SITC Immunoscore Validation Project

Society for Immunotherapy of Cancer

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Thank you to participating Immunoscore centers worldwide!

SITC Annual Meeting 2016
National Harbor, USA,
November 12th 2016

Immunoscore as a Prognostic Marker in Stage I-III Colon Cancer: Results of a SITC-led Global Validation Study

Jérôme GALON

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

SITC 2016
NATIONAL HARBOR, MD
NOVEMBER 9 - 13, 2016

SITC 2016
NATIONAL HARBOR, MD
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Disclosures

Co-founder and chairman of the scientific advisory board:

- *HalioDx*

Collaborative Research Agreement (grants) :

- *Perkin-Elmer, IObiotech, MedImmune, Astra Zeneca, Janssen*

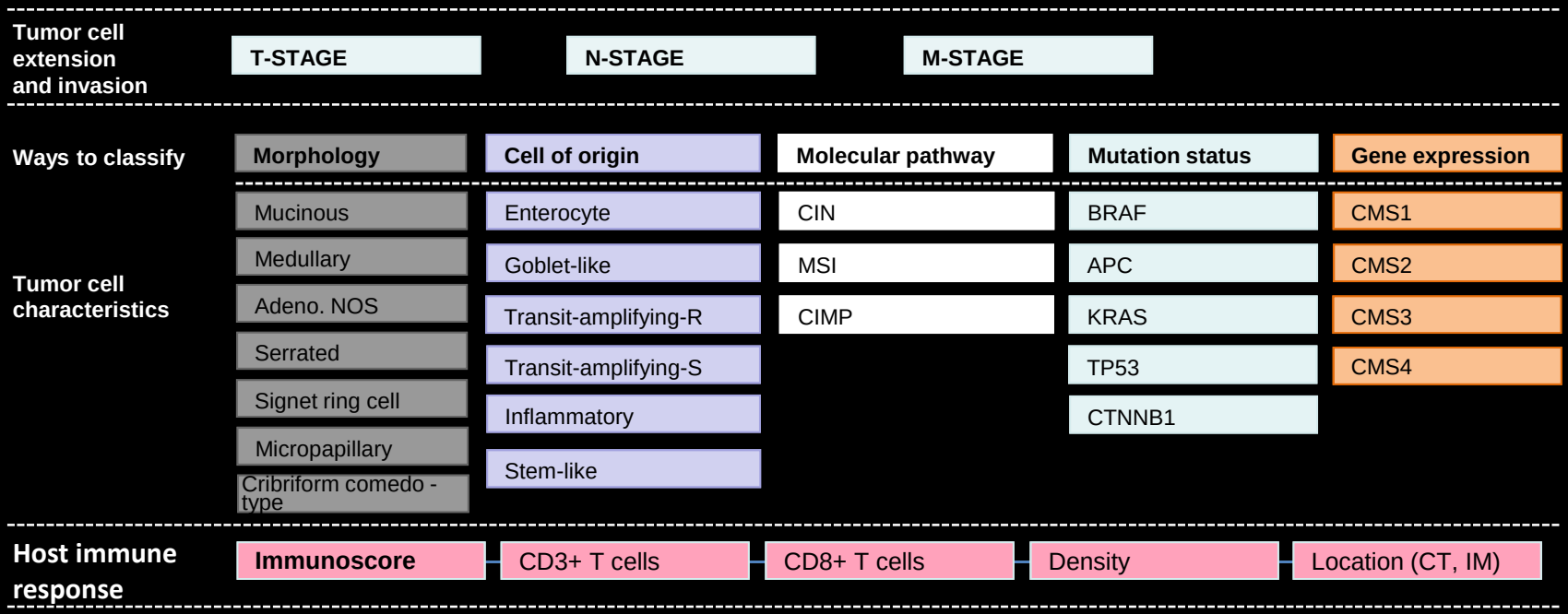
Participation to Scientific Advisory Boards:

- *BMS, MedImmune, Astra Zeneca, Novartis, Definiens, ImmunID, IObiotech, Northwest Biotherapeutics, Actelion, Amgen, Mologen, Kite Pharma*

Consultant :

- *BMS, Roche, Ventana, GSK, MedImmune, ImmunID, Nanostring, Compugen,*

Colorectal cancer classifications



> 80 publications showed the good prognostic value of T-cell infiltration

Galon et al. ***J Pathol***. 2014

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)

(21 Centers, 15 countries: >3000 patients)

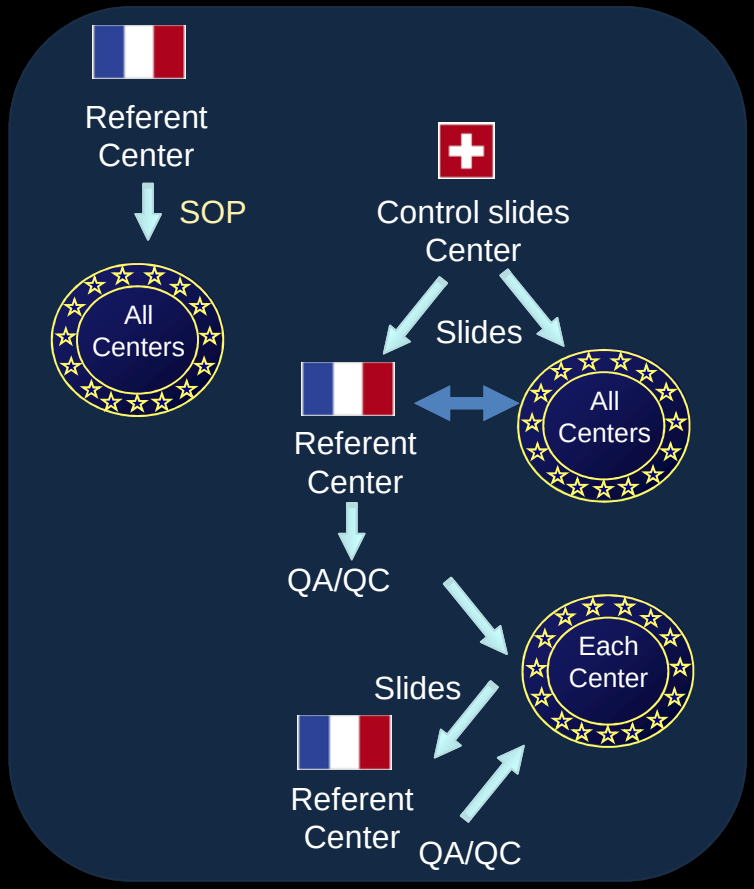
Assay harmonization

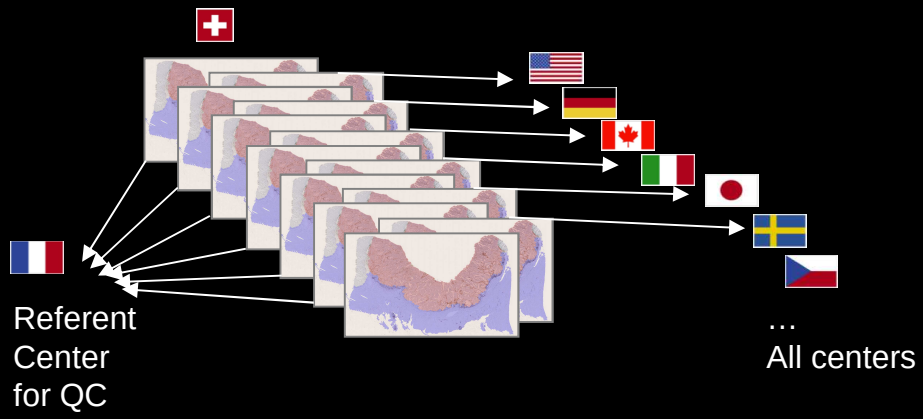
Immunoscore meetings :

