MINISTERIO
DE ECONOMIA
Y COMPETITIVIDAD

SECRETARÍA DE ESTADO
DE INVESTIGACIÓN, DESARROLLO E INNOVACIÓN

SECRETARIA GENERAL DE CIENCIA TECNOLOGÍA E INNOVACIÓN

DIRECCIÓN GENERAL
DE INVESTIGACIÓN CIENTÍFICA Y TÉCNICA

SUBDIRECCIÓN GENERAL
DE PROYECTOS DE INVESTIGACIÓN

CONSOLIDER-INGENIO 2010 PROGRAMME
2011 SCIENTIFIC ANNUAL REPORT

PROJECT REFERENCE NUMBER: CSD2009-00080
Coordinating Researcher: Juan Valcárcel
Project Title: An integrated approach to post-transcriptional regulation of gene expression and its role in human disease.
Managing Institution: Center for Genomic Regulation, Barcelona, Spain
Project Initiation Date: 17.12.2009
Project Completion Date: 16.12.2014

Consolider

1. Summary of key activities initiated by the project since the start of the funding period

The joint activities of the RNAreg Consortium have been pursued, completed, extended and fruitful during this project's second year. Building on the strong network of investigators, and on the collaborations established in 2010, the groups of the Consortium have consolidated and expanded their interactions, from 37 to 61. In the last year, RNAreg PIs have published more than a hundred papers, 29 of which within the aims of the Consolider grant, including the first 5 collaborative publications. 56 communications to international meetings have been presented and 4 patents have been filed. One previous patent has been licensed to a spin-off biotech company. Technology Transfer activities are monitored by institutional TT Offices and certain projects also by the Fundación Marcelino Botín.

Key collaborations of particular added value for the Consortium where significant progress has been made include:

a) application of high-throughput experimental and computational technologies to identify changes in RNA species (mRNA isoforms, miRNAs, snoRNAs and lincRNAs) in models of tumor progression, including melanoma, glioma, leukemias and colon cancer, as well as Hepatitic C Virus infection. Analysis of the impact of epigenetic alterations induced by chemotherapeutic agents.
b) analysis of the function of RNA binding proteins in cancer progression and viral infection, including the role of CEPB, UNR, EWS and G3BP in pancreatic cancer, melanoma, Ewing sarcoma and HCV infection.
c) technology development and exchange for RNA structural probing of viral and cellular translational control as well as miRNA target predictions.
d) generation and characterization of RNA-based regulatory molecules with potential therapeutic applications, including PNA-peptides to modulate alternative splicing, U1 snRNP-based gene silencing, inhibitors of CPE-CPEB interactions and IRES-dependent translation; characterization of the mechanism of anti-tumor drugs targeting the spliceosome and development of dsRNA-based nanocomplexes for the treatment of melanoma and glioma.

Below are a list of publications which can be highlighted as this year's main outcomes of the project (RNAREG PIs are indicated in **bold**):

- E. Ortiz-Zapater, D. Pineda, N. Martínez-Bosch, G. Fernández-Miranda, M. Iglesias, F. Alameda, M. Moreno, C. Eliscovich, E. **Eyras**, F. X. Real, R. **Méndez**, and P. Navarro, “Key contribution of CPEB4-mediated translational control to cancer progression,” *Nat. Med.*, vol. 18, no. 1, pp. 83–90, Jan. 2012 [1]
- N. Fernández, O. Fernandez-Miragall, J. Ramajo, A. García-Sacristán, N. Bellora, E. **Eyras**, C. Briones, and E. **Martínez-Salas**, “Structural basis for the biological relevance of the invariant apical stem in IRES-mediated translation,” *Nucleic Acids Res.*, vol. 39, no. 19, pp. 8572–8585, Oct. 2011 [2]
- N. Bitarte, E. Bandres, V. Boni, R. Zarate, J. Rodriguez, M. Gonzalez-Huarriz, I. Lopez, J. Javier Sola, M. M. **Alonso**, P. **Fortes**, and J. **Garcia-Foncillas**, “MicroRNA-451 is involved in the self-renewal, tumorigenicity, and chemoresistance of colorectal cancer stem cells,” *Stem Cells*, vol. 29, no. 11, pp. 1661–1671, Nov. 2011[3]
- M. P. Paronetto, B. Miñana, and J. **Valcárcel**, “The Ewing sarcoma protein regulates DNA damage-induced alternative splicing,” *Mol. Cell*, vol. 43, no. 3, pp. 353–368, Aug. 2011[16]
- A. Corrionero, B. Miñana, and J. **Valcárcel**, “Reduced fidelity of branch point recognition and alternative splicing induced by the anti-tumor drug spliceostatin A,” *Genes Dev.*, vol. 25, no. 5, pp. 445–459, Mar. 2011[4]

- M. Mihailovich, L. Wurth, F. Zambelli, I. Abaza, C. Militti, F. M. Mancuso, G. Roma, G. Pavesi, and F. **Gebauer,** "Widespread generation of alternative UTRs contributes to sex-specific RNA binding by UNR," RNA, vol. 18, no. 1, pp. 53–64, Jan. 2012[5]
- Tavanez JP, Madl T, Kooshapur H, Sattler M, **Valcárcel** J. hnRNP A1 Proofreads 3' Splice Site Recognition by U2AF. Mol. Cell. 2012;45(3):314–329 [6]
- Sánchez A, **Pedroso** E, Grandas A. Maleimide-dimethylfuran exo adducts: effective maleimide protection in the synthesis of oligonucleotide conjugates. *Org. Lett.* 2011;13(16):4364–4367[7]
- Fernández N, García-Sacristán A, Ramajo J, Briones C, **Martínez-Salas** E. Structural analysis provides insights into the modular organization of picornavirus IRES. *Virology*. 2011;409(2):251–261[8]
- P. J. A. Eichhorn, L. Rodón, A. Gonzàlez-Juncà, A. Dirac, M. Gili, E. Martínez-Sáez, C. Aura, I. Barba, V. Peg, A. Prat, I. Cuartas, J. Jimenez, D. García-Dorado, J. Sahuquillo, R. Bernards, J. Baselga, and J. **Seoane**, "USP15 stabilizes TGF-[beta] receptor I and promotes oncogenesis through the activation of TGF-[beta] signaling in glioblastoma," *Nature Medicine*, Feb. 2012. [9]
- A. Koornneef, R. van Logtenstein, E. Timmermans, L. Pisas, B. Blits, X. Abad, P. **Fortes**, H. Petry, P. Konstantinova, and T. Ritsema, "AAV-mediated in vivo knockdown of luciferase using combinatorial RNAi and U1i," *Gene Ther*, vol. 18, no. 9, pp. 929–935, Sep. 2011
- Mackereth CD, Madl T, Bonnal S, et al. Multi-domain conformational selection underlies pre-mRNA splicing regulation by U2AF. *Nature*. 2011;475(7356):408–411. [10]
- Zarate R, Boni V, Bandres E, **Garcia-Foncillas** J. MiRNAs and LincRNAs: Could They Be Considered as Biomarkers in Colorectal Cancer? *Int J Mol Sci*. 2012;13(1):840–865. [11]

In addition three major international conferences have been organized or are in the process of being organized by RNAREG PIs during 2011, including:

- International Conference on Alternative Splicing. Granada Feb 28-March 3, 2011 Organized by Juan Valcárcel jointly with the European Alternative Splicing Network of Excellence (EURASNET), with close to 300 participants and 27 top invited scientists in the field of RNA biology. RNAREG groups contributed 18 communications, 3 of them selected for platform presentations.

-International Symposium: New Frontiers in Hematological Malignancies Pamplona Nov 16-18, 2011 Organized by Felipe Prosper (Associate RNAREG group), with participation of invited speakers from RNAREG, brought some of the best international leaders in the field of hematological oncology.

- International Symposium: RNA Biology in Cancer and Other Diseases Barcelona May 3-4, 2012 Organized by Mayka Sánchez (RNAREG YI) and Juan Valcárcel (RNAREG Coordinator) jointly with the IMPPC (Institute of Predictive and Personalized Cancer Medicine of Barcelona). The event will bring international leaders in the field of RNA-based pathologies and therapies.

- Cold Spring Harbor Laboratory Asia "RNA Biology" meeting - Suzhou (China) October 8-12, 2012
Organized by Fátima Gebauer (RNAREG PI), Narry Kim, Adrian Krainer and Mistuhito Ohno, featuring world leaders in RNA research and covering major topics in RNA processing and RNA-based therapies.

The General assembly will meet again this year during the International symposium: RNA Biology in Cancer and Other Diseases - Organised by Mayka Sánchez (RNAREG YI) and Juan Valcárcel in Barcelona on May 3-4, 2012, organized jointly with the IMPPC (Institute of

Predictive and Personalized Cancer Medicine of Barcelona). All RNAREG groups will participate in the Symposium, which will be followed by the Annual Meeting of the Consortium, with participation of some of the invited speakers of the Symposium as ad hoc advisors. The Consortium will take advantage of the presence of some of the best experts in the field to get advice on the project's strategic objectives and key collaborations.

