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Beneath the Variants: Fragmentomics Unmasks Epigenomic Signal from Existing Mutation Profiling of cfDNA for Lung Cancer Classification and Prognosis

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Increasing use of liquid biopsies establishes cfDNA mutation calling as the clinical standard, but not its ceiling

Increasing use of liquid biopsy in clinical trials



Xie et al., *Nature Reviews Drug Discovery* 2023

Liquid biopsies show promise in lung cancer diagnosis, monitoring and risk assessment

- Detect actionable biomarkers at NSCLC diagnosis ^{1,2}
- Monitor mutations for treatment resistance post treatment ³
- Detect post adjuvant mutations for long-term risk assessment ⁴

¹ Page et al., *Clin Lung Cancer* 2022; ² Bayle et al., *Ann of Oncol* 2022; ³ Gray et al., *Clin Cancer Res.* 2023; ⁴ Black et al., *Cell* 2025

Mutation assays give a static glimpse of the tumor

CGP limitations and expression markers in the diagnostic toolkit

DNA testing can identify that a patient has a gene mutation

BRAF **TP53** **FGFR**
EGFR **KRAS** **MET**

GENE MUTATION EXAMPLES

However, it doesn't necessarily mean that the mutation is the disease driver or that the pathway is activated

Variable Response Rates in Patient with Mutations

Variable Response Rates

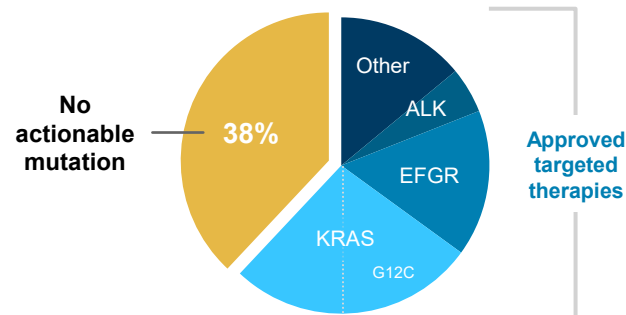


Disease	MELANOMA	COLORECTAL	
Mutation	B-RAF	B-RAF	Same mutation; same treatment
Therapeutic	Vemurafenib	Vemurafenib	
Response Rate	48%	5%	Very different response rates ^{1,2}

Across tumor types, typically, a minority of biomarker (+) patients respond, and response rates can be highly variable

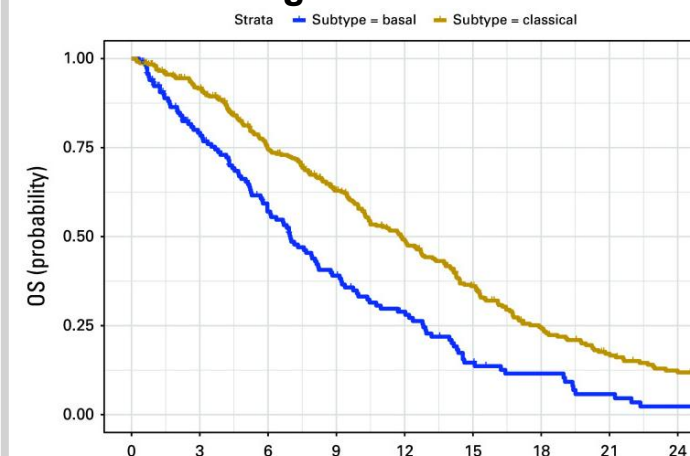
No Clear Targeted Therapy for Many Patients

