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### **Growth arrest-specific 5 lncRNA as a valuable biomarker of chemoresistance in osteosarcoma**

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(Article begins on next page)

## Anti-Cancer Drugs

# Growth arrest-specific 5 (GAS5) lncRNA as a valuable biomarker of chemoresistance in osteosarcoma

--Manuscript Draft--

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Dear Editor,

Here please find a copy of the manuscript entitled “**Title: Growth arrest-specific 5 (GAS5) lncRNA as a valuable biomarker of chemoresistance in osteosarcoma**” that we would like you to consider for publication on *Anti-cancer Drugs*; below we report the reasons why we consider this manuscript highly important for the scientific community and of broad interest for the readership of your journal.

Osteosarcoma (OST) is the most common bone tumour and it is more frequent in children and young adults affecting mostly the metaphysis of the long bones. Unfortunately, overall survival (OS) is only 20% when metastases occur. Doxorubicin, cisplatin and methotrexate are commonly used CT drugs in OST treatment with mixed results; indeed, patients treated with these drugs often undergo local recurrence and metastatic dissemination caused by the onset of drug resistant-tumour cells. Emerging evidence shows that lncRNAs may play complex and extensive roles in tumour development acting either as oncogenes or tumour suppressors. Similar to what occurs in many other tumours, lncRNAs might be implicated in OST chemoresistance mechanisms.

The well-characterised tumour suppressor GAS5 has been documented in a wide variety of human malignancies and loss of GAS5 expression is linked to tumorigenesis and disease progression, as well as in patients' prognosis. The potential role of GAS5 in OST is largely unknown. In the present study, for the first time, we have evaluated the clinical value of GAS5 tumour-suppressor lncRNA in improving patients' prognosis and prediction of response to chemotherapy in a pilot cohort of primary OST.

Based on these arguments we believe that our paper may be of great interest for the scientific community and hopefully worthy of publication on *Anti-cancer Drugs*.

*I declare that the manuscript in its submitted form has been read and approved by all authors and is not being considered for publication elsewhere. The authors declare no conflict of interest.*

Looking forward to hearing from you soon,  
Yours sincerely

Dr. Serena Pillozzi

Firenze, 12/07/2021

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**Title: Growth arrest-specific 5 (GAS5) lncRNA as a valuable biomarker of chemoresistance in osteosarcoma**

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**Keywords:** osteosarcoma (OST), long non coding RNA (lncRNA), biomarkers, GAS5, chemoresistance

## Abstract

**Introduction:** Osteosarcoma (OST) is the most common primary malignant bone tumour in children and teenagers, and it is characterized by drug resistance and high metastatic potential. Increasing studies have highlighted the critical roles of long noncoding RNAs (lncRNAs) as oncogenes or tumour suppressors as well as new biomarkers and therapeutic targets in OS. The growth arrest-specific 5 (GAS5) lncRNA can function as a tumour suppressor in several cancers. The present study aimed to validate GAS5 and other chemoresistance-associated lncRNAs in a cohort of primary OST samples.

**Methods:** The GAS5 and a panel of lncRNAs related to chemoresistance (SNGH1, FOXD2-AS1, DLEU2, LINC00963) were evaluated in a cohort of OST patients enrolled at the Orthopedic Oncology Unit, Careggi University Hospital. Total RNA was extracted from formalin fixed paraffin embedded (FFPE) tissue sections and the expression levels of the lncRNAs were quantified by qPCR. A bioinformatic analysis on deposited RNA-seq data was performed to validate the qPCR results.

**Results:** The expression levels of the tested lncRNAs vary greatly among the study population. Clustering analysis shows that GAS5 could be linked to the expression of isoforms 02 and 04 of the lncRNA DLEU2, while the DLEU2 isoform 08 is linked to the lncRNA LINC00963. Moreover, the evaluation of the expression of these lncRNAs involves a good separation according to gender and histology. Finally, GAS5 is significantly increased in patients with good prognosis; we did not observe any statistically significant association between the other lncRNAs and clinical data.

**Discussion:** We found GAS5 as a lncRNA expressed differently between chemosensitive and chemoresistant OST patients, however the results obtained are not concordant with *in-silico* analysis performed on TARGET OS dataset, probably due to inconsistency between the two case series. In the future we would enlarge the case series, including different disease settings. The final aim is to evaluate GAS5 expression as a biomarker, to obtain predictive information on resistance or sensitivity to treatment of OST patients.

## Introduction

OST is the most common bone tumour and it is more frequent in children and young adults affecting mostly the metaphysis of the long bones [1]. Its incidence rate is approximately one to three cases per million each year [2] and more than 20% of patients with OST develop metastases, frequently to the lung [3]. For patients with non-metastatic OST the survival rate has improved, reaching 60% at the fifth year after the introduction of neoadjuvant chemotherapy and the combination of surgery and chemotherapy (CT); unfortunately, overall survival (OS) is only 20% when metastases occur [4]. Doxorubicin, cisplatin and methotrexate are commonly used CT drugs in OST treatment with mixed results; indeed, patients treated with these drugs often undergo local recurrence and metastatic dissemination caused by the onset of drug resistant-tumour cells. Consequently, the development of novel therapies is one of the main goals of OST oncology research; this aim could be achieved with a better understanding of the molecular basis of OST development and acquisition of chemoresistance, that unfortunately are still largely unknown [5]. Long non coding RNAs (lncRNAs) are defined as non-protein coding RNAs longer than 200 nucleotides [6]; they can regulate gene expression through diverse mechanisms including epigenetic silencing, mRNA splicing, lncRNA-mRNA, lncRNA-miRNA and lncRNA-protein interaction [7]. Emerging evidence shows that lncRNAs may play complex and extensive roles in tumour development acting either as oncogenes or tumour suppressors [1]. Similar to what occurs in many other tumours, lncRNAs might be implicated in OST chemoresistance mechanisms [8].

The well-characterised tumour suppressor GAS5 has been documented in a wide variety of human malignancies and loss of GAS5 expression is linked to tumorigenesis and disease progression, as well as in patients' prognosis. The general consensus is that low GAS5 expression may predict poor survival and therapy resistance in many types of cancer [9-14]. The potential role of GAS5 in OST is largely unknown. In the present study, for the first time, we have evaluated the clinical value of GAS5 tumour-suppressor lncRNA in improving patients' prognosis and prediction of response to chemotherapy in a pilot cohort of primary OST.

## **Materials and Methods**

### **Primary OST**

A total of 10 OST formalin fixed paraffin embedded (FFPE) samples were obtained from patients enrolled at the Orthopaedic Oncology Unit of Careggi University Hospital and enrolled in this study after informed written consent. The main clinical and pathological characteristics of the patients are summarized in Table 1. FFPE sections (10 to 20  $\mu\text{m}$  thickness) of the tumours have been set up by the Histopathology and Molecular Diagnostic Unit, Careggi University Hospital, and were used for total RNA extraction.

