Nature Communications

https://doi.org/10.1038/s41467-026-73304-9

Article in Press

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Received: 30 May 2025

Accepted: 22 April 2026

[redacted]

Cite this article as: McKay, C.J., Mayoh, C., Power, P. *et al.* Precision-guided therapy in H3K27-altered diffuse midline glioma. *Nat Commun* (2026). https://doi.org/10.1038/s41467-026-73304-9

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Precision-guided therapy in H3K27-altered diffuse midline glioma

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Abstract

Precision-guided therapy (PGT) approaches have shown improved survival in children with high-risk cancer, but it is unclear whether children with H3K27-altered diffuse midline glioma (DMG) benefit from this approach. Here we report on the cohort of patients with DMG who were consecutively enrolled in the Australian Childhood Cancer PRecISion Medicine for Children with Cancer (PRSIM) clinical trial (NCT03336931), a prospective cohort study aimed at providing PGT recommendations based on comprehensive molecular profiling. A total of 68 patients with DMG were enrolled and 58 (85%) had WGS and RNAseq performed. PGT was recommended for 50 patients (74%). Eighteen patients (26%), who received 21 PGTs, were eligible for survival analysis. The overall response rate (CR or PR) was 52% (n=11). Median overall survival for the PGT group was 21.3 months versus 12.1 months for those that did not receive PGT (p= 0.34). Patients who received PGT after tumor progression had significantly improved overall survival (p=0.007) and subsequent progression-free survival (p=0.001) compared to those that received unguided therapy or reirradiation alone. These results highlight the feasibility of a precision-guided approach and demonstrate its potential clinical benefit in patients with DMG.

Introduction

H3K27-altered diffuse midline gliomas (DMG) represent a highly aggressive group of central nervous system (CNS) tumours associated with dismal outcomes. These tumours peak in incidence at 6-7 years of age and arise from, and diffusely infiltrate, midline structures of the CNS, such as the pons, thalamus and spinal cord. They are characterised by loss of histone H3K27 trimethylation, either through H3K27M mutation or overexpression of EZHIP, as defined in the most recent 2021 World Health Organisation (WHO) Classification[1]. Despite these advancements in understanding the biological underpinnings of DMG, treatment options remain limited. Complete surgical resection is impossible due to the location and infiltrative nature of the disease[2]. Radiation is the only proven therapy to slow the disease progression and remains the standard of care at diagnosis and is increasingly used at disease progression, if clinically appropriate[3,4,5]. Chemotherapy has not been shown to be of benefit in combination with radiation or as maintenance therapy[6,7]. The prognosis remains dismal, with a median overall survival of approximately 11 months and near universally fatal outcome[8,9]. Despite over 200 clinical trials conducted, there has been minimal improvement in patient outcomes in the past 30 years[10]. Historically, clinicians raised significant concerns regarding the biopsy of pontine tumours due to the risks and perceived lack of clinical benefit[11]. More recently, biopsies have been shown to be safe and feasible, and thanks to the advent of next-generation sequencing, both biopsies and post-mortem tumour analyses have helped increase our understanding of the biology of this disease. This has led to multiple attempts to treat DMG with novel and “targeted” therapies, albeit with limited success. The barriers to effective therapies include the identification of targetable tumour drivers, and the paucity of effective agents that penetrate the blood brain barrier.

We recently reported the results of the Australian Zero Childhood Cancer (ZERO) precision medicine program that showed that subsets of paediatric patients with high-risk cancer have improved survival when treated with precision-guided therapy (PGT)[12]. However, the role of PGT for children with H3K27-altered DMG remains unclear. Recent studies investigating the role of targeted therapy in DMG include the PNOC003 trial, which examined the use of upfront biology-guided therapy in DMG using comprehensive molecular profiling. This study showed this approach was feasible but that there was no benefit in survival outcomes. However, therapies were required to be approved by the U.S. Food and Drug Administration and included non-traditional antineoplastic agents[13]. One of the largest biology-driven trials for DIPG, the BIOMEDE study, examined the outcome of targeted agents based on protein expression assessed by immunohistochemistry (IHC) or gene amplification by FISH. This study did not show any significant improvement in outcomes compared to historical controls, but was also limited to only three biomarkers and three targeted therapies and did not use comprehensive molecular analysis to guide individualised treatment strategies[14]. The ZERO program's PRecISion Medicine for Children with Cancer (PRISM) trial enrolled patients with high-risk cancers (expected cure rate lower than 30%)[15]. The primary aim was to provide PGT recommendations based on comprehensive molecular profiling in a clinically relevant timeframe. Secondary and tertiary objectives included evaluating the treatment response in patients who had received a PGT and comparing the survival difference between patients receiving PGT and non-PGT.

Here, we report the clinical outcomes for patients with DMG enrolled in the PRISM study. We show that comprehensive molecular sequencing in this population is feasible and yields clinically actionable diagnostic and therapeutic insights. We further demonstrate that applying a precision medicine approach based on these findings is associated with improved outcomes for this patient group.

Results

Patient Characteristics

Seventy-three patients were consecutively enrolled with a suspected diagnosis of DMG from September 2017 to July 2023 across nine paediatric cancer centres (Figure 1). Of these, 66 ultimately had an integrated diagnosis of H3K27-altered DMG according to the 2021 WHO Classification diagnostic criteria. The seven patients for whom the diagnosis of H3K27-altered DMG was not confirmed are listed in Supplementary Table 1. A further four patients with a clinical diagnosis of high-grade glioma (HGG) were found to have a diagnosis of H3K27-altered DMG following comprehensive molecular analysis. Of the 70 patients with a confirmed diagnosis of H3K27-altered DMG, only two patients had inadequate tissue for molecular profiling and were excluded from further analysis. The primary outcomes of 31 patients from this cohort have previously been reported[12]. The mean follow-up time for the cohort of 68 patients was 15.8 months (0.87 - 75.2), with nine patients alive at the data cutoff date (December 31, 2023). Patients were predominantly enrolled at diagnosis (n=61, 90%) with a mean age of 8.8 years (1.7 - 22.6). Tumours were most commonly centred on the pons (n=45, 66%) and thalamus (n=17, 25%), with only one patient having metastatic disease at diagnosis (1%) (Table 1). Two patients had repeat biopsy, with their diagnosis sample used for results reporting.

Tissue samples for the cohort were predominantly fresh (n=48, 71%), but also included fresh frozen (n=10, 15%), cryopreserved (n=7, 10%) and formalin-fixed paraffin-embedded (FFPE) (n=3, 4%). Combined whole genome sequencing (WGS) (paired tumour-germline) and RNA sequencing (RNAseq)_were performed for 85% (n=58) of patients, including 82% (n=37) of patients with pontine tumours. WGS alone was performed in 9% (n=6) of patients, while panel sequencing with TruSight Oncology 500 DNA panel (TSO500) was performed in 6% (n=4) of patients, most often because only FFPE material was available. There was no difference in the type of analysis performed based upon the extent of resection (biopsy or resection/de-bulking) (p=0.86). The mean tumour mutational burden was 1.22 mutation per Mb (0.38 - 6.28). DNA methylation profiling was successfully performed in the majority of patients (n=61, 90%) (Figure 2a). Fifty-nine patients had a methylation class score classifying as DMG H3K27-altered. Fifty-five patients had a calibrated score >0.9 while four patients had a low calibrated score (>0.3 and <0.9). The remaining two patients did not have a methylation match (Supplementary Data 1).

Most tumours harboured an *H3-3A* (*H3F3A*) mutation (n=50, 74%), followed by *H3C2* (*HIST1H3B*) (n=11, 16%), *H3C3* (*HIST1H3C*) (n=2, 3%) and *H3C14* (*HIST2H3C*) (n=1, 1%). Four patients (6%) were H3K27M wild-type, three had pathogenic *EGFR* mutations associated with *EZHIP* overexpression, and one had isolated *EZHIP* overexpression. Thirty-one patients (46%) harboured *TP53* alterations and these co-occurred most frequently with *H3-3A* mutations. Commonly associated alterations were seen in the PI3K/mTOR pathway (n=24, 35%), along with mutations in receptor tyrosine kinases (n=18, 26%). Five patients (7%) harboured germline mutations, predominantly affecting DNA repair genes (Figure 2b), as described previously[16]. Of the 58 tumour samples that underwent RNAseq, 34 (59%) had differential gene expression reported. 21 patients (36%) did not have any copy number gain or structural variant to support the differential expression observed. Of note, the most common finding was upregulation of the MAPK pathway, which occurred in 5 patients (9%).

Precision Guided Therapy

PGT was recommended for 50 patients (74%). The mean time from enrolment to presentation at the national molecular tumour board (MTB) was 6.8 weeks (4.0 – 12.7). PGT recommendations were derived from actionable and/or reportable findings on each individual patient's molecular profiling as described[15]. The variant allele frequency (VAF) of the targetable mutation was taken into consideration by curators and the MTB when making treatment recommendations, but no strict cut-off was defined. Recommendations were tiered according to the strength of available evidence to support the recommendation (Methods). PGT was only recommended if age-specific drug safety data and dosing were available and there was a reasonable possibility of drug access in Australia via registered indication, clinical trials, compassionate access or off-label use.