- 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
- Dec 2015, Italy

Worldwide Immunoscore consortium (PI: J Galon)

Study design

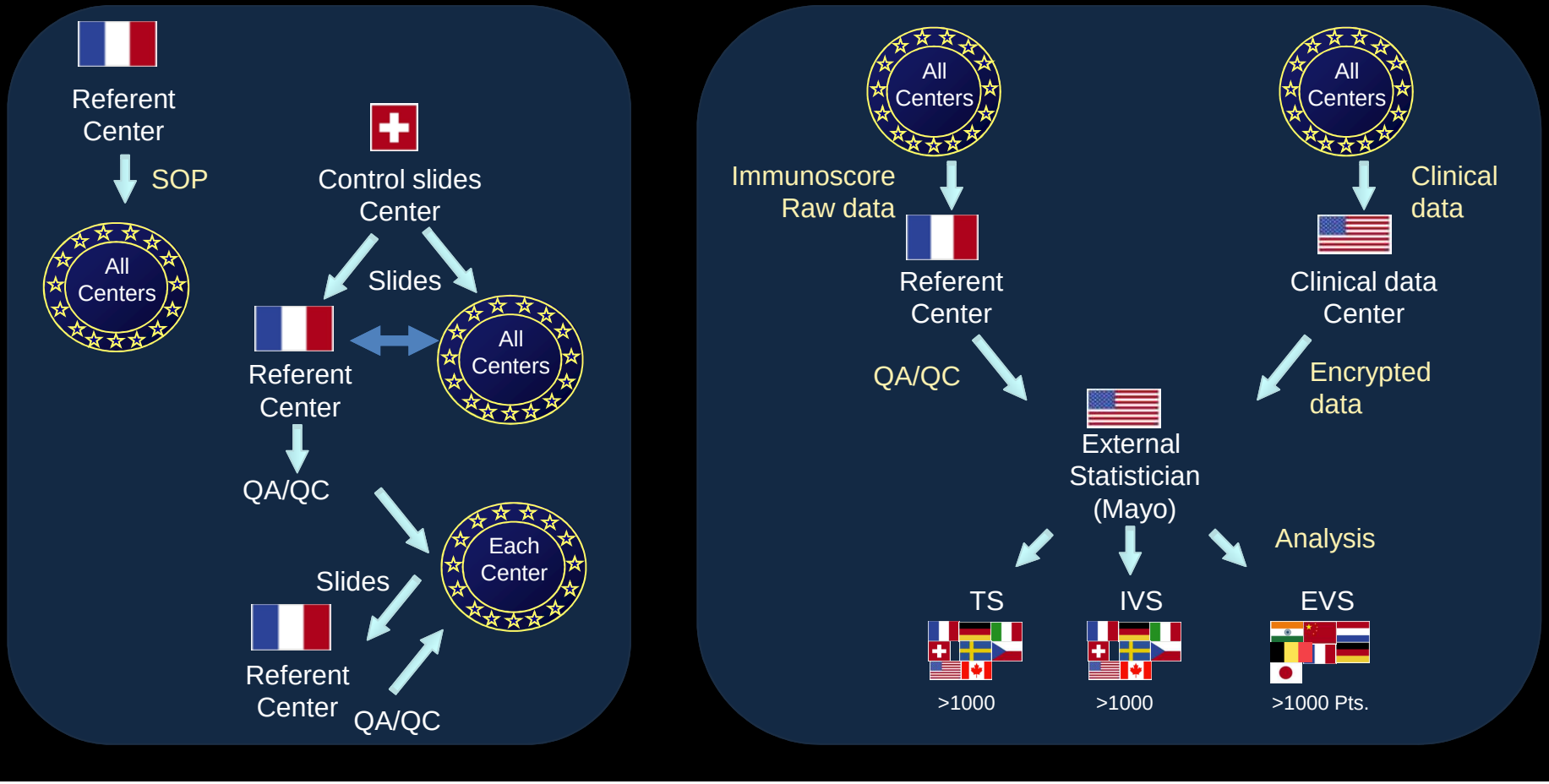




Quality Controls **Harmonization**

Worldwide Immunoscore consortium (PI: J Galon)

