BAG-1 as a biomarker in early breast cancer prognosis: a systematic review with meta-analyses

E S Papadakis¹, T Reeves*,¹, N H Robson¹, T Maishman³, G Packham¹ and R I Cutress¹,²

¹Cancer Research UK Centre Cancer Sciences Unit, University of Southampton Faculty of Medicine, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK; ²University Hospital Southampton, University of Southampton Faculty of Medicine, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK and ³Southampton Clinical Trials Unit, University of Southampton, Southampton SO17 1BJ, UK

Background: The co-chaperone protein Bcl-2-associated athanogene-1 (BAG-1) is overexpressed in breast cancer and has been incorporated in the oncotype DX and PAM50 breast cancer prognostic assays. Bcl-2-associated athanogene-1 exists as multiple protein isoforms that interact with diverse partners, including chaperones Hsc70/Hsp70, Ser/Thr kinase Raf-1 and Bcl-2, to promote cancer cell survival. The BAG-1L isoform specifically binds to and increases the transcriptional activity of oestrogen receptor in cells, and in some, but not all studies, BAG-1 expression is predictive of clinical outcome in breast cancer.

Methods: A systematic review of published studies reporting BAG-1 (mRNA and/or protein) expression and clinical outcome in early breast cancer. The REporting Recommendations for Tumour MARKer and Prognostic Studies (REMARK) criteria were used as a template against which data were assessed. Meta-analyses were performed for studies that provided a hazard ratio and 95% confidence intervals for clinical outcomes including disease-free survival or breast cancer-specific survival from univariate analysis.

Results: Eighteen studies used differing methodologies and reported on differing outcomes. Meta-analyses were only possible on results from a subset of reported studies. Meta-analyses suggested improved outcome with high BAG-1 mRNA and high BAG-1 nuclear expression by immunohistochemistry.

Conclusions: Increased levels of BAG-1 are associated with better breast cancer outcomes.

Keywords: BAG-1; breast cancer; clinical outcome; oestrogen receptor; apoptosis; prognosis; systematic review; meta-analyses

Breast cancer is the leading cause of cancer-related deaths in women worldwide (Ferlay et al., 2010). In 2012, 1.7 million women were diagnosed with breast cancer, and the incidence is predicted to continue to rise (Bray et al., 2012). Clinico-pathological parameters such as tumour grade, size and nodal burden used in combination with the four immunohistochemical biomarkers oestrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2) and Ki-67, known collectively as IHC4, are generally very effective in determining disease prognosis. Nevertheless, not all patients benefit from treatment and prediction of outcome could be improved. Therefore, additional molecular biomarkers might be used to effectively tailor therapies to specific breast cancer patient subgroups.

The anti-apoptotic protein Bcl-2-associated athanogene-1 (BAG-1) is frequently increased in breast cancer and pre-invasive breast disease compared to normal breast epithelium (Takayama et al, 1998; Brimmell et al, 1999). Bcl-2-associated athanogene-1 exists as three main isoforms, which are produced by alternative translation initiation from a single mRNA (Packham et al., 1997). Bcl-2-associated athanogene-1L is found in the nucleus, whereas BAG-1M and BAG-1S are generally found in the cytoplasm (Packham et al., 1997; Takayama et al., 1998; Brimmell et al., 1999; Schneikert et al., 1999; Yang et al., 1999; Knee et al., 2001), and the possibility of BAG-1 directed therapy has been suggested from laboratory studies (Sharp et al., 2009a, b; Enthammer et al., 2013). In the clinical setting, BAG-1 mRNA has been incorporated as a
prognostic biomarker in Oncotype DX (Paik et al., 2004) and PAM50 (Parker et al., 2009) multigene assays, which estimate prognosis following surgery, and can be used to assess the potential benefit of chemotherapy for breast cancer. Although the intensity and cytoplasmic/nuclear localisation of BAG-1 protein immunoreactivity has been related to tumour grade, disease subtype and clinical outcome, both positive and negative correlations with survival have been described.

To address the significance of BAG-1 as a biomarker in breast cancer, we have performed a systematic review against the REporting Recommendations for Tumour MARKer and Prognostic Studies (REMARK) criteria (McShane et al., 2005), which provide a framework for reporting of studies of cancer biomarkers. We have focused our critical appraisal on the most consistent findings in an attempt to clarify information relating BAG-1 protein expression and cellular distribution patterns to clinico-pathological parameters and early breast cancer clinical outcome, and have undertaken meta-analyses from the data where available.

MATERIALS AND METHODS

Search strategy and selection criteria. A computerised search according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) using MEDLINE and Embase databases through OVID Online (version: OvidSP_U103.17.00.125) was carried out in July 2016 using the MeSH terms ‘breast cancer’ AND 'Bag-1 protein’ with all appropriate subheadings included (Figure 1). Additional plain terms searches were performed and included a search of Scopus and Web of Science Core Collection databases. Reference lists within relevant articles were screened to identify articles not captured by the computerised search. Articles were screened for eligibility by two reviewers (ESP and TR) independently and any differences in judgment were resolved following discussion with a third reviewer (RIC). For inclusion in the review, articles had to report on the clinical significance of the level of BAG-1 expression in human studies and were assessed according to the criteria as set out in the REMARK guidelines (McShane et al., 2005). If articles had used the same data set, only the most recent article was included. No language barriers were imposed during our search and native speakers were used to overcome linguistic constraints.

Meta-analyses of data reporting clinical outcome according to BAG-1 status. Meta-analyses were performed for studies published in peer-reviewed journals that provided a hazard ratio (HR) and 95% confidence intervals (CIs) for disease-free survival (DFS) and/or breast cancer-specific survival (BCSS) from univariate analyses. Heterogeneity was assessed using the I² and χ² statistic. A fixed-effects models were used unless heterogeneity was significant (p<0.05), in which case random-effects models were used. Pooled effects were calculated using STATA 14.1 (StatCorp, College Station, TX, USA).

