Evaluation of Cell-Free DNA Approaches for Multi-Cancer Early Detection

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DECLARATION OF INTERESTS

Minetta C. Liu

MCL is an uncompensated consultant for GRAIL, Inc. The Mayo Clinic was compensated for MCL's institutional responsibilities for the Circulating Cell-Free Genome Atlas (CCGA) study protocol oversight and advisory board activities for GRAIL, Inc.

MULTI-CANCER EARLY DETECTION TESTS
Ideal Attributes for Use at Population Scale

Achieve benefits of early detection while minimizing harms:

- Low false positives: achieved through high specificity
- Localizing ability: predicts cancer signal origin to inform diagnostic work-up

Circulating Cell-Free Genome Atlas (CCGA) Study

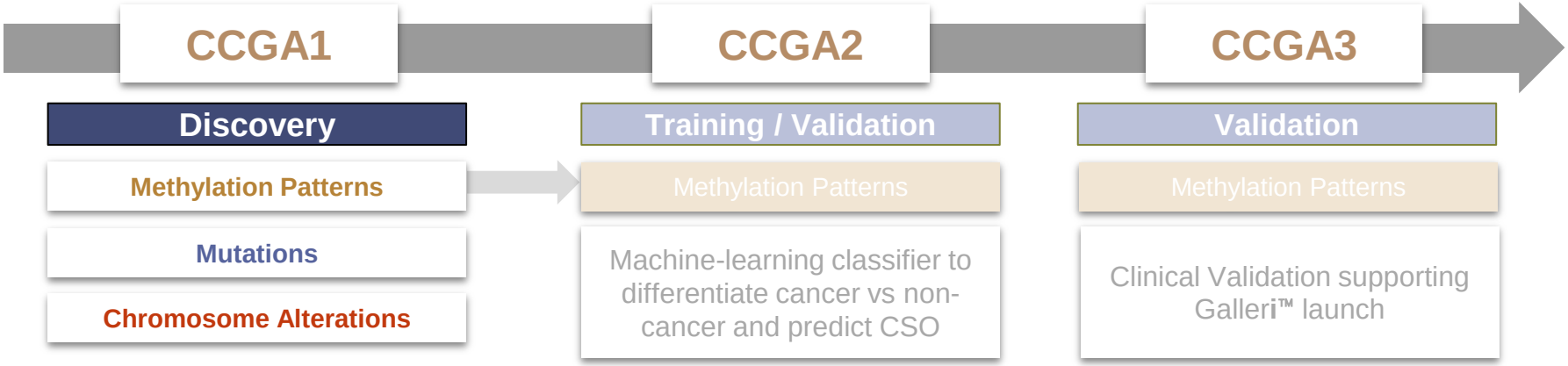
Observational case-control study designed to develop and validate a cfDNA-based MCED test

Study Design

CCGA, Circulating Cell-free Genome Atlas; cfDNA, cell-free DNA; MCED, Multi-Cancer Early Detection

THREE CCGA SUBSTUDIES

CCGA Substudy 1: Multiple Approaches to cfDNA Analysis for Cancer Signal Detection



- CCGA1 was designed to compare cfDNA approaches among the same set of samples
- Tumor tissue was sequenced when available to determine circulating tumor allele fraction (cTAF)

CCGA, Circulating Cell-free Genome Atlas; cfDNA, cell-free DNA; CSO, cancer signal origin; cTAF, circulating tumor allele fraction

