SPANISH NATIONAL CANCER RESEARCH CENTRE

CNIO - "LA CAIXA" FOUNDATION FRONTIERS MEETINGS 2017

Madrid 19th-22nd February 2017

PRIMARY AND SECONDARY BRAIN TUMORS

Organisers

Massimo Squatrito
Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Manuel Valiente
Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Richard Gilbertson
CRUK Cambridge Institute, UK

Michael Weller
University Hospital Zurich, Switzerland

GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA, INDUSTRIA Y COMPETITIVIDAD
Instituto de Salud Carlos III
EXCELENCIA SEVERO OCHOA
cnio Centro Nacional de Investigaciones Oncológicas
"la Caixa" Foundation

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Centro Nacional
de Investigaciones
Oncológicas

SPANISH NATIONAL CANCER RESEARCH CENTRE
CNIO - "LA CAIXA" FOUNDATION
FRONTIERS MEETINGS 2017

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PRIMARY AND SECONDARY BRAIN TUMORS

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SPANISH NATIONAL CANCER RESEARCH CENTRE
CNIO - "LA CAIXA" FOUNDATION
FRONTIERS MEETINGS 2017

Madrid 19th-22nd February 2017

PRIMARY AND SECONDARY BRAIN TUMORS

PROGRAMME

Madrid 19th-22nd February 2017

PRIMARY AND SECONDARY BRAIN TUMORS

Venue:
Spanish National Cancer Research Centre – CNIO Auditorium, Madrid, Spain

Organisers

Massimo Squatrito
Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Manuel Valiente
Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Richard Gilbertson
CRUK Cambridge Institute, UK

Michael Weller
University Hospital Zurich, Switzerland

Invited Journal:

Nature Medicine

Sponsors:

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19th February, 2017 - Sunday

20:00-21:30 *Welcome Cocktail for all participants*

Venue: Hotel Don Pío (Avenida Pío XII, 25, 28016 Madrid)
www.hoteldonpio.com/en/

20th February, 2017 - Monday

(venue: CNIO AUDITORIUM Melchor Fernandez Almagro street- Madrid www.cnio.es)

09:30 - 09:45 Opening Remarks **Michael Weller**

09:45 - 10:45 **Keynote lecture**
Chairperson: Michael Weller

Mapping cancer origins - beyond the brain
Richard Gilbertson, *Cambridge Cancer Center, University of Cambridge, UK*

10:45 - 11:00 *Coffee break (social room)*

11:00 - 12:50 Session#1 **PRIMARY ADULT BRAIN TUMORS (I)**
Chairperson: Massimo Squatrito

11:00 - 11:30 Modeling the landscape of human gliomas in the mouse
Eric Holland
Fred Hutchinson Cancer Research Center, US

11:30 - 12:00 Microenvironmental conditioning in glioma
Gabriele Bergers
Vesalius Research Center, Belgium

12:00 - 12:20 ATRX deficiency modulates differentiation state and migratory behavior by way of global epigenomic remodeling in glioma cells of origin


Jason Huse
MD Anderson Cancer Center, US

12:20 - 12:50 Mouse Models of Neural Cancer: Cancer Stem Cells & Therapeutic Insights
Luis Parada
Memorial Sloan Kettering Cancer Center, US

12:50 - 14:20 *Lunch break (cafeteria)*

14:20 - 18:00 Session#2 **PRIMARY ADULT BRAIN TUMORS (II)**
Chairperson: Eric Holland

14:20 - 14:50 Signaling pathways in glioma tumor formation and therapy resistance
Massimo Squatrito
Spanish National Cancer Research Centre, CNIO, Spain

14:50 - 15:10 

Mechanisms for EGFR signaling regulation by intracellular trafficking in Glioblastoma
Marta Portela Esteban
Cajal Institute, Spain

15:10 - 15:30 

TRF1 as a novel target in Glioblastoma
Maria Blasco
Spanish National Cancer Research Centre, CNIO, Spain

15:30 - 16:00 Next-generation brain tumor classification
Stefan Pfister
DKFZ, Germany

16:00 - 16:30 Molecular dissection of disease evolution in glioma
Roel Verhaak
The Jackson Laboratory, US

16:30 - 17:00 *Coffee break (social room)*

17:00 - 17:30 Immunotherapy for Glioblastoma
Michael Weller
University Hospital Zurich & University of Zurich, Switzerland

17:30 - 18:00 Next Generation Immune Therapeutics for GBM
Amy Heimberger
MD Anderson Cancer Center, US

18:00 - 20:00 *Poster Session & Wine and cheese (social room)*

21st February, 2017 - Tuesday

09:30 - 09:55 'Nature Medicine, meet the editor'
Navigating the publishing process in Nature Medicine
Javier Carmona, *Assistant Editor*

09:55 - 13:05 Session#3 **SECONDARY ADULT BRAIN TUMORS (I)**
Chairperson: Manuel Valiente

09:55 - 10:25 Cellular Networks: Implications for Brain Tumor Biology and Therapy
Frank Winkler
DKFZ, Germany

10:25 - 10:55 Genomic Evolution of Brain Metastases
Priscilla Brastianos
Massachusetts General Hospital, US

10:55 - 11:15 

New pericytes in glioblastoma (GBM) blood-vessels originate from a previously unrecognized, pericyte progenitor-like cell
Rainer Glass
Ludwig Maximilians University, Germany

11:15 - 11:50 *Group Picture & coffee break (social room)*

11:50 - 12:20 How to best design future clinical trials in brain metastasis
Riccardo Soffietti
University and City of Health and Science University Hospital of Turin, Italy

12:20 - 12:40 

Targeting carcinoma–astrocyte gap junctions in brain metastasis
Adrienne Boire
Memorial Sloan-Kettering Cancer Center, US

12:40 - 13:10 Evolving intratumor heterogeneity in primary and secondary brain tumor
Joan Seoane
Vall d´Hebron Institute of Oncology, VHIO, Spain

13:10 - 14:30 *Lunch break (cafeteria)*

14:30 - 18:10 Session#4 **SECONDARY ADULT BRAIN TUMORS (II)**
Chairperson: Riccardo Soffietti

14:30 - 15:00 The inflammatory microenvironment of brain metastases and its potential role as therapy target
Matthias Preusser
Comprehensive Cancer Center Vienna, Austria

15:00 - 15:30 On-site evolution of metastatic cells and surrounding environment during brain colonization
Manuel Valiente
Spanish National Cancer Research Centre, CNIO, Spain

15:30 - 15:50 

Induction of lysosomal membrane permeability as a novel glioma treatment
Pirjo Laakkonen
University of Helsinki, Finland

15:50 - 16:10 

EANO-ESMO recommendations for the diagnosis and treatment of leptomeningeal metastasis from solid tumors
Emilie Le Rhun
CHRU University Hospital, France

16:10 - 16:40 *Coffee break (social room)*

16:40 - 17:10 Targeting the inflammatory response for diagnosis and treatment of brain metastasis
Nicola Sibson
CRUK/MRC Oxford Institute for Radiation Oncology, University of Oxford, UK

17:10 - 17:40 Clinical trials using molecular precision concepts
Wolfgang Wick
German Cancer Research Center, DKFZ, Germany

17:40 - 19:30 *Posters Session & Wine and cheese (social room)*

22nd February, 2017 Wednesday

09:45 - 13:10 Session#5 **PRIMARY AND METASTATIC PEDIATRIC TUMORS**

Chairperson: Richard Gilbertson

09:45 - 10:15 Biology and therapy for pediatric brain tumors
Will A. Weiss
UCSF Helen Diller Family Comprehensive Cancer Center, US

10:15 - 10:35 Effective preclinical treatment of Diffuse Intrinsic Pontine Glioma by MELK inhibition
SHORT TALK
Michaël Meel
VU Medical Center, Netherlands

10:35 - 10:55 Combinatorial targeting of tumor-associated macrophages/microglia with radiotherapy in gliomas
SHORT TALK
Leila Akkari
Netherland Cancer Institute, Netherlands

10:55 - 11:30 *Coffee break (social room)*

11:30 - 12:00 The biology of medulloblastoma metastases
Michael Taylor
The Hospital for Sick Children, Canada

12:00 - 12:20 ATR inhibition induces DNA damage and apoptosis in medulloblastoma and attenuates tumorigenesis
SHORT TALK
Patrick Lang
University of North Carolina, US

12:20 - 12:40 Study of Otx2/Hoxa2 antagonistic functions in the control of medulloblastoma tumorigenesis
SHORT TALK
Thomas Di Meglio
Centre for Molecular Biology "Severo Ochoa", Spain

12:40 - 13:10 Oncohistones: epigenetic drivers of pediatric high-grade glioma
Suzanne Baker
St. Jude Children´s Research Hospital, US

13:10 - 13:20 *Poster Award Sponsored by Fisher Scientific*

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13:20 Closing remarks **Manuel Valiente** and **Massimo Squatrito**

Madrid 19th-22nd February 2017

PRIMARY AND SECONDARY BRAIN TUMORS

KEYNOTE LECTURE

Chairperson: Michael Weller

Mapping cancer origins - beyond the brain

Richard Gilbertson
Cambridge Cancer Center,
University of Cambridge, UK

20th February 2017 - Monday

20th February 2017 - Monday

Mapping cancer origins - beyond the brain

Richard Gilbertson
Cambridge Cancer Center,
University of Cambridge, UK

MAPPING CANCER ORIGINS - Beyond the Brain

Richard Gilbertson
Cancer Research UK Cambridge Institute, University of Cambridge, UK

Cancers are distributed unevenly across the body, but the importance of cell intrinsic factors such as stem cell function in determining organ cancer risk is unknown. Over the last 15 years we have developed the technique of cross-species genomics to map cells of origin of brain tumours in the developing nervous system. These studies have revealed that brain tumours arise from matched combinations of susceptible cell types and oncogenic mutations. More recently we have built on these data to use Cre-recombination of conditional lineage tracing, oncogene, and tumour suppressor alleles to define populations of stem and non-stem cells in multiple mouse organs and test their life-long susceptibility to tumorigenesis. We show that tumour incidence is determined by the life-long generative capacity of mutated cells. This relationship held true in the presence of multiple genotypes and regardless of developmental stage, strongly supporting the notion that stem cells dictate organ cancer risk. Using the liver as a model system, we further show that damage-induced activation of stem cell function markedly increases cancer risk. Therefore, we propose that a combination of stem cell mutagenesis and extrinsic factors that enhance the proliferation of these cell populations, creates a “perfect storm” that ultimately determines organ cancer risk.

Prof. Richard J. Gilbertson
Li Ka Shing Chair of Oncology, Head of Dept. of Oncology, Director, Cambridge Cancer Center, CRUK Cambridge Institute, Li Ka Shing Centre, Robinson Way Cambridge CB2 0RE

Madrid 19th-22nd February 2017

PRIMARY AND SECONDARY BRAIN TUMORS

Session #1
PRIMARY ADULT BRAIN TUMORS (I)

Chairperson: ***Massimo Squatrito***

20th February 2017 - Monday

Modeling the landscape of human gliomas in the mouse

Eric Holland
Nancy and Buster Alvord Brain Tumor Center,
Fred Hutchinson Cancer Research Center, UW Medicine, Seattle, US

In spite of our growing knowledge of glioma biology, the outcomes for this disease have shown little improvement over a long period of time. Critical issues that remain to be resolved include 1) a better understanding of molecular heterogeneity and diagnosis that can be addressed with big data on human gliomas 2) mouse modeling to investigate specific genomic questions in glioma biology and 3) a more complete understanding of the biology of glioma immunology and response to immunotherapy using immune-competent glioma modeling systems.

Microenvironmental conditioning in glioma

Gabriele Bergers
VIB Center for Cancer Biology, Leuven, Belgium

Glioblastoma multiforme (GBM) are aggressive brain tumors that are resistant to standard treatments. Extensive characterization of the genome, epigenome and transcriptome of tumor cells has provided a higher-resolution picture of their alterations revealing substantial inter- and intra-tumor heterogeneity which challenges attempts of targeted therapies. A further layer of complexity arises from the different normal host cell constituents within the tumor that comprise the tumor microenvironment. They are composed of a variety of non-neoplastic stromal cells, of which the vasculature, and the various infiltrating and resident immune cells are dominant inhabitants in all tumor types. Importantly, tumor cells and host cell constituents are located in distinct tumor niches that form microanatomical communication centers in which they dynamically interact to ensure maintenance, growth and protection of tumor cells and CSC from immune surveillance and therapeutic threats. Although tumor niches have been portrayed as conceptually similar in a variety of tumor types, there exist at least three specialized tumor niches in GBM with the vasculature as an integral regulatory part. In the perivascular tumor niche tumor stem cells nestle in close juxtaposition with the abnormal angiogenic vasculature and infiltrating innate immune cells while in the vascular-invasive tumor niche tumor cells co-opt normal blood vessels and interact with microglial cells enabling survival and migration. In contrast, the vasculature in the hypoxic tumor niche is either non-functional or regressed leading to necrotic areas. All three niches elicit specific features and functions as evidenced by the differing composition of host-cell constituents and functional status of the vasculature.

Here we present data relating to the interaction of immune cells, tumor cells and the vasculature in angiogenic and invasive tumor niches. We provide evidence that immune cells in these two tumor niches have different functions and that modulation and reconstruction of the immune-suppressive tumor vasculature could be a suitable strategy to enhance CTL infiltration and subsequent eradication of tumor cells.

SHORT TALK

ATRX deficiency modulates differentiation state and migratory behavior by way of global epigenomic remodeling in glioma cells of origin

Carla Danussi, Yuxiang Wang, Promita Bose, Prasanna T. Parthasarathy, Pedro Silberman, Adriana Heguy, David Picketts, Timothy A. Chan, Kasthuri Kannan, and **Jason T. Huse**

Memorial Sloan-Kettering Cancer Center
UT MD Anderson Cancer Center
NYU Langone Medical Center
Ottowa Hospital Research Institute, US

Comprehensive genomic profiling in cancer continues to reveal frequent alterations in epigenetic regulators, firmly implicating chromatin biology in oncogenesis. Inactivating mutations in the SWI/SNF family member ATRX invariably pair with mutations in TP53 and IDH1/2 and represent defining molecular alterations in diffusely infiltrating gliomas. While ATRX deficiency has been linked to a wide spectrum of physiological dysfunction, the precise molecular mechanism(s) promoting oncogenesis are still largely unknown. We inactivated Atrx in Tp53-/- murine neuroepithelial progenitor cells (NPCs), which prior studies have implicated as potential glioma cells of origin. Atrx loss induced a morphologic change in NPCs, while also upregulating their motility, the latter an established pathogenic feature of diffusely infiltrating gliomas. Moreover, Atrx-deficient cells displayed increased expression of astrocytic markers and reduced levels of both neuronal and oligodendrocytic markers, suggesting that Atrx directly regulates NPCs differentiation state and potential. Notably, the observed phenotypes correlated with altered gene expression profiles in functionally relevant molecular networks (e.g. cell differentiation and migration). Integrating these transcriptional changes with shifts in chromatin accessibility induced by Atrx deficiency and genome-wide Atrx distribution (ChIP-seq) revealed highly significant spatial correlations between differentially expressed genes, altered chromatin compaction, and genomic sites normally occupied by Atrx. Finally, we found altered H3.3 histone monomer enrichment at genes mediating specific phenotypes in the Atrx-deficient setting, consistent with the established role of Atrx as the core component of an H3.3 chaperone complex. Taken together, these findings demonstrate that Atrx deficiency disrupts chromatin organization, leading to dramatic shifts in gene expression and disease-relevant phenotypes in putative glioma cells of origin.

The role of cell of origin and cancer stem cells in GBM phenotype

Luis F. Parada
Brain Tumor Center, Albert C. Foster, MSKCC. New York, US

The mammalian brain is composed of a diversity of cell types. We have addressed whether there exists equivalent tumorogenic potential among the diverse cell types. Our results using genetically engineered mice indicate that adult neural stem and progenitor cells harboring tumor suppressor mutations readily give rise to GBM. In contrast, more differentiated neurons and glia harboring the same mutations do not create tumors. We have further extended these studies to examine whether different cell lineages can give rise to tumors and find that both and find SVZ derived as well as OPC derived progenitor cells harboring identical mutations can produce GBM. However, the GBMs arising from the two different lineages exhibit subtle but distinct tumor phenotypes and harbor transcriptomes that reflect their specific cells of origin. Evidence that these two GBM subtypes are present in the human GBM data-base suggest that GBM may be stratified based on cell of origin and may provide a functional premise for therapeutic evaluation.

Madrid 19th-22nd February 2017

PRIMARY AND SECONDARY BRAIN TUMORS

Session #2

PRIMARY ADULT BRAIN TUMORS (II)

Chairperson: ***Eric Holland***

20th February 2017 - Monday

Signaling pathways in glioma tumor formation and therapy resistance

Massimo Squatrito
Spanish National Cancer Research Centre (CNIO),
Madrid, Spain

Although the last decade highlighted enormous advances in treating other solid cancers the median survival for Glioblastoma (GBM) stayed nearly the same over the last 50 years, averaging 15 months. Therapy resistance is characteristic of various cancer types, however it is not clear if it is a consequence of tumour progression or it is intrinsically associated with the genetic events that lead to the tumour formation in the first place. Gaining insights into the pathways that determine this poor treatment response will be instrumental for the development of new therapeutic modalities. A decade of studies has underlined the complexity of the genetic events that characterize the GBM genome, however, the functional significance of the vast majority of these alterations still remains elusive.

I will discuss the identification of RAN Binding Protein 6 (RanBP6) as a novel regulator of the epidermal growth factor receptor (EGFR) and the response to tyrosine-kinase inhibitors. RanBP6 silencing raises EGFR levels and signal output and accelerates *in vivo* glioma growth. At a molecular level, RanBP6 represses EGFR transcription by promoting nuclear import of Signal transducer and activator of transcription 3 (STAT3). Our results establish a novel function of RanBP6 as a link between EGFR-STAT3 signaling and the Ran-mediated nuclear import pathway, and identify RanBP6 as candidate tumor suppressor on chromosome 9p, a common cancer locus.

Mechanisms for EGFR signaling regulation by intracellular trafficking in Glioblastoma

SHORT TALK

Marta Portela[1], Patricia Jarabo-Blázquez[1], Almudena Sáiz[1], Berta Segura[2], Esther Hernández SanMiguel[2], Irene Argudo[1], Pilar Sánchez-Gómez[2], Sergio Casas-Tintó[1]

[1] Instituto Cajal, CSIC, Madrid, Spain
[2] Neuro-oncology Unit, Instituto de Salud Carlos III-UFIEC, Madrid, Spain

The most frequent genetic lesions in GBM include EGFR which show constitutive kinase activity and Ras signaling to drive cellular proliferation and migration. We use a GMB model in Drosophila melanogaster based on the expression of constitutively active EGFR and PI3K in glial cells, this model reproduces the highly proliferative and invasive neoplastic cells that create transplantable tumor-like growths, mimicking human GBM.

A genetic screen to identify new modulators of GBM progression was performed. As a result two members of the secretory pathway were identified: Kish and Gryzun. Downregulation of kish or gryzun rescues glioma formation, proliferation, neurodegeneration, alterations of the circadian rhythms and viability of the animals. Our aim is to understand how Kish and Gryzun modulate EGFR signaling during its intracellular trafficking. The majority of EGFR signaling occurs at the plasma membrane, but it is known that activated EGFR-mediated signals continue from endosomes. With this goal we have measured EGFR accumulation in specific endosomes in GBM and GBM samples were kish or gryzun have been downregulated. The results show that when the secretory pathway is altered by the inhibition of kish in a GBM, EGFR accumulates in early endosomes. And when gryzun is downregulated in a GBM, EGFR accumulates in late endosomes probably targeted to degradation in the lysosome. Moreover, the inhibition of these two candidates in GBM reduced the total circulating EGFR to control levels. Experiments looking at other trafficking markers are still ongoing.

These and future results will provide basic information on the glioma mechanisms and may be key for the design of future therapeutic strategies against specific targets involving EGFR signaling.

SHORT TALK

TRF1 as novel target in Glioblastoma

Leire Bejarano, Méndez-Pertuz M, Schuhmacher AJ, Megías D, Martínez S, Blanco-Aparicio C, Squatrito M, Pastor J, **María A. Blasco**

Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Glioblastoma multiforme (GBM) is the most frequent and aggressive glioma, with a mean survival of 14-16 months. The poor prognosis of GBM has been linked to the existence of a group of cells within the tumour with stem-like properties, also term glioma stem-like cells (GSCs). Telomeres are considered potential anti-cancer targets, as telomere maintenance above a minimum length is necessary for cancer cell growth. TRF1 plays an essential role in maintaining telomere protection and stability in vivo and its depletion produces severe telomeric damage. It has been shown that TRF1 expression is maximum in stem cell compartments in the adult mice and its expression correlates with pluripotency. In addition, TRF1 ablation is able to supress tumour growth in a lung cancer mouse model. With this in mind, we hypothesize that disrupting telomere stability by targeting TRF1 could open a new therapeutic window for the treatment of glioblastoma. In our experiments, we use the RCAS-Tva system to model GBM, specifically overexpressing PDGFB in Nestin-TVA expressing cells (glial progenitors) in Ink4Arf null background. We show that TRF1 is upregulated in GBM compared to normal tissue in a telomere length independent manner. TRF1 genetic ablation delays both tumour initiation and tumour progression *in vivo*. TRF1 deficient mice show significant increase in survival and smaller tumour areas. This is accompanied by telomeric DNA damage induction, decrease in proliferation and reduced stemness. In addition, in vitro results show that TRF1 inhibition by small molecules is able to induce DNA damage and decrease proliferation and stemness in the U251 GBM cell line. Taken together, these results suggest that TRF1 could be a potential new therapeutic target for GBM.

Next-generation brain tumor classification

Stefan Pfister
DKFZ, Heidelberg, Germany

There is accumulating evidence that DNA methylation patterns in the tumor epigenome represent a very robust fingerprint of the cell of origin of the tumor that remains extremely stable throughout disease course and tumor evolution, thus representing an elegant tool for robust molecular tumor classification. To systematically address the issue of diagnostic uncertainty in CNS tumors, we aimed to set up a comprehensive reference cohort of DNA methylation fingerprints covering all known CNS tumor entities and initiated "Molecular Neuropathology 2.0" (MNP2.0), a national molecular diagnostics trial for all childhood brain tumors.