A total of 116 PGT recommendations were made among the 50 patients, with 12 patients receiving one recommendation, 18 patients with two recommendations and 20 patients with three or more recommendations. Most recommendations were Tier 1 or 2 (n=74, 64%), followed by Tier 3 or 4 (n=36, 31%) and Tier 5 (n=6, 5%). The most commonly recommended PGTs were PI3K/mTOR inhibitors or tyrosine kinase inhibitors (TKIs), consistent with the molecular profile of the cohort (Figure 3). Differential gene expression from RNAseq supported WGS-based PGT recommendations in 16 patients (28%), and provided independent novel PGT recommendations in 12 patients (21%).

Out of fifty patients with therapeutic recommendations, 23 patients received a PGT as either a single agent or combination therapy. The majority of PGTs utilised were from Tier 2 recommendations (n=13, 57%). Fourteen different PGT targeting eight different molecular pathways were used across the 23 patients (Figure 3).

Of 27 patients who received a PGT recommendation but were not treated, documentation was available for 25. Reasons were obtained from follow-up surveys completed by the treating oncologists as part of the study requirements. Ten patients instead received alternative therapy, mostly through open clinical trials. PGT was considered inappropriate in eight patients due to clinical status (e.g. stable disease with PGT treatment not considered necessary). In four cases, clinicians or families felt evidence was insufficient to proceed, and in three cases, lack of drug access was the barrier.

Response Assessment

Of the 23 patients receiving PGT, 18 were eligible for response assessment and survival analysis. Five patients were excluded from efficacy analysis due to discontinuation of PGT within 0-4 weeks of initiation without adequate evaluation of treatment response. In the 18 assessable patients there were 21 episodes of PGT treatment, 12 of these episodes commenced following initial disease progression. Imaging of patients receiving PGT were centrally reviewed for response according to Response Assessment in Pediatric Neuro-Oncology (RAPNO) DIPG criteria for pontine lesions and RAPNO HGG criteria for non-pontine tumours[17,18]. All patients had measurable disease prior to the commencement of PGT. The response rate (complete response (CR) + partial response (PR)) was 48% (n=10) (Figure 4). In addition, one patient (zccs418) had an initial major reduction in tumour volume that was not sustained so did not meet the criteria for PR. Two patients had progressive disease (PD) due to leptomeningeal dissemination, but their primary tumour volume remained stable (zccs392, zccs402). The objective clinical benefit (CR or PR or SD for >24 weeks) (OCB) for each episode of PGT treatment was 52% (n=11). There was no significant difference in response rate nor OCB if PGT was commenced within 12 weeks of radiation/reirradiation (50%, n=8/16) or later (60%, n=3/5) (p >0.99).

The majority of patients with OCB had PGT targeting a single nucleotide variant (n=9, 82%) with the remaining targeting increased RNA expression. No patients with PGT informed solely by copy number changes demonstrated OCB. Fifty-six percent (n=10/18) of patients who received Tier 1 or Tier 2 recommended PGT demonstrated OCB compared to 33% (n=1/3) of Tier 3-5 recommendations (p=0.59). Simple logistic regression did not show any association between VAF and OCB, though the sample size was not powered to draw any meaningful conclusions.

A summary of each patient’s treatment is demonstrated in Figure 5. One patient (zccs318) ceased PGT following two months of treatment due to toxicity. Two patients continued PGT following radiological progression due to perceived ongoing clinical benefit.

One patient (zccs1504), who was treated with trametinib (PGT) for 18 months for a *PTPN11* SNV associated with increased expression of *PTPN11* and *MAPK1* underwent repeat biopsy at metastatic progression. This demonstrated tumour evolution with new findings of a *PTPRD* disruption, *PPM1D* mutation and biallelic loss of *CDKN2A/B*, along with an increase in VAF of a *PIK3CA* SNV (from 25 to 50%). The previously described differential aberrant RNA expression of *PTPN11* and *MAPK1* was not detected.

Survival Analysis

On univariate analysis, *TP53* mutations were associated with inferior outcome (p<0.01). There was no survival difference between patients with pontine or thalamic tumour location (p=0.68) nor based on H3-mutation type (p=0.22). There was no survival difference in patients who underwent biopsy alone versus sub-total resection (p=0.41). Notably *BRAF* and *FGFR1* mutations were not associated with improved overall survival in our cohort (p=0.25) (Supplementary Figure 1).

We next compared patients who received PGT at any time point (PGT cohort, n=18) with those who did not (non-PGT cohort, n=45) (Table 2). Patients were excluded from the survival analysis if they had died prior to MTB presentation (n=3). There were no significant differences in the clinical or molecular characteristics between the two cohorts.

The overall survival (OS) from the time of diagnosis for patients in the PGT cohort was 21.3 months compared to 12.1 months for the non-PGT cohort (p=0.34) (Figure 6). We then performed further subgroup analysis to determine the impact of PGT prior to, or following initial disease progression. Nine patients received PGT as part of upfront therapy. The mean time to PGT commencement in upfront therapy was 2.76 months (0.30 – 4.33). There was no significant difference in progression-free survival (PFS) or OS for these patients compared to the non-PGT cohort (median PFS for the upfront PGT cohort 7.8 months versus 6.5 months for the non-PGT cohort, p=0.32; median OS for the upfront PGT-cohort 15.2 months versus 11.7 months for the non-PGT cohort, p=0.71) (Supplementary Figure 2). The remaining nine patients received PGT therapy following initial disease progression. We compared this group to all patients who experienced progression in our cohort, and the patients receiving PGT had improved OS from the time of progression. The median OS was 15.3 months for the group receiving PGT compared to 3.4 months for patients who did not receive PGT (p=0.02). Given the potential selection bias for patients able to receive treatment, we then compared patients receiving PGT to those patients treated with other therapies at progression. This included either unguided therapies (UGT), defined as novel or targeted therapies not guided by the tumour's molecular findings and therefore not recommended by the ZERO MTB, or re-irradiation. There was no significant difference in time from diagnosis to progression between the groups. The mean time to commencement of treatment was 1.50 months (0.03 – 5.17) for PGT compared to 1.31 months (0.10 – 11.57) for UGT or reirradiation. Patients receiving PGT following initial progression had a significant improvement in PFS and OS (Figure 7). Median overall survival was 15.3 months in the PGT cohort versus 4.3 months in the UGT/re-irradiation-alone cohort (p=0.007). On multivariate analysis of this cohort, while *TP53*-mutant status was associated with increased risk of death, PGT and reirradiation both remained associated with improved survival (Table 3).

Discussion

H3K27-altered DMG remains an incurable disease despite the testing of many innovative approaches. While precision medicine studies have previously been conducted in this tumour type, there are limited published reports on the role of PGT in DMG from large precision medicine platforms. The INFORM study has reported on the feasibility of PGT in a smaller cohort of DMGs but has not reported on treatment outcome[19]. The MATCH trial excluded CNS tumours while the MAPPYACTS trial did not specifically report DMG separate to HGG, nor report survival outcome[20,21]. This study is the first to demonstrate the potential clinical benefit of PGT in H3K27-altered DMG. Patients who received PGT following initial progression had both an improved PFS and OS when compared to patients treated with the current standard of care (re-irradiation or no treatment) and/or UGT. This benefit was seen regardless of tumour location. There was a trend towards improved survival outcomes for use of PGT across the entire cohort that did not reach statistical significance and, whilst we are cautious in drawing definitive conclusions given the small cohort, clinical benefit was demonstrated through objective responses to PGT on centrally reviewed imaging, and the impact on survival was shown in a multivariate analysis. Importantly, our data also supports the feasibility of this approach in patients with H3K27-altered DMG and highlights additional benefits of comprehensive molecular analysis.

Comprehensive molecular profiling was successfully performed in the majority of patients. WGS and RNAseq were successfully performed in 85% of the cohort, despite this being a national study incorporating centres with varying case volume, and a tumour type where tissue sample is often limited due to the biopsy size. Results were delivered within a clinically meaningful timeframe, with a mean time of six weeks from enrolment to presentation at the national MTB. Notably, 74% of patients in the cohort were able to receive a PGT recommendation based on their molecular profiling, highlighting the practical potential of incorporating precision medicine into the treatment of H3K27-altered DMG. Nearly half of the patients who received a recommendation proceeded to treatment with PGT, underscoring the clinical applicability of this approach. The OCB of 52% for those receiving PGT reflects a meaningful response, especially considering the historically poor prognosis of these patients. We observed that PGT targeting SNVs and Tier 1 or 2 recommendations were associated with higher OCB rate. Whilst not statistically significant in this cohort, this finding aligns with our previously published report in paediatric high-risk cancers[12]. This is unsurprising, as higher Tier recommendations are derived from clinical evidence in the same or comparable tumour types, rather than pre-clinical data. However, this association was not universal across drug targets and PGTs. Such variability may reflect both intra- and intertumoral heterogeneity, as well as differences in pharmacodynamics and pharmacokinetics across agents. Understanding these factors remains an active area of investigation to optimise the efficacy of PGT.

