# Circulating tumor DNA

# as a tool to predict response to immune therapy

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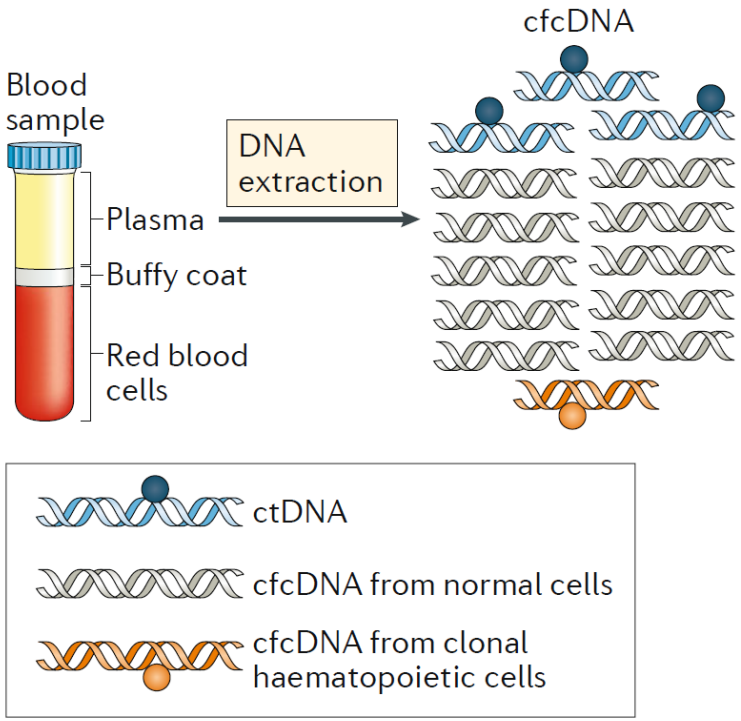
Professor of Medical Oncology

Institut Curie & Paris-Saclay University

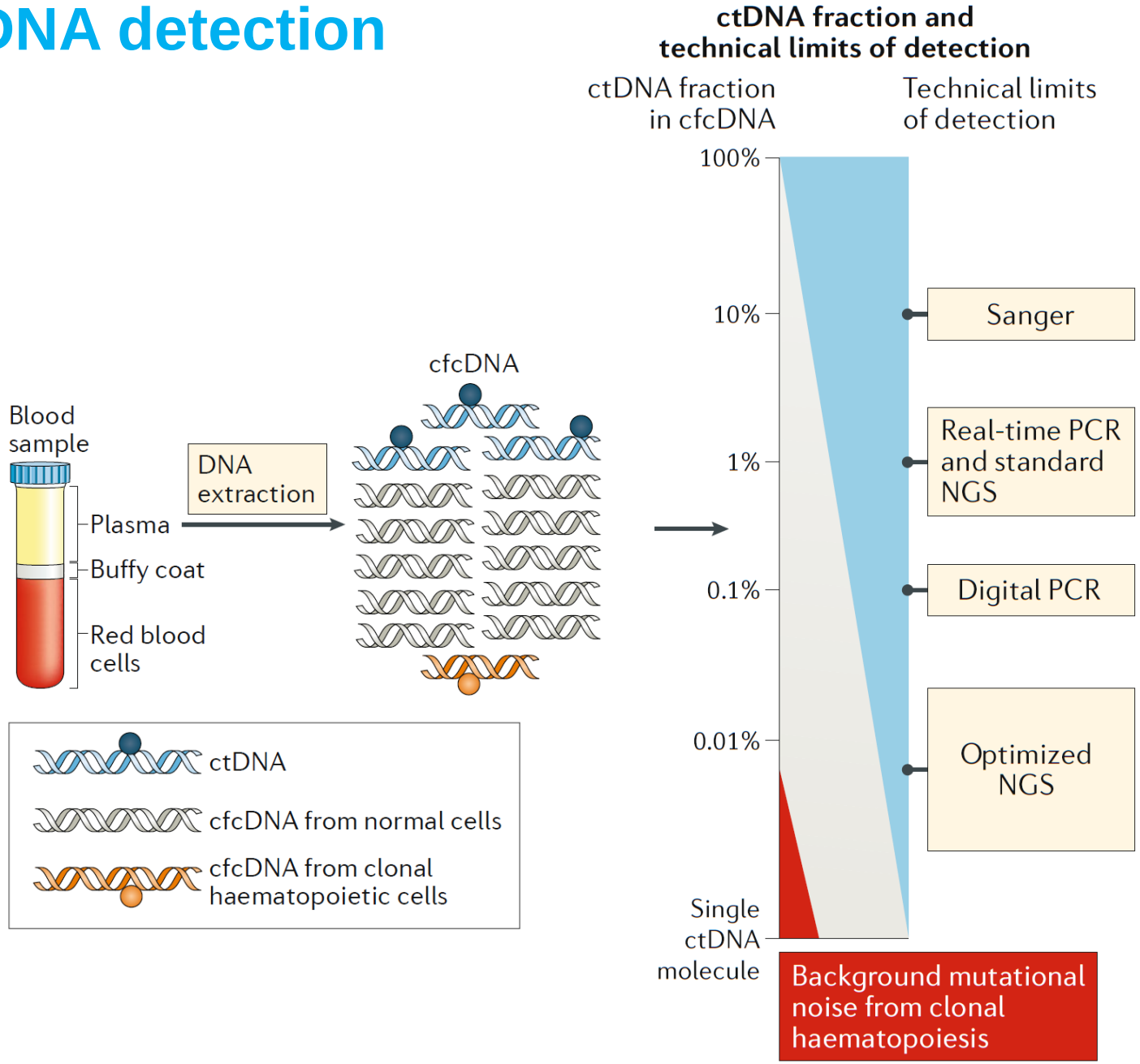
**COI:** ongoing patents related to ctDNA detection (MSI and others)

# ctDNA detection




Cabel *et al*, Nat Rev Clin Oncol 2018

# ctDNA detection




Cabel *et al*, Nat Rev Clin Oncol 2018

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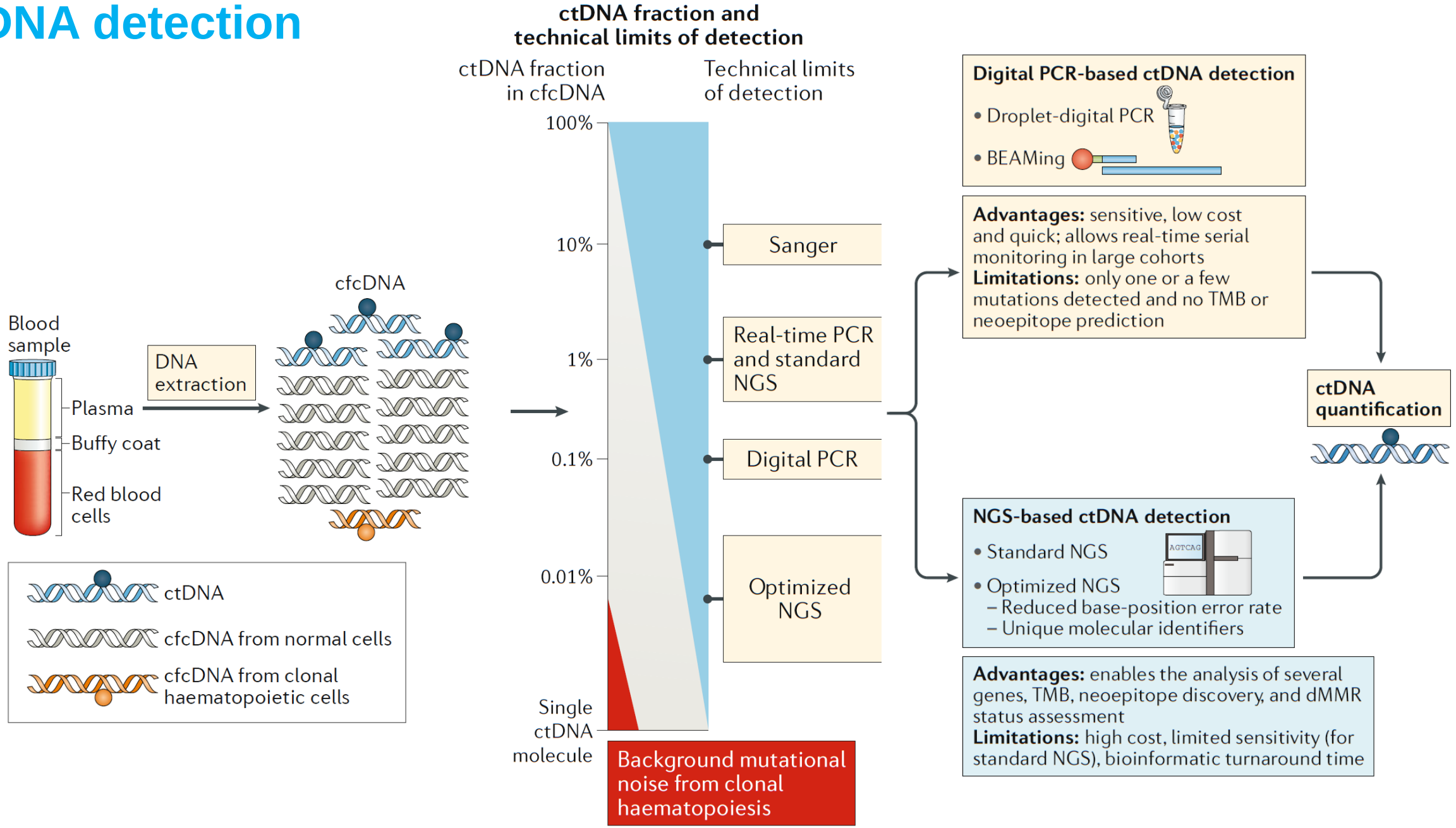


Cabel *et al*, Nat Rev Clin Oncol 2018

# Outline

ctDNA to capture genomic predictive markers in Immuno-Oncology (IO)

ctDNA to monitor immune therapy

Hypothesis: new windows of opportunity for better IO efficacy

Hypothesis: immune system monitoring through cell-free DNA sequencing

# ctDNA to capture genomic predictive markers in IO

## Genome-wide markers

- Tumor mutation burden
- Microsatellite instability
- other mutational signatures…

## Specific alterations

- IFNγ & HLA pathways
- PDL1 amplification

# Tumor Mutation Burden… in tissue

| Clinical trial (reference) | Assay | NSCLC patients (total number of patients) | Number of genes (for panels) | Covered Mb | Gene variants | Sample type | TMB cut-off‡ |
|---|---|---|---|---|---|---|---|
| KEYNOTE-001 (2) | WES | 34 | – | – | Nonsynonymous mutations | Tumor | 200 mut/tumor |
| CheckMate 026 (3) | WES | 312 | – | – | Total missense mutations | Tumor | Low <100; medium 100 to 242; high TMB ≥243 |
| CheckMate 012 (22) | WES | 75 | – | – | Nonsynonymous single nucleotide and indel variants | Tumor | TMB high >158 mut |
| (20) | WES, MSK–IMPACT | 49 with WES, 240 with MSK-IMPACT | 341, 410, 468† | 0.98, 1.06, 1.22† | Somatic nonsynonymous mutations | Tumor | Above versus below the 50th percentile of TMB |
| FIR/BIRCH/ POPLAR (33) | FoundationOne | 102 1L and 465 2L+ | 315 | 1.1 | Number of somatic, coding, base substitutions, indel mutations per Mb of genome examined | Tumor | Median TMB 9.9 mut/Mb or high TMB 16.2 mut/Mb |
| CheckMate 568 (34) | FoundationOne CDx | 98 | 324 | ~0.8 Mb | Number of somatic, coding, base substitutions, short indels per Mb of genome examined | Tumor | 10 mut/Mb |
| CheckMate 227 (21) | FoundationOne CDx | 1,004 | 324 | ~0.8 Mb | Number of somatic, coding, base substitutions, short indels per Mb of genome examined | Tumor | 10 mut/Mb |

