TEMPUS TECH SPOTLIGHT

IHC Prediction

Utilizing RNA expression to predict IHC positivity for HER2, TROP2 and Nectin-4

Introduction

Tempus AI has developed IHC Prediction to identify patients with solid tumors that are likely to test positive by immunohistochemistry (2+ or 3+/H-score ≥ 100) for HER2, TROP2 and/or Nectin-4. The algorithms utilize RNA data from the Tempus xR test to find patient samples that demonstrate moderate to high RNA expression of the target genes *ERBB2* (HER2), *TACSTD2* (TROP2), and *PVRL4* (Nectin-4). This new algorithm is intended to flag patients that may be eligible for clinical trial enrollment and/or potential treatment options, who may benefit from confirmatory IHC testing. Results from the algorithm should **not** be used as evidence of IHC status. IHC Prediction results alone do not qualify patients for trial inclusion or treatment eligibility.

The algorithm is designed to classify patient samples based on their RNA expression levels with transcripts per million (TPM) as the unit of measure. The classification is achieved by setting specific TPM cutoffs for each gene: *ERBB2*, *TACSTD2* and *PVRL4*. Patient samples whose RNA expression levels meet or exceed the cutoffs for one or more biomarkers are predicted to be 2+ or 3+/H-score ≥100 by IHC for each respective biomarker. These cutoffs have been characterized through representative pan-solid tumor validation cohorts. However not all cancer types included in the validation study will be eligible for delivery of results. Of note, prevalence rates of IHC positivity vary by cancer type, influencing assay performance measures. As such, respective prevalence rates and ranges have been provided in the tables below.

HER2 Validation and Performance

The HER2 validation study population consisted of tumor samples from the following cancer types: cancer of unknown primary, cervical, colorectal, endometrial, fallopian tube & primary peritoneal, gallbladder & biliary tract, gastroesophageal, head & neck squamous cell carcinoma, kidney, liver, melanoma, non-small cell lung cancer (NSCLC), ovarian, pancreatic, prostate, salivary gland, small cell lung cancer (SCLC), small bowel, thyroid, urinary tract, and other (composed of cancer types with <10 patient samples each).

ERBB2 (HER2) High Expressors

In cancer types with >3% prevalence of 3+ IHC (see table below), 67-73% of samples identified by IHC Prediction as *ERBB2* (HER2) High Expressors were 3+ by confirmatory IHC. Many cancer types in the validation study had a low prevalence of 3+ IHC (≤3%) for which this statistical estimate is more variable, and therefore were not included in the table below.

Cancer Types	**Prevalence of 3+ IHC**	**Number of High Expressors**	**Proportion of High Expressors that are 3+ IHC**
Gastroesophageal (n=158)	>3% to ≤ 6%	9	67%
Cervical (n=36), Gallbladder & Biliary Tract (n=81), Salivary Gland (n=21), Urinary Tract (n=94)	>6% to ≤ 10%	22	73%

ERBB2 (HER2) Moderate Expressors

In cancer types with >20% prevalence of 2+/3+ IHC (see table below), 40-80% of samples identified by IHC Prediction as *ERBB2* (HER2) Moderate Expressors were 2+/3+ by confirmatory IHC. Cancer types with <10 samples in the moderate range are not included in the table below.

Cancer Types	**Prevalence of 2+/3+ IHC**	**Number of Moderate Expressors**	**Proportion of Moderate Expressors that are 2+/3+ IHC**
Colorectal (n=361)	22%	45	40%
Endometrial (n=166)	27%	39	46%
Gallbladder & Biliary Tract (n=81)	28%	11	55%
Gastroesophageal (n=158)	34%	26	62%
Head & Neck* (n=152)	11%	11	27%
NSCLC (n=364)	39%	99	65%
Ovarian, Fallopian Tube & Primary Peritoneal (n=107)	21%	27	41%
Pancreatic (n=133)	33%	19	63%
Prostate* (n=81)	17%	24	33%
Urinary Tract (n=94)	51%	35	80%

*Head & neck and prostate cancer samples fell below the 20% prevalence rate but were included due to sample size.

TROP2 Validation and Performance

The TROP2 validation study population consisted of tumor samples from the following cancer types: breast, colorectal, gastroesophageal, NSCLC, prostate, other GI (pancreatic, gallbladder & biliary tract), and other (endometrial, urinary tract, ovarian, fallopian tube & primary peritoneal, thyroid, and cervical).

TACSTD2 (TROP2) High Expressors

In cancer types with >60% prevalence of 2+/ 3+ IHC (see table below), 80-100% of samples identified by IHC Prediction as *TACSTD2* (TROP2) High Expressors were 2+/ 3+ by confirmatory IHC.

Cancer Types	**Prevalence of 2+/3+ IHC**	**Number of High Expressors**	**Proportion of High Expressors that are 2+/3+ IHC**
Breast (n=19)	84%	19	84%
Colorectal* (n=33)	39%	13	62%
Gastroesophageal (n=10)	80%	10	80%
NSCLC (n=28)	89%	27	89%
Other GI combined (Pancreatic, Gallbladder & Biliary Tract) (n=11)	64%	8	88%
Other combined (Endometrial, Urinary Tract, Ovarian, Fallopian Tube & Primary Peritoneal, Thyroid, and Cervical) (n=43)	95%	37	100%
Prostate (n=40)	90%	38	92%

*Colorectal cancer samples fell below the 60% prevalence rate but were included due to sample size.

Nectin-4 Validation and Performance

The Nectin-4 validation study population consisted of tumor samples from the following cancer types: breast, gastroesophageal, HNSCC, NSCLC, ovarian, fallopian tube & primary peritoneal, other (pancreatic, CRC, prostate, biliary tract, other female genital tract, liver, ampulla of vater, cervical, and endometrial), and urinary tract.

PVRL4 (Nectin-4) High Expressors

Urinary Tract had the highest prevalence (38%) of H-score ≥100 while all other cancer types had 2-21% prevalence. In Urinary Tract and HNSCC cancers, *PVRL4* (Nectin-4) High Expressor status identified 88% and 43% of samples with H-score ≥100, respectively. However, in other cancer types, less than 20% of samples with H-score ≥100 were identified as High Expressors.

Cancer Types	**Prevalence of H-score ≥100**	**Number of High Expressors**	**Proportion of High Expressors among H-score ≥ 100***
Breast (n=89)	19%	13	18%
Gastroesophageal (n=54)	4%	1	0%
Head & Neck Squamous Cell Carcinoma (n=61)	12%	13	43%
NSCLC (n=80)	21%	5	18%
Other combined (Pancreatic, CRC, Prostate, Biliary Tract, Other female genital tract, Liver, Ampulla of Vater, Cervical, Endometrial) (n=93)	11%	1	10%
Ovarian, Fallopian Tube & Primary Peritoneal (n=56)	2%	0	0%
Urinary Tract (n=45)	38%	24	88%

*TPM distributions for *PVRL4* vary widely across cancer subtypes and may contribute to variable performance depending on the cancer type tested.