TEMPUS TECH SPOTLIGHT

The Tempus HRD Test

A New Companion Test for Detecting Homologous Recombination Deficiency From Next-generation Sequencing Data

INTRODUCTION

As part of our mission to provide precision medicine to cancer patients, Tempus Labs has recently developed a laboratory test to detect homologous recombination deficiency (HRD) from next-generation sequencing (NGS) data. Tempus HRD is a DNA-based test available as an additional assessment for patients who receive the Tempus|xT Solid Tumor + Normal Test. We offer the HRD test to ensure physicians and patients have every tool available to make informed treatment decisions without requiring additional specimens. The HRD test is designed and analytically validated to provide insights regarding patients who may be sensitive to Poly (ADP-ribose) Polymerase inhibitors (PARPi).

The Emergence of PARPi Therapy in Oncology

In recent years, PARPi have been established as a viable therapy for the treatment and maintenance of *BRCA*-mutated cancers. Besides the FDA approval of various PARPi in *BRCA*-mutated ovarian, breast, pancreatic, and prostate cancers,[1,2] emerging evidence also suggests therapeutic benefit in bladder, endometrial, gastric, colon, lung, and other solid tumors.[3-7] Furthermore, treatment with PARPi has demonstrated clinical utility beyond the setting of *BRCA*-mutated cancers,[8-10] including a recent FDA approval for the treatment of castration-resistant metastatic prostate cancers harboring mutations in any homologous recombination (HR)-related gene.[11]

Another notable example is the FDA approval of niraparib for the treatment of patients with HRD-positive ovarian cancer, regardless of germline *BRCA* mutation status. The approval was based on results from the PRIMA trial, wherein patients who progressed within ≥6 months of their last platinum-based treatment were selected for PARPi therapy solely based on HRD status. Compared to the control arm, PARPi treatment enhanced PFS significantly in HRD-positive patients with and without *BRCA*-mutated tumors (22.1 vs 10.9 months and 19.6 vs 8.2 months, respectively).[12,13]

Relevance of HRD for PARPi Therapy

Mechanistically, PARPi target the vulnerability that arises in tumor cells following breaks in DNA by inhibiting PARP-mediated DNA repair. However, backup mechanisms such as HR may salvage the integrity of tumor DNA and prevent the anti-tumor effects of PARPi. In cells with existing DNA repair defects, such as in the HR pathway, PARPi induces "synthetic lethality."[14] This makes HRD a valuable biomarker for identifying patients most likely to respond to PARPi therapy. While HRD is commonly associated with the presence of deleterious *BRCA* mutations, other markers may be used for HRD detection and identification of PARPi-sensitive patients, such as genomic loss of heterozygosity (LOH). The Tempus HRD Test was designed by combining the most reliable HRD detection techniques in the field.

THE CURRENT LANDSCAPE OF DNA-BASED HRD DETECTION METHODS

Mutations in the BRCA1/2 Pathway

As mentioned above, the presence of *BRCA1/2* mutations is strongly associated with HRD. BRCA1 and BRCA2 proteins are components of a complex that enables HR and, thus, BRCA1/2 defects are powerful markers of PARPi response.[15-18] In turn, copy number and variant analyses to identify germline *BRCA1/2* mutations are standard in calculating HRD, along with assessments of germline mutations in *BRCA1/2*-related genes involved in the HR pathway.[19] However, *BRCA1/2* and/or related pathway deficiencies may be inherited as germline mutations or acquired, in which case the detection of somatic (tumor-only) mutations enhances the identification of PARPi-sensitive patients. This distinction requires the assessment of sequencing results from both tumor and normal tissue, which is an integral step in Tempus' HRD assessment.

Loss of Heterozygosity (LOH)

In addition to *BRCA1/2* and related pathway mutations, genomic instability measured by LOH is another well-established marker of HRD. Detected through extensive copy number analysis of NGS data, LOH reflects the loss or replacement of a maternal or paternal chromosome or chromosomal region. A tumor with intact heterozygosity may recover from mutations in the *BRCA1/2* pathway by calling upon a functional copy of the same gene from the other parental chromosome, but tumors with high levels of LOH have limited access to functional copies of those genes and are more vulnerable to DNA repair inhibition. Results from the ARIEL-2 clinical trial, for example, suggest a relatively improved response to PARPi in patient tumors with high LOH, even within *BRCA1/2* wild-type populations.[20] Therefore, LOH is considered a measure of HRD severity and tumor vulnerability to PARPi therapy.

