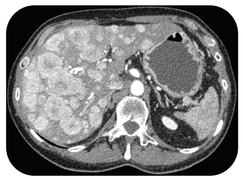
Identifying Biomarkers for Prognosis and Treatment Selection in Metastatic Neuroendocrine Tumors

Daniel Ackerman[1,2], Elise R. Seyferth[1,2,3] , Wuyan Li[1,2] , Daniel DePietro[1,2,3] , Abashai Woodard[1,2,3] , Michael Soulen[1,2,3] , Terence P. F. Gade[1,2,3,4]

[1]Penn Image-Guided Interventions Lab, University of Pennsylvania; [2]Department of Radiology, Hospital of the University of Pennsylvania;
[3]Division of Interventional Radiology, Department of Radiology, Hospital of the University of Pennsylvania; [4]Department of Cancer Biology, University of Pennsylvania

Background



Ronot et al. (2017)

Liver metastases are common in neuroendocrine neoplasms (NEN) and are often treated using locoregional therapies, including transarterial chemoembolization (TACE). While effective in many patients, treatments are rarely selected based on molecular tumor features and clinical outcomes are highly variable. Our study aims to identify urgently needed biomarkers of disease progression and treatment response to improve treatment selection and facilitate the development of new therapies for metastatic NEN.

Past molecular profiling studies of NEN have relied on short-read sequencing, which is well suited for detecting small genetic alterations such as single nucleotide variants but far less sensitive for the detection of larger, structural variants (SVs). As a result, the role of SVs remains poorly understood despite their recognized role as oncogenic drivers. To address this gap, our research employs a combination of optical genome mapping (OGM) and other molecular profiling methods to study a cohort of NEN liver metastases.

Patient Characteristics

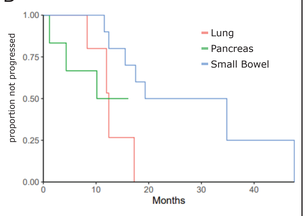
We collected biopsy tissue from liver metastases for 30 neuroendocrine cancer patients (11 Female, 19 Male). The anatomic locations of primary tumors included in small bowel (n=12), pancreas (n=8), lung/bronchus (n=5), colon/rectum (n=3) and two cases with unknown primary sites. Biopsies were collected from treated liver tumors at the time of TACE treatment.

Figure 3
(A) Summary of patient characteristics and pathology findings by primary subgroup.
(B) Kaplan-Meier plot of time to local progression for the treated liver tumor. Cases were censored at the date of last follow-up.

A

	Median Age (Q1-Q3)	Differentiation Well/Poorly	Grade 1/2/3	KI67 (Q1-Q3)
Colon/Rectum	66 (66-68)	3/0	0/3/0	5 (4-6)
Lung/Bronchus	70 (68-73)	5/0	0/4/1	12 (5-16)
Pancreas	70 (61-75)	7/1	1/4/3	24 (7-36)
Sm. Bowel	65 (58-72)	12/0	3/8/1	8 (4-8)
Unknown	66 (62-69)	2/0	0/2/0	11 (9-12)
Total	68 (59-73)	29/1	4/21/5	13 (5-14)

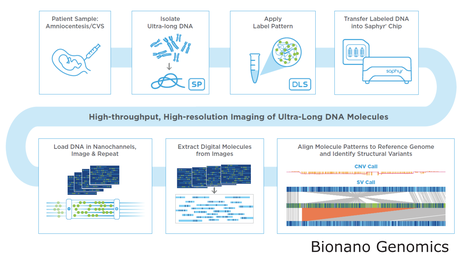
B



Optical Genome Mapping

Steps:

1. Extracting high molecular weight (HMW) DNA;
2. Enzymatically labeling the HMW DNA with fluorescent tags at specific sequence motifs abundantly distributed across the genome;
3. Loading DNA onto a microfluidic chip, where it is linearized in nanochannels;
4. Capturing high-resolution images, by recording the distinctive fluorescent patterns along the stretched DNA molecules;
5. Generating optical maps that are algorithmically aligned with reference genome maps.



Bionano Genomics

By mapping the entire genome with high-resolution imaging, OGM provides a comprehensive, genome-wide approach to the detection of structural variations such as deletions, insertions, and translocations.

