Precision Medicine for Pediatric Brain Tumors: Are We There Yet?

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No conflict of interest
Cancer Remains a Leading Causes of Death among 1-14 Year-Olds
U.S., 1991 vs. 1998

- Accidents
- Cancer*
- Birth Defects
- Homicide
- Heart Diseases
- Pneu & Influenza
- Cerebral Palsy
- Suicide
- HIV Infection
- Obstruc. Lung Dis.
- Dis. of Infancy
- Cerebrovasc. Dis.
- Septicemia
- Viral Diseases
- Meningitis

*includes fatal 'benign' tumors

1991: 5189
1998: 5389
More than 7000 new cases of childhood cancers are diagnosed each year in the United States

Central Nervous System (CNS) Tumors are the most common solid tumor of childhood

The morbidity caused by CNS tumors and their treatment exceeds that of most other childhood cancers
Early diagnosis and extent of surgical resection of CNS tumors in children are the strongest predictors of increased survival or cure

- Signs and Symptoms
- Role of Surgery and Chemotherapy
  - Advances leading to decreased Morbidity
- Precision Medicine and Personalized Modeling of Cancers
- Advances in the laboratory
Signs and Symptoms: can be fairly nonspecific...

- Seizures
- Ataxia/Dysmetria
- Vomiting
- Cranial Neuropaties
- Macrocephaly

- Nausea
- Headache
- Visual Disturbance
- Altered Mental Status
- Pain
- Weakness
Vomiting

- Occurs in 84% of pediatric patients with brain tumors
- May be due to generalized elevated ICP, or compression of vagal nuclei or vomiting centers in floor of fourth ventricle
- **Early morning** headaches and vomiting
- **Waxing and waning** symptoms often with improvement of headaches after emesis
Seizures

- Rare early indication of a mass lesion:
  - Initial presentation in 15% of children with supratentorial lesions
- Temporal lobe most common site for tumors producing seizures as an early sign
  - Can present as absence seizures
  - Difficult to detect: staring, Lack of awareness, sudden halt in activity, lip smacking etc.
  - behavioral issues and decline in school performance
Ataxia/Dysmetria

- Gait disturbances may suggest either a brain stem or cerebellar process
- Triad of long-tract signs, cranial neuropaties, and ataxia suggests brain stem pathology
- Cerebellar lesions
  - Midline lesions = truncal ataxia
  - Cerebellar hemisphere = Appendicular ataxia
Cranial Neuropaties

- Seen in multiple types of brain tumors
- Produced by direct compression/invasion or
- Indirectly due to increased ICP
  - Most commonly CN VI
    - Diplopia with complimentary strabismus or head tilt
Clinical Presentation of Brain Stem Gliomas

- **Most Common** -- Diplopia, asymmetric cranial nerve deficits and difficulty walking
- **Hydrocephalus Syndrome** -- Headaches, vomiting and ventriculomegaly
- **Psychiatric Syndrome 1** -- Apathy, depression, decline in school performance and memory loss
- **Psychiatric Syndrome 2** -- Behavioral change, nightmares and enuresis
- **Stroke or hemorrhagic Syndrome** -- Acute stroke like onset of hemiplegia, quadraparesis, intra-nuclear ophthalmoplegia and upbeat nystagmus
  - Cerebello-pontine angle presentation with involvement of cranial nerves V, VII and ataxia
Back pain in a child may be warning of underlying spinal cord lesion

- When presenting with intracranial signs or symptoms may represent a drop metastasis from intracranial tumor
Weakness

- Weakness of extremities commonly seen with supratentorial tumors
- Can be presenting sign of spinal cord compression
- Other associated signs and symptoms must be elicited to differentiate between the two types of presentation
SPINAL CORD TUMOR: clinical progression
Macrocephaly

- Young children can accommodate large tumors with minimal signs of raised ICP.
- Presence of open sutures or frontal bossing is a longstanding sign of increased ICP.
- Imperative to measure a child’s head circumference if any signs of a change in mental status, irritability or lethargy occurs.
Visual Disturbances

