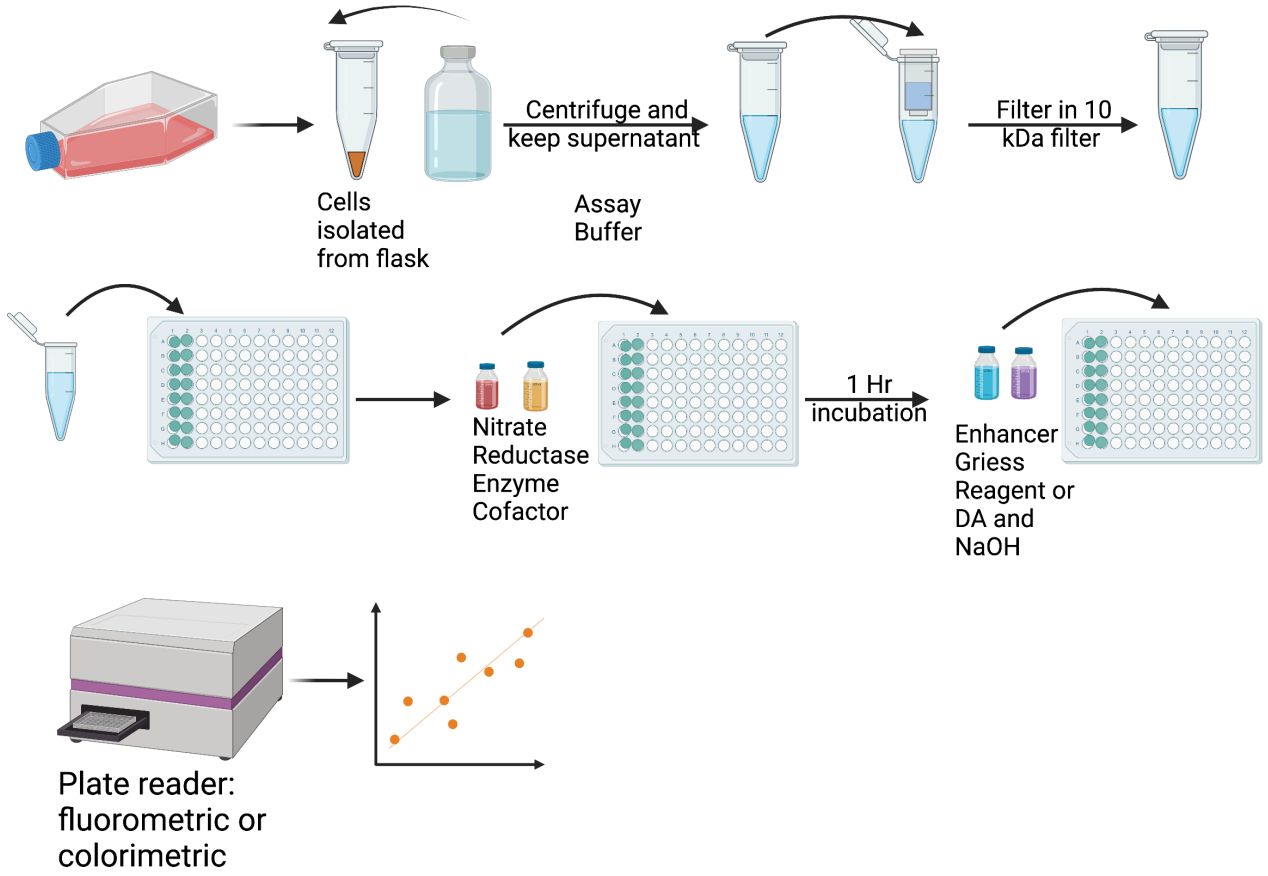



Milestones/Aims
Conduct baseline experiments on HUVECs
Isolate blood outgrowth endothelial cells
Edit endothelial cells to express eNOS
Design a way to engraft BOECs on stent
Implant stent into mouse model

Methods & Results

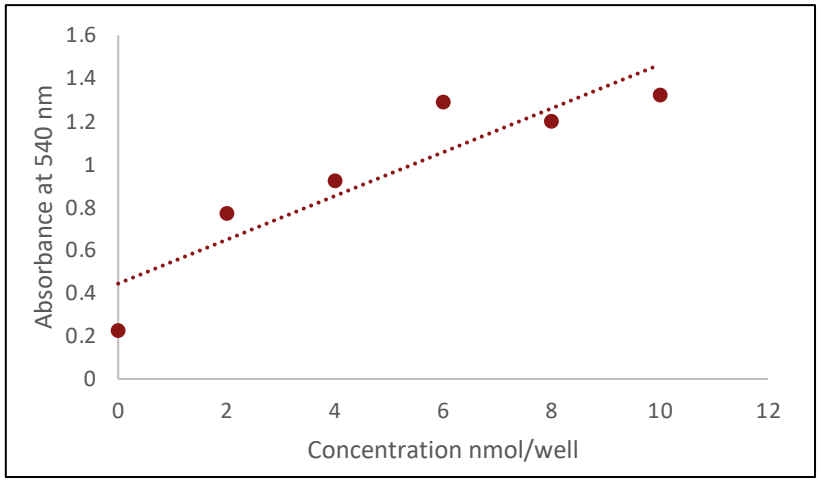
Milestone 1: Conduct baseline experiments on HUVECs

- Nitric Oxide Assay
- VE-Cadherin and VWF Staining
- VWF Optimization Staining
- Determining most effective AAV serotype for ECs
- Editing HUVECs using W3 and sgRNA11
- Editing HUVECs using different guides, MOIs, AZD, and cell count

