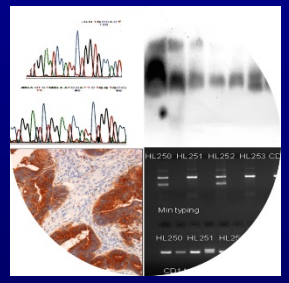
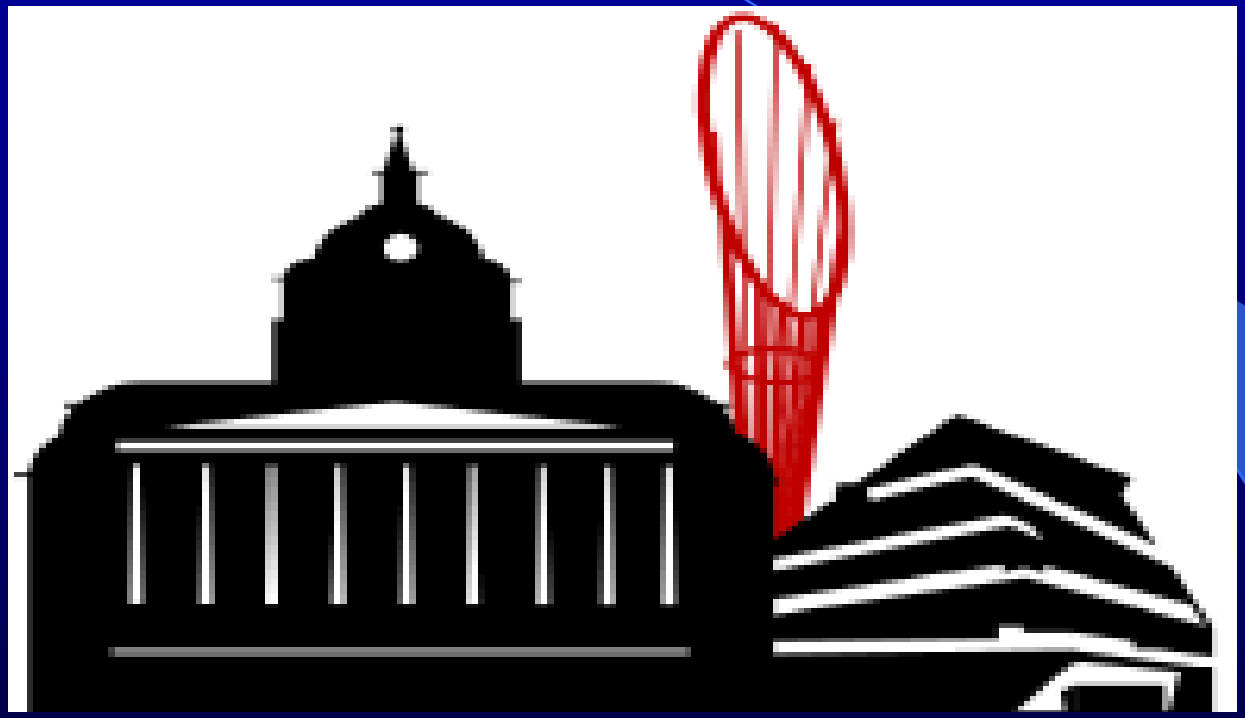
Practical Molecular Pathology of the lower GI tract





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Transforming the Pathology Landscape

Mohammad Ilyas

Speaker Declarations

Name of Speaker: Mohammad Ilyas

This presenter has the following declarations of relationship with industry

- Personal payments/honoraria/fees: None
- Research grants: None
- Educational grants: None

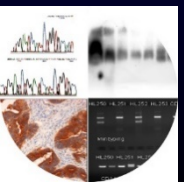
(sponsorship of **Molecular Diagnostics Training Course , Image Analysis Training Course, MSc projects**: Illumina, Roche, Merck, Thermofisher, Bristol Myers Squib, Sysmex, Biocartis, TissueGnostics, Source biosciences, Agilent)

- Travel grant or fellowship: None
- Equipment grant: None
- Sponsorship of fellow within department: None

[23rd November 2018]

Overview

- Ras testing
- Testing for Lynch Syndrome
- Loss of Mismatch Repair (MMR) function and leads to microsatellite instability (MSI)
- IHC versus PCR for loss of MMR function
- An algorithm for Lynch Syndrome screening
- Clinical implications of dMMR



Enough of the doom-mongering!

Genomics england

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Home > News > Posts > Secretary of State for Health and Social Care announces ambition to sequence 5 million genomes within five years

Secretary of State for Health and Social Care announces ambition to sequence 5 million genomes within five years

Posted on October 2, 2018 at 5:00 pm

Secretary of State for Health and Social Care, the Rt Hon Matt Hancock MP, today set out an ambitious vision for genomic medicine in the NHS – with plans to sequence 5 million genomes over the next five years.

The announcement, made as part of the Secretary of State's speech to the Conservative Party Conference in Birmingham, recognises the critical importance of genomic medicine to the future of the NHS. Mr Hancock announced:

- Expansion of the 100,000 Genomes Project to see 1 million

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- Sir John Chisholm · Surveys
- Vivienne Parry

Ras testing

Journal of
Clinical Pathology

Best practice

RAS testing of colorectal carcinoma—a guidance document from the Association of Clinical Pathologists Molecular Pathology and Diagnostics Group FREE

Newton ACS Wong[1], David Gonzalez[2], Manuel Salto-Tellez[3], Rachel Butler[4], Salvador J Diaz-Cano[5], Mohammad Ilyas[6], William Newman[7], Emily Shaw[8], Philippe Taniere[9], Shaun V Walsh[10]

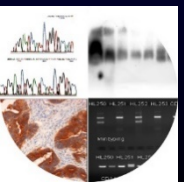
Author affiliations +

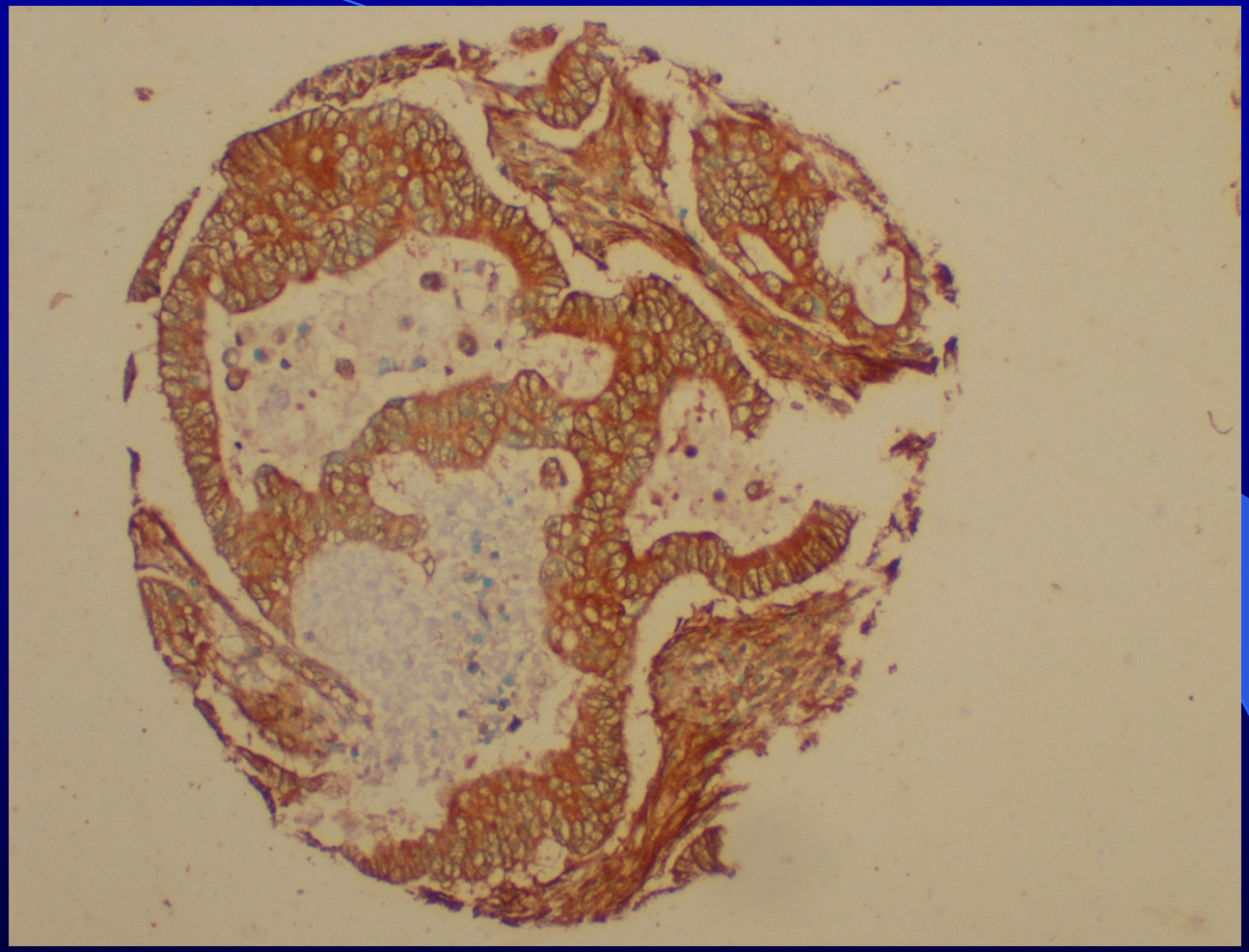
Abstract

Analysis of colorectal carcinoma (CRC) tissue for *KRAS* codon 12 or 13 mutations to guide use of anti-epidermal growth factor receptor (EGFR) therapy is now considered mandatory in the UK. The scope of this practice has been recently extended because of data indicating that *NRAS* mutations and additional *KRAS* mutations also predict for poor response to anti-EGFR therapy. The following document provides guidance on RAS (i.e., *KRAS* and *NRAS*) testing of CRC tissue in the setting of personalised medicine within the UK and particularly within the NHS. This guidance covers issues related to case selection, preanalytical aspects, analysis and interpretation of such RAS testing.

