Acta Neuropathologica Communications

CASE REPORT

Open Access

An exceptionally rare case of a diffuse midline glioma with concomitant H3.1 K27M and G34R mutations in the *HIST1H3C (H3C3)* gene

Zita Reisz[1], Rita Pereira[2], Smitha Nevis[1], Alan Mackay[2], Leena Bhaw[1], Yura Grabovska[2], Ross Laxton[1], Valeria Molinari[2], Anna Burford[2], Barnaby Clark[3], Cristina Bleil[4], Bassel Zebian[4], Erika Pace[5], Annette Weiser[6], Fernando Carceller[6,7], Lynley Marshall[6,7,8], Andrew King[1], Istvan Bodi[1], Safa Al-Sarraj[1†], Chris Jones[2†] and Matthew Clarke[2*†]

Abstract

Histone mutations (H3 K27M, H3 G34R/V) are molecular features defining subtypes of paediatric-type diffuse high-grade gliomas (HGG) (diffuse midline glioma (DMG), H3 K27-altered, diffuse hemispheric glioma (DHG), H3 G34-mutant). The WHO classification recognises in exceptional cases, these mutations co-occur. We report one such case of a 2-year-old female presenting with neurological symptoms; MRI imaging identified a brainstem lesion which was biopsied. Histology showed diffusely infiltrating pleomorphic astrocytes, multinucleated cells, and conspicuous mitotic activity; the diagnosis was DMG, H3 K27-altered (immunohistochemistry: H3K27me3 loss, H3K27M positivity). DNA methylation profiling (Illumina EPIC BeadArrays, brain tumour classifier (MNP v12.5 R package)) classified the tumour as 'DMG, H3 K27-altered' (calibrated score = 0.99). Further molecular studies (whole exome, whole genome sequencing) revealed concurrent H3.1 K27M and G34R mutations (clonal, in the same reads) of *H3C3*, *FGF11* and *PIK3CA* somatic variants, and a pathogenic germline *NBN* variant. The RNAseq profile clustered with H3K27M-mutant tumours. A patient-derived cell culture was established enabling unbiased in vitro drug screening; no selective sensitivities were identified. Chromatin immunoprecipitation assays with sequencing (ChIP-seq; H3K27ac, H3K27me3, H3K36me3, RNApol2 marks) showed features in keeping with DMG H3 K27M-mutant tumours (H3K27ac loci including *OLIG2*, *IRX1/2*, *PKDCC*). The patient was treated with adjuvant radiotherapy, but progressed and passed away 13 months post-diagnosis. This case is an exceptionally rare, complex variant of histone-mutant paediatric HGG, illustrating that the H3.1 K27M mutation demonstrates a dominance over the molecular and clinical profiles compared to G34R, and highlights the importance of broad molecular profiling to identify such examples for further study.

Keywords Diffuse midline glioma, Histone-mutant, High-grade glioma, Rare case

†Safa Al-Sarraj, Chris Jones and Matthew Clarke are contributed equally to this work.

*Correspondence:
Matthew Clarke
matthew.clarke01@icr.ac.uk

Full list of author information is available at the end of the article

Introduction

The fifth edition of the WHO Classification of the Central Nervous System Tumours recognises two clinicopathologically distinct paediatric-type high-grade gliomas (HGG) defined by mutations of histone H3 genes [1]. Diffuse midline glioma (DMG), defined by H3 K27 alterations, are located in midline structures (including pons, thalamus, spinal cord). They are characterised by a global decrease of H3 tri-methylation (H3 p.K28me3 (p.K27me3)), associated with either H3 p.K28M (K27M) or rarely p.K28I (K27I) substitutions in histone H3.3 or H3.1/H3.2 isoforms, abnormal overexpression of EZHIP, or *EGFR* alterations [1–4]. The second subtype is the H3 G34-mutant diffuse hemispheric glioma (DHG), typically occurring in adolescents and young adults [5–7]. They contain a mutation in the *H3F3A* gene that results in an amino-acid substitution on the histone H3.3 tail at codon 35, most commonly glycine to arginine (c.103G > A or c.103G > C p.G35R - G34R) or rarely valine (c.104G > T p.G35V - G34V) [8]; these mutations have not been reported to occur in histone H3.1, unlike the K27M mutations. Each histone-mutant subgroup has a distinct methylation profile [9]. According to the WHO classification, it is exceptional for H3 K27M mutations to co-occur with H3 G34R/V mutations [1]. We report the clinicopathological and molecular characteristics of one such rare case with dual K27M and G34R mutations in the *HIST1H3C (H3C3)* gene, which has not been reported before.

