PurIST℠ Validation

Tempus' PurIST℠ test is an algorithm that uses Tempus RNA sequencing data and a k-top scoring pair (k-TSP) method (8 top scoring pairs, 16 genes in total) to classify pancreatic ductal adenocarcinomas (PDAC) into one of two subtypes (basal-like or classical). Patients with a basal probability score of ≥50 are categorized as basal subtype, while those with basal probability score <50 are categorized as classical subtype.

Published literature using the PurIST algorithm with non-Tempus RNA sequencing data indicates that patients with the basal subtype have a worse prognosis and are less likely to benefit from FOLFIRINOX therapy than classical patients.[1] The clinical and biological significance of a higher or lower probability score within a subtype has not been assessed.

Validation of Tempus' PurIST test in Chicago, Illinois and Durham, North Carolina was performed in Tempus' CLIA-certified labs. The previously published association between the subtypes provided by PurIST and prognosis was replicated by Tempus, by conducting retrospective clinical validation of Tempus' PurIST test using previously sequenced de-identified Tempus patient data.[2] The clinical validity of PurIST is defined by its ability to prognosticate clinical outcomes between classical and basal subtypes using a probability score threshold of 50. In the retrospective clinical validation utilizing 232 PDAC patient samples, Tempus' PurIST test reached a statistical significance for its primary endpoint of measuring the differential 12-month survival rates in patients receiving FOLFIRINOX as first line therapy (33.33% survival for basal patients and 59.34% survival for classical patients; p=0.0236), and for its secondary endpoint of differentiating median overall survival of patients receiving FOLFIRINOX as first line therapy (8.98 months vs 14.1 months for basal and classical patients, respectively; p=0.015). In an analytical validation study the lowest evaluated tumor purity where PurIST yielded a 100% overall percentage agreement score was 20%, and therefore 20% is the lower limit of tumor purity for the PurIST test.[2]

Confidence scores for Tempus' PurIST test were calculated based on how close normalized gene expression values of the combined 8 PurIST gene pairs were to the basal-classical threshold, and the likelihood of discordant results on sample reruns (i.e. flipping from basal to classical or vice-versa). Such discordance arises from the stochastic nature of lab-based measurements, and is not due to poor sample quality or technical difficulties. To ensure high patient-level reproducibility, confidence in the subtype call of each sample was quantified and patients with confidence scores below 0.85 were flagged as indeterminate and omitted from validation analyses. Accordingly, clinical samples with confidence scores below 0.85 are deemed indeterminate and the subtype is not reportable. Tempus' PurIST test is anticipated to produce an indeterminate result in approximately 7% of samples based on prior preliminary analyses.

1 Rashid NU et al. Purity independent subtyping of tumors (PurIST), a clinically robust, single-sample classifier for tumor subtyping in pancreatic cancer. *Clinical Cancer Research*. 2020;26(1):82-92. **doi: 10.1158/1078-0432.CCR-19-1467**

2 Wenric S, Davison JM, Guittar J, et al. Real-world data validation of the purist pancreatic ductal adenocarcinoma gene expression classifier and its prognostic implications. *medRxiv*. Preprint posted online February 24, 2023. **doi:10.1101/2023.02.23.23286356**