GENETIC AND MOLECULAR CHARACTERIZATION
OF POOR RESPONDER PEDIATRIC BRAIN TUMORS

Genetics Unit
Neurosurgery Unit
Pathology Unit
Neuro-Oncology Unit

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Florence, Italy
GENETIC AND MOLECULAR CHARACTERIZATION
OF PEDIATRIC BRAIN TUMORS

✓ array-CGH (a-CGH)

✓ Next Generation Sequencing (NGS)
  - Target resequencing
  - Exome/Genome sequencing

✓ miRNA profiling
9 pediatric Glioblastoma Multiforme (pGBM)

Reccurent somatic CNVs

1q32.1-qter dup/amp (66%), 4q13.1 del (22%), 6q14.1 del (33%), 7q31.32 dup/amp (22%), 9p h-del/del (44%), 10q11.22-q21.31 del (44%), 13q del (44%), 15q del (33%), 17p13.2 del (33%), 18p del (22%) and 18q21.31-q22.1 del (44%)

Strong genomic instability
Next Generation Sequencing (NGS) in pGBMs

✓ Target resequencing of 433 interesting genes in Minimal Common Regions (MCRs) of recurrent somatic CNVs identified with a-CGH (454 Flx, Roche)

✓ Exome of three pGBMs families (proband, father and mother) and 11 pGBMs (HiSeq 2000, Illumina)
## Target resequencing in pGBMs

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gene</th>
<th>Variant</th>
<th>State of variants</th>
<th>PolyPhen</th>
<th>Mutation Taster</th>
<th>pMut</th>
<th>AA conservation among species</th>
<th>Parental origin</th>
<th>Constitutional origin (leucocyte)</th>
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<tbody>
<tr>
<td>P1</td>
<td>ST3RD1</td>
<td>c.499G&gt;A; V164M</td>
<td>Heterozygous</td>
<td>Possibly damaging</td>
<td>Disease causing</td>
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<td>++++</td>
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<td>nd</td>
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<td>Neutral</td>
<td>Pastoreno et al., 2008</td>
<td>paternal</td>
<td>present</td>
</tr>
</tbody>
</table>

**AA conservation among species** (Homo sapiens, mus musculus, ratus norvegicus, gallus gallus-columbia livia, macaca mulatta-callithrix jacchus-pan troglodytes, donio rerio):+++ highly conserved, ++ conserved and + less conserved.
miRNAs in chemoresistance of pGBMs

 ✓ 5 miRNAs study
 miR-21, miR-7, miR-124, miR-128 e miR-137

 ✓ 377 microRNA study
 *TaqMan® Human MicroRNA Array v2.0, Applied Biosystems*
in vitro experiments demonstrated the role of miR-21 in the drug resistance phenotype of T98G cells

✓ Transfection with miR-21 inhibitor reduces miR-21 expression in T98G cells

✓ Concomitant treatment with miR-21 inhibitor and doxorubicin shows an increased cytotoxic and pro-apoptotic effect of the drug

In conclusion Anti-miR21 oligonucleotide enhances chemosensitivity of T98G cell line to Doxorubicin by inducing apoptosis
**TaqMan® Human MicroRNA Array v2.0, Applied Biosystems**

**Overview of basic product workflow.** The A and B content is fixed, and each is comprised of a Megaplex RT pool, a Megaplex PreAmp Primer pool (optional), and a TaqMan MicroRNA Array enabling the quantitation of up to 381 miRNAs.

- **3 pGBMs:**
  - pGBM (no amp)
  - pGBM (amp1q)
  - pGBM (amp1q/ampX/amp7)

- **POOL:**
  - 5 no-tumor pediatric cerebral cortex

- **miRNAs differentially expressed (miR-137, miR-216a, miR-490, miR-501-3p, miR-521, miR-525-3p, miR-672, miR-873, miR-876-3p, miR-876-5p, miR-448)**

- **GRIA1, SORL1, NUCKS1, SOX11, SAP30L, HTT, PXMP4, THRB, PSD3, SPN, AGPAT4, USP31, GRIK3, POM121L8P, TNRC6B, SNX29, HIPK2, RIMKLA, ZNF738, LOC388692** (www.microrna.org database)

- **Are involved in chemoresistance?**
COLLABORATION PROPOSAL
Molecular characterization of Glioneuronal Tumor with Neuropil-like islands, GTNI

GTNI is a new tumor entity described the first time in 1999

At the moment we have studied 4 GTNI by array CGH (tumor and blood DNAs: 2 spinal, 1 frontoparietal, 1 intraventricular congenital)

✓ Increase the number of tumors
✓ Homogeneous case series (spinal or cerebrum GTNI)
✓ Expand technological methods
Glioneuronal Tumor with Neuropil-like Islands

World Health Organization (WHO) classification 2007

Glioneuronal tumor with neuropil-like islands

Papillary glioneuronal tumor

Rosette-forming glioneuronal tumor of the fourth ventricle

WHO grade II/III

WHO grade I

WHO grade I
Glioneuronal Tumor with Neuropil-like Islands

A Distinctive Glioneuronal Tumor of the Adult Cerebrum With Neuropil-Like (Including “Rosetted”) Islands
Report of 4 Cases

Jennifer G.C. Teo, F.R.C.P.A., S. Humayun Gultekin, M.D., Mark Bilsky, M.D., Philip Gutin, M.D., and Marc K. Rosenblum, M.D.


