Intérêt de l’étude des réponses immunitaires dans les cancers colorectaux
**Introduction**

**Immune cells are present within solid tumors**

(colorectal, prostate, breast, kidney, bladder, ovarian, mélanoma, .............)

ex: colorectal cancer

H&E sections

- tumor
- invasive margin
- at distance

CD3 T cells (brown)

Lymphoid islets

tumor cells (blue)
Introduction

• The immune reaction could influence the clinical outcome

The immune criteria is not included in clinical practice as a powerful indicator of clinical outcome

- TNM classification determine prognosis
Introduction

In clinical practice:

- colorectal cancers
- bad prognosis
- 5 years survival of 65%
- 1200 deaths per week in the USA

UICC TNM staging system
Introduction

Why are immune criteria not included in clinical practice?

1) The picture of the immune infiltration is complex
   -> type of cells, immune orientation,…

2) To provide a method available in routine practice to investigate
   the in situ immune reaction
Introduction

• The treatment of cancer

1) Clinical development of agents that target immune-checkpoint pathways
   - anti-CTLA4 (ipilimumab)
   - anti-PD1 / PD1L

2) Turn on the immune system with tumor vaccines
   - PROVENCE® (sipuleucel-T)
   - Prostvac

(Hodi FS et al, NEJM 2010)

(Topalian SJ et al, NEJM 2012)
• Complexity of the immune reaction

-> combination of large scale analyses (Integrative Cancer Immunology)

DNA MicroArrays (n=54000)
Low Density Arrays (LDA) (n=384)
microRNA expression (n=384)
Seldi-Tof Antibody arrays (proteomic)
FACS Phenotype Data (n=820)
FACS Functional Data (n=980)
Tissue MicroArrays (TMA) of Colorectal Tumors (n=750)

Database

Bioinformatics programs
Statistic Networks

 CNIL agreement

> 800 000 parameters

ARACNe (algorithm for the reconstruction of accurate cellular networks)
-> combination of large scale analyses (Integrative Cancer Immunology)
cohorts of colorectal cancer patients (> 600 patients)

(Galon J et al, Science 2006)
(Pagès F et al, J Clin Oncol 2009)
(Mlecnik B et al, J Clin Oncol 2011)
(Fridman H et al, Nat Rev Immunol 2012)

cytotoxic T lymphocytes (CD8) & memory T cells (CD45RO)
in tumor regions (core of the tumor and the invasive margin)

clinical outcome
Results

Each tumor region is informative

- The combined analysis of the two tumor regions improves the accuracy of prediction for survival [OS and DFS]
 Création of an immune score

Results

Immune score (CT+IM)

immunostaining

Tumor regions (CT&IM)

Immune score (CT+IM)

Pagès F et al, J Clin Oncol 2009
Mlecnik B et al, J Clin Oncol 2011
Results

- Importance of the immune score for all stages of the cancer

ex: localized CRC (stage I-II; n=411 pts)

classify 70% of the patients into groups of prognosis

(Pagès F et al, J Clin Oncol 2009)
(Mlecnick B et al, J Clin Oncol 2011)
Results

- Prognostic performance of the Immune Score (CRC stage I-III)

COX analysis for DFS

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>Log Rank P-values</th>
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<tbody>
<tr>
<td>Tumor (T) stage</td>
<td>1.24</td>
<td>0.29</td>
</tr>
<tr>
<td>N Stage</td>
<td>1.31</td>
<td>0.17</td>
</tr>
<tr>
<td>Gender</td>
<td>1.47</td>
<td>0.18</td>
</tr>
<tr>
<td>Number of total lymph nodes</td>
<td>1.13</td>
<td>0.68</td>
</tr>
<tr>
<td>Histological grade</td>
<td>0.69</td>
<td>0.29</td>
</tr>
<tr>
<td>Mucinous Colloid</td>
<td>1.29</td>
<td>0.47</td>
</tr>
<tr>
<td>Occlusion</td>
<td>1.03</td>
<td>0.94</td>
</tr>
<tr>
<td>Perforation</td>
<td>4.03</td>
<td>0.0084</td>
</tr>
<tr>
<td>Immune Score</td>
<td><strong>0.65</strong></td>
<td><strong>0.0003</strong></td>
</tr>
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</table>

(Mlechik et al. J Clin Oncol 2011)
Results

Immune cell infiltration is a common feature of many human solid tumors. Recently, increased infiltration of tumors with cytotoxic CD8-positive T cells has been correlated with prolonged survival in multi-institutional analyses in a variety of epithelial cell cancers, including small-cell lung carcinoma,18 carcinomas of the endometrium,22 kidney,16 esophagus,19 and hepatocellular carcinoma.23 In this issue of Journal of Clinical Oncology, Mlecnik et al.24 suggest that immune cell infiltration of colorectal cancer by cytotoxic CD8-positive and memory CD4+CD25-negative T cells has prognostic discriminatory power that is superior to standard staging systems (ie, American Joint Committee on Cancer International Union Against Cancer-TNM). The authors examined more than 400 patients with colorectal cancer, quantified the density of tumor-infiltrating T cells, devised an immune scoring system (higher scores reflecting greater CD8-positive and CD4+CD25-negative T-cell density), and correlated the immune scores with clinical outcomes. The results demonstrate two key findings: patients with high immune scores have increased disease-free and overall survival as compared with patients whose tumors demonstrate low immune scores, and the immune score is superior in predicting disease outcomes as compared with a host of important prognostic clinical parameters, including TNM staging. The authors further observed that there was an inverse correlation between immune cell density and tumor stage. In patient samples with the greatest immune cell density, the majority (80%) were tumor in situ or T1 stage tumors. In contrast, only 16% of T4 tumors demonstrated high density T-cell infiltrates. Moreover, there were no tumors in the T3 or T4 stage disease spectra showing weak immune cell density scores, whereas 64% of T4 tumors demonstrated low immune scores. The data suggest that even with minimal tumor invasion, patients with a low immune score will be likely to experience a disease relapse. For these patients, surgery may not be curative. Remarkably, the prognostic significance of the immune score was obtained regardless of whether the tumor tissue was derived from a patient with stage I or stage IV colorectal cancer.

