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Tesi di Laurea: "Personalized targeted therapy in diffuse intrinsic pontine glioma" (Sintesi)

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Diffuse Intrinsic Pontine Glioma (DIPG) is a highly aggressive paediatric brainstem tumour, with a yearly incidence of 2.32 per 1.000.000, no gender predilection and a median age at diagnosis of 6-9 years. Less than 10% of DIPG patients survive for 2 years, with a median overall survival of 9-12 months. Due to the anatomical location and the diffusely infiltrative nature of the tumour, current treatment options are limited and progress towards clinical improvement has been stationary throughout the last four decades. Until recently, a paucity of tissue samples to study led to a lack of understanding of the underlying molecular components of these tumours. Focal radiation therapy is the current standard-of-care for children with newly diagnosed DIPG. Nevertheless, this treatment provides significant but only temporary efficiency delaying tumour progression (Donaldson et al., 2006). Often alongside the radiotherapy, some forms of chemotherapy are provided in the context of a clinical trial to improve outcome, but no chemotherapeutic agent has demonstrated significant efficacy.

Patients with DIPG can present with a wide variety and usually with an acute onset of neurological symptoms and signs reflecting the anatomic localization and the extent of the lesion. Commonly reported symptoms include abnormal or limited eye movements, diplopia, facial asymmetry, clumsiness, disturbance of gait and loss of balance, and weakness. Symptoms are usually of rapid onset with short duration (1-2 months) prior to diagnosis. Cranial nerves VI and VII are the most affected and specific dysfunction of these is characteristic of DIPG.

The diagnosis of DIPG is based on typical clinical presentation and characteristic neuroimaging findings. MRI is the imaging modality of choice for diagnosis. On MRI, DIPGs demonstrate T1-hypointensity and hyperintensity on T2- weighted images, generally without contrast enhancement, and frequently appear relatively homogeneous on fluid attenuated inversion recovery (FLAIR) sequences. Contrast- enhancement is variable, but these lesions frequently do not significantly enhance at diagnosis. On imaging, the tumour core is centred in the pons and diffusely involves most of its axial diameter (Fig. 1).

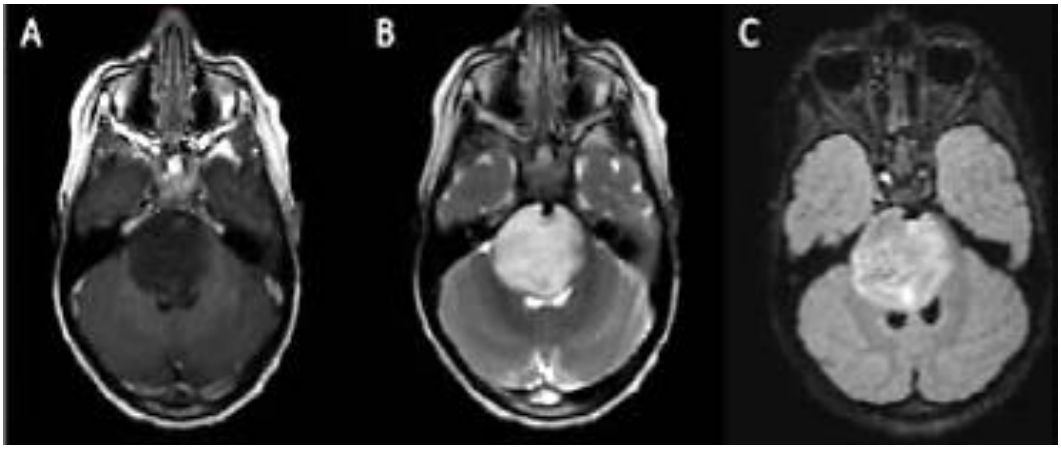

Fig. 1. *MRI appearance of DIPG. A: T1-weighted post contrast. B: T2-weighted. Brainstem demonstrates a diffuse expansile hyperintense lesion in the pons. C: FLAIR. These tumours are surgically unresectable due to their poorly circumscribed border and eloquent location (Warren, 2012).*

For decades, the role of neurosurgery in DIPG has been limited to evaluation and treatment of obstructive hydrocephalus from DIPG and rare biopsies for cases of uncertain diagnosis. However, with the improvements in the biological, molecular and genetic understanding of this disease, tissue acquisition, and therefore surgical biopsy, has developed an increasingly critical role. Developments in identification of potential treatment targets for these lesions, together with the increased safety of stereotactic brain stem biopsies, have helped move the field forward (Hamisch et al., 2017; Williams et al., 2020). Improvements in the safety and feasibility of brain stem biopsy have occurred coincident with improved understanding of DIPG biology. Stereotactic brainstem biopsy in all cases of newly diagnosed DIPG represents a means to better understand the disease and to characterize individual tumours based on molecular pathology in order to direct individualized treatment.

