Prediction of anti-tuberculosis treatment duration based on a 22-gene transcriptomic model

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A 22-gene RNA-based model predicts individual durations of antimicrobial therapy for patients treated for tuberculosis. Application of this model will potentially shorten treatment duration in the majority of patients with MDR-TB. https://bit.ly/36dZOq0

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Abstract

Background The World Health Organization recommends standardised treatment durations for patients with tuberculosis (TB). We identified and validated a host-RNA signature as a biomarker for individualised therapy durations for patients with drug-susceptible (DS)- and multidrug-resistant (MDR)-TB.

Methods Adult patients with pulmonary TB were prospectively enrolled into five independent cohorts in Germany and Romania. Clinical and microbiological data and whole blood for RNA transcriptomic analysis were collected at pre-defined time points throughout therapy. Treatment outcomes were ascertained by TBnet criteria (6-month culture status/1-year follow-up). A whole-blood RNA therapy-end

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model was developed in a multistep process involving a machine-learning algorithm to identify hypothetical individual end-of-treatment time points.

Results 50 patients with DS-TB and 30 patients with MDR-TB were recruited in the German identification cohorts (DS-GIC and MDR-GIC, respectively); 28 patients with DS-TB and 32 patients with MDR-TB in the German validation cohorts (DS-GVC and MDR-GVC, respectively); and 52 patients with MDR-TB in the Romanian validation cohort (MDR-RVC). A 22-gene RNA model (TB22) that defined cure-associated end-of-therapy time points was derived from the DS- and MDR-GIC data. The TB22 model was superior to other published signatures to accurately predict clinical outcomes for patients in the DS-GVC (area under the curve 0.94, 95% CI 0.9–0.98) and suggests that cure may be achieved with shorter treatment durations for TB patients in the MDR-GIC (mean reduction 218.0 days, 34.2%; p<0.001), the MDR-GVC (mean reduction 211.0 days, 32.9%; p<0.001) and the MDR-RVC (mean reduction of 161.0 days, 23.4%; p=0.001).

Conclusion Biomarker-guided management may substantially shorten the duration of therapy for many patients with MDR-TB.

Introduction

Tuberculosis (TB) remains a major global health threat, with emerging *Mycobacterium tuberculosis* drug-resistance being particularly worrisome [1]. Multidrug-resistant (MDR)-TB, defined by bacillary resistance against rifampicin and isoniazid, and extensively drug-resistant TB, defined by MDR-TB plus resistance against at least one fluoroquinolone and one of the second-line injectable drugs amikacin, capreomycin and/or kanamycin) are associated with high treatment costs [2], frequently occurring adverse events [3] and discouragingly poor outcomes [4] despite prolonged treatment duration of ⩾18 months [5, 6]. The World Health Organization (WHO) has endorsed a short-course MDR-TB treatment regimen lasting 9–12 months [7] for patients with fluoroquinolone-susceptible MDR-TB who also fulfil certain criteria. Nevertheless, the great majority of patients in several regions of the world, including Europe, are not eligible for the short-course regimen due to second-line *M. tuberculosis* drug resistance [8].

The treatment duration needed to achieve cure is highly variable between individual patients and depends on the host's immune status, the severity of disease and the pathogen's virulence and drug-resistance status, as well as drug availability [9, 10]. There is a growing interest and clinical need for a biosignature to guide individualised treatment duration [11]; this is especially relevant for the treatment of patients with drug-resistant TB in order to reduce the rate of adverse events and cost, and to improve compliance [9].

Due to rapid changes in expression profiles following the initiation of anti-TB drug treatment, host genome-wide RNA expression holds promise as a surrogate marker for the duration of treatment required for an individual to achieve cure [12]. RNA signatures that correlate with treatment response and predict individual patient outcome including disease recurrence have been described previously in patients with drug-susceptible (DS)-TB [13].

We prospectively analysed whole-blood RNA transcripts in patients from two identification cohorts including patients with DS- and MDR-TB. We developed an RNA-based model as a reference for relapse-free cure based on strict outcome criteria [14] in patients with DS-TB, which was then further applied to patients with MDR-TB to indicate individual end-of-therapy time points. Subsequently, this model was prospectively applied to three independent validation cohorts, one with DS-TB and two with MDR-TB.

Materials and methods

Study design and participants

Between March 2013 and March 2016, patients with culture-confirmed pulmonary DS-TB and MDR-TB identified by detection of *M. tuberculosis* DNA from sputum by the Xpert MTB/RIF test (Cepheid, Sunnyvale, CA, USA) were prospectively enrolled into the DS German identification cohort (GIC) and the MDR-GIC, at five clinical centres in Germany as described previously (supplementary material) [15, 16]. Between March 2015 and April 2018, patients with DS-TB and MDR-TB were prospectively enrolled into the DS German validation cohort (GVC) and MDR-GVC at the same centres and at two additional centres in Germany (supplementary material). Between May 2015 and March 2017, patients with MDR-TB were prospectively enrolled into the MDR Romanian validation cohort (RVC) at the Marius-Nasta-Institute in Bucharest, Romania.

Individuals were not included in the study if they were aged <18 years, under legal supervision or living with HIV.

In addition, adult healthy controls with no history of previous TB and without any known concurrent illnesses at the time point of blood sampling were enrolled at the Medical Clinic of the Research Center Borstel (Germany) between June 2015 and December 2015.

Study visits included clinical assessment and blood sampling for whole-blood RNA measurements from PAXgene tubes (Qiagen, Venlo, the Netherlands). Study visits were performed at (ideally) before treatment initiation, at 14 days of therapy, at the times of smear conversion and following culture conversion (not available in the MDR-RVC), at 6 months and/or therapy end in patients with DS-TB, and additionally at 10, 15 and 20 months of therapy in patients with MDR-TB. After completion of 4 weeks of therapy, an additional study visit was performed in patients from the MDR-RVC. All patients completed 12 months of evaluation following the end of therapy to capture disease recurrence. A subset of DS-/MDR-GVC participants provided specimens during this follow-up period. Sputum samples provided by German study participants were evaluated *via* smear microscopy and culture at the National Reference Center for Mycobacteria at the Research Center Borstel. Samples provided by study participants at the Marius-Nasta-Institute were analysed at the Romanian National Reference Center for Mycobacteria in Bucharest. Anti-TB therapy regimens were based on comprehensive drug-susceptibility testing and consistent with current therapy recommendations [5, 17, 18]. Treatment outcomes were assessed following the TBnet definitions, where relapse-free cure is defined by having a negative *M. tuberculosis* culture status at 6 months after treatment initiation without positive cultures thereafter and no disease recurrence during the follow-up period of 1 year after therapy end [14]. TBnet outcome criteria were preferred for this study, since WHO outcome definitions do not include 1-year follow-up post-treatment completion to exclude for recurrent disease (supplementary table S1) and the WHO definition for treatment success involves items that cannot be predicted by a biomarker since they depend on a patient's behaviour or clinical decisions in the course of therapy (*i.e.* treatment completion or change of drugs during the course of treatment) [19].

