AACR, Atlanta, GA, March 29-April 3, 2019 Abstract # 437

Contact: cmtang@creatvmicrotech.com
301-983-1650

Combining circulating stromal cells with cell free DNA for increased sensitivity in profiling oncogenic mutations and indicates highly aggressive non-small cell lung cancer

Daniel L Adams[1], Steven H Lin[2], Ashvathi Raghavakaimal[1,3], Glenn Weiss[4], Andrew Ford[4], Charmaine Brown[4], Chen-Hsiung Yeh[4]

[1] Creatv MicroTech, Inc., Monmouth Junction, NJ 08852, [2]MD Anderson Cancer Center, Houston, TX 77030,[3]Rutgers, State University of NJ, New Brunswick, NJ 08901,[4]Circulogene, Birmingham, AL 35209

Introduction

Circulating cell free DNA (cfDNA) in the plasma of cancer patients may provide oncogenic mutation status in late stage NSCLC. Recently, specific phagocytic stromal cells found in blood, i.e. cancer associated macrophage-like cells (CAMLs), have been shown to contain large quantities of tumor DNA. We hypothesized that a single blood sample may provide cfDNA and parallel CAML DNA, providing more sensitive tumor mutation screening on a broader array of patients. We screened untreated NSCLC patients with a range of stages (stage I=3, stage II=5, stage 3a=10, stage 3b=7, & stage IV=5), that had available primary tissue for NGS. Blood was drawn prior to induction of radiotherapeutic treatment and plasma was sequenced using a 50 gene oncopanel. Separately, CAMLs were isolated from the plasma cell pellet, lysed and sequenced. Our data suggests that CAMLs contain clinically relevant oncogenic variants that correspond to both the primary tumor and the matched plasma.

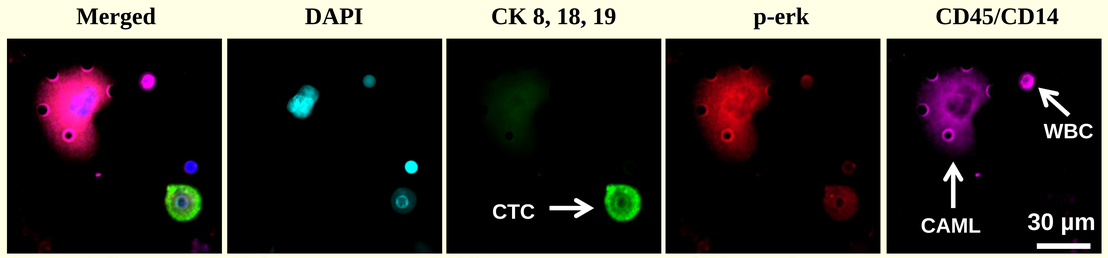
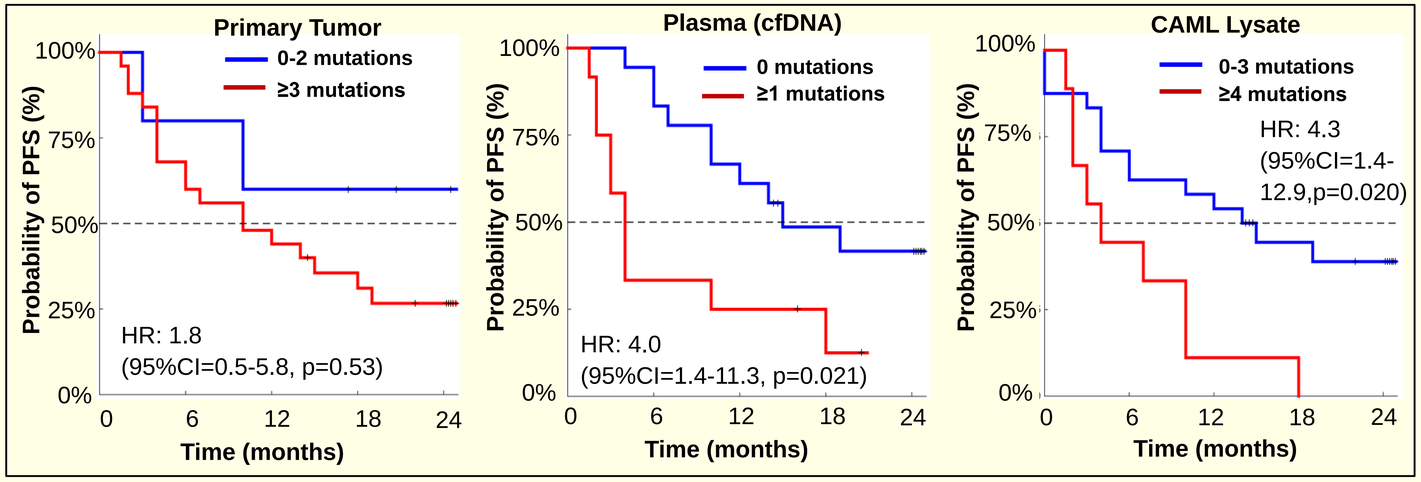


