Parameters guiding a site/histology - independent drug development

Workshop on Site and Histology – Independent Indications in Oncology
EMA, 14 December 2017

ANTONI VAN LEEUWENHOEK
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Disclosures

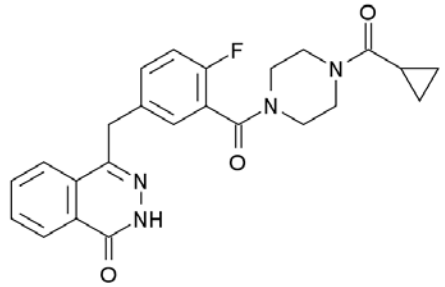
- Research relationships with pharmaceutical companies
- Commercial interest in Modra pharmaceuticals https://modrapharmaceuticals.com

Cases

Targeting:

- PARP (poly[ADP-ribose]polymerase)
- BRAF V600 mutation
- Mismatch repair deficiency

Olaparib – Phase I



- Olaparib is an example of an oral, selective PARP1 inhibitor
- First-in-man phase I study with a PARP inhibitor in NKI and Royal Marsden in patients with advanced solid malignancies
- Olaparib monotherapy showed mild, manageable toxicity profile (grade 1-2); not interfering with daily life or intake of study drug
- Unselected population: patients with all advanced solid malignancies → low response rate

permeabilized cells by double-stranded DNA oligomers. Anal. Biochem. 198, 236–239 (1991).
26. Lundin, C. et al. RAD51 is involved in repair of damage associated with DNA replication in mammalian cells. J. Mol. Biol. 328, 521–535 (2003).

Supplementary Information accompanies the paper on **www.nature.com/nature**.

Acknowledgements We wish to thank J. Lunec, J. Thacker, L. Thompson, M. Zdzienicka, Z. Hostomsky and Pfizer GRD, La Jolla for providing materials. The investigation was financed by grants to T.H. and M.M from Yorkshire Cancer Research. Additional support was financed through grants to T.H. from the Swedish Cancer Society and the Swedish Research Council and a grant to N.J.C. from Cancer Research UK.

Competing interests statement The authors declare that they have no competing financial interests.

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Targeting the DNA repair defect in *BRCA* mutant cells as a therapeutic strategy

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BRCA1 and BRCA2 are important for DNA double-strand break repair by homologous recombination[1], and mutations in these genes predispose to breast and other cancers[2]. Poly(ADP-ribose) polymerase (PARP) is an enzyme involved in base excision repair, a key pathway in the repair of DNA single-strand breaks[3]. We show here that BRCA1 or BRCA2 dysfunction unexpectedly and profoundly sensitizes cells to the inhibition of PARP enzymatic

sister chromatid exchange, causing chromatid aberrations and loss of viability. To examine the effects of PARP1 depletion, we transfected a plasmid expressing a short interfering RNA (siRNA) targeting mouse *Parp1* into wild-type embryonic stem (ES) cells and ES cells lacking wild-type *Brca1* or *Brca2* (refs 7, 8). These cells bear specific genomic mutations of *Brca1* or *Brca2* and like *BRCA1/2* tumours lack a wild-type allele and can be directly compared to their isogenic wild-type counterparts. The *Parp1* siRNA construct caused a clear reduction in clonogenic survival of BRCA1- and BRCA2-deficient cells compared with wild-type cells (Fig. 1a, b). This prompted us to test whether chemical inhibitors of PARP activity might have similar effects. We used two novel, specific and very potent small-molecule PARP inhibitors: KU0058684 (PARP1 half-maximal inhibitory concentration (IC_{50}) = 3.2 nM) and KU0058948 (PARP1 IC_{50} = 3.4 nM), as well as a much less active but chemically related compound KU0051529 (PARP1 IC_{50} = 730 nM) (Supplementary Fig. 1 and Supplementary Table 1).

Clonogenic cell survival assays showed that BRCA1- or BRCA2-deficient cells were extremely sensitive to KU0058684 and KU0058948 compared with heterozygous mutant or wild-type cells (Fig. 1c), and that these effects were rapid and irreversible (Supplementary Fig. 2). The SF_{50} (dosage at which 50% of cells survived) for KU0058684 was 35 nM for BRCA1-deficient ES cells and 15 nM for BRCA2-deficient ES cells; for wild-type cells this was approximately 2 μM. Notably, this represents factors of 57-fold and 133-fold enhanced sensitivity of cells lacking wild-type BRCA1 and BRCA2, respectively, compared with wild-type-cells. Similar results were obtained with non-embryonic cells such as BRCA2-deficient Chinese hamster ovary cells[9], which showed a greater than 1,000-fold enhanced sensitivity compared with a BRCA2-complemented derivative (Supplementary Fig. 3a). Similarly, depletion of *BRCA1* messenger RNA in MCF7 human breast cancer cells by RNA interference induced sensitivity to PARP inhibition (Supplementary Fig. 3b). In contrast, KU0051529, which does not effectively inhibit PARP1 or PARP2, had no selective effect on cells lacking wild-type BRCA1 or BRCA2. In conjunction with the siRNA data, this indicates that the mechanism of sensitivity is through inhibition of PARP. Although BRCA1 and BRCA2 mutant cells do show enhanced sensitivity to certain DNA-damaging agents, such as cisplatin, this was to a much lesser degree (approximately threefold) (data not shown), demonstrating the greater selectivity of PARP inhibition compared to chemotherapy. Notably, none of the inhibitors had any selective effect on cells heterozygous for *Brca1* or *Brca2* mutations (Fig. 1c).

After 24 h exposure, KU0058684 elicited a profound arrest of cells with a tetraploid DNA content, indicating arrest in the G2 or M phase of the cell cycle (Fig. 1d). Most (>85%) of the arrested cells did not become labelled with an anti-phospho histone H3 antibody, an M-phase marker, indicating predominant arrest at

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Competing interests statement The authors declare that they have no competing financial interests.

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Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase

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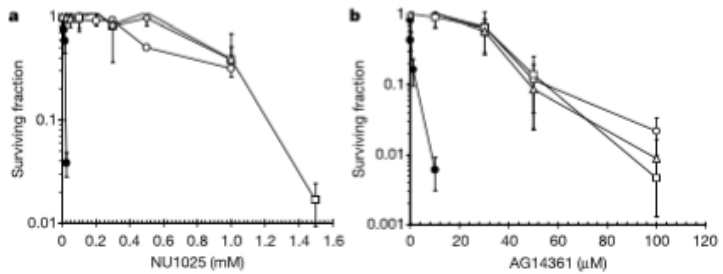
Poly(ADP-ribose) polymerase (PARP1) facilitates DNA repair by binding to DNA breaks and attracting DNA repair proteins to the site of damage[1–3]. Nevertheless, PARP1$^{-/-}$ mice are viable, fertile and do not develop early onset tumours[4]. Here, we show that PARP inhibitors trigger γ-H2AX and RAD51 foci formation. We propose that, in the absence of PARP1, spontaneous single-strand breaks collapse replication forks and trigger homologous recombination for repair. Furthermore, we show that BRCA2-deficient cells, as a result of their deficiency in homologous recombination, are acutely sensitive to PARP inhibitors, presumably because resultant collapsed replication forks are no longer repaired. Thus, PARP1 activity is essential in homologous recombination-deficient *BRCA2* mutant cells. We exploit this requirement in order to kill BRCA2-deficient tumours by PARP inhibition alone. Treatment with PARP inhibitors is likely to be highly tumour specific, because only the tumours (which are BRCA2$^{-/-}$) in BRCA2$^{+/-}$ patients are defective in homologous recombination. The use of an inhibitor of a DNA repair enzyme alone to selectively kill a tumour, in the absence of an exogenous DNA-damaging agent, represents a new concept in cancer treatment.

Despite its important role in the cellular response to genotoxic stress, PARP1 is not required for survival in the absence of such an insult, and PARP1$^{-/-}$ mice are viable and fertile[2,5,6]. These mice do not develop early onset tumours and tumour latency is increased in PARP1 knockout mice that are deficient for p53 (ref. 4). Nevertheless, it is generally accepted that loss of PARP1 activity is important in maintaining genetic stability, because PARP1$^{-/-}$ mice exhibit defective DNA single-strand break (SSB) repair and an increase in homologous recombination, sister chromatid exchange and micronuclei formation[1,2,5–7]. However, the elevated homologous recombination levels in PARP1$^{-/-}$ mice represent an error-free repair pathway, which may explain why the genetic instability in PARP1-deficient or inhibited cells is not associated with any accumulation of mutations or cancer.

PARP1 does not seem to be directly involved in homologous recombination, as RAD51 foci form normally in PARP1-deficient cells and homologous recombination-mediated repair of a DNA double-strand break (DSB) is unaffected by inhibition or loss of



Nature April, 2005

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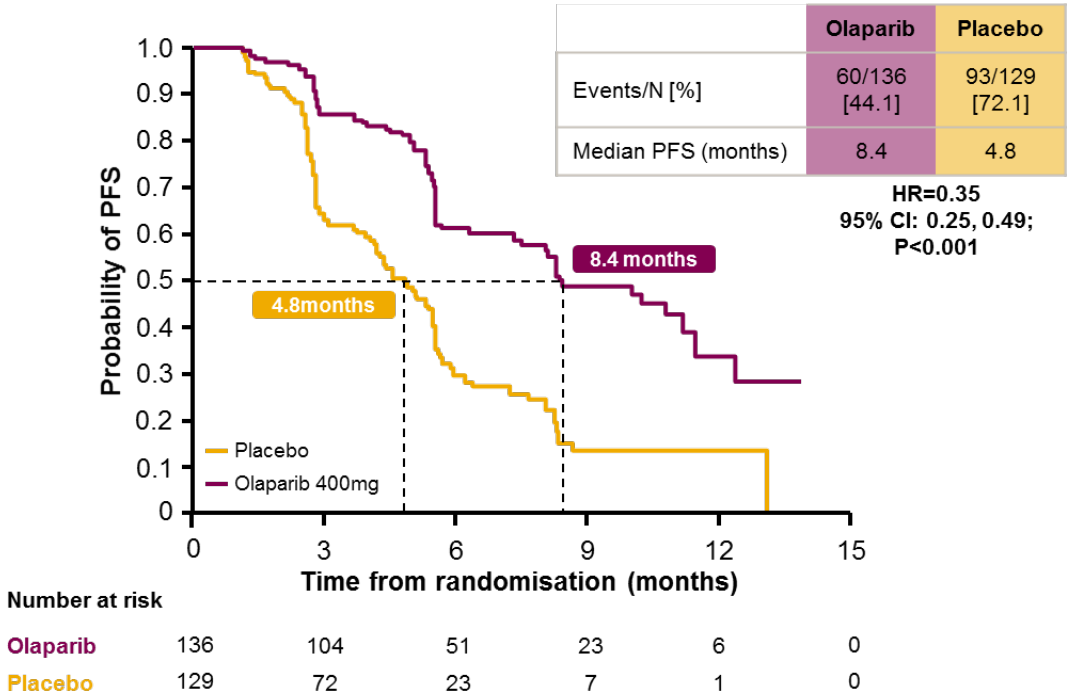
Olaparib – Phase I

Table 4. Clinical Responses in Study Patients for Whom the Response Could Be Evaluated.*

Subgroup and Dose	Total No. of Patients	Partial or Complete Radiologic Response	Radiologically Stable Disease	Tumor-Marker Response
				number of patients
All patients	60	9	7†	7
Patients with *BRCA1* or *BRCA2* ovarian, breast, or prostate cancer‡	19	9 (8 with ovarian cancer, 1 with breast cancer)	2 (1 with ovarian cancer, 1 with breast cancer)	7 (6 with ovarian cancer, 1 with prostate cancer)

Fong et al. N Engl J Med 2009

Registration trial olaparib ovarian cancer

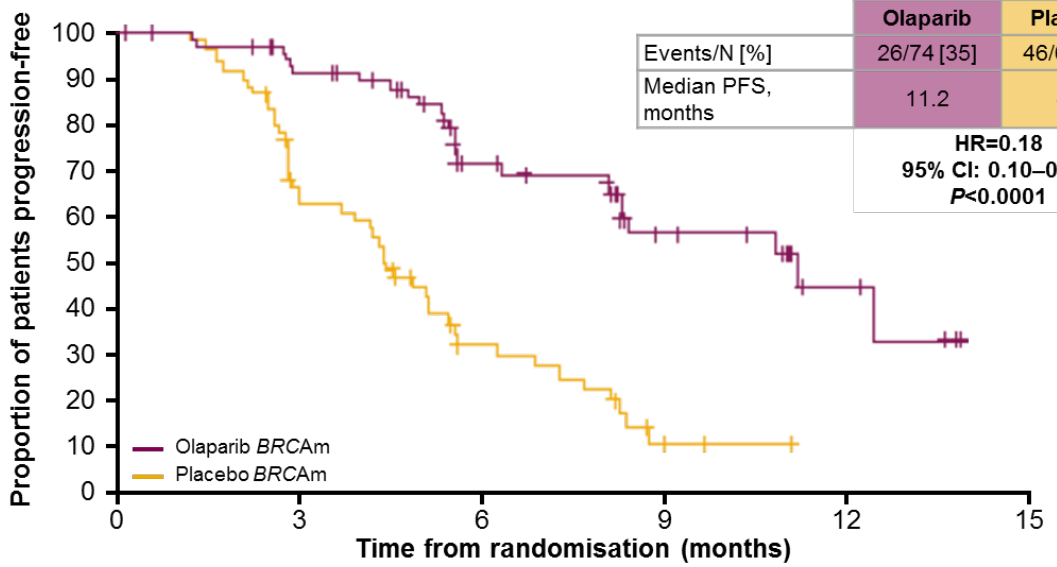


	Olaparib	Placebo
Events/N [%]	60/136 [44.1]	93/129 [72.1]
Median PFS (months)	8.4	4.8

HR=0.35
95% CI: 0.25, 0.49;
P<0.001

Number at risk

Olaparib	136	104	51	23	6	0
Placebo	129	72	23	7	1	0



	***BRCA*m (n=136)**	
	Olaparib	**Placebo**
Events/N [%]	26/74 [35]	46/62 [74]
Median PFS, months	11.2	4.3

HR=0.18
95% CI: 0.10–0.31;
***P*<0.0001**

Number at risk

Olaparib *BRCA*m	74	59	34	15	5	0
Placebo *BRCA*m	62	35	13	2	0	0

Ledermann J et al. N Engl J Med 2012; 366:1382-92

The NEW ENGLAND JOURNAL of MEDICINE
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Tumor agnostic approval within reach for targeted drugs?

