Evaluation of Cases with Neuroendocrine Cell Hyperplasias for Classification as Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia (DIPNECH) and Subsequent Whole Exome Sequencing Analysis

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Background

• Neuroendocrine cell hyperplasia (NECH) occurs in the bronchial epithelium of the airways. While this can happen as a reactive response to other processes in the lung, it can also happen as a primary process that can progress to carcinoid tumor. The latter is especially common in the relatively new disease, diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH). Patients with DIPNECH develop numerous foci of NECH throughout the lungs bilaterally. These NECH foci can be throughout the medium and small airways of the lung and can lead to significant airway obstruction sometimes causing the lung to threaten respiratory failure. Additionally, these patients almost always develop carcinoid tumors often at multiple sites. Thus, the NECHs of DIPNECH represent true premalignant lesions. The malignant and obstructive complications of DIPNECH are associated with significant morbidity and mortality. To date, no treatments have been developed, and an underlying cause of the NECHs that lead to the life-threatening complications of DIPNECH has not been identified. Some patients will require lung transplantation, but the efficacy of this approach has been limited.

• We hypothesize: DIPNECH results from a somatic mutation affecting pulmonary neuroendocrine cells resulting in proliferation and widespread dispersion within the bronchial epithelium.

• This type of change has been associated with other premalignant lesions in the lung including the demonstration of common TP53 mutations in the lung squamous cell carcinoma precursor bronchial dysplasia. Our project will incorporate histologic, clinical and radiographic evaluation of candidate cases to identify patients with true DIPNECH. Multiple NECHs will be tested for TP53 mutations.

Methods

• Case Identification: All cases from 2006 – 2021 with the terms “neuroendocrine cell hyperplasia,” “diffuse idiopathic pulmonary neuroendocrine cell hyperplasia” or “DIPNECH” either in the diagnosis or diagnosis comment of surgical pathology reports were identified in the University of Colorado Cancer Center pathology archives. The first 60 cases were reviewed and 53 of these were found to have adequate tissue as well as pathologic findings that could constitute DIPNECH. Additionally, potential DIPNECH cases have been identified giving a total of 60 cases currently being reviewed for classification.

• Microdissection and DNA Extraction: Reference tissue and bronchial biopsies were used to optimize the extraction of adequate yield DNA from small airway lesions for whole sequencing (WES). Laser capture microdissection (Mison Eppendorfika) was performed on formalin fixed paraffin or OCT frozen tissue specimens. DNA extraction was carried out using a QIAamp DNA FFPE Tissue Kit (Qiagen) with or without nucleic acid release protocols (NuNAIRE). DNA numbers that ranged from 1,000 to 20,000 cells were collected. Following Qubit and Tapestation quantification, selected samples were subjected to WES and somatic variants identified following subtraction of germline polymorphisms using sequencing data from matched peripheral blood DNA. "Somatic DNA from DIPNECH-associated carcinoids will have been sequenced in specific L." 1

• Histologic and Clinico-Radiologic Review: Histologic review (H. Yu) included assessment of cellularity as a marker of potential adequacy of NEC lesions and carcinoid tumors for WES. Using number of 400X fixed paraffin blocks traversed by the lesions (~400 NEC cells per 400X field), a total of 8,000 – 10,000 cells for collection by microdissection (assuming up to 20 lesions can be used per lesion) was used to assign adequacy. Clinical chart review was performed (Y. Miller) to document the presence of neuroendocrine cell hyperplasia.

• Complete clinical and radiographic review of all cases will be performed over the remainder of 2022 with ultimate classification of all cases as true DIPNECH vs. non-DIPNECH. Of ~30 DIPNECH cases that can be carried through WES of multiple sites will be sought. If our numbers are short of this, additional cases from prior to 2021 will be obtained from the pathology archive.

• Microdissection will begin with use of a few cases with many robust (> 1.4X 400X fields) NEC lesions and collection of combined lesions in a few cases with multiple but generally small lesions. DNA yield will be assessed and adjustments to microdissection and extraction protocols will be made if necessary. Necrotic lymph node carcinoid and/or tumor (including from non-DIPNECH cases also) will be microdissected for analysis.

• WES will be performed in batches with reference sequence being provided from benign lymph node tissue. Somatic variants will be identified and assessed for commonality between DIPNECH and non-DIPNECH cases for potential unique mutation patterns.

• Additional cases. Invasive carcinoid tumors will be assessed for mutations associated with progression as compared to NECH.

Conclusions

• Target collection of 8,000 – 10,000 lesional cells should reliably provide at least 50 ng of total DNA for WES. Carcinoid tumorlets will increase yield and provide high quality WES variant detection.

• Attempting to use tissue to support WES of multiple NEC lesions is present in 48 of 60 cases (80%), and additional neuroendocrine tumors and tumors are available in all but 3 of these cases.

• In some cases abundant NEC is present but overall small size (i.e. < 1.4X 400X fields) and WES will likely require combination of lesions to support informative WES sequence data.

• >50% of cases fully evaluated for histologic, clinical, and radiographic features qualify as DIPNECH cases (1/12 with 2 of remaining 3 having features that may support DIPNECH classification).

Future Directions

• Complete clinical and radiographic review of all cases will be performed over the remainder of 2022 with ultimate classification of all cases as true DIPNECH vs. non-DIPNECH. Of ~30 DIPNECH cases that can be carried through WES of multiple sites will be sought. If our numbers are short of this, additional cases from prior to 2021 will be obtained from the pathology archive.

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References


