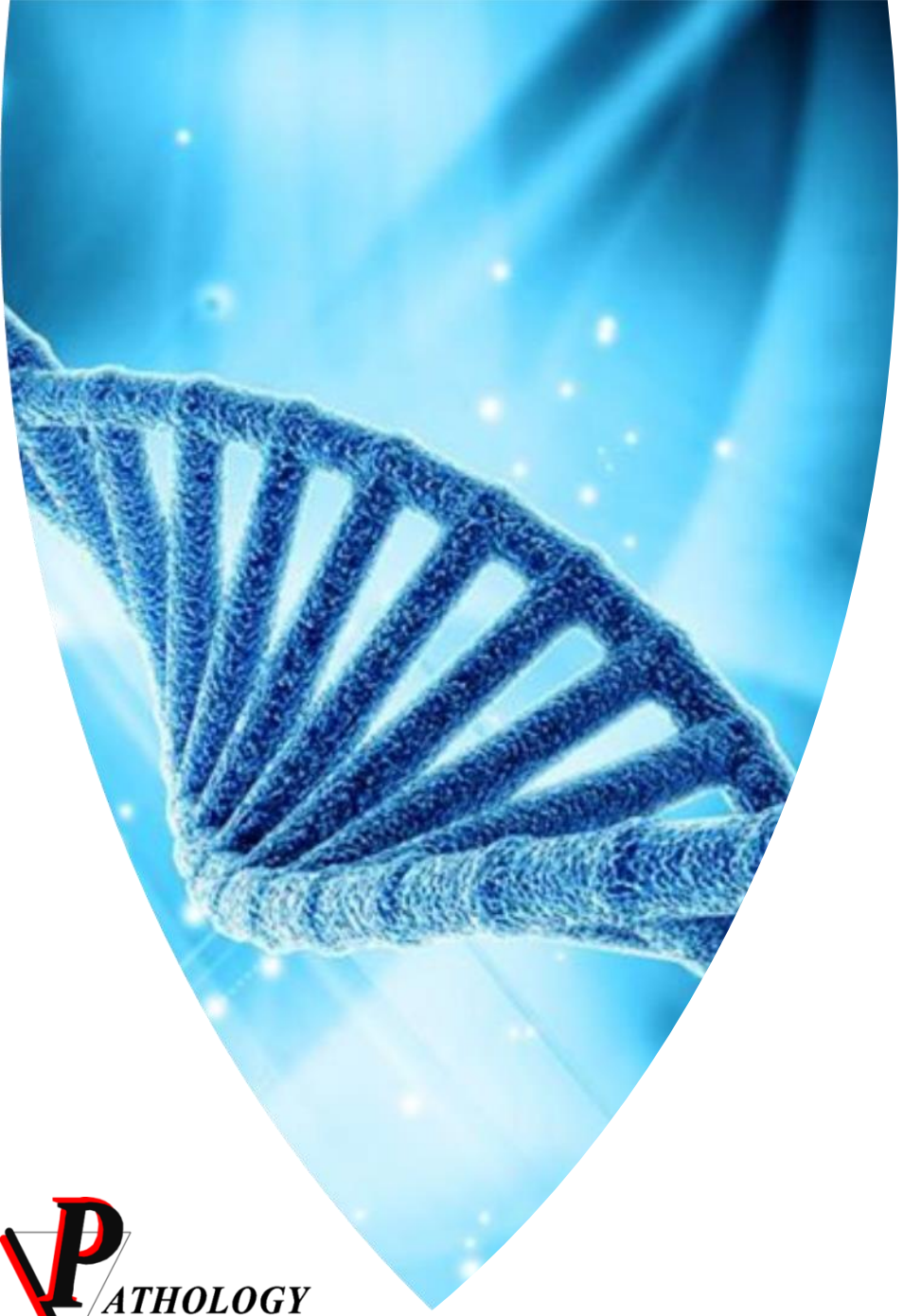

VERIFICATION OF NEXT GENERATION SEQUENCING IN THE PATHOLOGY DEPARTMENT CORK UNIVERSITY HOSPITAL

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Chief Medical Scientist

Molecular Pathology Laboratory

Cork University Hospital

VPATHOLOGY

HSE

CUH
Ospidéal na hOllscoile Corcaigh
Cork University Hospital

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Overview

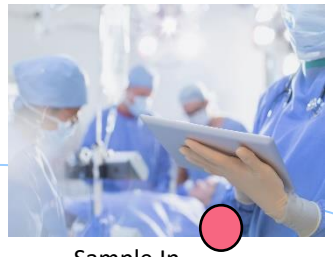
1. Pathology Department Cork University Hospital
2. Current Predictive Biomarker Portfolio
3. Justification for Insourcing NGS
4. Next Generation Sequencing; Why Genexus?

