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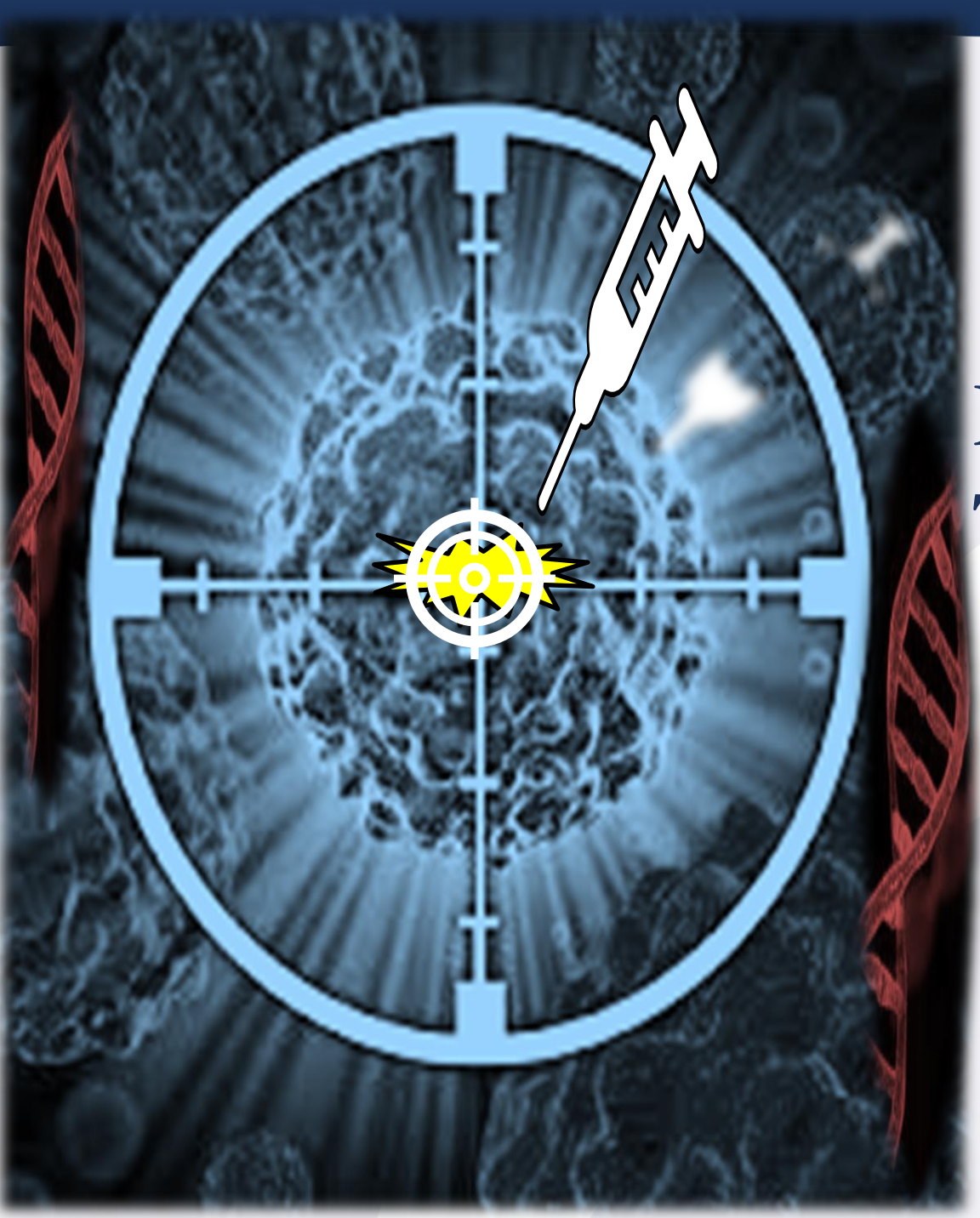
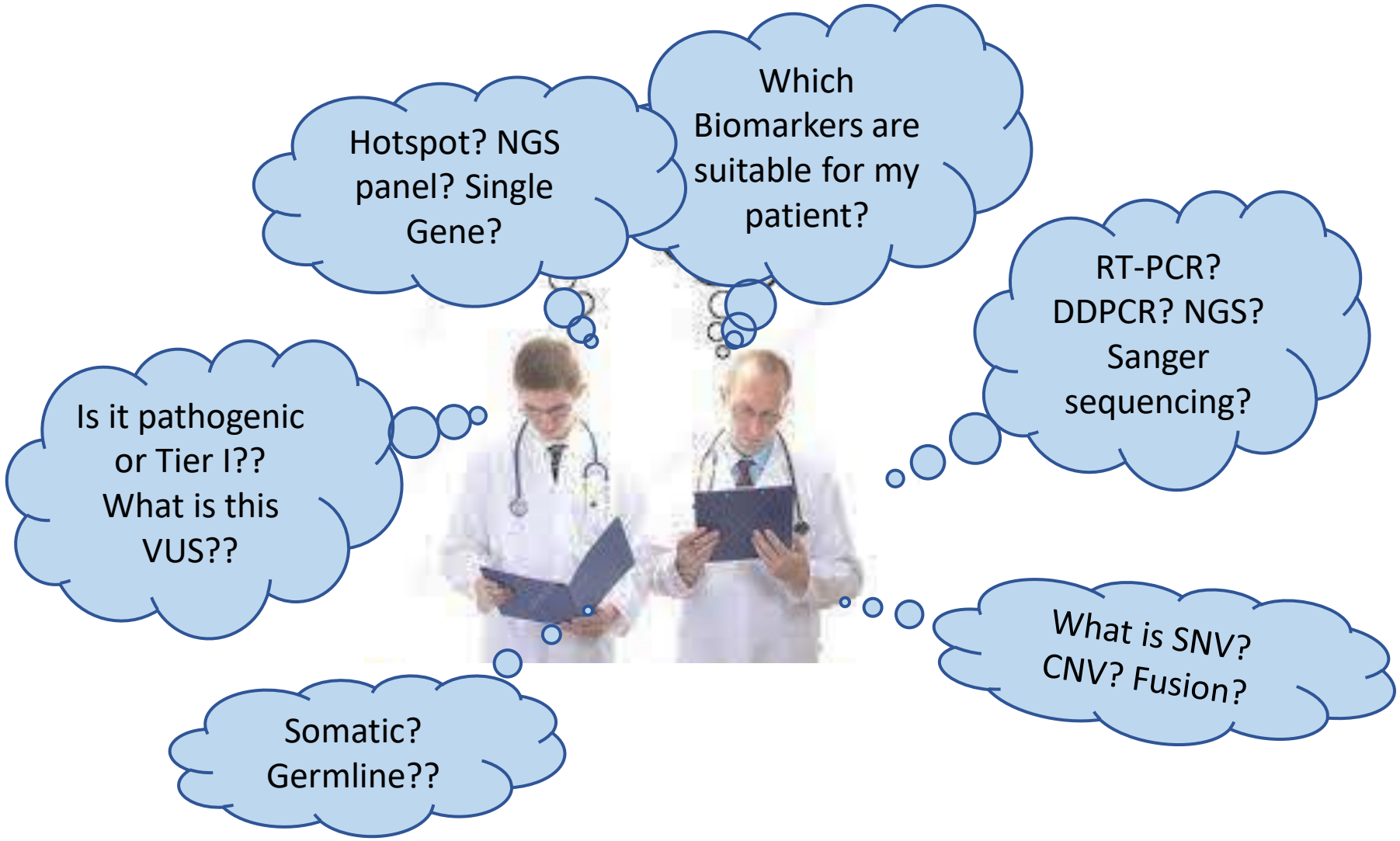