Driver Mutation in Lung Adenocarcinoma³



Many patients have no actionable mutations

Expression Subtyping Predicts Outcome Even in Genetically Homogenous Tumors

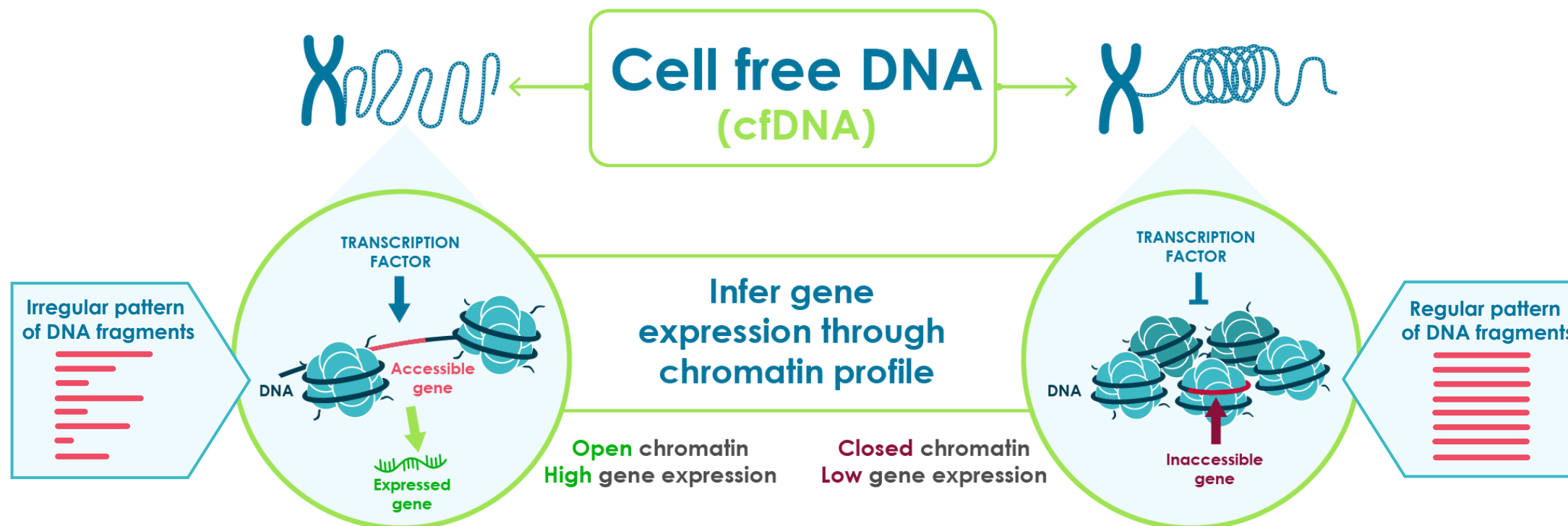


In PDAC, classical subtype has better survival than basal on FFX⁴

(1) Chapman PB et al. *N Engl J Med* 2011; (2) Kopetz et al. *J Clin Oncol* 2010; (3) Pakkala et al., *JCI insight* 2018; (4) Wenric et al. *JCO Precision Oncology* 2025

- DNA alteration biomarkers do not fully account for the complexity of tumor response
- Expression-based biomarkers provide additional information to guide treatment decisions

Fragmentomics technology offers a route to assess tumor gene expression in blood



Adapted from GeneCentric website

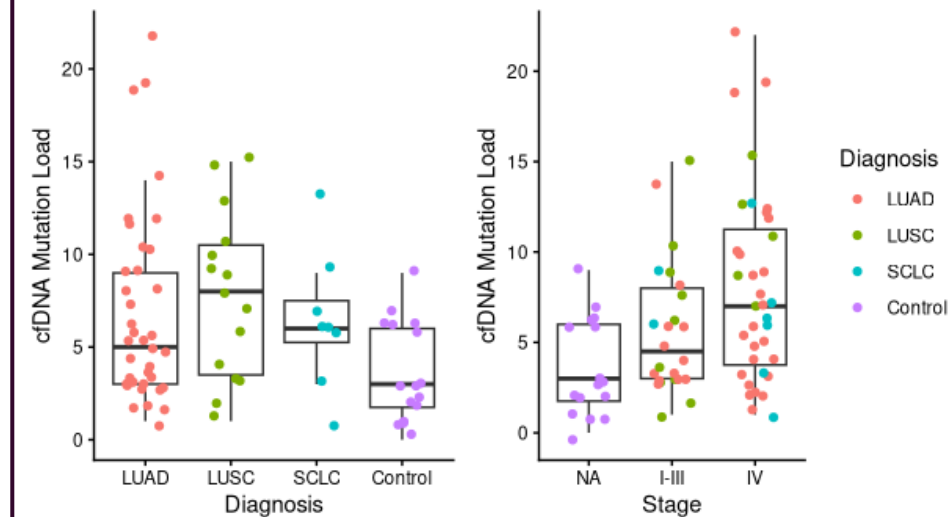
Fragmentomics can unlock a strong signal for quantitating gene expression in blood