Condition	Fulfilled?
Established Mechanism Of Action (MOA)	
MOA tumor tissue independent	
Preclinical Proof Of Principle (POP)	
Preclinical safety	
Validated biomarker	
Clinical POP	
Clinical safety	
Pivotal randomized study	
Activity in other tumor types	
No relevant competing strategies	
Unmet medical need	

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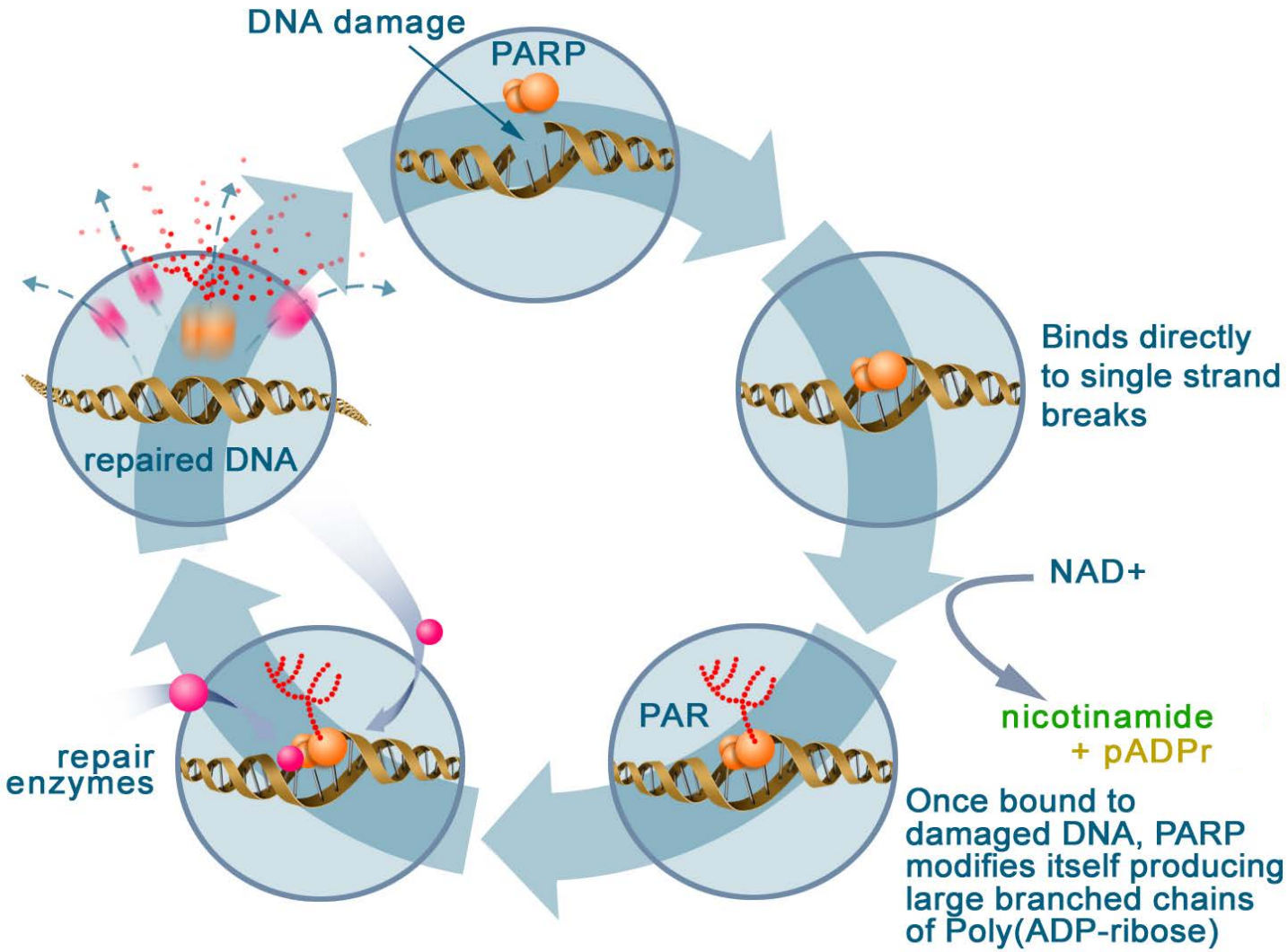
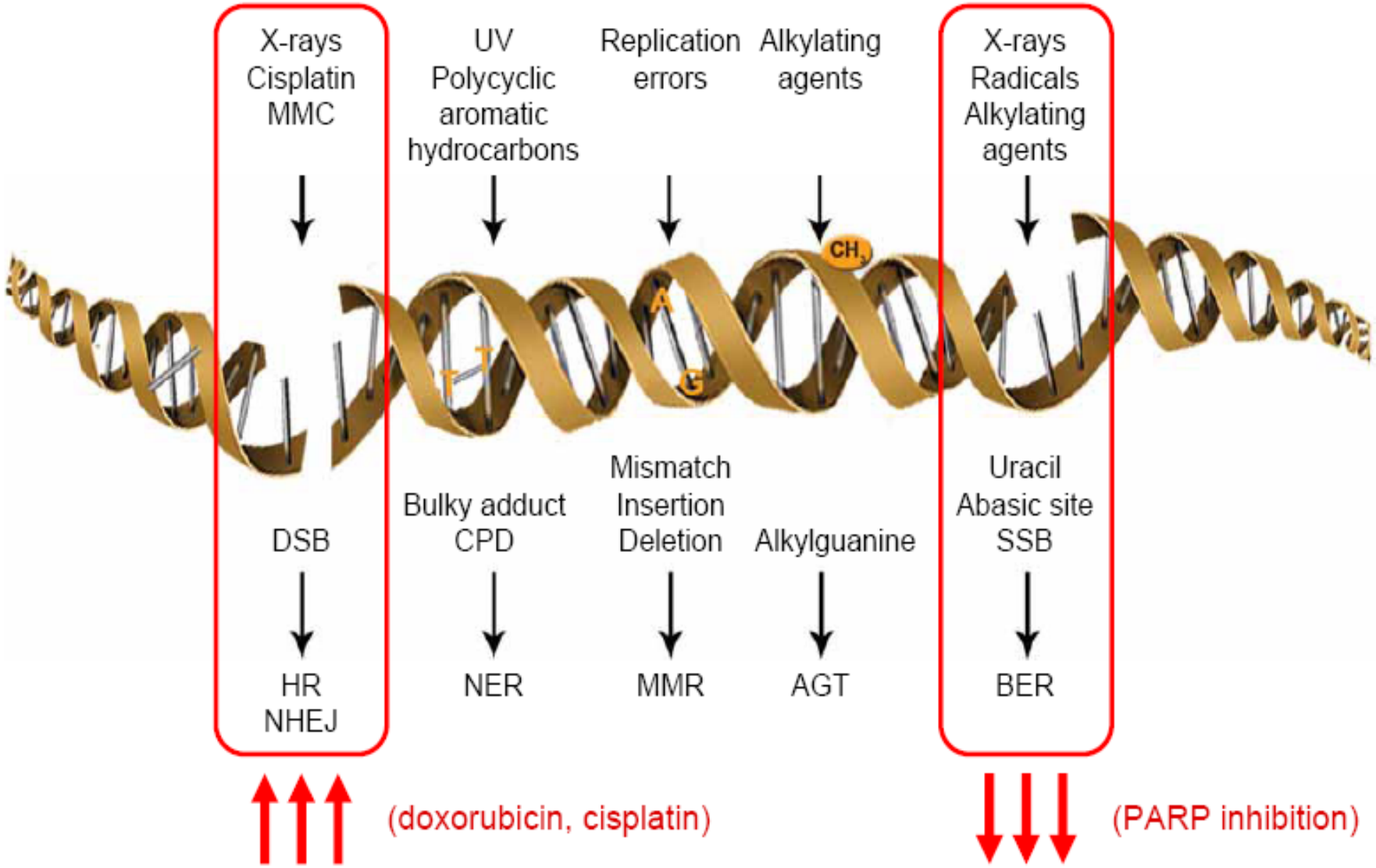
Poly (ADP-Ribose) Polymerase (PARP)



Increased understanding of DNA repair



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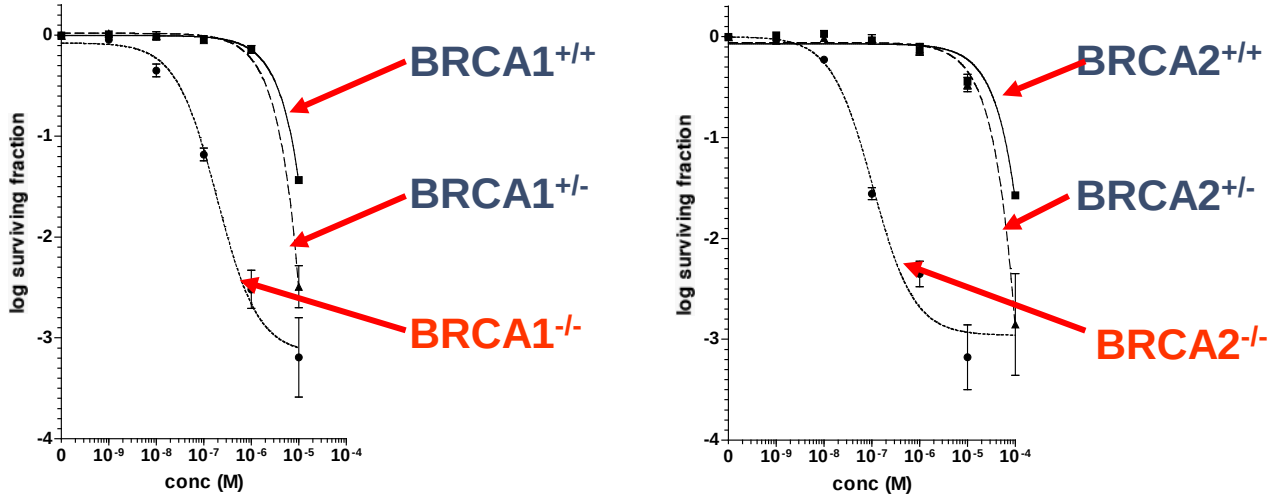
BRCA1$^{-/-}$ and BRCA2$^{-/-}$ cells are extremely sensitive to PARP inhibition



No difference in sensitivity between heterozygous and wild-type BRCA cells

Targeted inhibition ⟶ selective and less toxic therapy

Farmer *et al. Nature 2005 &* Bryant *et al. Nature 2005*

Consistency of *BRCA1* and *BRCA2* Variant Classifications Among Clinical Diagnostic Laboratories

ClinVar Submitter	No. Classified Variants	No. Comparable Variants	Full Name in ClinVar	Most Recent Classification	Evidence Provided	Note
Ambry	2,792	1,613	Ambry Genetics	February 2015		
SCRP/Myriad Genetics	2,327	1,351	Sharing Clinical Reports Project	December 2015		Benign and likely benign variants are under-reported
Invitae	1,998	1,367	Invitae	March 2016	Yes	
GeneDx	1,216	957	GeneDx	October 2015	Yes	
Counsyl	272	256	Counsyl	February 2015		No VUS submitted
CHEO	257	220	Molecular Genetics Diagnostic Laboratory, Children's Hospital of Eastern Ontario	Dates not provided		
Emory	203	183	Emory Genetics Laboratory	June 2015		
Total	5,124	2,006				

