Performance of a high-intensity 508-gene circulating-tumor DNA (ctDNA) assay in patients with metastatic breast, lung, and prostate cancer

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Background

- ctDNA assays can noninvasively assess tumor burden and biology by identifying tumor-derived somatic alterations
- To date, ctDNA studies have focused primarily on detecting driver mutations to inform treatment strategies in advanced disease or monitoring disease burden in patients with established cancer diagnoses. Platforms used for these purposes target individual variants or limited genomic regions informed by sequencing of tumor tissue. (Wan 2017)
- Analysis of plasma cell-free DNA may enable early cancer detection in previously undiagnosed individuals but will require *de novo* variant calling (in the absence of tissue) as well as sufficient genomic coverage to address the spectrum of variant profiles that are cancer-defining. (Aravanis 2017)
- We propose that a high-intensity approach (ultra-deep sequencing of plasma cell-free DNA with broad genomic coverage) will add a new dimension to our understanding of intra-patient and population-level heterogeneity.

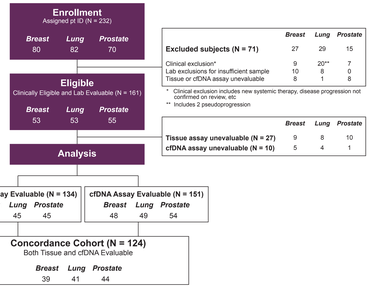
Objectives

- Assess concordance of variants detected in tissue with MSK-IMPACT™ versus detected in plasma cell-free DNA.
- Assess the cell-free DNA variant detection rate based on observing at least one MSK-IMPACT™ tissue variant in the same patient.
- Assess concordance using tissue as a reference according to:
  - Clinical actionability
  - Clonality

Methods: Patient Population

- Metastatic breast, lung, or prostate cancer, either de novo or with progressive disease on current therapy.
- All patients have provided written informed consent to an MSK institutional protocol (NCT01775072) allowing research of cfDNA and clinical tumor sequencing.
- Blood and tissue were prospectively collected within 6 weeks of each other with no intervening therapy change.
  - Two tubes of blood collected in Streck.
  - Tissue from surgical resection or biopsy.
- Blood and tissue were analyzed independently and blinded to the results of each.

Results: Patient Disposition



	Breast	Lung	Prostate
Excluded subjects (N = 71)	27	29	15
Clinical exclusion*	9	20**	7
Lab exclusions for insufficient sample	10	8	0
Tissue or cfDNA assay unevaluable	8	1	8

* Clinical exclusion includes new systemic therapy, disease progression not confirmed on review, etc
** Includes 2 pseudoprogression

	Breast	Lung	Prostate
Tissue assay unevaluable (N = 27)	9	8	10
cfDNA assay unevaluable (N = 10)	5	4	1

Methods: Analysis

cfDNA variant calling pipeline

- The variant calling pipeline includes the following steps:
  - Read alignment, error correction (consisting of read collapsing by position and UMI, as well as stitching paired reads), de novo assembly, variant calling, and variant filtering.
  - Variants are filtered using two approaches (1) heuristics applied based on the surrounding sequence context and type and quality of reads supporting the variant and (2) empirical noise levels observed in a set of healthy samples.
  - Blood samples from an independent cohort of 24 non-cancer (self-reported) individuals (vendor sourced) were used to set a baseline noise level in this study.
  - Variant calls are further filtered using matching WBC for each patient.
  - Bioinformatics filters included removing non-COSMIC dbSNP variants as well as restricting to protein coding regions.

MSK tissue variant calling pipeline has been described extensively in the past (Cheng 2015, Zehir 2017)

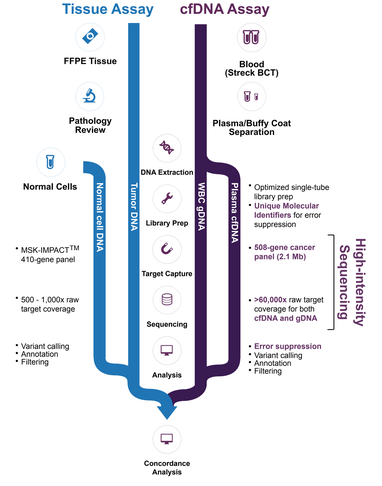
FACETS algorithm and clonality analysis:

- To classify the variants identified in tissue into clonal and subclonal mutations, the cancer cell fraction (CCF) was calculated based on mutant variant allele frequency (VAF) adjusting for tumor purity, ploidy, and local allele-specific copy number obtained from the FACETS algorithm (Shen 2016).
- Exact confidence intervals (CI) were calculated around the point estimate of CCF:
  - Mutations with lower bound of 95% CI ≥75% were classified as "clonal".
  - Mutations with CCF ≥ 80% and lower bound of 95% CI below 75% were classified as "likely clonal".
  - Mutations with CCF < 80% and lower bound of 95% CI below 75% were classified as "subclonal".

Statistical Methods

- Only regions covered by both panels were used for concordance analysis and synonymous variants were excluded from the concordance analysis since they are not called by the MSK-IMPACT™ pipeline.
- Concordance is calculated as positive percent agreement (PPA) with tissue as a reference (cfDNA/tissue).

Methods: Sample Workflows and Assays



cfDNA and tissue assays share 1.2 Mb of targeted sequence.

	cfDNA Assay	Tissue Assay
Inputs	Plasma WBC	FFPE tissue biopsy WBC
Genes	508	410
Breadth (Mb)	2.13	1.36
Raw sequencing coverage	>60,000X (~3-4000 error corrected depth)	500 - 1000X
Enrichment	Hybridization capture	Hybridization capture
Analytical Metrics	SNV/indel detection (30 ng input DNA) Sensitivity*: >95% @ 0.2% MAF >70% @ 0.1% MAF Specificity*: 99.992%	LOD of 2% for hotspot mutations and 5% for non-hotspot mutations

*Analytical sensitivity and specificity determined by cell line and cfDNA titrations, respectively

Patient Characteristics

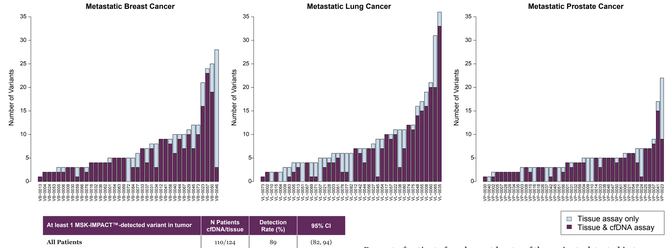
Patient Characteristics	Breast (n=39)	Lung (n=41)	Prostate (n=44)
Age at enrollment			
Mean (SD)	56.5 (11.55)	65.2 (11.18)	67.3 (10.03)
Median	60	67	67
Range	30, 79	33, 83	46, 87
Gender, N (%)			
Female	39 (100.0%)	28 (68.3%)	N/A
# of lines of therapy, N (%)			
0	20 (51.3%)	25 (61.0%)	12 (27.3%)
1	2 (5.1%)	9 (22.0%)	14 (31.8%)
2	2 (5.1%)	3 (7.3%)	9 (20.5%)
>=3	15 (38.5%)	4 (9.8%)	9 (20.5%)
Tissue Sampled for MSK-IMPACT™, N (%)			
Metastatic	35 (89.7%)	28 (68.3%)	44 (100.0%)
Primary	4 (10.3%)	13 (31.7%)	0
Receptor Status, N (%)			
HR+/HER2+	3 (7.7%)	N/A	N/A
HR+/HER2-	26 (66.7%)	N/A	N/A
HR-/HER2+	2 (5.1%)	N/A	N/A
Triple Negative	8 (20.5%)	N/A	N/A

Cancer Characteristics

Histology	N (%)
Metastatic Breast Cancer (n=39)	
Breast Invasive Ductal Carcinoma	32 (82.1%)
Breast Invasive Lobular Carcinoma	2 (5.1%)
Breast Mixed Ductal and Lobular Carcinoma	5 (12.8%)
Metastatic Lung Cancer (n=41)	
Lung Adenocarcinoma	38 (92.7%)
Lung Non-adenocarcinoma	3 (7.3%)
Metastatic Prostate Cancer (n=44)	
Prostate Adenocarcinoma	39 (88.6%)
Prostate Neuroendocrine	5 (11.4%)