Another notable finding was demonstrating the benefit of a comprehensive molecular approach. WGS identified mutational signatures (e.g. homologous recombination) and complex rearrangements that may not have been identified by panel sequencing for 7 patients (11% of patients that underwent WGS). These findings are particularly notable as they offered or supported therapeutic targets. The value of RNAseq and WGS over panel sequencing alone was demonstrated by the fact that 3 patients had OCB after being treated with PGT that were identified through the combination of RNAseq and WGS and would not have been recommended by a panel-based approach. Further work is still required in evaluating the broader utility of differential gene expression in isolation.

Beyond the immediate clinical benefit of PGT, the study also reinforces the advantages of a comprehensive molecular profiling approach. The diagnostic testing enabled by molecular profiling led to a revised diagnosis in 11 patients. This included seven patients (who all had typical imaging appearances of DMG) who were found to have alternate diagnoses, some of whom had highly targetable tumour drivers. In addition, four patients were identified as having H3K27-altered DMG that had not been appreciated through clinical testing. Together with the potential for identifying PGT, these results suggest that biopsy should be considered for all DMG patients.

While the study's results are promising, several limitations must be considered. The sample size of patients receiving PGT was relatively small, which limited the power of the subgroup analysis. This is particularly important when assessing the impact of PGT on clinical outcomes, as a larger cohort may yield more definitive conclusions. Additionally, the heterogeneity in the implementation of PGT and response assessments complicates the interpretation of the results. These factors may have contributed to the lack of clear benefit in patients who received PGT at initial diagnosis and cautions against the interpretation in the role or efficacy of PGT at a specific timepoint. The improvement in survival only reached statistical significance in the group with progressive disease. This likely relates to the fact that this is a more uniform group of patients, with shorter survival and who all received PGT treatment at a similar state of disease. Notably, four of nine patients in the progression cohort had a Tier 1 recommendation compared to only one of nine patients in the upfront cohort. Fewer types of PGT were used in the upfront cohort (n=4) compared with the progression cohort (n=9), which also included a greater number of combination therapies. Previous evidence in high-risk paediatric cancers has shown that PGT in upfront therapy is a favourable prognostic factor[12]. The response rates of PGT are also confounded by the use of radiation therapy, as many patients received radiation or re-irradiation close to the time at which they were treated with PGT. Although we attempted to address this by comparing PGT outcomes with those of patients receiving UGT and re-irradiation alone and by performing multivariate analysis, the impact of radiation therapy on survival and progression cannot be fully excluded. This remains a challenge in examining the impact of novel therapies for this patient group affected by a rare tumour and future studies with larger cohorts and more standardized treatment regimens and response assessments are needed to better define the role of PGT and its optimal timing. Umbrella trials informed by a precision medicine pipeline are a possible approach.

The varied response to PGT, irrespective of recommendation tier and targeted alteration type highlights the complexity of applying molecular profiling to guide therapy. Not all molecular alterations lead to actionable therapeutic targets, and the challenge remains to identify those alterations that truly drive tumourigenesis and which can be effectively targeted with existing or emerging therapies. Serial sequencing of H3K27-altered tumours has shown that these tumours gain additional mutations and varied expression profiles over the course of the disease, including following PGT treatment. This highlights the need for combination therapies and a refined approach in selecting targets and PGT[22]. It also raises the role of serial sample collection, including liquid biopsy, to assess treatment response and evolution of tumour biology. This evolution was seen in our cohort and highlights how PGT recommendations are also able to evolve with time, though this was not tested in our cohort. The blood-brain barrier remains an obstacle for the delivery of many targeted agents, and addressing this issue will be critical in enhancing the effectiveness of these therapies.

Lastly in approximately a quarter of our patients (n=18, 26%), no PGT recommendation was able to be made. For 11 patients the only clinically relevant molecular aberrations identified were H3K27M mutations, with or without TP53 mutation. This highlights a barrier in the use of PGT in a subset of patients due to the lack of currently druggable targets and reinforces the urgent need to identify new molecular targets and to develop novel effective targeted therapies.

In conclusion, precision guided approaches have the potential to improve outcomes for patients with H3K27-altered DMG. In depth molecular profiling is feasible for most patients, can change the diagnosis in a substantial proportion of patients, and in the majority may identify potential targeted treatment strategies. The use of PGT can lead to clinical benefit, including objective responses and improved survival. Further studies are needed to confirm these findings, refine therapeutic approaches, and better define the optimal timing for PGT administration. Ultimately the use of combination precision guided therapies incorporated into comprehensive treatment protocols may help further improve outcomes and ultimately lead to sustained clinical benefit.

Methods

Selection and description of participants

The PRISM trial (ClinicalTrials.gov registration: NCT03336931) was a multicentre prospective observational cohort study conducted by the Australian ZERO Childhood Cancer Precision Medicine Program. Patients were enrolled from a total of nine paediatric oncology centres in Australia. All clinical data were collected by designated clinical research associates and clinicians based at each hospital. The primary objective was to determine the proportion of patients for whom PGT could be recommended to the treating physician using a comprehensive precision medicine platform within a clinically relevant time frame. Secondary and tertiary objectives included evaluating the treatment response in patients who had received a PGT and the difference in survival between patients receiving PGT and non-PGT. Patients with a diagnosis of H3K27-altered DMG were enrolled on the PRISM trial from September 2017 to July 2023, data cutoff was set at December 31, 2023. Patients were included if they met the diagnostic criteria for H3K27-altered DMG as per the World Health Organisation (WHO) 2021 classification.

Study oversight

The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki and approved by the Hunter New England Human Research Ethics Committee of the Hunter New England Local Health District in Australia (reference no. 2019/ ETH00701). Written informed consent for all patients in this study were provided either by the parent or legal guardian for patients younger than 18 years or by patients older than 18 years. There was no participant compensation.

Patients and tumour samples

Patients younger than 21 years with suspected or confirmed diagnosis of a high-risk malignancy at diagnosis or relapse or refractory, defined as an estimated probability of cure lower than 30%, could be consented and registered on the study. Patients older than 22 years with high-risk paediatric-type cancers could also be registered on approval from the study chair. The sex of a patient was either reported by the guardian or parent or self-reported. No gender information was collected. Sex was not considered in the study design. After trial registration, patient samples were delivered to the central laboratory at the Children's Cancer Institute (CCI) (Sydney) for processing.

A patient was deemed eligible for enrollment when all criteria were satisfied: confirmed high-risk cancer; both tumour and germline samples received at CCI; and sufficient DNA could be extracted for sequencing. High-risk cancer (estimated cure rate of less than 30% based on the published literature) was confirmed by central review of clinical history, histopathology and imaging. Tumor tissue was fresh, snap-frozen or cryopreserved on receipt. An FFPE tumour sample was accepted with previous approval from the study chair if this was the only sample available. Clinical and demographic data at registration and follow-up were entered into the Labmatrix by Biofortis v.R7 3.2.0 laboratory information management system. Molecular profiling WGS (paired tumor-germline) was conducted for all patients, except when there was insufficient tumor DNA for WGS or only an FFPE tumour sample was available. Targeted panel DNA sequencing was performed for these patients (TruSight Oncology 500, Illumina). Whole transcriptome sequencing was conducted in non-FFPE tumour samples whenever RNA of adequate quantity and quality was available. DNA methylation analysis was performed on the Illumina EPIC array and all methylation samples were processed through the Molecular Neuropathology CNS classifier (DKFZ Brain tumour classifier version 12.5). The analytical pipelines for molecular profiling and variant curation for WGS, WTS and methylation and targeted panel sequencing, have been described previously[15].

MTB and PGT recommendations

Patients with molecular alterations that were potentially targetable or could lead to a change or refinement of diagnosis, and patients with reportable germline variants, were presented in the national MTB meeting held fortnightly. The MTB meetings were attended by oncologists, pathologists, clinical geneticists, genetic counsellors, basic scientists, bioinformaticians and study managers. The treating oncologist for the patient being discussed was invited to attend and provide a clinical update to facilitate MTB discussion. Cases for MTB presentation were prepared jointly by the clinical team (consisting of two molecular oncologists and oncology fellows) and the curation scientist team. The clinical team researched and reviewed evidence to support therapeutic options and presented reportable molecular findings and therapeutic options at the MTB. A five-tier system was used to assign the strength of PGT recommendations. Tier 1 referred to evidence from clinical studies of the same cancer type and tier 2 from clinical studies of different cancer types. Patients from these clinical studies can be molecularly selected or unselected. The evidence from tiers 3 and 4 was based on preclinical evidence in the same and different cancer type, respectively. Preclinical models in these studies can be molecularly selected or unselected. Tier 5 was based on the consensus opinion of the MTB. The final MTB report was generated after the meeting. The treating oncologist made the final treatment decision, in consultation with the family and patient, including consideration for treatments other than the MTB recommendations.

