Epstein-Barr virus as a marker of biological aggressiveness in breast cancer

C Mazouni*,1,2,4, F Fina1,6, S Romain1, L Ouafik1, P Bonnier4, J-M Brandone5 and P-M Martin1

1Laboratoire de transfert d’oncologie biologique, Assistance Publique – Hôpitaux de Marseille, Faculté de Médecine Nord, Marseille, France; 2Département de chirurgie générale, Institut Gustave Roussy, 114 rue Edouard Vaillant, Villejuif 94805, France; 3UMR 911 CRO2: Centre de Recherche en Oncologie biologique et Oncopharmacologie, Marseille F-13344, France; 4Département d’oncologie chirurgicale mammaire et gynécologique, clinique Beauregard, Marseille, France; 5Département de chirurgie gynécologique, clinique Bouchard, Marseille, France

PURPOSE: Although a potential role of the Epstein-Barr virus (EBV) in the pathogenesis of breast cancer (BC) has been underlined, results remain conflicting. Particularly, the impact of EBV infection on biological markers of BC has received little investigation.

METHODS: In this study, we established the frequency of EBV-infected BC using real-time quantitative PCR (RT–PCR) in 196 BC specimens. Biological and pathological characteristics according to EBV status were evaluated.

RESULTS: EBV DNA was present in 65 of the 196 (33.2%) cases studied. EBV-positive BCs tended to be tumours with a more aggressive phenotype, more frequently oestrogen receptor negative (P = 0.05) and with high histological grade (P = 0.01). Overexpression of thymidine kinase activity was higher in EBV-infected BC (P = 0.007). The presence of EBV was weakly associated with HER2 gene amplification (P = 0.08).

CONCLUSION: Our study provides evidence for EBV-associated BC undergoing distinct carcinogenic processes, with more aggressive features.

British Journal of Cancer (2011) 104, 332–337. doi:10.1038/sj.bjc.6606048 www.bjcancer.com

Keywords: breast cancer; Epstein-Barr virus; HER2; real-time quantitative PCR; thymidine kinase

A viral aetiology is one recently evoked theory behind the physiopathology of breast cancer (BC) (Glaser et al, 2004; de Villiers et al, 2005; zur Hausen, 2009). Even though, the mechanistic aspects of cancer induction by infectious agents sound multiples, that is, immunosuppressive, linked to animal–human transmission, direct or indirect oncogenic, there are epidemiological evidences of pathogens involvement in human cancer (zur Hausen, 2009).

Among the putative viruses observed in BC tissue, the presence of the Epstein-Barr virus (EBV), a γ-herpes virus, has been reported in a number of studies (Bonnet et al, 1999; Fina et al, 2001; Glaser et al, 2004). The implication of EBV in carcinogenesis associated with other cancers, such as Burkitt’s lymphoma, undifferentiated nasopharyngeal carcinoma, as well as Hodgkin’s disease, has been well documented (zur Hausen, 1991).

However, the presence and implication of EBV in BC remains controversial. The use of conventional technical approaches (in situ hybridisation, immunochemistry and standard PCR) for its detection may explain the conflicting results. Some groups have failed to detect EBV (Chang et al, 1992; Gaffey et al, 1993; Lespagnard et al, 1995; Chu et al, 1998; Glaser et al, 1998; Dadmanesh et al, 2001; Deshpande et al, 2002; Herrmann and Niedobitek, 2003; Perrigoue et al, 2005), whereas results from others show discrepancy and depended on the methodology used.

For instance, although Murray et al (2003) could detect EBV nuclear antigen-1 by immunochemistry using 2B4-1 monoclonal antibody, they failed to detect the EBV genome by quantitative PCR. The reasons behind these apparently conflicting results remain to be clarified; however, technical limitations of the assays, dissimilarities in the archival materials and heterogeneity among cluster cells contaminated by the EBV genome may be same. Moreover, EBV positivity has been linked to the presence of latently infected lymphocytes in the tumours (Horiuchi et al, 1994; Brink et al, 2000) thus, questioning the role of EBV in BC (Chu et al, 2001). However, in accordance with other groups (Labrecque et al, 1995; Luqmani and Shousha, 1995; Bonnet et al, 1999; Chu et al, 2001; Huang et al, 2003; Preciado et al, 2005; Arbach et al, 2006; Perkins et al, 2006; Tsai et al, 2007), we have shown the presence of EBV genetic information in a subset of BC tissue with a specific localisation in the epithelial malignant cells (Fina et al, 2001).

