Are gene signatures ready for use in the selection of patients for adjuvant treatment?

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“New Signatures”

- RNA signatures
  - Supervised signatures
    - (Oncotype®/Coloprint®…)
  - Non-supervised signatures
    - CRCSC Consensus Classification
    - EMT
- Microenvironment signatures
  - Inmunoscore ®
- Other molecular markers
  - CDX2
  - ctDNA MRD

“Old markers”

- MSI (dMMR) vs MSS (pMMR)
- Stage II
  - T3-4 (stage II)
  - N of nodes examined
- Stage III
  - T1-4
  - N1-2
- Others are not validated

Methodology*:
Training Set
Internal Validation Set
External Validation set
Multivariate Analysis

PROGNOSIS & PREDICTION

Clinical setting

*Salazar & Tabernero. Clin Cancer Res 2015

MSI Prognosis & Drug selection in adjuvant setting (stage II&III)

- 5-FU (Pooled Analysis Accent Data Base)
  - Stage III: Benefits both MSS than MSI
  - Stage II:
    - MSI no Need for Chemo
      - Good prognosis and NO BENEFIT from 5-FU
    - MSS small or uncertain benefit-Treat only high risk (T4)
      - N of nodes < 12 (Quality indicator)
- Oxaliplatin (Mosaic & NSABP-C07 trials)
  - Stage III: Benefits both MSS than MSI
  - Stage II: No benefit MSS or MSI

Sargent et al., ASCO Annual Meeting 2014
Gavin PG et al., Clin Cancer Res 2012;
Fléjou JF et al., ASCO Annual Meeting 2013
Dienstmann, Salazar & Tabernero. J Clin Oncol 2015

Gene signatures (tumor)

- CIN, MSI (dMMR)
- Individual mutations
  - (RAS/BRAF…)
- Supervised signatures
  - (Oncotype/Coloprint…)
- Itrinsec signatures
  - CRCSC Consensus Classification
  - TGF-B , EMT & Composite PC1-EMT
  - Emerging biomarkers
  - Inmunoscore
  - CDX2
  - ctDNA MRD

PROGNOSIS & PREDICTION

Clinical setting

Candidate gene signatures with independent and external validation

Modified from Santos et al., Current Colorectal Cancer Reports 2016

	No patients	Tumor stage	No of genes (training set)	No of genes (validation set)	Tissue sample	Outcome	HR
Oncotype DX® (rtPCR)							
Gray et al	1436	II	48	12 (7 recurrence)	FFPE	Recurrence	1.43
Di Narzo et al	668	II – III	--	7	Fresh frozen	RFS OS	1.30 1.34
Venook et al	1713	II	--	7	FFPE	Recurrence	1.68
Yothers et al	892	II – III	--	7	FFPE	RFS	1.57
ColoPrint® (Agilent)							
Salazar et al	206	I – III	18	18	Fresh frozen	RFS	2.69
Kopetz et al	416	II	--	18	Fresh frozen	RFS	2.16
Salazar et al	>600	II		18	Fresh frozen	RFS	pend
Veridex (Affimetrix)							
Jiang et al	Set 1: 123 Set 2: 110	II	--	7	Set 1: Fresh frozen Set 2: FFPE	Distant recurrence	Set 1: 2.87 Set 2: 14.2
Di Narzo et al	668	II – III	--	7	Fresh frozen	RFS OS	1.27 1.21
GeneFx® Colon (Affimetrix)							
Kennedy et al	144	II	634-probe set	634-probe set	FFPE	Recurrence	2.551
Di Narzo et al	688	II – III	--	482	FFPE	RFS OS	1.20 1.22
Niedzwiecki et al	393	II	--	634-probe set	FFPE	Recurrence	2.06
microRNA							
Zhang et al	Set 1: 137 Set 2: 460	II II	35 (6-miRNA prognostic)	6	FFPE	DFS	3.79

ONCOTYPE DX® IN **QUASAR**

Table 1. Analyses of Association of Single and Multiple Explanatory Variables With Risk of Recurrence in Patients Who Underwent Surgery Alone

Variable	No. of Patients	Single-Covariate Cox Regression* HR	95% CI	P	Multiple-Covariate Cox Regression† HR	95% CI	P	Direction
Dichotomous								
Tumor location: right *v* other	692	0.63	0.45 to 0.89	.008	0.66	0.44 to 0.97	.032	Right better
T stage: T4 *v* T3	707	1.94	1.35 to 2.79	< .001	1.87	1.25 to 2.81	.004	T4 worse
Tumor grade: high *v* low	711	0.73	0.51 to 1.04	.083	0.65	0.42 to 1.02	.050	High better
Sex: female *v* male	711	0.78	0.57 to 1.07	.12				Male worse
Nodes examined: < 12 *v* ≥ 12	657	1.38	0.97 to 1.96	.071	1.43	0.98 to 2.08	.058	< 12 worse
Lymphovascular invasion	711	1.46	0.95 to 2.23	.085	1.43	0.90 to 2.28	.150	Invasion worse
MMR: deficient *v* proficient	654	0.31	0.15 to 0.63	< .001	0.36	0.17 to 0.80	.004	Deficient better
Continuous								
Age, years	711	1.02	1.00 to 1.03	.089	1.02	1.00 to 1.04	.034	Older worse
Recurrence score per IQR	711	1.38	1.11 to 1.74	.004	1.43	1.11 to 1.83	.006	Higher worse

Abbreviations: HR, hazard ratio; IQR, interquartile range; MMR, mismatch repair.
*Cox regression with each covariate as the only explanatory variable in the model.
†Cox regression with tumor location, T stage, grade, nodes examined, lymphovascular invasion, MMR, age, and recurrence score included in the model.