Treatment and outcome data analysis

Patients who died between registration and MTB presentation were excluded from the outcome analyses. All treatments (including standard of care and experimental treatments) and response to treatment were recorded prospectively in Labmatrix. Receipt of a PGT was defined as the patient having received at least one dose of a drug in the same therapeutic class as the PGT recommendation. A treatment was included for the treatment outcome analysis when all three criteria were met: treatment duration lasting 4 weeks or longer; no disease progression within the first 4 weeks of treatment; and treatment response evaluation being available. These criteria were also applied to treatment outcome analysis of UGT. Treatment response was evaluated using the RAPNO DIPG criteria for pontine tumours and RAPNO HGG criteria for non-pontine tumours. To meet the criteria for SD, measurements must have met the SD criteria at a minimum interval of 8 weeks after commencing a treatment. Treatment response evaluation was conducted by central review of the imaging report and images by two neuro-oncologists and a neuro-radiologist. Measurable diseases were evaluated for CR, PR, MR, SD and PD as defined by the relevant RAPNO criteria. Measurable disease refers to lesions 10 mm or larger by magnetic resonance imaging and that can be accurately measured. Evaluable but non-measurable disease refers to lesions smaller than 10 mm or other sites of disease that cannot be accurately measured. The OR for measurable disease was defined as CR or PR as the best response. OCB was defined as CR, PR or SD lasting for 24 weeks or longer in measurable disease. Each eligible treatment was evaluated for PFS, defined as the time from the start of that specific treatment to disease progression for that treatment, or death from any cause, whichever occurred first. OS for individual patients in the entire cohort was defined as the time from diagnosis to death from any cause. For OS comparison between PGT and non-PGT, patients were categorized according to whether they received PGT treatment at any time point from diagnosis and was defined as the time from diagnosis to death from any cause. Patients that discontinued PGT within 0-4 weeks of initiation were excluded from analysis due to inadequate evaluation of treatment response. For OS comparison following initial progression, patients were assigned according to the first treatment they received prior to any further progression.

Statistics

The Kaplan-Meier method was used to analyse survival (OS and PFS) while comparisons were performed using the log-rank test. Multivariate survival analysis was performed using Cox proportional hazards regression analysis. Proportions were compared using a chi-squared test. A two-tailed $P \leq 0.05$ was considered statistically significant. Statistical analyses were performed using GraphPad Prism 10.

Data Availability

The WGS, RNAseq and DNA methylation data generated in this study have been deposited in the European Genome-phenome Archive (EGA) under ascension numbers EGAS00001007029 [https://ega-archive.org/studies/EGAS00001007029], EGAS00001004572 [https://ega-archive.org/studies/EGAS00001004572]. Access to and use of these data for research purposes is possible through an application to the ZERO Data Access Committee (ZERO DAC). Data access is restricted to protect the privacy and confidentiality of research participants. All submissions and enquiries should be addressed to the ZERO DAC via zeroDAC@ccia.org.au. The named investigator of each application will be notified in writing the outcome of their application within 4 weeks. The clinical data generated in this study are provided in the supplementary information/ source data file.

Code Availability

The code used for the molecular analysis has previously been reported and is available at https://github.com/CCICB/2020-hrPC-landscape

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Acknowledgements

D.S.Z. thanks A. Seifman, E. Jacobson and S. Masnick for their support and guidance. We thank patients, parents and clinicians at participating centres, Australian and New Zealand Children's Haematology/Oncology Group (ANZCHOG) for study sponsorship, the member biobanks of the ANZCHOG Biobanking Network, and staff of the CCI for their work. We thank the staff at Kinghorn Centre for Clinical Genomics, and the Garvan Institute for WGS and panel sequencing. We also thank Murdoch Children's Research Institute/Victorian Clinical Genetics Services and Australian Genome Research Facility for processing WGS, RNA-seq and methylation. We thank the Australian Government for National Computational Infrastructure and DNAnexus for data processing. The author(s) would also like to acknowledge Luminesce Alliance - Innovation for Children's Health for its contribution and support. Luminesce Alliance - Innovation for Children's Health, is a not for profit cooperative joint venture between the Sydney Children's Hospitals Network, the Children's Medical Research Institute, and the CCI. It has been established with the support of the NSW Government to coordinate and integrate paediatric research. Luminesce Alliance is also affiliated with the University of Sydney and the University of New South Wales Sydney. We thank J. Collins for project governance and advocacy. ZERO Childhood Cancer is a joint initiative led by the Children's Cancer Institute and Sydney Children's Hospital, Randwick.

Funding

D.S.Z. is funded by a National Health and Medical Research Council (NHMRC) Synergy Grant (no. APP2019056) an NHMRC Leadership Grant (no. APP2017898) and a Cancer Institute New South Wales (CINSW) Program Grant (no. TPG2037). M.C. is funded by the Priority-Driven Collaborative Cancer Research Scheme (no. APP1165556) and a CINSW Program Grant (no. 2021/TPG2112). G.M.M. and P.G.E. are supported by The Steven Walter Children's Cancer

Foundation and The Hyundai Help 4 Kids Foundation. M.C., M.K.M. and E.M.D. also received funding from the MRFF Emerging Priorities and Consumer Driven Research Scheme and the Australian Brain Cancer Mission/ NHMRC/ Lifting Clinical Trials and Registry Capacity (NHMRC MRF9500002). Funding was provided by: Australian Federal Government Department of Health; NSW Government; Australian Cancer Research Foundation; Kids Cancer Alliance; Cancer Therapeutics Cooperative Research Centre; Tour de Cure; Cure Brain Cancer Foundation; Samuel Nissen Charitable Foundation; the Lions Kids Cancer Genome Project, a joint initiative of Lions International Foundation; the Australian Lions Children's Cancer Research Foundation; Luminesce Alliance, Minderoo Foundation; ZERO Capacity Campaign; Kids Cancer Project; UNSW Sydney; W. Peters; Australian Genomics Health Alliance and Kinghorn Foundation.

Author contributions

M.H., G.M.M., M.C., P.G.E., T.N.T., and D.S.Z. conceived ZERO. V.T. is program manager of ZERO. C. McKay, P.P., S.J., S.S., J.S., S.T., N.Z., L.M.S.L., collected the clinical data. D.G., F.A., P.D., J.R.H., T.H., S.V., M.K.M, D.-A.K.-Q. were clinical leads at recruiting centres. A.J.G. led the pathology analysis. N.M., D.-A.K.-Q., C.Mignone, performed central review of imaging. C. Mayoh, M.W, M.C. performed computational genomics analysis. L.M.S.L., P.B., G.T., M.T., E.M.D. analysed the molecular findings. C.McKay wrote the original draft of the manuscript. N.M., D.S.Z., D.-A.K.-Q conceived, designed and edited the manuscript, with contributions from all authors.

Competing interests

D.S.Z. reports consulting / advisory board fees from Medison Pharma, Amgen, Novartis and Roche and research support from Accendatech. The remaining authors declare no competing interests.

Tables

Table 1.

Characteristics	**Number of patients (%)**
Number of patients	68
Mean age at diagnosis (years)	8.8 (± 5.4)
Sex	
Male	30 (44%)
Female	38 (56%)
Status at enrolment	
Diagnosis	61 (90%)
Post radiation	1 (1%)
Progression/ relapse	6 (9%)
Tumour location	
Pons	45 (66%)
Thalamus	17 (25%)
Spine	4 (6%)
Other	2 (3%)
Stage at diagnosis (radiological)	
Localised	67 (99%)
Metastatic	1 (1%)

Baseline clinical characteristics of patients with H3K27-altered diffuse midline glioma (DMG) enrolled on PRISM

Table 2.

Characteristics	**PGT cohort**	**Non-PGT cohort**	**p-value**
	No. of patients (%)	No. of patients (%)	
No. of patients	18	45	
Mean age at diagnosis (years)	9.6 (±4.9)	8.3 (±4.7)	p 0.26
Sex			
Male	7 (39%)	22 (49%)	
Female	11 (61%)	23 (51%)	p 0.58
Status at enrolment			
Diagnosis	16 (89%)	41 (91%)	
Post-radiation	0	1 (2%)	
Progression/ relapse	2 (11%)	3 (7%)	p 0.78

Tumour location			
Pons	12 (67%)	31 (69%)	
Thalamus	5 (28%)	10 (22%)	
Spine	1 (6%)	3 (7%)	
Other	0	1 (2%)	p 0.85
Stage at diagnosis (radiological)			
Localised	18 (100%)	45 (100%)	
Metastatic	0	0	p >0.99
H3K27me3			
H3-3A	12 (67%)	32 (71%)	
H3C2/H3C3/H3C14	5 (28%)	8 (18%)	
Wild-type	1 (6%)	5 (11%)	p 0.66
Other mutations			
TP53-mut	6 (33%)	22 (49%)	p 0.26
BRAF/FGFR1-mut	3 (17%)	3 (7%)	p 0.18

Baseline clinical and molecular characteristics of patients who received precision-guided therapy (PGT) at any time-point (PGT cohort) compared to patients who did not (non-PGT cohort). Abbreviations: No., number; RT, radiation-therapy; Wild-type, H3K27M wild-type.

Table 3.

Variable	**Hazard Ratio (95% CI)**	**p-value**
Age >12 years	0.64 (0.16 – 2.19)	0.491
Pontine tumor	4.16 (1.13 – 17.94)	0.031
Sub-total resection (versus biopsy)	3.14 (0.63 – 14.71)	0.159
H3-3A-mutant	2.22 (0.44 – 15.96)	0.355
TP53-mutant	4.19 (1.21 – 14.86)	0.025
BRAF/*FGFR1*-mutant	0.82 (0.11 – 5.90)	0.841
Radiation (versus no reirradiation)	0.08 (0.01 – 0.40)	0.002
PGT (versus reirradiation alone)	0.02 (0.00 – 0.35)	0.006
UGT (versus reirradiation alone)	0.37 (0.07 – 1.86)	0.227

Cox proportional hazards regression of overall survival for patients treated following progression (n=30).

