IMMUNOTHERAPY STRATEGIES FOR COLORECTAL CANCER

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LEARNING OBJECTIVES OF THE MODULE

- Understand the molecular basis of cancer immunotherapy

- To gain insight on the immunobiology and different therapeutic strategies for the different immune subtypes of colorectal cancer
  - MSI mCRC
  - MSS mCRC
MODULE OUTLINE

- Introduction to cancer immunotherapy
- Overview of immunologic characteristics of CRC
  - (Immunobiology of mCRC)
- Therapeutic immune strategies in CRC in MSI and MSS tumours
  - Immune checkpoint inhibitors
  - Adoptive cell therapies
  - Anti-tumour vaccines
  - Other agents
INTRODUCTION TO CANCER IMMUNOTHERAPY
INTRODUCTION TO IMMUNOTHERAPEUTICS

Immunotherapy is directed to the immune system compartment, not to the tumour.

As tumours progress on their tumourigenic cascades, the ability of the immune system to control tumour growth decreases.

Cancer immunotherapy seeks to induce the recovery of this ability.

The anticancer response is cell mediated and either adaptive (T-cell mediated) or innate (natural killer cell-dependent).
Natural killers are responsible for detecting and killing virally infected cells and cancer cells that do not express MHC class I

Failure of MHC class 1 to bind to the Killing Inhibitory Receptor (KIR) leads to tumour cell lysis

MECHANISM OF ACQUIRED CELL-MEDIATED IMMUNE RESPONSE

1. Release of cancer cell antigens
2. Cancer antigen presentation
3. Priming and activation
4. Trafficking of T cells to tumours
5. Infiltration of T cells into tumours
6. Recognition of cancer cells by T cells
7. Killing of cancer cells

Reprinted from Immunity, 39(1), Chen DS, Mellman I, Oncology Meets Immunology: The Cancer-Immunity Cycle, 1-10, Copyright 2013, with permission from Elsevier.
1. Tumour antigens of dying cells are released to tumour microenvironment
2. Antigen Presenting Cells (APC) process these antigens and present them through MHC class 2
3. Some populations of APC can cross-present the phagocytosed antigens through MHC class 1


Republished with permission of Annual Reviews, from Annu Rev Immunol, Kroemer G, et al. 31:51-72, copyright 2013, permission conveyed through Copyright Clearance Center, Inc.
Once activated through antigen capturing and incorporation to the MHC, type 2 APCs migrate to the loco-regional lymph nodes following chemokine gradients. Once in the lymph node, they interact with the different populations of lymphocytes looking for T cell receptor compatibilities.
Once the dendritic cells find a T helper cell or cytotoxic T cell with a compatible T cell receptor to the presented antigen, both cells interact through three levels of signalling:

**Signal 1**: the so-called MHC-TCR binding that depends on TCR specificity for the antigen

**Signal 2**: the interaction of the different activating and inhibitory immune checkpoint receptors and ligands

**Signal 3**: consisting of the exchanging of cytokines between both cells. The outcome of this signalling determines whether there will be an immune response against the presented antigen or not.
PRIMING IMMUNE SYNAPSE SIGNAL 2

Signal 2: The agonistic and inhibitory ligands and ligand receptors involved are referred to as immune checkpoints. Immune checkpoint inhibitors directed against PD-1/PD-L1, CTLA-4, LAG-3, ICOS, CD-40, OX-40 TIGIT constitute the main current therapeutic field in cancer immunotherapy.
Activated antigen reactive lymphocytes abandon the lymph node and enter into the circulation looking for zones of inflammatory endothelium.

Inflammatory endothelium expresses proteins that allow activated lymphocytes to cross the endothelial barrier and enter into the tissues.

The activated lymphocyte subsequently moves towards the inflammatory focus through a cytokine gradient that will direct the lymphocyte to the inflammatory stimuli, in this case the tumour.

This process may be hampered in case of aberrant tumour vasculature.

