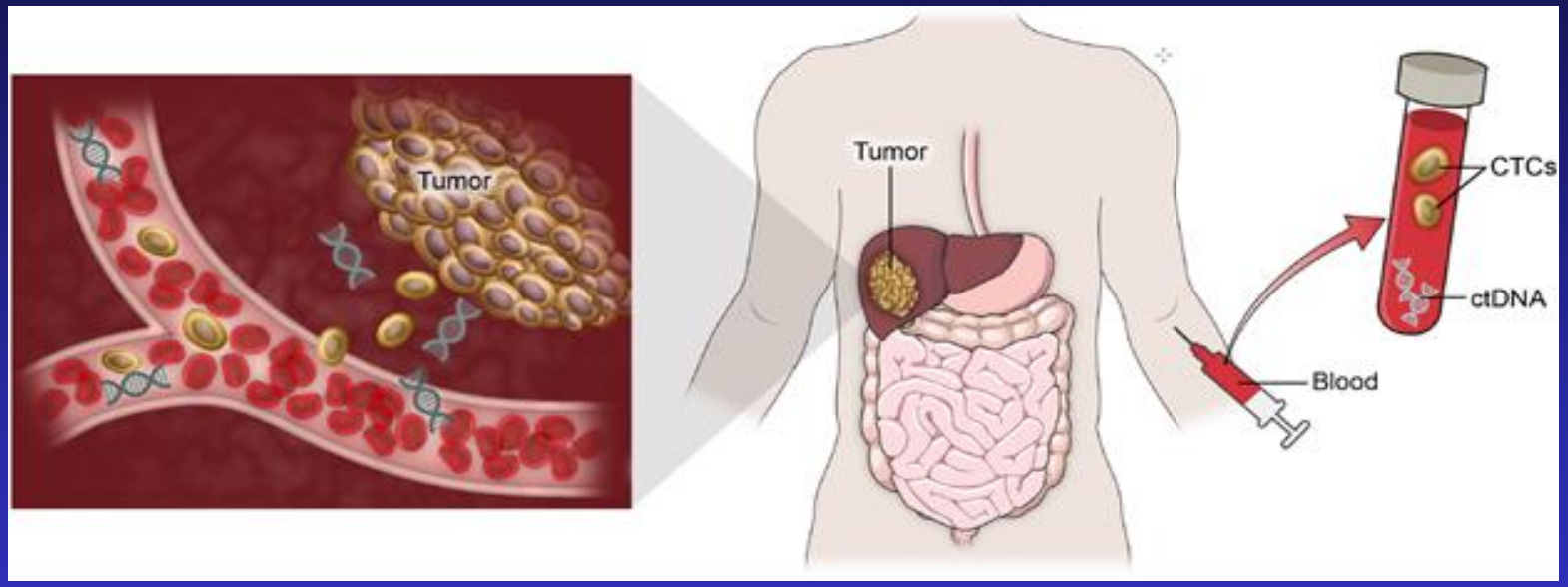
Liquid Biopsy in Colorectal Cancer: An Update



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Agenda

Updates on liquid biopsies in CRC:

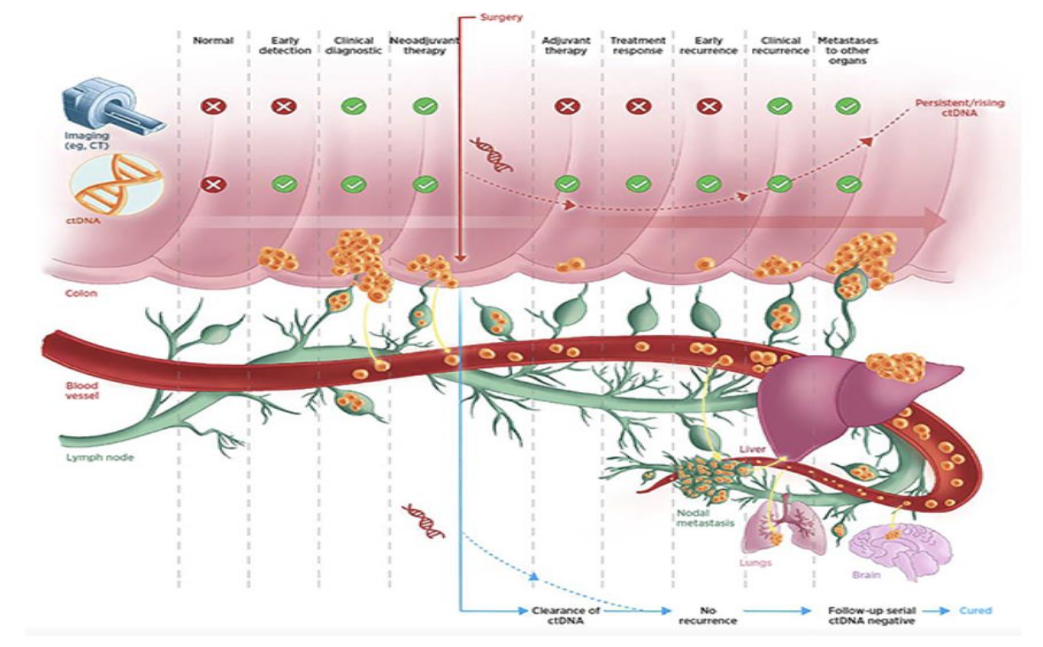
- Post-surgical resection with curative - detecting minimal residual disease (MRD)
- Post-surgical liquid biopsy in localized and locally advanced stages
- Post-surgical liquid biopsy in oligometastatic disease
- Neoadjuvant setting in locally advanced rectal cancer (LARC)
- Nonresectable advanced disease
- Anti-EGFR rechallenge by the introduction of interventional ctDNA assessment
- Liquid biopsies for immunotherapy

Introduction

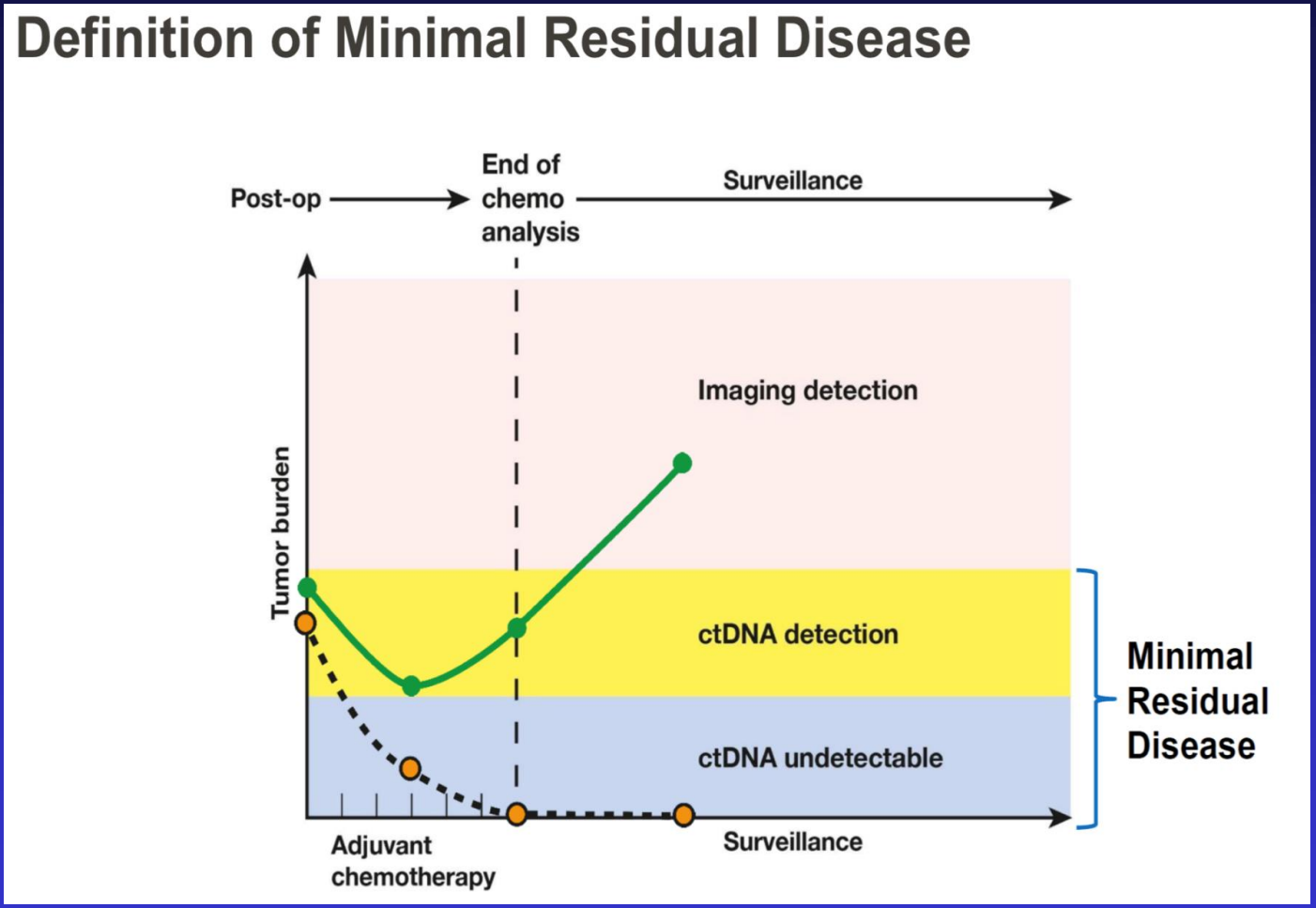
- CRC is one of the most prevalent and deadly cancers worldwide.
- Despite recent improvements in treatment and prevention, most of the current therapeutic options are weighted by E impacting patients' QOL.
- Currently, core tumor biopsy specimens represent the gold-standard biological tissue to identify such biomarkers.
    - However, technical feasibility, tumor heterogeneity and cancer evolution are major limitations of this single-snapshot approach.
- Liquid biopsy (LB) is increasingly gaining attention as a complementary and potentially alternative non-invasive tool to bypass these limitations.

Post-surgical resection with curative intent

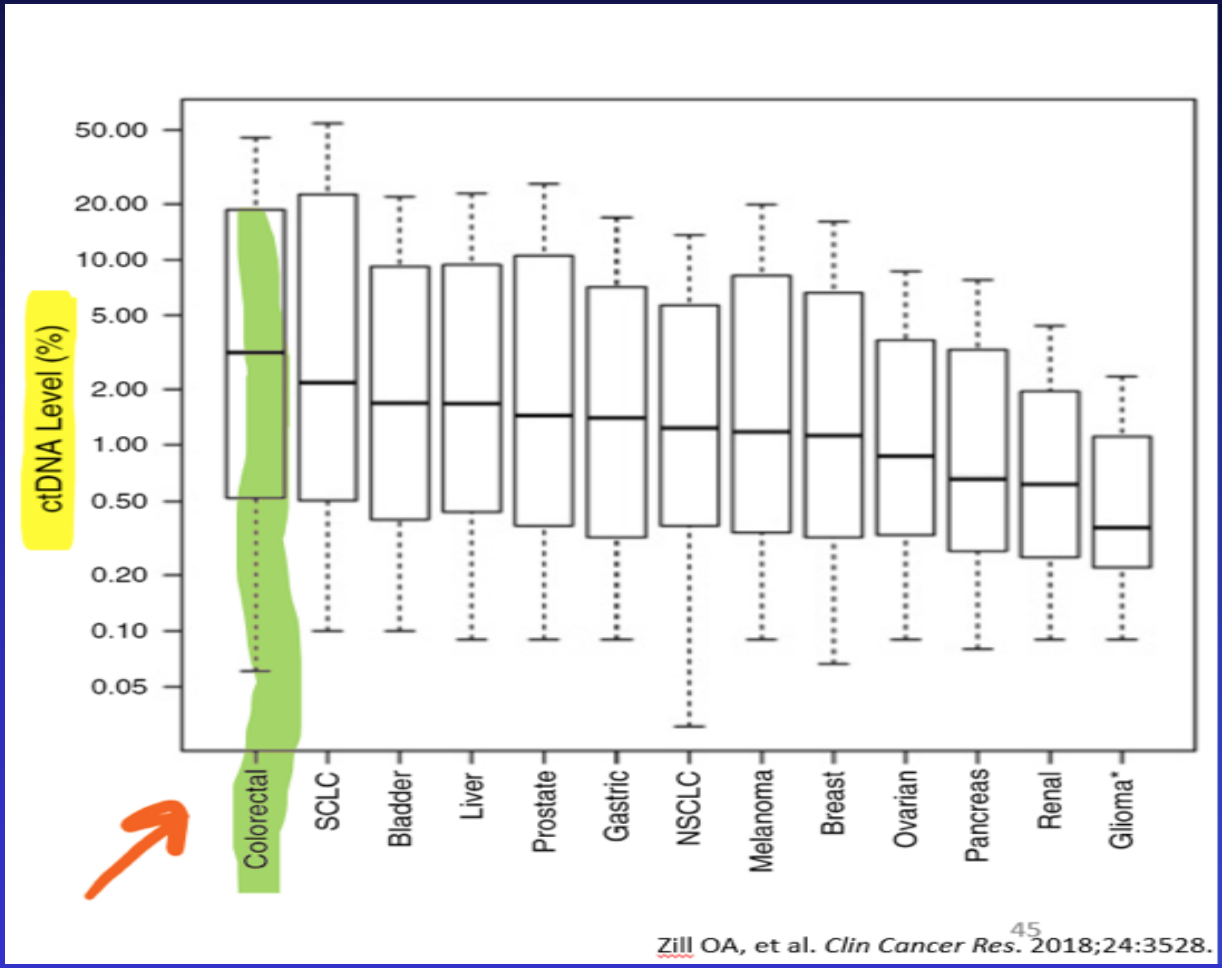
- Surgery represents the main curative treatment of CRC.
- In these patients the presence of ctDNA in the blood post-surgery can identify the existence of a minimal residual disease (MRD), invisible at radio-imaging and conceptually like the MRD in hematology.



