Leveraging the cfDNA fragmentome to predict immunotherapy response

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Disclosure Information

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Valsamo Anagnostou

I have the following relevant financial relationships to disclose:

Consultant for: Astra Zeneca, Neogenomics

Grant/Research support (active, to institution): Astra Zeneca, Personal Genome Diagnostics/Labcorp

Honoraria from: Foundation Medicine, Personal Genome Diagnostics/Labcorp, Guardant Health

- and -

My additional financial relationship disclosures are:

I am an inventor on patent applications (63/276,525, 17/779,936, 16/312,152, 16/341,862, 17/047,006 and 17/598,690) submitted by Johns Hopkins University related to cancer genomic analyses, ctDNA therapeutic response monitoring and immunogenomic features of response to immunotherapy that have been licensed to one or more entities. Under the terms of these license agreements, the University and inventors are entitled to fees and royalty distributions.

Opportunities and challenges with ctDNA response as an early endpoint for immunotherapy

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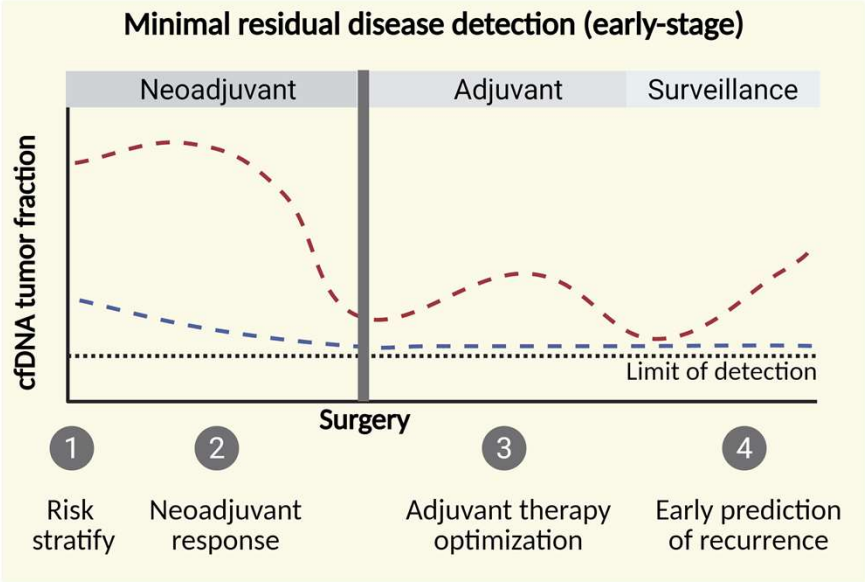

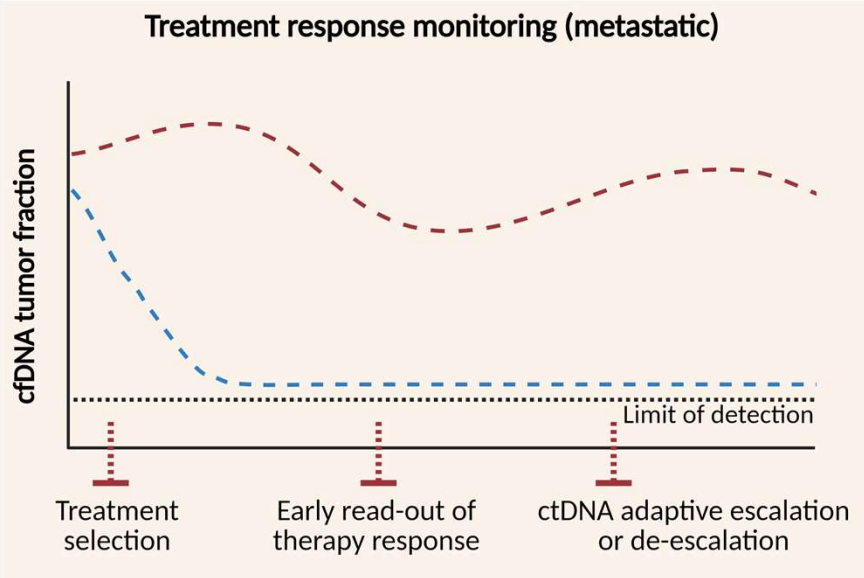

Aredo et al., *ASCO Educational Book*, 2025, *in press*

Mutation-based ctDNA molecular response predicts immunotherapy outcomes

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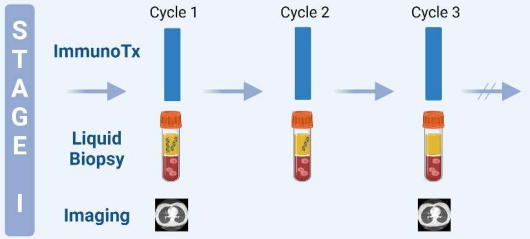
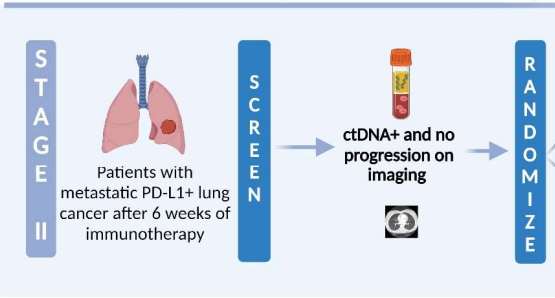
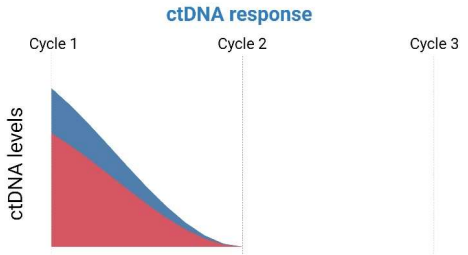


