CRC subtypes: from molecular signatures to therapeutic opportunities

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No conflicts of interest.
REALITY

Until recently, the evolution of biomarkers for matched targeted therapies in CRC has been restrictive.

From a therapeutic perspective, only the \textit{RAS mutated vs. RAS wild-type} disease classification had clinical utility.
CRC subtypes: from molecular signatures to therapeutic opportunities

PAST

Single gene – single marker – single drug

PRESENT

Multi gene – multi marker – multi drug

FUTURE

Multi omics – systems biomedicine – adaptive (immune) drug
CRC subtypes: from molecular signatures to therapeutic opportunities

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CRC subtypes: from molecular signatures to therapeutic opportunities

PAST

Vilar & Tabernero. Cancer Discov 2013
Matched Targeted Agent

- **KRAS** mutation → MEK inhibitor
- **BRAF** mutation → BRAF inhibitor
- **PIK3CA** mutation → PI3K inhibitor
- **MET** overexpression → MET inhibitor

*One test - one drug (companion diagnostics)*
CRC subtypes: from molecular signatures to therapeutic opportunities

Matched Targeted Agent

- KRAS mutation $\rightarrow$ MEK inhibitor
- BRAF mutation $\rightarrow$ BRAF inhibitor
- PIK3CA mutation $\rightarrow$ PI3K inhibitor
- MET overexpression $\rightarrow$ MET inhibitor

Response rate $<5\%$

1. DRUG?
2. PRIMARY-METASTASIS HETEROGENEITY?
CRC subtypes: from molecular signatures to therapeutic opportunities

Matched Targeted Agent

\[ \text{BRAF mutation} \quad \rightarrow \quad \text{BRAF inhibitor} \]

MELANOMA - dabrafenib

Hauschild et al, Lancet 2012
Is metastasis different from primary?

Brannon et al, Genome Biol 2014
CRC subtypes: from molecular signatures to therapeutic opportunities

HOW TO IMPROVE CLINICAL BENEFIT FURTHER?

Deeper biological understanding of the disease

- Genomic
  - Transcriptomic
  - Immune-Stromal
CRC subtypes: from molecular signatures to therapeutic opportunities

CO-OCCurring ALTERATIONS

TCGA, Nature 2012
CRC subtypes: from molecular signatures to therapeutic opportunities

DYNAMICS OF TARGET INHIBITION

**Cetuximab** inhibits the EGFR signaling pathway by disrupting the interaction with the ligand. **BYL719** targets the PI3Kα signaling node, which is involved in regulating the PIP2/PIP3 levels. **Encorafenib** acts on the BRAF signaling node, inhibiting its activity. The downstream effects include the regulation of proliferation, angiogenesis, survival, and invasion and metastasis of cancer cells.
CRC subtypes: from molecular signatures to therapeutic opportunities

CLONAL SELECTION – TEMPORAL HETEROGENEITY

Misale et al, Cancer Discov 2014
Innate anti-EGFR resistance

CRC subtypes: from molecular signatures to therapeutic opportunities

Dienstmann et al, Cancer J 2016
CRC subtypes: from molecular signatures to therapeutic opportunities

Innate anti-EGFR resistance

Responsive upfront

- All wild-type
- RAS mut
- RAS + PIK3CA mut
- BRAF V600E mut
- PIK3CA/PTEN mut
- ERBB2 ampl
- MET ampl

Acquired

- EGFR mut
- RAS mut
- RAS + PIK3CA mut
- ERBB2 ampl
- MEK1 mut, ERBB2 mut, others
- MET ampl

1/3 cases co-occurring alterations

Dienstmann et al, Cancer J 2016
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Dienstmann et al, J Clin Oncol 2013
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Matched Targeted Agent

- **KRAS, NRAS, BRAF, PIK3CA** wild-type → **Anti-EGFR mAb**
- **BRAF** mutation → **BRAF inh + anti-EGFR mAb + MEK inh**
- **ERBB2** amplification, **KRAS** wild-type → **Anti-HER2 mAb + pan-HER inh**
- **EGFR** mutation, **RAS/BRAF** wild-type → **Second generation anti-EGFR mAb**
Resistance to anti-EGFR mAb

CRC subtypes: from molecular signatures to therapeutic opportunities

Dienstmann et al, ASCO Ed Book 2015
CRC subtypes: from molecular signatures to therapeutic opportunities