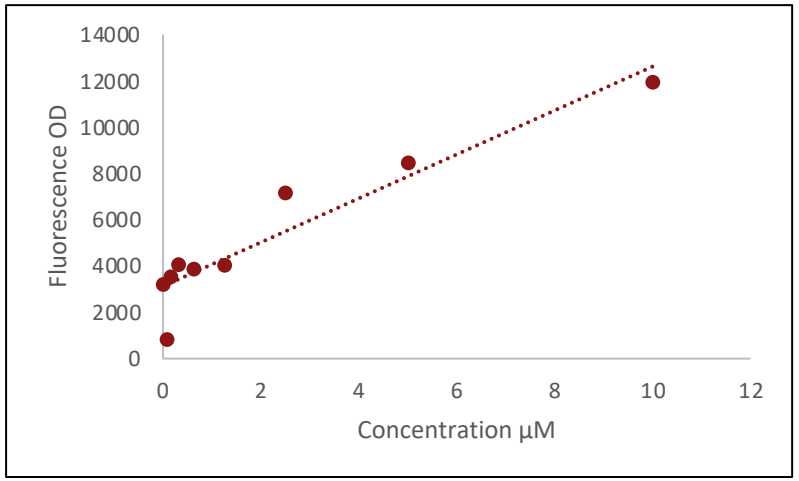


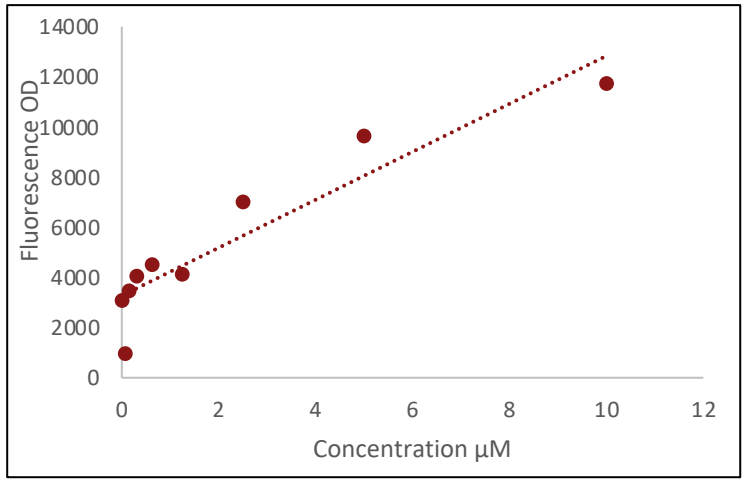
Milestone 1: Nitric Oxide Assay Fluorometric & Colorimetric

Colorimetric



Fluorometric

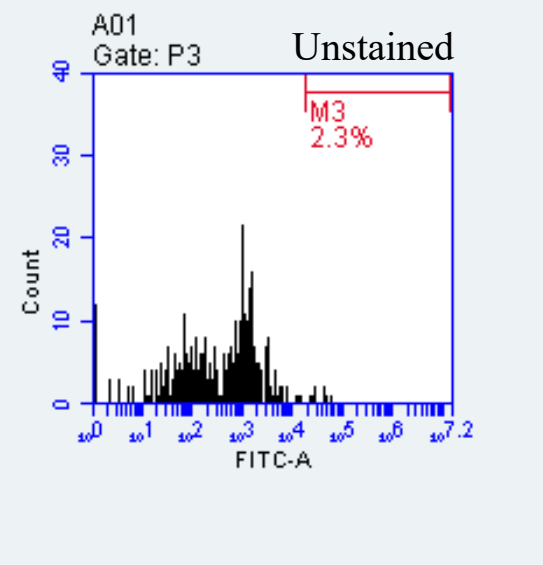


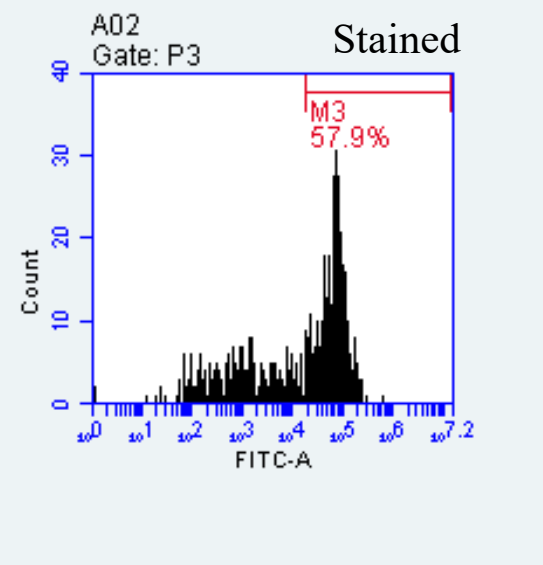


Nitric Oxide Concentrations	
Colorimetric	Fluorometric
0.884 nmol/well	0.41 µM
	0.48 µM

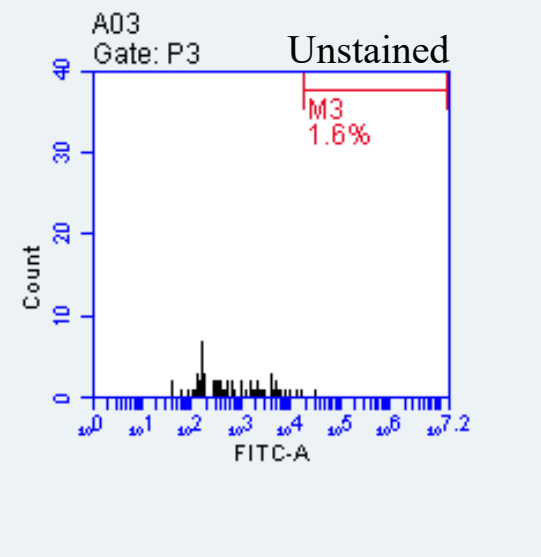
Milestone 1: Antibody Staining: VE-Cadherin and VWF in HUVECs

