RAS, BRAF, Microsatellite instability and other molecular markers: how useful are they? Pitfalls in diagnosis

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ESMO preceptorship, Valence, 17.05.19
DISCLOSURE OF INTEREST

Amgen, BMS, MSD, Merck, Sanofi
Content

- Colorectal cancer context
- CRC molecular classification
- Diagnostic value
- Prognostic value
- Therapeutic value
- Perspectives
- Conclusion
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Tis T1 T2 T3 T4

MUCOSA
Muscularis Muscosae -->
SUB-MUCOSA

MUSCULARIS

SUB-SEROSA -->
SEROSA -->

pT
Tis T1 T2 T3 T4

pN
N0 : no positive lymph node (LN)
N1 : ≤ 3 positive LN
N2 : ≥ 4 positive LN

pM
M0 : No distant metastasis
M1 : Distant metastasis

Organe infiltration and / or visceral peritoneal perforation

TNM UICC 2016 8th Classification
Early CRC treatment

N+
Stage III
Chemotherapy
(FOLFOX, 5-FU)

pT3-4 N0
Stage II
No chemotherapy
But rate of relapses: 20%

Need for additional prognostic factors
Metastatic CRC treatment

- Chemotherapy: 5FU/oxaliplatin/irinotecan
- Targeted therapies:
  - Cetuximab (Erbitux®) (IgG1)
  - Panitumumab (Vectibix®) (IgG2)
  - Bevacizumab (Avastin®) (IgG1)

Aflibercept (Zaltrap®), Regorafenib(Stivarga®)

Need for predictive factors
CRC context

Sporadic
(majority of cases)

Hereditary
(6% of cases)

Screening tools
Optimal management
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CRC tumour progression

Aberrant crypts → small polype → advanced polyp → cancer → metastases
CRC carcinogenesis

- **CIN pathway**
  - Chromosomal Instability

- **CIMP pathway**
  - CpG Island Methylator Phenotype

- **MSI pathway**
  - MicroSatellite Instability

**Epigenetic instability**

- 80-85%
  - \(\approx 20\%\)
  - 15-20%

**Gene Mutations**

- KRAS, TP53 mutation
- hMLH1, p16, MGMT methylation
- BRAF mutation
Molecular profile

Microsatellite Instability

Normal DNA

MSI tumour

Nucleotides
Loss or gain
Deficient MisMatch Repair (MMR) system

MSI CRC carcinogenesis
Terminology

MSI  (microsatellite instable)
    dMMR  (deficient mismatch repair)
        RER+ Phenotype  (Replication Error+)

MSS  (microsatellite stable)
    pMMR  (proficient mismatch repair)
        RER- Phenotype  (Replication Error-)
Immunohistochemistry
Stable tumour (MSS): 4 MMR proteins expressed
Immunohistochemistry

Instable tumour (MSI): extinction of MMR proteins

Loss of hML1

hMSH2 +

hMSH6 +

Parallel loss of PMS2

Negative tumour

Positive tumour
Immunohistochemistry pitfall

MSH6 decreased expression after chemoradiation!

Not MSI!
Immunohistochemistry pitfall

MSH6 normal expression on pre-treatment biopsy

Adapted from Bao et al. Am J Surg Pathol 2010; 34:1798-1804
CRC molecular classification

- Chromosomal instability (CIN pathway)
  - Conventional carcinoma
  - 80-85%

- Epigenetic instability (CIMP pathway)
  - Serrated tumours
  - ≈20%

- Microsatellite instability (MSI pathway)
  - Cancer of the elderly
  - Lynch syndrome
  - 15-20%

- Serrate tumours
- Lieberkühnian
- Médullary/lymphocytes
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Lynch syndrome screening

Early onset CRC and other cancers

Lynch syndrome spectrum

HNPCC: Hereditary Non-Polyposis Colorectal Cancer

Cancer risk: 75% CCR, 50% endometrium, 15% others
Lynch syndrome screening

Germile mutation

Time consuming
Highly specialized laboratories

MLH1
MSH2
MSH6
PMS2

Constant MSI

Mutation of the corresponding gene

DNA
RNA
PROTEINS
MLH1
MSH2
MSH6
PMS2
Extinction
Lynch syndrome screening

- CRC < 60 year-old
- Personal CRC history
- CRC familial context

↓

MSI +

↓

Clinical, endoscopic, and US (if woman) follow-up

+ Familial investigation

Lynch diagnosis

Oncogenetics team consultation
Germline mutation determination

Prophylactic surgery ...
Microsatellite instability context

MSI and hMLH1 loss

Sporadic cancer (15%)

Hypermethylation
  *MLH1* promotor

*BRAF* mutation

Elderly patient

Lynch syndrome (2%)

Absent

Absent

Young patient
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Identification of favorable stage II CRC

No adjuvant chemotherapy (5-FU)
Lack of 5-FU efficacy
Characterization of High risk CRC stage II

- Perforation
- Occlusion
- pT4
- Lymph node < 12
- Poorly differentiated tumour
- Venous/lymphatic Invasion
- Perineural invasion

MSS

Adjuvant chemotherapy: to be discussed (5-FU)
Characterization of aggressive stage III CRC

Intensified chemotherapy: clinical trials
Stratification according mutations?

MSS
KRAS mut.
BRAF mut.

TNM UICC 2009 7th Classification

*Taieb et al JAMA Oncol 2016
Identification of aggressive stage IV CRC

Metastatic setting

MSS
KRAS mut.
BRAF mut.

