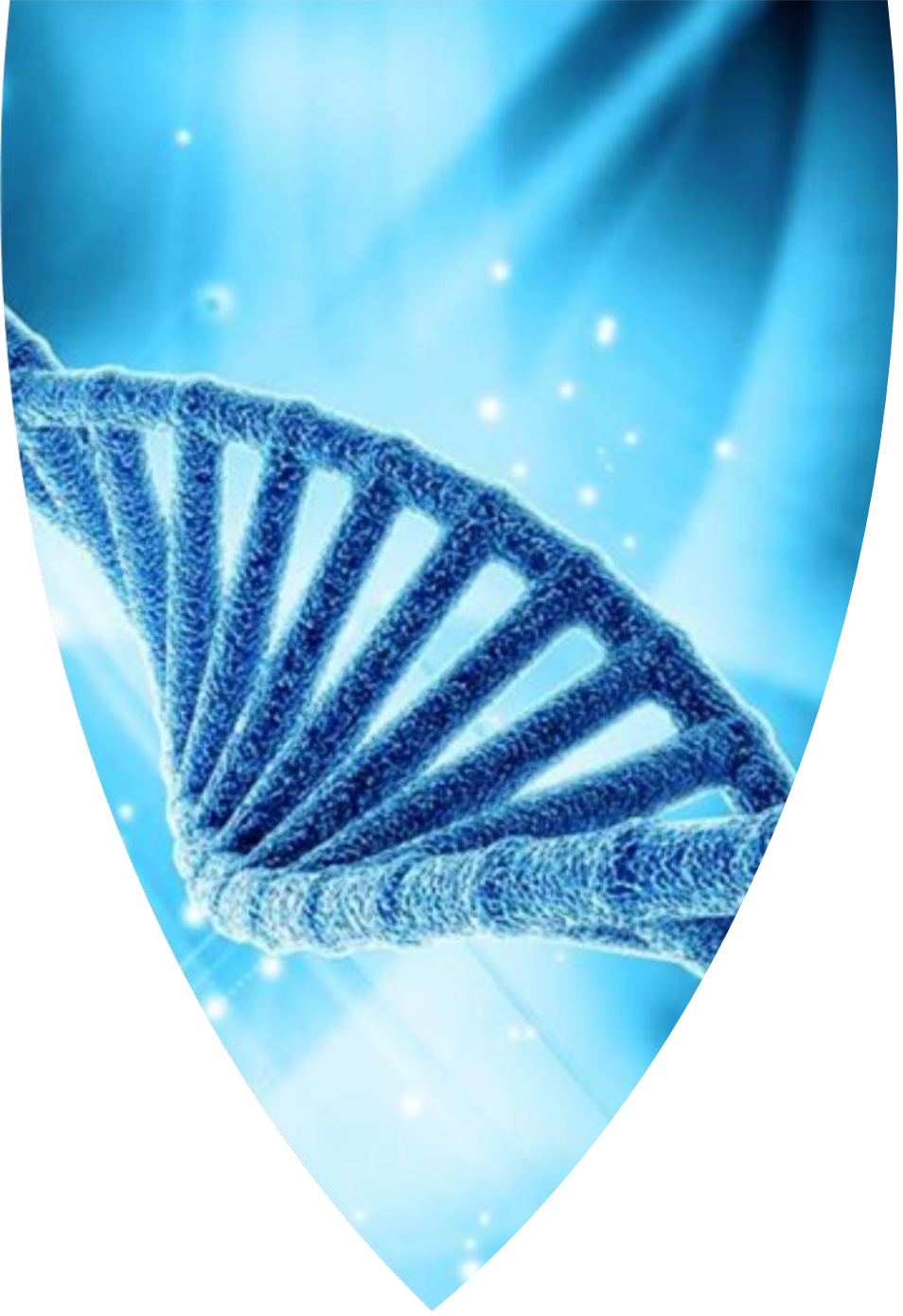


University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

HSE

CUH
Ospidéal na hOllscoile Corcaigh
Cork University Hospital

ISO15189 Next Generation Sequencing (NGS) testing at Cork University Hospital.

Réiltín Werner
Chief Medical Scientist
Dept. of Pathology, CUH/UCC
Email: reiltin.werner@hse.ie

35th European Congress of Pathology
9 – 13 September 2023
Convention Centre Dublin, Ireland
European Society of Pathology

Thermo Fisher Scientific and its affiliates are not endorsing, recommending or promoting any use or application of Thermo Fisher Scientific products by third parties during this seminar. Information and materials presented or provided by third parties as-is and without warranty of any kind, including regarding intellectual property rights and reported results. Parties presenting images, text and material represent they have the right to do so. Speaker was provided honorarium by Thermo Fisher Scientific for providing this presentation.

