	Total number of patients	Number positive for *BAT26* mutations in faecal DNA	Number negative for *BAT26* mutations in faecal DNA
No neoplasia	69	0	69
With adenoma	19	0	19
<1 cm	14	0	14
⩾1 cm	5	0	5
With cancer	46	17	29
Dukes' A	5	1	4
Dukes' B	22	11	11
Dukes' C	11	4	7
Dukes' D	8	1	7

Results of analysis of faecal DNA for *BAT26* alterations

Of 134 faecal DNA samples analysed, 17 were found to have *BAT26* alterations. Examples of the results from this assay are shown in the figure. All 17 faecal DNA samples yielding a positive *BAT26* test were subsequently found to have been derived from patients with colorectal cancer (table).

Among the cancer patients with proximal lesions, the clinical sensitivity of the *BAT26* faecal DNA test was 37% (17 of 46 [95% CI 23–52]), with no positives among 69 individuals with normal colonoscopies or among 19 individuals with adenomas. The specificity was therefore 100% (95% CI 95–100). None of the patients in our cohort had variant *BAT26* alleles in their germ line.[4]

To determine the concordance of *BAT26* alterations between faecal DNA and tumours, we microdissected neoplastic lesions from paraffin-embedded specimens of all 65 tumours (46 cancers plus 19 adenomas). DNA of adequate quality was recovered from 57 lesions, and 18 cases with *BAT26* alterations were seen, all among cancers. 17 of these 18 cases corresponded to those with positive faecal tests, and in each of these cases, the size of the *BAT26* alteration in tumour and faecal DNA was identical (figure).

The results recorded above have several important implications for faecal DNA testing. First, they provide compelling evidence that mutations in faeces can be used to identify patients with cancer. The fact that 17 of the 18 cases with *BAT26* mutations in their tumours gave rise to a positive faecal DNA test, coupled with the zero false-positive rate, was of particular note. Second, the results show that proximal cancers do not represent a barrier to faecal DNA analysis. Third, small samples of stool, rather than whole stools, could be analysed effectively, facilitating collection and storage of specimens for analysis. Finally, the proportion of mutant DNA molecules in faecal DNA ranged from 1·1% to 10·6%. Thus, techniques to assess faecal DNA mutations need be no more sensitive than this to detect most mutations. In the one sample that was a false negative, increasing the potential sensitivity five-fold by analysing an additional 2000 *BAT26* genes in faecal DNA did not result in detection of the mutation.

One practical application of these findings involves combination of *BAT26* with sigmoidoscopy. Cost-effectiveness modelling has indicated that sigmoidoscopy combined with unhydrated faecal occult blood tests can be more effective than colonoscopy for colorectal cancer screening.[1] The sensitivity of the *BAT26* assay is similar to that of the unrehydrated faecal occult blood tests but is more expensive. This cost disadvantage is counterbalanced by the fact that the *BAT26* test seems to be substantially more specific, thereby precluding the need for follow-up colonoscopies in many patients with false-positive faecal occult blood tests. Furthermore, the *BAT26* test does not require patients to change their dietary habits before testing, nor to provide several faecal samples, potentially increasing compliance. Prospective studies to validate the sensitivity and specificity in a screening context, and to compare efficacy and cost-effectiveness with other screening strategies, are justified by the results reported above.

Contributors

Giovanni Traverso, Kenneth W Kinzler, and Bert Vogelstein directed the molecular aspects of the paper, developed the digital BAT26 technology, and wrote the first draft of the paper. Giovanni Traverso also did the Digital BAT26 assays. Louise Olsson, Bernard Levin, and Constance Johnson directed the clinical aspects of the study, including the selection of patients and the collection of clinical samples. They also helped interpret the data and formulate the final paper. Anthony Shuber and Kevin Boynton purified the DNA from the faecal samples using hybrid capture, and participated in the interpretation of the data and the formulation of the final paper. Stanley R Hamilton assessed the histopathology of the studied tumors and helped formulate the final paper.

Conflict of interest statement

Under agreements between the Johns Hopkins University and Exact Sciences, Genzyme Molecular Oncology and Hoffmann-LaRoche, Kenneth W Kinzler and Bert Vogelstein are entitled to a share of the royalties received by the university on sales of products related to the use of stool DNA for cancer diagnosis. Kenneth Kinzler is a consultant to Genzyme and to Exact Sciences, and Bert Vogelstein has in the past consulted for Genzyme and Exact Sciences. Kenneth Kinzler and Bert Vogelstein also own stock in Exact Sciences, and the university, along with Kenneth Kinzler and Bert Vogelstein own stock in Genzyme, which are subject to certain restrictions under university policy. The terms of these arrangements are being managed by the university in accordance with its conflict of interest policies. Anthony Shuber and Kevin Boynton are employees of Exact Sciences and are stockholders in the company.

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Mammographic screening: no reliable supporting evidence?

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Much confusion is being generated by the conclusion of a recent review that "there is no reliable evidence that screening for breast cancer reduces mortality." In that review, however, there was no appreciation of the appropriate mortality-related measure of screening's usefulness; and correspondingly, there was no estimation of the magnitude of this measure. We take this measure to be the proportional reduction in case-fatality rate, and studied its magnitude on the basis of the only valid and otherwise suitable trial. We found reliable evidence of fatality reduction, apparently substantial in magnitude.

