**Edited Manuscript:**

**Expression Patterns of Galectins-1, -3, and -7 are Prognostic for Overall Survival in Ovarian Cancer**

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**Abstract:** There is a considerable need for the development of new prognostic factors in ovarian cancer. Galectins are carbohydrate-binding proteins that have been suggested to serve as prognostic factors for various cancer types. In this study, the expression of galectin (Gal)-1, -3, and -7 was investigated in 156 ovarian cancer specimens using immunohistochemical staining. Overall patient survival was compared among groups stratified by galectin expression. Gal-1 and -3 staining was observed in the peritumoural stroma as well as the nucleus and cytoplasm of tumour cells, while Gal-7 was only present in the cytoplasm. Patients with Gal-1 expression in the cytoplasm or high Gal-1 expression in the peritumoural stroma showed reduced overall survival. Nuclear Gal-3 staining correlated with better clinical outcomes. Cases with high Gal-7 expression exhibited significantly reduced overall survival, while Gal-7-negative cases exhibited improved survival. Our results indicate that tumour and stromal staining of Gal-1 and cytoplasmic staining of Gal-7 serve as negative prognostic factors for ovarian cancer, while nuclear Gal-3 staining may represent a new positive prognosticator for ovarian cancer. These findings suggest that galectins may represent promising new targets for ovarian cancer treatment.

**Introduction**

Ovarian cancer is the most lethal gynecological malignancy, ranking fifth in estimated cancer deaths among women in the USA1. First-line treatment consists of primary debulking surgery followed by platinum and paclitaxel chemotherapy2. Despite these treatments, the 5-year relative survival rate for epithelial ovarian cancer patients remains below 50%3. A lack of screening methods and frequent presentation with advanced stage disease are considered the main reasons for the poor outcomes of ovarian cancer patients.

Prognosticators in ovarian cancer include disease stage at diagnosis, extent of residual disease after surgery, histological subtype, and the volume of ascites4. Numerous studies have aimed to identify new biological prognostic factors in ovarian cancer. Recently, the carbohydrate stem cell marker TF1 has been proposed as a negative prognostic marker in ovarian cancer displaying wild-type p53, while estrogen receptor promoter methylation predicts overall survival in low-grade ovarian carcinoma patients5,6. Although the prognostic value independent of clinical parameters has been demonstrated for these and various other molecules, to date, with the exception of breast cancer gene (*BRCA*) status, no biological marker is commonly accepted4. Further specification of anti-cancer therapies necessitates an improvement in the biological prognostic markers for ovarian cancer.

Galectins belong to a family of proteins sharing two main characteristics: binding affinity for β-galactosides and significant similarity in the carbohydrate-recognition domain (CRD)7. The first member of this family to be described was galectin (Gal)-1, which can be isolated as a homodimer comprising two identical CRD subunits8. Since then, a growing number of galectin family members have been identified, but Gal-1–4, Gal-7–10, Gal-12, and Gal-13 are known to be present in humans9. Similar to Gal-1, Gal-7 typically occurs as a homodimer, while Gal-3 is the only galectin characterized as a chimeric protein that is known to form higher order oligomers10,11. In several types of cancer, galectins are known to affect tumour growth, metastasis, angiogenesis, cell migration, invasiveness, and progression, and they are therefore good candidates for proteins with prognostic value for patient survival9,12.

The role of Gal-1 in cancer has been studied by various groups. In patient sera and ovarian cancer tissues, it has been shown that a combination of CA-125 and Gal-1 serves as a possible two-marker combination for the preoperative discrimination of benign and malignant ovarian masses13. In addition, patients suffering from metastatic epithelial ovarian cancer were observed to exhibit higher serum Gal-1 levels than those with non-metastatic cancer. Elevated Gal-1 staining of the peritumoural stroma was shown to occur in advanced stages of epithelial ovarian cancer and is also associated with reduced progression-free survival in univariate analysis14. However, these results have not yet been reproduced for overall survival or confirmed by multivariate analysis15. Thus, the potential of Gal-1 as an independent prognostic marker in ovarian cancer requires further investigation.

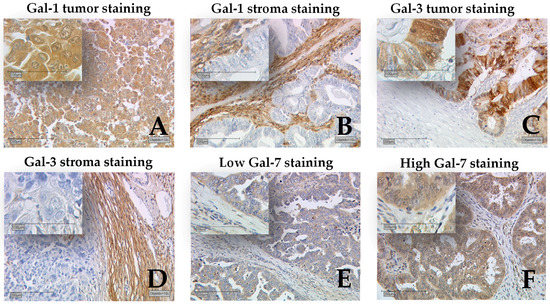
High cytoplasmic Gal-3 expression has been suggested as a negative prognostic factor, as it was shown to correlate with reduced progression-free survival in ovarian cancer16. However, in another study, Gal-3 expression did not correlate with reduced overall survival, though a cytoplasmic staining pattern was associated with poor outcome when compared to patterns including nuclear staining17. Although Gal-3 staining has been observed in the nucleus and stroma, its influence on overall survival remains unclear.

Finally, Gal-7 has been proposed by two independent groups to serve as a negative prognostic factor in ovarian cancer. In both studies, its influence on progression-free survival and overall survival was confirmed by univariate and multivariate analysis16,18. However, disagreement remains regarding whether Gal-7 staining occurs predominantly in the nucleus or the cytoplasm. In addition, it is currently unknown whether the expressions of different galectins are correlated in ovarian cancer, and there is a critical need for a comprehensive study of various galectins in a representative ovarian cancer panel. Therefore, in this study, we investigated the prognostic value of Gal-1, -3, and -7 in patients with epithelial ovarian cancer and analysed correlations among the expression patterns of the three proteins as well as with clinical and pathological parameters. Our results suggest that Gal-1, -3, and -7 are localization-dependent prognostic factors for overall survival in ovarian cancer patients.

**Results**

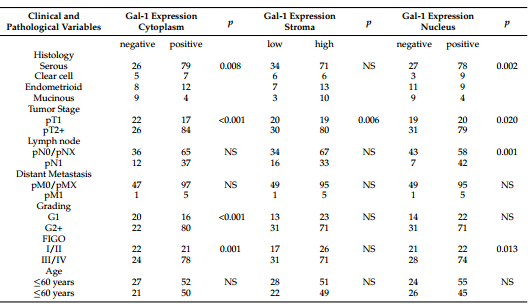
*Gal-1 tumour and stromal staining is a negative prognostic indicator of overall survival*

Gal-1 staining was conducted in 150 ovarian cancer specimens. Gal-1 was present in the cytoplasms and the nuclei of ovarian cancer cells, as well as in the peritumoural stromae (Fig. 1). In 102 cases (68.0%), the tumour cell cytoplasm was positive for Gal-1, with a median Remmele immunoreactive (IR) score of 3. The peritumoural stroma was positive for Gal-1 in 148 cases (98.0%), with a median IR score of 8. Gal-1 expression was significantly correlated with several clinical and pathological factors (Table 1).



