BRAF mutant colorectal cancer: A different entity

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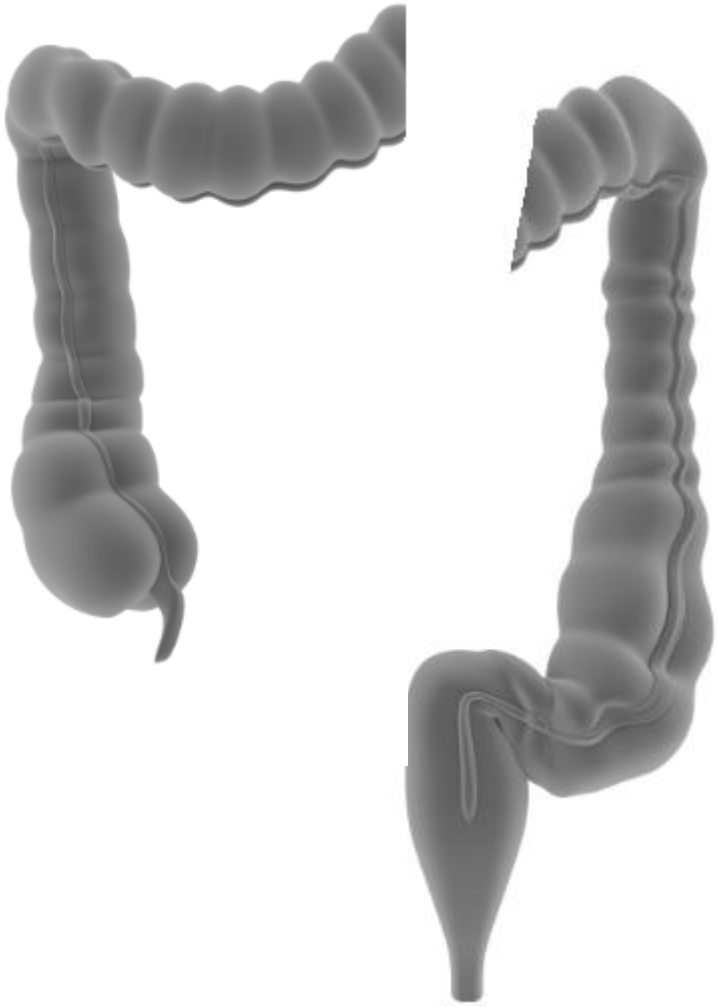
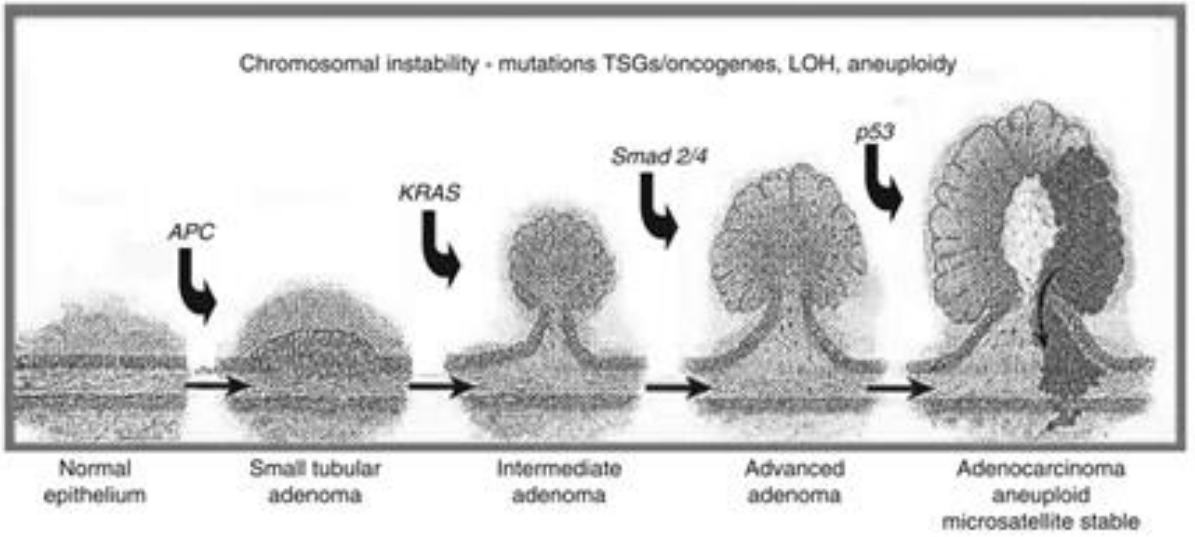
Colorectal cancer (CRC) is a heterogeneous disease but patients are not yet selected for individualized treatments



- Colorectal cancer is the second leading cause of cancer death
- Although several treatments exist, we do not have a good way to select patients for individualized treatments
- Only *RAS* status has been established as a predictor of anti-EGFR treatment activity
- New technologies that allow genetic definition of different types of colon cancer based on the expression, methylation, mutation rate of the genes might help in better understanding CRC biology
- The better understanding of CRC biology might drive personalized treatments

