

Plea for a systematic use of MMR protein IHC in CRCs

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MMR = Mismatch Repair
IHC = immunohistochemistry

PLAN

- I. History and common misconceptions about CRCs in Lynch syndrome
 - I. Amsterdam and Bethesda criteria
 - II. MMR proteins Immunohistochemistry in CRCs
- II. Presentation of an obvious Lynch syndrome family and two very ordinary CRC cases
- III. Chemotherapy in MSI-H CRC: overview
- IV. Conclusions :
 - I. MMR IHC should be a standard in any CRC
 - II. A very simple alternative to the standard family-based Lynch syndrome-criteria

1991 Henri Lynch commentary about the diagnosis of HNPCC

Recognition of Lynch syndromes is wholly dependant upon a well orchestrated family history. Unfortunately, in the usual clinical practice settings where **there is often a lack of attention to [...] the family history** the disorder frequently goes undiagnosed. All too often, HNPCC is not recognized until a family has suffered an inordinate amount of morbidity and mortality.

We have repeatedly encountered patients in whom the hereditary nature of their cancer-prone problem was either completely ignored and/or mismanaged by physicians.

HNPCC genetics was unknown in 1991!

Diagnosis

- ◆ Lynch syndrome lacks overt phenotypic markers, therefore, accurate family history is crucial.
- ◆ If the **clinical pattern of disease in the family** is positive, molecular studies are performed to detect microsatellite instabilities and germline mutations (MSH2, MLH1, MSH6) that segregate in affected family members.
- ◆ MSH6 accounts for 10% of Lynch syndrome mutations and is associated with milder disease, but an excess of endometrial cancer.
- ◆ Patients must meet the **Amsterdam criteria to be diagnosed**, and **the Bethesda criteria to undergo microsatellite testing**

Amsterdam criteria and Bethesda criteria

AMSTERDAM CRITERIA II⁸

Patient must meet ALL of the following criteria:

- Three or more relatives with a histologically verified hereditary nonpolyposis colorectal cancer-associated cancer (colorectal cancer, cancer of the endometrium, ovary, small bowel, ureter, or renal pelvis), one of whom is a first-degree relative of the other two (familial adenomatous polyposis should be excluded); AND
- Cancers involving at least 2 generations; AND
- One or more cancer cases diagnosed before the age of 50.

THE REVISED BETHESDA CRITERIA FOR TESTING COLORECTAL TUMORS FOR MICROSATELLITE INSTABILITY⁹

Tumors from individuals should be tested for microsatellite instability in the following situations:

- Colorectal cancer diagnosed in a patient who is less than 50 years of age.
- Presence of synchronous, metachronous colorectal, or other hereditary nonpolyposis colorectal cancer-associated

tumors, regardless of age. (Hereditary nonpolyposis colorectal cancer-related tumors include colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, and brain [usually glioblastoma as seen in Turcot syndrome] tumors, sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome, and carcinoma of the small bowel.)

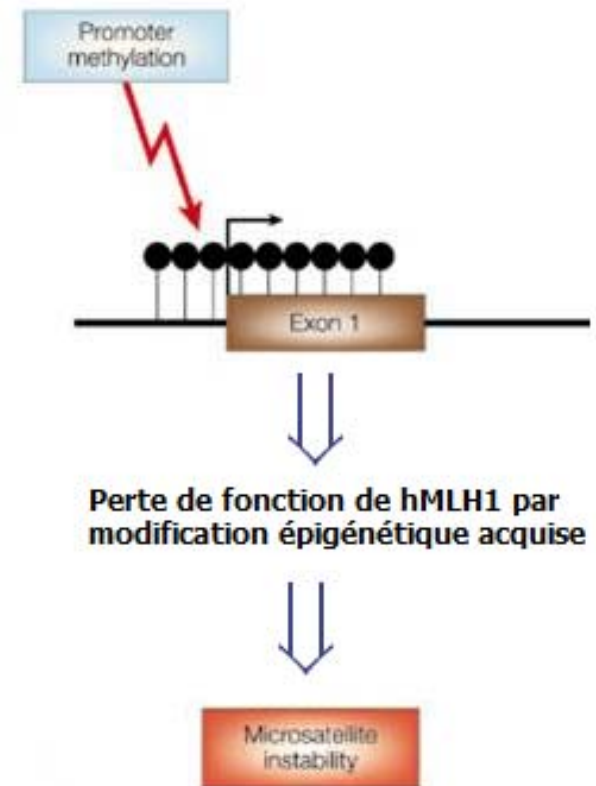
- Colorectal cancer with the microsatellite instability-high histology diagnosed in a patient who is less than 60 years of age. (Microsatellite instability-high in tumors refers to changes in 2 or more of the 5 National Cancer Institute-recommended panels of microsatellite markers. Histology indicated by presence of tumor-infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern.)
- Colorectal cancer diagnosed in one or more first-degree relatives with a hereditary nonpolyposis colorectal cancer-related tumor, with one of the cancers being diagnosed under age 50 years.
- Colorectal cancer diagnosed in 2 or more first- or second-degree relatives with hereditary nonpolyposis colorectal cancer-related tumors, regardless of age.

Today's knowledge and facts

- ◆ MSI-H CRCs are well-defined biologically and clinically very particular CRCs
- ◆ Lynch syndrome predisposes to MSI-H CRCs
- ◆ Amsterdam and Bethesda criteria are not easy to use in the every-day practice
- ◆ IHC became available in the late 90ies
- ◆ Misconceptions about sporadic MSI-H and Lynch-associated CRCs remain widespread

Loss of hMLH1 is the **only** cause of sporadic MSI-H CRCs

- ◆ Promoter hypermethylation = **the** mechanism of MLH1 loss of function in sporadic MSI-H CRC
- ◆ Loss of MSH2, MSH6 and PMS2 observed **ONLY** in Lynch syndrome – associated CRCs



