multiple transcriptional regulators, using small molecules known to control pancreatic and intestinal development, and hormone production. We chose small molecules instead of gene editing tools to avoid the potential pitfall of off-target mutagenesis.

We found that inhibition of FoxO1 in our organoid culture led to an increase in EE cell differentiation as assessed by EE-specific gene expression, with a 5-10 fold upregulation in expression of *ChgA*, *NeuroD1*, and *Neurog3* compared to whole mucosal biopsies (P<0.01 for all targets, n=3 per group). Flow cytometry data showed 6-8% of cells produced CHGA, compared to 0.2% in undifferentiated organoids (P<0.0001, n=3 per group), and the 1% typically seen in the duodenum. We also noted a corresponding increase in the production of EE hormones, including glucose-dependent insulinotropic peptide (GIP), serotonin and somatostatin, by qPCR and immunofluorescence. Analysis of conditioned media using ELISA, compared to undifferentiated organoids, revealed increased serotonin (362.6±52.3 vs 167.5±5.1 ng/mL, P=.0037, n=3 per group) and GIP (5.76±1.31 pg/mL vs undetectable, n=3 per group). Independently, upregulation of GATA4-Nkx2.5 also induced EE cell differentiation and hormone production, although to a lesser extent than FoxO1 inhibition. The exception to this was GIP, which showed increased expression and production with GATA4-Nkx2.5 compared to FoxO1 inhibition (20.8±7.4 vs 5.8±1.3 pg/mL, n=3 per group), with a much larger increase when FoxO1 inhibition was followed by GATA4-Nkx2.5 activation (53.4±4.8 pg/mL, n=3). Of note, all experiments were performed in a minimum of three human lines.

Taken together, our data have identified multiple factors, including inhibition of FoxO1 and activation of GATA4-Nkx2.5, that can drive *ex vivo* human EE cell differentiation, with unique hormone production profiles, when targeted via small molecules. This is a critical first step towards understanding the role of enteroendocrine cells in disease and the development of EE cell-based therapies.

Neuroendocrinology and Pituitary

PITUITARY TUMORS I

Pituitary Stem Cells May Drive Adenomas Causing Cushing's Disease

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SAT-304

Introduction:Cell rests of self-renewing Sox2+ progenitor cells have been identified in the normal pituitary glands[1], however their role in human pituitary tumorigenesis is not understood. Adrenocorticotropic hormone (ACTH) producing microadenomas that cause Cushing's disease frequently (~70%) lack pathogenic genetic mutations.[2] In mice, targeted expression of oncogenic β-catenin in Sox2+ cells generate microadenomas. Interestingly, the Sox2+ cells reside within the adjacent normal gland and drive adenomas in a paracrine fashion.[3] We hypothesized that Sox2+ progenitors in human pituitary gland may drive the formation of microadenomas that cause Cushing's disease (CD).

Methods:Four ACTH producing adenomas and two non-functional adenomas (NFPA) with separately annotated adjacent normal tissue (henceforward called 'microenvironment') were procured for this study (NCT00060541). We performed RNA deep sequencing (RNAseq) and compared expression of lineage-specific markers and progenitor markers using two-sample T-tests after testing for variance equality and using Welch's approximation for degrees of freedom.

Results:We found expected overexpression of ACTH preprohormone POMC in CD adenomas compared to adjacent microenvironment (?-fold) and NFPA (?-fold). The microenvironment in Cushing's disease showed increased expression of progenitor markers including *SOX2, SOX9, CDH1, GRFA2,* and *KLF4* compared with microenviroment in NFPA. Likewise, the Cushing's disease microenvironment showed increased expression of*POMC* (26.98 - fold, P = 0.004) as well as PRLR (FC 17.39, P = 0.006) and GH1 (FC 29.91, P = 0.003) implying that increased Sox2+ progenitors contribute to terminally differentiated corticotrope, lactotroph and somatotroph lineages in-vivo.

Conclusions:We report increased expression of several progenitor markers and concomitant elevation in tissues-specific markers in the microenvironment of Cushing's disease patients. Our results indicate that increased pituitary progenitors in the microenvironment of human corticotropinomas may signal in paracrine fashion and may contribute to the pathogenesis of Cushing's disease.

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Adrenal

ADRENAL - TUMORS

Status at 10 Years: Long-Term Follow-Up for a Phase 2a Study of High-Specific-Activity (HSA) I 131 Iobenguane in Patients (Pts) with Relapsed/ Refractory High-Risk Neuroblastoma

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SAT-163

Background: Metaiodobenzylguanidine (MIBG; iobenguane), a guanethidine derivative, is a substrate for norepinephrine reuptake transporter which is highly expressed on the surface of neuroblastoma cells. AZEDRA® (HSA I-131 MIBG) has been approved by the FDA for the treatment of pheochromocytoma and paraganglioma, in pts 12 years and older with MIBG avid, unresectable, locally advanced or metastatic PPGL who require systemic