Prognostic and predictive value of Immunoscore

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Disclosures

Co-founder and chairman of the scientific advisory board:
  - HalioDx

Collaborative Research Agreement (grants):
  - MedImmune, Janssen

Participation to Scientific Advisory Boards:
  - BMS, ImmunID, MedImmune, Astra Zeneca, Novartis

Consultant:
  - BMS, Roche, Ventana, GSK, MedImmune, ImmunID, Nanostring, Definiens, Compugen, Actelion
Redefining cancer

Primary Tumor → Early stages (N0M0)

Tis

T1

T2

Early-metastatic
Invasion (VELIPI+)

T3

Distant
Metastasis

(T+) Ref. 1, 15

T4

What drives metastasis?

How to explain metachronous metastasis?

Parameters associated with patients’ survival?

Tumor recurrence – Metachronous metastasis

Prognosis – Survival

Cancer treatment - immunotherapy

Ref. 11, 12, 20

Ref. 1, 2, 9, 10, 15, 16, 17

Ref. 13, 14, 19

Ref. 1, 2, 3, 4, 5

Ref. 6, 7, 8

Ref. 17, 18, 19, 20, 21

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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
Hallmarks 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

3) Our hypothesis: cancer is heterogeneous microenvironment, dynamic and communicating with the immune system
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, 2011)

-> 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 pre-existing immunity and on Immunoscore.

- Pre-existing immunity is determining the fate and survival of the patient.
- Pre-existing immunity is determining the likelihood of response to immunotherapy.
Tumor microenvironment

- Neutrophils
- Eosinophils
- Basophils
- Mast cells
- Red cells
- T-lymphocytes
- B-lymphocytes
- T-cytotoxic
- B-lymphocytes
- T-lymphocytes
- Neutrophils
- Eosinophils
- Basophils
- Mast cells
- Macrophages
- NK cells
- NKT cells
- NK cells
- Lymph vessels
- Blood vessels
- Plasma
- B cells
- Tumor cells
- mDC
- T_H1
- T_H2
- T_H17
- T_H3
- Treg
- T_memory
- T_EM
- T_EMRA
- T_memory
- T_EM
- T_EMRA
- T-cytotoxic
- T-H1
- T-H2
- T-H17
- T-H3
- Treg
- T-memory
- T_EM
- T_EMRA
- T-cytotoxic
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- Treg
- T-memory
- T_EM
- T_EMRA
- T-cytotoxic
- T-H1
- T-H2
- T-H17
- T-H3
- Treg
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

**Immunoscore**

**Immune contexture**
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<sub>CT</sub>/CD3<sub>IM</sub> evaluation
plus
CD45RO<sub>CT</sub>/CD45RO<sub>IM</sub> evaluation

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Coordinated adaptive immune reaction more than tumor invasion predicts clinical outcome

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

<table>
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<tr>
<th>Parameter</th>
<th>$HR$</th>
<th>$P$ value</th>
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<tr>
<td>• T-stage</td>
<td>1.2</td>
<td>0.25</td>
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<td>• N-stage</td>
<td>1.4</td>
<td>0.15</td>
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<td>• Differentiation</td>
<td>1.1</td>
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<td>• Immune</td>
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“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

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
Implications for cancer classification and therapies

From the **Immune contexture**

(Complexity of intratumor immune reaction)

\[ \downarrow \]

To the **Immunoscore**

(A simple and powerfull Immune Test)
How to explain “Hot” and “Cold” immune infiltrated tumors?

Patient 1 (weak)
- Immunoscore: Im0
- CD3/CD8: Center/Margin
- Median OS: < 2 years
- Median OS (death): 

Patient 2 (moderate)
- Immunoscore: Im2
- Median OS: 4.9 years

Patient 3 (strong)
- Immunoscore: Im4
- Median OS: > 15 years

Patient 1 (weak)
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**

![Graphs showing survival data](image)

Prognostic importance of the in situ immune reaction in patients with early-stage (Stage I/II) colorectal cancer

n=602 Stage I/II

**Stage I cancer**

\[ CD45RO_{CT/IM} \text{ } CD8_{CT/IM} \] \( P < .0001 \)

**Stage II cancer**

\[ CD45RO_{CT/IM} \text{ } CD8_{CT/IM} \] \( P < .0001 \)

THE IMMUNOSCORE AS A NEW POSSIBLE APPROACH IN THE CLASSIFICATION OF CANCER

Naples, Italy, Feb 2012
Organizer: P Ascierto, J. Galon,
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

World Immunotherapy Council inaugural meeting (Feb 2012)

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)

Assay harmonization

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
Worldwide Immunoscore consortium (PI: J Galon)

