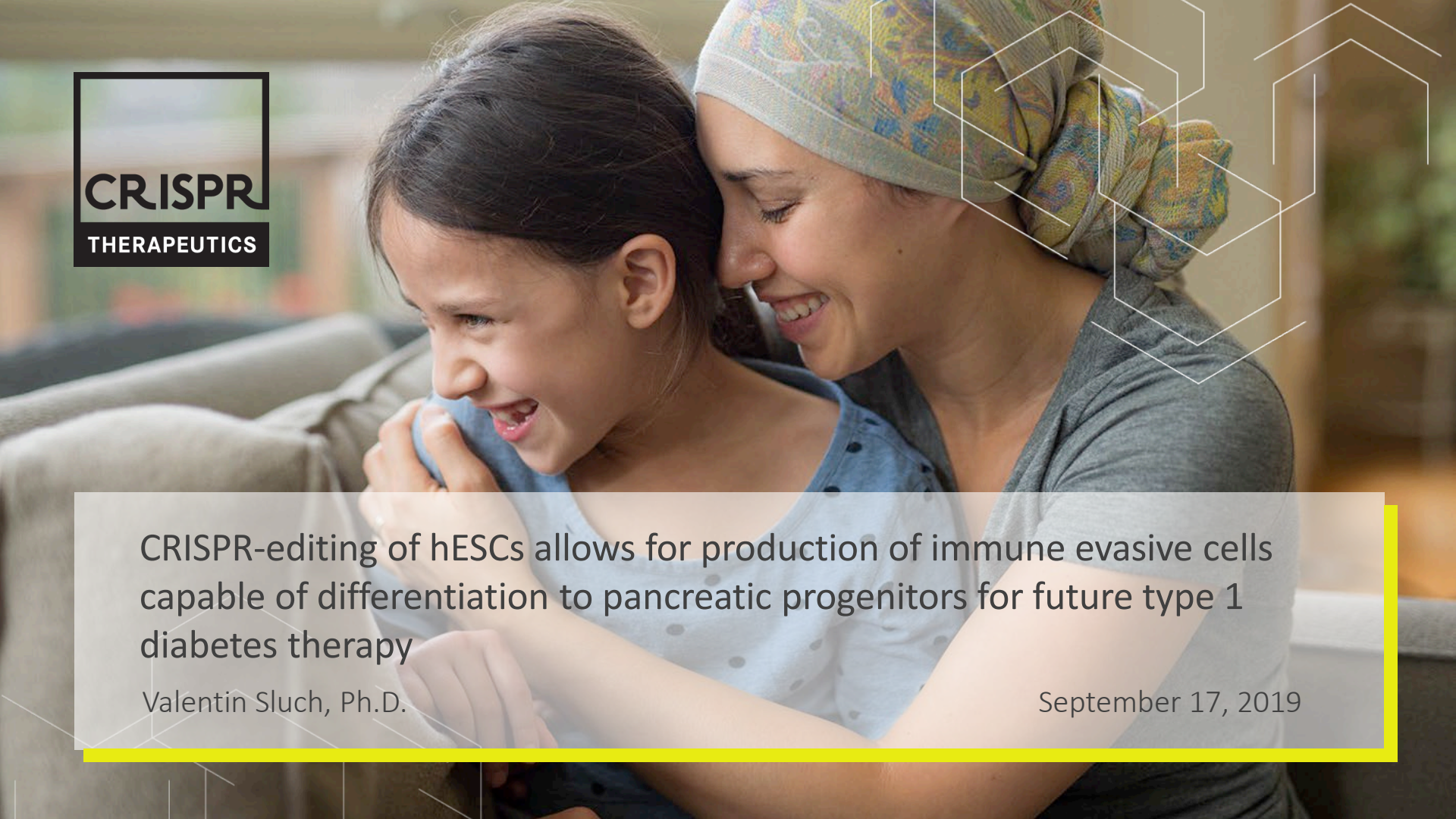


CRISPR THERAPEUTICS

CRISPR-editing of hESCs allows for production of immune evasive cells capable of differentiation to pancreatic progenitors for future type 1 diabetes therapy

Valentin Sluch, Ph.D.