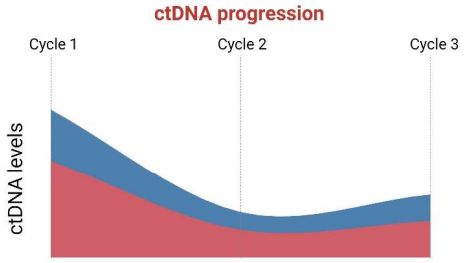
Key Questions

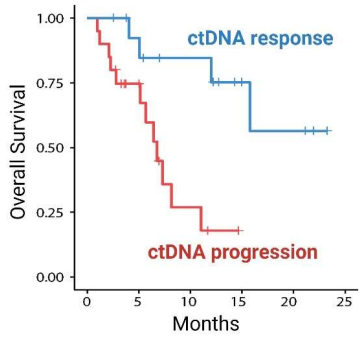
- What is ctDNA molecular response?
- When is molecular response best captured?
- How often do liquid biopsies agree with imaging?

Individuals with ctDNA progression on pembrolizumab can be rapidly and accurately identified followed by treatment escalation









Core Conclusions (BR.36 stage 1)

- Undetectable ctDNA 6 weeks on pembrolizumab provides an early measure of clinical response.
- Molecular responses overall agree with imaging (82% sensitivity).
- Liquid biopsies better predict overall survival.

Anagnostou et al., *Cancer Res*, 2019, Sivapalan et al., *Clin Can Res*, 2023, Murray et al., *Clin Can Res*, 2024, Anagnostou et al., *Nat Med*, 2023

Clinical sensitivity of mutation-based ctDNA molecular response by magnitude

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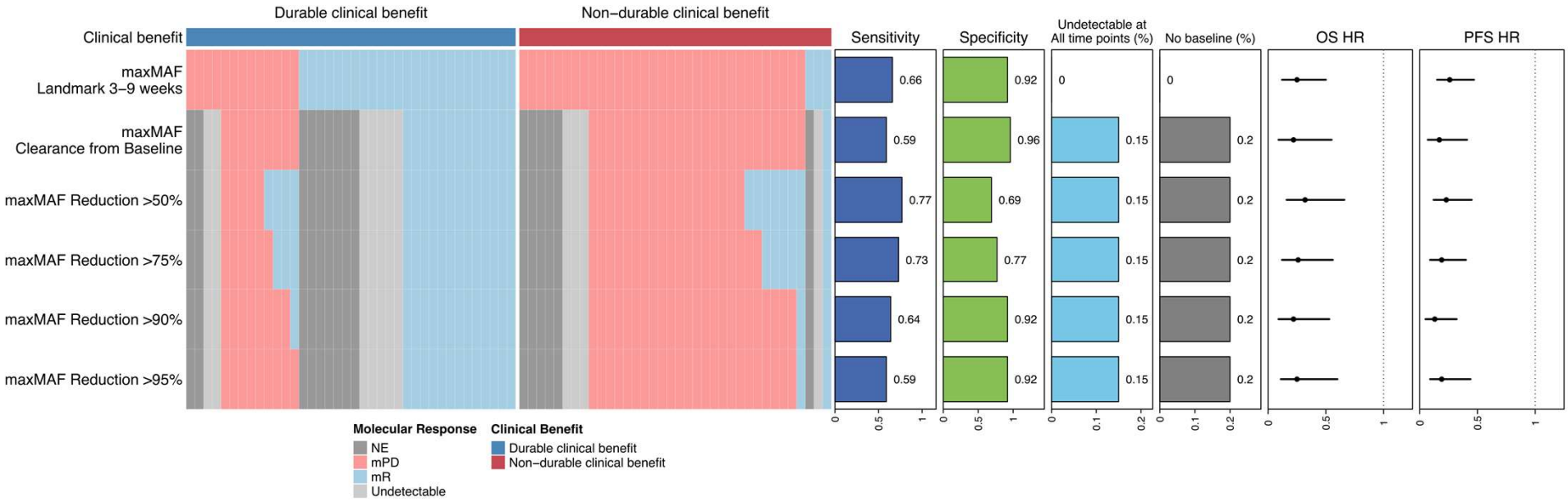

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Sivapalan et al., *under review*

Inherent challenges with tumor-agnostic WBC DNA-informed liquid biopsy approaches