The study by Mlecnik et al. is the culmination of several observations from a collaborative group of researchers who have been testing the hypothesis that the immune response to colorectal cancer is important in tumor progression and survival. Recent studies have shown that high density infiltrating T cells are associated with improved survival.18-19

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### Table: CD8 Density in CT Rechute

<table>
<thead>
<tr>
<th>Nb cellules/mm² tissu</th>
<th>Rechute</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>100</td>
<td>+</td>
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<tr>
<td>200</td>
<td>+</td>
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<tr>
<td>300</td>
<td>+</td>
</tr>
<tr>
<td>400</td>
<td>+</td>
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</tbody>
</table>

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![Image of TNM staging in colorectal cancer](https://example.com/tnm-staging.png)

**TNM Staging in Colorectal Cancer: T Is for T Cell and M Is for Memory**

Elizabeth K. Broussard and Mary L. Driscoll, Tumor Vaccine Group, Center for Translational Medicine in Women’s Health, University of Washington, Seattle, WA

See accompanying article doi: 10.1001/jco.2010.30.0425

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![Image of immune score](https://example.com/immune-score.png)
Results

**Meta-analysis:** 124 articles

20 different cancer types

From bench to bedside

To translate into the clinic

Immunomonitoring Plateform

to determine the immunoscore on tissue sections in a routine setting (whole slide analysis)

HEGP, Paris, France

From bench to bedside

To translate into the clinic

Immunomonitoring Plateform

to determine the immunoscore on tissue sections in a routine setting (whole slide analysis)
From bench to bedside
From bench to bedside

Segmentation
From bench to bedside

Tissue detection
From bench to bedside

Tumor region selected
From bench to bedside

Automatic proposition for the invasive margin
Validate the staining intensity of the IHC for the marker on the slide.
Determination of the immunoscore

- All the tumor is analyzed (500-700 tiles)
- Each region is determined by the **mean density of the 3 tiles the most infiltrated**

- The mean density is compared to the cut off value
  - Hi or Lo

- Faisability (>96%)
- Reliability (R=0.98)
- Repetability (>99%)
- Density Reproducibility (>96%)
- Immune Score Reproducibility (>99%)
From bench to bedside

retrospective cohort of 230 patients with stage II CRC

The Immune Score strongly predicts prognosis in stage II CRC patients

CD3 & CD8 (CT/IM)
From bench to bedside

To promote the Immunoscore in routine clinical settings

From bench to bedside

International Validation Study:

- Validate the immunoscore as a prognostic marker (stages I-III colon cancer)
  (300 patients /center ; 70 stage I; 140 stage II; 90 stage III)

- Test it's power to predict high-risk patients in stage II colon cancer.
  (300 patients with stage II colon cancer /center)
From bench to bedside

To promote the Immunoscore in routine clinical settings

French multi-institutional prospective study
(6 centers; > 900 CRC (stage I-IV) inclusion period 2012-1014)

- HEGP : Pr. A Berger, Pr P Bruneval, Dr T. Méatchi
- Avicenne : Pr. Ph Wind, Dr C. Lagorce-Pagès
- Besançon : Pr. C Borg, Pr. B Heyd, Pr. O Adotevi, Dr. S Valmary-Degano
- Dijon : Pr. C Lepage, Pr. F. Piard, Pr. L Martin.
- Rouen : Dr. F Di Fiore, F Le Pessot
- Poitiers : Dr. D Tougeron, P-H Haineaux, Dr. R. Gauzolino, Dr. S Milin
From bench to bedside

To promote the Immunoscore in routine clinical settings

French multi-institutional prospective study
(6 centers ; > 900 CRC (stage I-IV) included 2012-1014)

From bench to bedside

To promote the Immunoscore in routine clinical settings

Personalized medicine

Immune events in the course of the disease

AUTOIMMUNE DISEASE

ATOPIC DISEASE

Psychologic

relapse

death
From bench to bedside

analyses moléculaires
5 copeaux 20 microns

envoi postal HEGP

sélection lame et bloc

immunoscore
3 coupes 4 microns

étuve sèche ON

si biopsie(s)
Conclusion:

- The immune reaction at tumor site could be the most important parameter influencing the clinical outcome

  # Prognostic

  -> help the identification of high-risk patients

  -> better define patients for adjuvant therapy

  # Theranostic (companion diagnostic)

- The current multiinstitutional validation of the “immunoscore” may result in the implementation of this parameter as a new component for the classification of cancer.
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