ThermoFisher
SCIENTIFIC

CUH Pathology Overview

Sample Out

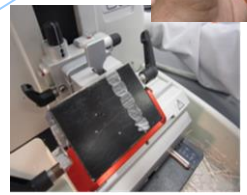
Sample In

Accessioning

Histodissection & Tissue Processing

Embedding

Microtomy



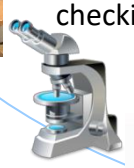
Primary Staining H&E

Advanced Staining

Molecular Pathology Testing

NGS

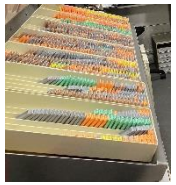
Quality checking

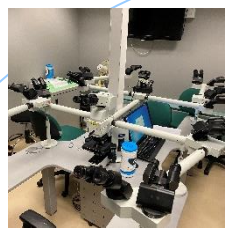


Digital Pathology

Pathologist Report

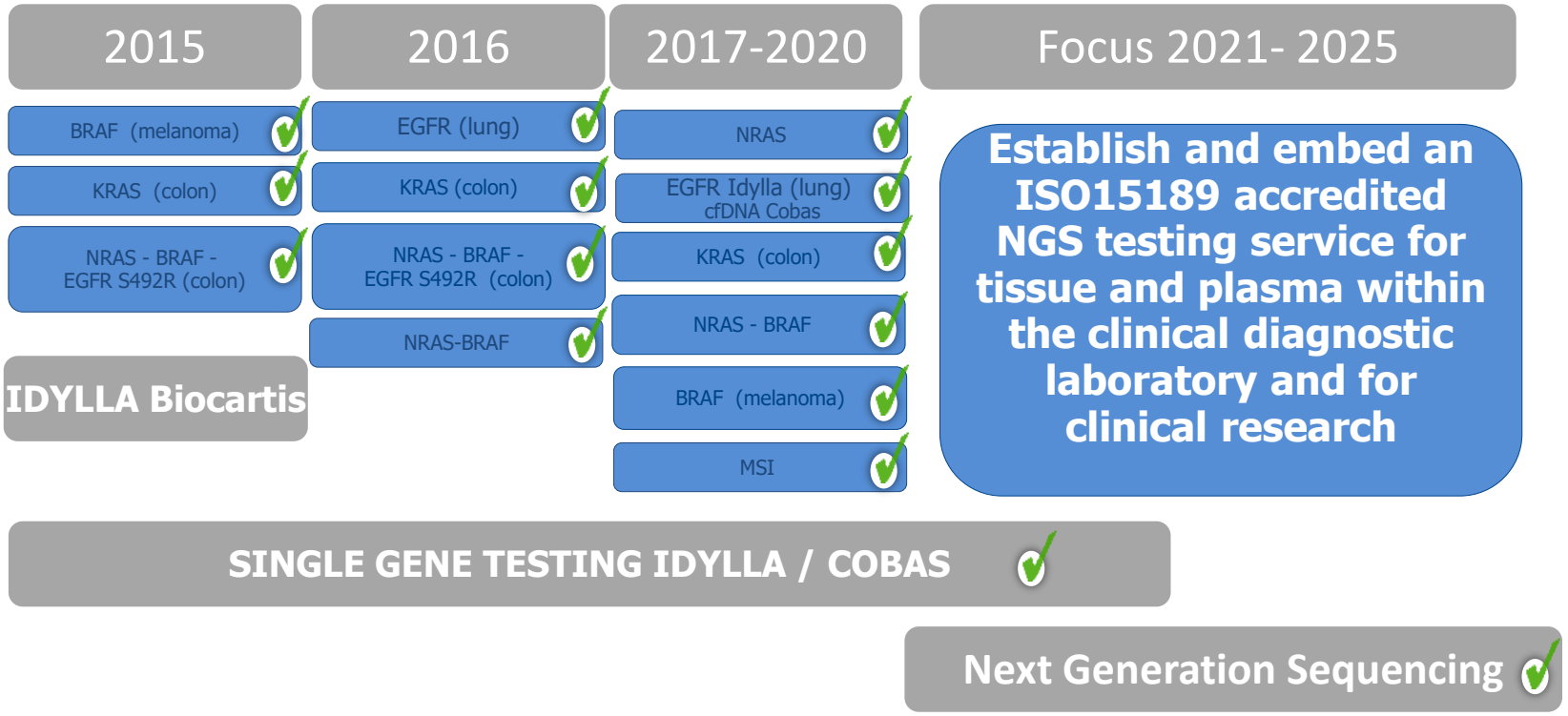
Archiving





90,000 samples/year
>100 staff
Sub-speciality pathology service - Cork/Kerry
Regional molecular pathology -
Cork, Kerry, Waterford, Limerick (Cyto)

CUH Pathology Molecular Testing in-house 2015-2025



Project aims for future compliance with best practice and NSCLC guidelines

- CAP/IASLC/AMP/ ESMO guidelines for NSCLC
- Targeted NGS Panel to include key biomarkers *EGFR,ALK,ROS-1, KRAS, BRAF, RET, MET, NTRK,ERBB2….*
- Assessment of multiple biomarkers simultaneously with a fast TAT on minimal tissue.
- Integrate into existing pathology workflow.

ESMO scale for clinical actionability of molecular targets (ESCAT)[1]

I-A evidence from randomized clinical trials	*ALK*	Fusions	NSCLC
	EGFR	Common mutations and T790M	NSCLC
	ERBB2	Amplifications	Metastatic gastric cancer
	BRAF	V600E mutations	Metastatic colorectal cancer
	PIK3CA	Mutations	Metastatic breast cancer
	*BRCA 1/2**	Somatic and/or germline	Metastatic breast cancer, advanced prostate cancer, advanced pancreatic ductal adenocarcinoma
	IDH1	Mutations	Advanced cholangiocarcinoma
I-B evidence from prospective nonrandomized clinical trials	*BRAF*	V600E	NSCLC
	MET	Exon 14 skipping	NSCLC
	ROS1	Fusions	NSCLC
	FGFR2	Fusions	Advanced cholangiocarcinoma
	EGFR	Uncommon mutations (Ex 20 ins)	NSCLC
I-C evidence from clinical trials across tumor types or basket clinical trials	*MET*	Fusions	NSCLC
	RET	Fusions	NSCLC
	NTRK1/2/3	Fusions	Squamous NSCLC, metastatic gastric cancer, metastatic colorectal cancer, metastatic breast cancer, advanced pancreatic ductal adenocarcinoma, advanced hepatocellular carcinoma, advanced cholangiocarcinoma
		MSI-H*	Metastatic gastric cancer, metastatic breast cancer, advanced prostate cancer, advanced pancreatic ductal adenocarcinoma, advanced hepatocellular carcinoma, advanced cholangiocarcinoma

Original Reports | Quality in Cancer Care

Compromised Outcomes in Stage IV Non–Small-Cell Lung Cancer With Actionable Mutations Initially Treated Without Tyrosine Kinase Inhibitors: A Retrospective Analysis of Real-World Data

Jeffrey A. Scott, MD[1]; Jochen Lennerz, MD, PhD[2]; Melissa Lynne Johnson, MD[3]; Lucio N. Gordan, MD[4]; Robert H. Dumanois, BA[5]; Luca Quagliata, PhD[5]; Lauren L. Ritterhouse, MD, PhD[2]; Federico Cappuzzo, MD, PhD[6]; Brandon Wang, MBA[1]; Mei Xue, MBA[1]; Anupama Vasudevan, PhD, MPH, BDS[1]; Prateesh Varughese, PharmD[1]; Varun Vaidya, PhD[7]; Mike Gart, MBA[1]; Natalie Dorrow, MS[1]; Hinco J. Gierman, MS, PhD[1]; and Rushir J. Choksi, MD[8]

DOI https://

Jeffrey A. Scott, et al, JCO Oncology Practice 2023 Aug 9;OP2200611.

Challenges to NGS Implementation in CUH

Too slow

- Results taking weeks to issue

Too complex

- Lack of expertise required to run NGS
- Workflows requiring multiple instruments, laboratory space and touchpoints

Too costly

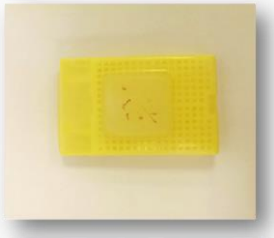
- Staffing
- Space
- Cost penalty when running small sample batches

