Early View

Original article

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

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Take home message (230/256 characters): 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.

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Abstract (248/250):

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

Methods: Adult patients with pulmonary tuberculosis were prospectively enrolled into 5 independent cohorts in Germany and Romania. Clinical and microbiological data, and whole-blood for RNA transcriptomic analysis were collected at pre-defined timepoints throughout therapy. Treatment outcomes were ascertained Treatment outcomes were ascertained by TBNET criteria (6-month culture status/one-year follow-up). A whole-blood RNA therapy end model was developed in a multi-step process involving a machine-learning algorithm to identify hypothetical individual end-of-treatment timepoints.

Results: Fifty patients with drug-susceptible (DS)-tuberculosis and 30 patients with MDR-tuberculosis were recruited in the German identification cohorts (DS- and MDR-GIC), 28 patients with DS-tuberculosis and 32 patients with MDR-tuberculosis in the German validation cohorts (DS- and MDR-GVC), and 52 patients with MDR-tuberculosis in the Romanian validation cohort (MDR-RVC). A 22-gene RNA model that defined cure-associated end-of-therapy timepoints was derived from the DS- and MDR-GIC data. The model was superior to other published signatures to accurately predict clinical outcomes for patients in the DS-GVC (AUC=0.94 [95%CI:0.9-0.98]) and suggests that cure may be achieved with shorter treatment durations for tuberculosis 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-tuberculosis.

Introduction

Tuberculosis remains a major global health threat with emerging *Mycobacterium tuberculosis* drug-resistance being particularly worrisome [1]. Multidrug-resistant (MDR)-tuberculosis, defined by bacillary resistance against rifampicin and isoniazid and extensively drug-resistant (XDR)-tuberculosis, defined by MDR-tuberculosis 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 or longer [5, 6]. The World Health Organization (WHO) has endorsed a short-course MDR-tuberculosis treatment regimen lasting 9-12 months [7] for patients with fluoroquinolone susceptible MDR-tuberculosis 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 among individual patients and depends on the host's immune status, the severity of disease, 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 individualized treatment duration [11]; this is especially relevant for the treatment of patients with drug-resistant tuberculosis in order to reduce the rate of adverse events, cost, and to improve compliance [9].

Due to rapid changes in expression profiles following the initiation of anti-tuberculosis 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 previously described in patients with drug-susceptible (DS)-tuberculosis [13].

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

Materials and Methods

Study design and participants

Between March 2013 and March 2016, patients with culture-confirmed pulmonary DS-tuberculosis and MDR-tuberculosis identified by detection of *M. tuberculosis* DNA from sputum by the Xpert MTB/RIF test (Cepheid, Sunnyvale, USA) were prospectively enrolled into the DS German identification cohort (DS-GIC) and the MDR German identification cohort (MDR-GIC), at 5 clinical centres in Germany as previously described [15, 16] (**Online Supplement**). Between March 2015 and April 2018, patients with DS-tuberculosis and MDR-tuberculosis were prospectively enrolled into the DS German validation cohort (DS-GVC) and MDR German validation cohort (MDR-GVC) at the same centres and at 2 additional centres in Germany (**Online Supplement**). Between May 2015 and March 2017, patients with MDR-tuberculosis were prospectively enrolled into the MDR Romanian validation cohort (MDR-RVC) at the Marius-Nasta-Institute (MNI) in Bucharest, Romania.

Individuals were not included to the study, if they were less than 18 years of age, under legal supervision, or living with human immunodeficiency virus (HIV).

In addition, adult healthy controls with no history of previous tuberculosis and without any known concurrent illnesses at the timepoint 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-tuberculosis, and additionally, at 10, 15, 20 months of therapy in patients with MDR-tuberculosis. 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 end-of-therapy to capture disease recurrence. A subset of DS/MDR-GVC participants also 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 MNI were analyzed at the Romanian National Reference Center for Mycobacteria in Bucharest. Anti-tuberculosis 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 one year after therapy end [14]. TBNET outcome criteria were preferred for this study since WHO outcome definitions do not include one-year follow-up post treatment completion to exclude for recurrent disease (**Online Supplement Table 1**) and WHO definition for treatment success involve items that cannot be predicted by

a biomarker since they depend on a patient's behavior 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, hybridization and microarray analysis, on data extraction, data normalization, data analysis, open access RNA data availability, the detailed steps for the model development, and the comparison with other published signatures or scores are shown in the **Online Supplement**.

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 tuberculosis-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 generalized 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 generalized linear model of 9 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 tuberculosis 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 generalizable generalized linear model and checked for validity in the external data set of DS-GVC patients and further applied to the MDR-tuberculosis patients of GIC, GVC and RVC. A detailed description of the statistical methods can be found in the **Online Supplement**.

Ethics

Study approval was granted by the Ethics Committee of the University of Lübeck, Germany (AZ 12-233), which was then approved by the corresponding local Ethic Committees of all participating centers 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 tuberculosis (**Table 1, Online Supplement Figure 1**) [15, 16]. In detail, fifty patients were enrolled into the DS-GIC and 30 patients to the MDR-GIC. Twenty-eight patients were enrolled in the DS-GVC, 32 patients in the MDR-tuberculosis MDR-GVC, and 52 patients in the MDR-RVC. Patients were followed up one year after therapy end to assess for disease recurrence after therapy end. Clinical and mycobacterial data as well as transcriptomic data from samples taken longitudinally throughout therapy from the cohorts' patients 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 (DS-GIC median 21 days, interquartile range [IQR]: 16.0–32.3 days vs. DS-GVC DS-tuberculosis median 10 days, IQR: 8.0–13.0 days; $p=0.080$). The median duration of therapy was 184 days (IQR: 182.5–246.0 days) in DS-GIC patients and 273 days (IQR: 202.6–365 days) in DS-GVC patients ($p=0.038$). MDR-GIC, MDR-GVC, and MDR-RVC patients were treated for a median duration of 638 days (IQR: 612.6–682.3 days), 641 days (IQR: 608.0–656.5 days), and 611 days (IQR 597.5–631.5 days), respectively ($p=0.729$).