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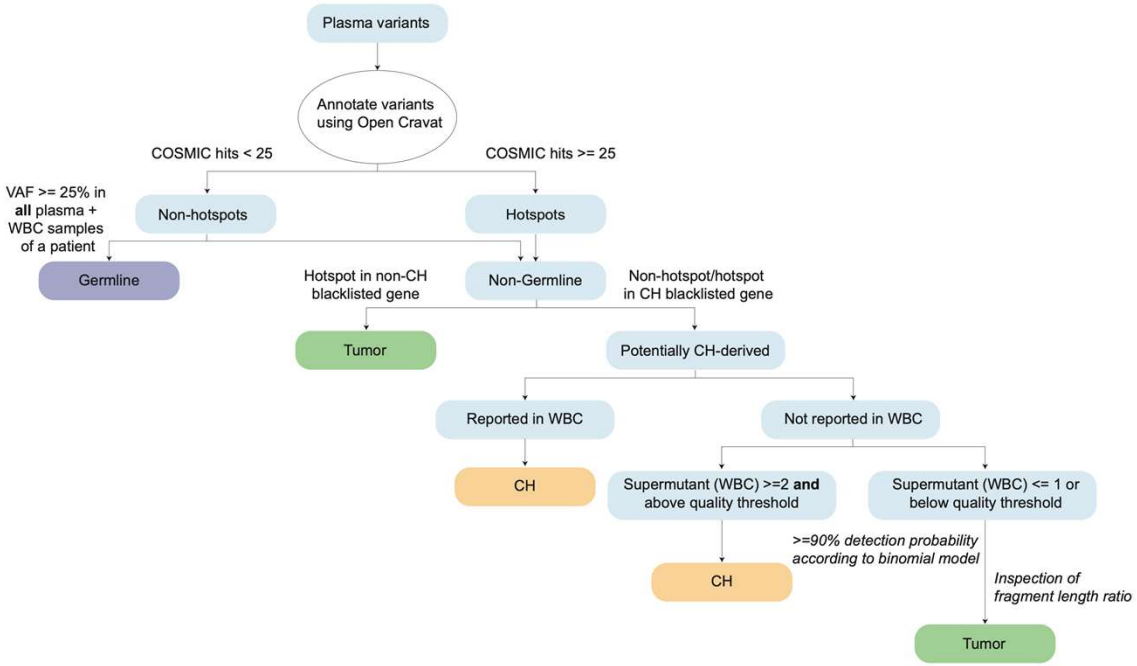

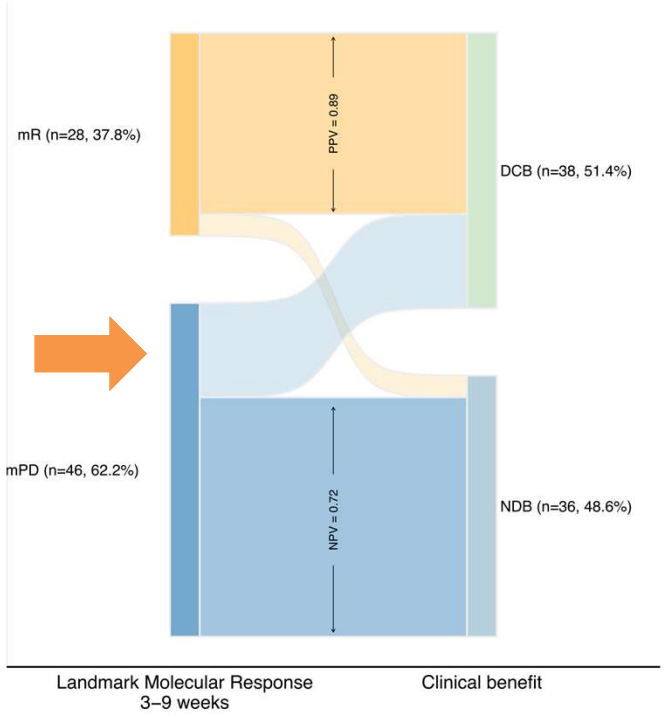

Sivapalan et al., *submitted*

Johns Hopkins Thoracic Oncology prospective minimally invasive biomarker clinical protocol

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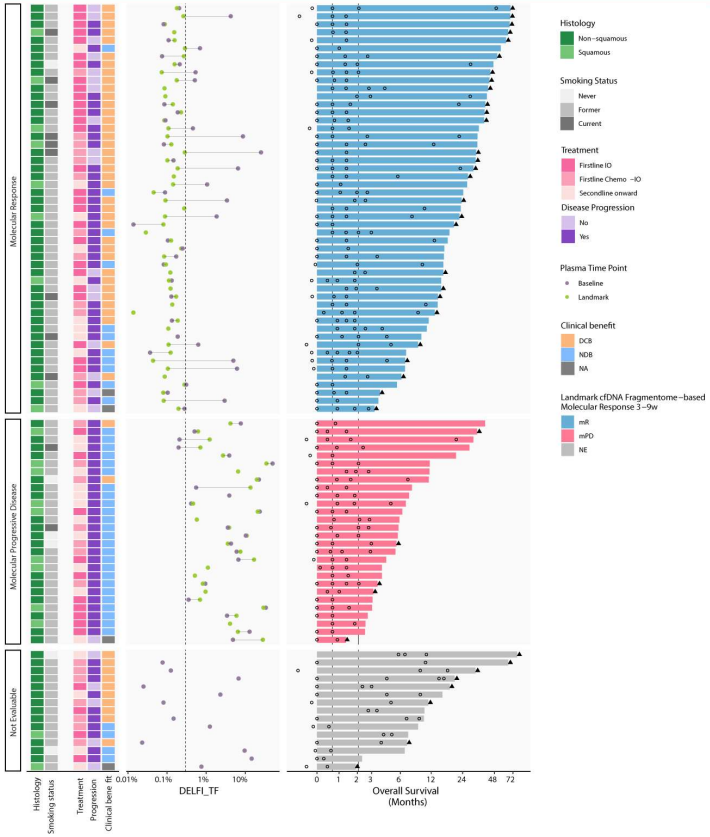

