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

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**Microsatellite stable (MSS) CRC**

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

**High mutation rate/tumor**

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

MSI + MSS POLE/D-mut CRC

Microsatellite stable (MSS) CRC

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
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 Hendlisz, 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>
<tr>
<td>Best overall response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete response</td>
<td>4 (3)</td>
<td></td>
</tr>
<tr>
<td>Partial response</td>
<td>61 (51)</td>
<td></td>
</tr>
<tr>
<td>Stable disease</td>
<td>39 (33)</td>
<td></td>
</tr>
<tr>
<td>Progression</td>
<td>15 (13)</td>
<td></td>
</tr>
<tr>
<td>Unasurable</td>
<td>1 (1)</td>
<td></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

• 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

• 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)

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

Retrospective cohort of CRC patients: n=80

Expression profile of HLA-E/β2m in relation with Density, phenotype and function of CD94+ TILs depending on clinicopathological and molecular features of CRC
Study design

Retrospective cohort 1
Monocentric (University Hospital of Nantes)
234 CRC patients (1998 to 2014)

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

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

Functional analyses of CD94+ TILs
Generation of TIL cell lines

Paired Normal mucosa
Primary tumor
Mechanical dissociation
Isolation of lymphocytes (TILs and LPL) and NK cells

Flow cytometry
Phenotype of CD94+ cells (TIL, NK, PBL, and LPL)

Paired Peripheral blood
Isolation of lymphocytes (PBL)

Eugene et al, Mod Pathol, 2019, in press
Results
Table 1 Clinicopathological and molecular features of CRC patients

<table>
<thead>
<tr>
<th></th>
<th>Cohort 1, n = 234</th>
<th>Cohort 2, n = 27</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSI</td>
<td>MSS</td>
<td></td>
</tr>
<tr>
<td>Age: mean (range)</td>
<td>72.2 (23–89)</td>
<td>69.7 (36–94)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>13 (27.6)</td>
<td>122 (65.3)</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>34 (72.3)</td>
<td>65 (34.7)</td>
<td>3.10^{-6}</td>
</tr>
<tr>
<td>Tumor location</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>
</tr>
<tr>
<td>Left</td>
<td>2 (4.2)</td>
<td>92 (49.2)</td>
<td>0.01</td>
</tr>
<tr>
<td>Transverse</td>
<td>2 (4.2)</td>
<td>6 (3.2)</td>
<td>0.03</td>
</tr>
<tr>
<td>Rectum</td>
<td>0</td>
<td>3 (1.6)</td>
<td></td>
</tr>
<tr>
<td>Histological subtypes</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>
</tr>
<tr>
<td>Mucinous adenocarcinoma</td>
<td>12 (25.5)</td>
<td>21 (14.4)</td>
<td>0.01</td>
</tr>
<tr>
<td>Medullary carcinoma</td>
<td>4 (8.3)</td>
<td>3 (1.6)</td>
<td>0.03</td>
</tr>
<tr>
<td>Mixed carcinoma</td>
<td>2 (4.1)</td>
<td>1 (0.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>Signet ring cell carcinoma</td>
<td>1 (2)</td>
<td>1 (0.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>Poorly differentiated carcinoma</td>
<td>1 (2)</td>
<td>2 (0.6)</td>
<td>0.03</td>
</tr>
<tr>
<td>Serrated carcinoma</td>
<td>0</td>
<td>1 (0.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>UICC Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>2 (1)</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1 (2)</td>
<td>16 (8.5)</td>
<td>2.10^{-8}</td>
</tr>
<tr>
<td>II</td>
<td>22 (46.8)</td>
<td>61 (32.6)</td>
<td>2.10^{-8}</td>
</tr>
<tr>
<td>III</td>
<td>18 (38.3)</td>
<td>52 (27.8)</td>
<td>2.10^{-8}</td>
</tr>
<tr>
<td>IV</td>
<td>6 (12.7)</td>
<td>56 (30)</td>
<td>2.10^{-8}</td>
</tr>
<tr>
<td>M1a</td>
<td>2 (33.3)</td>
<td>35 (62.5)</td>
<td>2.10^{-8}</td>
</tr>
<tr>
<td>M1b</td>
<td>0</td>
<td>1 (1.8)</td>
<td>2.10^{-8}</td>
</tr>
<tr>
<td>M1c</td>
<td>4 (66.7)</td>
<td>17 (30.3)</td>
<td>2.10^{-8}</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>3 (5.4)</td>
<td>2.10^{-8}</td>
</tr>
<tr>
<td>Mutational profile</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>
</tr>
<tr>
<td>RAS</td>
<td>4/35 (11.5)</td>
<td>35/100 (35)</td>
<td>0.001</td>
</tr>
<tr>
<td>WT</td>
<td>18/35 (47.5)</td>
<td>56/100 (56)</td>
<td>2.10^{-8}</td>
</tr>
</tbody>
</table>

Notes: MSI = microsatellite instability, MSS = microsatellite stable, UICC = Union for International Cancer Control, BRAF = BRAF V600E, RAS = RAS mutations
The ligand HLA-E/β2m is preferentially overexpressed in MSI and right-sided CRC.

**Eugene et al, Mod Pathol, 2019, in press**

**Immunohistochemistry on TMA**

HLA-E cut-off: 5% of strongly positive tumor cells (3 TMA spots)

**No relation with BRAF/RAS mutational status**

**Right-sided CRC**

28.7% of HLA-E/β2m + CRC

**Left-sided CRC**

14.9% of HLA-E/β2m + CRC
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
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)

CD8+ CD94/NKG2A+

CD8+ CD94/NKG2A-

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

Agonist anti-CD3

Agonist anti-CD94

FcyR

CD8+ TIL

TCR

Granz B perforine

Anti-CD94

Anti-CD3

Cr51-labelled P815

Lysis

% of specific lysis

OKT-3 (ng/mL)

CD94/NKG2A

$\rho = 0.092$

Anti-CD3 agonist

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.
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
Acknowledgments

University Hospital Nantes
- Emilie Duchalais
- Juliette Podevin
- Guillaume Meurette
- Tamara Matysiak
- Jaafar Bennouna
- Stéphane Bezieau
- Jean-François Mosnier
- Techniciens of Pathology department

ICO
- Frédéric Dumont
- Emilie Thibaudeau
- Olivier Kerdraon

NCN
- Pierre Fourquier

IHP
- Jerome Chettritt
- Wassila El Alami Thomas

MicroPicell platform, SFR
- Stéphanie Blandin

Biobank CHU
- Cécile Girard
- and its team

Patients
Thanks!

Welcome in Nantes!