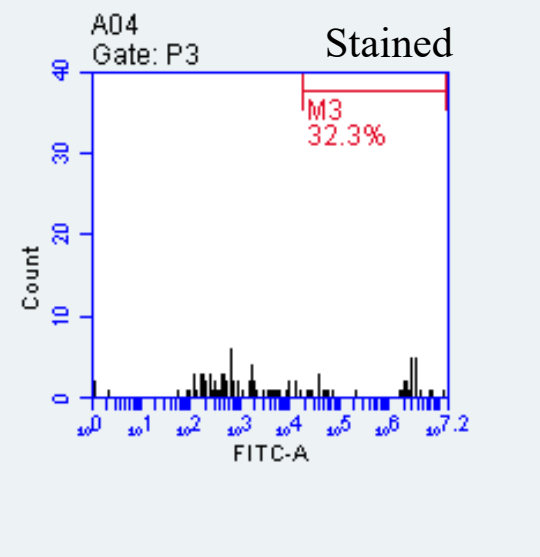
VE-Cadherin





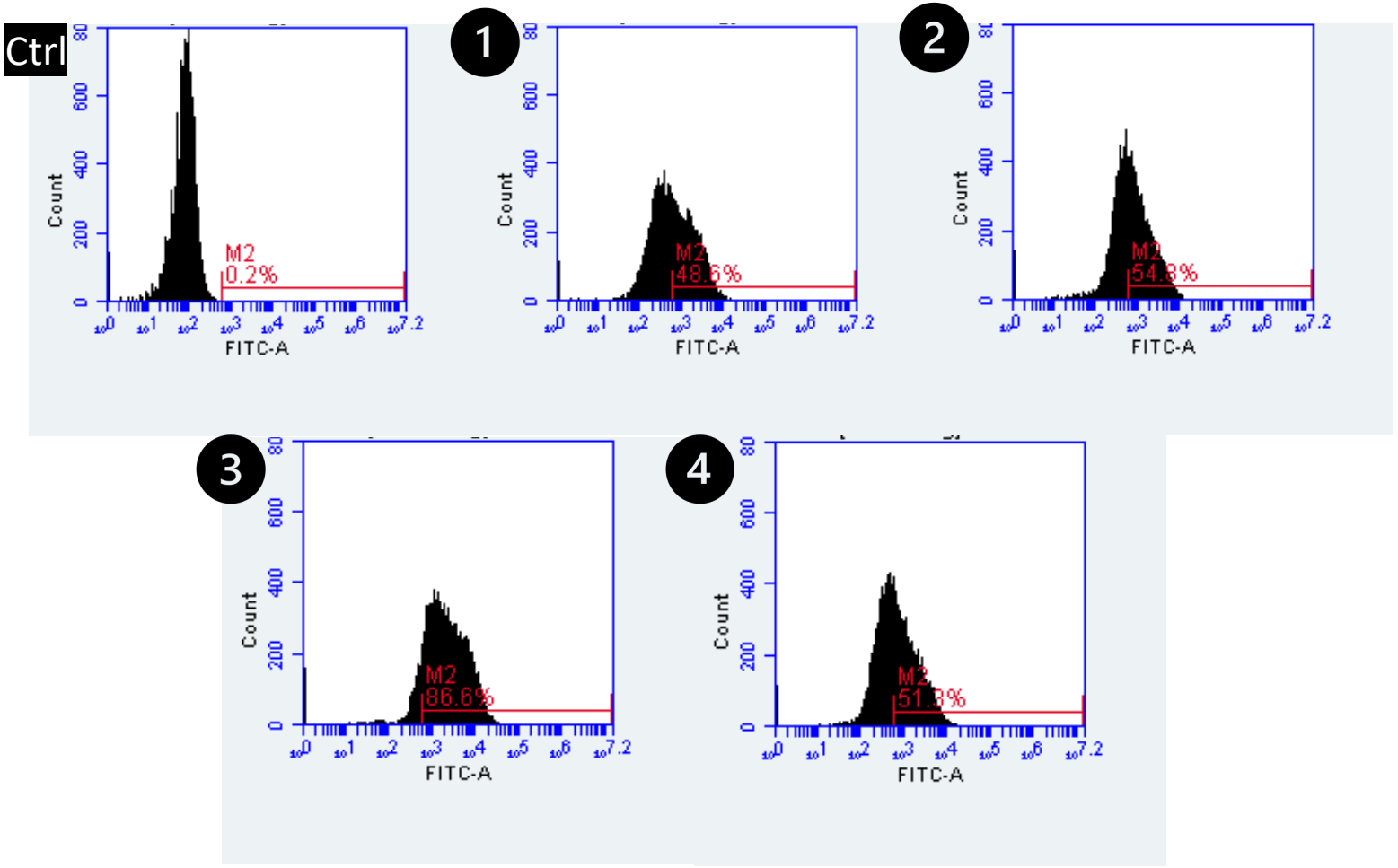
VWF



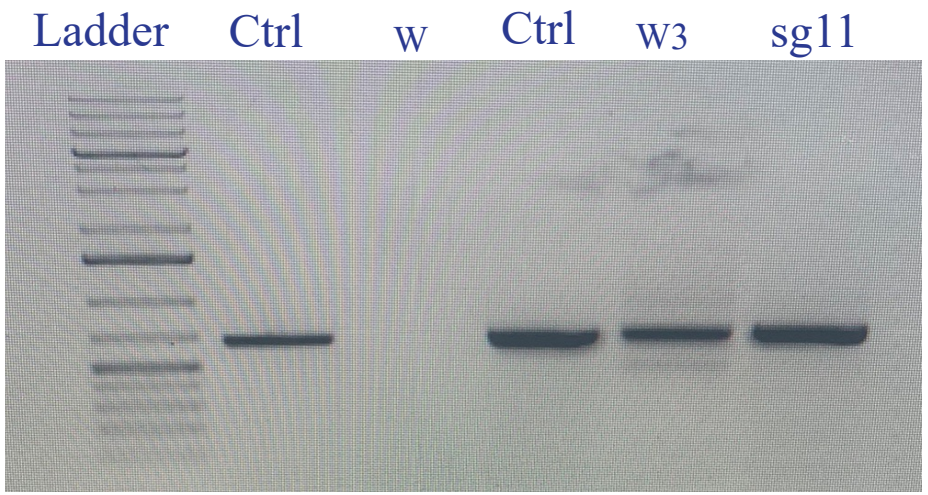


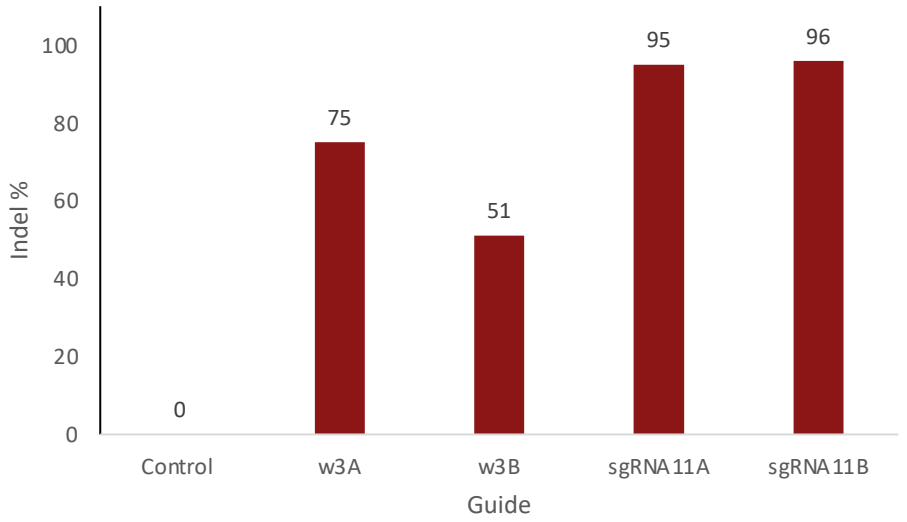
Milestone 1: VWF Optimization Staining

Condition	Primary Antibody	Secondary Antibody
Control	0	0
1	1:50	1:200
2	1:25	1:200
3	**1:50**	**1:100**
4	1:25	1:100



