Biomarkers for tuberculosis disease activity, cure, and relapse

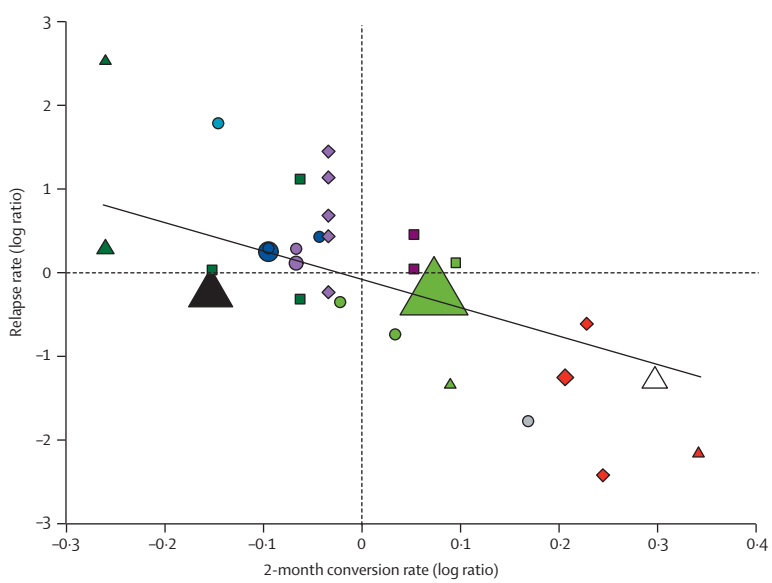
Our Review published in *The Lancet Infectious Diseases* last year[1] contained an analysis of the relation between rates of 2-month sputum-culture conversion and relapse in a small number of published trials on the treatment of tuberculosis. The findings supported a potential role for 2-month conversion in accelerating the approval process of new drugs for the treatment of tuberculosis. Assessment of that material during recent regulatory meetings revealed that one study should not have been included, because it contained an arm in which a new drug (thiacetazone) was added only in the continuation phase. We regret this error, as it called into question the validity of our conclusion because of the relatively small number of cited studies.

See Online for webappendix

	Estimate	SE	95% CI	*Z* value	p value
All data (30 pairs)*					
Intercept	−0·0870	0·1287	−0·3396 to 0·1655	−0·6760	0·4992
Slope	−3·3596	0·5516	−4·4420 to −2·2771	−6·0906	<0·00001
Pairs with identical continuation phases (16 pairs)†					
Intercept	0·0573	0·1867	−0·3091 to 0·4237	0·3068	0·7591
Slope	−3·8916	1·4141	−6·6666 to −1·1165	−2·7519	0·0060

*The variance estimate associated with study effect was 0·09847. †The variance estimate associated with study effect was 0·1341.

Table: **Meta-regression analyses of natural log relapse-rate ratio on natural log 2-month sputum conversion-rate ratio (experimental/control)**



Africa	Hong Kong	Singapore	India	Treatment modification
▲				Thiacetazone added
△				Pyrazinamide added
▲			◆	Rifampicin added
▲	●	■		Streptomycin added
▲		■		Intensive phase shortened
	●			Three times weekly
	●		◆	Twice weekly
	●			Pyrazinamide removed
	●			E changed to Z
		■		Streptomycin removed, intensive phase lengthened

Figure: **Relation between rates of relapse and 2-month sputum conversion in randomised controlled trials of treatment of tuberculosis**
Axes show natural log rate ratios (experimental/control). Dotted lines show equality (no effect). Solid line show the meta-regression estimates using all the data (table).

Our new analysis, which includes all randomised controlled trials in pulmonary tuberculosis done by the UK Medical Research Council from 1946–86, is intended to serve as a robust correction. All Medical Research Council trials into the treatment of tuberculosis reviewed by Fox and colleagues in 1999 were considered.[2] Studies were selected if the report included the rates of conversion of sputum culture at the end of the second month of treatment and relapse 18–24 months after completion of treatment, and the tested regimens differed either only in the first 2 months or consistently throughout treatment. Only data from patients with fully drug-sensitive isolates at baseline were included.

