Frederick National Laboratory/NCI Support of Friends of Cancer Research's Tumor Mutational Burden (TMB) and Homologous Recombination Deficiency (HRD) Harmonization Projects

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Tumor Mutational Burden (TMB) Harmonization Project

Tumor Mutational Burden (TMB)

- TMB is a measure of the number of somatic mutations per area of the tumor's genome (mut/Mb)
- High TMB occurs in numerous tumor types and evidence is growing for the association of TMB with neoantigen load [1]
- TMB is a predictive biomarker and has been shown to correlate with clinical benefit from cancer immunotherapies [1,2]

The problem

Methods of TMB estimation and reporting vary widely across clinical studies[3]

The solution

An initiative that would facilitate standardization and best practices for estimating and reporting TMB

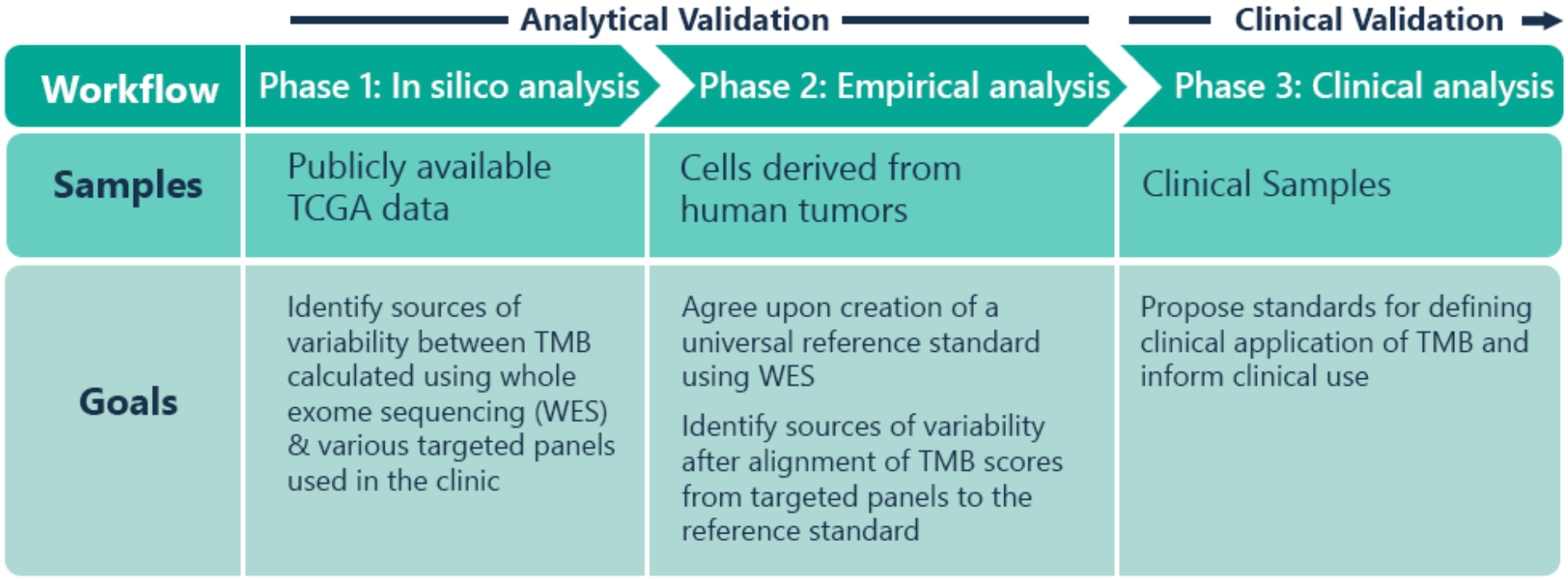
The approach

Convene multi-stakeholder working group to conduct studies that generate evidence to drive alignment & consensus solutions

1. Chan TA, et al. *Ann Oncol*. 2019. 2. Chalmers ZR, et al. *Genome Med.* 2017. 3. Stenzinger A, Allen JD, et al. *Genes Chromosomes Cancer*. 2019.

Friends of Cancer Research TMB Harmonization Project

Multi-stakeholder working group to align on and publish universal best practices for defining TMB, and analytic validation approaches including alignment against reference standards.



Merino et al. *J Immunotherapy Cancer*, 2020

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In silico analysis: 11 participating labs

Laboratory	Panel name	# genes	Total region covered (Mb)	TMB region covered* (Mb)	Type of exonic mutations included in TMB estimation	Published performance characteristics (ref.)
ACT Genomics	ACTOnco+	440	1.8	1.12	Non-synonymous†, synonymous	NA
AstraZeneca	AZ600	607	1.72	1.72	Non-synonymous, synonymous	NA
Caris	SureSelect XT	592	1.60	1.40	Non-synonymous	Vanderwalde *et al*[40]
Foundation Medicine	FoundationOne CDx‡	324	2.20	0.80	Non-synonymous, synonymous	Frampton *et al*[41] Chalmers *et al*[25] Fabrizio *et al*[42] US FDA SSED[31]
Guardant Health	GuardantOMNI§	500	2.15	1.00	Non-synonymous, synonymous	Quinn *et al*[43]
Illumina	TSO500 (TruSight Oncology 500)	523	1.97	1.33	Non-synonymous, synonymous	NA
Memorial Sloan Kettering Cancer Center	MSK-IMPACT¶	468	1.53	1.14	Non-synonymous	Cheng *et al*,[44] Zehir *et al*,[30] US FDA[32]
NeoGenomics	NeoTYPE Discovery Profile for Solid Tumors	372	1.10	1.03	Non-synonymous, synonymous	NA
Personal Genome Diagnostics	PGDx elio tissue complete	507	2.20	1.33	Non-synonymous, synonymous	Wood *et al*[45]
QIAGEN	QIAseq TMB panel	486	1.33	1.33	Non-synonymous, synonymous	NA
Thermo Fisher Scientific	Oncomine Tumor Mutation Load Assay	409	1.70	1.20	Non-synonymous	Chaudhary *et al*[46] Endris *et al*[35]

*Coding region used to estimate TMB regardless of the size of the region assessed by the panel.
†Non-synonymous mutations include single nucleotide variants, splice-site variants and short insertions and deletions (indels).
‡FoundationOne CDx assay has been approved by the US FDA as an IVD.[31]
§GuardantOMNI is a plasma-based circulating tumor DNA assay.
¶MSK-IMPACT assay has been authorized by the US FDA[32]
NA, not available.

Merino et al. *J Immunotherapy Cancer*, 2020

Methodological approach

Gold-standard

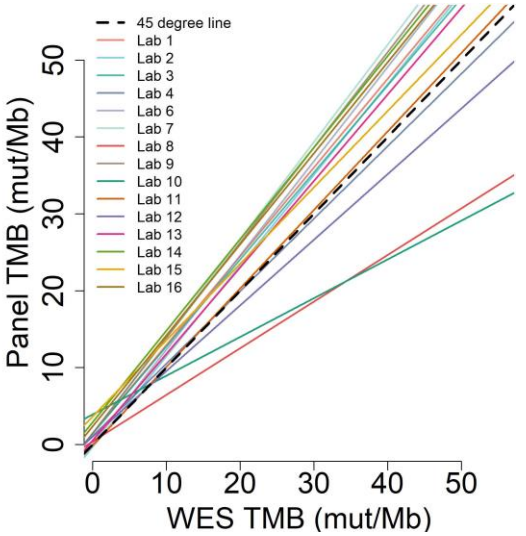
Consortium-agreed WES TMB algorithm → WES TMB score

Panel-specific

Partner Laboratory's TMB algorithm → Panel TMB score

Comparison of panel TMB to WES TMB

Fit regression lines to individual lab data

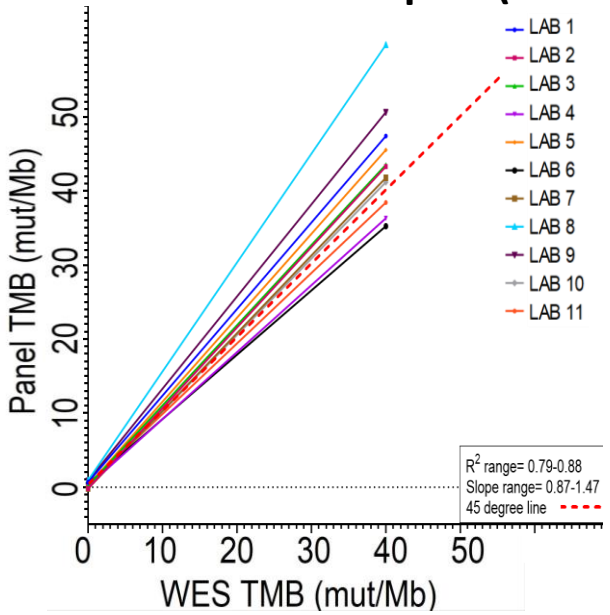


Tissue Source

In silico analysis	Cell lines	Clinical samples
TCGA validation cohort samples (N=4065) [Numerous tumor types]	Human tumor-derived cell lines (N=10) [Lung & breast cancer]	Tumor-normal matched FFPE clinical samples (N=29) [Lung, bladder, gastric and colon]

