Nanopore-based intraoperative brain tumour diagnosis

The harbinger of near-patient, ultra-rapid cancer genomic testing in the NHS

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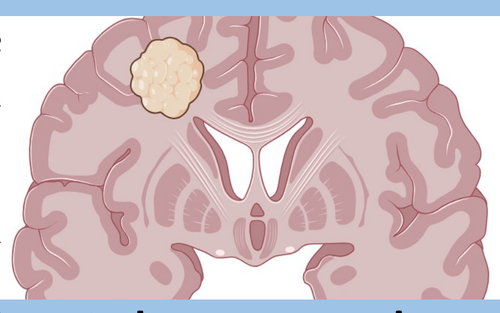
1. Rapid diagnosis is essential for cancer care

Intraoperative

Surgical decision making

Extent of resection



Post-operative

Prognosis

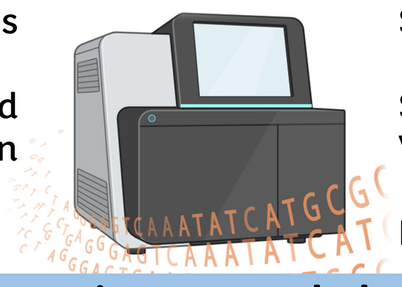
Treatment plan

Targeted therapy

...and increasingly relies on genomic testing

Copy Number Changes

Methylation-based Classification



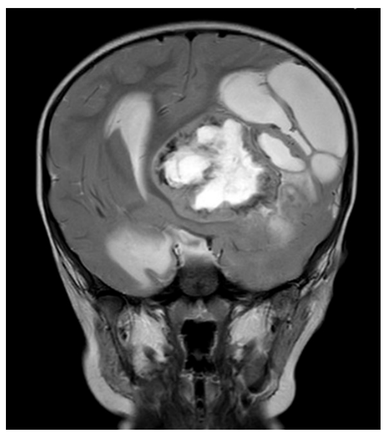
Structural Variation (SVs)

Small-nucleotide Variation (SNVs)

Fusions

2. Current testing takes several weeks, with grave consequences for patients and the health system

11-month old child with brain tumour



Day 0
Intraoperative pathology opinion **non-diagnostic**

Day 7
Sample sent for genomic testing.

Day 14
Classified as **Ependymoma, *RELA*-fused**

Day 21
Full report issued by pathology team

33 days avg. NHS time to diagnosis

Day 0
Subtotal resection; await definitive diagnosis

Day 28
MDT Discussion

>1 month
Reoperation

Delayed diagnoses matter

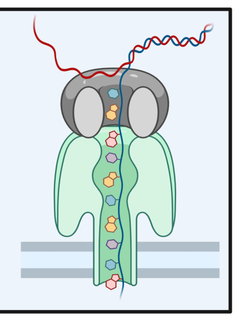
- Many vital clinical decisions must await diagnosis
- Deferred initiation of radio- & chemo-therapy
- Repeat discussion at MDT
- Weeks of uncertainty and stress for patients
- Access to clinical trials requires complete diagnosis
- Delayed discharge and wider system inefficiencies

3. We present an ultrafast sequencing assay, making *intraoperative* diagnosis a reality

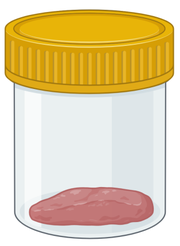
We developed a novel, nanopore-based sequencing assay which can deliver **methylation-based classification under 2 hours** and **complete molecular profiling within 24 hours**. We sequenced 100 brain tumour cases, evaluating performance against NHS standard-of-care testing.

Why Nanopore sequencing?

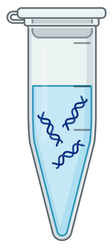
1. Can detect both DNA sequence and modifications
2. Data can be analysed live, in real-time
3. Long reads aid detection of complex genetic changes
4. Important genes of interest can be specifically targetted
5. The device has a small laboratory footprint and is low cost



1. Sample is received fresh direct from operating room.



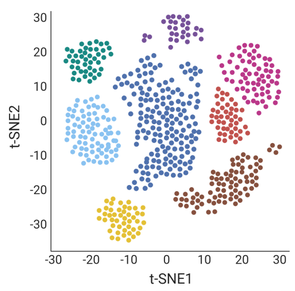
2. DNA is quickly purified from the tumour tissue.