Lincoln SE et al., *JCO Precision Oncology* 2017;1:1-10

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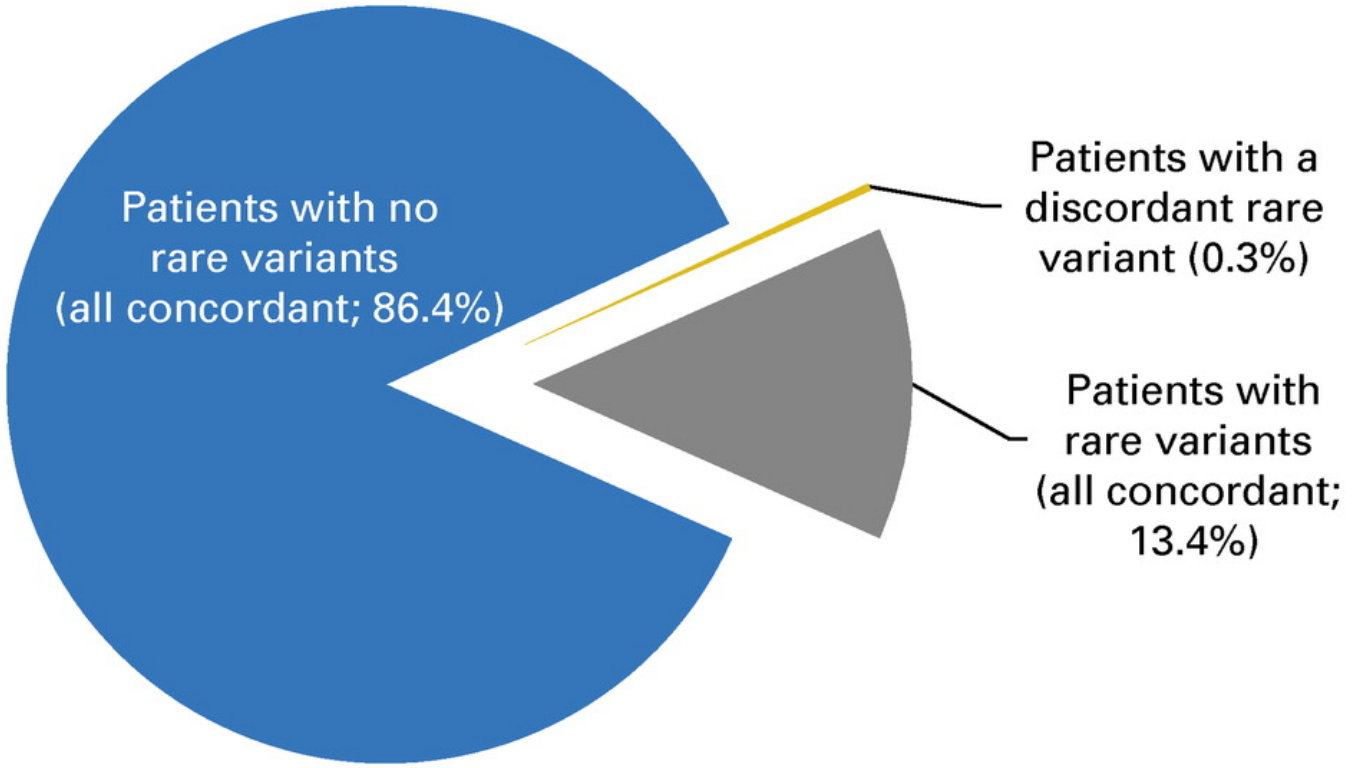
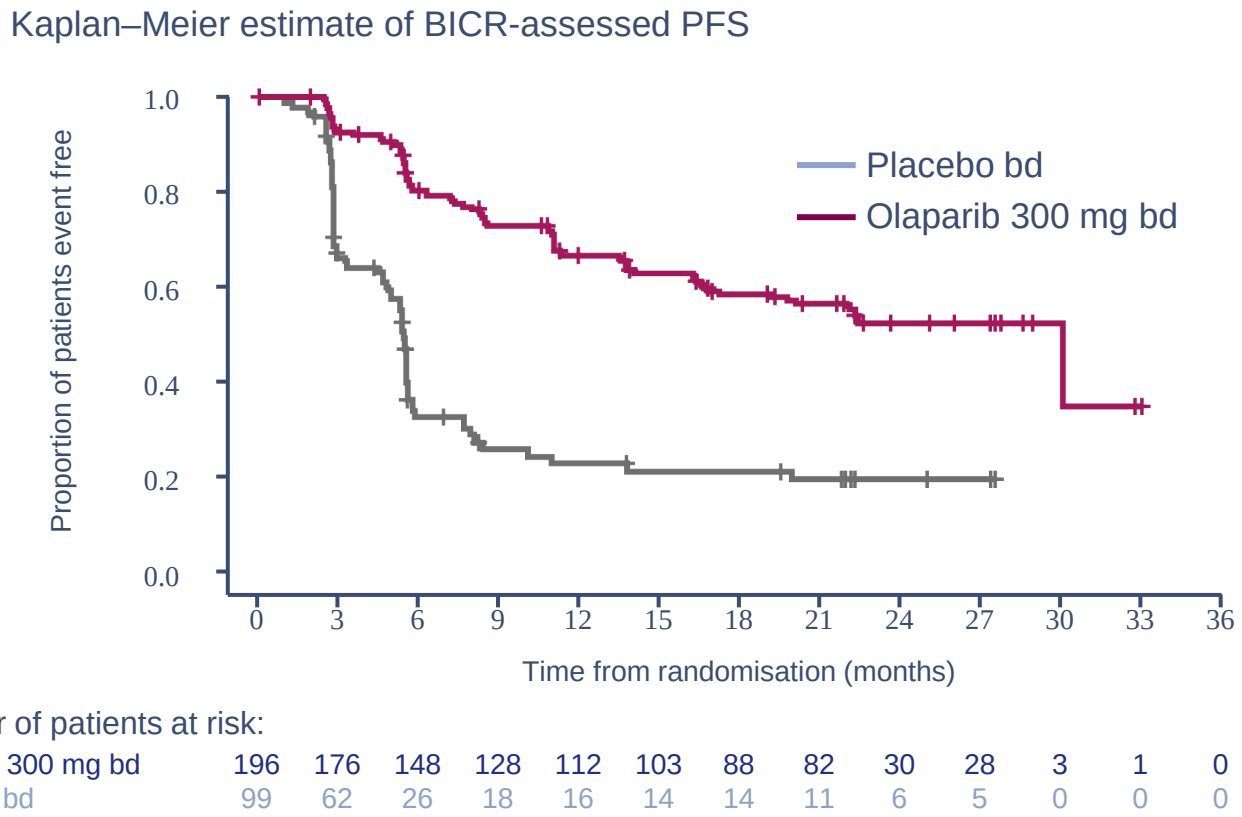
Per patient concordance



Lincoln SE et al., *JCO Precision Oncology* 2017;1:1-10

Confirmatory phase III study in BRCAm ovarian cancer



Number of patients at risk:

Olaparib 300 mg bd	196	176	148	128	112	103	88	82	30	28	3	1	0
Placebo bd	99	62	26	18	16	14	14	11	6	5	0	0	0

	Olaparib 300mg bd	Placebo
Events	81/196 (41.3%)	70/99 (70.7%)
Median	30.2 m	5.5 m
	HR = 0.25 95% CI (0.18,0.35) p<0.0001	

Pujade-Lauraine et al. SGO Annual Meeting 2017 (LBA2)

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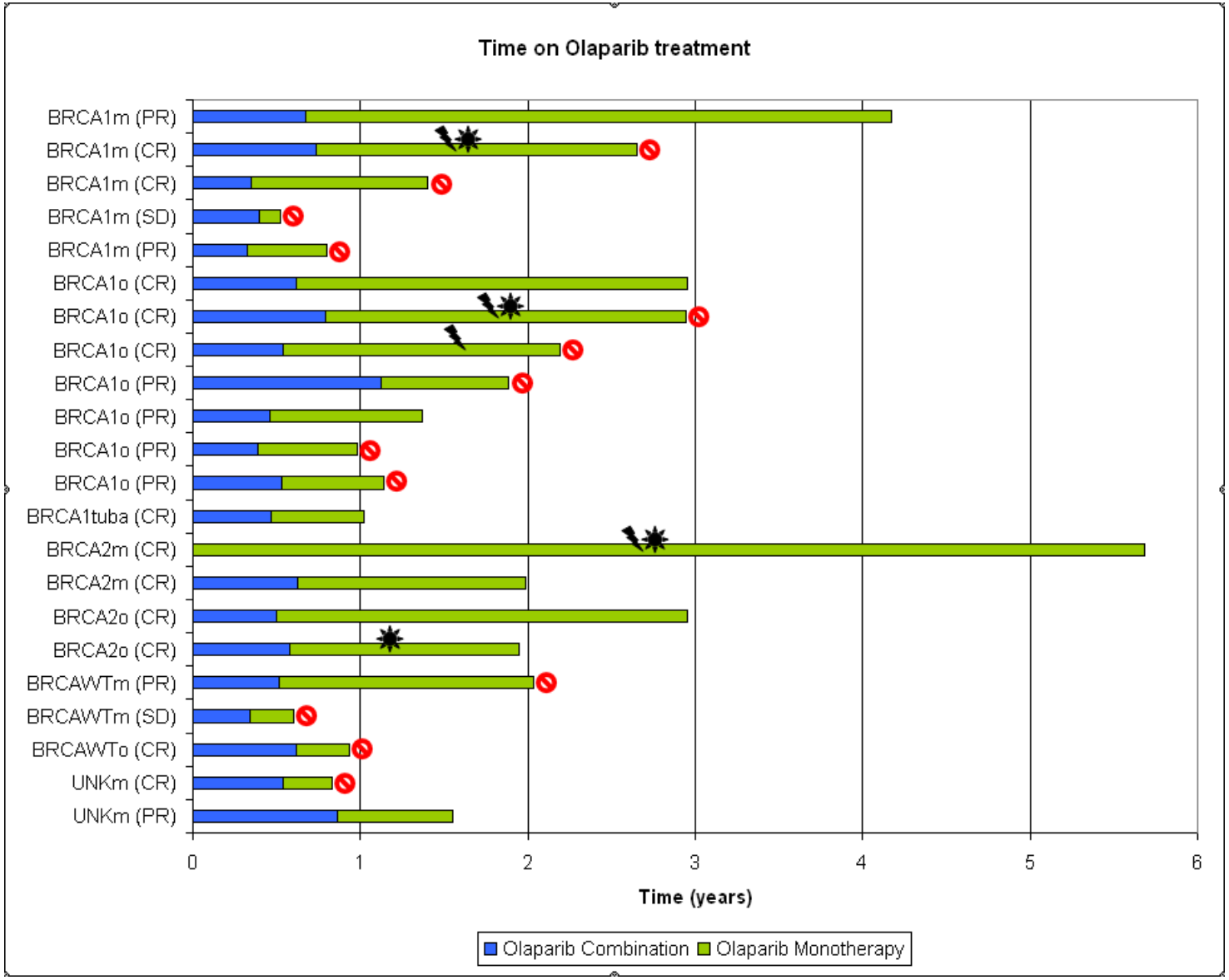
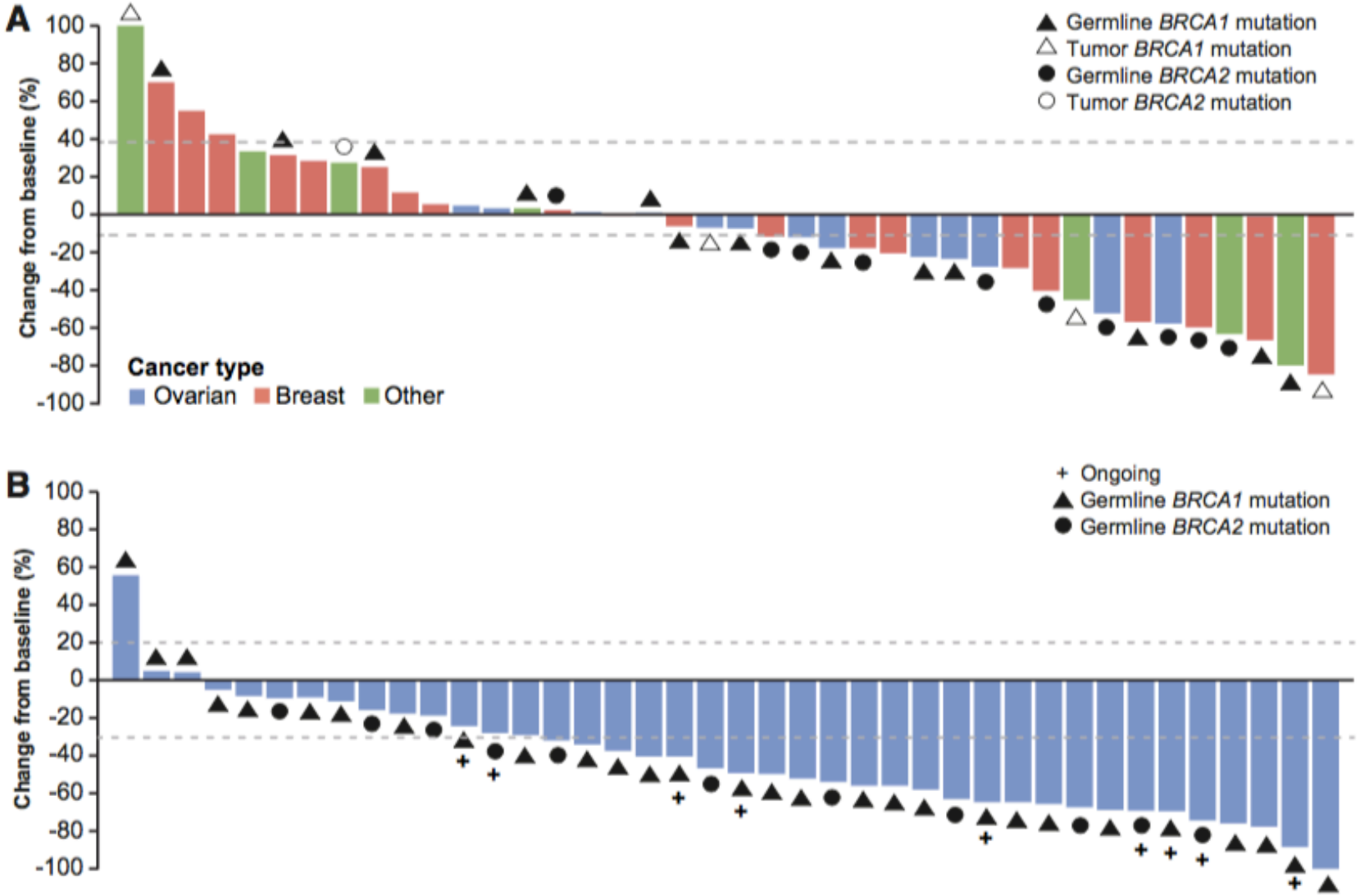
Efficacy of olaparib extended use in BRCA1/2 carriers



