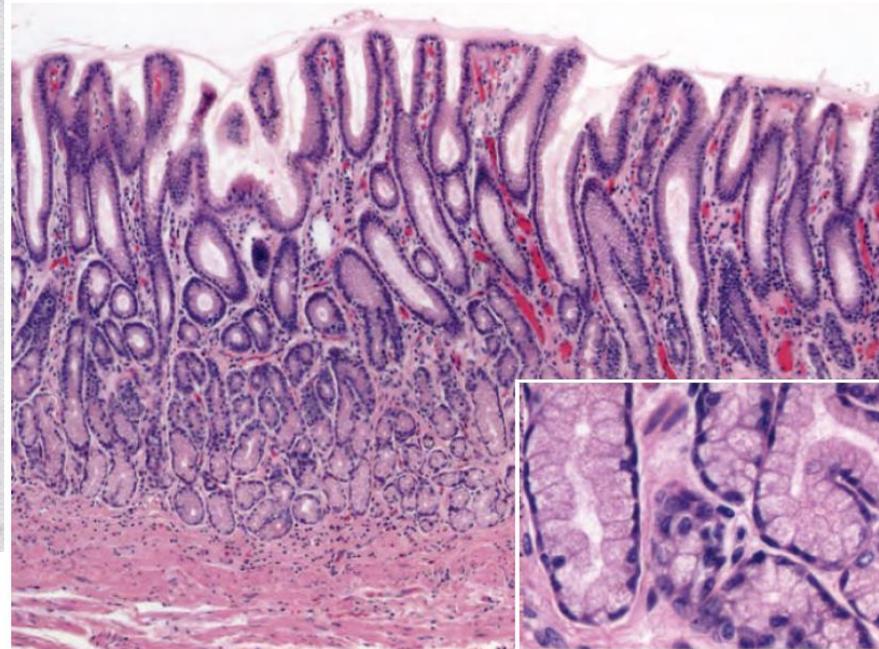
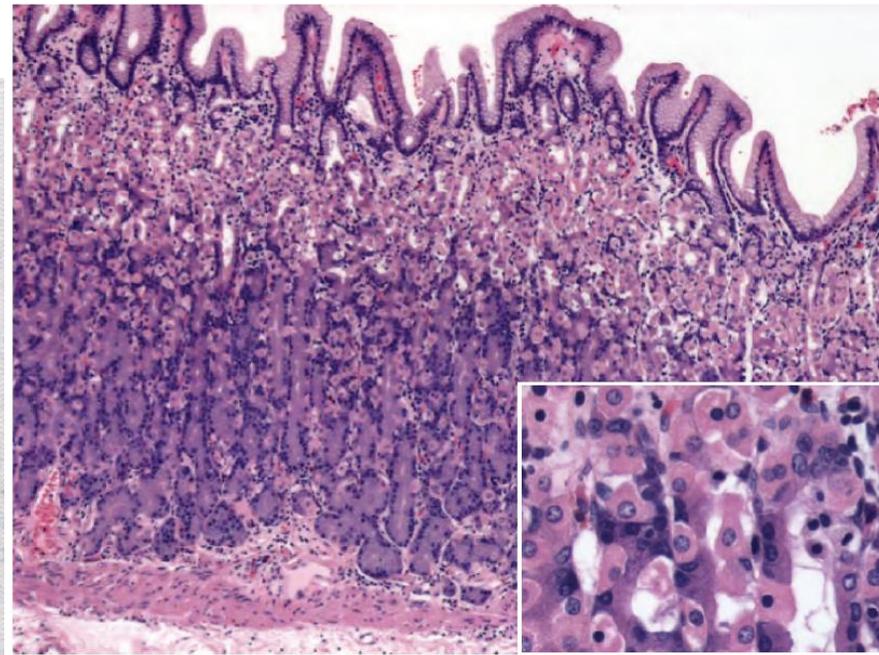
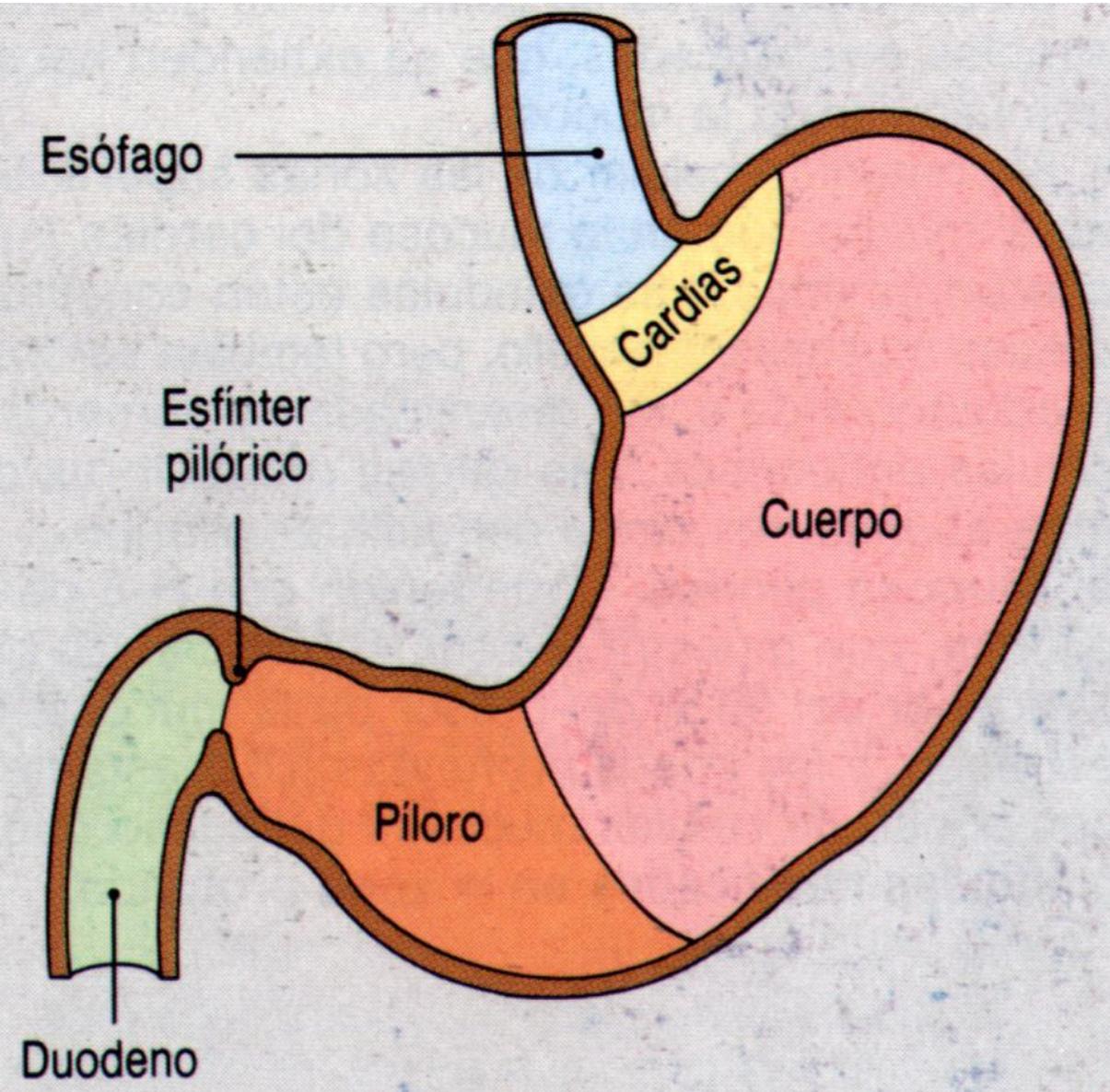


An anatomical illustration of the human torso, showing the internal organs. The pancreas is highlighted in yellow and orange, and a red, irregular mass representing a tumor is visible on its surface. The background is a blue gradient.

**Dr. Alejandro Corvalán**  
Departamento Hematología y Oncología  
Advanced Center for Chronic Diseases (ACCDIS)  
Facultad de Medicina,  
Pontificia Universidad Católica de Chile

Viernes 28 Agosto 2020  
Instituto Nacional del Cáncer

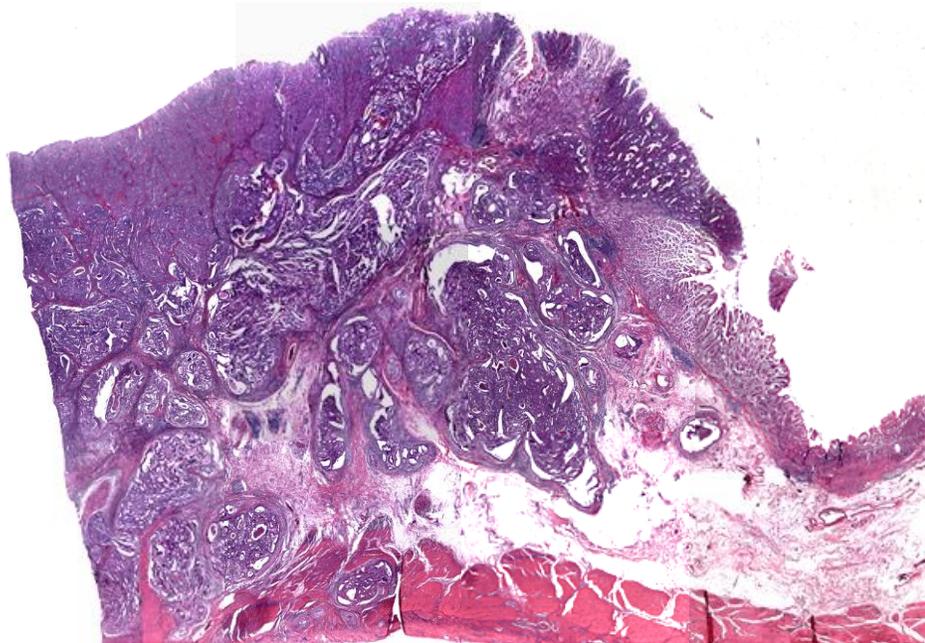
## Normal Stomach



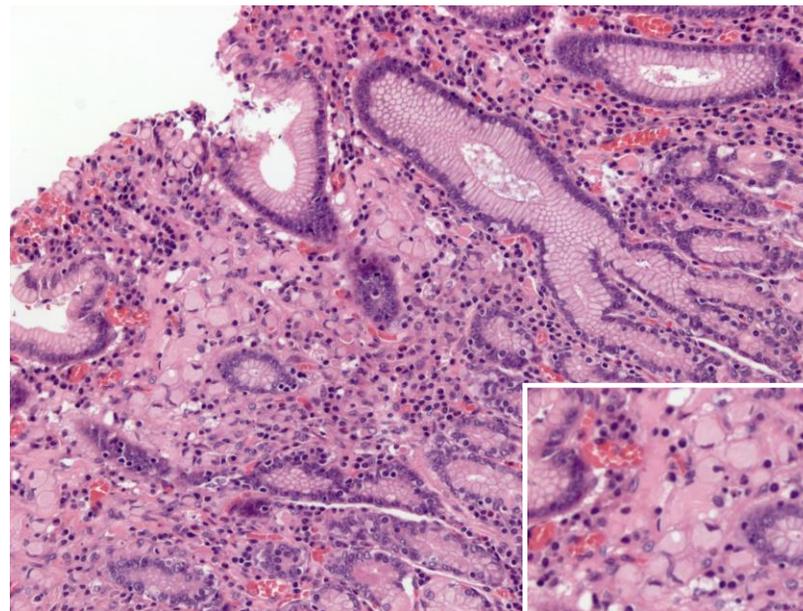
Fundic glands are simple, branched tubular glands that extend from the bottom of the gastric pits to the muscularis mucosae; the more distinctive cells are parietal cells. H&E 10x; square 40x

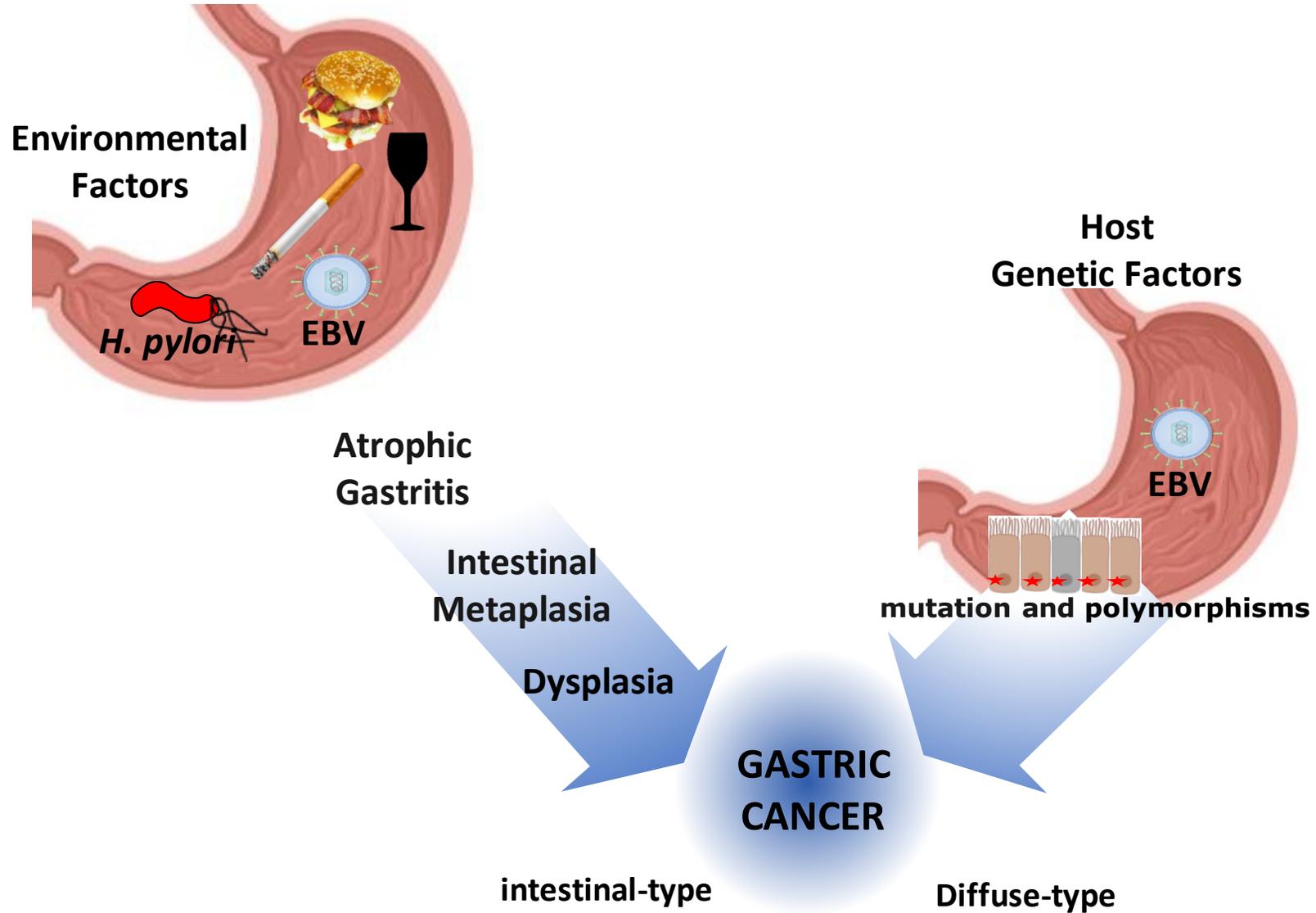
Antral mucosa is formed by branched coiled tubular glands lined by secretory cells similar in appearance to the surface mucus cells. H&E 10x; square 40x

A



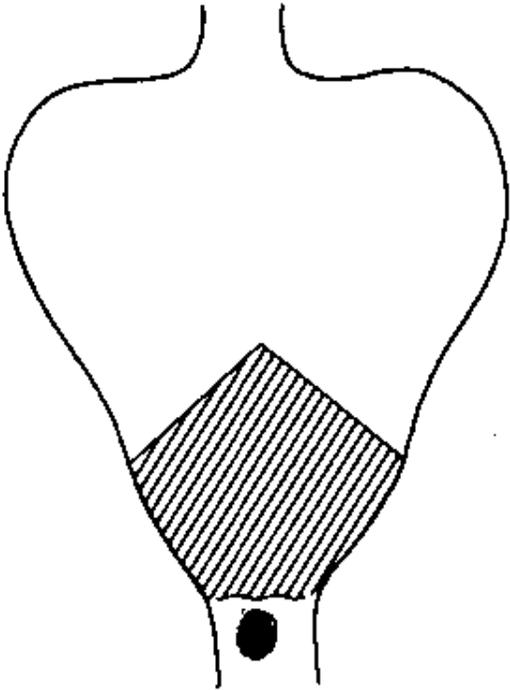
B





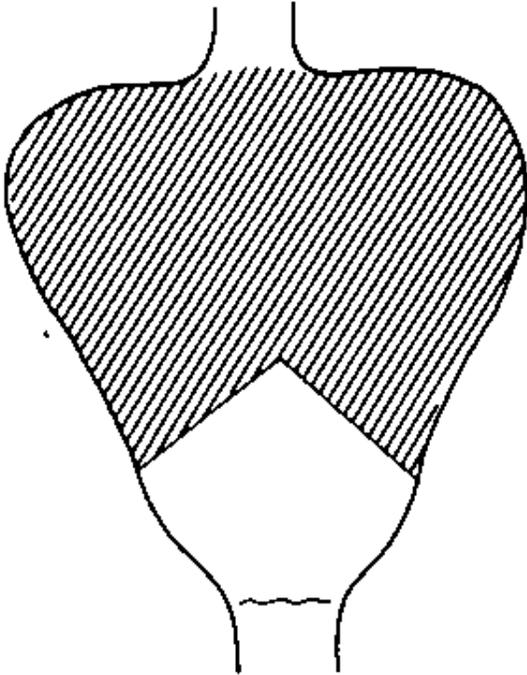
Gastritis

Superficial

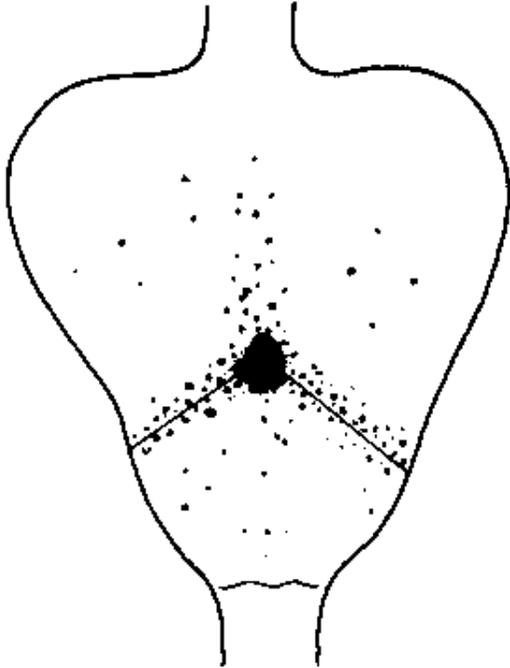


difusa antral  
(hipersecretora)