Currently, real-time PCR (RT–PCR) is increasingly being used for both research and clinical applications. For BC in particular, the detection of HER2 gene amplification has been validated by comparison with conventional methods, such as FISH (Lamy et al, 2001). Analysis using RT–PCR might also help to clearly identify the presence of EBV in BC. However, the use of whole tissue can result in the risk of contamination and this risk has been corrected with the introduction of laser-assisted microdissection (Fina et al, 2001). In studies on formalin-fixed sections, micro- and macro-dissected breast tumours have been tested for the presence of multiple regions of the EBV genome with few actually uncovering the viral sequence (McCall et al, 2001; Thorne et al, 2005). Interestingly, by in situ hybridisation using a (35S-labelled

*Correspondence: Dr C Mazouni; E-mail: chafika.mazouni@gfr

These authors contributed equally to this work.

Revised 12 October 2010; accepted 16 November 2010; published online 21 December 2010
Patients were consecutively recruited in Marseille France, between May 1996 and December 1998. Tumours were graded according to the Scarff Bloom and Richardson classification (Bloom and Richardson, 1957). Axillary lymph node status was assessed by histological examination. The local Medical Ethics Committee (IRB) approved this laboratory study on stored specimens.

### Tissue specimens

All tumour samples were histologically examined by a pathologist at the time of initial surgery and stored in liquid nitrogen. Frozen tissue (100 mg) was pulsed with a micro-disemembrator and the frozen powder subsequently used for DNA extraction (Sambrook et al, 1982). Cytosols were prepared using a Trix buffer (10 mM Tris-HCl, 1.5 mM EDTA, 10 mM Na2MoO4, 0.5 mM dithiothreitol, 10% glycerol, pH 7.4).

### Q-PCR analysis of HER2 gene

The Q-PCR reaction conditions used have already been published (Lamy et al, 2006). The calibration curve was prepared from normal human genomic DNA (Roche Molecular Biochemicals, Meylan, France). Data were expressed as the HER2/GAPDH and HER2/SSTR2 relative copy number ratio. The human SKBR3 and A431 cell lines, known to, respectively, display HER2 amplification or not, were used as controls.

### Q-PCR analysis of the EBV genome

The Q-PCR analysis of the EBV genome was performed as previously described (Fina et al, 2001). Briefly, primers for BamHIC were: sense, 5’-AAA-CAG-GAC-AGC-CGT-TGC-C-3’ (6935–6953); antisense, 5’-AAG-CCT-CTC-TTC-TTG-CCC-3’ (7036–7016) and the probe was 5’-FAM-TTT-CGG-ACA-CAC-CCG-CAG-TGC-C-3’ (6961–6983). The cycling conditions for both BamHICs were as follows: 95°C for 15 min; 45 cycles of 94°C for 20 s, and 55°C for 20 s. Amplification was performed in a 50-µl reaction volume with a buffer consisting of 10 mMol·L−1 Tris-HCl (pH 8.3; 25°C), 50 mMol·L−1 KCl, 10 mMol·L−1 ethylene-diamine tetracetate and 5 mMol·L−1 MgCl2 in the presence of 200 mMol·L−1 deoxy(d)-ATP, dCTP and dGTP, 400 mMol·L−1 dUTP, 200 mMol·L−1 of each primer, 200 mMol·L−1 probe, 1 U Amp EraseUNG (Perkin-Elmer Corp.), and 1.25 U AmpliTaq Gold polymerase (Perkin-Elmer Corp.). To quantify the EBV genome load in the tissues, genomic DNA prepared from the Raji cell line, containing 50 integrated EBV copies per cell was used. Serial dilutions of DNA were prepared from 1 ng to 0.1 pg equivalent to 15000–1.5 copies of EBV genome, respectively. Absolute quantification of the BamHIC standard curve involved comparison against normal human genomic DNA (Roche Molecular Biochemicals). The calibration curve for GAPDH was directly prepared from normal human genomic DNA (Roche Molecular Biochemicals, Mannheim, Germany).

### Biochemical assays

Oestrogen receptors and progesterone receptors (PRs) (EIA, Abbott Laboratories, Chicago, IL, USA), as well as...
urokinase plasminogen activator (UPA) and plasminogen activator inhibitor type 1 (PAI-1) (UPA Imubind no 894 and PAI-1 Imubind no 821, both from Enzyme Diagnostics, Greenwich, CT, USA) were measured with enzyme immunoassays. Thymidine kinase (TK) activity was determined by a radiometric phosphorylation assay (TK-REA, Sangtec Medical, Bromma, Sweden) optimised to detect the fetal TK1 isoenzyme, as previously described (Romain et al., 1994). Quality control was assured by frequent testing with internal controls. The EORTC standards were also used for oestrogen receptor and PR (Geurts-Moespot et al., 2000).