30 pairs of regimens were identified in trials of 5561 patients in four regions worldwide (webappendix). Meta-regression analyses were done with natural log relapse-rate ratio (experimental/control) as the response variable, and natural log 2-month sputum culture conversion-rate ratio (experimental/control) as the exploratory variable.[3] The model accounted for heterogeneity across studies by including study as a random effect. The model also incorporated dependences or covariances among the log relapse-rate ratios within study, where applicable. The residual covariance matrix was assumed fixed, whereas the random study variance was estimated by restricted maximum likelihood using the SAS MIXED procedure. Regression parameters were estimated via weighted least squares using the inverse of the sum of the residual and study covariance matrices as the weight matrix. When any of the arms within a study had zero relapse, 0·5 was added to the number of relapses and the number of non-relapses in each arm of that study to calculate the relapse rate and the log relapse-rate ratio.

The table shows the results of the meta-regression analyses. The slope of the natural log relapse rate ratio on the natural log 2-month sputum-culture conversion rate ratio using all the data was –3·3596 (SE 0·5516, 95% CI –4·4420 to –2·2771), which was statistically significant (p<0·00001). Adjusting for the effect of region yielded a similar statistically significant slope estimate (p<0·00001). The relation between the log ratios of relapse and conversion is illustrated in the figure, in which each symbol represents a pair of study arms, along with a straight line predicted from the meta-regression. The sizes of the symbols in the figure are proportional to the precision of the natural log relapse-rate ratios, which are the inverse of the variances and range from 0·38 to 27·23. Note that the regression line is primarily driven by data points that have greater precision (ie, smaller variance) as expected in the meta-regression. Meta-regression analyses restricted to 16 pairs of the data with identical continuation phases also yielded statistically significant slopes, both without region (table; p=0·006) and with region (p=0·0069) as an additional covariate.

These findings strongly support our original conclusion that 2-month culture conversion predicts relapse risk, and should be a surrogate endpoint for the registration of new drugs for the treatment of tuberculosis.

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We declare that we have no conflicts of interest.

1 Wallis RS, Doherty TM, Onyebujoh P et al. Biomarkers for tuberculosis disease activity, cure, and relapse. *Lancet Infect Dis* 2009; **9**: 162–72.
2 Fox W, Ellard GA, Mitchison DA. Studies on the treatment of tuberculosis undertaken by the British Medical Research Council tuberculosis units, 1946–1986, with relevant subsequent publications. *Int J Tuberc Lung Dis* 1999; **3:** S231–79.
3 Whitehead A. Meta-analysis of controlled clinical trials. Hoboken, NJ: John Wiley and Sons, 2002.

Biomarkers for tuberculosis disease activity, cure, and relapse

Robert S Wallis and colleagues present a timely and useful overview of the development of biomarkers and surrogate endpoints for the assessment of treatment success in tuberculosis.[1] They make the important distinction between the use of biomarkers in clinical trials evaluating new treatments and predicting the individual patient's response to treatment. However, the implications of these two connected but different applications of biomarkers are not fully discussed. Furthermore, the presentation of existing data on bacteriological biomarkers seems incomplete and potentially misleading.

As the investigators acknowledge, bacteriological biomarkers are likely to be the most directly relevant pharmacodynamic response during the treatment of tuberculosis. In our opinion, the key contribution of new biomarkers would be the accurate discrimination of the subpopulation of persisting organisms from which relapses arise,[2] and to track it in a clinically accessible specimen after culture conversion. The contribution of more indirect immunological biomarkers of the host during treatment remains speculative and incompletely studied. The high cost and lengthy timescale involved in the discovery and validation of improved biomarkers should be taken into account. Although the very continuation of clinical trials for the development of new drugs is under threat because of increasing regulation[3] and costs, the priority for the development of new biomarkers is necessarily uncertain. In the meantime, sputum bacteriology, whether semiquantitative or fully quantitative, has undergone quite extensive evaluation, and provides important lessons for the process that will need to be followed in the development of newer biomarkers.