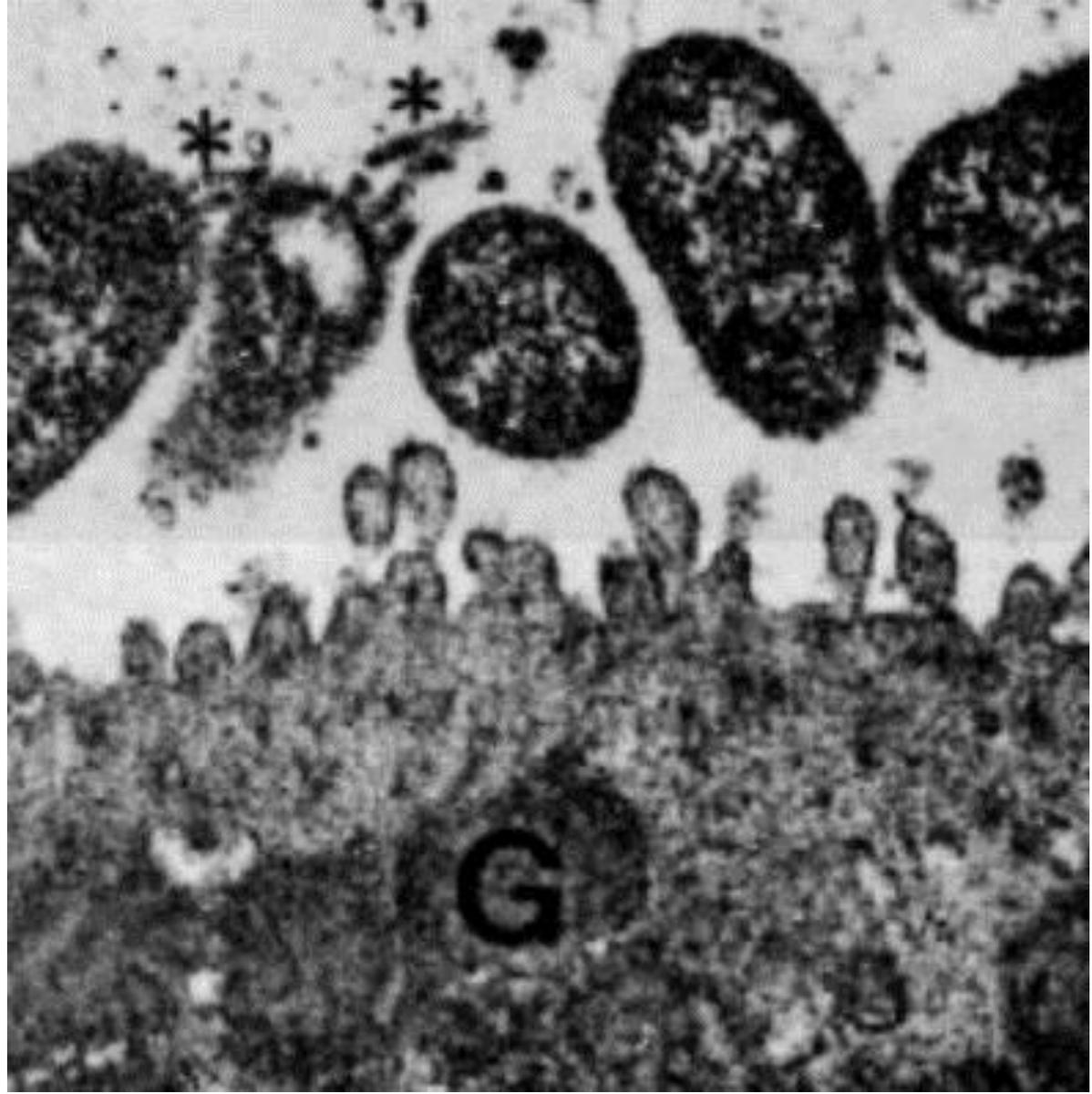
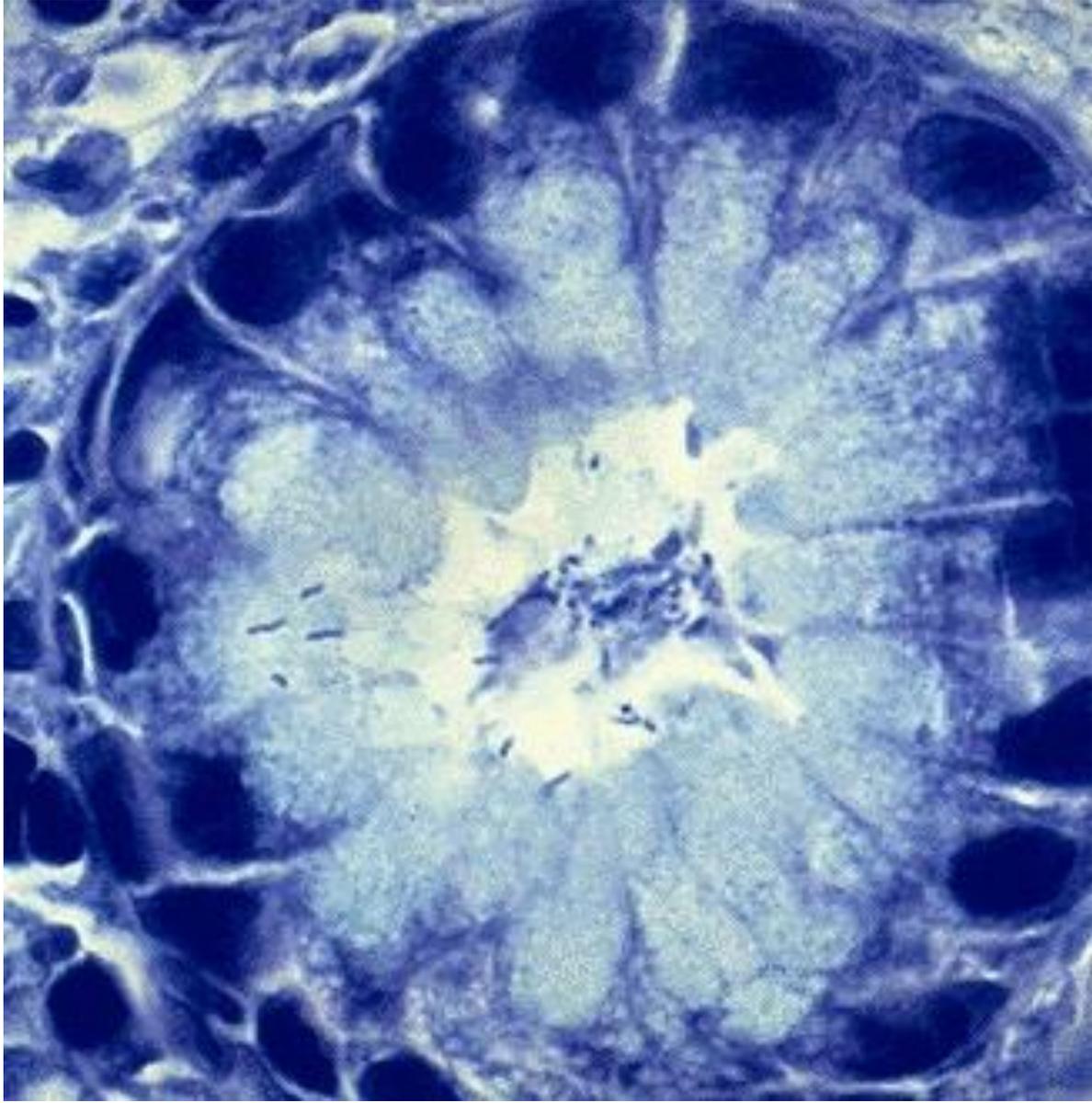
Atrophic



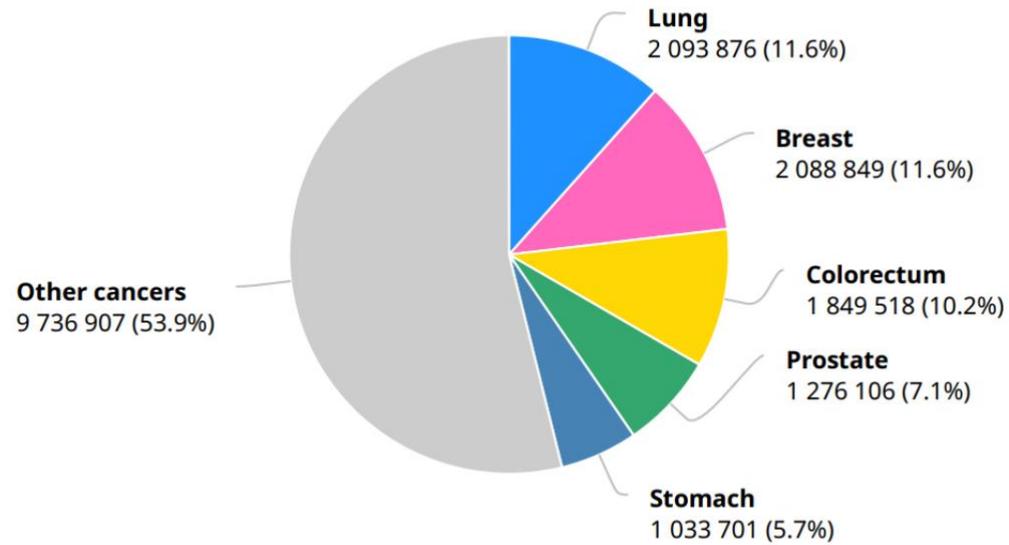
difusa corporal  
(autoimmune)



multifocal  
(ambiental)

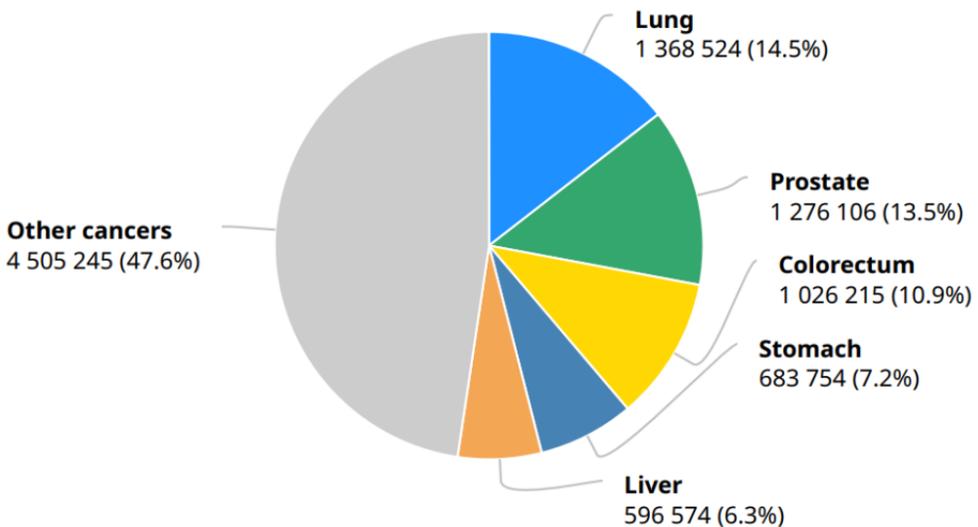


## Number of new cases in 2018, both sexes, all ages



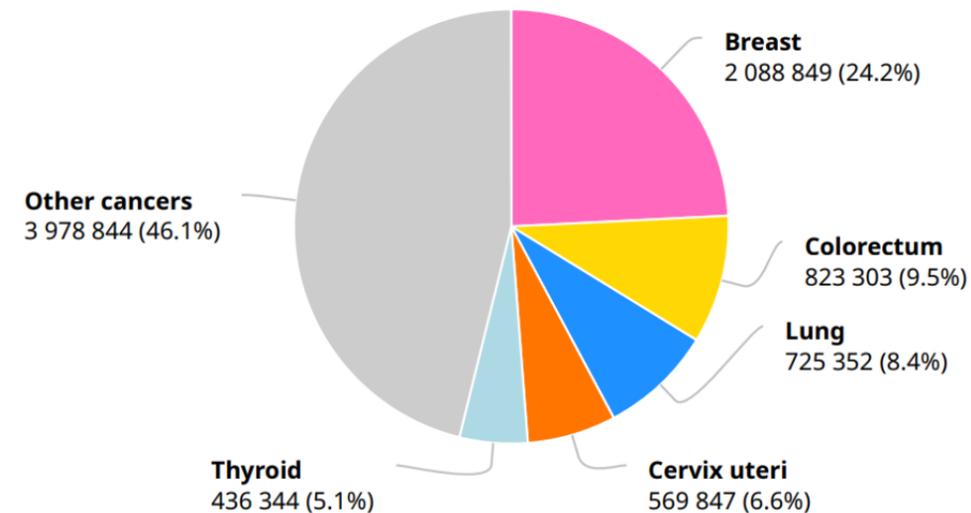
Total: 18 078 957

## Number of new cases in 2018, males, all ages



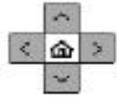
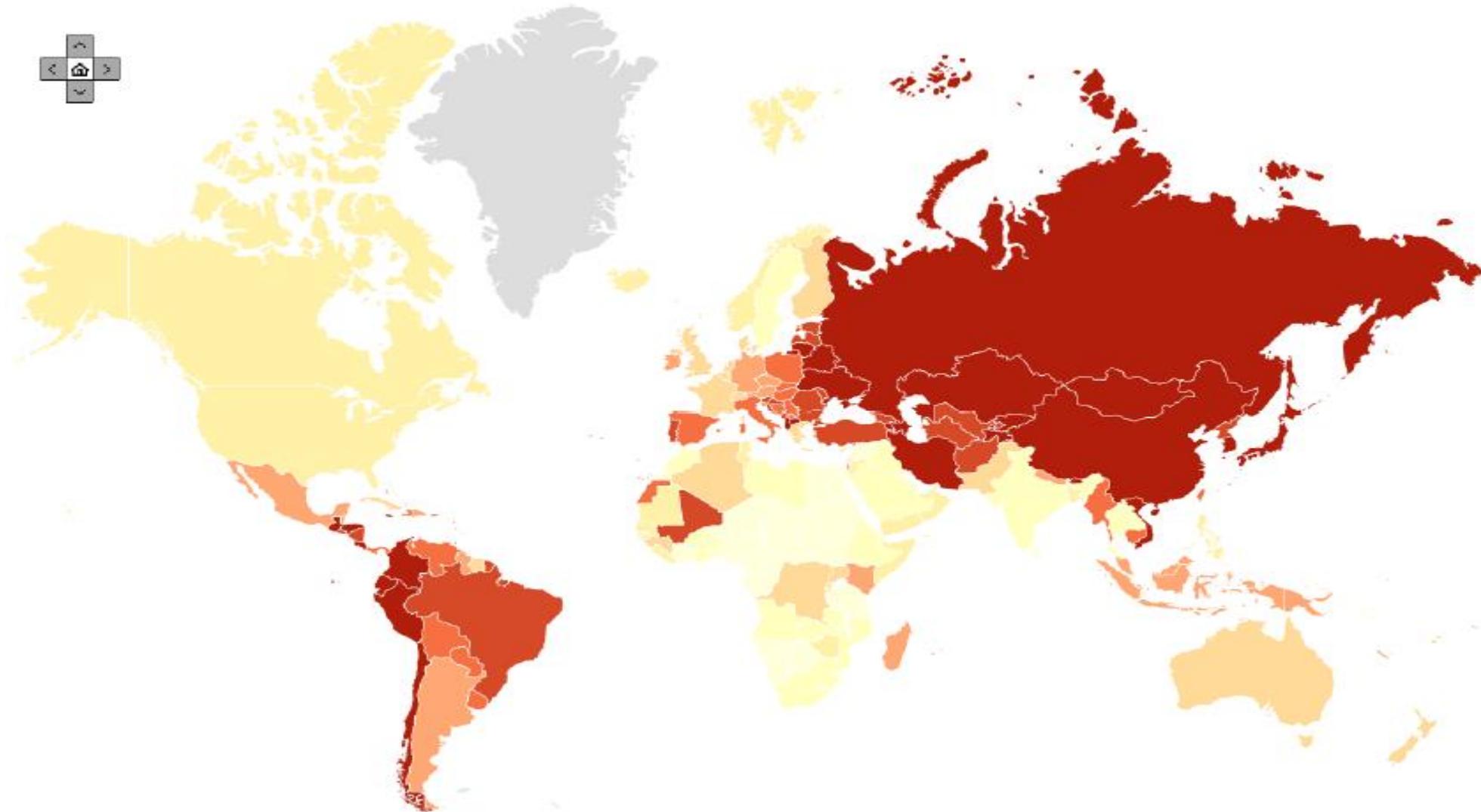
Total: 9 456 418

