Article:
Romanska, HM, Potemski, P, Krakowska, M et al. (8 more authors) (2015) Lack of CD151/integrin alpha 3 beta 1 complex is predictive of poor outcome in node-negative lobular breast carcinoma: opposing roles of CD151 in invasive lobular and ductal breast cancers. British Journal of Cancer, 113 (9). pp. 1350-1357. ISSN 0007-0920

https://doi.org/10.1038/bjc.2015.344

Lack of CD151/integrin α3β1 complex is predictive of poor outcome in node–negative lobular breast carcinoma: opposing roles of CD151 in invasive lobular and ductal breast cancers.

Hanna M. Romanska[1§], Piotr Potemski[2], Magdalena Krakowska[2], Magdalena Mieszkowska[3], Shalini Chaudhri[4], Radzisław Kordek[1*], Robert Kubiak[1], Valerie Speirs[6], Andrew M. Hanby[6], Rafał Sadej[3§], and Fedor Berditchevski[5]

[1]Department of Pathology, Medical University of Łódź, ul. Pomorska 251, 92-213 Łódź, Poland, [2]Department of Chemotherapy, Medical University of Łódź and M. Kopernik Memorial Hospital, ul. Paderewskiego 4, 93-509 Łódź, Poland; [3]Department of Molecular Enzymology, Intercollegiate Faculty of Biotechnology, Medical University of Gdańsk, ul. Dębinki 1, 80-210 Gdansk, Poland; [4]Department of Cellular Pathology, University of Birmingham, Birmingham UK; [5]School of Cancer Sciences of the University of Birmingham, Birmingham B15 2TT, UK; [6]Leeds Institute of Cancer and Pathology, University of Leeds, Leeds LS9 7TF, UK.

Running title: CD151/α3β1 in lobular breast carcinoma.

[§]Corresponding authors: Dr HM Romanska, Department of Pathology, Medical University of Łódź, 92-213 Łódź, Poland, e-mail: hanna.romanska@gmail.com; Dr R Sadej, Department of Molecular Enzymology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, 80-210 Gdansk, Poland, e-mail: rsadej@gumed.edu.pl

ABSTRACT

Background: The proposed involvement of CD151 in breast cancer (BCa) progression is based on findings from studies in invasive ductal carcinoma (IDC). IDC and invasive lobular carcinoma (ILC) represent distinct disease entities. Here we evaluated clinical significance of CD151 alone and in association with integrin α3β1 in patients with ILC in context of the data of our recent IDC study.

Methods: Expression of CD151 and/or integrin α3β1 was evaluated in ILC samples (No=117) using immunohistochemistry. The findings were analysed in relation to our results from an IDC cohort (No=182) demonstrating a prognostic value of an expression of CD151/integrin α3β1 complex in patients with HER2-negative tumours.

Results: Unlike in the IDCs, neither CD151 nor CD151/α3β1 complex showed any correlation with any of the ILC characteristics. Lack of both CD151 and α3β1 was significantly correlated with poor survival ($p = 0.034$) in lymph node negative ILC N(-) cases. $CD151^{-}/\alpha3\beta1^{-}$ patients had 3.12-fold higher risk of death from BCa in comparison to the rest of the ILC N(-) patients.

Conclusions: Biological role of CD151/α3β1 varies between ILC and IDC. CD151/α3β1 assessment might help to identify ILC N(-) patients with increased risk of distant metastases.

Key words: invasive lobular carcinoma, node-negative, CD151, integrin α3β1

INTRODUCTION

The tetraspanin protein CD151 (Tspan24) has recently emerged as a new candidate indicator of tumour cell invasiveness (Boucheix and Rubinstein, 2001; Hemler, 2005; Zoller, 2009). Elevated expression of CD151 protein and its involvement in tumour progression have been observed in various human malignancies (Romanska and Berditchevski, 2011). In breast cancer (BCa), in particular, high expression of tetraspanin CD151 was shown to correlate with axillary lymph node involvement and patient poor overall survival (Kwon *et al.*, 2012; Novitskaya *et al.*, 2014; Sadej *et al.*, 2009). The role of CD151 in tumour invasive and metastatic progression is thought to be relying on its ability to form complexes with laminin-binding integrin receptors (i.e. $\alpha6\beta1$, $\alpha3\beta1$, $\alpha6\beta4$) (Sadej *et al.*, 2014) and its involvement in the regulation of cell-cell and cell-matrix interactions (Johnson *et al.*, 2009). The underlying signalling pathways are likely to depend on adhesion-dependent and co-ordinated activation of small Rho and Ras GTPases, c-Akt and p38 (Novitskaya *et al.*, 2014). It was also proposed that the CD151/$\alpha3\beta1$ complex is directly linked to cadherin and catenin, thus regulating E-cadherin mediated cell-cell adhesion (Chattopadhyay *et al.*, 2003). A suppressive role of the CD151/$\alpha3\beta1$ complex, recently demonstrated in ovarian cancer, has been linked to stabilization of integrin $\alpha3\beta1-$ and E-cadherin-mediated cell-cell contacts (Baldwin *et al.*, 2014). In addition to its role in regulation adhesion-dependent outside-in signalling pathways, CD151 might contribute to mammary tumourigenesis via ErbB2/HER2 (Deng *et al.*, 2012; Novitskaya *et al.*, 2014). Interestingly, clinical analyses of breast cancer patients showed that the elevated expression of CD151 correlated with poor overall survival only of patients with HER2-negative (luminal A and quintuple-negative) tumours (Kwon *et al.*, 2012). Moreover, an impact of CD151/$\alpha3\beta1$ on IDC patients survival was shown to be inversely correlated with the level of HER2 expression (Novitskaya *et al.*, 2014).

Existing knowledge of CD151 involvement in mammary tumourigenesis is almost exclusively based on clinical and *in vitro* studies of the invasive ductal carcinoma (IDC). However, it is becoming increasingly apparent that CD151 might have diverse, even opposing roles, in different biological contexts and its clinical significance should be investigated in relation to the phenotypical and histological variants of the tumour (Voss *et al.*, 2011).

Invasive lobular carcinoma (ILC) accounts for approximately 10% to 15% of newly diagnosed breast carcinomas. Classical ILCs are characterized by an outgrowth of small, uniform discohesive neoplastic cells that invade the stroma in a single-file pattern (Sikora *et al.*, 2014). The majority of ILC tumours are of low histological grade and preferentially display a luminal A phenotype (ER+/PR+/HER2-) (Weigelt *et al.*, 2010). Classical ILCs lack E-cadherin expression, which is considered a defining feature of this BCa type (Sikora *et al.*, 2014). Recent studies demonstrated that ILCs differ from grade- and molecular subtype-matched IDCs in the transcriptomic profiles related to cell adhesion, cell-cell signalling and metastatic pattern indicating that IDCs and ILCs represent distinct disease entities (Sikora *et al.*, 2014; Weigelt *et al.*, 2010).

