Received: 11 September 2023 | Accepted: 10 October 2023

DOI: 10.1111/bjh.19167

BJHaem
BRITISH JOURNAL OF HAEMATOLOGY

LETTER TO THE EDITOR

MEK 1 inhibition and bleeding in hereditary haemorrhagic telangiectasia

Hereditary haemorrhagic telangiectasia (HHT) affects approximately 1.5 million individuals worldwide, results from a germline loss-of-function gene variant ('mutation') usually in *ENG*, *ACVRL1* or *SMAD4*, and causes a spectrum of vascular malformations, including mucocutaneous telangiectasia and visceral arteriovenous malformations (AVMs).[1–3] Expert consensus informs clinical management,[1] as randomised control trial (RCT) evidence for local and systemic approaches is limited. There is RCT evidence for tamoxifen and tranexamic acid, but management of severe haemorrhage causing transfusion-dependent iron deficiency anaemia remains challenging. Here, international guidance[2] proposes intravenous bevacizumab (monoclonal anti-vascular endothelial cell growth factor (VEGF), but this is not well supported by RCT evidence,[4] nor approved for HHT in the United Kingdom. There is an urgent need for new treatments.

At our institution, prospective characterisation of more than 1000 HHT patients over 24 years enables recognition and validated categorisation[5] when expected patterns are not followed. For the case presented, patterns of HHT nosebleeds and anaemia at clinical review differed markedly from multiple previous assessments, and on direct questioning, a relevant new drug was noted to have been taken for an unrelated gynaecologic low-grade cancer. Following a failure of cytotoxic drugs and hormone therapy, the MEK inhibitor trametinib[6] was given continuously, and the tumour has responded for more than 2 years. The initial dose of trametinib (2 mg/day) was not well tolerated due to hand oedema and it was reduced to 1 mg/day. This was well tolerated, with only Grade 1–2 adverse events (intermittent finger swelling) described at 10 and 18 months. The patient described no other adverse events, none of the lethargy, paraesthesia or pains described by HHT patients on other anti-angiogenics, and at 2 years there was continued regression of tumour deposits.

Prior to commencing trametinib, the patient (with a pathogenic variant in *ENG*) had nosebleeds at least once a week, and specialist ENT treatments had been required when at higher frequencies and severity. After 10 months of trametinib 1 mg, nosebleeds had reduced in intensity and frequency to a brief trickle less than once per month (Figure 1A–C). Prior to commencing trametinib, the patient had been receiving regular red cell transfusions for many years, at one point every 3 weeks, due to HHT bleeds and haemolytic anaemia. When reviewed after 10 months, transfusion rates were reduced to >12 weekly (Figure 1B). By 18 and 24 months, nosebleeds were occurring approximately once a year, with reduced iron requirements[8] (Figure 1D,E). Blinded analysis of thoracic CT scans taken for clinical purposes was conducted by an expert thoracic radiologist to address the question of 'whether the PAVMs have stayed the same, got better, or got worse?' examining images 10 years, 2 years and 1 month before trametinib, and images 4 months and 10 months after trametinib. As shown in Figure 1F, the natural history of the pulmonary AVMs was not observably altered.

The trametinib targets, mitogen-activated extracellular signal-regulated kinases 1 and 2 (MEK1 and MEK2), are gatekeepers for the extracellular signal-regulated kinase (ERK) pathway, one of the best characterised mitogen activated protein kinase (MAPK) signalling pathways (Figure 2A).[9,10] The pathways explain trametinib efficacy against cancers,[6] and against other vascular malformation syndromes where germline or somatic DNA variants constitutively activate MAPK signalling (Figure 2A; Table S1). However, previous literature did not provide a direct rationale for why trametinib should be effective in the heterozygous state of human HHT, since proteins encoded by HHT causal genes are not present on similar pathways (Figure 2A), and only complete blockade of ALK1–ENG signalling has been shown to impact the networks. To further emphasise genetic distinctions, we examined whole genome[6] sequence data from HHT patients recruited to the 100,000 Genomes Project and identified none of the activating mutations in MAPK pathway genes that cause other 'proliferative' vascular malformation syndromes (Table S2).

