Personalized Medicine in Colorectal Cancer: Molecular Classifications and Biomarkers

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Overview

Single marker molecular subtyping	RNA-based molecular subtyping	Immune subtyping
• KRAS/NRAS • BRAF • MSI-H • HER2 amplification • Fusions	• Consensus molecular subtypes • Intrinsic subtyping	• Immune quantification • Tumor mutation burden

Overview

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MSI-H

Prevalence:

Stage	MSI-H
II	22%
III	12%
IV	3.5%

Enrichment:

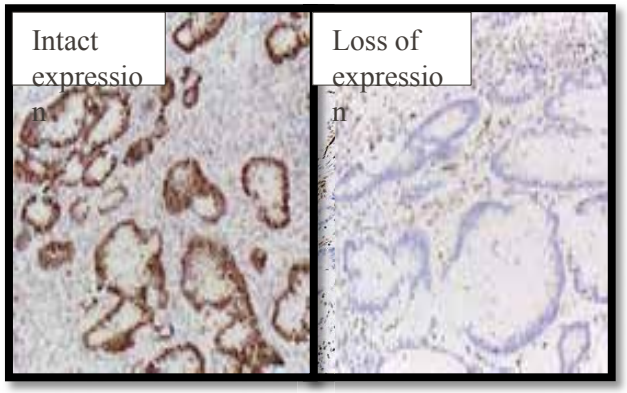
Right sided, bimodal age distribution

Recommendation:

Test all CRC patients of any stage

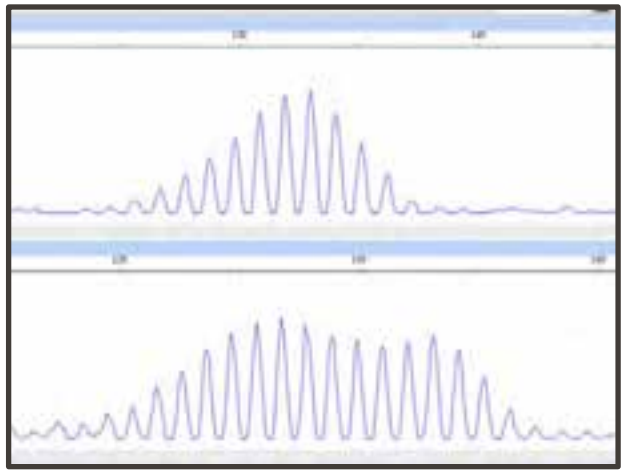
Immunohistochemistry

Complete loss of expression in one of the MMR proteins = MSI-high



Polymerase Chain Reaction

Panel of 5 or more microsatellites with allelic shift in 2 (>30%) or more markers = MSI-high

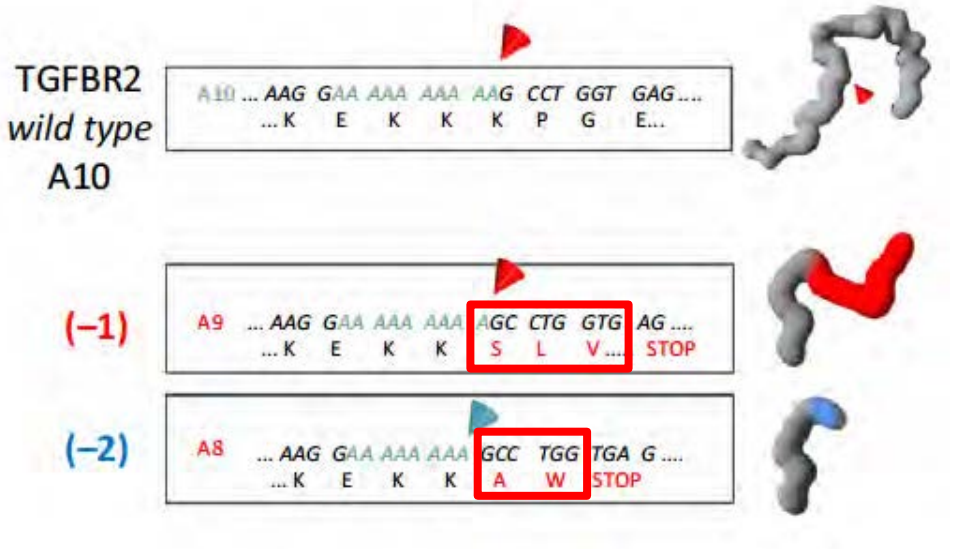


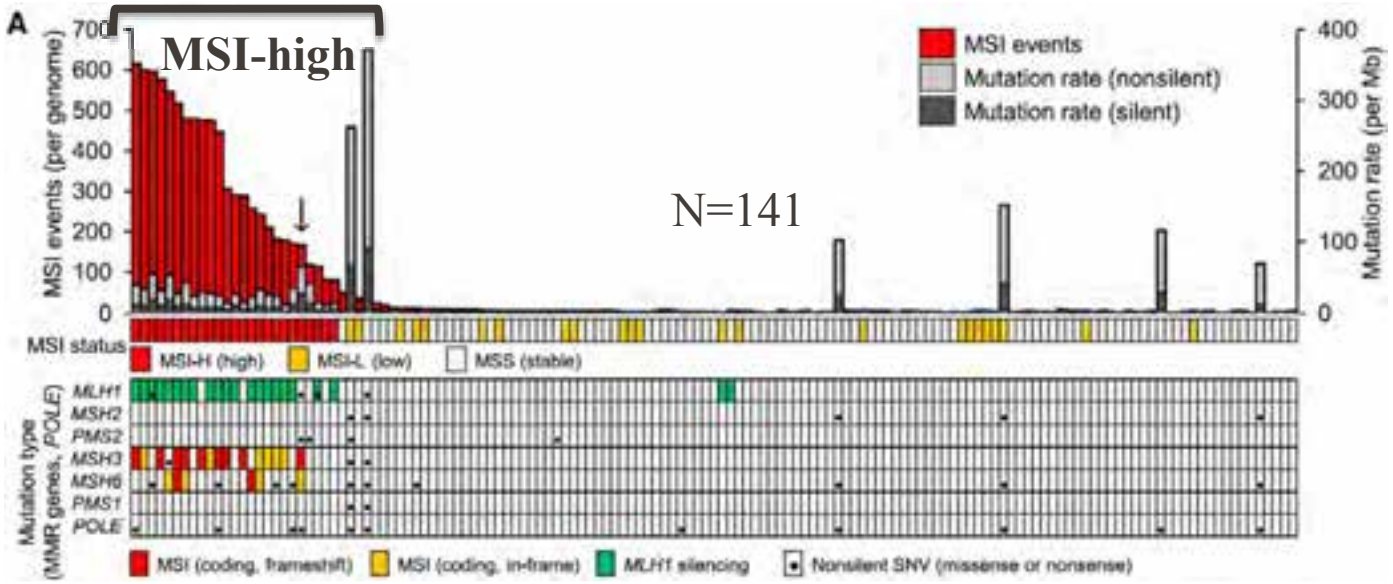
Tejpar et al BJC ‘09; Hall et al ASCO 2016 and GI ASCO 2016; Le Science 2017

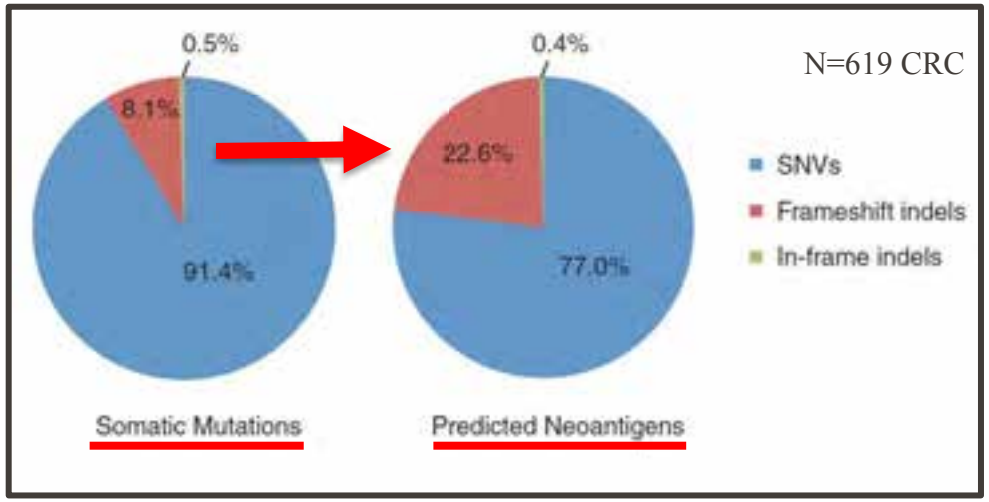
dMMR or MSI-H CRC: Frameshift Neoantigens

Tumor Antigens:

1. Differentiation (melanocyte differentiation antigens…)
2. Overexpressed (HER-2…)
3. Viral (HPV proteins…)
4. Cancer/testis (MAGE, NY-ESO-1…)
5. **Mutational (p53…)**





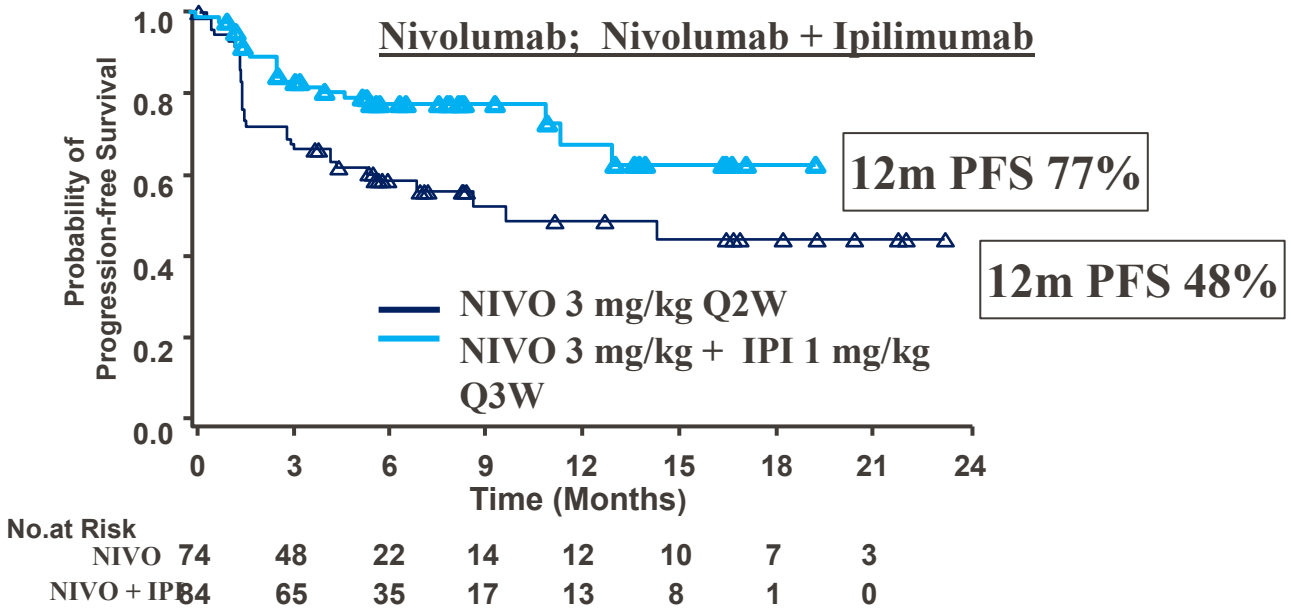


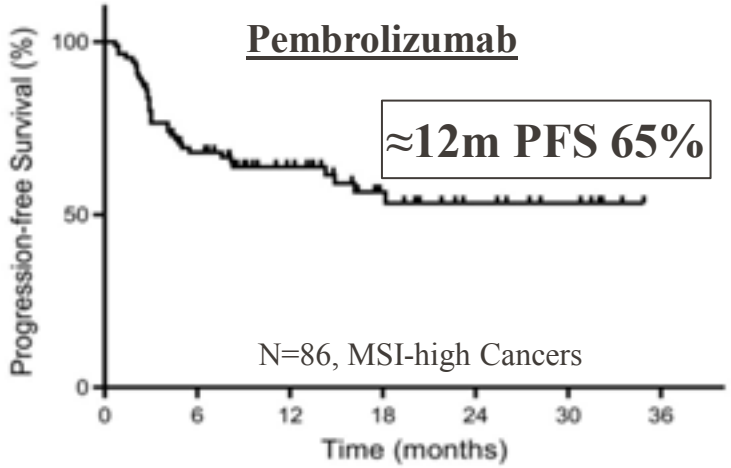
Slide from Michael Overman

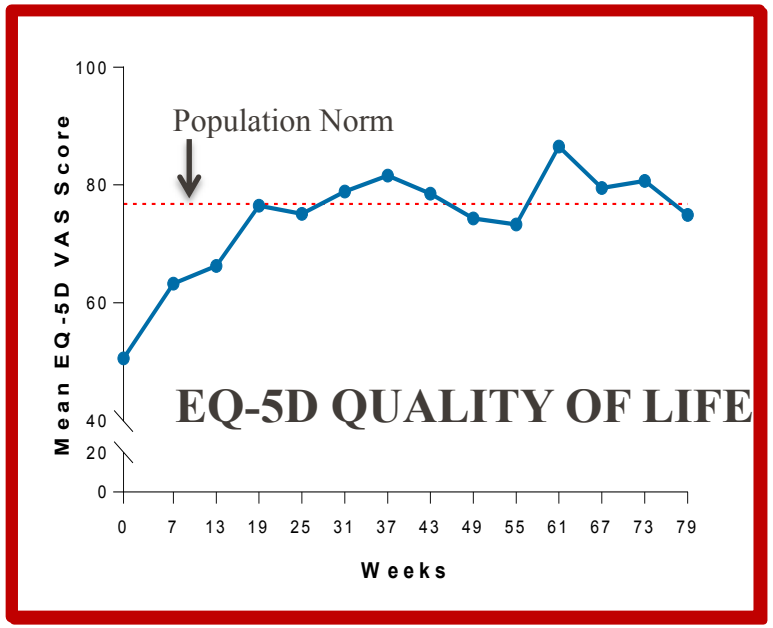
THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Giannakis et al. Cell Report 2016; Kloor et al. Trends in Cancer 2016; Chalmers et al. Genomic Medicine 2017; 2013 Kim et al. Cell

Durability of anti-PD1 +/- anti-CTLA-4 in dMMR







Pembrolizumab (mandatory stop at 2 years)

18 pts (11 with CR and 7 with residual disease)

Median time off tx is 8 months

None have recurred

Slide from Michael Overman

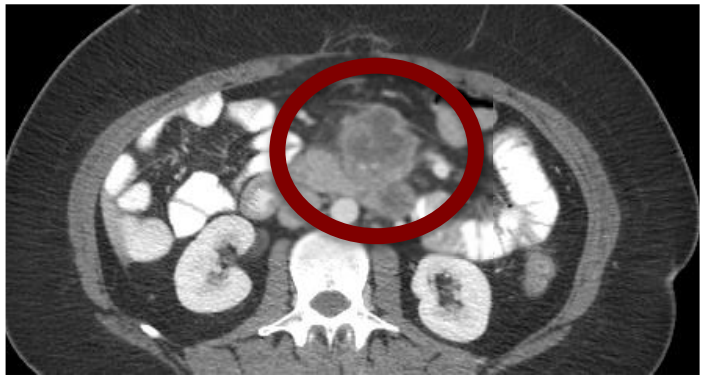
THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Overman Lancet Onc 2017; Overman JCO 2018; Lee et al. Science 2017

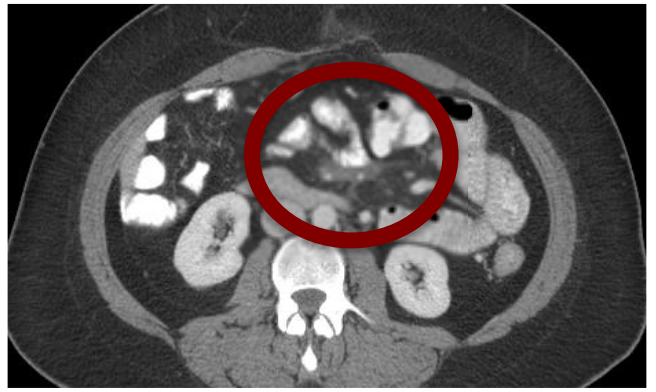
Locally Advanced/Recurrent dMMR CRC: Pathological Complete Response from anti-PD1

CASE 1

- Locally recurrent treated with irinotecan/cetuximab and then capox/ panitumumab
- Then pembrolizumab x 4 cycles

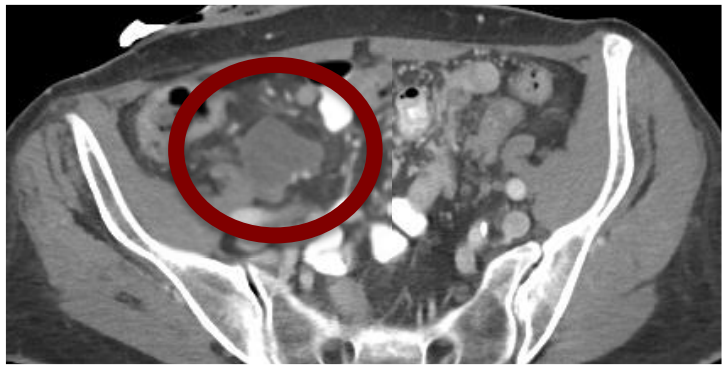


Pembro x4

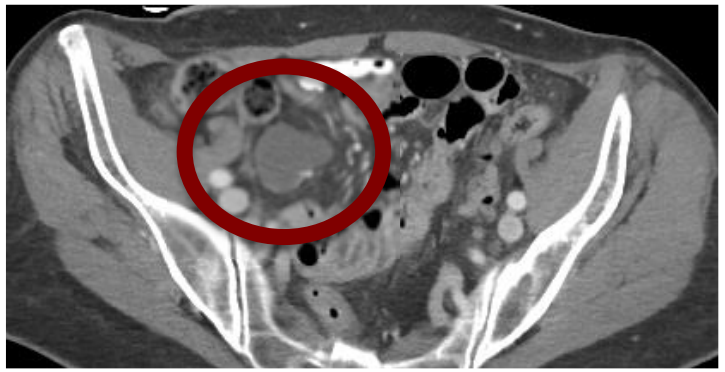


CASE 2

- Locally advanced treated with FOLFOX with progression
- Then Nivolumab x 6 cycles