Gong C, et al. Front Immunol 2014;5:57. Reproduced under the terms of the Creative Commons Attribution License (CC BY 3.0; https://creativecommons.org/licenses/by/3.0/)
Within the tumour microenvironment, activated lymphocytes usually have to cope with a myriad of mechanisms, such as PD-L1 overexpression in tumour cells, paucity of nutrients, low tissue pH and T-helper cell differentiation into regulatory T cells (Treg), which lead to a decreased anti-tumour immune response.
IMMUNOLOGIC CHARACTERISTICS OF COLORECTAL CANCER (CRC)
With regard to treatment with immune checkpoint inhibitors, it is important to discriminate between:

- Microsatellite Instable (MSI) CRC
- Microsatellite Stable (MSS) CRC
MICROSATELLITES

- Microsatellites are repeats of 1 to 10 nucleotides with a variable length (5–50 repeats), such as:
  - A A A A A A A A → 7 poly-A microsatellite
  - GT GT GT GT → 4 poly-GT microsatellite
  - ACGTCC-ACGTCC-ACGTCC → 3 poly ACGTCC

- Microsatellites are localised in coding or non-coding DNA regions
Microsatellites cause DNA polymerase slips in the replicative fork causing DNA mismatches and ultimately protein mutations.

- Zones of accumulations of mutations
- Commonly frame shifts
Evolution endowed eukaryotic cells with a dedicated system to repair mismatches in DNA.

**Human mismatch repair proteins**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Heterodimer</th>
<th>Repair function</th>
</tr>
</thead>
<tbody>
<tr>
<td>MutSα</td>
<td>MSH2 ♦ MSH6</td>
<td>Binds base-base mismatches and insertion-deletion mismatches</td>
</tr>
<tr>
<td>MutSβ</td>
<td>MSH2 ♦ MSH3</td>
<td>Binds insertion-deletion mismatches</td>
</tr>
<tr>
<td>MutLα</td>
<td>MLH1 ♦ PMS2</td>
<td>Early step before excision</td>
</tr>
</tbody>
</table>

**Diagram:**
- Error in newly synthesized strand
- Binding of mismatch proofreading proteins
- DNA scanning detects nick in new DNA strand (before sealing by DNA ligase)
- Strand removal
- Repair DNA synthesis
- Up to 1000 bp can be removed

**Reference:**
Alterations on microsatellite repairing system can be determined in two ways:

- By PCR against a group of microsatellites looking for mutations
  - Given the name of microsatellite instable tumours
- By immunohistochemistry looking for the expression of the different mismatch repair genes
  - Given the name of mismatch repair deficient tumours

- The overlap between both techniques is 90%
- The frequency of MSI-H status in metastatic CRC is 5%
THEORETICAL BASIS

For improved clinical benefit of treatment with checkpoint inhibitors in case of microsatellite instability

- The loss of mismatch repair system causes mutations and ultimately cancer
- Colon cancers harbouring a defective mismatch repair system have a higher number of neoantigens
- This increased mutational load increases the chance of a checkpoint inhibitor-mediated anti-tumour response
TUMOUR RESPONSE TO ANTI-PD1 AXIS AGENTS ACCORDING TO MICROSATELLITE STATUS

- MSI CRC → Higher mutation tumour burden → higher lymphocyte infiltration → Responses to anti-PD-1 inhibitor
- MSS CRC → LOW mutation tumour burden → low lymphocyte infiltration → No responses to anti-PD-1 inhibitor

Adapted from The Cancer Genome Atlas Network. Nature 2012;487:330–7. Reproduced under the terms of the Creative Commons Attribution-Non-Commercial-Share Alike licence (http://creativecommons.org/licenses/by-nc-sa/3.0/).
WATERFALL PLOT OF MS-STABLE AND INSTABLE PATIENTS TREATED WITH PEMBROLIZUMAB FOR MCRC

MS-instable patients presented dramatic responses in contrast with MS-stable patients

<table>
<thead>
<tr>
<th></th>
<th>MMR-deficient CRC</th>
<th>MMR-proficient CRC</th>
<th>MMR-deficient non-CRC</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>13</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>Objective Response Rate</td>
<td>62%</td>
<td>0%</td>
<td>10%</td>
</tr>
<tr>
<td>Disease Control Rate</td>
<td>92%</td>
<td>16%</td>
<td>70%</td>
</tr>
</tbody>
</table>

MMR, mismatch repair
CLINICAL TRIAL DESIGN: KEYNOTE-164

A Phase 2, single-arm study of pembrolizumab in pretreated patients to address significant patient unmet needs