Ras testing: the role of the pathologist

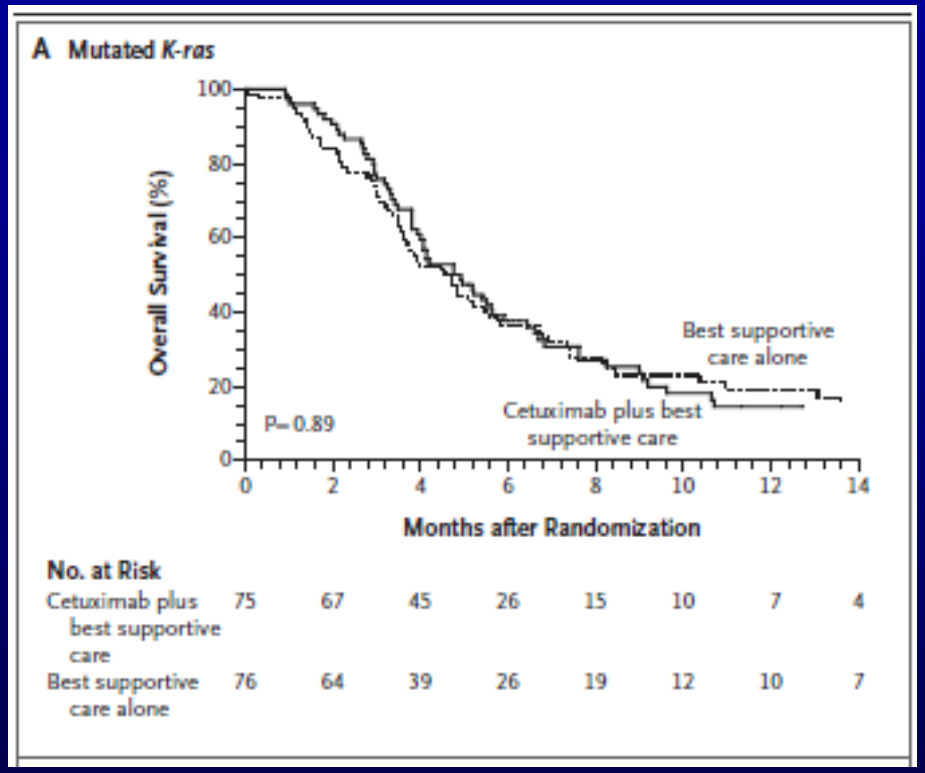
- Assess tumour burden in tissue sections
- (Evaluate the data)
- (Evaluate the results)
- Make intelligent comment on the results at the MDT

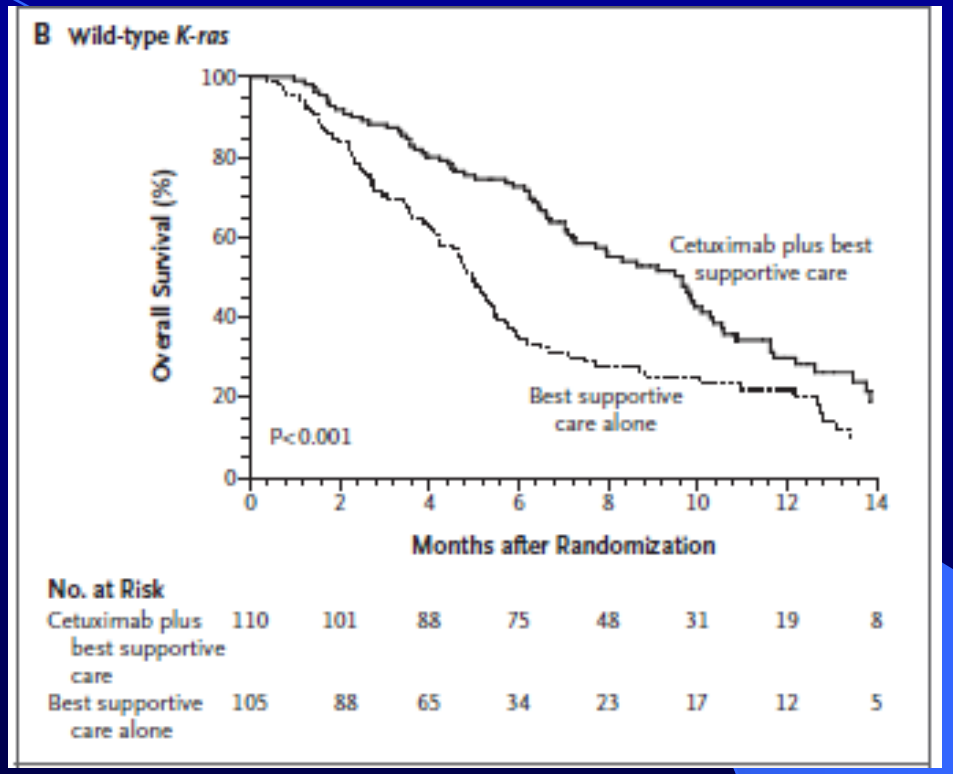




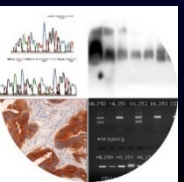
EGFR IHC

KRAS mutation in CRC

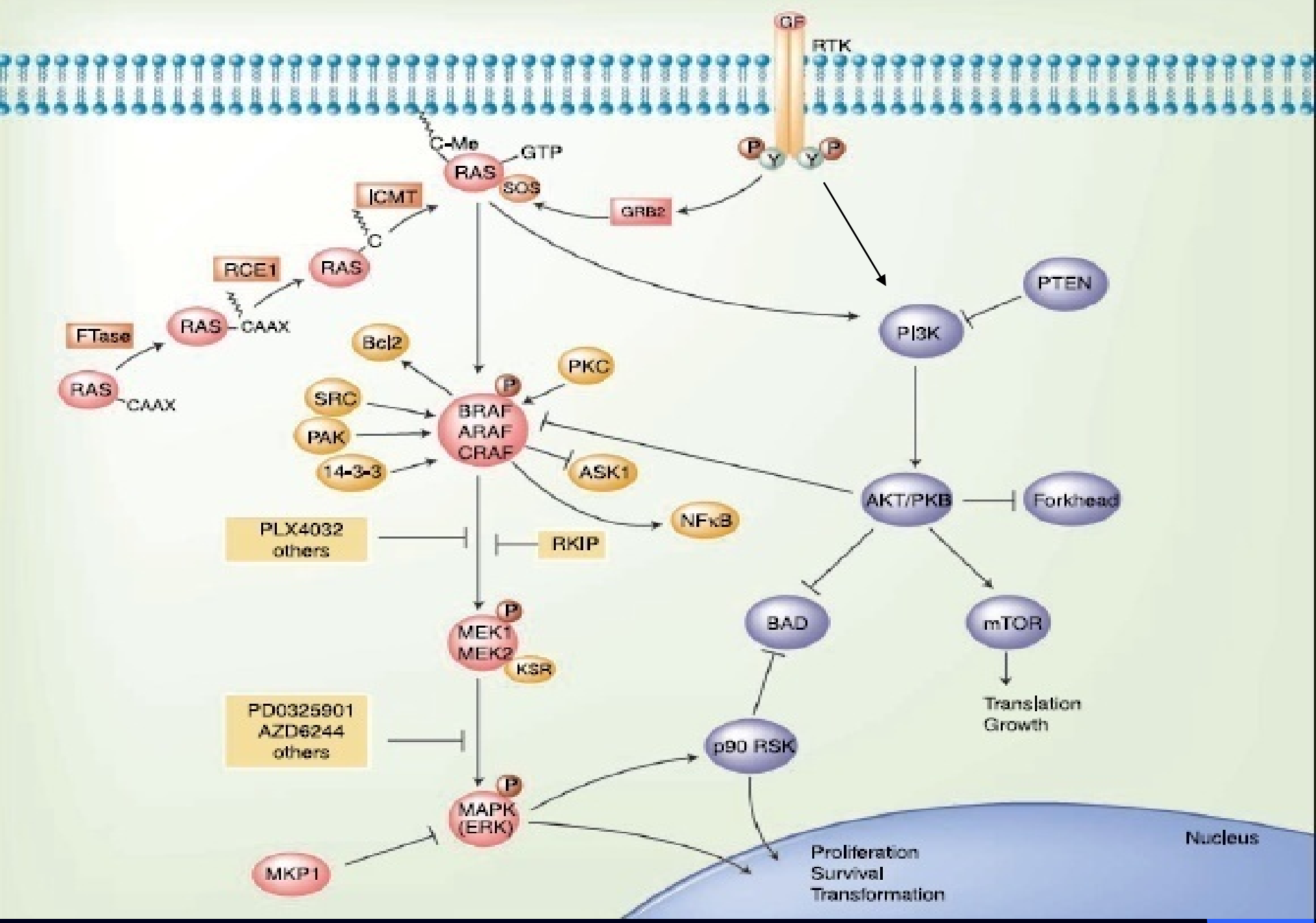




Patients whose tumours harbour a KRAS mutation will not respond to Cetuximab. Testing for KRAS mutation therefore stratifies patients into “treatment” and “non-treatment” groups