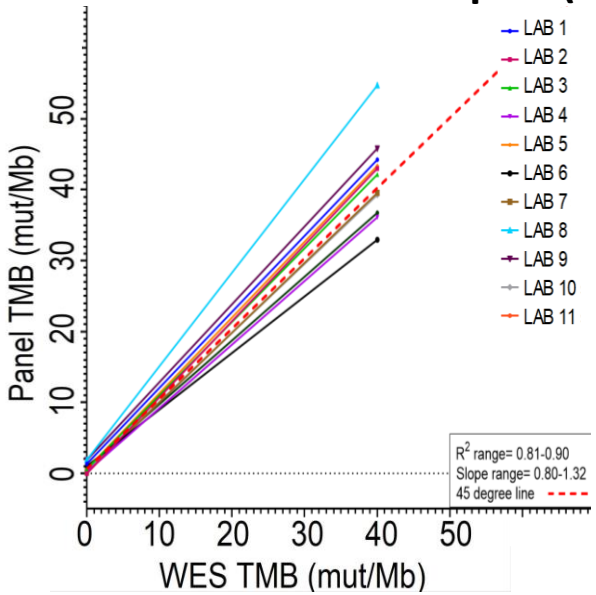
Association between panel TMB and WES TMB varied between participating laboratories

All TCGA Samples (N=4065)



32 tumor types

Stratum 1 TCGA Samples (N=1563)

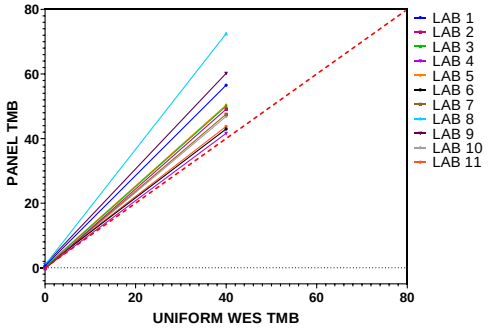


8 tumor types

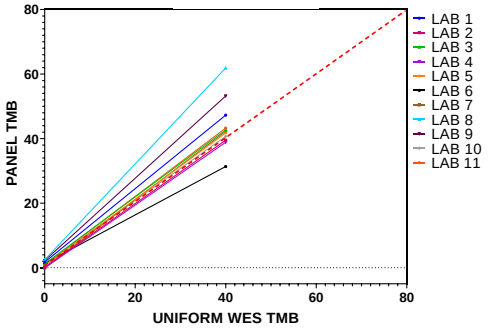
Merino et al., *J Immunother Cancer.* 2020

In silico analysis: A cancer-dependent relationship was observed

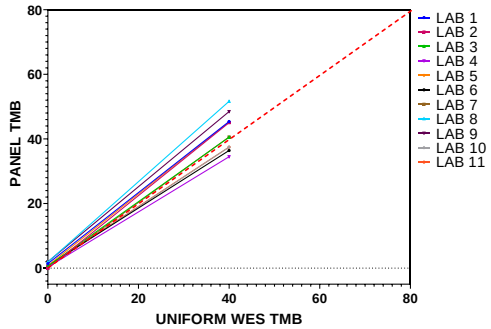
Bladder



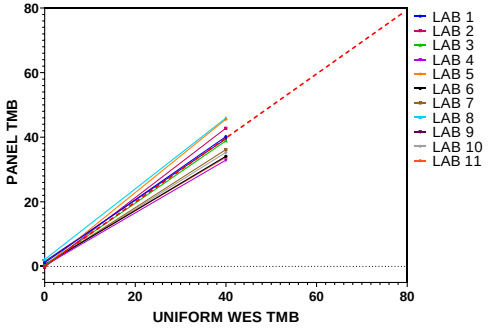
Colon



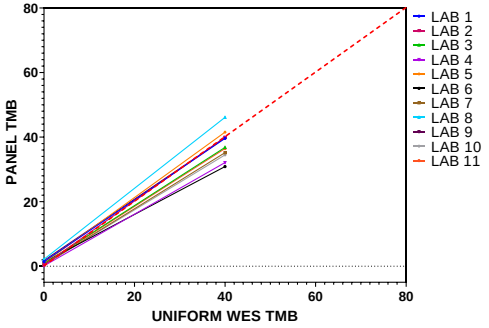
Head & Neck



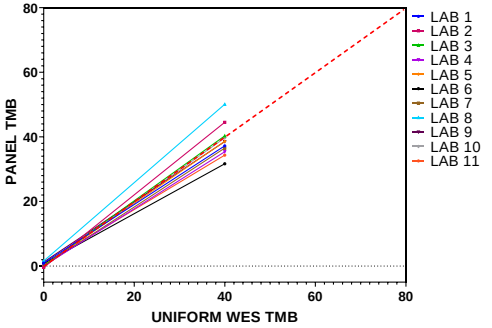
Lung Adenocarcinoma



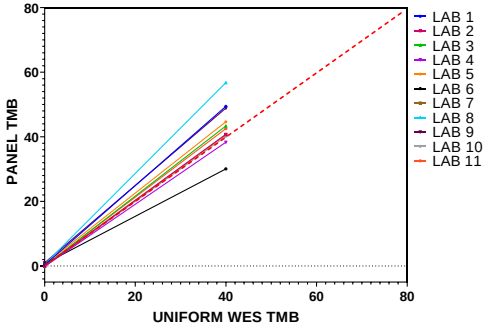
Lung Squamous



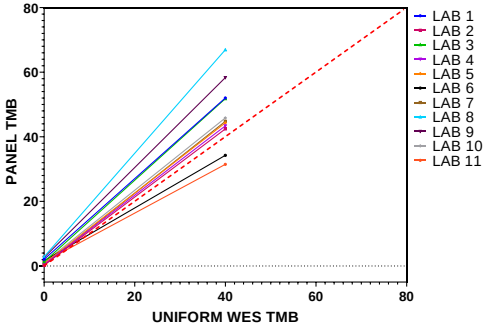
Melanoma



Stomach Adenocarcinoma



Endometrial



Stratum 1

- 8 tumor types
- N = 1563 total, 128 – 232 for each tumor type

Merino et al., *J Immunother Cancer.* 2020

Cell lines and clinical samples: Methodological approach

Tissue Source

TCGA validation cohort samples (N=4065)
[Numerous tumor types]

Human tumor-derived cell lines (N=10)
[Lung & breast cancer]

Tumor-normal matched FFPE clinical samples extracted at MoCha (N=29)
[Lung, bladder, gastric and colon]

Gold-standard

Consortium-agreed WES TMB algorithm → MoCha WES TMB score

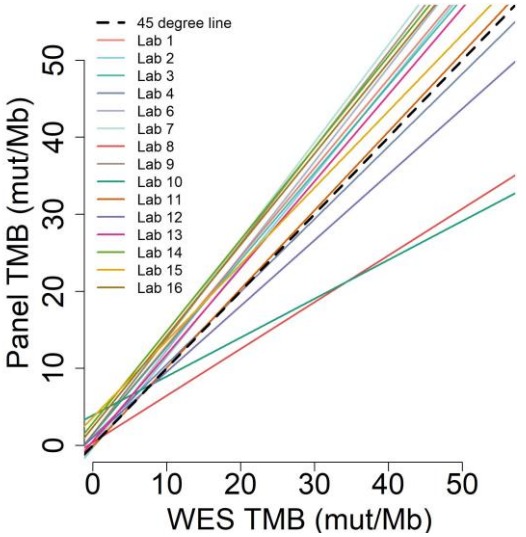
Panel-specific

Partner Laboratory's TMB algorithm → Panel TMB score

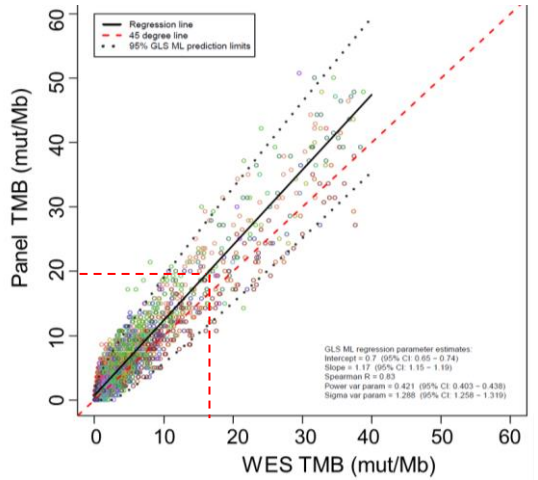
Apply calibration approaches to align across 16 participant NGS panels

Comparison of panel TMB to WES TMB

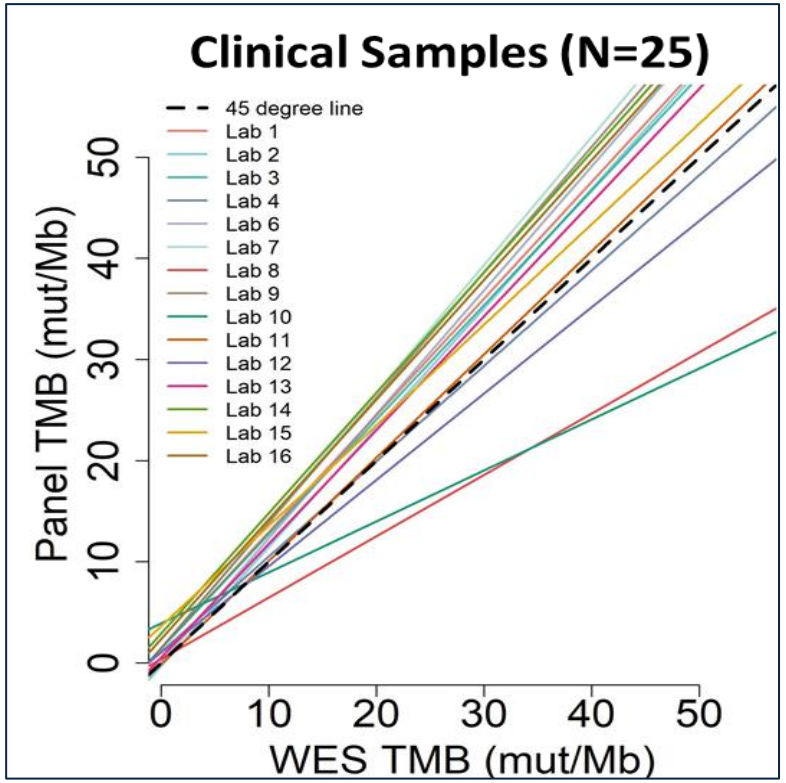
Fit regression lines to individual lab data

