LncRNA H19, HOTAIR AND MALAT1 AS MOLECULAR BIOMARKERS IN GIST

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Abstract

Long non-coding RNAs (IncRNAs) are emerging as essential regulators of genetic and epigenetic networks, and their deregulation may underlie complex diseases, such as carcinogenesis. Several studies have described IncRNAs alterations in patients with solid tumors. In particular, in Gastrointestinal Stromal Tumors (GIST), upregulation of HOTAIR has been associated with tumor aggressiveness and metastasis, and poor patients' survival. In order to gain more detailed insight on the molecular role of IncRNAs in GIST, we analyzed in vivo the expression levels of IncRNAs H19, HOTAIR and MALAT1 in tissue specimens of both surgically resected and metastatic GIST patient. The expression of the IncRNAs H19, HOTAIR and MALAT1 was evaluated in a total of 40 patients with GIST using quantitative real-time reverse transcriptase (qRT-PCR). H19 was overexpressed in 50% (20/34) GIST patients (p-value: 0.0496). MALAT1 was overexpressed in 45,15% (14/31) GIST patients (p-value: 0.032). Furthermore, the up-regulation of H19 has been found in 74% (17/23) patients harboring cKIT mutations compared to 4/7 (57%) wild type patients (p-value: 0.042). Conversely the up-regulation of MALAT1 has been found in 76% (13/17) patients harboring cKIT mutations compared to 4/4 (100%) wild type patients (p-value: 0.027). Finally, the up-regulation of H19 has been found in 100% (5/5) patients with TTP < 3 months compared to 25% (1/4) patients with TTP >3 months, while the up-regulation of MALAT1 has been found in 25% (1/4) patients with TTP < 3 months compared to 75% (4/5) patients with TTP >3 months.

H19 and MALAT1 appear upregulated in GIST patients according to the KIT-mutation status. These data would suggest a potential, opposite prognostic value of both H19 and MALT1 IncRNAs in these patients. Further analyses are needed to confirm these data, and evaluate the potential role of such IncRNAs, as prognostic/predictive biomarkers.
Background Rationale and Objectives

1.1 Introduction

“We are more than the simply sum of our genes”. Over the last decade, this is the idea that characterized the post-genomic era and it will likely allow us to understand the molecular cellular identity. Indeed every type of cell shows the same single genome but different epi-genomes able to determine the final cellular identity. Therefore the “personality of cells” becomes definite by its epigenome that is the result of both the classic Mendel’s laws of genetic inheritance and epigenetic, whose basic unit is the nucleosome. The nucleosome structure together with non-histone proteins and RNAs constitute the plasticity of chromatin architecture (Figure 1). Indeed, the set of ATP-dependent chromatin remodeling complexes covalent modifiers, and non-coding RNAs, seem to play a key role in the network of epigenetic regulation being able to make modification beyond the DNA sequences. Although these events take place physiologically, it was also observed that aberrations arising at RNA or protein levels, may influence the tumor phenotype without generating any changes in genes coding (Figure 2). Recent data have shown that several emerging classes of non-coding RNA, such as HOTAIR, Xist ed AIR long non coding RNA (lncRNA) interact with chromatin remodelling complexes in order to direct themselves toward gene targets. LncRNAs are emerging as essential components of gene regulatory networks, including cancer. In the current scenario, characterized by a spasmodic research of new biomarkers and the advent of advanced technologies, the lncRNAs represent a new, valid and largely unexplored field of investigation, that I like wildly think as “super-natural or epi-DNA”.
1.2 The little world of long non-coding RNA (LncRNA)

It is the versatile molecule of RNA that, always considered as a simple mediator between DNA and proteins, in the last decades has received favorable consensus, being recognized as a centerpiece of the complex world that goes around the life of the cell. Indeed, recently the scientific community has re-considered all the potential scenarios in which the LncRNAs and their related mechanisms of action could shed light on organismal complexity. LncRNAs belong to a class of regulatory RNA noncoding for proteins that, as it has been estimated, represent approximately 1.5% of the eukaryotic genome, almost entirely transcribed. The NONCODE human LncRNA database annotated 527,336 transcripts that are antisense, intergenic, sense intronic, and processed transcript. Differently from other transcriptome and epigenome data sets the LncRNAs are generated through a molecular pathway similar to that used for protein-coding genes. LncRNAs have been arbitrary defined according to their size, as transcribed RNA molecules greater than 200 nt in length in their mature form. In contrast to the small ncRNAs (siRNAs, miRNAs, and piRNAs), which are highly conserved in commonly studied species, and act as negative regulator of gene expression, LncRNAs are modestly conserved, and regulate gene expression through mechanisms that are mostly poorly understood.

1.3 LncRNAs as subject: features and functions

As mRNA, LncRNAs are often multi-exonic and lack an open reading frame of significant length (less than 100 amino acids). LncRNAs are preferentially localized in the chromatin and nuclear RNA fractions, but also in cytoplasm fractions. They may have or not 3'poly(A) motif and 5’ capped, showing canonical splices sites. LncRNAs are subjected to transcriptional and post-transcriptional regulation, thus lacking any protein-coding potential. Despite the majority of LncRNAs' functions remain unknown, it seems that their effects are likely due to a synergistic interaction with other components. Indeed their peculiar structure allows them to easily interact with DNA, RNA nucleic acids and proteins. Thus far, it has not been clarified yet the specific molecular mechanisms used by LncRNAs to regulate such processes and if they play an active or a passive role in this context. Thanks to their structure, they play a
critical role in a plethora of biological functions at transcriptional, post-transcriptional and translation levels, including also epigenetic processes\textsuperscript{27,28,35,38}.

This wide range of cellular functions are not mutually exclusive\textsuperscript{21} (Figure 3).

✓ As signal, the expression of lncRNAs responds to intra- and extracellular stimuli in a specific space-time (Figure 3 I).

In mammals, lncRNAs can act as signals for the regulation of gene expression during the development process, or in stress conditions, or during the splicing of pre-mRNA. They can recruit chromatin-modifying factors on their target gene promoters inducing transcriptional active euchromatin or silent heterochromatin status. They can also contribute merely in a signaling pathway\textsuperscript{21,39}. For example, the overexpression or down-regulation of lincRNAs induces high or low levels of induced pluripotent stem cells (iPSCs), respectively, and their absence is associated with dysregulation of p53, in response to a particular stress as DNA damage (confirming the key role of lincRNAs in the induction of pluripotency)\textsuperscript{40}.

