Haemophagocytic lymphohistiocytosis as a complication of combination anti-PD-1 and anti-CTLA-4 checkpoint inhibitor immunotherapy for metastatic melanoma, and the outcome of rechallenge with single-agent anti-PD-1 immunotherapy

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SUMMARY

A woman with metastatic melanoma was treated with immunotherapy induction with ipilimumab and nivolumab and radiotherapy to liver metastases. The patient deteriorated shortly thereafter, becoming febrile and hypotensive and requiring admission to the intensie care unit (ICU) for inotrope support. Failure to respond to antibiotics and a negative septic screen prompted further investigation, which ultimately led to a diagnosis of haemophagocytic lymphohistiocytosis (HLH). The patient improved on high dose steroids and was discharged home. Months later, in the context of progressive melanoma, the patient was re-challenged with nivolumab monotherapy and subsequently experienced recurrence of HLH, confirming the aetiology as being immunotherapy related. This case serves as a reminder to consider HLH where there are fevers of unknown origin in an unwell patient receiving immune checkpoint inhibitor therapy and also highlights immunotherapy as a potential cause for HLH, which has rarely been reported in the literature to date.

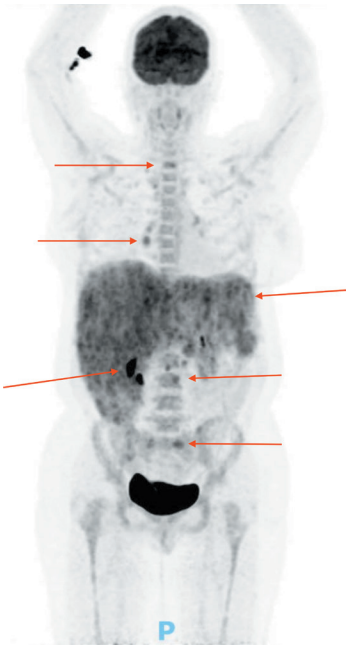

Figure 1 Positron emission tomography scan showing extensive metastatic disease involving the liver, spleen, lung, skeleton and nodes.

BACKGROUND

Recent advances in the use of immunotherapy have revolutionised the treatment of many cancers, including melanoma. Within the past decade, immune checkpoint inhibitors (ICIs) targeting programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) have been demonstrated to significantly prolong overall survival and produce durable responses in patients with metastatic melanoma.[1] However, due to the manner by which these drugs work by up-regulating the immune system, they have the potential to cause serious immune-related adverse events (irAE).[1]

Haemophagocytic lymphohistiocytosis is a rare and aggressive syndrome of excessive immune activation that is thought to result from the absence of normal downregulation driven by activated macrophages and lymphocytes.[2] It is primarily a paediatric illness, with an estimated incidence of 1.2 cases per million children each year, but it is also known to affect adults.[3] In adults, it is often associated with triggers such as infection, malignancy and rheumatological disorders. Clinical features include fever, organomegaly, cytopenias, elevated ferritin, elevated lactate dehydrogenase (LDH) and haemophagocytosis on bone marrow aspirate.[4] The diagnostic criteria from the HLH-2004 guidelines are commonly used to help confirm a diagnosis of HLH. Management involves addressing the underlying cause in addition to the use of corticosteroids and chemotherapeutic agents such as etoposide. However, even with best available treatment, the prognosis is poor, with only a 55% chance of survival.[5]

To date, there have been very few cases reported of ICIs causing HLH.[6–18] Additionally, data relating to outcomes of ICI rechallenge after an index episode of HLH are even scarcer.

CASE PRESENTATION

A woman in her 40s presented with a 1-month history of malaise, nausea, vomiting, fatigue, anorexia and 14 kg of unintentional weight loss on a background of a right shoulder melanoma excision in 1995.

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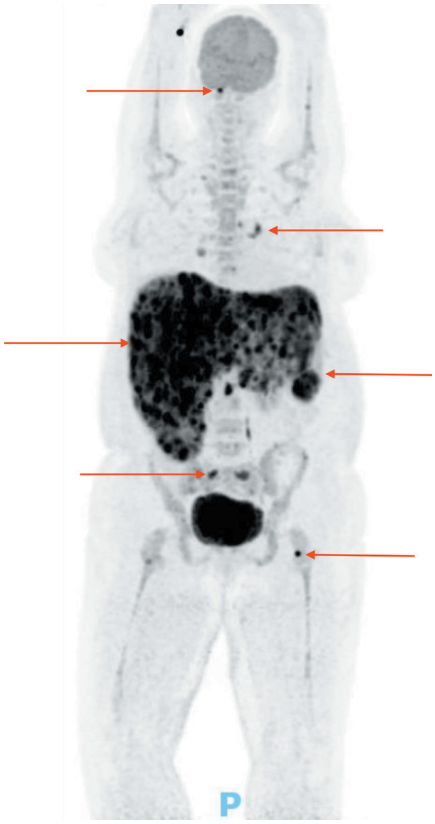

Figure 2 Positron emission tomography scan showing increased uptake compared with previous imaging, consistent with an interval response (pseudoprogression).

