Leading History Reshaping Future

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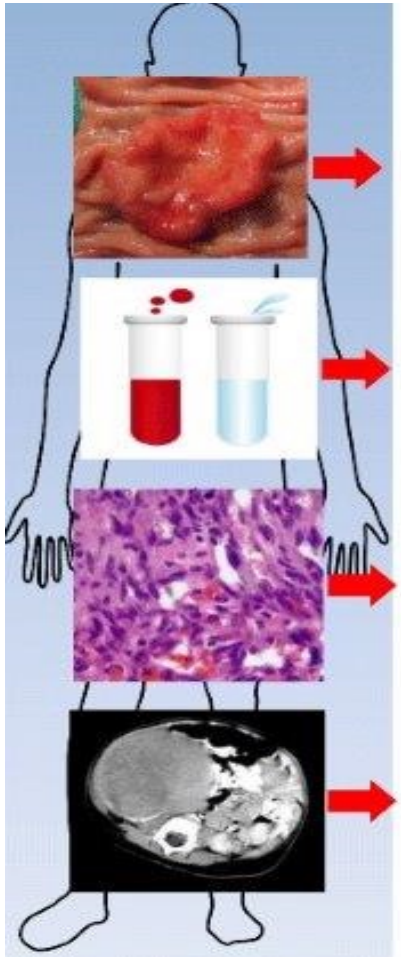
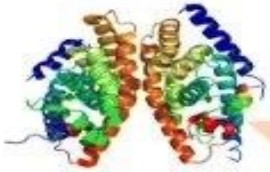
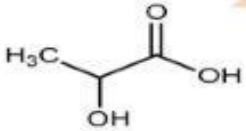


Genomics

Transcriptomics



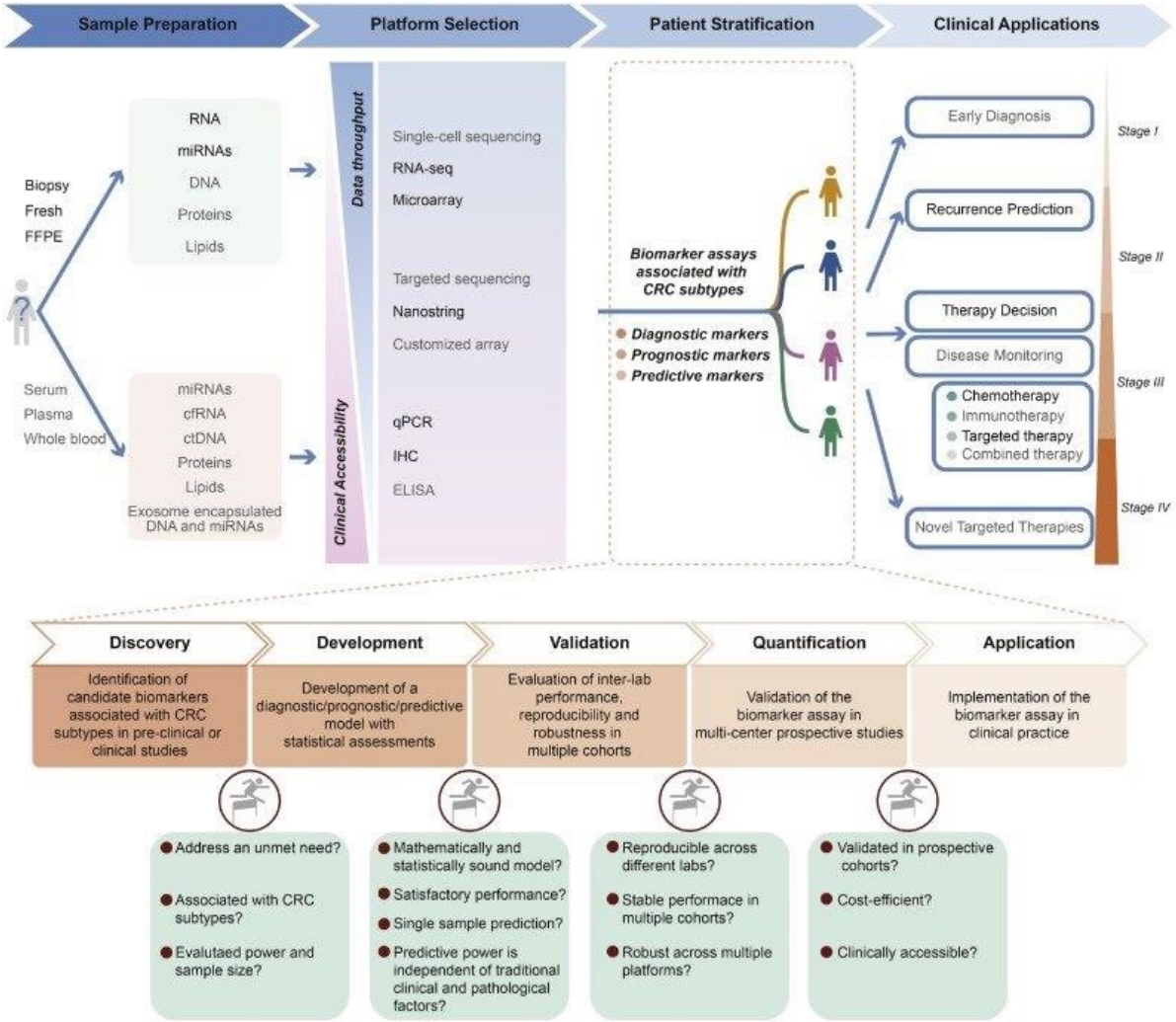
Proteomics



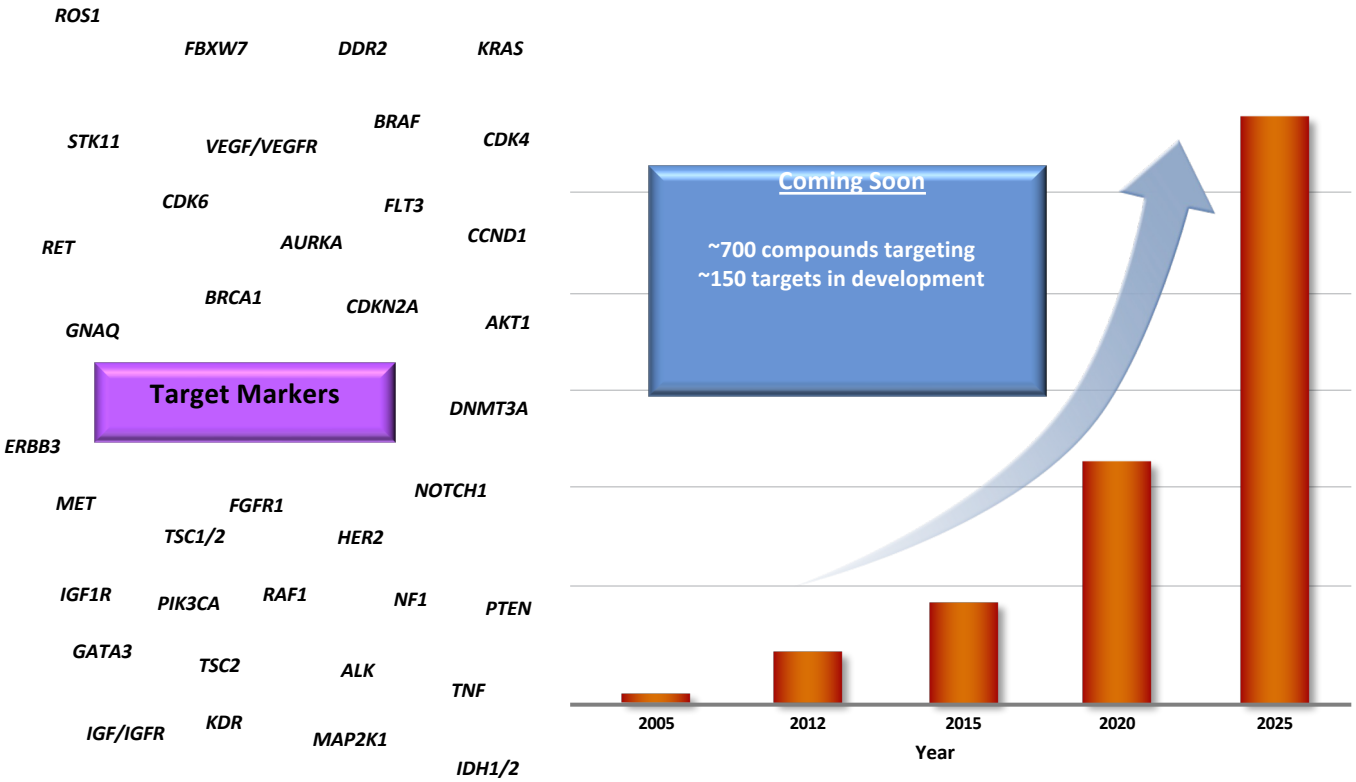
Metabolomics

Radiomics

Prediction
Prevention
Personal treatment

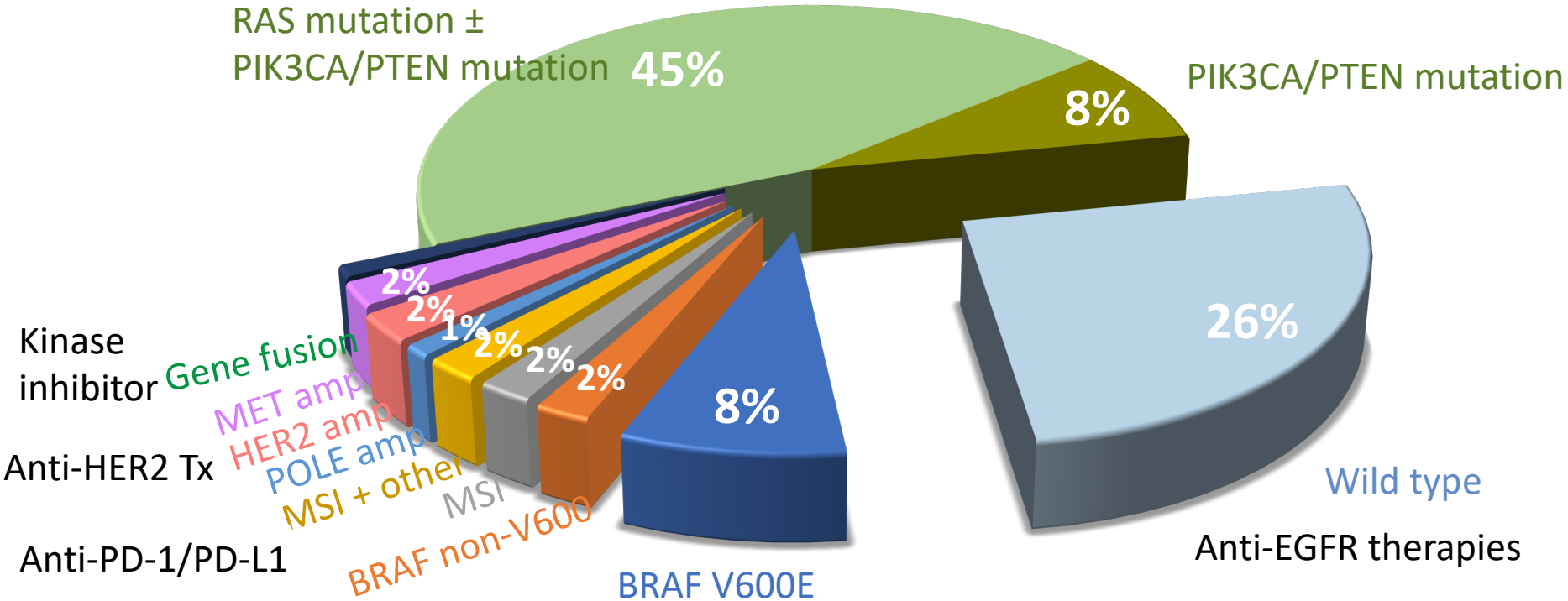

Sample Preparation
Platform Selection
Patient Stratification
Clinical Applications
Biopsy
Fresh
FFPE
RNA
miRNAs
DNA
Proteins
Lipids
Serum
Plasma
Whole blood
miRNAs
cfRNA
ctDNA
Proteins
Lipids
Exosome encapsulated DNA and miRNAs
Data throughput
Clinical Accessibility
Single-cell sequencing
RNA-seq
Microarray
Targeted sequencing
Nanostring
Customized array
qPCR
IHC
ELISA
Biomarker assays associated with CRC subtypes
Diagnostic markers
Prognostic markers
Predictive markers
Early Diagnosis
Recurrence Prediction
Therapy Decision
Disease Monitoring
Chemotherapy
Immunotherapy
Targeted therapy
Combined therapy
Novel Targeted Therapies
Stage I
Stage II
Stage III
Stage IV
Discovery
Identification of candidate biomarkers associated with CRC subtypes in pre-clinical or clinical studies
Development
Development of a diagnostic/prognostic/predictive model with statistical assessments
Validation
Evaluation of inter-lab performance, reproducibility and robustness in multiple cohorts
Quantification
Validation of the biomarker assay in multi-center prospective studies
Application
Implementation of the biomarker assay in clinical practice
Address an unmet need?
Associated with CRC subtypes?
Evalutaed power and sample size?
Mathematically and statistically sound model?
Satisfactory performance?
Single sample prediction?
Predictive power is independent of traditional clinical and pathological factors?
Reproducible across different labs?
Stable performace in multiple cohorts?
Robust across multiple platforms?
Validated in prospective cohorts?
Cost-efficient?
Clinically accessible?