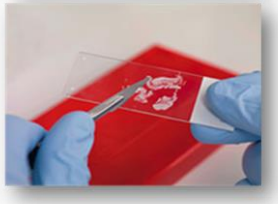
Too limited

- Tissue requirements

Next Generation Sequencing
CUH

Off instrument Pre-Processing





Nucleic acid purification and quantitation

Ion Torrent Genexus
Purification System

Library preparation to variant interpretation

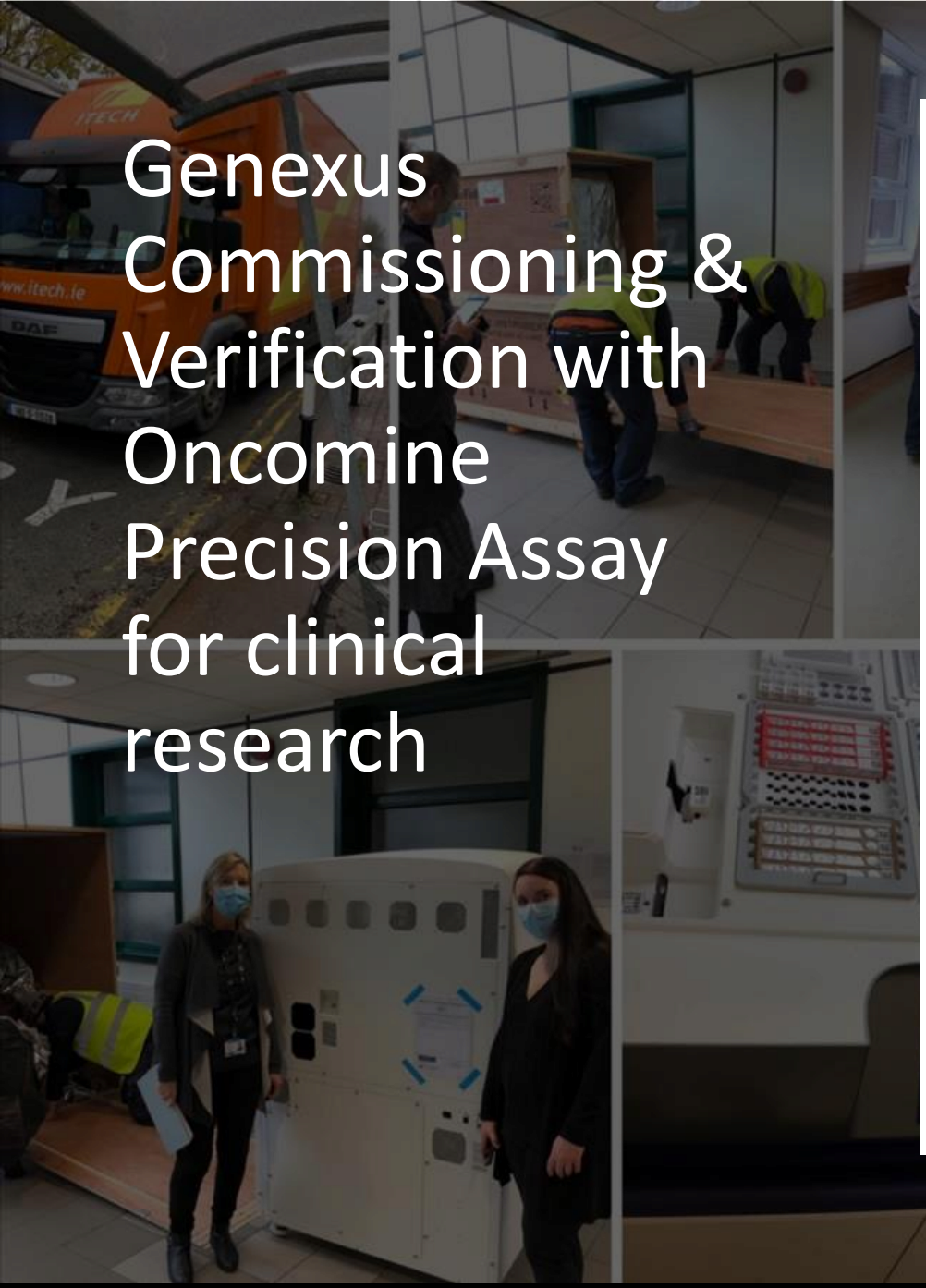
Report

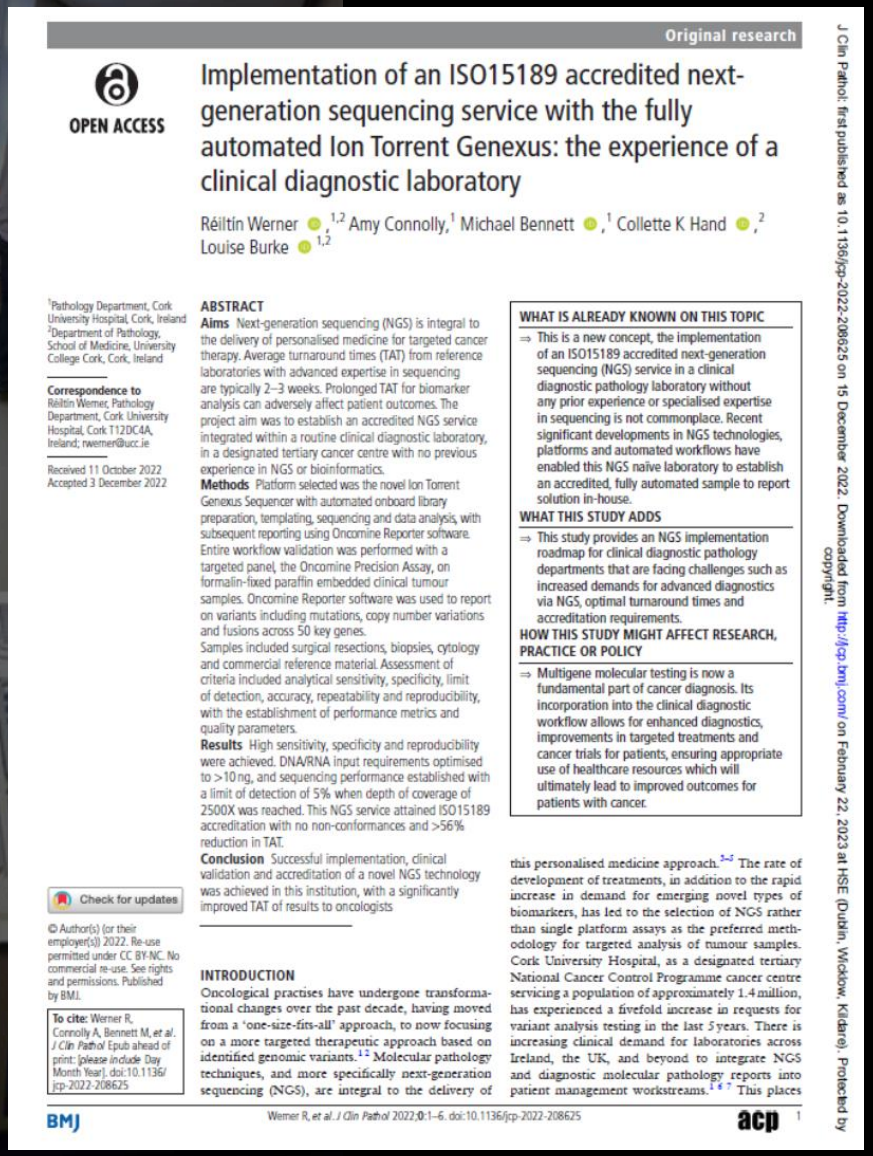
Ion Torrent Genexus
Integrated Sequencers

Ion Torrent™ GX5™ Chip:
12–15M reads/lane

14 hours for a single-lane run (approx. 24 to 30 hours for full chip) Up to 32 Samples per run

Genexus Commissioning & Verification with Oncomine Precision Assay for clinical research





Original research

OPEN ACCESS

Implementation of an ISO15189 accredited next-generation sequencing service with the fully automated Ion Torrent Genexus: the experience of a clinical diagnostic laboratory

Réiltín Werner [1,2] Amy Connolly,[1] Michael Bennett [1] Collette K Hand [2] Louise Burke [1,2]

[1]Pathology Department, Cork University Hospital, Cork, Ireland
[2]Department of Pathology, School of Medicine, University College Cork, Cork, Ireland

Correspondence to
Réiltín Werner, Pathology Department, Cork University Hospital, Cork T12DC4A, Ireland; rwerner@ucc.ie

Received 11 October 2022
Accepted 3 December 2022

ABSTRACT

Aims Next-generation sequencing (NGS) is integral to the delivery of personalised medicine for targeted cancer therapy. Average turnaround times (TAT) from reference laboratories with advanced expertise in sequencing are typically 2–3 weeks. Prolonged TAT for biomarker analysis can adversely affect patient outcomes. The project aim was to establish an accredited NGS service integrated within a routine clinical diagnostic laboratory, in a designated tertiary cancer centre with no previous experience in NGS or bioinformatics.

