別添４－２

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（分担研究報告書）

「多中心性細網組織球症のゲノム解析に関する研究」

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研究要旨
多中心性細網組織球症(MRH)の分子病態は明らかになっていない。本研究では、代表研究施設で経験した 2 症例に対して、Whole exome sequence, RNA シーケンス等を実施し、MRH の分子病態を解析した。2 症例においてそれぞれ KIF5B-FGFR1 融合および MAP2K1 変異が検出された。本研究結果は、MRH は腫瘍性疾患と考えるべきであり、その治療には化学療法が有用である可能性が示された。

A．研究目的

多中心性細網組織球症（以下 MRH）は特徴的な皮膚病変と進行性破壊性多発関節病変を示すきわめて希な疾患であり、歴史的に関節リウマチに対する治療が実施されることが多い。しかし治療抵抗性の症例があり、MRH がリウマチ性疾患なのか腫瘍性疾患なのか、あるいは他の病態を有するかは不明である。本研究では、MRH 症例の病変と血液より遺伝子を抽出し、Whole exome sequence, RNA シーケンス等を実施することにより MRH の病態を明らかにすることを目的とした。

B．研究方法

研究代表施設である名古屋大学で経験した MRH 症例 2 例に対して、遺伝子解析研究の倫理委員会で承認を得、また患者より研究の同意を得た後に、病変組織および血液より抽出した遺伝子に対して、全エクソームシーケンス（WES）と RNA シーケンスを実施した。また 2 症例について Gene Set Enrichment Analysis (GSEA)を実施した。

(倫理面への配慮)

ヒト遺伝子の取り扱いを含む研究については、ヒトゲノム・遺伝子解析研究に関する倫理指針を遵守する。患者のプライバシーの保護等を念頭においたインフォームドコンセントを得た上で研究を実施する。調査データと、氏名、年齢などの個人データは切り離して管理することにより、個人が特定できないように配慮する。

C．研究結果

WES の結果、症例 1 では明らかなドライバー変異は検出されず、一方症例 2 では MAP2K1 の in-frame deletion と TET2 の non-sense mutation を検出した。RNA シーケンスにより、症例 1 で、KIF5B と FGFR1 を含む新規の in-frame fusion を検出した。GSEA 解析により、症例 1 では KIF5B-FGFR1 融合タンパク質のチロシンキナーゼ活性上昇を示唆するキナーゼの活性化が、症例 2 では MAP2K1 による RAS-MAPK シグナル伝達経路の活性化を示唆する KRAS シグナル伝達の上昇が示された。

D．考察

1937 年以来、MRH は 200～300 例のみが報告されており、MRH の分子病態はよくわかっていなかった。臨床症状が関節リウマチに似ていることから、各種の抗リウマチ薬が使用されてきたが治療抵抗性を示す症例が少なからず存在した。これまで MRH の分子病態を示す解析は実施されていない。2 症例と解析症例数は少ないが、本研究により、MRH が、LCH、エルドハイムチェスター病（ECD）、および若年性黄色肉芽腫の患者に存在するものと同様の RAS-MAPK 経路またはチロシンキナーゼの異常な活性化によって引き起こされる腫瘍性疾患と見なされるべきであることを示唆している。MRH 患者で検出された KIF5B-FGFR1 融合および MAP2K1 変異は薬物治療が可能であり、FGFR1 または MEK 阻害剤を使用した標的療法は最近 ECD などの他の組織球性疾患で実証されているため MRH に対して有効な治療法となる可能性がある。

E．結論

本研究結果は、MRH は腫瘍性疾患と考えるべきであり、その治療には化学療法が有用である可能性が示された。 今後、分子標的療法を含めた MRH の最適な治療アプローチの研究を進めるべきであることが示唆された。

Ｆ．健康危険情報

該当なし

Ｇ．研究発表

１．論文発表

Murakami N, Sakai T, Arai E, Muramatsu H, Ichikawa D, Asai S, Shimoyama Y, Ishiguro N, Takahashi Y, **Okuno Y**, **Nishida Y**.
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２．国際学会発表

