Can we increase Immunogenicity in MSS Metastatic Colorectal Cancer (mCRC)?

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DISCLOSURE SLIDE:

- Personal financial interests, I received honoraria for advisory role, travel grants, research grants (past 5 years):
  Hoffman La-Roche, Bristol Myers Squibb, Bayer, Servier, Amgen, Merck Serono,

- Institutional financial interests, my institution received honoraria due to my investigator contribution in clinical trials from:
  Bayer, Servier, Novartis, Boehringer Ingelheim, Boston Pharmaceuticals, Hoffman la Roche, Genentech
Learning objectives:

• Understand the immune-biology of non-MSI colorectal cancer.

• Review & discuss the results of the recently reported initiatives to increase immunogenicity on this patient population.

• Know the rational behind the different strategies under development.
MSI/MSS: Does a lower Tumor Mutation Burden (TMB) make a difference?

CRC Samples ordered according TMB

TILs

Benefit from pembrolizumab

Adapted from TCGA Nat 2012 – Adapted from LE D NEJM 2015
Discrepancies between MTB and RR seen with immunotherapy

Tumor Mutational Burden and ORR Correlation with Anti–PD-1 axis cross-tumor type

MSS mCRC has a immune-suppressant biology:

- **WNT signalling**
  - Dickkopf 1 (DKK1)
  - LRP5
  - FZD10
  - AXIN2
  - APC
  - C/EBPβ
  - TCF7

- **TGF-β signalling**
  - TGF-β
  - Activin

- **PI3K signalling**
  - AKT2
  - PI3Kγ

- **RTK-RAS signalling**
  - EGFR

- **P53 signalling**
  - ATM

References:
- TCGA, Nat 2012
- Fearon, Cell 2000
- Walther, Nat Rev Can 2009
Wnt pathway activation leads to dendritic cell exclusion from the tumor:

Melanoma

Localized CRC

mCRC

MAPK-PI3K Pathway Activation impairs antigen presentation:

MHC class 1

CD8+ T cells per tumour cell

Proportion of CD8+ cells with low PD-1 expression

Proportion of IFNγ-producing CD8+ T cells

Ebert et al. Immunity 2016 – Bendell J ASCO 2015
TGF-Beta activation inhibits anti-cellular immunity and causes unspecific inflammation:

**Bladder Cancer:**

**CRC:**

Taurello Nat 2018 – Mariathasan Nat 2018
Need for immune-biology biomarkers:

First generation immune-biomarkers

- Tumor mutation burden
- Markers of hot tumors
- PD-L1

Second generation immune-biomarkers:

- Gene signatures

[Diagram showing relationships between biomarkers and tumor characteristics]
Transcriptomic classification of mCRC

**CMS1**
- MSI Immune
- 14%
- MSI, CIMP high
- Hypermutation
- SCNA high
- BRAF mutations
- Immune infiltration and activation
- Worse survival after relapse

**CMS2**
- Canonical
- 37%
- SCNA low, CIMP low
- WNT and MYC activation
- Better survival after relapse

**CMS3**
- Metabolic
- 13%
- Mixed MSI status
- Metabolic deregulation

**CMS4**
- Mesenchymal
- 23%
- SCN high
- Stromal infiltration
- TGF beta activation
- Worse relapse-free and overall survival

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Disclaimer for CMS:

- CMS classifier was built to describe the different biological backgrounds of colorectal cancer.

- The classification can not be used to take therapeutic decisions.

- For the purpose of this talk I am going to use CMS to depict the different CRC populations at the molecular level and propose rational-based therapeutic approaches.
Transcriptomic classification of mCRC

- **CMS1**
  - MSI Immune
  - 14%
- **CMS2**
  - Canonical
  - 37%
- **CMS3**
  - Metabolic
  - 13%
- **CMS4**
  - Mesenchymal
  - 23%

<table>
<thead>
<tr>
<th>Feature</th>
<th>CMS1</th>
<th>CMS2</th>
<th>CMS3</th>
<th>CMS4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSI, CIMP high</td>
<td>14%</td>
<td>37%</td>
<td>13%</td>
<td>23%</td>
</tr>
<tr>
<td>Hypermethylation</td>
<td></td>
<td>SCNA high</td>
<td>Mixed MSI status</td>
<td>SCN high</td>
</tr>
<tr>
<td>SCNA low, CIMP low</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>BRAF</em> mutations</td>
<td></td>
<td>WNT and MYC activation</td>
<td>Metabolic deregulation</td>
<td>TGF beta activation, Metastasis</td>
</tr>
</tbody>
</table>
| IMMUNE INFILTRATION AND ACTIVATION | Worse survival after relapse | Better survival after relapse | Worse relapse-free and overall survival | **CMS4**