Recurrence risk groups	% patients	3-year RR
Low RS	43.7%	12% (0% - 16%)
Int RS	30.7%	18% (13% - 24%)
High RS	25.6%	22% (16% - 29%)

CALGB 9581 (Edrecolumab vs observation) st II
NSABP C07 (FULV+/-Ox) st II & III

Kerr et al., ASCO Ann Meeting 2009
Gray et al., J Clin Oncol 2011

Oncotype Dx ® SUNRISE STUDY

- Stage II – III
  - Japanese (2000-2005)
- N = 630 patients
  - 210 with recurrence and 420 without recurrence)
- No adjuvant chemo
- 1º end-point RFI

Recurrence Score risk group	Stage II	
	% of patients	% risk (95% CI) at 5 years
Low	60	9 (7 to 12)
Intermediate	26	14 (11 to 17)
High	14	19 (13 to 24)

Recurrence Score risk group	Stage IIIA/B	
	% of patients	% risk (95% CI) at 5 years
Low	46	20 (14 to 25)
Intermediate	31	29 (23 to 35)
High	23	38 (29 to 47)

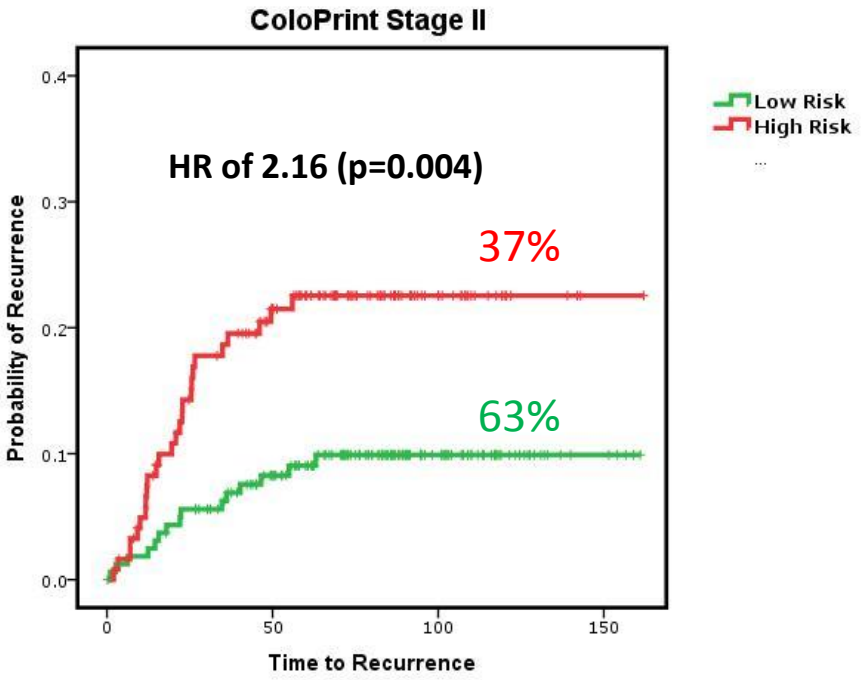
Recurrence Score risk group	Stage IIIC	
	% of patients	% risk (95% CI) at 5 years
Low	45	38 (25 to 50)
Intermediate	29	51 (38 to 64)
High	26	62 (48 to 77)

Yamanaka et al., JCO 2016. In press

ColoPrint ® Validation Set

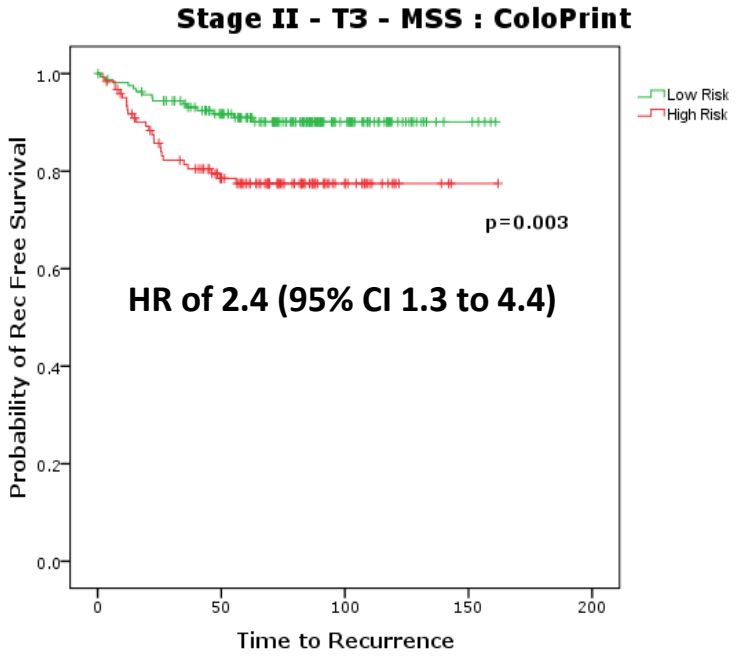
Trained in Whole Genome analysis on 44K Agilent microarrays, 18 genes selected for MFS