Minimal residual disease (MRD)



Can we reliably detect CTDNA in patients with CRC?



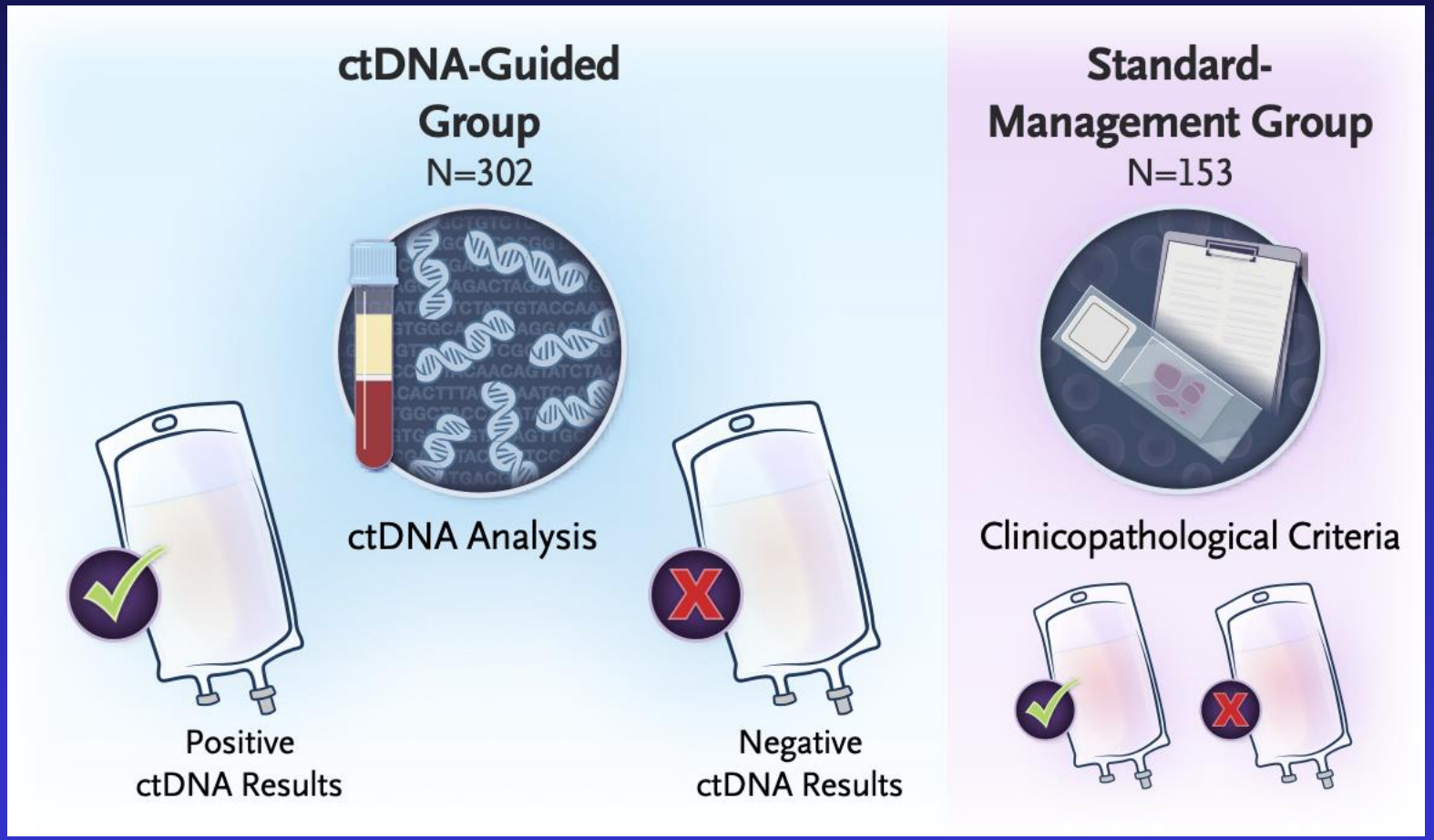
Zill OA, et al. *Clin Cancer Res*. 2018;24:3528.

Post-surgical liquid biopsy in localized and locally advanced stages

- Prognostic role of ctDNA is specifically dramatic in high-risk stage II (T4) and stage III CRC patients.

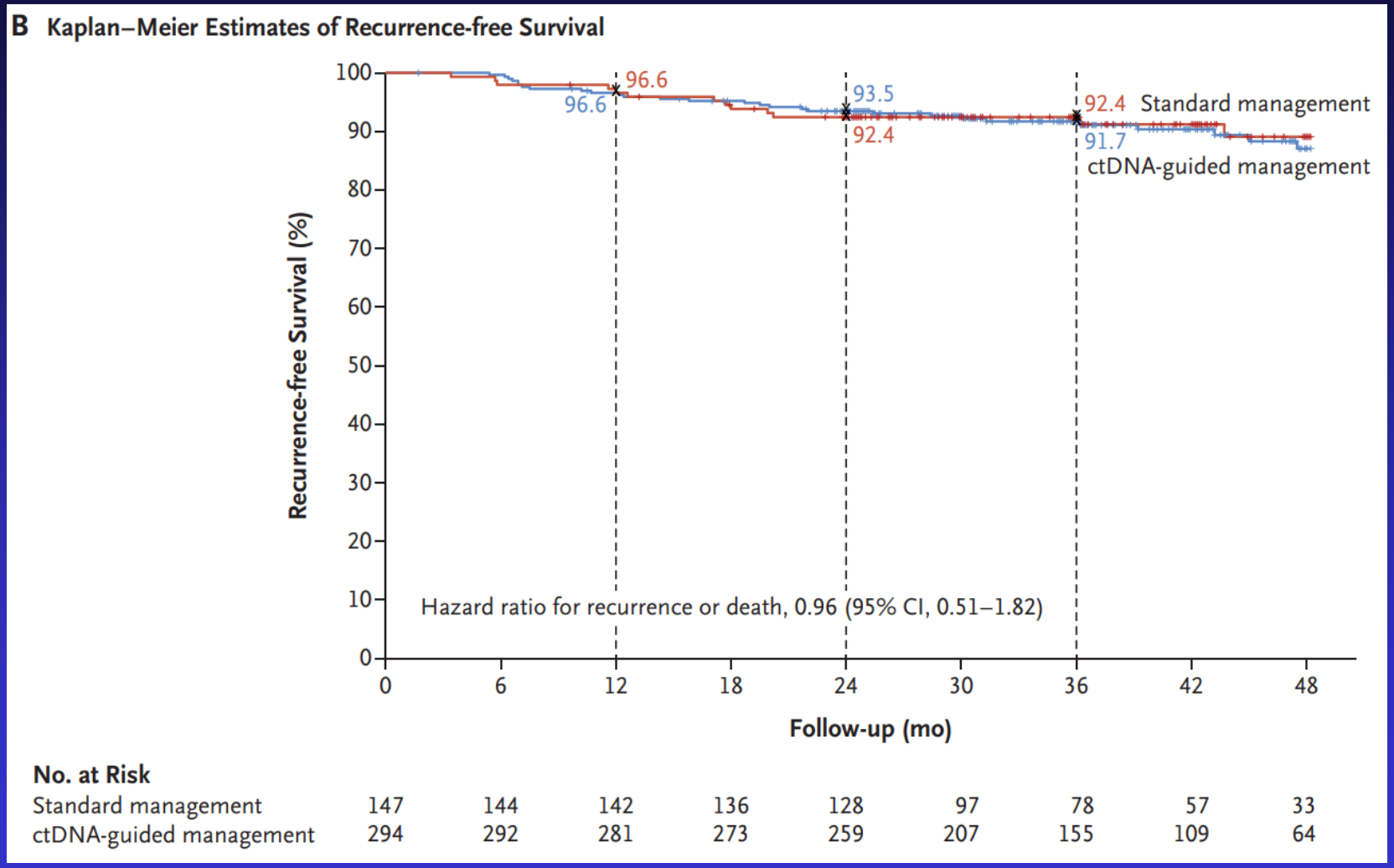
1. *Can ctDNA safely cherry-pick only patients that have a post-surgery MRD and thus should receive adjuvant treatment, while sparing treatment and toxicities to those already rendered disease-free by surgery alone?*
2. **ctDNA positivity is also associated** with **worse RFS** among those patients who had adjuvant treatment.
3. **ctDNA positivity** is remarkably prognostic also in stage III patients, predicting **disease relapse both post-surgery and post-adjuvant treatment**.

Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer



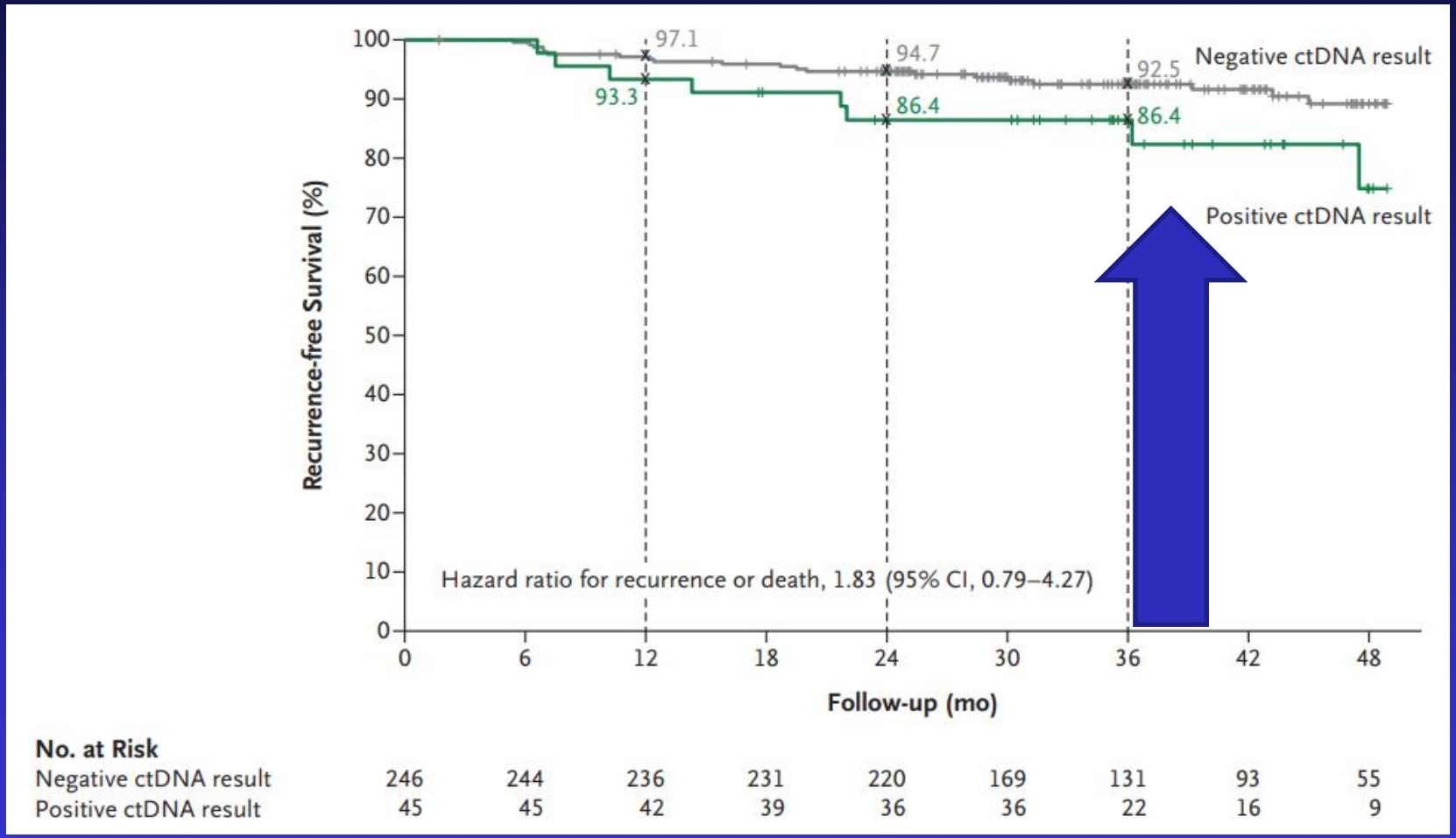
Tie et al. N Engl J Med 2022; 386:2261-2272