Fenizia *et al*, TLCR 2018

# Tumor Mutation Burden… in ctDNA

- Not invasive, can be repeated throughout therapy
- Better estimate of the tumor spatial heterogeneity

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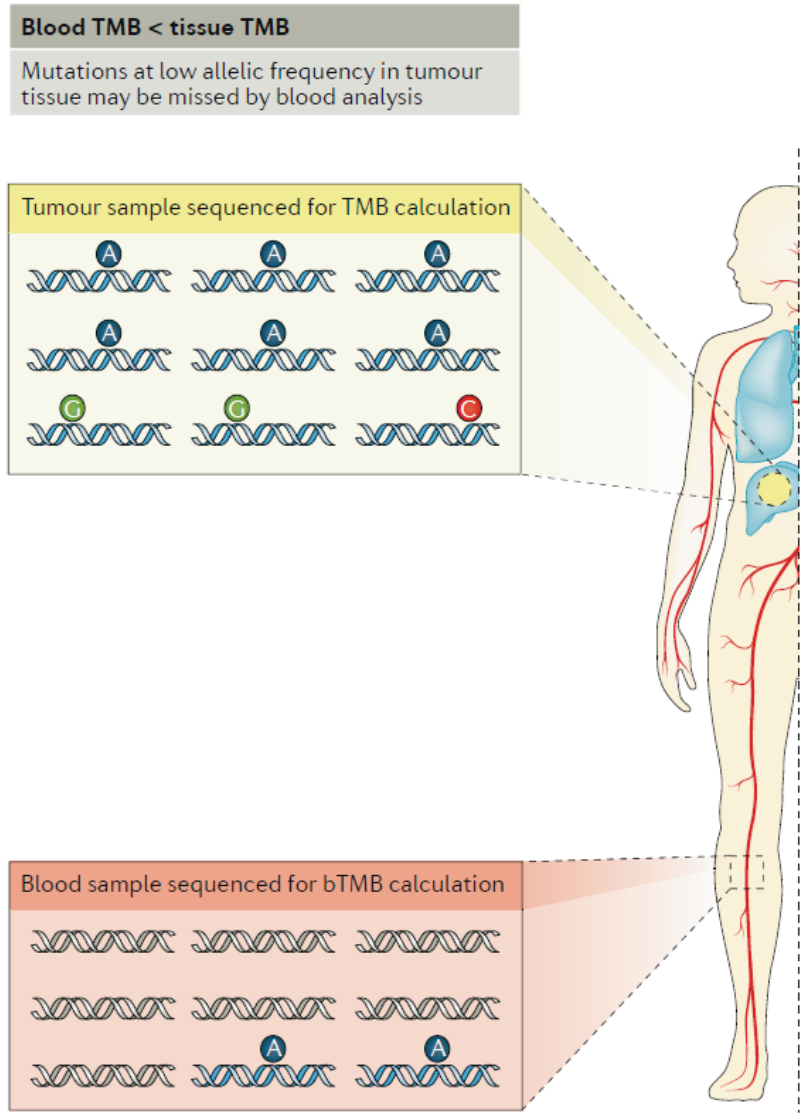
- ctDNA usually not detected in responding pts
- Shorts indels: difficult to detect
- SNV with low allelic frequency can be missed




Cabel *et al*, Nat Rev Clin Oncol 2018

# Tumor Mutation Burden… in ctDNA

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- ctDNA usually not detected in responding pts
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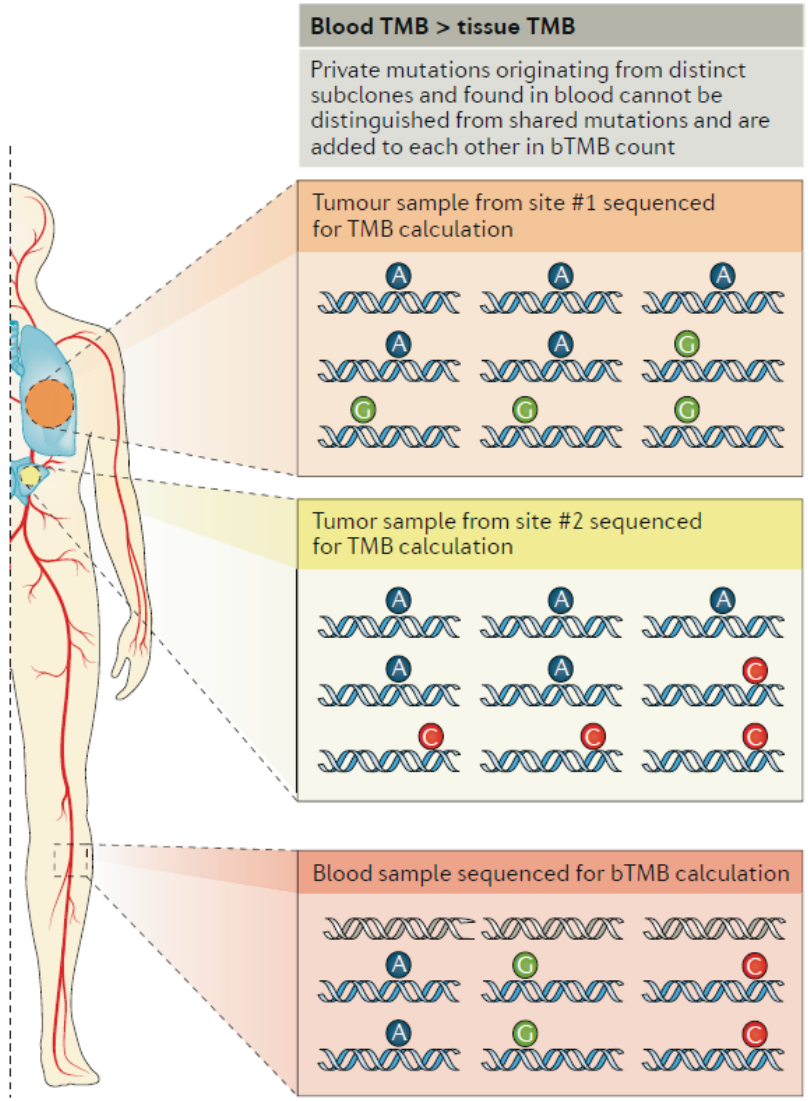
- Spatial heterogeneity *might be* misleading (?)




Cabel *et al*, Nat Rev Clin Oncol 2018

# Tumor Mutation Burden… in ctDNA

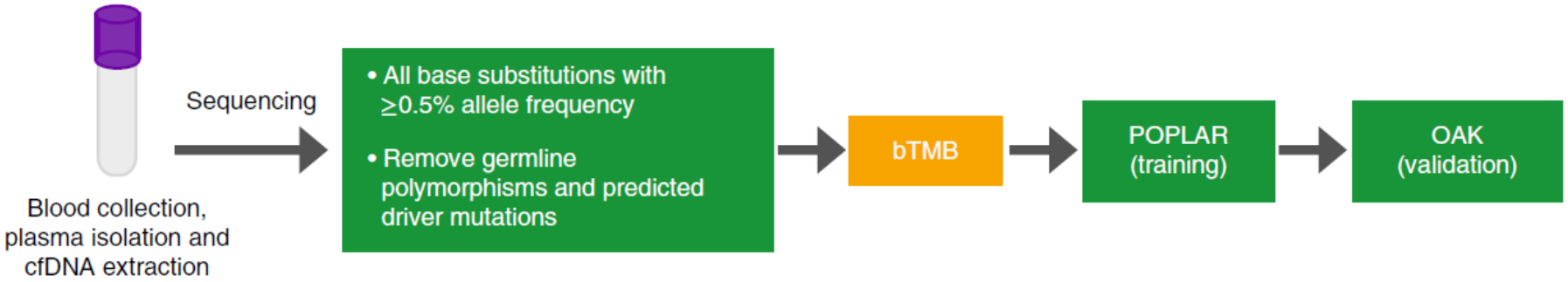
Table 1 NGS assays for TMB testing in tumor tissue and in liquid biopsy

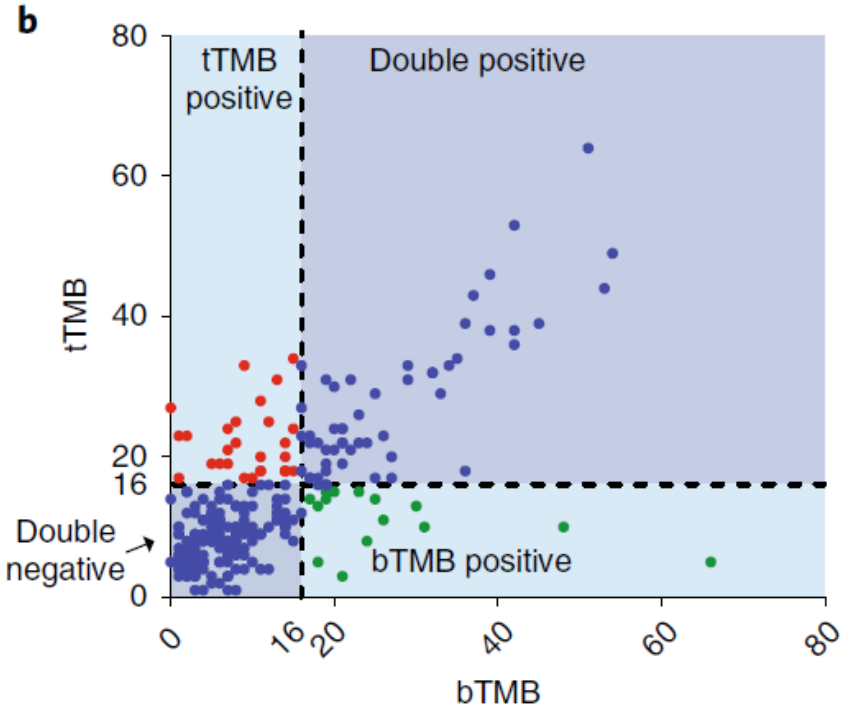
| Clinical trial (reference) | Assay | NSCLC patients (total number of patients) | Number of genes (for panels) | Covered Mb | Gene variants | Sample type | TMB cut-off‡ |
|---|---|---|---|---|---|---|---|
| MOSCATO 01/ MATCHR (35) | cfDNA-WES | 19 [32] | – | – | Single nucleotide variants and short indels | Blood | Not for TMB clinical use |
| (36) | NEO New Oncology NEOliquid | 82 | 39 | NA | Point mutations, small indels, CNA, rearrangements, gene fusions | Blood | Not for TMB clinical use |
| (37) | Guardant Health Guardant 360 | 53 [97] | 54–70† | NA | VUS and synonymous mutations with and without potentially functional/driver variants | Blood | >15 mutations |
| (38) | CAPP-Seq | In silico | 139 | ~125 Kb | Nonsynonymous mutations | Tumor/ blood | ≥5 mutations |
| POPLAR/OAK (39) | Foundation Medicine bTMB | 794 | 394 | NA | Single nucleotide variants | Blood | 16 mutations (14 mut/Mb) |
| POPLAR/OAK (40) | Foundation Medicine bTMB | 794 | 394 | NA | Somatic synonymous and non-synonymous base substitutions with at least 0.5% AF | Blood | 10 and 16 mutations (9 and 14 mut/Mb) |

