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Activity and clinical relevance of autotaxin and lysophosphatidic acid pathways in high-grade serous carcinoma

Running title: LPA in serous effusions

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Abstract

The aim of this study was to analyze the expression, biological role and clinical relevance of autotaxin (ATX), the enzyme synthetizing lysophosphatidic acid (LPA), and LPA receptors (LPAR) in high-grade serous carcinoma (HGSC). mRNA expression by qRT-PCR of *LPAR1-6* was analyzed in 155 HGSC specimens (88 effusions, 67 solid lesions). *ATX* mRNA expression was analyzed in 97 specimens. ATX, ERK and AKT protein expression was studied by Western Blotting. *LPAR2* mRNA was overexpressed in HGSC cells in effusions compared to solid lesions, with opposite findings for *LPAR3* and *LPAR6* mRNA and ATX protein. Higher *LPAR1* levels were significantly related to longer overall survival (OS) in pre-chemotherapy effusions (p=0.027). Conversely, higher expression of *LPAR1*, *LPAR2* and *LPAR5* in post-chemotherapy effusions was significantly associated with shorter OS (p=0.037, p=0.025 and p=0.021, respectively) and PFS (p<0.001, p=0.007 and p<0.001, respectively) in univariate survival analysis. *LPAR1* mRNA expression was an independent prognosticator of OS and PFS in patients with pre- and post-chemotherapy effusions, respectively (p=0.013 both). In conclusion, *LPAR* mRNA and ATX protein levels are anatomic site-dependent in HGSC and the former are informative of disease outcome.

Keywords: lysophosphatidic acid; autotaxin; high-grade serous carcinoma; effusion; survival

Introduction

Ovarian cancer, consisting predominantly of ovarian carcinoma (OC), is the most lethal gynecologic malignancy and ranks as the 5th most common and 6th most lethal cancer in women in developed countries [1]. The aggressive behavior of ovarian cancer owes mainly to diagnosis at advanced stage, tumor heterogeneity and intrinsic or acquired chemoresistance [2].

Lysophosphatidic acid (LPA) is a bioactive phospholipid present in many cells, as well as in plasma and serum [3,4]. It is involved in numerous physiological and pathological cellular activities, such as proliferation, platelet aggregation, wound healing, atherosclerosis and cancer [5-7]. It possesses a mitogenic, anti-apoptotic activity and regulates tumor angiogenesis and invasion through inducing the expression of numerous genes [5,8,9]. A group of six G protein-coupled receptors (GPCRs), of which LPAR1, LPAR2, and LPAR3 are the best characterized and most widely expressed, mediates the cellular responses of LPA [5,10,11]. Previous reports have suggested that an upregulated expression of LPARs may mediate tumor growth and metastasis [12-15]. LPA is produced via autotaxin (ATX), a secreted lysophospholipase D (lysoPLD) which hydrolyzes membrane-derived lysophosphatidylcholine (LPC) to produce LPA [8,16-18].

The activity of LPA has been extensively documented in ovarian cancer and shown to mediate tumor growth and survival, as well as induce motility via its receptors LPAR2 and LPAR3, and to correlate with poor prognosis [15,19,20]. Yet, little is known about each one of these receptors and its downstream effect in this disease. Elucidating the signaling pathway of each LPA receptor would help further understand the ATX-LPA axis and its involvement in the progression of this malignancy. Additionally, the identification of LPA as

a novel “ovarian cancer activating factor” present in ascites from ovarian cancer patients suggests that LPA may be a therapeutic target in ovarian cancer [5,21,22].

The present study analyzed the expression and potential prognostic role of LPAR family members and ATX in high-grade serous carcinoma (HGSC), the most common and clinically aggressive OC histotype, with focus on effusion specimens.

Materials and methods

Patients and specimens

Specimens were submitted for routine diagnostic purposes to the Department of Pathology at the Norwegian Radium Hospital during the period of 1998 to 2008. HGSC specimens and clinical data were obtained from the Department of Gynecologic Oncology, Norwegian Radium Hospital. As the fallopian tubes have not been adequately assessed in this cohort, tumors in the ovary are specified as such without reference to primary site. The diagnosis of HGSC was made based on the combination of morphology (obvious nuclear atypia and the presence of multiple mitoses) and the presence of aberrant (diffusely positive or entirely negative) p53 immunostaining.

HGSC effusions analyzed for *LPAR* mRNA expression using quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) consisted of 88 effusions (69 peritoneal, 19 pleural) from 88 patients. Clinicopathologic data for this cohort are presented in **Table 1**. Additionally, 38 solid ovarian carcinoma specimens and 29 solid metastases, the majority omental, were analyzed for comparative purposes. *ATX* mRNA expression was analyzed in 97 specimens (60 effusions, 20 ovarian tumors, 17 solid metastases).

Effusions were centrifuged immediately after tapping, and cell pellets were frozen at -70°C in equal amounts of RPMI 1640 medium (GIBCO-Invitrogen, Carlsbad, CA) containing 50% fetal calf serum (PAA Laboratories GmbH, Pasching, Austria) and 20% dimethylsulfoxide (Merck KGaA, Darmstadt, Germany). Supernatants were frozen at -70°C without any treatment. Effusions were diagnosed by an experienced cytopathologist (BD) based on morphology and immunohistochemistry performed on cell blocks prepared using the Thrombin clot method. Frozen sections from all solid tumors were reviewed by an experienced gynecopathologist (BD), and only specimens with tumor cell population >50% and minimal or no necrosis were included in this study.

Informed consent was obtained according to national and institutional guidelines. Study approval was given by the Regional Committee for Medical Research Ethics in Norway.

qRT-PCR

mRNA levels of the various LPA receptors and the housekeeping gene RPLP0 were analyzed by qRT-PCR. cDNA from the 155 above-described specimens (88 effusions, 67 surgical specimens were amplified using KAPA SYBR FAST qPCR kit (Kapa Biosystems, Wilmington MA). PCR specificity was confirmed by appropriate melting curves. mRNA levels were established by calculating the target molecule/RPLP0 ratio. RNA (1000 ng) was reverse-transcribed using qScript™ cDNA synthesis Kit (Quanta Biosciences, Gaithersburg, MD). The cDNA sample was further processed by real time PCR (CFX Connect™ Real-Time System, Bio-Rad, Hercules CA), using KAPA SYBR FAST Universal qPCR Kit. Primers used for qRT-PCR are listed in **Table 2-A**.

Western blotting (WB)

ATX protein expression by WB was analyzed in 146 specimens, including 70 effusions and 76 surgical specimens (38 ovarian carcinomas and 38 solid metastases). Cell-free effusion supernatants from 80 samples and exosomes from 24 samples were additionally studied for ATX. Protein from 92 specimens, including 49 effusions and 43 surgical specimens (22 ovarian carcinomas and 21 solid metastases) was studied for ERK, p-ERK, AKT and p-AKT expression.

25µg of protein from each specimen and 5µl of effusion supernatant or exosomes were resolved by 10% SDS-PAGE. The separated extracts were transferred onto Immobilon PVDF membrane (Millipore, Bedford, MA) in a transfer buffer. Membranes were then incubated for 1h in TBST containing 5% Difco™ skim milk (BD Biosciences, San Jose, CA) to block non-

specific binding. Blots were then incubated with a monoclonal antibody against ATX (E-12; sc-374222, Santa Cruz Biotechnology, Santa Cruz, CA), ERK monoclonal antibody (#4695, Cell Signaling Biotechnology, Danvers, MA), p-ERK (#4377, Cell Signaling Biotechnology), AKT monoclonal antibody (#4691, Cell Signaling Biotechnology) or p-AKT monoclonal antibody (#4060, Cell Signaling Biotechnology). GAPDH (14C10; Cell Signaling Biotechnology) was used as loading control. Proteins were detected using EZ-ECL Chemiluminescence detection kit for HRP (Biological Industries) according to the manufacturer's specifications using Image Lab 5.0 gel reader (Bio-Rad, Hercules CA). Densitometer analysis of blots was performed using a computerized image analysis program (Image-J, NIH, Bethesda, MD). Protein expression levels were established by calculating the target molecule/GAPDH ratio (all cases scored for band intensity compared with internal control). Expression intensity of 5% or less of control levels was interpreted as negative.

Statistical analysis

Statistical analysis was performed applying the SPSS-PC package (Version 24, Chicago IL). Probability of <0.05 was considered statistically significant. Comparative analysis of LPAR, ATX, ERK, p-ERK, AKT and p-AKT expression in effusions, ovarian tumors and solid metastases was performed using the Kruskal-Wallis H test. Analysis of the association between expression levels of LPAR and ATX in HGSC effusions and clinicopathologic parameters was executed using the Mann-Whitney U test. For this analysis, as well as for survival analysis, clinicopathologic parameters were grouped as follows: age: $\leq$60 vs. >60 years; effusion site: peritoneal vs. pleural; FIGO stage: III vs. IV; chemotherapy status: pre- vs. post-chemotherapy specimens; residual disease (RD): $\leq$1 cm vs. >1 cm; response to chemotherapy: complete response vs. partial response/stable disease/progressive disease.