ctDNA-guided management was noninferior to standard management



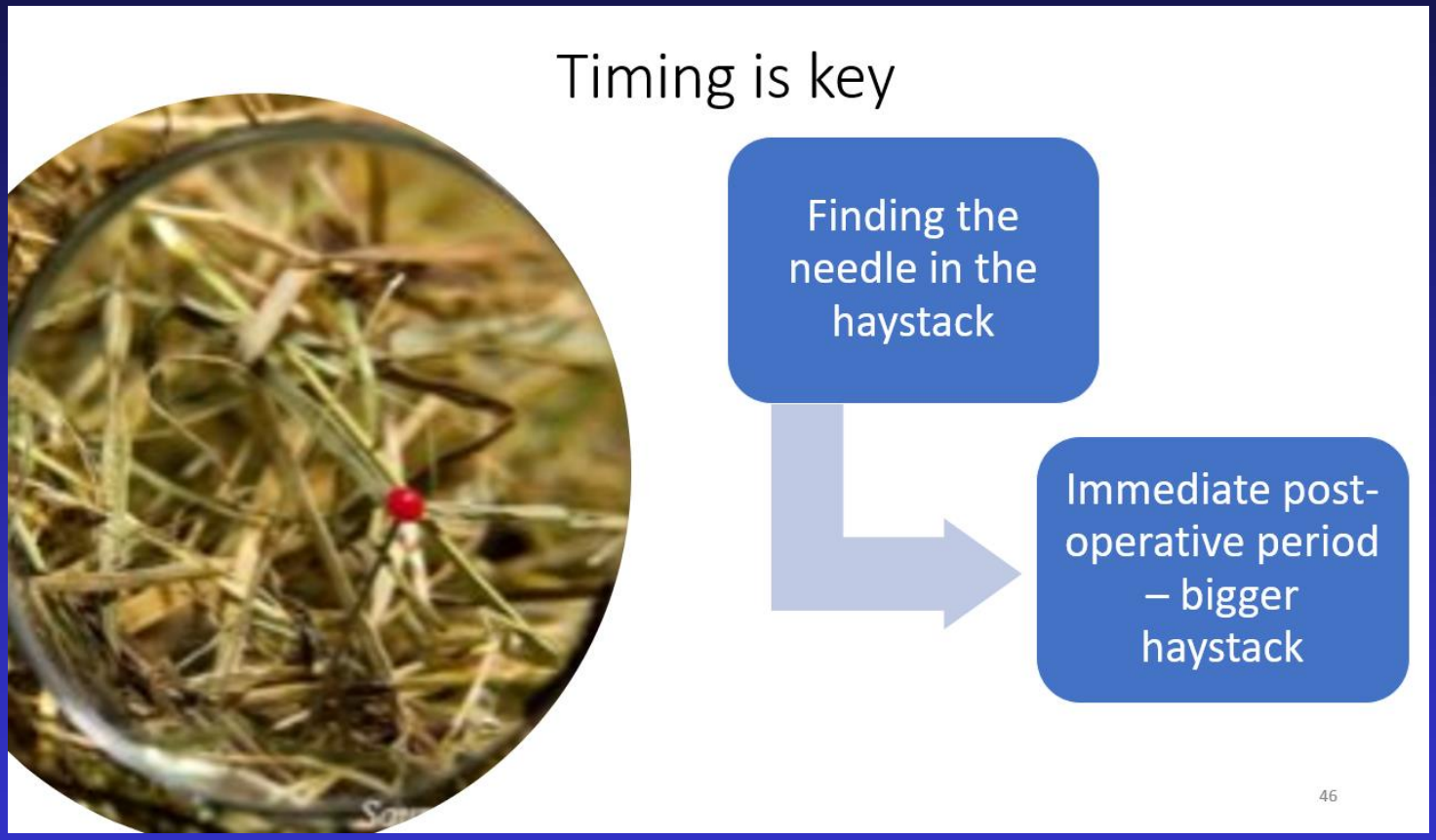
Tie et al. N Engl J Med 2022; 386:2261-2272

Three-year recurrence-free survival was 86.4% among ctDNA-positive patients who received ACT and 92.5% among ctDNA-negative patients who did not.



Tie et al. N Engl J Med 2022; 386:2261-2272

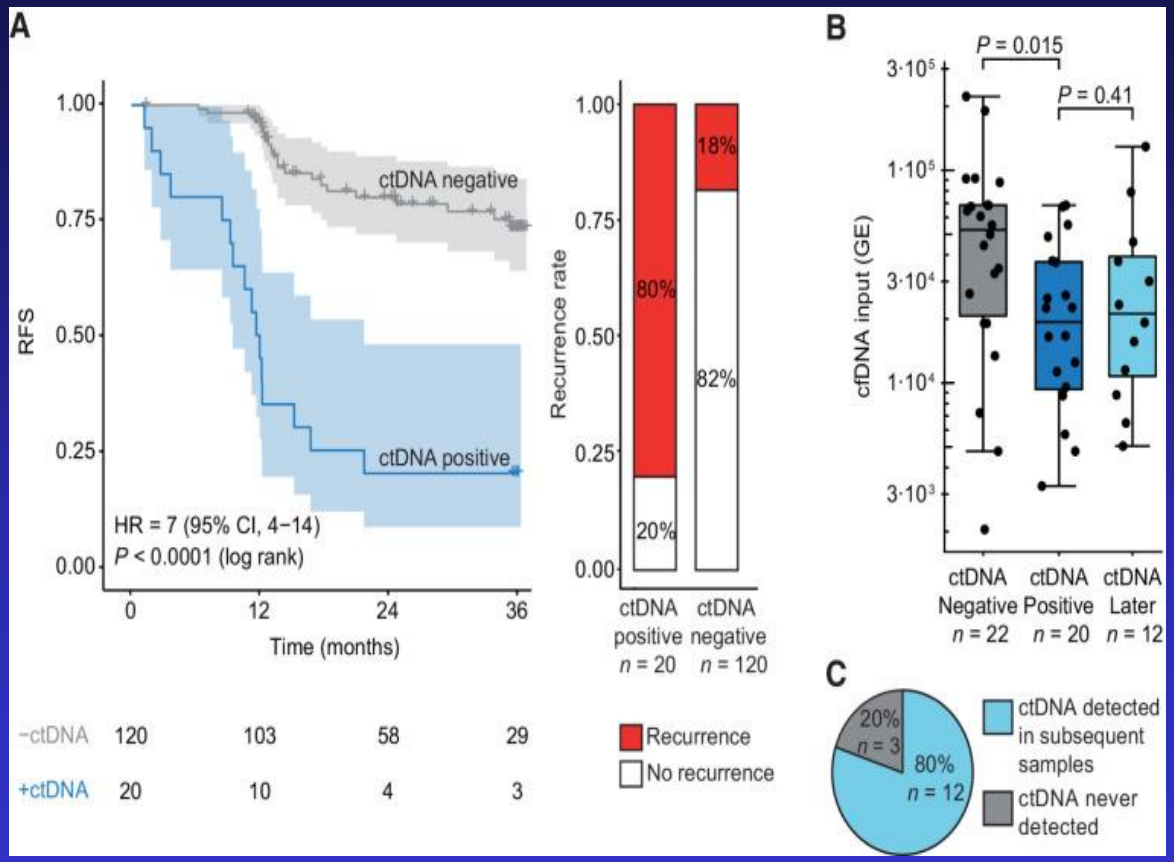
When do you need to make adjuvant therapy decisions?



Post-operative period (background cell-free DNA cfDNA "NOISE")

Circulating Tumor DNA in Stage III CRC, beyond MRD Detection, toward Assessment of ACT Efficacy and Clinical Behavior of Recurrences

A. Kaplan–Meier plot of RFS stratified for ctdna detection in blood samples collected within 2 months after surgery.

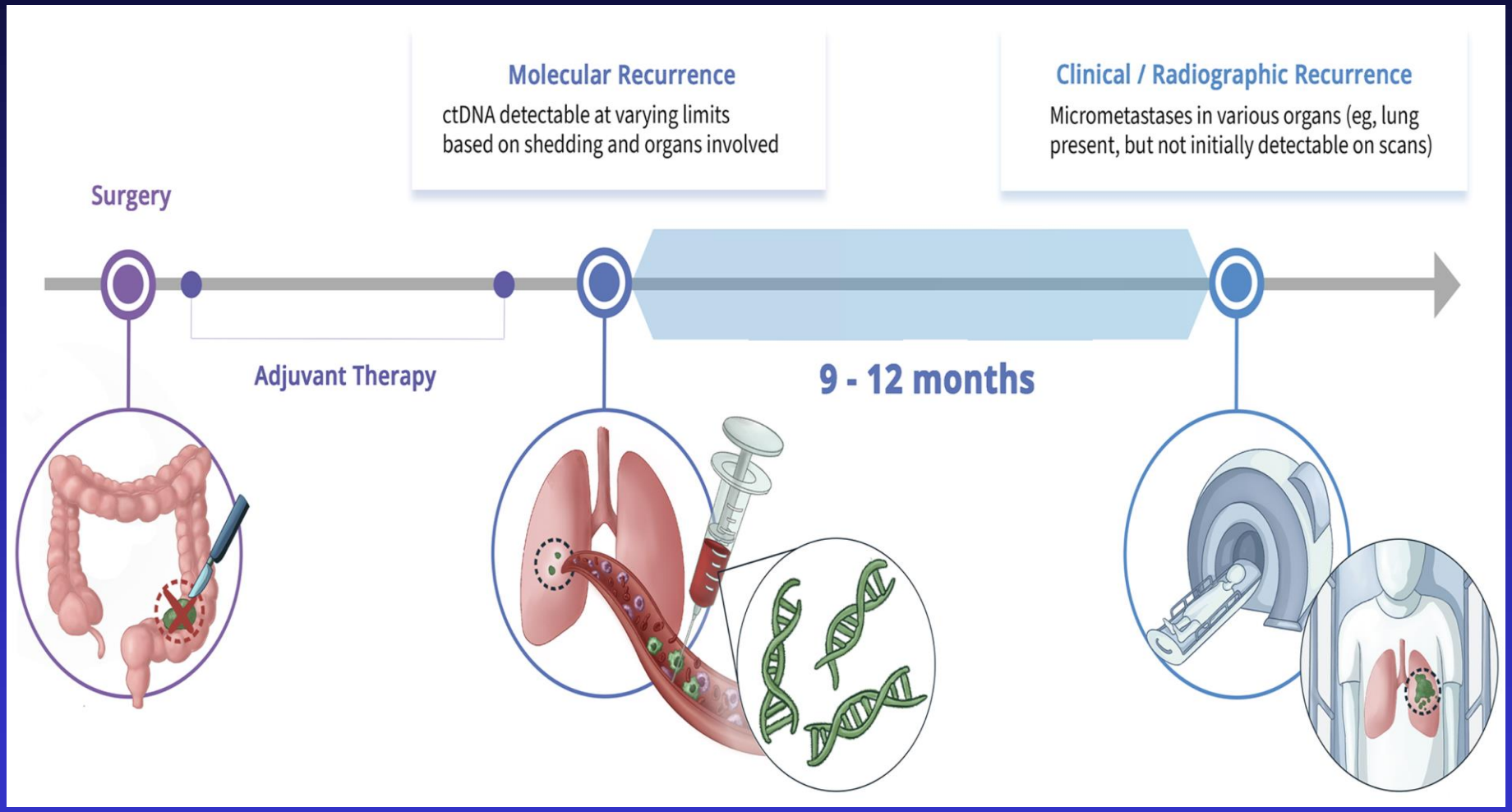


B. Levels of cfDNA in samples that were ctdna - immediately after surgery in recurrence patients; ctdna + immediately after surgery; or ctdna + >2 months after surgery in initially ctdna - recurrence patients.

C. Recurrence patients without detectable ctdna immediately after surgery and with samples collected >2 months after surgery.

Henricksen TV, et al. Clin Can Research 2022.

