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## 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 parafin 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 children<sup>1</sup>. Currently, efforts are focusing on molecular stratification of MB, which will allow patients to receive a more personalized treatment<sup>1</sup>. 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 subgroup<sup>1</sup>. 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-to-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 discriminatory 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.

	Patients
Male:Female	70 (67%):34 (33%)
Age	4 months – 23 years (mean 7 years and 2 months)
Mortality Rate	37%
Sequelae	68.27%
Relapse	29 cases
Time between symptom and diagnosis	Mean of 2,3 months
Chemotherapy	86,54%
Radiotherapy	82,7%
Clinical Staging	High Risk: 64 Standard Risk: 24 No information: 16

### 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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<sup>1</sup> NORTHCOTT, P. A. et al. Medulloblastoma comprises four distinct molecular variants. *J Clin Oncol*, v. 29, n. 11, p. 1408-14, Apr 2011. ISSN 1527-7755.