**RESULTS**

**Patient characteristics**

The demographics of the studied patients are reported in Table 1. The tumours had a diameter of more than 2 cm in 97 patients (49.5%). In all, 76 patients (38.8%) had positive axillary lymph nodes. The histological differentiation was determined as grade 3 in 56 tumours (28.6%).

HER2 gene amplification was detected in 15.3% (n = 30) of the analysed BCs, with the HER2/GAPDH ratio for amplified cases ranging from 2.0–22.1 (median 4.5). Among the HER2-amplified tumours, 43.3% (n = 13) showed moderate HER2 amplification (HER2/GAPDH ratio 2.0–4.0) and 56.7% (n = 17) a strong amplification (HER2/GAPDH ratio ≥ 4.0).

**Q-PCR analysis of the EBV genome**

To ensure that the presence of EBV was related to epithelial cells, as previously described (Fina et al., 2001). Tissue sections were microdissected with a PixCell II LCM system (Arcturus Engineering, Mountain View, CA, USA). For each tumour analysed, several epithelial areas (approximatively 5 × 10^3 cells) were independently captured; stromal areas without infiltrating malignant epithelial cells were pooled to provide a sufficient number of GAPDH copies. Cell populations were estimated to be homogeneous as determined by microscopic visualisation. DNA from laser-captured cells was extracted and subsequently used for Q-PCR.

The presence of the BamHIC sub-region of the EBV genome was used to define EBV positivity. EBV was detected in 65 (33.2%) of the 196 investigated BCs. Among the positive tumours, the load of EBV genome varied from 0.08 to 810.8 BamHIC copies per 100 ng GAPDH (median 1.4). Fibrocystic diseases (n = 3), fibroadenomas (n = 6), phyllode tumours (n = 4) and normal mammary tissue obtained from mammoplasty (n = 2) were also analysed. They were all found to be negative, with the exception of one phyllode tumour (0.43 BamHIC copies per 100 ng GAPDH).

**Detection of the EBV genome and patient characteristics**

Table 1 shows the frequency of the EBV genome according to the characteristics of the patient and tumour.

Tumour positive for EBV presented markers of proliferation. Thus, the proportion of EBV-positive samples was significantly higher among the high-grade tumours (16.2% for grade I, 32.0% for grade II and 46.4% for grade III, P = 0.01). EBV-positive samples were more frequent among those of ER-negative (45.4%) compared with ER-positive tumours (29.6%) (P = 0.05). Among the tumours with high TK, 49.0% displayed the EBV genome compared with 27.9% of those with low TK (P = 0.007). In contrast, no significant link was observed between the detection of the EBV genome and age at diagnosis, tumour size, lymph node involvement, PR, UPA or PAI-1 status.

To quantitatively assess the relation between the EBV presence and pathological markers, we have studied the load of EBV genome. We confirmed that BamHIC copy numbers were higher among high-grade tumours (P = 0.006) and between those ER-negative (P = 0.01) and high TK value (P = 0.009). Other relationships were not significant (Table 2).

**Detection of the EBV genome and amplification of HER2**

A weak association was observed between EBV genome presence and HER2 amplification. Subgroups with EBV– HER2– (n = 115)

**Table 2** Mean EBV load (BamHIC copies per 100 ng GAPDH) according to biological characteristics

| Characteristics          | Mean EBV load (BamHIC copies per 100 ng GAPDH) | P-value |
|--------------------------|-----------------------------------------------|---------|
| Age (years)              |                                               |         |
| <50                      | 0.79                                          | 0.42    |
| ≥50                      | 7.84                                          |         |
| Tumour size (pT)         |                                               |         |
| <2 cm                    | 1.28                                          | 0.70    |
| ≥2 cm                    | 10.60                                         |         |
| Nodal status (pN)        |                                               |         |
| N−                       | 1.40                                          | 0.34    |
| N+                       | 13                                            |         |
| Histological grade       |                                               |         |
| I                        | 0.32                                          | 0.006   |
| II                       | 2.58                                          |         |
| III                      | 15.7                                          |         |
| ER                       |                                               |         |
| Negative                 | 1.64                                          | 0.01    |
| Positive                 | 20.58                                         |         |
| PR                       |                                               |         |
| Negative                 | 1.83                                          | 0.20    |
| Positive                 | 14.67                                         |         |
| UPA                      |                                               |         |
| Low                      | 7.48                                          | 0.56    |
| High                     | 1.12                                          |         |
| PAI-1                    |                                               |         |
| Low                      | 7.21                                          | 0.75    |
| High                     | 1.93                                          |         |
| TK                       |                                               |         |
| Low                      | 1.87                                          | 0.009   |
| High                     | 17.95                                         |         |
| No HER2 amplification    |                                               |         |
| HER2 amplification       |                                               | 0.10    |