Cork University Hospital Pathology Laboratory

- One of the largest INAB accredited laboratories in Ireland delivering subspecialty pathology services for hospitals in the southern region Cork/Kerry, and a regional molecular pathology service for Cork, Kerry, and Waterford
- >70,000 samples generating > 45,000 IHC & Molecular requests / year
- Large team of > 100 staff in the department
- The laboratory is involved in both intradepartmental and collaborative research.

CUH Sample Journey

Sample Out

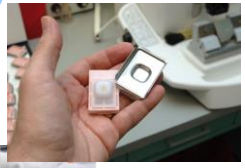


Sample In

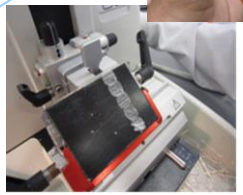
Accessioning

Histodissection & Tissue Processing

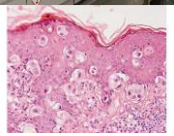
Embedding



Microtomy



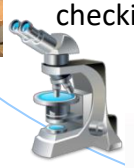
Primary Staining H&E



Advanced Staining

Molecular Pathology Testing

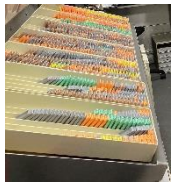
NGS



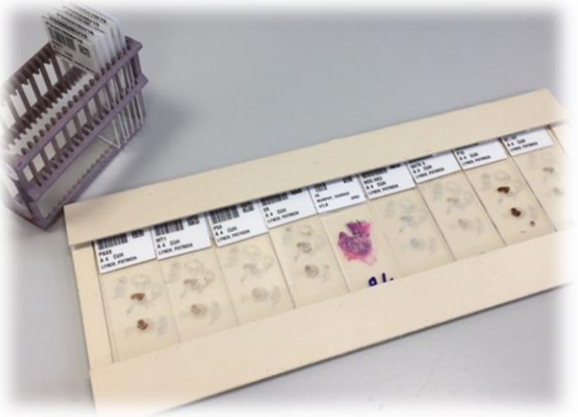
Quality checking

Digital Pathology

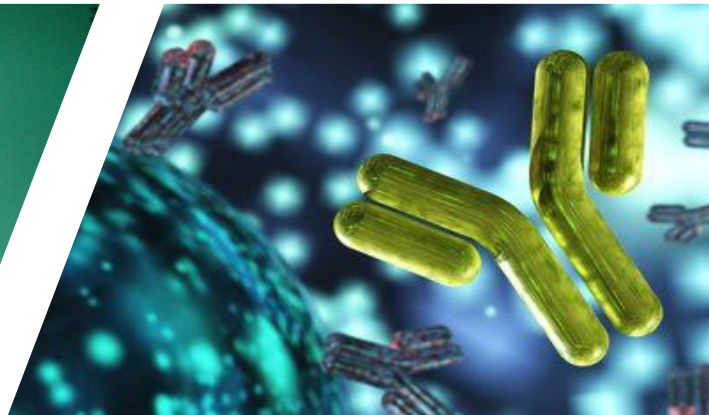
Pathologist Report

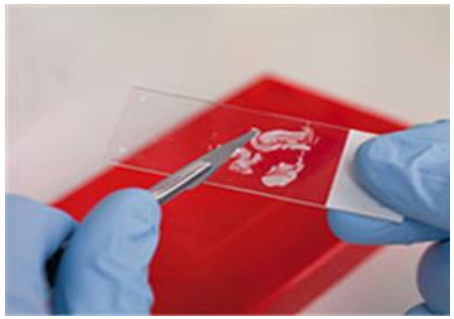


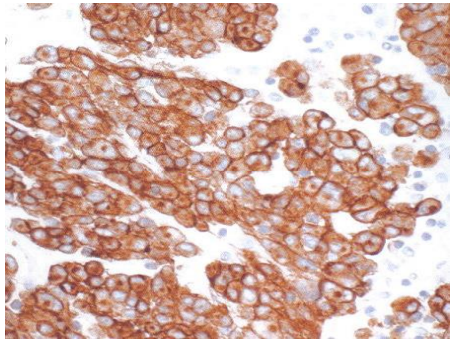
Archiving

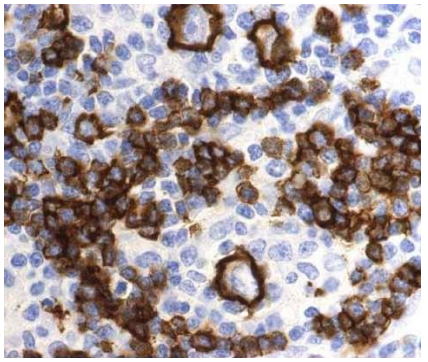


PRE-ANALYTICS









CUH Pathology biomarker testing is via

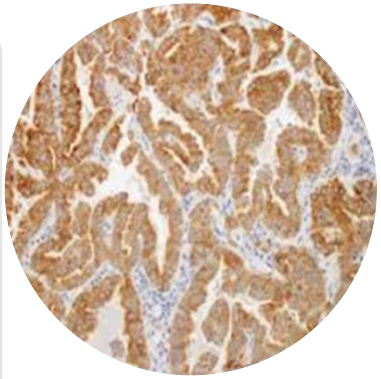
IHC / ISH / PCR

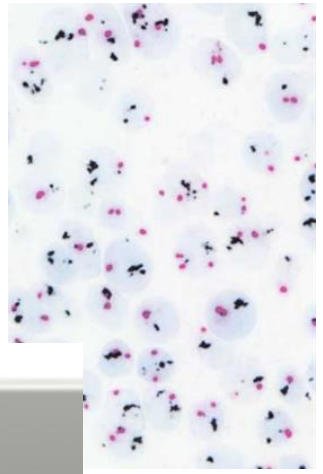
Current CUH Testing Paradigm

Relevant biomarkers assays

e.g., Immunohistochemistry (IHC) or In Situ Hybridisation (ISH) tests and more complex single gene PCR assays are carried out in house.

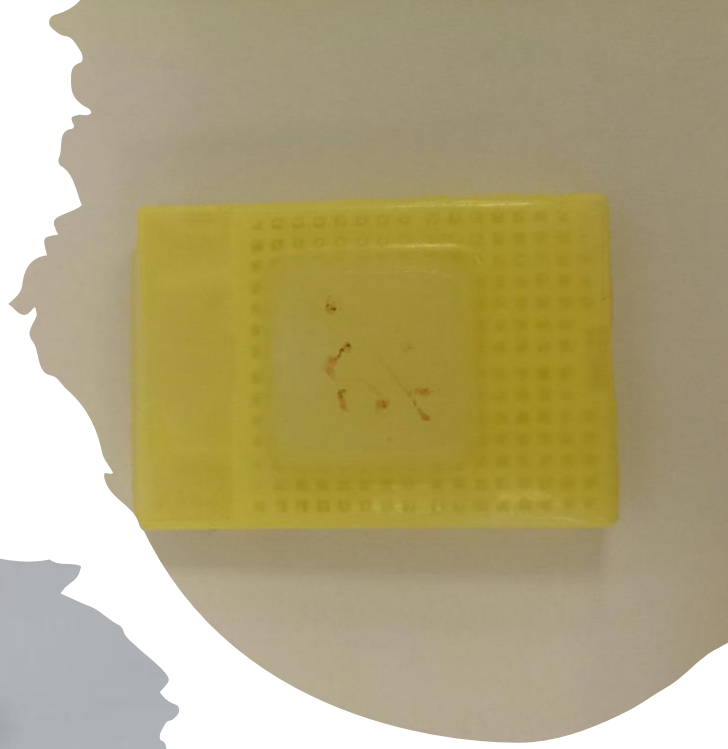
- IHC
  - ER/PR/Her2 IHC Breast Cancer
  - PD-L1 / Alk IHC Lung cancer
  - C-KIT IHC GIST
- ISH
  - Breast / Gastric cancer Her2 - ISH
- PCR
  - RAS/RAF/MSI Colorectal cancer
  - BRAF / NRAS Melanoma
  - EGFR Lung cancer
- NGS - OUTSOURCED