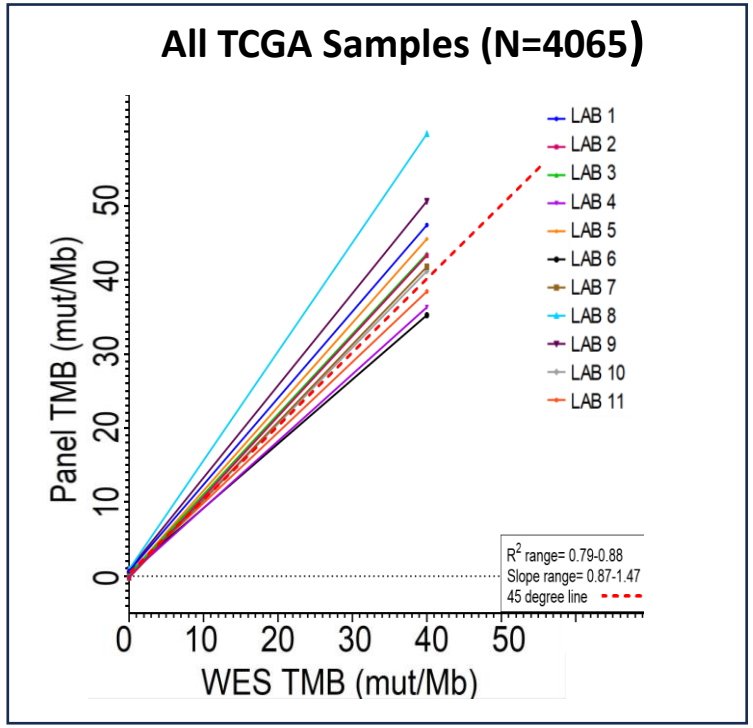


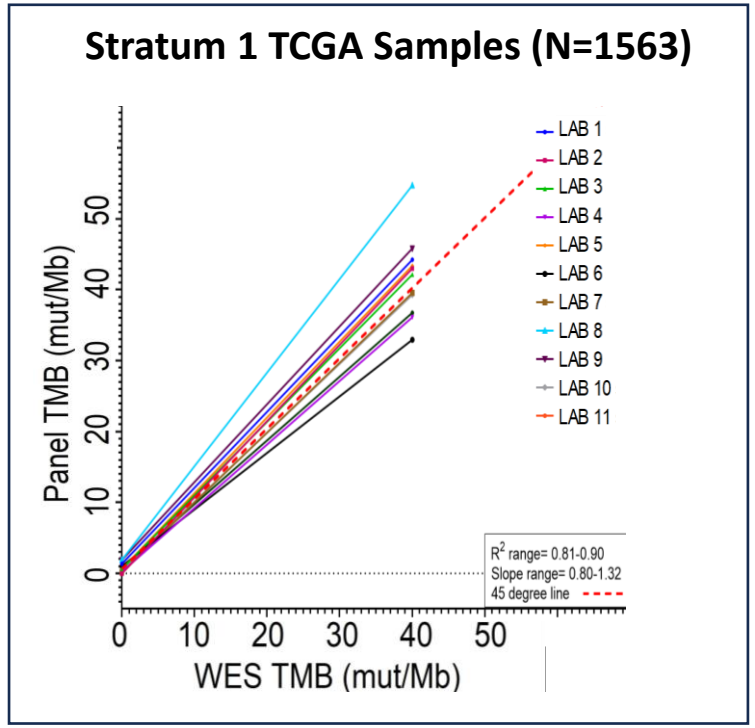
Calibration curves



Clinical sample analyses: Variability observed across 16 participating laboratories

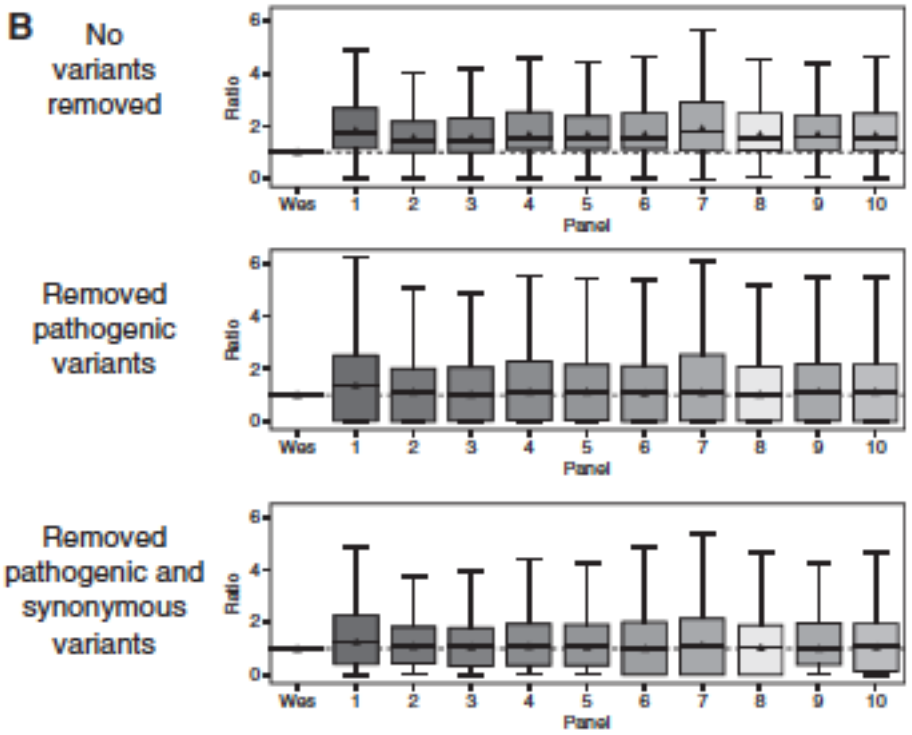






Vega et al., Ann Oncol 2021

Clinical sample analysis: Filtering pathogenic variants improves panel TMB relative to WES TMB



- In an in silico simulation conducted by 10 labs:
  - Removing known pathogenic cancer mutations (those seen in COSMIC) improved panel TMB estimates
  - Removing synonymous variants had minimal effect on panel TMB

Vega et al., Ann Oncol 2021

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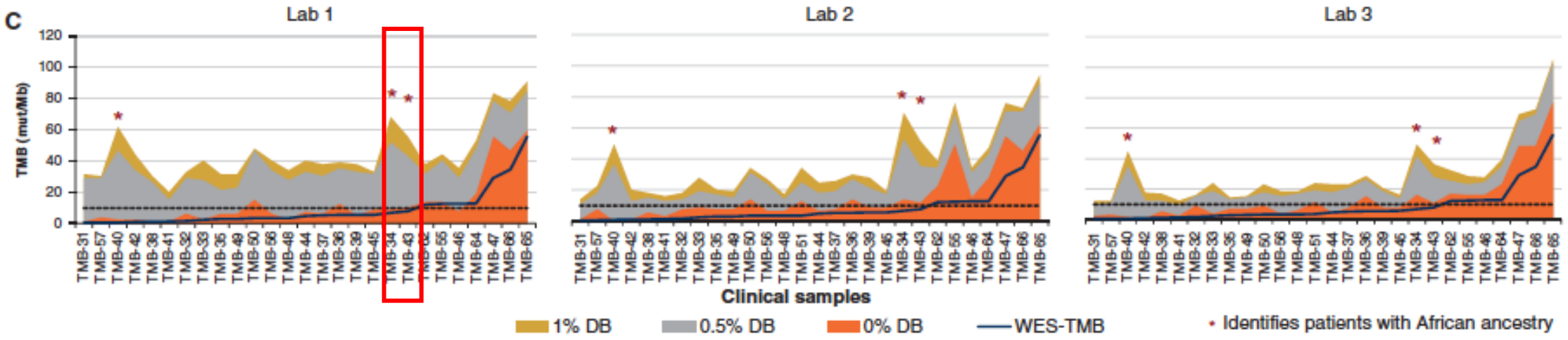
Clinical sample analysis: Approach to germline variant filtering can greatly impact panel TMB