### **RNA extraction and quantitative real-time reverse transcription PCR (qPCR)**

Total RNA was extracted from FFPE sections using the Tri Reagent (Millipore) solution. The quantity and the quality of RNA were evaluated using a Nanodrop spectrophotometer. Whenever possible, 500 ng of RNA from each sample was retro-transcribed with the “High Capacity cDNA Reverse Transcription Kit” (ThermoFisher); the resulting cDNA was then used for qPCR analysis with “GoTaq Sybr” (Promega) on a Rotor Gene Q (Qiagen). The relative quantification was performed using LinRegPCR software and the data were normalized to GAPDH. The primers used are listed in Table 2.

### **Statistical analysis**

Statistical analysis was performed using Jamovie and R on  $\log_2$  transformed data. The hierarchical clustering heatmaps were created with the pheatmap library [15] using the Euclidean distance. t-Test was used to evaluate statistical differences of qPCR data.

### ***In silico* gene expression analysis**

Open access RNA-seq raw-counts data and survival data from 92 OS patients have been downloaded from TARGET (<https://ocg.cancer.gov/programs/target/data-matrix>). Raw-counts (produced with Kallisto [16]) have been analyzed via Deseq2 [17], and normalised using the “vst” algorithm from the Deseq2 R package.

Vst normalised values have been used for survival analysis, performed via coxph R package with default settings.

## Results

### Clinicopathological features of OST patients

A total of 10 OST FFPE samples were obtained from patients enrolled at the Orthopaedic Oncology Unit of Careggi University Hospital and used in this study after informed written consent. The clinicopathological features of the ten enrolled patients (5 male and 5 female) are reported in Table 1. The mean age of the patients is 12.3 years (range 7-15), two patients had tumour localized in the proximal tibia, two in the proximal humerus and six in the distal femur; eight patients had osteoblastic OST while two were teleangiectatic OST; the mean value of follow-up was 99.5 months (range 7-166).

### *Expression analysis of GAS5 and other lncRNAs in OST patients by qPCR*

The tumour suppressor lncRNA GAS5 is linked to the chemoresistance of several cancers [18], while data on OST are limited (e.g. its association to the resistance to therapy is to date unreported) so we evaluated its expression in our cohort of OST patients. As reported in Table 3 and summarized in Figure 1 the lncRNA GAS5 is expressed in all OST samples analyzed with a mean expression value of -3.62 (range -5.52; -1.90).

We next evaluated the expression of a panel of lncRNAs (SNGH1, FOXD2-AS1, DLEU2, LINC00963) that have been reported in literature to be involved in chemoresistance mechanisms (Figure 2A and Table 3). For the DLEU2 gene we evaluated the expression of two isoforms (DLEU2 02-04; DLEU2 08). We have decided to study two isoforms to this because it was not possible to design a primer suitable for the DLEU2 isoforms that emerged from bioinformatics analysis and because DLEU2 has at least seven linear transcript variants, generated by alternative splicing [39], and their roles might be cancer specific.

Whereas GAS5 is detectable in all amplifiable samples, the other lncRNAs are often poorly expressed in the cohort. Specifically, the lncRNA FOXD2-AS1 is expressed in most of the samples (6 out of 8) analyzed with a mean expression value of -7.65 (range -15.11;-2.16); lncRNA SNGH1 has a mean expression value of -11.87 (range -21.81;-6.57), (6 out of 8 samples); the lncRNA LINC00963 is detectable at meaningful levels in half of the samples

(mean expression value of -4.24 with a range -6.78;-2.42). Finally, the two isoforms of lncRNA DLEU2 have a similar, although in a different scale, expression; specifically, the DLEU2 02-04 has a mean expression value of -4.70 (range -6.07;-1.52) and DLEU2 08 has a mean expression value of -6.00 (range -12.61;2.15), both were detectable in 60% of the samples.

With the R-studio software, using the Euclidean distance, we built a heatmap that clustered patients according to the gene expression values ( $\log_2(\text{RQ})$ ) found through the qPCR. The results obtained showed how genes that are reported in the literature as oncosuppressors (GAS5, DLEU2 02-04) cluster together. In addition a clear separation of patients according to gender and histology and finally a cluster association of GAS5 levels of expression with the 02-04 isoforms of lncRNA DLEU2, whose 08 isoform is instead associated to LINC00963 (Figure 2B).

#### ***Association between lncRNAs expression level and chemoresistance***

Given the importance of chemoresistance in the progression of OST, we evaluated the relation of these lncRNAs to the outcome in the patients. Given that all patients underwent the same therapeutic treatment, the population was divided into the two subgroups “poor responders” and “good responders” (Table 4). We observed a statistically significant difference only for GAS5, whose expression is increased in the “therapy-sensitive” group ( $p= 0.038$ ) (Figure 3A).

Hierarchical clustering of lncRNA in “therapy-sensitive” and “therapy-resistant” groups shows the association of GAS5 to LINC00963 (Figure 3B).

#### **In-silico analysis**

Subsequently, we retrieved RNA-seq data from 92 OST patients from the TARGET repository, with the aim of extending our analysis to larger datasets. Unfortunately, GAS5 over-expression is weakly associated with poorer progression free survival (PFS, HR=1.6,  $p=0.00065$ ) and overall survival (OS, HR=1.57,  $p=0.00261$ ), in discordance with our previous observations on primary samples of our cohort (Table 5). Considering the high heterogeneity of OST, we stratified the patients based on the recruitment protocol reported by the uploaders,

with the aim of obtaining cleaner subsets of patients. We separately evaluated PFS and OS in four protocols with at least 10 patients: AOST0331, AOST06B1, IHRT, P9851. Among these, GAS5 is significantly associated with poor survival only in AOST0331. Notably, AOST0331 and AOST06B1 show very large HR values compared to IHRT, P9851 (Table 5). Such differences among protocols suggests that GAS5 might play different roles in different disease settings, which could explain the disagreement between our wet-lab results and in-silico analysis.

## **Discussion**

Chemoresistance is associated with poor outcomes of OST patients and remains a major challenge in treatment. Many studies have shown the role of lncRNA in processes that confer resistance to treatment. After a literature analysis on the role of lncRNAs in cancer we identified GAS5 and other lncRNAs (SNGH1, FOXD2-AS1, DLEU2, LINC00963) as possible lncRNAs that were associated to OST survival and chemoresistance; hoping to find out novel biomarkers, their expression and association to clinical and pathological data were evaluated in primary OST. The analysis was carried out on a pilot cohort of 10 patients suffering from OST and enrolled at Careggi University Hospital. The limited number of patients is due to the rarity of the neoplasm studied and to the need of a homogeneous cohort characterized by patients who had followed the same therapeutic schedule. Literature analysis on the role of lncRNAs in cancer identified GAS5 as a possible lncRNA associated to OST survival. GAS5 plays the role of tumour suppressor gene in many types of cancer and is found under expressed in breast [9], prostate [10], pancreatic [11], colorectal [12], cervical [13] and gastric cancers [14]. It plays a role as tumor suppressor gene by disadvantaging processes such as cell migration and cell proliferation and promoting cell death by apoptosis [19]. In particular, it acts on cell proliferation by raising P21 levels and lowering CDK6, Cyclin D1 and E2F1 levels [20] as well as on apoptotic processes by lowering cIAP2 levels and raising P53 levels. The expression of GAS5 can be regulated by the interaction between mTOR and NMD (nonsense mediated decay) [21]. In addition, in the literature there are data on the possible role of GAS5 in OST; also in this case it plays the role of oncosuppressor interacting with some miRNA [22].

