Computational and Immunohistochemical Analyses Highlight AXL as a Potential Prognostic Marker for Ovarian Cancer Patients

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Abstract. *Background/Aim: The activation of the membrane tyrosine kinase AXL is implicated in the migration and invasion in several carcinomas, including ovarian cancer. Herein, we investigated the association of the expression of AXL transcript and protein to the aggressiveness of ovarian cancer, as well as to patient outcome. Materials and Methods: Overall and relapse-free survival were determined with respect to AXL transcript levels by computational analysis on two publicly available datasets containing data of gene expression from high-grade ovarian cancers (n=776). Immunohistochemical evaluation of AXL protein expression was then performed using a proprietary tissue microarray consisting of 62 ovarian cancers of different histology, grading and staging. Expression was analyzed for association with clinicopathological parameters, including survival. Results: In both analyzed datasets, AXL transcript expression was significantly associated to both overall and relapse-free survival in high-grade ovarian cancers. Membrane expression of AXL protein was observed in 89% of the analyzed ovarian cancers. A significant correlation was found between AXL expression and serous histologic subtype, higher tumor grade and type II tumors. No significant association between AXL protein expression and patient survival was found in our cohort. AXL is frequently expressed in high-grade serous ovarian cancers and its expression is significantly associated to tumors displaying poor prognosis. Conclusion: AXL is a potential prognostic marker for the most aggressive ovarian carcinomas.*

Ovarian cancer is one of the most aggressive neoplasms in females representing the fifth most common cause of cancer-related death in women in the United States (1).

Although significant progresses in the understanding of its biology have been made, the disease is frequently diagnosed in advanced stages (III-IV) when intraperitoneal carcinomatosis is already present. The main reasons for the delayed diagnosis are the non-specific symptomatology, absence of an efficient screening program and lack of specific diagnostic markers (2). A recent classification divided ovarian cancers into 2 broad categories designated type I, not very aggressive and genetically stable, and type II, very aggressive, genetically unstable and present at advanced-stage tumors. Type I tumors include low-grade endometroid and serous, mucinous and clear cell carcinomas. Type II tumors comprise of high-grade endometrioid and serous, malignant mixed mesodermal tumors (carcinosarcomas) and undifferentiated carcinomas. The serous histotype represents the majority of type II ovarian carcinomas (3).

Recently, complex expression profiles from ovarian cancer biopsies identified novel molecules likely associated to the progression of these tumors. These molecules could be components and modulators in the multi-step events that lead to transformation and dissemination, thus being potential prognostic or predictive factors (4). AXL is one of these molecules, presently under the scrutiny of several research

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groups interested in the identification and validation of biomarkers specific for ovarian cancer. AXL, whose name derived from the Greek word *anexelekto* meaning "uncontrolled", was originally characterized from chronic myelogenous leukemia cells and later also documented in the reproductive organs, nervous tissue, vascular structures and hematopoietic organs (5). AXL is potentially related to the *UFO* gene and its essential proven role is the regulation of erythropoiesis and other hematopoietic cell lineages (6).

AXL and the other members of the Tyro3/Axl/Mer (TAM) family of tyrosine kinase receptors (7) play a role in the transmission of signals leading to survival and/or migration and invasion of tumor cells. The three receptors have similar structures with shared ligands, such as GAS6, which has the highest affinity for AXL, as opposed to SKY and MER (8). GAS6 stimulation induces the auto-phosphorylation of AXL followed by various intracellular signals, which in turn promote adhesion, secretion of proinflammatory cytokines and tumor proliferation, as well as invasion (7).

In the last decade, AXL emerged as determinant of invasion in a number of solid tumors, including melanomas, non-small cell lung, breast, prostate, renal cell and ovarian carcinomas (9-14). Published evidence indicate the potential of AXL as therapeutic target in these latest solid tumors (15). Clinically, the analysis of the possible significance of AXL expression as prognostic indicator in ovarian cancer is limited to a study focused on immunohistochemical evaluation of AXL expression (16). Very recently, in high-grade ovarian carcinomas, we identified AXL as a driver of an adhesion-dependent signaling pathway activated during tumor progression (17). Furthermore, an *AXL*-associated molecular signature of 62 genes was also defined to be able to distinguish high-grade ovarian cancer with the worst prognosis (17).

Here, our research focused on the potential exploitation of AXL alone as a prognostic tool for ovarian carcinoma patients at the transcript (*AXL*) and protein (AXL) levels. Specifically, we investigated the prognostic value of *AXL* expression, by performing a computational analysis of gene expression data and validating these computational data by the immunohistochemical assessment of the protein expression using a proprietary tissue microarray (TMA).

