Expression of the inhibitory receptor CD94/NKG2A on CD8+ Tumour-Infiltrating Lymphocytes in colorectal cancer: a new promising druggable immune checkpoint in a context of HLA-E/β2microglobuline overexpression by tumor cells

Céline Bossard, Juliette Eugène, Nicolas Jouand, Kathleen Ducoin, Romain Oger, Juliette Podevin, Jaafar Bennouna, Stéphane Bezieau, Christelle Volteau, Wassila El Alami Thomas, Olivier Kerdraon, Pierre Fourquier, Emilie Thibaudeau, Anne Jarry, Jean-François Mosnier, Nadine Gervois
MSI + MSS POLE/D-mut CRC

Microsatellite stable (MSS) CRC

<table>
<thead>
<tr>
<th>CMS1</th>
<th>MSI immune</th>
<th>CMS2</th>
<th>Canonical</th>
<th>CMS3</th>
<th>Metabolic</th>
<th>CMS4</th>
<th>Mesenchymal</th>
</tr>
</thead>
<tbody>
<tr>
<td>14%</td>
<td></td>
<td>37%</td>
<td></td>
<td>13%</td>
<td></td>
<td>23%</td>
<td></td>
</tr>
</tbody>
</table>
- MSI, CIMP high, hypermutation
- BRAF mutations
- Immune infiltration and activation
- SCNA high
- Mixed MSI status, SCNA low, CIMP low
- KRAS mutations
- WNT and MYC activation
- Metabolic deregulation
- Stromal infiltration, TGF-β activation, angiogenesis

High mutation rate/tumor

↑ Immune-stimulating neoepitopes
↑ Tumor antigenicity
↑ Recruitment of cytotoxic T cells

Guinney et al, 2015, Nature Medicine; Llosa et al, 2015, Cancer Discov
Microsatellite stable (MSS) CRC

- **CMS1**: MSI immune
  - 14%
  - MSI, CIMP high, hypermutation
  - BRAF mutations
  - Immune infiltration and activation

- **CMS2**: Canonical
  - 37%
  - SCNA high
  - Mixed MSI status, SCNA low, CIMP low

- **CMS3**: Metabolic
  - 13%
  - WNT and MYC activation
  - Metabolic deregulation

- **CMS4**: Mesenchymal
  - 23%
  - KRAS mutations
  - Stromal infiltration, TGF-β activation, angiogenesis

**High mutation rate/tumor**

- ↑ Immune-stimulating neoepitopes
- ↑ Tumor antigenicity
- ↑ Recruitment of cytotoxic T cells

**T-cell inhibitory receptors or ligands** (PD1/PDL1, CTLA4...)

*Guinney et al, 2015, Nature Medicine; Llosa et al, 2015, Cancer Discov*
Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair–Deficient/Microsatellite Instability–High Metastatic Colorectal Cancer

Michael J. Overman, Sara Lonardi, Ka Yeung Mark Wong, Heinz-Josef Lenz, Fabio Gelsomino, Massimo Aglietta, Michael A. Morse, Eric Van Cutsem, Ray McDermott, Andrew Hill, Michael B. Sawyer, Alain Hendelisz, Bart Neyns, Magali Svrcek, Rebecca A. Moss, Jean-Marie Ledeine, Z. Alexander Cao, Shital Kamble, Scott Kopetz, and Thierry André

Table 2. ORR, Best Overall Response, and DCR per Investigator Assessment (N = 119)

<table>
<thead>
<tr>
<th>Response</th>
<th>No. (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR</td>
<td>65 (55)</td>
<td>45.2 to 63.8</td>
</tr>
</tbody>
</table>

Abbreviations: DCR, disease control rate; ORR, objective response rate.

40% of MSI patients and the majority of MSS patients remain non responders → need new immune targets for novel immunotherapeutic options
• HLA-E is a poorly polymorphic human non classical MHC class Ib molecule

• A narrow tissue distribution but a low cell surface expression.

