A lead-in safety study followed by a phase 2 clinical trial of dabrafenib, trametinib and hydroxychloroquine in advanced BRAFV600 mutant melanoma patients previously treated with BRAF-/MEK-inhibitors and immune checkpoint inhibitors

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Running title: Dabrafenib, trametinib and hydroxychloroquine in advanced melanoma

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Abstract:

Patients with advanced *BRAF*^{V600} mutant melanoma who progressed on prior treatment with BRAF-/MEK-inhibitors and programmed cell death 1 (PD-1) or cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) immune checkpoint inhibitors can benefit from retreatment with the combination of a BRAF- and a MEK-inhibitor ("rechallenge"). Hydroxychloroquine can prevent autophagy-driven resistance and improve the efficacy of BRAF-/MEK-inhibitors in preclinical melanoma models. This clinical trial investigated the use of combined BRAF-/MEK-inhibition with dabrafenib and trametinib plus hydroxychloroquine in patients with advanced *BRAF*^{V600} mutant melanoma who previously progressed on prior treatment with BRAF-/MEK-inhibitors and immune checkpoint inhibitors. Following a safety lead-in phase, patients were randomized in the phase 2 part of the trial between upfront treatment with dabrafenib, trametinib and hydroxychloroquine (experimental arm), or dabrafenib and trametinib, with the possibility to add-on hydroxychloroquine at the time of documented tumor progression (contemporary control arm). Ten patients and 4 patients were recruited to the experimental and contemporary control arm, respectively. The objective response rate was 20.0% and disease control rate was 50.0% in the experimental arm, while no responses were observed before or after adding hydroxychloroquine in the contemporary control arm. No new safety signals were observed for dabrafenib and trametinib. Hydroxychloroquine was suspected of causing an anxiety/psychotic disorder in 1 patient. Based on an early negative evaluation of the risk/benefit ratio for adding hydroxychloroquine to dabrafenib and trametinib when “rechallenging” *BRAF*^{V600} mutant melanoma patients, recruitment to the trial was closed prematurely.

Keywords:

Advanced melanoma; autophagy; BRAF-inhibitor; MEK-inhibitor; hydroxychloroquine; clinical trial

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INTRODUCTION

Patients with advanced (defined as unresectable or metastatic) *BRAF*^{V600} mutant melanoma who have progressed on prior treatment with BRAF-/MEK-inhibitors and programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) immune checkpoint inhibitors can derive benefit from retreatment with the combination of a BRAF- and a MEK-inhibitor ("rechallenge"). In a prospective phase 2 clinical trial in 25 patients who had interrupted prior BRAF-/MEK-inhibitor treatment at least 12 weeks before inclusion, the objective response rate (ORR) was 32% and the disease control rate (DCR) was 72% (1). Similar results were observed in a multicenter retrospective study (2).

Multiple resistance mechanisms against BRAF-/MEK-inhibitors have been described, including genomic alterations that lead to reactivation of the mitogen-activated protein kinase (MAPK) pathway (e.g. *NRAS* mutations, *BRAF* amplification, *CRAF* overexpression), activation of alternative oncogenic pathways (such as the phosphoinositide-3-kinase [PI3K] pathway), epigenetic and transcriptomic alterations leading to an epithelial-to-mesenchymal transition, and the process of autophagy (3). In physiologic circumstances, autophagy is a cellular process during which damaged intracellular proteins and organelles are collected in autophagosomes which subsequently fuse with lysosomes. The autophagosomic contents are degraded in these lysosomes, in order to sustain cellular homeostasis. Autophagy is particularly important in liver, brain and muscle tissue to prevent the accumulation of toxic damaged proteins and organelles (4). In the context of nutrient deprivation, which is a stress factor, the process of autophagy induces the degradation of normal intracellular proteins and organelles to promote metabolic homeostasis and survival (4).

Preclinical studies with *BRAF*^{V600} mutant melanoma cell lines have shown that mutant, and thus constitutively active, BRAF drives autophagy as a survival mechanism. By augmenting autophagy and metabolic turnover, mitochondrial metabolism and oxidative phosphorylation are activated which promote survival and increased tolerance of stress (autophagy addiction) (5).

