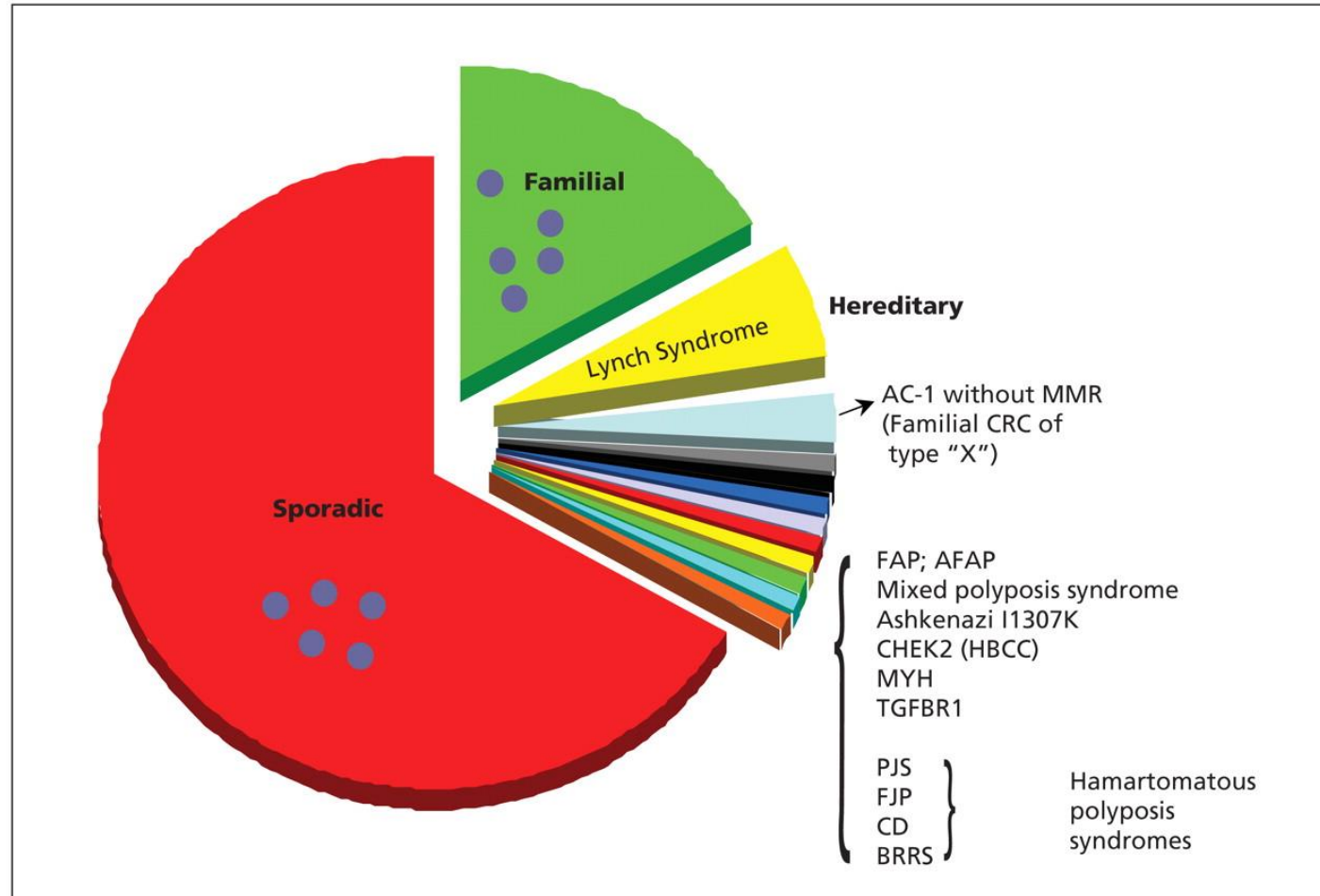


Patologens rolle ved opsporing af Lynch syndrom

MMR og MSI

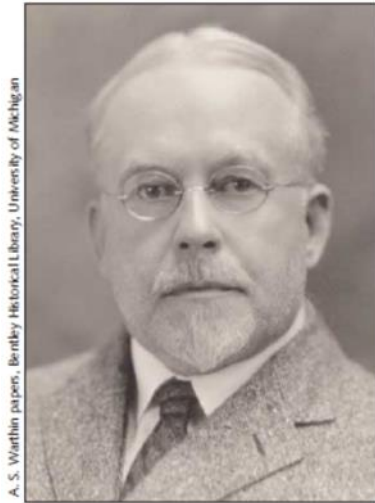
Louise Klarskov
Patologifdelingen
Herlev Hospital

Arvelig Colorectal Cancer

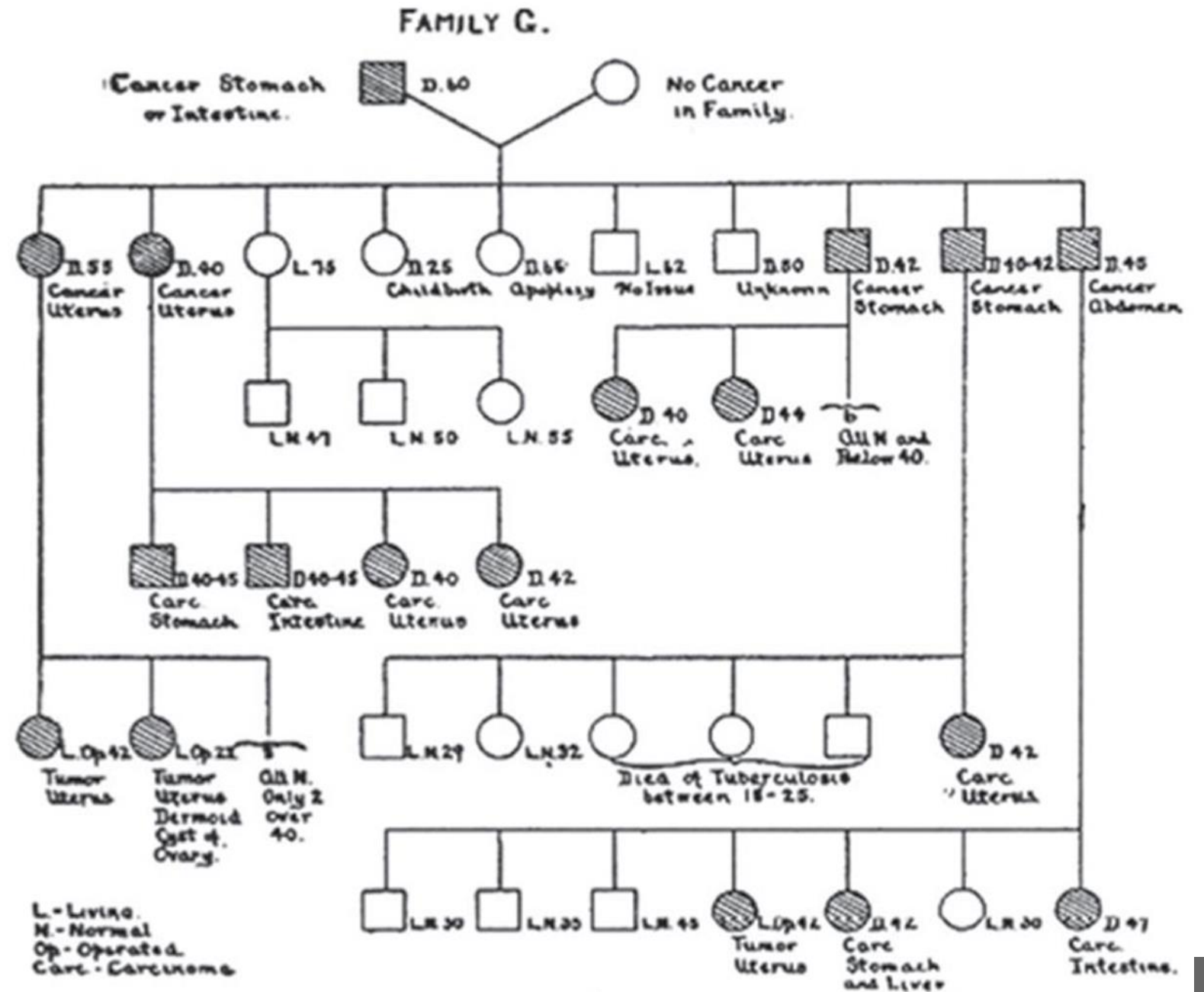


Lynch syndrom

- Arvelig defekt i et af mismatch repair generne, *MLH1*, *PMS2*, *MSH2* eller *MSH6*
- Autosomal dominant arvegang
- Penetrans ~ 80%
- 2-4% af kolorektal cancere
- Inkluderer også andre cancertyper (endometrie, øvre urinveje, tyndtarm m.fl)
- Tidligere kendt som HNPCC



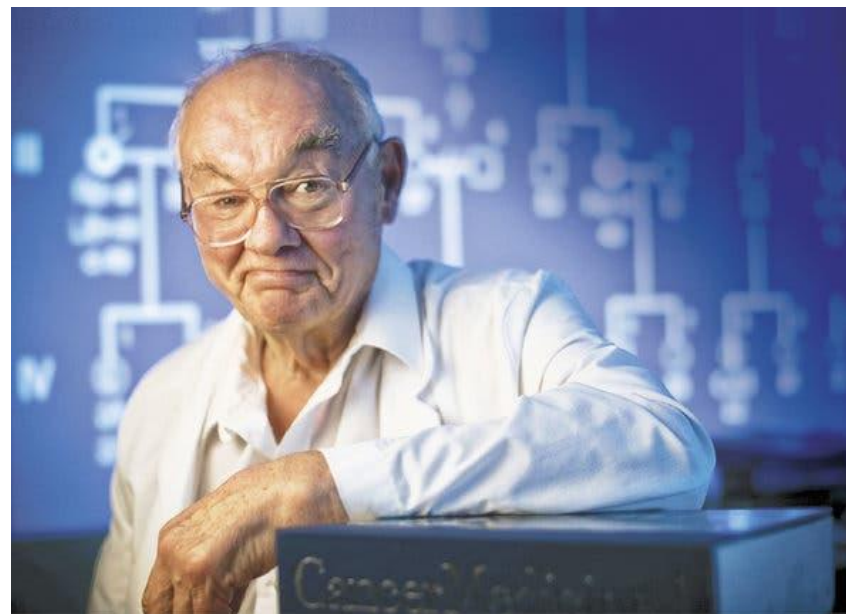
A.S. Warthin papers, Bentley Historical Library, University of Michigan