Milestone 1: Editing HUVECs
PCR and Indel Frequency Results

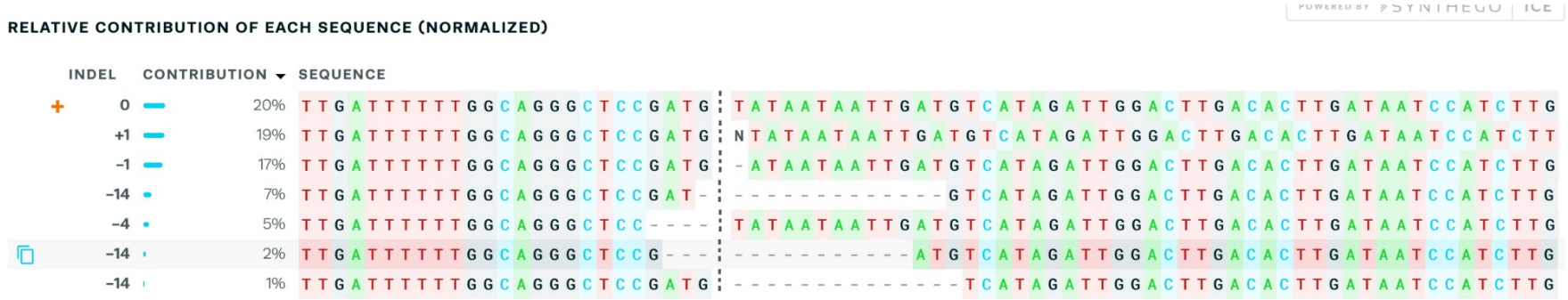




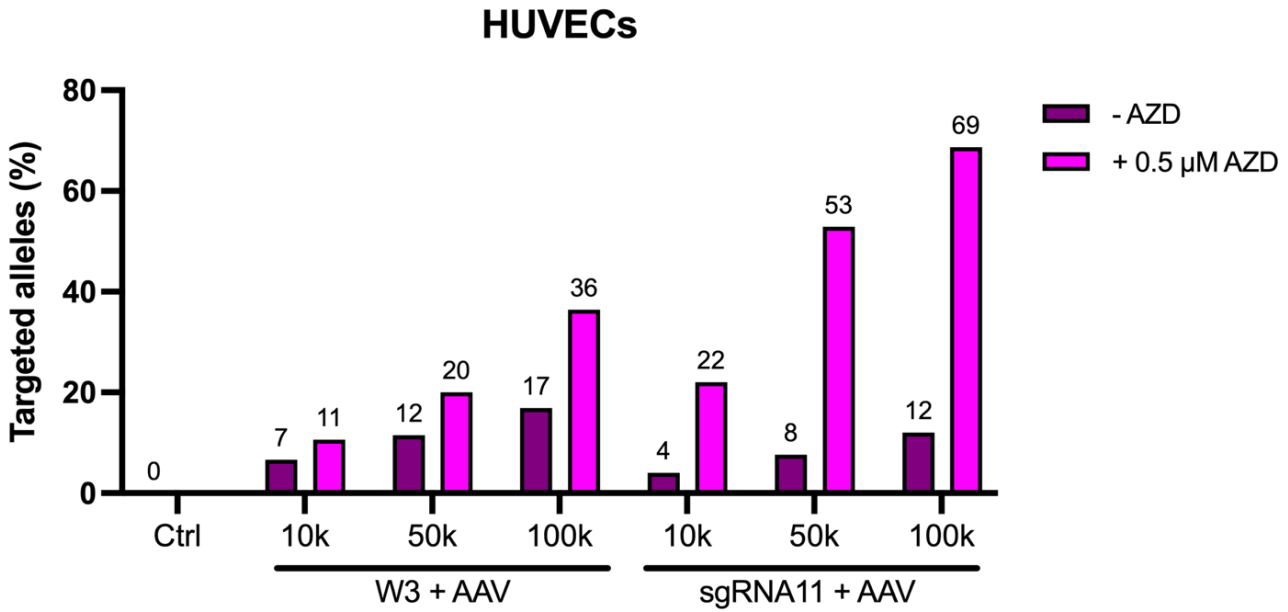
Milestone 1: Editing HUVECs
sgRNA11 has higher indel frequency than W3 and less editing events

