Despite our inability to validate our hypotheses, it is clear that CDWPs are epidemiologically ineffective although they remove bacteria in laboratory trials. In view of the aggressive marketing of CDWPs and the increasing willingness of people to buy them, our findings warrant further testing.

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Manic-depressive illness and tyrosine hydroxylase gene

SIR—Conflicting results have been obtained about the existence of a susceptibility locus for manic-depressive illness at the tyrosine hydroxylase (TH) gene, the rate-limiting enzyme for catecholamine synthesis. In French samples, positive associations between a tetranucleotide repeat polymorphism in the first intron of the TH gene and bipolar affective disorder were found.[1,2] However, Todd and colleagues (June 8, p 1634)[3] reported negative results in North American samples, using both case-control and haplotype relative-risk designs to avoid possible bias due to population stratification. Although their findings were in line with most previous reports, including our earlier report,[4] their results should be interpreted with caution because these studies used polymorphisms which may not be associated with the structure of the enzyme or its expression.

We have done an allelic association study between bipolar disorder and a polymorphism that results in an aminoacid change (Val81Met) located in the second exon of the TH gene.[5] We genotyped a sample of 95 Japanese patients (45 male) with bipolar disorder (DSM-III-R criteria) and 161 controls (80 male). All were unrelated and from the same geographical area. With informed consent, venous blood was drawn from each person, and genomic DNA was isolated. PCR amplification was done with primers 5'-GGAGAAGGAGGGGATGGCC-3' and 5'-ACCAGCT-CACCTCAAACACCT-3'. The PCR product was subsequently digested by the restriction enzyme *Bal*I which cleaves the 81Met allele but not the 81Val allele. The digested fragments were visualised by 10% polyacrylamide gel electrophoresis with ethidium bromide staining. The genotype distributions for both the patients and the controls were in Hardy-Weinberg equilibrium. Genotype and allele frequencies were similar in the two groups (table).

These results are congruent with those of Todd and colleagues, and several other studies, including our own, which reported no association between manic-depressive illness and variations which do not cause a change in the aminoacid sequence of the TH protein. The positive association observed in the French samples[1,2] should be re-evaluated, by examining polymorphisms that directly influence the function of the enzyme.

	Genotype frequency (%)				Allele frequency (%)		
	n	Met/Met	Met/Val	Val/Val	n	Met	Val
Patients	95	46·3	42·1	11·6	190	67·4	32·6
Controls	161	48·4	42·9	8·7	322	69·9	30·1

Table: **Genotype and allele frequencies among patients and controls**

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Riluzole and amyotrophic lateral sclerosis

SIR—Several points in Lacomblez and colleagues' report (May 25, p 1425)[1] require clarification before concluding that riluzole has a biological effect on survival in amyotrophic lateral sclerosis (ALS) and that its routine clinical use is warranted.

First, the intention-to-treat analysis assumes that the dropouts (table), roughly 25% in all groups, did not produce an imbalance—ie, the dropouts were equally distributed for accepted risk factors for survival at the time of their withdrawal as well as in the time of their withdrawal from the protocol. It would be safe to conclude that a particular drug effect has been shown if the proportion of dropouts is small and the statistical significance is large. However, if the proportion of dropouts is as large as a quarter of the starting population and the statistical significance is borderline ($p=0·048$ at 18 months, all doses *vs* placebo), then explanatory checks are essential before such a conclusion is drawn. A per-protocol analysis "confines analysis to patients who received therapy, ie, analysis of compliers only".[2] Lacomblez et al have done a per-protocol check "on evaluable patients defined before unblinding", but did their analysis exclude only the nine losses to follow-up and 35 protocol violations or did it exclude all 240 patients who did not comply with their treatment (table)? What were the unadjusted results of their per-protocol analysis as well as those excluding these 240 patients, and did this large number of dropouts produce an imbalance in the two groups?

Second, results adjusted with mathematical modelling need to be viewed with caution. Empirical validation of the prognostic variables in independent samples is important. Lacomblez and co-workers state that these variables and their relatives risks were consistent with those in their

	Placebo (242)†	Riluzole			Totals (959)
		50 mg (237)	100 mg (236)	200 mg (244)	
Protocol violation	7	10	9	9	35
Adverse events					
Clinical	19	20	25	27	91
Laboratory	8	3	9	9	29
Lack of efficacy/consent withdrawal	23	21	17	14	75
Unknown	0	4	3	3	10
Total number (% of entry)	57 (23·5%)	58 (24·4%)	63 (26·6%)	62 (25·4%)	240 (25%)

*Adapted from Lacomblez et al, 1996.[1]

Table: **Riluzole in ALS-dropouts***

previous study.[3] However, only four of the ten were given as significant in the 1994 study (age, vital capacity, visual analogue stiffness, and visual analogue tiredness). Which variables were introduced at the beginning of the stepwise procedure and why?[4] Was the assumption of a multiplicative rather than, for example, an additive effect on the hazard function tested for each prognostic variable? Were residuals checked? Were plotting procedures done to check validity? The Cox model they use also assumes that the hazard of a variable does not change with time, an assumption not applicable, for example, to vital capacity in ALS; how was this aspect taken care of in the modelling?