Phase I/II study with rucaparib in BRCA1/2 mutant cancers



Kristeleit R et al
CCR 2017; 23:4095-4106

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PARP inhibition in prostate cancer with BRCA2m

Key eligibility criteria

- Histologically confirmed mCRPC
- Progression after 1–2 taxane-based chemotherapy regimens (as per RECIST version 1.1 and/or PCWG2)
- ECOG performance status 0–2
- Naïve to platinum, mitoxantrone, cyclophosphamide or PARP inhibitors
- CTC count ≥5 cells/7.5mL blood
- Willing to provide fresh tumour biopsy samples for biomarker studies

Olaparib 400mg BID (N=50)

Treatment administered until radiological progression, unequivocal clinical progression, unacceptable toxicity, withdrawal of consent or death

Dose reductions to as low as 100mg BID permitted in the event of an initial or recurrent Grade 3 or 4 adverse event* (as per NCI CTCAE criteria)

Primary endpoint:

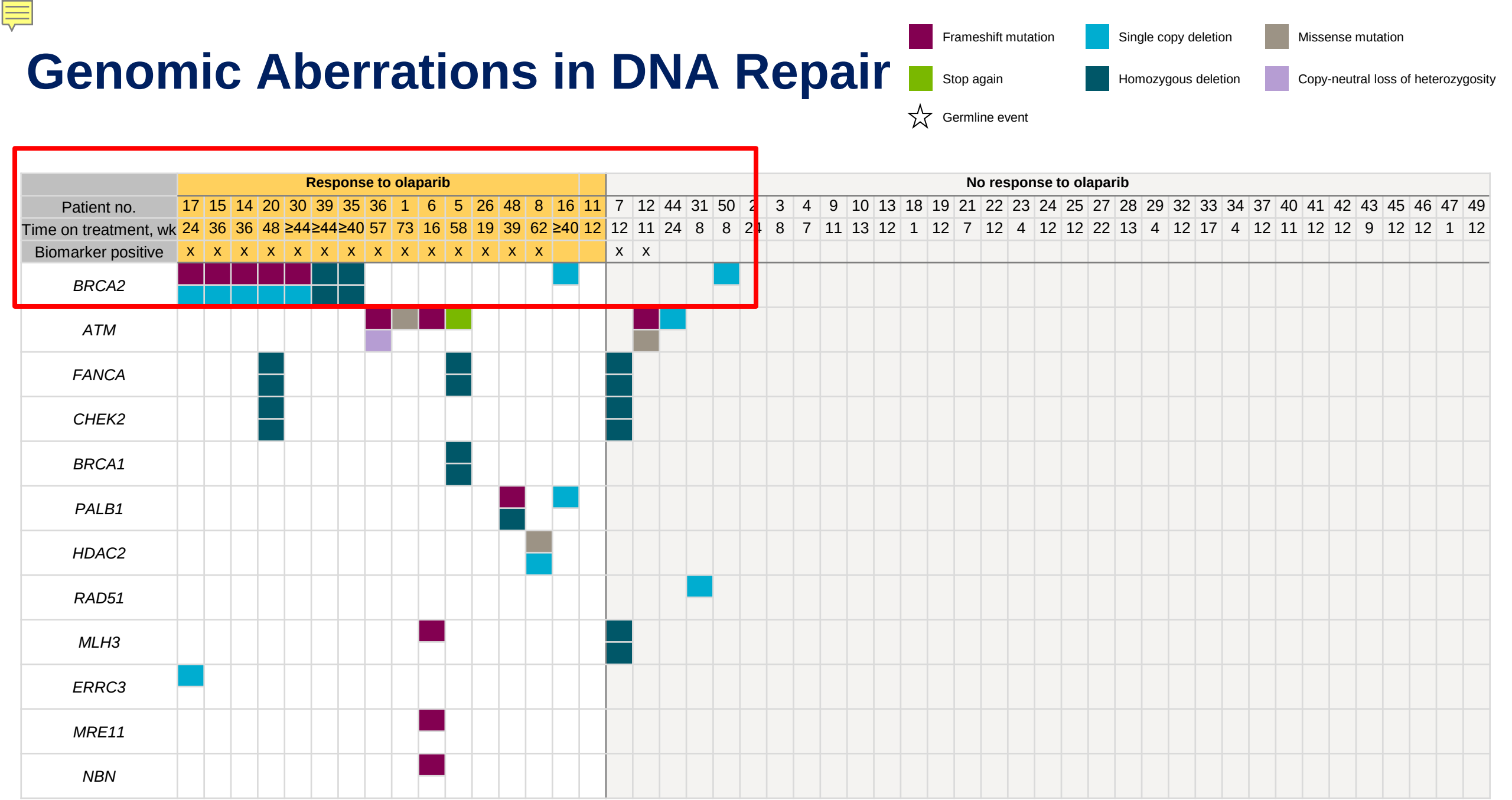
- Response
  - Radiological response (RECIST version 1.1)
  - PSA decline of ≥50% (PCWG2)
  - CTC conversion (≥5 to <5 cells/7.5mL blood)†

Secondary endpoints:

- rPFS‡
- PFS
- OS
- Time to PSA progression (25% increase in PSA)
- Rate of CTC conversion
- Safety and adverse events

Mateo J, et al. *N Engl J Med.* 2015;373:1697–1708

Genomic Aberrations in DNA Repair



wk, week.

Mateo J, et al. *N Engl J Med*. 2015;373:1697–1708.

Response to Olaparib – BRCA2 Aberrations

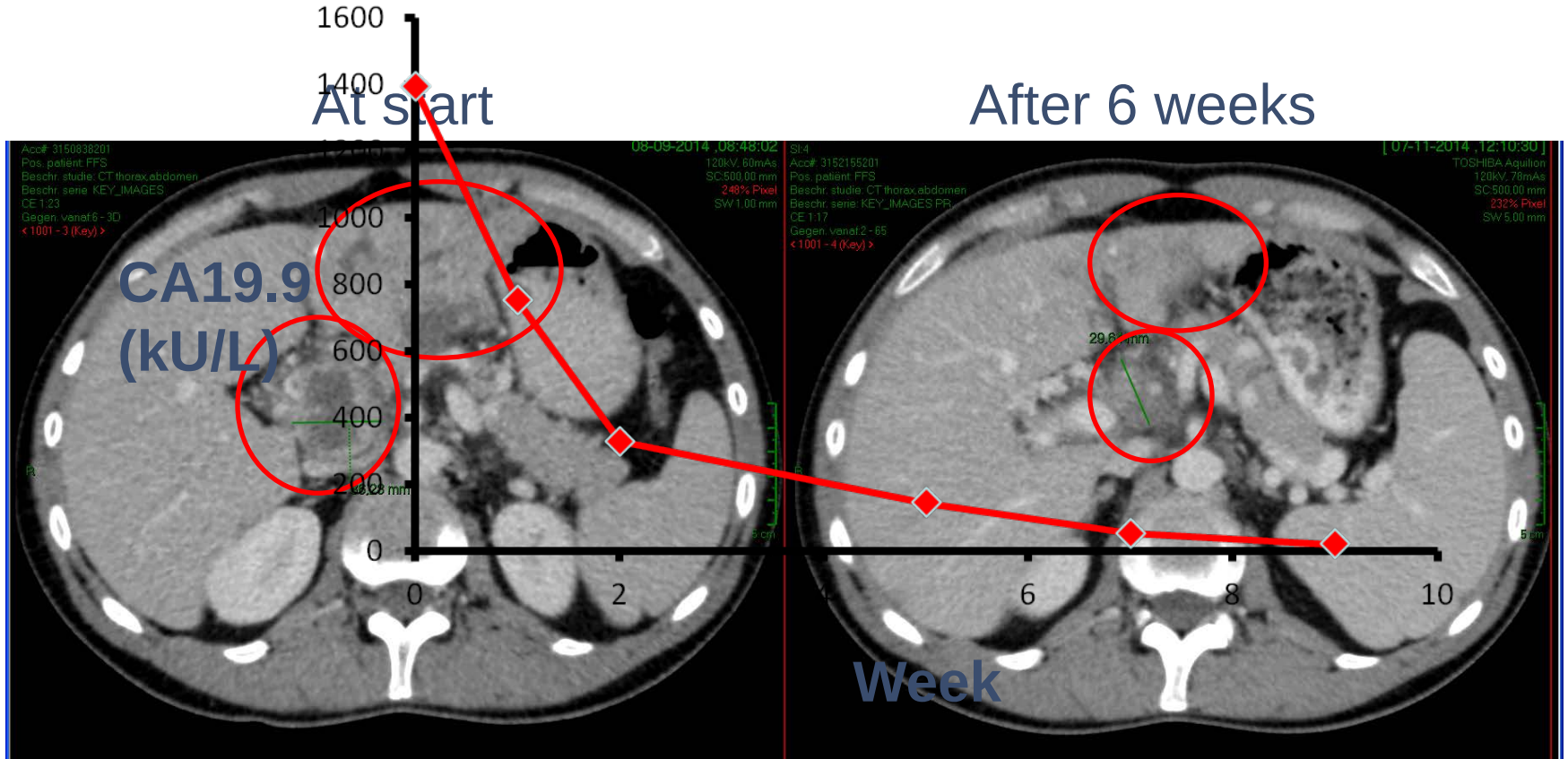
Patient no.	Germline hit?	Response Y/N	RECIST response	PSA fall >50%	CTC conversion	Dose reduced	Time on trial (weeks)
14	Yes	Yes	N/A	Yes	Yes	No	36
15	Yes	Yes	PR	Yes	Yes	Yes	36
17	No, somatic fs + het-del	Yes	PR	Yes	Yes	No	24
20	No, somatic fs + het-del	Yes	PR	Yes	NE	No	40+
30	Yes	Yes	N/A	Yes	Yes	No	40+
35	No, somatic fs + het-del	Yes	PR	Yes	Yes	Yes	24+
39	No, somatic fs + het-del	Yes	PR	Yes	Yes	No	40+

CTC, circulating tumour cell; fs, frameshift; het-del, heterozygous deletion; hom-del, homozygous deletion; N/A, not applicable; NE, not evaluable; PSA, prostate-specific antigen; PR, partial response; RECIST, Response Evaluation Criteria In Solid Tumors.

