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ORIGINAL ARTICLE

Pharmacokinetic and cytokine profiles of melanoma patients with dabrafenib and trametinib-induced pyrexia

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Abstract

Purpose The combination of a BRAF inhibitor dabrafenib and a MEK inhibitor trametinib (CombiDT) has improved outcomes compared with chemotherapy or BRAF inhibitor monotherapy in advanced BRAF V600E/K melanoma. However, CombiDT causes a high incidence of pyrexia and treatment interruptions. Pharmacokinetic analysis may provide an explanation for the pyrexia.

Methods 34 patients with Stage 3 BRAF V600 melanoma were treated with CombiDT on a clinical trial between August 2014 and June 2017. Plasma concentrations of drugs and metabolites were determined using validated LC–MS assays, in addition to analysis of a panel of cytokines.

Results Pyrexia was experienced by 71% of the patients, with an additional 17% requiring dose interruption related to a pyrexia-like prodrome. Dabrafenib concentrations ranged from 4.0 to 4628 ng/ml and trametinib from 1.0 to 45 ng/ml in 34 patients. *N*-desmethyl-dabrafenib was the most prevalent metabolite, followed by carboxy- and hydroxy-dabrafenib. No definitive association between pyrexia and AUC or C_{min} of the drugs, or metabolites could be observed. The level of IL-1B at the early during treatment (EDT) (as a % of pre-treatment) was higher in the pyrexia group (median 109% (range 32–681%) than in the no-incidence group [56% (26–79%)] ($p = 0.029$). Similarly, the level of IL-6 at EDT was higher in the pyrexia group [181% (34-3156%) vs 73% (57–101%)] ($p = 0.028$).

Conclusions No apparent associations between pyrexia and exposure to the drugs or metabolites could be observed. Greater elevations in IL-1B and IL-6 were observed in patients with pyrexia during the first week of treatment compared to those without pyrexia.

Keywords Dabrafenib · Trametinib · BRAF V600 melanoma · Pharmacokinetics · Pyrexia · Cytokines

Introduction

Targeted therapy with selective BRAF inhibitors such as dabrafenib or vemurafenib has improved survival outcomes compared with chemotherapy in BRAF V600E/K metastatic melanoma [1–3]. Furthermore, the combination of dabrafenib and the MEK inhibitor trametinib (CombiDT) provided a superior response rate, progression free survival and overall survival compared with dabrafenib [4–7] or vemurafenib alone [8].

Although adverse events due to paradoxical activation of mitogen-activated protein kinase (MAPK) pathway, such as cutaneous toxicity, are less frequent with CombiDT than with BRAF inhibition alone, the incidence of pyrexia (body temperature > 38.0 °C) is higher (50–70% of patients), recurrent and more severe [4–6, 8]. Such toxicity resulted

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in almost one quarter of patients on a clinical trial of adjuvant CombiDT discontinuing therapy within the planned 1-year schedule [9]. Symptoms of pyrexia are managed by interruptions to treatment, and in recurrent cases, a planned intermittent schedule or addition of corticosteroids is commonly used for prophylaxis.

To date, there are neither known clinical factors that associate with pyrexia nor are there data to suggest the etiology of CombiDT pyrexia. To our knowledge, only one study has examined CombiDT pharmacokinetics and pyrexia [10]. A trend towards a higher risk of pyrexia with increasing plasma concentration of dabrafenib and of its primary metabolite hydroxy-dabrafenib was identified [10]. Trough dabrafenib plasma concentrations (48 ng/mL) predictive of adverse effects were also reported in a small study of patients with metastatic melanoma, perhaps indicating that dabrafenib dose reduction may be of benefit [11]. However, interruption of treatment is preferred to dose reduction, due to concerns regarding potential disease resistance [12, 13]. Dose reductions also appear less effective in preventing recurrent pyrexia and may compromise efficacy [4, 14]. Further study is required to establish clinically significant predictive factors for the incidence of pyrexia.

To this end, the pharmacokinetics of dabrafenib and trametinib were investigated in BRAF-mutated Stage 3 melanoma patients receiving neoadjuvant CombiDT. Concentrations of drug and metabolites, together with an analysis of a panel of inflammatory cytokines were investigated in relation to the incidence of pyrexia.

Methods

Patients and treatment

CombiDT was given as part of an open-label, phase II neoadjuvant clinical trial in patients with resectable Stage 3 BRAF V600E/K melanoma (NeoCombi NCT01972347) between August 2014 and June 2017 [15]. Patients received a total of 52 weeks of CombiDT therapy (dabrafenib 150 mg twice daily, trametinib 2 mg daily given in 28-day cycle) with 12 weeks of this as a neoadjuvant treatment.

Patients were given self-administration diaries to record dosing times for dabrafenib and trametinib, and to document incidents of pyrexia, including raised temperature ($\geq$ 38.0 °C) or other relevant symptoms (e.g. chills, rigors). Patients were followed up in the clinic to document treatment interruptions. For each patient, drug administration times were obtained for dabrafenib and trametinib from patient diaries. Time after dose was calculated for each sample using the last drug administration time and the recorded sampling time. When there was a prolonged discontinuation of treatment, time after dose was still defined relative to the most recent documented administration time. All patients provided informed consent prior to the start of the study. This study was approved by the relevant ethics committee.

Drugs and metabolites in patient samples

EDTA blood samples for the preparation of plasma were collected at baseline, week 1 (day 4–7), 2, 4, 8, 12 and additionally during episodes of pyrexia. The exact sample time was recorded, but these were not at fixed times following the previous administration of drugs. Plasma from a total of 205 samples from 34 patients was collected and stored at − 80 °C prior to analysis.

Plasma samples were prepared for determination of drug and metabolite concentrations using liquid chromatography–mass spectrometry (LC–MS) (Thermofisher, TSQ Series). Samples were extracted using acetonitrile (60µ l sample: 90µ l acetonitrile containing 0.05µ g/ml vemurafenib as an internal standard (IS)). The LC–MS method used 0.1% formic acid in water (70%) and methanol (30%) as mobile phases, guard column (Agilent Zorbax Eclipse XDB 1 × 17 mm, 5 µm) and column (Agilent Zorbax Eclipse XDB C8 1 × 50 mm, 3.5 µm) at a flow rate of 0.2 ml/min, with a run time of 4 min.