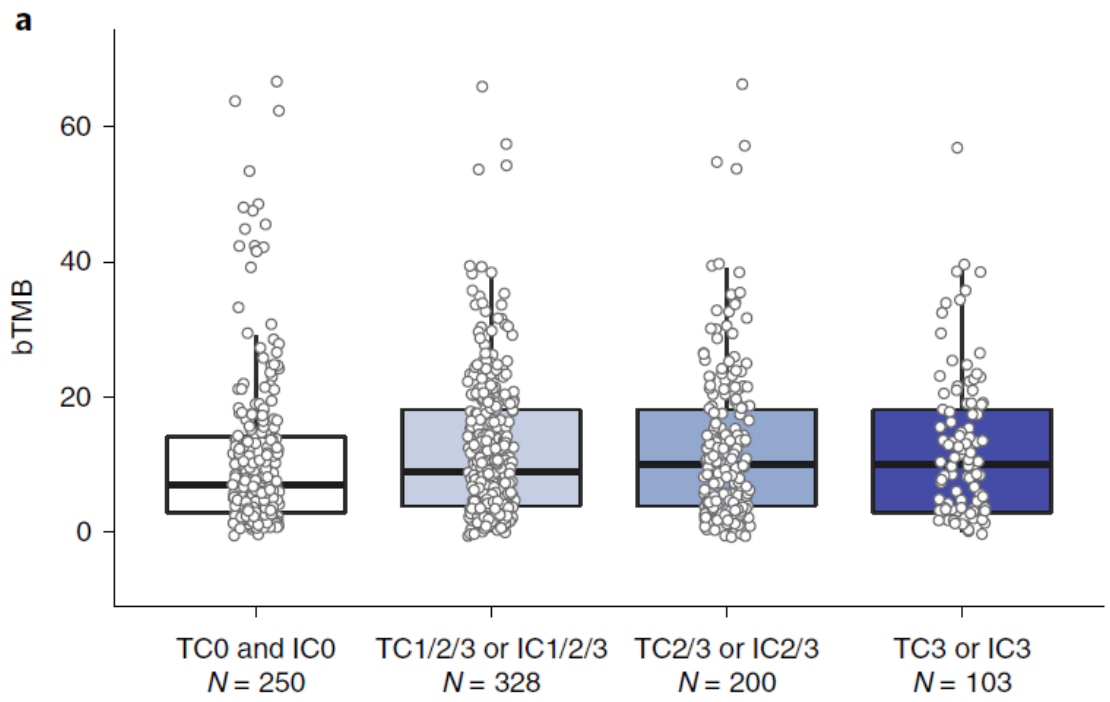
Fenizia *et al*, TLCR 2018

# POPLAR and OAK trials

Stage IV NSCLC, 2nd line



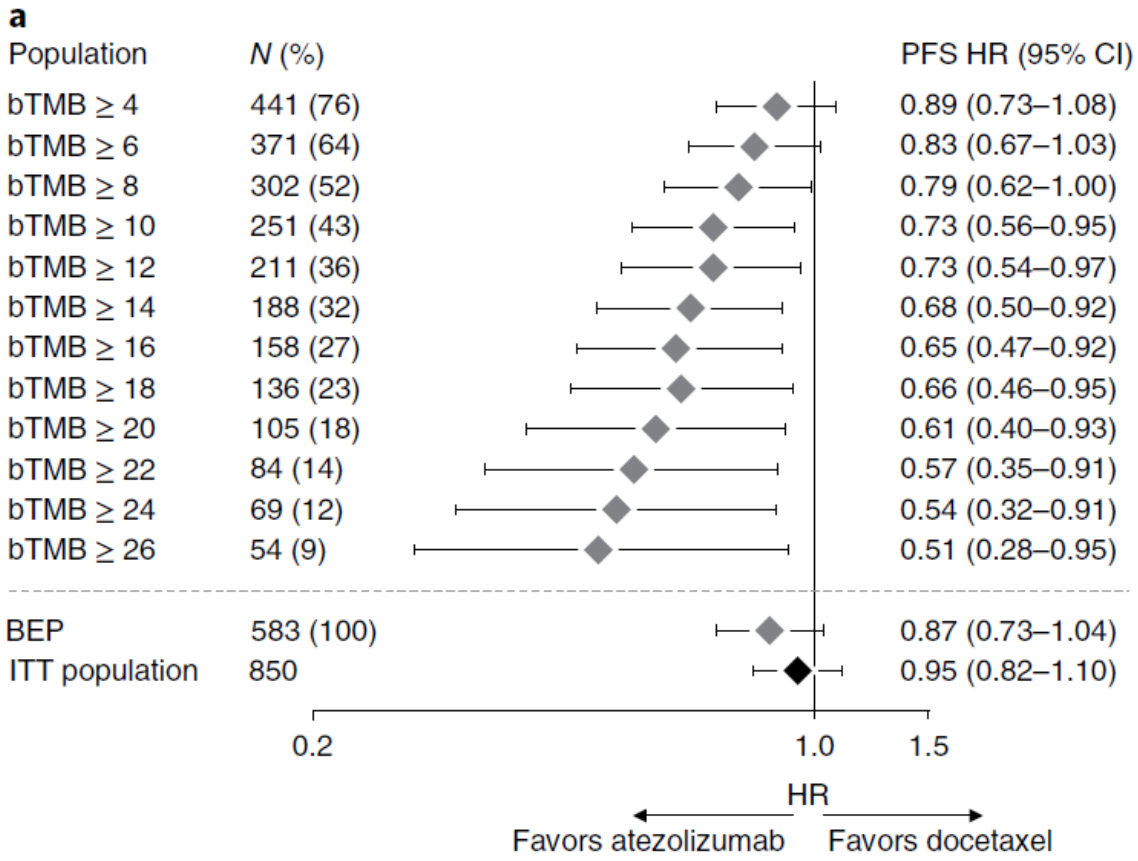



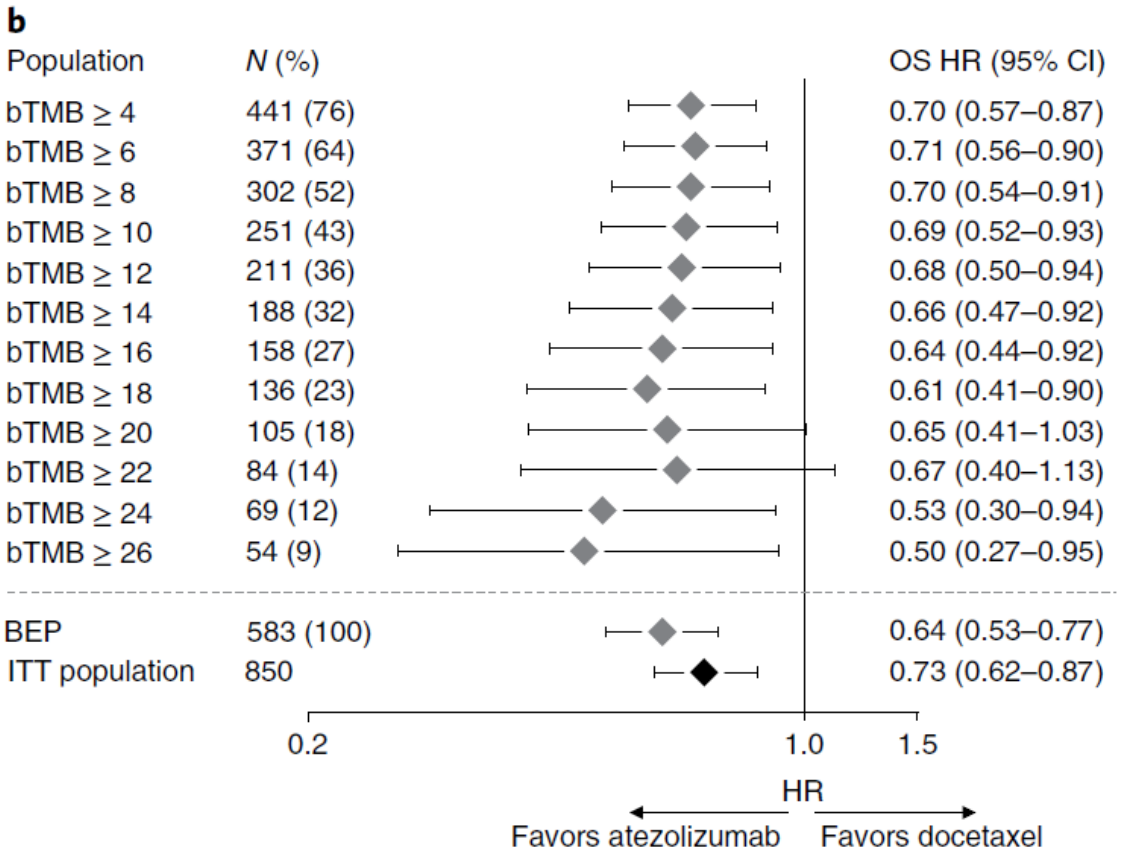

Gandara *et al*, Nat Med 2018

# POPLAR and OAK trials

PFS




OS



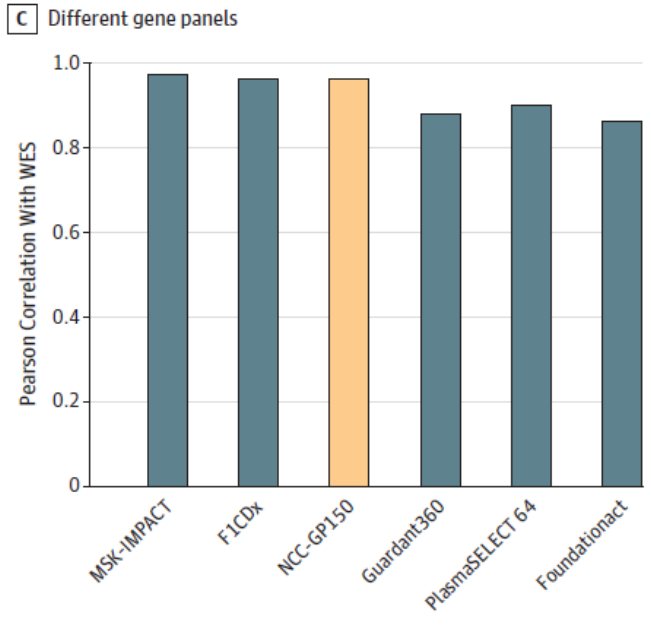

Gandara *et al*, Nat Med 2018

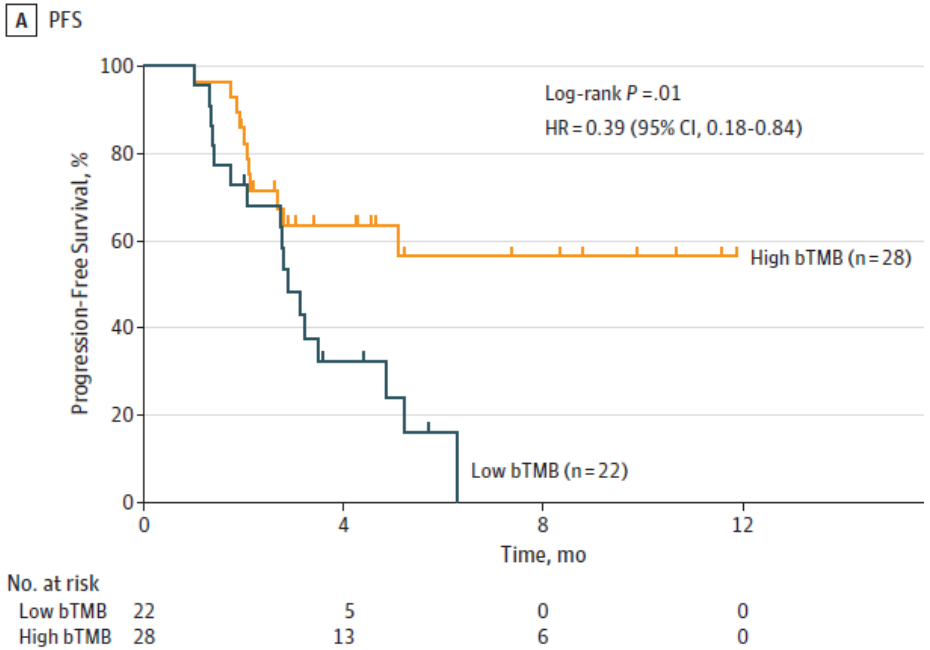
# National Cancer Center *(Beijing)* study