Sporadic MSI-H CRCs are very specific for the right colon

sporadic MSI-H CRCs occur in
 $\approx 10\%$ of cases/ distal colon

References

Samowitz W. 2001

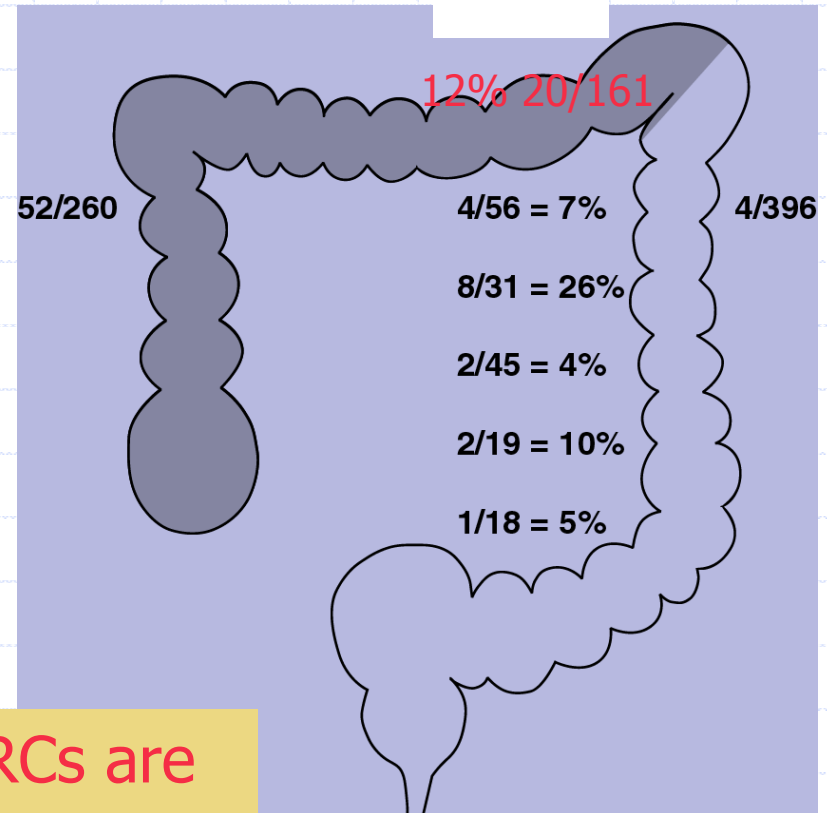
Elsaleh H. 2000

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Lanza G. 1999

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Kim H. 1994

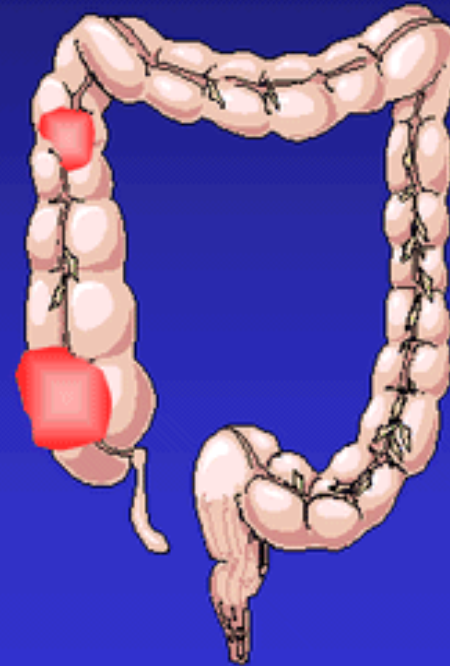


$\approx 90\%$ of sporadic MSI-H CRCs are located before the splenic flexure

ASCO teaching slide about HNPCC

Clinical Features of HNPCC

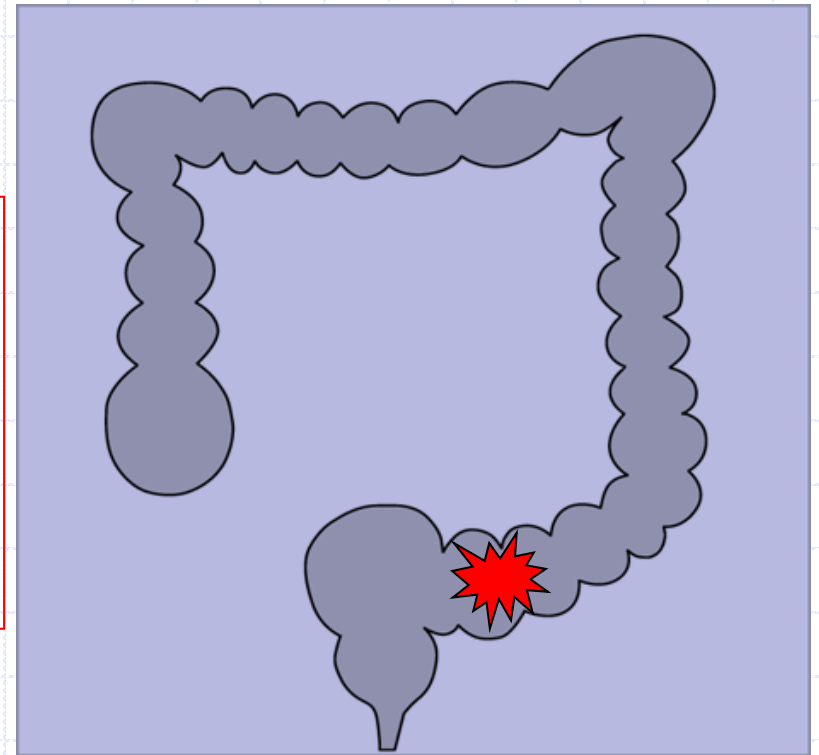
- Early, variable age at CRC diagnosis (~45 years)
- Proximal colon predominance
- Extracolonic cancers: endometrium, ovary, stomach, urinary tract, small bowel, other, sebaceous skin tumors



Distribution of CRCs within Lynch syndrome families

Lynch-associated CRCs: left-sided in 35-50% of cases

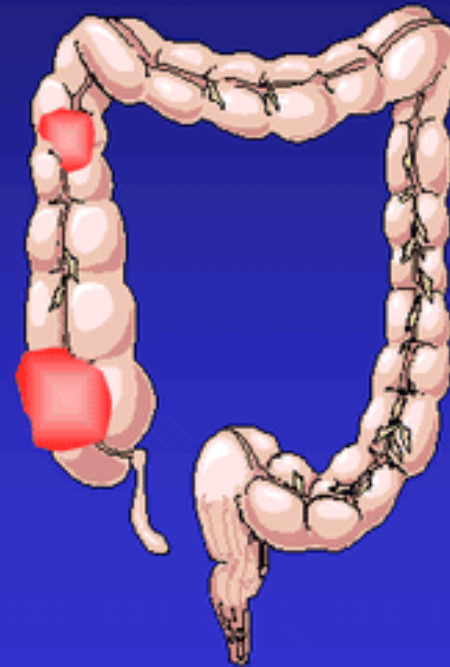
=> Left side location is an **obvious characteristic** of Lynch syndrome - associated MSI-H CRCs



Common presentation of Lynch syndrome CRCs

Clinical Features of HNPCC

- **Early** variable age at CRC diagnosis (~45 years)
- Proximal colon predominance
- Extracolonic cancers: endometrium, ovary, stomach, urinary tract, small bowel, other, sebaceous skin tumors