Nivo x 6



Slide from Michael Overman

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BRAF Mutations

Prevalence:

BRAF V600E : 4-6%

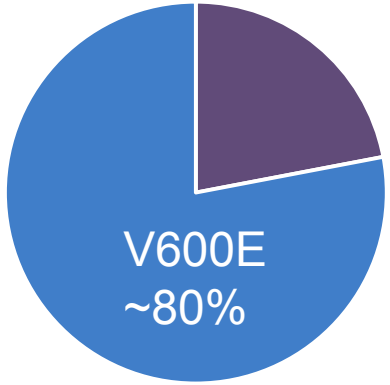
Atypical BRAF : 2%

Enrichment:

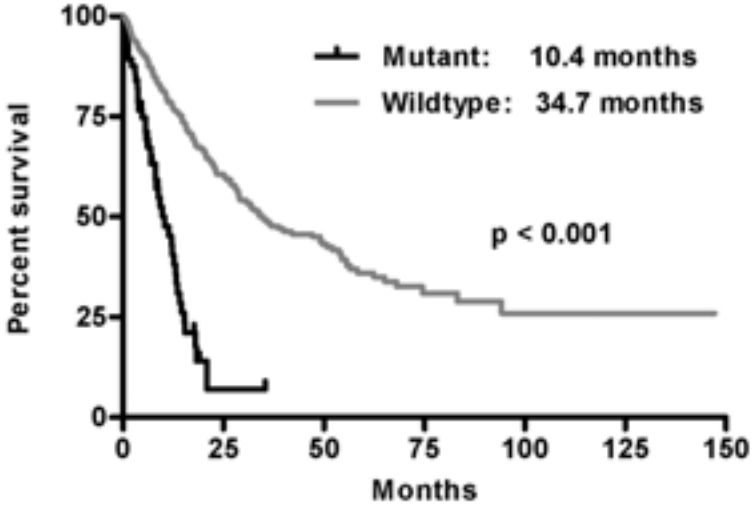
Right sided, older age

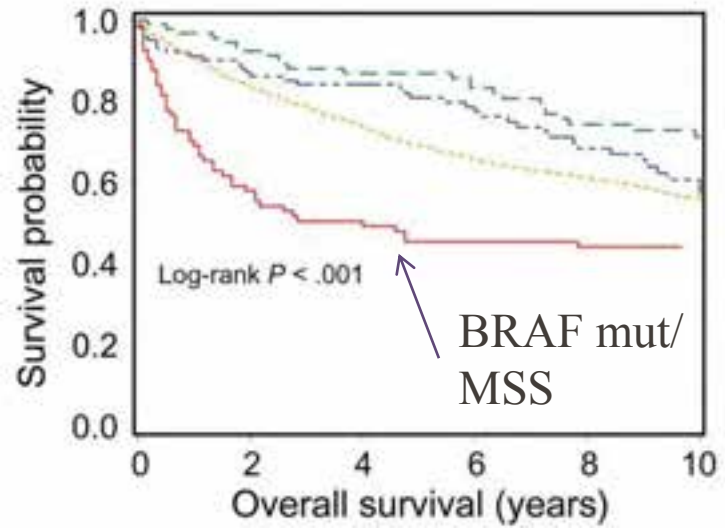
Recommendation:

Test all mCRC patients



Poor prognosis of BRAF V600E

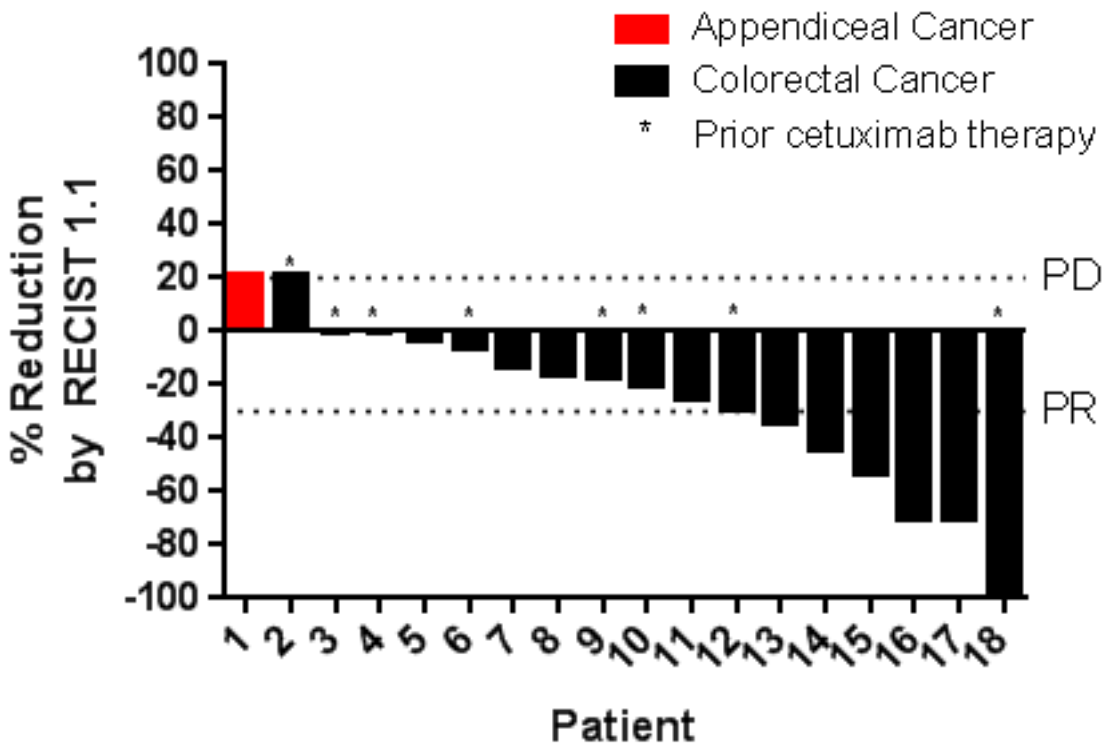




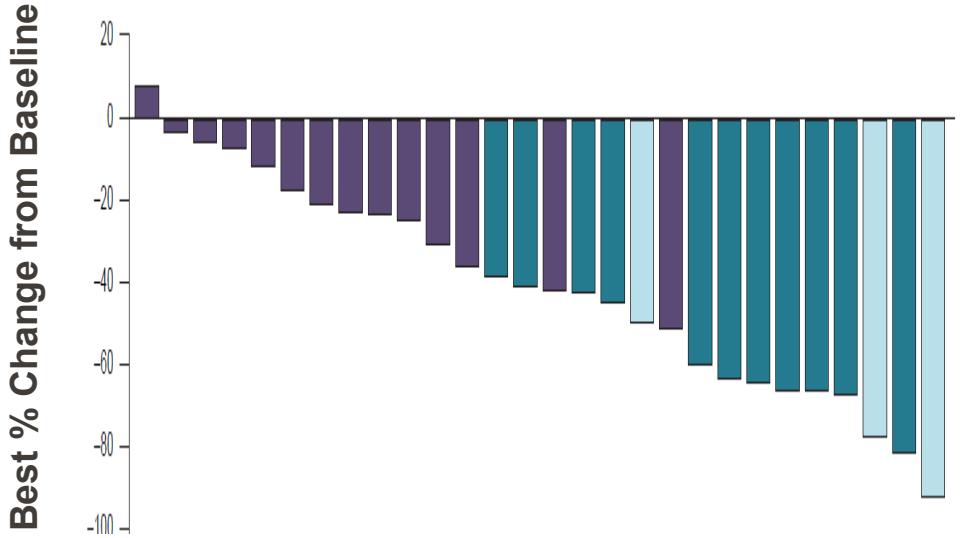
Jones et al JCO ‘17; Phipps et al Gastroenterology ‘15; Lockhead et al JNCI ’13; Sinicrope ASCO ’14; Tran, et al, Cancer ‘11

BRAF V600E: Impact on Treatment Options

Vemurafenib, Irinotecan, Cetuximab



Binimetinib + Encorafenib + Cetuximab



Kopetz et al GI ASCO ‘18; Kopetz et al GI ASCO ‘19

BEACON Phase 3: Study Design

Results of Safety Lead-In led to the introduction of an additional primary endpoint of ORR and an interim OS analysis to allow for early assessment

Patients with *BRAF*^{V600E} mCRC with disease progression after 1 or 2 prior regimens; ECOG PS of 0 or 1; and no prior treatment with any RAF inhibitor, MEK inhibitor, or EGFR inhibitor

Safety Lead-in

ENCO + BINI + CETUX
N = 30

Encorafenib 300 mg PO daily
Binimetinib 45 mg PO bid
Cetuximab standard weekly dosing

A separate Safety Lead-in cohort of n=7 in Japan was enrolled subsequently. Results will be reported at a later time.

Phase 3

R
1:1:1

- **Triplet therapy**
  ENCO + BINI + CETUX
  n = 205
- **Doublet therapy**
  ENCO + CETUX
  n = 205
- **Control arm**
  FOLFIRI + CETUX, or
  irinotecan + CETUX
  n = 205

Overall Survival

Primary Endpoints:

Triplet vs Control

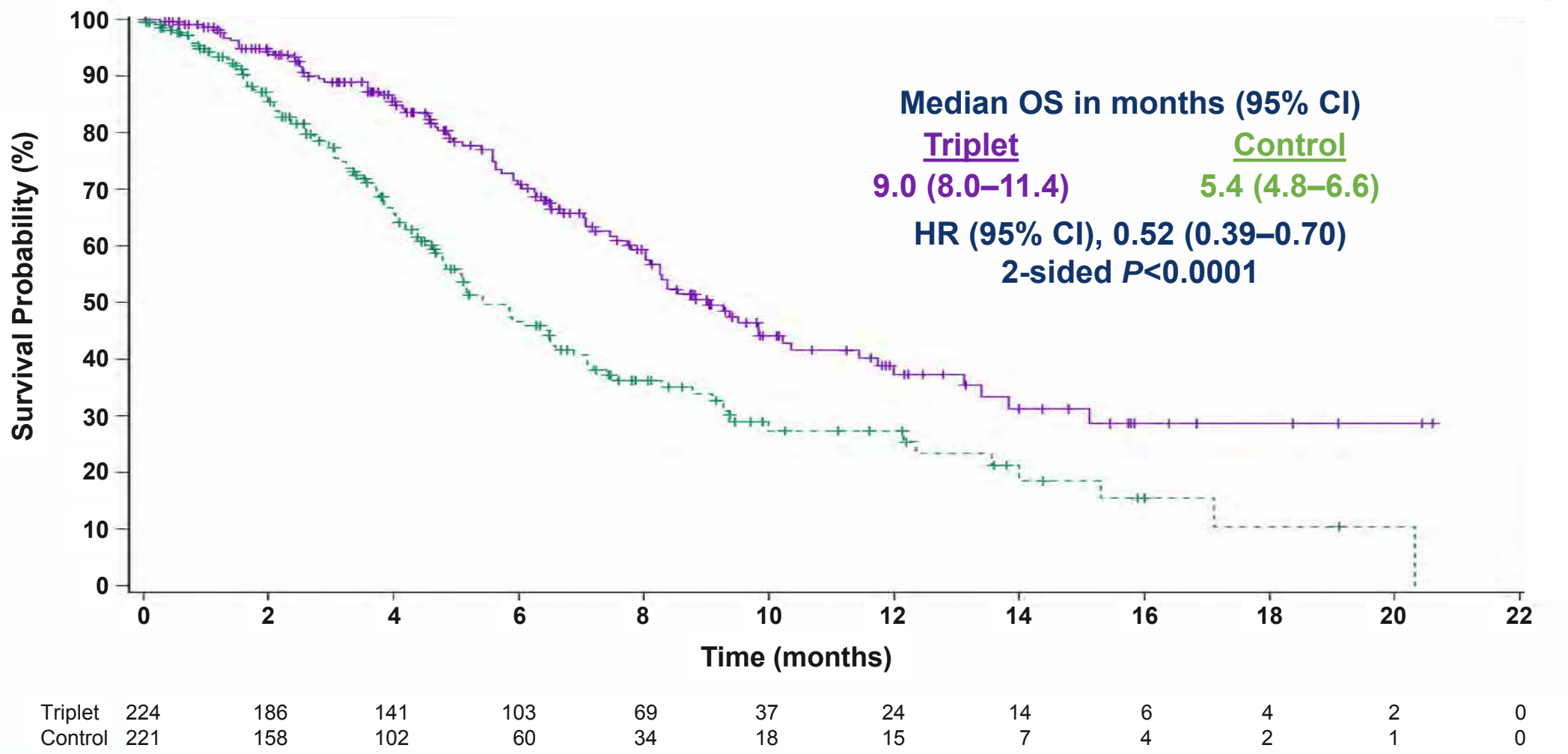
OS

ORR
(Blinded Central Review)

Secondary Endpoints: Doublet vs Control OS & ORR, PFS, Safety

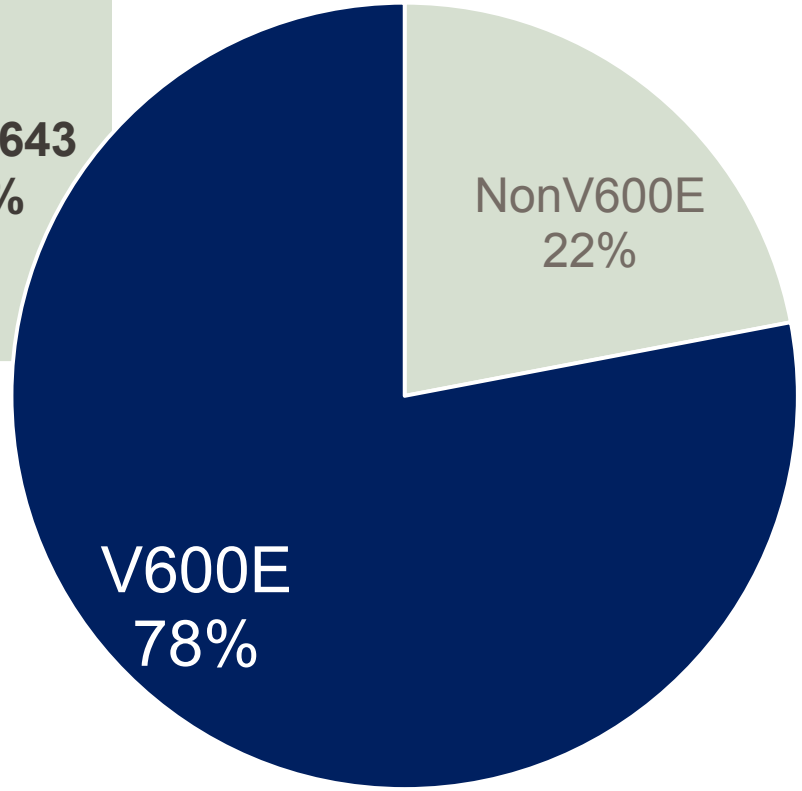
Randomization was stratified by ECOG PS (0 vs. 1), prior use of irinotecan (yes vs. no), and cetuximab source (US-licensed vs. EU-approved).

