DIPG: What to do with the tissue?

Chris Jones PhD FRCPPath
The Institute of Cancer Research / Royal Marsden Hospital
London (Sutton), UK
What do we do with the tissue now?

- “Inventory of European DIPG tissue”
  - Liaise with National Coordinators

- International repository of DIPG genomic data
  - Funded transatlantic collaboration
  - Retrospective (systematic literature review)
    - Eventual link to DIPG Registry
  - Prospective, pre-publication data
What to do with the tissue?

- Providing evidence for therapeutic efficacy
  - Retrospective, correlative
  - Treatment stratification

- Understanding the biology of the disease
  - Genomics, expression profiling, methylation, proteomics
  - Deep sequencing

- Developing models for preclinical screening
  - Primary / neurosphere / 3D / stem cell cultures *in vitro*
  - Orthotopic xenograft *in vivo*

Integrating biological knowledge with pharmacological response in order to drive novel therapies in DIPG
What to do with the tissue?

• **Source**
  – Biopsy
  – Autopsy

• **Destination**
  – Formalin-fixed paraffin-embedded
  – Flash frozen in liquid nitrogen
  – Collection in tissue culture media
  – Immediate implantation in pons of mice
What to do with the tissue?

• **Source**
  - Biopsy
  - Autopsy

• **Destination**
  - Formalin-fixed paraffin-embedded
  - Flash frozen in liquid nitrogen
  - Collection in tissue culture media
  - Immediate implantation in pons of mice
# Biopsy vs Autopsy

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<th><strong>Autopsy</strong></th>
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<tr>
<td>Pro</td>
<td>Con</td>
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<tr>
<td>No treatment-induced changes</td>
<td>End-stage, resistant disease</td>
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<td>Amenable to cell culture</td>
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<td>Dilution of resistant subclones</td>
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<tr>
<td>Intratumoral heterogeneity</td>
<td>Low cell culture success rate?</td>
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</table>
Disease biology

• Targeted analyses
  – Candidate mutations
  – Gene amplifications/deletions
  – Protein expression

• Genome-wide assays
  – DNA copy number
  – mRNA expression
  – (miRNA profiling)
  – (Global methylation patterns)
  – (Proteomics)
  – Deep sequencing
Biopsies can give sufficient quantity macromolecules for profiling
Title
Mesenchymal transition and PDGFRA amplification/mutation are key distinct oncogenic events in pediatric diffuse intrinsic pontine gliomas

Authors
Puget S1,2#, Philippe C2#, Bax DA3, Job B4, Varlet P5, Junier MP5, Andreiuolo F5, Jubert C2, Opolon P2, Carvalho D3,6,7, Reis R6, Guerrini-Rousseau L7, Roujeau T7, Dessen P4, Richon C5, Lazar V5, Le Teuff G9, Sainte-Rose C1, Vassal G2, Jones C3, Geoerger B2,10, Grill J2,10.

n=32
Clinical Relevance of Tumor Cells with Stem-Like Properties in Pediatric Brain Tumors


**HIGH-GRADE GLIAL TUMORS**

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<th>Patient</th>
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![Graph A](image1.png)  ![Graph B](image2.png)
Autopsies can give sufficient quality macromolecules for profiling.
Whole-Genome Profiling of Pediatric Diffuse Intrinsic Pontine Gliomas Highlights Platelet-Derived Growth Factor Receptor α and Poly (ADP-ribose) Polymerase As Potential Therapeutic Targets

Maryam Zarghooni, Ute Bartels, Eric Lee, Pawel Buczkowicz, Andrew Morrison, Annie Huang, Eric Bouffet, and Cynthia Hawkins

n=11

PDGFRA
Genome-Wide Analyses Identify Recurrent Amplifications of Receptor Tyrosine Kinases and Cell-Cycle Regulatory Genes in Diffuse Intrinsic Pontine Glioma


n=43
Genome-Wide Analyses Identify Recurrent Amplifications of Receptor Tyrosine Kinases and Cell-Cycle Regulatory Genes in Diffuse Intrinsic Pontine Glioma


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Abbreviations: FISH, fluorescent in situ hybridization; PI3K, phosphoinositide 3-kinase; RB, retinoblastoma protein; RTK, receptor tyrosine kinase.