Details on RNA processing, labelling, hybridisation and microarray analysis, data extraction, data normalisation, data analysis, open-access RNA data availability, the detailed steps for the model development and comparison with other published signatures or scores are shown in the supplementary material.

Statistical analysis

Using RNA microarray data from whole-blood PaxGene tubes, we identified genes that were significantly up- or downregulated between healthy controls and therapy-naïve TB patients using a moderated t-test with Benjamini–Hochberg correction. From this gene set, a gene signature consisting of six genes was shown to be suitable for predicting the outcome of therapy in DS-GIC and MDR-GIC patients. From these six genes a therapy outcome score was developed using generalised linear model. In a second step, we identified genes that correlated with the remaining therapy duration of DS-GIC and MDR-GIC patients using lasso regression techniques. We applied variable reduction steps to create a generalised linear model of nine genes to calculate the remaining therapy days, used as therapy progression score. In a third step, genes were identified *via* lasso regression that could significantly differentiate between ongoing therapy and successfully completed therapy in DS-GIC TB patients. This gene set, as well as the therapy outcome score and therapy progression score generated in the previous steps underwent variable reduction procedures in order to find a suitable random forest model that could distinguish between ongoing therapy and successfully completed therapy with high accuracy. This model was further translated into a generalised linear model and checked for validity in the external data set of DS-GVC patients and further applied to the MDR-TB patients of GIC, GVC and RVC. A detailed description of the statistical methods can be found in the supplementary material.

Ethics

Study approval was granted by the ethics committee of the University of Lübeck, (AZ 12-233; Lübeck, Germany), which was then approved by the corresponding local ethics committees of all participating centres in Germany, and by the ethics committee of the Marius Nasta Institute (3181/25.03.2015; Bucharest, Romania).

Results

All patients enrolled into these cohorts had culture-confirmed pulmonary TB (table 1, supplementary figure S1) [15, 16]. In detail, 50 patients were enrolled into the DS-GIC and 30 patients to the MDR-GIC. 28 patients were enrolled in the DS-GVC, 32 patients in the MDR-TB MDR-GVC and 52 patients in the MDR-RVC. Patients were followed-up 1 year after therapy end to assess for disease recurrence. Clinical

TABLE 1 Clinical characteristics of tuberculosis (TB) patients including the observed and predicted therapy durations in drug-susceptible (DS)- and multidrug-resistant (MDR)-TB patients from the German identification cohorts (GIC), the German validation cohorts (GVC) and the Romanian validation cohort (RVC)

	DS-TB (n=78)		MDR-TB (n=114)			p-value
	GIC	GVC	GIC	GVC	RVC	
Patients	50	28	30	32	52	
Baseline age years	48.2 (40.0–60.2)	34.6 (22.1–49.3)	36.2 (32.0–41.6)	33.2 (24.5–44.7)	37.0 (28.3–46.7)	0.083
Baseline TTP^{+} days	21.0 (16–32.3)	10 (8–13.0)	22.0 (11.8–32.5)	22.0 (11.8–32.5)	40.0 (27.5–56.0)	>0.001
Time to culture conversion days	47.5 (25.8–75.0)	46.0 (24.5–55.0)	38.0 (33.0–215.5)	50.0 (30.5–59.8)	32.0 (27.0–60.0)	0.861
Therapy outcome$^{\#}$						
Cure	29 (58.0)	20 (71.4)	17 (56.7)	20 (62.5)	34 (65.4)	
Failure	7 (14.0)	1 (3.6)	3 (10.0)	1 (3.1)	4 (7.7)	
Death	1 (2.0)	1 (3.6)	2 (6.6)	1 (3.1)		
Lost to follow-up/ undeclared	13 (26.0)	6 (21.4)	8 (26.7)	10 (31.3)	14 (26.9)	
Observed therapy duration days	184.0 (182.5–246.0)	273.0 (202.6–365)	638.0 (612.6–682.3)	641.0 (608.0–656.5)	611.0 (597.5–631.5)	<0.001
Predicted therapy duration days	175.0 (152.5–233.8)	225.0 (176.0–310.0)	420.0 (340.0–520.0)	430 (427.5–510.0)	450.0 (325.0–0.5)	<0.001

Data are presented as n, median (interquartile range) or n (%), unless otherwise stated. TTP^{+}: time to culture positivity. $^{\#}$: derived following the TBnet criteria [14].

and mycobacterial data as well as transcriptomic data from samples taken longitudinally throughout therapy from the patients in each cohort were available to conduct the analysis.

Baseline time to sputum culture positivity in therapy-naïve patients was not significantly different in the DS-GIC when compared to the DS-GVC (median 21 days, interquartile range (IQR) 16.0–32.3 days *versus* DS-TB 10 days, 8.0–13.0 days; p=0.080). The median (IQR) duration of therapy was 184 (182.5–246.0) days in DS-GIC patients and 273 (202.6–365) days in DS-GVC patients (p=0.038). MDR-GIC, MDR-GVC and MDR-RVC patients were treated for a median (IQR) duration of 638 (612.6–682.3) days, 641 (608.0–656.5) days and 611 (597.5–631.5) days, respectively (p=0.729).