Study design



IMMUNOSCORE: METHODS

-> Standardized Operating Procedure
-> Today's tools for modern pathologists

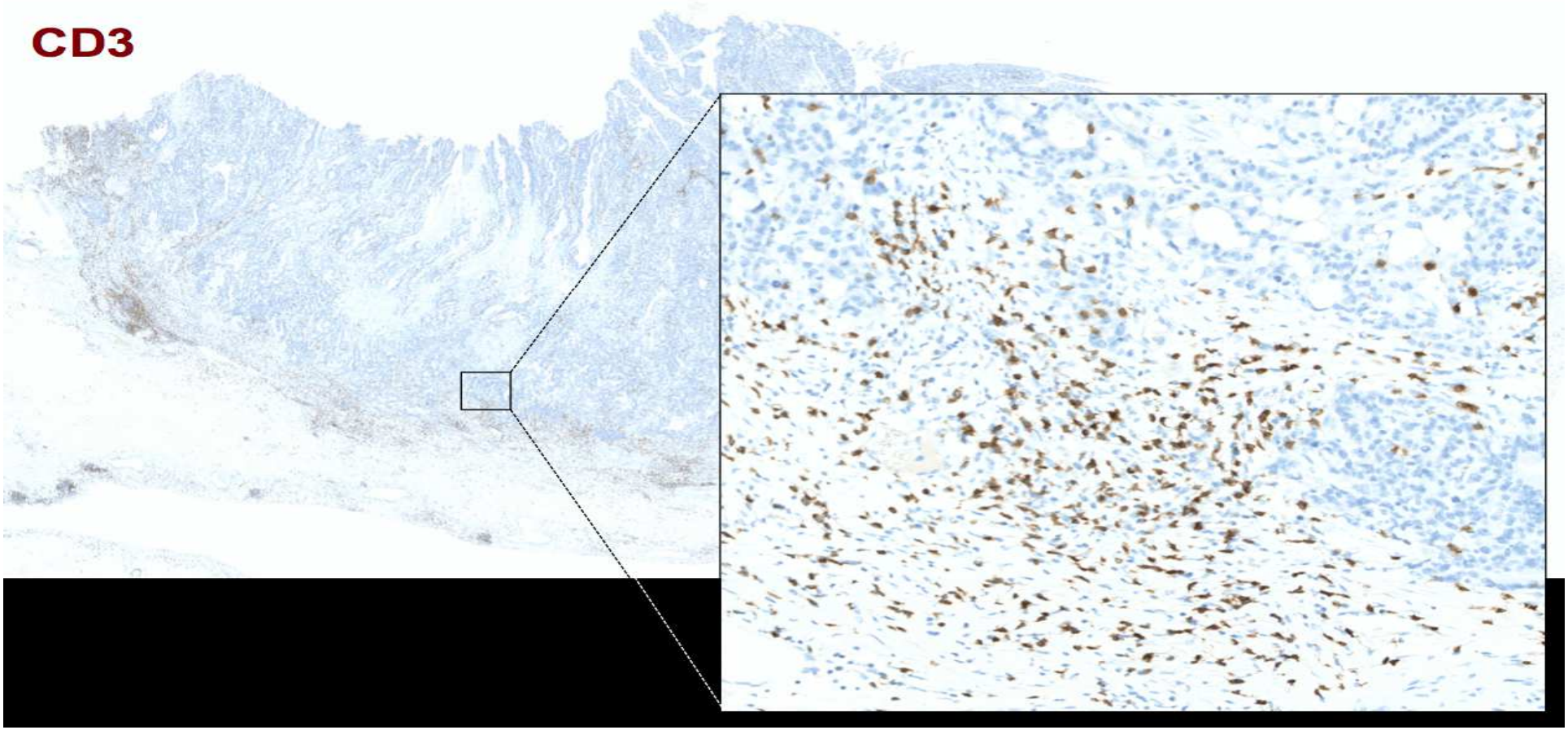
-> Conceptual and technological challenge

Immunoscore

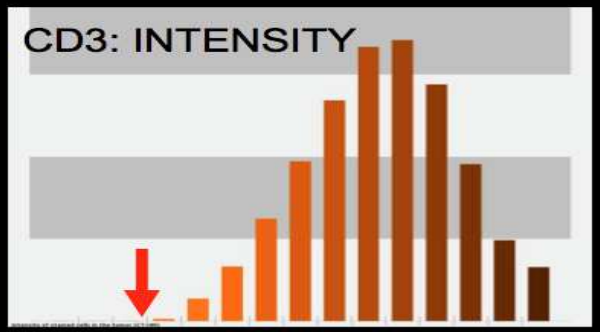
Galon J et al. ***J. Transl. Med.*** 2012
Galon J et al. ***J. Pathol.*** 2014

Immunoscore using whole slide FFPE

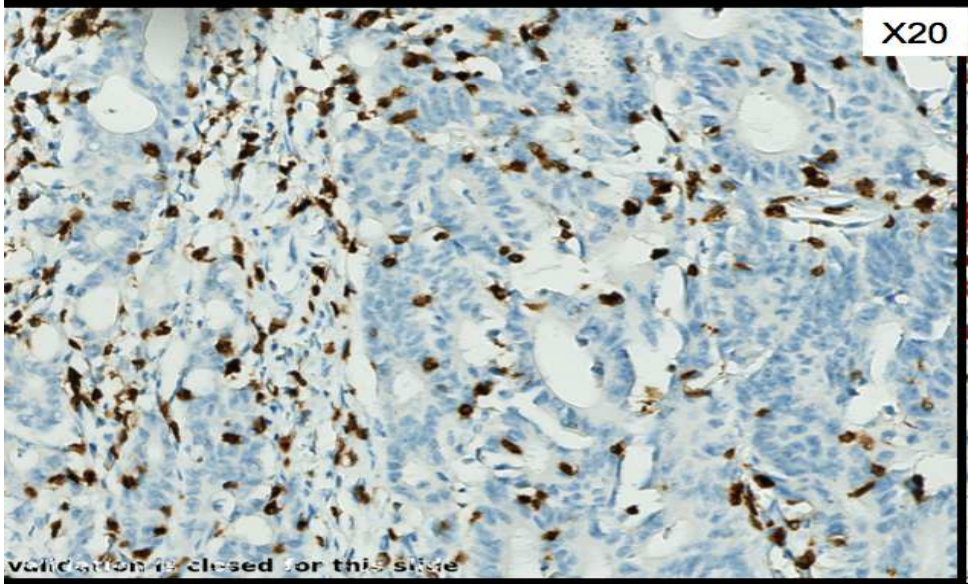
Routine whole slide stainings & full image quantification

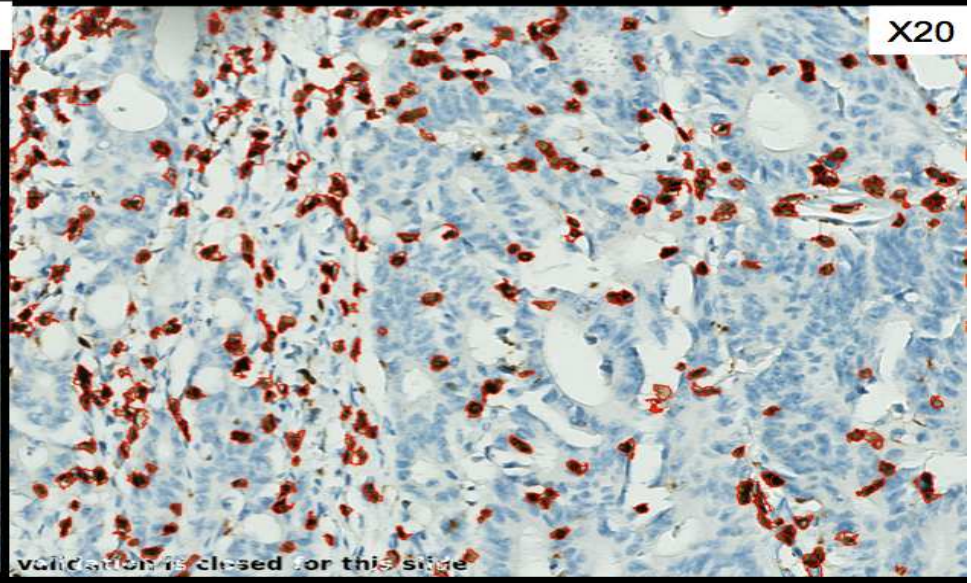


Digital quantification: Density (cells/mm^2)



