Molecular Variances Between Rectal and Left-Sided Colon Cancers

Mohamed E. Salem[1], Heinz-Josef Lenz[2], Joanne Xiu[3], Wafik S. El-Deiry[4], Zoran Gatalica[3], Jimmy J. Hwang[5], Philip A. Philip[6], Anthony F. Shields[6], and John L. Marshall[1]

[1]Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC;[2]Norris Comprehensive Cancer Center, Los Angeles, CA; [3]Caris Life Sciences, Phoenix, AZ; [4]Fox Chase Cancer Center, Philadelphia, PA; [5]Levine Cancer Institute, Charlotte, NC; [6]Karmanos Cancer Institute, Detroit, MI

PRESENTED AT: 2017 Gastrointestinal Cancers Symposium | #GI17

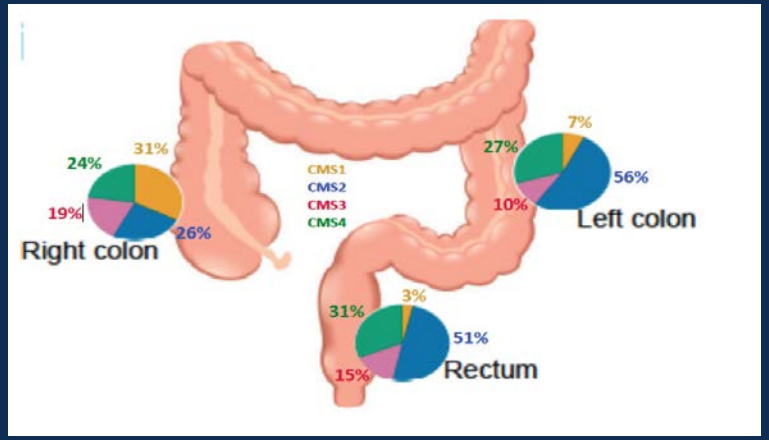
Disclosure

- Research grant funding from Bayer and Taiho
- Consultant for of Genentech, Bayer and Taiho
- Speaker’s Bureau Member: Genentech, Bayer and Taiho

Background

- Colorectal cancer (CRC) is a heterogeneous disease with different genetic alterations and clinical behavior
- CRC was recently classified into four consensus molecular subtypes (CMSs) with distinguishing features[1]
- CMS 1-4 tumors have different carcinogenic pathways and genomic patterns

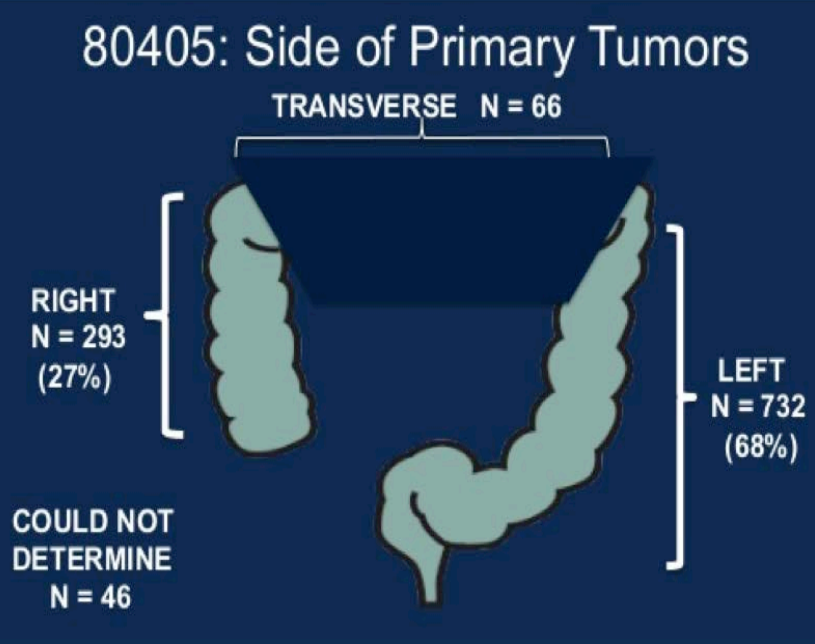
CMS1 MSI immune	CMS2 Canonical	CMS3 Metabolic	CMS4 Mesenchymal
14%	37%	13%	23%
MSI, CIMP high, hypermutation	SCNA high	Mixed MSI status, SCNA low, CIMP low	SCNA high
BRAF mutations		*KRAS* mutations	
Immune infiltration and activation	WNT and MYC activation	Metabolic deregulation	Stromal infiltration,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



[1]Guinney J et al. Nat Med. 2015;21(11): 1350-1356

Background

- Recent retrospective analysis of CALGB 80405 showed that left-sided colon tumors respond differently to biologics compared to right-sided colon tumors[1], likely due to molecular differences
- In the CALGB 80405 analysis, rectal cancers were included as part of the "left-sided" tumors
- However molecular variations between rectal and left-sided colon tumors are not well defined



[1]Venook AP et al. Clin Oncol. 2016;34 (suppl; abstr 3504)

Objective

- To identify the molecular variations among left-sided CRC tumors:
  - Rectal cancers
  - Sigmoid colon cancers
  - Descending colon cancers (plus splenic flexure)

Methods

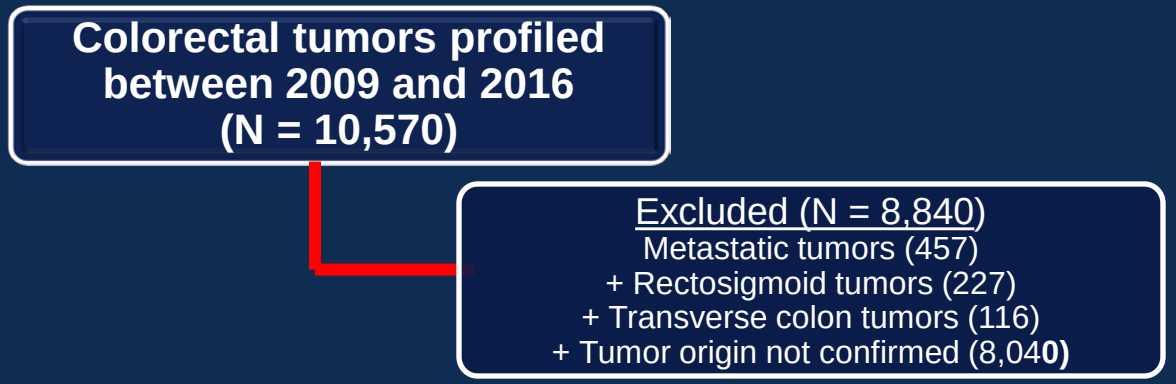
- Retrospective analysis of 1,730 CRC tumors that were profiled by Caris Life Sciences between 2009 and 2016 was performed
- All samples were independently reviewed by at least one pathologist, in addition to the local pathologist
- Only primary tumors were included in the current analysis
- Tumors without clearly defined origins were excluded
- Chi-square test was used for comparison between groups (IBM SPSS Statistics, Version 23) and significance was defined as $p < 0.05$

CONSORT Diagram

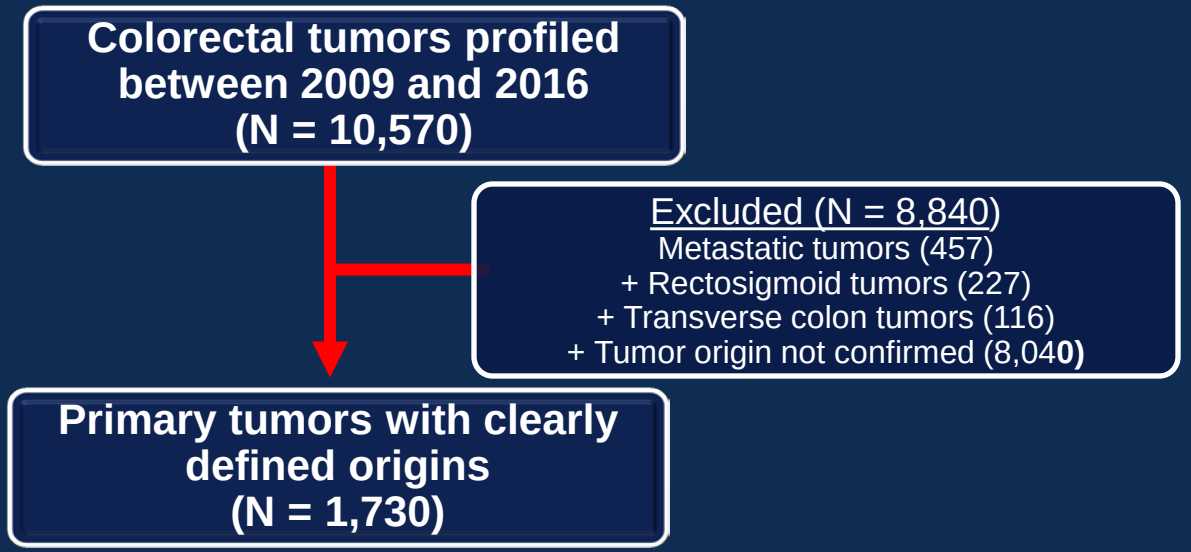
CONSORT Diagram

Colorectal tumors profiled between 2009 and 2016 (N = 10,570)