Results:

Per Patient Variant Detection | Primary Analysis – Independent cfDNA and Tissue Assessment



At least 1 MSK-IMPACT™-detected variant in tumor	N Patients cfDNA/tissue	Detection Rate (%)	95% CI
All Patients	110/124	89	(82, 94)
Metastatic Breast Cancer Patients	38/39	97	(87, 100)
Metastatic Lung Cancer Patients	35/41	85	(71, 94)
Metastatic Prostate Cancer Patients	37/44	84	(70, 93)

Percent of patients for whom at least 1 of the variants detected in tumor tissue was also detected in plasma:
Breast cancer - 97%, Lung cancer - 85%, Prostate cancer - 84%.

Pooled Variant Detection | Primary Analysis – Independent cfDNA and Tissue Assessment

	All Variants (SNV, indels, CNA, Fusions)		SNV/ indels ONLY		Clinically Actionable Mutations*	
	/tissue	PPA (95% CI)	cfDNA/tissue	PPA (95% CI)	cfDNA/tissue	PPA (95% CI)
Metastatic Breast Cancer Patient	221 / 300	74% (68, 79)	188 / 250	75% (69, 80)	24 / 27	89% (71, 98)
Metastatic Lung Cancer Patient	256 / 355	72% (67, 77)	243 / 321	76% (71, 80)	11 / 18	61% (36, 83)
Metastatic Prostate Cancer Patient	150 / 209	72% (65, 78)	122 / 168	73% (65, 79)	19 / 26	73% (52, 88)
All Patients	627 / 864	73% (69, 76)	553 / 739	75% (72, 78)	54 / 71	76% (64, 85)

PPA = positive percent agreement with tissue as a reference (cfDNA/tissue)

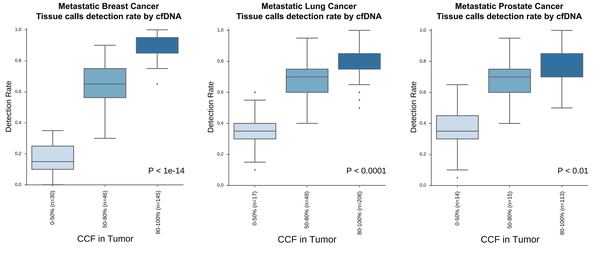
In tissue, pooled across patients, 864 variants were detected across the 3 tumor types, with 627 (73%) also detected in plasma: single nucleotide variants/indels - 75%, fusions - 67%, and copy number alterations - 58%.

*For breast and lung cancer, defined based on OncoKB, precision knowledge base maintained at MSKCC. In breast cancer, variants known to contribute to progression, level 1, 2, or 3 evidence. In lung cancer, variants shown to predict sensitivity or resistance to targeted therapy, level 1 or 2 evidence. In prostate cancer, variants shown to contribute to progression (Chang 2015, Robinson 2015).

Post-hoc Analysis on Clonal Mutations

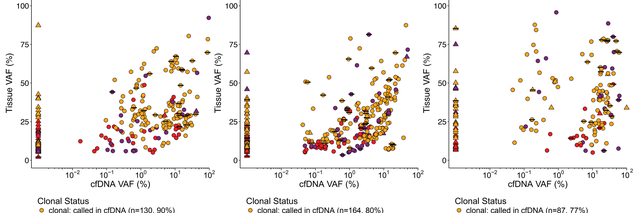
	Number of patients		
	Evaluable	With at least one clonal mutation detected in tissue	At least one clonal mutation detected in cfDNA
Metastatic Breast Cancer	39	36	35 (97%)
Metastatic Lung Cancer	41	37	31 (84%)
Metastatic Prostate Cancer	44	40	33 (83%)

Post-hoc Analysis | Association between Cancer Cell Fraction (CCF) in Tumor and Detection Rate in Plasma

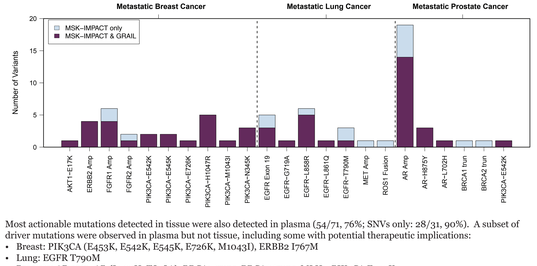


- Clonal variants in tissue were more likely to be detected in plasma than subclonal variants (p<.0001).
- The greater the representation of cancer cell fraction (CCF) from FACETS in the tumor, the higher the detection in plasma.