Furthermore, other lncRNAs with a possible role in cancer chemoresistance (SNHG1, FOXD2-AS1, DLEU2, LINC00963) have been selected from the literature analysis. FOXD2-AS1 has been reported to play the role of oncogene in many types of cancers, as it has been found to be abnormal expressed in gastric, lung, bladder, colon rectum, nasopharyngeal, oesophagus, liver, thyroid, and skin cancers [23]. Its role is related to cell proliferation that promotes through its involvement in both Wnt/ $\beta$  catenin and Notch signaling pathway; moreover, in bladder cancer FOXD2-AS1 plays a role in gemcitabine chemoresistance [23]. Regarding the role of this gene in OST, it has been reported that FOXD2-AS1, following the activation by transcription factor HIF-1 $\alpha$ , acts as an oncogene in the OST tumorigenesis and interacts with EZH2 to silence p21 protein [24].

SNHG1 is overexpressed in oesophageal squamous cell [25], lung squamous cell [26], hepatocellular carcinomas [27], colorectal [28], gastric [29], and liver cancers where it acts as a tumour promoting factor. In the liver, it promotes the development of tumour mass by inhibiting the expression of P53 [30] and the resistance to Sorafenib and Doxorubicin [31]. Regarding OST, it promotes cell proliferation, metastases and invasiveness while its silencing promotes cell death by apoptosis and the G0/G1 cell cycle arrest. In OST it plays its role of oncogene by inhibiting the expression of miRNA-101-3p and this favours the expression of ROCK1 [32].

LINC00963 has been reported as an oncogene in different types of cancer such as prostate cancer and hepatocarcinoma. In prostate cancer it has been shown that its over expression is linked to the malignant development by acting on EGFR [33]. In hepatocarcinoma, this lncRNA acts on the PI3K/AKT signalling pathway, favouring the tumour progression [34]. There is little data in the literature on the role of LINC00963 in OST but the available data suggest that its over expression is linked to an unfavourable prognosis and that it facilitates proliferation and invasiveness by suppressing the miR-204-3P/FN1 axis [35].

DLEU2 is a tumour suppressor gene since its down regulation is linked to malignant processes such as angiogenesis, metastasis and invasiveness. Its role as an onco-suppressant in laryngeal, oesophageal and pancreatic carcinoma has been verified, but above all it plays a

fundamental role in many types of leukaemia [36]. One of the isoforms of DLEU2 is an indicator of poor prognosis in patients with oesophageal adenocarcinoma [37]. In the literature, data on the correlation between DLEU2 and OST are limited and are related to the phenomenon of hypoxia. Low oxygen levels suppress the transcription of DLEU2 and miR-15a and this favours the proliferation and invasion of cancer cells [38]. Noteworthy the studies reported in the literature were carried out on cell lines while our study is the first on primary samples of OST.

Observing the clinicopathological features of the enrolled patients, we have decided to focus only on the chemoresistance. Some data on the correlation between some lncRNAs and chemoresistance in OST are reported in literature [40]. Recent studies demonstrated that a distinctly higher expression of lncRNA FOXC2-AS1 (FOXC2 antisense RNA 1, FOXC2-AS1) was associated with poor prognosis for OST patients. It was also revealed that FOXC2-AS1 may have contributed to doxorubicin resistance by increasing the expression of some classical MDR (multidrug resistance) associated genes, including ABCB1 and HIF1A [41]. Overexpression of lncRNA ODRUL (OS doxorubicin resistance related up-regulated lncRNA, ODRUL) was shown in tumour tissues of OST patients with lung metastasis and a low chemoresponse. It was reported that ODRUL may reduce sensitivity to doxorubicin in OS cells by inducing expression of ABCB1 [42]. lncRNA LINC00161 (long intergenic non-coding RNA 161, LINC00161) was revealed to play an essential role in cisplatin induced apoptosis, and attenuate OST chemoresistance by targeting the miR-645-IFIT2 (interferon-induced with tetratricopeptide repeats 2, IFIT2) signaling axis [43]. lncRNA HOTTIP participated in OST cellular resistance to cisplatin by the activation of Wnt/ $\beta$ -catenin signaling pathway, which indicates a potential therapeutic approach to targeting Wnt/ $\beta$ -catenin signaling pathway to reverse the resistance [44].

We compared therapy-sensitive and therapy-resistant patients and we found that the lncRNAs FOXD2-AS1, SNHG1, and LINC00963 tend to be more expressed in the group of therapy-resistant patients than in the group of sensitive patients; the lncRNA GAS5 and the isoforms of the lncRNA DLEU2 analyzed (DLEU2 02-04 and DLEU2 08) have a tendency to

be more expressed in patients sensitive to therapies. The trends we found and observed are in accordance with the oncological roles of the studied lncRNA in the literature; genes of which a possible role of oncogene is reported tend to be more expressed in patients with OST resistant to therapies and genes of which a possible role of tumour suppressor gene is reported tend to be more expressed in patients with OST sensitive to therapies. However, our data on GAS5 expression were not confirmed by bioinformatics analysis.

In conclusion we found a panel of lncRNAs expressed in primary OST tissues, their expression is different between chemosensitive and chemoresistant patients and the lncRNA GAS5 has a significantly different expression into the two groups. In the future we have hypothesized its use as a biomarker in OST and evaluate the expression of GAS5 could give predictive information on resistance or sensitivity to treatment of OST patients.

Confidential

## Tables

**Table 1:** Clinicopathological features of the patients enrolled with OS.

Patient ID	Sex	Age (years)	Histology	Location	Follow-up (months)	Response
1	F	15	T	PH	112	DOD
3	F	9	T	DF	19	DOD
4	M	15	O	DF	7	DOD
5	M	13	O	PT	64	DOD
6	F	13	O	PH	99	CDF
7	F	7	O	DF	166	CDF
8	M	12	O	DF	135	CDF
9	F	12	O	DF	135	CDF
10	M	14	O	DF	143	CDF
11	M	13	O	PT	115	CDF

T= teleangectasic; O= osteoblastic; PH= proximal humerus; DF= distal femur; PT= proximal tibia; DOD = died of disease; CDF = continuously disease free

**Table 2:** Sequences of qPCR primers.

<b>Gene name</b>	<b>Primer forward</b>	<b>Primer reverse</b>	<b>Lenght (bp)</b>
<i>FOXD2-AS1</i>	AAGCGATCAGCTCCCTTAGC	CAGACGCGTGGTGGTTATCT	184
<i>SNHG1</i>	TTGCTGCCTTTCTTACATGATC C	AGACACGAAGTGGAGTTATGGG AA	132
<i>GAS5</i>	CTTGCCTGGACCAGCTTAAT	CAAGCCGACTCTCCATACCT	122
<i>LINC00963</i>	GGTAAATCGAGGCCCAGAGAT	ACGTGGATGACAGCGTGTGA	100
<i>DLEU2 02-04</i>	GTCCGAGAGTATAGCGCCAC	ATTAAACCGACTGCGCCAGC	120
<i>DLEU2 08</i>	AAAGATGGTCCCTGTCAGCA	TCCTTCAAGCTTCTACCACAC	130
<i>GAPDH</i>	AGCCACATCGCTCAGACAC	GCCCAATACGACCAAATCC	66

**Table 3:** Expression levels (as  $\log_2(\text{RQ})$ ) of lncRNAs (FOXD2-AS1, SNHG1, GAS5, LINC00963, DLEU2 02-04, DLEU2 08) for OST patients. Data are represented as arbitrary fluorescence units normalized to GADPH.