## Number of new cases in 2018, females, all ages



Total: 8 622 539

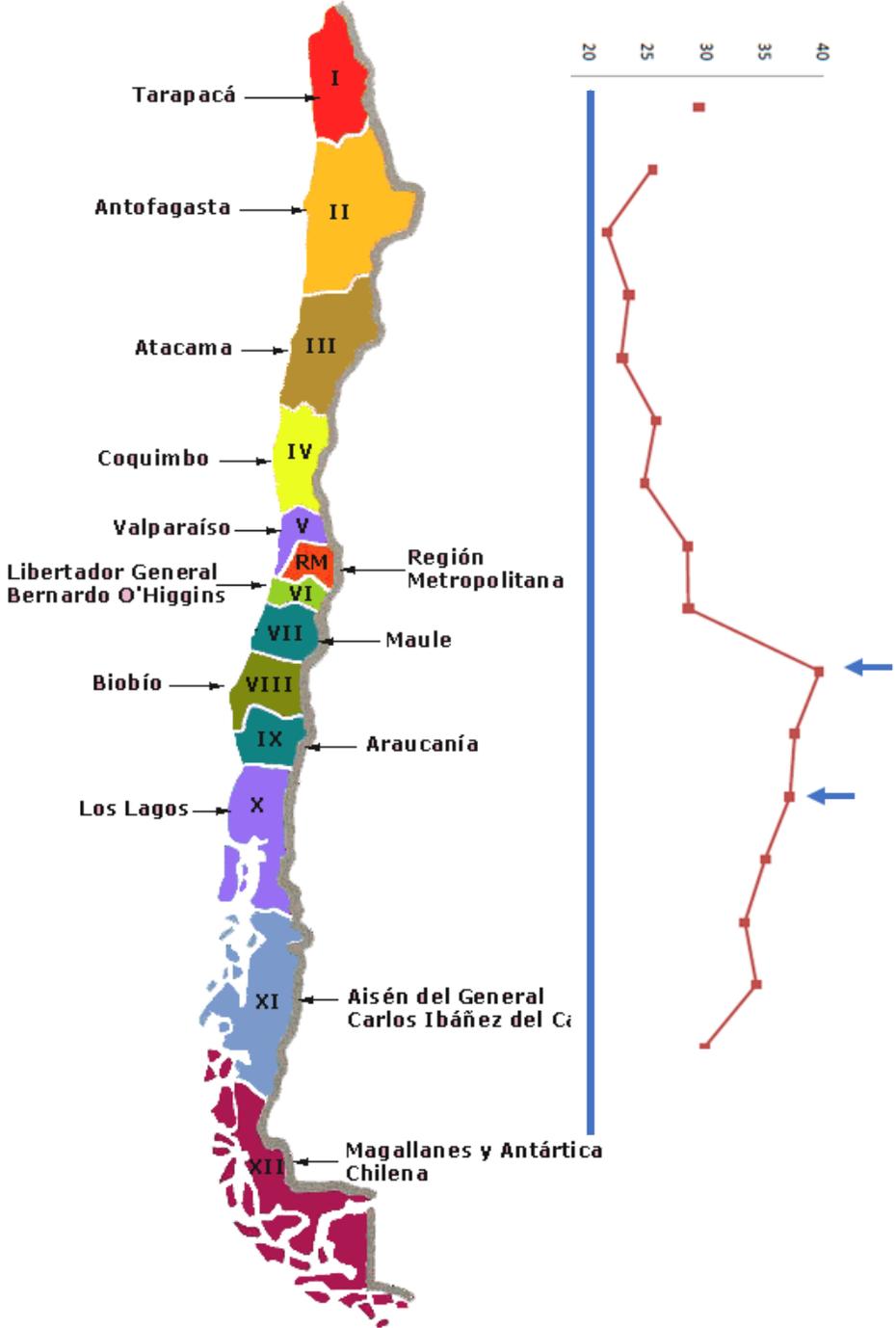
# Gastric Cancer: geographic variation of incidence and mortality worldwide



Globocan 2013

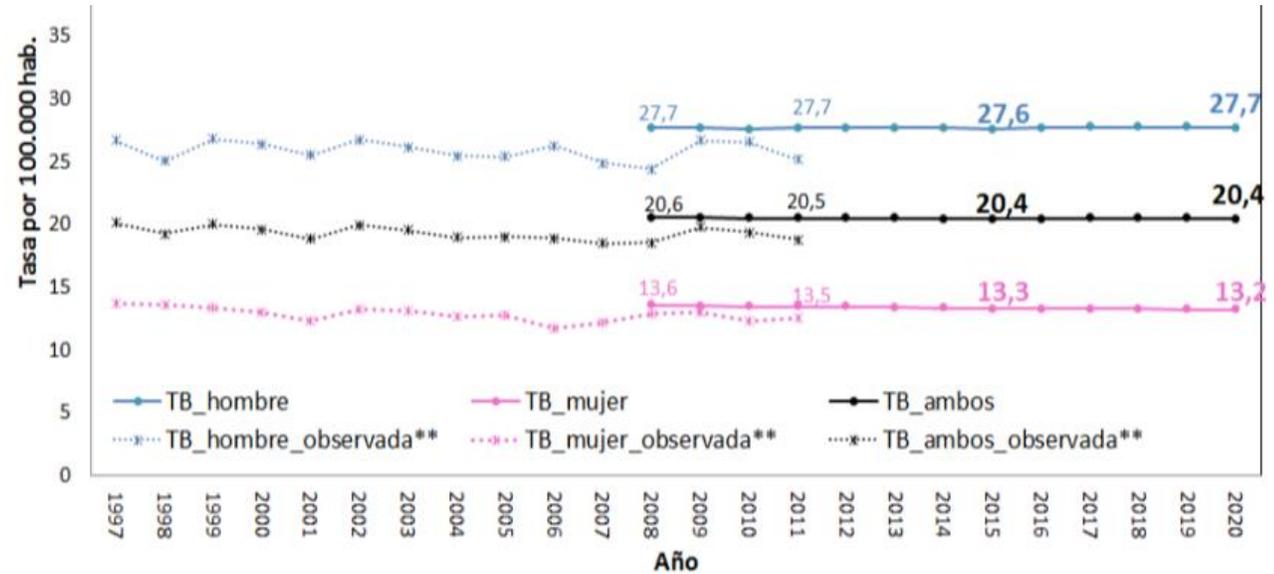
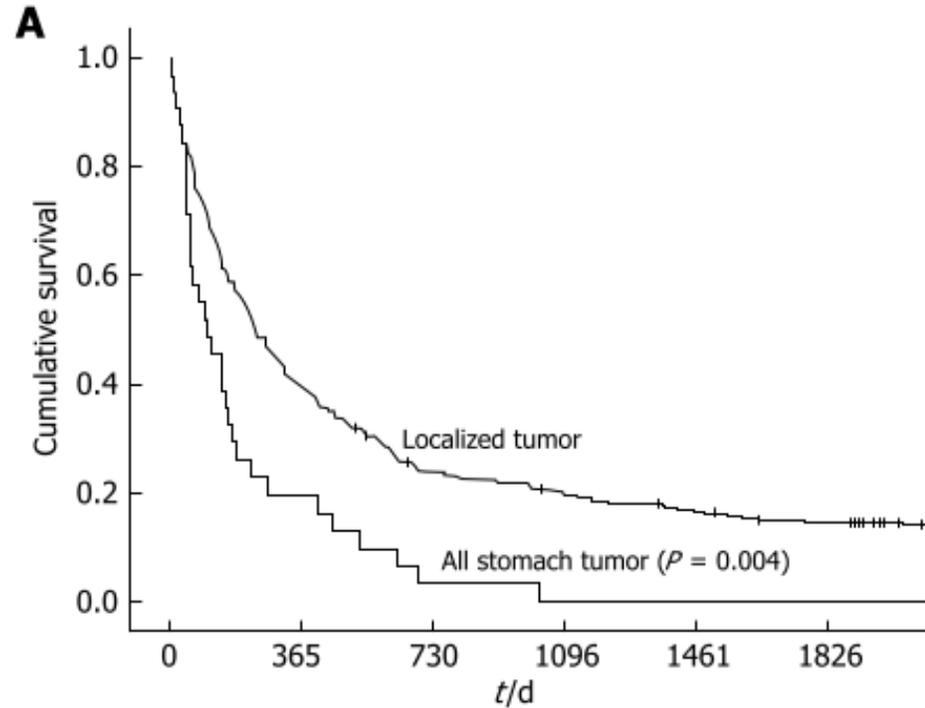


# Stomach Cancer Mortality by regions in Chile in 2015



# Gastric Cancer 5-year survival in Region de los Rios, Chile (1998-2005)

Five-year relative survival rate **12.3%** (95% CI: 9.1-16.1)



Heise *et al.*, World J Gastroenterol. 2009

Proyecciones de mortalidad en Chile 2011 al 2020, para algunas causas no transmisibles en vigilancia MINSAL 2013

# FONDECYT 1191928 (2019-2022)

## The upregulation of long-noncoding RNA, a novel opportunity to address the complexities of the pathogenesis of gastric cancer

**HYPOTHESIS:** The upregulation of the long non-coding RNA KCNQ1OT1 and TSPEAR-AS2 is implicated in the pathogenesis of gastric cancer through the dysregulation of competing endogenous miR-34a/p73, miR-335/CDH11-PLAUR and miR-597/MMP10-METAP2 RNA networks. Circulating long-noncoding RNA KCNQ1OT1 and TSPEAR-AS2 serve as potential biomarkers for non-invasive detection of advanced premalignant stages of gastric cancer.

### AIM

To evaluate the role of the dysregulation of the competing endogenous miR-335/CDH11-PLAUR, miR-34a/p73 and miR-597/MMP10-METAP2 RNA networks in gastric cancer and its premalignant cascade through the upregulation of the long non-coding KCNQ1OT1 and TSPEAR-AS2.

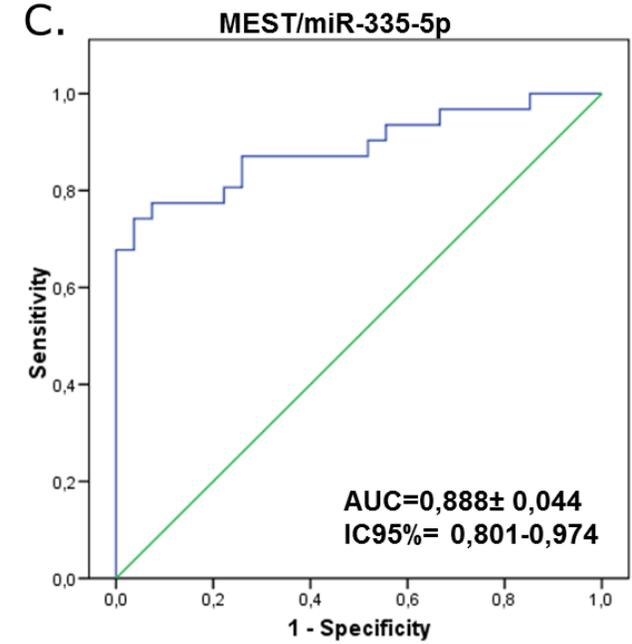
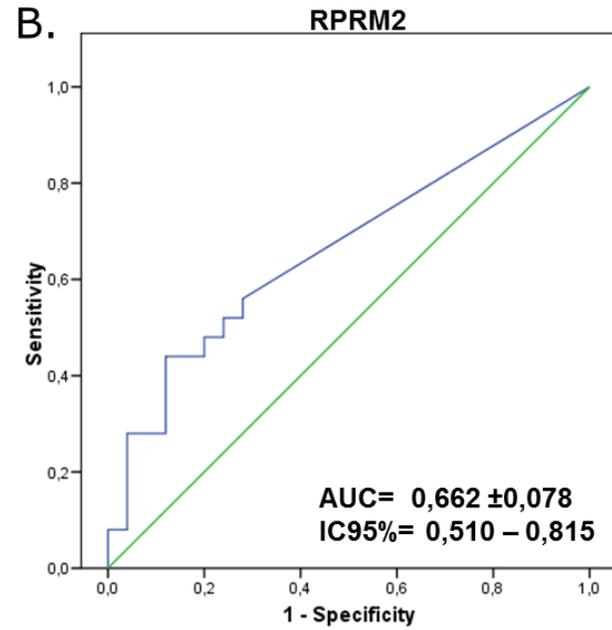
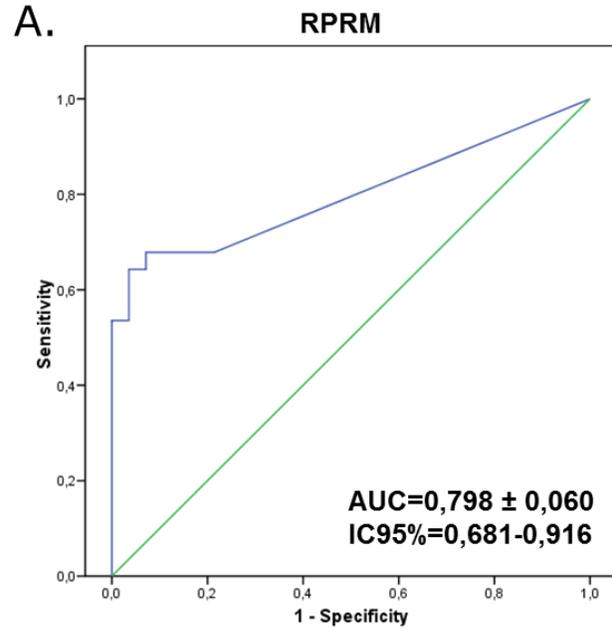
### SPECIFIC AIMS

Aim 1. To characterize and correlate the upregulation of KCNQ1OT1 and TSPEAR-AS2 transcripts in gastric cancer and premalignant lesions with clinico-pathological variables.

Aim 2. To characterize the tumorigenic effect of KCNQ1OT1 and TSPEAR-AS2 transcripts in gastric cancer cell lines.

Aim 3. To evaluate the competing endogenous activity of KCNQ1OT1 and TSPEAR-AS2 in the miR-335/CDH11-PLAUR, miR-34a/p73, and miR-597/MMP10- METAP2 RNA networks.

Aim 4. To determine the capacity of circulating KCNQ1OT1 and TSPEAR-AS2 transcript levels to discriminate among OLGA stages and gastric cancer.



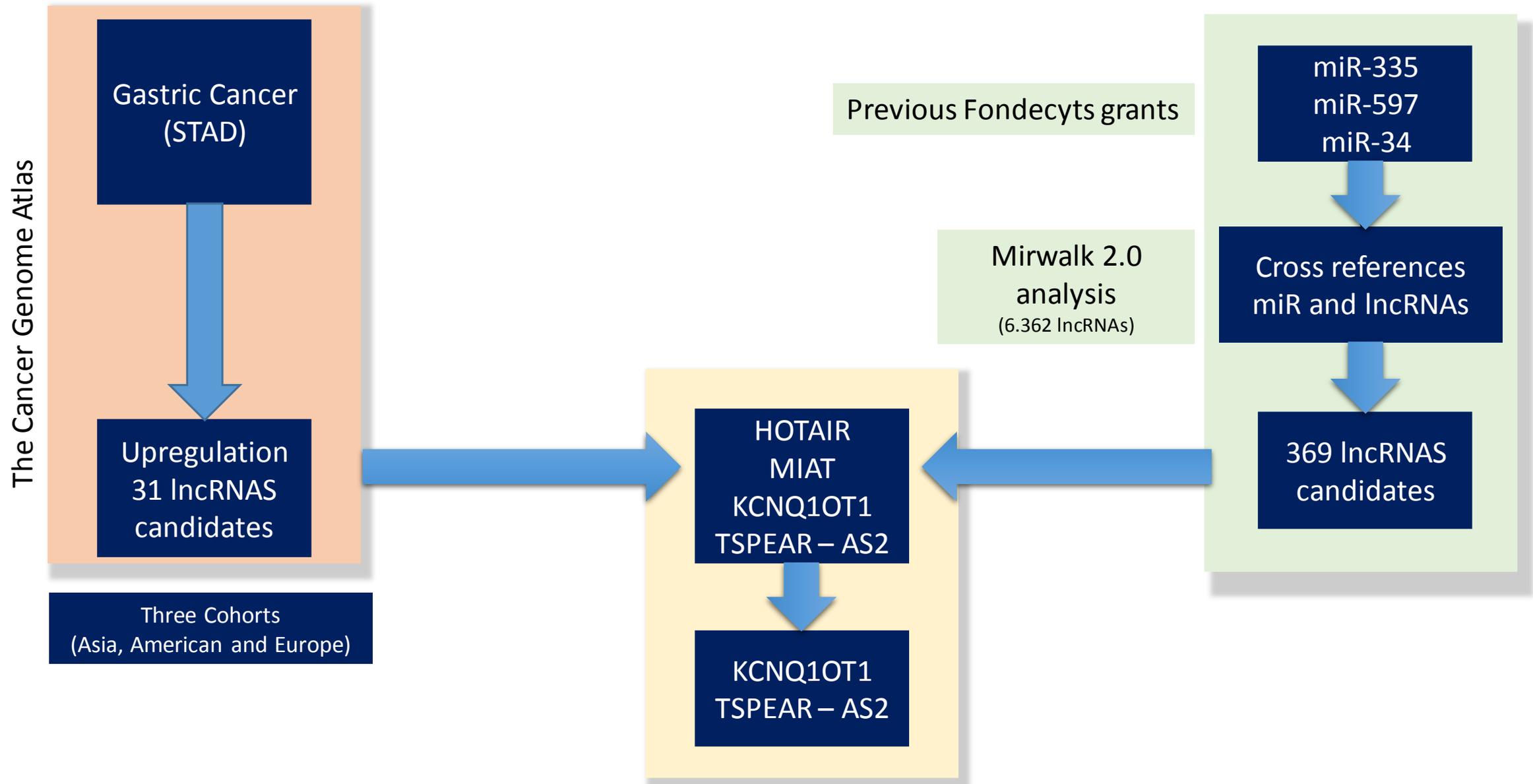
**D.**

Biomarcador	Sensibilidad	IC95%	Especificidad	IC95%	VPP	IC95%	VPN	IC95%
RPRM	67.86%	47.65% - 84.12%	96.86%	76.5% - 99.12%	90.48%	70.92% - 97.37%	74.29%	62.55% - 83.32%
RPRM2	56%	34.93% - 75.6%	72%	50.61% - 87.93%	66.70%	49.37% - 80.4%	62%	49.68% - 73.06%
MEST/miR-335-5p	87.33%	70.17% - 96.37%	74.07%	53.73% - 88.89%	79.40%	66.78% - 88.1%	83.33%	66.11% - 92.76%

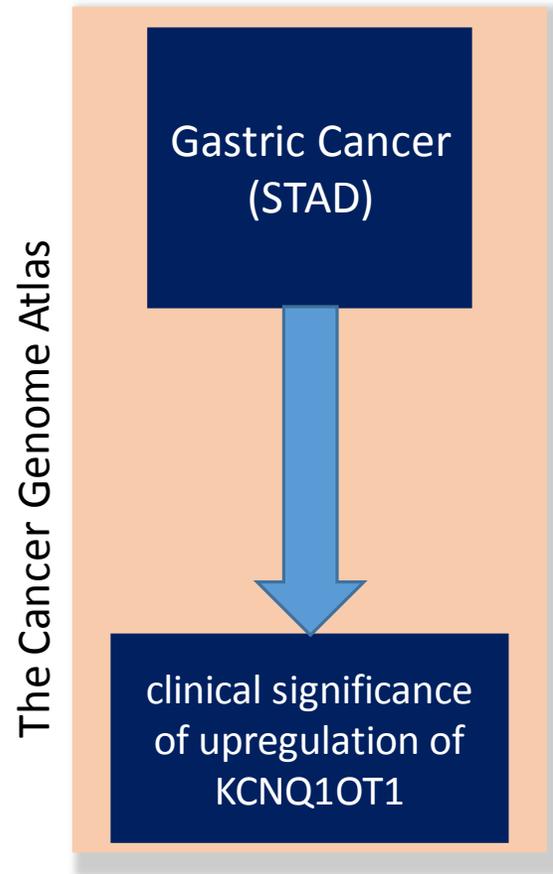
**E.**

Biomarcador	Sensibilidad	IC95%	Especificidad	IC95%	VPP	IC95%	VPN	IC95%
RPRM	42%	21.82% - 65.98%	72%	55.13% - 85%	45.00%	28.82% - 62.31%	70%	60.54% - 78.02%