Due to the relatively low incidence of ILC as well as the paucity of available research models (Sikora *et al.*, 2014), the mechanisms underlying its pathophysiology are poorly understood. It is likely that the role of CD151 in progression of E-cadherin-inactivated ILCs, and hence its impact on patients outcome differs from those documented in the more common IDCs. To date CD151 expression has been studied only in a few ILC cases, a small part (2.9%; 25/886) of a large cohort analysed according to the phenotypical, not the histological subtypes (Kwon *et al.*, 2012). More information is available regarding expression of integrins in ILC. Most reports are in agreement that malignant ILC phenotype is associated with decrease of

expression of a range of integrin subunits. However, independently, the integrins seem to be of limited clinical value as no correlation between their expression, histological grade, nodal involvement, proliferative activity or, above all, the overall survival has ever been found (Gonzalez-Angulo *et al.*, 2006; Gui *et al.*, 1995; Lanzafame *et al.*, 1996; Gonzalez *et al.*, 1999).

The α3β1 integrin forms a stoichiometric complex with the tetraspanin CD151, and the interaction with CD151 is functionally important in α3 integrin–dependent matrix remodeling and cell spreading (Kazarov *et al.*, 2002). The aim of our study was to assess clinical significance of CD151 alone and in association with integrin α3β1 in a group of patients with ILC. The findings were analysed in context of our recent IDC study demonstrating a prognostic value of an expression of integrin CD151/α3β1 complex in patients with HER2-negative tumours (Novitskaya *et al.*, 2014).

MATERIAL AND METHODS

Patient Selection and Samples

The study included radical surgery specimens of primary ILC from 117 patients treated between 2000 and 2008 at three sites: 1) the Oncology Department of Copernicus Memorial Hospital in Łódź, Poland, 2) the UHB NHSFT, Birmingham, UK and 3) the Leeds Teaching Hospitals NHS Trust, Leeds, UK. All samples were obtained according to the local ethical regulations (project ethics licence: # RNN/174/11/KE). The characteristics of the study population are summarized in Table 1. Follow-up period was defined as the time from surgery to the last observation for censored cases or death for complete observations.

Immunohistochemistry

Serial 5 μm paraffin sections of formalin-fixed blocks were processed for immunohistochemistry for CD151 (mouse anti-human; 1:100; Novocastra, UK) and α3β1 (INTA3) (goat anti-human; 1:200, Santa Cruz, UK) using protocols described previously (Novitskaya *et al.*, 2014). Immunostaining for E-cadherin (mouse anti-human, 1:50; Novocastra, UK) was used to confirm the initial pathological diagnosis of ILC (E-cadherin-positive samples were excluded from the study). As a negative control for the immunostaining, primary antibodies were replaced by non-immune sera.

Scoring of immunostaining for CD151 was based on Hofmann's method for membranous reactivity of ErbB2 (Hofmann *et al.*, 2008) and modified as follows: i) 0/negative – no reactivity or only partially membranous reactivity in ≤ 10% of tumour cells; ii) 1+/negative – faint membranous or partially membranous in ≥ 10% of tumour cells; iii) 2+/positive – weak to moderate complete membranous in ≥ 10% of tumour cells; iv) 3+/positive – strong complete membranous in ≥ 10% of the tumour cells. Immunoreactivity for α3β1 was scored as follows: i) 0/negative – no reactivity, ii) 1+/positive – faint membranous and/ or cytoplasmic staining in ≥ 10% of tumour cells; iii) 2+/positive – weak to moderate membranous and/or cytoplasmic staining in ≥ 10% of tumour cells; iv) 3+/positive – strong membranous and/or cytoplasmic staining in ≥ 10% of the tumour cells. Immunohistochemical staining was evaluated and scored independently by two observers (HMR and RK* or HMR and SC). The agreement on staining intensity was > 90%. Where there was disagreement, intensity was determined by consensus. Dichotomization of the final scores into: a)'negative' and b)'positive' for CD151/ 0-2; INTA3/ 0 and CD151/3 and INTA3/1-3, respectively, was guided by intensity of immunostaining in positive controls recommended by the manufacturer.

Statistical analysis

Overall survival was calculated from the date of surgery to the date of death or the last follow-up, as recommended by the Kaplan-Meier method. Differences in survival distributions were compared using log-rank test. Data for patients who died from other causes than breast cancer were censored at the time of death. Univariate analysis of overall survival was performed using the Cox proportional hazards regression model. Parameters showing a significant correlation ($p< 0.05$) were included in the multivariate logistic regression analysis. Pearson's χ^2 test or Fisher's exact test were used to assess the associations between expression of CD151 and $\alpha3\beta1$ alone and their co-expression and clinicopathological variables. The results were considered statistically significant when two-sided p was less than 0.05. The analyses were performed using the Statistica 9.1 and and Statistica 10 (StatSoft Inc. Tulsa, OK, USA) software.

Data on cancer recurrence were available in only four cases which precluded the DFS analysis.

RESULTS

An impact of CD151/α3β1 on tumour biology differs between ILC and IDC.

In normal gland, both CD151 and α3β1 showed moderate to strong, predominantly, membranous immunoreactivity, confined to the basal and lateral surfaces of the myoepithelial cells, with no or very weak staining in luminal epithelial cells (Fig. 1A). Similarly, in ILCs, CD151 and α3β1 were localized mainly to the membrane of tumour cells and there was a significant correlation ($p = 0.012$) between levels of their expression (Table 2A). We observed four distinct patterns of immunoreactivity for CD151/α3β1: 1) CD151+/α3β1+ in 55/117 (49.01%); 2) CD151-/α3β1- in 20/117 (17.09%); 3) CD151+/α3β1- in 28/117 (23.94%) and 4) CD151-/α3β1+ in 14/117 (11.96%) cases (Fig. 1B-E). Interestingly, the level of CD151 expression (but not expression of α3β1) on cancer cells was similar to that observed on endothelial cells of intratumoural vessels (Fig. 2).

There was no significant correlation between the level of α3β1 expression and any of tumour characteristics (Table 2A). On the other hand, expression of CD151 assessed alone was inversely associated with tumour size ($p = 0.047$) and stage ($p = 0.019$), thus indicating that CD151 might have an ability to suppress proliferation of cells and progression of disease. Interestingly, positivity for both CD151 and α3β1 (CD151+/α3β1+) was significantly associated only with grade ($p = 0.019$). These results suggest that phenotypic dedifferentiation and adopting of anaplastic features by epithelial cells in ILC are associated with CD151 acting in a complex with α3β1.