**Patient population**
- Locally advanced or metastatic MSI CRC
- Patients must have received at least 2 prior treatments
- Stage IV disease

**Pembrolizumab 200 mg q3w IV**

**Complete response**
- Discontinuation permitted

**Partial response or stable disease**
- Treat for up to 2 years or until progression or unacceptable toxicity

**Confirmed progressive disease**
- Discontinue

**Target enrollment:** 60

**Primary Endpoint**
- ORR by RECIST 1.1

**Assessments:** Radiological assessments using RECIST 1.1 and irRC every 9 weeks

Le TD, *et al.* Ann Oncol 2018;29(suppl_5). abstract O-021 Presented at ESMO 20th World Congress on Gastrointestinal Cancer 20–23 June 2018, Barcelona, Spain
BEST PERCENTAGE CHANGE FROM BASELINE IN TARGET LESION SIZE*

Prior lines of therapy
- 1
- 2
- ≥3

Median duration of follow-up:
12.6 months (range, 0.1-15.4 months)

+20% increase in tumor size
-30% decrease in tumor size

MSI mCRC patients presented 32% RR regardless of the number of prior therapy lines

*RECIST v1.1 per IRC. Data cutoff: September 12, 2017.
OVERALL SURVIVAL

76% of patients alive at 1-year follow-up

Data cutoff: September 12, 2017.
PROGRESSION-FREE SURVIVAL*


*RECIST v1.1 per IRC. Data cutoff: September 12, 2017.

...and 41% progression-free at 1-year follow-up
CHECKMATE 142

Nivolomab provided similar benefits (RR 31%, 48% free from progressing and 74% alive 12 months after treatment initiation)

Primary endpoint: ORR per investigator assessment
Secondary endpoint: ORR per blinded independent central review (BICR)
Other endpoints: PFS, OS, biomarkers, safety and tolerability

OVERALL SURVIVAL BY BEST OVERALL RESPONSE

Very interestingly, none of the responders died during the trial follow-up, which demonstrates the immunising effect of the treatment.

NE = not estimable; NR = not reached.

These ground-breaking results merited the approval of pembrolizumab and nivolumab for the MSI population in the refractory setting. The EMA, however, halted the approval until randomised data are analysed.
FUTURE GOALS OF IMMUNOTHERAPY IN ADVANCED MSI CRC

- Grant the approval in Europe
- Move the treatment to earlier lines
- Maximise the percentage of responding patients
KEYNOTE 177 IS THE FIRST RANDOMISED TRIAL EXPLORING ANTI-PD1 AXIS BLOCKADE IN MSI-H MCRC

A Phase 3 study of pembrolizumab monotherapy vs. standard chemotherapy in 1L MSI CRC

Patient population
- Locally advanced or unresectable or metastatic CRC
- MSI

Randomisation 1:1

Pembrolizumab
200 mg q3w
Up to 2 years

mFOLFOX6 + bevacizumab:
Bevacizumab 5 mg/kg
+ Oxaliplatin 85 mg/m² IV
+ Leucovorin 400 mg/m² IV
+ 5-FU 400 mg/m² IV bolus on Day 1, then 1,200 mg/m²/day for 2-day continuous infusion; repeat every 2 weeks until progressive disease

PD
Off study

Crossover

PD

Target enrollment: 270

Primary Endpoint
PFS
OS

Keynote 177 will make it clear whether anti-PD1 inhibitors should become common frontline practice in MSI mCRC and, if positive, could support final EMA approval for Europe

PD, disease progression
CHECKMATE 142: DOES CLINICAL \nBENEFIT IMPROVE IF NIVOLUMAB \nAND IPILUMUMAB ARE COMBINED?

