Druggable Targets in Colorectal Cancer

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Disclosures

- No relevant relationships to disclose
• Genomic alterations in CRC signalling pathways
• Emerging molecular determinants of resistance to EGFR targeted therapies in mCRC
• Validation of these molecular alterations as alternative ‘drivers’ of CRC tumorigenesis and progression
• Challenging molecular alterations as actionable therapeutic targets within the context of CRC molecular landscape
Deregulation of signalling pathways in CRC

Colorectal cancer progression

A genetic model for colorectal tumorigenesis.
Fearon ER, Vogelstein B.
Cell. 1990 Jun 1;61(5):759-67

Figure from Markowitz S., & Bertagnolli M N Engl J Med 2009; 361:2449-2460
What we have learnt on CRC genomics


• Several hundred molecular aberrations (not only mutations)
• Co-existence of genetic, epigenetic and transcriptional drivers of tumour progression

What we have not learnt so far

• Which are drivers?
• Co-existence of multiple drivers within the same tumour?
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Colorectal cancer preclinical models

GEMMs
Mouse distal colon tumors
Murine CRC cell lines from GEMMs

Human CRC cell lines

Cell-line xenografts, patient-derived tumorgrafts in immunodeficient mice

Biological and functional consequences of cancer alleles
Mechanisms of metastasis development
Test therapies to prevent metastasis formation and to treat established metastases
Mechanisms of primary and acquired resistance to anticancer drugs

Mouse models of Kras mutant colorectal cancer: valuable GEMMs for drug testing?
Colorectal cancer preclinical models

**FIGURE 1.** Di Nicolantonio F. Clin Cancer Res. 2013 Apr 23.
Mouse models of Kras mutant colorectal cancer: valuable GEMMs for drug testing?
Patient derived xenografts

From Tentler J et al., Nature Reviews Clinical Oncology 9, 338-350 (June 2012)
Cetuximab monotherapy mouse clinical trial in PDXs

SURGERY

p0 engraftment (2 mice)

p1 expansion (4-8 mice)

p2 treatment (12-24 mice)

NUMBER OF INITIAL SAMPLES

150 → >85% → 85

Biobank Archive
RNA extraction
Genomic DNA extraction

Bertotti & L. Trusolino, Molecular Pharmacology, IRCCS - Candiolo
Response to EGFR signalling inhibition in mCRC PDXs

KRAS ex.3/ex.4, NRAS, BRAF mutations are associated resistance to cetuximab in KRAS ex.2 wt PDXs

* Quadruple negative (unexplained) non responders
‘KRAS/NRAS/BRAF negative’ tumours

Screened for candidate genetic alterations by gene expression profile
HER2 and MET are actionable biomarkers that correlate with resistance to EGFR therapies in CRC

Genomic alterations in CRC signalling pathways

Emerging molecular determinants of resistance to EGFR targeted therapies in mCRC

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Challenging molecular alterations as actionable therapeutic targets within the context of CRC molecular landscape
Is MET a target in MET amplified CRC?

Is HER2 a target in CRC? Only a small molecule-antibody combination of anti-HER2 therapies has preclinical efficacy.

... But efficacy is variable

HER2 ampl. CRC186

Volume (mm$^3$)

Days from implantation
Heracles Studies

**HER2 Amplification for Colo-rectal Cancer Enhanced Stratification**

**Heracles Companion Diagnostic**

A retrospective study to establish a HER2 scoring system for CRC to identify suitable patients for enrollment in trial of trastuzumab and lapatinib in advanced metastatic colorectal cancer.

**Heracles Therapeutic Trial**

A single arm, Phase II, multi-center, trial designed to assess the objective response rate in an HER2 amplification-enriched population of mCRC patients receiving trastuzumab + lapatinib.
The HERACLES trial

• HERACLES (*HER2 Amplification for Colo-rectal Cancer Enhanced Stratification*)

• HER-2 amplified chemorefractory mCRC patients (Chair: Salvatore Siena, Niguarda Hospital, Milan, Italy)
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Response to EGFR signaling inhibition in CRC cell lines

Response to Cetuximab

Unpublished data
Response to EGFR signaling inhibition in CRC cell lines

Screened for candidate genetic alterations responsible for cetuximab resistance in RAS/BRAF wt cells by gene expression profile
Outlier kinase expression predicts resistance to EGFRi in CRC cell lines

Response to Cetuximab

- NTRK1
- ALK
- FGFR2
- PDGFRA
- KIT
- NTRK2

- KRAS mutations/ampl.
- NRAS mutations
- BRAF V600E/K
- RAS/BRAF wt
Prevalence of RTK overexpression in CRC specimens

Gene expression does not always correlate with gene amplification

TCGA CRC dataset (195 complete tumors) interrogated through [http://www.cbioportal.org/](http://www.cbioportal.org/)

Cerami E et al., Cancer Discov. 2012 May;2(5):401-4

Gao J et al., Sci Signal. 2013 Apr 2;6(269):pl1
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ALK translocation in CRC cells is associated with tumour sensitivity to ALK kinase inhibition

ALK gene fusions described in CRC – but lack of functional data.