September 17, 2019

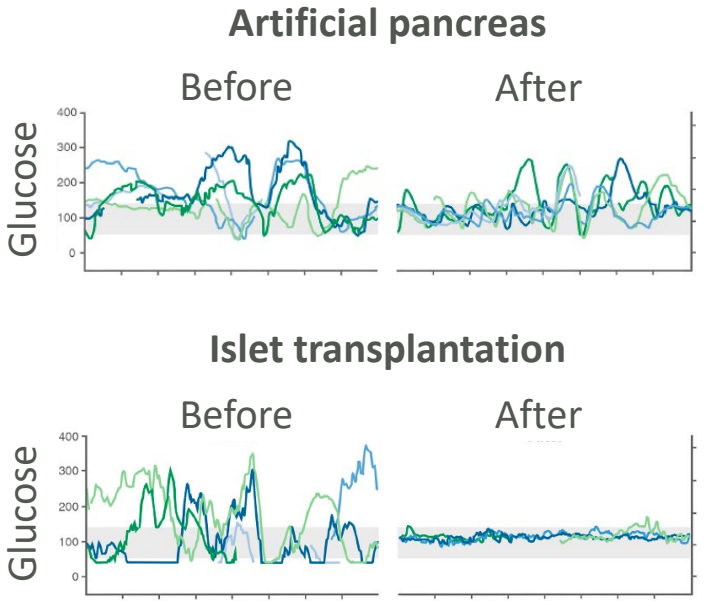
Cell Therapy for T1D Has Been Successful

Human proof of principle – Edmonton protocol:

- Currently ~1500 patients successfully transplanted with human cadaveric islets since 2000
- Insulin-independence commonly achieved for 5 years or longer; daily glucose excursions eliminated

Two main challenges:

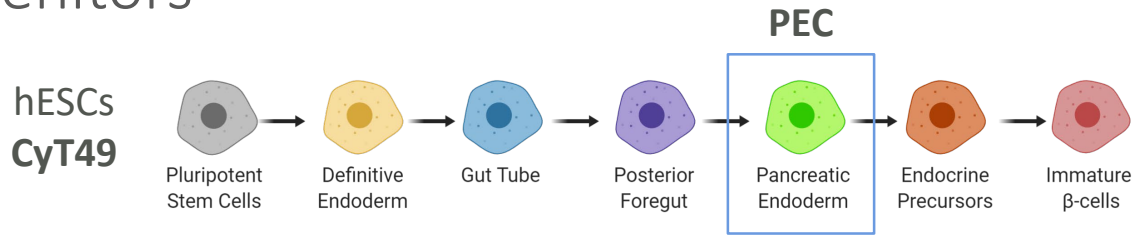
1. Very limited **supply** of suitable islets
2. Chronic **immunosuppression** is required

