REPORT
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# INHIBITION OF THE PI3K/AKT PATHWAY IN HIGH GRADE AND DIFFUSE BRAINSTEM GLIOMAS (PHGG/DIPG)

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## 1. Summary

Diffuse pediatric-type high-grade gliomas (pHGG), and especially its most frequent subclass, known as diffuse midline gliomas H3 K27-altered (DMG; which present cancer-driver alterations in histone 3), are pediatric brain cancers carrying very poor prognosis. Within the DMG subclass, those tumors located in the pons, known as diffuse intrinsic pontine gliomas (DIPG), are the most frequent, and the most aggressive. DMG do not have curative treatment because they infiltrate diffusely midline brain structures such as the brainstem and thalamus, which impedes surgery. The molecular analysis of more than 1000 pHGG patient samples identified frequent alterations in the genes *PDGFRα* and *PIK3CA*. These genes belong to the RTK-PI3K-MAPK axis, also known as the PI3K/Akt pathway, which is related to cancer initiation and progression. Overall, more than 60% of the pHGG carry alterations in this axis. In this study, we tested small molecule inhibitors of the PI3K/Akt pathway as therapeutic strategies for DMG. We studied the molecules GDC-0077 (inavolisib), a class I PI3K α isoform (p110α) inhibitor, and GDC-0068 (ipatasertib), a selective ATP competitive pan-Akt inhibitor, which are currently being evaluated in clinical trials for several types of cancers appearing in adult patients.

First, we analyzed our set of samples, composed of ten patient-derived preclinical cancer models (all of them DIPG cell lines). We observed that one of them, known as HSJD-DIPG-007 (abbreviated as DIPG-007), carried the mutation *PIK3CA H1047R*. Both drugs, ipatasertib and inavolisib, inhibited the proliferation of all DIPG cell lines in vitro, independently of they carrying mutations in the RTK-PI3K-MAPK axis. However, DIPG-007 was the most sensitive cell line to the activity of the drugs, with a sensitivity to inavolisib in the low nanomolar concentration range, and to ipatasertib in the low micromolar range. These results suggested a potential activity of this drug in animal models or in patients whose tumors bear this mutation.

We measured whether the new drugs inhibited the PI3K/Akt pathway. Inavolisib inhibited cell signaling through phospho-Akt Ser473 (pAkt) and its downstream effectors phospho-PRAS40 (PPRAS40) and phospho-S6 kinase (pS6). Ipatasertib increased the phosphorylation of Akt, consistently with its mechanism of action. We observed that caspase-3/7 and poly (ADP-ribose) polymerase protein (PARP) were

cleaved after both treatments, particularly after long times of incubation at high concentrations. Thus, PI3K/Akt inhibitors increased the apoptosis of DIPG cells.
We then tested the anticancer activity of ipatasertib and inavolisib in immunodeficient mice bearing intracranial DIPG-007 xenografts. Inavolisib (50 mg/kg) significantly prolonged the median survival of the treated mice, compared to untreated controls(75 versus 85 days, $P = 0.0072$). The analysis of the mouse brain and cerebrospinal fluid confirmed that the drug reached the target tissue at potentially active concentrations, for at least 8 hours. Following the quantification of cancer cells in the mouse brain using a digital droplet PCR method, we observed a significant decrease in the inavolisib-treated mice, compared to control mice. In contrast, ipatasertib (25 or 100 mg/kg) did not achieve therapeutic benefit in DIPG xenograft survival, regardless of the dose. Concentration-time curves showed that ipatasertib concentrations could not achieve sufficiently high concentration values in the mouse brain. Chemical conjugation of ipatasertib to blood-brain barrier-penetrating peptidic shuttles improved significantly (3-fold) the penetration of the drug through in vitro blood-brain barrier models, but resulted in loss of activity compared to the original drug, precluding the in vivo studies with the new conjugates.

In summary, this study found a very relevant preclinical activity of the PIK3CA inhibitor inavolisib against DIPG, related to the presence of PIK3CA mutations in the cancer cells, which could be relevant for the design of future clinical trials.

## 2. Relevance of the study

Our study provides the rationale for the design of a phase I trial of the PI3K inhibitor inavolisib for pediatric patients with pHGG carrying mutations in the gene PIK3CA, which are approximately 20% of all patients with pHGG.

The study confirmed the relationship between drug exposure and activity in brain cancers with intact blood-brain barrier. This result is important in order to prioritize medicines with the appropriate CNS distribution profiles for clinical trials.
The study emphasizes the need for the appropriate screening methods for patient selection for the design of new clinical trials. Only with an appropriate selection method patients will obtain the benefit of the treatments.

## 3. Scientific bibliography generated

Articles related to this project have not yet been published.
Two articles will be generated (one already sent for review, one in preparation) and a doctoral thesis (Leire Balaguer, to be presented in autumn 2024).