UGT1A1 Validation

The Tempus UGT1A1 test reports five specific variants in the *UGT1A1* gene, which may be associated with a patient's risk of developing toxicity to drugs metabolized by the UGT1A1 enzyme, during treatment with irinotecan, sacituzumab govitecan, and belinostat based on respective US drug labeling.[1–4] In addition, Gilbert syndrome and carrier status will also be reported as a concurrent finding if detected, in accordance with peer reviewed literature.[5] Gilbert syndrome is a heritable benign hyperbilirubinemia caused by alterations in the *UGT1A1* gene. Tempus UGT1A1 reports on some Gilbert variants also associated with drug toxicity. Some DNA variants in *UGT1A1* have been associated with therapeutic implications in drug metabolism and can be associated with hyperbilirubinemias. Results are not definitively diagnostic for Gilbert syndrome, clinical correlation and monitoring are recommended.

The assay reports on the gene alterations listed in Table 1 below, detected within the *UGT1A1* gene through analysis of the patient's germline DNA by next generation sequencing (NGS). The UGT1A1 assay is assessed based on the blood or saliva sample submitted as part of the Tempus xT test. It may only be ordered in conjunction with the Tempus xT Solid Tumor + Normal test and requires no additional sample.

Validation of the Tempus UGT1A1 test in Chicago, Illinois and Durham, North Carolina was performed in a CLIA-certified lab. The analytical validation cohort for test accuracy consisted of 45 samples from the Genetic Testing Reference Materials Coordination Program (GeT-RM) which have previously been genotyped and 5 samples from the Coriell Cell Repositories. The samples from the Coriell Cell Repositories were orthogonally confirmed by a CLIA/CAP lab using a fragment analysis by capillary electrophoresis. The analytic sensitivity and specificity was evaluated using blood and saliva from 72 internal clinical samples of which 23 were used as reference controls (i.e. samples with established results). There were 6 homozygous and 33 heterozygous combinations confirmed at the TA repeat region. Additionally, a total of 7 homozygous *6 and *27 SNVs were confirmed and 16 heterozygous combinations were confirmed. The TA repeat region was orthogonally confirmed by a CLIA/CAP lab using a fragment analysis by capillary electrophoresis and the SNVs were orthogonally confirmed using Sanger sequencing. Only variants listed in the table below are tested and reported.

The Tempus UGT1A1 test has an accuracy of >99% and a sensitivity and specificity of >99% for genotypes in the validated range at associated sequencing coverage depth for DNA input ranges between 25 ng and 300 ng.

TABLE 1: SAMPLES WERE SEQUENCED AT THE FOLLOWING LOCI

Clinical Annotation	TA repeat	Functional Status	rs_ID	Targeted SNP	Minimum Depth of Coverage
*1	TA(6)	Normal	rs3064744	NM_000463.2:c.-41_-40	70
*6	—	Decreased	rs4148323	NM_000463.3:c.211G >A:p.G71R	20
*27	—	Decreased	rs35350960	NM_000463.3:c.686C >A:p.P229Q	20
*28	TA(7)	Decreased	rs3064744	NM_000463.3:c.-41_-40dupTA	70
*36	TA(5)	Normal	rs3064744	NM_000463.3:c.-41_-40delTA	70
*37	TA(8)	Decreased	rs3064744	NM_000463.3:c.-41_-40dupTATA	70

*Note: Biochemical studies show an increase in promoter activity for *36, without significantly affecting function.*

REFERENCES

1. Camptosar (irinotecan) [package insert]. New York, NY: Pfizer, Inc.; 2022
2. Onivyde (irinotecan liposome) [package insert]. Basking Ridge, NJ: Ipsen Biopharmaceuticals, Inc.; 2015
3. Trodelvy (sacituzumab govitecan-hziy) [package insert]. Foster City, CA: Gilead Sciences, Inc.; 2022
4. Beleodaq (belinostat) [package insert]. East Windsor, NJ: Acrotech Biopharma, LLC; 2022
5. Nelson RS et al. UGT1A1 guided cancer therapy: Review of the evidence and considerations for clinical implementation. Cancers. 2021;13(7):1566. doi:10.3390/cancers13071566