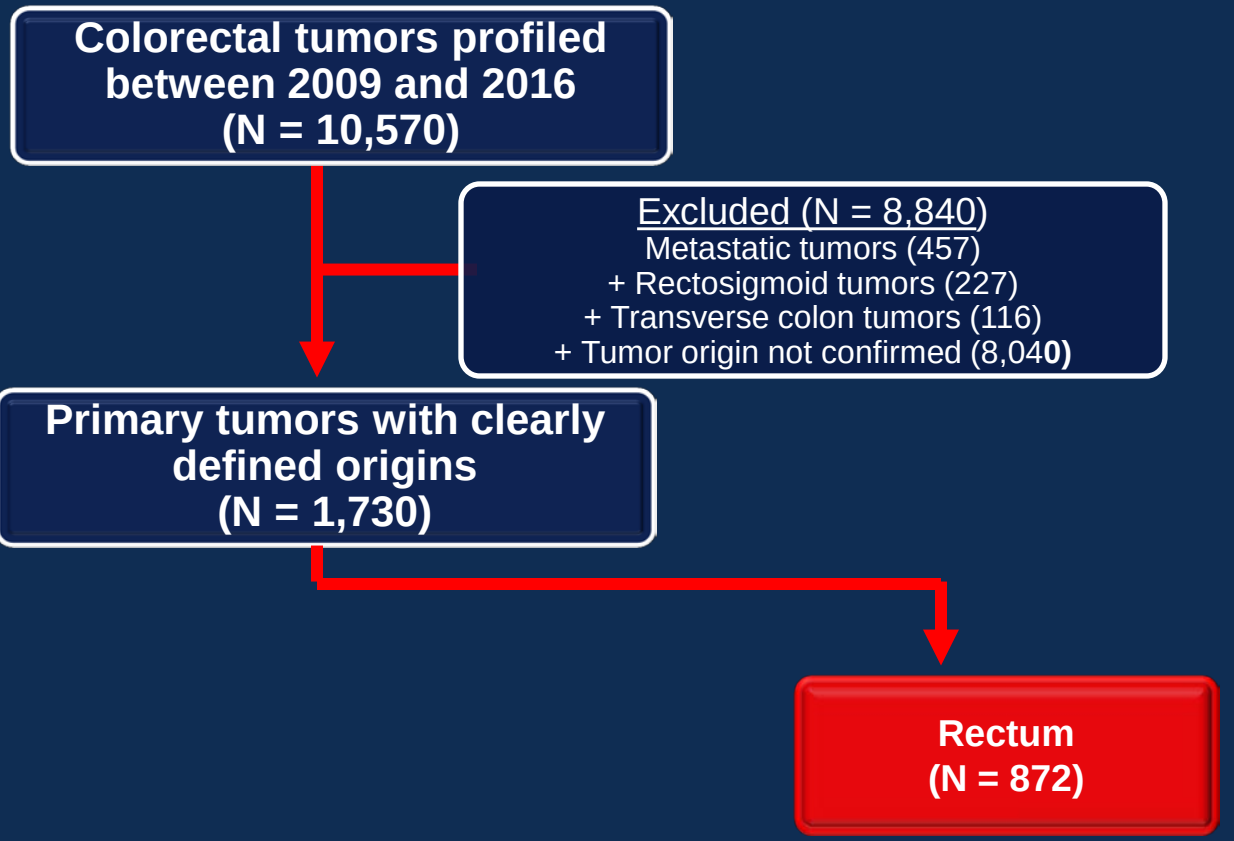
CONSORT Diagram



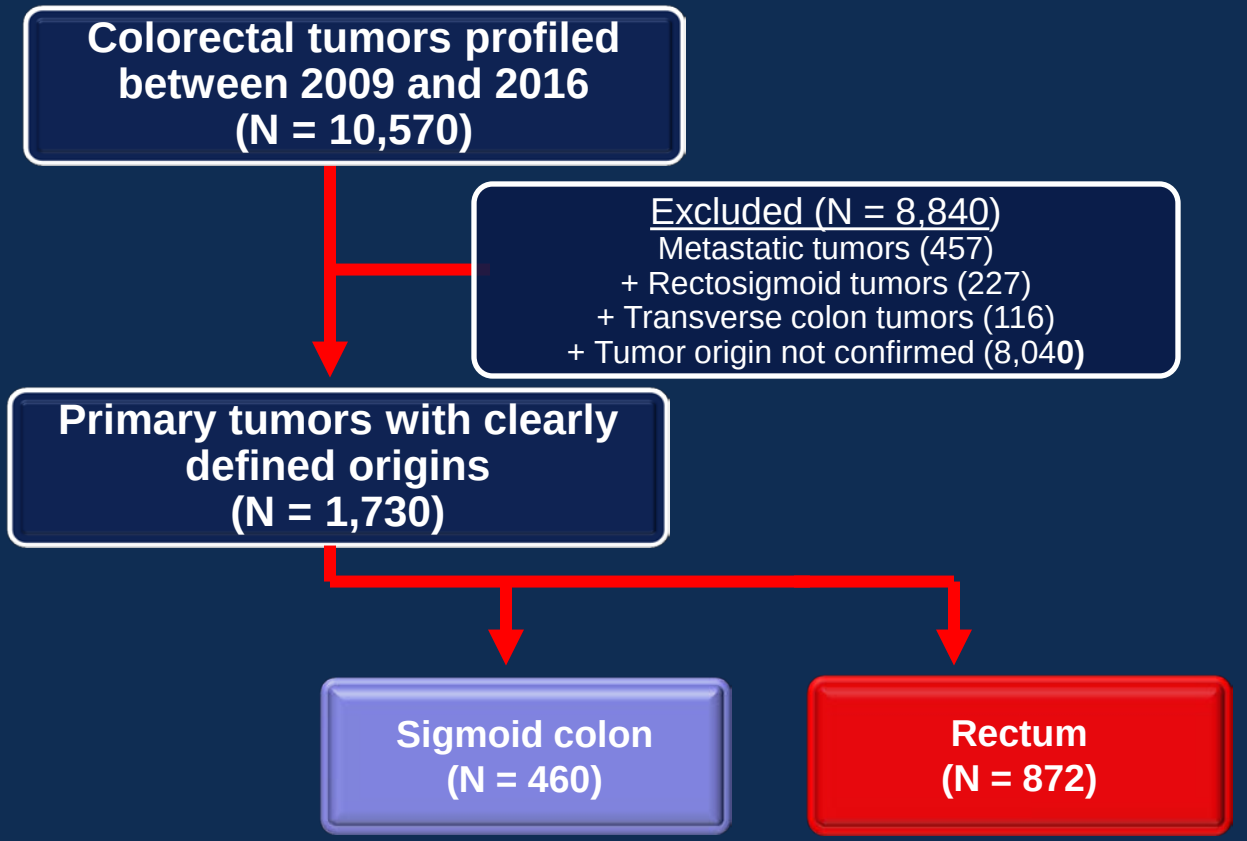
CONSORT Diagram



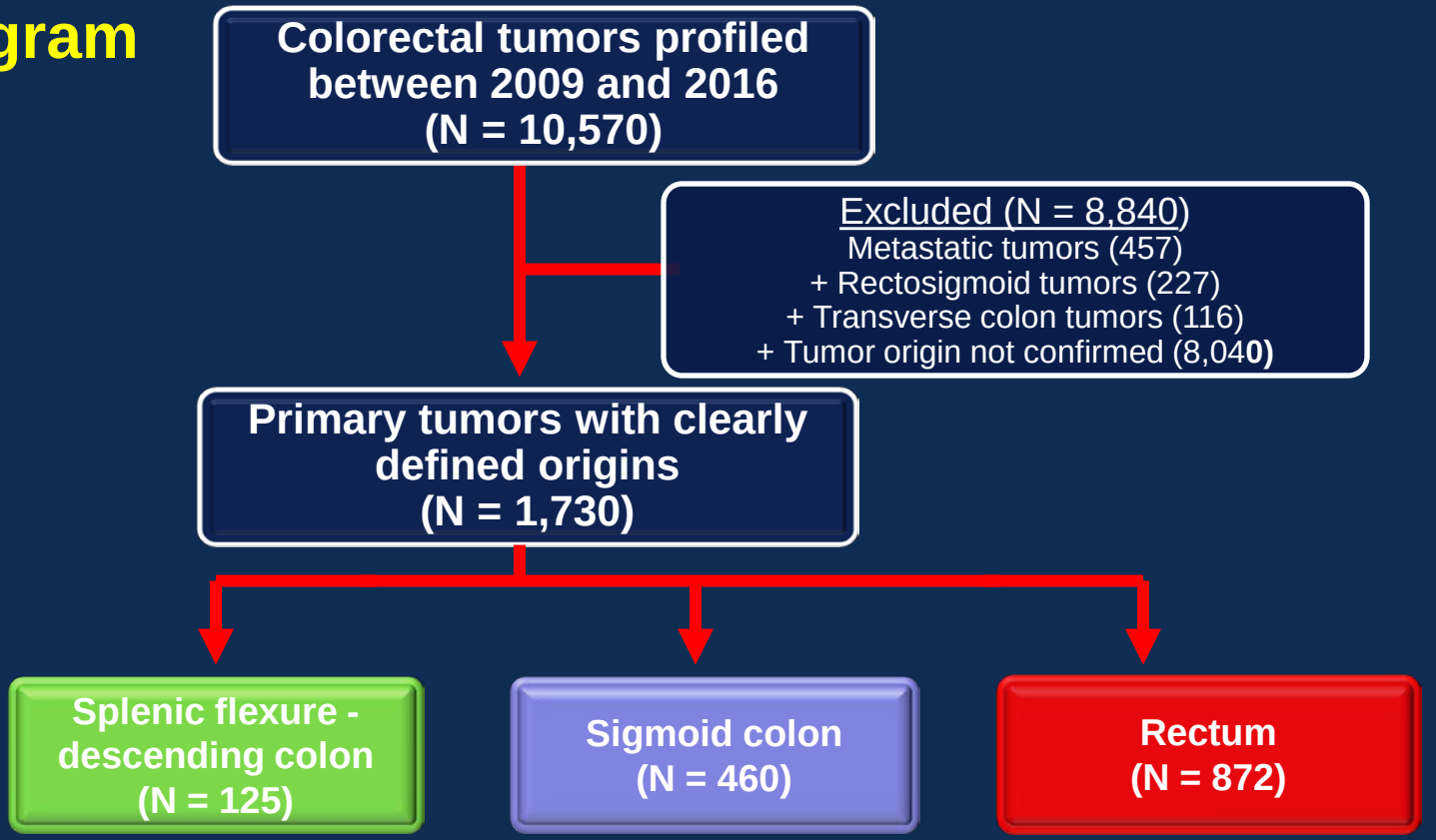
CONSORT Diagram



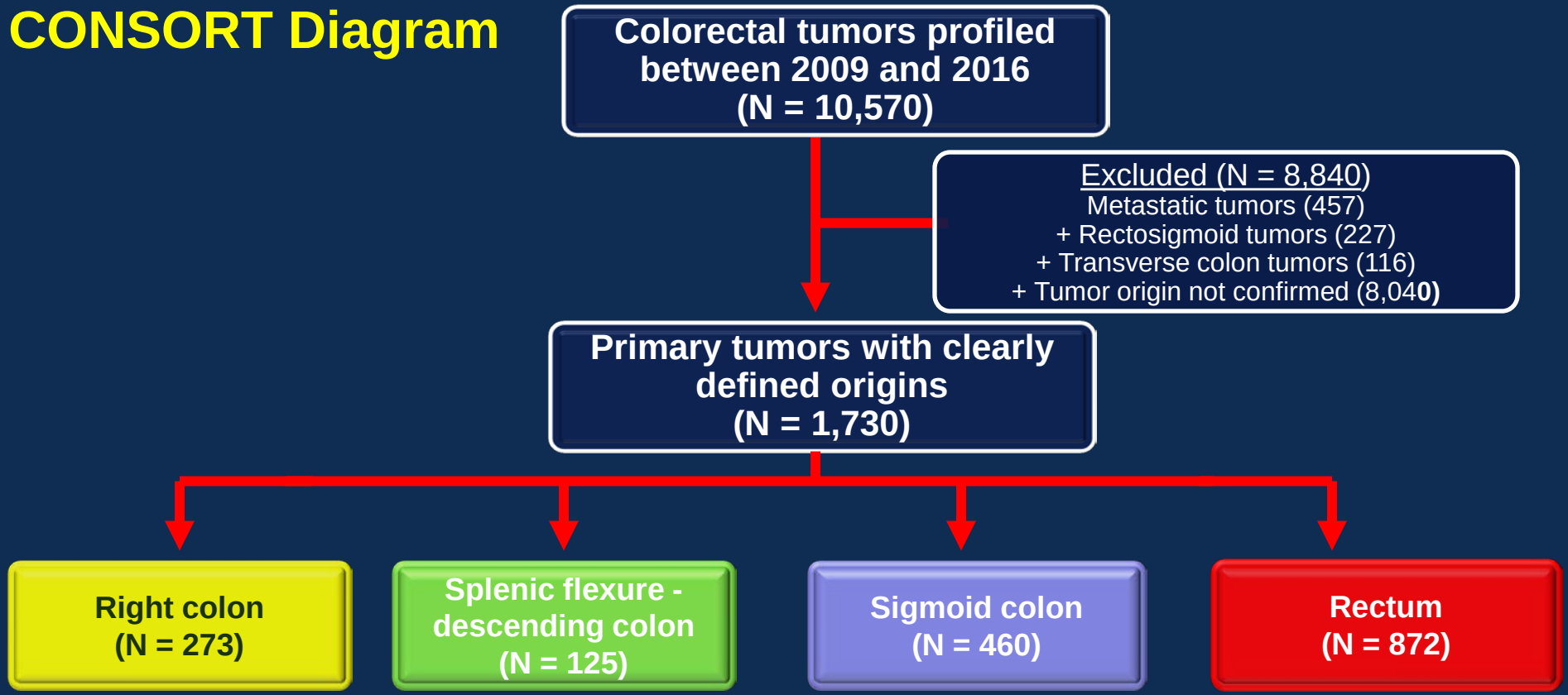
CONSORT Diagram



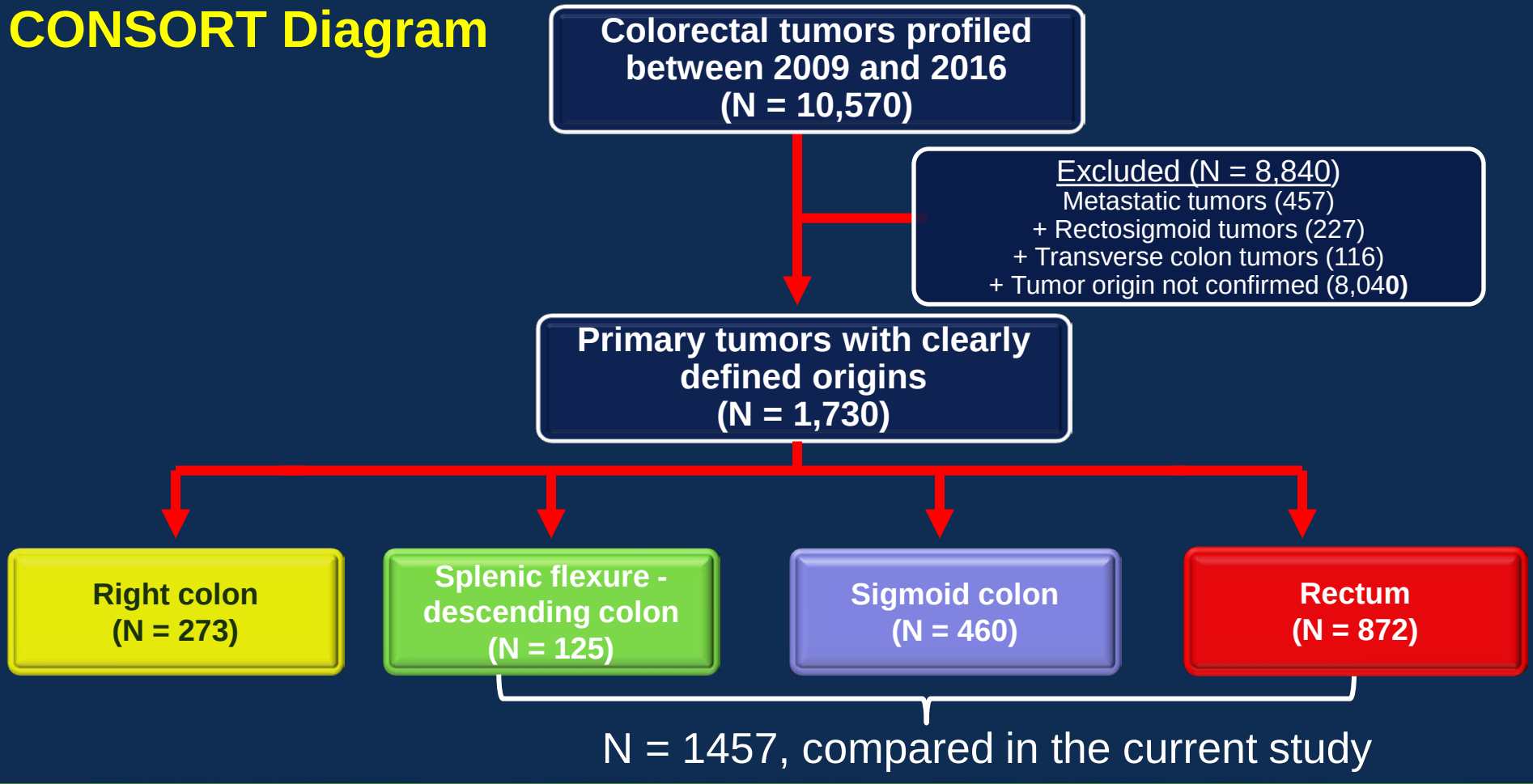
CONSORT Diagram



CONSORT Diagram



CONSORT Diagram



Multi-platform profiling

- Immunohistochemistry (IHC):

ALK	PGP
AR	PR
cMET	PTEN
EGFR	RRM1
ER	TLE3
ERCC1	TOP2A
Her2/Neu	TOPO1
MGMT	TS
PD-1	TUBB3
PD-L1	

PD-L1 antibody clone used: SP142

- Microsatellite Instability fragment analysis (Promega)
  - Microsatellite Instability
- In-situ hybridization (CISH or FISH)
  - Her2
  - cMET
  - EGFR