Furthermore, BRAF-inhibitors, with or without MEK-inhibitors, also induce increased autophagy in *BRAF*^{V600} mutant melanoma cell lines as an adaptive cytoprotective resistance mechanism induced by and against BRAF-/MEK-inhibitors (6). The combination of the autophagy inhibitor hydroxychloroquine (which acts by inhibiting the fusion between lysosomes and autophagosomes) and a BRAF- with or without a MEK-inhibitor led to a significant degree of growth inhibition in BRAF-inhibitor resistant melanoma cell lines.

The combination of chloroquine and vemurafenib has been used in pediatric cases with glioblastoma with remarkable success (7). In these cases, the equivalent daily dose of 400 mg hydroxychloroquine

was able to overcome acquired resistance to vemurafenib monotherapy, resulting in a renewed objective tumor response and improved survival.

Hydroxychloroquine has been investigated in combination with the alkylating agent temozolomide in a phase 1 trial in 40 patients with advanced solid tumors (73% melanoma) (8). Oral hydroxychloroquine was administered at a dose of 200 to 1200 mg daily in combination with dose-intense oral temozolomide (150 mg/m^2 daily for 7/14 days). A maximum tolerated dose was not reached for hydroxychloroquine and the recommended phase 2 dose was 600 mg twice daily in combination with temozolomide. Toxicities included grade 2 fatigue (55%), anorexia (28%), nausea (48%), constipation (20%) and diarrhea (20%). The DCR was 41% in evaluable patients with melanoma (9/22 patients, 3 patients with a partial response and 6 patients with stable disease). The combination of dabrafenib, trametinib and hydroxychloroquine has been investigated in a phase 1/2 trial in patients without previous exposure to BRAF-/MEK- inhibitors. In 7 patients in the phase 1 trial, no dose-limiting toxicity was observed and the recommended phase 2 dose of hydroxychloroquine was 600 mg twice daily. Six out of seven patients achieved an objective response (9).

The objective of this randomized phase 2 trial (COMBI-R 2) is to explore the efficacy and safety of the combination of dabrafenib, trametinib and the autophagy inhibitor hydroxychloroquine in patients with advanced *BRAF^{V600}* mutant melanoma who have been previously treated with BRAF-/MEK-inhibitors and immune checkpoint inhibitors and who developed progressive disease (PD) on these treatments. A lead-in safety assessment of the triple drug combination preceded the randomized part of the trial. We hypothesize that the addition of hydroxychloroquine can safely overcome or prevent autophagy-driven resistance to BRAF-/MEK-inhibitor therapy and thus augment efficacy of this targeted therapy in a “rechallenge” setting.

METHODS

Study design and patient population

This single-center lead-in safety study, followed by a two-center, open-label, asymmetrically randomized, double-arm, two-stage phase 2 clinical trial (NCT03754179) was conducted at the Universitair Ziekenhuis Brussel (Brussels, Belgium) and the Universitair Ziekenhuis Gent (Ghent, Belgium) and included patients with advanced *BRAF^{V600}* mutant melanoma who had progression of disease following treatment with PD-1 and/or CTLA-4 immune checkpoint inhibitors and BRAF-/MEK-inhibitors. Patients were considered for study participation not earlier than 12 weeks after the last dosing of the prior BRAF-/MEK-inhibitor therapy (to allow reversal of resistance of the melanoma cells to the prior treatment with BRAF-/MEK-inhibitors) and 4 weeks after the last dosing of immune checkpoint inhibitor therapy (to guarantee adequate drug washout).

Eligible patients must have had an Eastern Cooperative Oncology Group Performance Status of 0-2 and an adequate baseline organ function. Major exclusion criteria were patients with uveal or mucosal melanoma; prior treatment with hydroxychloroquine, chloroquine or other quinine derivatives; grade 4 or repetitive grade 3 adverse events (AE) to prior treatment with BRAF-/MEK-inhibitors; and presence of uncontrolled cardiovascular and/or ocular diseases.

Procedures and study treatment

In the lead-in safety study, patients were screened for eligibility by clinical examination, blood analysis, electrocardiography (ECG), transthoracic echocardiography (TTE), ophthalmic examination (including optical coherence tomography), whole-body 18-fluorodexoxyglucose positron emission tomography/computed tomography (^{18}F-FDG-PET/CT) and magnetic resonance imaging of the brain in case of brain metastases. A plasma sample was obtained for circulating tumor deoxyribonucleic acid (ctDNA) analysis (see below). If found eligible, patients were treated with the combination of dabrafenib (150 mg twice daily orally) and trametinib (2 mg once daily orally) with the autophagy inhibitor hydroxychloroquine (200 mg twice daily orally). Patients were evaluated on a regular basis with a clinical examination, blood analysis, ECG, TTE and ophthalmic examination. Blood plasma was collected at predefined endpoints for ctDNA analysis. Every 8 weeks, response assessments were performed. Study therapy was continued until confirmed PD, unacceptable toxicity or withdrawal of consent.

