Beyond BRCA: a journey into molecular diversity in ovarian cancer and beyond

Reinhard Büttner

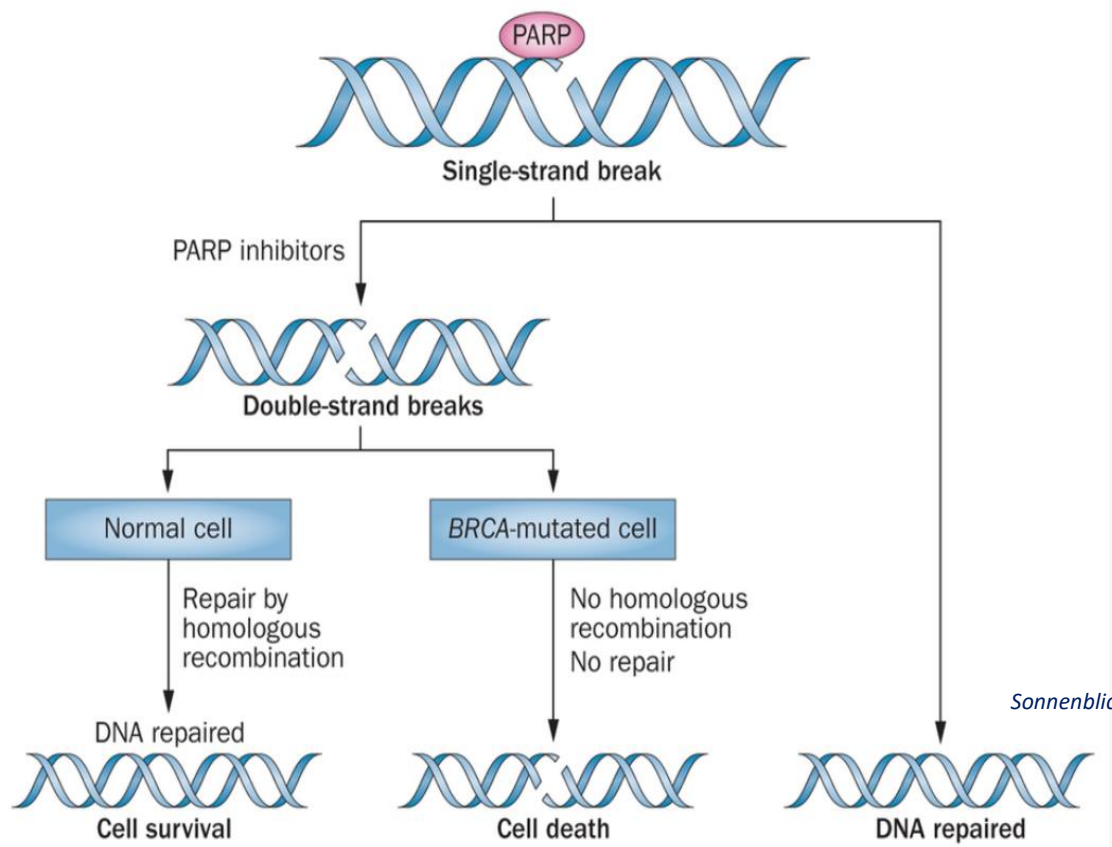
Cologne Institute for Pathology

BRCA-Diagnostics in Ovarian Cancer

- 2015: Approval of the first PARP-inhibitor (Olaparib) for *BRCA*-mutated, relapsed, platin-sensitive, serous high-grade cancer of Ovary, Fallopian tube or Peritoneum
- In ~30-40% of *BRCA*-mutated Ovarian Cancer mutations are somatic

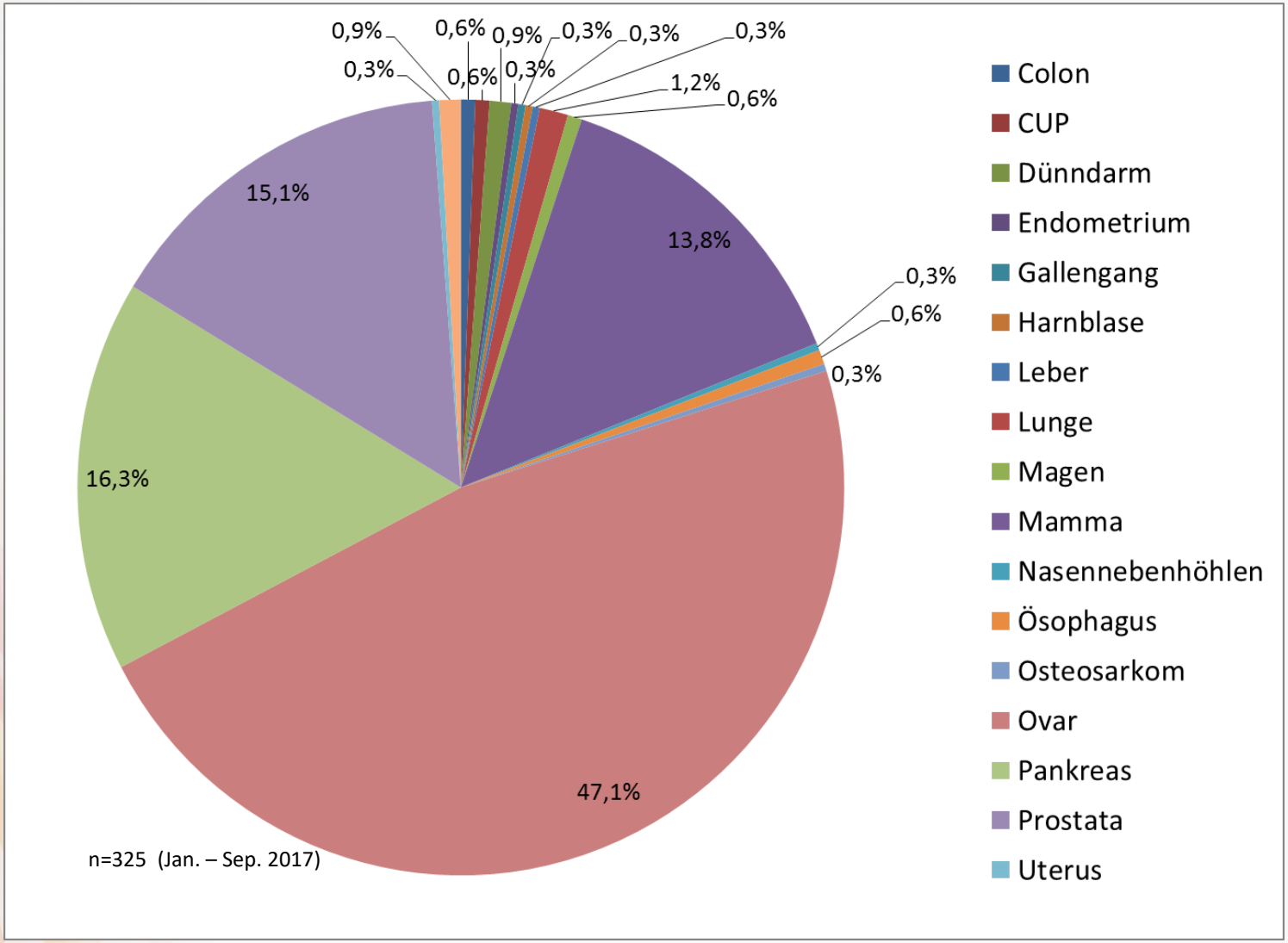
(*Cancer Genome Atlas Research, Nature 2011; Hennessy BT et al., J Clin Oncol 2010*)

How do PARP inhibitors work in HRD tumors ?



Sonnenblick et al., Nat Rev Clin Oncol 2015

BRCA-testing in Cologne (2017)
All tumor entities



Results in other tumour types

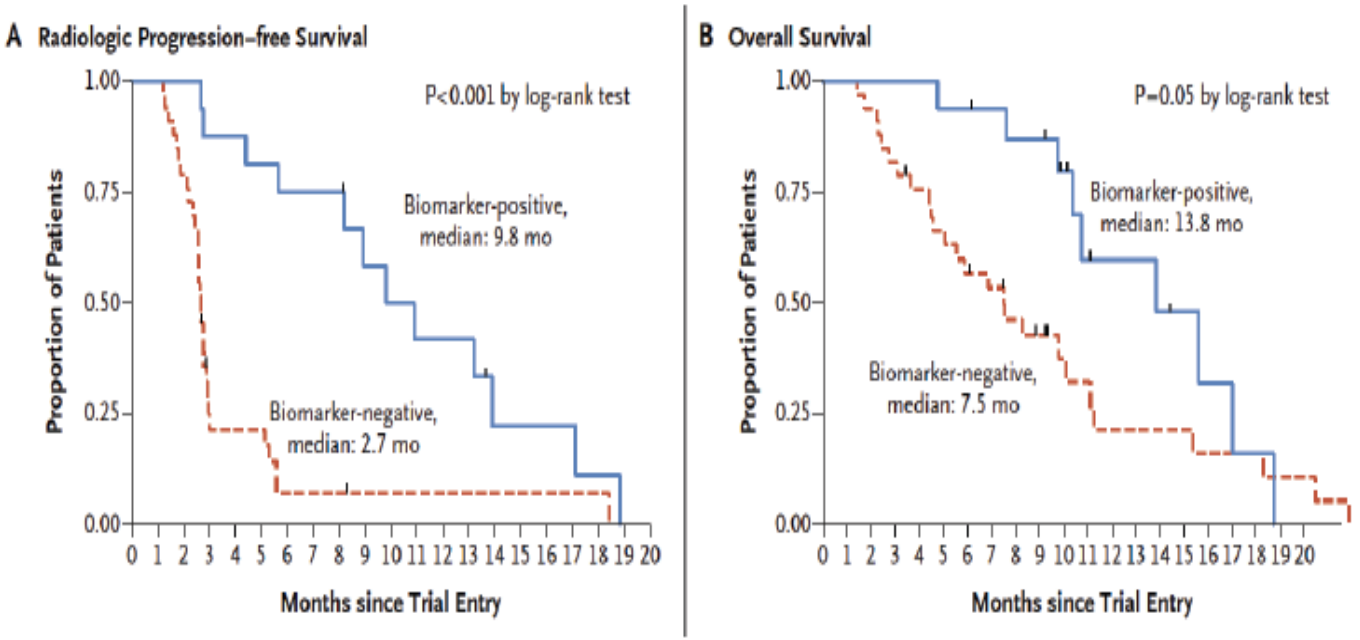
Table 2. Tumor Response Rates (full analysis set)