Third, Lacomblez argues that p values were less impressive at 18 than at 12 months because of recruitment differences, with more severely affected patients included at the beginning of the trial. If this were true more deaths would have occurred in the initial months, which did not happen. Other possible reasons for the difference in p values include absence of a drug effect on survival and reduced statistical power as the number of censored data increases.

Last, only the relative risk of surviving 12 or 18 months but not the magnitude of the effect on survival is shown. An increase in median survival of 3 months has been recorded,[3] without improvement in quality of life. Is this correct?

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Authors' reply

SIR—Most of Guiloff and colleague's criticisms relate to the absence of the results of secondary exploratory analyses. Intention-to-treat analysis (ITT) deals with the difficulty these workers raise—namely, that there may be a non-random distribution of patients excluded from the analysis. Excluding such patients could seriously bias the analysis. Omitting patients who stop treatment for a treatment-related adverse event could hide a toxic effect of the drug. Furthermore, many patients stop treatment shortly before death, which could apply to almost any drugs used in ALS, and there are no grounds for excluding such events from any type of analysis. We reported treatment withdrawals in the adverse events table as a way of indicating the severity of some of these adverse events. We defined, a priori, and before unblinding, a treatment dropout as being a documented minimum of 1 month without treatment. Patients meeting this criterion were censored at the time of treatment withdrawal (147 [15·3%] patients; 30, 39, 41, 37 for placebo and 50, 100, 200 mg riluzole dose groups, respectively). Results of this on-treatment analysis (table) shows that they are consistent with those of the ITT analysis. Our report makes clear that the per-protocol analysis did not exclude patients who were lost to follow-up. Patients should be counted for the time of their participation in the study, provided that they represent only a small number of the total population, as in our study (<1%), and are not subjected to systematic bias. Thus the per-protocol analysis was a priori defined as that excluding patients with protocol violations. As shown in the table, the results of these analyses are entirely consistent with that for ITT.

	Intention-to-treat (n=959)		On-treatment (n=959)		Per-protocol (n=924)	
	Relative risk (95% CI)	p	Relative risk (95% CI)	p	Relative risk (95% CI)	p
Unadjusted	0·81 (0·66–0·99)	0·048	0·79 (0·63–0·99)	0·041	0·82 (0·66–1·01)	0·061
Adjusted	0·67 (0·54–0·83)	0·0003	0·67 (0·53–0·85)	0·0008	0·69 (0·56–0·86)	0·001

Table: **18 months survival analyses—all dose pooled *vs* placebo**

In our original study, there were in fact five significant prognostic variables, the fifth being the presence or absence of bulbar signs at entry. Of the five additional prognostic factors entered in the second study, two could not be assessed in the first study by the nature of its design, and the three others (muscle testing, weight, and clinical global impression) are all relevant to ALS. We doubt that the clinical significance of the prognostic factors is contentious, but the statistical modelling might be. Empirical rules to evaluate the validity and stability of a Cox model relate the number of variables (V) in the model to a limit in the number of events (E) in the study such as $V^2 \leqslant E$. The number of events (431) was more than four times the limit (100). Our prognostic score summarising the results of the Cox model clearly illustrates the danger of failing to take these prognostic factors into account. Vital capacity was only tested twice yearly and several patients were not assessable because of disease progression.

Guiloff and colleagues misunderstand our interpretation of the slight difference in relative risk estimates at 12 versus 18 months in the unadjusted analysis. In survival studies, effects of covariates are assumed to be constant over the trial period, provided that accrual (hence censoring) is random with regard to prognostic factors. Until month 14, there was virtually no censoring, but after this time the level of censoring rose sharply (to 34%) at 18 months. As shown in figure 2 of our report, the level of censoring in the low-risk group is nearly twice as high as that in the high-risk group. After adjusting for the prognostic score at entry, relative risks for the treatment effect were constant over time.

We did not conclude that riluzole increases the median survival for 3 months without improvement in quality of life. In neither study was quality of life evaluated. Nor have we used the median, because when the proportion of patients surviving is close to 50%, the median survival time is not a valid measure of the magnitude of the treatment effect, because of its instability.

In assessing the clinical usefulness of any treatment, it is preferable to consider the absolute decrease in event rate that it provides,[1,2] and the inverse number of patients it is necessary to treat before a clinical benefit is observed. At 12 months (no censoring in either trial), the absolute decrease in death or tracheostomy ranges between 16% (first study) and 11% (second study). This means that six to nine patients have to be treated with riluzole to avoid one death or tracheostomy yearly. This estimate compares very favourably with treatments that are regarded as clinically relevant for other conditions.

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