sgRNA11

W3

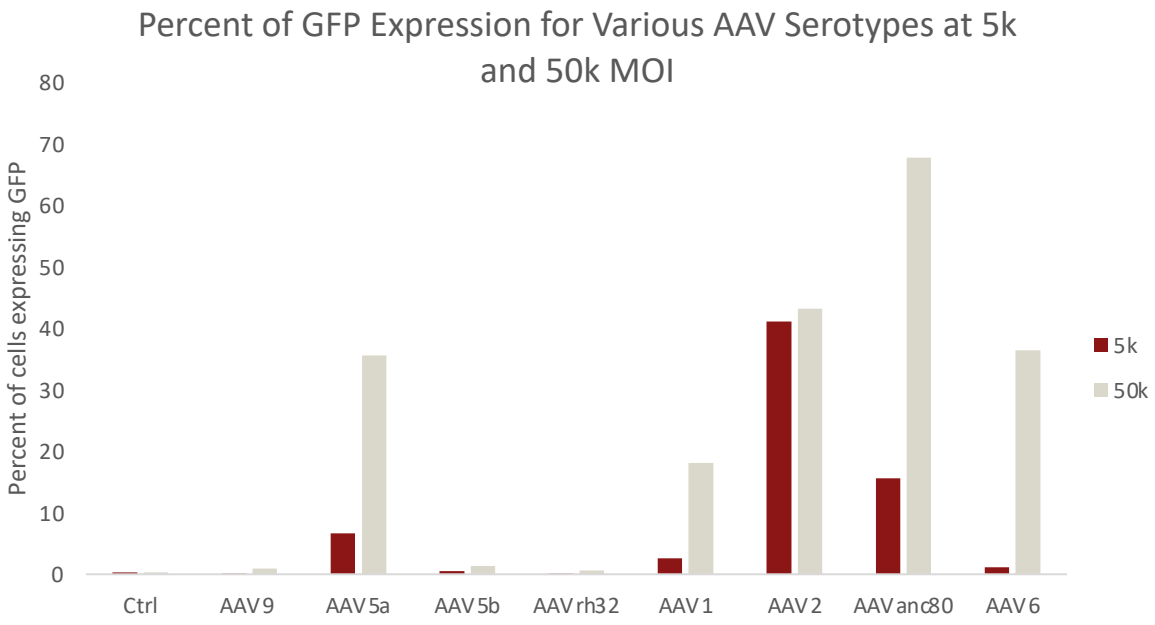


Milestone 1: Addition of AZD and Various MOI of AAV6

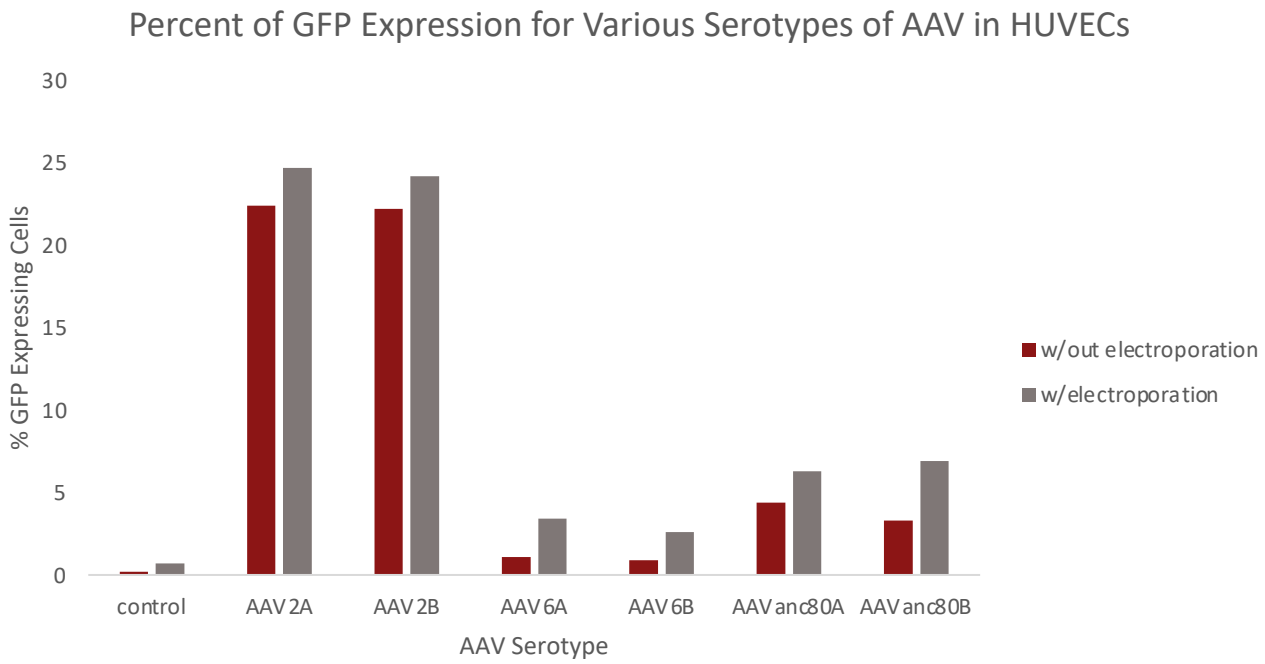


Milestone 1: Determination of most Effective AAV Serotype for Gene Integration

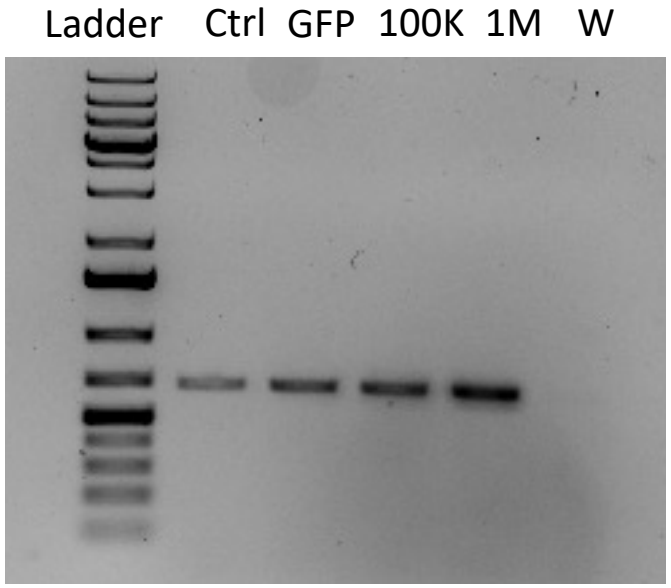
Experiment 1: Various Serotypes and MOIs

