ADJUVANT TREATMENT OF COLORECTAL CANCER
A MOLECULAR CLASSIFICATION: CURRENT STAGES AND FUTURE PERSPECTIVES

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DECLARATION OF INTERESTS

Josep Tabernero reports personal financial interest in form of scientific consultancy role for Array Biopharma, AstraZeneca, Bayer, BeiGene, Boehringer Ingelheim, Chugai, Genentech, Inc., Genmab A/S, Halozyme, Imugene Limited, Inflection Biosciences Limited, Ipsen, Kura Oncology, Lilly, MSD, Menarini, Merck Serono, Merrimack, Merus, Molecular Partners, Novartis, Peptomyc, Pfizer, Pharmacyclics, ProteoDesign SL, Rafael Pharmaceuticals, F. Hoffmann-La Roche Ltd, Sanofi, SeaGen, Seattle Genetics, Servier, Symphogen, Taiho, VCN Biosciences, Biocartis, Foundation Medicine, HalioDX SAS and Roche Diagnostics.

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VALL D'HEBRON Institute of Oncology

Vall d'Hebron Hospital

ADJUVANT TREATMENT IN COLON CANCER :

Do we have any effective biomarker?

INTRODUCTION

ADJUVANT CRC: Love and lost stories

VOLUME 27 · NUMBER 19 · JULY 1 2009

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

ET

Randomized Phase III Trial Comparing Biweekly Infusional Fluorouracil/Leucovorin Alone or With Irinotecan in the Adjuvant Treatment of Stage III Colon Cancer: PETACC-3

Eric Van Cutsem, Roberto Labianca, György Bodoky, Carlo Barone, Enrique Aranda, Bernard Nordlinger, Claire Topham, Josep Tabernero, Thierry André, Alberto F. Sobrero, Enrico Mini, Richard Greil, Francesco Di Costanzo, Laurence Collette, Laura Cisar, Xiaoxi Zhang, David Khayat, Carsten Bokemeyer, Arnaud D. Roth, and David Cunningham

ges 939-944

and folinic acid in colon

THE LANCET Oncology

Bevacizumab plus oxaliplatin-based chemotherapy as adjuvant treatment for colon cancer (AVANT): a phase 3 randomised controlled trial

Prof Aimery de Gramont, MD, Prof Eric Van Cutsem, MD, Prof Hans-Joachim Schmoll, MD, Josep Tabernero, MD, Prof Stephen Clarke, FRACP, Prof Malcolm J Moore, MD, Prof David Cunningham, MD, Thomas H Cartwright, MD, Prof J Randolph Hecht, MD, Fernando Rivera, MD, Prof Seock-Ah Im, MD, Prof György Bodoky, MD, Prof Ramon Salazar, MD, [illegible]que Maindrault-Goebel, MD, Einat Shacham-Shmueli, MD, Prof Emilio Bajetta, MD, Martina Makrutzki, MD, Aijing [illegible] MD, Thierry André, MD, Prof Paulo M Hoff, FACP

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

THE LANCET Oncology

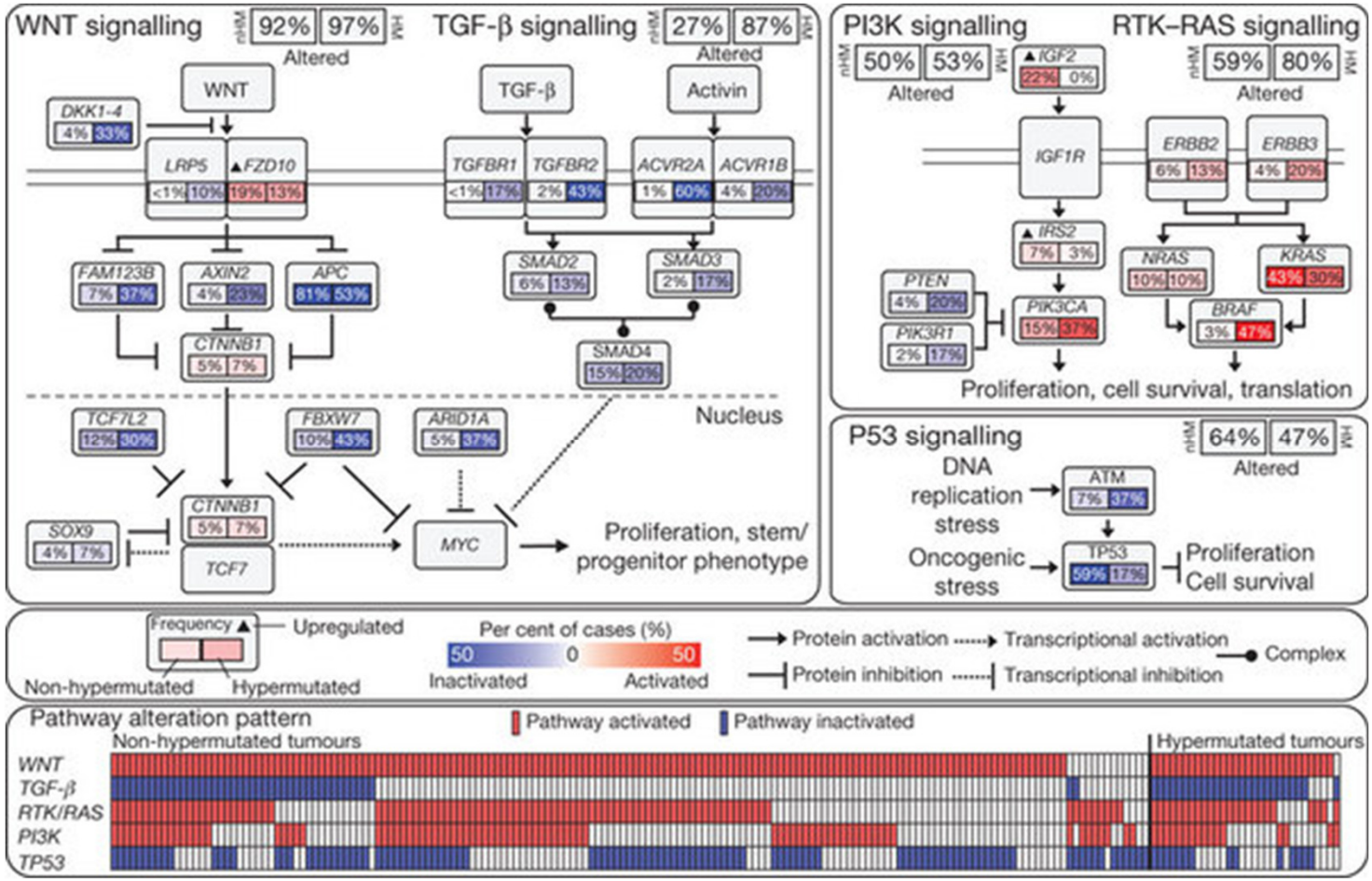
Oxaliplatin, fluorouracil, and leucovorin with or without cetuximab in patients with resected stage III colon cancer (PETACC-8): an open-label, randomised phase 3 trial

Prof Julien Taieb, MD, Prof Josep Tabernero, MD, Prof Enrico Mini, MD, Fabien Subtil, PhD, Gunnar Folprecht, MD, Prof Jean-Luc Van Laethem, MD, Prof Josef Thaler, MD, John Bridgewater, MD, Prof Lone Nørgård Petersen, MD, Hélène Blons, PhD, Laurence Collette, PhD, Prof Eric Van Cutsem, MD, Prof Philippe Rougier, MD, Prof Ramon Salazar, MD, Prof Laurent Bedenne, MD, Prof Jean-François Emile, MD, Prof Pierre Laurent-Puig, MD, Prof Come Lepage, MD

[illegible] Alfred Wong, M.D., Marek P. Nowacki, M.D., [illegible]loward Burris III, M.D., Alfredo Carrato, M.D., [illegible]rés Cervantes, M.D., Jan Fagerberg, M.D., Ph.D., [illegible].D., Fares Husseini, M.D., Duncan Jodrell, M.D., [illegible]., Hendrik Kröning, M.D., Jean Maroun, M.D., [illegible], Joseph McKendrick, M.D., Marek Pawlicki, M.D., [illegible]hannes Schüller, M.D., Jean-François Seitz, M.D., [illegible]D., Jerzy Tujakowski, M.D., Guy Van Hazel, M.D., [illegible] M.D., and Werner Scheithauer, M.D.*

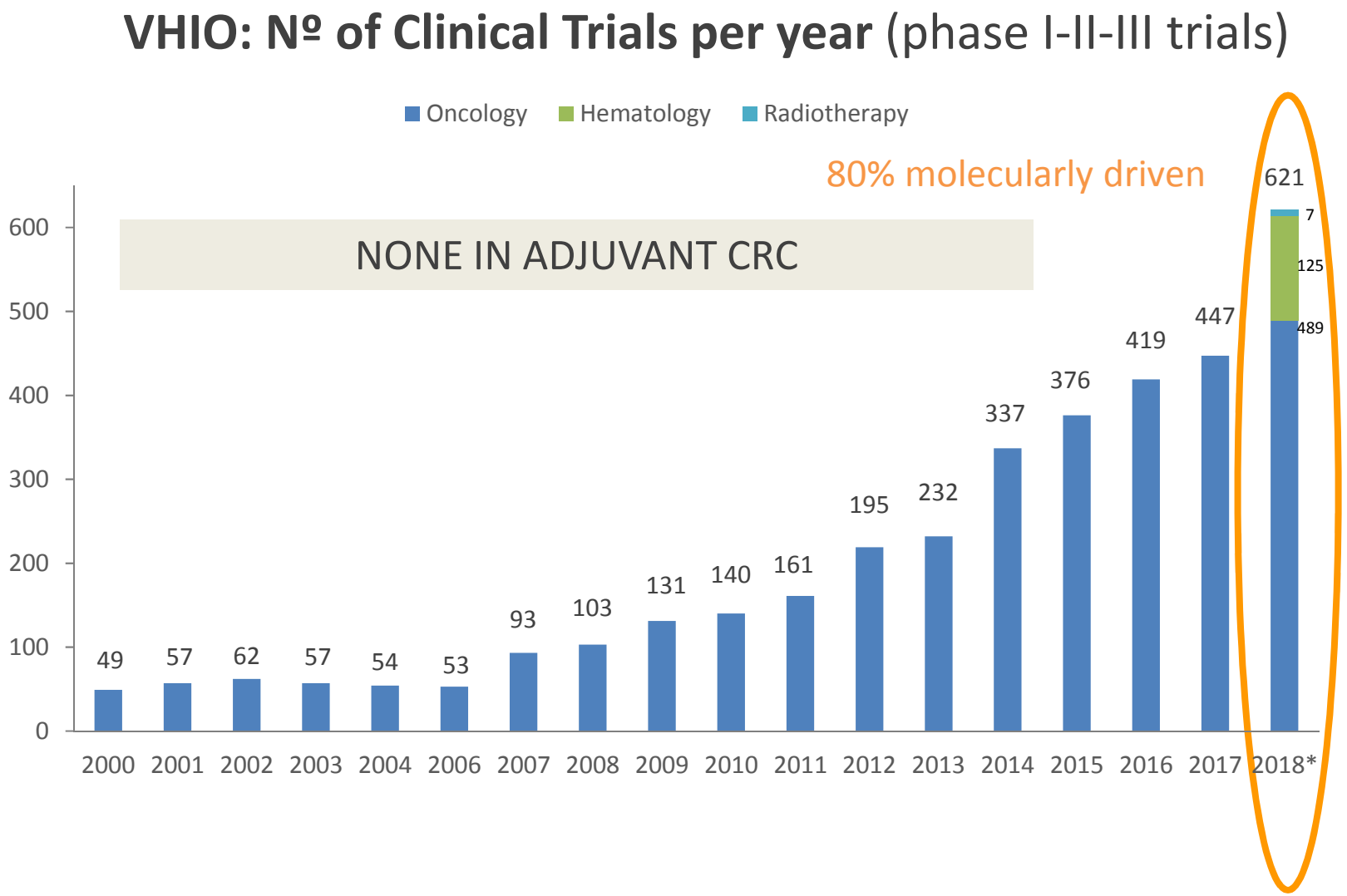
CRC BIOLOGY

It seems that limited and metastatic disease are not the same, CRC is a complex illness



TCGA, Nature 2012

BACKGROUND



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ADJUVANT TREATMENT IN COLON CANCER :

Do we have any effective biomarker?