In the phase 2 trial, eligible patients (assessed by the same screening examinations as in the lead-in safety study) were randomized to treatment with the combination of standard dosing of dabrafenib (150 mg twice daily orally) and trametinib (2 mg once daily orally) with hydroxychloroquine (200 mg twice daily orally) (experimental arm) or treatment with standard dosing of dabrafenib (150 mg twice daily orally) and trametinib (2 mg once daily orally) (contemporary control arm). Stratification occurred according to baseline lactate dehydrogenase level. Treatment was continued until PD, unacceptable toxicity or withdrawal of consent. At disease progression in the contemporary control arm, hydroxychloroquine (200 mg twice daily orally) could be added to dabrafenib and trametinib and treatment (if the patient's clinical status permitted this). Therapy was continued until PD, unacceptable toxicity or patient’s refusal to continue study treatment. Throughout their study participation, patients were continuously monitored for safety and evaluated for tumor response every 8 weeks or sooner if there was clinical suspicion of PD. The database was locked on 6th July 2021. The study was done in accordance with both the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines and was approved by the medical ethics committee of both institutions. All participants provided written informed consent.

Endpoints

The primary endpoint of the safety lead-in assessment was safety (AE, graded by the Common Terminology Criteria for Adverse Events [CTCAE] version [v] 4.03). Secondary endpoints included the confirmed ORR (per Response Evaluation Criteria In Solid Tumours [RECIST] v1.1), progression-free survival (PFS; time from treatment initiation until PD or death) and overall survival (OS; time from treatment initiation until death). The value of baseline and on-treatment plasma *BRAF*^{V600} mutant ctDNA as a predictive or prognostic marker was an exploratory endpoint.

The primary endpoint of the phase 2 trial was the confirmed ORR (per RECIST 1.1) in the experimental arm (per intention-to-treat analysis). Secondary endpoints were PFS (time from randomization until PD or death), OS (time from randomization until death) and safety (per CTCAE v4.03) in the experimental arm. Other secondary endpoints included the confirmed ORR prior to (ORR 1) and following the addition of hydroxychloroquine to dabrafenib and trametinib (ORR 2), PFS from randomization to dabrafenib and trametinib (time from randomization until PD or death while treated with dabrafenib and trametinib [PFS 1]), PFS from addition of hydroxychloroquine (time from addition of hydroxychloroquine to dabrafenib and trametinib until PD or death [PFS 2]), OS from randomization until death while treated with dabrafenib and trametinib (OS 1), OS from addition of hydroxychloroquine to dabrafenib and trametinib until death (OS 2), OS from randomization until death (OS 3), and safety (per CTCAE v4.03) in the contemporary control arm. The value of plasma *BRAF*^{V600} mutant ctDNA as a predictive/prognostic marker during treatment served as an exploratory endpoint in both arms.

Circulating tumor DNA analysis

The Idylla ctNRAS-BRAF Mutation Test (Biocartis, Mechelen, Belgium) was used for the analysis of plasma *BRAF*^{V600} mutant ctDNA. The method of analysis has been described in a previous article by our group and was dichotomized as detectable or undetectable (10).

Statistical analysis

The safety lead-in study included 4 patients and enabled us to investigate the safety of the triple drug combination of dabrafenib, trametinib and hydroxychloroquine and if necessary, detect unexpected toxicities or perform dose adjustments after trial amendment. The double-arm approach in the phase 2 trial enabled us to investigate the effect of addition of hydroxychloroquine upfront in combination with dabrafenib and trametinib or at progression after treatment with dabrafenib and trametinib.

Asymmetrical randomization was performed as the 4 patients in the lead-in safety study will be followed for efficacy in the experimental arm (dabrafenib, trametinib and hydroxychloroquine upfront). The sample size of this arm is calculated according to a Simon's two-stage optimal design.