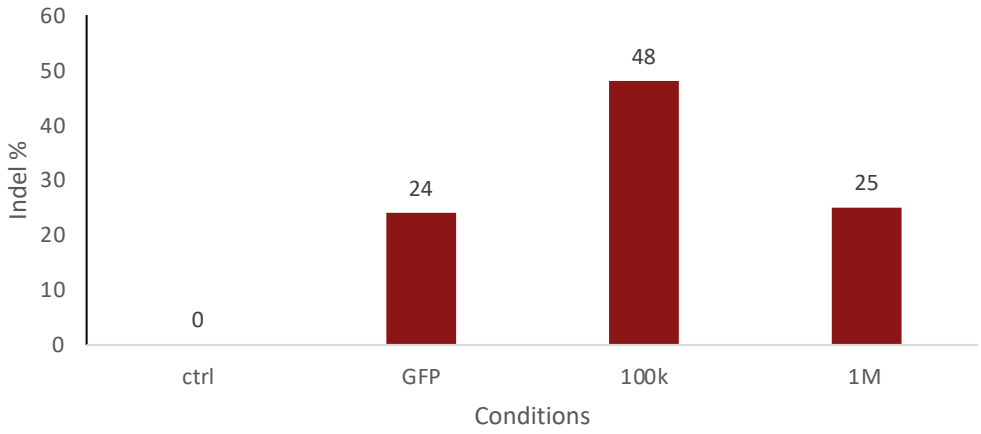


Experiment 2: Top Three AAVs with or without Electroporation

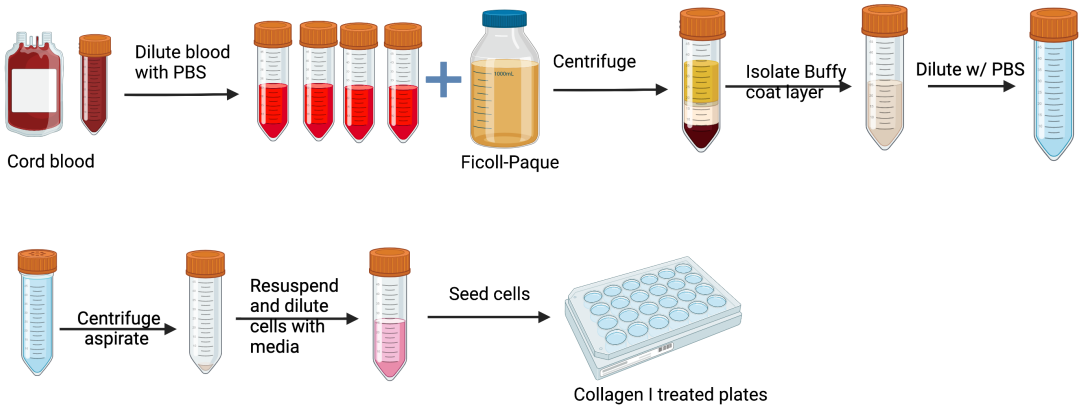


Milestone 1: Using same amount of Cas9+sgRNA for 100k and 1M Cells





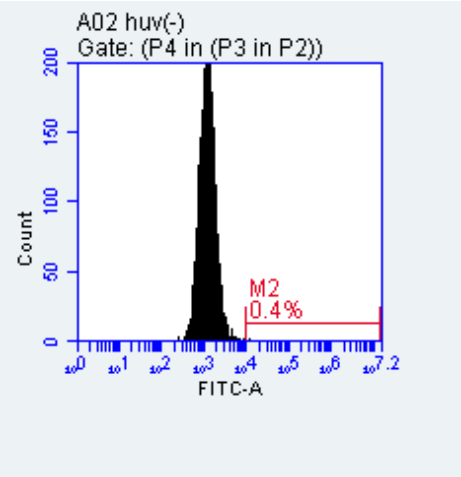
Milestone 2: Isolating and Culturing BOECs

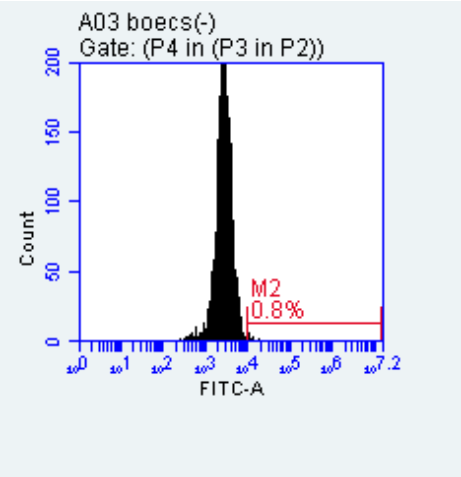


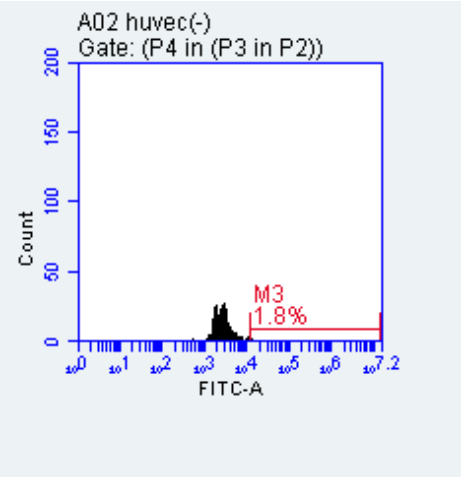
- Should start to see BOEC colonies between 14-28 days
- We're observing some cells, but they are becoming confluent further along after isolation than would be expected of ECs
- Resemble the same morphology that we see in HUVECs

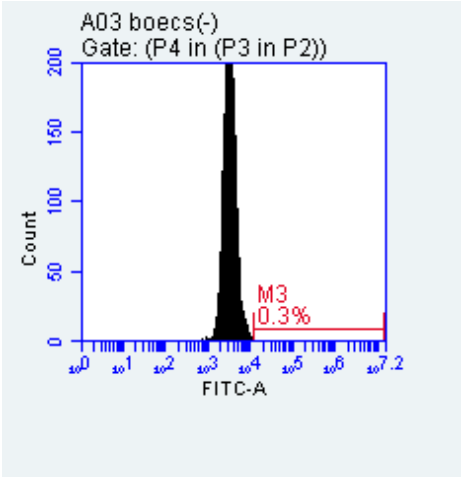
Milestone 2: Confirming Identity of Suspected BOECs