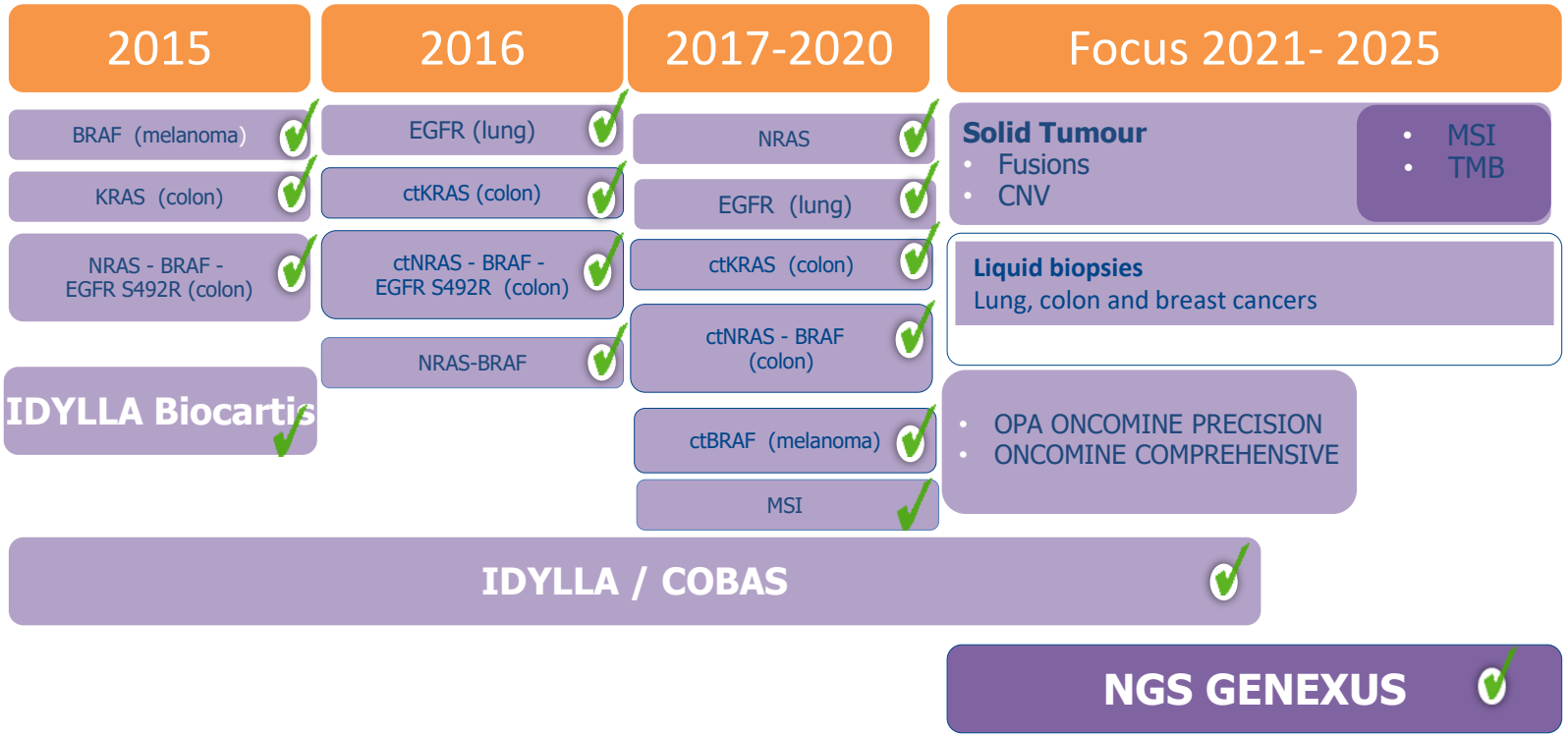


TISSUE IS THE ISSUE!

1. Single Gene Testing e.g., Lung Biomarkers as per CAP/IASLC/AMP Guidelines
   - EGFR – TKIs
   - ALK – ALK inhibitors
   - PD-L1 IHC – Programmed death ligand 1 inhibitor
   - ROS-1 – Ros inhibitors
2. Clinical Trials require more investigation with expanded panels : e.g., KRAS,RET,MET,NTRK,HER2
3. Lung cancer sample with EGFR T790M resisting mutation in FFPE & Plasma