The null hypothesis that the true response rate is 30% (P_0) will be tested against a one-sided alternative that the minimal response rate on the experimental therapy is 50% (P_1). In the first stage, 15 patients will be accrued (the four patients from the lead-in safety study will be included in this number). If there are 5 or fewer responses in these 15 patients, the study will be stopped for futility. Otherwise, 31 additional patients will be accrued for a total of 46 patients in the second stage. The null hypothesis will be rejected if 19 or more responses are observed in these 46 patients. This design yields a type I error rate of 0.05 and a power of 0.80.

The sample size of the contemporary control arm (dabrafenib plus trametinib with hydroxychloroquine added-on at progression) will be identical to the experimental arm (15 patients during the first stage). At progression after dabrafenib plus trametinib we will investigate the value of adding hydroxychloroquine using a Simon's two-stage optimal design. The null hypothesis that the true response rate is 0.05 (P_0) will be tested against a one-sided alternative that the minimal response rate on the experimental therapy is 25% (P_1). In the first stage, 9 patients will be accrued. If there are 0 responses in these 9 patients, the trial will be stopped for futility and patients initially treated with dabrafenib and trametinib will continue as a control arm for the experimental arm if this arm should continue to the second stage. Otherwise, 8 additional patients will be accrued for a final total number of 17 patients. The null hypothesis will be rejected if 3 or more responses are observed in these 17 patients. This design yields a type I error rate of 0.05 and a power of 0.80.

Median PFS, OS and time on therapy were estimated using the Kaplan-Meier method (SPSS Statistics version 27, IBM, Armonk, New York, USA).

RESULTS

Patient baseline characteristics

Between January 2018 and March 2021, 15 patients were screened for eligibility of whom one did not meet the eligibility criteria for study participation. The first 4 patients initiated dabrafenib, trametinib and hydroxychloroquine treatment as part of the safety lead-in phase. Subsequently, an additional 6 patients were randomized to upfront treatment with dabrafenib, trametinib and hydroxychloroquine in the experimental treatment arm (adding up to a total of 10 patients initiating the triplet combination therapy upfront), and 4 patients were randomized to the contemporary control arm (dabrafenib and trametinib upfront, with the possibility to add-on hydroxychloroquine at the time of first progression) (Fig. 1). The patient baseline characteristics are summarized in Table 1.

Treatment disposition

The median duration of treatment for the 10 patients treated with triplet therapy was 12.7 weeks (95% CI 9.6-15.8; range 1.9-33.4). Treatment interruptions due to AE were required in 7 out of 10 patients

while four patients required a dose reduction of dabrafenib and trametinib (Table 2). One patient permanently discontinued hydroxychloroquine treatment, while continuing dabrafenib and trametinib (because of a hydroxychloroquine-related anxiety/psychotic disorder that was reversible upon withdrawal of hydroxychloroquine). At the time of database lock, one patient was still on treatment, seven patients discontinued study treatment due to PD and 2 patients discontinued dabrafenib, trametinib and hydroxychloroquine due to AE (Fig. 1).

Four patients initiated dabrafenib and trametinib in the contemporary control arm. The median duration of treatment with dabrafenib and trametinib was 9.0 weeks (95% CI 0-20.8; range 4.0-16.1). Treatment interruptions due to AE were required in three patients, and one patient required a dose reduction due to AE (Table 2). Two patients eventually added-on hydroxychloroquine (after 9.0 and 16.1 weeks, respectively). Treatment duration on the triplet combination was respectively 1.9 and 5.6 weeks, and no treatment interruptions or dose reductions were required. All patients discontinued study treatment due to PD (Fig. 1).

Safety

All patients experienced AE (Table 2). Grade 3-4 AE and serious AE were observed in 70.0% and 60.0% of patients treated with dabrafenib, trametinib and hydroxychloroquine upfront, respectively. In the contemporary control arm, grade 3-4 and serious AE were observed in 25.0% of patients. The most frequent AE were fatigue and fever. Toxicities of special interest include grade 1 central serous retinopathy in two patients (dabrafenib/trametinib-related), grade 2 left ventricular ejection fraction decrease in one patient (dabrafenib/trametinib-related), and grade 2 hydroxychloroquine-related anxiety/psychotic disorder in one patient (that ceded after permanent hydroxychloroquine discontinuation). Two patients permanently discontinued triplet therapy due to AE (acute kidney injury, and fever and headaches, respectively). All treatment-related AE were reversible upon treatment interruption. There were no fatal AE.