We have previously reported that in IDC, in contrast to ILC, CD151 expression showed a positive association with stage ($p = 0.030$) and inverse with tumour grade ($p = 0.041$). We have also demonstrated that, when assessed in combination, positivity for CD151 and/or α3β1

correlated closer than CD151 alone with stage of disease ($p<0.001$ vs. $p = 0.03$ for CD151/α3β1 vs. CD151, respectively) (Novitskaya *et al.*, 2014). Here we have expanded our previous analyses and evaluated co-expression of CD151 and α3β1 in the context of histopathological and clinical characteristics of the IDC cohort. The results showed, that in contrast to ILCs, combined positivity for both CD151 and α3β1 showed no correlation with any of the clinicopathological features in IDCs (Table 2B). Taken together, these results suggest that the involvement of CD151 and its principal transmembrane partner, the integrin α3β1, in tumour development and progression is likely to differ between histological subtypes of breast cancer.

In ILC, in contrast to IDC, neither CD151 nor α3β1 hold prognostic value.

Neither CD151 nor α3β1 analysed alone were of any prognostic value in ILCs (Table 3A). In contrast, in IDC, as shown previously, CD151-positive patients had 1.88-fold higher risk of death from breast cancer in comparison with CD151-negative patients. Moreover, a multivariate statistical analysis identified CD151 as an independent marker ($p = 0.0172$) of poor prognosis in IDC (Table 4). In neither ILC nor IDC co-expression was significantly associated with patient survival. Furthermore, while in the IDC cohort, clinicopathological characteristics commonly recognized as independent prognostic factors (tumour size, lymph node status, stage), were significantly associated with poor survival, none of them held any prognostic value in our ILC study population (Table 3A/B).

Lack of CD151/α3β1 expression is associated with poor survival in node–negative ILC.

As CD151, acting in partnership with α3β1, is thought to affect invasive spread of tumour cells, we analysed prognostic values of CD151 and/or α3β1 in the ILC cohort in relation to the lymph node status. Results presented in Table 5 demonstrate that neither CD151 nor α3β1

assessed alone or in combination had any significant prognostic value in any of the subgroups. Only a trend towards statistical significance (p=0.082) was seen for CD151 in node–negative patients [ILCN(-)]. Furthermore, in the ILCN(-) subgroup, the data were suggestive that the presence of either CD151 or α3β1 could be favourable to the prognosis but their co-expression had no additive effect on patient survival. Thus, we looked next at the relationships between CD151 and/or α3β1 and tumour characteristics in the lymph node-negative subgroup (characteristics of the group are summarized in Table 6A). Table 6B demonstrates that, as in the whole group, significant correlations between: 1) expression of CD151 and α3β1 ($p = 0.018$); 2) CD151 and tumour size ($p = 0.035$) and 3) CD151/α3β1 and grade ($p = 0.029$) were maintained. Univariate analysis in ILC N(-) cases showed that lack of combined expression of CD151 and α3β1 was significantly correlated with poor patient survival ($p = 0.034$) and was the only prognostic factor in this group (Table 6C). CD151/α3β1– negative patients had 3.12-fold higher risk of death from breast cancer in comparison to the rest of the patients with no lymph node involvement and five-year estimated survival rates were 64% vs 91%, respectively (Fig. 3).

DISCUSSION

An overexpression of CD151/Tspan24 has been repeatedly reported as a negative predictor of overall survival of patients with invasive ductal breast carcinoma (IDC) (Sadej *et al.*, 2009; Yang *et al.*, 2008; Kwon *et al.*, 2012; Novitskaya *et al.*, 2014) but nothing is known about its clinical significance in invasive lobular breast cancer (ILC). Here we demonstrate that, in contrast to IDC, loss of CD151 expression in complex with that of its principal molecular partner, the integrin $\alpha3\beta1$, is significantly associated with poor survival in patients with lymph node-negative ILC. There have been a few attempts to establish an expression signature of a risk of distant metastasis in primary lymph-node–negative IDC (Tutt *et al.*, 2008; Wang *et al.*, 2007; Mirza *et al.*, 2002). To the best of our knowledge, this is the first study providing information on prognostic factors in a subgroup of lymph-node–negative ILC patients.

Results of our study provide support for the notion of biological diversity of CD151 in human cancer. Acting alone and/or in complex with laminin-binding integrins, CD151 has been linked with various aspects of carcinogenesis (Palmer *et al.,* 2014; Sadej *et al*., 2014). Whilst its pro-migratory and pro-invasive functions are well established, it was also reported that in certain cell types the presence of CD151 is associated with suppression of motility. For example, its downregulation induced by hypoxic stress at the primary site in colon cancer was shown to decrease adhesion of cells to the extracellular matrix and enhanced cell motility (Chien *et al*., 2008). Similarly, both up- and down-regulation of integrin $\alpha3\beta1$ expression have been correlated with tumour invasion and poor prognosis (Adachi *et al*., 1998; Fukushima *et al*., 1998; Gustafson *et al.,* 2013). Furthermore, the CD151/$\alpha3\beta1$ complex has been recently shown to suppress ovarian tumour growth (Baldwin *et al*., 2014). Although in our study no prognostic value could be ascribed to either CD151 or $\alpha3\beta1$ assessed alone, their

independent suppressor-like functions cannot be excluded. Further mechanistic investigation would be required to evaluate an actual impact of a loss of the CD151/α3β1 complex on ILC tumour bevaviour.

Increasing evidence suggests that ILCs and IDCs are biologically different. This is reflected in disparities in morphological and phenotypic characteristics, transcriptomic profiles related particularly to cell-cell and cell-matrix interactions (Weigelt *et al.*, 2010), as well as responsiveness to neoadjuvant therapy (Korkola *et al.*, 2003). As demonstrated by several genomic studies, the only consistent finding characterizing ILC tumours is inactivation of *CDH1* (E-cadherin, cadherin-1) (Sikora *et al.*, 2013; Weigelt *et al.*, 2010; Weigelt *et al.*, 2008; Bertucci *et al.*, 2008) and indeed, lack of E-cadherin expression is considered a hallmark of ILC. Although the role of E-cadherin in tumour onset and progression is still largely unknown, its inactivation alone is clearly not sufficient to induce neoplastic growth. It has been shown that combined loss of E-cadherin and p53, but not E-cadherin alone, in murine mammary epithelial cells induces metastatic carcinomas that resemble human ILC (Derksen *et al.*, 2006).