Number of Targeted Therapeutics is Rising
Knowing Which Tests to Order is the Challenge



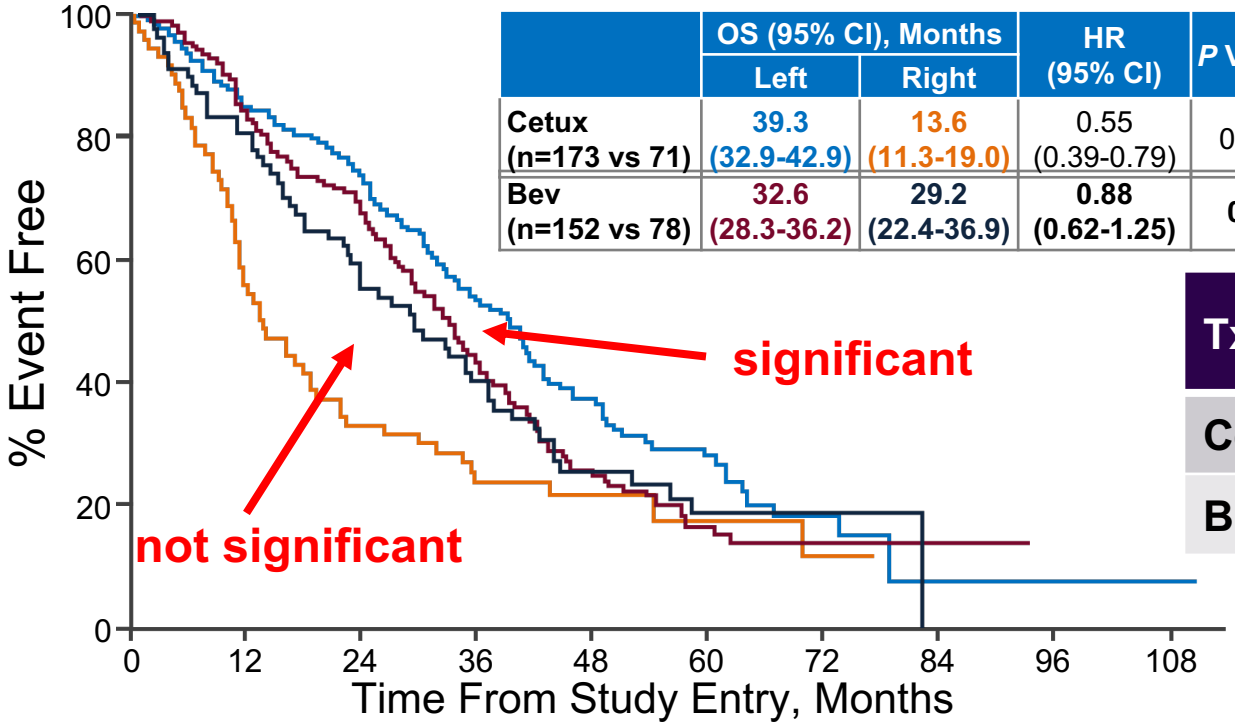
Extrapolated from BioCentury Online Intelligence Database

Genomic Markers in CRC



Dienstmann. ASCO Ed Book. 2018.

CALGB/SWOG 80405: OS by Tumor Location (*RAS* WT)



	OS (95% CI), Months		HR (95% CI)	*P* Value*
	Left	Right		
Cetux (n=173 vs 71)	39.3 (32.9-42.9)	13.6 (11.3-19.0)	0.55 (0.39-0.79)	0.001
Bev (n=152 vs 78)	32.6 (28.3-36.2)	29.2 (22.4-36.9)	0.88 (0.62-1.25)	0.50

Tx	ΔR vs L (mos)
Cetux	25.7
BEV	3.4

*Adjusted for biologic, protocol CT, prior adjuvant therapy, prior RT, age, sex, synchronous disease, in place primary, liver metastases.

Lenz et al presented at: ESMO. 2016.

Novel Approaches (Leading)

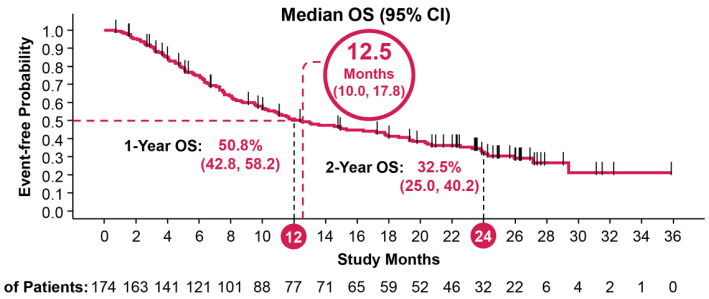
1. RAS/RAF (G12C, G12D, G12V)
2. Pan Inhibitor
3. Metabolic Targets
4. Novel Immune therapies for MSS CRC

Codebreak

- Sotorasib, a selective KRAS^{G12C} inhibitor, is approved in Europe, the US, and other countries for patients with previously treated *KRAS* p.G12C-mutated NSCLC[1–4]
- In Phase 1/2 of the CodeBreaK 100 study,[5,6] sotorasib monotherapy demonstrated:

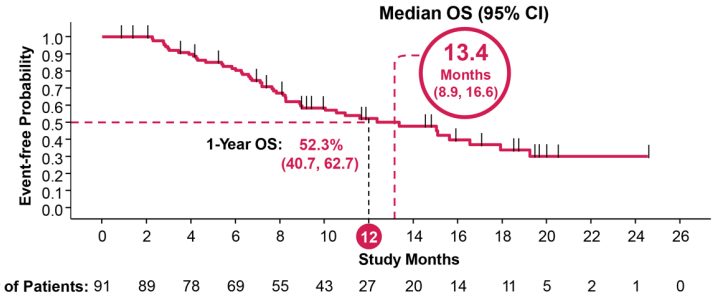
NSCLC

- **ORR: 41%**
- Median PFS: 6.3 months
- DCR: 84%



CRC

- **ORR: 12%**
- Median PFS: 4.2 months
- DCR: 82%

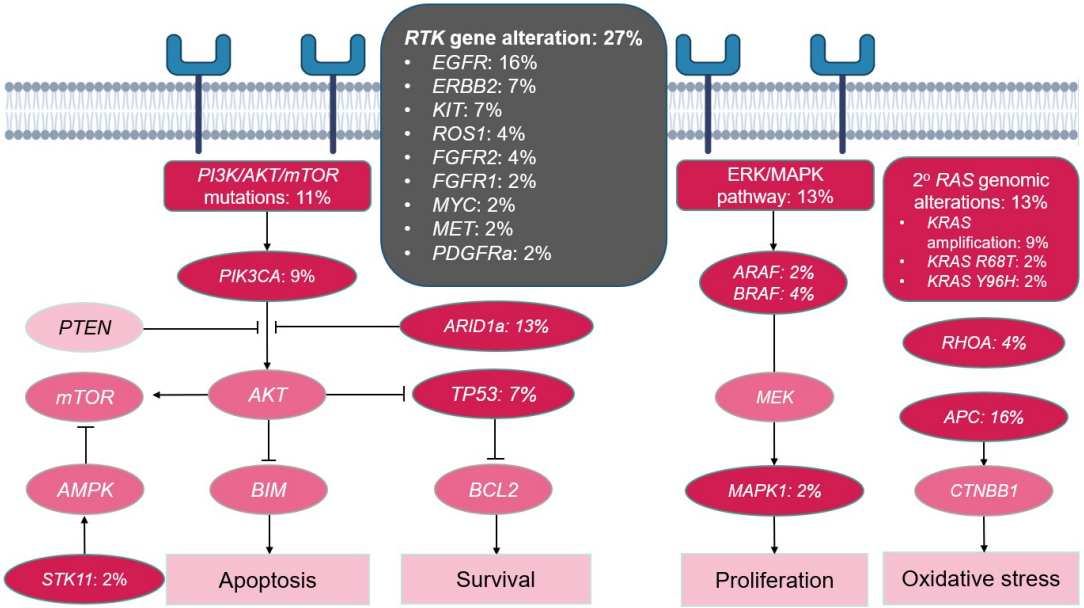


We describe putative mechanisms of acquired resistance to sotorasib in patients with CRC from the CodeBreaK 100 study

1. Canon J, et al. *Nature.* 2019;575:217–23; 2. Lumakras (sotorasib). Prescribing Information. Thousand Oaks, CA, Amgen Inc., 2021; 3. Lumykras (sotorasib). Summary of Product Characteristics, Cambridge, UK, Amgen Ltd, 2021; 4. Lumykras (sotorasib). European Medicines Agency, Amsterdam, Netherlands, Amgen Inc., 2021; 5. ClinicalTrials.gov. NCT03600883. Accessed 8 June 2022; 6. Dy GK, et al. Oral Presentation at AACR Annual Meeting 2022; April 8–13, New Orleans, LA. Abstract CT008.

CI, confidence interval; CRC, colorectal cancer; DCR, disease control rate; NSCLC, non-small cell lung cancer; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

Putative Acquired Resistance Mechanisms After Sotorasib[a]



OncoKB[1]

- **16/100 alterations were potentially targetable[b]**
- **Higher incidence of secondary *RAS* variants in CRC versus NSCLC**

***RTK* gene alterations were the most prevalent acquired genomic alteration in patients with CRC (12/45; 27%)**

1. Chakravarty D, et al. *JCO Precis Oncol*. 2017, doi:10.1200/PO.17.00011.

[a]Mutation rate presented based on 45 evaluable patients with CRC; [b]Actionability levels defined in full at https://www.oncokb.org/levels. Actionable variants: Level 1; *BRCA1* E352* (n = 1), *BRCA2* S196R (n = 1), *CDK12* G909* (n = 1), *PIK3CA* E542K (n = 2). Level 2; *PIK3CA* R38C (n = 1). Level 4; *ARID1A* Q1402* (n = 1), *ARID1A* R1721* (n = 1), *ARID1A* single nucleotide variant (n = 1), *CDKN2A* truncating mutation (n = 1), *EGFR* copy number variant (n = 6); *Termination or stop codon.

CRC, colorectal cancer; NSCLC, non-small cell lung cancer.

CRC patients with G12C with multiple acquired mechanisms of resistance Awad et al NEJM 2021

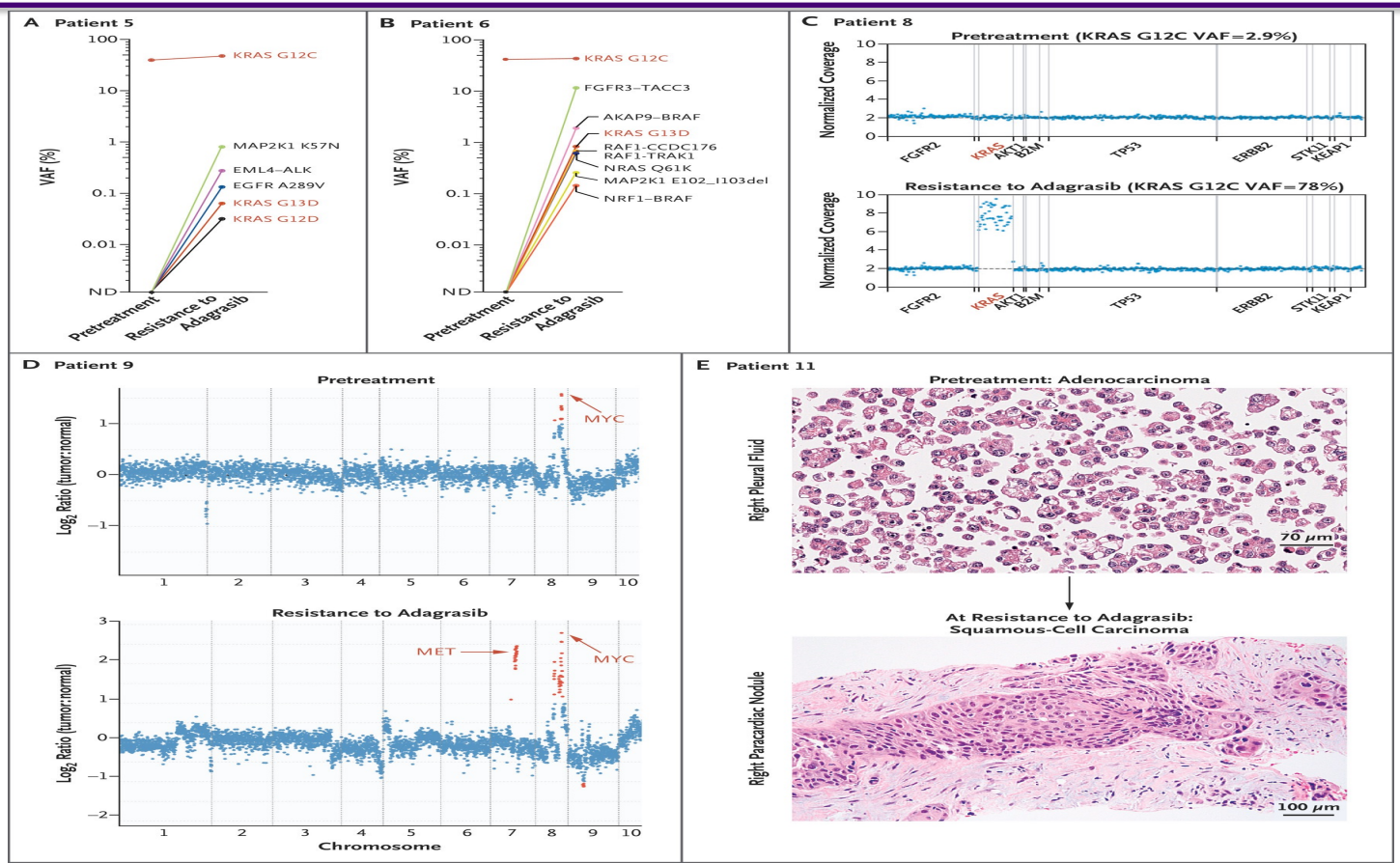


TABLE. Clinical Development of *KRAS* Mutant–Specific Inhibitors

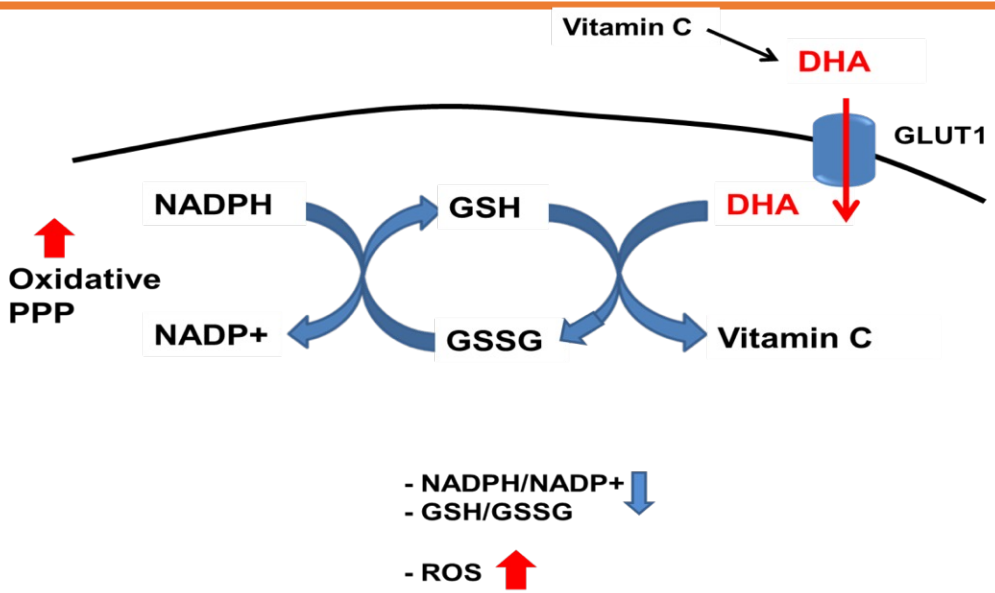
Drug (sponsor/collaborator)	Description	Phase Clinical trial name/ Clinicaltrials.gov identifier
KRAS G12C inhibitors		
Sotorasib (AMG 510) (Amgen)	Vs docetaxel in previously treated locally advanced and unresectable or metastatic *KRAS* G12C–mutant NSCLC	Phase 3 CodeBreak 200/ NCT04303780
	In *KRAS* G12C–mutant advanced nonsquamous NSCLC	Phase 2 Lung-MAP cohort/NCT04625647[a]
	+/- a PD-1/PD-L1 inhibitor in *KRAS* G12C–mutant advanced solid tumors	Phase 1/2 CodeBreak 100/NCT03600883
	+/- various different drugs in *KRAS* G12C–mutant advanced solid tumors	Phase 1 CodeBreak 101/NCT04185883
Adagrasib (MRTX849) (Mirati Therapeutics, Inc)	+ pembrolizumab in *KRAS* G12C–mutant advanced NSCLC	Phase 2 KRYSTAL-7/NCT04613596
	+/- cetuximab, afatinib, or pembrolizumab in *KRAS* G12C–mutant advanced solid tumors	Phase 1/2 KRYSTAL-1/NCT03785249
	+ TNO155 (SHP2 inhibitor) in *KRAS* G12C–mutant advanced solid tumors	Phase1/2 KRYSTAL-2/NCT04330664
GDC-6036 (Genentech, Inc)	+/- atezolizumab, cetuximab, bevacizumab, or erlotinib in *KRAS* G12C–mutant advanced or metastatic solid tumors	Phase 1 GO42144/NCT04449874
Exosomes engineered to deliver siRNA targeting *KRAS* G12D		
iExosomes (The University of Texas MD Anderson Cancer Center)	In *KRAS* G12D–mutant metastatic pancreatic cancer	Phase 1 2018-0126/NCT03608631[a]
mRNA vaccine targeting *KRAS* G12C, G12D, G12V, and G13D		
mRNA-5671 (Moderna Therapeutics/Merck)	+/- pembrolizumab in *KRAS* G12C–, G12D–, G12V–, or G13D–mutant advanced/ metastatic NSCLC, CRC, or pancreatic adenocarcinoma with specific HLA subtypes	Phase 1 V941-001/NCT03948763
T cells transduced with *KRAS* G12V–specific TCRs		
KRAS TILs (Changhai Hospital/Providence Cancer Center, Earle A. Chiles Research Institute)	In *KRAS* G12V–mutant advanced pancreatic cancer with a specific HLA subtype	Phase 1/2 ChanghaiH-PP06/NCT04146298

CRC, colorectal cancer; HLA, human leukocyte antigen; NSCLC, non–small cell lung cancer siRNA, small interfering RNA; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte.