	Ovarian (n = 193)		Breast (n = 62)		Pancreas (n = 23)		Prostate (n = 8)		Other (n = 12)		Total (N = 298)	
Response	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Tumor response rate	60	31.1	8	12.9	5	21.7	4	50.0	1	8.3	78	26.2
95% CI	24.6 to 38.1		5.7 to 23.9		7.5 to 43.7		15.7 to 84.3		0.02 to 38.5		21.3 to 31.6	
CR*	6	3	0	0	1	4	0	0	0	0	7	2
PR*	54	28	8	13	4	17	4	50	1	8	71	24
Stable disease ≥ 8 weeks	78	40	29	47	8	35	2	25	7	58	124	42
95% CI	33.4 to 47.7		34.0 to 59.9		16.4 to 57.3		3.2 to 65.1		27.7 to 84.8		36.0 to 47.4	
Stable disease	64	33	22	36	5	22	2	25	6	50	99	33
Unconfirmed PR	12	6	7	11	3	13	0	0	1	8	23	8
PD†	41	21	23	37	9	39	2	25	3	25	78	26
95% CI	15.7 to 27.7		25.2 to 50.3		19.7 to 61.5		3.2 to 65.1		5.5 to 57.2		21.3 to 31.6	
RECIST progression	33	17	16	26	6	26	1	13	3	25	59	20
Early death‡	8	4	7	11	3	13	1	13	0	0	19	6
Not evaluable	14	7	2	3	1	4	0	0	1	8	18	6
No follow-up assessments	12	6	2	3	1	4	0	0	0	0	15	5
Stable disease < 8 weeks	2	1	0	0	0	0	0	0	1	8	3	1

Abbreviations: CR, complete response; PD, progressive disease; PR, partial response.
*Response confirmed ≥ 4 weeks after initial observation of response.
†Progression events that occurred within 118 days of last evaluable assessment during first 182 days of treatment period or that occurred within 174 days of last evaluable assessment after first 182 days of treatment period.
‡Death in absence of evaluable RECIST assessment.

Kaufman B et al J Clin Oncol. 2015;33:244-50

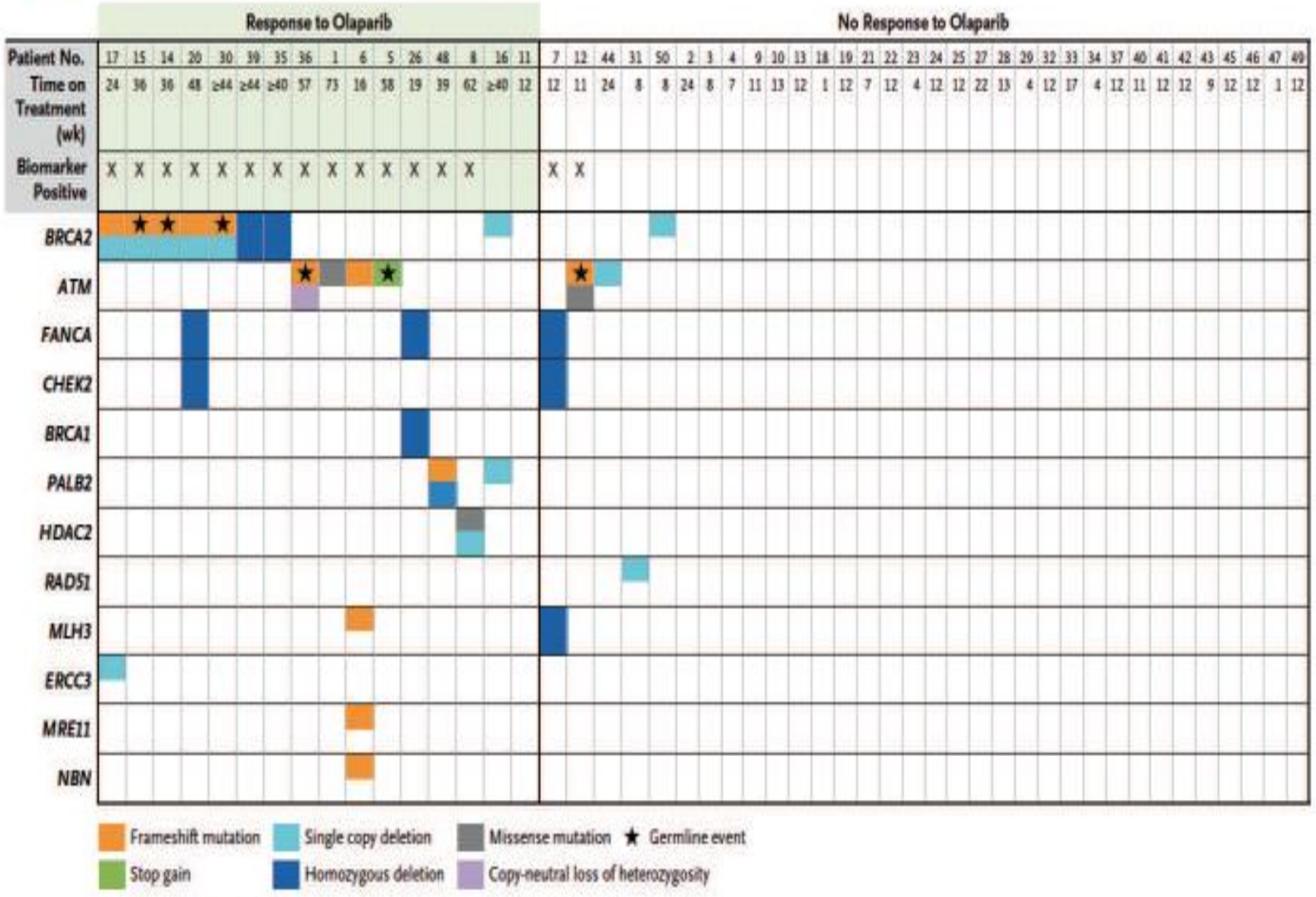
Results in other tumour types (prostate cancer)



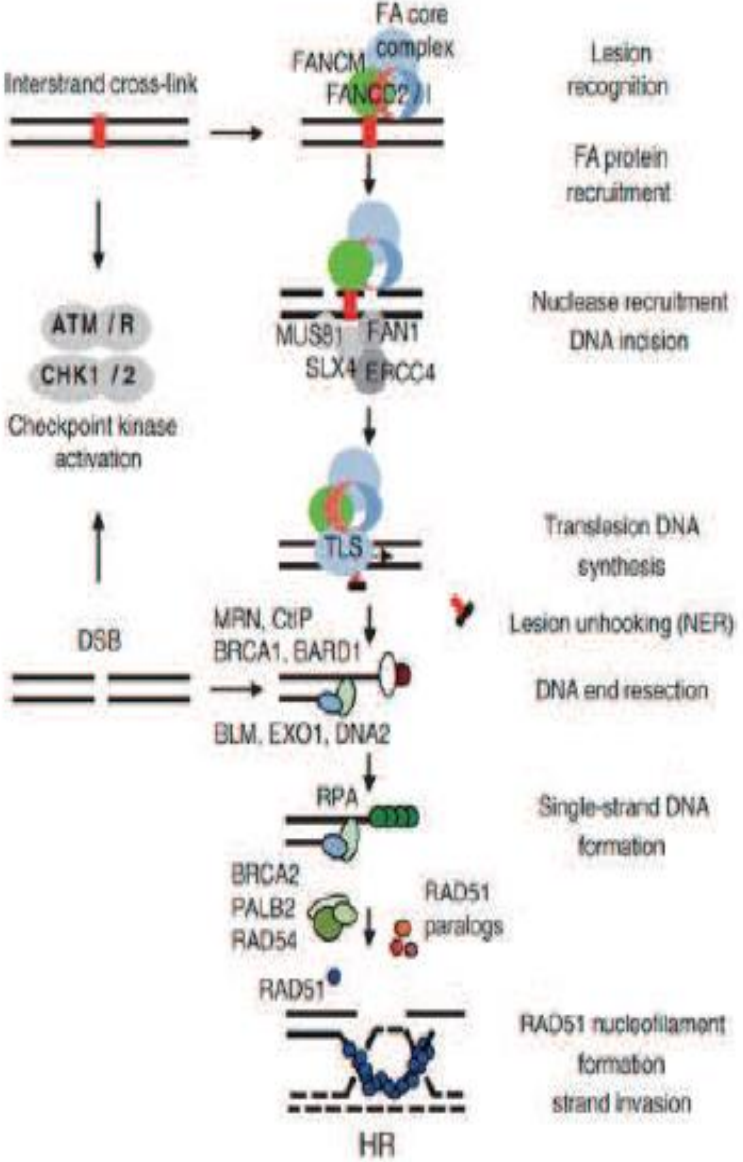
Mateo J et al N Engl J Med 2015;373:1697-708

Why gene panels???

Not just BRCA1/2 but HRD



Mateo J et al N Engl J Med 2015;373:1697-708



Panagiotis A. Konstantinopoulos et al. Cancer Discov 2015;5:1137-1154

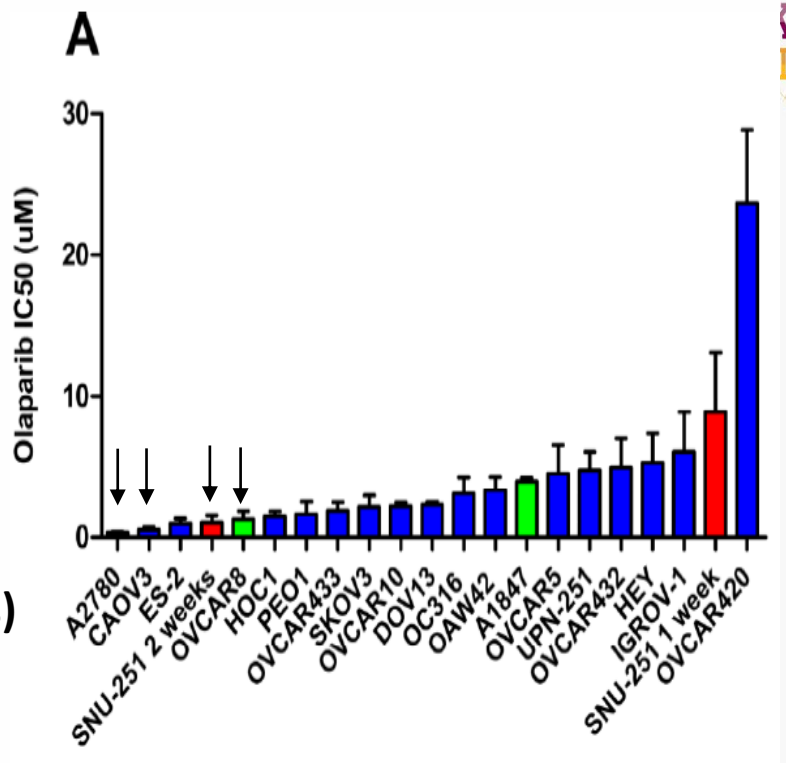
- *The National center for Familial Cancer*
- *Pathway 1: Fam. History positive*
- *Pathway 2: Fam. History negative*