Alternate mechanistic rationales were considered. The HHT-perturbed TGF-β and BMP signalling pathways are essential for development and viability, but our recent studies in blood outgrowth endothelial cells (BOECs) derived from HHT patients heterozygous for a pathogenic variant in each of the three major HHT genes have provided further evidence that both arms of the TGF-β canonical pathway are attenuated in HHT heterozygous endothelial cells.[11] One of us (CLS) hypothesised that in order to survive, HHT

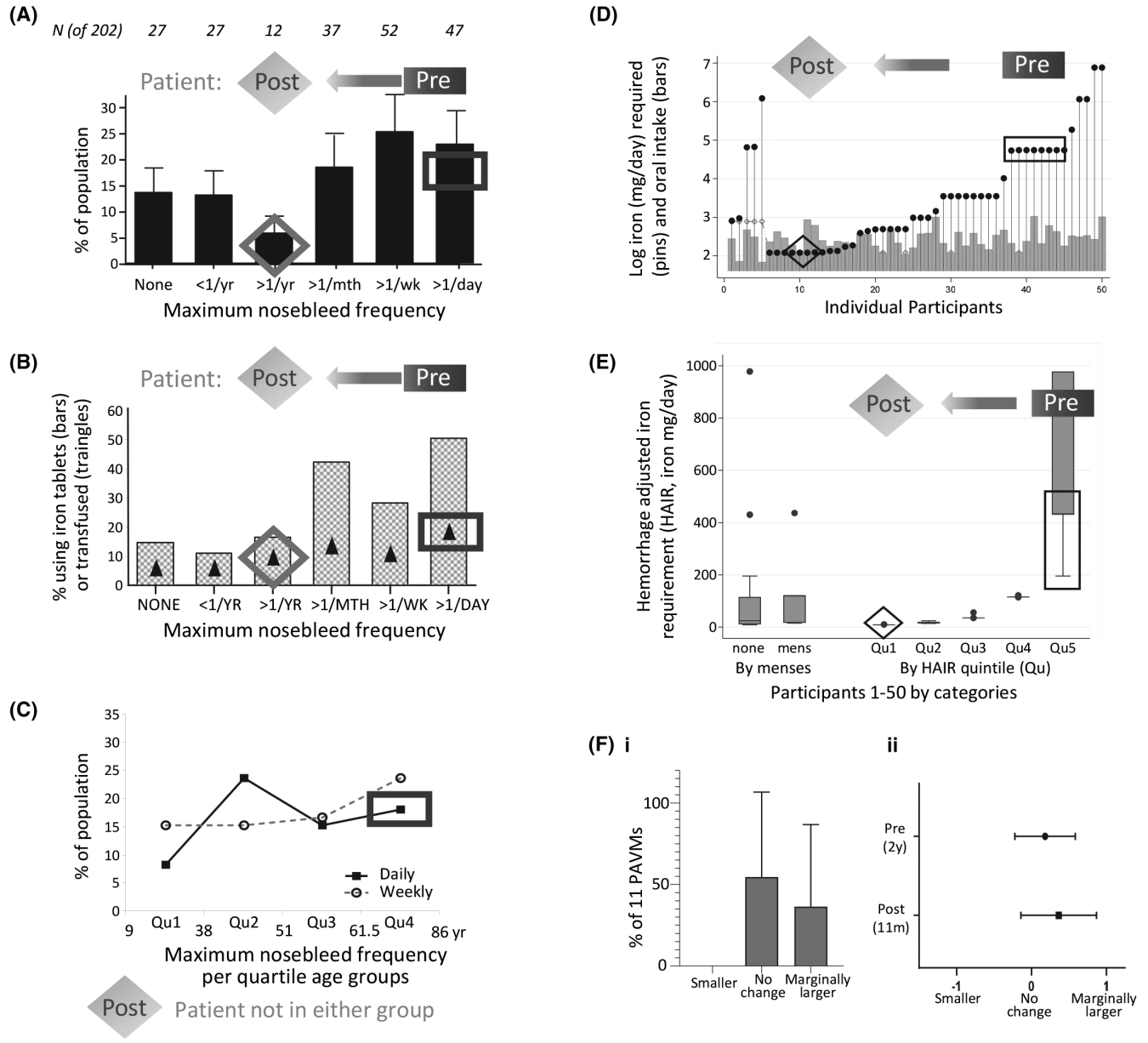

FIGURE 1 Hereditary haemorrhagic telangiectasia (HHT) symptoms and treatments pre- and post-trametinib compared to cohorts of previously reported HHT patients. (A–C): Comparison of trametinib-treated patient to 202 HHT patients illustrated on base graphs originally published by Shovlin[7] showing change in categories before ('Pre') and after ('Post') trametinib, with identical categories at 10, 18 and 24 months. (A) Maximum nosebleed frequency. (B) Oral iron (bars) and blood transfusion use (triangles). (C) Maximum nosebleed frequency by age quintiles. (D and E) Comparison to 50 HHT patients with base graphs originally published by Finnamore et al.,[8] to illustrate changes in (D) Haemorrhage-adjusted iron requirement (HAIR[8]) and (E) HAIR ranked by quintiles (Qu). (F) Quantitative representation of pulmonary (P)AVMs