**Figure 1.** Detection of galectins by immunohistochemistry. Representative photomicrographs are shown. Gal-1 was present in the cytoplasm and the nucleus of ovarian cancer cells (**A**) as well as the peritumoural stroma (**B**). Gal-3 staining was observed in the nucleus, cytoplasm (**C**), and stroma (**D**). Staining for Gal-7 was mainly observed in the cytoplasm (**E**), with only a few individual cases showing nuclear staining (**F**). Scale bars, 200 μm (10× magnification) in main images, 100 μm (50× magnification) in insets.

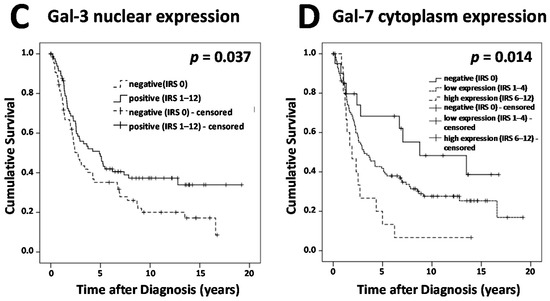
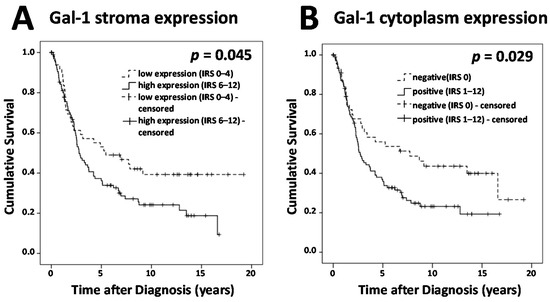
**Table 1.** Correlations between Gal-1 staining and clinical and pathological factors.



TNM staging was performed according to the standards of the Union for International Cancer Control (UICC); pT1 = tumour stage 1; pT2+ = tumour stage 2 or higher; pN0 = lymph node stage 0; pNX = lymph node stage not evaluated; pN1 = lymph node stage 1; pM0 = distant metastasis stage 0; pMX = distant metastasis not evaluated; pM1 = distant metastasis stage 1; G1 = grade 1; G2+ = grade 2 or higher; FIGO = Fédération Internationale de Gynécologie et d’Obstétrique; NS = Not significant (*p* > 0.05)

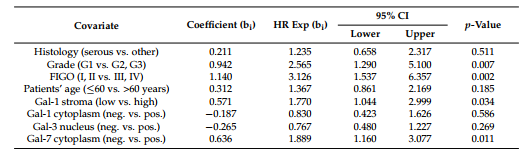
Gal-1 staining in the cytoplasm and nucleus differed among several histological subtypes (*p* = 0.008 and *p* = 0.002, respectively). Cytoplasmic Gal-1 staining was significantly stronger in serous, clear cell, or endometrioid subtypes, while for the mucinous subtype, we observed more negative cases. In addition, more cases with serous and clear cell subtypes exhibited Gal-1-positive nuclei, while the endometrioid and mucinous subtypes exhibited weaker nuclear Gal-1 staining. Furthermore, Gal-1 staining in the nucleus, cytoplasm, and stroma was significantly higher in cases with advanced tumour stage (*p* < 0.001, *p* = 0.006, and *p* = 0.02, respectively). Gal-1 expression in the cytoplasm was significantly higher in cases with higher grading (*p* < 0.001) and advanced FIGO (Fédération Internationale de Gynécologie et d’Obstétrique) stage (*p* = 0.001). The IR scores of nuclear Gal-1 staining were higher in lymph node-positive cases (*p* = 0.001) and those with advanced FIGO stage (*p* = 0.013).

The survival times of groups characterized by their Gal-1 expression in the nucleus, cytoplasm, and stroma were compared (Fig. 2). Cases with Gal-1 expression in the cytoplasm showed significantly reduced overall survival compared to cases without any Gal-1 expression in the cytoplasm (*p* = 0.029) Moreover, cases displaying high Gal-1 expression in the stroma showed significantly poorer outcomes than those with low Gal-1 expression in the stroma (*p* = 0.045). A comparison of cases negative and positive for Gal-1 expression in the nucleus did not reveal any difference in terms of overall survival. However, based on multivariate analysis, only Gal-1 stromal staining serves as an independent prognostic factor (Table 2).



**Figure 2.** Survival times were plotted as Kaplan-Meier graphs. Percentage of living patients (vertical axis) was plotted against time (horizontal axis). Patients without an observed event (death) who exited the study before the observation period ended have been censored, as indicated in the graphs. Survival times of different groups stratified by galectin expression are compared. the immunoreactive (**A**) Cases displaying high Gal-1 expression in the stroma showed significantly reduced survival compared to cases with low Gal-1 expression in the stroma. (**B**) Cases with Gal-1 expression in the cytoplasm showed significantly reduced overall survival compared to cases without Gal-1 expression in the cytoplasm. (**C**) Cases without Gal-3 expression in the nucleus showed significantly reduced overall survival compared to cases with nuclear Gal-3 expression. (**D**) Cases with high Gal-7 expression showed significantly reduced overall survival and Gal-7-negative cases showed better overall survival when compared to cases with low expression of Gal-7.