DNA methylation fingerprints are used to accurately classify brain tumors into biologically and clinically meaningful subgroups. Amongst a total of 20.000 analyzed CNS tumor specimens to date in Heidelberg, we have established a reference set of ~2.800 samples with very good histopathological and clinical annotation currently covering 82 different entities and subgroups. This reference cohort is now being used in a diagnostic application for individual prospective samples to identify the class with the best fit. A web interface to make this reference freely available to the community is now available online at www.molecularneuropathology.org. First evidence from ~1.500 diagnostic cases within the MNP2.0 study and outside suggests that in more than 10% of cases the histopathological diagnosis (in accordance with neuropathology) will be changed in a way that affects clinical management of the patient. In about an additional 20% of cases, the diagnosis is refined by revealing a meaningful subgroup that cannot be established by conventional neuropathology alone (e.g., molecular subgroups of medulloblastoma or ependymoma). 8% of cases are still unclassifiable using the current reference cohort (but highly enriched in cases with a hereditary predisposition background) indicating that many very rare molecular entities are yet to be identified.

In summary, this principle has turned out to be an extremely efficient, accurate, and cost-effective tool for discoveries as well as clinical applications.

David Capper, David TW Jones, Martin Sill, Volker Hovestadt, Andreas von Deimling, and Stefan M. Pfister.

Molecular dissection of disease evolution in glioma

Roel Verhaak
The Jackson Laboratory for Genomic Medicine, Farmington, US

The highly lethal nature of glioma results in a huge year of potential life lost cost. High throughput sequencing has identified somatic alterations in primary disease but these have been hard to target, and as a result improvement in clinical outcomes has lagged behind compared to other cancers. An improved understanding of the mechanisms of glioma progression is needed to develop higher efficacy therapies. Through sequencing of patient tumors and model systems, we show how the glioma genotype and phenotype evolves over time.

Immunotherapy for Glioblastoma

Michael Weller
University Hospital Zurich & University of Zurich, Switzerland

The current standard of care for glioblastoma of resection followed by involved-field radiotherapy and concomitant and maintenance temozolomide chemotherapy (TMZ/RT→TMZ) prolongs survival to a median of 16 months in clinical trial populations, but survival with glioblastoma is still below 12 months on a population level. Immune inhibition is one of the biological hallmarks of glioblastoma, prompting the clinical development of various immunotherapeutic strategies that are currently being explored in phase I-III clinical trials. Efforts focusing on the antagonism of glioma-associated immunosuppression alone, e.g., blocking the transforming growth factor (TGF)-β pathway, have not been successful. However, abrogating inhibitory signalling to T cells via cytotoxic T lymphocyte-associated protein (CTLA)-4 or programmed death (PD)-1 using various neutralizing antibodies has generated new hope not only for several solid cancers outside the brain, but also for glioblastoma. Various vaccination approaches are also being tested, including dendritic cell-based vaccines, using either crude tumor lysates (DCVax) or tailored mRNA or peptide stimulation (ICT-107). The most advanced approach explored in phase III (ACT IV) was based on the vaccination against a mutant variant of the epidermal growth factor receptor (EGFR), EGFRvIII, which is expressed in approximately 20-30% of all primary glioblastomas. This mutation results in inability to bind ligand and constitutive signalling activity. Moreover, EGFRvIII represents a unique tumor antigen exhibiting a novel peptide sequence and may thus represent one of the most specific tumor antigens in glioblastoma. Phase II trials in glioblastoma patients with EGFRvIII-positive tumors without progression after radiotherapy and concomitant temozolomide chemotherapy treated with the vaccine rindopepimut showed encouraging progression-free and overall survival compared with historical controls, but the ensuing randomized, double-blind phase III trial, ACT IV, failed to meet the primary endpoint of improving overall survival. Yet, somewhat paradoxically, the ReACT trial which explored bevacizumab plus rindopepimut and bevacizumab plus placeno in a non-comparative randomized phase II trial in patients with EGFRvIII-positive recurrent glioblastoma, provided early evidence for efficacy of this vaccine. High-throughput analyses involving genome, transcriptome, and proteome are likely to result in the delineation of novel glioma-specific targets for novel immunotherapy approaches in the near future.

Next Generation Immune Therapeutics for GBM

Amy B. Heimberger
The University of Texas MD, Anderson Cancer Center, Houston, US.

The field of immunotherapy has been embraced by the oncology community especially after meaningful sustained prolongation of survival has been achieved in subsets of melanoma and lung cancer patients. In the last several years there has been a flurry of FDA approvals for antibodies that inhibit immune checkpoints. Multiple steps are required in order to successfully harness the antitumor capacity of the immune system. Specifically, an immunologic target needs to be present and the immune system needs to be activated. Then, there must be sufficient trafficking to the tumor microenvironment and the effector function must be preserved in the setting of profound tumor-mediated immune suppression. Additionally, a potent immune attack will have to be counterbalanced with the inadvertent induction of autoimmune side effects. To make meaningful advances, two distinct approaches are being employed – identifying unique subsets of patients that may preferentially benefit (i.e. biomarkers) or using combinatorial strategies that can potentially overcome tumor heterogeneity and immune therapeutic resistance. From these premises, we will be discussing the evolution of immune therapeutic approaches, specifically for glioblastoma, including the initial attempts of precision medicine with peptide vaccines to the emerging efforts of chimeric antigen receptor techniques. Antibodies have had the longest history for therapeutic development and have been modified over the years to include toxins and now modified for bi-specificity. Adoptive immune therapeutics including dendritic cells, NK and T cell therapeutics have similarly increased in complexity to address the needs of full optimize anti-tumor immune responses. Perhaps one of the most rapid areas of evolution are biomarkers for immune checkpoint inhibitors which have included tumor expression of PD-1, PD-L1, mutational load, and defective mismatch repair, which are expressed in small subsets of glioblastoma patients. The data indicates that we are likely going to need to do comprehensive biomarker analysis to identify those subjects that may benefit from immune checkpoint inhibitors and devise new biomarkers that integrate the complexity of a successful anti-tumor immune interaction. Furthermore, the singular focus on a specific immune checkpoint family ligand interaction is an oversimplification of the complex immune suppressive biology that is emerging. Given the heterogeneity of glioblastoma and its ability to gain mutations throughout the disease course, multifaceted treatment strategies utilizing multiple forms of immunotherapy in combination will be most likely to succeed moving forward.

NOTES

Madrid 19th-22nd February 2017

PRIMARY AND SECONDARY BRAIN TUMORS

Session #3
SECONDARY ADULT BRAIN TUMORS (I)

Chairperson: ***Manuel Valiente***

21st February 2017 - Tuesday

Cellular Networks: Implications for Brain Tumor Biology and Therapy

Frank Winkler
Neurology Clinic and National Center for Tumor Disease University Hospital Heidelberg;
German Cancer Research Center, Heidelberg, Germany

The recent discovery of distinct, ultra-long, and highly functional membrane protrusions in gliomas, particularly in astrocytomas, extends our understanding of how these tumors progress in the brain and how they resist therapies. We have suggested the term “tumor microtubes” (TMs) for these striking cellular features (Osswald et al., Nature 2015). This talk will present the cellular and molecular characteristics and biological functions of TMs, and the malignant multicellular network they form. One focus will lie on the parallels between TM (network-) formation with normal CNS development, and what we can learn from it. A second focus will lie on clinical aspects: First, the connection between 1p/19q codeletion and the inability to form functional TMs via certain neurodevelopmental pathways is presented; this could provide an explanation for the distinct clinical features of oligodendrogliomas. Second, the role of TMs for primary and potentially also adaptive resistance to cytotoxic therapies, but also to surgery, is highlighted. Third, avenues for therapeutic approaches to inhibit TM formation and/or function are discussed, with a focus on disruption (or exploitation) of network functionality. An increasing understanding of TMs in clinical and preclinical settings will show us whether they really are the long-sought-after Achilles’ heel of treatment-resistant gliomas.

Genomic Evolution of Brain Metastases

Priscilla Brastianos
Massachusetts General Hospital, Harvard Medical School,
Boston, US

Brain metastases are a common and devastating complication of cancer. Historically, it has been unclear if intracranial progression is due to inadequate systemic therapeutic penetration of the blood-brain barrier versus different genetic drivers in the brain metastases. There is a limited understanding of the oncogenic alterations harbored by brain metastases and whether these are shared with their primary tumors. We performed whole-exome sequencing of more than 150 matched brain metastases, primary tumors and normal tissue, including cases with spatially and temporally separated brain metastasis sites and additional extracranial disease sites from the same patient. In all clonally related cases, we observed branched evolution, where all metastatic, extracranial and primary sites shared a common ancestor yet continued to evolve independently. In more than half of cases, we found clinically informative driver alterations in the brain metastases that were not detectable in the matched primary-tumor sample. In contrast, spatially and temporally separated brain metastasis sites were more genetically homogenous. We detected frequent alterations in the PI3K pathway, CDK pathway, and HER2/EGFR pathway. Decisions for individualized therapies in patients with brain metastasis are often made from primary-tumor biopsies. These observations demonstrate that brain metastasis tissue provides an opportunity to identify clinically important driver alterations that may be undetected in primary tumors and extracranial sites. Genetic divergence between primary tumors and brain metastases may underlie some of the difficulties encountered with the combined treatment of systemic disease and brain metastases in patients.

SHORT TALK

New pericytes in glioblastoma (GBM) blood-vessels originate from a previously unrecognized, pericyte progenitor-like cell

Roland E. Kälin[1], Jörg-Christian Tonn[2], Michael Synowitz[3], **Rainer Glass[1]**

[1] Neurosurgical Research, University Clinics Munich-Ludwig-Maximilians University, Munich, Germany
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GBM is characterized by an exorbitant neo-vascularization and pericytes play a pivotal role in regulating tumor-vessel permeability and stability. Previous studies suggested that newly generated pericytes derive from mitotically active, mature pericytes, which can be identified by the expression of markers including PDGFR-B, NG2, desmin or CD146. Using a newly established transgenic lineage-tracing model (for conditional and timed recombination of nestin-expressing cells) we show that mature pericytes largely originate from marker-negative cells. In an orthotopic glioma model we studied pericyte development over a time-course. Throughout tumor growth recombined cells expanded in the tumor mass and labelling-experiments with different thymidine-analogues revealed their continuous proliferation. Strikingly, the vast majority of recombined cells in early gliomagenesis was negative for all pericyte-markers investigated, but the recombined cells acquired multiple pericyte markers at later stages of tumor expansion. Pericyte progenitors were also identified in developmental models. This suggests that a large number of newly generated pericytes in physiological and pathological angiogenesis derive from a pericyte progenitor-like cell. Bone marrow exchange paradigms indicated that pericyte-progenitors are resident in the CNS. Lineage ablations studies revealed that pericyte progenitors control tumor-vascularization and glioma expansion. Transient reduction of pericyte progenitor cell-numbers reduced tumor-burden by 50%. All in all, we provide an entirely new view on the generation of pericytes in blood-vessels and indicate a new target for anti-angiogenesis.

Here we provide unpublished data on a new cell-type Controlling glioma vascularization. A small number of pericyte progenitors generates a large pericyte progeny which is mandatory to maintain glioma blood-vessels. Therefore, pericyte progenitor cells constitute a new and promising target for future anti-angiogenic therapies.

How to best design future clinical trials in brain metastasis

Ricardo Soffietti
University and City of Health and Science Hospital, Turin, Italy

A major issue in designing clinical trials in brain metastasis is the choice of the endpoints, and the definition of response and progression is particularly critical. The new RANO criteria will be described and discussed. They are based so far on the measurement of tumor area (in the two perpendicular diameters); however, the assessment and reporting of volumetric response is encouraged in order to better define an appropriate cutoff. A clear distinction between intracranial PFS, extracranial PFS and overall PFS should be done, depending on the type of trial (local treatment vs systemic treatment trials). The issue of treatment-related changes following either local or systemic treatments such as pseudoprogression and pseudoresponse, is challenging, and in this regard the role of modern neuroimaging techniques must be explored and validated. Increasingly, health-related quality of life and cognitive functions need to be evaluated, and in some trials could become the primary end-point. Trials on targeted agents should at least contain a translational part (phase 0) to measure the drug activity on the molecular target. More specific RANO criteria are being developed for clinical trials on local and systemic treatments, respectively.

SHORT TALK

Targeting carcinoma–astrocyte gap junctions in brain metastasis

Adrienne Boire[1,3], Qing Chen[2], Mariza Daras[1,3], Thomas Kaley[1,3], Krishnaben Patel[1,3], Lisa DeAngelis[1,3] and Joan Massagué[1,4]

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[2] The Wistar Institute, Philadelphia, PA, USA
[3] Department of Neurology
[4] Department of Cancer Biology and Genetics

Brain metastatic breast and lung cancer cells assemble carcinoma–astrocyte gap junctions composed of connexin 43 (Cx43) to engage with the astrocyte gap-junctional network. We recently demonstrated that brain metastatic cancer cells use these channels to transfer the second messenger cGAMP to astrocytes, activating the STING pathway and production of inflammatory cytokines such as interferon-α (IFNα) and tumor necrosis factor (TNF). These factors activate the STAT1 and NF-κB pathways in brain metastatic cells to support tumor growth and chemoresistance in a paracrine fashion. To target the mechanistic bottleneck, we employed the orally bioavailable modulators of gap junctions meclofenamate and tonabersat to treat established brain metastasis in preclinical mouse models. A pilot study of meclofenamate in patients with recurrent brain metastasis from solid tumor primary cancers is currently underway (NCT02429570). This trial has accrued 12 of an anticipated 25 patients, and focuses on primary outcomes of feasibility and safety. Interim analysis demonstrates that this treatment is well-tolerated and in select patients, efficacious. This bench-to-bedside strategy has uncovered a key interaction between metastatic cancer and the brain parenchyma as well as a novel therapeutic approach to brain metastasis.

Evolving intratumor heterogeneity in primary and secondary brain tumors

Joan Seoane
Vall d'Hebron Institute of Oncology, Barcelona, Spain

One of the most important challenges in the treatment of cancer is its evolving genomic intratumor heterogeneity. Tumors are composed of assortments of cells with subclonal genomic alterations that evolve following Darwinian selection. Glioblastoma (GBM) is a prototypical heterogeneous tumor and still little is known about intratumor heterogeneity in brain metastasis. Most importantly not much is known about how intratumor heterogeneity impacts on the response to treatments and tumor relapse. The study of the spatial and temporal genomic architecture of GBM is essential to understand the biology and improve the treatment of this dismal disease. Through the study of primary and relapsed tumors and patient-derived xenograft models (PDX) of GBM, we are studying the genomic subclones emerging after treatment since these subclones might be enriched in genomic alterations that confer a selective advantage and resistance to treatment. In addition, we are also developing non-invasive circulating biomarkers such as the analysis of circulating tumor DNA in the cerebrospinal fluid to be able to monitor the genomic evolution of primary and metastatic brain tumors.

Madrid 19th-22nd February 2017

PRIMARY AND SECONDARY BRAIN TUMORS

Session #4
SECONDARY ADULT BRAIN TUMORS (II)

Chairperson: ***Riccardo Soffietti***

21st February 2017 - Tuesday

The inflammatory microenvironment of brain metastases and its potential role as therapy target

Matthias Preusser
Medical University of Vienna, Austria

Immunotherapies have shown impressive activity in a large number of different tumor types. Recent evidence shows that the immune system has a role in the brain-metastatic cascade and that manifest brain metastases have an immunologically “hot” microenvironment with infiltration of various immune cell subsets and expression of various immune-related molecules including immune check-point molecules. Emerging clinical evidence seems to indicate clinically meaningful activity of immunomodulatory drugs in brain metastases and clinical data are being performed. Open questions remain with regard to combination and sequencing strategies of different immunotherapies with each other and with other therapeutic approaches. This lecture will summarize the current knowledge and open issues with regard to the role of the immune system in the biology and the treatment of brain metastases.

On-site evolution of metastatic cells and surrounding environment during brain colonization

Manuel Valiente
Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Recent data suggest the possibility that adaptation to the brain environment involves a selection of metastatic cell clones, which will subsequently evolve in a divergent way compared to extra-craneal tumors. Although a significant amount of work has been devoted to the initial stages of colonization, we have almost no knowledge regarding the clinically relevant advanced stages of the disease.

I will present data regarding the involvement of H1.2, a member of the H1 linker histones, in the emergence of heterogeneity within metastatic lesions *in situ* and how targeting it dramatically impairs brain metastasis formation as well as viability of established lesions. Isolation of single cell progenies allowed us to conclude about the cell hierarchy regarding H1.2 levels, being those with higher levels of the histone necessary for the maintenance of the others. This dynamic regulation of metastatic lesions also affects the surrounding microenvironment. Outgrowth of metastatic lesions occurs in a microenvironment that is being increasingly exposed to the influence of cancer cells. This sustained interaction modifies the initially hostile brain defenses into a pro-metastatic niche. How this transition is regulated remains unknown. We have detected a *de novo* generated subpopulation of reactive astrocytes during advanced stages of the disease. Targeting this subpopulation of reactive astrocytes using a conditional mouse model probes its crucial role to maintain the viability of established lesions. Characterization of the subpopulation of reactive astrocytes suggests the existence of direct and indirect influence on cancer cells, which might involve the immune system. Pharmacological intervention with blood-brain barrier permeable inhibitors targeting this subpopulation of reactive astrocytes reproduced the genetic approach in experimental models and was successfully translated to individual clinical cases. Both findings imply the need to understand the basis of such cancer cell evolution and microenvironment remodeling in order to target established brain metastasis more efficiently.

SHORT TALK

Induction of lysosomal membrane permeability as a novel glioma treatment

Maija Hyvönen[1], Pauliina Filppu[1], Pauliina Turunen[2], Harri Sihto[3], Heikki Joensuu[3], Olli Tynninen[4], Michael Weller[5], Kaisa Lehti[2], Vadim Le Joncour[1] and **Pirjo Laakkonen[1,6]**

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[3] Department of Oncology, Helsinki University Central Hospital, Helsinki, Finland
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[5] Department of Neurology and Brain Tumor Center, University Hospital Zurich and University of Zurich, Switzerland
[6] Laboratory Animal Centre, University of Helsinki, Finland

We have utilized the disease-specific molecular tags in peptide-based tumor targeting. Using the in vivo phage display we identified a homing peptide that specifically recognizes invasive gliomas. Systemically administered peptide-targeted delivery of chemotherapeutics prolonged lifespan of invasive glioma-bearing mice and significantly reduced the number of tumor satellites compared to the free drug. We also showed successful glioma imaging using the radiolabeled peptide in whole-body SPECT/CT and used our homing peptide to deliver porous silicon nanoparticles to tumors. We identified mammary derived growth inhibitor, MDGI, as the interacting partner for our peptide on glioma cells and glioma-associated vasculature. MDGI is as a novel marker for malignant gliomas and its expression positively correlates with the histological grade of the tumor in human brain tumor specimens. Our unpublished results show that MDGI expression correlates with poor 5-year overall survival of glioma patients. MDGI overexpression increases aggressive growth of gliomas, whereas it's silencing drastically reduces viability of glioma cells via induction of lysosomal membrane permeabilization (LMP) and caspase-independent apoptosis. LMP leads to irreversible leakage of lysosomal cathepsins and other hydrolases to the cytoplasm, where they digest vital proteins and intracellular organelles, leading eventually to cell death. Multiple triggers of the LMP are known but genes and molecular pathways that regulate the lysosomal membrane integrity are poorly understood. Although cancer cells very often escape spontaneous or therapy-induced apoptosis they still remain sensitive to the lysosomal cell death opening a possible therapeutic window for LMP inducing agents in cancer therapy. Currently, we are investigating the sensitivity of our patient-derived glioma cells and normal cells to LMP inducing drugs in vitro and validating their potential in treatment of gliomas in pre-clinical animal models.

SHORT TALK

EANO ESMO Recommendations for the Diagnosis and Treatment of Leptomeningeal Metastasis from Solid Tumors

Emilie Le Rhun, for the EANO ESMO Guideline Task Force on Leptomeningeal Metastasis

Neuro-oncology, Department of Neurosurgery, University Hospital, Lille, France;
Neurology, Medical Oncology Department, Oscar Lambret Center, Lille, France;
Lille University, Inserm U-1192, Villeneuve d'Ascq, France

Background: Leptomeningeal metastasis (LM) is commonly a late manifestation of systemic cancer, which affects up to 10% of all patients with solid tumors. Survival is overall poor, with a median survival of 2-3 months and a 1-year survival rate of 10%. Treatment strategies vary widely and there are very few prospective clinical trials to inform on evidence-based treatment strategies.

Methods: Here we provide multidisciplinary consensus-based recommendations for the diagnostic work-up of patients with suspected LM and delineate a new approach of classifying LM, which allow for evidence-based management algorithms.

Results: The diagnosis is based on clinical presentation, magnetic resonance imaging (MRI) findings, and cerebrospinal fluid (CSF) cytology including immunocytochemistry and molecular marker assessment. The detection of tumor cells in the CSF or rarely a leptomeningeal biopsy remain the gold standard for the diagnosis and define confirmed LM (type I) as opposed to type II LM which is supported by clinical findings and neuroimaging only. To facilitate treatment decisions, we define LM with typical diffuse MRI abnormalities as type A, LM with nodular disease only as type B, LM with both diffuse and nodular disease as type C, and LM without MRI abnormalities as type D.

Conclusion: Management strategies for individual patients should be derived by integrating general and neurological health status, CSF findings, neuroimaging findings, and absence or presence of solid brain and systemic metastases, histology of the primary tumor and previous treatments, and comprise intra-CSF chemotherapy, systemic pharmacotherapy, and focal or large volume radiotherapy.

