

2<sup>ND</sup> ANNUAL SYMPOSIUM  
*of the*  
FINNISH BRAIN TUMOR  
RESEARCH ASSOCIATION

November 1<sup>ST</sup> & 2<sup>ND</sup>, 2016  
*Helsinki, Finland*



*Symposium Booklet*



## Welcome to the second edition of the annual symposium *Finnish Brain Tumor Research Association*

Finnish Brain Tumor Research Association welcomes you to the 2<sup>nd</sup> Annual Finnish Symposium on Brain Tumors, which is held at Uunisaari, Helsinki, in November 1-2, 2016.

The symposium is an interdisciplinary meeting, where different aspects of brain tumor research and management are covered, including e.g. neuro-oncology, neuropathology, and molecular tumor biology.

We want to acknowledge that different disciplines are important for improving our understanding and management of various brain tumors. Hopefully the symposium will increase interaction between different experts working with these diseases, including both clinicians and researchers, and provide bases for future collaborations.

We look forward to have you at the 2<sup>nd</sup> Annual Finnish Symposium on Brain Tumors on Uunisaari, which is an atmospheric island with a fascinating history. May your attendance at the symposium be pleasant, beneficial, and inspiring.

On behalf of the organizing and the scientific committee,

*Kirsi Granberg*

Chairman, Finnish Brain Tumor Research Association

### *Organizing committee*

HELSINKI



Prof. Pirjo Laakkonen, Ph.D.  
Dr. Vadim Le Joncour, Ph.D.



Pauliina Filppu  
Abiodun Ayo  
Jayendrakishore T. Ramanathan



TAMPERE



Dr. Kirsi Granberg, Ph.D.  
Suvi Luoto



Dr. Maria Gardberg, M.D., Ph.D.  
Prof. Jukka Westermarck, M.D., Ph.D.

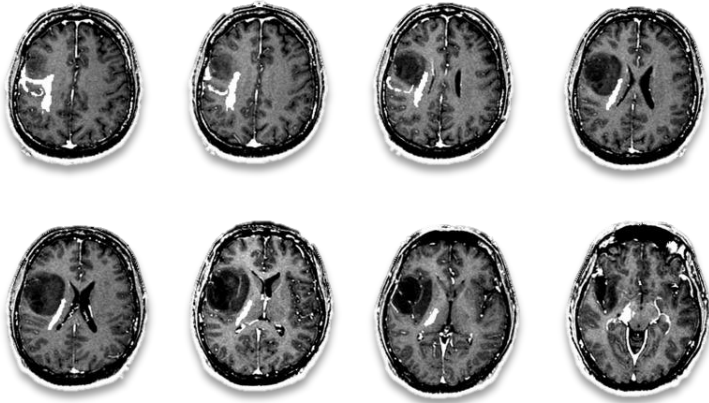


TURKU

**FIBTRA**  
-org-

# FiBTRA 2016 sponsors





*Symposium program*



## Tuesday November 1<sup>st</sup>

### 16:30 Welcome and Registration

- 17:00 – 17:15 **Opening words**  
*Short introduction of FiBTRA by the chair, Dr. Kirsi Granberg, Ph.D.*  

---

*Chair: Dr. Kirsi Granberg, Ph.D.*
- 17:15 – 18:00 **Keynote**  
*Patient-Derived Xenograft Models: Towards Personalized Treatment Strategies for Glioma Patients*  
Prof. Simone Niclou (Ph.D.)  
Luxembourg Institute of Health
- 18:00 – 18:30 **Invited Talk**  
*What's new in the WHO 2016 classification of brain tumors?*  
Docent Olli Tynninen (M.D., Ph.D.)  
Helsinki University Central Hospital; Finland
- 18:30 **Selected abstracts: Virotherapy**
- 18:30 – 18:45 *Continuous Administration of Valganciclovir Improves Lentiviral Vector Mediated Suicide Gene Therapy (p. 8)*  
Jubayer Hossain, University of Bergen; Norway
- 18:45 – 19:00 *Oncolytic features of clinical herpes simplex virus isolates in nervous system-derived cells (p. 9)*  
Jenni Lehtinen, University of Turku; Finland
- 
- 19:00 – 19:30 **Get together**

