The immune landscape of colon cancer

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Disclosure

Co-founder and Chairman of the Scientific Council:
- HalioDx

Collaborative Research Agreement (grant):
- MedImmune, Janssen, Perkin Elmer, IO biotech

Participation to Scientific Advisory Boards:
- ImmunID, MedImmune, Astra Zeneca, Definiens, Actelion, HalioDx, Incyte

Consultant:
- BMS, Roche, GSK, MedImmune, Janssen, ImmunID, Nanostring, Compugen
Lessons learned after 15 years of systems immunology in human tumors? And how important is the immune system against cancer?

How to explain “Hot” and “Cold” immune infiltrated tumors?

What is the relationship between tumor genotype and immunophenotype?
Anti-PD1 treatment in CRC patients

PD-1 Blockade in Tumors with Mismatch-Repair Deficiency


Table 2. Objective Responses According to RECIST Criteria.

<table>
<thead>
<tr>
<th>Type of Response</th>
<th>Mismatch Repair–Deficient Colorectal Cancer (N=10)</th>
<th>Mismatch Repair–Proficient Colorectal Cancer (N=18)</th>
<th>Mismatch Repair–Deficient Noncolorectal Cancer (N=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete response — no. (%)</td>
<td>0</td>
<td>0</td>
<td>1 (14)*</td>
</tr>
<tr>
<td>Partial response — no. (%)</td>
<td>4 (40)</td>
<td>2 (11)</td>
<td>4 (57)†</td>
</tr>
<tr>
<td>Stable disease at week 12 — no. (%)</td>
<td>5 (50)</td>
<td>2 (11)</td>
<td>0</td>
</tr>
<tr>
<td>Progressive disease — no. (%)</td>
<td>1 (10)</td>
<td>11 (61)</td>
<td>2 (29)</td>
</tr>
<tr>
<td>Could not be evaluated — no. (%)</td>
<td>0</td>
<td>5 (28)</td>
<td>0</td>
</tr>
<tr>
<td>Objective response rate (95% CI)</td>
<td>40 (12–74)</td>
<td>0 (0–19)</td>
<td>71 (29–96)</td>
</tr>
<tr>
<td>Disease control rate (95% CI)</td>
<td>90 (55–100)</td>
<td>11 (1–35)</td>
<td>71 (29–96)</td>
</tr>
<tr>
<td>Median duration of response — wk</td>
<td>Not reached</td>
<td>NA¶</td>
<td>Not reached</td>
</tr>
<tr>
<td>Median time to response (range) — wk</td>
<td>28 (13–35)</td>
<td>NA¶</td>
<td>12 (10–13)</td>
</tr>
</tbody>
</table>

Le et al. NEJM 2015
Cancer is one of the most complex biological system of all

“The whole is greater than the sum of its parts”, Aristotle

-> Systems biology in human cancer
Definition of cancer

1) A tumor cell DNA disease – Cell-centric paradigm
2) Due to the acquisition of secondary key behavioral characteristics following tumor genomic changes (Hanahan & Weinberg, Cell 2001)

- Tumor invasion
- N-Stage
- Early-metastasis (venous emboli)
- M-Stage

- Tumor progression
  - Tis
  - T1
  - T2
  - T3
  - T4

- Tumor grade differentiation
- Tumor aggressiveness
  (driver mutations, CIN, MSI, CIMP...)

- Tumor aggressiveness, progression, invasion and recurrence define early and late stage cancers, and the severity of the disease
Tumor progression, invasion and recurrence are dependent on immunity and the Immunoscore. Pre-existing immunity is determining the fate and survival of the patient, and the likelihood of response to immunotherapy.
Role of immune infiltrates in the promotion/control of early-metastatic invasion (VELIPI) in human cancers? Impact on clinical outcome?

959 colorectal cancer patients

Question

Lymphatic emboli / Tumor

Venous Emboli (VE)
Lymphatic Invasion (LI)
Perineural Invasion (PI)

---

Beneficial
Detrimental
No impact

Promotion
Control

-> Global analysis of tumor microenvironment in Human tumors
Memory T cells, in particular, $T_{EM}$ correlate with the absence of early-metastatic invasion, and improved clinical outcome in colorectal carcinoma.


Quantification of immune cell densities (n=415 Patients, 6640 IHC) revealed the major positive role of cytotoxic and memory T cells for patient’s survival.

