Digoxin Plus Trametinib Therapy Achieves Disease Control in BRAF Wild-Type Metastatic Melanoma Patients

Abstract
This is the first prospective study of a combination therapy involving a cardenolide and a MEK inhibitor for metastatic melanoma. Whereas BRAF mutant melanomas can exhibit profound responses to treatment with BRAF and MEK inhibitors, there are fewer options for BRAF wild-type melanomas. In preclinical studies, we discovered that cardenolides synergize with MEK inhibitor to promote the regression of patient-derived xenografts irrespective of BRAF mutation status. We therefore conducted a phase 1B study of digoxin 0.25 mg and trametinib 2 mg given orally once daily in 20 patients with advanced, refractory, BRAF wild-type melanomas. The most common adverse events were rash, diarrhea, nausea, and fatigue. The response rate was 4/20 or 20% with response durations of 2, 4, 6, and 8 months. The disease control rate (including partial responses and stable disease) was 13/20 or 65% of patients, including 5/6 or 83% of patients with NRAS mutant melanomas and 8/14 or 57% of NRAS wild-type melanomas. Patients with stable disease had disease control for 2, 2, 2, 4, 5, 6, 7, 10, and 10 months. Xenografts from four patients recapitulated the treatment responses observed in patients. Based on these pilot results, an expansion arm of digoxin plus MEK inhibitor is warranted for NRAS mutant metastatic melanoma patients who are refractory or intolerant of immunotherapy.

Key points
Digoxin plus trametinib is well tolerated and achieves a high rate of disease control in BRAF wild-type metastatic melanoma patients.

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Introduction
Whereas BRAF mutant melanomas often exhibit striking responses to treatment with BRAF and/or MEK inhibitors [7,14,15,30], BRAF wild-type melanomas generally do not respond. The MEK inhibitor trametinib extends progression-free survival of patients with BRAF mutant melanomas by 3.3 months relative to traditional chemotherapy [14,15] but not of patients with BRAF wild-type melanomas, irrespective of NRAS mutation status [13]. Only 10% of patients with BRAF wild-type melanomas respond to trametinib therapy [13].

Systemic therapy for inoperable or metastatic BRAF wild type melanoma was revolutionized with the introduction of CTLA-4 and/or PD-1 blockade. Response rates for single agents vary from 15% to 30% and for combinations from 40% to 60% [19]. Nevertheless,
many patients do not benefit, and the autoimmune complications are frequent and diverse (Horvat, 2015). Other approved immunotherapy treatments include recombinant human interleukin-2 and tamirolgene laherparepvec virotherapy [3,16]. They have low response rates and distinct cytokine storm-related side effects. Chemotherapy yields even lower response rates of 5% to 10% with no survival benefit. In this setting, there is an acute need for new therapies [25].

We previously screened 200,000 small molecules for increased toxicity against primary human melanoma cells as compared to normal human melanocytes [12]. Several cardiac glycosides, including digoxin and digitoxin, were found to exhibit increased toxicity against melanoma cells as compared to normal human melanocytes and umbilical cord blood cells. This reflected on-target inhibition of the ATP1A1 Na⁺/K⁺ pump, which maintains ion gradients across the plasma membrane that are critical for the transport of a variety of substrates in and out of cells. Cardiac glycosides were not sufficient to induce the regression of patient-derived xenografts, but they synergized with MAPK pathway inhibitors to induce regression. The combination of digitoxin plus MEK inhibitor induced cytoplasmic acidification, dysregulated mitochondrial calcium levels, and induced the death of melanoma cells but not normal human melanocytes or umbilical cord blood cells [12]. These responses were observed in patient-derived xenografts of both BRAF wild-type and BRAF mutant melanomas.

Based on these observations, we initiated a phase 1B trial of digoxin and trametinib in Stage IV BRAF wild-type metastatic melanoma patients refractory or intolerant to immune checkpoint blockade. Patients were stratified for NRAS mutation status and history of prior immunotherapy. Tumor samples were collected in a subset of patients. We report safety and efficacy in 20 patients and compared responses in patients to the responses observed in xenograft avatars from 4 patients.

