Are gene signatures better than traditional clinical factors?

In a recent Article in *The Lancet Oncology*, Bueno-de-Mesquita and colleagues assessed concordance between the 70-gene prognostic signature and commonly used clinicopathological indices in 427 patients with lymph-node-negative breast cancer from 16 hospitals in the Netherlands.[1] Overall, discordance between the 70-gene prognostic signature and any of the assessed clinical risk indices was noted in about one-third of patients. Although the Nottingham Prognostic Index (NPI) scored best of the indices studied, the 73% concordance rate with the 70-gene prognostic signature clearly shows that there is a need for improvement.

We disagree with the researchers' opinion that their findings relate to all node-negative breast cancers, and explain here why the data from the study by Bueno-de-Mesquita and coworkers might only relate to the subgroup of moderately differentiated (grade 2) tumours. On the basis of the NPI definition, all grade 1 tumours in this study were in the low NPI risk group and all grade 3 lesions were in the moderate or high NPI risk group[1]. Concordance between NPI and the 70-gene prognostic signature in grade 1 and grade 3 lesions was high (192 of 223 [86·1%] patients; kappa=0·7085 [95% CI 0·6135–0·8035]). By use of the NPI guidelines, only 15 of 223 (6·7%) patients would have been undertreated and only 16 of 223 (7·2%) patients would have been overtreated. The remaining 204 patients had grade 2 lesions and were distributed between low risk (n=161; pT1, grade 2 lesions) and moderate or high risk (n=43; pT2, grade 2 lesions) groups on the basis of tumour size. In this way, we were able to assess concordance between findings for the NPI risk index and the 70-gene prognostic signature for grade 2 lesions separately from those for grade 1 and grade 3 lesions (table). Our findings suggest that grade 2 lesions rather than grade 1 or 3 lesions are responsible for the poor concordance between NPI and the 70-gene prognostic signature that was noted in the study by Bueno-de-Mesquita and colleagues for the entire group because only 103 of 161 (64·0%) patients with a pT1 lesion and 28 of 43 (65·1%) patients with a pT2 lesion had a good prognosis according to findings with the 70-gene signature. About one in five of patients with grade 2 breast cancers (28 of 131) that had a good prognosis according to the 70-gene signature were classified as high risk by the NPI guidelines. By contrast, about 80% (58 of 73) of grade 2 breast cancers with a poor prognosis according to the 70-gene signature were classified as low risk by the NPI guidelines, which might indeed lead to substantial undertreatment. The kappa statistic for discordant findings confirms this observation (kappa=–0·0091 [–0·1370–0·1188]). We conclude that findings by use of the NPI guidelines compare fairly well with those from use of the 70-gene signature for grade 1 or grade 3 tumours, but not for grade 2 lesions. Furthermore, tumour size, at least on its own, does not differentiate between a good and poor prognostic 70-gene signature in patients with early node-negative breast cancer of an intermediate tumour grade.

Histological grade 2 tumours have a heterogeneous gene-expression profile that differs to that seen in grade 1 and grade 3 tumours.[2] Other researchers have suggested that gene-expression profiles specific for histological tumour grades can be used as a substitute for, or to complement, histological grade alone that is currently used in the NPI.[2] This is of particular importance given that pathologists and institutions often differ in their assessment of histological tumour grading of breast cancers.[3]

The prognostic value of NPI in lymph-node-negative breast cancer might be further improved when other markers—such as vascular-space involvement—are combined into the index.[4] Researchers have also shown that panels of proteins assessed by immunohistochemistry—such as Bcl-2, vascular endothelial growth factor, and Ki-67—might be useful to improve the accuracy of the NPI.[5-7] Additionally, early relapse in patients with breast cancer depends on increased expression and amplification of steroid-receptor and ERBB2.[8,9] In our own database at the

	70-gene prognosis signature (n=427)		Discordant findings, n (%), 95% CI, kappa
	Good (n=219)	Poor (n=208)	
Clinical risk (NPI guidelines) grade 1 and grade 3 lesions (n=223)			
Low (n=87)	72	15	31 (13·9), 10·0–19·1, 0·7085
Moderate or high (n=136)	16	120	..
Clinical risk (NPI guidelines) grade 2 lesions (n=204)			
Low (n=161)	103	58	86 (42·2), 35·6–49·0, 0·0091
Moderate or high (n=43)	28	15	..