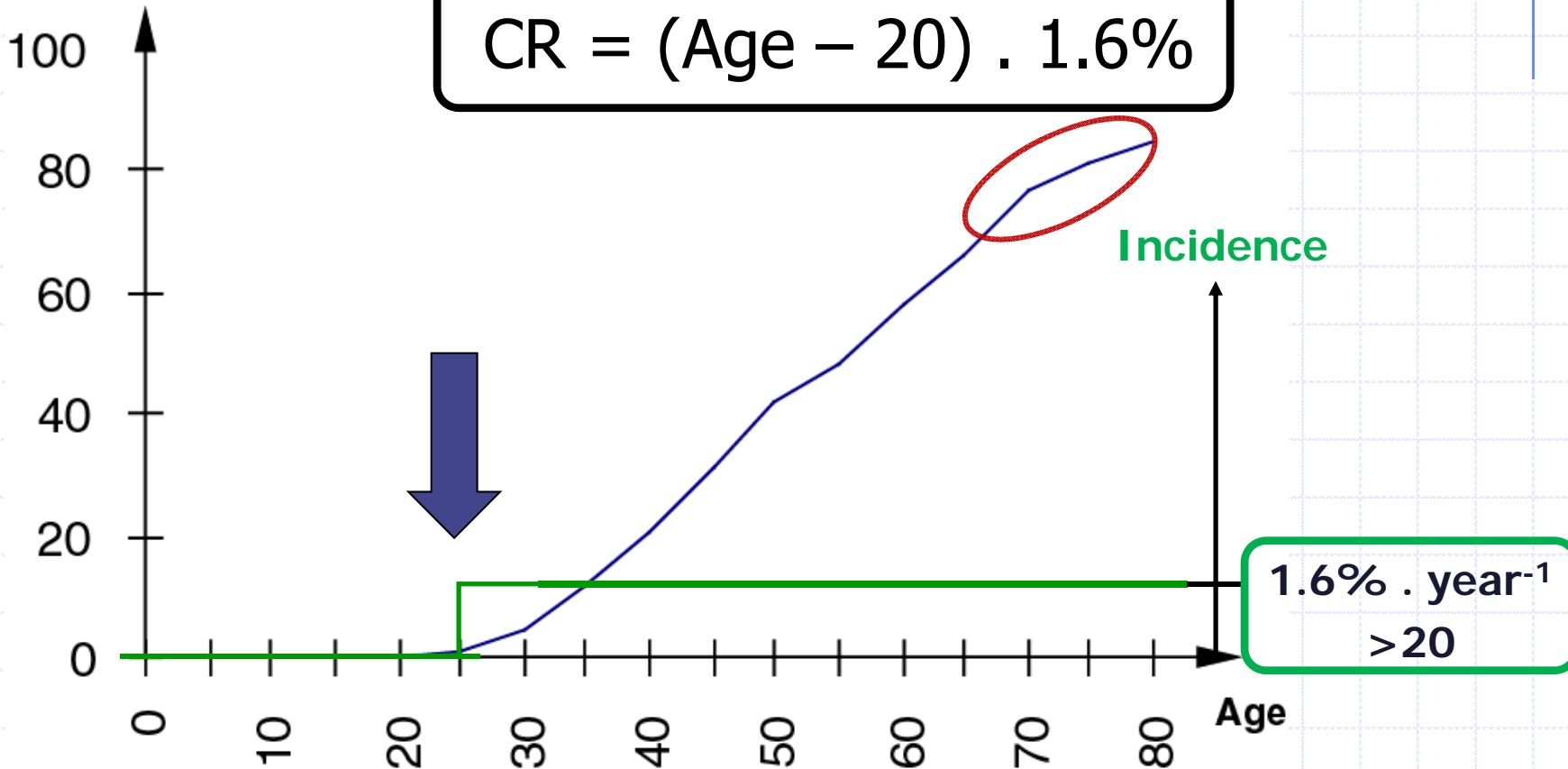


Evolution of CRC risk in Lynch syndrome

Voskuil D. W. et al. *Int. J. Cancer* 1997

Cumulative risk (%)

$$CR = (Age - 20) \cdot 1.6\%$$



Immunohistochemistry (IHC) of MMR proteins in CRCs

Tested protein:

MLH1

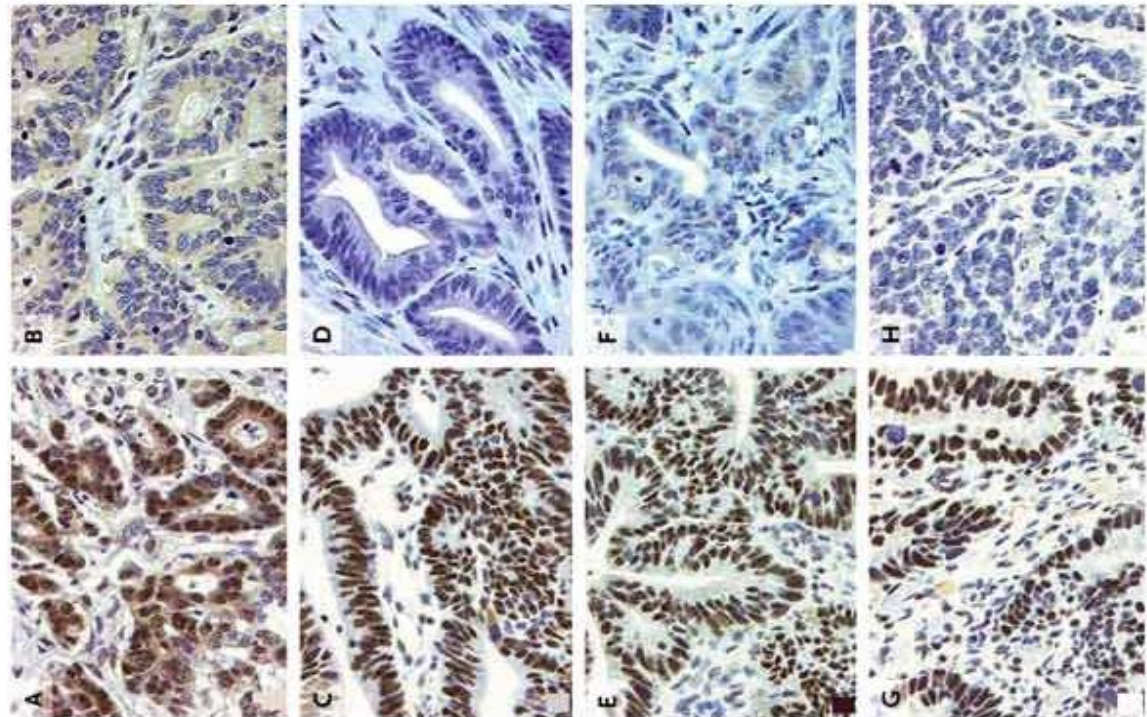
MSH2

MSH6

PMS2

CRCs MSI-H →
(loss of either one or
of a combination of
MMR protein)