Primary Endpoint - Overall Survival: Triplet vs Control
(all randomized patients)



Prevalence of Non-V600E BRAF mutations in CRC

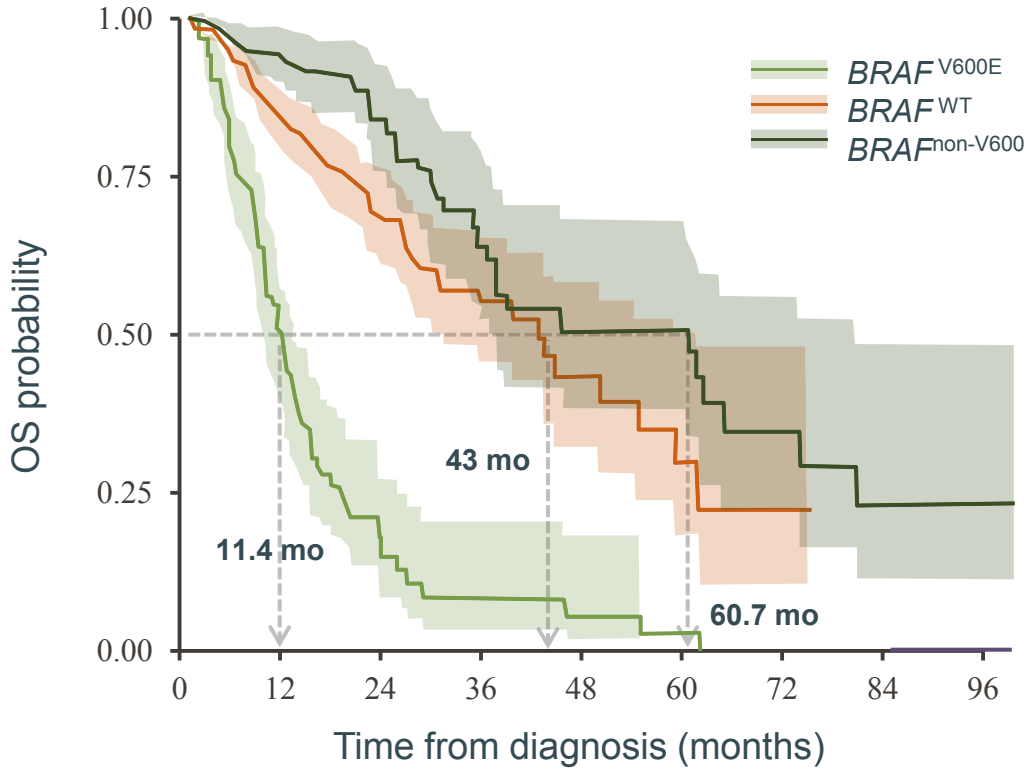
	MC	MDA	FM	Totals	All BRAF mut %	% of all BRAF mut which are non-V600	% of total CRC which are non-V600
Total CRC Cases	1014	2276	6353	9643	**1147/9643 11.9%**	**207/940 22%**	**207/9643 2.1%**
Total BRAF Mutations	137	334	469	940			
Non-V600 BRAF	27	54	126	207			



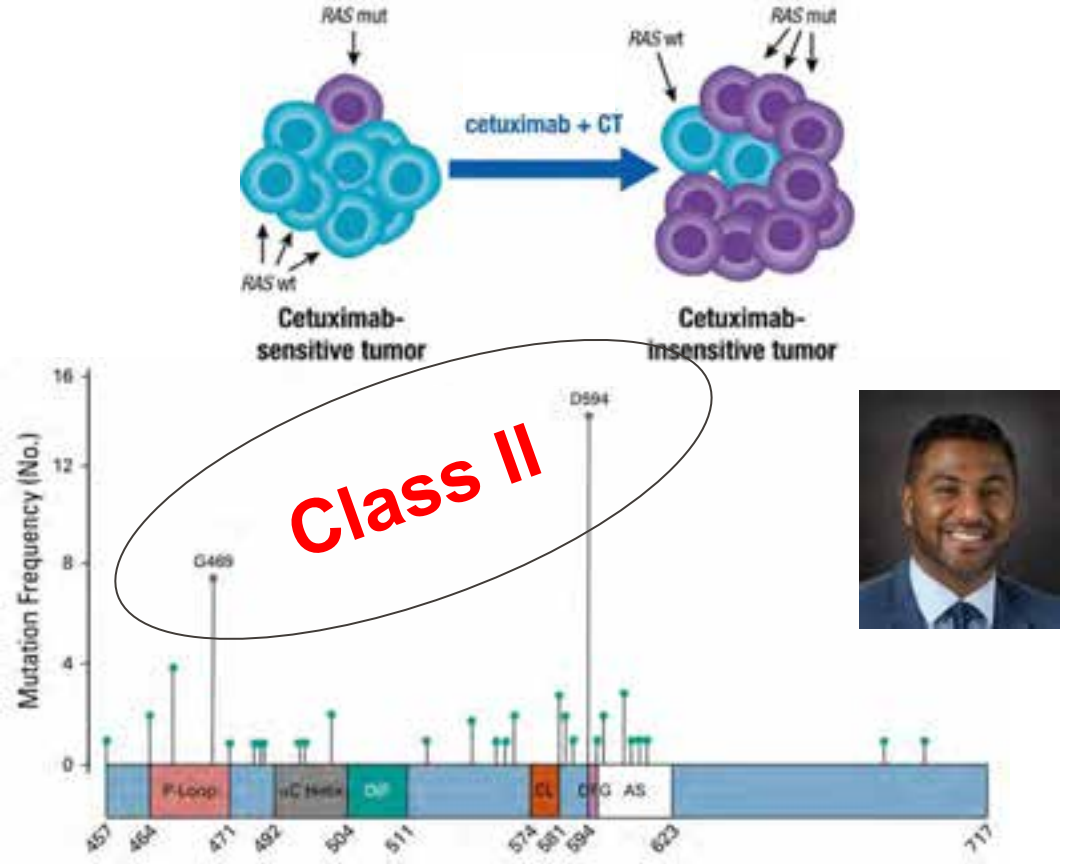
Jones et al., JCO ‘16

Atypical (Non-V600E) BRAF mutations

Prognosis is similar to BRAF wild-type



Recently identified as acquired alterations in post-EGFR inhibitor treated tumors

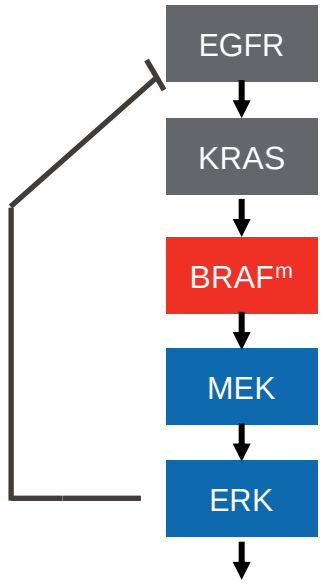


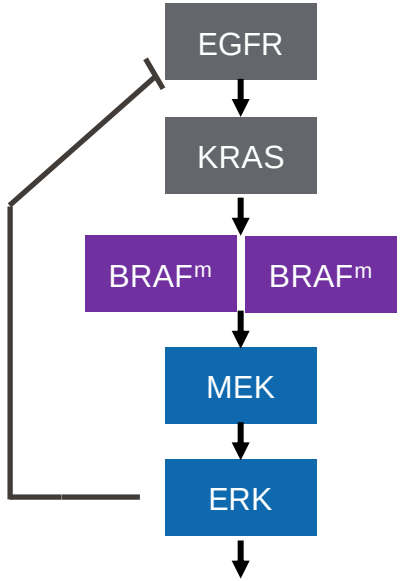
Jones JC, et al. J Clin Oncol 2017;35:2624–30.

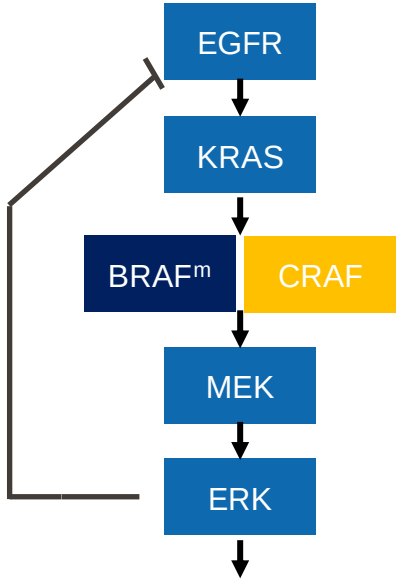
Johnson et al JCO PO ‘19

Understanding Class II and Class III Non-V600E *BRAF*^{mut}

	BRAF V600E Class I	Class II BRAF	Class III BRAF
Structure	BRAF monomer	BRAF dimers	BRAF/CRAF dimers
RTK (EGFR) Dependency	No	No	Yes
Kinase activity	High	High/Intermediate	Low
EGFRi sensitivity	No	Unlikely	Likely
Potential Strategy	BRAF, MEK, EGFR	RAF dimer inhibitors	RTK, MAPK combinations







Yao et al Nature '17

HER2 Amplification

Prevalence: 2-4%

Enrichment:

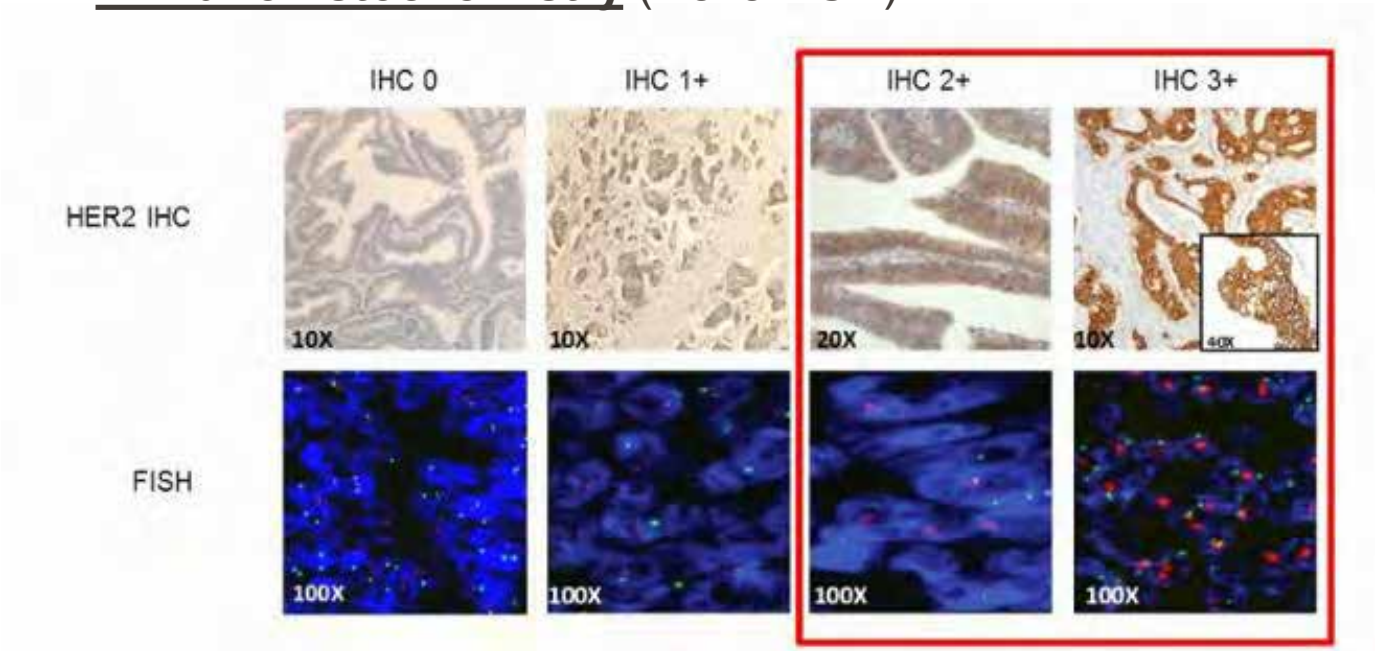
RAS/BRAF wild-type patients

Recommendation:

Consider testing all mCRC patients

*Not yet universally recommended on biomarker guidelines

Immunohistochemistry (Reflex ISH)



NGS Panels

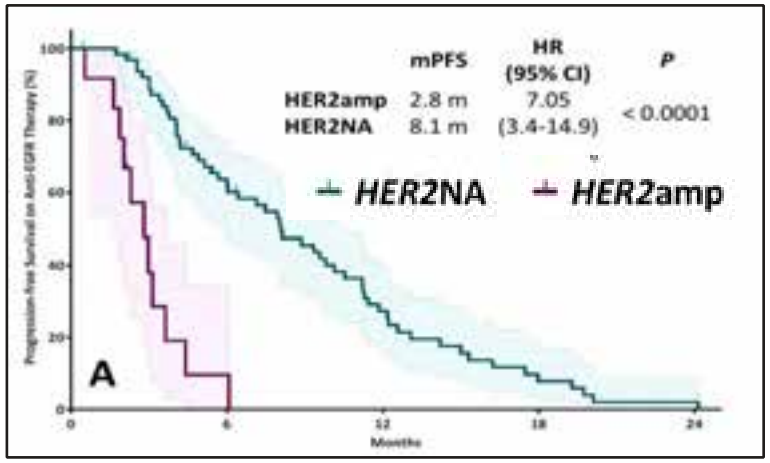
High concordance between NGS testing and IHC/FISH results

ctDNA testing can reliably detect and quantify amplifications (Raghav et al GI ASCO Poster #604)

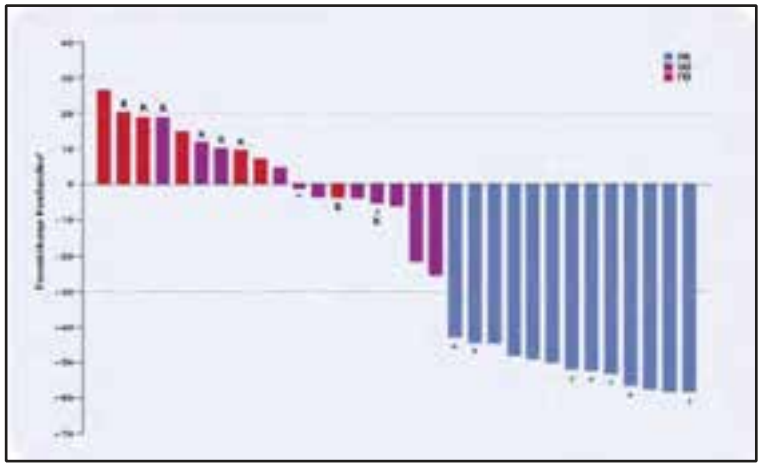
Marx et al.Human Path ’10; Siena et al GI ASCO ‘14

HER2 Amplifications: Potential predictive information

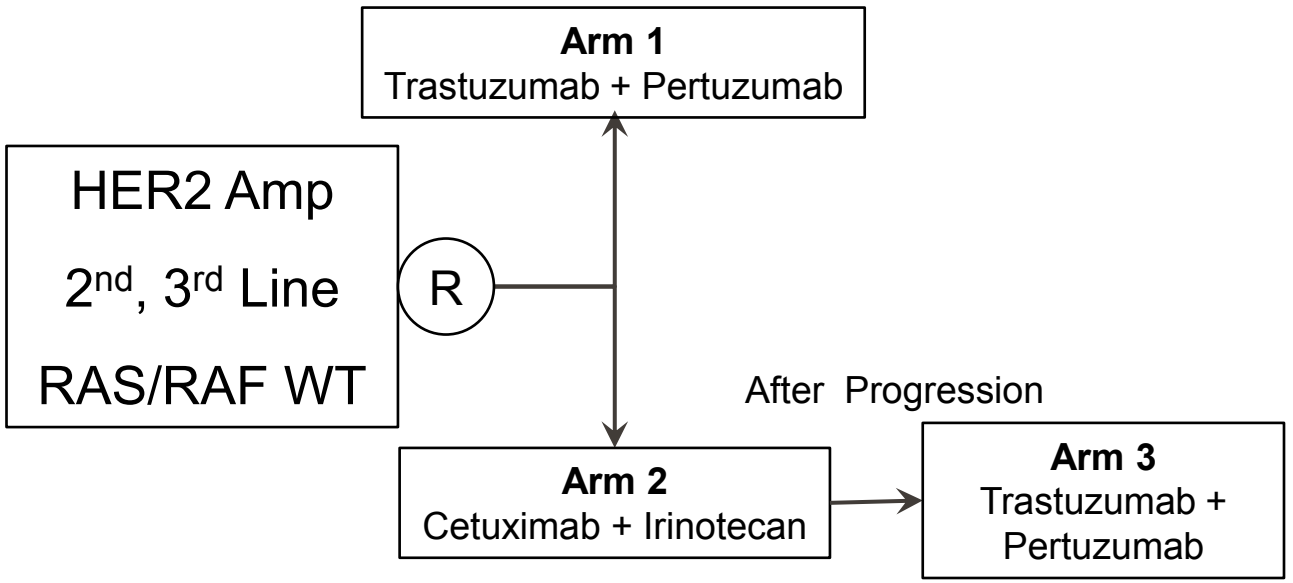
EGFR Inhibition



Trastuzumab + Pertuzumab



SWOG 1613



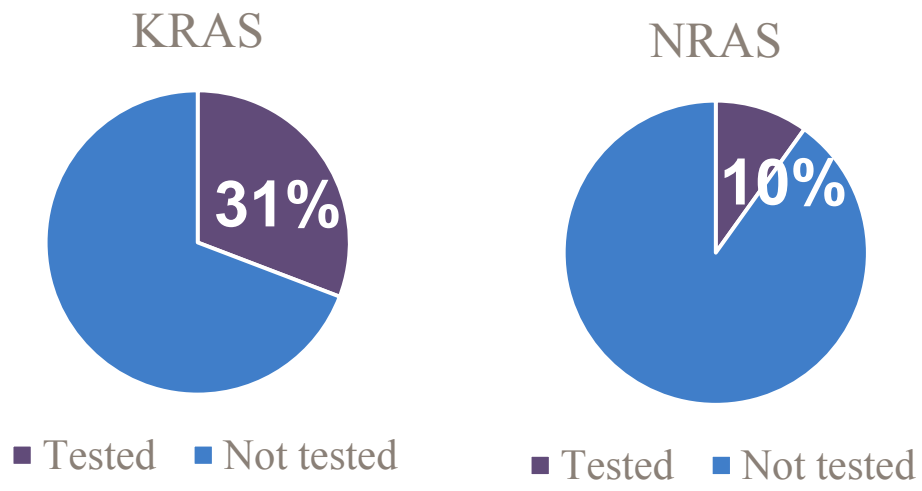
Raghav et al JCO PO, '18; Hurwitz GI ASCO '17;
Raghav, Fakih PI's NCT03365882

KRAS/NRAS testing: Barriers in dissemination of best-practices

Codons 12, 13, 59, 61, 117, 146

Low rate of initial biomarker testing

Flat Iron Health: 13,437 patients with mCRC from 2013 to 2017, testing with 1st line therapy



