xF Validation

The non-invasive Tempus xF liquid biopsy assay detects cell-free DNA (cfDNA) in blood specimens of patients with advanced solid tumors. The assay is capable of detecting mutations in two variant classes in 105 genes, including: Single Nucleotide Variants (SNVs) and insertions and deletions (INDELs), as well as Copy Number Gains (CNGs) in 6 genes, and gene rearrangements in 7 genes spanning ~0.3 Mb of genomic space. The assay spans clinically relevant coding exons for 35 genes and covers recurrent hotspot mutations in 70 genes. Insertions and deletions will be reported down to the lower limit of detection in clinically relevant regions in 97 genes (list available upon request). The panel is designed to provide clinical decision support for patients with solid tumors and is focused on the identification of oncologic, including resistance, mutations. The report includes therapy options and clinical trials matched to the patient's genomic profile, as well as clinical history. Microsatellite Instability High (MSI-H) status is reported when detected. Blood Tumor Mutational Burden (bTMB) status is also reported when detected for tests performed in the Tempus Chicago, IL laboratory.

CAP/CLIA validation of the Tempus xF panel at Tempus' Chicago, Illinois and Durham, North Carolina laboratories focused on the detection of actionable oncologic and resistance variants in blood plasma. The assay requires two 8.5 mL Streck tubes of peripheral blood. Clinical sequencing is performed to ~20,000x coverage (at least 5,000x unique reads). Performance specifications are listed in Tables 1 and 2 below. These results establish, as shown in the tables, high sensitivity and specificity for the Tempus xF assay.

Not intended for:

- Hematologic malignancies
- Early stage (stage I/II) cancers
- Primary CNS malignancies

xF PERFORMANCE SPECIFICATIONS—CHICAGO LAB

Variant Class	VAF	Sensitivity	Specificity	LOD
Single Nucleotide Variants (SNVs)	≥0.25% 0.10%	98.5% 66.7%	>99.9%	0.25%
Insertions and Deletions (INDELs)	≥0.5% 0.25%	98.5% 75.0%	>99.9%	0.5%
Copy Number Gains (CNGs)	≥0.5% 0.5%	>99.9% >99.9%	96.2%	0.5%
Rearrangements	≥1% 0.5%	94.4% 75.0%	>99.9%	1%
Microsatellite Instability High (MSI-H) Status	—	31.3%	>99.9%	—
Blood Tumor Mutational Burden (bTMB)	—	39.0%	95.3%	—

xF PERFORMANCE SPECIFICATIONS—DURHAM LAB

Variant Class	VAF	Sensitivity	Specificity	LOD
Single Nucleotide Variants (SNVs)	≥0.25% 0.10%	99.6% 75.6%	>99.9%	0.25%
Insertions and Deletions (INDELs)	≥0.5% 0.25%	99.5% 91.1%	>99.9%	0.5%
Copy Number Gains (CNGs)	≥2.5% 1%	>99.9% 66.7%	>99.9%	2.5%
Rearrangements	≥1% 0.5%	>99.9% 98.2%	99.2%	1%
Microsatellite Instability High (MSI-H) Status	—	85.7%	>99.9%	—

xF Gene List

AKT1	*BRAF*	*CDK6*	*FGFR1*	*HRAS*	*MAP2K1*	*MYCN*	*PDGFRA*	*RET*	*TERT*
AKT2	*BRCA1*	*CDKN2A*	*FGFR2*	*IDH1*	*MAP2K2*	*NF1*	*PDGFRB*	*RHEB*	*TP53*
ALK	*BRCA2*	*CTNNB1*	*FGFR3*	*IDH2*	*MAPK1*	*NF2*	*PIK3CA*	*RHOA*	*TSC1*
APC	*BTK*	*DDR2*	*FGFR4*	*JAK1*	*MET*	*NFE2L2*	*PIK3R1*	*RIT1*	*TSC2*
AR	*CCND1*	*DPYD*	*FLT3*	*JAK2*	*MLH1*	*NOTCH1*	*PMS2*	*RNF43*	*UGT1A1*
ARAF	*CCND2*	*EGFR*	*FOXL2*	*JAK3*	*MPL*	*NPM1*	*PTCH1*	*ROS1*	*VHL*
ARID1A	*CCND3*	*ERBB2(HER2)*	*GATA3*	*KDR*	*MSH2*	*NRAS*	*PTEN*	*SDHA*	
ATM	*CCNE1*	*ERRFI1*	*GNA11*	*KEAP1*	*MSH3*	*NTRK1*	*PTPN11*	*SMAD4*	
ATR	*CD274(PD-L1)*	*ESR1*	*GNAQ*	*KIT*	*MSH6*	*PALB2*	*RAD51C*	*SMO*	
B2M	*CDH1*	*EZH2*	*GNAS*	*KMT2A*	*MTOR*	*PBRM1*	*RAF1*	*SPOP*	
BAP1	*CDK4*	*FBXW7*	*HNF1A*	*KRAS*	*MYC*	*PDCD1LG2*	*RB1*	*STK11*	

GENE REARRANGEMENTS

ALK, BRAF, FGFR2, FGFR3, NTRK1, RET, ROS1

COPY NUMBER GAINS

CCNE1, CD274(PD-L1), EGFR, ERBB2(HER2), MET, MYC