Vemurafenib provides a rapid and robust clinical response in paediatric Langerhans cell histiocytosis with the BRAF V600E mutation but does not eliminate low-level minimal residual disease based on ddPCR using cell-free circulating DNA

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Abbreviation table

LCH	Langerhans cell hisitiocytosis
RO	Risk organs
2-CdA	Cladribine
Ara-C	Cytoside-arabinosine
DAS	Disease activity score
ddPCR	Digital droplet PCR
cfDNA	Cell-free DNA
PCR	Polymerase chain reaction
MRD	Minimal residual disease

Abstract

Background

Langerhans cell histiocytosis (LCH) involves abnormal proliferation of Langerhans cells (LC), which is typically driven by the BRAF V600E mutation. High-risk LCH has a poor prognosis.

Procedure

Fifteen children (5 girls, 10 boys) with BRAF V600E+ LCH received vemurafenib (initial dose median 40 mg/kg/day, range: 11–51.6 mg/kg/day) between March 2016 and February 2020. All patients had previous received LCH-directed chemotherapy. The median age at LCH onset was 2 months (range: 1–28 months) and the median age at the start of vemurafenib treatment was 22 months (range: 13–62 months). The median disease activity score (DAS) at the start of vemurafenib treatment was 12 points (range: 2–22 points).

Results

The median duration of vemurafenib therapy was 29 months (range: 2.4–45 months). All patients responded to treatment, with median DAS values of 4 points (range: 0–14 points) at week 4 and 1 point (range: 0–3 points) at week 26. Toxicities included skin/hair changes (93%) and non-significant QT prolongation (73%). Two patients died, including 1 patient who experienced hepatic failure after NSAID overdose and 1 patient who developed neutropenic sepsis. Electively stopping vemurafenib treatment resulted in relapse in 5 patients, and complete cessation was only possible for 1 patient. Digital droplet PCR for BRAF V600E using cell-free circulating DNA revealed that 7 patients had mutation statuses that fluctuated over time.

Conclusion

Our study confirms that vemurafenib treatment is safe and effective for young children with BRAF V600E+ multisystem LCH. However, treatment using vemurafenib does not completely eliminate the disease.

Introduction

Langerhans cell histiocytosis (LCH) is a rare disease that involves abnormal proliferation and dissemination of Langerhans cells. This disease typically occurs in infants and children, with manifestations ranging from a single bone or skin lesion to severe multisystemic disease. Involvement of the bone marrow, spleen, and/or liver (risk organs, RO) is associated with an especially unfavourable prognosis. A solitary bone lesion usually does not require any treatment and resolves spontaneously [1], although multisystem disease with RO involvement can have a 5-year survival rate of ≤50% in cases that are refractory to first-line treatment using vinblastine and steroids [2]. Salvage treatment can be performed using cladribine (2-CdA at 9 mg/m^2/day) and intermediate-dose cytosine-arabinoside (Ara-C)[3]. This regimen provides an impressive response in refractory/relapsed patients, especially with RO involvement, although it is also associated with high myelotoxicity and considerable infectious morbidity. Patients also have a poor prognosis if their disease activity score (DAS) does not decrease by ≥5 points after the first course of treatment using 2-CdA plus Ara-C. Allogeneic hematopoietic stem cell transplantation is a potentially curative option, but is also associated with toxicity and mortality[4].

In 2010, the BRAF V600E mutation was found to be the pathological substrate for 57% of archived LCH specimens, which confirmed the neoplastic nature of this disease [5]. This information guided targeted treatment using BRAF V600E inhibitors for patients with BRAF-mutant histiocytic disorders, which provided impressive efficacy among adult patients with Erdheim-Chester disease and LCH [6,7]. A retrospective international study also confirmed that vemurafenib was a powerful treatment for LCH among children[8]. However, the optimal dosage, treatment duration, side effect management, and safety of prolonged use remain unclear among paediatric patients. Therefore, we report our experience using vemurafenib, with or without chemotherapy, to treat paediatric patients with BRAF V600E+ relapsed or refractory multisystem LCH. Five patients from the current cohort have already been reported in the previous retrospective international study[8].

Patients and Methods

The study was approved by Local Ethics Committee (№ of approval 3e/1-18) and all patients' legal representatives provided written informed consents in accordance with Declaration of Helsinki.