DAY BY DAY BIOMARKERS

THE PATHOLOGICAL REPORT

T4	✓ Most robust indicator of poor prognosis in stage II and III ✓ Magnitude of risk conferred by these features relative to nodal status: not reliably estimated from available literature ✓ pT4 *vs* pT3 hazard ratios of DFS and OS (in stage II CC) are likely in the same range as those for pN1 versus pN0 disease
PNI/LVI/ VVI	✓ Pointed as high risk markers in early stage retrospective studies ✓ Node-negative/PNI-positive tumors can have 5-year survival rates significantly lower than stage III patients ✓ Under-reported phenomenon in CC (differences ranged from 1% to 20% by review)
Grade	✓ Not consistently associated with increased risk of recurrence in stage II disease ✓ Correlated with microsatellite instability (MSI) not negatively impact ✓ Restricted to MSS disease, and NCCN guidelines adopt this stratification for risk determination
LN	✓ Considered improperly staged and at greater risk of having microscopic residual disease ✓ Postoperative treatment in patients with stage II CC with less than 12 lymph nodes examined (ESMO/NCCN) ✓ The number of lymph nodes in a surgical specimen is associated with the level of the immunologic response, which is positively correlated with MSI

Dientsmann et al. J Clin Oncol 2015

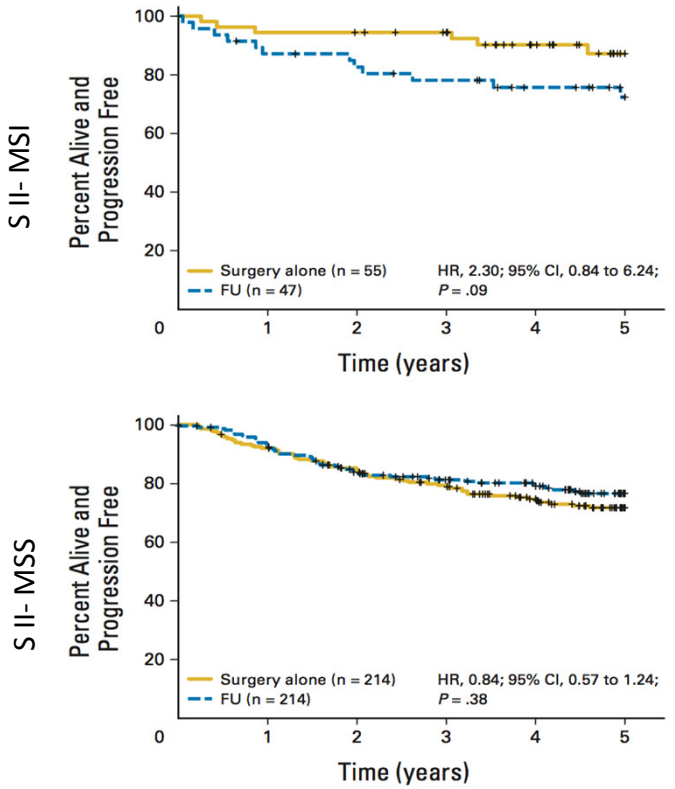
MICROSATELLITE STATUS

- Validated prognostic factor with positive impact on survival more pronounced in earlier stage disease
- Stage II-MSI patients:
  - Low recurrence rates and a good outcome without CT[1,2]
  - Distant recurrences not reduced by FU-based adjuvant treatment in MSI stage II patients[3]
  - In one study, chemotherapy was found to have a detrimental effect on OS (not confirmed)[4]
- Current data in MSI stage II CC support an approach of not administering adjuvant treatment

VOLUME 28 · NUMBER 20 · JULY 10 2010
JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Defective Mismatch Repair As a Predictive Marker for Lack of Efficacy of Fluorouracil-Based Adjuvant Therapy in Colon Cancer

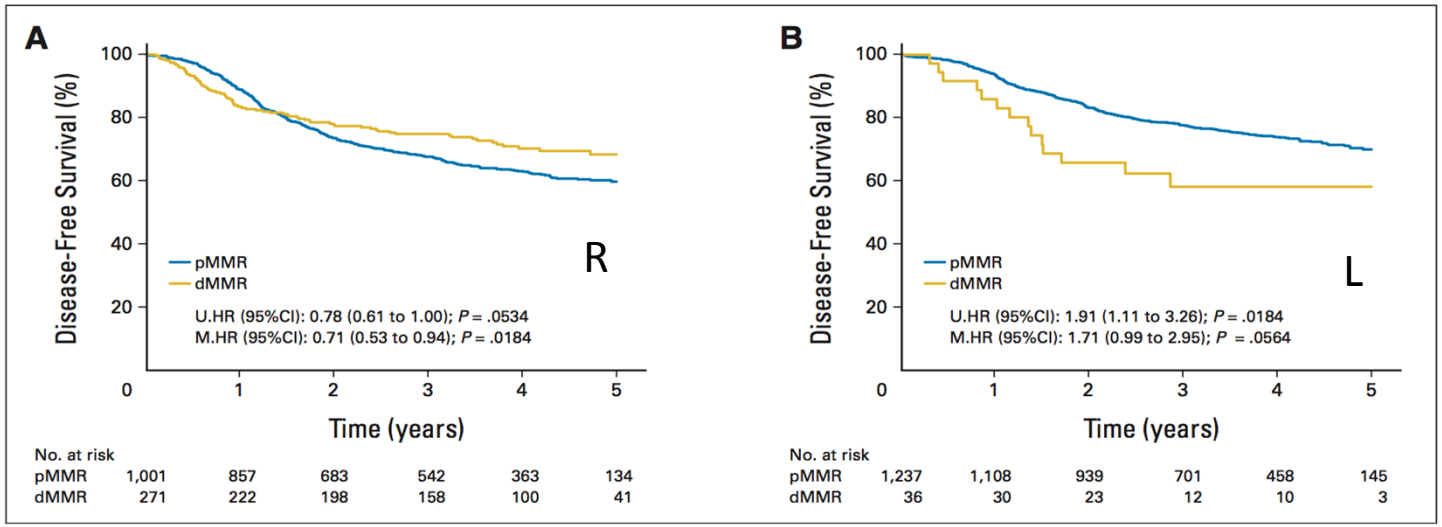
Daniel J. Sargent, Silvia Marsoni, Genevieve Monges, Stephen N. Thibodeau, Roberto Labianca, Stanley R. Hamilton, Amy J. French, Brian Kabat, Nathan R. Foster, Valter Torri, Christine Ribic, Axel Grothey, Malcolm Moore, Alberto Zaniboni, Jean-Francois Seitz, Frank Sinicrope, and Steven Gallinger



1. Hutchins G et al. JCO 2011 , 2. Sinicrope FA et al. JCO 2013, 3. Sinicrope et al. JNCI 2011, 4. Sargent et al. JCO 2010

MICROSATELLITE STATUS

- Stage III disease:
  - Favorable prognostic effect less robust[1-4]
  - MSI stage III: benefit from adjuvant CT with no differential benefit compared with MSS in clinical trials assessing FU or oxaliplatin-based regimens[2,5,6]
  - MSI status should not guide treatment decisions in stage III
  - Possible interaction between MSI status and site in stage III treated disease (right-side tumors do better)[4]



Impact of mismatch repair (MMR) status on disease-free survival by primary tumor site

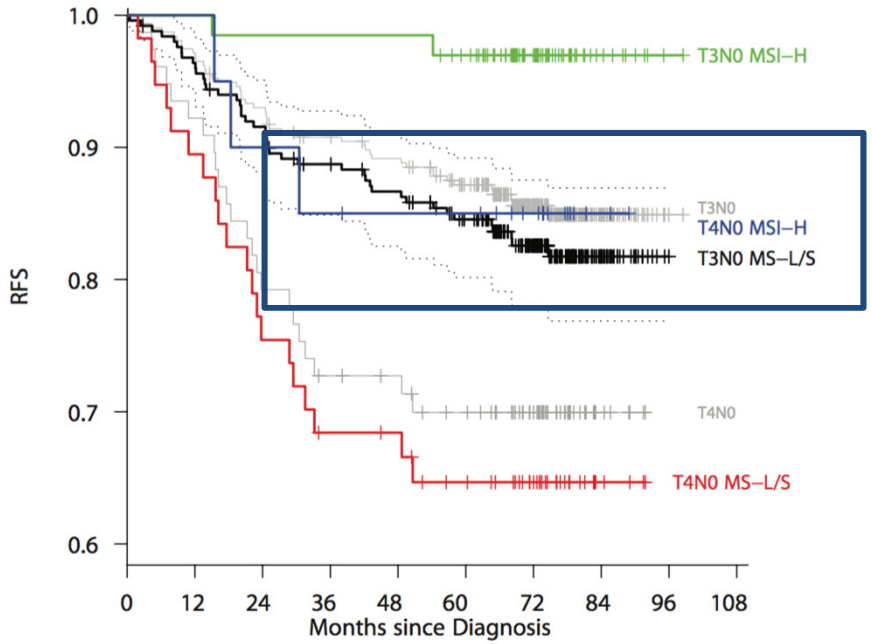
1. Roth et al. JNCI 2012, **2.** Gavin PG et al. Clin Cancer Res 2012, 3. Sargent D et al. J Clin Oncol 2104, 4. Sinicrope FA et al. J Clin Oncol 2013, 5. Sinicrope FA et al. JNCI 2012, 6. Flejou JF et al. J Clin Oncol 2013.

TNM *VS* MSI

Integrated Analysis of Molecular and Clinical Prognostic Factors in Stage II/III Colon Cancer

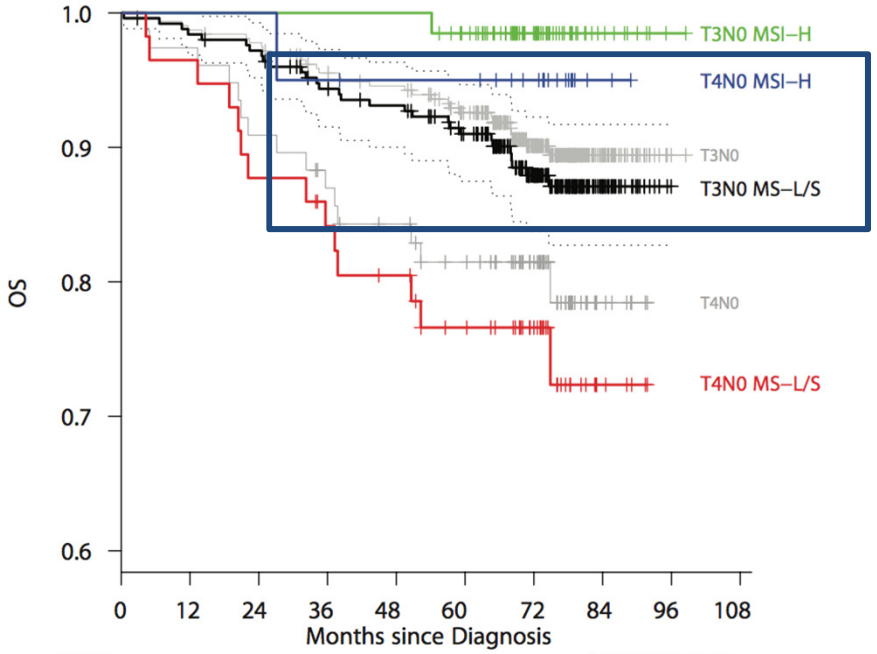
Arnaud D. Roth,* Mauro Delorenzi,* Sabine Tejpar,* Pu Yan, Dirk Klingbiel, Roberto Fiocca, Giovanni d'Ario, Laura Cisar, Roberto Labianca, David Cunningham, Bernard Nordlinger, Fred Bosman, Eric Van Cutsem

Recurrence free survival



At risk:									Number of events
T3N0 MS–L/S	251	240	227	216	209	194	130	30	43
T3N0 MSI–H	66	66	65	65	65	61	35	9	2
T4N0 MS–L/S	57	51	43	38	37	32	23	4	20
T4N0 MSI–H	20	20	18	17	16	16	12	2	3

Overall survival

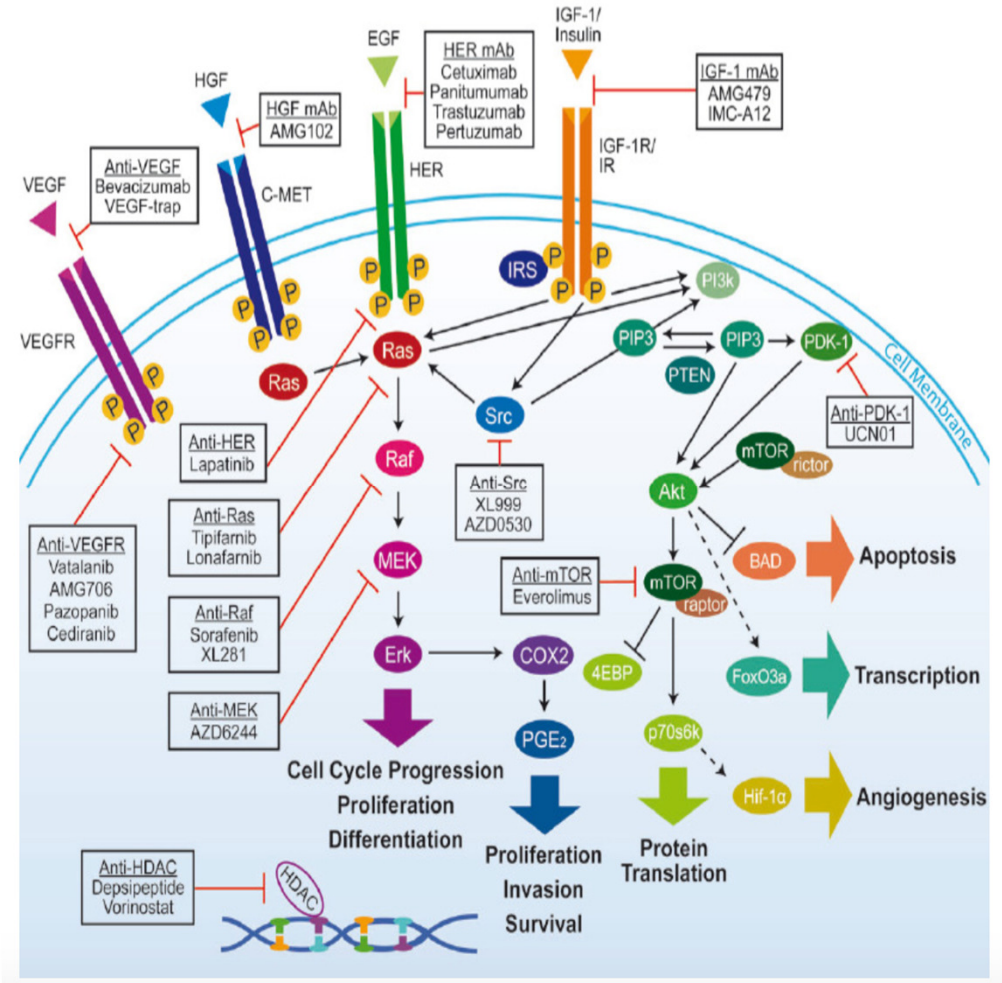


At risk:									Number of events
T3N0 MS–L/S	251	246	242	230	225	209	138	31	29
T3N0 MSI–H	66	66	66	66	66	61	35	9	1
T4N0 MS–L/S	57	55	50	46	43	37	26	5	14
T4N0 MSI–H	20	20	20	19	18	18	14	2	1

Roth A et al, J Natl Cancer Inst 2012

KRAS AS A PROGNOSTIC FACTOR

- The presence of *KRAS* mutations have been pointed to confer a worse prognosis, with reduced DFS and OS
- Small effect: codon 12 variants and in left-side tumors.
- Most studies evaluating *KRAS* mutation are limited to stage III and its prognostic impact in multivariable models in stage II is still unknown
- *KRAS* MT (12/13) lack predictive value for adjuvant cetuximab. Of note, in the C07 trial, it was associated with a detrimental effect in OS of stage III *KRAS* WT patients.