Mateo J, et al. AACR 2015 (abstr. CT322).

PARPi in cholangiocarcinoma and BRCA1 carrier

- 42 yr male, cholangiocarcinoma, BRCA1m
- PD after 6*cisPt-gemcitabine ➡ olaparib trial



Tumor agnostic approval within reach for PARPi ?

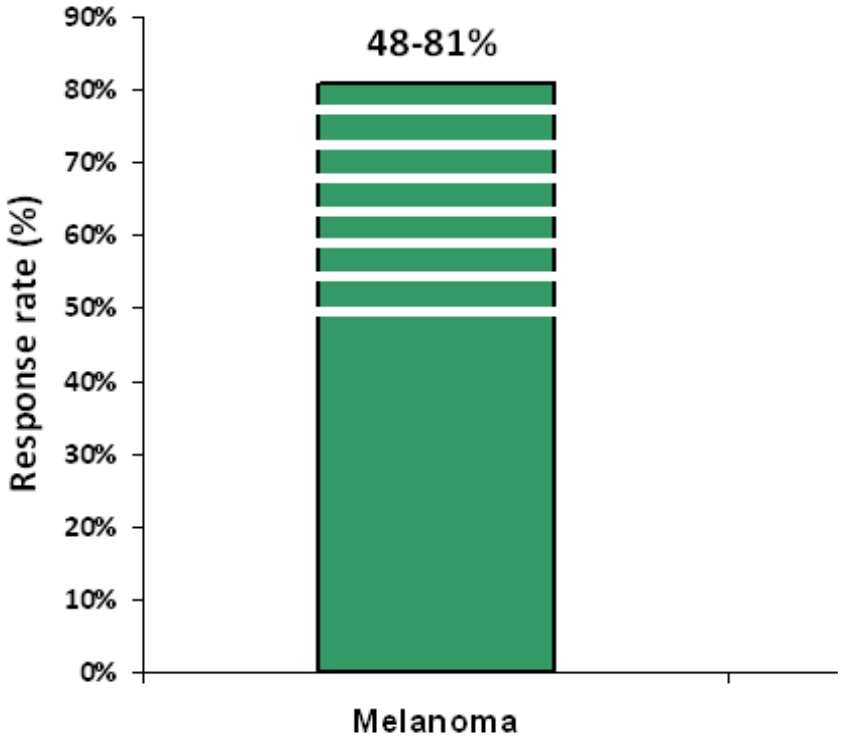
Condition	Fulfilled?
Established MOA	√
MOA tumor tissue independent	√
Preclinical POP	√
Preclinical safety	√
Validated biomarker	√ (but not every BRCAm responds)
Clinical POP	√
Clinical safety	√
Pivotal randomized study	√
Activity in other tumor types	√ (sufficient?)
No relevant competing strategies	√ ?
Unmet medical need	√

Cases

Targeting:

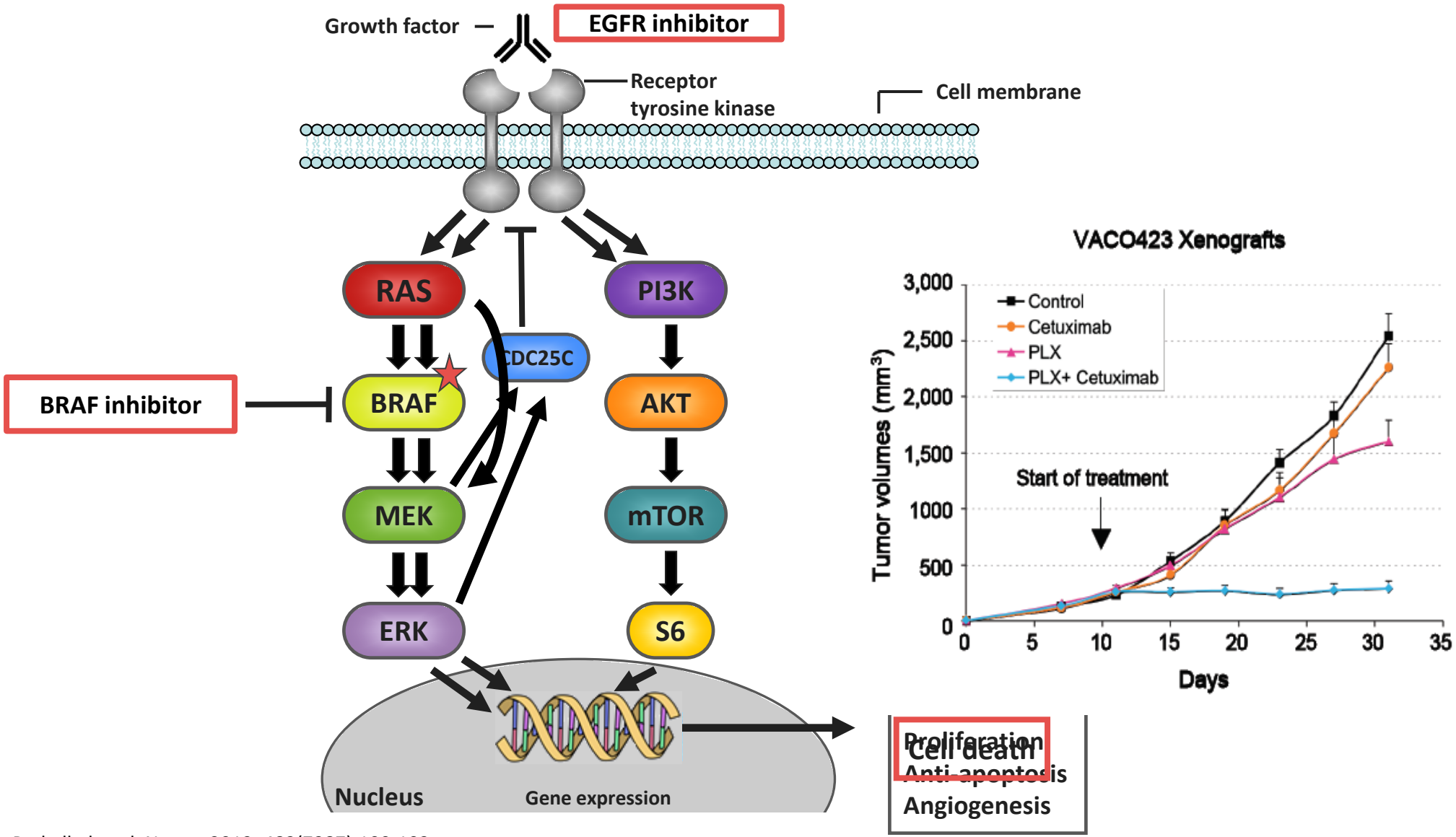
- PARP (poly[ADP-ribose]polymerase)
- BRAF V600 mutation
- Mismatch repair deficiency

BRAF V600E: does tissue context matter?



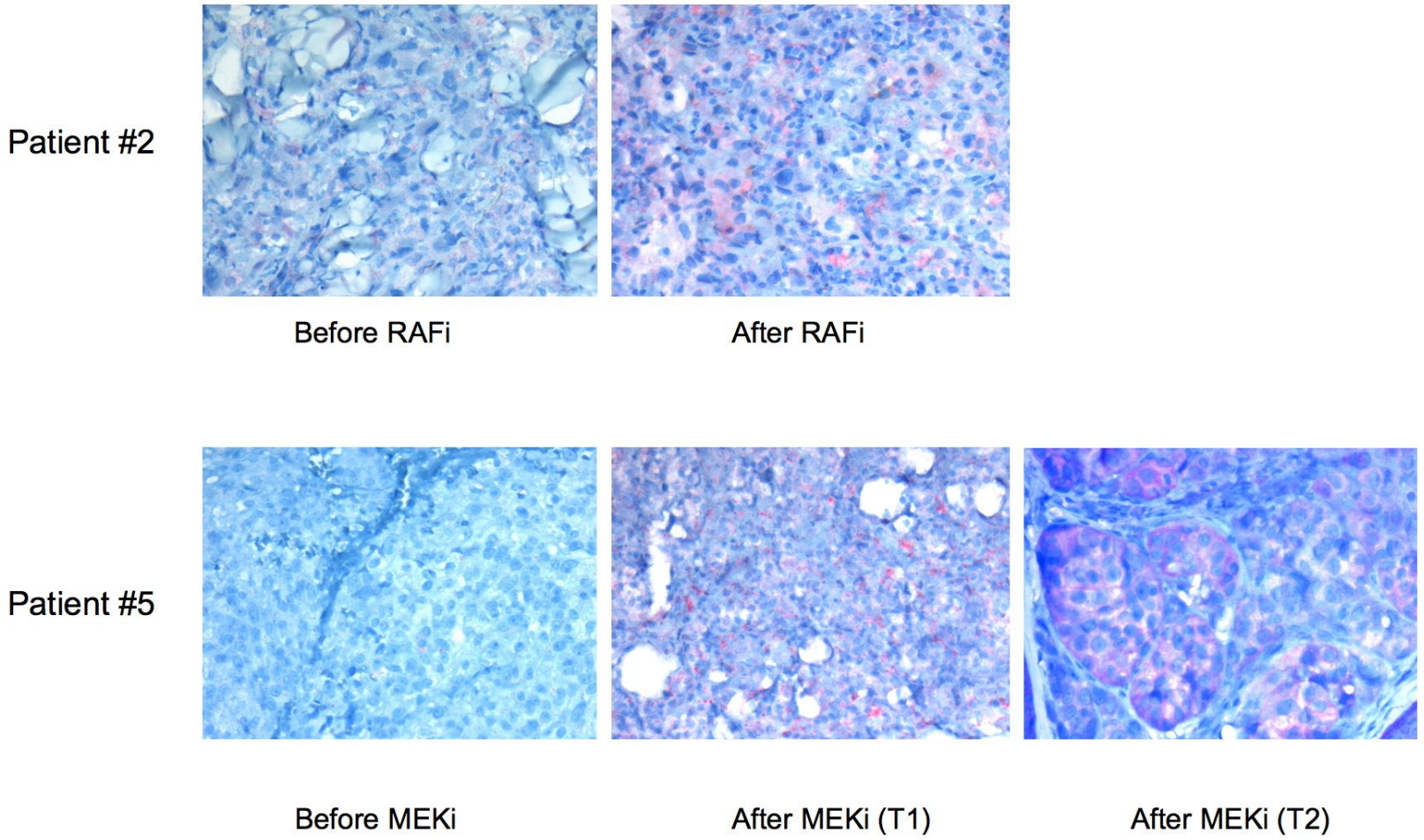
Flaherty et al. N Engl J Med 2010;363:809-19
Chapman et al. N Engl J Med 2011;364:2507-16

‘Feedback’ activation of EGFR by BRAF inhibition in *BRAF*m colon cancer



Prahallad et al. Nature 2012; 483(7387):100-103.

