Molecular pathology of Ewing sarcoma: From diagnosis and target to treatment

Enrique de Alava

University Hospital of Salamanca and Centro de Investigación del Cáncer
Salamanca, Spain

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Abstract

In this lecture we will first define Ewing sarcoma (ES) as a small round cell sarcoma showing consistent molecular findings, and varying degrees of neuroectodermal differentiation by light or electron microscopy or immunohistochemistry. The term PNET was classically used for ES with evidence of neuroectodermal differentiation. ES is characterized by recurrent balanced translocations involving, in almost all cases, the EWSR1 gene on chromosome 22 and a member of the ETS family of transcription factors; this leads to the formation of novel fusion oncogenes that are the key to pathogenesis. There is an emerging group of Ewing-like sarcomas with closely related clinical-pathological features to ES, and fusion genes which do not involve members of the FET family of RNA binding molecules (EWSR1 or FUS).

Despite the recent advances in targeted therapy, prognosis for ES patients with disseminated disease or early relapse remains dismal, and the presence of metastatic disease at diagnosis continues to be the major prognostic factor. The need to find and inhibit new targets is therefore extremely urgent. To achieve this aim, a large number of targeted therapeutic approaches have been evaluated and some of them are being assessed in early phase human clinical trials.

Nevertheless, targeted therapy design requires a deep understanding of the mechanisms of initiation and progression of ES. As detailed in the talk our current knowledge includes at least these evidences: There is a key molecular event, which acts on the putative cell of origin, together with additional mutations, in an appropriate microenvironment, and there is some degree of heterogeneity with a clear tumor cell hierarchy.

In this lecture we will then outline the main technical platforms required for a biomarker discovery program in Ewing sarcoma. Specifically, we will address the role of:

- Biobanks, as key instruments with samples and related data available for research.
- Cooperative genomic analyses (copy number variation, next generation sequencing), such as ICGC, to generate hypothesis for new translational projects.
- Bioinformatics. We are faced with an explosion of systems-level data. Integrating this information to stimulate the discovery of new cancer therapies and associated biomarkers is a great challenge.
- *In vitro* and preclinical *in vivo* models, including shRNA screens and new animal models of disease.
We will then focus on some of the most promising drug targets/therapy strategies. Cooperative use of technical platforms has identified key molecular events leading to the progression and development of ES which are good candidates to targeted therapy. These include:

- Targeting chimeric EWS-ETS fusion proteins
- Targeting Ewing sarcoma signaling: Inhibitors of the tyrosine kinase receptors, such as IGF-1R, c-kit, PDGFR, VEGFR, or the mTOR signaling pathway, proteasome, angiogenesis, and stress response proteins are under clinical evaluation against ES.
- Targeting CD99, an important actor in Ewing sarcoma pathogenesis.
- Targeting other mechanisms of tumor progression.

Lastly, the need for a pipeline of phase I-II clinical trials as well as the role of several cooperative initiatives will be discussed. Specifically, phase I-II trials designed under the European project EuroSarc for ES will be described, as well as the translational research strategies to be developed with the samples collected in the clinical trials.

Acknowledgements

Research at Enrique de Alava’s lab is supported by the European Commission (Networks of excellence on sarcomas EuroBoNet and EuroSarc), as well as the Spanish Ministry of Science and Innovation, the regional Government of Castilla y León, the María García-Estrada Foundation, and PharmaMar.

Selected references


Graham C, Chilton-Macneill S, Zielenska M, Somers GR. The CIC-DUX4 fusion transcript is present in a subgroup of pediatric primitive round cell sarcomas. Hum Pathol. 2011 Aug 1. [Epub ahead of print]


Majewski IJ, Bernards R. Taming the dragon: genomic biomarkers to individualize the treatment of cancer. Nat Med 2011;17:304-12

Molecular pathology of Ewing sarcoma: From diagnosis and target to treatment.