Targeting the inflammatory response for diagnosis and treatment of brain metastasis

Nicola R. Sibson
CRUK/MRC Oxford Institute for Radiation Oncology, University of Oxford, UK

Metastatic spread of a primary tumour to the brain remains one of the greatest hurdles in cancer therapy, and prognosis is poor. Magnetic resonance imaging (MRI) is commonly used for the diagnosis of brain cancer, but current techniques are sensitive only to larger, late stage tumours, when the blood-brain barrier (BBB) has become permeable. At the same time access of systemic therapies to early stage brain metastases is severley hampered by the presence of an intact BBB. We hypothesise that earlier detection of brain metastases would faciliate more effective treatment, but at the same time that strategies to improve therapeutic access to such micrometastatic tumours is essential. Over many years in Oxford, we have developed a number of targeted MRI contrast agents that enable detection of specific molecules expressed early in disease. We have shown that one of these contrast agents, targeting the endovascular cell adhesion molecule VCAM-1, enables early detection of metastases in mouse brain when they are undetectable by existing clinically-used methods. We have also demonstrated expression of VCAM-1 in human brain tissue containing micrometastases, supporting clinical translation of this diagnostic approach. In subsequet studies, we have identified a previously unknown phenotype of the metastasis-associated vasculature, specifically upregulation of the cytokine TNF type 1 receptor, and have further demonstrated that systemic administration of TNF selectively and specifically permeabilises the BBB at sites of micrometastases in the brain. This opening of the BBB at metastatic sites facilitates both localised entry of MRI-detecable contrast agents and relevant therapeutics. Finally, we have recently identified novel anti-inflammatory strategies, targeting cellular adhesion molecules and their signalling pathways, that may provide new therapeutic avenues in brain metastasis. Taken together, we believe that these new approaches will open a therapeutic window in brain metastasis that currently does not exist and could impact significantly on patient outcome.

Clinical trials using molecular precision concepts

Wolfgang Wick
Heidelberg University Hospital, Germany

Apart from *O6-methyl guanine O6-methylatransferase* (MGMT) promoter methylation there are no predictive biomarkers for patients with glioma. It is reasonable to assume that patients with glioblastoma without MGMT promoter methylation do not benefit from alkylating chemotherapy. Clinical trials aiming at replacing temozolomide (TMZ) with targeted agents in unselected patient populations have failed to demonstrate any benefit. Advances in the understanding of glioblastoma at a molecular level along with technological progress have led to the identification of key genetic alterations in a timely manner allowing for treatment decisions in newly diagnosed patients. In this setting, these alterations not only refine the sub-classification of glioblastoma, but also allow for subset specific treatments, with contemporary analyses allowing for differentiating prognostic and predictive biomarkers.

At the same level, immunotherapies applying checkpoint inhibitors or vaccines to an unselected group of patients are not likely to yield a relevant benefit. Also for immunotherapies concepts to select patients on the basis of a more or less immunogenic microenvironment or focusing on immunogenic mutations are more likely to provide a next step, although first concepts e.g. using the activated variant of the epidermal growth factor receptor (EGFR) vIII as a target are reported to have failed.

In the presentation, precision trials on peptide vaccines (GAPVAC) and targeted therapies (N^2M^2) as well as other international acitivities in this respect will be discussed.

Madrid 19th-22nd February 2017

PRIMARY AND SECONDARY BRAIN TUMORS

Session #5
PRIMARY AND METASTATIC PEDIATRIC TUMORS

Chairperson: ***Richard Gilbertson***

22nd February 2017 - Wednesday

Biology and therapy for pediatric brain tumors

Will A. Weiss
UCSF Helen Diller Family Comprehensive Cancer Center, US

Although genetically engineered mouse models (GEMMs) have been the gold standard to model diseases such as cancer, GEMMs are costly, time consuming, and require transformation of mouse cells that may not fully replicate the behavior of transformed human cells. We present human stem cell (hSC)-based model of medulloblastoma (MB) derived from human induced pluripotent stem cells (iPSC) using both established (MYCN, PTCH1) and new driver genes. Ongoing experiments to characterize genetic and epigenetic cytoarchitecture, as well as engineering and validating candidate cooperating genes and chromosomal deletions will be discussed.

Effective preclinical treatment of Diffuse Intrinsic Pontine Glioma by MELK inhibition

SHORT TALK

Michaël H Meel[1], Miriam G Navarro[1], Mark C de Gooijer[2], Piotr Waranecki[1], Levi Buijl[2], Olaf van Tellingen[2], Dannis G van Vuurden[1], Gertjan JL Kaspers[1,3], Esther Hulleman[1]

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[3] Princess Máxima Center of Pediatric Oncology, Utrecht, The Netherlands

Maternal embryonic leucine zipper kinase (MELK) is the second highest expressed kinase in diffuse intrinsic pontine glioma (DIPG). Inhibition of MELK by the small molecule OTSSP167 effectively inhibits migration, reduces proliferation and induces cell death and radiosensitization in primary DIPG cell lines at low nanomolar concentrations. RNA sequencing of DIPG cells treated with OTSSP167 reveals upregulation of genes associated with the PPAR transcription factors.

Western blot and immunofluorescence demonstrates reduced inhibitory phosphorylation and increased nuclear localization of PPARγ upon MELK inhibition. Combined treatment of DIPG cells with OTSSP167 and PPARγ agonists synergistically reduced cell survival. Given the importance of the blood-brain barrier in DIPG, brain penetration of OTSSP167 was investigated by administration of the compound to wild-type, Abcb1a/b -/-, Abcg2-/- and Abcb1a/b -/-; Abcg2-/- mice. Brain bioavailability was severely restricted by both Abcb1a/b and Abcg2 efflux activity. To demonstrate the potential of MELK inhibition for the treatment of DIPG, Abcb1a/b -/-; Abcg2-/- athymic nude mice were xenografted with primary DIPG tumors and treated with a low dose of OTSSP167 for six weeks. After 15 days of treatment, DIPG xenografts went into remission. To our knowledge, this is the first time treatment of DIPG xenografts resulted in remissions, urging the development of brain penetrable MELK inhibitors for clinical trials.

SHORT TALK

Combinatorial targeting of tumor-associated macrophages/microglia with radiotherapy in gliomas

Leila Akkari[1,2,3], Robert L. Bowman[3,4], Daniela F. Quail[3], Lucie Tillard[1,2], Jason T. Huse[5], James C. Sutton[6] and Johanna A. Joyce[1,2,3]

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[6] Novartis Institutes for Biomedical Research, Emeryvilles

Eighty percent of tumors that develop in the central nervous system are malignant gliomas, with over half being glioblastoma multiforme (GBM), the most aggressive form of this disease. Even following treatment with standard of care therapy, the overall 5-year survival rate of patients diagnosed with GBM is less than 5%.

In GBM patients and mouse models of the disease, the major non-cancerous cell type in the glioma microenvironment is tumor-associated macrophages/microglia (TAMMs). We previously used an inhibitor of colony stimulating factor 1 receptor (CSF-1R), BLZ945, to target macrophages in the PDG mouse model of gliomagenesis. CSF-1R inhibition dramatically improves survival in a long-term intervention trial and markedly regresses established high-grade lesions.

To determine the translational clinical potential for CSF-1R inhibition, we designed preclinical trials in combination with radiation. We treated established GBMs with fractionated radiation concomitant with acute, short-term BLZ945 treatment and observed that the overall survival of animals was significantly extended compared to single treatment modalities. We next examined how re-educating TAMMs could increase radiation efficacy, and our preliminary data support a mechanism by which CSF-1R inhibition sensitizes glioma cells to radiotherapy by modulating the DNA damage response pathway. Incorporating long-term treatment with BLZ945 in tumors treated with fractionated radiation led to a block in tumor recurrence, indicating that reversing cancer-induced macrophage 'education' has the potential to inhibit disease relapse. In recurrent irradiated tumors, the ratio of macrophages infiltrating from the periphery and tissue-resident microglia is significantly altered, suggesting that changes in TAMM sub-populations may support glioma recurrence. Additionally, treatment of IR-resistant gliomas with BLZ945 de novo resulted in efficient regression of the disease. Together these results identify critical roles.

The biology of medulloblastoma metastases

Michael Taylor
The Hospital for Sick Children, Canada

SHORT TALK

ATR inhibition induces DNA damage and apoptosis in medulloblastoma and attenuates tumorigenesis

Patrick Y. Lang[1,6], Jaclyn Wu[2], Jing Gao[3], Duhyeong Hwang[3], Alexander V. Kabanov[3,4], Marina Sokolsky-Papkov[3,5], and Timothy R. Gershon[6,7]

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We have found that the DNA repair protein ATR is essential for formation and maintenance of the sonic hedgehog (SHH) medulloblastoma subtype. Our prior developmental studies show that cerebellar granule neuron progenitors (CGNPs), the cells of origin for SHH medulloblastoma, require ATR for survival during postnatal neurogenesis. When Atr is deleted, CGNPs accumulate proliferation-dependent DNA damage from replicative stress that results in p53-activated, BAX/BAK-mediated apoptosis. These findings led us to explore whether cellular stress from ATR disruption in SHH medulloblastoma would have an anti-tumor effect. We first bred conditional Atr deletion into two primary tumor-prone mouse lines, Math1-Cre;SmoM2;Atr(loxP/loxP) and hGFAP-Cre;SmoM2;Atr(loxP/loxP). Atr loss greatly reduced tumor growth in the Math1-Cre;SmoM2;Atr(loxP/loxP) mice and completely blocked tumor formation in the hGFAPCre;SmoM2;Atr(loxP/loxP) animals. We then examined whether pharmacologic inhibition of ATR in mice with established medulloblastomas would similarly disrupt tumorigenesis. To do so, we developed a novel, nanoparticle formulation of the small molecule ATR inhibitor VE-822 (nanoVE-822). Our initial studies demonstrated that IP injection of nanoVE-822 (60mg/kg QD for 5 consecutive days in wild-type and medulloblastoma-bearing mice) produced detectable DNA double-strand breaks in CGNPs and medulloblastoma cells, proving effective blood-brain barrier penetration. Importantly, nanoVE-822 increased DNA damage and apoptosis in the medulloblastomas of SmoM2 mice and these effects were correlated with reduced tumor size. Our preliminary results support the therapeutic potential of ATR inhibition as a novel treatment for medulloblastoma.

Study of Otx2/Hoxa2 antagonistic functions in the control of medulloblastoma tumorigenesis

SHORT TALK

El Nagar S.[2], Nieto-López F.[1], Marcos Ramirez B.[1], Vilain N.[3], Ducret T.[3], Lamonerie T.[2], Rijli FM.[3], Billon N.[2], **Thomas Di Meglio[1]**

[1] Centro de Biología Molecular "Severo Ochoa" (CBMSO), Madrid, Spain
[2] Valrose Institute, Nice, France
[3] Friedrich Miescher Institute for biomedical research (FMI), Basel, Switzerland

Medulloblastomas (MB) are the most frequent and lethal pediatric brain tumors. Class 2 MB tumor stem cells originate in neural precursors derived from progenitors of the rhombic lips (RL) located in the developing hindbrain. The oncogenic transformation of these RL derivatives (RLd) is caused by a deregulation and consequent hyperactivation of the Sonic Hedgehog (SHH) pro-mitotic signaling.

We found that functional interactions between the transcription factors Otx2 and Hoxa2 co-regulate the normal abilities of the RLd to proliferate or not. We indeed performed comparative studies of mouse models for Otx2 or Hoxa2 conditional gain or loss of function, and obtain concordant results showing that Otx2 and Hoxa2 (i) cross-repress each other and (ii) show respective pro- and anti-mitotic activities in RLd. Importantly, Otx2 and Hoxa2 antagonistic functions inversely control RLd competence to transduce the Shh pro-mitotic signaling.

We thus further explored if Otx2 and Hoxa2 could respectively promote or repress class 2 MB carcinogenesis. The cerebellar and cochlear granule cells precursors (GCP), which are the only RLd sub-populations competent to initiate class 2 MB carcinogenesis, specifically express Otx2 while repressing Hoxa2. Using new mouse models for Shh tumorigenic signaling hyperactivation, we evidenced that Otx2 loss and Hoxa2 gain of function in GCP strongly disrupt class 2 MB tumor initiation. Our studies decipher the mechanisms mediating such anti-tumor effects. They also characterize the abilities of different chemical treatments to release Hoxa2 tumor suppressor activity in the developing GCP.

Overall, this work thus elucidates the abilities of Hoxa2 and Otx2 to control the Shh-dependent neurogenic and tumorigenic signaling, proving that they could be viable targets for new MB cancer therapies.

Oncohistones: Epigenetic Drivers of Pediatric High-Grade Glioma

Suzanne J. Baker
St. Jude Children's Research Hospital, Memphis, US

Diffuse high-grade gliomas (HGG) in children are rare and incurable childhood brain tumors that provide intriguing examples of the connections between epigenetics, development, and cancer. High frequency somatic mutations in histone H3, termed oncohistones, are a hallmark of pediatric HGG, distinguishing their molecular pathogenesis from adult HGG. Approximately half of all childhood HGG are diffuse intrinsic pontine glioma (DIPG), which arise in the brainstem. Mutually exclusive somatic heterozygous pK27M mutations in histone H3.3 or H3.1 are found in nearly 80% of DIPGs and more than half of non-brainstem HGG arising in midline structures such as the thalamus. In contrast, mutations encoding histone H3.3 pG34R or pG34V are found in approximately 15% of HGG arising in the cortex in older children through young adulthood, suggesting a distinct developmental origin compared with the HGGs carrying K27M mutation. We developed patient derived xenografts and genetically engineered mouse models that demonstrate the oncogenic activity and downstream consequences of oncohistone proteins on the epigenome and transcriptome.

Andre B Silveira, Jon D Larson, Lawryn H Kasper, Gang Wu, Yiping Fan, David Finkelstein, Hongian Jin, Barbara S Paugh, Xiaoyan Zhu, Junyuan Zhang, David W Ellison, Jinghui Zhang, **Suzanne J Baker**

NOTES

Madrid 19th-22nd February 2017

PRIMARY AND SECONDARY BRAIN TUMORS

SPEAKERS' BIOGRAPHIES

Suzanne J. Baker

St. Jude Children's Research Hospital, Director, Brain Tumor Research Division, Co-Leader, Neurobiology & Brain Tumor Program, Endowed Chair in Brain Tumor Research, Memphis, US

Dr. Suzanne Baker received her BS degree in Zoology from Miami University, and her PhD degree in Molecular Biology and Human Genetics from The Johns Hopkins University, where she trained with Dr. Bert Vogelstein. After postdoctoral training with Dr. Tom Curran at the Roche Institute of Molecular Biology, she joined the faculty at St. Jude Children's Research Hospital. She currently serves as the Associate Director of Basic Research and Co-Leader of the Neurobiology and Brain Tumor Program in the St Jude Comprehensive Cancer Center, and the Director of the Division of Brain Tumor Research.

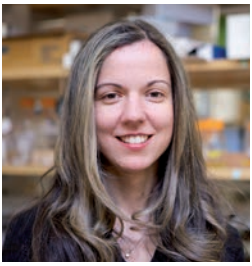
The Baker laboratory is focused on understanding the underlying molecular, cellular and genetic mechanisms driving high-grade gliomas, including diffuse intrinsic pontine glioma (DIPG) in children. We employ the latest genomic technologies to study primary human tumors, and incorporate genomic discoveries into the design and analysis of *in vitro* models employing primary neural cultures, and *in vivo* mouse models for mechanistic studies and preclinical testing, including xenografts established from primary tumors and genetically engineered mouse models. The unique genetic landscape of these tumors highlights critical connections between development, epigenetics and cancer.

Gabriele Bergers

Professor of Oncology, KU Leuven and VIB-Center for Cancer Biology, Leuven, Belgium
Professor of Neurosurgery, UCSF Helen Diller Family Cancer Research Center,
San Francisco, USA

Dr. Gabriele Bergers is a Professor of Oncology at the University of Leuven and a group leader at the Vlaams Instituut voor Biotechnologie (VIB)-Center for Cancer Biology in Leuven since 2016. She has been a Professor in the Department of Neurological Surgery, and a PI in the Brain Tumor Research Center (BTRC) at the Helen Diller Family Comprehensive Cancer Center at the University of California, San Francisco for more than 15 years. She has made seminal discoveries about tumor niches regarding both the interactions among tumor cells, the vasculature, and inflammatory cell constituents in regulating neovascularization and invasion in various mouse models of brain, breast and pancreatic cancer and in revealing and understanding intrinsic and evasive resistance mechanisms of tumors to antiangiogenic therapy.

Dr. Bergers has received numerous awards for her research, including the Sidney Kimmel, the Sandler Opportunity and the Judah Folkman award. She has been an External Advisory Board member for various universities and pharmaceutical companies, and is currently on the Editorial Board of Cancer Research and Science. She has served on the NIH Tumor Microenvironment Study Section since 2006 and was Chair from 2010-12. In addition, Dr. Bergers was the co-director of the TMEN (Tumor Microenvironment) Brain Tumor Center at UCSF, as well as an EAB member at the Max-Planck-Institute for Biomedicine (Vascular Biology focus) in Muenster, Germany.



Priscilla Brastianos

Massachusetts General Hospital, Harvard Medical School, Boston, US

Priscilla Kaliopi Brastianos, MD
Assistant Professor in Medicine, Director, Central Nervous System Metastasis Program, Divisions of Hematology/Oncology and Neuro-Oncology, Departments of Medicine and Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA USA

Originally from Vancouver, British Columbia, Dr. Priscilla Brastianos received her BSc in biochemistry and chemistry from the University of British Columbia, where she graduated as her class valedictorian. She received multiple awards, including the Science Scholar Award, the Canadian Society for Chemistry Prize and the Violet and Blythe Eagles Undergraduate Prize in Biochemistry. She completed her medical school training and her internal medicine residency training at Johns Hopkins School of Medicine. While at Hopkins, she received the Johns Hopkins Medical Student Award for Excellence in Research, the national Leah J. Dickstein, MD, award for leadership and scholarship, and the Bradley Benton Davis Research Award from the American Brain Tumor Association. Following her training at Johns Hopkins, she pursued her fellowship training in hematology/oncology and neuro-oncology at the Dana-Farber Cancer Institute and Massachusetts General Hospital. She is now director of the Central Nervous System Metastasis Program at Massachusetts General Hospital. She was recently named a 'NextGen Star' by the American Association for Cancer Research, and received a Susan G. Komen Career Catalyst Award, a Damon Runyon Clinical Investigator Award and an ASCO Young Investigator Award. Dr. Brastianos' research focuses on understanding the molecular mechanisms that drive brain tumors. She lead the studies which identified novel therapeutic targets in meningiomas, craniopharyngiomas and brain metastases. Her work in brain metastases demonstrates that brain metastases have branched evolution, and harbor clinically significant drivers that are distinct from clinically sampled primary tumors. She has translated her scientific findings to national multicenter trials. She also leads a multidisciplinary central nervous system metastasis clinic at Massachusetts General Hospital/Harvard Medical School.

Javier Carmona

Nature Medicine, New York, US

Dr. Javier Carmona started his studies at the University of Navarra and received a degree in Biology from the Autonomous University of Madrid. In 2013, he obtained his Ph.D. after working in Manel Esteller's Cancer Epigenetics and Biology Program in Barcelona. Javier continued his research as a postdoctoral fellow in the group of José Baselga at the Memorial Sloan Kettering Cancer Center in New York, where he studied the mechanisms of resistance to therapy in patients with breast cancer. In 2016 he joined *Nature Medicine* as an Assistant Editor.

Richard J. Gilbertson

Li Ka Shing Chair of Oncology, Head of Deptartment of Oncology, Director,
Cambridge Cancer Center, CRUK Cambridge Institute, Li Ka Shing Centre, UK

Prof. Richard Gilbertson trained as a pediatric oncologist in the UK before moving in 2000 to St. Jude Children's Research Hospital, USA where he served as Scientific and Comprehensive Cancer Center Director, Executive Vice President and Lillian R. Cannon Endowed Chair. In August 2015, he moved back home to England where he directs the Department of Oncology and the Cancer Centre at Cambridge University. His laboratory research is focused on understanding the link between normal development and the origins of cancer, particularly brain tumors.

Amy B. Heimberger
The University of Texas MD, Anderson Cancer Center, Neurosurgery Department, Houston, US

Dr. Amy Heimberger is a Professor in the Department of Neurosurgery at the University of Texas MD Anderson Cancer Center in Houston. She has an extensive research program focused on immune therapeutic strategies for glioma patients and studies tumor-mediated mechanisms of immune suppression. Dr. Heimberger's laboratory has: 1) co-developed from bench to bedside a peptide (PEP-3-KLH/CDX-110/ rindopepimut) vaccine that targets the epidermal growth factor receptor variant III (EGFRvIII); 2) clarified that the signal transducer and activator of transcription 3 (STAT3) pathway is a key molecular hub of gliomagenesis and tumor-mediated immune suppression and conducted the pre-clinical development of a novel small molecule inhibitor of STAT3, WP1066, which will be introduced into clinical trials for melanoma patients with CNS metastasis and glioblastoma patients; 3) showed that tumor-associated microglia/macrophages do not participate in anti-tumor immune responses but rather assist in potentiating gliomagenesis via STAT3; 4) established that glioblastoma-associated cancer stem cells exert immune suppressive properties on both the adaptive and innate arms of the immune system and showed this could be reversed with inhibitors of the STAT3 pathway; and 5) demonstrated that microRNAs could target immune suppressive mechanisms and be exploited as immune modulatory therapeutics. Dr. Heimberger is the only faculty member at MD Anderson that has been awarded the Presidential Early Career Award for Scientists and Engineers. She holds multiple NIH and foundation grants and is a project leader in the Brain SPORE and is the PI of the Glioblastoma Moonshot. Dr. Heimberger has a clinical interest in awake mapping and resection of gliomas within eloquent cortex. She has been named by the US News and World Report as a Top Doc.

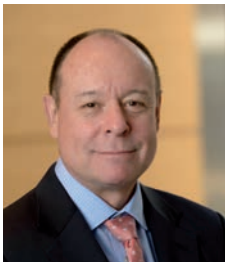
Eric Holland

Senior VP, Director, Human Biology Division,
FHCRC Director, Seattle Tumor Translational Research (STTR),
FHCRC & UW Professor, Department of Neurological Surgery,
UW Director, Nancy and Buster Alvord Brain Tumor Center,
FHCRC & UW Chap and Eve Alvord and Elias Alvord Chair in Neuro-Oncology, UW
Nancy and Buster Alvord Brain Tumor Center, Fred Hutchinson Cancer Research Center,
UW Medicine, US

Dr. Eric Holland earned a doctorate in biochemistry and molecular biology from the University of Chicago and a medical degree from Stanford University. He completed a neurosurgery residency at the University of California at Los Angeles School of Medicine and a fellowship at the National Cancer Institute in Bethesda, Md. His postdoctoral training included work with two Nobel laureates: Dr. Paul Berg, who pioneered recombinant DNA technology at Stanford, and Dr. Harold Varmus, director of the National Cancer Institute.