Overview of the experimental approach and bioinformatic analyses

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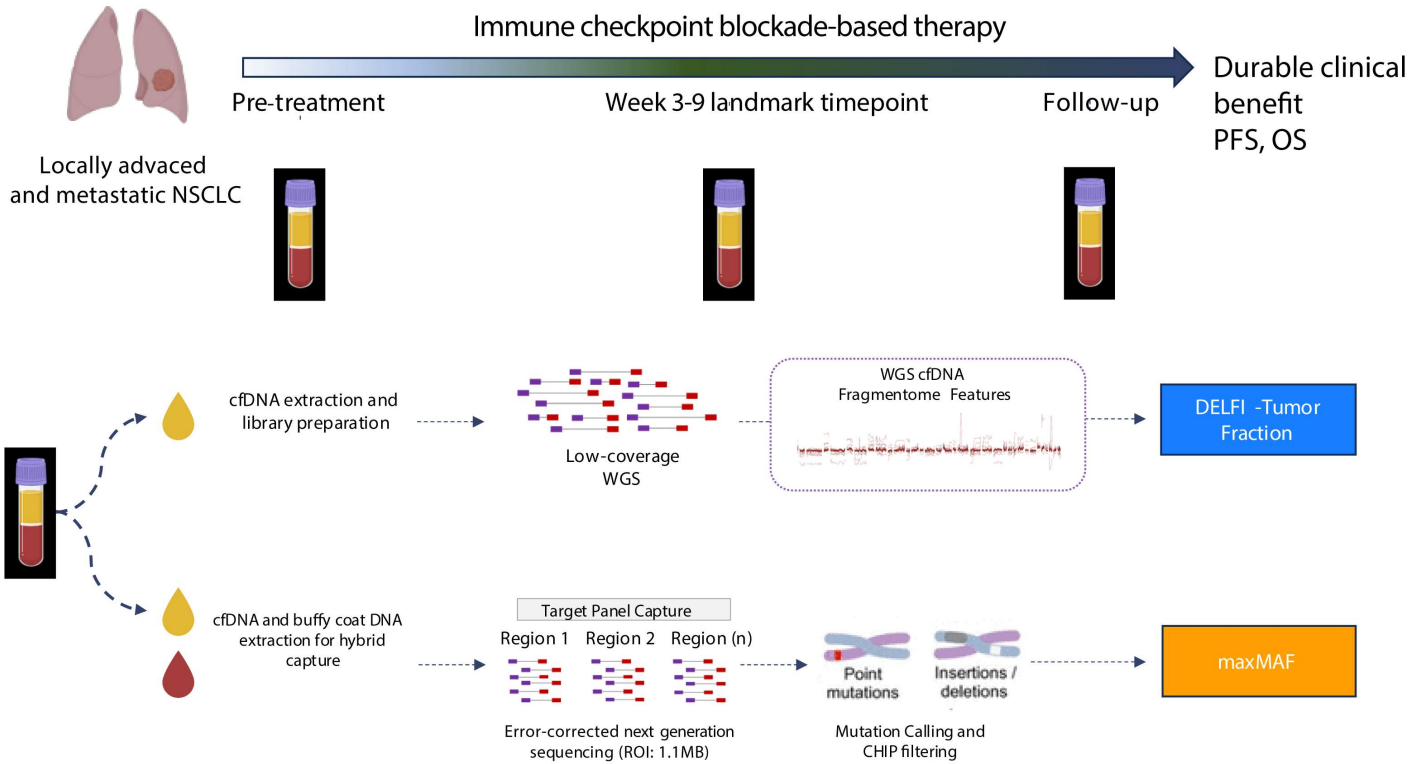

Concordance between cfDNA fragmentome- and mutation-derived tumor fraction estimates

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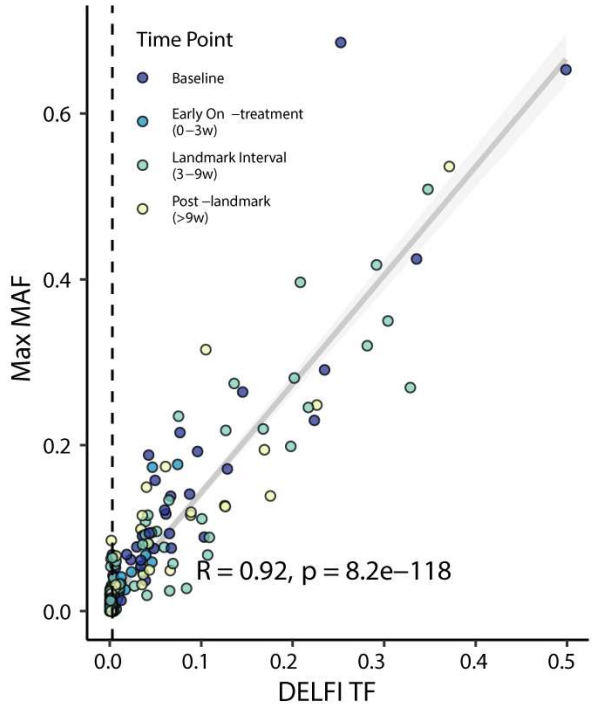