Efficacy

At the time of database lock, 9 patients (90.0%) treated with dabrafenib, trametinib and hydroxychloroquine upfront (experimental arm) were evaluable for efficacy. One patient who interrupted dabrafenib, trametinib and hydroxychloroquine early in the treatment course due to toxicity initiated a new treatment before undergoing a first CT-based tumor response evaluation.

Two patients (20.0%) obtained a confirmed partial response (first response was documented after 8 weeks of treatment in both patients) per intention-to-treat analysis (Table 3; Fig. 2). The duration of response was 24.0 and 25.4 weeks, respectively. Three patients (30.0%) obtained a stable disease as best response (including 1 patient with an unconfirmed partial response), and 4 patients (40.0%) had

PD. The disease control rate is 50.0%. The evolution in the sum of diameters of target lesions is depicted in Figure 3. Eight (of 9 evaluable) patients have progressed (median PFS is 11.3 weeks [95% CI 1.7-20.9]), one patient continued study therapy (Fig. 1, Fig. 2). Seven patients (70.0%) have died at the time of analysis (median OS is 41.4 weeks [95% CI 2.7-80.1]).

Among the 4 patients who initiated dabrafenib and trametinib in the contemporary control arm, one patient obtained a stable disease (duration 6.3 weeks) and 3 patients progressed on treatment (ORR 1 0%) (Table 3). The median PFS with dabrafenib and trametinib was 7.6 weeks (95% CI 3.8-11.4) (PFS 1) and the median OS from dabrafenib and trametinib initiation in these 4 patients was 17.4 weeks (95% CI 0-42.9) (OS 3). Both patients who added-on hydroxychloroquine were diagnosed with PD at the first subsequent response evaluation (after 0.6 and 5.1 weeks, respectively [PFS 2]; ORR 2 0%), and died after 16.0 and 8.4 weeks (OS 2), respectively. The two patients who did not add on hydroxychloroquine died 6.1 weeks and 43.9 weeks after dabrafenib and trametinib initiation, respectively (OS 1).

Circulating tumor DNA analysis

Of twelve patients who underwent a baseline plasma *BRAF*^{V600} mutant ctDNA assessment, all had detectable levels in the plasma. In 11 patients, ctDNA remained detectable after 2 weeks of treatment, while it became undetectable in 1 patient (this patient interrupted study therapy early in the treatment course due to AE, but a clinical benefit was observed in a regressed subcutaneous lesion). In 2 patients who obtained a confirmed partial response, ctDNA became undetectable, while in patients who had PD as best response, ctDNA remained detectable throughout the treatment. In one patient who was treated with the triplet upfront, an acquired *NRAS*^{Q61} resistance mutation was detected at the time of progression.

DISCUSSION

This study investigating the combination of dabrafenib, trametinib and hydroxychloroquine in patients with advanced *BRAF*^{V600} mutant melanoma who progressed after prior treatment with BRAF-/MEK-inhibitors and immune checkpoint inhibitors was stopped prematurely because of low activity and increased toxicity observed with the experimental triplet combination therapy.

Following the response evaluation in 10 patients, an ORR of 20.0% (90% CI 3-50%, per intention-to-treat analysis) almost excluded that the primary objective could be met. The observed efficacy in the experimental arm of this trial is lower than reported in a similar population of patients of our previous trial, where dabrafenib and trametinib was also investigated in the "rechallenge" setting (ORR 32%) (1). In the contemporary control arm (dabrafenib and trametinib upfront, with add-on of hydroxychloroquine in case of PD with dabrafenib and trametinib), no responses were observed prior to and after addition of hydroxychloroquine at first progression to dabrafenib and trametinib. Although

the patient numbers are low, the addition of hydroxychloroquine does not seem to efficiently reverse resistance to BRAF-/MEK-inhibitors either, which was the case in pediatric glioblastoma that progressed on treatment with vemurafenib (7).

It is important to highlight that the process of autophagy is not the only mechanism involved in resistance to BRAF-/MEK-inhibitors. Genomic alterations that reactivate the MAPK-pathway or activate other parallel oncogenic pathways have already been described as acquired resistance mechanisms to MAPK-pathway inhibitors (3). In our study population, we identified one patient who at the time of progression developed an *NRAS*^{Q61} mutation (detected on ctDNA). This mutation is known to reactivate the MAPK- (despite BRAF- and MEK-inhibition) and PI3K-pathways (11). Furthermore, non-genomic alterations (such to dedifferentiation to a neural crest-like phenotype) that lead to drug resistance have also been described (12). In this context, autophagy inhibition with hydroxychloroquine is probably less relevant.

