Immunogenetics (2019) 71:433–436
https://doi.org/10.1007/s00251-019-01108-x

SHORT COMMUNICATION

Prolonged stable disease in a uveal melanoma patient with germline *MBD4* nonsense mutation treated with pembrolizumab and ipilimumab

Peter A. Johansson[1] · Andrew Stark[2] · Jane M. Palmer[1] · Kieron Bigby[3,4,5] · Kelly Brooks[1] · Olivia Rolfe[2] · Antonia L. Pritchard[1,6] · Kevin Whitehead[7] · Sunil Warrier[2] · William Glasson[2] · Nicholas K. Hayward[1]

Received: 12 October 2018 / Accepted: 26 January 2019 / Published online: 4 February 2019

Abstract

There is currently no effective treatment for metastasised uveal melanoma (UM). Recently, it was reported that a UM patient was responsive to checkpoint inhibitor (CI) treatment, due to a high tumour mutation burden correlated with a germline loss-of-function *MBD4* mutation. Here, we report on another UM patient who carried an *MBD4* germline nonsense variant (p.Leu563Ter) and her tumour showed a fivefold higher than average mutation burden. We confirmed the association between germline loss-of-function variant in *MBD4* and CI response. The patient experienced stable disease (10 months) and survived 2 years with metastatic disease, which is twice as long as median survival. Additionally, the frequency of *MBD4* loss-of-function variants in reported UM cohorts was > 20 times higher than in an aggregated population genome database ($P < 5 \times 10^{-5}$), implying a potential role as UM predisposition gene. These findings provide a strong basis for the inclusion of *MBD4* in the screening of potential UM-prone families as well as stratification of immunotherapy.

Keywords MBD4 · Uveal melanoma · Mutation · Immunotherapy · Predisposition gene

In the Caucasian population, uveal melanoma (UM) has an annual incidence of 2–8 cases per million (Virgili et al. 2008). Currently, non-invasive primary UM is effectively controlled with radiotherapy (episcleral brachytherapy), transpupillary thermotherapy, enucleation, or combinations of these. However, despite intervention at the stage of primary disease, ~ 50% of UM cases metastasize within 15 years, which is invariably fatal with no effective treatments available (Damato and Heimann 2013). Recently, progress has been made for treating cutaneous melanoma (CM), including targeted therapies and immunotherapies (Polkowska et al. 2016), which have unfortunately not translated to successful treatments for UM (Oliva et al. 2016). Germline biallelic deactivation of *MBD4*, which encodes a DNA glycosylase, has been reported to predispose to acute myeloid leukaemia, presumably through compromising DNA repair, resulting in a high mutation load (Sanders et al. 2018). Recently, effective checkpoint inhibitor (CI) therapy for metastatic UM and glioblastoma patients with germline loss-of-function (LoF) mutations in *MBD4* has been reported (Rodrigues et al. 2018). We therefore sought to identify additional UM patients that had received CI and carried an *MBD4* germline LoF mutation. To this end, we interrogated germline genome and exome sequence data from a previous study of UM genomics, and of 28 patients available, one patient had a germline LoF mutation in *MBD4* (Johansson et al. 2016).

The patient was a female indoor worker with blue eyes, Fitzpatrick type 1 skin and few moles. She presented at 65 years with sudden-onset blurred left vision leading to a

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00251-019-01108-x) contains supplementary material, which is available to authorized users.

✉ Peter A. Johansson
peter.johansson@qimrberghofer.edu.au

[1] QIMR Berghofer Medical Research Institute, Brisbane, QLD 4006, Australia

[2] Queensland Ocular Oncology Service, The Terrace Eye Centre, Brisbane, QLD, Australia

[3] Montserrat Cancer Care, North Lakes Day Hospital, North Lakes, QLD, Australia

[4] Redcliffe Hospital, Anzac Ave, Redcliffe, QLD, Australia

[5] School of Medicine, University of Queensland, St Lucia, QLD, Australia

[6] The University of the Highlands and Islands, Inverness, UK

[7] Sullivan Nicolaides Pathology, Brisbane, QLD, Australia

diagnosis of choroidal melanoma. A whole body positron-emission tomography (PET) computed tomography (CT) investigation found no evidence of metastatic spread. Given these findings, iodine 125 episcleral brachytherapy was undertaken, after which the tumour initially regressed. However, 10 months later tumour growth of 3 mm was observed and she therefore underwent enucleation. Histopathological analysis showed a variable, darkly pigmented choroidal melanoma posterior in the globe measuring 10 mm in diameter and 4 mm in height of mixed spindle A (40%) and B (60%) types, with 1 mitosis per square millimetre. Tumour was present adjacent to the optic nerve head, with no invasion and minimal extension through the scleral envelope. Further testing revealed tumour cell nuclei were negative for BAP1 staining and FISH studies showed monosomy 3 and copy number gains of 8q. Together, these findings placed the patient in the high-risk category for metastatic progression.