The lower limit of quantification (LLQ) was 1 ng/ml for both dabrafenib and trametinib. Standard solutions in plasma for dabrafenib ranged from 1 ng/ml (CV 7.2%) to 1000 ng/ml (CV 13.0%) and for trametinib from 1 ng/ml (CV 9.8%) to 100 ng/ml (CV 10.1%), with a linear correlation of 0.98–0.99 from five separate assays. Quality control (QC) solutions of 50/5 ng/ml and 500/50 ng/ml dabrafenib/trametinib were also used to confirm consistent performance of LC–MS analysis. Samples with concentrations above the highest standard were diluted with plasma (50:50) before extraction and were re-analysed.

The extracted solutions from the patient samples were re-analysed for dabrafenib metabolites, identified by MS using published molecular weights [16]. Concentrations of hydroxy- carboxy and *N*-desmethyl dabrafenib metabolites were quantified as peak area ratios (Metabolite:IS).

Population pharmacokinetics analysis

Population PK analysis for dabrafenib and trametinib was performed using nonlinear mixed-effects modeling with NONMEM, Version 7.3 (ICON Development Solutions, USA). The first-order conditional estimation method with interaction (FOCE-I) was used for parameter estimation. Model development was managed using Perl-Speaks-NONMEM (PsN) 3.5.3 (Uppsala, Sweden;[17]), Pirana 2.8.1 (Amsterdam, The Netherlands;[18]) and R (Version 3.2.5, Vienna, Austria;[19]). Model improvement was assessed by objective function value (OFV, − 2log likelihood) changes. A decrease in OFV value

of at least 3.84 indicated a statistically significant improvement ($P < 0.05$, χ^2 distribution) for the inclusion of an additional parameter.

Associations with causes of pyrexia

Exposures to dabrafenib, trametinib and metabolites

From the population pharmacokinetic model, dabrafenib and trametinib AUC (area under concentration) and trough (C_{min}) values for each patient were calculated for each day of administration, and averaged over the 12-week neo-adjuvant treatment period. Exposure to each metabolite was measured as averaged peak-area ratio for each patient (for multiple samples for each patient, only those taken within 12 h of the most recent dose were included). These measures of exposure to the drugs were compared in patients with pyrexia, those with treatment interruptions (but no documented pyrexia) and in those who continued therapy with no documented pyrexia. The Mann–Whitney test was used for statistical comparison of differences in exposures between pyrexia, treatment interruption and no incidence groups.

Cytokines analysis

Peripheral blood samples (~ 10 ml) were collected from 32 patients in EDTA vacutainer tubes (BD Vacutainer Blood Collection Tubes) before treatment, early during treatment (EDT; day 4–7), at weeks 2, 4, 6, 8 and 12, and on disease progression. Blood samples were centrifuged at 1500 rpm (800×*g*) for 15 min at room temperature to separate plasma. Approximately 3–4 ml plasma were collected and centrifuged again at 4100 rpm (1600×*g*) for 10 min at room temperature. The collected plasma samples were stored at − 80 °C until analysis. Undiluted plasma samples were measured for cytokine concentrations using the 65-plex Human Cytokine/Chemokine Discovery assay (Cat no: HD65; Eve Technologies, Alberta, Canada). The 65-plex Discovery assay utilized 150 µl of undiluted plasma per run and each run was performed in duplicate; duplicates did not vary by more than 4% (data not shown). For each cytokine, the median and range of pre-treatment, early during treatment (EDT, day 4–7) and peak concentration was compared between the pyrexia, treatment-interruption, and no-incidence group. The Mann–Whitney test was used for statistical analysis of differences in each cytokine concentration between pyrexia, treatment-interruption and no-incidence groups.

Results

Patient characteristics

Thirty-four patients commenced CombiDT on trial and had blood samples available for assessment. Most were male (56%), and patients had a median age of 57 years (range 22 to 84), and median weight of 86 kg (range 51–134 kg) (Table 1).

Pyrexia and treatment interruptions

Pyrexia (≥ 38.0 °C) occurred in 71% (24/34) of patients, resulting in treatment interruption during the first 12 weeks of dabrafenib and trametinib combination therapy. An additional 4 patients (12%) had treatment interruption only, without documented pyrexia. These patients experienced symptoms of pyrexia such as chills, rigors or malaise without formal documentation of a raised body temperature. The remaining 6 (18%) patients received continuous treatment with no treatment-limiting side effects and no pyrexia or pyrexia-prodrome. Pyrexia was managed with frequent dose interruptions (intermittent regimen or treatment break), in some cases re-introducing treatment initially either as dabrafenib monotherapy or with temporary dose reductions. Anti-pyretics such as paracetamol (4/24 patients) or prophylactic prednisolone (9/24 patients) were used to control this side effect. The median time to onset of pyrexia was 15 days (range 1–69 days). Median duration of pyrexia was 1 day (range 1–7 days). Of those 24 patients who experienced pyrexia, 18 (75%) had recurrent episodes, with a median of 2 episodes (range 1–5). Patients with pyrexia and pyrexia symptoms were older compared to those without any of these incidence (median age 58 vs 43, $p = 0.008$).

Table 1 Patient characteristics for the 34 patients from whom plasma samples were available for analysis

Patient characteristics	Values	Number of patients ($n = 34$)
Gender		
Male		19
Female		15
Age		
Median (range)	57 (22–84) years old	
≥ 65 years old		25 patients
< 65 years old		25 patients
Age		
Median (range)	86 (51–134) kg	
≥ 70 kg		25 patients
< 70 kg		25 patients

Dabrafenib and trametinib concentrations in patient samples

Dabrafenib and trametinib concentrations were measurable in 139 (dabrafenib) and 162 (trametinib) plasma samples from the 34 patients, after excluding baseline measurements and samples with undetectable drug levels due to a prolonged time (~ 300–700 h) after the most recent previous dose (32 dabrafenib and 9 trametinib samples). Dabrafenib concentrations ranged from 4.0 to 4628.0 ng/ml and trametinib from 1.0 to 45.0 ng/ml. Both dabrafenib and trametinib concentrations showed high inter-patient variability, particularly for trametinib in the first 10 h after administration (Fig. 1a, b). Dabrafenib concentrations were generally detectable for up to 4 days (100 h) after the most recent previous dose, and trametinib concentrations were detectable for up to about 12 days (up to 300 h) after the most recent previous dose.