Vega et al., Ann Oncol 2021

Calibration approach using all TCGA samples

Observe panel TMB value y_0

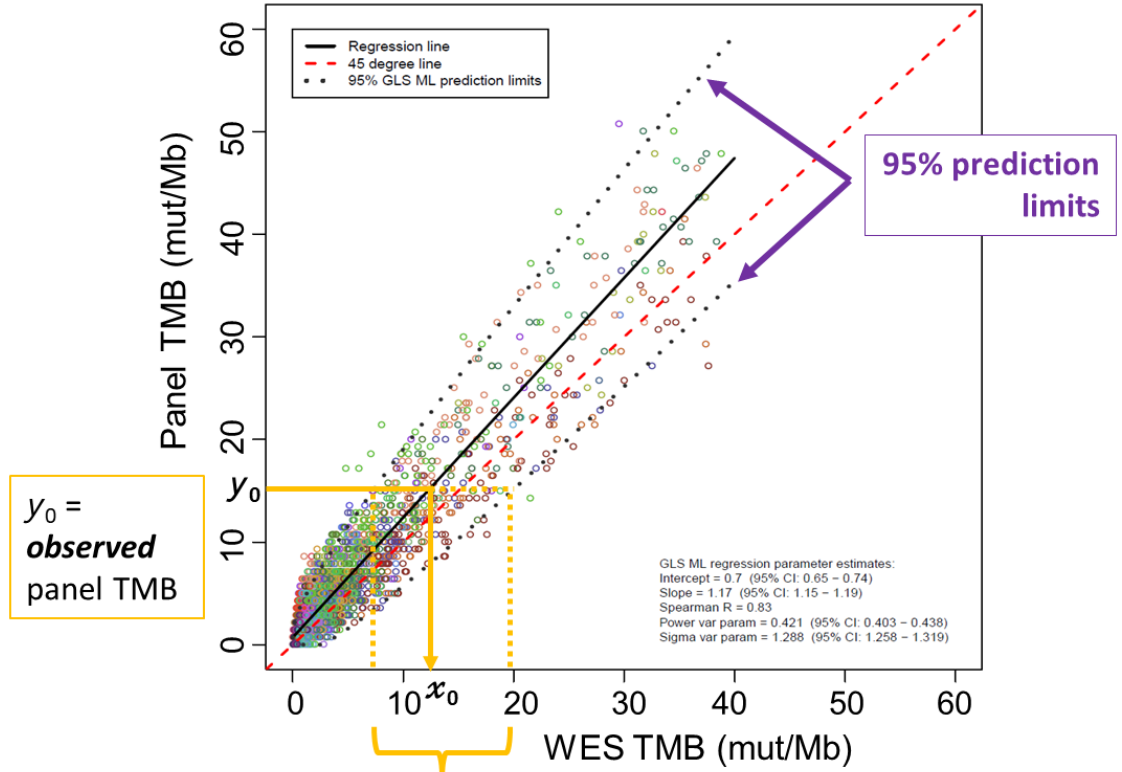
↓

Invert regression line to estimate WES TMB value x_0

↓

Interval of uncertainty ($LL_{95}(y_0)$, $UL_{95}(y_0)$):
Find x values where horizontal line $y=y_0$ intersects with 95% prediction limits

Calibration for individual laboratory informed by fitted regression line as well as scatter of points around the line (quantified by 95% prediction limits)



y_0 = ***observed*** panel TMB

x_0 = ***estimated*** *WES TMB calculated from calibration curve* at observed y_0
($LL_{95}(y_0)$, $UL_{95}(y_0)$) = **interval of uncertainty** around x_0

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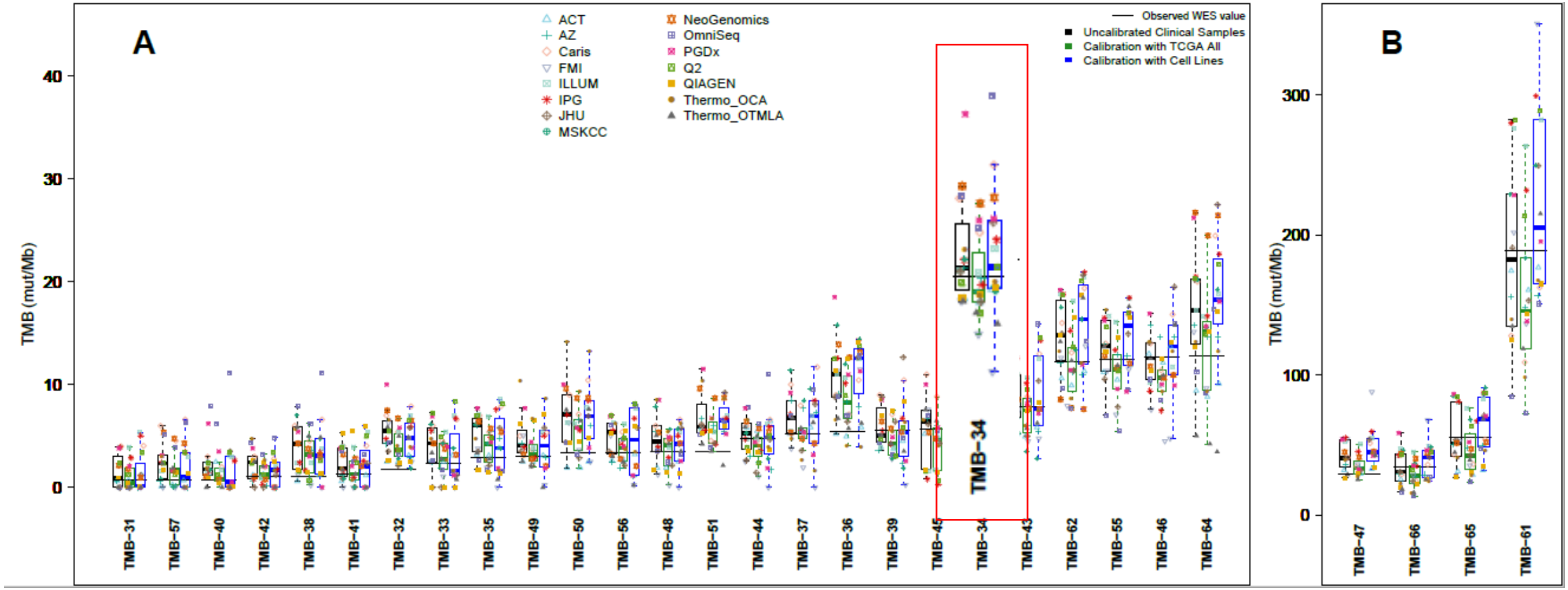
Application of two calibration approaches to clinical sample set



Vega et al., Ann Oncol 2021

TMB Harmonization Project Summary

- TMB estimates varied substantially between participating labs
- Variability in the association between panel TMB and WES TMB was similar for in silico TCGA, cell line and FFPE clinical samples
- Failure to filter pathogenic variants in a panel-based TMB resulted in overestimation of TMB relative to WES
- Germline filtering using only population databases may not be sufficient to remove germline variants for optimal TMB estimation
- Calibration approaches using TCGA data performed better than cell line samples

➢ *Calibration methods using TCGA data may be a viable approach to align across panel TMB scores*

SPECIAL THANKS

- NCI statisticians: Lisa McShane, Laura Yee
- NCI software development: Qian Xie, Ming-Chung Li, Yingdong Zhao
- MoCha Lab team: Mickey Williams, Tomas Vilimas, Lily Chen
- Diana Merino Vega, Friends of Cancer Research project lead and data coordinator
- The entire TMB Harmonization Consortium

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TMB Harmonization Consortium

Government: National Cancer Institute (NCI), U.S. Food and Drug Administration (FDA) **Academia:** Brigham & Women's Hospital, College of American Pathologists (CAP), Columbia University, EORTC, Genomic Testing Cooperative, Hartwig Medical Foundation, Johns Hopkins University, Massachusetts General Hospital, MD Anderson Cancer Center, Memorial Sloan Kettering Cancer Center, Quality in Pathology (QuIP), University of Heidelberg **Diagnostics:** ACT Genomics, Biodesix, Caris Life Sciences, Foundation Medicine, Inc., Guardant Health, Inc., Illumina, Inc., Intermountain Precision Genomics, NeoGenomics Laboratories, Inc., OmniSeq, Personal Genome Diagnostics (PGDx), Q^2 Solutions, QIAGEN, Inc., Quest Diagnostics, RocheDx, Thermo Fisher Scientific, Thrive **Industry:** AstraZeneca, Bristol-Myers Squibb Company, EMD Serono, Inc., Genentech, Merck & Co., Inc., Pfizer, Inc., Regeneron Pharmaceuticals **Operational:** precisionFDA, SeraCare

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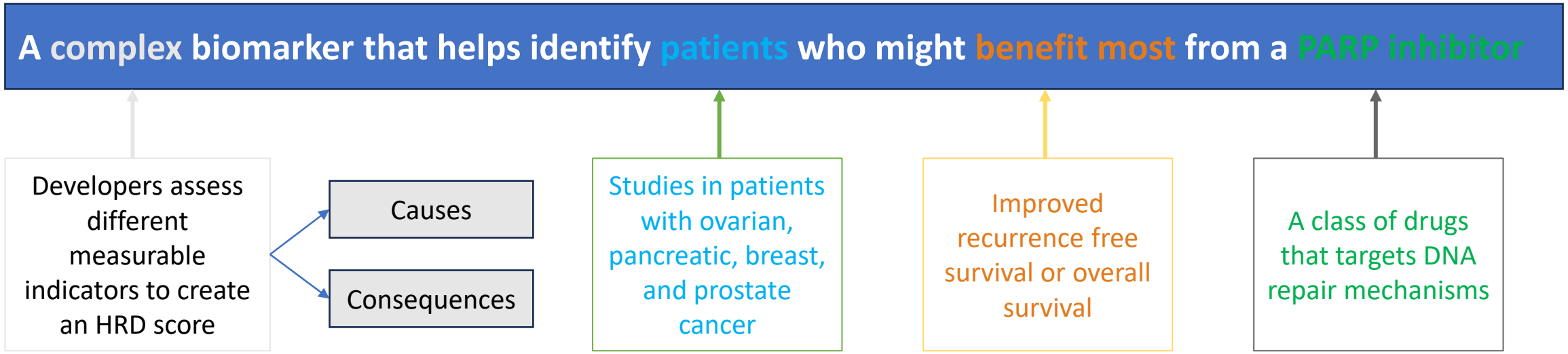
Homologous Recombination Deficiency (HRD) Harmonization Project