There are several reports suggesting that an association of CD151 with integrin α3β1 might be important for regulation of E-cadherin-dependent cell-cell interactions. In mouse kidney, the CD151/integrin α3β1, acting as both an organizer and a component of a large multimolecular complex containing E-cadherin–β-catenin, promoted its association with the actin cytoskeleton and cadherin-mediated cell–cell adhesion. Deletion of integrin α3β1 in this system was found to disturb E-cadherin localization and function (Chattopadhyay *et al.*, 2003). In highly expressing E-cadherin human keratinocytes (HaCat cell line), blocking of CD151 supported cell dispersal (Chometon *et al.*, 2006), while CD151 overexpression

enhanced carcinoma cell-cell association (Shigeta *et al.*, 2003). Similarly, in A431 epithelial carcinoma cells, near total silencing of CD151 destabilized E-cadherin-dependent cell-cell junctions . However, it was not the disruption 0f the E-cadherin regulatory complex but an excessive RhoA activation and disorganization of actin fibres at the cell-cell junctions, induced by loss of CD151, that led to the enhancement of cell migration (Johnson *et al.*, 2009). Through stabilization of E-cadherin based cell-cell interactions, CD151 was suggested to counteract the metastatic progression of endometrial cancer (Voss *et al.*, 2011). Activation of Rho/ROCK signalling axis triggered by loss of E-cadherin was recently demonstrated to be responsible for induction of anoikis resistance and invasive phenotype in a mouse model of human ILC (Schackmann *et al.*, 2011). Interestingly, results of our own study have shown that in IDC cells, depletion of CD151 and α3β1 caused increase of active RhoA (Novitskaya *et al.*, 2014). Taken together, these findings seem to suggest that although loss of E-cadherin is a pre-requisite of the lobular phenotype, other factors, including, perhaps, the CD151-α3β1 partnership, also contribute to invasive behaviour of cancer cells.

Results of our study demonstrated that unlike IDC, lack of both CD151 and integrin α3β1 correlated with decreased survival of patients with lymph-node-negative ILCs. Unlike IDC, ILC tumour cells are deprived of E-cadherin expression and the function and significance of the CD151/α3β1 complex in biology of E-cadherin–negative cells are poorly characterized. However, it is conceivable that in this particular biological context, as in the settings described above, the CD151/α3β1 complex is controlling cell-cell adhesion and loss of E-cadherin contributes but is not decisive in destabilization of cell-cell contacts and enhancement of cell migration.

The pathogenesis of IDC and ILC seems indeed to be governed by distinct mechanisms. Not only the high expression of CD151, but also the lymph–node status, one of the most clinically important indicators of poor prognosis in IDC, was not correlated with survival of patients in our ILC study population. Instead, our results showed that the combined absence of CD151 and α3β1 was a negative prognostic factor in patients with no lymph node involvement. This unexpected finding gives a new insight into the biology of ILC and possible contribution of CD151 and α3β1 to disease progression. CD151 is highly expressed in endothelial cells and is thought to regulate pathologic angiogenesis. It has also been demonstrated that CD151 plays an important role in maintaining integrity of endothelial layer (Zhang *et al.*, 2011). Our immunohistochemical analysis showed that the level of CD151 expression on endothelial cells was correlated with that seen on tumour-associated endothelium. While the mechanisms underlying this 'phenotypic unity' remains unknown, it is possible that the compromised integrity of CD151-negative endothelium would facilitate intravasation of CD151-negative cells. Whatever the underlying mechanisms, results of our study indicate that loss of CD151-α3β1 may serve as a potential prognostic marker of poor survival in a subgroup of patients deemed to carry a low risk of cancer-related death (i.e. lymph-node negative). This could have important clinical implications by helping to identify patients likely to benefit from adjuvant therapy.

In summary, results of our comparative analysis of clinical significance of CD151/integrin α3β1 in ILC and IDC highlight considerable differences in biology between these two BCa types. Furthermore, the study suggests that evaluation of the level of CD151/α3β1 expression might provide important information on behaviour of ILC tumours and identify patients with increased risk of distant metastases, commonly considered ineligible for routine adjuvant therapy.

ACKNOWLEDGEMENTS:

This work was supported by the National Science Centre (grant No. UMO-2013/09/B/NZ4/02512 to HR and UMO-2012/06/M/NZ3/00023 to RS).

DISCLOSURE STATEMENT:

Conflict of interest none.

REFERENCES:

Adachi M, Taki T, Huang C, Higashiyama M, Doi O, Tsuji T, Miyake M (1998) Reduced integrin alpha3 expression as a factor of poor prognosis of patients with adenocarcinoma of the lung. *J Clin Oncol* **16** (3): 1060-1067.

Baldwin LA, Hoff JT, Lefringhouse J, Zhang M, Jia C, Liu Z, Erfani S, Jin H, Xu M, She QB, van Nagell JR, Wang C, Chen L, Plattner R, Kaetzel DM, Luo J, Lu M, West D, Liu C, Ueland FR, Drapkin R, Zhou BP, Yang XH (2014) CD151-alpha3beta1 integrin complexes suppress ovarian tumor growth by repressing slug-mediated EMT and canonical Wnt signaling. *Oncotarget* **5** (23): 12203-12217, doi:2622.

Bertucci F, Orsetti B, Negre V, Finetti P, Rouge C, Ahomadegbe JC, Bibeau F, Mathieu MC, Treilleux I, Jacquemier J, Ursule L, Martinec A, Wang Q, Benard J, Puisieux A, Birnbaum D, Theillet C (2008) Lobular and ductal carcinomas of the breast have distinct genomic and expression profiles. *Oncogene* **27** (40): 5359-5372, doi: 10.1038/onc.2008.158.

Boucheix C, Rubinstein E (2001) Tetraspanins. *Cell Mol Life Sci* **58** (9): 1189-1205.

Chattopadhyay N, Wang Z, Ashman LK, Brady-Kalnay SM, Kreidberg JA (2003) alpha3beta1 integrin-CD151, a component of the cadherin-catenin complex, regulates PTPmu expression and cell-cell adhesion. *J Cell Biol* **163** (6): 1351-1362, doi:10.1083/jcb.200306067.

Chien CW, Lin SC, Lai YY, Lin BW, Lin SC, Lee JC, Tsai SJ (2008) Regulation of CD151 by hypoxia controls cell adhesion and metastasis in colorectal cancer. *Clin Cancer Res* **14** (24): 8043-8051, doi:1078-0432.CCR-08-1651.

Chometon G, Zhang ZG, Rubinstein E, Boucheix C, Mauch C, Aumailley M (2006) Dissociation of the complex between CD151 and laminin-binding integrins permits migration of epithelial cells. *Exp Cell Res* **312** (7): 983-995, doi: 0.1016/j.yexcr.2005.12.034.

Deng X, Li Q, Hoff J, Novak M, Yang H, Jin H, Erfani SF, Sharma C, Zhou P, Rabinovitz I, Sonnenberg A, Yi Y, Zhou P, Stipp CS, Kaetzel DM, Hemler ME, Yang XH (2012) Integrin-associated CD151 drives ErbB2-evoked mammary tumor onset and metastasis. *Neoplasia* **14** (8): 678-689.

Derksen PW, Liu X, Saridin F, van der Gulden H, Zevenhoven J, Evers B, van Beijnum JR, Griffioen AW, Vink J, Krimpenfort P, Peterse JL, Cardiff RD, Berns A, Jonkers J (2006) Somatic inactivation of E-cadherin and p53 in mice leads to metastatic lobular mammary carcinoma through induction of anoikis resistance and angiogenesis. *Cancer Cell* **10** (5): 437-449, doi:10.1016/j.ccr.2006.09.013.

