



Original Research

Rechallenge with BRAF-directed treatment in metastatic melanoma: A multi-institutional retrospective study[☆]



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KEYWORDS

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Abstract Background: Most metastatic melanoma patients treated with BRAF inhibitors (BRAFi) ± MEK inhibitors (MEKi) eventually progress on treatment. Along with acquired resistance due to genetic changes, epigenetic mechanisms that could be reversed after BRAFi discontinuation have been described. The purpose of this study was to analyse retrospectively outcomes for patients retreated with BRAF-directed therapy.

Patients and methods: One hundred sixteen metastatic melanoma patients who received BRAFi-based therapy and, after a break, were rechallenged with BRAFi ± MEKi at 14 centres in Europe, US and Australia were studied, respectively. Response rate (RR), overall survival (OS) and progression-free survival (PFS) from the start of retreatment were calculated.

Results: The median duration of treatment was 9.4 months for first BRAFi ± MEKi treatment and 7.7 months for the subsequent treatment (immunotherapy 72%, other 17%, drug holiday 11%) after BRAFi discontinuation. Brain metastases were present in 51 (44%) patients at BRAFi retreatment. The RR to rechallenge with BRAFi ± MEKi was 43%: complete response (CR) 3%, partial response (PR) 39%, stable disease (SD) 24% and progressive disease 30%, 4% missing. Of 83 patients who previously discontinued BRAFi due to disease progression, 31 (37.3%) responded (30 PR and 1 CR) to retreatment. The median OS from retreatment was 9.8 months, and PFS was 5 months. Independent prognostic factors for survival at rechallenge included number of metastatic sites (hazard ratio [HR] = 1.32 for each additional organ with metastases, $P < .001$), lactic dehydrogenase (HR = 1.37 for each multiple of the upper normal limit, $P < .001$), while rechallenge with combination BRAFi + MEKi conferred a better OS versus BRAFi alone (HR = 0.5, $P = .006$).

Conclusion: Rechallenge with BRAFi ± MEKi results in a clinically meaningful benefit and should be considered for selected patients.

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1. Background

BRAF inhibitors (BRAFi) and the combination of BRAFi (vemurafenib, dabrafenib, encorafenib) with MEK inhibitors (MEKi) (trametinib, cobimetinib, binimetinib) have revolutionised the treatment of patients with BRAF-mutant melanoma. However, most patients responding to targeted therapy will subsequently develop resistance and progress, with a median progression-free survival of 6–10 (BRAFi monotherapy) to 11–15 months (BRAFi + MEKi). Treatment options after the development of BRAFi resistance include immunotherapy. While many patients will benefit from this, the majority progress, and the median survival remains approximately 24 months [1].

In addition to the development of acquired resistance based on a classical clonal Darwinian selection [2], acquired epigenetic mechanisms have been proposed as important mechanisms of resistance. There is evidence that BRAFi may induce cancer cell ‘drug addiction,’ matrix remodelling and secretome adaptation resulting in temporary resistance to BRAFi [3–5]. In this scenario of a plastic phenotype which may be reversible on withdrawal of the driving stimulus, retreatment with BRAFi after a treatment break would have a rationale. Rechallenge with targeted therapy has been shown to be effective in other cancers including lung cancer, gastrointestinal

stromal tumours (GIST) and renal cell cancer [6–11]. At the start of this study, there was evidence of heterogeneous clinical and molecular patterns of resistance to BRAFi [12,13] and anecdotal reports of reversible resistance in melanoma patients [14–22]. More recently, a small prospective study in 25 patients showed a benefit for retreatment with BRAFi retreatment [22].

The aim of the present study was to draw together the experience of a large number of melanoma centres to understand if rechallenge with BRAFi therapy is a clinically useful approach and to identify factors to select patients with more probabilities to have a benefit.