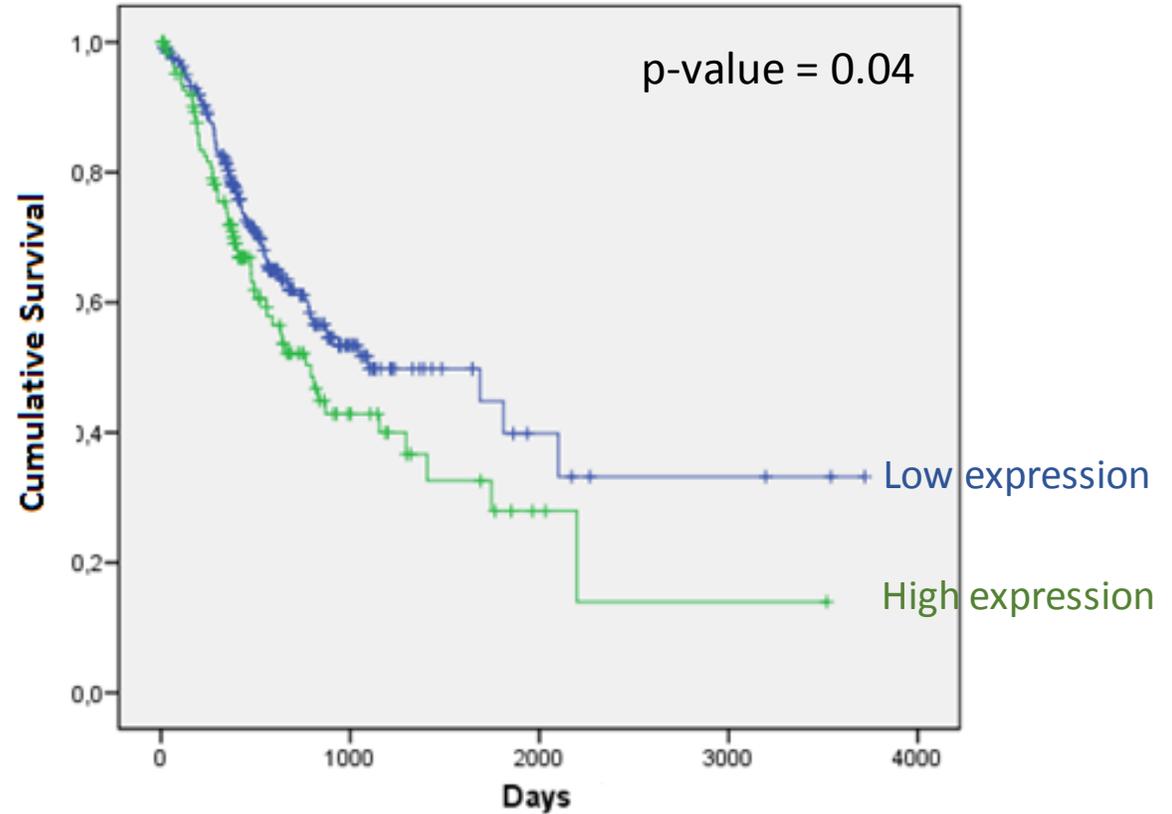
# *In silico* Discovery



# *In silico* Discovery



Global Survival by KCNQ10T1 expression (STAD TCGA)



# **A *ceRNA* Hypothesis: The Rosetta Stone of a Hidden RNA Language?**

**Leonardo Salmena,<sup>1</sup> Laura Poliseno,<sup>1,2</sup> Yvonne Tay,<sup>1</sup> Lev Kats,<sup>1</sup> and Pier Paolo Pandolfi<sup>1,\*</sup>**

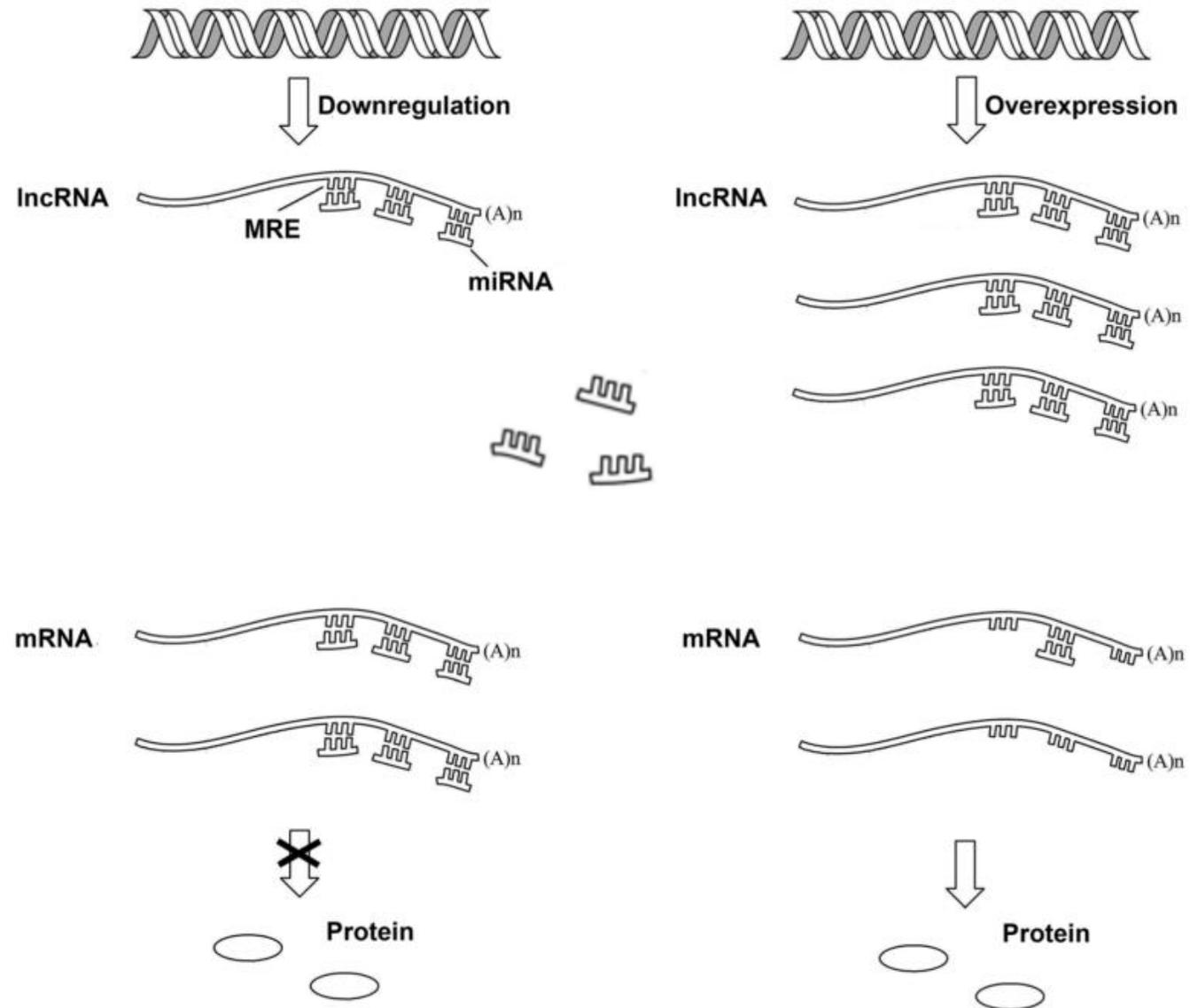
<sup>1</sup>Cancer Genetics Program, Beth Israel Deaconess Cancer Center, Departments of Medicine and Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA

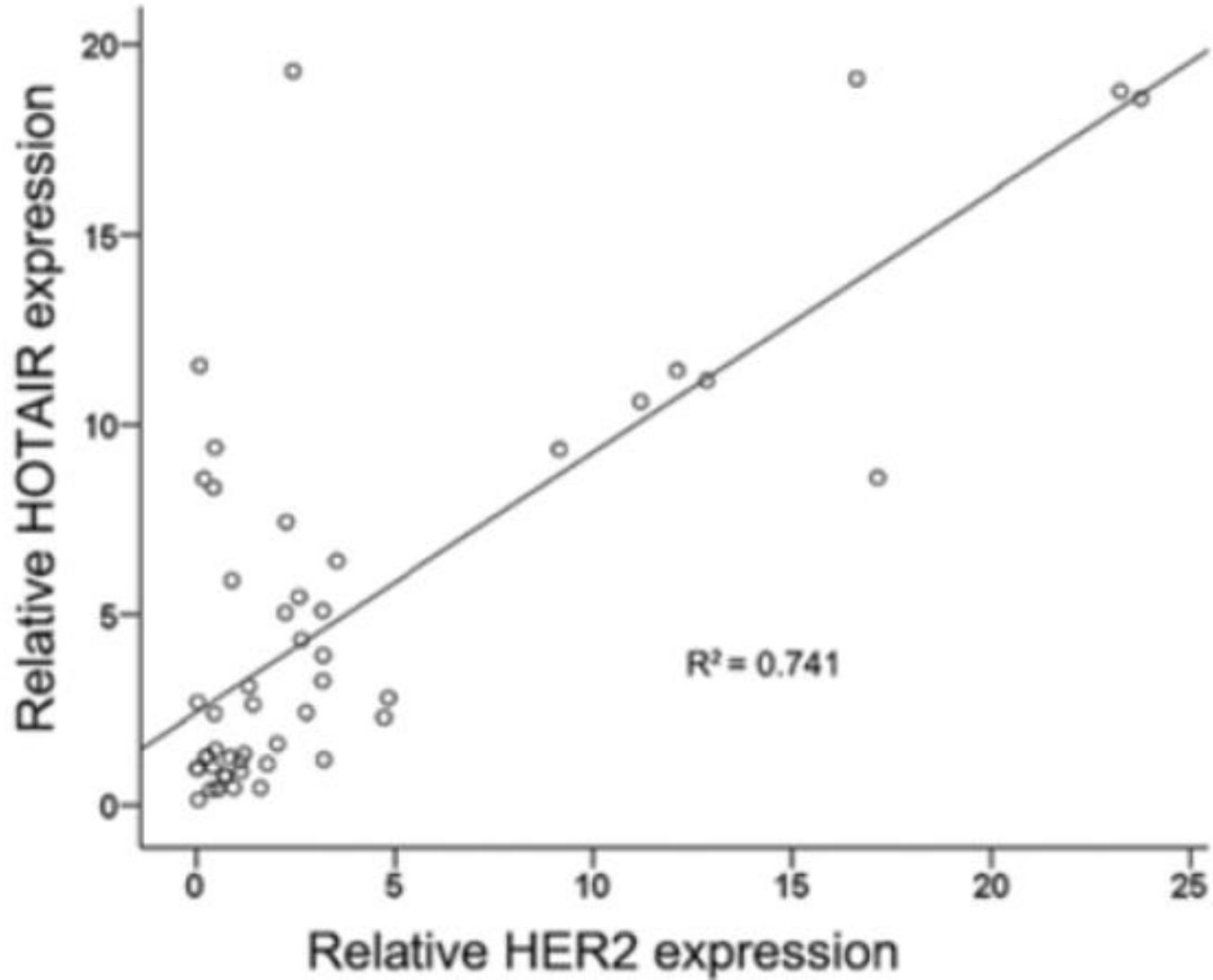
<sup>2</sup>Present address: Department of Dermatology, New York University School of Medicine, New York, NY 10016, USA

\*Correspondence: [ppandolf@bidmc.harvard.edu](mailto:ppandolf@bidmc.harvard.edu)

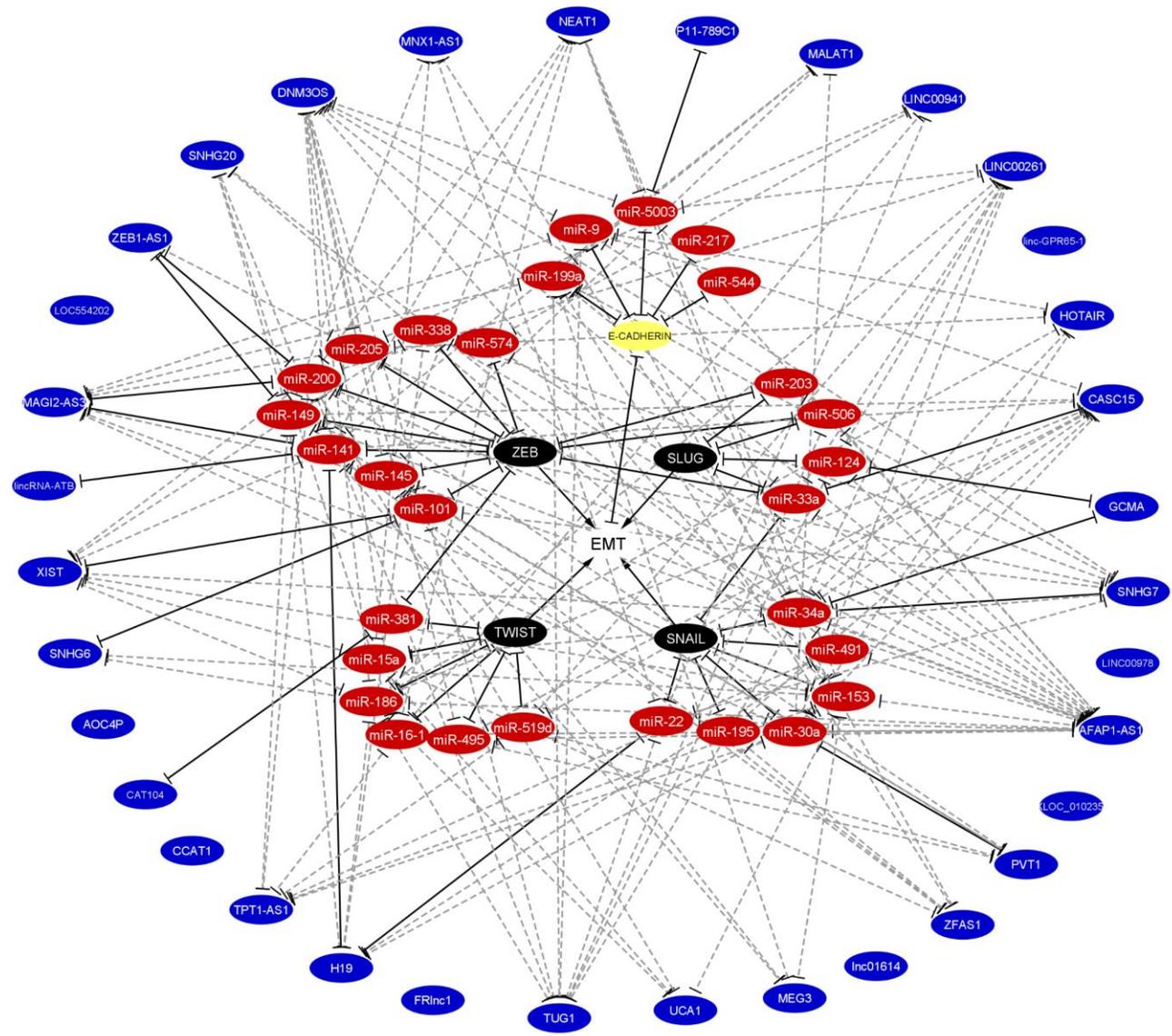
DOI [10.1016/j.cell.2011.07.014](https://doi.org/10.1016/j.cell.2011.07.014)

# competing endogenous RNA (ceRNA)





Liu, X.H., et al., *Lnc RNA HOTAIR functions as a competing endogenous RNA to regulate HER2 expression by sponging miR-331-3p in gastric cancer*. *Mol Cancer*, 2014. **13**: p. **92**.