2. Degree to which project objectives have been achieved, as measured by the indicators listed in Section 8 of the Implementation Agreement (4 pages)

1) Juan Valcárcel group

Progress has been made in understanding molecular mechanisms of splicing regulation, including the characterization of conformational changes important for splice site recognition and proofreading, mechanisms of sex-specific splicing and mechanisms relevant for genetic disease and cancer, including the characterization of splicing functions or the Ewing sarcoma protein and novel effects of anti-tumor drugs targeting splicing factors (see list of publications). In addition the following collaborations have been pursued:

1. with **Méndez** group: Characterization of a new function for CPEB1 in alternative processing of pre-mRNAs. **Manuscript submitted** in Nature: "A new function for the Cytoplasmic Polyadenylation Element Binding protein 1 in nuclear pre-mRNA processing and alternative 3' UTR formation." Alessio Bava, Carolina Eliscovich, Pedro G. Ferreira, Belén Miñana, Claudia Ben-Dov , Roderic Guigó, Juan Valcarcel and Raúl **Méndez**.
2. with **Eyras** group: mapping of CliP tags and combination with results from splicing-sensitive microarrays for the generation of regulatory maps for the splicing regulatory factors EWS, RBM5, RBM6 and RBM10.
3. with **Pedroso** group: generation of PNA-peptide libraries with diverse linkers, to be tested as reagents for modulation of Fas exon skipping.
4. with **Soengas** group: a) splicing microarray profiling of melanocytes vs melanoma cell lines; detected changes in expression/localization of splicing regulators Muscleblind and CUGBP, phenotypic effects of MBNL1 knock down on melanoma cells proliferation, additional effects under analysis. b) splicing microarray profiling of oncogene-driven senescence in melanocytes, comparison between different oncogenic mutations.
5. With **Seoane** group: splicing microarray analysis of CD44 high vs low glioblastoma cells, validation of splicing differences in the genes CROP, TGFBI and fibronectin, with potential relevance for the different tumorigenic capacity of these cell populations.
6. With **Roman** group: splicing microarray analysis of acute myeloblastic leukemia and acute lymphoblastic leukemia cell lines treated with decitabine (demethylating agent affecting WNT pathway and mir-124 and -9 expression). Validation of results pending.

2) Raúl Méndez group

The efforts of our group during 2011 have been directed towards three specific aims:
1. Mechanistic approaches into the function of the CPEB-family of protein in gene regulation, with an emphasis in genome-wide analysis. Collaboration with **Valcarcel group** described above.
2. Sequential functions of CPEB1 and CPEB4 in localized translation in mitotic chromosome segregation. In collaboration with **Eduardo Eyras group**, we have found that CPEB1 mediates localized translation in the mitotic spindles, which, in turn, is required for local CPEB4 synthesis and proper chromosome segregation. We have performed two genome wide identifications of the transcripts localized by CPEB1 to the spindles. CPEB depletion results in asymmetric chromosome segregation, a hallmark of tumoral cells.*CPEB1 function in*

cap modifications, we have identified, cloned and biochemically characterized a CPEB1 recruited cap-ribose methyltransferase required for the translational activation of, at least, some CPE-regulated transcripts. In collaboration with **Enrique Pedroso's** Group we have designed a set of 2'-O methylated and unmethylated cap sequences to test their contribution to the translational control of cytoplasmically polyadenylated mRNAs and the differential recruitment of translational initiation factors.

3. Functions of CPEBs in cancer, prognostic and therapeutical values.

In collaboration with **Eyras group** we have determined that CPEB4 is ectopically expressed in pancreatic tumors and gliomas, showing that CPEB4 is essential for tumoral growth and vascularization and performing a genome wide identification of CPEB4 targets in pancreatic tumors. This work has been **published in Nature medicine** [1] and resulted in a **patent**. As a direct continuation of this project we have further shown that CPEB4 regulates the translation of VEGF mRNA and is required for angiogenesis "in vivo" and in vitro. In collaboration with **Seoane group** we have found that CPEBs are differentially expressed in glioblastomas and we are analyzing the possibility of a CPEB-mediated translational control of TGFb. In collaboration with **Soengas group** we are studying the contribution of CPEB4 to malignancy, vascularization and metastasis in melanoma. Currently a PhD. Student from **Soengas**'s group (Eva Perez) is in our group performing RIP experiments for CPEB4 in melanoma cells. We have generated several tissue specific and conditional knock out mice models for CPEB1 and CPEB4 and we are studying the contributions of these two proteins to cell polarity, asymmetric cell divisions and stem cell activation in the context of tumor development (for CPEB4) or tumor protection (for CPEB1) and vascularization.

4. Development of CPEB-inhibitors with therapeutical potential.

We have developed a dual reporter cell based method to measure CPEB activity, in live cells, by live imaging and cell shorting. Using this system we will screen for CPEB-specific inhibitors. To design these compounds we will take advantage of the collaboration with **Macias group** and **Pedroso group** where we are studying the interaction between the RRMs and the RNA to design compounds that inhibit the interaction between the CPEs and the CPEBs.

3) Fatima Gebauer group

1. We have identified the RNA targets of *Drosophila* UNR in both male and female adult flies. We have found that UNR binds to mRNAs in a sex-specific fashion, largely due to sex-specific differences in alternative processing of the 5' and 3' UTRs of target transcripts.[6] Some these targets encode conserved regulators that, in mammals, are involved in tumor progression. We have subsequently found that mammalian UNR is over-expressed in melanoma (collaboration with **Soengas group**) and in ALL (collaboration with **Prosper group**). Furthermore, expression of UNR correlates with malignancy of colorectal tumors (collaboration with **García-Foncillas group**) and possibly of glioma (collaboration with **Seoane group**), and inversely correlates with HCV infection (collaboration with **Fortes group**). During this year, we have focused on melanoma to obtain and in-depth view of the possible role of UNR in tumor progression. Knock-down of UNR in melanoma cells dramatically decreases their ability to form colonies on agar and, conversely, over-expression of UNR in human primary fibroblasts confers invasive capacity and the ability to grow on agar. These results are striking, as normally a combination of oncogenes are required to transform fibroblasts. The results so far suggest that UNR is a potent oncogene, and that figuring out its targets and its modes of interaction has the potential to lead to novel therapeutic strategies.

2. We have also optimized RNA affinity chromatography methods to purify RNP complexes. We have applied these methods to the purification of *msl2*, *Toll* and *cyclin B1* RNPs in *Drosophila* and are currently testing the role of the identified proteins in translational control. Most of these proteins belong to conserved families with members involved in cancer progression.

4) Puri Fortes group

1. As described above, we have analyzed RNA regulatory proteins in hepatitis C virus (HCV) infected cells. Our results show that, out of several factors tested, both unr (collaboration with **Gebauer group**) and G3BP (collaboration with **Martinez-Salas group**) are posttranscriptionally down-regulated after HCV infection. Preliminary experiments suggest that overexpression of G3BP has a negative impact on the expression of HCV viral proteins.
2. We have also studied non-coding RNAs in different systems. We have identified a miRNA whose down-regulation is essential to maintain the tumorigenicity and the resistance to chemotherapy of colon cancer stem cells (collaboration with **García-Foncillas group**). Besides, we have analyzed the expression of small nucleolar RNA and host genes (collaboration with **Prosper group**). One of them, snora36C, and the snora host gene AAK are downregulated in all cell lines and patients of Acute Lymphoblastic Leukemia (ALL) tested. Downregulation does not result from epigenetic regulation and could reflect the decreased expression of some transcription factors in ALL cells. Besides, we have identified several long non-coding RNAs (lncRNAs) whose expression is altered in ALL (collaboration with **Prosper group**), upon HCV infection, interferon (IFN) treatment or both. Some lncRNAs upregulated in ALL have been described as oncogenic in other tumor cells. Surprisingly, other upregulated lncRNAs are antisense to described tumor suppressors. LncRNAs altered in HCV infection and/or IFN treatment have been identified by expression arrays and RNASeq. All lncRNA tested are induced after treatment with IFN alpha, beta or lambda, however, they show different kinetics. Some respond very fast while others are only activated at later times.
3. Finally, we have analyzed modified U1 snRNAs to target the expression of HBV or endogenous genes in a mouse model. The results show that some U1 snRNA-based inhibitors are efficient and specific in vivo. This system is being modified to address the efficacy of the technique to inhibit HBV expression in transgenic mice.

5) Encarna Martínez-Salas group

1. Structural and functional analysis of the IRES region: We have identified conserved motifs involved in tertiary interactions, in collaboration with **Eyras group** [2]
2. Analysis of the effect of overexpression or depletion of G3BP on viral IRES activity in cell-free systems and transfected cells, in collaboration with **Fortes group**. These results will be combined with the results obtained by P **Fortes** group in a **joint manuscript**
3. In collaboration with **Pedroso group**, we have determined the effect of the small RNA ligand AD2 on viral IRES activity in cell-free systems. The interaction site has been mapped by SHAPE footprinting. These results will be prepared in a **collaboration manuscript**
4. Demonstration of the specific proteolysis of Gemin5 in FMDV-infected cells. This work has allowed the identification of a novel motif to identify L protease targets in host factors.[23]
5. Functional characterization of Gemin5 as a novel IRES-transacting factor: mapping the protein region controlling translation repression and IRES-interaction (D. Piñeiro, N. Fernandez, J. Ramajo and E. Martinez Salas, **manuscript in preparation**).

6) José Román-Gómez group

1. With **Eyras group**: Upregulation of the reprogramming factor Lin28a in acute myeloid leukemia depends on genetic and epigenetic mechanisms involving the hsa-mir-9 family. Hypermethylation of the has-mir9 promoters and a common single-nucleotide polymorphism in the 3' UTR region of the Lin28a are associated with high levels of Lin28a. This upregulation has an adverse prognostic impact in leukemia patients**.**

2. With **Soengas group**: Pharmacological and genomic approach to reveal the possible existence of an aberrant epigenetic silencing pattern of large non-coding RNAs by treating leukemia cells with DNA-demethylating agents followed by hybridization to an expression microarray containing these sequences. We have detected 8 lincRNAs undergoing specific CpG island hypermethylation-associated silencing in leukemia cells compared with normal tissues. This methylation pattern has prognostic impact in leukaemia patients.