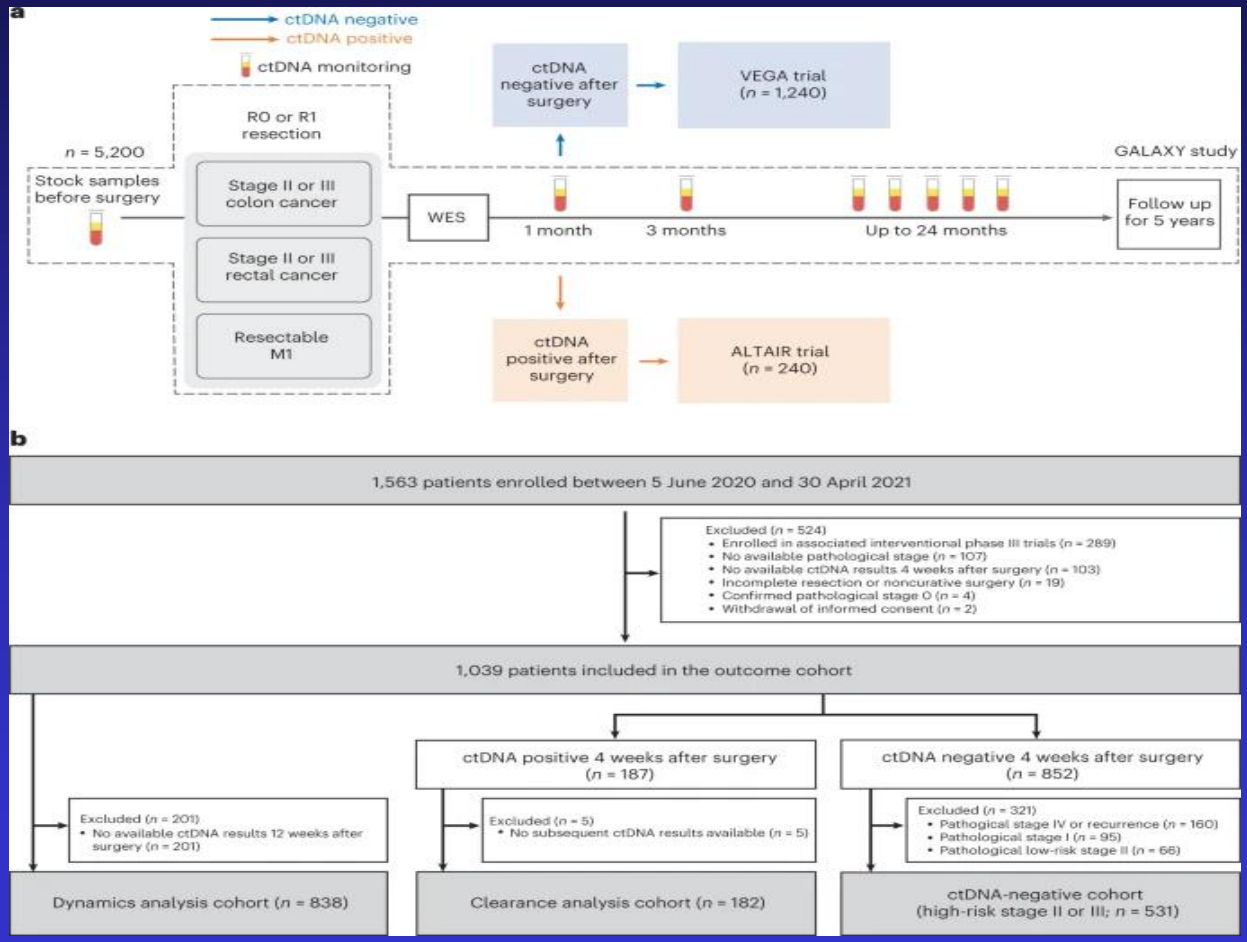
"Adjuvant-plus"



Kasi PM. Utility and Debate of Liquid Biopsy Assays in Surveillance Setting. March 2023. ASCO

GALAXY STUDY

- **GALAXY,** part of the CIRCULATE-Japan platform protocol.
- Patients with stage II to IV resectable CRC.
- Clinical utility of ctDNA analysis for detecting “molecular residual disease” (MRD).



Kotani D et al. Nature Medicine, 2023.

GALAXY Trial

Prognostic Impact of ctDNA Status on Disease-Free Survival

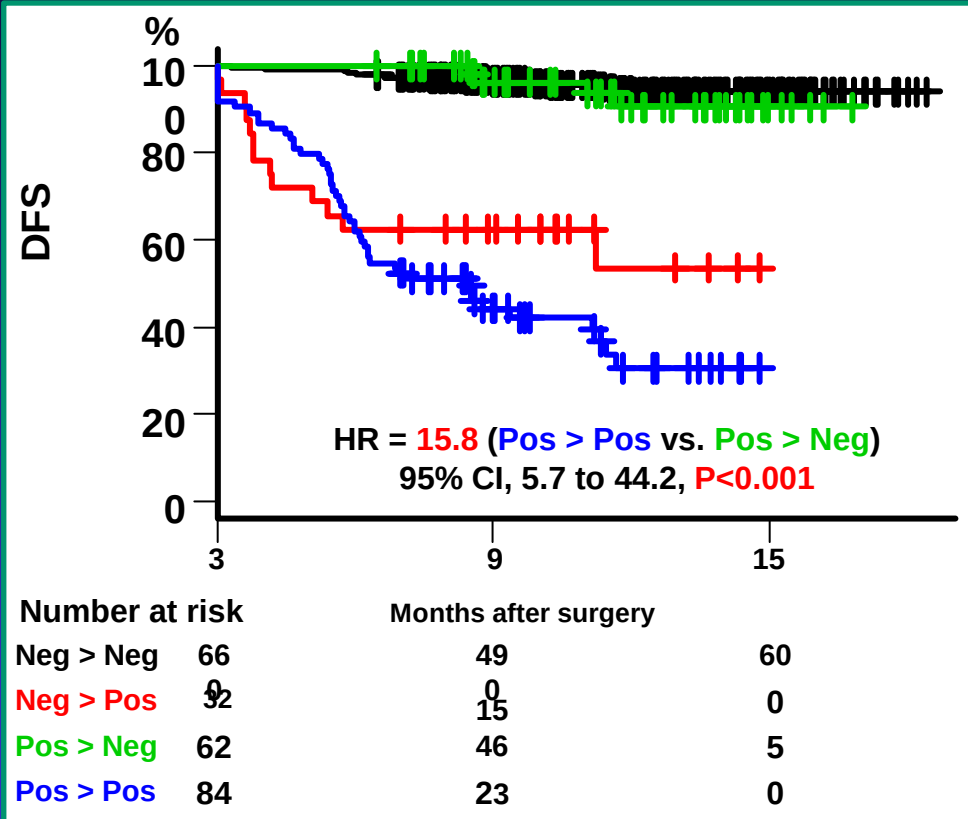
Outcome	ctDNA-Negative	ctDNA-Positive	Hazard Ratio
For Stage I–IV Disease			
6-month DFS	96.5%	62.8%	10.9 (P < .001)
12-month DFS	92.7%	47.5%	
For Stage II–III Disease			
6-month DFS	97.8%	73.0%	13.3 (P < .001)
12-month DFS	95.2%	55.5%	

Disease-Free Survival by cDNA Status at 4 Weeks After Surgery in GALAXY Trial

Kotani D et al. Nature Medicine, 2023.

GALAXY Trial

Dynamics Analysis: When ctDNA Status Changes



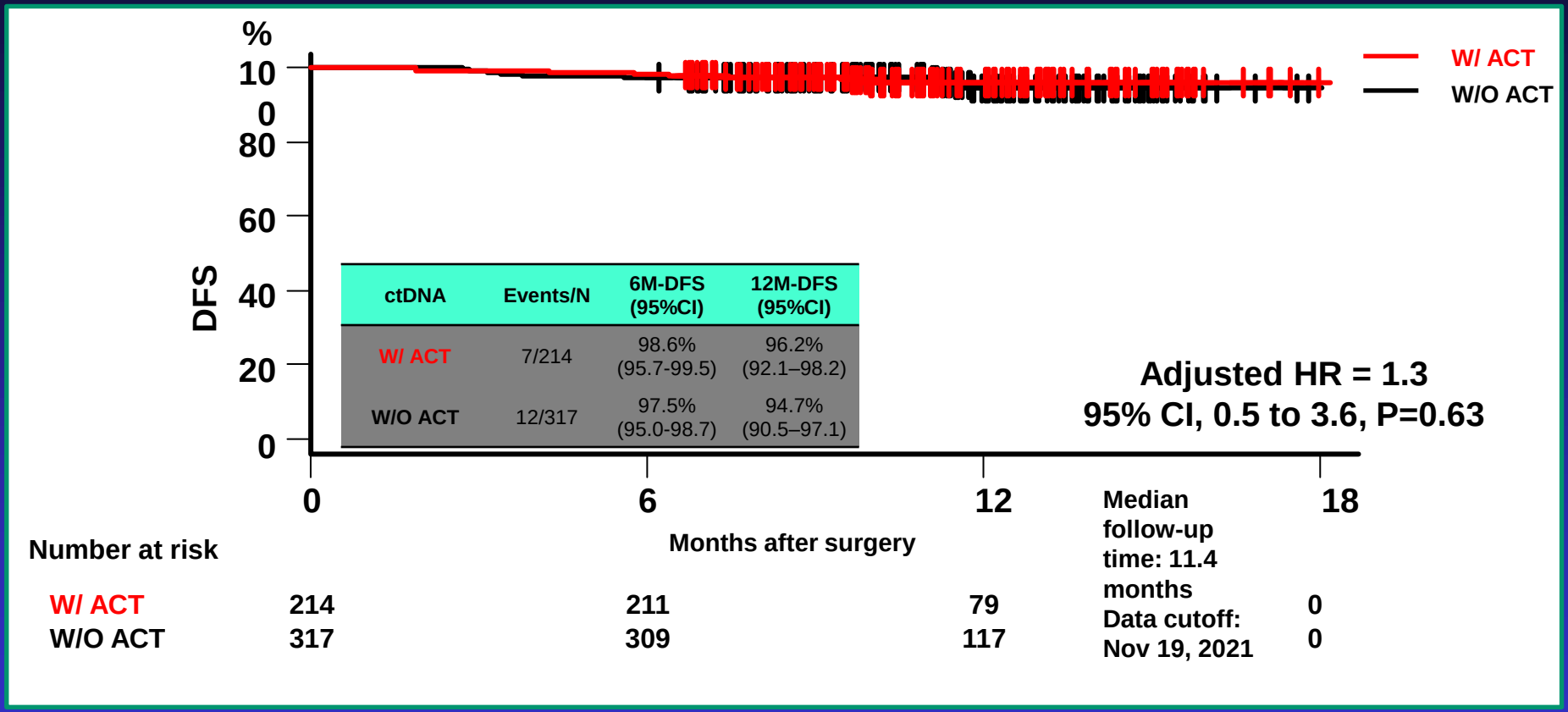
dynamics	Neg > Neg	Neg > Pos	Pos > Neg	Pos > Pos
Events/N	31/660	13/32	4/62	50/84
6M-DFS	98.0%	62.5%	100%	58.3%
HR	0.8	9.2	Reference	15.8
95%CI	0.27–2.15	3.0–28.4	-	5.7-44.2
P	0.60	<0.001	-	<0.001

- Patients with baseline ctDNA + who remained + over course of treatment had 16-fold increased risk of DFR
- Patients + to + during ACT had significantly worse outcomes (58.3%relative to those who + to – (100%) exhibiting a 15.8-fold risk of DFR
- Patients who remained - was 98% DFS rate
- Patients who turned to + 62.5% DFS rate

Kotani D et al. Nature Medicine, 2023.

