Evaluation of Cases with Neuroendocrine Cell Hyperplasia 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 rare disease, diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH). Patients with DIPNECH develop numerous foci of NECH throughout the lungs bilaterally. These NECH foci often involve the medium and small airways of the lung and can lead to significant airway obstruction sometimes causing life-threatening 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 signficant 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 wide dispersal 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 by whole exome sequencing as well as matched carcinoid tumorlets and tumors when present to identify any recurrent somatic mutation that may underlie DIPNECH. The identification of an underlying mutation would improve diagnosis of this condition and potentially point to a targeted therapeutic approach to treating this disease.

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 archive. The first 66 cases were reviewed and 53 of these were found to have adequate tissue as well as pathologic findings that could constitute DIPNECH. An additional seven potential DIPNECH cases have been identified giving a total of 60 cases currently being reviewed for classification. Full histologic review has been completed and lesion adequacy has been evaluated.
- 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 exome sequencing (WES). Laser capture microdissection (Nikon Eclipse/Arcturus NK) was performed on formalin fixed paraffin embedded or OCT frozen dysplastic tissue and extraction was carried out with a Qiagen DNA kit with or without carrier RNA (cRNA). Cell 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 derived monocytes. A variety of sequencing quality metrics were compared and fidelity of variant calling evaluated for cRNA vs. no cRNA extractions.
- Histologic and Clinico-Radiologic Review: Histologic review (H. Yu) included assessment of cellularity as a marker of potential adequacy of NECH lesions and carcinoid tumorlets for WES. Using number of 400X fields traversed by the lesions (~400 NECH cells per 400X field), a target of 8,000 – 10,000 cells for collection by microdissection (assuming up to 20 levels can be used per lesion) was used to assign adequacy. Clinical chart review was performed (Y. Miller) to document the presence of DIPNECH related symptoms (dyspnea, chronic cough) or abnormal pulmonary function testing results. Additionally CT exams were reviewed to document the presence of DIPNECH related diffuse multifocal nodules and/or air trapping as indicated by mosaicism.

Results

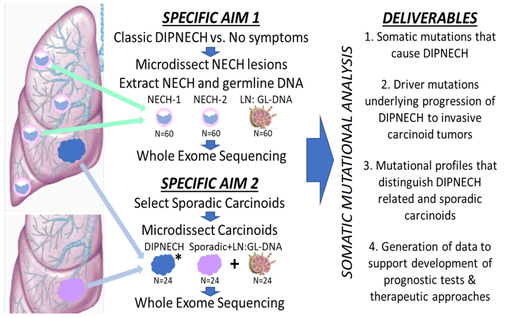

Figure 1. Schematic representation of studies planned in specific aims 1 & 2 and expected deliverables from analyses. Abbreviations - LN: GL-DNA = Lymph node derived germline DNA. *Germline DNA for DIPNECH associated carcinoids will have been sequenced in specific aim 1.

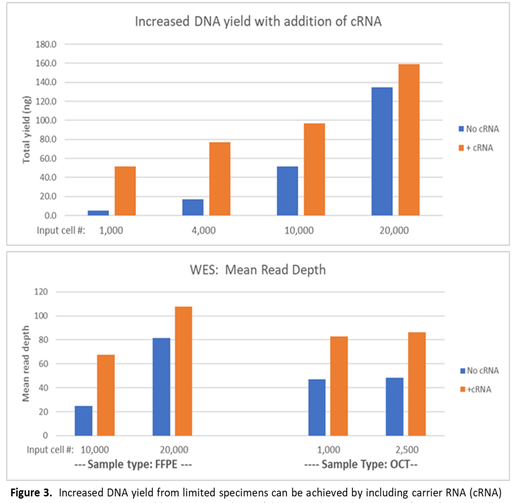

Figure 3. Increased DNA yield from limited specimens can be achieved by including carrier RNA (cRNA) in extraction protocol. A – Yields are increased 2-10 fold in samples with 10,000 or fewer lesional cells. B – Sequencing metrics are maintained or improved in samples extracted with cRNA with mean read depth approximately doubled. Improved reduction of duplicate reads as well as maintenance of variant identification were also maintained (data not shown).