RESULTS

Study selection and characteristics. The literature search yielded 594 publications (Figure 1). After excluding reports that were out of the scope of our systematic review (articles had to report on the prognostic value of BAG-1 in breast cancer patients), 89 abstracts were reviewed and 58 that had used non-human samples were excluded. Thirty-one articles that assessed the prognostic value of BAG-1 expression in breast cancer patients were considered eligible for inclusion in the systematic review. Five were excluded because they were non-clinical, five because they did not address BAG-1 as a prognostic biomarker, one was a review article and one had overlapping patients. A retrospective analysis of BAG-1 expression in metastatic breast cancer in a prospective randomised phase three study was not included further in this review of early breast cancer, and did not demonstrate a role for BAG-1 in predicting response to chemotherapy in later-stage disease (Sjostrom et al., 2002). Eighteen studies that met the inclusion criteria were used in this systematic review. An additional two studies (Paik et al., 2004; Parker et al., 2009) include BAG-1 as part of composite scores associated with clinical outcome and so are also discussed. Studies included were published between 1999 and

Figure 1. PRISMA flowchart illustrating the selection methodology for eligible studies.
2016 and comprised 6363 patients (sample range 70–1971 patients) with an average follow-up period of 8.2 years ranging between 3.3 and 12.8 years (Table 1).

**Clinical study design.** Sixteen studies utilised cohorts of 100 or more patients (Tang et al, 1999; Turner et al, 2001; Townsend et al, 2002; Cutress et al, 2003; O’Driscoll et al, 2003; Sirvent et al, 2004; Tang et al, 2004; Yun et al, 2005; Lin et al, 2008; Nadler et al, 2008; Millar et al, 2009; Afentakis et al, 2013; Wang et al, 2014; Dowsett et al, 2015; Davidson et al, 2016) and 2 studies (Yang et al, 2008; Athanassiadou et al, 2009) recruited <100 patients. With the exception of one prospective immuno-cytochemical study (Athanassiadou et al, 2009), and two retrospective analyses of a prospective clinical trial (Afentakis et al, 2013; Dowsett et al, 2015), the studies associating BAG-1 expression with clinicopathological variables involve retrospective analysis of patient survival data (Table 2a and b). Although most studies had rigorous methodology, some had heterogeneous patient cohorts and did not take into account the many histological, analytical, clinical and treatment subgroups encountered in the management of breast cancer, which could potentially influence biomarker evaluation and validation (de Gramont et al, 2015).

**Assay methodology.** Two studies reported analysis of mRNA expression from published data sets: Millar et al (2009) from two studies (van de Vijver et al, 2002; Naderi et al, 2007) and Papadakis et al (2016) from a third (Curtis et al, 2012). Of the 18 studies, all clearly stated inclusion and/or exclusion criteria. Two studies (O’Driscoll et al, 2003; Dowsett et al, 2015) used RT-PCR to examine BAG-1 mRNA expression, whereas 15 studies examined BAG-1 protein expression by immunohistochemical staining (Tang et al, 1999, 2004; Turner et al, 2001; Townsend et al, 2002; Cutress et al, 2003; O’Driscoll et al, 2003; Sirvent et al, 2004; Yun et al, 2005; Lin et al, 2008; Yang et al, 2008; Athanassiadou et al, 2009; Millar et al, 2009; Afentakis et al, 2013; Wang et al, 2014; Davidson et al, 2016). Full-face sections from formalin-fixed paraffin-embedded (FFPE) tumours were used in 11 studies (Tang et al, 1999; Turner et al, 2001; Townsend et al, 2002; Cutress et al, 2003; O’Driscoll et al, 2003; Sirvent et al, 2004; Tang et al, 2004; Yun et al, 2005; Athanassiadou et al, 2009; Dowsett et al, 2015; Papadakis et al, 2016), whereas 7 studies utilised FFPE tissue microarrays (Lin et al, 2008; Nadler et al, 2008; Yang et al, 2008; Millar et al, 2009; Afentakis et al, 2013; Wang et al, 2014; Davidson et al, 2016). To address the issue of tumour heterogeneity involved with the use of tissue microarrays, two studies included in their analyses at least 2 cores taken from different sites from within the same breast tumour (Naderi et al, 2008; Millar et al, 2009). Fourteen studies (Tang et al, 1999, 2004; Townsend et al, 2002; Cutress et al, 2003; Sirvent et al, 2004; Yun et al, 2005; Lin et al, 2008; Yang et al, 2008; Athanassiadou et al, 2009; Millar et al, 2009; Afentakis et al, 2013; Wang et al, 2014; Davidson et al, 2016) assessed staining intensity using a subjective scoring system based on the H-score technique (a summation of the percentage of area stained at 1, 2, or 3; in intensity level multiplied by the weighted intensity of staining 1, 2, or 3; where 0 is no staining, 1 is weak staining, 2 is moderate staining and 3 is strong staining; McCarty et al, 1986), whereas 1 used automated quantitative analysis (AQUA; Nadler et al, 2008). Nine studies examined the nuclear and/or cytoplasmic pattern of BAG-1 staining (Tang et al, 1999, 2004; Turner et al, 2001; Townsend et al, 2002; Cutress et al, 2003; Naderi et al, 2008; Athanassiadou et al, 2009; Millar et al, 2009; Afentakis et al, 2013), while six studies (Sirvent et al, 2004; Yun et al, 2005; Lin et al, 2008; Yang et al, 2008; Wang et al, 2014; Davidson et al, 2016) reported only on the total expression levels of BAG-1. Most studies did not use a separate control specimen of normal breast epithelium, but scored the normal cells adjacent to the tumour within biopsies. Only two studies (Yun et al, 2005; Millar et al, 2009) included normal breast epithelium specimens of which one (Millar et al, 2009) reported staining scores. Assay controls comprised specimens incubated with secondary but no anti-BAG-1 primary antibody or tumour sections that exhibited no BAG-1 immunoreactivity. Fifteen studies applied both univariate and multivariate analyses (Tang et al, 1999, 2004; Turner et al, 2001; Townsend et al, 2002; Cutress et al, 2003; O’Driscoll et al, 2003; Sirvent et al, 2004; Naderi et al, 2008; Athanassiadou et al, 2009; Afentakis et al, 2013; Wang et al, 2014; Davidson et al, 2016; Papadakis et al, 2016), whereas three studies used only univariate analysis (Yun et al, 2005; Lin et al, 2008; Dowsett et al, 2015). Seven studies included relative risk or HR with CIs for outcome (Cutress et al, 2003; Sirvent et al, 2004; Nadler et al, 2008; Millar et al, 2009; Afentakis et al, 2013; Dowsett et al, 2015; Papadakis et al, 2016).