Targetable driver mutations in multicentric reticulohistiocytosis (Poster)
Tomohisa Sakai, Norihiro Murakami, Eisuke Arai, Hideaki Muramatsu, Daisuke Ichikawa, Shuji Asai, Yoshie Shimoyama, Naoki Ishiguro, Yoshiyuki Takahashi, **Yusuke Okuno**, **Yoshihiro Nishida**
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Ｈ．知的財産権の出願・登録状況

該当なし

Targetable driver mutations in multicentric reticulohistiocytosis

Multicentric reticulohistiocytosis (MRH) is a very rare systemic disease, characterized by multiple destructive arthritic and papulonodular skin lesions that can also affect other organs including the lungs and heart.[1] MRH is classified as a group C histiocytosis (cutaneous and mucocutaneous histiocytosis)[2] with typical histopathological findings such as histiocytic infiltration, particularly of multinucleated giant cells with eosinophilic cytoplasms. From the first report in 1937, only 200-300 cases of MRH have been reported, and the molecular pathogenesis of MRH remains poorly understood. Given that its clinical manifestations are similar to those of rheumatoid arthritis (RA), it has been suspected that MRH is an autoimmune or inflammatory disease, and treatments similar to those for RA, including administration of corticosteroids, methotrexate, bisphosphonates, and several biological anti-inflammatory agents (etanercept, adalimumab, and infliximab), have been tried.[3,4] Although spontaneous remission is occasionally observed during the first ten years after diagnosis, functional prognosis is usually poor; joint replacement surgery has often been required because of the progression of destructive arthritis, and current treatment is inadequate, especially in severe cases.[4] In this study, we performed a comprehensive genetic analysis in two MRH patients to help elucidate its molecular pathogenesis.

We studied specimens from two patients with MRH and from 13 patients with Langerhans cell histiocytosis (LCH). One of our patients with MRH has been reported elsewhere.[5] Patients provided written informed consent. The Ethics Committee of the Nagoya University Graduate School of Medicine approved this study, which was conducted in accordance with the principles of the Declaration of Helsinki. We performed whole-exome sequencing (WES) and RNA sequencing in two patients with MRH. We performed PCR–amplicon-based targeted deep sequencing covering *BRAF* and *MAP2K1*, and RNA sequencing in 13 patients with LCH. Details of these analyses are provided in the *Online Supplementary Methods*.

A 60-year old female (unique patient number 1, UPN1) visited our hospital because of multiple skin lesions that had first appeared when she was 52 years old. She had a history of breast cancer at the age of 48. Her family history was unremarkable. Fluorodeoxyglucose (FDG) positron-emission tomography (PET) showed the accumulation of FDG in the polyarticular lesions of bilateral upper and lower extremities (Figure 1A). The histological findings of periarticular lesions showed infiltration of histiocytes and multinucleated giant cells (Figure 1C). Immunohistochemical analysis showed CD68 was positive in these cells, whereas LCH markers such as CD1a and Langerin were negative. No abnormalities were revealed in her blood test results, and no blasts appeared in her peripheral blood (*Online Supplementary Table S1*). We diagnosed the patient as having MRH based on the clinical and pathological features.

UPN2 was a 39-year old male who visited our hospital because of disturbances in his daily activities caused by polyarticular nodular lesions and arthralgia in addition to multiple skin lesions (erythematous papules) (*Online Supplementary Figure S1*).[5] He had no remarkable family history. At the age of 30, abnormal chest shadows were found on X-ray images at routine medical examination. A bronchoscopic biopsy revealed the infiltration of histiocytic cells. The disease was followed up without any intervention because of the lack of subjective symptoms. FDG-PET showed abnormal accumulation of FDG in the large and small joints of the body (including phalangeal joints) (Figure 1). We performed a biopsy of a periarticular lesion of the left elbow and diagnosed him as having MRH based on the typical histological findings similar to those of UPN1 (Figure 1C).

We performed WES using biopsy specimens containing histiocytic cells and peripheral blood mononuclear cells derived from the two patients with MRH. We identified eight non-synonymous somatic mutations in the histiocytic cells of each specimen, which were suggestive of clonal expansion (Table 1). While to the best of our

Table 1. Somatic mutations in multicentric reticulohistiocytosis.