Progression-free survival (PFS) and overall survival (OS) were calculated from the date of the last chemotherapy treatment/diagnosis to the date of recurrence/death or last follow-up, respectively. Univariate survival analyses of PFS and OS were executed using the Kaplan-Meier method and log-rank test. Platinum resistance was defined as PFS≤6 months according to guidelines published by the Gynecologic Oncology Group and progressive disease or recurrence was evaluated by RECIST criteria. Multivariate survival analysis was performed using the Cox regression model (Enter function).

Results

LPARs, ATX, p-ERK and AKT are differentially expressed at different anatomic sites in HGSC

Comparative analysis of LPAR expression in the ovarian carcinomas, solid metastases and effusions showed significantly higher expression of *LPAR2* mRNA in effusions compared to both groups of solid specimens ($p<0.001$), whereas the opposite was true for *LPAR3* ($p=0.001$) and *LPAR6* ($p<0.001$; **Figure 1-A**). *LPAR1* and *LPAR5* levels did not differ significantly at the different anatomic sites ($p=0.127$ and $p=0.231$, respectively). *LPAR4* was not expressed in any of the HGSC specimens.

ATX mRNA expression was comparable at the three anatomic sites ($p=0.447$; **Figure 1-B**). However, ATX protein expression was significantly higher in the ovarian carcinomas and solid metastases compared to effusions ($p<0.001$). A 5-tier comparative analysis of ATX protein expression in HGSC cells at the three above-mentioned anatomic sites, in effusion supernatants and in exosomes showed comparable expression levels in effusions and exosomes (mean rank=96.02 and 112.92, respectively), with higher expression in the ovarian tumors, solid metastases and effusion supernatants (mean rank=160.26, 153.09 and 143.34, respectively; **Figure 1-C**).

p-ERK levels ($p<0.001$), p-ERK/ERK ratio ($p=0.001$) and total AKT levels ($p=0.008$) were highest in solid metastases, intermediate in the ovarian tumors, and lowest in the effusions. No significant anatomic site-related differences were seen for total ERK, p-AKT and AKT ratio (**Figure 1-D**).

Association with clinicopathologic parameters and survival

The clinical relevance of the studied molecules was analyzed in the effusion cohort, which included the largest number of patients. *LPAR3* levels were significantly higher in pre-

chemotherapy effusions tapped at diagnosis (n=43) compared to post-chemotherapy specimens (n=44; p=0.025; 1 patient with no data regarding chemotherapy status). Higher *LPAR2* and *LPAR5* expression was seen in specimens from patients with RD>1 cm (p=0.008 and p=0.013, respectively). Higher *LPAR2* and *LPAR5* levels were measured in effusions from patients whose tumors showed primary resistance to chemotherapy (PFS≤6 months; p=0.034 and p=0.017, respectively).

ATX mRNA and protein expression was unrelated to any of the clinicopathologic parameters (p>0.05; data not shown).

The follow-up period for the 88 patients with HGSC effusions studied for *LPAR* mRNA expression ranged from 1 to 179 months (mean = 37 months, median = 26 months). PFS ranged from 0 to 81 months (mean = 10 months, median = 6 months). At the last follow-up, 82 patients were dead of disease, 4 were alive with disease and 1 was with no evidence of disease. One patient was lost to follow-up. The association between LPAR expression, as well as clinical parameters (age, FIGO stage and RD volume), and survival was analyzed. In univariate survival analysis of all cases, LPAR and LPA expression was unrelated to survival (p>0.05; data not shown). However, in analysis limited to patients with pre-chemotherapy effusions tapped at diagnosis, higher *LPAR1* levels were significantly related to longer OS (p=0.027; **Figure 2-A**). A trend for longer PFS was additionally observed for *LPAR1* (p=0.061). Conversely, in univariate survival analysis limited to patients with post-chemotherapy effusions, higher expression of *LPAR1* (p=0.037), *LPAR2* (p=0.025) and *LPAR5* (p=0.021) was significantly associated with shorter OS (**Figures 2-B to 2-D**). High expression of *LPAR1* (p<0.001), *LPAR2* (p=0.007) and *LPAR5* (p<0.001) was further strongly associated with shorter PFS (**Figures 2-E to 2-G**).

None of the clinicopathologic parameters was significantly associated with OS in patients with pre-chemotherapy effusions. Parameters with p-value <0.2, including patient age (p=0.184) and RD volume (p=0.149), were entered into the Cox multivariate analysis with *LPAR1*. *LPAR1* was the only parameter with independent prognostic role (p=0.013).

None of the clinicopathologic parameters was significantly associated with OS in patients with post-chemotherapy effusions. Parameters with p-value <0.2, including FIGO stage (p=0.078) and RD volume (p=0.155), were entered into the Cox multivariate analysis with *LPAR1*, *LPAR2* and *LPAR5*. FIGO stage was the only parameter with independent prognostic role in this patient group (p=0.013).

Among the clinicopathologic parameters, RD volume was significantly related to PFS (p=0.042), with a trend for FIGO stage (p=0.054), in patients with post-chemotherapy specimens. These parameters and expression level of *LPAR1*, *LPAR2* and *LPAR5* were included in Cox multivariate analysis. FIGO stage (p=0.005) and *LPAR1* expression (p=0.013) were independent predictors of PFS in this patient group.

ATX mRNA and protein expression was unrelated to survival in the entire cohort, as well as in separate analysis for patients with pre- and post-chemotherapy effusion (p>0.05; data not shown).

Discussion

OC is characterized by the presence of malignant effusions that are associated with poor prognosis. A growing number of studies have documented changes in the expression and activation of various molecular pathways in the transition from the solid tumor to effusion [23-25].

Westermann et al. found elevated levels of LPA in malignant effusions from ovarian cancer patients, along with elevated levels of ATX. The ATX-LPA axis is a signaling pathway involved in inflammation and cancer through diverse cellular processes, such as cell migration and proliferation. Progress has been made in recent years to uncover the role of this axis in ovarian cancer [17,18,26], yet the downstream signaling is still unclear, and many questions are yet to be answered.

Previous studies of LPAR in ovarian cancer have focused on comparative analysis of tumor tissue and normal ovaries and/or benign ovarian tumors [27-29]. In the present study, we chose instead to compare LPAR expression in HGSC effusion-derived cells to that in solid lesions, the majority from patients with advanced-stage disease. We observed significant anatomic site-related differences in the expression of 3 LPAR family members, with the most profound differences between solid lesions and effusions. *LPAR2* mRNA levels were higher in malignant effusions, while the opposite for *LPAR3* and *LPAR6*. Another finding was that *LPAR4* was not detected at any of the different anatomic sites, though it is reported to be present at elevated levels in the normal ovary. To the best of our knowledge, this is the first documentation of changes in LPAR expression along tumor progression in HGSC.

We additionally found significant association between LPAR expression and clinicopathologic parameters, including an association between higher *LPAR2* and *LPAR5* and primary resistance to chemotherapy. That would be in agreement with the recently

reported role for LPA-ATX signaling in mediating a cancer stem cell (CSC) phenotype in OC cell lines [30], particularly in view of the fact that OC cells in effusions possess CSC characteristics [2].

Data regarding the prognostic role of LPAR family members in OC are, to the best of our knowledge, unavailable to date. In the present study, we observed that higher *LPAR1* mRNA levels in pre-chemotherapy HGSC effusions tapped at diagnosis are associated with longer OS, whereas higher *LPAR1*, *LPAR2* and *LPAR5* levels in post-chemotherapy effusions, the majority tapped at disease recurrence, were associated with shorter OS and PFS. The opposite association between *LPAR1* expression and survival in pre- and post-chemotherapy effusions remained significant in Cox multivariate analysis. The reason for this change in clinical significance along disease progression is unclear at present. One may, however, speculate that the selection of chemoresistant tumor cell populations that occurs in recurrent disease may affect the clinical significance of LPAR expression. In chemoresistant cells, higher LPAR expression would then mediate aggressive behavior. The association between LPAR1 and aggressive disease in the metastatic setting is supported by its involvement in bone metastasis [31].