The foundation a new concept

**Immune contexture** ← **Immunoscore**
Importance of the distribution of the adaptive immune reaction compared to tumor invasion

Tumor Histopathologic Findings

AJCC/UICC-TNM
Current prognosis classification

Immune cells analysis

CD3\textsubscript{CT}/CD3\textsubscript{IM} evaluation
plus
CD45RO\textsubscript{CT}/CD45RO\textsubscript{IM} evaluation

\[ \text{Coordination adaptive immune reaction more than tumor invasion predicts clinical outcome} \]

Novel Paradigm

COX multivariate analysis (OS) in all stages I, II, III patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-stage</td>
<td>1.2</td>
<td>0.25</td>
</tr>
<tr>
<td>N-stage</td>
<td>1.4</td>
<td>0.15</td>
</tr>
<tr>
<td>Differentiation</td>
<td>1.1</td>
<td>0.84</td>
</tr>
<tr>
<td>Immune</td>
<td>1.9</td>
<td>0.00001</td>
</tr>
</tbody>
</table>

“Contexture: the act of assembling parts into a whole; an arrangement of interconnected parts”

“Immune Contexture”: nature, functional orientation, density, and location within distinct tumor regions, of a natural in situ immune reaction

# Immune Contexture

## Parameters with major positive impact on patient's survival

<table>
<thead>
<tr>
<th>Type</th>
<th>Density</th>
<th>Functional orientation</th>
<th>Location</th>
</tr>
</thead>
</table>
| • Cytotoxic T cells (CD3, CD8)  
• Memory T cells (CD45RO) | Number of cells/mm² | $T_H1$ cells  
Associated factors | • Center of the Tumor (CT)  
• Invasive Margin (IM)  
TLS (Presence and quality) |

- IFNG
- IL12
- TBX21
- IRF1
- STAT1
- GZMA
- GZMB
- GZMH
- PRF
- GLNY
- CXCL1
- CXCL9
- CXCL10
- CXCL13
- ICAM1
- VCAM1
- MADCAM1
- CCL5
- CCL2
- CCL10
- IL21
- IL15
- IL17
- T_reg
- T_H2 cells have variable impact on survival depending on tumor types |
Understanding the evolution of the immune response with tumor progression using systems biology

- Evolution of the tumor microenvironment with tumor progression?
- Immune escape mechanisms in human tumors?

-> Spatio-temporal dynamics of the immune response with tumor progression

Bindea G et al. *Immunity*, 2013
Tumor microenvironment
“Immunome” of purified immune cell subpopulations

Purified immune cell subpopulations: “Immunome”

Cell types (n=28)
- B cells
- T cells
- T helper cells
- Tcm
- Tem
- Th1 cells
- Th2 cells
- TFH
- Th17 cells
- Treg
- CD8 T cells
- Tgd
- Cytotoxic cells
- NK cells
- NK CD56dim cells
- NK CD56bright cells
- DC
- iDC
- aDC
- pDC
- Eosinophils
- Macrophages
- Mast cells
- Neutrophils
- SW480 cancer cells
- Normal mucosa
- Blood vessels
- Lymph vessels

Bindea G et al. *Immunity* 2013
Intratumor “immunome” clustering within CRC

Cluster 1
Cluster 2

NC

Survival (Months)

Cluster 1 (n=60)
Cluster 2 (n=45)

Expression of 372 significant genes within CRC tumors

HR = 1.996 (1.07-3.74)
* P < 2.49e-02
Intratumor “immunome” clustering within CRC

T cells
Cytotoxicity
Th1

B cells
MHC Cl II

T<sub>FH</sub> cells

MHC Cl I
Inflammation

- “Immunome” analysis from CRC tumors reveals functional association of T cells, cytotoxicity, B cells, MHC Cl II molecules, and T<sub>FH</sub> cells

Bindea G et al. *Immunity* 2013
Intratumor innate cells, adaptive cells, and vessels

IHC enzymatic stainings: -> Quantification (cells/mm²)
Cohort 1 (n=120 patients), cohort 2 (n=415 patients)

Bindea G et al. *Immunity* 2013
Intratumor B cells, T cells and $T_{FH}$ cells

Bindea G et al. *Immunity* 2013
Understanding the evolution of the immune response with tumor progression: The immune landscape

Bindea G et al. *Immunity* 2013

**tumor progression**

Cell density (cells/mm²)

Invasive margin (IM)
Center of the tumor (CT)
Understanding the evolution of the immune response with tumor progression: The immune landscape

Bindea G et al. *Immunity* 2013

**tumor recurrence**

- **Hazard ratio**
  - Green: Good
  - Red: Bad

- **Impact on DFS**

**Invasive margin (IM)**

**Center of the tumor (CT)**

- **x-y-Force**
  - Directed Topology

**B-cells**

**T**

**FH**

**T**

**H**

17
Immune contexture associated with prolonged survival

Bindea G et al. *Immunity* 2013
Relationship between molecular phenotypes and immunophenotypes of CRC tumors using systems biology

- Mutation, immunogenicity and immune microenvironment?
- Immune escape mechanisms in human tumors?