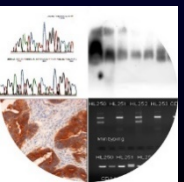
NMPN



EGFR signals through RAS/BRAF pathway

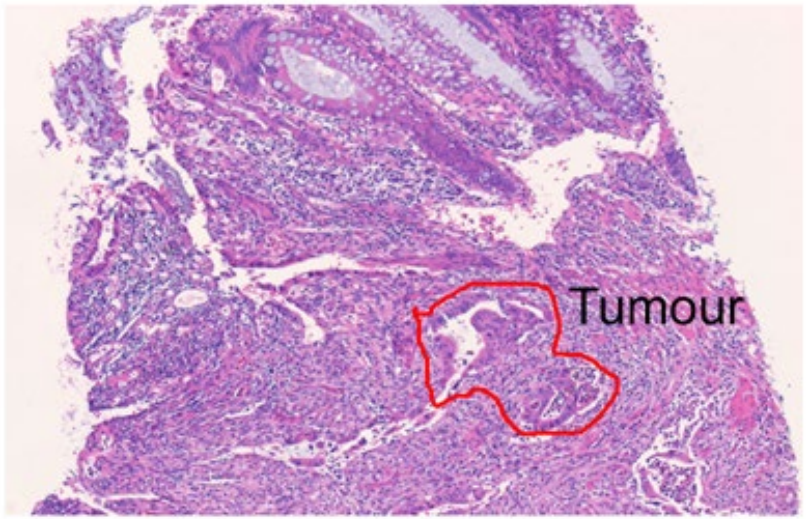
Ras testing

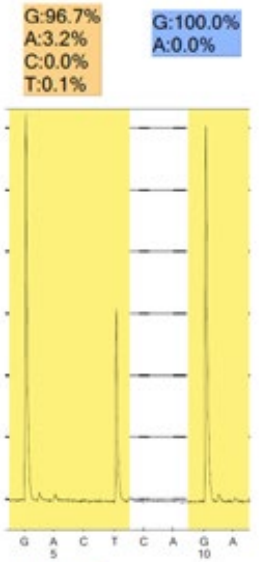
- Treatment with anti-EGFR biologics can improve survival in patients with CRC
- EGFR signals through the Ras/Raf signaling pathway
- Activation of this pathway through KRAS or BRAF mutation will negate the effect of the anti-EGFR treatment
- Ras testing is mandatory before administering anti-EGFR treatment

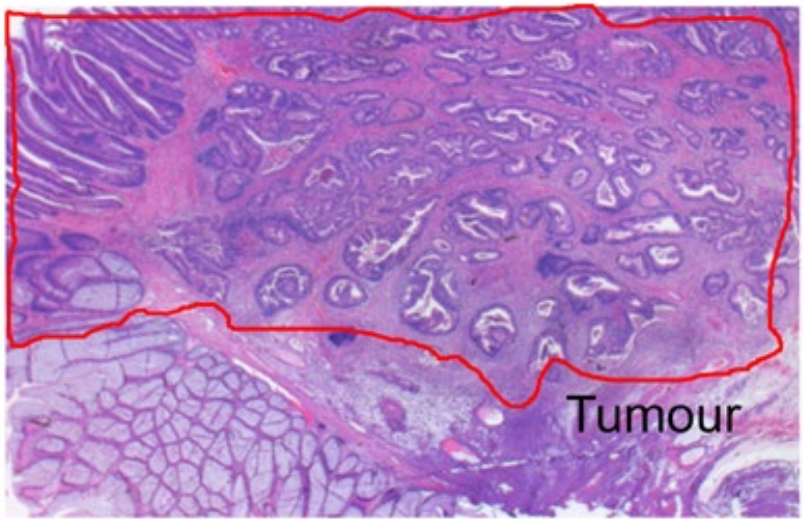


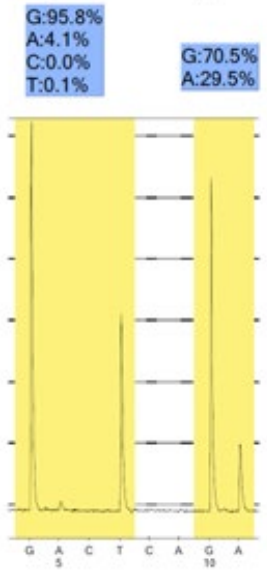
Ras testing: tumour burden

Figure 3



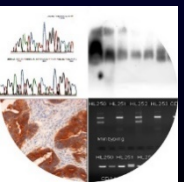






Ras testing: intelligent comments

- There was sufficient tumour present
- The limit of detection is 20% for Sanger Sequencing, 5% pyrosequencing and NGS
- There are co-incident KRAS and BRAF mutations – this is probably an artefact
- There is a discrepancy between tumour load and mutant allele frequency
- Although there is KRAS mutation, the site may mean it is still responsive
- Although it is wild-type, the profile suggests it will not respond