Abbreviations: PGT, precision-guided therapy; UGT, unguided therapy. P-values are two-sided.

Figure Legends/ Captions

Figure 1. Flow-chart of the study. Flow-chart describing the number of patients with suspected diagnosis of diffuse midline glioma (DMG) enrolled on PRISM and additional patients with H3K27-altered DMG diagnosed following PRISM analysis, culminating in **PGT-cohort** (n=18) and **non-PGT cohort** (n=45). Abbreviations: WGS, whole genome sequencing; RNAseq, RNA sequencing (whole transcriptome sequencing); TSO500, TruSight Oncology 500 DNA panel; PGT, precision guided therapy. *Ineligible due to PGT received for less than 4 weeks.

Figure 2. Molecular profile of the H3K27M-altered diffuse midline glioma (DMG) cohort. Figure 2a. t-SNE plot of DNA methylation profiles of H3K27-altered DMG cohort compared to other central nervous system (CNS) tumours from ZERO-PRISM cohort. Figure 2b. Oncoplot depicting individual molecular profile for each patient with a diagnosis of H3K27-altered DMG enrolled on PRISM. Each vertical line represents a patient and each horizontal line represents a single gene, while different colors indicate the type of mutation. Gender, age and tumour location for each patient are shown above the plot. Color legends are depicted on the right side of the figure. Superior X-axis, mutation number per patient. Left Y-axis, right y-axis, percentage and name of relevant mutation respectively. Plot characterised by H3-mutation type, with common driver mutations and germline mutations shown. Abbreviations: ATRT, atypical teratoid rhabdoid tumour; DMG, diffuse midline glioma; EPN-PFA, ependymoma posterior fossa type A; EPN-PFB ependymoma posterior fossa type B; EPN-ZFTA ependymoma with ZFTA fusion; ETMR, embryonal tumour with multilayered rosettes; HGG, high-grade glioma not otherwise classified; LGG, low grade glioma; MB-non WNT/non-SHH, medulloblastoma group 3/group 4; MB-SHH, medulloblastoma sonic hedgehog; MB-WNT, medulloblastoma wingless-related integration site.

Figure 3. Summary of drug target recommendations made by the ZERO molecular tumour board for patients with H3K27-altered diffuse midline glioma (DMG). The stacked bars show the total number of patients for whom the drug target was recommended. Patients receiving a PGT recommendation are in grey and patients treated with the recommended PGT are in black. Abbreviation: PGT, precision guided therapy; ICI, immune checkpoint inhibitor. Source data are provided as a Source Data file.

Figure 4. Waterfall plot for each episode of precision guided therapy (PGT) received in patients with measurable disease. Each bar represents a patient with study ID and tumour location (blue for pons and yellow for thalamus) beneath the bars. Plot is separated by whether PGT was given as part of upfront treatment or following initial progression. Treatment response was evaluated using RAPNO DIPG for pontine tumors and RAPNO HGG for non-pontine tumors. The dotted lines at 25%, -25%, -50% and -100% delineate the category of response (RAPNO DIPG: ≥25% PD, 25 to -25% SD, -25 to -100% PR) (RAPNO HGG: ≥25% PD, 25 to -25% SD, -25 to -50% ^MR, -50 to -100% ^PR). The color legends indicating the treatment received are depicted in the right side of the figure. *Radiation/reirradiation not given within 12 weeks of PGT commencement. Source data are provided as a Source Data file.

Figure 5. Swimmer plot of each patient who received precision guided therapy (PGT). Each bar represents a patient with study ID and tumour location (blue for pons and yellow for thalamus). PGT is described in black, other non-PGT combined treatment in grey, molecular alteration in red (SNV), green (increased RNA expression), and brown (CNV). Event legends (PGT start and treatment duration, tumour response, or radiation therapy) are depicted on the right side of the figure. Abbreviations: PGT, precision guided therapy; PR, partial response; SD, stable disease; PD, progressive disease; Alive, alive at time of data cutoff; RT, radiation therapy; SNV, single nucleotide variant; CNV, copy number variant. Source data are provided as a Source Data file.

Figure 6. Overall survival of patients treated with PGT. Overall survival (OS) of patients who received precision guided therapy (PGT) at any time-point (PGT cohort in black) versus those that did not (non-PGT cohort in red). Median OS 21.3 months for PGT cohort compared to 12.1 months for non-PGT cohort. Hazard ratio 0.74; 95% CI (0.41 – 1.34). The Kaplan-Meier survival curves were compared using a two-sided log-rank test. Source data are provided as a Source Data file.

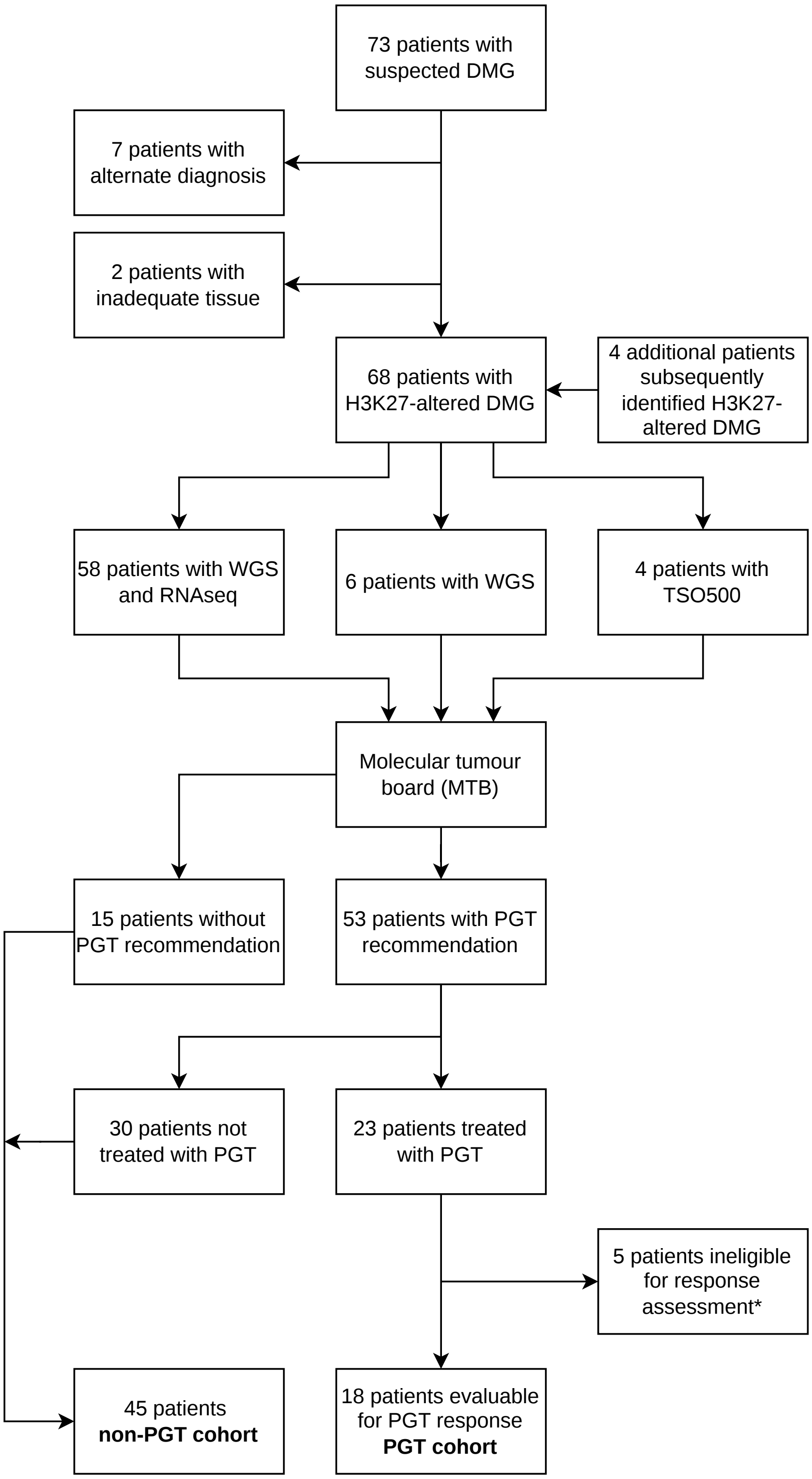
Figure 7. Superior clinical outcome of patients treated with PGT after initial progression a. Overall survival (OS) of patients following initial progression who received PGT +/- reirradiation in black (n=4/9 received reirradiation) versus UGT +/- reirradiation in red (n=3/8 received reirradiation) versus

reirradiation alone in green. Figure 7b. Secondary progression free survival (PFS) of patients who received PGT +/- reirradiation in black versus UGT +/- reirradiation in red versus reirradiation alone in green after initial progression. The Kaplan-Meier survival curves were compared using a two-sided log-rank test. Source data are provided as a Source Data file.