Case presentation

A 2-year-old girl was admitted as an emergency case with ataxia, left-sided weakness, new-onset strabismus, and increasing lethargy. MRI head revealed an expansile ventral brainstem lesion centred on the pons, with extension into the superior aspect of the medulla and right side of midbrain (Fig. 1A). There was mass effect in the surrounding parenchyma with partial effacement of the prepontine cistern and distortion of the fourth ventricle, but no hydrocephalus. Elective stereotactic needle biopsy was undertaken (Fig. 1B).

Histology showed an infiltrative highly cellular glial tumour comprising pleomorphic and angulated nuclei, coarse chromatin, scanty eosinophilic cytoplasm and rare multinucleated cells, embedded in a fibrillary stroma (Fig. 1C). Mitotic activity was prominent, but there was no definite microvascular proliferation. There were focal areas of incipient necrosis (Fig. 1C). Immunohistochemistry showed strong immunoreactivity for OLIG2, GFAP, and H3K27M, H3K27me3 expression was lost from tumour cells and ATRX expression was retained by the tumour cells (Fig. 1D). Immunohistochemistry for the H3G34R mutation (specific to histone H3.3), EZHIP and mutant p53 was negative (Fig. 1D), with moderate-high Ki67 labelling (Fig. 1D). The immunohistochemistry profile therefore confirmed the diagnosis of diffuse midline glioma, H3 K27-altered, CNS WHO grade 4 and did not show a profile reflective of G34R/V-mutated tumours. The DKFZ Brain tumour classifiers (v12.5, v12.8) assigned the tumour to the DMG class with calibrated scores of 0.92 and 0.99 respectively.

DNA and RNA panel sequencing identified concurrent p.K28M (c.83 A > T) and p.G35R (c.103G > C, AF 0.44) mutations in the *HIST1H3C (H3C3)* gene in *cis*, and a pathogenic *PIK3CA* variant (c.1031T > G, AF 0.46) (Fig. 1F). Whole genome sequencing (WGS) identified an *FGF11* variant and a pathogenic germline *NBN* variant (c.698_701delAACA p.Lys233SerfsTer5). Copy number profiles showed gains of chr1q and chr2 (Supp. Figure 1A). The final integrated diagnosis was diffuse midline glioma, H3 K27-altered, with concomitant H3.1 K27M and H3.1 G34R mutations, CNS WHO grade 4.

At biopsy, a tumour sample was collected for in vitro model establishment. 2D and 3D models (ICR-CXJ074) were established (Fig. 2A). DNA methylation profiling (passage 5), classified all of the cultures as diffuse midline glioma with high calibrated scores (0.74 and 0.72), clustering with the DMG group in our glioma reference cohort t-SNE ($n = 3357$) (Fig. 1E). Whole exome sequencing (WES) identified the same mutations as the primary tumour including the *PIK3CA* mutation (Fig. 1F). Copy number profiling, derived from the Illumina array data, showed a gain of chromosome 1q and 2, similar to that of the primary tumour (Supp. Figure 1A). Immunofluorescence showed expression of glial markers GFAP, OLIG2, stem-cell markers SOX2 and Nestin (Fig. 2B). RNA sequencing, for both the patient-derived models and the primary tumour, confirmed an expression profile consistent with other K27M-mutant DMGs (Supp. Figure 1B, C). Having established in vitro patient-derived models representative of the primary tumour, we undertook unbiased drug screening using the ICR drugs & tools library of over 800 compounds on cells cultured in 2D; no selective sensitivities were identified (Fig. 2C).