Four years after initial presentation, despite the patient being asymptomatic, routine liver function tests were noted to be raised. Subsequent abdominal ultrasound identified numerous liver lesions implicating metastases; CT and bone scan confirmed widespread liver metastases and a solitary rib metastasis. Liver biopsy revealed a heavily pigmented epithelioid malignancy consistent with metastatic melanoma; no activating mutations in *BRAF* or *NRAS* were detected by targeted sequencing. With her history, the temporal and characteristic patterns of spread were consistent with secondary UM.

Given the absence of suitable clinical trials and therapeutic availability, the humanised IgG4 programmed death 1 (PD-1) monoclonal antibody, pembrolizumab (Keytruda™; Merck) was commenced as monotherapy (2 mg/kg intravenously every 3 weeks). The patient experienced no dose-limiting toxicities or immune-related adverse event during this treatment. After 15 cycles (45 weeks), with stable disease her best response, progression was confirmed with increased liver and rib metastases as well as new cutaneous and subcutaneous lesions. The anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), ipilimumab (Yervoy™; Bristol-Myers Squibb) was given as follow-on therapy at 3 mg/kg intravenously at 3 week intervals for 4 doses only (initial induction course). The 4th cycle was delayed due to grade 1 colitis which settled without corticosteroids and no other immune-related adverse events were noted. However, further progression was noted at both the initial and delayed evaluation CT scans after the induction 4 cycles. No further systemic therapy was given beyond 13 months post initiation besides thoracic spine radiotherapy 3 months after therapy cessation to prevent spinal cord compression. With no further appropriate systemic therapy available, supportive care alone was continued until the patient died 2 years after diagnosis of disseminated disease.

Ethics approval was obtained from the Human Research Ethics Committees of the QIMR Berghofer Medical Research Institute. Written informed consent was obtained from the patient. We performed exome sequencing of the primary UM from this individual. Sequencing, read alignment, mutation calling, mutation signature analysis, and Sanger sequencing were performed as previously described (Johansson et al. 2016). We analysed chromosome copy number with a moving average over read depth in protein-coding regions. Bins were defined so each bin contained 2 million bases and median of coverage was used as metric. Allelic imbalances were identified by looking at variant allele frequency in tumour sample at loci being heterozygous in germline. Heterozygosity was defined as Phred-scaled genotype likelihood for homozygous genotypes being greater than 60 (for both wildtype and variant). Loci with read depth below 30 in the tumour were ignored.

Exome sequencing data from the patient revealed a germline LoF mutation in *MBD4* (p.Leu563Ter, rs200758755). Subsequent Sanger sequencing confirmed the *MBD4* variant as heterozygous in the germline and homozygous in the tumour, suggesting a loss of heterozygosity (LOH) in the latter. Copy number analysis of the tumour confirmed the FISH findings that chromosome 3, on which *MBD4* is located, was hemizygous and that there was a gain of chromosome 8. Additionally, the UM tumour had monosomy 13 and partial 1p and Xp loss (Fig. S1). The tumour (00028-001-CL) carried *GNA11* p.Q209L and *SF3B1* p.R625C hotspot mutations and a *BAP1* p.D73Vfs*4 frameshift deletion (Table 1) and exhibited a mutation burden of 2.52 exonic mutations per Mb, > fivefold higher than the other 27 samples (average 0.45/Mb, range 0.06–0.83/Mb), as previously reported (Johansson et al. 2016). LoF mutations in *MBD4* have been reported to be associated with COSMIC mutation signature 1 (Waszak et al. 2017). Mutation signature analysis showed that mutations were heavily dominated by signature 1 (cosine similarity 89%) and no other signature made a significant contribution. Signature 1 has been proposed to reflect spontaneous deamination of 5-methylcytosine (Alexandrov et al. 2013), which results in CpG>TpG transitions, and as expected, the majority of mutations (74%) were of this type. While no tissue from the metastasis was available for immune reactivity profiling, we obtained the primary UM and stained for CD3 (total tumour-infiltrating lymphocytes (TILs)) and PD1. There was a mild TIL count but no PD1-positive UM cells (Fig. S2).