The dose of hydroxychloroquine that was investigated in this trial was lower than the recommended phase 2 dose of 800 mg twice daily in combination with dabrafenib and trametinib in previous early-phase trials in the non-rechallenge setting (8, 9). However, we estimate that the dose of 200 mg twice daily was biologically active as it was effective in a case of pediatric glioblastoma (7). Furthermore, this is also the recommended dose in other non-neoplastic indications such as rheumatoid arthritis. Higher dosing of hydroxychloroquine increases the risk of retinal toxicity, which is also a specific toxicity of MEK-inhibitors (13). Finally, one patient developed an anxiety/psychotic disorder that recovered after hydroxychloroquine interruption, indicating the pharmacodynamic activity of hydroxychloroquine.

Ongoing studies are investigating this drug combination in a less heavily pretreated population (9). Furthermore, selecting patients for treatment with hydroxychloroquine could be based on the presence of expression of autophagy markers (e.g., LC3) on baseline or on-treatment tumor samples, while patients without this characteristic could be offered other treatments. This should be investigated as part of an explorative study.

A higher incidence of grade 3 or 4 toxicity was encountered in our study as compared to our previous trial in the “rechallenge” setting (1). Of interest is the observation that two patients permanently interrupted dabrafenib and trametinib due to acute kidney injury, and fever and headaches, respectively, while one patient discontinued hydroxychloroquine due to an anxiety/psychotic disorder. Previous research already suggests that tolerance of BRAF-/MEK-inhibitors is lower after previous treatment with PD-1 immune checkpoint inhibitors, illustrating the more complex toxicity management of these patients (14).

Although our patient numbers are low, the persistent detection of plasma *BRAF*^{V600} mutant ctDNA during study therapy appears to be predictive of early PD, while loss of the ctDNA signal appears to be associated with response to therapy. This confirms previous findings by our group (15).

In conclusion, adding hydroxychloroquine to dabrafenib and trametinib in patients with advanced melanoma who have previously been treated with BRAF-/MEK-inhibitors and PD-1 and/or CTLA-4 immune checkpoint inhibitors failed to provide an early strong signal for increased efficacy and is associated with increased toxicity in a pretreated population of advanced *BRAF*^{V600} mutant melanoma patients.

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FIGURES

Figure 1. CONSORT diagram. Abbreviations: AE: adverse events; DAB: dabrafenib; HCQ: hydroxychloroquine; pt(s): patient(s); PD: progressive disease; TRA: trametinib.

Figure 2. Panel A: swimmer plot in experimental arm. Legend: *: patients included in the lead-in phase 1 trial; black arrow: treatment ongoing; black cross: death; black X: treatment interruption; dark blue bar: progression-free survival on dabrafenib, trametinib and hydroxychloroquine; light blue bar: overall survival; PR: partial response; SD: stable disease. Panel B: swimmer plot in contemporary control arm. Legend: cross: death; black X: treatment interruption; dark blue bar: progression-free survival on dabrafenib and trametinib; grey bar: progression-free survival on dabrafenib, trametinib and hydroxychloroquine; HCQ: hydroxychloroquine add-on; light blue bar: overall survival; SD: stable disease.

Figure 3. Evolution of the sum of diameters of target lesions in 9 evaluable patients included in the experimental arm (red, blue and orange lines) and 4 evaluable patients included in the contemporary control arm (purple and green lines). Legend: blue line: partial response as best confirmed objective response (investigational arm); green line: stable disease as best confirmed objective response (contemporary control arm); orange line: stable disease as best confirmed objective response (investigational arm); purple line: progressive disease as best confirmed objective response (contemporary control arm); red line: progressive disease as best confirmed objective response (investigational arm); sphere: new lesions; square: progression of non-target lesions; triangle: progression of non-target lesions and new lesions. Abbreviations: SDTL: sum of diameters of target lesions.

TABLES

Table 1. Patient baseline characteristics.