Dabrafenib metabolite concentrations in patient samples

Three dabrafenib metabolites, hydroxy-, carboxy- and *N*-desmethyl-dabrafenib were measurable in 150 samples from the 34 patients. As with the parent drug, each of the metabolites showed a high degree of inter-patient variation. Based on LCMS peak-area ratios, *N*-desmethyl-dabrafenib was the most prevalent metabolite, followed by carboxy-dabrafenib and then hydroxy-dabrafenib. Most samples with detectable metabolites were taken within 24 h after drug administration (Fig. 2), but in some patients metabolites were detectable for up to 10 days (data not shown).

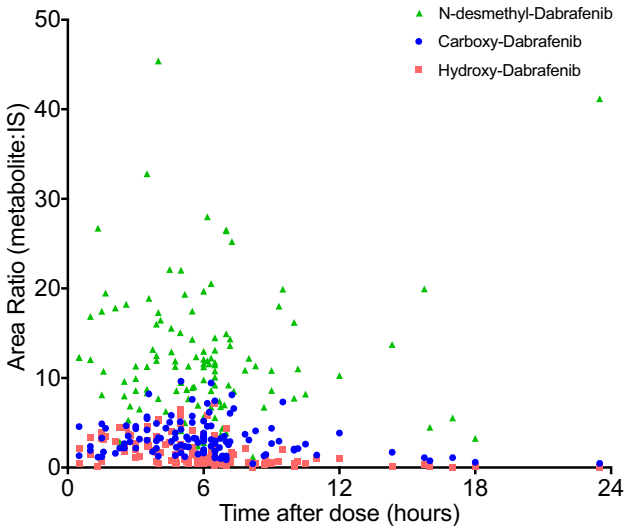

Fig. 2 Dabrafenib metabolites (*N*-desmethyl-, carboxy- and hydroxy-dabrafenib) area ratio [metabolite: internal standard (IS)] in 34 patients plotted against time after the most recent previous dose (plotted only up to 24 h). Data are drawn from more than one dosing occasion for each patient

Population pharmacokinetics analysis

Dabrafenib

From the 139 measurable dabrafenib concentrations, 117 concentrations from 33 patients were used in the final model. Exclusion of these observations in the final model building was due to outlier concentrations (e.g. too high or low concentration), which may be due to poor compliance with the study protocol or deviations from the assumptions of the

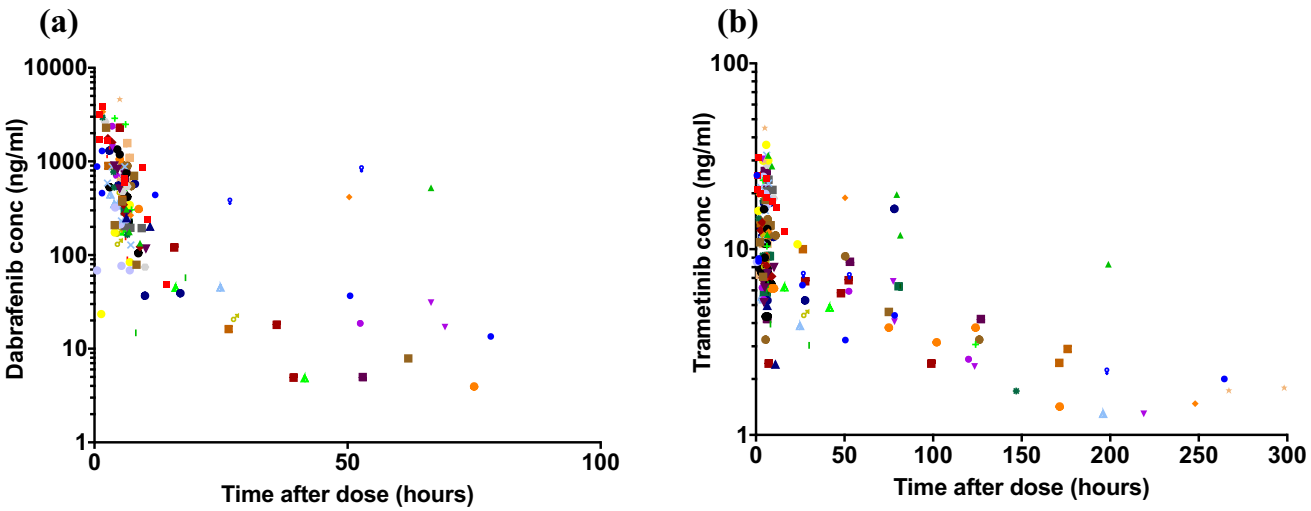

Fig. 1 Dabrafenib (**a**) and trametinib (**b**) concentrations in 34 patients (each symbol represents a patient) plotted against time after the most recent previous dose. Data are drawn from more than one dosing occasion for each patient

population analysis due to the intermittent nature of drug administration. Log-transformed concentrations were used as this improved the model. No significant effect of weight as a covariate was observed for any parameter in our dataset.

A two-compartment model, with first-order absorption, provided the best fit to the dabrafenib data. Dabrafenib CL/*F* (clearance) and V_c/F (central volume of distribution) were estimated to be 24.7 L/h and 88.3 L, respectively, by the model. K_a (absorption rate), *Q*/*F* (distributional clearance) and V_p/F (peripheral volume of distribution) were fixed to the literature values [20] as these parameters could not be estimated with the available data (Table 2).

The final model showed good agreement between the observations and the model predictions (Supplementary Figure S1) and good agreement between the observations and the model simulations (Supplementary Figure S2).

Trametinib

All 162 measurable trametinib concentrations from 34 patients were included in the final trametinib population PK model. Log-transformed concentrations were used. Trametinib PK was best described by a two-compartment with first-order absorption. CL/*F* and V_c/F were estimated to be 6.15L/h and 195L, respectively, and were comparable to literature values (Table 3). K_a, *Q*/*F* and V_p/F were fixed to literature values [21].