Patient ID	FOXD2-AS1	SNHG 1	GAS5	LINC00963	DLEU2 02-04	DLEU2 08
1	-7.22	-7.96	-5.52	-5.12	-5.80	-5.43
3	-2.16	-6.57	-4.43	-2.63	-4.14	-2.78
4	NA	NA	-3.92	NA	NA	-11.30
5	-8.62	-21.81	-3.59	-6.78	NA	NA
6	NA	-9.45	-3.21	NA	NA	2.15
7	-5.13	-13.93	-1.90	NA	-1.52	NA
8	-15.11	-11.51	-3.60	-2.42	-6.07	NA
10	NA	NA	-2.81	NA	-5.96	-12.61
<b>Mean</b>	-7.65	-11.87	-3.62	-4.24	-4.70	-6.00
<b>Median</b>	-7.22	-10.48	-3.60	-3.87	-5.80	-5.43
<b>St Dev</b>	4.83	5.52	1.08	2.09	1.94	6.10
<b>Minimum</b>	-15.11	-21.81	-5.52	-6.78	-6.07	-12.61
<b>Maximum</b>	-2.16	-6.57	-1.90	-2.42	-1.52	2.15

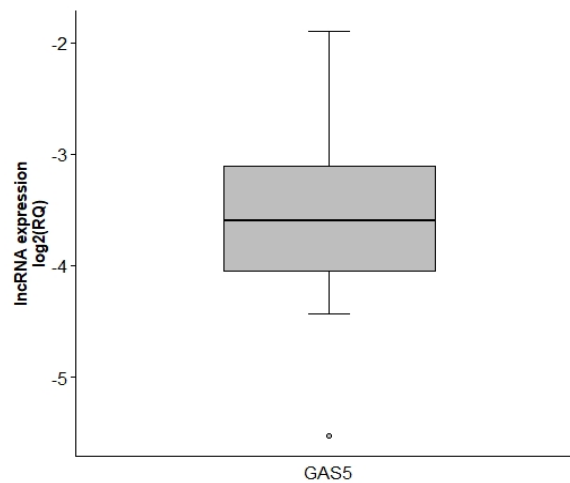
**Table 4:** Descriptive statistic of lncRNA expression in the study population grouped according to response to therapy.

	<b>Response</b>	<b>FOXD2-AS1</b>	<b>SNHG1</b>	<b>GAS5</b>	<b>LINC00963</b>	<b>DLEU2 02-04</b>	<b>DLEU2 08</b>
<b>N</b>	Resistant	4	4	4	4	4	4
	Sensitive	4	4	4	4	4	4
<b>Mean</b>	Resistant	-6.00	-12.1	-4.37	-4.84	-4.97	-6.51
	Sensitive	-1-.1	-11.6	-2.88	-2.42	-4.52	-5.23
<b>Median</b>	Resistant	-7.22	-7.96	-4.17	-5.12	-4.97	-5.43
	Sensitive	-10.1	-11.5	-3.01	-2.42	-5.96	-5.23
<b>Standard deviation</b>	Resistant	3.40	8.43	0.845	2.09	1.17	4.36
	Sensitive	7.06	2.24	0.729	NaN	2.60	10.4
<b>Range</b>	Resistant	6.46	15.2	1.93	4.15	1.65	8.52
	Sensitive	9.98	4.48	1.70	0.00	4.55	14.8

**Table 5:** Progression free survival (PFS) and overall survival (SUR) results based on GAS5 expression in the TARGET dataset patient cohort

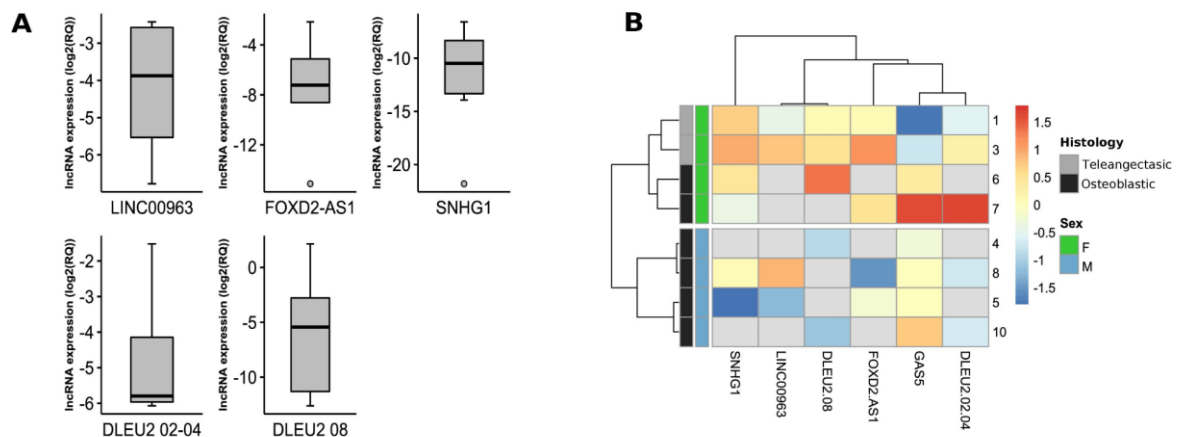
	<b>Patients</b>	<b>HR PFS</b>	<b>P value PFS</b>	<b>HR SUR</b>	<b>P value SUR</b>
<b>TOT</b>	92	1.60	0.0006	1.57	0.003
<b>AOST0331</b>	24	2.06	0.01	4.33	0.001
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## Figures

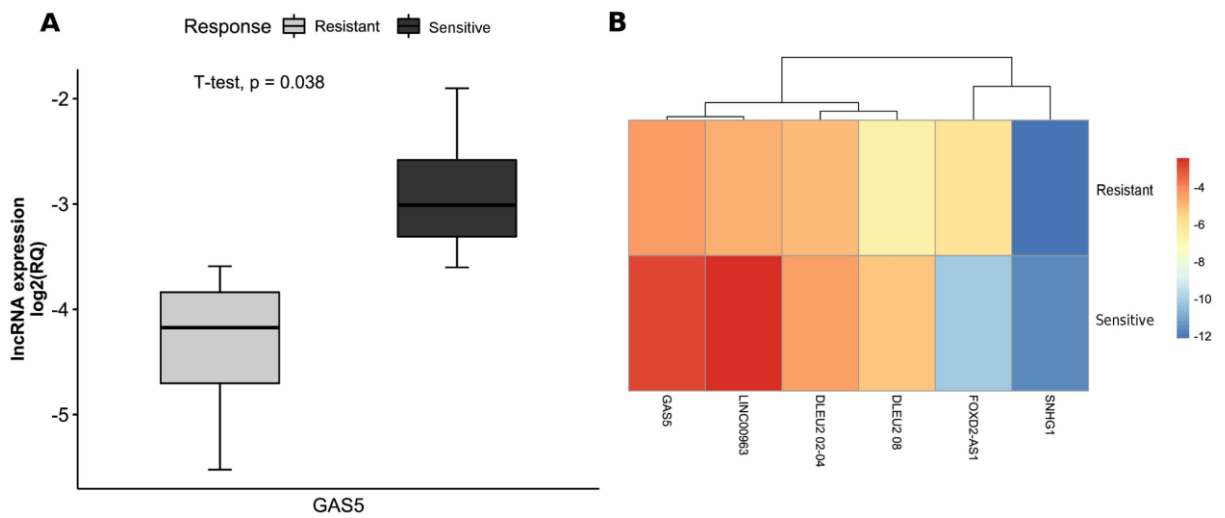


**Figure 1**

Expression boxplot showing trends of lncRNA GAS5 levels of expression in OST samples; the expression values are reported as  $\log_2(\text{RQ})$ ; gray dots represent outlier values.