- Direct involvement of visual pathway
  - Optic glioma, hypothalamic gliomas, visual cortex and craniopharyngioma: visual field cuts
  - Pineal and tectal plate tumors: perinaud’s sign
- Papilledema secondary to raised intracranial pressure
  - Present in up to 90% of patients with cerebellar astrocytoma
Endocrine Signs Indicating Tumor

- Growth disturbances
- Precocious puberty
- Amenorrhea
- Hypothyroidism
- Diabetes insipidus
42% of patients present with hormonal deficiency (D.I.) and precocious or delayed puberty.

Ophthalmologic examination is critical because patients rarely complain of visual disturbance.

Visual cuts are commonly found on exam.
Sophia
CRANIOPHARYNGIOMA

- Frequently present with hormonal deficiency
- Over 50% present with growth hormone deficiency
- Over 91% of patients have hypopituitarism with multiple hormonal deficiencies
- Diabetes Insipidus is a late sign and frequently underappreciated
Signs of a Pineal Tumor

- **Parinaud’s sign**
  - Paralysis of upward gaze with retraction nystagmus, pupillary light-near dissociation, lid retraction
- **Raised intracranial pressure: hydrocephalus**
- **Diplopia**
- **Diabetes insipidus**
- **Hypopituitarism**

Pre-op

Post-op
DOES CHOICE OF SURGEON IMPACT SURVIVAL?

CHOROID PLEXUS CARCINOMA IN 16 MONTH OLD TODDLER
Neurosurgeon - a new prognostic factor; resection by Pediatric Neurosurgeon vs. other

- Resection > 90%
- Residual < 1.5cc
- Neurological complications

Graph showing comparison of GNS, DPNS, and ASPN.
the Pediatric neurosurgeon be an agent of $2 \log_{10}$ tumor cytoreduction! $10^{11}$ to $10^{9}$ cells
Holocord Ependymoma

Surgical Resection
NEURONAVIGATION
Neuronavigation

Neuroanatomy
Microneurosurgery
Imaging
FUNCTIONAL MRI AND TRACTOGRAPHY: RESECTION OF TUMOR IN MOTOR CORTEX
Tractography: avoiding injury to white matter tracts in subcortical surgery
SURGICAL TUBES; avoiding resection of white matter
Use of operative microscope for posterior fossa tumor
INTRAOPERATIVE MRI

- Real time images
- Can account for brainshift
- Ideally suited for use in children
  - Smaller surgical field
  - Higher incidence of low-grade tumors
  - Do not need to use traditional head holding apparatus
Minimally Invasive Laser thermal Ablation of Recurrent or Inoperable Brain Tumors

- A Flexible Laser applicator is guided to intended targeted area

- In the MRI unit a physical precisely monitors treatment using software to measure temperature change

- Laser light heats and destroys target area. Temperature maps show extent of tumor tissue being destroyed, minimizing risk of damage to surrounding healthy tissue
Chemotherapy

- Determinants of Drug Efficacy
  - Sensitivity of tumor to the drug
  - Pharmoracokinetics of the drug
  - Drug’s ability to cross the BBB
  - Ability of tumor cells to accumulate and retain the drug
  - Ability of the tumor cells to repair damage caused by the drug
Malignant Glioma

- Increases the disease free survival in both anaplastic astrocytoma (30%) and Glioblastoma (20%) when used following radiotherapy as compared to those treated with postsurgical radiotherapy alone.