ENRIQUE DE ALAVA
Outline

• Round cell tumor

• Sarcomagenesis

• Therapeutic possibilities
Outline

• Round cell tumor
Ewing sarcoma.
The paradigm of a small round cell tumor
17 y/o Male

CD99
## Routine techniques to detect translocations

<table>
<thead>
<tr>
<th></th>
<th>RT-PCR</th>
<th>interphase FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Cytology/Touch p.</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Paraffin</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>
No Sarcoma:
• Small cell ca
• Melanoma
• Lymphoma
• Neuroblastoma
• …

IHC

Sarcoma

IHC

Sarcoma no RMS

IHC

RMS

FISH

E RMS

A RMS

IHC

Synovial?

Ewing?

DSRCT?

Others
No Sarcoma:
- Small cell ca
- Melanoma
- Lymphoma
- Neuroblastoma
- …

Sarcoma

RMS

E RMS

A RMS

IHC

FISH

Synovial?

Ewing!!

DSRCT?

Others

SRCT
Diagnosis (in the molecular era)

• Good medical record-clinical examination
• Good image study
• Good biopsy-cytology
  – Benign or malignant?
  – Differentiation?
  – IHC, EM, molecular
Molecular genetics

Strongly recommended for

1. Unfrequent morphological subtypes
2. Uncommon clinicopathological presentations

ESMO guidelines, 2012 (in preparation)
Ewing-like sarcoma

Graham et al., Hum Pathol 2011 (ePubl)
CIC DUX4 fusion transcript in round cell sarcomas

CIC

| HMG box |

CIC-DUX4 Variant 1 (Positive Control, USTS1, USTS2)

| HMG box |

CIC-DUX4 Variant 3 (USTS3)

| HMG box |

- MAPK phosphorylation sites
HD: Homeodomain
HMG: High-mobility group
Ewing-like sarcoma

Italiano et al., Gen Chrom Canc 2011

CIC-DUX4
Some molecular understanding is sometimes helpful for diagnosis.
Outline

• Round cell tumor
Outline

• Round cell tumor

• Sarcomagenesis

• Therapeutic possibilities
The main elements

1. There is a **key** molecular event
2. Which acts on the **cell of origin**
3. Together with additional **mutations**
4. In an appropriate **microenvironment**
5. There is a tumor cell **hierarchy**
Sarcomagenesis

• **Gene fusions:**

• **Mutations:**

• **Copy number alterations**
Sarcomagenesis

- **Gene fusions:** Transcriptional dysregulation
- **Mutations:** Key genes/signaling pathways
- **Copy number alterations**
Sarcomagenesis

• Gene fusions: Transcriptional dysregulation
Translocation
Chimeric transcription factors (i.e. EWS-ETS)

Transactivation  DNA-binding
Chimeric transcription factors (i.e. EWS-ETS)
EWS-FLI1 Fusion Protein Up-regulates Critical Genes in Neural Crest Development and Is Responsible for the Observed Phenotype of Ewing’s Family of Tumors

Siwen Hu-Lieskovan, Jingsong Zhang, Lingtao Wu, Hiroyuki Shimada, Deborah E. Schofield, and Timothy J. Triche

(Cancer Res 2005; 65(11): 4633-44)
Factors affecting EWS-FLI1 activity

- **Post translational modifications**
  - N-linked glycosylation

- **Direct protein-protein interactions**
  - e.g. RNA helicase A, NR0B1

- **Factors indirectly affecting activity**
  - Hypoxia, IGR1R loop, p53/INK4A

- **miRNAs**
  - miR-145

*Herrero D et al., Sarcoma 2011*
The cell of origin
Plasticity of adult mesenchymal stem cells of the bone marrow

Growth Factors, Cytokines, Matrix

Pluripotent stromal cells

- myo D
- myogenin
- others

Myotube

PPARY

CBFA1

Fat cell

Osteoblasts

VEGF

FGF2

Endothelial cells

Sox9

Chondroblasts

Robbins 7th Edition
Three clues

1. Permissive cell of origin
2. Secondary genetic features
3. Microenvironment
Table 1. Immunohistochemical expression of the diagnostic markers

<table>
<thead>
<tr>
<th>Immunohistochemical marker</th>
<th>Negative (%)</th>
<th>Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD99</td>
<td>4 (1)</td>
<td>612 (99)</td>
</tr>
<tr>
<td>HNK.1</td>
<td>292 (47)</td>
<td>329 (53)</td>
</tr>
<tr>
<td>FLI1</td>
<td>87 (15)</td>
<td>496 (85)</td>
</tr>
<tr>
<td>Caveolin1</td>
<td>29 (5)</td>
<td>516 (95)</td>
</tr>
</tbody>
</table>