Homologous Recombination Deficiency (HRD)



Challenge: There are different ways to measure HRD; unclear how they align

- Different assays apply different cut-offs or thresholds to their scores

Impact: Variability in HRD measurements could lead to different treatment decisions and ultimately patient outcomes

HRD Harmonization Project

Goal: *Compare different HRD assays and investigate reasons for variability between them*

Phase 1
Discovery and Definition

Landscape assessment published in the *Oncologist*

JOURNAL ARTICLE

Homologous Recombination Deficiency: Concepts, Definitions, and Assays

Mark D Stewart, Diana Merino Vega, Rebecca C Arend, Jonathan F Baden, Olena Barbash, Nike Beaubier, Grace Collins, Tim French, Negar Ghahramani, Patsy Hinson ... Show more

The Oncologist, Volume 27, Issue 3, March 2022, Pages 167–174, https://doi.org/10.1093/oncolo/oyab053

Published: 27 January 2022 **Article history**

Phase 2
Assay Alignment

Analysis of HRD assays assessing shared datasets

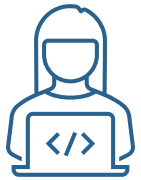
- In Silico Analysis
- Clinical Analysis

Phase 3
Clinical Contextualization

Interpreting and sharing findings

- AMP Conference 2022
- AACR OvCa Conf. 2023
- *Friends* Public Meeting
- Publication forthcoming

Phase 1 of Friends of Cancer Research HRD Project: *In silico a*nalysis using TCGA data

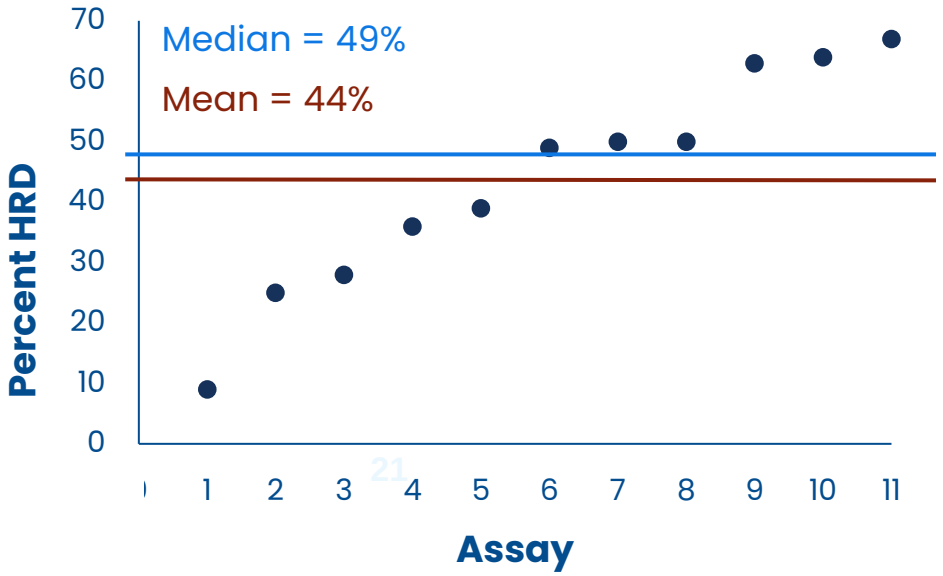


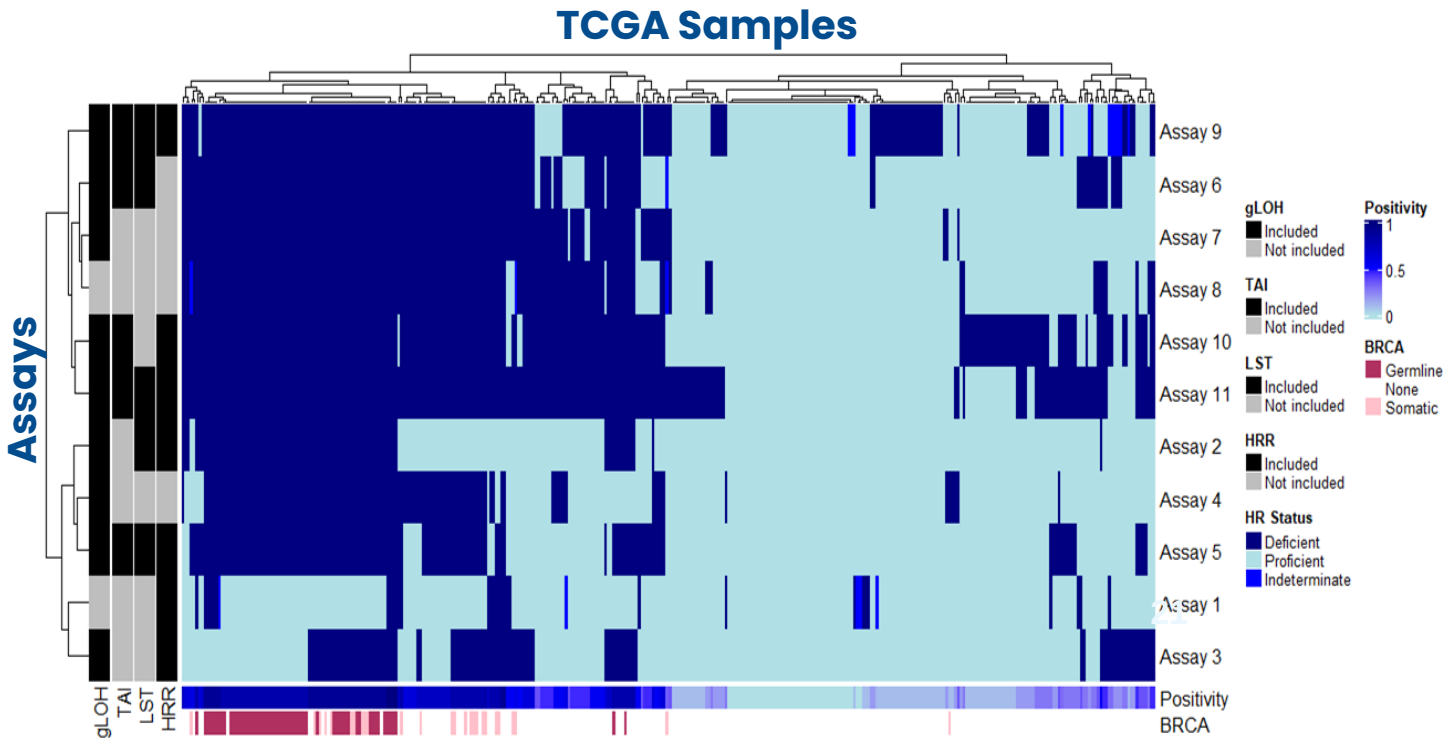
Shared 348 ovarian cancer TCGA sample files with assay developers*	11 Assay developers ran TCGA samples through their HRD pipeline and reported HRD or not	NCI BRP stats team* compared HR status calls to determine the agreement level	The HRD Harmonization Working Group reviewed and reported findings

*De-identified segmented files, MAF files, and BRCA germline mutation files

Friends of Cancer Research HRD Project: *In silico* results

Percent of samples that were called as HRD positive reported as the "percent HRD" for each assay





Clustered by relatedness using hierarchical clustering with complete linkage

Range of percent HRD positivity across labs is 9-67% with a median of 49% and a mean of 44%.

Substantial variability in HR status calls across assays. Samples with BRCA1/2 mutations were more uniformly called HRD.

(Results presented at AMP meeting, October 2022)

Phase 2 of Friends of Cancer Research HRD Project: Clinical analysis

MoCha extracted and distributed nucleic acids from 90 archival ovarian cancer samples

↓

Assay developers *independently sequenced* samples then measured and reported HRD

↓

NCI Biometric Research Program compared results to determine level of agreement

↓

The HRD Harmonization Working Group reviewed and aligned on findings

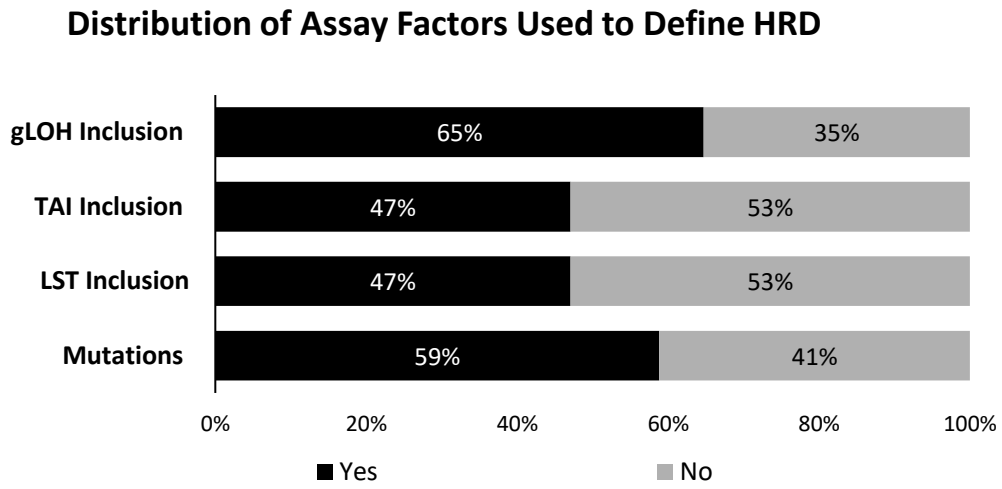
We lack a "gold standard" for HRD, so we focused on observed variability across assays