**CMS4**
- **CMS4**
  - MSI LIKE
  - 23%
  - T cells chemotaxis and activation
  - T cells specific inhibition
  - Myeloid cells chemotaxis
  - Angiogenesis
  - Immunosuppression
  - Complement
  - Tertiary lymphoid structures
  - Major Histocompatibility Complex

**CMS3**
- **CMS3**
  - Metabolic
  - 13%
  - TGF beta activation
  - Angiogenesis

**CMS2**
- **CMS2**
  - Canonical
  - 37%
  - WNT and MYC activation
  - Metabolic deregulation

**CMS1**
- **CMS1**
  - MSI Immune
  - 14%
  - T cells chemotaxis and activation
  - T cells specific inhibition
  - Myeloid cells chemotaxis

**BRCAness**
- BRCA mut.
- BRCAness.
- ARID 1

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Therapeutic alternatives based on molecular background:

- MoTriColor Clinical Trial 3: Atezolizumab + Bev in CMS1 pts.
- Anti-CTLA4- Anti-PD1 +/- Radiotherapy

CMS1:
- MSI 10%
- CMS1 non MSI 5%

CMS2:
- Block Wnt Pathway
- Allow Dendritic Cell infiltration
- Foster T-Cell anti-tumor reactivity
- Wnt-inhibitors + anti-PD1

CMS3:
- Block MAPK/PI3K pathway
- Increase MHC-Class 1 expression
- Foster T-Cell anti-tumor reactivity
- Mek inh + anti-PD1 ?
- Natural killer engaging therapies

CMS4:
- Block TGF-beta Pathway in CMS 4 mCRC
- Change the Inflammatory Microenvironment to a TH1 Immune response
- Foster T-Cell infiltration
- Sensitivity to Immune-checkpoint inh.
- TGF-beta inh + anti-PD-1

CMS1: CMS2: CMS3: CMS4:
Transversal strategies:

- Chemo or RT + Immune-Checkpoint Inhibitors (ICI)
- Tyrosine-Kinase Inh. + ICI combos
- ICI + ICI combos
- Metabolic disruptors + ICI
- T-Cell engaging bi-Specific Antibodies
- Personalized Cancer Vaccines
- Adoptive T-cell Therapy
  - TIL-Therapy
  - CAR-T Cells

Regorafenib – Nivolumab: REGONIVO trial

Rego 80 – Nivo 3mg/kg

Figure 1. Duration of Treatment

Adverse event, N (%) | All N = 50 | Regorafenib 80 mg N = 22 | Regorafenib 120 mg N = 25 | Regorafenib 160 mg N = 3

| All grades | Grade23 | All grades | Grade23 | All grades | Grade23 | All grades | Grade23 |
| All events | 50(100) | 20(40) | 22(100) | 10(45) | 25(100) | 11(44) | 3(100) | 3(100) |
| Palmar-plantar erythrodysesthesia | 8(16) | 5(10) | 13(59) | 1(9) | 0(0) | 20(80) | 5(20) | 2(67) | 1(33) |
| Hypertension | 24(48) | 2(4) | 10(46) | 2(9) | 14(56) | 6(24) | 0(0) | 0(0) | 0(0) |
| Fatigue | 23(46) | 0(0) | 10(46) | 0(0) | 12(48) | 0(0) | 1(33) | 0(0) | 0(0) |
| Rash | 21(42) | 6(12) | 8(36) | 0(0) | 11(44) | 9(20) | 2(67) | 1(33) |
| Fever | 20(40) | 0(0) | 8(36) | 0(0) | 11(44) | 0(0) | 1(33) | 0(0) | 0(0) |
| Proteinuria | 19(39) | 6(12) | 5(23) | 2(9) | 8(32) | 3(12) | 2(67) | 1(33) |
| Liver dysfunction | 14(28) | 3(6) | 5(23) | 0(0) | 8(32) | 1(4) | 1(33) | 0(0) | 0(0) |
| Oral mucositis | 11(22) | 0(0) | 3(14) | 0(0) | 6(24) | 0(0) | 2(67) | 1(33) |
| Diarrhea | 11(22) | 1(2) | 5(23) | 0(0) | 4(16) | 1(4) | 2(67) | 0(0) | 0(0) |
| Decreased appetite | 11(22) | 0(0) | 6(27) | 0(0) | 5(20) | 0(0) | 0(0) | 0(0) | 0(0) |
| Hypothyroidism | 6(12) | 0(0) | 4(18) | 0(0) | 2(8) | 0(0) | 0(0) | 0(0) | 0(0) |
| Hypothyroidism | 6(12) | 0(0) | 4(18) | 0(0) | 2(8) | 0(0) | 0(0) | 0(0) | 0(0) |
| Hoarseness | 5(10) | 0(0) | 4(18) | 0(0) | 1(4) | 0(0) | 0(0) | 0(0) | 0(0) |
| Platelet count decreased | 5(10) | 1(2) | 0(0) | 0(0) | 4(16) | 1(4) | 1(33) | 0(0) | 0(0) |

One treatment-related death was observed due to diabetic ketoacidosis

Fukuoka S, ASCO 2019- Hara WGI 2019
Biology behind REGONIVO:

Regorafenib reduced Tumor Associated Macrophages (M2) and induced M1 macrophages

Hoff S, et al. ESMO Annual Meeting 2017 - Fukuoka ASCO 2019
Open questions after REGONIVO:

• Can this impressive data be extrapolated to western populations?
  • There are trials ongoing exploring this combination in Europe and US.