[a]Trial is not yet recruiting participants.

KRAS or BRAF mutant CRC cells rewire glucose metabolism by upregulating GLUT1 expression

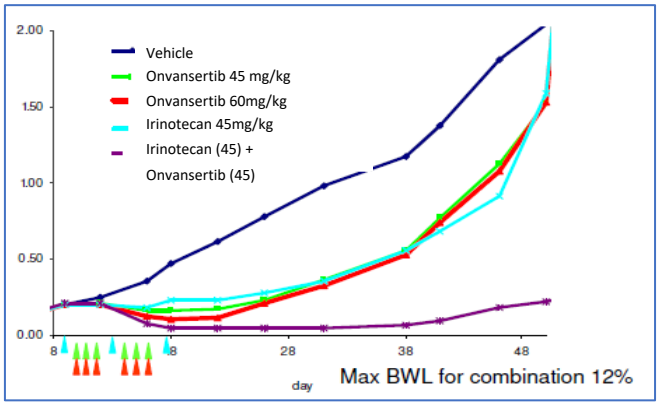
We developed a strategy for targeting KRAS or BRAF-mutant cancers by exploiting the selective high expression of GLUT1 and the high levels of reactive oxygen species (ROS) produced in these cells with vitamin C



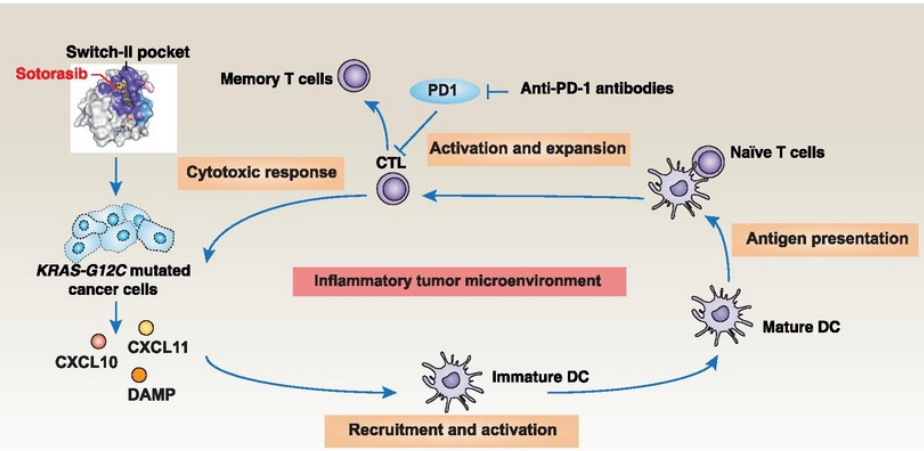
Our preclinical studies have shown that ascorbate selectively kills KRAS and BRAF mutant CRC cells in culture and genetically engineered mouse models

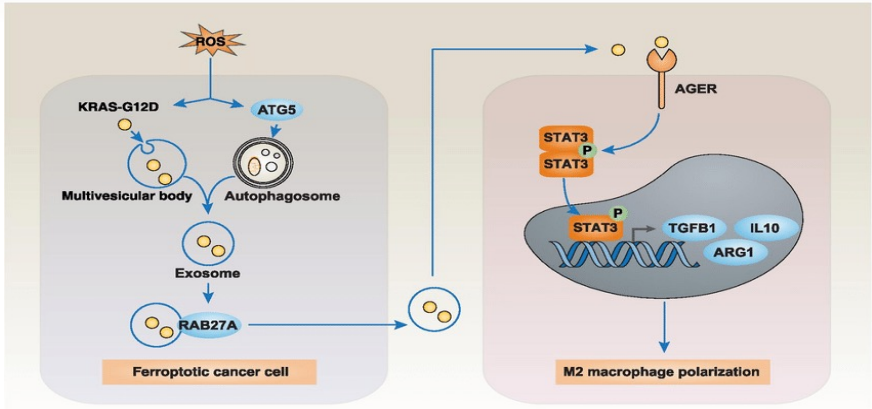
PCM-075 in Combination with Anticancer Agents in CRC

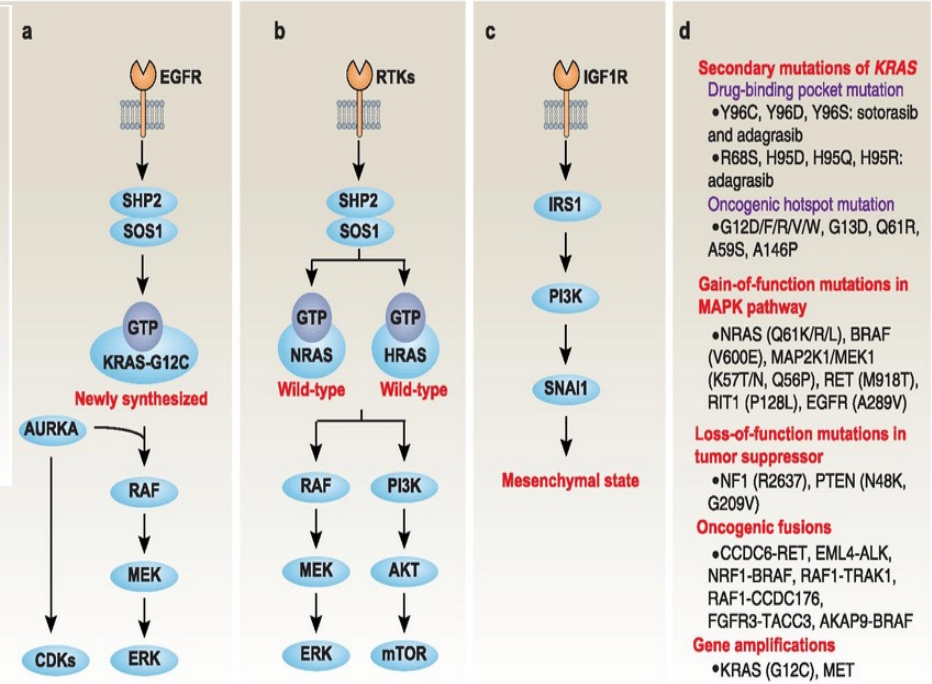
- **In the HCT116 cell line, PCM-075 was found to be synergistic in vitro with different class of drugs including:**
  - the chemotherapeutic agent cisplatin
  - the active metabolite of the topoisomerase inhibitor irinotecan (SN-38)
  - the microtubule inhibitor paclitaxel
- **In the HT29 xenograft model, PCM-075 was found to be:**
  - **Synergistic** with the topoisomerase inhibitor irinotecan
  - **Additive** with the chemotherapeutic agent fluorouracil (5FU) or the angiogenesis inhibitor bevacizumab



Kras mutations are immunosuppressive through AGER inducing STAT3, TFGB, IL10 and ARG1 leading to Polarization of M2 Makrophages

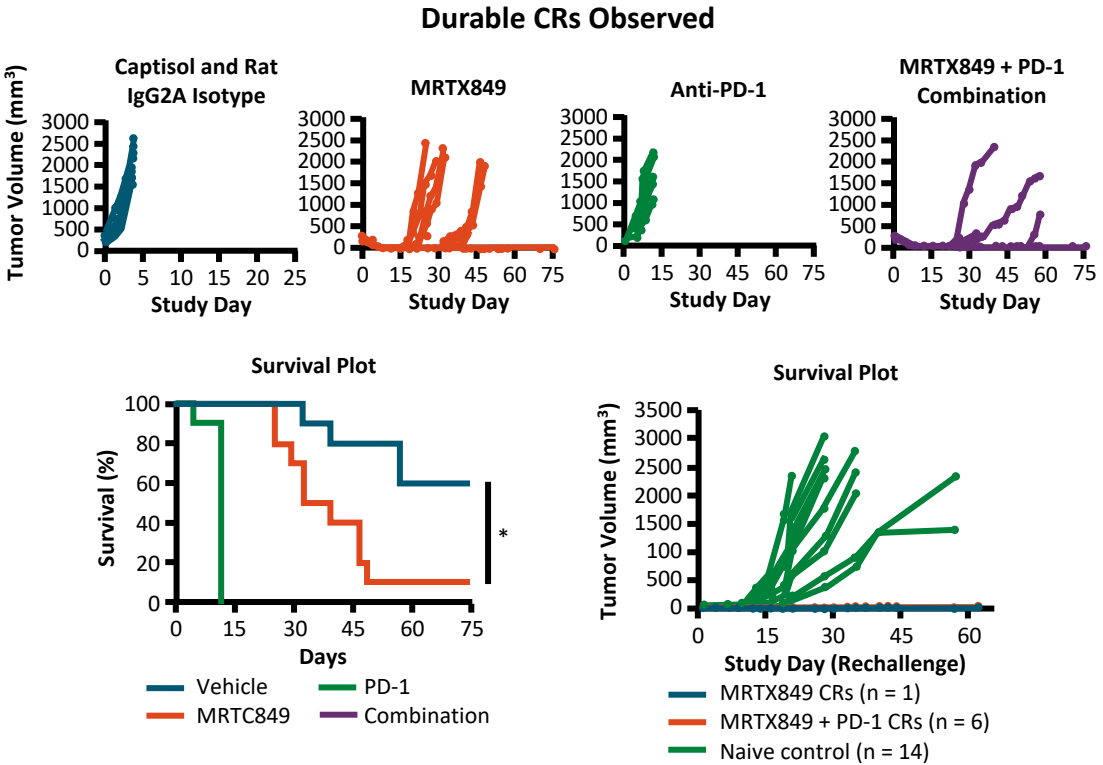






Tang et al Molecular Cancer 2021

KRAS^{G12C} Inhibition Enhances Immune Responses: MRTX849 and Anti-PD-1 in Syngeneic Mouse Model

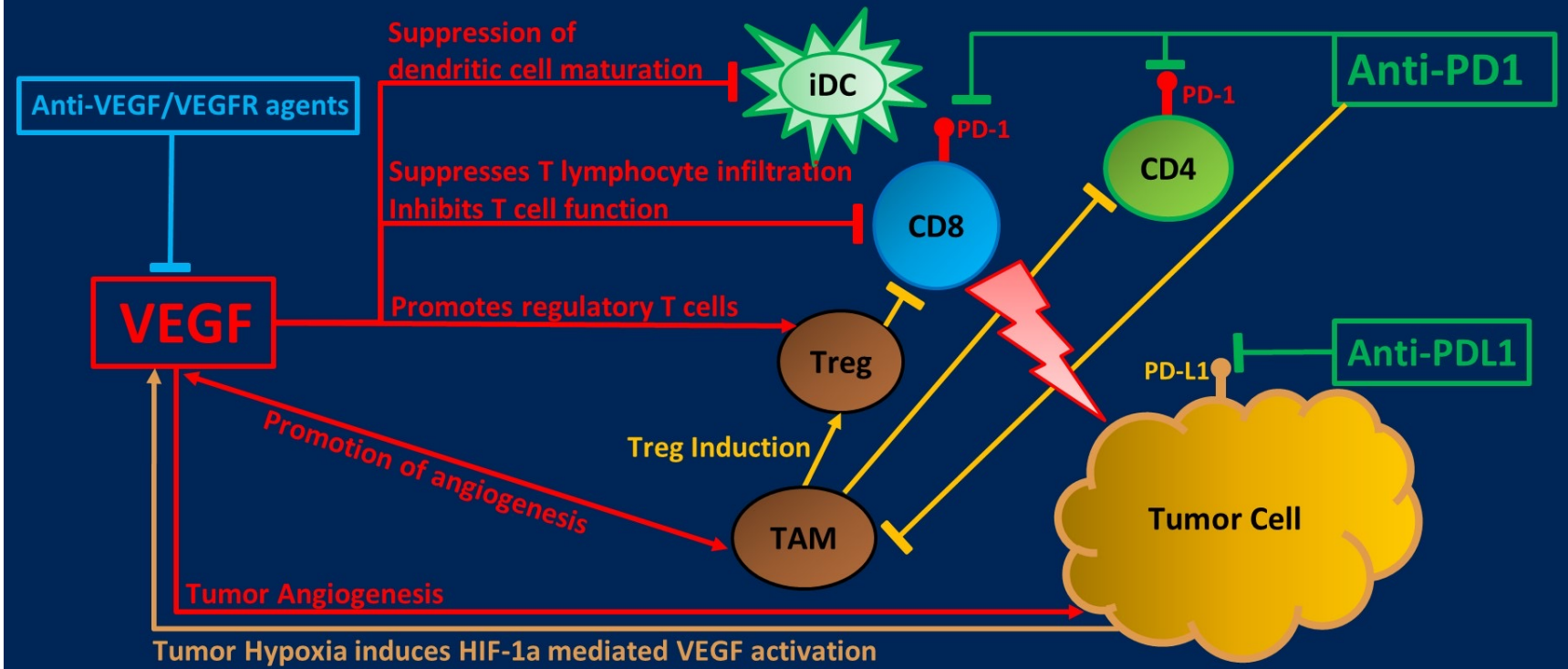


- MRTX849 plus anti–PD-1 leads to:
  - Decrease in M2-polarized macrophages, and M- and G-MDSCs
  - Increased immune promoting, macrophages, dendritic cells, CD4 and NK T-cells in CT26 KRAS^{G12C} tumors
  - Durable CRs, and a survival advantage relative to either single agent therapy observed in the majority of mice
    - Antitumor adaptive immune response, observed failure of implantation when rechallenged with CT26 KRAS^{G12C} cells compared with naive mice

Briere. AACR-NCI-EORTC 2019. Abstr LB-C09.