CUH Molecular Testing in-house 2015-2022



Today's Typical NGS Workflow

NGS workflow steps:	Nucleic acid purification	Quantitation	Library prep	Sequencing	Variant interpretation	Report
Ion GeneStudio™ S5 series instrument workflow • Instruments: 6 • Touchpoints: 7 • Total HOT: 75 min • Total TAT: 4 days	 Day 1		 Day 2	 Day 3	  Day 4	
Illumina™ MiSeq™ instrument workflow • Instruments: 5 • Touchpoints: >10 • Total HOT: ≥90 min • Total TAT: 6 to 7 days	 Day 1		 Day 2 Day 3	 Day 4 Day 5	 Day 6	 Day 7
Qiagen™ GeneReader™ instrument workflow • Instruments: 6 • Touchpoints: • Total HOT: >8 hrs • Total TAT: 5 to 6 days	  Day 1		 Day 2	 Day 3 Day 4	 Day 5	 Day 6

NGS Implementation in CUH
Challenges and Barriers

Too slow

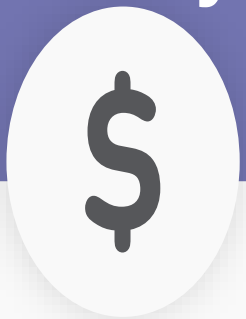
Requires days/ weeks to get the results

Too complex

High level of user expertise required to run NGS

Workflows requiring multiple instruments and touchpoints

Too costly



Staffing

Cost penalty when running small sample batches

Too limited

Tissue requirements

CUH Pathology Solution
Ion Torrent Genexus NGS System

Genexus System—Specimen-to-Report NGS Workflow

- CUH Ongoing Verification Q2/Q3 2021

FFPE tissue

- CUH Verification Q4 2021

Plasma

Nucleic acid purification and quantitation

Ion Torrent™ Genexus™
Purification System

(Expected Delivery CUH 2021)

Currently use KingFisher DuoPrime with Qubit

Library preparation to variant interpretation

Report

Ion Torrent™ Genexus™
Integrated Sequencer

(Currently Under Validation in Pathology CUH 2021)

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

Ion Torrent™ Genexus™ System in Pathology CUH

Benefits of the Genexus NGS system

- **Single-day turnaround time** *potential* to provide IHC and NGS results at the same time
- **Automated**, 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 – *however current limitations with the extraction process does require some level of batching for now.*
- 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**

Genexus Software—End-To-End Solution from Specimen to Report

Integrated

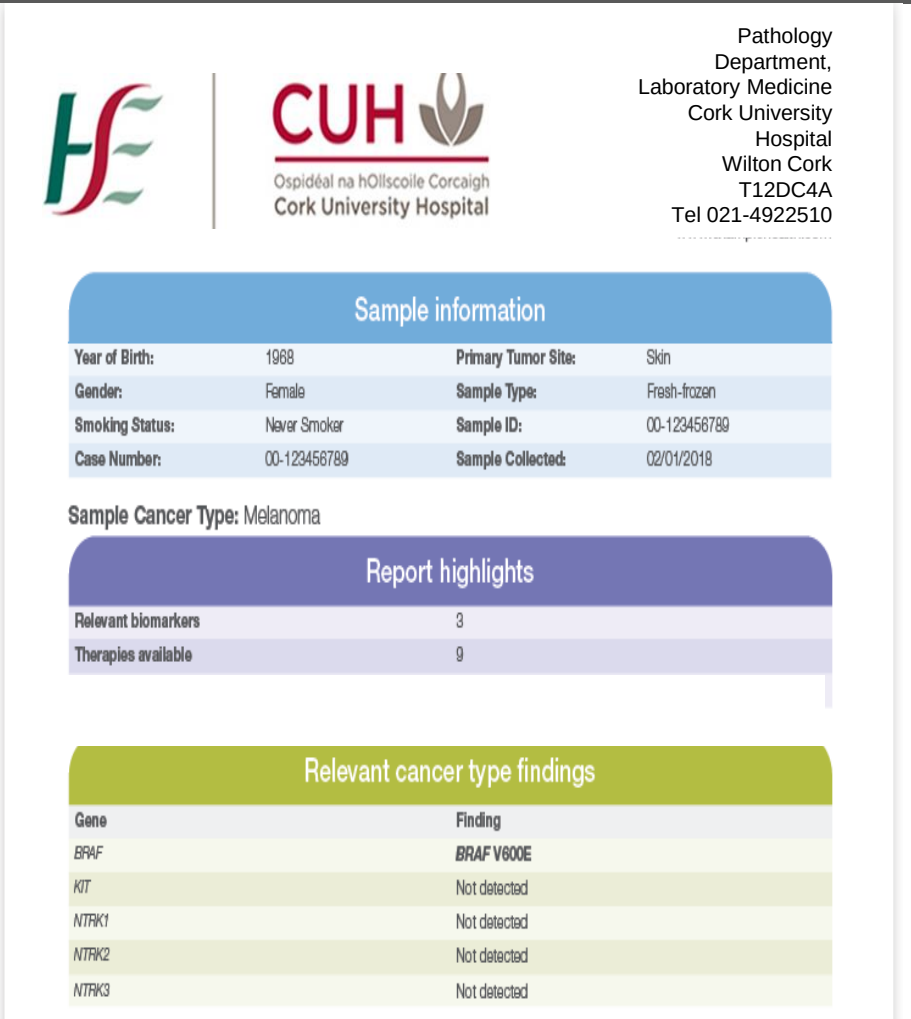
specimen-to-report workflow;
Integrated Bioinformatics