Immunotherapy and Targeted Therapy in Clinical Oncology: Commercial Perspective

Dr. Bhawna Dubey Ph.D.
Chief Scientific Officer

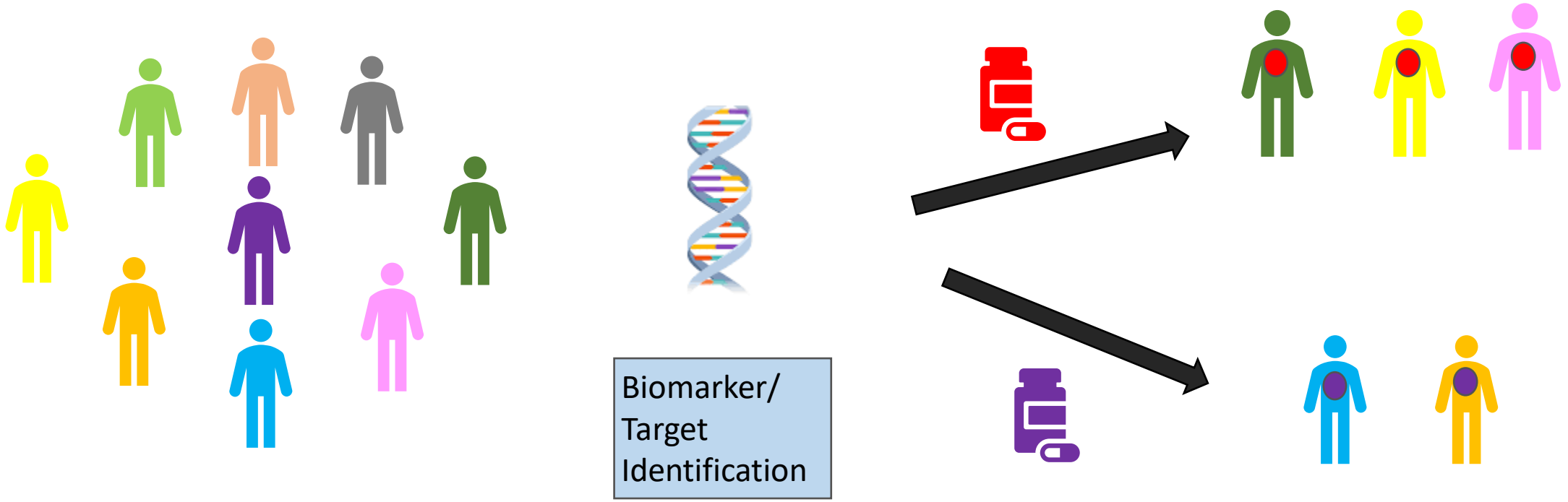
REPROCELL

Detection of Biomarkers- Physician's Conundrum

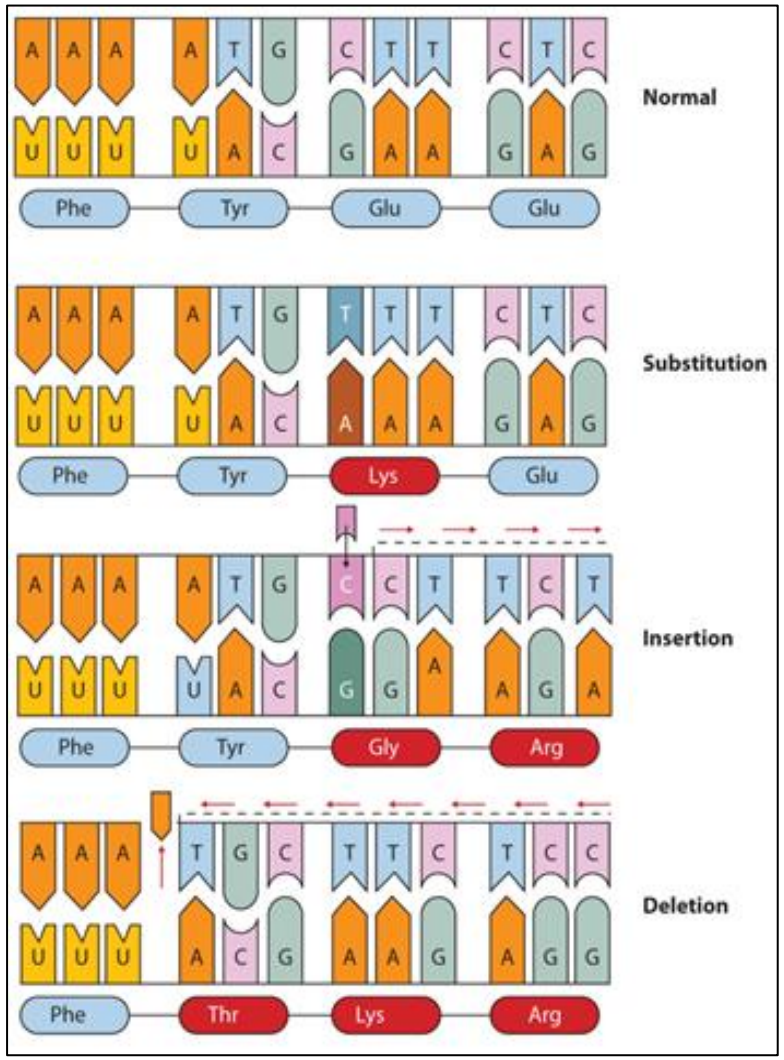


Why Target Testing

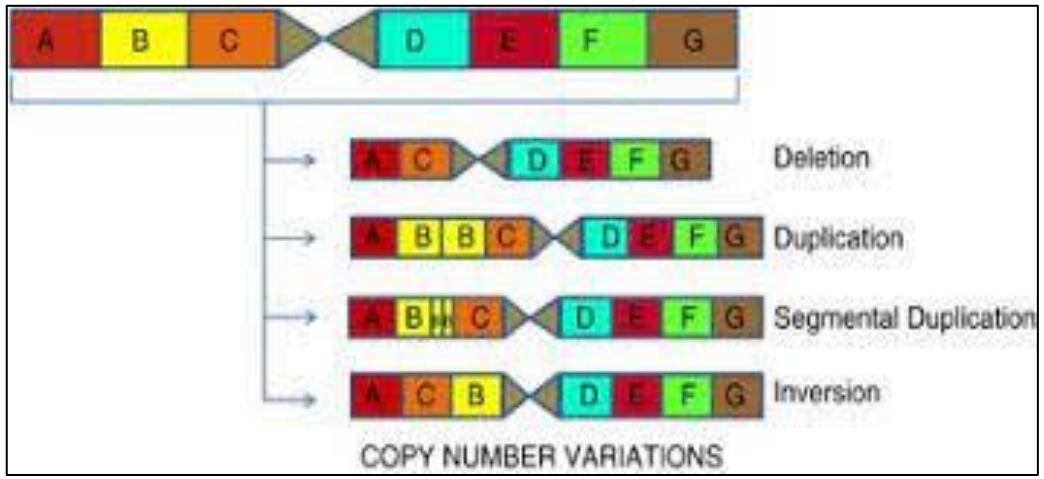
- Identify target in the patient to decide therapy options
- Enrich Patient pool which can benefit from target therapy



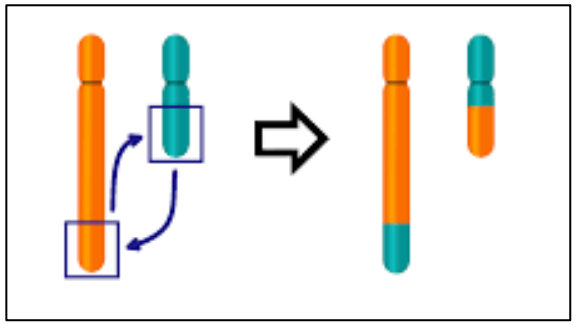
What are these “Targets”? What are their types?



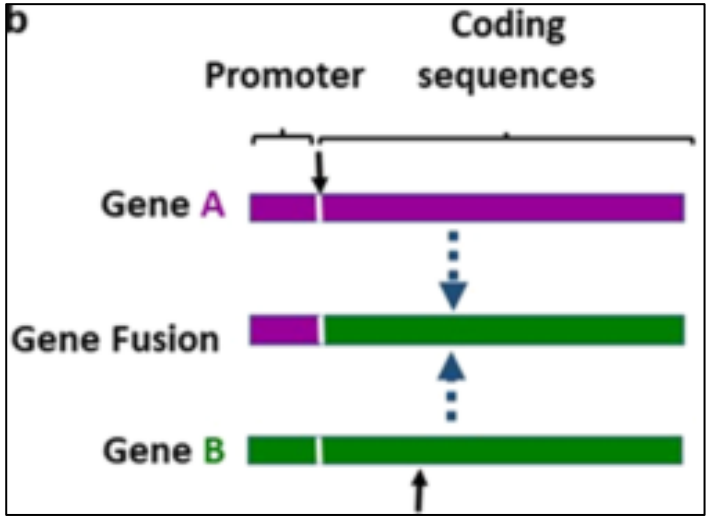
Single nucleotide Mutation (SNV) or
Point mutations
Ex: EGFR T790M



CNV –Copy Number Variants
Ex: MET amplification



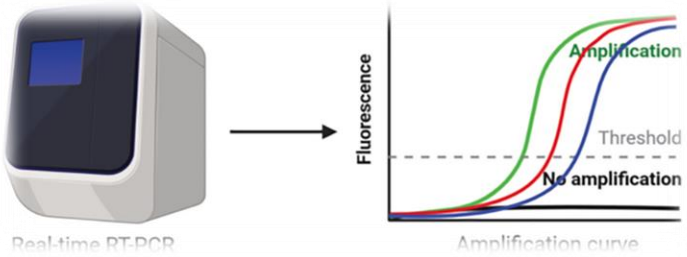
Translocations
Ex: BCR-ABL (Ph chromosome)

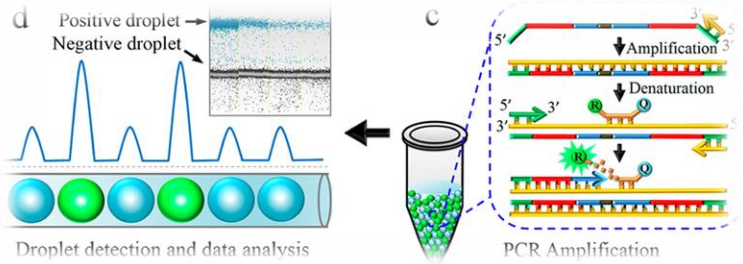


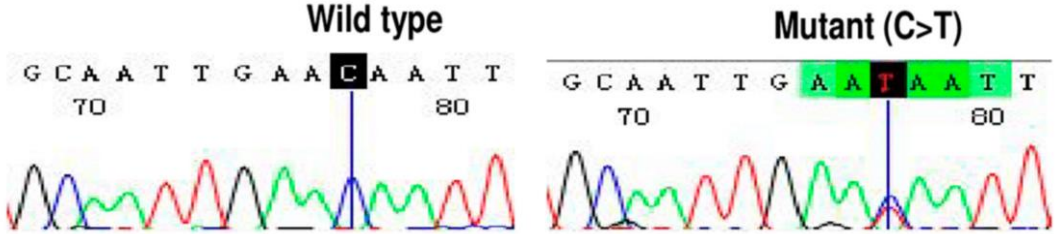
Gene Fusion
Ex: ALK/ROS

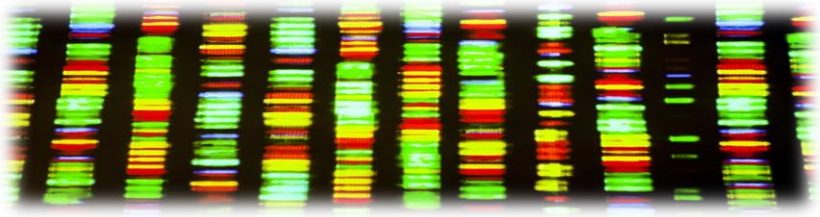
How are these Targets detected: Molecular Techniques

Technique	Application
RT-PCR	Detection/ Quantification of a marker eg. BCR/ABL IS %
DD-PCR	Highly sensitive detection/ Quantification of a marker eg. EGFR T790M mutation
Sanger Sequencing	Detection multiple mutations on a gene eg. JAK2 Exon 12, KRAS mutations
Next Generation Sequencing	Detection multiple mutations on multiple genes. Gene fusions/ CNVs eg. All genes involved in lung cancer