Therapy end model

A model to identify individual end-of-therapy timepoints for tuberculosis 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 **Online Supplement**. 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, Online Supplement Table 2, Online Supplement Figure 2**). The final therapy end model 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 timepoints (**Figure 1, Online Supplement Table 2**). Each measurement resembles an independent end-of-therapy calculation for a tuberculosis patient under therapy. All calculation results above the cut-off ≥ 0.5 indicate for hypothetical end-of-therapy timepoints with cure as final treatment outcome. The model identified end-of-therapy timepoints with high accuracy in DS-GVC patients (area under the curve [AUC] 0.94; 95% confidence interval [CI]:0.90-0.98; **Table 2 and Figure 4**). 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 2A-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 model's threshold for the calculated end-of-therapy at the end of clinical anti-tuberculosis 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 of 68 days, IQR: 50.0–126.0 days vs. median of 46.0 days, IQR: 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-tuberculosis patients having reached cure according to the model were 84.6% in the MDR-GIC, 40% in the MDR-GVC and 88.5% in the MDR-RVC.

Probabilities for end-of therapy for all patients from the different cohorts were below the threshold at baseline (**Figure 2A**). The majority of patients with DS-tuberculosis reached the end-of-therapy threshold at 6 months while drug-resistant tuberculosis patients did not (**Figure 2B**). Nearly all tuberculosis patients from the different cohorts reached the end-of-therapy threshold at the end of clinical therapy (**Figure 2C**). The model probabilities for end-of-therapy were also compared between patients with DS-tuberculosis and with MDR-tuberculosis at relevant bacteriologically defined endpoints such as the individual time of sputum culture and smear microscopy conversion. End-of-therapy probabilities were well below the threshold for both DS-tuberculosis and MDR-tuberculosis at these timepoints, but probability values were significantly lower for patients with MDR-tuberculosis when compared to DS-tuberculosis patients in the GICs (median probability at smear conversion: DS-GIC $P=0.21$ vs. MDR-GIC $P=0.06$, $p=0.038$; median probability at culture conversion: DS-GIC $P=0.29$ vs. MDR-GIC $P=0.04$, $p=0.007$) and the GVCs (median probability at smear conversion: DS-GVC $P=0.09$ vs. MDR-GVC $P=0.01$, $p=0.040$; median probability at culture conversion: DS-GVC $P=0.29$ vs. 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 probabilities for therapy end were stratified for drug-resistance status in pooled data from the different cohorts, they showed low probabilities for therapy end at baseline, after 2 weeks of therapy, but probabilities above the threshold at clinical therapy end timepoints (**Online Supplement Figure 4A and B**).

The calculated therapy durations did not differ significantly from observed durations for the DS-GIC patients (median calculated 175.0 days vs. observed 184.0 days, $p=0.104$) but they

do for the DS-GVC group (median calculated 225 vs. observed 273.0 days, $p=0.001$), which could be explained by the larger gaps between sampling timepoints, 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 vs. observed 638 days, $p=0.001$) and the MDR-GVC group (median calculated 430.0 days vs. observed 641 days, $p<0.001$). Calculated therapy durations in MDR-RVC patients were also significantly shorter than the observed durations (median calculated 450.0 vs. 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 for a median of 161 days in the MDR-RVC. According to the therapy end model, 32.9% of patients with MDR-tuberculosis who had a negative *M. tuberculosis* culture status at 6 months of therapy reached the therapy end model threshold after 10 months, 69.5% were above the therapy end model threshold after 15 months and 97.6% were above the therapy end threshold after 20 months of treatment, respectively. Also, in patients from the DS-GVC, 52.0% of patients who had a negative culture status at 2 months of anti-tuberculosis therapy had a therapy end model status at month 6 of anti-tuberculosis treatment indicating cure. In contrast, 10.0% of patients who had a positive culture status at 2 months of anti-tuberculosis therapy had a 22 gene-model status at months 6 of anti-tuberculosis treatment indicating cure.

We also compared this model to publicly available RNA signatures and scores (**Table 2, Figure 4 and 5**) [13, 20-31]. While the model yielded an area under the curve (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 4**). Importantly, other published gene sets did not yield plausible end-of-therapy timepoints in DS or MDR-GVC patients (**Figure 5**).

Discussion

Whole-blood-derived RNA transcriptomic analysis in samples from two cohorts of tuberculosis patients in Germany, one with DS-tuberculosis and one with MDR-tuberculosis, yielded a 22-gene RNA therapy end model that indicates individual therapy durations associated with one-year post end-of-treatment relapse-free cure. This model was subsequently applied to two independent validation cohorts of patients with DS-tuberculosis and MDR-tuberculosis from Germany and a third validation cohort of patients with MDR-tuberculosis from Romania. The model provides individual probabilities 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 timepoints.

Transcriptional signatures for the prediction of progression to active diseases, the diagnosis of tuberculosis, and early responses to anti-tuberculosis therapy in patients with DS-tuberculosis have been published [12, 27, 30]. Clinical therapy outcomes, including recurrent disease, have also been predicted by published RNA signatures [12, 13, 31], which were also compared to the therapy end model in this work. In contrast to these studies, hereby described model was able to indicate individual therapy durations among patients with DS-tuberculosis and MDR-tuberculosis. Compared to most other published marker combinations, our findings were affirmed by considering various established clinical endpoints such as smear and culture status, radiological findings, and strict outcome criteria [14, 15, 32], which include a follow-up period of one 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 timepoints, this distinction also 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 tuberculosis rather than for the conduction of individualized therapy durations [13, 20-31].

The genes included in the therapy end model are involved in different functional signaling pathways and cannot be connected to a single functional background. All genes that are part of the therapy end model, except KCNJ1-AS1, PDE4D, TMFRSF21 and A_33_P33271041, were previously described as part of host responses to TB [21, 33-42]. The genes that were identified for the therapy end model belong to several signaling cascades (e.g., related to metabolism, cell signaling, DNA repair and RNA transport), which reflects the complexity of individual treatment responses for the host. This can also be interpreted as a strength of the therapy end model since it does not depend on a single pathway only.

Therapy responses in patients with tuberculosis 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 individualized therapy durations [43]. Of note, the probability levels for cure classified by this model at defined biological timepoints, i.e., sputum smear or culture conversion, were comparable in patients with DS-tuberculosis and MDR-tuberculosis.