The final model showed good agreement between observed and predicted concentrations (Supplementary Figure S3) and good agreement between observed and simulated concentrations (Supplementary Figure S4).

Table 2 The population parameter estimates from the final dabrafenib model

Population PK parameter	Estimated value	Compared to literature
CL/*F*	24.7(L/h) (RSE 42%) (BSV 19.8%)	34.3 (L/h) [20]
V_c/F	88.3(L)	70.3 (L) [20]
K_a	1.8 (1/h)	Fixed to literature value [20]
Q/*F*	3.44 (L/h)	Fixed to literature value [20]
V_p/F	150 (L)	Fixed to literature value [20]

CL/F oral clearance, V_c/F central volume of distribution, V_p/F peripheral volume of distribution, K_a absorption rate, *Q*/*F* distributional clearance, *BSV* between-subject variability, *RSE* relative standard error

Associations with causes of pyrexia

Exposure to dabrafenib

Dabrafenib average AUC and C_{min} for the patients from the final model were calculated and analysed for their association with pyrexia. Incidents of pyrexia during the first 12 weeks (neo-adjuvant period) were included in the analysis.

The median average dabrafenib AUC was 5.55 mg.h/L (range 4.61–7.52 mg.h/L) in patients with no incidence of pyrexia or treatment interruption, 5.95 mg.h/L (5.17–7.21 mg.h/L) in patients with treatment interruption only, and 5.98 mg.h/L (4.99–7.83 mg.h/L) in patients with pyrexia: pyrexia *vs* no-incidence, $p = 0.23$; treatment-interruption *vs* no-incidence, $p = 0.91$.

The median average dabrafenib trough concentration was 416 ng/ml (222–497 ng/ml) in patients with no incidence of pyrexia or treatment interruption, 310 ng/ml (251–488 ng/ml) in patients with treatment interruption only, and 392 ng/ml (74.3–907 ng/ml) in patients with pyrexia: pyrexia *vs* no-incidence, $p = 0.90$; treatment-interruption *vs* no-incidence, $p = 0.61$.

There was no statistically significant difference in dabrafenib AUC or C_{min} comparing those patients with pyrexia, those with treatment interruption and those without pyrexia (Fig. 3).

Exposure to trametinib

Similarly, trametinib average AUC and C_{min} for the patients were calculated and analysed for their association with pyrexia.

The median average trametinib AUC was 0.223 mg.h/L (0.167–0.451 mg.h/L) in the no-incidence group, 0.343 mg.h/L (0.285–0.413 mg.h/L) in the

Table 3 Population parameter estimates from the final trametinib model

Population PK parameter	Estimated value	Compared to literature
CL/*F*	6.15(L/h) (RSE 7%) (BSV 39%)	4.91(L/h) [21]
V_c/F	195 (L) (RSE 23%) (BSV 69%)	214 (L) [21]
K_a	2 (1/h)	Fixed to literature value [21]
Q/*F*	60 (L/h)	Fixed to literature value [21]
V_p/F	568 (L)	Fixed to literature value [21]

CL/F oral clearance, V_c/F central volume of distribution, V_p/F peripheral volume of distribution, K_a absorption rate; *Q*/*F* distributional clearance, *BSV* between-subject variability, *RSE* relative standard error

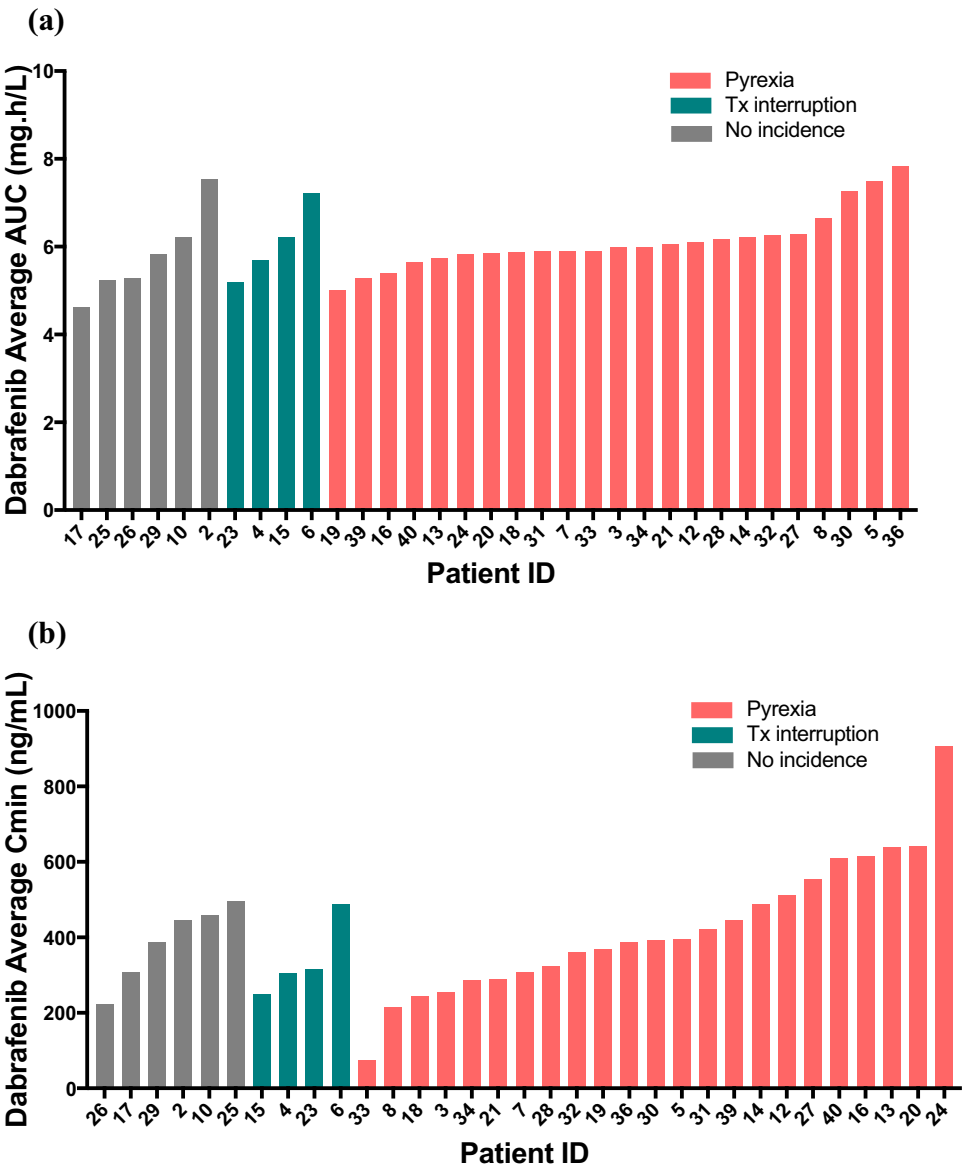

Fig. 3 Dabrafenib average AUC (**a**) and average C_{min} (**b**) from the final model for patients in pyrexia, treatment interruption and no incidence group. (patients ordered in ascending value for each variable within the groups)