Rodrigues and colleagues reported two UM, and here, we report a third case, all with heterozygous germline LoF mutations accompanied with somatic loss of *MBD4*. To further examine how common *MBD4* LoF mutations are in UM, we explored publicly available sequence data (Furney et al. 2013; Harbour et al. 2013; Martin et al. 2013). In the Harbour cohort, we identified a frameshift deletion (p.Leu482Trpfs; rs200758755) in MM138, which also had monosomy 3. Our analysis demonstrated a high mutation burden, predominantly from mutation signature 1 in MM138. Thus, in total, four UM cases with LoF mutations in *MBD4* have been identified from

Table 1 Key somatic mutations in patient 00028-001. The variant allele frequencies (VAF) show that activating hotspot mutations in *GNA11* and *SF3B1* were heterozygous while the deletion in *BAP1* was hemizygous due to monosomy 3

Gene	RefSeq	Nucleotide change	Amino Acid change	Ref allele	Var allele	VAF germline	VAF tumour
BAP1	NM_004656	c.218_221del	p.D73Vfs*4	TCAT		0 / 98	19/19
GNA11	NM_002067	c.626A>T	p.Q209L	A	T	0 / 41	9/24
SF3B1	NM_012433	c.1873C>T	p.R625C	G	A	0 / 21	32/64

161 that have undergone exome or genome sequencing (Furney et al. 2013; Harbour et al. 2013; Johansson et al. 2016; Martin et al. 2013; Robertson et al. 2017; Rodrigues et al. 2018), translating to a variant allele frequency (VAF) of 1.24%. In comparison, the genome aggregation database (http://gnomad.broadinstitute.org/gene/ENSG00000129071) reports a total of 132 *MBD4* LoF mutations in approximately 230,000 examined alleles (VAF = 5.7×10^{-4}; odds ratio, 21.6; Fisher's exact test $P < 5 \times 10^{-5}$) (Lek et al. 2016).

We describe a patient with metastatic UM that was treated with a combination of CI and had an unexpected long period of stable disease. Good response to CI is associated with high somatic mutation burden, as more somatic mutations increase the number of neoantigens formed and thereby the probability for recognition by immune cells (Rizvi et al. 2015). The generally low mutation burden in UM probably contributes to the lack of success with CI in metastatic UM, with median progression-free survival less than 3 months and median overall survival between 8 and 13 months(Yang et al. 2018). In comparison, the patient described here had prolonged stable disease, stayed progression free for more than 10 months and survived metastatic disease for more than 24 months. Exome sequencing revealed an unusually high somatic mutation burden, likely due to an *MBD4* LoF mutation, in keeping with a recent similar report (Rodrigues et al. 2018). MBD4 is involved in base excision repair of mismatches arising from spontaneous deamination of 5-methylcytosine, a mutation process typically resulting in CpG>TpG base changes (Hendrich et al. 1999). The majority (74%) of the somatic mutations were CpG>TpG transitions, which further supports the high mutation burden being caused by MBD4 loss. Rodrigues and colleagues observed an even higher percentage of CpG > TpG mutations (> 91%) in a patient with germline *MBD4* LoF mutation, as well as higher mutation burden (> 19-fold compared with average). A comparably lower mutation burden and less distinct CpG>TpG mutation profile observed in the primary UM from our patient is consistent with the hypothesis that the MBD4-related mutagenic process continues to cause somatic mutations during tumour progression. These observations of increased mutation burden in UM patients with deficient MBD4 suggest that CI is potentially a viable option, given that mutation burden correlates with response rates to CI in other cancers (Rizvi et al. 2015). To date, two UM patients with deleterious germline *MBD4* variants are known to have received CI, one who had complete response (Rodrigues et al. 2018) and our patient who did not, suggesting further investigations are required.

In total, four UM patients with *MBD4* LoF mutations have been reported, which corresponds to 2.5% of examined UM patients or more than 20 times more frequent than in the general population. This observation suggests that in addition to its loss of function being associated with response to CI, *MBD4* is also a UM predisposition gene. Sequencing of a large population-based series of UM patients is required to more accurately determine the relative frequency of germline *MBD4* mutations in UM cases versus controls. These findings together with those of Rodrigues and colleagues (Rodrigues et al. 2018) provide a strong basis for the inclusion of germline *MBD4* variants in the screening of potential UM-prone families as well as stratification for CI treatment in UM.

Sequence data has been deposited at the European Genome-phenome Archive (EGA), which is hosted at the EBI and the CRG, under accession number EGAS00001003362.