Therapy-end model

A model to identify individual end-of-therapy time points for TB patients was developed using data from the GICs and then independently validated in the DS- and MDR-GVC and in the MDR-RVC. Development of the model is described in detail in the supplementary material. It included several validation steps involving clinical, radiological and bacteriological data. In total, three steps were needed to arrive at a final therapy-end model (figure 1, supplementary table S2 and figure S2). The final therapy-end model (TB22) consists of a total of 22 gene targets (CD274 (PD-L1), FAM20A, LPCAT2, TRIM27, GYG1, HIST1H1B, RPAP3, A_33_P3281041, BATF2, C2, GK, IFIT2, IFITM1, KREMEN1, PDE4D, GBP5, IL27, KCNJ2-AS1, SERPING, STAT1, TNFRSF21, VAMP5) to calculate end-of-therapy scores at different time points (figure 1, supplementary table S2). Each measurement resembles an independent end-of-therapy calculation for a TB patient under therapy. All calculation results above the cut-off (⩾0.5) indicate for hypothetical end-of-therapy time points with cure as final treatment outcome. The model identified end-of-therapy time points with high accuracy in DS-GVC patients (area under the curve (AUC) 0.94, 95% CI 0.90–0.98; table 2 and figure 2). It was applied to MDR-GIC, and to patients from the independent DS- and MDR-GVCs, and patients from the MDR-RVC to calculate hypothetical therapy durations. Figure 3a–c shows the end-of-therapy probabilities of the different cohorts as a function of time under therapy.

The proportion of patients who reached the TB22 model's threshold for the calculated end of therapy at the end of clinical anti-TB treatment was 100% in the DS-GIC and 97.4% in the DS-GVC. Patients who did not reach the threshold indicating a relapse-free end of therapy at month 6 showed an increased time to *M. tuberculosis* sputum culture conversion when compared to those who did (median 68 days, IQR 50.0–126.0 days *versus* 46.0 days, 30.0–63.0 days; p=0.041). None of the patients in the MDR-GIC and MDR-GVC and only one patient (1.9%; culture conversion within 2 weeks) in the MDR-RVC reached the threshold for cure at 6 months. Following 15 months of therapy, the overall proportions of MDR-TB