treatment-interruption only group, and 0.349 mg.h/L (0.161–0.608 mg.h/L) in the pyrexia group: pyrexia *vs* no-incidence, $p = 0.19$; treatment-interruption *vs* no-incidence, $p = 0.35$.

The median average trametinib trough concentration was 10.1 ng/ml (7.39–19.0 ng/ml) in the no-incidence group, 10.1 ng/ml (3.55–10.3 ng/ml) in the treatment-interruption group, and 9.41 ng/ml (1.91–17.7 ng/ml) in the pyrexia group: pyrexia *vs* no-incidence, $p = 0.25$; treatment-interruption *vs* no-incidence, $p = 0.61$.

There was no significant difference in trametinib AUC or C_{min} comparing those patients with pyrexia, those with treatment interruption and those without pyrexia (Fig. 4).

Exposure to dabrafenib metabolites

Average metabolite peak-area ratios during the first 12 h after the most recent dose were obtained for three dabrafenib metabolites (Fig. 5) in 33 patients. One patient (patient ID: 24) had no detectable metabolites in samples taken within the 12-h period after dosing.

For N-desmethyl dabrafenib, the median peak area ratio was 11.3 (3.01–13.2) in patients with no incidence of pyrexia or treatment interruption, 13.4 (5.7–18.9) in patients with treatment interruption only, and 10.9 (4.0–24.3) in patients with pyrexia: pyrexia *vs* no-incidence, $p = 0.90$; treatment-interruption *vs* no-incidence, $p = 0.48$.

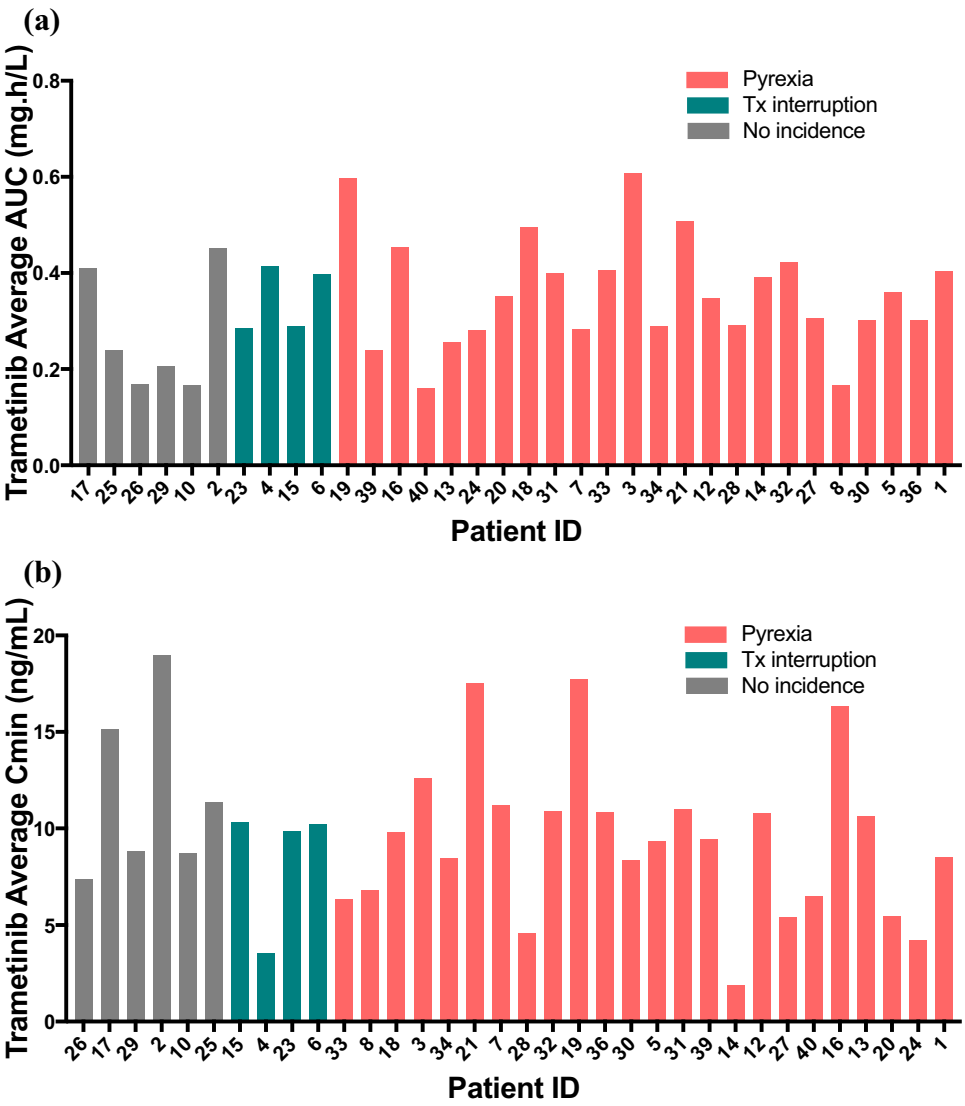

Fig. 4 Trametinib average AUC (**a**) and average C_{min} (**b**) from the final model for patients in pyrexia, treatment interruption and no incidence group (in the corresponding order of dabrafenib AUC and C_{min} data, respectively)

The median peak area ratio for carboxy-dabrafenib was 2.73 (1.09–4.19) in the no-incidence group, 4.88 (1.61–8.22) in treatment-interruption only group, and 3.16 (1.07–9.88) in the pyrexia group: pyrexia *vs* no-incidence, $p = 0.33$; treatment-interruption vs no-incidence, $p = 0.26$.