✓ As Decoy, lncRNAs tighten the interaction with ribonucleoproteins structure (RBPs) on chromatin, into nuclear subdomains or in the cytoplasm\textsuperscript{21,41} (Figure 3 II).

LncRNAs can recruit transcriptional factors, chromatin modifiers or other regulatory factors\textsuperscript{21}. Also, they can interact with DNA to cause a triple helix structure that block the maturation of pre-initiation mRNA complex\textsuperscript{42}. Instead, at post-transcriptional level, lncRNA-mRNA double helix complex can grow the stability of mRNA and it may prevent the degradation of mRNA\textsuperscript{43}. Furthermore, lncRNAs can bind regulatory microRNAs\textsuperscript{44}. The interaction microRNA-lncRNA can cause the degradation of lncRNAs or can promote the creation of small microRNA. Thanks to their “miRNA-sponge” action, lncRNAs can block the microRNA function or may act as endogenous competitor for the binding with mRNA targets\textsuperscript{45,46} (not shown on figure).

✓ As Guide, lncRNAs form RBP complexes in \textit{cis} or in \textit{trans} position of their production site (Figure 3 III).
IncRNAs can bind the proteins involved in the transport activity likely to facilitate the localization in the specific chromosome sites or to recruit chromatin-modifying enzymes.

- As Scaffold, in the same space and time (Figure IV),

IncRNAs can bring together several different proteins, to make cellular substructures or protein complexes that has an effect on chromatin inside or outside the cell.

So, they take part to both local and global changes in chromatin packaging.

Since IncRNAs regulate several biological processes, their overexpression may be essential in the switch towards pathological conditions. They may be involved in the development of different human diseases such as cancer. They may act as pro-oncogenic and/or tumor suppressor factors, modulating both tumor initiation, progression and metastatic pathways. The majority of validated hypothesis suggested that the aberrant expression of IncRNAs related to the activation of oncogenes such as Myc and p53.

Experimental evidences suggested that dysregulation of IncRNAs promotes tumorigenesis and metastasis of several human cancers (Figure 4). Among the different IncRNAs identified in solid tumors, H19, HOTAIR and MALAT1 represent those better studied and characterized as described below.

1.4 LncRNA H19

LncRNA H19 has been one of the first to be identified. It is mostly expressed in the embryonic and fetal tissues, but its expression is reduced in adult tissues. The H19 locus encodes a 2.3 kb noncoding and it maps on 11p15.5 chromosome in humans. This gene belongs to a subgroup of imprinted genes. The loss of H19 maternally expressed gene and its consecutive expression alteration has been observed in different solid tumors. qRT-PCR analyses on gastric cancer cells and tissues, obtained during surgical resection, has shown a higher expression of IncRNA H19 than that observed in adjacent normal gastric cancer (GC), non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), ovarian cancer (OC), gallbladder cancer (GBC), laryngeal squamous cell cancer (LSCC), colorectal cancer (CRC), esophageal cancer (EC) and hepatocellular carcinoma (HCC) tissues. Its
dysregulation has been correlated with worse survival, poor DFS, histological grade, positive lymph node metastasis and advanced TNM stage\textsuperscript{55}. Also, in plasma of pre-operative patients with GC, the H19 plasma levels were high far more than healthy control patients although in no way there were conformity with clinico-pathological characteristics. It’s different the situation/analyses using paraffin-embedded tissues. There was no different H19 expression levels between cancerous tissues and paired non-cancerous tissues\textsuperscript{56}. Despite the molecular mechanisms remain unclear, in GC tissue and cell lines, H19 acts rarely as tumor suppressor\textsuperscript{57,58}, more as an oncogene through its mature product mir-675\textsuperscript{59,60}. They directly or indirectly act on different target genes modulating different molecular pathways. Probably, the activation of Akt/mTOR pathway for the progression of GC seems to be mediated by inactivation of tumor suppressor RUNX1 (runt domain transcription factor 1), which is a target of mir-675\textsuperscript{61,62}. Even the inactivation of p53 seems to grow the H19 expression level in hypoxic condition, likely through HIF1a, supporting the proliferation of gastric cancer cells\textsuperscript{54}. The consequent epithelial–mesenchymal transition (EMT) phenomenon may be responsible of metastatic phenotype and/or pharmacoresistance events\textsuperscript{63}. From a meta-analysis results, high H19 level expression was inversely correlated with OS and prognosis in many types of cancer, that could make it a negative prognostic biomarkers\textsuperscript{55}.

\textbf{1.5 LncRNA HOX antisense intergenic RNA (HOTAIR)}

HOX antisense intergenic RNA (HOTAIR) encodes a large intergenic LncRNA. It is located within the HOXC locus in antisense orientation relative to the HOXC genes. Thanks to its peculiar architecture it plays a key role as scaffold for the formation of different complexes. HOTAIR consists of two modules and a linker sequence. Both this modules bind a chromatin remodelling factor at target locus: the Polycomb Repressive Complex 2 (PRC2) and the lysine-specific demethylase 1 (LSD1), promoting the gain of trimethylate lysine 27 of histone H3 (H3K27me3), which is a well-known repressive mark, and the loss of H3K4me2methylation, considered as an active transcription mark. Thereby, it is able to establish the silent euterochromatin state (essential to silence the locus HOXD located in trans on a different chromosome\textsuperscript{64} to alter other genes’ expression patterns\textsuperscript{65}. HOTAIR is considered a prognostic unfavorable marker in various kinds of cancer despite there has been not any significant association between
its abnormal expression and the clinical-pathological features of analyzed patients. Higher HOTAIR expression has been observed in breast, gastroenteric, liver, lung, prostate, pancreatic, sarcoma cancer tissues compared to the adjacent normal tissues\textsuperscript{12,66}. In some tumor types higher levels of HOTAIR have been detected in plasma of cancer patients\textsuperscript{66} compared to healthy controls, but in GC patients the plasma levels of such IncRNA ) were similar to the normal population\textsuperscript{56}.

1.6 LncRNA MALAT1

MALAT1 is the most expressed within the Inc transcripts. Its transcript is long 6.7 kb. It localizes into nuclear speckles\textsuperscript{67-69} reaching the citoplasmatic compartment as RNA of few nt\textsuperscript{70}. Nuclear-localized IncRNA MALAT1 shuttles to transcription start sites where it functions as scaffold to guide transcription and alternative pre-mRNA splicing in a target gene. It acts as oncogenic factor. In stress condition it has been found upregulated in common site of metastasis from different solid tumors first of all lung cancer\textsuperscript{71}. In this type of tumor it plays a role as negative prognostic marker\textsuperscript{72,73}. As shown by a recent meta-analysis, also in breast, ovarian, colon, pancreatic and digestive cancer, the high expression of MALAT1 positively correlated with worse patients’ prognosis while in B cell lymphoma the condition was the opposite. Indeed, high level of MALAT1 expression was associated to a good patients’ prognosis\textsuperscript{74}. Similarly to HOTAI, MALAT1 plasma levels in GC patients were no different compared to the healthy control\textsuperscript{56}.