Hutchins G et al. JCO 2011, Sinicrope et al. JCO 2014, Imamura Y et al. Clin Cancer Res 2012, Yoon HH et al. Clin Cancer Res 2014, Blons H et al. Ann Oncol 2014

MAP KINASE IMPACT

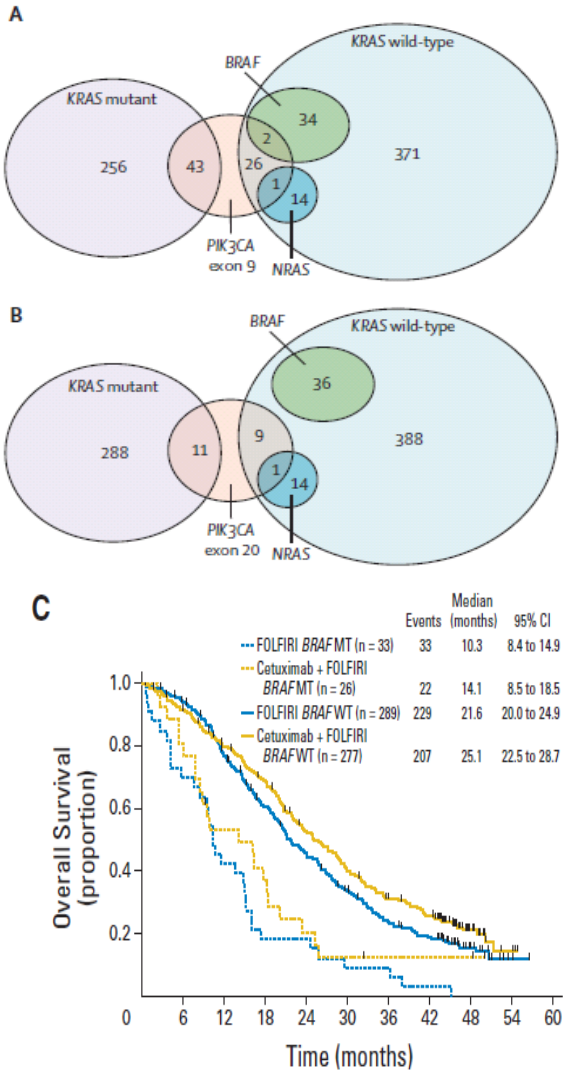
	Age	Gender (M vs F)	Tumor site (R vs L)	T stage		N stage		MSI status (MSI-H vs MS-L/S)	*KRAS* (mut vs wt)
				T3 vs T1/2	T4 vs T3	N1 vs N0	N2 vs N0		
QUASAR (Stage I, n = 42; Stage II, n = 1708; Stage III, n = 163)									
RR	--	--	--	--	--	--	--	RR = .53 (p = .003)	RR = 1.40 (p = .002)
PETACC 3 (Stage II, n = 420; Stage III, n = 984)									
RFS	HR = 1.11 (p = .03)	ns	Ns	HR = 2.08 (p = .009)	HR = 1.73 (p < .001)	HR = 1.73 (p < .001)	HR = 3.97 (p < .001)	HR = .54 (p = .003)	ns
OS	HR = 1.17 (p = .008)	ns	HR = 1.29 (p = .04)	HR = 2.22 (p = .02)	HR = 1.94 (p < .001)	HR = 1.83 (p < .001)	HR = 4.53 (p = .001)	HR = .43 (p = .001)	ns
NSABP C-07/C-08 (Stage II, n = 641; Stage III, n = 1658)									
TTR	--	--	--	--	--	--	--	HR = .48 (p = .0001)	ns
OS	--	--	--	--	--	--	--	ns	ns
SAR	--	--	--	--	--	--	--	ns	ns
MOSAIC (Stage II, n = 899; Stage III, n = 1347)									
OS	HR = 1.81 (p < .001)	HR = 0.70 (p = .007)	ns	ns		HR = 0.28 (N2 vs N0-1) (p = .001)		HR = 2.02 (p = 0.14) (MSS vs MSI)	--

Slide courtesy of Ramon Salazar (adapted)

Roth et al., J Natl Cancer Inst 2012; Hutchins et al., JCO 2011; Gavin et al., Clin Cancer Res 2012; André et al., JCO 2015

BRAF AS A PROGNOSTIC FACTOR

- Small population:
  - 8-10% early stage
  - 4-5% late stage
- *BRAF V600E* mutations as a biomarker?
  - Very poor prognosis in late stage (mCRC)
  - Predictive: negative predictive effect for anti-EGFR MoAbs in some studies:
    - Cetuximab: refractory (European cons.)[1,2] & first-line setting (CRYSTAL study)[3]
    - Panitumumab: 2nd line setting (PICCOLO study)[4]
    - No change in the label by any regulatory authority



[1] Di Nicolantonio F, JCO 2018; [2] De Roock et al. Lancet Oncol 2010;
[3]Van Cutsem et al, JCO 2011; [4]Seymour MT et al, Lancet Oncol 2013

BRAF AS A PROGNOSTIC FACTOR

- Strongly prognostic in terms of OS in part because of the association with poor survival after relapse[1-9]
- The interpretation of *BRAF* mutation as an additional risk factor has to be made in the context of MSI status and tumor location[2,4]
- The largest impact is seen in MSS and left-side tumors, a setting in which it also confers a worse prognosis in terms of DFS (x6 risk of death, MSS>MSI)[2,4]
- The protective effect of MSI status in early-stage overrides the poor prognosis conferred by *BRAF* mutations still giving a favorable prognosis, and the MSI *BRAF*-mutated population nevertheless has a favorable prognosis[1,3]

1. Lochhead P et al. JNCI 2013, 2. Opovici V et al. BMC Cancer 2013, 3. Gavin PG et al. Clin Cancer Res 2012, 4. Sinicrope FA et al,. JCO 2013, 5. Ogino S et al. Clin Cancer Res 2012, 6. Sinicrope FA et al. JCO 2014, 7. Roth et al. JCO 2010

MSI vs *KRAS* vs *BRAF*

Multivariate analysis, DFS

	NCCTG N0147 (N = 2686, stage III)			PETACC 8 (N = 1791, stage III)		
	HR	CI	P-value	HR	CI	P-value
MMR (dMMR vs pMMR)	0.82	0.64 – 1.07	0.1403	1.10	0.73 – 1.64	0.67
KRAS (mut vs wt)	**1.45**	1.22 – 1.71	< 0.001	**1.55**	1.23 – 1.95	< 0.001
BRAF (mut vs wt)	**1.35**	1.06 – 1.71	0.0147	1.22	0.81 – 1.85	0.34
MMR/*BRAF*						
pMMR/*BRAF* (mut vs wt)	**1.32**	1.01 – 1.73	0.0437	**1.74**	1.14 – 2.69	0.01
dMMR/*BRAF* (mut vs wt)	1.58	0.88 – 2.82	0.122	**0.23**	0.06 – 0.92	0.04
MMR/*KRAS*						
pMMR/*KRAS* (mut vs wt)	**1.45**	1.22 – 1.73	< 0.001	**1.64**	1.29 – 2.08	< 0.001
dMMR/*KRAS* (mut vs wt)	0.91	0.40 – 2.10	0.8338	0.94	0.32 – 2.74	0.91

Slide courtesy of Ramon Salazar (adapted)

Sinicrope et al. JCO 2013, Sinicrope et al. JCO 2014, Taieb et al. JAMA Oncol 2016

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ADJUVANT TREATMENT IN COLON CANCER :

Do we have any effective biomarker?

SIGNATURES

GENE EXPRESSION SIGNATURES

Table 4. Prognostic Value of Supervised Gene/microRNA Expression Signatures and Unsupervised Gene Expression Subtypes of Early-Stage Colon Cancer

Signature and Disease Setting	No. of Patients	End Point	HR	95% CI	Data Set
Supervised signatures					
Oncotype DX Colon Cancer					
Stage II treated and untreated	711	RR	1.43	1.11 to 1.83	Clinical trial (QUASAR)[31]
Stage II and III untreated	690	RR	1.68	1.18 to 2.38	Clinical trial (CALGB9581)[64]
Stage II and III treated	892	RFS	1.57	1.19 to 2.08	Clinical trial (NSABP C-07)[32]
Stage II and III treated	688	RFS	1.30	1.11 to 1.57	Clinical trial (PETACC-3)[65]
		OS	1.34	1.10 to 1.62	
ColoPrint					
Stage I-III treated and untreated	206	RFS	2.69	1.41 to 5.14	Prospective cohort[36]
Stage II MSS treated and untreated	286	RFS	3.16	1.50 to 6.88	Prospective cohort[66]
Veridex					
Stage II untreated	123	RR	2.87	1.04 to 7.92	Prospective cohort[67]
Stage II and III treated	688	RFS	1.27	1.07 to 1.51	Clinical trial (PETACC-3)[65]
		OS	1.21	0.99 to 1.48	
GeneFx Colon					
Stage II untreated	144	RFS	2.55	1.47 to 4.42	Prospective cohort[68]
		OS	2.21*	1.22 to 3.97	
Stage II and III treated	688	RFS	1.20	1.02 to 1.40	Clinical trial (PETACC-3)[65]
		OS	1.22	1.02 to 1.47	
microRNA					
Stage II treated and untreated	460	DFS	3.70	2.56 to 5.35	Prospective cohort[69]
Unsupervised signatures					
Mesenchymal *v* others					
Stage I-IV treated and untreated	775	RFS	2.00*	1.50 to 2.70	Prospective cohort[73]
Stage II and III treated	688	RFS	1.68	1.32 to 2.04	Clinical trial (PETACC-3)[71]
Stage II and III treated and untreated	543	DMFS	2.30	$P = .016$	Prospective cohort[74]

Dienstmann, Salazar and Tabernero, J Clin Oncol 2015

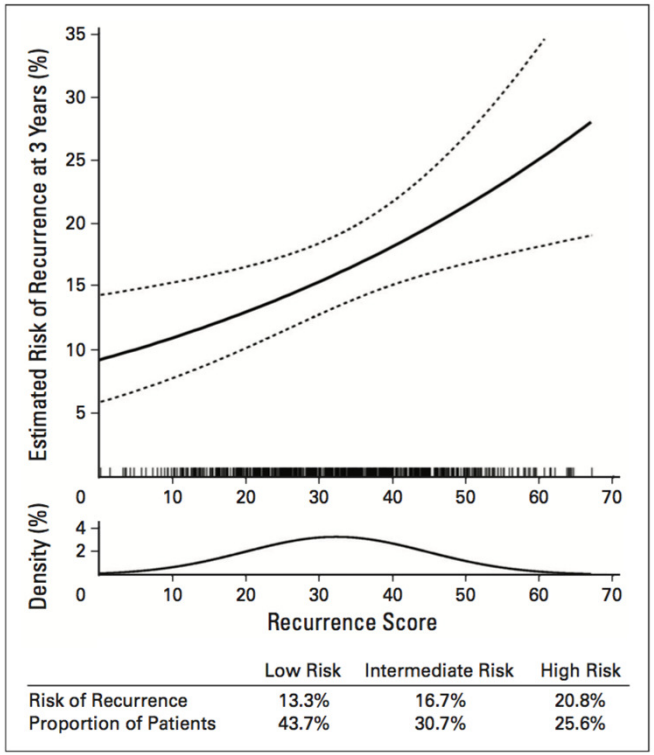
SUPERVISED GENE EXPRESSION SIGNATURES

VOLUME 29 · NUMBER 35 · DECEMBER 10 2011
JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

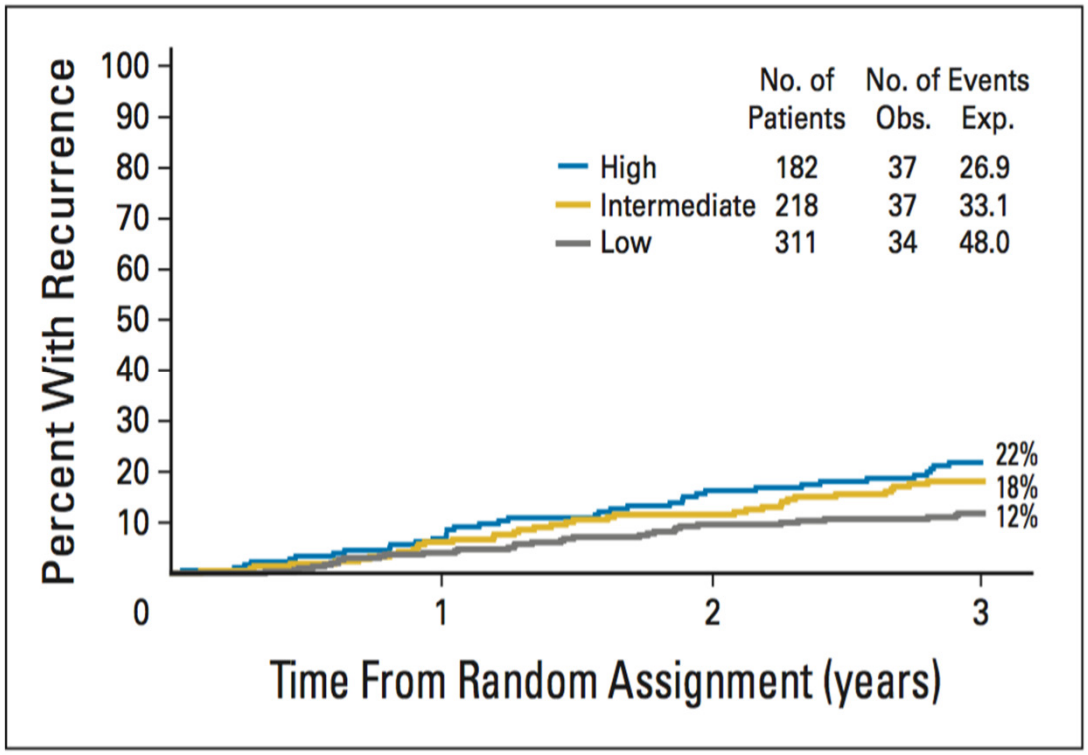
Oncotype DX®

Validation Study of a Quantitative Multigene Reverse Transcriptase–Polymerase Chain Reaction Assay for Assessment of Recurrence Risk in Patients With Stage II Colon Cancer

Recurrence risk at 3 years



	Low Risk	Intermediate Risk	High Risk
Risk of Recurrence	13.3%	16.7%	20.8%
Proportion of Patients	43.7%	30.7%	25.6%