This retrospective single-centre study included 15 patients (10 boys, 5 girls) with BRAF V600E+ LCH who received vemurafenib between March 2016 and February 2020. The study's retrospective protocol was approved by our institutional review board. All patients had previously received various chemotherapy regimens for the LCH, and the first-line therapy for all patients was ≥6 weeks of vinblastine plus prednisolone, according to the LCH-IV protocol. Seven patients received at least 1 course of treatment using Ara-C plus 2-CdA and 1 patient received monotherapy using 2-CdA. Disease activity was quantified using the DAS described by Donadieu et al.[9]. Most patients started vemurafenib treatment at a fixed dose of 480 mg/day (median: 40 mg/kg/day, range: 11–51.6 mg/kg/day), and the pills were crushed and administered orally with food. However, the dose was reduced to 240 mg/day throughout the maintenance period after the patient responded to treatment, with a median maintenance dose of 13.15 mg/kg/day (range: 10.9–20 mg/kg/day). No relapses occurred after the dose was lowered.

Samples from the LCH lesions were obtained using punch or surgical biopsy and were tested for the V600E mutation using standard PCR. Positive results were confirmed using Sanger sequencing. Droplet digital PCR (ddPCR) was performed using circulating free DNA (cfDNA) from blood samples that were collected in 10-mL PlasmaProtect tubes (Evrogen), which are designed to prevent cellular DNA from being released into the plasma. After centrifugation and plasma collection, cfDNA enrichment was performed in 4 steps using a QIAmp Circulating Nucleic Acid Kit. The PCR mixtures with two probes (wild type and V600E-specific) were analysed using a QX200 droplet generator with AutoDG (Bio-Rad), which automatically generates droplets with or without a piece of DNA and performs PCR for each generated droplet. The results were analysed using QuantaSoft software (Bio-Rad). Seven patients underwent serial testing of cfDNA during their vemurafenib monotherapy.

Complete response to the therapy was defined as no or minimal clinical signs of disease, which corresponded to DAS values of 0–1 points. Partial response was defined as minimal clinical signs of disease, which corresponded to DAS values of 2–4 points. Adverse events were identified and graded according to version 5.0 of the Common Terminology Criteria for Adverse Events.

Results

The patients' baseline characteristics are presented in Table 1. The median age at disease onset was 2 months (range: 1–28 months), the median age at diagnosis was 13 months (range: 5–31

months) and the median age at the start of vemurafenib treatment was 22 months (range: 13–62 months). Twelve of the 15 patients (75%) had RO involvement. The lesions were classified as skin lesions (14 patients), bone lesions (7 patients), lymph node lesions (3 patients), extensive lung lesions (1 patient), lacrimal gland lesions (1 patient), and central nervous system lesions (1 patient with diabetes insipidus and 1 patient with neurodegenerative lesions).
All patients had active disease at the start of vemurafenib therapy, with a median DAS value of 12 points (range: 2–22 points). Fourteen patients had the BRAF V600E mutation in at least one LCH lesion, based on the standard PCR result and confirmation using Sanger sequencing. In addition, all 12 patients who also underwent ddPCR analysis had BRAF V600E in their cfDNA (in addition to the confirmation using Sanger sequencing). Moreover, ddPCR revealed a positive result in the cfDNA for the 1 patient whose lesion was not positive for BRAF V600E based on the PCR and Sanger sequencing results.

Twelve of the 15 patients experienced dramatic improvements in their clinical condition and laboratory abnormalities. Fever resolved within 4 days after starting vemurafenib treatment and had subsided in <24 h for 2 patients. No patients required red blood cell or platelet transfusions after 1 week of vemurafenib therapy. Normalization of liver and spleen size generally occurred within several weeks, although mild hepatosplenomegaly persisted in 4 patients. Figure 1 shows the DAS changes over time, which generally improved early during treatment and then reached a plateau. The median DAS values were 4 points (range: 0–14 points) at 4 weeks and 1 point (range: 0–3 points) at 6 months. Twelve patients experienced complete or partial response during the first 3 months of therapy. However, 3 patients (cases 5, 13, and 14) did not experience remarkable improvements, and all of these patients had received vemurafenib as third-line therapy. Nevertheless, vemurafenib therapy was considered warranted for these patients, despite their relatively moderate disease, because of their refractoriness to previous therapies.