Materials and Methods

Computational analysis of gene expression data. Two publicly-available datasets of gene expression from ovarian cancer patients were downloaded from the web and their characteristics are reported (Table I) (18). Raw data of dataset I were downloaded from the NCBI Gene Expression Omnibus repository (ID GSE9891) and those of dataset II were downloaded from the proprietary repository (19). Both datasets comprised mainly high-grade serous cancers. To compare homogeneous tumor samples, dataset I was filtered for "malignant", primary site "ovary" and histological type "serous" and the presence of clinical outcome. Data were normalized by the RMA algorithm. *AXL* gene expression intensity (probe 202686_s_at) values were extracted and analyzed further. Overall or progression-free survival (defined as the time interval between the date of diagnosis and the first confirmed sign of disease recurrence) were used as the primary end-points. For survival analysis, we divided the patients in "high" and "low" *AXL* expression intensities based on the median value (half of the patients high and half low) or the quartiles (¼ higher than the 3rd quartile and ¼ lower than the 1st quartile). Curves were generated with the Kaplan-Meier method and hazard ratios (HRs) and 95% confidence intervals (CI) were also computed.

Case selection. The case material was composed of 62 cases of malignant ovarian tumors diagnosed between 2006 and 2011 at the "Sf. Spiridon" University Hospital and "Cuza – Vodă" Obstetrics and Gynecology University Hospital of Iassy, Romania. All the cases had been surgically treated without neoadjuvant therapy. The study was approved by the Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy Iassy, based on the patients' informed consent to use their biologic material for research after the diagnosis.

The main clinicopathological characteristics (tumor stage, tumor grade, histologic type and subtype) of the cases are summarized in Table II. The histologic type established according to the current World Health Organization (WHO) classification (20) was further refined according to the classification related to the presumed pathogenetic mechanism and classified as: type I (low-grade ovarian cancer; LG) and type II (high-grade ovarian cancer; HG) (3). The follow-up of the patients ranged from 4 months to 5 years, with an average of 18.20 months.

TMA and immunohistochemistry (IHC). The TMA preparation and the immunohistochemical investigation were performed at Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.

From the blocks corresponding to each case, we selected the most representative one as "donor" block. The technique consisted in the extraction of 1-4 cores of tumoral tissue, each with a diameter of 1.5 mm out of each "donor" block and their integration into a new and larger "recipient" block by using the semi-automated machine GALILEO TMA CK 3500 (Integrated System Engineering, Milan, Italy). Each "recipient" block contained several tumor cores from several cases. At the end of the procedure, the 62 selected cases were distributed on 4 TMA blocks from which 4-micron serial sections were cut.

The specimens were de-waxed and rehydrated. For antigen retrieval, we applied a pH6-HIER technique based on autoclave treatment for 6 min. The immunostaining procedure included the following steps: blocking of endogenous peroxidase (5 min, by using 3% hydrogen peroxide), incubation with primary antibody (anti-AXL, polyclonal, dilution 1:25, code AF 154; R&D Systems, Minneapolis, MN, USA) overnight at 4˚C, amplification of the immune reaction with the secondary antibody for 30 min at room temperature and the addition of the high sensitivity streptavidin-horseradish peroxidase (HRP) conjugate (Cell and Tissue Staining Kit; R&D Systems).

The immune reaction was developed with 3.3'-diaminobenzidine tetrahydrochloride chromogen and counterstaining was performed by using hematoxylin. In parallel, positive controls (by using colon cancer tissue) and negative (omitting the primary antibody controls) were run by applying the same protocols as for those used for the specimens included in the study.

Table I. *List of epithelial ovarian cancer (EOC) data sets of gene expression analyzed in the present study.*

Datasets	Platform	Array	No. of probes	N of ovarian cancer patients
I (Tothill 2008)	Affymetrix	HG-U133 Plus 2	54,675	198
II (TCGA 2011)	Affymetrix	HT_HG-U133A	22,277	578

Table II. *Clinicopathological profile of the studied group.*

Number of cases (n=62)	Tumor stage		Tumor grade			Histological subtype	Pathogenic type
	Stage I/II (n=23)	Stage III (n=39)	G1 (n=12)	G2 (n=22)	G3 (n=26)		
1	1	0	1	0	0	LGSC	Type I
4	2	2	4	0	0	LGEC	
9	7	2	5	3	1	MOC	
2	1	1	2	0	0	CC-OC	
42	11	31	0	18	24	HGSC	Type II
2	1	1	0	1	1	HGEC	
2		2	UNDIFFERENTIATED			UNDIFFERENTIATED	

LGSC, Low-grade serous carcinoma; LGEC, low-grade endometrioid carcinoma; MOC, mucinous ovarian carcinoma; CC-OC, clear cell ovarian carcinoma; HGSC, high-grade serous carcinoma; HGEC, high-grade endometrioid carcinoma.

Semi-quantitative assessment. The semiquantitative assessment was made on virtual images acquired by using the scanning facilities of the software APERIO SCAN SCOPE xT (Leica Microsystems, Nussloch, Germany). Each tissue core was examined with progressive magnifications (×4, ×5, ×10 and ×20). The IHC reaction was interpreted using an adapted / individualized evaluation system based on the only semiquantitative score reported in literature (21) for AXL expression that estimates the percentage of tumor cells with membrane staining. We assigned a score 0 for a negative staining of tumor cells, a score 1 for less than 50% of stained tumor cells and a score 2 for more than 50% of stained tumor cells.