1.7 LncRNAs and GIST

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumor belonging to the class of sarcoma. We usually find them in gastrointestinal tract at a percentage of 60-70% in the stomach or even of 30% in small bowel, with lower frequency up to esophagus. GISTs can develop sporadic mutations in the c-KIT receptor and platelet-derived growth factor receptor alpha (PDGFRα) proto-oncogenes, which encode both KIT and PDGFRα proteins, respectively. Mutations of c-KIT exons 9/11 are reported in about 80% of GISTs, making them sensitive to the targeted therapy with the multi-target tyrosine kinase inhibitor (TKI) imatinib mesylate, whose advent has revolutioned the natural history of such disease. Conversely PDGFRα mutations are described in about 5-8% of GISTs and are associated with the
occurrence of primary resistance to the TKI imatinib. Finally about 15% of all GISTs don’t report any mutations in both c-KIT and PDGFRα genes and are defined as “wild-type”. For GIST patients with localized disease who underwent the surgical treatment, the evaluation of cKIT/ PDGFRα molecular alterations together with other clinical-pathological factors, including tumor size/site, mitotic rate, and proliferation index, is crucial to predict the potential risk of recurrence and ultimately decide if patients are candidate to receive adjuvant therapy with imatinib. As regards patients with advanced disease harboring c-KIT activating mutations imatinib mesylate represents the standard first-line treatment, while sunitinib and regorafenib are two multi-target TKIs usually administered after imatinib failure. Currently both c-KIT and PDGFRα mutations represent the only approved molecular biomarkers taken into account by clinicians to decide the medical therapy. In this scenario the identification of IncRNA as new potential diagnostic, prognostic, and predictive molecular biomarkers represent a new challenge for current translational research, especially in rare tumors such as GISTs.

Few working groups have studied, in vivo and in vitro, the expression of IncRNAs in GISTs. Niinuma et al. in 2012 described the deregulation of the HOTAIR expression in GIST patients. In particular, HOTAIR was up-regulated in high-risk malignancy samples from frozen GIST tissues. The lncRNA tissue overexpression was associated with metastasis and poor overall survival, even it was not statistically significant. This analysis was not confirmed in FFPE specimens, likely due to the low quality of RNA. In vitro, knockdown of HOTAIR in GIST-T1 cells influences the expression of target genes and the invasive ability of cells though not the cell viability. The dysregulation of both HOTAIR and other downstream genes expression in cancer cells, need to be ascribed to an epigenetic mechanism. Looking to the chromatin status of GIST-T1 cells, the histone codes show an enrichment of a marker of active gene transcription, H4K4me3, in the transcription start sites of genomic regions of interest. Alternatively, the overexpression of HOTAIR can mediate the trimethylation of the histone H3 at lysine 27 and the subsequent silencing of the target gene. An additional study by Lee et al. has recently confirmed such evidences, showing that if the target gene subjected to silencing is a tumor suppressor as PCDH10, the final result will be the failure of the mechanisms which control both tumor invasion and progression. Even if very interesting, these are the only published data currently available regarding IncRNAs expression in GISTs.
1.8 Rationale and Objectives

An increasing number of studies described lncRNAs expression alteration in different solid tumors, suggesting their potential involvement in tumorigenesis and metastatic processes, as well as their role as prognostic/predictive biomarkers for clinical use. However very few data are currently available on lncRNAs dysregulation in rare tumors such as GISTs. Our work represents one of the few studies evaluating lncRNAs expression in GISTs. Indeed, in order to gain more detailed insight on the molecular role of lncRNAs in GIST, we analyzed in vivo the expression levels of lncRNAs H19, HOTAIR and MALAT1 in tissue specimens of both surgically resected and metastatic GIST patients. Identifying reliable biomarkers of both cancer risk development/recurrence and prognosis, and treatment sensitivity/resistance predictors, represents an area of intense investigation in the cancer research. Therefore we subsequently tried to assess the potential prognostic role of these lncRNAs in relation to other clinical-pathological parameters in GIST patients as well as the possible association between their upregulation and the targeted therapy efficacy.
CHAPTER 2

Materials and Methods

2.1 Study population

From January 2009 and November 2016 a total of 40 patients were enrolled within a single-institutional translational research study at the Oncology Department of Palermo University Hospital, including 35 patients with localized disease and 15 patients with advanced disease. Written informed consent was obtained from all patients before inclusion in such study and specimens collection. All patients with diagnosis of metastatic disease harboring cKIT activating mutations received oral imatinib mesylate at 400/800 mg daily until progression (PD) or unacceptable toxicity. Among the patients with localized disease subjected to surgical treatment, only those defined at high risk of recurrence according to the risk definition system proposed by Miettinen's et al. received oral imatinib mesylate at 400/800 mg daily for 3 years. All the patients underwent a CT-scan every 3 months and responses were classified according to RECIST criteria. Clinical and pathological characteristics of all GIST patients included in our study were retrieved from the clinical records available and were assessed retrospectively.

2.2 Tumor samples

A total of 40 pairs of disease formalin-fixed paraffin-embedded (FFPE) tissue and adjacent normal tissue from 40 patients with GIST were collected from Pathology Department of Palermo University Hospital between 2009 and 2016. Resected GISTs specimens were fixed in buffered formalin and embedded in paraffin for pathological examination. The same standard methods were used for pathological assessment of
the tissue biopsies from patients with metastatic onset. After that all sample sections (10 µm thick) were received at the laboratory of Genetic and Clinical Molecular Oncology, where they were subjected to the molecular analysis of KIT exon 9, 11, 13, 17 mutations and PDGFRα exon 12, 14 and 18 mutations. Furthermore, we have obtained other sections of the tumor and simultaneously of the counterparts healthy, which were used for gene expression analysis.