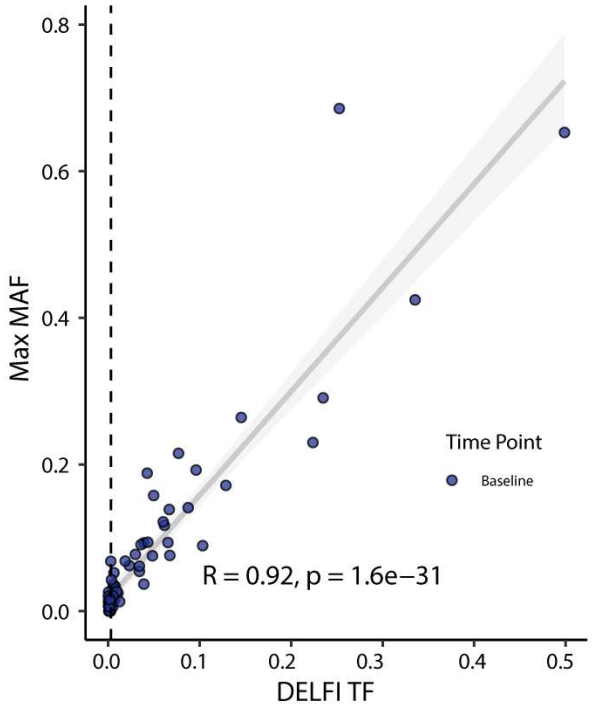

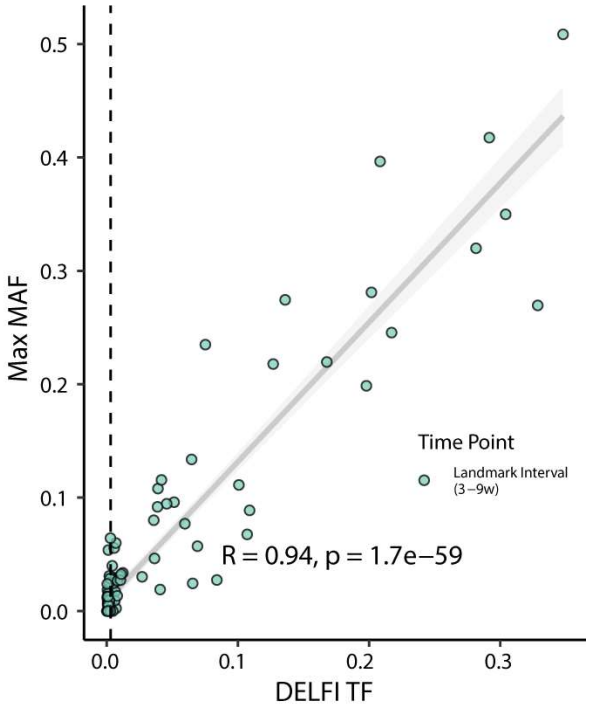

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Detectable rate by plasma-only, WBC-informed, tumor-informed and fragmentome approach

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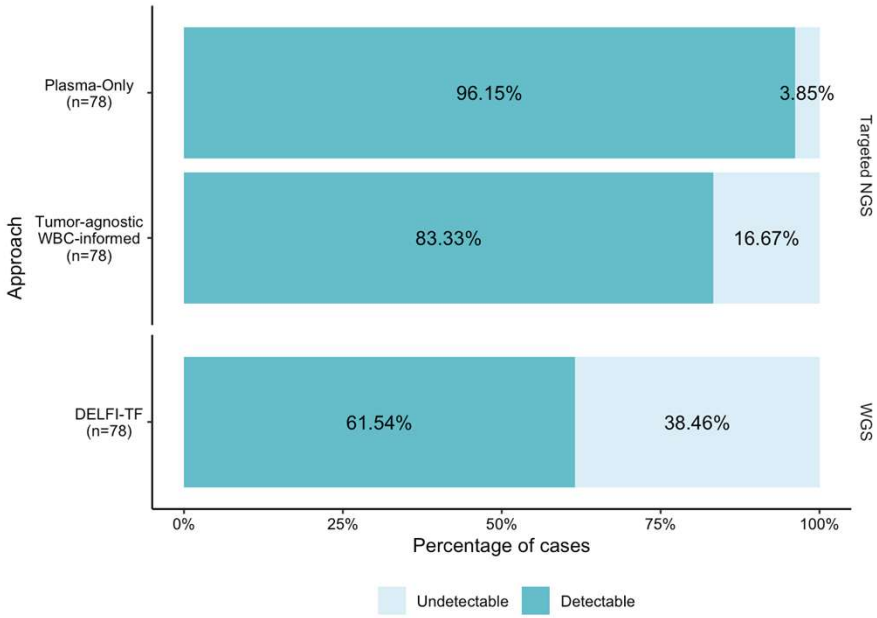

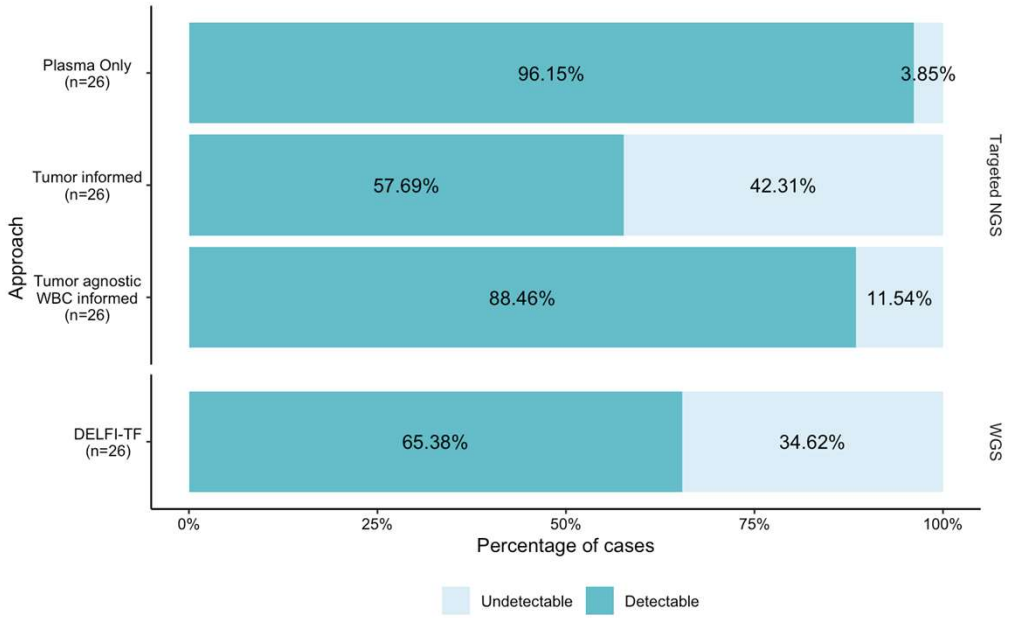

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Differential cfDNA fragmentation patterns by timepoint and molecular response groups