Our model yielded shorter treatment durations for most patients with MDR-tuberculosis enrolled in this study. There have been standardized approaches recommended for a shorter MDR-tuberculosis treatment regimen with therapy durations of 9-11 months [44]. This regimen has been utilized in patients with MDR-tuberculosis globally leading to successful outcomes in a high proportion of study patients [45]. However, *M. tuberculosis* isolates from European patients with MDR-tuberculosis frequently carry second-line drug-resistance against core drugs included in the shorter course MDR-tuberculosis regimen; hence, the

regimen's use among European patients is severely limited [8]. Standardized therapies have been shown to lead to higher proportions of treatment failure and disease relapse in settings with high proportions of drug-resistances when compared to individualized therapy regimens [46]. Therefore, tailored therapies informed by comprehensive drug-resistance testing may be a more promising approach to design effective MDR-tuberculosis drug regimens [15, 47]. The model described herein can add substantial value to the individualized therapy approach since the drug regimens' effect can be monitored and individual durations can be precisely identified. Individualized durations largely depend on the bacterial load, the host constitution, the pathogen's resistance pattern, and the availability of drugs. RNA signature-guided individualized therapy with shorter treatment duration can potentially avert disease recurrence, lessen adverse events, improve compliance and reduce overall cost for tuberculosis treatment, particularly among patients with MDR-tuberculosis. Future clinical evaluation of individualized therapy durations in patients with tuberculosis requires comparative studies such as a non-inferiority approach, which has demonstrated the general usefulness of shorter MDR-tuberculosis treatment regimens [45]. Importantly, the full impact of RNA signature-guided individual therapies cannot be realized 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-tuberculosis using the standard four-drug regimen are cured before completing 6 months of therapy [48]. This study was designed to identify and validate biomarkers for individualized therapy durations in patients with MDR-tuberculosis, not in patients with drug-susceptible disease. Therefore, the sampling schedule did not include fixed study visits between month 4 and 6 of anti-tuberculosis therapy to detect possible end-of-therapy timepoints in patients with drug-susceptible tuberculosis. A more

frequent sampling strategy could have provided data to calculate more precise end-of-therapy timepoints for patients with DS-tuberculosis possibly indicating shorter durations of therapy.

The therapy end 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 one 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" one year after the end of therapy and thus consider also relapse within this period [14].

Our study has several limitations. The study population lacks in 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. Also, the overall sample size of the study was modest. Still, patients from this prospective multi-center 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 tuberculosis. Application of this model may potentially shorten treatment duration in the majority of patients with MDR-tuberculosis. 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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Patent: The 22-gene model has been filed for patenting (EP20158652.6)

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Tables

Table 1: Clinical characteristics of tuberculosis (TB) patients including the observed and the 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). Therapy outcomes are derived following the TBNET criteria [14]. TTP+ = time to culture positivity, IQR = interquartile range.

	Drug-susceptible tuberculosis (n=78)		Multidrug-resistant tuberculosis (n=114)			p-value
	GIC n= 50	GVC n= 28	GIC n= 30	GVC n= 32	RVC n= 52	
Baseline Age (median, IQR)	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+ in days (median, IQR)	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 in days (median, IQR)	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 in days (median, IQR)	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 in days (median, IQR)	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-.5)	<0.001
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Table 2: Comparison of the 22-RNA gene therapy end model with published scores and signatures to identify the relapse-free end-of therapy in the German validation cohort (GVC). Area under the curve=AUC, 95% confidence interval=CI.

RNA Signature or Score	AUC (CI)	Figure 5 label	p-Value of ROC curve compared to 22-RNA gene therapy end model ROC curve
<i>22-RNA gene therapy end model</i>	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 [31]	0.69 (0.59 – 0.80)	I	0.001
Sambaray et al., 10 genes [25]	0.75 (0.65 – 0.84)	J	0.006
Singhania et al., 20 genes [26]	0.71 (0.61 – 0.80)	K	<0.001
Suliman et al., 4 genes [27]	0.65 (0.54 – 0.76)	L	<0.001
Sutherland et al., 4 genes [28]	0.56 (0.45 – 0.67)	M	<0.001

Sweeney et al., 3 genes [29]	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 [30]	0.78 (0.70 – 0.87)	R	0.041

Figures

Figure 1: Multi-step development of the therapy end model for tuberculosis treatment

Simplified flow chart showing the multi-step approach of transcriptomic and clinical data analysis to develop the Therapy end model (TEM) that identifies the optimal timepoint to stop anti-tuberculosis therapy. A. Development of therapy outcome score (TOS). Showing the volcano plot representing differentially expressed genes in healthy controls vs. therapy naïve drug-susceptible (DS)- and multidrug-resistant (MDR)-tuberculosis patients from the German identification cohorts (GICs). Genes that were significantly up- or down-regulated (significant ≥ 2 -fold change after Benjamini-Hochberg correction) form the basis for the Therapy outcome score development. B. Therapy progression score (TPS) development. Depiction of penalizing regression coefficient adjustment (y-axis) and the explained deviation as a function of $\text{Log-}\lambda$ (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 tuberculosis 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 data set with 44,000 gene targets. C. End-of-therapy list (EOT list). Showing penalizing regression coefficient adjustment (y-axis) and the explained deviation as a function of $\text{Log-}\lambda$ (x-axis) for variable selection to identify genes that classify between sample measurements in DS-GIC tuberculosis patients under therapy vs. timepoints at the end of relapse-free therapy in DS-GIC tuberculosis 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. Implementing the gene scores (Therapy outcome score and Therapy progression score) and the End-of-therapy list into a machine learning algorithm model (Random Forest), a final simplified Therapy end model for the calculation of end-of-therapy timepoints was developed via Generalized Linear Model (GLM). The initial Therapy end model evaluation was carried out on data from DS-GIC tuberculosis patients. The ROC-curve shows Therapy end model's classification accuracy in the independent data set of DS German validation cohort (GVC) tuberculosis patients (Area under the curve [AUC] 0.937, confidence interval [CI] 0.899-0.976)). Therapy end model was further applied to patients with multidrug-resistant tuberculosis from the GIC, GVC, and from the Romanian validation cohort (MDR-RVC).

Figure 2: Therapy end model scores for individual end-of-therapy timepoints over time for the five cohorts of patients with tuberculosis

Scores for end-of-therapy by the therapy end model (TEM) over the time of anti-tuberculosis treatment for the five cohorts of drug-susceptible (DS)-tuberculosis and multidrug-resistant (MDR)-tuberculosis patients of German Identification Cohort (GIC), German Validation Cohort (GVC) and Romanian Validation Cohort (RVC) following the therapy end model. Y-axis: Therapy end model scores for end-of-therapy, horizontal line: probability threshold ($P \geq 0.5$) for relapse-free end-of-therapy; X-axis: time under treatment (months), first vertical dotted line indicates 6 months of therapy, the common timepoint of therapy end (TE) in drug-susceptible tuberculosis, second vertical dotted line indicates the usual timepoint for TE in multidrug-resistant tuberculosis after 20 months of therapy. Blue line: smoothed mean line for DS-tuberculosis based on shown calculation (blue dots), Grey area: 95%-CI interval of DS patients smoothed mean line of Therapy end model Score. Orange line: smoothed mean line for MDR-tuberculosis based on shown calculation (orange triangles). Grey area: 95%-CI interval of MDR-tuberculosis patients smoothed mean line of Therapy end model Score.