**Patients and Methods**

The study design was a phase 1B, single-site, single-dose level, combination of digoxin and trametinib in 20 patients. Digoxin was purchased from Jerome Stevens Pharmaceuticals, Inc., and trametinib was provided by Glaxo-Smith Kline, Inc. The study was performed under FDA IND exemption #123040, registered in clinical trials.gov as NCT02138292, and approved by the University of Texas Southwestern Medical Center Institutional Review Board—IRB #01913. The study was conducted in accordance with the Declaration of Helsinki.

We enrolled patients with a histologic diagnosis of BRAF wild-type unresectable or metastatic melanoma that were ineligible or had failed immune checkpoint therapy, were age ≥ 18 years, had Eastern Cooperative Group performance status of <2, and gave informed consent according to institutional and federal guidelines. Other eligibility requirements included NRAS mutation assessment, adequate contraception for both men and women of child-bearing potential, and measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST) v1.1. Tumor sizes were evaluated within 4 weeks prior to beginning therapy. The patients could not have received chemotherapy, radiation therapy, or any melanoma systemic therapy within 3 weeks prior to entering the study. Patients must have recovered from adverse events due to agents administered more than 3 weeks earlier, could receive no concomitant other melanoma therapy, and have no active CNS disease and no active infection with hepatitis B or C or HIV. Patients could not have other malignancies within the last 2 years except for cured basal or squamous cell skin cancer or superficial bladder cancer or carcinoma in situ of the cervix. They could have no uncontrolled intercurrent illness such as heart disease or psychiatric disorder, no history of retinal vein occlusion or central serous retinopathy, no hypersensitivity to digoxin, no known cardiac metastases, no Wolf-Parkinson-White syndrome or AV heart block or intracardiac defibrillator, no history of interstitial lung disease or unresolved pneumonitis, and no treatment-refractory hypertension.

Patients were treated at the University of Texas Southwestern Medical Center with 8-week cycles of outpatient trametinib 2 mg orally and digoxin 0.25 mg orally with dose adjustments to maintain digoxin serum concentrations at 0.8 to 2.1 ng/ml and to reduce trametinib-related toxicities to CTCAE v4.2 grade 1 or less. Patients maintained a study medication diary. A day 4 digoxin level was obtained to make early dose adjustments.

Patients were seen weekly in clinic during the first cycle and prior to each new cycle to obtain history with performance status, physical exam with vital signs, electrocardiograms, chemistries, blood counts, digoxin levels, assessment of adverse events and concurrent medications, and troponin T levels. At week 4 of the first cycle, cardiac echocardiogram and serum magnesium and lactate dehydrogenase levels were obtained. Optional tumor biopsies were obtained from some patients pretreatment and at progression. Computed tomographic or magnetic resonance imaging scans were done prior to each cycle.

Adverse events were recorded and graded on the basis of the CTCAE v4.2 (http://ctep.cancer.gov/reporting/ctc.html). Antitumor effects were measured according to RECIST v1.1 criteria [11].

For patient-derived xenograft assays, melanoma specimens were obtained with informed consent from patients according to protocols approved by the Institutional Review Board of the University of Texas Southwestern Medical Center eIRB#012014-007. Tumors were dissociated in Kontes microtubes with VWR disposable pestles followed by enzymatic digestion for 20 minutes with 200 U/ml of Worthington collagenase IV, 5 mM CaCl₂, and 50 U/ml of DNase. To obtain a single cell suspension, cells were filtered through a 40-μm cell strainer. From each patient sample, equal numbers of cells (up to 1 million suspended in BD Biosciences Matrigel) were injected subcutaneously into the right flank of 20 NSG mice [27]. Treatment with digoxin and trametinib was initiated when tumors became palpable. Mice were randomized into groups and implanted with 42-day Braintree Scientific #AP-2006 osmotic pumps containing either 50% DMSO or digoxin (Sigma) at 10 mg/kg/day in 50% DMSO. Seven to 10 mice were orally gavaged daily with trametinib (Selleckchem) at 0.5 mg/kg/day in gavage solution containing 5% DMSO, 0.5% promethylcellulose, and 0.2% Tween 80, or control mice received 200 μl of gavage solution. Tumors were measured weekly with calipers. The study ended after 42 days of treatment. These experiments were performed according to protocol 2011-0118, approved by the Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center.