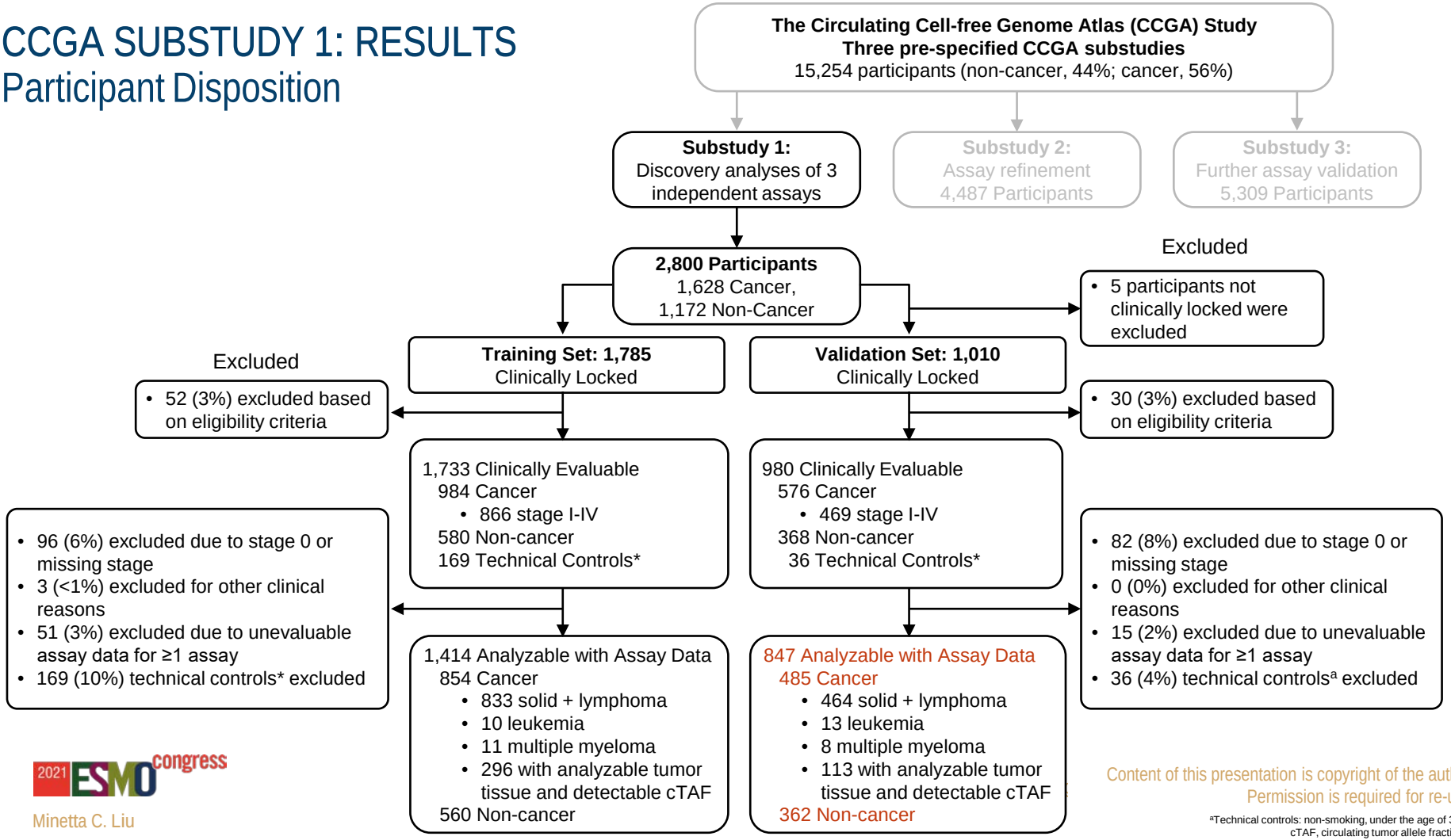
MAPPING ASSAYS TO SAMPLES, FEATURES, AND CLASSIFIERS

Assay	Sample	Feature	Classifier name	
Whole-genome bisulfite sequencing	cfDNA	Whole-genome methylation patterns (≈30 million CpGs)	**WG methylation***	Pan-feature[c]
Targeted mutations covering 507 genes	cfDNA	Single nucleotide variants	SNV	
	WBCs	Single nucleotide variants to remove noise	**SNV-WBC***	
Whole-genome sequencing	cfDNA	Somatic copy number aberrations	**SCNA***	
		Fragment endpoints	Fragment endpoints	
		Fragment lengths	Fragment lengths	
		Allelic imbalance	Allelic imbalance	
	WBCs	Somatic copy number aberrations to remove noise	SCNA-WBC	
	Tumor tissue	Variant calling for allele fraction estimation[a]	_	
None	Clinical data	Age, smoking, family history of breast/ovarian cancer	Clinical data[b]	

*Prototypes that were also evaluated for cancer signal origin prediction are underlined.