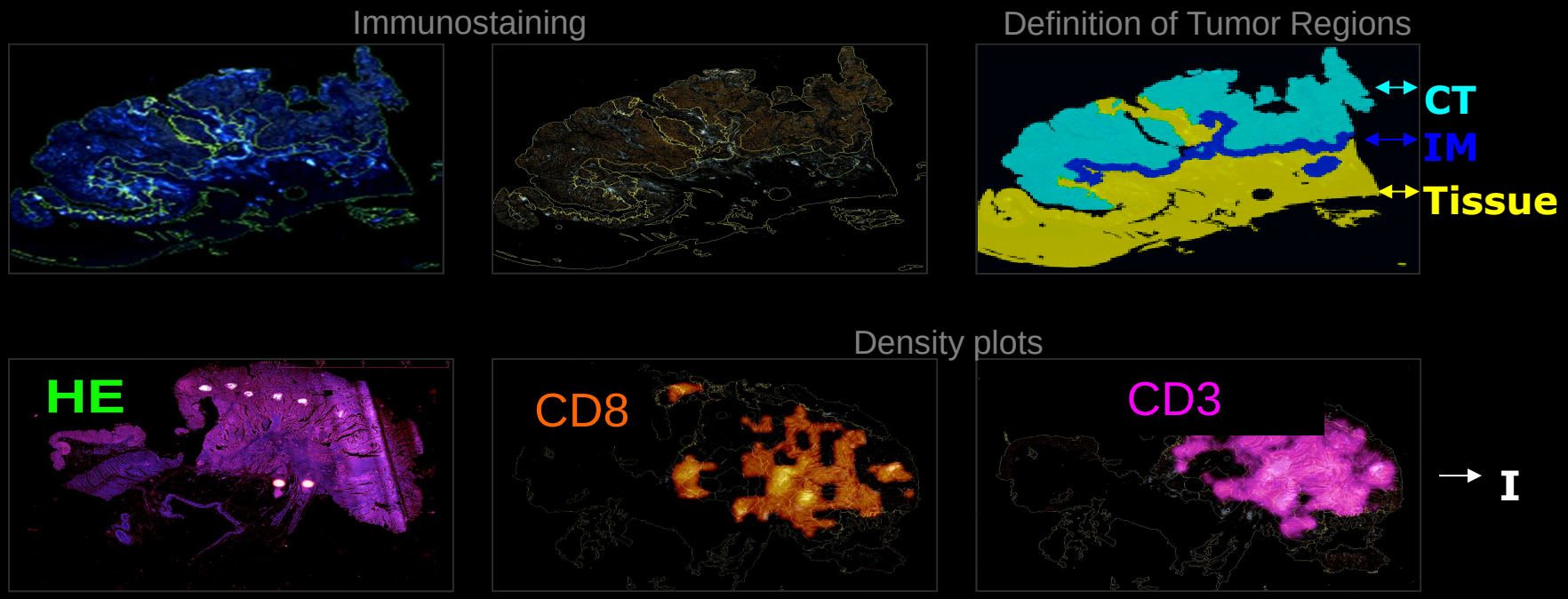
Mean brown Intensity: 264 [+/-61]
Median brown intensity: 265 [+/-61]
Minimum brown intensity: 100
Maximum brown intensity: 722





Immunoscore (I) using whole slide FFPE

Routine whole slide staining & precise image quantification



✓ Immunoscore is **Standardized, Objective, Quantitative**

Immunoscore worldwide consortium study

Methods:

- ✓ Statistical analysis plan was pre-defined
- ✓ All statistical analyses performed by blinded external statisticians
- ✓ Primary study endpoint was time-to-recurrence (TTR) for Immunoscore (High/Low)
- ✓ Analyses were performed by Cox models stratified by enrolling center

***Of note*: There were at least 4 big hurdles in this study:**

- ✓ Heterogeneity of patients between Centers
- ✓ Heterogeneity of patient-care between countries
- ✓ Heterogeneity of IHC staining between Centers (we did our best with QCs)
- ✓ Heterogeneity of clinical data and follow-up between Centers

The main objectives were to demonstrate feasibility, reproducibility, signifcance and **robustness** of Immunoscore in a Worldwide study

Patient population and clinical characteristics

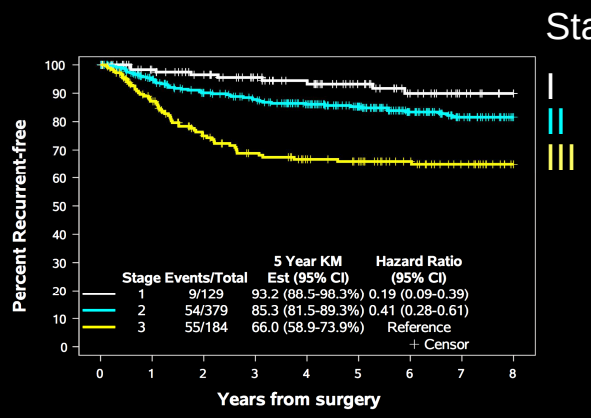
Inclusion criteria:

- ✓ Colon cancer
- ✓ Stages I/II/III (T1-T4, N0-N2, M0)
- ✓ No neo-adjuvant treatments
- ✓ Clinical data and follow-up

Exclusion criteria:

- ✓ Rectum cancer (n=255)
- ✓ Stages IV (M1) (n=81)
- ✓ Neo-adjuvant treatments (n=6)
- ✓ Missing Clinical data (n=45)
- ✓ Missing follow-up (n=127)
- ✓ Staining intensity <152 (n=86)
- ✓ Missing/incomplete biomarker data (n=490)

3855 patients were quantified for Immunoscore



2667 patients were analyzed after QC and exclusion

Following a pre-defined Statistical analysis workplan

Immunoscore in Colon Cancer
Statistical Analysis Plan — Society for Immunotherapy of Cancer/Mayo Clinic

Patient population and clinical characteristics

	TS	IVS	EVS
Time to end of Follow-up			
Median Survival Months:	143.6 (127.3-162.2)	180.7 (147.7-197.6)	160.1 (124.5-191.4)
5 Yr Survival Rate:	74.9% (71.6%-78.2%)	77.8% (74.5%-81.1%)	68.8% (65.6%-72.0%)
Recurrence-free Survival time			
Median Survival Months:	122.3 (107.6-132.8)	140.2 (116.6-150.4)	95.1 (80.0-106.9)
5 Yr Survival Rate:	68.3% (64.7%-71.9%)	71.3% (67.6%-75.0%)	58.3% (54.9%-61.8%)