Primary endpoint: ORR per investigator assessment
Secondary endpoint: ORR per blinded independent central review (BICR)
Other endpoints: PFS, OS, biomarkers, safety and tolerability

INVESTIGATOR-ASSESSED RESPONSE AND DISEASE CONTROL RATE COMBINATION VS. SINGLE AGENT

- DCR was 80% (95% CI: 71.5, 86.6) with combination therapy and 69% (57.1, 79.2) with monotherapy
- Combination therapy provided a numerically higher ORR, including CRs, and DCR relative to monotherapy during a similar follow-up period

PROGRESSION-FREE AND OVERALL SURVIVAL COMBINATION VS. SINGLE AGENT

<table>
<thead>
<tr>
<th></th>
<th>Nivolumab + ipilimumab&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>Nivolumab&lt;sup&gt;1,e,f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-month rate (95% CI), %</td>
<td>76 (67.0, 82.7)</td>
<td>54 (41.5, 64.5)</td>
</tr>
<tr>
<td>12-month rate (95% CI), %</td>
<td>71 (61.4, 78.7)</td>
<td>50 (38.1, 61.4)</td>
</tr>
<tr>
<td>9-month rate (95% CI), %</td>
<td>87 (80.0, 92.2)</td>
<td>78 (66.2, 85.7)</td>
</tr>
<tr>
<td>12-month rate (95% CI), %</td>
<td>85 (77.0, 90.2)</td>
<td>73 (61.5, 82.1)</td>
</tr>
</tbody>
</table>

- With similar follow-up, combination therapy provided improved PFS and OS relative to monotherapy<sup>a,e,f,2</sup>

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<sup>a</sup>Median follow-up 13.4 months (range, 9–25). <sup>b</sup>Median PFS not reached (95% CI, not estimable). <sup>c</sup>PFS per investigator assessment. <sup>d</sup>Median OS not reached (95% CI, 18.0, not estimable). <sup>e</sup>Median follow-up 13.4 months (range, 10–32). <sup>f</sup>CheckMate-142 monotherapy and combination therapy cohorts not randomised or designed for a formal comparison.

CONCLUSIONS

- Anti-PD-1 axis monoclonal antibodies produce dramatic and long-lasting response in MSI CRC and are the new standard of care in US
  - Same will happen in the EU once randomised data are available

- Current efforts aim to move the treatment to earlier lines and increase the % of patients who respond to treatment
  - Anti-CTLA4 + anti-PD-1 agents will soon become new standard of care
MSS CRC
Immune checkpoint inhibitors and specifically-designed immunotherapy combinations failed to improve outcome

**BACKGROUND**

- Pembrolizumab
  - Nivolumab + Ipilimumab
  - Cobimetinib + Atezolizumab

<table>
<thead>
<tr>
<th></th>
<th>Nivolumab 1 mg/kg +</th>
<th>Nivolumab 3 mg/kg +</th>
<th>Atezolizumab + cobimetinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR, n (%)</td>
<td>1 (10)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Median PFS, mo (95% CI)</td>
<td>2.28 (0.62, 4.40)</td>
<td>1.31 (0.89, 1.71)</td>
<td></td>
</tr>
</tbody>
</table>
CONSIDERING THE MUTATIONAL BURDEN OF MSS CRC RESPONSE RATE TO TREATMENT WITH ANTI-PD-1 AXIS AGENTS IS LOW

Tumour mutational burden and ORR correlation with anti–PD-1 axis agents in 27 tumour types¹

2. Reprinted from Cancer Discovery, 2018; 8(6):730–49, Grasso CS, et al., Genetic Mechanisms of Immune Evasion in Colorectal Cancer, with permission from AACR.
The described stepwise model of CRC carcinogenesis starts with WNT path activation.
WNT PATHWAY ACTIVATION LEADS TO DENDRITIC CELL EXCLUSION FROM TUMOUR MICROENVIRONMENT

Melanoma\(^1\)

Localised CRC\(^2\)

mCRC\(^3\)

2. Reprinted from Cancer Discovery, 2018; 8(6):730–49, Grasso CS, et al., Genetic Mechanisms of Immune Evasion in Colorectal Cancer, with permission from AACR;
MSS mCRC HAS AN IMMUNOSUPPRESSANT BIOLOGY

The second step is the activation of the MAPK-PI3K pathway

1. The Cancer Genome Atlas Network, Nature 2012490, pages 61–70; under the terms of the Creative Commons Attribution-Non-Commercial-Share Alike licence (http://creativecommons.org/licenses/by-nc-sa/3.0/),
MAPK-PI3K PATHWAY ACTIVATION IMPAIRS ANTIGEN PRESENTATION BY DECREASING MHC CLASS 1 EXPRESSION

MSS mCRC HAS AN IMMUNOSUPPRESSANT BIOLOGY

The third step is the intracellular loss of function of the TGF-beta pathway.