All Stage II (N = 416)



5-year ROR
Low Risk =10.3% (95%CI 6.6%-14%)
High Risk = 20.9% (95%CI 14.2%-27.6%)

T3-MSS (N=301)



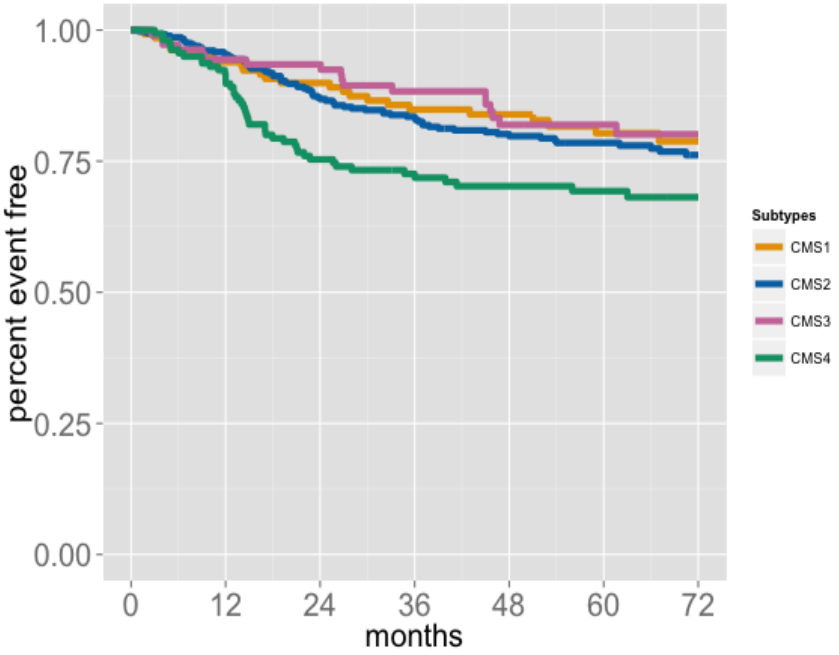
5-year ROR
Low Risk = 9.9% (95%CI 5.4%-14.4%)
High Risk = 22.4% (95%CI 14.8%-30%)

* No differences in relapse between low and high clinical risk using ESMO risk assessment

Salazar et al., J Clin Oncol 2011
Kopetz et al. The Oncologist 2015; 20:127–133

Colorectal Cancer Subtyping Consortium (CRCSC) identifies consensus molecular subtypes

RFS in stage II colorectal cancer (N= 1400)



CMS1 14%	Females, older age, right colon, MSI, hypermutation, *BRAF* mut, immune activation	Better RFS, intermediate OS, worse SaR
CMS2 37%	Left colon, epithelial, MSS, high CIN, *TP53* mut, WNT/MYC pathway activation	Intermediate RFS, better OS, better SaR
CMS3 13%	Epithelial, CIN/MSI, *KRAS* mut, *MYC* ampl, IGFBP2 overexpression	Intermediate RFS, OS and SaR
CMS4 23%	Younger age, stage III/IV, mesenchymal, CIN/MSI, TGFβ/VEGF activation, NOTCH3 overexpression	Worse RFS, worse OS Intermediate SaR

Rodrigo Dienstmann ASCO 2015
Guinney et al. Nature Medicine 2015

Alexandre Calon,., Eduard Batlle. **Dependency of Colorectal Cancer on a TGF-β-Driven Program in Stromal Cells for Metastasis Initiation**. Cancer Cell 2012: 22, 5, p571–584, 13

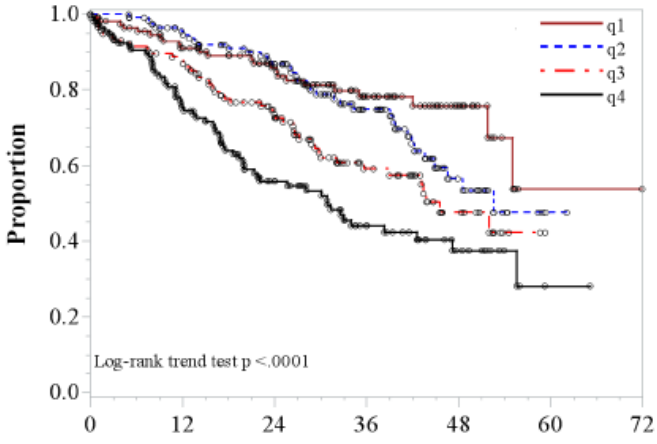
Loboda A, Nebozhyn MV, Watters JW, Buser CA, Shaw PM, Huang PS, et al. **EMT is the dominant program in human colon cancer**. BMC Med Genomics. 2011;4:9.

