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Validation process of a gene-expression signature of local recurrences after breast-conserving treatments

M.A. Bollet[1], N. Servant[2], B. Kreike[3], H. Halfwerk[4], S. Daoud[5], I. Lebigot[6], H. Bartelink[3], A. Fourquet[7], B. Sigal-Zafrani[6], M.J. van de Vijver[8]. *[1]Institut Curie, Radiation Oncology, Paris, France; [2]Institut Curie, Bio-informatics, Paris, France; [3]The Netherlands Cancer Institute, Radiation oncology, Amsterdam, The Netherlands; [4]The Netherlands Cancer Institute, Experimental therapy, Amsterdam, The Netherlands; [5]The Netherlands Cancer Institute, Central Microarray Facility, Amsterdam, The Netherlands; [6]Institut Curie, Tumour Biology, Paris, France; [7]Institut Curie, Radiation oncology, Paris, France; [8]Academic Medical Centre, Pathology, Amsterdam, The Netherlands*

Background: Kreike et al. have constructed a gene-expression (GE) profile predictive for local recurrence (LR) after breast-conserving treatment (BCT) from a series of 165 Dutch young (<51 yo), premenopausal patients (pts) using Human Genome Oligo Set version 3.0 arrays (Clinical Cancer Research, 2009). This study aimed to test this signature both internally (cross-platform) and externally on a large independent series of pts.

Material and Methods: GE profiles of primary invasive breast carcinomas were constructed, using version 3 Illumina microarrays, on 148 of the initial series of 165 Dutch pts (52 LR+, 96 LR−). A French series of 195 pts (67 LR+, 128 LR−) was used as an independent validation set. All pts, in both series, were treated with local excision of the tumour followed by whole-breast radiotherapy with or without a boost; followed by systemic treatment (hormone-therapy and/or chemotherapy) in part of the patients. The previously assessed LR signature was evaluated both in its ability to predict for the risk of developing a LR in the Dutch series (cross-platform validation) and in the French series (independent validation).

Twenty-one other gene-sets that could play a role in LR after BCT from the literature were also evaluated. We applied a diagonal linear discriminant analysis on both populations using a 10-fold cross-validation. The area under the receiver operating characteristic curve (AUC) was used as criterion of prediction quality. P values <0.05 were considered significant.

Results: There were significantly more pts in the French population >40 years old (82% vs. 71% in the Dutch population, $p = 0.02$), with free surgical margins (93% vs. 82%, $p = 0.003$), without axillary involvement (69% vs. 57%, $p = 0.03$), with Lobular Carcinoma In-Situ (12% vs. 5%, $p = 0.02$), with lympho-vascular involvement (7% vs. 0%, $p = 0.004$), with Ductal Carcinoma In-Situ (72% vs. 54%, $p = 0.001$) and who did not receive a radiotherapy boost (43% vs. 7%, $p < 0.0001$).

In the new Dutch series of 148 pts, our previously assessed LR signature achieved a higher performance than those of all the other 21 gene-lists tested (AUC of 0.7, Specificity = 0.67, Sensitivity = 0.71, 32% of mis-classification). In the French series, its performance was less (AUC = 0.57, Specificity = 0.49, Sensitivity = 0.73, 43% of mis-classification) and other signatures achieved as good or even better results.

Conclusion: We have tested a previously obtained GE profile associated with LR after BCT and found that it could be validated on a different platform than the one used to construct it (cross-platform validation). However, we were unable to validate the LR signature in an independent series of patients who underwent BCT. The independent validation series differed in a number of important characteristics, which may explain our results. We will continue our efforts to identify novel predictors for LR after BCT.

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HER2 (cerbB2) positivity: cross correlations, and effect on survival of a pre-Herceptin treated group

G. Ball[1], I.O. Ellis[2], A. Green[2], R. Blamey[3]. *[1]Nottingham Trent University, School of Science and Technology, Nottingham, United Kingdom; [2]University of Nottingham, Pathology, Nottingham, United Kingdom; [3]University of Nottingham, Surgery, Nottingham, United Kingdom*

Background: Here we present a detailed analysis of the expression of HER2 in 1524 cases of operable primary invasive breast cancer cases treated at Nottingham City Hospital 1990–1999. The primary focus is an analysis and cross tabulation of factors in the Nottingham Prognostic Index (NPI) and analysis of the impact of HER2 positivity (determined by Herceptest) on survival in a cohort of patients recruited prior to the regular use of Herceptin. The aim of this study was to identify patterns in factors associated with survival across the NPI groups and survival per se as a baseline prior to regular use of Herceptin.