TruRisk™ BC/OC gene panel (34 genes) by the German Consortium GC-HBOC

ATM *core gene*	***BRCA1*** *core gene*	***BRCA2*** *core gene*	***CDH1*** *core gene*	***CHEK2*** *core gene*	***NBN*** *core gene*	***PALB2*** *core gene*	***RAD51C*** *core gene*
RAD51D *core gene*	***TP53*** *core gene*	***MLH1*** *Lynch syndrome*	***MSH2*** *Lynch syndrome*	***MSH6*** *Lynch syndrome*	***PMS2*** *Lynch syndrome*	***ENIGMA*** *#1*	***ENIGMA*** *#2*
ENIGMA *#3*	***ENIGMA*** *#4*	***ENIGMA*** *#5*	***ENIGMA*** *#6*	***ENIGMA*** *#7*	***ENIGMA*** *#8*	***ENIGMA*** *#9*	***ENIGMA*** *#10*
ENIGMA *#11*	***ENIGMA*** *#12*	*candidate*	*candidate*	*candidate*	*candidate*	*candidate*	*candidate*
candidate	*candidate*						

Proof of principle experiment

- **Agilent SURE SELECT-Capture Library for target enrichment:**
  - **Ca. 350 genes involved in DNA repair**
  - **coding region + promoter**
  - **hg19**
  - **total probes: 43 724**
  - **total probes size: 2.77 Mbp**
  - **recommended minimum sequencing per sample: 554.186 Mbp (ca. 7x10^6 reads)**

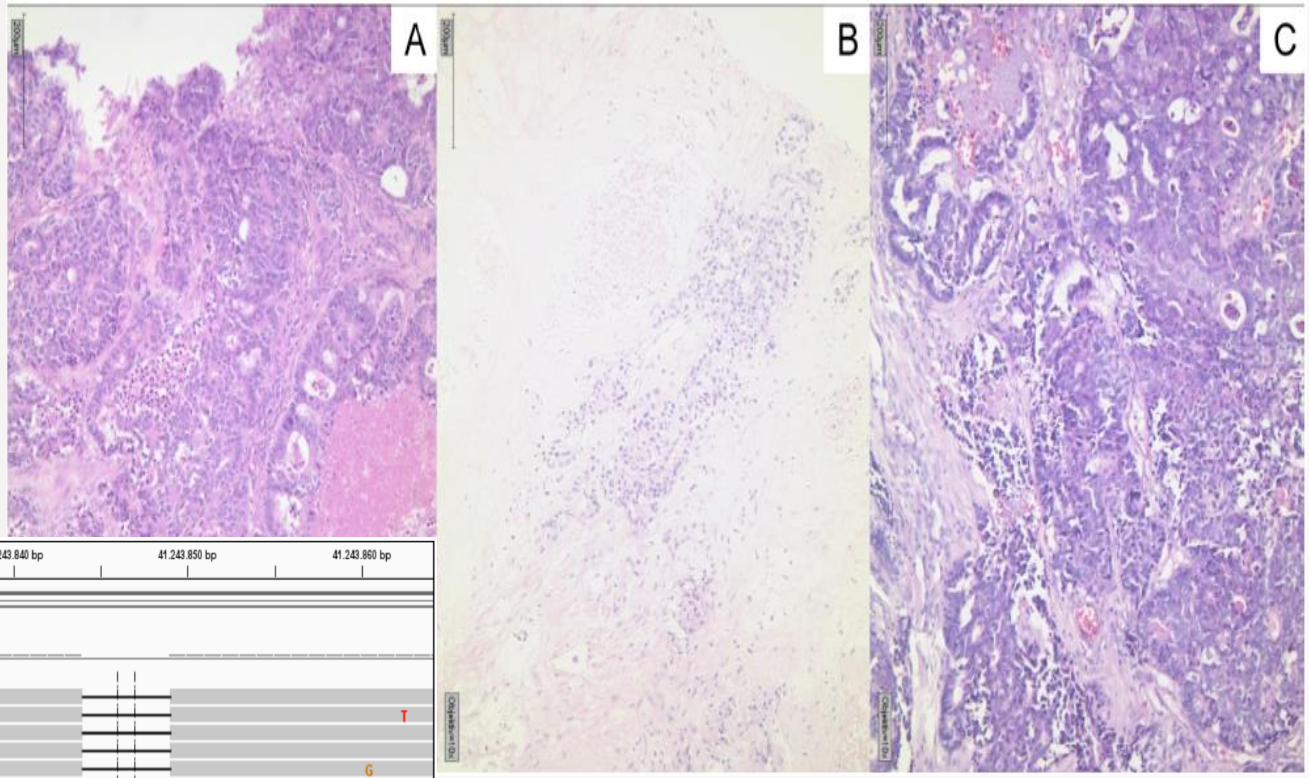


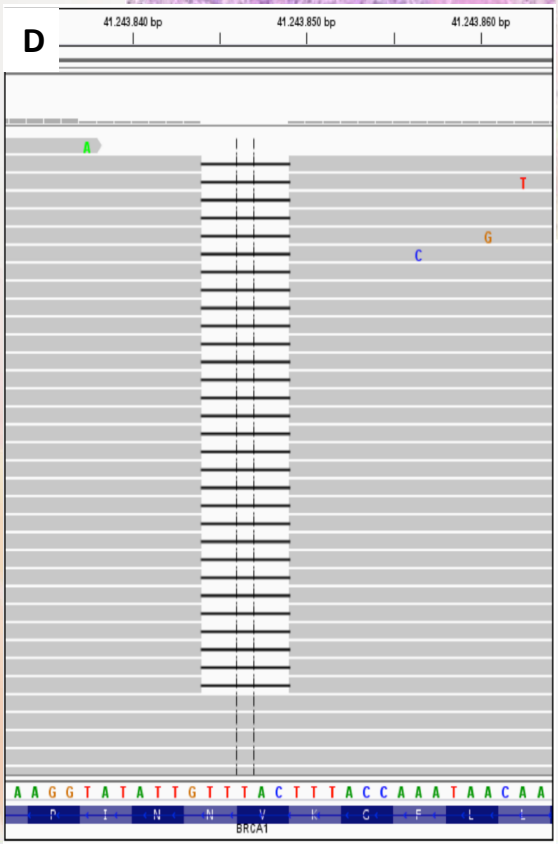
cell line	mutation/methylation result	sensitivity to Olaparib
A2780		TRUE
SNU-251	BRCA1, homozygous (5564 G>A; p.W>STOP)	TRUE
OVCAR8	BRCA1 promoter methylation	TRUE
CAOV-3	TP53, p.Q136* stop mutation	TRUE

Potentially druggable and highly homogeneous BRCA 1 mutation in small bowel adenocarcinoma

Alexander Quaas [1], Hakan Alakus [2], Thomas Zander [3], Carina Heydt [1], Dirk Waldschmidt [4], Anna Brunn [5], Heike Göbel [1], Reinhard Büttner [1], Sabine Merkelbach-Bruse [1]

1. Institute of Pathology; University of Cologne
2. Departement of Visceral Surgery; University of Cologne
3. Departement of Oncology and Hematology; University of Cologne
4. Departement of Hepato- and Gastroenterology; University of Cologne
5. Institute of Neuropathology; University of Cologne





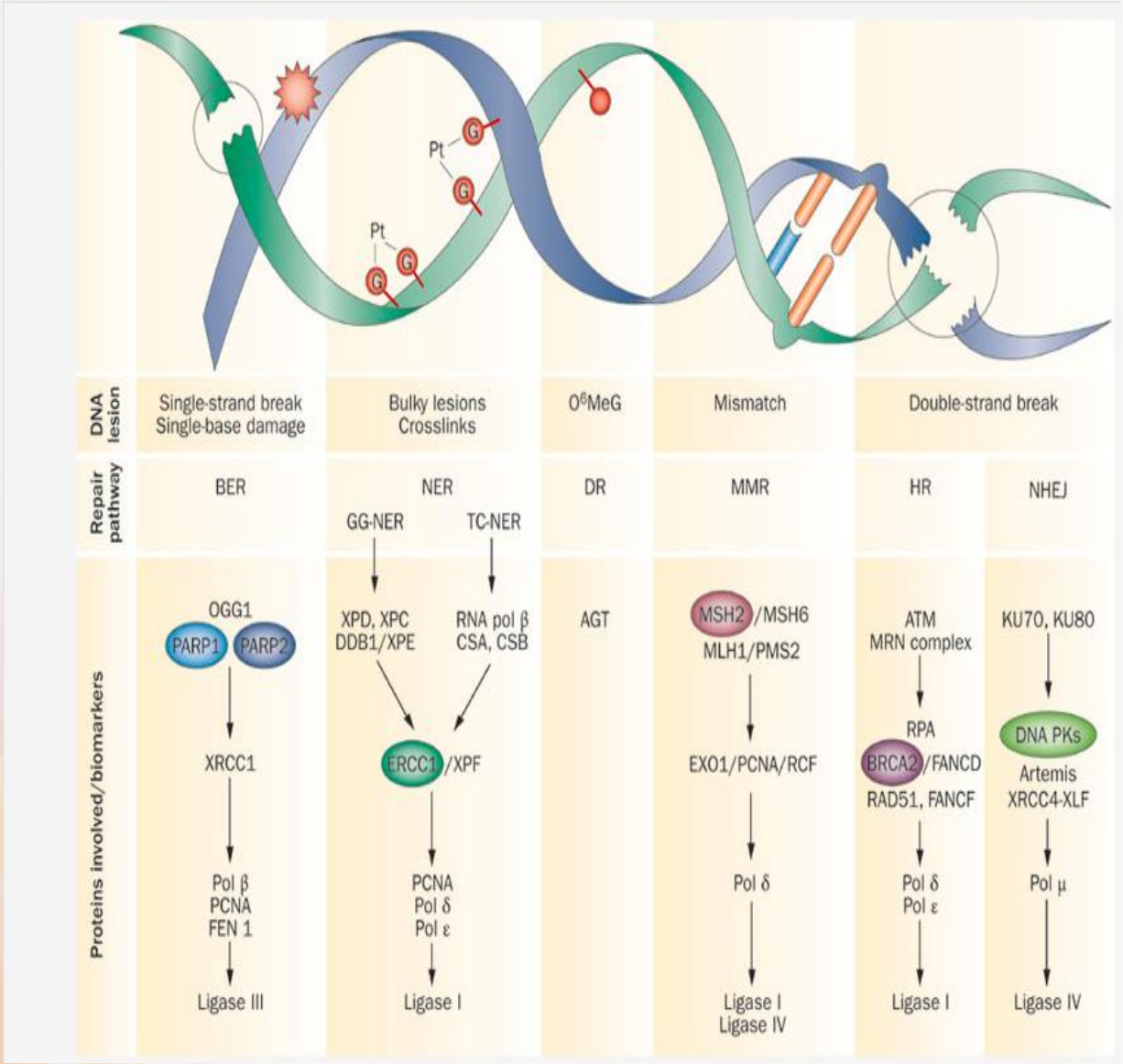
A: Primary Small Bowel AdenoCA
B: Liver Met
C: Brain Met
D: Class 5 Mutation in BRCA1

Squamous Cell Carcinoma of the Pancreas in a Patient with Germline BRCA2 Mutation-Response to Neoadjuvant Radiochemotherapy.