**Figure 2.** Graphical representation of expression values of a panel of selected lncRNA. **A)** Expression boxplots showing trends of individual lncRNAs in OST samples; the expression values are reported as  $\log_2(\text{RQ})$ ; gray dots represent the outliers. **B)** Expression heatmap of lncRNA among the patients, this heatmap was built using the Pheatmap function in R-studio software; the clustering between genes and between patients was created using the Euclidean distance; the color scale represents the expression values ( $\log_2(\text{RQ})$ ), normalized in the columns.



**Figure 3.** Graphical representation of OST cohort division based on response to treatment. **A)** Boxplots of expression levels of lncRNA GAS5 in the treatment-sensitive group and the resistant group. **B)** Expression heatmap of lncRNA among the sensitive and resistant groups; this heatmap was built using the Pheatmap function in R-studio software, the clustering between genes was created using the Euclidean distance; the color of each cell represents the mean expression value of that gene within the group.

## References

1. Wu PF, Dai ZT, Liu WD, Zhao ZX, Kong YH. Elevated long noncoding RNA HAGLROS expression correlates with clinical progression and prognosis in osteosarcoma. *Eur Rev Med Pharmacol Sci.* 2019 Feb;23(4):1428-1433. doi: 10.26355/eurrev\_201902\_17099. PMID: 30840263.
2. Pan Y, Lu L, Chen J, Zhong Y, Dai Z. Identification of potential crucial genes and construction of microRNA-mRNA negative regulatory networks in osteosarcoma. *Hereditas.* 2018 May 9;155:21. doi: 10.1186/s41065-018-0061-9. PMID: 29760609; PMCID: PMC5941338.
3. Xu J, Li D, Cai Z, Zhang Y, Huang Y, Su B, Ma R. An integrative analysis of DNA methylation in osteosarcoma. *J Bone Oncol.* 2017 May 19;9:34-40. doi: 10.1016/j.jbo.2017.05.001. PMID: 29234590; PMCID: PMC5715438.
4. Shi Z, Zhou H, Pan B, Lu L, Wei Z, Shi L, Yao X, Kang Y, Feng S. Exploring the key genes and pathways of osteosarcoma with pulmonary metastasis using a gene expression microarray. *Mol Med Rep.* 2017 Nov;16(5):7423-7431. doi: 10.3892/mmr.2017.7577. Epub 2017 Sep 21. PMID: 28944885; PMCID: PMC5865874.
5. Rosen G, Murphy ML, Huvos AG, Gutierrez M, Marcove RC. Chemotherapy, en bloc resection, and prosthetic bone replacement in the treatment of osteogenic sarcoma. *Cancer.* 1976 Jan;37(1):1-11. doi: 10.1002/1097-0142(197601)37:1<1::aid-cnrcr2820370102>3.0.co;2-3. PMID: 1082364.
6. Wang Y, Li W, Chen X, Li Y, Wen P, Xu F. MIR210HG predicts poor prognosis and functions as an oncogenic lncRNA in hepatocellular carcinoma. *Biomed Pharmacother.* 2019 Mar;111:1297-1301. doi: 10.1016/j.biopha.2018.12.134. Epub 2019 Jan 15. PMID: 30841443.
7. Li W, Xie P, Ruan WH. Overexpression of lncRNA UCA1 promotes osteosarcoma progression and correlates with poor prognosis. *J Bone Oncol.* 2016 May 10;5(2):80-5. doi: 10.1016/j.jbo.2016.05.003. PMID: 27335776; PMCID: PMC4908186.
8. Chen R, Wang G, Zheng Y, Hua Y, Cai Z. Long non-coding RNAs in osteosarcoma. *Oncotarget.* 2017 Mar 21;8(12):20462-20475. doi: 10.18632/oncotarget.14726. PMID: 28103585; PMCID: PMC5386777.
9. Mourtada-Maarabouni M, Pickard MR, Hedge VL, Farzaneh F, Williams GT. GAS5, a non-protein-coding RNA, controls apoptosis and is downregulated in breast cancer. *Oncogene.* 2009 Jan 15;28(2):195-208. doi: 10.1038/onc.2008.373. Epub 2008 Oct 6. PMID: 18836484.
10. Romanuik TL, Wang G, Morozova O, Delaney A, Marra MA, Sadar MD. LNCaP Atlas: gene expression associated with in vivo progression to castration-recurrent prostate cancer. *BMC Med Genomics.* 2010 Sep 24;3:43. doi: 10.1186/1755-8794-3-43. PMID: 20868494; PMCID: PMC2956710.
11. Lu X, Fang Y, Wang Z, Xie J, Zhan Q, Deng X, Chen H, Jin J, Peng C, Li H, Shen B. Downregulation of gas5 increases pancreatic cancer cell proliferation by regulating CDK6. *Cell Tissue Res.* 2013 Dec;354(3):891-6. doi: 10.1007/s00441-013-1711-x. Epub 2013 Sep 12. PMID: 24026436.
12. Yin D, He X, Zhang E, Kong R, De W, Zhang Z. Long noncoding RNA GAS5 affects cell proliferation and predicts a poor prognosis in patients with colorectal cancer. *Med Oncol.* 2014 Nov;31(11):253. doi: 10.1007/s12032-014-0253-8. Epub 2014 Oct 18. PMID: 25326054.
13. Cao S, Liu W, Li F, Zhao W, Qin C. Decreased expression of lncRNA GAS5 predicts a poor prognosis in cervical cancer. *Int J Clin Exp Pathol.* 2014 Sep 15;7(10):6776-83. PMID: 25400758; PMCID: PMC4230116.
14. Sun M, Jin FY, Xia R, Kong R, Li JH, Xu TP, Liu YW, Zhang EB, Liu XH, De W. Decreased expression of long noncoding RNA GAS5 indicates a poor prognosis and promotes cell proliferation in gastric cancer. *BMC Cancer.* 2014 May 6;14:319. doi: 10.1186/1471-2407-14-319. PMID: 24884417; PMCID: PMC4022532.