2. Materials and methods

2.1. Patients

We carried out a retrospective review of patients who had received BRAFi-based treatment (with or without MEKi) and, after a break, were rechallenged with BRAFi-containing regimen. Eligible patients were treated between February 2011 and September 2016 at 14 centres in Europe, the US and Australia. Inclusion criteria were patients with BRAF-mutated melanoma in which treatment with BRAFi-based treatment had been interrupted, with another therapy regimen initiation or drug holiday of minimum 4 weeks and subsequently

rechallenged with a BRAFi-containing regimen. Data included in the analysis were patient demographics, disease stage and extent, treatments received, lactic dehydrogenase (LDH) level, duration of first BRAFi-based treatment, reason for first BRAFi discontinuation and interval between first BRAFi stop and rechallenge. Factors analysed for prognostic value for overall survival (OS), progression-free survival (PFS) and response rate (RR) included the duration of the first-line treatment, best response, reason for discontinuation, interval treatment, duration of interval duration, number of metastatic sites, presence of brain metastases, LDH and rechallenge therapy.

2.2. Statistical analysis

Covariates were studied in univariate and multivariate Cox regression models for their prognostic value for death or progression. Hazard ratios (HRs) and 95% confidence intervals (CIs) for each risk factor were based on maximum likelihood estimates for each covariate. Cox-Snell residual-based methodology was used to check the proportional hazard assumption of the Cox models. The two-sided χ^2 *P* values from Wald test with Bonferroni correction for multiple testing was used to assess the significance of each covariate included in the full multivariate models. Fast-backward method (with Akaike information criterion as a stopping rule instead of *P* values, in order to favour parsimonious models) was used to select the covariates included in the final models for prognostic factors of OS and PFS. Model performance was measured with calibration and discrimination; the slope factor is the slope of the calibration plot when the model is fitted to new data and allows to estimate model overfitting (and thus allows to estimate the likelihood that the model will reliably predict new observations) and was calculated after 100 bootstrap replications. The shrinkage factor (slope) of the final prognostic model was 0.90 (range of the parameter 0–1, where 1 would be the ideally fitted model). Discrimination was determined with Harrell's C-index. The cohort was not large enough to provide a separate validation set; thus, the results were internally validated using bootstrap method (after 100 bootstrap replications). The prognostic model was validated internally with bootstrap (100 replications) and the C-index (the possible range of the parameter being 0–1, where 1 is the ideal model) resulted 0.66.

Multiple imputation methodology was used to compute missing values in the prognostic model analyses (first BRAFi-containing regimen data missing for 2 patients; best response at first BRAFi regimen was missing for 8 patients, best response at rechallenge was missing for 3 patients and baseline rechallenge LDH blood concentration missing in 7 cases). Performance status (PS) was missing for 20 patients (17%) and failed

the imputation procedure quality control, thus had to be omitted from the prognostic analysis.

In addition, the variables were then assessed for their interactions using exponential regression tree analysis for risk of death. This allowed for the identification of homogeneous prognostic subgroups.

Logistic regression was used to examine potential association with RR at rechallenge with BRAFi-based therapy. Spearman rank test was used to assess pairwise associations between covariates at first BRAFi regimen and rechallenge. PFS was defined as the time from rechallenge start to disease progression or last follow-up. Overall survival was defined as the time from rechallenge start to the date of death or last follow-up.

Survival rates were calculated using the Kaplan–Meier estimator, log-rank test being used to compare survival estimates across different groups.

The study was conducted in compliance to local ethics regulations, approved by the institutional ethics committees or audited by the review boards; informed consent procedures were conducted according to local regulations, and patient data were anonymised.

Statistical analyses were performed using R (v 3.3.3; CRAN project, R Foundation for Statistical Computing, Vienna, Austria). Anonymised raw data could be available upon reasonable request to the first author.

3. Results

A total of 116 patients fulfilling the eligibility criteria were identified from 14 centres. Patients' characteristics prior to rechallenge are shown in [Table 1](#), and [Supplemental Table 1](#) shows the details of first BRAF-directed therapy. BRAFi regimen was the first-line systemic treatment in the majority of patients (*N* = 90, 77%); BRAFi-based therapies were given after chemotherapy in 19 of the remaining patients. Locoregional treatment for brain metastases was administered before the first targeted therapy in 26 patients (22.4%), with 5 intracranial complete responses (CRs), 7 partial responses (PRs), 8 stable diseases (SDs) and 6 disease progressions (PD).