Dr. Holland was recruited to Seattle from Memorial Sloan-Kettering Cancer Center in New York, where he directed the Brain Tumor Center and built one of the nation's most successful research and clinical programs. As a neurosurgeon and physician–scientist, he addresses the molecular basis of brain tumors to develop new, more precise approaches to their treatment. He specializes in glioblastoma, the most common brain cancer in adults, has developed mouse versions of brain cancer that mimic how tumors behave in humans, and has identified tumor cells that are resistant to standard therapies. These research findings have led to clinical trials for new drugs and drug combinations. At UW Medicine and the Hutch, Dr. Holland and his colleagues will help usher in an era of precision treatment for cancer patients.

Dr. Holland has received the American Brain Tumor Association Research Award, among other honors. He is a member of the Institute of Medicine of the National Academies and a member of the editorial boards of *Virology*, *Molecular Cancer Research*, the *Journal of Molecular Medicine* and *Neoplasia*.



Luis F. Parada

Director, BTC & Member, Cancer Biology and Genetics,
Memorial Sloan Kettering Cancer Center, New York, US

Dr. Luis F. Parada obtained a BS from the University of Wisconsin and a Ph.D. in Biology from MIT, identifying oncogenes in human cancer. He was a Damon Runyon and Helen Hay Whitney Postdoctoral Fellow at the Pasteur Institute. He headed the Molecular Embryology Section at the National Cancer Institute in Frederick, MD from 1988 to 1994 when he moved to the University of Texas Southwestern Medical Center at Dallas as the inaugurating Diana and Richard C. Strauss Distinguished Chair in Developmental Biology, and was Director of the Kent Waldrep Foundation Center for Basic Neuroscience Research. During his time in Dallas, Dr. Parada advanced his studies of nerve cell survival and regeneration, mood disorders, and renewed his attention on cancer with emphasis on the nervous system. His laboratory uses genetic mouse models to study human disease including Neurofibromatosis, cancers of the nervous system, autism, and neural development. In 2015 Dr. Parada moved his laboratory to Memorial Sloan Kettering Cancer Center to assume leadership of the interdisciplinary Brain Tumor Center. In addition Dr. Parada holds the Albert C. Foster Chair and is Professor of Cancer Biology and Genetics. In recognition of his contributions to science, Dr. Parada has been elected to: The National Academy of Sciences; The American Academy of Arts and Sciences: The Institute of Medicine - National Academy of Sciences (National Academy of Medicine); The American Association for the Advancement of Science (AAAS), The Academy of Medicine, Engineering and Science of Texas TAMEST); and is a life-time American Cancer Society Professor.

Stefan M. Pfister

Head, Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ);
University Hospital Heidelberg Angelika Lautenschläger Children's Hospital
Department of Pediatric Hematology and Oncology, Heidelberg, Germany

Prof. Dr. med. Stefan Pfister was appointed acting head of the Division Pediatric Neurooncology at the German Cancer Research Center (DKFZ) in 2012. Since 2014 he is professor for pediatric neurooncology at the DKFZ and heading the department permanently. Being a pediatrician by training, Pfister received his MD from Tübingen University, and his clinical education at Mannheim and Heidelberg University Hospitals. As a physician-scientist, he completed postdoctoral fellowships with Christopher Rudd at the Dana-Faber Cancer Institute/Harvard Medical School, and with Peter Lichter at the German Cancer Research Center, Division of Molecular Genetics. Pfister´s research focuses on the genetic and epigenetic characterization of childhood brain tumors by applying next-generation profiling methods and subsequently translating novel findings into a clinical context. For his translational neurooncology projects, Pfister received amongst others the German Cancer Award in 2012.

Matthias Preusser
Medical University of Vienna, Department of Medicine I, Austria

Prof. Dr. Matthias Preusser is an Associate Professor of Medicine and a consultant in Medical Oncology at the Medical University of Vienna. He serves as Coordinator of the CNS Tumours Unit at the Comprehensive Cancer Centre Vienna, and his research focuses on biomarkers and therapy in brain tumours. Recent works focus on the characterization of inflammatory infiltrates and immune checkpoint molecules as well as clinical evaluation of immunotherapies in various types of brain tumours. Professor Preusser is co-chair of the European Organisation for Research and Treatment of Cancer Brain Tumour Brain Metastases Platform and is leading several international clinical trials in Neuro-Oncology.

Joan Seoane

Vall d'Hebron Institute of Oncology (VHIO), Vall d'Hebron University Hospital,
Director of Translational Research Programme, Barcelona, Spain

Dr. Joan Seoane is a Group Leader and Director of the Translational Research program at the Vall d'Hebron Institute of Oncology (VHIO) within the Vall d'Hebron University Hospital since 2011. In 1998, Joan obtained his PhD in Biochemistry and Molecular Biology from the University of Barcelona. Previously, in 1993, he obtained his BSc degree in Chemistry. Joan joined the Memorial Sloan-Kettering Cancer Center (MSKCC) in New York as a post-doctoral fellow in 1998. From 1998 to 2001, he worked as a Research Fellow and subsequently, from 2001 to 2003, as a Research Associate. There, he discerned the molecular mechanisms involved in the antiproliferative response to TGF-β in cancer.

He was appointed ICREA Research Professor in 2004 and joined VHIO. In 2007, he became a member of the EMBO Young Investigator program and the recipient of a European Research Council (ERC) grant in 2008. Later, he obtained two ERC Proof of Concept grants (2011, 2013). In 2008, he became Board member of the European Association of Cancer Research (EACR) and Associate Professor of the Autonomous University of Barcelona. In 2012, founded Mosaic Biomedicals as a spin-off company from his lab. In 2016, he became Secretary General of the EACR.

His main objective is to understand the molecular mechanisms involved in the initiation and progression of cancer in order to design rational, specific and successful therapeutic approaches. Specifically, his work is focused in the study of brain tumors aiming at improving the treatment of this dismal disease.

Nicola Sibson

Professor of Imaging Neuroscience, CR-UK and MRC Oxford Institute for Radiation Oncology, University of Oxford, UK

Nicola Sibson is Professor of Imaging Neuroscience and CRUK Senior Group Leader at the Oxford Institute for Radiation Oncology, within the Department of Oncology, at the University of Oxford. Nicola heads the Experimental Neuroimaging Group and her research focuses on the diagnosis and treatment of brain metastasis. On completion of her Ph.D. at the University of Cambridge, Nicola spent four years at Yale University, before joining the MRC Biochemical and Clinical Magnetic Resonance Unit in Oxford. Subsequently, she moved to the Department of Physiology, Anatomy and Genetics as a University Research Lecturer, and was recruited to the Institute in 2007. Nicola has authored over 100 publications, filed 8 patents and attracted over £17m in research funding. She sits on numerous national and international boards, including a Helmholtz Alliance Scientific Advisory Board (Germany), an *Aeres* Evaluation Committee (France) and The Brain Tumour Charity Biomedical Scientific Advisory Board (UK). For more information, please go to *http://www.radiationoncology.ox.ac.uk/research/nicola-sibson*.

Ricardo Soffietti

University and City of Health and Science Hospital, Turin, Italy

Present Position
Professor of Neurology and Neuro-Oncology, Dept. Neuroscience 'Rita Levi Montalcini' , University of Turin, Italy.
Head, Dept. Neuro-Oncology, City of Health and Science University Hospital, Turin, Italy.
Appointments and Positions
- Founding Member of the European Association for Neuro-Oncology (EANO).
- Past President of EANO
- Chairperson of the Scientific Panel of Neuro-Oncology of the European Academy of Neurology (EAN).
- Chairperson of the Research Group Neuro-Oncology of the World Federation of Neurology (WFN).
- Member of the Steering Committee of the Brain Tumor Group of the European Organization for Research and Treatment of Cancer (EORTC)
- Past President of the Italian Association for Neuro-Oncology (AINO).
- Member of the Society for Neuro-Oncology (SNO, US).
- Member of the Neuro-Oncology Section of the American Academy of Neurology.
- Cancer Expertise for research projects of the European Community and of Ministry of Health and Research of Italy, France, Switzerland and Netherlands.
- Advisor of the European Medicines Agency (EMA).
- Member of the International Group Response Assessment in Neuro-Oncology (RANO).
- Member of Cochrane Group for Brain Tumors

Research and clinical experiences related to the proposed project :
- Longstanding experience in Clinical Neuro-Oncology (i.e. management of primary and secondary brain tumors, and neurological complications of systemic cancer).
- Specific research experience in designing, coordinating and conducting clinico-translational trials of phase II and III in glioblastomas and lower grade gliomas, lymphomas, ependymomas, medulloblastomas, brain metastasis and neoplastic meningitis with new drugs (including targeted agents, such as EGFR inhibitors, m-TOR inhibitors, anti-VEGF compounds).
- Specific research experience in monitoring treatment effects with advanced neuroimaging techniques (MRS Spectroscopy, PET with FDG and amino acids).
- Specific research experience in studies on prognostic and predictive factors (clinical, radiological, pathological and molecular) in gliomas and ependymomas.

Editorial Activity
- Member of the Editorial Board : Neuro-Oncology, Neuro-Oncology Practice, Anticancer Drugs, Journal of Neurology, Current Cancer Therapy Reviews, CNS Oncology and Neurological Sciences.
- Referee: Neurology, Lancet Neurology, Brain, European Journal of Neurology, Lancet, Lancet Oncology, Journal of Clinical Oncology, Clinical Cancer Research, Cancer, European Journal of Cancer, The Oncologist, Journal of Neuro-Oncology, Critical Review in Hematology and Oncology, Expert Opinion on Pharmacotherapy, Oncology Research, Tumori, Expert Review of Neurotherapeutics, Expert Review of Anticancer Therapy, Future Oncology, British Medical Cancer Journal.
- Executive Editor Neuro-Oncology, Oxford University Press, (IF : 6.76).
- Section Editor (Neuro-Oncology) Current Treatment Options in Neurology, Springer (IF : 2.175).
- Consulting Editor Journal of Neurosurgical Sciences (IF : 1.158).
- Managing Editor of EANO Online Magazine.
- Co-Editor Module "Tumor and cysts" for E-Learing Project of EFNS and ENS.
- Member of the International Editorial Liason Committee of Advances in Clinical Neuroscience and Rehabilitation

Awards
- Award for Excellence in Clinical Research (Society for Neuro-Oncology, US, 2009)

Massimo Squatrito

Spanish National Cancer Research Centre (CNIO),
Junior Group Leader and Director of the Seve Ballesteros Foundation Brain Tumor Group,
Madrid, Spain

Dr. Massimo Squatrito, born in Italy, obtained his PhD in Applied Genetics from the University of Milan, working at the European Institute of Oncology (IEO) in the laboratory of Giulio Draetta.

After a short postdoctoral training in the laboratory of Bruno Amati at IEO, at the end of 2006 he joined the laboratory of Eric Holland, first as postdoctoral fellow and then as a Research Associate, at the Memorial Sloan-Kettering Cancer Center, New York (USA). Since then he has been focusing his research on the role of the role of the DNA damage response in the process of brain tumorigenesis and response to therapy.

At the end of October 2012 Massimo joined the CNIO Cancer Cell Biology Programme as Junior Group Leader and director of the Seve Ballesteros Foundation Brain Tumor group.

Michael Taylor

The Athur and Sonia Labatt Brain Tumour Research Centre, The Hospital for Sick Children, Division of Neurosurgery, Ontario, Canada

Dr. Taylor was born in Calgary, Alberta and was educated at The University of Western Ontario where he obtained his MD in 1994. He entered the University of Toronto Neurosurgery residency program in 1994. He then did a PhD in Molecular Pathology at the University of Toronto (1998-2002), and completed his residency training in 2003. In 2003 Michael was awarded a Detweiler Travelling Fellowship from the Royal College of Physicians and Surgeons of Canada for fellowship training in paediatric neurosurgery and paediatric neuro-oncology at St. Jude Children's Research Hospital in Memphis Tennessee. Dr. Taylor also did a post-doctoral fellowship in the Department of Developmental Neurobiology at SJCRH.

Dr. Taylor joined The Hospital for Sick Children (SickKids), Division of Neurosurgery in 2004. He has an appointment in the Developmental & Stem Cell Biology Program at the SickKids Research Institute. He is a principal investigator at the Arthur and Sonia Labatt Brain Tumour Research Centre. He also has cross-appointments to the Departments of Surgery & Laboratory Medicine and Pathobiology at the University of Toronto. His research is supported by the Canadian Institutes of Health Research (CIHR), Genome Canada, National Cancer Institute of Canada, National Institutes of Health (USA), American Brain Tumor Association and SickKids Foundation. He has published close to 100 peer reviewed publications. Dr. Taylor's laboratory focuses on the genetics of paediatric medulloblastoma and ependymoma. Clinically, he has a special interest in paediatric neuro-oncology.

Manuel Valiente

Spanish National Cancer Research Centre (CNIO), Head of the Brain Metastasis Group, Madrid, Spain

Dr. Valiente is the Head of the Brain Metastasis Group (CNIO, Madrid), where he leads a team of scientists whose goal is to discover critical aspects of brain metastasis biology in order to develop new therapeutic opportunities. His laboratory is validating novel brain metastasis mediators, characterizing the microenvironment associated with brain metastasis, improving experimental models to incorporate therapies and exploring novel methods to target established brain metastasis. Central to all these aims is the brain environment, which is considered by Dr. Valiente and his group as a critical component to understand the biology of this progression of cancer. Dr. Valiente has described critical mediators of brain metastasis specifically required for the initial steps during colonization. His most significant contribution includes the discovery of the molecular basis for the high inefficiency of brain metastasis and the identification of a cell adhesion molecule critical for vascular co-option. More recently he has participated in a proof-of-concept validating experimental therapies against brain specific survival mechanisms of metastasis, now in a clinical trial.

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Roeland Verhaak

Professor and Associate Director of Computational Biology,
The Jackson Laboratory for Genomic Medicine, Farmington, US

Roel Verhaak's research focuses on the analysis of cancer genomics data to improve understanding of cancer biology. He has a specialized research interest in understanding disease progression of brain tumors, particularly glioblastoma and glioma. He mostly uses high throughput sequencing and computational analysis in our research.

William A. Weiss
UCSF, Helen Diller Family Comprehensive Cancer Center, San Francisco. US

William A. Weiss MD, PhD is Professor of Neurology, Pediatrics, and Neurological Surgery at UCSF and Co-Director of the Pediatrics Malignancies Program in UCSF's Helen Diller Family Comprehensive Cancer Center. Dr. Weiss is deputy editor of *Cancer Research, and serves on the editorical board of Molecular and Cellular Biology, NeuroOncology*, and Scientific Reports; an is an external advisor to Brain Tumor Program at Saint Jude Children's Research Hospital. Dr. Weiss has developed a number of models for cancer based on recapitulating cardinal genetic abnormalities in transgenic mice and in human stem cells, and has developed therapeutic programs focused on EGFR/PI3K/mTOR and myc pathways.

Michael Weller

University Hospital Zurich, Laboratory of Molecular Neuro-oncology, Zurich, Switzerland

Dr. Michael Weller has been Chairman of the Department of Neurology at the University Hospital Zurich, Switzerland, since 2008. He has received several awards in recognition of his contributions to cancer research, including the German Cancer Award in 2007. He served as Chairman of the Neuro-Oncology Group of the German Cancer Society from 2001-2008. He is the Chairman of the German Glioma Network of the German Cancer Council, joined the Executive Board of the European Association for Neuro-Oncology (EANO) in 2010 and was elected President of EANO for 2014-2016. He is also the Chairman of the Brain Tumor Group of the European Organization for Research and Treatment of Cancer (EORTC) (2015-2018) and will host the World Federation of Neuro-Oncology Societies (WFNOS) Meeting 2017 in Zurich, Switzerland.

Dr. Weller was involved in major practice-changing clinical trials in Neuro-Oncology. He has a research focus on the immunology of gliomas and serves as the PI of the phase III immunotherapy trials ACT IV (Rindopepimut) and STING (ICT-107). He has co-authored more than 550 original publications in peer-reviewed journals, including *The New England Journal of Medicine*, *Science*, *Nature*, *Nature Medicine*, *Lancet Oncology*, *PNAS*, *The Journal of Clinical Investigation*, and *The Journal of Clinical Oncology*.

Wolfgang Wick

Heidelberg University Hospital, Department of Neurology, Heidelberg, Germany
Chairman:Germany Neurology Clinic, Heidelberg University Medical Center
Head: Clinical Cooperation Unit Neurooncology

ACADEMIC EDUCATION & QUALIFICATION

10/90 – 08/93	University of Bonn: Medicine
09/93 – 06/94	King´s College London, Great Britain
07/94 – 08/96	University of Bonn
09/96 – 05/97	Harvard Medical School, Boston, USA
06/97 – 11/97	University of Bonn
11/97	Examination xx
04/03	Board certification in Neurology

SCIENTIFIC EDUCATION & QUALIFICATION

05/98	Doctoral thesis at the Institute for Neuropathology (Prof. Dr. O.D. Wiestler) at the University of Bonn (Summa cum laude)
07/03	Habilitation and venia legendi for Neurology

PROFESSIONAL EXPERIENCE

01/98 – 06/99	Internship at the Department of Neurology, University of Tübingen (Chairman: Prof. Dr. J. Dichgans)
07/99 – 12/01	Resident at the Department of Neurology, University of Tübingen
01/02 – 12/02	Resident at the Department of Psychiatry, University of Tübingen (Chairman: Prof. Dr. G. Buchkremer)
since 08/04	Attending in Neurology
04-12/06	Vice Chairman, Dep. of General Neurology, University of Tübingen, Germany (Chairman: Prof. Dr. M. Weller)
01/07 – 09/14	Chairman Dep. of Neurooncology/NCT and Professor of Clinical Neurooncology, University of Heidelberg
10/2014	Chairman General Neurology Heidelberg

OTHER QUALIFICATIONS/ROLES/RESPONSIBILITIES

since 10/2005	Steering Committee of the Brain Tumor Group of the EORTC
since 07/2007	Steering Committee of the Neurooncology Working Group (NOA) of the German Cancer Society
02/2008-02/2009	Chair-elect of the Brain Tumor Group of the EORTC
09/2008	Member of the EANO Board
03/2009-06/2015	Chairman of the EORTC Brain Tumor Group
03/2009	Member of the of the Grant Application Council for Young Scientists of the German Cancer Aid
01/2010	Member of the Board of Directors at the NCT
01/2010-04/2014	Member of the Board of Directors European Cancer Organization (ECCO)
06/2014	Chair of the NOA
10/2016	President EANO

Frank Winkler

Professor of Experimental Neurooncology,
Neurology Clinic and National Center for Tumor Disease University Hospital Heidelberg;
Clinical Cooperation Unit Neurooncology, German Cancer Research Center, Heidelberg, Germany

Prof. Dr. med. Frank Winkler is a Neurologist and a brain tumor expert. He is managing senior physician of the Dpt of Neurology, University Hospital Heidelberg, and was appointed Professor of Neurooncology in Heidelberg in 2012 (jointly by Heidelberg University and German Cancer Research Center, DKFZ). In his lab in the DKFZ, a better understanding of the development of gliomas and brain metastasis, and neuroinflammatory diseases is sought, primarily by use of dynamic in vivo two-photon microscopy of pathological CNS structures, combined with other technologies of advanced imaging and molecular biology. The ultimate goal is to illuminate the cellular and molecular dynamics of these diseases, and thus to identify novel therapeutic approaches that can readily be translated into clinical trials.

NOTES

Madrid 19th-22nd February 2017

PRIMARY AND SECONDARY BRAIN TUMORS

POSTER SESSIONS

The peptide transporter K16ApoE increases drug-delivery across the blood brain barrier in an animal model of melanoma brain metastases

Synnøve Nymark Aasen[1], Heidi Espedal[2], Olivier Keunen[3], Christopher Florian Holte[2], Habib Baghirov[4], Rolf Bjerkvig[2], Tine Veronica Karlsen[2], Olav Tenstad[2], Dag Erlend Olberg[5], Frits Thorsen[2]

[1] Haukeland University Hospital, Bergen, Norway
[2] University of Bergen, Bergen, Norway
[3] Luxembourg Institute of Health, Luxembourg, Luxembourg
[4] Norwegian University of Science and Technology, Trondheim, Norway
[5] Translational Nanoformulation Core Facility, Eshelman School of Pharmacy, UNC, Chapel Hill, USA
[6] Oslo University Hospital, Oslo, Norway

Patients with brain metastases usually anticipate a poor prognosis. Regardless of the constant progress in novel drug establishments, a crucial obstacle is the delivery of drugs across the blood brain barrier (BBB) and into the metastatic neoplasms. Different study designs to achieve temporary BBB opening have previously been studied. Here, we describe a peptide transporter called K16ApoE, which is able to transiently open the BBB for drug-delivery into experimental brain metastases.

A systemic study of the ability of the peptide to open the BBB was conducted by dynamic contrast enhanced magnetic resonance imaging (DCE MRI) in nonobese diabetic/severe combined (nod/scid) mice. Further, cellular effects after treatment with the peptide was investigated in vitro using confocal microscopy, flow cytometry and impedance experiments. The biodistribution of the peptide was studied in blood plasma and several organs using I-125 labeled K16ApoE. Finally, a treatment study using the peptide in combination with the B-RAF inhibitor Dabrafenib, only Dabrafenib or vehicle was conducted.

An opening of the BBB for up to 4 hours was clearly demonstrated by DCE-MRI. Microscopy showed that the peptide disrupted brain endothelial cell monolayers by reducing the barrier properties of the cells. Through impedance experiments it was observed that the permeability through endothelial cell barriers was elevated after treatment with K16ApoE, and dose-dependent cell death was seen at higher concentrations of K16ApoE. The treatment study demonstrated that the group of animals receiving K16ApoE followed by Dabrafenib had smaller tumor volumes than the other two animal groups.

