Identification of genetic profile associated with response to treatment with protein Smoothened (SMO) inhibitors in paediatric medulloblastoma patients.

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Abstract
Medulloblastoma is the most common malignant brain tumor in children. Recent reports have shown that this tumor is not a single disease, but comprises at least four subgroups, named Wingless, Sonic-Hedgehog, group 3 and group 4, each with its particular molecular profile and clinical features. Although current treatment protocols lead to 70-85% survival rate, there are many neurological side effects due the therapy. For those patients with medulloblastoma of Sonic-Hedgehog subgroup, an option using Smoothened inhibitors has been considered to target Sonic-Hedgehog pathway. However, primary and acquired resistance to the treatment with these inhibitors are a limiting factor for its clinical use. The present study aims to identify mutations in Sonic-Hedgehog pathway genes on tumor samples of medulloblastoma to identify genetic profiles predictive of the response to Smoothened inhibitors as well assess whether formalin-fixed paraffin embedded samples are suitable for this type of genetic analysis. Furthermore, we intend to establish if there is a correlation between tumoral genetic profile and clinical progression of disease.

Key words: Medulloblastoma, Sonic Hedgehog, Smoothened.

Introduction
Medulloblastoma (MB) is the most common malignant brain tumor in children¹. Currently, efforts are focusing on molecular stratification of MB, which will allow patients to receive a more personalized treatment¹. In this way, Smoothened (SMO) inhibitors has been considered in the treatment of Sonic-Hedgehog (SHH) patients, once this pathway is frequently hyperactive in tumors of this subgroup¹. However, primary and acquired resistance to the treatment with these inhibitors are a limiting factor for its clinical use. The present study aims to identify mutations in SHH pathway genes on tumor samples of medulloblastoma to identify genetic profiles predictive of the response to SMO inhibitors. Furthermore, we intended to establish if there is any correlation between tumoral genetic profile and clinical progression of disease.

Results and Discussion
Clinical data were obtained through medical records review of all 104 selected patients and they are resumed at Chart 1. Male outnumber female 2:1 and mortality rate is 37%, while other studies have shown same rate in males and females and lower mortality rate.

In order to check whether formalin-fixed paraffin embedded (FFPE) samples could be used in genomic studies retrieving similar results to that obtained for frozen samples, we compared the yield and integrity between these two types of samples. We observed that the genetic material extracted from FFPE samples were moderately deteriorated, so its usefulness is limited. To perform the molecular stratification, gene expression microarray data were analyzed, a set of 14 genes was selected and primers and probes were designed for quantitative real-time PCR targeting exons which have greater discriminative power to classify the 4 subgroups. To assess the genetic profile predictive of response to SMO inhibitors, screening of mutations in gene SUFU by Sanger sequencing is in progress. MYCN and GLI2 amplification will be assessed by quantitative PCR. After these steps have been completed, we will proceed to statistical analysis to establish the putative relationship between clinical and genetic data.

Chart 1. Clinical data.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Male:Female</th>
<th>Age</th>
<th>Mortality Rate</th>
<th>Sequelae</th>
<th>Relapse</th>
<th>Time between symptom and diagnosis</th>
<th>Chemotherapy</th>
<th>Radiotherapy</th>
<th>Clinical Staging</th>
</tr>
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<tbody>
<tr>
<td>70 (67%):34 (33%)</td>
<td>4 months – 23 years (mean 7 years and 2 months)</td>
<td>37%</td>
<td>68.27%</td>
<td>29 cases</td>
<td>Mean of 2,3 months</td>
<td>86.54%</td>
<td>82.7%</td>
<td>High Risk: 64, Standard Risk: 24, No information: 16</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions
The results obtained from FFPE samples showed that this type of sample is not suitable for genomic studies, once the genetic material is degenerated. The gold standard for genetic analysis is the frozen tissue. SUFU sequencing in addition to MYCN and GLI2 amplification will allow us to identify the subset of patients responsive to SMO inhibitors.

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