Sample Characteristics (n=90)

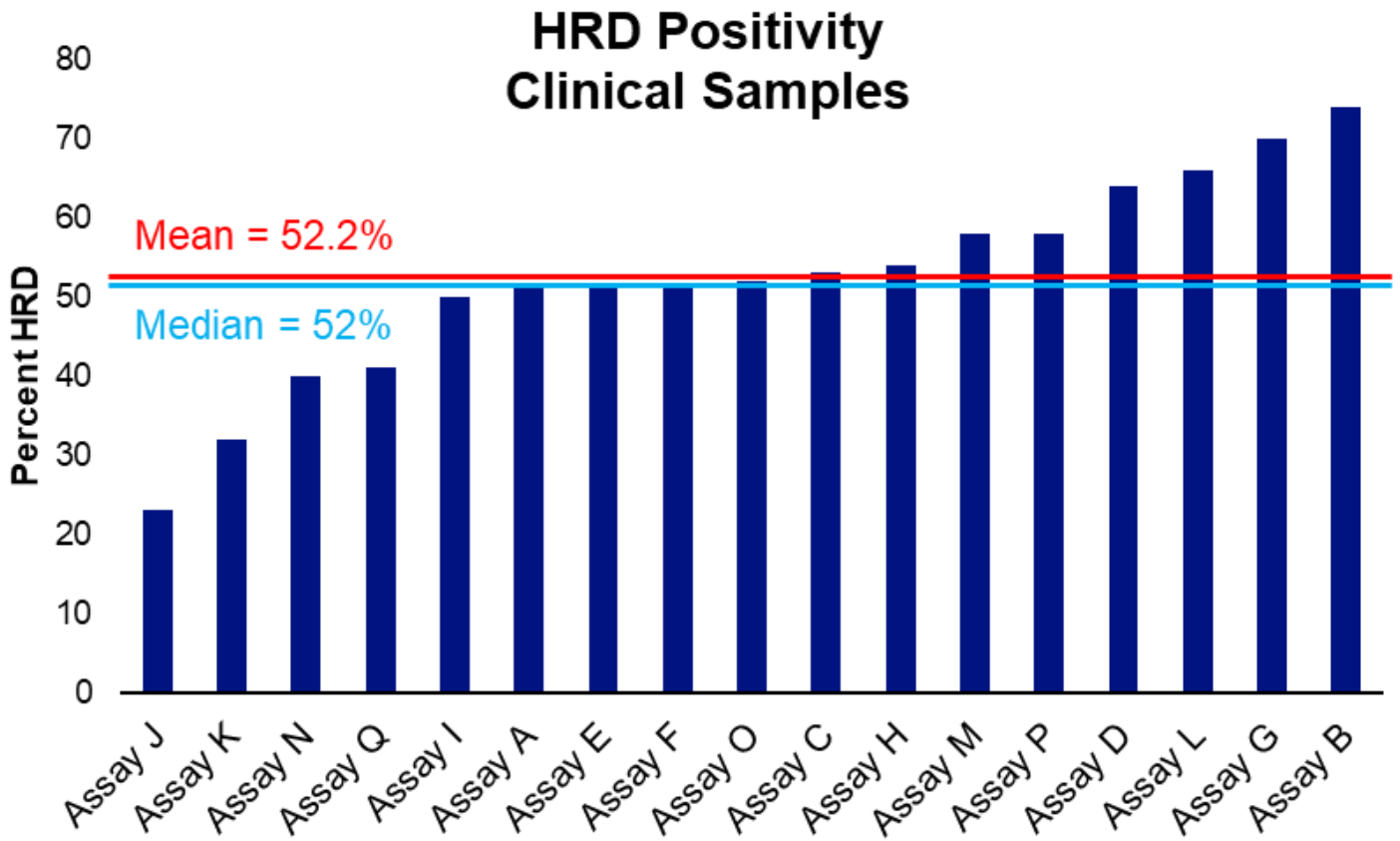
- Stage III or IV high grade serous ovarian cancer
- Treatment-naïve, subsequently treated with platinum-based chemotherapy

Assay Characteristics (n=17)

- All assessed *BRCA1* and *BRCA2* mutations as part of defining HRD
- Range of reported continuous scores and cut-offs for determining HRD status (+/-) varied



Percentage of samples called HRD varied widely



Range of percent HRD positivity across labs is 23-74% with a median of 52% and a mean of 52.2%

Approach to assess concordance

Positive Percent Agreement (PPA)

The percentage of samples that test ***positive*** by one test (Assay A) that are found ***positive*** by a second test (Assay B).

Also calculated:

Negative Percent Agreement (NPA)

Average Positive percent Agreement (APA)

Average Negative percent Agreement (ANA)

HRD = Positive
Not HRD = Negative

EXAMPLE

Patient	1	2	3	4	5	6	7
Assay A	HRD	HRD	Not	HRD	HRD	Not	Not
Assay B	HRD	HRD	HRD	Not	Not	Not	Not
Assay C	HRD	HRD	Not	Not	Not	HRD	Not

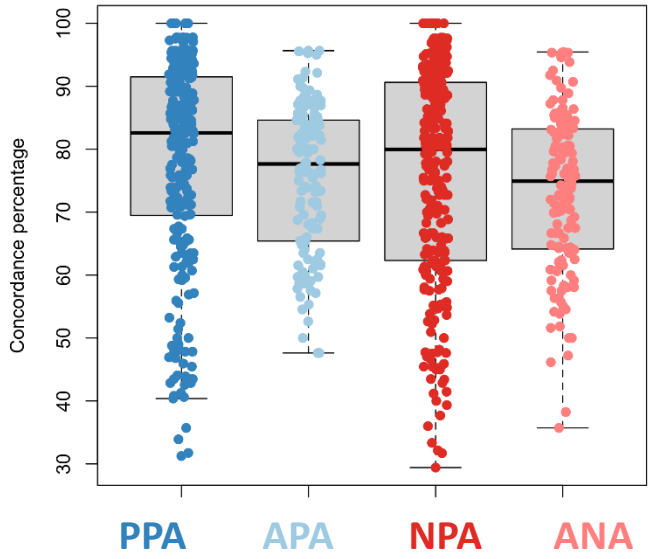
Agreement analyses performed over all possible pairings of 17 assays (136 pairs x 2 directions).

Comparison	PPA
A to B	50%
B to A	66%
A to C	50%
C to A	66%
B to C	66%
C to B	66%

FDA Guidance: Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests

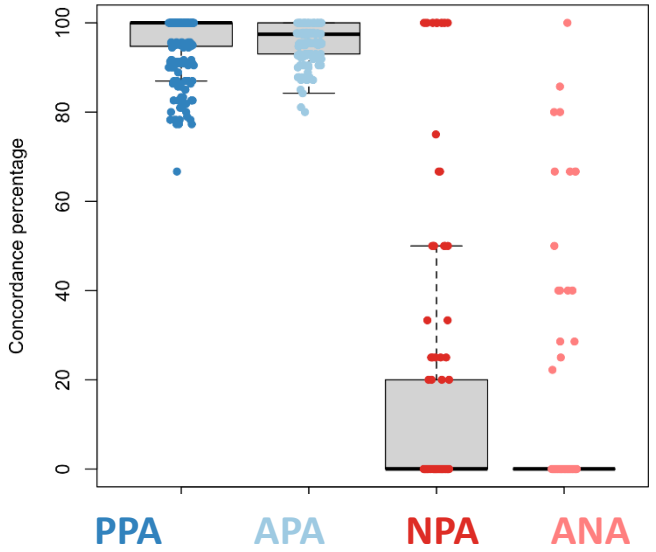
Concordance for HRD calls was best for *BRCA*

All Samples (n=90)



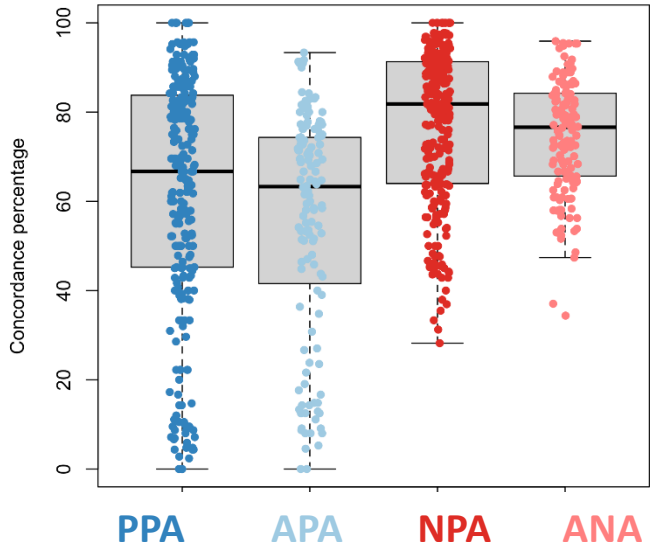
	PPA	APA	NPA	ANA
Median (IQR)	83 (71-91)	78 (65-85)	80 (62-91)	75 (64-83)

Mutated *BRCA1* and *BRCA2* (n=23)



PPA	APA	NPA	ANA
100 (95-100)	97 (93-100)	0 (0-20)	0 (0-0)

Wild-Type *BRCA1* and *BRCA2* (n=67)