For hydroxy-dabrafenib, the median value was 1.17 (0.32–2.35) for the no-incidence group, 3.08 (0.62–4.16) for the treatment-interruption group, and 1.68 (0.35–5.03) in the pyrexia group: pyrexia *vs* no-incidence, $p = 0.28$; treatment-interruption *vs* no-incidence, $p = 0.17$.

No apparent association between metabolite exposures and incidence of pyrexia or treatment interruptions was observed.

Cytokines

Concentrations of 65 cytokines in the plasma of 32 patients were measured. For each cytokine, the median value for each patient group was compared to identify any apparent differences in pre-treatment values, peak values, and early during treatment (EDT) absolute values. None of these were significantly different between patients with or without pyrexia (data not shown), with the exception noted below.

When expressed as a percentage of matched pre-treatment samples (all 65 cytokines shown in Supplementary Table S1), the level of IL-1B at EDT was higher in the pyrexia group (median 109% (range 32–681%) than in the no-incidence group [56% (26–79%)] ($p = 0.029$) (Fig. 6a). Similarly, the level of IL-6 at EDT was also higher in the pyrexia group (181% (34–3156%) than in the no-incidence group [73% (57–101%)] ($p = 0.028$) (Fig. 6b).

Combining the treatment-interruption group with the pyrexia group reduced the magnitude of these differences for both IL-1B [median 88% (range 32–681%), $p = 0.04$] and IL-6 [170% (34-3156%), $p = 0.06$].

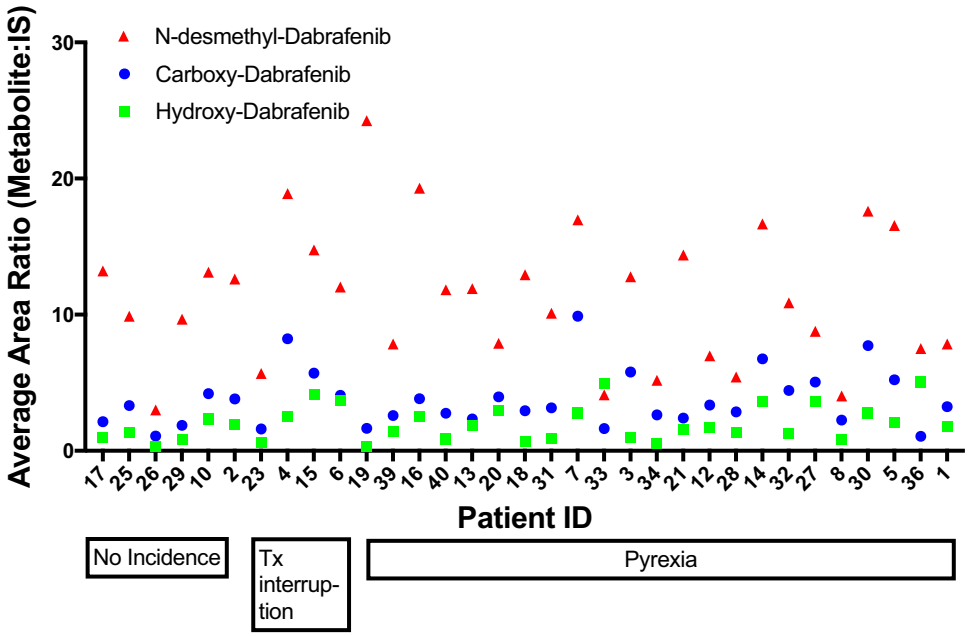

Fig. 5 Average metabolite ratio for three dabrafenib metabolites for the samples taken within 12 h after the most recent dose in 33 patients (In the corresponding patient ID order for dabrafenib AUC data)