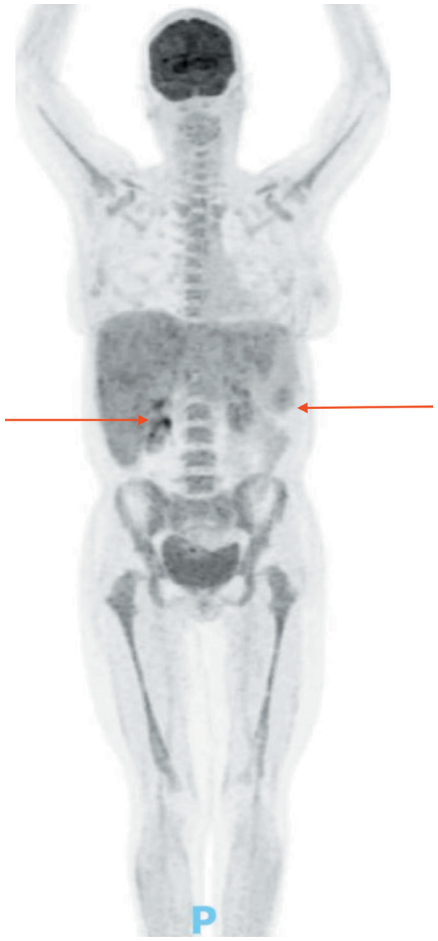

Figure 3 Positron emission tomography scan showing marked metabolic response, with no new sites and only small volume disease in the liver and spleen.

Her medical history was otherwise significant for coeliac disease, which was well controlled with a gluten-free diet. She previously smoked from age 16 to 30 and infrequently drank alcohol. There was no known relevant family history.

CT showed innumerable metastases in the liver, as well as metastases to bone and spleen. MRI of the brain was unremarkable. Subsequent positron emission tomography (PET) showed extensive fluorodeoxyglucose-avid (FDG) metastatic disease involving the liver, spleen, lung, skeleton and lymph nodes, consistent with stage IV melanoma (see figure 1). Liver biopsy confirmed metastatic melanoma with a positive mutation in the B-rapidly accelerating fibrosarcoma (BRAF) V600E gene.

Due to the high volume of disease and the desire to achieve prompt tumour debulking, she was given a 2 week course of dabrafenib upfront, before receiving her first cycle of combination immunotherapy in the form of ipilimumab 3 mg/kg and nivolumab 1 mg/kg. Additionally, because of her particularly heavy burden of liver metastases causing significant symptoms, she was offered palliative radiotherapy to the liver, at a dose of 12 grey in four fractions. Two weeks after her first dose of immunotherapy, on the same day of receiving first fraction of radiotherapy to liver mets, the patient deteriorated, becoming febrile and hypotensive requiring an admission to the intensive care unit (ICU) for inotropic support. She was initially treated with broad-spectrum antibiotics for presumed Gram-negative sepsis from a hepatobiliary source on the basis of fevers, raised C reactive protein and deranged liver function tests (LFTs). Following an initial response to antibiotics and supportive care, the patient was transferred back to the ward haemodynamically stable. Due to concern about the melanoma continuing to rapidly progress in the background, the patient was commenced on encorafenib and binimetinib at this time. Shortly after return to the ward, she deteriorated again with recurrent hypotension, ongoing fevers and type I respiratory failure, and returned to ICU for non-invasive ventilation and vasopressors. Repeat imaging was significant only for pleural effusions, with no focus of infection identified. Multiple sets of blood, urine and faecal cultures were all clear of micro-organisms. A pleural fluid sample was negative for infection, but cytology was consistent with malignancy. Ongoing lack of response to antibiotics, in addition to an entirely negative septic screen, prompted further investigation into the cause of fevers.