In conclusion, the K16ApoE transporter peptide in combination with Dabrafenib decreased the number of experimental brain metastases. Thus, the current strategy could have the potential to improve the treatment of patients with brain metastatic disease.

A novel therapeutic strategy in Glioma: Discovering the mechanism of action of anti-tumor drug 2-Hydroxyoleic acid

Laura Arbona-González[1], Maitane Ibarguren, David J López[2], Roberto Beteta-Göbel[1,2], Javier Fernández-Díaz[1,2], Xavier Busquets[1,2], Pablo V Escribá[1]

[1] Laboratory of Molecular and Cell Biomedicine, University of the Balearic Islands
[2] Lipopharma Therapeutics, S.L., Palma de Mallorca, Spain

The most common of primary brain tumor is generally associated with very high rates of mortality (ca. 90%) and low survival (about 1 year). Moreover, glioma have a poor prognosis and limited treatment options. The reference drug is temozolomide (TMZ) which only increases the patients' life expectancy about 2.5 months.

Our studies have shown that changes in membrane lipid content fulfils a critical role in the propagation of tumorigenic signals. 2-hydroxyoleic acid (2OHOA), it's an antitumor drug and currently being studied in a phase I/IIa clinical trials. The greater efficacy than TMZ and the lack of toxicity at therapeutic doses has been acknowledged by the European Medicines Agency (EMA) to designate 2OHOA orphan drug for the treatment of glioma.

Despite its promising effectiveness, its mechanism of action is not fully understood. 2OHOA induces cell cycle arrest in lung cancer cells, apoptosis in leukemia cells and differentiation followed by autophagy in glioma cells. It produces changes in the levels, activity or interactions of lipids and proteins (and/or their corresponding mRNAs): (1) reorganization of membrane microdomains; (2) modulation of the transition temperatures in model membranes; (3) induction membrane-to-cytoplasm Ras translocation followed by inhibition of the MAPK, Cyclin/CDK and Akt signaling pathways; (4) stimulation cytoplasm-to-membrane PKC translocation, (5) increase of sphingomyelin levels, (6) selective induction of several key effectors of endoplasmic reticulum stress; (7) marked downregulation of E2F-1, a key transcription factor that regulates the expression of a large number of cell cycle-related genes; (8) lowering in EGFR phosphorylation; (9) reduction of β-catenin expression; (10) induction of Fas Receptor capping and activation of the intrinsic apoptosis pathway. The aim of this work is to summarize all the known effects produced by 2OHOA in different cell lines of glioma find connections among them and shed light on its mechanism of action.

This work has been supported by the Spanish Ministerio de Economía y Competitividad (MINECO) (BIO2013-49006-C2-1-R; RTC-2015-3542-1; RTC-2015-4094-1) cofinanced by FEDER funds"Una manera de hacer Europa" and Lipopharma Therapeutics S.L.

LAG is supported by the University of Balearic Islands (UIB) by RTC-2015-4094-1 contract. DJL supported by Torres-Quevedo Research Contracts (PTQ-09-02-02113). RBG is supported by MINECO. JFD is supported by the Balearic Conselleria d'Innovacio, Recerca i Turisme cofinanced by the FSE"Invertimos en tu futuro".

Targeting Brain Metastases of Breast Cancer

Abiodun Ayo, Vadim Le Joncour, Maija Hyvönen, Pauliina Filppu, Tanjore Ramanathan and Pirjo Laakkonene

Research Programs Unit, Translational Cancer Biology, University of Helsinki, Helsinki, Finland

Mammary derived growth inhibitor (MDGI) is a member of the small fatty acid binding proteins, expressed by various tissues such as the heart and skeletal muscle, the adipose tissue, and mammary fat pad. Classically, MDGI binds to long chain fatty acids in order to facilitate their uptake, metabolism and transport. Nevertheless, it has been recently demonstrated that MDGI is overexpressed in many cancers, and more notably in malignant central nervous system (CNS) tumors. Moreover, in glioblastoma multiforme (GBM), MDGI overexpression is correlated with accelerated progression and greatly reduced patient survival. Consistent with these findings, our research group has shown that upregulation of MDGI expression occurs in patient gliospheres, and it can be selectively found in invasive glioma cells *in vivo*. Silencing of MDGI induces dramatic death of glioma cells due to major cell metabolism disturbance. In addition, we used the in vivo phage display to identify a peptide (CooP) that targets the MDGI expressing gliomas after intravenous injection. The aim of this work is to examine whether the MDGI dependence on cell survival was glioma specific or could it be used as a therapeutic target for other cancers. MDGI silencing was performed and analyzed in MDA-MB-231 breast cancer cell lines. The knockdown of MDGI halts cell proliferation. Currently, we are validating the role of MDGI in formation of CNS metastases of breast cancer using MDA-MB-231 cells overexpressing MDGI and their wild type counterparts as a model. Moreover, we aim to validate the usage of the CooP homing peptide for the selective therapeutic intervention of CNS metastases of breast cancer.

2-Hydroxylated free fatty acids and their antitumoral effect against Glioma

Roberto Beteta-Göbel[1,2], Javier Fernández-Díaz[1,2], Paula Fernández-García[2], Raquel Rodríguez-Lorca[1], Catalina Ana Rosselló[1,2], Laura Arbona-González[1], Xavier Busquets[1,2], Pablo V. Escribá[1], Victoria Lladó[1]

[1] Laboratory of Molecular and Cell Biomedicine, University of the Balearic Islands, Palma de Mallorca, Spain
[2] Lipopharma Therapeutics SL, Palma de Mallorca, Spain

Malignant brain tumors are devastating despite aggressive treatments, such as surgical resection, chemotherapy, and radiation therapy. The average life expectancy of patients diagnosed with glioblastoma, the most advanced and aggressive form of glioma, is approximately 1.5 years. The first-line chemotherapeutic drug Temozolomide (TMZ) is widely used for treating primary and recurrent high-grade gliomas notwithstanding its efficacy is often limited by the development of resistances. For these reasons, developing new therapeutic modalities is crucial.

The so-called Membrane Lipid Therapy is an innovative therapeutic approach that aims to regulate the membrane lipid structure with the subsequent modification of the interaction and activity of membraneinteracting proteins which propagate proliferation signals. According to this novel therapy, we designed a family of lipidic molecules, including 2-hydroxylated free fatty acids, which showed a high anticancer activity.

In this present work, we used two novel hydroxylated free fatty acids: 2OHOA (hydroxylated-derivative of Oleic Acid) and LP226 (hydroxylatedderivative of DHA). In vivo experiments were performed in nude mice bearing human glioblastoma cells (U-118 MG) and showed decreased tumor growth when treated with these compounds compared to untreated mice. Moreover, no apparent toxicity was appreciated in nude mice. Regarding to *in vitro* experiments, inhibition of proliferation was achieved using hydroxylated free fatty acids in U-118 MG cells. Furthermore, LP226 induced the activation of JNK and eIF2 signaling pathways and endoplasmic stress proteins, BiP and Chop, induction. These events induced by LP226 seem to lead to autophagy initiation as indicated by an increase in LC3B-II levels.To sum up, hydroxylated free fatty acids present anticancer effects against glioma and exhibit an appealing potential to be exploited in the treatment of patients that develop this disease with unmet effective therapy.

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MYCN overexpression and stabilization drives medulloblastoma from human neuro-epithelial stem cells

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Medulloblastoma (MB) is the most common type of malignant pediatric brain tumor. MB is a highly heterogeneous disease that is molecularly divided into four subgroups, with WNT subgroup having the best prognosis, while the remaining subgroups SHH, Group 3 and Group 4 have rather poor outcome. Amplification and/or overexpression of MYC gene family can be found in all four subgroups of MB. We have previously shown that overexpression of mutationally stabilized MYCN (MYCN-T58A) can drive MB development from mouse neural stem cells. Here we demonstrated MB formation by overexpressing MYCN-T58A in human hindbrain neuro-epithelial stem (NES) cells and induced pluripotent stem cell-derived NES (iPS-NES) cells. NES cells overexpressing MYCN-T58A (NES-M) and iPSC-NES cells overexpressing MYCN-T58A (iPS-NES-M cells) formed rather invasive tumors with high penetrance when injected into the mouse cerebella. NES-M and iPS-NES-M tumors where primitive (Nestin positive), showed high degree of proliferation (30% and 28% Ki67+ nuclei) and had negligible rate of apoptosis (0.3% and 0.9% relative Cleaved Caspase 3 density). Based on RNA-Seq analysis, iPS-NES-M tumors clustered together with MB rather than with other common types of brain tumors. The expression analysis showed 500-1000 fold upregulation of MYCN, about 50-fold upregulation of TUBB3 (Tuj1) and SYP (Synaptophysin), and had very low expression of GFAP, which was also confirmed by immunohistochemistry. These results suggest that iPS-NES-M tumors are indeed primitive and of neuronal origin. Collectively, our data suggests a new MB model driven from a human cell of origin that can be further utilized in studying how MB develops and as a platform for drug evaluation.

Keywords: medulloblastoma, neuro-epithelial stem cells, MYCN

Epigenetic regulation of ZBTB18 promotes glioblastoma progression

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Over the past decade, glioblastoma subclasses with distinct differentiation characteristics and associated clinical outcome were identified. The mesenchymal subtype is characterized by inherent resistance to radiotherapy. The association with patient survival instead has been more controversial, although it is possible that an association with survival could be masked by mixed subtype profiles of many tumors. ZBTB18 is a transcription factor that belongs to the BTB/POZ-ZF protein family and plays a crucial role in brain development and neuronal differentiation. Consistent with our previous study, which provided preliminary evidence of a role for ZBTB18 in a network of mesenchymal transformation in glioblastoma, our new data have discovered epigenetic silencing through promoter methylation as a mechanism to downregulate ZBTB18 in these tumors. ZBTB18 promoter analysis by pyrosequencing identified a region which is specifically methylated in the mesenchymal subgroup and negatively correlated with ZBTB18 expression. Restitution of ZBTB18 reverses the phenotype and impairs tumor-forming ability. We propose that loss of ZBTB18 contributes to the aggressive phenotype of glioblastoma through regulation of poor prognosis-associated signatures possibly by interfering with the TGFβ pathway. Overall, our results support a tumor suppressor role of ZBTB18 in the brain and identify promoter hypermethylation as a mechanism to silence ZBTB18 in the mesenchymal subtype of glioblastoma, which provides a new mechanistic opportunity to specifically target this tumor subclass.

Glioblastoma neurodegenerative strategies in a Drosophila model

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Malignant astrocytic gliomas such as glioblastoma (GBM) are the most common, aggressive and lethal central nervous system (SNC) tumors. These tumors are characterized by diffusely infiltrative growth and great invasiveness, which leads to neurological dysfunction and death despite intensive radio and chemotherapy. Recently, it has been discovered that these types of tumors extend highly functional, ultra-long membrane protrusions (tumor microtubes-TM) that interconnect tumoral cells forming a glial network.

This network is responsible for the infiltrative growth and therapy resistance, giving a positive correlation between the length and number of TMs and unfavorable prognosis. The neuronal growth-associated protein 43 (Gap43) is necessary in the formation of these TMs, here we demonstrate that suppression of Gap43 expression specifically in glial cells of a Drosophila Melanogaster brain tumor model prevents the network formation and promotes survival.

Moreover, we show that the projections of glial cells that form the network overexpress wg/WNT receptor frizzled1 but no frizzled2 to deplete wingless to neighboring neurons. As a consequence, wg/WNT pathway is active in GBM cells which enhance proliferation and invasiveness. On the other side, wg depletion from neurons causes a synapse loss and neurodegeneration.

The final goal of this Project is to elucidate mechanisms through which glial cells induce neurodegeneration and find mechanisms to prevent it and to extend life span and improve life quality.

Role of Cyclin D1 in the regulation of glioblastoma invasiveness

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Glioblastoma is a highly invasive brain neoplasia with an elevated percentage of recurrences after chirurgical resection. The axis D1/Cdk4–RB1 is frequently altered in Glioblastoma releasing proliferation by retinoblastoma (RB1) deletions or by D1/Cdk4 over-activation. High levels of Cyclin D1 promote not only proliferation, but also glioblastoma cell invasion by not well understand mechanisms. In previous works we have described in mouse fibroblasts that cytoplasmic D1/Cdk4 regulates cell attachment and invasion indirectly triggering Ral and Rac1 activities. Regarding Rac1, D1/cdk4 phosphorylates Paxillin at serines 83 and 178 which in turn promote Rac1 activation. In this work we show that cytoplasmic CyclinD1-Cdk4 activity also triggers cell invasion and detachment in primary human glioblastoma cells via targeting Paxillin and Ral GTPases. This is also true for RB1-negative glioblastoma cells. In those cells: 1) Cyclin D1 was co-localized in the cytoplasm and membranes with RalA and Paxillin; 2) the overexpression of cyclin D1 promoted Ral and Rac1 activation; 3) Cyclin D1 cannot induced cell detachment in the presence of a S83A S178A allele of Paxillin or a dominant-negative allele of RalB; and 4) a hyperactive allele of Ral or a S83E S178E allele of Paxillin subverted the drop of invasion efficiency due to the inactivation of cyclinD1-Cdk4 activity after Palbociclib treatment. Finally, we are using a mouse model of glioma-genesis to analyze the complete expression pattern of cyclinD1 in the tumor. Preliminary, we have detected tumor regions with cytoplasmic Cyclin D1 and phosphopaxillin. In conclusion, we propose that cyclinD1-associated activity in the cytoplasm regulates glioblastoma invasion through paxillin and Ral activities and independently of retinoblastoma protein. Then, chemical inhibitors of Cyclin D1, phosphopaxillin and Ral GTPase may be useful as therapy to reduce glioblastoma invasion and avoid recurrences.

Study on Autophagy and UPR in primary and chemoresistant Glioblastoma

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GlioblastomaMultiforme (GBM) is the most common and highly invasive brain tumor, with a poor prognosis and average survival of the patientsbelow 14 months. The standart treatment involves surgery, radiotherapy and chemotherapy with Temozolomide (TMZ), an alquilant agent. Despite of the enforcement of this therapeutic scheme the recurrence of GBM is at that moment inevitable.

Among the mechanisms for tumoral resistance, the study of the endoplasmic reticulum stress (Unfolded Protein Response, UPR) and autophagy as adaptative processes activated in response to nutrient deprivation, chemo and radiotherapy has gained interest in recent years.

Here we have investigated the regulation of autophagy and UPR after TMZ treatment in GBM cells. We have generated a GBM cell line resistant to TMZ (A172-R), derivedfrom the A172 cell line. Finally, we have analyzed the autophagy and UPR markersin paired biopsies obtained from patients (primary tumor vs. recurrence of the same patient).

Results indicate that TMZ raises the autophagy markers (LC3-II, p62,Beclin, P-AMPK, Atg5 and the dimer Atg5-Atg12), some of them already at 6h afterTMZ, and the UPR marker P-eIF4. Similar results were obtained in the chemoresistantcell line. Moreover, resistant cells showed an increasedautophagic flux,as revealed by the expression of ptfLC3 construct. Together these findings demonstrate that TMZ is an autophagy inductor. Analysis of the paired biopsies of primary and recurrent GBMs confirmedan increase of the mentioned markers in the recurrences.

On the other hand, we have observed that A172-R overexpresses a T-type isoform of voltage gated calcium channel (Cav3.1)previously associated to a high autophagic flux and which inhibition deregulates autophagy promotingcell death. Ongoing experiments will define if the regulation of Cav3.1 and autophagy in primary and chemoresistant GBM could be a good therapeutic strategy for this tumor.

Developing in a new CRISPR/Cas9-based mouse model for the study of gliomas

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Mouse models are a powerful strategy that allows elucidating functions of genes, analyzing genetic pathways, and manipulating the cellular or biochemical properties of proteins.

For the study of gliomas, the most common and lethal primary central nervous system tumor in adults, one widely-used model is based on the RCAS-Tva System that is highly reproducible, feasible, and displays the high-grade features. This model can be controlled in a time and space manner by targeting either astrocytes (Gtv-a) or glioneural progenitors (Ntv-a). This system is versatile and allows the incorporation of different genetic tools including RNAi and recombinases. These days, the CRISPR/Cas9 technology has achieved an enormous development and it is one of the most promising tools for the study of the diseases, including cancer.

We have developed a new RCAS-CRISPR mouse model that combines the power of RCAS-Tva model and genome editing by CRISPR/Cas9. To do this, we generated the compound mice [Ntv-a/Gtv-a; Rosa26- LSL-Cas9-GFP; NCre/GCre] and novel RCAS-gRNA vector that includes specific gRNAS against well-known tumor suppressor genes (TSGs) for gliomagenesis.

We have successfully knockout in vivo the expression of TSGs cells producing high-grade gliomas, suggesting that our RCAS-CRISPR is a new powerful and quick tool to study the loss-of-function role of genes in gliomas.

In addition to gene ablation, alone or in combination, this mouse model also allows to study complex genetic alterations such as fusion events, point mutations or chromosomal deletions.

Brain drug delivery with the assistance Of LRP-1 targeted self-assembled nanocarriers

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Introduction

Drug discovery and development towards brain diseases (including brain tumors and neurological disorders) is a challenging task due to the presence of the most impenetrable biological barrier in the human body, the blood brain barrier (BBB) [1] that does not allow for effective drug concentrations at the brain parenchyma within the therapeutic window [2]. In this project we aimed to overcome such limitation by applying novel nanosystems able to bypass the BBB by means of active targeting strategies based on LRP-1 receptor [3].

Results and Discussion

We have developed a strategy for the preparation of novel drug delivery systems (DDS) with higher MW [4, 5] that would allow longer circulation times in vivo, adequate functionalities [6] to enhance BBB crossing and subsequently accumulate therapeutics as well as imaging probes in the brain. Those systems are biocompatible and biodegradable self-assembled star-shaped polyglutamates [7,8]. The preliminary biological evaluation of our constructs against selected cell lines revealed their absence of toxicity and exhibited a significantly enhanced cell internalization rate when compared with the linear-PGA and star unimers under the conditions tested, as shown by flow cytometry and confocal microscopy. This newly described architectures, decorated with imaging agents and targeting units (ANG-2) by surface modifications, showed interesting *in vivo* biodistributions in wild-type animals with relevant brain accumulations (up to ~1.5 % ID) [8]. Furthermore, we have recently tested this compound in a well-stablished metastatic brain cancer [9] where we could clearly observed a selective accumulation in the tumor area.

Conclusion

So far, the data obtained up to date with these systems highlights their promising potential for their use as nanomedicine in the treatment of brain diseases. Further experiments in order to validate these structures in different models (including brain tumors, and neurological disorders such as Alzheimer's Disease and neuroinflammation) are being conducted.

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The mucin-like glycoprotein podoplanin in glioblastoma

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The transmembrane protein podoplanin (PDPN) is expressed in various organs including the brain where it is restricted to the choroid plexus, ependymal and neural stem cells. In primary glioblastoma (GB) strong PDPN expression, due to loss of the PTEN tumor suppressor, correlates with shorter overall survival. To assess the function of PDPN in a model closely reflecting all features of GB we used primary patient-derived GB cells. Gene set enrichment analyses of a panel of these samples revealed the association of high PDPN expression with genes involved in cell adhesion, migration and inhibition of apoptosis. Consistently, the expression of PDPN increased upon serial xenotransplantation of the cells into immune compromised mice paralleled by a shorter survival time. To decipher the functional role of PDPN on tumor progression we deleted PDPN in these cells using the CRISPR/Cas9 technology. However, we do neither observe a significant survival benefit in recipients injected with PDPNKO tumor cells nor do these tumors display an altered histology compared to control tumors. Taken together, our current in vivo and complementary in vitro data support the notion that PDPN is a valuable marker for poor prognosis but does not constitute a major driver for malignant progression of GB. Interestingly, in the course of our studies, we detected Pdpn expression not only in tumor cells, but also in the tumor microenvironment, raising the question whether this might cause the poor prognosis for GB patients exhibiting high PDPN levels. Thus, we first sought to determine the source of Pdpn expression in the microenvironment. Employing stainings of tumor sections and primary cell isolations, we found Pdpn expression in tumor associated-myeloid cells and reactive astrocytes. Currently we are engaging mouse models that harbor a specific Pdpn loss in these cells as recipients for orthotopic tumor cell transplantations to elucidate the impact of microenvironmental Pdpn on GB progression.

A first-in-man phase I/IIa clinical trial with 2OHOA in patients with advanced solid tumors including grade III/IV glioblastoma multiforme (GBM).

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2-Hydroxyoleic acid (2OHOA) is a synthetic hydroxylated lipid that activates sphingomyelin synthase (SGMS) and regulates the membranes lipidic structure, resulting in translocation of Ras to the cytoplasm and Ras/MAPK, PI3K/Akt and PKC/cyclin/CDK signaling pathways inactivation. This firstin-man trial was designed to determine 2OHOA's safety, tolerability, and recommended Phase IIb dose, alongside its PK, PD and anti-tumor efficacy.

54 patients with advanced solid tumors have participated in the Phase I/IIa clinical study with 2OHOA (NCT01792310), half of them with malignant glioma. The study comprised two parts: 1) a dose-escalation phase where 7 cohorts with a total of 32 patients (15 with glioma) have been orally treated with doses ranging from 0.5 to 16 g/day and in which the Maximum Tolerated Dose (MTD) was defined as 12 g/day; and 2) an expanded safety part with two cohorts, one with malignant glioma patients (n=12) and another with other advanced solid tumors (n=10) treated at the MTD. Interestingly, no drug-related serious adverse events have been reported in any of the 54 patients treated.

2OHOA anti-tumoral activity as single agent has been confirmed in 10 glioma patients according to RANO criteria. 6 responses have been observed in refractory malignant glioma patients, including one patient that has had a marked shrinkage (>91% over baseline) of the lesion for 3 years. Other 4 refractory malignant glioma patients have shown stable disease (SD) for at least 6 months.

In conclusion, 2OHOA can be safely administered up to doses of 12 g/day. Clinical benefit was observed in 10 glioma patients. These results indicate that 2OHOA is a safe and efficacious antitumoral lipid molecule. The positive results of this first phase warrants further clinical development of 2OHOA. Lipopharma Therapeutics S.L. is now planning a Phase IIb study, if it is positive, Lipopharma will seek conditional approval as a first line treatment in combination with SoC for GBM in EU.