LOW: -/+ , +--
HIGH: +++-/++++

256/274
93% positive cases

193 High
63 Low
18 Neg
Smaller
• Have a higher expression of EWS-FLI1
• Show a higher expression of stemness and drug resistance proteins
• Good sarcoma xenograft model
• No expression of stem cell markers…

Whether this population represents cancer stem cells or intrinsically drug resistant tumour cells: the SP cells represent a subtype of sarcoma cells with highly malignant features.
How do these players interact?

• hMSC from normal donors and Ewing sarcoma patients are quite similar, lack translocations and a significant CD99 expression (Unpublished Eurobonet results).

• EWS-FLI1 upregulates CD99 and IGF1 (Herrero D, Br J Canc 2009; Rocchi et al., J Clin Inv 2010).

• There is a cellular hierarchy, including a side population (Dirksen et al., unpublished)
Sarcomagenesis

• Gene fusions: Transcriptional dysregulation

• Mutations: Key genes/signaling pathways

• Copy number alterations
Sarcomagenesis

• Gene fusions: Transcriptional dysregulation

• Mutations: Key genes/signaling pathways

• Copy number alterations
Magic Cancer Bullet

How a Tiny Orange Pill Is Rewriting Medical History

DANIEL VASHELLA, M.D.

Chairman and CEO, Novartis

with ROBERT SLATER
Ewing Sarcomas With \( p53 \) Mutation or \( p16/p14ARF \) Homozygous Deletion: A Highly Lethal Subset Associated With Poor Chemoresponse

Hsuan-Ying Huang, Peter B. Illei, Zhiquan Zhao, Madhu Mazumdar, Andrew G. Huvos, John H. Healey, Leonard H. Wexler, Richard Gorlick, Paul Meyers, and Marc Ladanyi

*J Clin Oncol 23:548-558. © 2005 by American Society of Clinical Oncology*

### Table 1. \( p53 \) and \( p16/p14ARF \) Alterations in Uncultured Ewing Sarcoma/Peripheral Neuroectodermal Tumor Samples

<table>
<thead>
<tr>
<th>Study Site</th>
<th>Publication Date</th>
<th>( p53 ) Alteration*</th>
<th>( p16/p14ARF ) Homozygous Deletion</th>
<th>Either Alteration (or both)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vienna, Austria</td>
<td>1993, 1997</td>
<td>2/37</td>
<td>7/27</td>
<td>—†</td>
<td>16,17</td>
</tr>
<tr>
<td>Tokyo, Japan</td>
<td>1993</td>
<td>2/14</td>
<td>—</td>
<td>—</td>
<td>18</td>
</tr>
<tr>
<td>Paris, France</td>
<td>1994</td>
<td>2/12</td>
<td>—</td>
<td>—</td>
<td>19</td>
</tr>
<tr>
<td>Boston, MA</td>
<td>1995</td>
<td>3/38</td>
<td>—</td>
<td>—</td>
<td>20</td>
</tr>
<tr>
<td>Pamplona, Spain</td>
<td>1997</td>
<td>1/5</td>
<td>0/5</td>
<td>1/5</td>
<td>21</td>
</tr>
<tr>
<td>Magdeburg, Germany</td>
<td>1998</td>
<td>1/24</td>
<td>—</td>
<td>—</td>
<td>22</td>
</tr>
<tr>
<td>Birmingham, UK</td>
<td>1999</td>
<td>7/52</td>
<td>—</td>
<td>—</td>
<td>23</td>
</tr>
<tr>
<td>Saitama, Japan</td>
<td>2000</td>
<td>1/24</td>
<td>3/24</td>
<td>3/24</td>
<td>26</td>
</tr>
<tr>
<td>Valencia, Spain</td>
<td>2001</td>
<td>3/19</td>
<td>4/19</td>
<td>7/19</td>
<td>27</td>
</tr>
<tr>
<td>Compilation of published data</td>
<td>28/280 (10%)</td>
<td>21/114 (18%)</td>
<td>11/48 (23%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present study</td>
<td>8/60 (13%)</td>
<td>8/60 (13%)</td>
<td>15/60 (25%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Relationship of p53 and/or p16/p14ARF Alterations to Chemotherapy Response