**Table 2.** Multivariate analysis of prognostic factors for overall survival in ovarian cancer.

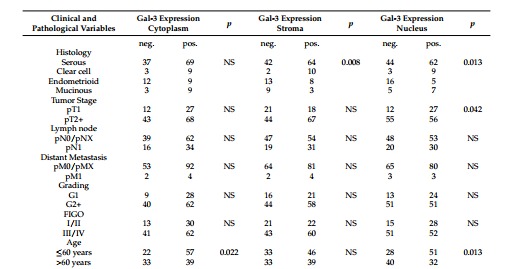


HR = hazard ratio; CI = confidence interval

*Presence of Gal-3 in the nucleus is a positive prognostic indicator in ovarian cancer*

Gal-3-positive nuclei were observed in 83 (55%) out of 151 cases, while 96 cases (63.6%) showed cytoplasmic Gal-3 staining and 85 cases (56.3%) presented with Gal-3-positive peritumoural stromae (Fig. 1). Median IR scores for Gal-3 in the nucleus, cytoplasm, and stroma were 1, 2, and 1, respectively. Gal-3 staining was correlated with clinical and pathological variables (Table 3). Gal-3 expression in the stroma and nucleus differed among different histological subtypes (*p* = 0.008 and *p* = 0.013, respectively). Gal-3 stromal staining was stronger in the serous and clear cell subtypes but weaker in the endometrioid and mucinous subtypes, while nuclear Gal-3 staining was stronger in the serous, clear cell, and mucinous subtypes but weaker in the endometrioid subtype. Tumours rated as pT1 presented with significantly stronger nuclear Gal-3 staining than those rated pT2 or higher (*p* = 0.042). We observed correlations between Gal-3 staining in the nucleus and cytoplasm with patient age (*p* = 0.022 and *p* = 0.013, respectively), observing higher IR scores for patients younger than 60. In our study panel, Gal-3 overexpression in the cytoplasm was not correlated with poorer outcomes in ovarian cancer patients. Similarly, Gal-3 staining in the peritumoural stroma was not observed to be a prognostic factor. In contrast, nuclear Gal-3 expression could serve as a positive prognostic factor (Fig. 2). Cases without Gal-3 expression in the nucleus showed significantly reduced overall survival compared to cases with nuclear Gal-3 expression (*p* = 0.034). According to the results of multivariate analysis, however, nuclear Gal-3 staining was not an independent prognostic factor, probably due to its strong correlations with patient age, tumour stage, and histology (Table 2).

**Table 3.** Correlations between Gal-3 staining and clinical and pathological factors.

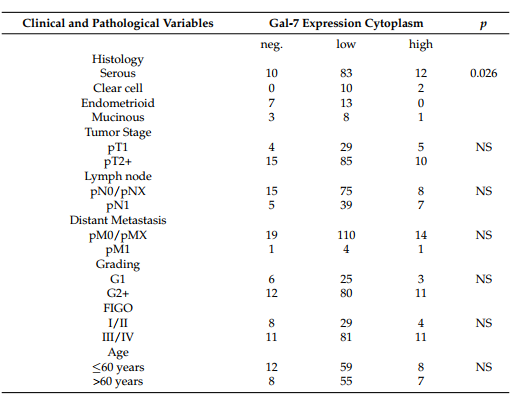


TNM staging was performed according to the standards of the UICC; pT1 = tumour stage 1; pT2+ = tumour stage 2 or higher; pN0 = lymph node stage 0; pNX = lymph node stage not evaluated; pN1 = lymph node stage 1; pM0 = distant metastasis stage 0; pMX = distant metastasis not evaluated; pM1 = distant metastasis stage 1; G1 = grade 1; G2+ = grade 2 or higher; NS = Not significant (*p* > 0.05).

*Gal-7 expression levels predict overall survival in ovarian cancer*

Staining for Gal-7 was mainly observed in the cytoplasm; only a few individual cases showed nuclear staining (Fig. 1). Cytoplasmic Gal-7 staining was present in 129 (86.6%) out of 149 specimens, with a median IR score of 3. In total, 20 cases were negative for Gal-7, while 114 cases showed low and 15 cases showed high expression of Gal-7. Gal-7 expression appeared to differ among different histological subtypes (*p* = 0.026). The strongest Gal-7 staining was found in the serous subtype, and the weakest in the endometrioid subtype (Table 4). No other correlations between Gal-7 staining and pathological data were found. Survival times of Gal-7-negative cases and those with high Gal-7 expression were compared to those with low Gal-7 expression (Fig. 2). We observed significantly reduced overall survival for cases with high Gal-7 expression and improved survival for Gal-7-negative cases compared to that of cases with low expression of Gal-7 (*p* = 0.014). In addition, according to the results of multivariate analysis, Gal-7 expression can be confirmed as an independent prognostic factor for overall survival in ovarian cancer (Table 2).

**Table 4.** Correlations between Gal-7 staining and clinical and pathological factors.

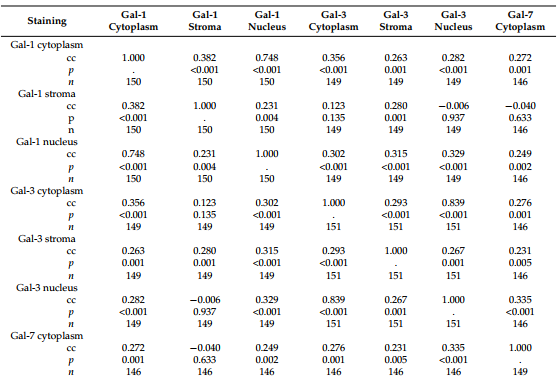


TNM staging was performed according to the standards of the UICC; pT1 = tumour stage 1; pT2+ = tumour stage 2 or higher; pN0 = lymph node stage 0; pNX = lymph node stage not evaluated; pN1 = lymph node stage 1; pM0 = distant metastasis stage 0; pMX = distant metastasis not evaluated; pM1 = distant metastasis stage 1; G1 = grade 1; G2+ = grade 2 or higher; NS = Not significant (*p* > 0.05).

*Correlations among galectin expression patterns*

Results of the analysis of the correlations among galectin expression patterns are shown in Table 5. For Gal-1 staining, we observed positive correlations among staining results in the cytoplasm, nucleus, and stroma. Similarly, the staining results of Gal-3 in the cytoplasm, nucleus, and stroma were positively correlated with each other. Furthermore, we observed correlations between Gal-1 and -3 staining in the nucleus, cytoplasm, and stroma. Gal-7 staining was positively correlated with Gal-1 staining in the cytoplasm and nucleus and all types of Gal-3 staining.

**Table 5.** Correlation analysis of galectin expression patterns.



Correlations among IR scores of Gal-1, -3, and -7 staining in different compartments were assessed using Spearman’s correlation analysis. cc = correlation coefficient, *p* = two-tailed significance, *n* = number of patients.