Results

OGM identifies extensive structural variation in NET samples

Genomic rearrangements indicating chromothripsis are common in pancreatic NEN (pNEN) samples

- see chr 2, Figure 4B showing a pattern of focal translocations and fluctuations between different copy number states, indicating chromothripsis

Inter- and intrachromosomal translocations are commonly seen in NET samples

- see Figure 4A (middle) showing the number of translocations and Figure 4C showing the frequently affected chromosomes

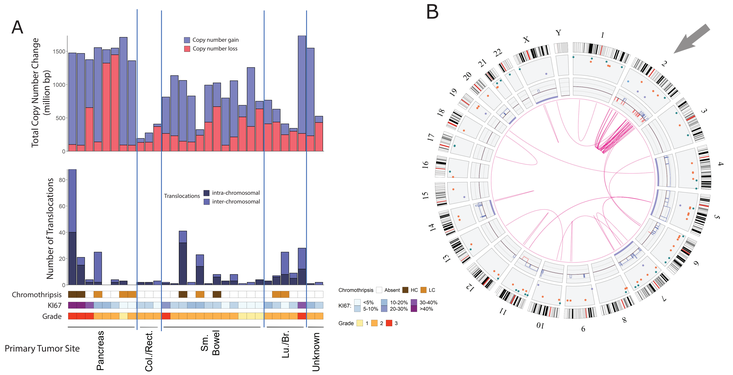


Figure 4 (A) (Top) Total CN gains and losses across samples. (Middle) Total number of translocations per sample. (Bottom) Samples showing evidence of chromothripsis (HC = high confidence; LC = low confidence), KI67 index and tumor grade from pathology reports (B) Circos plot showing cytoband (outermost), SV, copy number and translocation (innermost) tracks for S12.

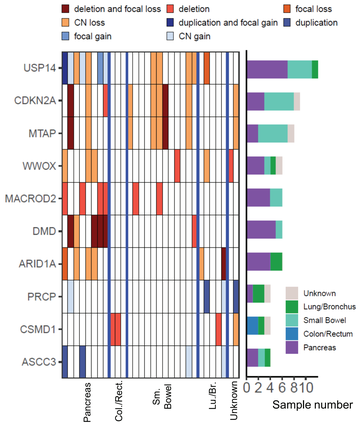


Figure 5: Oncogenes and tumor suppressor genes affected by SVs

OGM identifies subtype-specific structural variations

- see DMD, ARID1A, CSMD1

Clinically meaningful biomarkers are frequently affected by structural variation

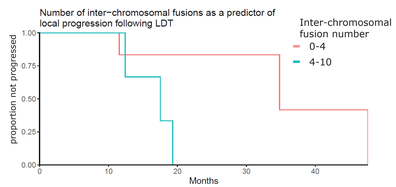
- Loss of MTAP sensitizes cancer cells to PRMT5 inhibitors and other therapeutics
- Mutations in ARID1A are known to correlate with treatment response
- CDKN2A loss can sensitize cells to CDK inhibition

OGM detects structural variants at fragile genomic sites (see *DMD, MACROD2,* and *WWOX*)

- a high frequency in pNENs points to genomic instability as a critical factor in this subtype
- OGM is ideally suited for detecting variations in challenging genomic regions, which include fragile genomic regions.

In Progress

1. Associating genomic features with clinical outcomes for liver directed therapy



- We are generating a carefully curated clinical response dataset including measures of local progression following locoregional treatment of liver metastases

- Testing the association of local treatment response with molecular features uncovered a potential relationship between the number of interchromosomal translocations and treatment response in small bowel NETs

2. Expanding genetic dataset to include additional data types

- Obtaining single nucleotide variant (SNV) alterations and additional copy number data through targeted sequencing of a panel of genes frequently altered in solid tumors using the Illumina TSO500 assay.

- Examining additional genomic features, such as alternative lengthening of telomeres (ALT) positivity, using targeted assays.

3. Validating alterations in known tumor suppressors and biomarkers using orthogonal assays

4. Expanding the patient cohort to include primary tumors, thereby improving the statistical power for associating molecular features with clinical outcomes.

Conclusions

Neuroendocrine neoplasms carry extensive structural variation and exhibit subtype-specific genomic changes

- pNEN liver metastases show high levels of aneuploidy and chromothripsis, while siNETs, colon and rectal NET liver metastases show much lower frequencies of these alterations

- subtype-specific gene loss (eg. *DMD, ARID1A*) suggests different oncogenic pathways are driving tumor development

OGM is a highly sensitive and cost effective approach for genome wide structural variant detection

- While unable to detect small alterations, OGM surpasses traditional sequencing methods in identifying large-scale insertions, deletions, inversions, and translocations.

- Combining OGM with short read sequencing is a highly sensitive approach to whole genome profiling of somatic mutations in cancer, providing a richer and more detailed understanding of the cancer genome's complexity.

Penn IR | PIGI LAB Penn Image-Guided Interventions Laboratory