Nine patients received concomitant chemotherapy (Figure 2 and Table 1). Four patients received vinblastine plus prednisolone followed by 6-mercaptopurine plus methotrexate without any changes in disease activity. Vemurafenib withdrawal was attempted for 3 patients, who all experienced disease relapse. Seven patients (including 2 patients who had previously received vinblastine, prednisone, methotrexate, and 6-mercaptopurine) received several courses of low-dose Ara-C plus low-dose 2-CdA, although vemurafenib withdrawal without relapse was only possible for 1 patient at the time of this report. In 5 cases, vemurafenib withdrawal resulted in relapse (3 patients experienced prompt relapse in ROs, 1 patient experienced relapse as a central

nervous system lesion, and 1 patient experienced relapse as a skin rash). One patient died during the concomitant therapy.

Adverse events were common and generally involved skin rash (93% of patients) or QTc prolongation (73% of patients), although 3 patients experienced complete hair loss, including the eyebrows and eyelashes. All events were considered Grade ≤3 according to the Common Terminology Criteria for Adverse Events. Skin toxicities varied from small papulae to extensive septal panniculitis, which was confirmed via biopsy. In most patients, the skin rash persisted in mild form during continued vemurafenib treatment, although 2 patients required analgesics because of painful skin rash. The QTc elongation was mild (corrected index: <0.5 s) and never required vemurafenib withdrawal. The severity of the adverse events did not seem to be related to specific treatment factors (e.g., dose).

Two patients died. One patient (case 2) was not able to tolerate the vemurafenib treatment, because of intractable vomiting caused by viral gastroenteritis and probably by vemurafenib itself. That patient received salvage therapy using clofarabine but died because of neutropenic sepsis shortly after the chemotherapy. The second patient (case 5) developed an odontogenic infection after returning home between courses of low-dose Ara-C and low-dose 2-CdA. The patient consumed a large amount of nonsteroidal anti-inflammatory drugs (including paracetamol), which led to severe liver failure and death because of lung haemorrhage. Autopsy revealed no LCH lesions in the liver, spleen, or bone marrow, as well as negative PCR results for the V600E mutation.

Serial testing was performed using 26 ddPCR samples that were obtained from 7 patients during their vemurafenib monotherapy. Most patients had their V600E allelic load in the cfDNA decrease by several fold during the first 6 months of treatment and then stabilize at low but still detectable levels (Figure 3).

Discussion

There is no single explanation for the heterogeneity of LCH, its clinical diversity, and the observation of self-healing in some cases. A popular theory that was raised several decades ago considers LCH an inflammatory disorder that is mostly mediated by cytokines and intercellular interactions[10,11]. However, in 2010, Badalian-Very et al. proposed that LCH was a neoplastic

disease based on the gain-of-function BRAF V600E mutation being detected in 57% of archived LCH samples [5]. This mutation leads to constitutive activation of the Ras/Raf/MEK/ERK pathway, and is one of the most common oncogenic mutations that is observed in approximately 30 neoplasm types[12]. The MAP2K1 mutation was also discovered in 50% of patients with wild-type BRAF[13]. The remaining 25% of patients have a broad range of mutations that affect ARAF, MAP3K1, NRAS, PI3CA, and other targets[14]. These data suggest that LCH is best characterized as a clonal myeloid neoplasm that is driven by Ras/Raf/MEK/ERK pathway activation with a prominent inflammatory component, and this information has generated interest in using targeted therapy to treat LCH.

Mutation-specific or pathway-directed blockade was considered before the role of BRAF in LCH was clarified, as patients with high-risk or refractory LCH and other histiocytic disorders had been empirically treated, albeit with limited effect, using tyrosine-kinase inhibitors since 2004[15,16]. However, the introduction of targeted therapy ushered in a new era for treating various neoplasms[17], and in 2013 Haroche et al. reported the "dramatic efficacy" of vemurafenib treatment for a patient with Erdheim-Chester disease [6]. Subsequent reports described the successful use in paediatric cases of LCH [18–20], which generally involved critically ill infants who experienced fever resolution after the first vemurafenib dose and achieved transfusion independence after a week of therapy. However, similar to our findings, attempts to stop vemurafenib rapidly led to complete relapse, which could not be prevented even using concomitant chemotherapy[21]. Most of our patients were young children or infants with aggressive multisystem LCH, which was refractory to first-line or second-line conventional chemotherapy, and had active disease at the start of vemurafenib treatment. In this context, LCH with RO involvement that is refractory to disease-specific chemotherapy is thought to have a poor prognosis, with high risks of late sequelae, severe irreversible disability, and death. Thus, a dramatic improvement in the DAS after just 4 weeks of therapy, and stably low DAS values at 3 months, suggest that vemurafenib treatment is an important advance in the treatment of LCH.