Figure 4. Histologic evaluation of candidate DIPNECH cases for tissue adequacy. Two neuroendocrine cell hyperplastic (NECH) lesions or at least one NECH plus a carcinoid tumorlet are required for inclusion. Assessment of NECH size is also included with NECH that span at least one 40X field (~400 lesional cells) being considered adequate (up to 20 sections from each NECH would be used in microdissection to reach the 8,000 – 10,000 NECH cells required to give adequate DNA yield). Yellow boxes in the NECH tissue adequacy column denotes cases where no NECH spans a full 40X field, but numerous NECH would allow for combining lesions to reach adequate tissue input. Green indicates qualifying tissue is present. Red indicates adequate lesional tissue not identified.

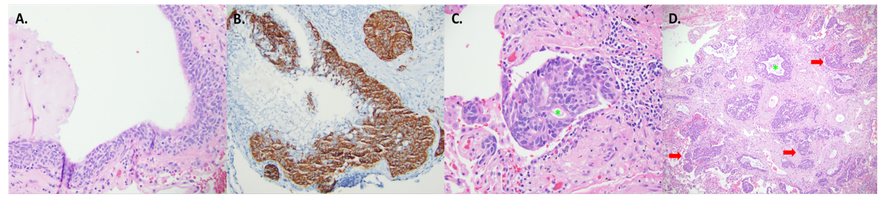

Figure 2. Histologic appearance of NECH focus with prominent nodular intra-epithelial proliferation evident on H&E stain (A) and highlighted by immunohistochemical stain for neuroendocrine marker synaptophysin (B), 200X. (C) Small airway obliteration by NECH focus with near complete obstruction of airway lumen (green asterisk), 400X. H&E image of a carcinoid tumorlet (D) showing airway with NECH (green asterisk) and invasion of peri-bronchial tissue (red arrows) by a small tumor < 0.5 cm in greatest diameter, 100X.

NETRF DIPNECH Pt ID	Include in analysis	NECH tissue adequacy	Carcinoid tumorlet(s) present	Carcinoid tumor(s) present
ND001				
ND002				
ND003				
ND004				
ND005				
ND006				
ND007				
ND008				
ND009				
ND010				
ND011				
ND012				
ND013				
ND014				
ND015				
ND016				
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ND058				
ND059				
ND060				

Pathologic Features (≥ 2 present)	Clinical Features* (≥ 2 present)	Radiologic Features (≥ 1 present)
≥ 4 NECHs OR # NECH per tissue section > 0.5	Dyspnea	Multiple nodules
Airway obstruction by NECH	Persistent cough	Parenchymal mosaicism
Multiple carcinoid tumors or tumorlets	Pulmonary function test abnormalities	Diffuse, bilateral changes

Table 1. Diagnostic features of DIPNECH. Qualifying findings expected in two of three categories. *Respiratory symptoms must not be attributable to another respiratory process; some symptoms, if convincing, may qualify patient as an isolated finding (i.e. persistent cough for 6 months in a never smoker without other causes).

NETRF DIPNECH Pt ID	DIPNECH case	Pathology c/w DIPNECH	Clinical c/w DIPNECH	CT c/w DIPNECH
ND001				
ND002				
ND003				
ND004				
ND005				
ND006*				
ND007				
ND008				
ND009*				
ND010				
ND011				
ND012				

Figure 5. DIPNECH cases as determined by combined histologic, clinical and radiographic (CT) assessment. DIPNECH status is established by meeting the criteria listed in table 1 in at least two of the three categories. *ND006 was not considered DIPNECH by clinical/radiographic evaluation but may qualify based on histologic findings. *ND009 showed possible DIPNECH by clinical radiographic features, but also shows DIPNECH features histologically so likely qualifies as DIPNECH.

Conclusions

- Target collection of 8,000 – 10,000 lesional cells should reliably provide at least 50 ng of total DNA for WES
- Carrier RNA significantly increases yield and provides quality WES variant detection
- Adequate lesional tissue to support WES of multiple NECH is present in 48 of 60 cases (80%), and additional carcinoid tumorlets and tumors are available in all but 3 of these cases.
- In some cases abundant NECH are present but all of small size (i.e. < 1.0 400X fields) and will likely require combination of lesions to support informative NECH sequence data.
- >50% of cases fully evaluated for histologic, clinical and radiographic features qualify as DIPNECH cases (7/12 with 2 of remaining 5 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 case as true DIPNECH vs. non-DIPNECH. A goal 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 2006 will be obtained from the pathology archive.
- Microdissection will begin with use of a few cases with many robust (i.e. > 1.0 400X fields) NECH 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. Lymph node and carcinoid tumorlet 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 and within DIPNECH cases and for potential unique mutation patterns versus non-DIPNECH cases. Invasive carcinoid tumors will be assessed for mutations associated with progression as compared to NECH.

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