UPN	Gene	Nucleic acid change	Amino acid change	VAF
UPN1	*TRPV3*	c.916G>A	p.V306M	0.15
UPN1	*BEND2*	c.1069G>A	p.V357I	0.13
UPN1	*PANK4*	c.512C>T	p.P171L	0.12
UPN1	*NOX1*	c.107C>T	p.A36V	0.12
UPN1	*CLUH*	c.713A>T	p.Y238F	0.11
UPN1	*ADH7*	c.811G>A	p.G271S	0.09
UPN1	*AKAP1*	c.335C>G	p.P112R	0.09
UPN1	*DOCK1*	c.689A>G	p.K230R	0.02
UPN2	*CDK2*	c.431C>G	p.P144R	0.13
UPN2	†*TET2*	c.2890C>T	p.Q964*	0.12
UPN2	SYNE1	c.12137C>T	p.T4046M	0.11
UPN2	†*MAP2K1*	c.305_310delAGATCA	p.103_104delIK	0.07
UPN2	*LIPG*	c.1276C>A	p.L426M	0.04
UPN2	*ZNF233*	c.16-2A>T	(exon 3)	0.04
UPN2	*LRIG2*	c.1576G>C	p.D526H	0.02
UPN2	*OTOF*	c.3614C>T	p.A1205V	0.02

†Mutations for which identical mutations were reported as drivers in the literature or databases. UPN: unique patient number; VAF: variant allele frequency.