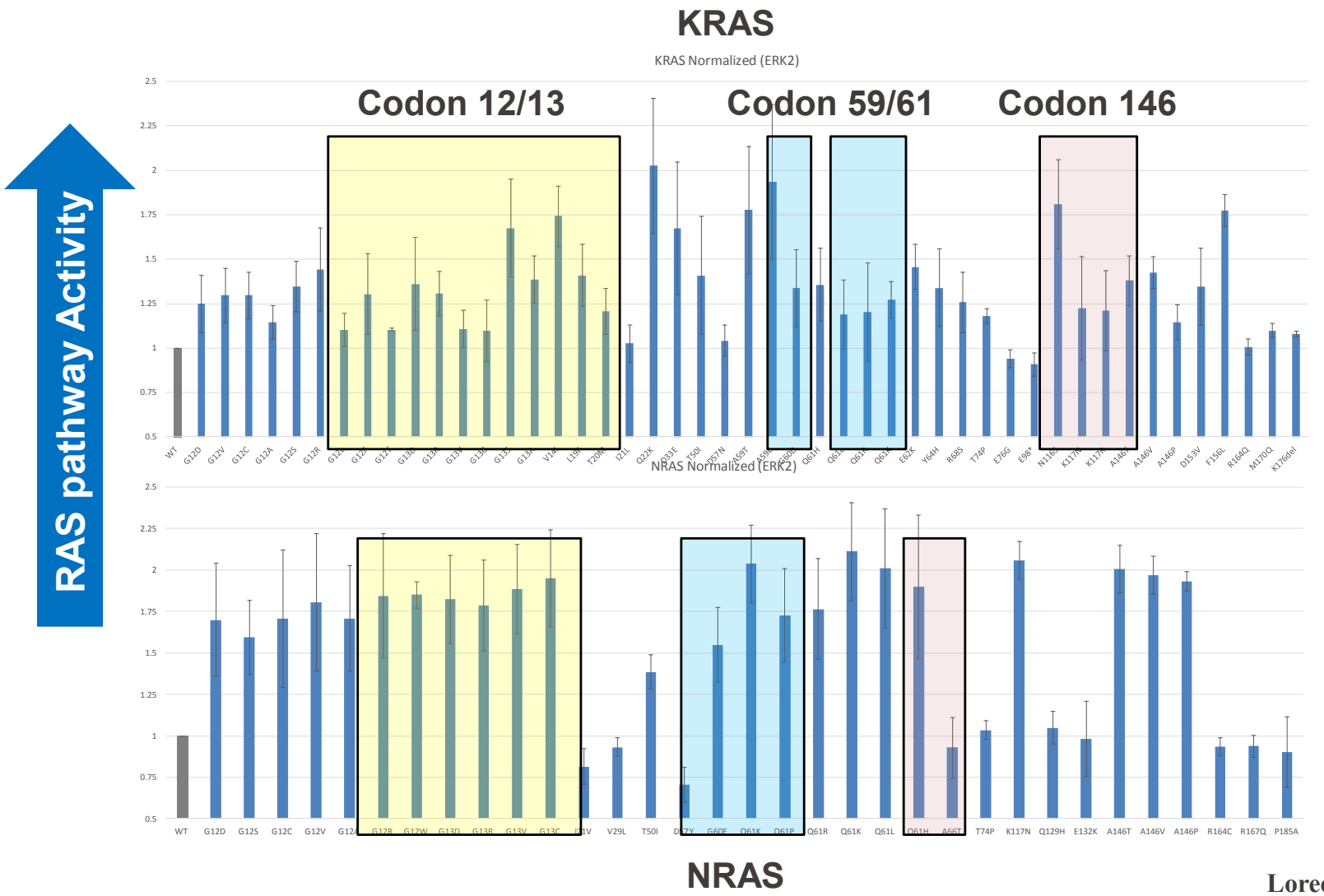
Median time to obtain testing results: 26 days

Need for education/awareness

The best biomarker is one that is actually tested

Florea et al GI ASCO '18

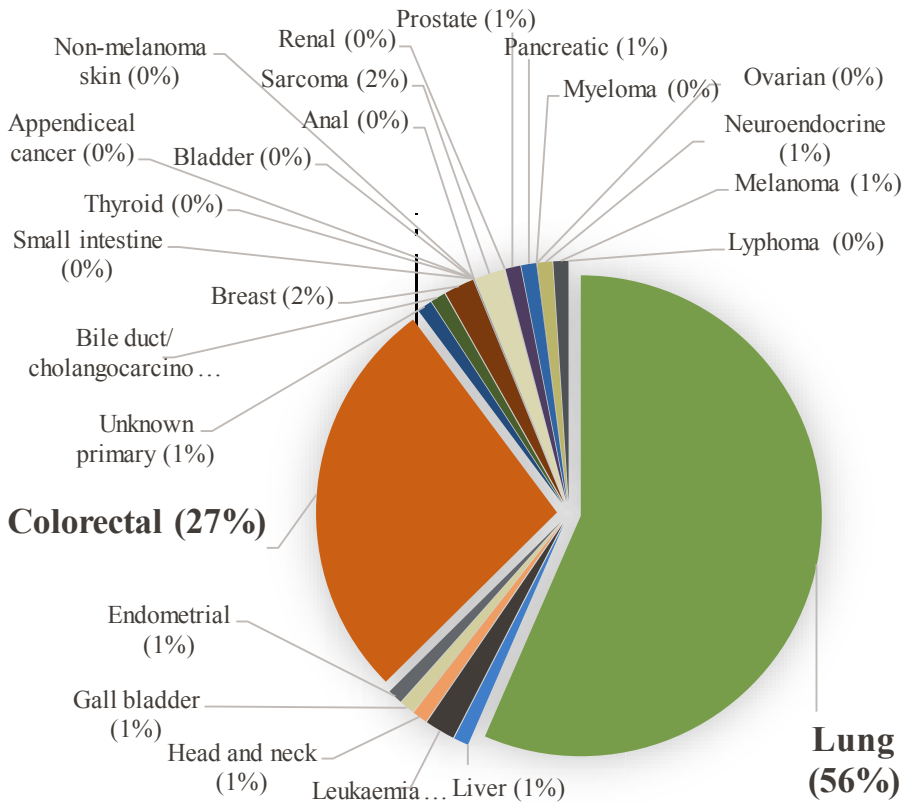
Atypical KRAS and NRAS: What to do With the Rare Variant?



- Several notable atypical *RAS* mt with high activity included *KRAS* V14I, Q22K, D33E, N116S, and F156L (all >165% of WT activity).
- Conversely, within the typical mutations, *KRAS* G13C and K117R were not shown to increase activity above WT.
  - *(However, these two mutations are very rare)*

Loree JM, et al. *J Clin Oncol.* 2017;35(Suppl 4):Abstract 3589.

Direct targeting of KRAS: G12C inhibitors entering clinic



Distribution of KRAS^{G12C}

Inhibitors bind to the P2 pocket of KRAS adjacent to the mutant cysteine

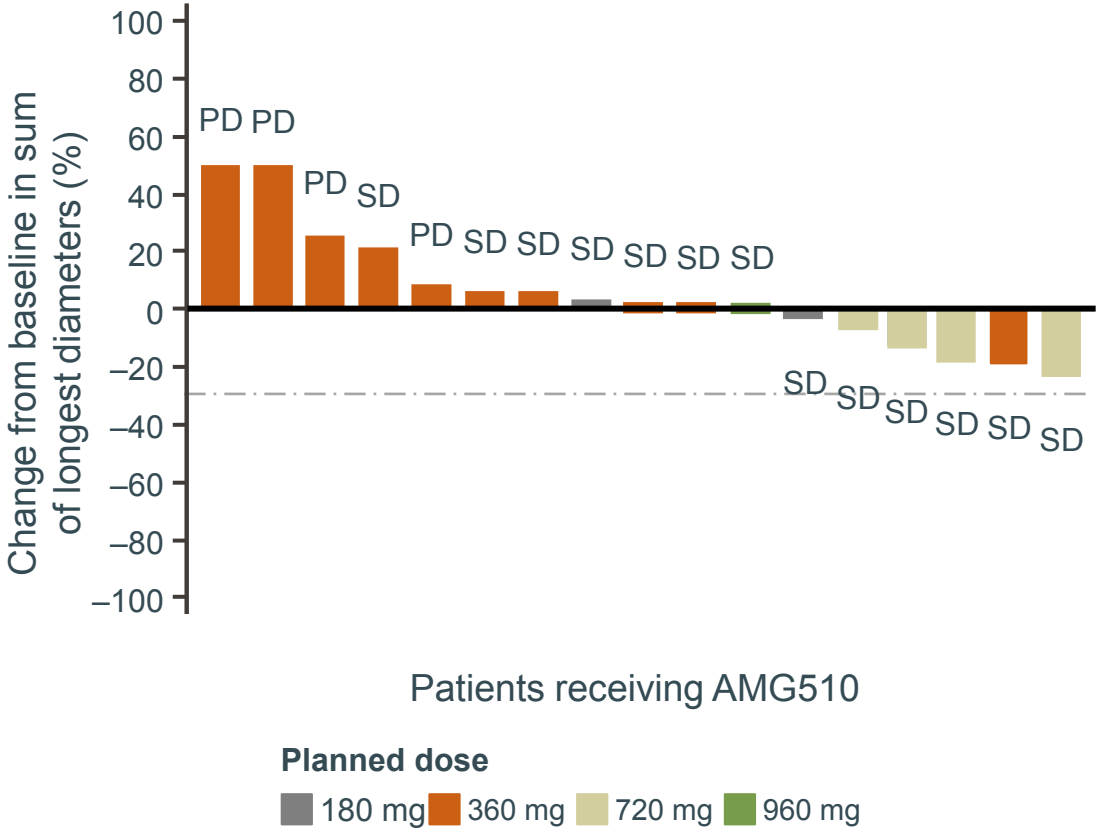
The inhibitor **covalently** modifies the cysteine residue

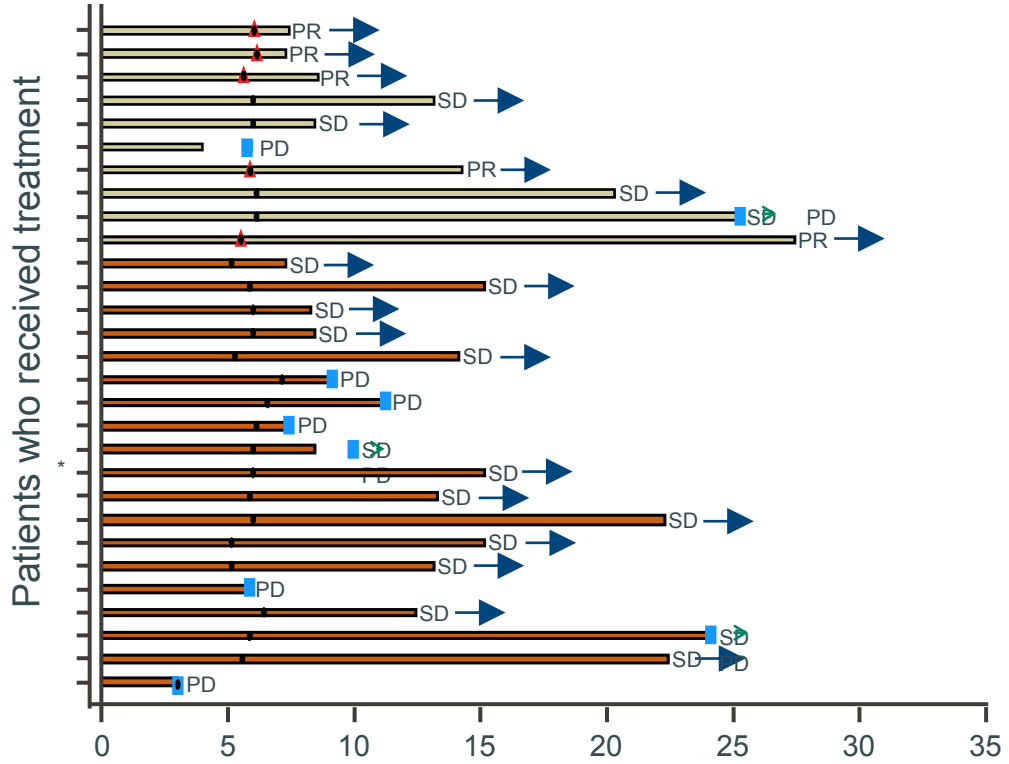
Results in KRAS^{G12C} locked in an inactive, GDP-bound conformation

GDP, guanosine diphosphate

Unpublished data

AMG510 in CRC and other solid tumours





Phase 2 study in CRC is starting to define RR/PFS at MTD

CRC, colorectal cancer; MTD, maximum tolerated dose; PD, progression of disease; PFS, progression-free survival; RR, response rate; SD, stable disease.

Fakih M, et al. J Clin Oncol 2019;37(suppl):Abstr 3003.

Fusions

Prevalence: <1% collectively

Enrichment:

MSI-H; low rates of APC, TP53, KRAS mutations

Recommendation:

Consider testing all refractory mCRC patients, especially MSI-H

*Not yet universally recommended on biomarker guidelines

NGS testing on 21,000 CRC pts including fusion, MSI

Kinase	N cases	% MSI-H
ALK	17	14
FGFR2	4	75
NTRK1	26	85
NTRK3	3	100
RET	27	50

Overall prevalence:

~0.4%

Number needed to screen

250

Prevalence in MSI-H:

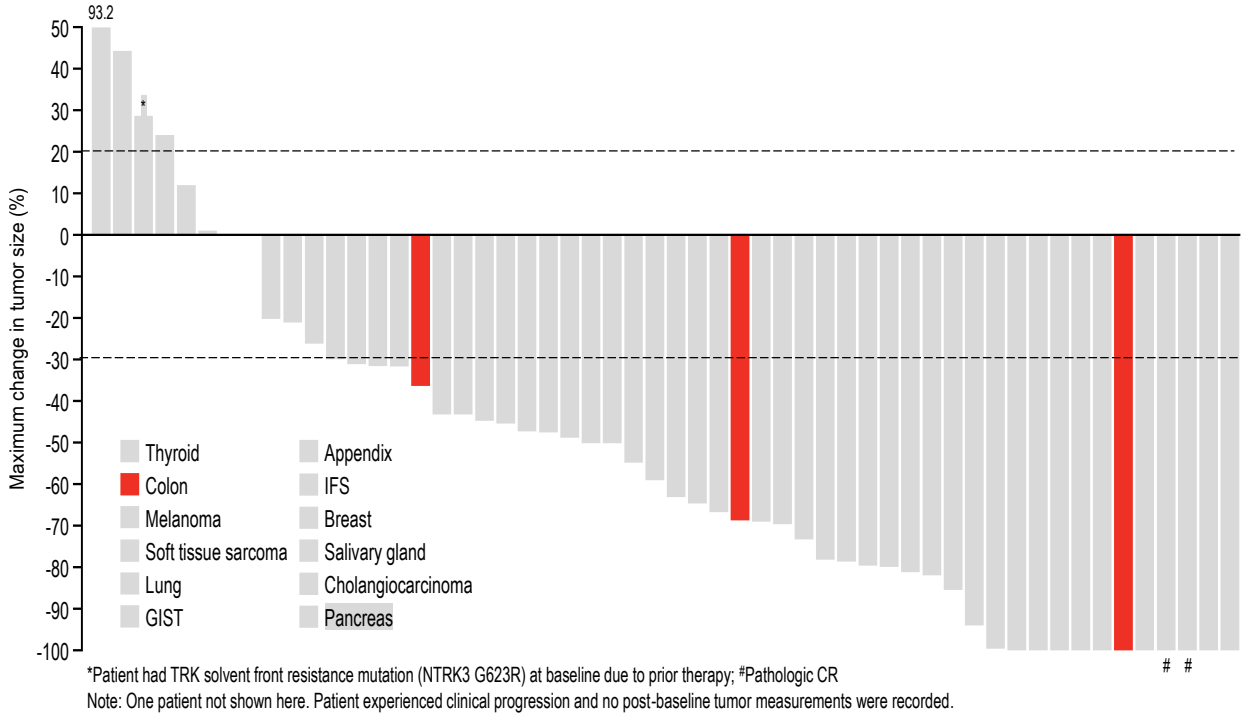
~10%

Number needed to screen

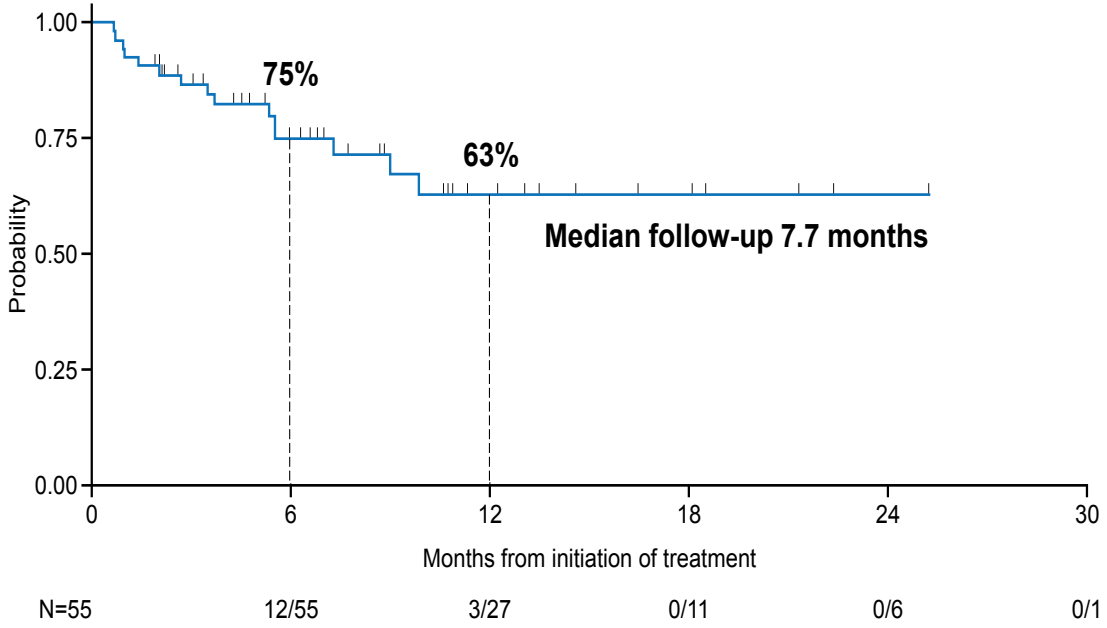
10

Madison et al ESMO ’18, #3509

Larotrectinib FDA Approved for TRK Fusion, Including CRC



*Patient had TRK solvent front resistance mutation (NTRK3 G623R) at baseline due to prior therapy; #Pathologic CR
Note: One patient not shown here. Patient experienced clinical progression and no post-baseline tumor measurements were recorded.