GALAXY Trial

Impact of Adjuvant Chemotherapy

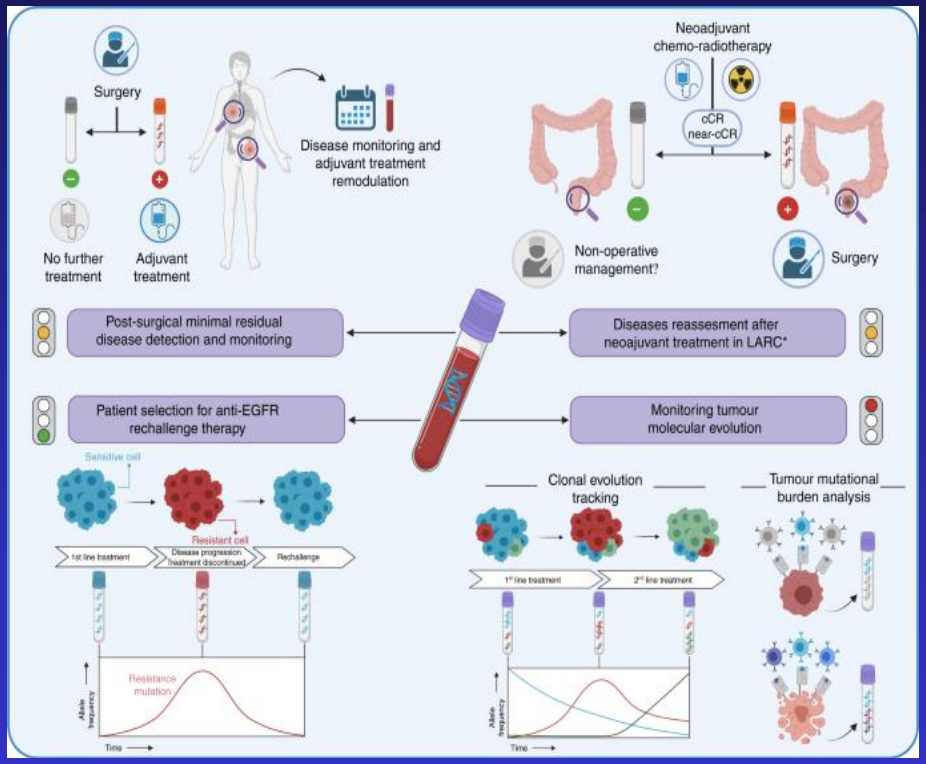


- + patients at 4 weeks post-surgery were administered ACT, impact of treatment assessed as ctDNA clearance was 68% vs 10% in patients who did not receive ACT (HR = 9.3; *P* < .001).

Kotani D et al. Nature Medicine, 2023.

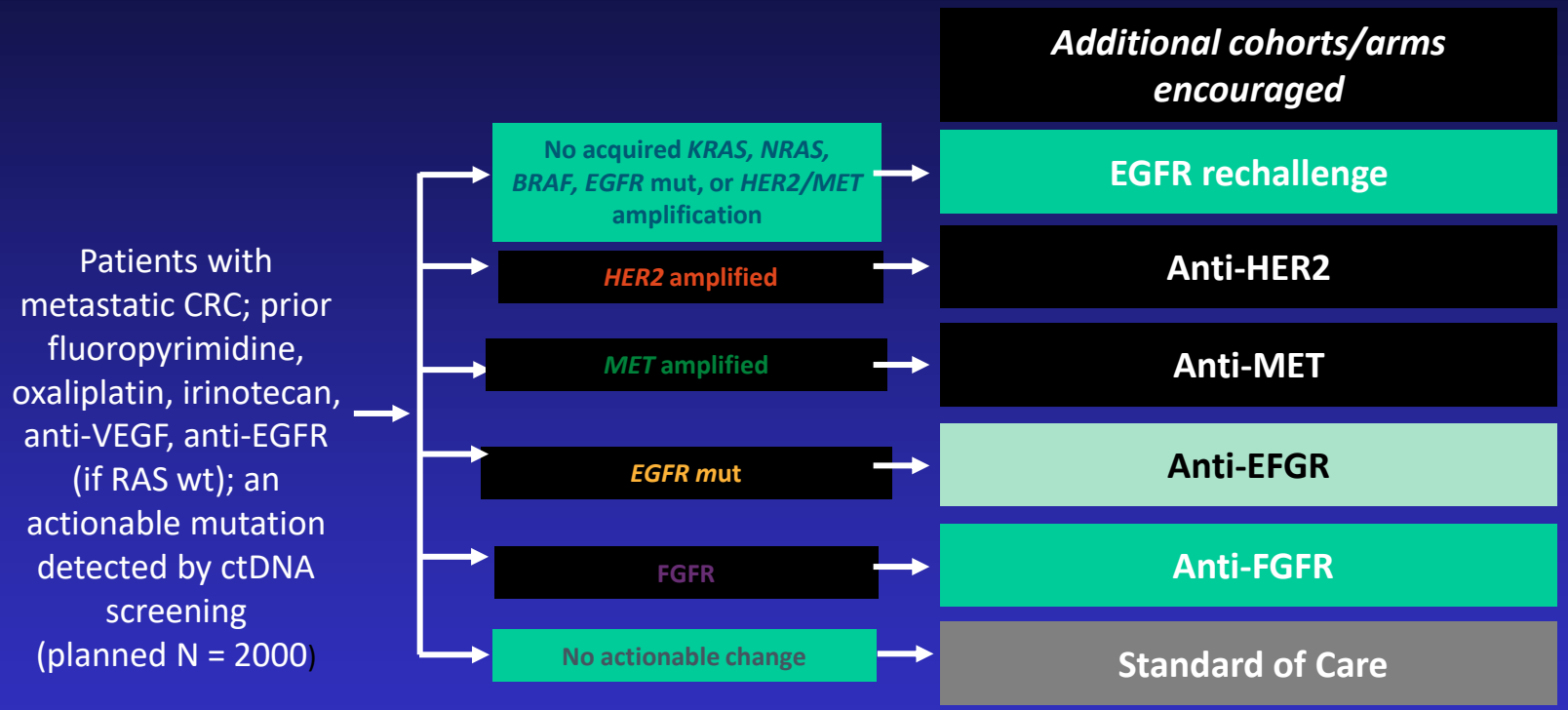
Post-surgical liquid biopsy in oligometastatic disease

- In selected stage IV oligometastatic patients, surgical resection of metastasis can be pursued with curative intent.
- In this setting as well, ctDNA provided striking results comparable to those described above for non-metastatic disease.
- More recently, Tie and co-workers demonstrated that patients with liver-only metastases undergoing surgical resection had a lower RFS and survived less in the case of ctDNA positivity.
- Despite this exciting amount of retrospective evidence suggesting that ctDNA is a potential predictive marker of disease recurrence in radically resected stage I–IV CRC patients, the actual clinical benefit is yet to be proven in prospective interventional trials.



Mauri G. British J of Cancer, 2022.

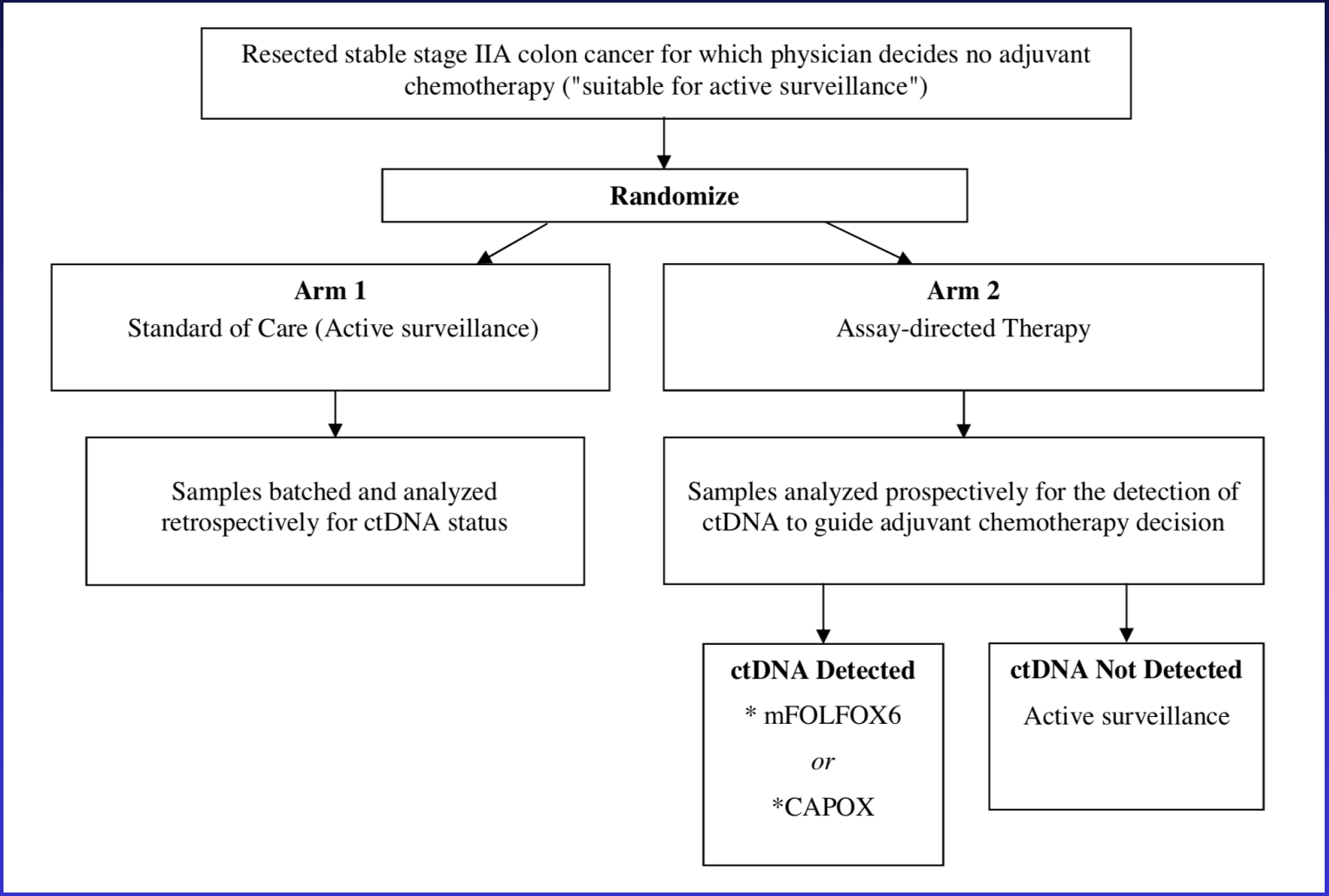
COLOMATE: Colorectal and Liquid Biopsy Molecularly Assigned Therapy



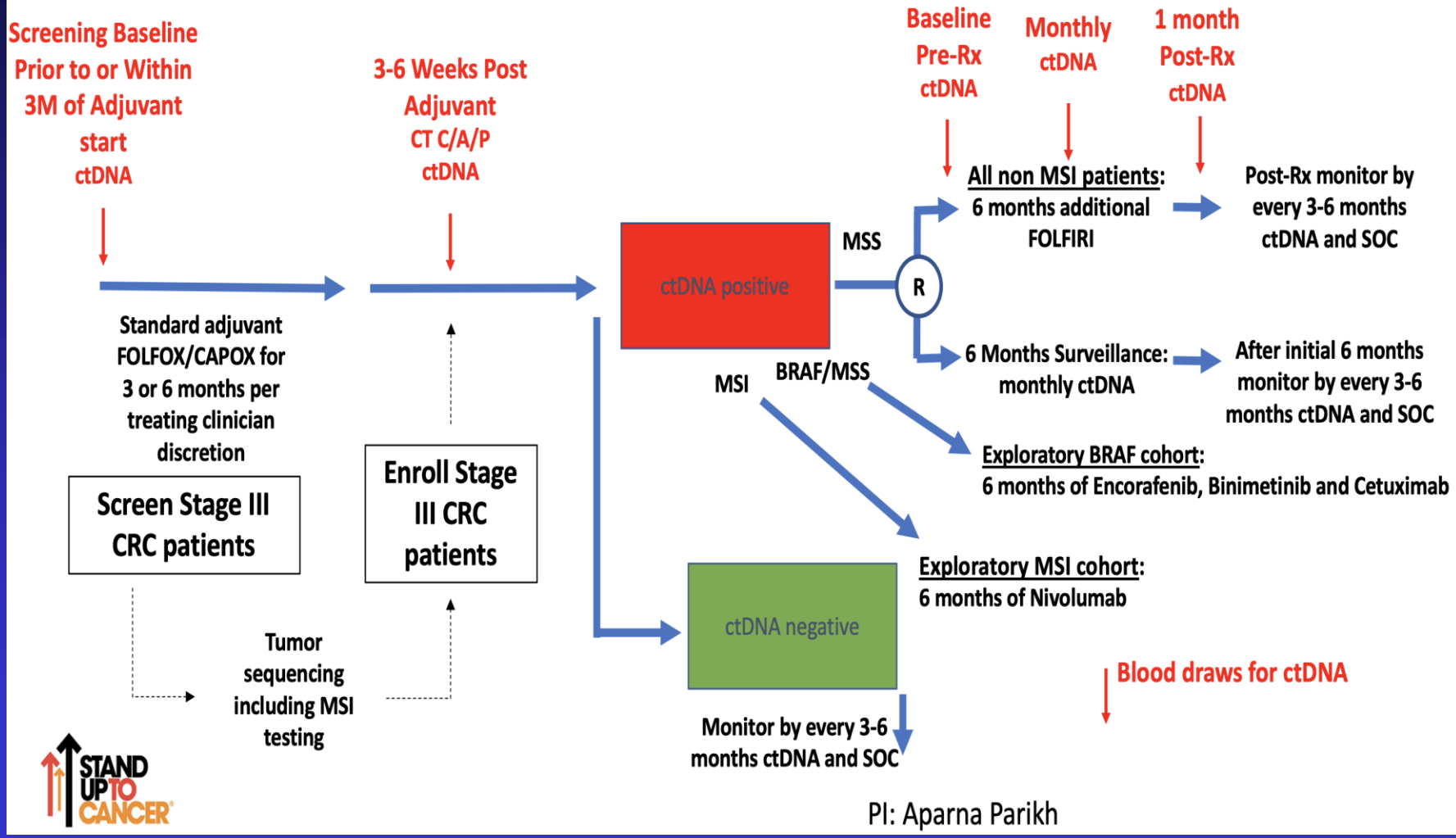
- Endpoints dependent on MoA of investigational agent; flexible study design:
  arms open and close with best available science