Colon carcinogenesis is a multi step process

The chromosomal instability (CIN) pathway

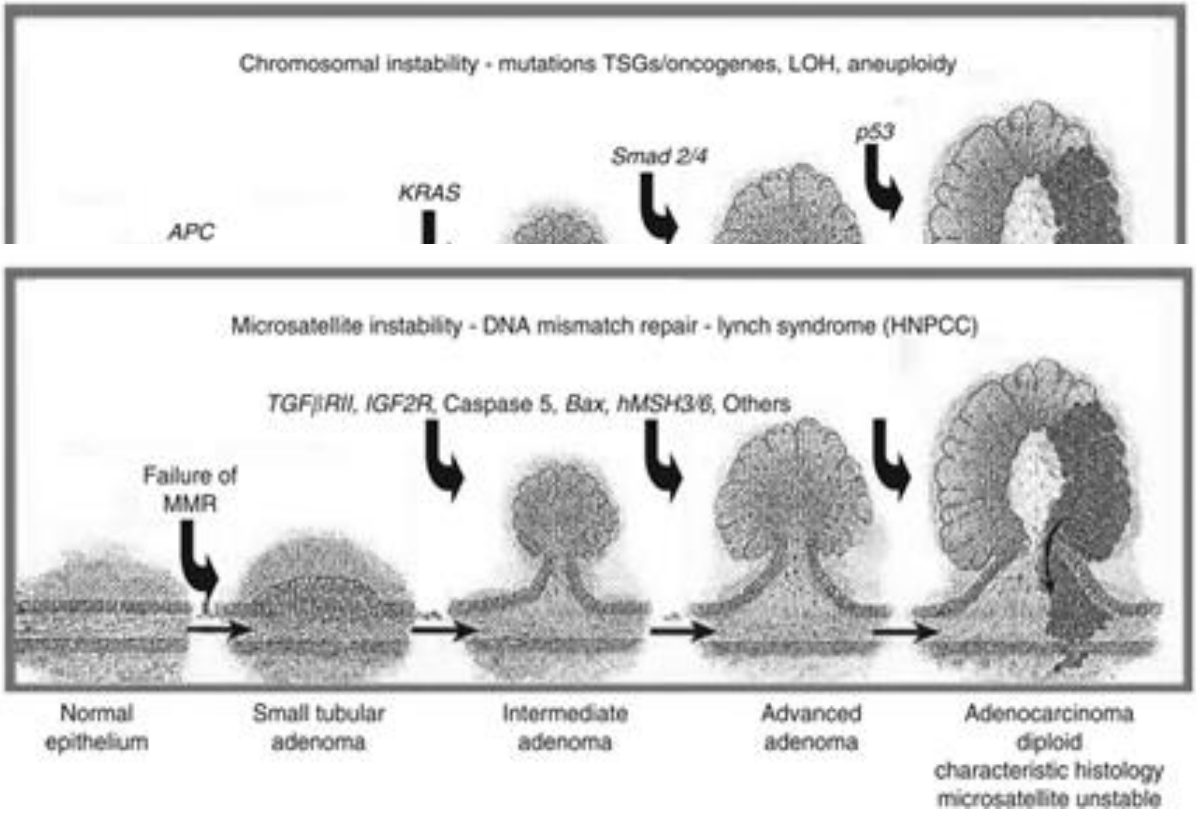


Adapted from Ahnen DJ: The American College of Gastroenterology Emily Couric Lecture—The Adenoma–Carcinoma Sequence Revisited: Has the Era of Genetic Tailoring Finally Arrived? ***The American Journal of Gastroenterology*** **106, 190-198 (February 2011)**

Colon carcinogenesis is a multi step process

The chromosomal instability (CIN) pathway

The microsatellite instability (MIN) pathway



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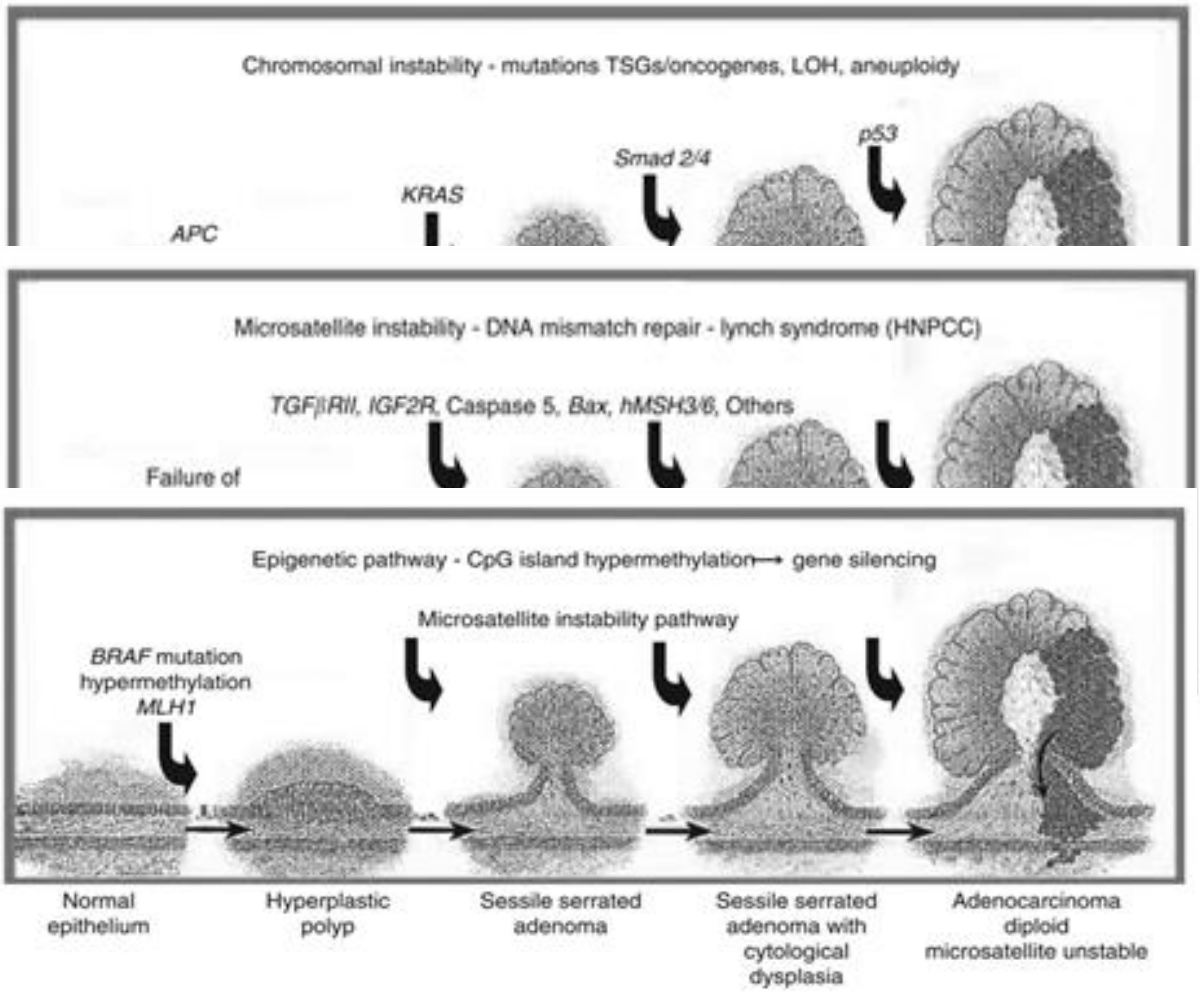
Colon carcinogenesis is a multi step process