Matched Targeted Agent

KRAS wild-type
NRAS wild-type
BRAF wild-type

Second generation anti-EGFR mAb SYM004

*D RAS/BRAF mutation
* EGFR mutation

Dienstmann et al, Cancer Discov 2015
Matched Targeted Agent

*ERBB2* amplification  
*KRAS* wild-type  

Anti-HER2 mAb + pan-HER inhibitor
CRC subtypes: from molecular signatures to therapeutic opportunities

Matched Targeted Agent

BRAF mutation \rightarrow\text{ BRAF inhibitor + anti-EGFR mAb + MEK inhibitor}

Van Cutsem et al, ESMO-WGIC 2015
CRC subtypes: from molecular signatures to therapeutic opportunities

Matched Targeted Agent

NTRK1 fusion → Pan-TRK inhibitor

De Braud et al, ASCO 2014
HOW TO IMPROVE CLINICAL BENEFIT FURTHER?

Deeper biological understanding of the disease

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Guinney, Dienstmann et al, Nat Med 2015
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Guinney, Dienstmann et al, Nat Med 2015
CRC subtypes: from molecular signatures to therapeutic opportunities

Overall survival

Relapse-free survival

Guinney, Dienstmann et al, Nat Med 2015
**CMS1** and **CMS3** enrichment

- EGFR overexpression
- EGFR ligand overexpression
- BRAF mut
- NF1/MEK1/PIK3CA/PTEN mut
- MSI and CIMP<sub>high</sub>

**CMS2** enrichment

- EGFR overexpression
- EGFR ligand overexpression
- IRS2 amplification
- BRAF mut

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Guinney, Dienstmann et al, Nat Med 2015
CRC subtypes: from molecular signatures to therapeutic opportunities

- Genomic
  - MSI
  - CIN
- Epigenomic
  - Copy number
  - Methylation
- Transcriptomic
  - CMS1
  - CMS3
  - CMS2
  - CMS4

Driver genes
- MAPK mutations

Clinical
- Proximal (Tumor Location)
- Distal
CRC subtypes: from molecular signatures to therapeutic opportunities

- **Genomic**
  - MSI
  - CIN

- **Epigenomic**
  - Copy number
  - Methylation

- **Transcriptomic**
  - CMS1
  - CMS3
  - CMS2
  - CMS4

- **Microenvironment?**

- **Driver genes**

- **Clinical**

- **MAPK mutations**

- **Distal** (Tumor Location)

- **Proximal**
Supervised immune and stromal infiltration analysis

Supervised immune infiltration analysis

CRC subtypes: from molecular signatures to therapeutic opportunities

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Immune-activated

- dMMR – MSI
- Hypermutation

- Th1 cells
- IFNγ
- CXCL9/10/13
- PDL1
- IFNγ
- Cytotoxic T cells
- Macrophages
- NK cells

Immune-tolerant

- Inflamed

- TGFβ
- Complement
- Stromal cells
- Monocytes
- IL-17
- CCL2
- IL-23

Immune-ignorant

Cancer cell

Cancer cell

Th1 cells

Cytotoxic T cells

Macrophages

NK cells

MDSC

Th17 cells

Stromal cells

CCL2

IL-23

Cytotoxic T cells

Macrophages

NK cells
Intra-CMS prognosis as per tumor infiltrating lymphocytes (TILs)

*QuanTILfy* in 98 MSS samples
CRC subtypes: from molecular signatures to therapeutic opportunities

- **Genomic**: MSI, CIN
- **Epigenomic**: Copy number, Methylation
- **Transcriptomic**: CMS1, CMS3, CMS2, CMS4
- **Stroma – Immune Microenvironment**: Highly immunogenic, Poorly immunogenic, Inflamed (immune-tolerant)
- **Driver genes**: MAPK mutations
- **Clinical**: Proximal (Tumor Location), Distal
Transcriptomic classification of CRC is strongly associated with:

i. pathway activation patterns;

ii. immune and stromal contexts.