Out of 116 patients, 68 (58.6%) were treated with single-agent BRAFi as first BRAFi regimen, 41 (35.3%) received a combination of BRAFi and MEKi and the remaining (*N* = 5, 4.3%) had a combination of single-agent BRAFi and immunotherapy; data were missing for 2 patients. Most patients received treatment at full dose, while 31 (27%) required a dose reduction for toxicity.

The median duration of first-line BRAFi was 9.4 months (range 0.5–42.9, interquartile range 4.8–16.22). The most common reasons for stopping treatment was disease progression in 83 patients (71.6%), toxicity in 16 (13.8%), treatment break after CR in 9 patients (7.8%)

Table 1
Patients demographics and disease characteristics at rechallenge.

Patient demographics prior to rechallenge	N = 116
Age, years (range)	51.9 (28.6–80.3)
Metastatic organs number (range)	3 (1–8)
Brain metastases	51 (44%)
Stage	
M1a	14 (12%)
M1b	6 (5%)
M1c	96 (83%)
LDH	
<UNL	45 (41%)
≥UNL	64 (59%)
Missing	7 (6%)
PS	
0	34 (35%)
1	37 (39%)
2	20 (21%)
3–4	5 (5%)
Missing	20 (17%)
BRAF^{v600E} rechallenge drugs	
Dabrafenib	19 (16%)
Dabrafenib + trametinib	54 (47%)
Encorafenib	1 (1%)
Encorafenib + binimetinib	14 (12%)
Encorafenib + binimetinib + ribociclib	2 (2%)
Vemurafenib	19 (16%)
Vemurafenib + cobimetinib	7 (6%)

LDH, lactic dehydrogenase.

or other reasons in 5 patients (4.3%); information was missing in 3 patients.

The most frequent sites of treatment failure in the 83 patients who stopped first BRAF^{v600E} due to disease progression were lymph nodes (32.5%), soft tissue (27.7%) and central nervous system (26.5%); 28 (33.7%) patients were treated beyond progression, for a median of 2.2 months (range 0.9–17.6 months).

The median time from first BRAF^{v600E} regimen cessation to rechallenge was 7.7 months (range 0.9–34.9), and progression was the reason for discontinuation of the interval regime in the majority of patients (55.1%). Immunotherapy was the most commonly administered treatment between first and rechallenge BRAF^{v600E} treatment (71.5%) (Table 1). One third of patients (33.6%) had more than one line of interval therapy, with 22 (18.9%) receiving sequential immunotherapy with anti-CTLA4 followed by anti-PD1 treatment.

At the time of BRAF^{v600E} rechallenge, 83% of patients were stage M1c, 59% had a raised LDH (median value, 1.1 × upper normal limit (UNL); range, 0.5–6.9 × UNL) and 44% had brain metastases.

Most patients (62.9%) had single-agent BRAF^{v600E} at first BRAF-directed therapy, whereas a combination regimen was the most commonly used treatment at rechallenge (66.3%), reflecting the changing standard of care. The overall RR to BRAF^{v600E} rechallenge was 42.3% for evaluable patients: CR in 3 patients (2.6%), PR in 46 (39.7%), SD in 28 (24.1%), PD in 36 (31%) and data