*** All Multivariate models**

Adjusted for: Immunoscore, age, gender, T-Stage, N-Stage

Stratified by: City Center

Biomarker characteristics: Results

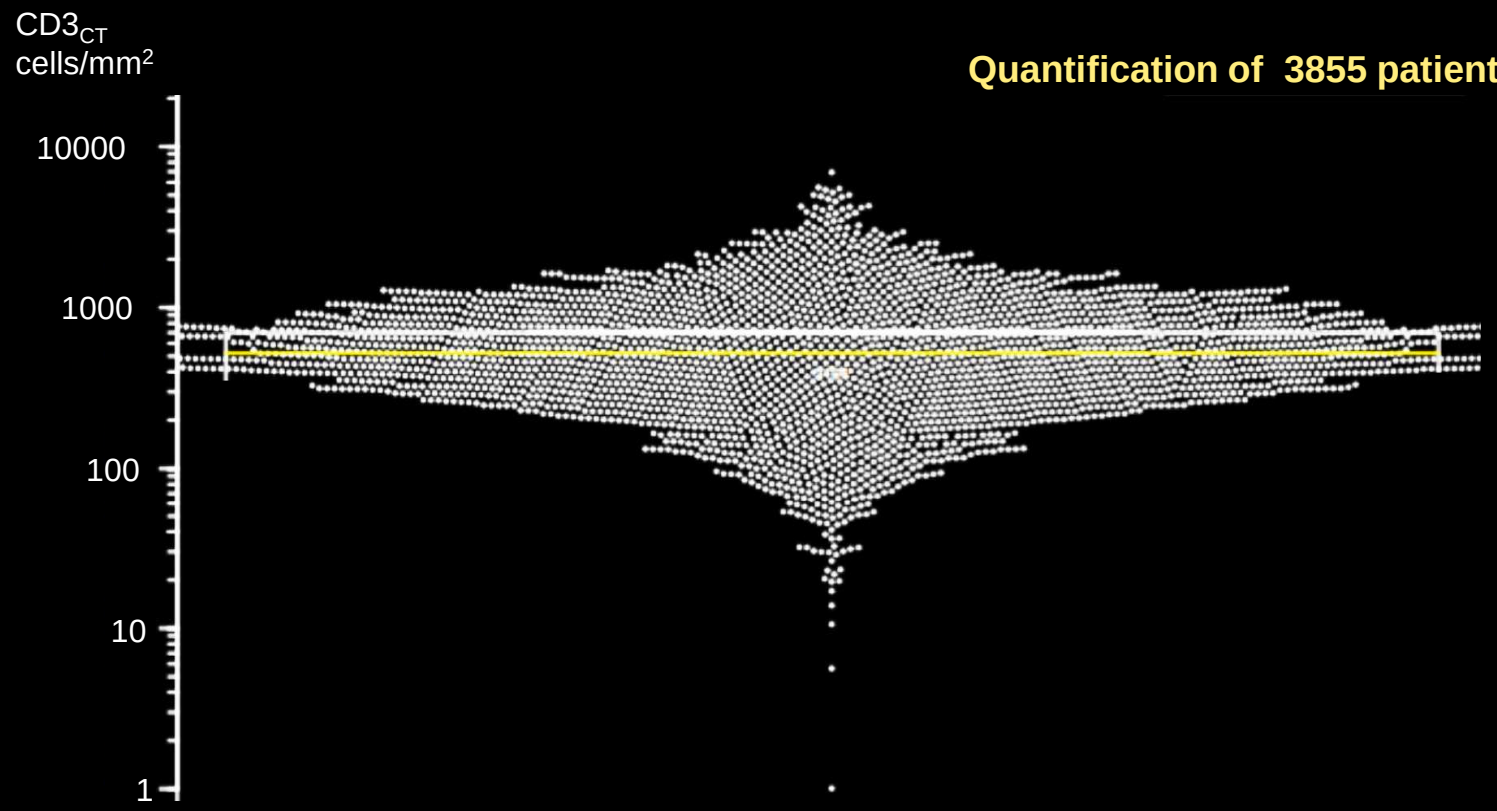
- ✓ More than 352,000,000 CD3+ T cells were counted by all Centers

	Number of CD3+ T cells / slide	Whole slide density of CD3+ (cells / mm^2)	Whole slide density of CD8+ (cells / mm^2)
Center (CT)	64,537 ± 80,962	685 ± 1297	239 ± 534
Margin (IM)	23,643 ± 23,524	1174 ± 1985	436 ± 832
Total	88,180		

Distribution of Immunoscore across all Centers

- ✓ High Immunoscore: 26%
- ✓ Int. Immunoscore: 49%
- ✓ Low Immunoscore: 25%

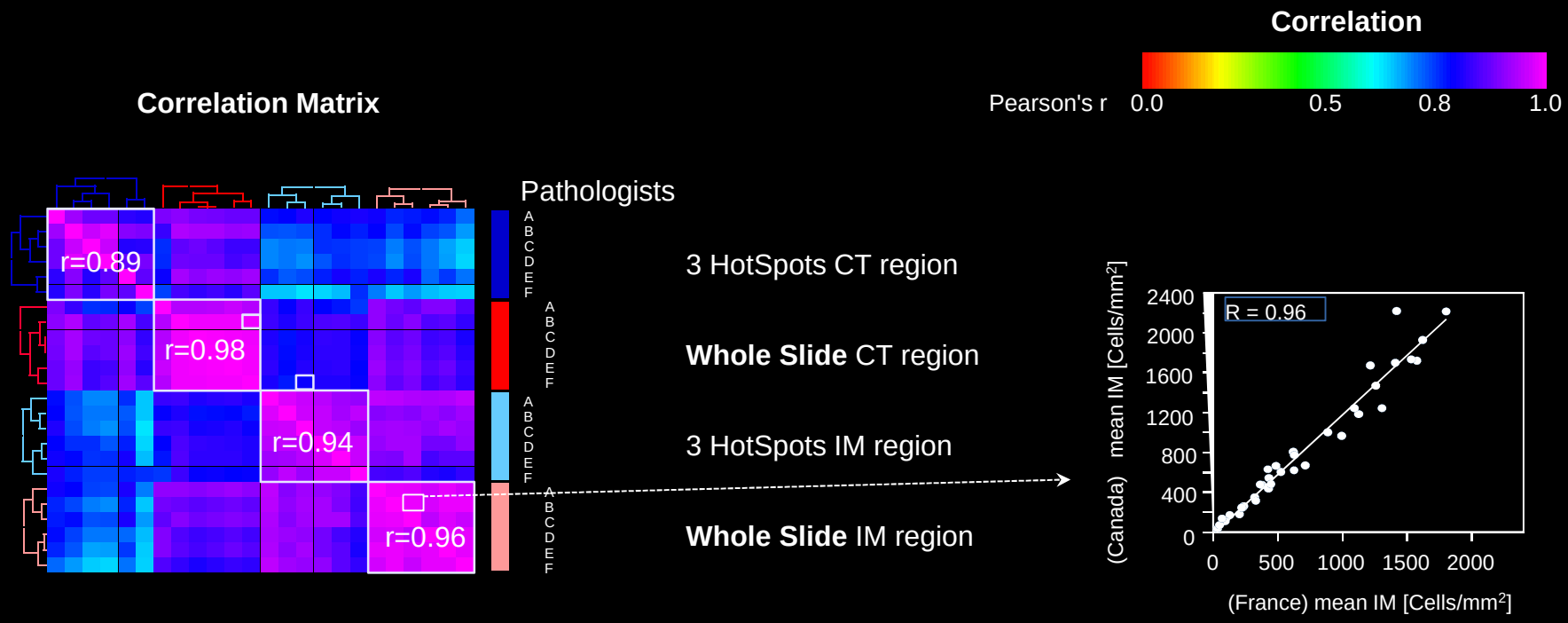
Densities of $CD3_{CT}$ (cells/mm^2) within tumors