Fukushima Y, Ohnishi T, Arita N, Hayakawa T, Sekiguchi K (1998) Integrin alpha3beta1-mediated interaction with laminin-5 stimulates adhesion, migration and invasion of malignant glioma cells. *Int J Cancer* **76** (1): 63-72, doi:10.1002/(SICI)1097-0215(19980330)76:1<63::AID-IJC11>3.0.CO;2-H.

Gonzalez MA, Pinder SE, Wencyk PM, Bell JA, Elston CW, Nicholson RI, Robertson JF, Blamey RW, Ellis IO (1999) An immunohistochemical examination of the expression of E-cadherin, alpha- and beta/gamma-catenins, and alpha2- and beta1-integrins in invasive breast cancer. *J Pathol* **187** (5): 523-529, doi:10.1002/(SICI)1096-9896(199904)187:5<523::AID-PATH296>3.0.CO;2-3.

Gonzalez-Angulo AM, Sahin A, Krishnamurthy S, Yang Y, Kau SW, Hortobagyi GN, Cristofanilli M (2006) Biologic markers in axillary node-negative breast cancer: differential expression in invasive ductal carcinoma versus invasive lobular carcinoma. *Clin Breast Cancer* **7** (5): 396-400, doi:10.3816/CBC.2006.n.056.

Gui GP, Wells CA, Browne PD, Yeomans P, Jordan S, Puddefoot JR, Vinson GP, Carpenter R (1995) Integrin expression in primary breast cancer and its relation to axillary nodal status. *Surgery* **117** (1): 102-108

Gustafson-Wagner E, Stipp CS (2013) The CD9/CD81 tetraspanin complex and tetraspanin CD151 regulate alpha3beta1 integrin-dependent tumor cell behaviors by overlapping but distinct mechanisms. *PLoS One* **8** (4): e61834, doi:10.1371/journal.pone.

Hemler ME (2005) Tetraspanin functions and associated microdomains. *Nat Rev Mol Cell Biol* **6** (10): 801-811, doi:10.1038/nrm1736.

Hofmann M, Stoss O, Shi D, Buttner R, van d, V, Kim W, Ochiai A, Ruschoff J, Henkel T (2008) Assessment of a HER2 scoring system for gastric cancer: results from a validation study. *Histopathology* **52** (7): 797-805, doi:10.1111/j.1365-2559.2008.03028.x.

Johnson JL, Winterwood N, DeMali KA, Stipp CS (2009) Tetraspanin CD151 regulates RhoA activation and the dynamic stability of carcinoma cell-cell contacts. *J Cell Sci* **122** (Pt 13): 2263-2273, doi:10.1242/jcs.045997.

Kazarov AR, Yang X, Stipp CS, Sehgal B, Hemler ME (2002) An extracellular site on tetraspanin CD151 determines alpha 3 and alpha 6 integrin-dependent cellular morphology. *J Cell Biol* **158** (7): 1299-1309, doi: 10.1083/jcb.200204056.

Korkola JE, DeVries S, Fridlyand J, Hwang ES, Estep AL, Chen YY, Chew KL, Dairkee SH, Jensen RM, Waldman FM (2003) Differentiation of lobular versus ductal breast carcinomas by expression microarray analysis. *Cancer Res* **63** (21): 7167-7175.

Kwon MJ, Park S, Choi JY, Oh E, Kim YJ, Park YH, Cho EY, Kwon MJ, Nam SJ, Im YH, Shin YK, Choi YL (2012) Clinical significance of CD151 overexpression in subtypes of invasive breast cancer. *Br J Cancer*, doi:10.1038/bjc.2012.11.

Lanzafame S, Emmanuele C, Torrisi A (1996) Correlation of alpha 2 beta 1 integrin expression with histological type and hormonal receptor status in breast carcinomas. *Pathol Res Pract* **192** (10): 1031-1038

Mirza AN, Mirza NQ, Vlastos G, Singletary SE (2002) Prognostic factors in node-negative breast cancer: a review of studies with sample size more than 200 and follow-up more than 5 years. *Ann Surg* **235** (1): 10-26

Novitskaya V, Romanska H, Kordek R, Potemski P, Kusinska R, Parsons M, Odintsova E, Berditchevski F (2014) Integrin alpha3beta1-CD151 complex regulates dimerization of ErbB2 via RhoA. *Oncogene* **33** (21): 2779-2789, doi:10.1038/onc.2013.231.

Palmer TD, Martinez CH, Vasquez C, Hebron KE, Jones-Paris C, Arnold SA, Chan SM, Chalasani V, Gomez-Lemus JA, Williams AK, Chin JL, Giannico GA, Ketova T, Lewis JD, Zijlstra A (2014) Integrin-free tetraspanin CD151 can inhibit tumor cell motility upon clustering and is a clinical indicator of prostate cancer progression. *Cancer Res* **74** (1): 173-187, doi:0008-5472.CAN-13-0275.

Romanska HM, Berditchevski F (2011) Tetraspanins in human epithelial malignancies. *J Pathol* **223** (1): 4-14, doi:10.1002/path.2779.

Sadej R, Grudowska A, Turczyk L, Kordek R, Romanska HM (2014) CD151 in cancer progression and metastasis: a complex scenario. *Lab Invest* **94** (1): 41-51, doi:10.1038/labinvest.2013.136.

Sadej R, Romanska H, Baldwin G, Gkirtzimanaki K, Novitskaya V, Filer AD, Krcova Z, Kusinska R, Ehrmann J, Buckley CD, Kordek R, Potemski P, Eliopoulos AG, Lalani e, Berditchevski F (2009) CD151 regulates tumorigenesis by modulating the communication between tumor cells and endothelium. *Mol Cancer Res* **7** (6): 787-798, doi:10.1158/1541-7786.MCR-08-0574.

Schackmann RC, van AM, Haarhuis JH, Vlug EJ, Halim VA, Roodhart JM, Vermaat JS, Voest EE, van der Groep P, van Diest PJ, Jonkers J, Derksen PW (2011) Cytosolic p120-

catenin regulates growth of metastatic lobular carcinoma through Rock1-mediated anoikis resistance. *J Clin Invest* **121** (8): 3176-3188, doi:10.1172/JCI41695.

Shigeta M, Sanzen N, Ozawa M, Gu J, Hasegawa H, Sekiguchi K (2003) CD151 regulates epithelial cell-cell adhesion through PKC- and Cdc42-dependent actin cytoskeletal reorganization. *J Cell Biol* **163** (1): 165-176, doi:10.1083/jcb.200301075 .

Sikora MJ, Cooper KL, Bahreini A, Luthra S, Wang G, Chandran UR, Davidson NE, Dabbs DJ, Welm AL, Oesterreich S (2014) Invasive lobular carcinoma cell lines are characterized by unique estrogen-mediated gene expression patterns and altered tamoxifen response. *Cancer Res* **74** (5): 1463-1474, doi:10.1158/0008-5472.CAN-13-2779.