missing in 3 patients. There was no correlation between best response to first BRAF^{v600E} and best response at rechallenge ($P = .331$). Of note, 4 patients who had PD as best response for first BRAF^{v600E} (3 BRAF^{v600E} monotherapy and 1 BRAF^{v600E} + MEKi) achieved a PR at rechallenge (with BRAF^{v600E} + MEKi). For the 83 patients who previously interrupted first BRAF^{v600E} because of disease progression, 30 (36.1%) had PR and 1 CR (1.2%) at rechallenge. RR did not significantly differ between the combination and monotherapy group ($P = .392$); however, all patients achieving CR ($N = 3$) had received combination treatment and of them, 2 had monotherapy (one partial response and one complete response) and one had combination at first BRAF^{v600E} (with complete response). The duration of first-line BRAF^{v600E} therapy was associated with the RR to BRAF^{v600E} rechallenge, with a median duration of first-line therapy of 14.8 months for responders versus 9.7 months for non-responders ($P = .014$). Responders to rechallenge had a longer median interval between initial and retreatment BRAF^{v600E} (8.8 versus 6.7 months, respectively, $P = .011$) but patients with an interval as short as 0.9 months also responded. Of note, the duration of the interval between first BRAF^{v600E} and rechallenge in the 3 patients who achieved CR from the rechallenge spanned from 5.9 months (a patient who had first-line dabrafenib) to 20.8 months (a patient who had first-line dabrafenib plus trametinib), while in patients who had PD as rechallenge best response the interval of BRAF^{v600E} drugs was from 0.9 (dabrafenib plus trametinib as first BRAF^{v600E}) to 24.6 months (a patient treated with dabrafenib as first BRAF^{v600E}). Furthermore, response to the interval treatment was not associated with response to BRAF^{v600E} rechallenge (not shown). Supplemental Fig. 1 shows an example of successful rechallenge in a patient who, after an initial benefit from single-agent dabrafenib, progressed, did not respond to immunotherapy and then had a partial response to the rechallenge with dabrafenib and trametinib.

With a median follow-up of 15.7 months from the start of retreatment, 68 (58.6%) patients have died and 89 (76.7%) have progressed. Median OS for rechallenge was 9.8 months (0.2–34.4), and median PFS was 5 months (0.2–31.7). The OS and PFS at 1 year were 42.8% and 23.1%, respectively. Of note, PFS from rechallenge was significantly longer than the PFS reported for first-line chemotherapy (2.7 months, $P = .005$) and similar to PFS for first-line dabrafenib (5.1 months, $P = .9$) in treatment-naïve patients [23].

The results of the full model for prognostic factors of OS are shown in Supplemental Table 2. The proportional hazard verification by means of Cox-Snell residuals for the OS and PFS prognostic models are represented in Supplemental Fig. 2A and 2B, respectively. The significant prognostic factors were the number of metastatic sites, LDH level and the treatment administered during rechallenge. In particular, patients

with more organs involved by metastatic disease (HR = 1.32 for each additional metastatic site, 95% CI 1.09–1.59, $P < .001$) and high LDH (HR = 1.37 for every additional multiple of the UNL, 95% CI 1.07–1.57, $P < .001$) had a worse prognosis, while patient receiving combination of BRAF plus MEKi (BRAFi plus MEKi or MEKi plus ribociclib) had a better prognosis (HR = 0.5, 95% CI 0.24–0.76, $P = .006$).

The regression tree analysis for risk of death identified 5 prognostic subgroups (Fig. 1A). The worst prognostic group was patients treated with single-agent BRAFi, 3 or more metastatic organs and high LDH (median OS = 4.0 months), while patients treated with combination therapy and less than 3 metastatic organs had the best prognosis (OS not reached). Fig. 1B shows the survival curves for the 5 subgroups, while the survival curves for each single prognostic factor are represented in Fig. 2.

The prognostic factors identified for PFS were similar to those for OS: the number of metastatic sites (patients with more metastatic organs had a worse prognosis; HR = 1.44 for each added metastatic site, 95% CI 1.24–1.67, $P < .001$, Fig. 3A) and the rechallenge therapy (patients treated with combination of BRAF plus MEKi or MEKi + ribociclib had a better prognosis; HR = 0.45, 95% CI 0.28–0.70, $P < .001$) (Fig. 3B); prognostic factors not retained in the final model are reported in Supplemental Table 3. OS and PFS prognostic analyses were repeated, in the subgroup of patients who had interrupted first BRAFi-based therapy because of progression, with consistent results (not shown).