[a]Variant allele fraction was used to estimate circulating tumor allele fraction, not in a classifier; [b]The clinical data classifier used only clinical data (no assay data); [c]The pan-feature classifier was trained using scores from each of the cfDNA classifiers. cfDNA, cell-free DNA; SCNA, somatic copy number alterations; SCNA-WBC, somatic copy number alterations with correction for clonal hematopoiesis noise; SNV, single nucleotide variants; SNV-WBC, single nucleotide variants with correction for clonal hematopoiesis noise; WBC, white blood cell; WG, whole-genome.

CCGA SUBSTUDY 1: RESULTS
Participant Disposition



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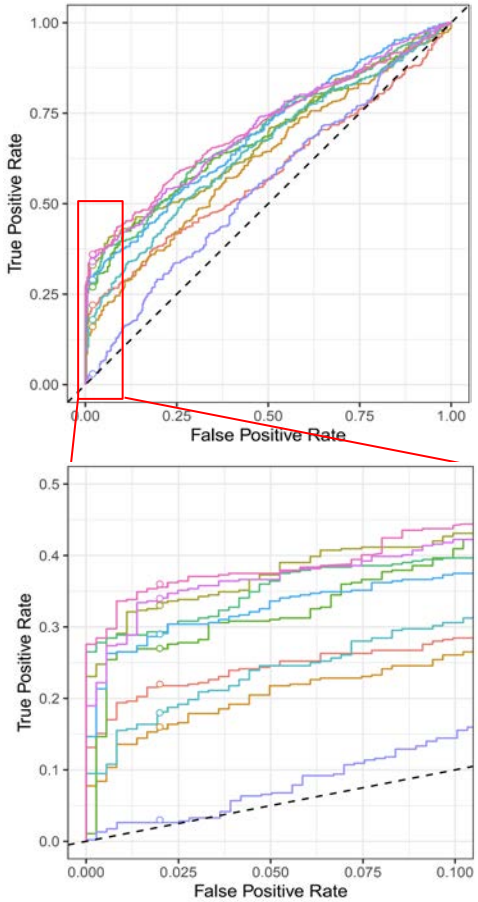
[a]Technical controls: non-smoking, under the age of 35.
cTAF, circulating tumor allele fraction

CCGA SUBSTUDY 1: RESULTS
Cancer Signal Detection

Assay	Classifier	Sensitivity at 98% specificity	
		% (95% CIs)	TP/Total samples, n
WGBS	**WG methylation**	**34% (30%-39%)**	**158/464**
TS	SNV	16% *** (13%-20%)	73/464
	SNV-WBC	**33% (29%-38%)**	**155/464**
WGS	SCNA	27% *** (23%-31%)	125/464
	SCNA-WBC	30% * (26%-34%)	139/464
	Fragment endpoints	18% *** (15%-22%)	84/464
	Fragment lengths	29% * (25%-34%)	135/464
	Allelic Imbalance	22% *** (18%-26%)	101/464
All three	**Pan-feature**	**36% (31%-40%)**	**165/464**
None	Clinical Data	2.6% ***(1.4%-4.5%)	12/457

* p<0.01; ** p<0.001; *** p< 0.0001. p-values were only computed for the validation set and represent paired McNemar analysis versus WG methylation.

Performance



CI, confidence interval; SCNA, somatic copy number alteration; SCNA-WBC, somatic copy number alterations with correction for clonal hematopoiesis noise; SNV, single nucleotide variant; SNV-WBC, single nucleotide variants with correction for clonal hematopoiesis noise; TP, true-positive; TS, targeted sequencing; WG, whole-genome; WGS, whole-genome sequencing

CCGA SUBSTUDY 1: RESULTS
Cancer Signal Origin Prediction Accuracy

Assay	Classifier	Accuracy	Comparison to WG Methylation[a]
WGBS	**WG methylation**	**75% (95/127)**	--
TS	**SNV-WBC**	**35% (44/127)**	$p=6.5x10^{-12}$
WGS	**SCNA**	**41% (52/127)**	$p=8x10^{-9}$

- WG methylation predicted CSO with significantly more accuracy than either SNV-WBC or SCNA
- CSO prediction accuracy of the SNV-WBC classifier versus the SCNA classifier was not statistically different

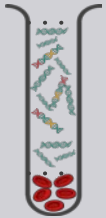
[a]Statistical significance measured using McNemar's two-sided test

CSO, cancer signal origin; SCNA, somatic copy number alterations; ; SNV-WBC, single nucleotide variants with correction for clonal hematopoiesis noise; TS, targeted sequencing; WBC, white blood cell; WG, whole-genome; WGBS, whole-genome bisulfite sequencing

CIRCULATING TUMOR ALLELE FRACTION
Relationship Between cTAF and Classifier Performance

What?