Despite its efficacy, targeted therapy must also be considered from the perspective of whether it can be discontinued, when that can be attempted, and the quality of the supporting data. The prototypic example is chronic myeloid leukaemia, in which several continuous years of minimal residual disease (MRD) negativity during BCR/ABL kinase inhibitor treatment allows for safe drug discontinuation and durable treatment-free remission in nearly one-half of patients[22]. However, we have encountered rapid disease recurrence in all of our patients after stopping vemurafenib treatment, which agrees with findings from a European and Mediterranean

collaborative group[8]. To overcome that, we opted for the combination of the vemurafenib treatment and concomitant chemotherapy.

Nine of our patients received chemotherapy plus targeted therapy. In some cases, the vemurafenib treatment was combined with first-line chemotherapy (vinblastine plus prednisolone induction followed by 6-mercaptopurine plus methotrexate maintenance), and we attempted to stop the vemurafenib treatment after 6 months while continuing maintenance therapy. However, that approach was ineffective as 3 of 4 patients experienced relapse, which ranged from limited skin disease to severe multisystem disease with cytopenia, hepatosplenomegaly and other LCH-specific symptoms. One patient stopped the maintenance therapy while continuing the vemurafenib treatment. We also combined vemurafenib with cytarabine and cladribine, which is a popular second-line chemotherapy that is effective for refractory/relapsed LCH. However, we slightly modified the protocol designed by Rosso et al. [23] and used cytarabine at 100 mg/m^2 every 12 h on days 1–5 and cladribine at 6 mg/m^2/day on days 1–5 (3 courses), with 3 maintenance courses of cladribine monotherapy. Among the 7 patients who received this regimen, 1 patient died after experiencing combined vemurafenib toxicity and nonsteroidal anti-inflammatory drug overdose, 4 patients experienced relapse soon after stopping vemurafenib and promptly re-started treatment, 1 patient experienced stable treatment-free partial remission, and 1 patient discontinued chemotherapy because it did not resolve persistent disease (hepatosplenomegaly and mild cytopenia).

Our previous experience, as well as the experience of other groups, indicates that higher dose cytarabine (500–1,000 mg/m^2/day for 5 days) plus standard dose 2-CdA (9 mg/m^2/day for 5 days) resulted in long-term relapse-free survival and cure for most patients with refractory multisystem LCH. Thus, conventional doses of cytarabine with lower doses of 2-CdA seem to be unable to eradicate early the LCH-initiating progenitors. This may be related to decreased sensitivity to conventional drug doses or possibly related to decreased drug penetration at those doses into the sanctuaries where the disease-initiating progenitors reside.

At the time of this report, our patients had a median treatment duration of 29 months (range: 2.4–45 months) and only 1 patient was able to stop vemurafenib treatment without reactivation. The other patients are still receiving vemurafenib and have no signs of active disease. Nevertheless, according to the chronic myeloid leukaemia paradigm, the treatment duration may be too short to have eradicated the myeloid progenitors of LCH. This is important because, unlike the various assays that can be used to detect MRD in acute leukaemia, there has historically been no reliable

method for detecting LCH because of the focal lesion distribution and lack of suitable genetic markers. Promisingly, ddPCR can be used to measure MRD in the peripheral blood by assessing tumour-specific mutations in cfDNA, even at very low amounts that are released during natural tumour cell turnover or chemotherapy-related apoptosis[24]. However, ddPCR positivity is not correlated with the DAS (Pearson's correlation coefficient = 0.56) and appears to reflect the persistence of disease and potential for relapse, rather than disease activity.

All of our patients with the BRAF V600E mutation based on standard PCR also had positive results from ddPCR using cfDNA. Furthermore, 1 patient (case 11) had the mutation detected in cfDNA using ddPCR, despite negative results from standard PCR using samples from a skin biopsy and bone marrow aspirate. This patient exhibited prompt clinical and biological responses to vemurafenib treatment. Interestingly, some of our patients had negative cfDNA findings for BRAF V600E during vemurafenib therapy but subsequently had positive findings later in their treatment. This agrees with the results reported by Heritier et al.[25], who noted that relapse was possible after vemurafenib withdrawal even in patients with mutant cfDNA levels of <0.001%. Thus, stable long-lasting complete clinical response and ddPCR negativity without low-level fluctuations will be needed to safely consider treatment discontinuation.