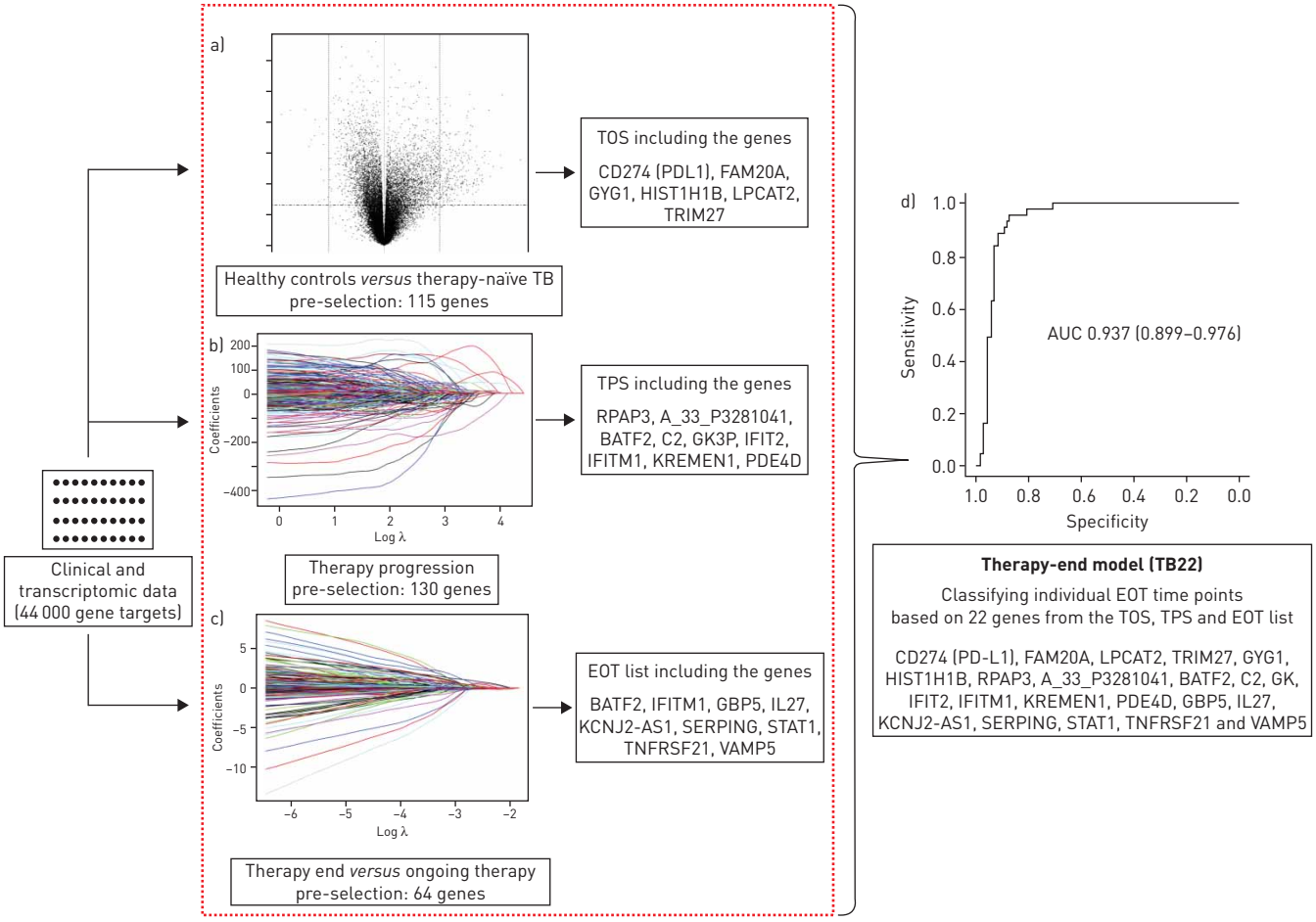

FIGURE 1 Multistep development of the therapy-end model for tuberculosis (TB) treatment. Simplified flow chart showing the multistep approach of transcriptomic and clinical data analysis to develop the therapy-end model that identifies the optimal time point to stop anti-TB therapy. a) Development of therapy outcome score (TOS) showing the volcano plot representing differentially expressed genes in healthy controls *versus* therapy-naïve drug-susceptible (DS)- and multidrug-resistant (MDR)-TB patients from the German identification cohorts (GIC). Genes that were significantly up- or down-regulated (significant two-fold or greater change after Benjamini–Hochberg correction) form the basis for the TOS development. b) Therapy progression score (TPS) development depicting penalising regression coefficient adjustment (y-axis) and the explained deviation as a function of log-ʎ (x-axis) for variable selection to identify genes that predict the remaining days of therapy that has been conducted in reality in all sample measurements from DS- and MDR-GIC TB patients. Each line represents one gene of interest and the genes shown in the plot were pre-selected by the initial lasso regression step. The initial data selection was carried out on the entire dataset with 44 000 gene targets. c) End-of-therapy (EOT) list showing penalising regression coefficient adjustment (y-axis) and the explained deviation as a function of log-ʎ (x-axis) for variable selection to identify genes that classify between sample measurements in DS-GIC TB patients under therapy *versus* time points at the end of relapse-free therapy in DS-GIC TB patients. Each line represents one gene of interest and the gene targets shown in the plot were pre-selected by the initial lasso regression to reduce the number of genes of interest. d) Therapy-end model (TB22). Implementing the gene scores (TOS and TPS) and the EOT list into a machine-learning algorithm model (random forest), a final simplified therapy-end model for the calculation of EOT time points was developed *via* a generalised linear model. The initial therapy-end model evaluation was carried out on data from DS-GIC TB patients. The receiver operating characteristic curve shows the therapy-end model's classification accuracy in the independent dataset of DS German validation cohort (GVC) TB patients (area under the curve (AUC) 0.937, 95% CI 0.899–0.976). The therapy-end model was further applied to patients with MDR-TB from the GIC, GVC and from the Romanian validation cohort.

patients having reached cure according to the TB22 model were 84.6% in the MDR-GIC, 40% in the MDR-GVC and 88.5% in the MDR-RVC.

TB22 scores for all patients from the different cohorts were below the threshold at baseline (figure 4a). The majority of patients with DS-TB reached the TB22 threshold at 6 months while drug-resistant TB patients did not (figure 4e). Nearly all TB patients from the different cohorts reached the TB22 threshold at the end of clinical therapy (figure 4f). In addition, the model probabilities for TB22 were compared between patients with DS-TB and with MDR-TB at relevant bacteriologically defined end-points such as

TABLE 2 Comparison of the 22-RNA gene therapy-end model (TB22) with published scores and signatures to identify the relapse-free end of therapy in the German validation cohort

	AUC (95% CI)	Figure 5 label	p-value of ROC curve compared to 22-RNA gene therapy-end model ROC curve
TB22	0.94 (0.90–0.98)	a	
Anderson *et al.*, 43 genes [20]	0.77 (0.68–0.85)	b	0.007
Berry *et al.*, 87 genes [21]	0.68 (0.57–0.78)	c	<0.001
Kaforou *et al.*, 27 genes [22]	0.81 (0.74–0.89)	d	0.047
Kaforou *et al.*, 44 genes [22]	0.74 (0.64–0.84)	e	0.023
Kaforou *et al.*, 53 genes [22]	0.79 (0.70–0.88)	f	0.043
Laux da Costa *et al.*, 3 genes [23]	0.79 (0.70–0.88)	g	0.045
Maertzdorf *et al.*, 3 genes [24]	0.76 (0.66–0.86)	h	0.008
Penn-Nicholson *et al.*, 6 genes [25]	0.69 (0.59–0.80	i	0.001
Sambarey *et al.*, 10 genes [26]	0.75 (0.65–0.84)	j	0.006
Singhania *et al.*, 20 genes [27]	0.71 (0.61–0.80)	k	<0.001
Suliman *et al.*, 4 genes [28]	0.65 (0.54–0.76)	l	<0.001
Sutherland *et al.*, 4 genes [29]	0.56 (0.45–0.67)	m	<0.001
Sweeney *et al.*, 3 genes [30]	0.75 (0.65–0.84)	n	0.002
Thompson *et al.*, 9 genes [13]	0.81 (0.71–0.89)	o	0.048
Thompson *et al.*, 13 genes [13]	0.62 (0.51–0.73)	p	<0.001
Thompson *et al.*, 32 genes [13]	0.65 (0.54–0.76)	q	<0.001
Zak *et al.*, 16 genes [31]	0.78 (0.70–0.87)	r	0.041

AUC: area under the curve; ROC: receiver operating characteristic.

the individual time of sputum culture and smear microscopy conversion (figure 4c and d). TB22 scores were well below the threshold for both DS-TB and MDR-TB at these time points, but scores were significantly lower for patients with MDR-TB when compared to DS-TB patients in the GICs (median TB22 score at smear conversion DS-GIC p=0.21 *versus* MDR-GIC p=0.06, p=0.038; median TB22 score at culture conversion DS-GIC p=0.29 *versus* MDR-GIC p=0.04, p=0.007) and the GVCs (median TB22 score at smear conversion DS-GVC p=0.09 *versus* MDR-GVC p=0.01, p=0.040; median TB22 score at culture conversion DS-GVC p=0.29 *versus* MDR-GVC p=0.04, p=0.007). Of note, no patient with positive sputum culture result reached the threshold for end of therapy as classified by the model. When the model scores for therapy end were stratified for drug-resistance status in pooled data from the different cohorts, they showed low TB22 scores for therapy end at baseline and after 2 weeks of therapy, but scores above the threshold at clinical therapy-end time points (supplementary figure S4).

The calculated therapy durations did not differ significantly from observed durations for the DS-GIC patients (median calculated 175.0 days *versus* observed 184.0 days, p=0.104), but they did for the DS-GVC group (median calculated 225 *versus* observed 273.0 days, p=0.001), which could be explained by the larger gaps between sampling time points, or higher bacillary burden at baseline. Calculated therapy durations were significantly shorter compared to those observed in patients of the MDR-GIC (median calculated 420.0 days *versus* observed 638 days, p=0.001) and the MDR-GVC group (median calculated 430.0 days *versus* observed 641 days, p<0.001). In addition, calculated therapy durations in MDR-RVC patients were significantly shorter than the observed durations (median calculated 450.0 *versus* observed 611.0 days, p=0.001). For patients in the MDR-GIC, this would have resulted in a median reduction of therapy duration by 218 days. In the MDR-GVC, therapy would have been reduced by a median of 211 days, and a median of 161 days in the MDR-RVC. According to the TB22 model, 32.9% of patients with MDR-TB who had a negative *M. tuberculosis* culture status at 6 months of therapy reached the TB22 threshold after 10 months; 69.5% were above the TB22 threshold after 15 months; and 97.6% were above the TB22 threshold after 20 months of treatment. Furthermore, in patients from the DS-GVC, 52.0% of patients who had a negative culture status at 2 months of anti-TB therapy had a TB22 status at month 6 of anti-TB treatment indicating cure. In contrast, 10.0% of patients who had a positive culture status at 2 months of anti-TB therapy had a TB22*+60 status at months 6 of anti-TB treatment, indicating cure.