4. Discussion

In this study, we show that melanoma patients treated with BRAF-directed therapy, who discontinue due to progression or other causes, can benefit from retreatment at a later stage. This translates to a clinically meaningful survival benefit from the time of retreatment, with a median overall OS of 9.8 months and median PFS of 5 months, respectively.

Targeted therapy has revolutionised the outcome for melanoma patients with a BRAF V600 mutation. The first phase III study with vemurafenib showed an improvement in OS from 9.7 to 13.6 months, with a HR of 0.70 coupled with a high RR and significant benefit in PFS [24]. Dual BRAF and MEK inhibition has extended the median OS to greater than 2 years and is now a standard of care [25]. The evolution of treatment from single to combination therapy is reflected in the treatments received by patients in this study. Single-agent therapy was the most common treatment administered initially (62.9%), and combination therapy was the preferred option (66.3%) on retreatment. Our results show that responses occur

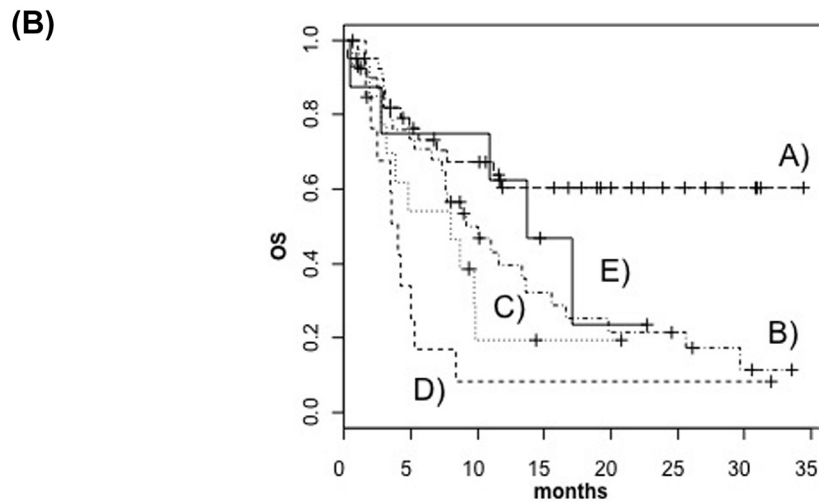
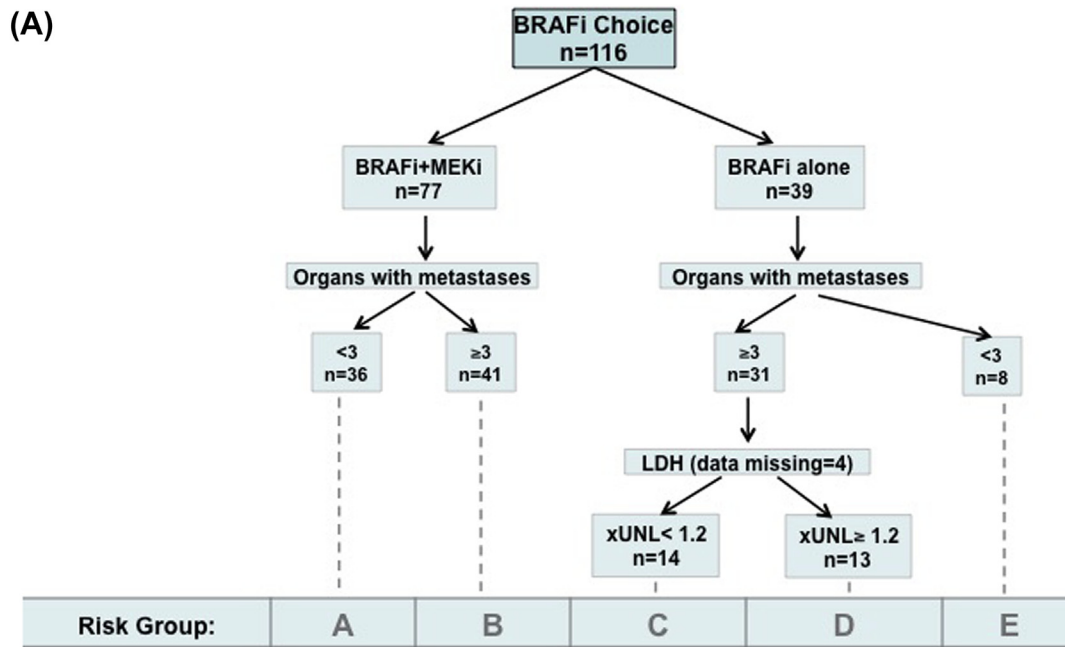
and survival may be extended with retreatment, with a median overall survival of 9.8 months from retreatment in patients that had exhausted standard therapeutic options.