Statistical analysis. We applied the Chi-square (χ^2) test for the analysis of the relationship of AXL expression (present/positive *versus* absent/negative) with the clinicopathological features (tumor stage, tumor type, tumor grade, histologic subtype) and the Kaplan-Meier test for the survival analysis. This statistical analysis was performed by the GraphPad Prism software 5.0d (San Diego, CA, USA).

For all statistical analyses, the assessment of the differences was performed according to the log-rank test, with the standard interpretation of statistical significance for $p<0.05$.

Results

Correlation between AXL transcript levels and survival. We first analyzed the correlation of AXL expression with relapse-free and overall survival in two large public available datasets (Table I) containing gene expression data from 198 and 578 patients, respectively.

In dataset I, the association between AXL gene expression and survival was significant both for overall (median value: $p=0.0002$; quartile value: $p=0.0030$) (Figure 1A) and relapse-free (median value: $p=0.0016$; quartile value: $p=0.003$) survival (Figure 1B). In dataset II, we found significant differences for overall survival (median value: $p=0.042$; quartile value: $p=0.0033$) (Figure 2A), while for the relapse-free survival only the quartile analysis was significant (median value: $p=0.13$; quartile value: $p=0.044$) (Figure 2B). Since the majority of these patients were diagnosed with high-grade serous ovarian cancer, we can argue that high AXL transcript levels strongly identify ovarian cancer patients with the shortest relapse-free and overall survival.

Correlation between AXL protein expression, clinicopathological characteristics and survival. In order to confirm, at the protein level, the significance of AXL on ovarian cancer samples, AXL expression was evaluated on TMA containing 62 ovarian cancers from different histotypes, tumor grade and stage (Table III). Anti-AXL antibody stained the cell membrane as reported in representative images of Figure 3. In all these cells, the staining intensity was high. Among the 62 cases examined, tumor cells showed AXL positivity in 50 cases (81%) and negativity in 12 cases (19%). Among the positive cases, the scoring value was 1 in 12 cases and 2 in the other 38 cases.

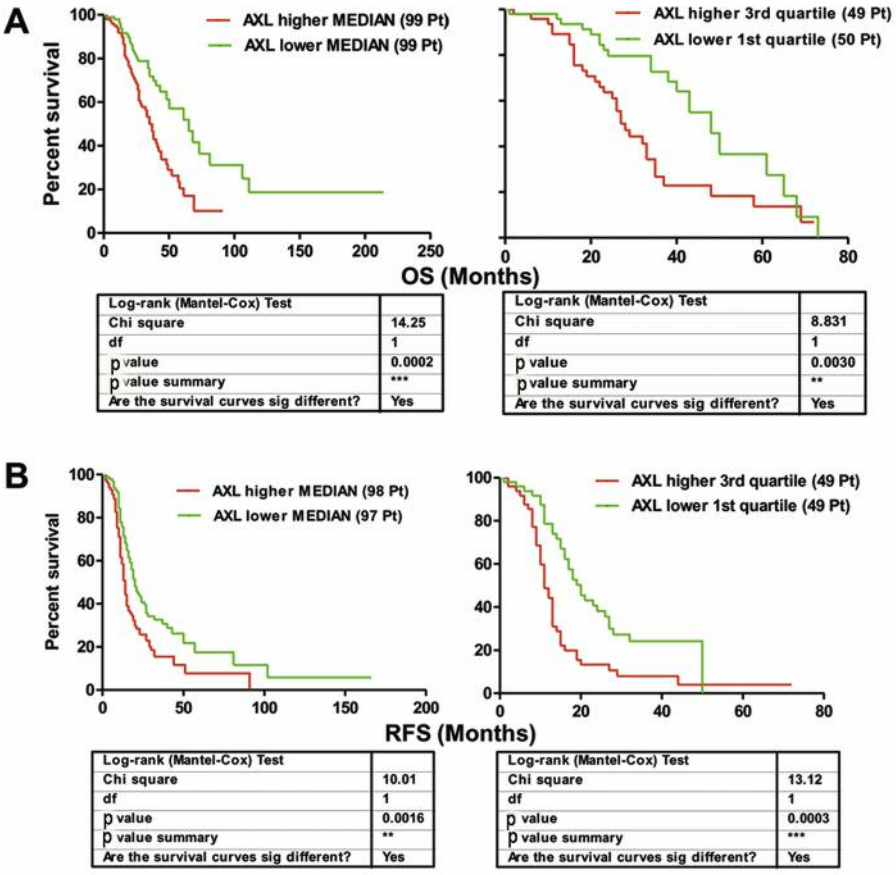


Figure 1. *Dataset I. (A) Association between AXL gene expression and overall survival (OS). (B) Association between AXL gene expression and relapse-free survival (RFS). In parenthesis the number of patients (Pt) is reported.*