We compared this model to publicly available RNA signatures and scores (table 2, figures 2 and 5) [13, 20–31]. While the model yielded an AUC of 0.94 (95% CI 0.90–0.98) to identify correct therapy durations in DS-GVC patients, the performance for other published markers was in the range of 0.56–0.81 only (table 2 and figure 2). Importantly, other published gene sets did not yield plausible end-of-therapy time points in DS- or MDR-GVC patients (figure 5).

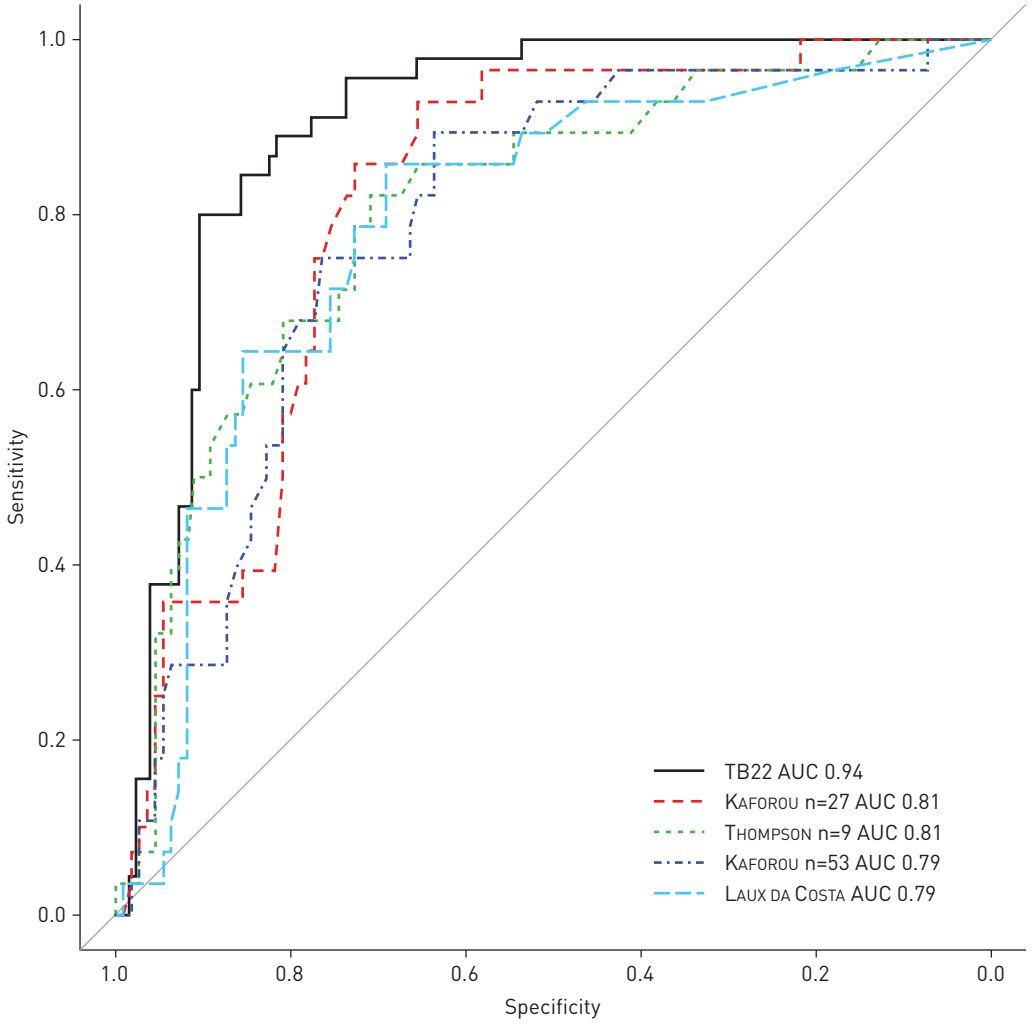

FIGURE 2 Receiver operating characteristic curve for the therapy-end model classification in drug-susceptible TB patients from the German validation cohort. Receiver operating characteristic curve analysis showing the performance of the therapy-end model (TB22) and the five published signatures/scores with the highest areas under the curve (AUC) (table 2) for the identification of optimal end-of-therapy time points when compared to clinical therapy-end time points in drug-susceptible German validation cohort patients. Therapy-end model: AUC 0.937 (95% CI 0.899–0.976); Kaforou *et al.* [22], 27 genes: AUC 0.81 (95% CI 0.74–0.89); Thompson *et al.* [13], nine genes: AUC 0.81 (95% CI 0.71–0.89); Kaforou *et al.* [22], 53 genes: AUC 0.79 (95% CI 0.70–0.88), Laux da Costa *et al.* [23], three genes: AUC 0.79 (95% CI 0.70–0.88).

Discussion

Whole-blood-derived RNA transcriptomic analysis in samples from two cohorts of TB patients in Germany, one with DS-TB and one with MDR-TB, yielded a 22-gene RNA therapy-end model (TB22) that indicates individual therapy durations associated with 1-year post-end-of-treatment relapse-free cure. This model was subsequently applied to two independent validation cohorts of patients with DS-TB and MDR-TB from Germany and a third validation cohort of patients with MDR-TB from Romania. The TB22 model provides individual scores for cure-associated end-of-therapy time points at any given moment throughout therapy, therefore providing unique data for therapy monitoring. Additionally, the comparison of the model to presently published RNA signatures or scores showed superiority in identifying end-of-therapy time points.