Toxicities in patients were dichotomized as none versus any, or none and mild versus moderate to severe. The rates of toxicity, disease control rate (DCR), partial response (PR) rate, and fraction of patients with stable disease (SD) as well as their 95% confidence intervals were estimated using an exact binomial method. Fisher’s exact test was used to assess the significance of differences in DCR for different treatment groups and different age groups. Response duration was estimated using a Kaplan-Meier method and was
Table 1. Characteristics of the Patients and Their Disease

| Characteristics               | Number of Subjects (N = 20) |
|-------------------------------|-----------------------------|
| Age, median (range), years    | 68 (36-84)                  |
| Gender (male/female)          | 8/12                        |
| Race                          |                             |
| Caucasian                     | 19                          |
| Hispanic                      | 1                           |
| Disease                       |                             |
| Cutaneous                     | 11                          |
| Unknown                       | 4                           |
| Vulvar                        | 3                           |
| Uveal                         | 1                           |
| Subungual                     | 1                           |
| Prior therapy                 |                             |
| Lines, median (range)         | 1 (0-2)                     |
| Ipilimumab                    | 6                           |
| High-dose interleukin 2       | 2                           |
| Temozolomide                  | 2                           |
| Pembrolizumab                 | 1                           |
| Cisplatin                     | 1                           |
| Interferon                    | 1                           |
| GM-CSF                        | 1                           |
| Active sites of metastases    |                             |
| Lung                          | 12                          |
| Soft tissues                  | 7                           |
| Nodes                         | 6                           |
| Liver                         | 2                           |
| NRAS                          |                             |
| Wild type                     | 14                          |
| Mutant                        | 6                           |

defined as the time from treatment initiation to time of progression. Analysis of variance was used to test whether treatment with digoxin plus trametinib significantly affected tumor diameter in xenografts.

Results

Fifty-six patients were screened, and 20 patients were treated in the study. All 20 patients were evaluable for safety analysis and response. All patients received at least one 8-week cycle of treatment. The patients’ demographic data and prior treatment information are presented in Table 1 and Supplementary Table S1. There were 12 females and 8 males. The median age was 68 years (range, 36 to 84 years). The patients had received an average of 1 prior therapy, although 11 patients had received no prior regimens and 6 patients had been treated with 2 modalities. Eleven patients had cutaneous melanoma; three patients had vulvar melanoma, one patient had a subungual melanoma, one patient had uveal melanoma, and four patients had melanoma of unknown origin. Active sites of metastases at therapy onset were lung (n = 12), liver (n = 2), soft tissues (n = 7), and lymph nodes (n = 6). Fourteen patients were NRAS wild type, and six patients were NRAS mutant.

Adverse events attributed to drug treatments are listed in Table 2 and Supplementary Table S2. Toxicities were mild to moderate in most cases and consisted of rash (n = 19), diarrhea (n = 12), nausea (n = 9), and fatigue (n = 4). Three patients showed transient confusion, and one patient had an episode of syncope. These were expected based on prior clinical results with trametinib alone. Further, with patient education and early symptomatic intervention, patients were able to tolerate the regimen. No toxicities attributable to digoxin were observed.

There were 4 PRs and 9 SDs for an overall DCR of 13/20 or 65% with a 95% confidence interval of 41% to 85%. Table 3 and Supplementary Table S3 detail the response and response duration (defined as time from treatment initiation to progression of disease) for each patient. PRs persisted for 2, 4, 6, and 8 months. SDs lasted for 2, 2, 2, 4, 5, 6, 7, 10, and 10 months. Median duration of response for 13 patients with PR and SD was 5 months. NRAS mutant patients had a DCR of 5/6 or 83%. NRAS wild-type patients had a DCR of 8/14 or 57%. Prior immunotherapy was associated with a DCR of 7/8 or 88%. Immunotherapy-naive patients had a DCR of 6/12 or 50%. Females had a DCR of 9/12 or 75%, and males had a DCR of 4/8 or 50%. There was no difference in DCR between cutaneous and noncutaneous primaries or between patients greater than 70 years old and those less than 70 years old. Figure 1 shows a waterfall plot of RECIST measurements.

We had shown previously that the metastatic behavior of patient-derived xenografts in NSG mice correlates with the metastatic behavior of the same melanomas in patients treated only with surgery [29]. To begin to assess whether treatment responses also correlate between NSG mice and patients, a subset of trial patients had biopsies from metastatic sites that were transplanted subcutaneously into immunocompromised NSG mice (avatars). Avatars treated with digoxin plus trametinib exhibited tumor growth inhibition that correlated with patient response. Digoxin plus trametinib controlled disease in patient #2 (SD), #9 (PR), and #15 (SD) and significantly reduced the growth of xenografts from the same patient (Figure 2). In contrast, patient #16 exhibited progressive disease in response to digoxin plus trametinib, and xenografts from the same patient also did not respond (Figure 2). These results suggest that xenograft responses to targeted agents in NSG mice can reflect responses in patients and raise the possibility that responsiveness to this therapy is primarily determined by intrinsic differences among melanomas.