7) Marisol Soengas group

Collaborative activities between the CNIO Melanoma Group and members of this RNAREG Consolider have focused on the contribution of RNA binding factors and splicing modulators to melanoma initiation, progression and response to therapy. Specifically, main efforts were dedicated to the validation of candidate genes which we had been identified on customized splicing-sensitive arrays to be express in a differential manner in normal melanocytes and in aggressive melanoma cells. In addition, we made great progress in defining the contribution of polyadenylation and translation regulators on melanoma cell proliferation. Finally, from a translational perspective, we have demonstrated that dsRNA nanocomplexes have a broad spectrum of activity even against tumor stem cells. Highlights of our results are as follows:
1. As described above, in collaboration with the **Valcárcel group** we have found that CUGBP1 and MBNL1 (mRNA splicing and mRNA stability modulators) are expressed in an isoform-specific manner in melanoma cells, and are required to sustain cell proliferation. With this group we have also identified an unexpected tumor suppressive role of the RNA helicase DDX46, preventing malignant transformation by BRAF and NRAS oncogenes.
2. As mentioned above with **Mendez group**, we unveiled a strict requirement of the Cytoplasmic Polyadenylation Element Binding Protein 4 (CPEB4) for proper mitosis of melanoma cells. This function of CPEB4 was not shared by pancreatic tumor cells, suggesting tumor-type restricted functions of this protein in cancer.
3. Additional dependencies of melanoma cells on UNR are being described by **Gebauer group**. We are collaborating with her group to define the mechanism of action of this factor and to assess its contribution to melanoma growth and metastasis in vivo (mouse melanoma models).
4. In the context of anti-cancer treatment, we have demonstrated a potent killing activity of bioavailable nanocomplexes of dsRNA (named BO-110 for simplicity) in various tumor models, ranging from melanoma to cancers of the pancreas, bladder or glioblastoma, among others. Working with **Seoane group**, we found that stem-cell like tumor cells (from glioblastoma) are also highly sensitive to BO-110. These results are clinically relevant considering that the failure of classical therapies is in part related to the inability to induce cell death in subpopulations that repopulate tumor development.

8) Joan Seoane Group

We have followed our studies on glioma-initiating cells (GICs) or glioma stem cells isolated from tumor samples obtained from patients that undergo surgery in our Hospital.
1. We have continued the collaboration with **Valcárcel group** with the objective of analyzing the splicing variants found in GICs and non-GICs. We have obtained RNA from GICs and non-GICs from 3 different patients and we have analyzed the change in the levels of the splicing variants present in the two cell types using a microarray-based technology developed by Dr. Valcárcel's group. We have identified several events (differential spicing of b-catenin, axin, fibronectin, CD44, CROP and others) that we have validated through RT-qPCR and RT-PCR.
We have identified a splicing variant of b-catenin that is present in the GIC compartment of some patients. Interestingly, the splicing form of b-catenin skips an exon that codifies for a

protein region that contains phosphorylation sites for Src and Alk kinases. We are studying the functional relevance of this splicing variant of b-catenin.

2. In collaboration with **Méndez group** we are in the process of assessing the role of the CPEB family members in the context of our model of study. We have analyzed the level of CPEB expression in GICs and non-GICs. We have found that CPEB2 is overexpressed in GIC compartment whereas CPEB4 is overexpressed in the non-GIC compartment. In addition, we are evaluating whether the TGFb2 gene is regulated by CPEBs. We have already identified CPEB binding sites in the 3'UTR of the TGFb2 gene and we are in the process of functionally validating those sites.

3. In collaboration with **Gebauer group** we have analyzed the levels of UNR in glioma and GICs. No major differences have been observed so far.

4. In collaboration with **Fortes group** we have analyzed the differential expression of long non-coding RNAs in our GBM samples and we are in the process of analyzing and validating the results.

9) Jesús García-Foncillas group

1. Objective 1 and 2: miRNAs as molecular markers of progression in gastrointestinal cancer. MicroRNA as biomarkers of response to chemotherapy in gastrointestinal cancer.

Many antitumor therapies affect rapidly dividing cells. However, tumor proliferation may be driven by cancer stem cells (CSCs), which divide slowly and are relatively resistant to cytotoxic drugs. Thus, many tumors may progress because CSCs are not sensitive to the treatment. we have searched for target genes whose expression is involved in proliferation and chemoresistance of CSCs. Both of these processes could be controlled simultaneously by cell regulators such as microRNAs (miRNAs). Therefore, colonospheres with properties of CSCs were obtained from different colon carcinoma cells, and miRNA profiling was performed. The results showed that miR-451 was downregulated in colonspheres versus parental cells. Surprisingly, expression of miR-451 caused a decrease in self-renewal, tumorigenicity, and chemoresistance to irinotecan of colonspheres. We identified cyclooxygenase-2 (COX-2) as an indirect miR-451 target gene involved in sphere growth. Our results indicate that miR-451 downregulation allows the expression of the direct target gene macrophage migration inhibitory factor, involved in the expression of COX-2. In turn, COX-2 allows Wnt activation, which is essential for CSC growth. Furthermore, miR-451 restoration decreases expression of the ATP-binding cassette drug transporter ABCB1 and results in irinotecan sensitization. These findings correlate well with the lower expression of miR-451 observed in patients who did not respond to irinotecan-based first-line therapy compared with patients who did. Our data suggest that miR-451 is a novel candidate to circumvent recurrence and drug resistance in colorectal cancer and could be used as a marker to predict response to irinotecan in patients with colon carcinoma.

2. Collaboration with **Alonso's** group

We undertook this study to understand how the transcription factor Sox2 contributes to the malignant phenotype of glioblastoma multiforme (GBM), the most aggressive primary brain tumor. We initially looked for unbalanced genomic rearrangements in the Sox2 locus in 42 GBM samples and found that Sox2 was amplified in 11.5% and overexpressed in all the samples. These results prompted us to further investigate the mechanisms involved in Sox2 overexpression in GBM. We analyzed the methylation status of the Sox2 promoter because high CpG density promoters are associated with key developmental genes. The Sox2 promoter presented a CpG island that was hypomethylated in all the patient samples when compared to normal cell lines. Treatment of Sox2-negative glioma cell lines with 5-azacitidine resulted in the re-expression of Sox2 and in a change in the methylation status of the Sox2 promoter. We further confirmed these results by analyzing data from GBM cases generated by The Cancer Genome Atlas project. We observed Sox2 overexpression (86%; N = 414), Sox2

gene amplification (8.5%; N = 492), and Sox 2 promoter hypomethylation (100%; N = 258), suggesting the relevance of this factor in the malignant phenotype of GBMs. To further explore the role of Sox2, we performed in vitro analysis with brain tumor stem cells (BTSCs) and established glioma cell lines. Downmodulation of Sox2 in BTSCs resulted in the loss of their self-renewal properties. Surprisingly, ectopic expression of Sox2 in established glioma cells was not sufficient to support self-renewal, suggesting that additional factors are required. Furthermore, we observed that ectopic Sox2 expression was sufficient to induce invasion and migration of glioma cells, and knockdown experiments demonstrated that Sox2 was essential for maintaining these properties. Altogether, our data underscore the importance of a pleiotropic role of Sox2 and suggest that it could be used as a therapeutic target in GBM.

10) Eduardo Eyras group

1. The collaboration with **Mendez group** and Pilar Navarro group (IMIM, Barcelona) about the role of CPEB4 in cancer has been published.[1]
2. With E. **Martinez-Salas group** we concluded a collaboration on the RNA structural analysis of the IRES region. This has been published in NAR[2]
3. With the group of **Valcarcel,** we studied the function of EWS, whose altered regulation is related to tumorigenesis. We have analyzed the data from an experiment of CLIP-Seq (Cross-linking immunoprecipitation followed by deep-sequencing). We obtained new targets and have validated the predicted binding site for EWS. This work is currently under preparation for submission.

11) Enrique Pedroso group

1. We have synthesized ISIS-11 and a novel analog, dehydro-ISIS-11, in the context of the collaboration with **Martínez group**. ISIS-11 is an inhibitor of HCV replication that induces a structural change in the HCV IRES subdomain IIa. The hypothesis is that ISIS-11, and hopefully its dehydro analog, could inhibit FMDV FMDV IRES by interacting with a bulge region of its domain 3, which has some structural similarities with the HCV IRES.
2. With **Méndez group** we have designed a set of 2'-O methylated and unmethylated cap sequences to see if there is a differential binding of eIF4E to activate translation or eIF4Eb to repress translation. The synthesis of such products will soon be finished (beginning 2012) and the cap sequences will be tested in **Méndez** group.
3. In a collaborative project with **Méndez and Macías groups** we have performed the large-scale preparation of a 15-mer RNA sequence to study using NMR its interaction with a CPE-binding protein.
4. With **Valcárcel group** we have undertaken the preparation of a series of PNA-peptide conjugates to be employed as artificial splicing regulators by either promoting exon inclusion or exon exclusion. The conjugation is carried out by a Michael reaction between a cysteine-containing peptide and a maleimido-derivatized PNA. The first set of eight conjugates is addressed to Fas exon 6 and includes peptides having the terminal cysteine residue either at the C-terminal or at the N-terminal position, and PNAs with different polyethyleneglycol linkers between the maleimido unit and the PNA sequence.

12) María Macias group

1. The **C**ytoplasmic **P**olyadenylation **E**lement **B**inding (CPEB) proteins are RNA binding proteins, which amongst other functions regulate polyadenylation-induced translational control. To gain insight into the mechanisms of CPEB4 interaction with CPE site in the mRNAs, we expressed the **R**NA **R**ecognition **M**otifs (RRM) of CPEB4 single and as a tandem and in collaboration with the **Pedroso group** we have started to investigate the RNA recognition site. During 2011 we have obtained soluble samples of the CPEB4 RRM

domains including triple labeled samples. Interactions between the RRM1-RRM2 tandem of CPEB4 and the RNA template containing a CPE (consensus UUUUUAU) have been detected using HSQC – based titration experiments.
2. We have finished the work related with the interaction of Transcription Elongation Regulator 1 and its localization to the periphery of speckles. This work is the result of a collaboration with the group of Carlos Suñé (Instituto de Parasitología y Biomedicina "López Neyra" (IPBLN-CSIC)).The FF4 and FF5 domains of Transcription Elongation Regulator 1 (TCERG1) form a structural unit that directs proteins to the periphery of speckles. The FF4 and FF5 domains constitute a novel speckle periphery-targeting signal. This speckle periphery-targeting signal might participate in the coordination of transcription and splicing.