NCT03765788

Circulating tumOr DNA as a Predictive BiomaRker in Adjuvant Chemotherapy in Patients with Stage IIA Colon Cancer (COBRA)



SU2C ACT3 Trial

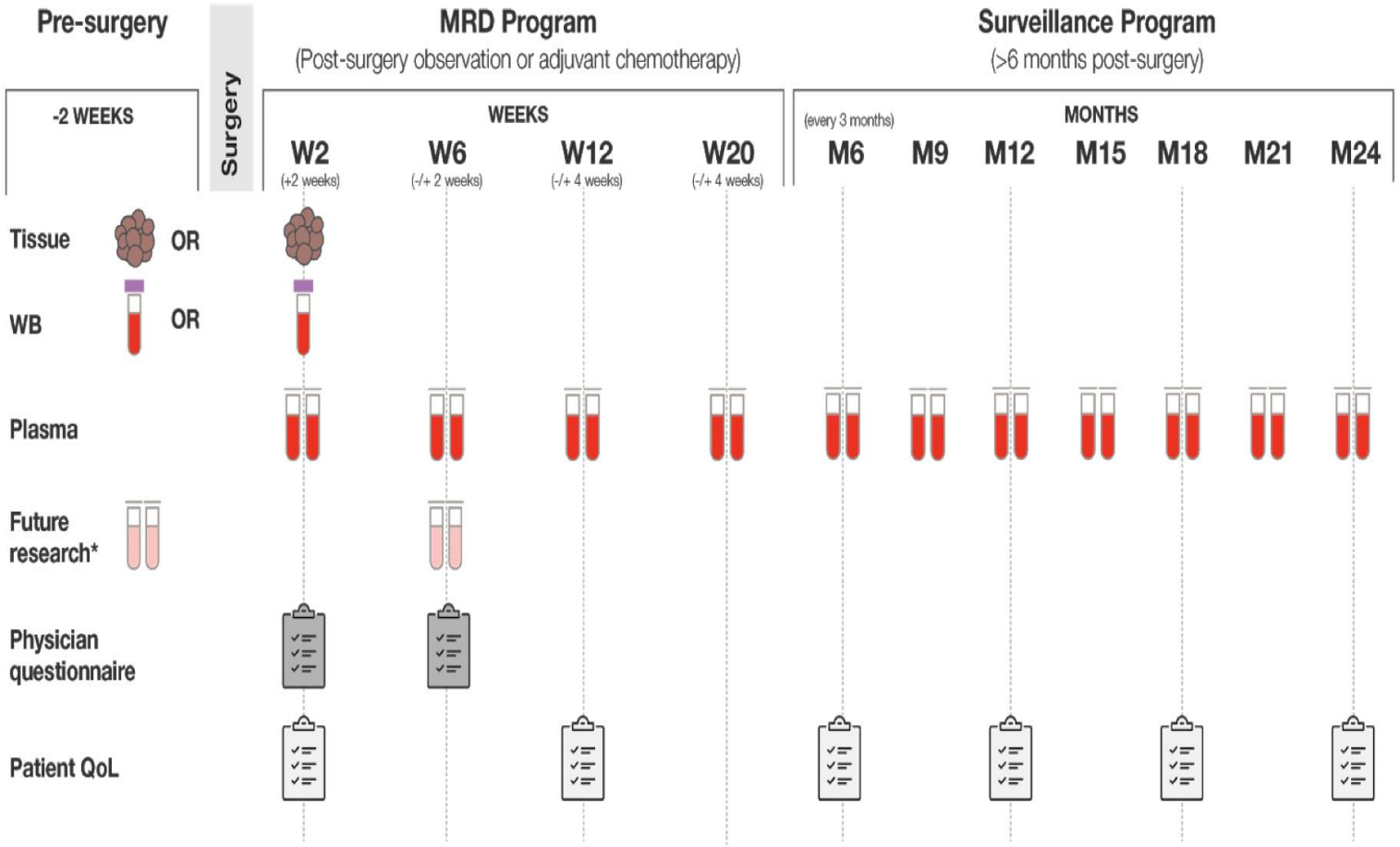


STAND UP TO CANCER

PI: Aparna Parikh

BESPOKE study schema

NCT04264702



Kasi PM, et al. BESPOKE study protocol: a multicentre, prospective observational study to evaluate the impact of circulating tumour DNA guided therapy on patients with colorectal cancer. BMJ Open. 2021 Sep 24;11(9):e047831. doi: 10.1136/bmjopen-2020-047831. PMID: 34561256; PMCID: PMC8475162.

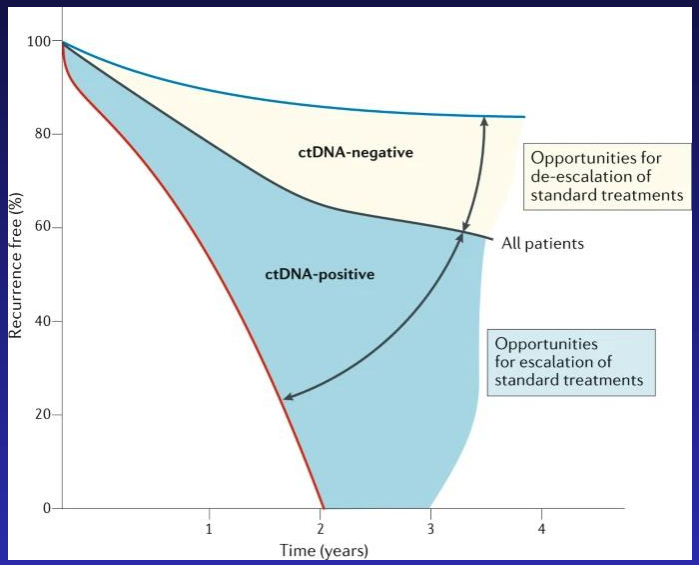
CIRCULATE-US Study Schema

Stage III (T1-3, N1/N1c) Resected Colon Cancer or ctDNA +ve
Stage II or Stage IIIC Resected Colon Cancer

- R0 resection
- pMMR / MSS

Step 1-Registration*
Central ctDNA Testing for all patients

Cohort A ctDNA-ve	Cohort B* ctDNA+ve
Stratification • Stage (IIIA vs IIIB) • Intended chemo (5-FU vs Capecitabine)	Stratification • Intended chemo (5-FU vs Capecitabine) • Post-op ctDNA status (+ve vs. -ve)
Step 2-Randomization	Step 2-Randomization
Arm 1 mFOLFOX6 for 3-6 months or CAPOX for 3 months	Arm 3 mFOLFOX6 or CAPOX for 6 months
Arm 2 Monitored with serial ctDNA testing every 3 months**	Arm 4 mFOLFIRINOX for 6 months



Nat Rev Clin Oncol. 2020 Dec;17(12):757-770

*Patients with completely resected stages II or IIIC colon cancer who are ctDNA +ve as determined by a Signatera ctDNA test performed outside of the trial through routine clinical care and who otherwise meet all eligibility criteria for Step 1-Registration are eligible for enrollment into Cohort B.

**Patients in Cohort A (Arm 2) who develop a ctDNA +ve assay during serial monitoring may transition to the ctDNA+ve cohort (Cohort B) and undergo a second randomization.

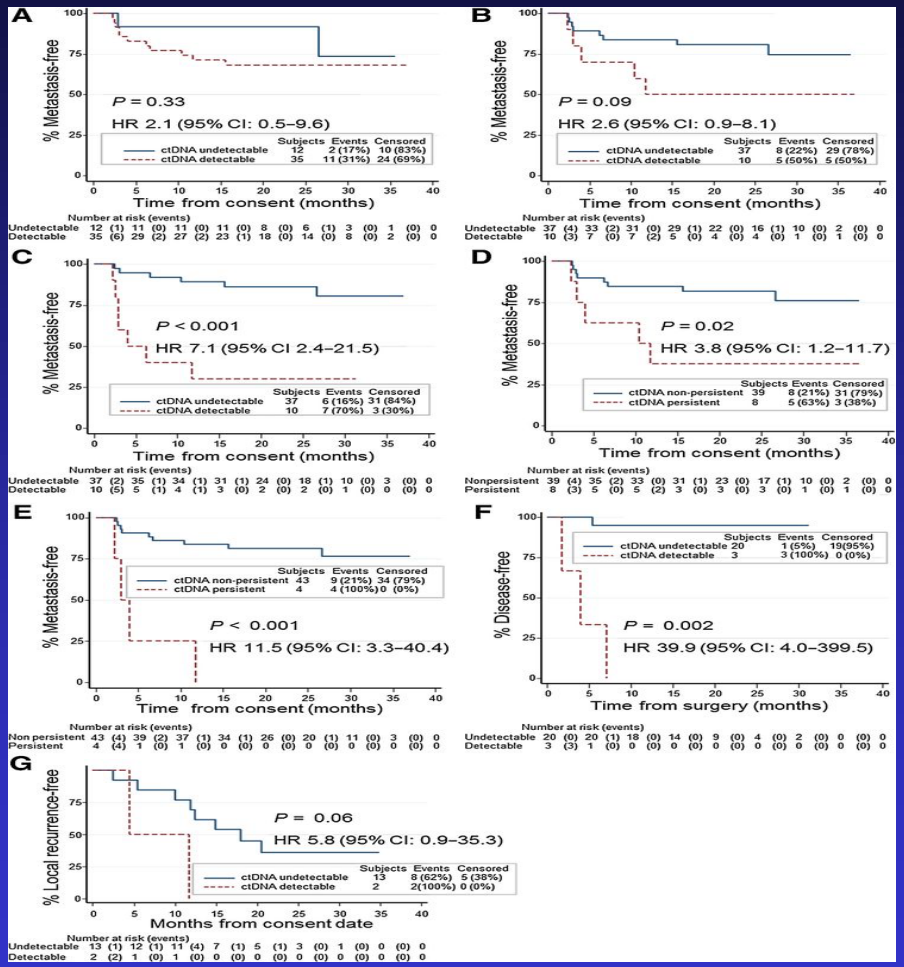
Neoadjuvant setting in locally advanced rectal cancer

- Current consensus on the management of LARC below peritoneal reflection consists of a multimodality treatment of NCRT.
- Randomized trials have shown that pre-operative CT intensification as part of TNT strategy doubles pCR rate vs. conventional NCRT (25 vs 12%).
- Doubling in pCR rate suggests that through TNT, surgery might be avoided in a higher proportion of cases, paving way towards a safer surgery-free “watch-and-wait” approach.
- This expanding complexity in the management of LARC, poses pressing clinical questions including patient’s selection for different pre-operative treatments and early disease reassessment.
- In the study by ***Khakoo and colleagues***, ctDNA detection after pre-operative CRT was associated with primary tumor regression by magnetic resonance tumor regression grade (mrTRG).