We recently presented a comprehensive evaluation of clinical trials of short-course chemotherapy sponsored by the UK Medical Research Council during the 1970s and 1980s[4] using a modern meta-analytic framework for evaluating surrogate endpoints.[5] This comprises data on 6974 people participating in 15 trials with 37 treatment comparisons in both Africa and Asia. Sputum bacteriology was available at 1, 2, 3, and

4 months in all these trials but at none of these time points was it a reliable predictor of long-term outcome of treatment in the individual patient. Nevertheless, there was a stronger relation between treatment effect in the 2-month culture result and treatment effect on long-term outcome in Hong Kong trials (R^2 value of 0·86), and between treatment effect on the 3-month culture result and treatment effect on long-term outcome in trials in east Africa (R^2 value of 0·81). The trial-level R^2 values compare favourably with surrogate endpoints in common use in other specialties.

Although it is true that prediction of relapse by use of these methods is imperfect in individual patients, this property is neither logically nor practically required for efficient evaluation of new regimens in clinical trials.[6]

We believe that a more careful distinction between surrogacy in trials and individual patients is central to a clearer debate about the usefulness of improved biomarkers for these different purposes. In particular, it has implications for the design of evaluation studies. The evaluation of surrogate endpoints in trials needs to focus on quantity and diversity of the treatment comparisons included, whereas comparisons between individual patients need to maximise the number of participants across different study contexts.

We agree that to assemble cohorts similar in scope to the Medical Research Council dataset will need closer collaboration in terms of study design and data sharing between all those involved in drug development and the evaluation of surrogate endpoints in tuberculosis, including statisticians and methodologists. As yet no forum or framework for such an enterprise in tuberculosis exists, but this task is becoming all the more urgent while new clinical trial activity accelerates. Innovative approaches will surely be crucial to success, but it is also important that we think ahead clearly about how and for what they will be evaluated.

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1 Wallis RS, Doherty TM, Onyebujoh P, et al. Biomarkers for tuberculosis disease activity, cure, and relapse. *Lancet Infect Dis* 2009; **9:** 162–72.
2 Garton NJ, Waddell SJ, Sherratt AL, et al. Cytological and transcript analyses reveal fat and lazy persister-like bacilli in tuberculous sputum. *PLoS Med* 2008; **5:** e75.
3 Griffin GE, Brown M, Gilmore I, et al. UK research trials are on verge of extinction. *The Times* (London), Jan 14, 2009. http://www.timesonline.co.uk/tol/comment/letters/article5511067.ece (accessed Dec 15, 2009).
4 Phillips PJ, Fielding K. Surrogate markers for poor outcome to treatment for tuberculosis: results from extensive multi-trial analysis. *Int J Tuberc Lung Dis* 2008; **12:** S146–47.
5 Prentice RL. Discussion: surrogate endpoint definition and evaluation. In: Molenberghs G, Buyse ME, Burzykowski T, eds. The evaluation of surrogate endpoints. Berlin: Springer, 2005: 341–48.
6 Baker SG, Kramer BS. A perfect correlate does not a surrogate make. *BMC Med Res Methodol* 2003; **3:** 16.

Authors' reply

We thank Patrick Phillips and colleagues for their comments. As they suggest, the distinction between surrogacy as an endpoint for clinical trials and a predictor for the outcomes in individual patients is an important one. Federal regulations in the USA (subpart H of 21CFR314) permit accelerated approval of new drugs for serious or life-threatening illnesses on the basis of a surrogate endpoint that is "reasonably likely, based on epidemiologic, therapeutic, pathophysiologic, or other evidence, to predict clinical benefit". We strongly agree with Phillips and colleagues that 2-month culture conversion meets this standard, on the basis of the highly significant inverse relation between effects on rates of conversion and relapse that is evident in randomised trials of tuberculosis chemotherapy.[1] This mechanism could shorten the time needed for approval of new drugs for multidrug-resistant tuberculosis by many years.