Sikora MJ, Jankowitz RC, Dabbs DJ, Oesterreich S (2013) Invasive lobular carcinoma of the breast: patient response to systemic endocrine therapy and hormone response in model systems. *Steroids* **78** (6): 568-575, doi:10.1016/j.steroids.2012.11.002.

Tutt A, Wang A, Rowland C, Gillett C, Lau K, Chew K, Dai H, Kwok S, Ryder K, Shu H, Springall R, Cane P, McCallie B, Kam-Morgan L, Anderson S, Buerger H, Gray J, Bennington J, Esserman L, Hastie T, Broder S, Sninsky J, Brandt B, Waldman F (2008) Risk estimation of distant metastasis in node-negative, estrogen receptor-positive breast cancer patients using an RT-PCR based prognostic expression signature. *BMC Cancer* **8** 339, doi:10.1186/1471-2407-8-339.

Voss MA, Gordon N, Maloney S, Ganesan R, Ludeman L, McCarthy K, Gornall R, Schaller G, Wei W, Berditchevski F, Sundar S (2011) Tetraspanin CD151 is a novel prognostic marker

in poor outcome endometrial cancer. *Br J Cancer* **104** (10): 1611-1618, doi: 10.1038/bjc.2011.80.

Wang Y, Klijn J, Zhang Y, Atkins D, Foekens J (2007) Gene expression profiles and prognostic markers for primary breast cancer. *Methods Mol Biol* **377** 131-138, doi:10.1007/978-1-59745-390-5_7.

Weigelt B, Geyer FC, Natrajan R, Lopez-Garcia MA, Ahmad AS, Savage K, Kreike B, Reis-Filho JS (2010) The molecular underpinning of lobular histological growth pattern: a genome-wide transcriptomic analysis of invasive lobular carcinomas and grade- and molecular subtype-matched invasive ductal carcinomas of no special type. *J Pathol* **220** (1): 45-57, doi:10.1002/path.2629.

Weigelt B, Horlings HM, Kreike B, Hayes MM, Hauptmann M, Wessels LF, de JD, van de Vijver MJ, Van't Veer LJ, Peterse JL (2008) Refinement of breast cancer classification by molecular characterization of histological special types. *J Pathol* **216** (2): 141-150, doi:10.1002/path.2407.

Winterwood NE, Varzavand A, Meland MN, Ashman LK, Stipp CS (2006) A critical role for tetraspanin CD151 in alpha3beta1 and alpha6beta4 integrin-dependent tumor cell functions on laminin-5. *Mol Biol Cell* **17** (6): 2707-2721, doi:10.1091/mbc.E05-11-1042.

Yang XH, Richardson AL, Torres-Arzayus MI, Zhou P, Sharma C, Kazarov AR, Andzelm MM, Strominger JL, Brown M, Hemler ME (2008) CD151 accelerates breast cancer by

regulating alpha 6 integrin function, signaling, and molecular organization. *Cancer Res* **68** (9): 3204-3213, doi:10.1158/0008-5472.CAN-07-2949.

Zhang F, Michaelson JE, Moshiach S, Sachs N, Zhao W, Sun Y, Sonnenberg A, Lahti JM, Huang H, Zhang XA (2011) Tetraspanin CD151 maintains vascular stability by balancing the forces of cell adhesion and cytoskeletal tension. *Blood* **118** (15): 4274-4284, doi: 10.1182/blood-2011-03-339531.

Zoller M (2009) Tetraspanins: push and pull in suppressing and promoting metastasis. *Nat Rev Cancer* **9** (1): 40-55, doi:10.1038/nrc2543.

TITLES AND LEGENDS TO FIGURES

Figure 1. Expression of CD151 and α3β1 in:

A. Normal gland (x100; insets x400). Both CD151 and α3β1 show moderate to strong, predominantly, membranous immunoreactivity, confined to the basal and lateral surfaces of the myoepithelial cells, with no or very weak staining in luminal epithelial cells.
B. ILC – cells of similar areas of the tumour display various levels of CD151/α3β1 immunoreactivity representing four predominant phenotypes: B) CD151+/α3β1+ (55/117; 49.01%); C) CD151+/α3β1- (28/117; 23.94%) D) CD151-/α3β1- (20/117; 17.09%), and E) CD151-/α3β1 + (14/117; 11.96%)

Figure 2. Expression of CD151 in intratumoural vessels (x400). Level of expression in endothelial cells of intratumoural vessels (asterisks) is consistent with that in epithelial cells of adjacent tumour (arrows) (A - high; B - moderate; C - negative).

Figure 3. Kaplan-Meier curves of overall survival for patients with invasive lobular breast cancer.

Overall survival of patients with tumours negative for both CD151and α3β1 (1) in relation to the rest of the cohort (0).

Figure1.