In respect to the histologic subtype, positive reaction was recorded as follows: in 39 cases (90.7%) out of 43 cases of low- and high-grade serous carcinoma (LGSC and HGSC), in both cases of clear cell ovarian carcinoma (CC-OC) (100%), in 5 cases (83%) out of 6 cases of low- and high-grade endometrioid carcinoma (LGEC and HGEC) and in 2 cases (22%) from 9 cases mucinous ovarian carcinoma (MOC), as well as in both cases (100%) diagnosed as undifferentiated carcinoma. Eight (50%) of the 16 cases designated as type I carcinoma and 42 (91%) of the 46 cases designated as type II carcinoma were AXL-positive. In regard to tumor grading, we noticed an increase of the percentage of AXL positive cases from G1 to G3: the AXL expression was positive in 7 cases (58%) from the 12 cases graded as G1, in 17 cases (74%) from the 22 graded as G2, in 24 cases (92%) from the 26 graded as G3 and in both cases of undifferentiated carcinoma. Overall, we recorded AXL positivity in 11 out of the 17 stage I cases (65%); 5 out of the 6 stage II cases (83%); and 34 out of the 39 stage III cases (87%). Our data revealed the existence of significant differences between AXL expression and histological subtype (p=0.0003), tumor grade (p=0.0231) and tumor type (type I *vs*. type II) (p<0.0001) (Table IV). These data strongly indicate that high AXL protein expression characterizes serous high-grade ovarian cancers with low cellular differentiation and type II.

We also evaluated the impact of AXL expression on the survival of these patients.

Although the Kaplan-Meier curves showed no statistically significant differences (p=0.2763) (Figure 4) between AXL expression and overall survival, they indicated a trend that could indeed mean that ovarian cancer patients with the poorest outcome harbor AXL protein expression, as also resulted by the computational analysis above.

Discussion

Ovarian cancer comprises of a diverse group of neoplasms, exhibiting a wide range of morphological characteristics and clinical manifestations that are characterized by a broad

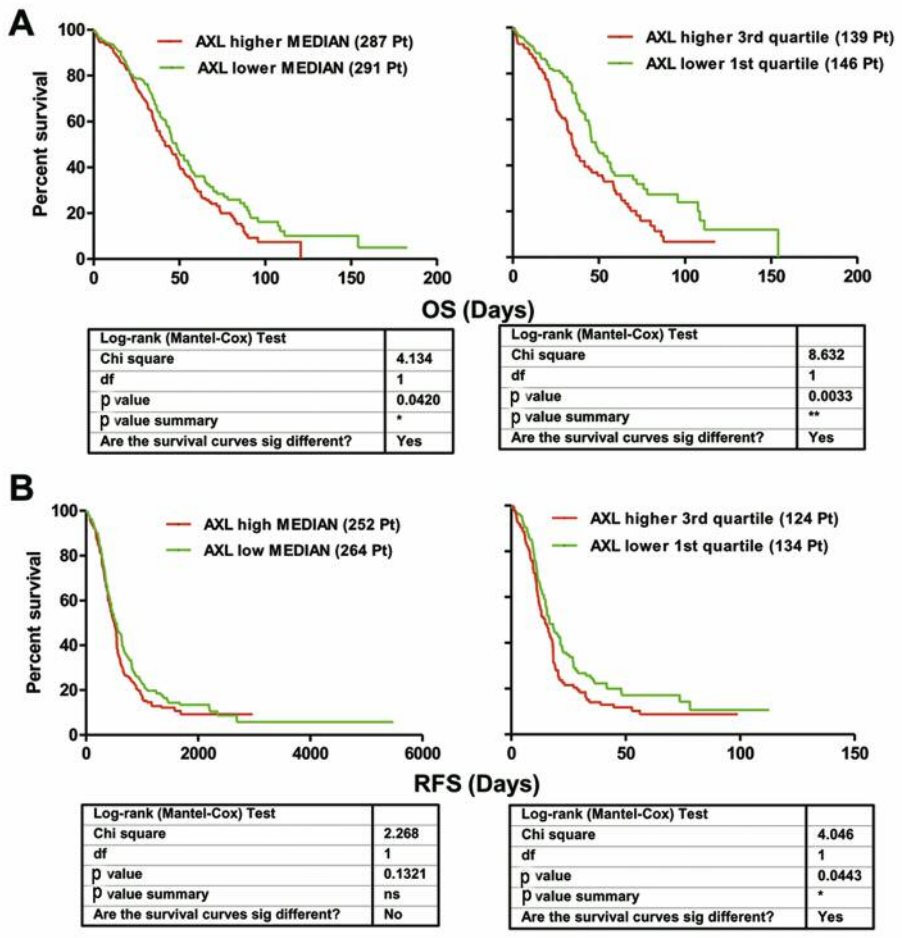


Figure 2. *Dataset II. (A) Association between gene AXL expression and overall survival (OS). (B) Association between gene AXL expression and relapse-free survival (RFS). In parenthesis the number of patients (Pt) is reported.*

spectrum of biological behavior ranging from tumors that have an excellent prognosis and high likelihood of cure to those that progress rapidly with a very poor prognosis. Nevertheless, all ovarian cancers are equally treated with a platinum-based chemotherapy. Despite of the numerous molecular knowledge acquired during the last decade, no molecular markers are now recommended for estimation of prognosis. Here, we reported data on AXL expression both at the protein and transcript levels indicating this receptor tyrosine kinase a good candidate as prognostic marker. Our data on the relationship between AXL expression and the clinicopathological features open attractive perspectives for the validation of AXL as a valuable marker of ovarian tumor progression.