Clinical Cancer Research

A composite gene expression signature optimizes prediction of colorectal cancer metastasis and outcome

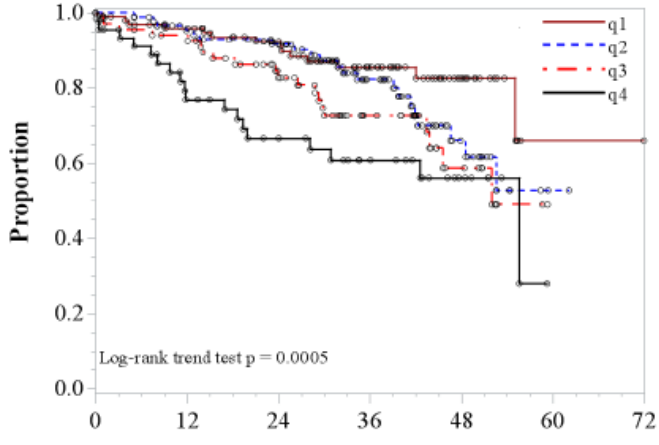
Michael J. Schell, Mingli Yang, Edoardo Missiaglia, et al.

Clin Cancer Res Published OnlineFirst October 7, 2015.



PC1.EMT	N	Event	Censored	Median os_time (95% CI)
q1	117	24 (21%)	93 (79%)	NA (51.8, NA)
q2	117	35 (30%)	82 (70%)	52.6 (43.5, NA)
q3	117	47 (40%)	70 (60%)	45.5 (35.6, NA)
q4	117	61 (52%)	56 (48%)	30.8 (20.0, 47.1)

(N=306)



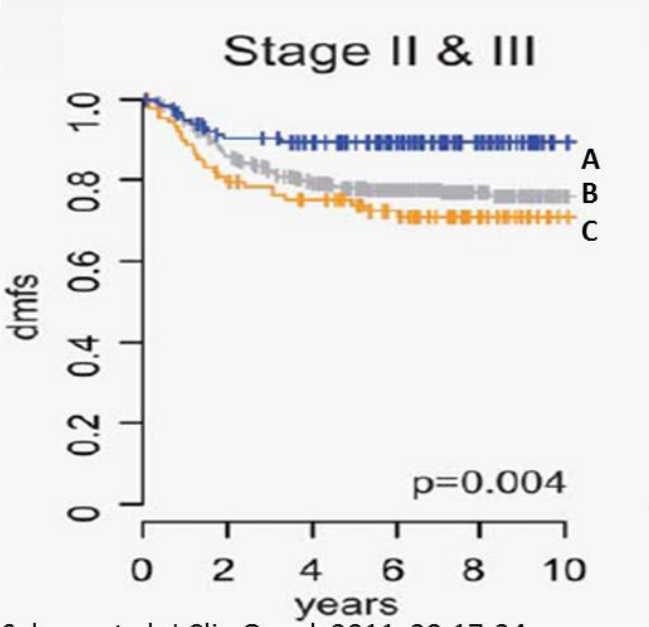
PC1.EMT	N	Event	Censored	Median os_time (95% CI)
q1	102	14 (14%)	88 (86%)	NA (55.1, NA)
q2	90	21 (23%)	69 (77%)	NA (48.5, NA)
q3	69	20 (29%)	49 (71%)	52.0 (43.7, NA)
q4	45	18 (40%)	27 (60%)	55.6 (28.1, NA)

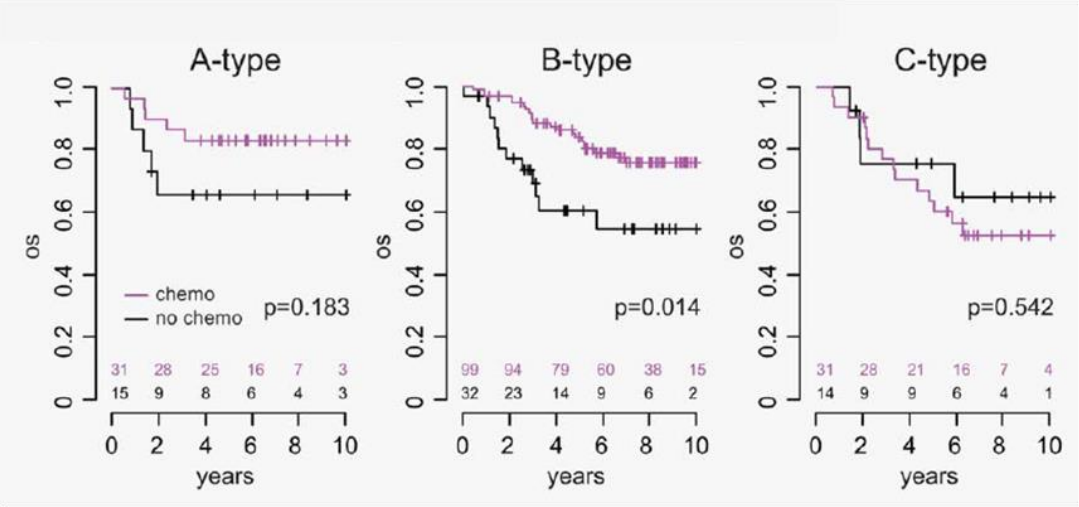
Colorectal cancer intrinsic subtypes predict chemotherapy benefit, deficient mismatch repair and epithelial-to-mesenchymal transition

Int. J. Cancer: 00, 00–00 (2013) © 2013 UICC

Paul Roepman[1], Andreas Schlicker[2], Josep Tabernero[3], Ian Majewski[2], Sun Tian[1], Victor Moreno[4,5], Mireille H Snel[1], Christine M Chresta[6], Robert Rosenberg[7], Ulrich Nitsche[7], Teresa Macarulla[3], Gabriel Capella[5], Ramon Salazar[5], George Orphanides[6], Lodewyk FA Wessels[2,8], Rene Bernards[1,2] and Iris M Simon[1]