The chromosomal instability (CIN) pathway

The microsatellite instability (MIN) pathway

The epigenetic (CIN) pathway



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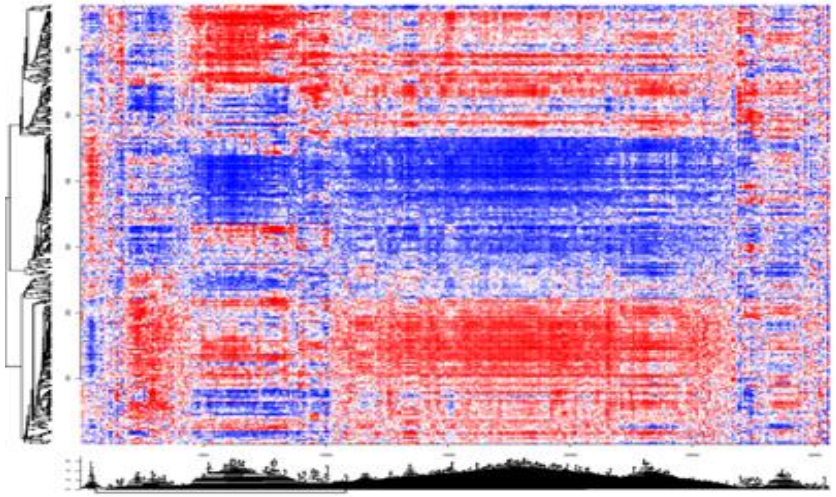
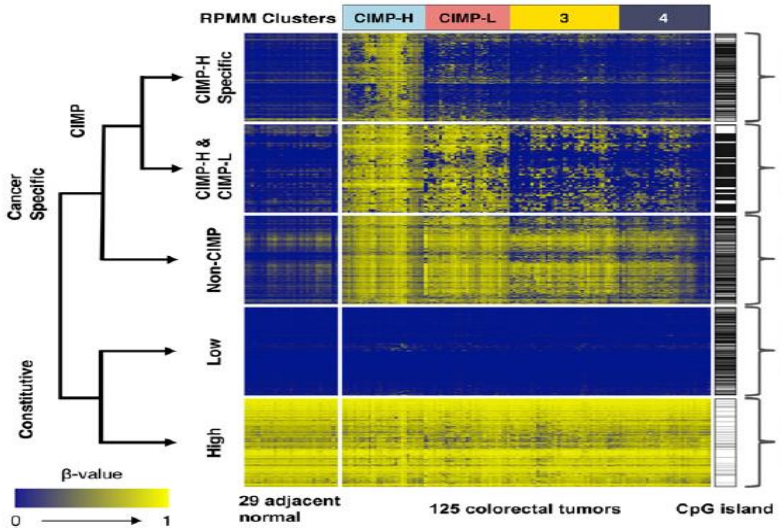
CRC is a heterogeneous disease at …

…gene expression level



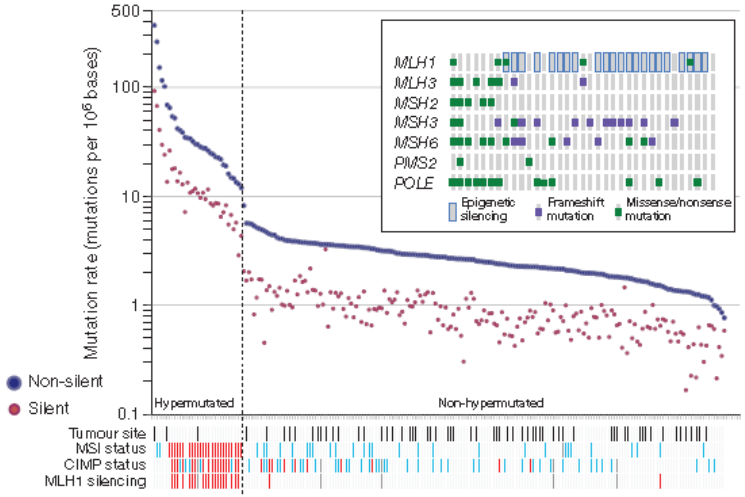
Adapted from Tejpar et al, PETACC 3 trial, ASCO 2010

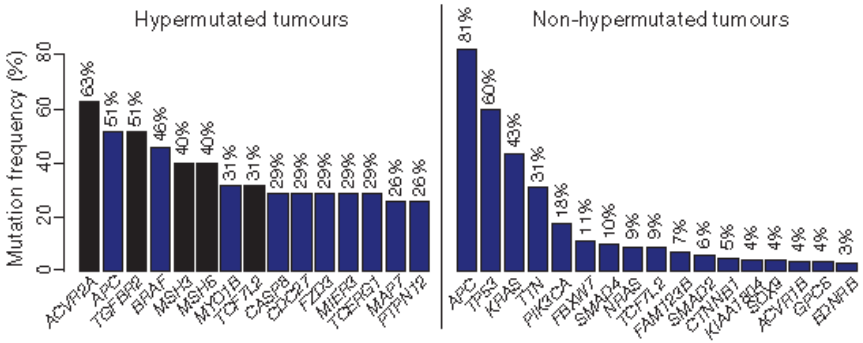
…methylation level



Adapted from Hinoue T et al: Genome-scale analysis of aberrant DNA methylation in colorectal cancer. Genome Res. 2011 Jun 9