- Next-Generation Sequencing
  - Illumina MiSeq platform Illumina TruSeq Amplicon Cancer Hotspot panel
    - All tumor samples micro-dissected
    - Average depth of coverage > 1500X
    - Analysis of tumor tissue,
    - 45 gene panel

ABL1	CSF1R	FGFR2	IDH1	PIK3CA
CDH1	FGFR1	HRAS	cMET	SMARCB1
FBXW7	HNF1A	KRAS	PDGFRA	BRAF
GNAS	cKIT	NRAS	SMAD4	ERBB4
KDR	NPM1	RET	ATM	GNAQ
NOTCH1	RB1	VHL	ERBB2	JAK3
PTPN11	TP53	APC	GNA11	MPL
STK11	ALK	EGFR	JAK2	PTEN
AKT1	CTNNB1	FLT3	MLH1	SMO

  - 10% of tumors were tested with NextSeq platform: Agilent SureSelect XT, 592 gene panel, which were used to calculate tumor mutation load

Testing was performed under accreditation from CLIA, CAP and ISO 15189:2012

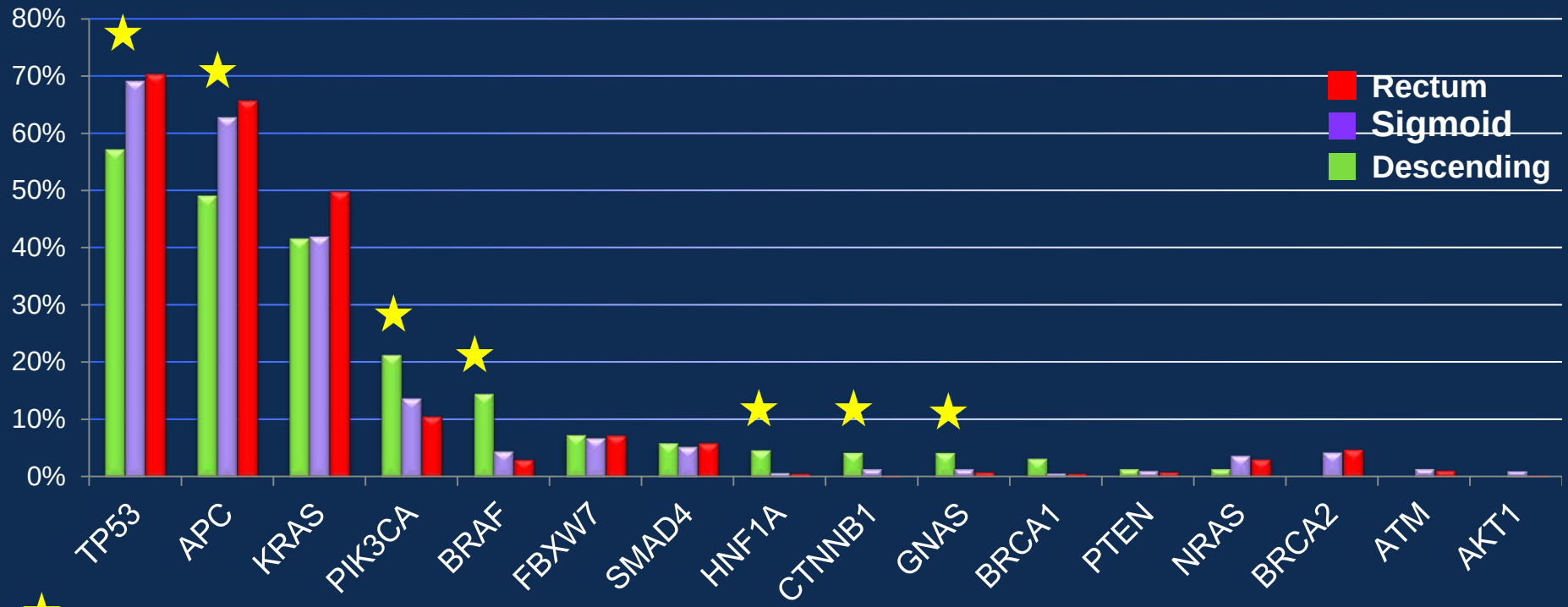
Results

Patient characteristics

Primary tumor location		Splenic flexure - descending colon (N=125)	Sigmoid colon (N=460)	Rectum (N=872)
Median Age (yr.)		62	60	60
Sex (%)	Female	50%	44%	37%
	Male	50%	56%	63%

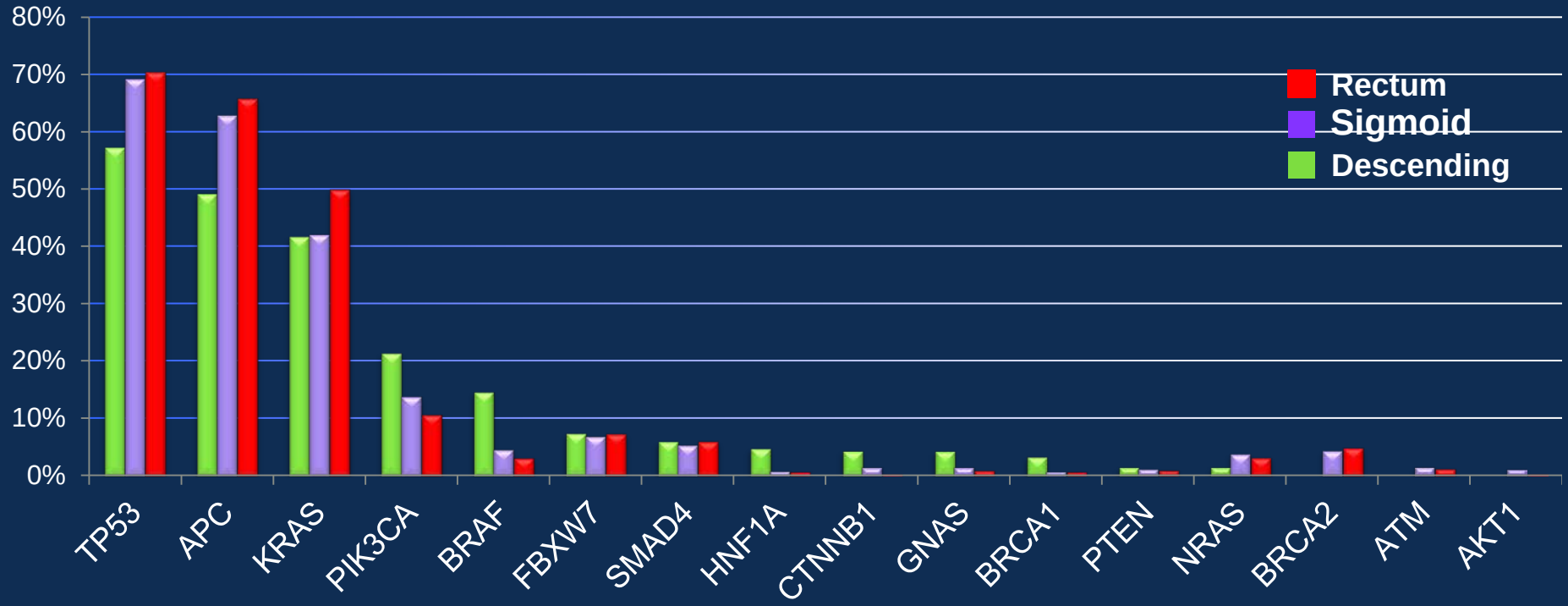
Next-Generation Sequencing

Mutation Frequency Comparison Between Rectal and Descending Colon Tumors



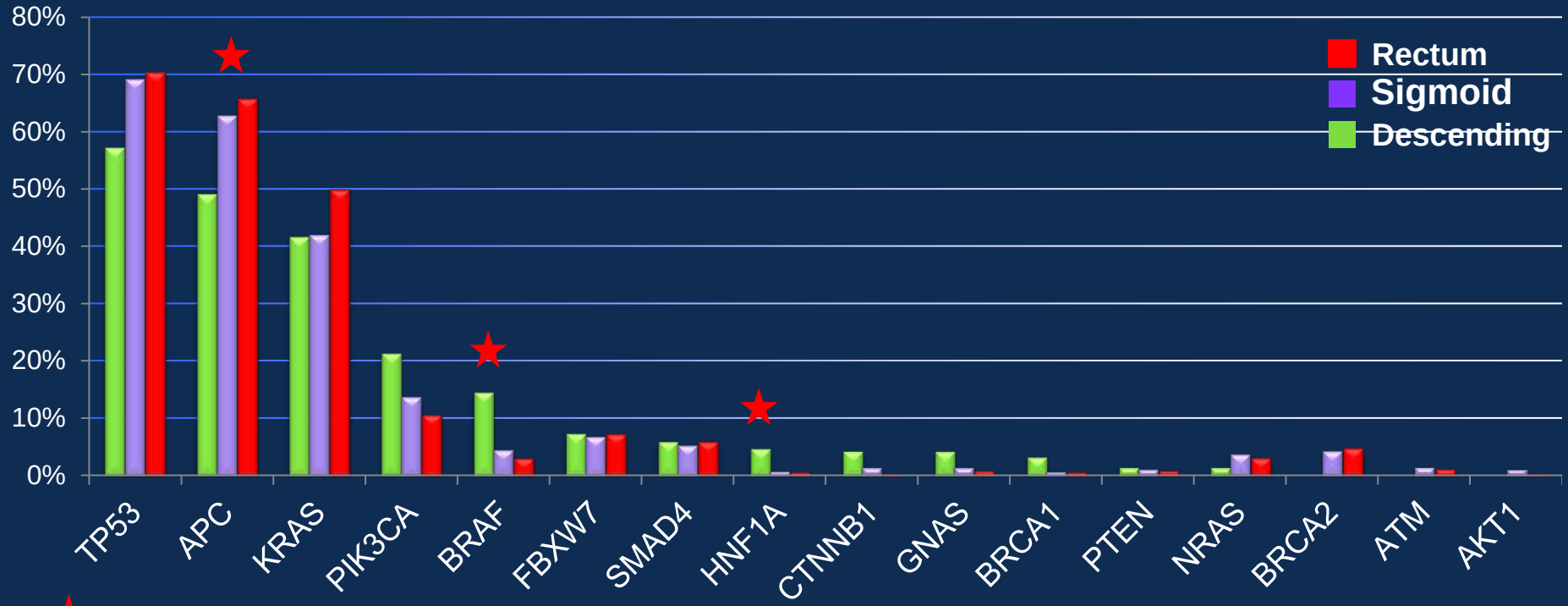
★ indicates a significant difference between rectal and descending colon tumors ($p < 0.05$)