**Discussion**

In this study, we assessed the prognostic value of Gal-1, -3, and -7 expression on overall survival in ovarian cancer patients. According to our data, Gal-1 staining in the cytoplasm and stroma predicts poor overall survival in ovarian cancer. Consistent with this, *in vitro* experiments have shown that the overexpression of Gal-1 significantly increases migration and invasion behaviours in ovarian cancer cells19. Furthermore, Gal-1 knockdown experiments in ovarian cancer cells result in reductions in cell growth, migration, and invasion. Possible mechanisms for this include the interaction of Gal-1 with H-Ras to activate the Raf/extracellular signal-regulated kinase (ERK) pathway, as well as downregulate matrix metalloproteinase-9 (MMP-9) and c-Jun. Moreover, Gal-1 overexpression may significantly decrease the sensitivity of ovarian cancer cells to cisplatin, reflecting a possible explanation for the reduced survival of ovarian cancer patients with increased Gal-1 expression14. Thus, Gal-1 represents a promising new target for ovarian cancer therapy, and several compounds targeting Gal-1 have been introduced20. OTX008, for instance, is a new compound able to bind non-covalently to Gal-1 on the side back face, inhibiting the proliferation and invasion of various cancer cells lines21. The anti-proliferative effects of OTX008 correlated with Gal-1 expression across a large panel of cell lines. Moreover, OTX008 efficiently inhibited the growth of ovarian cancer xenografts *in vivo*22.

According to the results of multivariate analysis in this study, only Gal-1 stromal staining serves as an independent prognostic factor for overall survival. The accumulation of Gal-1 in the peritumoural stroma has been described for various other tumour entities23-25. Some groups have investigated the mechanisms responsible for this phenomenon. *In situ* hybridization experiments showed that fibroblasts adjacent to malignant cells express *GAL1* mRNA, suggesting a possible explanation for peritumoural Gal-1 accumulation. In addition, it was demonstrated that ovarian cancer cells produce Gal-1 and release it into the medium. Furthermore, conditioned medium obtained from ovarian carcinoma cells induces elevated Gal-1 expression in fibroblasts. These experiments suggest that ovarian cancer cells may be primarily responsible for stromal Gal-1 expression26. Our findings regarding the positive correlation between Gal-1 staining in the peritumoural stroma and malignant cells is consistent with this hypothesis. However, further investigations are required to explain cases of Gal-1 expression in the stroma but not in cancer cells, and *vice versa*.

Several groups have suggested that higher Gal-3 expression is associated with reduced progression-free survival in ovarian cancer17,27. However, in these studies, detection of Gal-3 expression was limited to the cytoplasm, and the prognostic value of nuclear Gal-3 staining has not been further studied. We could not confirm a negative influence of cytoplasmic Gal-3 overexpression on overall survival in our study panel. On the contrary, nuclear Gal-3 staining served as a positive prognostic factor, although it was not independent of the influence of clinical and pathological parameters. Thus, it is apparently nuclear and not cytoplasmic Gal-3 expression that has a major influence on patients’ outcomes. In line with this, Gal-3 has been observed to play an important role in nuclear physiology, as it is involved in the processes of pre-mRNA splicing and mRNA transport28,29. Furthermore, cell culture experiments using human cervix adenocarcinoma (HeLa) cells demonstrated delayed activation of the DNA damage repair response and a decrease in G2/M cell cycle checkpoint arrest in the absence of Gal-330. A similar mechanism is conceivable in ovarian cancer, predisposing cells for further mutations in the absence of nuclear Gal-3. To our knowledge, reduced Gal-3 expression as an indicator of poor prognosis has only been observed in gastric cancer thus far31. In cholangiocarcinoma, Gal-3 expression is associated with a poorly differentiated type, while *in vitro* experiments show significantly increased cell migration and invasion after suppression of Gal-3 expression32. However, for ovarian cancer, *in vitro* experiments have shown that knockdown of Gal-3 inhibits migration and invasion of cancer cells, while increasing apoptosis and sensitivity to carboplatin33. Moreover, paclitaxel and additional treatment with a Gal-3 inhibitor resulted in synergistic cytotoxic effects and increased apoptosis in an ovarian cancer cell line34. Due to the discrepancies in previous research and to the fact that our data are not consistent with either previous studies on progression-free survival or recent *in vitro* research, further investigation into the prognostic role of Gal-3 in ovarian cancer is required.

As recently proposed by other groups, we were able to confirm Gal-7 as a negative prognosticator for overall survival in ovarian cancer according to both uni- and multivariate analyses. Cell culture experiments have demonstrated that Gal-7 expression is induced by a mutant form of p53. In addition, Gal-7 was shown to increase the proliferation16, invasiveness, and motility of ovarian cancer cells, while acting as an immunosuppressant by killing Jurkat T cells and human peripheral T cells18. Together, these investigations confirm Gal-7 as a promising new target for specific therapeutic treatment of epithelial ovarian cancer.

We observed a variety of positive correlations among the expression patterns of Gal-1, -3, and -7. This observation, along with the fact that galectins share binding affinities and exhibit similarities in protein structure, suggests that galectins might also share common functions in ovarian cancer molecular biology. However, as these observations are rather descriptive, further investigations into the biological characteristics and functions of different galectins are required to determine their similarities and differences, specifically in regards to their role(s) in ovarian cancer.

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**Methods**

*Patients*

Formalin-fixed, paraffin-embedded (FFPE) ovarian cancer samples from 156 female patients who underwent surgery at the Department of Obstetrics and Gynecology, Ludwig-Maximilians-University (LMU) of Munich, Germany between 1990 and 2002 were analysed in this study. Women diagnosed with benign or borderline tumours of the ovary were excluded, and no patient had received neo-adjuvant chemotherapy. Tumour grading [G1 (*n* = 38), G2 (*n* = 53), G3 (*n* = 53)], and histological characterization [serous (*n* = 110), endometrioid (*n* = 21), clear cell (*n* = 12), mucinous (*n* = 13)] were performed by a gynecological pathologist. Tumour staging was performed using FIGO classifications [I (*n* = 35), II (*n* = 10), III (*n* = 103), IV (*n* = 3)]. TNM classification was performed according to the UICC. Data on the extension of the primary tumour were available in 155 cases [T1 (*n* = 40), T2 (*n* = 18), T3 (*n* = 93), T4 (*n* = 4)], data on lymph node involvement were available in 95 cases [N0 (*n* = 43), N1 (*n* = 52)], and data on the presence of distant metastasis were available in 9 cases [M0 (*n* = 3), M1 (*n* = 6)]. Clinical data were retrieved from patients’ charts, and follow-up data were requested from the Munich Cancer Registry. Patient age at surgery ranged from 31 to 88 years, with a median age of 62 ±12 years. Mean overall survival was 3.2 ± 3.0 years, and 104 deaths were observed in total. The mean follow-up period was 5.1 ± 4.8 years.