Abbreviations: ER = oestrogen receptor; PAI-1 = plasminogen activator inhibitor 1; PR = progesterone receptor; TK = thymidine kinase; UPA = urokinase plasminogen activator.
and EBV + HER2+ (n = 14) status represented 65.8% of the investigated patients (P = 0.09). When using the Mann–Whitney test, EBV-positive tumours showed the highest HER2 copy numbers though the difference did not reach significance (P = 0.08).

DISCUSSION
The aim of this study was to investigate the presence of EBV in BC, alongside possible associations with clinicopathological factors and biological tumour features that either mark the natural history of the disease or determine the therapeutic outcome. The analysed biological factors were selected on the basis of their high utility score in the tumour marker grading system (Isaacs et al, 2001), with evidence coming either from prospective trials or meta-analysis (ER, PR, HER2, UPA and PAI-1), or at least from large retrospective studies (TK).

The implication of EBV in the aetiology of BC has been addressed in other series, including a multicentric study carried out by our group (Fina et al, 2001). In accordance with our analysis, the presence of EBV was showed to be restricted in the epithelial cells (Fawzy et al, 2008; Trabelsi et al, 2008; Joshi et al, 2009). EBV has been evocated along with other viruses, such as the papillomavirus (de Villiers et al, 2005; Kulka et al, 2008) or polyomavirus (Berebbi et al, 1990), as well as cytomegalovirus (Richardson et al, 2004). One of the controversies surrounding EBV as a causative agent in BC has been its potential coincidental presence as no virus-related physiopathological effects have emerged from pathological observations. However, one interesting epidemiological study provided some arguments in favour of a role and a potential explanation relating to the stage of mammary gland development. Indeed Yasui et al (2001) showed a correlation between the incidence of infectious mononucleosis and the risk of BC. Particularly, an increase in age corresponding to a later stage of mammary gland development at infectious mononucleosis onset seemed to increase the risk for BC. We have also observed this potential link between the incidence of BC and hormonal status in one of our previous studies with the polyomavirus (Berebbi et al, 1990). Our evaluation of oncogenicity in nude mice showed that mammary tumour induction was oestrogen-dependent during a short period after polyomavirus injection. This sensitivity of the mammary gland to virus exposure corresponds to an oestradiol-mediated modification of the target organ occurring during ductal development (Berebbi et al, 1990). After this developmental period, the mitogenic stimulus induced by hormones is no longer necessary. The influence of hormonal environment during the critical period of mammary gland development thus determines the future carcinogenesis process and the pool of hormone-responsive epithelial cells (Nandi et al, 1995). However, the analysis of changes in EBV immunoglobulins (Ig) showed discrepant results; thus, although Cox et al (2010) failed to show an association with the risk of BC in Ig taken before and after the development of BC, in contrast Joshi et al (2009) observed no difference that mean anti-EBNA-1 IgG levels were significantly higher in BC patients as compared with benign breast disease.

The Q-PCR method has been used here to investigate EBV in a large series of BCs. Overall, 33.2% of the 196 frozen tumours analysed contained the BamHI C sub-region of the EBV genome that encodes the Epstein-Barr encoded RNAs. In our previous multi-centre study (Fina et al, 2001), the samples from our centre showed a positive ratio of 26.7% by standard PCR. The higher percentage of EBV-positive tumours observed in the present study may be related to the size of the tumours samples analysed, (100 mg), and to the sensitivity of Q-PCR. Two other investigators have also found EBV by PCR in frozen tissues by PCR (Labrecque et al, 1995; Bonnet et al, 1999). The absence of detection (Gaffey et al, 1993; Lespagnard et al, 1995) or the low detection rate even by RT – PCR (McCall et al, 2001) that has elsewhere been reported elsewhere could be due to result from the use of fixed tissues. Indeed, it has been demonstrated that fixation generates both PCR-inhibitory components (Satoh et al, 1998; Kösel et al, 2001; kalkan et al, 2005) and sequence alterations (Williams et al, 1999; Amaranthe and Watanabe, 2009). Inhibition of the viral DNA PCR amplification was most likely the case for the study of McCall and colleagues (McCall et al, 2001). Kalkan et al (2005) and Thorne et al (2005) detected EBV genome in epithelial and also in normal cells. In these studies, low amounts of template DNA was probably used as the tissues were microdissected and DNA amplifiability was controlled by HER2 detection. In our BC samples, the loads of EBV genomic information (EBV DNA per 100 ng DNA) ranged far below the range of HER2 values (HER2/GAPDH copy number). The high heterogeneity in EBV detection that has been shown within individual tumours also needs to be considered (Fina et al, 2001).