1. The Cancer Genome Atlas Network, Nature 2012490, pages 61–70; under the terms of the Creative Commons Attribution-Non-Commercial-Share Alike licence (http://creativecommons.org/licenses/by-nc-sa/3.0/),
TGFβ ACTIVATION

TGFβ activation in the tumour microenvironment is a primary mechanism of immune evasion that promotes T-cell exclusion and blocks acquisition of the TH1-effector phenotype.

TGFβ blockade in combination with PD-1 axis inhibition can reverse this process and induce anti-tumour immune responses.

MSS mCRC HAS AN IMMUNOSUPPRESSANT BIOLOGY

The fourth step is the P53 pathway inactivation

1. The Cancer Genome Atlas Network, Nature 2012490, pages 61–70; under the terms of the Creative Commons Attribution-Non-Commercial-Share Alike licence (http://creativecommons.org/licenses/by-nc-sa/3.0/),
Wild type p53 in tumours unleashes the CTL response via inhibition of PD-L1 and enhances their effectiveness by upregulating Fas/APO-1 and MHC I.

Given that p53 is mutated in approximately 50% of human cancers and also impacts the immunoreactivity of cancer cells, a significant number of patients can be affected by the impaired CTL response that results from non-functional p53.

An attenuated CTL response due to p53 mutations could decrease response rates to immunotherapeutic drugs, leading to poor patient prognoses.
Contrary to normal cancer therapeutics, cancer immunotherapy is based on a bi-compartment model where tumour and immune system interact constantly.

Biologic compartments implied in normal cancer therapeutics

One-compartment model

Biologic compartments implied in immunotherapeutics

Two-compartment model

1. Adapted from Immunity, 39(1), Chen DS & Mellman I. Oncology Meets Immunology: The Cancer-Immunity Cycle, 1-10, Copyright 2013, with permission from Elsevier.
### Transcriptomic Classification of CRC

<table>
<thead>
<tr>
<th>CMS1</th>
<th>CMS2</th>
<th>CMS3</th>
<th>CMS4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSI Immune</td>
<td>Canonical</td>
<td>Metabolic</td>
<td>Mesenchymal</td>
</tr>
<tr>
<td>14%</td>
<td>37%</td>
<td>13%</td>
<td>23%</td>
</tr>
<tr>
<td>MSI, CIMP high</td>
<td>SCNA high</td>
<td>Mixed MSI status</td>
<td>SCNA high</td>
</tr>
<tr>
<td>Hypermutation</td>
<td></td>
<td>SCNA low, CIMP low</td>
<td></td>
</tr>
<tr>
<td>BRAF mutations</td>
<td></td>
<td>KRAS mutations</td>
<td></td>
</tr>
<tr>
<td>Immune infiltration and activation</td>
<td>WNT and MYC activation</td>
<td>Metabolic deregulation</td>
<td>Stromal infiltration TGF beta activation Angiogenesis</td>
</tr>
<tr>
<td>Worse survival after relapse</td>
<td>Better survival after relapse</td>
<td></td>
<td>Worse relapse-free and overall survival</td>
</tr>
</tbody>
</table>

- Gene signatures can capture the bi-compartmentation of cancer immunotherapy
- The most validated gene-signature for mCRC is the Consensus Molecular Subtype Classifier that divides CRC in four molecular subtypes.

### Transcriptomic Classification of CRC

<table>
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<tbody>
<tr>
<td>MSI Immune (MSI Immune)</td>
<td>Canonical (CMS2)</td>
<td>Metabolic (CMS3)</td>
<td>Mesenchymal (CMS4)</td>
</tr>
<tr>
<td>14%</td>
<td>37%</td>
<td>13%</td>
<td>23%</td>
</tr>
</tbody>
</table>

- **CMS1**: MSI, CIMP high
  - Hypermutation
  - Immune infiltration and activation
  - Worse survival after relapse
  - CMS1 behaves like MSI tumours at an RNA level

- **CMS2**: Canonical
  - SCNA high
  - WNT and MYC activation
  - Better survival after relapse

- **CMS3**: Metabolic
  - Mixed MSI status
  - SCNA low, CIMP low
  - Metabolic deregulation
  - CMS3 harbours MAPK pathway activation and metabolic reprogramming

- **CMS4**: Mesenchymal
  - SCN high
  - Stromal infiltration
  - TGFβ activation
  - Angiogenesis
  - Worse relapse-free and overall survival

- **CMS4**: Is characterised by stromal TGFβ activation and diffuse inflammation

---

CMS1 behave like MSI tumours. CMS2 and CMS3 are cold tumours, while CMS4 harbour a non-anti-tumoral inflammatory microenvironment.