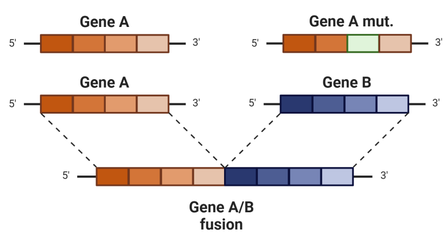
3. We prepare DNA for sequencing using a tailored protocol incorporating a rapidly-acting fragmentation enzyme.

4. Sequencing starts and data is analysed in real-time.

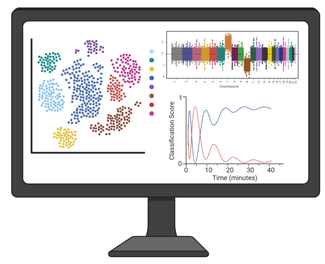
5. Classification compares tumour methylation signature against reference brain tumour data.



6. Sequencing continues, generating additional data to detect events such as gene mutations and fusions.



7. We visualise live results in a web-based tool, enabling viewing by the neuropathologist.



0hrs Receipt of sample

30min DNA extraction

60min Sample preparation

90min Commence sequencing

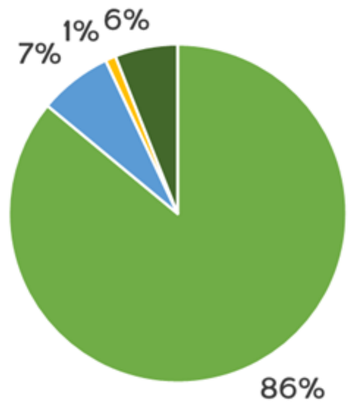
2hrs Methylation classification

24hrs Full molecular profiling

We estimate **per-patient cost at £400**, a figure comparable to the cost of methylation array alone. However, by integrating further molecular testing that is currently performed by multiple tests into a single assay, our pipeline is **cost-saving, by sparing the need for any additional ancillary testing**.

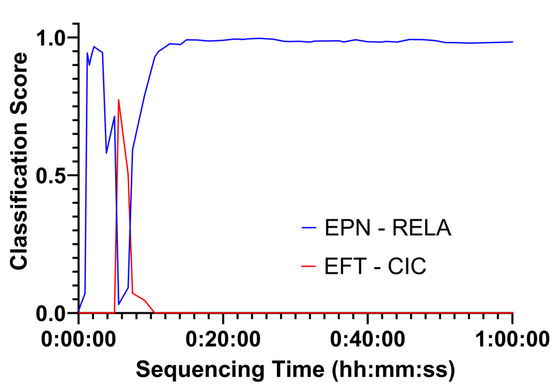
4. This pipeline is reliable and readily adoptable in the NHS

100 Cases: ~90% Concordance



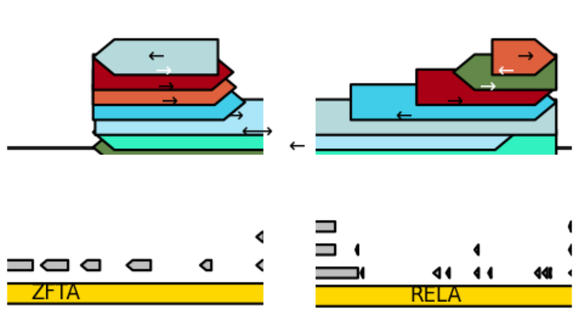
- Concordant with WHO diagnosis
- Novel entities not in classifier version
- Sampling error
- Unclassifiable by array and nanopore

Intraoperative Diagnosis



Nanopore sequencing quickly classified the above case as "Ependymoma *ZFTA::RELA* fused". Intraoperative diagnosis would have spared the patient a second operation.

Full Molecular Profile at 24hrs



Continued sequencing can detect additional events such as SNVs, SVs, and CNVs, that further aid diagnosis. For example, data confirming a *ZFTA::RELA* fusion event in this ependymoma, consistent with the classification.

Next Steps

Early adoption has been encouraging, with testing now ongoing at **multiple hospitals within the UK.**

Funding for a multicentre clinical trial is required to embed the technology within the NHS and further validate its clinical use.

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