

Rational targeting of very high-risk medulloblastoma of childhood - 1st year progress report

Purpose of the research

Medulloblastoma is the most common malignant brain tumor of childhood and treatments for this devastating disease are very limited. A major roadblock in developing new treatments has been a lack of an efficient system to evaluate new approaches. My project aims to generate an accurate pre-clinical pipeline of drug development for medulloblastoma, which reproduces, with precision, the treatment currently given to children with brain cancer.

Background

While MB was historically thought to represent one morphologically identical disease, more recently, integrated genomics has shown that there exists considerable intertumoral heterogeneity. MB consists of several different diseases. Work from the Taylor lab and others has shown that MB is comprised of at least four distinct molecular subgroups: Shh, Wnt, Group 3 and Group 4 each with its own distinct demographics, presentation, transcriptomics, genetics, epigenetics, and outcomes¹.

Within the Shh subgroup, a subset harbor inactivating mutations of tumor protein 53 (TP53), which is usually a germline mutation (Li-Fraumeni Syndrome), and represents a very high risk group with an almost universally fatal outcome. Indeed, TP53 mutated Shh tumors are the major cause of death in irradiated children between the ages of 3-17 and represent a major challenge in pediatric neuro-oncology^{2,3,4}. TP53 mutated Shh tumors have a high degree of genomic instability with frequent chromothripsis and are completely resistant to the current generation of Shh pathway inhibitors due to downstream genomic aberrations such as GLI2 and MYCN amplification^{4,5}.

In addition to TP53 inactivating mutations at diagnosis, the Taylor lab has recently demonstrated in both human and mouse Shh MB, that there is an acquisition of TP53 inactivating lesions at the time of recurrence post-radiation, resulting in genomic instability^{6,7}. This suggests that germline TP53 inactivating mutations at diagnosis result in radiation resistance, or tumors acquire TP53 inactivating mutations which then drive recurrence.

As a therapeutic strategy to treat these highly malignant tumors and to prevent recurrences in Shh MB I am using two different compounds from a new generation of agents that exploit aneuploidy of tumor cells to prevent recurrences after radiation treatment, the PLK4 inhibitor and ATM inhibitor.

The **specific aims** I proposed for this project were:

Aim #1: To characterize the genomic spectrum of MB aneuploid tumors, as well as the drivers of aneuploidy.

Aim #2: To test the experimental drugs PLK4 inhibitor (CFI-400945) and ATM kinase inhibitor (AZ32) in our mouse model of recurrent MB with and without radiation to evaluate their efficacy in preventing MB relapse.

Aim #3: To test the experimental drugs PLK4 inhibitor (CFI-400945) and ATM kinase inhibitor (AZ32) in sporadic mouse models harboring TP53 inactivating alleles (TP53 null mice and TP53 DN) with and without radiation.

In the first year of the project I have analyzed the levels of PLK4 in human tumor datasets and I found that PLK4 is aberrantly expressed in many tumors, including MB. I found in two independent, non-overlapping datasets that higher *PLK4* levels correlate with a trend toward worse outcome, however, when restricting this analysis only to Shh tumors, there is a significant survival difference between high and low *PLK4* expression. As the majority of treatment failures within the Shh subgroup are associated with TP53 mutations, it is logical that we observe this clear association between high *PLK4* expression and poor outcome. As such, PLK4 inhibition represents a novel and rational target for therapy for a subset of very high risk MB. Moreover, in neuroblastoma, the most common extracranial solid tumor of childhood, high PLK4 expression is strongly correlated with a poor outcome independent of MYCN amplification, further supporting this approach in a wide range of pediatric cancers. In addition, I analyzed *PLK4* expression across a dataset of paired primary-metastatic MB and found that the expression is conserved in the metastatic compartment, suggesting PLK4 can be targeted in both compartments. The expression of ATM is also higher in Shh tumors.

I have also performed the pharmacokinetic characterization of the novel PLK4 inhibitor in our mouse model. I found through the pharmacokinetic assays with CFI-400945 demonstrated excellent brain penetration; concentrations were higher in tumors than in the brain of mice (**Figure 1**).