cfDNA detected mutation burden is prognostic in lung cancer

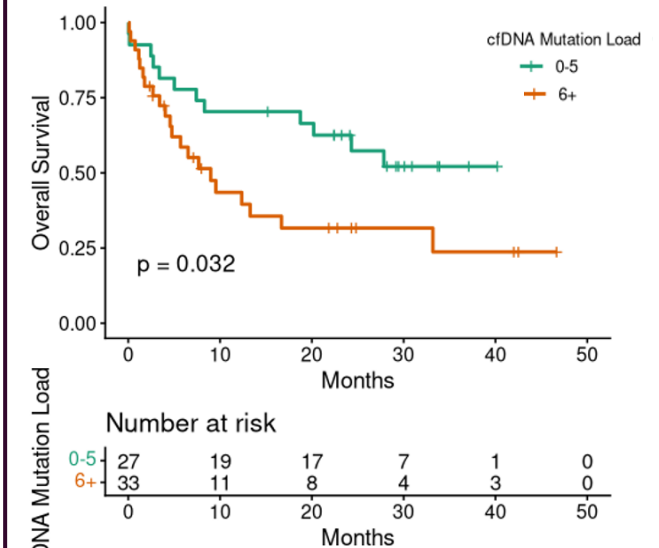
Evaluation of Avenio cfDNA NGS in lung cancer patients

Patient Details		N
Lung cancer		58
Diagnosis		
	Adenocarcinoma (LUAD)	36
	Squamous Cell (LUSC)	14
	Small Cell (SCLC)	8
	Benign (Control)	16
Stage		
	I	8
	II	5
	IIIa	5
	IIIb	4
	IV	36
ECOG Performance Score		
	0	14
	1	35
	2	11
	3	8
	4	1
Survival (median follow up 11.0 mo.)		
	Deceased	33
	Censored	25