MSS CRCs express
all MMR proteins →



Result of IHC of MMR proteins in CRCs: three possibilities

1. All MMR proteins normally expressed
⇒ Lynch syndrome very unlikely
2. Loss of MSH2 and/or MSH6 or PMS2
⇒ Lynch syndrome is very likely
3. Loss of MLH1 : sporadic case is likely if
 1. the individual is very old
 2. CRC is right-sided
 3. NO family history

Cases where a loss of MLH1 expression is suspicious of possible Lynch syndrome

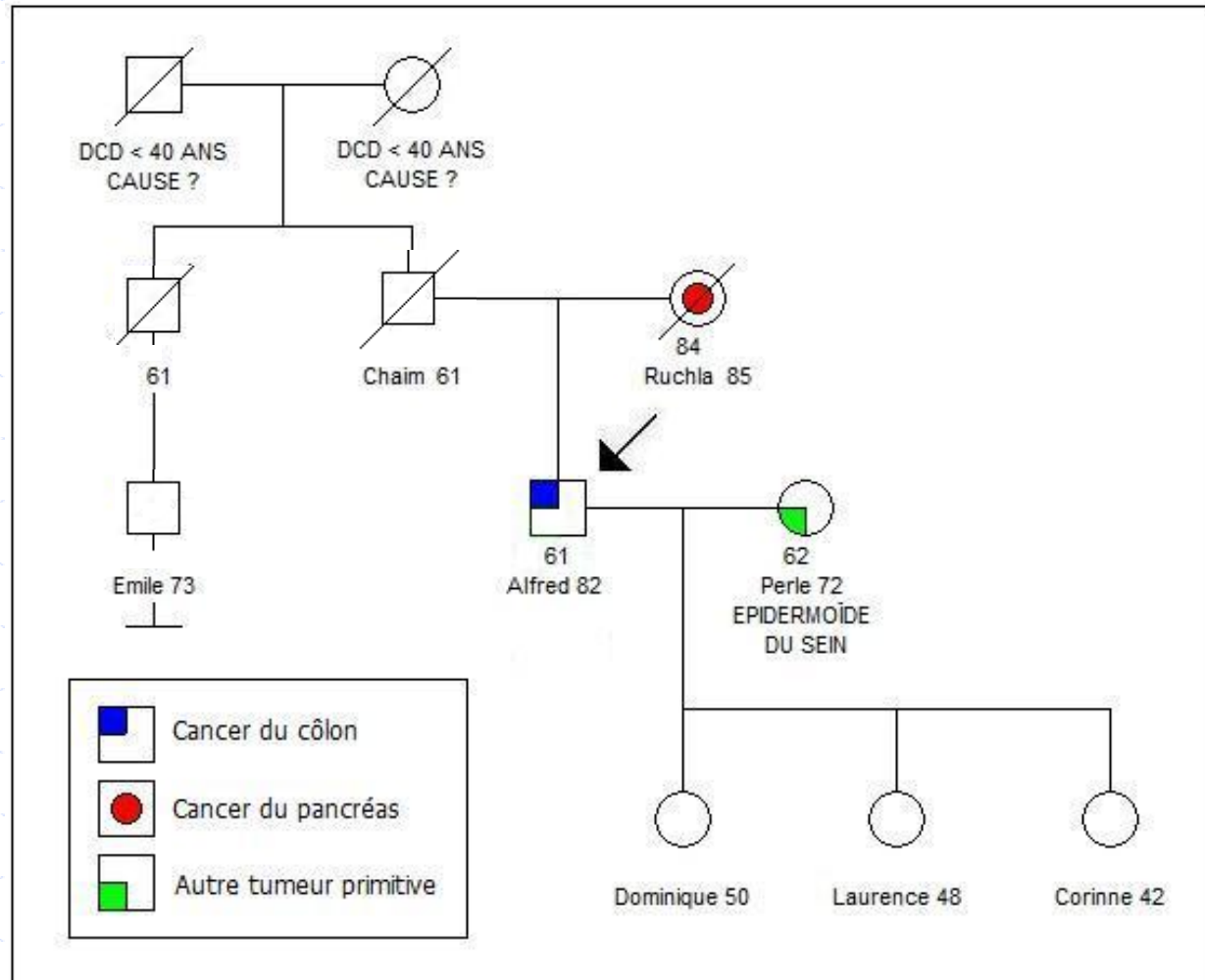
1. MSI-H CRC is **left-sided**
2. “Early” age at diagnosis of CRC is (<70)
3. Personal and/or familial history of other tumors belonging to the spectrum of Lynch-associated tumors

NB: an investigation should be undertaken if **any** of these criteria is present

Case n°1.

- ◆ Extremely fit couple of Jewish Ashkenazy origin
- ◆ ♂ 82, colon cancer at age 61
- ◆ ♀ 73, breast cancer at age 62
- ◆ Three healthy daughters