Transcriptional signatures for the prediction of progression to active diseases, the diagnosis of TB and early responses to anti-TB therapy in patients with DS-TB have been published [12, 28, 31]. In addition, clinical therapy outcomes, including recurrent disease, have been predicted by published RNA signatures [12, 13, 25], which were also compared to the TB22 model in this work. In contrast to these studies, the model described

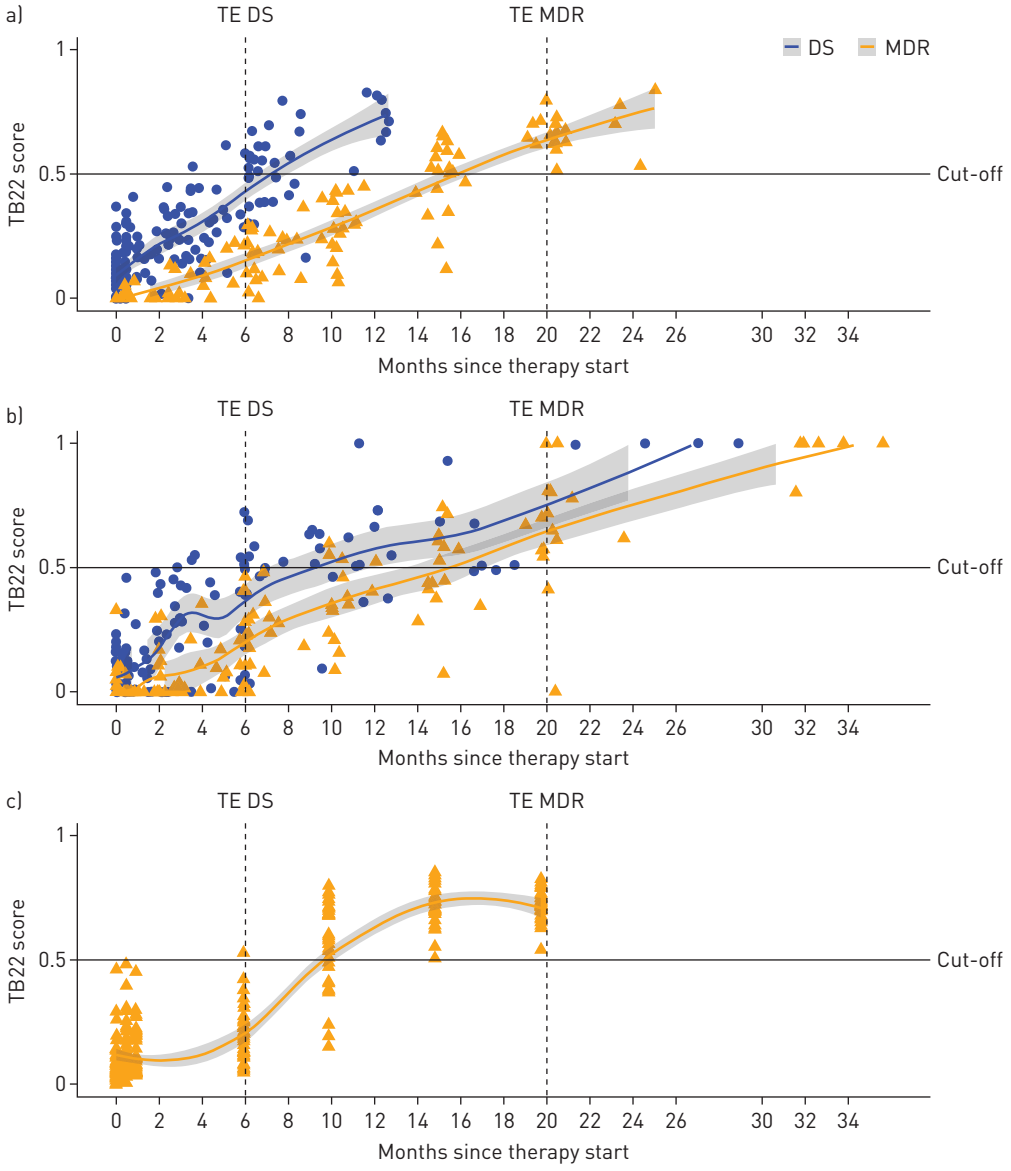

FIGURE 3 Therapy-end model (TB22) scores for individual end-of-therapy time points over time for the five cohorts of patients with tuberculosis (TB). Scores for end of therapy by the TB22 over the time of anti-TB treatment for the five cohorts of drug-susceptible (DS)-TB and multidrug-resistant (MDR)-TB patients of a) German identification cohort (GIC), b) German validation cohort (GVC) and c) Romanian validation cohort (RVC) following the TB22. 6 months of therapy is the common time point of therapy end (TE) in DS-TB; 20 months of therapy represents the usual time point for TE in MDR-TB. Data are presented as smoothed mean lines based on shown calculation and 95% CI. Cut-off: TB22 threshold ($p \geqslant 0.5$) for relapse-free end of therapy.

here was able to indicate individual therapy durations among patients with DS-TB and MDR-TB. Compared to most other published marker combinations, our findings were affirmed by considering various established clinical end-points such as smear and culture status, radiological findings and strict outcome criteria [14, 15, 32], which include a follow-up period of 1 year after completion of therapy to capture disease recurrence. In contrast to the other signatures included in the comparison, the model was specifically trained to identify end-of-therapy time points; this distinction explains the model's superior performance when compared to other published signatures that were mainly developed to predict the future onset of disease and to diagnose active TB rather than for the conduct of individualised therapy durations [13, 20–31].

The genes included in the TB22 model are involved in different functional signalling pathways and cannot be connected to a single functional background. All genes that are part of the TB22 model, except