cfDNA mutation load varies by lung cancer type and stage



High mutation load is associated with poor survival

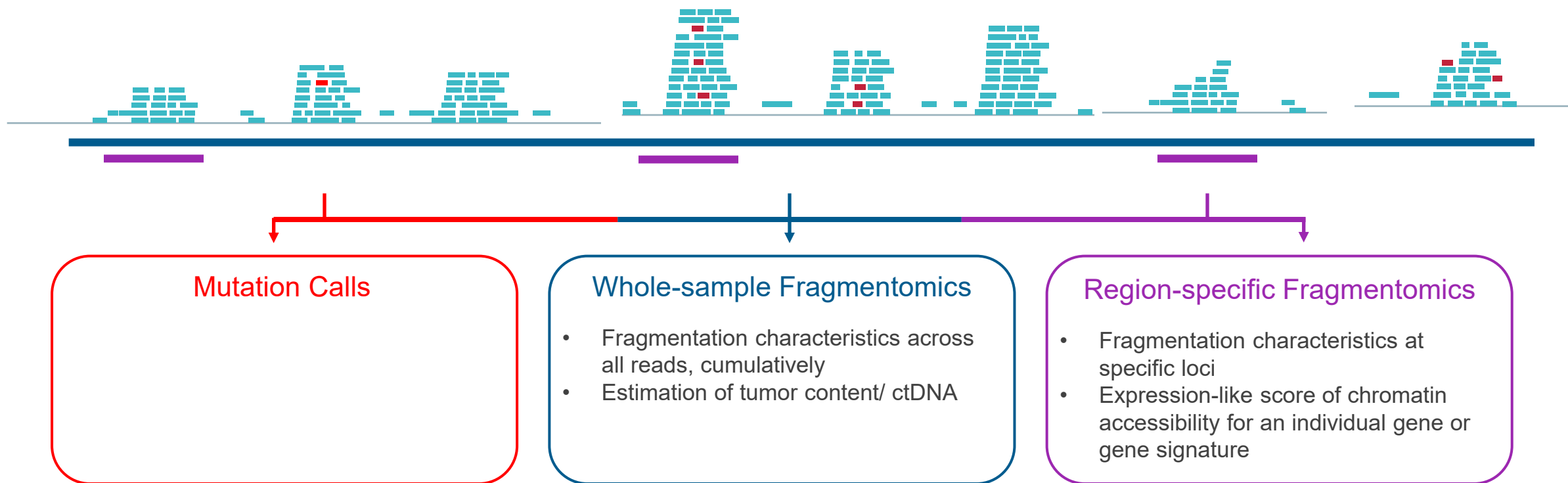


cfDNA library prep using AVENIO ctDNA Surveillance kit
Evaluate 197 genes, across 471 hypermutated regions

What additional insights can we derive from orthogonal fragmentomics – even on this small hybrid capture gene panel?

Fragmentomics goes beyond mutation data from the same cfDNA library

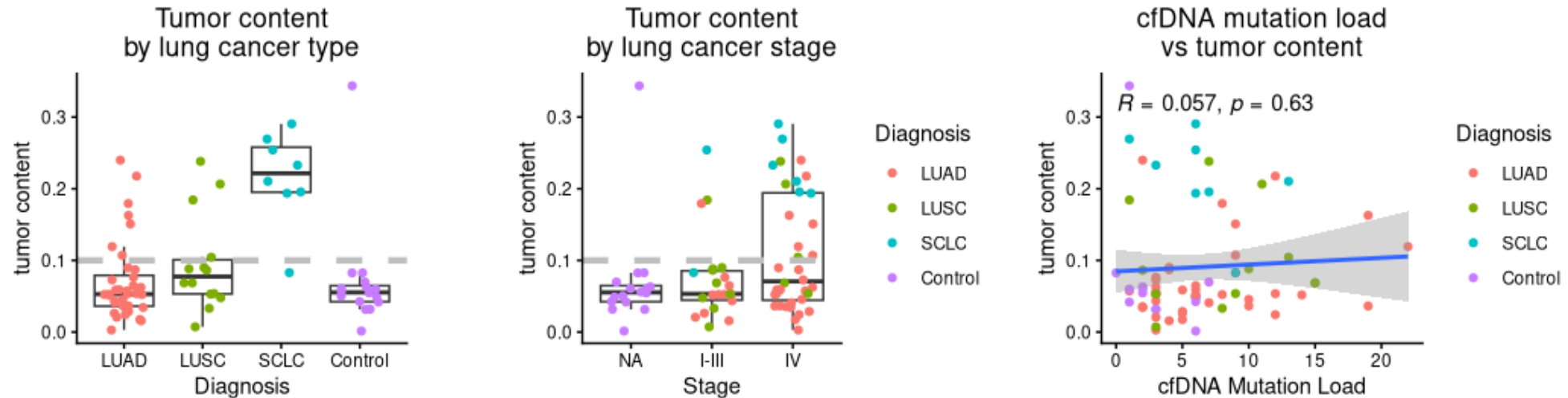
One cfDNA NGS library — Same sequencing reads — Multiple levels of data



