ORAL PRESENTATION/POSTER ABSTRACT FORM

To be considered for oral presentation and trainee travel award: Due May 1, 2013
All Others: Due June 1, 2013

(This information may be emailed directly to Dr. Weiss (Daniel.Weiss@uvm.edu), or may be submitted on-line as you register.)

The information below will be printed in the course booklet:

1st Author Name and Institution: [1st author name]
[name of institution]

☐ Check here if 1st Author is a Trainee who wishes to be considered for oral presentation and a Trainee Travel Award. *(Previous Travel Award Winners may apply)*

Other Author Names: [author name], [author name], [author name], [author name]

☐ Check here if you wish to identify your source of funding: _____

Check Category: O Endogenous Lung Progenitors ■ ESCs/iPS O Lung Bioengineering
O EPCs/MSCs/Clinical Trials

Poster Title: Generation of human ESC and iPSC *NKX2-1* knock-in reporter lines for guiding *in vitro* lung cell differentiation

Poster boards and pushpins will be available at the Davis Conference Center. Recommended poster size is 3'x5'. Please use the standard ATS format for abstract presentation

Background: Transplantation of corrected, patient-specific, iPSC-derived lung stem/progenitor cells represents a potential therapeutic approach for various inherited monogenic lung diseases. Although our laboratory has demonstrated correction of such mutant iPSCs (e.g. Cystic fibrosis), there remains the significant challenge to derive lung-specific stem/progenitor cells suitable either to generate lung epithelial tissue in vitro or for transplantation in vivo. NKX2-1 is a key transcription factor active during embryonic development of lung, thyroid and forebrain; importantly a murine ESC NKX2-1 knock-in reporter has previously enabled purification of lung/thyroid progenitors capable of subsequent development of lung tissue (Longmire 2012). NKX2-1 is haplo-insufficient and mutations can cause brain-lung-thyroid disease.
Methods: Using TALENs, we introduced a site-specific DNA break into the second intron of NKX2-1 in hESCs/iPSCs with subsequent integration of a splice acceptor, NKX2-1 exon 3 and 2A-GFP containing donor matrix by homologous recombination. The objective was to obtain NKX2-1 specific reporter expression without creating haploinsufficiency.
Results: We have verified that expression of GFP by the targeted reporter lines accurately recapitulates NKX2-1 expression in the development of ventral forebrain neurons. Furthermore, in a step-wise differentiation assay designed to derive anterior foregut endoderm (Green 2011, Longmire 2012), we were able to generate cells co-expressing NKX2-1 and GFP. Sorted GFP positive cells exclusively express early lung/thyroid specific markers.
Conclusions: ESC and iPSC 'knock-in' reporter lines engineered with TALENs-based methods to express GFP under regulatory control of the NKX2-1 gene locus provides a powerful human platform for the potential tracking and purification of progenitors undergoing the earliest stages of lineage specification to lung, thyroid, or forebrain.