The impact of *AXL* on survival of ovarian cancer patients was performed by a computational analysis on the two largest and most relevant datasets of gene expression from high stage ovarian cancers (summing up to a total of 776 cases). The significant association of *AXL* gene expression levels with both overall and relapse-free survival clearly indicates that *AXL* might be relevant for the prognosis of ovarian cancer. Noteworthy, the association between AXL protein expression and overall survival of the Kaplan-Meier curve, although non-significant (Figure 4), showed the same trend observed for *AXL* transcript expression. The differences observed between the computational data and those obtained by IHC is likely due to the difference in sample number; in addition, we cannot exclude a difference between protein and RNA levels due to different transcriptional and post-transcriptional controls.

The fact that, in dataset II, the relapse-free analysis using the median expression value was not significant, could be due to the reasonable batch effects known to affect it and to the importance of the fine evaluation of the level of AXL expression, semi-quantitatively evaluable in an easier way at the transcription level. On the other side, when we stratified the patients based on the quartile values, the

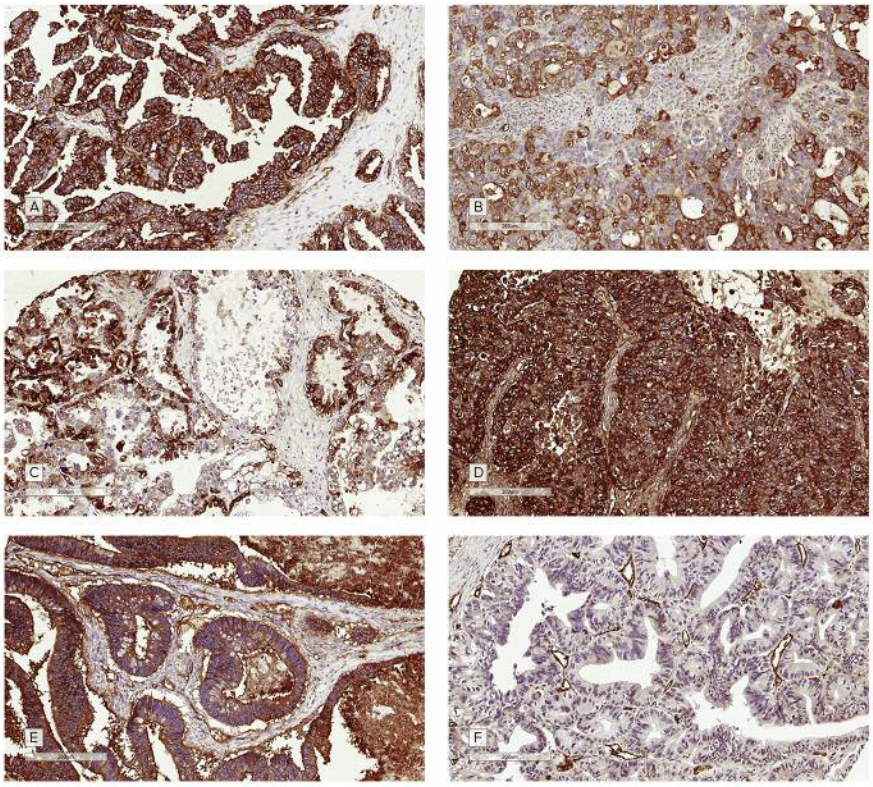
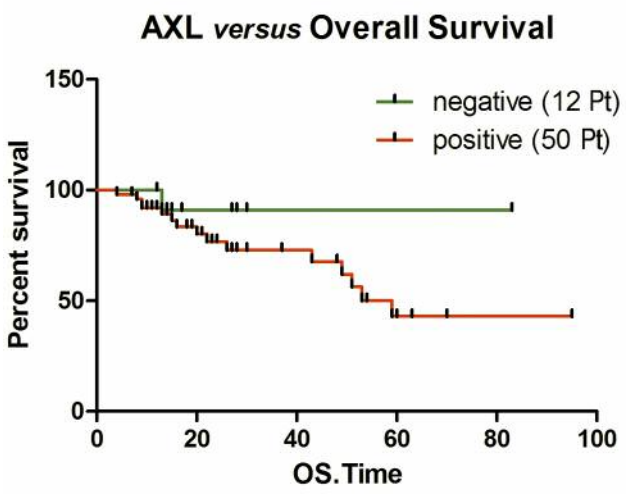


Figure 3. *AXL immunohistochemical expression in different histologic subtypes of ovarian carcinoma (OC) (original magnification: ×200). (A) High-grade serous carcinoma (HGSC)-positive reaction, score 2; (B) High-grade endometrioid carcinoma (HGEC)-positive reaction, score 1; (C) Clear cell ovarian carcinoma (CC-OC)-positive reaction, score 1; (D) Undifferentiated OC-positive reaction, score 2; (E) Mucinous ovarian carcinoma (MOC)-positive reaction, score 1; (F) MOC-negative reaction.*



Log-rank (Mantel-Cox) Test	
Chi square	1.185
df	1
p value	0.2763
p value summary	ns
Are the survival curves sig different?	No

Hazard Ratio	
Ratio	0.4718
95% CI of ratio	0.1220 to 1.824

Figure 4. *Kaplan-Meier curve showing the association between the AXL expression and the overall survival (hazard ratio (HR)=0.47, p=0.2763).*

analysis was statistically significant, thus demonstrating the robustness of the method employed and the consistency with trend in our cohort.