Adapted from Clinical Cancer Research, © 2016, 22(16), 4057–66, Becht E, et al. Immune and Stromal Classification of Colorectal Cancer Is Associated with Molecular Subtypes and Relevant for Precision Immunotherapy, with permission from AACR.
CMS1: MSI-LIKE SUBGROUP WITH HEALTHY TILS

Strategy: PD1 blockade

Immune CMS1

Anti-PD-1 therapies could achieve the same results in CMS1, as seen in MSI tumours. Motricolor CT3 trial (atezolizumab + bevacizumab) is exploring this hypothesis.

Adapted from Clinical Cancer Research, © 2016, 22(16), 4057–66, Becht E, et al. Immune and Stromal Classification of Colorectal Cancer Is Associated with Molecular Subtypes and Relevant for Precision Immunotherapy, with permission from AACR.
CMS2 is characterised by Wnt pathway activation and by having a cold tumour microenvironment. WNT inhibition may foster anti-tumoral immune response in these tumours.

Adapted from Clinical Cancer Research, © 2016, 22(16), 4057–66, Becht E, et al. Immune and Stromal Classification of Colorectal Cancer Is Associated with Molecular Subtypes and Relevant for Precision Immunotherapy, with permission from AACR.
Wnt pathway inhibitors are, however, often not effective due to downstream APC mutations

- LRP 5/6 Inh
- Anti-RSPONDIN MoAb
- Porcupine inhibitor

Giannakis M, Nat Genetics 2014

1. Reprinted from Developmental Cell, 17, MacDonald BT, et al. Wnt/β-Catenin Signaling: Components, Mechanisms, and Diseases, 9–26; Copyright 2009, with permission from Elsevier; 2. Reprinted from Cell, 149(6), Clevers H, et al. Wnt/β-Catenin Signaling and Disease 192–205, Copyright 2012, with permission from Elsevier.
CMS3: DEPENDS ON MAPK PATHWAY ACTIVATION

Rational approach should be MEK + PD-1 axis inhibition

Unresectable mCRC patients
Received at least 2 regimens in metastatic setting (not including maintenance)

2:1:1
N=360

ARM A Cobimetinib + atezolizumab
n=180

ARM B Atezolizumab
n=90

ARM C Regorafenib
n=90

Treatment to continue until loss of clinical benefit

1. Adapted from Clinical Cancer Research, ©2016, 22(16), 4057–66, Becht E, et al. Immune and Stromal Classification of Colorectal Cancer Is Associated with Molecular Subtypes and Relevant for Precision Immunotherapy, with permission from AACR;
COTEZO TRIAL
OVERALL SURVIVAL

<table>
<thead>
<tr>
<th></th>
<th>Atezo + cobi (n=183)</th>
<th>Atezo (n=90)</th>
<th>Rego (n=90)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median OS, mo</td>
<td>8.9 (7.00, 10.61)</td>
<td>7.1 (6.05, 10.05)</td>
<td>8.5 (6.41, 10.71)</td>
</tr>
<tr>
<td>(95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR vs. rego</td>
<td>1.00 (0.73, 1.38)</td>
<td>1.19 (0.83, 1.71)</td>
<td>N/A</td>
</tr>
<tr>
<td>(95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.9871</td>
<td>0.3360*</td>
<td>N/A</td>
</tr>
<tr>
<td>12-mo OS, %</td>
<td>38.5</td>
<td>27.2</td>
<td>36.6</td>
</tr>
</tbody>
</table>

The COTEZO trial was negative for the overall population. Outcomes of the CMS3 population need to be determined to validate the hypothesis.