The patient was noted to have a bicytopenia, with a haemoglobin of 65 g/L and platelets of 21×10^9/L. A haemolytic screen showed a bilirubin of 82 umol/L, lactate dehydrogenase of 1981 U/L, haptoglobins of 0.3 g/L, reticulocytes of 0.1% and a negative direct Coombs' test. A blood film showed did not demonstrate prominent features of haemolysis or leucoerythroblastosis. Serum ferritin was 19 917 μg/L, which raised the index of suspicion for HLH. A bone marrow biopsy was taken from the right superior iliac spine. This showed a marked increase in macrophages with a proportion demonstrating haemphagocytosis, but significantly, immunohistochemistry was negative for melanoma infiltration, thus supporting a diagnosis of HLH. The patient was treated with methylprednisolone 500 mg intravenously for 3 days before being switched to prednisolone at a dose of 1 mg/kg, which was then gradually tapered. She responded both

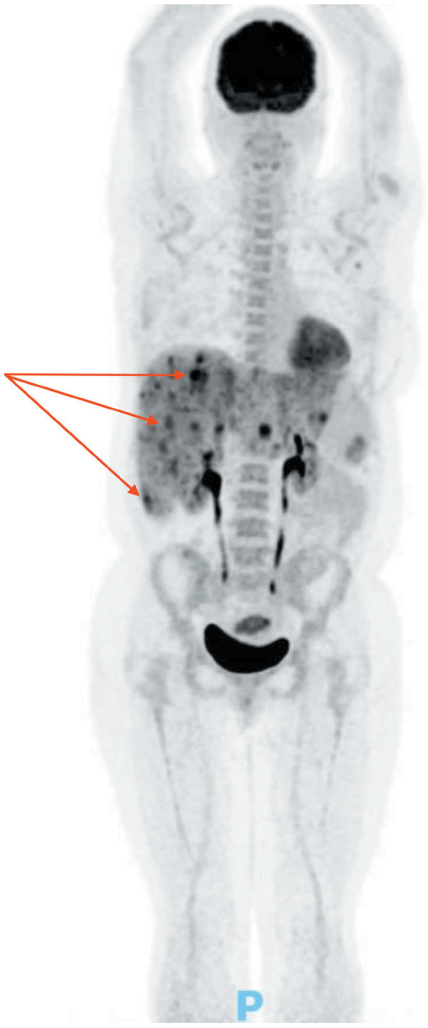

Figure 4 Positron emission tomography scan disease recurrence in the liver, stable disease in the spleen, and persistent complete response in osseous and nodal mets while on encorafenib and binimetanib.

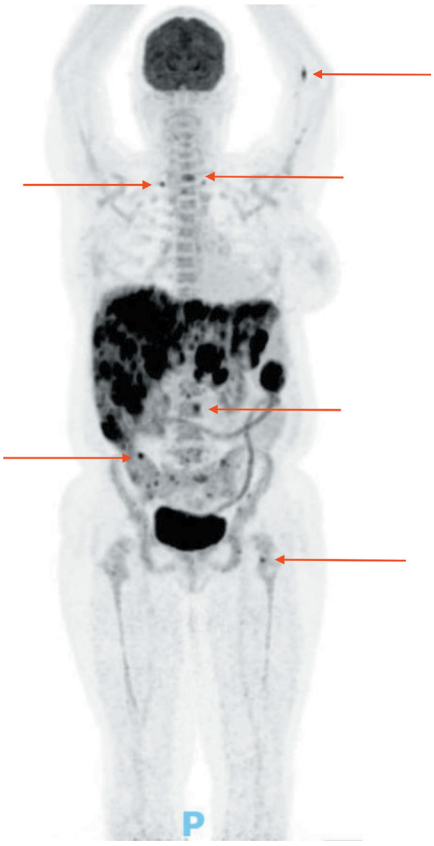

Figure 5 Positron emission tomography scan showing disease progression with new foci in marrow in the upper spine, upper ribs and bony pelvis post nivolumab plus encorafenib and binimetanib.

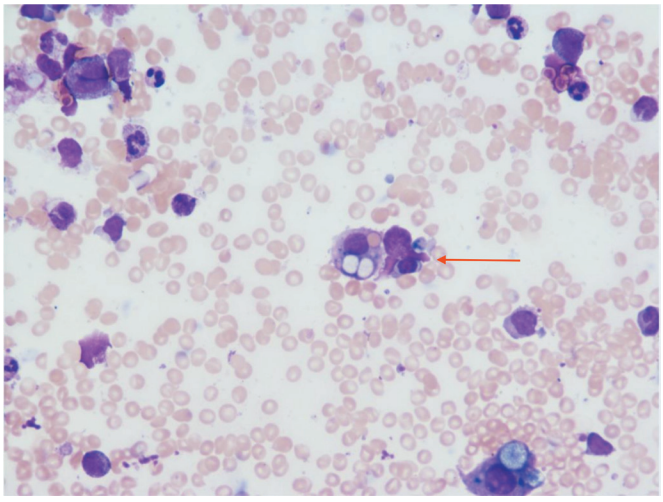

Figure 6 Microscopic image of bone marrow aspirate showing macrophages exhibiting haemophagocytosis.

clinically and biochemically, with a gradual reduction in ferritin levels back to normal range prior to discharge home.