LOH is often measured across all genes to illustrate the percentage of the genome exhibiting phenotypic LOH, which is called genome-wide LOH (GW LOH), but it may also be evaluated only at specific loci. In the case of HRD detection, *BRCA1/2*-specific LOH is a useful metric for determining PARPi-sensitive patient populations.[21] The Tempus HRD test incorporates both GW and *BRCA1/2*-specific LOH into the HRD score reported to physicians. Uniquely, for ovarian, breast, and pancreatic tumors, the test identifies samples with high LOH based on thresholds specific to those cancer types, while for other tumors a generalized threshold is used.

Telomeric Allelic Imbalance and Large-scale State Transitions

Other markers of genomic instability besides LOH have been implicated in HRD, including telomeric allelic imbalance (TAI) and large-scale state transitions (LST). Allelic imbalance is a measure of unequal contribution from paternal and maternal DNA regardless of changes in overall copy number, which may occur following improper repair of DNA breaks. Accordingly, these imbalances often represent downstream effects of *BRCA1/2* defects. Previous studies have identified an allelic imbalance extending from the telomere to the sub-telomere, or TAI, as a relevant measurement of HRD.[22]

Genomic stability may be evaluated from a different perspective by measuring LST, which describe the number of chromosomal breaks resulting in fragments of DNA >10 Mb across a genome. LST may contribute to HRD detection in breast and ovarian cancers, because their presence is associated with inter-chromosomal translocations and germline *BRCA1* mutations.[23-25] While TAI and LST may be used in HRD status classification, Tempus HRD alternatively relies upon somatic GW LOH to measure genomic instability. The Tempus HRD GW LOH percentage is highly concordant with a combined TAI, LST, and LOH approach, as described in an analysis below.

THE TEMPUS HRD TEST

The Tempus HRD detection approach is a model used in conjunction with data from the Tempus|xT Solid Tumor + Normal test, a 648-gene NGS panel spanning 3.6 Mb of DNA. Therefore, if a physician orders xT to identify actionable mutations in their patient's tumor, no additional tissue is required to process an HRD status. The laboratory developed test is performed using Tempus DNA sequencing data to determine deleterious *BRCA1/2* mutations, *BRCA1/2*-specific LOH, and somatic GW LOH, culminating in an HRD score that indicates each patient's likelihood for PARPi response. Additionally, the Tempus HRD report includes results

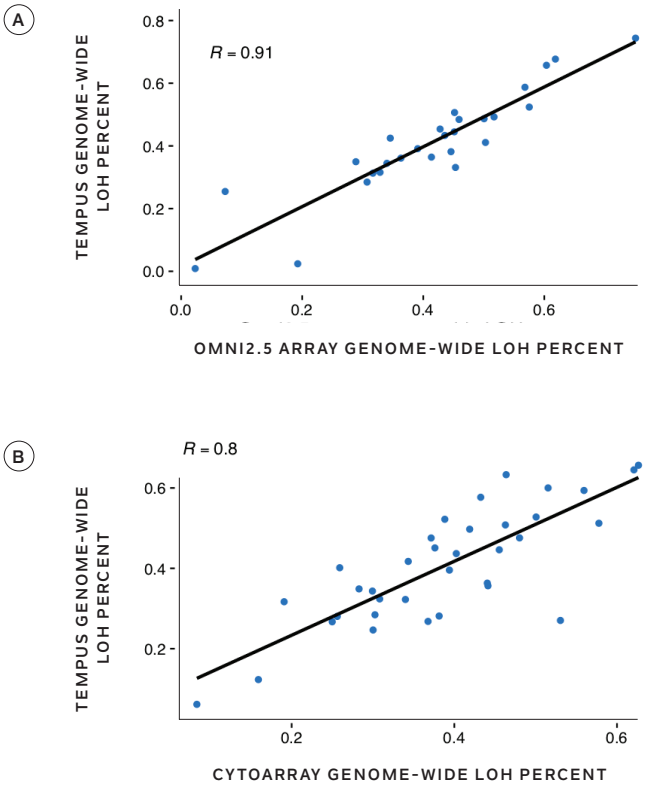
> "... if a physician orders xT to identify actionable mutations in their patient's tumor, **no additional tissue is required to process an HRD status**."