The MEK inh./ PD-L1 inh-combination did not achieve a better outcome

Future directions for CMS 3

NCT02876224: Cobimetinib + atezolizumab + bevacizumab for 2nd line MSS mCRC

NCT02060188: Cobimetinib + Ipilimumab + Nivolumab for MSS mCRC

NCT03271047: Binimetinib + Nivolumab +/- Ipilimumab for RAS mut mCRC

Natural killer cell therapy
Natural Killers are the cells that kill those cells that do not express MHC class 1

MAPK/PI3K activation

Change in Tumor Size in MSS-CRC Patients in the Expansion Phase

3/39 PRs

Monalizumab + Durvalumab
MSS mCRC

CMS4: TGFβ ACTIVATION

Block TGFβ pathway in CMS 4 mCRC

Change the inflammatory microenvironment to a Th1 immune response

Foster T-cell infiltration

Increased sensitivity to Immune checkpoint inhibitors

NCT03436563: M7824 (bi-specific TGFβ/PD-L1 MoAb) for CMS 4 mCRC

1. Adapted from Clinical Cancer Research, © 2016, 22(16), 4057–66, Becht E, et al. Immune and Stromal Classification of Colorectal Cancer Is Associated with Molecular Subtypes and Relevant for Precision Immunotherapy, with permission from AACR;
CRC IMMUNE CLASSIFICATION AT TRANSCRIPTOMIC LEVEL

TREATMENTS FOR ALL SUBGROUPS

Adapted from Clinical Cancer Research, © 2016, 22(16), 4057–66, Becht E, et al. Immune and Stromal Classification of Colorectal Cancer Is Associated with Molecular Subtypes and Relevant for Precision Immunotherapy, with permission from AACR.
## CHEMOTHERAPY OR RADIOTHERAPY–IMMUNE CHECKPOINT INHIBITOR COMBINATIONS

<table>
<thead>
<tr>
<th>Target</th>
<th>Therapy</th>
<th>Phase</th>
<th>Trial Design</th>
<th>Trial ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-PDL1</td>
<td>Atezolizumab (engineered IgG1, no ADCC)</td>
<td>I</td>
<td>Solid tumours</td>
<td>NCT01375842</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ib</td>
<td>Solid tumours (+ bevacizumab ± FOLFOX)</td>
<td>NCT01633970</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II</td>
<td>mCRC (+ bevacizumab + fluoropyrimidine)</td>
<td>NCT02291289</td>
</tr>
<tr>
<td></td>
<td>MEDI4736 (modified IgG1, no ADCC)</td>
<td>II</td>
<td>mCRC</td>
<td>NCT02227667</td>
</tr>
<tr>
<td>Anti-PD-1</td>
<td>Nivolumab (IgG4)</td>
<td>I/II</td>
<td>mCRC (± ipilimumab) (CheckMate 142)</td>
<td>NCT02060188</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I/II</td>
<td>Solid tumours (+ INCB24360)</td>
<td>NCT02327078</td>
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<tr>
<td></td>
<td></td>
<td>I/II</td>
<td>Solid tumours (+ chemotherapy)</td>
<td>NCT02423954</td>
</tr>
<tr>
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<td>I/II</td>
<td>Solid tumours (+ varilimumab)</td>
<td>NCT02335918</td>
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<tr>
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<td>Pembrolizumab (IgG4, humanised)</td>
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<td>Solid tumours (+ aflibercept)</td>
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<tr>
<td></td>
<td></td>
<td>I/II</td>
<td>GI cancers (+mFOLFOX6)</td>
<td>NCT02268825</td>
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<td></td>
<td></td>
<td>I/II</td>
<td>WT mCRC (+ cetuximab)</td>
<td>NCT02318901</td>
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<tr>
<td></td>
<td></td>
<td>II</td>
<td>mCRC (+ radiotherapy or ablation)</td>
<td>NCT02437071</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II</td>
<td>mCRC (+ chemotherapy)</td>
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<tr>
<td></td>
<td></td>
<td>II</td>
<td>mCRC (+ azacitidine and/or romidepsin)</td>
<td>NCT02512172</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II</td>
<td>MSI-positive/-negative CRC</td>
<td>NCT01876511</td>
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*Recruiting studies Clinicaltrials-gov
BI-SPECIFIC T-CELL ENGAGING ANTIBODIES

CEA-TCB structure\(^{1,2}\)

