MASSACHUSETTS GENERAL HOSPITAL
CANCER CENTER

HARVARD MEDICAL SCHOOL

An introduction to liquid biopsy (cell free DNA sequencing): clinical applications

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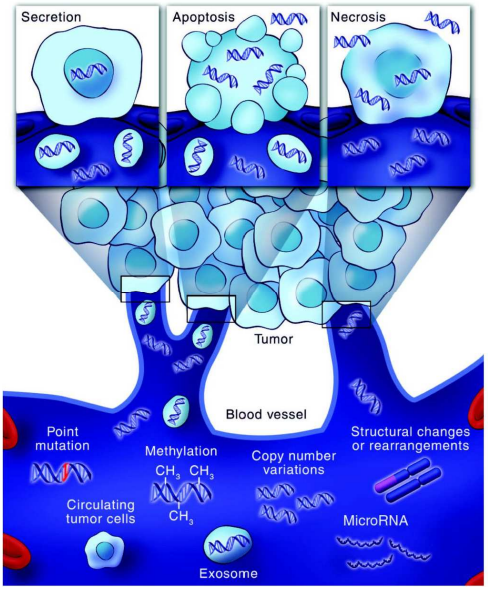
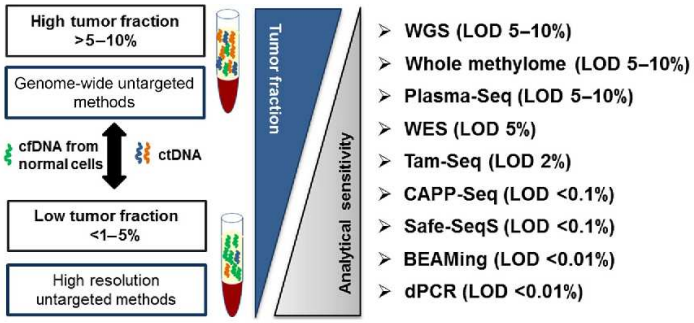
Declaration of Conflict of Interest

Ely Lilly: *advisor board member*

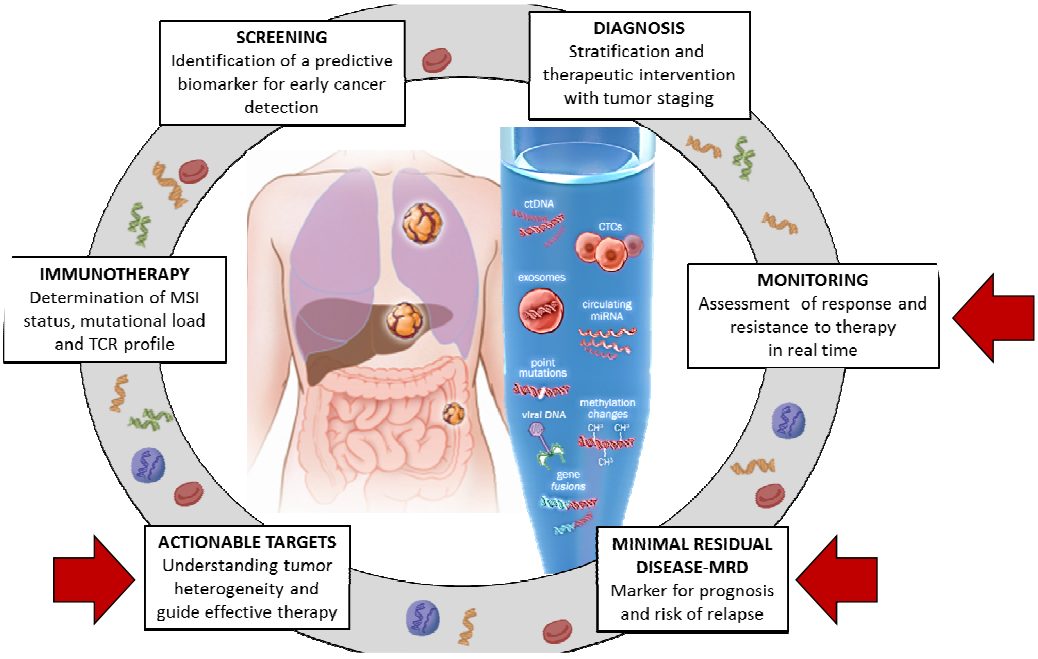
Liquid biopsies – genotyping circulating tumor DNA (ctDNA)





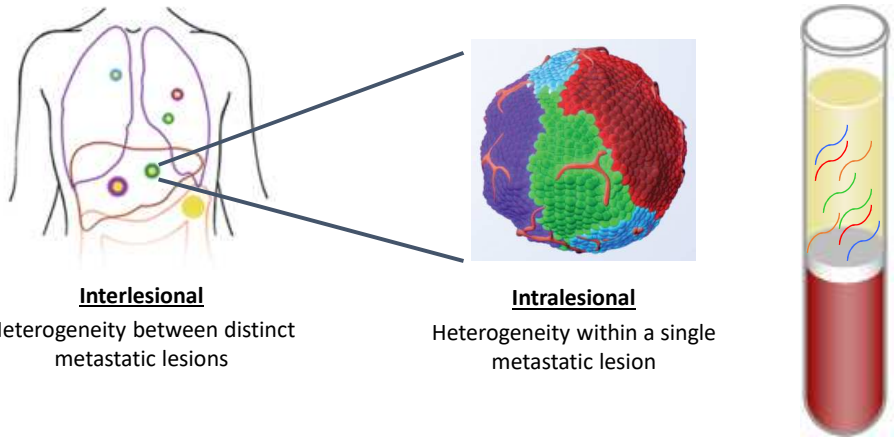
- WGS (LOD 5–10%)
- Whole methylome (LOD 5–10%)
- Plasma-Seq (LOD 5–10%)
- WES (LOD 5%)
- Tam-Seq (LOD 2%)
- CAPP-Seq (LOD <0.1%)
- Safe-SeqS (LOD <0.1%)
- BEAMing (LOD <0.01%)
- dPCR (LOD <0.01%)