Acknowledgments We are indebted to the patient and her family and acknowledge the contribution of Dr. Grant Cameron, along with the many clinicians and allied health professionals involved in her care. We are grateful to Dr. Robert Rawson, Dr. Michael Walsh, Dr. Ken Dutton-Regester, Dr. Lauren Aoude, Madeleine Howlie and Hayley Hamilton for input into this study.

Funding information The work was funded by the National Health and Medical Research Council of Australia (NHMRC 1093017). N.K.H. is supported by a fellowship from the NHMRC (1117663).

Compliance with ethical standards

Ethics approval was obtained from the Human Research Ethics Committees of the QIMR Berghofer Medical Research Institute. Written informed consent was obtained from the patient.

References

Alexandrov LB et al (2013) Signatures of mutational processes in human cancer. Nature 500:415–421. https://doi.org/10.1038/nature12477

Damato B, Heimann H (2013) Personalized treatment of uveal melanoma. Eye (Lond) 27:172–179. https://doi.org/10.1038/eye.2012.242

Furney SJ, Pedersen M, Gentien D, Dumont AG, Rapinat A, Desjardins L, Turajlic S, Piperno-Neumann S, de la Grange P, Roman-Roman

S, Stern MH, Marais R (2013) SF3B1 mutations are associated with alternative splicing in uveal melanoma. Cancer Discov 3:1122–1129. https://doi.org/10.1158/2159-8290.CD-13-0330

Harbour JW, Roberson ED, Anbunathan H, Onken MD, Worley LA, Bowcock AM (2013) Recurrent mutations at codon 625 of the splicing factor SF3B1 in uveal melanoma. Nat Genet 45:133–135. https://doi.org/10.1038/ng.2523

Hendrich B, Hardeland U, Ng HH, Jiricny J, Bird A (1999) The thymine glycosylase MBD4 can bind to the product of deamination at methylated CpG sites. Nature 401:301–304. https://doi.org/10.1038/45843

Johansson P, Aoude LG, Wadt K, Glasson WJ, Warrier SK, Hewitt AW, Kiilgaard JF, Heegaard S, Isaacs T, Franchina M, Ingvar C, Vermeulen T, Whitehead KJ, Schmidt CW, Palmer JM, Symmons J, Gerdes AM, Jönsson G, Hayward NK (2016) Deep sequencing of uveal melanoma identifies a recurrent mutation in PLCB4. Oncotarget 7:4624–4631. https://doi.org/10.18632/oncotarget.6614

Lek M et al (2016) Analysis of protein-coding genetic variation in 60,706 humans. Nature 536:285–291. https://doi.org/10.1038/nature19057

Martin M, Maßhöfer L, Temming P, Rahmann S, Metz C, Bornfeld N, van de Nes J, Klein-Hitpass L, Hinnebusch AG, Horsthemke B, Lohmann DR, Zeschnigk M (2013) Exome sequencing identifies recurrent somatic mutations in EIF1AX and SF3B1 in uveal melanoma with disomy 3. Nat Genet 45:933–936. https://doi.org/10.1038/ng.2674

Oliva M, Rullan AJ, Piulats JM (2016) Uveal melanoma as a target for immune-therapy. Ann Transl Med 4:172. https://doi.org/10.21037/atm.2016.05.04

Polkowska M, Czepielewska E, Kozlowska-Wojciechowska M (2016) Drug combinations as the new standard for melanoma treatment. Curr Treat Options in Oncol 17:61. https://doi.org/10.1007/s11864-016-0436-y

Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, Lee W, Yuan J, Wong P, Ho TS, Miller ML, Rekhtman N, Moreira AL, Ibrahim F, Bruggeman C, Gasmi B, Zappasodi R, Maeda Y, Sander C, Garon EB, Merghoub T, Wolchok JD, Schumacher TN, Chan TA (2015) Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 348:124–128. https://doi.org/10.1126/science.aaa1348