Gray et al. J Clin Oncol 2011

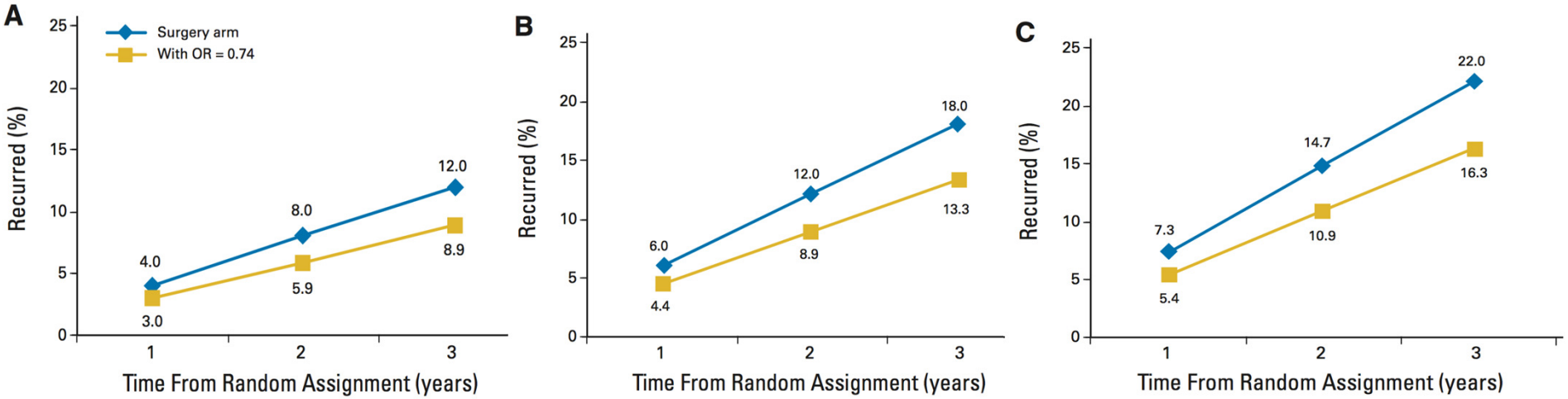
SUPERVISED GENE EXPRESSION SIGNATURES

VOLUME 29 · NUMBER 35 · DECEMBER 10 2011
JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Oncotype DX®

Validation Study of a Quantitative Multigene Reverse Transcriptase–Polymerase Chain Reaction Assay for Assessment of Recurrence Risk in Patients With Stage II Colon Cancer

Estimated absolute risk of recurrence at 3 years with and without FUFA chemotherapy, assuming the overall treatment effect for all stage II



LOW RISK (-3,1%) INTERMEDIATE RISK (-4,7%) HIGH RISK (-5,7%)

- Signature also validated on CALGB 9581 (S II, edrecolumab vs observation), NSABP C07 (S II/III, 5FU-LV +/- Ox) and SUNRISE (S II/III, no chemo) datasets with similar results
- Limitations: The signature does not predict benefit from adjuvant chemotherapy

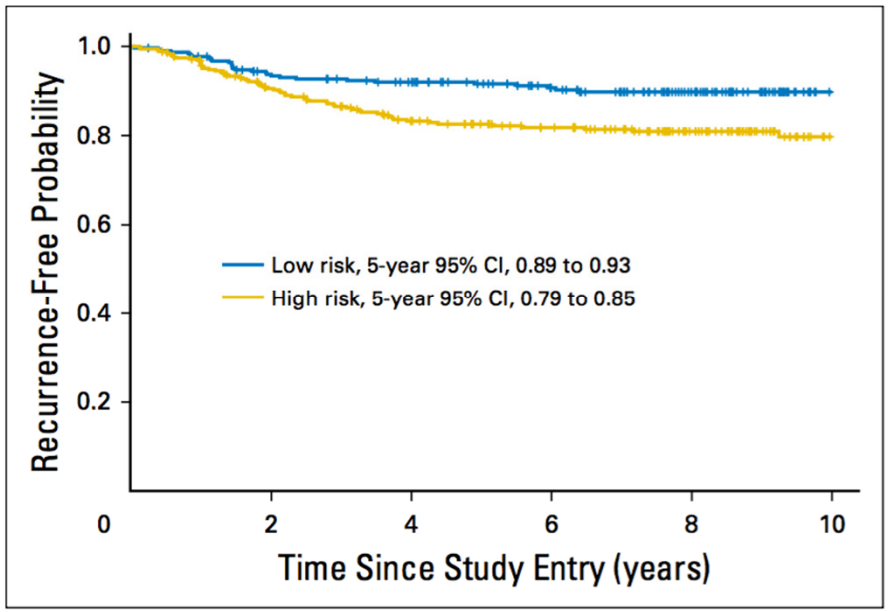
Gray et al. JCO 2011, Venook et al. JCO 2013, O'Connell et al, JCO 2013, Yamanaka et al. JCO 2016

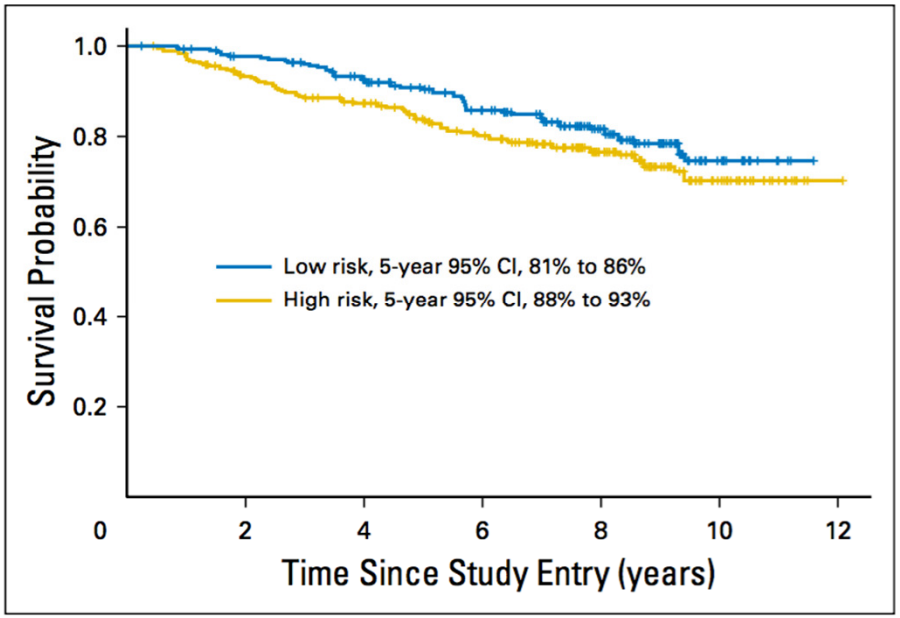
SUPERVISED GENE EXPRESSION SIGNATURES

VOLUME 34 · NUMBER 25 · SEPTEMBER 1, 2016
JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

GeneFx®Colon
(previously Colo-Dx®)

Association Between Results of a Gene Expression Signature Assay and Recurrence-Free Interval in Patients With Stage II Colon Cancer in Cancer and Leukemia Group B 9581 (Alliance)





ColDx is associated with RFI in the C9581 subsample in the presence of other prognostic factors, including MMR deficiency. ColDx could be incorporated with the traditional clinical markers of risk to refine patient prognosis.

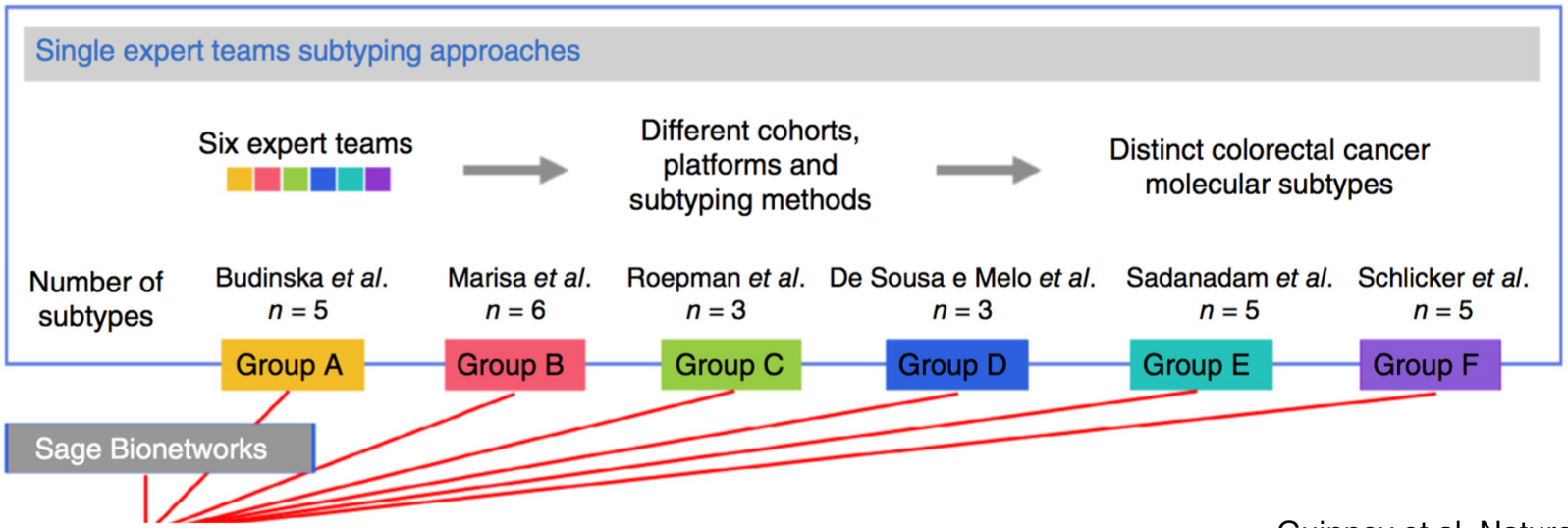
Niedzwiecki et al. J Clin Oncol 2016

UNSUPERVISED GENE EXPRESSION SIGNATURES

nature medicine

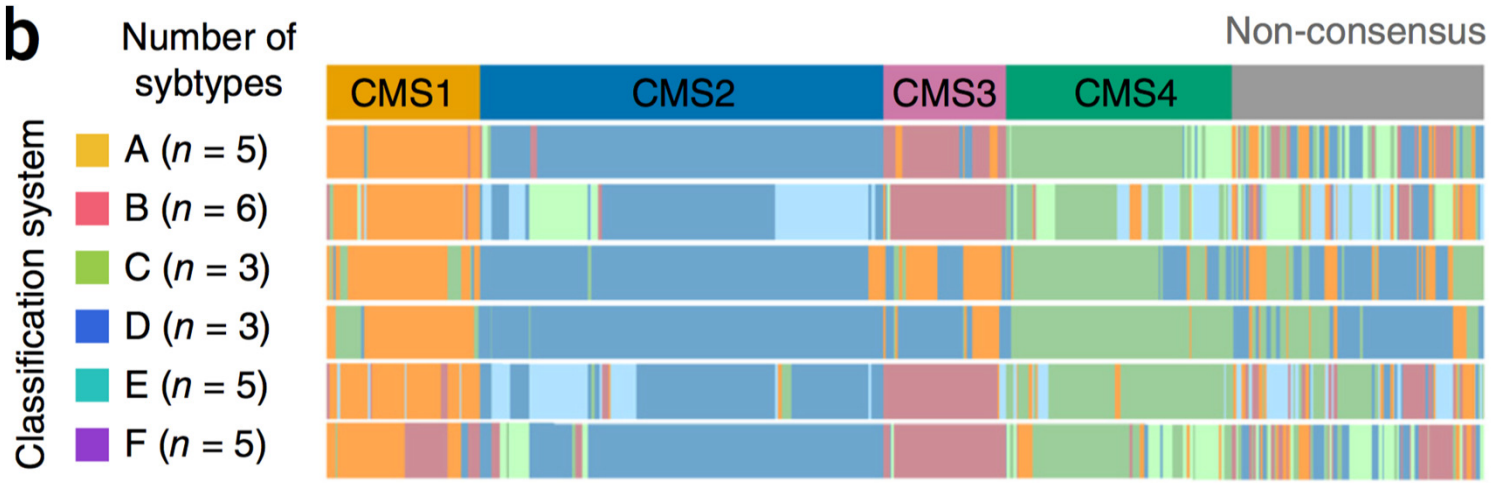
The consensus molecular subtypes of colorectal cancer

Justin Guinney[1,21], Rodrigo Dienstmann[1,2,21], Xin Wang[3,4,21], Aurélien de Reyniès[5,21], Andreas Schlicker[6,21], Charlotte Soneson[7,21], Laetitia Marisa[5,21], Paul Roepman[8,21], Gift Nyamundanda[9,21], Paolo Angelino[7], Brian M Bot[1], Jeffrey S Morris[10], Iris M Simon[8], Sarah Gerster[7], Evelyn Fessler[3], Felipe De Sousa E Melo[3], Edoardo Missiaglia[7], Hena Ramay[7], David Barras[7], Krisztian Homicsko[11], Dipen Maru[10], Ganiraju C Manyam[10], Bradley Broom[10], Valerie Boige[12], Beatriz Perez-Villamil[13], Ted Laderas[1], Ramon Salazar[14], Joe W Gray[15], Douglas Hanahan[11], Josep Tabernero[2], Rene Bernards[6], Stephen H Friend[1], Pierre Laurent-Puig[16,17,22], Jan Paul Medema[3,22], Anguraj Sadanandam[9,22], Lodewyk Wessels[6,22], Mauro Delorenzi[7,18,19,22], Scott Kopetz[10,22], Louis Vermeulen[3,22] & Sabine Tejpar[20,22]