The xenografts of patients #9 and #15 began to show a small but progressive increase in tumor diameter after approximately 30 days of treatment that was statistically significant for patient #9 (paired t-test, day 30 vs 43, P = .0035). Of note, patient #9 also demonstrated a short-lived response in the actual clinical trial. One possible explanation for this observation could be the acquisition of trametinib resistance in a subpopulation of tumor cells. Alternatively, some tumor cells may become less sensitive to digoxin therapy due to downregulation of ATP1A1 during the course of treatment. We have observed this phenomenon previously in mouse xenografts, but it is unknown whether this affects treatment efficacy or occurs in the patients in this clinical trial.

Discussion

We developed a xenograft assay in which melanomas obtained from patients engraft efficiently in NOD/SCID IL2Rγnull (NSG) mice [27,28]. Melanoma metastasis in this assay is predictive of clinical outcome in patients [29]. Stage III melanomas that metastasize efficiently in NSG mice form distant metastases in patients despite surgical resection, whereas melanomas that metastasize inefficiently in mice are generally cured by surgery in patients [29]. We used this
assay to test new therapies and determined that cardiac glycosides, including digitoxin and digoxin, synergize with MAPK pathway inhibitors, including trametinib, to promote the regression of patient-derived xenografts [12]. Trametinib and digitoxin additively or synergistically inhibited NHE proton pumps, leading to intracellular acidification, dysregulated mitochondrial calcium levels, a failure of mitochondrial function, and cell death. We observed these effects in melanoma cells but not in normal human melanocytes or hematopoietic cells, each of which exhibits lower levels of ATP1A1 expression and MAPK pathway activation.

The clinical trial described in this study suggests that digoxin plus trametinib induces partial responses in 20% and controls disease in 65% of patients with BRAF wild-type metastatic melanomas. These values are significantly greater than observed for trametinib alone in BRAF wild-type metastatic melanomas (10% PR and 50% DCR) [13]. Our data further suggest that therapy responses of patient-derived xenografts correlate with responses in the actual patients (Figure 2). This finding suggests there are tumor-intrinsic properties that confer sensitivity and resistance to combination therapy with cardenolides and MEK inhibition. Along with our prior studies of metastatic behavior [29], the behavior of xenografts in NSG mice is consistent with the clinical outcome in patients. Progression after initial response to therapy in the NSG xenografts and in patients may reflect tumor escape from targeted pathway inhibition as expected from mutation and evolution of a population of melanoma cells in vivo under selective pressure. Although xenograft studies may take too long to impact treatment decisions for individual patients, they appear to be a promising model for studying mechanisms of therapy responsiveness.

Retrospective studies show that patients taking cardiac glycosides for a heart indication exhibited a 25% reduction in prostate cancer incidence [25], reduced breast cancer recurrence after mastectomy [32], and better survival outcomes for various carcinomas (breast, colon, liver, and head and neck) [21]. Cardiac glycoside use increased the risk of breast cancer or death from prostate cancer in other studies [2,5,23]. Several phase I and II clinical trials have tested digoxin as a single agent or in combination with chemotherapy or targeted agents in multiple cancers [22]. These included a phase II trial in melanoma that combined digoxin with cisplatin, IL-2, IFNα, and vinblastine [22]. To our knowledge, no results have yet been reported from these trials. Our results suggest that it will be necessary to combine cardiac glycosides with targeted agents to achieve disease control but that they can synergize with MAPK pathway inhibitors in at least some cancers [12].

Digoxin plus trametinib was tolerated at full doses of digoxin and trametinib. The study was designated phase 1B as this was the first clinical experience with this combination. The major toxicities of rash, diarrhea, and fatigue were expected based on the prior clinical results with trametinib alone [1,13,20]. With patient education and early symptomatic intervention as described by others [38], patients were able to tolerate the regimen and exhibited fewer side effects. Four patients on study discontinued treatment because of side effects, but the majority tolerated the treatment well. No cardiac or ocular toxicities were observed as had been seen in others receiving trametinib therapy, perhaps because of strict patient selection criteria in this study [24,33]. No toxicities attributable to digoxin were observed. Thus, most patients were able to remain on study with the oral medications for months without complications.