Clin Cancer Res. 2020;26(1):183-192. doi:10.1158/1078-0432.CCR-19-1996

ctDNA identified patients at risk of developing metastases during the neoadjuvant period and post-surgery

Kaplan–Meier estimates of MFS by ctDNA status

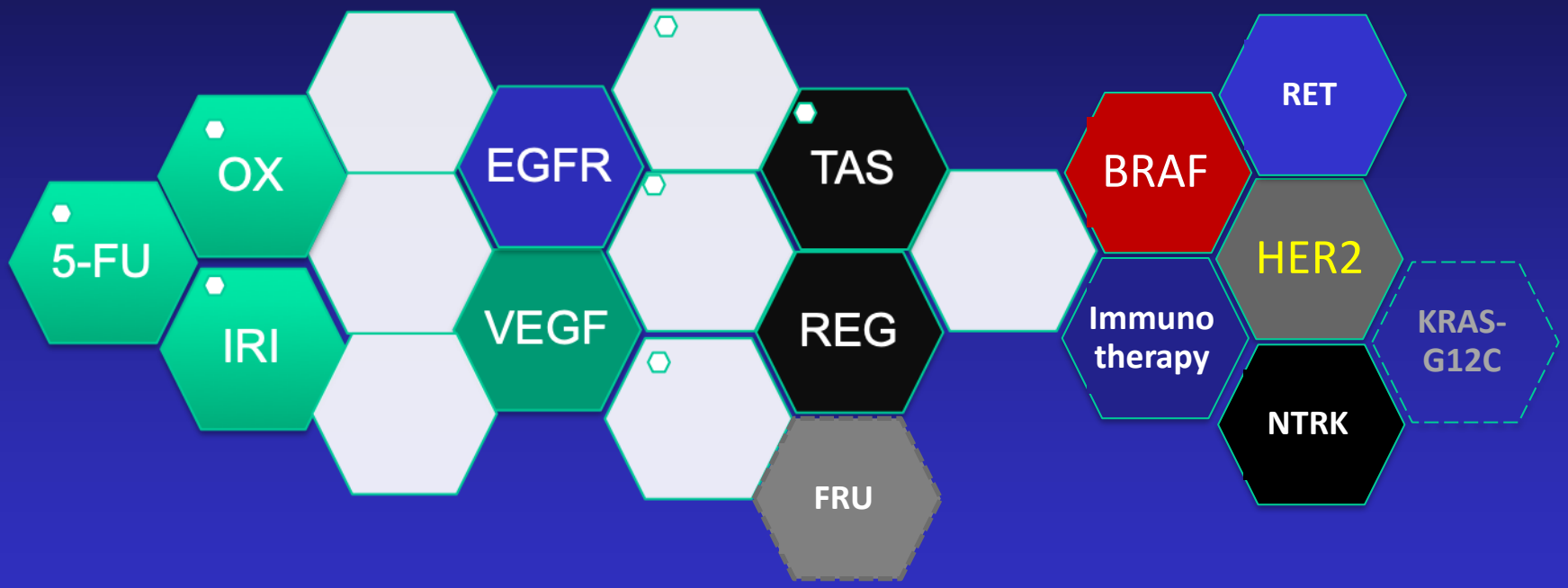


Kaplan–Meier estimates of MFS by ctDNA status: pretreatment (**A**), mid CRT (**B**), and on completion of CRT (**C**). **D,** Persistence of ctDNA pretreatment and mid CRT compared with not persistent. **E,** Persistence of ctDNA pretreatment, mid CRT, and on completion of CRT compared with not persistent throughout. **F,** DFS from surgery by ctDNA status post-surgery. **G,** LRFS in patients deferring surgery by ctDNA status on completion of CRT

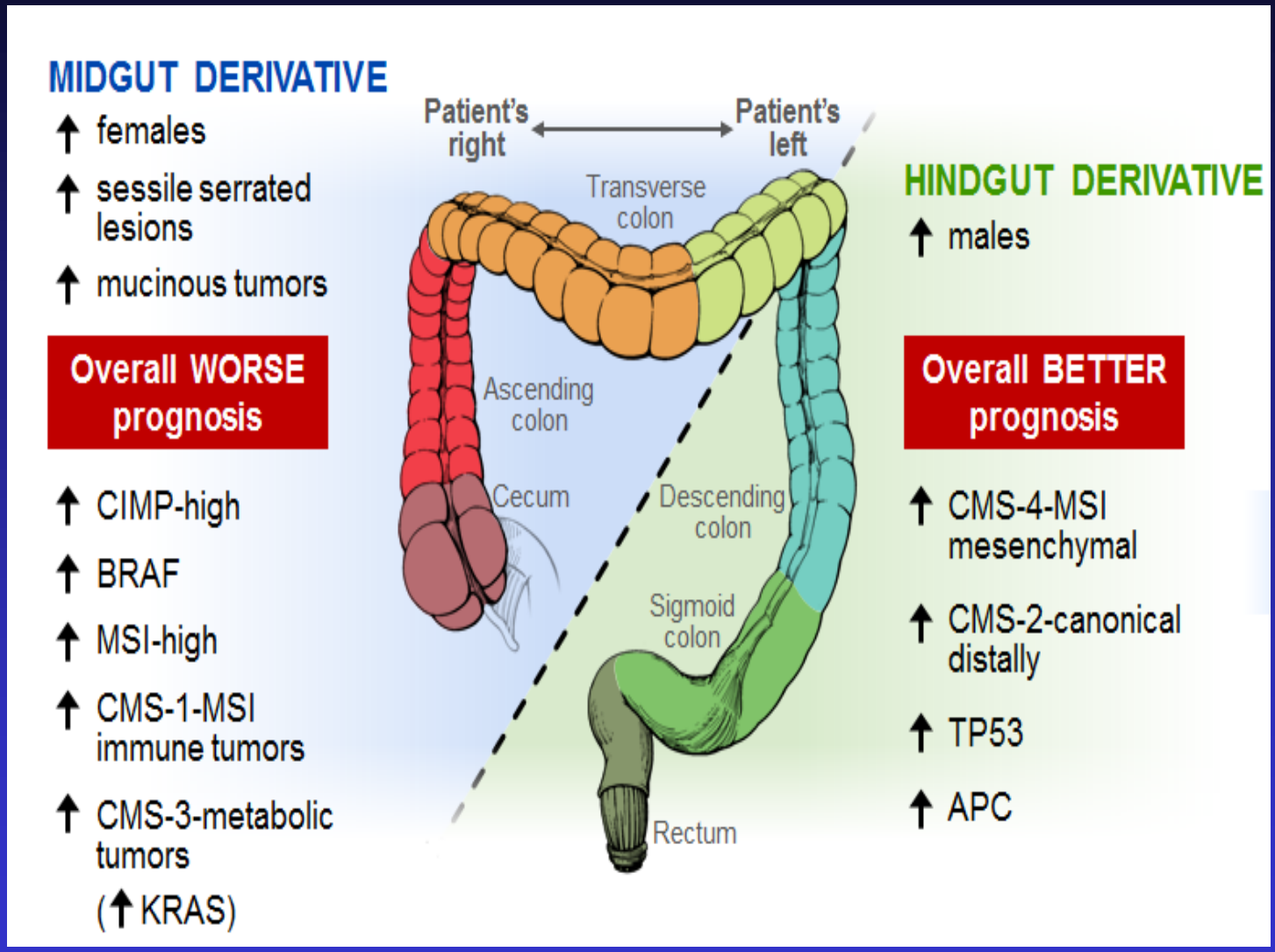
Non-resectable advanced disease

- A liquid biopsy has the added advantage that ctDNA captures alterations occurring in multiple genes, specifically EGFR, ERBB2, PIK3CA or MAP2K1, unshadowing new potential targets for treatment as well as putative mechanisms of resistance to SoC targeted therapies such as anti-EGFR, anti-BRAF and anti-HER2 agents.
- In a cohort of 232 CRC patients both solid tumor tissue and ctDNA were genotyped and an overall high concordance (84.9–100.0%) increased to near 100% (97.0–100.0%) when considering only clonal alterations.
- GI-SCREEN network demonstrated that ctDNA genotyping significantly shortens biomarker evaluation turnaround time (3 days versus 11 in standard pathological assessment) and increases screening efficiency for targeted agents trial enrolment (9.5% enrollment versus 4.1%).

Treatment options for patients with mCRC



RIGHT vs. LEFT



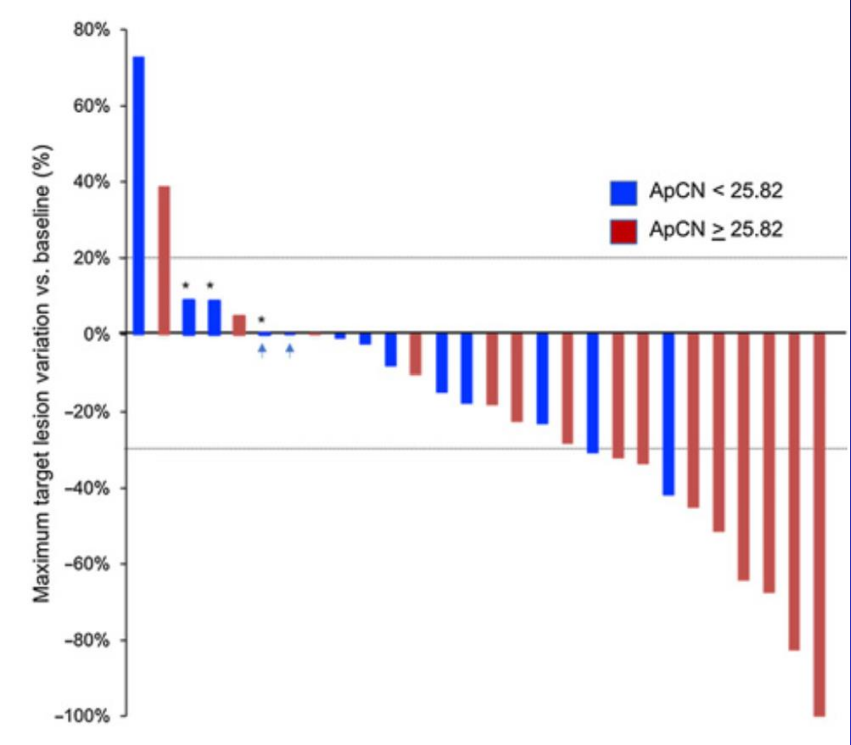
1st line Anti-EGFR therapy selection

- **Selection** of the patient for anti-EGFR – tissue
  - LEFT
  - RAS-wildtype
  - BRAF-wildtype
  - HER2-negative
- Role for **liquid biopsies (YES)**

	Anti-EGFR OS (months)	Anti-VEGF OS (months)
NCDB	42.9	27.5
CALGB 80405	39.3	32.6
PEAK	43.4	32.0
FIRE-3	38.3	28.0
PARADIGM	37.9	34.7
PARADIGM (ctDNA hyper-selected)	42.1	35.5

Shitara K et al.
Negative hyperselection of patients with RAS wild-type metastatic colorectal cancer for panitumumab: A biomarker study of the phase III PARADIGM trial.
DOI: 10.1200/JCO.2023.41.4_suppl.11 Journal of Clinical Oncology 41, no. 4_suppl (February 01, 2023)

HER2/ERBB2 - Plasma



Results:

- 47 of 48 samples had detectable ctDNA
- **46 of 47 samples were ERBB2-amplified** on the basis of cfDNA [2.55–122 copies];
- **97.9% sensitivity** (95 CI, 87.2%–99.8%)].
- An adjusted ERBB2 pCN of 25.82 copies correlated with ORR and PFS (P = 0.0347)

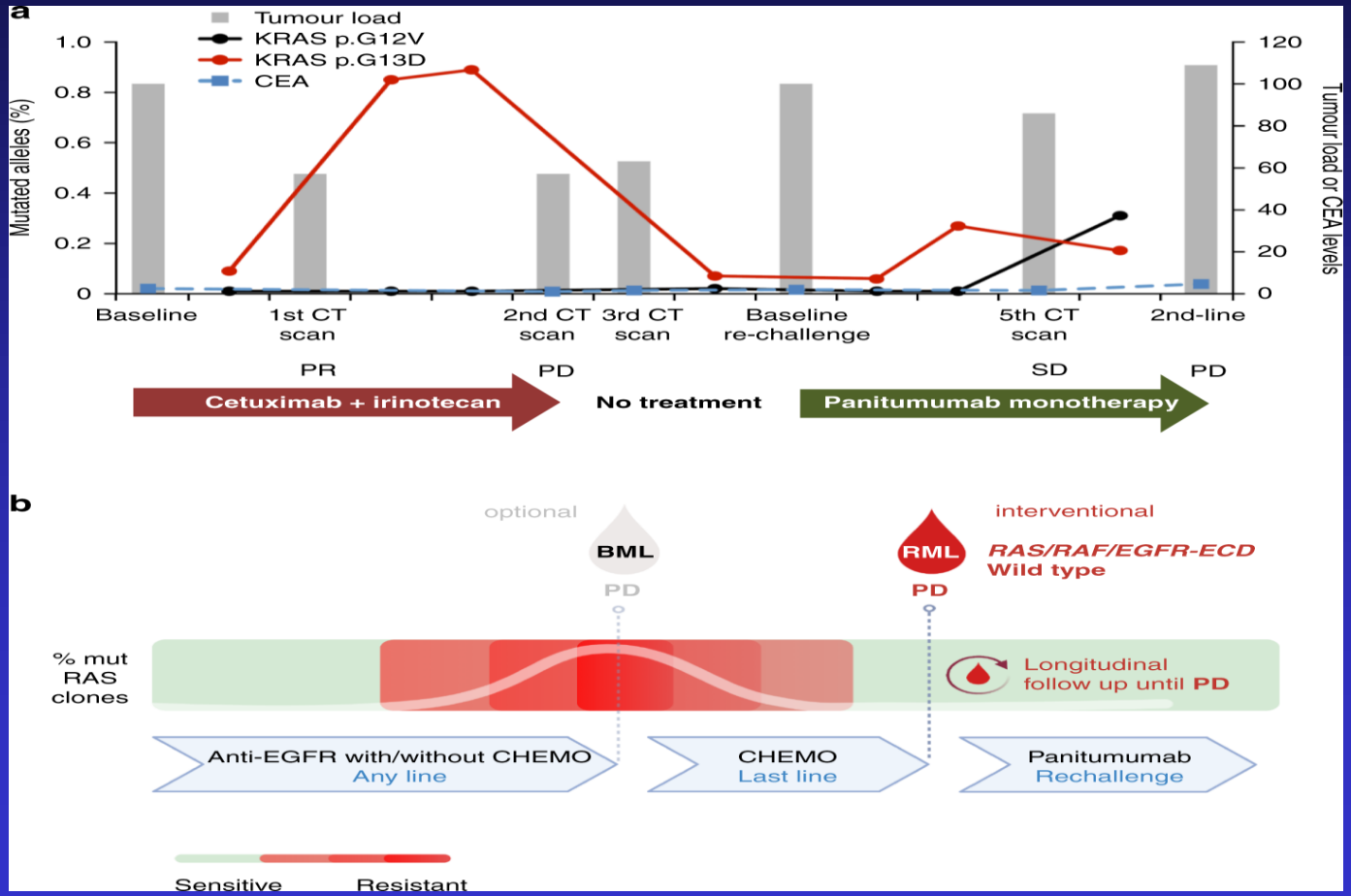
Plasma HER2 (*ERBB2*) Copy Number Predicts Response to HER2-targeted Therapy in Metastatic Colorectal Cancer. Clin Cancer Res. 2019 May 15;25(10):3046-3053.