Slide credit: clinicaloptions.com

Rationale for Anti-VEGF + ICI



Abbreviations: iDC, immature dendritic cell; HIF-1a, hypoxia inducible factor-1a; TAM, tumor-associated macrophages; Treg, T-regulatory cell; VEGF, vascular endothelial growth factor.

PRESENTED BY: Richard Kim M.D.

References 1. Chen Y et al. J Biomed Sci. 2019;26(1):78.
2. Manegold C et al. J Thorac Oncol. 2017;12(2):194-207.
3. Saeed A et al. J Hematol Oncol. 2021;14(1):13.

Colorectal Liver Metastases- Mechanisms of Immune Resistance??

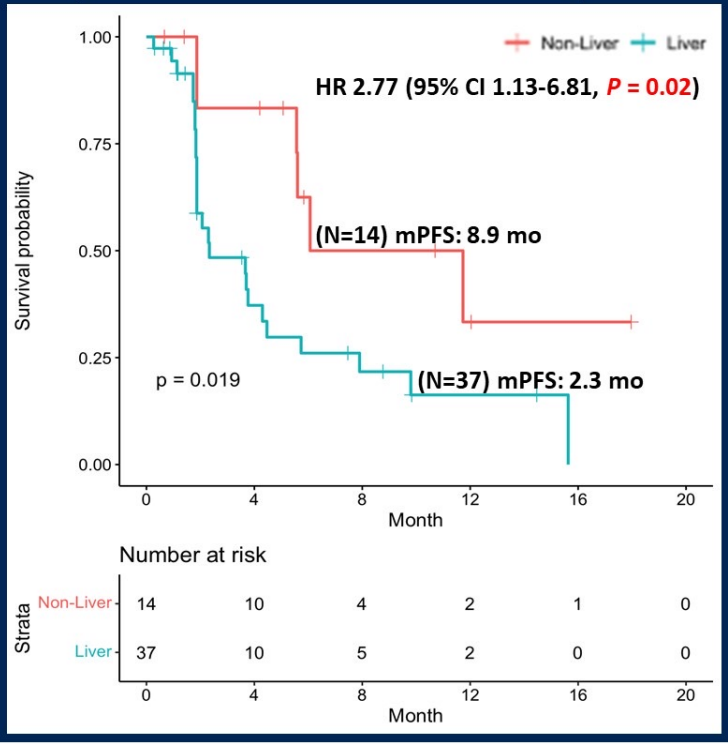
- Liver microenvironment is immunosuppressive which need to be elucidated.
- Liver metastases may induce a systemic immunosuppressive effect, thereby inhibiting antitumor immunity- ie. lower CD8+ T-cell infiltration[1].
- Liver metastasis attracts immunosuppressive macrophages that induce apoptosis of tumor antigen–specific T cells within the liver [2].
- Liver metastasis and accumulation of Tregs[3].

PRESENTED BY: Richard Kim M.D.

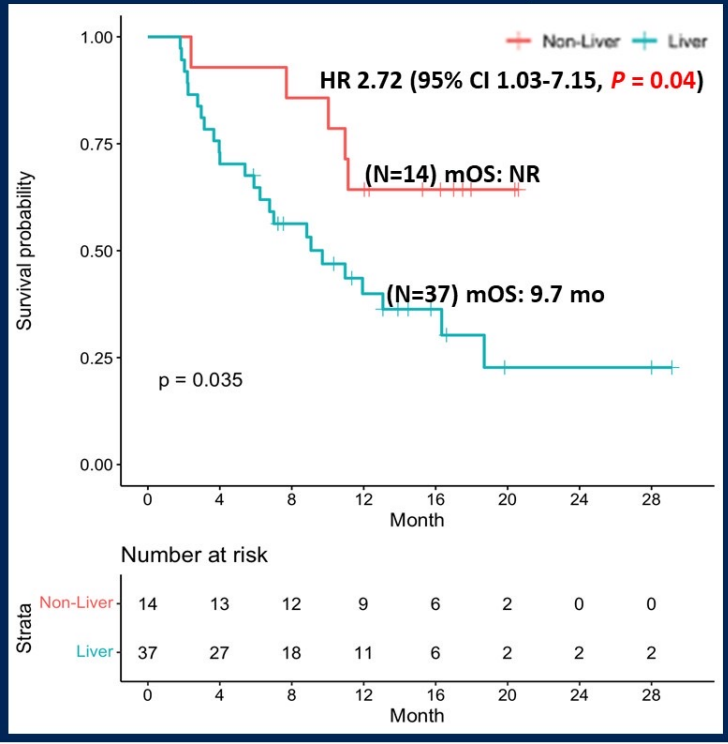
References: 1. Tumeh PC et al Cancer Immunol Res. 2017.
2. Yu J et al Nat Med. 2021
3. Katz, S.C. et al Ann. Surg. Oncol. 2013

PFS and OS by Presence and Lack of Liver Metastases in MSS MCRC

Progression Free Survival



Overall Survival

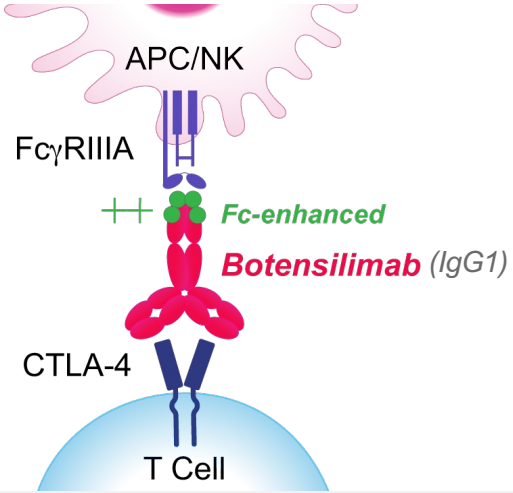


PRESENTED BY: Richard Kim M.D.

References: 1. Kim R et al. EJOC 2022 in-press.

Novel Immunotherapy Agents

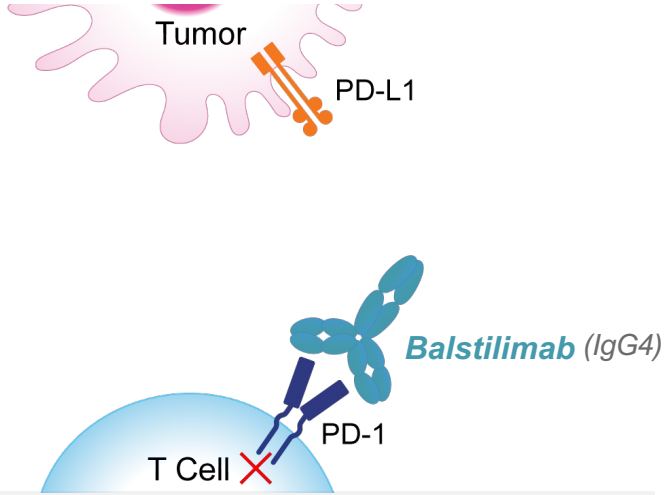
botensilimab
Fc-enhanced CTLA-4 Inhibitor



Active in cold and IO refractory tumors:[1]

- ↑ T cell priming, expansion, memory
- ↑ Treg depletion
- ↓ Complement mediated toxicity[4]

balstilimab
PD-1 Inhibitor

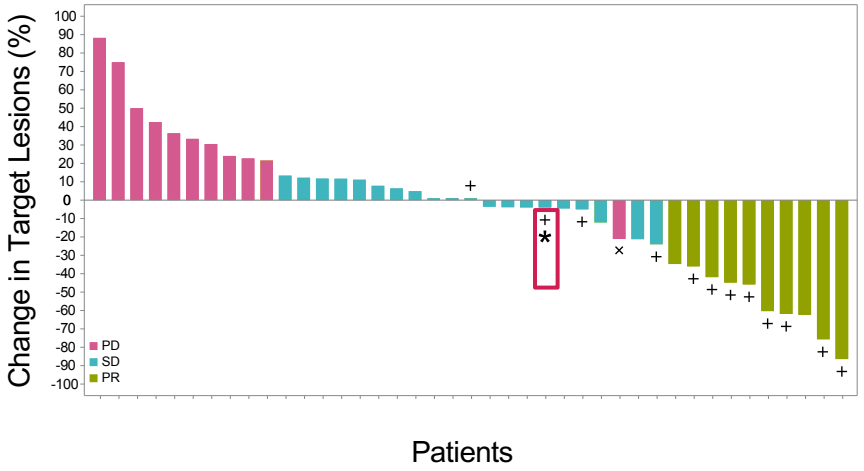


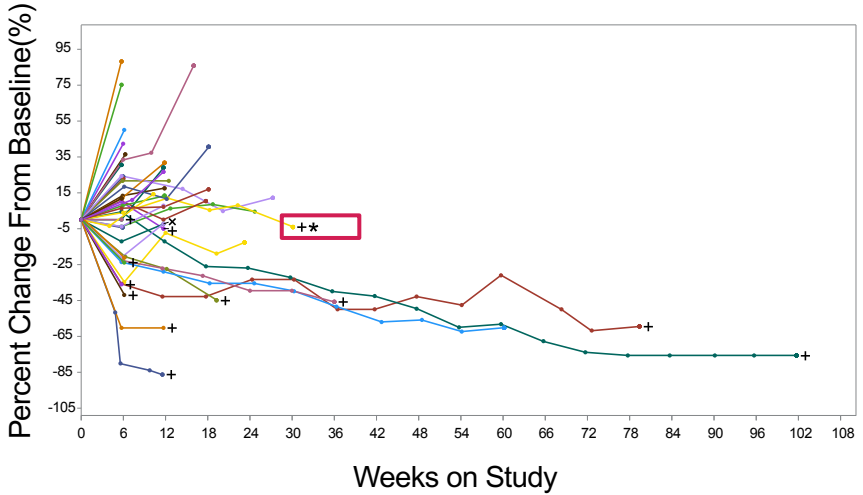
Safety and efficacy analogous to approved anti-PD-1 mAbs[2,3]

- >650 patients treated
- X ongoing trials / X completed[refs-clinical trials.gov]

1. Waight et al. Cancer Cell. 2018;33(6): 1033-1047. 2. O'Malley, et al. Gynecol Oncol. 2021; 163: 274-280. 3. O'Malley et al, J Clin Oncol. 2022; 40(7): 762-771. 4. El-Khoueiry AB. SITC 2021 Annual Meeting. Poster #479.

Durable Objective Responses (N=41)





- **8/10 objective responses ongoing**
- DOR range: 0.0+ to 17.0+ months
- Median follow-up: 5.8 months (range, 1.6-24.4)

ORR 24%

DCR 73%

+Ongoing PR/SD. *Complete metabolic response by PET. xProgression of non-target lesions.

Safety

TRAEs in ≥10% of Patients (N=41)

TRAE, n (%)	Any Grade	Grade 1-2	Grade 3
Any	31 (76)	21 (51)	10 (24)
GASTROINTESTINAL			
Diarrhea/colitis	16 (39)	12 (29)	4 (10)
Nausea	7 (17)	7 (17)	0
Vomiting	4 (10)	4 (10)	0
SKIN			
Pruritus	4 (10)	4 (10)	0
Rash	4 (10)	4 (10)	0
INVESTIGATIONS			
Alanine aminotransferase increased	5 (12)	5 (12)	0
Aspartate aminotransferase increased	4 (10)	3 (7)	1 (2)
MUSCULOSKELETAL			
Arthralgia	5 (12)	4 (10)	1 (2)
Myalgia	5 (12)	5 (12)	0
GENERAL			
Fatigue	9 (22)	8 (20)	1 (2)
Chills	7 (17)	7 (17)	0
Pyrexia	6 (15)	5 (12)	1 (2)
METABOLISM			
Decreased appetite	9 (22)	9 (22)	0

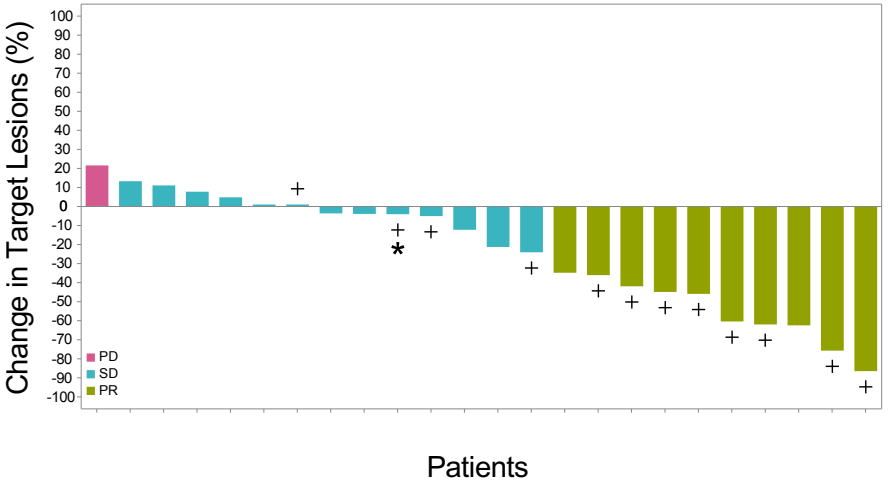
No grade 4 or 5 TRAEs
No hypophysitis

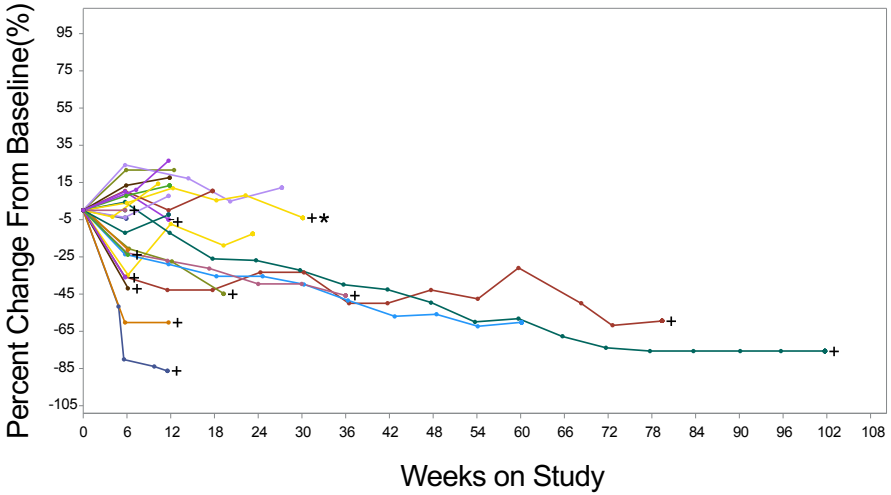
Discontinuation due to a TRAE:
- 10% Bot only
- 10% Bot and Bal

irAEs (investigator-assessed):
- 46% any grade
- 17% grade 3

Median duration of treatment:
- 2.8 months (95% CI, 1.2-22.8)

No Active Liver Metastases (n=24)





Definition of no active liver metastases:
- no history of liver metastases **OR**
- resected or ablated without recurrence

ORR 42%

DCR 96%

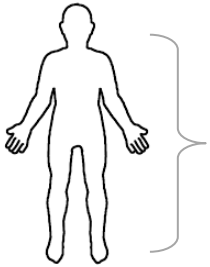
+Ongoing PR/SD. *Complete metabolic response by PET.