- Binds simultaneously with one arm to CD3 on T cells and with two arms to CEA on tumour cells
- Flexible 2-to-1 format enables high-avidity binding and selective killing of CEA-overexpressing tumour 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 tumour (CEA) and T cells (CD3)
- Killing of tumour cells independent of pre-existing immunity
- T-cell proliferation at site of activation

Fab, fragment antigen-binding region; IRR, infusion-related reaction.
CEA-TCB AT DOSES ≥60 MG + ATEZOLIZUMAB IN 3L+ PATIENTS WITH MSS MCRC

Study 2: CEA-TCB + atezolizumab (n=11, 80 and 160 mg of CEA-TCB)

Data reported by investigators, cut-off: March 3, 2017.

Radiological signs of tumour inflammation seen at ≥ 60 mg (safety data cut-off is ≥ 40 mg).

A PHASE I STUDY OF ENADENOTUCIREV

An oncolytic Ad11/Ad3 chimeric group B adenovirus, in combination with nivolumab in tumours of epithelial origin

A PHASE I STUDY OF ENADENOTUCIREV

An oncolytic Ad11/Ad3 chimeric group B adenovirus, in combination with nivolumab in tumours of epithelial origin

Personalised immune therapies are a good option in expert hands

**Personalised peptide vaccines**¹

**Adoptive T-cell therapy**²

PERSONALISED IMMUNE THERAPIES

- Personalised immune therapies can be good options for patients not responding to other available therapies.
- However, their complexity makes these options only available in highly experienced centres, since their success depends on the experience of the team.
### EXAMPLES OF VACCINES FOR IMMUNOSTIMULATION IN CRC

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Antigen</th>
<th>Enhancer</th>
<th>Phase</th>
<th>Study population</th>
<th>Trial ID</th>
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</thead>
<tbody>
<tr>
<td>Anti-tumour vaccines</td>
<td>Autologous tumour cells</td>
<td>BCG</td>
<td>II</td>
<td>Adjuvant CRC</td>
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<td></td>
<td>III</td>
<td>Adjuvant CRC</td>
<td>PMID: 15755632</td>
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<tr>
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<td>III</td>
<td>Adjuvant Stage II CRC</td>
<td>NCT02448173</td>
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<tr>
<td></td>
<td>Newcastle Disease Virus</td>
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<td>II</td>
<td>Liver resected CRC</td>
<td>PMID: 18488223</td>
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<tr>
<td>Dendritic cell vaccines</td>
<td>CEA</td>
<td>Dendritic cells</td>
<td>I</td>
<td>Adjuvant Stage III CRC</td>
<td>NCT01890213</td>
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<tr>
<td></td>
<td>MUC 1</td>
<td>Dendritic cells</td>
<td>II</td>
<td>Liver or lung resected CRC</td>
<td>NCT00103142</td>
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</table>

Pubmed - Clinicaltrials.gov
### EXAMPLES OF ADOPTIVE CELL THERAPY FOR IMMUNOSTIMULATION IN CRC

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Target</th>
<th>Enhancer</th>
<th>Phase</th>
<th>Trial Design</th>
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<tbody>
<tr>
<td>TILS</td>
<td>Autologous tumour cells</td>
<td>IL-2 Pembrolizumab</td>
<td>II</td>
<td>GI tumours</td>
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<td>CAR T cells</td>
<td>CEA</td>
<td></td>
<td>I</td>
<td>Tumours expressing CEA</td>
<td>NCT02349724</td>
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<tr>
<td></td>
<td>CEA</td>
<td>Yttirum 90</td>
<td>I</td>
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<tr>
<td></td>
<td>EGFR</td>
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<td>I/II</td>
<td>Tumours expressing EGFR</td>
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<tr>
<td>Cytokine-induced-killers cells</td>
<td>Autologous tumour cells</td>
<td></td>
<td>II</td>
<td>Adjuvant CRC in combination with XELOX</td>
<td>NCT01929499</td>
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</tbody>
</table>
Immune checkpoint inhibitors and MEK-based combinations have failed in MSS mCRC, potentially reflecting the lack of implementation of tumour biology knowledge into study design.

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

Deeper understanding of tumour biology is crucial to ensure their success and further implementation in clinical practice.
THANK YOU!