Table: Grade 1 and 3 and grade 2 breast cancers assessed by NPI and the 70-gene signature

University Hospital of Leuven (Leuven, Belgium), where new patients with breast cancer have been registered since January, 2000, 30 of 573 women with grade 2 node-negative breast cancer had a relapsed after a mean follow-up of 3·5 years (median 3·47 [range 0·06–6·64]; data not shown). In these grade 2 breast cancers, an early event (relapse) was three times more likely in those patients with a ERBB2-amplified tumour than in those patients with an oestrogen-receptor (ER)-positive, progesterone-receptor (PR)-positive, and ERBB2-negative lesion (4 of 27 [14·8%] patients *vs* 26 of 546 [4·8%] patients; p=0·055 [χ^2]). However, the number of early events in each group were too small to reliably use the log-rank assess to further test the time to events. Although ERBB2 overexpression as an early prognostic marker might be affected by adjuvant treatment with trastuzumab, interestingly, nine of 48 (18·8%) ERBB2-amplified breast cancers in the Bueno-de-Mesquita study were classified by the 70-gene signature in the good-prognosis group.

Therefore, we urge the Dutch researchers not only to centrally review data for all 204 patients with grade 2 lesions for consistency in tumour grading, because some individuals might be reclassified on the basis of conventional assessments, but also to combine traditional clinical factors with immunohistochemical expression of ER, PR, and ERBB2, and other surrogate markers of proliferation, such as Ki-67. This will optimise the value of the traditional clinical prognostic markers.[10] For now, whether microarray gene-expression profiling for breast-cancer prognosis is better than an optimised panel of clinical, objectively measured, prognostic markers remains an open question.

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1 Bueno-de-Mesquita JM, van Harten WH, Retel VP, et al. Use of 70-gene signature to predict prognosis of patients with node-negative breast cancer: a prospective community-based feasibility study (RASTER). *Lancet Oncol* 2007; **8**: 1079–87.
2 Sotiriou C, Wirapati P, Loi S, et al. Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. *J Natl Cancer Inst* 2006; **98**: 262–72.
3 Robbins P, Pinder S, de Klerk N, et al. Histological grading of breast carcinomas: a study of interobserver agreement. *Hum Pathol* 1995; **26**: 873–79.
4 Lee AH, Pinder SE, Macmillan RD, et al. Prognostic value of lymphovascular invasion in women with lymph node negative invasive breast carcinoma. *Eur J Cancer* 2006; **42**: 357–62.
5 Callagy GM, Pharoah PD, Pinder SE, et al. Bcl-2 is a prognostic marker in breast cancer independently of the Nottingham Prognostic Index. *Clin Cancer Res* 2006; **12**: 2468–75.
6 de Azambuja E, Cardoso F, de Castro G Jr, et al. Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12 155 patients. *Br J Cancer* 2007; **96**: 1504–13.
7 Coradini D, Boracchi P, Daidone MG, et al. Contribution of vascular endothelial growth factor to the Nottingham prognostic index in node-negative breast cancer. *Br J Cancer* 2001; **85**: 795–97.
8 Suen D, Chow LW. Prognostic contribution of the HER-2 oncogene overexpression to the Nottingham Prognostic Index in breast cancer. *Biomed Pharmacother* 2006; **60**: 293–97.
9 Ponzone R, Montemurro F, Maggiorotto F, et al. Clinical outcome of adjuvant endocrine treatment according to PR and HER-2 status in early breast cancer. *Ann Oncol* 2006; **17**: 1631–36.
10 Edén P, Ritz C, Rose C, Fernö M, Peterson C. "Good Old" clinical markers have similar power in breast cancer prognosis as microarray gene expression profilers. *Eur J Cancer* 2004; **40**: 1837–41.

Authors' reply

Neven and coworkers emphasise in their letter that findings with use of the 70-gene prognostic signature and Nottingham Prognostic Index (NPI) are mainly discordant for patients with histological grade 2, node-negative breast cancers. These data are, of course, in agreement with our findings. As can be seen from table 1 in our original article,[1] the 70-gene prognostic signature adds more prognostic information to the histological grade-2 subgroup and to the oestrogen-receptor-positive breast cancers than to other categories. Neven and colleagues state that only 6·7% would be undertreated and 7·2% overtreated with use of the NPI instead of the 70-gene signature. They calculated these percentages by dividing the number of patients with low NPI risk and poor prognosis according to the 70-gene signature (n=15) or those with high NPI risk and good prognosis according to the 70-gene signature (n=16) by the number of grade 1 and grade 3 tumours combined (n=223). However, we propose that these calculations should be done for grade 1 and grade 3 tumours separately, resulting in 17% (15 of 87) undertreatment for patients with grade 1 tumours and 12% (16 of 136) overtreatment for patients with grade 3 tumours. Based on these numbers, we suggest that use of the 70-gene prognostic signature might also provide substantial additional information for patients with grade 1 and 3 tumours, albeit the findings are less informative than in the group with grade 2 tumours.

We agree with Neven and colleagues that the accuracy of histological tumour grading is affected by large inter-observer (and potentially also intra-observer) variations, especially when distinguishing grade 1