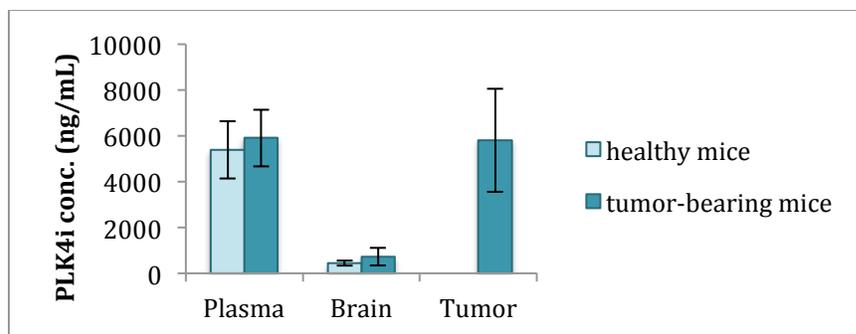
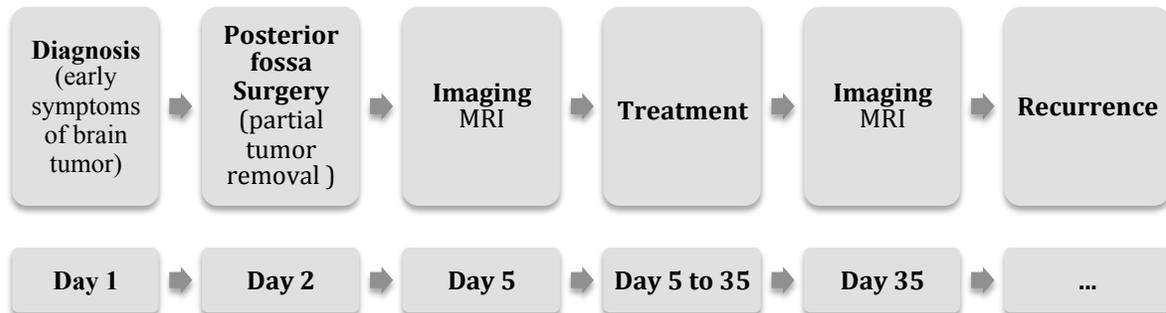


Figure 1: Pharmacokinetic Analysis (PK). PK analysis was performed by treating *Ptch* heterozygous mice with 100 mg/kg single oral administrations of CFI-400945. Plasma, brain tissue and tumor were obtained 4 hours after administration. The dose and treatment schedule were based on previous studies. Concentrations of CFI-400945 were determined by liquid chromatography-tandem mass spectrometry.

The bioavailability of the ATM inhibitor, AZ32, has been demonstrated by Astra Zeneca. The ATMi probe given orally penetrates the blood-brain barrier (BBB) providing sufficient mouse brain PK exposures over the ATM IC₅₀ (data not shown).

Also, during the first year of this project I established the clinical trial in the humanized mouse model for the study of the two novel compounds on the prevention of MB recurrence, exemplified by the workflow below.



Briefly, when the mice were symptomatic for brain tumor, I performed the partial resection of the tumor and collected the sample for further analysis. Mice that recovered from brain surgery were submitted to radiation and or experimental compound treatment as follows:

- Group (1) 5 mice treated with CFI-400945 alone
- Group (2) 5 mice treated with CFI-400945 and radiotherapy (36Gy)
- Group (3) 5 mice treated with vehicle and radiotherapy (36Gy)

The total dose of radiation was given in 18 fractions of 2Gy to the brain and 6 fractions of 4.76Gy to the spine for a focal therapeutic craniospinal radiation, CFI-400945 was given daily before radiation treatment. After treatment, the mice were imaged by MRI for tumor recurrence monitoring. Using this dose of radiation, only one mouse showed a recurrent tumor in the radiation only arm, while all the mice showed a recurrent brain tumor in the CFI-400945 alone arm and none of the mice showed any recurrence in the combination arm. The recurrences on the drug only arm were expected as CFI-400945 is meant to be effective on the very aneuploidic cells spared after radiation and not to affect the bulk tumor.