Schultheis AM, Nguyen GP, Ortmann M, Kruis W, Büttner R, Schildhaus HU, Markiefka B.

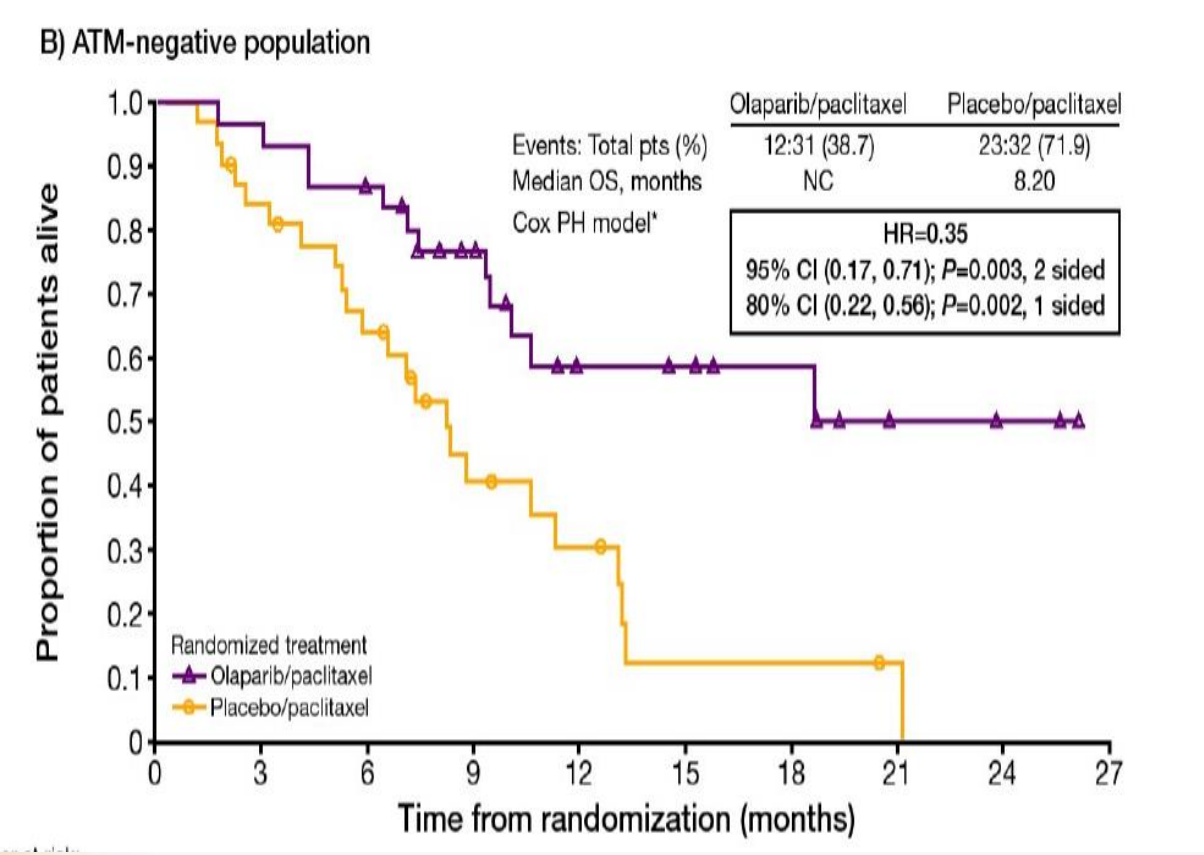
Case Rep Oncol Med. 2014;2014:860532.

Intrinsic DNA Repair Deficiency as Therapeutic Target



Postel-Vinay et al., Nat Rev Clin Oncol 2012

DBR als target in CRC



→DNA-Reparatur als wichtiger neuer Angriffspunkt

Bang et al, AACR 2016, #4013

BRCAmut and Immune Checkpoint Inhibitors

CTLA-4 Blockade Synergizes Therapeutically with PARP Inhibition in BRCA1-Deficient Ovarian Cancer.

Higuchi T, Flies DB, Marjon NA, Mantia-Smaldone G, Ronner L, Gimotty PA, Adams SF.

Cancer Immunol Res. 2015 Nov;3(11):1257-68.

DNA-repair deficient tumors respond to therapies with immune-checkpoint inhibitors

BRCA, ATM, RAD51C (DBR-deficient)

MSI-H (Mismatch repair-deficient)

PolE-deficient

- *Many high-grade serous ovarian cancers have alterations in HR repair genes*
- *The vast majority of mutations are germ-line*

But

- *HRD is also present in*
- *Small bowel carcinoma (~20%)*
- Pancreatic carcinoma (~10-12%)
- Prostate cancer (~10-12%)

- BRCAness in almost all tumours

Composition of *aCIO* (= all cancers in one) panel *LTCGv3.0*

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Gene	target	Gene	target	Gene	target
ABL1	exons	IDH1	exons	RHOA	Exon 2,3
ALK	breakpoints and exons	IDH2	exons	RICTOR	exons
APC	exons	IGF2R	exons	ROS1	breakpoints and exons
AR	exons	JAK2	exons	RPTOR	exons
ARAF	exons	KDR	exons	SMO	exons
ATM	exons	KEAP1	exons	STK11	exons
ATR	exons	KIF5B	breakpoint only	TGFBR2	exons
BCL6	exons	KIT	exons	TP53	exons
BRAF	breakpoints and exons	KNSTRN	Exon1	TSC1	exons
BRCA1	exons	KRAS	exons	TSC2	exons
BRCA2	exons	MAP2K1	Exon 2	VHL	exons
CCND1	exons	MDM2	exons		
CCNE1	exons	MET	whole gene		
CD74	breakpoints	MSH3	exons		
CDK4	exons	MTOR	exons		
CDK6	exons	MYC	exons		
CDKN2A	exons	MYCL1	exons		
CDKN2B	exons	MYCN	exons		
CTNNB1	exons	NF1	exons		
EGFR	whole gene	NF2	exons		
EML4	breakpoint	NFE2L2	exons		
ERBB2	exons	NOTCH 1	exons		
FGFR1	whole gene	NOTCH 2	exons		
FGFR2	breakpoints and exons	NOTCH 3	exons		
FGFR3	whole gene	NRAS	exons		
FLT1	exons	NRG1	breakpoint only		
FLT4	exons	NTRK1	breakpoints and exons		
GNA11	exons	OXA1L	Exon 1		
GNA13	exons	PDGFRa	breakpoints and exons		
GNAI2	exons	PDGFRb	breakpoints and exons		
GNAQ	exons	PIK3CA	exons		
GNAS	exons	PTCH1	exons		
GNAT2	exons	PTEN	exons		
GNG2	exons	RAC1	Exon2		
HDAC2	exons	RB1	exons		
HRAS	exons	RET	breakpoints and exons		

83 genes and gene regions

Mutation analysis

Rearrangement analysis

Size of *aCIO*
(= all cancers in one) panel:

- estimated total target region:
  2.26 Mb
- suggested mean coverage:
  200-400 x

S Merkelbach Bruse

Identification of large deletions/duplications in *BRCA1/2* by MLPA*

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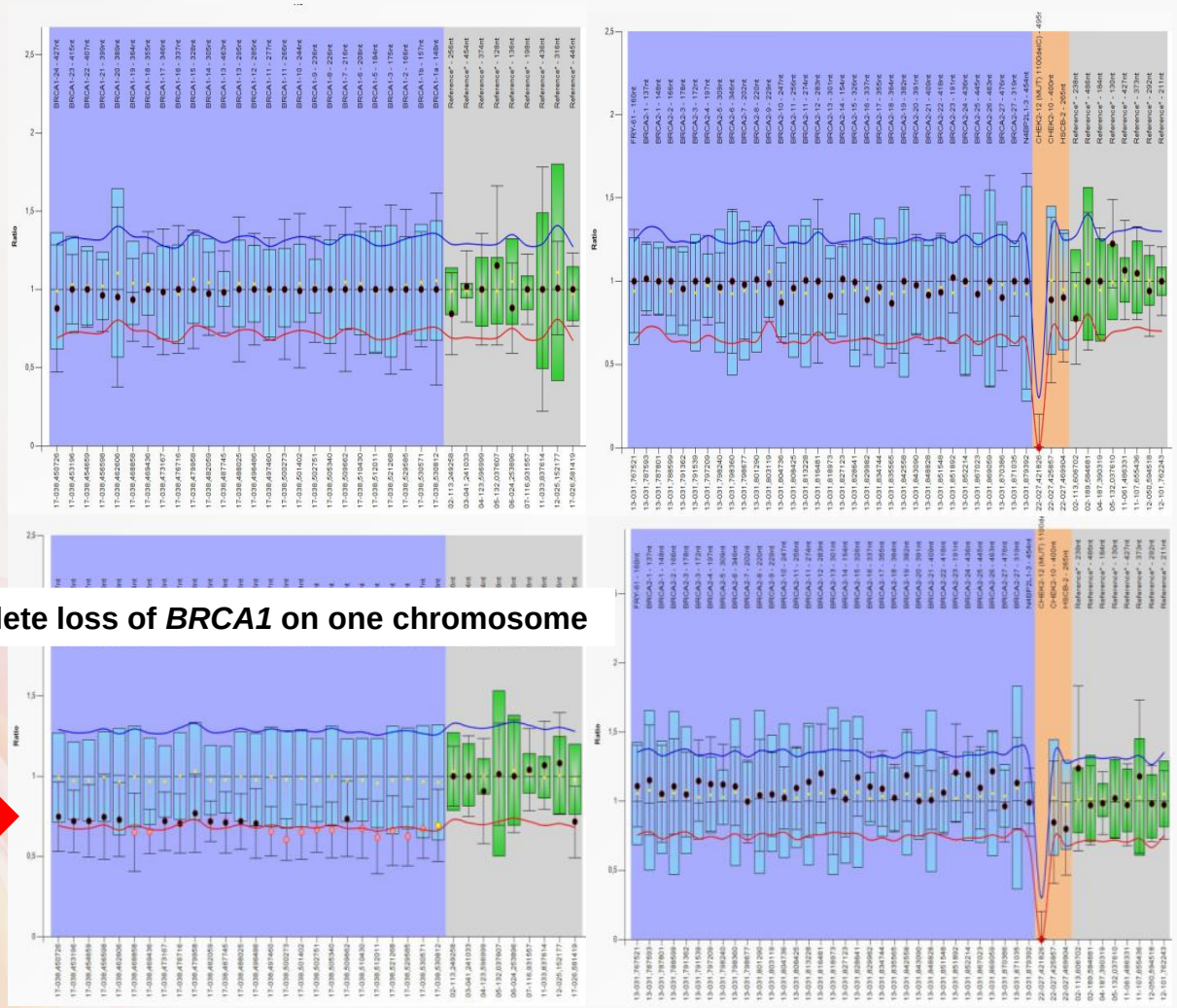
the biomarker national meeting by ONCOACADEMIA