Novel Approaches (Future)

Do we need Liquid Biopsies to Predict Metastatic Disease

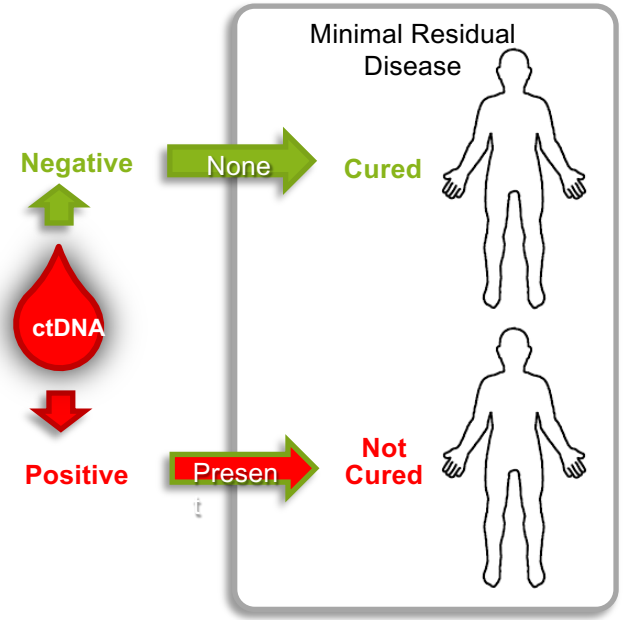
Minimal Residual Disease: The Clinical Problem (Metastatic?)

Stage III CRC:
All patients get adjuvant chemo
>50% cured by surgery alone



Curative Intent Surgery

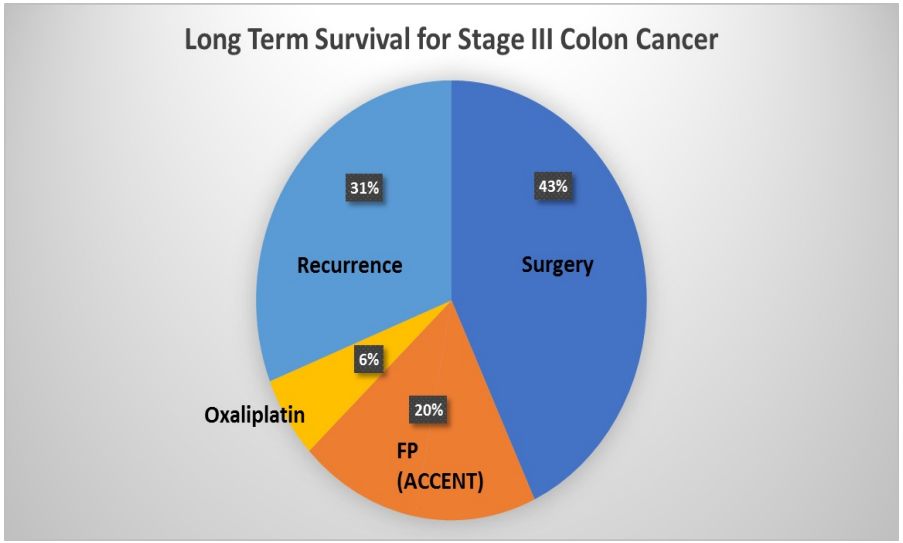
Stage II CRC:
SOC is NO adjuvant chemo
10-15% of patients recur



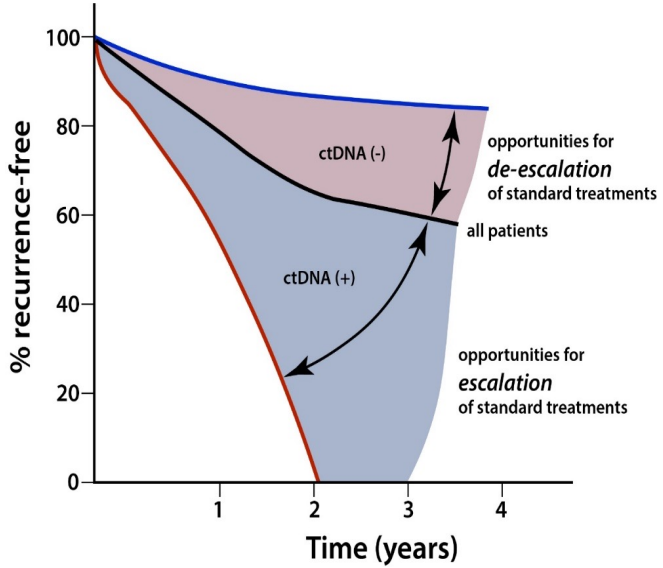
We have no way to determine who is cured and who will recur

Adjuvant Therapy in Stage III CC : Room for Improvement

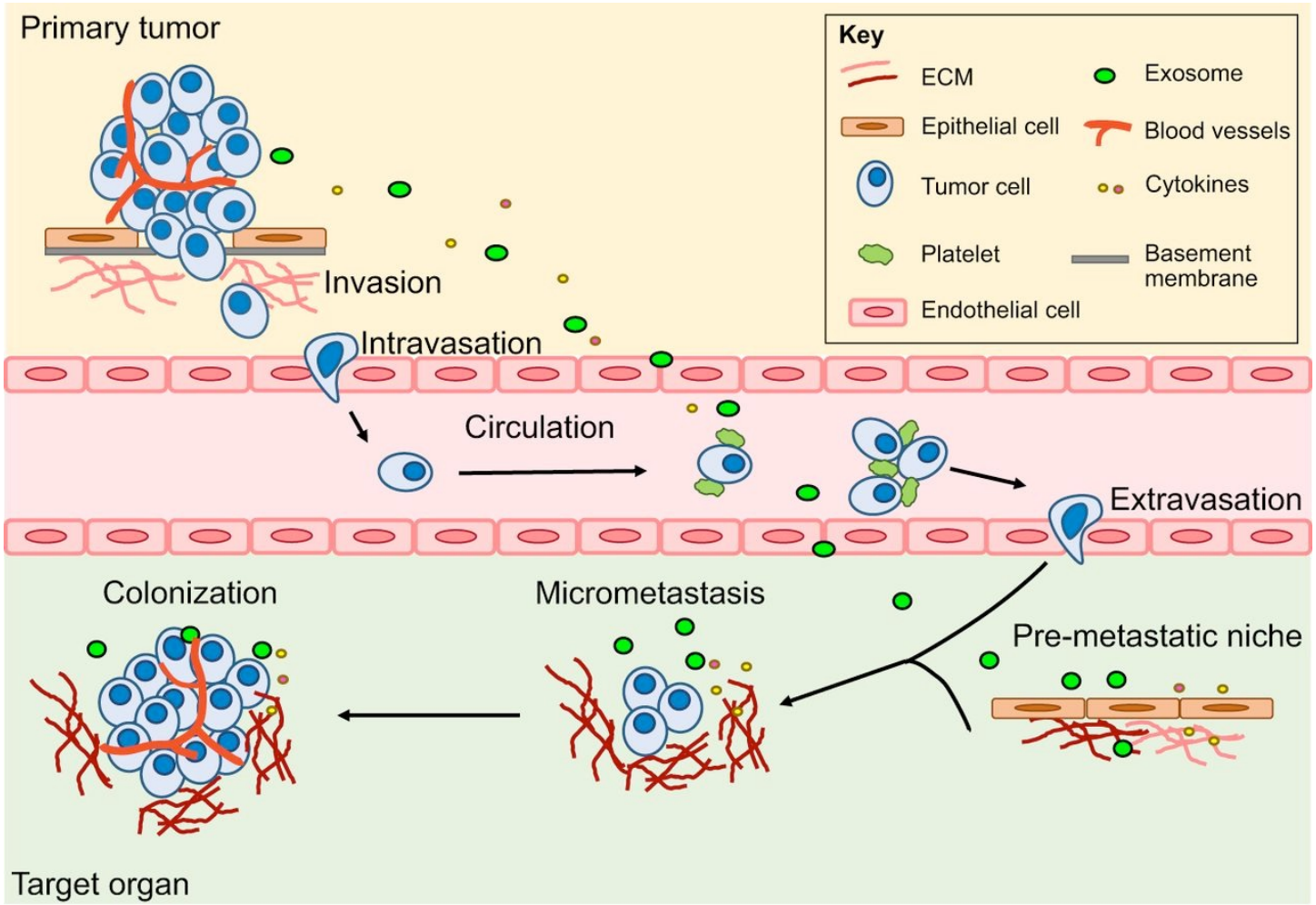
CURRENT (TNM):



FUTURE (ctDNA):