Testing for Lynch Syndrome

NICE National Institute for Health and Care Excellence

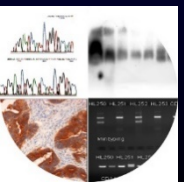
NICE guidance

Molecular testing strategies for Lynch syndrome in people with colorectal cancer

Diagnostics guidance

Published: TBC

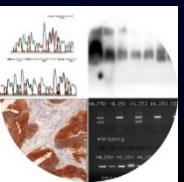
nice.org.uk/guidance/dg27



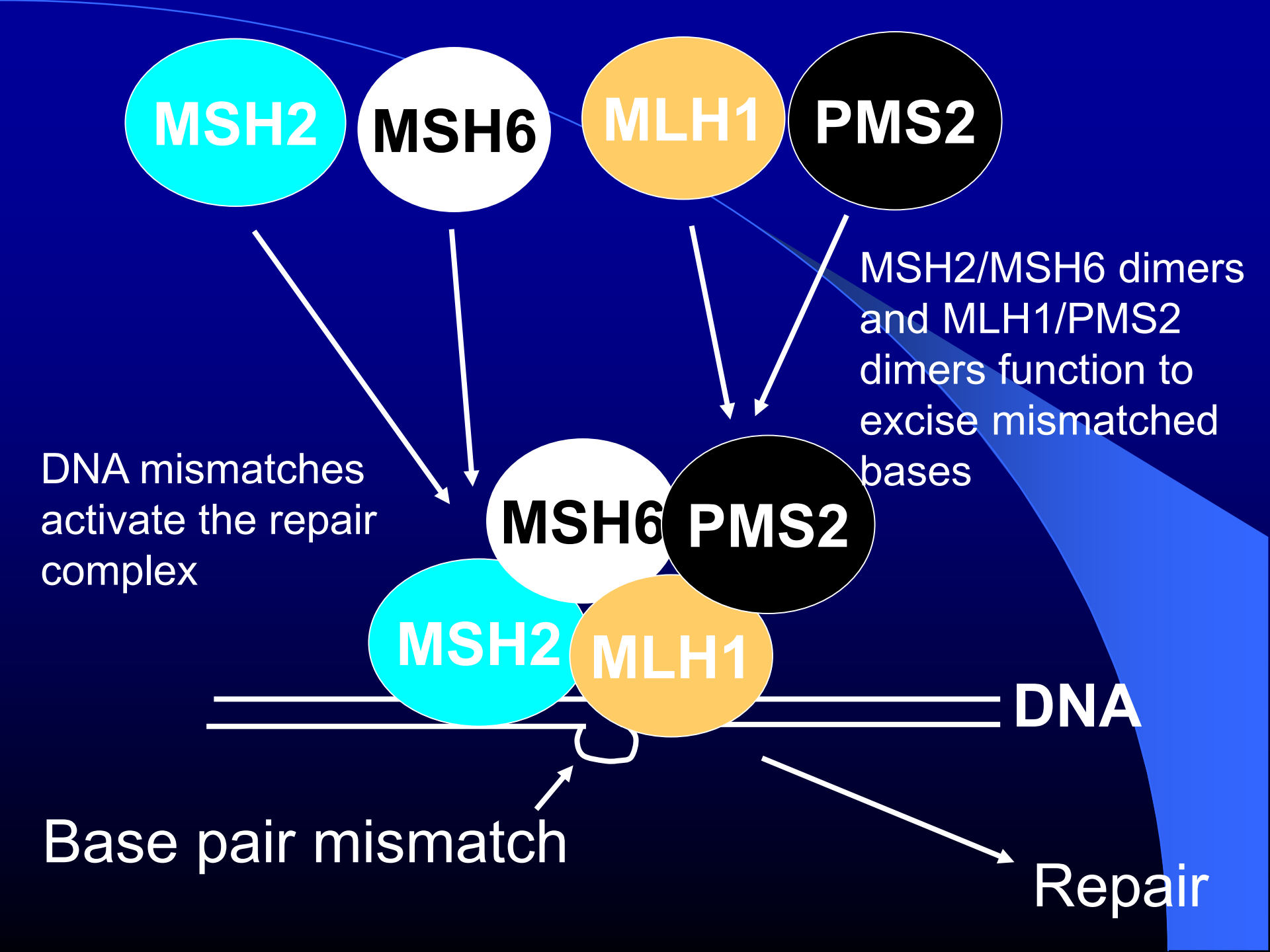
NMPN

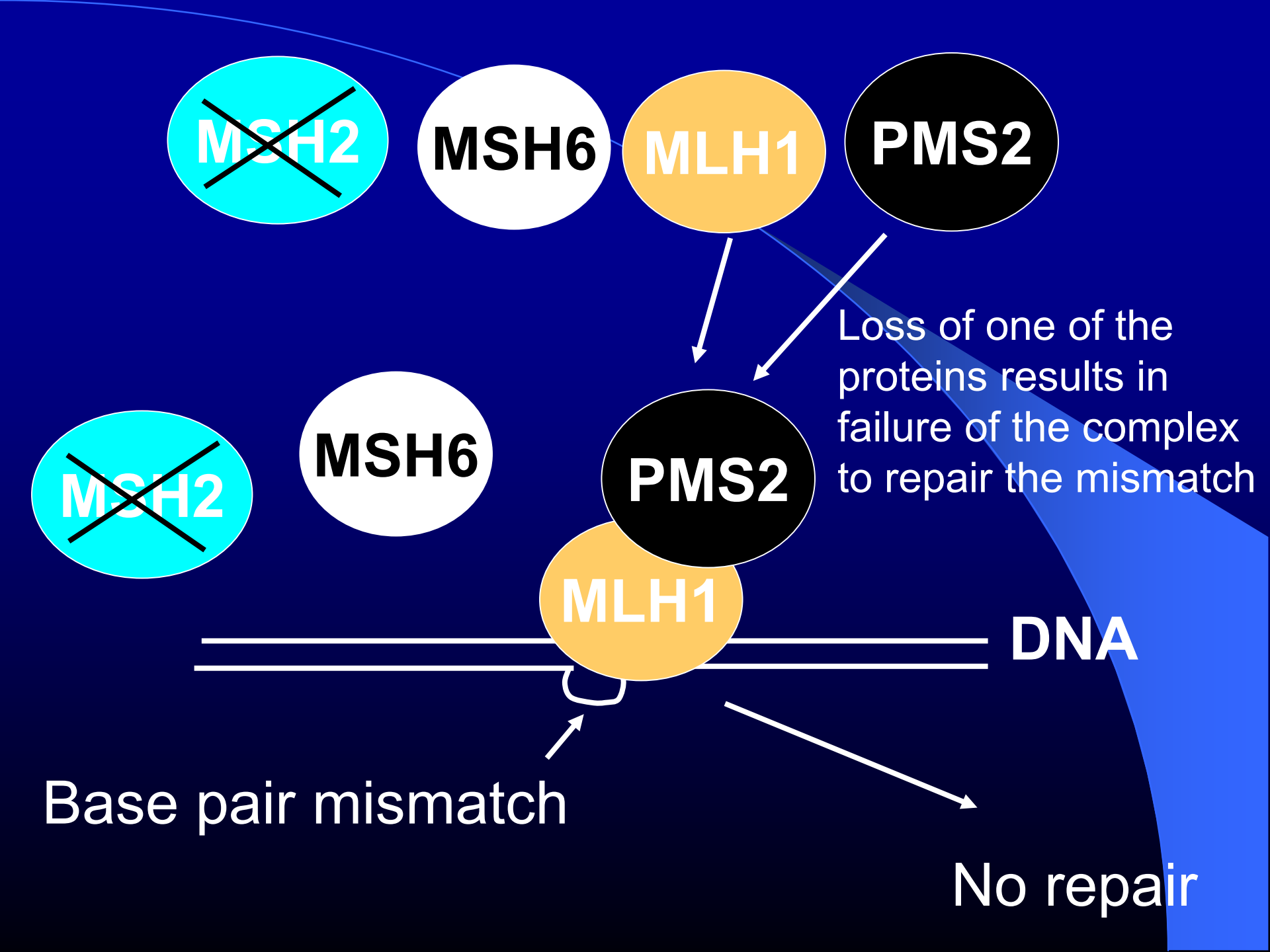
Lynch Syndrome

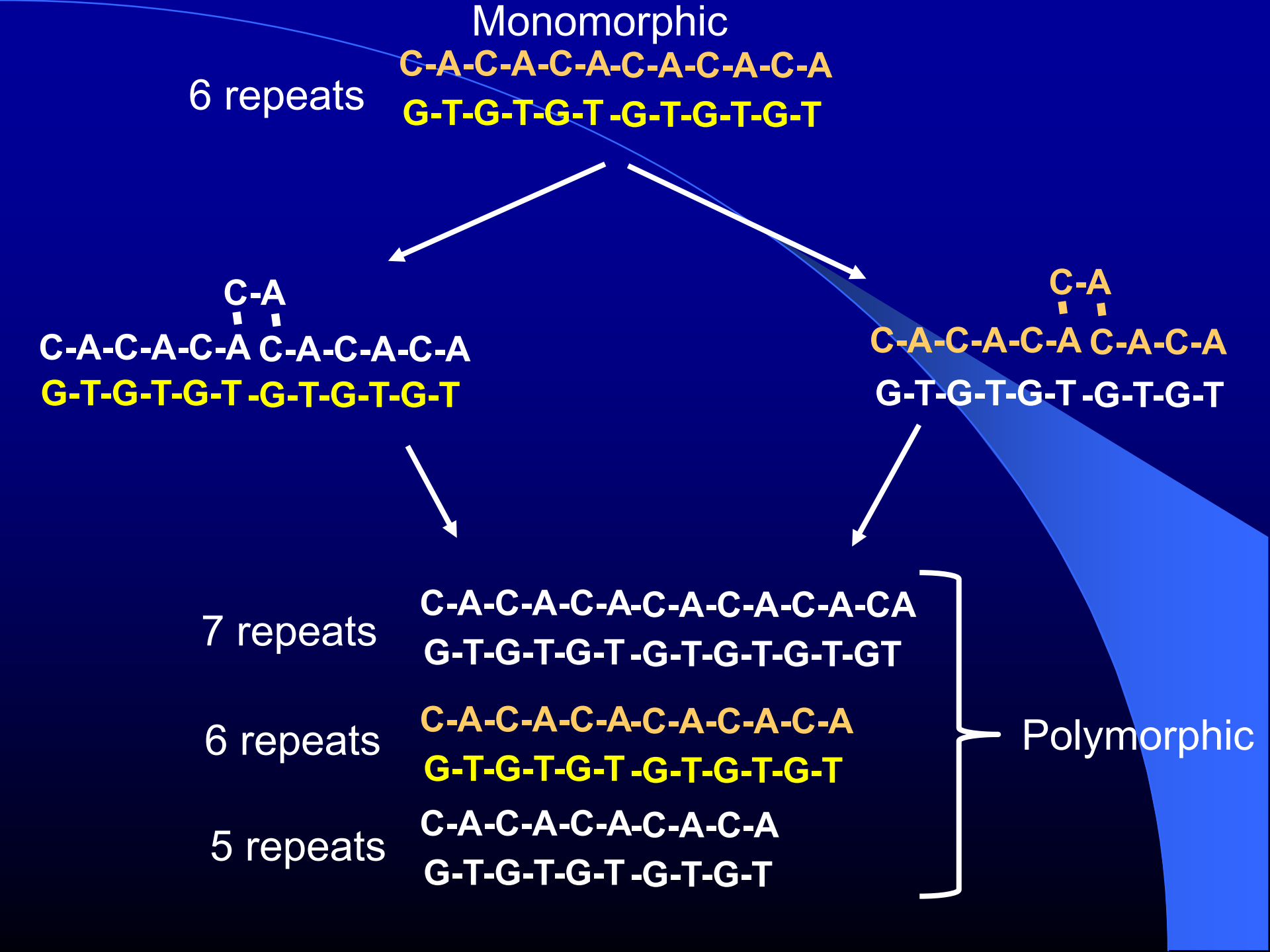
- Also called Hereditary Non-Polyposis Colorectal Cancer (HNPCC)
- Rapid development of polyps into cancer rather than increased polyp numbers
- Penetrance variable
- Due to mutation of any one of several mismatch repair genes: *MSH2, MLH1, PMS2, MSH6, (EpCAM)*




Mis-
Match
Repair

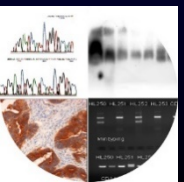

MSH2
MSH6
MLH1
PMS2
MSH2/MSH6 dimers and MLH1/PMS2 dimers function to excise mismatched bases
DNA mismatches activate the repair complex
MSH6
PMS2
MSH2
MLH1
DNA
Base pair mismatch
Repair


MSH2
MSH6
MLH1
PMS2
Loss of one of the proteins results in failure of the complex to repair the mismatch
MSH2
MSH6
PMS2
MLH1
DNA
Base pair mismatch
No repair


Monomorphic
6 repeats
C-A-C-A-C-A-C-A-C-A-C-A
G-T-G-T-G-T-G-T-G-T-G-T
C-A
C-A-C-A-C-A C-A-C-A-C-A
G-T-G-T-G-T-G-T-G-T-G-T
C-A
C-A-C-A-C-A C-A-C-A
G-T-G-T-G-T-G-T-G-T
7 repeats
C-A-C-A-C-A-C-A-C-A-C-A-CA
G-T-G-T-G-T-G-T-G-T-G-T-GT
6 repeats
C-A-C-A-C-A-C-A-C-A-C-A
G-T-G-T-G-T-G-T-G-T-G-T
5 repeats
C-A-C-A-C-A-C-A-C-A
G-T-G-T-G-T-G-T-G-T
Polymorphic

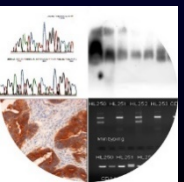
MMR function testing

- Loss of MMR proteins can result in loss of MMR function
- Loss of MMR function results in an *increased mutation rate*
- Microsatellites are highly prone to mutation
- Loss of MMR function can be tested either:
  - by IHC for loss of protein expression
  - by PCR for microsatellite instability