Figure 2A: Therapy end model scores in DS-GIC and MDR-GIC over time (blue: DS-GIC, orange: MDR-GIC) **Figure 2B:** Therapy end model probabilities in DS-GVC and MDR-GVC patients over time (blue DS-GVC, orange: MDR-GVC) **Figure 2C:** Therapy end model scores in MDR-RVC patients over time (orange).

Figure 3: Therapy end model scores for the different cohorts of patients with tuberculosis at relevant clinical timepoints

Violin plot with therapy end model (TEM) scores for drug susceptible (DS)-tuberculosis patients from the German identification cohort (DS-GIC) and the German validation cohort (DS-GVC), and for multidrug-resistant (MDR)-tuberculosis patients from the GIC, the GVC, and the Romanian validation cohort (RVC) at different time points during therapy. Y-axis: Therapy end model score, dotted horizontal line: threshold for relapse-free end-of-therapy (Scores (P) $\geq$ 0.5). All values below the threshold indicate an ongoing need for anti-therapy; X-axis showing the different cohorts. **Figure 3A:** Therapy end model scores at therapy start for DS-GIC, DS-GVC, MDR-GIC, MDR-GVC, and MDR-RVC tuberculosis patients, **Figure 3B:** Therapy end model scores after 14 days of therapy for DS-GIC, DS-GVC, MDR-GIC, MDR-GVC, and MDR-RVC tuberculosis patients, and **Figure 3C:** Therapy end model scores at smear conversion for DS-GIC, DS-GVC, MDR-GIC, MDR-GVC, and MDR-RVC tuberculosis patients. **Figure 3D:** Therapy end model scores at culture conversion for DS-GIC, DS-GVC, MDR-GIC, MDR-GVC, and MDR-RVC patients, **Figure 3E:** Therapy end model scores at 6 months of therapy for DS-GIC, DS-GVC, MDR-GIC, MDR-GVC, and MDR-RVC tuberculosis patients, and **Figure 3F:** Therapy end model scores at individual therapy ends for DS-GIC, DS-GVC, MDR-GIC, MDR-GVC, and MDR-RVC tuberculosis patients.

Figure 4: Receiver operating characteristic curve for the therapy end model classification in drug-susceptible tuberculosis patients from the German Validation Cohort

Receiver operating characteristic curve (ROC) analysis showing the performance of the therapy end model (TEM) and the five published signatures/scores with the highest (Table 2) areas under the curve (AUC) for the identification of optimal end-of-therapy timepoints

when compared to clinical therapy end timepoints in drug susceptible (DS) German Validation Cohort patients (GVC). Therapy end model: AUC 0.937 (95% confidence interval (CI) 0.899–0.976), Kaforou et al., 27 genes [22]: AUC 0.81 (CI 0.74–0.89), Thompson et al., 9 genes [13]: AUC 0.81 (CI 0.71–0.89), Kaforou et al., 53 genes [22]: AUC 0.79 (0.70–0.88), Laux da Costa et al., 3 gene [23]: AUC 0.79 (CI 0.70–0.88).

Figure 5: Comparison of the 22-RNA gene signature model with published RNA-signatures and scores to identify end-of-therapy timepoints in drug-susceptible and multidrug-resistant tuberculosis patients from the German Validation Cohort (GVC).

Calculated end-of therapy timepoints in drug-susceptible (DS)-German Validation Cohort (GVC) patients (blue) and multidrug-resistant (MDR)-GVC patients (orange) for different RNA signatures and scores previously published in the literature. The Therapy end model and the other signatures were used to derive end-of-therapy timepoints. Results are depicted for both patient groups over the course of treatment where probabilities ≥ 0.5 would be associated with successful therapy end. Y-axis: probabilities (P(TE)) from the different models for the classification of the end-of-therapy timepoints with a cut-off of ≥ 0.5 , X-axis: time under therapy in months. **Figure 5A:** 22-RNA gene therapy end model (TEM). **Figure 5B:** Anderson et al., 43 genes [20]. **Figure 5C:** Berry et al., 87 genes [21]. **Figure 5D:** Kaforou et al., 27 genes [22]. **Figure 5E:** Kaforou et al., 44 genes [22]. **Figure 5F:** Kaforou et al., 53 genes [22]. **Figure 5G:** Laux da Costa et al., 3 genes [23]. **Figure 5H:** Maertzdorf et al., 3 genes [24]. **Figure 5I:** Penn-Nicholson et al., 6 genes [31]. **Figure 5J:** Sambaray et al., 10 genes [25]. **Figure 5K:** Singhania et al., 20 genes [26]. **Figure 5L:** Suliman et al., 4 genes [27]. **Figure 5M:** Sutherland et al., 4 genes [28]. **Figure 5N:** Sweeney et al., 3 genes [29]. **Figure 5O:** Thompson et al., 9 genes. [13]. **Figure 5P:** Thompson et al., 13 genes [13]. **Figure 5Q:** Thompson et al., 32 genes [29]. **Figure 5R:** Zak et al., 16 genes [30].

ROC curves for therapy end detection - DS-GVC

Online Supplement Materials:

Supplement Methods

Online Supplement Tables 1 and 2

Online Supplement Figures 1-4

Online Supplement File

Original article submission

European Respiratory Journal

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

ONLINE SUPPLEMENT

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Supplement Methods and Results

RNA processing and data analysis

Whole blood RNA isolation from PAXgene (Qiagen®, Venlo, the Netherlands) was handled according to the manufacturer's instructions and stored at -80°C until RNA isolation using the PAXgene blood RNA isolation kit (Qiagen®, Venlo, the Netherlands). Aliquots of isolated RNA were used for quality control to analyze the RNA integrity with the RNA Nano 6000 Kit on an Agilent Bioanalyzer (Agilent®, Böblingen, Germany) according to the manufacturer's instructions. In case of an insufficient RNA Integrity Number (RIN) as a measure of sample quality and number or signs of degradation, samples were excluded from further analysis.