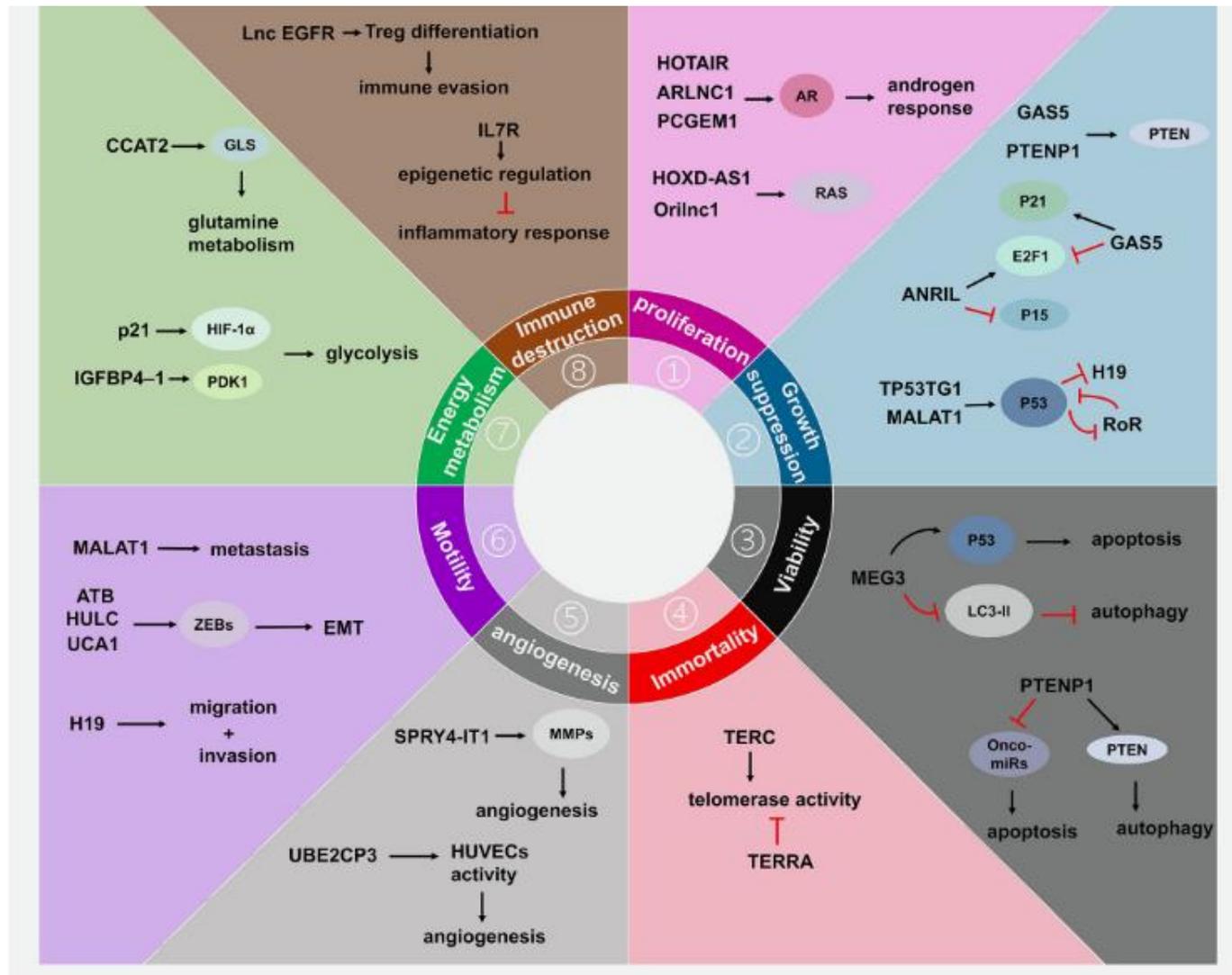
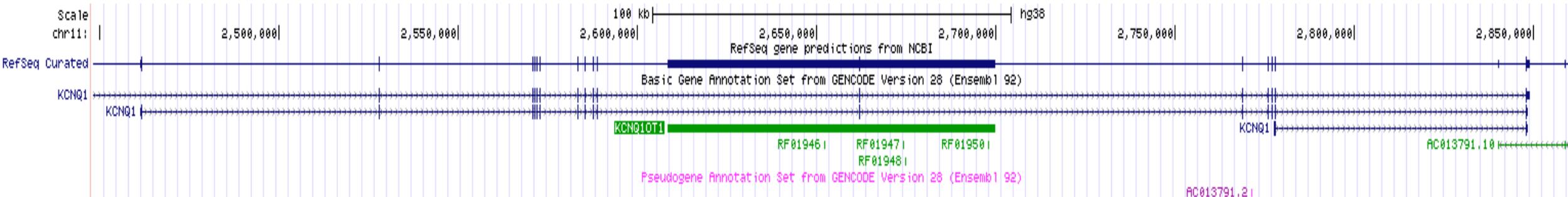
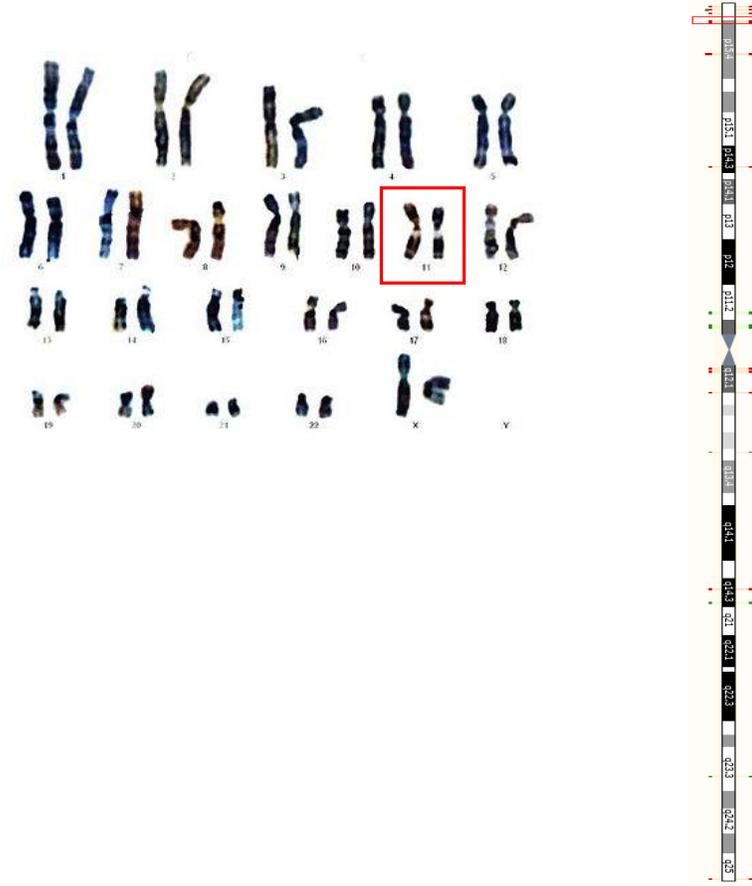


Figure 1. LncRNAs in Cancer Hallmarks. LncRNAs contribute to each of the eight hallmarks of cancer (diagram adapted from Hanahan and Weinberg, *Cancer: Principles & Practice of Oncology*, 2015, 28–57, ©Wolters Kluwer). Selected examples of lncRNAs and their molecular partners or genomic targets are shown for proliferation, growth suppression, immune destruction, immortality, motility, angiogenesis, viability and energy metabolism.<sup>132–136</sup> [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

# KCNQ10T1

- Located in chromosome 11
- Antisense from intron 10 of KCNQ1 gene
- Imprinting domain in maternal allele
- 92.000 nt
- Largest monoexomic gene of human genome
- 700 predicted union sites for miRNAs

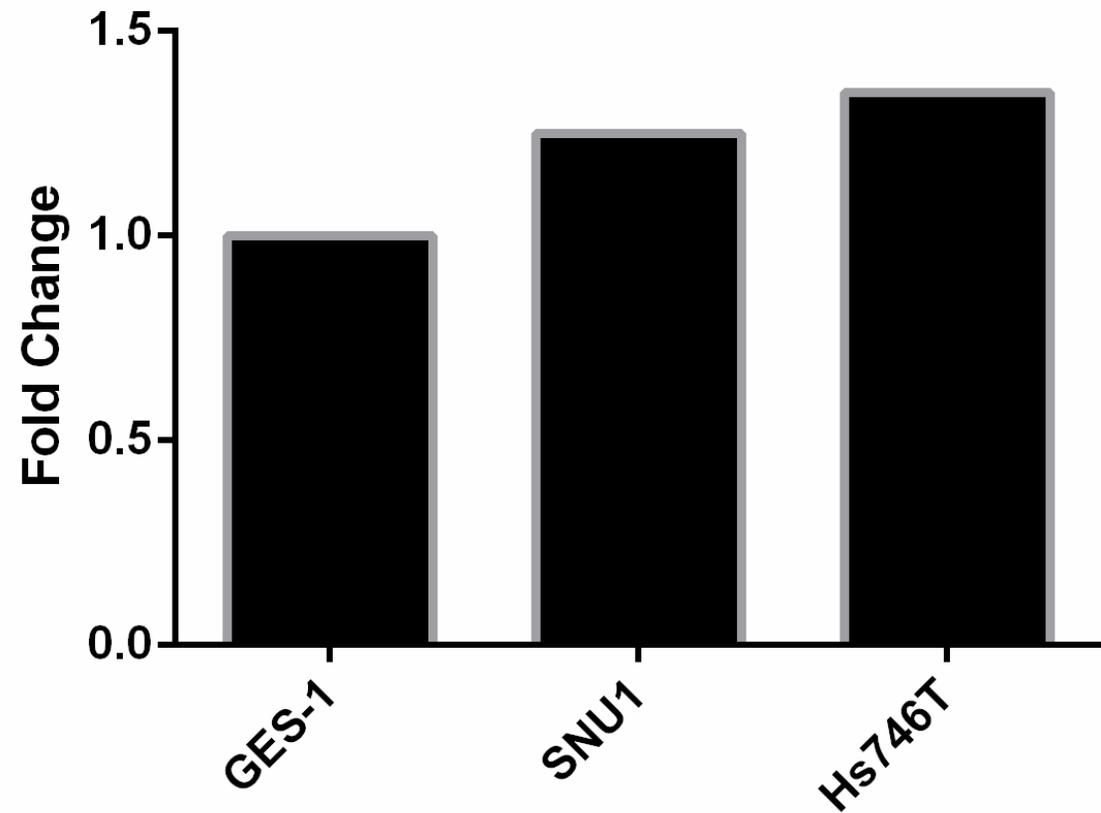


# RESULTS

## Transcript expression of KCNQ10T1 in gastric cancer cell Lines

---

- Ges-1: Normal gastric cell line
- SNU1: Primary tumor, carcinoma
- Hs746T: Derived from metastatic site: left leg



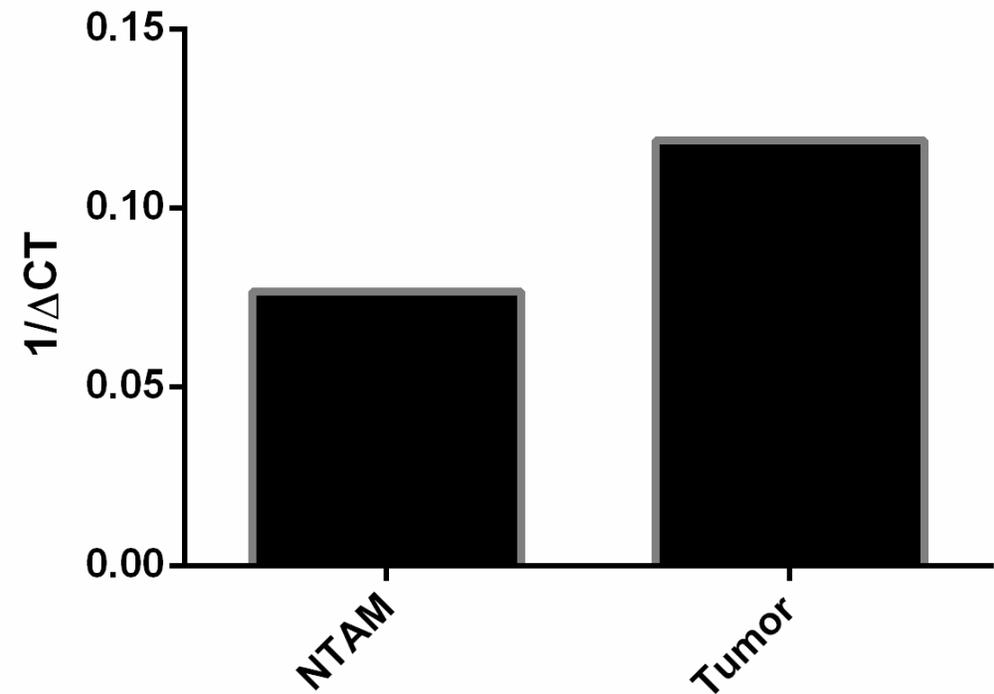
# RESULTS

## Expression of KCNQ10T1 in clinical samples (tissue biopsies)

---

### Clinical Lesions

- Four paired normal and tumor samples from gastric cancer patients
- Pooled samples



NTAM: Normal Tissue Adjacent Mucosa

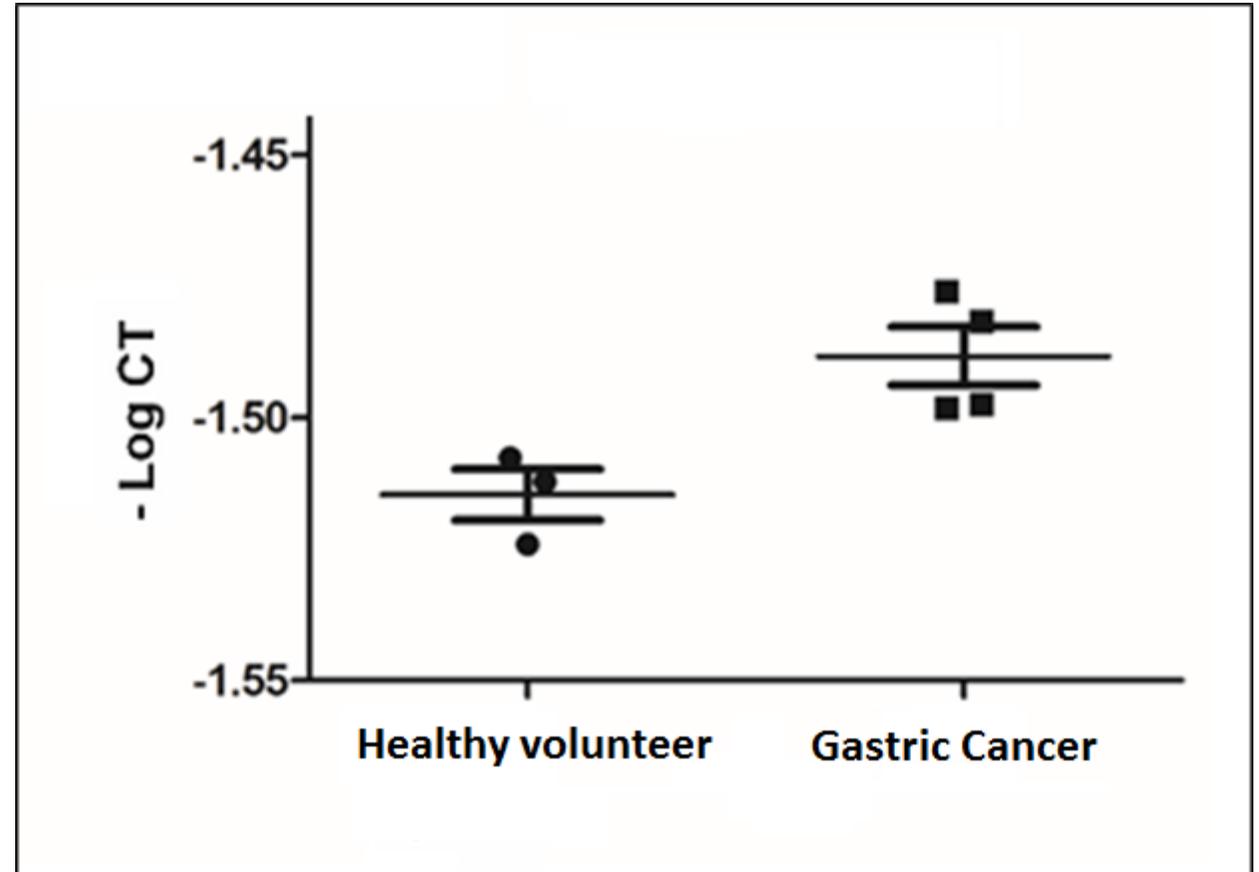
# RESULTS

## Cell free RNA of KCNQ10T1 in peripheral blood samples (plasma)

---

Peripheral blood samples:

- Three healthy asymptomatic volunteer
- Four gastric cancer



Differences in the logarithmic value (-log) of KCNQ10T1 transcripts in peripheral blood (plasma) between OLGA stage 0 and GC cases.

**Advanced Center for Chronic Diseases (ACCDiS)  
Research Line 4**

# **Epigenomic Landscapes in Gastric Cancer**

## Principal Investigator:

### Associated PI:

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Faculty of Biological Sciences,  
Pontificia Universidad Católica de Chile

**Alejandro Corvalan, M.D.**

**Iva Polakovicova, Ph.D.**

Faculty of Medicine,  
Pontificia Universidad Católica de Chile

### Associated PI:

**Francisco Aguayo, Ph.D.**

Virology Program, Institute of Biomedical Sciences,  
University of Chile

### PhD. Students:

Maximiliano Arce (shared AQ)  
Andrés Valdivia (shared AC)  
Miguel Cordova (shared MG)  
Gabriel Mingo

### PostDoc:

Keila Torres (shared CF)  
Jenny Ruedlinger (shared CF)  
Graciela Molina (shared L Carvajal, UC Davis)  
Natalia Landeros (shared L Jara)

### **Ph.D. students**

Christian Sotomayor (shared SL)  
María Fernanda González (shared AQ)  
Alejandra Alarcón (Dissertation approved)  
Ignacio Wichmann (shared EF)  
Héctor Ramos (shared MA and IP)

### **Staff:**

Maria Jose Maturana, Wilda Olivares,  
Andres Rodriguez, Carolina Castro,  
Alvaro Neira, Pablo Santoro

**PostDocs:** Juan Pablo Munoz, Ramses  
Blanco

**Ph.D. students:** Diego Carrillo

**M.Sc students:** Daniela Baez (shared with  
D. Bravo,  
AQ group)

## National Research Collaborations:

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Hosp. Sotero del Rio,  
PUC

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Pathology Dept, Hospital Clinico  
and Faculty of Medicine, UCH

Marcelo Garrido, M.D.,  
Department Hematology and Oncology,  
Faculty of Medicine, PUC

Iva Polakovicova, Ph.D. Dep. Hematology  
and Oncology, Faculty of Medicine, PUC

Anne Marie Ziegler, Ph.D. Faculty of Medicine,  
Universidad Del Desarrollo

Lilian Jara, Ph.D. Department of Genetics,  
Faculty of Medicine, Universidad de Chile

Marcelo Andia, M.D., Ph.D., Department of  
Radiology, Pontificia Universidad Católica de Chile

Bettina Muller GOCCHI/INCANCER



# International Research Collaborations



Charles Rabkin  
NIH/NCI, USA



Luis Carvajal  
UC Davis, USA



Wael El-Rifai  
U.Miami, USA



Elmer Fernandez  
Universidad Catolica de Cordoba,  
Argentina



Pilar Blancafourt  
The University of Western Australia,  
Australia

Parry Guiford  
Otago, New Zealand

# Research line 4: Epigenomic landscapes in gastric cancer (2018-2023)

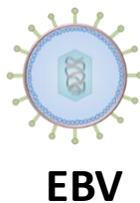
**Hypothesis:** Non-coding RNA modulates the pathogenesis of gastric cancer and tumor dissemination.