### 2.2 DNA preparation and mutation screening

Genomic DNA was extracted from formalin-fixed, paraffin-embedded sections using a QIAamp DNA FFPE Tissue Kit (Qiagen). To detect hotspot mutations, we amplified exons 9, 11, 13, 17, and 18 of the KIT gene by PCR in a preparation of genomic DNA. The primer sequences are listed in Table 2. We purified PCR products with PureLink® PCR Purification Kit (Thermo Fisher SCIENTIFIC), and directly sequenced them using BigDye XTerminator® Purification Kit (Thermo Fisher SCIENTIFIC) on an ABI 3130 XL Genetic Analyzer automated sequencer (Applied Biosystems). Sequence data were analyzed using Sequencing Analysis software 5.2 (Applied Biosystems).

### 2.3 IncRNA isolation

Formalin-fixed, paraffin-embedded tissues samples were deparaffinized and underwent total RNA and IncRNA extraction using miRNeasy FFPE Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer’s instructions. LncRNA yield was determined through a Qubit™ 3.0 Fluorometer (Thermo Fisher SCIENTIFIC), and the quality assessed by agarose gel electrophoresis. The IncRNA concentration and quality were assessed with the Bioanalyzer 2100 (Agilent Technologies, CA) using the Agilent Small RNA Analysis kit (Agilent, CA).

### 2.4 Reverse Transcription (RT) and quantitative Real time Polymerase chain reaction (qRT-PCR)

Quantitative real-time PCR was used to measure IncRNA expression levels in 40 disease/normal-paired GISTs samples. 500 nanograms of total RNA were reverse transcribed using High-Capacity cDNA Reverse Transcription Kit (ThermoFisher SCIENTIFIC) according to manufacturer's instructions. RT reactions contained RNA sample, 1 × 1 mL of 10X RT Buffer, 1 × 1 mL of 10X RT Random Primers, 1 × 0.2 mL of
25X dNTP Mix (100 mM), 2 × 0.1 mL of MultiScribe® Reverse Transcriptase (50 U/µL) (all from ThermoFisher SCIENTIFIC) and nuclease-free water. The 50-µl reactions were incubated in a Thermocycler (Eppendorf, North Ryde, New South Wales, Australia) for 10 min at 25°C, 120 min at 37°C, 5 min at 85°C and then held at 4°C.

The following Applied Biosystems assays were used for TaqMan analysis of *H19* (Assay ID Hs00399294_g1); *HOTAIR* (Assay ID Hs03296631_m1); *MALAT1* (Assay ID Hs00273907_s1) as potential prognostic biomarkers. The reactions were incubated in a 96-well plate at 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The quantitative PCR was performed on an Applied Biosystems 7900HT fast RT-PCR system, and data were collected and analyzed using ABI SDS version 2.3. Triplicate reactions were performed on all samples. To normalize qRT-PCR reactions, parallel reactions were run on each sample for *GAPDH* (Assay ID Hs03929097_g1). Changes in IncRNA expression levels were determined using a comparative CT method.

**2.5 Statistical Analysis**

Statistical analysis was performed using Microsoft Excel and Prism GraphPad software (GraphPad software, CA).

A Fold Change > 1 (logarithmic scale) cut-point was used to define IncRNA up-regulation in analyzed tumor tissues.

Time to progression (TTP) was calculated from the date of admission to the date of first radiologic progression. Survival analysis was performed using Kaplan–Meier method, providing median and 95% confidence interval (CI).

To compare two independent samples, X² test was used for intergroup comparison of categorical variables, while the Mann Whitney test was used for statistical analysis of continuous variables. A p-value < 0.05 was used as a threshold for statistical significance.
CHAPTER 3

Results

3.1 Clinical characteristics of the patients

Forty patients with histopathological diagnosis of GIST were included into the study. Median age of the patients was 59 years. Majority of the patients were male (n.26, 65%). More than half (n.25, 64%) of the cases had localized disease at diagnosis, so 36% represented with stage IV disease. C-KIT mutations were detected in 27/40 (67%) of patients, 17/25 patients with localized disease and 10/15 patients with metastatic disease at baseline. Consequently 8/25 (32%) patients with localized disease harboring c-KIT mutations received adjuvant treatment with imatinib for 3 years because of high risk of relapse. Conversely 10/15 (66%) patients with metastatic disease received first-line therapy with imatinib mesylate until PD or unacceptable toxicity. Clinical and pathological characteristics of patients included in the study are summarized in Table 1.

3.2 Expression of IncRNAs H19, HOTAIR, MALAT1 in GISTs

Among the 40 patients included 34 were evaluable for IncRNA H19 expression analysis in tumor tissue. As shown in figure 5 the up-regulation of H19 has been found in 20/34 (50%) patients with GIST with median log₂ fold-change of 5 (Table 3). Furthermore the up-regulation of H19 was significantly higher in tumor tissue of GIST patients than in normal tissue of the same individuals with p-value: 0.0496.

Among the 40 patients included 31 were evaluable for IncRNA MALAT1 expression analysis in tumor tissue. As shown in figure 6 the up-regulation of MALAT1 has been
found in 14/31 (45.15%) patients with GIST with median fold-change of 4 (Table 3). Furthermore the up-regulation of MALAT1 was significantly higher in tumor tissue of GIST patients than in normal tissue of the same individuals with p-value: 0.032.

Among the 40 patients included none of them was evaluable for IncRNA HOTAIR expression analysis in tumor tissue (Table 3).

No statistically significant difference has been detected for the up-regulation of both IncRNAs H19 and MALAT1 in relation to the different clinical-pathological characteristics analyzed, including site/size of tumor, mitotic index, stage at diagnosis, and risk stage according to Miettinen's criteria (Table 4, 5).

A statistically significant difference has been detected for the up-regulation of both IncRNAs H19 and MALAT1 between the KIT-mutated and wild type tumors. Indeed the up-regulation of H19 has been found in 17/23 (74%) patients harboring cKIT mutations compared to 4/7 (57%) wild type patients (p-value: 0.042) (Table 4). Conversely the up-regulation of MALAT1 has been found in 13/17 (76%) patients harboring cKIT mutations compared to 4/4 (100%) wild type patients (p-value: 0.027) (Table 5).

3.3 Relation of IncRNA upregulation and treatment efficacy

To investigate the predictive value of both IncRNAs H19 and MALAT1 in GIST patients we evaluated the up-regulation of such IncRNAs in tumor tissues of 10/40 patients with advanced disease who received first-line therapy with imatinib.

A statistically significant difference has been detected for the up-regulation of both IncRNAs H19 and MALAT1 between patients with early PD compared to those with late PD. Indeed the up-regulation of H19 has been found in 5/5 (100%) patients with TTP < 3 months compared to 1/4 (25%) patients with TTP >3 months (Table 4). Conversely the up-regulation of MALAT1 has been found in 1/4 (25%) patients with TTP < 3 months compared to 4/5 (75%) patients with TTP >3 months (Table 5).