### 19:30 Gala Dinner at Uunisaaari

## Wednesday November 2<sup>nd</sup>

### 08:30 Registration

- 
- Chair: Prof. Jukka Westermarck, M.D., Ph.D.*
- 09:00 – 09:45 **Keynote**  
*Immunotherapy for glioblastoma*  
Prof. Michael Weller (M.D., Ph.D.)  
University Hospital Zurich; Switzerland

09:45 – 10:30 **Keynote**  
*Understanding and targeting the abnormal DNA damage response in glioblastoma stem-like cells*  
Prof. Anthony Chalmers (Ph.D.)  
University of Glasgow; United Kingdom

10:45 **Selected abstract: Nanotherapies I**

10:45 – 11:00 *The Good Drug, The Bad Barrier and the Ugly Glioma. A story of novel Nanocompounds targeting brain tumors (p. 10)*  
Dr. Vadim Le Joncour (Ph.D.) University of Helsinki; Finland

11:00 – 11:15 **Sponsor talk**

Mikko Ketonen, **medac**; Finland



11:15 **Lunch**

*Chair: Dr. Vadim Le Joncour, Ph.D.*

12:30 – 12:55 **Invited Talk**  
*Peptide-based targeting of glioblastoma multiforme*  
Prof. Pirjo Laakkonen (Ph.D.)  
University of Helsinki; Finland

12:55 – 13:15 **Invited Talk**  
*Isocitrate dehydrogenase 1-mutated gliomas rely on import of the neurotransmitter glutamate as a rescue mechanism for defective isocitrate processing: consequences for studies on IDH function*  
Prof. William P.J. Leenders (Ph.D.)  
Radboud University Medical Center; Netherlands

13:15 **Selected abstract: Nanotherapies II**

13:15 – 13:30 *Immune modulation and drug delivery: The missing link in cancer therapy (p. 11)*  
Dr. Oommen Podiyan Oommen (Ph.D.)  
Tampere University of Technology; Finland

13:30 **Selected abstracts: *Molecular biology of brain tumors I***

13:30 – 13:45 *Synthetic lethality through combination of multikinase inhibitors and PP2A activation in glioblastoma* (p. 12)  
Joni Merisaari, University of Turku, Finland

13:45 – 14:00 *Molecular characterization of choroid plexus and atypical teratoid/rhabdoid tumors* (p. 13)  
Joonas Tuominen, University of Tampere; Finland

14:00 **Coffee break**

Chair: Prof. Pirjo Laakkonen, Ph.D.

14:30 – 15:00 **Invited Talk**

*Herpes Simplex Virus Thymidine Kinase Gene Therapy*  
Prof. Seppo Ylä-Herttua (M.D., Ph.D.)  
University of Eastern Finland

15:00 **Selected abstract: *Molecular biology of brain tumors II***

15:00 - 15:15 *Actin regulating formins INF2 and FHOD1 are upregulated in migrating glioblastoma cells* (p. 14)  
Dr. Maria Gardberg (M.D., Ph.D.)  
Turku University Hospital; Finland

15:15 **Selected abstracts: *Genetics of brain tumors***

15:15 - 15:30 *Identification of Predisposing Germline Variants in Familial Glioma* (p. 15)  
Ebrahim Afyounian, University of Tampere; Finland

15:30 - 15:45 *Gatekeeper inactivation drives glioma progression into secondary glioblastoma* (p. 16)  
Matti Annala, University of Tampere, Finland

15:45 - 16:00 *Different immune cell responses are associated with typical genetic alterations in glioblastoma* (p. 17)  
Suvi Luoto, University of Tampere; Finland

16:00 **Closing words**



## *Selected Abstracts*





## Continuous Administration of Valganciclovir Improves Lentiviral Vector Mediated Suicide Gene Therapy

J.A. Hossain<sup>1,2</sup>, L. Rømo Ystaas<sup>1</sup>, K.M. Talasila<sup>1</sup>, K. Riecken<sup>3</sup>, Boris Fehse<sup>3</sup>, R. Bjerkvig<sup>1,4</sup> and H. Miletic<sup>1,2</sup>

### Author affiliations:

1 K. G. Jebsen Brain Tumour Research Centre, Department of Biomedicine, University of Bergen, Norway;

2 Department of Pathology, Haukeland University Hospital, Norway;

3 Research Department Cell and Gene Therapy, Department of Stem Cell

Transplantation, University Medical Centre Hamburg-Eppendorf, 20246 Hamburg, Germany;