• Its biological activity requires the coexpression of the β2 microglobulin chain (β2m) to form a stable heterodimer - HLA-E/β2m –

• Interacts either with an activating CD94/NKG2C or an inhibitory CD94/NKG2A heterodimeric receptor

Wischhusen et al, 2005; Derré et al, 2006; Levy et al, 2008
• HLA-E is a poorly polymorphic human MHC class Ib molecule

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• Interacts either with an activating CD94/NKG2C or an inhibitory CD94/NKG2A heterodimeric receptor

• In vitro, tumor cells overexpressing HLA-E activate the inhibitory receptor CD94/NKG2A on effector cells and induce a tolerogenic effect

Wischhusen et al, 2005; Derré et al, 2006; Levy et al, 2008
Monalizumab: a new immune checkpoint inhibitor targeting the inhibitory receptor NKG2A

NK and T cell inhibition by NKG2A

Activation by NKG2A blockade
HLA-E/β2m is overexpressed by tumor cells in 20% of CRC (TMA) (clone MEM-E/02)

Normal colonic mucosa
Colon cancer

HLA-E/β2m overexpression = A strong membranous +/- cytoplasmic expression of HLA-E and β2m, at least by 5% of tumor cells

CD94+ TIL in the tumor microenvironment

HLA-E/β2m overexpression is associated with a poor overall survival

Expression profile of HLA-E/β2m in relation with Density, phenotype and function of CD94+ TILs depending on clinicopathological and molecular features of CRC
Retrospective cohort 1
Monocentric (University Hospital of Nantes)
234 CRC patients (1998 to 2014)

Prospective cohort 2
Multicentric (University Hospital, Institut Cancérologie de l’Ouest, Nouvelles Cliniques Nantaises, Nantes)
27 CRC patients

Immunohistochemistry on Tissue Micro-arrays
- Expression profile of HLA-E/β2m by tumor cells
- Density of Intraepithelial CD94+ cells

Eugene et al, Mod Pathol, 2019, in press
Results
<table>
<thead>
<tr>
<th></th>
<th>Cohort 1, $n = 234$</th>
<th></th>
<th>Cohort 2, $n = 27$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSI $N = 47$</td>
<td>MSS $N = 187$</td>
<td>$p$ Value</td>
<td>MSI $N = 11$</td>
</tr>
<tr>
<td></td>
<td>$N$ (%)</td>
<td>$N$ (%)</td>
<td></td>
<td>$N$ (%)</td>
</tr>
<tr>
<td>Age: mean (range)</td>
<td>72.2 (23–89)</td>
<td>69.7 (36–94)</td>
<td></td>
<td>77.4 (63–88)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>13 (27.6)</td>
<td>122 (65.3)</td>
<td></td>
<td>2 (18)</td>
</tr>
<tr>
<td>Women</td>
<td>34 (72.3)</td>
<td>65 (34.7)</td>
<td>$3.10^{-6}$</td>
<td>9 (82)</td>
</tr>
<tr>
<td>Tumor location</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>44 (91.6)</td>
<td>86 (46)</td>
<td>$2.10^{-8}$</td>
<td>10</td>
</tr>
<tr>
<td>Left</td>
<td>2 (4.2)</td>
<td>92 (49.2)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Transverse</td>
<td>2 (4.2)</td>
<td>6 (3.2)</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Rectum</td>
<td>0</td>
<td>3 (1.6)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Histological subtypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma not otherwise specified (NOS)</td>
<td>27 (56.2)</td>
<td>158 (84.5)</td>
<td>$4.10^{-5}$</td>
<td>8</td>
</tr>
<tr>
<td>Mucinous adenocarcinoma</td>
<td>12 (25.5)</td>
<td>21 (14.4)</td>
<td>0.01</td>
<td>2</td>
</tr>
<tr>
<td>Medullary carcinoma</td>
<td>4 (8.3)</td>
<td>3 (1.6)</td>
<td>0.03</td>
<td>0</td>
</tr>
<tr>
<td>Mixed carcinoma</td>
<td>2 (4.1)</td>
<td>1 (0.5)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Signet ring cell carcinoma</td>
<td>1 (2)</td>
<td>1 (0.5)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Poorly differentiated carcinoma</td>
<td>1 (2)</td>
<td>2 (0.6)</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Serrated carcinoma</td>
<td>0</td>
<td>1 (0.5)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>UICC Stage</td>
<td>0</td>
<td>2 (1)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>1 (2)</td>
<td>16 (8.5)</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>II</td>
<td>22 (46.8)</td>
<td>61 (32.6)</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>III</td>
<td>18 (38.3)</td>
<td>52 (27.8)</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>IV</td>
<td>6 (12.7)</td>
<td>56 (30)</td>
<td>0.03</td>
<td>1</td>
</tr>
<tr>
<td>M1a</td>
<td>2 (33.3)</td>
<td>35 (62.5)</td>
<td></td>
<td>1/1 (100)</td>
</tr>
<tr>
<td>M1b</td>
<td>0</td>
<td>1 (1.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1c</td>
<td>4 (66.7)</td>
<td>17 (30.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>3 (5.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutational profile</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRAF</td>
<td>13/35 (37)</td>
<td>9/100 (9)</td>
<td>0.003</td>
<td>4/6</td>
</tr>
<tr>
<td>RAS</td>
<td>4/35 (11.5)</td>
<td>35/100 (35)</td>
<td>0.01</td>
<td>2/6</td>
</tr>
<tr>
<td>WT</td>
<td>18/35 (47.5)</td>
<td>56/100 (56)</td>
<td></td>
<td>2/6</td>
</tr>
</tbody>
</table>