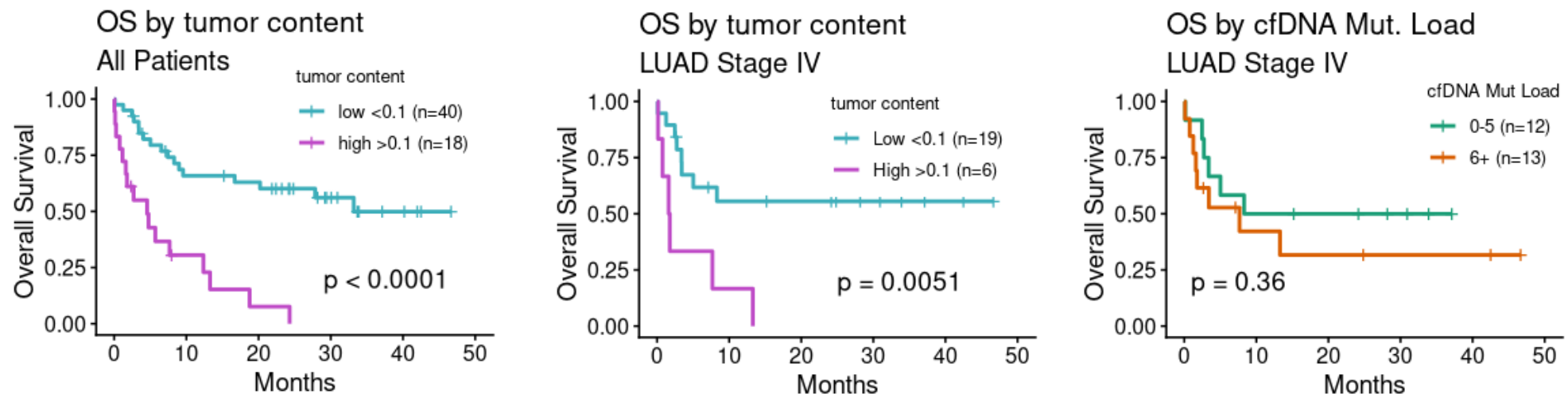
Same library, added layers: **mutation calls** plus **tumor content** and **expression-level insight**

Fragmentomics estimated tumor content: Tumor content is associated with lung cancer type, stage and prognosis

ctDNA tumor content varies by lung cancer type and stage, but does not correlate with mutation load



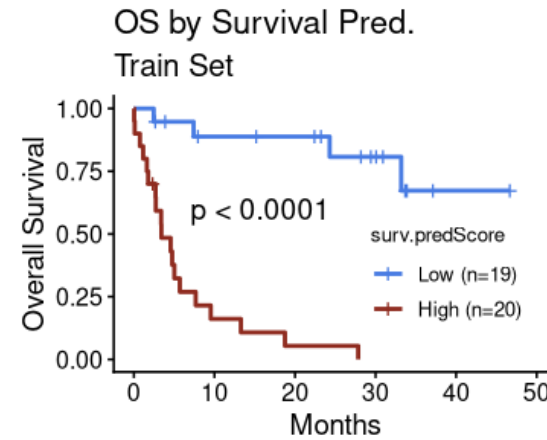
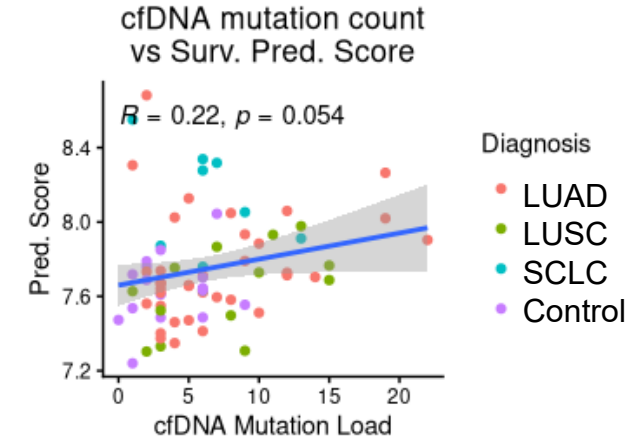
High ctDNA tumor burden is associated with worse survival, even within stage IV LUAD



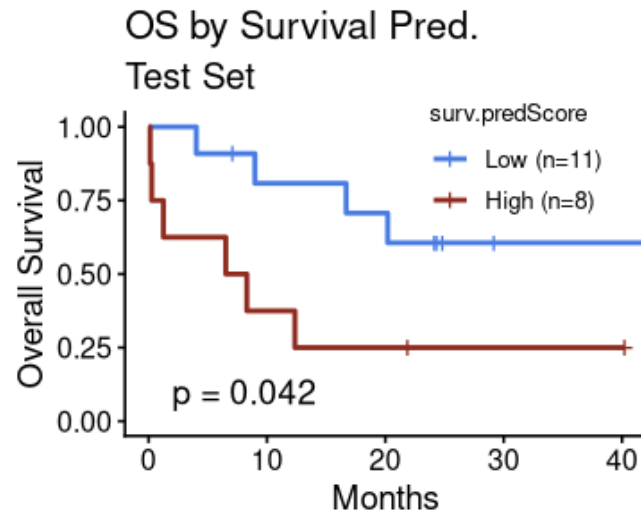
Region-specific fragmentomics: Lung cancer prognostic signature identifies high risk lung cancer patients