Diaz and Bardelli, JCO 2013



Modified from Siravegna and Bardelli, Genome Biology 2014

Tumor heterogeneity and acquired resistance

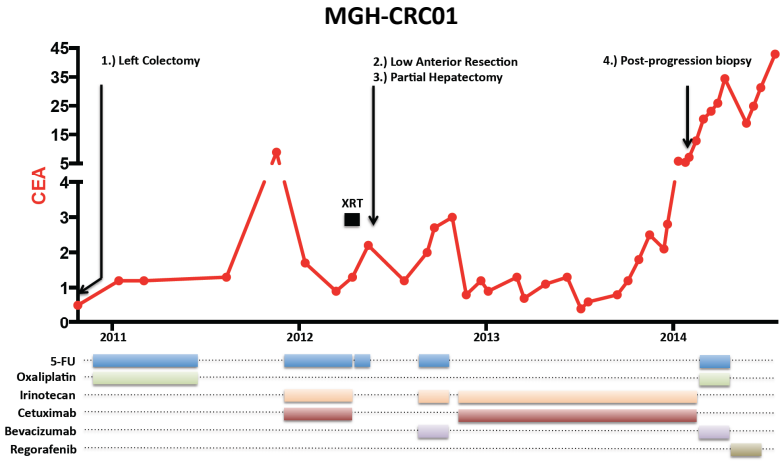


Interlesional
Heterogeneity between distinct metastatic lesions

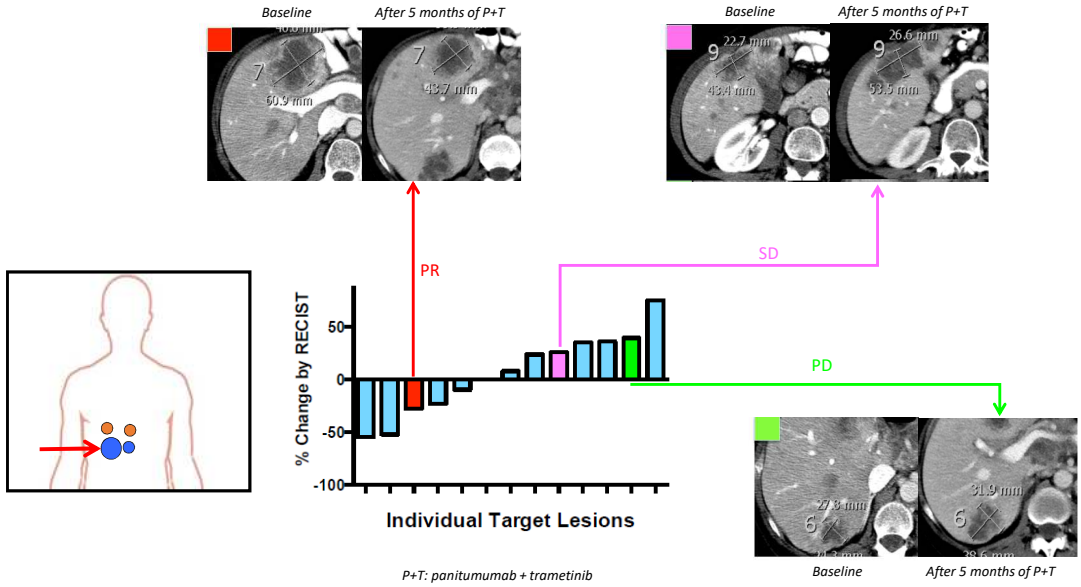
Intralesional
Heterogeneity within a single metastatic lesion

- A single needle biopsy may vastly underrepresent molecular heterogeneity
- Liquid biopsy may detect alterations in ctDNA shed by tumor cells throughout the body

Plasma ctDNA analysis capture tumour heterogeneity underlying lesion-specific responses in colorectal cancer