To further characterise this tumour, chromatin immunoprecipitation assays with sequencing (ChIP-Seq) were undertaken looking at loci associated with the H3K27ac, H3K27me3, H3K36me3, RNApol2 marks, comparing with published data for H3.3 K27M- and H3.3 G34R-mutant patient-derived cell lines. The ICR-CXJ074 H3K27me3 peaks were in keeping with that of H3.3 K27M-mutant lines with a similar pattern observed for H3K27ac peaks (Fig. 2D). At the gene level, H3K27ac loci highlighted *OLIG2*, *PKDCC* and *CBLN1* genes and H3K27me3 loci were associated with *DLX1*, *DLX2*, *DLX5* and *OTX1* genes (Fig. 2E, Supp. Figure 2A). The H3K27ac super-enhancer profile of ICR-CXJ074 clustered with other K27M-mutant tumours (Supp. Figure 2B). Overall,

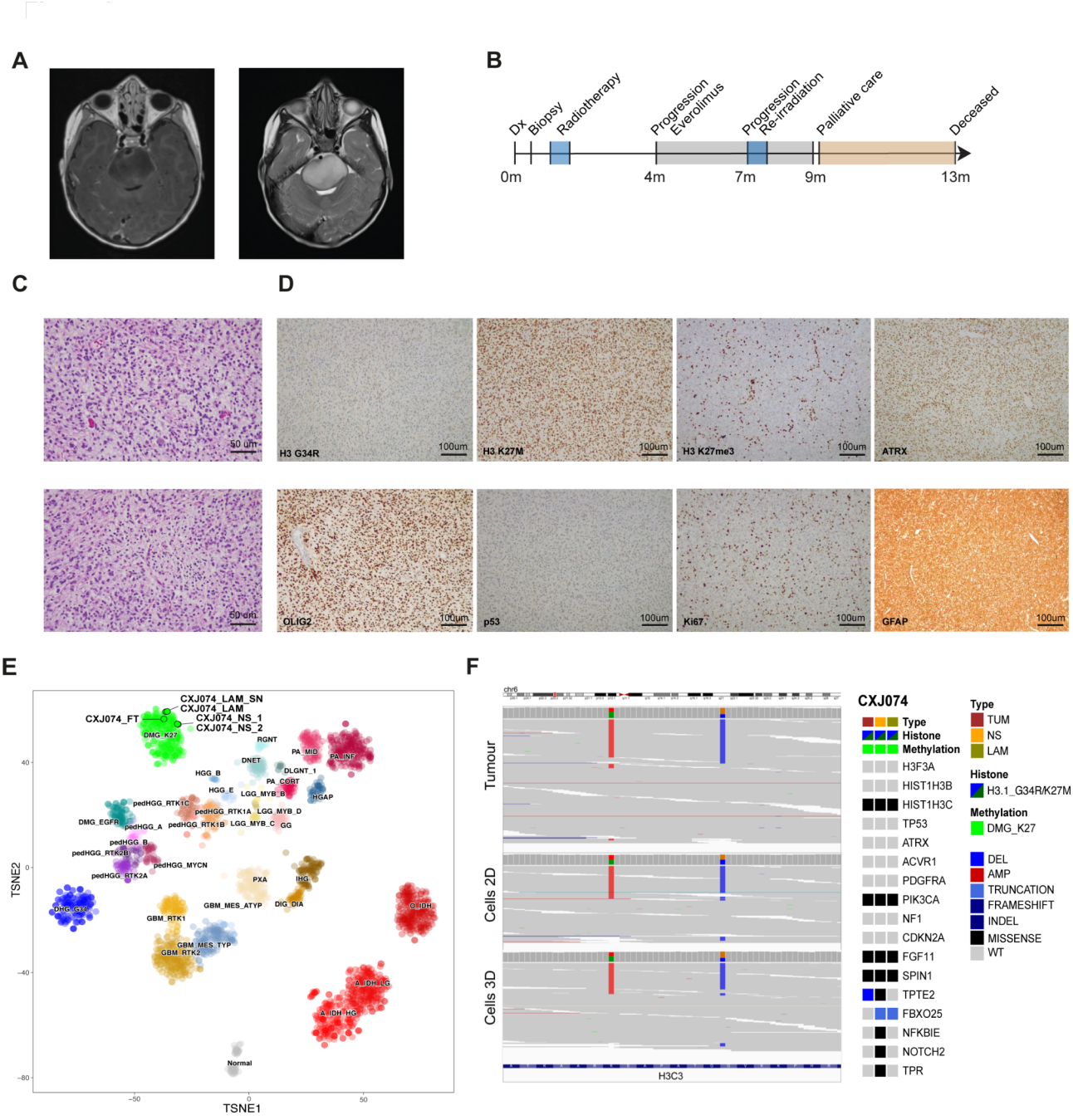

Fig. 1 Characterising the tumour. (**A**) T1-weighted axial MRI image (left) and T2-weighted axial MRI image (right). (**B**) Clinical timeline showing the key timepoints of the patient's diagnosis, treatment and progress. The timeline corresponds to the months post-diagnosis. (**C**) H&E sections of the tumour. (**D**) Panel of immunohistochemistry used to diagnose the tumour. The name of each stain is labelled in the bottom left corner of the image. (**E**) t-SNE plot of the Jones Lab glioma reference set ($n = 3357$). The primary tumour and the cell cultures are indicated by a black circle. Different glioma subgroups are represented by different colours and labelled with the group name. (**F**) IGV plot of DNA panel sequencing data showing the dual histone H3.1 *HIST1H3C* (H3C3) K27M and G34R mutations, occurring in the same reads, across the different cell cultures and the primary tumour. Oncoprint showing the variants identified in the primary tumour and cell cultures

ICR-CXJ074 showed genetic, epigenetic and transcriptomic features in keeping with DMG H3 K27M-mutant tumours.

The patient received radiotherapy (39 Gy, 13 fractions) [10] and subsequently the mTOR inhibitor everolimus (5-month duration) due to the identification of a somatic *PIK3CA* alteration, a relevant molecular target within the PI3-kinase/AKT/mTOR signalling pathway. Initial follow-up scans after radiotherapy showed shrinkage of the tumour on treatment. However, areas of expansion