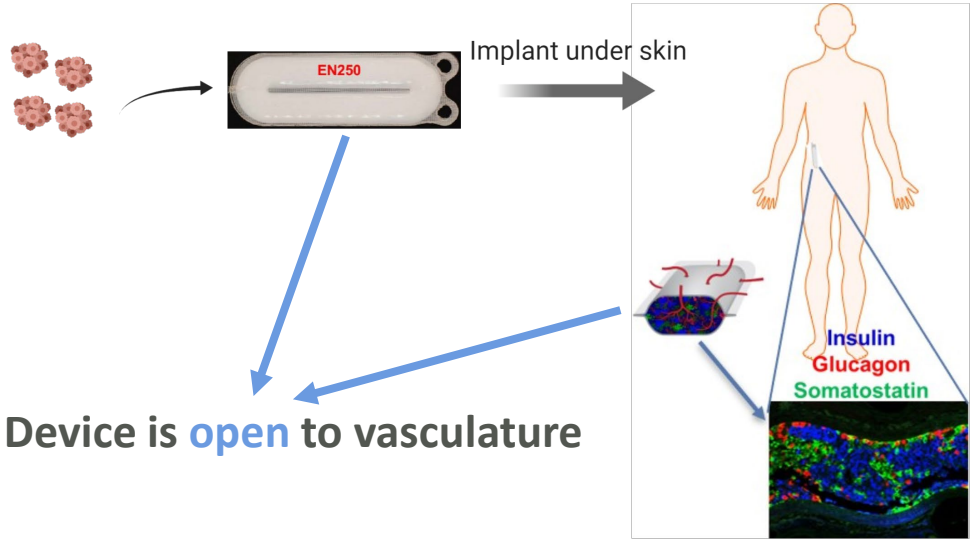


Glucose excursions eliminated after transplant

Almehthel et al., US Endocrinology, 2015
Moassesfar et al., Am J Transplant., 2016
Schuetz and Markmann, Curr Transplant Rep., 2016
Latres et al., Cell Metabolism, 2019

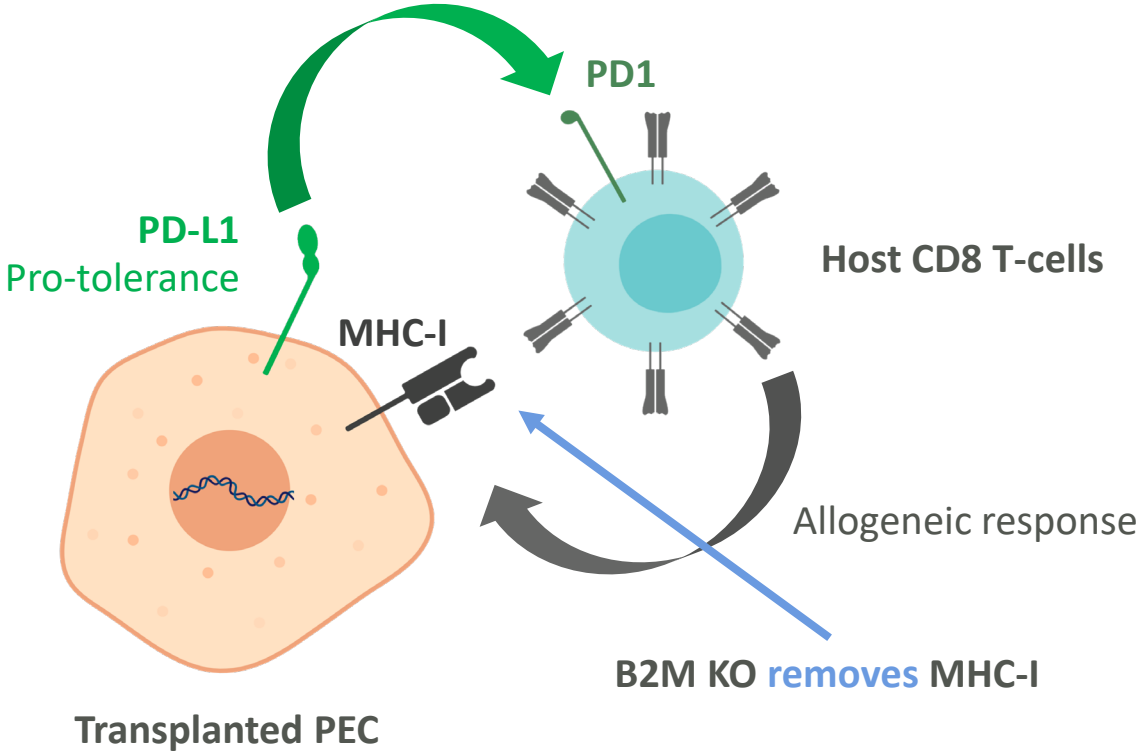
Solving the Supply Issue – Stem Cell-Derived Pancreatic Progenitors