Robertson AG, Shih J, Yau C, Gibb EA, Oba J, Mungall KL, Hess JM, Uzunangelov V, Walter V, Danilova L, Lichtenberg TM, Kucherlapati M, Kimes PK, Tang M, Penson A, Babur O, Akbani R, Bristow CA, Hoadley KA, Iype L, Chang MT, Cherniack AD, Benz C, Mills GB, Verhaak RGW, Griewank KG, Felau I, Zenklusen JC, Gershenwald JE, Schoenfield L, Lazar AJ, Abdel-Rahman MH, Roman-Roman S, Stern MH, Cebulla CM, Williams MD, Jager MJ, Coupland SE, Esmaeli B, Kandoth C, Woodman SE, Abdel-Rahman MH, Akbani R, Ally A, Auman JT, Babur O, Balasundaram M, Balu S, Benz C, Beroukhim R, Birol I, Bodenheimer T, Bowen J, Bowlby R, Bristow CA, Brooks D, Carlsen R, Cebulla CM, Chang MT, Cherniack AD, Chin L, Cho J, Chuah E, Chudamani S, Cibulskis C, Cibulskis K, Cope L, Coupland SE, Danilova L, Defreitas T, Demchok JA, Desjardins L, Dhalla N, Esmaeli B, Felau I, Ferguson ML, Frazer S, Gabriel SB, Gastier-Foster JM, Gehlenborg N, Gerken M, Gershenwald JE, Getz G, Gibb EA, Griewank KG, Grimm EA, Hayes DN, Hegde AM, Heiman DI, Helsel C, Hess JM, Hoadley KA, Hobensack S, Holt RA, Hoyle AP, Hu X, Hutter CM, Jager MJ, Jefferys SR, Jones CD, Jones SJM, Kandoth C, Kasaian K, Kim J, Kimes PK, Kucherlapati M, Kucherlapati R, Lander E, Lawrence MS, Lazar AJ, Lee S, Leraas KM, Lichtenberg TM, Lin P, Liu J, Liu W, Lolla L, Lu Y, Iype L, Ma Y, Mahadeshwar HS, Mariani O, Marra MA, Mayo M, Meier S, Meng S, Meyerson M, Mieczkowski PA, Mills GB, Moore RA, Mose LE, Mungall AJ, Mungall KL, Murray BA, Naresh R, Noble MS, Oba J, Pantazi A, Parfenov M, Park PJ, Parker JS, Penson A, Perou CM, Pihl T, Pilarski R, Protopopov A, Radenbaugh A, Rai K, Ramirez NC, Ren X, Reynolds SM, Roach J, Robertson AG, Roman-Roman S, Roszik J, Sadeghi S, Saksena G, Sastre X, Schadendorf D, Schein JE, Schoenfield L, Schumacher SE, Seidman J, Seth S, Sethi G, Sheth M, Shi Y, Shields C, Shih J, Shmulevich I, Simons JV, Singh AD, Sipahimalani P, Skelly T, Sofia H, Soloway MG, Song X, Stern MH, Stuart J, Sun Q, Sun H, Tam A, Tan D, Tang M, Tang J, Tarnuzzer R, Taylor BS, Thiessen N, Thorsson V, Tse K, Uzunangelov V, Veluvolu U, Verhaak RGW, Voet D, Walter V, Wan Y, Wang Z, Weinstein JN, Wilkerson MD, Williams MD, Wise L, Woodman SE, Wong T, Wu Y, Yang L, Yang L, Yau C, Zenklusen JC, Zhang J, Zhang H, Zmuda E (2017) Integrative analysis identifies four molecular and clinical subsets in uveal melanoma. Cancer Cell 32:204–220 e215. https://doi.org/10.1016/j.ccell.2017.07.003

Rodrigues M, Mobuchon L, Houy A, Fiévet A, Gardrat S, Barnhill RL, Popova T, Servois V, Rampanou A, Mouton A, Dayot S, Raynal V, Galut M, Putterman M, Tick S, Cassoux N, Roman-Roman S, Bidard FC, Lantz O, Mariani P, Piperno-Neumann S, Stern MH (2018) Outlier response to anti-PD1 in uveal melanoma reveals germline MBD4 mutations in hypermutated tumors. Nat Commun 9:1866. https://doi.org/10.1038/s41467-018-04322-5

Sanders MA et al (2018) MBD4 guards against methylation damage and germline deficiency predisposes to clonal hematopoiesis and early-onset AML. Blood. https://doi.org/10.1182/blood-2018-05-852566

Virgili G et al (2008) Survival in patients with uveal melanoma in Europe. Arch Ophthalmol 126:1413–1418. https://doi.org/10.1001/archopht.126.10.1413

Waszak SM et al (2017) Germline determinants of the somatic mutation landscape in 2,642 cancer genomes. bioRxiv. https://doi.org/10.1101/208330

Yang J, Manson DK, Marr BP, Carvajal RD (2018) Treatment of uveal melanoma: where are we now? Ther Adv Med Oncol 10:1758834018757175. https://doi.org/10.1177/1758834018757175