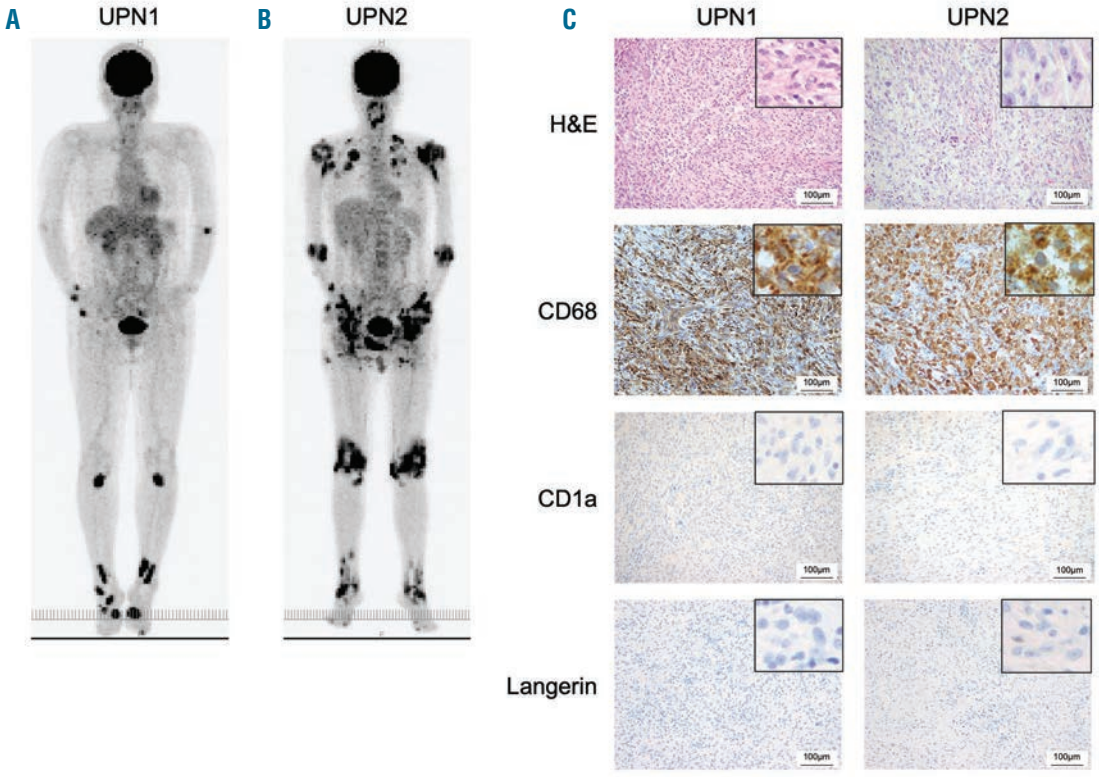

Figure 1. Fluorodeoxyglucose positron-emission tomography (FDG-PET) and histopathological findings of patients with multicentric reticulohistiocytosis (MRH). (A and B) FDG-PET images of Patient 1 (UPN1) (A) and Patient 2 (UPN2) (B). (C) Histopathological and immunohistochemical analyses of biopsy samples. Each inset shows higher magnification. H&E: Hematoxylin & Eosin.

knowledge UPN1 carried no identifiable driver point mutations, we detected an in-frame deletion of *MAP2K1* (encoding dual specificity mitogen-activated protein kinase 1, or MEK1, c.305_310delAGATCA, p.103_104delIK) and a nonsense mutation of *TET2* (encoding methylcytosine dioxygenase TET2, c.2890C>T, p.Q964*) in UPN2. The *MAP2K1* mutation was located in the protein kinase domain of *MAP2K1*, and identical mutations have been reported as gain-of-function driver mutations in melanoma.[6] Taken together, the presence of driver and non-driver mutations suggested neoplastic clonal proliferation of histiocytic cells in MRH.

We identified a novel in-frame fusion involving *KIF5B* (encoding kinesin-1 heavy chain) and *FGFR1* (encoding fibroblast growth factor receptor 1 tyrosine kinase) in UPN1 (Figure 2A, *Online Supplementary Table S2* and *Online Supplementary Figure S3*) by RNA sequencing. The fusion protein contained the dimerization domain derived from KIF5B, which is known to activate RET (*KIF5B-RET*)[7] or ALK (*KIF5B-ALK*)[8] tyrosine kinases, and the tyrosine kinase domain derived from FGFR1, which drives breast cancer (*ERLIN2–FGFR1*),[9] lung squamous cell carcinoma (*BAG4-FGFR1*),[9] and hematologic malignancies (*BCR-FGFR1*).[10] This protein structure strongly suggests gain-of-function tyrosine kinase activity, and we analyzed the differential gene expressions between UPN1 and UPN2 to examine the function of the KIF5B-FGFR1 fusion protein. We found 69 and 191 genes significantly over-expressed in UPN1 and UPN2 with adjusted *P*-values <0.1, respectively (*Online Supplementary Table S3*). A gene set enrichment analysis revealed marked enrichment of genes up-regulated in response to tyrosine kinase activation in UPN1, suggesting upregulation of tyrosine kinase activity of the KIF5B-FGFR1 fusion protein. On the other hand, UPN2 showed enrichment of genes up-regulated in KRAS signaling, indicating the activation of RAS-MAPK signal transduction pathway by the mutated *MAP2K1* (Figure 2B and C).

Because patients with MRH harbored a *MAP2K1* mutation similar to that observed in patients with LCH, and an *FGFR1* tyrosine kinase fusion, which had not been reported in LCH, we guessed that the tyrosine kinase fusions like the *KIF5B-FGFR1* might be present in LCH. To test this hypothesis, we performed a mutational analysis including targeted deep sequencing and RNA sequencing in 13 patients with LCH (*Online Supplementary Table S4*). Among 13 patients, eight and two harbored a *BRAF* p.V600E mutation and a *MAP2K1* p.102_103delEI mutation, respectively. The remaining three patients carried no *BRAF* mutations, *MAP2K1* mutations, or fusion genes.