Therapy currently in clinical trials and requires immuno-suppression

CRISPR Engineering a Universal Donor Cell Line

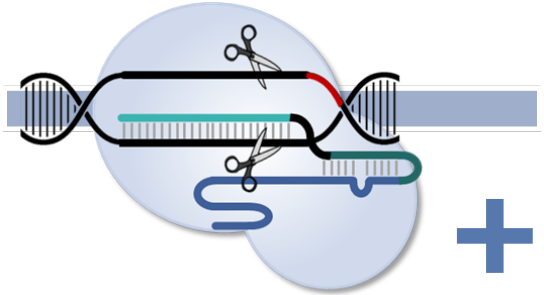


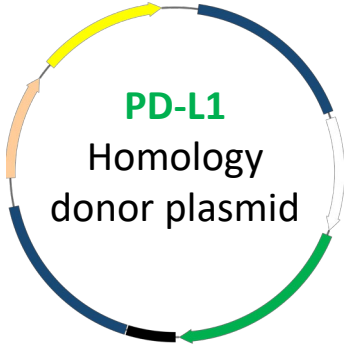
PD-L1 is protective in T1D mouse models

Li et al., Diabetes, 2015
Wang et al., Diabetes, 2008
El Khatib et al., Gene Ther., 2015

Genome Editing Strategy to KO B2M and KI PD-L1

CRISPR-Cas 9 allows for precise gene editing





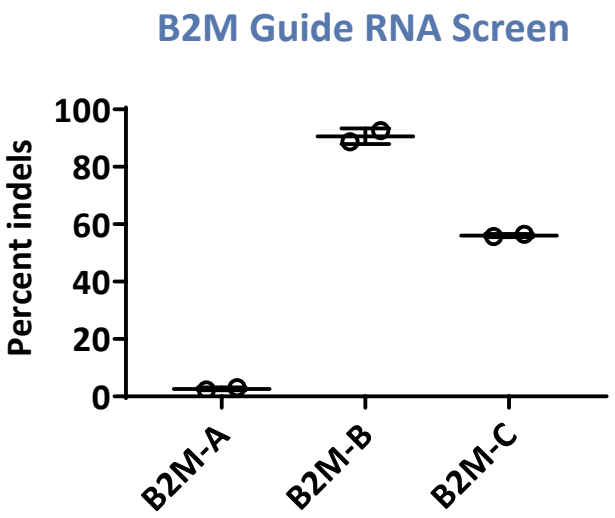
Homology-directed repair (HDR)