**Specific aim 1:** To identify germline genetic variants in miRNA genes targeting CDH1 genes in cases which tested negative for CDH1 mutations



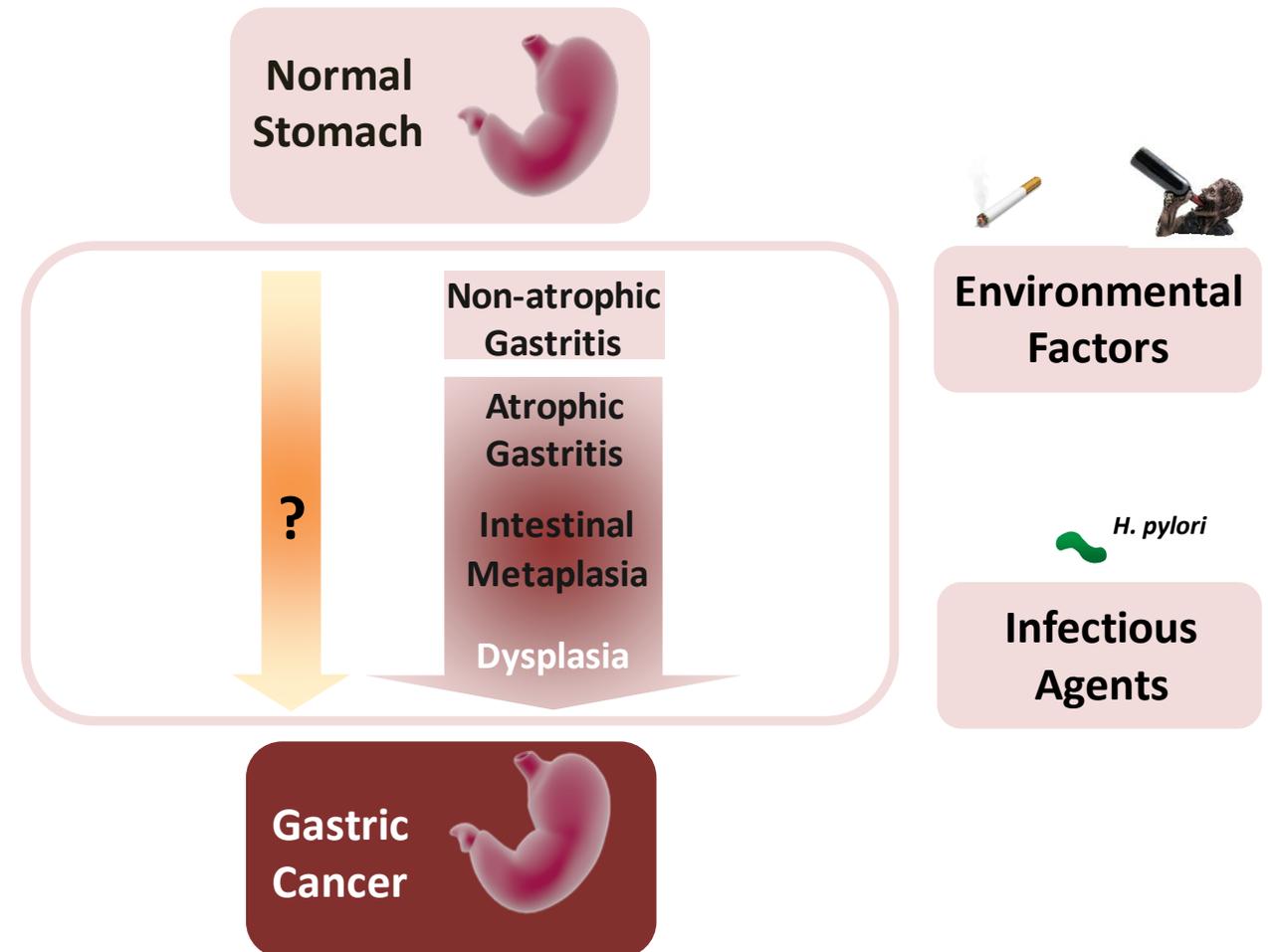
**Genetic Factors**

**Specific aim 2:** To identify epigenomic dysregulation associated with interactions between EBV coding and non-coding genes and environmental factors in EBV-associated GC



**Infectious Agents**

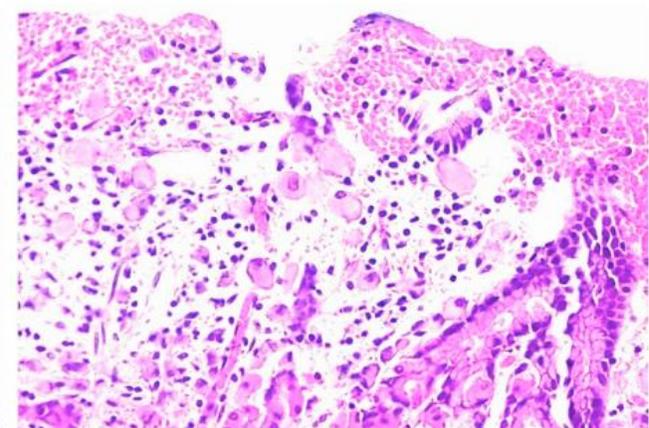
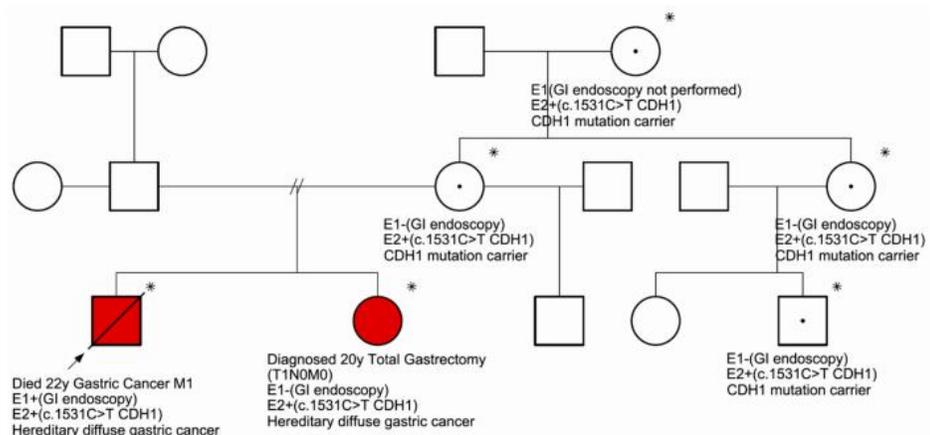
**Specific aim 3:** To investigate the role of exosomes and their non-coding RNA cargo in gastric cancer dissemination



**Table 2.** CDH1 variants identified and pathologic significance.

SNP ID	Sequence Variant	Protein Change	Probands (n)	Location	Class	Significance <sup>α</sup>
rs16260	c.-285C>A		19	Promoter	Promoter	Non-coding
rs28372783	c.-197A>C		11	Promoter	Promoter	Non-coding
rs3743674	c.48+6C>T		36	Intron 1	Splice site	Benign
rs139866691	c.88C>A	p.Pro30Thr	1	Exon 2	Missense	Benign
rs33963999	c.531+10G>C		1	Intron 4	Splice site	Benign
rs61756284	c.1272A>T	p.Thr424Thr	1	Exon 9	Synonymous	Benign
→ rs1131690810	c.1531C>T	p.Gln511 *	1	Exon 10	Nonsense	Pathogenic
rs786201452	c.1893A>T	p.Thr631Thr	1	Exon 12	Synonymous	Likely benign
rs764379691	c.2052C>T	p.Ser684Ser	1	Exon 13	Synonymous	Likely benign
rs1801552	c.2076T>C	p.Ala692Ala	30	Exon 13	Synonymous	Benign
rs33964119	c.2253C>T	p.Asn751Asn	4	Exon 14	Synonymous	Benign

<sup>α</sup> Significance according to ClinVar [20]. \* Incomplete protein.



(a)



## HOSPITAL SOTERO DEL RIO HEREDITARY TUMOR GROUP



- E.Norero – Familial Gastric Cancer
- M.Petric – Hereditary Breast Cancer
- Paulina Gonzalez - Nurse
- MJ Mullins – Genetic counseling



Sistema de Información de Exámenes, SINFEX

### GEN *CDH1*, CÁNCER GÁSTRICO DIFUSO HEREDITARIO, ESTUDIO GENÉTICO MOLECULAR POR SECUENCIACIÓN

Actualizado en Octubre de 2017 por TM Eliana Romeo

Revisado por TM Ligia Valdivia y Aprobado por Dra. Marcela Lagos

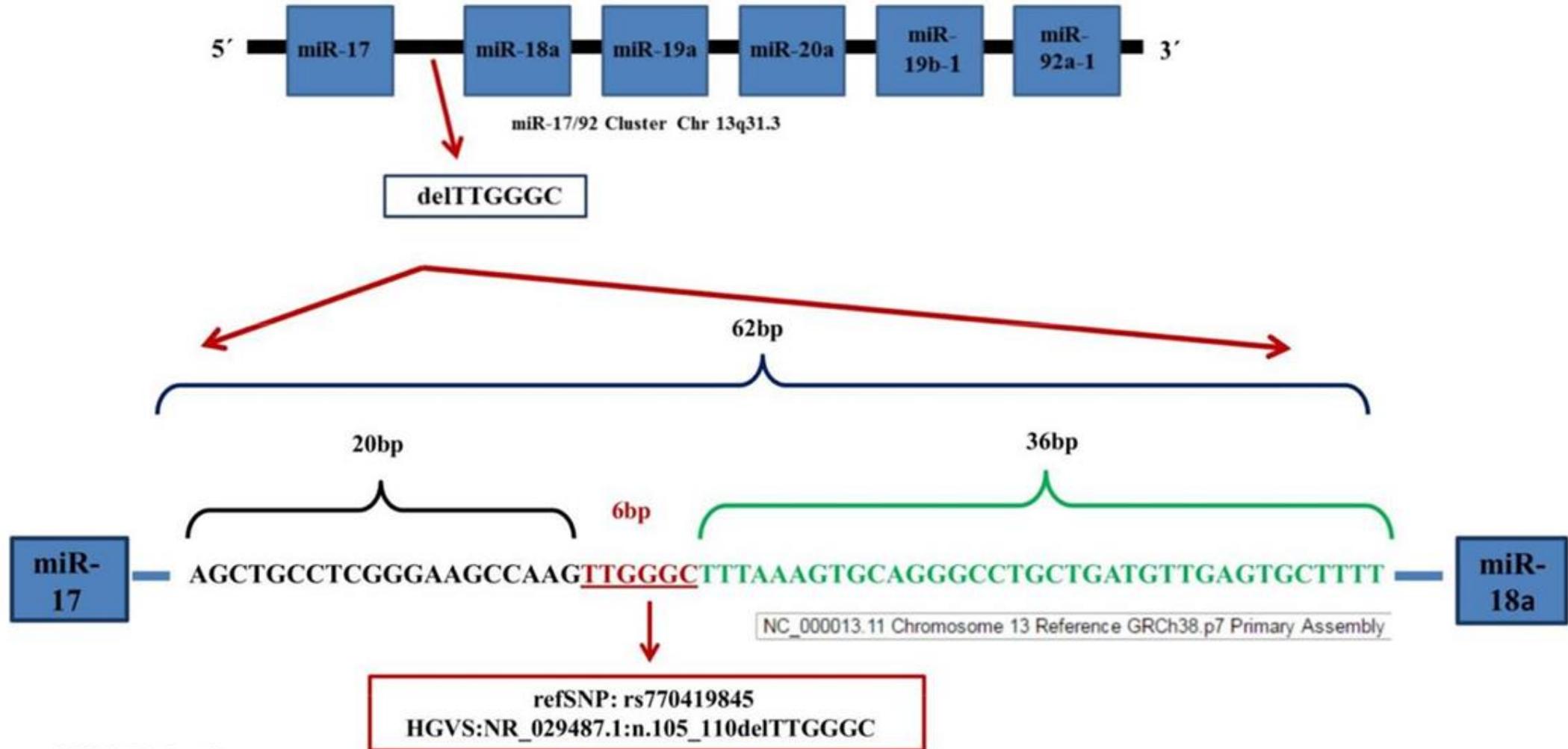
Código del Examen	: 2690						
Nombres del Examen	: Estudio Genético Molecular del Cáncer difuso hereditario por secuenciación, gen <i>CDH1</i>						
Laboratorios de Procesamiento	: <table border="1" style="display: inline-table; border-collapse: collapse;"> <thead> <tr> <th>Laboratorio</th> <th>Días de Procesamiento</th> <th>Plazo de Entrega de Resultados</th> </tr> </thead> <tbody> <tr> <td>Biología Molecular</td> <td>Lunes a Viernes</td> <td>15 días hábiles</td> </tr> </tbody> </table>	Laboratorio	Días de Procesamiento	Plazo de Entrega de Resultados	Biología Molecular	Lunes a Viernes	15 días hábiles
Laboratorio	Días de Procesamiento	Plazo de Entrega de Resultados					
Biología Molecular	Lunes a Viernes	15 días hábiles					
Preparación del Paciente	: No requiere						
Muestra Requerida	: <ul style="list-style-type: none"> <li>■ Sangre completa</li> </ul> Recolectar un tubo tapa lila (EDTA), volumen mínimo: 1 mL de sangre						

## Clinical Experience 2019-2020

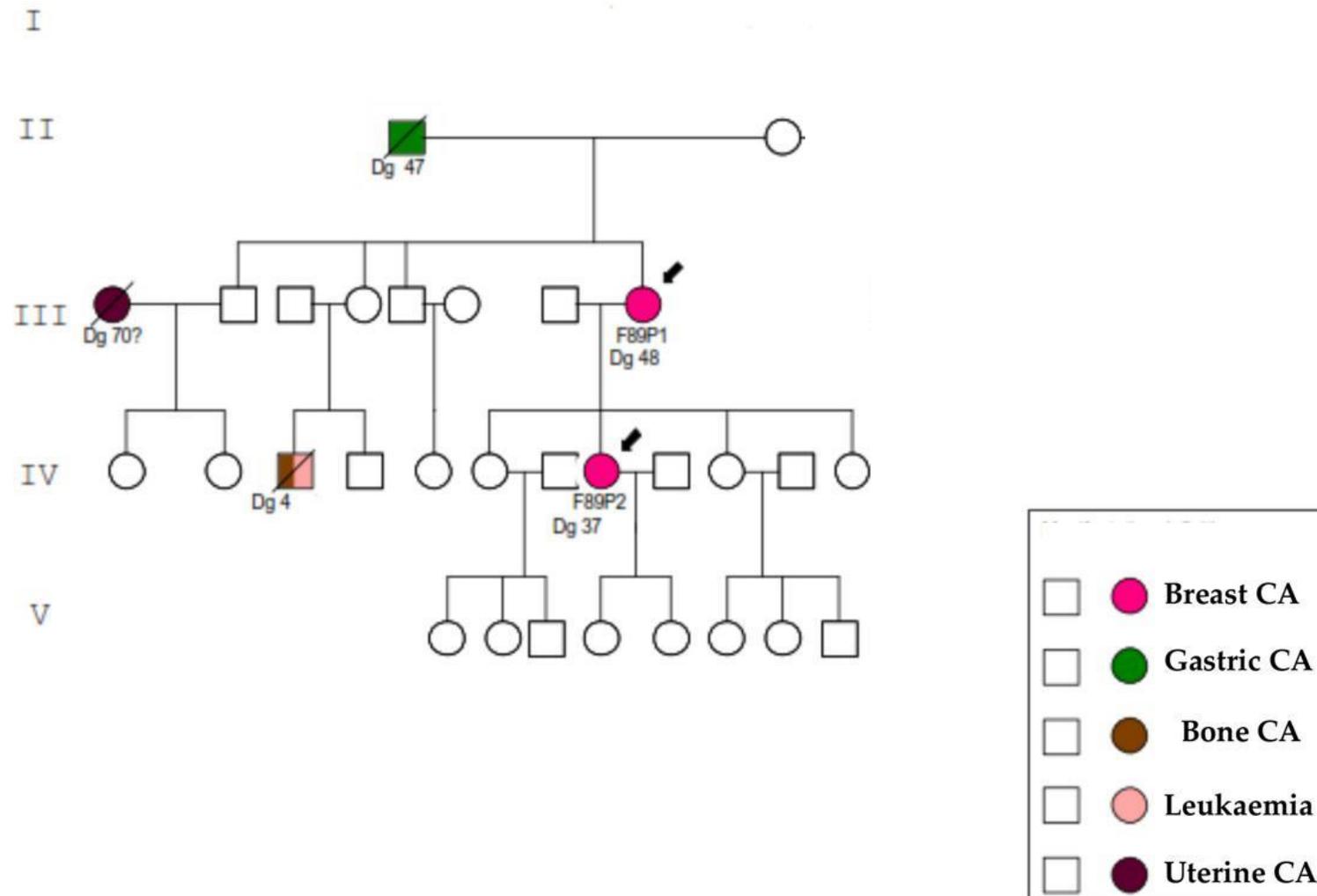


- Cancer Gástrico y criterios de inclusión 49 pacientes estudiados por CDH1 en contexto de estudio clínico UC-HSR
  - 1 paciente con mutación patogénica y estudio familiar (5 familiares positivos y 4 gastrectomías profilácticas)
  - 1 paciente con VUS y con familia estudiada. VUS reclasificada como benigna
- Hospital Sótero del Río realiza convenio con Laboratorio Biología Molecular Red Salud UC-Christus para estudio de pacientes seleccionados para mutaciones CDH1
  - 15 pacientes con Cancer Gástrico y criterios de inclusión (abril 2018 y mayo 2019)
  - Ningún paciente resultó con mutaciones patogénicas,
  - Muchos pacientes con las mismas VUS, en pacientes no relacionados
- Proyecto de Cancer Hereditario HSR para pacientes de Cancer Gástrico y Cancer de Mama
  - Convenio Laboratorio INVITAE – GENOMETRICS: Panel para Cancer Gástrico y Cancer de Mama
  - Diplomado en Asesoramiento Genético, U. de Chile.
  - Resultados:
    - Cancer Gastrico: 10 pacientes (1 rechazó estudio, 2 pendientes por pandemia, 1 con mutación patogénica en ATM, 6 estudios negativos)
    - Cancer Gástrico Familiar: 1 familia (2 familiares) con ATM negativo.
    - Cancer de Mama Familiar: 32 pacientes derivados (4 pacientes con mutaciones patogénicas, 3 familias estudiadas)

# Identification of a Rare Germline Heterozygous Deletion Involving the Polycistronic miR-17–92 Cluster in Two First-Degree Relatives from a BRCA 1/2 Negative Chilean Family with Familial Breast Cancer: Possible Functional Implications



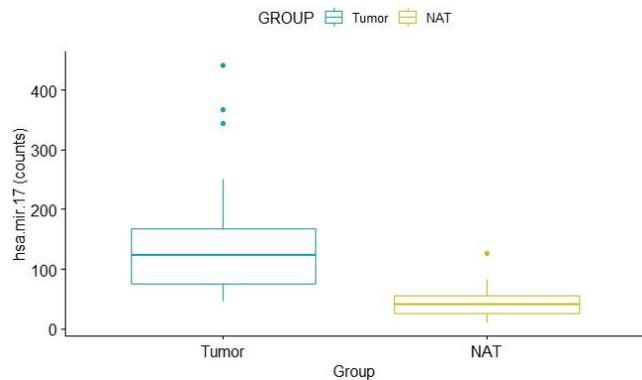
# Identification of a Rare Germline Heterozygous Deletion Involving the Polycistronic miR-17–92 Cluster in Two First-Degree Relatives from a BRCA 1/2 Negative Chilean Family with Familial Breast Cancer: Possible Functional Implications