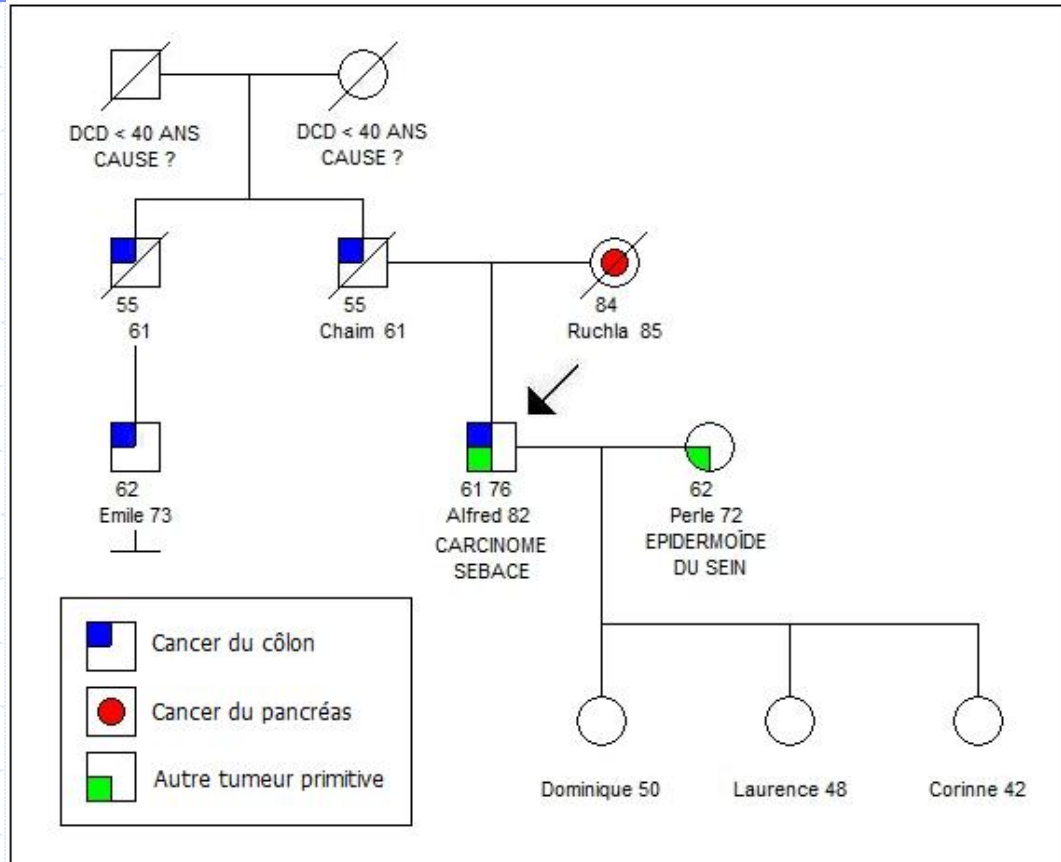
Familial anamnesis



Alfred's personal anamnesis

- ◆ One polyp removed at age 40
- ◆ Colon cancer age 61
 - Right-sided
 - Well differentiated
 - No polyp seen on right hemicolectomy
- ◆ Three skin tumors > 70 (not mentioned by A)
 - Kerato-acanthoma
 - Basal cell carcinoma
 - Sebaceous carcinoma

Summary of Alfred's personal and familial anamnesis



No polyposis \Rightarrow typical Lynch syndrome with Muir-Torre syndrome

A typical Lynch syndrome ?

Amsterdam II criteria

1. Three relatives with an HNPCC-associated cancer (colorectal, endometrial, small bowel, ureter, or renal pelvis), one a first-degree relative of the other two ✘
2. Cases that span at least two generations ✘
3. At least one cancer case diagnosed before age 50 years NO

Modified Amsterdam criteria

1. In very small families, two colon cancer cases in first-degree relatives spanning at least two generations, one case diagnosed before age 55 years NO
2. In families with two first-degree relatives with colon cancer, a third relative with an unusual early-onset cancer or endometrial cancer NO

Laboratory analyses

- ◆ Best guess: germline hMSH2 mutation
 - Muir-Torre is strongly associated with hMSH2
- ◆ First analysis performed: immunohistochemistry of available sebaceous carcinoma
 - loss of hMLH1
- ◆ ⇒ analysis of germline hMLH1
 - → hMLH1 c.1739-40delCA
 - an obviously deleterious mutation

Reflexions about case n°1 :

- ◆ An obvious Lynch syndrome although none of the colon cancers has been diagnosed before the age of 50.
- ◆ HT Lynch in 1991: "Recognition of Lynch syndromes is wholly dependant upon a well orchestrated family history. Unfortunately, in the usual clinical practice settings [...] **the disorder frequently goes undiagnosed.**"
- ◆ This remains true today

Case n°2. An 82 year-old lady affected by colon cancer

- ◆ An apparently sporadic large cancer in the cæcum
- ◆ Pathological report:
 - Diameter 7,5 cm
 - Poorly differentiated adenocarcinoma
 - Focal mucinous differentiation (<50%)
 - No polyp seen on right hemicolectomy
- ◆ Good prognosis: pT2N0M0

No one suspects there might be a problem

- ◆ Her doctors do not worry, on the contrary!
- ◆ The patient does not worry:
 - she suffers from senile dementia,
 - she is of Spanish origin and has been living in Belgium since the early fifties
- ◆ Her three healthy children do not worry
 - They were raised in Belgium and have lost contact with their Spanish relatives

Results of immunohistochemistry (IHC) of the tumor

- ◆ Analysis to be performed on each new colon cancer operated in the CHU
- ◆ Results:
 - → specific loss of MSH2 and MSH6
 - MLH1 and PMS2 are normally expressed
- ◆ IHC showing MMR loss are transmitted to cancer geneticist

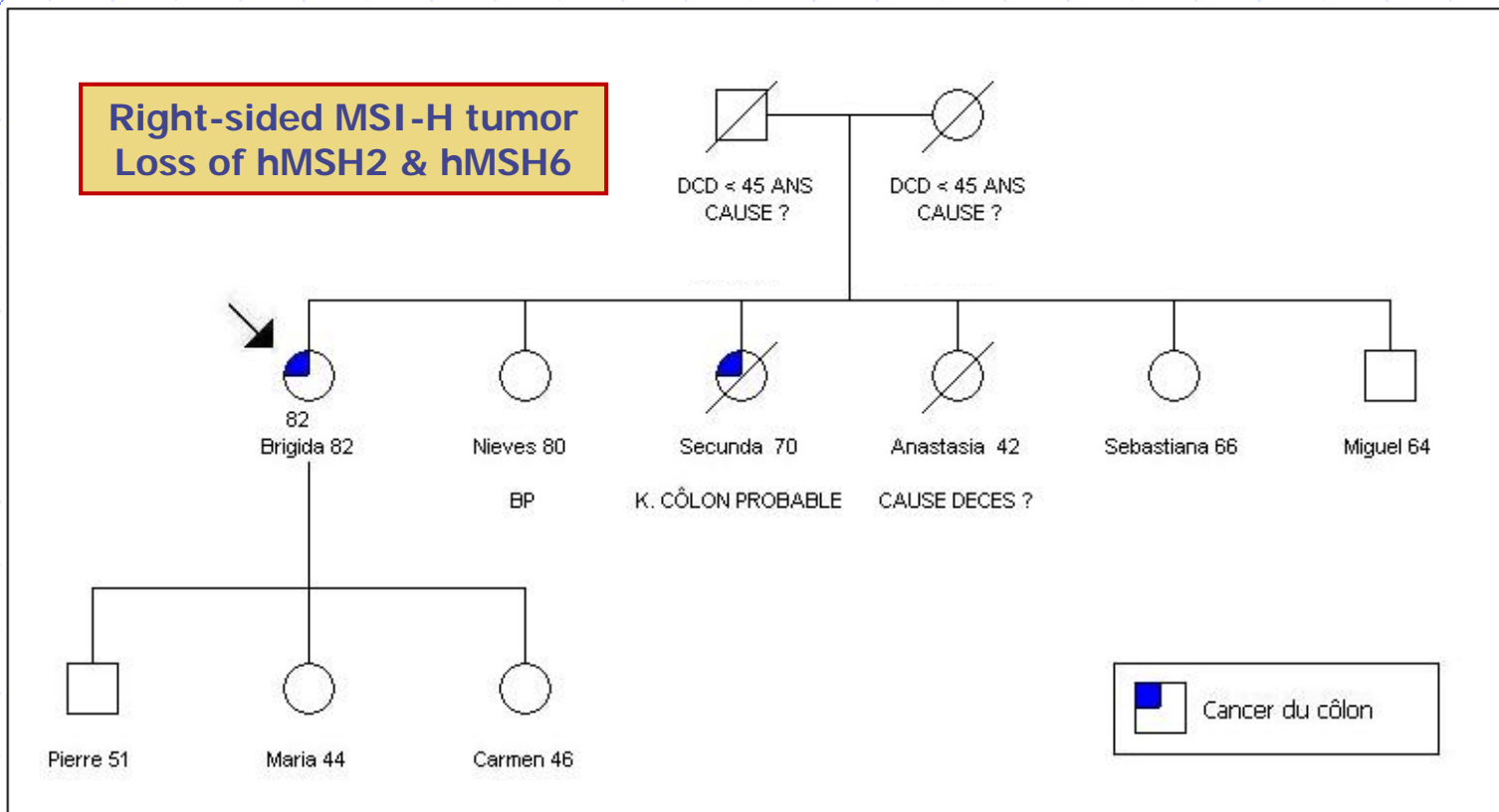
A right-sided MSI-H tumor in an old lady. So what?