	Safety lead-in DAB + TRA + HCQ upfront *n* = 4	**Experimental arm phase 2 DAB + TRA + HCQ upfront *n* = 6**	**Contemporary control arm phase 2 DAB + TRA (HCQ add-on) *n* = 4**
Sex (*n* [%])			
Male	2 (50.0)	5 (83.3)	3 (75.0)
Female	2 (50.0)	1 (16.7)	1 (25.0)
Age (median [range])	43 (27-56)	65 (30-82)	59.5 (52-77)
ECOG PS (*n* [%])			
0	1 (25.0)	0 (0)	1 (25.0)
1	3 (75.0)	6 (100.0)	2 (50.0)
2	0 (0)	0 (0)	1 (25.0)
Melanoma subtype (*n* [%])			
Superficial spreading	1 (25.0)	0 (0)	3 (75.0)
Nodular	0 (0)	3 (50.0)	0 (0)
Cutaneous NOS	1 (25.0)	1 (16.7)	0 (0)
Unknown primary lesion	2 (50.0)	2 (33.3)	1 (25.0)
AJCC stage (*n* [%])			
IV-M1a	0 (0)	1 (16.7)	0 (0)

IV-M1b	0 (0)	2 (33.3)	0 (0)
IV-M1c	3 (75.0)	4 (66.7)	2 (50.0)
IV-M1d	1 (25.0)	0 (0)	2 (50.0)
Number of affected organs (median [range])	4.5 (2-6)	4.5 (1-5)	3.5 (3-7)
Lactate dehydrogenase (*n* [%])			
Normal	1 (25.0)	3 (50.0)	2 (50.0)
Elevated	3 (75.0)	3 (50.0)	2 (50.0)
***BRAF* mutation subtype (*n* [%])**			
V600E/D	3 (75.0)	5 (83.3)	2 (50.0)
V600K/R	0 (0)	1 (16.7)	1 (25.0)
V600 NOS	1 (25.0)	0 (0)	1 (25.0)
Prior lines of therapy			
Median (range)	2.5 (2-6)	3 (3-6)	3.5 (3-4)
2 (*n* [%])	2 (50.0)	0 (0)	0 (0)
3 (*n* [%])	1 (25.0)	4 (66.7)	2 (50.0)
>3 (*n* [%])	1 (25.0)	2 (33.3)	2 (50.0)
BRAF-/MEK-inhibitor (*n* [%])	4 (100.0)	6 (100.0)	4 (100.0)
***BRAF*^{V600} mutant ctDNA present (*n* [%])**	3 (100.0)*	6 (100.0)	3 (100.0)*

*1 patient in each arm did not undergo baseline ctDNA analysis. Abbreviations: AJCC: American Joint Committee on Cancer; ctDNA: circulating tumor DNA; DAB: dabrafenib; ECOG PS: Eastern Cooperative Oncology Group performance status; HCQ: hydroxychloroquine; NOS: not otherwise specified; trametinib: TRA.

Table 2. Adverse events in the study population.