15. Raivo Kolde (2019). pheatmap: Pretty Heatmaps. R package version 1.0.12. <https://CRAN.R-project.org/package=pheatmap>
16. Near-optimal probabilistic RNA-seq quantification. *Bray NL, Pimentel H, Melsted P, Pachter L Nat Biotechnol.* 2016 May; 34(5):525-7.
17. Love MI, Huber W, Anders S (2014). "Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2." *Genome Biology*, **15**, 550. doi: [10.1186/s13059-014-0550-8](https://doi.org/10.1186/s13059-014-0550-8).
18. Lambrou GI, Hatzigiapiou K, Zaravinos A. The Non-Coding RNA GAS5 and Its Role in Tumor Therapy-Induced Resistance. *Int J Mol Sci.* 2020 Oct 15;21(20):7633. doi: 10.3390/ijms21207633. PMID: 33076450; PMCID: PMC7588928.
19. Qiao HP, Gao WS, Huo JX, Yang ZS. Long non-coding RNA GAS5 functions as a tumor suppressor in renal cell carcinoma. *Asian Pac J Cancer Prev.* 2013;14(2):1077-82. doi: 10.7314/apjcp.2013.14.2.1077. PMID: 23621190.
20. Liu Z, Wang W, Jiang J, Bao E, Xu D, Zeng Y, Tao L, Qiu J. Downregulation of GAS5 promotes bladder cancer cell proliferation, partly by regulating CDK6. *PLoS One.* 2013 Sep 17;8(9):e73991. doi: 10.1371/journal.pone.0073991. PMID: 24069260; PMCID: PMC3775789.
21. Kino T, Hurt DE, Ichijo T, Nader N, Chrousos GP. Noncoding RNA gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor. *Sci Signal.* 2010 Feb 2;3(107):ra8. doi: 10.1126/scisignal.2000568. PMID: 20124551; PMCID: PMC2819218.
22. Xu L, Xia C, Xue B, Sheng F, Xiong J, Wang S. A promoter variant of lncRNA GAS5 is functionally associated with the development of osteosarcoma. *J Bone Oncol.* 2018 Mar 6;12:23-26. doi: 10.1016/j.jbo.2018.03.001. PMID: 30013899; PMCID: PMC6045496.
23. Hu Q, Tai S, Wang J. Oncogenicity of lncRNA FOXD2-AS1 and its molecular mechanisms in human cancers. *Pathol Res Pract.* 2019 May;215(5):843-848. doi: 10.1016/j.prp.2019.01.033. Epub 2019 Jan 29. PMID: 30723052.
24. Ren Z, Hu Y, Li G, Kang Y, Liu Y, Zhao H. HIF-1 $\alpha$  induced long noncoding RNA FOXD2-AS1 promotes the osteosarcoma through repressing p21. *Biomed Pharmacother.* 2019 Sep;117:109104. doi: 10.1016/j.biopha.2019.109104. Epub 2019 Jun 19. PMID: 31228799.
25. Zhang Y, Jin X, Wang Z, Zhang X, Liu S, Liu G. Downregulation of SNHG1 suppresses cell proliferation and invasion by regulating Notch signaling pathway in esophageal squamous cell cancer. *Cancer Biomark.* 2017 Dec 12;21(1):89-96. doi: 10.3233/CBM-170286. PMID: 29081407.
26. Zhang HY, Yang W, Zheng FS, Wang YB, Lu JB. Long non-coding RNA SNHG1 regulates zinc finger E-box binding homeobox 1 expression by interacting with TAp63 and promotes cell metastasis and invasion in Lung squamous cell carcinoma. *Biomed Pharmacother.* 2017 Jun;90:650-658. doi: 10.1016/j.biopha.2017.03.104. Epub 2017 Apr 14. PMID: 28415044.
27. Zhang H, Zhou D, Ying M, Chen M, Chen P, Chen Z, Zhang F. Expression of Long Non-Coding RNA (lncRNA) Small Nucleolar RNA Host Gene 1 (SNHG1) Exacerbates Hepatocellular Carcinoma Through Suppressing miR-195. *Med Sci Monit.* 2016 Dec 9;22:4820-4829. doi: 10.12659/msm.898574. PMID: 27932778; PMCID: PMC5167104.
28. Tian T, Qiu R, Qiu X. SNHG1 promotes cell proliferation by acting as a sponge of miR-145 in colorectal cancer. *Oncotarget.* 2017 Dec 14;9(2):2128-2139. doi: 10.18632/oncotarget.23255. PMID: 29416759; PMCID: PMC5788627.
29. Hu Y, Ma Z, He Y, Liu W, Su Y, Tang Z. LncRNA-SNHG1 contributes to gastric cancer cell proliferation by regulating DNMT1. *Biochem Biophys Res Commun.* 2017 Sep 30;491(4):926-931. doi: 10.1016/j.bbrc.2017.07.137. Epub 2017 Jul 25. PMID: 28754593.
30. Li SJ, Wang L, Sun ZX, Sun SJ, Gao J, Ma RL. LncRNA SNHG1 promotes liver cancer development through inhibiting p53 expression via binding to DNMT1. *Eur Rev Med*