A-type (22%): BRAFm, MSI/dMMR, epithelial proliferative	B-type (62%): low mutation, MSS, epithelial proliferative, benefit adjuvant CT	C-type (16%): mesenchymal, no benefit CT





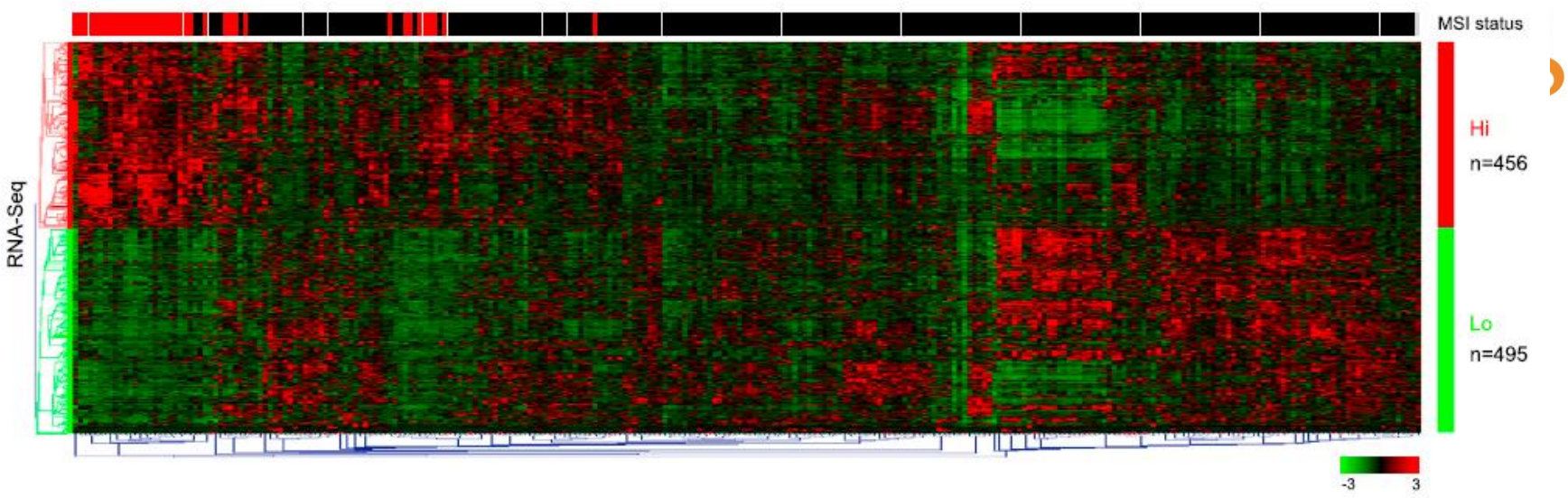
Salazar et al, J Clin Oncol 2011: 29:17-24

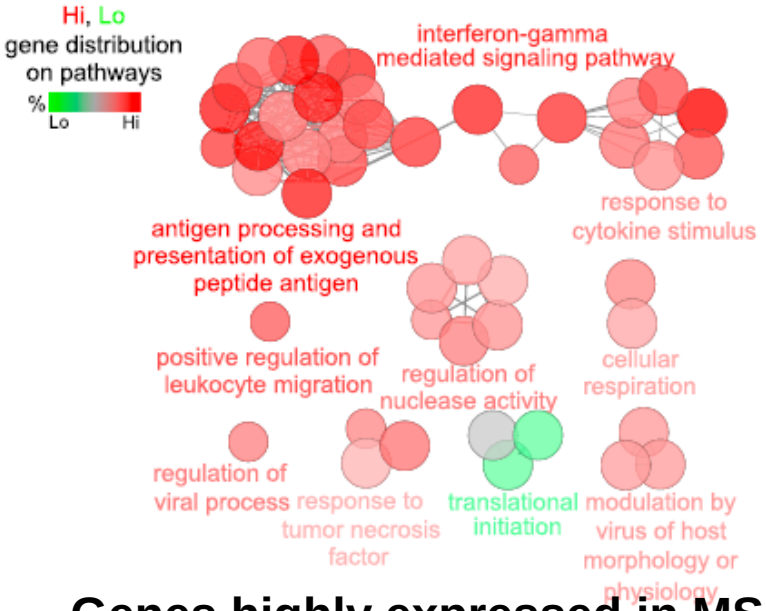
New signatures (stroma)

- CIN, MSI (dMMR)
- Individual mutations
  - (RAS/BRAF…)
- Microenvironment signatures
  - Inmunoscore ®
- Other Emerging biomarkers
  - Inmunoscore
  - CDX2
  - ctDNA MRD