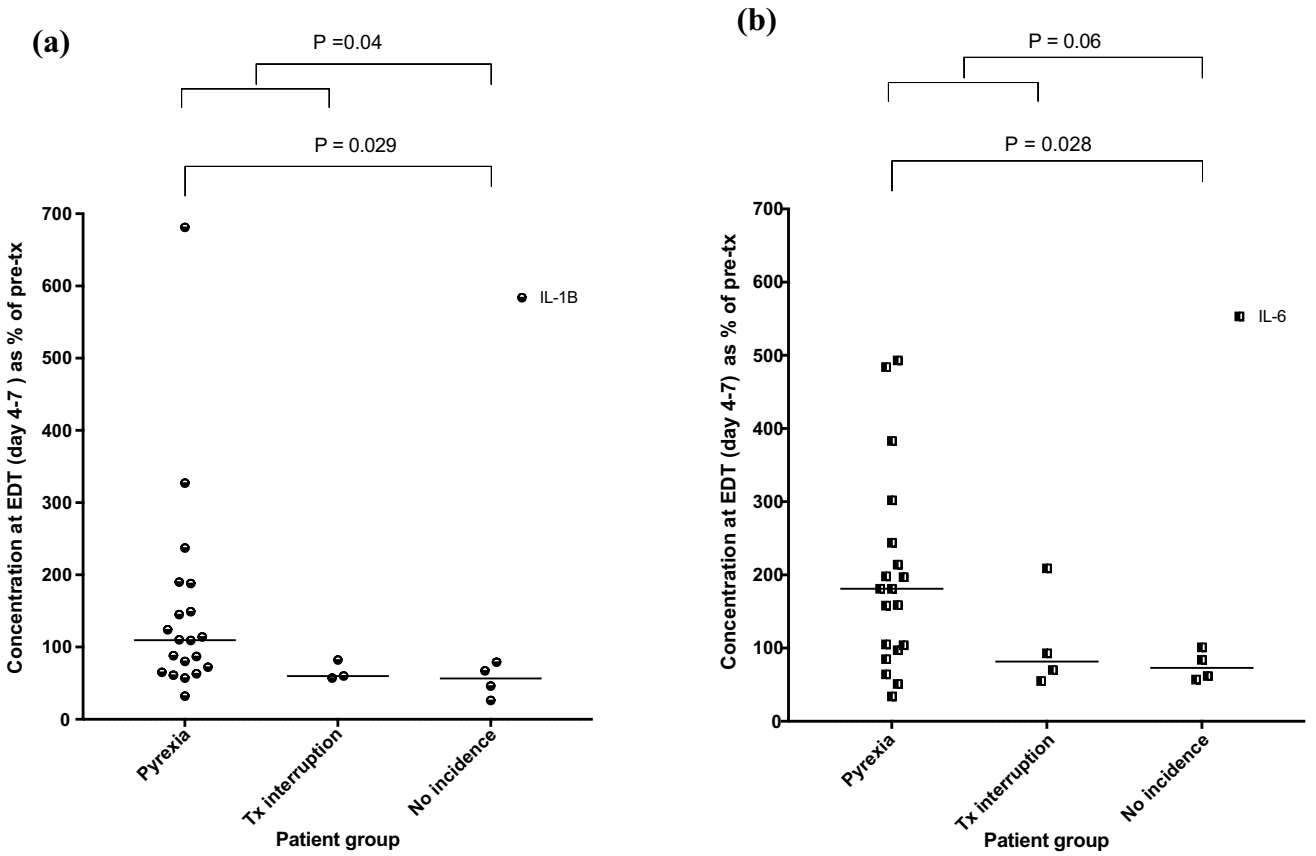

Fig. 6 Concentrations of **a** IL-1B and **b** IL-6 at EDT (early during treatment: day 4–7) as % of pre-treatment, in patients with pyrexia, treatment (Tx) interruption and no incidence. Horizontal bars represent median. **a** $n=27$ patients with available IL-1B data, **b** $n=28$ patients with available IL-6 data [note: one patient in the pyrexia group with IL-6 concentration of >3000% (not plotted)]. *p* values are stated comparing pyrexia *vs* no-incidence group, and between combined pyrexia plus treatment-interruption group *vs* no-incidence group (using the Mann–Whitney test)

Discussion

Constitutively activating mutations in the BRAF serine–threonine kinase gene have been reported in 33–47% of primary melanomas [22–24] and 41–55% of metastatic melanomas [22, 25, 26], resulting in cancer cell growth and proliferation. The most common BRAF mutation is V600E (valine to glutamic acid at position 600) in 74–95% of patients, followed by V600K (valine to lysine) in 5–20% of patients [26–29].

Selective inhibitors of BRAF V600E/K, such as vemurafenib and dabrafenib significantly improved response rates and survival outcomes compared to conventional chemotherapy [1, 2, 30]. However, skin toxicities were common with BRAF inhibitor monotherapy [4, 5], and resistance to BRAF inhibition occurred largely due to MAPK pathway re-activation [31–33]. This led to the combination use of BRAF inhibitors with MEK inhibitors such as trametinib [4]. The combination of dabrafenib and trametinib (CombiDT) has significantly improved outcomes and demonstrated superior long-term clinical benefit compared to dabrafenib monotherapy, with a 3-year PFS of 22% versus 12% and OS, 44% versus 32% [7]. CombiDT is currently a standard treatment for stage 4 melanoma in patients with the V600 BRAF mutation, at a dose of 150 mg twice daily for dabrafenib and 2 mg daily for trametinib.

As opposed to the improved treatment outcome and reduced hyperkeratosis (6% in CombiDT compared to 33% in dabrafenib monotherapy), the most common and treatment-limiting side effect of CombiDT is pyrexia, observed in 52–59% of patients in contrast to only 25–33% of patients receiving dabrafenib monotherapy in Phase III trials [6, 7]. In the earlier Phase I/II study, the incidence of pyrexia in CombiDT treatment group was 71% compared to 26% in the dabrafenib monotherapy group [4]. The onset of the first pyrexia during CombiDT therapy is usually in the first 3 weeks of treatment, with a median duration of 9 days. The toxicity was episodic but recurrent, often resolving with dose interruption [10].

No significant association has been observed between patient or disease-related characteristics (e.g. age, gender, lactate dehydrogenase (LDH), ECOG performance status, renal function, baseline tumour burden, BRAF genotype, metastasis stage) and pyrexia, and the prevalence of this side effect is not significantly associated with clinical outcome [10]. A genome-wide study combining data from five CombiDT clinical studies found no significant associations between common SNPs or HLA alleles and pyrexia [34]. A trend of higher dabrafenib C_{avg} and hydroxy-dabrafenib C_{min} levels being associated with higher proportion of patients with pyrexia has been reported [10]. These findings suggested that a more systematic analysis of pharmacokinetic associations between pyrexia and exposure to the drugs or metabolites would be useful.

Exposure to dabrafenib is dose-proportional up to 300 mg twice daily following a single dose, and is less than dose-proportional after repeat dosing (up to day 15) [35, 36]. Following a single dose of 150 mg twice daily, the peak dabrafenib concentration is reached 2 h after dose, and the terminal half-life is approximately 5 h [36, 37]. Dabrafenib is primarily metabolised by CYP2C8 and CYP3A4 to a hydroxy-dabrafenib, which is further oxidized to carboxy-dabrafenib, and decarboxylated via non-enzymatic process to form desmethyl-dabrafenib [16]. Carboxy- and *N*-desmethyl-dabrafenib have longer half-lives than dabrafenib and hydroxy-dabrafenib (18–20 h *vs* 5–6 h) [16]. Of the administered dose, 71% undergoes faecal excretion primarily as unchanged drug, and urinary excretion accounts for 23% of the administered dose primarily as metabolites [16]. In previous pre-clinical and clinical pharmacokinetic studies, dabrafenib seemed to induce its own metabolism [35, 36, 38], which is consistent with observed decrease in exposure after repeat dosing [35], but steady-state concentrations were achieved within 14 days of dosing [20]. A change from gelatin to hypromellose (HPMC) capsules during clinical development resulted in higher dabrafenib absorption (up to twofold increase in exposure) [39] and a population pharmacokinetic identified capsule shell as the most significant covariate (55%), while gender and weight had only a modest influence (< 20%) on dabrafenib exposure [20]. Accounting for these factors would reduce the between-subject variability of pharmacokinetic parameters. Despite this, our estimates of the PK parameters are consistent with previous studies [20]. Weight was not a significant covariate in the current dabrafenib dataset, which is consistent with a recent dabrafenib PBPK model (physiologically based pharmacokinetic model) [40].