- ◆ The only details that raise suspicion are the **loss of hMSH2 and hMSH6**
- ◆ This is most unusual for an apparently sporadic colon cancer case:
 - sporadic MSI-H CRCs are caused by hMLH1 loss of function due to hypermethylation of hMLH1 promoter
- ◆ **IHC → germline hMSH2 mutation?**

Consequence of IHC results

- ◆ A letter is sent to the gastroenterologist taking care of the patient
- ◆ A molecular analysis of the tumor is done that confirms the MSI-H profile
- ◆ The patient comes with her oldest son
 - Very difficult anamnesis
 - A very likely colon cancer in first-degree relative

Summary of Brigida's personal and familial anamnesis



Family anamnesis neither confirms nor excludes diagnosis of Lynch syndrome

Germline DNA analysis: a new hMSH2 unknown variant

◆ hMSH2 c.211G>C (p.Gly71Arg)

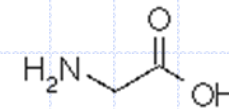
◆ Never reported

◆ Question: is this VUS

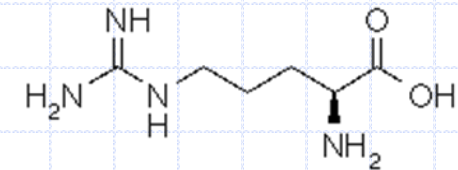
1. a very rare polymorphism?
2. or a pathogenic mutation?

◆ Possible impact if deleterious:

- at protein level?
- at RNA level?
 - ◆ Adequate software predicts splicing defect (Jean-François Vanbellinghen)



gly g Glycin



arg r Arginin

Further molecular analysis

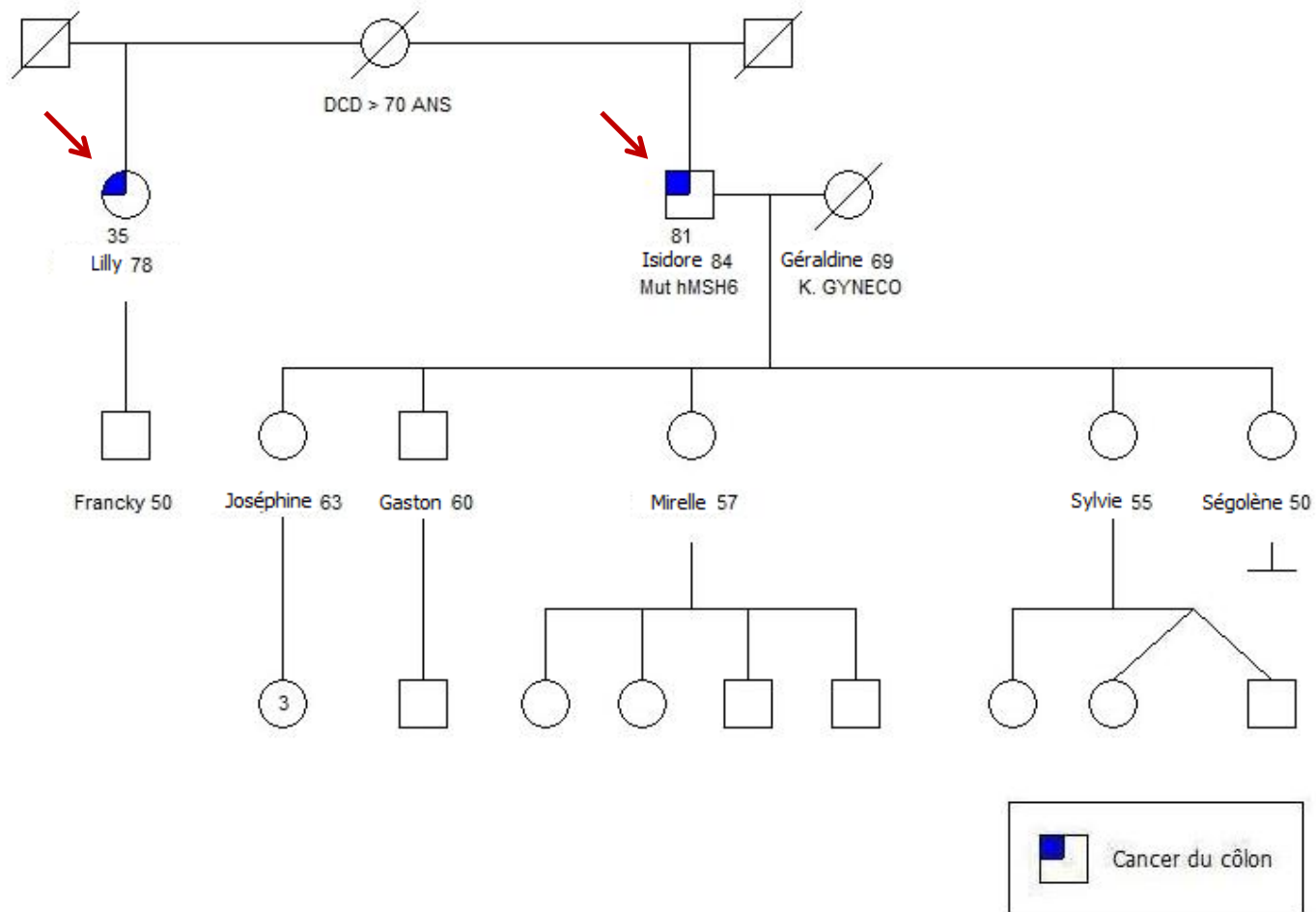
- ◆ A polymorphism allows identification of transcripts from each allele
- ◆ Analysis of WBC cells cDNA
 - **Only the wt transcript is present**
- ◆ hMSH2 c.211G>C blocks splicing of first intron