by thoracic CT scans across an 11-month period incorporating 10 months of trametinib treatment (see also Figure S1). (i) PAVMs are categorised by percentage as smaller, unchanged and marginally larger, and (ii) with individual PAVMs assigned to a scale of −1 (possibly smaller), 0 (no change) and +1 (possibly larger). Error bars indicate the mean and standard deviation. Additional images are shown in Figure S1. HAIR, haemorrhage-adjusted iron requirement[8]; mth, month; Qu quintile; wk, week; yr, year.

endothelial cell compensatory mechanisms would include reductions in TGF-β/BMP pathway inhibitors,[12] increases in alternate pathways that phosphorylate the final common pathway SMAD4, and noted a constitutively active form of MEKK1 (encoded by *MAP3K1*) selectively and independently activated SMAD-dependent transcription.[13] These hypotheses were tested and confirmed: Compared to control BOECs, HHT BOECs did display reduced transcript levels for pathway inhibitors[12] (Figure 2Bi; Figure S3). Crucially, whether examined as raw data or normalised either to total read counts per library (Figure 2Bii), or a panel of eight GINI housekeeping genes[11] (Figure 2Biii), *MAP3K1* encoding MEKK14 was the only major endothelial MAP3K increased in HHT BOECs.

While these early data need to be confirmed in greater numbers of patients and endothelial cells, they suggest