Earlier research had documented that peritoneal fluid from OC patients also contains high levels of ATX [32,33]. In the present study, we compared ATX levels in effusions to those of surgical specimens. Higher levels of ATX were found in HGSC cells in solid lesions compared to their counterparts in effusions, where ATX was almost completely depleted. However, high ATX levels were detected in effusion supernatants and in exosomes, indicating that ATX is secreted from HGSC cells in effusions into the effusion fluid through the secretion of exosomes.

In summary, we report on anatomic site-related differences in expression of LPARs in HGSC, as well as prognostic role for LPARs in metastatic HGSC in effusion. These results can contribute to our understanding the role of ATX and LPA in HGSC progression. Inhibition of specific LPA receptors may be a novel molecular therapy for this malignancy.

Compliance with Ethical Standards: The study was approved by the Regional Committee for Medical Research Ethics in Norway.

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Conflict of interest: None declared

Author contributions:

HO: Performed the experiments and wrote the manuscript.

LJC: Participated in performing the experiments, critically read the manuscript.

CGT: Provided clinical data, critically read the manuscript.

TEHF: Provided clinical data, critically read the manuscript.

BD: Designed the study, performed the statistical analysis, and supervised the writing of the manuscript.

RR: Designed the study, supervised the experiments, and participated in writing the manuscript.

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Table 1: Clinicopathologic parameters of the effusion cohort (88 patients)

Parameter	Distribution
Age (mean)	38-81 years (62)
FIGO stage	
II	2
III	47
IV	39
Residual disease [a]	
≤1 cm	26
>1 cm	25
NA [b]	2
CA 125 at diagnosis (range; median)	11-43800 (877) [c]
Chemoresponse after primary treatment	
CR	44
PR	21
SD	7
PD	10
NA [d]	6

Abbreviations: NA = not available; CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease

[a] For 53 patients who received surgery a upfront treatment.

[b] Not available

[c] Available for 61 patients

[d] Disease response after chemotherapy could not be evaluated because of normalized CA 125 after primary surgery or missing CA 125 information and no residual tumor.

Table 2-A: Primer sequences

Gene		**Primer sequence (5'→3')**
LPAR1	Forward	GGCTATGTTCGCCAGAGGACTAT
	Reverse	TCCAGGAGTCCAGCAGATGATAA
LPAR2	Forward	GTTGTCATCATCCTGGGGGC
	Reverse	GAAGCATGATGCGAGTGCTG
LPAR3	Forward	AACGTGAGCGGATGTTCACT
	Reverse	ACAGGCAGAAAAACGTCCCA
LPAR4	Forward	AAAGATCATGTACCCAATCACCT
	Reverse	CTTAAACAGGGACTCCATTCTGA
LPAR5	Forward	CGCCATCTTCCAGATGAAC
	Reverse	TAGCGGTCCACGTTGATG
LPAR6	Forward	GGTAAGCGTTAACAGCTCCCACT
	Reverse	TTTGAGGACGCAGATGAAAATGT
RPLP0	Forward	CCAACTACTTCCTTAAGATCATCCAACTA
	Reverse	ACATGCGGATCTGCTGCA
ATX	Forward	GCCAGAGGAAGTTACCAGACC
	Reverse	TT GTATGAAGCCGTTTGTTGAG

Figure legends

Figure 1: LPAR, ATX and ERK mRNA and protein levels in HGSC at different anatomic sites

A: qRT-PCR of LPARs mRNA in the ovarian tumor, solid metastases and effusions. LPAR2 is overexpressed in effusions compared to solid lesions ($p<0.001$), whereas LPAR3 ($p=0.001$) and LPAR6 ($p<0.001$) are overexpressed in solid lesions. No significant change was found relating to LPAR1 and LPAR5 levels.

B: mRNA mean ranks values for ATX. ATX mRNA expression was comparable at the three anatomic sites.

C: Western Blot for ATX showing representative examples of HGSC cell expression in effusions, ovarian tumors and solid metastases, as well as effusion supernatants and exosomes. Comparable expression levels are seen in effusions and exosomes, with significantly higher expression in the ovarian tumors, solid metastases and effusion supernatants

D: Western Blot for ERK, p-ERK, AKT and p-AKT showing representative examples of HGSC cell expression in effusions, ovarian tumors and solid metastases. Expression of p-ERK and total AKT, as well as the p-ERK/ERK activation ratio, was significantly higher in solid lesions compared to effusions, with highest levels in solid metastases.

* $p<0.05$, ** $p<0.001$; Kruskal-Wallis nonparametric test.

Figure 2: LPAR mRNA expression in HGSC effusions is significantly associated with survival

A. Kaplan-Meier survival curve showing the association between LPAR1 mRNA expression in pre-chemotherapy effusions (n=43) and overall survival (OS). Patients with effusions with high (above median) LPAR1 mRNA expression levels (n=22; solid line) had mean OS of 62

months compared to 31 months for patients with effusions having low LPAR1 mRNA levels (n=21, dashed line; p=0.027).

B. Kaplan-Meier survival curve showing the association between LPAR1 mRNA expression in post-chemotherapy effusions (n=43; one patient with missing value) and OS. Patients with effusions with high (above median) LPAR1 mRNA expression levels (n=20; dashed line) had mean OS of 24 months compared to 37 months for patients with effusions having low LPAR1 mRNA levels (n=23, solid line; p=0.037).

C. Kaplan-Meier survival curve showing the association between LPAR2 mRNA expression in post-chemotherapy effusions (n=43; one patient with missing value) and OS. Patients with effusions with high (above median) LPAR2 mRNA expression levels (n=19; dashed line) had mean OS of 24 months compared to 37 months for patients with effusions having low LPAR2 mRNA levels (n=24, solid line; p=0.025).

D. Kaplan-Meier survival curve showing the association between LPAR5 mRNA expression in post-chemotherapy effusions (n=44) and OS. Patients with effusions with high (above median) LPAR5 mRNA expression levels (n=18; dashed line) had mean OS of 23 months compared to 38 months for patients with effusions having low LPAR5 mRNA levels (n=26, solid line; p=0.021).

E. Kaplan-Meier survival curve showing the association between LPAR1 mRNA expression in post-chemotherapy effusions (n=43; one patient with missing value) and progression-free survival (PFS). Patients with effusions with high (above median) LPAR1 mRNA expression levels (n=20; dashed line) had mean PFS of 4 months compared to 9 months for patients with effusions having low LPAR1 mRNA levels (n=23, solid line; p<0.001).

F. Kaplan-Meier survival curve showing the association between LPAR2 mRNA expression in post-chemotherapy effusions (n=43; one patient with missing value) and PFS. Patients with effusions with high (above median) LPAR2 mRNA expression levels (n=19; dashed

line) had mean PFS of 4 months compared to 9 months for patients with effusions having low LPAR2 mRNA levels (n=24, solid line; p=0.007).

G. Kaplan-Meier survival curve showing the association between LPAR5 mRNA expression in post-chemotherapy effusions (n=44) and PFS. Patients with effusions with high (above median) LPAR5 mRNA expression levels (n=18; dashed line) had mean PFS of 4 months compared to 9 months for patients with effusions having low LPAR5 mRNA levels (n=26, solid line; $p<0.001$).