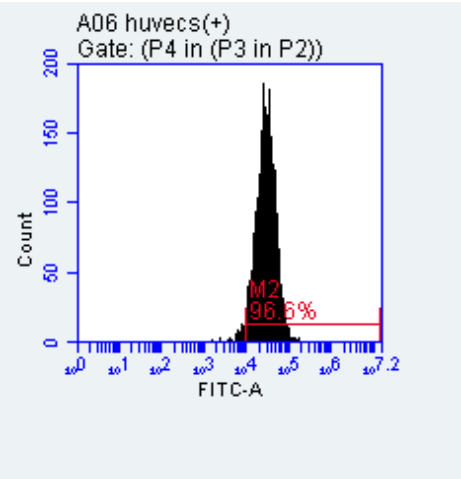
VE-Cadherin

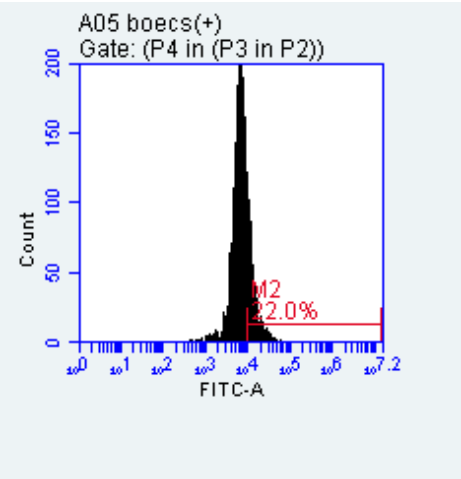


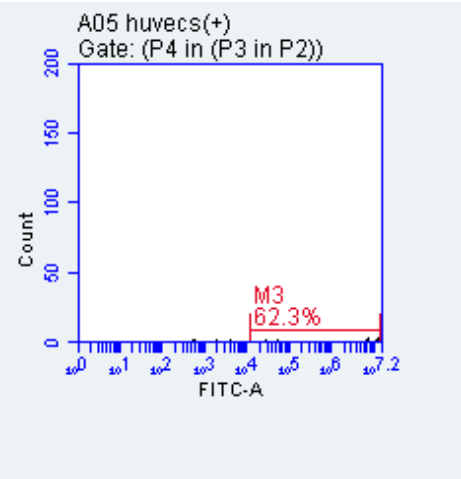


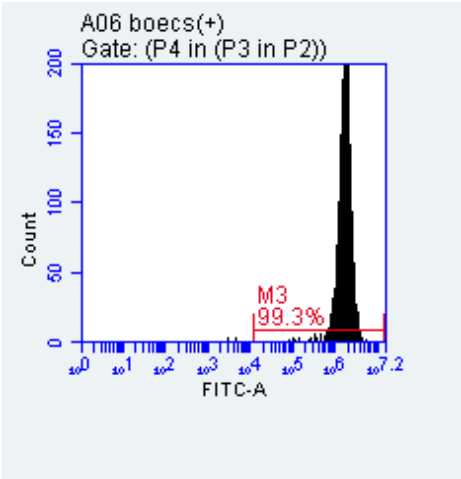






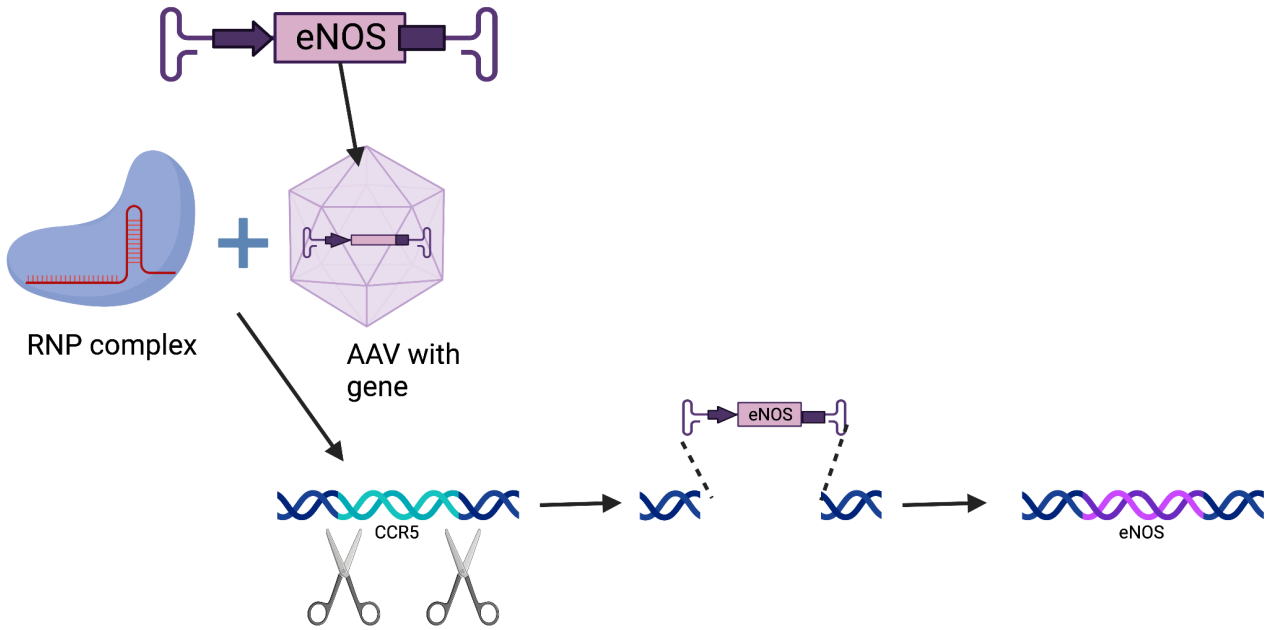






Looking Towards the Future

Milestone 3: eNOS Expression in Endothelial Cells



Summary

- **Nitric Oxide concentrations in the HUVECs is in the detectable range**
- **VE-Cadherin stains exclusively for ECs, VWF can be used as second confirmation**
- **Increasing the concentration of the secondary antibody, in VWF staining, increases the percent of endothelial cells that are stained**
- **AAV2, AAVanc80, and AAV6 are viable options for gene integration in ECs**
  - AAV2 has the highest percent of gene integration
- **sgRNA11 has a higher indel frequency than W3 and fewer editing events**
- **Using AZD and higher MOI of AAV6 produces higher frequency of targeted allele editing in ECs**
- **Editing with the same volume of RNP for 100k and 1M cells shows that 100K cells is optimal for 1uL Cas9/0.6uL guide**
- **Some confirmation that the cells we isolated are EC's**
- **There are a few options for stents and mouse models, yet to be determined which is overall better for this project**

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CIRM
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LELAND STANFORD JUNIOR UNIVERSITY
DIE LUFT DER FREIHEIT WEHT
1891

HUMBOLDT STATE UNIVERSITY
1913
ΦΩΣ ΑΛΗΘΕΙΑ

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RENDER

Citations

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