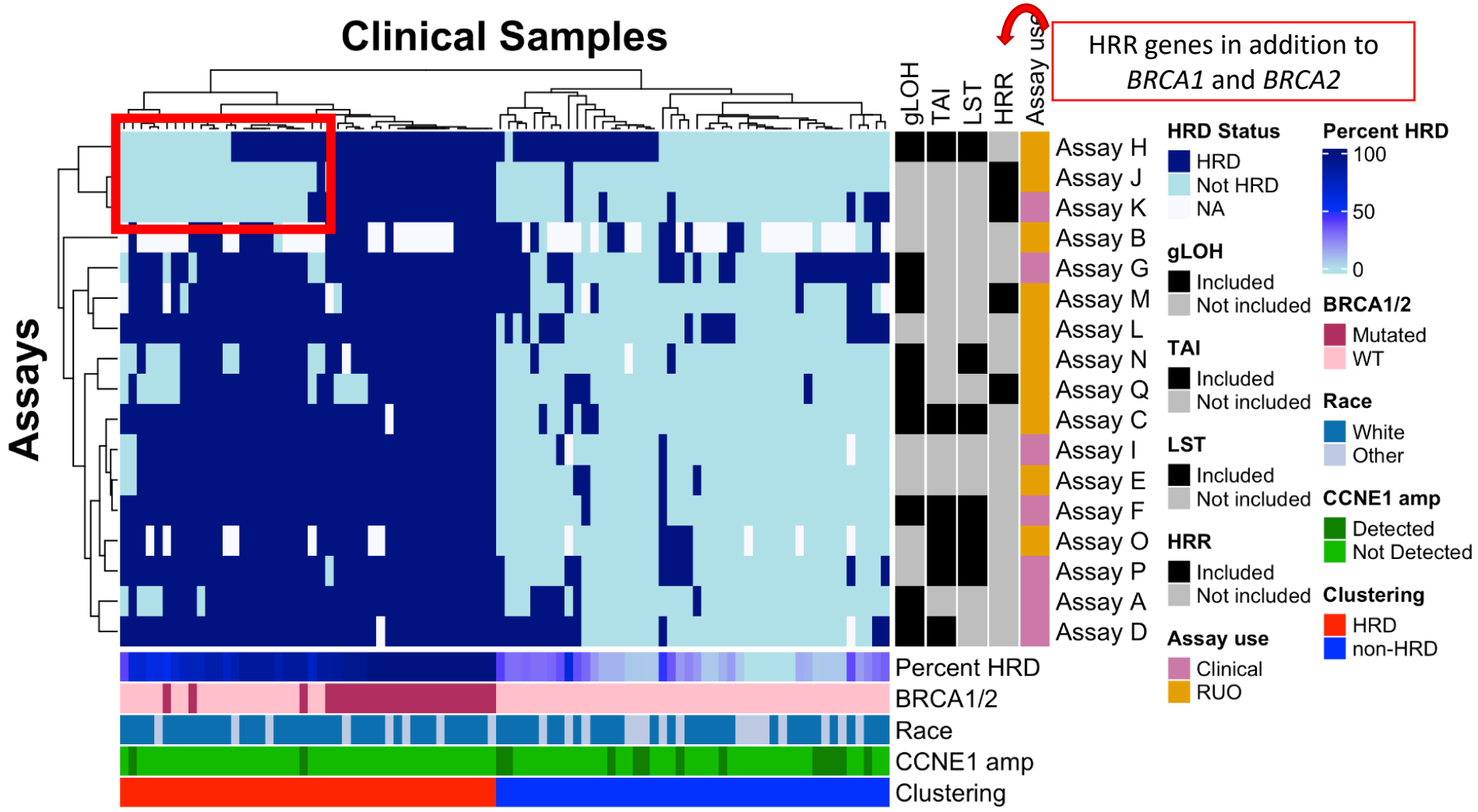


PPA	APA	NPA	ANA
67 (45-84)	63 (42-74)	82 (64-91)	77 (66-84)

Level of agreement varied depending on the pair of assays compared (one dot per pair).

Agreement is better for samples with mutated *BRCA1* and *BRCA2* compared to WT *BRCA1* and *BRCA2*.

Factors potentially associated with agreement between labs



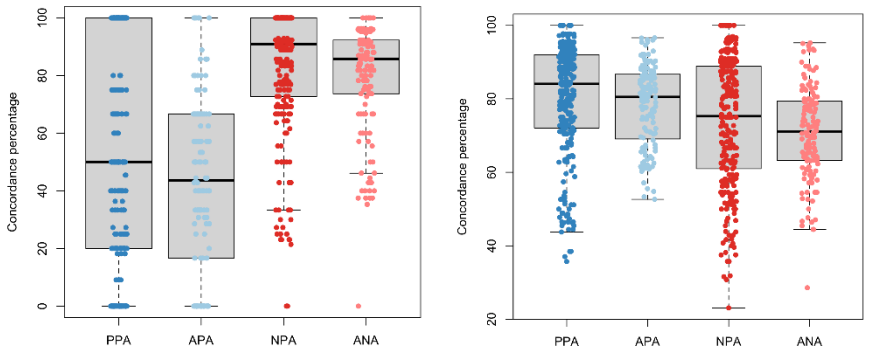
Factors potentially associated with agreement

Category	Factors Assessed
Clinical	CCNE1 Amplification
	Race
	Debulking Status
Sample	Tumor Purity*
	DNA Quality**
	Age of Block
Assay	Use (RUO vs. Clinical)
	HRD Cutoff

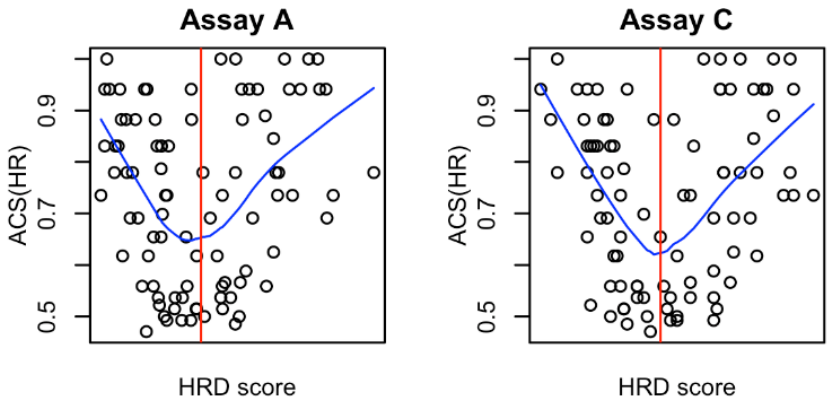
*Pathologist-assessed tumor content > 70% required
**DIN > 2.0 required

CCNE1 Amplified (n=14 samples)

CCNE1 Non-Amplified (n=76 samples)



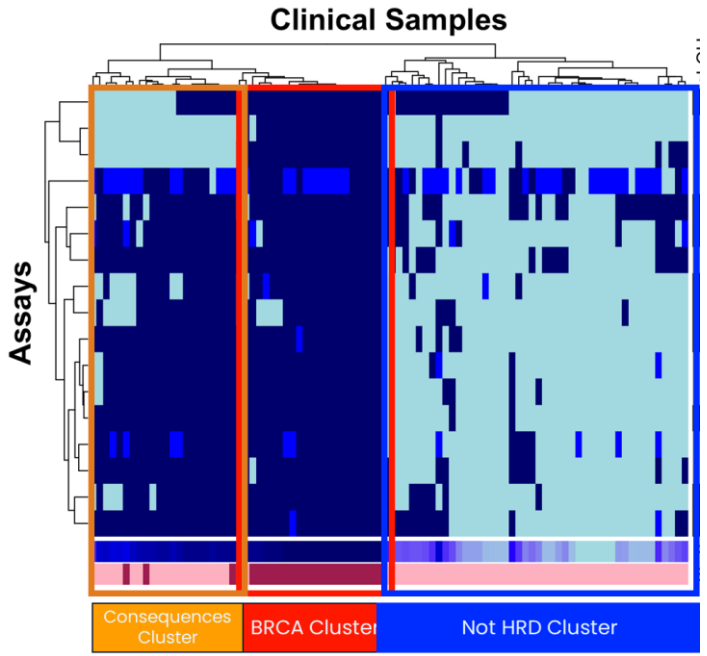
Samples with CCNE1 amplification have better agreement for not HRD calls.



Less agreement near HRD cut-off.

Survival analyses

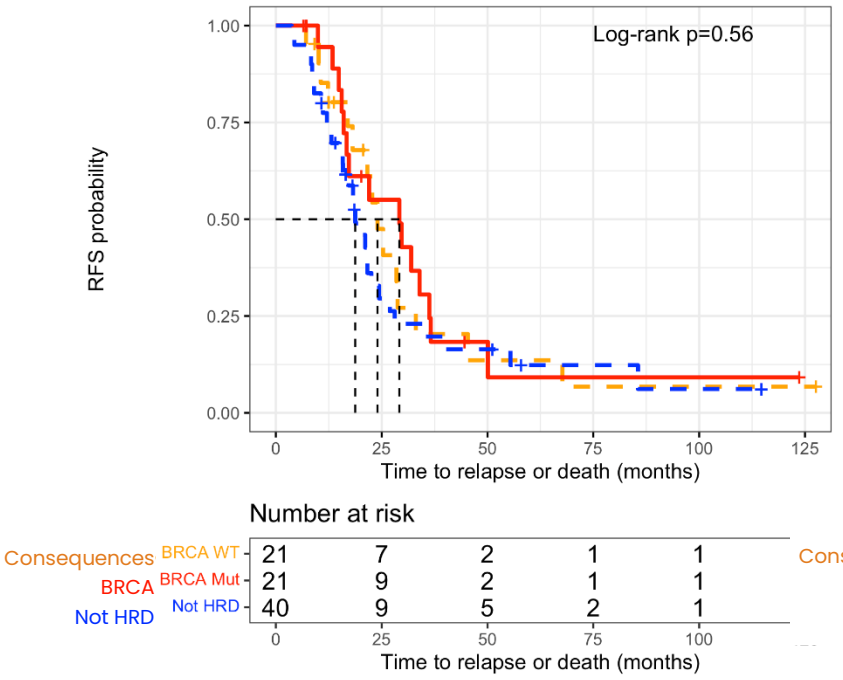
(Platinum-based therapy*)