Young Investigators:

Huarte Group

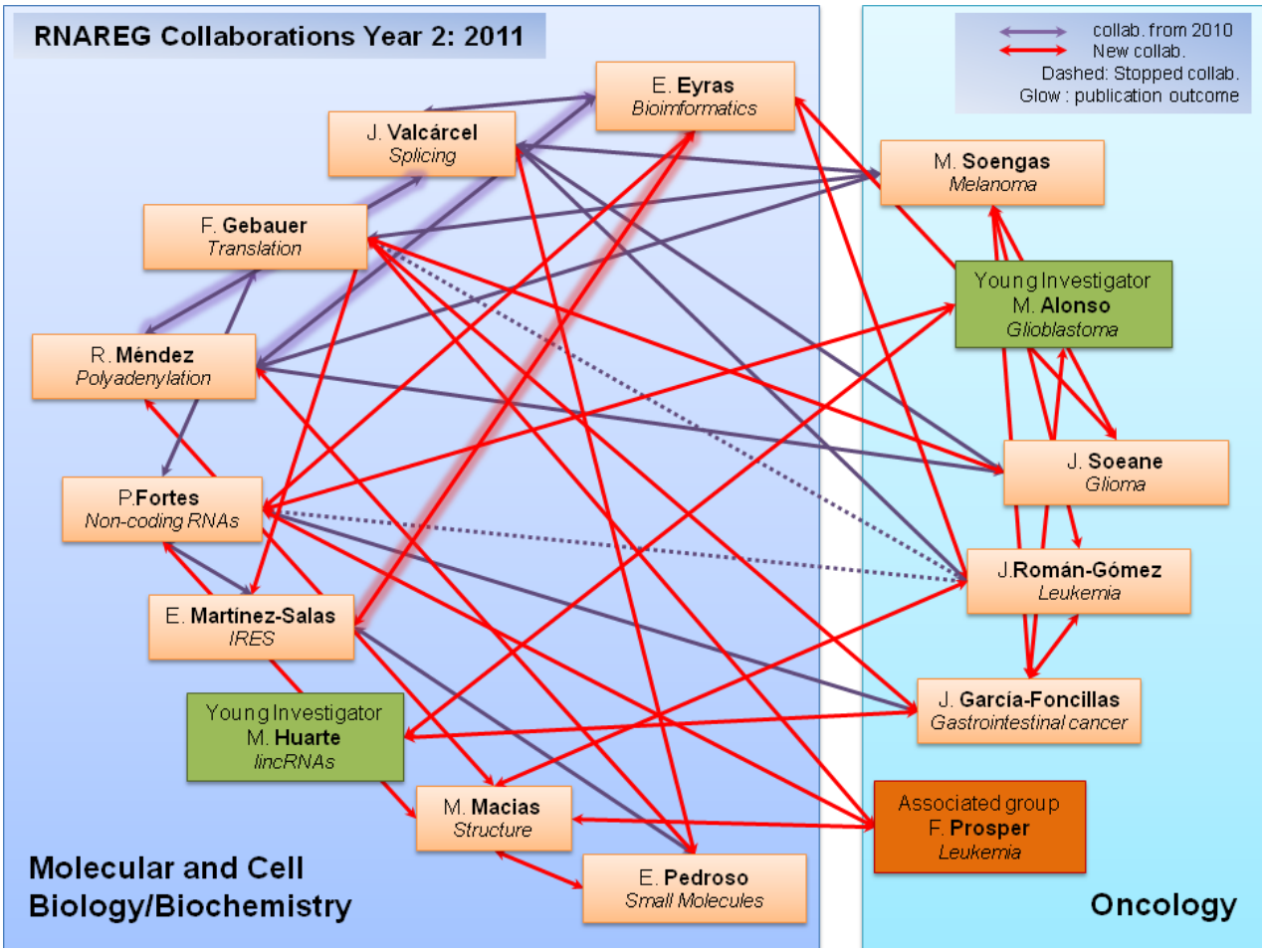
1. with **García-Foncillas group**: microarray analysis of lincRNA expression in colorectal cancer stem cells. We have found a cluster of lincRNAs that are specifically associated with the stem cell phenotype in colorectal cancer cell.
2. with **García-Foncillas group**: Analysis of lincRNA expression in colorectal cancer patient samples: Determination of the levels of specific lincRNAs involved in the p53 pathway by RT-PCR and global analysis of lincRNA expression by microarray . Characterization of the function of a lincRNA with a expression pattern as tumor suppressor.
3. with **Alonso group**: Analysis of LncRNAs in Gliomas regulated by Sox2. Our results indicate that Sox2 controls directly or indirectly several LncRNAs. We are currently validating these results and deciphering the functional implications of these observations.

Alonso group

1. with **Huarte group**: Analysis of LncRNAs in Gliomas regulated by Sox2. Our results indicate that Sox2 controls directly o indirectly several LncRNAs. We are currently validating these results and deciphering the functional implications of these observations. In addition we are evaluatin the role on linc-p21 in autophagy.
2. with **Fortes group**: We are working in the elucidation of the role of Dicer and/or specific miRNAs in the replication potential of adenovirus. We have evidence that Dicer attenuates adenoviral replication. We are knocking down Dicer and other molecules involved in miRNA processing (in different models) to evaluate the impact in the viral cycle.
3. with **García Foncillas** group: We have characterized exosomes isolated from serum of peripheral blood of glioblastoma patients and isolated miRNAs from these microvesicles. We have identified a profile of miRNAs that is able to distinguish healthy donors from patients.

Status of RNAREG collaborations.

During the two years of the grant, the 12 groups have initiated 37 collaborations, 24 of which in 2011, as illustrated by the following scheme:



This extensive network has been established through interactions between PIs/group members at the two Annual meetings of the Consortium and follow-up discussions by conference calls, web site intranet, email and participation in PhD thesis follow-up committees. As pointed out in their report by the members of our Scientific Advisory Board attending the II Annual Meeting “it was abundantly clear that the RNAREG consortium members had identified specific areas of complementation, cooperation, and synergy, and were working enthusiastically to accomplish these joint scientific goals”.

3. Description of project-related scientific and administrative management activities

The RNAreg Website, as well as the intranet has been maintained and regularly updated with information on new project publications, and relevant documentation to the project.
The Young Investigators selected in 2011 have been successfully added to the communication chain within the RNAreg project. The RNAreg mailing lists are used to enhance an efficient communication within the Consortium.

4. Description of budget expenditures, in relation to project objectives and the activities undertaken during the period covered by this report, including a distribution of partners' budgets

5. Brief description of the Research Activity Plan to be carried out between January 1, 2012 and December 31, 2013, as stated in Section 7 of the Implementation Agreement (2 pages)

1) Juan Valcárcel group

We will continue the characterization of targets and mechanisms of splicing regulation by RNA binding factors with oncogenic or tumor suppressor actvities, using a combination of high-throughput RNA binding and transcriptome profiling technologies, in collaboration with the **Eyras, Soengas and Seoane's groups**. The focus will be on the validation of the functional relevance of splicing changes observed accompanying tumor progression and the subsequent identification of key regulatory factors. We will continue the characterization of targets and molecular mechanisms of splicing regulation by CPEB proteins (in collaboration with **Méndez**). We will further develop and test the efficiency of PNA-peptide chimeras (in collaboration with **Pedroso**) to modulate exon skipping at will, a technology with significant potential applications for gene expression control. We will also compare the effects on splicing of a variety of antitumor drugs targeting splicing factors and compare the results with those of Spliceostatin A reported in 2011 by our group (Corrionero et al, 2011).

2) Raúl Méndez group

For the current year we will continue all the indicated ongoing projects. We will focus on the study of the "in vivo" models (i.e. CPEBs knock-outs), characterizing the tumor and angiogenesis related functions of CPEB1 and CPEB4 and completing the knockouts of CPEB2 and 3 (for which we are targeting ES cells). We will also continue our studies in the melanoma and glioblastoma models, studying the possible tumor protective function of CPEB1 and tumor inducer/facilitator of CPEB4. Genome-wide identification of mRNA targets for CPEB1 and CPEB4 and the competition between these two proteins as well as their function y metastasis and tumor relapse will be a priority. Finally, we hope to start the design and synthesis of small compounds to be tested in the indicated screening for CPEB-inhibitors.

3) Fatima Gebauer group

We plan to over-express a battery of UNR mutants in fibroblasts to identify which of its 5 RNA-binding domains is responsible for transformation. We will next identify the RNAs recognized by this domain in fibroblasts and melanocytes, as well as those recognized by full-length UNR in melanoma, using iCLIP. We will determine the transcriptome (RNA-Seq) and proteome (SILAC) changes of melanoma cells after UNR depletion, and will integrate the data to identify the targets regulated by UNR in this cancer system. In collaboration with Marisol **Soengas**, we will test the capacity of UNR-depleted melanoma cells to form tumors in mice, and will perform tissue-arrays to monitor the expression and subcellular location of UNR in tissues from patients. We will also progress in the basic analysis of UNR in ALL, glioma, colorectal cancer and HCV infection in order to extend our RNA network analysis to other tumor models.
We plan to analyze the role of the conserved regulators we have identified in translational control and try to dissect the molecular mechanisms involved.