Quantification of 3855 patients

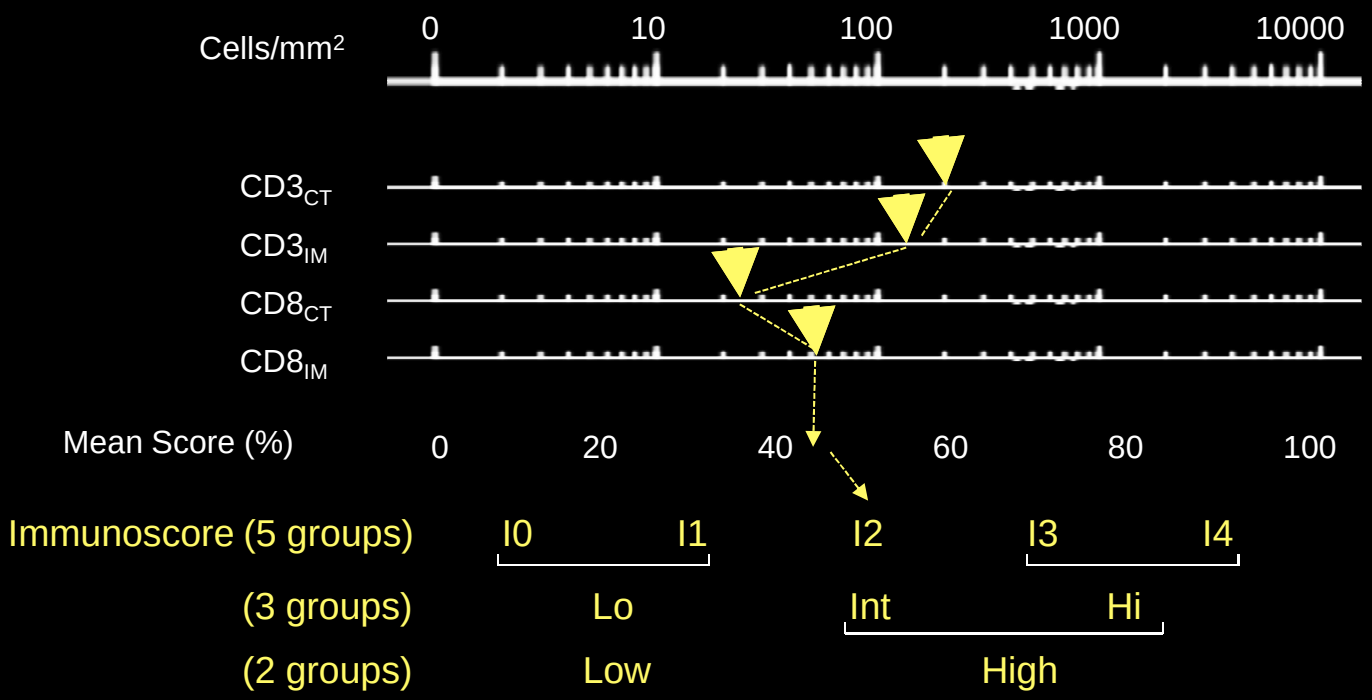
- ✓ Whole slide quantification within the CT region
- ✓ Similar quantification were performed for $CD3_{CT}$, $CD3_{IM}$, $CD8_{CT}$, $CD8_{IM}$

High reproducibility of Immunoscore



- ✓ Whole slide quantification shows the best correlation and reproducibility
- ✓ R=0.96 in IM region and R=0.98 in CT region
- ✓ Immunoscore is **quantitative, reproducible and robust**

Densities of each marker in each region were determined



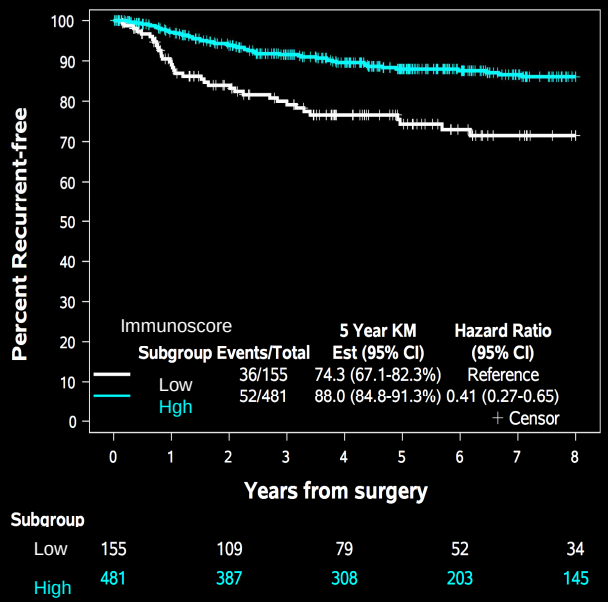
Mean score and Immunoscore were defined on Training Set (TS), blinded to clinical outcome, and applied to validation sets (IVS and EVS)

Primary Objective: Time to recurrence (TTR) for Immunoscore (High/Low)

TS

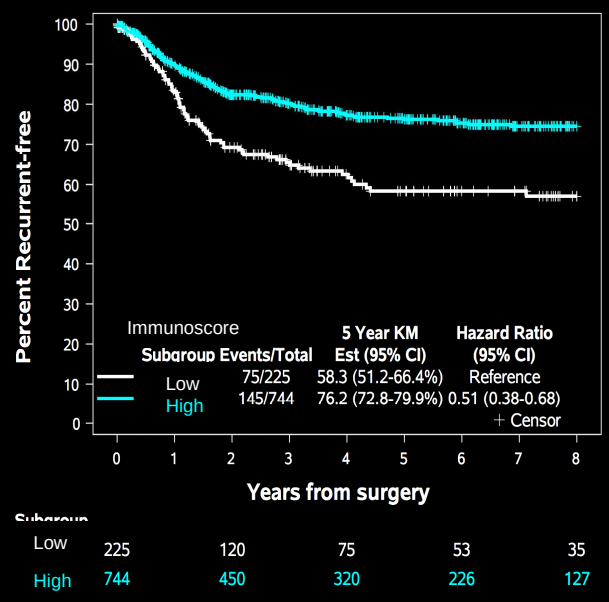
***P*< 0.0001**
HR = 0.41
C-index = 0.60