Udviklingen i Lynch syndrom

1895-1913: Dr Warthin kortlægger "Family G"

1960'erne: Dr Lynch genopdager koblingen mellem arvelighed og cancer



Udviklingen i Lynch syndrom

1895: Dr Warthin kortlægger "Family G"

1960: Dr Lynch genopdager koblingen mellem arvelighed og cancer

1990: Amsterdam I kriterierne



Amsterdam kriterierne

Amsterdam I, 1990

- CRC hos mindst tre slægtninge. Én afficeret skal være 1.grads slægtning (direkte blodsbeslægtede) til de to andre
- CRC forekommer i mindst to generationer.
- Ét tilfælde af CRC skal være diagnosticeret før patienten er fyldt 50 år
- FAP, Familiær Adenomatøs Polypose skal være udelukket

Amsterdam II, 1999

- Opfylder Amsterdam I kriterierne, men CRC kan erstattes af cancer i endometriet, øvre urinveje (urothelcelle) eller tyndtarmene

Udviklingen i Lynch syndrom

1895: Dr Warthin kortlægger "Family G"

1960: Dr Lynch genopdager koblingen mellem arvelighed og cancer

1990: Amsterdam I kriterierne

1993: Mikrosatellit instabilitet kobles til Lynch syndrom

Udviklingen i Lynch syndrom

1895: Dr Warthin kortlægger "Family G"

1960: Dr Lynch genopdager koblingen mellem arvelighed og cancer

1990: Amsterdam I kriterierne

1993: Mikrosatellit instabilitet kobles til Lynch syndrom

1993: Mutationer i *MSH2* kobles til Lynch syndrom

Udviklingen i Lynch syndrom

1895: Dr Warthin kortlægger "Family G"

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1990: Amsterdam I kriterierne

1993: Mikrosatellit instabilitet kobles til Lynch syndrom

1993: Mutationer i *MSH2* kobles til Lynch syndrom

1994: Mutationer i *MLH1* og *PMS2* kobles til Lynch syndrom

Udviklingen i Lynch syndrom

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1993: Mutationer i *MSH2* kobles til Lynch syndrom

1994: Mutationer i *MLH1* og *PMS2* kobles til Lynch syndrom

1996: Bethesda guidelines, revideret 2002

Revised Bethesda Guidelines for Testing Colorectal Cancers for Microsatellite Instability

Colorectal tumors should be tested for MSI in individuals meeting any of the following

CRC diagnosed at age **<50 years**

Presence of synchronous , metachronous CRC, or other HNPCC-associated tumors **regardless of age** (*Endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, and brain tumors, sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome, and carcinoma of the small bowel*)

CRC with the **MSI-H histology** diagnosed at age **<60 years** (*Presence of tumor infiltrating lymphocytes, Croh's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern.*)

CRC or tumors associated with HNPCC-related tumors diagnosed in ≥ 1 first-degree relative at age **<50 years**

CRC or tumors associated with HNPCC-related tumors diagnosed in ≥ 2 first- or second-degree relatives **at any age**

- * Intended to ID patients for MSI testing. NOT a diagnostic criteria for LYNCH.
- * If a tumor is not available, germline DNA testing should be considered.

Udviklingen i Lynch syndrom

1895: Dr Warthin kortlægger "Family G"

1960: Dr Lynch genopdager koblingen mellem arvelighed og cancer

1990: Amsterdam I kriterierne

1993: Mikrosatellit instabilitet kobles til Lynch syndrom

1993: Mutationer i *MSH2* kobles til Lynch syndrom

1994: Mutationer i *MLH1* og *PMS2* kobles til Lynch syndrom

1996: Bethesda guideline

1997: Mutationer i *MSH6* kobles til Lynch syndrom

Udviklingen i Lynch syndrom

1895: Dr Warthin kortlægger "Family G"

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1993: Mutationer i *MSH2* kobles til Lynch syndrom

1994: Mutationer i *MLH1* og *PMS2* kobles til Lynch syndrom

1996: Bethesda guideline

1997: Mutationer i *MSH6* kobles til Lynch syndrom

2000: Mulighed for rutine sekventering af MMR-generne (PMS2 først i 2009)

Udviklingen i Lynch syndrom

1895: Dr Warthin kortlægger "Family G"

1960: Dr Lynch genopdager koblingen mellem arvelighed og cancer

1990: Amsterdam I kriterierne

1993: Mikrosatellit instabilitet kobles til Lynch syndrom

1993: Mutationer i *MSH2* kobles til Lynch syndrom

1994: Mutationer i *MLH1* og *PMS2* kobles til Lynch syndrom

1996: Bethesda guidelines

1997: Mutationer i *MSH6* kobles til Lynch syndrom

2000: Mulighed for rutine sekventering af MMR-generne (PMS2 først i 2009)

2009: Studier viser at det er cost-effektivt at reflex-screene alle nye CRC for Lynch syndrom

Status i DK

DCCG har anbefalet MMR testning af alle nye CRC siden 2009

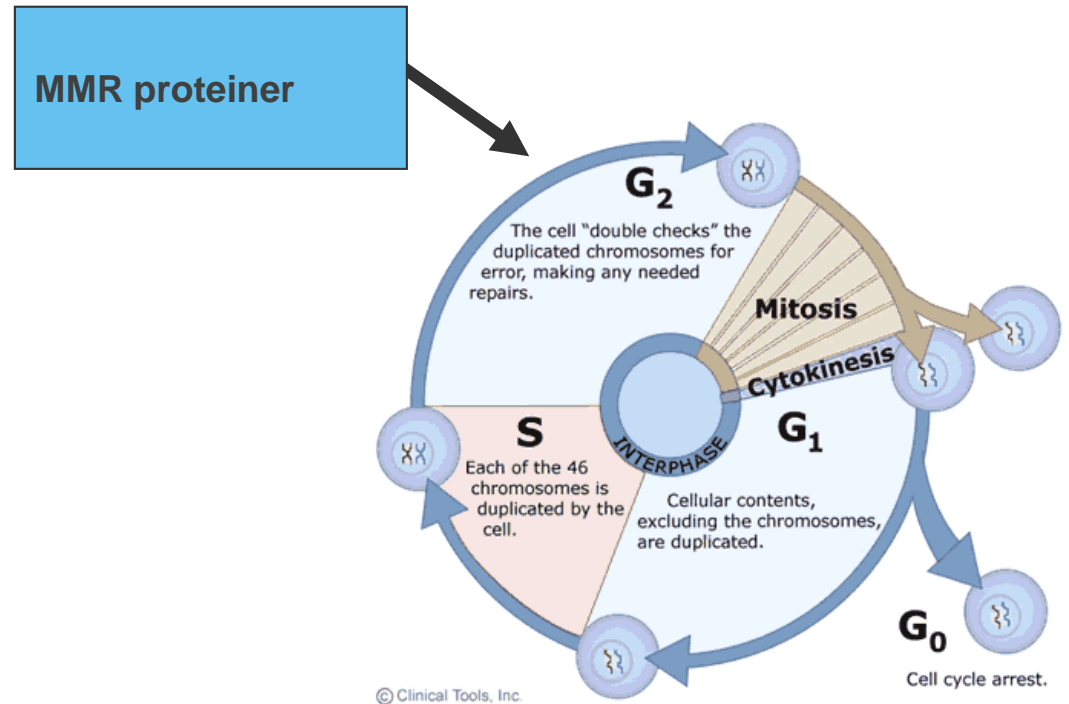
DCCG 2017: 88,1% af CRC har kendt MMR-status

DCCG 2017: 13,6% af CRC har defekt MMR



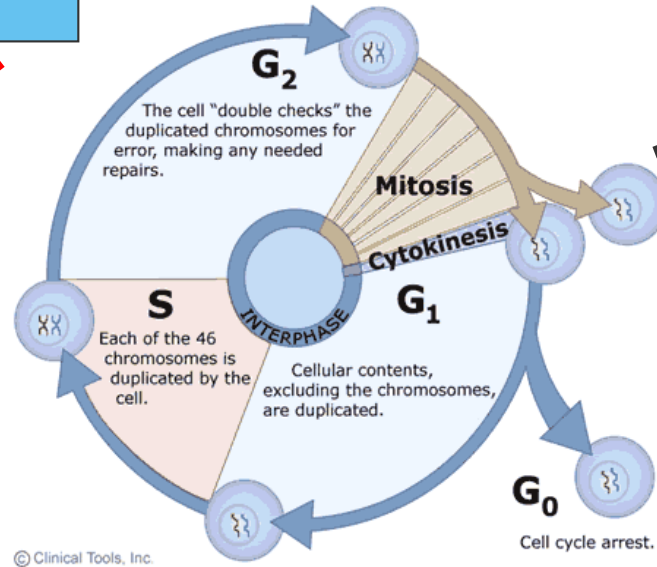
Mismatch repair systemet

- 4 proteiner: MLH1 + PMS2
MSH2 + MSH6
- Scanner og reparerer nydannet DNA ved celledeling