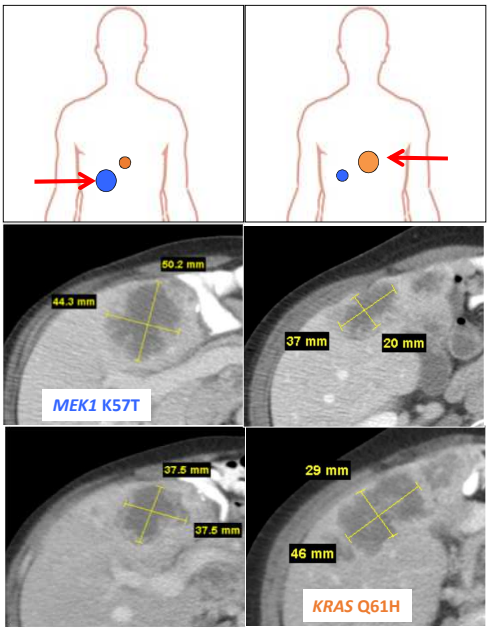


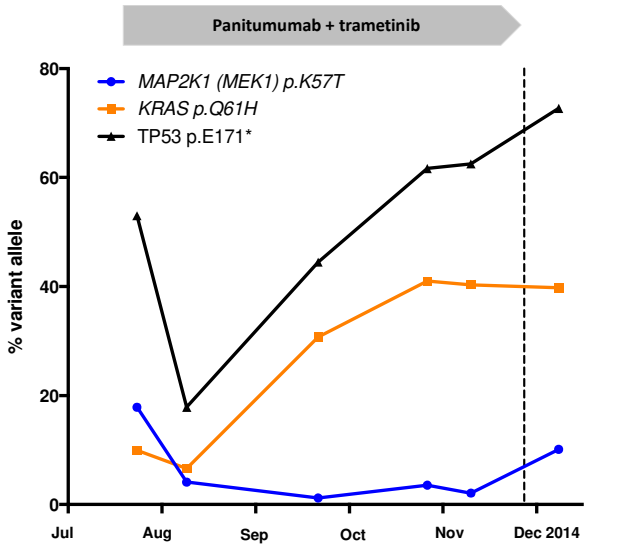
Targeted therapy drives clonal evolution and lesion-specific responses in colorectal cancer



P+T: panitumumab + trametinib

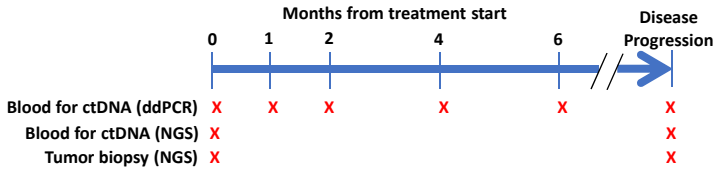
Single tumor biopsies are not sufficient to guide therapy

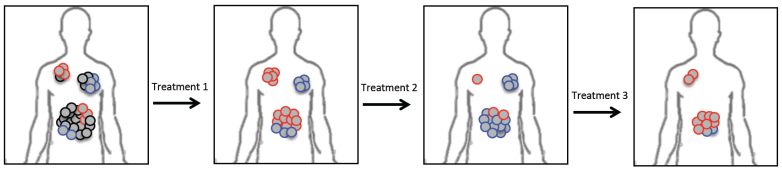


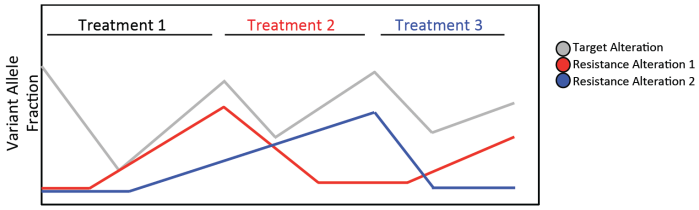


Russo, Siravegna et al, Bardelli and Corcoran Cancer Discovery 2016

Systematic liquid biopsy collection during targeted therapy

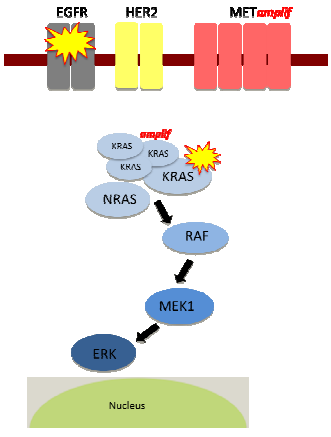






Plasma ctDNA analysis uncovers gene alterations driving acquired resistance in patients receiving anti-EGFR therapies

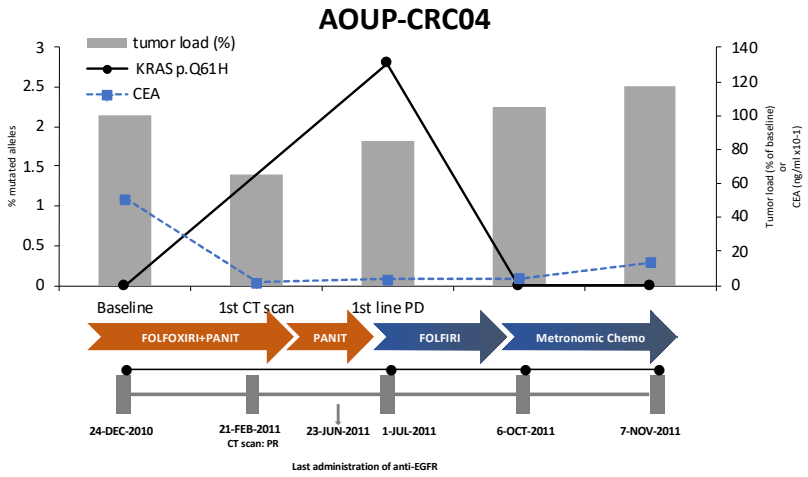
Patient ID	Therapy	Plasma cfDNA mutation at progression
ONCG-CRC57	Panit	*KRAS* p.G12A
		KRAS p.G12D
		KRAS p.G13D
AOUP-CRC04	Panit + folfoxiri	*KRAS* p.Q61H
ONCG-CRC69	Cetux; then panit	KRAS p.G12D
		KRAS p.G13D
MOLI-CRC04	Cetux + folfiri	*KRAS* p.Q61H
ONCG-CRC67	Panit	*MET* amplification
AOUP-CRC05	Panit + folfoxiri	*KRAS* p.G12D
AOUP-CRC01	Cetux + folfoxiri	*KRAS* p.Q61L
AOUP-CRC06	Cetux + folfoxiri	*KRAS* p.Q61L
AOUP-CRC03	Panit + folfoxiri	~~*KRAS* p.Q61L~~
ONCG-CRC72	Panit	*MET* amplification
ONCG-CRC70	Panit + irino	*KRAS* p.Q61H
		EGFR p.S464L
		EGFR p.G465R
ONCG-CRC71	Panit	*KRAS* p.Q61H
ONCG-CRC73	Panit	*MET* amplification
MGH-CRC02	Cetux	*KRAS* amplification
AOUP-CRC02	Panit + folfoxiri	*KRAS* p.Q61H
ONCG-CRC72	Panit	*EGFR* p.G465R
		EGFR p.G465E