Figure 1-A

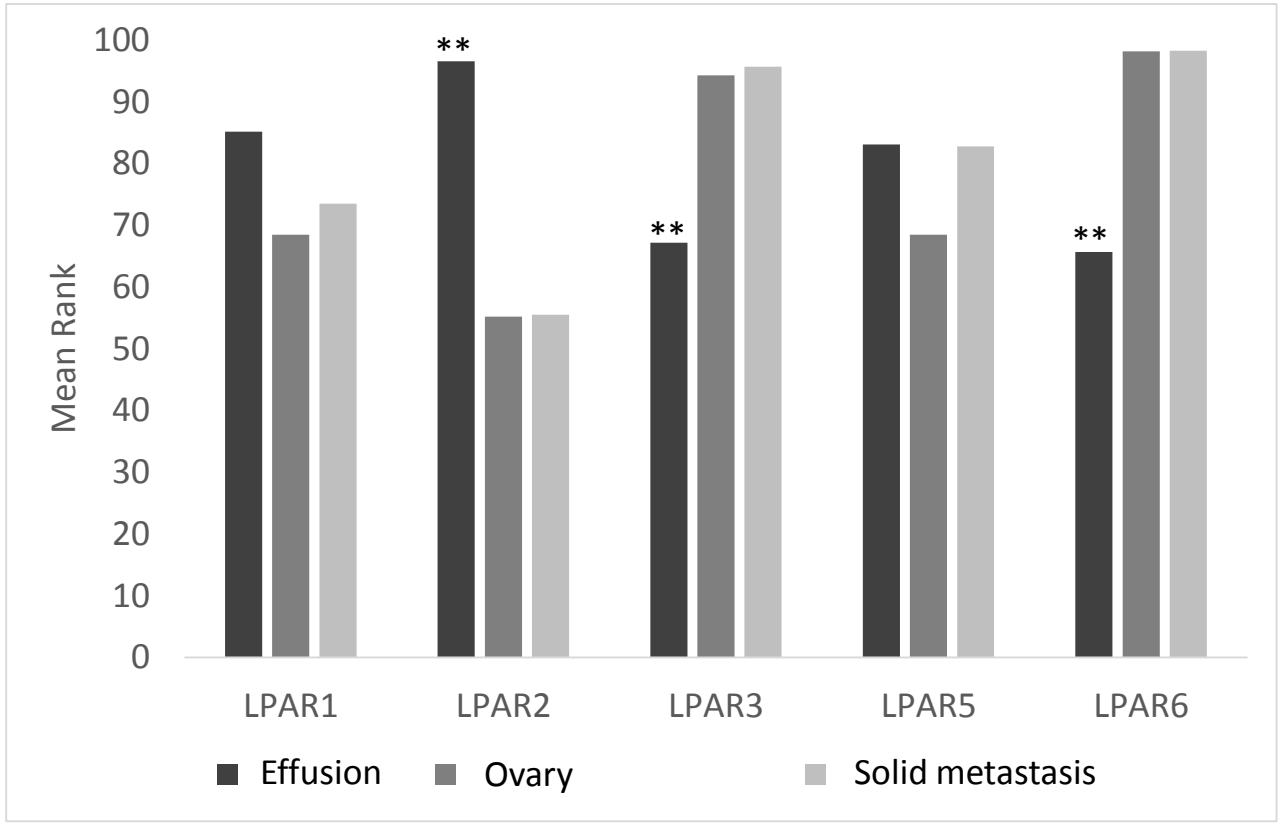

100
90
80
70
60
50
40
30
20
10
0
Mean Rank
**
**
**
LPAR1
LPAR2
LPAR3
LPAR5
LPAR6
Effusion
Ovary
Solid metastasis

Figure 1-B

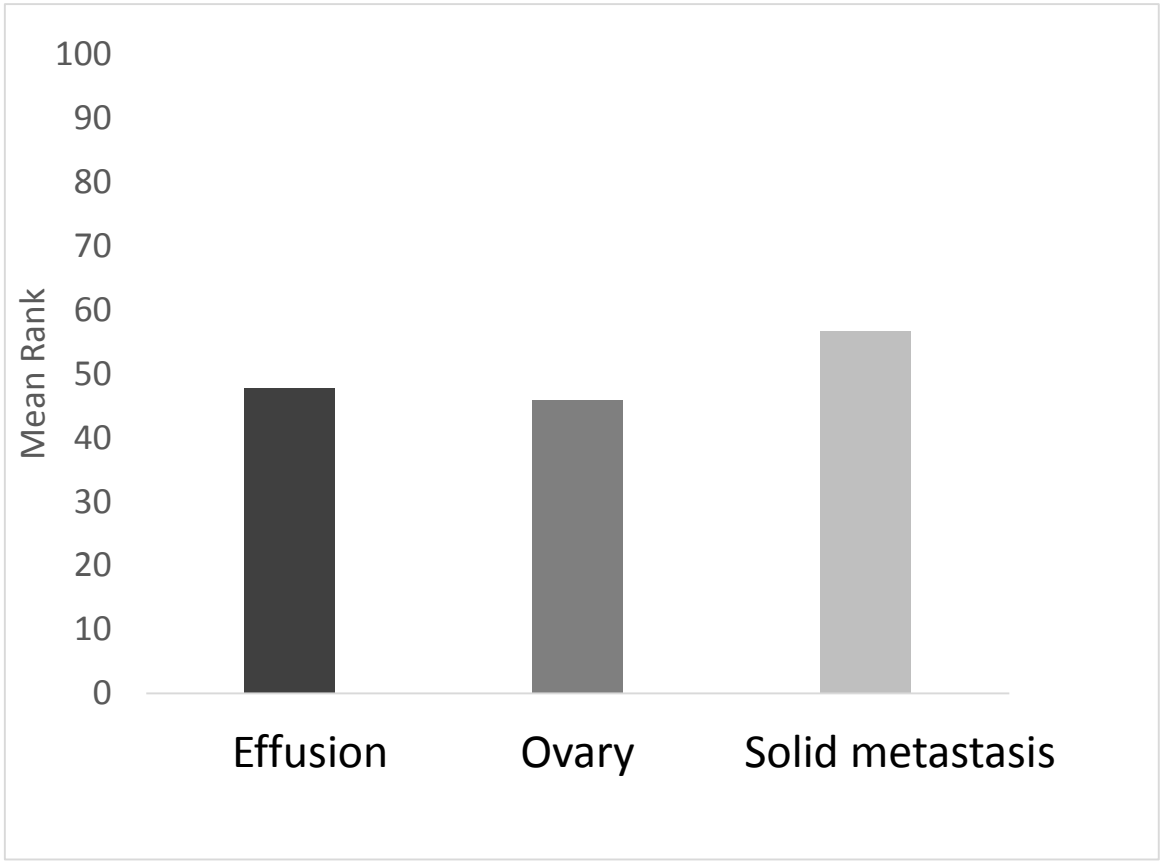
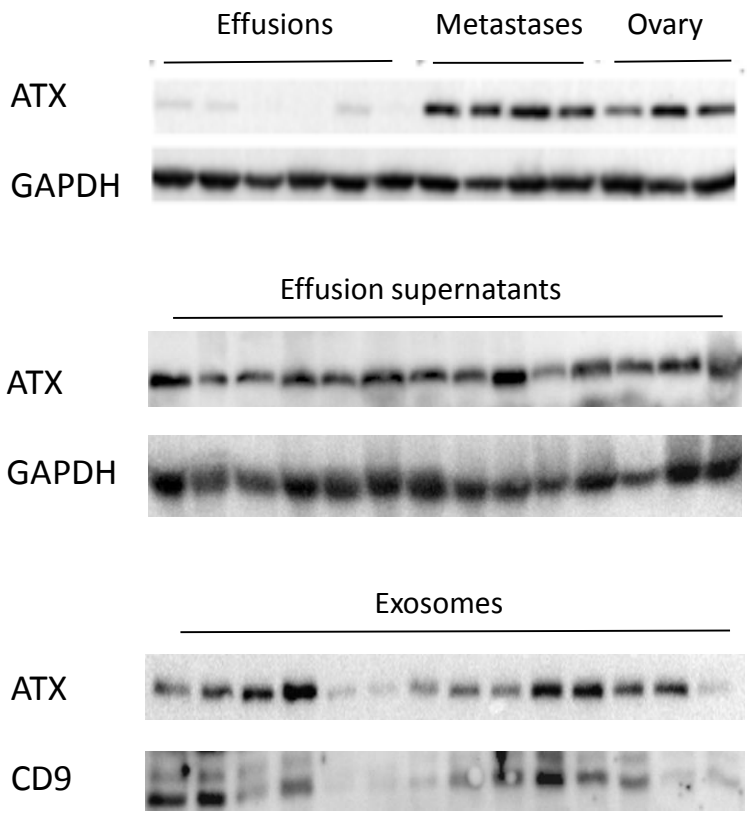

100
90
80
70
60
50
40
30
20
10
0
Mean Rank
Effusion
Ovary
Solid metastasis

Figure 1-C


Effusions
Metastases
Ovary
ATX
GAPDH
Effusion supernatants
ATX
GAPDH
Exosomes
ATX
CD9