Intensified chemotherapy: FOLFIRINOX+ Bevacizumab (*BRAF* mut.)

Ongoing clinical trials (combined targeted therapies)
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Anti-EGFR targeted therapies

*RAS mutations = marker of resistance*
Résistance: mutations **KRAS**

Normal différenciation, proliferation and growth

abnormal différenciation, proliferation and growth

Adapted from Van Krieken et al. Virchows 2008;453:417-431
Recommendations

*RAS testing mandatory before anti-EGFR therapy*

Primary CRC

Metastasis

Or
Molecular techniques

exhaustive
- Sanger sequencing
- HRM screening sequencing

targeted
- allele specific PCR
- Snap shot
- Pyrosequencing

Quick turn around time
Quality of the pre-analytique step

Selection

Macrodissection

Mutation ?

Quality (age) of the paraffin, type of fixatives...

pitfall !
Quality of the pre-analytique step

**KRAS genotyping in rectal adenocarcinoma specimens with low tumor cellularity after neoadjuvant treatment**

Florence Boissière-Michot\textsuperscript{1,}\textsuperscript{*}, Evelyne Lopez-Crapez\textsuperscript{2,}\textsuperscript{*}, Hélène Frugier\textsuperscript{1}, Marie-Laurence Berthe\textsuperscript{3}, Alexandre Ho-Pun-Cheung\textsuperscript{2}, Eric Assenat\textsuperscript{4}, Thierry Maudelonde\textsuperscript{3}, Pierre-Jean Lamy\textsuperscript{2} and Frédéric Bibeau\textsuperscript{1}

Use the pretreatment biopsy
CRCm molecular biomarkers and targets

Amplifications: 2.5%
Mutations: 1.9%

Anti-EGFR resistance?

Anti-HER2 Targeted therapies?
Trastuzumab + lapatinib (HERACLES)
Trastuzumab + pertuzumab

Raghac ASCO 2016

Sartore-Bianchi Lancet Oncol 2016
Hurwitz ASCO GI 2016
Marsoni AACR 2017

HER-2
40%

RAS and BRAF WT

RAS mutated
50%

MSI
5%

BRAF
10%

Mut KRAS ex 3, 4

Mut NRAS

Mutation KRAS exon 2

SPECTAcolor: Folprecht ESMO 2016, abst 4580
Immunotherapy

MSI CRC: immunogenic tumour

Metastatic MSI CRC

Immune escape

Crohn-like reaction

Lymphocytic infiltrate

CD3
Immunotherapy

Check-points immunity inhibitors

MSI CRC: immunogenic tumour

High response rate (anti-PD1)

Immunotherapy

Anti-PD-1 treatment: overall survival

Selection of patients based on MSI status

Association of Primary Resistance to Immune Checkpoint Inhibitors in Metastatic Colorectal Cancer With Misdiagnosis of Microsatellite Instability or Mismatch Repair Deficiency Status

Romain Cohen, MD; Elisabeth Hain, MD; Olivier Buhard, MSc; Agathe Guilloux, PhD; Armelle Bardier, MD; Rachid Kaci, MD; Philippe Bertheau, MD, PhD; Florence Renaud, MD, PhD; Frédéric Bibeau, MD, PhD; Jean-François Fléjou, MD, PhD; Thierry André, MD; Magali Svrcek, MD, PhD; Alex Duval, MD, PhD
### MSI Testing MSI: only in expert centres?

False positive vs cases: 10% of cases! Mainly PCR interpretation errors!

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Local assessment</th>
<th>Central review</th>
<th>Best response under ICKi</th>
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<tr>
<td></td>
<td>IHC</td>
<td>PCR</td>
<td>IHC</td>
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<td><strong>Prospective cohort (N = 36)</strong></td>
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<tr>
<td>#47</td>
<td>pMMR</td>
<td>MSI</td>
<td>pMMR</td>
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<td><strong>Retrospective cohort (N = 92)</strong></td>
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</tbody>
</table>

MSI Testing MSI: only in expert centres?

**Cohen R et al. Jama Oncol 2018**

Performing the 2 methods: IHC and PCR according the authors...

pitfall!
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RAS and BRAF mutationnal status determination

- Non invasive technique
- Monitoring
  (cf Pierre Laurent Puig et Clara Montagut 2017 lectures)

Circulating tumour DNA ?
Pitfalls

*RAS* mutation analysis in circulating tumor DNA from patients with metastatic colorectal cancer: the AGEO RASANC prospective multicenter study

Accuracy: 94.8%

But...

Absence of liver metastases (peritoneal carcinomatosis): main clinical factor associated with inconclusive circulating tumor DNA results

CRC molecular profile

In primary CRC!

- Predictive impact?
- Bevacizumab: CMS 1? 2-3?
- Anti-EGFR: CMS 2-4?
KRAS mutation testing for predicting response to anti-EGFR therapy for colorectal carcinoma: proposal for an European quality assurance program
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Molecular CRC classification - Useful biomarkers

- **CIN pathway**: 80-85%
  - Conventional carcinoma
  - RAS mutation
    - Anti-EGFR resistance (predictive factor)

- **CIMP pathway**: ≈20%
  - Serrated tumours
  - BRAF mutation
    - Pronostic factor

- **MSI pathway**: 15-20%
  - Cancer of the elderly
  - MSI
    - Lynch diagnosis
    - Pronostic
    - No 5-FU efficacy
    - Anti-PD-1 efficacy

- **Lynch syndrome**