Interval PET scan showed pseudoprogression (see figure 2), a phenomenon where imaging changes mimic tumour progression but are actually due to other causes such as inflammation related to therapy. A further repeat PET 1 month after discharge showed a marked response, with considerable improvement in the volume of metastatic disease (see figure 3). She continued encorafenib and binimetanib. Unfortunately, a PET scan 2 months later showed new uptake in the liver and spleen, consistent with progression of melanoma, and an MRI of the brain demonstrated multiple supratentorial brain metastases (see figure 4). The decision was made to rechallenge with single-agent nivolumab while continuing encorafenib and binimetanib, balancing the risk of HLH recurrence with the hope of achieving another oncological response. At this stage, prednisolone had been reduced to 10 mg/day, and HLH remained quiescent at this dose.

Approximately 2 weeks after the first cycle of nivolumab, the patient was readmitted to the hospital with fevers, lethargy and hypotension requiring return to ICU for vasopressor support. She was treated with piperacillin/tazobactam for sepsis, with a suspected urinary source in view of a preceding 4-week history of dysuria and malodorous urine, for which she had received multiple courses of oral antibiotics. However, urine culture as well as multiple blood cultures were negative. Similar to her previous admission, she failed to respond to antibiotics and she was unable to be weaned off norepinephrine while continuing to have recurrent fevers. Ferritin was again found to be significantly elevated at 11 816 ug/L, consistent with an HLH recurrence

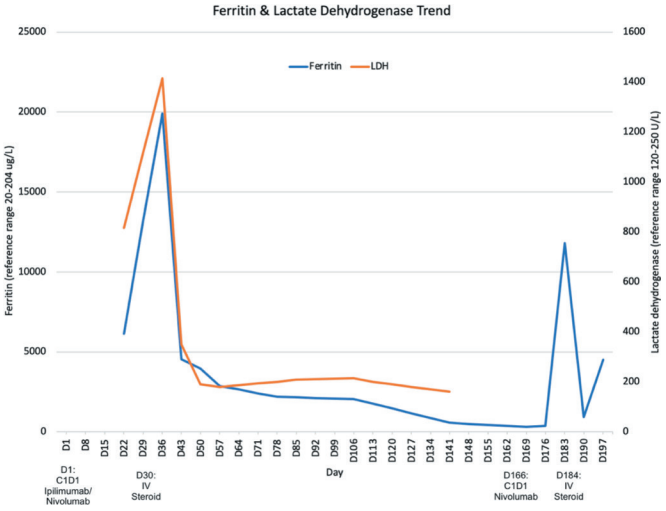

Figure 7 Graph showing ferritin and LDH trend, their relationship to immunotherapy and response to steroids. Created by Zachary Holmes. LDH, lactate dehydrogenase.

secondary to immunotherapy. The patient was treated with intravenous dexamethasone 20 mg/day and improved rapidly.

Restaging PET imaging performed 1 month after nivolumab rechallenge confirmed further marked progression despite nivolumab plus encorafenib and binimetanib (see figure 5). Due to her intolerance of immunotherapy and failure to respond to targeted therapy, the patient was commenced on temozolamide. Less than a month later, the patient represented with seizures and right-sided hemiparesis. CT imaging showed clear enlargement of brain metastases, particularly in the left frontal region, in keeping with the pattern of weakness. Unfortunately, the patient continued to deteriorate and treatment focus was shifted to comfort care shortly before she passed away in the hospital.

INVESTIGATION

Melanoma recurrence was suspected on initial presentation of the patient. CT of the chest, abdomen, pelvis was in keeping with metastases in the liver, bone and spleen. MRI of the brain revealed no central metastases, but PET imaging showed FDG avid disease involving liver, spleen, lung, skeleton and lymph nodes (see figure 1). A subsequent liver biopsy confirmed BRAF V600E mutant-positive metastatic melanoma.