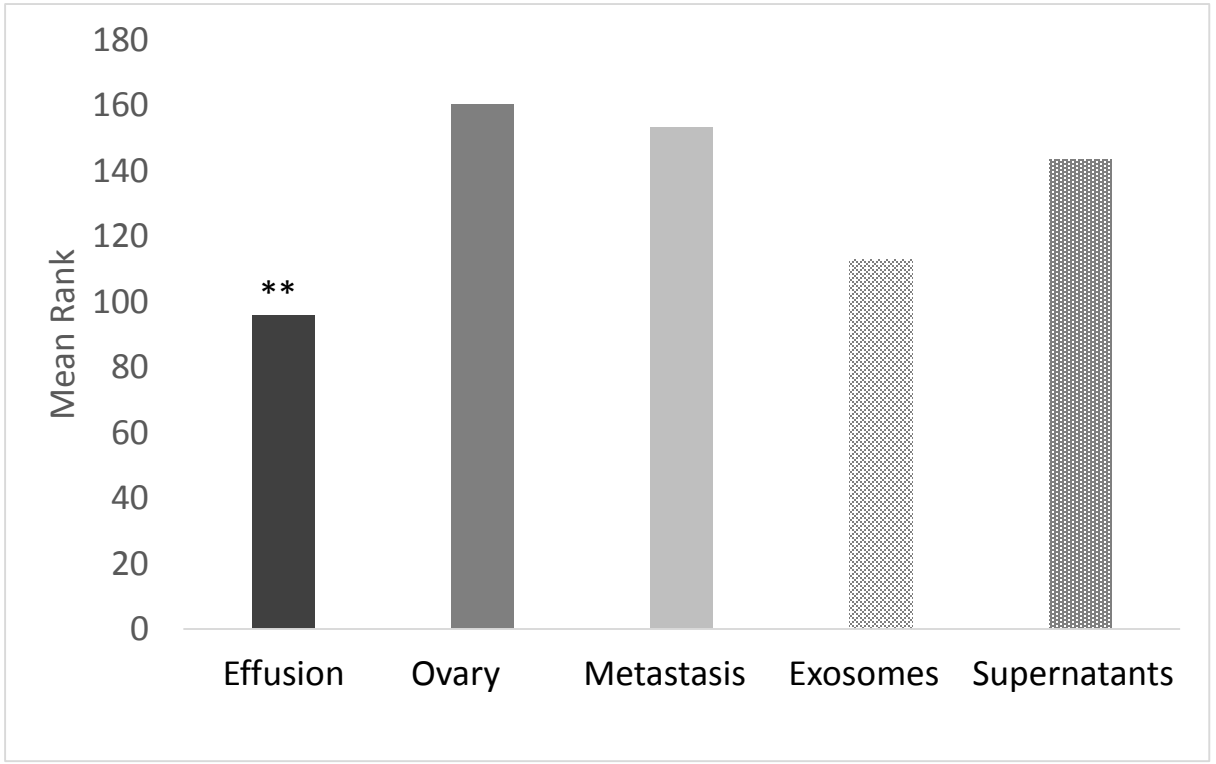
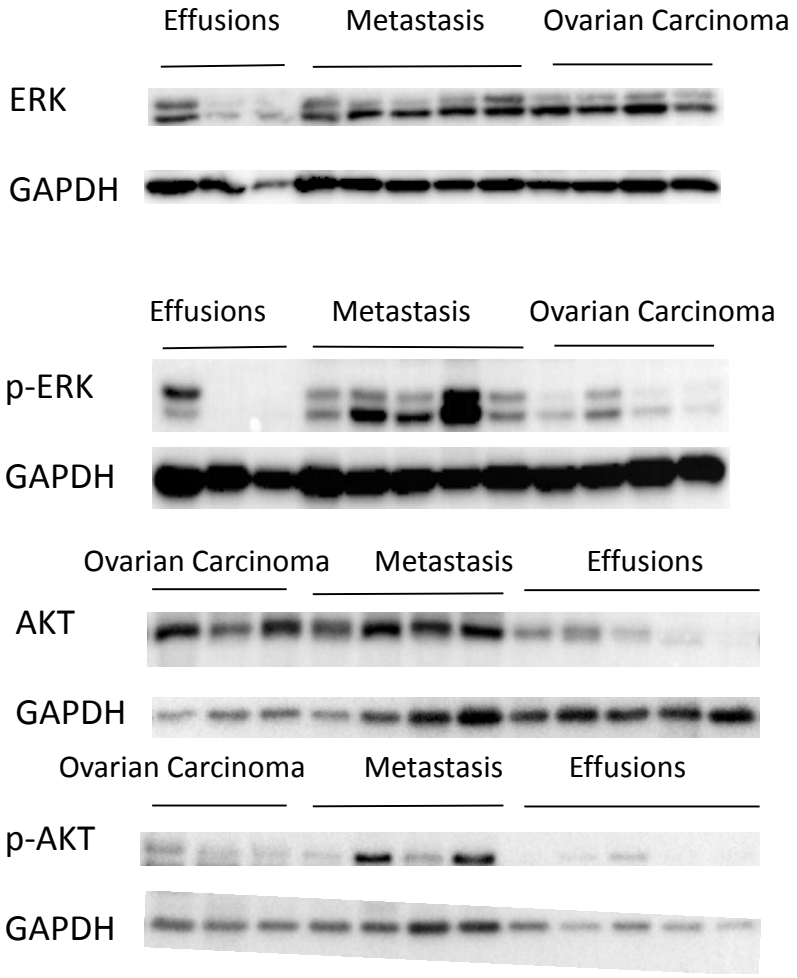

180
160
140
120
100
80
60
40
20
0
Mean Rank
**
Effusion
Ovary
Metastasis
Exosomes
Supernatants

Figure 1-D


Effusions
Metastasis
Ovarian Carcinoma
ERK
GAPDH
Effusions
Metastasis
Ovarian Carcinoma
p-ERK
GAPDH
Ovarian Carcinoma
Metastasis
Effusions
AKT
GAPDH
Ovarian Carcinoma
Metastasis
Effusions
p-AKT
GAPDH

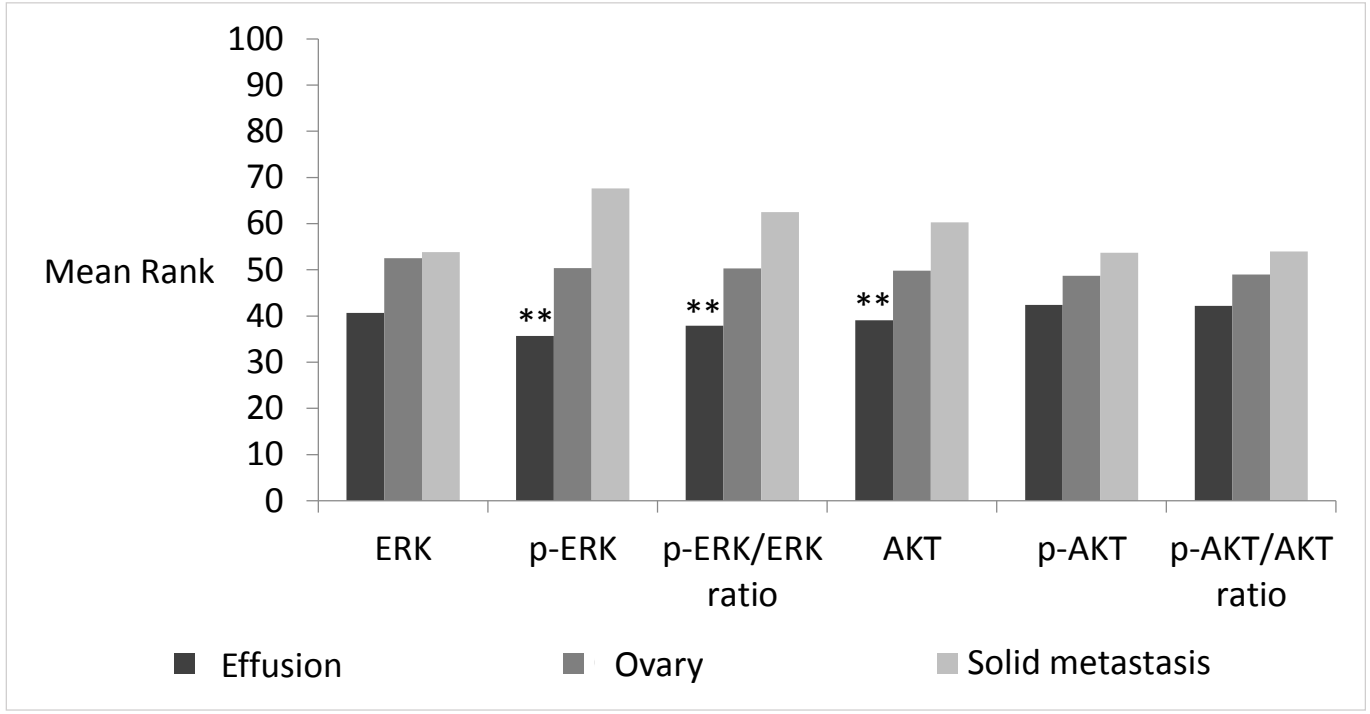

Mean Rank
100
90
80
70
60
50
40
30
20
10
0
**
**
**
ERK
p-ERK
p-ERK/ERK ratio
AKT
p-AKT
p-AKT/AKT ratio
Effusion
Ovary
Solid metastasis