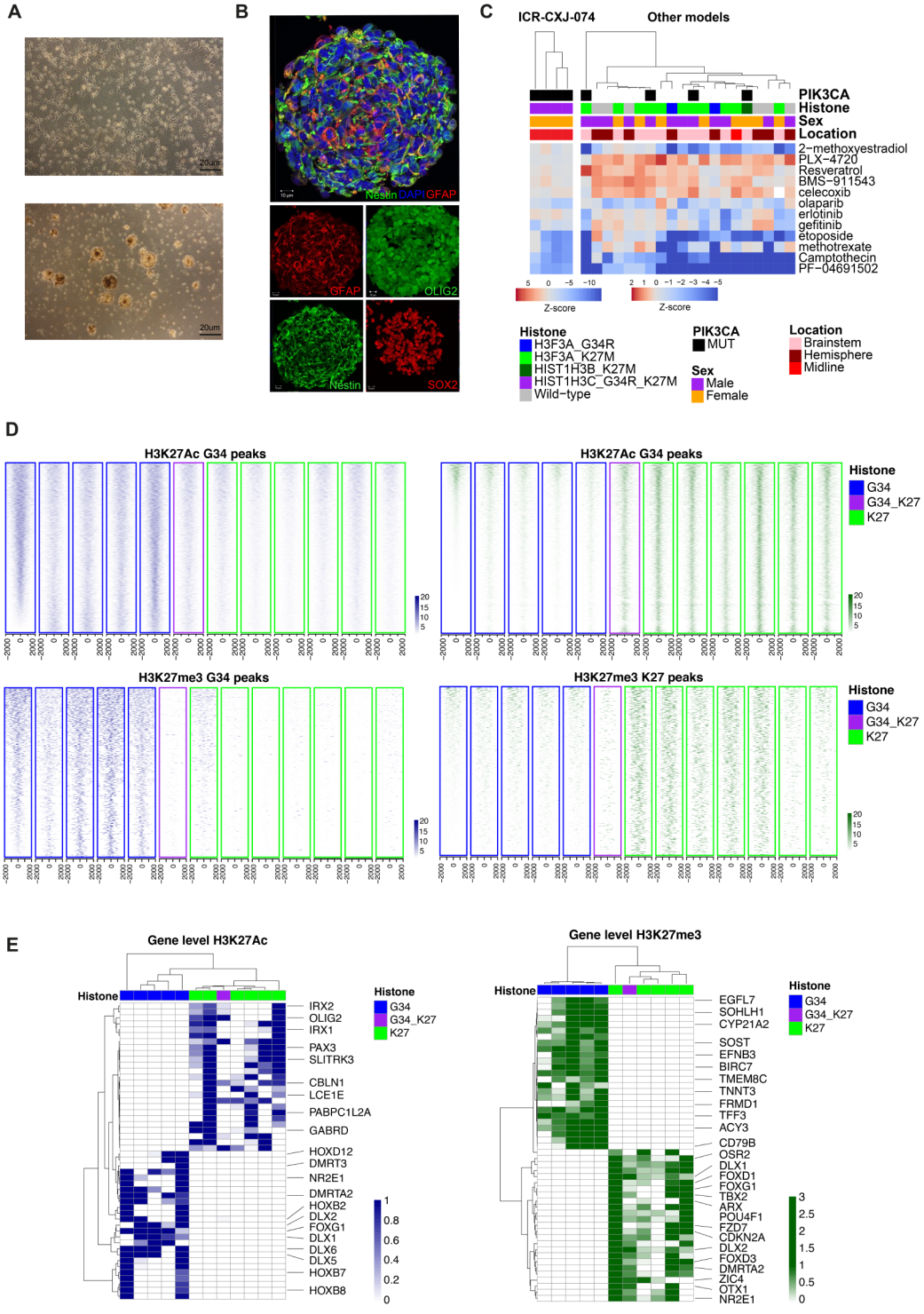

Fig. 2 In vitro patient-derived modelling. (**A**) Photo of ICR-CXJ074 2D culture, grown on laminin, passage 5 (top). Photo of ICR-CXJ074 3D neurosphere culture, passage 5 (below). (**B**) Immunofluorescence panel exploring the profiles of ICR-CXJ074 (3D). GFAP (red), Nestin (green), OLIG2 (green), SOX2 (red). (**C**) Heatmap showing screen results from the ICR drugs & tools library, compared with other screens using histone-mutant and wild-type models. ICR-CXJ74 results are shown in the heatmap on the left. (**D**) Heatmaps showing profiles of the H3K27me3 (left) and H3K27ac (right) peaks for ICR-CXJ074 compared to other histone-mutant tumours (the different histone subtypes are highlighted by a coloured outline according to the key provided). (**E**) Oncoprints showing ChIP-Seq gene level comparisons for H3K27ac (left) and H3K27me3 (right) compared to other histone-mutant models

and foci of enhancement in the pons were identified on repeat scans. The tumour extended into the medulla, and posterior limb of the internal capsule, with increased enhancement in keeping with tumour progression 6 months post-diagnosis. These matched evolving clinical symptoms of disease progression – increasing ataxia, weakness, deteriorating speech and eventually unsafe swallow. The patient was re-irradiated (20 Gy, 10 fractions) in keeping with national and international guidelines [11], but the disease continued to progress and the patient sadly passed away 13 months post-diagnosis (Fig. 1B).

Discussion and conclusions