Furthermore a statistically significant difference has been detected for the median TTP between patients with IncRNA H19 up-regulation vs those with IncRNA H19 down-regulation (7.5 weeks vs 22.5 weeks; p-value: 0.025) (Table 4). Similarly a statistically significant difference has been detected for the median TTP between patients with
IncRNA MALAT1 up-regulation vs those with IncRNA MALAT1 down-regulation (15 weeks vs 3 weeks; p-value: 0.036) (Table 5).
Several evidences identified IncRNAs dysregulation in tumor pre-clinical models, suggesting their potential involvement in cancer development\textsuperscript{79,80}. We have evaluated the expression of H19, HOTAIR, MALAT1 because they were better studied among the different IncRNAs identified in solid tumors as a hallmark of poor prognosis. They contribute to oncogenesis in cancer as oncogenic and/or tumor suppressor factors\textsuperscript{81}. They play essential biological functions including chromatin modification, transcriptional and post-transcriptional processing\textsuperscript{82,83}. Dysregulation of H19, HOTAIR and MALAT1 was observed in many kind of cancers. Their upregulation was associated with tumor cell proliferation, invasion and metastasis suggesting that these IncRNAs may be potential prognostic biomarkers. Indeed, studies \textit{in vitro} showed that their knockdown could inhibit invasions and metastasis. Therefore the identification of IncRNAs as new biomarkers for clinical use could represent an important finding in the context of rare tumors as GISTs. Although the IncRNAs have already attracted the attention of the scientific community\textsuperscript{77}, however the clinical significance of the IncRNA expression has been not yet understood. In this study, for the first time, we found that both IncRNAs H19 and MALAT1 were upregulated in tumor specimens of patients with GIST and that the up-regulation of both IncRNAs H19 and MALAT1 was significantly associated with the KIT-mutation status. Indeed the percentage of H19 upregulation was significantly higher in mutated vs wild-type patients, while the percentage of MALAT1 upregulation was lower in mutated vs wild-type patients. According to the known negative prognostic role of c-KIT mutations in GIST, these data would suggest a potential, opposite prognostic value of both H19 and MALT1 IncRNAs in these patients. This suggestion has been confirmed by the analysis of the TTP in the subgroup of patients with advanced disease who received first-line therapy with
imatinib. Indeed the percentage of H19 upregulation was significantly higher in patients with TTP < 3 months, suggesting a negative prognostic role of H19, which seems to be associated with an early PD to imatinib. By contrast, the percentage of upregulation of MALAT1 was significantly higher in patients with TTP > 3 months, suggesting a positive prognostic role of MALAT1, which seems to predict a longer treatment efficacy. Also, we demonstrated a significant lower median TTP in patients with H19 up-regulation as well as a significantly higher median TTP in patients with MALAT1 upregulation vs those with H19 and MALAT1 down-regulation, respectively.

According to these evidences the evaluation of lncRNAs expression could allow to select among all c-KIT mutated GIST patients eligible to receive first-line therapy with imatinib those patients who could really benefit from this treatment reserving a more effective therapy to the others, with interesting implications for their clinical management.

However the low number of patients included in the study limits of course the scientific validity of our results, which need to be explored and confirmed in prospective studies including larger patients’ cohort.

Furthermore the determination of H19, MALAT1 and HOTAIR could has been partially influenced by the following factors: FFPE tissues stability, formalin-fixation and paraffin-embedding may have influenced the stability of lncRNAs; heterogeneity of included population and their clinicalpathological parameters; heterogeneity of information and low amount of lncRNA expression levels to correlate with the clinicalpathological characteristics. Determination principle was the same, analysis type was qRT-PCR. As regards the lncRNA HOTAIR, it has a longer amplicon (152 bp) than H19 and MALAT1. Analysis with bioanalyzer, a software tool designed to help scientists in estimating the integrity of total RNA samples, have shown a RNA Integrity Number < 7, because of the poor quality of RNA, extracted from paraffin tissue. The results of HOTAIR expression levels were indeterminate in all analyzed tumor samples. Through qRT-PCR method, the probe couldn’t bind, amplify and express its target, probably because HOTAIR has been degraded during its isolation. By contrast, we tried to analyze the HOTAIR expression levels through digital real time PCR. We have compared a FFPE GIST sample to its healthy counterpart, using H19 expression level as control. Although we have confirmed the expression of lncRNA H19, obtained
by qRT-PCR, we have not detected any expression of HOTAIR with this third generation method.

Our results indicate that upregulation of H19 and MALAT1 could contribute to oncogenesis of GIST suggesting their potential role as prognostic/predictive biomarkers for clinical use.

Even if very interesting, these preliminary observations need to be confirmed by subsequent larger prospective studies. Of course it will be necessary to expand the study population, establishing collaborations with other centers of reference for rare tumors, to fine tuning the analysis of expression levels through third generation machineries and to confirm our data in fresh tissue. Recent evidences identified lncRNAs in plasma exosome or also in complex with circulating microRNAs. Although these studies clearly demonstrate that there are many functional circulating lncRNA, key questions remain to be solved\textsuperscript{84,85}. As recently shown in other tumor types, it would be also interesting evaluating the expression levels of circulating lncRNAs in plasma of GIST patients and comparing them with the results obtained in tumor tissue. In the last years, groups of reasercher have walked roads to identify biomarkers which could help the early detection and screening, the choice of surgical or medical treatments, and the monitoring during the follow-up period. This represents a small but interesting contribute to this research which aims to further personalize the management and treatment of patients with GIST.
Figure 1 | The triple-code hypothesis. The triple-code model includes genetic, epigenetic and nuclear architecture and it determines the functional biological identity of each cell. The genetic code respects Mendel’s laws of heredity, but a wrong letter of a gene’s nucleotides or other genetic mutation can encodes an aberrant protein. Finally, the epigenetic code is the combination of changes in epigenetic marks (DNA methylation, histone tail modifications) that occur on the nucleosome structure. Moreover, the action of noncoding RNAs molecules contributes to epigenetic phenotype. Image reproduced by Lomberk GA et al., 2015, Surg Clin North Am.3
**Figure 2** | Gen-epigenetic tetris model. Each cell accumulates genetic and epigenetic events that may or may not cause the aberrant state. As in Tetris game, the human organism is planned to correct the genetic errors. If these are not repaired, they may cause the tumor phenotype (left). Instead, the epigenetic events occur physiologically. They tear down the wall of incorrect combination because they are essential for our correct development (right vs left). Otherwise, the tumor phenotype is a resultant of genetics and epigenetics mechanisms. The latter can participate with the arrival of epigenetic marks which can contribute to the development and tumor progression without necessarily notching the gene sequence (right).