BRAF mutant melanomas upregulate EGFR during development of drug resistance



EGFR IHC in red; Sun et al. Nature 2014;508:118-22

Tumor agnostic approval not justifiable for BRAF V600 mutation

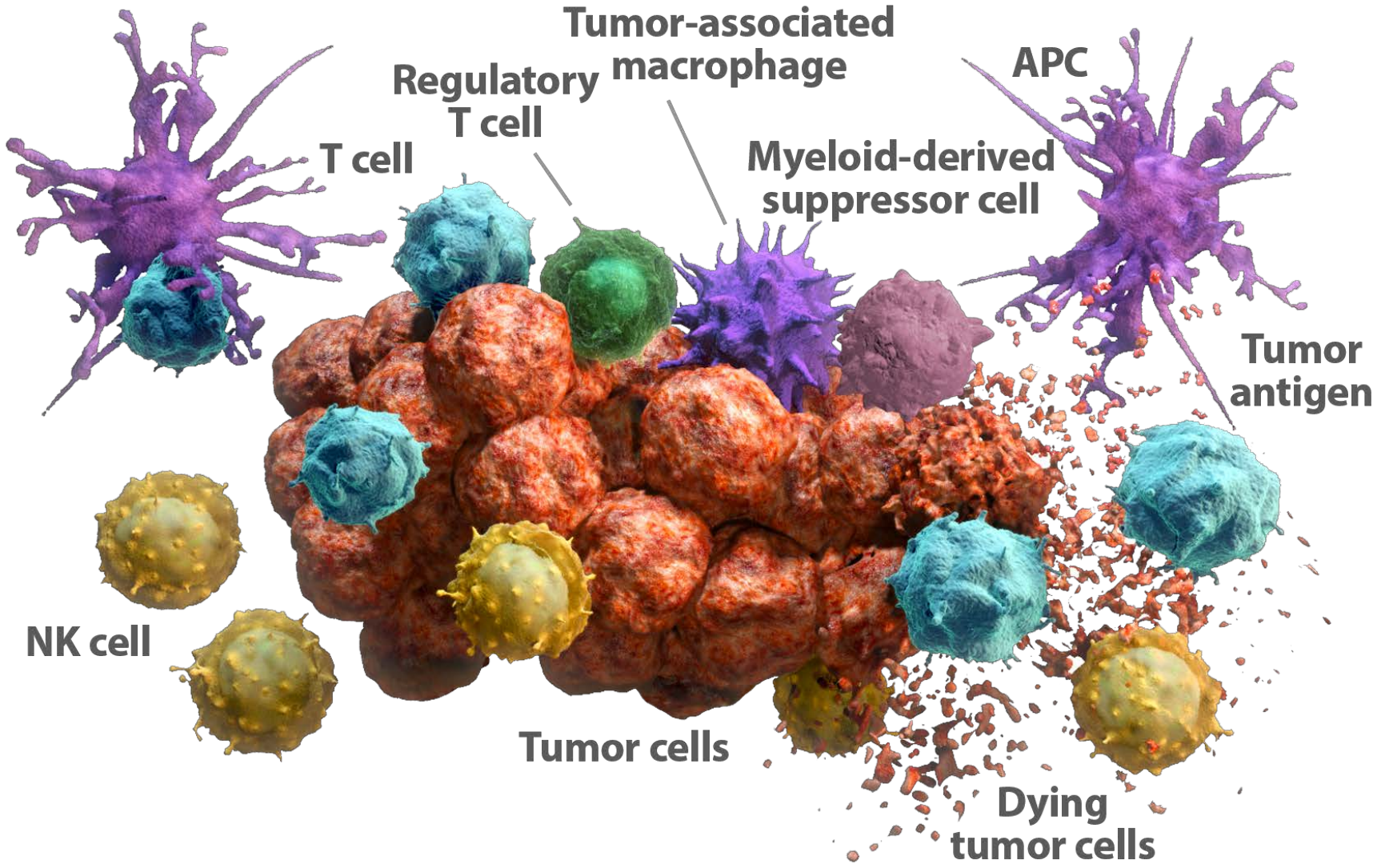
Condition	Fulfilled?
Established Mechanism Of Action (MOA)	
MOA tumor tissue independent	☐☐
Preclinical Proof Of Principle (POP)	
Preclinical safety	
Validated biomarker	
Clinical POP	
Clinical safety	
Pivotal randomized study	
Activity in other tumor types	
No relevant competing strategies	
Unmet medical need	

Cases

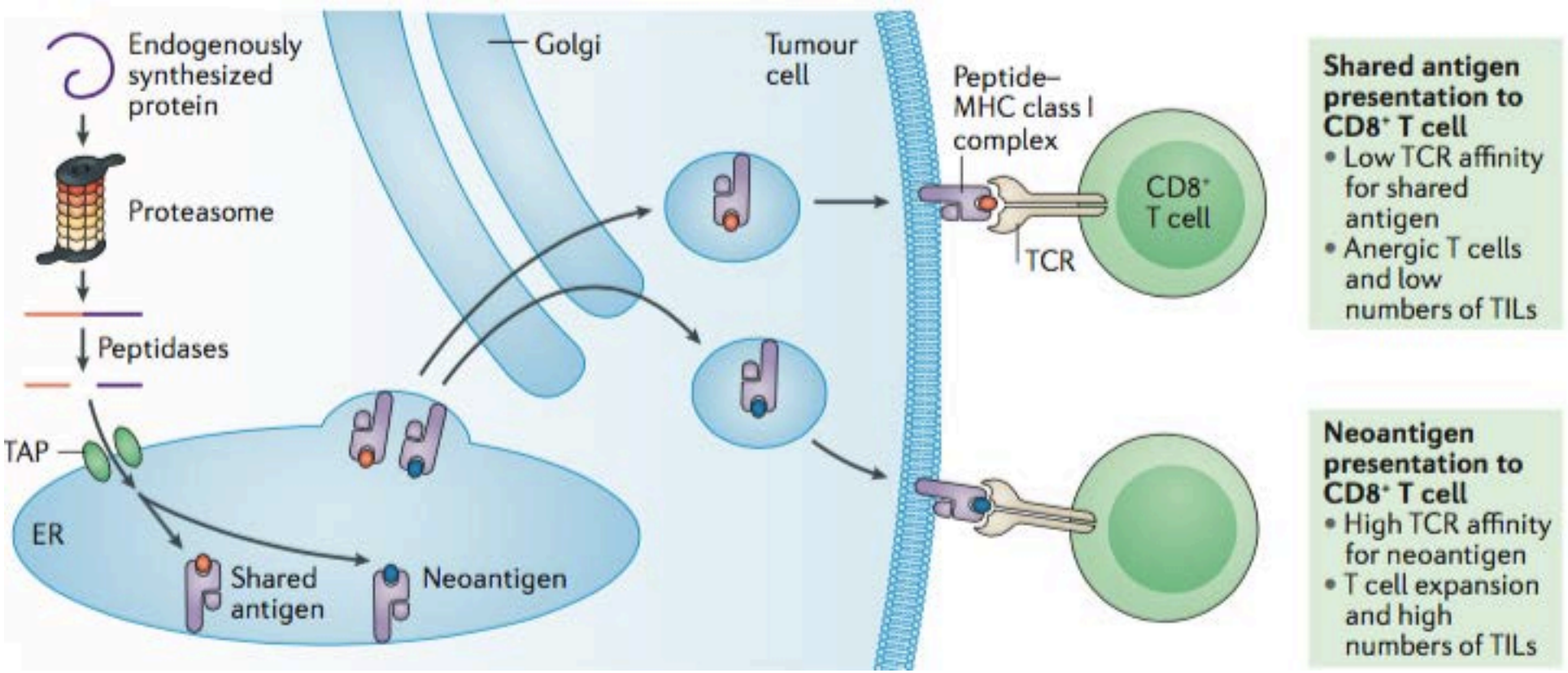
Targeting:

- PARP (poly[ADP-ribose]polymerase)
- BRAF V600 mutation
- Mismatch repair deficiency

Neo-antigens trigger the immune response

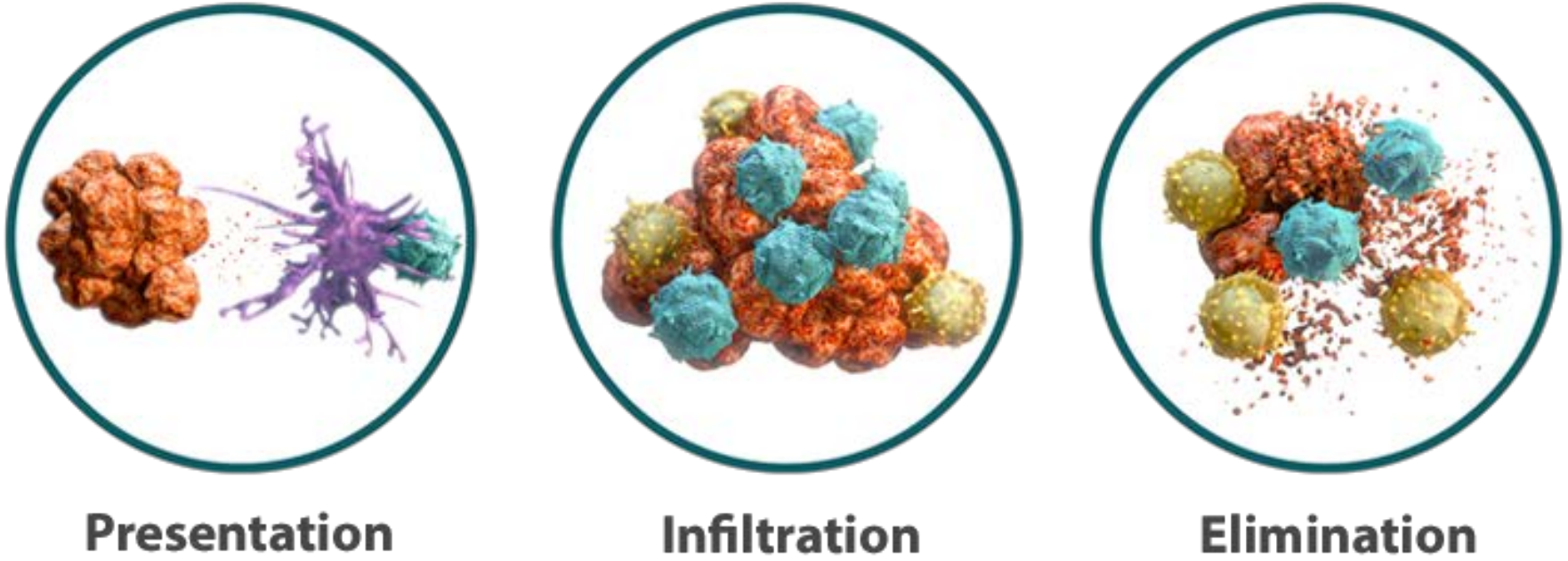


Tumor antigen processing and presentation on MHC class I



Yarchoan M et al., Nature Rev Cancer 2017;17:209-222

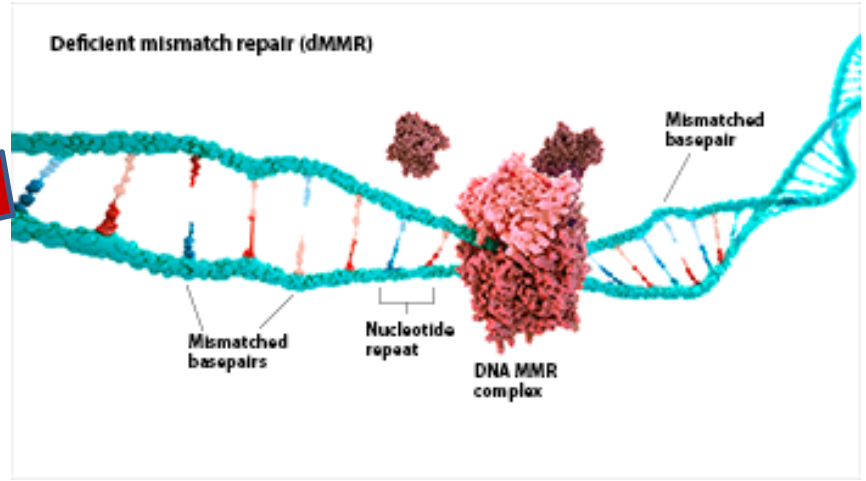
Three key stages of the antitumor immune response