hESCs
CyT49

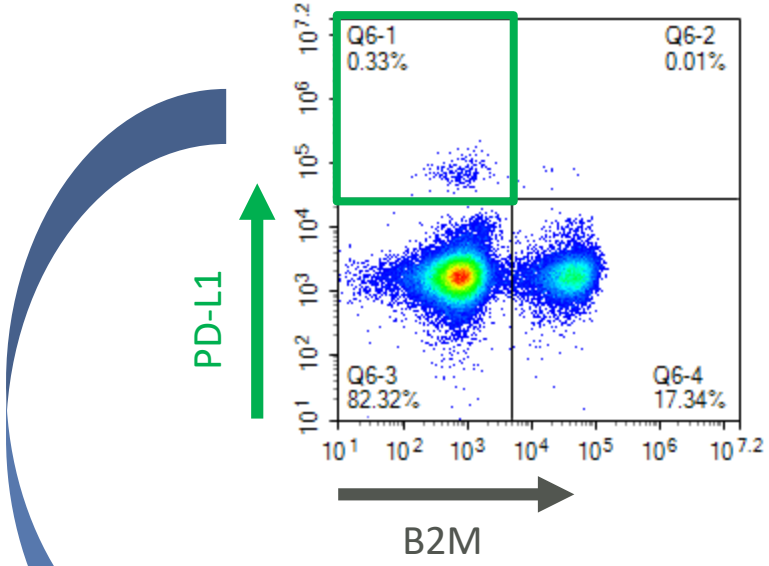
B2M negative
PD-L1 positive

B2M gRNA

Stem Cell Editing is Successful

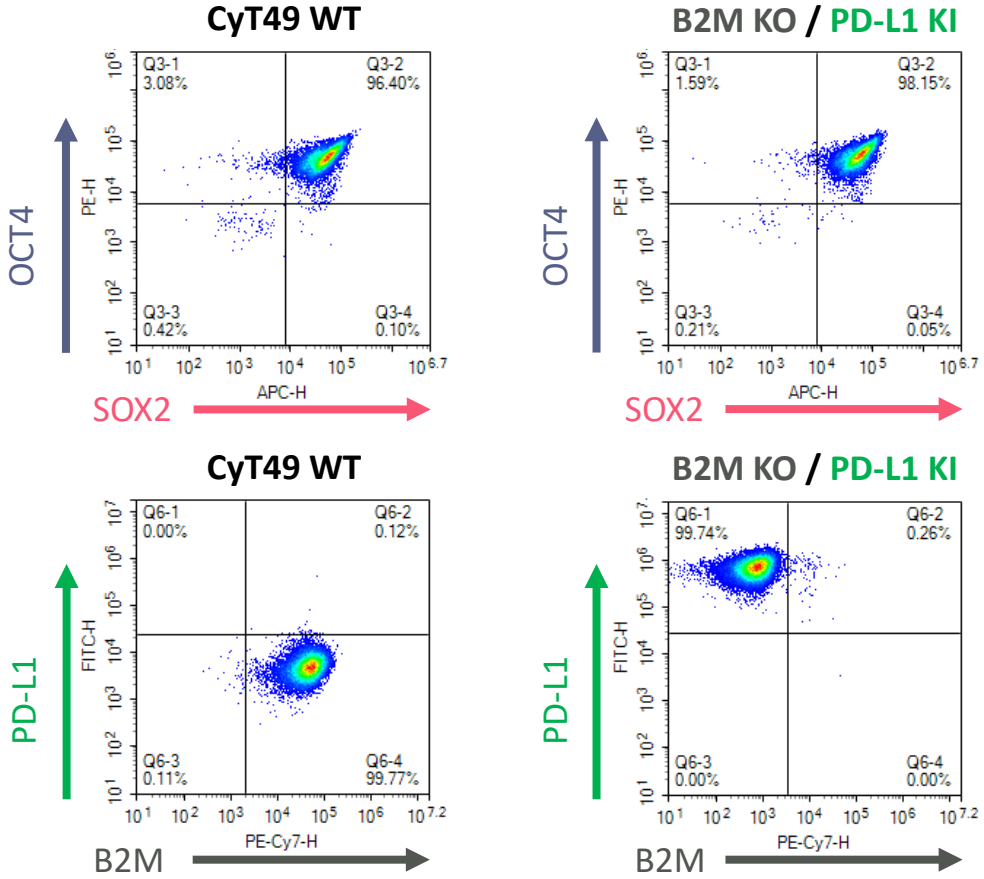


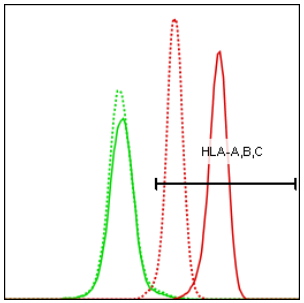
B2M-B guide produced on-target indels in stem cells with up to **90% efficiency** with **no detected off-targets**