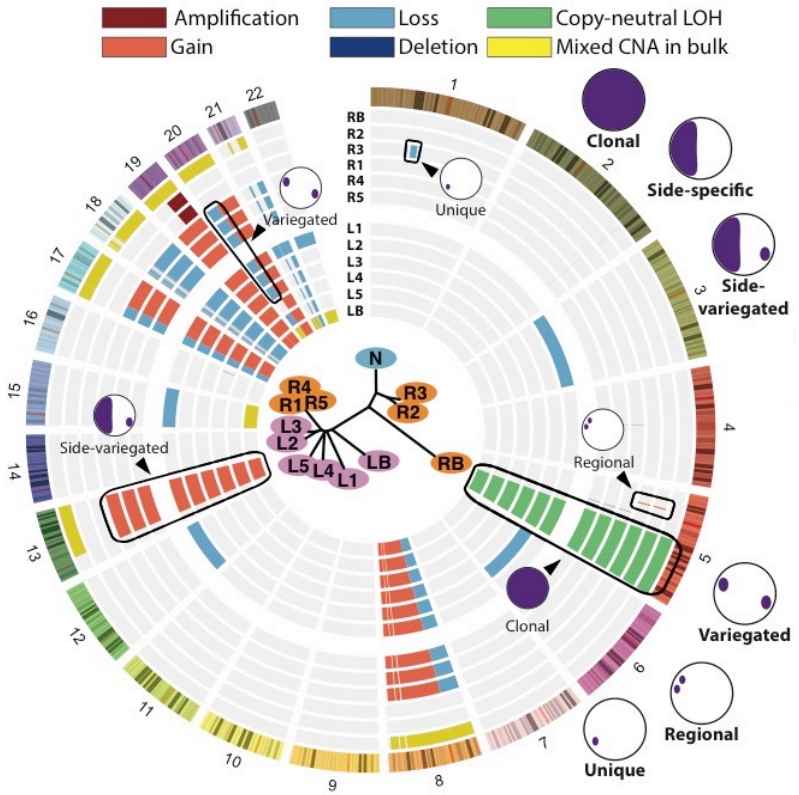


Adapted from
Sargent et al, JCO 2009
Andre et al, JCO 2015

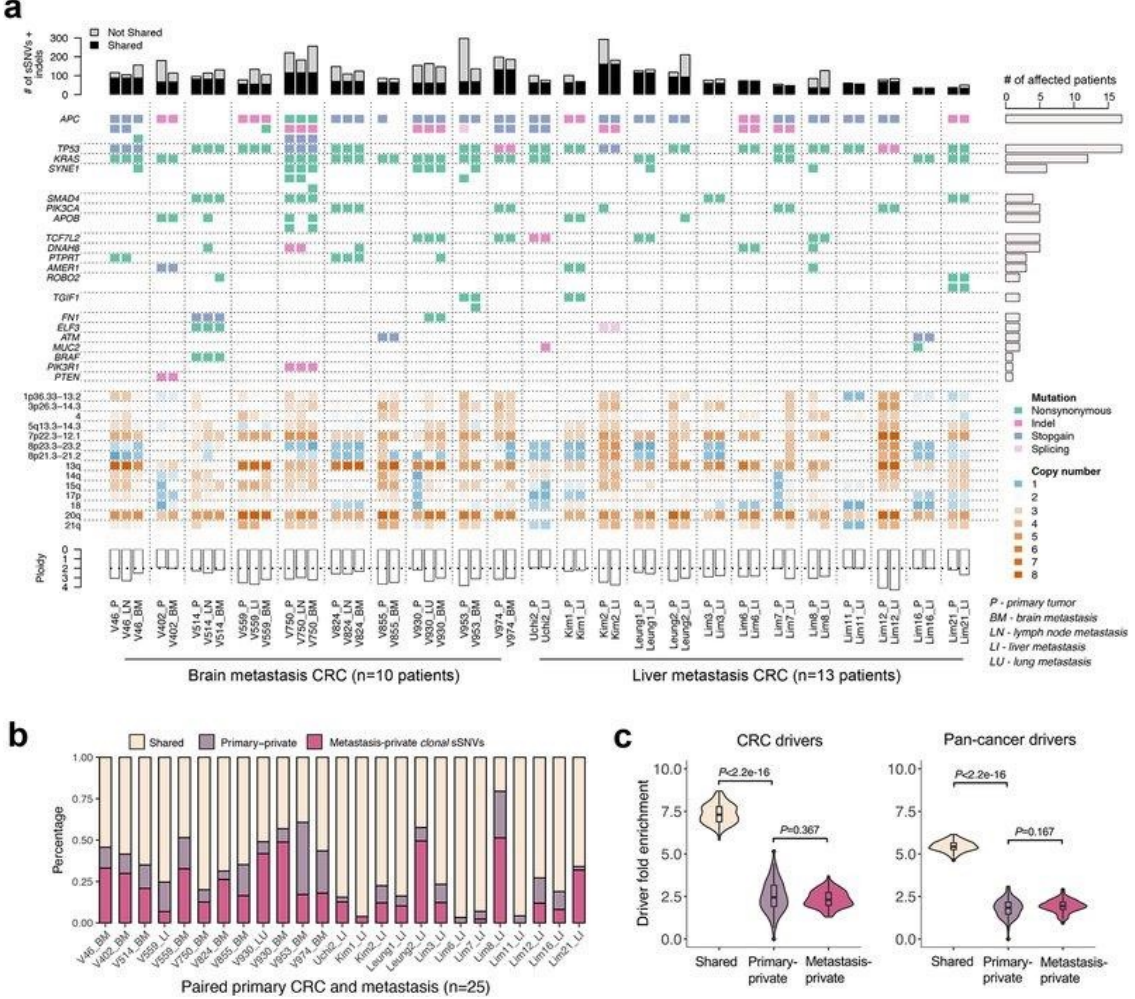


Gomez-Cuadrado, et al. 2017

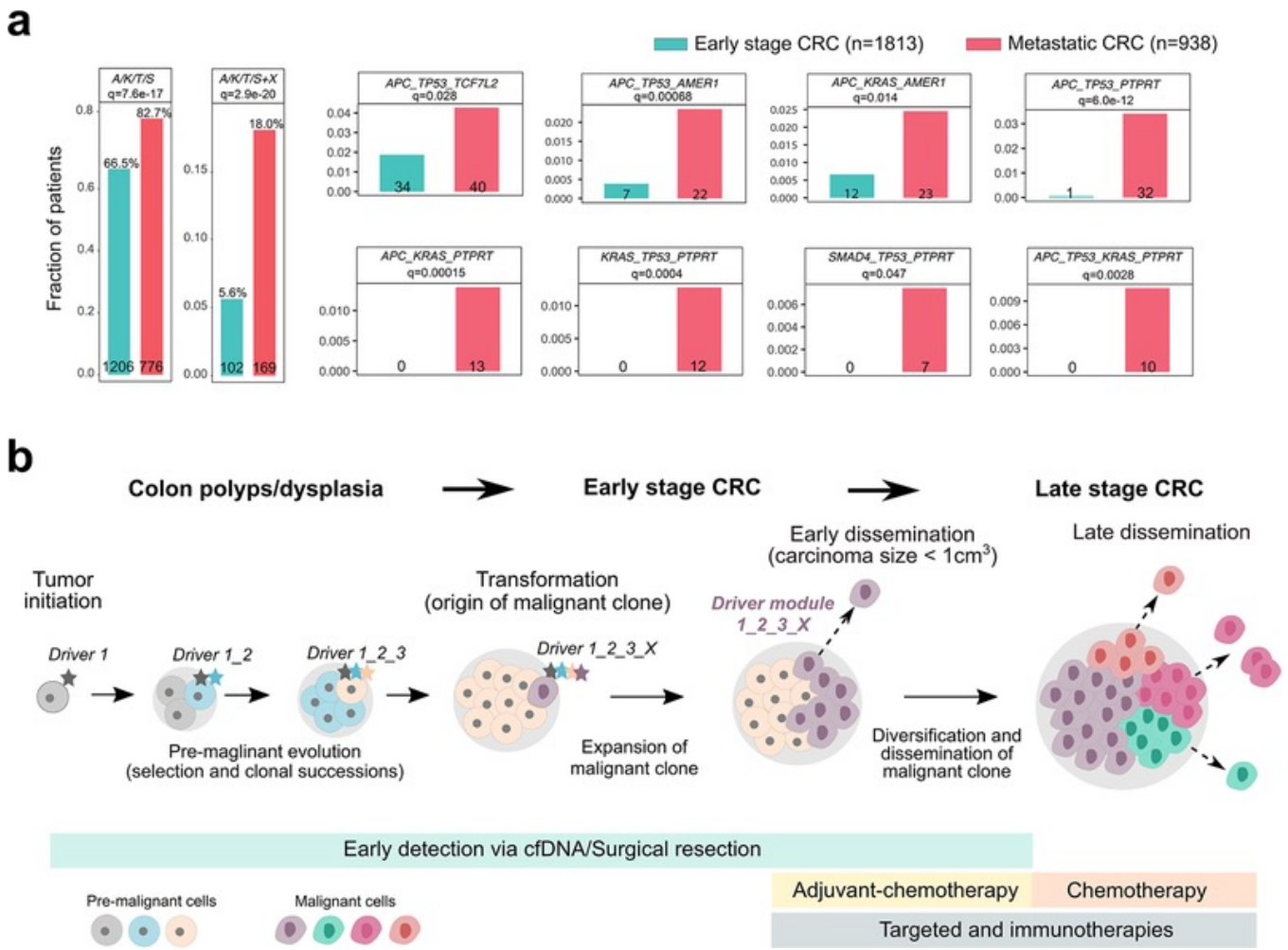
Intra-tumor copy number heterogeneity in CRC at the single gland level



Curtis et al Nature Genetics 2019

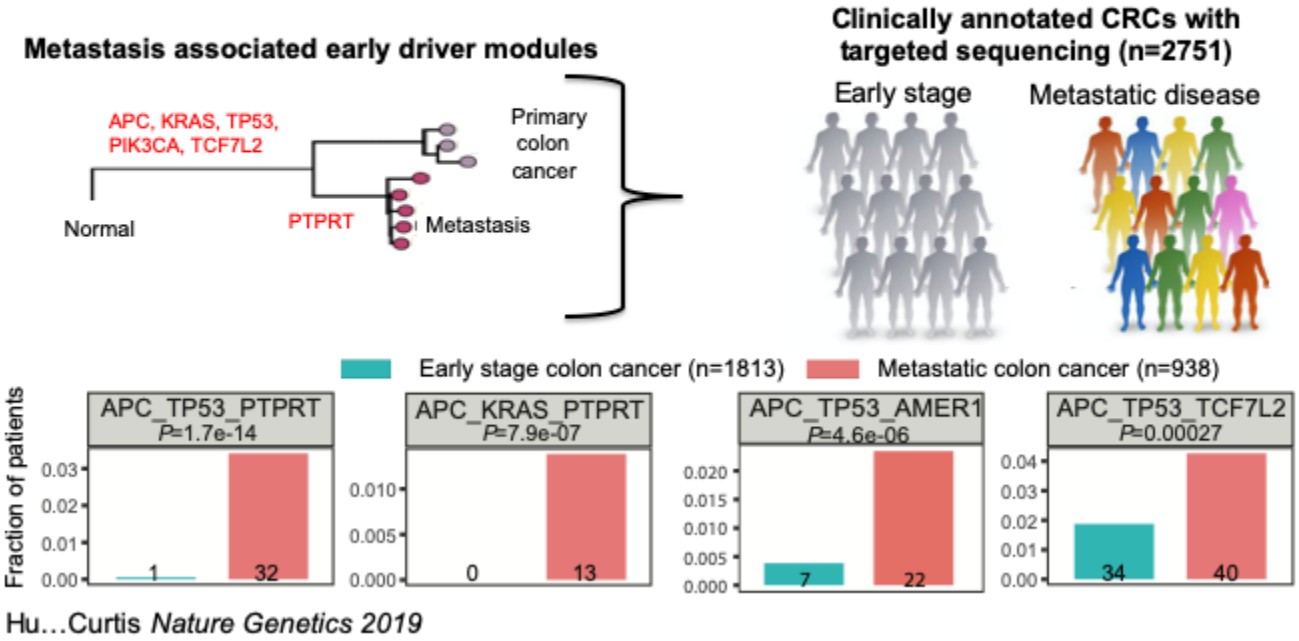


Hu et al Nature Genetics 2019



Hu et al Nature Genetic 2019

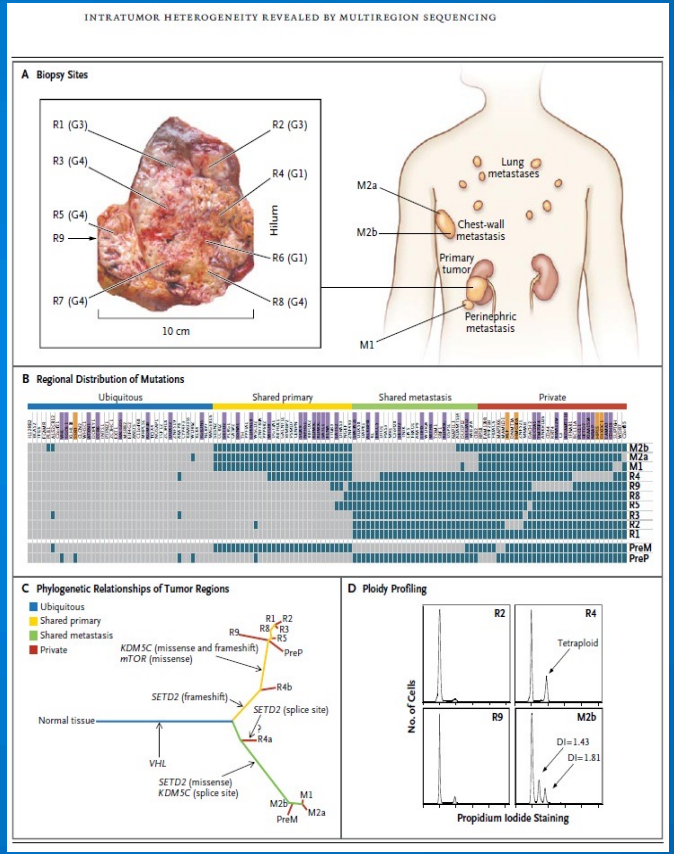
Validation of metastasis driver modules

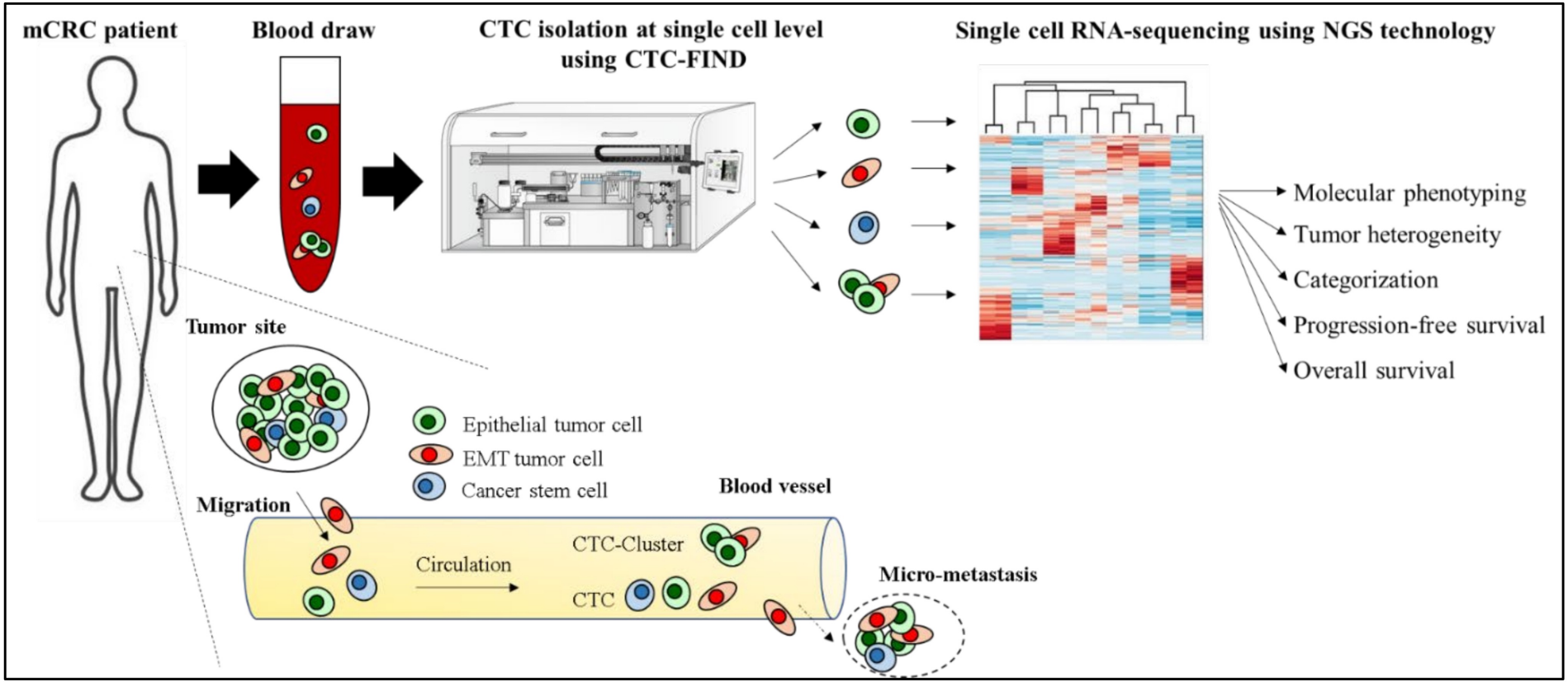


Hu…Curtis *Nature Genetics 2019*

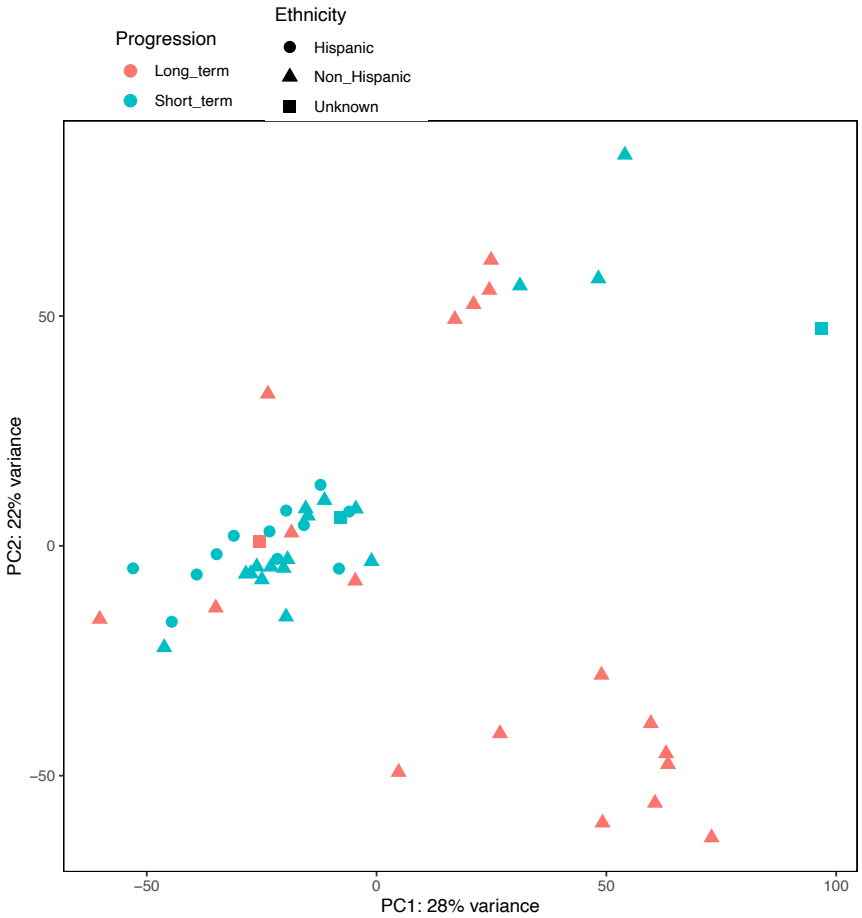
Heterogeneity also exists within individual tumors