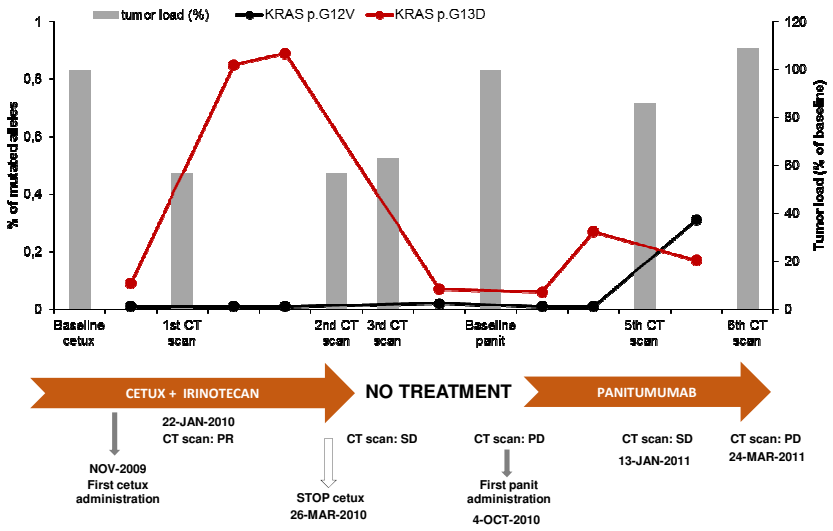
What happens to resistant clones upon progression?

Siravegna et al., Nature Medicine 2015

Follow tumor clonal evolution with plasma ctDNA: KRAS clones decline upon withdrawal of EGFR antibodies