4) Puri Fortes Group

We plan to overexpress and downregulate unr (collaboration with **Gebauer group**) and G3BP (collaboration with **Martinez-Salas group**) to evaluate their effect on HCV viability. We need to study in more detail the transcriptome of cells infected with HCV and/or treated with IFN obtained by RNASeq. Besides, we have just received the RNASeq results of healthy, cirrhotic and treated rat livers. The analysis of these transcriptomes should lead to the identification of

novel lncRNAs whose functionality could be relevant for the development or the treatment of these diseases. Thus, we plan to validate the expression of novel lncRNAs in ALL (collaboration with **Prosper group**), HCV infection, IFN treatment and liver cirrhosis. We will test the proliferation of ALL cells after overexpression of snora36C and AAK. Similarly, we will downregulate the expression of induced lncRNAs and analyze the effect of this downregulation in the proliferation of ALL cells, HCV viability or IFN response. Besides, we plan to perform bioinformatic analysis to obtain clues about the functionality of lncRNAs. We will built networks with our results from expression arrays and RNASeq and those obtained in glyoma (J**. Seoane, M. Alonso)** colon cancer stem cells (**J. García Foncillas, M. Huarte**) or released in public databases.
Finally, we will try to improve U1-based technology with novel molecular analysis and bioinformatic (collaboration with **Eyras group**) or structural (collaboration with **Macias group**) approaches. We would like to address the molecular mechanism of synergy between U1i and RNAi using molecular biology. We will perform specificity studies with U1 inhibitors of HBV expression in transgenic mice. These inhibitors will be expressed from gene therapy vectors to evaluate the functionality and toxicity of the system and its putative translation to the clinic.

5) Encarna Martínez-Salas group

In collaboration with **Pedroso group**, we will analyze the translation inhibitory capacity of two small molecules ISIS-11 and dehydro-ISIS-11, as well as changes in the RNA structure of viral IRES induced by these drugs.
The inhibitory capacity of a small molecules compounds library (nucleoside derivatives, heterocyclic compounds, peptides and peptoids, short PNAs containing unnatural nucleobases, and other compounds that are known to interact with nucleic acids) provided by E. **Pedroso** group potentially interacting with viral IRES.
Structural analysis by NMR of the C-terminal region of Gemin5 in complexes with domain 5 RNA (Collaboration with **Macias group**)
RNA SHAPE footprint of IRES-protein interactions. Role of RNA-binding protein G3BP in IRES-independent translation. Collaboration with P **Fortes** group.
Functional analysis of the effect RNA-binding proteins in cap-independent translation (Collaboration with **Fortes, and Soengas groups**)

6) Marisol Soengas group

1. In vivo studies of RNA regulators: Isogenic series of melanoma cell lines have been generated by transduction of specific shRNAs to deplete candidate genes (CUGBP1, MBNL1, CPEB4, UNR). These lines will be used to identify direct RNA targets of these factors (i.e. by pull downs and CLIP-Seq,). These assays will exploit the reagents and expertise of the groups of **Valcárcel, Gebauer** and **Méndez**. In parallel, shRNA transduced cells (and their controls) will be implanted in immunocompetent mice genetically modified to visualize tumor development and metastatic dissemination by non invasive methods. In particular, we will use a knock in system whereby a GFP-luciferase cassette has been inserted in the 3' UTR of the lymphatic modulator VEGRF3. These animal models are unique and will allow for the first comprehensive functional analysis of RNA regulators in physiologically relevant mouse tumor models.
2. Exploring novel melanoma-associated genes. In this context, ongoing collaborations include the analysis of Gemin (**Martínez-Salas**) and miRNAs (**García-Foncillas**). Support by **Eyras** and N. Sánchez-Bigas will help in an ambitious system analysis of global mechanisms of RNA processing, transcription and translation in melanoma progression.
3. Molecular mechanisms underlying dsRNA-based therapies. In collaboration with J. **Seoane**, we will define characterize the response to BO-110 of populations enriched in stem cell-like

cells. Gene-expression networks are also under analysis and will be further supported by the computational tools developed by N. Sánchez-Bigas. Altogether, we expect that next year will be highly successful in our efforts of identifying alternative strategies for treatment of otherwise highly chemoresistant melanoma tumors.

7) Joan Seoane group

We will pursue the functional analysis of the b-catenin splicing variant and its implications in cancer and cancer treatment.
We will also pursue the functional analysis of the splicing variant of fibronectin (there is a very dramatic change in the splicing of fibronectin when we compare GICs vs non-GICs).
We will follow the analysis of the differential expression fo CPEBs in GICs and assess the functional implication by knocking down CPEBs in GICs.
We will test whether the TGFb2 gene is regulated by CPEBs. The post-transcriptional regulation of the TGFb2 gene has not been described yet.

8) Jesús García-Foncillas group

The aims of our research for this year include to analyze the potential targets of microRNAs responsible of CSC phenotype and their role in the acquisition of a more aggressive behavior in the metastasis generation. In the same way, we will analyze the potential different miRNA profile shown by metastasis from primary colorectal cancer regarding the metastatic site (lung versus liver).

9) Eduardo Eyras group

In collaboration with the group of Juan Valcarcel, we have studied the family of RBM proteins, in particular, RBM5, RBM6 and RBM10, which are known to be involved in apoptosis, using CLIP-Seq, we have mapped the binding regions of these three proteins and have obtained their characteristic binding motifs. We plan to conclude this collaboration during 2012.
In collaboration with **Méndez's group**, we're studying the role of CPEBs in spindle formation. **Méndez**'s lab has produced RNA-Seq samples from whole cells and from isolated spindles from synchronized cells. We're calculating the genes that are differentially localized in Spindles. In particular, we will study the 3'UTR structure of these genes and their possible regulation by the CPEB family of proteins.
With P. **Fortes** group we will do a machine learning analysis of functional and non-functional mutant U1 snRNPs to extract the properties of their binding to RN molecules. Final predictions will be tested experimentally.

10) Enrique Pedroso group

In the context of the collaboration with **Martínez group**, we will undertake biophysical studies (UV and CD) to assess the interaction of ISIS-11 and dehydro-ISIS-11 with RNA sequences reproducing the secondary structure of a stem-loop and the bulge of domain 3 of FMDV IRES.
Additionally, in the search of small molecules that can interact and inhibit FMDV IRES we will provide **Martínez** group with a library of compounds. Such library will contain, among others: nucleoside derivatives, heterocyclic compounds, peptides and peptoids, short PNAs containing unnatural nucleobases, and other compounds that are known to interact with nucleic acids.
Using biophysical techniques we will try to unravel if there is a significant change in the structure of the cap sequences depending on base composition and degree of 2'-O methylation (collaboration with **Méndez group**). **Macias** group will use NMR to determine the nature of the interaction. The results obtained in Mendez group with the differently methylated

cap sequences in repressing or activating translation will most probably be followed by the synthesis of a new set of sequences.
To be tested in **Valcárcel group**, and bearing in mind the results obtained with the conjugates addressed to Fas exon 6, we will prepare various peptide-PNA conjugates initially aiming at modulating the alternative splicing of Tau exon 10 and Her-2 exon 19.

11) Maria Macias group

During the course of 2012 we will continue with the structural work on CPEB4 and 1. Using NMR we plan to obtain the information of the binding site in the RRM domains at an atomic detail and also start with the design of inhibitors.

6. National and international project visibility

The project RNAREG was mentioned in a Research News page of the Universitat Pompeu Fabra: http://www.upf.edu/enoticies-recerca/1112/1202.html

Meetings organized in relation to the Project

RNAREG members have been involved in the organization of 4 major international conferences that have featured RNAREG research:

1. **International Conference on Alternative Splicing -** Granada Feb 28-March 3, 2011
Organized by Juan Valcárcel (RNAREG Coordinator) jointly with the European Alternative Splicing Network of Excellence (EURASNET), with close to 300 participants and 27 top invited scientists in the field of RNA biology. RNAREG groups contributed 18 communications, 3 of them selected for platform presentations.
2. **International Symposium: New Frontiers in Hematological Malignancies -** Pamplona Nov 16-18, 2011.
Organized by Felipe Prosper (Associate RNAREG group), with participation of Juan Valcárcel as invited speaker, brought some of the best international leaders in the field of hematological oncology.
3. **International Symposium: RNA Biology in Cancer and Other Diseases -** Barcelona May 3-4, 2012
Organized by Mayka Sánchez (RNAREG YI) and Juan Valcárcel (RNAREG Coordinator) jointly with the IMPPC (Institute of Predictive and Personalized Cancer Medicine of Barcelona). The event will bring international leaders in the field of RNA-based pathologies and therapies. All RNAREG groups will participate in the Symposium, which will be followed by the Annual Meeting of the Consortium, with participation of some of the invited speakers of the Symposium as *ad hoc* advisors.

4. **Cold Spring Harbor Laboratory Asia "RNA Biology" meeting** - Suzhou (China) October 8-12, 2012
Organized by Fátima Gebauer (RNAREG PI), Narry Kim, Adrian Krainer and Mistuhito Ohno, featuring world leaders in RNA research and covering major topics in RNA processing and RNA-based therapies.

Award

Raúl Méndez group
Premio Carmen y Severo Ochoa de investigación en Biología Molecular, 2010 (2011)

7. Problems and suggestions

Raul Mendez group

In January of 2011 our group moved from the CRG (Barcelona) to the IRB (Barcelona). Among other things, this change of institutes meant that in November of 2010 our grants were frozen to be audited by the CRG before they could be transfer to the IRB. While most of our grants were readily transfer within the first two months of 2011 the funds provided by the Consolider did not arrive to the IRB until 2012. Thus, for the last 14 months, including the whole period corresponding to the present progress report, we did not have access to these funds. Although, as clearly shown in the present report, we have continued working in

the proposed project it has been at the expenses of additional funding sources and an extraordinary effort by our group and our hosting institution (the IRB). Doubtlessly this has affected the normal flow of the project and we would like to request a proportional extension of the duration of the funding period. An official request will be formally issued from the Consortium later on in the project.