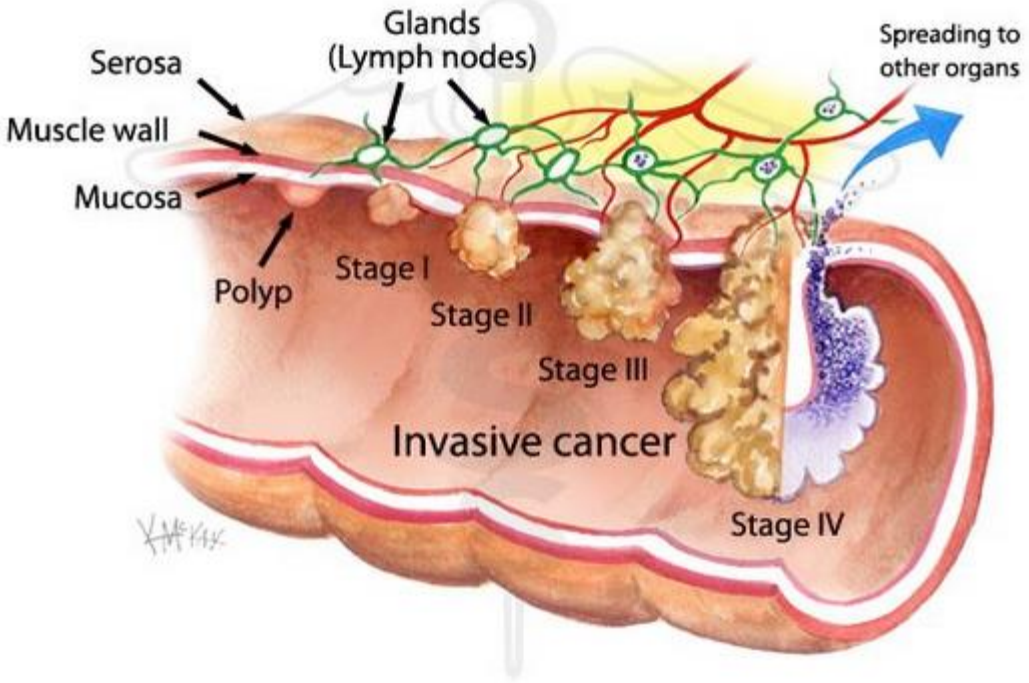
CRC is a heterogeneous disease at mutational rate level





Adapted from "Comprehensive molecular characterization of human colon and rectal cancer" The cancer genome atlas Network Nature, 2012 Jul 18;487(7407):330-7

The biology of CRC tells us that CRC is a heterogeneous disease at different levels but we still treat our patients by only considering the TNM classification



VOLUME 30 · NUMBER 12 · APRIL 20 2012
JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Identification of a Poor-Prognosis *BRAF*-Mutant–Like Population of Patients With Colon Cancer

Vlad Popovici, Eva Budinska, Sabine Tejpar, Scott Weinrich, Heather Estrella, Graeme Hodgson, Eric Van Cutsem, Tao Xie, Fred T. Bosman, Arnaud D. Roth, and Mauro Delorenzi

A combined oncogenic pathway signature of *BRAF*, *KRAS* and *PI3KCA* mutation improves colorectal cancer classification and cetuximab treatment prediction

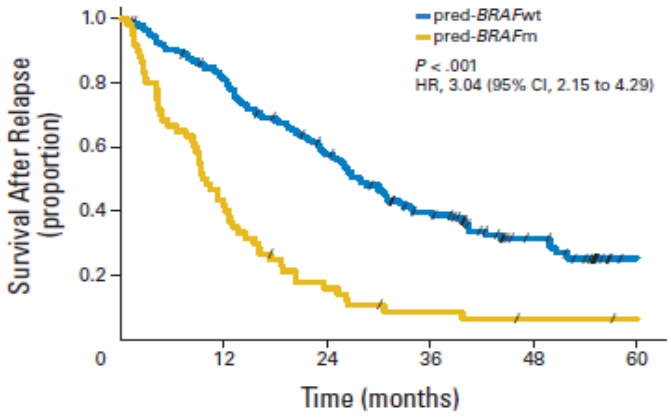
Sun Tian,[1] Iris Simon,[1] Victor Moreno,[2,3] Paul Roepman,[1] Josep Tabernero,[4] Mireille Snel,[1] Laura van't Veer,[1] Ramon Salazar,[2] Rene Bernards,[1,5] Gabriel Capella[2]

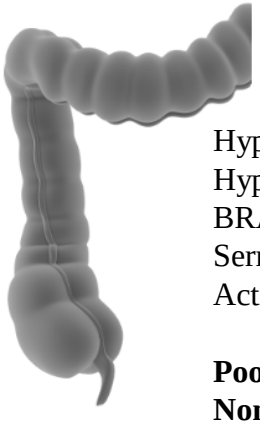
The BRAF - signature identifies:

1) Tumors that carry the BRAF V600E gene mutation with 96% sensitivity and 86% specificity

2) 30% of KRAS mutant CC
   13% of double wild type CC
   which carry the same gene expression profile as BRAF V600E

BRAF V600E and BRAF like CC tumors have poor outcome as compared to non BRAF like tumours





Hypermutation (MSI)
Hypermethylation (CIMP)
BRAF status (BRAF –like)
Serrated pathway
Activation of MAPK pathway

Poor SAR
Non responsive to chemotherapy

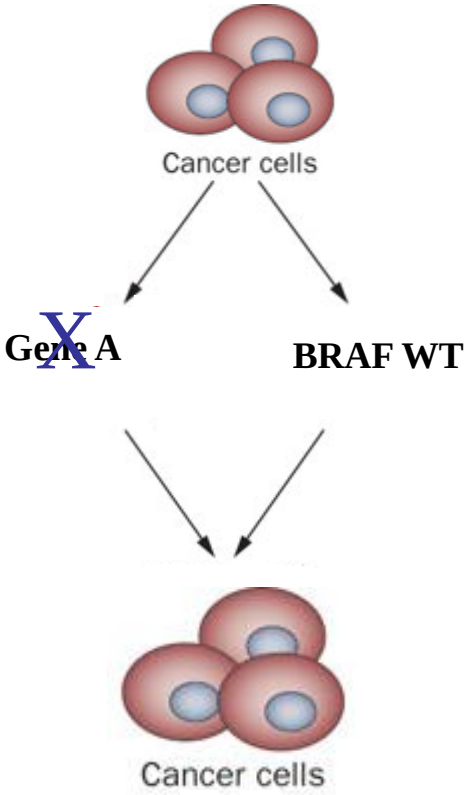
Adapted from Popovici et al, JCO March 2012