Figure 1. Example of CTC isolated with a CAML in a cancer patient. CTCs are Cytokeratin positive (green) and CD45/CD14 negative. In contrast, CAMLs are CD45/CD14 positive (purple) and may be weakly positive for Cytokeratin (green). White blood cells (WBCs) are normal sized CD45/CD14 positive cells.

Table 1 List of mutations found in primary tumor, plasma, and CAML cell lysate

ID #	Stage	Primary Tumor	Plasma	CAML Lysate
308	1a	EGFR S768I	No mutation detected	No mutation detected
315	1b	TP53 R282W	No mutation detected	No mutation detected
302	1c	CDKN2A H83Y; TP53 F858L	TP53 D281G (2%)	TP53 Y234H 4%
281	2a	KRAS G12V	TP53 L265P 4%; TP53 N131S 11%	KIT K558E 3%; I653T 5%; & V825A 3%; MET D1117G 3%
282	2a	KRAS G12C	No mutation detected	KIT M541L >50%; PTEN C124R 5%
312	2a	NRAS G12C; TP53 V272M	No mutation detected	PIK3CA S405F 3%
329	2b	TP53 V225	**KDR Q472H 10%,** FGFR2 I381V 6%, PDGFRA G829E 4%, PTEN R15G 19%	ABL1 A426V 5%, **KDR Q472H 43%,** KIT K818R 3%, TP53 P72R >50%, & L194P 4%
563	2b	EGFR E709K, EGFR L858R	MET D1117G 3%	MET D1117G 6%, SMAD4 F339S 6%
334	3a	KRAS G12D	APC Q1123* 46% CTNNB1 S45F 7.1%, FBXW7 D440N 46%, PTEN W111R 2%, TP53 D208G 10%, & Y205H 15%, VHL L158P 13%	APC E1577* 14%, EGFR G796S 44%, ERBB2 G776S 7%, PTEN K128N 3%, TP53 P72R >50%
9	3a	No mutation detected	No mutation detected	APC R1105W (>50%), ERBB4 Y285C (2.7%), FBXW7 S462F (3.9%), VHL W117R (2.7%), & F119L (5.7%)
10	3a	TP53 R181P	KIT M541L >50%	PTEN W111R 2%, SMAD4 R445* 37%, TP53 P72R >50%
15	3a	**TP53 P278A**; HNF1A G207C	KIT M541L >50%, EGFR P596L 4%	ATM R337C 6%, PIK3CA H1047R 2%, & I391M 43.8%, PTEN Y174* 5%, RET C634R 2%, **TP53 P278A6%,** P72R >50%, V272M 2%, & c376 2A>G 2%
256	3a	KRAS Q61L; ERBB4 1357V	TP53 K291R 3%, **VHL D121G 2%**	ATM N2875S 17%; KIT M541L >50%; **VHL D121G 3%**
261	3a	EGFR E476-T751del; APC N1108K; APC R337C	No mutation detected	No mutation detected
311	3a	KRAS G12V	No mutation detected	ERBB4 Y285C 4%; PTEN C124R 4% & D331G 7%
347	3a	MET V1007I	**PIK3CA F909L 2%**	**PIK3CA F909L 5%**
547	3a	TP53 R306*, BRAF G469V, PIK3CA E545K	No mutation detected	RB1 L158S 7%, TP53 P72R >50%
561	3a	TP53 T125K; EGFR L747-T751del; PIK3CA N345S	No mutation detected	No mutation detected
284	3b	KRAS G12A	No mutation detected	No mutation detected
289	3b	KRAS G12R	No mutation detected	KDR Q472H (>50%)
293	3b	BRAF V600E; TP53 P151H	No mutation detected	FBXW7 W486* 12%; MET H1112R 2%; MET M1268T 4%; PTEN K66E 2%; & C105R 5%; TP53 P72R >50%
297	3b	KRAS G12D; TP53 R248Q; STK11 P203R	No mutation detected	HRAS G12A >50%
300	3b	No mutation detected	No mutation detected	ATM H2872R 8%; TP53 F270L 9%
341	3b	No mutation detected	No mutation detected	PTEN L57S 12%
344	3b	TP53 E271K	No mutation detected	No mutation detected
2	4	APC G1312V; KRAS G12C; CDKN2A E61*	SMO T640A 2%	CTNNB1 T41A 3%, MET D1117G 5%, SMAD4 F339S 5%, TP53 R290C 18%
7	4	No mutation detected	**KIT M541L >50%,** NOTCH1 V1578del 4%, STK11 G180V >50%	ALK R1275Q 6%, ATM H2872R 4%, EGFR V769M 51%, & E114K 4%, **KIT M541L 50%,** MET K183E 5%, PTEN G165R 10%, SRC Q537* 3%, TP53 R158C 3%
260	4	EGFR T790M; TP53R273C	No mutation detected	ERBB4 D609N 5%; SMAD4 R100G 21%
323	4	EGFR E746-A250 del	**KIT M541L >50%**	CDH1 D402N 3%, **KIT M541L >50%,** RB1 R661W 3%, SMAD4 F339S 7%
504	4	**KRAS Q61H**	APC Y1102H 4%, KIT M541L 4%	**KRAS Q61R 2%;** MET K1262R 5%; PIK3CA K111R 3%