A major concern regarding long-term vemurafenib treatment is its potential toxicity, which has been observed in patients with Erdheim-Chester disease[7]. Fortunately, the safety profile of vemurafenib is more favourable, even with prolonged use, in infants and young children. Furthermore, the addition of vemurafenib treatment does not appear to increase the toxicity of the vinblastine/prednisolone/6-mercaptopurine/methotrexate and cytarabine/cladribine regimens.

Vemurafenib monotherapy does not appear to eradicate clonogenic LCH progenitors, which suggests that a combination of vemurafenib and low-intensity chemotherapy might be needed to cure the patient and shorten their vemurafenib exposure. Although our findings do not support that theory, it is important to note that our cohort included refractory patients who were heavily pre-treated with different chemotherapy regimens. Thus, well-designed studies are needed to develop appropriate combination therapy that incorporates vemurafenib, especially based on the mutant allele load as an MRD-like marker. Nevertheless, we observed dramatic and rapid clinical improvements in most of our patients, who had severe multisystemic LCH with RO involvement, which suggests that vemurafenib might be a useful part of first-line treatment in this population. Considering that and the high incidence of BRAF-mutant disease in that cohort

we suggest that BRAF inhibition could be initiated before the BRAF V600E status confirmation, just like all-trans retinoic acid in patients with acute promyelocytic leukaemia.

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Author Contribution

MM, AMa, DE designed the study. DE, IK, ZA, AI collected and aggregated the data. ER and DO did molecular analysis and interpreted the data. AMi did the pathology analysis. GN, MM, AMa, DE, KI, AI analysed the data. DE wrote the article. All authors critically revised the article.

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Table 1. Patient characteristics.

Case number/ sex	AaM, months	AaDx, months	AaV, months	Organs involved	Previous therapy	Vemurafenib		Concomitant therapy	Attempted to stop vemurafenib treatment	Status	Vemurafenib treatment, months
						Starting dose, mg/kg/day	Current dose mg/kg/day				
1/M	2	10	13	L, S, BM, Bo	VBL+PRED	48	12.3	VBL + PRED+ MTX + 6-MP; LD Ara-C + LD 2-CdA	Yes	Alive, receiving combined therapy	39.5
2/M	2	7	17	L, S, BM, Sk	VBL+PRED, ARA-C + 2-CdA	39.7	None	None	No	Dead (sepsis)	2.4
3/M	3	5	22	L, S, BM, LN, Sk	VBL+PRED, ARA-C + 2-CdA	34.8	13.3	LD Ara-C + LD 2-CdA	No	Alive, receiving vemurafenib monotherapy	45
4/M	1	6	18	L, S, BM, LN, Sk	VBL+PRED	41.7	12.9	VBL+PRED+MTX+6-MP	Yes	Alive with relapsed disease, receiving vemurafenib	29
5/F	1	22	26	L, S, BM, Sk, Bo, Lu	VBL+PRED, ARA-C + 2-CdA	51.6	15.8	VBL+PRED+MTX+6-MP; LD Ara-C + LD 2-CdA	Yes	Alive with skin-only relapsed disease, receiving vemurafenib monotherapy	30
6/F	9	22	24	L, S, BM, DI, Bo, Sk	VBL+PRED	44.4	None	LD Ara-C + LD 2-CdA	No	Dead (toxicity)	2.6

7/M	4	17	18	L, S, BM, Sk	VBL+PRED	47	17.4	LD Ara-C + LD 2-CdA	Yes	Alive, receiving vemurafenib monotherapy	35.8
8/F	2	13	14	L, S, BM, Sk	VBL+PRED	48	16.3	LD Ara-C + LD 2-CdA	Yes	Alive, receiving vemurafenib monotherapy	34.3
9/M	1	13	23	L, S, BM, Sk, Bo	VBL+PRED, ARA-C + 2-CdA	40	13.0	None	No	Alive, receiving vemurafenib monotherapy	36.5
10/M	2	5	21	L, S, BM, Sk	VBL+PRED+MTX	40	None	LD Ara-C + LD 2-CdA	Yes	Alive with partial response, no therapy	3.9
11/F	10	19	40	L, S, BM, Sk	VBL+PRED, ARA-C + 2-CdA	14	7.5	None	No	Alive, receiving vemurafenib monotherapy	17.9
12/M	2	7	23	Sk, Bo, LN	VBL+PRED, ARA-C + 2-CdA	21.8	20	None	No	Alive, receiving vemurafenib monotherapy	19.1
13/M	28	31	62	Sk, Bo, lacrimal glands	VBL+PRED+MTX, mono 2-CdA	11	10.9	None	No	Alive, receiving vemurafenib monotherapy	17.4
14/M	5	13	59	Sk, Bo, CNS	VBL+PRED+MTX+6-MP, ARA-C + 2-CdA	16	11.5	None	No	Alive, receiving vemurafenib monotherapy	29.3
15/F	9	18	18	L, S, BM, Sk	VBL+PRED+6-MP	24.7	17	VBL+PRED+MTX+6-MP	No	Alive, receiving vemurafenib monotherapy	24.9