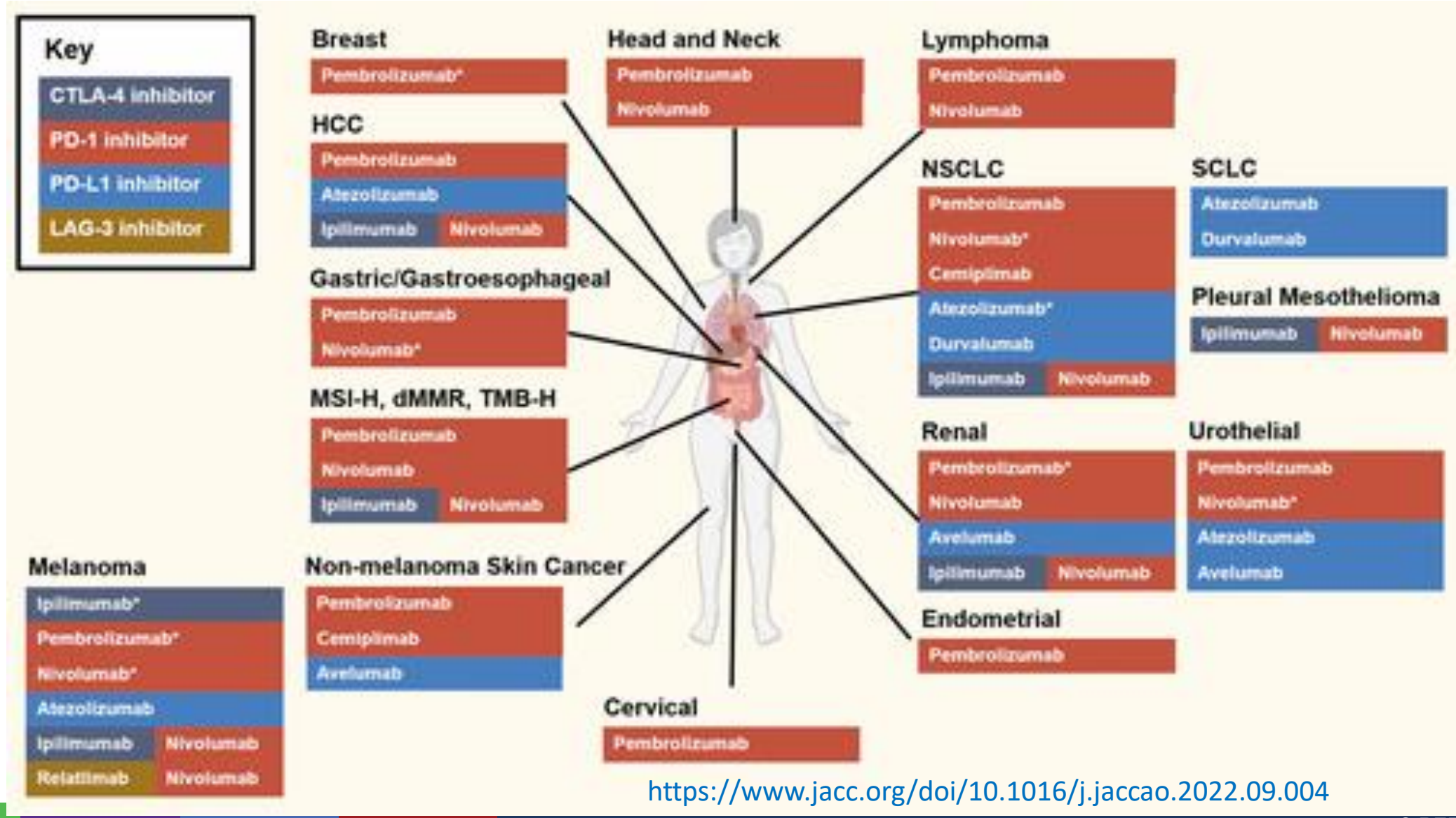








Immunotherapy: Immune checkpoint inhibitors



https://www.jacc.org/doi/10.1016/j.jaccao.2022.09.004

Targets/ Biomarkers for Immunotherapy

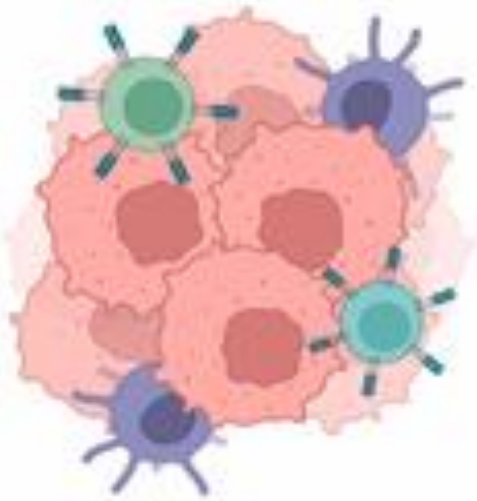
Biomarkers in Use

PD-L1 Expression
- Useful only in certain tumors
- Predictive for anti-PD-1/PD-L1

Tumor Mutational Burden
- Increases neoantigen expression
- Predictive for anti-CTLA-4 and anti-PD-1/PD-L1

Microsatellite Instability
- Reflects hypermutability
- Predictive for anti-CTLA-4 and anti-PD-1/PD-L1

Predictive Biomarkers of ICI Response



Biomarkers Under Investigation

Tumor Microenvironment
- Immune and angiogenesis profiles around tumor cells

Genomic Mutational Profile
- Defects in oncogenes, tumor suppressors and signalling

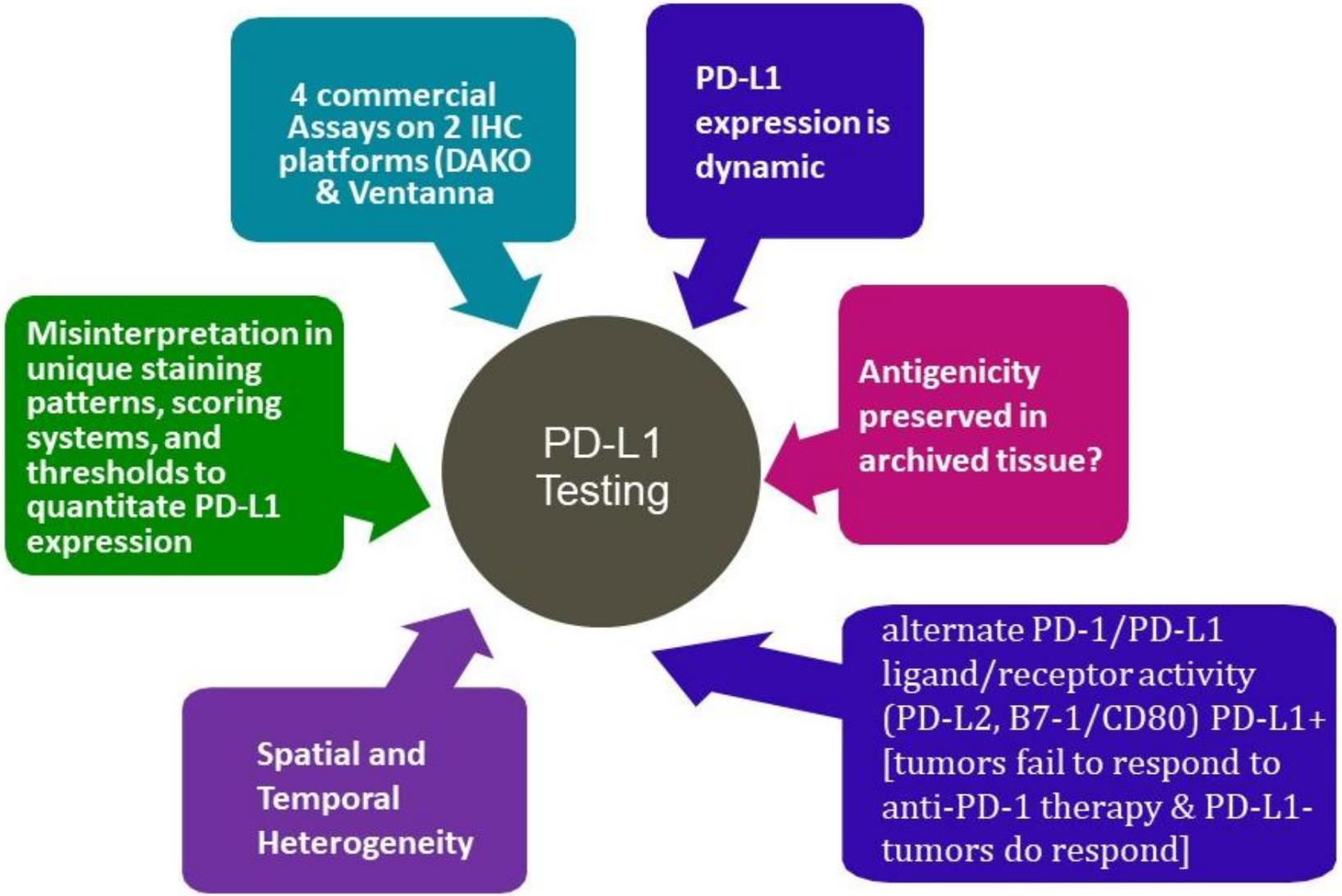
Gut Microbiome
- Better response with increased diversity, anabolic pathways and specific bacteria

Neutrophil/Lymphocyte Ratio
- Obtained from peripheral blood
- Imperfect and lacks standardized threshold

Diagnostic tools for immunotherapy

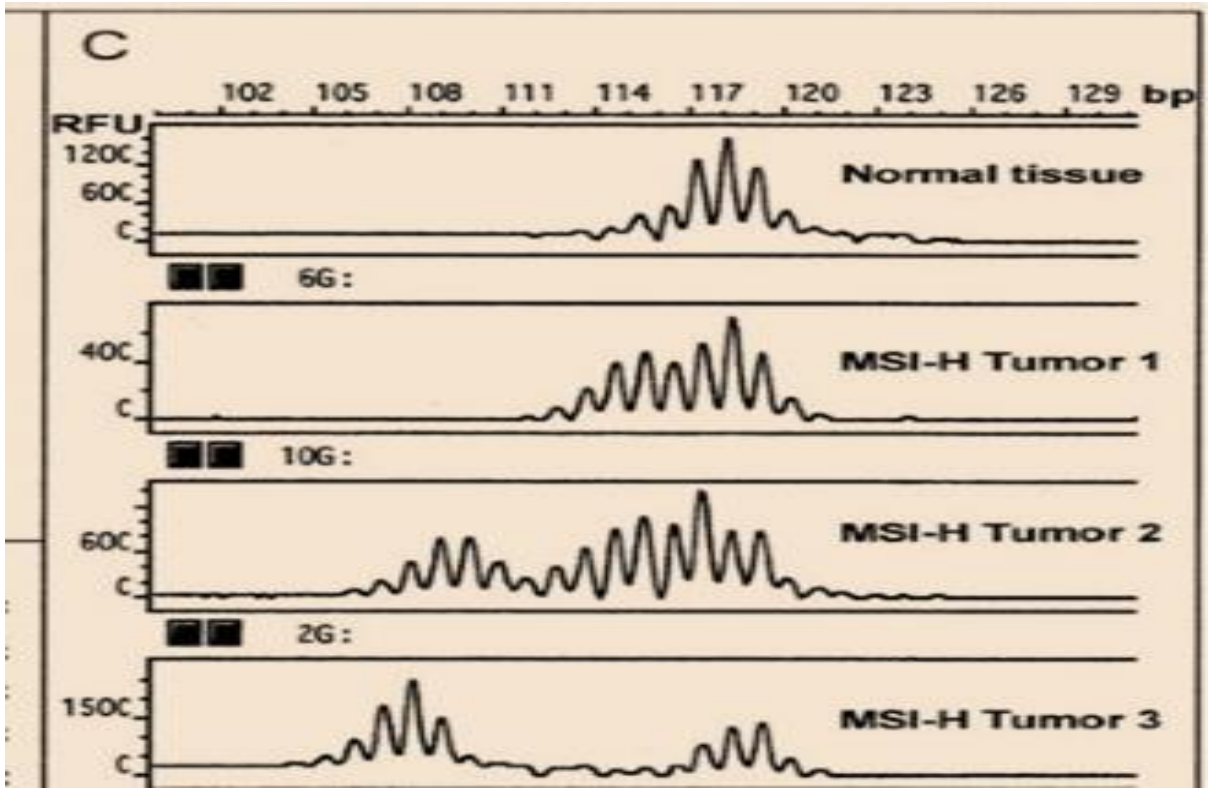
- PD-L1 staining
  - IHC-based
  - Companion diagnostics for some indications, complimentary for others
- Microsatellite instability (MSI) or mismatch repair deficiency (MMRd)
  - PCR- or sequencing-based microsatellite assay
  - IHC or sequencing of MMR genes (MLH1, MSH6, MSH2 & PMS2)
- Tumor mutational burden (TMB)
  - A measure of the total number of somatic mutations per million bases of coding sequence in a tumor genome
  - WES or panel sequencing

PD-L1 alone is not enough!!!