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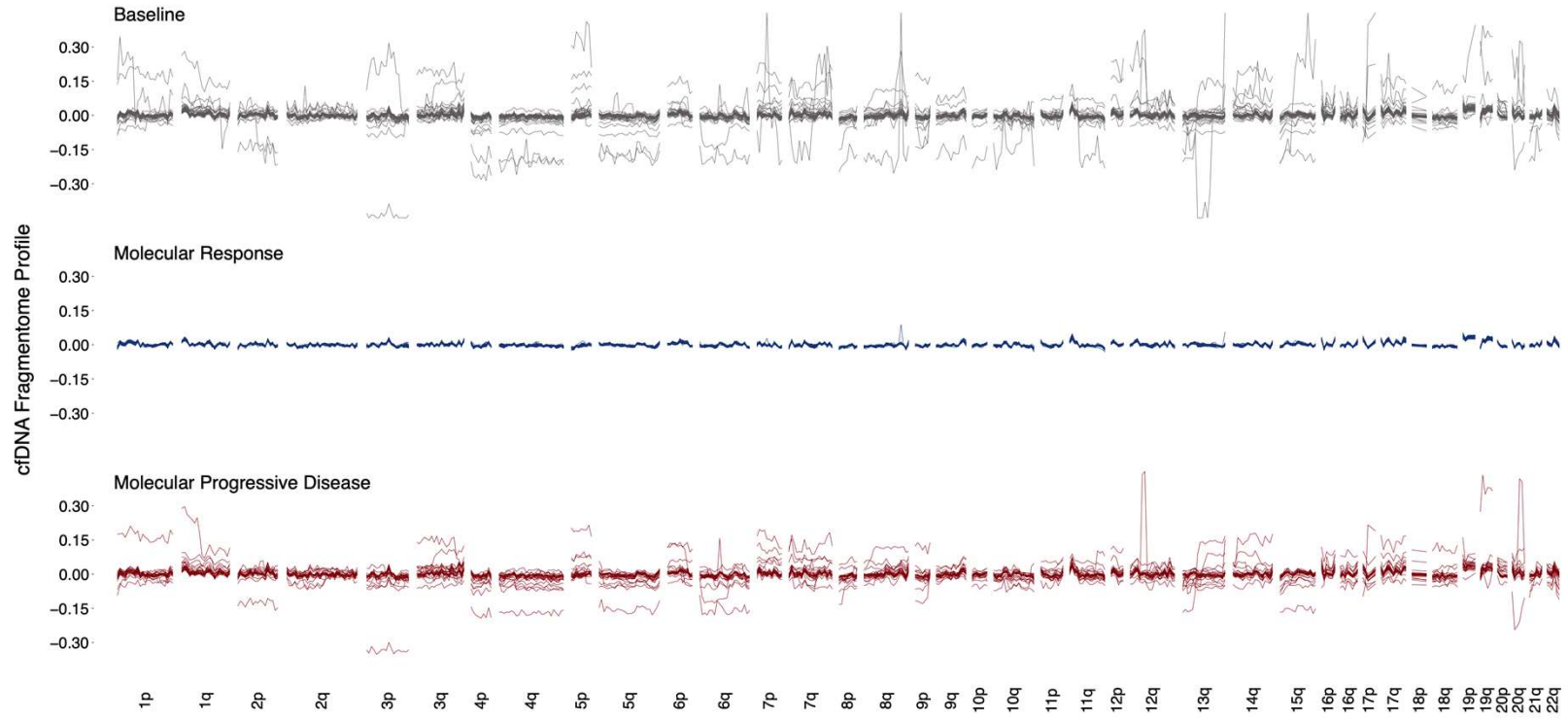

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Concordance between fragmentome-TF and mutation-based ctDNA response

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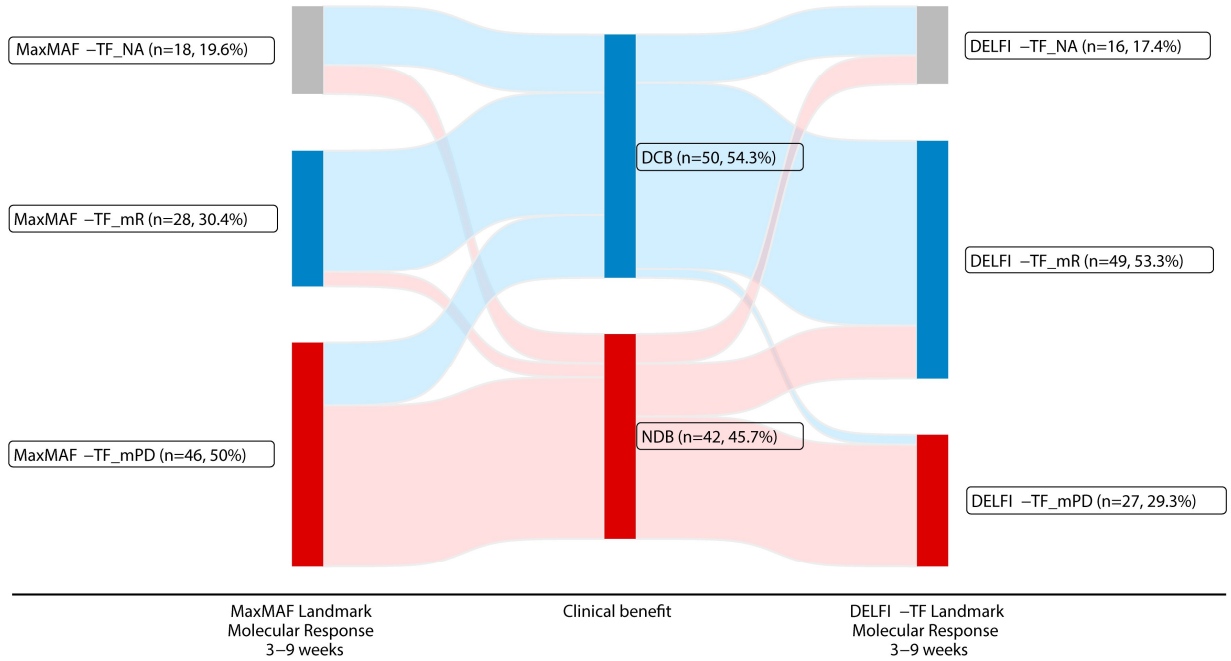