Mutation Frequency Comparison Between Rectal and Sigmoid Colon Tumors



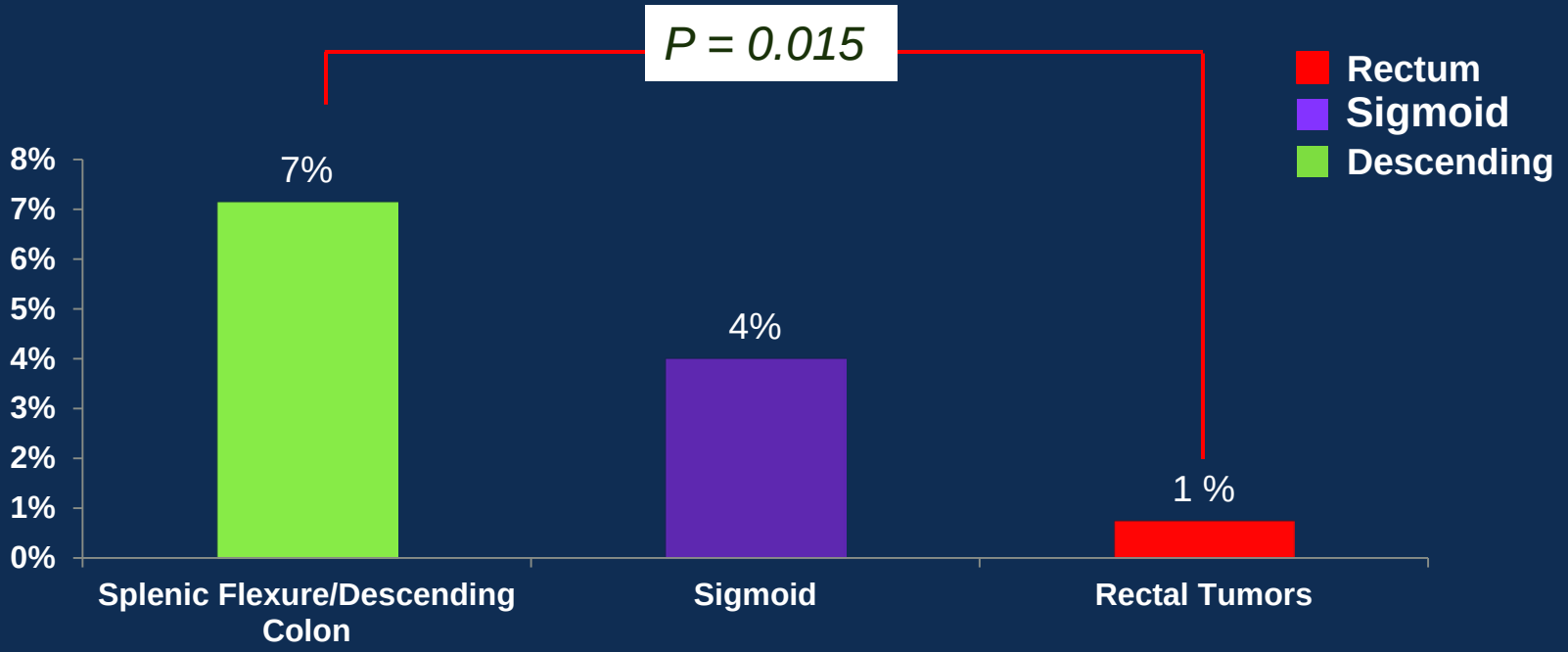
No significant differences were found between rectal and sigmoid colon tumors

Mutation Frequency Comparison Between Sigmoid Colon and Descending Colon Tumors



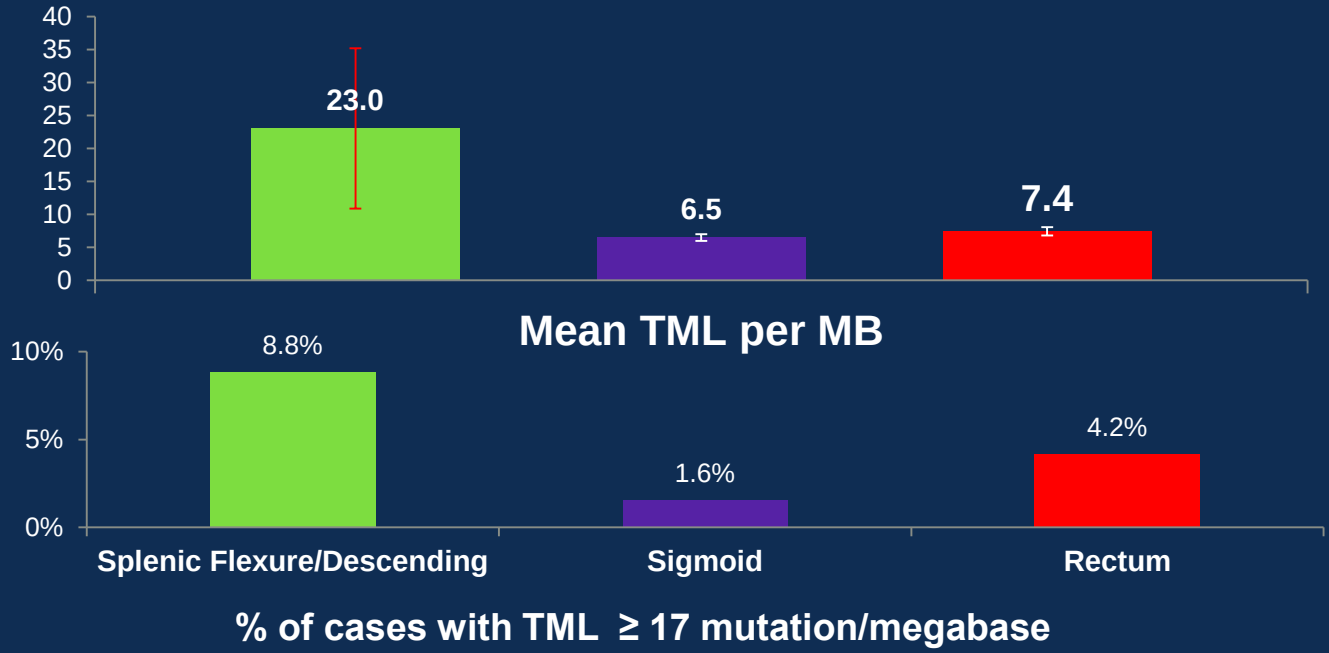
★ indicates a significant difference between Sigmoid Colon and Descending colon tumors ($p < 0.05$)

Frequency of microsatellite instability in left-sided CRC



Microsatellite instability was tested with Microsatellite Instability fragment analysis (Promega)

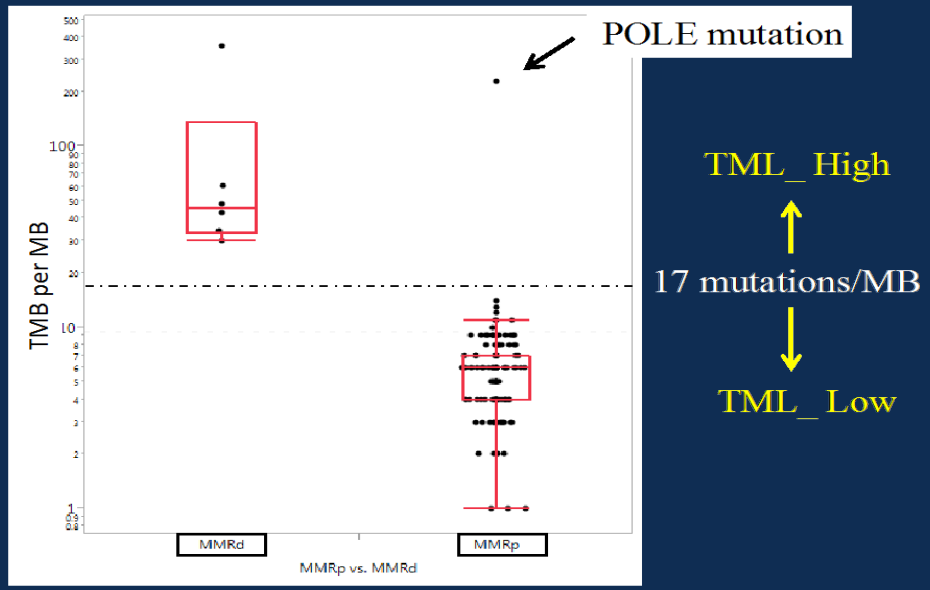
Tumor Mutation Load (TML)