Real-time adaptation of therapy guided by ctDNA

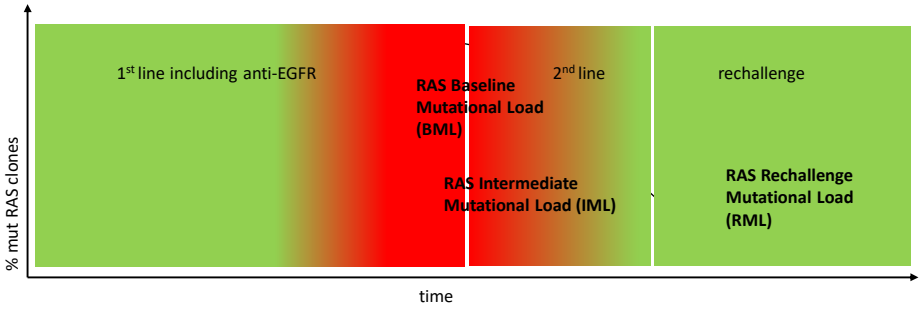


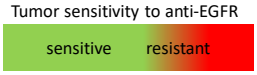
CHRONOS trial

Siravegna et al, Nature Medicine 2015

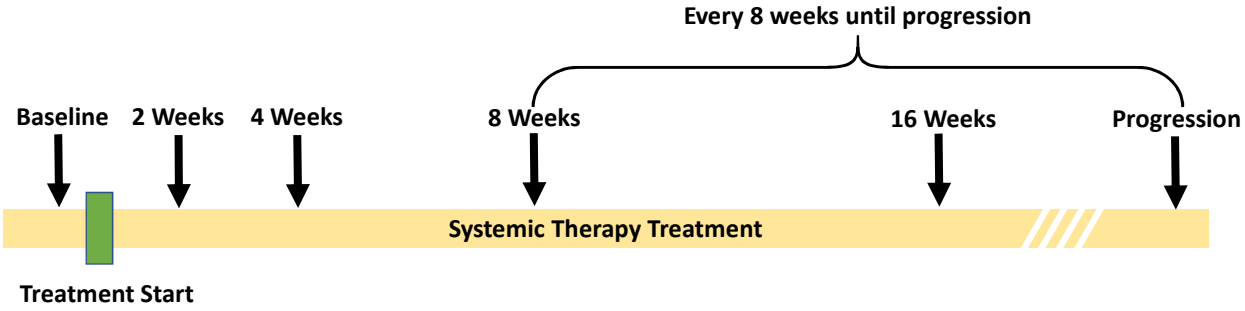
The CHRONOS trial

A PHASE II TRIAL OF RE**CH**ALLENGE WITH PANITUMUMAB D**R**IVEN BY RAS CL**ON**AL-MEDIATED DYNAMIC **O**F RE**S**ISTANCE





Serial ctDNA to predict treatment response

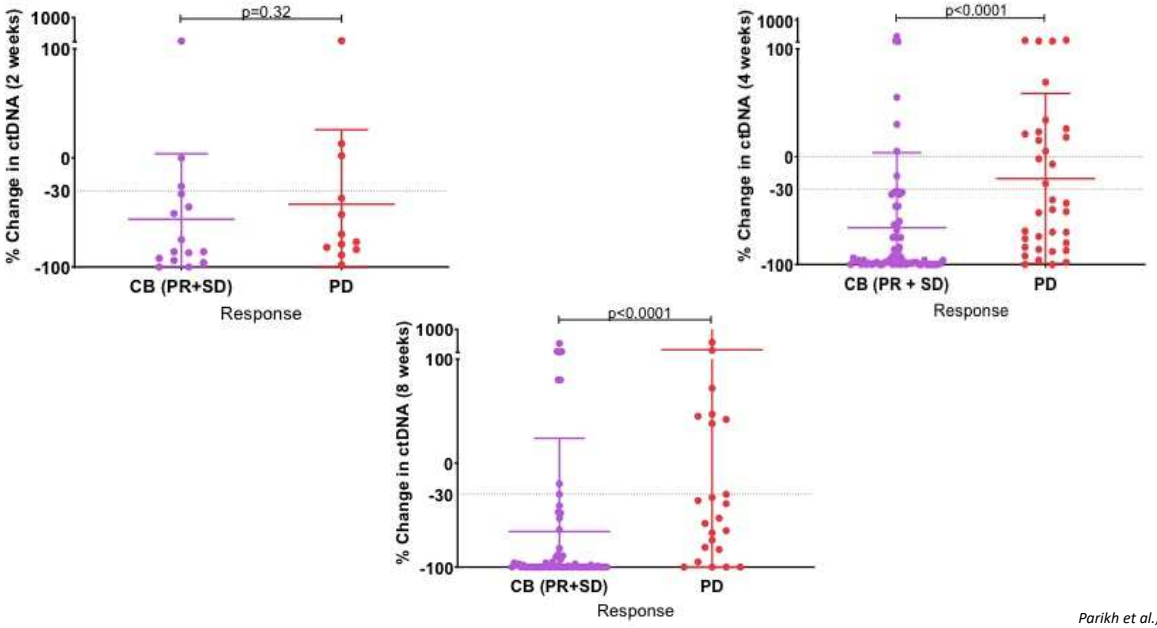


138 patients enrolled

- 50% colorectal cancer
- 29% pancreatic cancer
- 13% biliary cancers
- 12% esophagogastric cancer
- 6% other gastrointestinal primaries

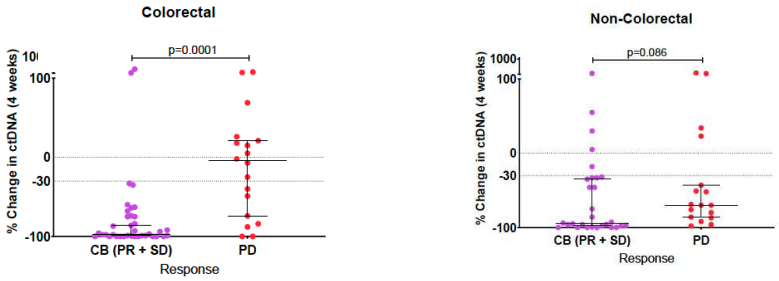
- 70% received cytotoxic chemo
- 17% received targeted agents
- 13% received both combined

