Next generation sequencing-based biomarkers in molecular pathology

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Introduction

The enormous analytical capacity of **next generation sequencing** (NGS) and the associated cost reduction opened a variety of important new application scenarios in medical diagnostics, particularly in the context of **personalized medicine**. Information on the multitude of genetic alterations that cause and influence the outcome of diseases might be considered as one of the key **biomarker** approaches of future medicine.

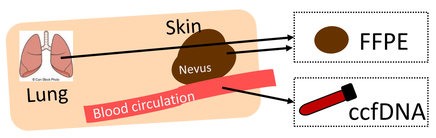
Aims

The project aims to develop methodology and complete **"Sample-to-Insight" NGS workflows** for diagnostic NGS, including the pre-analytical workflow steps such as sample collection, preservation, transport, storage and preparation as well as quality control of **melanoma, lung and colon** samples.

Methods

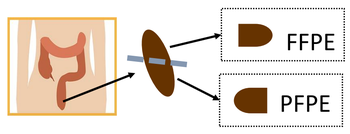
- Formalin-fixed paraffin-embedded (FFPE) and PAXgene-fixed paraffin-embedded (PFPE) tissue genomic DNA and/or ccfDNA from blood (A, B) was processed, sequenced and analyzed according to **the QIAGEN GeneReader NGS Workflow** (C).
- **Quality** was assessed with the QIAxcel® and QuantiMIZE Systems which are a part of the GeneReader workflow.
- Targeted amplification of actionable cancer hotspots was performed using the **GeneRead® Actionable Insights Tumor Panel** and the **GeneRead® QIAact Lung DNA Panel** for tissue and plasma (D).
- Pathogenic variants were identified using the **QCI-Analyze** software and interpreted with the **QCI-Interpret** software (E).

A



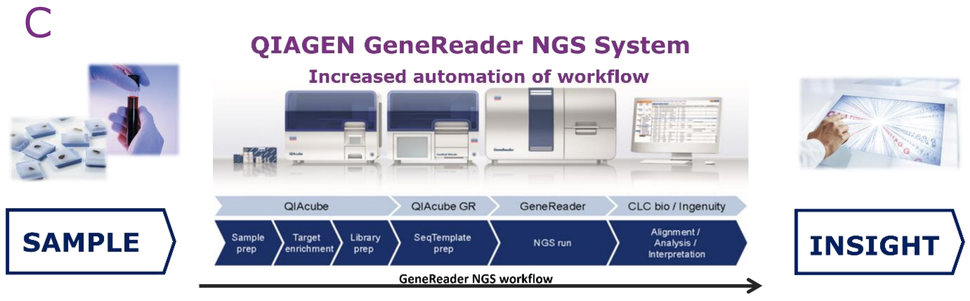
Matched tissue and blood samples were collected from **melanoma and lung cancer** patients.

B



Matched tissue samples from **colorectal cancer** were subjected to fixation by formalin and the PAXgene® Tissue System and embedded in paraffin.

C



D

Actionable Insights

GeneReader Actionable Insights Tumor Panel (GRTP-101X)

Breast | **Ovarian** | **Colorectal** | **Lung** | **Melanoma**

12 genes including 773 unique variant positions

KRAS, NRAS, KIT, BRAF, PDGFRA, ALK, EGFR, ERBB2, PIK3CA, ERBB3, ESR1, RAF1

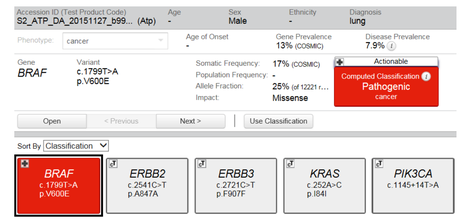
E

Qiagen Clinical Insight Analysis QCI-A

- Track viewer and report with variant table
- Occurrence of each variant of the reads mapped to a reference sequence
- Detection of single nucleotide variants (SNV)

Qiagen Clinical Insight Interpretation QCI-I

- Identify clinically relevant variants for reporting
- Treatment options through automated matching of genetic results to approved treatments and clinical trials



Results

- Despite different sample types (tissue, plasma) and different pre-analytical treatments (formalin fixation and formalin-free fixation for tissue), all samples showed high quality scores in the QC step of the NGS-workflow (data not shown).
- This was followed by successful amplification of target regions. All variants were correctly identified and interpreted.
- These data show the robustness of the results obtained with the GeneReader NGS System and its flexibility and reliability to handle different sample types and sources.