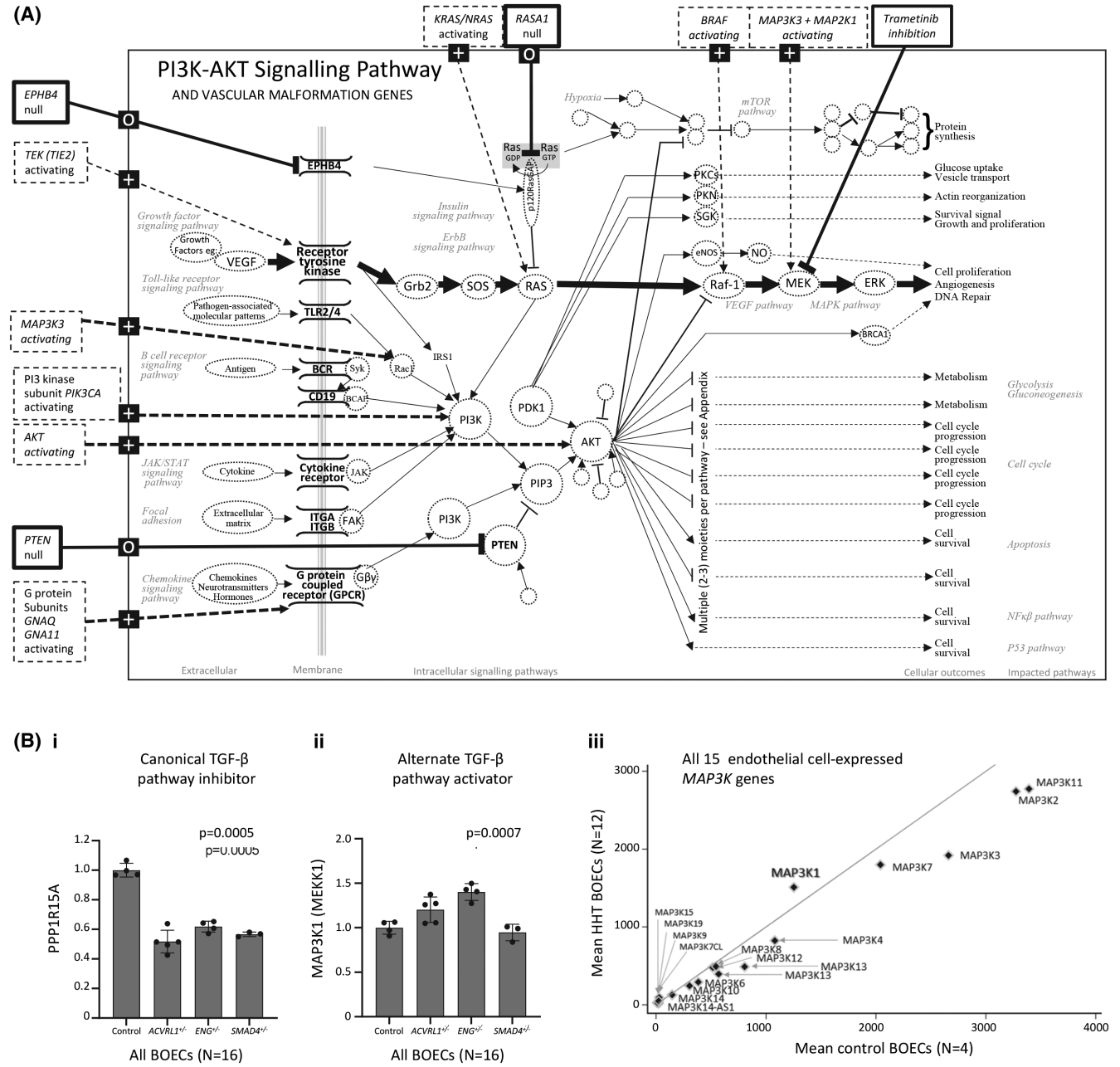

FIGURE 2 Mechanistic considerations. (A) Trametinib-relevant pathways, simplified from the Kanehisa Laboratories' KEGG pathway (Figure S2), and used with permission. Normal activators are shown by thin arrows, except for the trametinib target pathway, highlighted by bold arrows from VEGF. Normal inhibitors are shown by thin lines and bars, pathway names by grey italics and receptors flanked in bold at membranes. Vascular malformation genes are listed outside of the main box: thick bold boxes and barred lines indicate pathway inhibitors where inactivating mutations lead to pathway activation; thick dotted boxes and arrows indicate where pathway activation is caused by rare activating mutations (for further details, see Table S1). None of the HHT gene protein products (ALK1, ENG, SMAD4, BMP9) appear on the simplified or original (Figure S2) map. (B) RNASeq data in blood outgrowth endothelial cells (BOECs) isolated from control and hereditary haemorrhagic telangiectasia (HHT) donors, where HHT donors were heterozygous for nonsense variants in *ACVRL1, ENG* or *SMAD4*. (i) Alignments to *PPP1R15A* encoding TGF-β signalling pathway inhibitor GADD34 (for further examples of pathway inhibitors, see Figure S3) and (ii) *MAP3K1* in the BOECs. *p* values were calculated by Kruskal Wallis. (iii) All 15 MAP3Ks detected in BOECs by RNASeq, normalised to a panel of eight GINI genes as described elsewhere.[11] Findings were robust to normalisation methods and showed *MAP3K1* encoding MEKK14 to be the only highly expressed MAP3K increased in HHT BOECs.

trametinib/MEK1 inhibition offers potential for therapeutic benefit in HHT, delivered in a well-tolerated oral formulation better suited to regular lifestyles than requirements for intravenous medications.