- TML was calculated using only somatic nonsynonymous missense mutations sequenced with a 592-gene panel.
- On a separate cohort of 331 tumors tested with 592-gene panel (both primary tumors and metastasis included). Descending colon, N = 34; Sigmoid colon, N = 129; Rectum, N = 168
- No significant difference was seen between the three cohorts

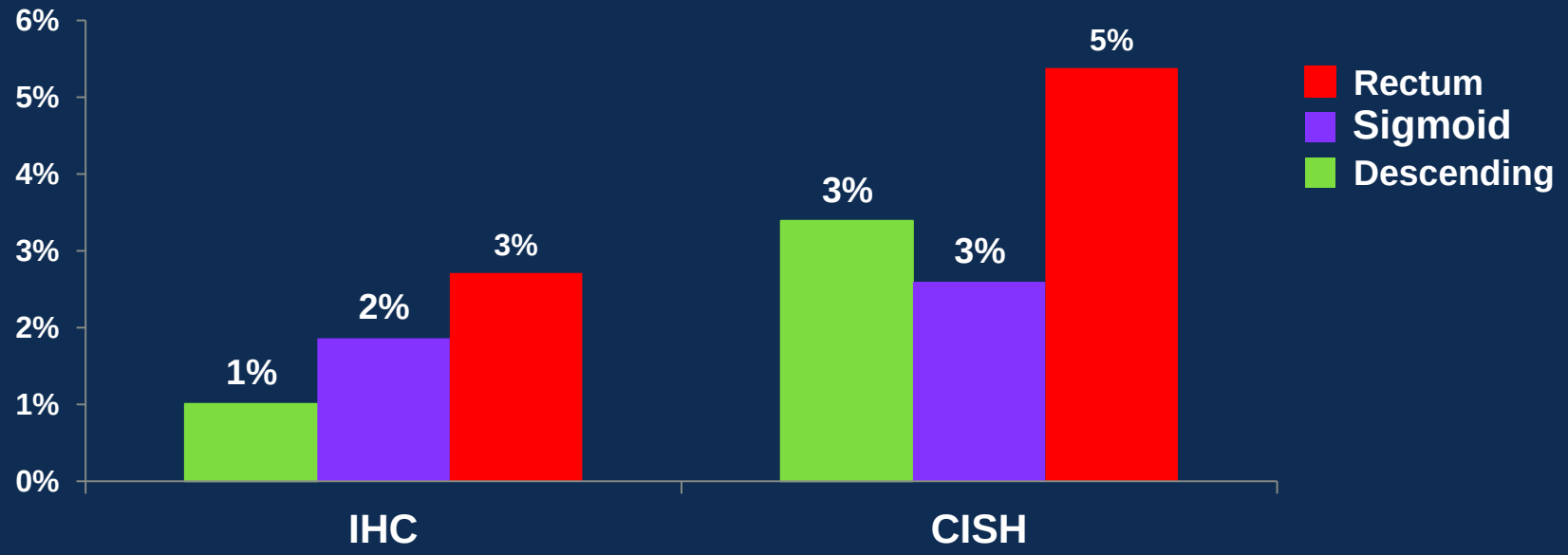
Presented by:

Correlation of MSI with TML



Stadler, et. al., (2016) J of Clin Oncol, 34(18):2141-7
Salem et al. Comparative molecular analyses of left-sided colon, right-sided colon, and rectal cancers. Unpublished data

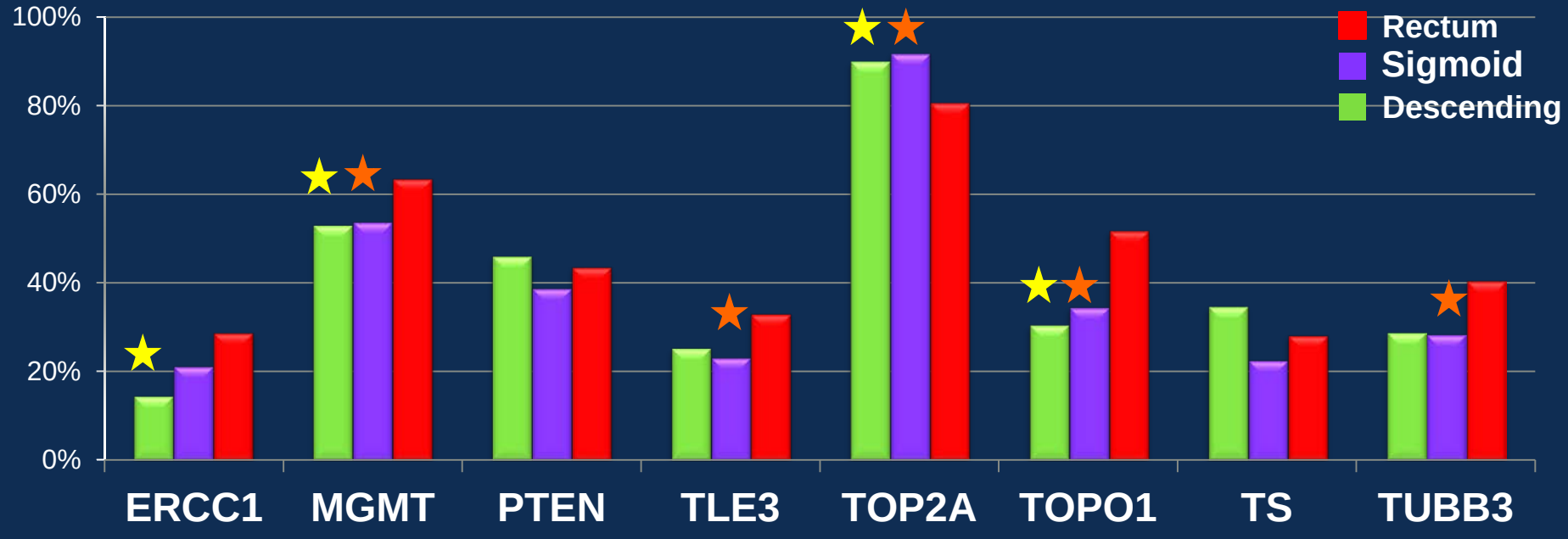
Her2/Neu: Overexpression and Amplification



- There were no significant differences in Her2 overexpression or amplification between rectal, sigmoid colon and descending colon cancers

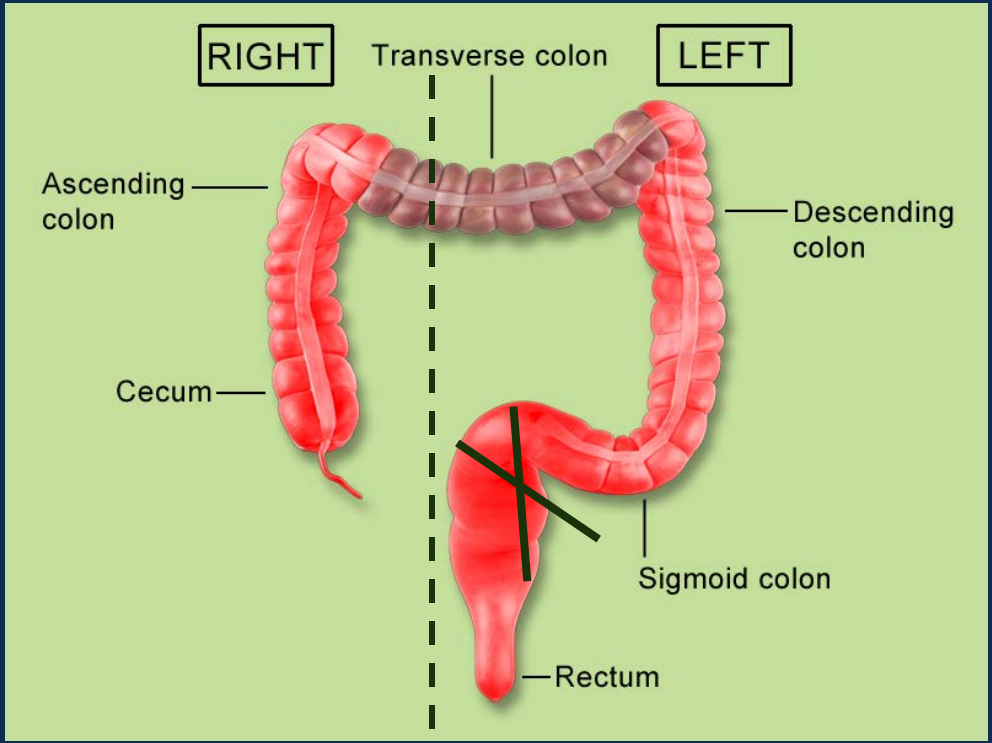
- Threshold for positivity
  - -Her2 IHC: ≥ 3+ and > 10%
  - -Her2 ISH: Her2/Neu:CEP 17 signal ratio of >= 2.0