*Immunohistochemistry*

Resected ovarian cancer tissue samples were fixed in formalin and embedded in paraffin after surgery. For histopathological investigations, sections were dewaxed in xylol for 20 min and immersed in 3% hydrogen peroxide (Merck, Darmstadt, Germany) to quench endogenous peroxidase. Then, slides were rehydrated in a descending series of alcohol (100%, 75%, and 50%) and cooked in a pressure cooker for 5 min in sodium citrate buffer (0.1 mol/L citric acid, 0.1 mol/L sodium citrate, pH 6.0) to ensure epitope retrieval. Afterwards, slides were washed in distilled water and phosphate-buffered saline (PBS), followed by a specific procedure for staining each galectin. For Gal-1 staining, slides were blocked using Power Block (BioGenex, San Ramon, CA, USA) for 3 min at room temperature and incubated with anti-Gal-1 primary antibody (goat, polyclonal; R&D Systems, Minneapolis, MN, USA) at a final concentration of 0.033 µg/mL in Power Block for 16 h at 4 °C. Gal-3 staining was performed by blocking specimens with 1.5% horse serum (Vector Laboratories, Burlingame, CA, USA) for 30 min at room temperature and incubating with anti-Gal-3 primary antibody (mouse, monoclonal; Novocastra Reagents, Leica Biosystems, Wetzlar, Germany) at a final concentration of 4.6 µg/mL in PBS for 16 h at 4 °C. For Gal-7 staining, specimens were blocked with Blocking Solution [Reagent 1, ZytoChem Plus HRP Polymer System (Mouse/Rabbit); Zytomed Systems GmbH, Berlin, Germany] for 5 min at room temperature. Slides were then incubated with anti-Gal-7 (rabbit, polyclonal; Abcam, Cambridge, UK) at a final concentration of 2.5 µg/mL in PBS for 16 h at 4 °C. Afterwards, for Gal-1 and -3 staining, slides were incubated with isotype-matched anti-goat/mouse IgG secondary antibody and avidin-biotin-peroxidase complex, both for 30 min at room temperature, according to the instructions of the ABC Vectastain kit (Vector Laboratories). For Gal-7 staining, specimens were incubated in Post-Block reagent (Reagent 2, Zytomed Systems GmbH) and HRP-Polymer (Reagent 3, Zytomed Systems GmbH) for 30 min at room temperature, according to the manufacturer’s protocol for the ZytoChem Plus HRP Polymer System (Mouse/Rabbit) (Zytomed Systems GmbH). All slides were washed twice in PBS for 2 min after every incubation step. For visualization, specimens were stained with 3,3′-diaminobenzidine chromogen (DAB; Dako, Glostrup, Denmark). The reaction was stopped after 30 s–2 min with tap water, and specimens were counterstained in Mayer acidic hematoxylin, dehydrated in an ascending series of alcohol followed by xylol, and covered with Consul Mount (Thermo Shandon, Pittsburgh, PA, USA). Tissue sections that had been previously incubated with isotype-matched rabbit-/mouse-/goat- IgG (Dako) instead of the primary antibody served as negative controls. For positive controls, tissue slides of placental (Gal-1, -3) or breast cancer (Gal-7) tissues were used. Primary antibodies were chosen due to the high expected staining specificities according to the results of positive-control staining, as well as descriptions and example pictures on the manufacturers’ homepages. The semi-quantitative Remmele IR score was determined by two independent observers in consensus to obtain staining results. For this purpose, the predominant staining intensity (0 = negative, 1 = low, 2 = moderate, and 3 = strong) and the percentage of stained cells (0 = 0%, 1 = 1–10%, 2 = 11–50%, 3 = 51–80%, and 4 = 81–100% stained cells) are multiplied, resulting in values from 0 to 12. Staining intensity was measured in the cytoplasm and the nucleus of cancer cells and in the peritumoural stroma. Cut-off points for IR scores were chosen specifically for each staining with regard to the distribution pattern of IR scores in the collective sample. For Gal-1 staining in the cytoplasm and nucleus of cancer cells, an IR score = 0 was considered negative and an IR score ≥ 1 as positive. For stromal staining, Gal-1 groups with low expression (IR score < 5) and high expression (IR score ≥ 5) were compared. For analysis of Gal-3 staining, negative cases with an IR score = 0 were compared to positive cases with an IR score ≥ 1. Gal-7 expression was grouped as negative (IRS = 0), low (1 ≤ IRS ≤ 4), and high (IRS ≥ 6).

*Statistical analysis*

Statistical analyses were performed using SPSS 23.0 (IBM, Armonk, NY, USA) Distributions of clinicopathological variables were tested with chi-square tests. Mann-Whitney *U*-tests were used to compare the IR scores of galectins among different clinical and pathological subgroups. Correlations among immunohistochemical staining results were calculated using Spearman’s correlation analysis. Kaplan-Meier curves and log-rank tests (Mantel-Cox) were used to compare survival times among different groups. Data are presented as the mean ± standard deviation. Values of *p* < 0.05 were considered significant.

*Ethics statement*

All tissue samples used for this study were left-over material from the archives of the LMU Munich Department of Gynecology and Obstetrics, which were initially collected for histopathological diagnostics. All diagnostic procedures had already been fully completed at the time the histopathological investigations for the current study were performed. Patients’ data were fully anonymized. The study was approved by the Ethics Committee of LMU Munich. All experiments were performed according to the standards set forth in the Declaration of Helsinki, 1975.

**References**

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**Competing Financial Interests**

*Source:* [*Galectins-1, -3, and -7 Are Prognostic Markers for Survival of Ovarian Cancer Patients*](https://doaj.org/article/8157f6db55a04e9eb5ce56e81cc541da) *by H. Schulz, E. Schmoeckel, C. Kuhn*[*, et al.*](https://doaj.org/article/701adfbb1df44e7793e127cfe239d56f)*, used under*[*CC-BY*](https://creativecommons.org/licenses/by/4.0/)

**Cover letter:**

[Date of submission]

Editor

*Scientific Reports*

Dear Editor:

I wish to submit an article for publication in *Scientific Reports,* titled “Expression Patterns of Galectins-1, -3, and -7 are Prognostic for Overall Survival in Ovarian Cancer.” The paper was coauthored by \_\_\_\_\_.