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Targeting heparan sulfate in the brain tumor microenvironment

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Malignant BTs are invasive and infiltrate healthy brain tissue, which is the main reason they remain fatal despite resection since cells that have already migrated away lead to rapid regrowth of the tumor. During the evolution of BT, the tumor is likely to progress from an initial, contained, environment to a more diffuse, infiltrative disease dependent on an as yet undefined invasive niche.

Heparan sulfate proteoglycans (HSPGs), main components of the niche, modulates the activities of other factors e.g. growth factors and we have identified a vital role for HS biosynthesis in neural stem cell differentiation. The major enzyme that degrades HS, heparanase (HPSE), is an important regulator of ECM remodeling and has recently been shown by us to promote the growth of teratoma and glioma, and correlate to patient survival in GBM. We now report that expression of HPSE is higher in pediatric tumors than in healthy brain tissue. Conditioned medium from CHO cells overexpressing HPSE contains the active enzyme and stimulates proliferation of MB cells and PNET (cell line PFSK, previously classified as PNET). Pediatric brain tumor cells were also treated with recombinant HPSE (rHPSE) in the form of latent 65kDa, which requires intracellular activation but may also exert direct effects. rHPSE increases the number of viable cells, and interestingly, rapidly activates ERK and AKT pathways, even before enzymatically active HPSE was detected. Thus, rHPSE has direct effects on MB and PFSK cells. Furthermore, we used a HPSE inhibitor (PG545) that efficiently killed pediatric brain tumor cells but not normal human astrocytes, and was found to potently reduce the size of flank tumors derived from these tumor cells.

Heparan sulfate proteoglycans and the enzymes that synthesize and degrade heparan sulfate are part of the malignant brain tumor signature. Our results support the hypothesis that HPSE affects both tumor cells and their ECM, thereby influencing tumor growth.

Multiplex genome engineering of mouse neural stem cells to create a novel set of glioblastoma-initiating stem cell lines

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Glioblastoma (GBM) is the most malignant form of primary brain cancer. Tumours comprise a heterogeneous population of cells, including those that express neural stem cell (NS) markers, and these are thought to drive tumour growth. Patient-derived tumour-initiating cells can be propagated in culture and have been termed glioma neural stem cells (GNS); these have the capacity to initiate tumours following orthotopic transplantation into immunocompromised mice.

Genetically engineered mouse models (GEMMs) have been extremely important in helping to define cell of origin and the key genetic drivers. However, GEMMs are costly to produce and maintain. To complement GEMM studies we have explored an alternative and more direct approach at generating mouse glioblastoma-initiating NS cells (GNS) cells. Engineering of candidate driver mutations stepwise and in combination directly into cultured mouse NS cells would provide a set of isogenic matched pairs without the widespread karyotypic alterations seen in late stage human tumours. This has recently become possible in mouse NS cells as we can now perform efficient genome editing using CRISPR/Cas technologies. Engineered mouse GNS cells can be transplanted into syngeneic mice, providing an immunomatched model with experimental control of both the site and timing of tumour initiation. This approach complements traditional GEMM, but is has significant advantages in terms of costs, flexibility and multiplexing. Here I present progress with generation of validated CRISPR/Cas reagents for efficient deletion of the most common tumour suppressors in GBM: Tp53, Pten, Cdkn2a and Nf1. Co-deletion of these reagents concomitantly with oncogenes such as EGFRVIII and PDGFRalpha, enables us to rapidly generate GNS cells across the spectrum of primary GBM subtypes. Characterization of this novel cellular genetics ‘toolkit’ for GBM is presented. This opens up opportunities for defined molecular cellular analysis of tumour growth and evolution.

Using a mouse glioma model to study the participation of tumor vasculature in the response to therapy

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Glioblastoma (GBM) is the most common primary brain tumor. Standard treatment for GBM consist of an aggressive surgery, followed by radiotherapy and Temozolomide concomitant (TMZ) treatment. However, GBM is a very resilient tumor and the overall survival is 15 months. Forty percent of GBMs show amplification of EGFR and, in half of those, the extracellular domain of the receptor is deleted, giving rise to a truncated isoform (EGFRvIII). We have participated in a Phase II trial with dacomitinib, a second-generation tyrosine kinase inhibitor that binds irreversibly to the receptor and inhibits EGRFR and EGFRvIII. However, despite the good results obtained in patient-derived xenograft (PDX), only a very small percentage of patients responded in a preclinical study.

One of the aspects that complicates GBM treatment is the brain location and the presence of a specialized blood brain barrier (BBB) that prevents drug delivery. However, profuse endothelial proliferation and disruption of the BBB are some of the histological features of GBM. Still, the crosstalk between human glioma cells and the mouse microenvironment is far from being optimal. In order to evaluate the role of vascular cells in the innate chemoresistance of GBM, we have generated a new glioma model by overexpressing EGFRwt or EGFRvIII in p16/p19 KO mouse neural progenitors. Co-culture studies in the presence of a mouse endothelial cell line suggest a protective role of these cells in response to chemotherapy. Moreover, analysis of the response of subcutaneous and intracranial allografts to TMZ and dacomitinib, show striking differences between EGFRwt and EGFRvIII expressing tumors. Preliminary data suggest that the vasculature of these two models is behind these differential results. We are confident that our model could help us to understand the poor response of GBM to chemotherapy, and probably to find predictive markers associated with the tumor-vessel architecture and/or function.

Different immune cell responses are associated with typical genetic alterations in glioblastoma

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Interactions between various components in tumor microenvironment and immunosuppression are thought to play important roles in cancer development. To better understand the role of immune cells in tumor pathogenesis and destruction, we computationally model the microenvironment of an aggressive brain tumor glioblastoma. Utilizing glioblastoma RNA-seq data from the Cancer Genome Atlas, we clustered the genes and identified 8 immune response related clusters based on Gene ontology and KEGG enrichment. We constructed a regression model to characterize the expression profiles of glioblastoma samples in these clusters of interest as linear combinations of normal cell and reference glioblastoma expression profiles. Based on the regression analysis, we were able to uncover high variability in the composition of microenvironment across the cohort, suggesting diverse immune responses in tumors. We showed that immune cell compositions are associated to typical alterations and subclassification in glioblastoma. Furthermore, using clustering we identified three subgroups of samples presenting different immune responses and linked to alterations and subclassification in glioblastoma. Taken together, our analysis provides a characterization of the immunomicroenvironment in glioblastoma and identified groups of samples with different immune responses. More detailed characterization of diverse immune responses will facilitate patient stratification and might provide tools for personalized immunotherapy in the future.

MELK is a regulator of PPARγ function in diffuse intrinsic pontine glioma

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Diffuse intrinsic pontine glioma (DIPG) is an infiltrative pediatric high grade glioma with very poor prognosis, which urges the development of new therapeutic approaches. Maternal Embryonic Leucine Kinase (MELK) is the second highest upregulated kinase in DIPG at the mRNA level. Since MELK is known to be involved in malignant transformation as well as in the maintenance of brain cancer stem cell features, we investigated the effects of MELK inhibition in DIPG cell cultures. In the present study, we confirm that MELK is highly expressed in DIPG neurosphere cell cultures. MELK inhibition using the small molecule inhibitor OTSSP167 had a strong antiproliferative effect in primary DIPG cell lines at low nanomolar concentrations *in vitro*. RNA sequencing analysis demonstrated enrichment of the PPAR signaling pathway gene set in DIPG cells following MELK inhibition by OTSSP167. Additionally, we found predicted PPAR response elements, mostly for PPARγ, in the promotor region of the majority of upregulated genes, as well as a significant upregulation of known PPARγ target genes. We demonstrated that MELK is involved in the phosphorylation of PPARγ at serine 112, a post-translational modification that is known to control the intracellular localization and transcriptional activity of the PPAR complex. As expected, MELK inhibition increased nuclear localization of PPARγ in vitro, suggesting a role for this kinase in regulating the subcellular localization of PPARγ. Finally, we showed that OTSSP167 and the PPARγ agonists troglitazone and pioglitazone synergystically decrease DIPG neurosphere survival *in vitro*. Based on these pre-clinical results, we propose MELK as a promising molecular target for the treatment of DIPG, and suggest the combination of a MELK inhibitor and PPARγ agonist for future in vivo and clinical trials.

This abstract describes research related to the project described in the abstract of Michael H Meel.

Ocoxin® oral solution (OOS) inhibits the growth of primary glioblastoma cells *in vivo*

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Glioblastoma (GBM) is one of the most aggressive primary brain tumors. It has a very poor prognosis, with an overall survival of around 15 months. The only improvement in therapy in the last 20 years has been the addition of oral doses of temozolomize (TMZ) to surgery and radiotherapy. This is known as the Stupp protocol and has become the standard treatment, although the improvement in survival is only of 2.5 months in average. So it is necessary to find new compounds that may help in the treatment of the GBM.

GBMs are characterized by regions of endothelial proliferation although the new vessels formed are aberrant and often associated with inefficient blood flow, which increases tumor hypoxia. Thrombotic vessels are also frequent in GBM, and they lead to hypoxic and necrotic regions. Paradoxically, hypoxia has been associated with an increase in the levels of reactive oxygen species (ROS), which could be participating in several aspect of the tumor biology: angiogenesis, cell survival, undifferentiation, chemoresistance, invasion and metabolic changes. Therefore, anti-oxidant agents, as nutritional supplements, might have antitumor activity, as it has been proposed for other types of cancer.

Ocoxin® oral solution (OOS) is a dietary supplement composed by green tea extract, vitamin C, B6 and B12, minerals and amino acids, with anti-inflammatory and anti-oxidant properties. It has been previously described that OOS slows tumor growth, inhibits tumor cells proliferation and reduces tumor incidence in experimental models of colorectal, hepatic or breast cancer an acute myeloid leukemia, with no side effects. The aim of this study was to analyze the antitumor effect of OOS in a panel of primary lines of GBM grown as flank xenografts in Nude (athymic Nude-Foxn-1nu mice, Harlan ibérica) mice. Our preliminary results suggests that OOS reduces the growth of different GBM cell lines, so it could be a good candidate to be used as an adjuvant during GBM treatment.

Glioma-induced neurodegeneration disrupts circadian rhythms

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Glioblastoma (GBM) is the most common tumor from the central nervous system (Ostrom et al., 2015). GBM is an infiltrative, invasive and highly aggressive tumor with a poor-prognosis. Currently, there is no efficient treatment against it. GMB causes neurological symptoms in patients such as sleep disturbances (Nymmudi & Jalali, 2014). Our group has proposed a novel approach to study GMB, considering it as a neurodegenerative disease. We use a GMB model based on the expression of constitutively active EGFR and PI3K in glial cells (Read et al., 2009). Our results indicate that GBM affects neuronal function by inducing synapse loss, one of the first events in neurodegeneration (Scheff & Price, 2003). Specifically, we focus our analysis on the effect of GMB in circadian clock neurons and disturbances in circadian rhythms. Our preliminary results show that the progression of GBM triggers a progressive disruption of circadian rhythmicity. Moreover, in absence of GBM, a decrease in the number of synapses in clock neurons induces changes in circadian rhythms. Our final aim is to protect the neurons from neurodegeneration and prevent phenotypes associated to the neurodegeneration linked to GBM, as a consequence, to improve the life expectancy and quality of life. Furthermore, we want to understand the role of circadian disturbances in GBM disease through the study of clock neurons. To perform these experiments, new genetic tools were created during this study.

The role of phosphofructokinase-1 in Glioblastoma maintenance and motility

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BACKGROUND: Glioblastoma (GBM) remains one of the most lethal tumors and is associated with a median survival of only approximately 15 months, despite aggressive combination radio-chemo-therapy. One distinct challenge in GBM management includes diffuse location of the tumor within the brain and migration of cancerous cells into the healthy surrounding tissue preventing complete surgical removal and promoting recurrence. It is commonly known that cancer cells utilize glucose as their main energy source, a phenomenon known as the Warburg Effect. Phosphofructokinase-1 (PFK-1) is one of two rate-limiting enzymes in glycolysis and presents a potential critical check point for these glucose "addictive" cells. Additional to its glycolytic role we here propose a regulatory role for PFK-1 in motility in GBM cells.

METHODS: To elucidate the role of PFK-1 in GBM, we abrogated its function using shRNA-mediated knockdown or chemical inhibition. Next, we evaluated the cellular responses utilizing a broad range of in vitro assays (cell viability, apoptosis, cytoskeleton assembly, migration assay). RESULTS: Depletion of PFK-1 led to decreased viability and increased rates of apoptosis. Furthermore, impaired function of PFK-1 affected cytoskeleton assembly and decreased migration capacity of GBM cells, a phenotype we were able to mimic with a chemical PFK-1 inhibitor.

CONCLUSION: The novel role of PFK-1 identified in our studies, together with successful therapeutic targeting in vitro and *in vivo*, furthers our understanding of PFK-1 in GBM maintenance and cell migration.

Modulators of temozolomide-induced citotoxicity in Glioblastoma multiforme

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Resistance to chemotherapy represents a major challenge for the development of durable therapeutic strategies in Glioblastoma multiforme (GBM). Recent findings suggest that the genetic alterations that characterize GBM genomes also contribute to the poor treatment response of this tumor type, by modulating the activity of the DNA damage checkpoints and the DNA repair machinery (DDR).

To understand and overcome glioma cell resistance to the alkylating agent temozolomide (TMZ) we have performed in vitro genetic screens with a customized shRNA library against "DDR genes". We have identified a series of genes that are able to modulate the response to TMZ. As previously described in patients, we have found in our screenings that impairment of the mismatch repair (MMR) genes represents a major mechanism of resistance in the absence of O6-methylguanine DNA methyltransferase. To identify synthetic lethal interactions with MMR impairment we are running a series of shRNA and compound screens in MMR deficient cell lines. In addition, we are performing several bioinformatic analysis of molecular and drug response transcriptional signatures to identify novel targets and compounds that could overcome MMR-deficiency mediated TMZ resistance in GBM. Gaining insights into the genes that determine this poor treatment response will be instrumental for the development of new therapeutic modalities and the identification of biomarkers for GBM patients.

The Good drug, the Bad barrier and the Ugly glioma: Targeting brain tumors with novel nanocompounds

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High grade malignant gliomas (GBM) represent the most common and aggressive brain tumors in adults. They are classically characterized by a strong invasiveness and a dense but disorganized angiogenesis causing changes in the tumor microenvironment (hypoxia/necrosis) and intracerebral hemorrhages. Till date, GBM is an incurable and deadly disease. Current treatment strategies suffer of modest efficiency and fail to prevent tumor relapse. In addition, poor permeability of therapeutics through the Blood-Brain-Barrier (BBB) and their inability to target distant invasive cells reduces the efficacy. Moreover, most of the therapeutics are cytotoxic for both tumor and normal tissue, causing numerous and severe adverse effects. Novel Nanotherapeutics (NT) represent potential players to treat GBM by limiting adverse effects of drugs, providing specific tumor cell targeting properties as well as ensuring delivery through the BBB. We validated an *in vitro* model of the gliovascular niche to dissect the NT capability to cross BBB and to efficiently target neoplastic cells. Thus, the best NP candidates were then tested in vivo, using orthotopic xenografts of patients' GBM cells in immunocompromised mice. This preclinical study reveals that NT size and presence of a targeting peptide on the surface greatly favor penetration of the NT within the brain parenchyma and selective homing to GBM cells. Altogether, our results demonstrate that NT constructs enriched with a targeting peptide can efficiently bind to tumor cells, with modest to none toxicity toward endothelial cells and astrocytes. We are currently evaluating the therapeutic value of our NT when enriched with Taxol, to specifically target and kill the tumor cells with limited side effects for the normal brain tissue.

Keywords: Glioma, Nanoparticles, Blood Brain Barrier, Targeted Therapies, preclinical.

C3G: a new player in the control of invasiveness, stemness and tumorigenesis in glioblastoma via ERKs and p38MAPK regulation

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C3G is a guanine-nucleotide exchange factor (GEF) for some Ras family members, although it can act through GEF independent mechanisms. The role of C3G in human cancer is controversial, acting as either a tumour suppressor or mediator. Its function in migration also depends on the context. Recently, we found that C3G acts as a migratory and invasive repressor in colon carcinoma cells through down-regulation of p38α MAPK activity. Thus, we have studied the role played by C3G in human glioblastoma through permanent gene silencing, using the U87 cell line as an *in vitro* model.

We found that C3G knock-down enhances migration and invasion and reduces cell adhesion, inducing the actin cytoskeleton re-organization. These functional and morphological changes correlate with the expression of transcription factors and proteins involved in the epithelial-mesenchymal transition (EMT). Moreover, C3G knock-down induces anchorage-dependent and -independent growth of U87 cells. C3G also controls its stemness, so that, its silencing favours spheres formation and the acquisition of a glioblastoma initiating cell-like phenotype.

C3G silencing also induces changes in different signalling pathways, such as p38 MAPKs, ERKs, STAT3 or c-Met, which contribute to mediate C3G effects. In particular, C3G inhibits anchorage-dependent and independent growth through ERKs inactivation and/or p38 MAPK regulation. These pathways are also involved in C3G effects on cell adhesion, invasion and proliferation.

All these data indicate that C3G is a new regulator of glioblastoma progression. Thus, its down-regulation would promote tumour growth, the generation of metastasis through a mechanism involving an EMT process and the acquisition of stemness properties. More studies are required to fully characterize C3G function in glioblastoma, as well as the molecular basis of this regulation and its relevance in clinic.

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The role of Fra-1 and Fra-2 in gliomagenesis

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AP-1 transcription factors are implicated in several biological processes including oncogenic transformation and its components have been described to be abnormally expressed and/or activated in several human cancers. Our study is focused on the Fos family members, Fra-1 and Fra-2, in gliomas. Fra-1 is overexpressed in brain tumors and it has been shown to modulate the malignant properties of glioma cells. Fra-2 has been described as a regulator of the Mesenchymal signature, one of the glioblastoma (GBM) subtypes as defined by gene expression analysis. Analysis of different human datasets showed Fra-1 and Fra-2 higher expression in high-grade tumors compared low-grade tumors and normal brain. In order to characterize the role of Fra-1 and Fra-2 in glioma tumor formation we are combining *in vivo* and *in vitro* strategies. Using the RCAS-tva system, we are generating tumors in different genetic backgrounds in a Fra-1/-2 loss of function context. This system also allows us to grow *in vitro* primary tumor cells and perform several assays *in vitro* as well as *in vivo*, using syngeneic models. We are also studying Fra-1/-2 roles in human established GBM cell lines and primary neurosphere cultures. Preliminary results show that Fra-1 might have a role in tumor cell growth and in mice survival but further studies, also in the context of treatment response, would be crucial to understand the relevance of this transcription factors in GBM.

(1) Garros-regulez L et al., Expert Opinion on Therapeutic Targets 2016
(2) Martin-Martin N et al., Nature Communications 2016

Identification of critical molecular pathways underlying glioblastoma stem cell activity

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Glioblastoma multiforme (GBM) is the most frequent and lethal primary brain tumor, which exhibits an extremely poor prognosis characterized by an average survival of 15 months. GBM contains a subpopulation of glioma stem cells (GSC) that are crucial drivers of tumor initiation, recurrence and resistance to therapies.

The dysregulation of genes essential for embryo development and stem cell maintenance seems to be a critical feature in GBM development and and GSC activity. Therefore, these genes could become potential therapeutic targets in the treatment of GBM. In this sense, we have recently identified that SOX9 (sex-determining region Y (SRY)-box 9 protein) is a relevant player in glioma stem cell activity and temozolomide resistance1.

To characterize SOX9 underlying molecular mechanisms we have performed transcriptomic analysis in patient-derived GSC with SOX9 knockdown Notably, JAK2/STAT3 and PML expression were within the genes significantly downregulated in SOX9 Knockdown cells. These results are in agreement with recent data in breast cancer where we have recently shown that STAT3-PML-SOX9 is a relevant axis for the metastatic features of breast cancer stem cells2.

In glioblastoma, our results show that SOX9, STAT3 and PML are enriched in the population of GSCs and are important for its maintenance. Indeed, Individual silencing of SOX9, STAT3 and PML severely impairs self-renewal and tumor initiation in patient-derived GSCs. Moreover, we found that there is a genetic regulatory loop between them. In line with this idea, we observed that Arsenic Trioxide, a PML inhibitor, and STX-0119, a STAT3 inhibitor, impairs GSCs activity through SOX9.We also tested their clinical relevance finding that their expression is elevated in biopsies from a subset of patients with overall poor prognosis. In summary, these results demonstrate that SOX9-STAT3-PML is a critical axis for cancer stem cell maintenance, postulating that its inhibition is a promising strategy to combat chemotherapy resistance in glioblastoma, but also in other types of aggressive cancers.

Exploring the astrocyte-dependent growth of glioblastoma cells through a high-throughput screening (HTS) of small molecules and a gene-expression analysis

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Glioblastoma multiforme (GBM) is among the most lethal tumor types. The pathological tissue contains different tumor-associated cell types, including astrocytes, which contribute to tumour behaviour. Our work aims at exploring the potential role(s) of crosstalk between astrocytes and malignant cells in GBM with regard to growth and drug response of the malignant cells. According to completed studies, GBM cell lines, as well as a panel of primary GBM cultures, indeed show increased growth upon direct co-culture with astrocytes.

Ongoing studies are performed with the double intention of identification of the molecular details of the underlying pathogenic paracrine crosstalk and identification of small molecule inhibitors able to interfere with astrocyte-dependent GBM growth.

An HTS has been established to identify low molecular weight compounds specifically blocking the astrocyte-dependent GBM growth. After a pilot screening of 1200 compounds, 15 have been identified which interfered with the astrocyte-dependent growth of GBM, but not with the astrocyteindependent GBM growth. Efficacy of selected compounds has been validated in independent experiments on the GBM cell line used in the screen and in other GBM cultures.