José Román-Gómez group

In January 2012, the RNAREG Coordinator was informed by the Scientific Director of the IMIBIC (Instituto Maimónides de Investigación Biomédica de Córdoba) and by the Director of the Hospital Universitario Reina Sofía and president of FIBICO (Fundación para la Investigación Biomédica de Córdoba), about the voluntary resignation of the RNAREG PI Dr. Román-Gómez (dated December 15 2011) from his position as researcher of the IMIBIC. The Coordinator approached Dr. Román-Gómez, who informed him that he cannot conduct the research he was doing for the RNAREG Consolider until a legal appeal that he has filed against his institute is resolved in court next November. The Coordinator then contacted the Director General de Investigación, Dr. Juan M. Vázquez Rojas, who suggested to proceed replacing Dr. Román-Gómez (who will need to resign as an RNAREG PI) by a new PI. Dr. Román-Gómez has agreed to resign and at present we are in the process of identifying a replacement.

Date:

Coordinating Researcher Representative of the Managing Institution

SRA. SUBDIRECTORA GENERAL DE PROYECTOS DE INVESTIGACIÓN
Calle Albacete, 5, 28027 MADRID

<u>NOTE</u>: Please be advised that any change representing a modification of the grant concession conditions requires that the funding recipient submit an application stating a valid reason for the change. This application must be submitted before the project completion deadline has expired and requires the express approval of the designated administrative organ, likewise to be obtained before the project finalization deadline has expired.

These applications will be processed separately from the follow-up reports.

GASTOS REALIZADOS (EN LA ANUALIDAD Y ACUMULADO)

Nota: *Debe cumplimentarse este apartado independientemente de la justificación económica que se haya podido enviar por el organismo.*

1. Indique el gasto realizado (importes aproximados)

entidad		total gasto anualidad (€)			total gasto acumulado (€)		
denominación / centro	tipo (gestora /participante)	personal	otros g ejecución	total	personal	otros g ejecución	total
total							

(añádanse tantas filas como sean necesarias)

2. Comente brevemente si ha habido algún tipo de incidencia en este apartado, indicando si ha sido comunicada previamente y autorizada por esta Subdirección general.

MODIFICACIONES DE CONCEPTOS DE GASTOS DE EJECUCIÓN CON RESPECTO A LA SOLICITUD ORIGINAL

Descripción de gastos de ejecución no contemplados en la solicitud original, (si ha realizado algún gasto no contemplado en la solicitud original, justifique la necesidad en este apartado)

DIFUSION DE LOS RESULTADOS DEL PROYECTO

Relacione únicamente los resultados derivados de este proyecto

Publicaciones científico-técnicas (con peer-review) derivadas del proyecto				
	Referencia		Tipo de publicación	Autores
	Title	Journal Reference		
1	Key contribution of CPEB4-mediated translational control to cancer progression	Nat. Med., vol. 18, no. 1, pp. 83–90, Jan. 2012.	Artículo científico	E. Ortiz-Zapater, D. Pineda, N. Martínez-Bosch, G. Fernández-Miranda, M. Iglesias, F. Alameda, M. Moreno, C. Eliscovich, E. Eyras, F. X. Real, R. Méndez, and P. Navarro
2	Structural basis for the biological relevance of the invariant apical stem in IRES-mediated translation	Nucleic Acids Res., vol. 39, no. 19, pp. 8572–8585, Oct. 2011.	Artículo científico	N. Fernández, O. Fernandez-Miragall, J. Ramajo, A. García-Sacristán, N. Bellora, E. Eyras, C. Briones, and E. Martínez-Salas,
3	MicroRNA-451 Is Involved in the Self-renewal Tumorigenicity and Chemoresistance of Colorectal Cancer Stem Cells	STEM CELLS, vol. 29, no. 11, pp. 1661–1671, Nov. 2011.	Artículo científico	N. Bitarte, E. Bandres, V. Boni, R. Zarate, J. Rodriguez, M. Gonzalez-Huarriz, I. Lopez, J. Javier Sola, M. M. Alonso, P. Fortes, and J. Garcia-Foncillas,
4	The Ewing sarcoma protein regulates DNA damage-induced alternative splicing	Mol. Cell, vol. 43, no. 3, pp. 353–368, Aug. 2011.	Artículo científico	M. P. Paronetto, B. Miñana, and J. Valcárcel,
5	Reduced fidelity of branch point recognition and alternative splicing induced by the anti-tumor drug spliceostatin A	Genes Dev., vol. 25, no. 5, pp. 445–459, Mar. 2011.	Artículo científico	A. Corrionero, B. Miñana, and J. Valcárcel,
6	Widespread generation of alternative UTRs contributes to sex-specific RNA binding by UNR	RNA, vol. 18, no. 1, pp. 53–64, Jan. 2012.	Artículo científico	M. Mihailovich, L. Wurth, F. Zambelli, I. Abaza, C. Militti, F. M. Mancuso, G. Roma, G. Pavesi, and F. Gebauer,
7	hnRNP A1 Proofreads 3' Splice Site Recognition by U2AF	Mol. Cell, vol. 45, no. 3, pp. 314–329, Feb. 2012.	Artículo científico	J. P. Tavanez, T. Madl, H. Kooshapur, M. Sattler, and J. Valcárcel,
8	Maleimide-dimethylfuran exo adducts: effective maleimide protection in the synthesis of oligonucleotide conjugates	Org. Lett., vol. 13, no. 16, pp. 4364–4367, Aug. 2011.	Artículo científico	A. Sánchez, E. Pedroso, and A. Grandas,
9	Structural analysis provides insights into the modular organization of picornavirus IRES .	Virology, vol. 409, no. 2, pp. 251–261, Jan. 2011	Artículo científico	N. Fernández, A. García-Sacristán, J. Ramajo, C. Briones, and E. Martínez-Salas,

10	USP15 stabilizes TGF-[beta] receptor I and promotes oncogenesis through the activation of TGF-[beta] signaling in glioblastoma	Nature Medicine, Feb. 2012.	Artículo científico	P. J. A. Eichhorn, L. Rodón, A. Gonzàlez-Juncà, A. Dirac, M. Gili, E. Martínez-Sáez, C. Aura, I. Barba, V. Peg, A. Prat, I. Cuartas, J. Jimenez, D. García-Dorado, J. Sahuquillo, R. Bernards, J. Baselga, and J. Seoane,
11	AAV vectors transduce hepatocytes in vivo as efficiently in cirrhotic as in healthy rat livers	Gene Therapy, Aug. 2011.	Artículo científico	L. Sobrevals, M. Enguita, C. Rodriguez, J. Gonzalez-Rojas, P. Alzaguren, N. Razquin, J. Prieto, and P. Fortes,
12	Adenovirus and miRNAs	Biochim. Biophys. Acta, vol. 1809, no. 11–12, pp. 660–667, Dec. 2011.	Artículo científico	E. Carnero, J. D. Sutherland, and P. Fortes,
13	Conjugation reactions involving maleimides and phosphorothioate oligonucleotides	Bioconjug. Chem., vol. 23, no. 2, pp. 300–307, Feb. 2012.	Artículo científico	A. Sánchez, E. Pedroso, and A. Grandas,
14	Cytoplasmic polyadenylation and translational control	Curr. Opin. Genet. Dev., vol. 21, no. 4, pp. 452–457, Aug. 2011.	Artículo científico	A. Villalba, O. Coll, and F. Gebauer,
15	Distinct regulatory programs establish widespread sex-specific alternative splicing in Drosophila melanogaster	RNA, vol. 17, no. 3, pp. 453–468, Mar. 2011.	Artículo científico	B. Hartmann, R. Castelo, B. Miñana, E. Peden, M. Blanchette, D. C. Rio, R. Singh, and J. Valcárcel
16	Inside Back Cover: Fine-tuning the π–π Aromatic Interactions in Peptides: Somatostatin Analogues Containing Mesityl Alanine	Angewandte Chemie International Edition, vol. 51, no. 8, p. 1977–1977, Feb. 2012.	Artículo científico	P. Martín-Gago, M. Gomez-Caminals, R. Ramón, X. Verdaguer, P. Martin-Malpartida, E. Aragón, J. Fernández-Carneado, B. Ponsati, P. López-Ruiz, M. A. Cortes, B. Colás, M. J. Macias, and A. Riera,
17	Posttranscriptional control of X-chromosome dosage compensation	Wiley Interdiscip Rev RNA, vol. 2, no. 4, pp. 534–545, Aug. 2011.	Artículo científico	A. Graindorge, C. Militti, and F. Gebauer,
18	Strict 3′ splice site sequence requirements for U2 snRNP recruitment after U2AF binding underlie a genetic defect leading to autoimmune disease	RNA, vol. 17, no. 3, pp. 401–411, Mar. 2011.	Artículo científico	A. Corrionero, V. A. Raker, J. M. Izquierdo, and J. Valcárcel
19	The gluttonous side of malignant melanoma: basic and clinical implications of macroautophagy	Pigment Cell & Melanoma Research, vol. 24, no. 6, pp. 1116–1132, Dec. 2011.	Artículo científico	A. Checinska and M. S. Soengas,