At the same time, it is important to note the shortcomings of this biomarker. About half of relapses arise from patients whose sputum cultures appropriately convert to negative by 2 months.[2] Extrapolation from the rate at which slowly metabolising (so-called fat and lazy) bacilli are cleared from sputum suggests this population is eliminated from the lungs of people with the infection by the third month of standard treatment.[3] These observations raise the strong likelihood that a substantial proportion of relapses arise from a minority bacterial population not readily detected in sputum by standard culture. Indeed, a recent study to establish whether treatment could be shortened to 4 months in patients with tuberculosis with non-cavitary disease whose sputum cultures convert to negative by the second month was stopped prematurely because of excess relapses in the 4-month treatment group.[4]

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Supplementary webappendix

This webappendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Robert S Wallis, Cunshan Wang, T Mark Doherty, Phillip Onyebujoh, Mahnaz Vahedi, Hannu Laang, Ole Olesen, Shreemanta Parida, Alimuddin Zumla. Biomarkers for tuberculosis disease activity, cure, and relapse. *Lancet Infect Dis* 2010; **10:** 68–69.

Webappendix: Relation between rates of relapse and 2-month sputum culture conversion in published randomized controlled tuberculosis treatment trials

	Modification to regimen*		Subjects	Relapse rate	2-month conversion rate	Natural log relapse rate ratio**	Natural log 2-month conversion rate ratio
Studies conducted in Africa[1–8]							
6SH	–	–	112	29%	49%	–	–
6SHT	T added	All	104	22%	42%	-0.2561	-0.1542
6SHZ	Z added	All	153	8%	66%	-1.2928	0.2978
6SHR	R added	All	152	3%	69%	-2.1617	0.3423
6HR	–	–	164	7%	64%	–	–
6SHR	S added	All	171	2%	70%	-1.3411	0.0896
2HRZ/4H	–	–	100	40%	79%	–	–
2SHRZ/4H	S added	2 mo	105	30%	85%	-0.2719	0.0732
2SHRZ/4TH	–	–	75	13%	87%	–	–
1SHRZ/5TH	IP ↓	2 mo	79	18%	67%	0.2845	-0.2612
2SHRZ/6TH	–	–	81	0%	87%	–	–
1SHRZ/7TH	IP ↓	2 mo	58	7%	67%	2.5264	-0.2612
Studies conducted in Hong Kong[9–14]							
6SHZ	–	–	60	18%	77%	–	–
$6S_3H_3Z_3$	3x/wk	All	68	24%	70%	0.2495	-0.0953
$6S_2H_2Z_2$	2x/wk	All	39	21%	72%	0.1123	-0.0671
9SHZ	–	–	65	5%	77%	–	–
$9S_3H_3Z_3$	3x/wk	All	65	6%	70%	0.2877	-0.0953
$9S_2H_2Z_2$	2x/wk	All	49	6%	72%	0.2826	-0.0671
6HRZE	–	–	163	1%	94%	–	–
$6H_3R_3Z_3E_3$	3x/wk	All	160	2%	90%	0.4240	-0.0435
$6H_3S_3R_3Z_3E_3$	S added *	All	152	1%	88%	-0.3542	-0.0225
$6H_3S_3R_3E_3$	Z removed *	All	166	8%	76%	1.7837	-0.1466
$6H_3S_3R_3Z_3$	E->Z *	All	151	1%	90%	-1.7771	0.1691
$6H_3R_3Z_3$	–	–	199	6%	88%	–	–
$6S_3H_3R_3Z_3$	S added	All	208	3%	91%	-0.7374	0.0335
Studies conducted in Singapore[15,16]							
$2HRZ/4H_3R_3$	–	–	109	1%	90%	–	–
$2HSRZ/4H_3R_3$	S added	2 mo	97	1%	99%	0.1166	0.0953
$1HSRZ/5H_3R_3$	IP ↓ *	2 mo	94	1%	85%	0.0314	-0.1525
$2SHRZ/4H_3R_3$ (FDC)	–	–	46	7%	98%	–	–
$1SHRZ/4H_3R_3$ (FDC)	IP ↓	2 mo	42	5%	92%	-0.3145	-0.0632
$2HRZ/3H_3R_3$ (FDC)	S removed, IP ↑ *	2 mo	40	8%	97%	0.4543	0.0529
$2SHRZ/4H_3R_3$	–	–	47	0%	98%	–	–
$1SHRZ/4H_3R_3$	IP ↓	2 mo	46	2%	92%	1.1197	-0.0632
$2HRZ/3H_3R_3$	S removed, IP ↑ *	2 mo	44	2%	97%	0.0435	0.0529
Studies conducted in India[17–20]							
$2SHZ/5S_2H_2Z_2$	–	–	129	4%	72%	–	–
$2SHRZ/5S_2H_2Z_2$	R added	2 mo	132	0%	92%	-2.4207	0.2451
7SHZ	–	–	140	4%	74%	–	–
7SHRZ	R added	All	129	2%	93%	-0.6113	0.2285
$3SHZ/2S_2H_2Z_2$	–	–	199	13%	74%	–	–
$3SHRZ/2S_2H_2Z_2$	R added	2 mo	187	4%	91%	-1.2500	0.2068
$2S_3H_3R_3Z_3/4S_2H_2R_2$	–	–	111	2%	89%	–	–
$2S_2H_2R_2Z_2/4S_2H_2R_2$	2x/wk	2 mo	108	3%	86%	0.4329	-0.0343
$2S_3H_3R_3Z_3/4S_1H_1R_1$	–	–	111	5%	89%	–	–
$2S_2H_2R_2Z_2/4S_1H_1R_1$	2x/wk	2 mo	117	4%	86%	-0.2350	-0.0343
$2S_3H_3R_3Z_3/4H_2R_2$	–	–	101	3%	89%	–	–
$2S_2H_2R_2Z_2/4H_2R_2$	2x/wk	2 mo	102	6%	86%	0.6833	-0.0343
$2S_3H_3R_3Z_3/4H_1R_1$	–	–	116	2%	89%	–	–
$2S_2H_2R_2Z_2/4H_1R_1$	2x/wk	2 mo	109	7%	86%	1.4485	-0.0343
$2S_3H_3R_3Z_3/4S_2H_2$	–	–	151	3%	89%	–	–
$2S_2H_2R_2Z_2/4S_2H_2$	2x/wk	2 mo	155	10%	86%	1.1370	-0.0343