Septic screen was extensive and found no source of infection. CT imaging on admission, in addition to repeat CT of the chest, showed no focus of infection. A total of eight blood cultures, three urine cultures, two faecal cultures, one pleural fluid sample and a respiratory viral swab including COVID-19 were all negative.

Haemolytic screen was performed to investigate bicytopenia. It was significant for anaemia, hyperbilirubinaemia, elevated LDH and low haptoglobins, but the lack of any relevant features on blood film, in addition to a low reticulocyte count and a negative direct Coombs test, outruled a haemolytic process.

HLH diagnosis was aided by the presence of bicytopenia, hepatitis, hypertriglyceridaemia, elevated ferritin, elevated LDH and a bone marrow aspirate showing a marked increase in macrophage activity, with a proportion exhibiting haemophagocytosis (see figure 6). Natural Killer (NK) cell activity and soluble CD 25 were not tested.

Imaging prior to discharge showed a significant oncological response, likely due to immunotherapy (see figure 3). However, follow-up imaging a month after discharge was in keeping with progression, despite treatment with encorafenib and binimetanib (see figure 4). The patient progressed further despite nivolumab rechallenge (see figure 5).

A timeline of ferritin and LDH levels, their relation to immunotherapy and response to steroids is illustrated in figure 7.

DIFFERENTIAL DIAGNOSIS

The patient's initial deterioration, characterised by fevers, tachycardia and hypotension, was highly suspicions for infection, with a hepatobiliary source felt to be likely due to a derangement in LFTs and the known presence of extensive liver metastases, which could theoretically impede biliary outflow. However, the patient did not respond to antibiotics, and an extensive septic screen identified no focus of infection. Cancer-related fevers were considered but would not have explained the persistent and severe hypotension. Fever and constitutional symptoms are common side effects of BRAF and mitogen activated protein kinase kinase (MAP2k/MEK) inhibitors, but the onset of deterioration preceded the commencement of encorafenib and binimetanib in our patient. A haemolytic process was thought

Table 1 Summary of cases of HLH secondary to immune checkpoint inhibitor therapy published in the literature to date

Author	Demographic	Cancer	Immunotherapy	HLH treatment	HLH outcome	Cancer outcome
Sadaat and Jang[6]	58M	Melanoma	Pembrolizumab	Steroids	CR	PD
Hantel *et al*[7]	35F	Melanoma	Ipilimumab/nivolumab	Steroids	CR	CR
Mizuta *et al*[9]	69F	Melanoma	Ipilimumab/nivolumab	Steroids	CR	PR
Chin *et al*[10]	69F	Melanoma	Ipilimumab/nivolumab	Steroids	CR	PD
Kalmuk *et al*[11]	61M	HNSCC	Pembrolizumab	Steroids+etoposide	CR	PR
Al-Samkari *et al*[12]	58F	Breast	Pembrolizumab	Steroids	CR	CR
Malissen *et al*[13]	42M	Melanoma	Ipilimumab	Steroids	CR	Unknown
	81F	Merkel cell carcinoma	Avelumab	Steroids	RIP	RIP
	77M	Melanoma	Nivolumab	Steroids	RIP	RIP
Shah *et al*[14]	76M	Bladder	Pembrolizumab	Steroids+etoposide	CR	Unknown
Takeshita *et al*[15]	63F	NSCLC	Nivolumab	Steroids	CR	PR
Satzger *et al*[16]	26F	Melanoma	Ipilimumab/nivolumab	Steroids+MMF	CR	CR
Lorenz *et al*[17]	68M	Prostate	Pembrolizumab	Steroids+plasmapheresis	CR	CR
Okawa *et al*[18]	78M	NSCLC	Pembrolizumab	Steroids	CR	PR

CR, complete response; F, female; HLH, haemophagocytic lymphohistiocytosis; HNSCC, head and neck squamous cell carcinoma; M, male; MMF, mycophenolate mofetil; NSCLC, non-small cell lung cancer; PD, progressive disease; PR, partial response; RIP, died.

to be possible. Bicytopenia, hyperbilirubinaemia, elevated LDH and low haptoglobins were all in keeping with this, but low reticulocyte count, a negative direct Coombs test and a blood film with no features of haemolysis effectively ruled this out. The results of the haemolysis screen, in addition to the ongoing bicytopenia, pointed towards a bone marrow pathology, with clinical suspicion for HLH increased by a significantly elevated ferritin. The diagnosis was further supported on bone marrow aspirate, with the patient fulfilling five of the eight HLH-2004 diagnostic criteria, namely, fever, bicytopenia, hypertriglyceridaemia, elevated ferritin and haemophagocytosis in bone marrow.