Assessment of antimelanoma activity is limited by the small sample size and heterogeneous patient population. We evaluated only 20 patients, and these patients had metastatic melanomas with different sites of origin including cutaneous, mucosal, and uveal tissues. Nevertheless, some preliminary findings merit discussion.

Digoxin plus trametinib had activity in BRAF wild-type metastatic melanoma patients regardless of primary site or patient age. The preliminary PR rate of 20% and DCR of 65% for the combination were somewhat better than the 10% PR rate and 50% DCR of single agent trametinib [13]. Interestingly, NRAS mutant patients in our study appeared to have better disease control rates than historical controls, with a DCR of 83% for digoxin plus trametinib compared to 29% for trametinib alone or 58% for binimetinib alone [4,10,13]. Thus, we hypothesize enhanced activity of digoxin plus trametinib therapy in the NRAS mutant metastatic melanoma population. The mean duration of DCR (independent of NRAS mutation status) was 5+ months for the trametinib/digoxin combination as compared to 7 months for trametinib alone or binimetinib alone. The variable depth and duration of response were most consistent with a primarily

**Table 3. Digoxin Plus Trametinib Responses**

| Response Type | Metastatic Sites | NRAS Status | Primary | Response Duration (mo) |
|---------------|-----------------|-------------|---------|-----------------------|
| PR            | Lung, SQ, Lung, Lung & Nodes | Q61K, G13D, WT, WT | C, C, V, C | 4, 2, 8, 6 |
| SD            | Lung, SQ, Lung & SQ, Lung, Lung & SQ & Liver & Nodes, Nodes, Muscle, Lung & Liver, Nodes | WT, WT, Q61K, WT, WT, WT, Q61K, WT, WT | C, U, C, O, S, C, V, U, C | 10, 2, 6, 8+, 2, 2, 4, 7, 5 |
| PD            | Lung, Lung, Lung & Nodes, SQ, Nodes, SQ, Lung | WT, WT, WT, Q61L, WT, WT, WT | C, C, U, U, C, G, V | — |

PR, partial response; SD, stable disease; PD, progressive disease; SQ, subcutaneous; C, cutaneous; V, vaginal; U, unknown; O, uveal; S, subungual.
cytostatic mechanism of action. This suggests that although more patients seem to respond to combination therapy with trametinib and digoxin, response durability remains a challenge.

The predominance of stable disease rather than partial remissions in the DCR merits caution. Fluctuations in computed tomographic scans or exams may overestimate activity. A focus on remissions in expanded studies of patient cohorts is critical.

Among the seven patients with a history of ipilimumab treatment, there was an 86% DCR. The prior study of single agent trametinib in BRAF wild-type patients did not reference prior immune checkpoint inhibitor therapy [13]. Thus, the role of recent ipilimumab on response is difficult to quantitate. Some of the activity observed in the current study may be due to the combination of MEK inhibition with anti-CTLA4 inhibition. Nevertheless, there were six patients in the current study who responded without prior ipilimumab. Thus, we hypothesize that there is added benefit to the digoxin combination.

There are few other clinical reports of MEK inhibitor combinations for BRAF wild-type metastatic melanoma. The tubulin polymerization stabilizer paclitaxel was given intravenously at 80 mg/m² on days 1, 8, and 15 every 4 weeks in combination with trametinib (2 mg po daily) [9]. The combination was well tolerated, and among eight NRAS mutant patients, there were a 50% PR rate and a 75% DCR. The median duration of response was 3.6 months. The depth of cytoreduction was better than with digoxin and trametinib, but the disease control duration was similar. Chemotherapy-related myelosuppression was common, however. The CDK4/6 inhibitor ribociclib was given orally (200-300 mg daily for 21 of 28 days) in combination with binimetinib (45 mg orally twice daily) [34]. Twenty-two patients were treated. The combination showed severe toxicities in some patients with high-grade creatine phosphokinase elevations and a fatal case of cardiomyopathy. Nine of 22 or 41% of patients experienced PRs, and 18/22 or 82% experienced disease control. Median duration of benefit was 6.7 months. Again, the CDK4/6 inhibitor regimen yielded greater antitumor activity but with greater toxicity than the digoxin combination. Two BRAF wild-type metastatic melanoma patients received the AKT inhibitor afuresertib (50 mg orally daily for 10 days per month) plus trametinib (1.5 mg orally daily). One of the two patients achieved a PR lasting 10 months. However, the regimen was associated with severe dyspnea, pulmonary embolism, headaches, nausea, vomiting, colitis, transaminasemia, and bowel obstruction or hemorrhage. Similarly, the AKT inhibitor uprosertib (50 mg orally daily) with trametinib (1.5 mg orally daily) in metastatic uveal melanoma patients produced significant adverse events including high-grade transaminasemia, rash, nausea, and diarrhea and only 1/20 PR.