- Extremely rare (approximately 35 cases)
- Most intracranial (23 cases) and rarely spinal (12 cases)
- Male > Female
- Rare in pediatric age (10 cases)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PGNT (n = 71)</th>
<th></th>
<th>RGNT (n = 85)</th>
<th></th>
<th>GNTNI (n = 26)</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
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<tr>
<td>Gender</td>
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<tr>
<td>Male</td>
<td>33</td>
<td>46.5</td>
<td>39</td>
<td>45.9</td>
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<tr>
<td>Female</td>
<td>38</td>
<td>53.5</td>
<td>46</td>
<td>54.1</td>
<td>9</td>
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<tr>
<td>Age at diagnosis</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Median (range)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Children (&lt;18 years)</td>
<td>25</td>
<td>35.2</td>
<td>18</td>
<td>21.2</td>
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<tr>
<td>Adults (&gt;18 years)</td>
<td>46</td>
<td>64.8</td>
<td>67</td>
<td>78.8</td>
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<tr>
<td>Patients &lt;26 years</td>
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<td>59.2</td>
<td>40</td>
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<tr>
<td>Patients &gt;26 years</td>
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<td>40.8</td>
<td>45</td>
<td>52.9</td>
<td>18</td>
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<td>Tumor location</td>
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<td>Supratentorial</td>
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<td>97.2</td>
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<td>15.3</td>
<td>18</td>
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<td>Posterior fossa</td>
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<td>68</td>
<td>80.0</td>
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<td>Spinal</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>1.2</td>
<td>6</td>
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<tr>
<td>More than one area</td>
<td>1</td>
<td>1.4</td>
<td>3</td>
<td>3.5</td>
<td>2</td>
</tr>
</tbody>
</table>

Glioneuronal Tumor with Neuropil-like Islands
Glioneuronal Tumor with Neuropil-like Islands

Treatment
There are no specific and universally accepted protocols for postsurgical treatment (RT+HDCT)

Prognosis
Aggressive behaviour despite a relatively indolent histology

Schlamann et al, PLoS One 2014;3;9(7):e101211

Agarwal et al, Neuropathology 2008;29,96-100
Glioneuronal Tumor with Neuropil-like islands, GTNI

✓ No recurrent somatic CNVs

✓ Two spinal cases did not show any difference in the CNV pattern between blood and cancer

✓ Congenital case has a duplication of the entire maternal chromosome 8 (mosaicism of 15-20%)

✓ One case presented an about 291 kb maternal amplification in 5q14.1 (chr5: 78,316,935-78,508,370)
Glioneuronal Tumor with Neuropil-like islands, GTNI

- **DMGDH**: encodes a mitochondrial dimethylglycine dehydrogenase (juvenile papillary thyroid carcinoma)
- **BHMT**: encodes a cytosolic enzyme that catalyzes the conversion of betaine and homocysteine to dimethylglycine and methionine (human hepatocarcinoma)
- **BHMT2** (betaine homocysteine S-methyltransferase 2): encodes a methyl transferases that can catalyze the transfer of the methyl group from betaine to homocysteine (tumorigenesis?)

- The distal breakpoint falling at 23 kbp from the 5’UTR of *JMY* gene encoding for a p53 cofactor.
Concerning the 5q rearrangement inheritance, literature data report different inherited genomic regions that influence susceptibility to cancer.

For example, recurrent inherited rearrangements in 9p21, including the \textit{CDKN2A/CDKN2B} genes, with expression variability/incomplete penetrance are reported in multiple types of cancer (including breast cancer, melanoma, glioma, and leukemia).
No clear data have been so far reported for the role of inherited amplification in cancer, although incomplete penetrance/expression variability is documented for some constitutional disorders (amp 7q, 16p ampl)
Glioneuronal Tumor with Neuropil-like islands, GTNI

- Mosaics trisomy 8 is a rare condition that could predispose to hematologic neoplastic disorders and childhood cancer such as Wilms tumor.
- MT8 has never been described in brain tumor.
- Could MT8 have a role in the onset of congenital GTNI?
Understanding the biological and molecular features of GTNI will lead to a better therapeutic approach.