-> Rationale for an immune classification of tumors

Angelova M et al. *Genome Biol*, 2015

Characterization of the immunophenotypes and antigenomes of colorectal cancers reveals distinct tumor escape mechanisms and novel targets for immunotherapy

High-resolution genomic analysis: the tumor-immune interface comes into focus

Angelova M et al. *Genome Biol*, 2015
Molecular phenotypes and immunophenotypes of CRC tumors

Spin chart from (n=460 patients)

Molecular phenotype
- Microsatellite instability
  - MSI-H
  - MSS
- Mutation rate
  - Hypermutated
  - Non-hypermutated
- Methylation subtype
  - CIMP-H
  - CIMP-L
  - CIMP-Neg

Immunome of CRC tumors

Angelova M et al. *Genome Biol.* 2015
Molecular phenotypes and immunophenotypes of CRC tumors

Spin chart from (n=460 patients)

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  - CIMP-L
  - CIMP-Neg

Angelova M et al. Genome Biol. 2015
Molecular phenotypes and immunophenotypes of CRC tumors

Enrichment of immune subpopulations: TILs in 97% of tumors, and T-cells in 69% of tumors

Spin chart from (n=460 patients)

Innate immunity

Adaptive immunity

metagenes expression profiles
Enriched and depleted immune subpopulations in hypermutated (MSI-H and MSS^)

Volcano plots for enriched (blue) and depleted (yellow) TIL subpopulations in the distinct patient groups compared to normal samples (n=50).

Tregs and MDSCs are depleted

Volcano plots for enriched (blue) and depleted (yellow) TIL subpopulations in the distinct patient groups compared to normal samples (n=50).

Angelova M et al. Genome Biol. 2015
How to explain “Hot” and “Cold” immune infiltrated tumors?

<table>
<thead>
<tr>
<th>Immunoscore</th>
<th>Patient 1 (weak)</th>
<th>Patient 2 (moderate)</th>
<th>Patient 3 (strong)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Im0</td>
<td>CD3</td>
<td>Tumor</td>
<td>Im4</td>
</tr>
<tr>
<td>CD3/CD8</td>
<td>Median OS &lt; 2 years</td>
<td>4.9 years</td>
<td>&gt; 15 years</td>
</tr>
<tr>
<td>Center/Margin</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Mechanisms associated with T cells infiltration

**Attraction**
- CXCL9
- CXCL10
- CCL2
- CCL5
- CX3CL1
- CXCL13

**Adhesion**
- MADCAM1
- VCAM1
- ICAM1

- T cells
- Memory T cells
- TH1 Cytotoxic
- TFH B cells

Mlecnik et al. *Gastroenterology* 2010
Bindea et al. *Immunity* 2013
IL15 and intratumoral immune reaction

*In situ* densities of proliferating B and T cells

<table>
<thead>
<tr>
<th>CD20+</th>
<th>CD3+</th>
<th>Ki67+</th>
</tr>
</thead>
</table>

-> Quantification in tumor core (CT), invasive margin (IM), and lymphoid ilets (LI)
Mechanisms associated with T cells infiltration

**Attraction**
- CXCL9
- CXCL10
- CCL2
- CCL5
- CX3CL1

**Adhesion**
- MADCAM1
- VCAM1
- ICAM1

**Local lymphocyte proliferation**
- IL15

**T cells**
- Memory T cells
- TH1 Cytotoxic
- TFH B cells

Mlecnik et al. *Gastroenterology* 2010
Bindea et al. *Immunity* 2013
Implications for cancer classification and therapies

From the **Immune contexture**

(Complexity of intratumor immune reaction)

To the **Immunoscore**

(A simple and powerful immune test)
## Colorectal cancer classifications

<table>
<thead>
<tr>
<th>Tumor cell extension and invasion</th>
<th>T-STAGE</th>
<th>N-STAGE</th>
<th>M-STAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ways to classify</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell of origin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular pathway</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutation status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gene expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunoscore</td>
<td>Immunoscore</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3+ T cells</td>
<td>CD3+ T cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD8+ T cells</td>
<td>CD8+ T cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>Density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location (CT, IM)</td>
<td>Location (CT, IM)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Ways to classify**