from a variant assessment of 16 other HR-related genes (18 total) conducted as part of the xT testing.

For breast, ovarian, and pancreatic samples, the Tempus HRD test uniquely determines LOH-high status using thresholds specific to each tumor type, while a generalized threshold is applied for other cancer types. Furthermore, the test incorporates data from both tumor and normal tissues to distinguish between germline and somatic mutations. While some commercial assays only measure germline mutations, the Tempus tumor/normal-matched approach ensures the provided HRD score is calculated from somatic mutations and specifically reflects tumor susceptibility to PARPi therapy.

Analytical Validation of Tempus HRD

The Tempus HRD test was developed alongside a rigorous analytical validation to confirm the resulting HRD score is accurate and meaningful to clinicians. Here, we present a brief overview of these validation efforts and results.



Figures 1A and 1B. Tempus GW LOH Estimate Concordance with Microarray Data.

Validation of the Tempus GW and *BRCA* LOH estimates included orthogonal comparisons with results from two different microarrays. These comparative analyses were conducted to evaluate concordance between completely independent, non-NGS-based methods and the Tempus HRD NGS-based test. The first analysis included 53 samples exclusively from tumor tissue analyzed by Tempus HRD and the Omni2.5 BeadChip array. Among the tumor-only samples, *BRCA1* and *BRCA2* LOH agreement for samples with tumor purity >60% were 91% (31/34) and 94% (32/34), respectively. Furthermore, GW LOH was highly concordant between the two methods (R= 0.91; ***Figure 1A***). Next, 35 tumor/normal-matched sample pairs with ≥50% tumor purity were analyzed by Tempus HRD and the CytoSNP-850K BeadChip array, demonstrating a relatively higher concordance of 97% for both *BRCA1* and *BRCA2* LOH (34/35 sample pairs). Similar to the tumor/normal-matched analyses, GW LOH estimates were highly concordant between the two methods (R = 0.8; ***Figure 1B***).

Due to the higher accuracy of somatic and germline variant classification observed, however, the commercially available version of Tempus HRD only evaluates NGS data from tumor/normal-matched samples.[26] Sensitivity, specificity, and limit of detection for single-nucleotide variants was 98.2%, 99.999%, and 5%, respectively, suggesting a high accuracy for identifying pathogenic somatic mutations. For copy number variants and gene rearrangements, the sensitivity calculated from Horizon Discovery reference DNA was 100% across titrations performed between 100% and 25% tumor purity.

Defining Cancer-specific Thresholds for LOH Status

Following technical validation of the LOH estimate methods, we sought to determine the appropriate threshold for GW LOH classification across multiple cancer types. True HRD positives were defined as samples with biallelic inactivation of *BRCA*, true HRD negatives as samples with wild-type *BRCA*, and ambiguous HRD as those with 1 hit in *BRCA1* and/or *BRCA2*. After identifying true-positive (n=235), true-negative (n=12,179), and ambiguous (n=7,586) HRD samples within the validation dataset, cohort-specific GW LOH thresholds were determined. These thresholds were designed to maximize the sensitivity and precision of HRD prediction from the peak F3 scores within each cancer type. F scores are a statistical measure of accuracy in machine learning models and, compared to F1 or similar statistics, F3 scores prioritize recall over precision. While a stringent precision (F1) may have excluded *BRCA* wild-type samples that were still HRD-positive, emphasizing recall parameters (F3) ensures that more potential PARPi-sensitive patients are captured by the defined LOH

thresholds. Based on the optimization of F3 scores for each tumor type, Tempus HRD incorporates individualized LOH thresholds for the most commonly targeted cancers (***Figure 2***).