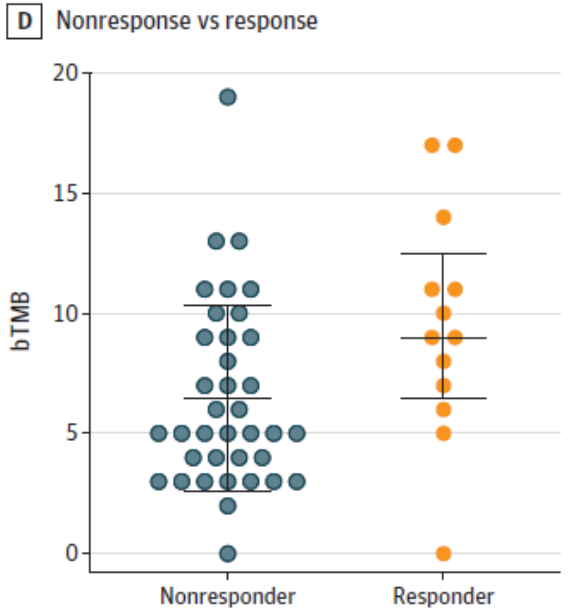
JAMA Oncology | Original Investigation

## Assessment of Blood Tumor Mutational Burden as a Potential Biomarker for Immunotherapy in Patients With Non–Small Cell Lung Cancer With Use of a Next-Generation Sequencing Cancer Gene Panel

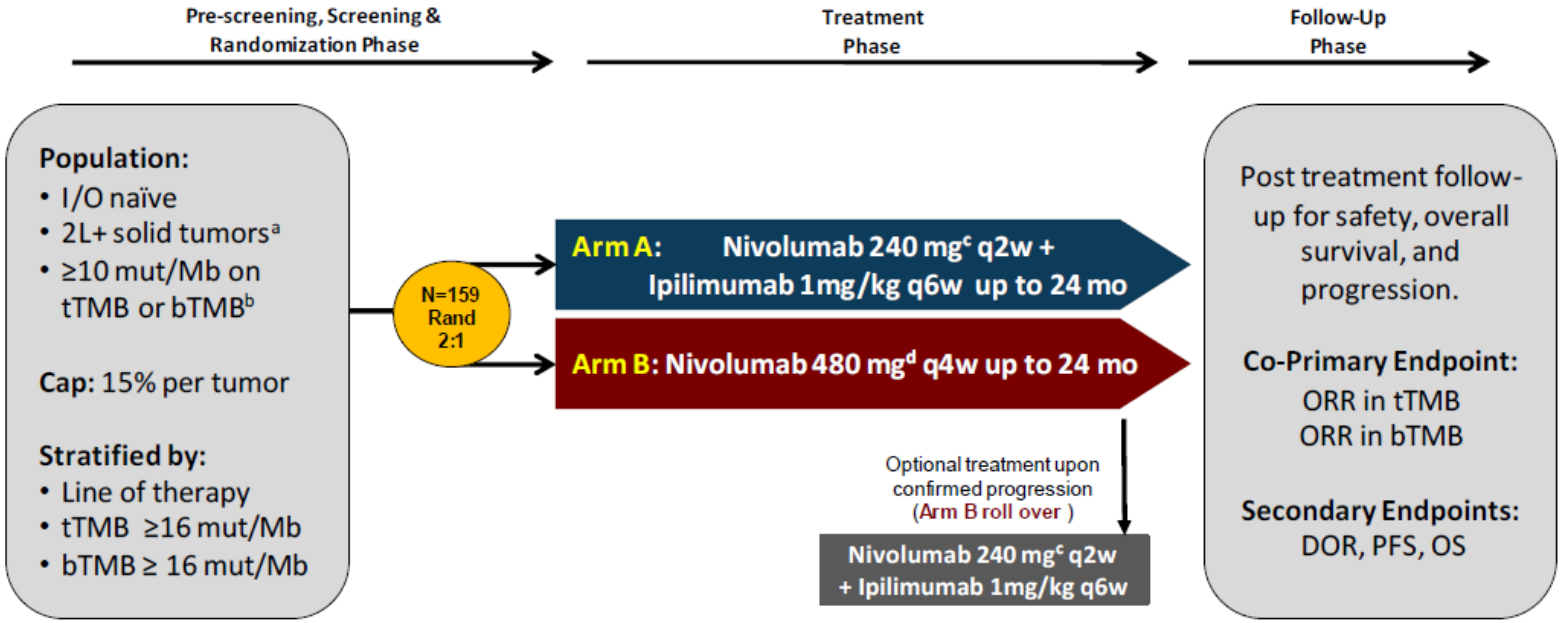
Zhijie Wang, MD; Jianchun Duan, MD; Shangli Cai, PhD; Miao Han, PhD; Hua Dong, PhD; Jun Zhao, MD; Bo Zhu, MD; Shuhang Wang, MD; Minglei Zhuo, MD, PhD; Jianguo Sun, MD; Qiming Wang, MD; Hua Bai, MD; Jiefei Han, MD; Yanhua Tian, MS; Jing Lu, PhD; Tongfu Xu, PhD; Xiaochen Zhao, MD; Guoqiang Wang, PhD; Xinkai Cao, PhD; Fugen Li, PhD; Dalei Wang, PhD; Yuejun Chen, PhD; Yuezong Bai, PhD; Jing Zhao, PhD; Zhengyi Zhao, PhD; Yuzi Zhang, MD; Lei Xiong, PhD; Jie He, MD, PhD; Shugeng Gao, MD; Jie Wang, MD, PhD



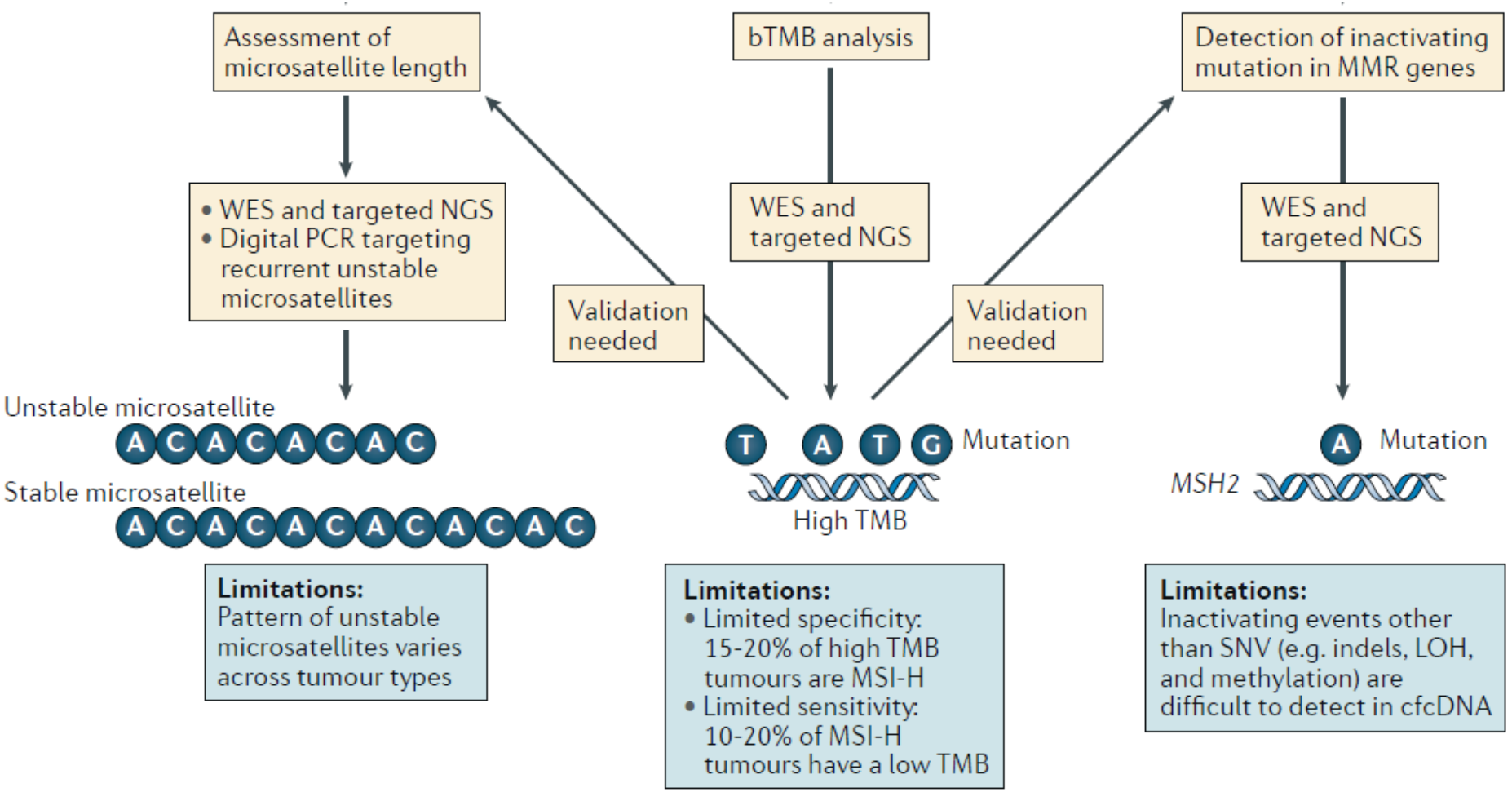



# Ongoing study: CheckMate 848 (NCT03668119)




[a]: excluding melanoma, NSCLC, RCC
[b]: both bTMB and tTMB results are required as part of screening procedures
[c]: 3 mg/kg for adolescents with body weight <40 kg
[d]: 6 mg/kg for adolescents with body weight <40 kg