- Morphology
  - Mucinous
  - Medullary
  - Adeno. NOS
  - Serrated
  - Signet ring cell
  - Micropapillary
  - Cribriform comedo-type

**Tumor cell characteristics**

- Cell of origin
  - Enterocyte
  - Goblet-like
  - Transit-amplifying-R
  - Transit-amplifying-S
  - Inflammatory
  - Stem-like

- Molecular pathway
  - CIN
  - MSI
  - CIMP
  - CIMP

- Mutation status
  - BRAF
  - APC
  - KRAS
  - TP53

- Gene expression
  - CCS1
  - CCS2
  - CCS3

- Host immune response
  - Immunoscore
  - CD3+ T cells
  - CD8+ T cells
  - Density
  - Location (CT, IM)

Galon et al. *J Pathol.* 2014
Prolonged survival in patients with high *Immunoscore* (Im) based on the evaluation of CD45RO-CT/IM and CD8-CT/IM

**AJCC/UICC-Stage I-III**

**AJCC/ UICC-Stage I-IV**

Multivariate proportional hazard COX analysis among all patients with AJCC/UICC-TNM Stage I/II/III colorectal cancer

According to clinical parameters and immune parameters

<table>
<thead>
<tr>
<th>COX analysis for DFS</th>
<th>HR</th>
<th>Log Rank P-values</th>
</tr>
</thead>
<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 Colloide</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><strong>Immunoscore</strong></td>
<td><strong>0.65</strong></td>
<td><strong>0.0003</strong></td>
</tr>
</tbody>
</table>

According to AJCC/UICC-TNM classification and immune score

<table>
<thead>
<tr>
<th>COX analysis</th>
<th>DFS</th>
<th>OS</th>
<th>DSS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>HR</td>
<td>HR</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>P-value</td>
<td>P-value</td>
</tr>
<tr>
<td>AJCC/UICC-TNM</td>
<td>1.38</td>
<td>1.18</td>
<td>1.43</td>
</tr>
<tr>
<td></td>
<td>0.09 ns</td>
<td>0.29 ns</td>
<td>0.10 ns</td>
</tr>
<tr>
<td>Immunoscore</td>
<td>0.64</td>
<td>0.71</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td><strong>&lt;0.0001</strong></td>
<td><strong>&lt;0.0001</strong></td>
<td><strong>&lt;0.0001</strong></td>
</tr>
</tbody>
</table>

-> Validation in 2 independent cohorts of colorectal cancer patients

Metastasis analysis

One primary tumor
Colorectal cancer

Multiple metastatic sites
Liver Metastasis
Lung Metastasis

- Immunoscore within multiple metastases at different sites
- Death at years: Low Immunoscore: 76%  vs High Immunoscore: 15%
THE IMMUNOSCORE AS A NEW POSSIBLE APPROACH IN THE CLASSIFICATION OF CANCER

Naples, Italy, Feb 2012
Principal investigator: J. Galon

The IMMUNOSCORE

- Standardized Operating Procedure
- Today’s tools for modern pathologists

Immunoscore (I) using whole slide FFPE

Routine whole slide stainings & precise image quantification

Immunostaining

Definition of Tumor Regions

Density plots

HE  CD8  CD3

CT  IM  Tissue

I
The Immunoscore as a New Possible Approach for the Classification of Cancer

Support (moral) from the World Immunotherapy Council (WIC), and support from societies including, EATI, BDA, CCIC, CIC, CRI, CIMT, CSCO, TIBT, DTIWP, ESCII, NIBIT, JACI, NCV-network, PIVAC, ATTACK, TVACT...