To elucidate the molecular basis for the astrocyte-dependent GBM growth, gene-expression of astrocytes and glioblastoma, alone or in co-culture, have been analysed. Ongoing analyses have identified approximately 400 genes that show differential expression in astrocytes kept in mono- or co-culture and 7 genes in the GBM cells. These genes are in the process of validation and knock-down/ overexpression studies will clarify their role in the astrocyte-GBM crosstalk.

Future studies will continue efforts to develop in vivo-active inhibitors of astrocyte-dependent GBM growth. Inhibitors will also be used as chemical biology tools to help identifying molecular pathways involved in the growthsupportive effect of astrocytes.

Synthetic lethality through combination of multikinase inhibitors and PP2A activation in glioblastoma

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Prognosis of the most common and devastating brain cancer in adults, glioblastoma multiforme (GBM), is very poor. Survival after the diagnosis is only up to few years with the current treatment thus a demand for new and innovative treatment targets is truly evident. Even though dysregulated kinase pathways are drivers of malignant progression in GBM, glioma cells exhibit intrinsic resistance towards many kinase inhibitors, and the molecular basis of this resistance remains poorly understood. Here we propose to study protein phosphatase 2A (PP2A) as a potential target for GBM therapy. PP2A has been identified as a tumor suppressor and is one of the most abundant phosphatases within cells. It regulates multiple cellular signaling pathways e.g. oncogenic signaling cascades, such as Raf, MEK and AKT, hence its dysfunction can lead to cancer. We recently demonstrated that overexpression of the PP2A inhibitor PME-1 drives resistance of glioma cells to various multikinase inhibitors (MKIs) (Kaur et al., Cancer Res, 2016). In *in vivo* studies we showed significant tumor growth inhibition in PME-1 silenced GBM xenografts treated with MKIs. In order to model therapeutic potential of PP2A reactivation for glioma therapy, we have tested the synthetic lethal activity of MKIs in combination with small molecule activators of PP2A. The results demonstrate that also this combination has a very potent cell killing effect in various GMB cell lines with different genetic backgrounds in vitro. Additional challenge in the treatment of GBM is blood-brain barrier passage. For this we have developed a slowly degrading nanoparticle releasing the drug combination in a regulated manner. Our goal is to eventually use these nanoparticles for modelling the efficacy of the combination therapy in an intracranial model of GBM. In summary, these results introduce a novel combination therapy approach for kinase inhibitor resistant glioma cells to be used as a secondary treatment after surgery.

Novel epigenetic targets in pediatric high-grade glioma

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Pediatric high-grade gliomas (HGGs) are the leading cause of cancer related death during childhood. Recent insights revealed that the epigenetic landscape of HGGs in children shows a distinct pattern from their adult counterparts, as has been shown with pediatric HGG associated H3K27M and H3G34V/R histone modifications. These findings highlight the importance of tailored therapeutic strategies in pediatric HGG. Our research group recently showed that pediatric diffuse midline gliomas are prone to epigenetic targeting via histone deacetylase inhibitors (HDACi). However, single epigenetic targeting therapies lack the effectiveness needed for clinical treatment. Therefore, we hypothesized that the effect might be dampened by rescue mechanisms. In this project we firstly aim to identify epigenetic drugs as potential candidates for pediatric HGG treatment. Secondly, we aim to combine the identified epigenetic drugs with a selection of relevant conventional chemotherapeutics to increase their effectiveness.

15 primary human HGG cell lines were cultured both as adherent monolayers and neurospheres. Subsequently, all cultures were treated with over 150 epigenetic drugs. All drugs were tested in absence and presence of conventional fractionated irradiation. Potential drug candidates were selected based on cell growth and viability parameters. The respective treated cells were subjected to total mRNA sequencing in order to identify drug-induced rescue mechanisms.

We will give an overview of the targets that have thus far been identified while highlighting their underlying mechanisms and potential as anti-cancer targets in pediatric HGG.

Epigenetic regulation of brain metastasis by linker histone H1.2

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Determinants of metastatic disease have been classically studied within specific cancer types. However cancer cells targeting a given organ will face a number of common barriers during their colonization. Thus, it is possible that commonly co-opted genes by different types of cancer cells targeting the same secondary organ are required independently of the primary tumor type. Previous work from our lab described a reduced gene list commonly upregulated in brain tropic cancer cells derived from breast and lung adenocarcinomas. The linker histone HIST1H1C (H1.2) is part of this gene signature however its contribution to brain metastasis has not been addressed.

In order to interrogate its functional role and the possibility to address its effects on the epigenetics of brain metastasis we performed brain metastasis assays. Knock down of H1.2 dramatically decreased brain metastasis burden and extended overall survival in a triple negative breast cancer and an EGFR mutant lung cancer models.

Mechanistically, loss of H1.2 impairs H3K27me3 *in vivo* suggesting a potential regulation on the Polycomb-mediated repression of as yet unknown brain metastasis suppressor genes. In this sense removal of H1.2 at initiation or in established metastasis impairs proliferation. This phenotype is not found under normal *in vitro* cultured conditions but only when brain tropic cancer cell lines are challenged in oncospheres or colony forming assays, which are molecularly linked to metastasis initiating capabilities. Although H1.2 levels in situ are heterogeneous at the intra and inter-metastasis levels, isolation of established metastasis from the brain invariably correlates with higher expression values of the histone, suggesting that adaptation to the brain microenvironment could be achieved by epigenetic regulation.

The role of the immune system in facilitating the formation of brain metastasis of breast cancer

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Brain metastases are clinically devastating and one of the most faired complications of cancer. They are the most common malignancy of the brain and 25-40% of cancer patients will develop brain metastases. The formation of cerebral metastases depends on the capacity of circulating tumor cells to successfully penetrate the blood-brain barrier (BBB). Elucidating the underlying mechanisms of crossing the BBB is crucial for the prevention of cerebral metastases.

We compared the gene expression profiles of ER- primary breast cancer biopsy samples of women with metastasized disease, with and without brain involvement. We found genes related to T cell response as most prominently associated with the development of brain metastases. In functional studies using an *in vitro* BBB model breast cancer cells that were co-cultured with T cells passed the artificial BBB with a 300-600 fold acceleration. We identified 11 differentially expressed proteins in breast cancer cell lines that were co-cultured with T lymphocytes. After matching these proteins with RNA expression data of the original patient datasets, we found that the gene for guanylate binding protein 1 (GBP1) was upregulated in the samples that were associated with cerebral metastasis. Silencing of GBP1 in breast cancer cells resulted in a 30-70 fold decrease of the passage of the breast cancer cells through the BBB model.

The results point to expressional imprinting of breast cancer cells by T lymphocytes, resulting in accelerated passage through the BBB. Proteins upregulated by the T cells like GBP1 are candidate biomarkers for estimating the risk of the formation of cerebral metastases.

Inhibition of Wnt/β-Catenin signalling upregulates P62 and autophagy and promotes glioblastoma cell death by autophagy blockers

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Wnt/β-catenin signaling regulates cancer cell proliferation and stemness. Furthermore, recent evidence indicate that Wnt signalling and autophagy inversely correlate. Here we investigated the impact of inhibiting Wnt signalling on autophagy in glioblastoma (GBM), a devastating brain tumor. Inhibiting or silencing T Cell-specific Factors (TCFs) that together with β-catenin regulate expression of Wnt targets or silencing β-catenin, upregulated p62 in GBM at transcriptional and protein levels and in turn, autophagy. Treatment with Dickkopf, a canonical Wnt receptor antagonist, also induced autophagic flux. Importantly, TCF inhibition regulated autophagy through mTOR. Additionally, TCF inhibition/silencing affected GBM cell proliferation and migration.

Autophagy induction followed by its blockade can be exploited to promote cancer cell death. In agreement with this notion, dual inhibition of TCF and autophagic flux with autophagy blockers decreased cell viability and induced apoptosis of GBM cells. Together, combined targeting of TCF and autophagy promotes cell death and in vivo experiments suggest that it could be a putative therapeutic strategy in GBM.

Plasticity of clonal glioma-initiating cell populations in GBM

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Intratumoral heterogeneity is substantial in glioblastoma multiforme (GBM), as the name implies. To study this in detail, we single-cell sorted patient GBM samples and established clonal glioma-initiating cell (GIC) culture libraries. Each individual clone library demonstrated a wide variety in drug and radiation responses (Segerman et al., Cell Reports, Dec 13, 2016). These responses were observed as a continuum of phenotypes, from multisensitive to multiresistant, and it resembled a normal distribution. The multitherapy resistance phenotype of the GIC clones was linked to proneural-mesenchymal transition in the transcriptome. Furthermore, multitherapy resistance was associated with low DNA methylation grade in promoter regions of mesenchymal (MES) master regulators (FOSL2, RUNX1). Our data thus implied that the transition of GIC clones is bi-directional and epigenetically regulated.

Our current work focuses on the plasticity of clonal populations. To investigate if spontaneous changes in drug and radiation response occur we long-term cultured (20 passages) a sensitive and a resistant clone; both clones showed stable therapy response. Next, we derived subclones from a resistant clone and again tested their therapy responsiveness and analyzed their transcriptomes. Both subclones with higher and lower therapy resistance than the parental resistant clone were generated, similar to the original tumor clone library. Also molecularly the subclones largely reconstituted the original clonal variation, showing a gradient along a proneural-mesenchymal axis. Our data shows that GICs are highly plastic and support the development of new treatment strategies aimed to sensitize drug and radiation resistant cells through antagonizing gene expression programs associated with a MES profile.

GBM on chip: Mimicking the glioblastoma microenvironment within microfluidic devices

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Background: Glioblastoma (GBM) is one of the most lethal tumor types. This tumor is distinguished by necrosis foci with a highly cellularized surrounding area, named pseudopalisades. This characteristic structure has been hypothesized to be promoted by thrombotic events inducing waves of migrating GBM cells. Although the universal presence of these structures in GBM tumors suggests that they may play an instrumental role in GBM's spread and invasion, the recreation of these structures in vitro has remained challenging. Recently, microfabrication and microfluidic technologies have arisen as interesting alternatives for creating biomimetic cell culture systems.

Methods: Novel microfluidic models of GBM based on a central chamber flanked by two lateral microchannels have been designed and fabricated to resemble the necrotic core formation and also the dynamics of pseudopalisade formation. Different human and rat GMB cells have been embedded within a collagen hydrogel in our microfluidic chip. By controlling the medium flow through lateral microchannels, we can mimic the oxygen and nutrient diffusion and also the blood-vessel obstruction events associated with this disease. Also, a mathematical model was developed incorporating the main parameters of pseudopalisade formation to predict the cellular behavior.

Results and discussion: When cultured within our microdevices, GBM cells can induce the appearance of a necrotic core, a hypoxic middle layer and a normoxic outer layer resembling the different GBM histological zones. This self-induced biomimetic gradient has be studied to determine drug response and cell metabolism in those areas. Moreover, when placed mimicking thrombotic conditions, GBM cells invaded towards the oxygen source, creating a migratory front which behaved very similar to the pseudopalisades found on patients. Once this migrating GBM cells reached nutrient-enriched regions, they became more aggressive and proliferation was dramatically increased.

Restoring the oncosuppressor activity of MicroRNA-34a in Glioblastoma using a Polyglycerol-based Polyplex

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Glioblastoma is the most common and aggressive primary neoplasm of the brain. Poor prognosis is mainly attributed to tumor heterogeneity, invasiveness, and drug resistance. microRNA-based therapeutics represent a promising approach due to their ability to inhibit multiple targets. In this work, we aim to restore the oncosuppressor activity of microRNA-34a (miR-34a) in glioblastoma. We developed a cationic nanocarrier system, dendritic polyglycerolamine (dPG-NH2), which remarkably improves miRNA stability, intracellular trafficking, and silencing activity. dPG-NH2 carrying mature miR-34a targets C-MET, CDK6, Notch1 and BCL-2, consequently inhibiting cell cycle progression, proliferation and migration of glioblastoma cells. Following complexation with dPG-NH2, miRNA is stable in plasma and able to cross the compromised blood brain barrier in glioblastoma while not extravasating from normal blood vessels leading to selective accumulation in brain tumors. We further show inhibition of tumor growth following treatment with dPG-NH2-miR-34a in a human glioblastoma mouse model. We hereby present a promising technology using dPG-NH2-miR-34a polyplex for brain-tumor treatment, with enhanced efficacy and no apparent signs of toxicity.

Opposite roles of Notch signaling in the formation of distinct glioma subtypes

Elena Parmigiani, Verdon Taylor, Claudio Giachino

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Neural stem cells in the postnatal brain are believed to be one origin of brain tumors such as gliomas. The Notch signaling pathway is required for neural stem cell maintenance and, accordingly, promotes a self-renewing stem cell-like state in glioma cells. Therefore, Notch signaling is believed to be oncogenic in glioma, primarily by virtue of its stem cell promoting activity. However, inactivating mutations in Notch pathway components and low Notch signaling activity have been identified in glioma subtypes in humans, suggesting a tumor suppressive role. We addressed the role of Notch signaling in glioma formation using conditional genetics and lineage tracing in mouse models of human brain tumors and discovered a context dependent function for the Notch pathway in distinct glioma subtypes. We found that gliomas driven by either p53 loss of function or Akt gain of function can originate from a cell population with high Notch signaling activity. However, surprisingly, Notch has distinct functions in these glioma subtypes. Genetic deletion of core Notch pathway components accelerates growth of gliomas driven by p53 loss of function and, conversely, genetic activation of the Notch pathway reduces glioma formation. In stark contrast, genetic deletion of Notch pathway components delays the development of gliomas driven by gain of Akt signaling. Interestingly, individual Notch receptors have distinct functions during glioma development, and only specific Notch receptors or receptor combinations can activate a tumor suppressive signal. Hence, Notch receptor paralog and glioma subtype dictate the tumor suppressive versus oncogenic role of Notch signaling.

Measuring the residual error in patient set-up during fractionated brain radiotherapy

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Introduction: Fractionated radiotherapy is the standard of care for patients with high grade gliomas (HGG). Modern radiotherapy (IMRT/VMAT) achieves better dosimetry than older techniques, but carries a risk of geometric miss and so accurate patient set-up remains important. Kilovoltage orthogonal planar imaging (KV-OPI) using bony skull anatomy is the standard for patient verification, but Cone beam CT (CBCT) has been used in a wide range of cancers. This study uses CBCT to assess residual bone and soft-tissue set-up error in patients undergoing IMRT for HGG.

Methods: We identified 18 patients with high-grade glioma treated with fractionated radiotherapy (60Gy; 30 fractions) delivered using IMRT. Each patient underwent CBCT and KV-OPI imaging on days 1,2,3 and weekly thereafter. A total of 123 paired images were assessed. The setup error in both modalities were assessed by calculating the three-dimensional iso-displacement vector (IDV). This was determined by superimposing the Digitally Reconstructed Radiographs on the KV-OPI images, and the planning CT on the CBCT images. We calculated shifts in 3 dimensions based on bone (using skull) and soft tissue (based on pineal gland and choroid plexi). The difference in IDV for KV-OPI and bone matching with CBCT and the difference in IDV for KV-OPI and soft tissue matching with CBCT were assessed using the Wilcoxon Signed Rank Test.

Results: The mean difference between KV-OPI and bone (0.35mm) and soft-tissue (0.49mm) IDVs was small, but statistically significant. ($p=0.0014$ & 0.004). 14 pairs (11%) showed more than 2mm difference in bone set-up (CBCT versus KV-OPI). 19 pairs (15%) showed more than 2mm difference in set-up error using soft tissue match on CBCT vs. KV-OPI.

Conclusion: Although the average additional error detected using CBCT is small, 15% of images have >=2mm error when comparing soft-tissue CBCT to KV-OPI. Further attempts to reduce the CTV-PTV margins in patients with HGG should be treated with caution.

GBM-microglia interaction modulated by Wnt pathway

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Glioblastoma (GBM), an extremely aggressive and deadly brain tumor, possess striking heterogeneity and capability of communicate with microenvironment components such as microglia. The interaction tumor cell-microglia can contribute to invasiveness. The Wnt pathway is one of the most crucial signaling cascades in tumor progression, playing a part in regulating cancer cells migration and invasiveness. We ought to elucidate the role of Wnt/β–catenin signaling in microglia and GBM crosstalk. We analyzed and observed the interaction and modulation of tumor cells invasiveness in the presence of microglia using GBM-microglia co-cultures. For this purpose, we used time-lapse imaging, immunofluorescence and western blot. We observed that the conditioned medium obtained from glioblastoma cells induces translocation of β-catenin from cytosol to nuclei in microglial cells. This observation is even more prominent in microglial cells treated with GBM conditioned medium from cells pre-treated with Wnt3a. Moreover, microglia enhanced the expression of proteins regulated by WNT such as Slug and ARG-I, and proteins involved in proliferation of tumor cells as STI1. Our evidences revealed that Wnt/β-catenin pathway plays important role in GBM-microglia crosstalk and that Wnt3a belong to the arsenal of factors that recruits microglia cells to tumor cells.

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Modeling glioma cell invasion in adult murine organotypic brain slices

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Glioblastoma WHO grade IV (GB) comprises the most frequent and most malignant form of brain tumors in adults. Despite the current excessive treatment combining surgical resection, radio- and chemotherapy the median survival time of glioblastoma patients still does not exceed 16 months. The aggressive growth and invasiveness, which enable tumors to escape complete surgical resection and lead to tumor recurrence, is one of the major causes of treatment failure. Tumor cell invasion is mediated by the cross-talk between tumor cells and the normal brain parenchyma. Glioma cells migrate along pre-existing Scherer´s structures, which include the neuropil, blood vessels, white matter tracts and the subarachnoid space. Our aim was to define an experimental setting which closely mimics the environment glioma cells encounter *in vivo*. To this end, we have optimized an *ex-vivo* assay assessing the ability of glioma cells to invade the parenchyma of adult murine brain. This approach allows a much more veridical analysis of glioma cell invasion compared to two-dimensional assays as the cytoarchitecture of the brain tissue is preserved. Moreover, the easy manipulation of the tumor cell environment by treatment with pharmacological inhibitors or by the use of brain slices from genetically modified mice permits the investigation of determining factors of cell migration in an easy-to-assess and reliable context. Taken together, this model will help to decipher the molecular mechanisms underlying glioma cell invasion and, eventually, to devise novel therapeutic strategies aimed at interfering with tumor cell spread in the brain.

CD44 differentially regulates HIF-1a and HIF-2a to control glioma stemness and hypoxic response

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Hypoxia-inducible factors (HIF-1a, HIF-2a) orchestrate the cellular response to hypoxia. In high-grade glioma, HIF-1a expression is elevated primarily in a hypoxic, perinecrotic niche (PN). HIF-2a is stabilized in a perivascular niche (PVN) in addition to the hypoxic niche, thereby conferring a pseudohypoxic phenotype on perivascular, stem-like glioma cells. HIF-2a has been implicated as a key transcription factor maintaining the stem-like and aggressive state of perivascular tumor cells, but little is known about the normoxic regulation of HIF-2a in these well-vascularized tumor areas. Using a mouse model of GBM, we demonstrate that the stem cell marker CD44 tunes the hypoxic and pseudo-hypoxic response of CD44+ glioma cells by interacting specifically with HIF-2a independently of the presence of oxygen, whereas CD44—HIF-1a interactions were blocked by a HIF-1a-specific hydroxylation under normoxia. CD44 expression was restricted to both the PN and PVN in PDGF-induced murine gliomas. CD44—HIF-2a interactions were dependent on the CD44 intracellular domain (CD44ICD), release of which was enhanced at hypoxia and could be pharmacologically blocked by inhibiting ADAM10/17 in primary glioma cultures. Inhibition of ADAM10/17 resulted in decreased HIF-2a (but not HIF-1a) stabilization at hypoxia, and consequently diminished activation of HIF downstream target genes, in cultured glioma cells. Finally, HIF-dependent stem cell characteristics including self-renewal, side population, and stem cell marker expression were diminished, and differentiation induced, by inhibiting CD44ICD release in glioma cells cultured under hypoxic conditions. Our data indicate that the stem cell marker CD44 regulates the hypoxic response of glioma cells, and the pseudo-hypoxic phenotype of perivascular stem-like glioma cells, by stabilizing HIF-2a independently of oxygen – and that this novel signaling pathway can be used to target HIF-dependent phenotypes *in vivo*.

A novel subpopulation of reactive astrocytes is required for the maintenance of brain metastasis

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In spite of major breakthroughs in the therapy of disseminated disease, treating brain metastasis is not efficient with current systemic approaches. Emerging evidences point towards an important role of the brain microenvironment to support brain metastasis progression. Reactive astrocytes (RA) are present surrounding metastatic lesions in the brain. Although during the early stages of brain colonization RA play an important role eliminating many of the potential brain metastasis initiating cells (Valiente et al. Cell 2014), with time their activity becomes altered and more permissive (Chen et al. Nature 2016). Understanding the complexity of this brain cell type in the context of advanced stages of brain metastasis is our ultimate goal in order to consider the development of brain specific therapies that could be combined with existing systemic approaches.

We have detected a subpopulation of RA in the immediate vicinity of established brain metastases characterized by de novo expression of the transcription factor Stat3. This finding applies to multiple brain metastasis experimental models as well as the majority of human brain metastasis samples from different cancer types. Based on these findings, we have generated a genetic engineered mouse model to specifically eliminate Stat3 from RA. The use of established and novel brain tropic syngeneic cell lines from lung adenocarcinoma and melanoma in this model shows that this subpopulation of astrocytes is required for the viability of established brain metastasis. Isolated Stat3+ and Stat3- RA behave differently and we have identified downstream Stat3 genes that are enriched in Stat3+ RA *in vitro* and in situ surrounding metastasis. Interestingly several of these pro-metastatic components are established modulators of the immune system, suggesting that targeting this subpopulation of reactive astrocytes in the context of brain metastasis could be a viable strategy to increase response rates to immunotherapies.

