Analytical validation of the RESOLUTION ctDx FIRST plasma assay as a companion diagnostic for adagrasib and its application to longitudinal monitoring

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ASCO 2022
POSTER # 384060

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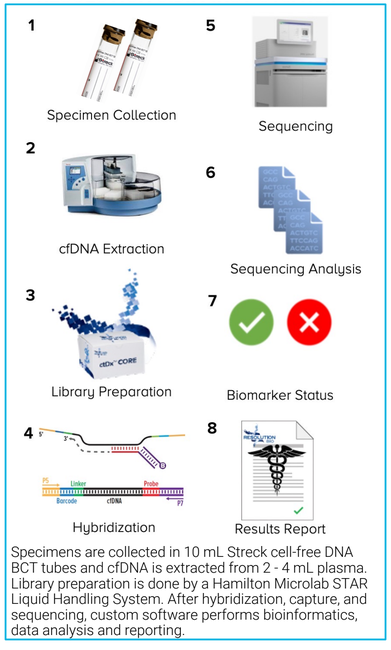
Introduction

RESOLUTION ctDx FIRST (IUO) is a targeted-hybrid capture, next generation sequencing (NGS) liquid biopsy assay which uses circulating cell-free DNA (cfDNA) isolated from plasma.

It is under development as a CDx in the identification of NSCLC patients who may potentially benefit from adagrasib: an investigational, highly selective, oral small molecule inhibitor of *KRAS* G12C (Mirati Study 849-001).

RESOLUTION ctDx FIRST assay allows sensitive detection of alterations in the genes listed in Fig 1. Isolating cfDNA from blood is minimally invasive and can inform clinical decisions and is of special benefit to NSCLC patients, many without lesions accessible by tissue biopsy testing.

RESOLUTION ctDx FIRST Overview



Specimens are collected in 10 mL Streck cell-free DNA BCT tubes and cfDNA is extracted from 2 - 4 mL plasma. Library preparation is done by a Hamilton Microlab STAR Liquid Handling System. After hybridization, capture, and sequencing, custom software performs bioinformatics, data analysis and reporting.

RESOLUTION ctDx FIRST

Main Features	Intended Use
113 Genes	Identify NSCLC patients with *KRAS* G12C mutations as candidates for treatment with adagrasib
74 genes with full CDS coverage	
Targeting ~2096 pathogenic hotspots	
LPWG	
MSI	
dMMR	
23 genes with deletion detection	

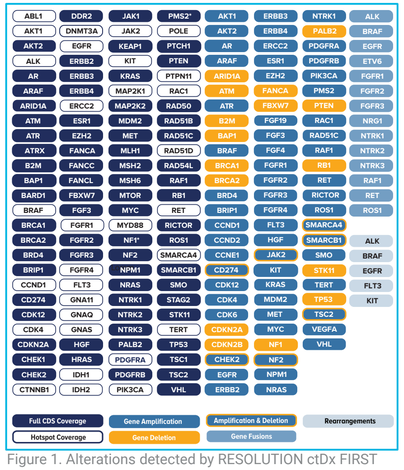


Figure 1. Alterations detected by RESOLUTION ctDx FIRST

Limit of Blank (LOB)

False positive rate was 0% for KRAS G12C, EGFR exon 19 deletions, EGFR L858R, and EGFR T790M.

LOB was established by profiling plasma from 30 individual risk-matched healthy donors, tested 6 times each for a total of 180 results, with multiple lots of reagents, instruments, and operators. Libraries were prepared from 50 ng cfDNA input.

Precision from DNA

PPA: 97.2%-100%. NPA/ANA: 100%

LOD study data were evaluated across multiple reagent lots, sequencers, and operators.

For each variant, the MAF level that was closest to 1.5X and 2–3X LOD was assessed for variant agreement. PPA for all variants and LOD levels: 97.2%-100%. PPAs for each level of the experimental components along with the average percent agreements (APAs) between the different components ranged from 96.7%-100%.

NPA and average negative agreement (ANA) were analyzed from the LOB study. There were no false positives in any of the variant positions, therefore, no evidence of differences between operators, reagent lots or sequencers. Therefore, the best estimates of ANA matched the NPA estimates = 100%.

cfDNA Input

Assay Input Range: 15 – 50 ng cfDNA

6 NSCLC clinical cfDNA sample blends with *KRAS* G12C, *EGFR* L858R or *EGFR* exon 19 deletions.

Each sample was tested 2X below assay minimum input (7.5 ng) to 1.5X above maximum input (75 ng), 50 ng was used as reference condition.

For all variants tested, PPA and NPA was 100% at each input level.

Limit of Detection (LOD)

LOD: Analytical Sensitivity

LOD values were established using NSCLC clinical samples with *KRAS* G12C, *EGFR* exon 19 deletions, *EGFR* L858R, and *EGFR* T790M variants in cfDNA from healthy donor plasma. Samples were serially diluted to six MAF levels: 0.0375 – 4.0% MAF. Each level was tested with 35-36 replicates at input of 15 ng. LoD estimates are listed in Table 1.