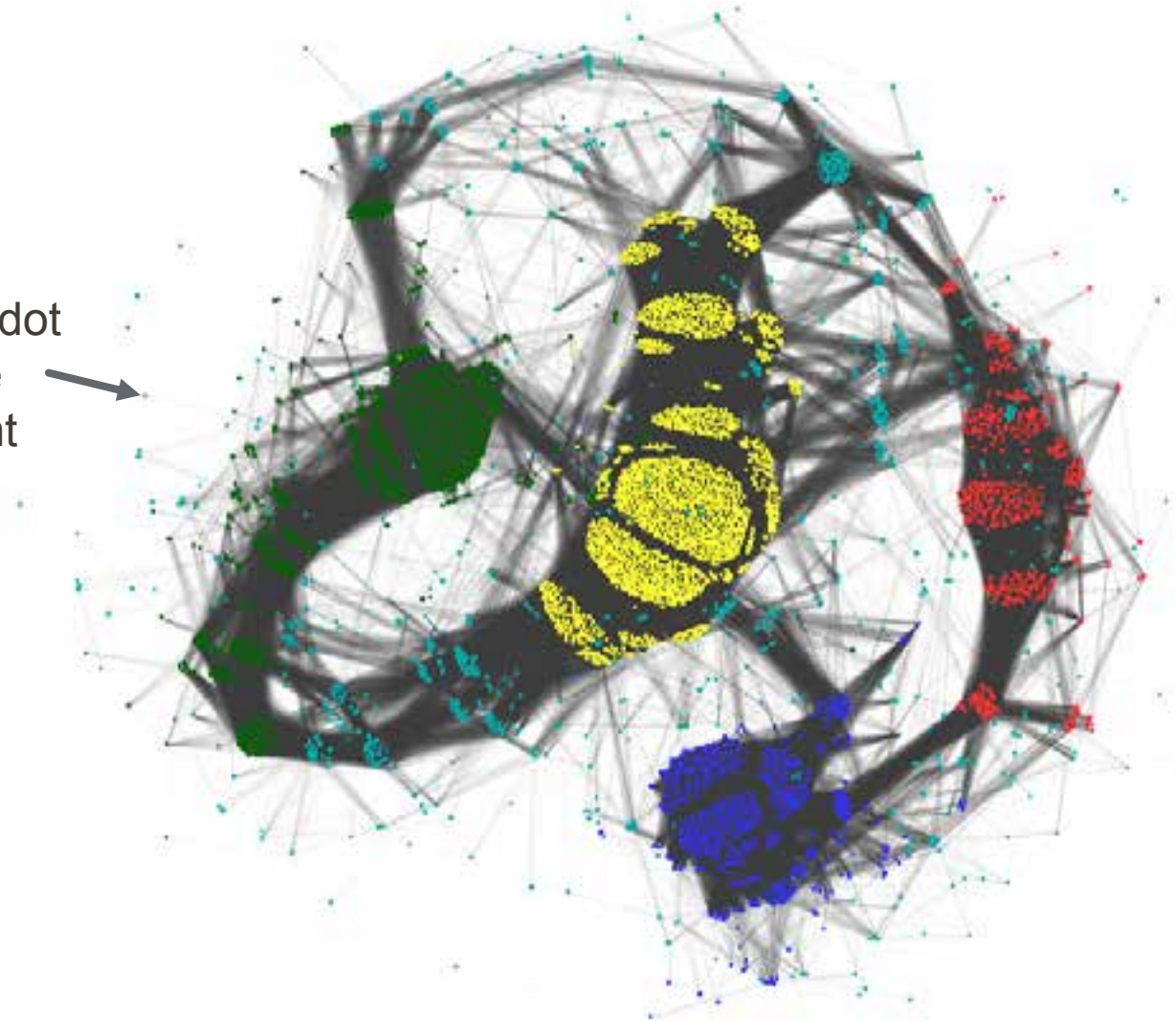
Hyman DM, et al. *J Clin Oncol.* 2017;35(Suppl 4):Abstract LBA2501. Hyman DM, et al. *N Engl J Med.* 2018 [Epub ahead of print].

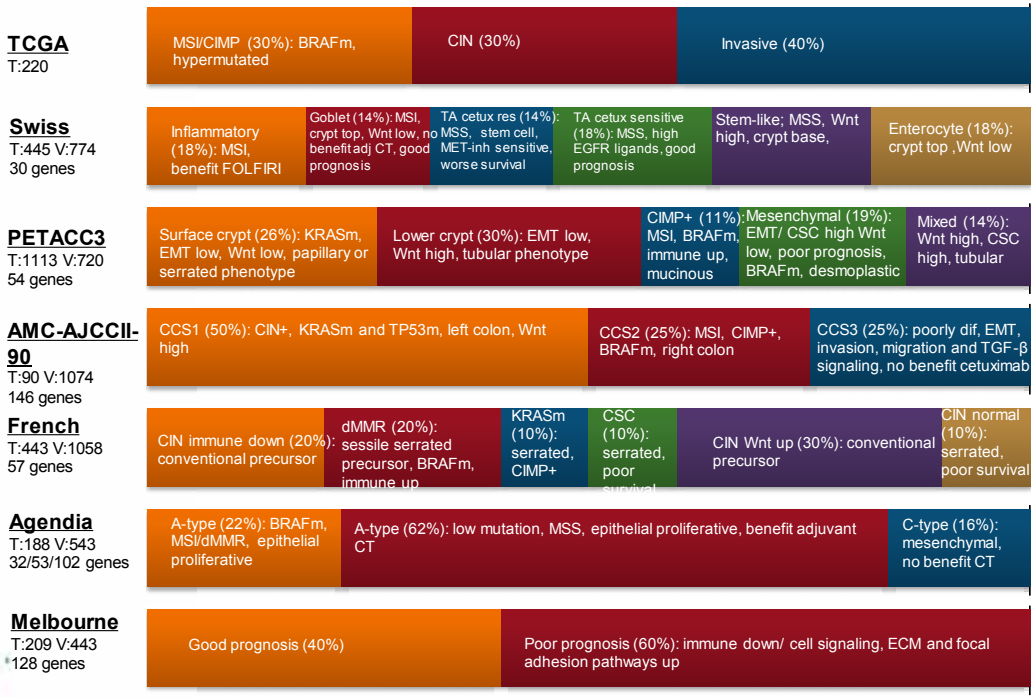
Overview

Single marker molecular subtyping	RNA-based molecular subtyping	Immune subtyping
• KRAS/NRAS • BRAF • MSI-H • HER2 amplification • Fusions	• Consensus molecular subtypes • Intrinsic subtyping	• Immune quantification • Tumor mutation burden

How do CRC differ by gene expression? Are there similarities in biology?

Each dot is one patient





Guinney et al, Nat Med ‘15

Philosophy: Consensus is required in order to move the field forward and transition to clinical application.

PIs: Justin Guinney
Rodrigo Dienstmann

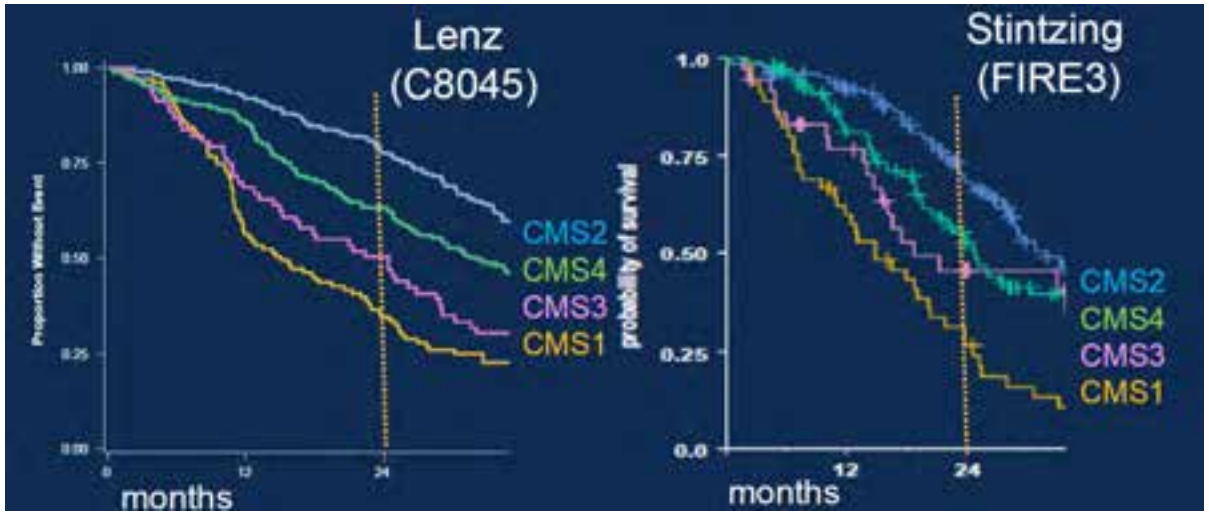
Key Features of the CMS Subtypes

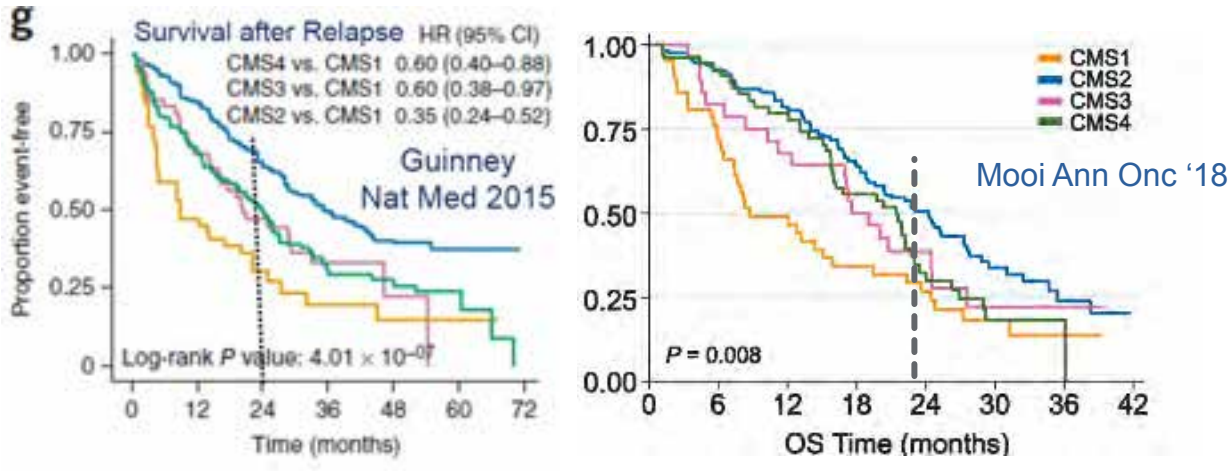
CMS1 MSI Immune	CMS2 Canonical	CMS3 Metabolic	CMS4 Mesenchymal
14%	37%	13%	23%
MSI, CIMP high Hypermutation	SCNA high	Mixed MSI status SCNA low, CIMP low	SCN high
BRAF mutations		*KRAS* mutations	
Immune infiltration and activation	WNT and MYC activation	Metabolic deregulation	Stromal infiltration TGF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

Guinney et al, Nat Med ‘15

CMS: Consistent Prognostic Information in mCRC

Despite being designed agnostic to outcomes, strong prognostic information.





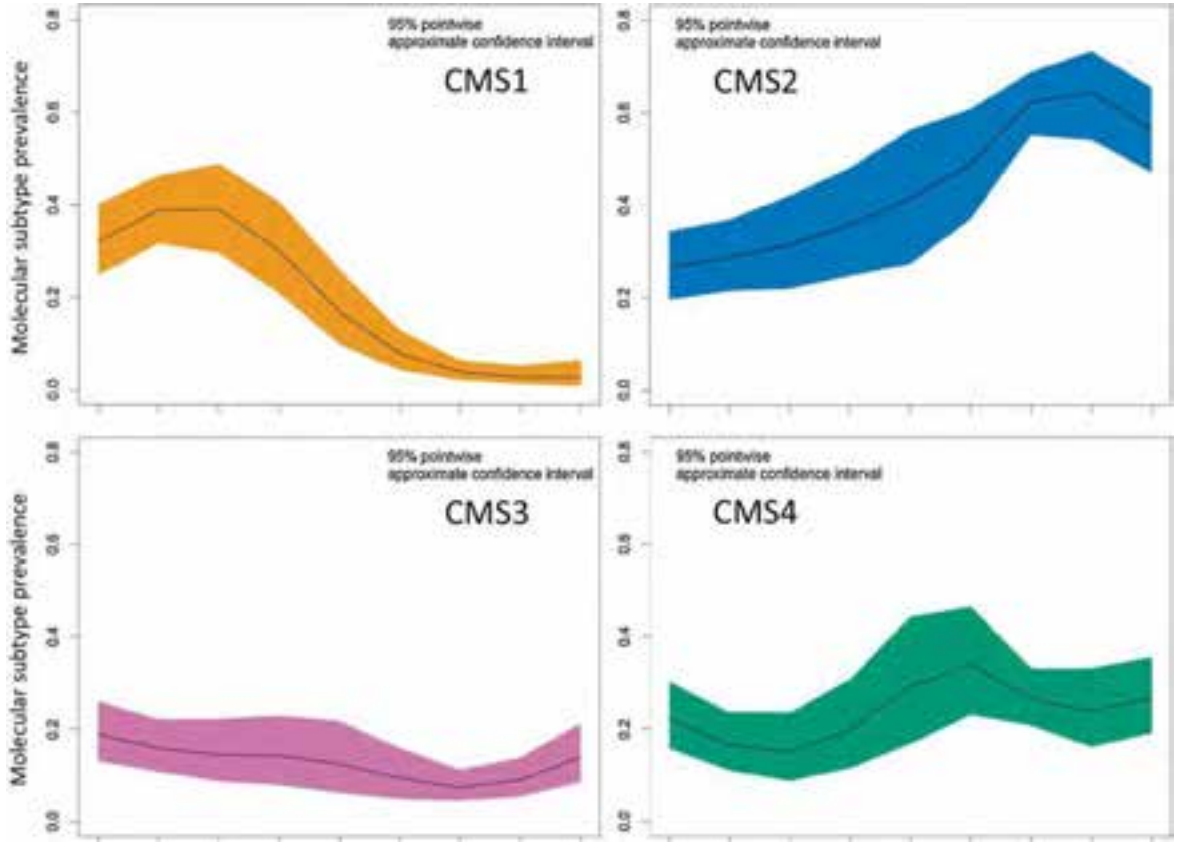
Mooi Ann Onc '18

Median overall survival: Differs from 15 months (CMS1) to 40 months (CMS2)

Progression-free survival: Differs from 5.7 months (CMS1) to 14.1 months (CMS2)

Slide courtesy of Wells Messersmith, ASCO '17

CMS Varies by Tumor Location

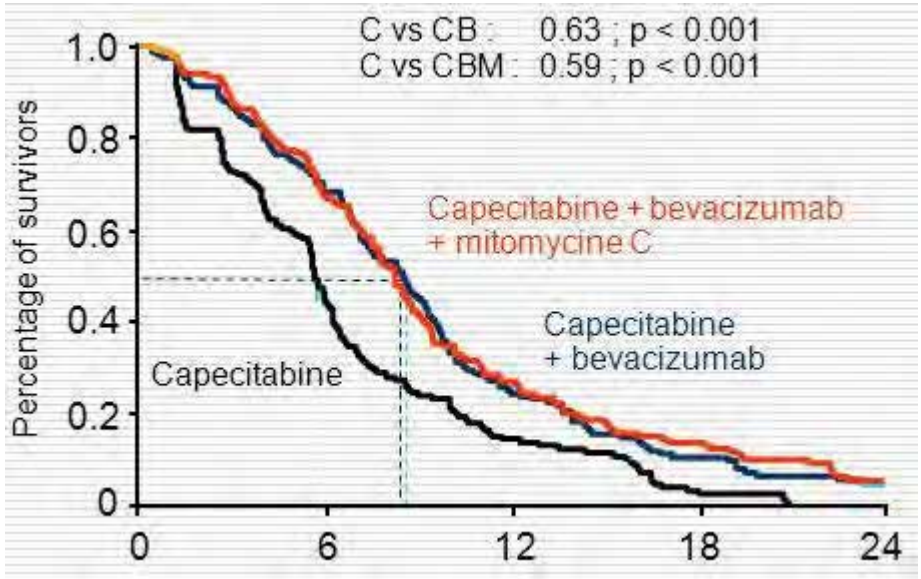


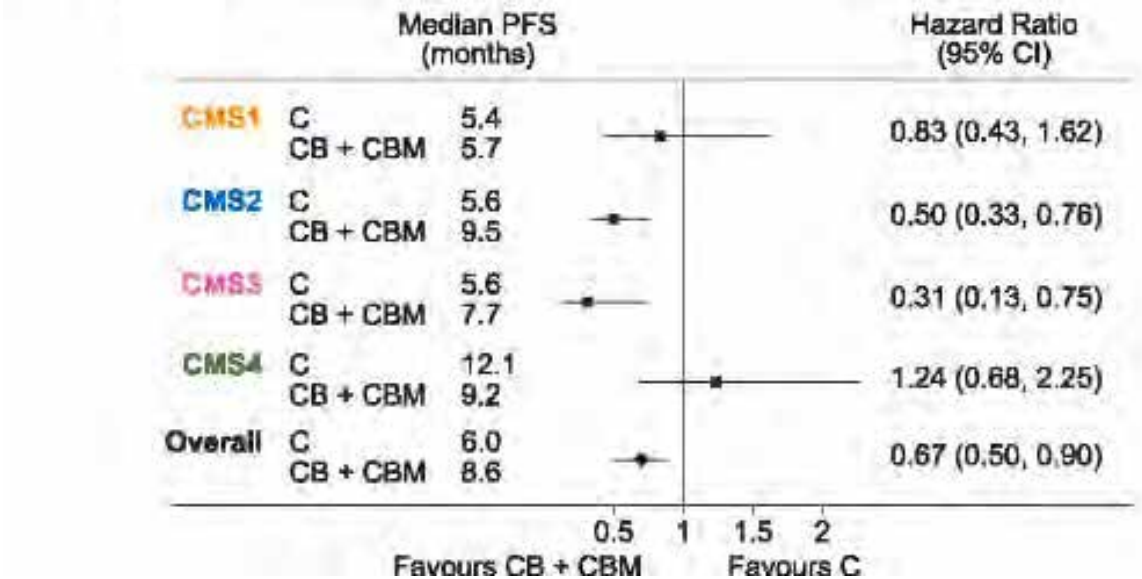
Further integrated analyses are needed to understand contributions of CMS and sidedness to prognosis, but appears to be independent information