E=ethambutol; FDC=fixed dose combination; H=isoniazid; IP=intensive phase; R=rifampicin; S=streptomycin; T=thiacetazone; Z=pyrazinamide. Numbers indicate months of treatment except for subscripts, which indicate number of doses per week. * Each regimen is compared to the first listed regimen its cell except for marked with a single asterisk,

which are compared to regimen immediately above. ** For studies in which any of the arms had zero relapses, 0.5 was added to the number of both relapses and non-relapses in each arm of that study to permit calculation the relapse rate and the log relapse rate ratio.

References

1 East African-British Medical Research Councils. Results at 5 years of a controlled comparison of a 6-month and a standard 18-month regimen of chemotherapy for pulmonary tuberculosis. *Am Rev Respir Dis* 1977; **116**: 3-8.

2 East African-British Medical Research Councils. Controlled clinical trial fo four short-course (6-month) regimens of chemotherapy for treatment of pulmonary TB. Third report. *Lancet* 1974; **2**: 237-240.

3 East African-British Medical Research Councils. Controlled clinical trial of four short-course (6-month) regimens of chemotherapy for treatment of pulmonary tuberculosis. Second report. *Lancet* 1973; **1**: 1331-1338.

4 East African-British Medical Research Councils. Controlled clinical trial of short-course (6-month) regimens of chemotherapy for treatment of pulmonary tuberculosis. *Lancet* 1972; **1**: 1079-1085.