Mismatch repair defekt

~~MMR proteiner~~

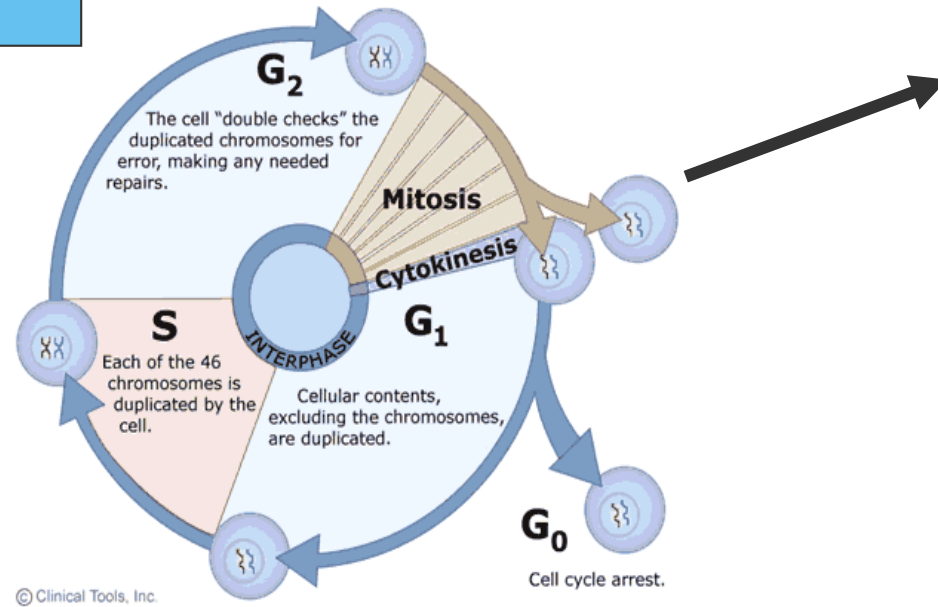


Ændringer i mikrosatellitter, MSI

Microsatellites	Unique sequence	Repeat units	Unique sequence
Mononucleotide	--GGTAGCCAA	A A A A (A) _n	CGATCCA---
Dinucleotide	--TCGCATGCA	CA CA CA (CA) _n	ATTCGCA---
Trinucleotide	--TTAGCATCAG	CAG CAG (CAG) _n	CCAGTGA---
Tetranucleotide	--AATGGTACCGG	(CCGG) _n	GTCAAGT---
Pentanucleotide	--CGATGATCCAAG	(CCAAG) _n	TTACGTA---
Hexanucleotide	--GCTAAGGCCATTG	(CCATTG) _n	ACTGTCA---

Mismatch repair defekt

~~MMR proteiner~~



Ændringer i mikrosatellitter, MSI



Frameshift mutationer

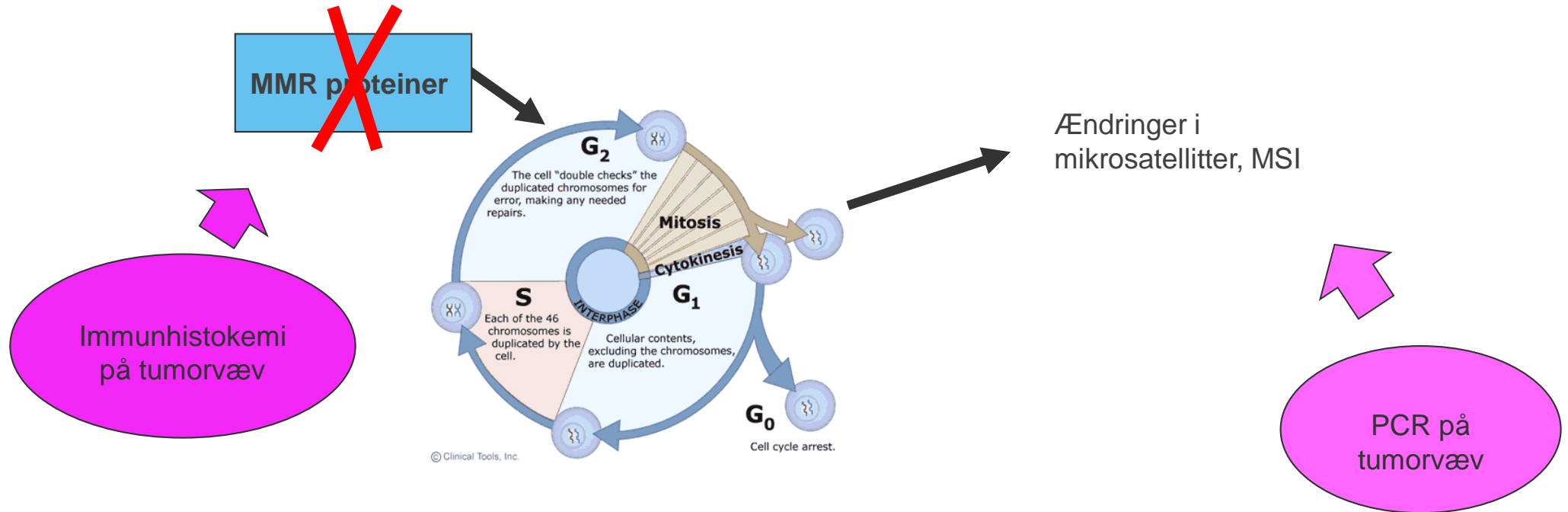


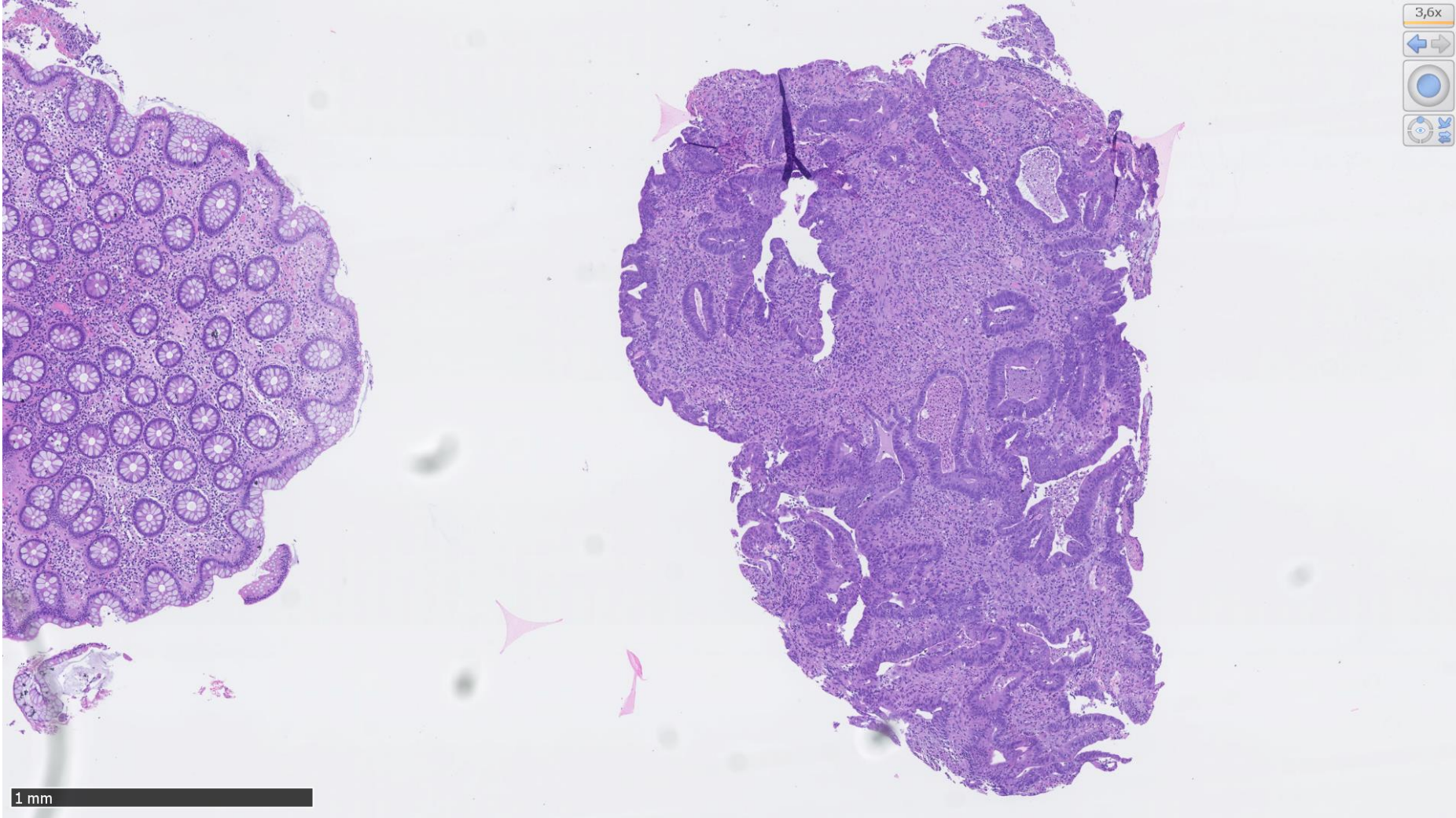
Neo-antigener x >20
"Hypermuteret fænotype"