Medulloblastoma

- Up to 80% five year disease free survival following gross total resection, radiation and chemotherapy compared to about 40% without chemotherapy
Chemotherapy

- Ependymoma
  - Chemotherapy has not improved survival
- Oligodendroglialomas
  - Very little data exist regarding use of chemotherapy for children with newly diagnosed or recurrent disease
- Germ Cell Tumors
  - Depends on the type of germ cell tumor
Diffuse Leptomeningeal Astrocytoma with Drop Metastasis
TUMOR PAINT
Optides; targeted treatments to kill cancer cells while sparing patients from toxic effects of chemotherapy

- 1993 team at Harvard identified component of scorpion venom that blocks chloride channels

- 1994 Nicole Ullrich and Harald Sontheimer used Cholorotoxin to block movement of Glioma cells and found compound only binds malignant cells

- Transmolecular Phase 1 and Phase 2 trials

- Jim Olson and Blaze Bioscience with tumor paint to visualize tumor in surgery

- Make surgeons able to remove more tumor without disturbing normal tissue

- Attach chemotherapeutic drugs to a binding agent that only attaches to tumor cells
What is Precision Medicine?

- Matching the right patient, to the right medicine, at the right time.
- Most tumors don’t have a single gene mutation or single abnormal pathway
- Most brain tumors are heterogenous and constantly evolving

Need to gain a deeper understanding of molecular characteristics that are driving tumor growth and matching treatment to those specific abnormalities
Pediatric and adult brain tumors are distinct entities

- Pediatric gliomas are associated with unique mutations
- Pediatric gliomas often resemble “normal” developmental programs (e.g. EGL progenitors in medulloblastoma)
- Pediatric disease more frequently presents as low grade while adult presents as GBM
- Notably pediatric disease presents unique challenges due to coincident brain development
Recurrent somatic mutation discovery is one of the hottest fields of investigation.

Next-gen sequencing has lead to rapid determination of the somatic mutations in low and high grade gliomas.

**Pediatric Glioma**
- Zhang et al. 2013, *Nature Genetics*

**Adult Glioma**
- Brennan et al. 2013, *Cell*
- Williams Parsons et. al. 2008, *Science*
- Frattini et. al. 2013, *Nature Genetics*
- Verhaak et al. 2010, *Cancer Cell*
We need models of brain tumors that closely mimic human disease

- Identify target
- Find specific drugs or combinations of drugs that attack those targets
- Identify biomarkers that can predict which combination of drugs will be effective for subgroup of patient(s)
There are an average of 15 somatic mutations in pediatric glioma—relevance of each mutation not clear.

For adult this number rises to 36 somatic mutations.

Many mutations do not have genetically engineered mouse models.

Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma.
Current tumor models

- Engineered Mouse Models
  - Costly
  - Take months/years to design
  - More time to cross
  - Even with a transgenic promoter such as Nestin, a “non-physiological” amount of cells express the mutation throughout development

- Viral Models
  - Safety concerns (pantropic)
  - Need skill in achieving high titers
  - Breeding needed for Rcas-Tva models (ecotropic)

- Transplant Models
  - Immune interactions an issue
  - Not autochthonic and likely does not recapitulate tumorigenic process
The piggyBac Brain tumor platform

Development of a novel methodology for rapid and efficient generation of brain tumor models
Electroporation is the application of current across the head to drive plasmid DNA into the nuclei of dividing cells.

Postnatal electroporation is a simple, “non-invasive” procedure taking less than 1 hour per litter to create somatic transgenics.
Postnatal Electroporation
Schematic of Electroporation

A. Injection site

B. 3 mm, 7 mm, 10 mm

C. Electrode gel, positive pole, current direction
Expression of plasmids in cells
Primary downside of electroporation

- Very few plasmids stably integrate
PiggyBac Transposition

- Employs “pBase” transposase to stably integrate the “gene of interest” into the genome in a targeted fashion
- Adapted by Loturco et al. for use in the embryonic CNS
Merging Postnatal Electroporation and PiggyBac Transposition into a model of pediatric glioma

- Postnatal electroporation allows for temporal and spatial control of somatic transgenesis
- PiggyBac transposition provides for permanent transgene integration
CRISPR/Cas9 system can be used for gene editing