**BAG-1 mRNA and outcome.** Association between BAG-1 mRNA levels with survival suggested correlation between increased expression and better survival in most studies (Table 2b). Interestingly, Townsend et al (2002) found no correlation between BAG-1 mRNA levels by in situ hybridisation and BAG-1 protein levels. Millar et al (2009) examined publically available gene expression data sets from studies by van de Vijver et al (2002) and Naderi et al (2007) to demonstrate a strong correlation between BAG-1 mRNA levels and improved survival outcome. Papadakis et al (2016) examined a publically available gene expression data set from a study by Curtis et al also demonstrating a correlation between BAG-1 mRNA and improved outcome. In contrast, O’Driscoll et al (2003) showed no significant correlation with tumour stage or treatment, and disease outcome. Dowsett et al (2015), in a study investigating individual genes of Oncotype Dx in 1125 patients from the ATAC study, found that BAG-1 expression was associated with better outcome in all patients over 10 years both in terms of all recurrences (HR: 0.70; 95% CI: 0.58–0.85) and distant recurrences (HR: 0.66; 95% CI: 0.53–0.83).

Parker et al (2009) included BAG-1 mRNA in a 50 gene classifier (PAM50) of breast cancer intrinsic subtype, and Paik et al (2004) included BAG-1 as 1 of 16 cancer-related genes in a multigene (Oncotype Dxs) assay to predict recurrence in node-negative patients treated with tamoxifen. In both studies, BAG-1 mRNA was part of a composite score (PAM50 for intrinsic subtypes, or Recurrence score), that was correlated to outcome. In the recurrence score, BAG-1 carries a minus sign in the algorithm, indicating that it is associated with a reduced risk of recurrence (Paik et al, 2004), and this is consistent with the data of Dowsett et al (2015).

**Bag-1 protein expression pattern.** Overall, the studies reported a high percentage of cells expressing BAG-1 within breast carcinomas, with five exhibiting positive staining for BAG-1 in >70% of tumours (Tables 2a and 2b). Six of the fourteen immunohistochemical studies showed a pattern of higher cytoplasmic than nuclear BAG-1 expression with 5 of these exhibiting at least a twofold difference. Only two studies gave a value for mixed staining (Tang et al, 1999; Athanassiadou et al, 2009). One study carried out on a relatively homogeneous cohort of 3 patients treated with surgery, followed by adjuvant hormone therapy but not chemotherapy, showed higher nuclear than cytosolic BAG-1 staining (Cutress et al, 2003). Sixty per cent of the tumours from this cohort were positive for ER and PgR, which is a clinical indicator of ER function. One study failed to give any precise subcellular analysis, but stated that staining for BAG-1 was mixed, with more cytosolic than nuclear BAG-1 in breast carcinomas (Naderi et al, 2008).

Turner et al (2001) reported staining for nuclear but not cytosolic BAG-1 in 25 of 88 (28%) normal breast epithelium specimens with H-scores ≥150. In the same study, high levels of cytoplasmic or nuclear BAG-1 immunostaining were present in 9 of 14 (64%) and 7 of 14 (50%) ductal carcinoma in situ (a pre-invasive form of breast cancer) specimens, respectively. Positive
BAG-1 staining is also found in the ductal carcinoma in situ component of some ER+ tumours, suggesting that upregulation of BAG-1 can occur relatively early in tumourigenesis and may be dependent on hormonal status.

Association of Bag-1 protein with clinicopathological features and outcome. In most studies that found significance (Table 2a), high levels of BAG-1 protein expression in invasive breast carcinoma positively correlate with improved patient survival outcomes or improved prognosis (Turner et al, 2001; Catress et al, 2003; Yun et al, 2005; Lin et al, 2008; Nadler et al, 2008; Millar et al, 2009; Mentakis et al, 2013). Preliminary observations by Krajewski et al (1999) were superseded by a subsequent study from

The same group. In 122 patients (41% ER+), Turner et al (2001) reported upregulation of immunoreactivity for cytoplasmic BAG-1 staining in early-stage breast cancers compared to normal breast epithelium (Turner et al, 2001). High cytoplasmic but not nuclear BAG-1 levels also associated significantly with improved overall survival and distant metastasis-free survival overall (stages I and II) and in node-negative (stage I) only patients based on univariate and multivariate analyses using Cox proportional hazards models with variables including BAG-1, Bcl-2, ER and stage (Turner et al, 2001). Bcl-2-associated athanogene-1 remained a strong predictor of overall survival independently of adjuvant therapy. In addition, stage was significantly associated with distant metastasis-free survival and overall survival in multivariate analysis. There was