Guinney et al. Nature Med 2015

UNSUPERVISED GENE EXPRESSION SIGNATURES

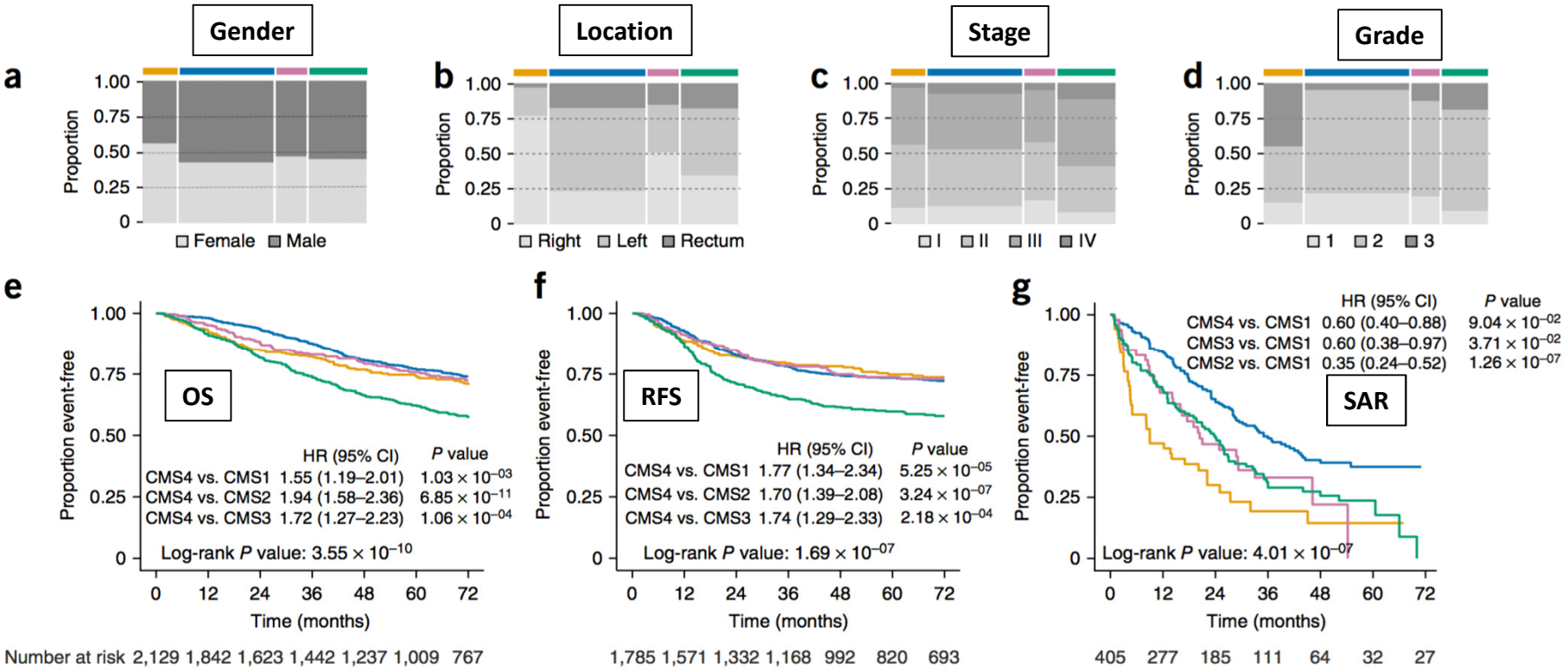


CMS1 MSI immune	CMS2 Canonical	CMS3 Metabolic	CMS4 Mesenchymal
14%	37%	13%	23%
MSI, CIMP high, hypermutation	SCNA high	Mixed MSI status, SCNA low, CIMP low	SCNA high
BRAF mutations		*KRAS* mutations	
Immune infiltration and activation	WNT and MYC activation	Metabolic deregulation	Stromal infiltration, TGF-β activation, angiogenesis
Worse survival after relapse			Worse relapse-free and overall survival

Guinney et al. Nature Med 2015

UNSUPERVISED GENE EXPRESSION SIGNATURES

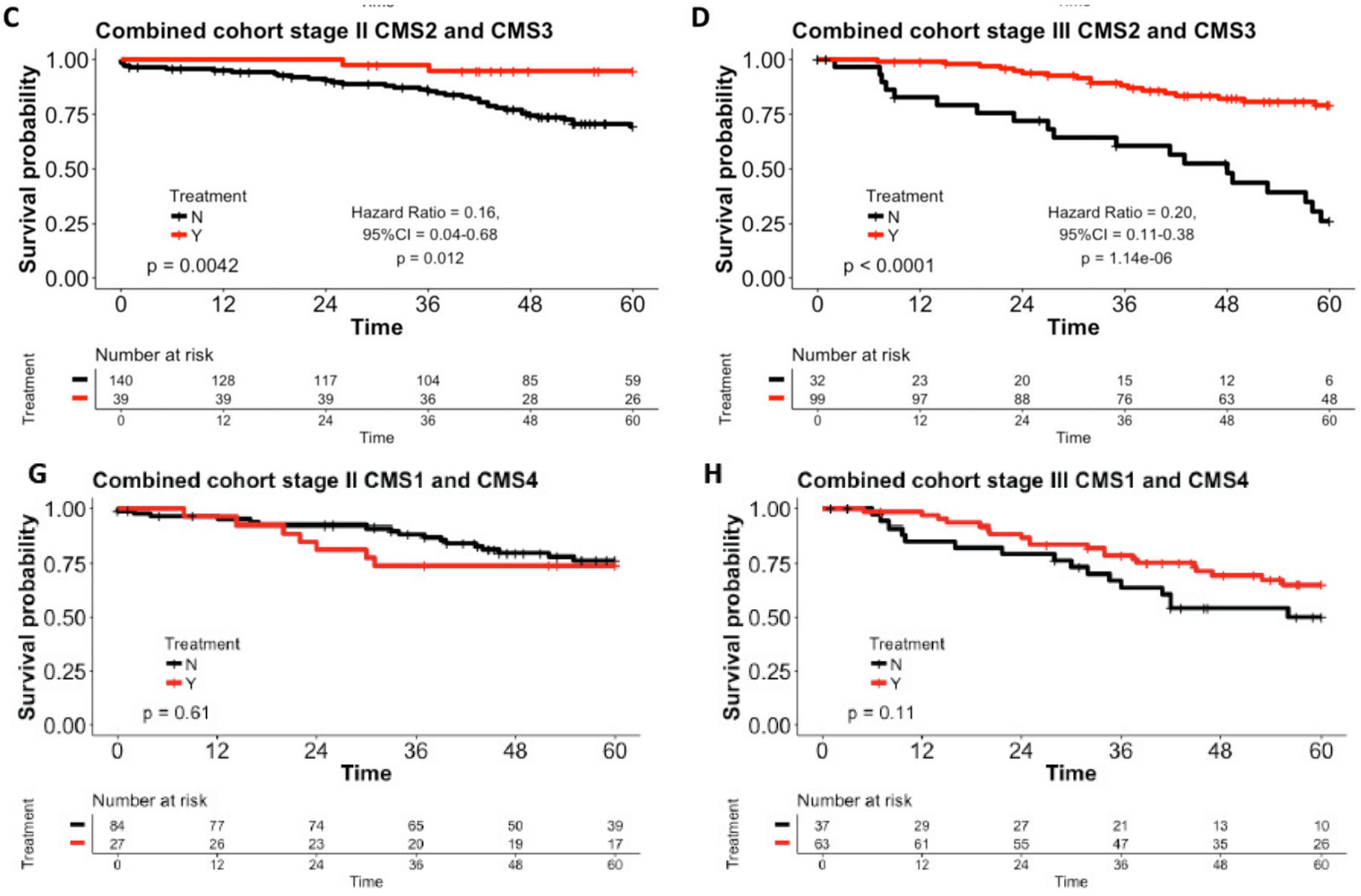
Clinical-pathological and prognostic associations of consensus molecular subtype groups



CMS1	14%	Females, older age, right colon, MSI, hypermutation, BRAF mut, immune +	Better RFS, intermediate OS, worse SaR
CMS2	37%	Left colon, epithelial, MSS, high CIN, TP53 mut, WNT/MYC +	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

Guinney et al. Nature Med 2015

PREDICTIVE ROLE OF CMS IN THE ADJUVANT SETTING (Treatment vs follow-up)



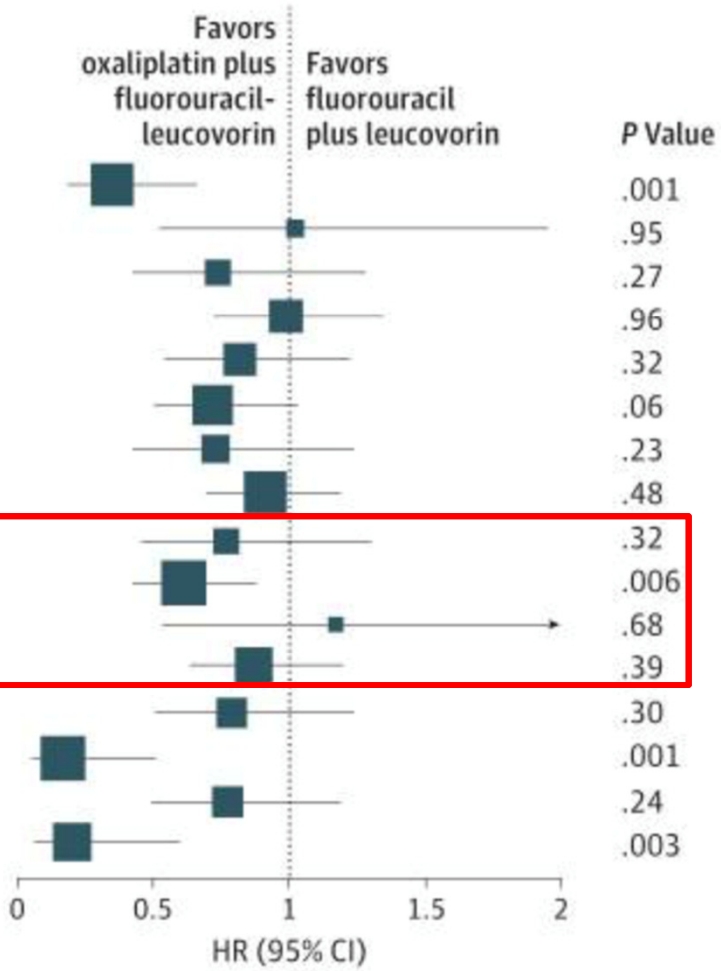
Training set: 156 samples stages II/III CRC
Validation sets: 2 stage II/III CRC cohorts: GSE39582 and GSE14333 (479 and 185, respectively)

Allen WL et al, JCO Precis Oncol 2018

PREDICTIVE ROLE OF CMS IN THE ADJUVANT SETTING (5-FU vs FOLFOX)

NSABP-C07/NRG

Classifiers	Subtypes	No. of Patients	HR (95% CI)	P Value
CRCA	Enterocyte	135	0.35 (0.19-0.65)	.001
	Gobletlike	103	1.02 (0.53-1.94)	.95
	Inflammatory	239	0.74 (0.43-1.27)	.27
	Stemlike	367	0.99 (0.73-1.34)	.96
	TA	307	0.82 (0.55-1.21)	.32
CCS	CCS1	410	0.72 (0.51-1.02)	.06
	CCS2	245	0.73 (0.43-1.23)	.23
	CCS3	496	0.91 (0.70-1.18)	.48
CMS	CMS1	231	0.77 (0.46-1.29)	.32
	CMS2	382	0.61 (0.43-0.87)	.006
	CMS3	86	1.17 (0.54-2.53)	.68
	CMS4	334	0.87 (0.64-1.19)	.39
Stratify CCS1 to TA and enterocyte	CCS1-TA	251	0.79 (0.51-1.23)	.30
	CCS1-enterocyte	74	0.17 (0.06-0.50)	.001
Stratify CMS2 to TA and enterocyte	CMS2-TA	261	0.77 (0.50-1.18)	.24
	CMS2-enterocyte	57	0.20 (0.07-0.59)	.003



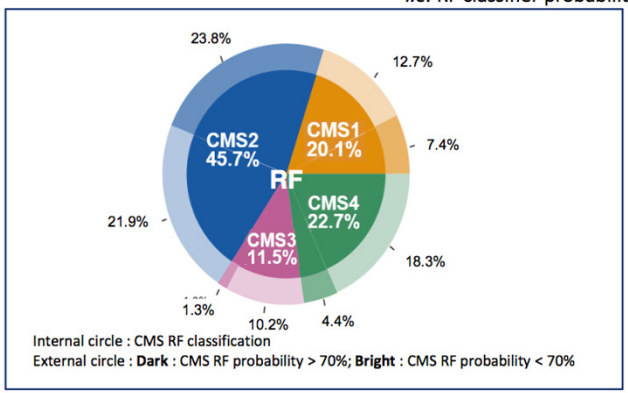
Song N et al, JAMA Oncol 2016

UNSUPERVISED GENE EXPRESSION SIGNATURES

Colon Cancer Molecular Subtype Intratumoral Hetrerogeneity and its prognostic impact (PETACC-8 Analysis)

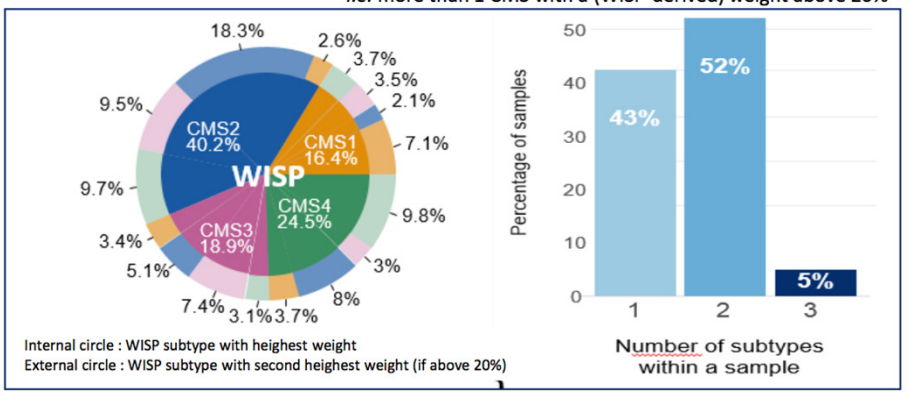
CMS attribution is uncertain* for most samples (63%)