Retreatment with chemotherapy after a drug holiday is an established practice in many cancers, with the chance of a benefit being related to the initial response to treatment, duration of response and length of treatment break. However, in these instances, patients have usually completed a set course of treatment and were responding at the time of discontinuation. In this study, the majority of patients discontinued targeted therapy due to acquired resistance and disease progression. Of particular interest is that 37.8% of patients who had discontinued BRAFi initially due to progressive disease went on to respond to a rechallenge. Resistance to BRAF- and MEK-directed therapy has been shown to be mediated by a number of different mechanisms, largely due to reactivation of the MAPK signalling pathway, for example, spliced variants of BRAF, emergence of NRAS mutation of other multilignage kinases, loss of feedback inhibition leading to BRAF dimerisation and resistance to BRAFi; however, other pathways are also involved, including the PI3K-PTEN-AKT pathway [26–29].

Our results that were also corroborated by a prospective study in 25 patients who had progressed on BRAFi-based therapy and, after receiving immunotherapy for at least 12 weeks followed by progression, were rechallenged with dabrafenib and trametinib [22] where the RR was 32%, demonstrate that acquired resistance to targeted therapy is not always irreversible. The simple Darwinian model of selective pressure resulting in new mutations and selection of a resistant clone conferring survival advantage does not explain our observations. Similarly, the findings for PFS and RR recapitulated the observations from Schreuer *et al.* confirming the evidence of a clinical benefit from the rechallenge with BRAFi in metastatic melanoma patients. In our cohort, where the minimum duration of the break was less stringent, the length of the interval between first BRAFi cessation and rechallenge was not associated with survival, indicating that resensitisation can occur quickly.

Preclinical models suggest that clones resistant to BRAFi may have a fitness disadvantage relative to those sensitive to BRAFi, and the selective growth advantage in the face of BRAFi therapy could be lost on discontinuation of the BRAFi [3]. These findings would also support the evaluation of intermittent treatment regimens in melanoma, and these are already established in other cancers including renal cell carcinoma [3]. Other models for transient acquired resistance include signalling plasticity [27,30,31], phenotype switching [32], quiescence [33,34] or epigenetic changes [35].

Prognostic factors for patients treated with combination BRAF and MEK inhibition are now well



Risk Group	N	Deaths	Median	95% CI
A) BRAFi+MEKi (+/- LEE011), metastatic organs <3	36	12	NA	11.7 - NA
B) BRAFi+MEKi (+/- LEE011), metastatic organs ≥3	41	28	10.0	7.6 – 16.6
C) BRAFi monotherapy, metastatic organs ≥ 3 and normal LDH	14	10	8.0	3.2 - NA
D) BRAFi monotherapy, metastatic organs ≥3 and high LDH	13	11	4.0	2.5 - NA
E) BRAFi monotherapy, metastatic organs <3	8	5	13.7	10.9 - NA

Fig. 1. **Regression tree and risk classes for overall survival.** (A) Shows the regression tree and the 5 different risk classes identified for overall survival from the start of retreatment; (B) shows the Kaplan–Meier curves and corresponding survival data for the 5 risk classes.

established and include PS, number of metastatic sites and LDH level [36]. This study shows that number of metastatic sites and LDH level is prognostic for survival with retreatment. The performances of the prognostic studies were comparable to previously published models

for rare tumours [37–39]. These allowed the identification of prognostic factors and risk classes, similarly to those observed for first-line BRAF-targeted therapy. Low tumour burden (less than 3 metastatic sites), the combination of BRAFi plus MEKi and low LDH were