Train model to predict prognostic signature in lung cancer

Dataset Details	Train (n= 39)	Test (n=19)	P-val
Diagnosis	39	19	0.12
LUAD	27	9	
LUSC	6	8	
SCLC	6	2	
Stage			0.39
I-III	13	9	
IV	26	10	
ECOG			0.25
0-1	23	15	
2+	16	4	
cfDNA mut. load (mean)	7.1	7.1	0.98
Tumor content (mean)	0.10	0.08	0.31



Survival signature significantly contributes prognostic signal in test set



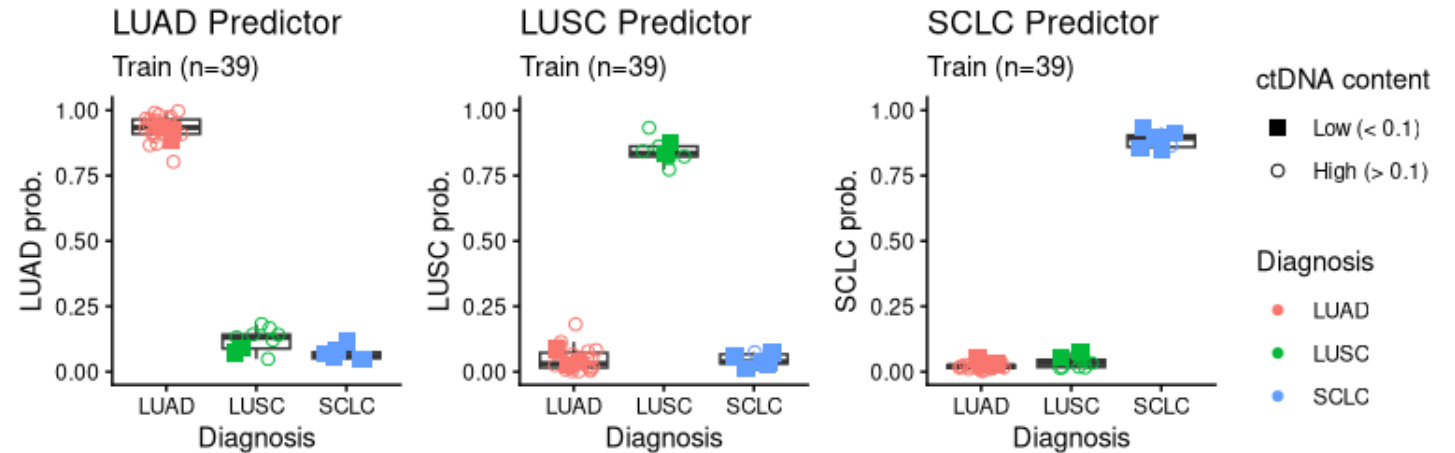
Multivariate CoxPH	HR (95% CI)	P-val
Diagnosis		
LUAD	Ref	-
LUSC	1.73 (0.24-12.48)	0.59
SCLC	0.76 (0.03-18.74)	0.87
Stage		
I-III	Ref	-
IV	4.69 (0.54-40.46)	0.16
ECOG		
0-1	Ref	
2+	10.23 (1.38-75.67)	0.023*
Survival Prediction Score		
Low	Ref	
High	7.31 (1.22-43.70)	0.029*

Log Rank Test Model	Log likelihood	P-val
Diagnosis + Stage + ECOG	-21.675	
Diagnosis + Stage + ECOG + Mut load	-21.427	0.48
Diagnosis + Stage + ECOG + Mut load + Surv Pred	-19.049	0.029*