Labelling, hybridization and scanning of microarrays

Total RNA was used for reverse transcription and subsequent Cy3-labelling with the Low-Input Quick Amp Labeling Kit (Agilent®, Böblingen, Germany) according to the One-Color Microarray-Based Gene Expression Analysis Protocol version 6.9.1 (Agilent®, Böblingen, Germany) with RNA Spike-In controls. 1650ng of Cy3-labelled cRNA was hybridized to human 4x44K V2 gene expression microarrays according to manufacturer's instructions. Arrays were scanned on a SureScan microarray scanner (Agilent®, Böblingen, Germany) at a resolution of 5 µm.

Data extraction and normalization

Raw expression data from scanned microarray slides were extracted from tiff files using the Feature Extraction Software version 11 (Agilent®, Böblingen, Germany). Raw data files were imported into Agilent GeneSpring software version 13 (Agilent®, Böblingen, Germany). Percentile Shift was used as normalization method with the 75th percentile as a target and baseline transformation was applied to the median of all samples. Prior to data analysis, quality

control was performed and compromised probes removed from further analysis. “Normalized expression” will refer in the manuscript to Log2 transformed expression values.

Data analysis

Comparisons between cohorts were performed by Kruskal-Wallis Test. The data analyses were performed with the software R (versions 3.4.2 to 4.00). The general transcriptomic data preparation was performed with the packages limma, reshape2, and tidyr. The package ggplot2 was used for graphical data presentation. Therapy outcome estimations and the transformation of this factor to a numerical score for the development of the therapy outcome score were performed using the R packages caTools, tidyr, reshape2, randomForest, dplyr and pROC. The development of the therapy progress score was executed by using the R packages MASS, tidyr, reshape2, dplyr, glmnet and caTools. All available time points were included for the therapy progression score and the therapy end model regardless of therapy outcome status. The R packages car, caTools, tidyr, dplyr, glmnet, randomForest and pROC were used to develop the therapy end model. In general, the outcome “lost to follow-up” was not an exclusion criterion to calculate hypothetical end-of-therapy scores.

Data availability

Microarray data have been deposited at Gene Expression Omnibus database (GSE147690, GSE147689, GSE147691).

Therapy end model development

We conducted three steps to develop a unified model to calculate hypothetical end-of-therapy timepoints for DS-GVC patients (**Figure 1 and Online Supplement Figure 2**). This was then further validated in independent data sets from the MDR-GIC, MDR-GVC, and MDR-RVC. This process also involved the use of clinical and mycobacterial data to evaluate the model's

plausibility. Therapy durations calculated by the model were hypothetical and retrospective, but were compared to the durations that were conducted in clinical reality. The workflow that was followed to analyze transcriptome data is described in **Figure 1 and Online Supplement Figure 2**. Transcriptome and clinical data from DS- and MDR-GIC were analyzed to build a model to predict therapy outcomes and to assess therapy response. The resulting scores and a list of genes that characterized the end of successful therapy best in DS-GIC patients were integrated into a final model to determine individual end-of-therapy timepoints. This algorithm was then validated on transcriptome data of the independent GVCs and the RVC (See **Figure 1 and Online Supplement Figure 2**). This validation process also involved the validation of the model's results with clinical data such as times of culture and smear conversion (TCC, TSC), time of mycobacterial culture positivity (TTP+), the radiological extent of disease as well as presence of cavities in chest X-ray imaging.

Therapy outcome score (step 1)

A score correlating with therapy outcome at any given sampling timepoint was developed by identifying genes that were significantly up- or downregulated in therapy-naïve DS-GIC and MDR-GIC patients compared to healthy controls (HCs) in a moderated T-test with a fold-change cut-off of ≥ 2 and a Benjamini-Hochberg multiple testing correction of $p \leq 0.01$ (**Online Supplement Figure 2, Online Supplement Table 2, Online Supplement File**). Next, this gene list was further analyzed to identify genes with the potential to predict patient therapy outcomes according to TBNET definitions [1] (outcome groups: cure, failure, death; For outcome definitions see **Online Supplement Table 1**; for further details regarding the analysis see **Figure 1 and Online Supplement Figure 2**) by using hierarchical clustering and cross-validated Random Forest model fitting in DS-GIC and MDR-GIC patients with available final therapy

outcome data. The expression levels of six genes - CD274 (PDL1), FAM20A, GYG1, LPCAT2, HIST1H1B, and TRIM27 - were identified as an ideal model to distinguish between patients experiencing cure, failure or death following the TBNET outcome criteria [1]. This procedure included the numerical translation of the outcome cure and non-favorable outcomes such as failure and death (dependent variable), which were described by the independent variables (expression of the six genes as described above) in a Generalized Linear Model (GLM) to form the therapy outcome score (see **Online Supplement Table 2, Supplement Figure 2**).

Further validation of this finding was performed by using the patients' culture conversion status, the drug-resistance status, and the radiographic extent of disease at baseline to re-assure the plausibility of the therapy outcome score. Kruskal-Wallis test showed highly significant differences of the therapy outcome score between outcome groups at baseline in the GICs ($p < 0.001$). MDR- and DS-GIC patients with prolonged times of culture conversion (2-month culture conversion status) also showed higher baseline score values (median: 3.4, IQR: 1.6–5.0) when compared to those with early culture conversion (median: 1.8, IQR: 0.5–3.4, $p = 0.111$). Therapy naïve MDR- and DS-GIC patients experiencing cure had a median score of 0.86 (IQR: -0.37–2.3) while patients with therapy failure exhibited a median score of 1.3 (IQR: 0.34–4.61) and deceased patients a value of 5.83 (IQR: 5.08–10.04; **Online Supplement Figure 3**). The performance of this outcome score to predict therapy outcome in therapy naïve patients was 0.85 (95% CI 0.78–0.92).

Therapy progression score (Step 2)

To identify genes that correlate with the proximity (distance in days) to the clinical end-of-therapy for any given sample timepoint, we performed a pre-selection from the total transcriptomic data set of DS- and MDR-GICs by using penalizing regression model (Lasso;

Figure 1, Online Supplement Table 2, Supplement Figure 2). Using this list of genes (**Online Supplement File**), variable reduction steps were conducted by applying Bonferroni correction and stepwise Akaike information criterion (AIC; both directions with 1.000 steps) to focus on genes that highly correlated with the progress of therapy leading to a GLM-model consisting of nine genes (RPAP3, A_33_P3281041, BATF2, C2, GK3P, IFIT2, IFITM1, KREMEN1, PDE4D) and the patients' drug resistance status (DS-TB vs. MDR-TB, binary variable). The time under therapy (in days) is subtracted from the GLM result to calculate the therapy progression score for each patient at any given sample timepoint, which correlated with the days of remaining therapy that was conducted in reality ($r=0.59$ ($p<0.001$); see **Online Supplement Table 2, Online Supplement Figure 2**).