Our present immunohistochemical data and those previously reported are summarized in Table V. Rankin *et al*. (21) defined AXL as a marker for type II ovarian cancer

Table III. *AXL immunohistochemical expression.*

Clinicopathological characteristics	Total number of cases (%)	AXL-negative cases (%)	AXL-positive cases (%)
Total	62 (100.00)	12 (19.35)	50 (80.64)
Tumor stage			
I	17 (28.57)	6 (35.29)	11 (64.70)
II	6 (9.52)	1 (16.67)	5 (83.33)
III	39 (61.90)	5 (12.82)	34 (87.17)
Tumor grade			
1	12 (19.05)	5 (41.66)	7 (58.33)
2	22 (35.48)	5 (22.72)	17 (77.27)
3	26 (41.27)	2 (7.69)	24 (92.30)
Undifferentiated	2 (3.17)	0 (0)	2 (100)
Histologic subtype			
LGSC	1 (1.59)	0 (0)	1 (100)
LGEC	4 (6.35)	1 (25)	3 (75)
MOC	9 (14.29)	7 (77)	2 (22)
COC	2 (3.17)	0 (0)	2 (100)
HGSC	42 (66.67)	4 (9.52)	38 (90.47)
HGEC	2 (3.17)	0 (0)	2 (100)
Undifferentiated	2 (3.17)	0 (0)	2 (100)

LGSC, Low-grade serous carcinoma; LGEC, low-grade endometrioid carcinoma; MOC, mucinous ovarian carcinoma; HGSC, high-grade serous carcinoma; HGEC, high-grade endometrioid carcinoma.

Table IV. *Correlations between AXL expression and clinicopathological characteristics.*

Clinicopathological characteristics (n=62)	AXL expression			χ^2	*p*-Value	Total
	AXL-negative N (%)	AXL-positive N (%)				
	Score 0 (n=12)	Score 1 (n=12)	Score 2 (n=38)			
Histologic subtype				29.55	0.0003	
Serous	4 (9)	6 (14)	33 (77)			43
Endometrioid	1 (17)	2 (33)	3 (50)			6
Clear cell	0 (0)	1 (50)	1 (50)			2
Mucinous	7 (78)	2 (22)	0 (0)			9
Undifferentiated	0 (0)	1 (50)	1 (50)			2
Stage (FIGO)				8.037	0.09	
I	6 (35)	4 (22)	7 (39)			17
II	1 (16)	0 (0)	5 (84)			6
III	5 (13)	8 (20)	26 (67)			39
Tumor grade				14.66	0.0231	
1	5 (42)	4 (33)	3 (25)			12
2	5 (23)	5 (22)	12 (52)			22
3	2 (8)	2 (8)	22 (85)			26
Undifferentiated	0 (0)	1 (50)	1 (50)			2
Tumor type				18.41	<0.0001	0
I	8 (50)	5 (29)	3 (18)			16
II	4 (9)	7 (15)	35 (76)			46

FIGO, International Federation of Gynecology and Obstetrics.

and metastasis by analyzing AXL expression in the normal ovary, primary ovarian tumors and ovarian metastases. Another study showed the negative relationship between AXL and overall survival in ovarian cancer, thus designating AXL-positive expression as an independent poor prognostic factor (16). Interestingly, we obtained statistically significant differences between AXL expression and histologic subtype (p=0.0003), as opposed to Chen *et al*. In relation to histologic subtype, our study confirms the predominance of AXL positivity in the serous subtype, while the smallest percentage of AXL-positive cases is recorded for the mucinous subtype. In our opinion, some contrasting results are probably due to the different composition of the study groups, with large differences in the number of cases corresponding to the specific histologic subtypes.

If we take into account the classification of the cases as tumor type I *versus* II, our data are in accord with those reported by Rankin *et al*. (21) with statistically significant differences between the two types (p=0.0001) – a fact that should be interpreted in the direction of the association of AXL with increased aggressiveness of type II ovarian tumors. Since the chemoresistant cases are frequent within this type of tumors, AXL activation may constitute a chemoresistance mechanism as observed for other tumors (22). Here, we also reported that the increase in the number of AXL-positive cases is associated with the increase in tumor grade (p=0.0231). We also noted an increase in the number of AXL-positive cases in parallel with the increase of the tumor stage.