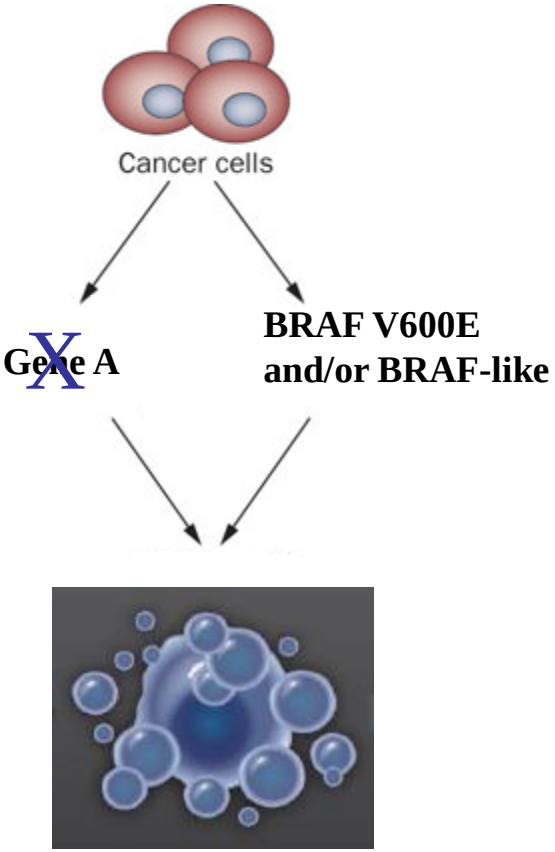
Identification of synthetic lethal interactions with the BRAF oncogene in CC

- to provide additional drug targets for therapeutic exploration

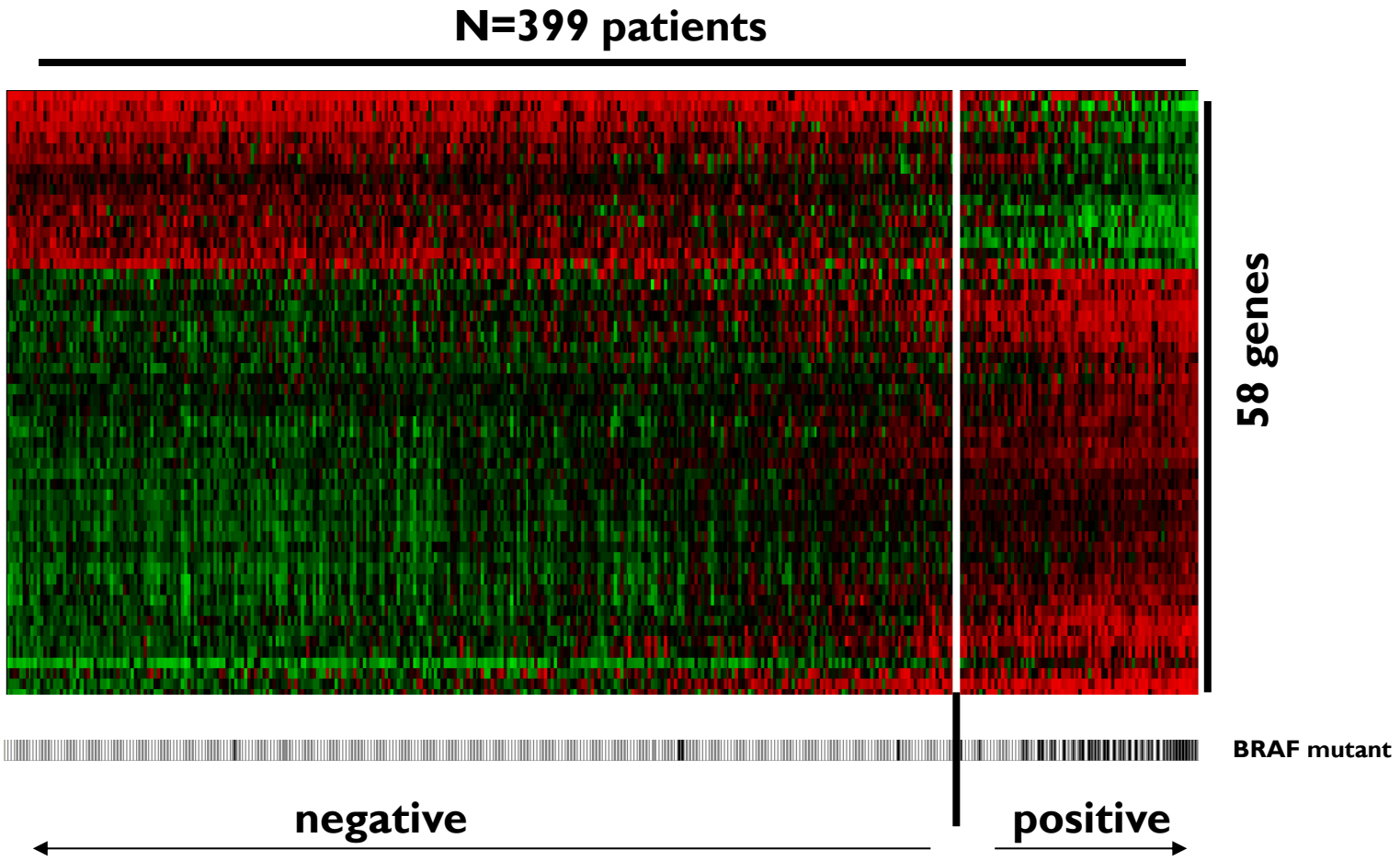
- to shed new light on BRAF mechanisms of action

Which are the genes synthetically lethal with BRAF V600E in CC?





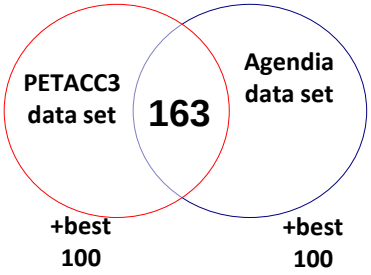
BRAF signature up-regulated genes are most likely to be candidates for synthetic lethality with BRAFV600E in CC



Adapted from: Tian S et al. Gut. 2013 Apr;62(4):540-9.