Table 1. Studies of BAG-1 expression in breast cancer

| Patients | Disease stage | Disease subtype | Co-morbidities | Inclusion/ exclusion criteria | Treatment received | Treatment randomised | Specimen | Type | Controls | Assay | Antibody | Histopathology score | Controls | Blinded? | Study | Type | Period of follow-up in years (median) | Clinical endpoints defined | Randomisation for sample size | Statistical analysis |
|----------|---------------|----------------|---------------|-------------------------------|-------------------|---------------------|----------|------|----------|-------|----------|---------------------|----------|---------|------|------|------------------------------|-------------------------|------------------------|---------------------|
|          |               |                |               |                               |                   |                     |          |      |          |       |          |                     |          |         |      |      |                             |                        |                       |                     |
no significant relationship in univariate analysis between ER, PgR or HER2 and survival, although there was a trend towards better survival rates in women with ER+ tumours. A statistically significant positive correlation of cytosolic BAG-1 immunostaining with Bcl-2 expression was found in 62 of 76 (82%) breast tumours coexpressing these proteins, suggesting that BAG-1 and Bcl-2 may be coregulated to some extent in early-stage invasive breast cancers (Turner et al., 2001).

Tang et al. (2004) (Paik et al., 2004) also reported strong immunoreactivity for BAG-1 in the cytoplasm but low in the nucleus in high-grade tumours. The authors reasoned that weak nuclear BAG-1 expression observed in this cohort may be due to the presence of a high proportion (58%) of poorly differentiated tumours. No correlation was found between cytoplasmic BAG-1 expression with disease-free or overall survival, and further subgroup analysis was precluded as the power to detect any real difference was deemed quite low. Spearman’s ρ analysis revealed a correlation between BAG-1 expression and that of Bcl-2, p53, ER and PgR, and the better differentiation of breast carcinoma. Correlation was significant between BAG-1 expression and that of ER, Bcl-2 pattern and intensity and differentiation in univariate analysis, whereas expression of BAG-1 significantly correlated only with that of ER in multivariate analysis. In contrast, previously published data from the same group (Tang et al., 1999) showed no correlation between the expression patterns of BAG-1 and that of ER or PgR in invasive breast carcinoma; this could be due to the lower proportion of ER+ and PgR+ tumours, missing data about receptor status, or the smaller cohort size. However, BAG-1 staining correlated with differentiation. Total BAG-1 staining significantly correlated with shorter disease-free and overall survival in multivariate analysis. Moreover, patients whose tumours had high nuclear BAG-1 expression had a trend towards shorter disease-free and overall survival (Tang et al., 1999). These findings are supported by Athanassiadou et al. (2009), who showed that nuclear expression of BAG-1 was associated with lower survival rates compared to positive staining. Positive overall staining for BAG-1 was associated with lower 5-year survival rates compared to negative staining. In univariate analysis, nuclear BAG-1 staining was correlated with worse prognostic indicators (stages III–IV, tumour size > 5 cm and presence of four or more positive lymph nodes) compared to cytoplasmic staining (stage II, tumour size 2–5 cm and one to four positive lymph nodes). No significant correlation was found between ER and PgR status and BAG-1 staining pattern (Athanassiadou et al., 2009).

Nadler et al. (2008) found no difference in all prognostic variables between nuclear and cytoplasmic BAG-1, but correlated total BAG-1 with significantly improved survival outcomes in node-positive patients by univariate analysis, whereas in multivariate analysis, BAG-1 did not retain its independent prognostic value. Histological grade and treatment information were not given. Moreover, Spearman’s ρ analysis revealed a significant association between BAG-1 with Bcl-2, ER and PgR prognostic markers.
Millar et al (2009) also found that high levels of nuclear and cytoplasmic BAG-1 were significantly associated with improved prognosis for local recurrence, distant metastases and cancer-specific death in univariate analysis. Nuclear and cytoplasmic BAG-1 expression was associated with low-grade tumours, ER and PgR positivity, and improved overall survival but was negatively correlated with HER2 and the triple-negative phenotype. Subtype analysis revealed that high nuclear BAG-1 expression alone is an independent predictor of outcome of ER+ tumours and correlates strongly with a luminal A intrinsic phenotype in both univariate and multivariate analyses; nuclear BAG-1 staining did not associate with outcome in univariate analysis of ER-negative tumours. Treatment of patients with tumours exhibiting high nuclear BAG-1 expression with tamoxifen showed an improved outcome for local recurrence, distant metastases and breast cancer-specific death (Millar et al, 2009). Similar findings were reported by Cutress et al (2003), who showed that high nuclear BAG-1 staining is a marker of good prognosis in a relatively homogeneous cohort of node-negative, ER+ patients treated with hormonal therapy (tamoxifen or anastrozole) but not chemotherapy after tumour resection. A strong inverse correlation was found between nuclear BAG-1 expression and tumour size, whereas ERz and PgR expression moderately correlated with nuclear and (to a lesser extent) with cytoplasmic BAG-1 expression. Taken together, the data by Cutress et al (2003) and Millar et al (2009) are consistent with the role of BAG-1 as a prognostic biomarker in the oncotype DX assay, and demonstrate that a high nuclear BAG-1 expression identifies a group of breast cancers with good prognosis and with enhanced sensitivity to hormonal therapy.