Trametinib is rapidly absorbed, and median time to C_{max} is 1.5 h after the recommended phase II dose of 2 mg daily [41]. Pharmacokinetics of trametinib is characterized by a dose-proportional increase in exposure up to day 15 with repeat dosing [41], which is also consistent with the later reports from a population pharmacokinetic study [21]. Oral bioavailability is 72% when fasted, but the absorption is delayed when administered with food [42]. Trametinib has a long half-life of about 4 days [41] and reaches steady-state after about 3 weeks [43]. Consistent with the literature, trametinib concentration was detected up to 300 h after administration in our study. The trametinib pharmacokinetic parameter estimates in our study were comparable to those of the previous population pharmacokinetic study [21]. In that study, trametinib clearance was lower in females than in males, and increased with weight. No other covariates, such as age, renal or hepatic impairment, had any effect on

clearance [21]. A small mass-balance study in two patients reported that trametinib predominantly undergoes faecal excretion, accounting for up to 39% of the administered dose, mostly as unchanged drug, and urinary recovery accounted for up to 9% of the administered dose mainly as deacetylated and/or hydroxylated metabolites [44]. However, the detection of trametinib metabolites in plasma was challenging due to low recovery (> 50% of samples having lower level of quantification) for samples taken during 2–48 h period post-dose. Indeed, during LC–MS analysis in our study, peaks for trametinib metabolites from the plasma samples could not be detected.

The aim of the population pharmacokinetic parameterisation, including estimated or fixed values based on previous models [20, 21], was to quantify dabrafenib and trametinib exposure in individual patients based on the sparse data available. Using model-predicted values, associations between the exposure to the drugs and incidents of pyrexia or treatment interruption were investigated. However, there was no apparent difference in exposure or trough level of either drug between patients with or without pyrexia. Exposure to metabolites also had no relationship with pyrexia incidence, although an association with higher exposure to hydroxy-dabrafenib has been reported [10].

A greater risk of adverse effects (including flu syndrome, fatigue and myalgia) was reported to occur during CombiDT therapy in patients with a dabrafenib C_{min} above 48 ng/mL, requiring in dose reduction [11]. In our study, the incidence rate for pyrexia was much higher (70% vs 30%), as was the range of estimated dabrafenib trough concentrations (median 388.2 (range 74.29–907.2 ng/ml) vs median 118.6 (range 33.9–203.3 ng/ml). Our study also had a more formal definition of pyrexia (temperature above 38 °C in the presence or absence of other pyrexia symptoms), as opposed to non-specific symptoms of pyrexia. In addition, the study by Menzies et al. [10] reported an increased trend towards pyrexia incidence for patients in the higher dabrafenib C_{avg} tercile, however, dabrafenib trough (C_{min}) level and AUC were used in our analysis.

Trametinib trough concentrations (median 9.7 (range 1.9–19.0 ng/ml)) were lower in our patients compared to those from the previous study (median 13.9 (8.4–19.4 ng/ml) for patients with adverse effects requiring dose reduction, and median 11.3 (7.1–15.5 ng/ml) in no dose reduction group [11]). Although trametinib trough concentration above 10 ng/mL was associated with greater clinical response and PFS [21], no data about its predictive role in toxicity have been reported. Menzies et al. also did not observe any association between trametinib C_{min} and pyrexia [10].

Other than the pharmacokinetics of dabrafenib and trametinib, other factors were also considered as potential contributing causes to the side effect of pyrexia. Cytokines are signalling molecules having a role in regulating immune and inflammatory responses to various triggers [45]. Pyrexia may be triggered by an excess of inflammatory cytokines, which could be used as a predictive marker of toxicity. In this study, a panel of cytokines, including chemokines, interleukins, interferons, lymphokines and tumour necrosis factor were investigated. The levels of IL-1B and IL-6 were higher during the first week of treatment in patients with pyrexia and pyrexia symptoms than in those with no incidence of these side effects. Cytokines such as IL-6 and IL-1B have been identified previously as major endogenous pyrogens and pro-inflammatory cytokines [46–51]. In our study, the elevation in IL-6 and IL-1B was observed early during treatment (EDT = day 4–7), preceding the onset of pyrexia, which had a median onset of 15 days post-treatment, similar to 19 days reported in the previous study [10].

In our study, the prevalence of CombiDT-induced pyrexia was higher than in the previously reported phase III studies [6–8]. A high proportion of patients (71%) with pyrexia and only a small number of patients in the no pyrexia group was a major limitation to observing any further apparent relationships between the investigated factors and the incidence of this side effect.

Furthermore, unlike previous studies by Menzies et al. [10] and Rousset et al. [11], our study was conducted in resectable Stage III B/C melanoma patients only, rather than Stage IV (metastatic). Upcoming clinical trial data from which our study was based on (NCT01972347), may further inform about toxicity and inter-patient variability observed in our patients.

Alternative hypotheses for the etiology of drug-induced pyrexia are possible, for instance, effect of trametinib on dabrafenib metabolism or non-pharmacokinetic mechanisms that affect the temperature set point in the hypothalamus of some individuals. Further studies are required to explore these other mechanisms and to provide definitive pyrexia management strategies rather than empirical treatment interruptions and delays.

Conclusion

Potential causes of drug-induced pyrexia were investigated. However, no apparent relationship between exposure to drugs or metabolites, and pyrexia was observed. Greater levels in IL-1B and IL-6 were observed in patients with pyrexia during the early treatment period compared to those without pyrexia. The small proportion of patients without pyrexia was a limitation to observing other potentially significant pharmacokinetic associations.

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Compliance with ethical standards

Conflict of interest A.M. Menzies is a consultant/advisor for Bristol-Myers Squibb, MSD, Novartis, Pierre-Fabre, and Roche. G.V. Long is a consultant/advisor for Amgen, Array, Bristol-Myers Squibb, Merck, Novartis, Pierre-Fabre, and Roche. All other authors have declared no conflicts of interest.

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