Ovarian cancer is the most lethal gynecological malignancy, ranking fifth in estimated cancer deaths among women in the USA. A lack of screening methods and frequent presentation with advanced stage disease are considered the main reasons for the poor outcomes of ovarian cancer patients. To date, with the exception of breast cancer gene (*BRCA*) status, no biological marker is commonly accepted as a prognostic indicator of overall survival in ovarian cancer. Galectins are known to affect tumour growth, metastasis, angiogenesis, cell migration, invasiveness, and progression in various types of cancer, and they are therefore good candidates for proteins with prognostic value for patient survival. Therefore, in this study, we investigated and confirmed the localization-dependent prognostic value of Gal-1, -3, and -7 expression in patients with epithelial ovarian cancer. We believe that our study makes a significant contribution to the literature because our findings have potential clinical implications for the treatment of ovarian cancer.

Further, we believe that this paper will be of interest to the readership of your journal because our study presents high-quality scientific research of broad interest to cancer researchers and clinicians.

Please consider, as potential referees, \_\_\_\_\_\_.

This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal. The study design was approved by the appropriate ethics review board. We have read and understood your journal’s policies, and we believe that neither the manuscript nor the study violates any of these. There are no conflicts of interest to declare.

Thank you for your consideration. I look forward to hearing from you.

Sincerely,

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**Scientific Review Report:**



# Scientific Review Report

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[Prepared by our professional reviewer, senior science editor, and managing editor]

Summary

It was a pleasure working on your document. This study on the value of galectin-1, -3, and -7 expression patterns in predicting overall survival in ovarian cancer presented findings that are likely to be of substantial interest to cancer researchers and clinicians. Overall, the title, abstract, and keywords give the readers a good idea of the paper and the methodology applied in the study is adequate to answer the research question. The reporting of the results, however, has several weaknesses with respect to the structure of the section and the description of the findings. I also noted some ambiguities in data presentation in the figures and tables. In addition, analysis related to paired expression of the studied galectins is incomplete. I have made recommendations to help you address these focus areas.

Scientific Review Report

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[Professional Reviewer's Comments 3](#_Toc46245031)

[Senior Science Editor’s Comments on Language and Paper Structure 7](#_Toc46245032)

[Senior Science Editor’s and Managing Editor’s Comments on the Paper’s Journal Readiness 9](#_Toc46245033)

## Major issues (likely to be raised by the journal peer reviewer and cause rejection) and corresponding next steps the author should take.

* One of the aims stated for the study is to investigate whether the expression levels of different galectins are correlated in ovarian cancer” because “there is a critical need for a comprehensive study of various galectins in a representative ovarian cancer panel.” However, you have not evaluated the correlation between combinations of different galectins and the outcome (survival). This aspect of the study needs to be clarified.
* The number of patients does not seem to be justified by power analysis, and it is unclear whether the sample size was sufficient to achieve statistical significance. A power analysis should be performed, and whether it confirmed that the results of the study were statistically conclusive should be indicated.
* There seem to be no control samples, i.e., those from cancer-free individuals. This aspect needs to be addressed in the manuscript.
* Histological groups (serous, endometrioid, clear cell, mucinous) should be briefly characterized, especially with regard to their comparative malignancy.
* Control tissue staining for all galectins should be presented in Fig. 1 and in Table 5.
* The result that “Gal-1 stromal staining serves as an independent prognostic factor for overall survival” has already been obtained in a previous study (Kim et al. High galectin-1 expression correlates with poor prognosis and is involved in epithelial ovarian cancer proliferation and invasion. Eur J Cancer. 2012 Aug;48(12):1914-21). This fact should be indicated, and the previous study must be appropriately cited.
* The statement that “it is apparently nuclear and not cytoplasmic Gal-3 expression that has a major influence on patients’ outcomes” in lines 260-261 of the Discussion is premature and does not correspond to the facts. Other studies (17, 27) obtained the opposite results both in terms of Gal-3 localization and cancer prognosis, as they showed that cytoplasmic Gal-3 had a negative correlation with cancer prognosis. This statement should be deleted, as you do not present enough evidence for total dismissal of the previous findings. Instead, the reason for this discrepancy between the present and earlier studies should be discussed.
* Correlations between expression patterns of Gal-1, -3, and -7 should be interpreted in view of your own findings regarding the distinct influence of these galectins on survival of ovarian cancer patients, i.e., the fact that nuclear Gal-3 indicates good prognosis and cytoplasmic Gal-1 and -7 indicate poor prognosis, whereas their expression showed positive correlation. Given these data, the statement that “This observation … suggests that galectins might also share common functions in ovarian cancer molecular biology” (lines 286-288 in the Discussion) is not supported by the results, as high expression of Gal-3 inhibits cancer progression, while that of Gal-1 and -7 promotes it, indicating that their functions are far from common in ovarian cancer according to your data.
* The Discussion should serve to emphasize the contribution of the study to the understanding of the prognostic potential of galectins in ovarian cancer. This is currently not the case; you should add a few lines to highlight this.

## Minor issues (likely to be raised by the journal peer reviewer for consideration but not cause rejection) and corresponding next steps the author should take.

* The Introduction does not provide a sufficient background of the problem studied. Mechanisms underlying the oncogenic effects of galectins should be outlined in view of their localization. Distinct functional activities of galectins in intracellular compartments should also be presented and appropriate references must be cited.
* The information not relevant to the study, such as Gal oligomerization or the number of CRD domains (lines 40-45 in the Introduction), should be removed.
* The Results section is not appropriately organized, and the presentation of data does not correspond to the data structure in the illustrations. As a rule, the data shown in a single illustration should be described in the same paragraph. However, the Results are structured according to individual galectins, and certain illustrations present the data related to all studied galectins (Fig. 1 – IF results; Fig. 2 – Survival; Table 2 - Multivariate analysis of prognostic factors), which complicates comparative analysis of the data and decreases the coherence and readability of the text.

## Does the paper present novel ideas/a novel direction with regard to the field of research?

In clearly stating the gaps in the existing literature on the topic of your study and presenting the rationale for your study, you have established the novelty of the study in the Introduction section. However, the novelty of the study should also be discussed in the abstract. It should also be highlighted in the Discussion by stating how the study furthered understanding of the prognostic value of the investigated galectins in ovarian cancer.