In line with these data, recent analysis of the TransATAC clinical trial cohort (Afentakis et al, 2013) and retrospective (Gee et al, 2010) studies in ER+ early breast cancer treated with hormonal therapy but not chemotherapy, show that expression of BAG-1 significantly associates with that of ER and PgR, and correlates with tumour grade. Bcl-2-associated athanogene-1 status is a more powerful marker than either Ki-67 or HER2 in relation to disease-free interval and than HER2 for survival in multivariate analysis (Gee et al, 2010). Moreover, nuclear BAG-1 immunoreactivity exhibits significant value for estimating a residual risk that is independent of standard clinical and immunohistochemical parameters, particularly in node-positive patients (Afentakis et al, 2013).

Meta-analyses of BAG-1 expression and outcome. In general, data were too heterogeneous, and outcome measures were too varied to perform meta-analyses for the majority of studies. Meta-analyses of mRNA expression from the two data sets analysed in Millar et al (2009) and the data set analysed in Papadakis et al (2016) including a total of 2422 patients produced a HR of 0.55 (95% CI 0.36–0.85) favouring improved BCSS with high expression of BAG-1 (Figure 2a). Similarly of the two studies (336 patients;...
Figure 2b) reporting pathologist assessment of nuclear BAG-1, improved BCSS was observed with high BAG-1 (HR 0.36; 95% CI 0.23–0.55). Nadler et al (2008) was not included in this analysis, as a different (automated) method of assessment of BAG-1 expression was used to the other immunohistochemical studies. Sensitivity analysis suggests that the result for nuclear BAG-1 and BCSS becomes non-significant with the inclusion of this study. Of the two studies (1239 patients; Figure 2c) reporting nuclear BAG-1 and BCSS, improved outcome was seen with high BAG-1 (HR 0.70; 95% CI 0.59–0.84).

DISCUSSION

As previous review (Cutress et al, 2002), evidence supporting the hypothesis that BAG-1 plays an important role in breast cancer has increased. The development of high-throughput assays, such as Oncotype DX and PAM50, reveal that increased BAG-1 mRNA is associated with a low risk of recurrence and improved prognosis. In addition, a large RT-PCR study of a clinical trial cohort is also consistent with this (Dowsett et al, 2015). Similarly, recent retrospective and clinical trial immunohistochemical studies of large patient cohorts show that increased BAG-1 expression associates significantly with that of ER and PgR and with histological grade. Moreover, high nuclear BAG-1 immunoreactivity is an independent predictor of outcome particularly in patients with ER+ early breast cancer receiving adjuvant hormonal therapy, and enhances the predictive power of IH4 staining. Including BAG-1 immunohistochemical staining as a standard biomarker in the clinic may therefore help to better stratify patients according to their risk of disease recurrence and determine their probability of responding to therapy.

A recent study assessed the possibility of performing immunohistochemical staining on a panel of 10 gene products included in Oncotype DX to reduce the number of patients requiring testing due to the increased cost of using this assay (Ingoldsby et al, 2013). Classification and regression tree analysis correctly classified 77% of cases into TAILORx categories based on nuclear pleomorphism, survivin, cyclin B1 and BAG-1. Staining ER+ breast cancer subtypes in a clinical pathology laboratory for BAG-1 may therefore help to identify individuals who will respond better to hormonal therapy without the need for unnecessary chemotherapy.

The concept that BAG-1, a protein that supports cancer cell survival, is related to improved patient survival may seem paradoxical. This observation, however, is not without precedent as both the ER, and Bcl-2, another anti-apoptotic protein, is also associated with good prognosis in breast cancer.

The controversy surrounding expression patterns and intensity of BAG-1 staining is apparent. Nevertheless, differences introduced by patient cohort heterogeneity in terms of histological type and number, tumour grade and treatment, the different antigen retrieval methods and antibodies used and the threshold chosen for judging positive staining may account for some of the differences between studies. For example, of studies that utilised an anti-BAG-1 monoclonal antibody, the majority demonstrated either a positive correlation between BAG-1 expression and outcome (Turner et al, 2001; Cutress et al, 2003; Lin et al, 2008; Millar et al, 2009; Afentakis et al, 2013) or a trend to this that was not significant (Sirvent et al, 2004), or a positive correlation in node-positive patients (Nadler et al, 2008). In contrast, three immunohistochemical monoclonal antibody studies did not demonstrate a correlation (Tang et al, 2004; Yang et al, 2008; Wang et al, 2014).

Although the meta-analyses, consistent with inclusion of BAG-1 in Oncotype DX and PAM50, suggested association between BAG-1 and clinical outcome, it was not possible to include many of the reported studies in the meta-analyses due to the lack of available data. However, studies that could not be included in the meta-analyses included four that reported significant positive correlations between BAG-1 expression and outcome (Turner et al, 2001; Sirvent et al, 2004; Afentakis et al, 2013) and two with a trend towards a positive correlation that was not significant (Townsend et al, 2002; Sirvent et al, 2004), consistent with the meta-analyses. In contrast, five reported no correlation with BAG-1 expression and outcome (O’Driscoll et al, 2003; Tang et al, 2004; Yang et al, 2008; Wang et al, 2014; Davidson et al, 2016), and two reported a negative correlation with outcome (Tang et al, 1999; Athanassiadou et al, 2009), one of which was an immunocytochemical rather than immunohistochemical study (Athanassiadou et al, 2009). Overall, from all studies, and consistent with the meta-analyses and RT-PCR studies, our interpretation is that the most consistent finding appeared to be a positive correlation with outcome in those with high BAG-1 levels.

To explain the clinical association observed between BAG-1 expression and localisation in breast cancer with other clinicopathological parameters such as ER expression, sensitivity to tamoxifen and prolonged patient survival, some studies have used breast cancer cell line models. The impact of BAG-1 on patient survival may depend partly on the regulation of ER function, particularly at an early stage of the disease. Targeting BAG-1S or BAG-1M to the nucleus fails to enhance ER transcriptional activity; however, BAG-1L is capable of achieving this particularly in the presence of oestrogens. As lifetime exposure to oestrogens is a significant risk factor for breast cancer development, BAG-1L may increase this through its sensitising effects on ERα and ERβ.