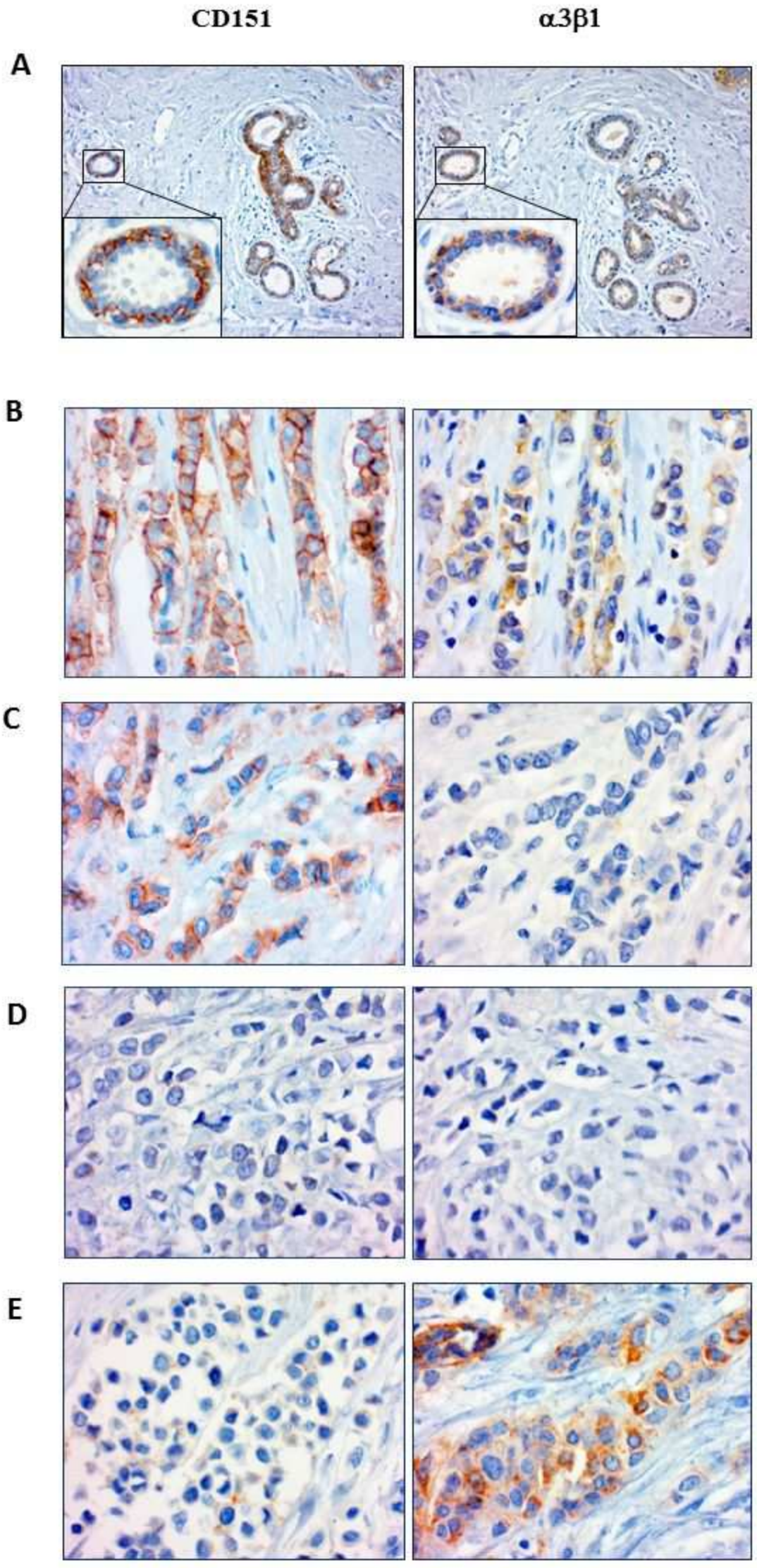


Figure 2.

CD151

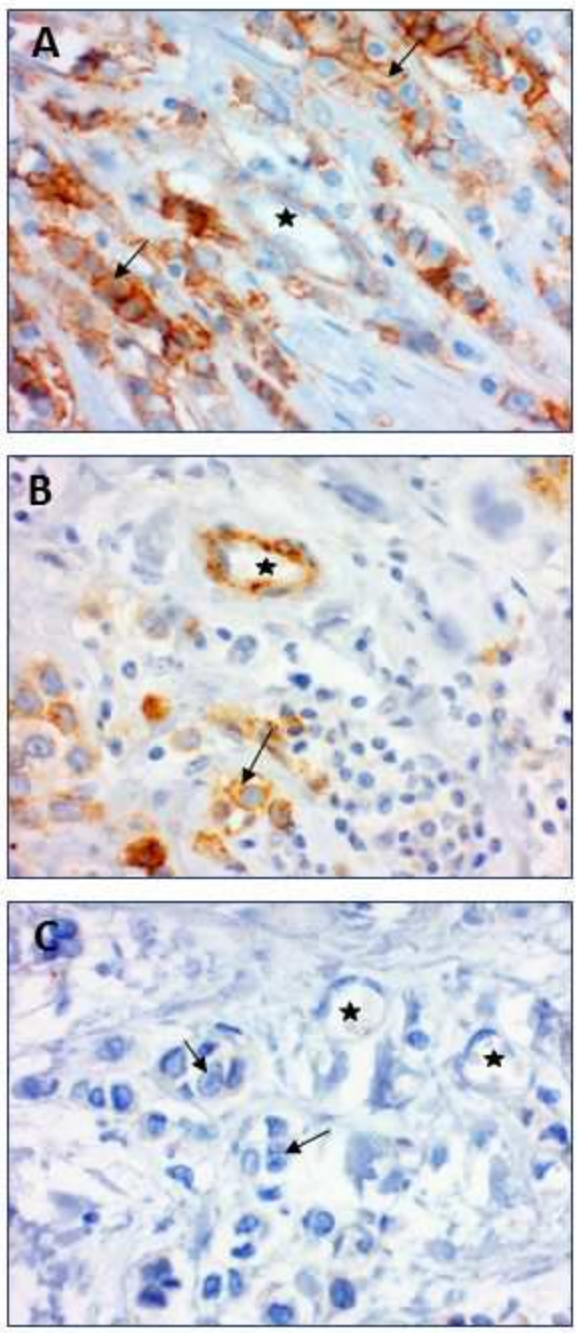
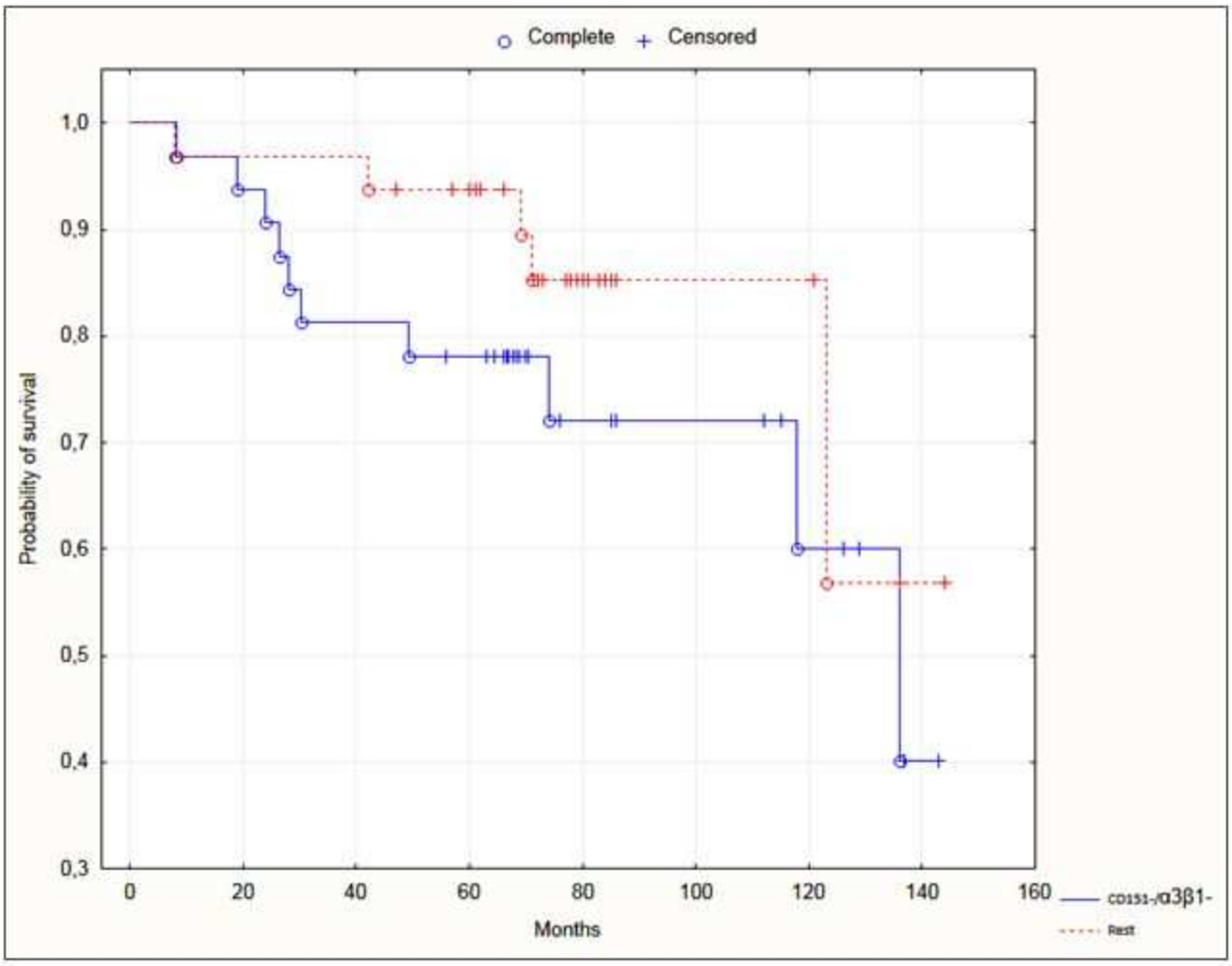