4 Norlux Neuro-Oncology Laboratory, Department of Oncology, Luxembourg Institute of Health, Luxembourg

Lentiviral vector mediated herpes simplex virus thymidine kinase (HSV-Tk) / ganciclovir (GCV) therapy is a very promising therapeutic option for GBM treatment. By using a patient derived xenograft model we have previously reported complete remission on MRI after lentiviral HSV-Tk/GCV therapy. However, remission was followed by recurrence of tumors and interestingly a fraction of recurrent glioma cells were found to be expressing tk-GFP suggesting that some of the transduced cells had survived the 3-week prodrug administration period. We hypothesize that short-term prodrug delivery fails to eliminate a fraction of gliomas cells, which are slow-proliferating; and thus, a longer period of prodrug administration would provide better survival benefit. As long-term prodrug we used valganciclovir (valGCV), which is similar to GCV, but tailored for oral administration. After orthotopic implantation of patient derived glioma spheroids and visible growth on MRI, we intratumorally injected lentiviral vectors expressing HSV-Tk.007-GFP, a highly active mutant of HSV-tk. Animals were treated with either short-term GCV (3 weeks) or long-term valGCV (3 months). Although both treatment groups showed remission and subsequent tumor recurrence, animals in the valGCV treatment group survived significantly longer and had a longer recurrence-free time period. Hereby we report that valGCV is a compatible prodrug in the modus operandi of HSV-Tk mediated suicide gene therapy that can effectively cross the blood brain barrier and upon long-term administration results in better therapeutic benefit compared with short-term GCV treatment. We are currently characterizing the recurrent tumors to identify new targets that potentially could lead to further development of the suicide therapy for GBM.

## Oncolytic features of clinical herpes simplex virus isolates in nervous system-derived cells

J. Lehtinen<sup>1,2</sup>, H. Paavilainen<sup>1,2</sup>, V. Hukkanen<sup>1</sup>

Author affiliations:

1 Department of Virology, University of Turku;

2 Drug Research Doctoral Programme, UTUGS

Herpes simplex virus (HSV) is a neurotropic virus and has recently become the pioneering oncolytic virotherapy. The knowledge on the innate immune responses, induced by circulating clinical HSV strains, is vital for understanding the pathogenesis of HSV, as well as for the development of vaccines and HSV-based virotherapies. We have studied the innate immunity induction by low-passage HSV-1 and HSV-2 field isolates, derived from clinical specimens, in cell lines representing the natural host tissues of HSV. We included laboratory wt strains of HSV-1 (17+) and 2 (G) in our studies as references. The growth properties, plaque morphology, and sensitivity for aciclovir were also studied. We used HaCaT epithelial cells, U373MG astrocytoma cells, T98G glioblastoma multiforme cells, and SH-SY5Y neuroblastoma cells for the study. HSV-1 strains showed more prominent replication in these human cell lines compared to HSV-2 strains, except in T98G glioma cells, in which HSV-2 strains replicated stronger. HSV-2 strains also induced stronger interferon alfa, beta, lambda1 (IL-29), PKR and ISG54 responses in T98G than did the HSV-1 strains. Clinical HSV isolates had diverse profiles in viral shedding and varying immunostimulatory properties. Although the wt strain HSV-1 (17+) replicated most efficiently, the innate responses it induced were modest in comparison with those to clinical HSV-1 isolates. One HSV-1 isolate strain induced especially strong type I and type III interferon responses in U373MG cells. Our aim is to develop gene therapy vectors for treatment of cancers of the nervous system, derived from selected clinical HSV isolates. Based on our studies, HSV-2 strains seem to be more immunogenic and suitable for oncolytic immuno-virotherapies of glioma than HSV-1 strains.