# In Silico análisis of miR 17-92 cluster in Tumor vs NAT Analysis based on STAD-TCGA paired samples

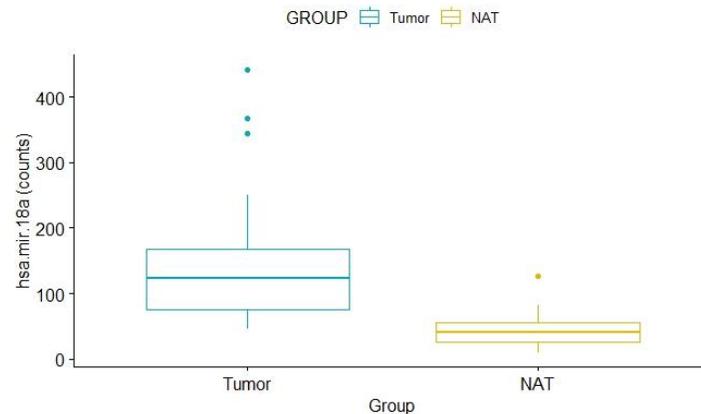
R. Artigas, Core Biodata-UC

## miR17



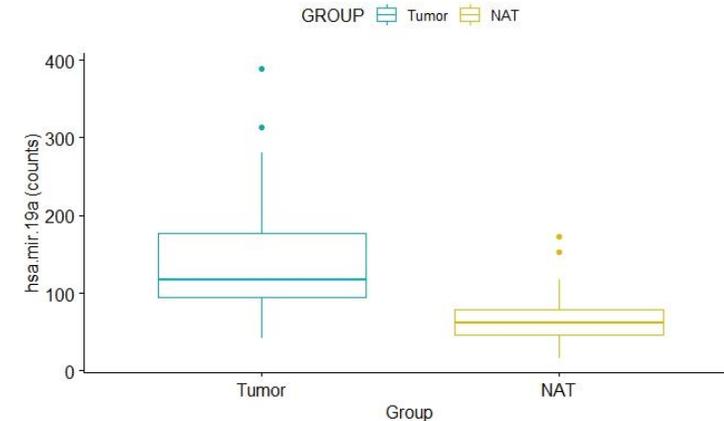
p-value = 0.0001909

## miR18-a



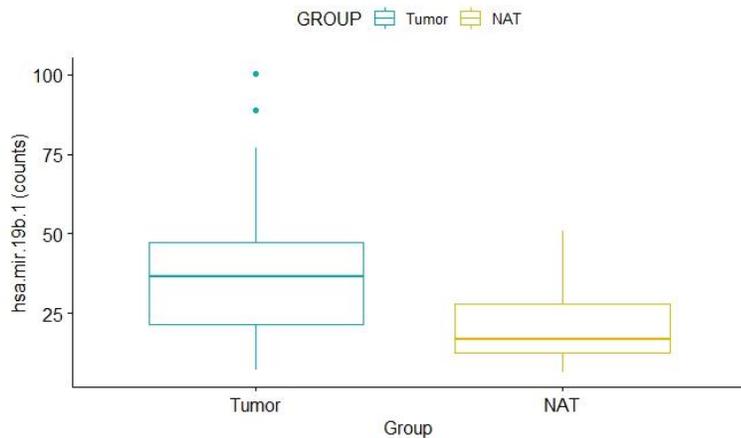
p-value = 2.498e-06

## miR 19-a



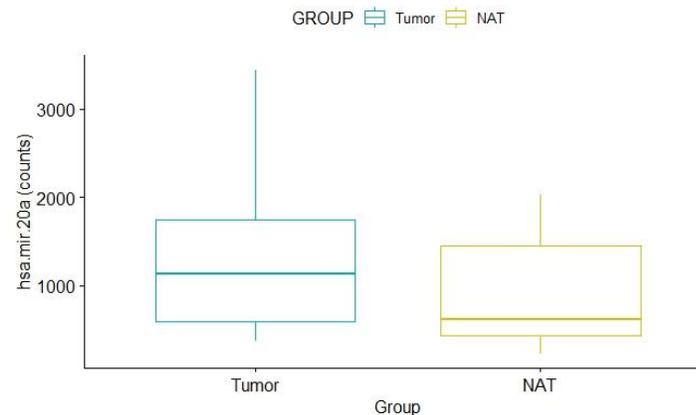
p-value = 2.235e-05

## miR 19-b1



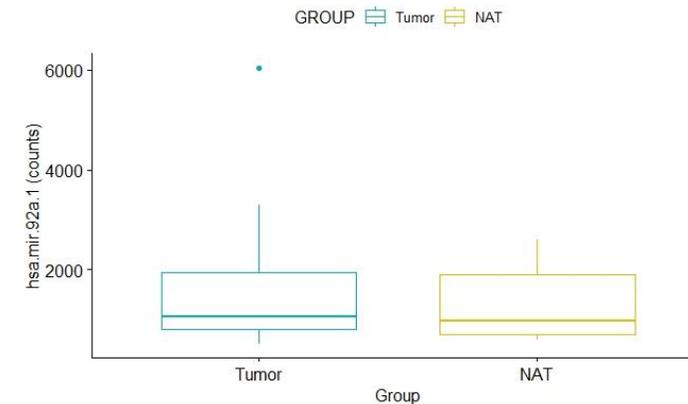
p-value = 0.0001731

## miR 20-a



p-value = 0.0001731

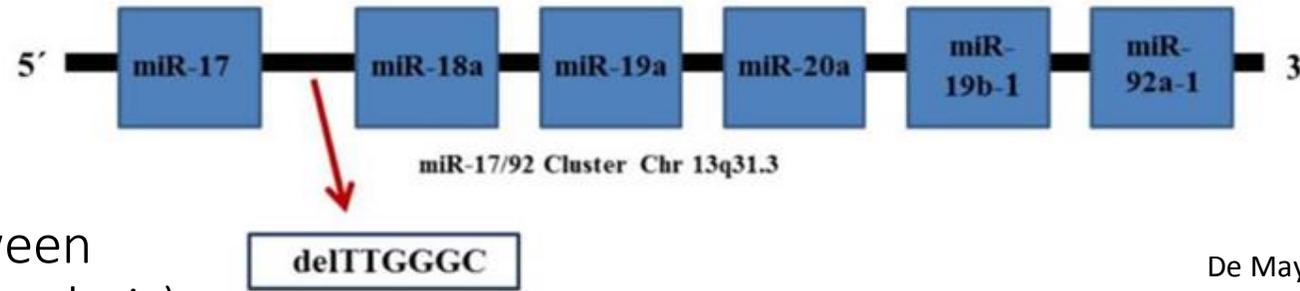
## miR 92-a1



p-value = 0.1873

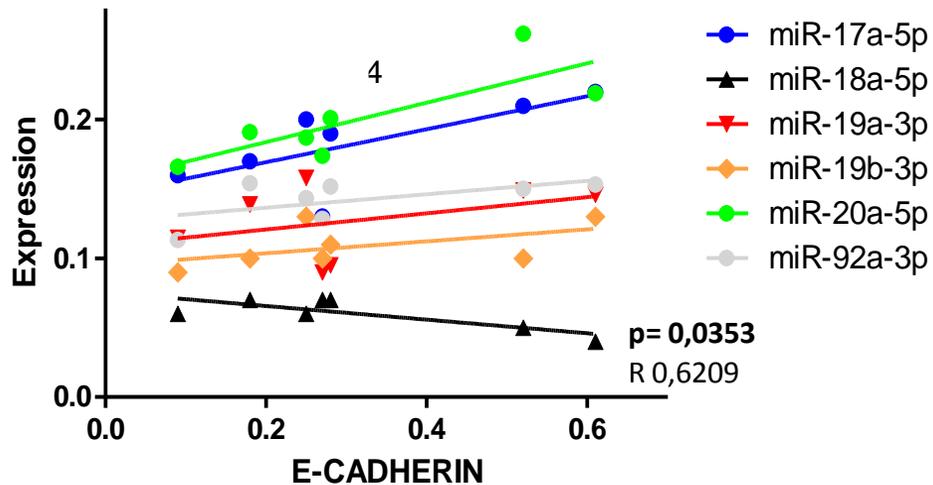
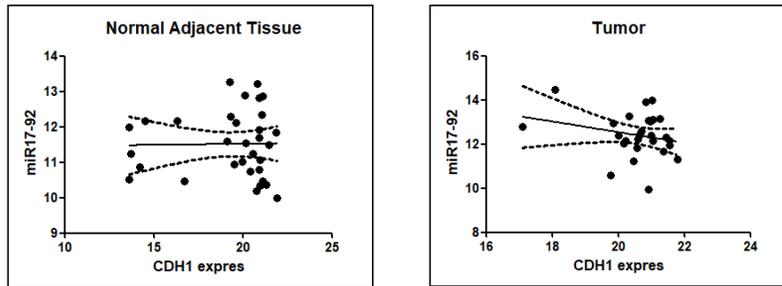
# Germline Heterozygous Deletion Involving the Polycistronic miR-17-92 Cluster (miR-17GH)

miR17HG



De Mayo *et al.* 2018 Int J Mol Sci (L. Jara's group)

An inverse relation between CDH1/miR17HG (TCGA analysis)



E.Norero, MD, Hospital Sotero del Rio  
50 Familial Gastric Cancer (CDH1 wild type)  
DelTTGGGC not detected

G.Molina, MD, PhD (postdoc L.Carvajal, UC Davis, US)  
35 Familal Gastric Cancer  
DelTTGGGC not detected

P. Gonzalez, PhD  
25 Early-onset gastric cancer  
DelTTGGGC not detected

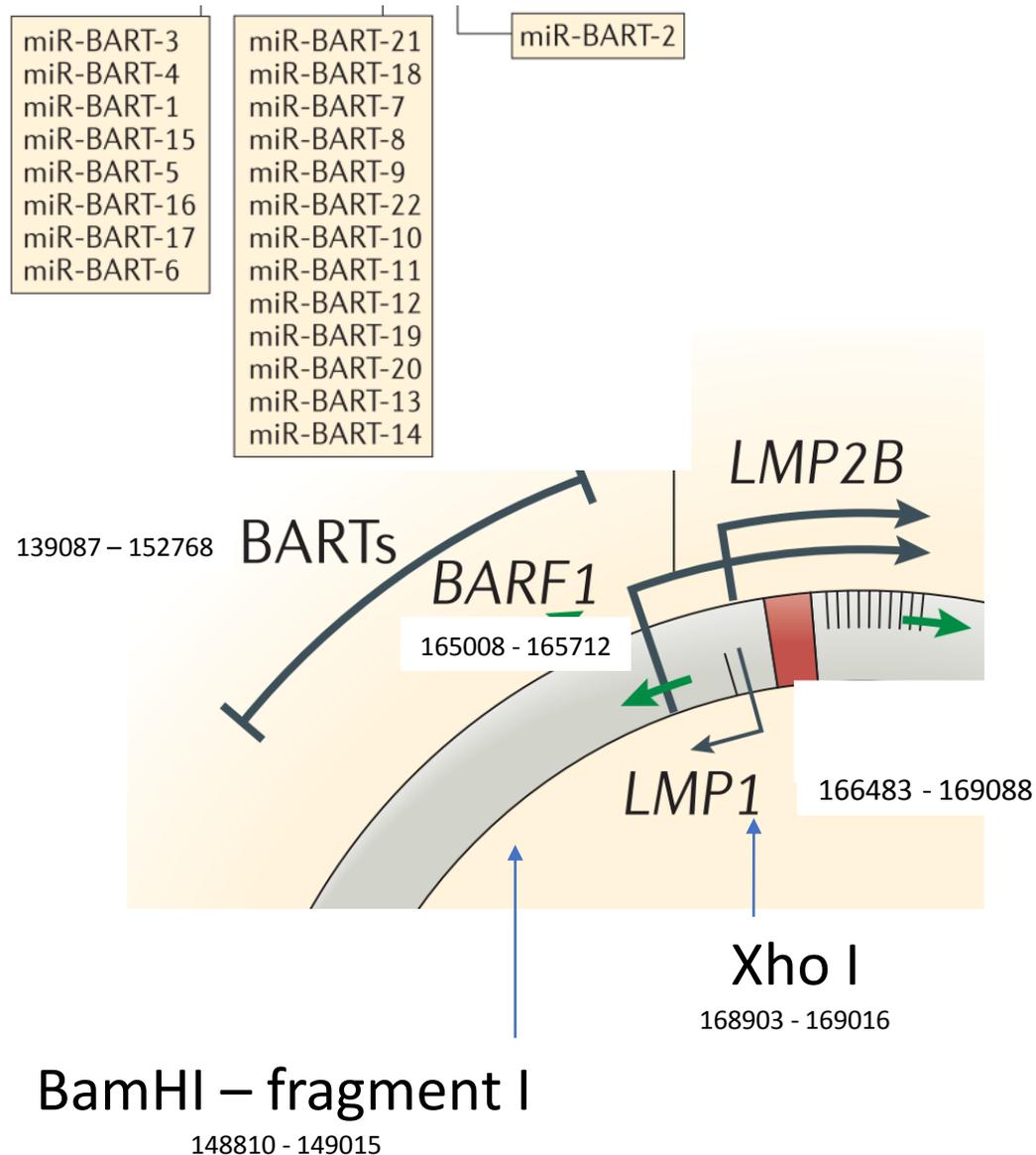
Next step: Germ line sequencing of miR-17HG

Landeros *et al.*, Virtual AACR meeting 22-24 June 2020 - Abstract # 6082.  
Loss of expression of wild-type transcript of CDH1 gene is associated with overexpression of microRNA-20 in diffuse-type of gastric cancer

## VALIDATION

- miLinker miR 17-92 cluster expression analysis in tissues samples of Familial Gastric Cancer cases
- DNA mutational analysis in tissue samples of Familial Gastric Cancer cases
- DNA mutational analysis in germ line samples of Familial Gastric Cancer cases

## Hypothesis 2: The interaction between Epstein-Barr virus (EBV) oncoproteins (EBNA-1 and LMP2A) and environmental exposures (i.e. tobacco and diet) induces dysregulation of the gastric cancer epigenome

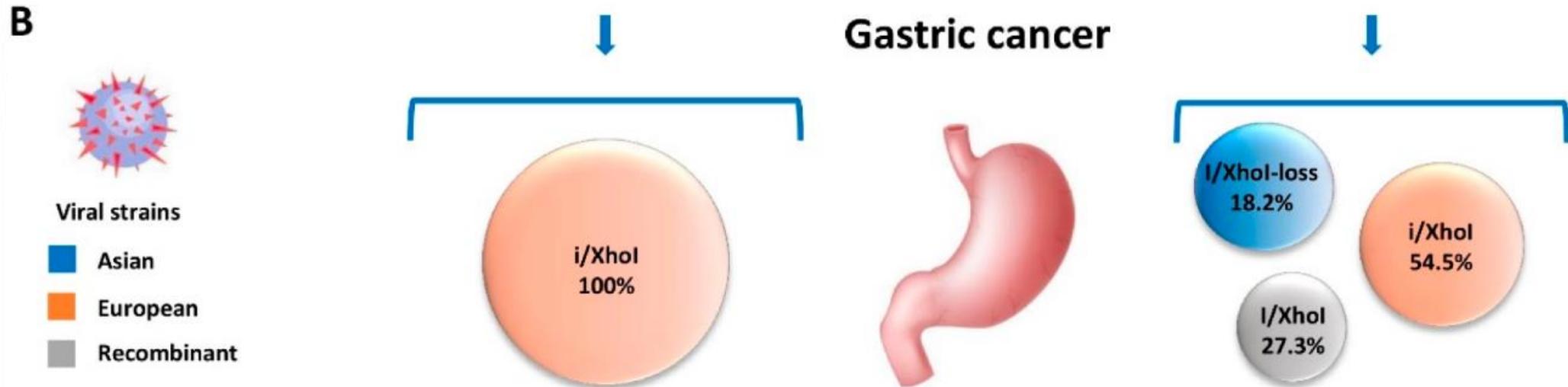
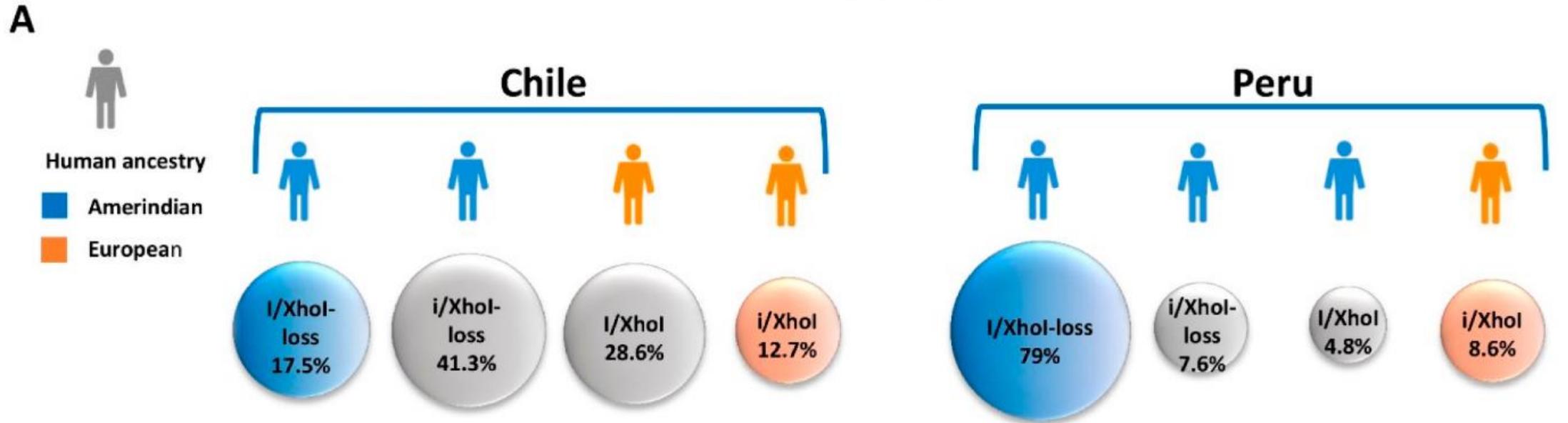


### Specific aim 2

To identify epigenomic dysregulation associated with interactions between EBV oncoproteins and environmental factors in EBV-associated GC (EBVaGC)