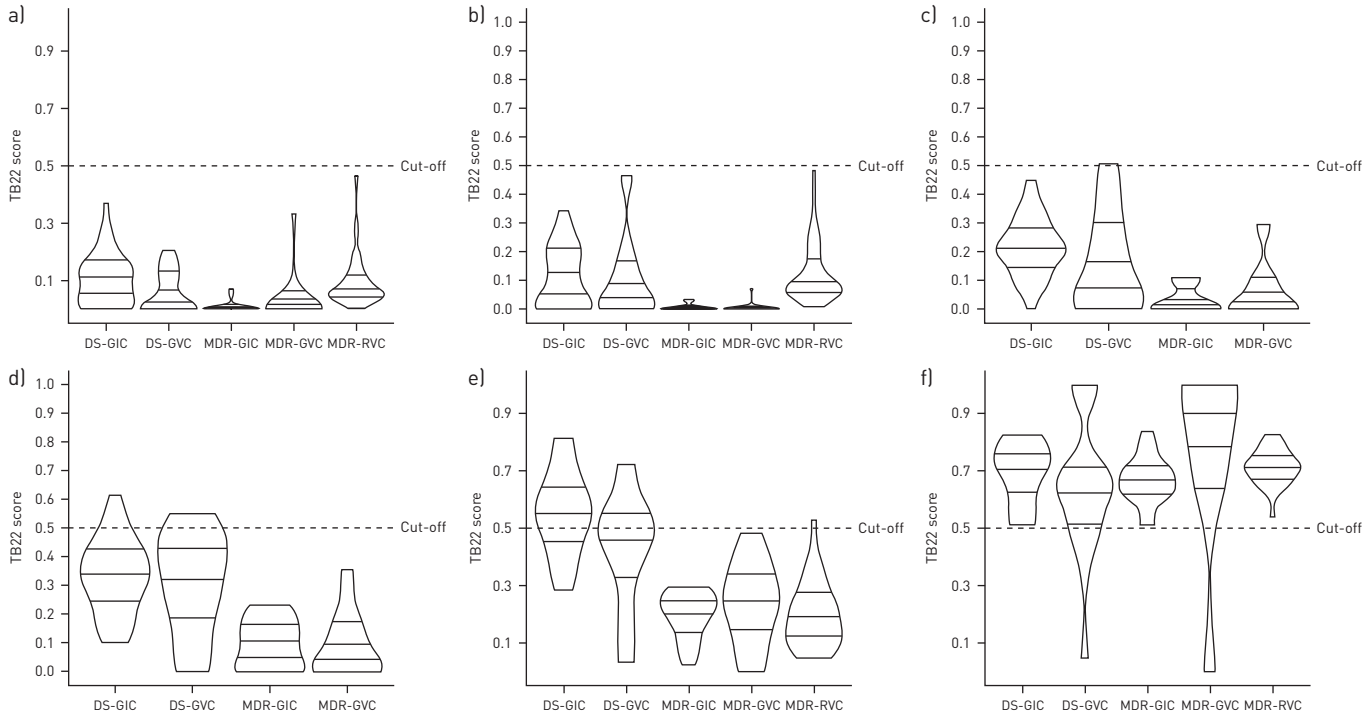

FIGURE 4 Therapy-end model (TB22) scores for the different cohorts of patients with tuberculosis (TB) at relevant clinical time points. Violin plots with TB22 scores for drug susceptible (DS)-TB patients from the German identification cohort (GIC) and the German validation cohort (GVC), and for multidrug-resistant (MDR)-TB patients from the GIC, the GVC and the Romanian validation cohort (RVC) at different time points during therapy: a) at therapy start; b) after 14 days of therapy; c) at smear conversion; d) at culture conversion; e) at 6 months of therapy; and f) at individual therapy ends. Cut-off: TB22 threshold ($p \geq 0.5$) for relapse-free end of therapy; all values below the threshold indicate an ongoing need for anti-TB therapy. There were no smear or culture conversion data for the MDR-RVC.

KCNJ1-AS1, PDE4D, TMFRSF21 and A_33_P33271041, were previously described as part of host responses to TB [21, 22, 27, 33–40]. The genes that were identified for the TB22 model belong to several signalling cascades (*e.g.* related to metabolism, cell signalling, DNA repair and RNA transport), which reflects the complexity of individual treatment responses for the host. This can be interpreted as a strength of the TB22 model, since it does not depend on a single pathway only.

Therapy responses in patients with TB are usually evaluated by serial sputum culture sampling, which are not accessible during later stages of therapy in most cases. Therefore, culture is not an accurate marker to guide individualised therapy durations [41]. Of note, the score levels for cure classified by this model at defined biological time points, *i.e.* sputum smear or culture conversion, were comparable in patients with DS-TB and MDR-TB.

Our model yielded shorter treatment durations for most patients with MDR-TB enrolled in this study. There have been standardised approaches recommended for a shorter MDR-TB treatment regimen with therapy durations of 9–11 months [42]. This regimen has been utilised in patients with MDR-TB globally leading to successful outcomes in a high proportion of study patients [43]. However, *M. tuberculosis* isolates from European patients with MDR-TB frequently carry second-line drug-resistance against core drugs included in the shorter MDR-TB regimen; hence, the regimen's use among European patients is severely limited [8]. Standardised therapies have been shown to lead to higher proportions of treatment failure and disease relapse in settings with high proportions of drug resistance when compared to individualised therapy regimens [44]. Therefore, tailored therapies informed by comprehensive drug-resistance testing may be a more promising approach to design effective MDR-TB drug regimens [15, 45]. The model described herein can add substantial value to the individualised therapy approach since the drug regimens' effect can be monitored and individual durations can be precisely identified. Individualised durations largely depend on the bacterial load, the host constitution, the pathogen's resistance pattern and the availability of drugs. RNA signature-guided individualised therapy with shorter treatment duration can potentially avert disease recurrence, lessen adverse events, improve compliance and reduce overall cost for