IVS



***P*< 0.0001**
HR = 0.41
C-index = 0.60

EVS



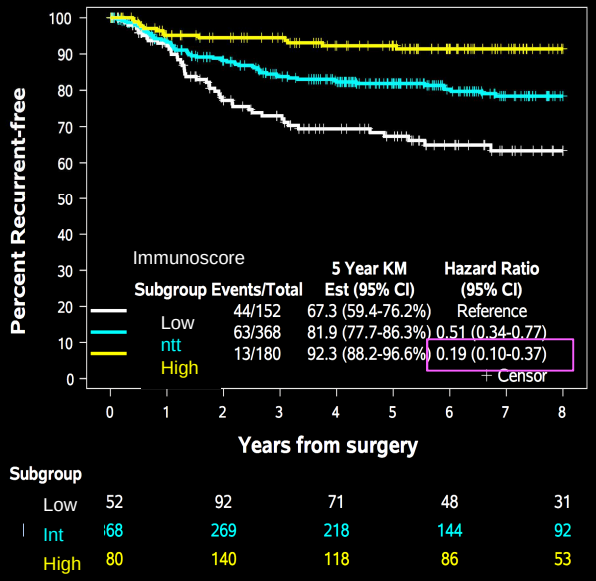
***P*< 0.0001**
HR = 0.51
C-index = 0.56

Primary objective is reached

Immunoscore predicted time to recurrence on Training Set (TS), and on 2 independent validation sets (IVS and EVS), blinded to clinical outcome.

Secondary Objective: Time to recurrence for Immunoscore (High/Int/Low)

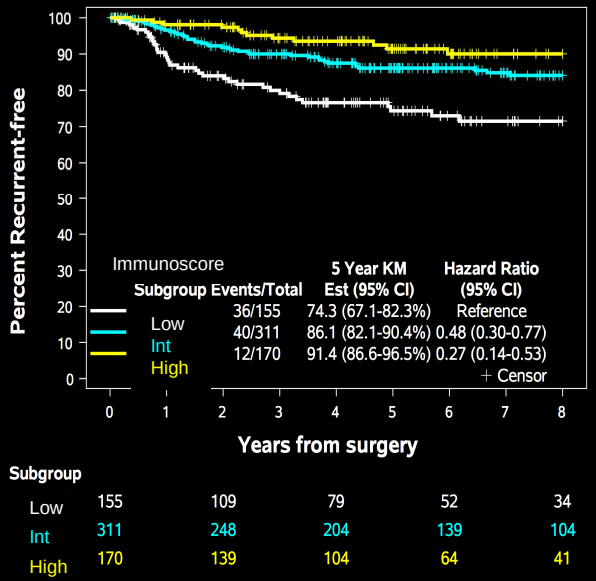
TS



Subgroup					
Low	52	92	71	48	31
Int	168	269	218	144	92
High	80	140	118	86	53

***P*< 0.0001**
HR (0-2)= 0.19
C-index= 0.64

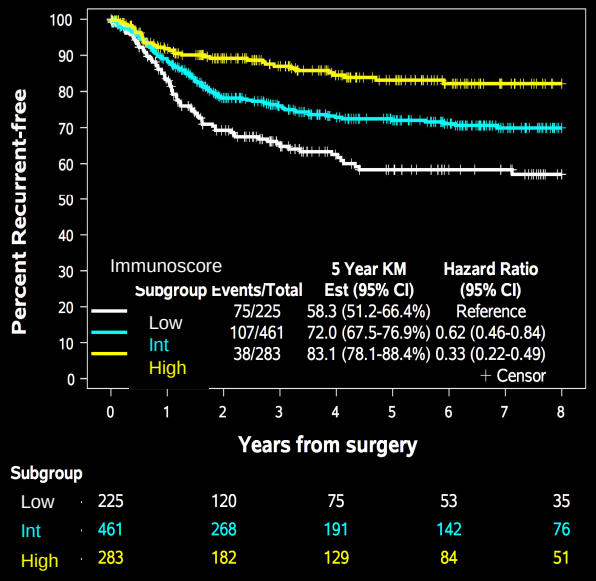
IVS



Subgroup					
Low	155	109	79	52	34
Int	311	248	204	139	104
High	170	139	104	64	41

***P*= 0.0001**
HR (0-2)= 0.27
C-index= 0.63

EVS



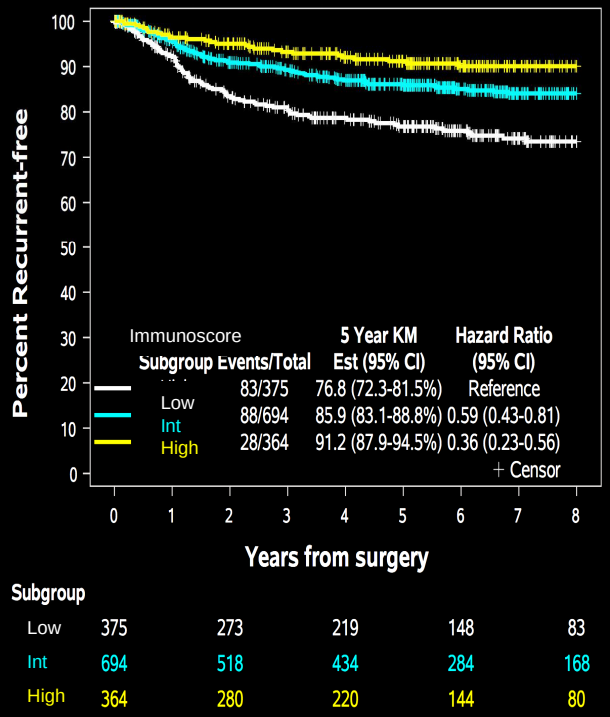
Subgroup					
Low	225	120	75	53	35
Int	461	268	191	142	76
High	283	182	129	84	51

***P*< 0.0001**
HR (0-2)= 0.33
C-index= 0.60

Secondary objective is reached

Immunoscore **3 groups** (**and 5 groups**) predicted time to recurrence on Training Set (TS), and on 2 independent validation sets (IVS and EVS), blinded to clinical outcome.

