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# Shallow Nanopore sequencing reveals genomic and epigenomic signatures of circulating tumor DNA in neuroendocrine tumor patients

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## Background

**Shallow whole-genome sequencing** (sWGS) can detect circulating tumor DNA (ctDNA) using **copy number** and **fragmentomic** signatures.

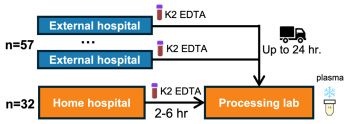
**Oxford Nanopore (ONT)** sWGS is PCR-free and adds native detection of **5-methylcyotsine (5mC)** and **5-hydroxymethylcytosine (5hmC)**

ONT sWGS is already used in Neuro-oncolgy to provide **rapid**, near-patient tumor biopsy classification in **2 hours or less**.

## Methods

**89 neuroendocrine tumor (NET) patients** from a clinical trial[1] requiring a well-differentiated NET tumor grade 1-3 from the pancreas or lung.

10 mL of whole blood **prior to any treatment** collected in K2 EDTA tubes.




**32 *Home hospital*** blood samples were transported within the same medical center for pre-analytical processing within a maximum of 6 hours.

**57 *External hospital*** samples were transported from other cities in France, with transfer at room temperature taking up to 24 hours.

**1-5mL of plasma** was used to extract 1.5-100ng of cfDNA per sample using the QIAamp® Circulating Nucleic Acid Kit (Qiagen, catalogue # 55114).

ONT library construction with Native Barcoding Kit v. 14 (SQK-NBD114-96), and sequencing using PromethION R10.4.1 flow cells on a P2 Solo sequencer at **1-2x genomic sequencing coverage.** Joint 5mC/5hmC modification calling performed by Dorado.

## Results - Effects of prolonged blood transit

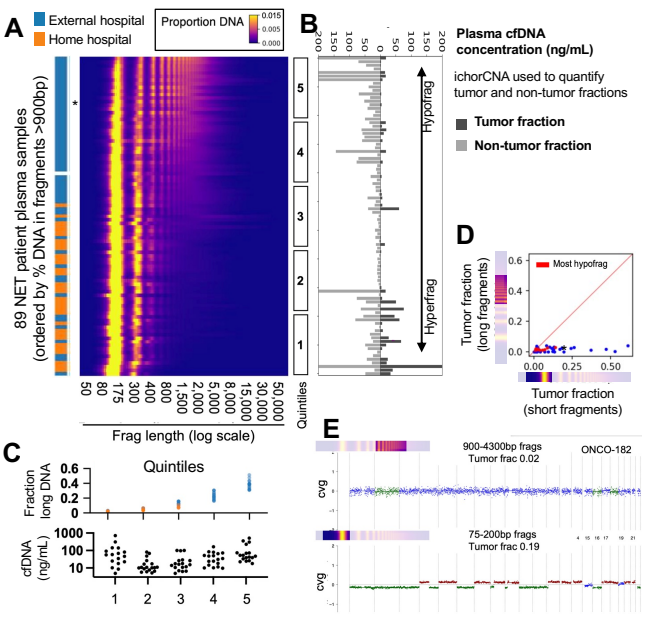


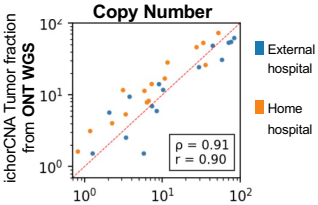

**Figure 1: Prolonged blood transit resulted in long fragments lacking cancer DNA. (A)** Samples were ordered by their fraction of cfDNA contained in long (>900bp) fragments, revealing a high percentage in many "external hospital" samples. **(B-C)** Hypofragmented samples had exessive cfDNA concentrations but very little tumor fraction as estimated by ichorCNA. **(D-E)** Long fragments were devoid of cancer DNA by ichorCNA. *marks same sample (ONCO-182) in A,D, and E. All plots comes from our preprint[2].

## Conflicts of interest

BPB has consulted for VolitionRx. CW and TKK are employees and hold stock.