<table>
<thead>
<tr>
<th>Alteration of p53 and/or p16/p14ARF</th>
<th>Chemoresponse*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good</td>
</tr>
<tr>
<td>Present</td>
<td>0</td>
</tr>
<tr>
<td>Absent</td>
<td>9</td>
</tr>
</tbody>
</table>

Univariate $P < .0001$
Multivariate $P < .001$
Sarcomagenesis

- **Gene fusions**: Transcriptional dysregulation
- **Mutations**: Key genes/signaling pathways
- **Copy number alterations**
Sarcomagenesis

- **Gene fusions**: Transcriptional dysregulation
- **Mutations**: Key genes/signaling pathways
- **Copy number alterations**
Genomic profile by aCGH
amount of tumor DNA vs normal control
CINSARC
PREDICTION OF CLINICAL OUTCOME IN SARCOMAS
BASED ON A GENE-EXPRESSION SIGNATURE
RELATED TO GENOME COMPLEXITY
(Compexity INdex of SARComas)

Frédéric Chibon
Department of Pathology
INSERM U916
Bergonie Institute
Bordeaux, France

French Sarcoma Group
Multivariate analysis

HR = 3.1; 95% CI [1.8 – 5.4]

CINSARC is an independent prognostic factor
1q gain and \textit{CDT2} overexpression underlie an aggressive and highly proliferative form of Ewing sarcoma

C Mackintosh\textsuperscript{1}, JL Ordóñez\textsuperscript{1}, DJ García-Domínguez\textsuperscript{1}, V Sevillano\textsuperscript{1}, A Llombart-Bosch\textsuperscript{2}, K Szuhai\textsuperscript{3}, K Scotlandi\textsuperscript{4}, M Alberghini\textsuperscript{5}, R Sciot\textsuperscript{6}, F Sinnaeve\textsuperscript{7}, PCW Hogendoorn\textsuperscript{8}, P Picci\textsuperscript{4}, S Knuutila\textsuperscript{9}, U Dirksen\textsuperscript{10}, M Debiec-Rychter\textsuperscript{11}, K-L Schaefer\textsuperscript{12} and E de Alava\textsuperscript{1,13}
Impact of CNAs and 1qG on survival of ES patients
Impact of CNAs and 1qG on survival of ES patients

Multivariate analysis: Independent factor of survival
- Metastasis
- Tumor size
- 1qG
Impact of CNAs and 1qG on survival of ES patients

Multivariate analysis:

Independent factor of survival
- Metastasis
- Tumor size
- 1qG
Functional relevance of candidate gene

Mackintosh et al., Oncogene 2011
Sarcomagenesis

- Gene fusions: Transcriptional dysregulation
- Mutations: Key genes/signaling pathways
- Copy number alterations
Outline

• Round cell tumor

• Sarcomagenesis

• Therapeutic possibilities
Outline

• Round cell tumor

• Sarcomagenesis

• Therapeutic possibilities
Sarcoma therapies

• Surgery
• Radiotherapy
• Chemotherapy
Molecular understanding is also needed for treatment
Primary Disseminated Multifocal Ewing Sarcoma: Results of the Euro-EWING 99 Trial

Ruth Ludenstein, Ulrike Pötschger, Marie-Cécile Le Deley, Jeremy Whelan, Michael Paulussen, Odile Overdin, Henk van den Berg, Uta Dirksen, Lars Hohls, Jan Mico, Ian Lewis, Alan Craft, and Herbert Jürgens

<table>
<thead>
<tr>
<th>Group</th>
<th>Patients</th>
<th>Events</th>
<th>3-yr pEFS ± SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 14 years</td>
<td>99</td>
<td>61</td>
<td>0.40 ± 0.05</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>&gt; 14 years</td>
<td>182</td>
<td>145</td>
<td>0.19 ± 0.03</td>
<td></td>
</tr>
</tbody>
</table>
Prospective clinical trial