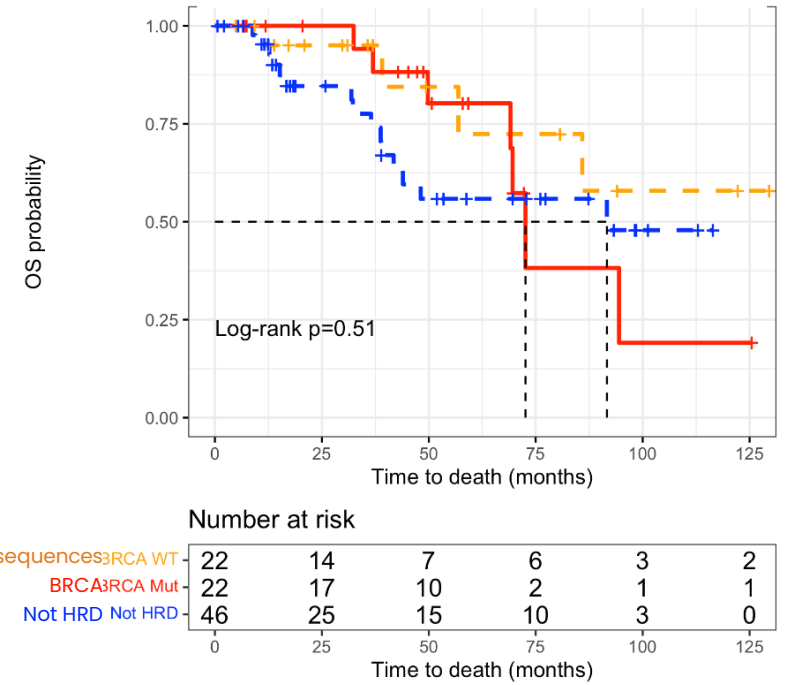
	Consequences Cluster
	BRCA Cluster
	Not HRD Cluster

*Only 15/90 patients received PARPi as maintenance therapy

Recurrence Free Survival



Overall Survival

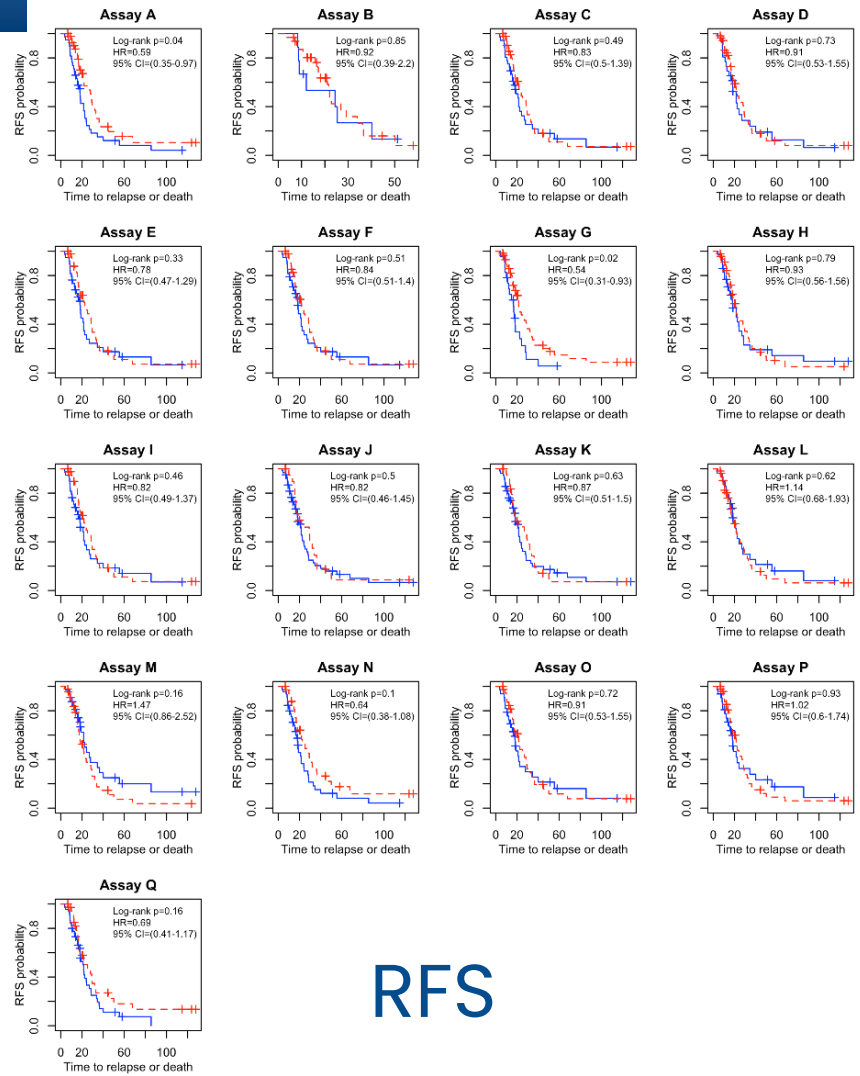


	Consequences Cluster	BRCA Cluster	Not HRD Cluster
Median RFS	24.0 months	29.2 months	18.7 months
Median OS	NA	72.6 months	91.6 months

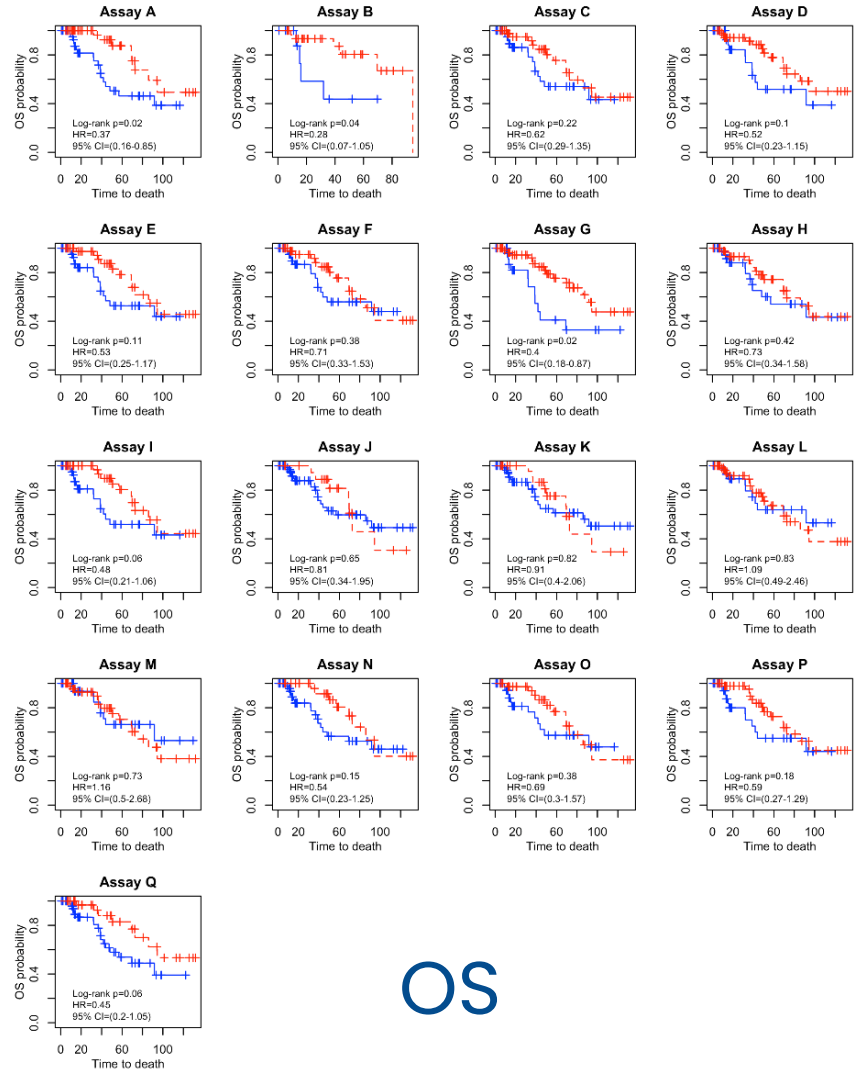
"Consequences" cluster trends toward more favorable OS compared to "Not HRD cluster" (not statistically significant)

Survival analyses by assay

Red = HRD
Blue = not HRD



RFS



OS

(Time scale in months)

Summary and Additional Thoughts

- Level of agreement between HRD assays varied depending on the pair of assays compared
  - Assay approaches varied
  - No difference based on RUO vs. clinical use

- Patient and sample characteristics were not associated with concordance but higher quality samples were used
  - Tumor content > 70%
  - DIN > 2.0

- Could not assess clinical performance of assays for selecting patients likely to benefit from PARPi
  - Only 15/90 patients received PARPi

Recommendations for assay development

- Identify the best approach for assays to report HRD to enhance consistency
- Align on expectations for analytical validation
- Consider approaches for developing a biological “gold standard,” including use of reference materials
- Consider use of supplemental “in silico” comparisons
  - Results of TCGA-based “In Silico Analysis” were broadly similar to Clinical Analysis

Thanks to the project partners!

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