Change in ctDNA over time

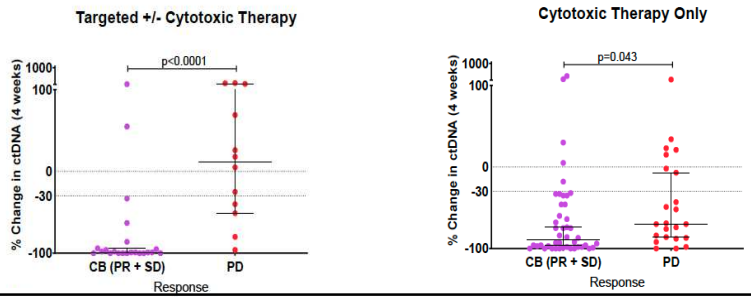


Parikh et al., CCR 2020

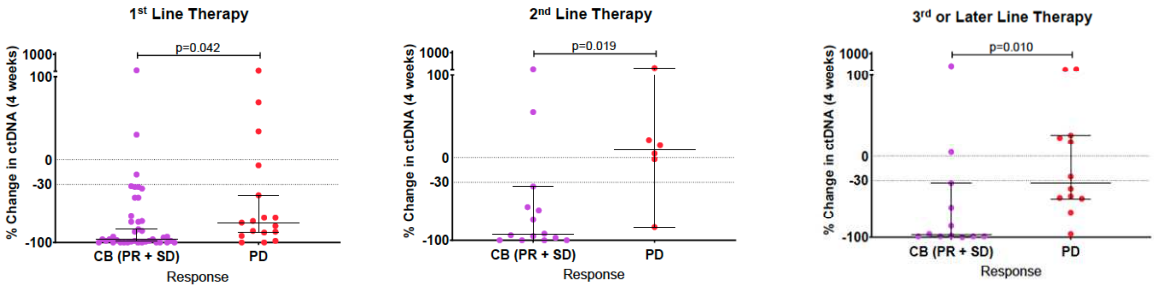
ctDNA change at 4 weeks predicted response and clinical benefit in CRC patients but not in other cancer types…



… and receiving targeted therapy with/without chemotherapy

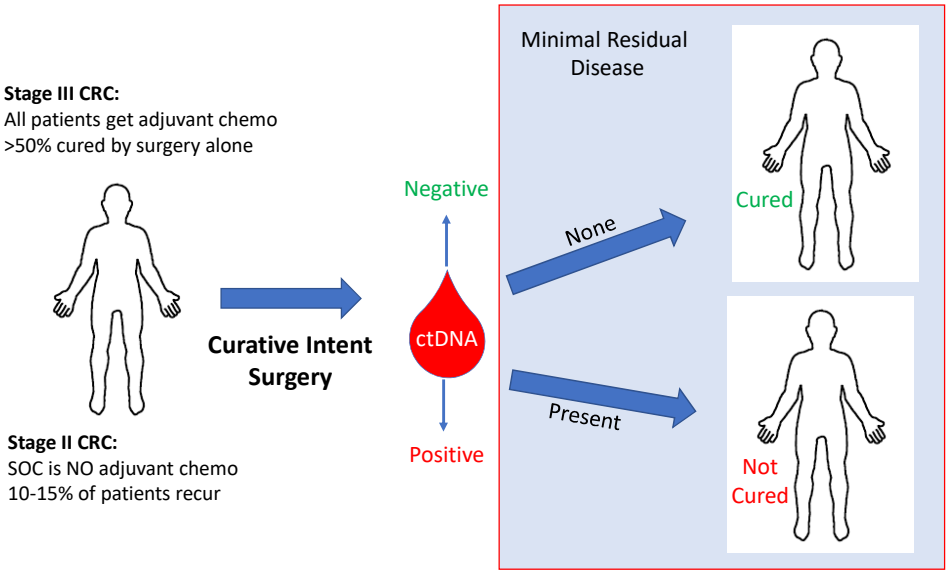


ctDNA change at 4 weeks is predictive of clinical benefit across different lines of therapy

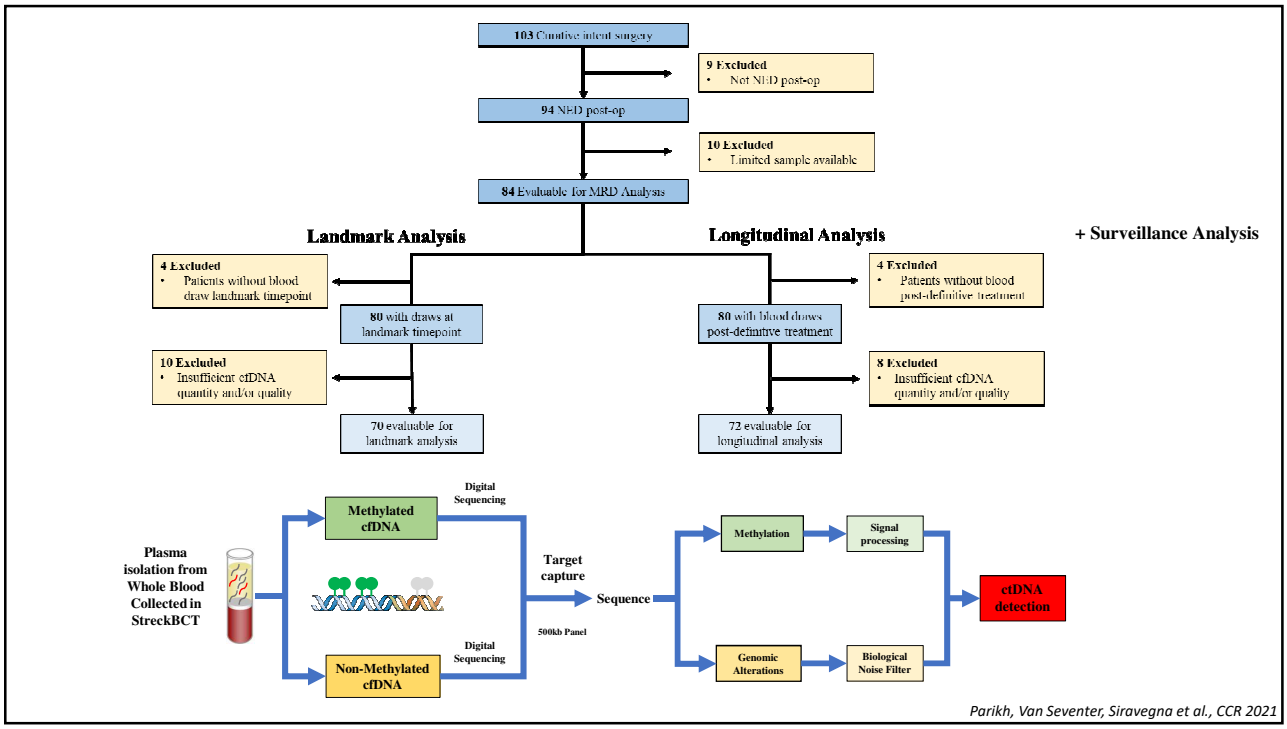


Serial ctDNA monitoring may provide early indication of response to systemic therapy in metastatic GI cancer patients prior to radiographic assessments and regardless of the treatment