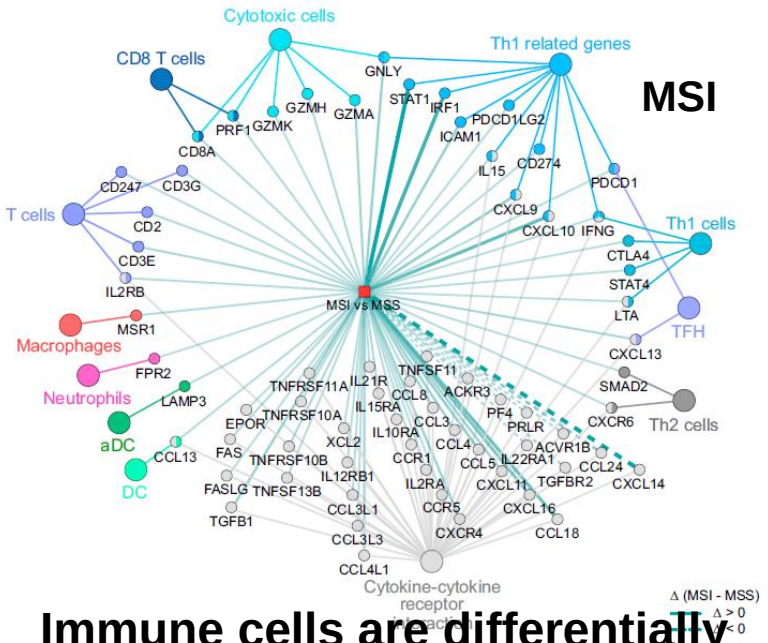
PROGNOSIS & PREDICTION

Clinical setting





Genes highly expressed in MSI tumors are associated with immune pathways



Immune cells are differentially expressed in MSI and MSS tumors

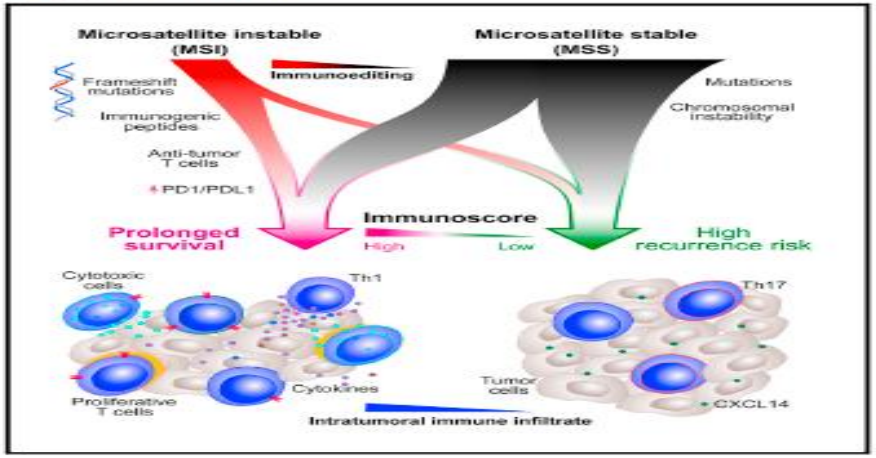
Immunoscore ® vs MSI –stage I-III

Article

Immunity

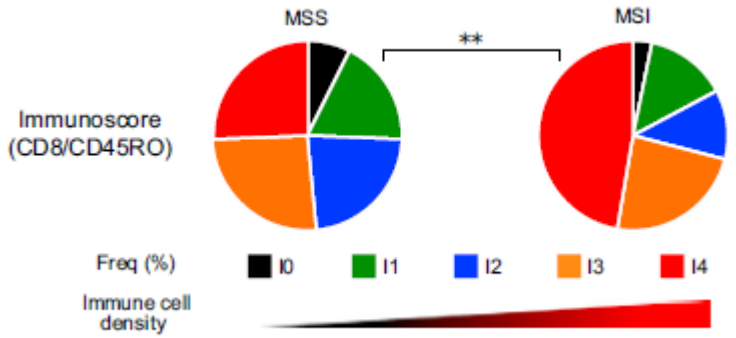
Integrative Analyses of Colorectal Cancer Show Immunoscore Is a Stronger Predictor of Patient Survival Than Microsatellite Instability

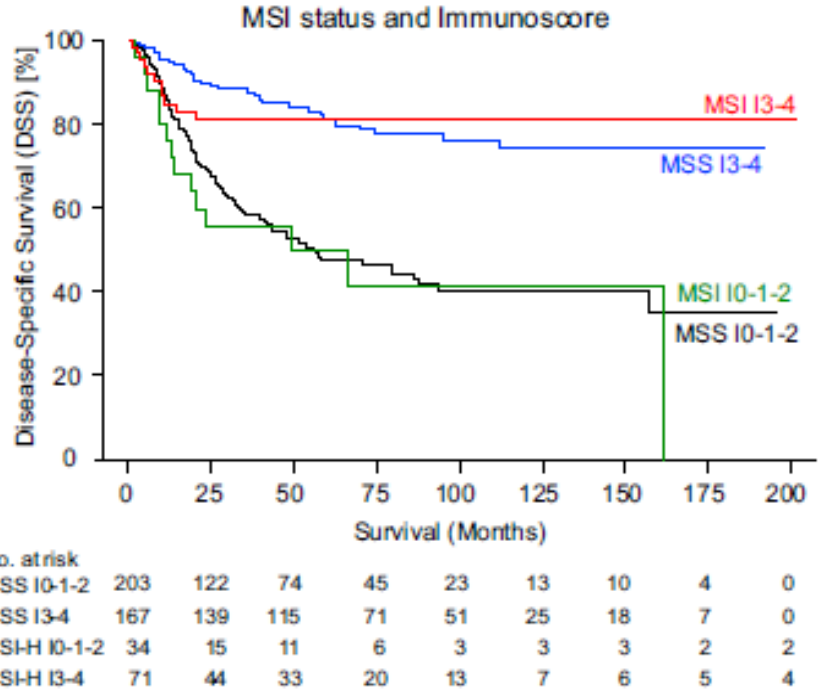
Graphical Abstract



Authors

Bernhard Mlecnik, Gabriela Bindea, Helen K. Angell, ..., Viia Valge-Archer, Jean-Baptiste Latouche, Jérôme Galon