Easy to use

Simplified, new user experience helps reduce the learning curve and human error

Reporting – ongoing Q2 2021

CUH currently verifying the reporting solution



HSE | CUH Ospidéal na hOllscoile Corcaigh Cork University Hospital

Pathology Department,
Laboratory Medicine
Cork University Hospital
Wilton Cork
T12DC4A
Tel 021-4922510

Sample information

Year of Birth:	1968	**Primary Tumor Site:**	Skin
Gender:	Female	**Sample Type:**	Fresh-frozen
Smoking Status:	Never Smoker	**Sample ID:**	00-123456789
Case Number:	00-123456789	**Sample Collected:**	02/01/2018

Sample Cancer Type: Melanoma

Report highlights

Relevant biomarkers	3
Therapies available	9

Relevant cancer type findings

Gene	Finding
BRAF	***BRAF* V600E**
KIT	Not detected
NTRK1	Not detected
NTRK2	Not detected
NTRK3	Not detected

Oncomine Precision Assay in Pathology CUH

Detects relevant variants

Curated pan-cancer content

- Mutations, CNVs, and fusion variant types across 50 key genes

Tissue and plasma samples

- One test, one workflow, multiple sample types

Molecular tagging

- Enhanced low-level variant detection
- Key for liquid biopsy testing

FusionSync™ Detection Technology

- Sensitive and specific—targeted isoform designs
- Novel fusion detection

One Kit, Multiple Applications – OPA Assay

DNA or RNA-only
testing for FFPE tissue samples

Up to **32** FFPE tissue samples
with DNA **OR** RNA only input

Simultaneous DNA + RNA
testing for FFPE tissue samples

Up to **16** FFPE tissue samples
with DNA **AND** RNA inputs

cfTNA (total nucleic acid)
CUH Pathology Validation Q3/Q4 2021

Up to **32** liquid biopsy samples with cfTNA* input

** Cell Free Total Nucleic Acid input includes detection of both DNA and RNA based variants with a single nucleic acid input*

Evolving Paradigm in Testing for Advanced NSCLC

Initial testing	At Progression

Old paradigm

FFPE tissue testing

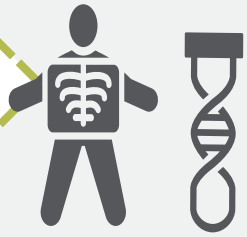
- *EGFR*
- *ALK*
- *ROS1*
- *BRAF*
- *PD-L1*

- Radiology
- FFPE Tissue – if available

FFPE tissue or liquid biopsy testing of tissue not available

New paradigm

- *EGFR*
- *ALK*
- *ROS1*
- *BRAF*
- *PD-L1*
- *NTRK*
- *RET*



- Liquid biopsy
- Radiology
- FFPE Tissue NGS – if available

Verification REPORT

Current Oncomine Precision Assay Gene Content

DNA hotspots

AKT1	ESR1	MAP2K2
AKT2	FGFR1	**MET**
AKT3	FGFR2	MTOR
ALK	FGFR3	NRAS
AR	FGFR4	**NTRK1**
ARAF	FLT3	**NTRK2**
BRAF	GNA11	**NTRK3**
CDK4	GNAQ	PDGFRA
CDKN2A	GNAS	PIK3CA
CHEK2	HRAS	PTEN
CTNNB1	IDH1	RAF1
EGFR	IDH2	**RET**
ERBB2	KIT	**ROS1**
ERBB3	**KRAS**	SMO
ERBB4	MAP2K1	TP53