Minimal Residual disease: The Problem



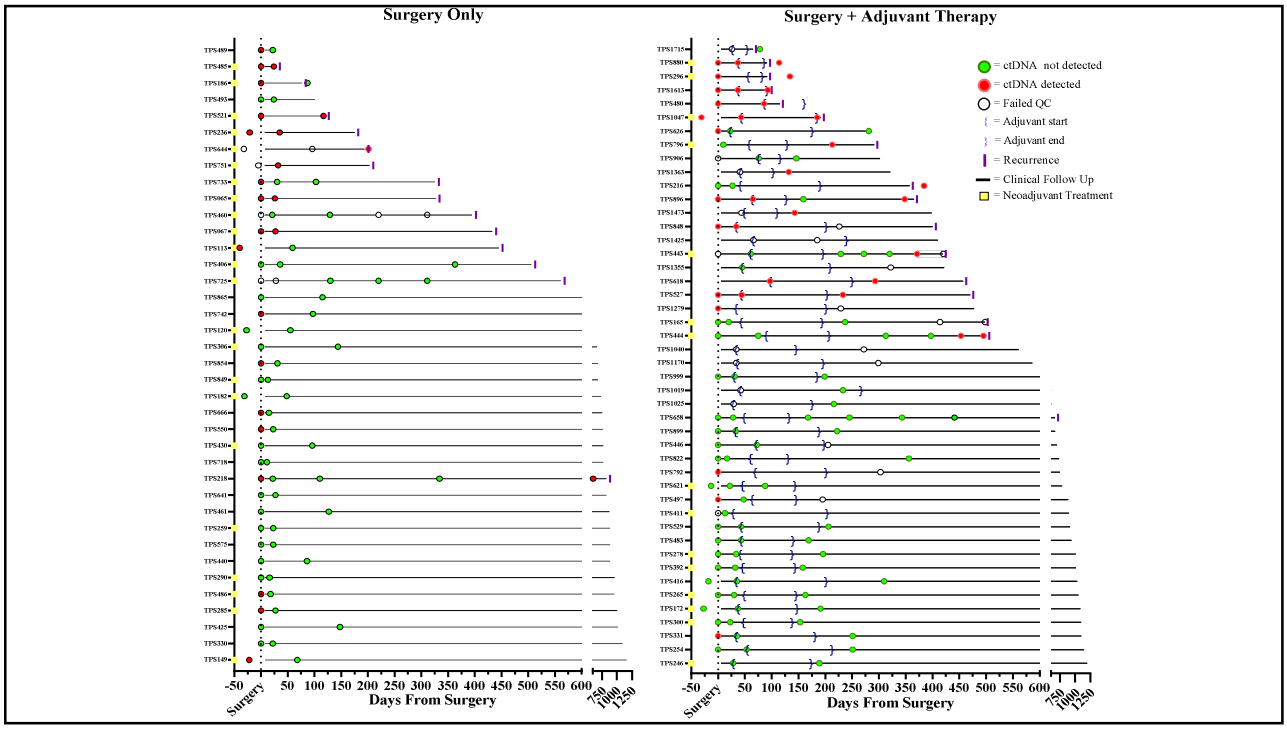
We have no way to determine who is cured and who will recur



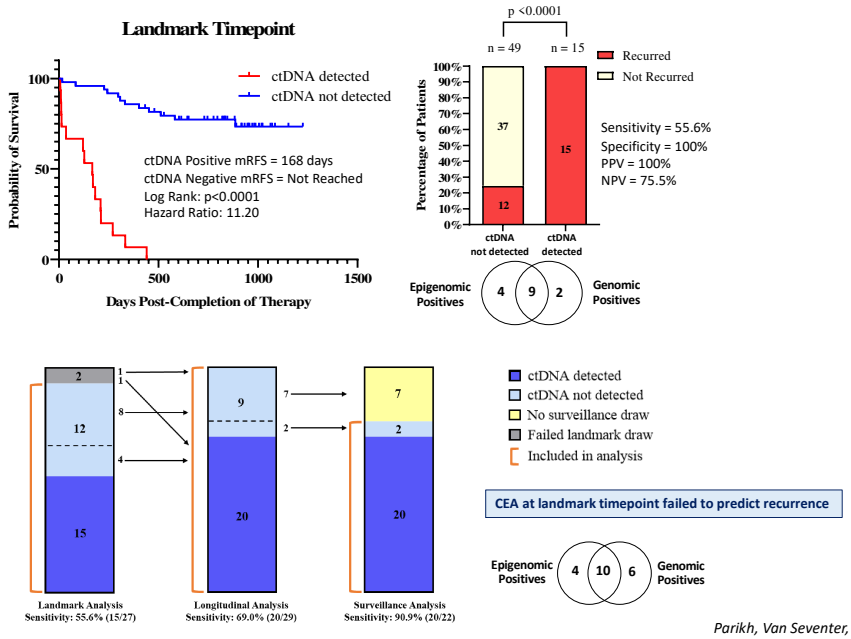
Parikh, Van Seventer, Siravegna et al., CCR 2021