Worldwide Immunoscore consortium (PI: J Galon)

(23 Centers, 17 countries: >3000 patients)

Immunoscore meetings:
- Feb 2012, Italy
- Dec 2012, Italy
- Nov 2013, SITC, USA
- Dec 2013, Italy
- Jan 2014, Qatar
- Jul 2014, Paris, France
- Nov 2014, SITC, USA
- Nov 2015, SITC, USA
- Dec 2015, Italy
Ongoing international consortia on Immunoscore

Worldwide Immunoscore Consortium

- PI: J Galon
- SITC, EATI
- 23 Centers, 17 countries

European Immunoscore Consortium

- PI: J Galon
- ERAnet H2020
- 5 European countries

National Prospective Immunoscore Consortium

- PI: F Pagès
- PHRC, INCa
- 6 hospitals
The immune landscape in human tumors, and definition of the immune contexture

Immune contexture

Type: Adaptive immunity, cytotoxic, memory T cells

Density: Quantification (cells/mm²)

Location: Tumor center, Margin, Tertiary lymphoid ilets

Functional orientation:
- IFNG
- IL12
- TBX21
- IRF1
- STAT1
- GZMA
- GZMB
- GZMH
- PRF
- GLNY
- CX3CL1
- CXCL9
- CXCL10
- CCL5
- CCL2
- MADCAM1
- ICAM1
- VCAM1
- ITGAE

T<sub>FH</sub> cells, B cells

CXCL13

IL21, IL15

Galon J et al. *Immunity* 2013
The overlap between the immune contexture, the immunologic constant of rejection and the Immunoscore

Immune contexture

Type: Adaptive immunity, cytotoxic, memory T cells

Density: Quantification (cells/mm²)

Location: Tumor center, Margin, Tertiary lymphoid ilets

Functional orientation: IFNG, IL12, TBX21, IRF1, STAT1, IFNG, IL12, TBX21, IRF1, STAT1

Immunologic Constant of Rejection: CX3CL1, CXCL9, CXCL10, CCL5, CCL2, CXCL13, MADCAM1, ICAM1, VCAM1, ITGAE

Immunoscore: GZMA, GZMB, GZMH, PRF, GLNY, CXCL1, IL21, IL15

Galon J et al. *Immunity* 2013
Stratification of cancer based on the immune status

Tumor classification

- MSI-H
- MSS^
- MSS/CIMP.hi
- MSS
- MSS-CIMP.lo

Immune classification

IMMUNE

-> Importance of having standardized immune Assays
Towards the future

1) Prognostic: Immunoscore™ - “I”
   -> Novel cancer classification
   - Cytotoxic & Memory T cells
   - Prognostic value: “I” > TNM
   - Tumor invasion parameters (T, N, …) are statistically dependent on “I”

2) Predictive markers
   - Current clinical trials

3) Patient stratification
   Based on immune contexture defect

4) Novel immunotherapies
   Adapted to patient’s immune defect

Galon J et al. *Immunity* 2013
Join Keystone Symposia for the 2016 conference on:

Cancer Immunotherapy: Immunity and Immunosuppression

Meet Targeted Therapies

January 24–28, 2016
Fairmont Hotel Vancouver | Vancouver, British Columbia | Canada

Scientific Organizers:
Barbara Seliger, Jerome Galon and Francesco M. Marincola
Galon lab.
INSERM, CRC, Paris, France
Franck Pagès
Tessa Fredriksen
Stéphanie Mauger
Florence Marliot
Lucie Lafontaine
Amélie Bilocq
Amos Kirilovsky
Marie Tosolini
Angela Vasaturo
Helen Angell
Mihaela Angelova
Sarah Church
Pauline Maby
Marc Van den Eynde
Bernhard Mlecnik
Gabriela Bindea

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Innsbruck, Austria
Pornpimol Charaoetong
Zlatko Trajanoski

Institute for Genetics, Graz, Austria
Anna Obenauf
Michael Speicher

Clinical Division of Oncology, Medical University
of Vienna, Austria
Anna Berghoff
Matthias Preusser

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Worldwide

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Christopher Becker
Maximilian Waldner

Team 10, Cordeliers Research Center, France
Gauthier Stoll
Guido Kroemer

Team 13, Cordeliers Research Center, France
Hervé Fridman

Rouen University, France
Jean Baptiste Latouche

Dpt. of General and Digestive Surgery,
HEGP, Paris, France
Anne Berger

Dpt. of Pathology, HEGP, Paris, France
Tchao Meatchi
Christine Lagorce
Patrick Bruneval

Dpt. Digestive Surgery and
Pathology, Avicenne, Bobigny, France
Philippe Wind

Dpt. Pathology, Graz hospital, Graz, Austria
Martin Asslaber

SITC, EATI and all supportive societies
Definiens, PathForce, MedImmune
The immune landscape of colon cancer

Jérôme GALON

ESMO Immuno-Oncology
Prague, Czech Republic,
October 2nd 2015

INSERM, Laboratory of Integrative Cancer Immunology
Cordeliers Research Center, Paris, France