Amplicon-based next-generation sequencing (NGS) of plasma cell-free DNA (cfDNA) for detection of driver and resistance mutations in advanced NSCLC

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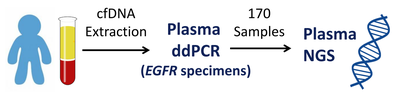
Background

- Several studies have evaluated hybrid-capture NGS for cfDNA genotyping, but this approach has some limitations[1]
- **Amplicon-based NGS** is an attractive alternative with the potential to be faster and less expensive
- We performed a **blinded evaluation** of this approach for the characterization and monitoring of the molecular profile of advanced NSCLC during genotype-directed therapy

Methods

- 170 specimens from 47 pts were studied:
  - 145 from 31 advanced *EGFR* mutant NSCLC with **T790M** acquired resistance to EGFR-TKI
  - 25 specimens from 16 patients with other **rare genotypes**
- **ddPCR** for *EGFR* mutations was performed previously as part of ongoing research (DF/HCC protocol #14-147)[2]
- For each case, up to 8 specimens were analyzed, blinded to tumor genotype:
  - Pre-treatment
  - Initial follow-up draw
  - Subsequent follow-up draw(s)
  - Progression on TKI

- **Plasma NGS** was performed using the Inivata Liquid Biopsy Platform, Invision™[3,4], which uses a combination of tagged amplicon sequencing and statistically-based analysis algorithms to identify and quantify low frequency tumor-derived single nucleotide variants (**SNVs**), short insertions/deletion (indels), copy number variants (**CNVs**) as well as structural variants of *EML4/ALK* fusions and *ROS1* **fusions**. 36 cancer-related genes are examined using gene specific primers designed to hotspots and entire coding regions of interest.
- Plasma NGS results were reported blinded to any existing information on tumor and plasma genotyping
- Diagnostic accuracy was compared to tumor genotyping (including tumor NGS when available)



Inivata NGS panel

Genes established to be relevant to *EGFR* acquired resistance

EGFR	*MET*	*BRAF*	*ERBB2*	*PIK3CA*	*PTEN*

Other key genes relevant to lung cancer biology

ALK	*ROS1*	*RET*	*KRAS*	*MYC*	*FGFR1*
FGFR 2	*AKT1*	*FGFR3*	*NRAS*	*TP53*	*STK11*

Other cancer-related genes

GNAS	*HRAS*	*CTNNB1*	*U2AF1*	*FOXL2*	*CCND1*
IDH1	*GNA11*	*GNAQ*	*KIT*	*MED12*	*ESR1*
IDH2	*PP2R1A*	*PDGFRA*	*NFE2L2*	*CDKN2A*	*NTRK3*

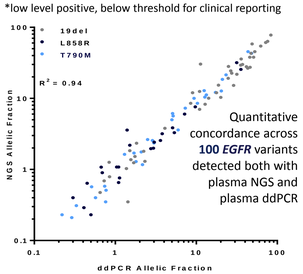
Results

A. Amplicon-based Plasma NGS Is Highly Sensitive For The Detection Of A Full Range Of Genotypes

Tissue Genotype	Driver (AF%)		T790M(AF%)	
	ddPCR	NGS	ddPCR	NGS
	n=26		n=26	
L858R + T790M	1.1	0.65	0	0.28
L858R + T790M	0.7	1	0.45*	0
Del19 + T790M	0.65	0.47	0	0
L858R + T790M	0.5	0.23	0.4	0.57
L858R + T790M	0	0.057	0	0
Del19 + T790M	0	0.2	0.35*	0
Del19 + T790M	0	0.05	0	0
Sensitivity	**88.5%**	**100%**	**84.5%**	**77%**

Sensitivity of plasma NGS and plasma ddPCR for the detection of low AF ***EGFR*** mutations, compared to tissue. All other variants were detected by both ddPCR and NGS.