Recent studies have shed light on the molecular makeup of diffuse intrinsic pontine gliomas and identified the H3K27M mutation in nearly 80% of DIPGs, leading to the 2016 WHO classification of diffuse midline glioma H3K27M-mutant, a grade IV brain stem tumour. The histone mutation H3K27M results in the substitution of lysine with methionine at position 27 in the isoforms H3.1 and H3.3, encoded by genes HIST1H3B and H3F3A respectively (Wu et al., 2012). This substitution occurs at a critical position within the N-terminal histone tail submitted to regulatory post-translational modification associated with transcriptional repression (K27). This mutation leads to the loss of histone trimethylation, via inhibition of polycomb repressive complex 2 (PRC2), with global hypomethylation of the lysine at position 27 of the H3 protein (H3K27), ultimately producing epigenetic silencing and dysregulation of gene expression (Bender et al., 2013).

There are subtle differences between the histone mutations in H3.1 and H3.3, particularly regarding survival, phenotype, and clinical outcomes (Castel et al., 2015; Khuong-Quang et al., 2012). K27M-H3.1 mutations are specific to the pons with a significantly longer survival (median 15.0 months), whereas K27M-H3.3 are found alongside midline structures *i.e.* pons, thalamus and spinal cord (Mackay et al., 2017) (Fig. 2). Patients who harbor the K27M-H3.3 mutation have worse overall survival in comparison with other H3 wild-type cases (Khuong-Quang et al., 2012) (Fig. 3).

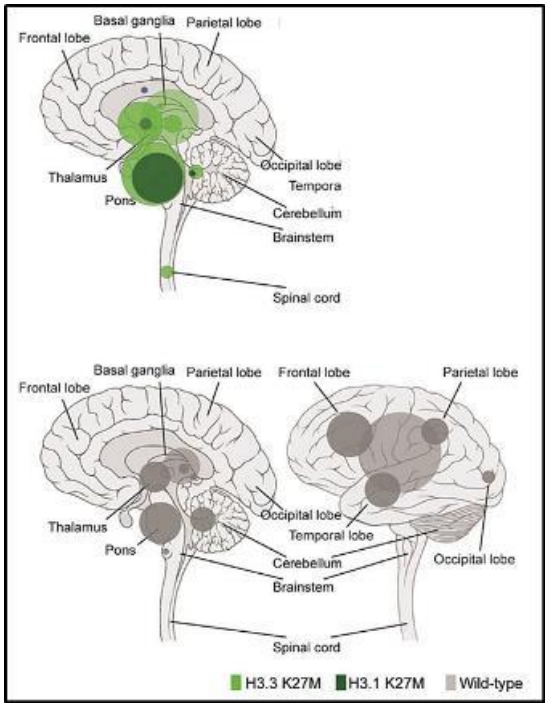

Fig. 2. *Anatomical location of H3K27M mutations and histone wild type. H3-K27M mutations are found exclusively into the pons, midline structures and spinal cord (Mackay et al., 2017).*

In addition, clonal missense ACVR1 mutations were found exclusively in DIPGs (32%) and co-segregate with H3.1 (Wu et al., 2014). ACVR1 gene encodes a bone morphogenetic protein (BMP) type I receptor and ACVR1 mutations in DIPG activate BMP signaling. ACVR1 mutations cause a constitutive ligand-independent activation of the TGFβ/BMP signaling pathway, resulting in increased levels of SMAD phosphorylation, as well as overexpression of downstream targets ID1 and ID2 (Buczkowicz et al., 2014; Wu et al., 2014). All the ACVR1 residues affected by mutation in DIPGs clustered around either the inhibitory glycine-serine-rich (GS) domain or the ATP-binding pocket of the kinase domain and would be expected to shift the kinase to an active conformation (Wu et al., 2014) (Fig. 4).