- Estimated fraction of the total cfDNA isolated from a blood sample that contained variant alleles



How?

- Computed from the detectable tumor-tissue-identified mutations in matched cfDNA

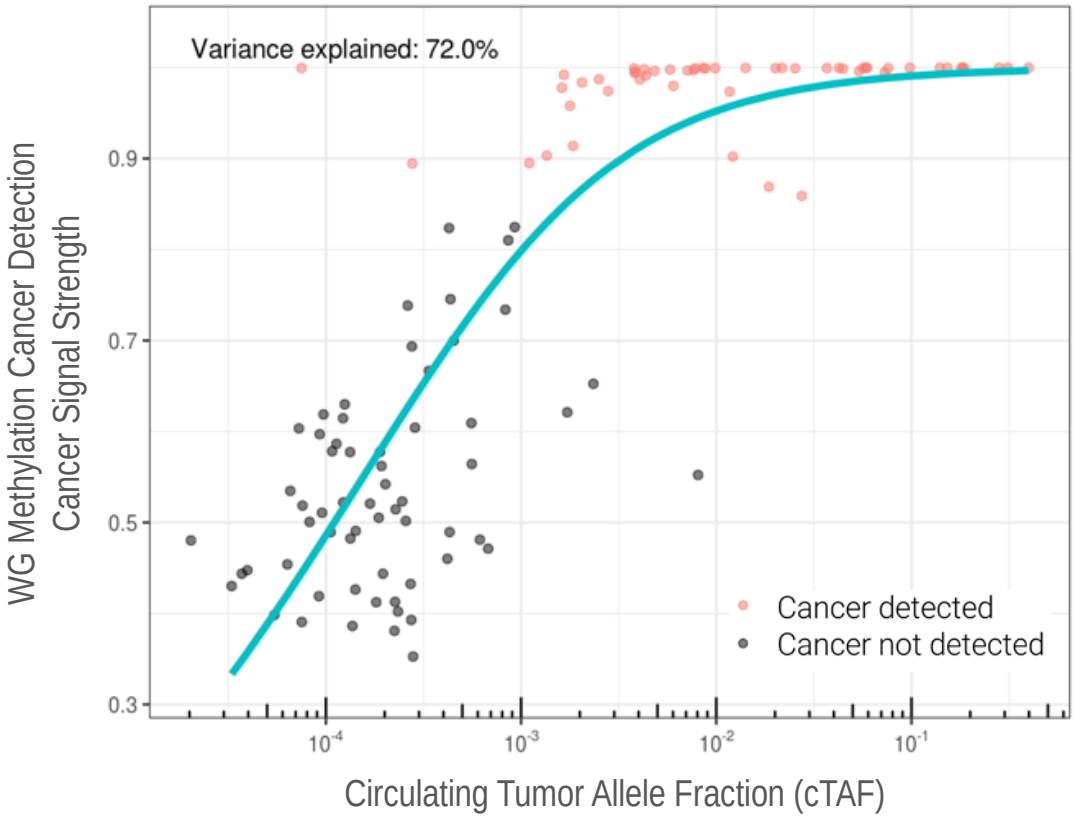
Why?

- Allows for detection performance to be assessed against shedding behavior
- Requires a fixed specificity for comparison

cfDNA, cell-free DNA; cTAF, circulating tumor allele fraction

CCGA SUBSTUDY 1: RESULTS
cTAF Explains the Majority of Cancer Signal Detection Performance

- cTAF accounted for 72% of the variance in WG methylation cancer signal detection classifier scores
- cTAF was an independent predictor of cancer signal strength in a multivariate analysis (clinical stage and cancer type were not)