CRC cells
(EML4-ALK)

Unpublished data
FGFR2 amplification in CRC cells is associated with tumour sensitivity to FGFR kinase inhibition.
NTRK1 rearrangements in CRC cells are associated with tumour sensitivity to TRKA kinase inhibition

- **NTRK1**, **NTRK2** and **NTRK3** genes encode for TRKA, TRKB and TRKC receptors
- Cell line found to have TPM3-NTRK1 translocation
- TRK inhibition by RXDX-101 shows activity *in vitro* and *in vivo* in NTRK1 rearranged CRC cells

Partial Response in Patient with TrkA Rearranged Colorectal Cancer

Pre Treatment
March 20, 2014

Cycle 1
April 23, 2014

Patient screened and treated at Ospedale Niguarda Ca’ Granda, Milan, Italy

De Braud F et al., 2014 ASCO Annual Meeting, J Clin Oncol 32:5s, 2014 (suppl; abstr 2502)
Prevalence of NTRK1 overexpression in CRC

1/66 (1.5%) CRC specimens (by RNA expression and IHC, rearrangement confirmed by FISH)


1/195 (0.5%) CRC specimens (by RNA Seq)

TCGA CRC dataset (195 complete tumors) interrogated through http://www.cbioportal.org/

Cerami E et al., Cancer Discov. 2012 May;2(5):401-4

Gao J et al., Sci Signal. 2013 Apr 2;6(269):pl1
Deregulation of RTK-RAS-PI3K signaling in CRC

- **ALK**, **ROS1**, **TRKs**, **FGFRs**, **EGFR**, **ERBB2**, **ERBB3**, **IGF1R**, **PDGFRs**, **MET**

- **BRAF** mut (5-8%), **CRAF** ampl (0.5%)
- **KRAS** mut (45%), **NRAS** mut (6%), **HRAS** mut (0.5%)
- **PIK3CA** mut (15%), **PTEN** mut/del (10%)
- **MAP2K1** mut (1%)
- **PTEN**
- **SOS**, **GRB2**, **IRS1/IRS2**, **PI3K**, **PIK3CA** mut (15%)

- **ERBB2**, **FGFR**, **ALK**, **ROS1**, **TRK**
- **S6**
Genomic classification of CRC: not there yet?

- **KRAS** 45%
- **BRAF** 5%
- **PIK3CA** 5%
- **NRAS** 4%
- **GNAS** 0.3%
- **PTEN** 5%
- **BRCA1/2** 2%
- **STK11** 1.5%
- **AKT1** 0.3%
- **MLK4** 2%
- **IDH1** 0.3%
- **JAK2** 0.3%
- **FLT3** 0.3%
- **KIT** 0.5%
- **ALK** 1%
- **TRKA** 1%
- **TRKB** 1%
- **RET** 1%
- **ROS** 1%
- **EGFR** 1%
- **HER2 amplified** 2%
- **HER3** 1%
- **HER4** 1%
- **FGFRs amplified** 4%
- **CDK8 amplified** 4%
- **MET amplified** 1%
- **HER2** 1%
- **HER4** 1%
- **HER3** 1%
- **HER2 amplified** 2%
- **HER3** 1%
- **HER4** 1%
- **KRAS** 45%
- **Others** 7%
Challenging drivers as targets

- Pinpointing exceptions in one tumour type needs big numbers:
  - Collect many cases and establish/find relevant preclinical models for functional validation (avoid mistake of translating from other tumour lineages)

- Identifying exceptions (and the contextual genetic milieu) needs integrated approaches with reliable outcomes:
  - Multi-dimensional genomic exploration of high-quality tumour material
Defining clinically actionable targets

- Explore cancer genomics & study functional relevance in suitable preclinical models
- Find the biomarker(s) to best describe biologically distinct subtypes
- Design POC trial with the appropriate drug(s)
Outline

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Deregulation of signalling pathways in CRC

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