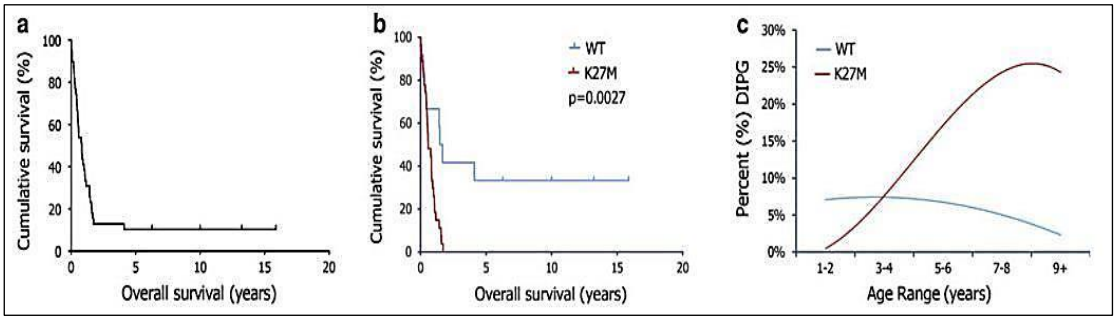

Fig. 3. *K27M-H3.3 is associated with worse overall survival and higher age of diagnosis in DIPG. a) Kaplan–Meier curve of overall survival for all DIPG patients. b) DIPG patients with K27M-H3.3 mutation have worse overall survival compared to patients wild-type for this histone as determined by Kaplan–Meier analysis. c) Age distribution of DIPG patients based on K27M-H3.3 mutational status (Khuong-Quang et al., 2012).*

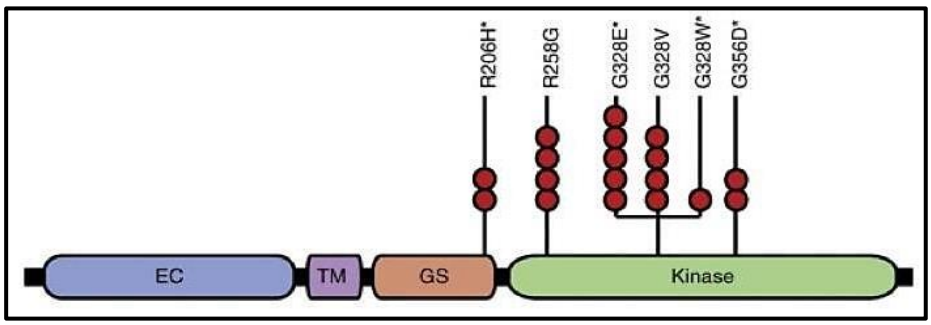

Fig. 4. *Missense ACVR1 substitutions in DIPG were clustered in the GS or kinase domains. Each red circle indicates a DIPG carrying the specified alteration (Wu et al., 2014).*

The standard of care of newly diagnosed DIPG patients is focal radiation therapy (RT), using a 1 cm margin to cover microscopic disease, to a total dose of 54 Gy administered over 6 weeks, usually in daily 1.8-2 Gy fractions. Glucocorticoids are frequently prescribed at the time of diagnosis to reduce and control edema associated with the tumour and radiation treatment. While high-dose steroids offer early relief, their adverse effect profile, including impaired sleep, wound healing, behavior, and endocrine and metabolic functional effects, limits their long-term utility (Curtis et al., 2006). Although large numbers of therapeutic approaches have been tested, no significant progress has been made in treating these high-grade gliomas. Therefore, the identification of new specific targeted therapies is of great importance for innovative and effective treatments.

In this context, the aim of my thesis was to evaluate molecular aspects and heterogeneity, in a cohort of DIPGs patients, and to use this information for the choice of a personalized targeted therapy. The goal of this study was to assess the overall survival and progression-free survival of children with DIPGs receiving a personalized approach with molecularly targeted agents based on tumour molecular profiling. The study retrospectively identified a cohort of 25 patients treated at Bambino Gesù Children's Hospital in Rome from 2014 to 2019, with a radiologic diagnosis of DIPG. Treatment at diagnosis was focal radiotherapy (54 Gy administered in daily 1.8 Gy fractions 5 days a week) associated with systemic chemo-immunotherapy as by institutional guidelines based on Massimino et al (2014) indications. Second line treatment included heterogeneous patients-adapted administration regimens.