BRCA1 ***BRCA2***

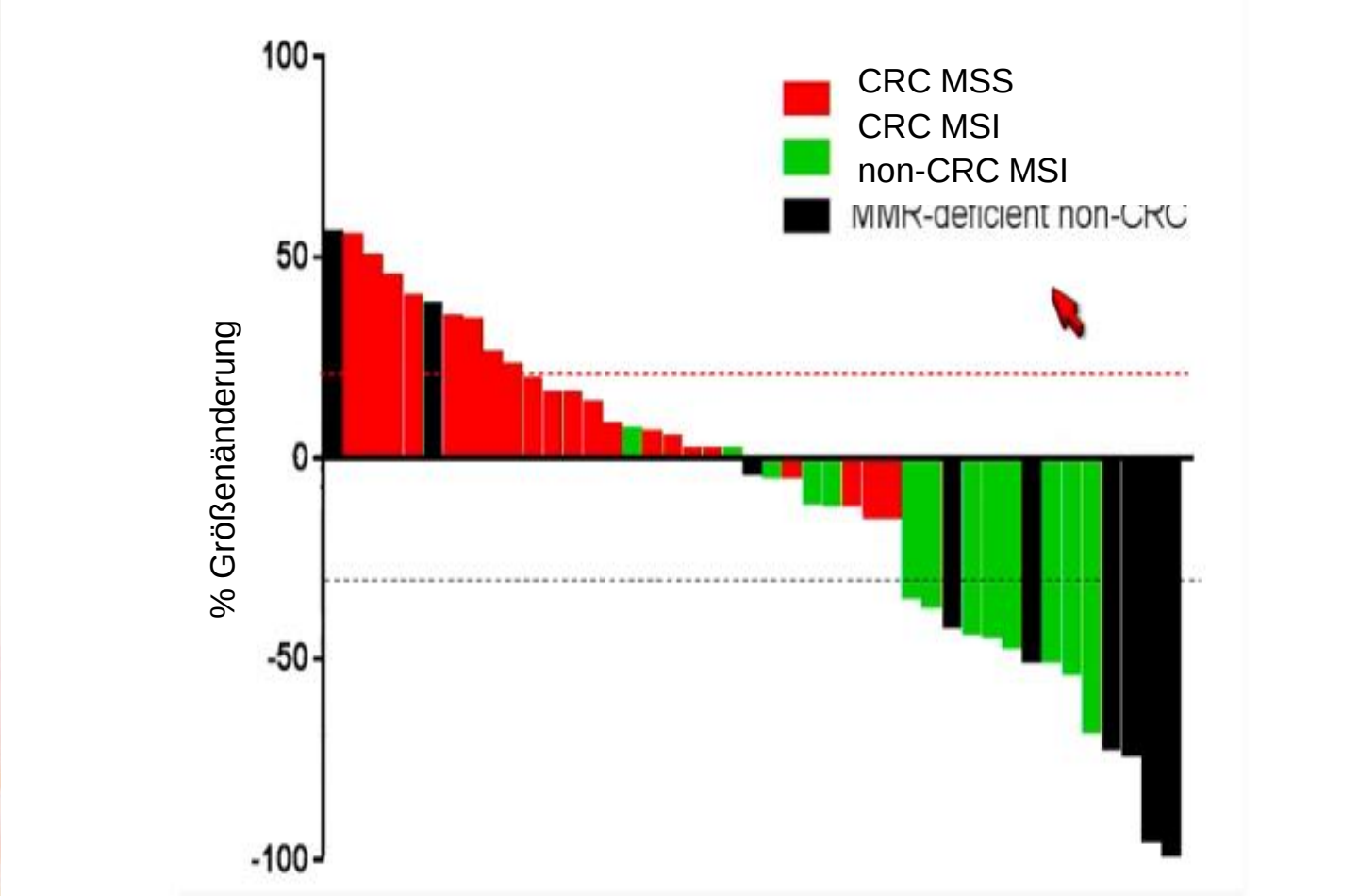


Complete loss of *BRCA1* on one chromosome

Immune
Checkpoint Inhibitors

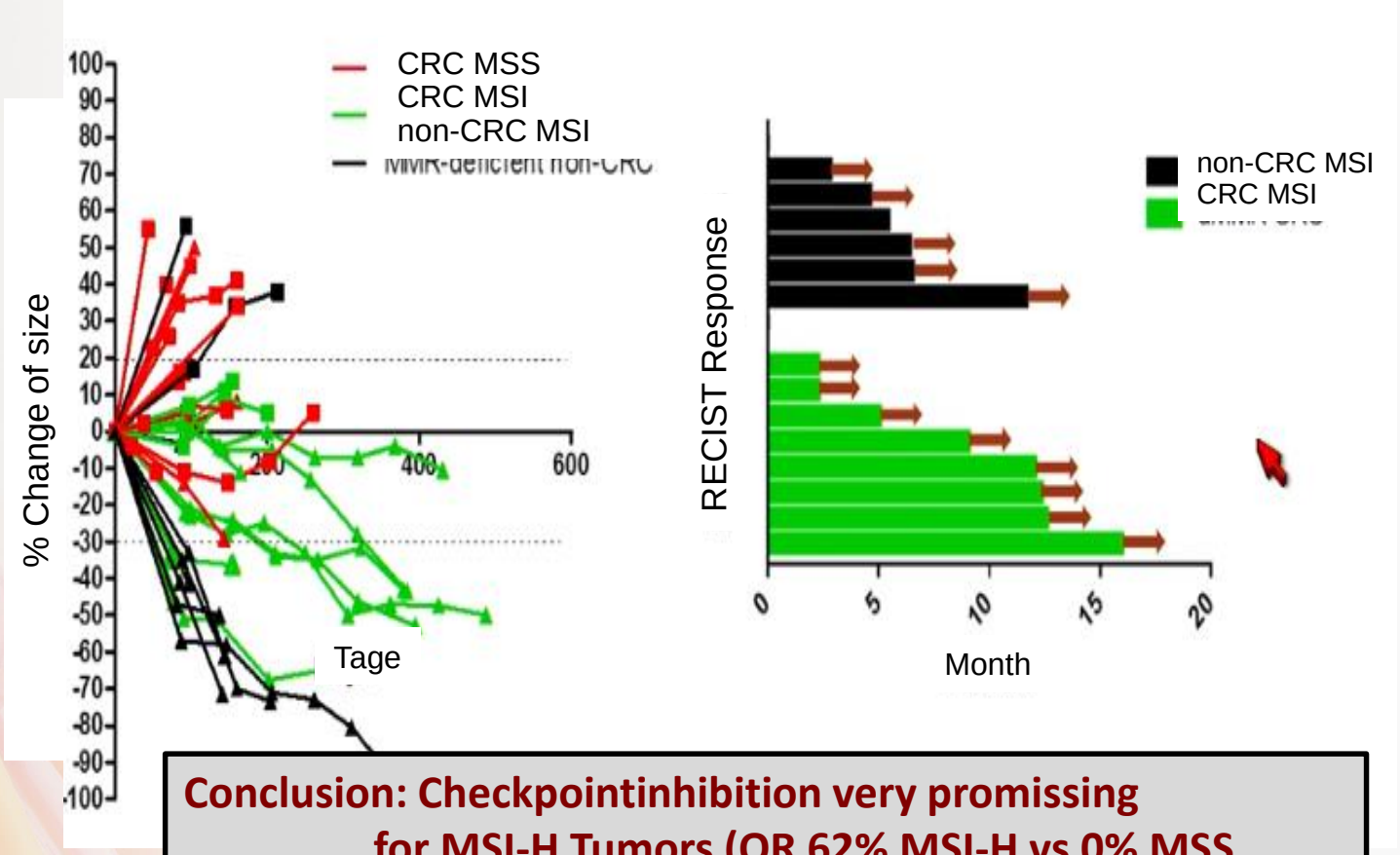
Intrinsic DNA replication error

Immune Checkpoint Inhibition in CRC



Dung T. Lee LBA 100 ASCO 2015

Immune Checkpoint Inhibition in CRC



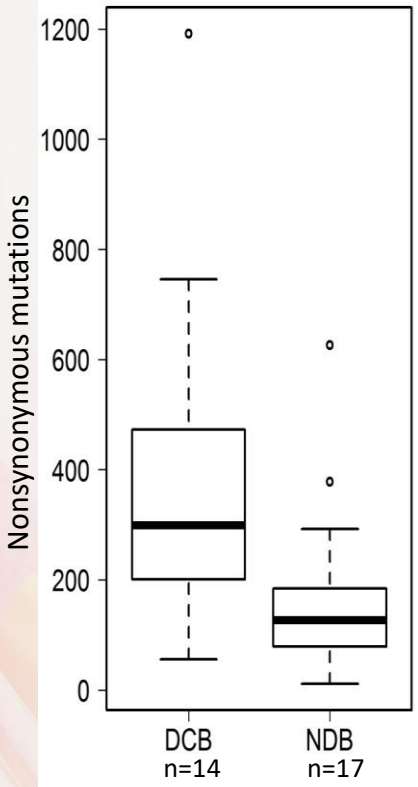
Conclusion: Checkpointinhibition very promissing for MSI-H Tumors (OR 62% MSI-H vs 0% MSS