Genomics
Proteomics

Functional validation
(in vitro/in vivo)
Tissue Validation

Cell lines
Biobanks
GE Animal models

Bioinformatics
shRNA screens
xenografts

Prospective clinical trial
Prospective clinical trial

Genomics
Proteomics

Functional validation (in vitro/in vivo)
Tissue Validation

Cell lines
Biobanks
GE Animal models

Bioinformatics
shRNA screens
xenografts

Prospective clinical trial
ICGC Cancer Genome Projects

Committed projects to date: 39

Sort by: Project

ICGC Goal: To obtain a comprehensive description of genomic, transcriptomic and epigenomic changes in 60 different tumor types and/or subtypes which are of clinical and societal importance across the globe.

Read more »

Launch Data Portal »

Apply for Access to Controlled Data »

Announcements

5/June/2011 - The ICGC Data Coordination Center is pleased to announce the release of version 5 of the ICGC data portal. This release includes the first data submission from the Chronic Lymphocytic Leukaemia (CLL) project, whose work was published in Nature on June 9th. (Read the article) The addition of this new project brings the total number of cancer projects in the release to 25. With this release, the CLL project also becomes the first to host its own ICGC data portal server. Users visiting the ICGC data portal now have the option of choosing to access all ICGC datasets through the portal hosted in Canada at CISCA, or through the portal hosted at EBI in the United Kingdom.
Problems for targeted therapies in Ewing sarcoma

• No preneoplastic lesion
• No “normal tissue” counterpart
• No genetically modified animal model available
• Sample availability
• Regulatory constraints
Chimeric transcription factors (i.e. EWS-ETS)
Sarcoma translocations as therapeutic targets: two caveats

- Fusion proteins are mostly intracellular molecules that are not expressed on the tumor cell surface.
- Many are not enzymes (i.e. kinases, like BCR-ABL) but rather are involved in protein-protein or protein-nucleic acid interactions, making them more challenging drug targets.
- Better to target the targets?
EWS-FLI1 is the perfect target

Without

a therapeutic agent
A small molecule blocking oncogenic protein EWS-FLI1 interaction with RNA helicase A inhibits growth of Ewing’s sarcoma

Hayriye V Erkizan¹, Yali Kong¹, Melinda Merchant², Silke Schlottmann¹, Julie S Barber-Rotenberg¹, Linshan Yuan¹, Ogan D Abaan¹, Tsu-hang Chou², Sivanesan Dakshanamurthy¹, Milton L Brown¹, Aykut Üren¹ & Jeffrey A Toretsky¹,³,⁴
## Targeting EWS-FLI1 activity

<table>
<thead>
<tr>
<th>Drug</th>
<th>targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mevalonate</td>
<td>N-linked glycosilation</td>
</tr>
<tr>
<td>YK-4-279</td>
<td>Interaction EWS-FLI1 with RNA helicase A</td>
</tr>
<tr>
<td>Nutlin 3a</td>
<td>Antagonizes interaction p-53/MDM2</td>
</tr>
<tr>
<td>Mithramycin</td>
<td>EWS-FLI1</td>
</tr>
<tr>
<td>ARA-C</td>
<td>EWS-FLI1</td>
</tr>
</tbody>
</table>
High-throughput luciferase-based primary screen and multiplex polymerase chain reaction secondary screen of a library of more than 50,000 compounds.

---

Grohar P J et al. JNCI 2011;103:962-978
Therapies targeting mechanisms of pathogenesis

- CD99
- TRAIL receptor
- sRNA
- Fenretinide
- IGFR
- Caspase 8
- Cytochrome C
- RIBOSOME
- EWS-FLI1
- MITOCHONDRIA
- ROS
- LYSOSOMES
- antioxidants
- p38
- ceramide
- RAS
- PI3K
- RAF
- MEK
- AKT
- ERK
- mTOR

Cell death vs. Cell survival
Both IGF/IGF-IR system and CD99 are required to create permissive condition for EWS-FL1 transformation.
IGF1R inhibitors in Ewing sarcoma
Clinical experience

- Massive response to anti-IGF1R inhibitors (30%)
- Vast majority lasts less than 12 weeks
- Lack of Predictive factors
  - No mutations of IGF1R
  - No IGF1R amplifications
- Drug combinations?