In order to design personalized treatments, potentially targetable alterations were screened using a combination of immunohistochemistry, Sanger sequencing and Next generation sequencing (NGS). The most frequently targetable mutations were mTOR/pmTOR pathway mutations known to increase sensitivity to mTOR inhibitors such as everolimus and activating ACVR1 mutations. Other potentially actionable alterations included BRAF mutation and a mutation of PDGFRA known to increase sensitivity to kinase inhibitors such as pazopanib.

Guided by comprehensive molecular profiling on tumour tissue, 9/25 patients were subjected to personalized treatment at primary diagnosis (1/9) and at disease progression and received backbone therapy including focal irradiation. Personalized treatments included inhibition of the mTOR pathway, immunotherapy, retinoic receptor agonist, receptor tyrosine kinase inhibition.

Median overall survival (OS) of the whole cohort was 17.6 months whereas median progression free survival (PFS) was 7.63 months (Fig. 5).

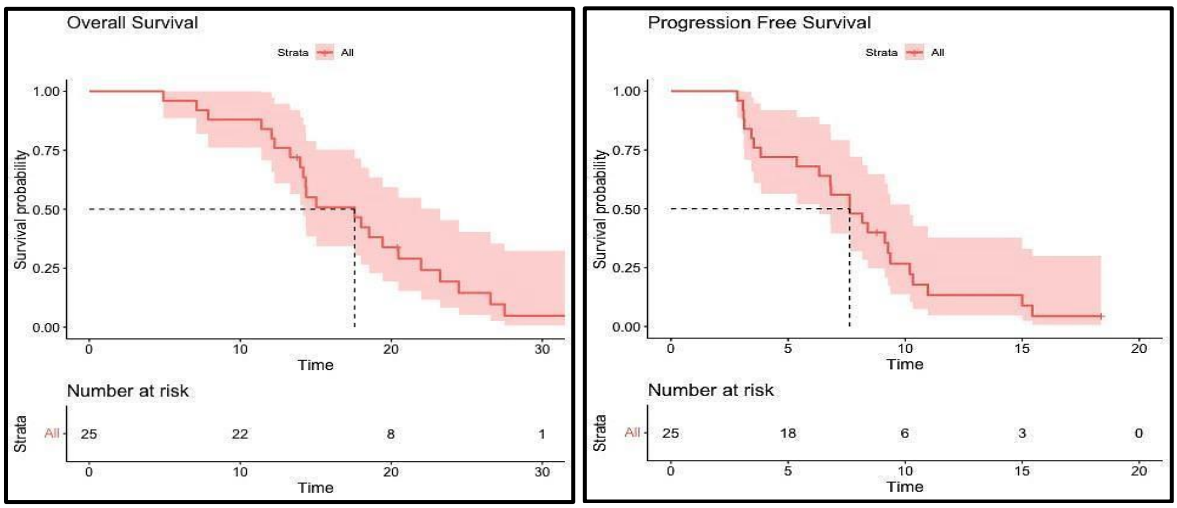

Fig. 5. *PFS and OS curves for the whole cohort.*

We found a significant association between TP53 staining and OS. Patients with positive TP53 staining (> 50%) showed a significant (p= 0.022) shorter overall survival (HR=3.15 CI:1.123-8.834) as shown in Figure 6. Median survival for patients with positive immunostaining of TP53 was 13.2 months versus 20.2 months median survival for patients with p53 < 50%.

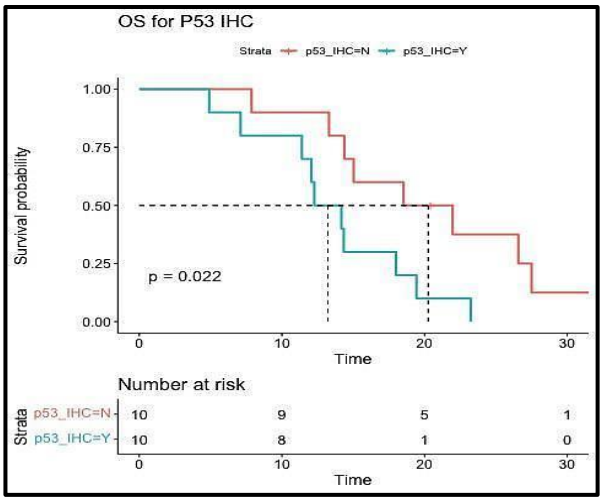

Fig. 6. *TP53 immunostaining (> 50%) is associated with OS.*

Similarly, we confirmed in our cohort that H3F3A mutation confers worst survival. Patients harboring H3F3A mutations had a median OS of 14.2 months versus a median of 24.5 months for patients with mutations in HIST1H3B (HR: 3.41 CI:1.231-9.443, p= 0.01) (Fig. 7).