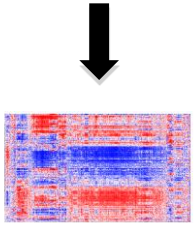
Set up experiment

Upregulated genes in BRAF V600E CC as compared with WT2



In vitro models

BRAF V600E and WT2 CC cell lines



Lim1215, Vaco432, Widr

Infect with the BRAF library

pool n.1, 2, 3, 4
MOI= 0.5
Coverage 400X

Negative selected genes in BRAF V600E vs WT2 CC cell lines

Criteria for selecting hits:

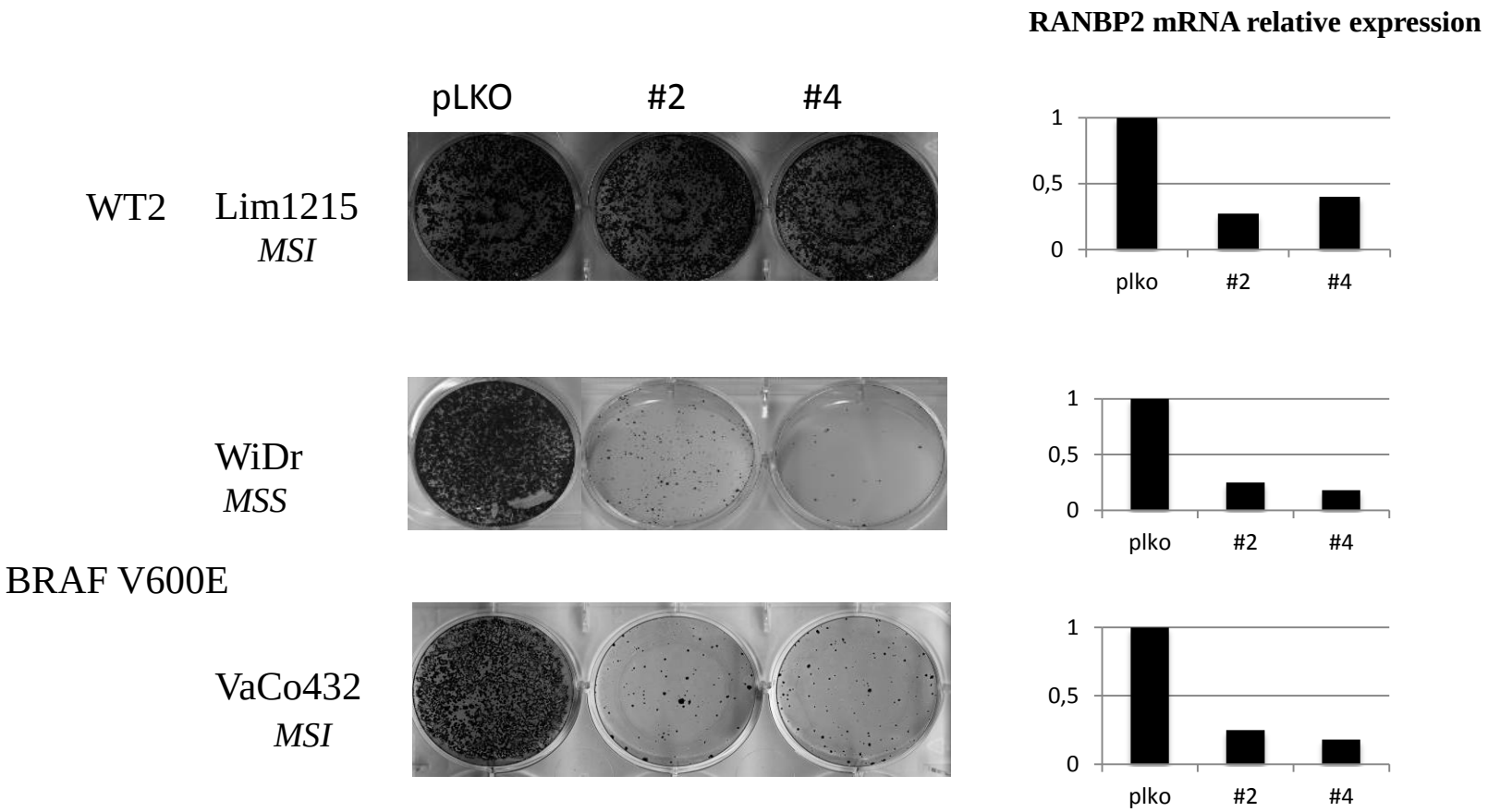
Depletion of BRAF mut cells over time must be at least 50% as compared to Time 0
log2 (Mut T13/T0) ≤ -1

Depletion of BRAF mut cells must be at least 1.5 fold higher than LIM1215
(LIM1215 T13/T0 fold change)/(BRAF Mut T13/T0 fold change) ≥ 1,5