Olmos et al., 2010
HSP90 expression could be a predictive factor of response to IGF1R therapies and a possible therapeutic target.

Ecteinascidin 743 Interferes with the Activity of EWS-FLI1 in Ewing Sarcoma Cells

Patrick J. Grohar*, Laurie B. Griffin*, Choh Yeung*, Qing-Rong Chen†, Yves Pommier‡, Chand Khanna§, Javed Khan† and Lee J. Helman*

*Molecular Oncology Section, Pediatric Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA; †Oncogenomics Section, Pediatric Research Program, Dana-Farber Cancer Institute, Boston, MA, USA; §Department of Medicine, Dana-Farber Cancer Institute; *Deceased

Neoplasia Vol. 13, No. 2, 2011
Yondelis in Ewing sarcoma

- Yondelis and HSP90 inhibitors
- Yondelis and IGF1R inhibitors
Yondelis in Ewing sarcoma

• Yondelis and HSP90 inhibitors

• Yondelis and IGF1R inhibitors
EuroBoNeT
European Network to Promote Research into Uncommon Cancers in Adults and Children: Pathology, Biology and Genetics of Bone Tumours

Network of Excellence

Life sciences, genomics and biotechnology for health
LSH-2004-2.2.8.1: Uncommon cancers in adults and children

TRIAL PROTOCOL PROPOSAL

Phase I/II study of Trabectedin (ET-743) combined with IGF1 receptor blockade (CP-751, 871) in patients with relapsed and/or refractory Ewing's sarcoma family of tumours

Version 1:

Date of preparation: 30th August 2009

Proposed start date of study: 1st quarter of 2010
Duration: 18 months

Study PI
Prof A B Hassan, on behalf of EuroBoNeT Translational Research Workpackage
University of Oxford and Oxford Cancer & Haematology Centre
Why combining Trabectedin with IGF1R/IR inhibitors in Ewing sarcoma?

1. EWS-FLI1 triggers Ewing sarcoma dependency on IGF1R signaling

2. Sensitivity to Trabectedin in t-sarcomas

3. High IGF1R signaling in Trabectedin-resistant ES cell lines
Functional pathways involved in 1qG 40-gene signature

Mackintosh et al., Oncogene 2011
Ongoing work: preclinical validation in ES of a specific inhibitor of the CRL protein-ubiquitin-ligase complexes

An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer

Teresa A. Soucy¹, Peter G. Smith¹, Michael A. Milhollen¹, Allison J. Berger¹, James M. Gavin¹, Sharmila Adhikari¹, James E. Brownell¹, Kristine E. Burke¹, David P. Cardin¹, Stephen Critchley¹, Courtney A. Cullis¹, Amanda Doucette¹, James J. Garnsey¹, Jeffrey L. Gaulin¹, Rachel E. Gershman¹, Anna R. Lublinsky¹, Alice McDonald¹, Hirotake Mizutani¹, Usha Narayanan¹, Edward J. Olhava¹, Stephane Peluso¹, Mansoureh Rezaei¹, Michael D. Sintchak¹, Tina Talreja¹, Michael P. Thomas¹, Tary Traore¹, Stepan Vyskocil¹, Gabriel S. Weatherhead¹, Jie Yu¹, Julie Zhang¹, Lawrence R. Dick¹, Christopher F. Claiborne¹, Mark Rolfe¹, Joseph B. Bolen¹ & Steven P. Langston¹

¹Discovery, Millennium Pharmaceuticals, Inc., 40 Landsdowne Street, Cambridge, Massachusetts 02139, USA.
Outline

• Round cell tumor

• Sarcomagenesis

• Therapeutic possibilities
Lab. PMD-BT
Teresa Hernández
Susana Fraile
Jairo Nieto
Telmo Teixeira

Lab. PMS
Ana Pastora Otero
Ana Sofía Martins
Ana Teresa Amaral
Carlos Mackintosh
Daniel J García
José Luis Ordóñez
Victoria Barbado
Victoria Sevillano

Thanks!