Adverse events (*n* [%])	Safety lead-in DAB + TRA + HCQ upfront *n* = 4		Experimental arm phase 2 DAB + TRA + HCQ upfront *n* = 6		Contemporary control arm phase 2 DAB + TRA (HCQ add-on) *n* = 4	
	All-grade	Grade 3-4	All-grade	Grade 3-4	All-grade	Grade 3-4
Any adverse event	4 (100.0)	2 (50.0)	6 (100.0)	5 (83.3)	4 (100.0)	1 (25.0)
Fatigue	4 (100.0)	2 (50.0)	5 (83.3)	0 (0)	2 (50.0)	0 (0)
Fever	2 (50.0)	0 (0)	4 (66.7)	0 (0)	2 (50.0)	0 (0)
Abdominal pain	2 (50.0)	0 (0)	1 (16.7)	0 (0)	1 (25.0)	0 (0)
Anorexia	2 (50.0)	0 (0)	2 (33.3)	0 (0)	0 (0)	0 (0)
Anemia	1 (25.0)	0 (0)	2 (33.3)	1 (16.7)	0 (0)	0 (0)
Arthralgia	1 (25.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
AST increased	1 (25.0)	0 (0)	2 (33.3)	0 (0)	0 (0)	0 (0)
Back pain	1 (25.0)	1 (25.0)	0 (0)	0 (0)	0 (0)	0 (0)
Chills	1 (25.0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)
Constipation	1 (25.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
CPK increase	1 (25.0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)
Diarrhea	1 (25.0)	0 (0)	0 (0)	0 (0)	1 (25.0)	0 (0)
Dyspnea	1 (25.0)	1 (25.0)	0 (0)	0 (0)	1 (25.0)	0 (0)
Flu-like symptoms	1 (25.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Hyperhidrosis	1 (25.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Hyponatremia	1 (25.0)	0 (0)	2 (33.3)	0 (0)	1 (25.0)	1 (25.0)
Ileocecal invagination	1 (25.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Nausea	1 (25.0)	0 (0)	1 (16.7)	0 (0)	2 (50.0)	0 (0)
Platelet count decreased	1 (25.0)	0 (0)	3 (50.0)	0 (0)	0 (0)	0 (0)
Lymphocyte count decreased	0 (0)	0 (0)	4 (66.7)	1 (16.7)	0 (0)	0 (0)
Acute kidney injury	0 (0)	0 (0)	2 (33.3)	1 (16.7)	0 (0)	0 (0)
Central serous retinopathy	0 (0)	0 (0)	2 (33.3)	0 (0)	0 (0)	0 (0)
Lipase increase	0 (0)	0 (0)	2 (33.3)	1 (16.7)	0 (0)	0 (0)
Alopecia	0 (0)	0 (0)	2 (33.3)	0 (0)	0 (0)	0 (0)
White blood cell decreased	0 (0)	0 (0)	3 (50.0)	0 (0)	1 (25.0)	0 (0)
Acneiform dermatitis	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)
ALT increase	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)
Anxiety/psychotic disorder	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)
Chest wall pain	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)
Eczema	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)
Left ventricular ejection fraction decreased	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)
GGT increased	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)
Headache	0 (0)	0 (0)	1 (16.7)	1 (16.7)	1 (25.0)	0 (0)
Hypercalcemia	0 (0)	0 (0)	1 (16.7)	1 (16.7)	0 (0)	0 (0)

Lung infection	0 (0)	0 (0)	1 (16.7)	1 (16.7)	0 (0)	0 (0)
Neutrophil count decreased	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)
Photophobia	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)
Skin infection	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)
Urinary tract infection	0 (0)	0 (0)	1 (16.7)	1 (16.7)	0 (0)	0 (0)
Xerostomia	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)
Urticaria	0 (0)	0 (0)	0 (0)	0 (0)	1 (25.0)	0 (0)
Vomiting	0 (0)	0 (0)	0 (0)	0 (0)	1 (25.0)	0 (0)
Serious adverse event	3 (75.0)	2 (50.0)	3 (50.0)	2 (33.3)	1 (25.0)	0 (0)
Adverse events leading to temporary treatment interruption	2 (50.0)	1 (25.0)	5 (83.3)	2 (33.3)	3 (75.0)	0 (0)
Adverse events leading to treatment discontinuation	0 (0)	0 (0)	3 (50.0)*	2 (33.3)	0 (0)	0 (0)
Adverse events leading to dose reduction	1 (25.0)	1 (25.0)	3 (50.0)	0 (0)	1 (25.0)	0 (0)

* One patient only interrupted hydroxychloroquine but continued dabrafenib and trametinib. Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; CPK: creatine phosphokinase; DAB: dabrafenib; GGT: gamma-glutamyltransferase; HCQ: hydroxychloroquine; TRA: trametinib.

Table 3. Best objective response in the intention-to-treat population.

	Safety lead-in DAB + TRA + HCQ upfront *n* = 4	**Experimental arm phase 2 DAB + TRA + HCQ upfront *n* = 6**	**Contemporary control arm phase 2 DAB + TRA *n* = 4**	**Contemporary control arm phase 2 DAB + TRA + HCQ add-on *n* = 2**
Best objective response (*n* [%])				
Complete response	0 (0)	0 (0)	0 (0)	0 (0)
Partial response	0 (0)	2 (40.0)	0 (0)	0 (0)
Stable disease	2 (50.0)	1 (20.0)*	1 (25.0)	0 (0)
Progressive disease	2 (50.0)	2 (40.0)	3 (75.0)	2 (100.0)
Not evaluable	0 (0)	1 (20.0)	0 (0)	0 (0)
Objective response rate (*n* [%])	2 (20.0)		0 (0)	0 (0)
Disease control rate (*n* [%])	5 (50.0)		1 (25.0)	0 (0)

*This is 1 patient with an unconfirmed partial response. Abbreviations: DAB: dabrafenib; HCQ: hydroxychloroquine; TRA: trametinib.