Figure 2-A

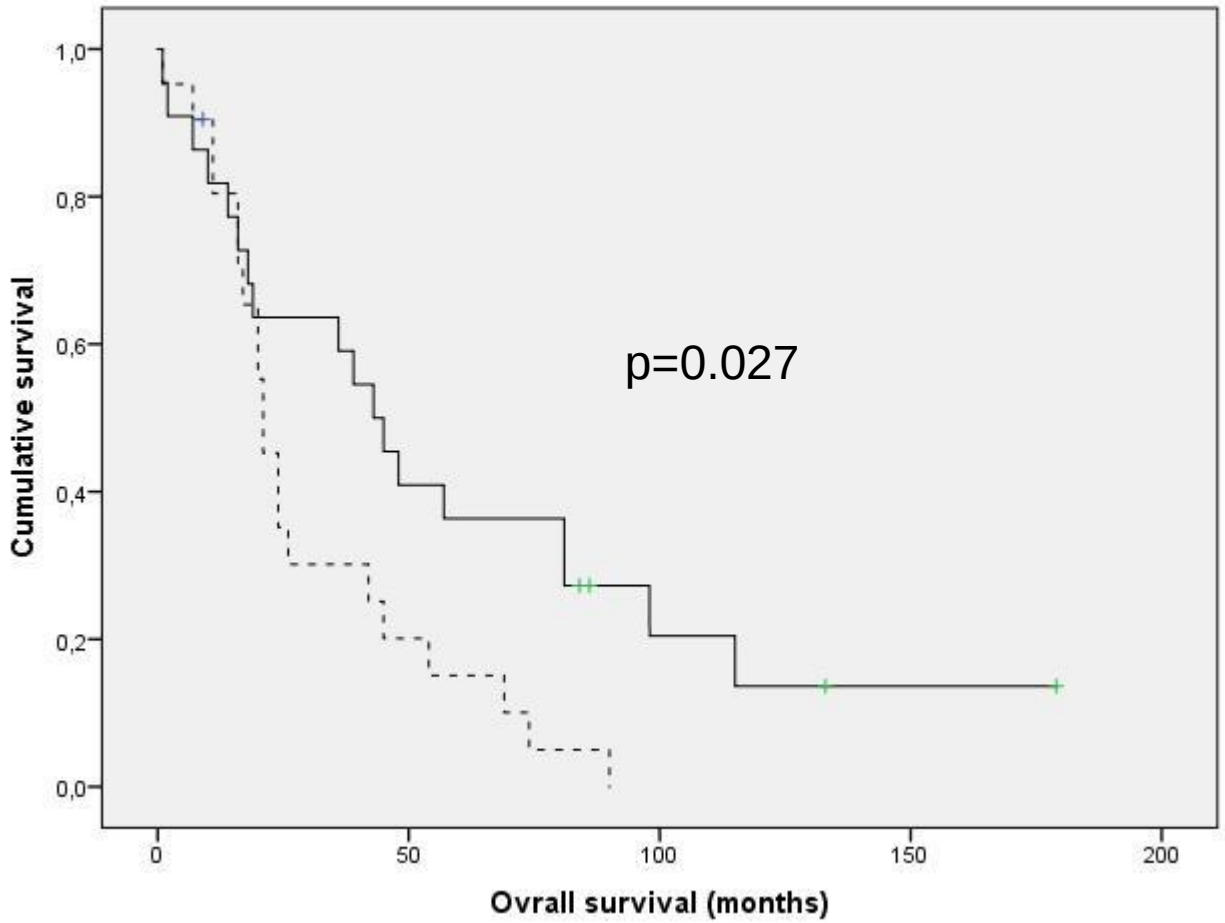

1,0
0,8
0,6
0,4
0,2
0,0
Cumulative survival
p=0.027
0
50
100
150
200
Ovrall survival (months)

Figure 2-B

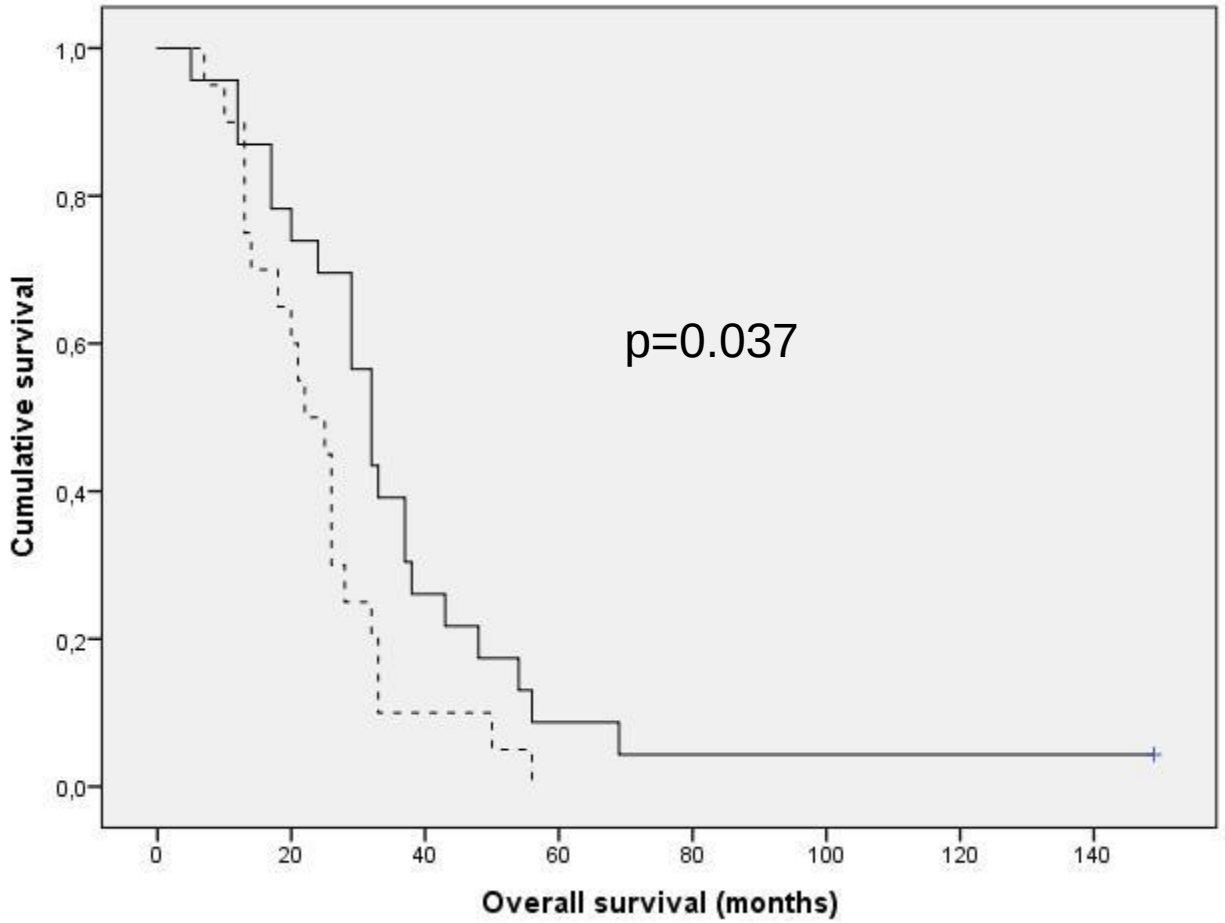

1,0
0,8
0,6
0,4
0,2
0,0
Cumulative survival
p=0.037
0
20
40
60
80
100
120
140
Overall survival (months)