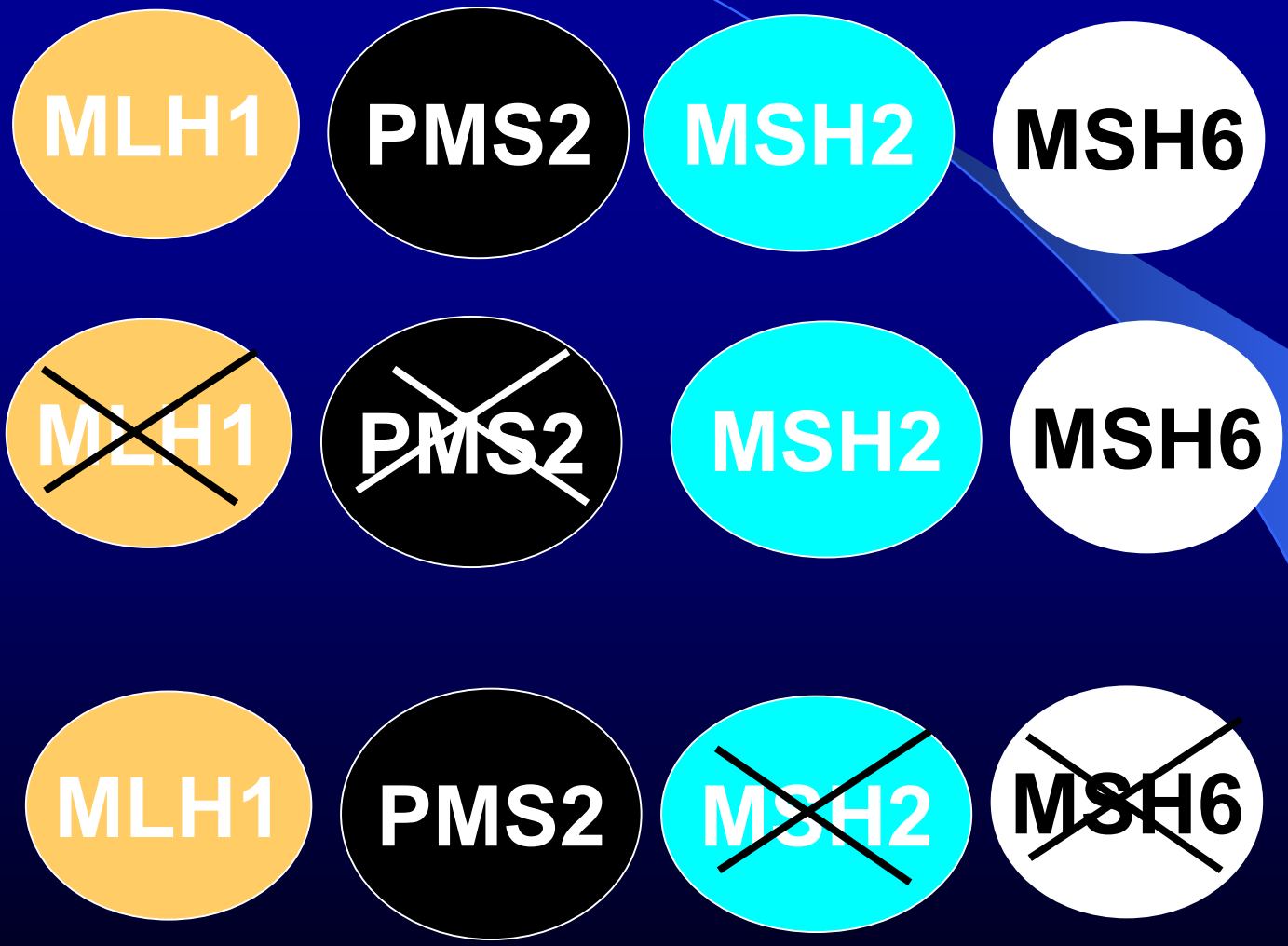


Immunostaining for MMR

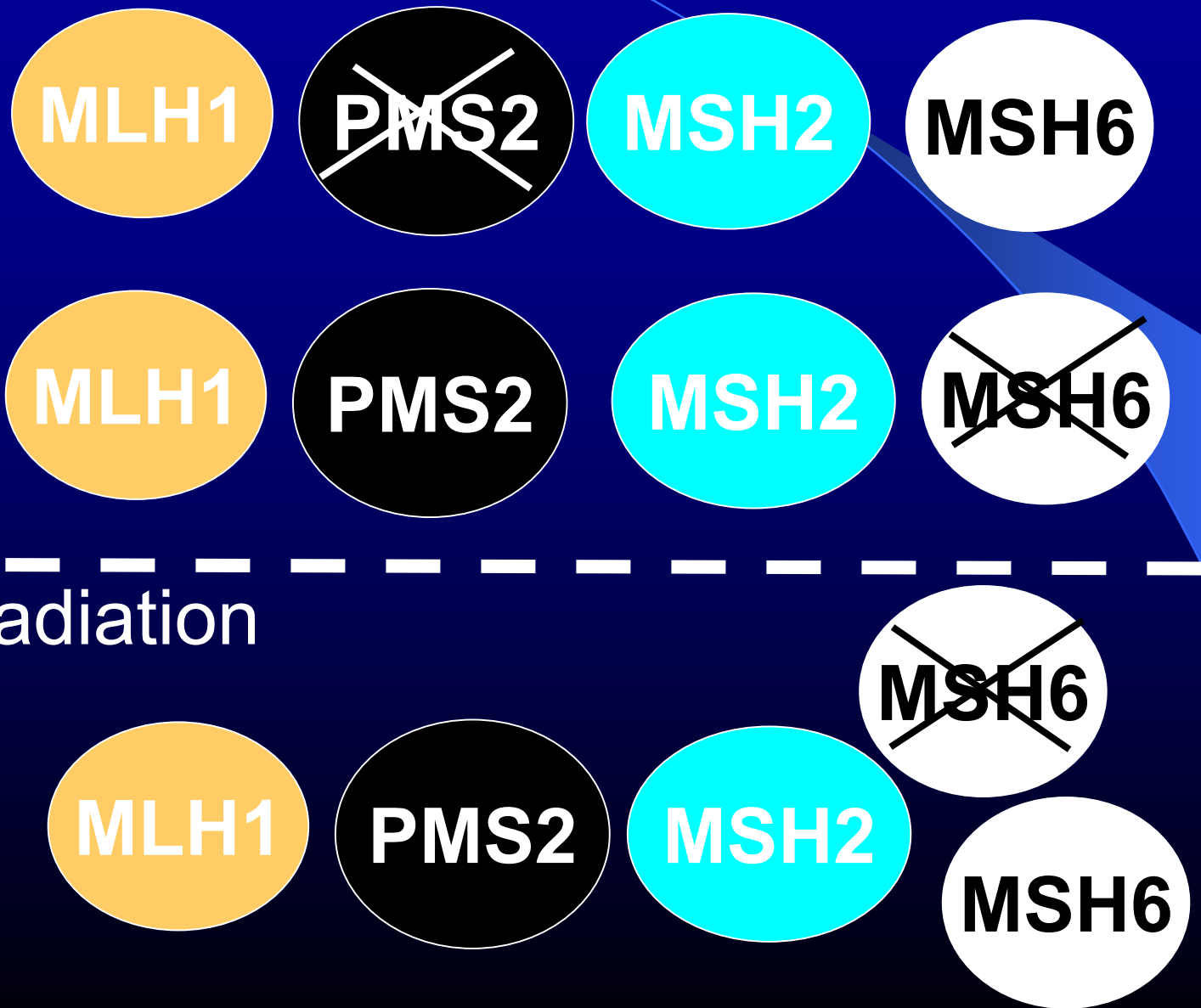
- Antibodies can be fixation sensitive (thus biopsies are better than whole WTS)
- Stromal cells act as internal positive controls
- Score only nuclear staining
- Specific patterns are seen: don’t forget the dimers!