- Ding et al., Nature 2010
  - Mutations present in 5–90% of sequencing reads from one tumor
- Navin et al., Nature 2011
  - Independent subclones coexisting in a single anatomic site in breast
- Gerlinger et al., NEJM 2012
  - Two-thirds of mutations in single biopsies were not uniformly detectable throughout all sampled regions
  - Both sensitive and resistant RNA expression patterns

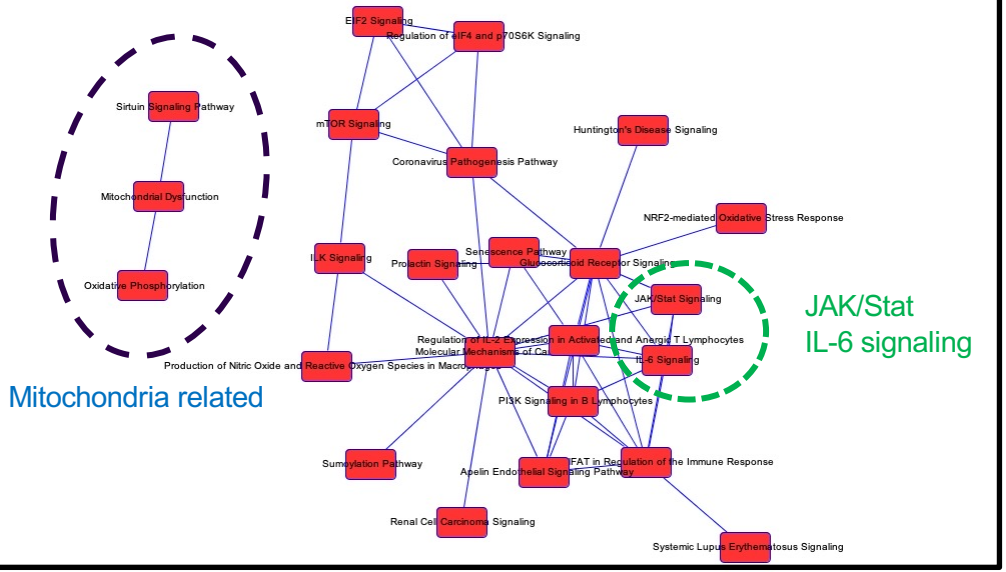




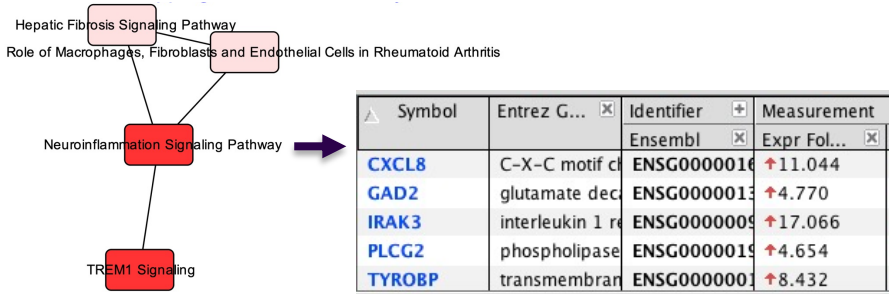
Kozuka et al Cancers 2021



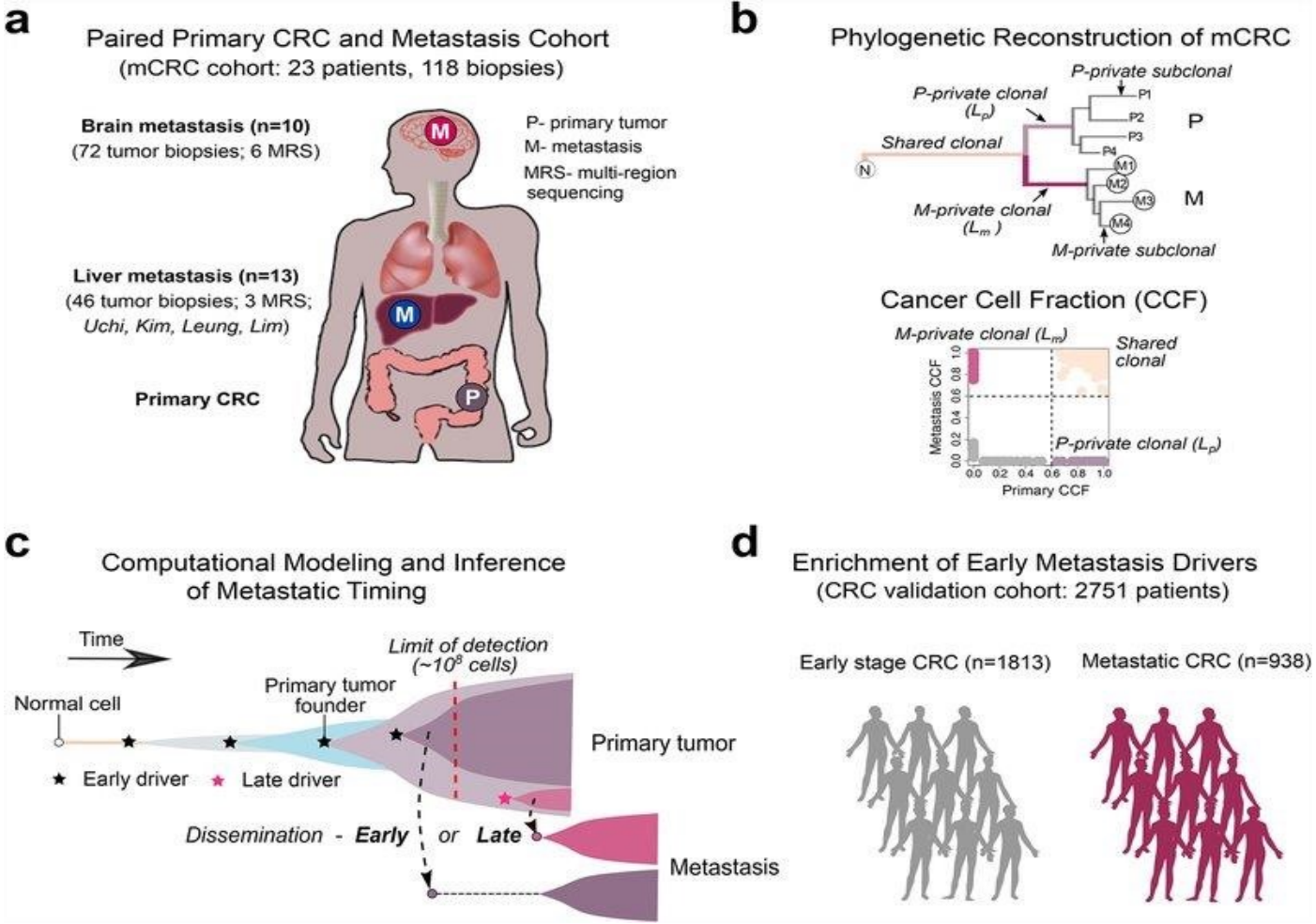
Top 40 enriched pathway network upregulated in short term vs. long te



Top enriched pathway network upregulated in Hispanic vs. non-hispan



Symbol	Entrez G...	Identifier Ensembl	Measurement Expr Fol...
CXCL8	C-X-C motif ch	ENSG0000016	↑11.044
GAD2	glutamate deca	ENSG0000013	↑4.770
IRAK3	interleukin 1 re	ENSG0000009	↑17.066
PLCG2	phospholipase	ENSG0000019	↑4.654
TYROBP	transmembran	ENSG0000001	↑8.432

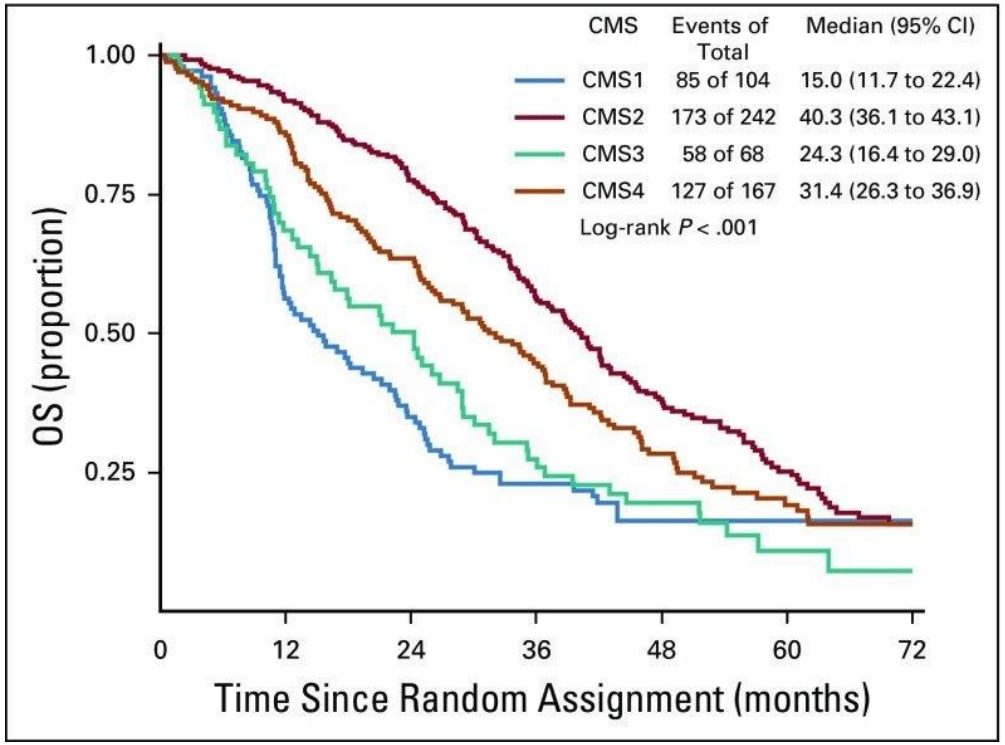


Hu et al Nature Genetics 2019

What is Unique about CRC and what we can learn from it

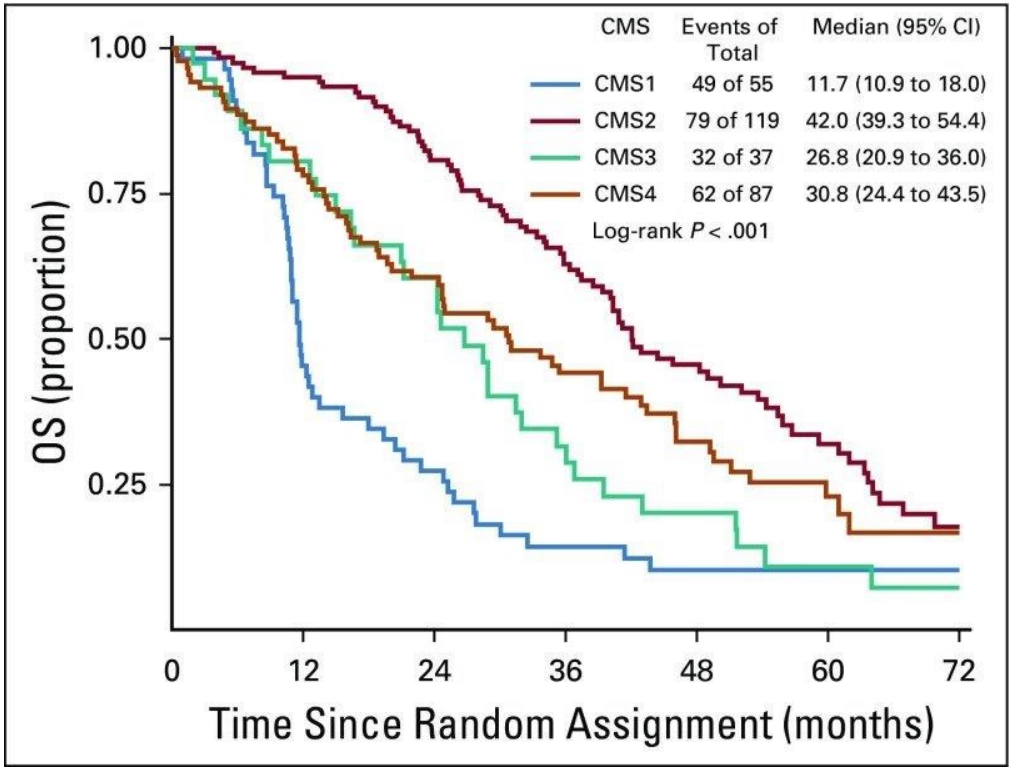
1. CMS
2. Tumor Heterogeneity
3. Single Cell Classification
4. NGS/RNA seq/New Targets