* *i.e.* RF classifier probability < 70%

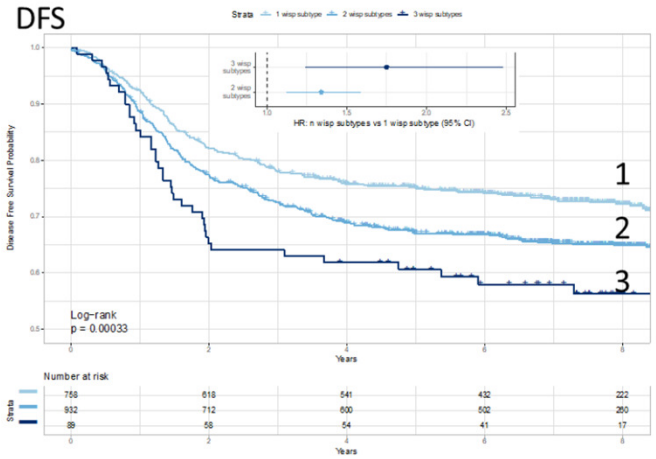


…and most samples (57%) show intra-tumor heterogeneity*

* *i.e.* more than 1 CMS with a (WISP-derived) weight above 20%

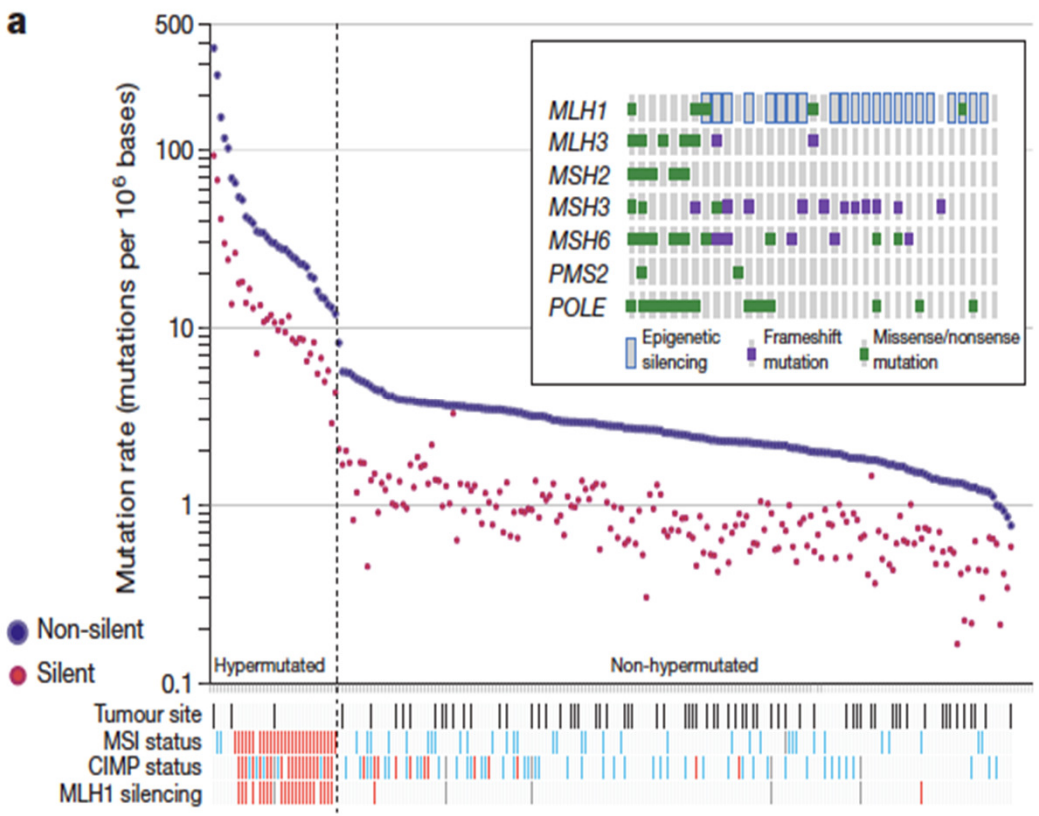


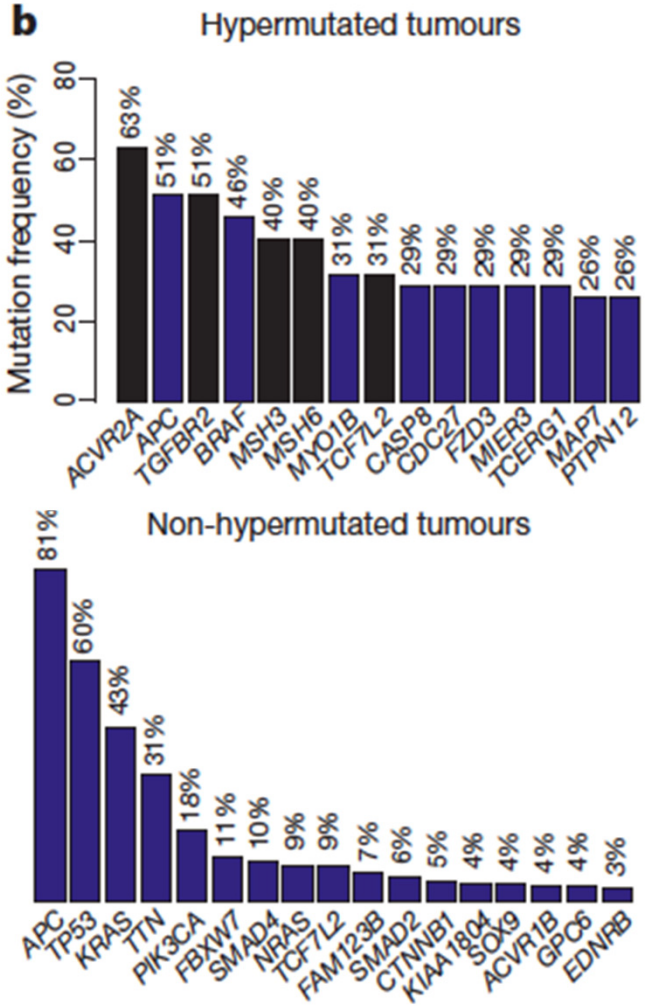
Intra-tumor heterogeneity has a prognostic impact



Marisa L et al. Ann Oncol 2018

UNSUPERVISED GENE EXPRESSION SIGNATURES



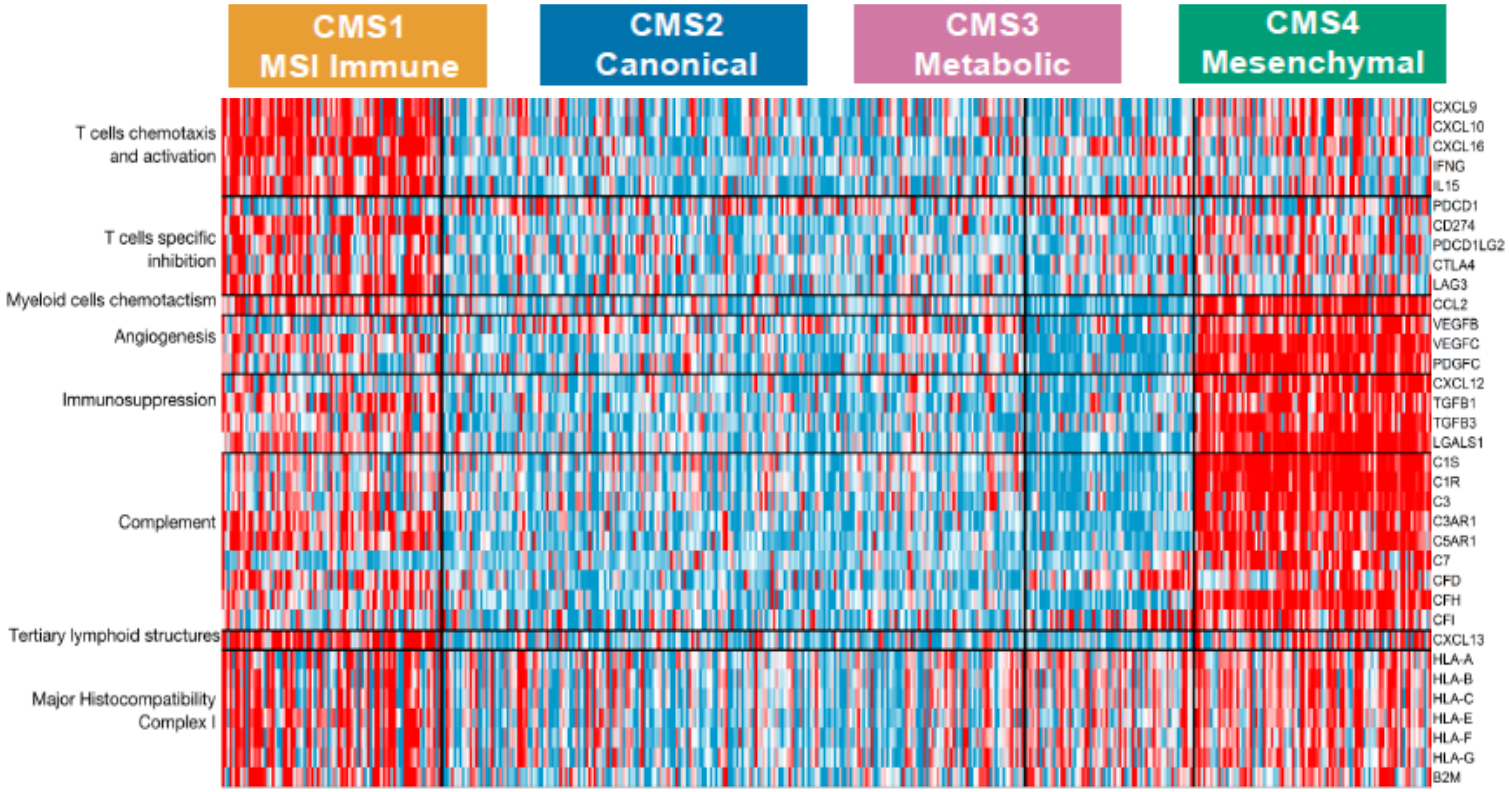


Altered proteins contain new epitopes for immune recognition, providing a common denominator for cancer immunotherapy

TCGA. Nature 2012

UNSUPERVISED GENE EXPRESSION SIGNATURES

Immune vs Transcriptomic subtypes of CRC



Becht et al, Clin Cancer Res 2016

UNSUPERVISED GENE EXPRESSION SIGNATURES

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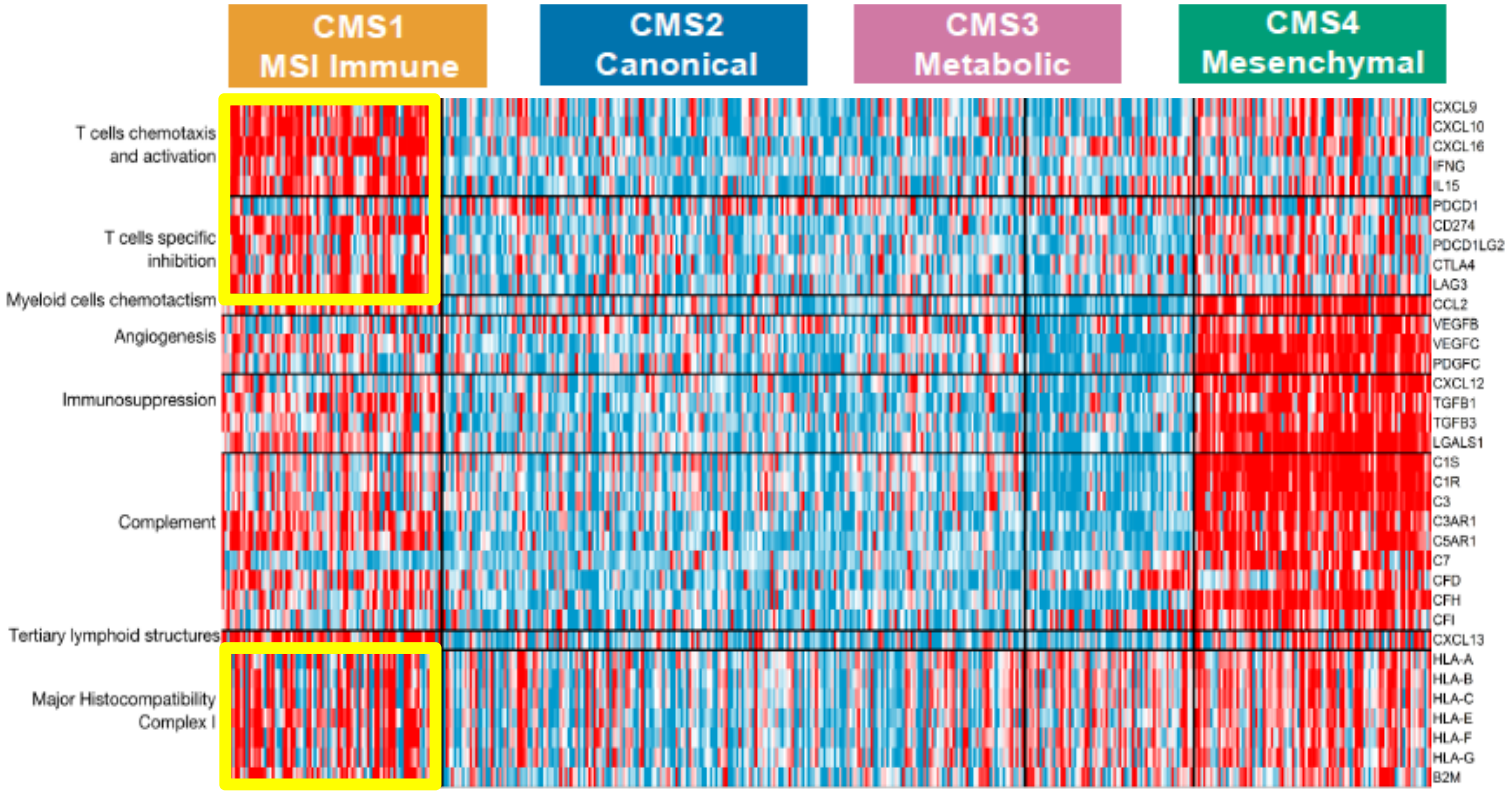