Editorial Summary

H3K27-altered diffuse midline gliomas are a highly aggressive form of paediatric brain cancer. Here, the authors present molecular profiling of 68 patients, and show the efficacy of precision-guided therapy based on this profiling.

Peer Review Information: *Nature Communications* thanks Amanda Saratsis and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. A peer review file is available.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2 patients with inadequate tissue
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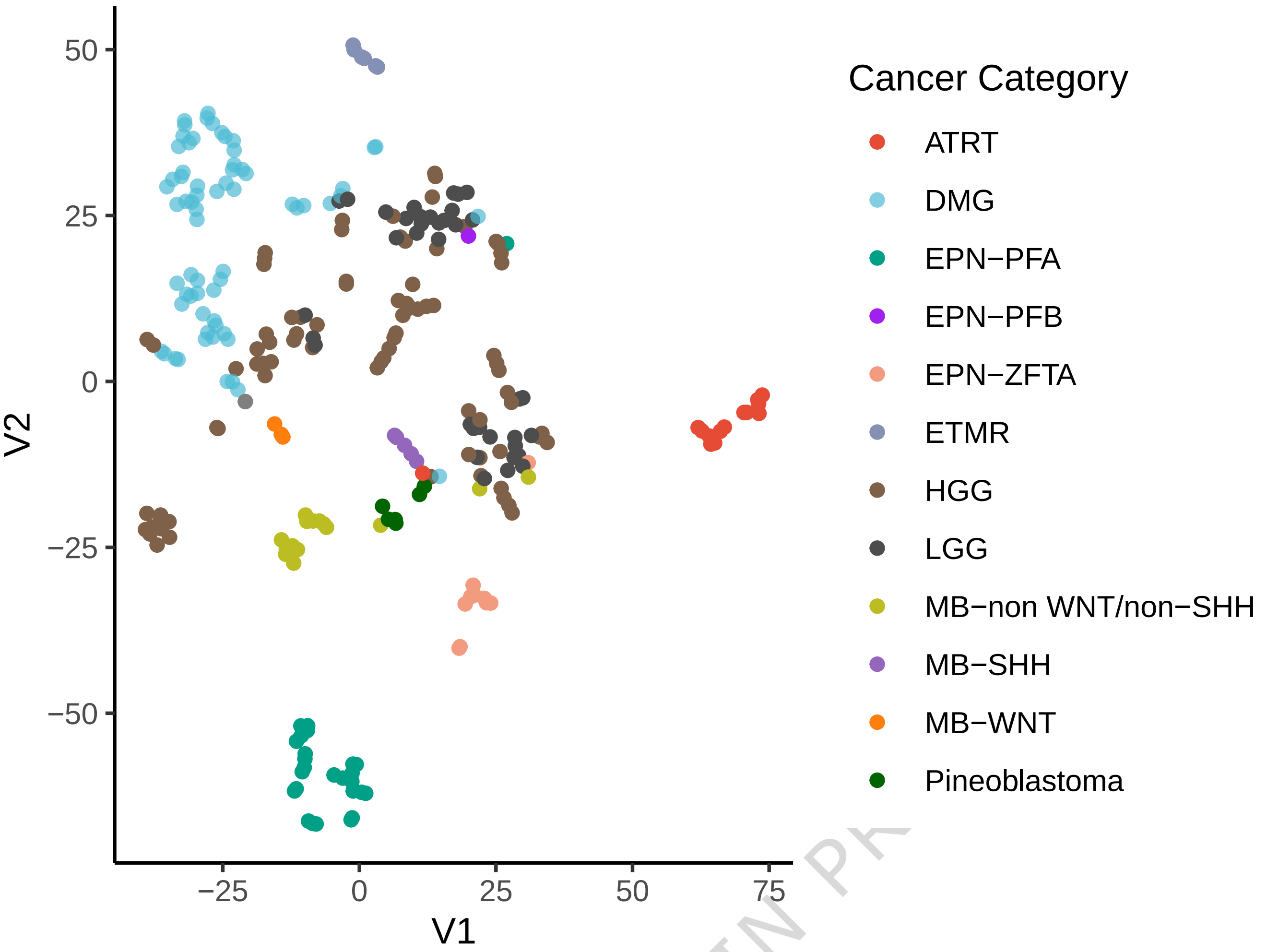
4 additional patients subsequently identified H3K27-altered DMG
58 patients with WGS and RNAseq
6 patients with WGS
4 patients with TSO500
Molecular tumour board (MTB)
15 patients without PGT recommendation
53 patients with PGT recommendation
30 patients not treated with PGT
23 patients treated with PGT
5 patients ineligible for response assessment*
45 patients **non-PGT cohort**
18 patients evaluable for PGT response **PGT cohort**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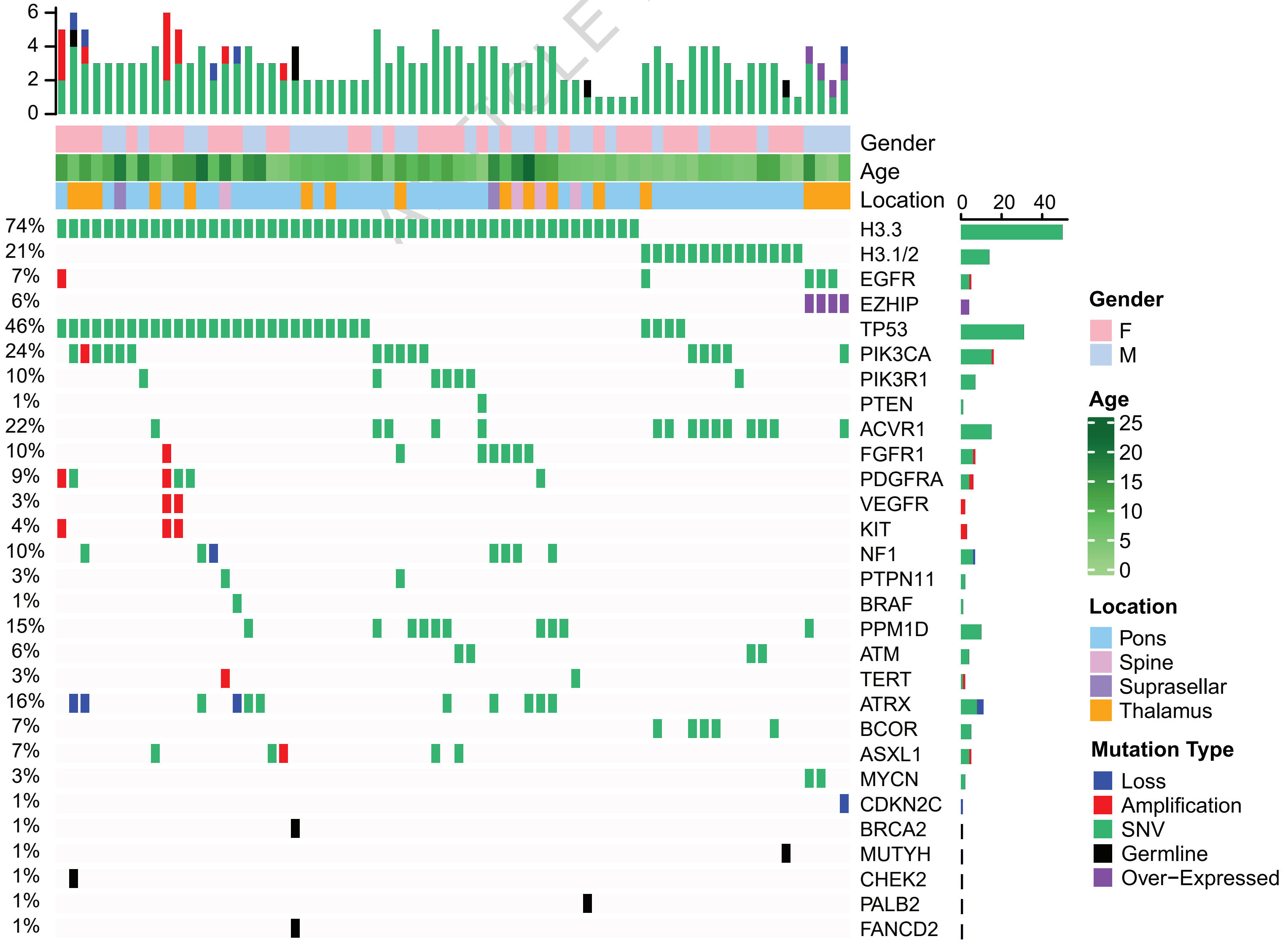
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10% PIK3R1
1% PTEN
22% ACVR1
10% FGFR1
9% PDGFRA
3% VEGFR
4% KIT
10% NF1
3% PTPN11
1% BRAF
15% PPM1D
6% ATM
3% TERT
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1% CHEK2
1% PALB2
1% FANCD2
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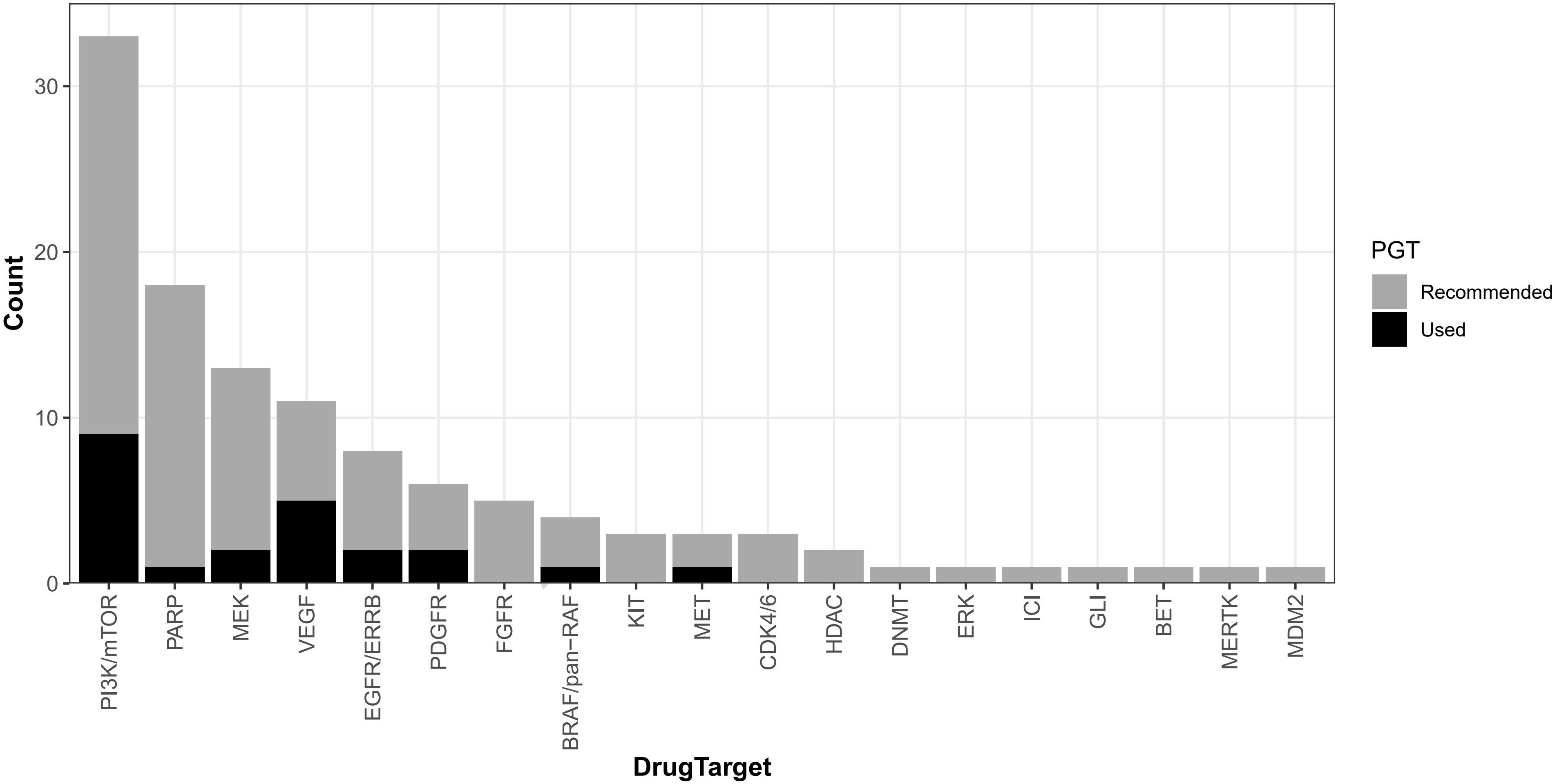
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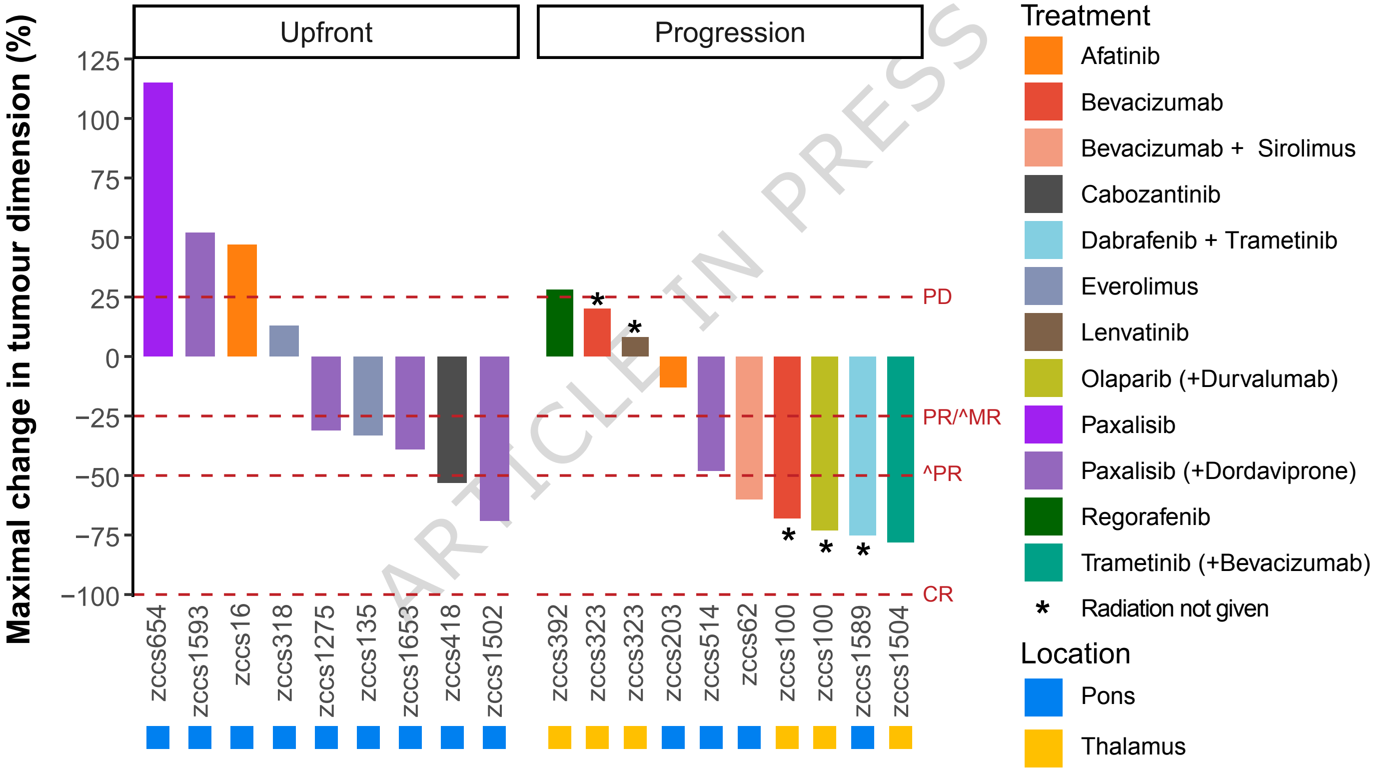
