Time to Stable Sputum Culture Positivity as a Surrogate Endpoint in Phase 2/3 Tuberculosis Trials

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BACKGROUND

- Tuberculosis (TB) remains a global health challenge, with an estimated 10.8 million cases reported annually.
- Although the standard 6-month regimen-comprising isoniazid, rifampicin, pyrazinamide, and ethambutol (HRZE)-is effective in clinical trials, its long duration leads to poor adherence and adverse outcomes in practice.
- Positive culture at month 2 (PCM2) has traditionally served as a milestone endpoint, but its predictive value for novel regimens is limited.
- Alternative culture-based biomarkers, such as time to stable sputum culture conversion (TSCC) and longitudinal time to positivity (TTP), may correlate more strongly with clinical outcomes and serve as surrogate markers, though their role is not yet established.
- **Objective**: identify a robust surrogate culture biomarker in phase 2/3 TB clinical trials.

Time to stable culture conversion (TSCC) was associated with TB-related unfavorable outcomes in 63.1% of treatment arms, indicating the potential utility of TSCC as a potential predictive marker in phase 2 and 3 TB trials.

METHODS

- Individual participant data from seven phase 2 and 3 clinical trials (Study 31/A5349 [S31], RIFAQUIN, REMoxTB, and OFLOTUB, SimpliciTB, STAND, DMID01-009), comprising 15 distinct treatment arms, were analyzed.
- Six culture readouts were evaluated: PCM2, TSCC, TTP at baseline, and TTP slopes over 0–2, 0–4, and 0–8 weeks.
- Association with TB-related unfavorable outcomes were assessed using multivariate Cox proportional hazard (Cox PH) models, stratified by treatment arm.
- RisksetROC package (v.1.0.4.1) with the Cox proportional hazard method was used to estimate the area under the receiver operating characteristic (AUC ROC) curve to evaluate the extent to which culture biomarkers explain TB-related unfavorable outcomes at 1 year.

Table 1. Baseline characteristics of patients in the dataset

Categories	Total
Participant #	7,897
Age, y (min-max)	30.0 (13.0-81.0)
Weight, kg (min-max)	53.0 (31.6-137.2)
BMI, kg/m² (min-max)	18.7 (12.0-50.9)
Male, n (%)	5,495 (69.6)
HIV status, n (%)	951 (12.0)
Diabetes status, n (%)	138 (1.7)
RACE, n (%)	
- Black	5,539 (70.1)
- Asian	988 (12.5)
- White	135 (1.7)
- Pacific islanders	44 (0.6)
- Mixed	874 (11.1)
- Missing	301 (3.8)
XPERT, value (min-max)	17.6 (4.2-35.2)
Chest X-ray aggregate cavity size, n (%)	
- No cavities	970 (12.3)
- < 4 cm	1,123 (14.2)
- ≥ 4 cm	1,048 (13.3)
- Missing	4,756 (60.2)
Chest X-ray extent of disease classification, n (%)	
- Minimal	780 (9.9)
- Moderate	2,072 (26.2)
- Advanced	2,117 (26.8)
- Missing	2,762 (35.0)
SMEAR, n (%)	
- 0	267 (3.4)
- 0.5	397 (5.0)
- 1	1,537 (19.5)
- 2	2,069 (26.2)
- 3	2,991 (37.9)
- 4	95 (1.1)
- missing	541 (6.9)
Clinical phenotype, n (%)[‡,§]	
- Easier-to-treat	5,056 (64.0)
- Harder-to-treat	2,841 (36.0)

Clinical phenotypes were determined using the SPECTRARISK algorithm, which calculates a risk score; individuals with harder-to-treat disease were defined as having a score above 0.64, whereas those with scores ≤ 0.64 were considered easier to treat

Table 2. Performance of Cox proportional hazard model for TB-related unfavorable outcomes at 1 year

Treatment arm	AUC ROC (95% CI)					
	Without clinical factor			With clinical factors		
	Base	+ TSCC	+ PCM2	clinical factor	+ TSCC	+ PCM2
2HRZE/4HR (Control-S31)	0.50	0.61	0.62	0.67	0.70	0.70
2PHZE/2PH (S31)	0.50	0.64	0.63	0.62	0.66	0.66
2PHM2PHM (S31)	0.50	0.68	0.62	0.52	0.68	0.63
2HRZE/4HR (Control-REMoxTB)	0.50	0.59	0.58	0.50	0.59	0.58
2MHRZ/2MHR (REMoxTB)	0.50	0.53	0.53	0.50	0.53	0.56
2EMRZ/2MR (REMoxTB)	0.50	0.57	0.52	0.53	0.57	0.54
2HRZE/4HR (Control-RIFAQUIN)	0.50	0.65	0.70	0.59	0.68	0.73
2EMRZ/2P2M2 (RIFAQUIN)	0.50	0.57	0.52	0.50	0.57	0.52
2EMRZ/4P1M1 (RIFAQUIN)	0.50	0.67	0.53	0.50	0.67	0.64
2HRZE/4HR (Control-OFLOTUB)	0.50	0.67	0.63	0.58	0.70	0.67
4HRZG (OFLOTUB)	0.50	0.59	0.61	0.55	0.62	0.64