• Why elimination of tumor associated macrophages and T-REGs is so important in mCRC?
  • They have never been described as important factors in mCRC immune-microenvironment.
Anti-CTLA-4 + Anti-PD1 combination:

CCTG CO.26 study schema:

Patients with advanced CRC refractory to all available therapy

Randomize

Durvalumab: 1500 mg IV q 28 days
Tremelimumab: 75 mg IV q 28 days, cycles 1-4
+ Best Supportive Care

Primary endpoint:
- OS

Secondary endpoints:
- PFS
- Safety and toxicity
- ORR

Tertiary endpoints:
- QoL
- Correlative studies

Sample Size: 180

Stratify:
- ECOG (0, 1)
- Side of tumor

Exploratory analysis: ctDNA sampling for TMB calculation

Results: overall survival

Median BSC = 4.1 months; 90% CI (3.3-6.0)
Median Durva+Treme = 6.6 months; 90% CI (6.0-7.4)
Stratified Hazard Ratio = 0.72; 90% CI (0.54-0.97); p=0.07
Unadjusted HR = 0.70; 90% CI (0.53-0.92); p=0.03
Anti-CTLA-4 + Anti-PD1 combination:

- 20% of patients had High TMB significantly higher than what reported in other series.

Eric X. Chen ASCO 2019
Open questions after Durva-treme combination:

- 6.6 months OS seems quite modest in light of the toxicity profile of the combination
  - Perhaps the effect can be fostered using radiotherapy...

- Can TMB increase during the course of the disease evolution?

Aparna Parikh WGI 2019
Monalizumab + durvalumab in mCRC:

- Monalizumab is an anti-NKG2A MoAb
- CRC overexpress HLA-E, the ligand of NKG2A
- NKG2A Blocks the action of NK and CD8 lymphocytes

Phase 2: Monalizumab-Durvalumab

Phase 2 expansion in mCRC in combination with Durvalumab

Seagal N ASCO 2018
Monalizumab + durvalumab in mCRC:

**Table 2. Safety Summary (MSS-CRC Expansion Cohort)**

<table>
<thead>
<tr>
<th>Patients With AEs, Preferred Term</th>
<th>MSS-CRC Expansion (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 1 AE</td>
<td>37 (92.5%)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>10 (25.0%)</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>7 (17.5%)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>7 (17.5%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>7 (17.5%)</td>
</tr>
<tr>
<td>≥ 1 treatment-related AE</td>
<td>22 (55.0%)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>AST increased</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>Pruritus</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>Rash</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>≥ 1 grade 3/4 or SAE</td>
<td>13 (32.5%)</td>
</tr>
<tr>
<td>≥ 1 treatment-related SAE</td>
<td>1 (2.5%)</td>
</tr>
<tr>
<td>≥ 1 treatment-related grade 3/4 AE</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>Fatal AE</td>
<td>0</td>
</tr>
<tr>
<td>≥ 1 AE leading to discontinuation of monalizumab and/or durvalumab</td>
<td>0</td>
</tr>
</tbody>
</table>

AE, adverse event; SAE, serious adverse event.

Seagal N ASCO 2018
Bi-Specific T-Cell engaging antibodies

**CEA-TCB structure**

- Binds simultaneously with 1 arm to CD3 on T cells and with 2 arms to CEA on tumor cells
- Flexible 2-to-1 format enables high-avidity binding and selective killing of high CEA-expressing tumor cells
- Longer half-life compared with other TCB formats
- Silent Fc results in reduced risk of FcγR-related cytokine release/IRRs

**Direct T-cell activation skipping antigen recognition upon binding to CEA protein.**

- Simultaneous binding of TCB to tumor (CEA) and T cells (CD3)
- Killing of tumor cells independent of pre-existing immunity
- T-cell proliferation at site of activation

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Personalized immune therapies are a good option in expert hands.

**Personalized peptide vaccines**

- DNA/RNA analysis and massively parallel sequencing
- Neoantigen discovery and NetMHC/RNA-seq inference

**Adoptive T-Cell Therapy**

- Therapeutic decisions
  - Choice of immune checkpoint blockade therapies
  - Use of neoantigen vaccines?

Conclusion:

• Immunotherapy is not for all-comers in MSS mCRC

• Deep understanding of tumor biology will be crucial to ensure their success and further implementation in clinical practice.

• A new generation of promising compounds and combinations specifically designed for this disease are currently initiating clinical development.
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