B2M negative PD-L1 positive stem cell clones are produced

Clones Express PD-L1, Lack MHC-I and are Pluripotent

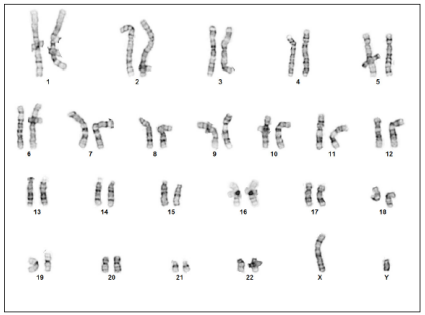




HLA-A,B,C
MHC-I

WT = red
B2M KO/PD-L1 KI = green

Dotted line = no IFNγ
Solid line = plus IFNγ

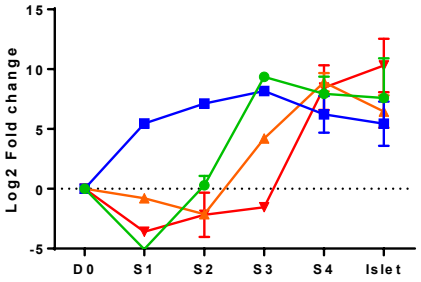


Clones have normal karyotype

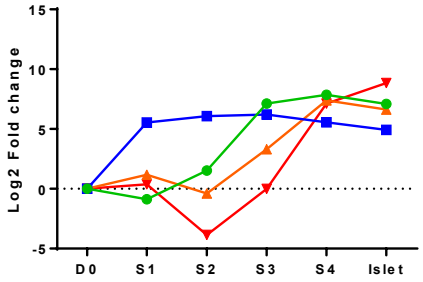
Gene Editing Does Not Affect Differentiation to PEC