Methods Platform selected was the novel Ion Torrent Genexus Sequencer with automated onboard library preparation, templating, sequencing and data analysis, with subsequent reporting using Oncomine Reporter software. Entire workflow validation was performed with a targeted panel, the Oncomine Precision Assay, on formalin-fixed paraffin embedded clinical tumour samples. Oncomine Reporter software was used to report on variants including mutations, copy number variations and fusions across 50 key genes.
Samples included surgical resections, biopsies, cytology and commercial reference material. Assessment of criteria included analytical sensitivity, specificity, limit of detection, accuracy, repeatability and reproducibility, with the establishment of performance metrics and quality parameters.

Results High sensitivity, specificity and reproducibility were achieved. DNA/RNA input requirements optimised to >10 ng, and sequencing performance established with a limit of detection of 5% when depth of coverage of 2500X was reached. This NGS service attained ISO15189 accreditation with no non-conformances and >56% reduction in TAT.

Conclusion Successful implementation, clinical validation and accreditation of a novel NGS technology was achieved in this institution, with a significantly improved TAT of results to oncologists

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ This is a new concept, the implementation of an ISO15189 accredited next-generation sequencing (NGS) service in a clinical diagnostic pathology laboratory without any prior experience or specialised expertise in sequencing is not commonplace. Recent significant developments in NGS technologies, platforms and automated workflows have enabled this NGS naïve laboratory to establish an accredited, fully automated sample to report solution in-house.

WHAT THIS STUDY ADDS

⇒ This study provides an NGS implementation roadmap for clinical diagnostic pathology departments that are facing challenges such as increased demands for advanced diagnostics via NGS, optimal turnaround times and accreditation requirements.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Multigene molecular testing is now a fundamental part of cancer diagnosis. Its incorporation into the clinical diagnostic workflow allows for enhanced diagnostics, improvements in targeted treatments and cancer trials for patients, ensuring appropriate use of healthcare resources which will ultimately lead to improved outcomes for patients with cancer.

Check for updates

To cite: Werner R, Connolly A, Bennett M, *et al*. *J Clin Pathol* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/jcp-2022-208625

INTRODUCTION

Oncological practises have undergone transformational changes over the past decade, having moved from a 'one-size-fits-all' approach, to now focusing on a more targeted therapeutic approach based on identified genomic variants.[1 2] Molecular pathology techniques, and more specifically next-generation sequencing (NGS), are integral to the delivery of this personalised medicine approach.[3–5] The rate of development of treatments, in addition to the rapid increase in demand for emerging novel types of biomarkers, has led to the selection of NGS rather than single platform assays as the preferred methodology for targeted analysis of tumour samples. Cork University Hospital, as a designated tertiary National Cancer Control Programme cancer centre servicing a population of approximately 1.4 million, has experienced a fivefold increase in requests for variant analysis testing in the last 5 years. There is increasing clinical demand for laboratories across Ireland, the UK, and beyond to integrate NGS and diagnostic molecular pathology reports into patient management workstreams.[1 4 7] This places

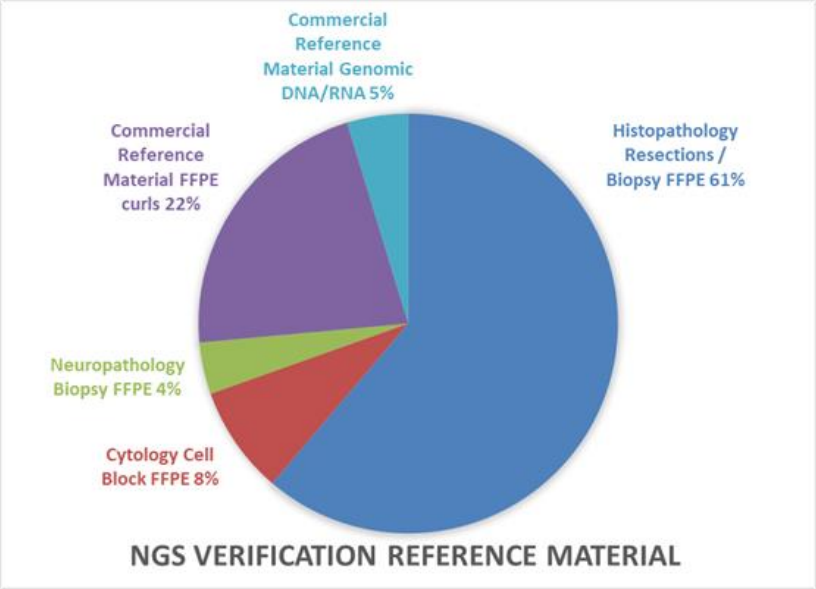
Werner R, et al. J Clin Pathol 2022;0:1–6. doi:10.1136/jcp-2022-208625

NGS Verification

- 276 assays - controls and real-world clinical tumour samples e.g.: NSCLC, colorectal cancer, sarcoma, melanoma, breast, brain, liver, urothelial and cervical cancers.

- Range of specimen types : surgical resections and biopsies (n=169), cytology cell blocks (n=23) and neuropathology samples (n=11).

- Commercial control material procured from External Quality Assessment (EQA) organisations GENQA, EMQN, QUIP and a variety of commercial suppliers (AcroMetrix™, Horizon, and Seraseq)



Manufacturer	Reference Name	Product Code
HORIZON	ALK /ROS/RET	HD784
HORIZON	EGFR	HD300
HORIZON	KRAS	HD301
HORIZON	MULTIPLEX	HD789
HORIZON	ONCOSPAN	HD832
ACROMETRIX	HOTSPOT ONC	969056
SERASEQ	RNA FUSION	0710-0496
SERASEQ	NTRK RNA	0710-1031