Microsatellite Instability (MSI)

Microsatellites are regions of repeated DNA that change in length (show instability) when mismatch repair is not working properly.



Detect a dMMR in cancer

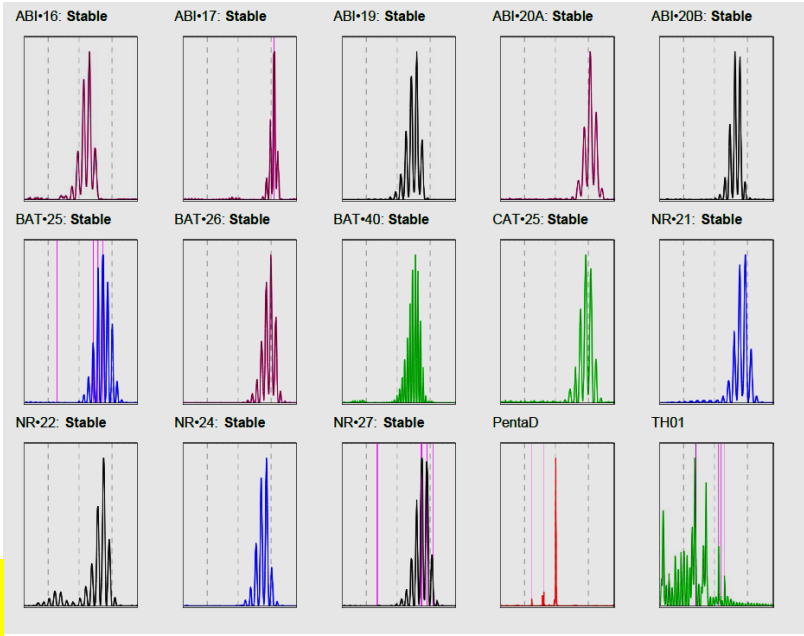
MSI

- Identification of MSI **by molecular testing of microsatellites**
- Represents direct proof of dMMR
- DNA from fresh, frozen, or paraffin-embedded tumour tissue using a PCR-based assay for detection of MSI. The highest specificity and sensitivity is reached using a panel of three or more polyA mononucleotide markers (BAT25, BAT26, NR-21, NR-22, NR-24, NR-27)
- **MSI-high (MSI-H), is defined as two or more microsatellite markers showing instability**

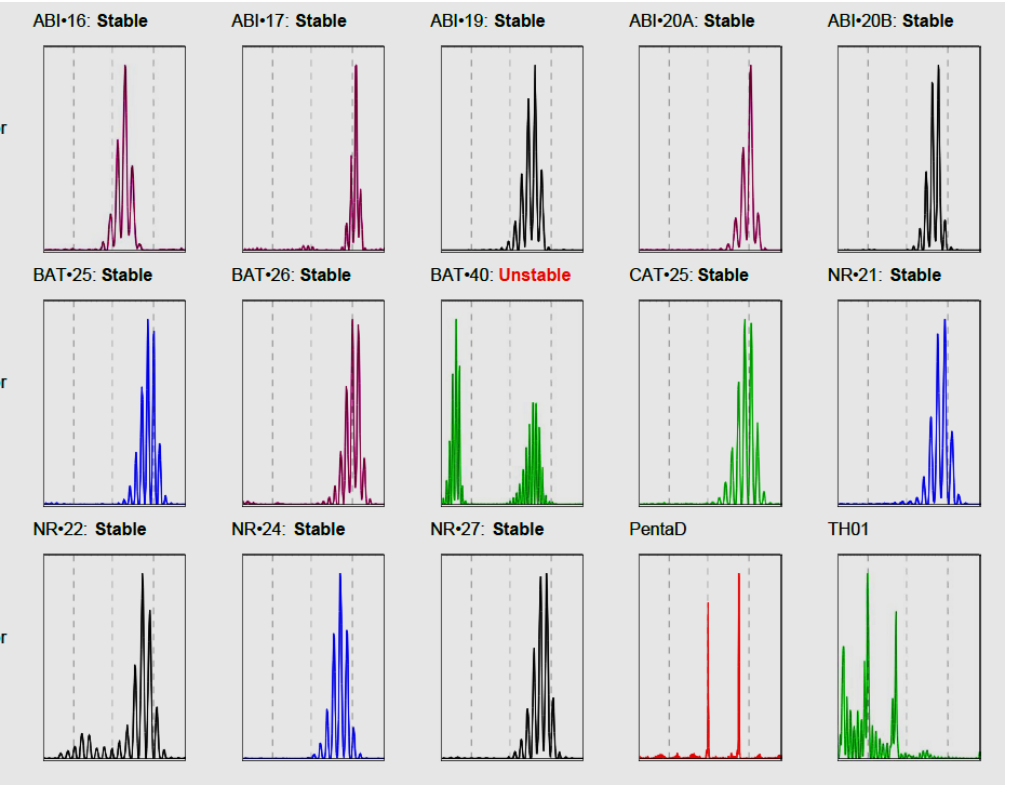
MMR

- Test Lack of **expression of MMR proteins** using IHC
- indirect suggestion of a dMMR system
- Antibodies against the four MMR proteins are commercially available and IHC is used to detect the expression of the **four MMR proteins (MLH1, MSH2, MSH6, and PMS2)**
- **dMMR is defined as at least 1 protein (MSH 2, MSH 6, PMS 2 and MLH 1) showing loss of expression-IHC**

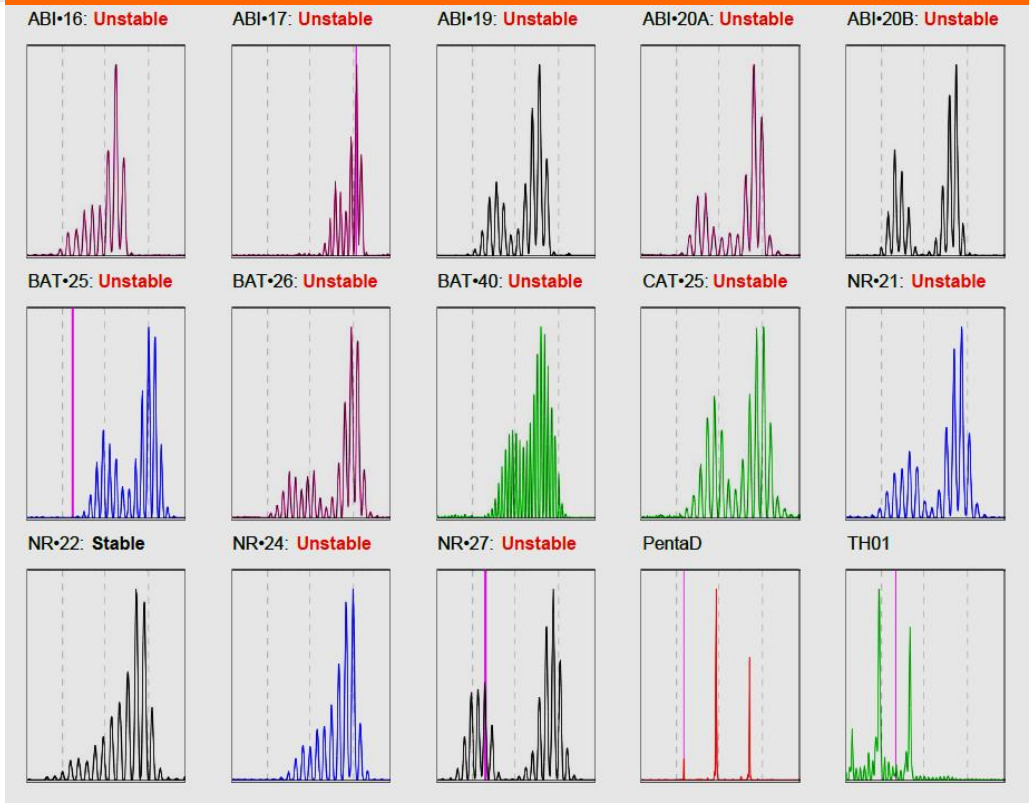
MSI-Stable



MSI-Low



MSI-HIGH



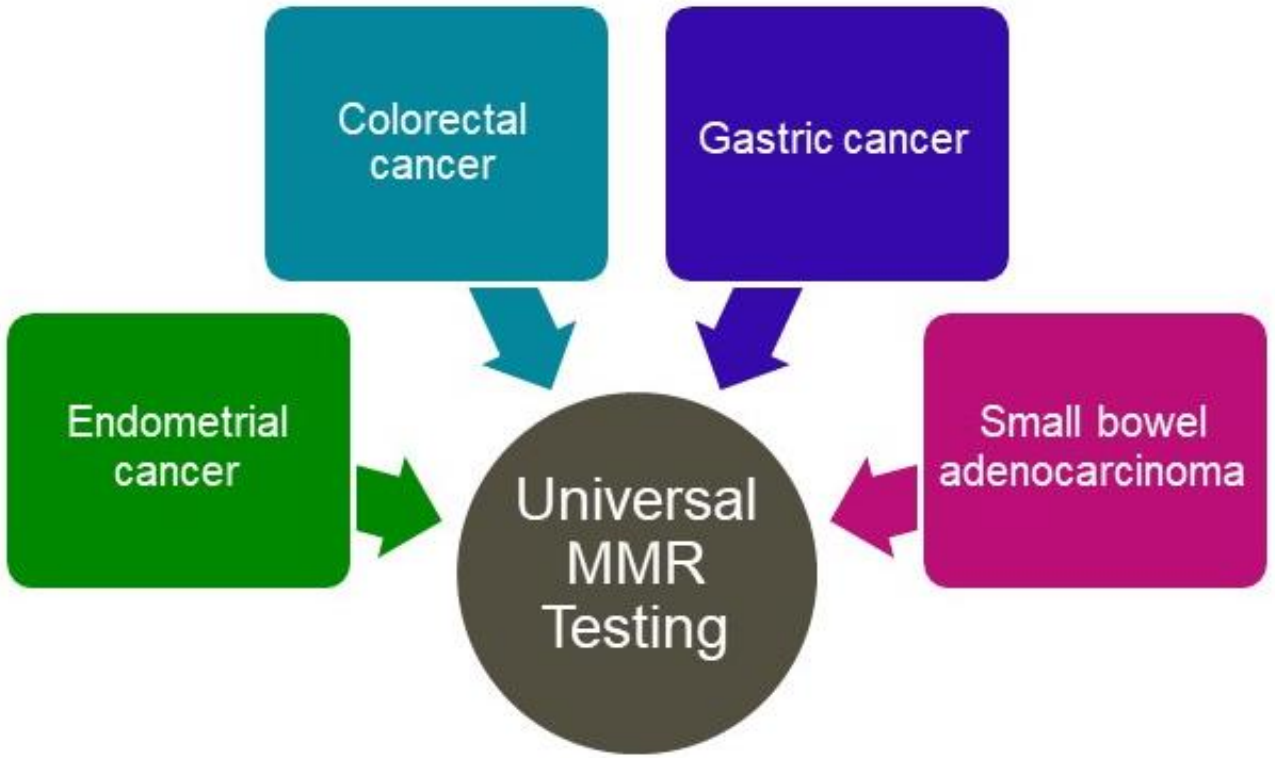
MSI-Clinical Significance

- **MSI-H = mismatch repair deficient (dMMR)**
- **MSI-H: Predictive for ICI therapy**
- **MSI-H: eligibility to Pembrolizumab, ipilimumab, nivolumab treatment of colorectal cancer patients**
- **MSI-H: Diagnostic- Lynch Syndrome.** MSI is the hallmark of Lynch syndrome and constitutional mismatch repair deficiency (CMMRD).