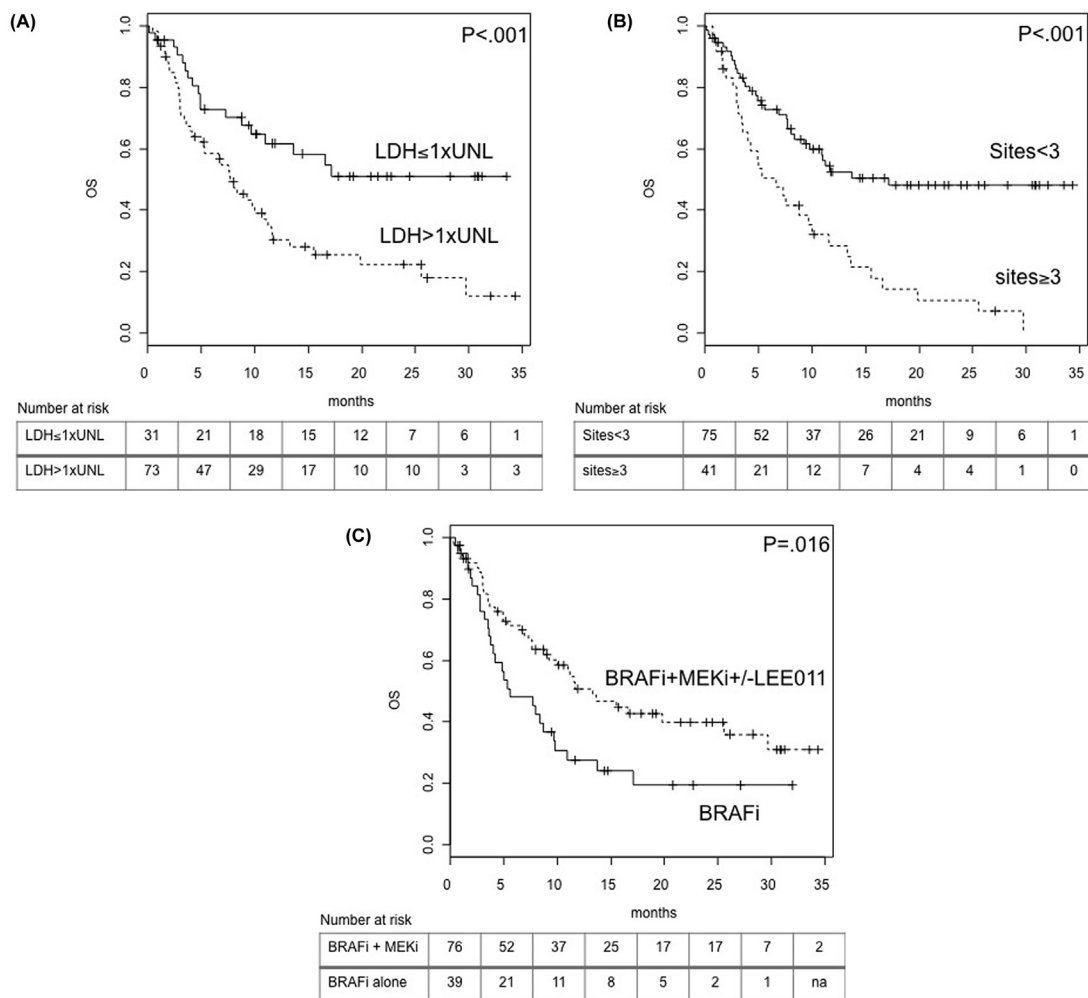


Fig. 2. Kaplan–Meier curves for the study cohort according to the prognostic factors for overall survival from the start of retreatment: (A) LDH above or below the upper normal limit, (B) more or less than 3 metastatic sites and (C) therapy with monotherapy BRAF inhibitors or combination of BRAF inhibitors and MEK inhibitors (plus ribociclib in 3 patients). LDH = lactic dehydrogenase; LEE011 = ribociclib.