Dung T. Lee LBA 100

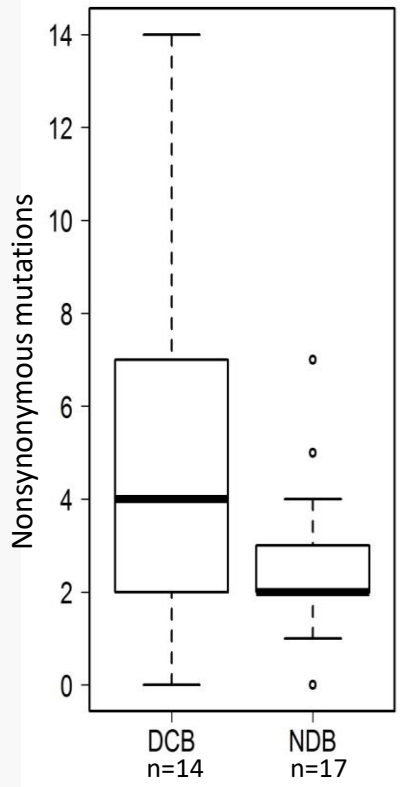
NSCLC - Nonsynonymous mutations

('Mutational load')

Published:
Whole exome seq
(20,500 genes)



Reanalysis:
'CAIO panel' Cologne
(93 genes)



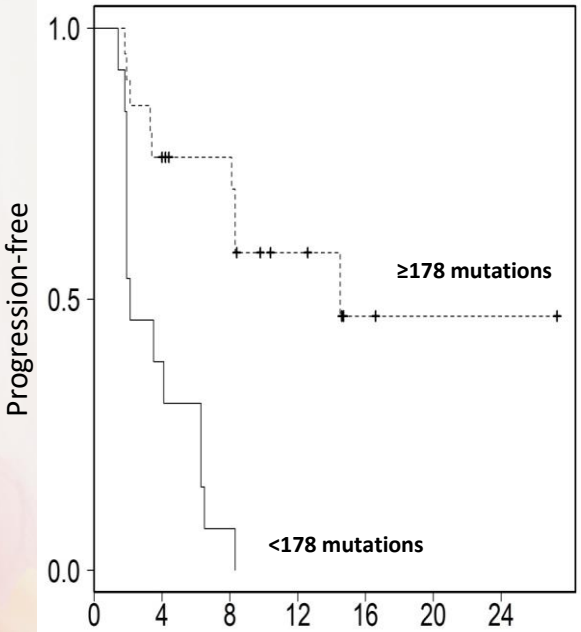
Rizvi et al Science 2015:
n=31 patients with NSCLC; Pembrolizumab (anti-PD-1). Number of nonsynonymous mutations determined by whole exome sequencing (published data) and 'CAIO panel' (reanalysis of same dataset).

DCB: Durable clinical benefit
NDB: No durable benefit

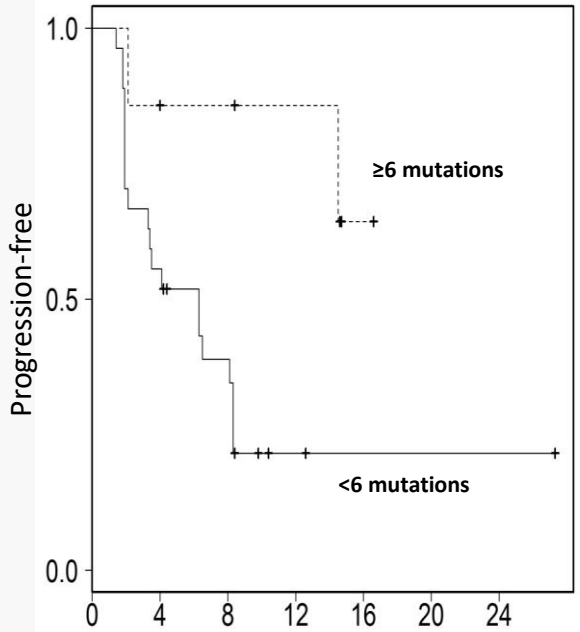
Data: Rizvi NA et al, Science 2015. 348(6230):124-8

NSCLC - Progression-free survival

Published:
Whole exome seq (20,500 genes)

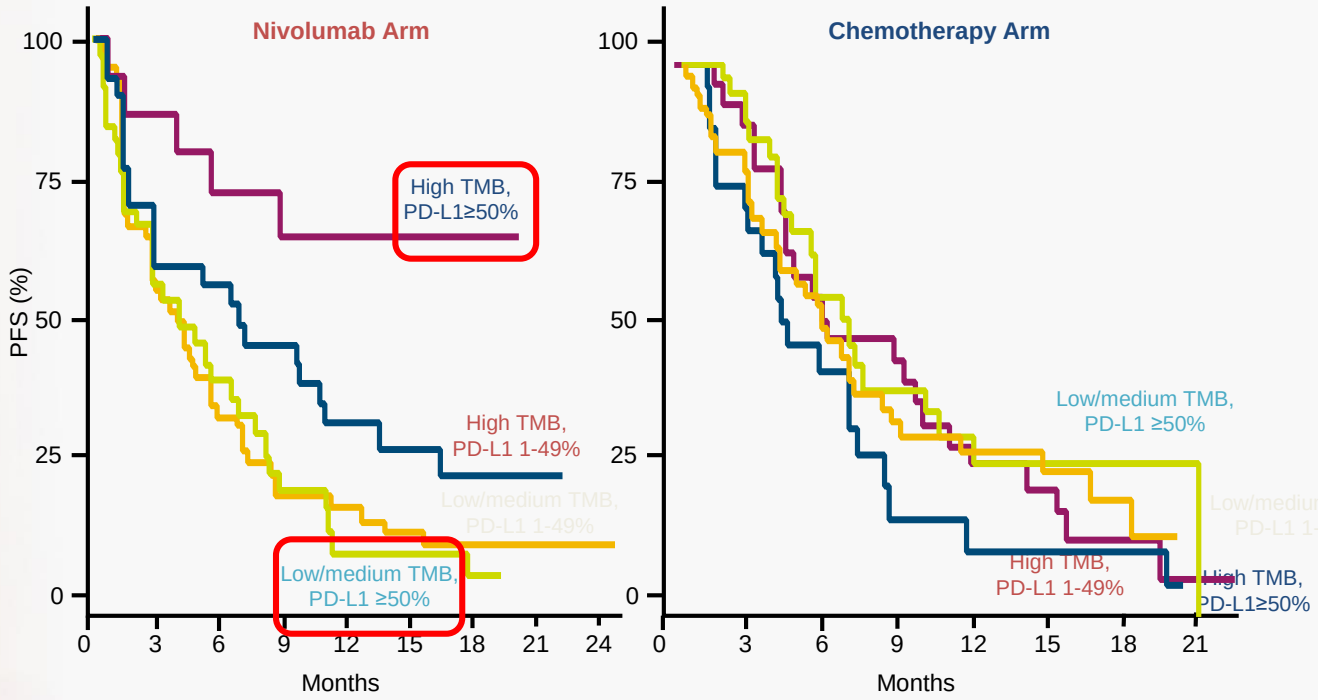


Reanalysis:
'CAIO panel' Cologne (93 genes)



Data: Rizvi NA et al, Science 2015. 348(6230):124-8

PFS by TMB Subgroup and PD-L 1 Expression
CheckMate 026 TMB Analysis: Nivolumab in First-line NSCLC



No. at risk

High TMB, PD-L1 ≥50%	16	13	10	8	8	6	2	0	0	32	24	13	12	7	5	2	1
High TMB, PD-L1 1-49%	31	17	16	13	8	6	2	1	0	28	18	9	3	2	2	2	0
Low/medium TMB, PD-L1 ≥50%	41	21	12	6	2	2	1	0	0	41	30	14	10	5	4	2	0
Low/medium TMB, PD-L1 1-49%	70	33	18	9	7	5	1	1	1	53	35	23	13	10	8	3	0

Peters S, et al. Presented at AACR. 2017, Abstract CT082

Cancer Gene Panel: Overview

	academic	Illumina	Thermo	NEOplus	Qiagen	F one
exonic region	1.1 Mb	1.9 Mb	1.7 Mb	1.3 Mb	1.3 Mb	1.1 Mb
drivers	✓	✓	-	✓	(✓)*	✓
microsatellites	✓	✓	-	✓	✓	✓
input	50 ng	40 ng (DNA+RNA each)	20 ng	50 ng	10-40 ng	200 ng
neg. predictors	✓	✓	unknown	(✓)*	✓	✓
resistance mutations	✓	(✓)*	unknown	(✓)*	✓	(✓)*
repair pathway genes	✓	(✓)*	unknown	unknown	✓	✓
sensitivity	unknown	unknown	unknown	unknown	unknown	78,6%§
specificity	unknown	unknown	unknown	unknown	unknown	70,6%§
wet lab tested	not yet	not yet	✓	✓	not yet	✓
kit locally available	(✓)*	✓	✓	✓	✓	-

* not all requirements met

§ data from Campesato et al., Oncotarget 2016

- *DNA-repair deficient tumors carry high mutational loads*
  - *MSI*
  - *BRCAness*
  - *Polymerase E-deficient*
  - *Smoking signatures*
  - *UV-signatures*

- Test for BRCA and BRCAness

- Current studies explore PARP1inhib in synergy with PD1/PD-L1mabs

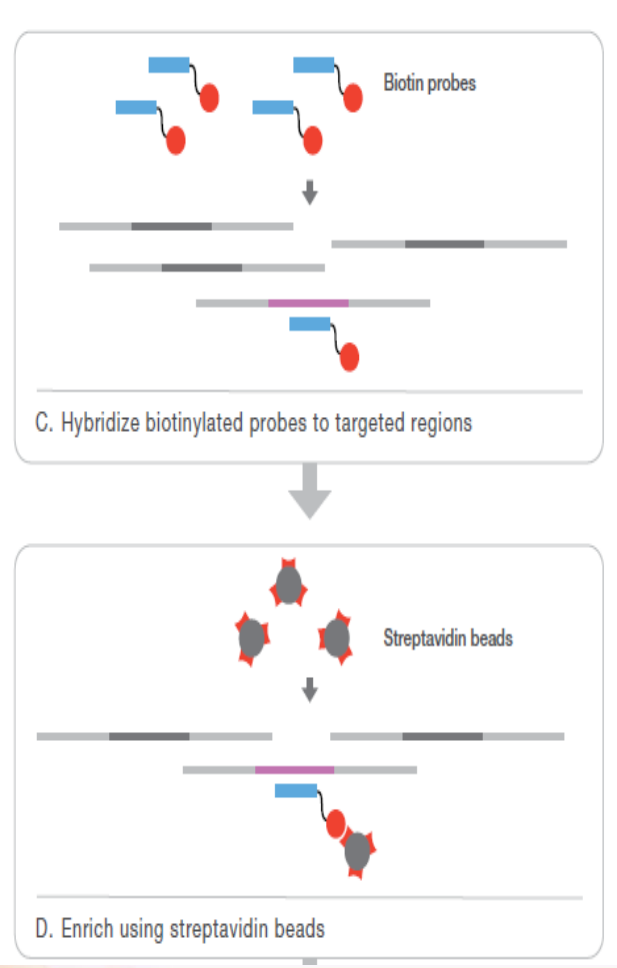
- Every pathologist has to be aware of HRD and provide
- comprehensive panel testing for stage IV cancer patients