Molecular analysis finally allows the diagnostic of Lynch syndrome and counseling the children

Case n°3. An 81 year-old man affected by rectal cancer

- ◆ A T2N0 rectal cancer
- ◆ Pathological report :
 - Diameter 1,5 cm (after radiotherapy)
 - Mucinous adenocarcinoma
 - No polyp seen on the 23 cm-long sigmoid and rectal mucosa
- ◆ IHC → **specific loss of MSH6...**

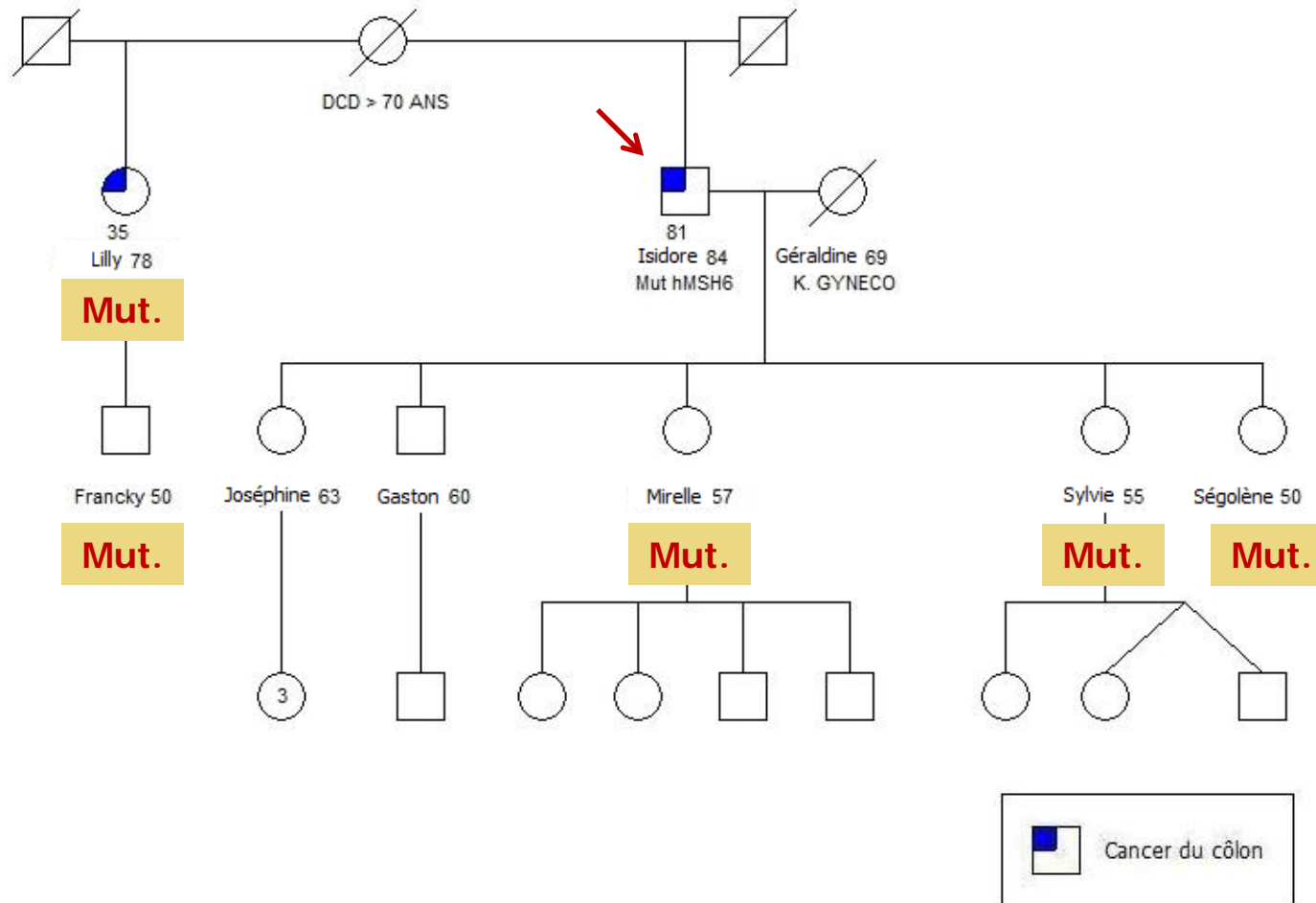
Family history



Germline DNA analysis: hMSH6 c.1118_19insTT

- ◆ An obviously pathogenic frameshift mutation
- ◆ that confirms the diagnostic of Lynch syndrome in this octogenarian
- ◆ a diagnosis that allows a useful genetic counseling in the family

Family counseling



Reflections about case n°1

- ◆ Case n°1 was a clinically obvious Lynch syndrome in which **none** of the colon cancers had been diagnosed before the age of 50.
- ◆ Systematic MMR-immunohistochemistry of all CRCs and all sebaceous carcinoma might have helped the physician to do a better job.