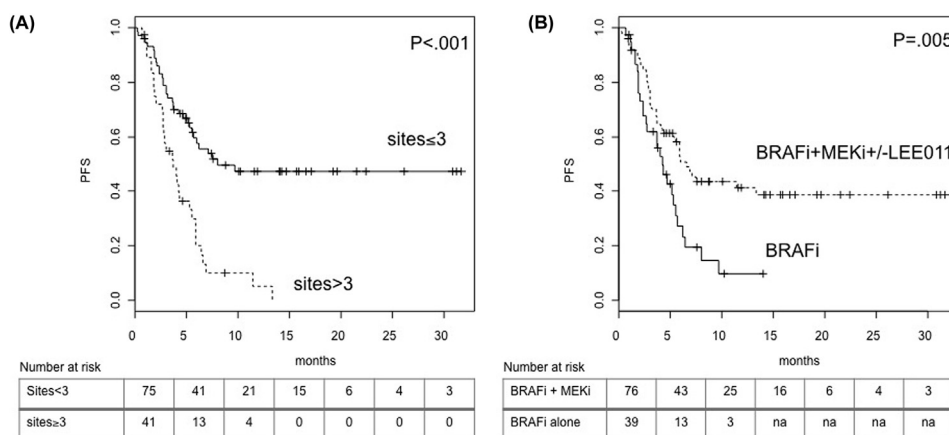


Fig. 3. Kaplan–Meier curves for the study cohort according to the prognostic factors for progression-free survival: (A) more or less than 3 metastatic sites and (B) therapy with monotherapy BRAF inhibitors or combination of BRAF inhibitors and MEK inhibitors (plus ribociclib in 3 patients). LEE011 = ribociclib.

associated with better overall survival, and the magnitude of the survival benefit was greater than the expected 6–9 months survival for treatment-naïve patients in the pretargeted drugs and pre-immunotherapy era.

This study has a number of limitations, being a retrospective series of patients managed heterogeneously, although it reflects the heterogeneity of melanoma patients and its sample size is comparable to similar studies in rare tumours [37,38,40]. The frequency of tumour assessments was not standardised and so data on PFS are less robust. Patient selection will have contributed to the outcome, though it is interesting to note that many of these patients would have been expected to have a very poor prognosis, considering that most had a raised LDH (59%), and the cohort was enriched for patients with brain metastases (44%). Of note, PFS from rechallenge in this heavily pretreated population with adverse prognostic factors were similar to PFS for first-line dabrafenib [23]. While the absence of a control arm makes it difficult to quantify the benefits of the rechallenge, the results seen for PFS and OS are unexpected for heavily pretreated patients who have progressed through multiple lines of treatment [41].

In conclusion, our results support retreatment as a new therapeutic option for selected patients with BRAF-mutated melanoma who have progressed on BRAFi and have completed a subsequent treatment. In addition, the identification of different prognostic groups has implications for the design and stratification of clinical trials evaluating intermittent dosing strategies or rechallenge therapy with BRAFi after a different treatment.

Conflict of interest statement

A.M.M. is a member of the advisory board of MSD, Novartis and Pierre Fabre and received honoraria from BMS and Roche. G.V.L. is a consultant/advisor for Amgen, Array, BMS, Merck MSD, Novartis, Pierre Fabre and Roche. P.A.A. plays a consultant/advisory role in BMS, Roche-Genentech, MSD, Array, Novartis, Amgen, Merck-Serono, Pierre Fabre and Incyte and received research funds from BMS, Roche-Genentech and Array. A.D.G. plays a consultant/advisory role in BMS, Incyte and Pierre Fabre. D.S. plays a consultant/advisory role in BMS, Roche-Genentech, MSD, Array, Novartis, Amgen, Merck-Serono, Pierre Fabre and Incyte and received research funds from BMS and Novartis. S.M.G. has an intermittent advisory relationship with Roche, Novartis, BMS and MSD and received research support from the University of Zurich and medAlumni.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.ejca.2017.12.007>.

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