Mlecnik et al., Immunity 2016

Inmunoscore ® International Consortium

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%

Stages I-III
3855 patients quantified for IS
2667 analyzed after QC and exclusion
pre-defined statistical analysis workplan
Divided in 3 sets
-Training set
-Internal validation set
-External validation set

Inmunoscore ® in Stage II

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

ASCO 2016
J Clin Oncol 34, 2016 (suppl; abstr 3500)

Inmunoscore ® in Stage II

Pooled training and validation sets
MSI unknown
T4?

ASCO 2016
J Clin Oncol 34, 2016 (suppl; abstr 3500)

Other Emerging biomarkers

- CIN, MSI (dMMR)
- Individual mutations
  - (RAS/BRAF…)
- Gene signatures
  - Supervised signatures
    - (Oncotype/Coloprint…)
  - CRCSC Consensus Classification
    - (Intrinsec subtypes)
  - Stromal signatures

- CDX2
- ctDNA MRD

PROGNOSIS & PREDICTION

Clinical setting

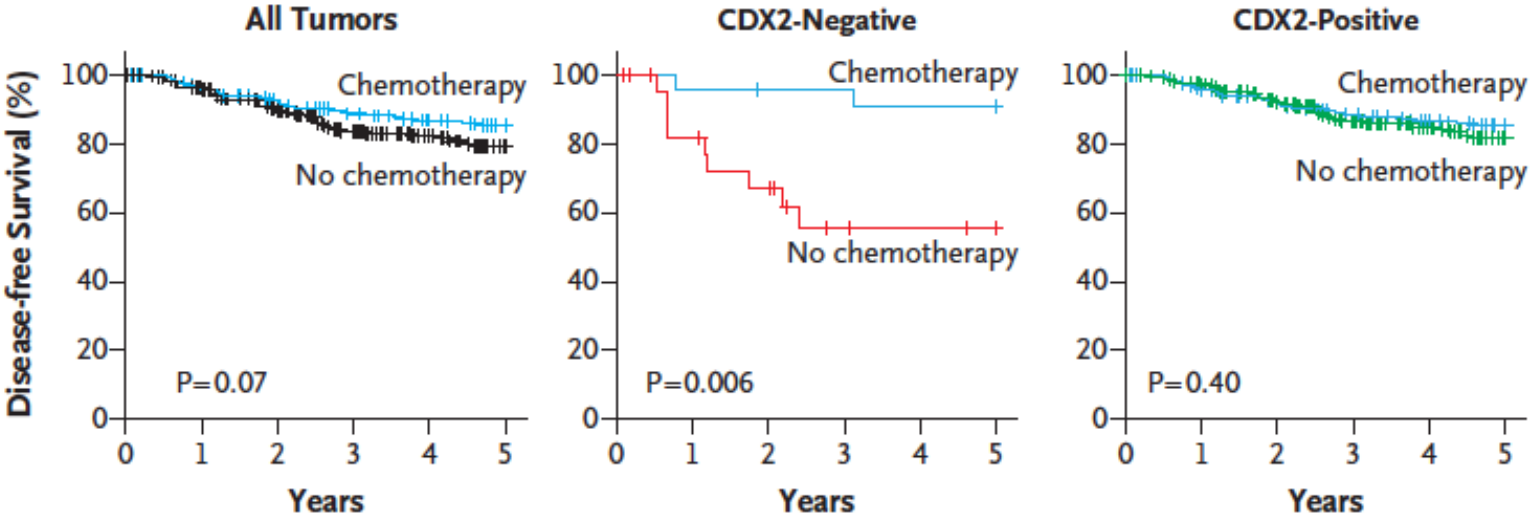
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JANUARY 21, 2016 VOL. 374 NO. 3

CDX2 as a Prognostic Biomarker in Stage II and Stage III Colon Cancer

Piero Dalerba, M.D., Debashis Sahoo, Ph.D., Soonmyung Paik, M.D., Xiangqian Guo, Ph.D., Greg Yothers, Ph.D., Nan Song, Ph.D., Nate Wilcox-Fogel, M.S., Erna Forgó, M.D., Pradeep S. Rajendran, B.S., Stephen P. Miranda, B.A., Shigeo Hisamori, M.D., Ph.D., Jacqueline Hutchison, Tomer Kalisky, Ph.D., Dalong Qian, M.D., Norman Wolmark, M.D., George A. Fisher, M.D., Ph.D., Matt van de Rijn, M.D., Ph.D., and Michael F. Clarke, M.D.