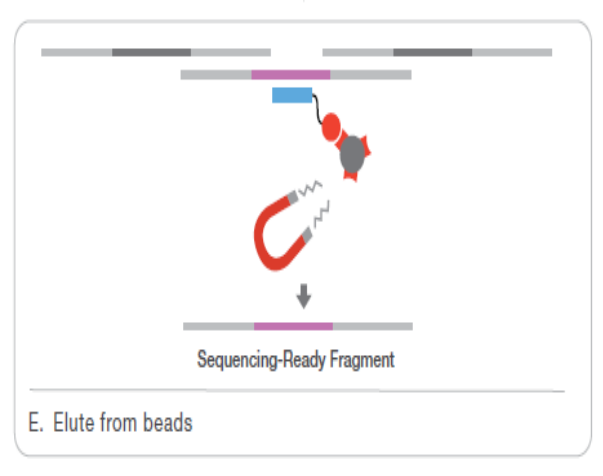

Thankyou

Foto: Jörg Engels–Fotolia.com

Hybrid Capture - New enrichment technology

Hybrid Selection instead of Multiplex PCR:
- Fragmentation of DNA (Covaris)
- Ligation of Adapter and Barcodes





Advantage:

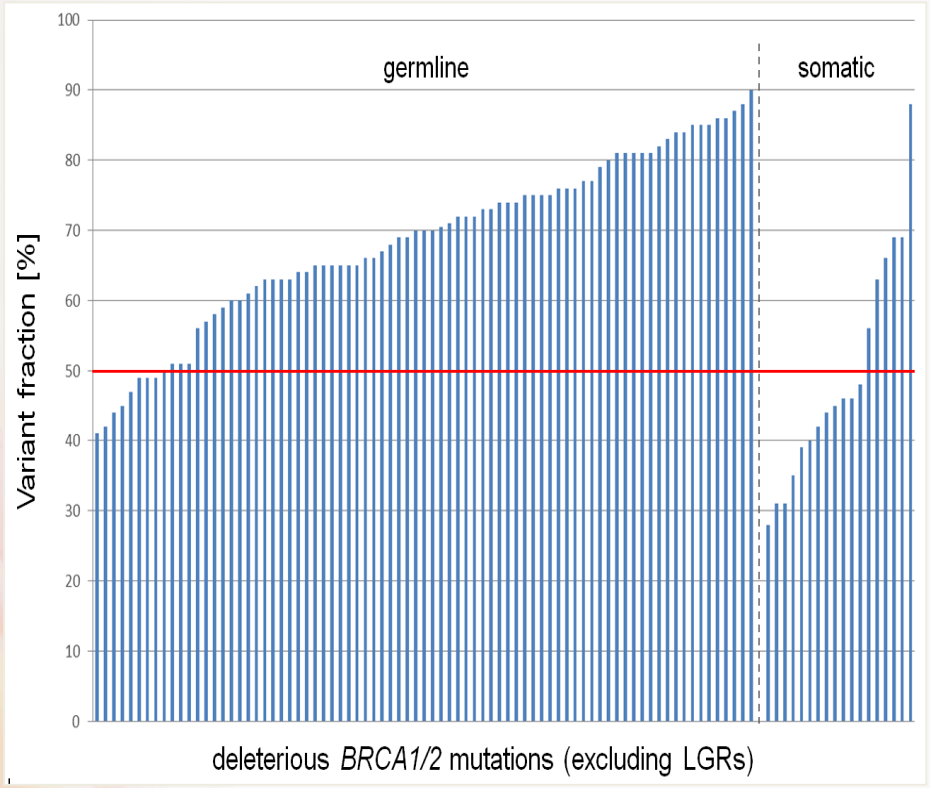
fusions can be integrated

Disadvantage:

more sample input (10x)

more data output

Prevalence of somatic mutations in risk genes including *BRCA1/2* in consecutive ovarian cancer patients (AGO-TR-1 study)



Clinical Trial Registration Number: NCT02222883

Rita Schmutzler 2017

Multiplex PCR-based panels

Lung Panel

for all NSCLC

189 Amplicons

NRAS	Exon2-3
DDR2	Exon1-18
PTEN	Exon7
FGFR2	Exon5-17
HRAS	Exon2-3
KRAS	Exon2-3
AKT1	Exon4
MAP2K1	Exon2
ERBB2	Exon19-20
STK11	Exon1-9
KEAP1	Exon1-6
ALK	Exon19-28
NFE2L2	Exon1-5
PIK3CA	Exon1-2,9,20
EGFR	Exon18-21
MET	Exon16-19
BRAF	Exon11,15
JAK2	Exon12,14

DDR2 Panel

for squameous

35 Amplicons

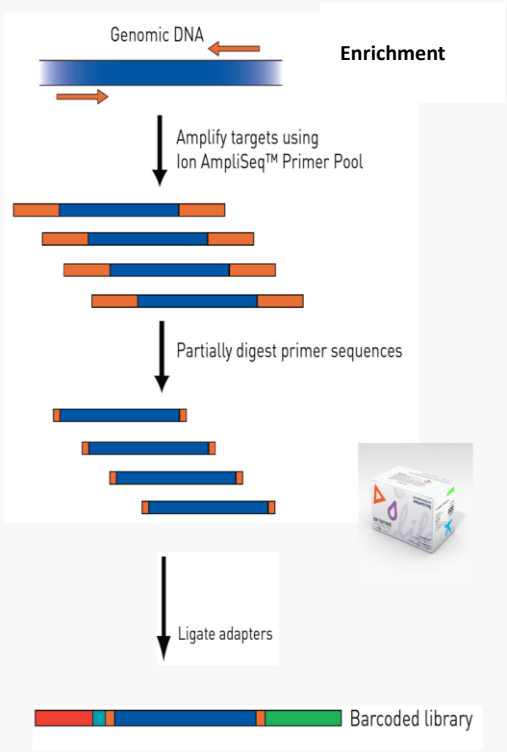
BRAF	Exon11, 15
DDR2	Exon1-18

80% of DNA Extracts have the minimal required amount of material

Multiplex PCR
- 10 to 50ng of gDNA
- DDR2 Panel
- Lung Panel

Library Preparation
- Adapter ligation incuding BC
- Enrichment (10 cycles)

MiSeq (Illumina)
- 48 Patients are loaded (DDR2 Panel)
- 24 Patients loaded (Lung Panel)
- Minimal coverage 500x

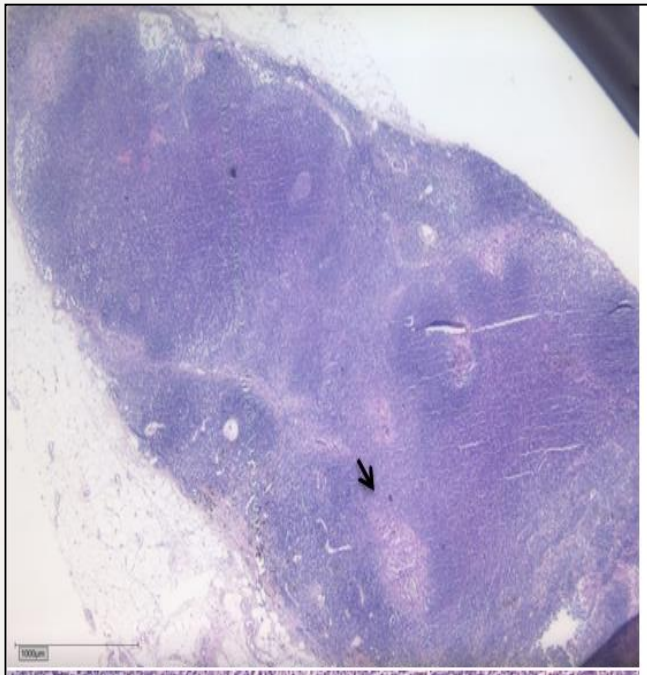


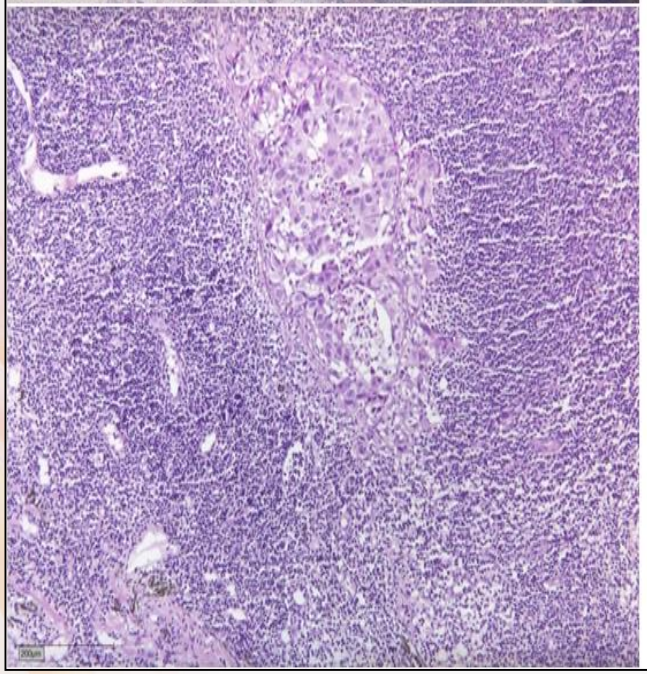
Quantification (Quibit)
Normalization
Pooling