HER2-targeted therapies in patients with HER2+ mCRC

Regimen	Trial (n) – year	ORR	PFS	OS	Most common Grade 3+ AEs
Trastuzumab + lapatinib	HERACLES-A (n=32) – 2016	**28%**	**4.7m**	**10m**	Fatigue 16% Decreased LVEF 6%
Trastuzumab + pertuzumab	MyPathway (n=84; 57 evaluable) – 2019	**32%**	**2.9m**	**11.5m**	Hypokalemia 5% Abdominal pain 5%
Pertuzumab and T-DM1	HERACLES-B (n=31) – 2020	**9.7%**	**4.1m**	**Not reported**	Thrombocytopenia 7%
Trastuzumab deruxtecan	DESTINY-CRC01 (N=78; 53 HER2+) – 2021	**45.3%**	**6.9m**	**15.5m**	Neutropenia 15% Anemia 13%
Tucatinib + trastuzumab	MOUNTAINEER (n=117) *FDA Approved	**38.1%**	**8.2m**	**24.1m**	Hypertension 7% Diarrhea 3.5%

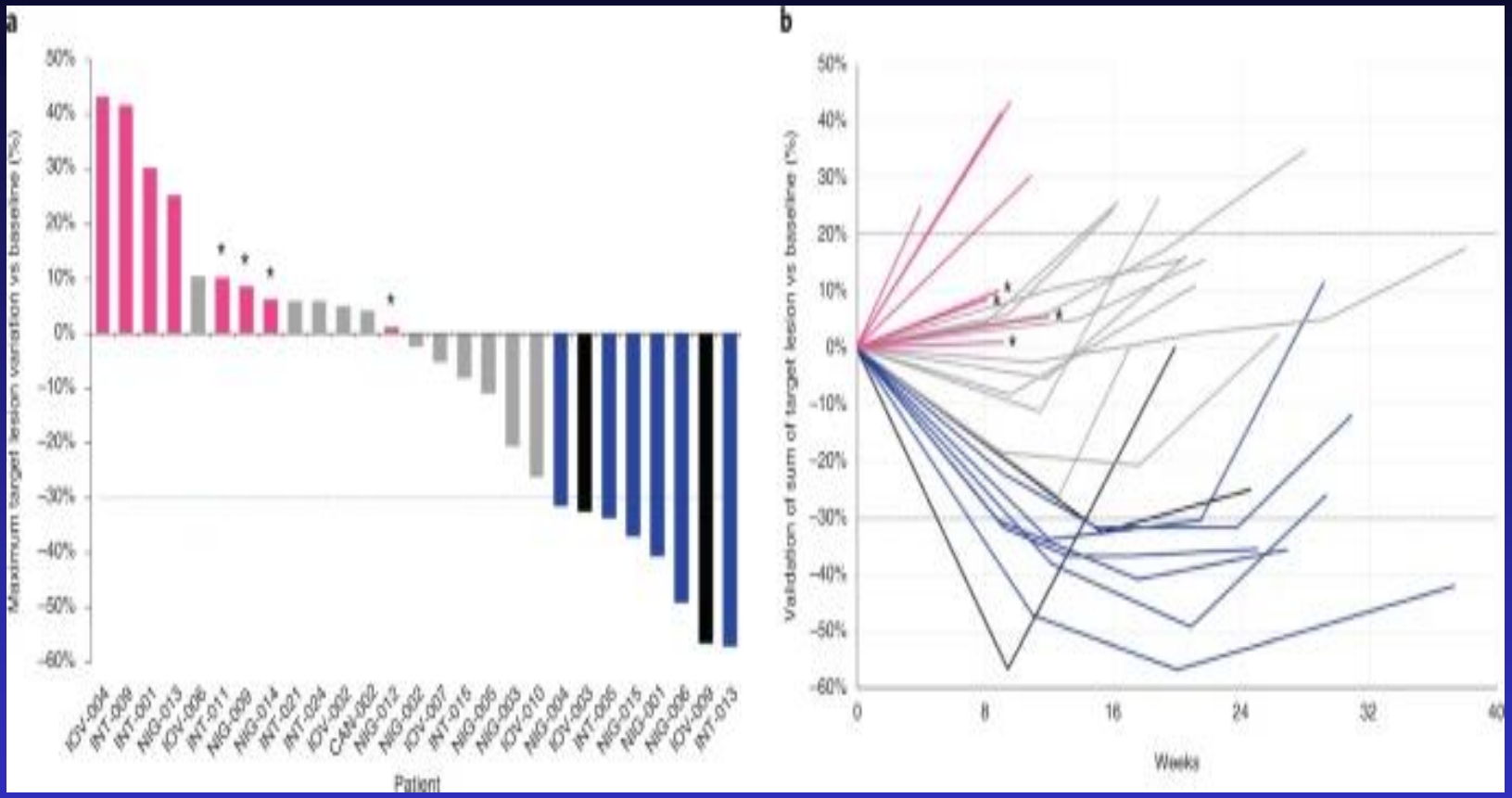
Disease monitoring and the Darwinian evolution model of CRC clones

Circulating tumor DNA to guide rechallenge with panitumumab in mCRC: CHRONOS trial



Sartore-Bianchi, A., Pietrantonio, F., Lonardi, S. *et al.* Circulating tumor DNA to guide rechallenge with panitumumab in metastatic colorectal cancer: the phase 2 CHRONOS trial. *Nat Med* **28**, 1612–1618 (2022)

CHRONOS trial



Waterfall plot depicts best responses to panitumumab rechallenge within the CHRONOS trial according to RECIST 1.1 (**a**). Spider plot displays best responses according to RECIST 1.1 and DOR to panitumumab rechallenge (**b**). Magenta, progressive disease; gray, stable disease; blue, partial response; black, unconfirmed partial response; * progressive disease exclusively due to the onset of a new metastatic lesion.

Liquid biopsies for immunotherapy and beyond

- TMB is approved by FDA as an agnostic biomarker to access cancer immunotherapy.
- The gold standard for TMB evaluation is tumor tissue specimens even if intra-tumor heterogeneity constitutes a relevant limit to its exact estimation, thus supporting the role of a ctDNA-based evaluation, as it already achieved in non-small cell lung cancer (NSCLC).
- However, similarly to TMB, MSI status is subjected to both spatial and temporal heterogeneity, making its monitoring through LB therapeutically valuable.

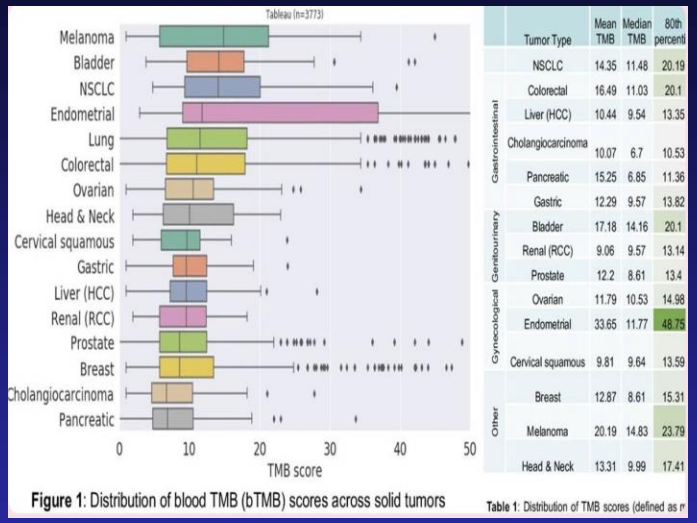
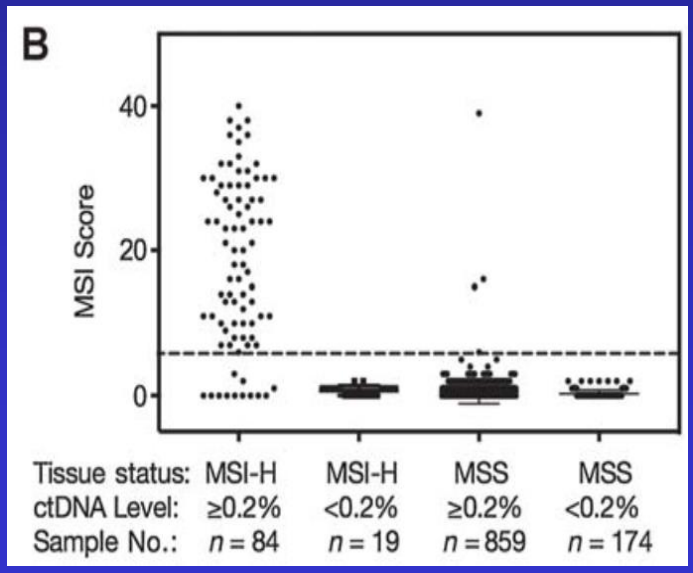


Figure 1: Distribution of blood TMB (bTMB) scores across solid tumors

	Tumor Type	Mean TMB	Median TMB	80th percenti
	NSCLC	14.35	11.48	20.19
Gastrointestinal	Colorectal	16.49	11.03	20.1
	Liver (HCC)	10.44	9.54	13.35
	Cholangiocarcinoma	10.07	6.7	10.53
	Pancreatic	15.25	6.85	11.36
	Gastric	12.29	9.57	13.82
Genitourinary	Bladder	17.18	14.16	20.1
	Renal (RCC)	9.06	9.57	13.14
	Prostate	12.2	8.61	13.4
Gynecological	Ovarian	11.79	10.53	14.98
	Endometrial	33.65	11.77	48.75
	Cervical squamous	9.81	9.64	13.59
Other	Breast	12.87	8.61	15.31
	Melanoma	20.19	14.83	23.79
	Head & Neck	13.31	9.99	17.41

Table 1: Distribution of TMB scores (defined as n



Clin Cancer Res. 2019 Dec 1;25(23):7035-7045.

Conclusions

1. Liquid biopsy is increasingly gaining traction in the clinical management of CRC patients in several clinical settings.
2. Retrospective data indicate that ctDNA can identify CRC patients requiring adjuvant treatments or conversely, not needing surgery after neoadjuvant treatment for LARC.
3. Once confirmed prospectively, the use of LB to detect MRD post-surgery with curative intent will likely be widely used in the management of early-stage CRC.
4. Recently, the CHRONOS clinical trial demonstrated that ctDNA-based anti-EGFR rechallenge treatments can improve the therapeutic index of this therapeutic regimen.

Future

- At the present time, the ability to detect mutations using ctDNA is superior to that using CTCs; however, the value of CTCs might improve if massive amounts of CTCs can be captured.
- Tumor educated platelets are also candidates for liquid biopsy.
- MRD could replace TMN classification as a method of judging the need for adjuvant therapy. Lymph node metastasis only indicates the possibility of MRD; in other words, it provides indirect proof of MRD. In contrast, the presence of ctDNA or CTC after surgery is a direct proof of MRD.
- Molecular volume could replace the Response Evaluation Criteria in Solid Tumors as a way to monitor the effect of chemotherapy. Measure of molecular volume does not