Figure 2-C

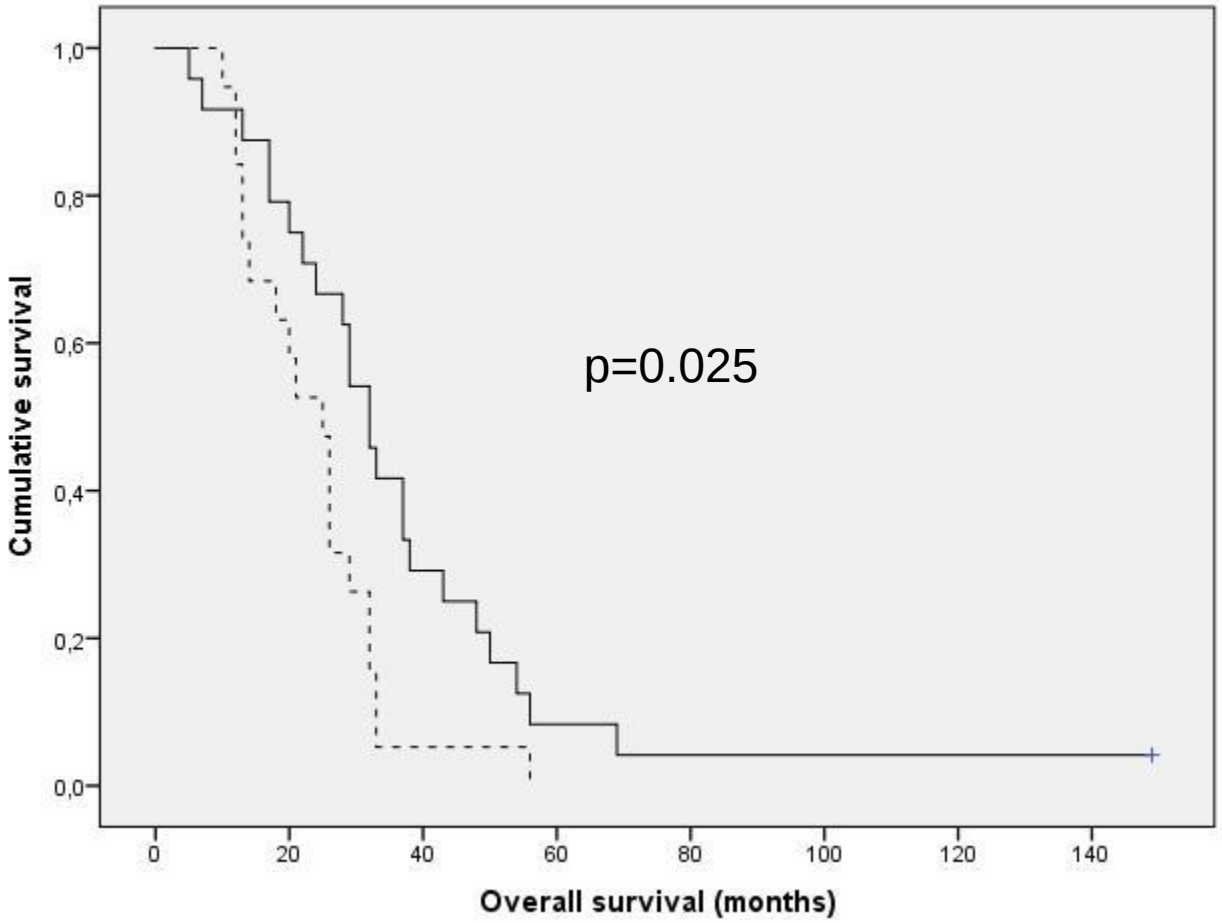

1,0
0,8
0,6
0,4
0,2
0,0
Cumulative survival
p=0.025
0
20
40
60
80
100
120
140
Overall survival (months)

Figure 2-D

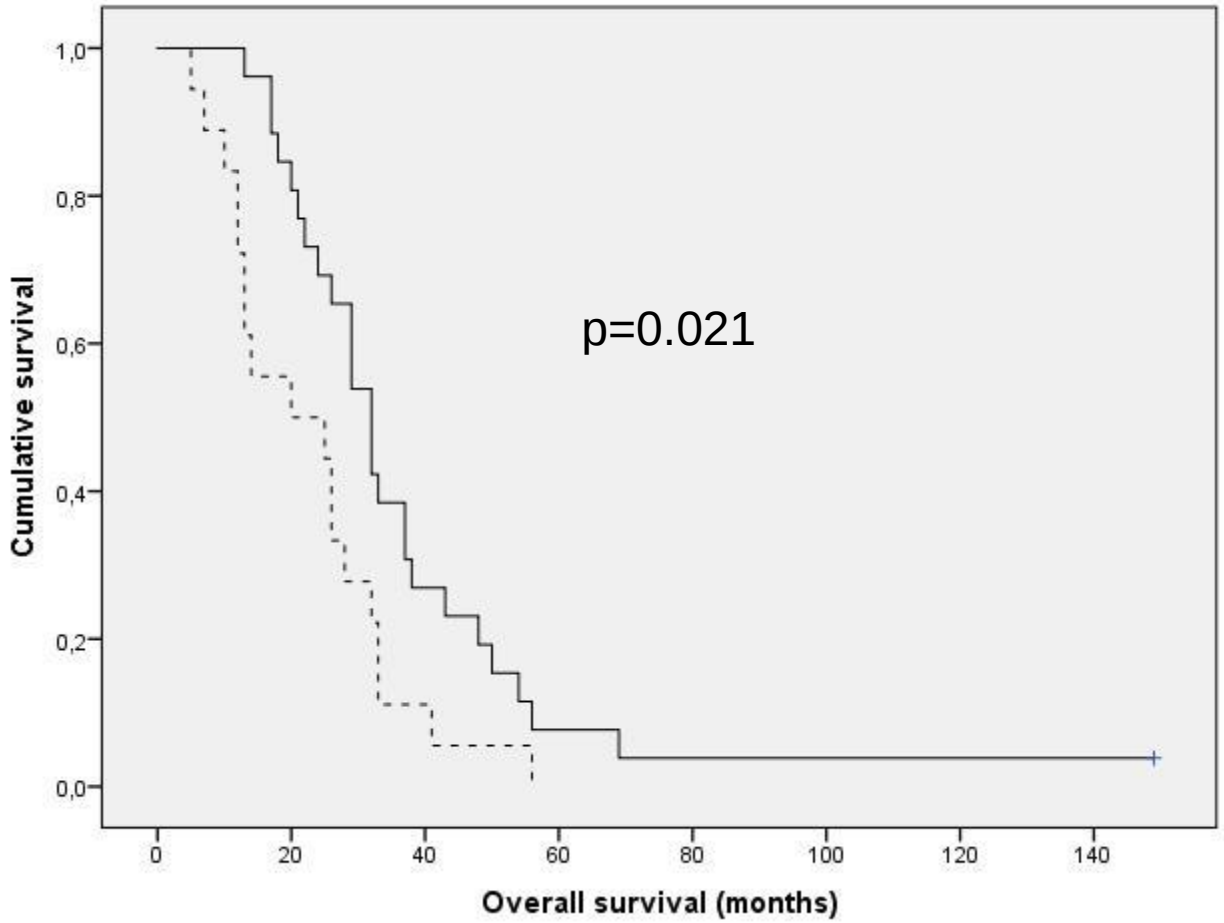

1,0
0,8
0,6
0,4
0,2
0,0
Cumulative survival
p=0.021
0
20
40
60
80
100
120
140
Overall survival (months)

Figure 2-E

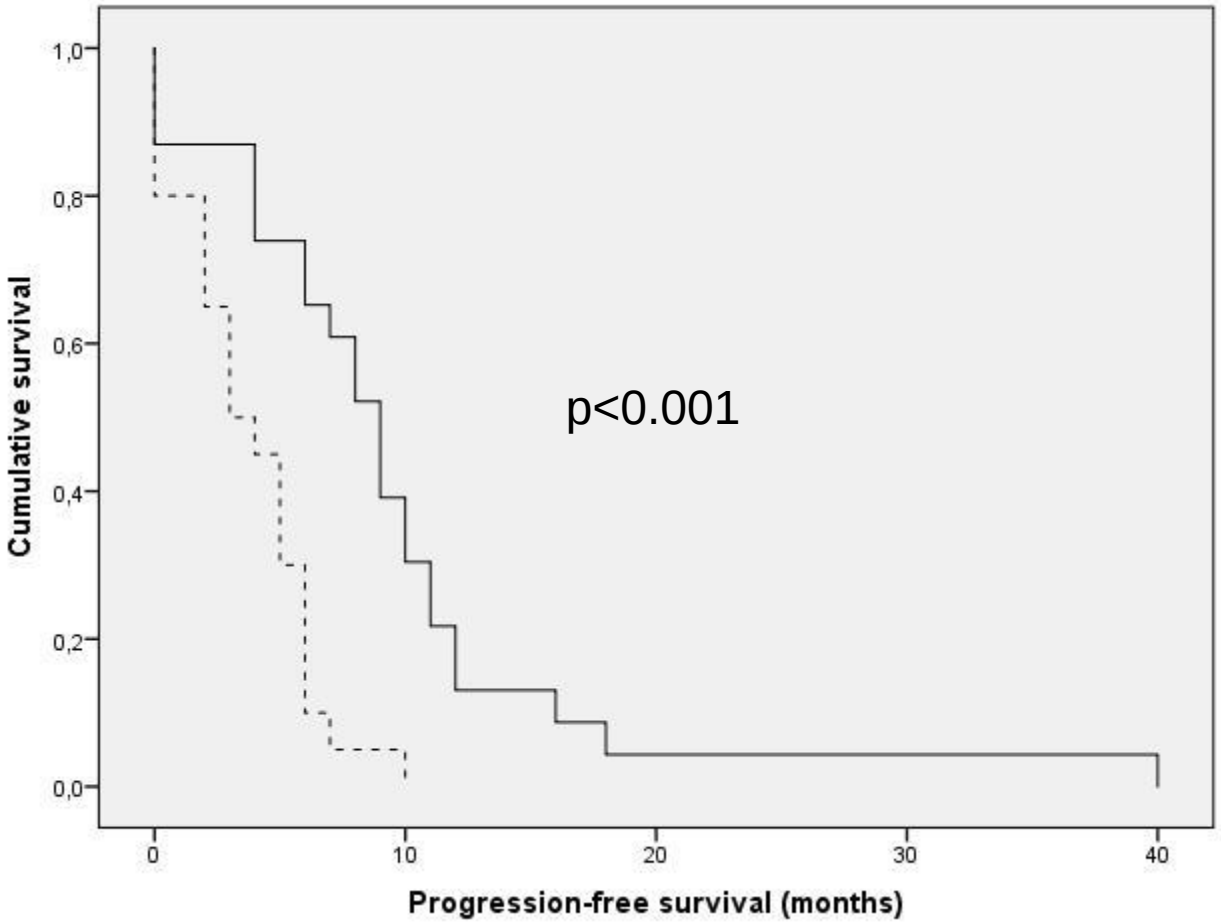

1,0
0,8
0,6
0,4
0,2
0,0
Cumulative survival
p<0.001
0
10
20
30
40
Progression-free survival (months)