**Figure 3** | Schematic cellular functions of lncRNAs. I (signal): in a specific space and time, lncRNAs can regulate gene expression recruiting chromatin-modifying factors (colored ovals) or participating in a molecular pathway; II (decoy): lncRNAs can tear off proteins from chromatin, into nuclear subdomains or cytoplasm; III (guide): lncRNAs can recruit, in cis or in trans, RBP complexes or chromatin modifying enzymes to target genes; IV (scaffold): lncRNAs can form ribonucleoprotein complexes. Image reproduced by Wang et al., 2011, Mol Cell.21
Figure 4|LncRNAs in a different tumoral hystotypes. Even before next generation sequencing, RNA-seq. and successive overlap of sequencing IncRNAs libraries have shown a large number of IncRNAs differentially expressed, both up (red) and down (blue), in different tumoral hystotypes if compared with normal tissues. (Image riproduced by Bartonicek N. et al., 2016, Mol Cancer)
Table 1 | Clinical features of the GIST samples

<table>
<thead>
<tr>
<th>Feature</th>
<th>Male</th>
<th>Female</th>
<th>Median age, years</th>
<th>Mitotic rate</th>
<th>Tumor size</th>
<th>Tumor site</th>
<th>Risk classification (Miettinen's criteria)</th>
<th>Analysis mutation</th>
<th>Onset</th>
<th>Imatinib 400/800 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>26</td>
<td>14</td>
<td>59</td>
<td>&lt;5/50 HPF:</td>
<td>10</td>
<td>Gastro-intestinal</td>
<td>Low risk-very low risk</td>
<td>Wild type</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥5/50 HPF</td>
<td>12</td>
<td>Colon-rectal</td>
<td>Intermediate-high risk</td>
<td>Mutated cKIT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age, years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cKIT</td>
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</tr>
<tr>
<td>Mitotic rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>PDGFR</td>
<td></td>
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<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor site</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk classification (Miettinen's criteria)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analysis mutation</td>
<td>Wild type</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imatinib 400/800 mg</td>
<td>Mutated cKIT</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Ajuvant</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>1st line</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 | Primers used for analysis of KIT and PDGFRα genetic aberrations

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primer Set</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>F:5'-AGC CAG GGC TTT TGT TTT CT-3'</td>
</tr>
<tr>
<td></td>
<td>R:5'-CAG AGC CTA AAC ATC CCC TTA-3'</td>
</tr>
<tr>
<td>11</td>
<td>F:5'-CCT TTG CTG ATT GGT TTC GT-3'</td>
</tr>
<tr>
<td></td>
<td>R:5'-ACC CAA AAA GGT GAC ATG GA-3'</td>
</tr>
<tr>
<td>13</td>
<td>F:5'-GTT CCT GTA TGG TAC TGC ATG CG-3'</td>
</tr>
<tr>
<td></td>
<td>R:5'-CAG TTT ATA ATC TAG CAT TGC C-3'</td>
</tr>
<tr>
<td>17</td>
<td>F:5'-CTG AAT ACT TTA AAA CAA AAG TAT TGG-3'</td>
</tr>
<tr>
<td></td>
<td>R:5'-TTA TGA AAA TCA CAG GAA ACA ATT T-3'</td>
</tr>
<tr>
<td>12</td>
<td>F:5'-AAG CTC TGG TGC ACT GGG ACT T-3'</td>
</tr>
<tr>
<td></td>
<td>R:5'-ATT GTA AAG TTG TGT GCA AGG GA-3'</td>
</tr>
<tr>
<td>14</td>
<td>F:5'-CAG CAT TAG TCA TAT CCT TGG TTT TT-3'</td>
</tr>
<tr>
<td></td>
<td>R:5'-TTT ATT ATC ATC ATT TAC GCA TGT GT-3'</td>
</tr>
<tr>
<td>18</td>
<td>F:5'-TAC AGA TGG CCT GAT CCT GAG T-3'</td>
</tr>
<tr>
<td></td>
<td>R:5'-AGT GTG GGA GGA TGA GCC TG-3'</td>
</tr>
</tbody>
</table>
Table 3: up, down, undetermined lncRNA H19, MALAT1 and HOTAIR in GIST patients and mean of their relative expression values.

<table>
<thead>
<tr>
<th>lncRNA-ID</th>
<th>Up regulated n (%)</th>
<th>mean log₂(fold change)</th>
<th>Down regulated n (%)</th>
<th>Mean log₂(fold change)</th>
<th>Undetermined n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H19</td>
<td>20 (50%)</td>
<td>5</td>
<td>14 (35%)</td>
<td>-3</td>
<td>6 (15%)</td>
</tr>
<tr>
<td>MALAT1</td>
<td>14 (45,15%)</td>
<td>4</td>
<td>10 (32,25%)</td>
<td>-0,65</td>
<td>7 (22,6%)</td>
</tr>
<tr>
<td>HOTAIR</td>
<td>N.A</td>
<td>N.A</td>
<td>N.A</td>
<td>N.A</td>
<td>40 (100%)</td>
</tr>
</tbody>
</table>