Two cases of dual-histone mutant tumours have been previously reported in the literature, and both displayed low-grade histological features and a global decrease of H3K27me3. The first, a 7-year-old male with a tumour in the spinal cord, harboured an *H3F3A* K27M mutation co-occurring with a novel G34W variant on the same allele [12]. Although novel in HGG, this G34W variant is known to characterise giant cell tumours of bone which are a rare group of bone tumours occurring more frequently in long bones at the meta-epiphyseal region [13]. The second, a thalamic tumour in a 38-year-old male, showed low-grade gemistocytic histological features with concomitant K27M and G34R mutations in the *HISTH3B* gene [14]. Accompanying mutations in the *FGFR1*, *NF1*, *PTPN11*, *PPM1D* and *ATRX* genes were also seen [14]. Our case represents the first reported DMG with concomitant H3 K27M and G34R mutations involving the *HIST1H3C (H3C3)* gene and high-grade histology; the molecular profile reflects that of a K27M-mutant DMG rather than a G34R-mutant DHG and suggests that the K27M mutation is the dominant driver over G34R on histone H3.1 and when in *cis*. It also should be noted that we are comparing the data with H3.3 G34 tumours and we do not know the role and function of the H3.1 G34 mutation at this stage. The germline pathogenic *NBN* variant is associated with autosomal recessive Nijmegen breakage syndrome (NBS), but there were no suggestive clinical features in this patient upon review by a clinical geneticist; variants in this gene have been reported in other astrocytic tumours [15, 16], but the possible role and implications are yet be determined.