## Results – Multiple ONT features reveal ctDNA fraction

ONT genomic features were **extracted from 75-200 bp fragments only**, and compared to Variant Allele Fraction (in percentages) **from matched Illumina cancer gene panel sequencing** for 29 cases that had a clonal somatic MEN1 mutation. **Log scale** highlights differences among low tumor fraction samples (1-10%)



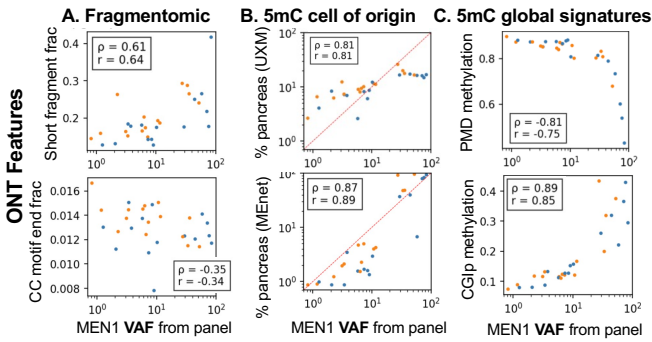
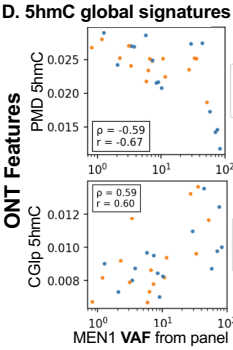




**Figure 2: Multiple ONT features associated with ctDNA fraction.**

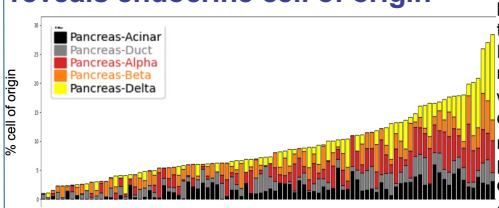
**Fragmentomic features (A)** were taken from[3]. Cell of origin was calculated from DNA methylation (5mC) using UXM[4,5] or MEnet[6].

For **global 5mC signatures (B)**, Partially Methylated Domain (PMD) methylation was averaged at CpGs from [7] and CpG Island Promoter Methylation (CGIp) was averaged at CpGs from a subset of CGIps from [8] that are hypermethyated in neuroendocrine cancer.

The same PMDs and CGIp CpGs are used to calculate **(C) global signatures for 5-hydroxymethylcytosine (5hmC)**.




## Results – Methylation-based deconvolution reveals endocrine cell of origin




Individual sorted pancreatic cell types from the Hebrew University methylation atlas[4] were used for deconvolution, revealing a **primarily endocrine cell of origin**, as expected for this cancer type.

## Conclusions

- Cellular release from **prolonged blood processing resulted in a hypo-fragmentation pattern** and higher cfDNA concentrations
- **Hyper-fragmented cases also had high cfDNA concentrations**, likely the result of cancer-related inflammation[9]
- **Multiple independent ONT features agreed with deep mutational sequencing** – could be combined for higher accuracy
- **Global loss of 5hmC** at PMDs was among the best predictors of ctDNA fraction.
- **Methylation-based cell-type deconvolution** revealed endocrine cell of origin

## References

**1** Walter et al., *JCO* 2025 (10.1200/JCO.23.02724), **2** Berman et al., *bioRxiv* 2025 (10.1101/2024.05.02.592182), **3** Katsman et al. *Genome Biol.* 2022 (10.1186/s13059-022-02710-1), **4** Unterman et al., *Genome Biol.* 2024 (10.1186/s13059-024-03275-x), **5** Loyfer et al., *Nature* 2023 (10.1038/s41586-022-05580-6), **6** Yasumizu et al., *NAR Cancer* 2024 (10.1093/narcan/zcae022), **7** Zheng et al. *Genome Biol.* 2023 (10.1186/s13059-023-03035-3), **8** Zheng et al., *Nat. Comm.* 2021 (10.1038/s41467-021-22720-0), **9** Curtis et al., *PNAS* 2025 (10.1073/pnas.2426890122)