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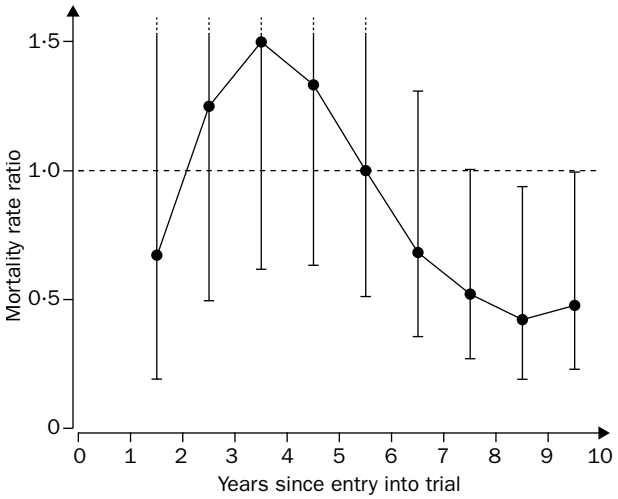
Cancer is malignant in the sense that its natural course is fatal, meaning that its case–fatality rate in the absence of curative treatment would be 100% if there were no role for other causes of death. Given the opportunity, it would kill every person with the disease. With screening, the idea is to achieve early diagnosis and, thereby, early treatment, which is presumed to be curative in more cases than later treatment. The idea, therefore, is to reduce the case–fatality rate. The authors of the Malmö study[1]—one of two accepted as valid by Olsen and Gøtzsche in their recent review[2]—refer to substantial reduction in breast-cancer mortality after a 6-year delay. They also mention that such a delay in the mortality gain is to be expected in randomised controlled trials that compare screening with no screening, since the reduced case–fatality rate presumed to be a consequence of screening tends to result in fewer deaths from the cancer only after a suitable delay. Analysis should therefore focus on deaths in the appropriate segment of follow-up—ie, not too early on study entry and not too late—after discontinuation of screening. Number of deaths divided by population-time in the appropriate time interval is the proper meaning of mortality (mortality rate) in this context.

Olsen and Gøtzsche did not address the case-fatality benefit of screening-associated early intervention, which, if it exists, becomes apparent only after a delay of several years. As a result, they concluded that "there is no reliable evidence that screening for breast cancer reduces mortality".[2] We set out to examine the results of the Malmö study more closely, allowing for the requisite delay. This analysis was possible because two requirements were met: the yearly numbers of deaths from breast cancer as of the time of study entry were reported for a sufficient number of years, and the screening was not discontinued prematurely.

The figure shows, for successive years after entry into the Malmö study, the corresponding mortality rate ratios for women 55 years of age or older at study entry. During the first 5 years after study entry, the rates in the screened cohort exceeded those in the control cohort; identity was reached in the sixth year; and from the seventh year onward, the rates of death from breast cancer in the screened cohort were lower than in the control cohort. On the basis of years 8–11, year 11 being the last one with information available, the point estimate for the rate ratio is 0·45 (95% CI 0·24–0·84).

The abstract of the Malmö study report shows the total numbers of breast-cancer deaths during 10 years of screening and documentation after entry into the study. It gives overall numbers (63 in the screening group *vs* 66 in the control group) and numbers stratified according to age (at least 55 years or less than 55 years) at entry into the study. An allusion is made to the temporal pattern of cause-specific mortality, but with no indication that focus on this pattern is essential to any genuine understanding of the usefulness of the screening regimen under study. Olsen and Gøtzsche refer only to the overall result (63 *vs* 66) and its associated "relative risk" and 95% CI (0·96 [0·68–1·35]), supplementing this information with the corresponding even more inclusive all-cause mortality ratio (0·98 [0·93–1·04]). Moreover, since they did not examine the studies for characteristics other than "methodological quality", they pooled the overall result from Malmö with that of a Canadian study,[3,4] despite very different regimens and durations of screening and follow-up.



Breast-cancer mortality ratio for women at least 55 years of age in the Malmö study
Shown are point estimates and 95% CI, based on the deaths in the year at issue together with those in the preceding and following years.

Screening in the Canadian study continued for only 3–4 years after study entry, and follow-up stopped at the point at which follow-up in the Malmö study started to show fewer breast-cancer deaths among those screened. In Malmö, the screening continued throughout the 10–11 years of follow-up. When the duration of screening in a trial that compares screening with no screening (rather than early intervention with late intervention) is too short, nowhere during follow-up does the mortality ratio decline all the way to the case–fatality ratio (which characterises early intervention relative to late intervention). For the fatality ratio to become fully apparent, in the appropriate interval of follow-up, the duration of screening must exceed the difference between the maximum and the minimum of the time lag from screening-associated early diagnosis to the death in the prevention of which early intervention is essential.

The delay principle addressed above is not in dispute. In its spirit, then, and also accepting Olsen and Gøtzsche's conclusion[2] that valid evidence derives mainly from the Malmö trial, we call attention to our figure. Screening in older women seems to have provided for a 100%–45%=55% reduction in case–fatality rate and thereby, after the requisite delay, in cause-specific mortality.

Contributors
O S Miettinen, C I Henschke, and D F Yankelevitz initiated the study, O S Miettinen did the analysis, and all authors participated in the writing and editing of the paper.

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None declared.

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The complete version of this paper can be found at http://image.thelancet.com/extras/1093web.pdf

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