Material and Methods: Breast Cancer cases matching the inclusion criteria (operable, Primary breast cancer Age $\leqslant 70$) were accrued consecutively over the period 1990–1999. For these cases parameters associated with the NPI were determined. Histological grade was determined using the Elston Ellis criteria, lymph node status determined by number of nodes positive at surgery and size determined as diameter post surgery. Estrogen Receptor (ER) status was also measured by Immuno-histochemistry and an H score determined.

Statistical cross-tabulation with factors across the NPI was conducted with the frequency of membership determined and significance determined by CHI squared analysis or Fischer's Exact test where appropriate. Regression analysis was conducted for size. The survival in NPI groups was determined and the Implications of HER2 positivity at different levels investigated by Log Rank tests. Multivariate Cox regression was also conducted to determine whether HER2 status had independents prognostic value over existing NPI factors.

Results: Significant correlations were seen between histological grade, lymph node status, size and ER status. HER positivity was more prevalent in higher grade, larger, lymph node positive tumors and as such more prevalent in tumors with a higher NPI score. Thus the investigation of HER2 positivity was most relevant for NPI groups of MPG1 or greater where overall significant Effects were seen on survival.

Conclusions: Significant correlations with HER2 status were seen across a number of key parameters associated with the NPI. Significant effects were observed with respect to survival in higher NPI groups and in these cases HER2 positivity was an independent prognostic factor. Prior to the use of Herceptin HER2 status was associated with a worse prognosis. After the introduction of Herceptin this is likely to change a comparison with long term follow up will determine this in the future.

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A validation study of 68 single nucleotide polymorphisms which have been associated with radiation toxicity: preliminary results of the RAPPER study

G.C. Barnett[1], C. Baynes[2], C. Luccarini[2], D. Conroy[2], C.M.L. West[3], R.M. Elliott[3], N.G. Burnet[1], C.E. Coles[1], J. Wilkinson[1], A.M. Dunning[2]. *[1]Addenbrookes Hospital, Department of Oncology, Cambridge, United Kingdom; [2]Strangeways Research Laboratory, Department of Oncology, Cambridge, United Kingdom; [3]University of Manchester, School of Cancer & Imaging Sciences, Manchester, United Kingdom*

Background: RAPPER is a large radiogenomics study designed to test for associations between common genetic variation (reported by single nucleotide polymorphisms − SNPs) and patient variability in radiation toxicity. Radiogenomics studies published to date have studied candidate genes encoding DNA repair proteins, antioxidant enzymes and cytokines such as TGFβ1. This study aimed to confirm previously reported associations between SNPs in these genes and radiation toxicity.

Materials and Methods: 96 SNPs were genotyped in 943 breast cancer patients who had received conformal or intensity-modulated radiotherapy to the breast. Toxicity was assessed two years after radiotherapy using a validated photographic technique, clinical assessment and patient questionnaires. Regression analysis was used to assess the relationship between genotype and radiation toxicity; reported by a standardised total average toxicity (STAT) score and by individual endpoints. Multivariate analysis of cumulative toxicity score including all patient- and treatment-related risk factors thought to affect toxicity was performed.

Results: Most of the previously reported associations were not confirmed in this much larger study. We find evidence that common variants in two genes may be associated with the total toxicity score (STAT score). However, after a conservative Bonferroni adjustment for multiple testing these results were no longer significant. The minor alleles of *MRE11* SNPs rs569143 & rs2155209 and *ATM* SNP rs1800057 (P1054R) were associated with differences in toxicity (P-trend= 0.001, 0.0009 and 0.003 respectively). On analysis of the individual endpoints, *HIF1A* SNP rs230113 was associated with altered breast shrinkage ($P = 0.003$) and *XRCC3* SNP rs1799794 pigmentation ($P = 0.007$). The rare allele of rs1805386 in the Lig4 gene was associated with poorer overall cosmesis ($P = 0.009$). The effect sizes associated with each SNP are small.

Conclusions: Similar to most genetic traits examined previously by this methodology, reported candidate gene SNP associations for radiation toxicity have proved difficult to confirm. Three of the six results reported in this study were confirmations of previous associations. Genome Wide Association Studies (GWAS) have proved more effective than candidate gene studies at identifying common genetic variants associated with traits and diseases. GWAS will be used in the RAPPER study, ensuring that there is good coverage of genes highlighted in this study.