- Pharmacol Sci. 2019 Apr;23(7):2768-2776. doi: 10.26355/eurrev\_201904\_17550. PMID: 31002127.
31. Li W, Dong X, He C, Tan G, Li Z, Zhai B, Feng J, Jiang X, Liu C, Jiang H, Sun X. LncRNA SNHG1 contributes to sorafenib resistance by activating the Akt pathway and is positively regulated by miR-21 in hepatocellular carcinoma cells. *J Exp Clin Cancer Res.* 2019 May 3;38(1):183. doi: 10.1186/s13046-019-1177-0. PMID: 31053148; PMCID: PMC6499991.
  32. Deng R, Zhang J, Chen J. lncRNA SNHG1 negatively regulates miRNA-101-3p to enhance the expression of ROCK1 and promote cell proliferation, migration and invasion in osteosarcoma. *Int J Mol Med.* 2019 Mar;43(3):1157-1166. doi: 10.3892/ijmm.2018.4039. Epub 2018 Dec 20. PMID: 30592267; PMCID: PMC6365036.
  33. Wang L, Han S, Jin G, Zhou X, Li M, Ying X, Wang L, Wu H, Zhu Q. Linc00963: a novel, long non-coding RNA involved in the transition of prostate cancer from androgen-dependence to androgen-independence. *Int J Oncol.* 2014 Jun;44(6):2041-9. doi: 10.3892/ijo.2014.2363. Epub 2014 Apr 2. PMID: 24691949.
  34. Wu JH, Tian XY, An QM, Guan XY, Hao CY. LINC00963 promotes hepatocellular carcinoma progression by activating PI3K/AKT pathway. *Eur Rev Med Pharmacol Sci.* 2018 Mar;22(6):1645-1652. doi: 10.26355/eurrev\_201803\_14574. PMID: 29630107.
  35. Zhou Y, Yin L, Li H, Liu LH, Xiao T. The LncRNA LINC00963 facilitates osteosarcoma proliferation and invasion by suppressing miR-204-3p/FN1 axis. *Cancer Biol Ther.* 2019;20(8):1141-1148. doi: 10.1080/15384047.2019.1598766. Epub 2019 Apr 12. PMID: 30975024; PMCID: PMC6605988.
  36. Wu DM, Wen X, Han XR, Wang S, Wang YJ, Shen M, Fan SH, Zhang ZF, Shan Q, Li MQ, Hu B, Chen GQ, Lu J, Zheng YL. Role of Circular RNA DLEU2 in Human Acute Myeloid Leukemia. *Mol Cell Biol.* 2018 Sep 28;38(20):e00259-18. doi: 10.1128/MCB.00259-18. PMID: 30037980; PMCID: PMC6168983.
  37. Ma W, Zhang CQ, Dang CX, Cai HY, Li HL, Miao GY, Wang JK, Zhang LJ. Upregulated long-non-coding RNA DLEU2 exon 9 expression was an independent indicator of unfavorable overall survival in patients with esophageal adenocarcinoma. *Biomed Pharmacother.* 2019 May;113:108655. doi: 10.1016/j.biopha.2019.108655. Epub 2019 Mar 5. PMID: 30849637.
  38. Leng J, Song Q, Zhao Y, Wang Z. miR-15a represses cancer cell migration and invasion under conditions of hypoxia by targeting and downregulating Bcl-2 expression in human osteosarcoma cells. *Int J Oncol.* 2018 Apr;52(4):1095-1104. doi: 10.3892/ijo.2018.4285. Epub 2018 Feb 23. PMID: 29484432; PMCID: PMC5843390.
  39. Chen Z, Zhang J, Zhang Z, Feng Z, Wei J, Lu J, Fang Y, Liang Y, Cen J, Pan Y, Huang Y, Zhou F, Chen W, Luo J. The putative tumor suppressor microRNA-30a-5p modulates clear cell renal cell carcinoma aggressiveness through repression of ZEB2. *Cell Death Dis.* 2017 Jun 1;8(6):e2859. doi: 10.1038/cddis.2017.252. PMID: 28569782; PMCID: PMC5520909.
  40. Chen R, Wang G, Zheng Y, Hua Y, Cai Z. Long non-coding RNAs in osteosarcoma. *Oncotarget.* 2017 Mar 21;8(12):20462-20475. doi: 10.18632/oncotarget.14726. PMID: 28103585; PMCID: PMC5386777.
  41. Zhu KP, Zhang CL, Shen GQ, Zhu ZS. Long noncoding RNA expression profiles of the doxorubicin-resistant human osteosarcoma cell line MG63/DXR and its parental cell line MG63 as ascertained by microarray analysis. *Int J Clin Exp Pathol.* 2015 Aug 1;8(8):8754-73. PMID: 26464619; PMCID: PMC4583851.
  42. Zhang CL, Zhu KP, Shen GQ, Zhu ZS. A long non-coding RNA contributes to doxorubicin resistance of osteosarcoma. *Tumour Biol.* 2016 Feb;37(2):2737-48. doi: 10.1007/s13277-015-4130-7. Epub 2015 Sep 25. PMID: 26408180.
  43. Wang Y, Zhang L, Zheng X, Zhong W, Tian X, Yin B, Tian K, Zhang W. Long non-coding RNA LINC00161 sensitises osteosarcoma cells to cisplatin-induced apoptosis by regulating the miR-645-IFIT2 axis. *Cancer Lett.* 2016 Nov 28;382(2):137-146. doi: 10.1016/j.canlet.2016.08.024. Epub 2016 Sep 5. PMID: 27609068.

44. Li Z, Zhao L, Wang Q. Overexpression of long non-coding RNA HOTTIP increases chemoresistance of osteosarcoma cell by activating the Wnt/ $\beta$ -catenin pathway. *Am J Transl Res.* 2016 May 15;8(5):2385-93. PMID: 27347346; PMCID: PMC4891451.

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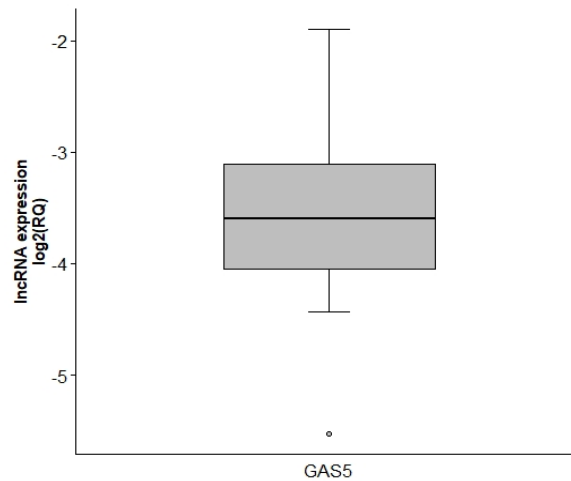
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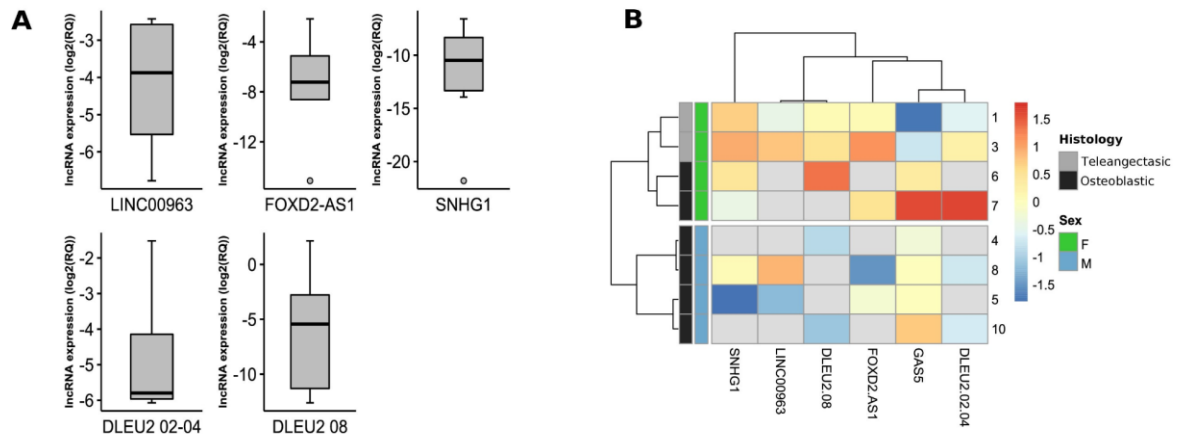
Our special memory goes to Alessio Cerasola, an unforgettable guy who fights the disease with his ever-present smile.