Table 2b. RT-PCR studies showing relationship between BAG-1 expression and prognostic markers

| Reference | BAG-1-positive samples (%) | Correlation | Univariate P (N/C/B) | Multivariate P (N/C/B) |
|-----------|----------------------------|-------------|----------------------|------------------------|
| O’Driscoll et al, 2003 | 80.9 | NS | NS | NS |
| Millar et al, 2009 (using data from van de Vijver et al, 2002) | 79.3 | Positive | OS; \( P = 0.005 \) | NS |
| Millar et al, 2009 (using data from Naderi et al, 2007) | 80.0 | Positive | OS; \( P = 0.0120/0.0151 \) | NS |
| Dowsett et al, 2015 | NG | Positive | AR; HR: 0.70; 95% CI: 0.58–0.85 | NG |
| Papadakis et al, 2016 (using data from Curtis et al, 2012) | NG | Positive | BCSS; \( P = 0.001 \) | BCSS; \( P = 0.022 \) HR: 0.81; 95% CI: 0.67–0.97 |

Abbreviations: AR = any recurrence; BCSS = breast cancer-specific survival; CI = confidence interval; DR = distant recurrence; HR = hazard ratio; NG = not given; NS = not significant; OS = overall survival.

www.bjcancer.com | DOI:10.1038/bjc.2017.130
notion is supported by evidence that anti-oestrogen therapies alter the sensitivity of BAG-1 overexpressing ER+ cells to cell cycle arrest, whereas downregulation of BAG-1 expression enhances the sensitivity of tamoxifen-resistant MCF-7 cells to tamoxifen (Liu et al., 2014). It should be noted that all BAG-1 isoforms are produced from a single mRNA, and all antibodies used in these studies recognise all BAG-1 isoforms so it is not possible to comment on the significance of the individual BAG-1 isoforms.

**Figure 2.** Meta-analyses of: (A) BAG-1 mRNA (high v low) for BCSS. Data for van de Vijver et al., and Naderi et al., are from analyses published in Millar et al (2009), and for Curtis et al., are from analysis included in Papadakis et al., and 95% CI obtained from the authors of Papadakis et al (2016) (B) nuclear BAG-1 protein by immunohistochemistry (high v low) for BCSS; and (C) nuclear BAG-1 protein by immunohistochemistry (high v low) for DFS.

| Study          | Number | HR (95% CI) | Weight |
|----------------|--------|-------------|--------|
| Curtis et al   | 1992   | 0.75 (0.62, 0.89) | 45.90  |
| van de Vijver et al | 295   | 0.44 (0.28, 0.70) | 31.94  |
| Naderi et al   | 135    | 0.41 (0.21, 0.84) | 22.16  |
| Overall (I-squared = 69.2%, P = 0.039) |        | 0.55 (0.36, 0.85) | 100.00 |
| Note: Weights are from random-effects analysis |

| Study          | Number | HR (95% CI) | Weight |
|----------------|--------|-------------|--------|
| Cutress et al  | 122    | 0.33 (0.14, 0.81) | 24.17  |
| Miller et al   | 214    | 0.36 (0.22, 0.60) | 75.83  |
| Overall (I-squared = 0.0%, P = 0.849) |        | 0.36 (0.23, 0.55) | 100.00 |

| Study          | Number | HR (95% CI) | Weight |
|----------------|--------|-------------|--------|
| Afentakis et al | 963    | 0.72 (0.60, 0.87) | 90.36  |
| Miller et al   | 276    | 0.56 (0.32, 0.99) | 9.64   |
| Overall (I-squared = 0.0%, P = 0.407) |        | 0.70 (0.59, 0.84) | 100.00 |
isofoms. It is tempting to speculate based on the cell line evidence that the nuclear localised BAG-1L isoform could be a more powerful prognostic and predictive biomarker than total or nuclear BAG-1 expression. Studies utilising BAG-1L-specific antibodies in large patient cohorts stratified based on disease subtype, treatment and clinicopathological characteristics should address this hypothesis.

Although the findings should be interpreted with caution due to the number of studies that could not be included in the meta-analyses, overall and despite heterogeneity between studies, this systematic review and meta-analyses suggest that increased expression of BAG-1 mRNA and BAG-1 protein, and in particular nuclear expression, appears associated with improved breast cancer outcomes.

ACKNOWLEDGEMENTS

This work was funded by Cancer Research UK and Breast Cancer Campaign (grant reference number: 2011NoPR39). T Reeves was supported by the University of Southampton National Institute of Health Research Academic Foundation Programme. We thank Dr L Forse for critical reading of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Afentakis M, Dowsett M, Sestak I, Salter J, Howell T, Buzdar A, Forbes J, Cuzick J (2013) Immunohistochemical BAG1 expression improves the estimation of residual risk by IHC4 in postmenopausal patients treated with anastrozole or tamoxifen: a TransATAC study. Breast Cancer Res Treat 140(2): 253–262.

Athanassiadou P, Grapsa D, Gonidi M, Athanassiadou AM, Tsipis A, Patsouris E (2009) CD24 expression has a prognostic impact in breast carcinoma. Pathol Res Pract 205(8): 524–533.

Bray F, Jemal A, Grey N, Ferlay J, Forman D (2012) Global cancer transitions according to the Human Development Index (2008–2030): a population-based study. Lancet Oncol 13(8): 790–801.

Brimmell M, Burns JS, Munson P, McDonald L, O’Hare MJ, Lakhani SR, Packham G (1999) High level expression of differentially localized BAG-1 isoforms in some oestrogen receptor-positive human breast cancers. Br J Cancer 81(6): 1042–1051.

Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, Speed D, Lynch AG, Samarajiwa S, Yuan Y, Graf S, Ha G, Hafiri G, Bashashati A, Russell R, McKinney S, Langerod A, Green A, Provenzano E, Wishart G, Pinder S, Watson P, Markowitz F, Murphy L, Ellis I, Purushotham A, Borresen-Dale AL, Brenton JD, Tavare S, Caldas C, Aparicio S (2012) The genomic and transcriptomic architecture of 2000 breast tumours reveals novel subgroups. Nature 486(7403): 346–352.

Cutrell RS, Townsend PA, Brimmell M, Bateman AC, Hague A, Packham G (2002) BAG-1 expression and function in human cancer. Br J Cancer 87(8): 834–839.

Cutrell RS, Townsend PA, Sharp A, Maison A, Wood L, Lee R, Brimmell M, Millea MA, Johnson PW, Royle GT, Bateman AC, Packham G (2003) The nuclear BAG-1 isoform, BAG-1L, enhances oestrogen-dependent transcription. Oncogene 22(32): 4973–4982.

Davidson B, Valborg Reintertsen K, Trinh D, Reed W, Bohler PJ (2016) BAG-1/SODD, HSP70 and HSP90 are potential prognostic markers of poor survival in node-negative breast carcinoma. Hum Pathol 54: 64–73.

de Gramont A, Watson S, Ellis LM, Rodon J, Tabernero J, de Gramont A, Hamilton SR (2015) Pragmatic issues in biomarker evaluation for targeted therapies in cancer. Nat Rev Clin Oncol 12(4): 197–212.

Dowsett M, Sestak I, Buus R, Lopez-Knowles E, Mallon E, Howell A, Forbes JF, Buzdar A, Cuzick J (2015) Estrogen receptor expression in 21-gene recurrence score predicts increased late recurrence for estrogen-positive/HER2-negative breast cancer. Clin Cancer Res 21(12): 2763–2770.

Enthammer M, Papadakis ES, Salome Gachet M, Deutsch M, Schwaiger S, Kharel K, Ashraf MI, Khalid S, Wolger G, Packham G, Cutrell RS, Stuppner H, Troppmair J (2013) Isolation of a novel thioloavin S-derived compound that inhibits BAG-1-mediated protein interactions and targets BRAF inhibitor-resistant cell lines. Mol Cancer Ther 12(11): 2400–2414.

Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 127(12): 2893–2917.

Gee JM, Aleskandarany MA, Finlay P, Farrow L, Nicholson RI, Habashy HO, Green AR, Rahman EA, Powe DG, Jasani B, Barrett-Lee PJ, Robertson JF, Shaw VE, Ellis IO (2010) Immunohistochemical markers progesterone receptor, HER2, Ki67 & bcl-2 associated athanogene 1 and prediction of adjuvant tamoxifen treatment outcome in ER+ early breast cancer. Cancer Res 70: (abstract P2-09-37).

Goeldeby H, Webber M, Wall D, Scarlett C, Newell J, Callagy G (2013) Prediction of Oncotype DX and TAILORx risk categories using histopathological and immunohistochemical markers by classification and regression tree (CART) analysis. Breast 22(5): 879–886.

Knee DA, Froesch BA, Nuber U, Takayama S, Reed JC (2001) Structure-function analysis of Bag1 proteins. Effects on androgen receptor transcriptional activity. J Biol Chem 276(16): 12718–12724.

Krajewski S, Krajewska M, Turner BC, Pratt C, Howard B, Zapata JM, Frenkel V, Robertson S, Ionov Y, Yamamoto H, Perucho M, Takayama S, Reed JC (1999) Prognostic significance of apoptosis regulators in breast cancer. Endocr Relat Cancer 6(1): 29–40.

Lin YD, Wang X, Yang YR, Wang MY, Wu DZ, Du JL (2008) Expression of BAG-1 and its clinical significance in breast cancer. [Chinese]. Chin J Clin Oncol Prev Treat 15(19): 1466–1468 + 1480.

Liu H, Lu S, Gu L, Gao Y, Wang T, Zhao J, Rao J, Chen J, Hao X, Tang SC (2014) Modulation of BAG-1 expression alters the sensitivity of breast cancer cells to tamoxifen. Cell Physiol Biochem 33(2): 365–374.

Mccarty Jr KS, Szabo E, Flowers JL, Cox EB, Leight GS, Miller L, Konrath J, Soper JT, Budwit DA, Creasman WT, Seigler HF, Mccarty Sr KS et al. (1986) Use of a monoclonal anti-estrogen receptor antibody in the immunohistochemical evaluation of human tumors. Cancer Res 46(8 Suppl): 4244s–4248s.

McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM (2005) Reporting recommendations for tumor MARker prognostic studies (REMARK). Nat Clin Pract Oncol 2(8): 416–422.

Miller EK, Anderson LR, McNeil CM, O'Toole SA, Pinese M, Crea P, Morey AL, Biankin A, Henshall SM, Musgrove EA, Sutherland RL, Butt AJ (2009) BAG-1 predicts patient outcome and tamoxifen responsiveness in ER-positive invasive ductal carcinoma of the breast. Br J Cancer 100(1): 123–133.

Naderi A, Thesendorf AR, Barbosa-Morais NL, Pinder SE, Green AR, Powe DG, Robertson JF, Aparicio S, Ellis IO, Brenton JD, Caldas C (2007) A gene-expression signature to predict survival in breast cancer across independent data sets. Oncogene 26(10): 1507–1516.

Nadler T, Camp RL, Giltnane JM, Moeder C, Rimm DL, Kluger HM, Kluger Y (2008) Expression patterns and prognostic value of Bag-1 and bcl-2 in breast cancer. Breast Cancer Res 10(2): R35.