IHC - Protein Overexpression



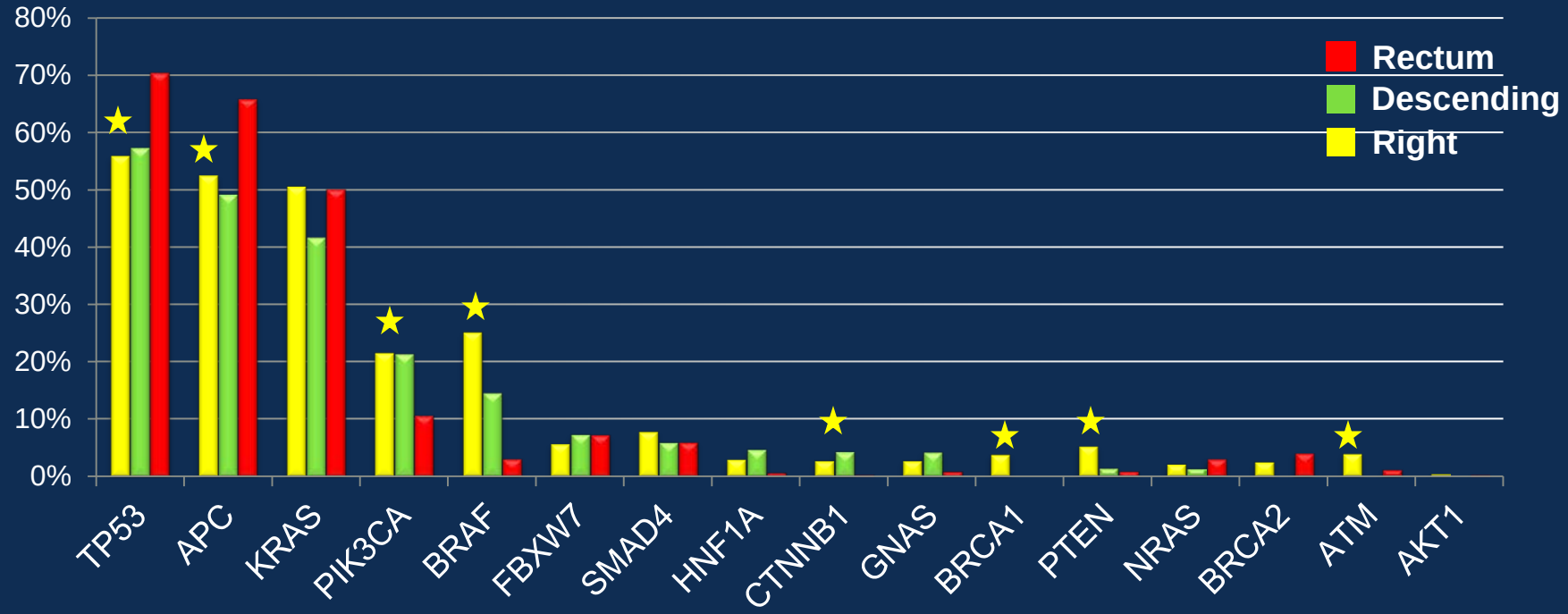
★ indicates a significant difference between rectal and descending colon tumors ($p < 0.05$)
★ indicates a significant difference between rectal and sigmoid colon tumors ($p < 0.05$)

Rectal vs. Descending Colon vs. Right-Sided Colon Cancers



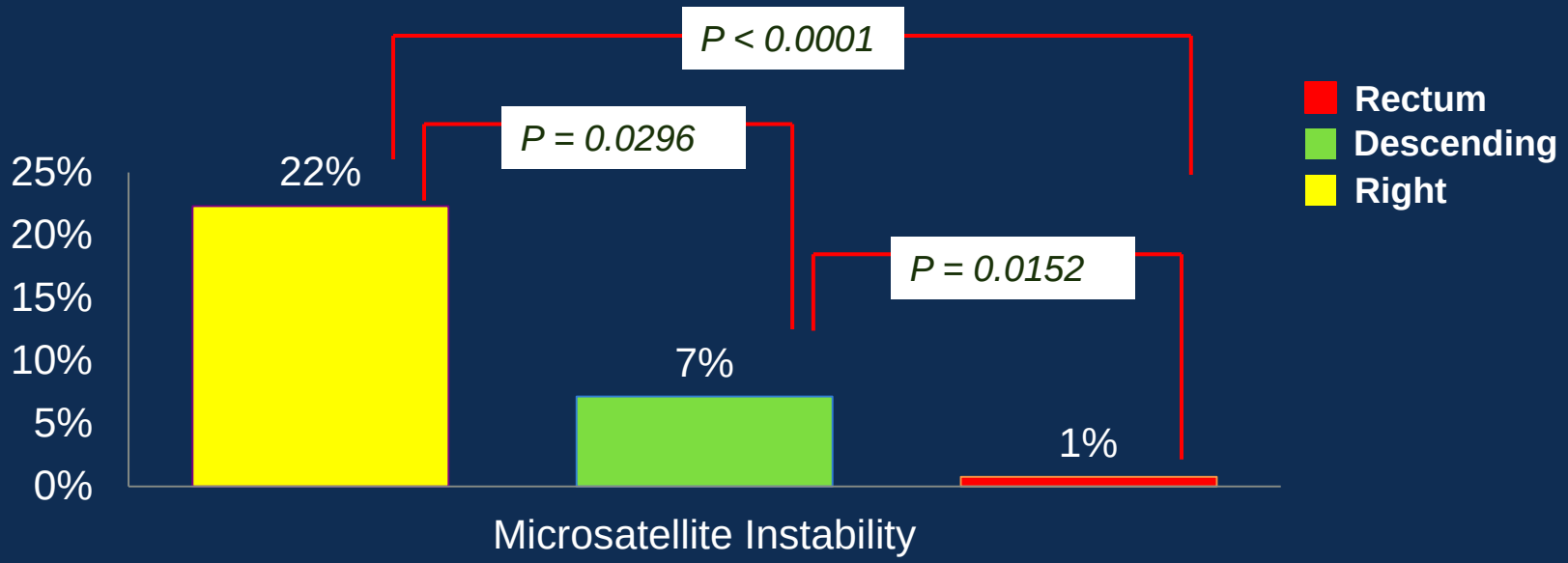
Presented by:

Mutation Frequency Comparison Between Rectal and Right-sided Colon Cancers



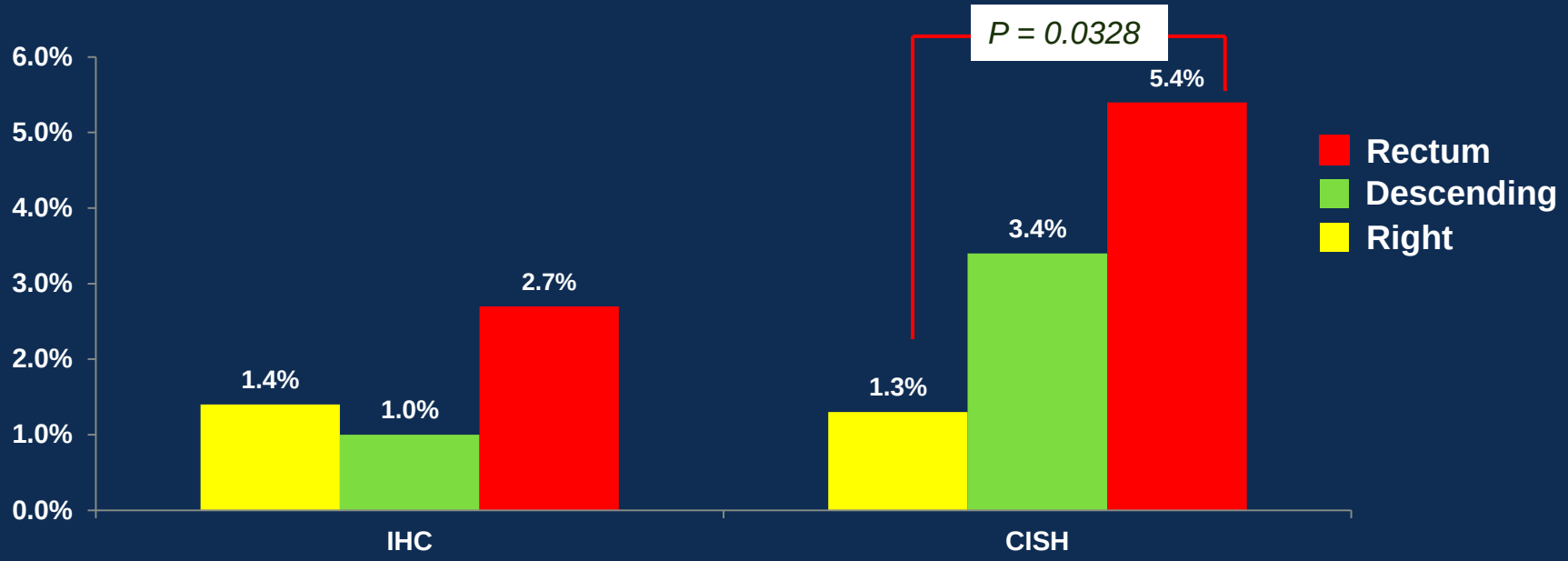
★ Indicates a significant difference between rectal cancers vs. right-sided colon tumors ($p < 0.05$)

Frequency of Microsatellite Instability



		Right Colon (N = 112)	Descending Colon (N = 42)	Rectum (N = 134)
MSI	Deficient	25	3	1
	Proficient	87	39	133