When the expression profiles were compared between MRH and LCH, LCH showed upregulation of the genes associated with maturation of dendritic cells in response to inflammatory signals (LINDSTEDT_DENDRITIC_CELL_MATURATION_A and LINDSTEDT_DENDRITIC_CELL_MATURATION_B) (*Online Supplementary Table S5* and *Online Supplementary Figure S4*). This finding suggests that the source of tumor cells in MRH might be different from that in LCH, in which marked enrichment of genes associated with DC progenitors and late DC has been reported.[11]

The mutational analysis in our study suggests that MRH should be considered a neoplastic disease caused by the activation of the RAS-MAPK pathway similar to

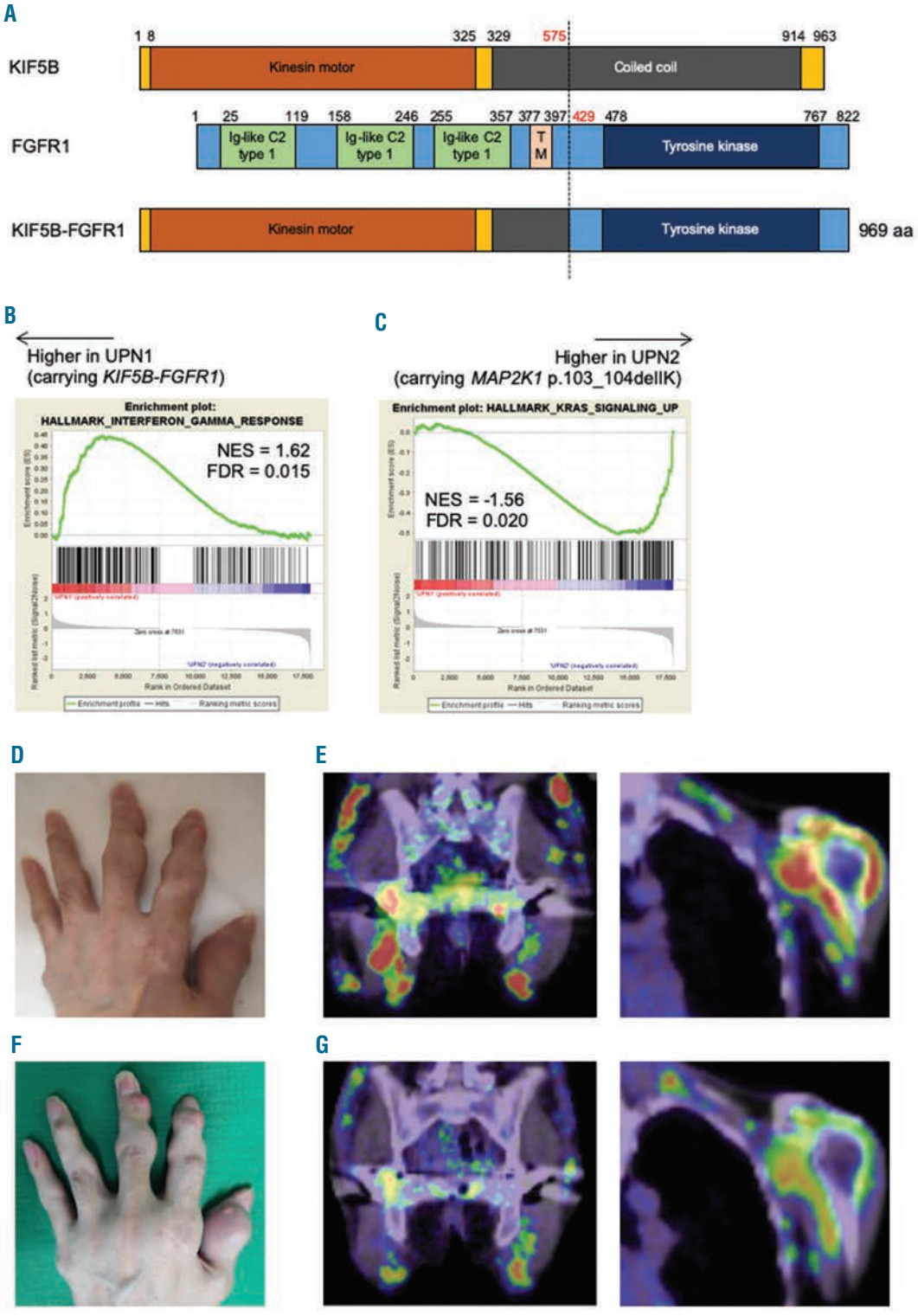

Figure 2. Genetic lesions and treatment response in patients with multicentric reticulohistiocytosis (MRH). (A) Structure of predicted KIF5B-FGFR1 fusion protein. Numbers indicate amino acid residues. Kinesin motor: kinesin motor domain; coiled coil: coiled coil domain; Ig-like C2 type1: immunoglobulin-like C2-type1 domain; TM: transmembrane domain. (B and C) Gene set enrichment analysis comparing the expression profiles obtained from Patient 1 (UPN1) and Patient 2 (UPN2). The genes in the HALLMARK_INTERFERON_GAMMA_RESPONSE gene set were up-regulated in UPN1, whereas those in the HALLMARK_KRAS_SIGNALING_UP were up-regulated in UPN2. NES: normalized enrichment score; FDR: false discovery rate. (D and F) Picture of the left hand of UPN2 before (D) and after (F) chemotherapy showing shrinkage of the periarticular masses, a reduced redness of the skin, and increased wrinkles in the joints. (E and G) Positron emission tomography-computed tomography findings of periarticular lesions of pelvis and left shoulder in UPN2 before (E) and after (G) chemotherapy.

that present in patients with LCH, Erdheim-Chester disease (ECD), and juvenile xanthogranuloma, all of which are classified as L group or C group histiocytosis.[11] We hypothesized that chemotherapy resembling that for LCH might be effective in these patients, and upon approval by our institutional review board, we performed chemotherapy used for LCH (JLSG-02 induction A chemotherapy) (*Online Supplementary Table S6*) to patient UPN2 who harbored *MAP2K1* and *TET2* mutations in his histiocytes.