In this study, we observed a difference in clinical and biological profiles between EBV-positive and EBV-negative cancers. In accordance with Tsai et al (2007) though in contrast to Murray et al (2003), we found no correlation between the presence of EBV and nodal status. Here, we have investigated markers of epithelial cells (ER, PR, grade and TK), whereas UPA and PAI-1, which are markers of stromal-epithelial interactions, associated with tumoural invasion process. In line with this result, no association was observed with the biological factors (UPA or PAI-1) related to tumour invasion. However, we did confirm that the proportion of EBV-positive samples is higher among the high-grade and the ER-negative cancers (Bonnet et al, 1999; Murray et al, 2003). These latter biological factors related to differentiation status (Rose et al, 1985; McGuire et al, 1986; Murray et al, 2003) were strongly associated with EBNA-1 as detected by immunostaining. Altogether these results confirm the epithelial presence of EBV as only correlations with epithelial markers were observed and not with the markers of stromal compartment. Interestingly, we showed a positive association between the presence of EBV and high cytosolic TK enzyme activity. High expression of this enzyme involved in the DNA synthesis salvage pathway has previously been associated with large tumour size, high histological grade and steroid hormone receptor negativity (Romain et al, 2000). The TK enzyme is encoded by the EBV genome and expressed in the cytosolic TK enzyme activity, which is expressed independently or in the context of lytic EBV infection (Gill et al, 2007). Although EBV TK is an early gene, it was nevertheless found to be transcribed with a significant delay compared with other early-class RNAs (Yuan et al, 2006). The commercial assay used to access TK activity has been optimised for the TK1 isoenzyme. The link between EBV and TK supports the notion that EBV is associated with fast-growing tumours. It agrees with data suggesting that DNA tumour viruses suppress the transcriptional downregulation of TK activity during the eukaryotic cell cycle (Hengsädla et al, 1994), and that nasopharyngeal carcinomas with detectable EBV LMP1 protein grow faster than the non-expressing ones (Hu et al, 2000). Interestingly, EBV positive tumours and high TK expression were sequenced mRNA and found that the expression of TK gene found in EBV-positive tumours is of human origin, not viral (data not shown). This human TK differs from the one deposited by Bradshaw and Deininger in the Genbank database (Gilles et al, 2003). In a previous study, Huang et al (2003), detected the EBV-encoded lytic transactivator protein ZTA in 7 out of 10 BCs. Interestingly, ZTA specifically binds the CCAAT motif (C/EBPδ, enhancer binding protein α) of the TK1 human gene promoter, which suggests a functional role in the activation of TK1 transcription.

In this study, the presence of EBV was only weakly associated with HER2 amplification. This result, together with the fact that

© 2011 Cancer Research UK
British Journal of Cancer (2011) 104(2), 332 – 337
EBV and HER2 correlate differently with other tumour features, suggests that the viral infection and the gene amplification occur at different times during BC progression. In BCs, both EBV (Bonnet et al, 1999) and HER2 (Révillion et al, 1998; Yamauchi et al, 2001) have been associated with a lack of oestrogen receptors.