Our study also indicated a trend for a better survival for AXL protein-negative cases. These results were obtained by the Kaplan-Meier test. However, the lack of statistical significance regarding survival is unexpected because analysis of AXL protein expression in association with clinicopathological characteristics highlighted the correlation of AXL with tumor aggressiveness and a reserved prognosis implicitly. On the other hand, the inconsistencies related to an association between the immunohistochemical data and survival is likely due to the small size of the sample available for testing so many groups and parameters, as well as by the characteristics of each patients' cohort. In our case, material from only 12 out of 62 cases were AXL-negative (19.35%), while in the above mentioned study (16) AXL-negative cases were 31 out of 80 (39%). Another possible reason is the different follow-up time – shorter in our study; since 46 cases (74.19%) were still alive, the algorithm cannot definitely discriminate the difference between patients with better or worse prognosis.

Table V. *Comparison of the data on AXL expression in different histological types of ovarian carcinoma obtained by different studies.*

Authors & number of cases	Histological subtype N (%)											
	SC				EOC							
	HGSC		LGSC		HGEC		LGEC		CO-CC		MOC	
	AXL expression											
	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos
Rankin *et al*., 2010 313 cases	20 (12)	139 (88)	32 (43)	42 (57)	11 (48)	12 (52)	3 (33)	6 (67)	11 (44)	14 (56)	17 (74)	6 (26)
Personal data 62 cases	4 (9.52)	38 (90.47)	0 (0)	1 (100)	0 (0)	1 (100)	1 (25)	3 (75)	0 (0)	1 (100)	7 (77)	2 (22)
	SC				EOC				CO-CC		MOC	
	AXL expression											
Chen *et al*., 2013 80 cases	Neg		Pos		Neg		Pos		Neg	Pos	Neg	Pos
	14 (35)		26 (65)		-		-		7 (46.66)	8 (53.33)	10 (40)	15 (60)

SC, Serous carcinoma; EOC, epithelial ovarian cancer; LGSC, low grade serous carcinoma; MOC, mucinous ovarian carcinoma; CC-OC, clear cell ovarian carcinoma; HGSC, high grade serous carcinoma; Neg, negative; Pos, positive.

Noteworthy, our data, obtained from the analysis of a large number of ovarian cancer patients by the computational approach, strongly indicate *AXL* transcript as poor prognostic factor.

It is widely recognized that AXL (and probably also the other members of the tyrosine-kinase receptors family (MER, SKY)), can be phosphorylated, thus inducing the activation of important signaling pathways, such as PI3K/Akt, RAS, MAPK, SRC, which, in turn, control the expression of a variable number of proteins thought to be direct contributors to cancer cell invasion and metastasis (23). *AXL* has been recently identified as one of the genes and proteins (AXL) whose expression increased in high-grade ovarian cancer cells competent for mesothelial cell clearance (24), a mechanism that governs the re-attachment of ovarian cancer cell to the peritoneum or omentum generating secondary lesions. Our evidence of higher *AXL* transcript expression in aggressive ovarian cancers associated to an earlier relapse indicates that its activation might impinge a plethora of intracellular signals leading to mechanisms fundamental to the peritoneal dissemination of ovarian cancer cells but are not yet fully understood. On this regard, we have recently assessed that in high-grade ovarian cancers, AXL activation leads to invasion through a crosstalk with an integrin-dependent signaling pathway (17).

Overall, our findings suggest the potential of AXL as a marker associated to the most aggressive ovarian cancers. Although this possibility needs to be further evaluated by analyzing AXL protein expression on a larger patient cohort, our data represent a challenge in the elucidation of AXL role in the mechanism occurring during the peritoneal dissemination and the resistance to chemotherapy of high-grade ovarian carcinomas.

Conflicts of Interest

The Authors declare no conflicts of interest.

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References

1 Cancer Facts & Figures, American Cancer Society, Atlanta, Ga, USA, 2013.

2 Coleman RL, Monk BJ, Sood AK and Herzog TJ: Latest research and treatment of advanced-stage epithelial ovarian cancer. Nat Rev Clin Oncol *10*: 211-224, 2013.