- Gene silencing
- Replacing normal gene with mutant gene

Advantages of novel tumor model

- Stable, somatic transgenic mutation
- Label and mutate simultaneously (i.e. tumor cells are fluorescent against a wild type, unlabeled background)
- Flexible-Multiple genes/shRNAs can be delivered simultaneously (i.e. patient mutation signatures)
- Autochthonic – from endogenous cells, allowing natural progression of tumors
- Non-viral – no safety/immune concerns
- Rapid – requires only PiggyBac-based donor plasmids
- Defined initial magnitude of somatic transgenesis as well as the spatial and temporal genesis of the tumor
Postnatal EP targets VZ stem and progenitor cells
Choosing mutations

- The MAPK pathway is highly implicated in gliomagenesis.
- Ras mutations are seen in 20-25% of cancers.
- Erbb2 (HER2/Neu) forms a heterodimer with other Erbb2 family members and mutations are found in glioma.

[Link to Image: http://www.biooncology.com/images/therapeutic-targets/]
Tumorigenesis using electroporation with PiggyBac transgenesis
High penetrance of malignancy

[A] Control  Hras G12V

[B] Hras G12V

[C] Hras G12V

[D] Survival over time for different groups:
- Ctrl (n=20)
- Hras G12V (n=20)
- Erbb2-CA (n=20)

Time (d) after Electroporation
High grade tumor development
High Grade Tumorigenesis in EP Model

- The critical pathological hallmarks are noted
Tumors mimic human glioma subtypes
Transplanted tumor cells generate secondary malignancies
miR-E knockdown technology

- miR-E technology is an evolution of mir30 microRNA technology
- Can be used with PolII promoters (CAG, TRE-Bi, tissue specific promoters, etc.)
- Nf1 functions to inhibit Ras activation and is a frequent mutation in pediatric glioma
### Spontaneous secondary Mutations

#### Spontaneous mutations observed in tumor cell lines

<table>
<thead>
<tr>
<th></th>
<th>Erbb2 mRNA</th>
<th>Nucleotide</th>
<th>Protein</th>
<th>Hras mRNA</th>
<th>Nucleotide</th>
<th>Protein</th>
<th>Kras mRNA</th>
<th>Nucleotide</th>
<th>Protein</th>
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<tr>
<td>Trp53</td>
<td>510 c to g</td>
<td>S118C</td>
<td></td>
<td>561 c to t</td>
<td>A135V</td>
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<td></td>
<td>794 g to a</td>
<td>V213M</td>
<td></td>
<td>665 g to a</td>
<td>V170M</td>
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<td></td>
<td>899 a to t</td>
<td>I248F</td>
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<tr>
<td>p16</td>
<td>134 a to c</td>
<td>H to P</td>
<td>silent</td>
<td>134 a to c</td>
<td>H to P</td>
<td>silent</td>
<td>134 a to c</td>
<td>H to P</td>
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<td></td>
<td>142 c to a</td>
<td></td>
<td>V to I</td>
<td>142 c to a</td>
<td></td>
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<td>142 c to a</td>
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<tr>
<td></td>
<td>232 g to a</td>
<td></td>
<td></td>
<td>232 g to a</td>
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<td>p19</td>
<td>311 g to a</td>
<td>R to H</td>
<td></td>
<td>301 c to a</td>
<td>Q to K</td>
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<td>311 g to a</td>
<td>R to H</td>
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<td></td>
<td></td>
<td>315 c to a</td>
<td>S to R</td>
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<td>527 c to t</td>
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</table>
Finding therapeutic targets from Patient-specific models

Schwartzentzrober et al. 2012, Nature

Table 1 - Comparison of mRNA Expression between control NSCs and Hras G12V Tumor Progenitors