In spite of the promising preliminary data, I encountered a caveat of the radiation treatment, which was more effective in this mouse model than previously expected. Thus, I am currently working on a de-escalation dose of radiation in order to better evaluate the effectiveness of the compound (CFI-400945). This new protocol has begun and I am currently waiting for mice to relapse after the radiation treatment of 18Gy while analyzing the DNA/chromosome structure of samples before and after treatment.

Once I define the new dose of radiation I will proceed to the second compound AZ32, as follows:

- Group (4) 5 mice treated with AZ32 alone
- Group (5) 5 mice treated with AZ32 and radiotherapy (18Gy)

Evaluation of the drug effect will also be assessed by estimation of survival probability.

After confirming the advantage of survival on the drug plus radiation arm for both compounds, I will proceed to the analysis of the mechanism of action of each drug.

As for the effect of CFI-400945, tumors obtained from **Aim#2** and **Aim #3** will be retrieved as primaries and recurrences, cultured *in vitro* and analyzed for signs of genomic instability, such as chromosome number, phospho-PLK4 Ser305 immunofluorescence analysis and analysis of the percentage of spindle poles in mitotic cells indicating the number of centrioles by staining cells with centrin2 (centriole marker), α -tubulin, and DAPI, for a proof of principle of the drug. Tumors will be profiled using low coverage whole genome sequencing to determine the degree of genomic instability and response to therapy.

And for the evaluation of the ATM kinase inhibitor AZ32 I will perform IF and western blot analysis on frozen primary and recurrent tumors collected from **Aim#2** and **Aim#3** using phospho ATM ser1981, a direct measure of ATM activation and activity. As a master regulator of DNA damage, ATM has several substrates and phosphorylates the amino acid motif of [pS/pT]Q on ATM substrates. Using an ATM/ATR substrate specific antibody that recognizes this motif, I will perform IF and western blot analysis to compare tumor cells from mice treated with ATMi compared to controls. I hypothesize that ATMi will lead to a global reduction of [pS/pT]Q. As additional validation, I will measure specific ATM substrates using phospho specific antibodies for Chk1(ser345), CHK2(Thr 68) and p53(ser15) where appropriate. Lastly, as functional evaluation of ATM inhibition and increased DNA damage as result, I will perform an alkaline Comet assay, which detects both single and double strand breaks (SSBs and DSB).

References

¹Cavalli FMG, Remke M, Rampasek L, Peacock J, Shih DJH, Luu B, et al. Intertumoral Heterogeneity within Medulloblastoma Subgroups. *Cancer Cell* 2017; 31: 737-754.

²Ramaswamy, Remke M, Adamski J, Bartels U, Tabori U, Wang X et al. Medulloblastoma subgroup-specific outcomes in irradiated children: who are the true high-risk patients? *Neuro-Oncology*, 2015 [Epub ahead of print]

³Zhukova N, Ramaswamy V, Remke M, Pfaff E, Shih DJ, Martin DC et al. Subgroup-specific prognostic implications of TP53 mutation in medulloblastoma. *J Clin Oncol* 2013; 31:2927-35.

⁴Kool M, Jones DT, Jäger N, Northcott PA, Pugh TJ, Hovestadt V et al. *Cancer Cell* 2014; 25:393-405.

⁵Rausch T, Jones DT, Zapatka M, Stütz AM, Zichner T, Weischenfeldt J et al. Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations. *Cell* 2012; 148:59-71.

⁶Morrissy SA, Garzia L, Shih DJH, Zuyderduyn S, Remke M, Cavalli FMG et al. Dramatic Divergent clonal selection dominates medulloblastoma at recurrence. *Nature* 2016; 529, 351–357.

⁷Hill RM, Kuijper S, Lindsey JC, Petrie K, Schwalbe EC, Barker K et al. Combined MYC and P53 defects emerge at medulloblastoma relapse and define rapidly progressive, therapeutically targetable disease. *Cancer Cell* 2014; 27:72-84.