Study design

- Immunoscore Raw data
  - Referent Center
    - QA/QC
      - External Statistician (Mayo)
        - TS
          - >1000
        - IVS
          - >1000
    - Clinical data
      - Encrypted data
        - Analysis
          - EVS
            - >1000 Pts.
Impacting all cancers

- Bladder cancer
- Brain cancer
- Breast cancer
- Cervical cancer
- Colorectal cancer
- Kidney cancer
- Liver cancer
- Lung cancer
- Melanoma cancer
- Ovarian cancer
- Pancreatic cancer
- Prostate cancer

Images depict various cancer types.
Metastasis analysis

Multiple primary tumors

- Melanoma
- Breast cancer
- Kidney cancer
- Lung cancer

Other cancers

One metastatic site

Brain Metastasis

Berghoff A. et al. *Oncolimmunol*. In press

➢ Immunoscore within brain metastasis
Immunoscore in brain metastasis and survival

Immunoscore quantification (CD3, CD8, in CT and IM regions) within Brain Metastases (n=116 patients)

Immunoscore predicts overall survival and long-term survival in patients with Brain Metastases

Berghoff A. et al. *OncoImmunol.* In press
The carcinoma sequence and tumor development

**Genetic alteration**

- APC
- KRAS
- BRAF
- SMAD2/3
- 18q LOH
- TP53
- 17p LOH

**Carcinoma sequence**

- Dysplasia
- Hyperplasia
- Adenoma
- Carcinoma

Primary Tumor → Early stages (N0M0)

- Tis
- T1
- T2
- T3
- T4

Distant Metastasis

What drives metastasis?
What drives metastasis?

- Early-Metastasis (venous emboli, perineural invasion)
- Synchronous Metastasis (M1)
- Metachronous Metastasis (recurrence)

Mlecnik et al. Science Transl Med. accepted
What drives metastasis?

Chromosomal Instability (CIN) ?

Cohort 1: n=276

M0

M1

Cohort 2: n=205

M0

M1

➢ CIN are similar in M0 and M1 patients
Genomic alterations in tumors

Chromosomal instability pattern (CIN)

Mutation pattern

Tumor gene expression pattern

-> No significant difference between M0 and M1 cancer patients
What drives metastasis?

M1 stage

Mlecnik et al. *Science Transl Med.* accepted
Metastasis risk depending on lymph vessels and GZMB densities

Quantification of blood and lymph vessel densities and GZBM+ cell densities within primary tumors (CT and IM regions)

- Risk of metastasis increases:
  - from 0% (blue) for tumors with High lymph vessels (IM) + high High GZMB (CT)
  - to 49% (red) for tumors with Low lymph vessels (IM) + Low High GZMB (CT)

❖ Decreasing density of 2 markers
❖ Analysis of metastasis frequency
Specific genotype and gene profiling in MSI-H patients

Chromosomal instability, mutation patterns, and gene expression profiling in 270 MSI-H and MSS patients
Patients with MSI-H have multiple Frameshift mutations (FSmut)

ExomeSeq

Multiplex FSmut validation

Mlecnik et al. accepted
MSI-H patients with TGFBR2 FSmut have anti-TGFBR2-FSmut T-cells in their tumor

MHC-dextramer Specific stainings
Anti-TGFBR2mut Specific T-cells

HLA-B*0801 (AAKGRGAAL) Negative Control
HLA-A*0201 (ALIAPVHAV) Negative Control
HLA-A*0201 (RLSSCVPVA)

Mlecnik et al. accepted
MSI-H patients with TGFBR2 FSmut have anti-TGFBR2-FSmut T-cells able to kill APC^{A2.1/FSmut^{P2}} cells

Mlecnik et al. accepted
Immunoscore high (I3, I4) patients have prolonged survival regardless of the MSI status.

Cox multivariate analysis for DSS
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, GZMA, GZMB, GZMH, PRF, GLNY, CXCL1, CXCL9, CXCL10, CCL5, CCL2, MADCAM1, ICAM1, VCAM1, ITGAE

**Immunologic Constant of Rejection** (other diseases)
- CXCL13
- IL21, IL15

**Immunoscore**

Galon J et al. *Immunity* 2013
The overlap between prognostic, predictive and mechanistic immune signatures

NON-Immune signatures

IMMUNE signatures

Prognostic

Predictive

Mechanistic

Galon J et al. *Immunity* 2013
Church S & Galon J *Immunity* 2015

Immunoscore
Th1
Cytotoxicity
Chemokines
Cytokines
Adhesion

Immune contexture
The continuum of cancer immunosurveillance: predictive, prognostic and mechanistic signatures

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

Tumor classification

Immune classification

-> Importance of having standardized immune Assays
Galon lab.
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Dpts. of Pathology from the 23 Centers
Worldwide
SITC and all supportive societies
Definiens, PathForce, MedImmune
Prognostic and predictive value of Immunoscore

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