Her2/Neu : Overexpression and Amplification

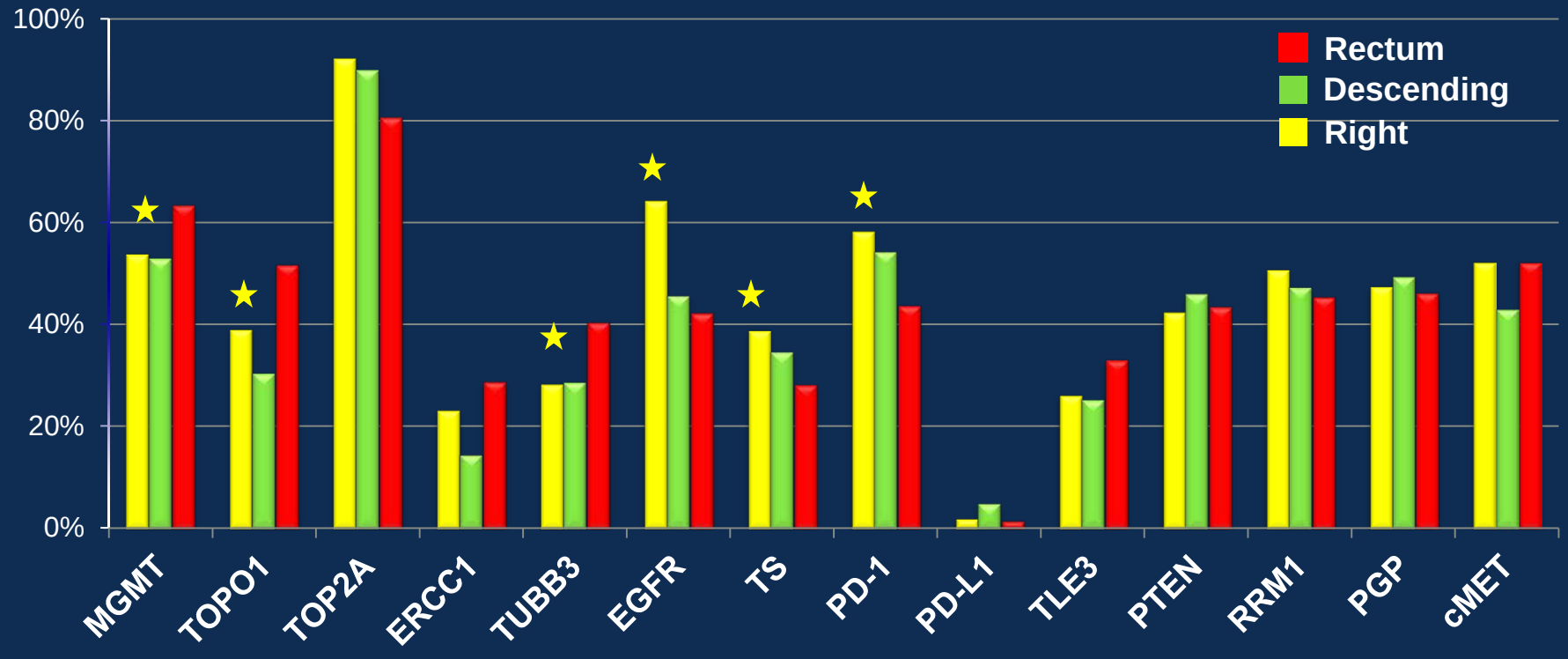


		Right Colon	Descending Colon	Rectum
IHC-Her2/Neu	Positive N	3	1	16
	Negative N	218	98	574
	Total N	221	99	590
p value	Left vs. Right	ns		
	Left vs. Rectum	ns		
	Right vs. Rectum	ns		

		Right Colon	Descending Colon	Rectum
CISH-Her2/Neu	Positive N	2	2	15
	Negative N	156	57	264
	Total N	158	59	279
p value	Left vs. Right	ns		
	Left vs. Rectum	ns		
	Right vs. Rectum	0.0328		

Presented by:

IHC - Protein Overexpression



★ indicates a significant difference between right-sided colon vs. rectal tumors ($p < 0.05$)

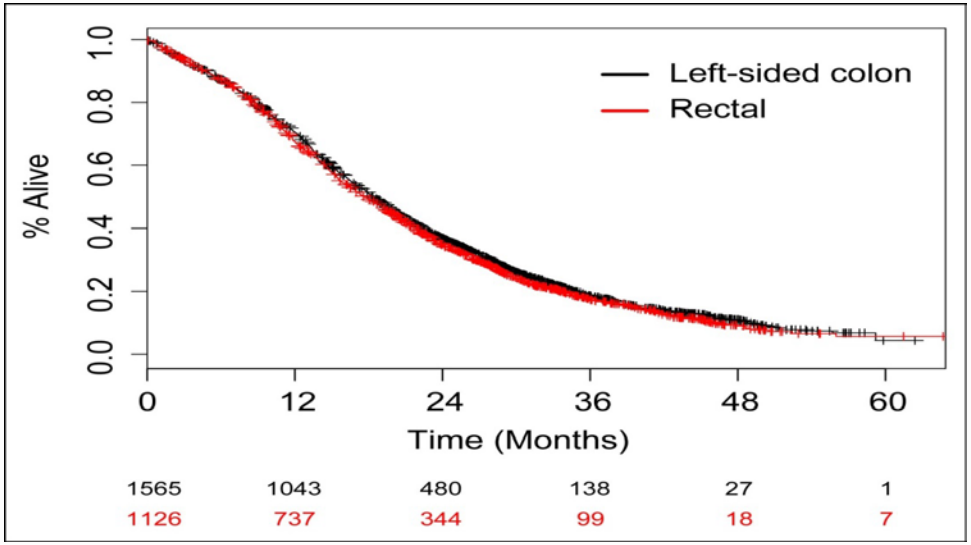
Presented by:

Limitations

- This was a retrospective analysis
- Potential effects of treatments including chemoradiation are unknown
- Limited clinical information was available for analyzed tumors
- A large number of samples were excluded due to a lack of definitive tumor location information

Primary Site Effects (left colon vs. rectal)

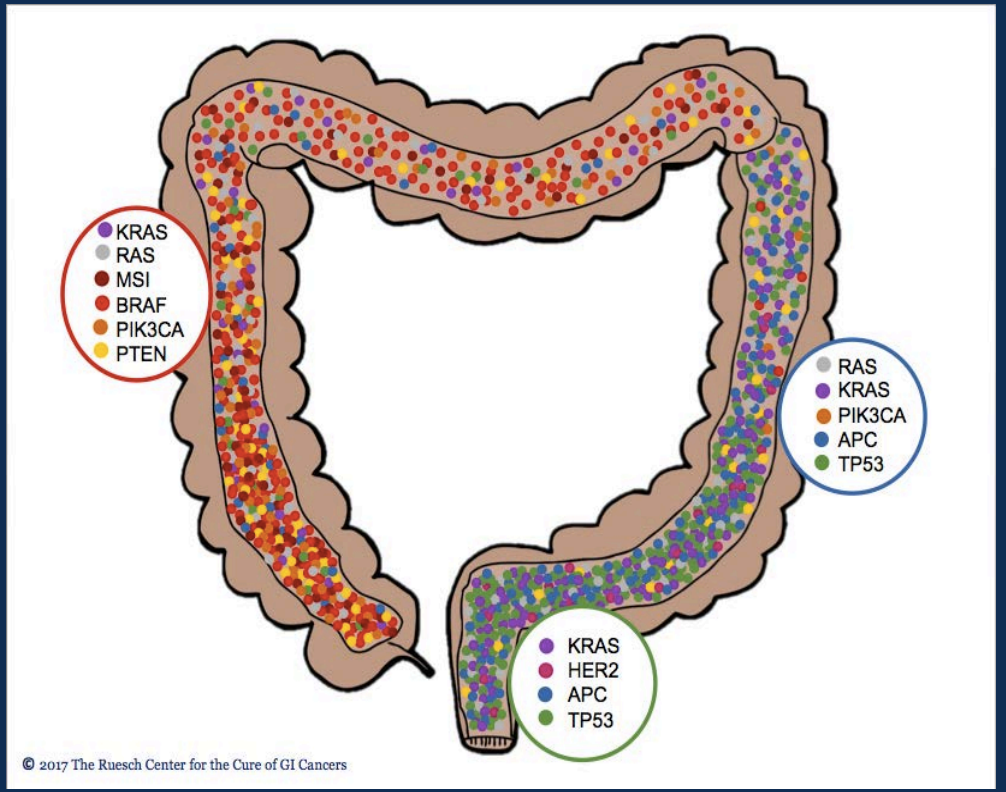
Figure 1. Overall survival among all pts



OS of all pts. Left-sided colon pts had similar OS as rectal pts
Median OS 18.7 mos left-sided colon versus 18.1 mos rectal
Adjusted HR 1.02 (0.95-1.10) p=0.559

Conclusions

- CRCs carry a continuum of molecular alterations from right to left, rather than having a sharp, clear-cut distinction



Conclusions

- Rectal cancers have molecular features that are different from left-sided colon tumors
- Clinical trials should stratify patients based on the location of the primary tumor (right vs. left) as well as molecular features
- Better understanding of disease biology may help to identify therapeutic targets and advance precision medicine

THANK YOU