## The Good Drug, The Bad Barrier and the Ugly Glioma. A story of novel Nanocompounds targeting brain tumors

V. Le Joncour<sup>1</sup>, M. Hyvönen<sup>1</sup>, E. Casals<sup>2</sup>, J.K. Tanjore Ramanathan<sup>1</sup>, P. Filppu<sup>1</sup>, A. Ayo<sup>1</sup>, J. Rosenholm<sup>2</sup>, J. Westermarck<sup>3</sup> & P. Laakkonen<sup>1</sup>

Author affiliations:

1 Research Programs Unit, Translational Cancer Biology, Biomedicum Helsinki, University of Helsinki;

2 BioNanoMaterials research group, Åbo Academy University;

3 Finnish Cancer Institute, Turku Center for Biotechnology

High grade malignant gliomas (GBM) represent the most common and aggressive brain tumors in adults. They are commonly characterized by a highly invasive tumor cell population and a dense but disorganized vascularization causing hypoxia/necrosis and intracerebral hemorrhages. 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 infiltrative cells and metastatic nodes reduces the efficacy. Moreover, most of the therapeutics are cytotoxic for both tumor and healthy cells, causing numerous and severe adverse effects. Novel Nanotherapeutics (NT) represent promising candidates to treat GBM, by limiting adverse effects of drugs, allowing 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. After this first selection, 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 binding 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 endothelial cell and astrocyte retention and toxicity. By loading the NT with conventional cytotoxic drugs and/or targeted therapies we could thus efficiently target and kill the tumor cells with minimal toxicity for the surrounding brain tissue.

## Immune modulation and drug delivery: The missing link in cancer therapy

Oommen P. Oommen<sup>1,2</sup>

Author affiliations:

1 Biomaterials and Tissue Engineering group, Tampere University of Technology, Finland;

2 Department of Chemistry, Ångström laboratory, Uppsala University, Sweden

Over the past few decades, the major thrust of anticancer research is aimed at developing drug delivery systems that could target the malignant cells. The efforts lead to the discovery of targeted drug delivery systems such as drug-loaded nanoparticles (NPs) and antibody drug conjugates. However, mounting evidence suggests that different nanoformulations adversely activate complement and coagulation cascades (J. Controlled Rel. 2014, 190, 556). Such activation of innate immunity evokes chronic inflammation, which in turn promotes tumor progression, instead of tumor suppression. Several clinically used nanoformulations are liposomal-based and most of them are coated with hydrophilic polymers such as PEG (to prevent opsonization). Recently it has been shown that PEGylated agents elicit antibody formation against PEG (anti-PEG) in animals and humans (Expert Opin. Drug Deliv. 2012, 9, 1319). I aim to design nanocarriers that could address this issue and promote targeted drug delivery. One of the most promising polymers that could be exploited to fulfill the need for biocompatible drug delivery system is glycosaminoglycans derived from our extracellular matrix. We find that chondroitin sulfate (CS) coated gold nanoparticles conjugated to DOX (CS-Au-DOX) offer a safe delivery platform for targeted delivery of the chemotherapeutic agent. DOX was conjugated to the nanoparticle via pH-responsive hydrazone linkages, which yielded sustained drug release profile at acidic pH. Unlike other colloidal gold particles, CS-Au-DOX is stable and can be stored as a lyophilized powder. This nanoparticle exhibits higher toxicity towards CD44 overexpressing human colon cancer cells (HCT116 and GP5D) compared to free DOX. Interestingly, CS-Au-DOX overcomes multidrug resistance in ovarian cancer cell line A2780, which is a result of p-glycoprotein overexpression. This nanoparticle mitigates DOX mediated toxicity to human platelets and suppresses thromboinflammation, demonstrating the significance of nanoparticle design for the delivery of toxic drugs. Currently, I am investigating an RNA interference approach synchronized with targeted drug delivery as a therapeutic modality for glioblastoma multiforme (GBM) an aggressive central nervous system malignancy with a dismal prognosis. At the conference, I will discuss more about my research and ambitions to bridge the gap in nanoparticle design for treating malignant diseases.

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

J. Merisaari<sup>1,2,3</sup>, A. Kaur<sup>1,2</sup>, O. Denisova<sup>1</sup>, D. Desai<sup>4</sup>, E. Casals<sup>4</sup>, J. Rosenholm<sup>4</sup>, J. Westermark<sup>1,2</sup>