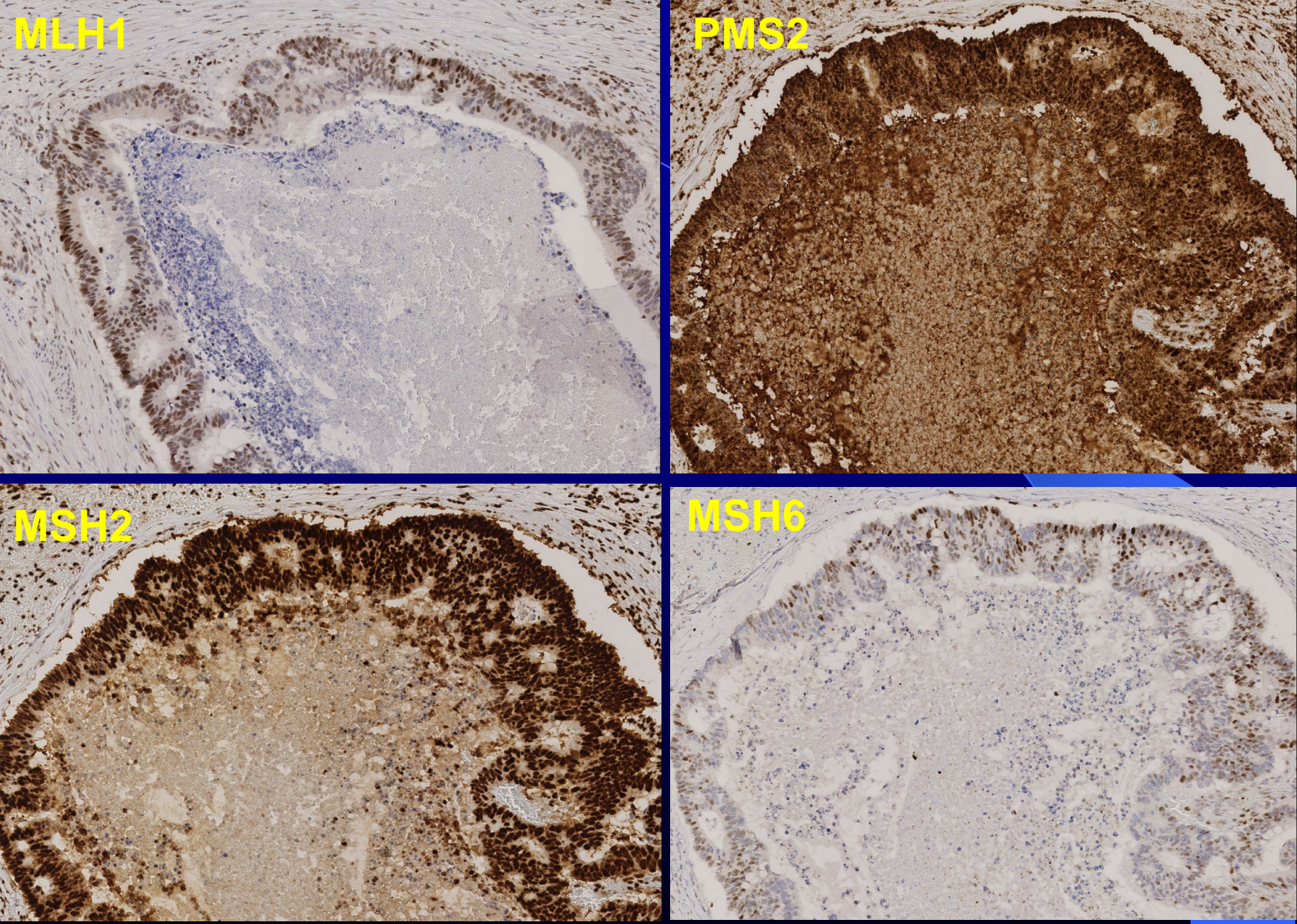


Immunostaining for MMR

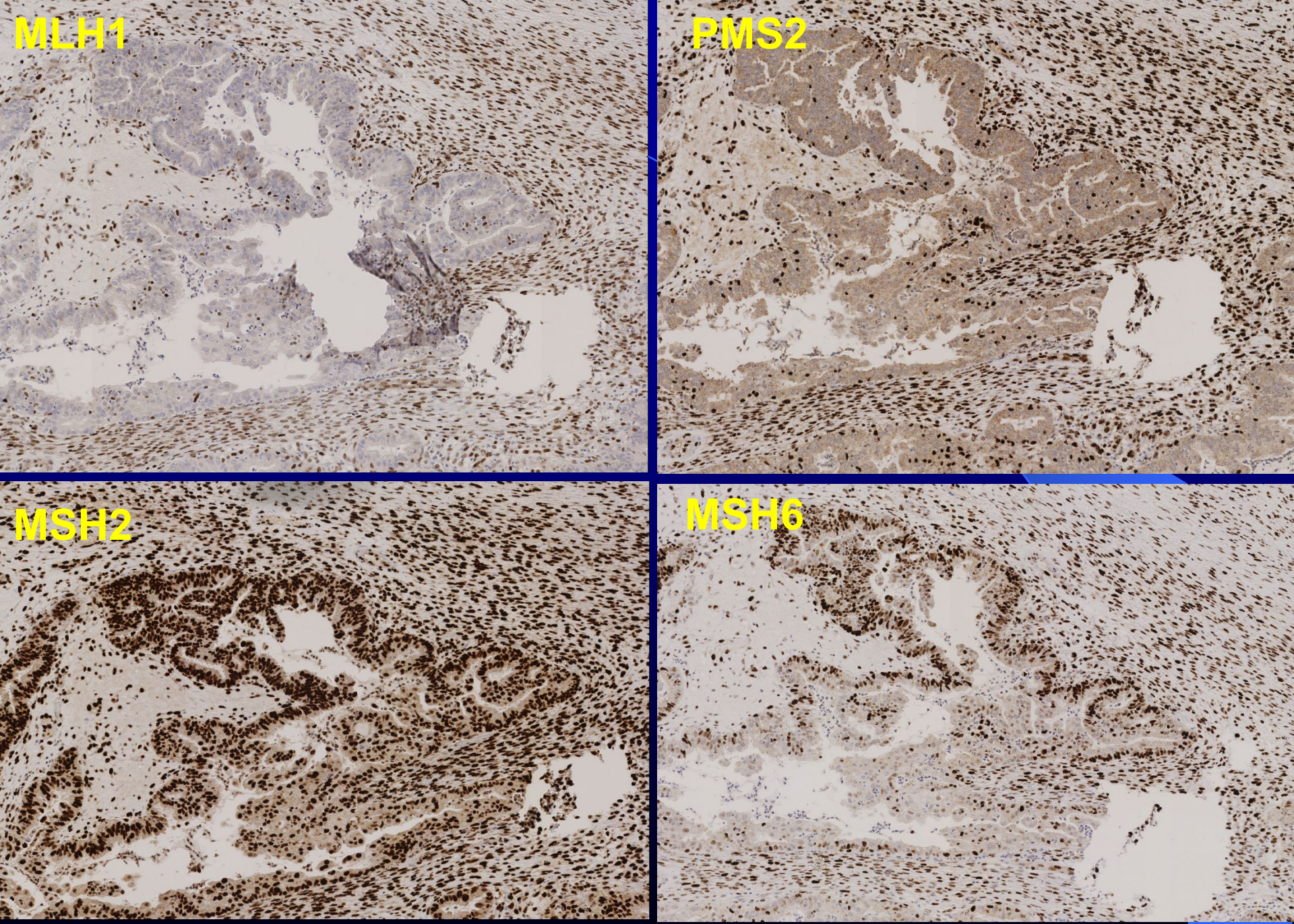


Immunostaining for MMR



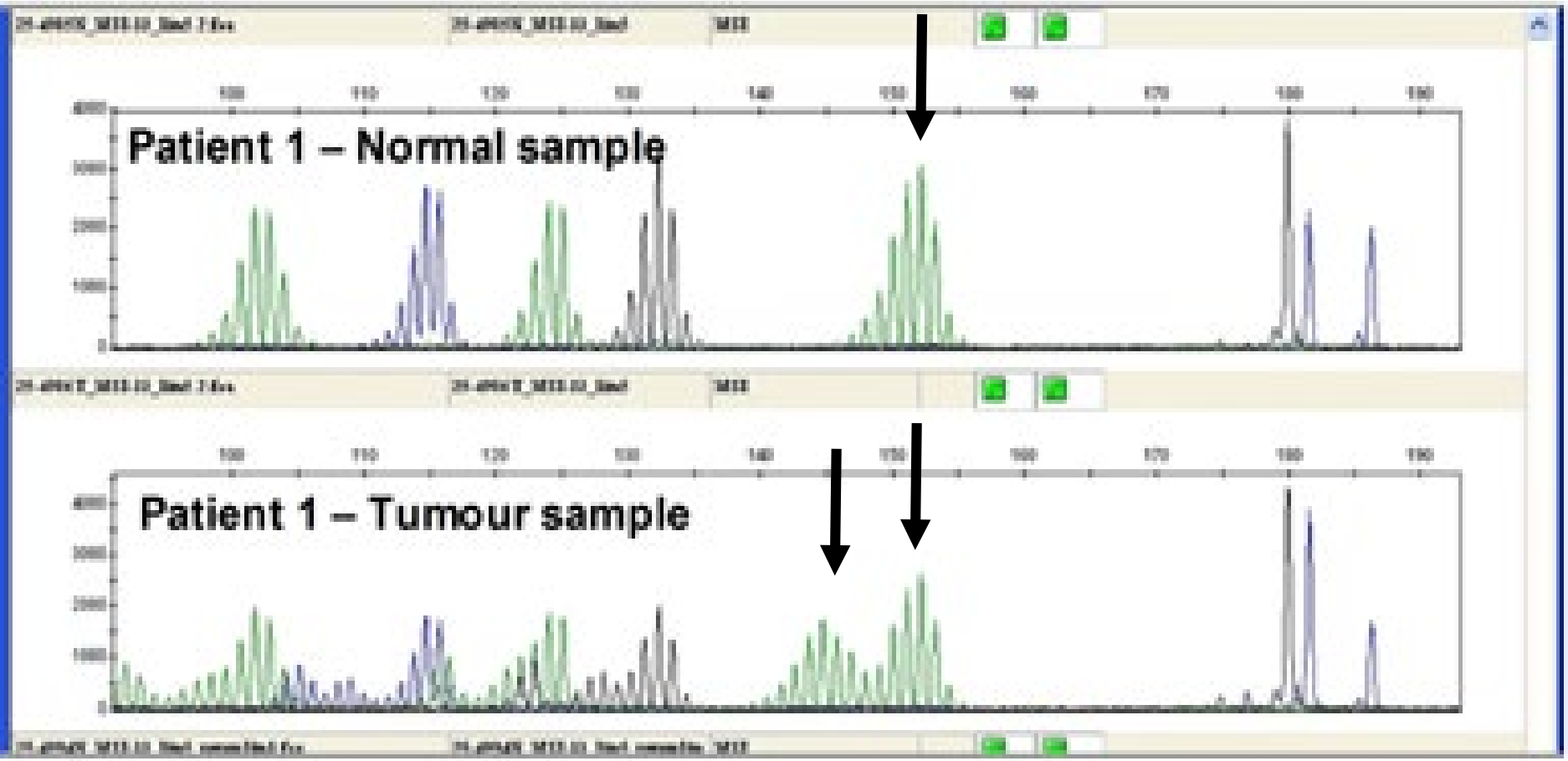


MMR proficient (pMMR)