*low level positive, below threshold for clinical reporting



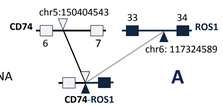
Quantitative concordance across **100 *EGFR*** variants detected both with plasma NGS and plasma ddPCR

Tissue Genotype (SNVs)	NGS
BRAF V600E	*KRAS* G12D (0.12)**
BRAF V600E	none
BRAF G469A	*BRAF* G469A (12.8)
MET exon 14	MET exon 14 (1.4)
MET exon 14	MET exon 14 (12.1)
HER2 exon 20 ins	HER2 exon 20 ins (0.3)
Sensitivity	**66.7%**

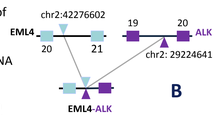
Sensitivity for the detection of **rare genotypes**

** secondarily tested negative by ddPCR for *KRAS* and *BRAF*

A: Example of a *CD74/ROS1* **fusion** gene detected in cfDNA



B, C: Examples of a *EML4/ALK* **fusion** gene detected in cfDNA



Plasma NGS can detect structural variants of *EML4/ALK* and *ROS1* **fusion** genes with a **89%** sensitivity (6/7 ***ALK***, 2/2 ***ROS1***).

B. False Positives Are Rare With Amplicon-based Plasma NGS

Tables list all mutations detected in genes besides the driver, limited to genes regions sequenced with both assays

Driver	Baseline tissue NGS	Baseline plasma NGS
EGFR	*TP53* A161T (55%)	none
EGFR	*TP53* R110L (56%)	*TP53* R110L (7.5%)
EGFR	*TP53* 166_167GA>A (64%)	*TP53* frameshift (2.3%)
EGFR	none	none
EGFR	*TP53* F134L (37%)	TP53 F134L (6.6%)
EGFR	none	*PIK3CA* E545K (0.6%)*
EGFR	*TP53* L43* (32%)	*TP53* L43 (8%)
EGFR	*TP53* C242F (76%)	*TP53* C242F (7.8%)
BRAF	*STK11* p.D343N (30%)	*STK11* p.D343N (50%)
MET	*TP53* R248W	*TP53* R248W (0.6%)
ROS1	none	none
ROS1	none	none
ALK	none	none
ALK	*TP53* R337C	*TP53* R337C (1.2%)

Driver	Resistance Tissue NGS	Resistance Plasma NGS
EGFR	TP53 R282W (44%)	TP53 R282W (27%)
EGFR	KRAS Q61K (30%) TP53 Q104* (53%)	KRAS Q61K (2.8%) TP53 Q104* (12%)
EGFR	none	none
EGFR	TP53 H193R (10%)	TP53 H193R (27%) CTNNB1 (S37F 0.3%, S45C 1%, S45F 0.7%)
EGFR	TP53 del (53%)	TP53 frameshift (1.21%) IDH1 (0.33%)**

Composite specificity calculated at **99.6%** (i.e. the maximum number of false positive calls was 3 of 684 genes sequenced)

*The one suspected false positive, ***PIK3CA*** E545K, was then confirmed with ddPCR (AF 0.6%) and could be due to tumor heterogeneity

Detection of a ***IDH1* mutation at low and constant AF, not following the same kinetics of other tumor-derived mutations, suggests a hematopoietic origin (e.g. CHIP).

C. Detection Of Acquired Resistance Mechanisms, Including Copy Number Variations

EGFR Patients with loss of T790M (n=7/15 (47%))

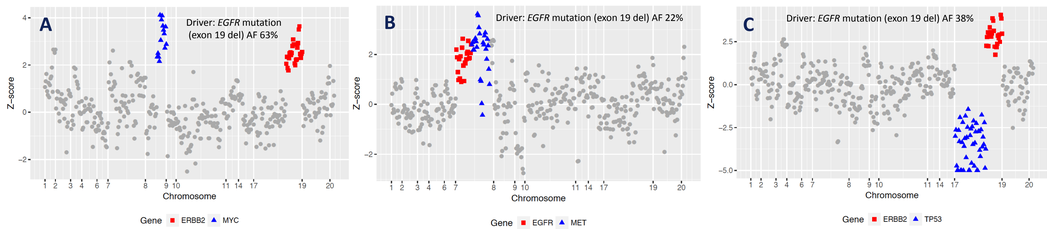
driver	Baseline NGS	Resistance NGS
Del19 Fig. A,D	***BRAF*** V600E (0.60%)	***BRAF*** V600E (12.4%) ***HER2*** amp
Del19	***BRAF*** V600E (6.5%) ***PTEN*** del (0,2%)	***BRAF*** V600E (AF: 0.4%)
Del19 Fig. E	***PIK3CA*** E545K (2.75%)	***PIK3CA*** E545K (1.17%)
Del19 Fig. B	none	***MET*** amp
Del19 Fig. C	***PIK3CA*** E545K (0.6%)	***HER2*** amp
Del19	none	***FGFR1*** amp
L858R	none	***KRAS*** G12S (0.21%)