Preclinical studies with human or dog melanoma cell line xenografts in immunocompromised mice demonstrated synergy between trametinib or binimetinib and the ERBB inhibitor afatinib [17], metformin [36], vincristine [26], the ROCK inhibitor fasudil [35], the PKC inhibitor AEB071 [8], the PI3K inhibitor BEZ235 [37], and radiotherapy [31]. However, none of these combinations have been tested clinically.

There are additional opportunities for digoxin plus trametinib therapy. In our preclinical study, this drug combination significantly prolonged the survival of mice xenografted with NRAS mutant acute myeloid leukemia cell lines [12]. Two recent reports documented complete responses of NRAS mutant myeloid leukemias to trametinib.
alone [6,18]. One patient with NRAS G12D atypical chronic myeloid leukemia achieved a durable (14+ months) hematologic remission with trametinib (2 mg orally daily). Thirteen of 61 or 21% of RAS mutant myeloid malignancy patients treated with trametinib (2 mg orally daily) had hematologic and marrow remissions. Median response duration was 2 months. The mild to moderate side effects and oral outpatient regimen suggest that the digoxin plus trametinib combination has the potential to be a good therapeutic option for elderly NRAS mutant myeloid leukemia patients.

In summary, this pilot study supports the advancement of the digoxin plus MEK inhibitor combination therapy into pivotal phase 2 trials in NRAS mutant melanomas as well as potentially in other NRAS mutant malignancies.

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References

[1] Abdel-Rahman O, EIHalawami H, Ahmad H, and Ellithy M (2015). Risk of selected gastrointestinal toxicities in cancer patients treated with MEK inhibitors. Exp Rev Gastroenterol Hepatol 9(11), 1433–1445.

[2] Ahern T, Lash T, Sorensen H, and Pedersen L (2008). Digoxin treatment is associated with an increased incidence of breast cancer: a population-based case-control study. Breast Cancer Res 10, R102.

[3] Andtbacka RH, Kaufman HL, Collichio F, Amatruda T, Senzer N, and Chesney J, et al (2015). Talimogene laherparepvec improves durable response rate in stage IV melanoma. J Clin Oncol 33(25), 2780–2788.

[4] Ascierto PA, Schadendorf D, Agarwala SS, van Herpen CM, and Queirolo P, et al (2013). MEK162 for patients with advanced melanoma harbouring NRAS or V600E BRAF mutations: a non-randomised, open-label phase 2 study. Lancet Oncol 14(3), 249–256.

[5] Borthakur G, Popplewell L, Boyiadzis M, Foran J, Platzbecker U, and Vey N, et al (2009). Combined BRAF and MEK inhibition in melanoma with BRAF V600E mutations. Lancet Oncol 10, 1143–1149.

[6] Chen X, Wu Q, Tan L, Porter D, Jager MJ, and Emery C, et al (2014). Cardiogenic shock and respiratory failure in a patient with metastatic melanoma receiving trametinib. J Clin Oncol 32(2), 363–364.

[7] D’Incalci M, D’Ascola P, Pugliese M, Gennari L, and Martinelli P, et al (2011). Combined BRAF and MEK inhibition in melanoma with RAF150 mutations: a phase II dose-escalation trial. J Clin Oncol 29(17), 2236–2240.

[8] Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, and Ford R, et al (2000). New response evaluation criteria in solid tumours: revised RECIST guidelines (version 1.1). Eur J Cancer 45(2), 228–247.

[9] Falchuk GS, Lewis RW, Rinn KN, and Grant R, et al (2014). Synergistic effects of ion transporter and MAP kinase pathway inhibitors in melanoma. Nature Commun [in press].