AaM: age at manifestation, AaDx: age at diagnosis, AaV: age at the start of vemurafenib treatment, L: liver, S: spleen, BM: bone marrow, Bo: bones, Lu: lungs, LN: lymph nodes, CNS: central nervous system, DI: diabetes insipidus, Sk: skin, LD Ara-C: cytosine-arabinoside at 100 mg/m^2 every 12 h

for 5 days, Ara-C: cytosine-arabinoside at 500 mg/m^2 every 12 h for 5 days, LD 2-Cda: cladribine at 6 mg/m^2/day for 5 days, 2-CdA: cladribine at 9 mg/m^2/day for 5 days.

Figure 1. Temporal changes in the disease activity scores according to vemurafenib treatment duration.

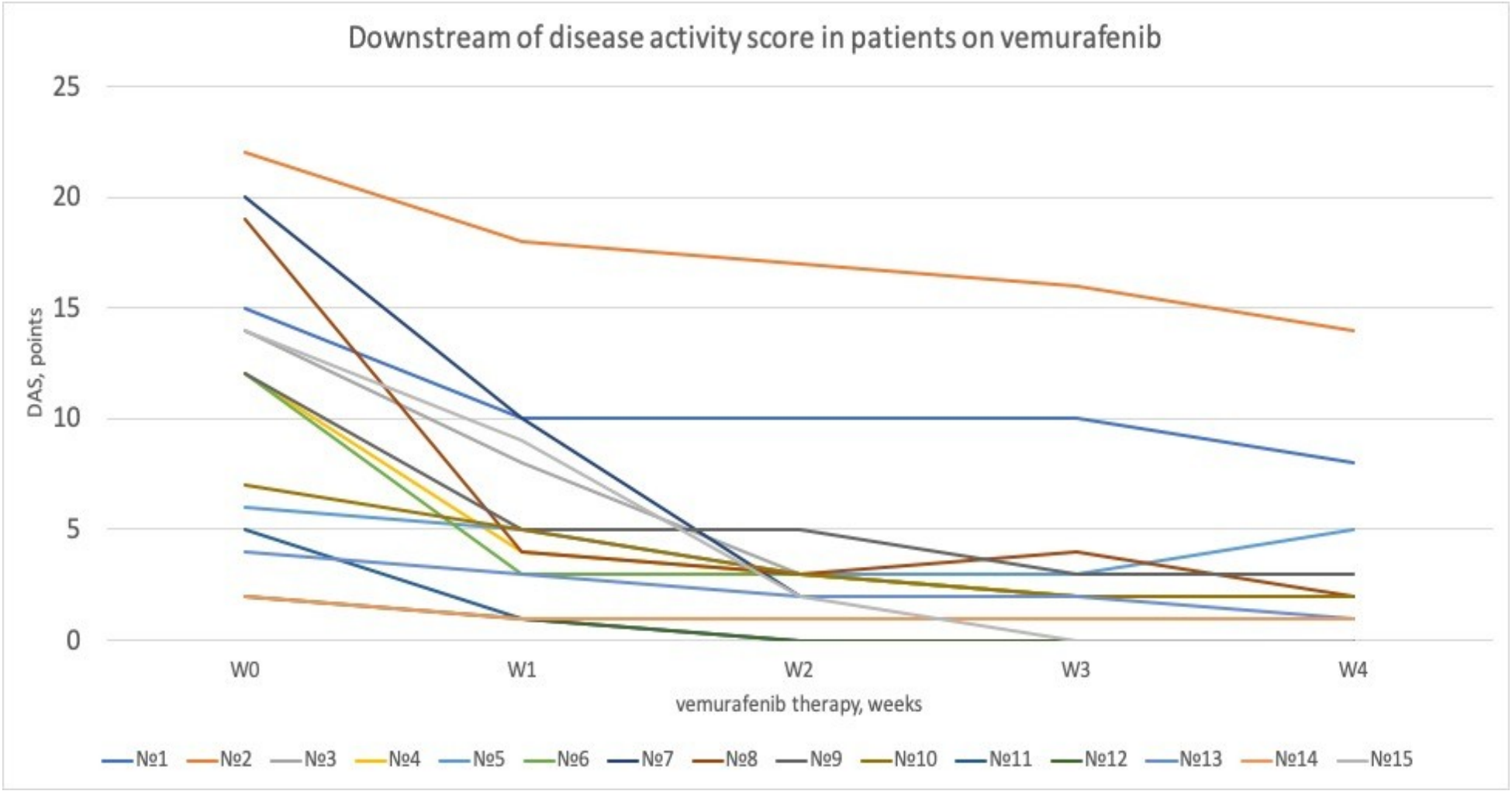


Figure 2. Swimmer plots showing the different concomitant therapies.