Post-hoc Analysis | ctDNA VAF versus Tissue VAF by Clonal Status



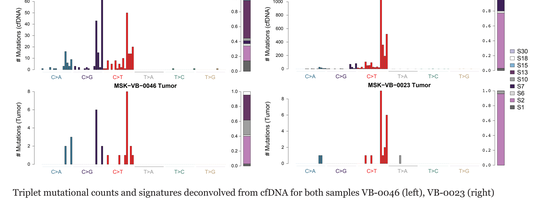
Actionable Mutations in Tissue and Plasma



Most actionable mutations detected in tissue were also detected in plasma (54/71, 76%; SNVs only: 28/31, 90%). A subset of driver mutations were observed in plasma but not tissue, including some with potential therapeutic implications:
- Breast: PIK3CA (E453K, E542K, E545K, E726K, M1043I), ERBB2 I767M
- Lung: EGFR T790M
- Prostate: AR amp, AR (L702H, T878A), BRCA1 trunc, BRCA2 trunc, MLH1, PIK3CA E545K

Consistency of Mutational Signature | APOBEC Hypermutation in Both Tissue and Plasma

- Patient mutational signatures were identified by deconvolving the observed triplet mutation profile of patients using constrained linear regression onto COSMIC mutational signatures (Alexandrov 2013). Patients with evidence for signatures compatible with APOBEC hypermutation were prioritized for cfDNA signature analysis.
- Seven patients (6 breast, 1 prostate) exhibited evidence for increased signature 2 and/or 13 in both tissue and plasma, which are both associated with APOBEC hypermutation; Two patients (MSK-VB-0023 and MSK-VB-0046) shown here.



Triplet mutational counts and signatures deconvolved from cfDNA for both samples VB-0046 (left), VB-0023 (right)

Summary

- Tumor tissue variants identified by MSK-IMPACT™, a validated tumor tissue profiling platform, enabled a demonstration of high overall detection rates (>70%) of the same variants in cfDNA.
- In the majority of patients, at least one mutation detected in tissue was also detected in plasma cfDNA of that same patient (97%, 85%, and 84% in breast, lung, and prostate cancer patients).
- Post-hoc analysis focused on the subset of patients with clonal variants in tissue:
  - Based on de-novo variant calls, at least one clonal mutation was detected in cfDNA: 97%, 84%, and 83% in breast, lung, and prostate cancer patients.
- The majority of clinically actionable mutations detected in tissue were also detected in plasma (54/71, 76%; SNVs only: 28/31, 90%).
- The breadth of detected variants in plasma cfDNA enables greater insight into tumor biology, including observation of hypermutation signatures.

Conclusions

- This novel, high-intensity cell-free DNA sequencing assay incorporates unprecedented breadth (10X number of genes) compared to previous assays at these sequencing depths, and demonstrated high levels of concordance for both clonal and non-clonal variants between plasma and tissue.
- By interpreting concordance as strong evidence for tumor DNA detection, an extremely high level of tumor DNA detection in plasma was demonstrated.
- Clinically actionable non-biopsy somatic alterations were detected, which may represent tumor heterogeneity not detectable in a single tissue biopsy. Ongoing work is being conducted to distinguish technical noise from the assay and biological signal for the variants detected in plasma but not in tissue.
- The breadth of the panel enabled the first exploration of mutational signature analysis in plasma, revealing samples with APOBEC signatures.
- This study is part of a larger program to evaluate high-intensity sequencing approaches (e.g. whole genome) to characterize potential cancer-defining signals in cell-free nucleic acids, with an ultimate goal of enabling detection of cancer at early curable stages.

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