## Does the paper present novel ideas or build on the research published in the target journal?

Yes, the paper presents novel ideas regarding the importance of galectin-1, -3, and -7 expression patterns in predicting overall survival in ovarian cancer.

## Is the research rationale sound? (is the reason for conducting the research explained clearly in the paper?)

The study rationale is discussed to some extent. The rationale for studying the effect of galectins on survival in ovarian cancer depending on their cellular localization should be elaborated. Moreover, the second aim stated for the study is to investigate whether the expression levels of different galectins are correlated in ovarian cancer because “there is a critical need for a comprehensive study of various galectins in a representative ovarian cancer panel.” The purpose of this analysis is unclear, as you have not evaluated the correlation between combinations of different galectins and the outcome (survival).

## Is the literature review complete? Which other papers can the author cite?

The literature review is not complete. The biological functions of galectins related to tumorigenesis, including malignant transformation, invasion, and metastasis, are not described, and it is unclear how galectins are involved in all these processes. As the study specifically focused on the correlation of galectin expression in different cellular compartments (extracellular, cytoplasmic, and nuclear) with ovarian cancer, you should include an outline of localization-dependent functional activity of galectins. Thus, it should be indicated that extracellular galectins mediate cell–cell and cell–ECM contacts via binding to mucins, including cancer antigen 125, which promotes tumor cell adhesion, migration, and invasion. Through interaction with glycosylated cell surface receptors, galectins induce the expression of oncogenes, thereby promoting cell proliferation. In contrast, intracellular galectins regulate signaling pathways and gene transcription by interacting with cytoplasmic and nuclear proteins. Consider citing the following papers along with presenting this information:

* Funasaka et al. Nuclear transport of galectin-3 and its therapeutic implications. Semin Cancer Biol. 2014 Aug; 0: 30–38.
* Bhat et al. Nuclear repartitioning of galectin-1 by an extracellular glycan switch regulates mammary morphogenesis. Proc Natl Acad Sci U S A. 2016 Aug 16; 113(33): E4820–E4827.
* Patterson et al. Understanding the biochemical activities of galectin-1 and galectin-3 in the nucleus. Glycoconj J. 2002; 19(7-9): 499–506.

## Are the research implications clearly mentioned? If they are mentioned, are they sound? If they are not mentioned, what tips should the author follow?

The implications of the results in the context of ovarian cancer are appropriately described in the Discussion section. However, as Scientific Reports caters to a broad audience, I recommend including any additional implications of your findings for other fields of research. For example, it appears that galectins have been implicated in a broad range of pathological conditions such as inflammation and fibrosis. A brief mention of these in the Discussion or Conclusions sections would, therefore, improve the multidisciplinary appeal of your manuscript.

## Are the concluding statements clear, and do they mention the contributions, limitations, and next steps for other researchers in the field?

A clear Conclusion section is provided. However, it can better emphasize the contribution of this study to the prognostic potential of galectins in ovarian cancer. Moreover, the limitations of the study need to be listed at the end of the Discussion before the scope for further research in the field is discussed.

## Is the research design appropriate? What are the gaps, and what should be done to fill these gaps?

Overall, the study design is appropriate. There are two major potential issues with the design as currently reported. First, the number of patients does not seem to be justified by a power analysis, and it is unclear whether the sample size was sufficient to achieve statistical significance. A power analysis should be performed, and whether it confirmed that the results of the study were statistically conclusive should be indicated. Second, there were no control samples, i.e., samples from cancer-free individuals. This also needs to be addressed in the manuscript. Finally, the correlation between different combinations of galectins and survival should be analyzed.

## Is the research methodology sound and relevant to the field?

Overall, the methodology applied in the study is adequate to answer the research question. The two points mentioned above should be addressed to ensure that the reporting of the methodology is without errors. The histological groups (serous, endometrioid, clear cell, mucinous) should also be briefly characterized, especially with regard to their comparative malignancy.

## Does the data appear accurate, and has it been interpreted appropriately? Flag cases of insufficient or insignificant data with the author.

* The Results should be restructured. First, IF data should be presented and interpreted for all galectins (Fig. 1). Second, each galectin should be described for its correlations with clinical and pathological factors (Tables 1, 3, and 4), and compared. Third, overall survival depending on galectin expression (Fig. 2) should be presented. Fourth, multivariate analysis of prognostic factors for overall survival in ovarian cancer (Table 2) should be analyzed. Finally, correlations among galectin expression patterns (Table 5) should be described.
* In Fig. 1, nuclear and cytoplasmic staining for Gal-3 and -7 is not clearly visible and should be indicated by arrows or asterisks.
* In Table 1, the last line (≤ 60) should be changed to > 60.
* It is unclear what statistical significance (*p*-value) is related to in Histology (Tables 1, 3, and 4). There are four histological tumor types in these tables and three levels of expression (negative, low, and high) in Table 4, but only one p-value is shown, and it is unclear which groups were compared. It should be clearly indicated in the column (*p* versus …) or in a footnote to each table.
* The purpose of performing the analysis presented in Table 5 is unclear, as correlations between galectin expression patterns and their significance in ovarian cancer are not interpreted. Nuclear Gal-3 correlated with cytoplasmic Gal-1 and -7; however, nuclear Gal-3 indicated good prognosis, whereas cytoplasmic Gal-1 and -7 indicated poor prognosis. Please explain this contradiction. Did any of the galectin combinations presented in Table 5 correlate with patient survival? What were such combinations in normal control samples? These issues must be addressed.

## Should the author get their data verified by a statistician or submit analyzed datasets to the journal?

Further data verification and submission are not required. However, the journal does require a Data Availability Statement to be included in the Methods section of submitted manuscripts, and this should be added.

1. **Does the journal accept this article type?**

*Scientific Reports* publishes original research in only one format: Article. This manuscript is an original research article and follows the journal-recommended structure for articles.

## Does the research in this article lie within the target journal’s scope?

*Scientific Reports* caters to a broad scientific audience and welcomes research from all areas across the natural and clinical sciences. Your paper will definitely fit this broad scope.

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## How was the paper's overall language quality prior to editing?

The language of the manuscript needed several improvements to make it submission-ready. Changes were made to correct grammatical errors related to article use, to improve word choice and sentence construction, and to ensure the use of formal language.

## What were the top 3 recurring grammar and language issues found and edited for native tone?

1. Poor article use: Definite and indefinite articles were added wherever missing in the file. Editorial changes made in this regard are presented in boldface in the following examples.