A Patients with Stage II Disease



No. at Risk

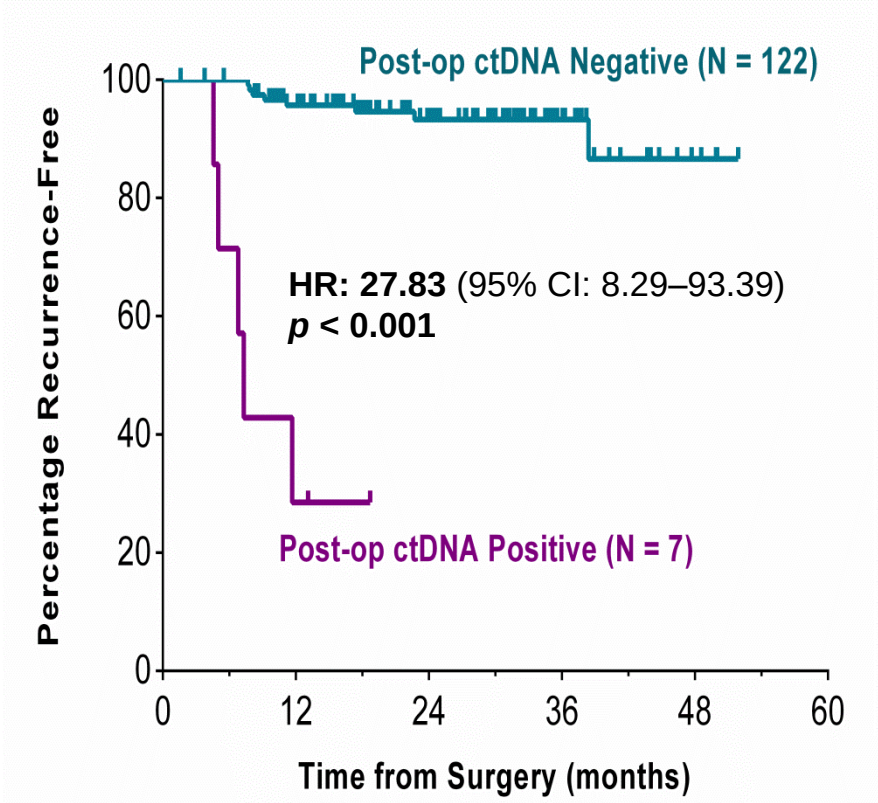
Chemotherapy	412	388	365	344	326	310	23	22	21	21	20	20	389	366	344	323	306	290
No chemotherapy	257	230	199	150	114	84	25	18	14	8	7	6	232	212	185	142	107	78

MRD in resected CRC

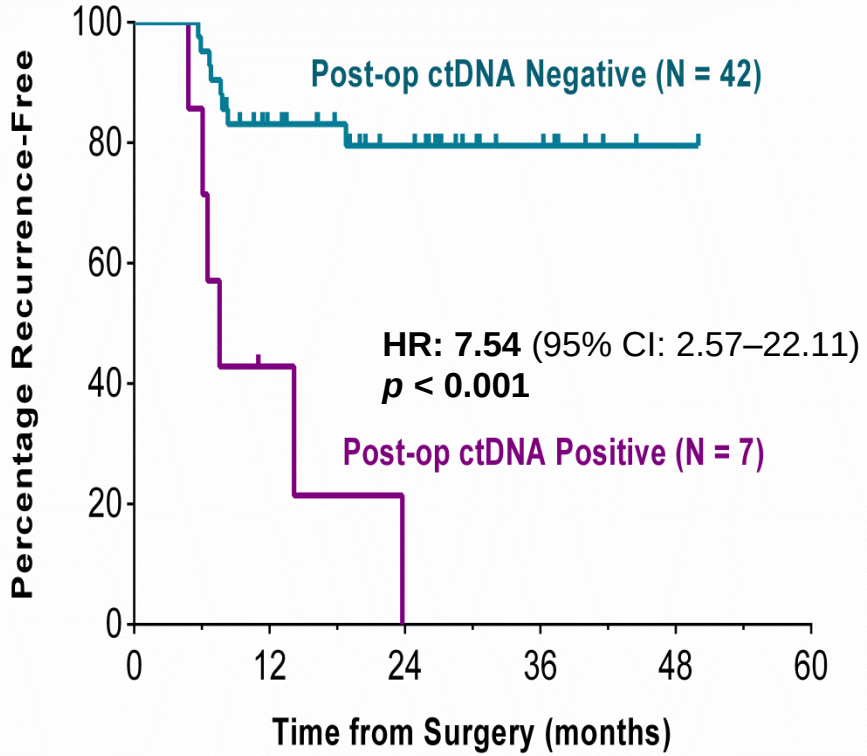
Tie et al. ASCO Ann Meeting 2016

MRD in stage II post-op ctDNA mutation assay

Clinical Low-Risk
(no poor prognostic features)



Clinical High-Risk



Tie et al. ASCO Ann Meeting 2016

MRD in stage II

Clinical Low-Risk (no poor prognostic features)	Clinical High-Risk (pMMR + at least one poor prognostic features)

- Is this the end of other Px markers in Stage II?
  - T4 remained in the multivariate analysis
  - MSI did not, but prone to low numbers effect

Tie et al. ASCO Ann Meeting 2016

My interpretation
Stage II:

- Who to treat?
  - T4 MSS
  - T3 MSS &
    - High risk signatures (not predictive)
    - or low IS (not predictive, MV in st II validation set?)
    - or CDX2 negative (more validation needed)
  - T4 MSI?
  - MRD + (ctDNA test not ready)

My interpretation
Stage III:

- Who to avoid treatment?
  - IIIa &
    - Low Risk Biomarkers? (Not Yet There)
    - Predictive Biomarkers? (Not Yet There)

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