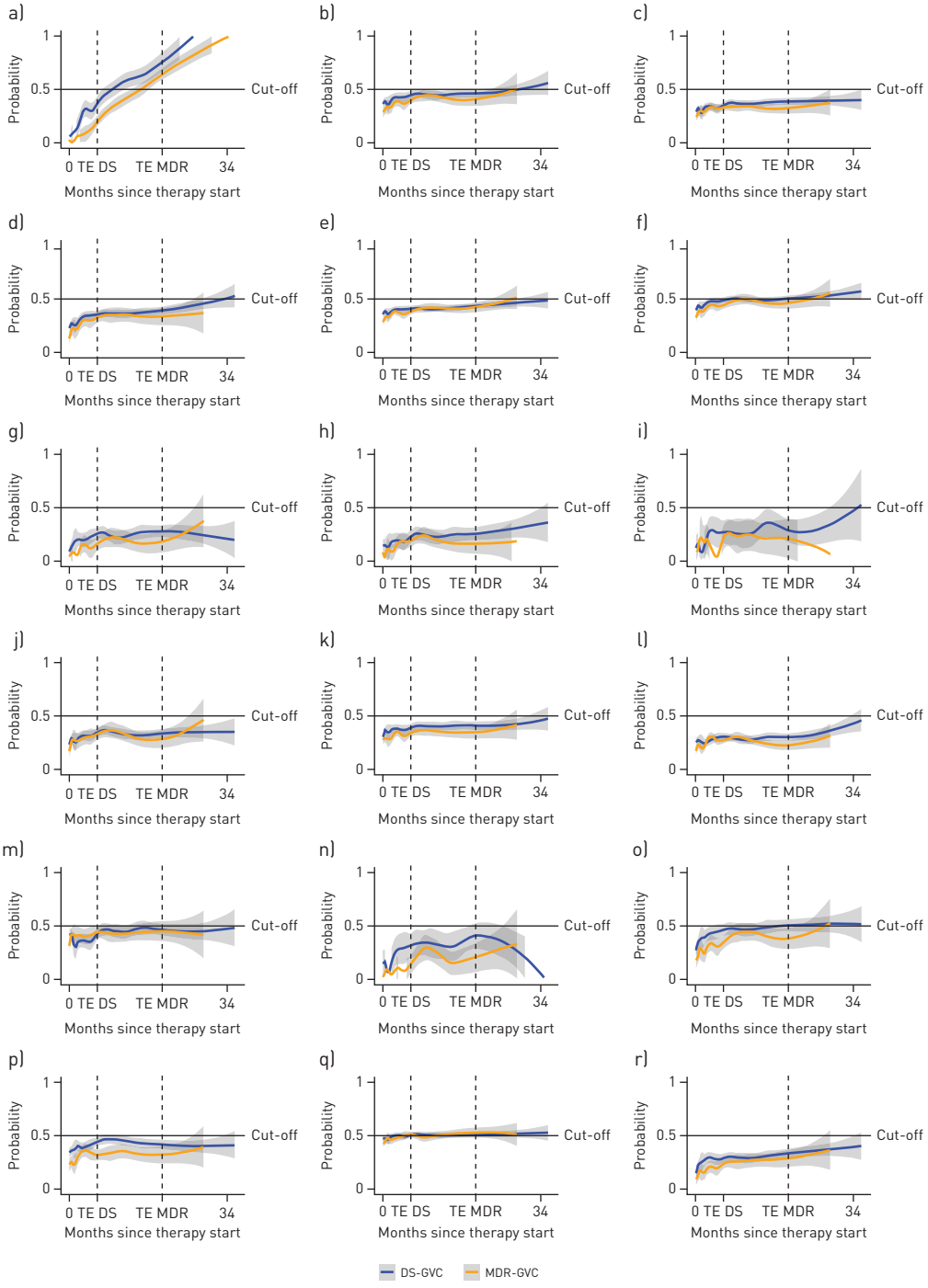

FIGURE 5 Comparison of the therapy-end model (TB22) with published RNA signatures and scores to identify end-of-therapy (TE) time points in drug-susceptible (DS) and multidrug-resistant (MDR)-tuberculosis (TB) patients from the German validation cohort (GVC). The therapy-end model and the other signatures were used to derive TE time points. Results are depicted for both patient groups over the course of treatment where probabilities ⩾0.5 would be associated with successful therapy end. a) TB22; b) Anderson *et al.* [20], 43 genes; c) Berry *et al.* [21], 87 genes; d) Kaforou *et al.* [22], 27 genes; e) Kaforou *et al.* [22], 44 genes; f) Kaforou *et al.* [22], 53 genes; g) Laux da Costa *et al.* [23], three genes; h) Maertzdorf *et al.* [24], three genes; i) Penn-Nicholson *et al.* [25], six genes (risk 6 score); j) Sambarey *et al.* [26], 10 genes; k) Singhania *et al.* [27], 20 genes; l) Suliman *et al.* [28], four genes; m) Sutherland *et al.* [29], four genes; n) Sweeney *et al.* [30], three genes; o) Thompson *et al.* [13], nine genes; p) Thompson *et al.* [13], 13 genes; q) Thompson *et al.* [13], 32 genes; r) Zak *et al.* [31], 16 genes.

TB treatment, particularly among patients with MDR-TB. Future clinical evaluation of individualised therapy durations in patients with TB requires comparative studies such as a noninferiority approach, which has demonstrated the general usefulness of shorter MDR-TB treatment regimens [43]. Importantly, the full impact of RNA signature-guided individual therapies cannot be realised without the development of an affordable point-of-care assay and platform amenable to implementation in high-burden, low-income countries.

In clinical practice, most patients treated for DS-TB using the standard four-drug regimen are cured before completing 6 months of therapy [46]. This study was designed to identify and validate biomarkers for individualised therapy durations in patients with MDR-TB, not in patients with drug-susceptible disease. Therefore, the sampling schedule did not include fixed study visits between months 4 and 6 of anti-TB therapy to detect possible end-of-therapy time points in patients with drug-susceptible TB. A more frequent sampling strategy could have provided data to calculate more-precise end-of-therapy time points for patients with DS-TB possibly indicating shorter durations of therapy.

The TB22 model was mainly based on the outcome definitions provided by the TBnet criteria [14, 15, 32]. The model's performance to discriminate between cure and failure in this study was limited to those patients who had a negative or positive *M. tuberculosis* culture status at 6 months of therapy [14] since we did not observe patients experiencing relapse within 1 year of post-treatment follow-up. One of the advantages of the TBnet treatment outcome definitions, in contrast to WHO treatment outcome definitions, is that they evaluate the parameter "cure" 1 year after the end of therapy and thus also consider relapse within this period [14].

Our study has several limitations. The study population lacks heterogeneity since it was mainly conducted in Caucasian patients and did not include people living with HIV, where RNA expression data analysis may yield different results. In addition, the overall sample size of the study was modest. Nonetheless, patients from this prospective multicentre trial had an in-depth clinical and bacteriological observational follow-up schedule with complete transcriptomic data for all patients involved.

In conclusion, we prospectively identified and validated a host 22-gene RNA-based model that may predict individual treatment durations for patients treated against TB. Application of this model may potentially shorten treatment duration in the majority of patients with MDR-TB. The model's translation into clinical practice will require further clinical evaluation in large studies and the development of an implementable platform to support feasibility in resource-limited settings.

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This work is registered at ClinicalTrials.gov as NCT02597621. RNA data is publicly available on the Gene Expression Omnibus database (GSE147690, GSE147689, GSE147691).

The 22-gene model has been filed for patenting (EP20158652.6).

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