HRD Validation

Tempus HRD is a laboratory developed test available as an additional test for patients who are tested with Tempus xT. For ovarian and breast cancer, the Tempus HRD test (version 2) provides a result based on DNA genome-wide loss-of-heterozygosity (GWLOH) or evidence of biallelic BRCA1 or BRCA2 loss. For patients with other cancers in which there is no established or accepted method for HRD measurement, Tempus HRD provides a whole transcriptome RNA expression score. The RNA version uses mRNA expression to predict the probability that a tumor's gene expression profile correlates with well characterized benchmarks of the HRD phenotype such as biallelic BRCA loss.

The GWLOH method was developed to control for non-HRD sources of GWLOH, such as stable aneuploidy. To validate the ability to remove the effect of aneuploidy on GWLOH, NGS-based methods were tested for detecting aneuploid chromosome arms in brain cancer patients tested for loss of chromosome arm 1p by fluorescence in situ hybridization (FISH). The NGS-based method identified 1p loss with accuracy of 89.3% and positive predictive value of 100%, using FISH results as ground truth.

This method for quantifying GWLOH was then used for establishing a threshold for HRD positivity using similar approaches as the prior version of the HRD assay for ovarian and breast cancer. Acceptance criteria for the validation assay included concordance and non-inferiority with the original assay. Those criteria were met. F1-score, which combines and balances sensitivity and positive predictive value, was 0.848 vs 0.806 in breast cancer and 0.938 vs 0.934 in ovarian cancer.

The RNA-based method uses a logistic regression model, trained on normalized RNA-seq gene counts (~20,000 genes) and BRCA status from tens of thousands of patients. Similar to the approach taken with the GWLOH-based HRD assay discussed above, for HRD-RNA a pan-cancer threshold of HRD based on analytical performance (maximum F1-score) was trained and validated to distinguish bi-allelic BRCA-positive from BRCA-negative samples. The Tempus HRD-RNA test outputs an HRD score between 0 to 100, with 50 being the cutoff for HRD positivity, while samples with RNA-HRD scores below 50 will receive a result of HRD-Not Detected.

The RNA-based method was evaluated on its ability to predict BRCA1/2 status. BRCA-positive status was defined by a biallelic loss of BRCA1 or BRCA2; BRCA wild-type was defined as no evidence of any alteration in BRCA1 or BRCA2. Data from 1,743 tumor samples from 42 cancer types sequenced using the Tempus xT.v4 assay were used to determine BRCA status. The Tempus RNA-based method outperformed the DNA-based method used in the prior version of the HRD test at predicting BRCA-positivity as measured by ROC-AUC (0.923 vs. 0.720) and positive predictive value (0.066 vs. 0.018), and had a similar negative predictive value (0.998 vs. 0.997).

Feature	GWLOH in DNA-Based Version	RNA-Based Version
Calculated Score	Genome Wide LOH **OR** Evidence of biallelic loss of BRCA1 or BRCA2	HRD score, which is a logistic equation calculated using all gene expressions in the transcriptome
Threshold values	21% for breast cancer; 17% for ovarian cancer	50 for all other cancers
Thresholds optimized for	F1, which weighs both sensitivity and specificity equally	F1, which weighs both sensitivity and specificity equally