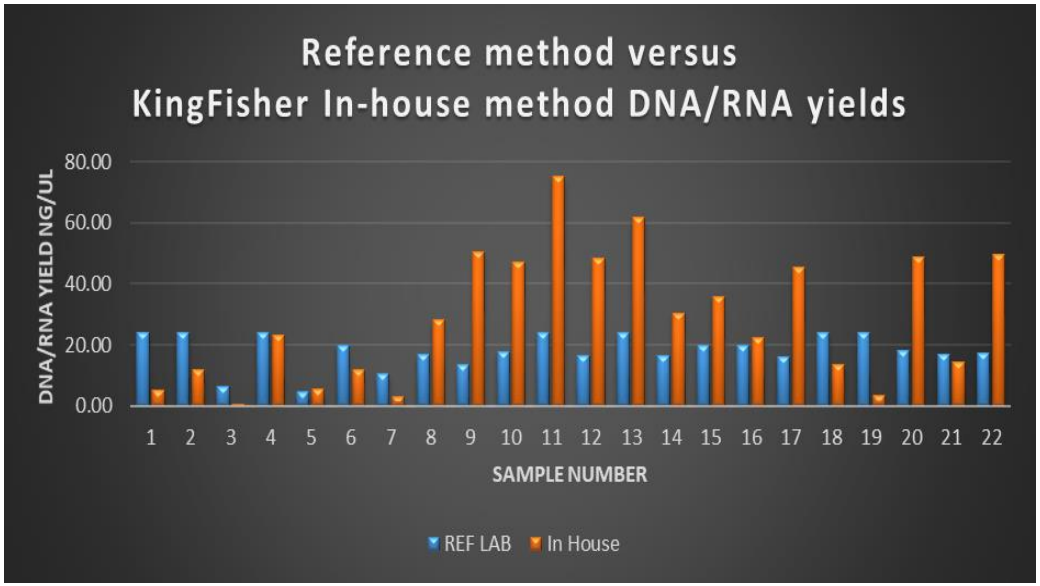
Extraction / Purification verification

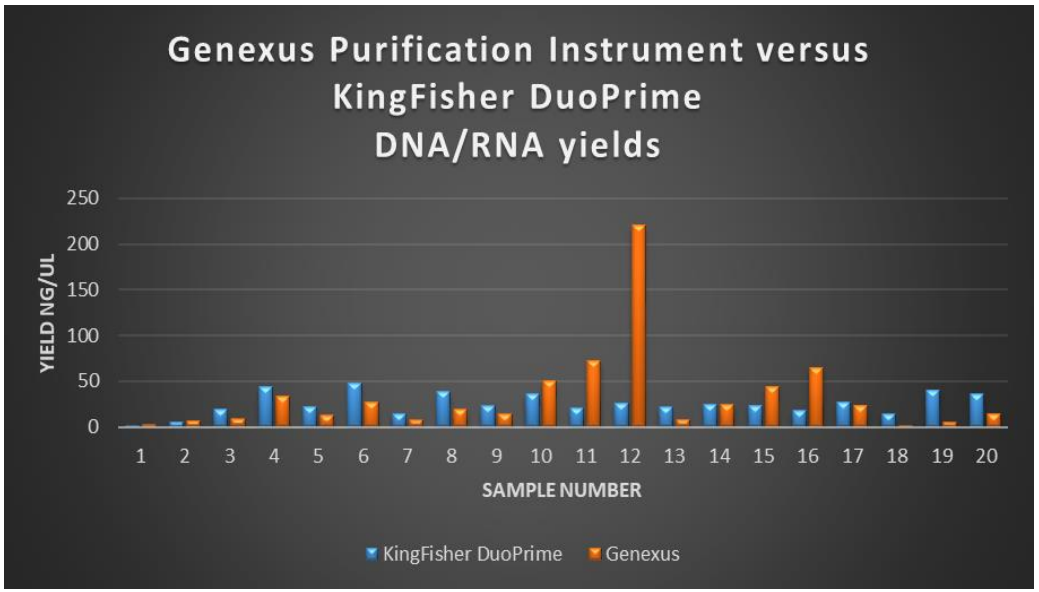
KingFisher DuoPrime.

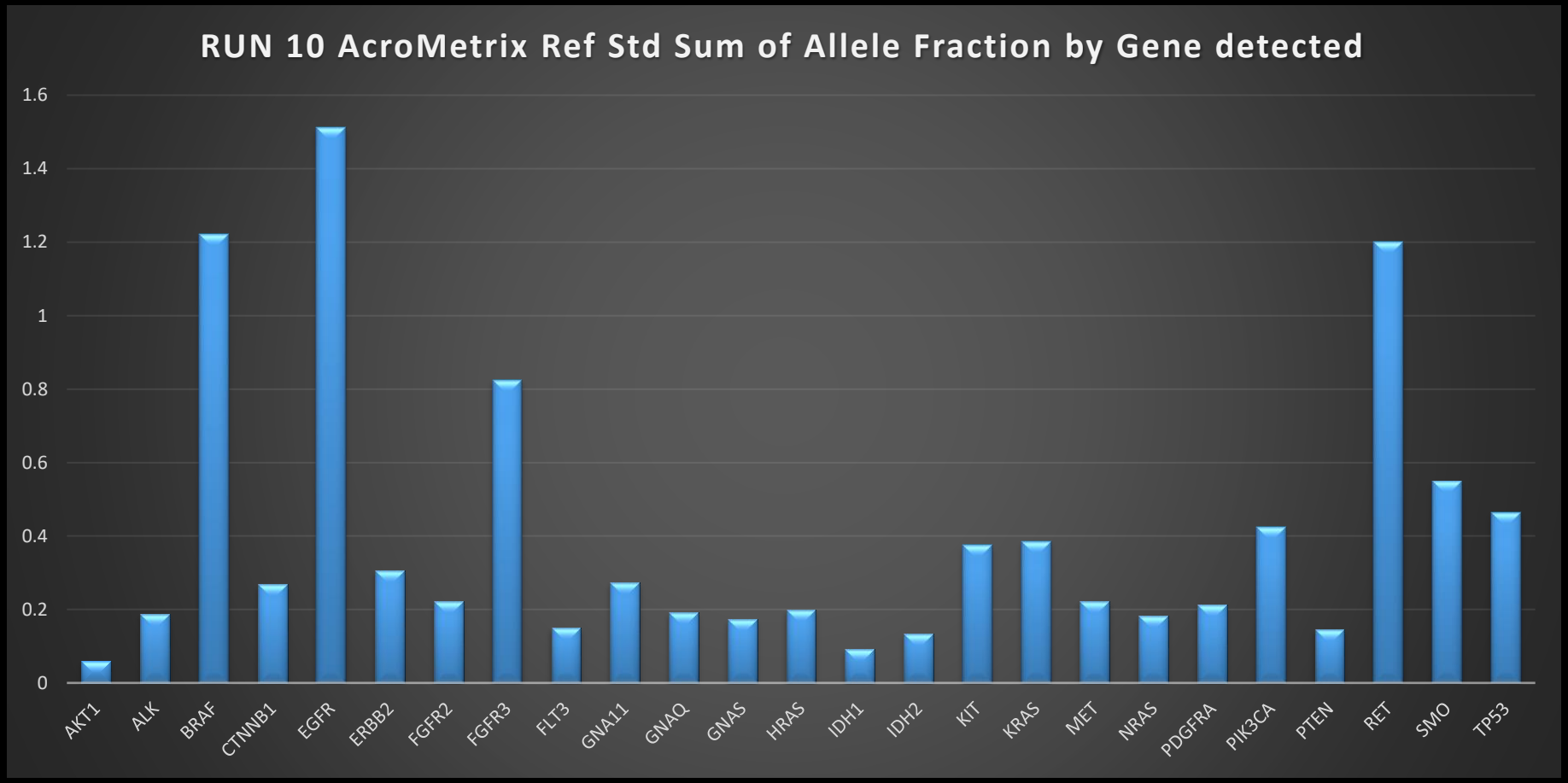
- Samples previously characterised by an accredited reference method were re-extracted with the MagMax kit on the KingFisher DuoPrime.
- Genexus minimum input requirements of 10ng of DNA / RNA
- 181 nucleic acid extractions tested with the Oncomine Precision Assay on the Genexus sequencer.