EGFR Patients with maintained T790M (n=8/10, 80%)

Driver	Baseline NGS (beside driver and T790M)	Resistance NGS (beside driver and T790M)
Del19 Fig. F	none	***EGFR*** C797S 26.6%
Del19 Fig. G	***KRAS*** Q61K (0.12%)	***EGFR*** C797S 7.9% ***KRAS*** Q61K (2.8%)
Del19	none	***EGFR*** C797S 0.4% ***PTEN*** Y27C (21.9%) ***KRAS*** G13D (0.25%)
L858R	none	***EGFR*** C797S T_A 1.27% ***EGFR*** C797S G_C 1.11%
L858R	none	***EGFR*** C797S T_A 2.7% ***EGFR*** C797S G_C 1% ***EGFR*** Q791P A_C 2.6%
del19	none	***BRAF*** V600E 0.4% ***EGFR*** C797S T_A 13.5%
del19	none	***EGFR*** C797S T_A 2.9%
del19	none	***EGFR*** C797S T_A 6.2% ***EGFR*** C797S G_C 0.9%

Patients with rare genotypes (n=1/7 (14%))

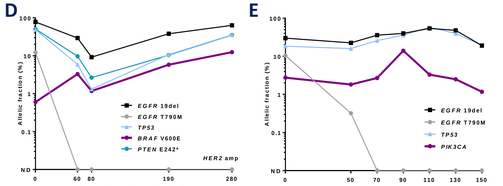
Driver	Baseline NGS	Resistance NGS
ALK	*EML4-ALK*	*ALK* p.C1156Y 0.55



Patients with acquired **amplifications** at the time of resistance to osimertinib. **A**: Patient with acquired ***ERBB2*** and ***MYC*** amplification (driver AF 63%) **B**: Patient with acquired ***ERBB2*** amplification and ***TP53*** deletion at resistance (driver AF 38%). **C**: Patient with *EGFR* amplification and acquired ***MET*** amplification (driver AF 22%)

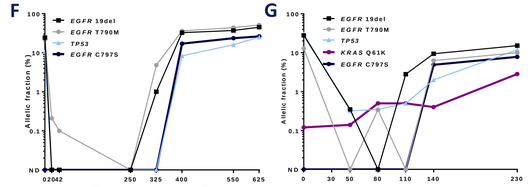
D. Early Detection Of Resistance in *EGFR* patients

Early detection of non-EGFR competing mutations



Patients with **mixed response**, defined by rapid and complete response on the T790M clone and incomplete response on the driver. Plasma NGS can detect at **baseline** competing mutations: ***BRAF*** V600E mutation in **patient D**; ***PIK3CA*** E545K mutation in **patient E**.

Early detection of EGFR C797S tertiary mutations



Patients with acquired ***EGFR C797S*** tertiary mutations detected several months before progression. Plasma NGS additionally early detect a competing ***KRAS*** mutations in **patient G**. This KRAS mutation can be detected at low AF at baseline and re-emerge at resistance.

Conclusion

- In this blinded retrospective validation, amplicon-based plasma NGS could detect a full range of genotypes, including gene rearrangements with an **exquisite sensitivity** and a high **specificity**.
- This is in particular the first report of the detection of ***ALK*** **and *ROS1* fusions** using amplicon sequencing, with a high sensitivity
- Quantitative concordance with ddPCR is excellent
- Serial plasma NGS can detect **emergence of common resistance mutations**
- Acquired ***KRAS* mutation** appears to be a newly identified recurring resistance mechanism to osimertinib
- In a subset of patients, resistance mutations can be detected at baseline, creating an opportunity for the study of **targeted therapy combinations**

References

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Acknowledgments

This research was supported in part by the Expects Miracle Foundation, the Damon Runyon Cancer Research Foundation, the National Cancer Institute at the National Institutes of Health (R01 – CA135257), the U.S. Department of Defense (LCRP Career Development Award), the Anna Fuller Fund, and the Stading-Younger Cancer Research Foundation Thoracic Oncology Fund.