RESISTANCE MECHANISMS TO BRAF INHIBITION IDENTIFIED BY SINGLE CIRCULATING TUMOR CELL AND CELL-FREE TUMOR DNA MOLECULAR PROFILING IN *BRAF*-MUTANT NON-SMALL-CELL LUNG CANCER

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Abstract N#598

BACKGROUND

- Combination therapy with dabrafenib + trametinib demonstrated robust activity in patients (pts) with *BRAF*-V600E-mutant advanced non-small cell lung cancer (NSCLC)[(1)], but its resistance mechanisms are poorly known [(2,3)].
- Non-invasive methods including circulating tumor cells (CTCs) are crucial to develop for the implementation of precision medicine in the treatment of NSCLC.
- Liquid biopsy components such as CTCs and cell-free (cf) tumor DNA can provide a comprehensive genomic picture of tumor content [(4)].

AIM

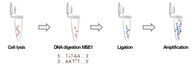
- Molecular profiling of single CTCs from patients with *BRAF*-V600E-mutant NSCLC was performed to carry out a pilot study to identify resistance mutations at failure to dabrafenib + trametinib and to compare the mutations detected on CTCs to the mutations found on cfDNA and tumor biopsies

METHODS

- Eight patients with **advanced *BRAF*-V600E-mutant NSCLC** at failure to dabrafenib plus trametinib were prospectively enrolled between Jul 2018 and Mar 2019 at Gustave Roussy (IDRCB2008-A00585–50). Bloods samples (30-50mL) were collected and matched tissue-cfDNA were available for some patients
- **Single CTC isolation strategy** included RosetteSep enrichment, immunofluorescent staining (Hoechst33342/CD45/pan-cytokeratins) and fluorescence activated cell-sorting (**Fig. 1**)

Figure 1. CTC isolation process

- The process **to identify CTC mutations** included **Ampli1 whole-genome amplification**, quality controls, multiplex targeted PCR with the **Ampli1 CHPCustomBeta cancer panel** developed by (Menarini Silicon Biosystems) and next-generation sequencing (NGS) (**Fig. 2**)



	Gene	Chrom	Amplicon Length (nt)
A	KRAS	12p	91 bp
B	D582117	6q	147 bp (106-166 bp)
C	TP53	17p	301 bp
D	CK19	6q	614 bp

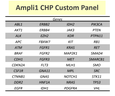


Figure 2. CTC molecular profiling

- **Liquid biopsies (cfDNA)** were analyzed using InVisionFirst®-Lung
- **Tissue samples** were analyzed using targeted NGS in the MATCH-R trial (Recondo G; NPJ Precis Oncol 2020)
- **Clinical data**: clinical and molecular data were collected

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RESULTS

Study population & Samples

- **A total of 7 patients were studied**
- **The median of Hoechst33342+/CD45-/pan-cytokeratins+ CTCs isolated by patient was 20 (8-28)**
- Matched tissue-CTCs for 4 patients
- Matched tissue-cfDNA-CTC were available for 4 patients (**Table 1**)
- Baseline characteristics of the study population is summarized in **Table 2**

	CTC	cfDNA	Tissue
N#1	Yes	-	Yes
N#2	Yes	Yes	Yes
N#3	Yes	Yes	Yes
N#4	Yes	Yes	-
N#5	Yes	Yes	-
N#6	Yes	Yes	Yes
N#7	Yes	-	-

Table 1. Type of samples available for analysis at the same timepoint of CTC collection

	Age (years)	Gender	Smoking	Histology	N# mts sites	Line of therapy	Progression to therapy	PFS (months)	Treatment duration (months)	CT60C, cellSearch (/7.5mL)	CTCs, FACs (/30mL)
N#1	65	Female	Non-smoker	Adeno	≤2	1st line	Dabrafenib + Trametinib	35	46	0	25
N#2	69	Female	Smoker	Adeno	≤2	2nd line	Dabrafenib + Trametinib	13	17	14	16
N#3	58	Male	Smoker	Adeno	≤2	1st line	Dabrafenib + Trametinib	10	13	NA	23
N#4	62	Male	Smoker	Adeno	≤2	2nd line	Dabrafenib + Trametinib	49	30	0	17
N#5	68	Male	Smoker	Adeno	>2	2nd line	Dabrafenib + Trametinib	6,4	7	0	8
N#6	81	Female	Non-smoker	Adeno	≤2	1st line	Dabrafenib + Trametinib	14	16,4	3	26
N#7	69	Male	Non-smoker	Adeno	≤2	2nd line	Dabrafenib + Trametinib	60	ongoing	0	23

Table 2. Baseline characteristics of the study population.