Genexus Purification Instrument

- Extraction and purification of samples (n=96) run on the GPI were also sequenced on the Genexus Integrated sequencer.
- Available data on previous extraction yields (n=20) average yield increased from 25.4ng/ul to 33.1ng/ul.







AcroMetrix Oncology Hotspot control

100% Specificity and 100% sensitivity across all 25 genes detected that are included in OPA assay

Run Performance Metrics

Performance metrics established over 20 runs utilising commercial controls and clinical FFPE samples

Optimal results achieved with sample input volume as low as 10ng DNA/RNA (routinely 10-30ng)

Overall concordance >99% (PPA/PPV) with orthogonal methods and controls.

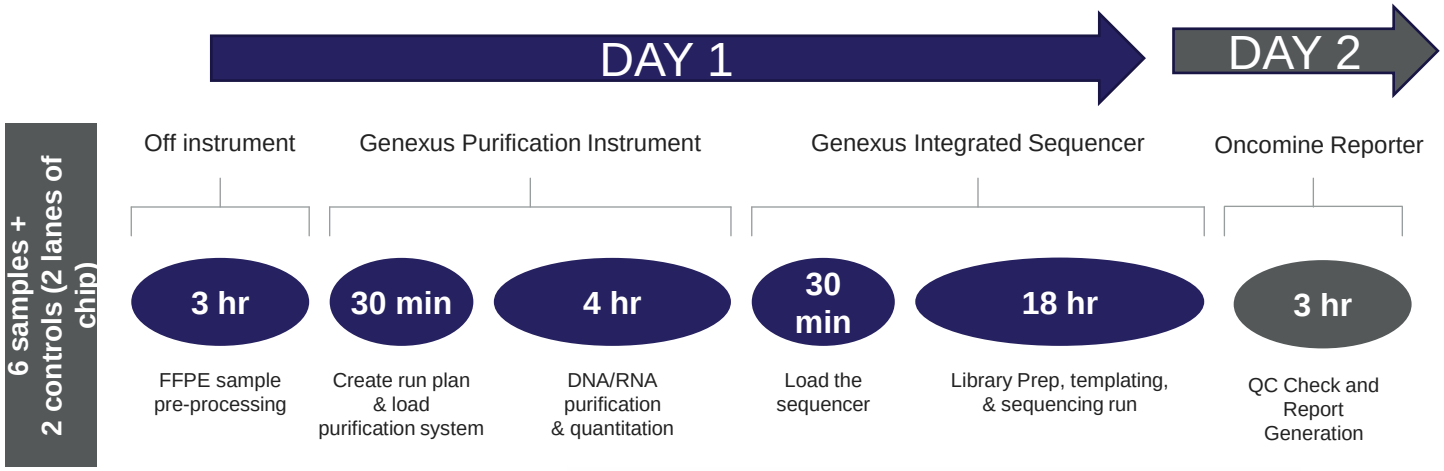
Run performance metrics are assessed prior to releasing results as per ongoing QC protocols and trend analysis.

FFPE OPA PERFORMANCE METRICS

Metric	Target
Final Reads	10-12M
Raw read accuracy	98-99%
% Loading	87-92%
Enrichment	99.90%
Library	99.90%
Mapped reads / DNA library	> 500K (>800 for 5% LOD)
Mapped reads /RNA library	> 100K
% Reads on Target	>90%
Base Coverage Depth	>1000(>2,500 for 5% LOD)
Uniformity	97-99% (>90% for 90%)
End to End reads	>90%
Reads / Amplicon	>500
AF	> 5% / 0.05
MAPD	<0.5 (0.18-0.24)
RNA detection	>5/7
Mean AQ20 Read length	85-95
Mean Read Length DNA	85-100
Mean Read Length RNA	70-100
Base Call Accuracy	97-99%

Optimised CUH NGS Workflow
Specimen-to-Report with TAT reduction of >56%

TAT Pre Implementation ➡ 16 days; 56% reduction of NGS 'request to report' current TAT ➡ 5 Days

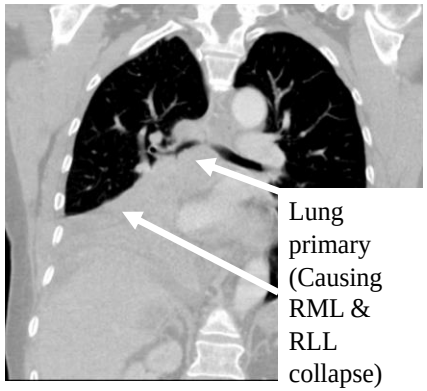


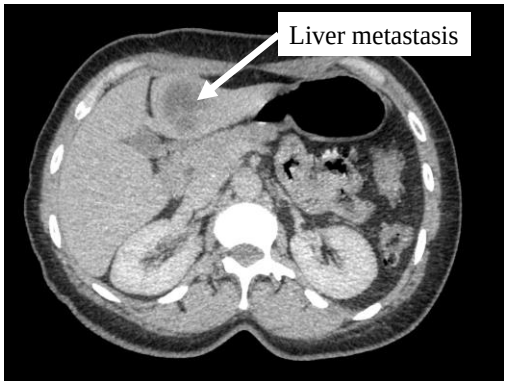
Benefits fully automated workflow for Pathology CUH

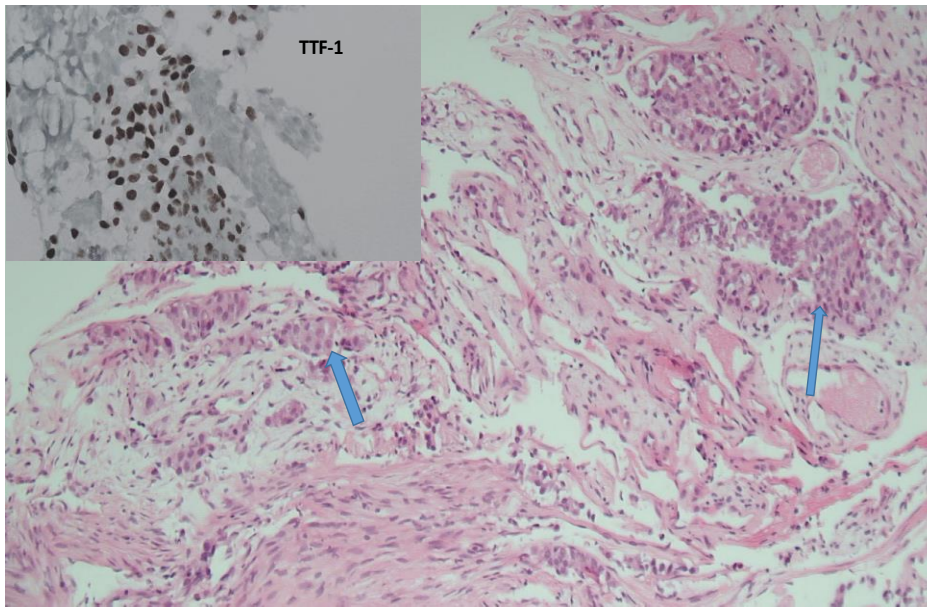
- **Single-day turnaround time** potential to provide IHC and NGS results at the same time
- **Automated**, sample prep, library prep, sequencing, analysis and reporting , reducing Medical Scientist time on the bench
- **Flexibility of economically running few or one sample** reduces the need for batching and helps deliver results faster.
- System manufactured at a facility registered with FDA and ISO 13485 certified **– CE IVD Marking / IVDR compliance in progress: This is important to Pathology CUH with Accreditation & INAB regulations**