* According to our data, Gal-1 staining in **the** cytoplasm and stroma… (Body parts are one-of-a-kind entities and their names are preceded by the definite article)
* Recently, **the** carbohydrate stem cell marker TF1 has been proposed as **a** negative prognostic marker in ovarian cancer displaying wild-type p53… (Specific, countable nouns take the definite article and non-specific, countable nouns take the indefinite article)

2. Word choice: “also” at the start of a sentence was replaced with the more formal alternative “In addition.” In a few instances, terms were replaced with more appropriate alternatives based on the context. For example, “reduced outcomes” was changed to “poorer outcomes.”

3. Sentence construction: The intended meaning did not come through very clearly in some sentences. For example, “However, it requires further investigations to explain cases without Gal-1 expression in cancer cells but in the stroma or vice versa” was changed to “However, further investigations are required to explain cases of Gal-1 expression in the stroma but not in cancer cells, and vice versa.”

## Does the paper adhere to the target journal's language preference?

The journal prefers British English usage. The manuscript has been edited accordingly. The journal also asks authors to avoid the use of technical jargon without it being explained. The manuscript meets this requirement.

## Do the main ideas in the paper flow well? Was the flow of ideas/the main argument natural?

The flow of ideas in the paper was appropriate for the most part and relevant information was provided under each section. The results section needs reorganization as explained earlier.

## What types of changes were made for improvements to paper flow and how has the paper's readability improved because of these?

A concluding statement was added to the abstract. Since the Discussion should ideally begin with a summary of the main aims and findings of the study to improve the flow of ideas from the Results, a statement to this effect was added. Finally, the Conclusions section should ideally follow the Discussion section. This has, thus, been moved from after the Methods to after the Discussion.

## Does the target journal have a word count limit, and does the paper adhere to this limit after editing?

The title is within the 20-word limit. The target journal requires the abstract to be within 200 words; the edited abstract adheres to this limit. Finally, the main text is required to be no more than 4,500 words (not including Abstract, Methods, References and figure legends). This limit has also been met.

## List out all the author preferences and instructions that could not be followed and why.

All author preferences and instructions have been followed.

## What were the major formatting requirements of the journal for this paper, and what changes have been made to meet these requirements?

* The journal requires the author names, affiliations, and contact information to be included on the title page with the corresponding author indicated with an asterisk. Placeholders have been created for this information.
* Keywords are not requested by the journal, so these have been removed.
* The journal does not require sections to be numbered, so section numbering was eliminated in the manuscript.
* In-text citations were to be in the form of sequential superscript numbers. They have been formatted to comply with this requirement.
* Figure callouts are to be mentioned using the abbreviation Fig. except at the start of a sentence. This change has been made in all relevant instances.

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## What details or documents are missing in the paper submission package based on the target journal's formatting and submission requirements?

The journal requires a separate cover letter to be provided with the submission, and one has been created for you. In the manuscript itself, please remember to add the author information on the title page and the reference list. A Data Availability Statement must be included at the end of the main text, before the References.

## Does the paper need to be split for submission?

Typically, the journal requires individual figure files. However, for first submissions (i.e. not revised manuscripts), you may incorporate the manuscript text and figures into a single file up to 3 MB in size in either a Microsoft Word, LaTeX, or PDF format. Since your manuscript is a first submission, it does not need to be split.

## Does the paper need to be blinded for review, and has it been blinded?

The paper does not need to be blinded for review.

## Have all the formatting guidelines, including the right file format for submission, been addressed? Mention any that have not and why they have not been addressed.

The paper is currently in MS Word format, which is one of the accepted file formats (the journal accepts either a Microsoft Word, LaTeX, or PDF format).

* The format for author information could not be checked, as this was not provided. Placeholders with instructions have been added on the title page.
* References could not be formatted, as these are not provided for editing.
* Tables need to be converted into an editable format. This was not done as file type conversion is beyond the scope of the service.

## Have ethical and financial declarations been provided? If not, alert the author to do so and explain why.

Ethical and financial declarations have been provided.

## Is a conflict of interest statement provided? If not, alert the author to do so and explain why.

A conflict of interest statement has been provided.

## Has a data availability statement been provided? If not, alert the author to do so and explain why.

A data availability statement has not been provided. As per journal guidelines, you must include a Data Availability Statement in all submitted manuscripts (at the end of the main text, before the References section). Data availability statements should include, where applicable, accession codes, other unique identifiers and associated web links for publicly available datasets, and any conditions for access of non-publicly available datasets. Where figure source data are provided, statements confirming this should be included in data availability statements. Please refer to <https://www.nature.com/srep/journal-policies/editorial-policies#availability> for examples of such statements.

## Has the corresponding author been identified for journal interaction?

The corresponding author has not been identified. Placeholders for author information have been added on the title page.

## Are all the references, tables, and figures present?

The references have not been provided for editing. All figures and tables are present.

## Are the references in the right format and the figures and tables labelled appropriately?

* The references have not been provided. Please ensure that a reference list is provided and formatted per the requirements of the target journal. These are the following formats that you should be aware of for different types of references:

**Published papers**

Printed journals

Schott, D. H., Collins, R. N. & Bretscher, A. Secretory vesicle transport velocity in living cells depends on the myosin V lever arm length. J. Cell Biol. 156, 35-39 (2002).

Online only

Bellin, D. L. et al. Electrochemical camera chip for simultaneous imaging of multiple metabolites in biofilms. Nat. Commun. 7, 10535; 10.1038/ncomms10535 (2016).

For papers with more than five authors include only the first author’s name followed by ‘et al.’.

**Books**

Smith, J. Syntax of referencing in How to reference books (ed. Smith, S.) 180-181 (Macmillan, 2013).

**Online material**

Manaster, J. Sloth squeak. Scientific American Blog Network http://blogs.scientificamerican.com/psi-vid/2014/04/09/sloth-squeak (2014).

Hao, Z., AghaKouchak, A., Nakhjiri, N. & Farahmand, A. Global integrated drought monitoring and prediction system (GIDMaPS) data sets. figshare http://dx.doi.org/10.6084/m9.figshare.853801 (2014).

* The figure callouts have been edited to be in the right format. Figure legends are within 350 words.
* Table callouts have been edited to be appropriate. The tables must be submitted in an editable format (Word or TeX/LaTeX, as appropriate), and not as images.