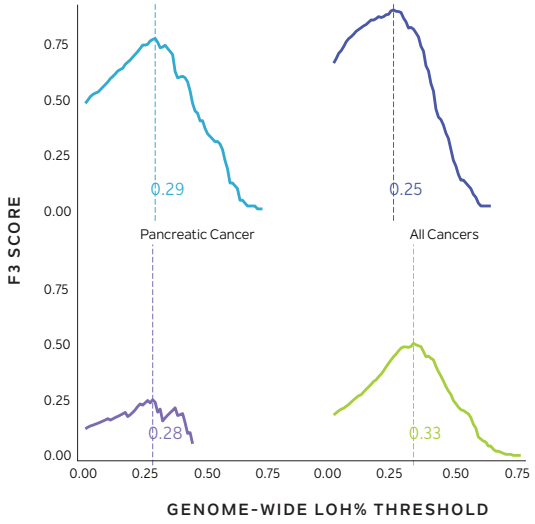


Figure 2. Tempus HRD GW LOH Thresholds by Cancer Type

Tempus HRD Association with PARPi Treatment in Organoid Models

To provide a direct evaluation of the relationship between Tempus HRD scores and PARPi response, an experiment was conducted in the Tempus organoid modeling laboratory. Three-dimensional tumor organoids provide an avenue to assess drug response in multiple tumor cells while recapitulating the inter- and intratumoral heterogeneity lacking in 2D cell culture experiments. Here, patient-derived organoids from breast tumors (n=3) were grown for 72 hours and incubated with niraparib, rucaparib, olaparib, talazoparib, pamiparib, or veliparib for an additional 72 hours. To visualize cell death, the organoids were then incubated in 2.5 ug/ml Hoechst 33342 (Fisher Scientific) and 300 nM TO-PRO-3 Iodide (Invitrogen) for 1.5 hours before imaging at 10x magnification.

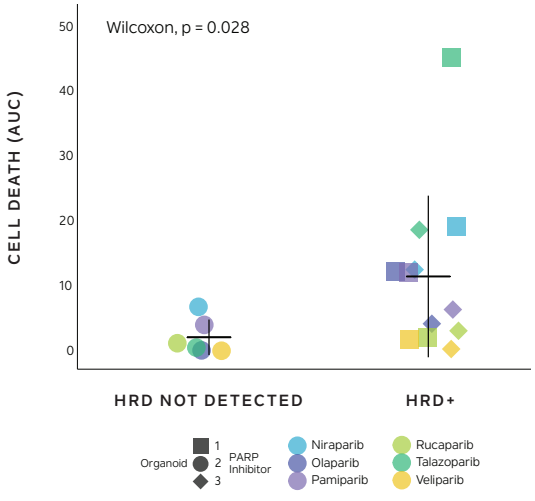


Figure 3. Tempus HRD in Patient-derived Tumor Organoids Treated with PARPi

Following image analysis with MetaXpress software (Molecular Devices), cell death was quantified for each PARPi-treated organoid sample and evaluated alongside their corresponding Tempus HRD scores. A Wilcoxon Rank-Sum Test revealed that cell death was significantly increased in HRD-positive tumor organoids relative to those without HRD detected ($P = 0.028$; ***Figure 3***).

TEMPUS HRD RESULTS IN A REAL-WORLD PATIENT COHORT

The Tempus platform extends beyond the laboratory with a real-world, clinicogenomic database of longitudinal structured and unstructured data from geographically diverse oncology practices. Using a collection of patient data from the Tempus clinicogenomic database, we sought to examine the Tempus HRD results and methodology in a real-world cohort. Clinical data were abstracted and structured into the database, including data from integrated delivery networks, academic institutions, and community practices. After clinical data abstraction and structuring, 20,000 patients with Tempus HRD results were selected as a representative sample population.

Distribution of Tempus HRD Results in a Real-world Patient Cohort

The sample population of tumor sequencing data (n=20,000) from the Tempus database was compiled across more than 20 cancer types, including ovarian (n=1,793), breast (n=2,706), pancreatic (n=1,523), prostate (n=1,174), and non-small cell lung cancer (n=3,200).