End-of-therapy list (Step 3)

To further characterize the correlations with successful end-of-therapy for TB patients on the transcriptional level, we identified genes that were differentially expressed in patients under anti-tuberculosis therapy when compared to patients at the end of successful therapy (cure; see **Figure 1, Online Supplement Table 2, Online Supplement Figure 2**) [1]. For this purpose, Lasso regression was performed throughout the cross-validated total gene expression data set of DS-GIC data to identify genes corresponding to sampling timepoints that would correspond to cure (**Figure 1, Online Supplement Table 2, Online Supplement Figure 2**). The genes identified by this process (**Online Supplement File**) went through a further variable reduction process (Bonferroni correction and stepwise AIC method in both directions with 1.000 steps) resulting in a list of nine relevant gene targets from the end-of-therapy list (BATF2, GBP5, IFITM1, IL27, KCNJ2-AS1, SERPING1, STAT1, TNFRSF21, VAMP5). Two of these targets were also part of the therapy progression score (BATF2 and IFITM1).

In summary, first, a set of six genes (CD274 [PDL-1], FAM20A, HIST1H1B, LPCAT2, GYG1, TRIM27) was identified with significant up- or downregulation in therapy-naïve DS-GIC and MDR-GIC patients compared to HC that were translated into the therapy outcome score. In the second step, a set of 9 gene targets (RPAP3, A_33_P3281041, BATF2, C2, GK, IFIT2, IFITM1, KREMEN1, PDE4D) was identified to determine genes that correlated with the remaining time of therapy in patients of the DS-GIC and MDR-GIC in longitudinal timepoints, which were implemented into the therapy progression score. The third step resulted in a list of target genes that characterized the transcriptional difference between patients under therapy and at the end-of-therapy, which included 9 genes (again the targets BATF2 and IFITM1, and additionally GBP5, IL27, KCNJ2-AS1, SERPING, STAT1, TNFRSF21, VAMP5; see **Figure 1, Online Supplement Table 2, Online Supplement Figure 2**). The numerical values from the treatment outcome score, the therapy progress score and the end-of therapy list were then integrated into the therapy end model, which, after further reduction of variables using cross-validation and Mean Decrease Gini Index, resulted in a Random Forest (RF) model to classify for relapse-free cure end-of-therapy timepoints (**Figure 1, Online Supplement Table 2, Online Supplement Figure 2**). 5000-fold RF iterations resulted in average probabilities for the ideal end-of-therapy timepoint in individual DS-GIC patients at any given sample timepoint and validated in MDR-GIC. The threshold value for the successful end of therapy was set at $P \geq 0.5$ (standard RF classification threshold). The model's results were then further validated and simplified by applying a Generalized Linear Model (GLM) to calculate the end-of-therapy score again with a cut-off of ≥ 0.5 . The GLM was developed using the same variables as for the RF model, which yielded the same probability results and then served as final therapy end model (**Online Supplement Table 2**).

Performance comparison with existing signatures

In order to further validate the discriminative performance of the model, published RNA signatures and scores were also assessed for their capability to identify end-of-therapy timepoints and compared with the model [2-14]. For this purpose, TB associated signatures or scores with less than 100 genes were identified. The gene sets or scores were trained in the training set of the DS-GICs (split ratio 0.7) using random forest algorithms to classify between for the probability of cure associated end-of-therapy timepoints. Duplicate genes in single signatures and unspecific sample names were not considered for the models. The resulting models were then applied to the independent DS-GVC data set to test for their discriminative performance in an external data set (**Table 2 and Figure 4**). In a subsequent step, the application of the models was extended to both DS and MDR-GVC data sets to test for plausibility (**Figure 5**).

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Online Supplement Tables

Online Supplement Table 1

Treatment outcome definitions in multidrug-resistant tuberculosis according to WHO 2014 [15] and TBNET simplified definitions 2016 [1]. n/a: not applicable.

	WHO Definition - revised 2014	Simplified definitions (TBNET)
Cured	Treatment completed as recommended by the national policy without evidence of failure AND three or more consecutive cultures taken at least 30 days apart are negative after the intensive phase	A negative culture status 6 months after treatment initiation, no positive cultures thereafter, and no relapses within 1 year after treatment completion
Treatment completed	Treatment completed as recommended by the national policy without evidence of failure BUT no record that three or more consecutive cultures taken at least 30 days apart are negative after the intensive phase.	n/a
Treatment failed	Treatment terminated or need for permanent regimen change of at least two anti-TB drugs because of: <ul style="list-style-type: none"> – lack of conversion by the end of the intensive phase, or – bacteriological reversion in the continuation phase after conversion to negative, or – evidence of additional acquired resistance to fluoroquinolones or second-line injectable drugs, or – adverse drug reactions (ADRs). 	A positive culture status 6 month after treatment initiation or thereafter, or a relapse within 1 year after treatment completion.
Died	A patient who dies for any reason during the course of treatment	A patient who dies for any reason during the course of observation

Lost to follow-up	A patient whose treatment was interrupted for 2 consecutive months or more	Non-receipt of care 6 months after treatment initiation.
Not evaluated	A patient for whom no treatment outcome is assigned. (This includes cases “transferred out” to another treatment unit and whose treatment outcome is unknown)	n/a
Undeclared	n/a	<ul style="list-style-type: none"> - no culture status at 6 months while the patient was receiving care, or - no post-treatment assessment
Treatment success	The sum of cured and treatment completed	n/a

Online Supplement Table 2

Model parameters of three steps leading to the end-of-therapy model for patients with tuberculosis. TOS= therapy outcome score, TPS = Therapy Progression Score, RF= Random Forest, DS= drug-susceptible, MDR= multidrug-resistant, TB= tuberculosis, GIC= German validation cohort, AUC= area under the curve, TE=Therapy End, GLM = Generalized linear model.