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Fragmentome-TF more accurately predicts outcomes in mutation-based molecular progressors

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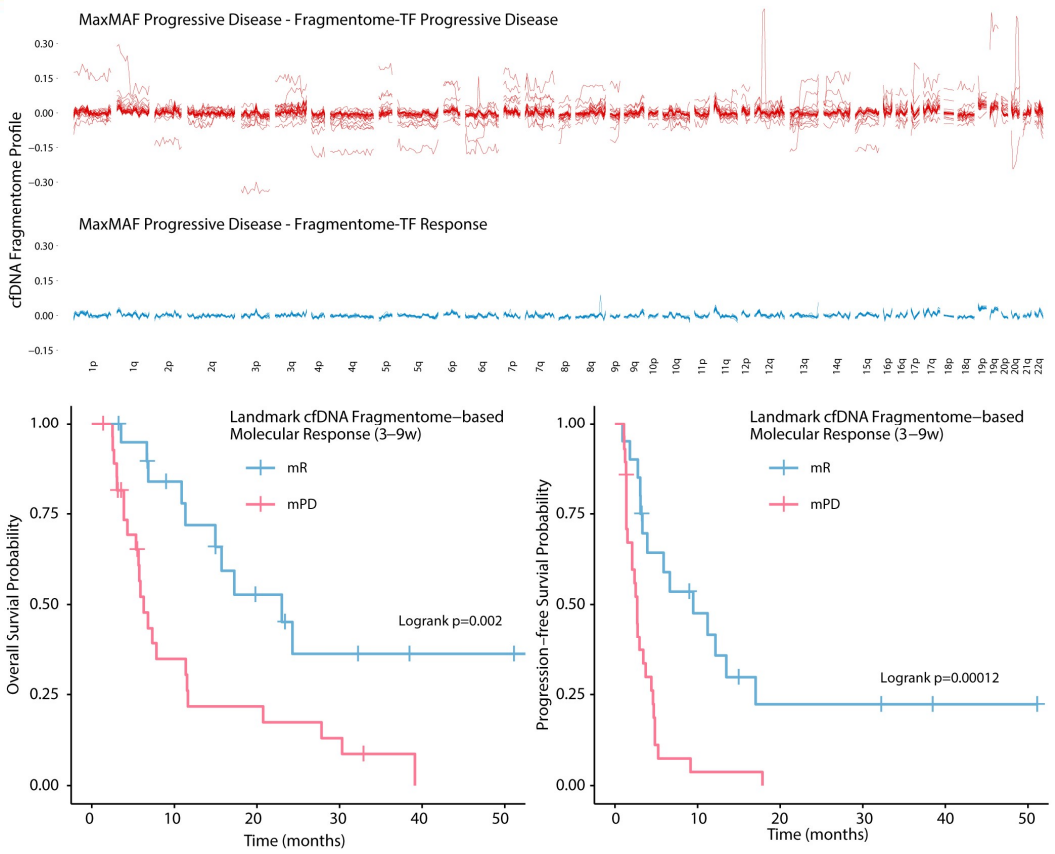

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Landmark fragmentome-TF molecular response predicts progression-free survival

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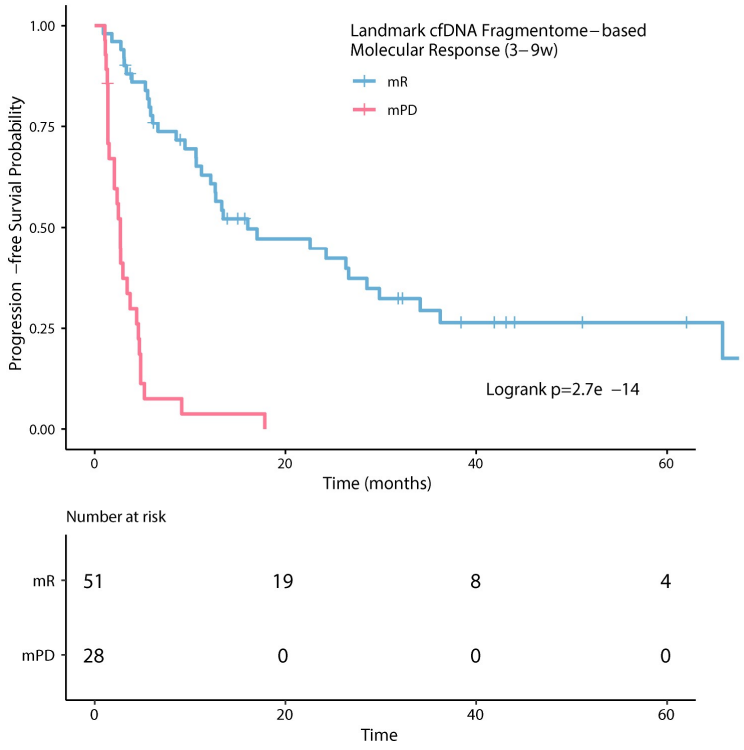
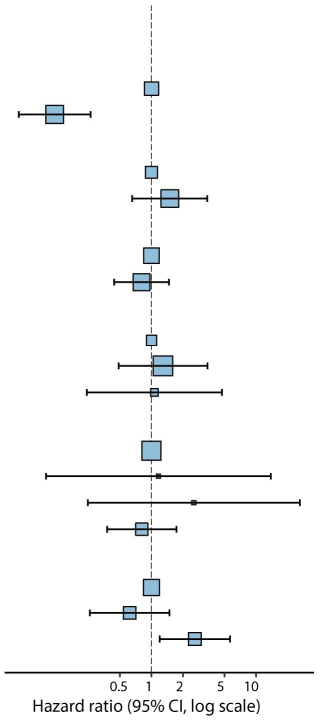