Difusión de resultados							
	Referencia				Tipo	Autores	Grupo
	Title/Description	Meeting	Place	Date			
1	A new function for CPEB1: nuclear processing of pre-mRNAs	Second International Conference on Alternative Splicing	Granada	Mar-11	Platform presentation	Bava FA, Eliscovich C, Ferreira PG, Ben-Dov C, Guigó R, Valcárcel, J and Méndez R	Valcárcel, Méndez
2	hnRNP A1 proofreads 3' splice site recognition by U2AF	Second International Conference on Alternative Splicing	Granada	Mar-11	Platform presentation	Tavanez, J. and Valcárcel, J	Valcárcel
3	Chromatin epigenetics and alternative splicing	Second International Conference on Alternative Splicing	Granada	Mar-11	Platform presentation	Schor IE, Alló M, Fiszbein A, Bertucci P, Buggiano V, Gómez Acuña L, Lières D, Agirre E, Lamond A, Eyras E, Valcárcel J and Kornblihtt AR	Valcárcel
4	Deciphering genetic networks of splicing regulation in vivo	Second International Conference on Alternative Splicing	Granada	Mar-11	Poster presentation	Barberán-Soler S, Lehner B and Valcárcel, J	Valcárcel
5	CLIP-Seq of RBM5, 6 and 10 reveals cooperative/competitive roles in alternative splicing regulation of cell proloferation and apoptosis related genes	Second International Conference on Alternative Splicing	Granada	Mar-11	Poster presentation	Bechara EG, Bernardis I, Eyras E and Valcárcel J	Valcárcel, Eyras
6	Structure-function analysis of OCRE, a novel protein fold involved in alternative splicing regulation	Second International Conference on Alternative Splicing	Granada	Mar-11	Poster presentation	Bonnal S, Miñana B, Mourao A, Sattler M and Valcárcel J	Valcárcel
7	Reduced fidelity of branch point recognition and alternative splicing induced by the anti-tumor drug Spliceostatin A.	Second International Conference on Alternative Splicing	Granada	Mar-11	Poster presentation	Corrionero A, Miñana B and Valcárcel J	Valcárcel
8	Exploring the role of nucleosome positioning in alternative splicing	Second International Conference on Alternative Splicing	Granada	Mar-11	Poster presentation	Iannone C, Gaveglia L, Castellano G, Miñana B, Beato M and Valcárcel J	Valcárcel
9	Exploring the role of nucleosome positioning in alternative splicing	Cold Spring Harbor Laboratory Eukaryotic RNA Processing meeting	Cold Spring Harbor	Aug-11	Poster presentation	Iannone C, Gaveglia L, Castellano G, Miñana B, Beato M and Valcárcel J	Valcárcel
10	A computational framework for integrated analysis of high-throughput chromatin and splicing data	Second International Conference on Alternative Splicing	Granada	Mar-11	Poster presentation	Papasaikas P and Valcárcel J	Valcárcel

11	The Ewing Sarcoma protein (EWS) regulates DNA damage-induced alternative splicing.	Second International Conference on Alternative Splicing	Granada	Mar-11	Poster presentation	Paronetto MP, Miñana B, Bernardis I, Eyras E and Valcárcel J	Valcárcel
12	A single nucleotide polymorphism in the orl1 gene that is a risk factor in coronary disease regulates alternative splicing	Second International Conference on Alternative Splicing	Granada	Mar-11	Poster presentation	Tajedor JR and Valcárcel J	Valcárcel
13	A single nucleotide polymorphism in the orl1 gene that is a risk factor in coronary disease regulates alternative splicing	Cold Spring Harbor Laboratory Eukaryotic RNA Processing meeting	Cold Spring Harbor	Aug-11	Poster presentation	Tajedor JR and Valcárcel J	Valcárcel
14	High-throughput genetic screen for regulators of Fas alternative splicing	Second International Conference on Alternative Splicing	Granada	Mar-11	Poster presentation	Valcárcel J	Valcárcel
15	Bioinformatic analysis of ENCODE data suggests a weak but widespread influence of chromatin organization on alternative splicing	Second International Conference on Alternative Splicing	Granada	Mar-11	Poster presentation	Tilgner H, Iannone C, Curado J, González, D, Djebali D, Merkel A, Valcárcel J and Guigó R	Valcárcel
16	CLIP-Seq of RBM5, 6 and 10 reveals cooperative/competitive roles in alternative splicing regulation of cell proloferation and apoptosis related genes	16th Annual Meeting of the RNA Society	Kyoto	Jun-11	Platform presentation	Bechara EG, Bernardis I, Eyras E and Valcárcel J	Valcárcel
17	CLIP-Seq of RBM5, 6 and 10 reveals cooperative/competitive roles in alternative splicing regulation of cell proloferation and apoptosis related genes	Cold Spring Harbor Laboratory Eukaryotic RNA Processing meeting	Cold Spring Harbor	Aug-11	Poster presentation	Bechara EG, Bernardis I, Eyras E and Valcárcel J	Valcárcel
18	Multi-domain conformational selection underlies pre-mRNA splicing regulation by U2AF	Cold Spring Harbor Laboratory Eukaryotic RNA Processing meeting	Cold Spring Harbor	Aug-11	Platform presentation	Mackereth C, Madl T, Bonnal S, Simon B, Zanier K, Gasch A, Rybin V, Valcárcel J and Sattler M.	Valcárcel
19	Domains requirement for the function of RBM5, RBM6 and RBM10 in alternative splicing regulation.	Cold Spring Harbor Laboratory Eukaryotic RNA Processing meeting	Cold Spring Harbor	Aug-11	Platform presentation	Bonnal S, Mourao A, Sattler M and Valcárcel J	Valcárcel
20	Project acknowledged	16th Annual Meeting of the RNA Society	Kyoto	Jun-11	Poster presentation		Gebauer
21	Project acknowledged	EMBO Conference on Protein Synthesis and Translational Control	Heidelberg	Sep-11	Poster presentation		Gebauer
22	Project acknowledged	Institut fur Molekularbiologie und Biophysik ETH	Zurich	Jun-11			Mendez
23	Project acknowledged	The 3rd EMBO Meeting	Vienna	Sep-11	Session chair		Mendez
24	Project acknowledged	“Severo Ochoa conference” at the Sociedad de	Valdivia	Sep-11			Mendez

		Bioquímica y Biología Molecular de Chile meeting					
25	La expresión de IGF-1 a partir de vectores basados en virus adenoasociados reduce el estrés oxidativo y produce una reversión total de la fibrosis en ratas cirróticas	Asociación española para el estudio del hígado (AEEH)	Madrid	Feb-11	Talk	L. Sobrevals, M. Sanchez Aragó, M. Enguita, C. Rodríguez, S.J. González-Rojas, N. Razquin,J.M. Cuezva, J. Prieto and P. Fortes	Fortes
26	Increased in vivo inhibition of HBV expression by combining RNA interference and U1 inhibition	Keystone Symposia: Mechanism and Biology of silencing	Monterey	Mar-11	Poster presentation	S.J. Gonzalez-Rojas, L. Blazquez, A. Abad, N. Razquin, X. Abad and P. Fortes	Fortes
27	IGF-I expresión from AAV vectors reduces oxidative stress and leads to total fibrosis reversion in cirrhotic rat livers	The International Liver Congress™ 2011 by EASL (46th annual meeting)	Berlin	Mar-11	Poster presentation	L. Sobrevals, M. Sanchez Aragó, M. Enguita, C. Rodríguez, S.J. González-Rojas, N. Razquin,J.M. Cuezva, J. Prieto and P. Fortes	Fortes
28	Analisis of the inhibition of HCV replication with different RNAi mediators	HCV Meeting	Seattle	Nov-11	Poster presentation	S.J Gonzalez-Rojas, A Ely, M García-Valdecasas, I Fernandez-Perez, E. Carnero, P. Arbuthnot and P. Fortes	Fortes
29	Increased in vivo inhibition of expression by combining RNA interference and U1 inhibition	XI Congreso Nacional de Virología	Granada	May-11	Talk	S.J. Gonzalez-Rojas, L. Blazquez, A. Abad, N. Razquin, X. Abad and P. Fortes	Fortes
30	Functional characterization of Gemin5 as a novel IRES-transacting factor	EMBO Conference on Protein Synthesis and Translational Control, EMBL	Heidelberg	Sep-11	Poster presentation	D Piñeiro, J Ramajo and E. Martinez-Salas	Martinez Salas
31	Caracterización estructural de dominios funcionales de RNAs genómicos virales mediante microarrays de DNA	XI Congreso de la Sociedad Española de Virología	Granada	May-11	Talk	Garcia-Sacristán, A, Fernández, N, Diaz-Gonzalez R, Fernandez-Algar, M., Gomez, J, Martínez-Salas, E. and Briones, C	Martinez Salas
32	Estudio funcional y estructural del tallo apical del IRES de FMDV	XI Congreso de la Sociedad Española de Virología	Granada	May-11	Talk	Fernández, N, Fernandez-Miragall, O, Garcia-Sacristán, A, Ramajo, J, Briones, C. and Martínez-Salas, E	Martinez Salas
33	Gemin5 es una nueva diana de la proteasa Lb de FMDV	XI Congreso de la Sociedad Española de Virología	Granada	May-11	Talk	Piñeiro, D, Ramajo J, and Martínez-Salas, E	Martinez Salas
34	Picornavirus IRES accessibility to 2´O methyl antisense oligonucleotides: implications in the inhibition of viral gene expression	XI Congreso de la Sociedad Española de Virología	Granada	May-11	Talk	Fajardo T, Rosas MF, Sobrino, F and Martinez Salas, E	Martinez Salas