Figure 2-F

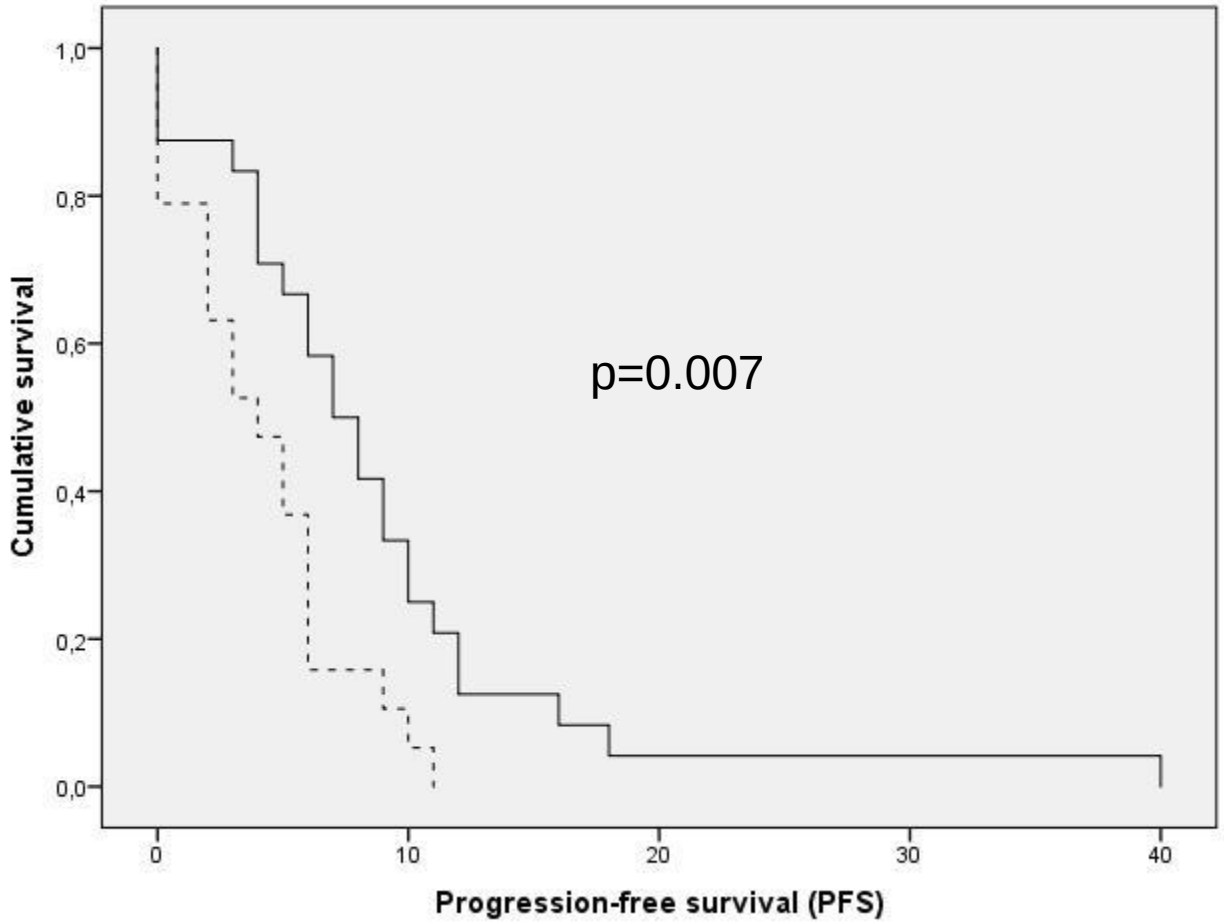
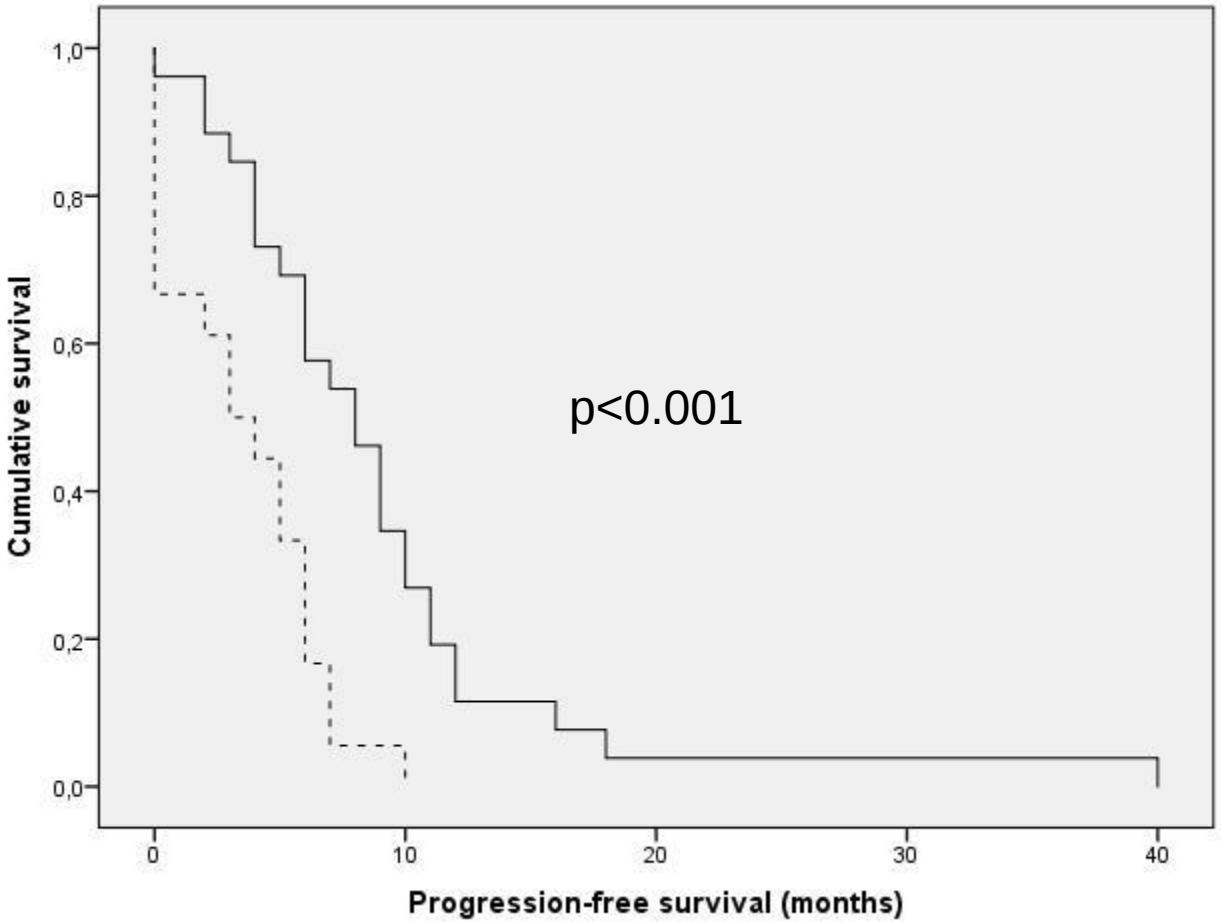

1,0
0,8
0,6
0,4
0,2
0,0
Cumulative survival
p=0.007
0
10
20
30
40
Progression-free survival (PFS)

Figure 2-G


1,0
0,8
0,6
0,4
0,2
0,0
Cumulative survival
p<0.001
0
10
20
30
40
Progression-free survival (months)