multiple hairpins

Tag	GeneSymbol	Lim1215	foldChange	log2FoldChange	pval	BRAF V600E	foldChange	log2FoldChange	pval	Lim1215/BRAF V600E
TRCN0000059338	ABCA3		0.53608408	-0.899468812	0.0547169		0.1450598	-2.785280313	1.4972E-08	3.695607399
TRCN0000063415	ANTXR2		0.54127007	-0.885579478	0.05597073		0.31181298	-1.681247088	0.00027389	1.735880472
TRCN0000063417	ANTXR2		0.51748765	-0.950403671	0.03729236		0.26577127	-1.911742931	3.3463E-05	1.947116572
TRCN0000074800	ANXA2;NHP2L1		0.05869392	-4.090645015	3.6043E-07		0.02583538	-5.274507823	5.1352E-07	2.27184248
TRCN0000074798	ANXA2;NHP2L1;OPRS1		0.03341995	-4.903146666	1.1005E-11		0.00317431	-8.299342605	6.4759E-38	10.52826599
TRCN0000074799	ANXA2;NHP2L1;OPRS1		0.26570725	-1.91209049	0.02156795		0.12355796	-3.016740192	1.9426E-09	2.150466568
TRCN0000074801	ANXA2;NHP2L1;OPRS1		0.46228346	-1.113150346	0.02261798		0.27722097	-1.850891678	0.00072582	1.667563076
TRCN0000083105	APOL2		0.34527875	-1.534166565	0.00130329		0.1643842	-2.604856433	4.1613E-08	2.100437515
TRCN0000083107	APOL2		0.09595975	-3.381426864	7.5823E-05		0.02397165	-5.382526681	7.0819E-22	4.003050501
TRCN0000153292	BTNL9		0.21809577	-2.196966304	8.3777E-05		0.03127433	-4.998877312	5.0673E-19	6.973635741
TRCN0000077965	C5orf32		0.47956559	-1.060199956	0.03684837		0.30988741	-1.690183944	0.00022628	1.547547819
TRCN0000077967	C5orf32		0.50460816	-0.986764566	0.03874484		0.24180128	-2.048106227	1.3136E-05	2.086871344
TRCN0000053717	CALB2		0.25679807	-1.961293759	0.09472819		0.14858215	-2.750667305	2.6457E-08	1.728323818
TRCN0000057891	CCL4;CCL4L1;CCL4L2		0.26035686	-1.941437684	0.06801713		0.12001761	-3.058682004	2.2629E-09	2.169322162
TRCN0000073652	CD109		0.38542634	-1.375472933	0.00496915		0.20167873	-2.309869122	1.1945E-06	1.911090619
TRCN0000057167	CD55		0.21244013	-2.23487179	0.00217426		0.132227	-2.918911306	7.8848E-10	1.606631999
TRCN0000000982	DAPK1		0.3972114	-1.33202106	0.00577843		0.18189931	-2.458788	4.3248E-07	2.183688299
TRCN0000059755	ERO1L		0.37484565	-1.415631422	0.00354685		0.16037071	-2.640517434	3.1731E-08	2.337369813
TRCN0000116119	GBP1		0.55539951	-0.848402187	0.0994408		0.29975247	-1.738156435	0.0002266	1.852860477
TRCN0000116120	GBP1		0.16610092	-2.589868061	0.00856215		0.10354114	-3.271723922	4.1751E-09	1.60420205
TRCN0000116184	GBP2		0.46333341	-1.109877375	0.04201693		0.23555286	-2.085877218	1.8116E-05	1.967003933
TRCN0000036226	GLRX		0.36613834	-1.449539237	0.05217314		0.21245507	-2.234770322	1.7076E-05	1.723368339
TRCN0000044724	KCNK1		0.46243259	-1.11268501	0.01905082		0.25869181	-1.950693731	2.6255E-05	1.787581131
TRCN0000059231	KITLG		0.27557196	-1.859499002	0.00059798		0.04381821	-4.512325745	4.6229E-18	6.288983029
TRCN0000116511	MAPRE2		0.51596158	-0.954664442	0.08625369		0.29458316	-1.763253142	0.00011775	1.751497222
TRCN0000063649	MS4A8B		0.49784442	-1.006233147	0.0429053		0.27770401	-1.84838008	4.5971E-05	1.792715971
TRCN0000047691	RAB27B		0.27784757	-1.847634448	0.01461385		0.18084369	-2.467184809	6.1348E-07	1.536396264
TRCN0000003451	RANBP2		0.16630518	-2.588095019	4.9878E-06		0.08120005	-3.622375559	5.7674E-13	2.048092025
TRCN0000003453	RANBP2		0.44259261	-1.175948719	0.02141394		0.13208078	-2.920507587	5.0253E-09	3.350923772
TRCN0000072968	RASGRF2		0.39284838	-1.3479555	0.09463595		0.21278578	-2.232526356	5.0956E-06	1.846215365
TRCN0000145544	SAMD9		0.43103282	-1.214130378	0.027978		0.17064814	-2.550903428	3.4886E-07	2.525857149
TRCN0000061193	SEMA4B		0.3768738	-1.407846581	0.00795911		0.20316891	-2.299248436	2.3546E-06	1.854977715
TRCN0000043987	SLC36A4		0.2406411	-2.055045	0.00209442		0.14587176	-2.777227517	0.00017129	1.649675786
TRCN0000038265	SLC4A11		0.1535094	-2.703601137	0.02745181		0.08225547	-3.603744599	4.4074E-06	1.866251554
TRCN0000015757	SPOCD1		0.43645696	-1.196088705	0.07909248		0.09772762	-3.355089791	7.1644E-08	4.46605521
TRCN0000056708	SPRED1		0.35876794	-1.478877135	0.00193224		0.12654216	-2.982309911	5.29E-10	2.83516515
TRCN0000059474	SYT13		0.22641469	-2.142960516	1.1751E-05		0.11638088	-3.103074088	2.4475E-10	1.945463039
TRCN0000059541	TC2N		0.28924929	-1.789614663	0.03648613		0.18443703	-2.438799778	7.4037E-07	1.568282125
TRCN0000073151	UBASH3B		0.42317468	-1.240674796	0.06331345		0.18158263	-2.461301862	1.1421E-06	2.330479894
TRCN0000007429	USP14		0.34196659	-1.548072703	0.00165015		0.15199466	-2.717907428	3.2644E-08	2.249859211

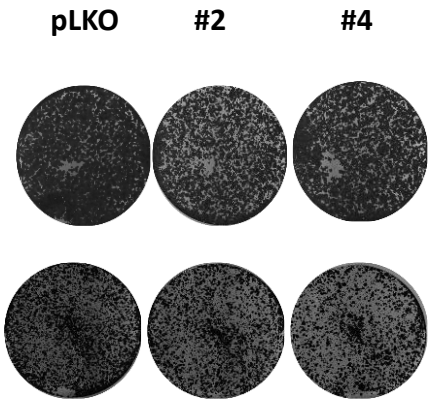
RANBP2 is selectively synthetic lethal with BRAF V600E in CC



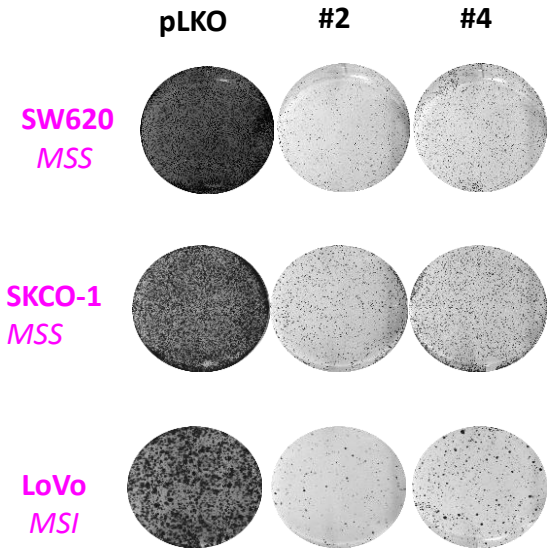
RANBP2 is selectively synthetic lethal with BRAF V600E in CRC