The patient's disease had been resistant to RA-like immunosuppressive therapies, and he had undergone total joint replacements of the left knee and bilateral hips because of intractable pain emanating from destructive arthritis. After starting chemotherapy, the periarticular masses gradually decreased in size and became soft (Figure 2D and F) and the subjective symptoms including the right knee pain improved. As a result of this response, the patient's right knee did not require the joint replacement surgery that had been considered inevitable before starting chemotherapy. Moreover, positron emission tomography-computed tomography (PET-CT) scan after the treatment showed decreased metabolic activity of tumorous and inflammatory cells (Figure 2E and G). The maximum standardized uptake value (SUVmax) of the right femoral mass decreased from 15.11 to 6.89. However, we had to stop the treatment course because of peripheral neuropathy and reversible cerebral vasoconstriction syndrome (RCVS) that were thought to be chemotherapy adverse-associated events.

Our results suggest that MRH is not an autoimmune or an inflammatory disease but a neoplastic one that may be caused by an aberrant activation of the RAS-MAPK pathway or tyrosine kinases. Both pathways are implicated in the molecular pathogenesis of several histiocytic neoplasms.[11] Both genetic alterations detected in the patients with MRH (*KIF5B-FGFR1* fusion and *MAP2K1* deletion) are druggable, and targeted therapies using FGFR1 or MEK inhibitors may be effective treatments for patients with MRH, as their efficacies have been recently demonstrated in other histiocytic diseases such as ECD.[11]

In current clinical practice, the diagnosis of MRH is completely based on clinical presentation and not on genetic abnormalities. Because our study is limited in terms of the number of patients, the genetic background of MRH still needs to be explored. Further identification of mutations in MRH will clarify the relationships with other hematologic malignancies that share similar mutations with MRH, including myeloproliferative neoplasm or acute leukemia with *FGFR1* rearrangement.[12]

On the basis of the results of our mutational analysis, we administered chemotherapy therapy similar to that used to treat LCH in a patient with the *MAP2K1* mutation. Although we had to stop the chemotherapy because of adverse events (peripheral neuropathy and RCVS), especially attributed to vincristine,[13] we observed improvement in both subjective and objective symptoms, and we think our patient had a partial but clinically substantial good response.

In summary, our results indicate that MRH should be considered a neoplastic disease and suggest promising effects of chemotherapy for its treatment. Further studies are warranted to contribute to the development of optimal therapeutic approaches for MRH, possibly including molecular targeted therapies.

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