35	Structure, Dynamics and function of biomacromolecules by solution NMR	EMBO Practical Course at the Bavarian NMR Center at the TU Munich	Garching	Jul-11			Macias
36	Project acknowledged	CCPN Europa 2011 ITQB	Qeiras	Sep-11			Macias
37	Project acknowledged	BioMed Conference Macromolecular Dynamics	Barcelona	Oct-11			Macias
38	Project acknowledged	2nd IRB Barcelona PhD Student Symposium: Life in Motion: Dynamics of Molecules&Systems	Barcelona	Nov-11			Macias
39	Autophagy, senescence and innate immunity in melanoma therapy	Cellular Therapy Symposium	Erlangen	Mar-11	Talk	Alonso-Curbelo D., Checinska A, Calvo T.G., Cañon E, Tormo D and Soengas MS	Soengas
40	Innate sensors of dsRNA in anticancer therapy	Immunity and Cancer Symposium	Istambul	May-11	Talk	Alonso-Curbelo D., Checinska A, Calvo T.G., Cañon E, Tormo D and Soengas MS	Soengas
41	Autophagy in Meanoma Progression and Drug Response	International Symposium in Translational Oncology	Barcelona	May-11	Talk	Soengas MS	Soengas
42	Avances en la biología y el tratamiento del melanoma. Jornadas divulgativas	Jornadas divulgativas. Asociación Española Contra el Cáncer	Tarragona	Jun-11	Talk	Soengas MS	Soengas
43	New animal models for the analysis and visualization of lymphangiogenesis and metastasis of malignant melanoma	International Melanoma Workshop	Milan	Jun-11	Talk	Soengas MS	Soengas
44	Regulation and therapeutic impact of endo/lysosomal vesicle trafficking in malignant melanoma	XXVI Annual Meeting of Brazilian Federation of Societies of Experimental Biology (FesBe)	Rio de Janeiro	Aug-11	Talk	Alonso-Curbelo D., Riveiro-Falkenbach E, Olmeda D.., Calvo T., Perez-Guijarro E, Canon E., and Soengas MS	Soengas
45	New animal models for the analysis and visualization of lymphangiogenesis and metastasis of malignant melanoma	Signal Rewiring and Addiction in Cancer	Barcelona	Sep-11	Talk	7. Olmeda D., Alonso-Curbelo D. Perez-Guijarro E., Calvo T., Ortega S and Soengas MS	Soengas
46	RAB-dependent endo/lysosomal vesicle trafficking in melanoma progression	XXIst International Pigment Cell Conference	Bordeaux	Sep-11	Talk	8. Alonso-Curbelo D., Olmeda D., Perez-Guijarro E., and Soengas MS	Soengas
47	New animal models for the analysis and visualization of lymphangiogenesis and metastasis of malignant melanoma	Graduate Student Seminar Series	Wurzburg	Oct-11	Talk	Soengas MS	Soengas

48	Endo/Lysosomal vesicle trafficking as a new rheostat in melanoma progression	International Melanoma Congress	Tampa	Nov-11	Talk	10. Alonso-Curbelo D., Riveiro-Falkenbach E, Perez-Guijarro E., Ortiz-Romero JL., Rodriguez-Peralto. JL, and Soengas MS	Soengas
49	Understanding and Treating Malignant Melanoma	CABIMER Seminar Series	Sevilla	Dec-11	Talk	Soengas MS	Soengas
50	Design and validation of a prognostic index score for pediatric acute lymphoblastic leukemia based on clinical and epigenetic data	16th Congress of the European Hematology Association	London	Jun-11	Talk		Roman Gomez
51	Diseño y validación de un nuevo índice de puntuación pronóstica para pacientes diagnosticados de leucemia aguda linfoblástica en edad pediátrica basado en datos clínicos y epigenéticos.	XXXI Reunión de la AAHH	Córdoba	May-11	Talk		Roman Gomez
52	La desregulación epigenética de la familia de microRNAs hsa-mir-9 afecta la expresión de múltiples oncogenes y genes supresores y tiene impacto pronóstico en la leucemia aguda mieloblástica.	XXXI Reunión de la AAHH. Córdoba, 13-14 Mayo 2011.	Córdoba	May-11	Talk		Roman Gomez
53	Diseño y validación de un nuevo índice de puntuación pronóstica para pacientes diagnosticados de leucemia aguda linfoblástica en edad pediátrica basado en datos clínicos y epigenéticos.	II Reunión IMIBIC Jóvenes Investigadores	Córdoba	May-11	Talk		Roman Gomez
54	La desregulación epigenética de la familia de microRNAs hsa-mir-9 afecta la expresión de múltiples oncogenes y genes supresores y tiene impacto pronóstico en la leucemia aguda mieloblástica.	LIII Reunión de la SEHH	Zaragoza	Oct-11	Talk		Roman Gomez
55	Diseño y validación de un nuevo índice de puntuación pronóstica para pacientes diagnosticados de leucemia aguda linfoblástica en edad pediátrica basado en datos clínicos y epigenéticos.	LIII Reunión de la SEHH	Zaragoza	Oct-11	Talk		Roman Gomez
56		Strategy Discussion Forum of TIDES 2011 meeting	Boston	May-11	Invited speaker	A. Granadas	Pedroso
57	Maleimido-oligonucleotides: solid-phase synthesis and applications	6th Cambridge Symposium on Nucleic Acids Chemistry and Biology	Cambridge	Sep-11	Poster presentation	A.Sánchez, E.Pedroso, A.Grandas	Pedroso

Otras publicaciones derivadas de colaboraciones mantenidas durante la ejecución del proyecto y que pudieran ser relevantes para el mismo

	Referencia		Tipo de publicación	Autores
	Title	**Journal Reference**		
1	AAV-mediated in vivo knockdown of luciferase using combinatorial RNAi and U1i	Gene Ther, vol. 18, no. 9, pp. 929–935, Sep. 2011.	Artículo científico	A. Koornneef, R. van Logtenstein, E. Timmermans, L. Pisas, B. Blits, X. Abad, P. Fortes, H. Petry, P. Konstantinova, and T. Ritsema
2	Artificial skin in perspective: concepts and applications	Pigment Cell & Melanoma Research, vol. 24, no. 1, pp. 35–50, Feb. 2011.	Artículo científico	C. A. Brohem, L. B. da Silva Cardeal, M. Tiago, M. S. Soengas, S. B. de Moraes Barros, and S. S. Maria-Engler
3	E2F1-dependent oncogenic addiction of melanoma cells to MDM2	Oncogene, vol. 31, no. 7, pp. 828–841, Jul. 2011.	Artículo científico	M. Verhaegen, A. Checinska, M. B. Riblett, S. Wang, and M. S. Soengas
4	Gemin5 proteolysis reveals a novel motif to identify L protease targets	Nucleic Acids Research, Feb. 2012.	Artículo científico	D. Piñeiro, J. Ramajo, S. S. Bradrick, and E. Martínez-Salas
5	Genetic and Epigenetic Modifications of Sox2 Contribute to the Invasive Phenotype of Malignant Gliomas	PLoS One, vol. 6, no. 11, Nov. 2011.	Artículo científico	M. M. Alonso, R. Diez-Valle, L. Manterola, A. Rubio, D. Liu, N. Cortes-Santiago, L. Urquiza, P. Jauregi, A. L. de Munain, N. Sampron, A. Aramburu, S. Tejada-Solís, C. Vicente, M. D. Odero, E. Bandrés, J. García-Foncillas, M. A. Idoate, F. F. Lang, J. Fueyo, and C. Gomez-Manzano
6	Increased in vivo inhibition of gene expression by combining RNA interference and U1 inhibition	Nucleic Acids Res., vol. 40, no. 1, p. e8, Jan. 2012.	Artículo científico	L. Blazquez, S. J. Gonzalez-Rojas, A. Abad, N. Razquin, X. Abad, and P. Fortes
7	MiRNAs and LincRNAs: Could They Be Considered as Biomarkers in Colorectal Cancer?	Int J Mol Sci, vol. 13, no. 1, pp. 840–865, Jan. 2012.	Artículo científico	R. Zarate, V. Boni, E. Bandres, and J. Garcia-Foncillas
8	Multi-domain conformational selection underlies pre-mRNA splicing regulation by U2AF	Nature, vol. 475, no. 7356, pp. 408–411, Jul. 2011.	Artículo científico	C. D. Mackereth, T. Madl, S. Bonnal, B. Simon, K. Zanier, A. Gasch, V. Rybin, J. Valcárcel, and M. Sattler
9	PP2A-B56|[alpha]| controls oncogene-induced senescence in normal and tumor human melanocytic cells	Oncogene, Aug. 2011.	Artículo científico	S. Mannava, A. R. Omilian, J. A. Wawrzyniak, E. E. Fink, D. Zhuang, J. C. Miecznikowski, J. R. Marshall, M. S. Soengas, R. C. Sears, C. D. Morrison, and M. A. Nikiforov
10	Targeted activation of innate immunity for therapeutic induction of autophagy and apoptosis in melanoma cells	Cancer Cell, vol. 16, no. 2, pp. 103–114, Aug. 2009.	Artículo científico	D. Tormo, A. Chęcińska, D. Alonso-Curbelo, E. Pérez-Guijarro, E. Cañón, E. Riveiro-Falkenbach, T. G. Calvo, L. Larribere, D. Megías, F. Mulero, M. A. Piris, R. Dash, P. M. Barral, J. L. Rodríguez-Peralto, P. Ortiz-Romero, T. Tüting, P. B. Fisher, and M. S. Soengas

PATENTES							
	Título	Nacional o internacional (países)	Fecha presentación	*Inventores*	*Grupo*	*Application number*	*Comment*
1	Método para el diagnóstico o pronóstico del cáncer de pancreas	Nacional - ES	20-05-11	Elena Ortiz-Zapater , Francisco X. Real, Pilar Navarro and Raúl Méndez.	*Raúl Méndez*	*P 201130819*	
2	Método in Vitro de pronóstico de la supervivencia de enfermos adultos con leucemia linfoblástica aguda.	Nacional - ES	27-12-10	I Roman J, Martín V, Torres A	*Jose Roman Gomez*	*P201031955*	
3	Método in Vitro de pronóstico de la supervivencia de enfermos pediatricos con leucemia linfoblástica aguda.	Nacional - ES	27-12-10	Roman J, Martín V, Torres A	*Jose Roman Gomez*	*P201031957*	
4	Compuestos maleimido-furanilo útiles en un procedimiento general de preparación de derivados maleimido-oligonucleótido	Nacional - ES	27-08-10	A.Grandas, A.Sánchez, E.Pedroso	*Pedroso*	*PCT/ES 2011/070311*	*Owner: Universitat de Barcelona - Licensed by the University of Barcelona to Glen Research Corporation, VA, USA*
5	Process for the identification of compounds for treating cancer	Internacional - AL, AT, BE, BG, CH,CY, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LI, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR.	04-07-09	Tormo D, Soengas MS	*Soengas*	*EP20100595993*	*PRIORITY: ES/04.07.09/ESA 200939417 Licensed to Bioncotech Therapeutics. Titular: Centro Nacional de Investigaciones Oncológicas CNIO*

(*) En cualquier caso, se deberán reflejar todos los indicadores comprometidos