Confidential

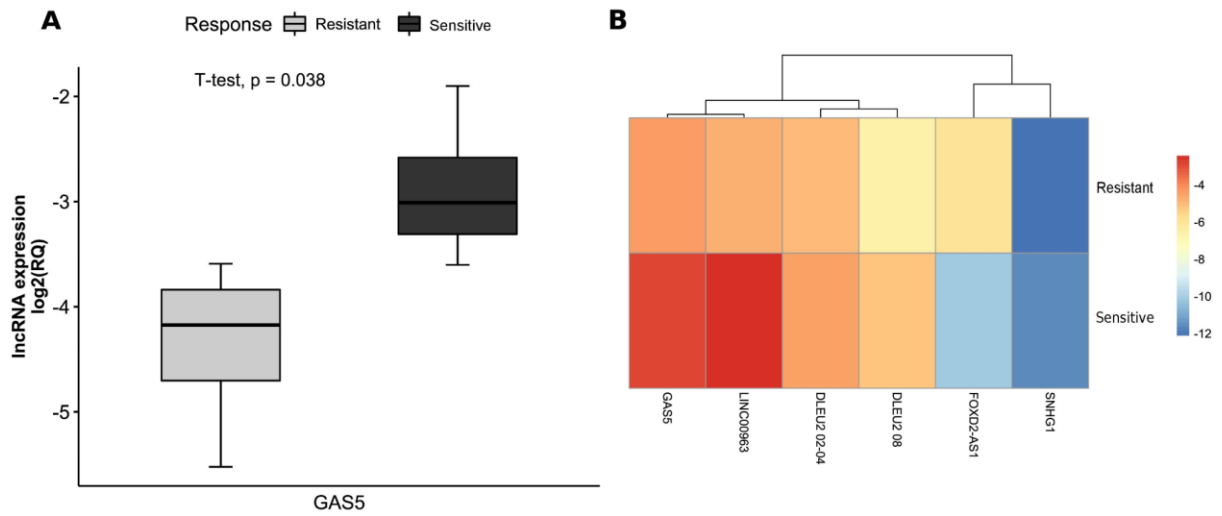
## Figures

**Figure 1**

Expression boxplot showing trends of lncRNA GAS5 levels of expression in OST samples; the expression values are reported as  $\log_2(\text{RQ})$ ; gray dots represent outlier values.



**Figure 2.** Graphical representation of expression values of a panel of selected lncRNA. **A)** Expression boxplots showing trends of individual lncRNAs in OST samples; the expression values are reported as  $\log_2(\text{RQ})$ ; gray dots represent the outliers. **B)** Expression heatmap of lncRNA among the patients, this heatmap was built using the Pheatmap function in R-studio software; the clustering between genes and between patients was created using the Euclidean distance; the color scale represents the expression values ( $\log_2(\text{RQ})$ ), normalized in the columns.



**Figure 3.** Graphical representation of OST cohort division based on response to treatment. **A)** Boxplots of expression levels of IncRNA GAS5 in the treatment-sensitive group and the resistant group. **B)** Expression heatmap of IncRNA among the sensitive and resistant groups; this heatmap was built using the Pheatmap function in R-studio software, the clustering between genes was created using the Euclidean distance; the color of each cell represents the mean expression value of that gene within the group.

## Tables

**Table 1:** Clinicopathological features of the patients enrolled with OS.

Patient ID	Sex	Age (years)	Histology	Location	Follow-up (months)	Response
1	F	15	T	PH	112	DOD
3	F	9	T	DF	19	DOD
4	M	15	O	DF	7	DOD
5	M	13	O	PT	64	DOD
6	F	13	O	PH	99	CDF
7	F	7	O	DF	166	CDF
8	M	12	O	DF	135	CDF
9	F	12	O	DF	135	CDF
10	M	14	O	DF	143	CDF
11	M	13	O	PT	115	CDF

T= teleangectasic; O= osteoblastic; PH= proximal humerus; DF= distal femur; PT= proximal tibia;  
DOD = died of disease; CDF = continuously disease free

**Table 2:** Sequences of qPCR primers.

<b>Gene name</b>	<b>Primer forward</b>	<b>Primer reverse</b>	<b>Lenght (bp)</b>
<i>FOXD2-AS1</i>	AAGCGATCAGCTCCCTTAGC	CAGACGCGTGGTGGTTATCT	184
<i>SNHG1</i>	TTGCTGCCTTTCTTACATGATC C	AGACACGAAGTGGAGTTATGGG AA	132
<i>GAS5</i>	CTTGCCTGGACCAGCTTAAT	CAAGCCGACTCTCCATACCT	122
<i>LINC00963</i>	GGTAAATCGAGGCCAGAGAT	ACGTGGATGACAGCGTGTGA	100
<i>DLEU2 02-04</i>	GTCCGAGAGTATAGCGCCAC	ATTAAACCGACTGCGCCAGC	120
<i>DLEU2 08</i>	AAAGATGGTCCCTGTCAGCA	TCCTTCAAGCTTCTACCACAC	130
<i>GAPDH</i>	AGCCACATCGCTCAGACAC	GCCCAATACGACCAAATCC	66

**Table 3:** Expression levels (as  $\log_2(\text{RQ})$ ) of lncRNAs (FOXD2-AS1, SNHG1, GAS5, LINC00963, DLEU2 02-04, DLEU2 08) for OST patients. Data are represented as arbitrary fluorescence units normalized to GAPDH.

Patient ID	FOXD2-AS1	SNHG 1	GAS5	LINC00963	DLEU2 02-04	DLEU2 08
1	-7.22	-7.96	-5.52	-5.12	-5.80	-5.43
3	-2.16	-6.57	-4.43	-2.63	-4.14	-2.78
4	NA	NA	-3.92	NA	NA	-11.30
5	-8.62	-21.81	-3.59	-6.78	NA	NA
6	NA	-9.45	-3.21	NA	NA	2.15
7	-5.13	-13.93	-1.90	NA	-1.52	NA
8	-15.11	-11.51	-3.60	-2.42	-6.07	NA
10	NA	NA	-2.81	NA	-5.96	-12.61
<b>Mean</b>	-7.65	-11.87	-3.62	-4.24	-4.70	-6.00
<b>Median</b>	-7.22	-10.48	-3.60	-3.87	-5.80	-5.43
<b>St Dev</b>	4.83	5.52	1.08	2.09	1.94	6.10
<b>Minimum</b>	-15.11	-21.81	-5.52	-6.78	-6.07	-12.61
<b>Maximum</b>	-2.16	-6.57	-1.90	-2.42	-1.52	2.15

**Table 4:** Descriptive statistic of lncRNA expression in the study population grouped according to response to therapy.

	<b>Response</b>	<b>FOXD2-AS1</b>	<b>SNHG1</b>	<b>GAS5</b>	<b>LINC00963</b>	<b>DLEU2 02-04</b>	<b>DLEU2 08</b>
<b>N</b>	Resistant	4	4	4	4	4	4
	Sensitive	4	4	4	4	4	4
<b>Mean</b>	Resistant	-6.00	-12.1	-4.37	-4.84	-4.97	-6.51
	Sensitive	-1-.1	-11.6	-2.88	-2.42	-4.52	-5.23
<b>Median</b>	Resistant	-7.22	-7.96	-4.17	-5.12	-4.97	-5.43
	Sensitive	-10.1	-11.5	-3.01	-2.42	-5.96	-5.23
<b>Standard deviation</b>	Resistant	3.40	8.43	0.845	2.09	1.17	4.36
	Sensitive	7.06	2.24	0.729	NaN	2.60	10.4
<b>Range</b>	Resistant	6.46	15.2	1.93	4.15	1.65	8.52
	Sensitive	9.98	4.48	1.70	0.00	4.55	14.8

**Table 5:** Progression free survival (PFS) and overall survival (SUR) results based on GAS5 expression in the TARGET dataset patient cohort

	<b>Patients</b>	<b>HR PFS</b>	<b>P value PFS</b>	<b>HR SUR</b>	<b>P value SUR</b>
<b>TOT</b>	92	1.60	0.0006	1.57	0.003
<b>AOST0331</b>	24	2.06	0.01	4.33	0.001
<b>AOST06B1</b>	15	1.85	0.24	3.96	0.07
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