This case highlights that there are rare but complex genetic aberrations of histone mutant tumours which are important to identify and research in order to understand more about their underlying biology.

Abbreviations

HGG High-grade glioma
DMG Diffuse midline glioma
DHG Diffuse hemispheric glioma
WGS Whole genome sequencing
DKFZ German cancer research center
WES Whole exome sequencing
NBS Nijmegen breakage syndrome

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40478-024-01899-5.

Supplementary Material 1

Supplementary Material 2: **Supplementary Fig. 1**: Copy number and expression. (**A**) Copy number plots derived from the Illumina array data showing the profiles of the patient-derived cell cultures and the primary tumour. Gains/amplifications highlighted in red, losses/deletions highlighted in blue. (**B**) Heatmap showing the RNA expression profiles of the ICR-CXJ074 cultures (highlighted in black) compared to other histone-mutant and wildtype models. (**C**) Heatmap showing RNA sequencing expression data for the primary tumour compared to other histone-mutant and wild-type tumours. ICR-CXJ074 is highlighted in black. **Supplementary Fig. 2**: ChIP-Seq results. (**A**) Gene level H3K27ac and H3K27me3 loci highlighting profiles for genes DLX5 and *OLIG2*. (**B**) Heatmaps showing the H3K27ac super-enhancer profiles of ICR-CXJ074 compared with other K27M-mutant and G34-mutant tumours. H3K27ac left, recurrent H3K27ac super enhancers right. ICR-CXJ074 is highlighted in purple.

Acknowledgements

Not applicable.

Author contributions

Z.R., M.C. and C.J. wrote the manuscript and constructed the figures. R.P. established the patient derived cell culture and supported the molecular characterisation of this model. S.N., L.B., R.L., and B.C., conducted the molecular analysis of the primary tumour. A.M. and Y.G. provided bioinformatic analytical support to all steps of the molecular characterisation process. V.M. performed the immunofluorescence studies. A.B. performed additional DNA panel sequencing of both the primary tumour and the cell culture samples. C.B and B.Z. were the neurosurgeons who undertook the biopsy and provided clinical data. E.P. provided the radiology review. A.W., F.C., and L.M. were the neuro-oncology team involved in treating the patient and provided clinical data regarding treatment and management. A.K., Z.R., I.B., and S.A-S. provided a neuropathological review of the diagnostic samples. All authors reviewed and provided edits to the paper and figures.

Funding

Programme Grant, Cancer Research UK – "Targeting cellular diversity and interactivity in paediatric diffuse glioma" (DRCRPG-Nov21\100002) – Chris Jones.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This is a statement to confirm that the investigators in this study have obtained informed consent to publish information and images from this case, from the patient's parents.
This project comes under the over-arching REC ethical approval in place for the Jones Lab encompassing the translational genomics of brain tumours in children and young adults.
REC reference: 18/LO/0514.

Consent for publication

This is a statement to confirm that the investigators in this study have obtained informed consent to publish information and images from this case, from the patient's parents.

Competing interests

The authors declare no competing interests.

Statement

This is a statement to confirm that the investigators in this study have obtained informed consent to publish information and images from this case, from the patient's parents.

Author details

[1]Department of Clinical Neuropathology, King's College Hospital NHS Foundation Trust, Denmark Hill, London, UK
[2]Institute of Cancer Research, London, UK
[3]Precision Medicine, King's College Hospital NHS Foundation Trust, Denmark Hill, London, UK
[4]Neurosurgery, King's College Hospital NHS Foundation Trust, London, UK
[5]Department of Radiology, The Royal Marsden NHS Foundation Trust, London, UK
[6]Department of Paediatric and Teenager & Young Adult Neuro-Oncology, The Royal Marsden Hospital NHS Foundation Trust, London, UK
[7]Paediatric and Adolescent Oncology Drug Development Unit, The Royal Marsden NHS Foundation Trust, London, UK
[8]Division of Clinical Studies, The Institute of Cancer Research, London, UK