### Author affiliations:

1 Turku Centre for Biotechnology, University of Turku and Åbo Akademi University;

2 Department of Pathology, University of Turku;

3 TuDMM Doctoral Programmes;

4 Pharmaceutical Sciences Laboratory, Faculty of Science and Engineering, Åbo Akademi University

Prognosis of the most common and devastating brain cancer in adults, glioblastoma multiforme (GBM), is very poor. With current therapy survival after the diagnosis is only up to few years and 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. Therefore, it is reasonable to believe that re-activation of PP2A complexes inhibited in cancer would result as a decrease in activity of oncogenic pathways. We recently demonstrated that overexpression of the PP2A inhibitor protein PME-1 drives resistance of glioma cells to various multikinase inhibitors (MKIs) (Kaur et al., *Cancer Res.*, in press, 2016). The PME-1-elicited resistance was dependent on specific PP2A complexes and was mediated by a decrease in cytoplasmic HDAC4 activity. Importantly, both PME-1 and HDAC4 associated with human glioma progression, supporting clinical relevance of the identified mechanism. Additionally, synthetic lethality induced by both PME-1 and HDAC4 inhibition was dependent on the co-expression of pro-apoptotic protein BAD. In *in vivo* studies we showed that significant tumor growth inhibition in PME-silenced GBM xenografts treated with MKIs. Thus, PME-1-mediated PP2A inhibition is a novel mechanistic explanation for multikinase inhibitor resistance in glioma cells. 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 results in very potent cell killing effect in various GBM cell lines with different genetic backgrounds *in vitro*. Additional challenge in the treatment of GBM is blood-brain barrier (BBB) 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 therapeutic efficacy of the strategy in an intracranial model of GBM. In summary, these results introduce a novel combination therapy approach for kinase inhibitor resistant glioma cells. In addition to the potential for new glioma therapy modality to be used as a secondary treatment after surgery, the superior sensitivity of PME-1 and HDAC4 negative glioma cells to MKIs may help to develop patient stratification strategies for future clinical trials with selected kinase inhibitors in GBM.

### Molecular characterization of choroid plexus and AT/RT tumors

J. Tuominen<sup>1</sup>, V. Kytölä<sup>2</sup>, K. Nordfors<sup>3</sup>, J. Haapasalo<sup>4</sup>, S. Häyrynen<sup>5</sup>, E. Afyounian<sup>6</sup>, H. Haapasalo<sup>7</sup>, O. Lohi<sup>8</sup>, M. Nykter<sup>9</sup>, K. Granberg<sup>10</sup>

Author affiliations:

1 Institute of Biosciences and Medical Technology (BioMediTech), University of Tampere, Tampere, Finland;

2 Department of Signal Processing, Tampere University of Technology, Tampere, Finland;

3 Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA;

4 Science Center, Tampere University Hospital, Tampere, Finland;

5 Fimlab Laboratories Ltd., Tampere University Hospital, Tampere, Finland;

6 Unit of Neurosurgery, Tampere University Hospital, Tampere, Finland;

7 Department of Pediatrics, Tampere University Hospital; Tampere Center for Child Health Research, 33014 University of Tampere, Tampere, Finland;

8 Department of Pathology, University of Tampere, Tampere, Finland

It is principally thought that cancer is driven by numerous mutations which are essential for the tumor growth. To obtain information about oncogenic drivers, we analyzed five choroid plexus tumors and three atypical teratoid/rhabdoid tumors (AT/RT) by using exome sequencing and reduced representation bisulfite sequencing. It is known that mutation rates in both tumor types are very low compared to other tumors. Indeed, we determined only few somatic mutations in both sample sets. In choroid plexus tumors, we had 2 cases carrying TP53 mutations and altogether 8 genes were mutated (ZNF283 in two samples) in tumor samples with matched blood DNA control. In rhabdoid tumors, we found altogether 28 genes to be mutated. Copy number analysis revealed several known copy number gains such as +5q, +7q, +9q, 15q and +18q in choroid plexus tumors. We also detected novel gains of chromosomes 12 and 17. All the rhabdoid tumor samples carry homozygous deletion of SMARCB1 which is characteristic for these tumors. Furthermore, we were able to detect some chromosomal instability in AT/RT samples mostly in form of small gains. Analysis of differentially methylated regions between AT/RTs, choroid plexus tumors, and 3 control medulloblastoma samples showed clear methylation differences between disease groups. Principal component analysis was able to separate different tumor types from

each other. A trend of increasing promoter and CpG island methylation was observed from medulloblastomas to choroid plexus tumors and further on to AT/RT samples. Our results support the notion that these tumors are driven rather by copy number alterations and epigenetic events, such as methylation, than mutations. More research is still needed to find the driver alterations among the various alterations and to understand why they drive the development of these tumor types.