Reflect the relationships between cancer cell phenotype and corresponding tumor microenvironment.
HYPOTHESIS

Intrinsic gene expression subtyping plus immune and stromal characterization:

- increase the biological understanding of the disease;
- optimize patient stratification based on differences in outcome and response patterns to targeted agents.
CRC subtypes: from molecular signatures to therapeutic opportunities

PAST
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FUTURE
Multi omics – systems biomedicine – adaptive (immune) drug
Molecular-driven therapeutic hypothesis

Progression-free survival
Cetuximab 3rd line in RAS/RAF wt

CRC subtypes: from molecular signatures to therapeutic opportunities
LETTER

Mutant \textit{Kras} copy number defines metabolic reprogramming and therapeutic susceptibilities

Emma M. Kerr\textsuperscript{1}, Edoardo Gaude\textsuperscript{1}, Frances K. Turrell\textsuperscript{1}, Christian Frezza\textsuperscript{1} & Carla P. Martins\textsuperscript{1}

Low glucose + glutathione biosynthesis inhibitor $\rightarrow$ Redox/ROS – DNA damage
CRC subtypes: from molecular signatures to therapeutic opportunities

Cancer Therapeutics Research Portal v2

Unpublished
BRAF mut-like cells are sensitive to vinorelbine
Predictive value – *hypotheses chemotherapies*

Strategy: repurposing of DNA damaging agents? microtubule inhibitors?

CRC subtypes: from molecular signatures to therapeutic opportunities
OX40 agonist + TGFBR inhibitor in CMS4 *in vivo* model

Figure A: Tumor CD8+ cell infiltration in different treatment groups (Control, αOX40, SM16, SM16 + αOX40).

Figure B: Percent survival of CT26 tumor-bearing mice across different treatment groups (Control + Rat IgG, Control + αOX40, SM16 + Rat IgG, SM16 + αOX40).
OX40 agonist in hypermutated MSS \textit{in vivo} model
CRC subtypes: from molecular signatures to therapeutic opportunities

Predictive value – *hypotheses immune therapies*

**Strategy:**
PD1 blockade + OX40 agonist?
CRC subtypes: from molecular signatures to therapeutic opportunities

Predictive value – *hypotheses immune therapies*

**Mesenchymal TGFβ activated CMS4**

*Strategy: combination of immuno-stimulatory drugs and inhibitors of immune suppression?*

**Epithelial Canonical CMS2**

*Strategy: epigenetic immune modulation and PD1 blockade? MEK inhibitors and PD1 blockade?*
Atezolizumab (PDL1 block) + cobimetinib (MEK inh) in mCRC

- 4 patients had partial responses (confirmed per RECIST v1.1)
- MSI status of CRC patients was examined by NGS-based scoring: 3 of 4 responders were mismatch-repair proficient (not MSI-H); 1 responder had unknown MSI status and was not evaluable
- Tumor volume reduction was not associated with PD-L1 status: TC3 (n = 1; PD), TC0 (n = 18), NA (n = 4)

Bendell et al, ASCO 2016
CRC subtypes: from molecular signatures to therapeutic opportunities

MoTriColor project

Total: 540 samples

Clinical Sites

5FFPE cuts per sample

Batched 10 days

Total: 126 Plasma Samples

Agenda

Batched 10 days

Eligible for MoTricolor Clinical Trials?

Total: 126 samples

Yes

TGFB active

Patient Included Clinical Trial 1
TGF-B

46 patients

BRAF mut-like

Patient Included Clinical Trial 2
Vinorelbine

40 patients

MSI-like

Patient Included Clinical Trial 3
anti-PD-L1

40 patients

UNITO

Total: 126 Tumor Blocks (primary tumors/biopsies)

Tumor Block
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**PRESENT**

Multi gene – multi biomarker – multi drug

- Genomic – clonal perspective

**FUTURE**

Multi omics – systems biology – adaptive (immune) drug

- Stromal – immune perspective
CRC subtypes: from molecular signatures to therapeutic opportunities

PAST

Single gene – single biomarker – single drug

PRESENT

Multi gene – multi biomarker – multi drug

TARGET CLONAL EVOLUTION

Genomic – clonal perspective

FUTURE

Multi omics – systems biology – adaptive (immune) drug

TARGET MICROENVIRONMENT DEPENDENCIES

Stromal – immune perspective

Intermittent use of targeted therapies, vertical inhibition of convergent pathway alterations, drugging ‘truncal’ genomic events

Combining immune and targeted therapies
CRC subtypes: from molecular signatures to therapeutic opportunities

Differential benefit adjuvant oxaliplatin in NSABP C-07 trial

1,035 patients

Song et al, JAMA Oncology 2016
Still unknown what combination of genomic features (mutation + MSI + gene expression + immune + stromal) will provide the best prediction of drug response.
CRC subtypes: from molecular signatures to therapeutic opportunities

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