Colorectal cancer (CRC) and endometrial cancer (EC) are the most common cancers in Lynch syndrome

- CRC: individuals with Lynch syndrome have a 50-80% lifetime risk of developing CRC compared to 2% in the general population.
- EC: Women with Lynch syndrome carry a 40-60% lifetime risk of developing EC

NCCN Guidelines Testing Recommendations



GI Cancers (CRC[a], gastric, esophageal, small bowel)

- Test all CRC, all newly diagnosed gastric cancers and small bowel adenocarcinoma
- Consider testing locally advanced, recurrent, or metastatic esophageal and EGJ cancers who are candidates for PD-1 inhibitors
- Available at nccn.org

Gynecologic Cancers (EC[a], ovarian)

- Test all EC and endometrioid carcinoma; test refractory/relapsed ovarian cancer
- Available at nccn.org

The NCCN Guidelines recommend universal MMR testing in CRC, EC, gastric cancer, and small bowel adenocarcinoma.
MMR testing is also recommended for certain patients with other solid tumors (e.g., ovarian, esophageal, EGJ).

MSI Testing suggestions

Cancer type	Testing suggestions	MSI prevalence
Colorectal	All cancers	15%
Gastric	All cancers	15%
Duodenal and ampulla of Vater	All cancers	Up to 10%
Esophageal	Barrett's associated cancers	5%
Endometrial	All cancers	Up to 33%
Ovarian	All cancers	10%
Cervical	Advanced stage cancers	5%
Breast	None	<1%
Hepatocellular	None	No evidence
Pancreatic and periampullary	Medullary histotype, cancers of periampullary area	<1% in pancreatic cancer, up to 10% in cancers of periampullary area
Sebaceous skin tumour	All tumours	25%
Melanoma	None	Inconsistent data
Lung cancer	None	<1%
Glioma	Paediatric, young adults	Controversial data 0%-33%
Prostate cancer	Advanced stage cancers	Up to 12%
Thyroid cancer	None	No evidence
Head and neck cancer	None	1%
Renal cell carcinoma	None	No evidence
Sarcoma	None	No evidence

Prevalence of MSI-H Tumors by Stage[2]

Gynecological Tumors	MSI-H (%) Stages 1-2	MSI-H (%) Stages 3-4
Endometrial Cancer	27%	26%
Ovarian Cancer	17%	20%

Gastrointestinal Tumors	MSI-H (%) Stages 1-2	MSI-H (%) Stages 3-4
Colorectal Cancer	20%	9%
Gastric Cancer	13%	10%
Esophageal Cancer	NR	18%

https://oncologypro.esmo.org/education-library/factsheets-or biomarkers/microsatellite-instability-defective-dna-mismatch-repair

ESMO: Choice of test

Molecular testing of polyA microsatellites is the choice to detect MSI as direct proof of dMMR in a given cancer. Immunohistochemistry can be used as an efficient indirect test for dMMR when a molecular laboratory is not available

Microsatellite instability detection methods

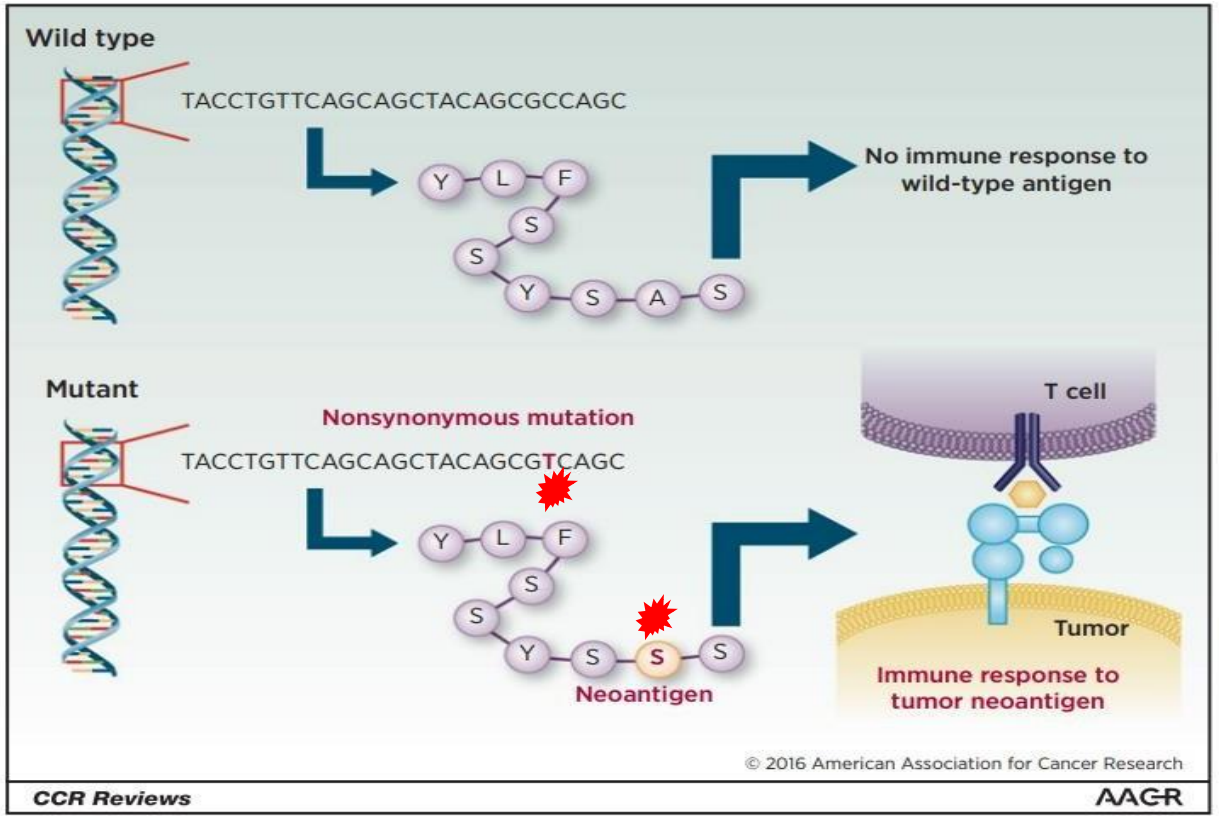
Detection method	Characteristics	Test items	Accuracy	Refs.
NGS	Accurate results were obtained from a small amount of sample	Nearly 100 MS loci	IMPACT™: 92% F1CDx: 94.6%	Hempelmann et al. [6]
Fluorescent multiplex PCR and CE	Only MSI results are obtained MSI analysis system is based on this method	5 MS sites: BAT-26, NR-21, BAT-25, MONO-27 and NR-24	**Gold standard, 100%**	Arulananda et al. [11]
IHC	Wide application and strong practicability, but only get the MMR results	The MMR protein: hMLH1, hPMS2, hMSH2, hMSH6	89–95%	Cheah et al. [12]

https://oncologypro.esmo.org/education-library/factsheets-on-biomarkers/microsatellite-instability-defective-dna-mismatch-repair

Tumor Mutation Burden- TMB

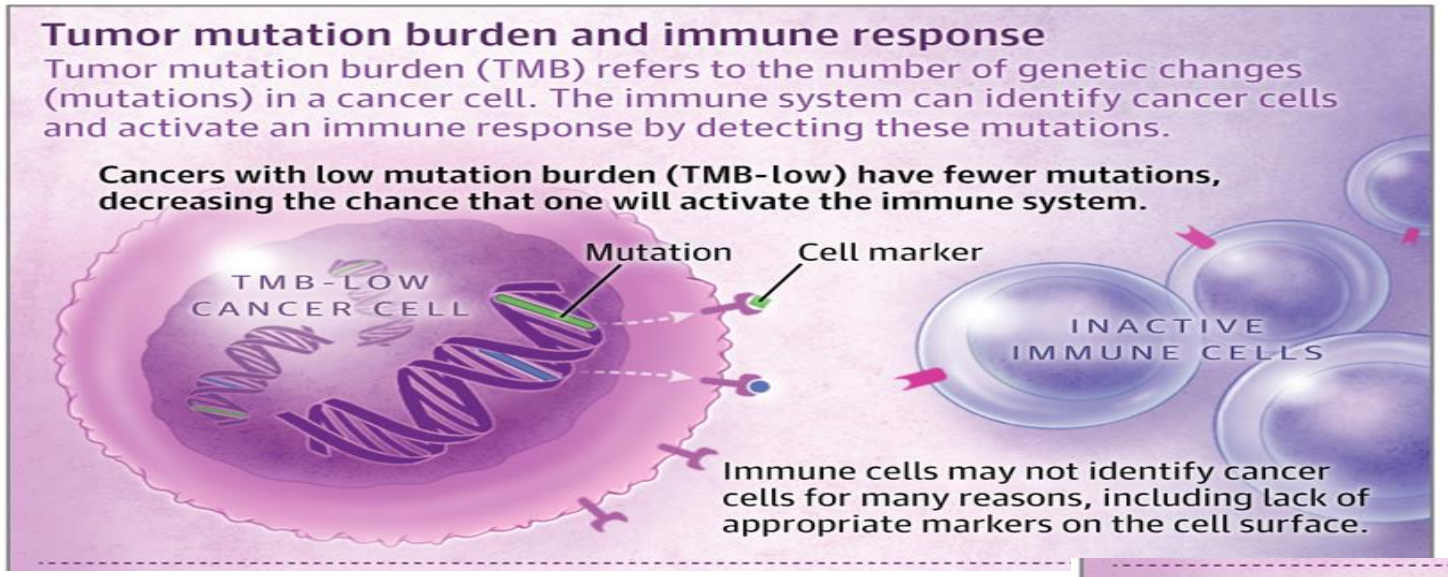
Tumour mutational load or mutation burden is a measure of the number of mutations within a tumour genome, defined as the total number of mutations per coding area of a tumour genome.

Higher tumour mutational load = higher levels of neoantigens.