Reflections about case n°2

- ◆ The best possible clinical practice never would have been sufficient to diagnose this case that was anything but obvious!
- ◆ **Only** routine immunohistochemistry allowed diagnosis

Reflections about case n°3

- ◆ Suppose the proband had died of an heart attack at the age of 80...
- ◆ No cancer at the age of 80 DOES NOT exclude Lynch syndrome
 - Late-onset CRCs have been known for a long time in hMSH6 associated Lynch s.
- ◆ Only routine MMR protein IHC allowed diagnosis of Lynch syndrome

Routine MMR IHC in CRCs

- ◆ Diagnosis of Lynch syndrome remains difficult in 2010
- ◆ Historical family-based diagnostic criteria remain relevant but have a very serious lack of sensitivity
- ◆ MMR IHC alone allows diagnosis of Lynch syndrome **in > 50% of cases**
 - Loss of MSH2, loss of MSH6, loss of PMS2
- ◆ hMLH1 germline mutation-associated CRCs can be very suggestive of Lynch syndrome if
 - **Left-sided** and/or diagnosed at an “early” age (<70?)
- ◆ **Late-onset CRC never excludes Lynch syndrome (not even an absence of cancer at 80)**

Drug sensitivity of MSI-H CRCs

1. MSI-H CRCs are **intrinsically resistant to the action of 5-FU**

1. Biological involvement of MMR system in the triggering of apoptosis cause by 5-FU
2. Multiple clinical data

2. MSI-H CRCs are **very sensitive to**

1. CDDP
2. irinotécan

Table 1 MMR genes associated with Lynch syndrome

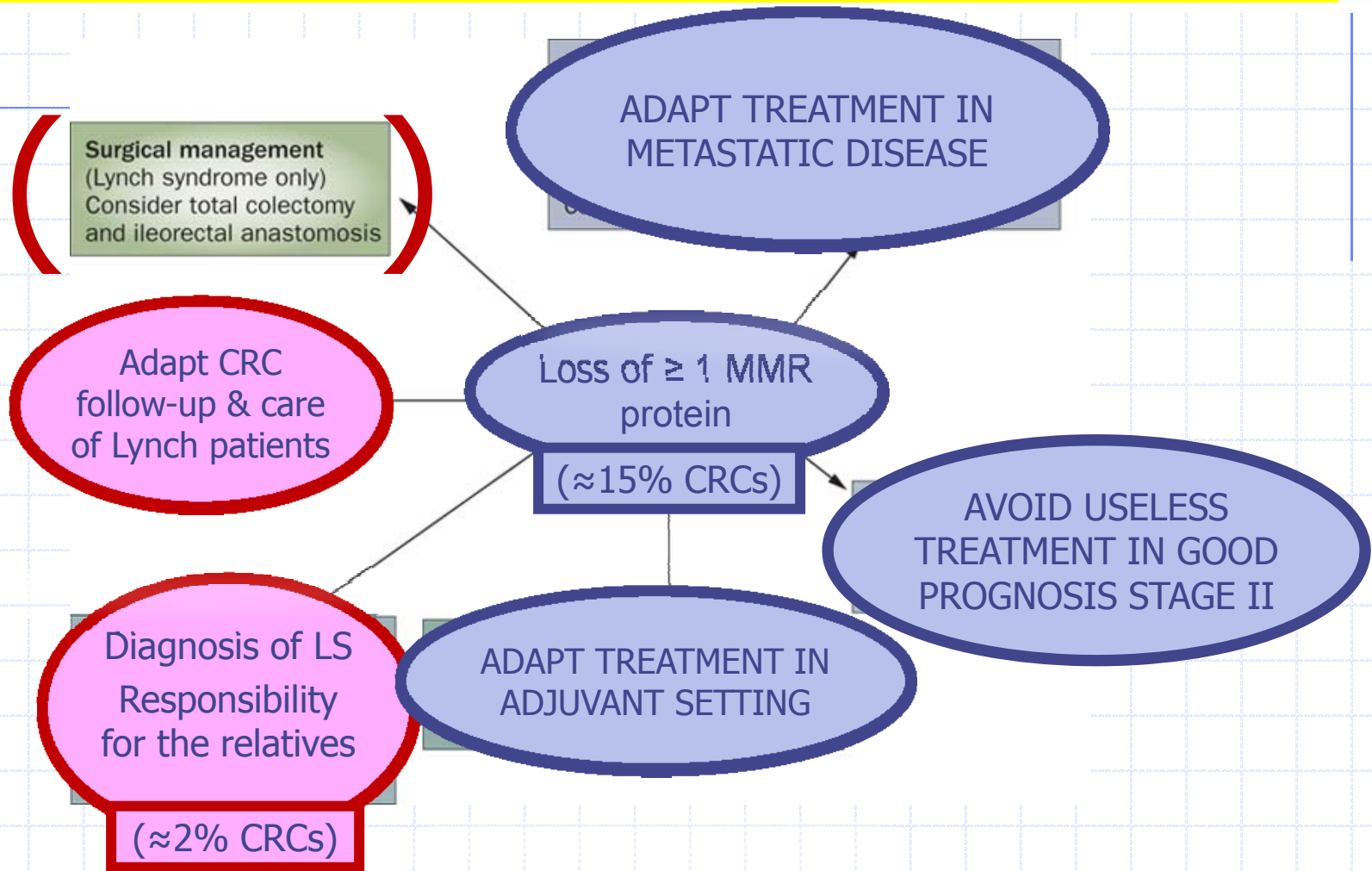
Table 1 | MMR genes associated with Lynch syndrome^{14,30,34,132}

Affected gene	Contribution to Lynch syndrome cases (%)	Median age at presentation (years)	Features of IHC	Sensitivity of IHC in germline mutation detection (%)	Sensitivity of MSI testing in germline mutation detection (%)
<i>MLH1</i>	32	45	Loss of PMS2 expression	92	92
<i>MSH2</i>	39	45	Loss of MSH6 expression	93	93
<i>MSH6</i>	14	56	Isolated protein loss	100 >>	25
<i>PMS2</i>	15	59	Isolated protein loss	100 >>	67
All	100	40–60	–	83–94	73–85

Abbreviations: IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability.

Hewish, M. *et al.* (2010) Mismatch repair deficient colorectal cancer in the era of personalized treatment
Nat. Rev. Clin. Oncol. doi:10.1038/nrclinonc.2010.18

Why do you need MMR IHC in the management of every CRC?



Hewish, M. *et al.* (2010) Mismatch repair deficient colorectal cancer in the era of personalized treatment *Nat. Rev. Clin. Oncol.* 2010.18

Conclusion : MMR protein IHC should be mandatory

- ◆ Amsterdam and Bethesda criteria belong to the history of Lynch syndrome identification.
- ◆ It should not be allowed to treat a CRC without the result of the IHC of MMR proteins:
 1. It is a great help in the identification of persons and families affected by Lynch syndrome
 2. MMR-deficient CRCs require specially-tailored treatments

THE END

Special thanks

- ◆ To the patients and their family
- ◆ To Jean-François Vanbellinghen
 - who is always ready to implement a new technique that may help the clinician to understand
 - who is presently searching for a job as molecular biologist
- ◆ To Irène Scagnol who read most of the routine MMR IHC