In conclusion, we confirmed the presence of EBV in one third of BC. Moreover, EBV-positive tumours presented with a more aggressive phenotype that could be useful when considering potential therapeutic targets. In particular, the high TK levels could confer resistance to chemotherapy.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Assistance Publique de Marseille (AORC 1998; UF 2843). A Durand, and M Soullière are acknowledged for their skilful technical assistance.
Luqmani Y, Shousha S (1995) Presence of Epstein-Barr virus in breast carcinoma. Int J Oncol 6: 899 – 903
McCall SA, Lichy JH, Bijwaard KE, Aguilera NS, Chu WS, Taubenberger JK (2001) Epstein-Barr virus detection in ductal carcinoma of the breast. J Natl Cancer Inst 93: 148 – 150
McGuire WL, Clark GM, Dressler LG, Owens MA (1986) Role of steroid hormone receptors as prognostic factors in primary breast cancer. NCI Monogr 1: 19 – 23
Murray PG, Lissauer D, Junying J, Davies G, Moore S, Bell A, Timms J, Rowlands D, McConkey C, Reynolds GM, Ghataura S, England D, Caroll R, Young LS (2003) Reactivity with a monoclonal antibody to Epstein-Barr virus (EBV) nuclear antigen 1 defines a subset of aggressive breast cancers in the absence of the EBV genome. Cancer Res 63: 2338 – 2343
Nandi S, Guzman RC, Yang J (1995) Hormones and mammary carcinogenesis in mice, rats, and humans: a unifying hypothesis. Proc Natl Acad Sci USA 92: 3650 – 3657
Perkins RS, Sahm K, Marando C, Dickson-Witmer D, Pahnke GR, Perrigoue JG, den Boon JA, Friedl A, Newton MA, Ahlquist P, Sugden B (2001) DNA-synthesizing enzyme activities (thymidine kinase and thymidylate synthase) in 908 T1-T2, N0-N1, M0 breast cancers: a retrospective multicenter study. Int J Cancer 87: 860 – 868
Romain S, Spyratos F, Guirou S, Deytieux S, Chabay P, Martin PM (1994) Epstein-Barr virus DNA in DNA extracted from paraffin-embedded gastric carcinoma tissue. J Clin Microbiol 36: 3423 – 3425
Romain S, Thorpe SM, Andersen KW, Pedersen BV, Mouridsen HT, Blüchert-Toft M, Rasmussen BB (1985) Beneficial effect of adjuvant tamoxifen therapy in primary breast cancer patients with high oestrogen receptor values. Lancet 1: 16 – 19
Sambrook J, Fritsch EF, Maniatis T (1982) Molecular cloning: a laboratory manual. Harbor Laboratory: Cold Spring Harbor Laboratory: Cold Spring
Sato Y, Takasaka N, Hoshikawa Y, Osaki M, Ohsu S, Ito H, Kaibara N, Kurata T, Sairenji T (1998) Pretreatment with restriction enzyme or bovine serum albumin for effective PCR amplification of Epstein-Barr virus DNA in DNA extracted from paraffin-embedded gastric carcinoma tissue. J Clin Microbiol 36: 3423 – 3425
Thorne LB, Ryan JL, Elmore SH, Glaser SL, Golley ML (2005) Real-time PCR measures Epstein-Barr virus DNA in archival breast adenocarcinomas. Diagn Mol Pathol 14: 29 – 33
Trabesi A, Rammeh S, Sitia W, Mokni M, Mourou A, Korbi S (2008) Detection of Epstein-Barr virus DNA in breast cancers with lymphoid stroma. Ann Biol Clin 66: 59 – 62
Tsai JH, Hsu CS, Tsai CH, Su JM, Liu YT, Cheng MH, Wei JC, Chen FL, Yang CC (2007) Relationship between viral factors, axillary lymph node status and survival in breast cancer. J Cancer Res Clin Oncol 133: 11 – 21
Williams C, Pontén F, Möberg C, Söderkvist P, Uhlen M, Pontén J, Sitbon G, Lundberg J (1999) A high frequency of sequence alterations is due to formalin fixation of archival specimens. Am J Pathol 155: 1467 – 1471
Yamauchi H, Stearns V, Hayes DF (2001) When is a tumor marker ready for prime time? A case study of c-erbB-2 as a predictive factor in breast cancer. J Clin Oncol 19: 2334 – 2356
Yasui Y, Potter JD, Stanford JL, Rossing MA, Winget MD, Bronner M, Daling J (2001) Breast cancer risk and ‘delayed’ primary Epstein-Barr virus infection. Cancer Epidemiol Biomarkers Prev 10: 9 – 16
Yuan J, Cahir-McFarland E, Zhao B, Kieff E (2006) Virus and cell RNAs expressed during Epstein-Barr virus replication. J Virol 80: 2548 – 2565
Zur Hausen H (2001) Viruses in human cancers. Science 254: 1167 – 1173
Zur Hausen H (2009) The search for infectious causes of human cancers: where and why. Virology 392: 1 – 10