Figure 2. Optimal PFS of mutation number for primary tumor, plasma (cfDNA), and CAML lysate



MATERIALS & METHODS

Whole peripheral blood was taken from 30 newly diagnosed NSCLC patients with confirmed stage I-IV disease in a cfDNA preservative tube. Blood cells were separated from the plasma. CAMLs were purified from the cell pellet using a standard CellSieve™ Assay, which isolates CAMLs based on size separation. Purified CAMLs were enumerated, then removed from the filter and lysed in 150 uL of buffer. The primary tumor biopsies, plasma and lysed CAMLs were blinded, anonymized then sent separately for sequencing against a standard 50 oncogene (~3,000 mutations) NGS panel (Birmingham, AL). Somatic variants were reported based those found in COSMIC database and in frequencies ≥1%. Total mutations in tumor/plasma/CAMLs were used to evaluate progression free survival (PFS) by censored univariate analysis.

RESULTS

- Primary tumor had 43 mutations (average=1.4 variants in 87% of patients). (**Table 1**)
- cfDNA had 28 mutations (average=0.9 variants in 47% of patients)
  - One patient (#302) had TP53 mutations in both cfDNA and tumor, though different variants
- CAML lysate had 78 mutations (average=2.6 variants in 80% of patients)
  - Two patients had variants in TP53 & KRAS that exactly matched in CAMLs and primary tumor
- Patients with higher cfDNA or CAML mutation numbers had lowered PFS, with ≥1 variants in cfDNA (HR=4.0, 95%CI=1.4-11.3, p=0.021) and ≥4 variants in CAMLs (HR=4.3, 95%CI=1.4-12.9, p=0.020) (**Figure 2**).

CONCLUSIONS

- CAMLs represents a population of tumor stroma cells that may promote tumor progression
- Monitoring the mutations of giant CAMLs may predict cancer progression or death
- A single blood sample can screen for oncogenic tumor mutations in plasma and phagocytic stromal cells, increasing sensitivity and specificity in comparison to cfDNA alone.
- High numbers of mutational variants in cfDNA or CAMLs may be indicative of highly aggressive NSCLC.

References

1. Adams DL, et al “Circulating giant macrophages as a potential biomarker of solid tumors.” Proc Natl Acad Sci, 111(9):3514-3519. 2014
2. Cristofanilli M, “Liquid Biopsies in Solid Tumors” *Springer Intl Publish.* 2017
3. Adams DL, et al. “Sequential tracking of PD-L1 expression and RAD50 induction in circulating tumor and stromal cells of lung cancer patients undergoing radiotherapy” Clin Can Res, 23(19): 5948-5958. 2017

Funding Sources

This work was supported by a grant R43CA206840 from the U.S. Army Research Office (ARO) and the Defense Advanced Research Projects Agency (DARPA) (W911NF-14-C-0098). The content of the information does not necessarily reflect the position or the policy of the US Government.