Current status of analysis (sample collection and analysis ongoing)

- Melanoma cases – FFPE and ccfDNA → 24 matched cases sampled, 9 analyzed
- Colon cancer cases – FFPE, PFPE and normal tissue → 15 matched cases sampled, 5 analyzed
- Lung cancer cases – FFPE and ccfDNA (including follow up blood samples) → 30 matched cases sampled, additional 50 blood samples collected, 5 matched cases analyzed

Sample Type	Preanalytical handling	Pathogenic genes	Likely pathogenic	Uncertain sig.	Alles frequency
Case 1 Melanoma					
Melanoma tissue	FFPE	*NRAS (p.Q61K)*		*3x EGFR*	NRAS 43%
Melanoma blood	ccfDNA	*NRAS (p.Q61K)*	*KRAS (V14I)*	*EGFR, PIK3CA, PIK3CA*	NRAS 6%
Case 2 Melanoma					
Melanoma tissue	FFPE	*NRAS (Q61R)*	*BRAF (S467L)*		NRAS 40%,
Melanoma blood	ccfDNA	*KRAS (A59T)*			KRAS 0.56%
Case 3 Melanoma					
Melanoma tissue	FFPE	*NRAS (p.Q61R)*		*2xEGFR, ALK, ERBB3*	NRAS 57%
Melanoma blood	ccfDNA	*NRAS (p.Q61R)*		*EGFR*	NRAS 17%
Case 4 Melanoma					
Melanoma tissue	FFPE	*KRAS (Q61R)*			KRAS 62%
Melanoma blood	ccfDNA	*KRAS (Q61R)*			KRAS 64%
Case 1 CRC					
Colon tumor (TU)	FFPE	*NRAS (Q61R)*		*KRAS*	NRAS 44%
Colon tumor (TU)	PFPE	*NRAS (Q61R)*		*KRAS, KIT*	NRAS 48%
Colon normal tissue	FFPE	*no pathogenic*		*KRAS, KIT*	
Colon normal tissue	PFPE	*no pathogenic*			
Case 2 CRC					
Colon tumor (TU)	FFPE	*KRAS (G12D), PIK3CA (Q546K)*		*ESR1*	KRAS 53%, PIK3CA 52%
Colon tumor (TU)	PFPE	*KRAS (G12D), PIK3CA (Q546K)*			KRAS 45%, PIK3CA 43%
Colon normal tissue	FFPE	*no pathogenic*			
Colon normal tissue	PFPE	*no pathogenic*			
Case 3 CRC					
Rectum tumor (TU)	FFPE	*KRAS (G12D)*		*KIT*	KRAS 43%
Rectum tumor (TU)	PFPE	*KRAS (G12D)*		*KIT*	KRAS 50%
Rectum normal tissue	FFPE	*no pathogenic*			
Rectum normal tissue	PFPE	*no pathogenic*			

QCI Interpret (QCI-I) analysis of selected melanoma and colorectal cancer dataset. Among three matched tissue and blood samples of the melanoma patients, QCI-I classified *NRAS* or *KRAS* mutations as pathogenic variants. Different pathogenic variants of matched FFPE and ccfDNA were detected in melanoma case 2. In the three colorectal cancer cases QCI-I identified concordant pathogenic variants of *NRAS*, *KRAS* or *PIK3CA* in matched FFPE and PFPE tumor tissue. No pathogenic variants were detected in all colorectal normal tissue samples used as negative controls. Likely pathogenic variants and/or variants with uncertain significance were more frequent in the melanoma cases. Allele frequencies of tissue samples ranged between 40-62%. Blood samples showed a broader range between 0,56-64%. Data of lung cancer samples are not shown.

Conclusion

In clinical research, the development of new prognostic and predictive biomarkers often depends on archived or prospectively collected samples that may have been treated using different pre-analytical protocols. The data presented show the advantages of a fully integrated Sample-to-Insight workflow including initial quality control steps and prove the versatility of the GeneReader System for different types of sample materials.

Acknowledgments Work performed by "CBmed" was funded by the Austrian Federal Government within the COMET K1 Centre Program, Land Steiermark and Land Wien.

funded by:

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