AUC ROC, Area Under the Receiver Operating Characteristic Curve; **CI**, confidence interval; **TSCC**, time to stable culture conversion. Blue and Red text denote the higher and lower ROC AUC values, respectively, for TB-related unfavorable outcomes at 1 year across models.
*Clinical factors indicate the effects of age on the Cox proportional hazard (Cox PH) model.
†TSCC indicates the inclusion of the effect time to stable culture conversion on the Cox PH model.
‡ Month 2 conversion status indicates the effect of month 2 culture conversion state on the Cox PH model.

(A) Association of culture biomarkers with TB-related unfavorable outcomes without stratifying by treatment arm

Treatment arm	Biomarker	No. unfavorable outcomes/ No. study participants (%)	Hazard ratio (95 % CI)	P-value
All treatment arms	TSCC (per 1 week increase)	368/4364 (8.4)	1.04 (1.03 – 1.04)	< 0.001
	PCM2	358/4285 (8.4)	3.23 (2.61 – 4.00)	< 0.001
	Observed TTP at baseline (per 1 week increase)	139/2248 (6.2)	0.70 (0.49 – 0.98)	0.040
	Observed TTP slope 0-2 weeks (per unit increase)	115/1891 (6.1)	0.95 (0.91 – 0.99)	0.024
	Observed TTP slope 0-4 weeks (per unit increase)	117/1902 (6.2)	0.91 (0.85 – 0.97)	0.005
	Observed TTP slope 0-8 weeks (per unit increase)	118/1950 (6.1)	0.85 (0.73 – 0.98)	0.030

0.1 1.0 10.0 50.0
Hazard ratio (95% CI)

(B) Association of time to stable culture conversion and positive culture at month 2 with TB-related unfavorable outcomes with stratifying by treatment arm