Multivariate Analysis of Progression–free Survival (n=66)

Variable	Levels	PFS HR (95% CI, p value)
Molecular_response	mPD	–
	mR	0.12 (0.05 –0.26, p<0.001)
Baseline_maxMAF	<1%	–
	>=1%	1.50 (0.66 –3.43, p=0.338)
Sex	Female	–
	Male	0.80 (0.44 –1.47, p=0.480)
Smoking_status	Current	–
	Former	1.29 (0.48 –3.43, p=0.611)
	Never	1.07 (0.24 –4.73, p=0.931)
Histology	Adenocarcinoma	–
	Large cell carcinoma	1.17 (0.10 –13.85, p=0.904)
	NSCLC –NOS	2.54 (0.25 –26.26, p=0.434)
	Squamous cell carcinoma	0.81 (0.38 –1.73, p=0.583)
Treatment	Firstline IO	–
	Firstline Chemo –IO	0.62 (0.26 –1.49, p=0.284)
	Secondline onward	2.60 (1.20 –5.65, p=0.016)



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Landmark fragmentome-TF molecular response independently predicts overall survival

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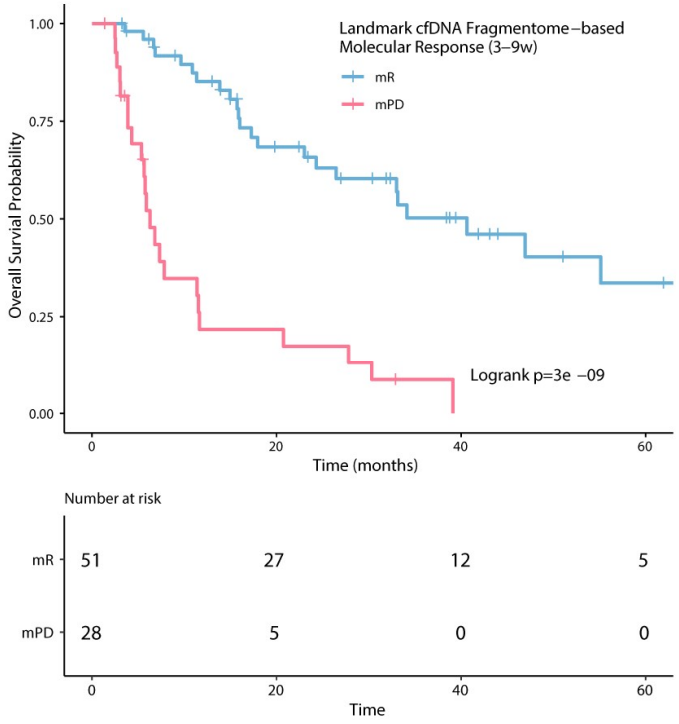
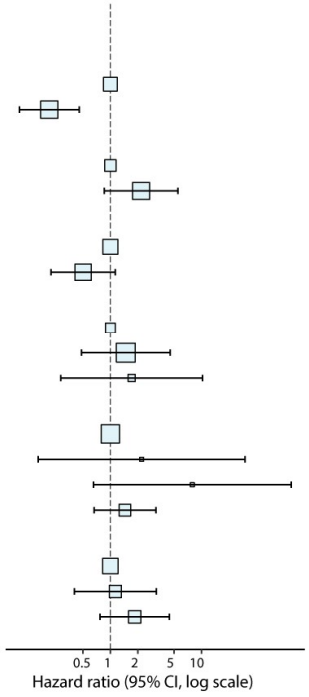


Multivariate Analysis of Overall Survival (n=66)

Variable	Levels	OS HR (95% CI, p value)
Molecular_response	mPD	–
	mR	0.21 (0.10 −0.46, p<0.001)
Baseline_maxMAF	<1%	–
	>=1%	2.17 (0.86 −5.51, p=0.102)
Sex	Female	–
	Male	0.50 (0.22 −1.13, p=0.097)
Smoking_status	Current	–
	Former	1.48 (0.48 −4.53, p=0.495)
	Never	1.71 (0.29 −10.23, p=0.556)
Histology	Adenocarcinoma	–
	Large cell carcinoma	2.20 (0.16 −29.97, p=0.553)
	NSCLC −NOS	7.91 (0.65 −95.71, p=0.104)
	Squamous cell carcinoma	1.45 (0.66 −3.15, p=0.355)
Treatment	Firstline IO	–
	Firstline Chemo −IO	1.13 (0.40 −3.20, p=0.812)
	Secondline onward	1.85 (0.77 −4.43, p=0.168)



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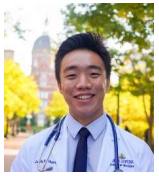
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