The role of Spt6 in glioblastoma maintenance and therapeutic resistance

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Glioblastoma (GBM) is among the most lethal of solid cancers in adults. It is associated with a median survival of only approximately 15 months despite aggressive radio- and chemotherapies and recurrence is inevitable. Despite recent advances in our understanding of this deadly disease, the molecular mechanism and/or genes that cause high recurrence rates and treatment resistance in GBM are poorly understood. Histone chaperones affect the structure of the chromatin and expression of genes through interaction with histones and RNA polymerase II (PolII). Spt6 is a highly conserved transcription elongation factor and histone chaperone that has been found to counteract tri- methylation of the histone variant H3, at various lysines, an otherwise epigenetic mark which is associated with repression of transcription. We have identified a novel role of Spt6 in glioblastoma cells. Our data show that siRNA-mediated knockdown of Spt6 in GBM cells results in decreased proliferation rates and progression through the cell cycle, increased levels of DNA damage (measured by γH2AX) as well as delayed capacity for DNA repair after exposure to ionizing radiation. Altogether, our data imply an important role of Spt6 in glioblastoma maintenance and therapeutic resistance.

Kish/TMEM167A, a molecule that regulates vesicular machinery, controls EGFR-dependent glioma growth

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Glioblastoma (GBM) is a rare but very aggressive tumor of the brain. It has a median overall survival of 15 months, regardless of an extensive surgery followed by combinatorial treatment with radiotherapy and oral doses of Temozolomide. Since nearly 50% of primary GBMs show overexpression of EGFR, its inhibition is one of the most attractive therapeutic strategies. However, tyrosine-kinase inhibitors have produced poor results in clinical trials with GBM patients, with no clear explanation for the lack of response. Animal models of brain tumors have served to study the molecular mechanisms that contribute to oncogenesis, and to evaluate potential therapies. Although the most commonly used are mouse xenografts (PDX) and genetically engineered models, gliomas have also been recently obtained in Drosophila brains by coactivation of EGFR and PI3K. They provide and excellent framework to analyze the interaction of the different signaling pathways participating in tumor growth, and to search for new targets. In fact, a genetic screen using this model have identified Kish as a key modulator of GBM growth after EGFR activation, with no effect in tumors generated by an oncogenic Ras isoform. Kish is a small transmembrane protein, associated with vesicular transport and secretion. We have observed that its human orthologue, TMEM167A, is overexpressed in gliomas. Interestingly, downregulation of this molecule in GBM cell lines has a mild proliferative effect *in vitro*, but severely hampers tumor growth in PDX models. Regarding mechanism, we have observed that levels of membrane EGFR are diminished, *in vitro* and *in vivo*, although we have also measured a strong decrease in tumor vessel density, which suggest autocrine and paracrine functions for this molecule in GBM. Therefore, our results indicate that targeting TMEM167A could be a good alternative strategy to downregulate EGFR in gliomas, but also to affect the dialogue between tumor cells and their surrounding stroma.

Kish/TMEM167A, a molecule that regulates vesicular machinery, controls EGFR-dependent glioma growth

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Glioblastoma (GBM) is the most common and most aggressive intrinsic human brain tumor. GBMs are also highly resistant to chemotherapy and radiotherapy, and they show a very poor prognosis with median survival of 12 to 18 months. New therapies, oriented to counteract the principal molecular alterations of GBMs, are also under study. One of the most promising ones, given the prevalence of Epidermal Growth Factor Receptor (EGFR) overexpression in GBMs, is the use of tyrosine kinase inhibitors against this receptor. However, the results have been disappointing because only a small percentage of patients benefit from these strategies. Therefore, there is an urge need to find new venues to treat GBM and to circumvent the resistance of tumor cells to cytotoxic or molecularly directed therapies.

A novel Drosophila GBM model has been developed by activation of a combinatorial genetic network composed, in part, of the pathways commonly mutated in human GBMs. In this fly model, neoplastic cells originate from committed glial and show signs of malignancy like proliferation, invasion and neurotoxicity. Using this model, we have performed a genetic screen and we have identified Kish as key modulator of GBM growth, but only after EGFR activation. Kish is a small transmembrane protein, associated with vesicular transport and secretion. Here we show that its human orthologue, TMEM167A, is overexpressed in gliomas. Moreover, its downregulation affects the stability of EGFR and reduces tumor cell proliferation *in vivo*, both in orthotropic and heterotropic xenografts. Interestingly, tumors with less TMEM167A show a clear phenotype associated with defects in angiogenesis, which suggest autocrine and paracrine functions for this molecule in GBM. Therefore, our results indicate that targeting TMEM167A could be a good alternative strategy for EGFR-dependent gliomas and that it could affect as well the dialogue between tumor cells and their protective niche.

Radioresistance in mesenchymal glioblastoma initiating cells

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BACKGROUND: Glioblastoma (GBM) still remains an incurable disease being radiotherapy (RT) the common mainstay treatment. GBM intra-tumoral heterogeneity and Glioblastoma-Initiating Cells (GICs) challenge the design of effective therapies. Given the controversy on GICs role in GBM radioresistance and the importance to uncover mechanisms underlying radioresistance, we investigated GICs and non-GICs response to RT and addressed molecular programs activated in GICs after RT.

METHODS: We performed a paired *in-vitro* comparison at both functional and molecular level of primary GICs and non-GICs response to clinically relevant fractionated RT, by means of clonogenic assay and microarray high-throughput evaluation.

RESULTS: Established GICs heterogeneously expressed the cancer stem-cell markers and displayed a mesenchymal signature. Upon fractionated RT, GICs reported higher surviving fraction at 2 and 8 Gy when compared to non-GICs. According to the Linear Quadratic Model interpretation of the survival curves, GICs showed lower α- and β-values than paired non-GICs. More importantly, a significant correlation was observed between GICs radiosensitivity and patient disease-free survival. Lastly, transcriptome analysis of GICs after acquisition of a radioresistant phenotype showed upregulation of genes involved in Proneuralto-Mesenchymal transition and pro-inflammatory pathways being STAT3 and IL6 the mayor players.

CONCLUSIONS: GICs are heterogeneous in nature and display higher intrinsic radioresistance compared to matching non-GICs cultures. Moreover, GICs radiosensitivity strikingly correlates to patient response to treatment, thus supporting the leading role of GICs in defining patient response to RT and clinical outcome. Finally, mesenchymal GICs preferentially upregulate pro-inflammatory programs and enhance mesenchymal traits upon fractionated RT.

Expression of Prostate Specific Membrane Antigen (PSMA) in gliomas and brain metastasis

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Prostate-specific membrane antigen (PSMA) is a type II transmembrane glycoprotein which is over expressed in the prostate cancer and neovasculature of various solid tumors. PSMA expression has been reported in benign glioma (grade I), malignant glioblastoma multiforme (GBM, grade IV) and breast cancer brain metastases (BM) (Nomura et al., 2014) with a limited patient cohort. Since PSMA expression in GBM is limited to new blood vessels, it represents a potential molecular target for therapy and/or brain tumor detection. In collaboration with Finnish and Hungarian hospitals, we are currently evaluating the expression of PSMA via immunohistochemistry in tissue microarray samples. Till date, our study contains more than 370+ glioma samples ranging from grade I to IV gliomas and 90+ primary lung and associated BM. Analysis consists of PSMA expression scoring and histological distribution associated with correlations with other major mutation signatures of glioma such as Isocitrate Dehydrogenase 1 and alpha-thalassemia/ mental retardation syndrome X-linked mutations, epidermal growth factor receptor amplification, p53 status and Heat Shock Proteins. In addition, other clinical data such as tumor relapse, sex/age ratio and survival of the patients are assessed and correlated to PSMA status. Initial data suggests, that glioblastoma (grade IV) neovasculature expresses high levels of PSMA compared to the low-grade gliomas. Eventually we, for the first time, report the expression of PSMA in Non-Small Cell Lung Carcinoma and Small Cell Lung Carcinoma metastasis to brain. Overall, despite a well-known association of PSMA with prostate cancer, this new molecular target of gliomas and BM could lead to the development of novel therapeutic/diagnostic (theranostic) agents in the clinics.

A locally translated Lck pathway regulates human glioma stem cell migration, tumor volume and cancer stemness gene expression

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Migration of tumor propagating glioma stem cells (GSCs) within the brain parenchyma makes glioblastoma one of the most aggressive and lethal human tumors. Studies of the cellular and molecular mechanisms underlying human GSC migration are hindered by the lack of efficient migration models. Here we developed a DRG axon-oligodendrocyte co-culture method to study in real time the migration and interaction of GSCs with axons, which occurs through the extensive formation of pseudopodia. Isolation of pseudopodialocalized polysome-bound RNA reveals local transcripts of Lck, Paxillin, Crk-II and Rac1 that undergo eIF4-dependent translation. Inhibition of Lck blocks the activation of this pathway, the formation of pseudopodia and the migration of GSCs. In vivo administration of a highly specific Lck inhibitor using an orthotopic xenograft mouse model results in significant inhibition of tumor formation, GSC migration and significant down-regulation of cancer stemness-specific gene expression. Targeting this locally translated Lck-dependent pathway constitutes a novel treatment paradigm for human glioblastomas.

Nuclear Receptor Binding Protein 2 is down-regulated in Medulloblastoma, and reduces tumor cell survival when overexpressed in Medulloblastoma cells *in vitro*

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Pseudokinases, comprising 10% of the human kinome, are emerging as new regulators of canonical kinases and their functions are starting to be defined. We previously identified the pseudokinase Nuclear Receptor Binding Protein 2 (NRBP2) in a screen for genes regulated during neural differentiation. During mouse brain development NRBP2 is expressed in the cerebellum. In the adult brain it is mainly confined to specific neuronal populations, such as cerebellar Purkinje cells and olfactory bulb mitral cells. In histological sections of medulloblastoma (MB), NRBP2 expression is found in a subset of tumor cells, co-staining with neuronal markers, but not with astrocyte lineage markers. To study the role of NRBP2 in brain tumors, we stained a brain tumor tissue array for NRPB2, and found its expression to be low or absent in a majority of the tumors. Using database mining of publically available expression data we found that NRBP2 was expressed at a lower level in MB than in the normal cerebellum. Furthermore, the most malignant forms of MB (SHH group, Group 3 and Group 4) expressed lower levels than the WNT group which has a better prognosis. Recent studies indicate that MB exhibits frequent epigenetic alternations and we therefore treated MB cell lines with drugs inhibiting DNA methylation or histone methylation. In all cell lines, these treatments led to an up-regulation of NRBP2 mRNA expression, showing that it is under epigenetic regulation in MB cells. Forced overexpression of NRBP2 in MB cell lines led to a dramatic decrease in cell proliferation, increased cell death, impaired cell migration and inhibited cell invasion *in vitro*. Taken together we propose an anti-tumor function for the pseudokinase NRBP2 in MB.

Brain microenvironment-secreted cytokines facilitate glioblastoma proliferation and invasion

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Glioblastoma is the most common and lethal type of brain cancer with a median survival of under fifteen months. It is a highly angiogenic tumor exhibiting an extremely invasive nature. It is well-known that the brain microenvironment plays a crucial role in glioblastoma progression although the large multitude of interactions between the cancer cells and their microenvironment are yet to be fully unraveled. Astrocytes are the most abundant glial cells in the brain and have been shown to be involved in many types of brain pathologies as well as metastatic colonization in the brain. Hence, we investigated the influence of astrocytes on the migratory and infiltrative abilities of glioblastoma cells. Using in vitro assays, we found that in the presence of either astrocytes or their conditioned media, the migration rate of glioblastoma cells is significantly increased. Microglia are macrophages-like cells which possess antigen-presenting and phagocytic abilities that serve as the brain immune system. In a co-culture proliferation assay, we observed that microglia increased glioblastoma cells proliferation at a concentration-dependent manner. Furthermore, co-culture of glioblastoma cells with either astrocytes, microglia or brain endothelial cells resulted in elevated levels of several similar cytokines. Moreover, in immunohistochemistry analysis of several brain tumors inoculated orthotopically in mice, activated astrocytes and microglia and high density of blood vessels within the tumor site were found. Our findings indicate that the brain microenvironment facilitate glioblastoma proliferation and invasion by cytokines secretion leading to novel paths for investigation of their use as targets for glioblastoma therapy.

NOTES

Madrid 19th-22nd February 2017

PRIMARY AND SECONDARY BRAIN TUMORS

Previous CNIO Frontiers Meetings and CNIO Cancer Conferences

2016

CANCEROMATICS III – TUMOR HETEROGENEITY

13/11/2016 – 16/11/2016

Organisers: Fátima Al-Shahrour, Núria Malats, Alfonso Valencia, Chris Sander

2015

METASTASIS INITIATION: MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES

28/09/2015 - 30/09/2015

Organisers: David Lyden, Yibin Kang, Gemma Alderton, Victoria Aranda, Li-kuo Su, Héctor Peinado

NEW TRENDS IN ANTICANCER DRUG DEVELOPMENT

22/03/2015 - 25/03/2015

Organisers: Manuel Hidalgo, Alberto Bardelli, Lillian Siu, Josep Tabernero

2013

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

27/05/2013 - 29/05/2013

Organisers: Robert Benezra, Ana Losada, Marcos Malumbres, René Medema

2012

ALLOSTERIC REGULATION OF CELL SIGNALLING

17/09/2012 - 19/09/2012

Organisers: Francesco Gervasio, Ermanno Gherardi, Daniel Lietha, Giulio Superti-Furga

2011

RECAPTURING PLURIPOTENCY: LINKS BETWEEN CELLULAR REPROGRAMMING AND CANCER

07/11/2011 - 09/11/2011

Organisers: Maria A. Blasco, Konrad Hochedlinger, Manuel Serrano, Inder Verma

CANCEROMATICS II : MULTILEVEL INTERPRETATION OF CANCER GENOME

28/03/2011 - 30/03/2011

Organisers: Søren Brunak, Stephen Chanock, Núria Malats, Chris Sander, Alfonso Valencia

BREAST CANCER

07/02/2011 - 09/02/2011

Organisers: Joaquín Arribas, José Baselga, Miguel Ángel Piris, Lajos Pusztai and Jorge Reis-Filho

2010

CANCER PHARMACOGENETICS: PERSONALIZING MEDICINE

22/11/2010 - 24/11/2010

Organisers: Javier Benítez, William E. Evans, Miguel Martín and Magnus Ingelman-Sundberg

MOLECULAR CANCER THERAPEUTICS

08/03/2010 - 10/03/2010

Organisers: Gail Eckhardt, Roy S. Herbst and Manuel Hidalgo

2009

THE ENERGY OF CANCER

02/11/2009 - 04/11/2009

Organisers: Toren Finkel, David M. Sabatini, Manuel Serrano and David A. Sinclair

CANCER-OM-ATICS : MULTILEVEL INTERPRETATION OF CANCER GENOME DATA

06/07/2009 - 08/07/2009

Organisers: Søren Brunak, Núria Malats, Chris Sander and Alfonso Valencia

STEM CELLS AND CANCER

23/02/2009 - 25/02/2009

Organisers: Elaine Fuchs, Maria A. Blasco, Eduard Batlle and Mirna Pérez-Moreno

2008

SIGNALLING UPSTREAM OF mTOR

03/11/2008 - 05/11/2008

Organisers: Dario R. Alessi, Tomi P. Mäkelä and Montserrat Sánchez-Céspedes

STRUCTURE AND MECHANISMS OF ESSENTIAL COMPLEXES FOR CELL SURVIVAL

23/06/2008 - 25/06/2008

Organisers: Niko Grigorieff, Eva Nogales and Jose María Valpuesta

DEVELOPMENT AND CANCER

04/02/2008 - 06/02/2008

Organisers: Konrad Basler, Ginés Morata, Eduardo Moreno and Miguel Torres

2007

LINKS BETWEEN CANCER, REPLICATION STRESS AND GENOMIC INTEGRITY

05/11/2007 - 07/11/2007

Organisers: Oskar Fernández-Capetillo, Jiri Lukas, Juan Méndez and André Nussenzweig

MYC AND THE TRANSCRIPTIONAL CONTROL OF PROLIFERATION AND ONCOGENESIS

11/06/2007 - 13/06/2007

Organisers: Robert N. Eisenman, Martin Eilers and Javier León

MOLECULAR MECHANISMS IN LYMPHOID NEOPLASM

19/02/2007 - 21/02/2007

Organisers: Elias Campo, Riccardo Dalla-Favera, Elaine S. Jaffe and Miguel Angel Piris

2006

TELOMERES AND TELOMERASE-CNIO / JOSEF STEINER CANCER CONFERENCE

13/11/2006 - 15/11/2006

Organisers: Maria A. Blasco and Jerry Shay

MEDICINAL CHEMISTRY IN ONCOLOGY

02/10/2006 - 04/10/2006

Organisers: Fernando Albericio, James R. Bischoff, Carlos García-Echeverria and Andrew Mortlock

INFLAMMATION AND CANCER

22/05/2006 - 24/05/2006

Organisers: Curtis Harris, Raymand Dubois, Jorge Moscat and Manuel Serrano

PTEN AND THE AKT ROUTE

08/05/2006 - 10/05/2006

Organisers: Ana Carrera, Pier Paolo Pandolfi and Peter Vogt

2005

CANCER AND AGING

07/11/2005 - 09/11/2005

Organisers: Maria A. Blasco, Kathy Collins, Jan Hoeijmakers and Manuel Serrano

MAP KINASES AND CANCER

30/05/2005 - 01/06/2005

Organisers: Philip Cohen, Roger Davis, Worcester, Chris Marshall and Ángel Nebreda

ANIMAL TUMOUR MODELS AND FUNCTIONAL GENOMICS

07/03/2005 - 09/03/2005

Organisers: Allan Balmain, Mariano Barbacid, Anton Berns and Tyler Jacks

2004

CADHERINS, CATENINS AND CANCER

29/11/2004 - 01/12/2004

Organisers: Amparo Cano, Hans Clevers, José Palacios and Franz Van Roy

STRUCTURAL BIOLOGY OF CANCER TARGETS

27/09/2004 - 29/09/2004

Organisers: Ernest Laue, Guillermo Montoya and Alfred Wittinghofer

2003

APOPTOSIS AND CANCER

01/12/2003 - 03/12/2003

Organisers: Gabriel Nuñez, Marisol Soengas and Scott Lowe

SMALL GTPases IN HUMAN CARCINOGENESIS

16/06/2003 - 18/06/2003

Organisers: Juan Carlos Lacal, Channing Der and Shuh Narumiya

TARGETED SEARCH FOR ANTICANCER DRUGS

17/03/2003 - 19/03/2003

Organisers: Amancio Carnero and David H. Beach

2002

MECHANISMS OF INVASION AND METASTASIS

18/11/2002 - 20/11/2002

Organisers: Joan Massagué and Richard Hynes

THE CELL CYCLE AND CANCER

30/09/2002 - 02/10/2002

Organisers: Marcos Malumbres, Charles Sherr and Jiri Bartek

CANCER EPIGENETICS : DNA METHYLATION AND CHROMATIN

29/05/2002 - 31/05/2002

Organisers: Manel Esteller and Stephen B. Baylin

SPANISH NATIONAL CANCER RESEARCH CENTRE

CNIO - "LA CAIXA" FOUNDATION FRONTIERS MEETINGS 2017

Madrid 19 — 22 February 2017

Application deadline January 19th

Abstract submission deadline December 19th

PRIMARY AND SECONDARY BRAIN TUMORS

Organisers

Massimo Squatrito
Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Manuel Valiente
Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Richard Gilbertson
CRUK Cambridge Institute, UK

Michael Weller
University Hospital Zurich, Switzerland

Confirmed Speakers

Suzanne Baker
Brain Tumor Research Division, St. Jude Children's Research, Hospital, Memphis, US

Gabriele Bergers
Vesalius Research Center, Leuven, Belgium

Priscilla Brastianos
Massachusetts General Hospital, Harvard Medical School, Boston, US

Amy B. Heimberger
The University of Texas MD Anderson Cancer Center, Houston, US

Eric Holland
Nancy and Buster Alvord Brain Tumor Center, Fred Hutchinson Cancer Research Center, UW Medicine, US

Luis F. Parada
Brain Tumor Center, Albert C. Foster, MSKCC, New York, US

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Joan Seoane
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Riccardo Soffietti
University and City of Health and Science University Hospital of Turin, Italy

Michael Taylor
The Arthur and Sonia Labatt Brain Tumour Research Centre, The Hospital for Sick Children, Ontario, Canada

Roeland Verhaak
The University of Texas MD Anderson Cancer Center, The Jackson Laboratory for Genomic Medicine, US

William A. Weiss
UCSF, Helen Diller Family Comprehensive Cancer Center, San Francisco, US

Wolfgang Wick
DKFZ, Heidelberg, Germany

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For further information and to apply please go to www.cnio.es/events
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Melchor Fernández Almagro 3, 28029 Madrid

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CNIO - "LA CAIXA" FOUNDATION FRONTIERS MEETINGS 2017

Madrid 2—4 May 2017

Abstract Submission deadline 28 February, 2017

Application deadline 3 April, 2017*

MOLECULAR CHAPERONES IN CANCER

IN MEMORY OF SUSAN LINDQUIST

Organisers

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Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Wilhelm Krek
Institute for Molecular Health Sciences, Zurich, Switzerland

Paul Workman
The Institute of Cancer Research, London, UK

Xiaohong Helena Yang
Cancer Cell, Cambridge, US

Confirmed Speakers

Udai Banerji
The Institute of Cancer Research, London, UK

Johannes Buchner
Technical University Munich, Germany

Bernd Bukau
Center for Molecular Biology of Heidelberg University, German Cancer Research Center (DKFZ), Germany

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Memorial Sloan Kettering Cancer Center, New York, USA

Ana Maria Cuervo
Albert Einstein College of Medicine, New York, USA

Erica A. Golemis
Fox Chase Cancer Center, Philadelphia, US

Mathias Heikenwälder
DKFZ - German Cancer Research Center, Heidelberg, Germany

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College of Biological Sciences, University of Minnesota, St. Paul, US

Michael Karin
University of California, San Diego, USA

Randal J. Kaufman
Stanford Burnham Prebys Medical Discovery Institute, San Diego, USA

Oscar Llorca
Centre for Biological Research (CIB-CSIC), Madrid, Spain

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Center for Molecular Biology of Heidelberg University, Heidelberg, Germany

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University of New Southwales, Sydney, Australia

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Stony Brook University, New York, US

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Whitehead Institute for Biomedical Research, Cambridge, US

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Abo Akademi University, Finland

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University of Leeds, UK

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School of Crystallography, Birkbeck College, London, UK

*Limited capacity of 130 people. CNIO reserves the right to close registration and abstract submission when we reach full capacity.

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