Case study 03/2023

- 58 year old Female
- Non-smoker
- No Past medical history
- CT thorax and upper abdomen
- Multiple LN and liver metastasis (Stage 4)
- Right main bronchus biopsy
- Non-small cell carcinoma, favour adenocarcinoma
- H&E NCC 30%
- ALK and PDL1 IHC & Molecular requested
- NGS run as a part of feasibility study
- Macrodissection performed to enrich NCC







Non-Small Cell Carcinoma, favour Adenocarcinoma

Case Study 03/23 NGS Run Review

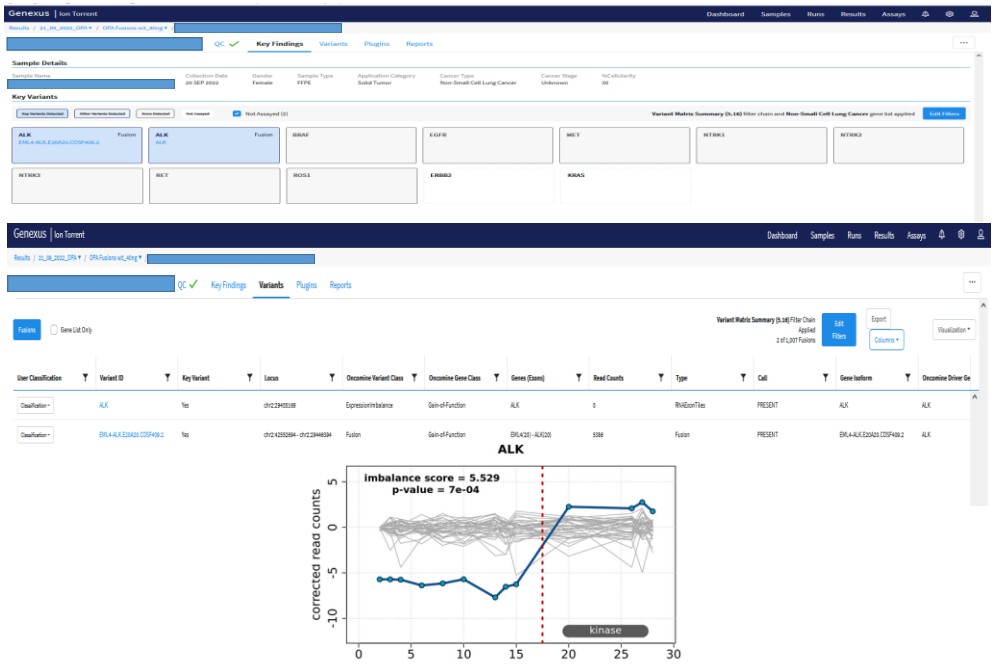
- NGS Run 1: Extraction and Purification QC Pass but NGS RNA QC failure: no test fragments or inline controls visible for the run.
- *NB careful handling of strips at sequencing set up*
- NGS Run 2 : QC Pass Repeat RNA run set up **same day** with material from GPI archive plate.

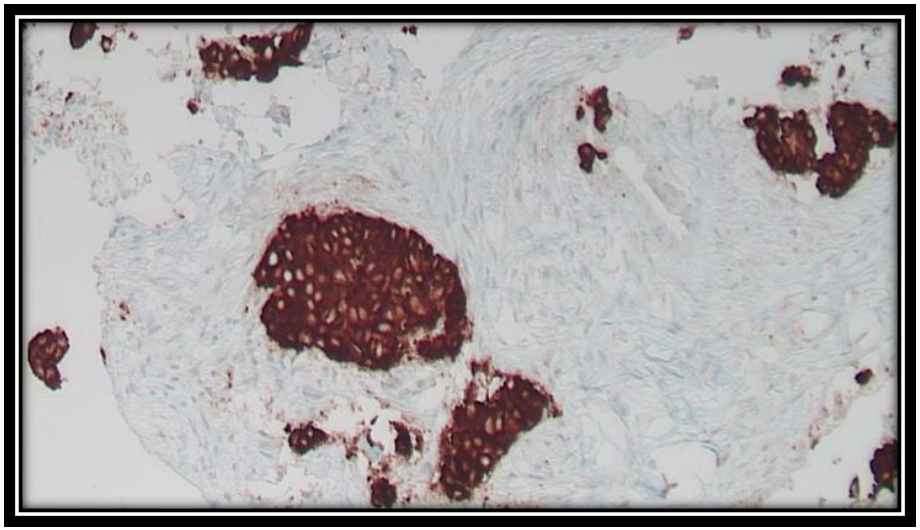
FFPE OPA PERFORMANCE METRICS		Run No.	Sample Name	Run No.	Sample Name
Metric	Target	20_09_2022_OPA_GPI		21_09_2022_OPA_	
Final Reads	10-12M	25,560,076		9,277,938	
Raw read accuracy	98-99%	99.32		99.04	
% Loading	87-92%	92.58		85.96	
Enrichment	99.90%	99.86		99.85	
Library	99.90%	99.92		99.93	
Mapped reads / DNA library	>500K (>800 for 5% LOD)		2,051,778		N/A - RNA only run
Mapped reads /RNA library	>100K		39		591,230
% Reads on Target	>90%		92.6		N/A - RNA only run
Base Coverage Depth	>1000(>2,500 for 5% LOD)		7,450		N/A - RNA only run
Uniformity	97-99% (>90% for 90%)		98.84		N/A - RNA only run
End to End reads	>90%		97.23		N/A - RNA only run
Reads / Amplicon	>500		7,600		N/A - RNA only run
AF	>5% / 0.05		N/A		N/A - RNA only run
MAPD	<0.5 (0.18-0.24)		0.23		N/A - RNA only run
RNA detection	>5/7		0		7
Mean AQ20 Read length	85-95		95		N/A - RNA only run
Mean Read Length DNA	85-100		102		N/A - RNA only run
Mean Read Length RNA	70-100		30		101
Base Call Accuracy	97-99%		98.4		98.2
Mean AQ20 Read length TC QC	97-99		112		102
21/09/2022: RNA QC Fail - repeat Fusion run as ALK IHC pos					
EML4-ALK fusion detected from 21_09_2022_OPA					