WT, wild type; MSS, microsatellite stable; MSI, microsatellite unstable.
The ligand HLA-E/β2m is preferentially overexpressed in MSI and right-sided CRC

Eugene et al, Mod Pathol, 2019, in press
The density of intra-epithelial TILs expressing the specific HLA-E/β2m receptor - CD94 – is higher in HLA-E/ β2m + CRC

Immunohistochemistry on TMA
Density of intra-epithelial CD94+ TILs = number of CD94+ cells per 100 tumor cells (mean of 3 TMA spots)

Eugene et al, Mod Pathol, 2019, in press
CD94+ cells predominantly co-express the NKG2A inhibitory chain and mainly correspond to CD8+ αβ T lymphocytes in tumor, regardless of the microsatellite status.

Eugene et al, Mod Pathol, 2019, in press
The receptor CD94/NKG2A is functional and its engagement inhibits the TCR-dependent lytic activity of CD8+ TILs.

Culture of tumor fragments with IL2

Expansion of polyclonal TILs

Cell-sorting (FACS)

Co-cultures with mouse P815 cell line + anti-CD3 + anti-CD94

Agonist anti-CD3

Agonist anti-CD94

Anti-CD3-mediated Redirected cytolytic assay

Lysis

Granz B perforine

CD8+ TIL

Cr⁵¹ - labelled P815

Anti-CD3

Anti-CD94

Eugene et al, Mod Pathol, 2019, in press
The receptor CD94/NKG2A is functional and its engagement inhibits the TCR-dependent lytic activity of CD8+ TILs.
The receptor CD94/NKG2A is functional and its engagement inhibits the TCR-dependent lytic activity of CD8+ TILs.

Culture of tumor fragments (1 mm³) with IL2 (3 weeks)

Expansion of polyclonal TILs

Cell-sorting (FACS)

Co-cultures with mouse P815 cell line + anti-CD3 + anti-CD94

Agonist anti-CD3

Agonist anti-CD4

Anti-CD3-mediated Redirected cytolytic assay

Cr⁵¹-labelled P815

Anti-CD3

Anti-CD94

CD94⁺NKGA⁻

CD94⁺NKGA⁺

% of specific lysis

OKT-3 (ng/mL)

Eugene et al, Mod Pathol, 2019, in press
Take home messages

✔ HLA-E/β2m ligand is preferentially overexpressed by tumor cells in MSI CRCs (45%), but also in 19% of MSS CRCs

✔ HLA-E/β2m + CRCs carry the highest density of CD94+ intraepithelial TILs, irrespective of the microsatellite status

✔ CD94+ TILs mainly correspond to CD8+ αβ T cells, but also NK cells, that preferentially co-express a functional inhibitory NKG2A chain

Taking into account the additive effects of monalizumab with anti-PDL1 or anti-EGFR antibodies in vitro, our results open new immunotherapeutic options in metastatic MSI and MSS CRC patients respectively

HLA-E/β2m expression as a new predictive biomarker?

Andre et al. Cell, 2018
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- Cécile Girard
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- Nicolas Jouand
- Kathleen Ducoin
- Romain Oger
- Edouard Leveque
- Anne Jarry
- Cécile Deleïne

**La Ligue Contre le Cancer**

**Cancéropôle Grand Ouest**

**AMGEN**

**Centre Hospitalier Universitaire de Nantes**
Thanks!

Welcome in Nantes!