	Therapy outcome score (TOS)	Therapy progression score (TPS)	End-of-therapy (EOT) list	Therapy end model (TEM)
Type	RF for initial gene selection, GLM for score creation, logistic regression	GLM	Lasso	RF GLM
Outcome information	Therapy outcome prediction	Progression of therapy	Clinical therapy end timepoint reached	1. Binary classification for therapy end (yes/no. Probability (P) threshold for end-of therapy ≥ 0.5) 2. Numeric probability value for monitoring therapy response (0-1)
Variables	Dependent: Score Value Independent: CD274, FAM20A, LPCAT2, TRIM27, GYG1, HIST1H1B	Dependent: Remaining days of therapy at sampling timepoint Independent: RPAP3, A_33_P3281041, BATF2, C2, GK, IFIT2, IFITM1, KREMEN1, PDE4D, multidrug-resistant (yes/no) strain. Days since therapy start subtracted from calculated value.	Clinical therapy end timepoint reached Independent: 44.000 genes in transcriptomic data	Dependent: Clinical therapy end timepoint reached /probability of therapy end Independent: sub-model 1 and 2; BATF2, GBP5, IFITM1, IL27, KCNJ2-AS1, SERPING, STAT1, TNFRSF21, VAMP5
Model building data set	All GICs patients with available outcome data (cross validation, split ratio: 0.7:0.3) including DS-TB and MDR-TB patients	All GICs patients (cross validation, split ratio: 0.7:0.3) including DS-TB and MDR-TB patients	All patients from DS-GIC	All patients from DS-GIC (cross validation, split ratio: 0.7:0.3), first validation in MDR-GIC

Formula	$TOS = \alpha + \beta_1 * CD274 + \beta_2 * CD274 + \beta_3 * FAM20A + \beta_4 * GYG1 + \beta_5 * HIST1H1B + \beta_6 * LPCAT2 + \beta_7 * TRIM27 + \varepsilon_i TOS = \alpha + \beta_1 * CD274 + \beta_2 * CD274 + \beta_3 * FAM20A + \beta_4 * GYG1 + \beta_5 * HIST1H1B + \beta_6 * LPCAT2 + \beta_7 * TRIM27 + \varepsilon_i TOS = \alpha + \beta_1 * CD274 + \beta_2 * C274 + \beta_3 * FAM20A + \beta_4 * GYG1 + \beta_5 * HIST1H1B + \beta_6 * LPCAT2 + \beta_7 * TRIM27 + \varepsilon_i$	$TPS = (\alpha + \beta_1 * A_{33P3281041} + \beta_2 * \square ATF2 + \beta_3 * C2 + \beta_4 * GK3P + \beta_5 * IFIT2 + \beta_6 * IFITM1 + \beta_7 * KREMEN1 + \beta_8 * PDE4D + \beta_9 * RPAP3 + \beta_9 * Resistance (MDR = yes) + \varepsilon_i) - Days since therapy start TPS = (\alpha + \beta_1 * A_{33P3281041} + \beta_2 * BATF2 + \beta_3 * \square 2 + \beta_4 * GK3P + \beta_5 * IFIT2 + \beta_6 * IFITM1 + \beta_7 * KREMEN1 + \beta_8 * PDE4D + \beta_9 * RPAP3 + \beta_9 * Resistance (MDR = yes) + \varepsilon_i) - Days since therapy start TPS = (\alpha + \beta_1 * A_{33P3281041} + \beta_2 * BATF2 + \beta_3 * C2 + \beta_4 * GK3P + \beta_5 * IFIT2 + \beta_6 * IFITM1 + \beta_7 * KREMEN1 + \beta_8 * PDE4D + \beta_9 * RPAP3 + \beta_9 * Resistance (MDR = yes) + \varepsilon_i) - Days since therapy start$	$End - of - therpay (yes; no) = \min_{\alpha, \beta} \frac{1}{n} \sum_{i=1}^N f(xi; yi; \alpha, \beta) End - of - therpay (yes; no) = \min_{\alpha, \beta} \frac{1}{n} \sum_{i=1}^N f(xi; yi; \alpha, \beta) End - of - therpay (yes; no) = \min_{\alpha, \beta} \frac{1}{n} \sum_{i=1}^N f(xi; yi; \alpha, \beta)$	$P \left(TE \sum_j \text{var} \left(P\theta(h((BATF2, GBP5, IFITM1, IL27, KCNJ2AS1, SERPING, STAT1, TNFRSF21, VAMP5, TOS, TPS), \theta) = Y) - P\theta(h((BATF2, GBP5, IFITM1, IL27, KCNJ2AS1, SERPING, STAT1, TNFRSF21, VAMP5, TOS, TPS), \theta) = j) \right) \right) = \alpha + \beta_1 BATF2 + \beta_2 GBP5 * TOS + \beta_3 IFITM1 + \beta_4 IL27 + \beta_5 KCNJ2AS1 + \beta_6 SERPING + \beta_7 STAT1 + \beta_8 TNFRSF21 + \beta_9 VAMP5 + \beta_{10} TOS + \beta_{11} TPS + \beta_{12} TOS * TPS + \beta_{13} GBP5$ $P \left(TE \sum_j \text{var} \left(P\theta(h((BATF2, GBP5, IFITM1, IL27, KCNJ2AS1, SERPING, STAT1, TNFRSF21, VAMP5, TOS, TPS), \theta) = Y) - P\theta(h((BATF2, GBP5, IFITM1, IL27, KCNJ2AS1, SERPING, STAT1, TNFRSF21, VAMP5, TOS, TPS), \theta) = j) \right) \right) = \alpha + \beta_1 BATF2 + \beta_2 GBP5 * TOS + \beta_3 IFITM1 + \beta_4 IL27 + \beta_5 KCNJ2AS1 + \beta_6 SERPING + \beta_7 STAT1 + \beta_8 TNFRSF21 + \beta_9 VAMP5 + \beta_{10} TOS + \beta_{11} TPS + \beta_{12} TOS * TPS + \beta_{13} GBP5 P \left(TE \sum_j \text{var} \left(P\theta(h((BATF2, GBP5, IFITM1, IL27, KCNJ2AS1, SERPING, STAT1, TNFRSF21, VAMP5, TOS, TPS), \theta) = Y) - P\theta(h((BATF2, GBP5, IFITM1, IL27, KCNJ2AS1, SERPING, STAT1, TNFRSF21, VAMP5, TOS, TPS), \theta) = j) \right) \right) = \alpha + \beta_1 BATF2 + \beta_2 GBP5 * TOS + \beta_3 IFITM1 + \beta_4 IL27 + \beta_5 KCNJ2AS1 + \beta_6 SERPING + \beta_7 STAT1 + \beta_8 TNFRSF21 + \beta_9 VAMP5 + \beta_{10} TOS + \beta_{11} TPS + \beta_{12} TOS * TPS + \beta_{13} GBP5$
Model performance	Overall GLM model's p-value <0.001	Overall GLM model's p-Value: <0.001	64 out of 44.000 targets with $\beta > 0$	Out of bag estimate of error rate: 5.78%

