The Idylla Molecular Testing System; A Useful Option for the Detection of Clinically Actionable Variants in Challenging FFPE

Hasan Samra, MD; Stephanie Springborn, MS, Alexander Mackinnon, MD,PhD;

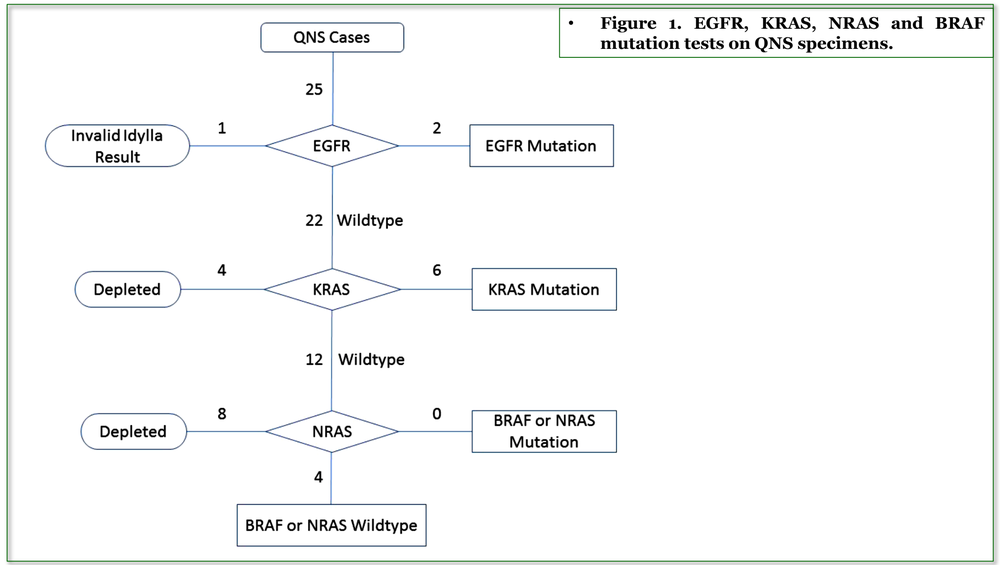
Department of Pathology, Medical College of Wisconsin , Milwaukee, Wisconsin

Introduction

- Optimal care of cancer patients often requires precise pairing of chemotherapeutics with the specific underlying genetic variants present in their tumors.
- To achieve this, DNA is isolated from tumor cells and molecular analysis is used to identify clinically actionable genetic variants. Next generation sequencing (NGS), and to a lesser extent, Sanger sequencing are broadly used for this analysis.
- Often, FFPE specimens submitted for testing are suboptimal and rejected due to insufficient quantity (QNS). Examples include small biopsies with scanty tissue, decalcified bone specimens, and samples with a low percent of tumor.
- To address this common problem, we evaluated the Idylla Molecular Testing System on 46 patient FFPE samples that were suboptimal for clinical NGS or Sanger testing
- .

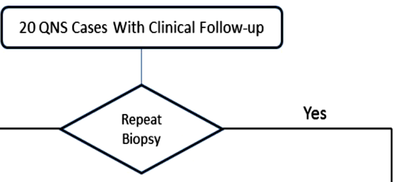
Materials and Methods

- The study cohort consists of 46 FFPE cancer specimens (9 decalcified bone, 37 FNA) previously evaluated and assessed by pathologist as QNS for conventional NGS or Sanger testing.
- Of the bone specimens, 8 had previous NGS results with the remaining case having unsuccessful library preparation.
- Of the QNS specimens, none were previously analyzed due to either paucicellularity or malignant cells representing <5% of the total cellularity.
- We utilized the Idylla™ (Biocartis) System to test these specimens. FFPE tissue sections were directly loaded onto Idylla™ cartridges (KRAS, BRAF or EGFR assay). DNA was isolated and fluorescence-based real-time PCR was performed. Results were evaluated with the Idylla Explore Software.