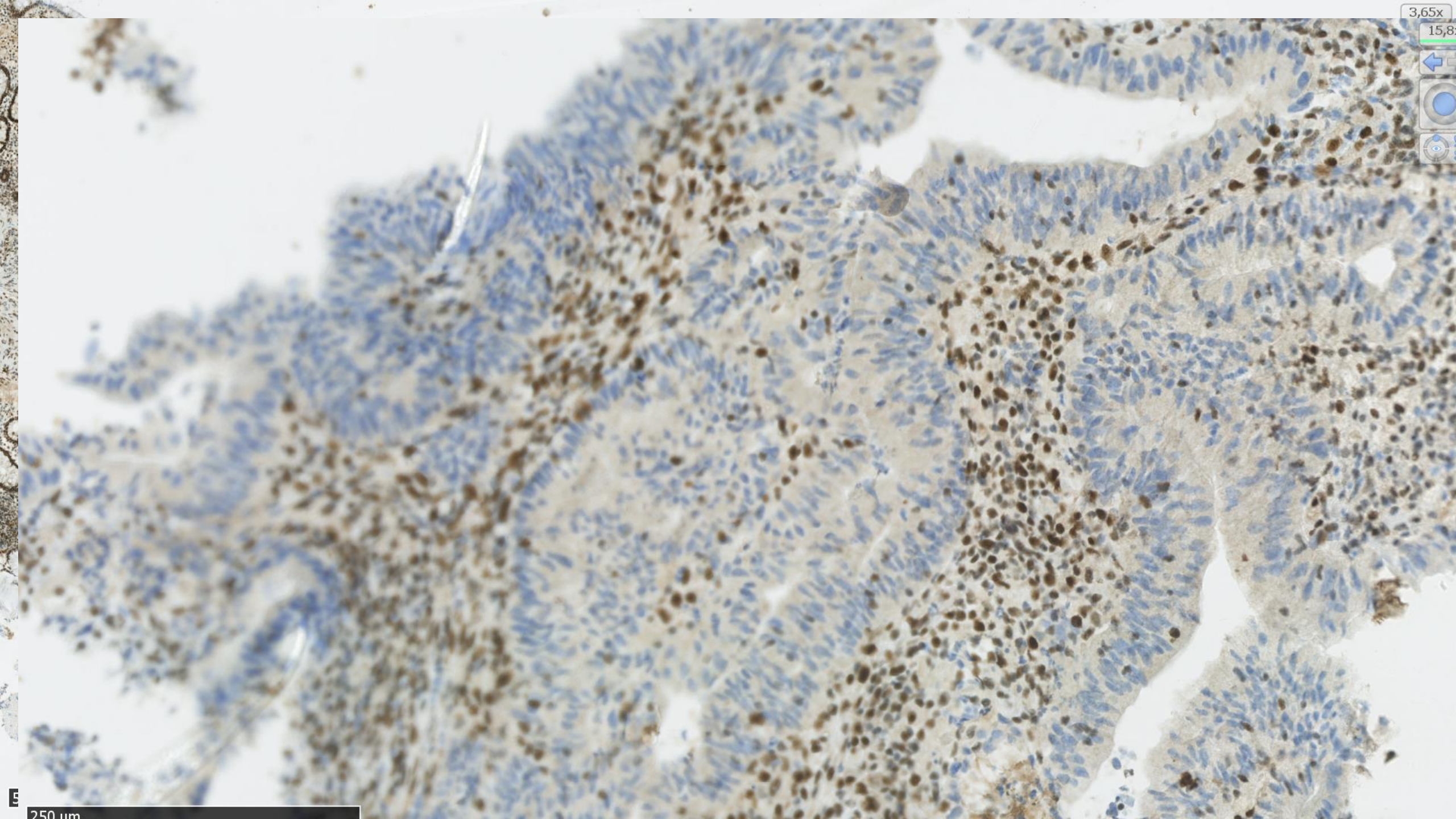


Immunologisk respons

Detektions metode?







3,65x
15,8
←
⊙
👁

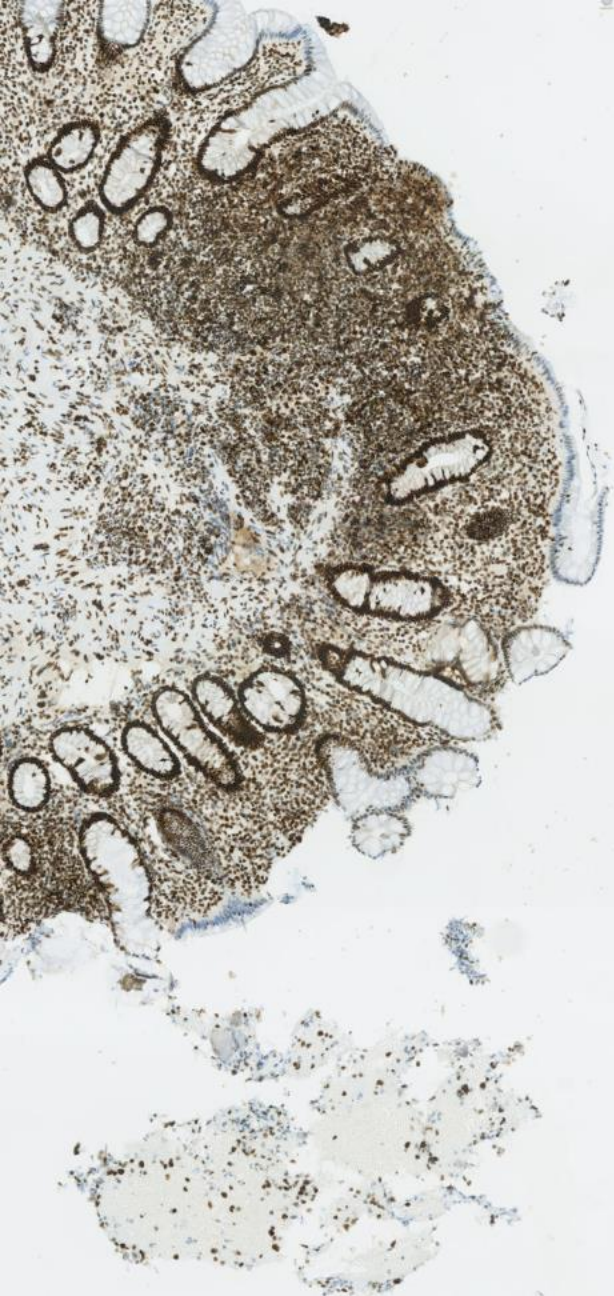
5
250 μm

MSH2



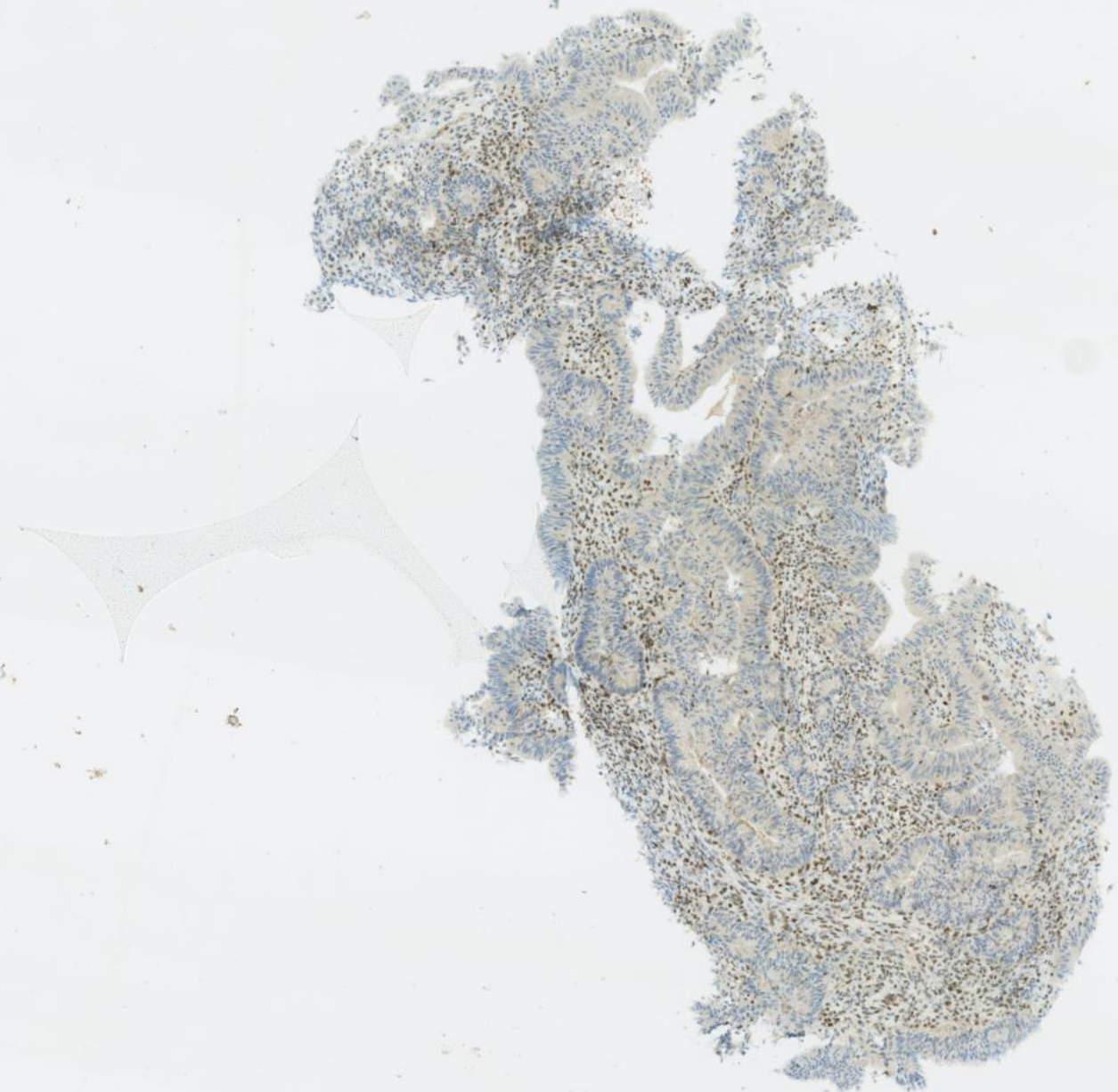
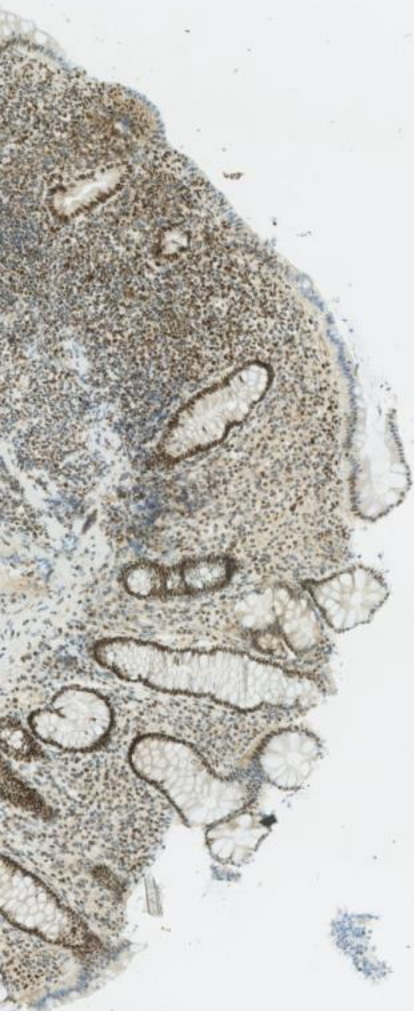
500 μ m