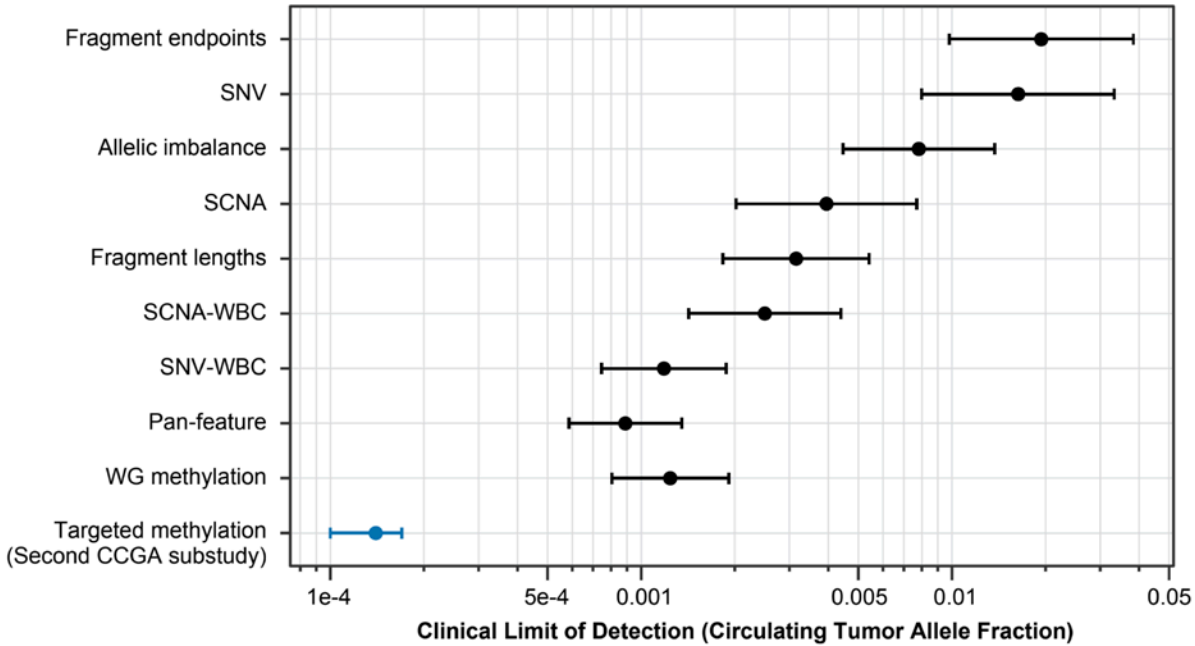


cTAF, circulating tumor allele fraction; WG, whole-genome
N = 113 participants with analyzable tumor tissue and detectable cTAF from the validation set

CCGA SUBSTUDY 1: RESULTS
Clinical Limit of Detection for Each Cancer Signal Detection Classifier[a]

- Clinical LOD mirrored relative sensitivity performances
- WG methylation, SNV-WBC, and pan-feature classifiers provided the lowest clinical LOD in this study
- A refined targeted methylation method (CCGA2)[1] had the lowest LOD

Clinical LOD: the cTAF where the probability of detecting a cancer signal was 50%



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[a]N=113 participants with tumor tissue and SNVs detected in both tissue and cfDNA. Error bars indicate 95% confidence intervals. cfDNA, cell-free DNA; CCGA, Circulating Cell-free Genome Atlas; CCGA2, second CCGA substudy; LOD, limit of detection; SCNA, somatic copy number alteration; SCNA-WBC, somatic copy number alterations with correction for WBC background; SNV, single nucleotide variants; SNV-WBC, single nucleotide variants with correction for WBC background; WBC, white blood cell; WG, whole-genome;

1. Liu MC, et al. Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA. Ann Oncol. 2020;31(6):745-759. doi:10.1016/j.annonc.2020.02.011

CHOOSING A CANDIDATE FOR FURTHER DEVELOPMENT
Why WG Methylation?