# Microsatellite Instability



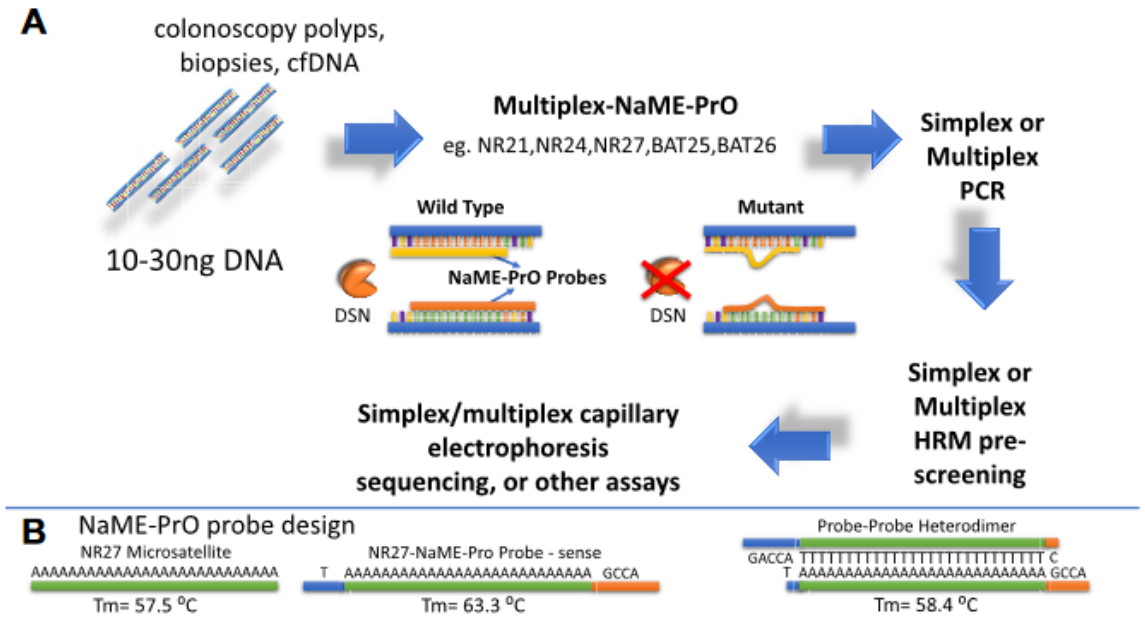

Cabel *et al*, Nat Rev Clin Oncol 2018

# Microsatellite Instability

Repeated sequences ➔ higher error rate with NGS (no published report so far)

Standard pentaplex assay: requires at least 10% allelic frequency

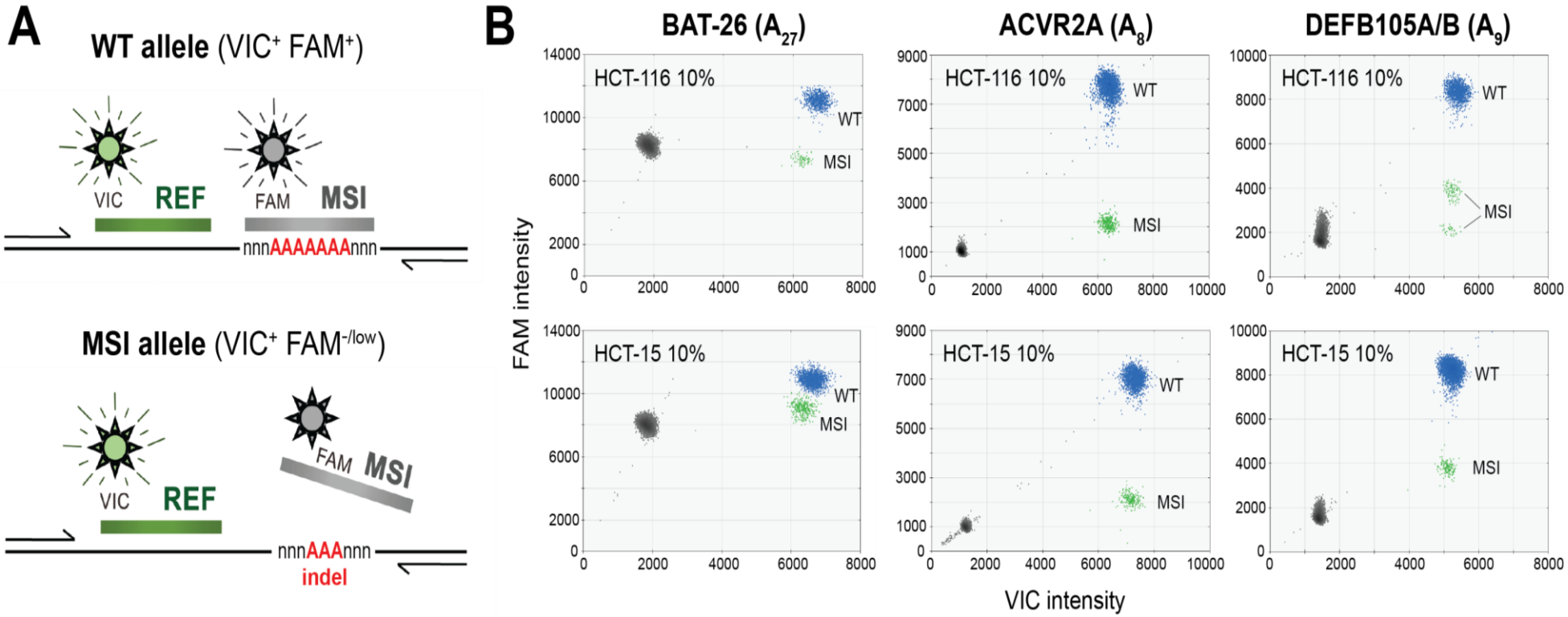
- DNAse-based enrichment of unstable MS:




Ladas *et al*, Nucleic Acids Res 2018

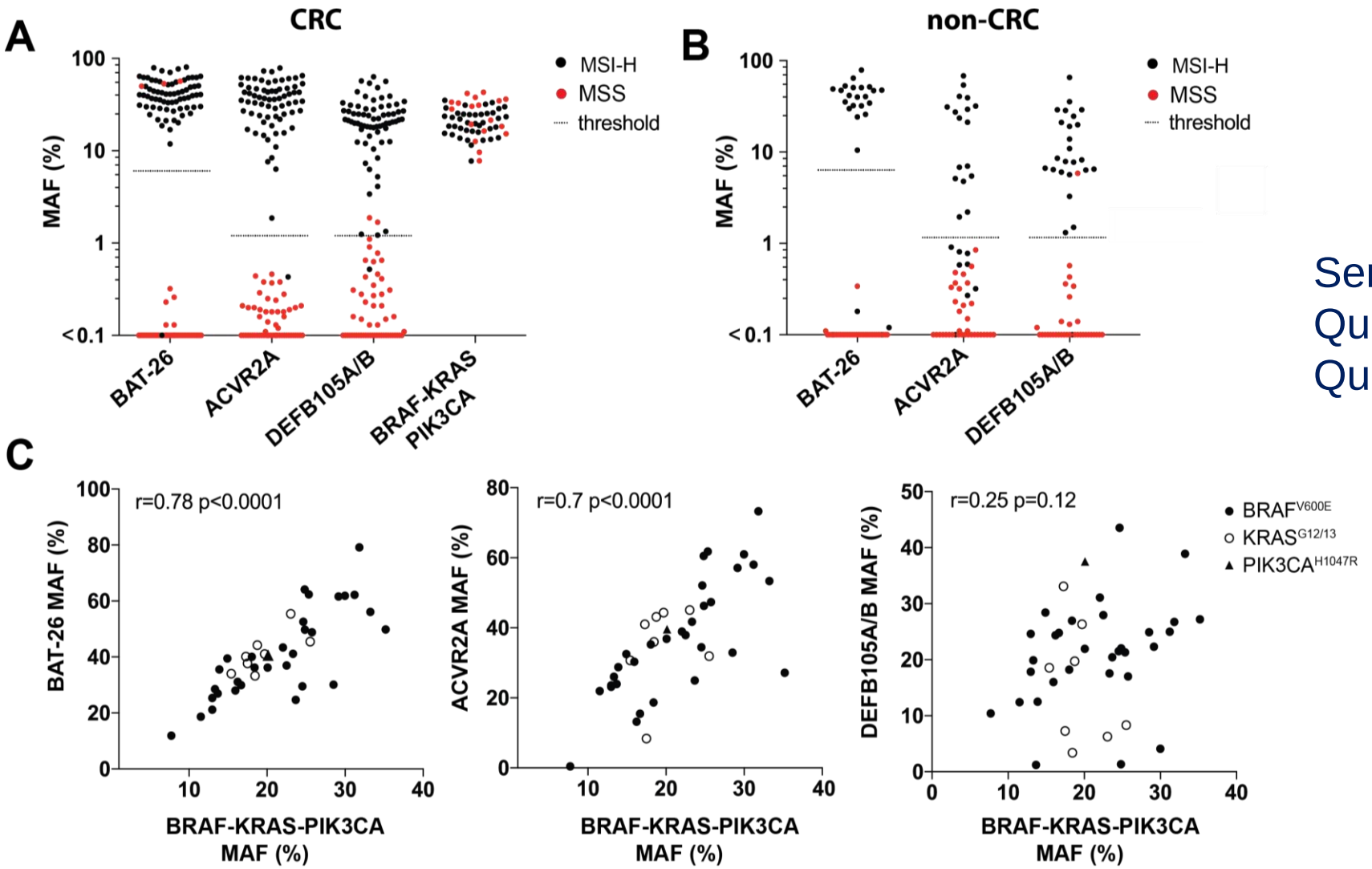
# Microsatellite Instability:

## Designing a drop-off ddPCR assay




Silveira *et al*, unpublished

# Microsatellite Instability:




Sensitive
Quantitative
Quick *and cheap* !