Case Study 03/2023 Report

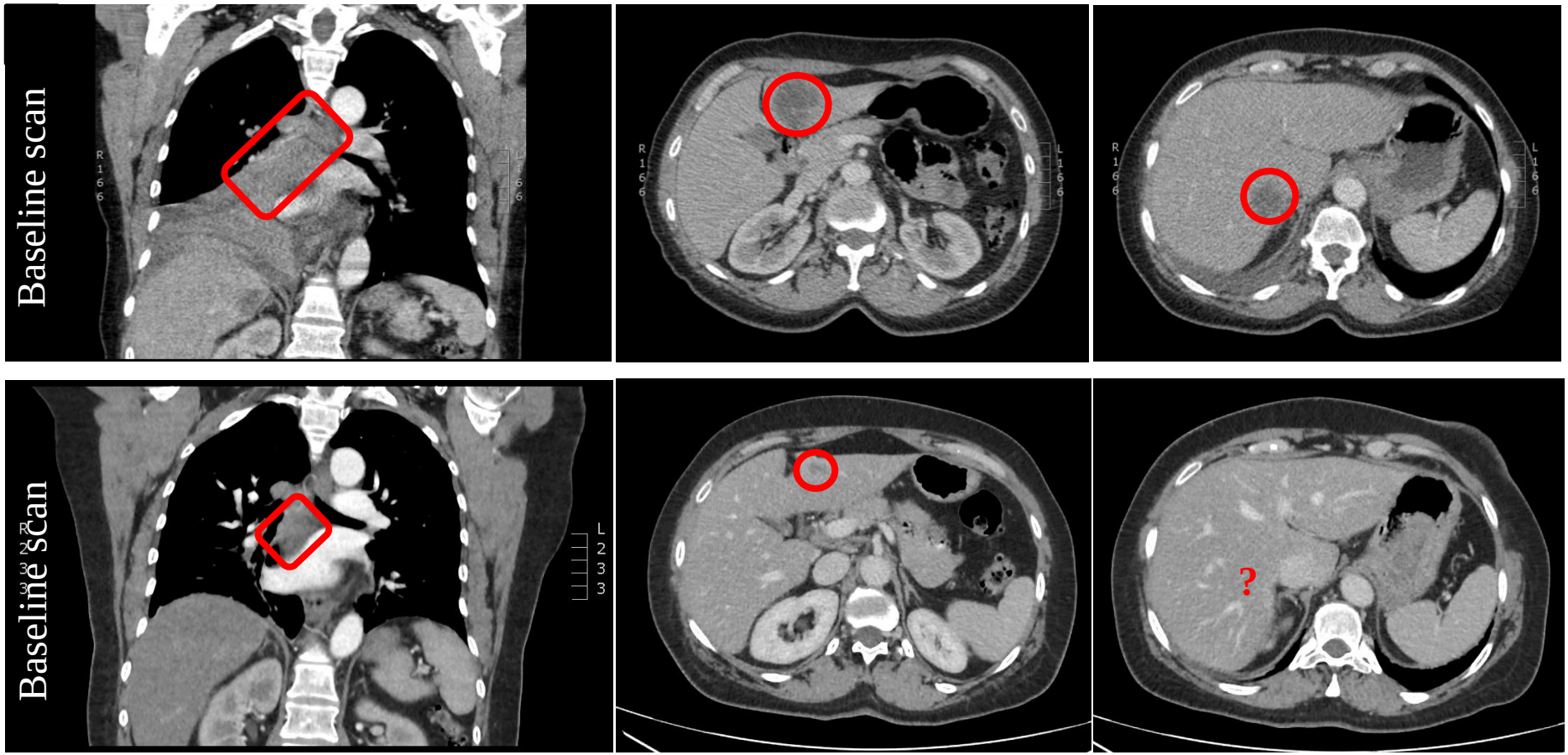
Complete integrated report – TAT 7 Days

- **NGS:** ALK fusion reported
- NGS **concordant** with ALK IHC





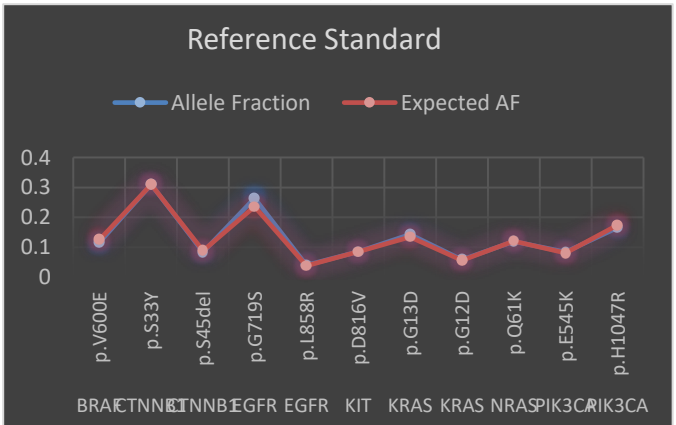
FISH+ ALK NSCLC case study follow up

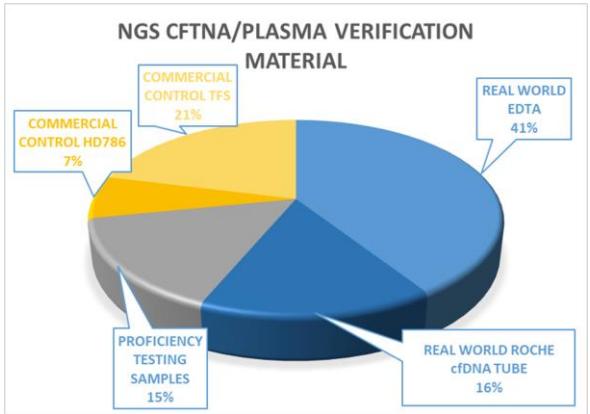


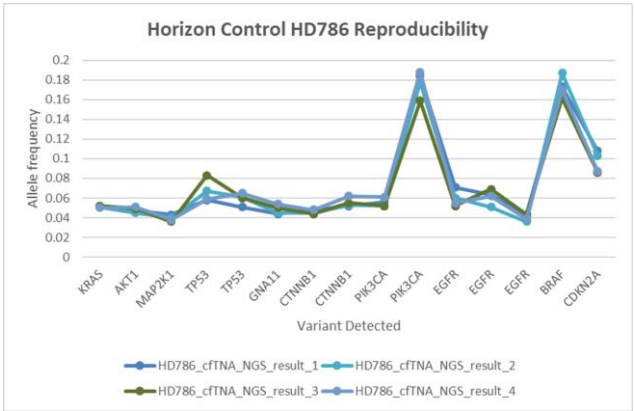
Ongoing Verification Projects include expansion to NGS in liquid biopsy

- Expansion of NGS panel to other tissue streams
- Verification of Liquid Biopsy (cfTNA) across 2 Genexus platforms and GPI Q.2 2023
- Extension to scope for Liquid Biopsy ISO15189 INAB assessment Q 3/4 2023
- cfTNA research study Q.3 2023

ONCOMINE PERFORMANCE METRICS cfTNA	
Metric	Target
Final Reads	10-12M / Lane
Raw read accuracy	97-99%
% Loading	88-92%
Enrichment	99.90%
Library	99.90%
Mapped reads / DNA library	8M - 12M
% Reads on Target	>90%
Mean Read Coverage	22,000 - 40,000
Uniformity	97-99%
Mean Molecular Coverage	1,000 - 3,000
AF	> 1.2% / 0.012
MAPD	<0.4 (0.14-0.25)
Mean Read Length DNA	99-100
Mapped reads /RNA library	> 150,000 - 400,000
Mean Read Length RNA	97-104
RNA detection	>2/7
Base Call Accuracy	97-99%







Karen Fenton
Ovarian Cancer Fund

CUH Charity
Cork University Hospital
'Saving and Changing Lives'

Thank You