3 Nik NN, Vang R, Shih IeM and Kurman R.J: Origin and pathogenesis of pelvic (ovarian, tubal, and primary peritoneal) serous carcinoma. Annu Rev Pathol *9*: 27-45, 2014.
4 Barter RL, Schramm SJ, Mann GJ and Yang YH: Network-based biomarkers enhance classical approaches to prognostic gene expression signatures. BMC Syst Biol *8*: S5, 2014.
5 O'Bryan JP, Frye RA, Cogswell PC, Neubauer A, Kitch B, Prokop C, Espinosa R 3rd, Le Beau M.M, Earp HS and Liu ET: Axl, a transforming gene isolated from primary human myeloid leukemia cells, encodes a novel receptor tyrosine kinase. Mol Cell Biol *11*: 5016-5031, 1991.
6 Axelrod H and Pienta KJ: Axl as a mediator of cellular growth and survival. Oncotarget *5*: 8818-8852, 2014.
7 Linger RM, Keating AK, Earp HS and Graham DK: Taking aim at Mer and Axl receptor tyrosine kinases as novel therapeutic targets in solid tumors. Expert Opin Ther Targets *14*: 1073-1090, 2010.
8 Nagata K, Ohashi K, Nakano T, Arita H, Zong C, Hanafusa H and Mizuno K: Identification of the product of growth arrest-specific gene 6 as a common ligand for Axl, Sky, and Mer receptor tyrosine kinases. J Biol Chem *271*: 30022-30027, 1996.
9 Dugo M, Nicolini G, Tragni G, Bersani I, Tomassetti A, Colonna V, Del Vecchio M, De Braud F, Canevari S, Anichini A and Sensi M: A melanoma subtype with intrinsic resistance to BRAF inhibition identified by receptor tyrosine kinases gene-driven classification. Oncotarget *6*: 5118-5133, 2015.
10 Shieh YS, Lai CY, Kao YR, Shiah SG, Chu YW, Lee HS and Wu CW: Expression of Axl in lung adenocarcinoma and correlation with tumor progression. Neoplasia *7*: 1058-1064, 2005.
11 Zhang YX, Knyazev PG, Cheburkin YV, Sharma K, Knyazev YP, Orfi L, Szabadkai I, Daub H, Kéri G and Ullrich A: AXL is a potential target for therapeutic intervention in breast cancer progression. Cancer Res *68*: 1905-1915, 2008.
12 Jacob AN, Kalapurakal J, Davidson WR, Kandpal G, Dunson N, Prashar Y and Kandpai RP: A receptor tyrosine kinase, UFO/Axl, and other genes isolated by a modified differential display PCR are overexpressed in metastatic prostatic carcinoma cell line DU145. Cancer Detect Prev *23*: 325-332, 1999.
13 Gustafsson A, Martuszewska D, Johansson M, Ekman C, Hafizi S, Ljungberg B and Dahlbäck B: Differential expression of Axl and Gas6 in renal cell carcinoma reflecting tumor advancement and survival. Clin Cancer Res *15*: 4742-4749, 2009.
14 Sun W, Fujimoto J and Tamaya T: Coexpression of Gas6/Axl in human ovarian cancers. Oncology *66*: 450-457, 2004.
15 Wu X, Liu X, Koul S, Lee CY, Zhang Z and Halmos B: AXL kinase as a novel target for cancer therapy. Oncotarget *5*: 9546-9563, 2014.
16 Chen PX, Li QY and Yang Z: Axl and prostasin are biomarkers for prognosis of ovarian adenocarcinoma. Ann Diagn Pathol *17*: 425-429, 2013.
17 Rea K, Pinciroli P, Sensi M, Alciato F, Bisaro B, Lozneanu L, Raspagliesi F, Centritto F, Cabodi S, Defilippi P, Avanzi GC, Canevari S and Tomassetti A: Novel Axl-driven signaling pathway and molecular signature characterize high-grade ovarian cancer patients with poor clinical outcome. Oncotarget *6*: 30859-30875, 2015.
18 Tothill RW, Tinker AV, George J, Brown R, Fox SB, Lade S, Johnson DS, Trivett MK, Etemadmoghadam D, Locandro B, Traficante N, Fereday S, Hung JA, Chiew YE and Haviv I, Australian Ovarian Cancer Study Group, Gertig D, DeFazio A and Bowtell DD: Novel molecular subtypes of serous and endometrioid ovarian cancer linked to clinical outcome. Clin Cancer Res *14*: 5198-5208, 2008.
19 Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature *474*: 609-615, 2011.
20 Kurman RJ, Carcangiu ML, Herrington CS and Young RH: WHO Classification of Tumours of the Female Reproductive Organs (IARC WHO Classification of Tumours). 4th ed. Lyon, IARC Press, 2014.
21 Rankin EB, Fuh KC, Taylor TE, Krieg AJ, Musser M, Yuan J, Wei K, Kuo CJ, Longacre TA and Giaccia AJ: AXL is an essential factor and therapeutic target for metastatic ovarian cancer. Cancer Res *70*: 7570-7579, 2010.
22 Li Y, Jia L, Liu C, Gong Y, Ren D, Wang N, Zhang X and Zhao Y: Axl as a downstream effector of TGF-β1 via PI3K/Akt-PAK1 signaling pathway promotes tumor invasion and chemoresistance in breast carcinoma. Tumour Biol *36*: 1115-1127, 2014.
23 Graham DK, DeRyckere D, Davies KD and Earp HS: The TAM family: phosphatidylserine sensing receptor tyrosine kinases gone awry in cancer. Nat Rev Cancer *14*: 769-785, 2014.
24 Davidowitz RA, Selfors LM, Iwanicki MP, Elias KM, Karst A, Piao H, Ince TA, Drage MG, Dering J, Konecny GE, Matulonis U, Mills GB, Slamon DJ, Drapkin R and Brugge JS: Mesenchymal gene program-expressing ovarian cancer spheroids exhibit enhanced mesothelial clearance. J Clin Invest *124*: 2611-2625, 2014.

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