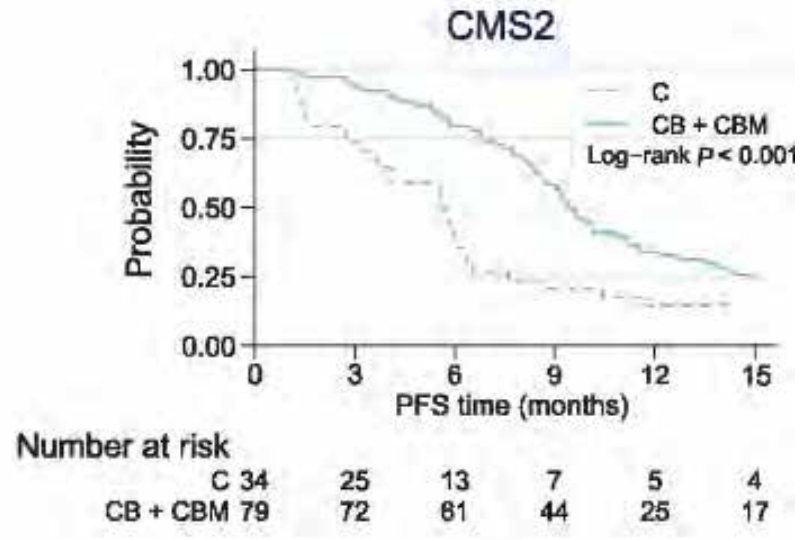
Loree et al CCR ‘18

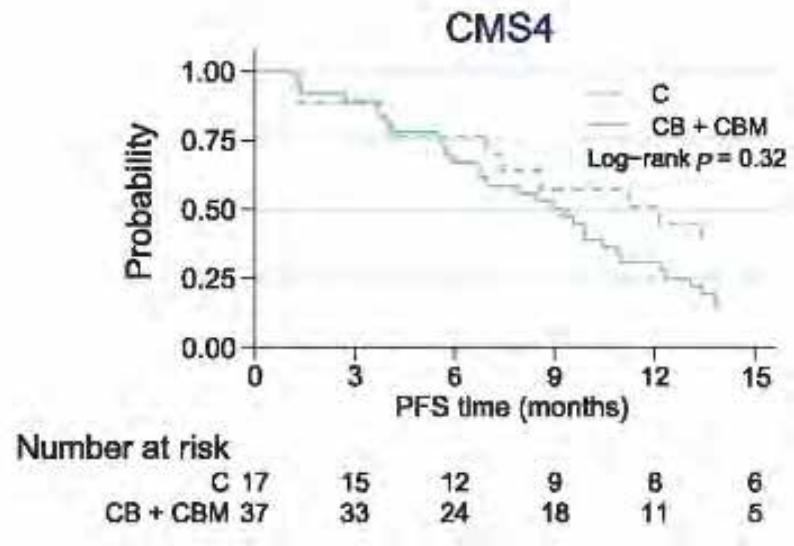
CMS2/3 may Benefit from Addition of Bevacizumab

AGITG MAX Trial







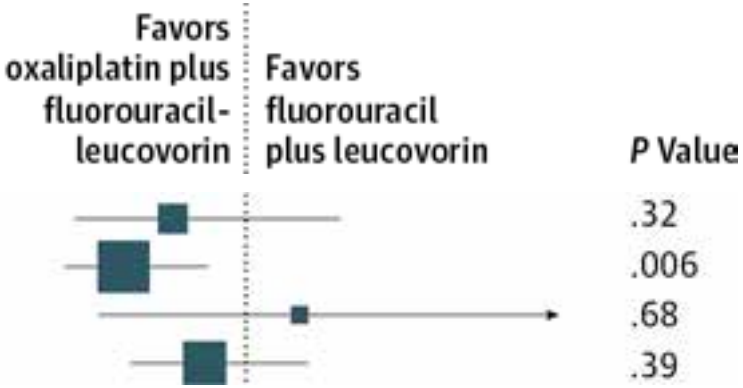


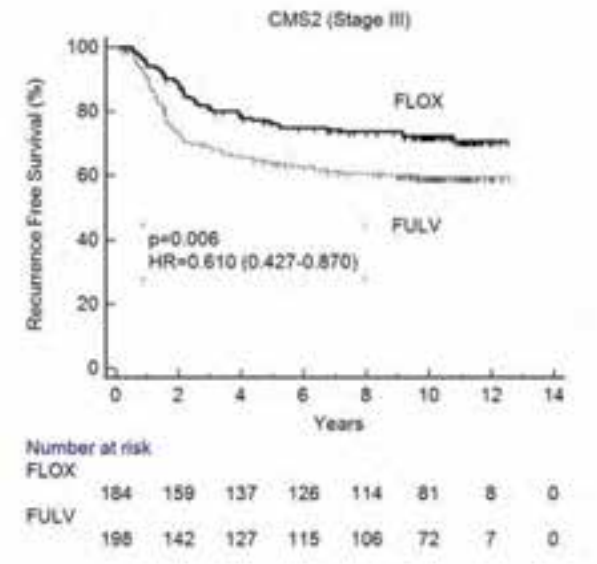
Mooi et al Annals Onc '18

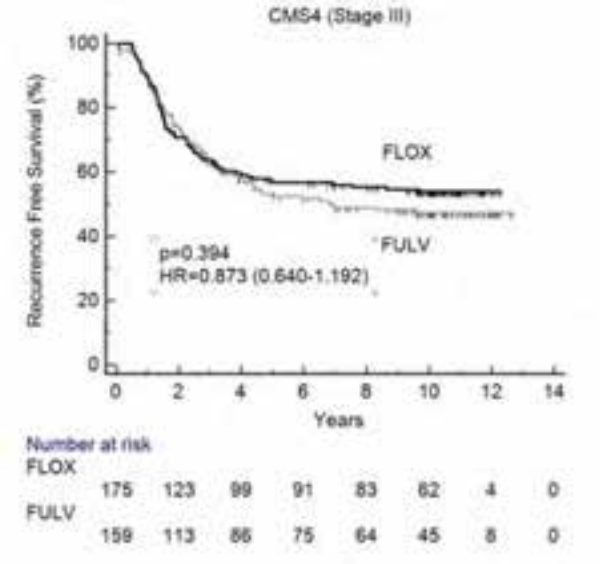
Mesenchymal CMS4 : Limited Benefit with Oxaliplatin?

C-07 study of FLOX vs FULV

Subtypes	No. of Patients	HR (95% CI)
CMS1	231	0.77 (0.46-1.29)
CMS2	382	0.61 (0.43-0.87)
CMS3	86	1.17 (0.54-2.53)
CMS4	334	0.87 (0.64-1.19)



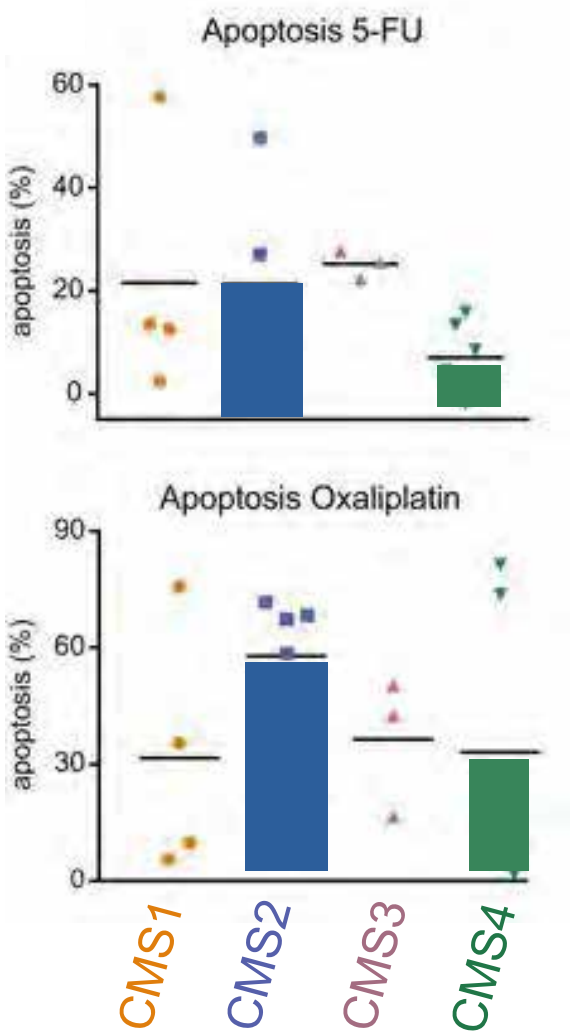




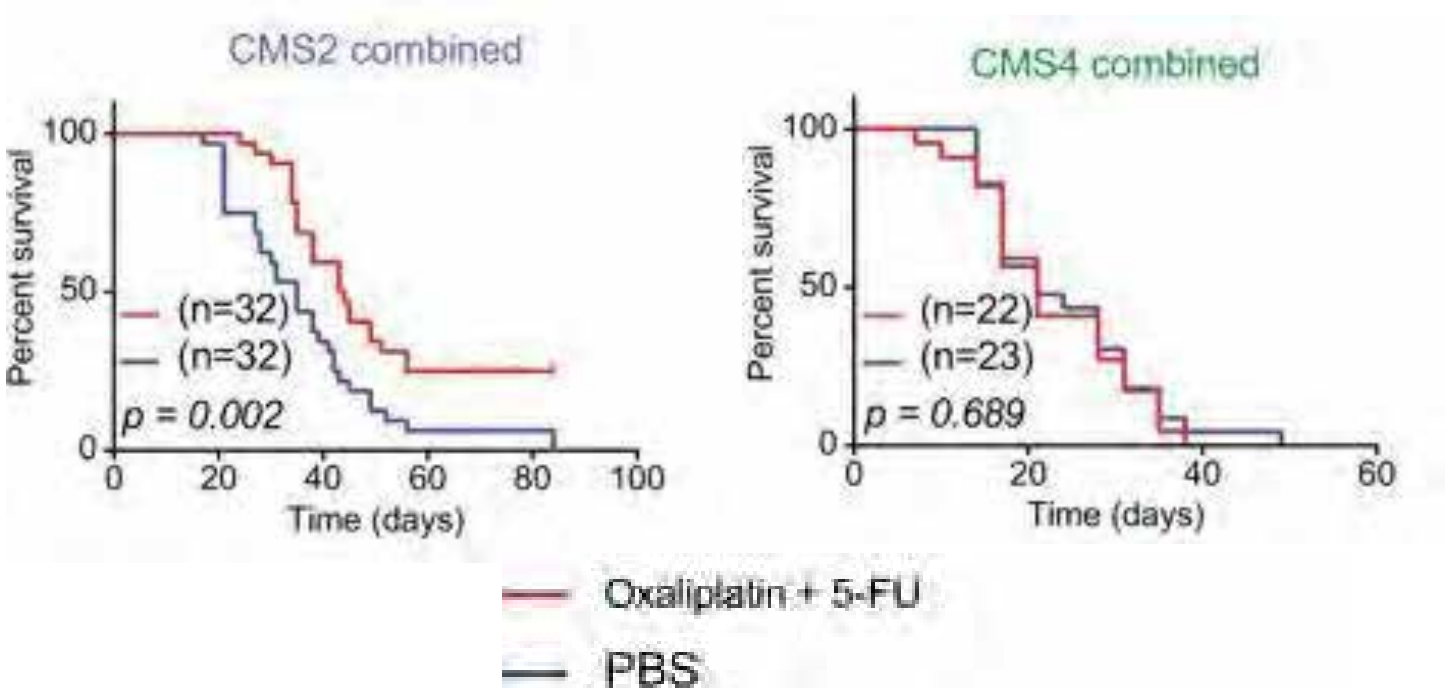
Are there other subgroups or oxali-specific signatures that would perform better?

Song et al JAMA Onc '17

Differential Sensitivity to Oxaliplatin: Preclinical Data



Mouse co-clinical trial



Needs validation, but limited retrospective specimens available.