Silveira *et al*, unpublished

# ctDNA to capture genomic predictive markers in IO

Genome-wide markers
- Tumor mutation burden
- Microsatellite instability
- other mutational signatures…

Specific alterations
- IFNγ & HLA pathways
- PDL1 amplification

Usual limitations apply

Sensitivity of most ctDNA assays: ~70%/80% for SNV
CNV are harder to detect

# ctDNA to monitor IO drugs

Early kinetics…

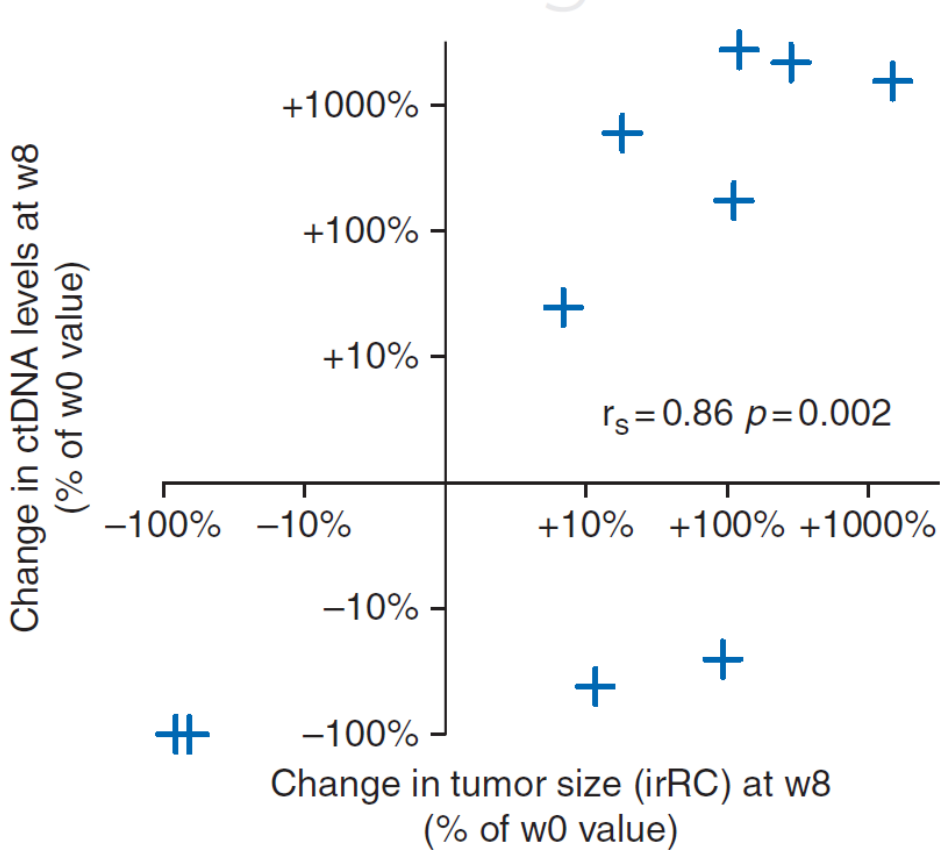
response

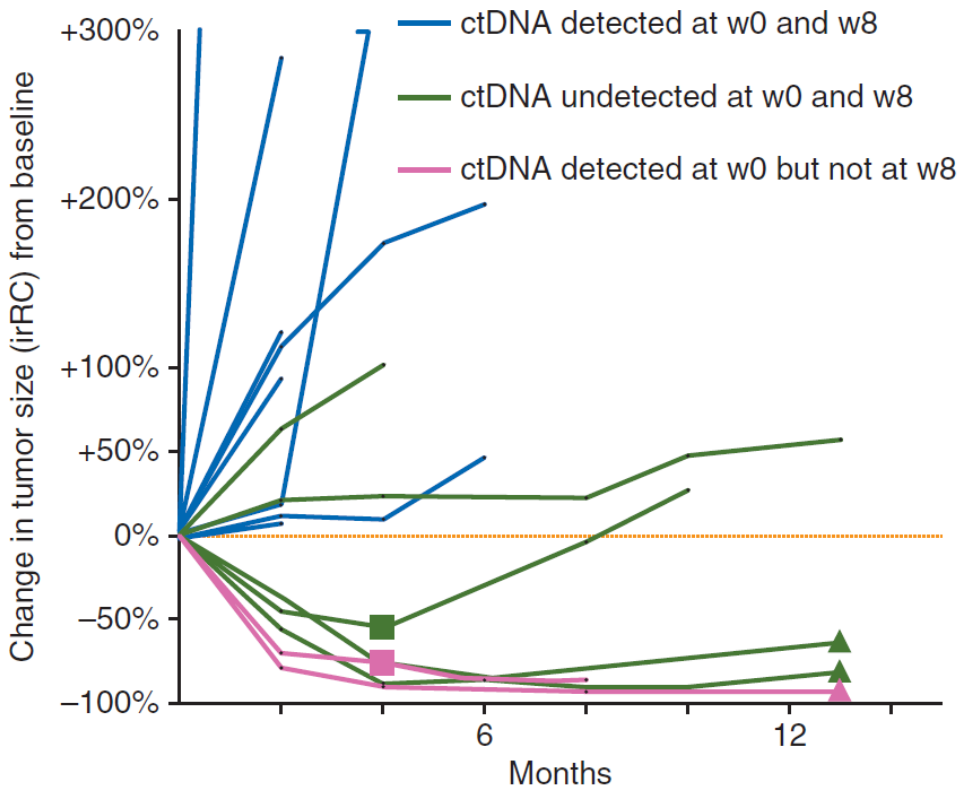
pseudo-progression

Long term monitoring

pre-clinical detection of resistance

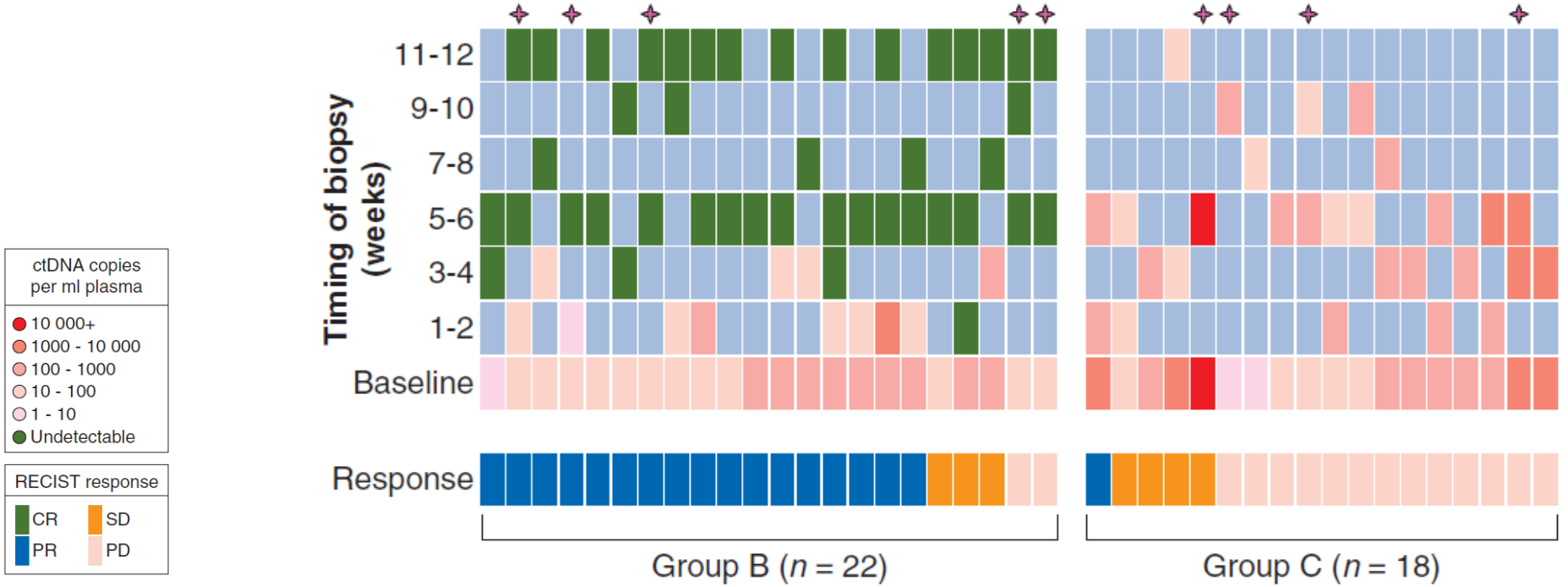
# Early kinetics & response to anti PD-1/PD-L1 mAbs: proof of concept