Francisco Aguayo (co-Inv) and Keila Torres (postdoc): in vitro assays and exosomes in EBV transfected gastric cancer cell lines. National Collaborators: Gareth Owen (co-Inv) and Marcelo Garrido (col): clinical samples with annotated data and FFPE DNA/RNA available and plasma, Catterina Ferreccio MAUCO samples, International Collaborators Charles Rabkin, NIH, USA

# Healthy population

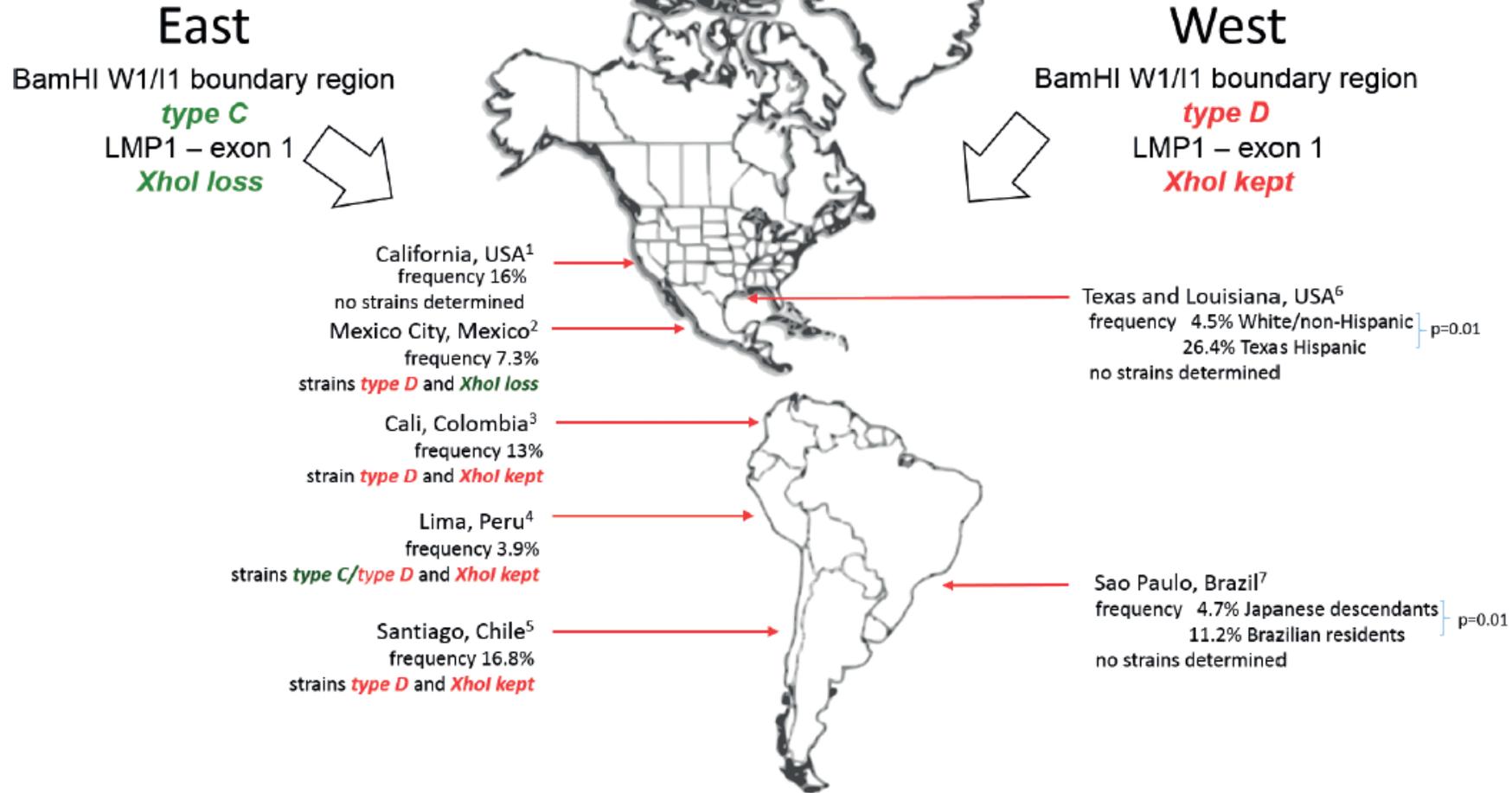


# High Proportion of Potential Candidates for Immunotherapy in a Chilean Cohort of Gastric Cancer Patients: Results of the FORCE1 Study

Table 2. Immunohistochemistry tumor analysis (*n* = 90).

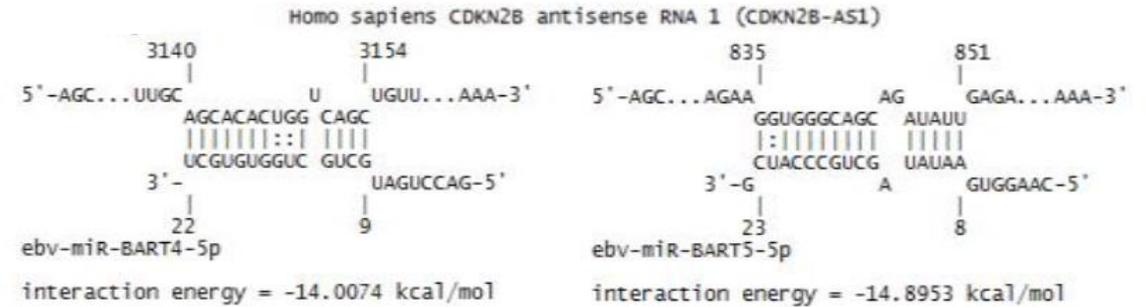
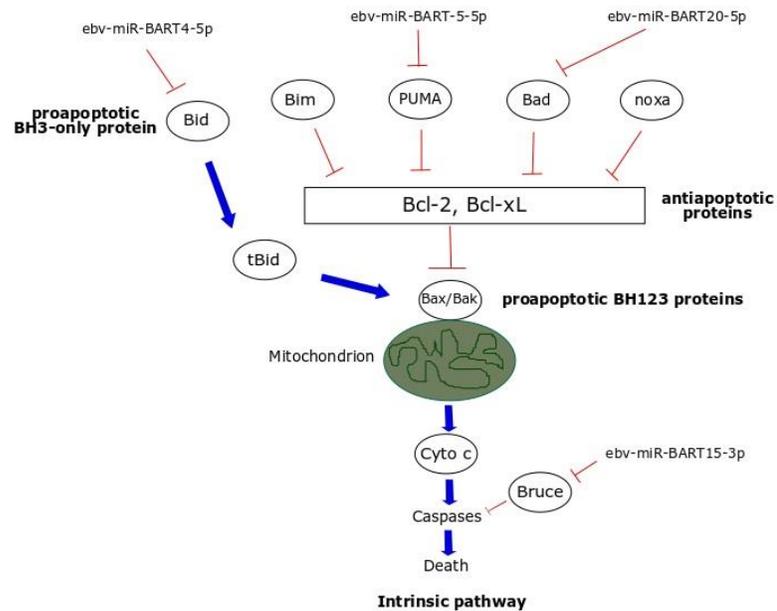
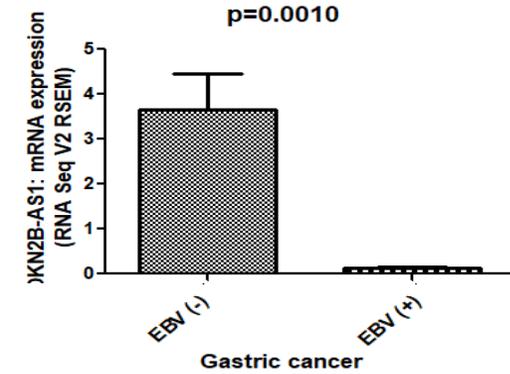
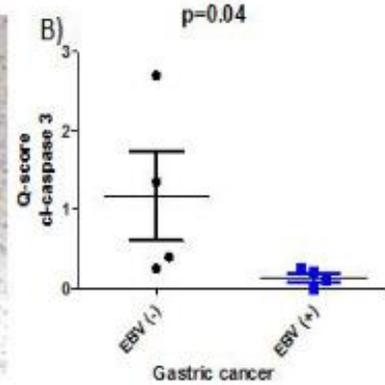
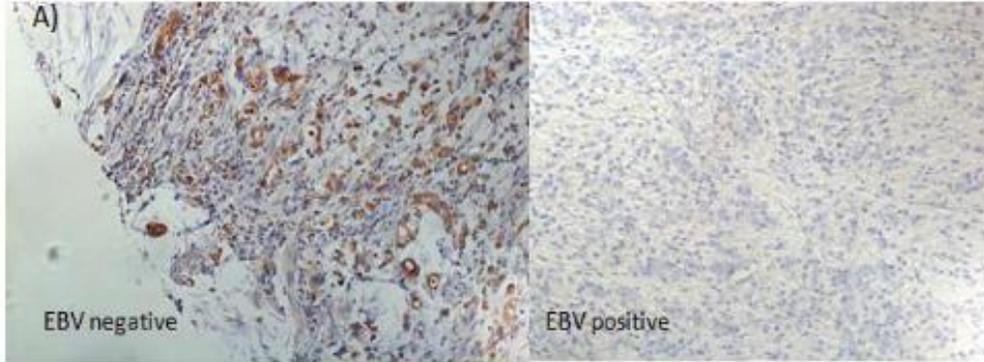
IHC Analysis	<i>n</i> (%)
<b>PDL-1 <sup>a</sup></b>	
Negative	64 (71.1)
Positive	26 (28.9)
<b>MSI+ (MMR deficient)</b>	13 (14.4)
<b>MLH-1</b>	
Intact	78 (86.7)
Lost	12 (13.3)
<b>PMS-2</b>	
Intact	78 (86.7)
Lost	12 (13.3)
<b>MSH-2</b>	
Intact	90 (100)
Lost	0
<b>MSH-6</b>	
Intact	88 (97.8)
Lost	2 (2.2)
<b>HER-2</b>	
Negative	78 (86.7)
Positive	12 (13.3)
<b>CISH-EBV</b>	
Negative	78 (86.7)
Positive	12 (13.3)
<b>p16</b>	
Absence	33 (36.7)
Presence	57 (63.3)
<b>p53</b>	
Intact	42 (46.7)
Mutated	48 (53.3)

# Epstein-Barr Associated Gastric Carcinoma in the Americas



# The loss of long non-coding RNAs CDKN2B-AS1 favor the inhibition of apoptosis by ebv-miR-BARTs in EBV-associated gastric cancer

(PostDoc grant awarded by Keila Torres 2020-2023)



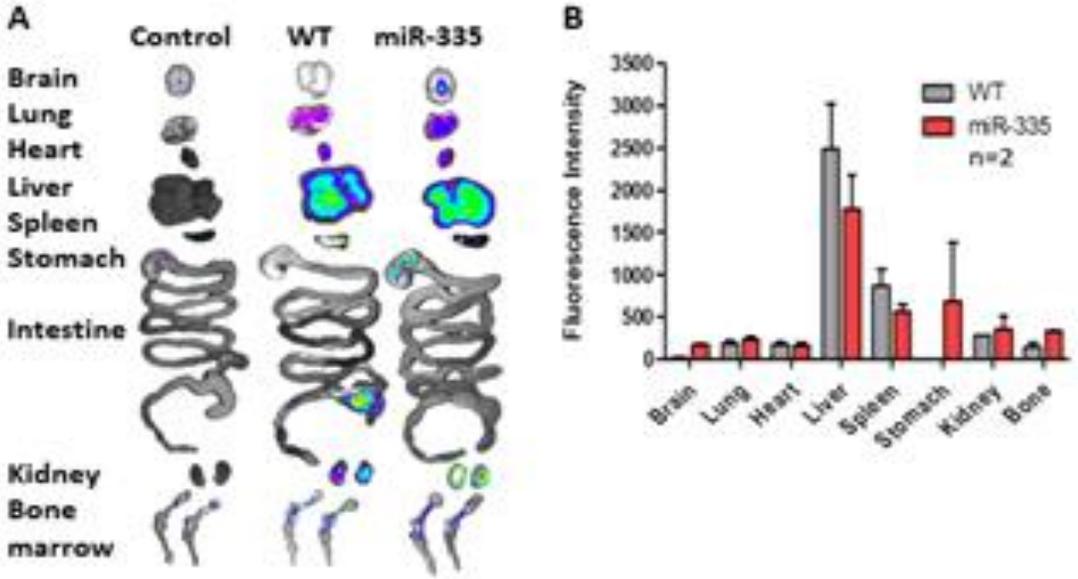
Pair Wise Sequence Comparison	Color Key	Score
ebv-miR-BART5 hsa-miR-18a cons	CAAGGGUGAAGUAGCUGCCCAUGG UAAGGGUGCAUCGAG-UCCAGAUAG *****	98

Torres et al., Virtual AACR meeting 22-24 June 2020 - Abstract # 5015  
 An additive effect of ebv-miR-BART5-5p and hsa-miR18a-5p in the downregulation of CDH1 transcript in Epstein-Barr virus associated gastric cancer

# Hypothesis 3: Exosomes cross the blood-brain barrier and influence the tumor growth in brain

## Specific aim 3

To investigate the role of exosomes and miR-335 in GC tumor dissemination in the brain



Biodistribution of exosomes in mice.

- A. Exosomes isolated from gastric cancer cells (WT) and cells transfected with miR-335 mimics (miR-335)
- B. Quantification of the fluorescence intensity for each organ

Collaborators: Iva Polakovicova (PUC) and Marcelo Andia (PUC),

Ph.D. student: Hector Ramos

Aim 3: To investigate the role of exosomes and their non-coding RNA cargo in gastric cancer dissemination

### IntraPeritoneal injection

Cell type	Number of mice	Tumor	Ascitis
Hs746T only	4	3	3
Hs746T + EV-335M	6	5	0
Hs746T + EV-WT	6	6	6

Abdominal Solid Tumor



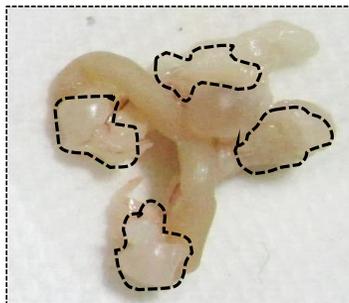
Associated Solid Spleen



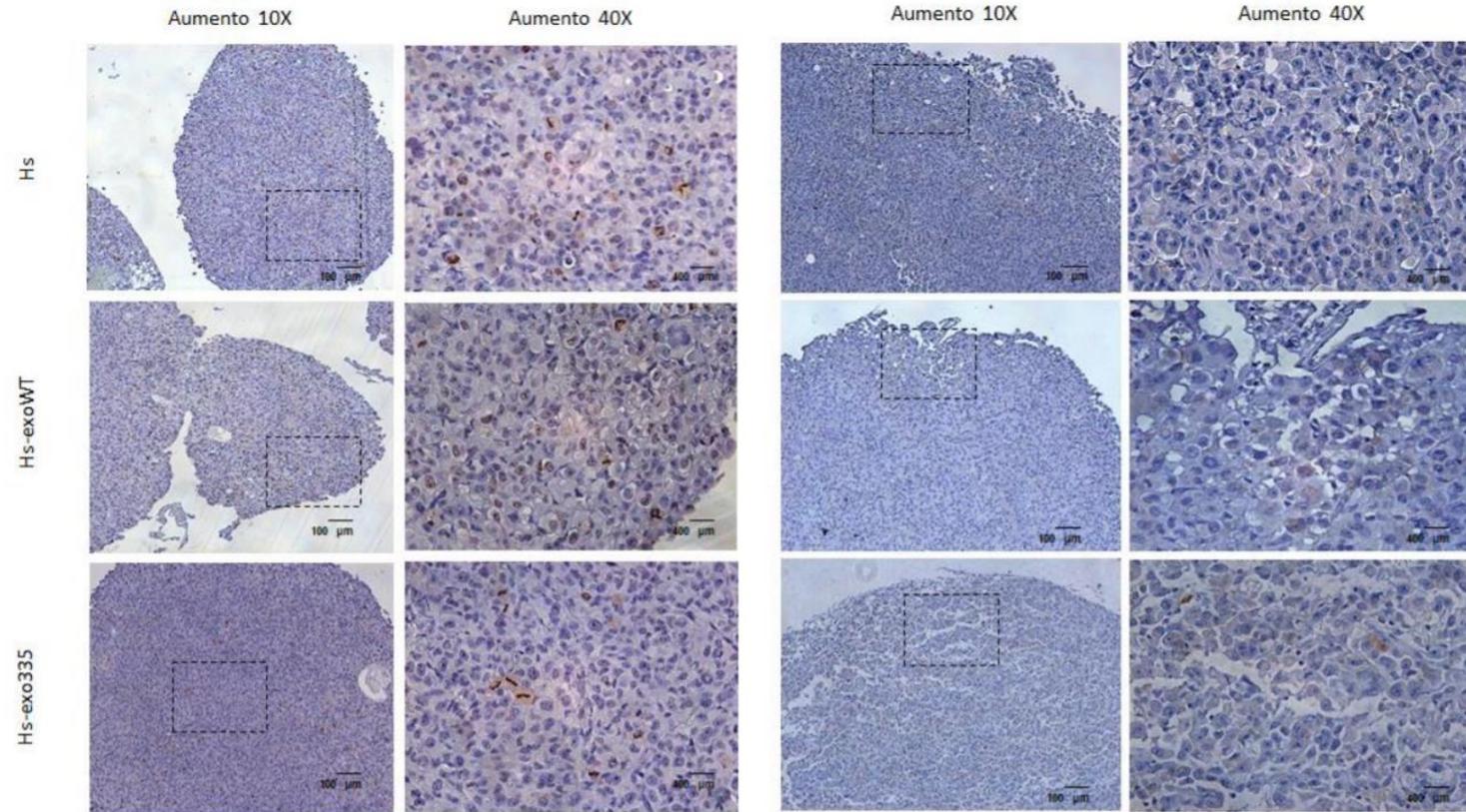
Mesenteric MultiTumor Nodules



Mesenteric Associated Intestine Nodules Formation



**Ki67 score in Hs-exo335 is lower, while the CASP-3 score tends to be higher**



Ki67 in tumor

CASP-3 in tumor

Alejandro Gottlieb and Paulina Gonzalez, Thesis UNAB 2019

*Polakovicova et al, manuscript in preparation*

# Acknowledgments

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**Fondecyt**  
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Pregun

**FONDAP**  
Fondo de Financiamiento de Centros de Investigación en Áreas Prioritarias