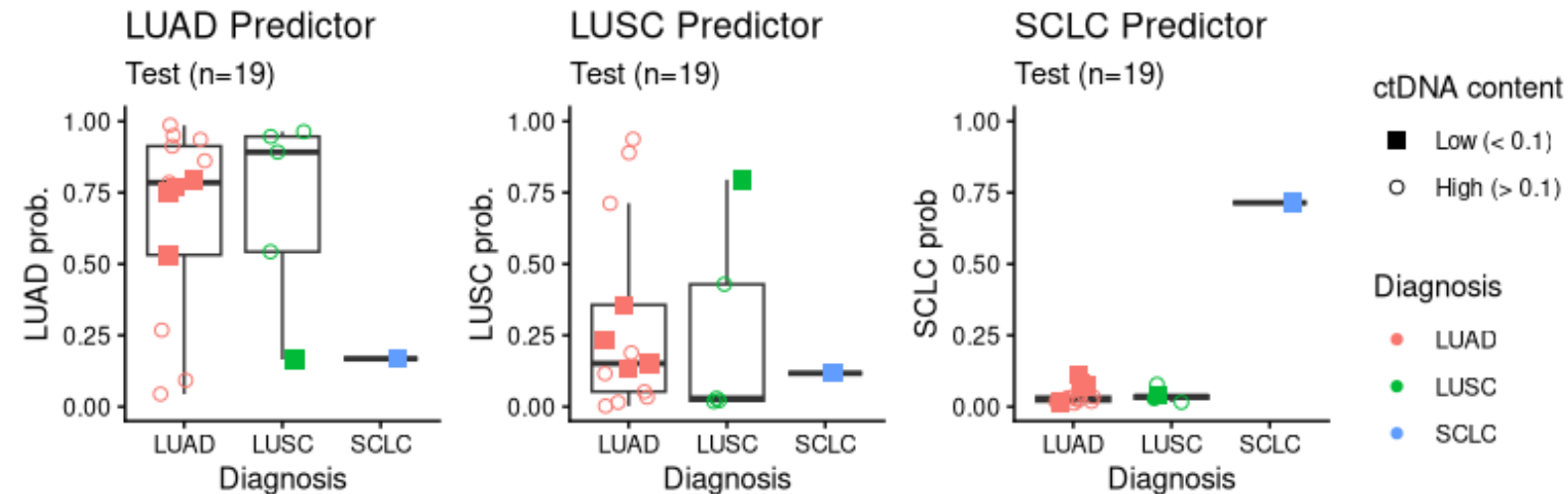
Region-specific fragmentomics: Lung cancer classifier distinguishes cancer types

Train models to distinguish LUAD, LUSC and SCLC

Dataset Details	Train (n= 39)	Test (n=19)	P-val
Diagnosis	39	19	0.47
LUAD	23	13	
LUSC	9	5	
SCLC	7	1	
Stage			0.39
I-III	13	9	
IV	26	10	
ECOG			0.25
0-1	23	15	
2+	16	4	
cfDNA mut. load (mean)	7.1	7.1	0.98
ctDNA content (mean)	0.093	0.098	0.83



Tumor type predictors show 100% accuracy in high ctDNA samples



ALL samples		Predicted		
Actual		LUAD	LUSC	SCLC
	LUAD	10 (0.77)	3 (0.23)	
	LUSC	4 (0.8)	1 (0.2)	
	SCLC			1 (1.0)

High tumor content samples		Predicted		
Actual		LUAD	LUSC	SCLC
	LUAD	4 (1.0)		
	LUSC		1 (1.0)	
	SCLC			1 (1.0)

Unlock new layers of information from cfDNA with fragmentomics

- **Fragmentomics provides additional data out of existing cfDNA mutation profiling** – one NGS library, same sequencing reads, no additional sample or assay
- **Whole-sample fragmentomics tumor content:**
 - High tumor content associates with worse survival, even within stage IV LUAD
- **Region-specific fragmentomics expression-like scores:**
 - Overall survival signature with independent prognostic value when adjusting for tumor type, stage and ECOG performance status
 - 3-subtype lung cancer classifier correctly classified test samples with sufficient tumor content

Applying fragmentomics to **hybrid capture sequencing** of cfDNA can unlock phenotypic and prognostic information from **retrospective studies** without additional clinical collection.

Thank You!

GeneCentric Therapeutics, Inc

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- Jeff Burdine
- Yoshi Shibata
- Gabe Milburn
- Kirk Pappan
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