[10] Falchuk GS, Lewis RW, Rinn KN, and Grant R, et al (2014). Combined BRAF and MEK inhibition in melanoma with V600E mutations. New England Journal of Medicine 367, 1694–1703.

[11] Falchuk GS, Lewis RW, Rinn KN, and Grant R, et al (2014). Combined BRAF and MEK inhibition in melanoma with V600E mutations. New England Journal of Medicine 367, 107–114.

[12] Heng HH, Kuo Y, Ong WY, and Tan WH, et al (2015). Prognostic significance of stable disease following high-dose interleukin-2 (IL-2) treatment in patients with metastatic melanoma and renal cell carcinoma. Cancer Immunol Immunother 64(4), 459–465.

[13] Hushchinson KE, Johnson DB, Johnson AS, Sanchez V, Kuba M, and Lu P, et al (2015). ERBB activation modulates sensitivity to MEK1/2 inhibition in a subset of driver negative melanoma. Oncoarget 6(26), 22348–22360.

[14] Khanna V, Pierce ST, Divo KHT, Tognon CE, Hunt DE, and Junio B, et al (2015). Durable disease control with MEK inhibition in a patient with NRAS-mutated atypical chronic myeloid leukemia. Cancer 7(12), e1414.

[15] Kim YJ, Chariot-Silen S, Vlamos G, Rasa H, Grob JJ, Cano C, and Lao CD, et al (2015). Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med 373(1), 23–34.

[16] Movarpoulos JC and Wang TS (2014). Managing the skin toxicities from new melanoma drugs. Curr Treat Options Oncol 15(2), 281–301.

[17] Nair A, Strippolis S, Alvino G, Shargla L, Recchimurzo N, and Guida M (2015). Ocular toxicity in metastatic melanoma patients treated with mitogen-activated protein kinase kinase inhibitors: a case series. Am J Ophthalmol 160(5), 959–967.

[18] Patel PM, Suciu S, Mortier L, Kruit WH, Robert C, and Schadendorf D, et al (2011). Cardiogenic shock and respiratory failure in a patient with metastatic melanoma receiving trametinib. Lancet Oncol 6(7), 1549–1550.

[19] Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, and Morrison SJ (2008). Efficient tumour formation by single human melanoma cells. Nature 456(7222), 593–598.

[20] Quintana E, Shackleton M, Foster HR, Fullen DR, Sabel MS, Johnson TM, and Morrison SJ (2011). Phenotypic heterogeneity among tumorigenic melanoma cells from patients that is reversible and not hierarchically organized. Cancer Cell 18, 510–523.

[21] Quintana E, Pikounova E, Shackleton M, Weinberg D, Edickouk F, Ulker DR, Johnson TM, and Morrison SJ (2012). Human melanoma metastasis in NGS mice correlates with clinical outcome in patients. Sci Transl Med 4(159).

[22] Robert C, Karaszewska B, Schachter J, Rutkowski P, Mackiewicz A, and Schadendorf D, et al (2015). Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med 372, 30–39.

[23] Schick U, Kyula J, Barker H, Patel R, Zaidi S, and Gregory C, et al (2015). ERBB activation modulates sensitivity to MEK1/2 inhibition in a subset of NRAS-mutated atypical chronic myeloid leukemia. Sci Transl Med 7(301), 034.

[24] Tseng D, Mason XL, Neilan TG, and Sullivan RJ (2016). Cardiogenic shock and respiratory failure in a patient with metastatic melanoma receiving trametinib therapy. Oncologist [Epub Jul 7].

[25] Van Herpen C, Postow MA, Carlino MS, Kalkavan H, Weise A, and Amaria RN, et al (2015). Cardiogenic shock and respiratory failure in a patient with metastatic melanoma receiving trametinib therapy. Oncologist [Epub Jul 7].

[26] Vujic I, Saniorenzo M, Posch C, Esteve-Puig R, Yen AJ, and Kwong A, et al (2015). Combined BRAF and MEK inhibition in melanoma with NRAS-mutated atypical chronic myeloid leukemia. Cureus 8, 2165–2170.

[27] Wang TS, Movarpoulos JC, and Lai M, et al (2013). ERBB activation modulates sensitivity to MEK1/2 inhibition in a subset of NRAS-mutated atypical chronic myeloid leukemia. Cancer 7(12), e1414.