### Actin regulating formins INF2 and FHOD1 are upregulated in migrating glioblastoma cells

M. Gardberg<sup>1</sup>, V.D. Heuser<sup>1</sup>, S. Munthe<sup>2,3</sup>, B. Winther Christensen<sup>2</sup>, O. Carpén<sup>1,4</sup>

Author affiliations:

1 Department of Pathology, Turku University Hospital and University of Turku, Turku, Finland;

2 Department of Pathology, Odense University Hospital, Odense, Denmark;

3 Department of Neurosurgery, Odense University Hospital, Odense, Denmark;

4 Department of Pathology, Helsinki University Hospital and University of Helsinki, Helsinki, Finland

The prognosis of glioblastoma remains poor due to the wide infiltration of tumour cells at diagnosis. Infiltration requires substantial remodelling of cellular actin filaments. The formins are a protein family that orchestrates actin cytoskeletal rearrangements in many dynamic cellular processes. Formin activity is controlled by Rho GTPases. Several formins are associated with cancer-related functions in cancer cell lines, such as invasion, proliferation and altering transcription. The formin family of proteins has fifteen members that are generally expressed in a tissue-or cell-type specific manner. The aim of this study is to elucidate whether formins contribute to the invasion of glioblastoma cells, and to test formins as treatment targets.

To test whether formins are expressed differentially in immotile vs. migrating primary glioblastoma cells, a newly described migration model was used. The method utilizes patient derived glioblastoma spheroids to model the switch from non-migrating to invasive cells. When a subpopulation of the tumour cells differentiated to an elongated morphology and migrated out of the spheroids, they were manually separated from the remaining spheroid cells. Transcriptomic analysis of the two populations revealed that the expression of several formins was increased in migrating vs. spheroid cells. Notably, inverted formin 2 (INF2) and formin homology domain 2 containing 1 (FHOD1) were widely upregulated 8-120-fold, in three out of five primary glioblastoma cell lines. All five cell lines upregulated either INF2 or FHOD1, or both.

In normal glial cells, INF2 and FHOD1 expression is low or absent. To characterize their expression in human glioma, a tissue microarray with 200

gliomas of grades II-IV was immunohistochemically stained. Preliminary analysis shows that FHOD1 expression in glioma is generally low. In contrast, INF2 is expressed heterogeneously within samples. A minority of cells express moderate to high levels of INF2, while the majority of tumour cells express little INF2. These results support the idea that FHOD1 and INF2 expression may be highest / restricted to motile cells, while the expression in the tumour bulk is relatively low.

We conclude that expression of INF2 or FHOD1 is markedly increased in migrating primary glioblastoma cells. INF2 and FHOD1 expression can be detected in a small cell population within human glioma samples. Future studies will show whether inhibition of formin activity can reduce glioma cell infiltration. This will be tested in patient-derived, low passage primary glioblastoma cells.

### Identification of Predisposing Germline Variants in Familial Glioma

E. Afyounian<sup>1</sup>, K. Granberg<sup>1</sup>, J. Tuominen<sup>1</sup>, M. Saarinen<sup>1</sup>, M. Annala<sup>1</sup>, N. Paunu<sup>2</sup>, H. Haapasalo<sup>3,4,5</sup>, M. Nykter<sup>1</sup>

Author affiliations:

1 University of Tampere; Institute of Biosciences and Medical Technology (BioMediTech);

2 Department of oncology, Tampere University Hospital, Tampere, Finland;

3 Fimlab Laboratories Ltd.;

4 Tampere University Hospital, Tampere, Finland;

5 Department of Pathology, University of Tampere, Tampere, Finland

Gliomas are generally sporadic but occasionally several family members are affected by these tumor types. In addition to Li-Fraumeni syndrome, little is known about the predisposing factors for familial forms of the disease. In this study, we aimed at identifying glioma susceptibility variants. We thus performed exome sequencing of 14 persons within four families with 50X coverage using blood-derived DNA. Eight of the samples were from the affected persons and the remaining six samples were the other family members. The raw sequencing data was mapped to human reference genome (hg19) using Bowtie2. Resulting BAM file was sorted, indexed, and duplicates were removed. An in-house pipeline Pypette was used to call the germline variants. Next, common variants in the Finnish population of the 1000 genomes project (n=100) and the SISU (sequencing Initiative Suomi) project were discarded while retaining the rare variants (<1%) in each of the databases. Then, the identified variants were annotated and the prospective variants based on their annotation (e.g. nonsynonymous, frameshift, stop-gain, and stop-loss) were retained. This resulted in the identification of 1160 prospective germline variants. The results were further filtered by applying a bimodality test for each variant and filtering the low scoring variants (<1) resulting in 1081 variants. Furthermore, variants were annotated using