MMR deficient (dMMR): MLH1/PMS2

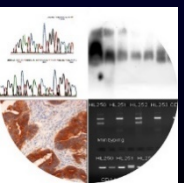
Figure 1



Microsatellite instability
Very good correlation with IHC

NICE guidance for CRC

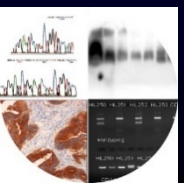
- NICE guidance is that *all* CRC should be tested for Lynch Syndrome
- Health economics modelling shows there will be benefit to society
- However, 10-15% of sporadic CRCs will also have loss of MMR or MSI
- These need to be discriminated from Lynch Syndrome



Separating syndromic and sporadic

- There are some molecular differences between Lynch Syndrome tumours and sporadic tumours with MSI:

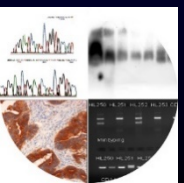
- MSH2 / PMS2 / MSH6 are rarely mutated in sporadic tumours
- BRAF mutations are rare in LS but occur in 40 – 70% of sporadic CRCs
- MLH1 promoter methylation almost never occurs in Lynch Syndrome



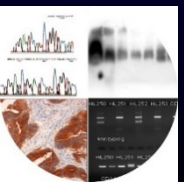
Separating syndromic and sporadic

- There are some molecular differences between Lynch Syndrome tumours and sporadic tumours with MSI:

- *CTNNB1* mutation only occurs in LS
- *RNF43* and *ZNRF3* mutations occur more frequently in sporadics (and possibly form part of the serrated pathway)

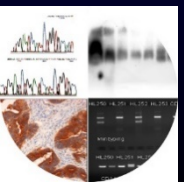


Screening for Lynch Syndrome



Clinical implications of dMMR

- Risk of Lynch Syndrome
- Good prognosis if there is no metastatic spread
- Poor prognosis if there is metastatic spread
- Resistance to 5FU and sensitivity to Irinotecan
- Sensitivity to Immunotherapy



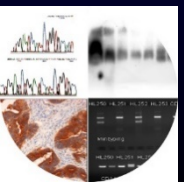
Immunotherapy

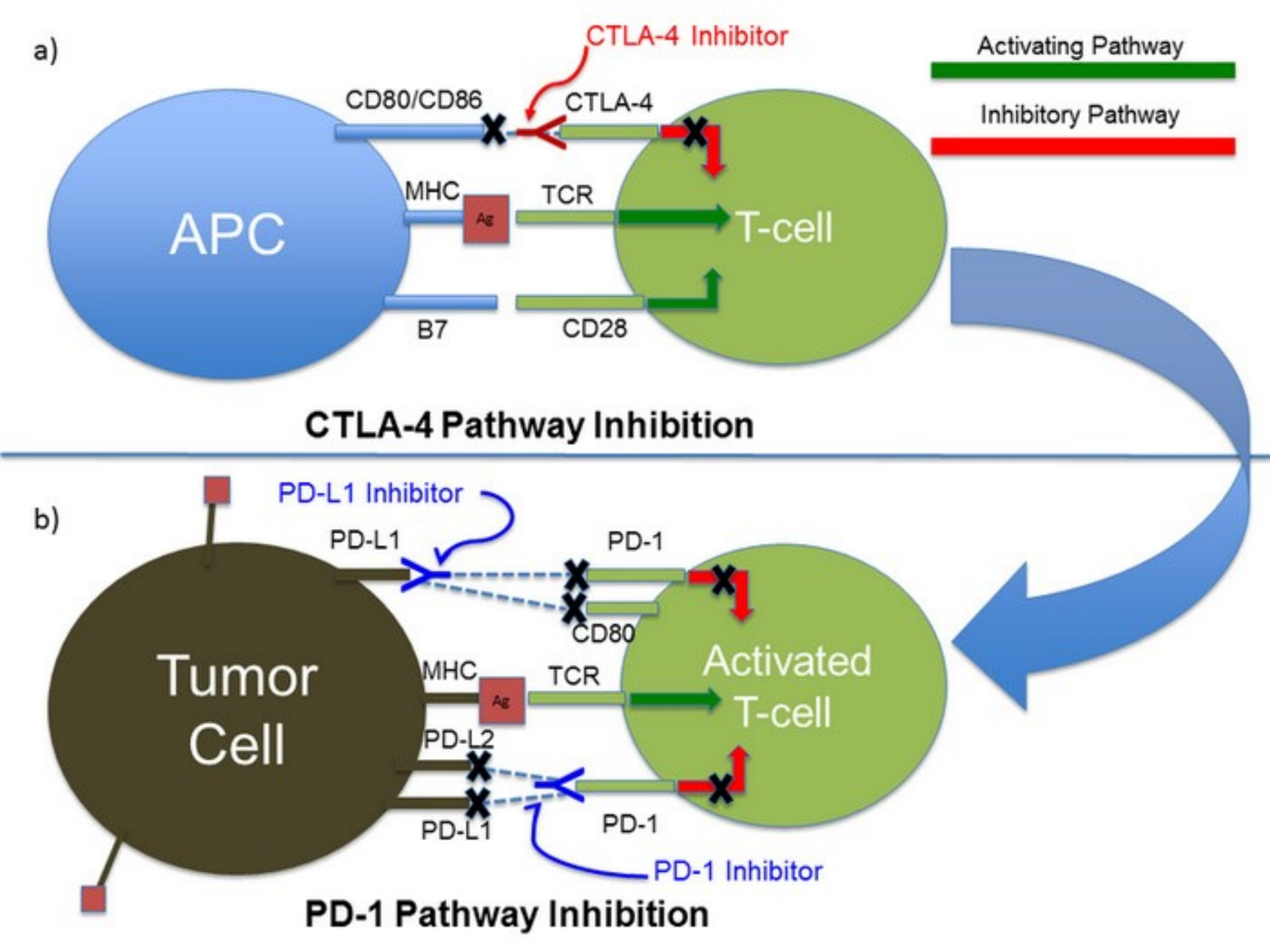
Immunotherapy

Immunotherapy

The rationale for immunotherapy

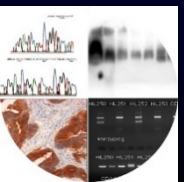
- Immunology is very complicated and nobody really understands it
- Immune response can be inhibited at several checkpoints to prevent auto-immunity
- There are two main targetable checkpoints:
  - Activation of T-cells by antigen presenting cells
  - T-cell mediated cytotoxicity

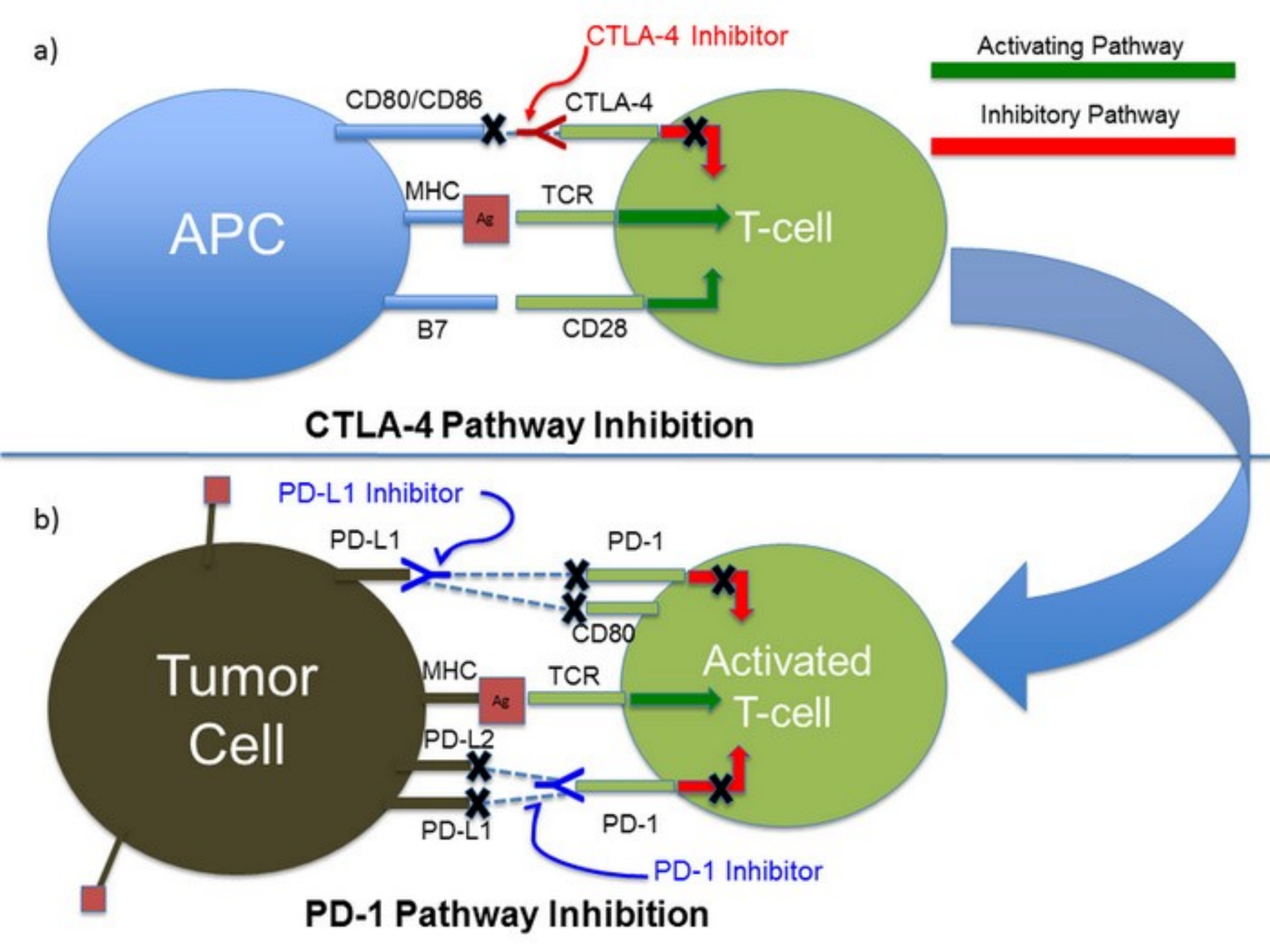



a)
CTLA-4 Inhibitor
Activating Pathway
Inhibitory Pathway
CD80/CD86
CTLA-4
APC
MHC
Ag
TCR
T-cell
B7
CD28
CTLA-4 Pathway Inhibition
b)
PD-L1 Inhibitor
PD-L1
PD-1
CD80
Tumor Cell
MHC
Ag
TCR
Activated T-cell
PD-L2
PD-L1
PD-1
PD-1 Inhibitor
PD-1 Pathway Inhibition