RNA seq

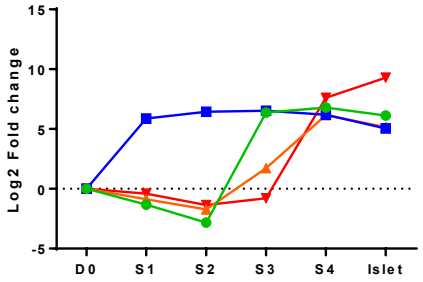
WT

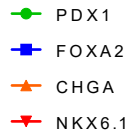


B2M KO

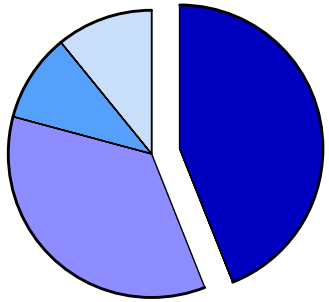


B2M KO / PD-L1 KI

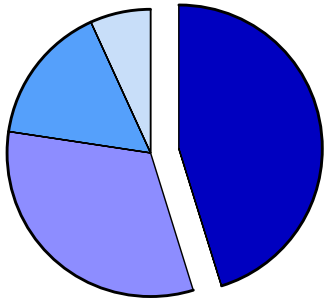




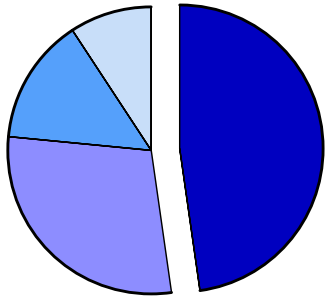
Flow cytometry



44% Progenitors
35% Early Endocrine
10% PDX1+ only
11% Others



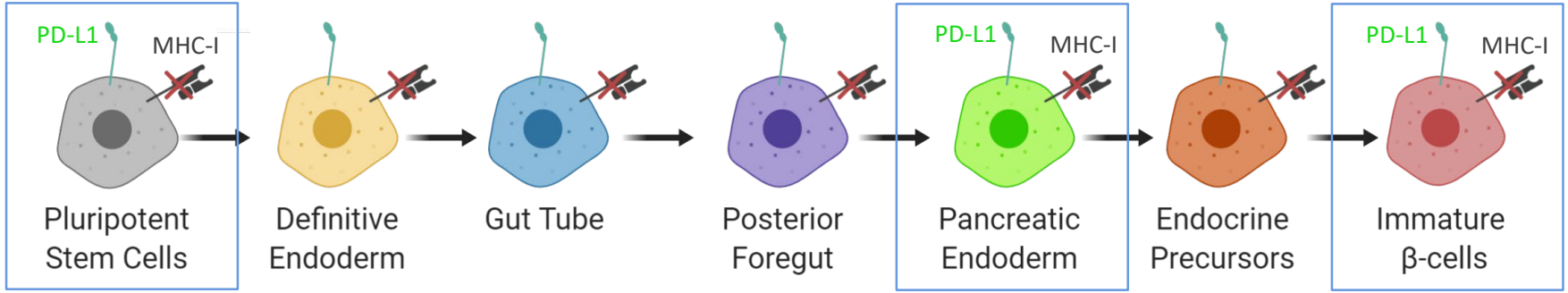
45% Progenitors
32% Early Endocrine
16% PDX1+ only
7% Others



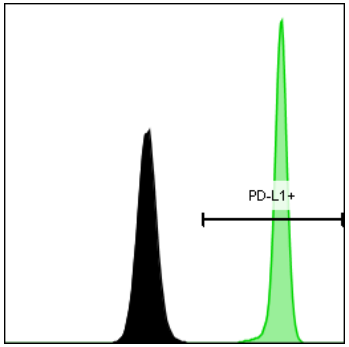
48% Progenitors
29% Early Endocrine
14% PDX1+ only
9% Others

Similar fractions of pancreatic progenitors are produced

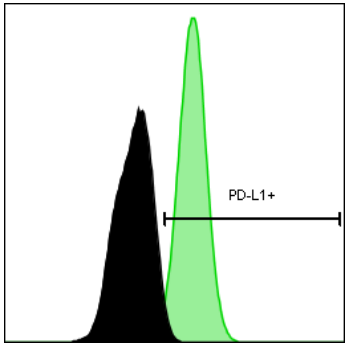
PD-L1 Expression is Retained with Maturation