Annovar. Using a majority test based on the result of Annovar analysis, each variant was assigned a label (pathogenic, non-pathogenic) and a score. Furthermore, gene expression profiles in glioblastoma (GBM) and low grade glioma (LGG) were extracted, normalized, and assigned to each of the variants. The results were then manually inspected and filtered by removing sequencing artefacts as well as variants which are not shared by the affected family members. This resulted into a list of 98 interesting germline variants. Finally, the identified variants were validated in the lab by Sanger sequencing. No significant familial glioma associated DNA copy number variants were detected. This study will be furthered by targeted sequencing of a panel of 95 patients probing for identified variants.

### Gatekeeper inactivation drives glioma progression into secondary glioblastoma

M. Annala\*<sup>1</sup>, K.J. Granberg\*<sup>1,2</sup>, J. Haapasalo<sup>3,4</sup>, O. Yli-Harja<sup>2</sup>, H. Haapasalo<sup>4,5</sup>, W. Zhang<sup>6</sup>, M. Nykter<sup>1</sup>

Author affiliations:

1 Department of signal processing, Tampere University of Technology, Tampere, Finland;

2 Institute of Biosciences and Medical Technology, University of Tampere, Tampere, Finland;

3 Unit of Neurosurgery, Tampere University Hospital, Tampere, Finland;

4 Fimlab Laboratories, Tampere University Hospital, Tampere, Finland;

5 Department of Pathology, University of Tampere, Tampere, Finland;

6 Department of Cancer Biology, Comprehensive Cancer Center of Wake Forest Baptist Medical Center, Winston-Salem, NC, USA;

\* Equal contribution

Glioblastoma (GBM) is the most common and lethal form of brain cancer in humans. Median survival is 15 months with best available treatment. Most GBMs arise de novo (primary GBM), but 5-10% progress from lower grade gliomas (secondary GBM). As progression of low grade glioma into secondary GBM significantly impacts prognosis, a better understanding of this process is paramount for treatment and monitoring of affected patients. In this study, we applied whole genome and transcriptome sequencing to primary glioma and relapsed secondary GBM tissue from seven patients with progression. All primary gliomas carried IDH1 mutations, and in all cases the mutation was inherited by the secondary GBM. ATRX alterations in all five astrocytomas and TERT promoter mutations in both 1p19q-codeleted oligoastrocytomas were also inherited in progressed tumors. In five patients, progression was associated with increased genomic instability, whereas mutation load was significantly increased in two other patients. One of them exhibited a hypermutation signature caused by a mutation in the proofreading domain of DNA polymerase epsilon, while the second had lost both copies of the DNA

mismatch protein MSH2. In addition, both oligoastrocytomas had acquired focal inactivating deletions of the protein tyrosine phosphatase PTPRD at progression, suggesting a novel driver mechanism for GBM progression. The most common progression-related genomic alterations were CDKN2A deletions, TP53 mutations, RB1 deletions, PTEN deletions, and deletions of genes crucial to the double strand break repair pathway. Taken together, progression into secondary GBM was significantly related to deletions in tumor suppressor genes as well as TP53 mutations. Disruption of these gatekeepers appears to be a significant mechanism for glioma progression.

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

S. Luoto<sup>1</sup>, J. Kesseli<sup>1</sup>, M. Nykter<sup>1</sup>, K.J. Granberg<sup>1,2</sup>

Author affiliations:

1 Institute of Biosciences and Medical Technology, University of Tampere, Tampere, Finland;

2 Department of signal processing, Tampere University of Technology, Tampere, Finland

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.

# Notes

---

# Notes

---

# FiBTRA info and contact

Details about the association and the members can be found on



Or by simply scanning the following Qr code



Join us on Facebook!



Meet us on LinkedIn!



An axial MRI scan of a human brain, showing the cerebral cortex, white matter, and ventricles. The image is in grayscale. Overlaid on the center of the brain is the text "LET'S MAKE THIS BETTER TOGETHER". The words "LET'S MAKE THIS" are in white, and "BETTER TOGETHER" is in a light red color.

LET'S MAKE THIS  
BETTER TOGETHER