Table 3: N.A.: not available

Figure 5: H19 expression levels (log₂ 2^{ΔΔCT}) in GIST groups. Relative expression levels are represented for each group. Each sample was normalized using the corresponding normal counterpart.
Figure 6] MALAT1 expression levels (log2 $2^{-\Delta\Delta CT}$) in GIST groups. Relative expression levels are represented for each group. Each sample was normalized using the corresponding normal counterpart.
<table>
<thead>
<tr>
<th>Variable</th>
<th>H19 expression level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>34</td>
</tr>
<tr>
<td>Mitotic rate: n.(%)</td>
<td></td>
</tr>
<tr>
<td>&lt;5/50 HPF:</td>
<td>9 (45%)</td>
</tr>
<tr>
<td>≥5/50 HPF:</td>
<td>4 (36,4%)</td>
</tr>
<tr>
<td>Tumor size: n.(%)</td>
<td></td>
</tr>
<tr>
<td>&lt;5 cm:</td>
<td>12 (60%)</td>
</tr>
<tr>
<td>≥5 cm:</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>Tumor site</td>
<td></td>
</tr>
<tr>
<td>Gastro-intestinal:</td>
<td>22 (91,66%)</td>
</tr>
<tr>
<td>Colon-rectum:</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>Risk classification (Miettinen’s criteria)</td>
<td></td>
</tr>
<tr>
<td>Low risk-very low risk:</td>
<td>7 (50%)</td>
</tr>
<tr>
<td>Intermediate-high risk:</td>
<td>7 (50%)</td>
</tr>
<tr>
<td>Onset</td>
<td></td>
</tr>
<tr>
<td>Localized:</td>
<td>24 (100%)</td>
</tr>
<tr>
<td>Metastatic:</td>
<td>10(100%)</td>
</tr>
<tr>
<td>Analysis mutation</td>
<td></td>
</tr>
<tr>
<td>Wild type.:</td>
<td>7 (23,3%)</td>
</tr>
<tr>
<td>Mutated.:</td>
<td>23 (76,7%)</td>
</tr>
<tr>
<td>TTP:</td>
<td></td>
</tr>
<tr>
<td>Early (&lt; 3 months):</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>Late (&gt; 3 months):</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>TTP: median (weeks)</td>
<td>7,5</td>
</tr>
</tbody>
</table>
Table 5: Results of the association of clinical-pathological parameters with IncRNA-MALAT1 expression level according to group of study

<table>
<thead>
<tr>
<th>Variable</th>
<th>MALAT1 expression level</th>
<th>&gt;1</th>
<th>&lt;1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>24</td>
<td>10 (41.6%)</td>
</tr>
<tr>
<td>Mitotic rate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5/50 HPF: 5 (38.5%)</td>
<td></td>
<td>3 (60%)</td>
<td>2 (40%)</td>
</tr>
<tr>
<td>≥5/50 HPF: 8 (61.5%)</td>
<td></td>
<td>5 (63.5%)</td>
<td>3 (37.5%)</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 cm: 7 (58.4%)</td>
<td></td>
<td>6 (85.7%)</td>
<td>1 (14.3%)</td>
</tr>
<tr>
<td>≥5 cm: 5 (41.6%)</td>
<td></td>
<td>3 (60%)</td>
<td>2 (40%)</td>
</tr>
<tr>
<td>Tumor site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastro-intestinal: 13 (%)</td>
<td></td>
<td>7 (%)</td>
<td>6 (%)</td>
</tr>
<tr>
<td>Colon-rectum: 2 (%)</td>
<td></td>
<td>2 (100%)</td>
<td>0</td>
</tr>
<tr>
<td>Risk classification (Miettinen’s criteria)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low risk-very low risk: 4(50%)</td>
<td></td>
<td>2 (50%)</td>
<td>2 (50%)</td>
</tr>
<tr>
<td>Intermediate-high risk: 4(50%)</td>
<td></td>
<td>3 (75%)</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>Onset</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localized: 24 (60.6%)</td>
<td></td>
<td>13 (54.2%)</td>
<td>11 (45.8%)</td>
</tr>
<tr>
<td>Metastatic: 10 (29.4%)</td>
<td></td>
<td>5 (50%)</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>Analysis mutation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type: 4 (19%)</td>
<td></td>
<td>4 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Mutated.: 17 (80.9%)</td>
<td></td>
<td>13 (76.5%)</td>
<td>4 (23.5%)</td>
</tr>
<tr>
<td>TTPI</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Early (&lt; 3 months): 5 (50%)</td>
<td></td>
<td>1 (20%)</td>
<td>4 (80%)</td>
</tr>
<tr>
<td>Late (&gt; 3 months): 5 (50%)</td>
<td></td>
<td>3 (60%)</td>
<td>2 (40%)</td>
</tr>
<tr>
<td>TTP: median (weeks)</td>
<td></td>
<td>15</td>
<td>3</td>
</tr>
</tbody>
</table>


60. Cai X, Cullen BR. The imprinted H19 noncoding RNA is a primary microRNA precursor. RNA. 2007;13(3):313-316.


In extenso:

  "Back to a false normality": new intriguing mechanisms of resistance to PARP inhibitors.
  Incorvaia L1, Passiglia F1, Rizzo S1, Galvano A1, Listì A1, Barraco N1, Maraglino R1, Calò V1, Natoli C2, Ciaccio M3, Bazan V1, Russo A1.
  1Department of Surgical, Oncological and Oral Sciences, University of Palermo, Palermo, Italy.
  2Department of Medical, Oral and Biotechnological Sciences, Centre of Ageing Sciences and Translational Medicine - CESI-MeT University "G. D'Annunzio", Chieti, Italy.
  3Section of Clinical Biochemistry and Clinical Molecular Medicine, Department of Biopathology and Medical Biotechnology, University of Palermo - U.O.C. Laboratory Medicine - CoreLab, Policlinico University Hospital, Palermo, Italy.

  Non-coding RNAs Functioning in Colorectal Cancer Stem Cells.
  Fanale D1, Barraco N1, Listì A1, Bazan V1, Russo A2.
  1Department of Surgical, Oncological and Oral Sciences, Section of Medical Oncology, University of Palermo, Via del Vespro 129, 90127, Palermo, Italy.
  2Department of Surgical, Oncological and Oral Sciences, Section of Medical Oncology, University of Palermo, Via del Vespro 129, 90127, Palermo, Italy. antonio.russo@usa.net.

  Nintedanib in NSCLC: evidence to date and place in therapy.
  Bronte G1, Passiglia F1, Galvano A1, Barraco N1, Listì A1, Castiglione M1, Rizzo S1, Fiorentino E1, Bazan V1, Russo A2.
  1Oncological and Oral Sciences, Section of Medical Oncology, University of Palermo, Italy.
  2Department of Surgical, Oncological and Oral Sciences, Section of Medical Oncology, University of Palermo, Via del Vespro 129, 90127 Palermo, Italy.

  Can the microRNA expression profile help to identify novel targets for zoledronic acid in breast cancer?
  Fanale D1, Amodeo V1, Bazan V1, Insalaco L1, Incorvaia L1, Barraco N1, Castiglia M1, Rizzo S1, Santini D2, Giordano A3, Castorina S4,5, Russo A1.
  1Department of Surgical, Oncological and Oral Sciences, Section of Medical Oncology, University of Palermo, Via del Vespro 129, 90127 Palermo, Italy.
  2University Campus Bio-Medico, Department of Medical Oncology, Rome, Italy.
  3Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, College of Science and Technology, Temple University, Philadelphia, PA, USA.
  4Fondazione Mediterranea "G.B. Morgagni", Catania, Italy.
  5Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy.