Baseline patient and disease characteristics

Characteristic	Overall Cohort	
	N = 84	%
Age (years)– median (range)	60 (35-84)	
Sex		
Female	33	39.3
Male	51	60.7
Stage at Surgery		
I	8	9.5
II	20	23.8
III	40	47.6
IV	16	19.0
Sidedness		
Right	18	21.4
Transverse	5	6.0
Left	31	36.9
Rectal	30	35.7
Neoadjuvant Treatment	38	45.2
Adjuvant Treatment	46	54.8
Type of Adjuvant Treatment		
FOLFOX	31	67.4
CAPOX	7	15.2
FOLFOX + chemoxRT	3	6.5
5FU/LV	3	6.5
Other	2	4.3
Days on Adjuvant Treatment – median (range)	134.5 (28-463)	
Experienced Disease Recurrence	30	35.7
Days from Surgery to Recurrence – median (range)	348.5 (35-887)	
Days of Clinical Follow Up from Surgery – median (range)	632.5 (33-1246)	

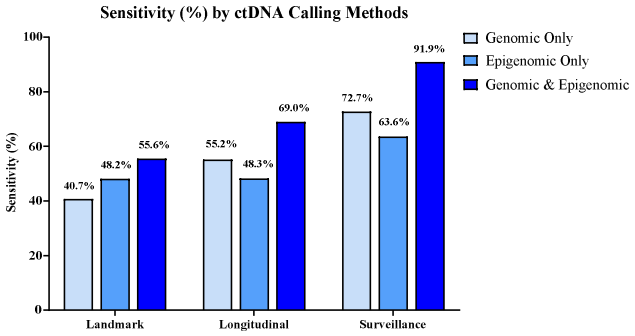


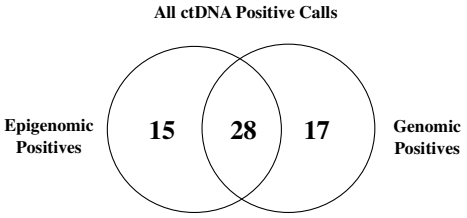
A plasma-only assay to detect MRD after surgery: results



Parikh, Van Seventer, Siravegna et al., CCR 2021

The combination of genomic and epigenomic calls is key to improve ctDNA detection



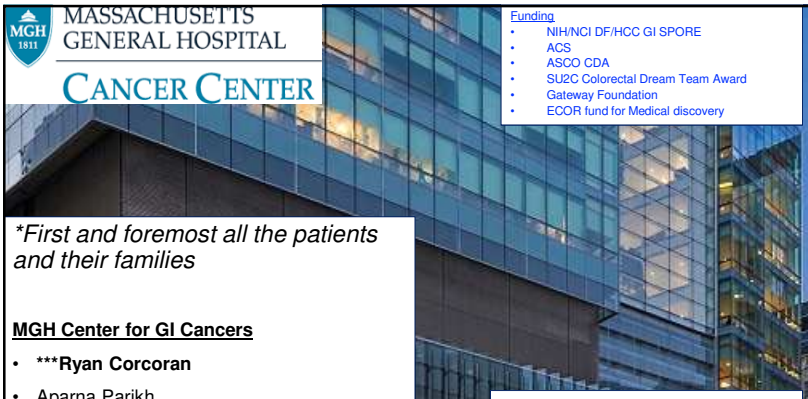


Parikh, Van Seventer, Siravegna et al., CCR 2021

Conclusions

- Molecular profiling through ctDNA can be used to guide treatment decisions, particularly when inadequate tissue is available
- ctDNA can uncover molecular heterogeneity in the same patient, reflecting lesion-specific responses
- Liquid biopsy may offer the ability to monitor emergence of resistance mechanisms in real-time and adjust therapy accordingly
- ctDNA at 4 weeks is a better predictor of radiologic response and clinical benefit to targeted agents and cytotoxic drugs in patients with mGI cancers
- A plasma ctDNA only assay, integrating genomic and epigenomic alterations assessment, can identify MRD in CRC patients at risk of recurrence after surgery with curative intent

Funding
- NIH/NCI DF/HCC GI SPORE
- ACS
- ASCO CDA
- SU2C Colorectal Dream Team Award
- Gateway Foundation
- ECOR fund for Medical discovery



First and foremost all the patients and their families

MGH Center for GI Cancers

- ***Ryan Corcoran**
- Aparna Parikh
- Sam Klepmner

ALL THE GI CANCER TEAM

MGH Pathology

- John Iafrate
- Joe Lennerz

MGH GI Research Assistant Team

- Emily Van Seventer

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- Meagan Ryan
- Jun Tian
- Katerina Fella
- Noritaka Tanaka
- Princy Sindurakar
- Stephanie Mc Queen
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- Andrea Sartore-Bianchi

IFOM

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