Table 1. LOD Estimation

Variant	LOD95 (%MAF)	95% CI (%MAF)
KRAS G12C	0.503%	0.379%, 0.667%
EGFR exon 19 deletions	0.339%	0.241%, 0.476%
EGFR L858R	0.377%	0.289%, 0.493%
EGFR T790M	0.820%	0.680%, 0.989%

Plasma Concordance (Analytical Accuracy)

KRAS G12C: PPA 98.1%, NPA 96.4%

Variant detection of 230 NSCLC plasma samples compared against an externally validated ddPCR assay.

- 76 *KRAS* G12C+ from Mirati Study 849-001 (enrolled with tissue result).
- 154 commercially procured and representative of trial population.

Table 2. Concordance of *KRAS* G12C Between RESOLUTION ctDx FIRST and ddPCR Assays

Variant	PPA (N) [95% CI]	NPA (N) [95% CI]
KRAS G12C	**98.1%** (53/54) [90.1, 99.9]	**96.4%** (163/169*) [92.4, 98.7]

*Variants observed in the RESOLUTION ctDx FIRST but not in the ddPCR assay were very low frequency (< 0.4% MAF) and enrolled with a tissue result.

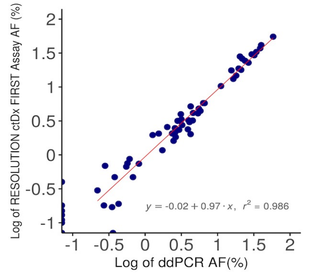


Figure 2. MAF correlation between RESOLUTION ctDx FIRST assay and ddPCR assay for *KRAS* G12C

EGFR: PPA 88.9 – 100%, NPA 97.8 – 100%

Detection of *EGFR* exon 19 deletions, L858R, and T790M variants was compared against the results of the externally validated ddPCR assay.

- 178 NSCLC plasma samples generated a total of 330 comparative valid results.

Table 3. Concordance of *EGFR* variants Between RESOLUTION ctDx FIRST and ddPCR Assays

Variant	PPA (N) [95% CI]	NPA (N) [95% CI]
EGFR L858R	**100%** (29/29) [88.1, 100]	**97.8%** (88/90*) [92.2, 99.7]
EGFR T790M	**88.9%** (8/9) [51.8, 99.7]	**100%** (89/89) [95.9, 100]
EGFR exon 19 Deletions	**92.9%** (26/28) [76.5, 99.1]	**100%** (85/85) [95.8, 100]

*Variants observed in the RESOLUTION ctDx FIRST but not in the ddPCR assay were very low frequency (< 0.3% MAF).

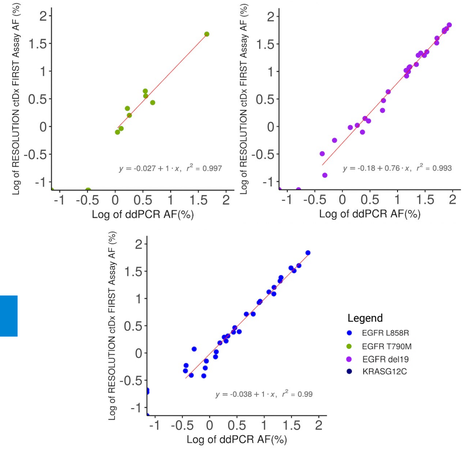


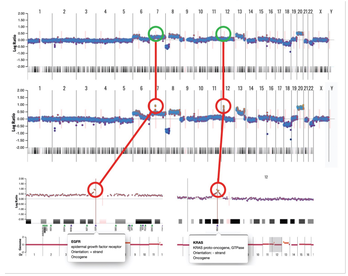
Figure 3. MAF correlation between RESOLUTION ctDx FIRST assay and ddPCR assay for *EGFR* variants

Longitudinal Monitoring: CRC Patients at progression developed resistance and new driver mutations

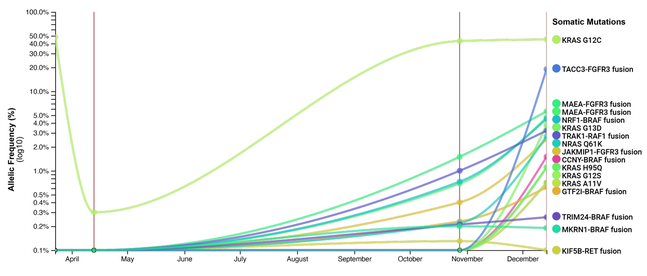
Patient 1 developed emergent high-level copy gain of *KRAS* and *EGFR* which can potentially enable a treatment decision at progression for the initiation of an EGFR inhibitor (e.g. cetuximab).

Patient 2 developed resistance (*KRAS* H95Q) as well as multiple new drivers including 11 functional gene fusions in multiple genes.

Patient 1: EGFR/KRAS Amplifications



Patient 2: Resistance and Emerging Drivers



Tissue Concordance

KRAS G12C Concordance: PPA 71.8%, NPA 100%

Variant detection of NSCLC plasma samples compared against tissue results.

- 71 *KRAS* G12C+ from Mirati Study 849-001 with previous tissue result.
- 74 commercially procured and representative of trial population.

CAUTION:

Investigational device. Limited by Federal (or United States) law to investigational use.

Supported by funding from The Expect Miracles Foundation and Mirati Therapeutics, Inc.

Conclusions

The analytical performance of the RESOLUTION ctDx FIRST assay offers highly sensitive, specific, and robust test results and meets analytical requirements for the intended use.