The aetiology of HLH was initially unclear and was thought to be likely due to the immunotherapy, cancer disease burden or a combination of the two. A subsequent recurrence of the HLH, although at a lower severity, shortly after the patient was rechallenged with a single checkpoint inhibitor, confirmed immunotherapy as the cause. We applied Naranjo's algorithm and the WHO–Uppsala Monitoring Centre system for adverse event causality assessment, which both regarded this case as a definite adverse event to immunotherapy.

TREATMENT

Treatments used for melanoma included ipilimumab and nivolumab combination, nivolumab monotherapy, dabrafenib, encorafenib and binimetanib combination and temozolamide. Radiotherapy was given for symptomatic liver metastases.

Treatments used for HLH included intravenous methylprednisolone, intravenous dexamethasone and oral prednisolone.

OUTCOME AND FOLLOW-UP

HLH on both occasions was responsive to steroids. Unfortunately, both combination and single-agent checkpoint inhibitors were shown to cause HLH in this patient. There was also evidence of progression with nivolumab, despite also receiving BRAF inhibitors, leaving limited treatment options for this patient. She was trialled on temozolamide but died approximately 1 month later.

DISCUSSION

Checkpoint inhibitors target transmembrane proteins such as PD-1, PDL-1 and CTLA-4 in order to induce a native T-cell response and ultimately promote tumour apoptosis. The use of combination checkpoint blockade has been proven to be more effective in terms of response rates when compared with monotherapy in metastatic melanoma. However, they frequently cause irAEs such as dermatitis, thyroiditis, colitis, hepatitis and pneumonitis. Rarer, but more severe irAEs related to checkpoint inhibitors include autoimmune haemolytic anaemia, pure red cell aplasia and HLH.

Searching the pharmacovigilance databases found that 92 cases of HLH secondary to nivolumab and 61 cases secondary to ipilimumab had been reported to WHO's Vigibase, while 93 cases secondary to nivolumab and 65 cases secondary to ipilimumab have been reported to the EudraVigilance database.[19 20] It is unclear from these databases whether these immunotherapies were given in combination or if any of them were a rechallenge. A small number of cases of HLH secondary to checkpoint inhibitor therapy have been published in the literature to date and are summarised previously (table 1). All patients received steroids as treatment for HLH, with four patients also receiving etoposide, mycophenolate or plasmapheresis. The majority of patients had a complete response with regard to HLH, which is a strikingly better outcome than those seen in primary HLH.[5] Most patients had at least a partial response to their cancer, with one study, by Kalmuk *et al*, describing a patient who was successfully rechallenged with pembrolizumab for head and neck squamous cell carcinoma progression without HLH recurrence.[11] Notably, no patients with HLH secondary to ipilumab and nivolumab were rechallanged with nivolumab monotherapy, making our case unique.

Learning points

- Consider haemophagocytic lymphohistiocytosis (HLH) in an unwell patient with fevers of unknown source.
- Immune checkpoint inhibitors cause a wide range of immune-related adverse events, including HLH.
- Single-agent and combination checkpoint inhibitors can cause HLH.
- Immune-related HLH is likely more susceptible to steroids than primary HLH.
- There is more to be learnt about the optimal management of this entity and the risk of HLH relapse after rechallenging with immunotherapy.

Patients perspective

The whole experience was quite frightening and frustrating. My main concerns with trying the immunotherapy was will it work and what side effects will I get. I had heard that some people respond really well to this type of immunotherapy though so I was eager to give it a try.

My first stay in hospital was really scary because I felt awful and they didn't know what was causing it. Eventually they found something rare called HLH, which they thought was due to the immunotherapy for my melanoma. This was obviously disappointing, especially considering I had such a good response on my initial scans. The risks and benefits of trying the immunotherapy again were explained to me. My priority was to be able to spend as much time as possible with my family, so I thought taking the risk and going for the immunotherapy again would give me the best chance. Unfortunately, it didn't work out and I got HLH again. This was devastating as I knew that there are limited options left to treat my melanoma.

Contributors ZH provided care to the patient while she was in the hospital and was also the principal contributor to the drafting of the report. AH also provided care to the patient during her admission and also contributed to the writing and editing of the report. AC contributed significantly to researching and editing the article.

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Case reports provide a valuable learning resource for the scientific community and can indicate areas of interest for future research. They should not be used in isolation to guide treatment choices or public health policy.

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