The rationale for immunotherapy

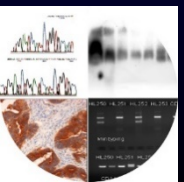
- T-cell activation and T-cell mediated cytotoxicity both require presentation of tumour antigens
- CD80/CTLA-4 interaction causes T-cell anergy
- PD-L1/PD1 interaction inhibits tumour cell killing
- The greater the antigenic diversity, the greater the likelihood of an immune response




a)
CTLA-4 Inhibitor
Activating Pathway
Inhibitory Pathway
CD80/CD86
CTLA-4
APC
MHC
Ag
TCR
T-cell
B7
CD28
CTLA-4 Pathway Inhibition
b)
PD-L1 Inhibitor
PD-L1
PD-1
CD80
Tumor Cell
MHC
Ag
TCR
Activated T-cell
PD-L2
PD-L1
PD-1
PD-1 Inhibitor
PD-1 Pathway Inhibition

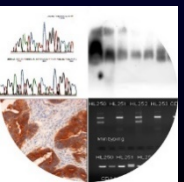
The rationale for immunotherapy

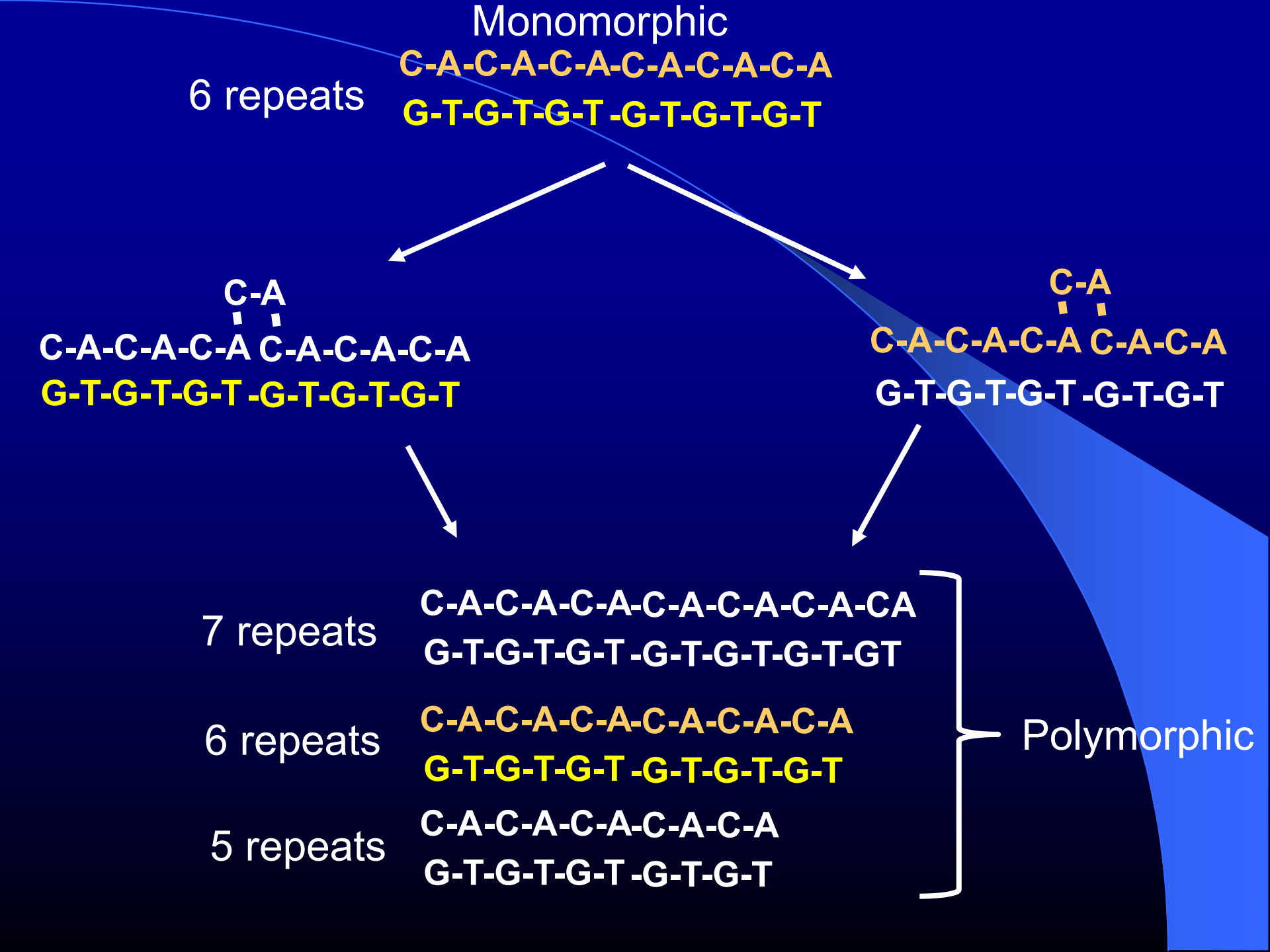
- Immunotherapy enhances the endogenous immune response
- Checkpoint inhibitors allow T-cell activation and T-cell mediated cytotoxicity
- The greater the number of antigens, the more likely there is to be an immune response
- A higher mutation rate will result in a greater number of *neo-antigens*



The rationale for immunotherapy

- Immunostaining for the checkpoint molecules is not easy (either in application or interpretation)
- An alternative method it to look at *Tumour Mutation Burden*
- This requires extensive sequencing to look for random mutations
- These are a reflection of the mutation rate and hence the antigenicity in a tumour




Monomorphic
6 repeats
C-A-C-A-C-A-C-A-C-A-C-A
G-T-G-T-G-T-G-T-G-T-G-T
C-A
C-A-C-A-C-A C-A-C-A-C-A
G-T-G-T-G-T-G-T-G-T-G-T
C-A
C-A-C-A-C-A C-A-C-A
G-T-G-T-G-T-G-T-G-T
7 repeats
C-A-C-A-C-A-C-A-C-A-C-A-CA
G-T-G-T-G-T-G-T-G-T-G-T-GT
6 repeats
C-A-C-A-C-A-C-A-C-A-C-A
G-T-G-T-G-T-G-T-G-T-G-T
5 repeats
C-A-C-A-C-A-C-A-C-A
G-T-G-T-G-T-G-T-G-T
Polymorphic

CANCER BIOMARKERS

Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade

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The genomes of cancers deficient in mismatch repair contain exceptionally high numbers of somatic mutations. In a proof-of-concept study, we previously showed that colorectal cancers with mismatch repair deficiency were sensitive to immune checkpoint blockade with antibodies to programmed death receptor–1 (PD-1). We have now expanded this

The genomes of mismatch repair–deficient tumors all harbor hundreds to thousands of somatic mutations, regardless of their cell of origin. We therefore sought to investigate the effects of PD-1 blockade (by the anti–PD-1 antibody pembrolizumab) in mismatch repair–deficient tumors independent of the tissue of origin. In the current study, we prospectively evaluated the efficacy of PD-1 blockade in a range of different subtypes of mismatch repair–deficient cancers (ClinicalTrials.gov number NCT01876511).

Eighty-six consecutive patients were enrolled between September 2013 and September 2016 (table S1). The data cutoff was 19 December 2016. All patients received at least one prior therapy and had evidence of progressive disease prior to enrollment. Twelve different cancer types were enrolled in the study (Fig. 1). All enrolled patients had evidence of mismatch repair deficiency as assessed by either polymerase chain reaction or immunohistochemistry. For most cases, germline sequencing of *MSH2*, *MSH6*, *PMS2*, and *MLH1* was performed to determine whether the mismatch repair deficiencies were associated with a germline change in one of these genes (i.e., whether the patients had Lynch syndrome) (table S2). Germline sequence changes diagnostic

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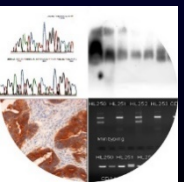
The rationale for immunotherapy

- Tumours with MSI have a high TMB and enhanced IR to CIs
- Tumours with microsatellite instability have a high tumour mutation burden and enhanced immune response to checkpoint inhibitors
- Tumours which are not MSI may still respond if there is high TMB
- This will be tested using NGS panels



Overview

- Ras testing
- Testing for Lynch Syndrome
- Loss of Mismatch Repair (MMR) function and leads to microsatellite instability (MSI)
- IHC versus PCR for loss of MMR function
- An algorithm for Lynch Syndrome screening
- Clinical implications of dMMR