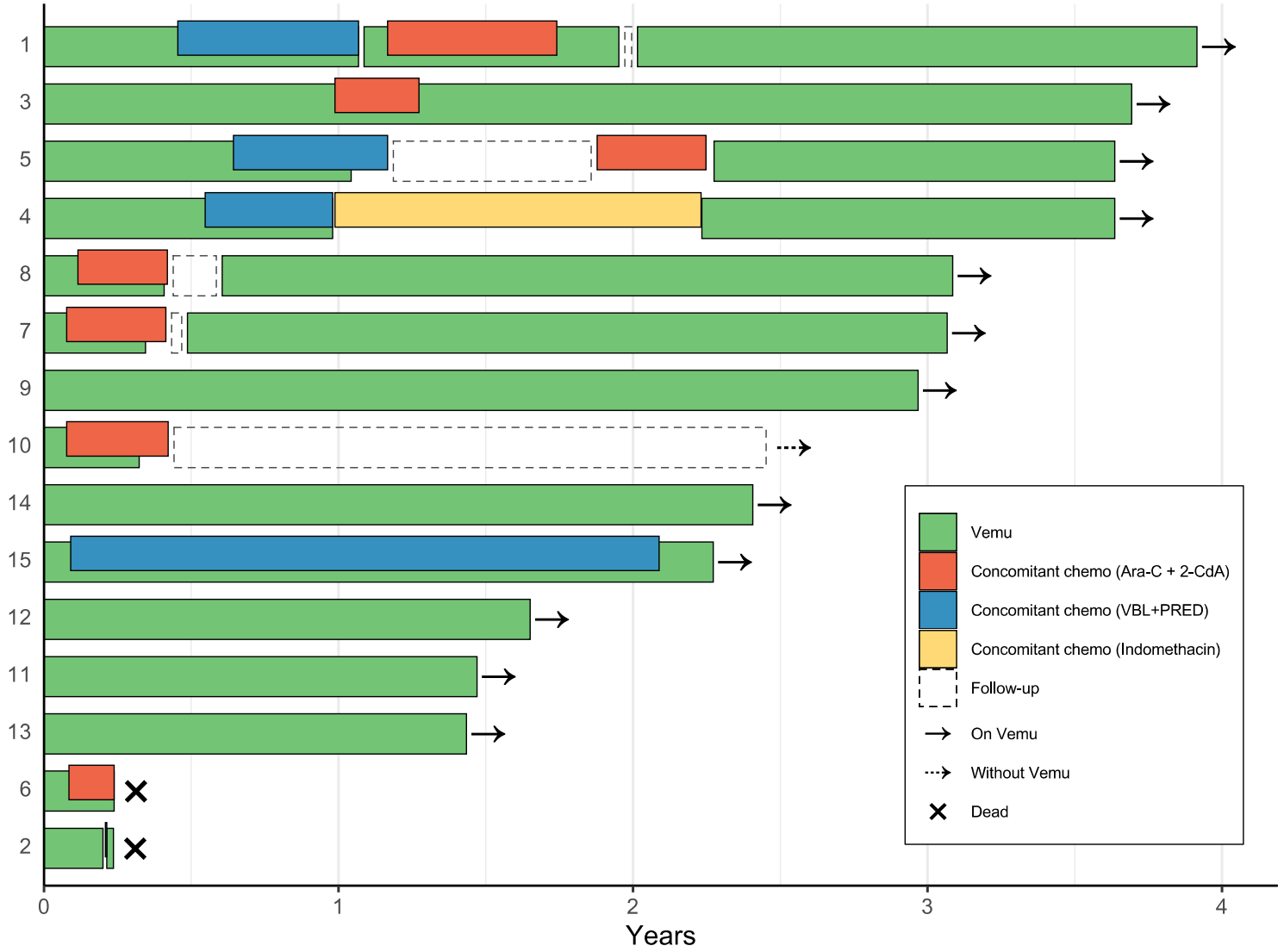
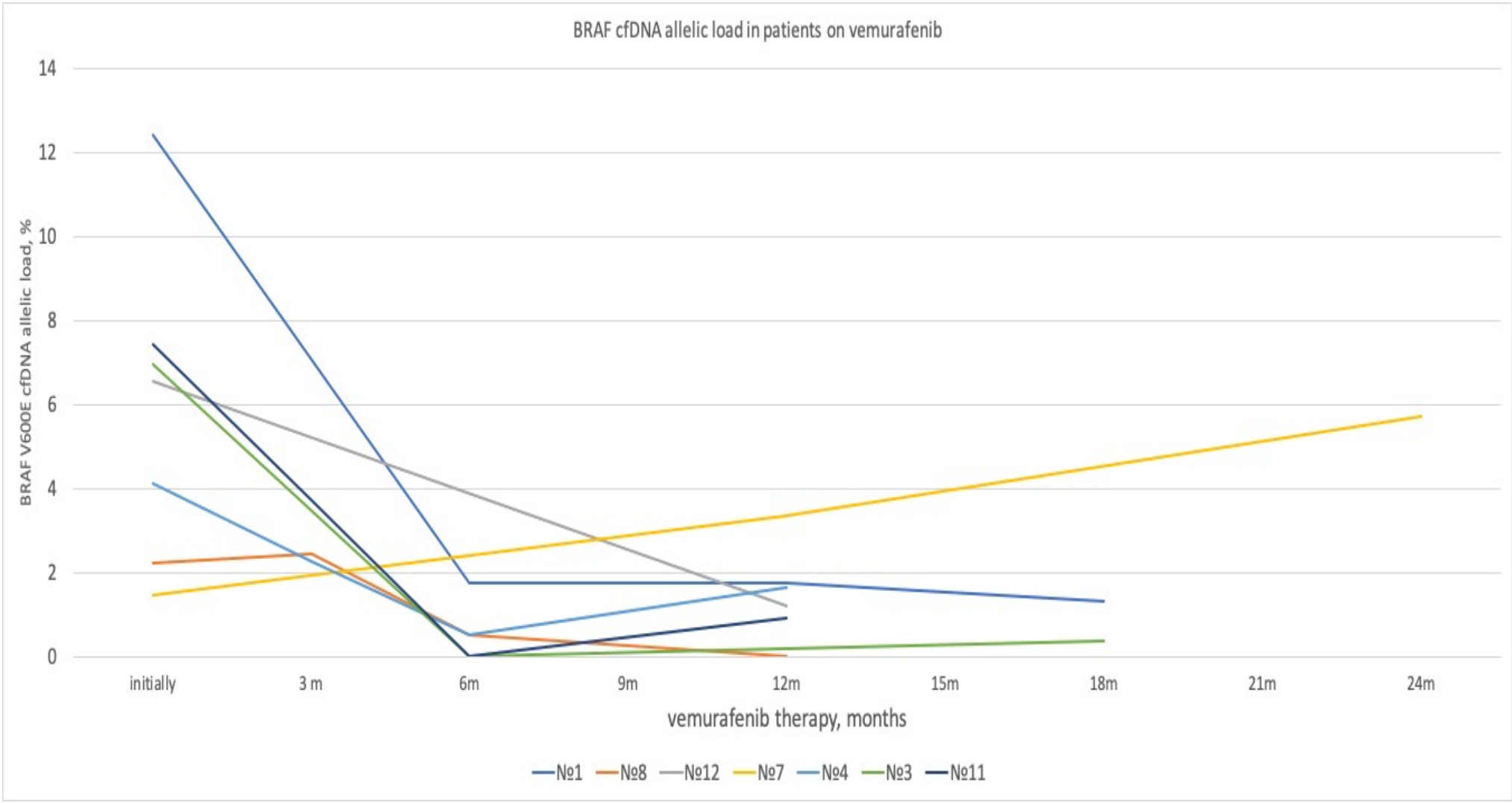


Figure 3. Changes in BRAF V600E allelic load in cell-free plasma DNA during vemurafenib therapy.


BRAF cfDNA allelic load in patients on vemurafenib
BRAF V600E cfDNA allelic load, %
14
12
10
8
6
4
2
0
initially
3 m
6m
9m
12m
15m
18m
21m
24m
vemurafenib therapy, months
№1
№8
№12
№7
№4
№3
№11