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Fold Changes</th>
</tr>
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<tbody>
<tr>
<td>Cspg4</td>
<td>chondroitin sulfate proteoglycan 4 (aka NG2)</td>
<td>13.7</td>
</tr>
<tr>
<td>Sox10</td>
<td>SRY-box containing gene 10</td>
<td>67.9</td>
</tr>
<tr>
<td>Pdgfra</td>
<td>platelet-derived growth factor receptor, alpha polypeptide</td>
<td>80.8</td>
</tr>
<tr>
<td>Erbb3</td>
<td>v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)</td>
<td>19.5</td>
</tr>
<tr>
<td><strong>Astrocyte Markers</strong></td>
<td></td>
<td></td>
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<tr>
<td>Aldh1a1</td>
<td>aldehyde dehydrogenase 1 family, member L1</td>
<td>-12.7</td>
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<tr>
<td>Fgfr3</td>
<td>fibroblast growth factor receptor 3</td>
<td>-8.0</td>
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<tr>
<td>Slc1a3</td>
<td>solute carrier family 1 (glial high affinity glutamate transporter), member 3 (aka Glast)</td>
<td>-6.6</td>
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<tr>
<td>Acsbg1</td>
<td>acyl-CoA synthetase bubblegum family member 1</td>
<td>-2.9</td>
</tr>
<tr>
<td>Aqp4</td>
<td>aquaporin 4</td>
<td>-3.2</td>
</tr>
<tr>
<td><strong>Metabolism Related</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasn</td>
<td>fatty acid synthase</td>
<td>3.1</td>
</tr>
<tr>
<td>Thrsp</td>
<td>thyroid hormone responsive (aka Spot14)</td>
<td>-29.5</td>
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Upregulation of multiple Ets factors in glioma cells

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Fold Changes over NSCs</th>
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<td>Etv1</td>
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<tr>
<td>Etv2</td>
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<td>Ets1</td>
<td>2.511168</td>
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<tr>
<td>Ets2</td>
<td>4.207538</td>
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</tbody>
</table>

**Figure B**

Fold change in mRNA abundance normalized to Rps29 for different genes:

- Ufek
- Hras
- Kras
- Erbb2
Er81 protein is upregulated in tumor cells
Ets blockade rescues acute RG phenotypes
Ets blockade inhibits gliomagenesis

Graph showing survival over time after electroporation.

- Blue line: DN-Etv5-TagBFP2 P2A Hras G12V (n=14)
- Green line: TagBFP2nls P2A Hras G12V (n=10)
Residual populations resemble hypertrophic astrocytes
DN-Etv5 induction after tumor initiation leads to differentiation

Ki67  EGFP  HA (BgIA)

pB-rtTA-V10-P2A-BgIA-HA-P2A-Kras G12V::Tet-On EGFP/DN-Etv5 PB

A

+Dox

Ctx  LV  Str

B

+Dox

C

+Dox

+Dox
Confirmation in Humans

- Inhibition of Fatty Acid Synthase Decreases Expression of Stemness Markers in Glioma Stem Cells. Yasumoto et al. PloS One 2016 Jan 25;11(1)

Pre-clinical validation of therapies derived from microarray screening

- Small molecules
  - Fasn
- Genetic proof of principle
  - Ets family
- Immunotherapy
  - Tgfb1-3
ADDRESSING TUMOR RECURRANCE
**Cell Reports**

*Ets Factors Regulate Neural Stem Cell Depletion and Gliogenesis in Ras Pathway Glioma*

**Graphical Abstract**

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**In Brief**
Breunig et al. report that increased Ras signaling functions to deplete neural stem cells and expand glial progenitors in gliomagenesis. Inhibition of the upregulated Ets signaling downstream of Ras is sufficient to inhibit glioma formation by attenuating the gliogenesis necessary for tumor propagation.

**Highlights**
- Rapid brain tumor modeling with postnatal electroporation and transposon methodology
- Modeling methodology allows for extensive interrogation of tumor growth mechanisms
- Ras pathway mutations deplete neural stem cells and upregulate Ets factors
- Ets signaling block rescues Ras-mediated stem cell loss and prevents tumor formation

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