MSH6



1 mm

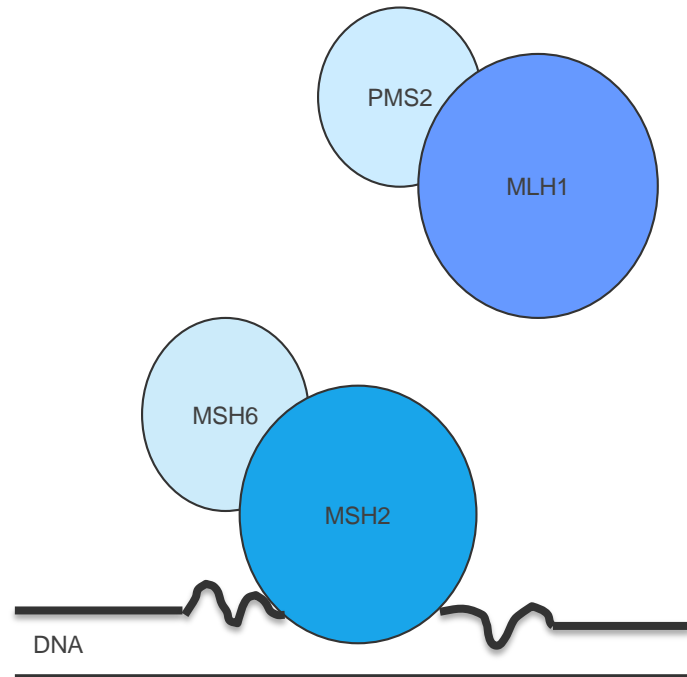
PMS2



500 μ m

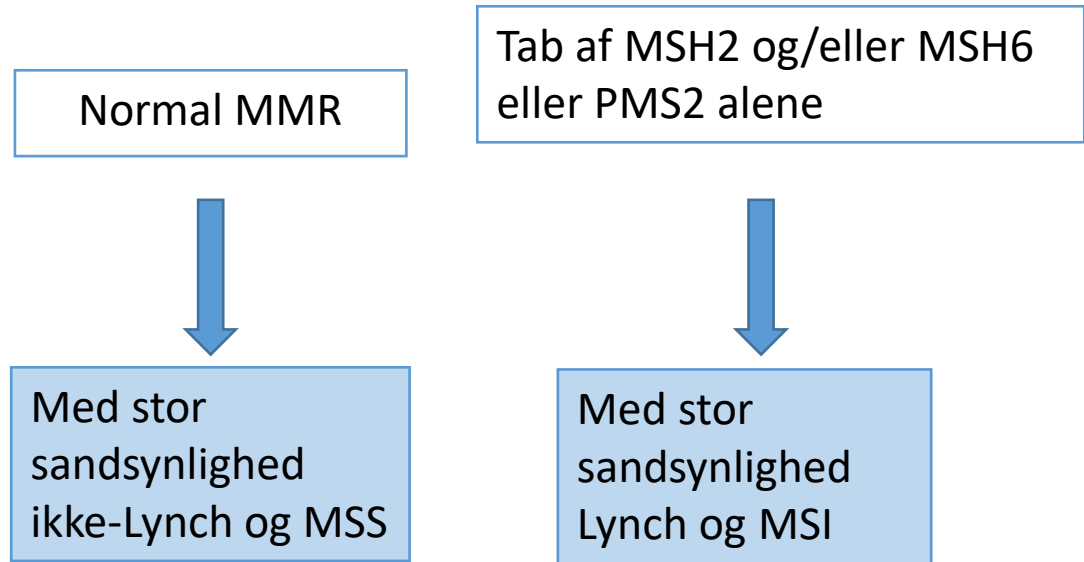


Immunhistokemi

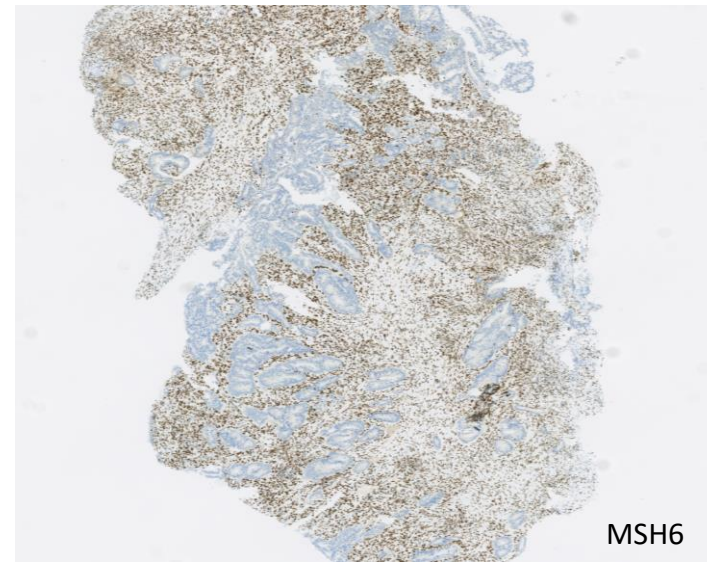
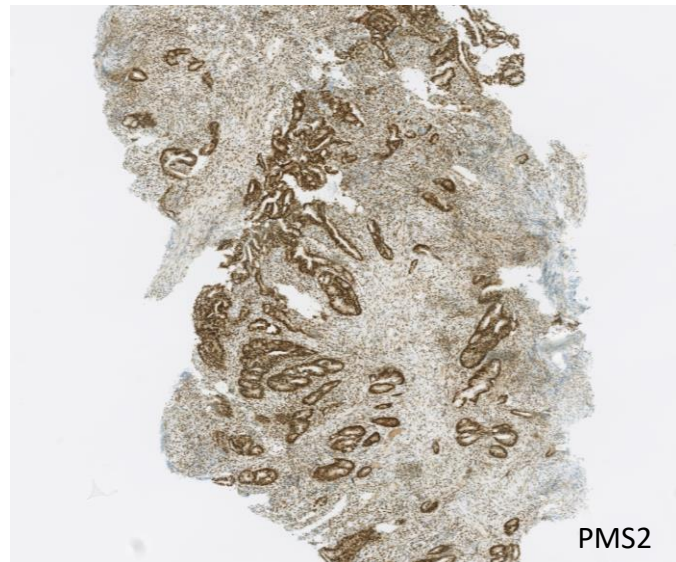
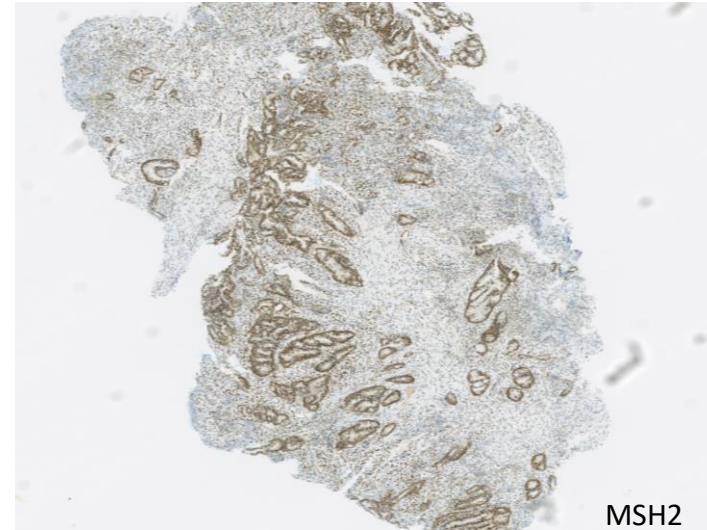
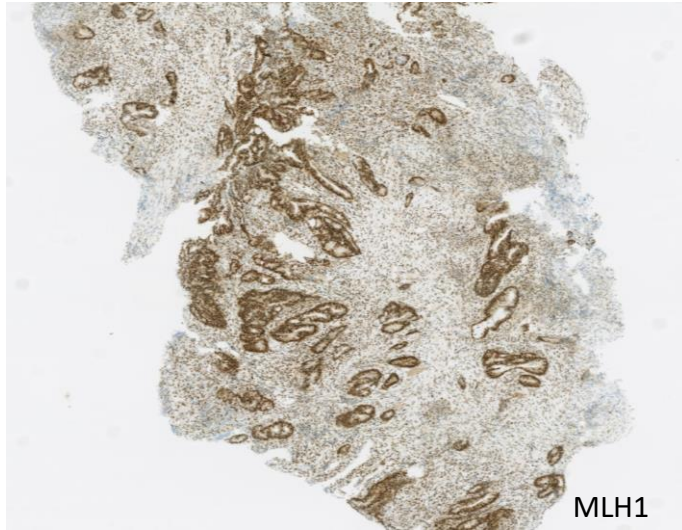