- Wild-type antigens are recognized as “self”, and do not generate an immune response.
- Nonsynonymous mutations may lead to an altered peptide sequence that is ultimately presented on MHC molecules.
- This altered peptide sequence therefore produces a new or “neoantigen”, which may then be recognized by the host immune system, leading to an anti-tumor immune response. Neoantigens are a type of cancer-specific antigen that may allow for a more robust immune response

Low and High TMB



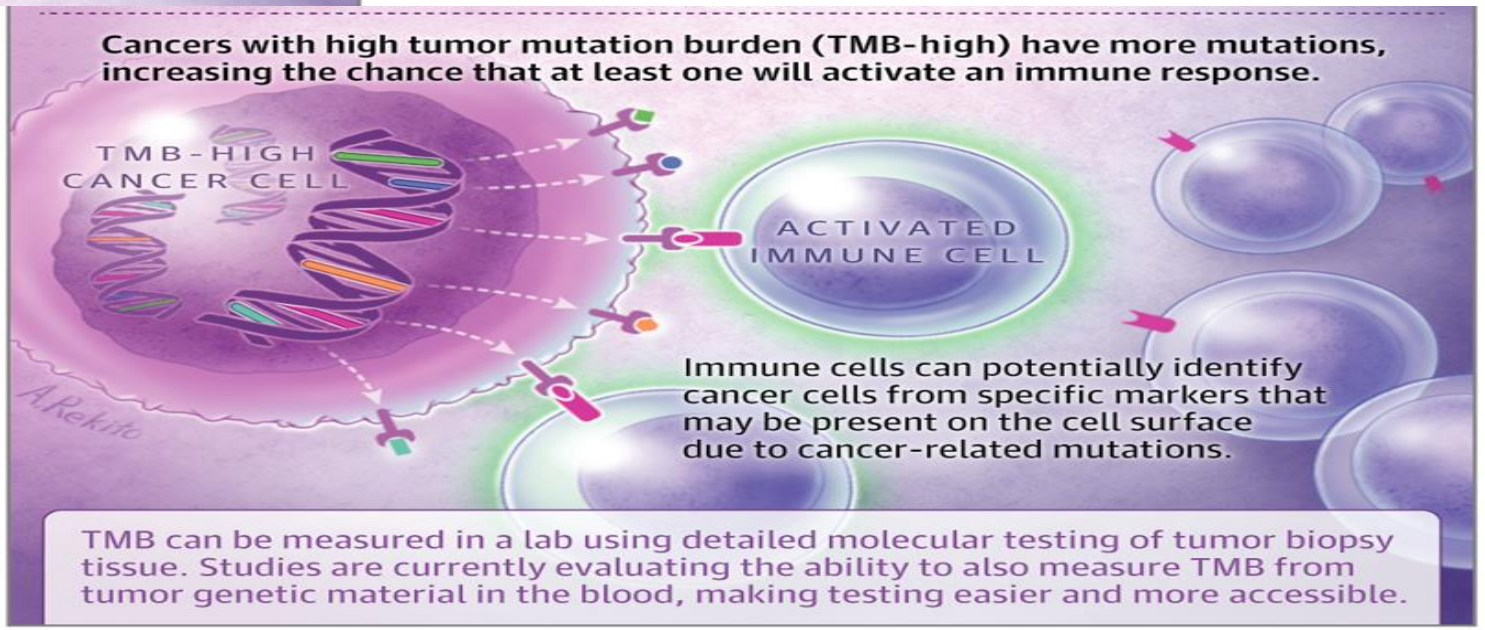
Low TMB

Cold Tumor

High TMB

Hot Tumor

Better ICI response



NGS Panels in TMB

Diagnostic Partner	Panel Name	Gene No	Size (Mb)
ACT Genomics	ACTOnco	440	1.12
AstraZeneca	AZ600	607	1.72
Caris Life Sciences	SureSelect XT	592	1.40
Foundation Medicine	FoundationOne CDx	324	0.80
Guardant Health	GuardantOMNI	500	1.00
Illumina	TSO500	523	1.33
MSKCC	MSK-IMPACT	468	1.14
NeoGenomics	NeoTYPE	372	1.03
Personal Genome Diagnostics	PGDx elio	507	1.33
QIAGEN	QIAseq TMB	486	1.33
Thermo Fisher Scientific	Oncomine TML	409	1.70

Panel Size cut off: 1.1 Mb

What is High TMB?

High TMB= > 10mut/mb

cancers

MDPI

Article

In-house Implementation of Tumor Mutational Burden Testing to Predict Durable Clinical Benefit in Non-small Cell Lung Cancer and Melanoma Patients

Simon Heeke [1,2,3,4], Jonathan Benzaquen [1,2,5], Elodie Long-Mira [1,2,3,4], Benoit Audelan [1,6], Virginie Lespinet [1,3], Olivier Bordone [1,3], Salomé Lalvée [1,3], Katia Zahaf [1,3], Michel Poudenx [1,7], Olivier Humbert [1,8], Henri Montaudié [1,4,9], Pierre-Michel Dugourd [1,9], Madleen Chassang [1,10], Thierry Passeron [1,4,9,11], Hervé Delingette [1,4,6], Charles-Hugo Marquette [1,2,3,4,5], Véronique Hofman [1,2,3,4], Albrecht Stenzinger [12,13], Marius Ilié [1,2,3,4] and Paul Hofman [1,2,3,4,*]

Tissue TMB

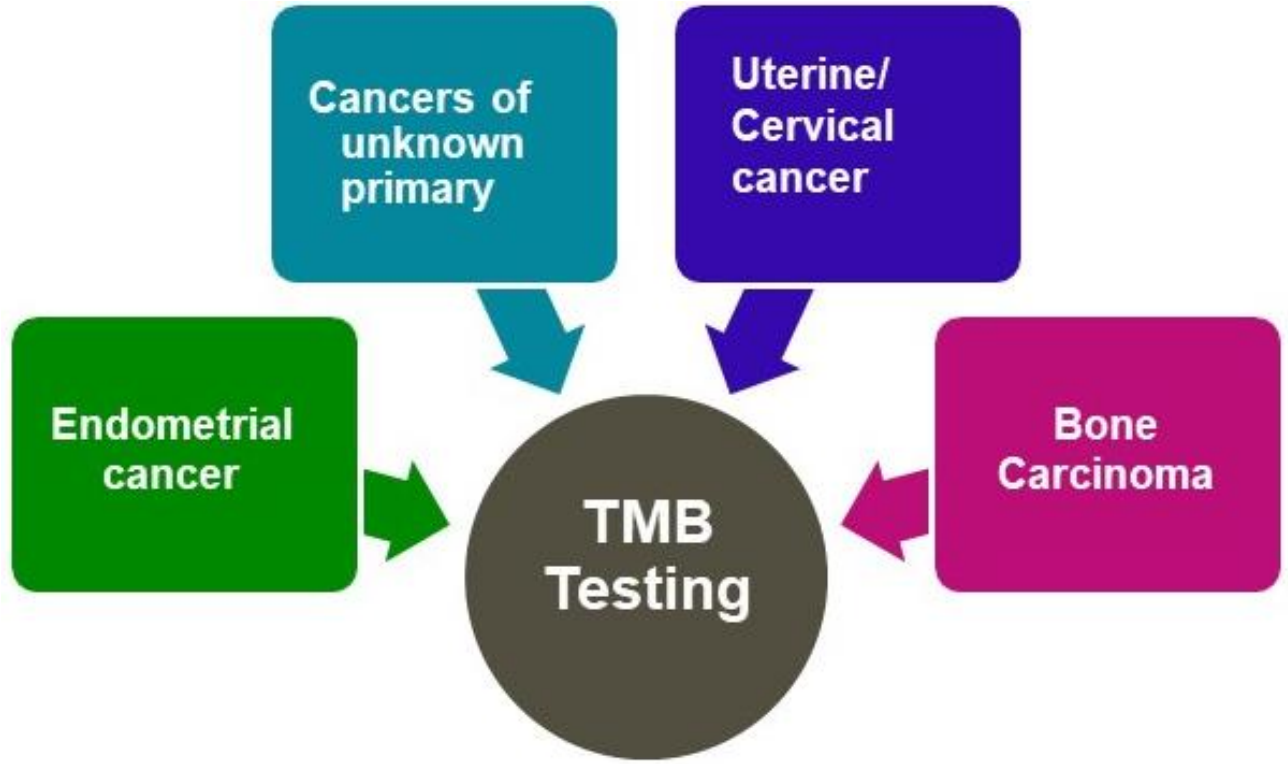
Oncomine TML panel Cutoff: 9.4 mut/Mb

F1Cdx panel Cutoff: 15 mut/mb

Cancers (2019) 11:1271

NCCN Guidelines Testing Recommendations

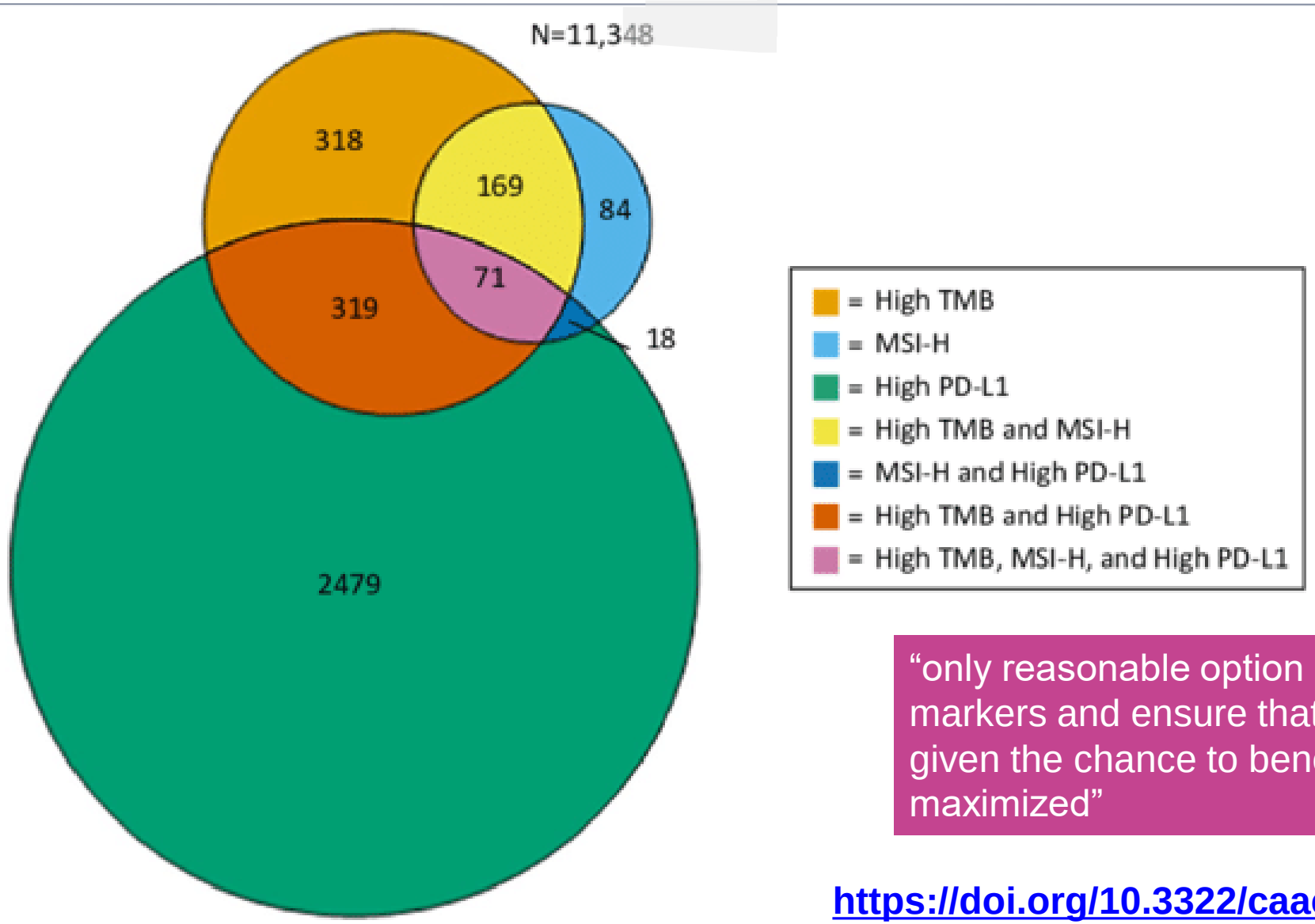
ESMO Precision Medicine Working Group & NCCN: TMB analysis using an FDA-approved test is recommended ahead of second-line treatment decisions for:



Solid Tumors; when no alternative treatment options, NCCN recommends immune checkpoint inhibition for patients with these **TMB-high** tumors.

https://www.360dx.com/sponsored/new-tmb-recommendations-help-guide-treatment-decisions-oncology

Relationships Between High-TMB, MSI-H and High PDL-1



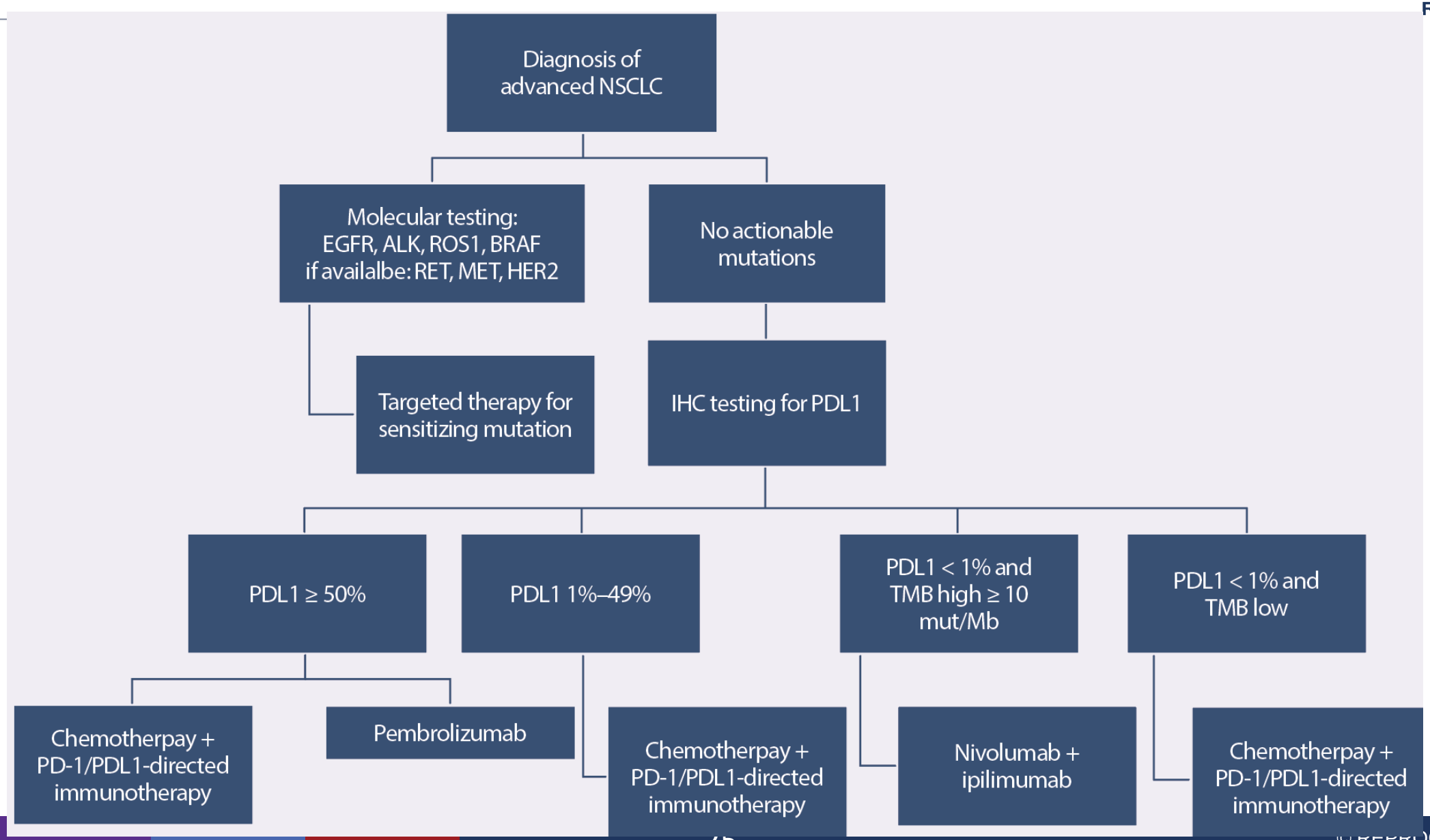
"only reasonable option is to continue to assay for all 3 markers and ensure that the number of patients who are given the chance to benefit from these drugs is maximized"

https://doi.org/10.3322/caac.21560

Applying Biomarker/ Target testing in Cancers

- **Lung Cancer**
- **Colorectal Cancer**

Treatment Algorithm for Non-Small Cell Lung Cancer



ESMO consensus guidelines CRC patient Management

Recommendation 4: RAS testing

- *RAS* mutational status is a negative predictive biomarker for therapeutic choices involving EGFR antibody therapies in the metastatic disease setting [I, A]
  - *RAS* testing should be carried out on all patients at the time of diagnosis of mCRC [I, A]
- *RAS* testing is mandatory before treatment with the EGFR-targeted monoclonal antibodies cetuximab and panitumumab [I, A]
- A network of arrangements should be established to ensure the rapid and robust transit of tissue samples from referral centres to testing laboratories, to minimise the turnaround time and avoid delays in having this information available for all patients with mCRC
- Primary or metastatic colorectal tumour tissue can be used for *RAS* testing (see also *Recommendation 3*)
- *RAS* analysis should include at least *KRAS* exons 2, 3 and 4 (codons 12, 13, 59, 61, 117 and 146) and *NRAS* exons 2, 3 and 4 (codons 12, 13, 59, 61 and 117)

Recommendation 5: BRAF testing

- Tumour *BRAF* mutation status should be assessed alongside the assessment of tumour *RAS* mutational status for prognostic assessment (and/or potential selection for clinical trials) [I, B]

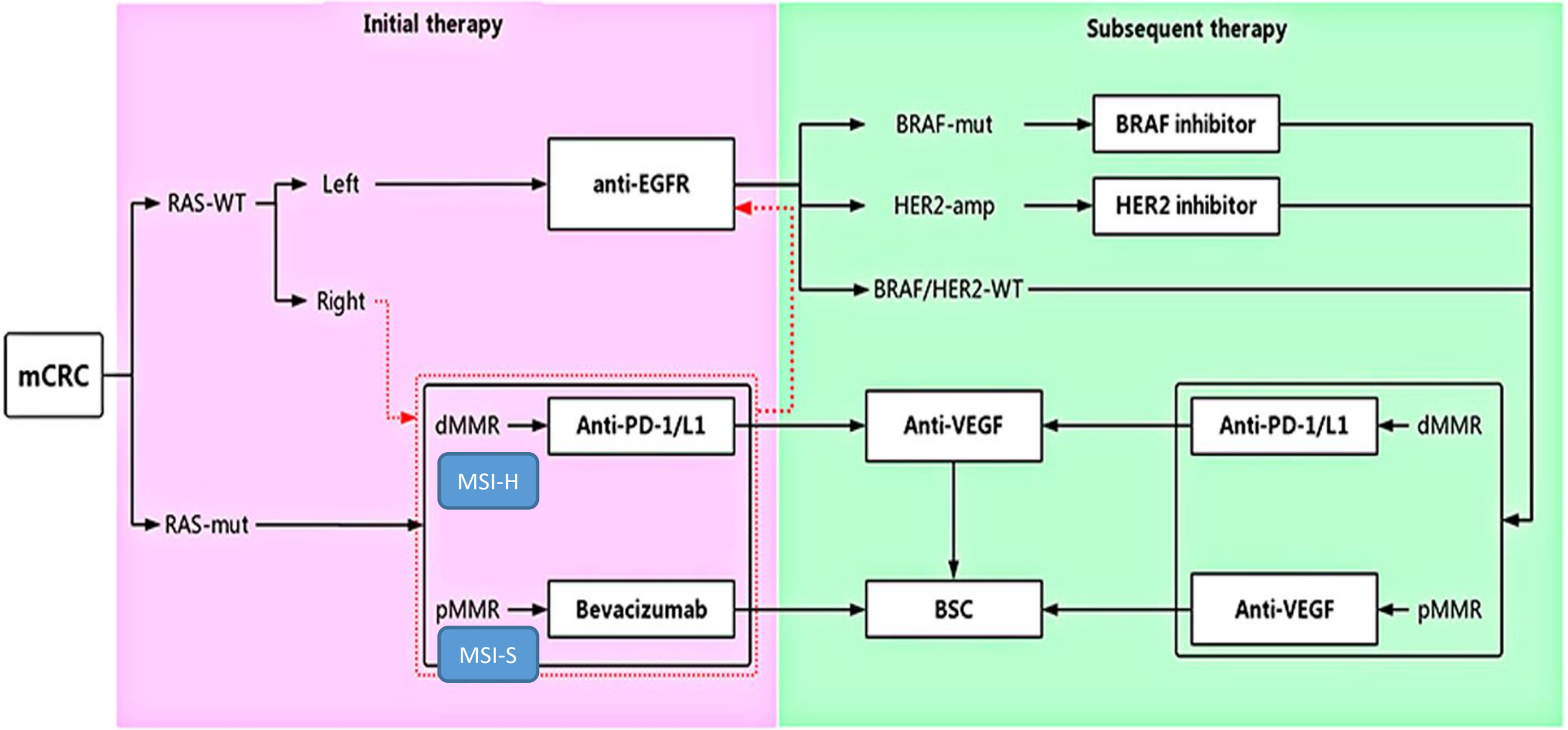
Recommendation 6: Microsatellite instability testing

- MSI testing in the metastatic disease setting can assist clinicians in genetic counselling [II, B]
- MSI testing has strong predictive value for the use of immune check-point inhibitors in the treatment of patients with mCRC [II, B]