parameter	<p>-AUC for TOS in therapy naïve patients for the ability to differentiate between cure and failure: 0.85 (95% CI: 0.78 – 0.92)</p> <p>Estimator: 0.35, $p > 0.001$</p> <p>-AUC for TOS in therapy naïve patients for the ability to differentiate between survivors and deceased: 0.96 (0.88 – 1)</p> <p>Beta-estimator: 0.80, $p = 0.008$</p> <p>-AUC for TOS in therapy naïve patients to predict smear conversion before and after 2 months: 0.59 (0.44 – 0.73)</p> <p>Beta-estimator: 0.17, $p = 0.011$</p> <p>-AUC for TOS in therapy naïve patients to predict culture conversion before and after 2 months: 0.70 (0.55 – 0.85)</p> <p>Beta-estimator: 0.12,</p>	<p>Coefficient of determination R^2: 0.53</p>		<p>Classification error specificity: 0.20</p> <p>Classification error sensitivity: 0.02</p> <p>Mean decrease in Gini:</p> <p>TPS: 23.4</p> <p>TNFRSF21: 4.74</p> <p>BATF2: 4.73</p> <p>IFITM1: 4.62</p> <p>IL27: 3.32</p> <p>GBP5: 3.17</p> <p>SERPING1: 2.81</p> <p>STAT1: 2.32</p> <p>KCNJ2-AS1: 2.20</p> <p>VAMP5: 2.1</p> <p>TOS: 1.89</p> <p>Overall GLM model's p-value: < 0.001</p> <p>Coefficient of determination R^2: 0.64</p> <p>Difference between RF-Probabilities ($\mu P = 0.244$) and GLM</p>
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	<p>p=0.057</p> <p>-AUC for TOS in therapy naïve patients to predict culture conversion before and after 6 months: 0.56 (0.36 – 0.77)</p> <p>Beta-estimator: 0.34, p>0.001</p> <p>-AUC for TOS to correlate with the presence of positive culture at sampling timepoint: 0.66 (0.53 – 0.80)</p> <p>Beta-estimator: 0.25, p>0.001</p>			Model ($\mu P=0.234$) not significant with p=0.686
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Online Supplement Figures

Online Supplement Figure 1

Cohorts of patients with drug-susceptible tuberculosis and multidrug-resistant tuberculosis

Flow chart showing recruitment for the model development of the five different cohorts. Patients with drug-susceptible (DS) and multidrug-resistant (MDR) tuberculosis (TB) were enrolled in the German Identification Cohorts (GICs), German Validation Cohorts (GVCs) and the Romanian Validation Cohort (RVC). Inclusion criteria were culture proven tuberculosis, sputum positivity at baseline, and age above 18 years at study inclusion. All patients were HIV negative. The blood sampling schedule for the different cohorts is depicted under the inclusion criteria. Therapy outcomes were assessed following the simplified TBNET definitions [1], which include a one year follow up after the end of therapy. Additionally, healthy controls (HC) were enrolled at the Research Center Borstel. The red dotted boxes indicate the use of the cohort's data for either model identification (left) or validation (right).

Online Supplement Figure 2

Development of therapy end model for tuberculosis treatment

Flow chart illustrating the therapy end model (TEM) development. A. Therapy outcome score (TOS). Identification of genes corresponding to therapy outcome (Cure/Failure/Death) according to TBNET criteria [1] by cross-validation in all drug susceptible (DS) and multidrug-resistant (MDR)-tuberculosis (TB) patients of the German Identification Cohorts (GICs). B. Therapy Progression Score (TPS). Identification of genes that correlate with the remaining therapy by cross-validation in DS- and MDR-tuberculosis patients of the GIC via Lasso regression. C. End-of-therapy (EOT) list. Identification of genes that are differently expressed previously to and at successful completion of therapy. The combination of TOS, TPS and the EOT list were included into the TEM in cross-validated DS-GIC patients. This model was then applied to independent data sets from DS-TB patients of the German validation cohort (GVC), and subsequently in patients from the MDR-GIC, the MDR-GVC, and the MDR-Romanian Validation Cohort (RVC).

Online Supplement Figure 3

Therapy outcome score values in therapy-naïve patients with tuberculosis

Boxplot graph showing the comparison of logarithmic therapy outcome score (TOS) values in therapy naïve tuberculosis patients, irrespectively of drug-resistance status, from the German identification cohorts (GIC) with regard to their therapy outcome (cure, failure, or death according to the TBNET-criteria) [1].

Online Supplement Figure 4

Therapy end model scores in patients with drug-susceptible tuberculosis and multidrug-resistant tuberculosis compared to healthy controls at relevant timepoints

Violin plots comparing calculated end-of-therapy scores of the therapy end model (TEM) for healthy controls, multidrug-resistant (MDR)- and drug-susceptible (DS) tuberculosis (TB) patients at relevant timepoints. Y-axis: TEM scores, dotted horizontal line: score threshold ($\text{Score (P)} \geq 0.5$) for relapse-free end-of-therapy; X-axis showing TEM scores at therapy start, after 14 days of therapy, at therapy end, and for healthy controls. **Supplement Figure 4A:** TEM scores for patients with DS tuberculosis at therapy start, after 14 days of therapy, at therapy end, and for healthy controls, **Supplement Figure 4B:** TEM scores for patients with MDR tuberculosis at therapy start, after 14 days of therapy, at therapy end, and for healthy controls.

Online Supplement File

List of genes of the different steps contributing to the therapy end model.

The first Excel file tab shows the preselection of genes contributing to the therapy outcome score (TOS), the second tab the ones contributing to the therapy progression score (TPS), and the third one shows the genes identifies for the end-of-therapy (EOT) list. The TOS tab lists the identified differentially expressed genes of healthy controls when compared to drug-susceptible (DS) and multidrug-resistant (MDR) tuberculosis patients of the German Identification Cohort (GIC) at therapy start. The TPS tab shows genes that correlate with the remaining days of therapy of DS- and MDR-GIC patients as basis for the therapy progression score. The EOT tab shows genes that are differentially expressed in all time points of drug-susceptible GIC patients under anti-tuberculosis therapy when compared to the end-of-therapy as basis end-of-therapy list.