High heterogeneity in CTCs at PD to BRAFi + MEKi

- **A wide spectrum of mutations in CTCs was observed at treatment failure that were involved in the main cancer pathways**
- Among them,
  - MAPK pathway (n=3; *NRAS, KRAS,…*)
  - Protein kinase pathways (n=4; *EGFR, ALK,…*)
  - DNA repair pathways (n=2; *AKT1, ATM,…*),
  - Tumor suppressor gene (n=5; *TP53*)

	CTCs, FACs (/30mL)	Molecular profiling	BRAF^{V600E} detected	MAPK pathway	PI3KCA pathway	DNA repair	Protein kinases	Signal transducers	Chromatin remodeling	Tumor suppressor genes	JAK-STAT pathway
N#1	25	3 CTC	-				■		■		■
N#2	16	5 CTC	-	■			■	■		■	
N#3	23	3 CTC	Yes			■				■	
N#4	17	5 CTC	-				■			■	
N#5	8	1 CTC	-							■	
N#6	26	2 CTC	-					■		■	
N#7	23	6 CTC	-	■	■	■	■				

Table 3. Cancer pathways involved in CTC samples by patient (molecular profiling of 1-6 CTC/patient)

Cancer Pathways altered in CTCs in *BRAF*^{V600E}NSCLC

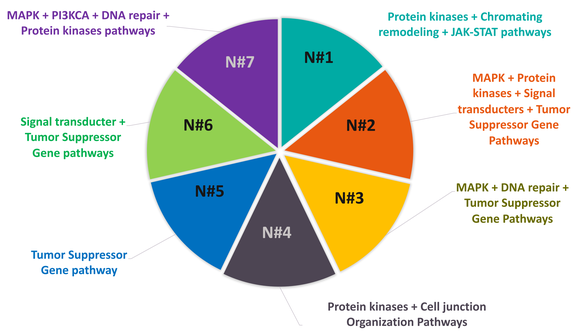

Figure 3. Cancer pathways involved, according to the genomic alterations identified in each patient (molecular profiling of 1-6 CTC/patient); each sector correponds to one patient.

Genomic alterations detected in CTCs in *BRAF*^{V600E}NSCLC

- **In the same CTC, several mutations were observed in 5/7 patients, commonly involving more than one cancer pathways**
- The most common genomic alterations were ***TP53*, followed by *EGFR, ATM*** and genes involved on the **MAPK pathway** *(NRAS, KRAS, BRAF)*

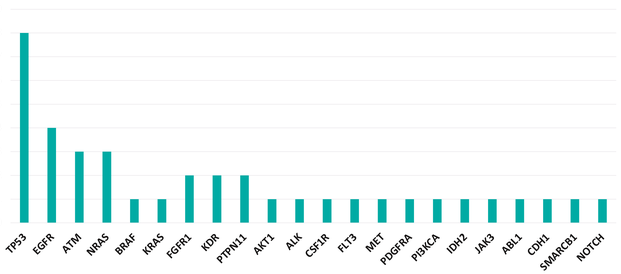

Figure 4. Description of the genomic alterations identified in the overall population (Number of cases)

CTC, cf-DNA & tissue analysis in *BRAFV600E*NSCLC

- A **higher degree of mutational diversity** was also observed in CTCs compared to tumor tissue biopsies and cfDNA
- **BRAF^{V600E} was only detected in one CTC (N#3)**
- In the **3 patients** with an available tumor/liquid biopsy, only one shared mutations between CTCs & matched tumor and cfDNA
- In the **4 patients** with an available liquid biopsy for CTC/cfDNA analysis, only one share mutations between CTCs & matched cfDNA

	CTC	cfDNA	Tissue (NGS)	Concordance for *BRAF* mutation	Concordance for other aterations
N#1	**BRAFV600E:** not detected **Other mutations:** *FGFR, JAK3, ABL1, SMARCB1*	-	**BRAFV600E:** detected **Other mutations:** *NRAS, AKT1, NRAS*	Non	Non
N#2	**BRAFV600E:** not detected **Others:** *EGFR, NRAS, KRAS, PTPN11, FLT3, MET, TP53, FBXW7*	**BRAFV600E:** detected **No other mutations**	**BRAFV600E:** detected **No other mutations**	Non	Non
N#3	**BRAFV600E** detected **Others:** *TP53, ATM*	**BRAFV600E:** detected **Others:** *TP53 (ATM: not covered)*	**BRAFV600E:** detected **Others:** *TP53*	Yes	Yes *(for TP53 variant)*
N#4	**BRAFV600E:** not detected **Others:** *EGFR, FGFR1, CSF1R, MET, TP53, CDH1*	**None detected**	-	Non	Non
N#6	**BRAFV600E:** not detected **Others:** *IDH2, TP53*	**BRAFV600E:** detected **Others:** *KRAS, TP53*	-	Non	Yes *(for TP53 variant)*
N#7	**BRAFV600E:** not detected **Others:** *KDR, AKT1, ALK, PDGFRA, PI3KCA, ATM*	-	-	NA	NA

Table 4. CTCs, cfDNA and tissue concordance in the study population

CONCLUSION

- **Single CTC profiling reveals a wide spectrum of therapeutic resistance mutations not detected by other analyses in pts with *BRAF*^{V600E}-mutant NSCLC at failure to dabrafenib plus trametinib**
- **Importantly, our results also highlighted the high CTC mutational heterogeneity present at resistance to dabrafenib plus trametinib in patients with *BRAF*^{V600E}-NSCLC**
- **Integration of single CTC sequencing to tumor & cfDNA analysis, provides important perspectives to assess heterogeneous resistance mechanisms and to guide precision medicine in *BRAF*^{V600E}- NSCLC**

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