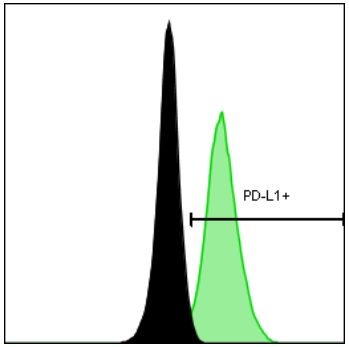
hESC



PEC/S4

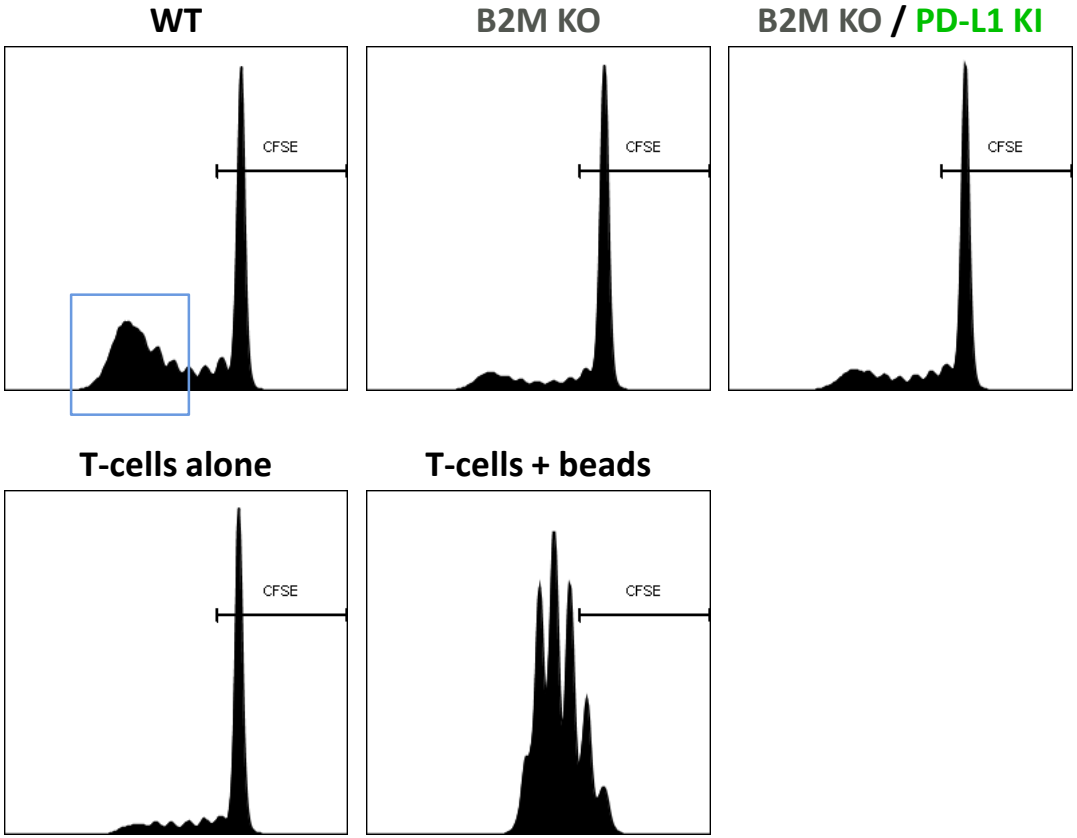


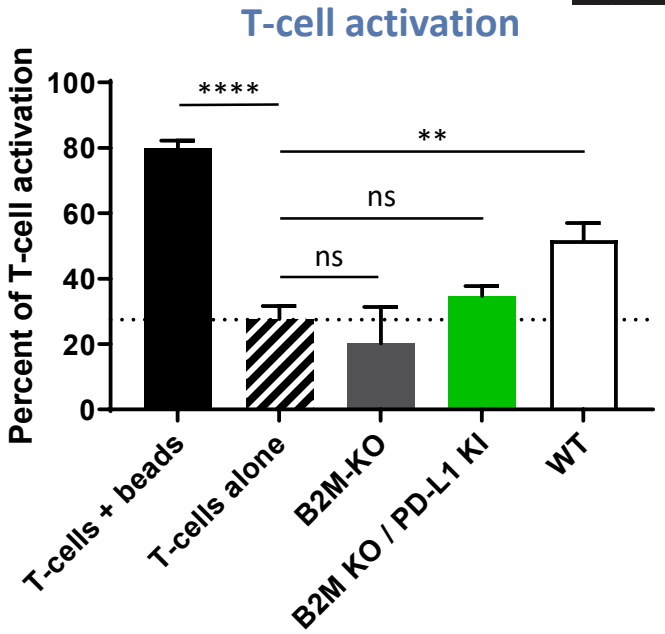
Stage 6



IgG PD-L1 KI

Gene Edited Cells Do Not Activate T-Cells





WT-PEC activated T-cells

B2M KO-PEC did not

PD-L1 KI + B2M KO-PEC did not

Summary and Future Directions

- Multiple B2M KO PD-L1 KI CyT49 hESC clonal lines have been generated
- These lines do not express MHC-I and still differentiate to PEC
- PD-L1 expression is retained with continued differentiation to immature β-cells
- Preliminary *in vitro* data suggests edited cells are immune evasive

In vivo testing of human insulin production:

- Ongoing *in vivo* transplantation study in nude rats to test glucose-stimulated insulin secretion (GSIS) from edited PEC

In vivo testing of edited PEC for immune system evasion:

- Humanized mouse models have been transplanted with edited PEC and human donor PBMCs that are allogeneic to the PEC transplant

Acknowledgements

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Alan Agulnick
Kevin D'Amour

CRISPR Therapeutics
www.crisprtx.com