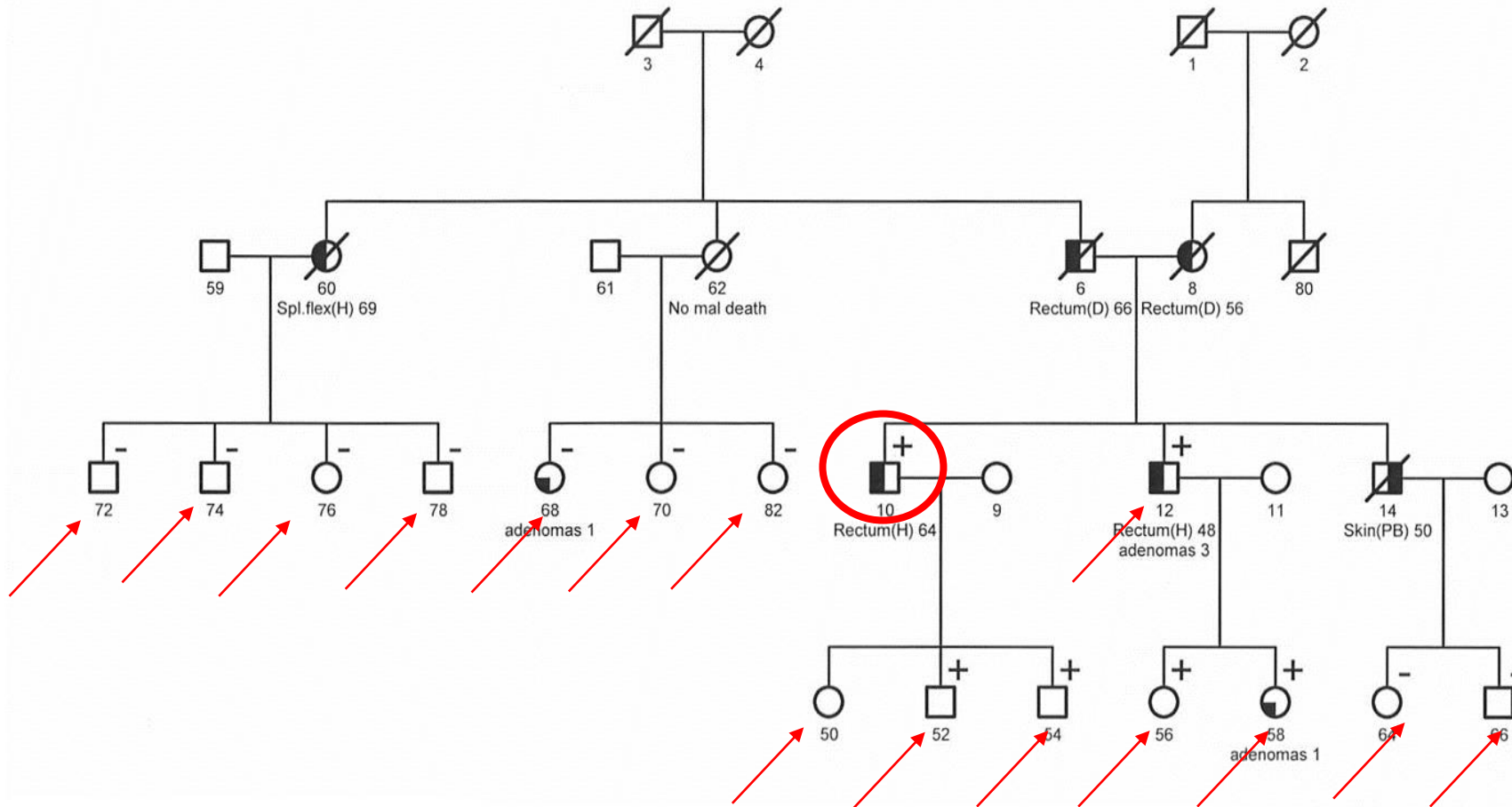
Protein ekspresion				Fortolkning, gen inaktivering
MLH1	PMS2	MSH2	MSH6	
-	-	+	+	<i>MLH1</i>
+	-	+	+	<i>PMS2</i>
+	+	-	-	<i>MSH2</i>
+	+	+	-	<i>MSH6</i>

Udrednings flowchart



Manglende MSH6





Normal MMR



Med stor sandsynlighed ikke-Lynch og MSS

Tab af MSH2 og/eller MSH6 eller PMS2 alene



Med stor sandsynlighed Lynch og MSI

Tab af MLH1 og PMS2



BRAF mut



Med stor sandsynlighed ikke-Lynch og MSI



BRAF wt



Methyleringsanalyse



Methyleret:
Med stor sandsynlighed ikke-Lynch og MSI



Ikke-methyleret:
Med stor sandsynlighed Lynch og MSI

Arvelig eller sporadisk?

MSI vha. PCR + kapillær elektroforese

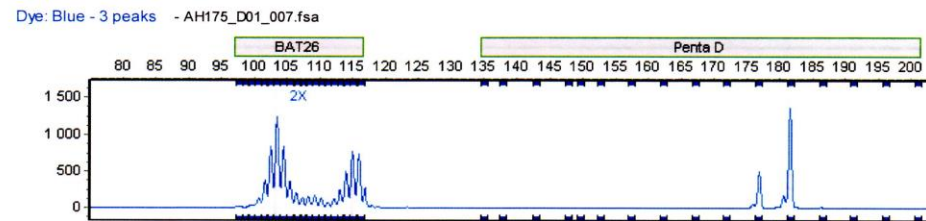
Panel af typisk 5 mikrosatellit markører

Normalt væv: 5'-AAAAAAAAAAAAAAAAAAAA-3'

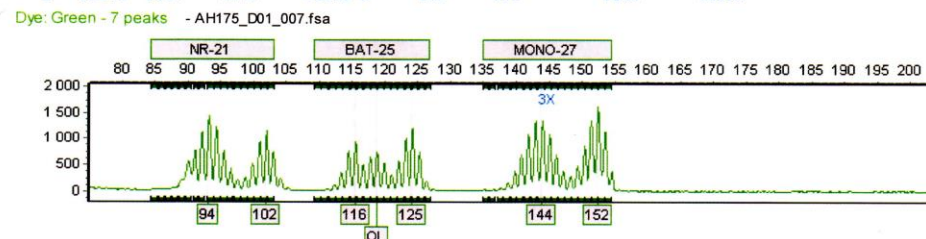
pMMR tumor: 5'-AAAAAAAAAAAAAAAAAAAA-3'

dMMR tumor: 5'-AAAAAAAAAAAAAAAAAAAA-3'

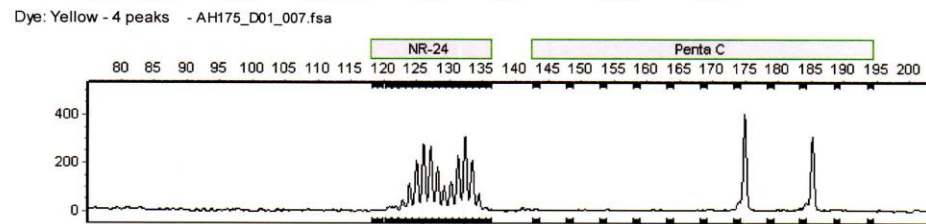
- MSS (alle 5 markører normale)
- MSI-Low (1 markør abnorm)
- MSI-High (≥ 2 markører abnorme)



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	103.5	617	3539	BAT26	104	0.1	Pass	77.8	
2	115.0	387	2167	BAT26	115	0.2	Pass	40.9	
3	181.6	1349	6870	Penta D	182	0.1	Pass	315.8	



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	93.3	1452	8225	NR-21	94	0.2	Pass	300.6	
2	102.0	1145	6481	NR-21	102	0.3	Pass	211.9	
3	115.6	937	4930	BAT-25	116	0.4	Pass	176.3	
4	118.9	753	3938	BAT-25	OL	0.5	Undetermined	127.9	
5	124.3	1197	6200	BAT-25	125	0.3	Pass	258.2	
6	144.0	450	2421	MONO-27	144	0.2	Pass	55.1	
7	152.5	538	3050	MONO-27	152	0.4	Pass	64.7	



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	126.0	279	1519	NR-24	126	0.1	Pass	24.8	
2	132.4	311	1710	NR-24	132	0.4	Pass	28.8	
3	174.9	405	2104	Penta C	OL	1.0	Undetermined	49.0	
4	185.2	311	1624	Penta C	OL	1.0	Undetermined	31.8	



Andre MSI metoder

- Idylla (PCR + smeltepunkts analyse)
- NGS





Klinisk performance

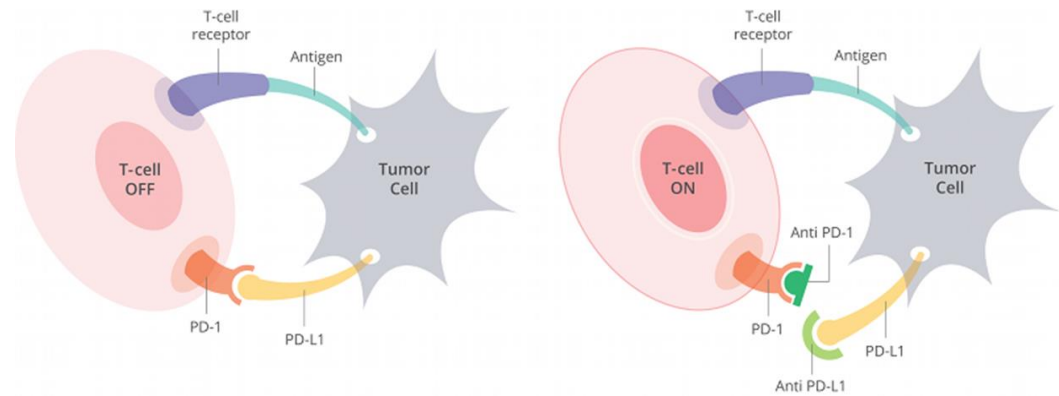
- Sensitivitet af IHC 94%, MSI 83% (Shia, review, 2008)
- Konkordans mellem IHC og MSI > 95%