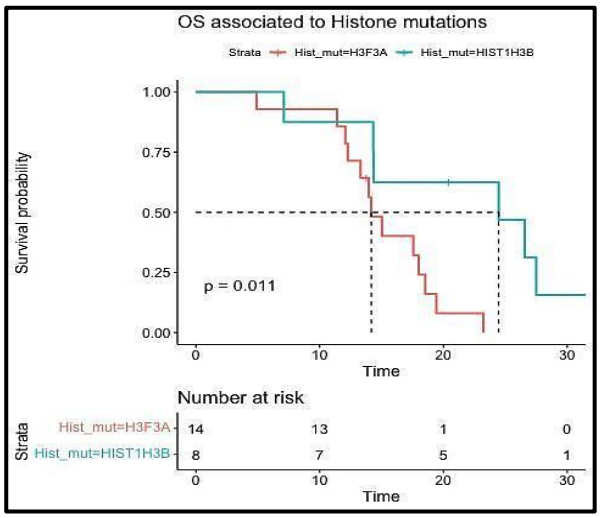

Fig. 7. *Histone modifications are associated with OS. H3F3A mutation is related to shorter survival compared to HIST1H3B mutation (p=0.01).*

Then, we evaluated the potential impact of personalized treatment approaches on survival compared to the control group. Kaplan-Meier survival analysis revealed significantly better overall survival for patients treated with targeted therapies versus control group with a median survival of 20.05 vs 14.3 months. The difference in survival was significant (p= 0.03) with an HR= 0.345 (CI:0.1236-0.9667) (Fig. 8).

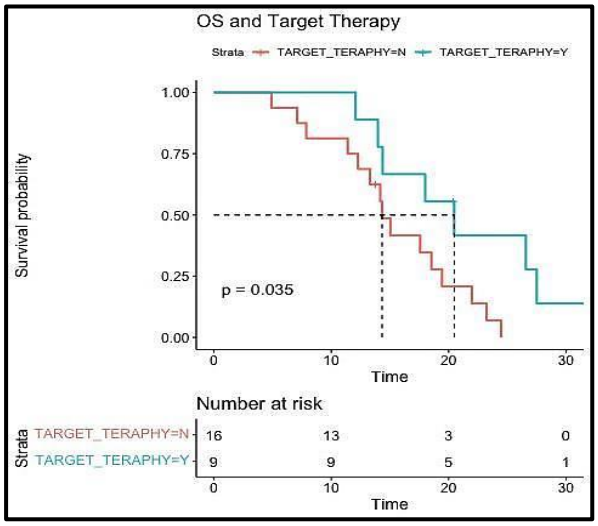

Fig. 8. *Kaplan-Meier curves showing difference in survival of patients receiving personalized treatment and control cohort. OS, overall survival. Patients treated with targeted treatment had better survival (blue curve) than patients treated with standard of care (control group) (red curve) (p=0.03).*

Major limitations of the study were reduced patient number, non-prospective design of the study and heterogeneity of population in terms of molecular biology and treatments.

Overall, this study details the institutional experience at Bambino Gesù Children's Hospital performing personalized targeted approaches in paediatric neuro-oncology patients. We highlighted the feasibility of genomic profiling in both the primary and recurrent disease setting. Moreover, we presented the significant impact this testing is having on the identification of potential actionable molecular alterations and the improved

patient outcome through a personalized approach in H3K27M gliomas.

Based on the results described in our study, we propose to consider biopsy in all patients with DIPG at diagnosis, in order to detect potential molecular drivers alterations that can be targetable and, subsequently, to add personalized agents in first-line treatment in DIPG patients along with the backbone treatment. We envision that this testing will serve as the basis for future clinical trials of personalized targeted therapy, leading to improved outcomes for children with brain tumours.

Nevertheless, large series and future clinical trials are needed to validate these interesting data.

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Sintesi della Tesi di Laurea discussa il 28 giugno 2021

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