O’Driscoll L, Linehan R, Kennedy S, Cronin D, Purcell R, Glyns S, W McDermott E, D Hill A, J O’Higgins N, Parkinson M, Clynne M (2003) Lack of prognostic significance of survivin, survivin-deltaEx3, survivin-2B, galecin-3, bag-1, bax-alpa and MRP-1 mRNAs in breast cancer. Cancer Lett 212(2): 225–236.

Packham G, Brimmell M, Cleveland JL (1997) Mammalian cells express two differently localized Bag-1 isoforms generated by alternative translation initiation. Biochem J 328(Pt 3): 807–813.

Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, Baehner FL, Walker MG, Watson D, Park T, Hiller W, Fisher ER, Wickerham DL, Bryant J, Wolmark N (2004) A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 351(27): 2817–2826.

Papadakis E, Robson N, Yeomans A, Bailey S, Laversin S, Beers S, Sayan AE, Ashton-Key M, Schwaiger S, Stuppner H, Troppmair J, Packham G, Cutrell R (2016) A combination of trastuzumab and BAG1 inhibition synergistically targets HER2 positive breast cancer cells. Oncotarget 7(14): 18851–18864.

Parkers JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, Davies S, Forbes C, He X, Hu Z, Quackenbush P, Stillman JJ, Pizzolo J, Mordon JS, Nobel AB, Mardis E, Nielsen TO, Ellis MJ, Perou CM, Bernard PS (2009) Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 27(8): 1160–1167.
Schneikert J, Hubner S, Martin E, Cato AC (1999) A nuclear action of the eukaryotic cochaperone RAP46 in downregulation of glucocorticoid receptor activity. J Cell Biol 146(5): 929–940.

Sharp A, Crabb SJ, Johnson PW, Hague A, Cutress R, Townsend PA, Ganesan A, Packham G (2009a) Thioflavin S (NSC71948) interferes with Bcl-2-associated athanogene (BAG-1)-mediated protein-protein interactions. J Pharmacol Exp Ther 331(2): 680–689.

Sharp A, Cutress RI, Johnson PW, Packham G, Townsend PA (2009b) Short peptides derived from the BAG-1 C-terminus inhibit the interaction between BAG-1 and HSC70 and decrease breast cancer cell growth. FERS Lett 583(21): 3405–3411.

Sirvent JJ, Aguilar MC, Olona M, Pelegri A, Blazquez S, Gutierrez C (2004) Prognostic value of apoptosis in breast cancer (pT1-pT2). A TUNEL, p53, bcl-2, bag-1 and Bax immunohistochemical study. Histol Histopathol 19(3): 759–770.

Sjoström J, Blomqvist C, Von Boguslawski K, Bengtsson NO, Mjaaland I, Malmstrom P, Ostenstad B, Wist E, Valvere V, Takayama S, Reed JC, Saksela E (2002) The predictive value of bcl-2, bax, bcl-xl, bag-1, fas, and fasL for chemotherapy response in advanced breast cancer. Clin Cancer Res 8(3): 811–816.

Takayama S, Krajewski S, Krajewska M, Kitada S, Zapata JM, Kochel K, Knee D, Scudiero D, Tudor G, Miller GI, Miyashita T, Yamada M, Reed JC (1998) Expression and location of Hsp70/Hsc-binding anti-apoptotic protein BAG-1 and its variants in normal tissues and tumor cell lines. Cancer Res 58(14): 3116–3131.

Tang SC, Beck J, Murphy S, Chernenko G, Robb D, Watson P, Khalifa M (2004) BAG-1 expression correlates with Bcl-2, p53, differentiation, estrogen and progesterone receptors in invasive breast carcinoma. Breast Cancer Res Treat 84(3): 203–213.

Tang SC, Shehata N, Chernenko G, Khalifa M, Wang X, Shahata N (1999) Expression of BAG-1 in invasive breast carcinomas. J Clin Oncol 17(6): 1710–1719.

Townsend PA, Dublin E, Hart IR, Kao RH, Hanby AM, Cutress RI, Poulson R, Ryder K, Barnes DM, Packham G (2002) BAG-1 expression in human breast cancer: interrelationship between BAG-1 RNA, protein, HSC70 expression and clinico-pathological data. J Pathol 197(1): 51–59.

Turner BC, Krajewski S, Krajewska M, Takayama S, Gumbs AA, Carter D, Rebbeck TR, Haffty BG, Reed JC (2001) BAG-1: a novel biomarker predicting long-term survival in early-stage breast cancer. J Clin Oncol 19(4): 992–1000.

van de Vijver MJ, He YD, van’t Veer LJ, Dai H, Hart AA, Voskuil DW, Schreiber GJ, Peterse JL, Roberts C, Marton MJ, Parrish M, Atsma D, Witteveen A, Glas A, Delahaye L, van der Velde T, Bartelink H, Rodenhuis S, Rutgers ET, Friend SH, Bernards R (2002) A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 347(25): 1999–2009.

Wang R, Liu X, Lu S, Gu L, Xiang R, Liu H (2014) BAG-1, EGFR, and PARP-1 expressions in breast cancer and their clinical significance. [Chinese]. Chin J Clin Oncol 41(13): 866–871.

Yang X, Hao Y, Ding Z, Pater A, Tang SC (1999) Differential expression of antiapoptotic gene BAG-1 in human breast normal and cancer cell lines and tissues. Clin Cancer Res 5(7): 1816–1822.

Yun J, Wang L, Wang T, Ling R, Yi J (2005) Effect of bag-1 expression on the occurrence of breast cancer and its 5-year survival rate. Chin J Clin Rehabil 9(22): 248–249.