Andre indikationer

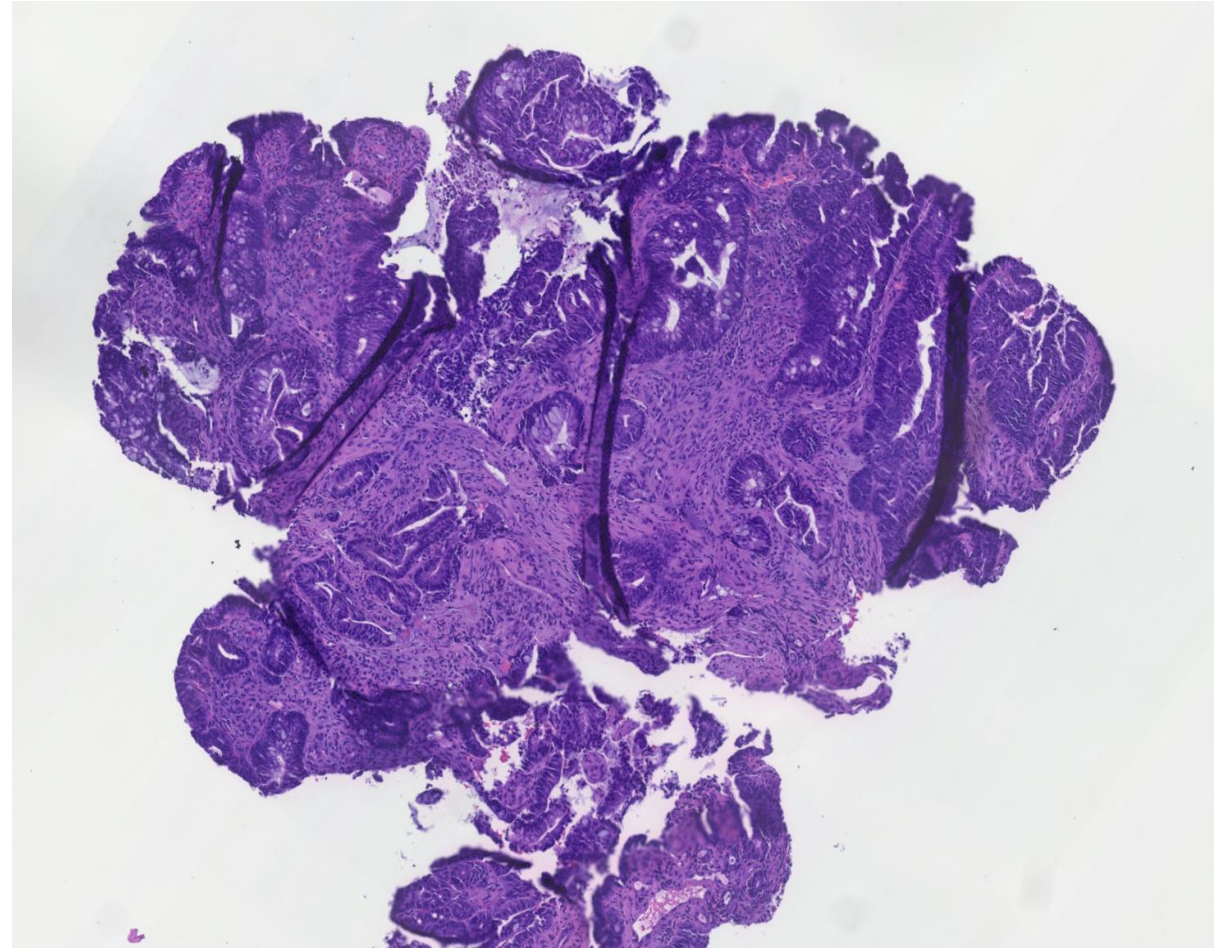
- Prognose ved CRC
- Prædiktiv markør for immunterapi

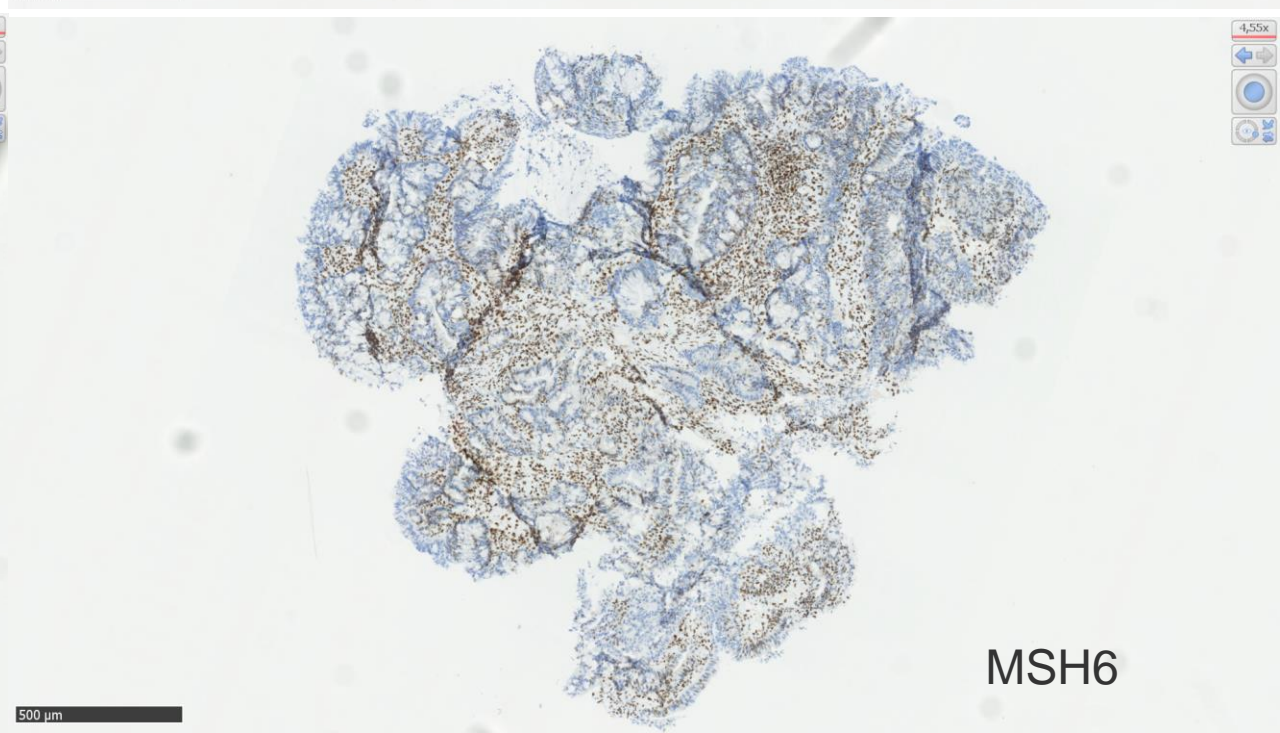
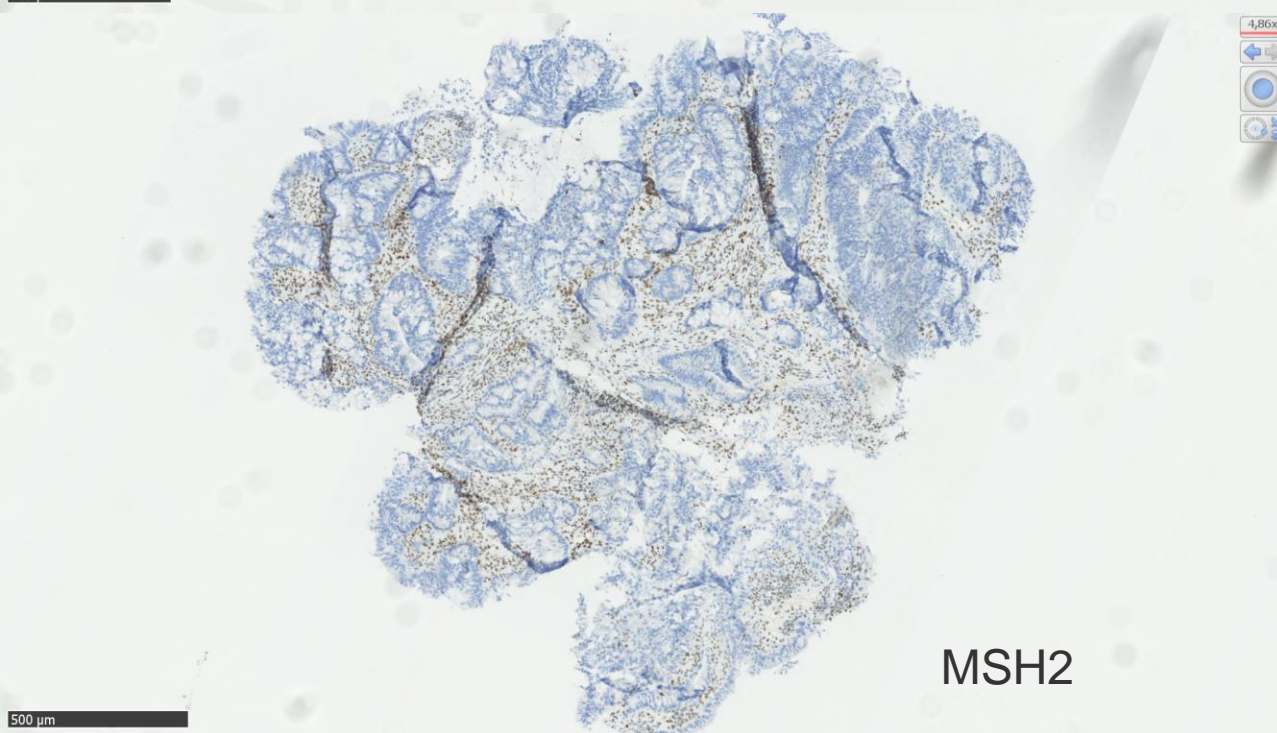
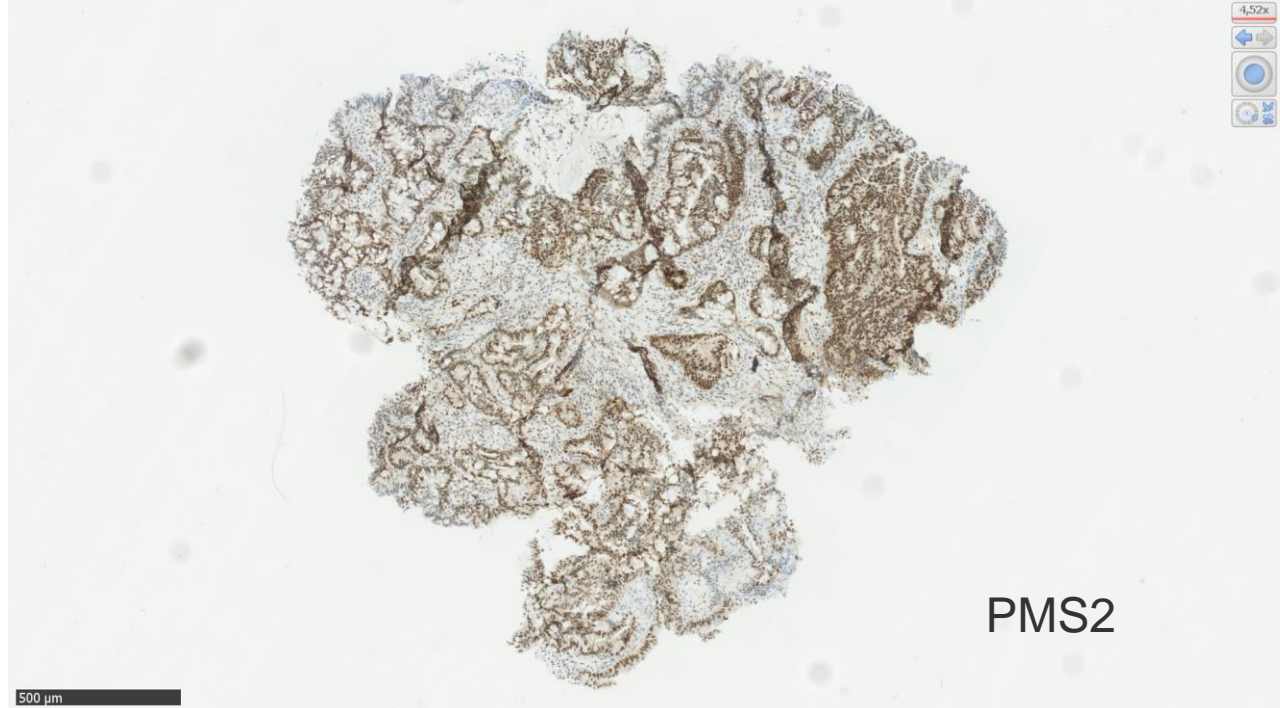
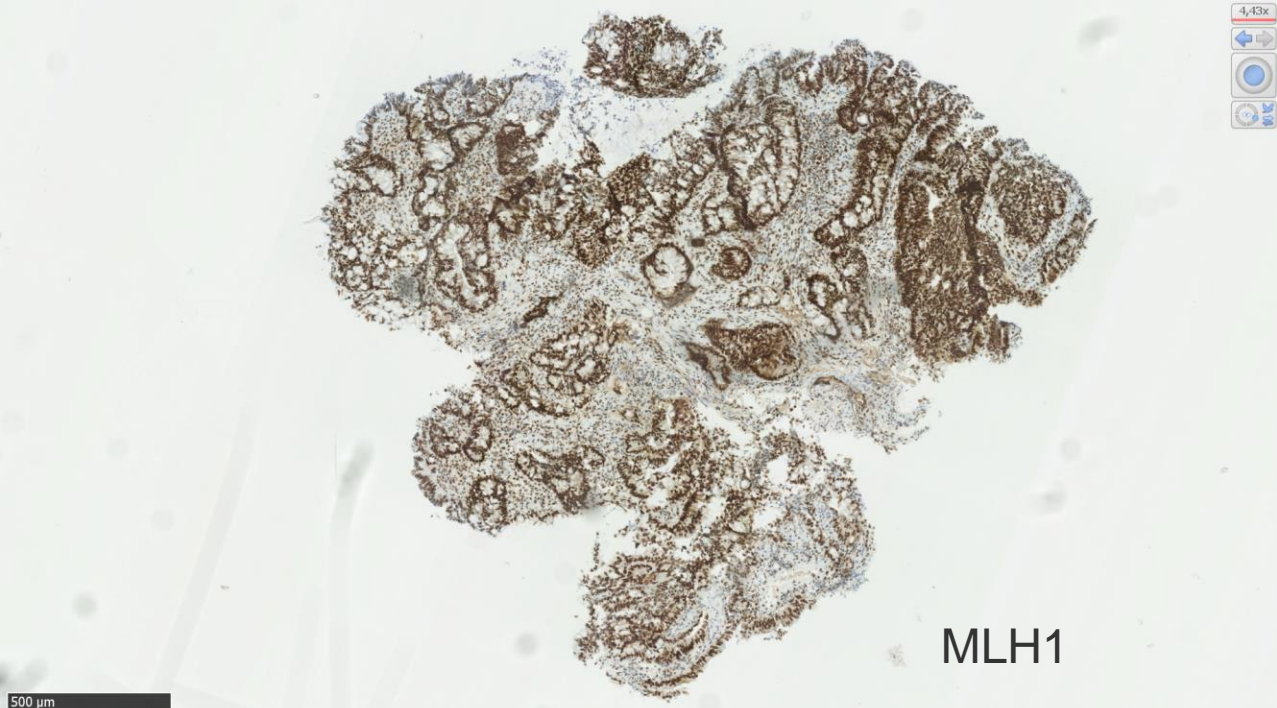