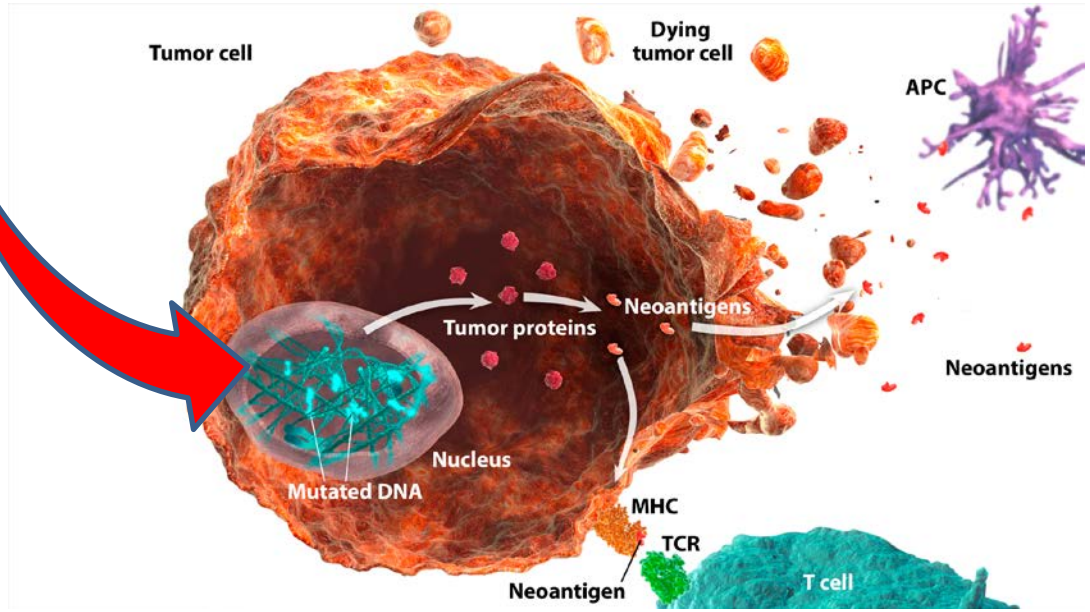


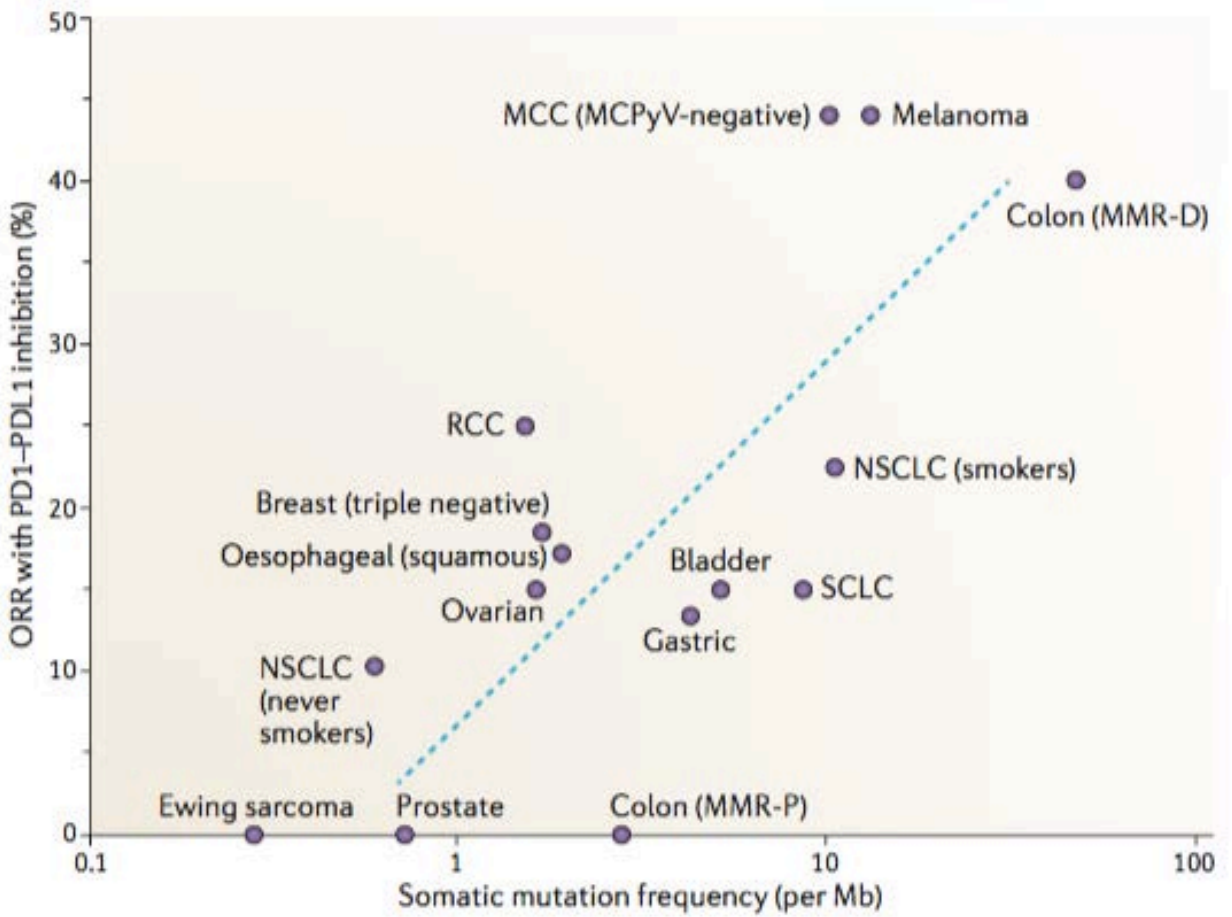
- **Presentation:** tumor cell death releases tumor antigens, which can activate the cytotoxic T cells of the adaptive immune system
- **Infiltration:** tumor antigens and other factors attract immune cells to the tumor site, where they invade and attack
- **Elimination:** activated cytotoxic T cells recognize tumor cells as the source of the antigen and target them for elimination

Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity*. 2013;39:1-10

Microsatellite instability high/deficient mismatch repair (MSI-H/dMMR) are indicators of genomic instability

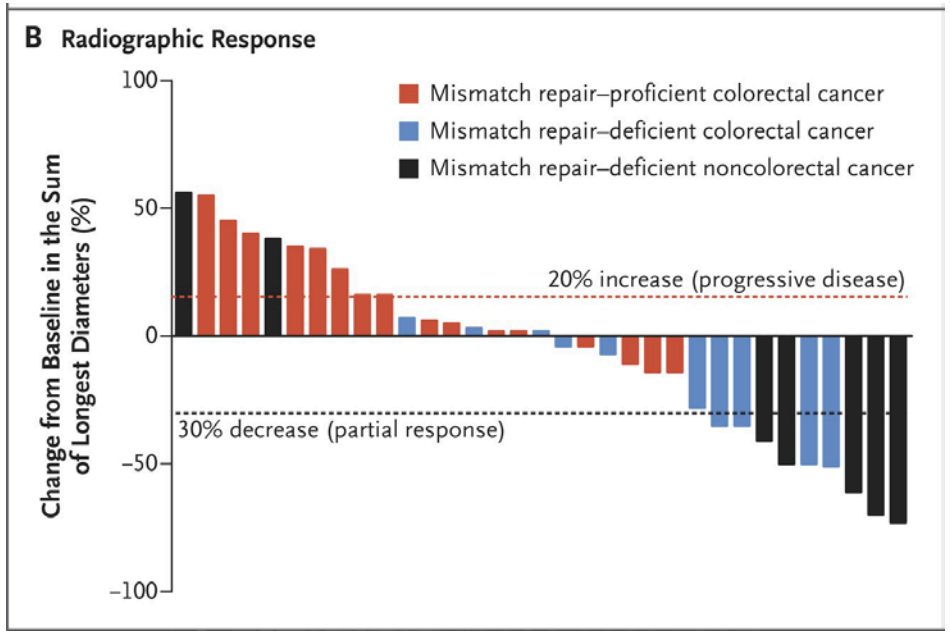


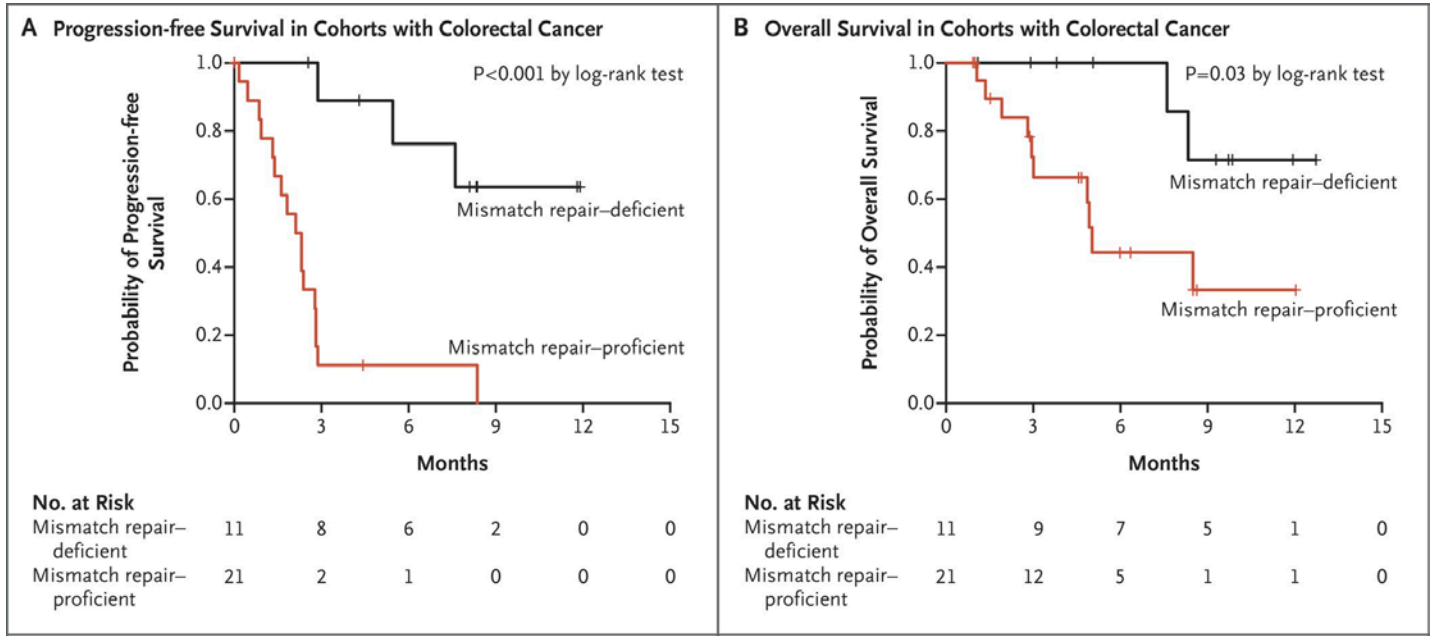




Hypothesis: mismatch repair (MMR)-deficient tumors

- MMR-deficient tumors are more likely than MMR-proficient tumors to benefit from anti-PD1 therapy probably because MMR–deficient tumors express more neoantigens

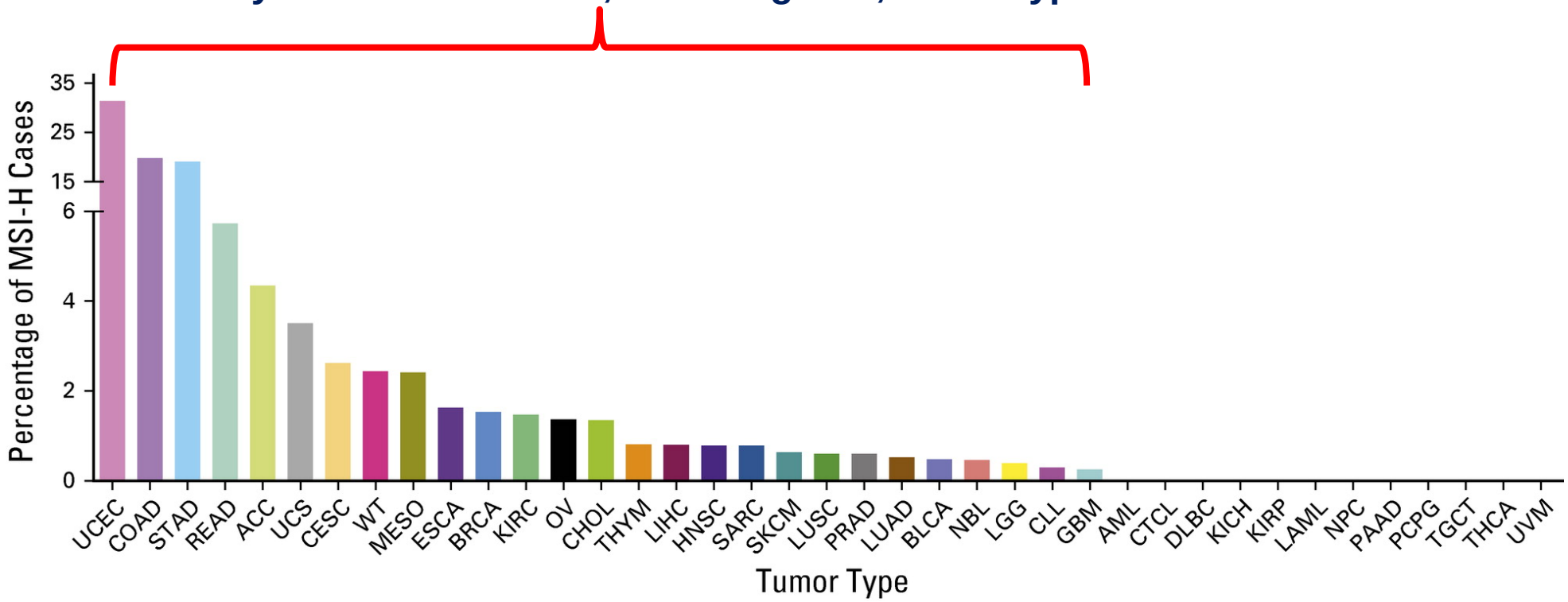




Le DT et al. N Engl J Med 2015;372:2509-20

PREVALENCE OF MICROSATELLITE INSTABILITY (MSI) ACROSS 39 HUMAN CANCER TYPES

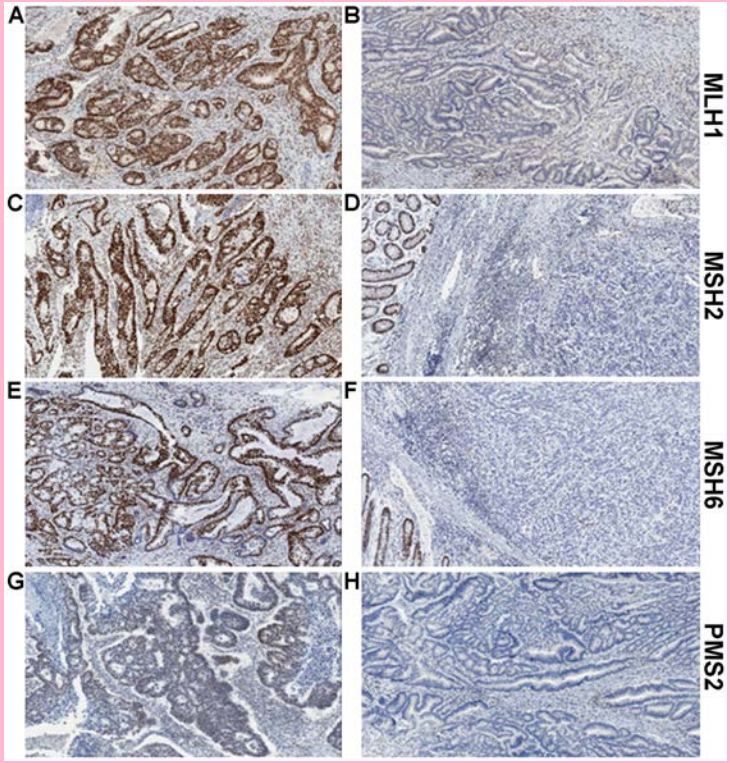
Proof activity of treatment in all, including rare, tumor types?