Linnekamp et al, Cell Death & Differentiation '18

CMS Strengths: Insights into Biologic / Immune Context

CMS1: Immunogenic Tumors

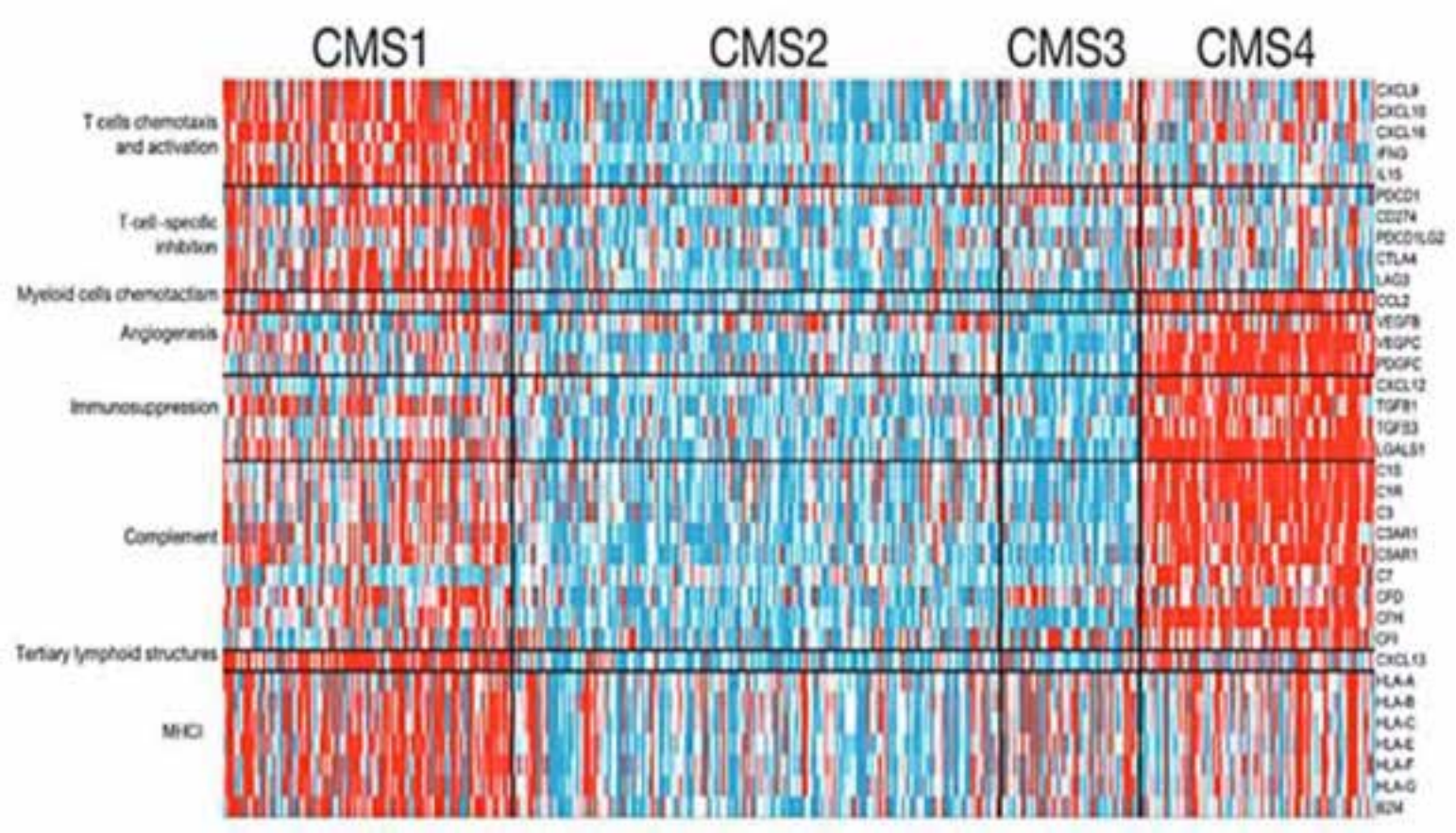
Infiltrating activated lymphocytes

CMS2/3: Immune Desert

No evidence of immune activation

CMS4: Immune Excluded

Immune system is engaged, but microenvironment prevents activity



Becht et al CCR '16

CMS Strengths: Insights into Biologic / Immune Context

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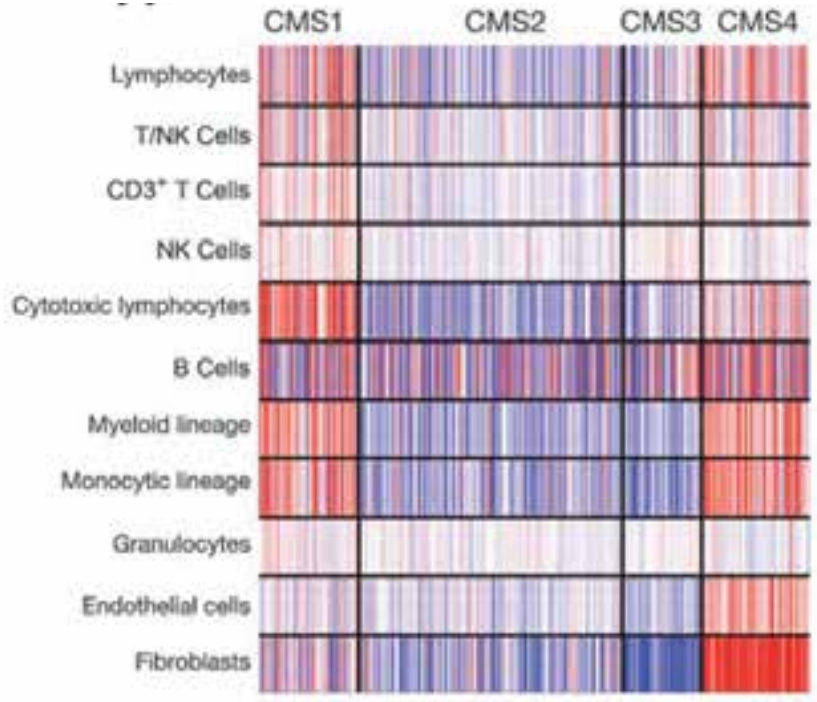
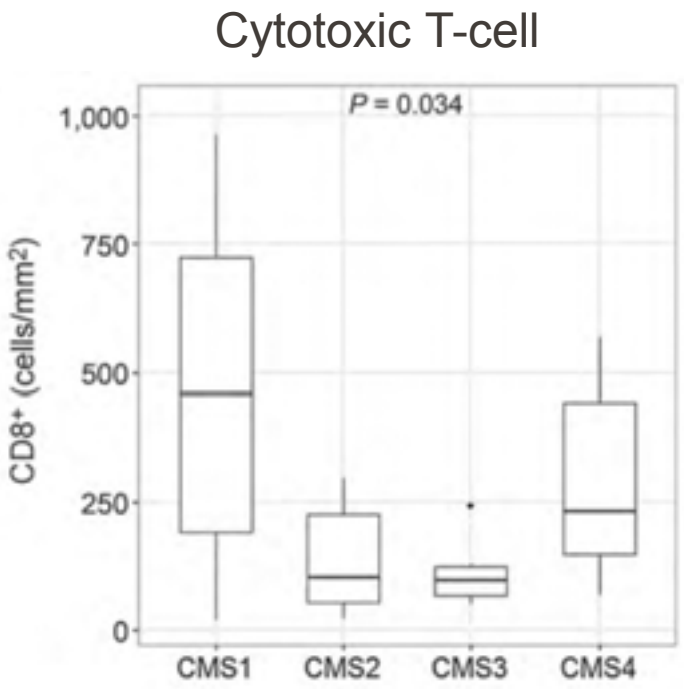


CMS2/3: Immune Desert

No evidence of immune activation



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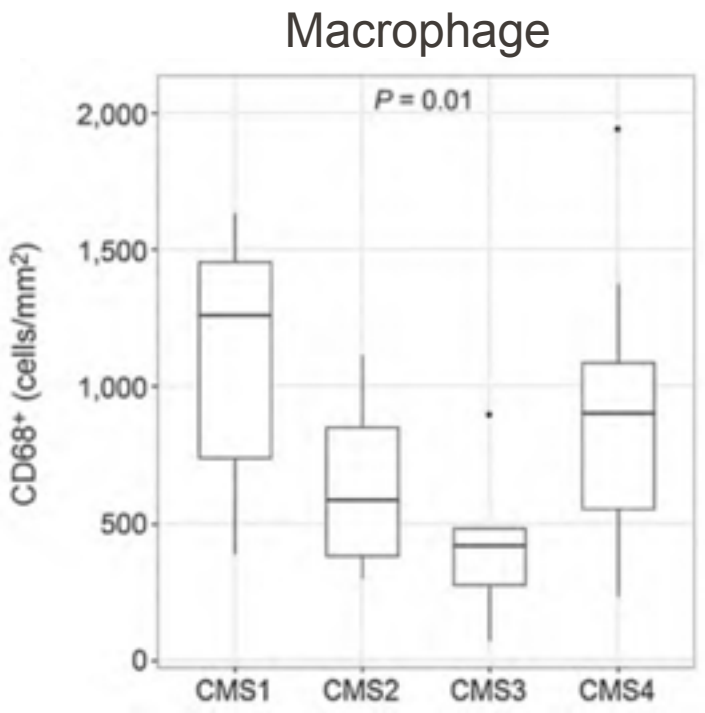
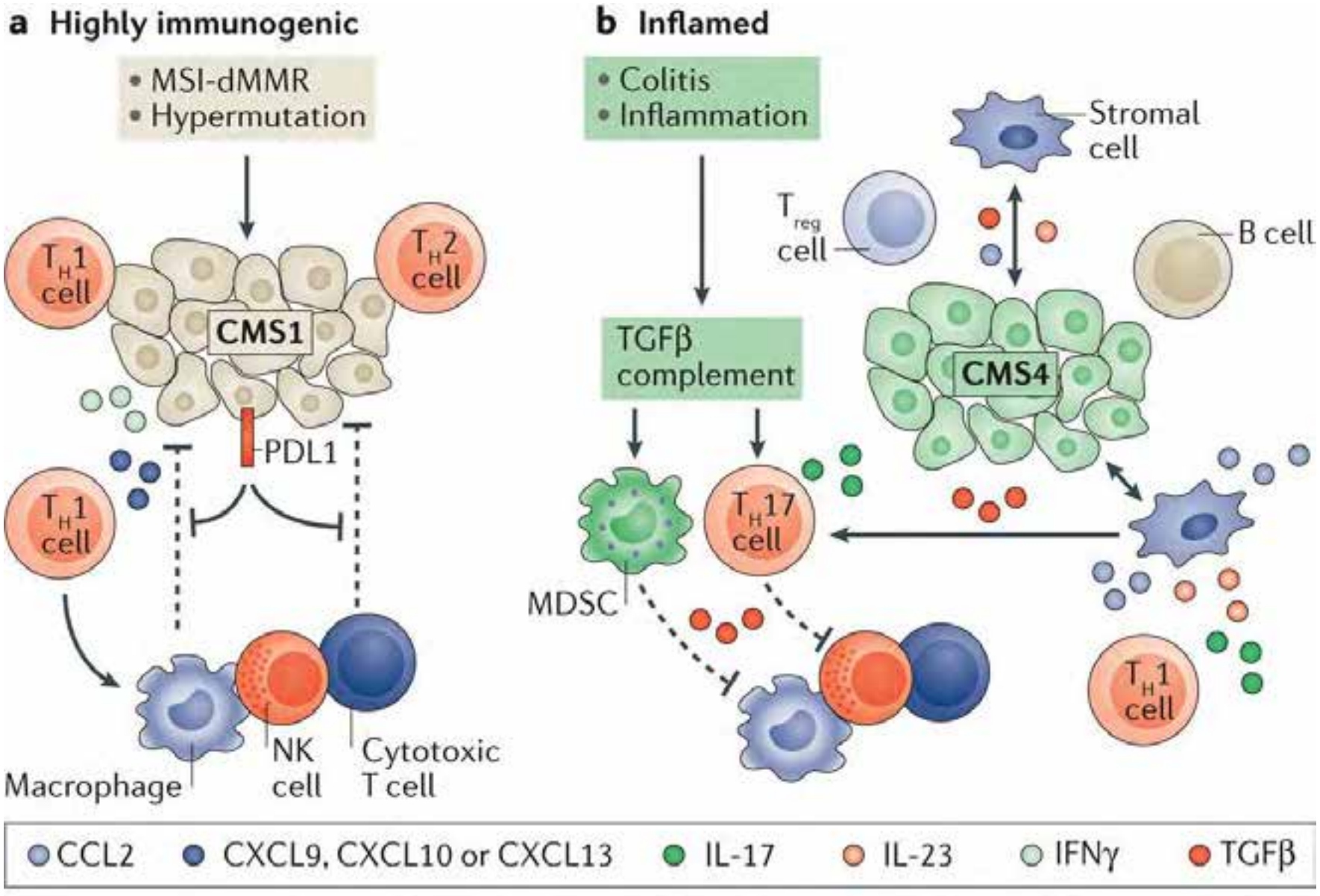


CMS4 has a moderate cytotoxic T-cell infiltrate, but high myeloid, TGF-β signaling