Becht et al, Clin Cancer Res 2016

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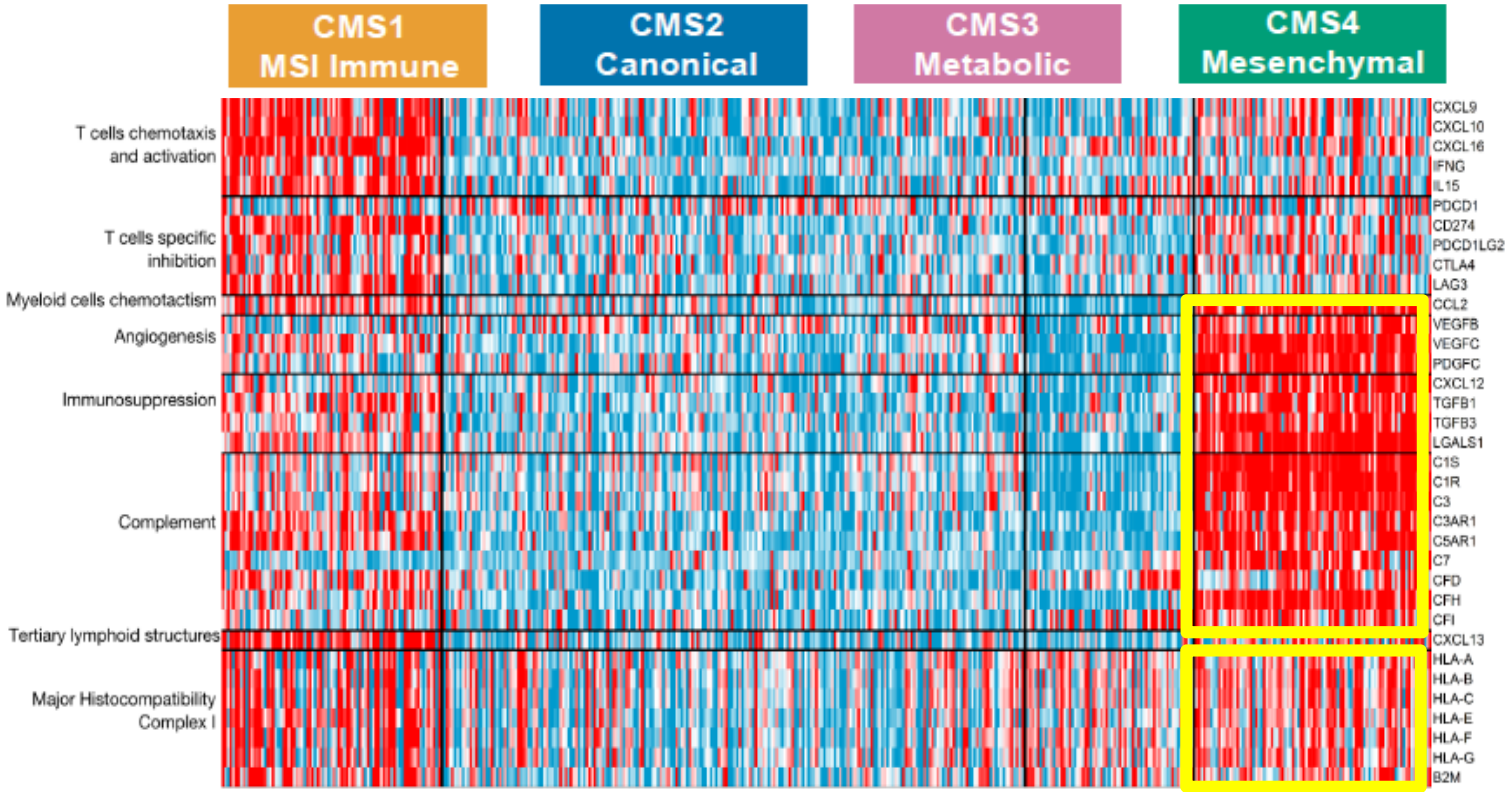

Becht et al, Clin Cancer Res 2016

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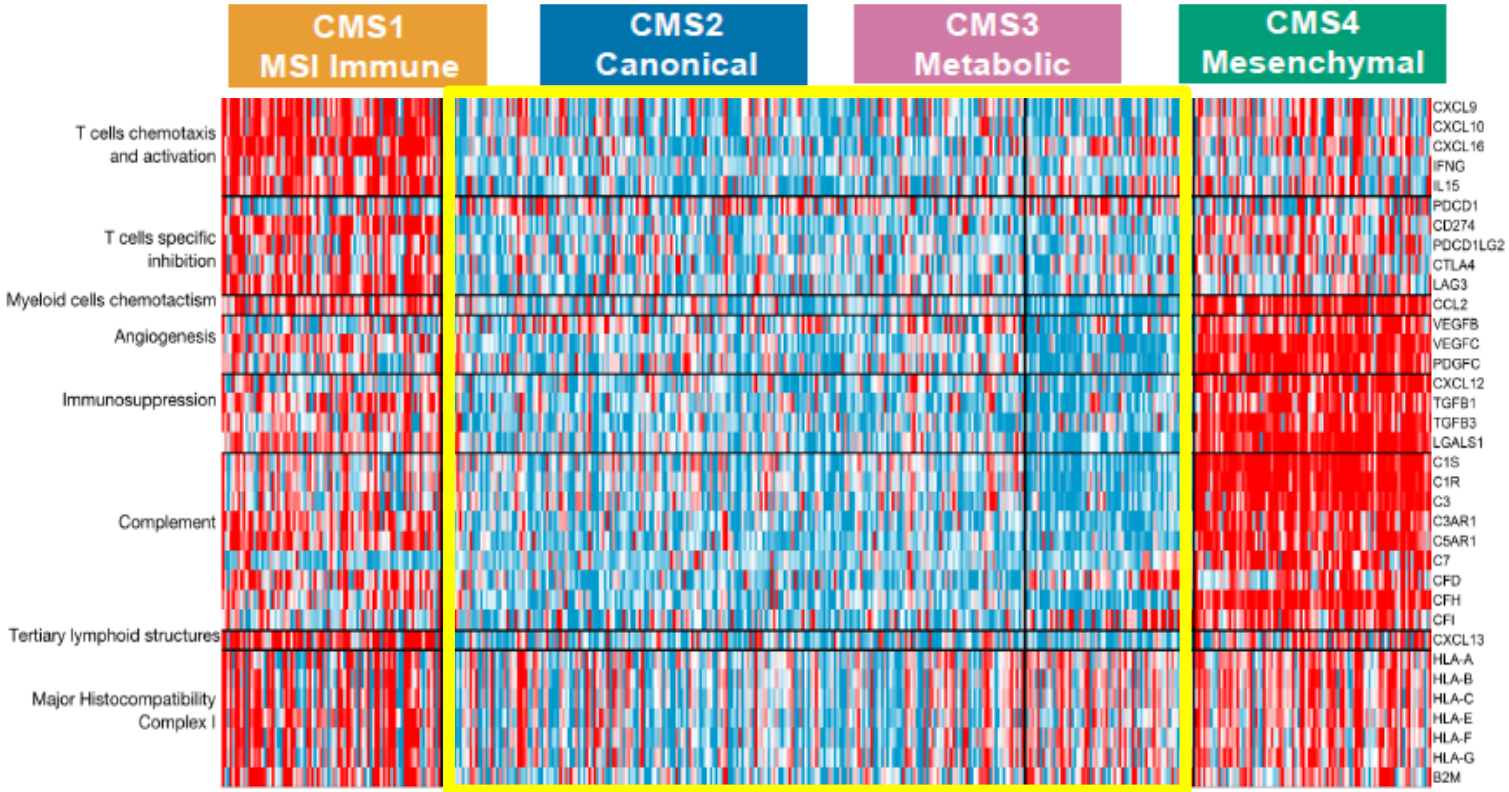


Becht et al, Clin Cancer Res 2016

INTERNATIONAL VALIDATION OF IMMUNOSCORE: STAGES I-III

✓ 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%

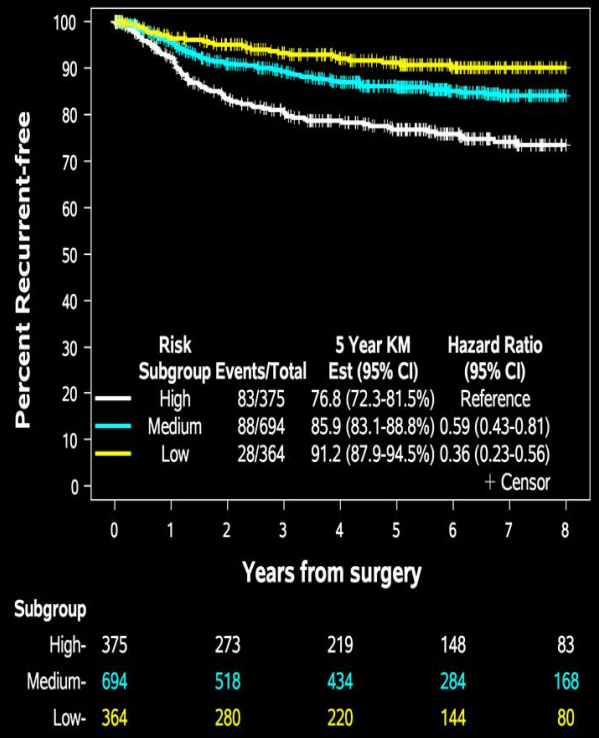
Stages I-III
3855 patients quantified for IS
2667 analyzed after QC and exclusion pre-defined statistical analysis workplan

Divided in 3 sets:
- Training set
- Internal validation set
- External validation set

Pages F et al. Lancet 2018

INTERNATIONAL VALIDATION OF IMMUNOSCORE: STAGES I-III

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

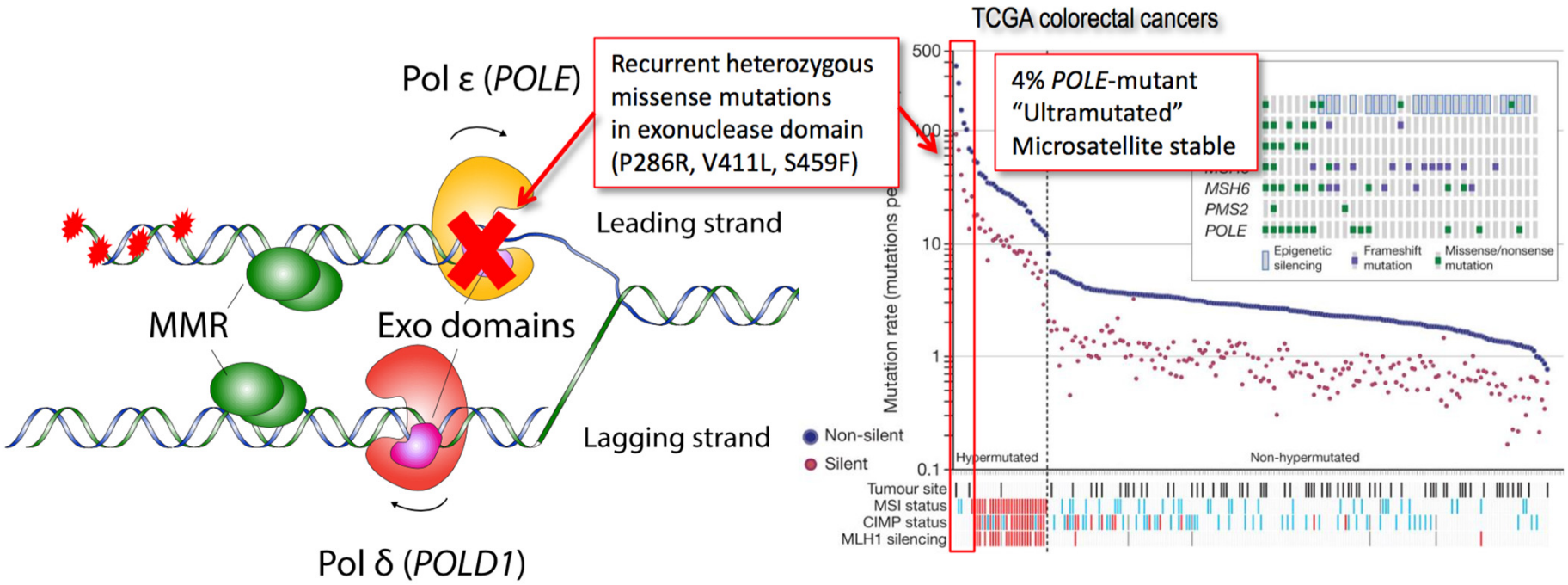
Pages F et al. Lancet 2018

ADJUVANT TREATMENT IN COLON CANCER :

Do we have any effective biomarker?

ADDITIONAL AIMS

POTENTIAL ROLE OF POL-E

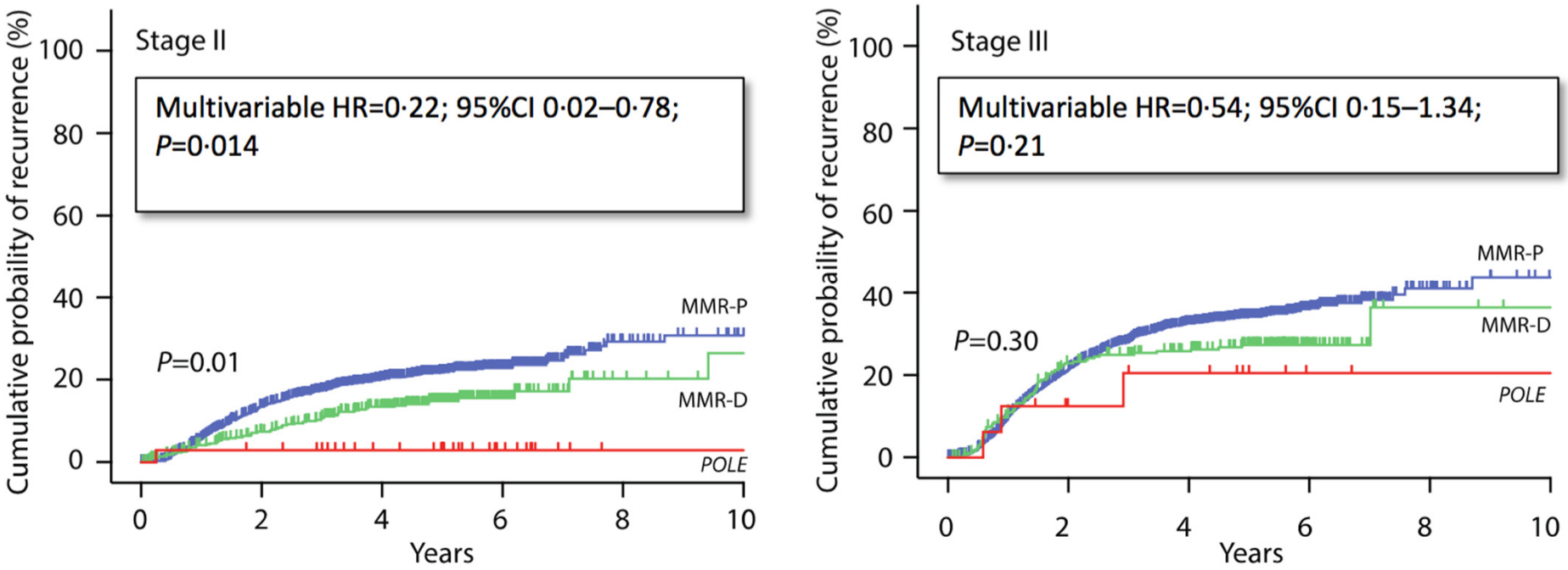


- Genomic stability depends on:
  - ✓ Proofreading of newly synthesized DNA strand by exonuclease domains in replicative DNA polymerases (*POLE* & *POLD1*)
  - ✓ Post-replication surveillance by DNA mismatch repair (MMR) system
- Defects in either cause increased mutation rate in model systems
- 1 study in CRC with a high number of *POLE* mutations (12%) not all pathogenic.