5 East African-British Medical Research Councils. Controlled clinical trial of four 6-month regimens of chemotherapy for pulmonary tuberculosis. Second report. *Am Rev Respir Dis* 1976; **114**: 471-475.

6 Controlled clinical trial of four short-course (6-month) regimens of chemotherapy for treatment of pulmonary tuberculosis. *Lancet* 1974; **2**: 1100-1106.

7 Controlled clinical trial of five short-course (4-month) chemotherapy regimens in pulmonary tuberculosis. First report of 4th study. East African and British Medical Research Councils. *Lancet* 1978; **2**: 334-338.

8 East African-British Medical Research Councils. Controlled clinical trial of four short-course regimens of chemotherapy for two durations in the treatment of pulmonary tuberculosis. Second report. *Tubercle* 1980; **61**: 59-69.

9 Controlled trial of 6- and 9-month regimens of daily and intermittent streptomycin plus isoniazid plus pyrazinamide for pulmonary tuberculosis in Hong Kong. *Tubercle* 1975; **56**: 81-96.

10 Hong Kong Chest Service/British MRC. Controlled trial of 6-month and 9-month regimens of daily and intermittent streptomycin plus isoniazid plus pyrazinamide for pulmonary tuberculosis in Hong Kong. The results up to 30 months. *Am Rev Respir Dis* 1977; **115**: 727-735.

11 Five-year follow-up of a controlled trial of five 6-month regimens of chemotherapy for pulmonary tuberculosis. Hong Kong Chest Service/British Medical Research Council. *Am Rev Respir Dis* 1987; **136**: 1339-1342.

12 Controlled trial of 4 three-times-weekly regimens and a daily regimen all given for 6 months for pulmonary tuberculosis. Second report: the results up to 24 months. Hong Kong Chest Service/British Medical Research Council. *Tubercle* 1982; **63**: 89-98.

13 Controlled trial of four thrice-weekly regimens and a daily regimen all given for 6 months for pulmonary tuberculosis. *Lancet* 1981; **1**: 171-174.

14 Hong Kong Chest Service/British Medical Research Council. Controlled trial of 2, 4, and 6 months of pyrazinamide in 6-month, three-times-weekly regimens for smear-positive pulmonary tuberculosis, including an assessment of a combined preparation of isoniazid, rifampin, and pyrazinamide. Results at 30 months. *Am Rev Respir Dis* 1991; **143**: 700-706.

15 Singapore Tuberculosis Service/British Medical Research Council. Clinical trial of three 6-month regimens of chemotherapy given intermittently in the continuation phase in the treatment of pulmonary tuberculosis. *Am Rev Respir Dis* 1985; **132**: 374-378.

16 Five-year follow-up of a clinical trial of three 6-month regimens of chemotherapy given intermittently in the continuation phase in the treatment of pulmonary tuberculosis. Singapore Tuberculosis Service/British Medical Research Council. *Am Rev Respir Dis* 1988; **137**: 1147-1150.

17 Tripathy SP, Ramakrishnan CV, Nazareth O et al. Study of chemotherapy regimens of 5 and 7 months' duration and the role of corticosteroids in the treatment of sputum-positive patients with pulmonary tuberculosis in South India. *Tubercle* 1983; **64**: 73-91.

18 Santha T, Nazareth O, Krishnamurthy MS et al. Treatment of pulmonary tuberculosis with short course chemotherapy in south India--5-year follow up. *Tubercle* 1989; **70**: 229-234.

19 A controlled clinical trial of 3- and 5-month regimens in the treatment of sputum-positive pulmonary tuberculosis in South India. Tuberculosis Research Centre, Madras, and National Tuberculosis Institute, Bangalore. *Am Rev Respir Dis* 1986; **134**: 27-33.

20 Balasubramanian R. Fully intermittent six month regimens for pulmonary tuberculosis in south India. *Indian J Tuberc* 1991; **38**: 51-53.