Case

52-årig mand, TKS

Tumor i venstre flexur

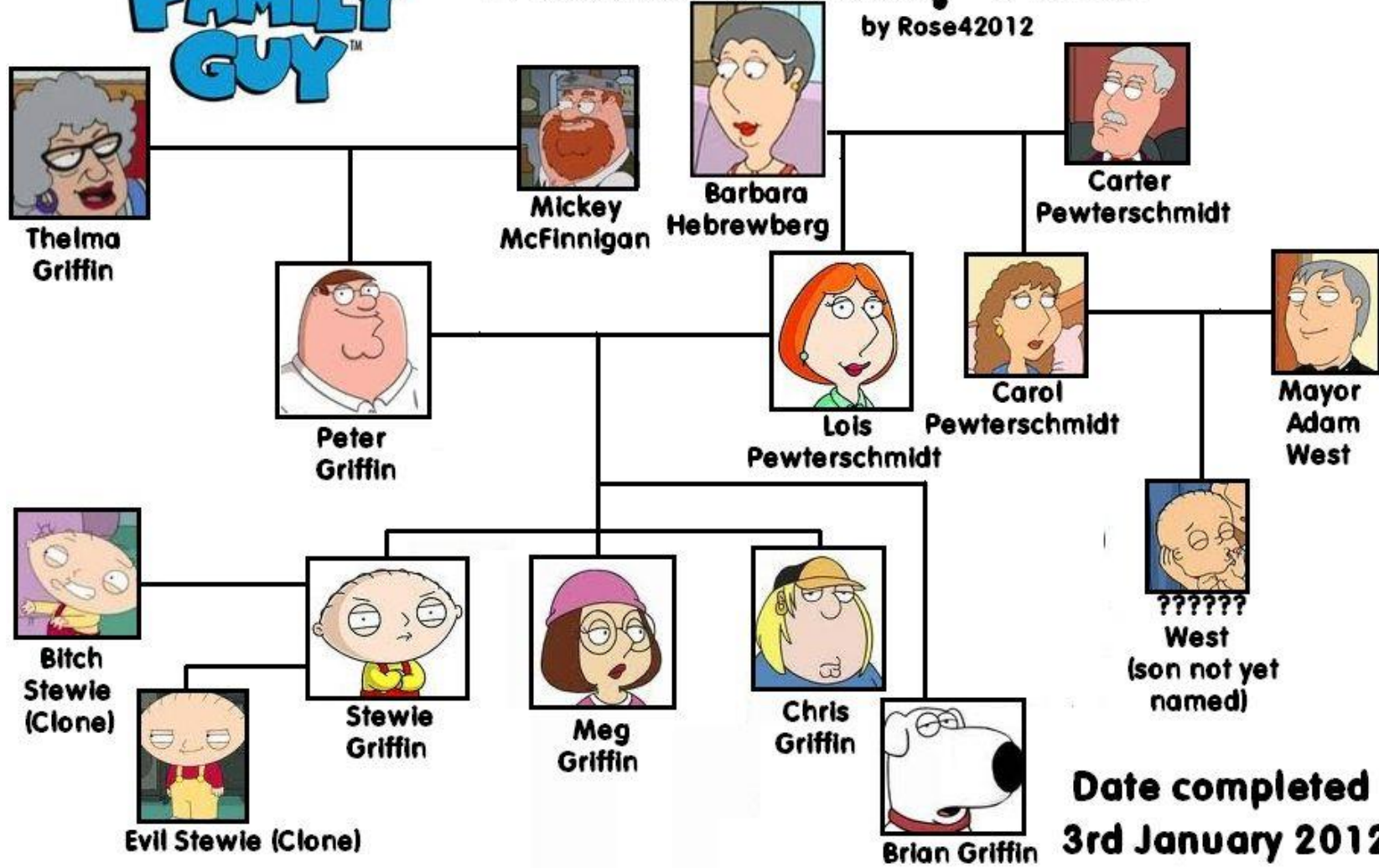






Griffin Family Tree

by Rose42012



Date completed
3rd January 2012