*Gain identified by FISH.
Mosaic Amplification of Multiple Receptor Tyrosine Kinase Genes in Glioblastoma

Matija Snuderl,1,8 Ladan Fazlollahi,1,8 Long P. Le,1 Mai Nitta,1 Boryana H. Zhelyazkova,1 Christian J. Davidson,2 Sara A. Fazlollahi,1 Daniel P. Cahill,1,3,4 Kenneth D. Aldape,1,3,4 Rebecca A. Betensky,2 David N. Louis,1 and A. John Iafate1,*
1Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA
2Department of Neurosurgery
3Department of Pathology
4MD Anderson Cancer Center, Houston, TX 77030, USA
5Department of Biostatistics, Harvard School of Public Health, Boston, MA 02115, USA

Intratumoral heterogeneity of receptor tyrosine kinases EGFR and PDGFRA amplification in glioblastoma defines subpopulations with distinct growth factor response

Nicholas J. Szerlip,6,7 Alicia Pedraza,6 Debyani Chakravarty,6 Mohammad Azim,4 Jeremy McGuire,1 Yuqiang Fang,5 Tatsuya Ozawa,6 Eric C. Holland1,4,5, Jason T. Huse,4,5 Suresh Jhanwar,6 Margaret A. Leversha,6 Tom Mikkelsen,6 and Cameron W. Brennan1,4,5,7,8

Molecular and Cellular Pathobiology

Receptor Tyrosine Kinase Genes Amplified in Glioblastoma Exhibit a Mutual Exclusivity in Variable Proportions Reflective of Individual Tumor Heterogeneity

Suzanne E. Little1,2, Sergey Pospov1,2, Alexa Jung,1,2 Dorine A. Bax1,2, Lawrence Doey3, Safa Al-Sarraj3, Juliane M. Jurgensmeier4, and Chris Jones1,2
Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas

Gang Wu1,8, Alberto Broniscer2,8, Troy A McEachron3,8, Charles Lu4, Barbara S Paugh5, Jared Becksfort5, Chunxu Qu5, Li Ding5, Robert Huether5, Matthew Parker5, Junyuan Zhang5, Amar Gajjar6, Michael A Dyer7, Charles G Mullighan6, Richard J Gilbertson3, Elaine R Mardis8, Richard K Wilson4, James R Downing6, David W Ellison6, Jinhui Zhang6 & Suzanne J Baker1 for the St. Jude Children’s Research Hospital–Washington University Pediatric Cancer Genome Project2

n=7

![Image of DNA sequencing results showing differences between normal and tumor samples for H3F3A and HIST1H3B genes.]

**Table 1** Frequency of recurrent somatic mutations in DIPG and GBM

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<tr>
<th>Gene</th>
<th>Amino acid change</th>
<th>DIPG (%)</th>
<th>non-BS-PG (%)</th>
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<td>All H3</td>
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<td>39 (78)</td>
<td>13 (36)</td>
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aFor DIPGs, total n = 50. bFor non-BS-PGs, total n = 36.
Deep sequencing strategies

• Thresholds for quantity and quality are falling rapidly
  – Already <1μg for whole genome, <500ng for exome
  – Soon to include FFPE

• Availability of matched normal DNA
  – Somatic vs germline variants

• Whole genome vs targeted exome vs transcriptome
  – Balance of cost, time, analysis
  – Single nucleotide vs structural variants vs splice variants

• Read depth critical to identify low abundance variants
  – Longitudinal studies of matched biopsy and autopsy to track tumour evolution and the selection of resistant subclones
Model development

• **Use of viable tumour cells**
  – Recapitulate disease biology
  – Reflect intertumoral heterogeneity of disease

• *In vitro* neurosphere cultures
  – Allow for serial passaging
  – Amenable to high-throughput screens

• *In vivo* orthotopic xenografts
  – Diffusely infiltrative growth
  – Mimic drug delivery?
  – Intratumoral heterogeneity?
Hedgehog-responsive candidate cell of origin for diffuse intrinsic pontine glioma