Treatment arm	Biomarker	No. unfavorable outcomes/ No. study participants (%)	Hazard ratio (95 % CI)	P-value
2HRZE/4HR (Control-S31)	TSCC (per 1 week increase)	20/755 (2.6)	1.15 (1.03 – 1.27)	0.009
	PCM2	20/757 (2.6)	2.61 (1.05 – 6.45)	0.038
2PHZE/2PH (S31)	TSCC (per 1 week increase)	75/771 (9.7)	1.17 (1.12 – 1.23)	< 0.001
	PCM2	75/776 (9.7)	3.86 (2.43 – 6.13)	< 0.001
2PHMZ/2PHM (S31)	TSCC (per 1 week increase)	45/770 (5.8)	1.18 (1.11 – 1.26)	< 0.001
	PCM2	45/780 (5.8)	3.38 (1.80 – 6.35)	< 0.001
2HRZE/4HR (Control-REMoxTB)	TSCC (per 1 week increase)	133/526 (25.3)	1.02 (1.01 – 1.03)	< 0.001
	PCM2	142/545 (26.1)	2.12 (1.52 – 2.95)	< 0.001
2MHRZ/2MHR (REMoxTB)	TSCC (per 1 week increase)	160/513 (31.2)	1.01 (1.00 – 1.02)	0.064
	PCM2	177/545 (32.5)	1.64 (1.18 – 2.26)	0.003
2EMRZ/2MR (REMoxTB)	TSCC (per 1 week increase)	168/521 (32.2)	1.02 (1.01 – 1.03)	< 0.001
	PCM2	182/535 (34.0)	1.36 (0.97 – 1.92)	0.076
2HRZE/4HR (Control-RIFAQUIN)	TSCC (per 1 week increase)	9/142 (6.3)	1.03 (0.99 – 1.07)	0.173
	PCM2	8/127 (6.3)	7.09 (1.55 – 32.34)	0.011
2EMRZ/4P1M1 (RIFAQUIN)	TSCC (per 1 week increase)	8/138 (5.8)	1.02 (0.93 – 1.12)	0.658
	PCM2	8/125 (6.4)	1.08 (0.12 – 9.61)	0.945
2EMRZ/2P2M2 (RIFAQUIN)	TSCC (per 1 week increase)	15/123 (12.2)	1.15 (1.07 – 1.23)	< 0.001
	PCM2	15/116 (12.9)	4.95 (1.66 – 14.73)	0.004
2HRZE/4HR (Control-OFLOTUB)	TSCC (per 1 week increase)	51/735 (6.9)	1.11 (1.08 – 1.15)	< 0.001
	PCM2	48/709 (6.8)	2.78 (1.54 – 5.02)	< 0.001
4HRZG (OFLOTUB)	TSCC (per 1 week increase)	108/730 (14.8)	1.03 (1.02 – 1.05)	< 0.001
	PCM2	104/704 (14.8)	3.60 (2.39 – 5.44)	< 0.001
2HRZE/4HR (Control-SimpliciTB)	TSCC (per 1 week increase)	4/144 (2.8)	1.04 (1.00 – 1.07)	0.051
4BPaMZ (SimpliciTB)	TSCC (per 1 week increase)	3/128 (2.3)	1.03 (0.98 – 1.08)	0.246
	PCM2	3/124 (2.4)	3.47 (0.29 – 42.23)	0.329
2HRZE/4HR (Control-STAND)	TSCC (per 1 week increase)	10/29 (34.5)	1.04 (1.00 – 1.08)	0.034
	PCM2	10/29 (34.5)	1.39 (0.34 – 5.60)	0.644
4MPa100Z (STAND)	TSCC (per 1 week increase)	12/29 (41.4)	1.03 (1.00 – 1.07)	0.034
	PCM2	12/29 (41.4)	3.57 (0.75 – 16.87)	0.109
4MPa200Z (STAND)	TSCC (per 1 week increase)	13/33 (39.4)	1.16 (1.03 – 1.31)	0.017
	PCM2	13/33 (39.4)	1.33 (0.42 – 4.20)	0.633
6MPa200Z (STAND)	TSCC (per 1 week increase)	12/32 (37.5)	1.03 (0.99 – 1.06)	0.178
	PCM2	12/32 (37.5)	4.33 (1.00 – 18.82)	0.051
DR-6MPa200Z (STAND)	TSCC (per 1 week increase)	12/32 (37.5)	1.03 (0.99 – 1.06)	0.178
	PCM2	12/32 (37.5)	4.33 (1.00 – 18.82)	0.051
2HRZE/2HR (DMID01-009)	TSCC (per 1 week increase)	9/106 (8.5)	1.35 (1.14 – 1.59)	< 0.001

0.1 1.0 10.0 50.0
Hazard ratio (95% CI)

Figure 1. Multivariable Cox proportional hazards regression of culture biomarkers with TB-related unfavorable outcomes: (A) without stratification by treatment arm, and (B) with stratification by treatment arm.
TSCC, time to stable sputum culture conversion; **PCM2**, positive culture at month 2; **H**, isoniazid; **R**, rifampicin; **Z**, pyrazinamide; **E**, ethambutol; **P**, rifapentine; **M**, moxifloxacin; **G**, gatifloxacin; **CI**, confidence interval. Blue lines with diamonds represent significant predictors while red lines with diamonds represent non-significant predictors. Each biomarker was adjusted for the weight, sex, age, extent of lung disease on chest X-ray, and HIV status.

While associations were generally consistent across several treatment arms, observed exceptions highlight the need for further validation

RESULTS

- A total of 7,897 participant records were analyzed; the median age and weight of the study population were 30.0 years and 53.0 kg, respectively (**Table 1**).
- Each culture biomarker was adjusted for weight, age, sex, extent of lung disease on chest X-ray, and HIV status.
- TSCC and PCM2 were associated with TB-related unfavorable outcomes in 63.1% (12/19) and 52.9% (9/17) of treatment arms, respectively (**Figure 1**).
- The Cox proportional hazards model including TSCC demonstrated higher AUC ROC for predicting TB-related unfavorable outcomes at 1 year (highlighted in **blue**) compared with a model including month 2 conversion status (**Table 2**)
- These findings suggest that both TSCC is a promising prognostic biomarker for phase 2 and 3 clinical trials.

CONCLUSIONS

- This study provides supportive evidence for the **potential use of TSCC as a predictive biomarker in phase 2 and 3 TB trials**. While associations were generally consistent across treatment arms, observed exceptions highlight the need for further validation. Establishing TSCC as a reliable surrogate could facilitate earlier assessment of treatment efficacy, optimize trial design, and accelerate the development of novel TB regimens.

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