Recommendation 7: Biomarkers of chemotherapy sensitivity and toxicity

- DPD testing before 5-FU administration remains an option but is not routinely recommended [II, D]
- UGT1A1 phenotyping remains an option and should be carried out in patients with a suspicion of UGT1A1 deficiency as reflected by low conjugated bilirubin and in patients where an irinotecan dose of >180 mg/m^2 per administration is planned [95] [III, C]

NCCN-recommended strategy for m-Colorectal cancer targeted therapy



https://www.nature.com/articles/s41392-020-0116-z/figures/4

ASCO treatment guideline Late-Stage CRC

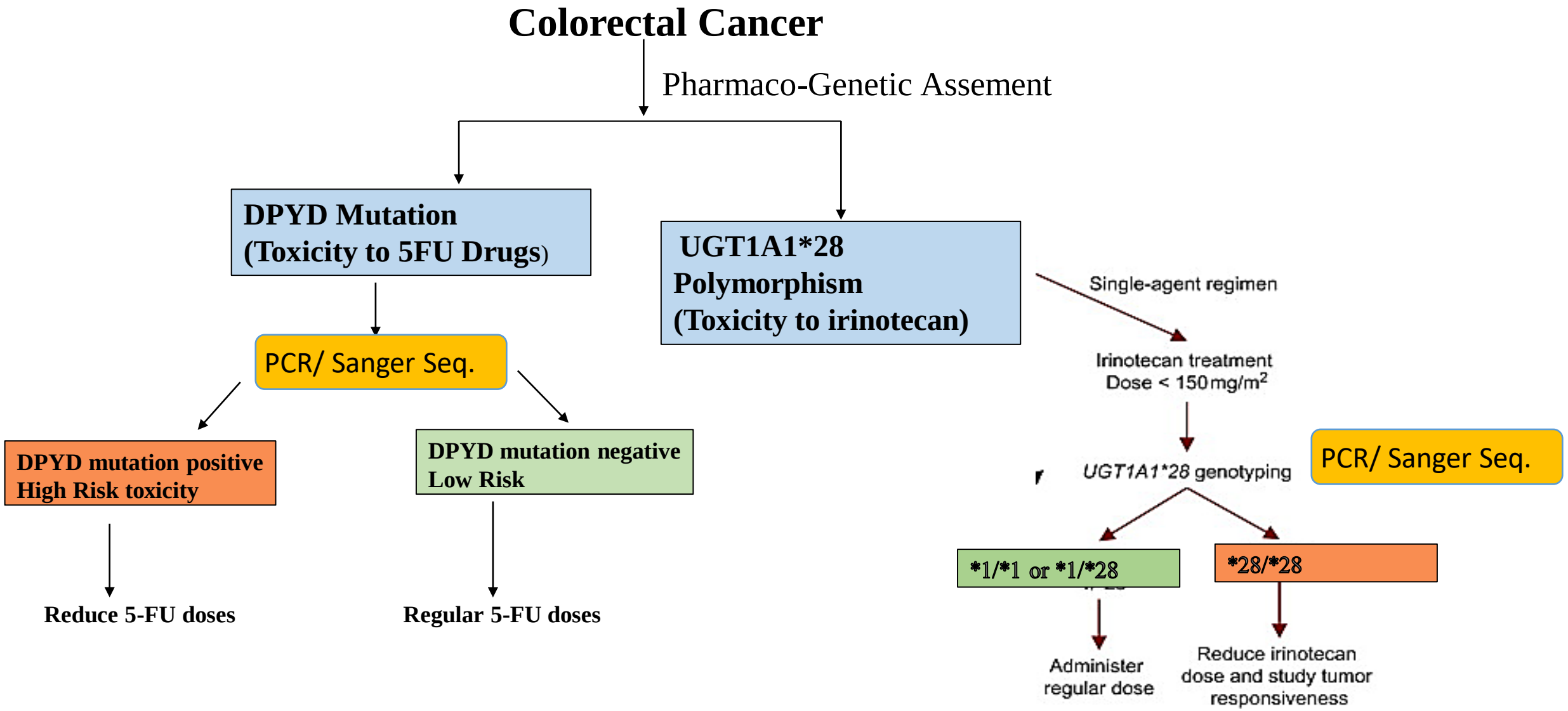
Rec	Population	ASCO Resource Levels: Maximal	Strength of Recommendation
First-Line Treatment			
2.8	Any *RAS* status and dMMR or MSI-H and patients not candidates for intensive chemotherapy	Immune checkpoint inhibitors[a]	Moderate
Recommendations on Second-Line Systemic Colorectal Metastatic Treatment			
3.7	dMMR or MSI-high	Immune checkpoint inhibitors (if not previously given)	Moderate
Recommendations on Third-Line and Fourth-Line Systemic Colorectal Metastatic Treatment			
4.3	dMMR/MSI-H	Immune checkpoint inhibitors (if not previously given)	Moderate

[a]Qualifying statement for first-line immunotherapy: At the time of this writing, the US Food and Drug Administration had not approved the use of immune checkpoint inhibitors (eg, single-agent pembrolizumab or nivolumab or the combination of nivolumab plus ipilimumab) in first-line treatment of patients with mCRC.

MED--US-6908 v7.0

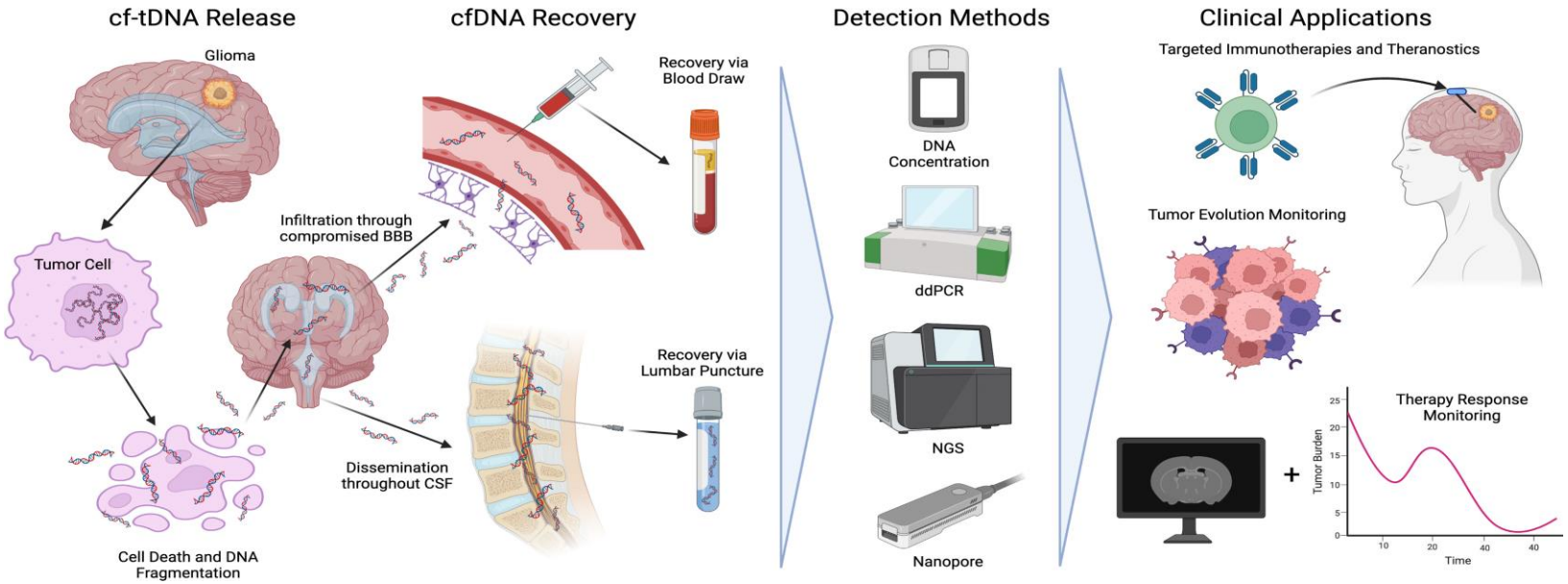
Chiorean EG, Nandakumar G, Fadelu T, et al. *JCO Global Oncol*. 2020;6:414-438.

Biomarkers for CT sensitivity/ Toxicity- CRC



Future Directions

- **Liquid Biopsy for Biomarker Detection and residual Disease monitoring**



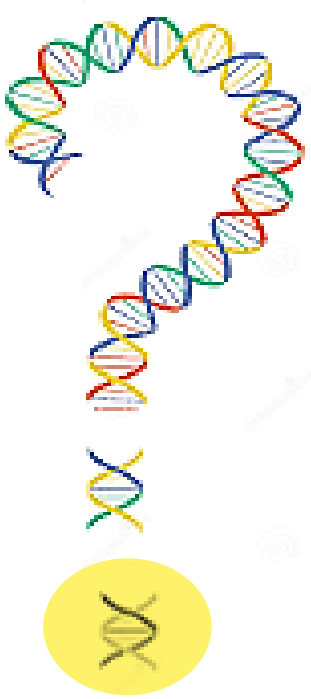
- **Gut microbiome- predict immune therapy response**
  - gut microbiota influences anti-tumor immunity
  - thereby impacting the clinical responses and outcomes of the patients receiving cancer immunotherapy
  - FMT in combination with checkpoint inhibitors are able to reprogram the tumor microenvironment and activate host immunity with favorable changes in immune cell infiltrates in patients with prostate cancer, melanoma, gastrointestinal and prostate cancer.
  - NCT04758507, NCT03353402, NCT04130763, NCT05094167

Bioserve: Biomarker Testing

Marker	Cancer	Technique
EGFR, KRAS,NRAS, BRAF	LUNG, CRC, MELANOMA	Sanger sequencing, RT-PCR
Met Exon 14 skipping	Lung	PCR
RET/ ROS/ ALK/NTRK FUSIONS	LUNG, CRC	FISH, NGS
BRCA 1/ 2 MUTATIONS	BREAST	NGS
PIK3CA MUTATIONS	BREAST	Sanger Sequencing
IDH1/ 2 MUTATIONS	GIOMA, AML	Sanger Sequencing
KIT MUTATIONS	GIST	Sanger Sequencing
BCR/ABL TRANSCRIPT/FUSION	CML	FISH, RT-PCR
JAK2 MUTAIONS	MPN	Sanger Sequencing
TUMOUR MUTATION BURDEN	**SOLID TUMORS**	**NGS**

Marker	Cancer	Technique
PAN-CANCER LIQUID BIOPSY PANEL	**PAN CANCER**	**NGS**
CEBPA, NPM1, FLT3 mutations	AML	Sanger sequencing, RT-PCR
TERT mutation	Melanoma	Sanger Sequencing
Inherited/ Hereditary Cancer	**PAN Cancer**	**NGS**
IMANITIB RESISTANCE	CML	Sanger Sequencing
HER 2 AMPLIFICATION	BREAST, CRC	FISH, RT-PCR
MGMT METHYLATION ASSAY	GLIOMA	RT-PCR
CALR, MPL MUTATIONS	MPN	Sanger Sequencing
Comprehensive genomic profiling	Solid tumors	NGS
MSI	**CRC, LUNG, BREAST**	**PCR+ Fragment Analysis**

Thank You!



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