RANBP2 is also synthetic lethal with BRAF likeness in CRC

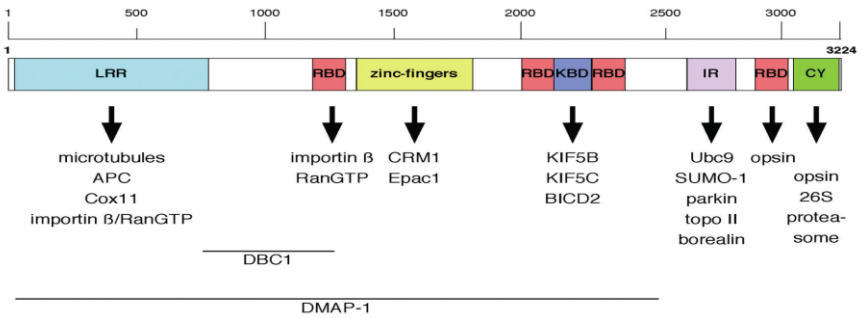
KRAS mut non BRAF like



KRAS mut BRAF like



RANBP2 (Nup358)



Schematic representation of RANBP2 domain organization and binding partners

Nucleus 3:2, 162–171; March/April 2012; G 2012 Landes Bioscience

J Biol Chem. 2012 Jan 6;287(2):819-31. Epub 2011 Nov 21.

Specific armadillo repeat sequences facilitate β-catenin nuclear transport in live cells via direct binding to nucleoporins Nup62, Nup153, and RanBP2/Nup358.

Sharma M, Jamieson C, Johnson M, Molloy MP, Henderson BR.

Westmead Institute for Cancer Research, The University of Sydney, Westmead, New South Wales 2145, Australia. manisha.sharma@sydney.edu.au

Gastroenterology. 2008 Jun;134(7):1961-71, 1971.e1-4. Epub 2008 Mar 10.

Regulation of Wnt signaling by the nuclear pore complex.

Shitashige M, Satow R, Honda K, Ono M, Hirohashi S, Yamada T.

Chemotherapy Division and Cancer Proteomics Project, National Cancer Center Research Institute, Tokyo, Japan.

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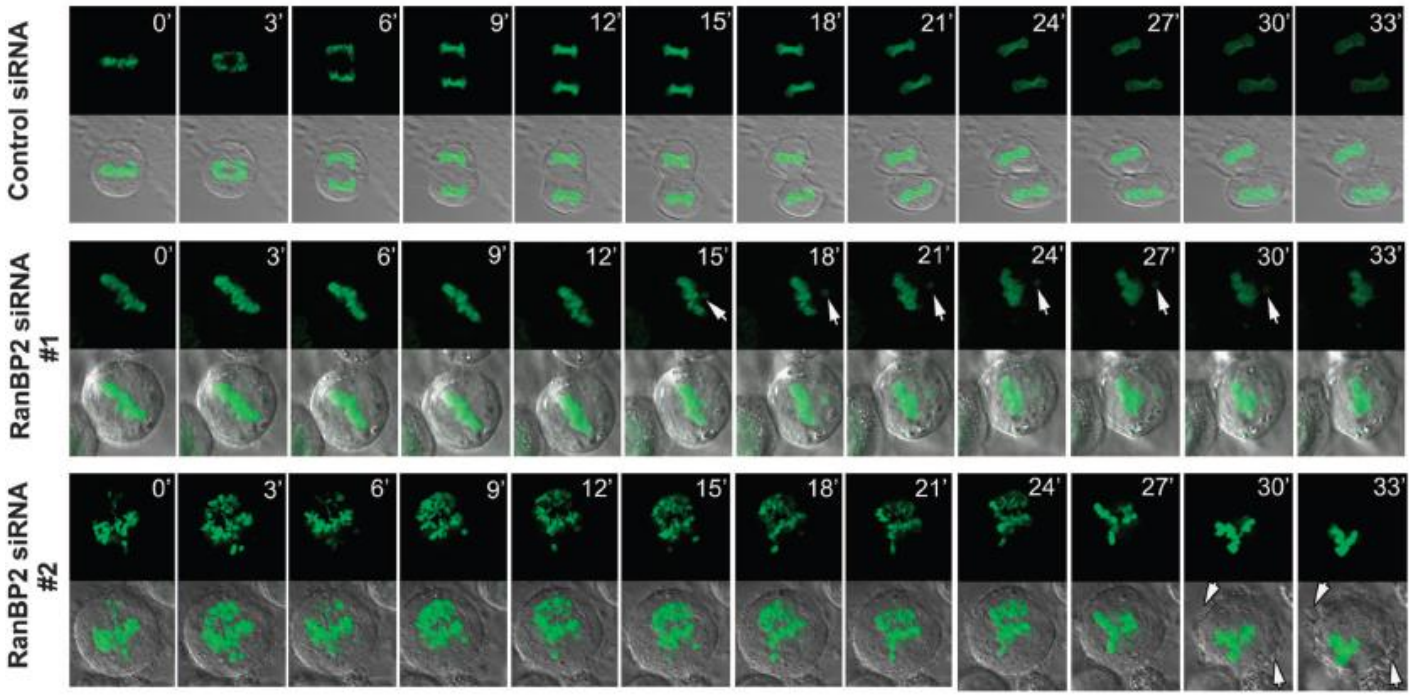
Sumoylation of Mdm2 by Protein Inhibitor of Activated STAT (PIAS) and RanBP2 Enzymes*

RANBP2 depletion induces abnormal chromosomal segregation



Hashizume et al *Cell Death and Disease* (2013) 4, e854

Conclusions

- The BRAF-like signature identifies BRAF V600E tumors with 96% sensitivity and 86% specificity and also a group of BRAF wild type tumors which share the same gene expression profile
- The BRAF-like tumors share a common poor prognosis
- The BRAF-like tumors belong to a specific CC subtype: hypermutated (MSI), hypermethylated, right sided location, serrated and MAPK pathway activated tumors.
- RANBP2 is selectively synthetic lethal with BRAF V600E and BRAF likeness in CC
- Ongoing research is focused on better defining the role of RANBP2 and its depletion in BRAF V600E tumors and on identifing treatments which could mimic RANBP2 KD

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SIB
Giovanni d'ario
Mauro Delorenzi

KUL
Sabine Tejpar

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