Bonneville R. et al., *JCO Precision Oncology* 2017;1:1-15

How to demonstrate MMR-deficiency?

IHC on four MMR- proteins



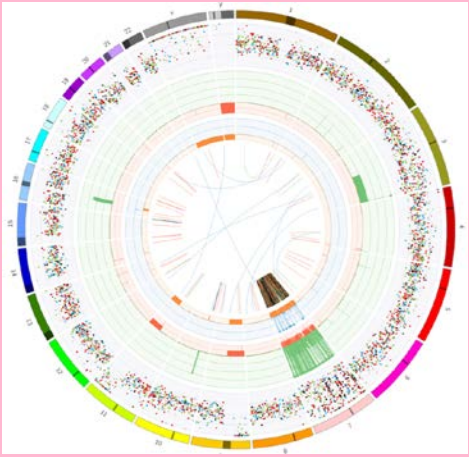
editorial

Microsatellite Instability Testing Using Next-Generation Sequencing Data and Therapy Implications

With the FDA's recent approval of immunotherapy in MSI/dMMR advanced cancers, and the finding of this molecular signature across a broad range of tumor types, screening all metastatic cancer cases for MSI should become standard practice. This could be done by examining microsatellites that are present in genes included in NGS panels. This would eliminate the need for separate testing by immunohistochemistry or MSI by PCR. Companies and/or institutions who offer multiple gene sequencing panels should strongly consider integrating MSI analysis into their existing NGS protocols and include this as part of their reporting. Some panels are already reporting this, such as the Foundation One platform.

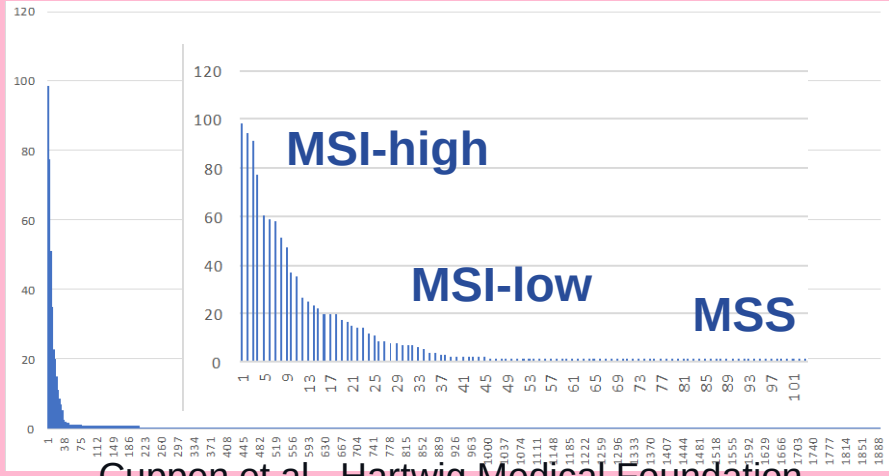
Haraldsdottir JCO Prec Oncol 10 Dec 2017

Comprehensive mutation analysis by WGS



Hartwig MEDICAL FOUNDATION

No. of indels at repeat sequences per Mb



Cuppen et al., Hartwig Medical Foundation

Tumor agnostic approval justifiable for anti-PD1 in MSI-h?

Condition	Fulfilled?
Established MOA	√ (with large extrapolations)
MOA tumor tissue independent	only circumstantial evidence
Preclinical POP	√
Preclinical safety	√
Validated biomarker	√ (IHC, but not optimal)
Clinical POP	√
Clinical safety	√
Pivotal randomized study	No, but OK for FDA; EMA however?
Activity in other tumor types	√ (sufficient?, guidance needed)
No relevant competing strategies	√ ?
Unmet medical need	√

Notes for guidance: take home messages

- Presented template with ‘Conditions’ may guide approval process
- Solid proof that MOA is tumor context independent is vital
- Such proof may be delivered employing representative preclinical models
- These models should be rigorously validated
- One positive pivotal trial that new therapy improves outcome is essential
- Design of the pivotal trial depends a.o. on rarity of the condition
- Selected biomarker(s) should be rigorously validated and cross-validated
- These biomarkers should guide pt selection in the development process

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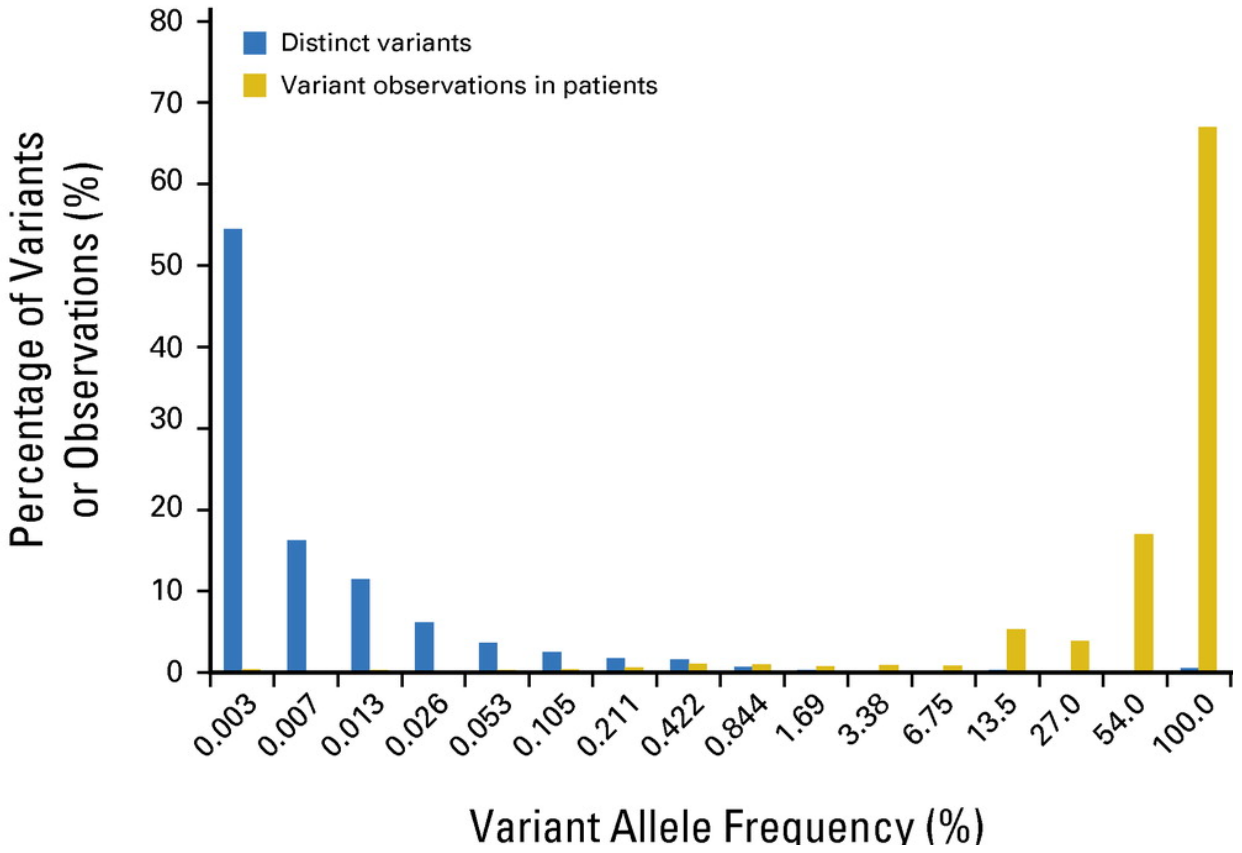
- Hans Bos, UMC Utrecht
- CBG-MEB:
  - Ton de Boer, Bert Leufkens, Marjon Pasmooij, Violeta Stoyanova,

Backup

Prevalence of mutations (n>5000 variants)



Lincoln SE et al., *JCO Precision Oncology* **2017,** 1, 1-10

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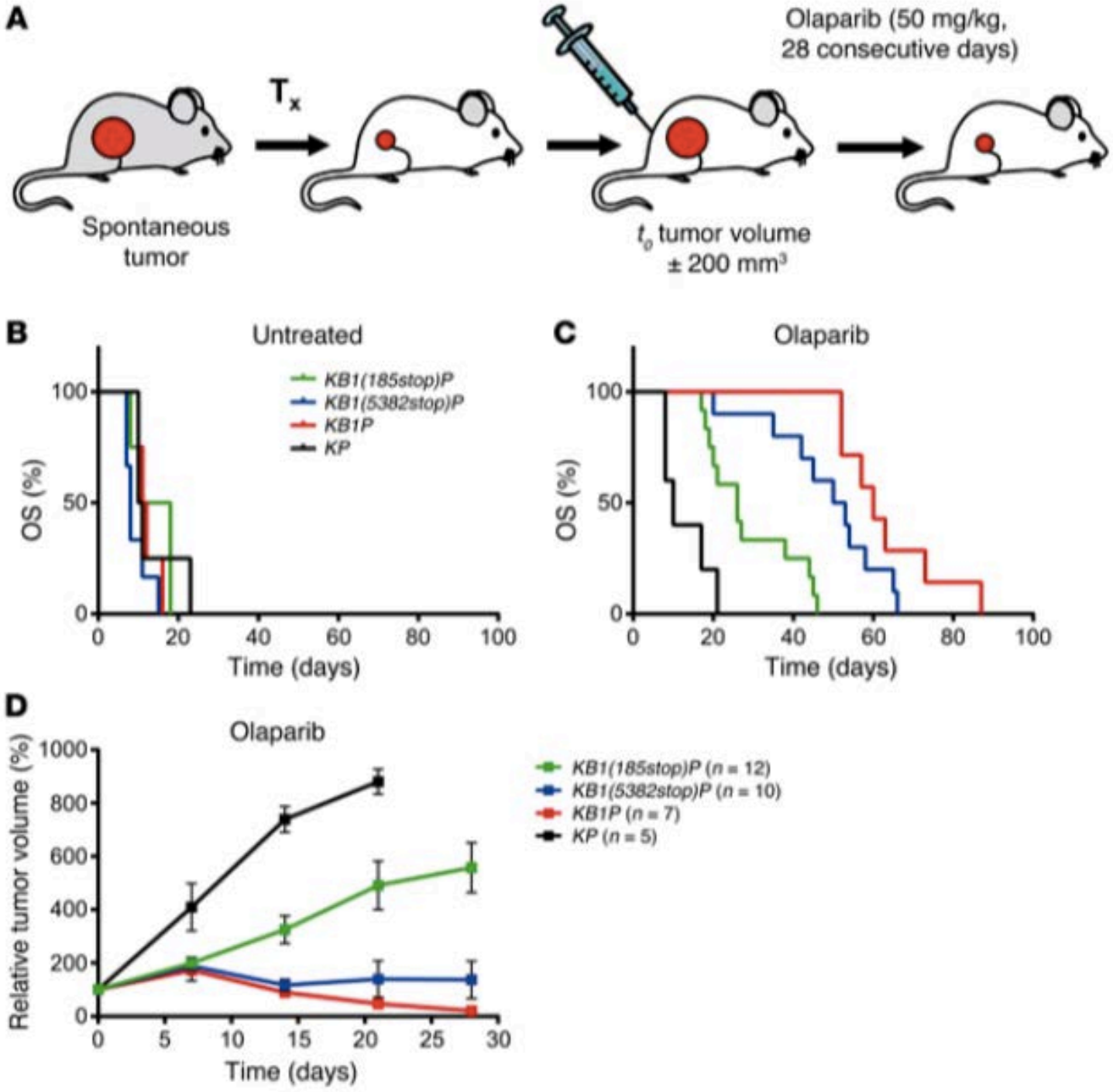

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