  Dietary restriction: could it be considered as speed bump on tumor progression road?
  Cangemi A1, Fanale D1, Rinaldi G1, Bazan V1, Galvano A1, Perez A1, Barraco N1, Massihnia D1, Castiglia M1, Vieni S1, Bronte G1, Mirisola M1, Russo A2.
  1Department of Surgical, Oncological and Oral Sciences, Section of Medical Oncology, University of Palermo, Via

A headlight on liquid biopsies: a challenging tool for breast cancer management.
Massihnia D1, Perez A1, Bazan V1, Bronte G1, Castiglia M1, Fanale D1, Barraco N1, Cangemi A1, Di Piazza F1, Calò V1, Rizzo S1, Cicero G1, Pantuso G1, Russo A2.
1Department of Surgical, Oncological and Oral Sciences, Section of Medical Oncology, University of Palermo, Via del Vespro 129, 90127, Palermo, Italy.
2Department of Surgical, Oncological and Oral Sciences, Section of Medical Oncology, University of Palermo, Via del Vespro 129, 90127, Palermo, Italy.


Stabilizing versus destabilizing the microtubules: a double-edge sword for an effective cancer treatment option?
Fanale D1, Bronte G1, Passiglia F1, Calò V1, Castiglia M1, Di Piazza F1, Barraco N1, Cangemi A1, Catarrella MT1, Insalaco L1, Listi A1, Maragliano R1, Massihnia D1, Perez A1, Toia F2, Cicero G1, Bazan V1.
1Department of Surgical, Oncological and Oral Sciences, Section of Medical Oncology, University of Palermo, 90127 Palermo, Italy.
2Department of Surgical, Oncological and Oral Sciences, Section of Plastic Surgery, University of Palermo, 90127 Palermo, Italy.

Book:

Advances in Experimental Medicine and Biology Volume 937 Ondrej Slaby • George A. Calin Editors
Non-coding RNAs in Colorectal Cancer Chapter 5: Non-coding RNAs Functioning in Colorectal Cancer Stem Cells.
Fanale D1, Barraco N1, Listi A1, Bazan V1, Russo A2.
1Department of Surgical, Oncological and Oral Sciences, Section of Medical Oncology, University of Palermo, Via del Vespro 129, 90127, Palermo, Italy.
2Department of Surgical, Oncological and Oral Sciences, Section of Medical Oncology, University of Palermo, Via del Vespro 129, 90127, Palermo, Italy.

Abstract:

Annals of Oncology 27, 2016
The role of microRNAs in driving EGFR-TKI resistance in NSCLC cell lines
1AOUP “Paolo Giaccone”, Palermo
2AOUP “Paolo Giaccone”, DICHIRONS Palermo
The prognostic role of KRAS and BRAF in patients undergoing surgical resection of colorectal cancer liver metastasis: a systemic review and meta-analysis


AOUP "Paolo Giaccone", Palermo

SICILIAN PATIENTS WITH familial MALIGNANT melanoma SHOW A very low frequency of germline CDKN2A mutations.


1 Department of Surgical, Oncological and Oral Sciences, Section of Medical Oncology, University of Palermo, 90127 Palermo, Italy;
2 Department of Surgical, Oncological and Oral Sciences, Section of Plastic Surgery, University of Palermo, 90127 Palermo, Italy

MiRNAs in metaplastic transformation of the esophagus: something in the way toward carcinogenesis


1 Department of Science for Promotion of Health and Mother and Child Care, Section of Human Pathology, University of Palermo, 90127 Palermo, Italy;
2 Department of Surgical, Oncological and Oral Sciences, Section of Medical Oncology, University of Palermo, 90127 Palermo, Italy;
3 Department of Surgical, Oncological and Oral Sciences, Section of Surgical Oncology, University of Palermo, 90127 Palermo, Italy

Potential miRNAs involved in molecular pathways mediating the anticancer effects of zoledronic acid in breast cancer cells


Department of Surgical, Oncological and Oral Sciences, Section of Medical Oncology, University of Palermo, 90127 Palermo, Italy.

KRAS and BRAF as prognostic biomarkers in patients undergoing surgical resection of colorectal cancer liver metastasis: a systematic review and meta-analysis

Francesco Passiglia1, Giuseppe Bronte1, Sergio Rizzo1, Antonio Galvano1, Angelo Listi1, Nadia Barraco1, Marta Castiglia1, Valentina Calò1, Viviana Bazan1, Giuseppe Cicero1, Christian Rolfo2, Antonio Russo1.

1Medical Oncology Unit, Department of Surgical, Oncological and Oral Sciences, University of Palermo, Palermo, Italy
Into the Wild of long non-coding RNAs in Gastrointestinal Stromal Tumors (GISTs) to explore new prognostic/predictive biomarkers

Barraco Nadia¹; Listì Angela; Maragliano Rossella; Bazan Viviana; Badalamenti Giuseppe; Fulfaro Fabio; Incorvaia Lorena; Calò Valentina; Castiglia Marta; Bronte Giuseppe; Cangemi Antonina; Perez Alessandro; Insalaco Lavinia; Bronte Enrico; Russo Antonio.

¹ Medical Oncology Unit, Department of Surgical, Oncological and Oral Sciences, University of Palermo, Palermo, Italy.

Study of mutational status of Sicilian GISTs patients

M. Castiglia, G. Badalamenti, F. Fulfaro, L. Incorvaia, V. Calò, V. Bazan, N. Barraco, D. Massihnia, G. Bronte, A. Russo

Policlinico Universitario “Paolo Giaccone”, Palermo

IASLC (September 9, 2015)

PD-L1 expression as predictive biomarker in patients with NSCLC: a pooled analysis

Francesco Passiglia¹, Giuseppe Bronte¹, Sergio Rizzo¹, Antonio Galvano¹, Giovanni Sortino¹, Emmanuela Musso¹, Angela Listì¹, Nadia Barraco¹, Marta Castiglia¹, Valentina Calò¹, Viviana Bazan¹, Giuseppe Cicero¹, Christian Rolfo², Antonio Russo¹.

¹ Medical Oncology Unit, Department of Surgical, Oncological and Oral Sciences, University of Palermo, Palermo, Italy

² Phase I- Early Clinical Trials Unit, Oncology Department and Multidisciplinary Oncology Center (MOCA), Antwerp University Hospital, Edegem, Belgium
“Bisogna sempre credere alle nostre osservazioni, per quanto bizzarre possano essere.

forse stanno cercando di dirci qualcosa” (Barbara McClintock)