Similar to data from previous clinical trials, 66.4% of ovarian tumors (n=1,191/1,793) and 50% of breast tumors (n=1,353/2,706) were classified as HRD-positive.[9,12,20,27] Furthermore, relatively high proportions of HRD-positive samples were detected among other tumor types such as esophageal (43.1%, n=137/318), non-small cell lung (38.3%, n=1,225/3,200), and tumors of unknown origin (33.4%, n=258/772) (***Figure 4***).

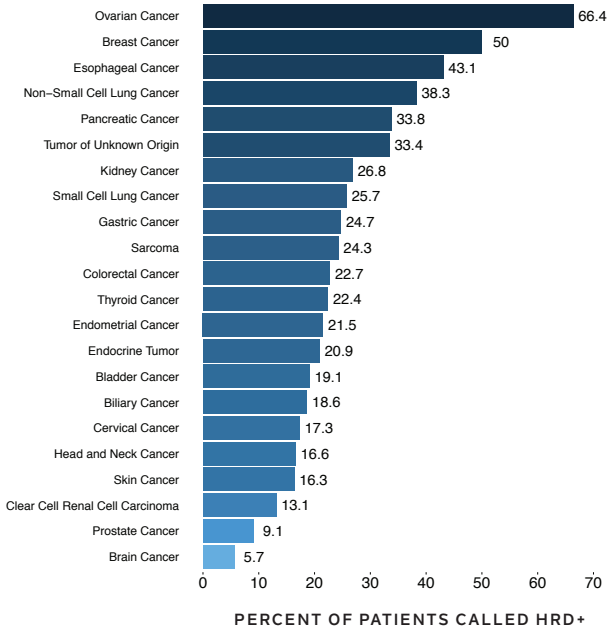


Figure 4. Distribution of HRD-positive Status Across a Sample Population From the Tempus Clinicogenomic Database.

Comparison Between Tempus HRD and an Alternative Commercially Available Method in a Real-world Patient Cohort

After establishing that the real-world cohort was representative of HRD results from previous studies, we compared the Tempus HRD approach with a method designed to mimic a popular commercially available HRD test. This alternative method includes assessments of LOH, LST, and TAI to measure genomic instability and classify HRD status.

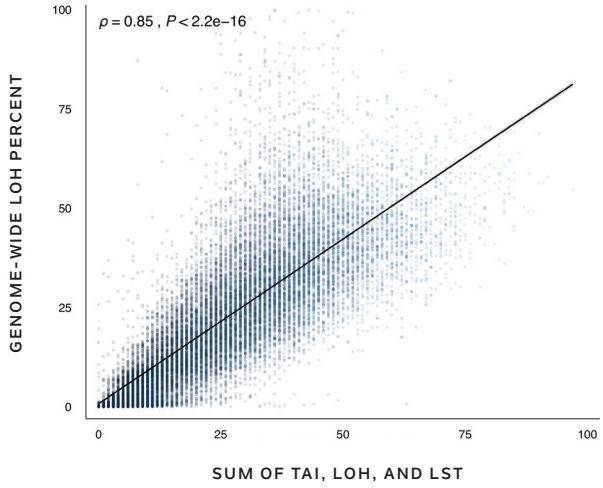


Figure 5. Tempus HRD Concordance with a Popular Alternative Method

The Tempus HRD GW LOH calculation and the alternative method were independently applied to the dataset (n=20,000) for a comparative analysis, demonstrating a strong correlation between the results (Spearman's ϱ = 0.85, $P < 2.2e-16$). ***Figure 5*** depicts the high concordance observed between the two methods.

CONCLUSION

PARPi are becoming an essential tool for oncologists in the treatment of breast, ovarian, prostate, and pancreatic cancers, and continue to demonstrate promise in a wide variety of other solid-tumor malignancies. The Tempus HRD detection test is an analytically validated, clinically useful approach for identifying patient candidates for PARPi therapy that seamlessly accompanies any Tempus|xT Solid Tumor + Normal Test ordered by a physician.

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