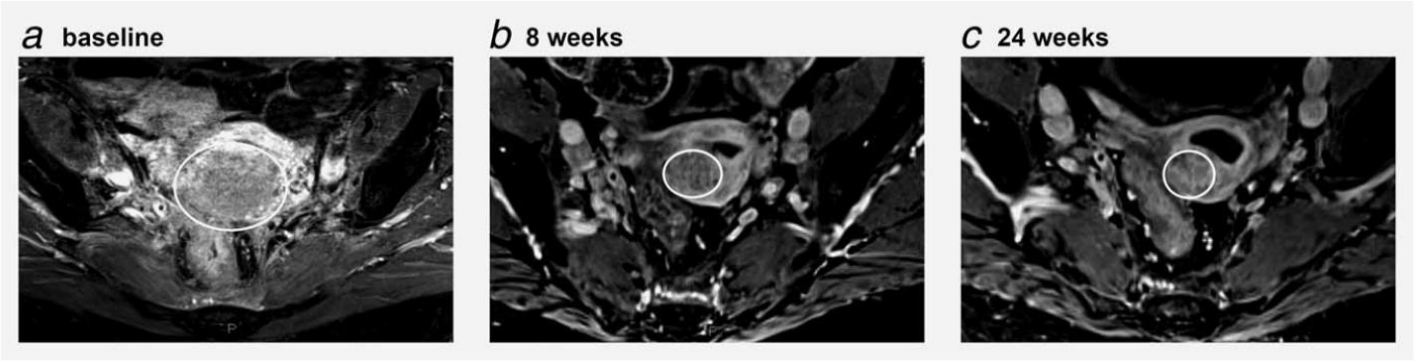

Cabel *et al*, Ann Oncol 2017

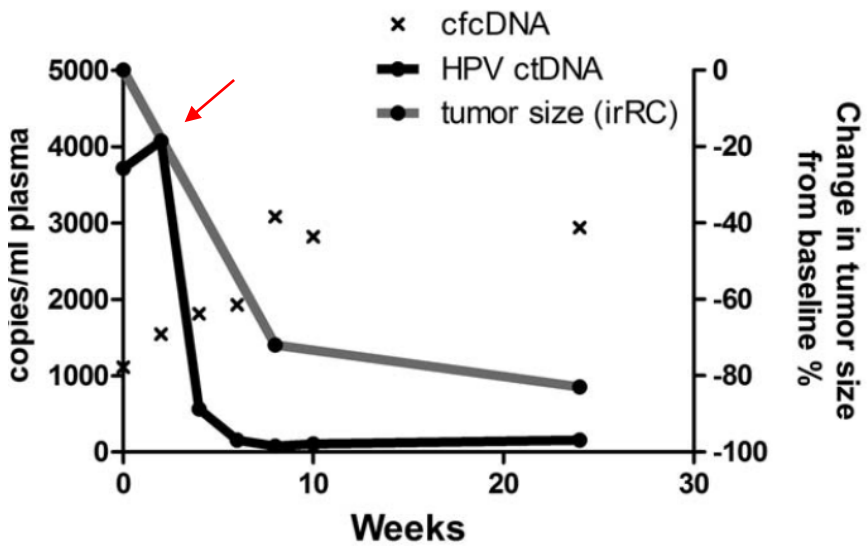
# Early ctDNA kinetics: timing



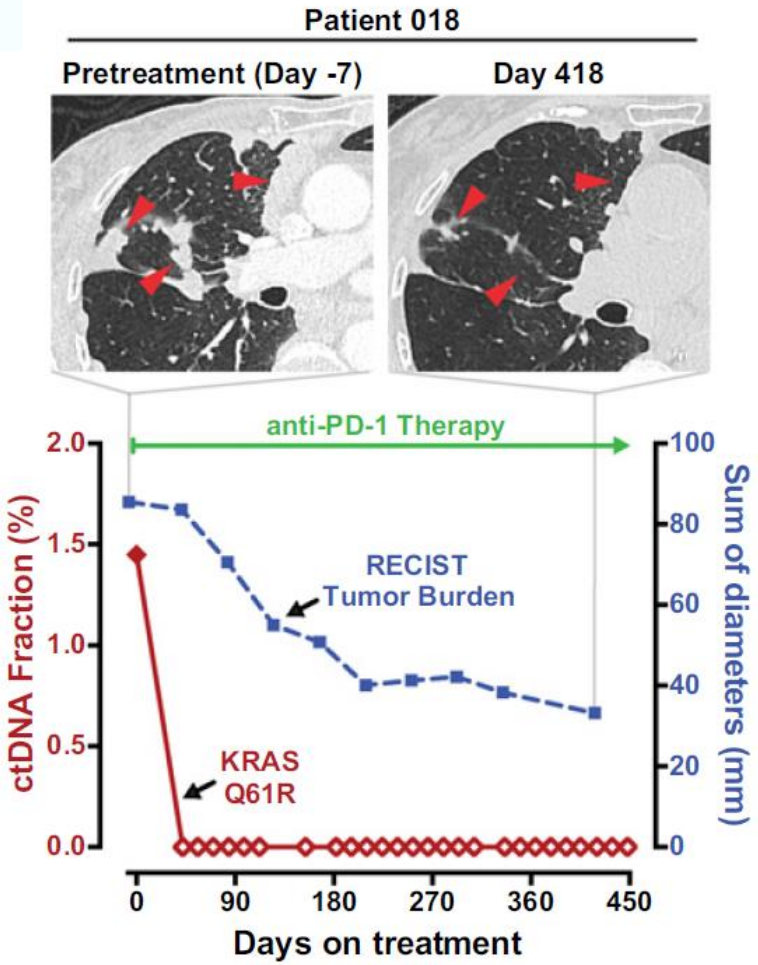

Lee *et al*, Ann Oncol 2017

# Early ctDNA kinetics: further studies…



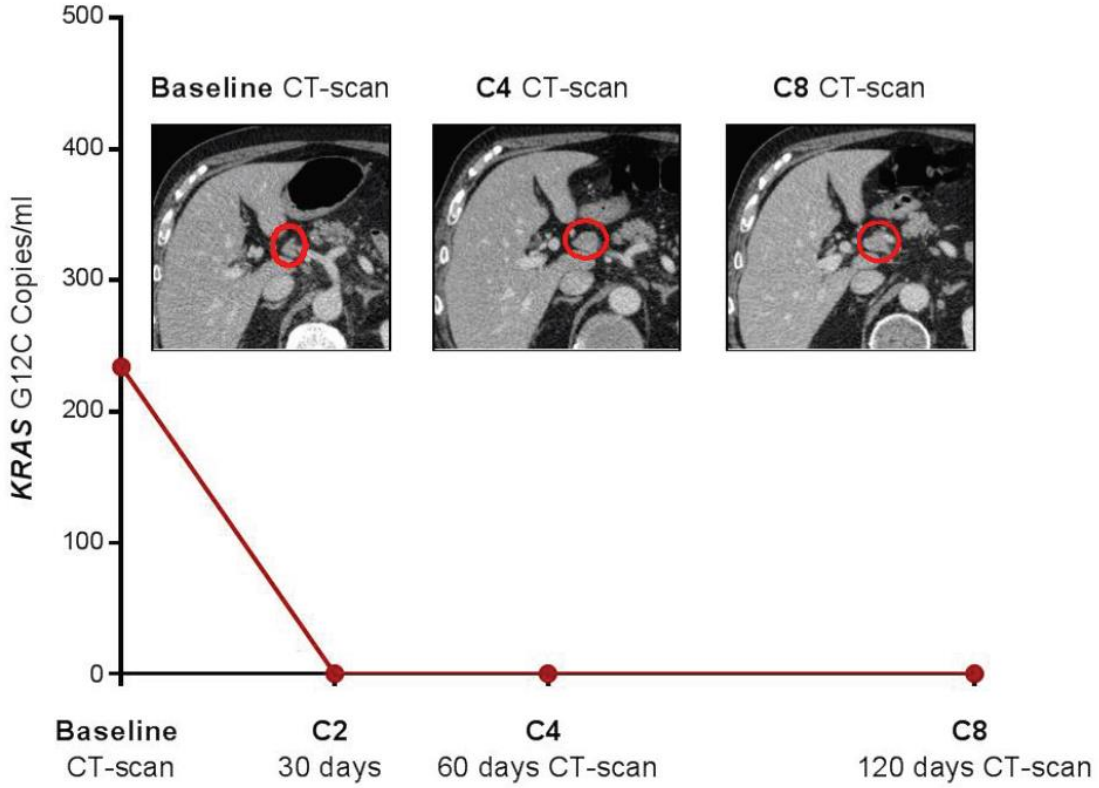



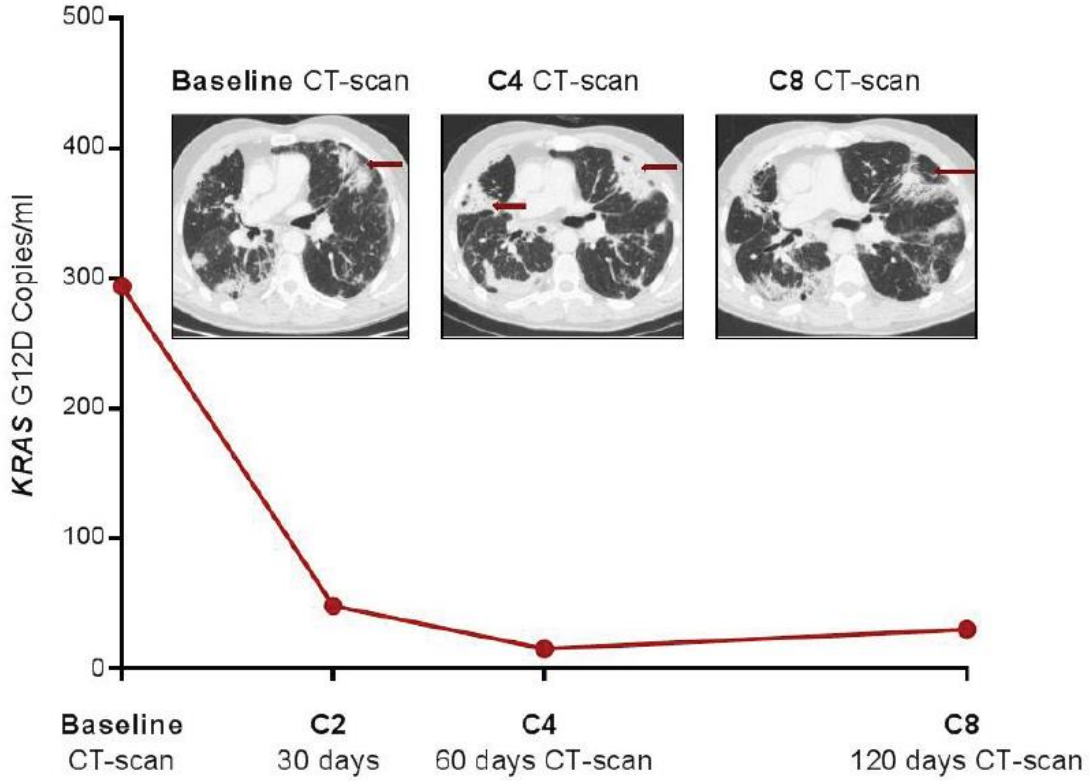

Cabel *et al*, Int J Cancer 2017




Goldberg *et al*, Clin Cancer Res 2018

# Early ctDNA kinetics: pseudoprogression




Guibert *et al*, Oncotarget 2017

# Early ctDNA kinetics: assessing sequential treatments

Article in Press

## Phase 2 trial of nivolumab combined with stereotactic body radiotherapy in patients with metastatic or locally advanced inoperable melanoma

Nora Sundahl, MD[1,2,#], Teofila Seremet, MD PhD[3], Jo Van Dorpe, MD PhD[2,4], Bart Neyns, MD PhD[3], Liesbeth Ferdinande, MD PhD[4], Annabel Meireson, MSc[5], Lieve Brochez, MD PhD[2,5], Vibeke Kruse, MD PhD[2,6,*], Piet Ost, MD PhD[1,2,*]

ctDNA was detected in 8 patients and changes corresponded to clinical response and suggested that a subset of patients, with a low PD-L1 score, only started responding after SBRT.

# Hypothesis: new windows of opportunity for better IO efficacy

IO drugs more efficient when used in patients with a limited tumor burden

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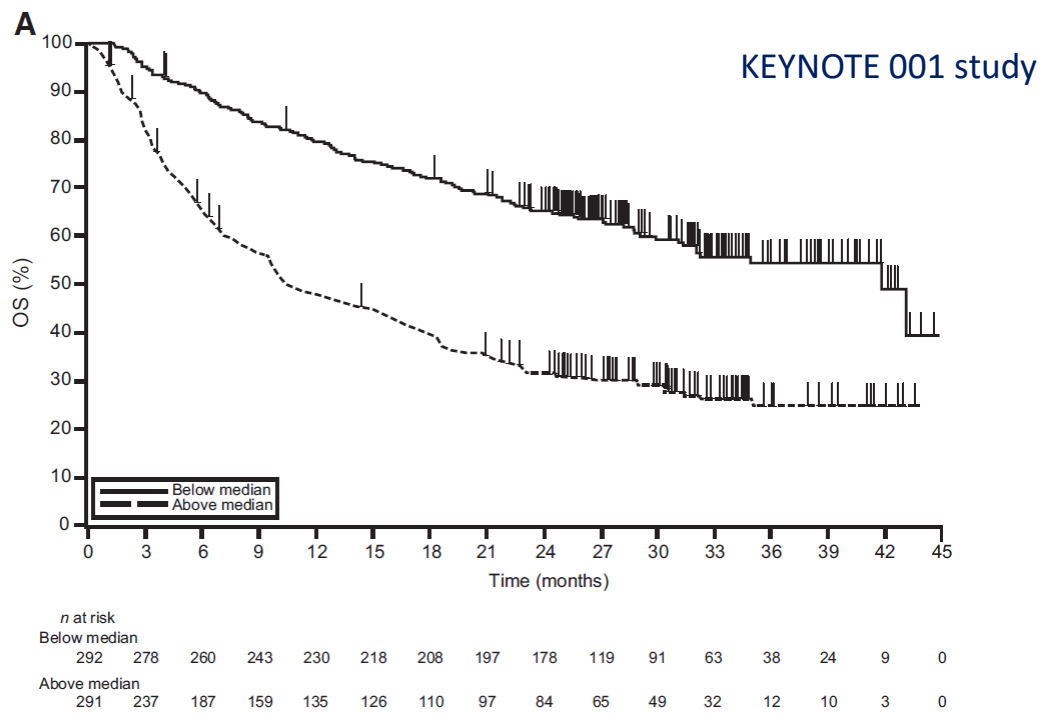
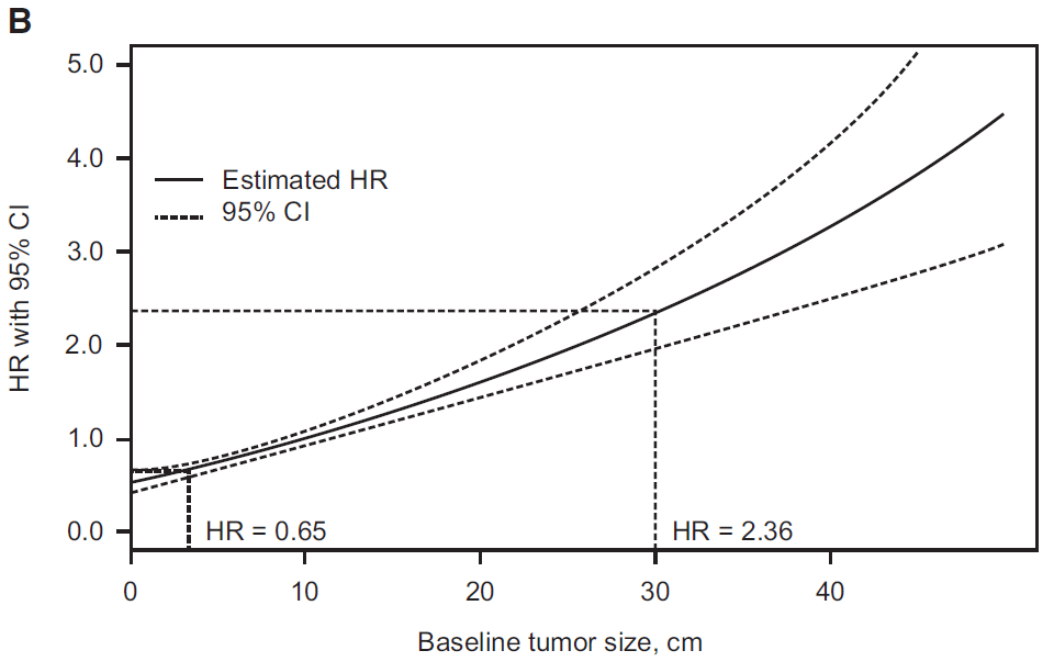
IO drugs more efficient when used in patients with a limited tumor burden