Received: 1 August 2024 / Accepted: 25 November 2024
Published online: 16 January 2025

References

1. Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D et al (2021) The 2021 WHO classification of tumors of the central nervous system: a summary. Neuro Oncol 23(8):1231–1251
2. Perez A, Huse JT (2021) The Evolving Classification of Diffuse Gliomas: World Health Organization Updates for 2021. Curr Neurol Neurosci Rep. 21(12). https://doi.org/10.1007/s11910-021-01153-8
3. Castel D, Kergrohen T, Tauziède-Espariat A, Mackay A, Ghermaoui S, Lechapt E et al (2020) Histone H3 wild-type DIPG/DMG overexpressing EZHIP extend the spectrum diffuse midline gliomas with PRC2 inhibition beyond H3-K27M mutation. Acta Neuropathol. 139(6):1109–13. https://doi.org/10.1007/s00401-020-02142-w
4. Mondal G, Lee JC, Ravindranathan A, Villanueva-Meyer JE, Tran QT, Allen SJ et al (2020) Pediatric bithalamic gliomas have a distinct epigenetic signature and frequent EGFR exon 20 insertions resulting in potential sensitivity to targeted kinase inhibition. Acta Neuropathol. 139(6):1071–88. https://doi.org/10.1007/s00401-020-02155-5
5. Wu G, Broniscer A, McEachron TA, Lu C, Paugh BS, Becksfort J et al (2012) Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. Nat Genet 44(3):251–253
6. Mackay A, Burford A, Carvalho D, Izquierdo E, Fazal-Salom J, Taylor KR et al (2017) Integrated Molecular Meta-Analysis of 1,000 Pediatric High-Grade and diffuse intrinsic pontine glioma. Cancer Cell 32(4):520–537e5
7. Kasper LH, Baker SJ (2020) Invited review: emerging functions of histone H3 mutations in paediatric diffuse high-grade gliomas. Neuropathol Appl Neurobiol 46(1):73–85
8. Schwartzentruber J, Korshunov A, Liu XY, Jones DTW, Pfaff E, Jacob K et al (2012) Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. Nature 482(7384):226–231
9. Capper D, Jones DTW, Sill M, Hovestadt V, Schrimpf D, Sturm D et al (2018) DNA methylation-based classification of central nervous system tumours. Nature 555(7697):469–474
10. Coleman C, Chen K, Lu A, Seashore E, Stoller S, Davis T et al (2023) Interdisciplinary care of children with diffuse midline glioma. Neoplasia (United States). 35(C):100851. https://doi.org/10.1016/j.neo.2022.100851
11. Standard Clinical (2023) Practice Recommendations for Paediatric High-Grade Glioma
12. Sloan EA, Cooney T, Oberheim Bush NA, Buerki R, Taylor J, Clarke JL et al (2019) Recurrent non-canonical histone H3 mutations in spinal cord diffuse gliomas. Acta Neuropathol 138(5):877–881
13. Lim J, Park JH, Baude A, Yoo Y, Lee YK, Schmidt CR et al (2017) The histone variant H3.3 G34W substitution in giant cell tumor of the bone link chromatin and RNA processing. Sci Rep 7(1):1–14
14. Morris M, Driscoll M, Henson JW, Cobbs C, Jiang LQ, Gocke CD et al (2021) Low-grade gemistocytic morphology in H3 G34R-mutant gliomas and concurrent K27M mutation: clinicopathologic findings. J Neuropathol Exp Neurol 79(10):1038–1043
15. Belhadj S, Khurram A, Bandlamudi C, Palou-Márquez G, Ravichandran V, Steinsnyder Z et al (2023) NBN Pathogenic Germline Variants Are Associated with Pan-cancer susceptibility and in Vitro DNA damage response defects. Clin Cancer Res 29(2):422–431
16. Piekutowska-Abramczuk D, Ciara E, Popowska E, Grajkowska W, Dembowska-Bagińska B, Kowalewska E et al (2010) The frequency of NBN molecular variants in pediatric astrocytic tumors. J Neurooncol 96(2):161–168

Publisher's note