Secondary Objective:
Time to recurrence for Immunoscore (High/Int/Low) in Stage II



Stage II patients (n=1433)

***P*< 0.0001**

HR(0-2)= 0.36
C-index= 0.65 (0.54-0.75) *

Objective is reached

Immunoscore predicted time to recurrence in Stage II colon cancer

Multivariate anlayses for Immunoscore (2, 3, or 5 groups)

Multivariate Analysis for TTR

Immunoscore	TS		IVS		EVS	
	P-values	***c-index***	**P-values**	***c-index***	**P-values**	***c-index***
2 groups	0.0008	0.72 (0.60-0.84)	0.0007	0.72 (0.60-0.85)	0.0076	0.75 (0.69-0.82)
3 groups	<0.0001	0.73 (0.61-0.85)	0.0019	0.73 (0.60-0.85)	0.0025	0.75 (0.69-0.851
5 groups	<0.0001	0.73 (0.62-0.85)	0.0007	0.74 (0.61-0.86)	0.0048	0.76 (0.69-0.82)

All patients

Immunoscore	P-values	*c-index*
2 groups	<0.0001	0.73 (0.66-0.80)
3 groups	<0.0001	0.73 (0.67-0.80)
5 groups	<0.0001	0.73 (0.67-0.80)

- ✓ Immunoscore (2, 3, or 5 groups) is significant in multivariate analyses in TTR
- ✓ Similar results are found for DFS and OS

Secondary Objective: Immunoscore (High/Int/Low)

TTR

***P*< 0.0001**
C-index= 0.73 (0.67-0.80) *

DFS

***P*< 0.0001**
C-index= 0.65 (0.60-0.70) *

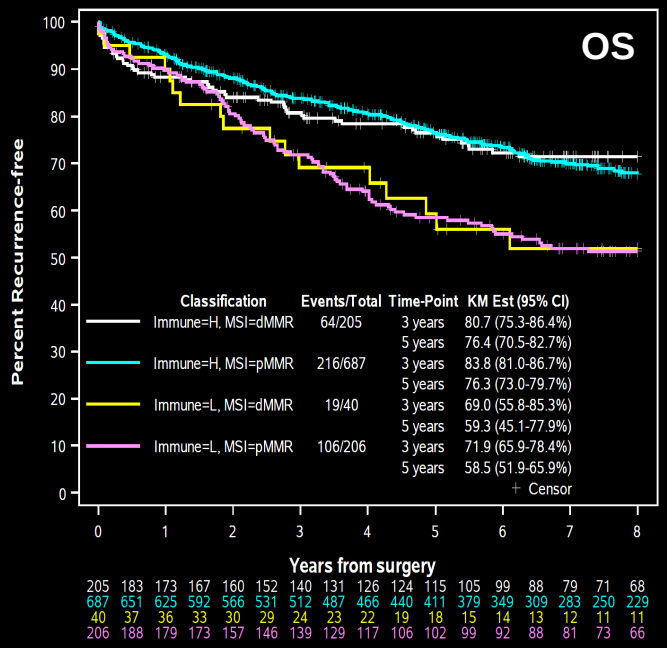
OS

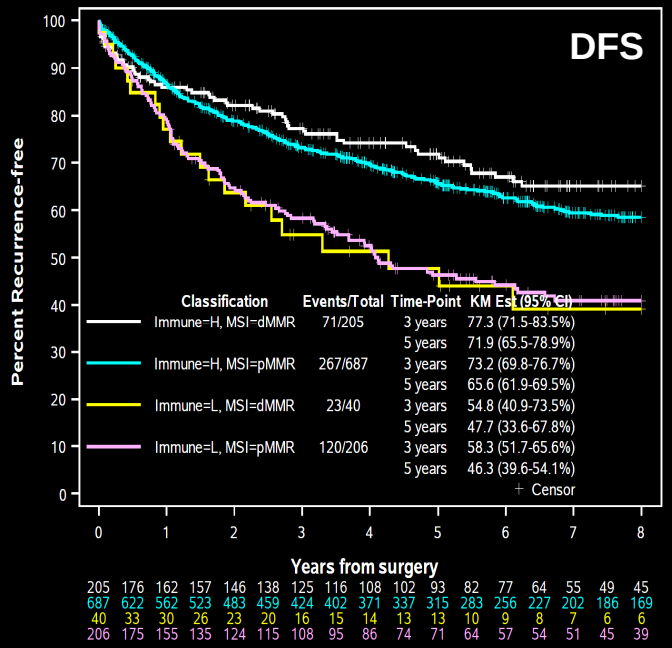
***P*< 0.0001**
C-index= 0.63 (0.58-0.68) *

Secondary objective is reached

Immunoscore (3 groups) predicted time to recurrence, TTR, DFS and OS.

Secondary Objective: Immunoscore & MSI





Immunoscore High & MSI
Immunoscore High & MSS

Immunoscore Low & MSI
Immunoscore Low & MSS

Multivariate Model: Immunoscore
***P*< 0.0001**
HR (95% CI)= 0.62 (0.50-0.77) *

Multivariate Model: Immunoscore
***P*< 0.0001**
HR (95% CI)= 0.57 (0.47-0.70) *

N = 1326 patients

Secondary objective is reached

✓ Immunoscore is significant in multivariate analyses in OS, DFS, TTR (including MSI, T-stage, N-stage, Age, Gender)

Conclusions:

- ✓ The primary endpoint of the Worldwide pre-specified Immunoscore study was reached
- ✓ TTR was significantly longer in patient's stages I/II/III with High-Immunoscore
- ✓ Low-Immunoscore identified a subgroup of patients with high-risk stage II colon cancer
- ✓ Immunoscore is significant in multivariate analysis in all cohorts, TS, IVS and EVS,
- ✓ Immunoscore is stronger than MSI
- ✓ Immunoscore predicts TTR, DFS and OS

Perspective:

- ✓ The results of this international consortium may result in the implementation of the Immunoscore as a new component for the classification of cancer, designated TNM-I (TNM-Immune)
- ✓ This will represent the first standardized immune-based assay for the classification of cancer
- ✓ In the era of immunotherapy, it is becoming essential to start classifying cancer patients based on immune parameters

Ways to routinely classify CRC based on:

Tumor cell characteristics

- **T-STAGE**
- **N-STAGE**
- **M-STAGE**
- **Morphology**
  Mucinous, Serrated, Signet ring, …
- **Cell of origin**
  Enterocyte, Gobelet, Stem-like, …
- **Molecular pathway**
  CIN, MSI, CIMP, …
- **Mutation status**
  BRAF, KRAS, TP53, …
- **Gene expression**
  CMS1, CMS2, CMS3, CMS4

Host-immune characteristics

-> Currently none

Hurdles for biomarker	TILs evaluation	**Immunoscore quantification**
• Routine	☑	☑
• Feasible	☑	☑
• Simple	☑	☑
• Rapid	☑	☑
• **Robust**	☐	☑
• Objective	☐	☑
• Specific	☐	☑
• Reproducible	☐	☑
• Quantitative	☐	☑
• Standardized	☐	☑
• Powerful	☐	☑
• Pathology-based	☑	☑

Additional Support for the Introduction of Immune Cell Quantification in Colorectal Cancer Classification

Robert L. Ferris and Jérôme Galon JNCI, 108(8) May 2016

Characteristics of good biomarker

Thanks (1)

Society for ImmunoTherapy of Cancer
Bernard Fox, Francesco Marincola, Howard Kaufman, Lisa Butterfield, Tara Withington, Chelsey Meier

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…

Independent external statisticians
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Alessandro Lugli, Inti Zlobec, Tilman Rau

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Immunoscore as a Prognostic Marker in Stage I-III Colon Cancer: Results of a SITC-led Global Validation Study

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