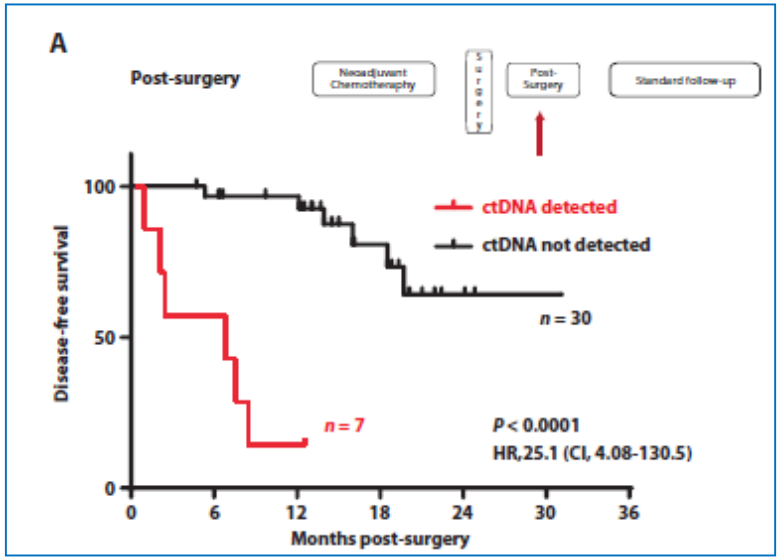




Joseph *et al*, Clin Cancer Res 2017

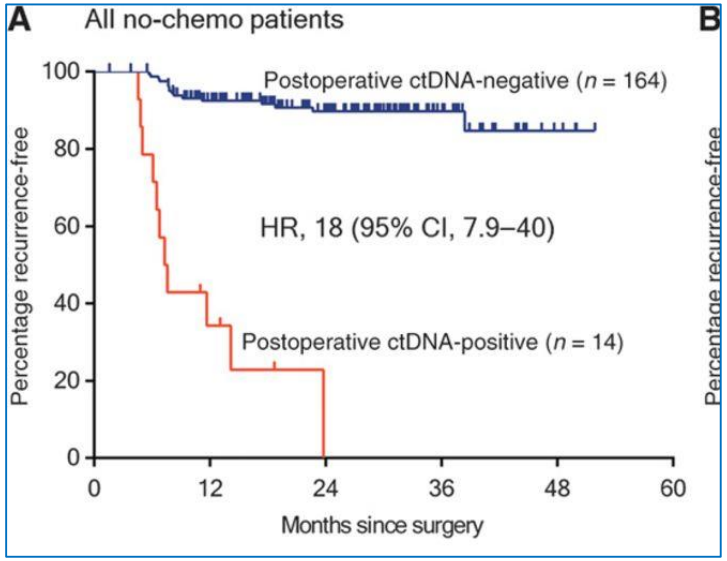
# Hypothesis: new windows of opportunity for better IO efficacy

IO drugs more efficient when used in patients with a limited tumor burden

## Minimal Residual Disease (??)




**Breast (all subtypes)**: Garcia-Murillas *et al*, 2015



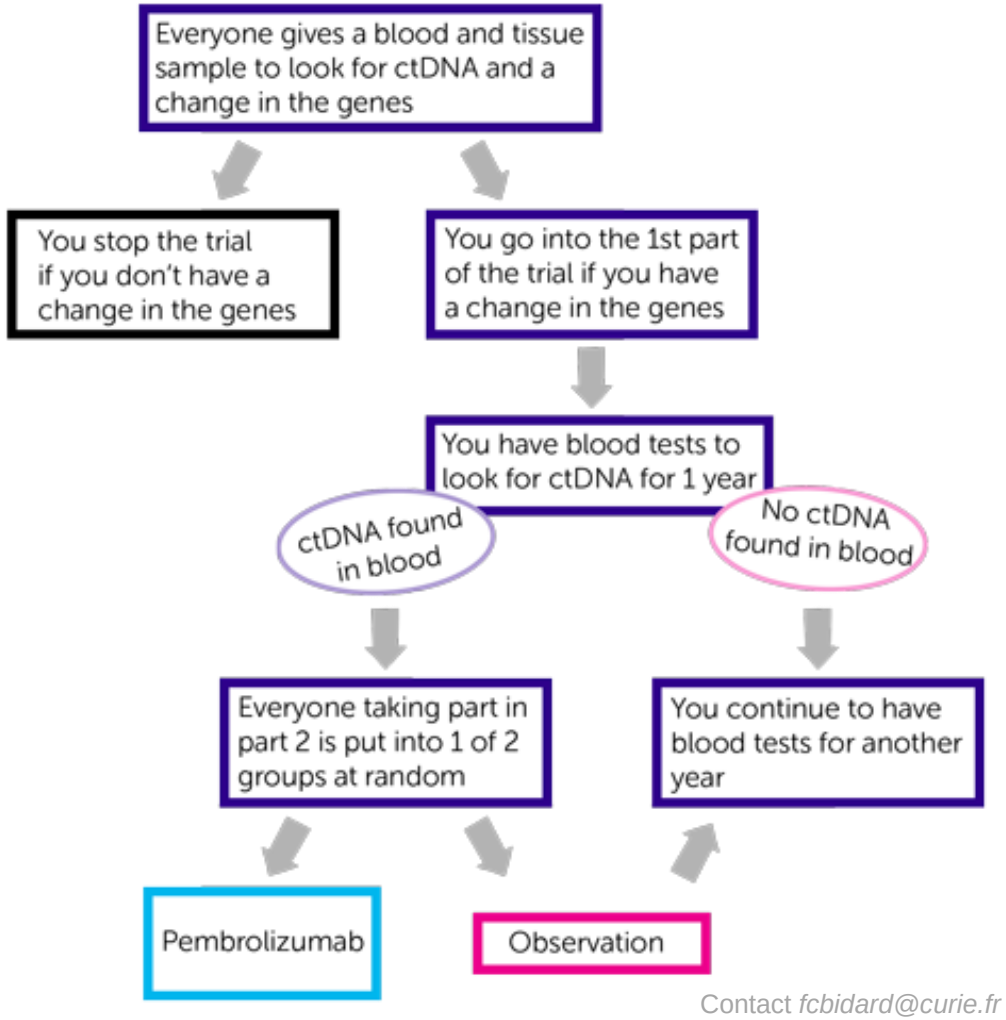

**Stage 2 CRC** : Tie *et al*, 2016

# Hypothesis: new windows of opportunity for better IO efficacy

IO drugs more efficient when used in patients with a limited tumor burden

## Early detection of relapse

A first trial is ongoing
(NCT03145961)



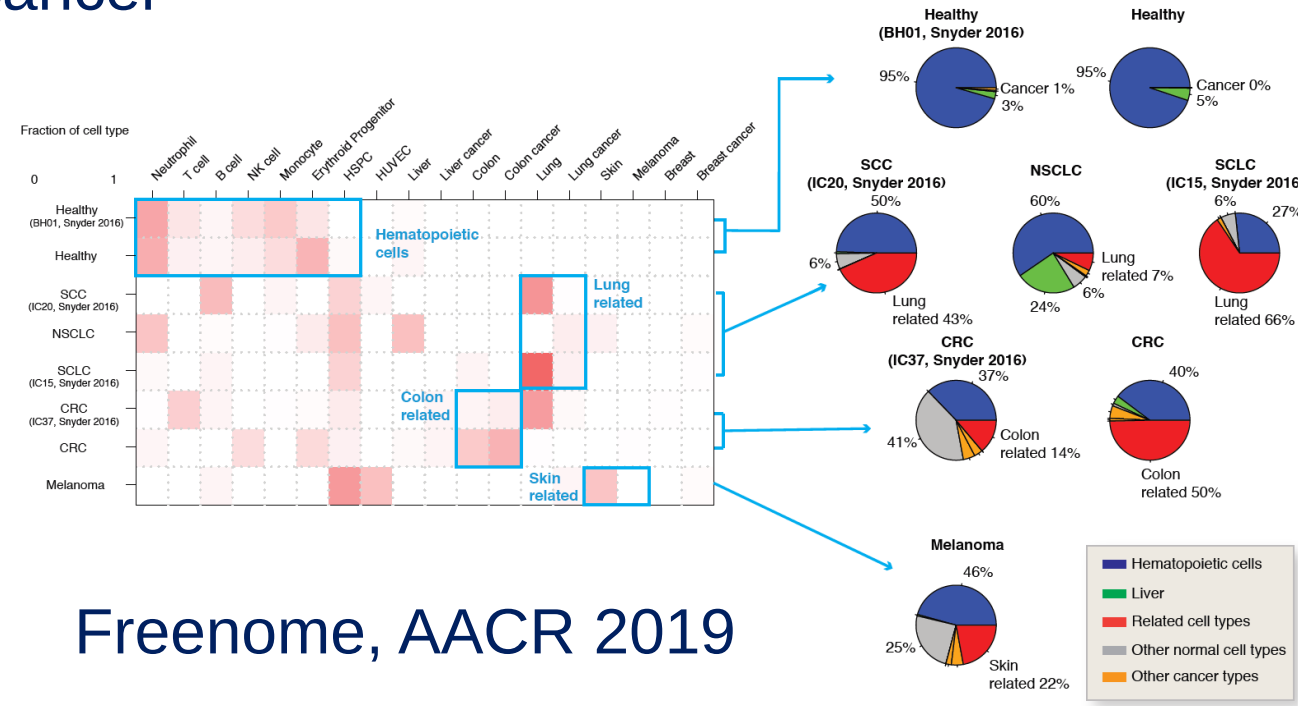

rising PSA has been cleared by FDA
In the future: rising ctDNA ?

# Hypothesis: immune system monitoring through cell-free DNA sequ.

Transcriptomic profiles can be inferred from cfcDNA fragment length

➔ Tracking back the tissue of origin

➔ Looking at *dying immune cells* in cancer




Freenome, AACR 2019

# Thank you

**Questions / comments / collaborations / *post-doctoral position***

**francois-clement.bidard@curie.fr**

**FREE course on breast cancer in Paris**

**Registration until May15 :**
**https://training.institut-curie.org/courses/BC2019**