- **Figure 1. EGFR, KRAS, NRAS and BRAF mutation tests on QNS specimens.**

If Idylla testing been performed on the initial QNS sample



7 Patients

	Initial Testing		If Idylla Testing had been performed on initial QNS sample		
Patient	InitialResult	Therapy	Idylla Result	Actionable result	Affect on Treatment
11	QNS	Standard Therapy	EGFR T790M not detected	Yes	T790M targeted therapy avoided
20	QNS	Standard Therapy	EGFR L858R	Yes	EGFR Targeted Therapy
10	QNS	Standard Therapy	KRAS G12C	No	Standard Therapy
30	QNS	Standard Therapy	KRAS A146	No	Standard Therapy
27	QNS	Standard Therapy	EGFR,KRAS,BRAF WT	No	Standard Therapy
23	QNS	Standard Therapy	EGFR, KRAS WT	No	Standard Therapy
13	QNS	Standard Therapy	EGFR WT	No	Standard Therapy

13 Patients

	Repeat Biopsy and NGS Testing		If Idylla Testing had been performed on initial QNS sample			
Patient	Result	Therapy	Idylla Result	Actionable result ?	Affect on Treatment ?	Perform Repeat Biopsy ?
21	No target Identified	Standard	KRAS G12D	No	No	No
15	No target Identified	Standard	KRAS Q61H	No	No	No
31	ALK Fusion	ALK directed	EGFR, KRAS WT	No	No	Yes
34	BRAF V600E	BRAF directed	EGFR, KRAS WT	No	No	Yes
12	EGFR exon21	EGFR directed	EGFR, KRAS WT	No	No	Yes
16	HER2 amp	HER 2 directed	EGFR, KRAS WT	No	No	Yes
19	KRAS	Standard	EGFR WT	No	No	Yes
28	RET fusion	Standard	EGFR, KRAS WT	No	No	Yes
24	No target Identified	Standard	EGFR, KRAS WT	No	No	Yes
32	No target Identified	Standard	Failed Testing	No	No	Yes
4	No target Identified	Standard	EGFR WT	No	No	Yes
18	No target Identified	Standard	EGFR, KRAS WT	No	No	Yes
36	QNS on repeat	Standard	EGFR, KRAS WT	No	No	Yes

Results

- The indication for testing was BRAF (4 cases) and EGFR (42 cases). Amplification was observed in all cases. The three bone cases with previously known KRAS mutations were also identified with the Idylla assay.
- The previously untested bone case demonstrated amplification with Idylla, and an actionable variant was not identified. Of the QNS samples that were previously rejected for NGS testing, clinically actionable variants were identified in 12% of these cases

Conclusions

- The Idylla Molecular Testing System is an accessible, rapid, and effective testing option for challenging FFPE specimens that are suboptimal for NGS or other conventional methods.
- A limitation of this system is the inability to discern true negative results from false negative results.
- In addition, true positive results are difficult to confirm by alternative methods or by repeat testing due to lack of sensitivity and the potential lack of residual tissue, respectively.

References:

[1] Benson AB, Venook AP, Cederquist L, Chan E, Chen Y-J, Cooper HS, et al. Colon Cancer, Version
1.2017, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2017;15:370–98.
[2] Lindeman NI, Cagle PT, Aisner DL, Arcila ME, Beasley MB, Bernicker EH, et al. Updated Molecular
Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine
Kinase Inhibitors. J Mol Diagnostics 2018;20:129–59. doi:10.1016/j.jmoldx.2017.11.004.
[3] Coit DG, Thompson JA, Algazi A, Andtbacka R, Bichakjian CK, Carson WE, et al. NCCN Guidelines
Insights: Melanoma, Version 3.2016. J Natl Compr Canc Netw 2016;14:945–58.
[4] Tan LY, Walker SM, Lonergan T, Lima NE, Todd AV, Mokany E. Superior Multiplexing Capacity of
PlexPrimers Enables Sensitive and Specific Detection of SNPs and Clustered Mutations in qPCR.
PLoS One 2017;12:e0170087. doi:10.1371/journal.pone.0170087.
[5] de Biase D, de Luca C, Gragnano G, Visani M, Bellevicine C, Malapelle U, et al. Fully automated
PCR detection of KRAS mutations on pancreatic endoscopic ultrasound fine-needle aspirates. J Clin Pathol 2016;69:986–91. doi:10.1136/jclinpath-2016-203696.
[6] Weyn C, Van Raemdonck S, Dendooven R, Maes V, Zwaenepoel K,