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Upfront
Progression
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100
75
50
25
0
−25
−50
−75
−100
PD
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^PR
CR
*
zccs654
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Treatment
Afatinib
Bevacizumab
Bevacizumab + Sirolimus
Cabozantinib
Dabrafenib + Trametinib
Everolimus
Lenvatinib
Olaparib (+Durvalumab)
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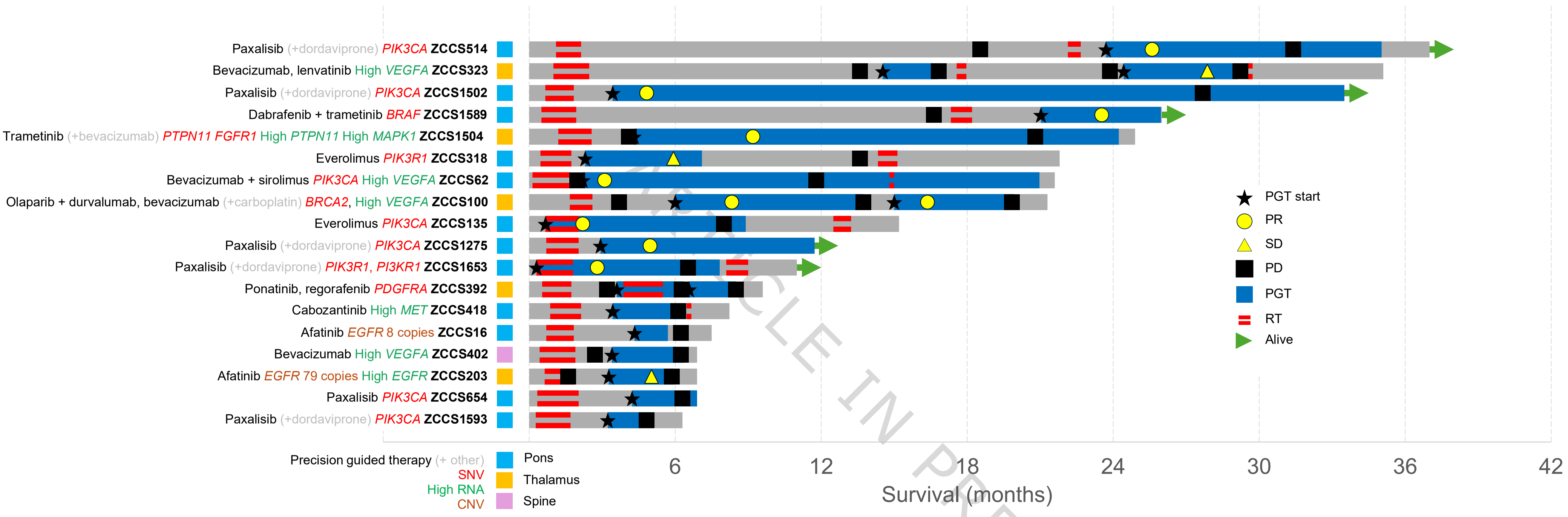
Location
Pons
Thalamus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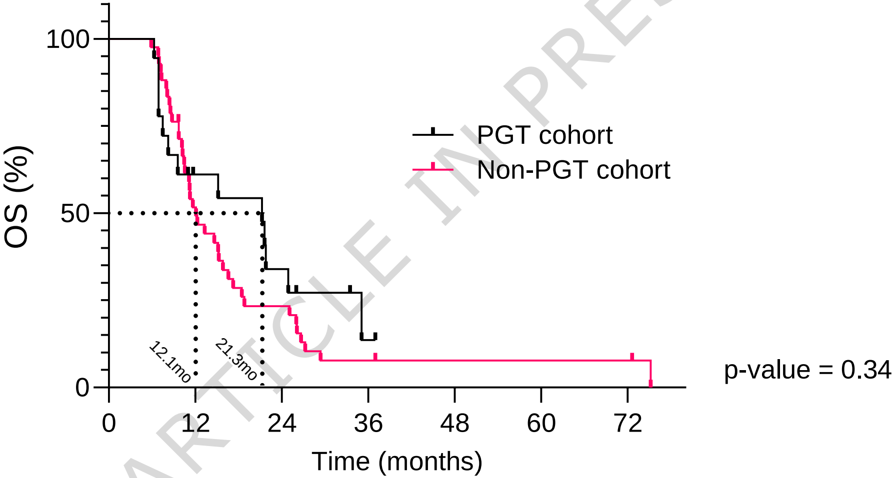
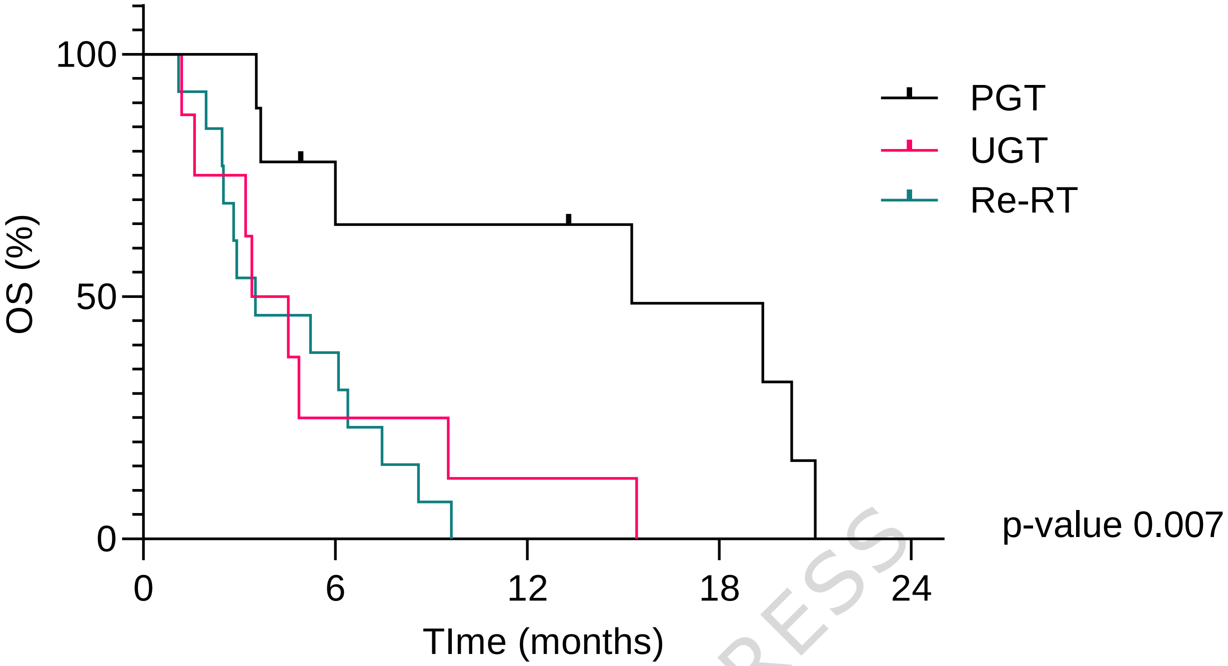
Bevacizumab, lenvatinib High VEGFA ZCCS323
Paxalisib (+dordaviprone) PIK3CA ZCCS1502
Dabrafenib + trametinib BRAF ZCCS1589
Trametinib (+bevacizumab) PTPN11 FGFR1 High PTPN11 High MAPK1 ZCCS1504
Everolimus PIK3R1 ZCCS318
Bevacizumab + sirolimus PIK3CA High VEGFA ZCCS62
Olaparib + durvalumab, bevacizumab (+carboplatin) BRCA2, High VEGFA ZCCS100
Everolimus PIK3CA ZCCS135
Paxalisib (+dordaviprone) PIK3CA ZCCS1275
Paxalisib (+dordaviprone) PIK3R1, PI3KR1 ZCCS1653
Ponatinib, regorafenib PDGFRA ZCCS392
Cabozantinib High MET ZCCS418
Afatinib EGFR 8 copies ZCCS16
Bevacizumab High VEGFA ZCCS402
Afatinib EGFR 79 copies High EGFR ZCCS203
Paxalisib PIK3CA ZCCS654
Paxalisib (+dordaviprone) PIK3CA ZCCS1593
Precision guided therapy (+ other)
SNV
High RNA
CNV
Pons
Thalamus
Spine
6
12
18
24
30
36
42
Survival (months)
PGT start
PR
SD
PD
PGT
RT
Alive