Mechanistically, the presented data suggest that MAPK pathways are impacted secondary to cellular compensations, whereby MEKK-1, which is a RAF-independent MAP3 kinase (MAP3K) that phosphorylates SMAD2

independently to TGF-β, exhibits increased basal cellular transcript levels. This should not constitutively activate MAPK pathways or constitutively up-regulate MEKK1-MEK1/2 signalling as in other vascular malformation syndromes detailed in Figure 2A and Table S1 since the primary function of MEKK1 is as a MAP3K operating under stress conditions.[13,14] However, signalling through the trametinib targets would be greater if cellular stress stimuli were cascading through MEKK1, and conversely, lower if any concurrent mitogenic signals (e.g. from angiogenic VEGF) were reduced either naturally or iatrogenically. These considerations appear highly relevant to the development of dynamic HHT telangiectasia, and to recently described HHT variant-stress relationships,[11,15] but less relevant to established AVMs. Indeed, in the case presented, there was no discernible effect on pre-existing pulmonary AVMs.

In conclusion, we have presented a clinical case and HHT patient-derived endothelial cell RNASeq data that together provide support for further examination of potential roles for MEK1 inhibition to reduce morbidity from HHT-associated haemorrhage and anaemia. We anticipate that attention to stress stimuli mediated by MEKK1 will further inform optimal pharmaceutical dosing regimens to minimise potential adverse events while maximising therapeutic opportunities.

AUTHOR CONTRIBUTIONS

Claire L. Shovlin conceptualised and designed the research study. Claire L. Shovlin, Dilip Patel, Adrianna Bielowka, Atieh Modarresi, Maria E. Bernabeu-Herrero, Micheala A. Aldred and Ali Alsafi performed the research. Claire L. Shovlin, Atieh Modarresi and Ali Alsafi analysed the data. Jonathan A. Ledermann and Genomics England Research Consortium contributed essential materials. Claire L. Shovlin generated the Figures and Tables, and wrote the paper. All authors have read and approved the manuscript.

ACKNOWLEDGEMENTS

The authors thank the patient for their development and approval of this manuscript, as well as the BOEC donors and 100,000 Genomes Project participants, all of whom provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki and approved by the Hammersmith Local Research Ethics Committee (LREC 2000/5764) and the East of Scotland Research Ethics Service (16/ES/0095).

FUNDING INFORMATION

The blood outgrowth endothelial cells were established with funding support from the National Institute for Health Research Imperial Biomedical Research Centre, London, UK (grant to CLS) and earlier, from the Congressionally Directed Medical Research Programme of the United States Department of Defense (PR152260, 09/01/2016-02/28/2019 grant to MA and CLS). RNA Sequencing was performed with funding from the Imperial College Healthcare NHS Trust (grant to CLS). Whole genome sequencing was made possible through access to the data and findings generated by the 100,000 Genomes Project. The 100,000 Genomes Project is managed by Genomics England Limited (a wholly owned company of the Department of Health and Social Care). The 100,000 Genomes Project is funded by the National Institute for Health Research and NHS England. The Wellcome Trust, Cancer Research UK and the Medical Research Council have also funded research infrastructure. The views expressed are those of the authors and not necessarily those of funders, the NHS, the NIHR or the Department of Health and Social Care.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare. The use of trametinib for the treatment of HHT bleeding is the subject of a patent application by Imperial College London.

DATA AVAILABILITY STATEMENT

Primary sequence data and BOECs used in this research were collected subject to the informed consent of the participants. The non-sensitive data underlying this article are available at 10.5281/zenodo.5201823 and can be used under the Creative Commons Attribution license. Further access to these data and cells will only be granted in line with patient consent, subject to approval by the project ethics board and under a formal Data Sharing Agreement. Primary data from the 100,000 Genomes Project, which is held in a secure research environment, are available to registered users. Please see https://www.genomicsengland.co.uk/about-gecip/for-gecip-members/data-and-data-access for further information.

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