CNV

ALK
AR
CD274
CDKN2A
EGFR
ERBB2
ERBB3
FGFR1
FGFR2
FGFR3
KRAS
MET
PIK3CA
PTEN

Inter-genetic fusions

ALK	**NTRK2**
BRAF	**NTRK3**
ESR1	NUTM1
FGFR1	**RET**
FGFR2	**ROS1**
FGFR3	RSPO2
MET	RSPO3
NRG1	
NTRK1	

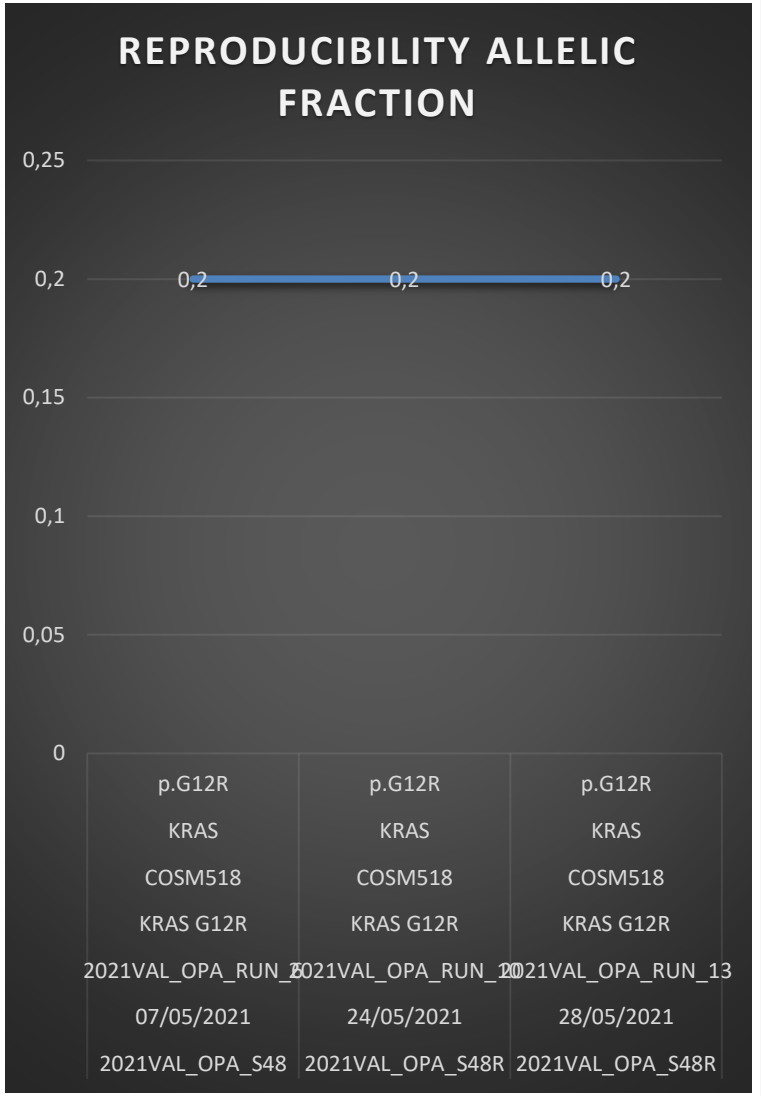
Intra-genetic fusions

AR
BRAF
EGFR
MET

Inclusion in the CUH Pathology Verification Plan

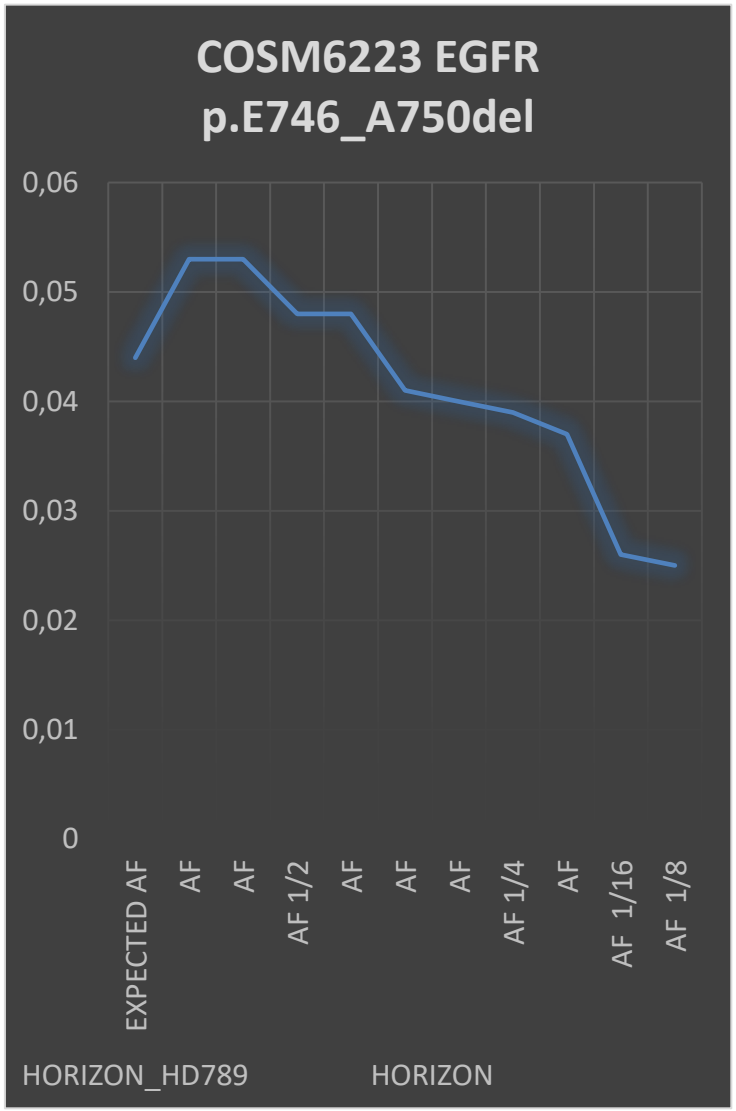
CUH Pathology Verification

- Initial verification for the actionable Lung cancer variants included 86 anonymised real world clinical research samples, Previously tested in a reference laboratory or confirmed on another accredited platform in-house.
- We also included 45 control samples in this initial verification phase (commercial controls from Horizon diagnostics, Seraseq, accrometrix and EQA samples).
- See 2021VAL_OPA_SAMPLE_48 run 3 times on 3 different days consistently gave an AF of 20%



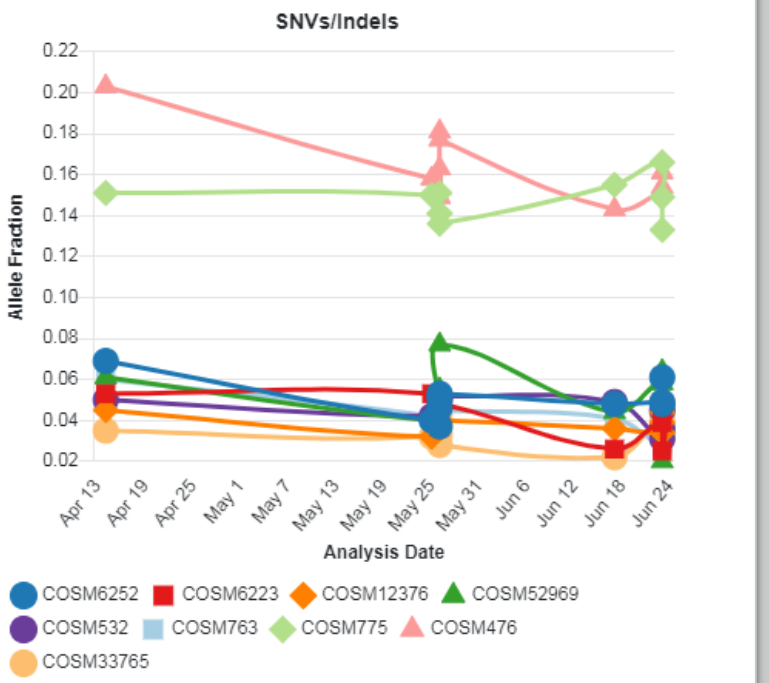
Reference Controls

- A range of Horizon controls included in the verification
- Seraseq Fusion controls, CNV Brain/Lung Mix
- Accrometrix controls
- The Horizon Structural Multiplex FFPE DNA Reference Standard includes 9 digital PCR-verified variants with allelic frequencies ranging from 3.5% to 9.7%
- Serial dilutions of this HD789 were also tested over a number of runs.



Ongoing Verification

- The 45 commercial controls and EQA samples were tested with the OPA assay on the Genexus over 16 runs between April 13 and June 24.
- See here at the different concentrations, multiple variants were detected at the expected AF with anticipated variants also evident at the lowest concentrations.
- The next phase of the verification is in establishing the report templates and structure, using the Ion Torrent Oncomine Reporter software.
- An application to the Irish National Accreditation Board (ISO15189) for extension to our scope has also been submitted.

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