Cancer Signal Detection
- Among the top-performing cancer signal detection classifiers of those tested

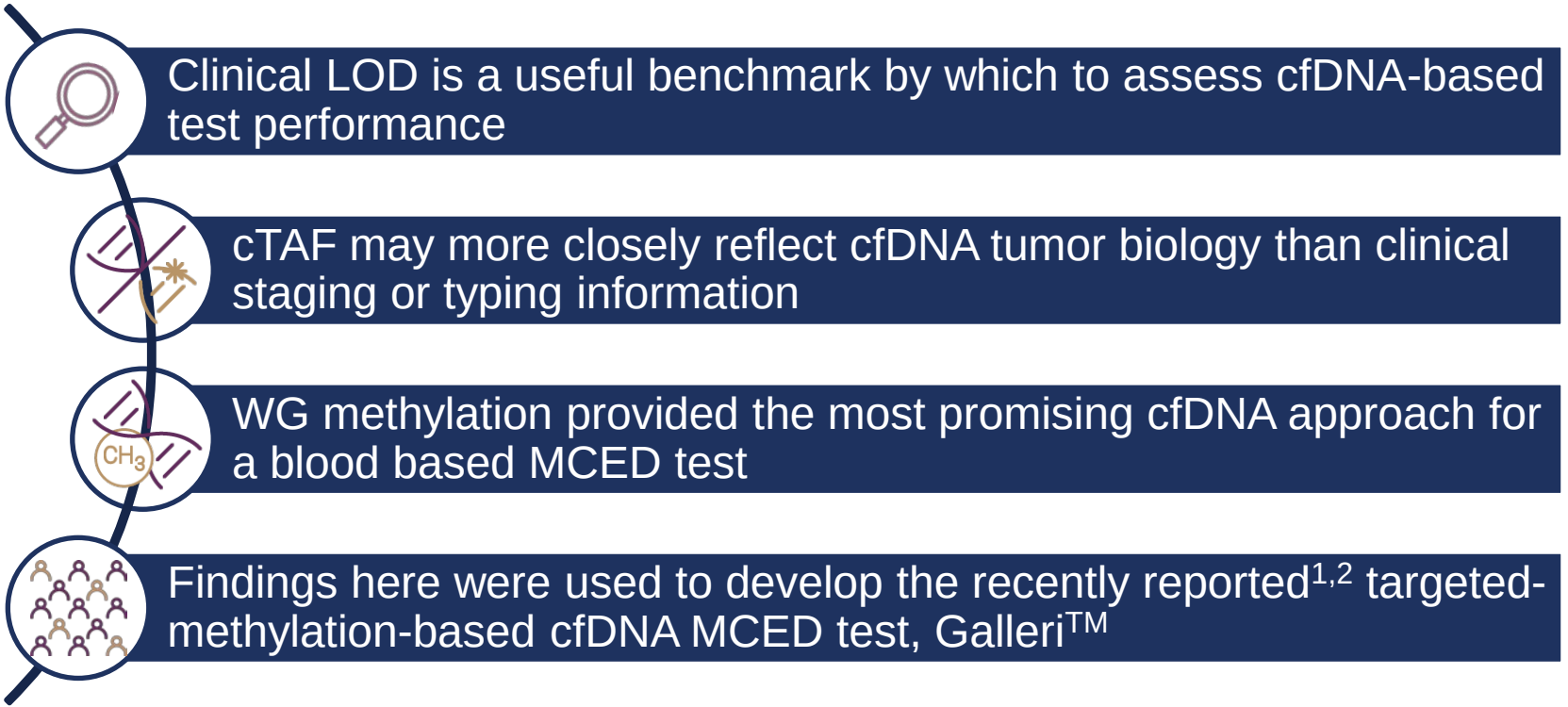
Accuracy of CSO Prediction
- Predicted CSO with significantly higher accuracy compared with representative classifiers from the other two assays tested

Clinical Limit of Detection
- Among the lowest clinical LOD measured
- Unlike SNV-WBC and pan feature, does not require removal of biological background from paired WBCs

Assay Potential
- Significant potential for further improvement to achieve higher performance through a targeted methylation assay approach

CSO, cancer signal origin; LOD, limit of detection; SNV, single nucleotide variants; WBC, white blood cell; WG, whole-genome

CONCLUSIONS
CCGA Substudy 1 Demonstrates:



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cfDNA, cell-free DNA; CCGA, Circulating Cell-free Genome Atlas; cTAF, circulating tumor allele fraction; LOD, limit of detection; MCED, multi-cancer early detection; WG, whole-genome

1. Liu MC, et al. Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA. Ann Oncol. 2020;31(6):745-759. doi:10.1016/j.annonc.2020.02.011
2. Klein EA, et al. Clinical validation of a targeted methylation-based multi-cancer early detection test using an independent validation set, Ann Oncol. 2021. doi:10.1016/j.annonc.2020.02.01