Stetzinger et al. Cancer Medicine 2014

POTENTIAL ROLE OF POL-E

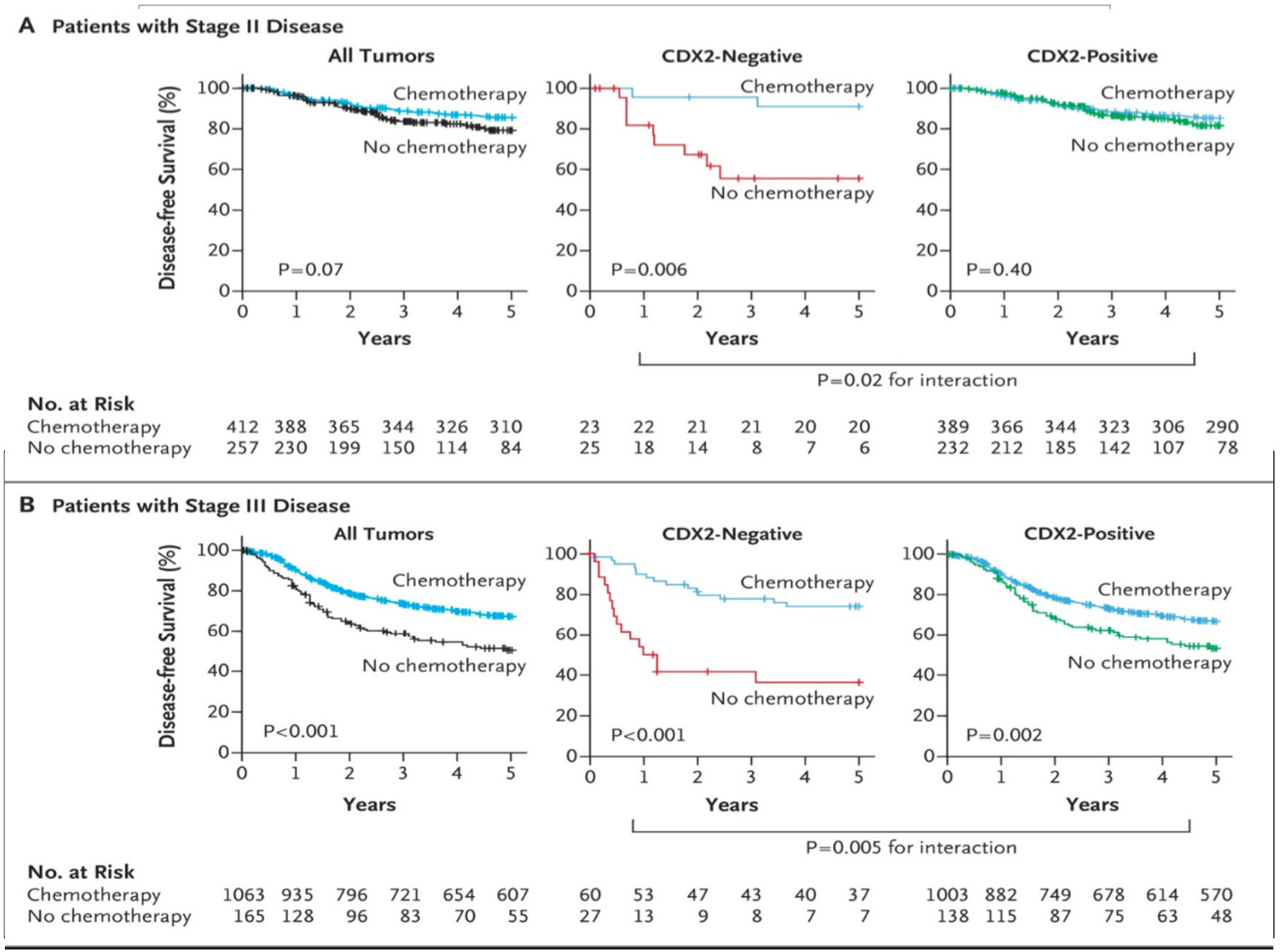
Stage II/III CRC: VICTOR, QUASAR2 and PETACC-3 trials, 5 CRC cohorts: LUMC, Oslo, Bern, AMC-AJCC-II & Epicolon1



- Pathogenic *POLE* proofreading domain mutations occur in 1-2% colorectal cancers, where they cause ultramutation
- Strong tendency to mutual exclusivity with MMR-D
- Evidence of robust anti-tumor cytotoxic T cell response and upregulation of immune checkpoints
- Favorable prognosis, particularly in stage II disease

Domingo E et al. Ann Oncol 2016; Domingo E et al, Lancet Gastroenterol Hepatol. 2018

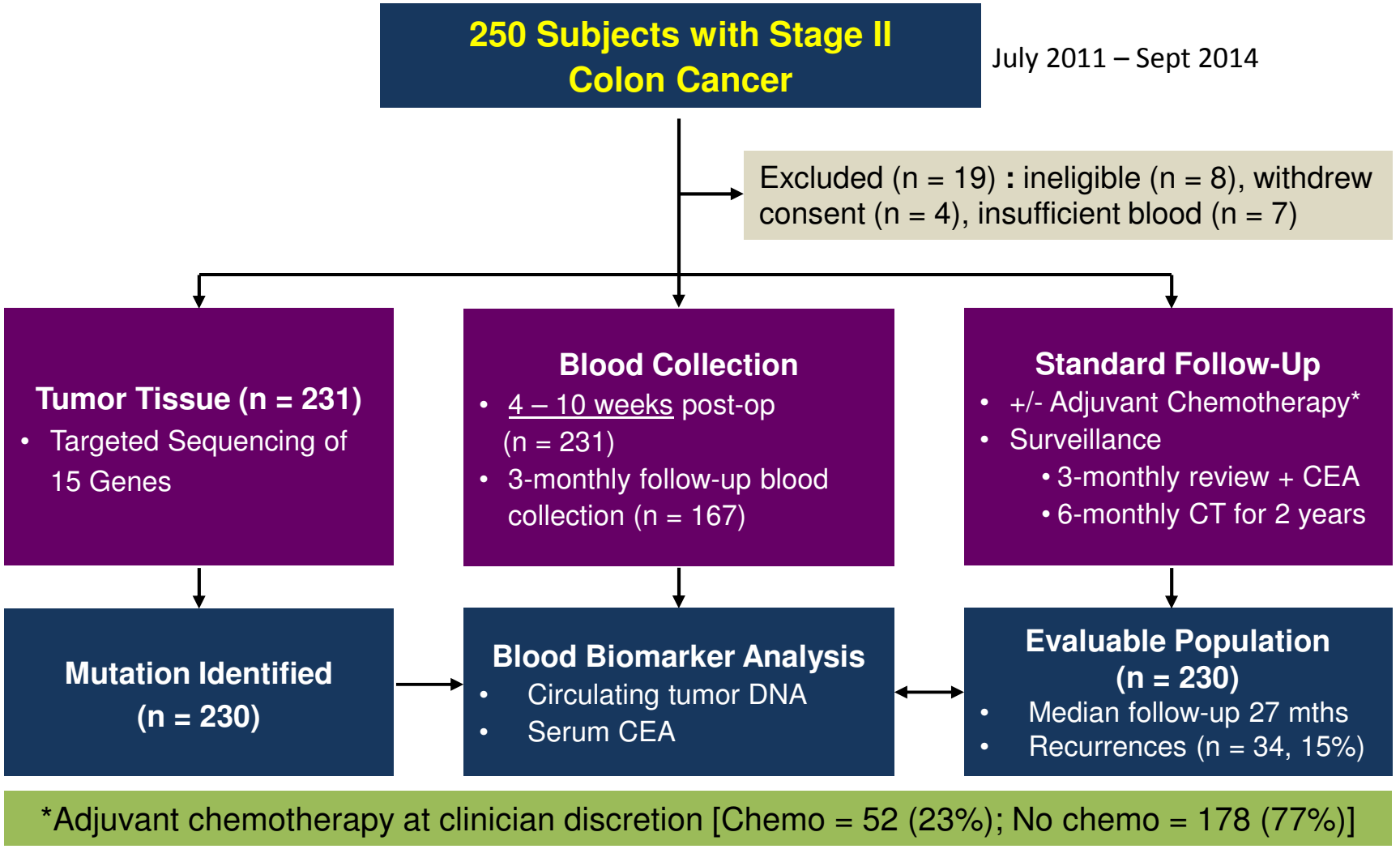
CDX2 IN RESECTED CCR



Dalerba P et al. NEJM 2016

Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer

Jeanne Tie[1,2,3,4,*,†], Yuxuan Wang[5,†], Cristian Tomasetti[6,7], Lu Li[6], Simeon Springer[5], Isaac Kinde[8], Natalie Silliman[5], Mark Tacey[9], Hui-Li Wong[1,3,4], Michael Christie[1,3,10], Suzanne Kosmider[2], Iain Skinner[2], Rachel Wong[1,11,12], Malcolm Steel[11], Ben Tran[1,2,3,4], Jayesh Desai[1,3,4], Ian Jones[4,13], Andrew Haydon[14], Theresa Hayes[15], Tim J. Price[16], Robert L. Strausberg[17], Luis A. Diaz Jr.[5], Nickolas Papadopoulos[5], Kenneth W. Kinzler[5], Bert Vogelstein[5,*,†], and Peter Gibbs[1,2,3,4,17,*,†]



Tie J et al. Sci Transl Med. 2016

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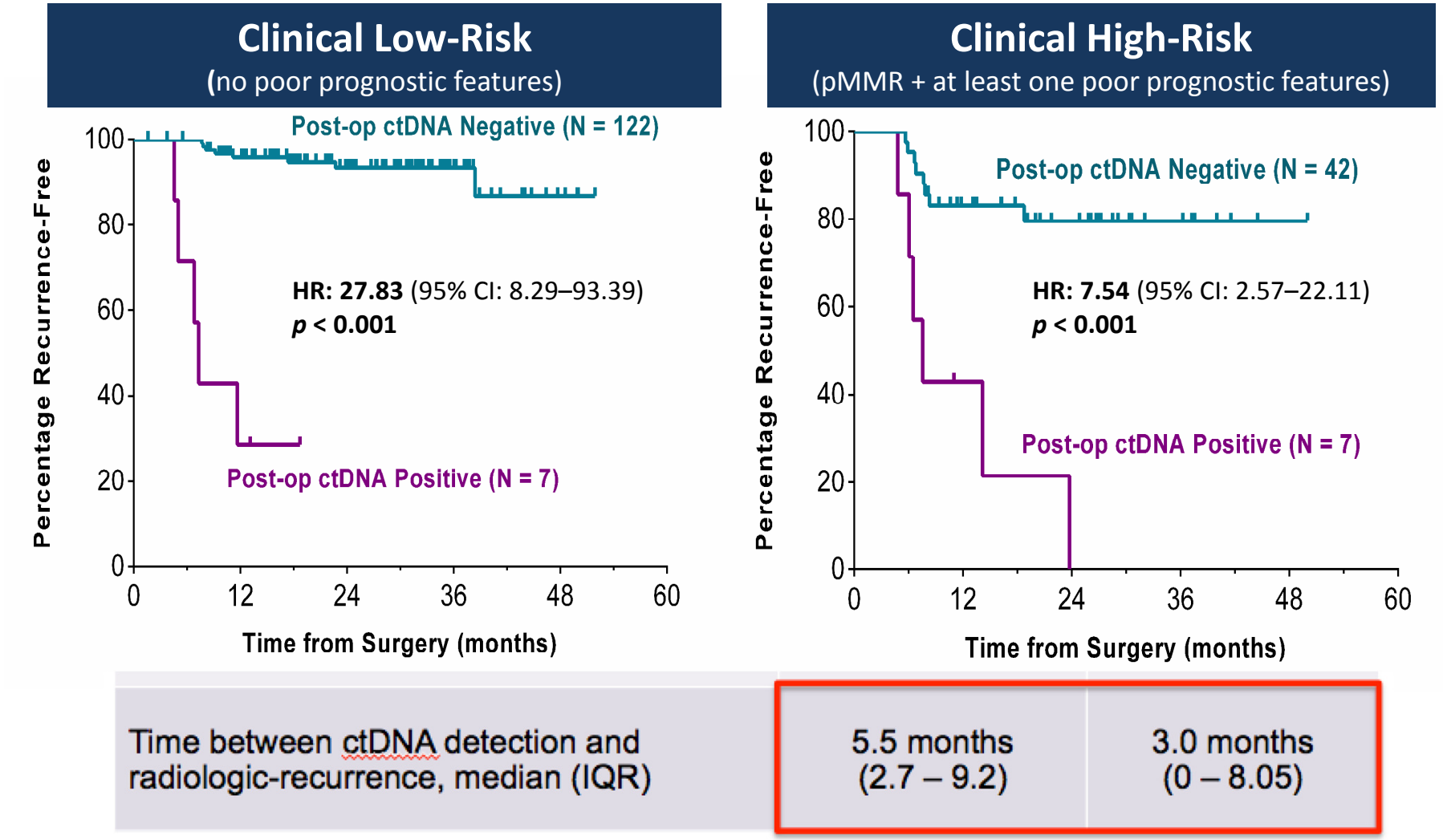

Time between ctDNA detection and radiologic-recurrence, median (IQR)	5.5 months (2.7 – 9.2)	3.0 months (0 – 8.05)

Tie J et al. Sci Transl Med. 2016

ADJUVANT TREATMENT IN COLON CANCER :

Do we have any effective biomarker?

CONCLUSIONS

CONCLUSIONS

- pTNM is still the best validated prognostic marker in colorectal cancer and the basis for therapeutic decision making
- Prognostic impact of *BRAF* depends on MSI-status
- Stage II:
  - *"Benefit of adjuvant therapy must be considered in 2016 and no place for oxaliplatin except may be T4 (MSS)"(T Andre)*
  - T3 MSS/T4 MSI: Slot for biomarker testing
    - High risk signatures: Not predictive
    - Low Immunoscore: Not predictive
    - Role of CDX2: To be determined
    - POL-E positive: Ready for that?
- Stage III: Benefit of ox-based therapy in all sub-stages
- Tumor location ? Treatment duration?
- Liquid biopsies (ctDNA) will have a role in this area
- CRC is not an homogeneous disease, gene expression classification suggests four major subgroups, partly matching the carcinogenesis pathways

THANK YOU