Michelle Monje\textsuperscript{a,b,c,1,2}, Siddhartha S. Mitra\textsuperscript{c,d,1}, Morgan E. Freret\textsuperscript{a,b,c}, Tal B. Raveh\textsuperscript{5}, James Kim\textsuperscript{b,c}, Marilyn Masek\textsuperscript{6}, Joanne L. Attema\textsuperscript{c,3}, Gordon Li\textsuperscript{d}, Terri Haddix\textsuperscript{6}, Michael S. B. Edwards\textsuperscript{d}, Paul G. Fisher\textsuperscript{3}, Irving L. Weissman\textsuperscript{c,e}, David H. Rowitch\textsuperscript{f,g}, Hannes Vogel\textsuperscript{6}, Albert J. Wong\textsuperscript{d,h}, and Philip A. Beachy\textsuperscript{b,c,2}
DIPG preclinical consortium

16? DIPG *in vitro* cell cultures

Charles Keller, OHSU  
Cynthia Hawkins, SickKids  
Xiao-Nan Li, Baylor
 Pediatric Preclinical Testing Initiative

SATURDAY, DECEMBER 3, 2011

Open Science Forum: DIPG Preclinical Consortium

Check back frequently to this blog post, which gives a week to week account of the project entitled, "Rapid Preclinical Development of a Targeted Therapy Combination for DIPG" described here and here. This study is made possible by a $100,000 grant from TheCureStartsNow. An additional $28,000 supplement from The Lyle Nisoul Foundation for Children's Brain Cancer Research has allowed our project to expand our 2 participating European collaborators.

The full international team is:

Charles Keller MD, Kellie Nazem MD, Nate Seiden MD, PhD and Dan Guillaume MD, PhD at the Oregon Health & Science University
(cell lines: OHSU-DIPG1)

Oren Becher MD, Duke University Medical Center
(cell line, from mouse: 11.10032, which is GFAP tva/foxed PDGF p53)

Michelle Morje MD, PhD, Stanford University
(cell line: SU-DIPG-I, SU-DIPG-II, SU-DIPG-III, SU-DIPG-IV, SU-DIPG-V, SU-DIPG-VI)

Maryam Foulad MD, Cincinnati Children's Hospital Medical Center

Cynthia Hawkins, MD, PhD, University of Toronto
(cell lines: HSC-DIPG27, HSC-DIPG58)

Xiao-Nan Li MD, PhD, Baylor College of Medicine
(cell lines: IBs-1215DIPG, IBs-A1204DIPG, IBs-AB1202DIPG, IBs-C1220DIPG, IBs-J1204DIPG, IBs-W1202DIPG)

Dennis G. van Vuuren MD, MSc, A. Esther Huitema, VU Cancer Center Amsterdam,
(cell lines: VUMC-DIPG-A, VUMC-DIPG-B)

Jacques Grill, Institut Gustave-Roussy, Villejuif, France
(cell line names not specified; sample not to be received for sequencing)

and

Marko DeWire, Nationwide Children's Hospital, Columbus, OH
(cell line name TBA)

WEBSITES WE LIKE

Breast-Notable Cancer Cell Line Encyclopedia
DrugPath
New Approaches in Neuroblastoma Therapy
Pediatric Brain Tumor Consortium
Pediatric Oncology Experimental Therapeutics Investigators' Consortium
Therapeutic Advances in Childhood Leukemia & Lymphoma

THE KELLER LABORATORY AT OHSU

VIP Visitor I
Scientific Writer needed
Our thanks to the Ethan Jostad Foundation

PEDIATRIC CANCER BIOLOGY PROGRAM AT OHSU

Patient Groups and Researchers Join Forces to Speed Treatments for Rare Pediatric Brain Tumor
Knight Cancer Institute Seminar Series speaker, Dr. Robin Jones (Seattle)

Remembrance

What to do with the tissue?

• Guidance for targeted therapy in the clinic
  – Making molecular profiling data available at relapse
  – Treatment stratification at diagnosis

• Linking disease biology to model development and preclinical screening \textit{in vitro} and \textit{in vivo}
  – Provide the evidence base for stratified therapy

• Collaborative pooling of resources
  – Data
  – Banked samples
  – Prospective collection of viable cells