OS for all patients in SWOG/Alliance 80405



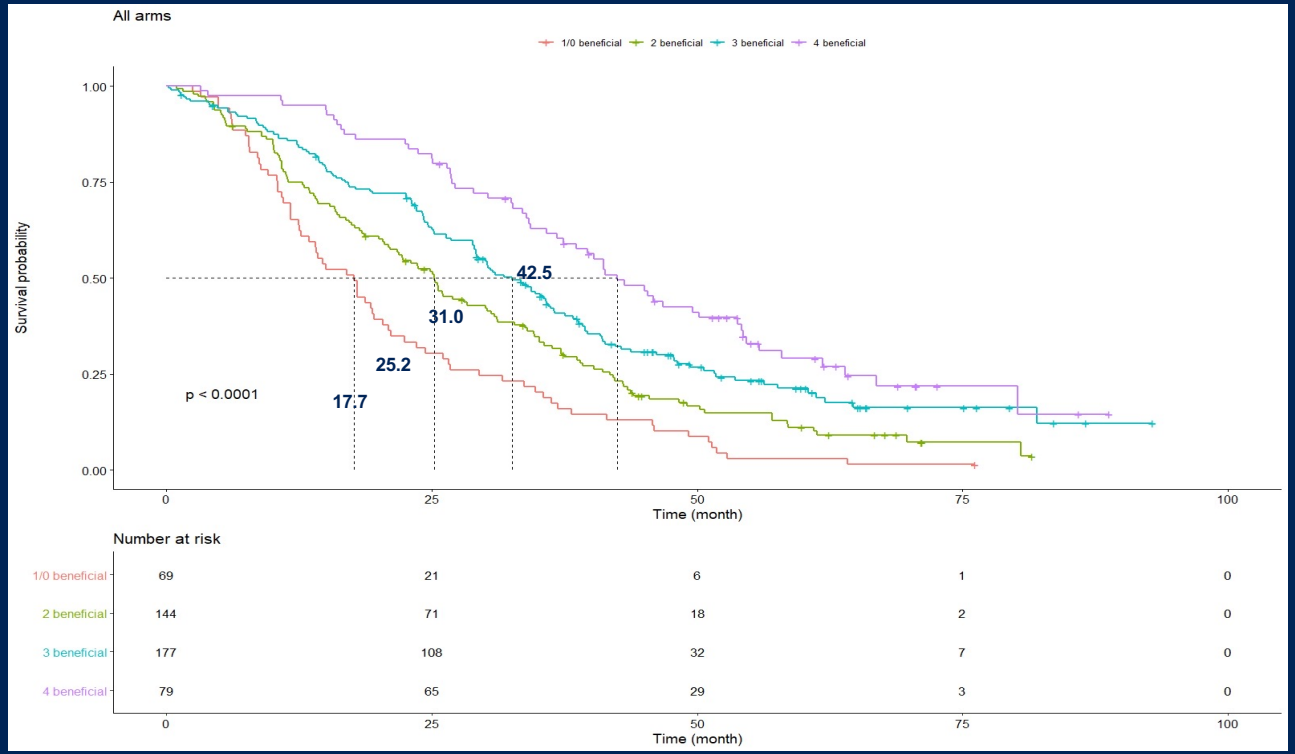
Lenz et al JCO 2019

OS for patients treated with Cetuximab

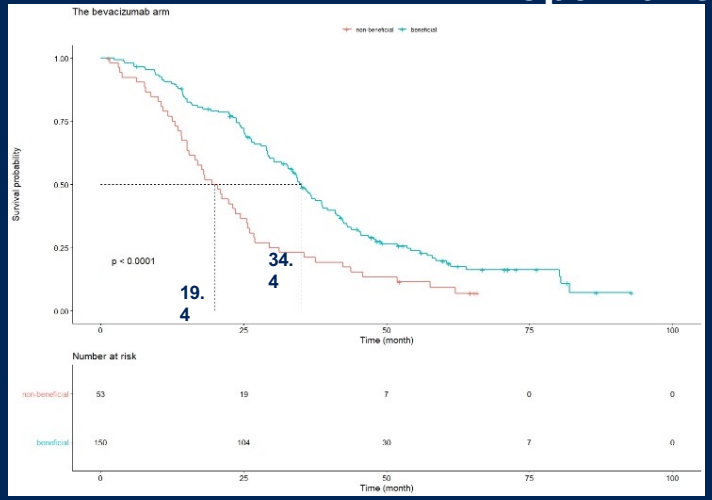


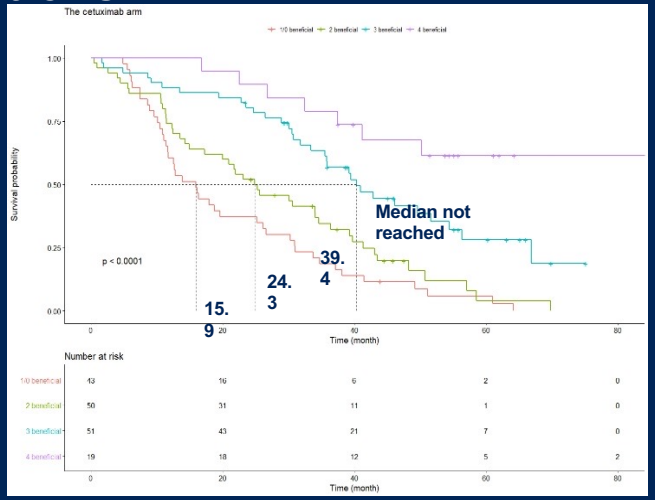
Lenz et al JCO 2019

In the three arms, combined effect of M2 macrophage, TGF-β, plasma cell, and memory activated CD4+ T cell signatures on OS, using optimal cut-offs



Effect of M2 macrophage, TGF-β, plasma cell, and memory activated CD4+ T cell signatures on OS for Cet (right) and of M2 macrophages for Bev (left), using optimal cut-offs





Conclusions

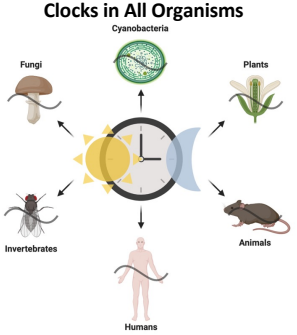
- Immune signatures affect prognosis and response to standard-of-care targeted therapy
- These results provide new markers for treatment selection and for the development of novel active combinations including immune checkpoint inhibitors

Federico Innocenti, MD, PhD
University of North Carolina at Chapel Hill

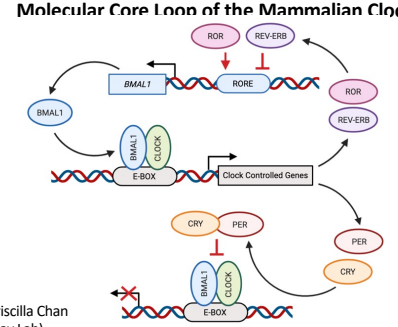
Novel Approaches

1. Novel Targets

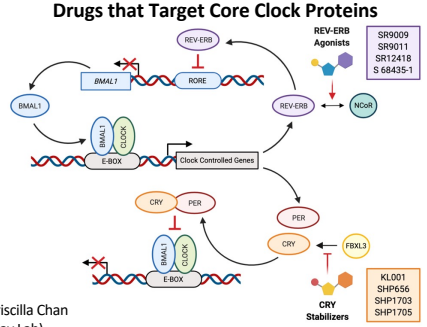
REV-ERB agonists as a novel therapeutic approach to colorectal cancer



Priscilla Chan (Kay Lab)



Priscilla Chan (Kay Lab)

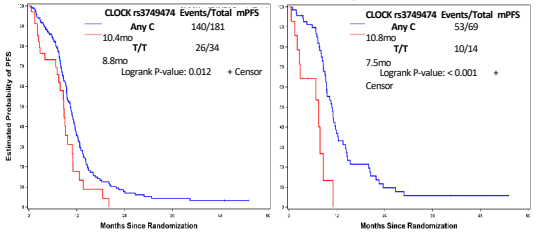


Priscilla Chan (Kay Lab)

mCRC Clinical Trials GWAS

1) First-line - Bevacizumab-based treatment: TRIBE + FIRE-3 trials (n tot = 451)

PFS results in TRIBE FOLFIRI-Bevacizumab cohort, overall and females

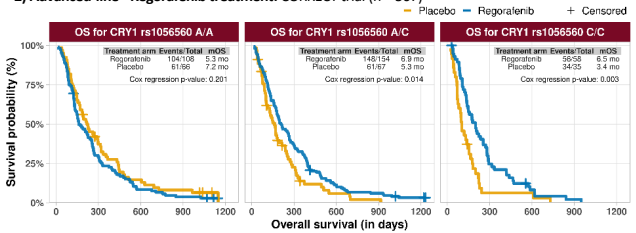


SNPs*	Progression-free Survival		
	P-value for FE	*Q* Statistics	*P*-value for *Q*
CLOCK rs3749474	0.005 (0.055)	7.276	0.064
BMAL1 rs2279287	0.025 (0.095)	3.312	0.346
RORA rs7164773	0.009 (0.070)	2.552	0.466
HTIM rs774034	0.014 (0.079)	0.152	0.985
HTIM rs4630333	0.004 (0.055)	1.426	0.699

Meta-analysis results across TRIBE and FIRE-3 treatment cohorts.

FE indicates fixed effects model based on inverse-variance-weighted effect size. Adjusted *P*-values after FDR (false discovery rate) are shown in parentheses (significant < 0.1)

2) Advanced-line - Regorafenib treatment: CORRECT trial (n = 507)



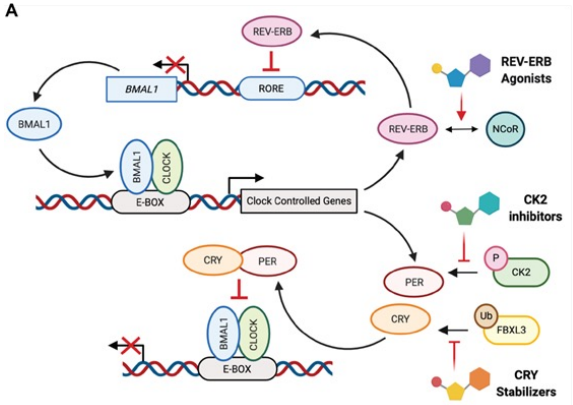
PFS results Rego vs placebo for *CRY1* rs1056560

Genotype Subset	n	HR (95%-CI)	*P*-value
A/A	174	0.71 (0.52-0.97)	**0.031**
A/C	221	0.49 (0.36-0.66)	**<0.001**
C/C	93	0.38 (0.24-0.61)	**<0.001**

*HR estimates for PFS from unadjusted models using the Cox-proportional hazard-model

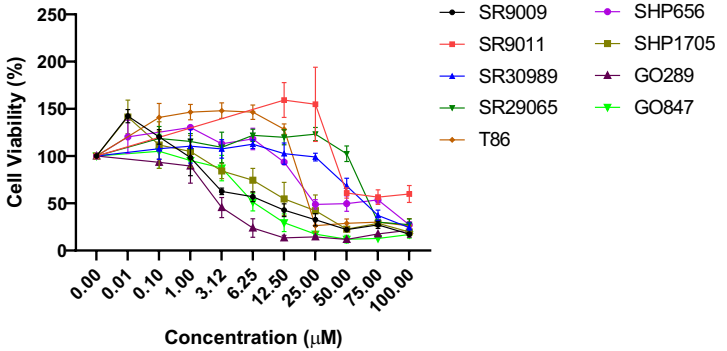
Battaglin et al. ASCO 2018 (Lenz Lab)
Battaglin et al. ESMO 2020 (Lenz Lab)

Drugging the Circadian Clock

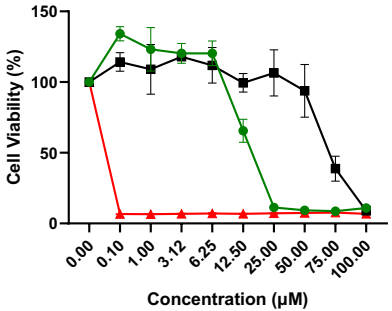


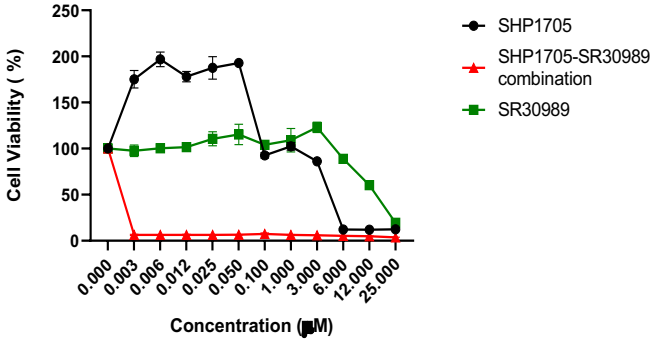
Compound	Expected Target	Mode of Action	Source	References
SR9009	REV-ERBα (also targets REV-ERBβ)	Agonist	Thomas Burris (purchased from Sigma-Aldrich)	Solt et al., 2012
SR9011	REV-ERBα (also targets REV-ERBβ)	Agonist	Thomas Burris (purchased from Sigma-Aldrich)	Solt et al., 2012
SR12418	REV-ERBα/β	Agonist	Laura Solt & Ted Kamenecka	Amir et al., 2018
S 68435-1	REV-ERBα	Agonist	Xavier Barril (provided by Laura Solt & Ted Kamenecka)	Westermaier et al., 2017
SR29065	REV-ERBα	Agonist	Laura Solt & Ted Kamenecka	-
SR30989	REV-ERBα	Agonist	Laura Solt & Ted Kamenecka	-
T86	REV-ERBα	Agonist	Laura Solt & Ted Kamenecka	-
KL001	CRY1/2	Stabilizer	Tsuyoshi Hirota & Steve Kay (purchased from Sigma-Aldrich)	Hirota et al., 2012
SHP656	CRY1/2	Stabilizer	Synchronicity	Dong et al., 2019
SHP1703	CRY1/2	Stabilizer	Synchronicity	-
SHP1705	CRY1/2	Stabilizer	Synchronicity	-
GO289	CK2	Inhibitor	Tsuyoshi Hirota	Oshima et al., 2019
GO847	CK2	Inhibitor	Tsuyoshi Hirota	-
CX-4945 (Silmitasertib)	CK2	Inhibitor	David M. Ryckman	Pierre et al., 2011

Discovery phase using 2D system
(representative data shown for HCT 116 (MSI, KRAS mutant, BRAF wildtype cell line)



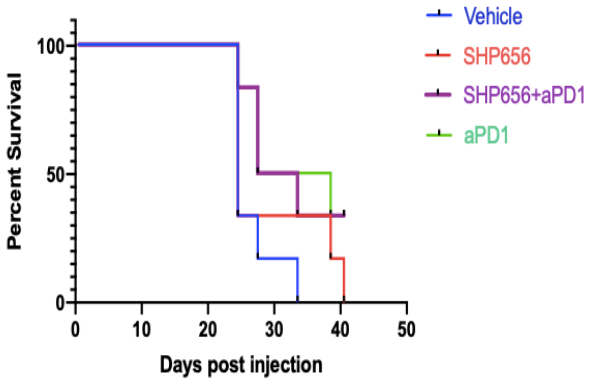
Compounds	EC_{50}
SR9009	3.241
T86	14.44
SHP656	15.61
SHP1705	8.889
GO289	2.539
GO847	5.792

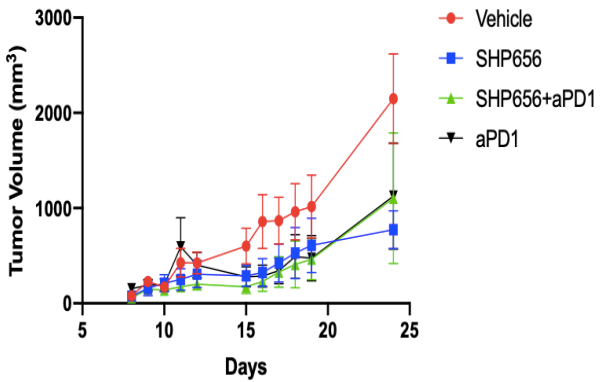




Original data: Lenz Lab(unpublished)

Anti-PD1 Treatment and/or Pharmacological Targeting of the Clock Prolongs Survival and slows tumor growth in an MSI high MC38 syngeneic mouse model (Proof of Principle)





In responders, treatment with SHP or SHP+aPD1, seems to be more effective in tumor growth suppression as compared to aPD1 alone

Our Goal: Right Treatment Right Time

- Genetic Testing of Tumor at time of diagnosis and if possible again at time of growth NGS/transcriptome
- Germline Testing of patients if evidence of Predisposition
- Active Monitoring with Liquid Biopsies
- Accelerating Access to Clinical Trials
- Identification of drugable Novel Targets
- Multi Omics Approach in the future (AI)

Conclusions

1. Detecting circulating tumor DNA technologies have been a tremendous progress to detect MRD and risk for recurrence. Need to increase sensitivity as about 50% of recurrences are detected with LB
2. Single cell technologies and understanding Tumor heterogeneity using AI are critical to potential predict presence of metastatic disease
3. Characterization of the TME will be the next frontier in Cancer Research to find novel therapies to prevent and treat colorectal cancer.

Immanuel Kant (Photo from a steel engraving)

The one who knows more, may decide better