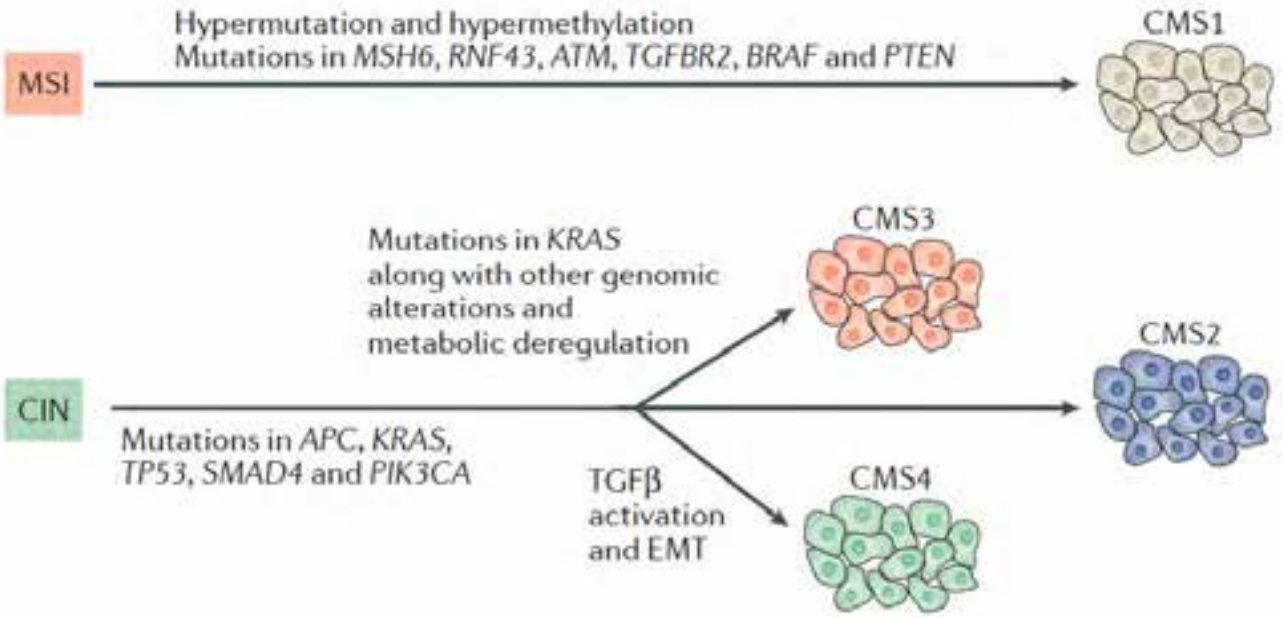
Becht et al CCR ’16, Lai et al CCR ‘18

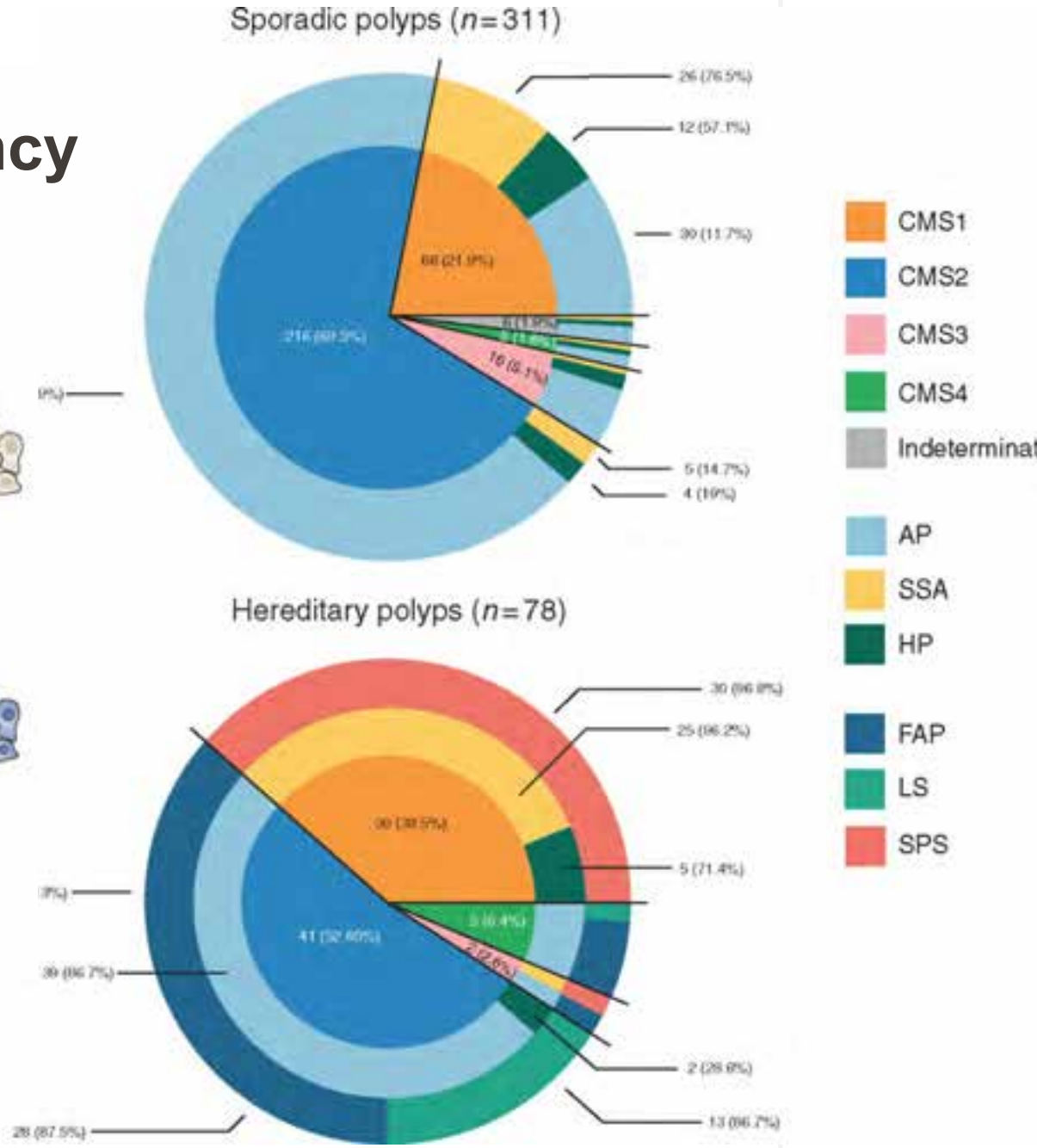


Dienstmann et al Nat Review Cancer ‘17

Molecular Subtypes in Premalignancy

Absence of CMS4 / Mesenchymal

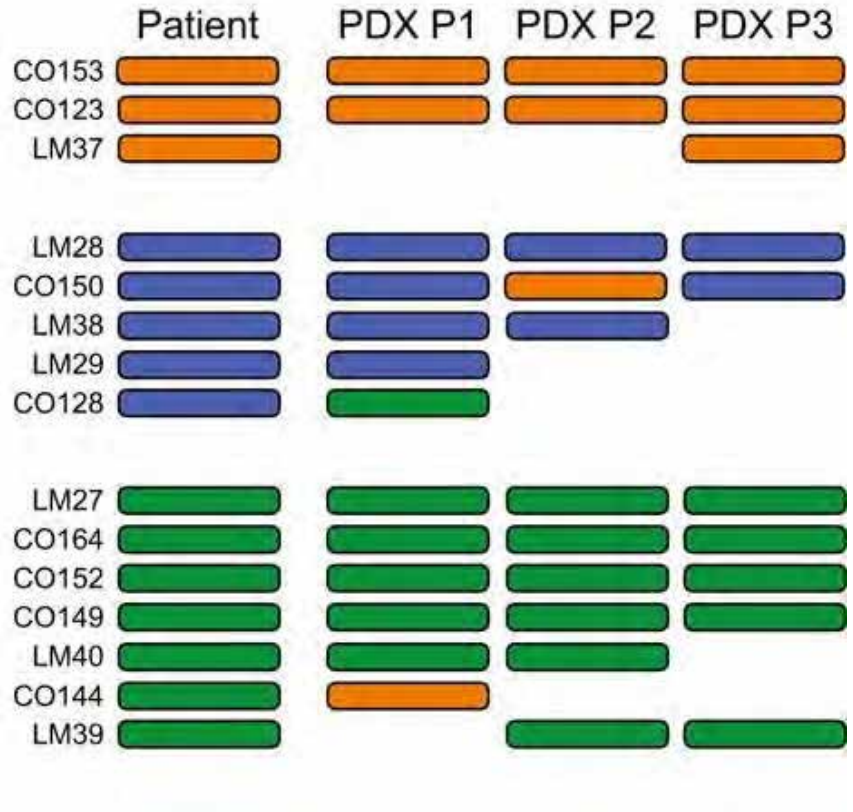


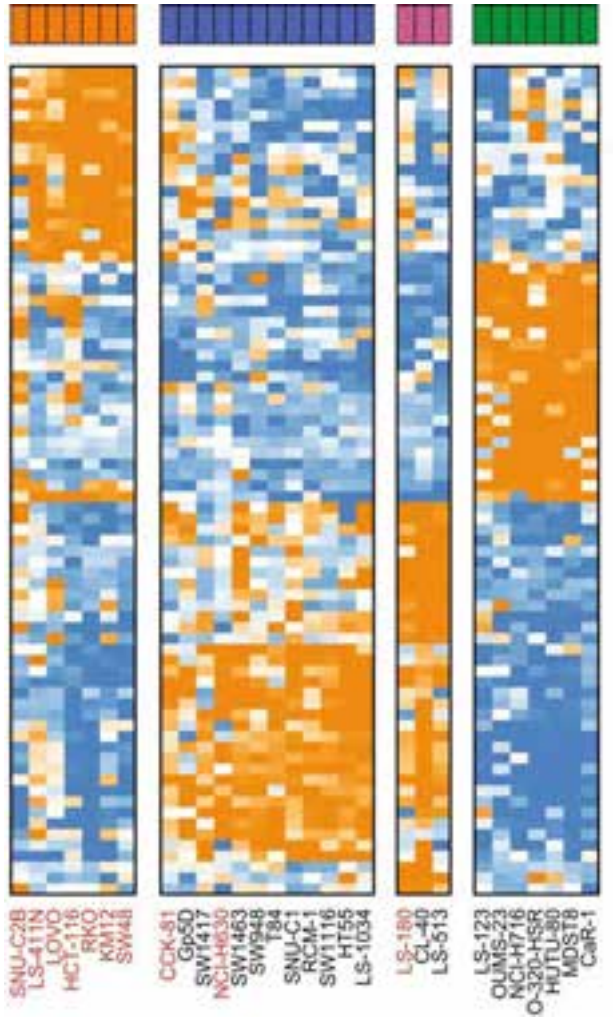


Chang et al Annals Onc '18, Dienstmann R. et al, Nat Rev Cancer '17

Molecular Subtype Tools: PDXs and Cell Lines

Annotated models now available to support preclinical research



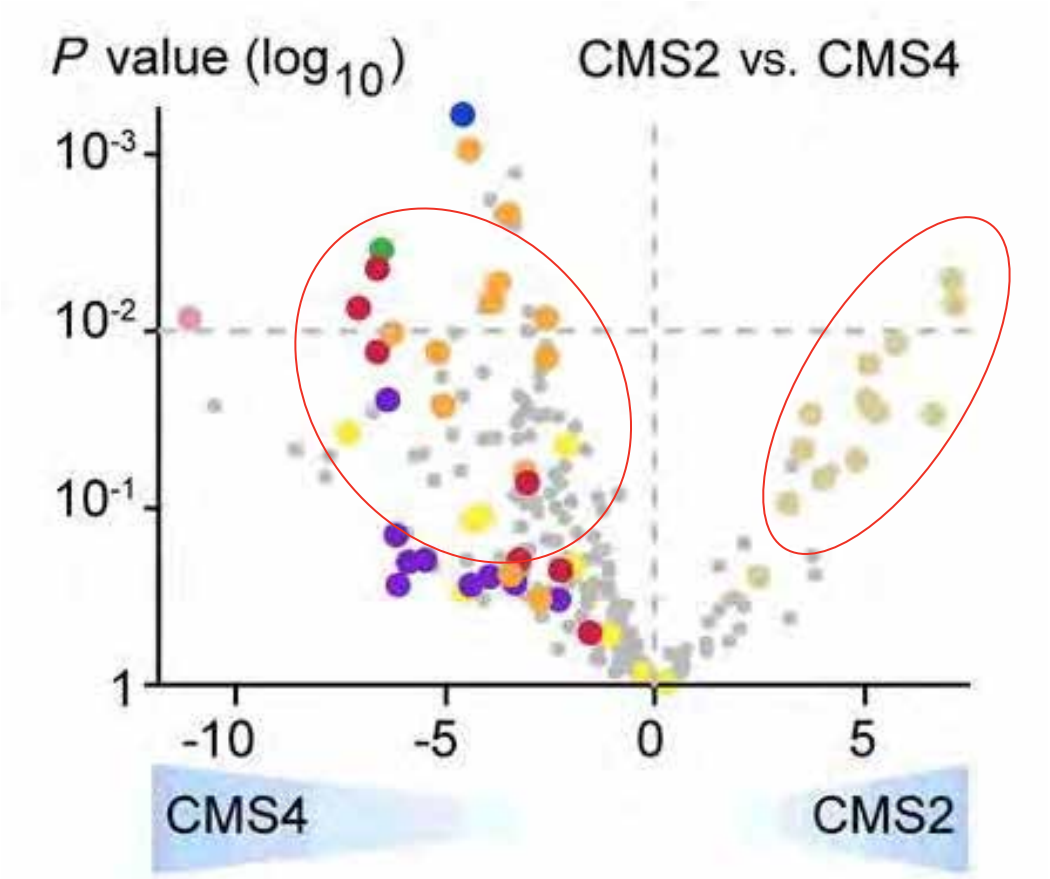


Linnekamp et al, Cell Death & Differentiation ‘18

Example: Screens for subtype-specific vulnerabilities

Topoisomerase inhibitors

Heat-shock proteins



EGFR and HER2 inhibitors

Sveen et al CCR ’18, Del Rio M Eur J Cancer ’17, Sadanandam Nat Med ‘13

What is needed to move RNA classifiers into the clinic?

Clinical utility

- Validation of findings across multiple retrospective cohorts
- Integration into prospective studies

Clinical-grade, parsimonious assay

- To date, there is no broadly available CLIA assay
- MDACC and other academic labs have established FFPE-robust classifiers

Classifier robust to real-world sampling

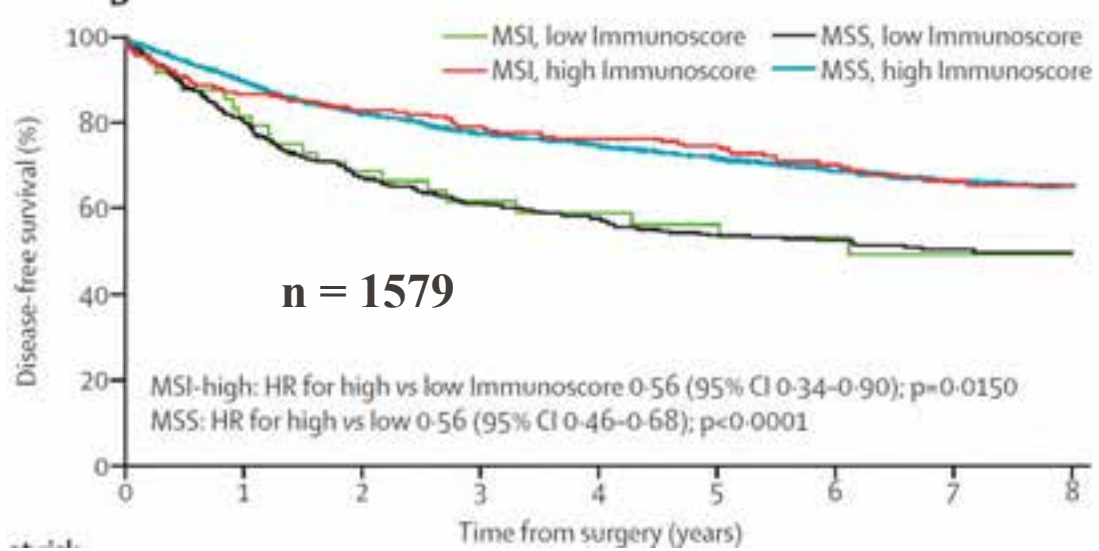
- Works on small tissue and biopsies from metastatic sites

Overview

Single marker molecular subtyping	RNA-based molecular subtyping	Immune subtyping
• KRAS/NRAS • BRAF • MSI-H • HER2 amplification • Fusions	• Consensus molecular subtypes • Intrinsic subtyping	• Immune quantification • Tumor mutation burden

Immunoscore and MSI/MSS subgroups

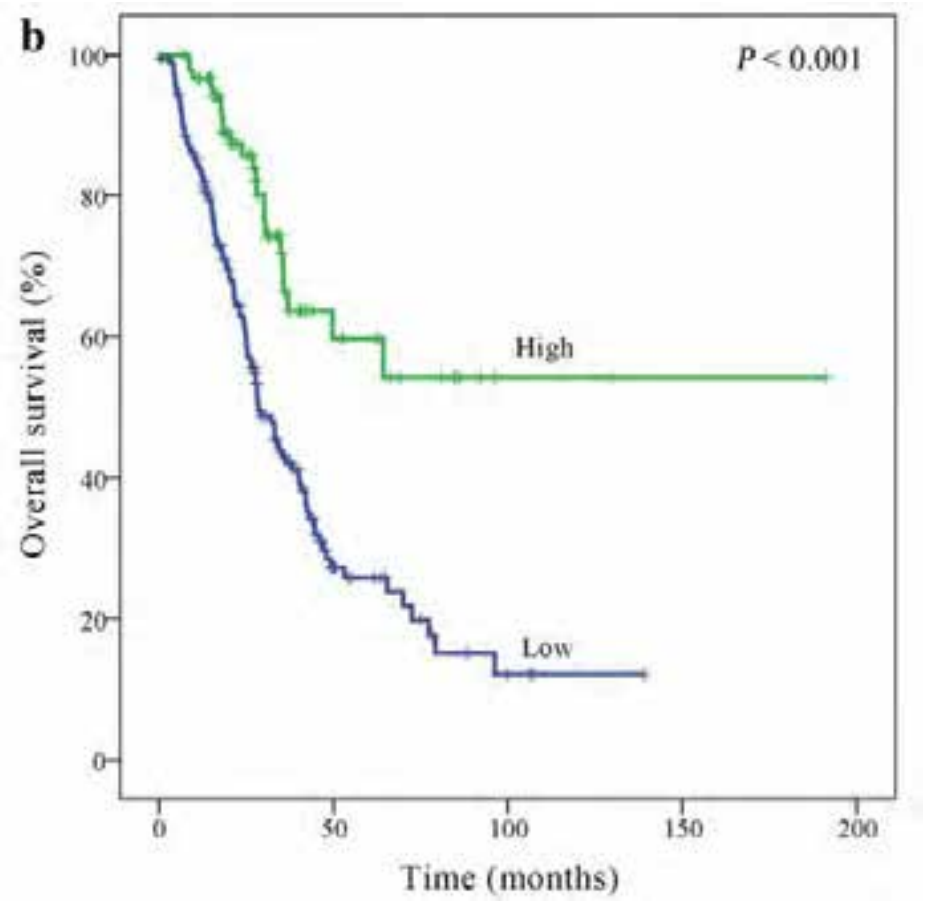
Stage II/III



High CD3+

Low CD3+

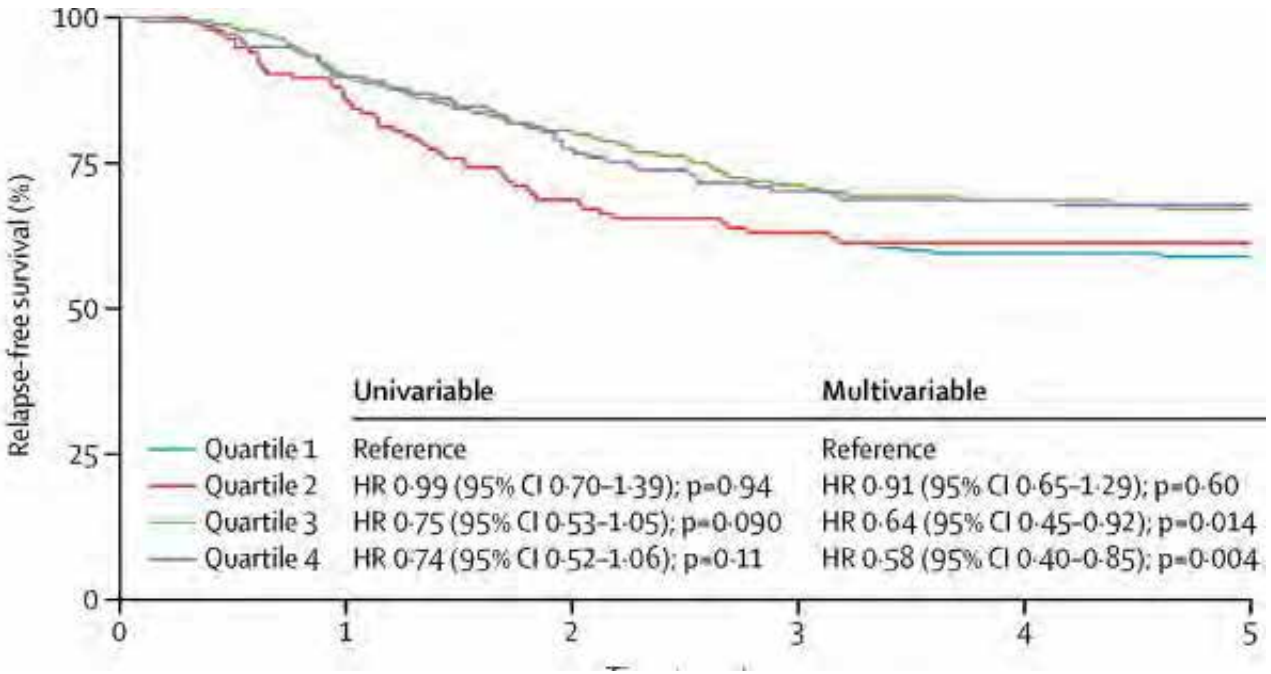
Stage IV, hepatectomy



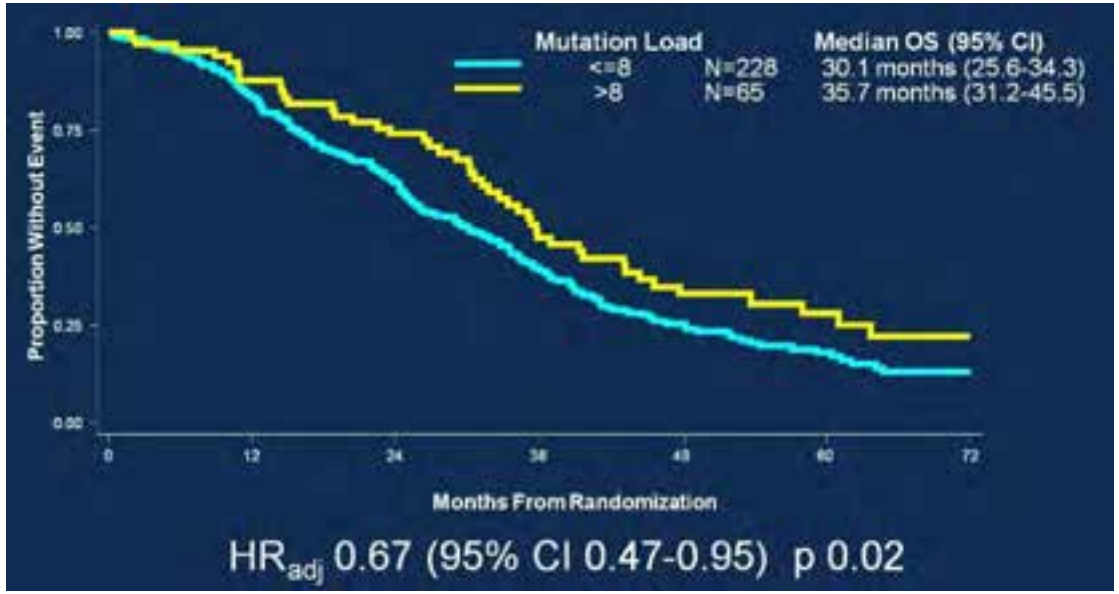
Pagès et al Lancet May 2018; Wnag et al Cancer Immun ‘17

Tumor mutation burden as a molecular classification

QUASAR 2



CALGB/SWOG 80405: MSS



The data to date are only (modestly) prognostic, which limits the potential clinical utility. These will not be routinely utilized unless predictive applications can be identified.

Innocenti et al ASCO ‘17; Domingo et al Lancet G&H ‘18

Conclusions

- Molecular subtyping is a key mechanism to improve patient outcomes.
- Current molecular subtypes with clinical activity:
  - BRAF *V600E* mutation: Dual EGFR and BRAF inhibition (+/- MEK)
  - HER2 amplification: Trastuzumab with Lapatinib or Pertuzumab
  - NTRK fusions: Larotrectinib
  - MSI-H: Nivolumab/Ipilimumab, Pembrolizumab
- Future precision therapies may incorporate RNA-based classification
  - We shouldn’t be discouraged by lack of immediate clinical applications
- Education and dissemination of existing best practices is critical !

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- Jack Lee, PhD

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- John Strickler, MD Duke
- Curt Harris, NKI
- Aaron Schetter, NKI
- Chloe Atreya, UCSF
- Ryan Corcoran, MD, PhD

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