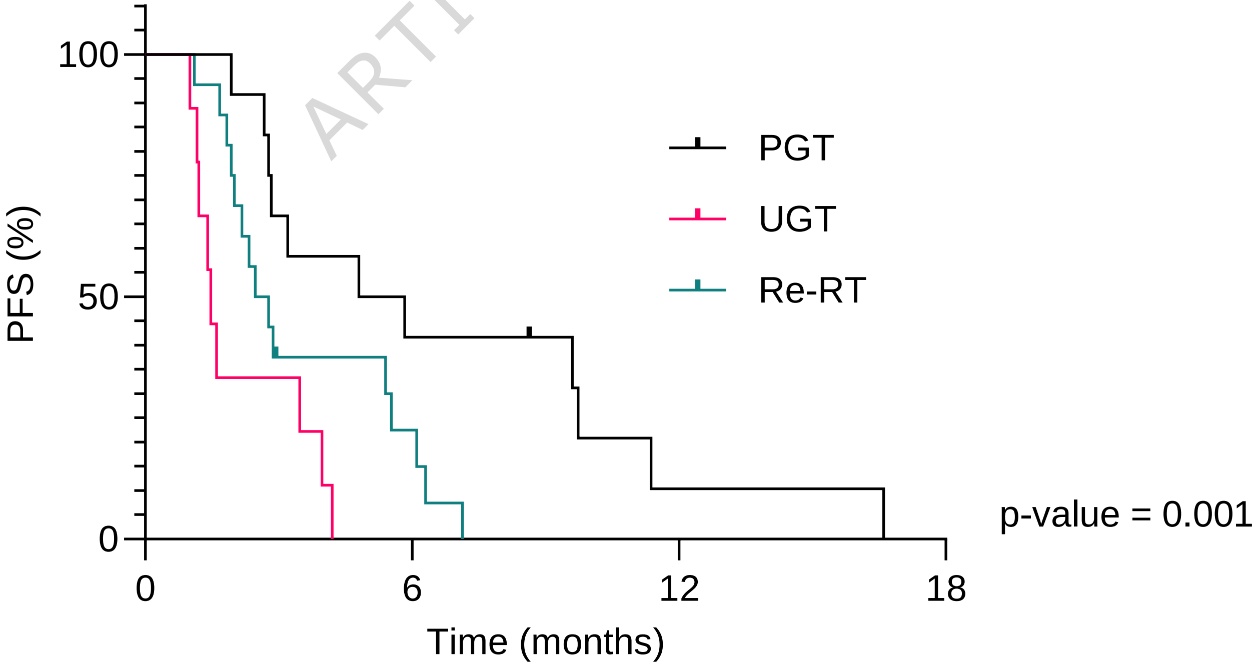
Number at risk

PGT	18	9	5	1	0	0	0
Non-PGT	42	21	9	3	2	2	2



Number at risk

PGT	9	5	5	3
UGT	8	2	1	0
Re-RT	13	5	0	0



Number at risk

PGT	12	5	1
UGT	9	0	0
Re-RT	15	2	0