Figure 3.



Log-rank: P = 0.0266

Table 1. Patient characteristics.

Feature	ILC	IDC
Number of patients	117	182
Age (years)		
< 50	21	56
≥ 50	96	127
Disease stage [1)]		
I	34	44
II	60	93
III	13	45
T status [2)]		
T1	52	61
T2	48	112
T3	15	1
T4	1	8
Grade [3)]		
1	21	105
2-3	94	77
Nodal status [4)]		
Negative	64	92
Positive	44	90
Steroid receptor status [5)]		
Negative	11	79
Positive	102	103
HER2 status [6)]		
Negative	102	151
Positive	4	31

In the ILC group data available for:

[1)] 107 patients

[2)] 116 patients

[3)] 115 patients

[4)]108 patients

[5)] 113 patients

[6)] 106 patients

Table 2.

Association between CD151 and/α3β1 expression and clinicopathological features.

A.

Feature	P value		
	ILC		
	α3β1 (high: n=69)	**CD151 (high: n=83)**	**CD151 /α3β1 (high: n=55)**
α 3		**0.012**	
CD151	**0.012**		
Size	0.574	**0.047**	0.381
Nodes	0.464	0.277	0.101
Stage	0.656	**0.019**	0.387
Grade	0.093	0.343	**0.019**
ErbB2	0.092	0.881	0.249
ER/PR	0.694	0.471	0.425

B.

Feature	P value		
	IDC		
	α3β1 (high: n=32)	**CD151 (high: n=87)**	**CD151 /α3β1 (high: n=26)**
α 3β1		<0.001	
CD151	<0.001		
Size	0.599	0.497	0.499
Nodes	0.271	0.076	0.413
Stage	0.545	**0.030**	0.174
Grade	0.163	**0.041**	0.162
ErbB2	>0.999	**0.015**	0.995
ER/PR	0.663	0.819	0.797

Table 3. Univariate analysis of prognostic factors.

A.

Factor	ILC		
	HR	**95%CI**	**P value**
α 3β1	0.80	0.39-1.61	0.750
CD151	0.56	0.28-1.14	0.109
CD151/ α 3β1	0.72	0.35-1.49	0.380
(T1 vs T2-4)	1.78	0.84-3.78	0.165
Nodal status	1.61	0.78-3.31	0.195
Stage (I vs II vs III)	2.08	0.85-5.11	0.111
ER/PR	0.46	0.17-1.19	0.110
HER2	NA[#]	NA[#]	NA[#]
Grade (G1-2 vs G3)	1.46	0.55-3.87	0.441

B.

Factor	IDC		
	HR	**95%CI**	**P value**
α 3β1	1.10	0.60-2.03	0.750
CD151	1.88	1.15-3.08	**0.012**
CD151/ α 3β1	0.98	0.48-1.98	0.952
(T1 vs T2-4)	1.77	1.30-2.40	**<0.001**
Nodal status	3.01	1.78-5.10	**<0.001**
Stage (I vs II vs III)	1.81	1.27-2.57	**<0.001**
ER/PR	0.53	0.33-0.87	**0.011**
HER2	2.07	1.18-3.64	**0.012**
Grade (G1-2 vs G3)	1.24	0.76-2.01	0.383

NA – not analyzed

[#] only 4 HER2 – positive

Table 4. Multivariate analysis of prognostic factors.

Factor	IDC		
	HR	**95%CI**	**P value**
CD151	1.92	1.12-3.31	**0.0172**
(T1 vs T2-4)	1.98	0.94-4.15	0.0711
Nodal status	4.11	2.04-8.29	**<0.0001**
Stage (I vs II vs III)	0.68	0.38-1.23	0.2063
ER/PR	0.53	0.32-0.91	**0.0198**
HER2	1.20	0.71-2.04	0.1927

Table 5. Univariate analysis of prognostic value of CD151 and/or α3β1 in relation to lymph node-status.

Factor	ILC N(+)			ILC N(-)		
	HR	**95%CI**	**P value**	**HR**	**95%CI**	**P value**
α 3β1	1.28	0.45-3.60	0.641	0.46	0.16-1.31	0.146
CD151	0.64	0.22-1.87	0.412	0.40	0.14-1.22	0.082
CD151/ α 3β1	1.02	0.35-3.00	0.970	0.72	0.35-1.49	0.187

Table 6. Lymph node – negative subgroup.

A. Patients characteristics

Feature	ILC
Number of patients	64
Age (years)	
< 50	10
≥ 50	54
Disease stage [1)]	
I	34
II	28
III	1
T status [1)]	
T1	34
T2-T4	29
Grade [2)]	
1	13
2-3	49
Steroid receptor status [3)]	
Negative	5
Positive	58
HER2 status	
Negative	64

Data available for:

[1)] 63 patients

[2)] 62 patients

[3)] 63patients

B. Association of CD151 and/or α3β1 and tumour characteristics.

	α3β1 (high: n=38)	**CD151 (high: n=47)**	**CD151 /α3β1 (high: n=32)**
α 3β1		**0.018**	
CD151	**0.018**		
Tumour size (T1 vs T2-4)	0.312	**0.035**	0.892
Grade	0.079	0.342	**0.029**
HER2	NA*	NA*	NA*
ER	0.377	0.999	0.192
PR	NA#	NA#	NA#

* - all tumors were HER2-

\# - lack of data for 45 cases

C. Univariate analysis of prognostic factors

Factor	**ILC N(-)**		
	HR	**95%CI**	**P value**
α 3β1	0.462	0.16-1.31	0.146
CD151	0.402	0.14-1.22	0.082
CD151/ α 3β1	0.724	0.35-1.49	0.187
CD151-/α 3β1-	3.125	1.09-8.99	**0.034**
Tumour size (T1 vs T2-4)	1.780	0.61-5.18	0.290
Grade	1.361	0.36-5.14	0.650
ER	0.372	0.08-1.72	0.206