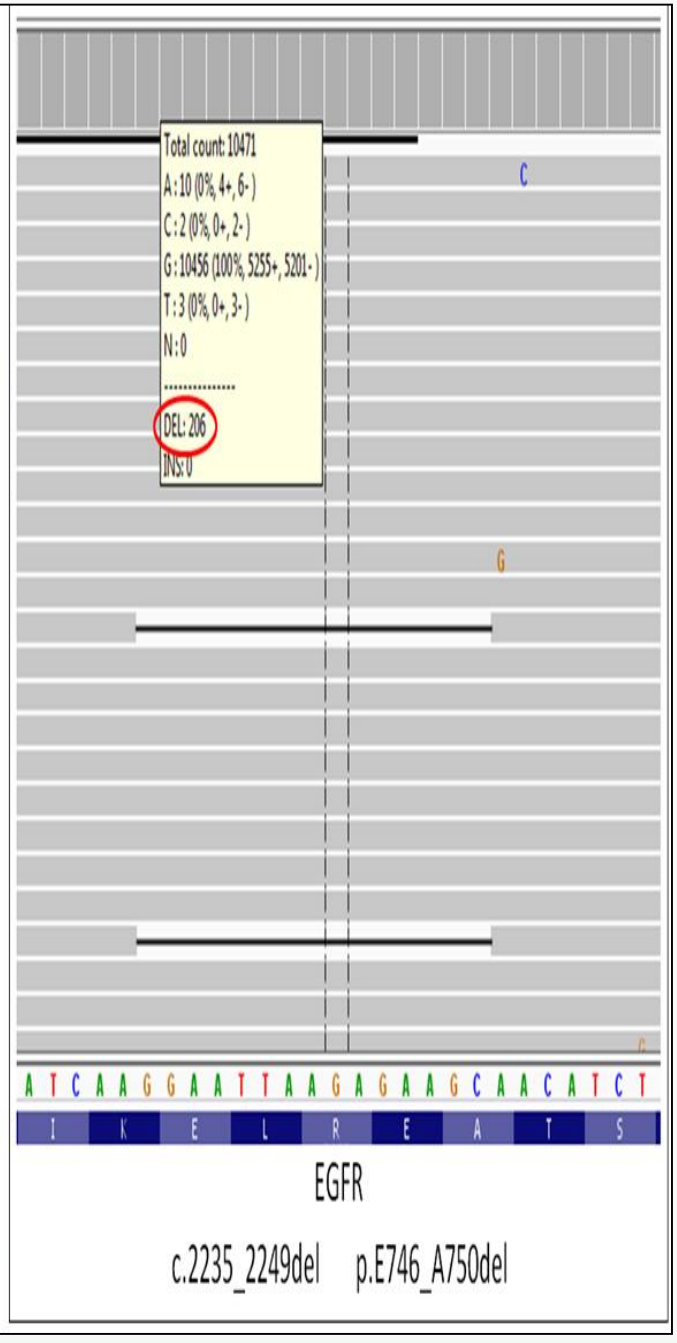
Timeline

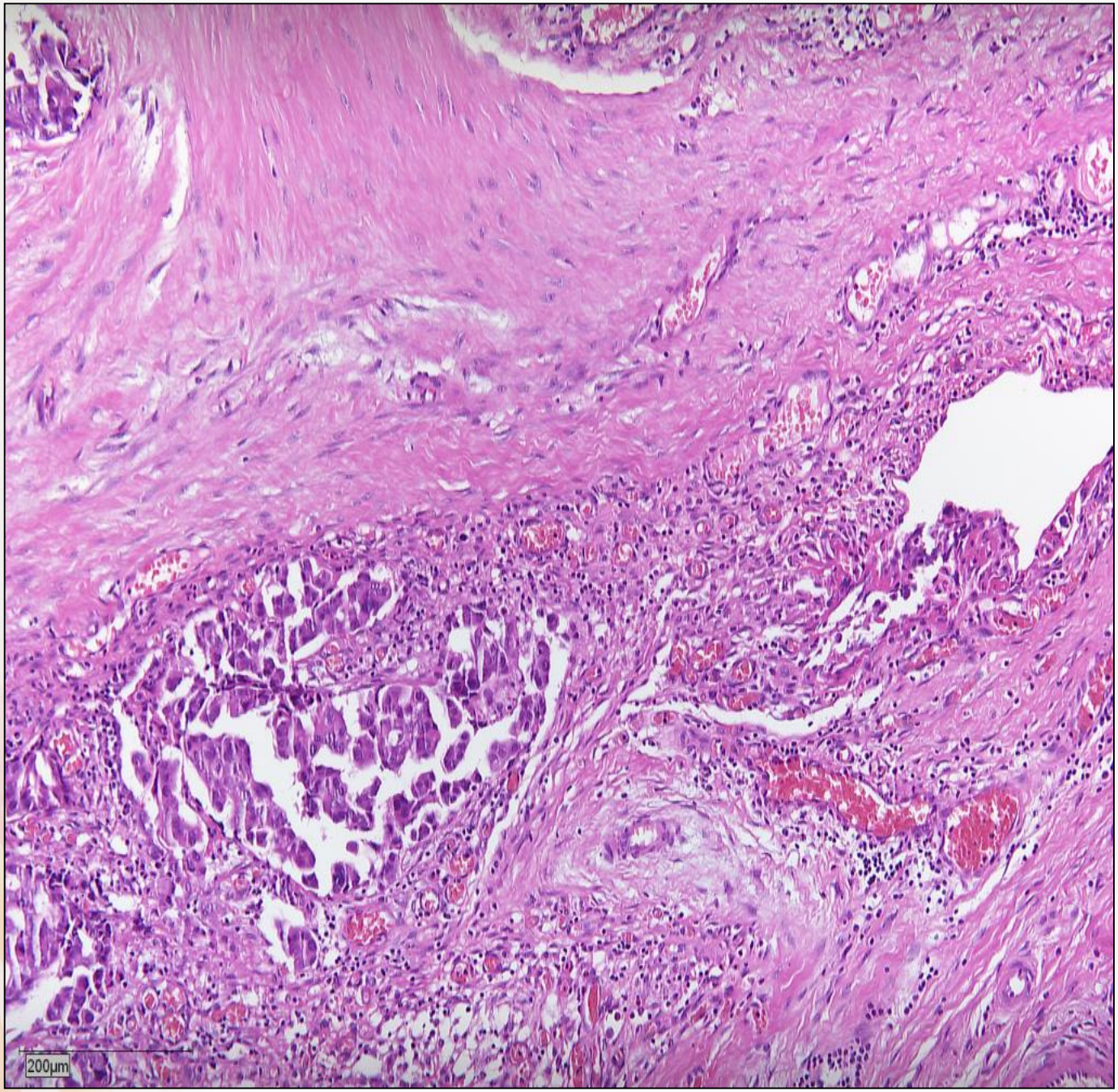
Day 1:	DNA → Multiplex PCR
Day 2:	Library Prep → MiSeq loading
Day 3:	MiSeq ready → Fastq files
Day 4:	Alignment, BAM → Data

König K, JTO 2015









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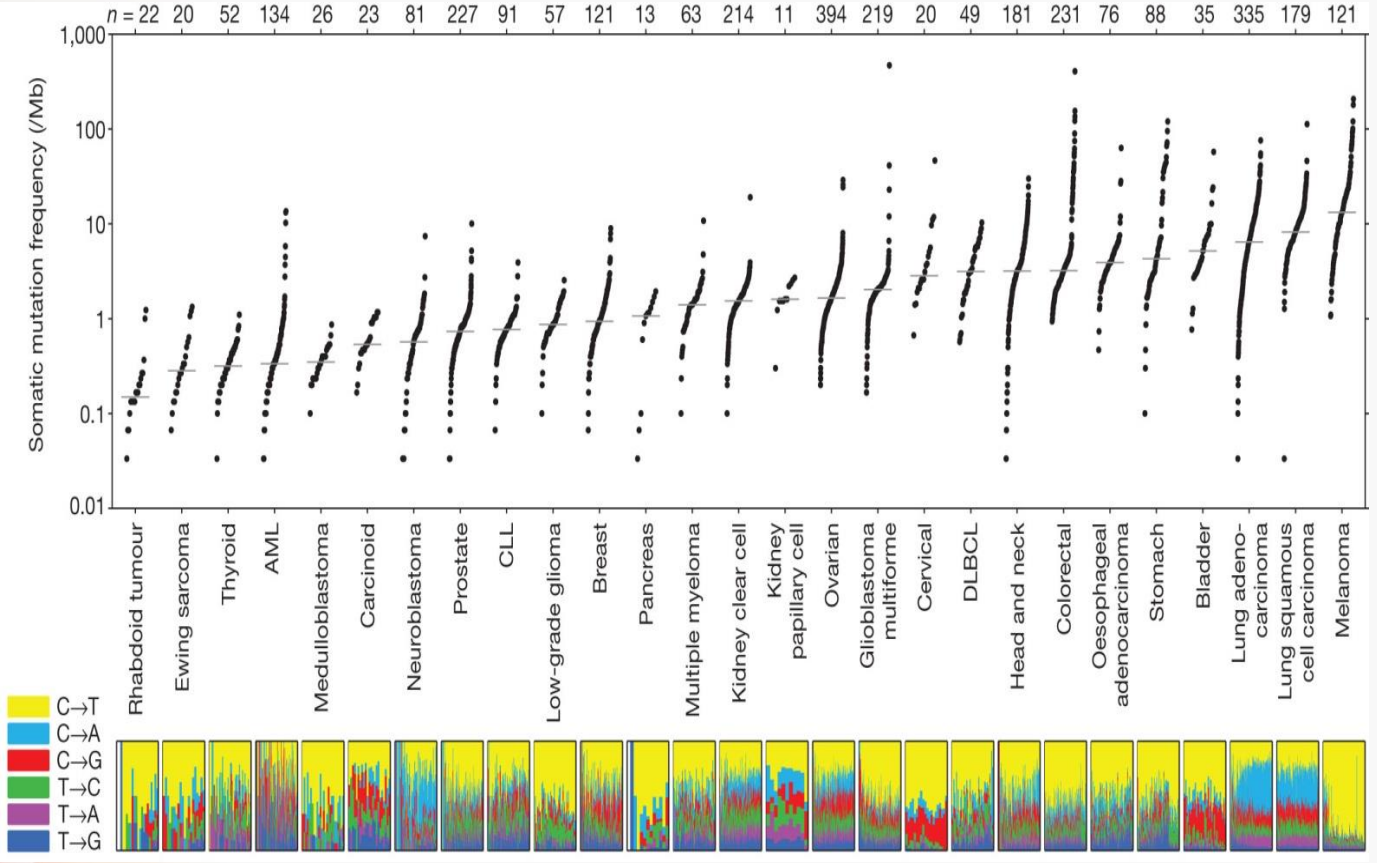
the biomarker national meeting by ONCOACADEMIA

Somatic genetic alterations in cancer correlate with response to PD1 therapies



MS Lawrence et al. Nature, 1-5 (2013)

Van Allen EM[1], et al., Science. 2015 Sep 10.

Genomic correlates of response to CTLA4 blockade in metastatic melanoma.

Overall mutational load, neoantigen load, and expression of cytolytic markers in the immune microenvironment were significantly associated with clinical benefit.