Trametinib

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Abstract

The mitogen-activated protein kinase (MEK MAPK/ERK kinase) signaling pathways play a critical role in regulation of diverse cellular activities, including survival, differentiation, proliferation, motility, and angiogenesis. Therefore, MEK inhibition was recognized as a promising target for antineoplastic therapy. While multiple MEK inhibitors have been tested clinically only trametinib (GSK1120212), an oral MEK inhibitor which is selective for MEK1 and MEK2 has shown promising activity in several clinical trials on melanoma and colorectal cancer and it is being evaluated by the FDA for the treatment of metastatic melanoma. Mechanistically it was shown that trametinib induces cell cycle arrest in vitro. In this overview, important preclinical and clinical data for trametinib are presented including mechanism-based in vitro studies as well as findings from different clinical studies. Future clinical trial in different solid tumor entities will define the therapeutic role of this targeted therapy approach, possibly as a combination with other targeted therapies such as BRAF inhibitors.

Contents

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1 Structure and Mechanism of Action

The mitogen-activated protein kinase (MAPK) signaling pathways involve a family of protein kinases that play critical roles in regulation of diverse cellular activities, including cell proliferation, survival, differentiation, motility, and angiogenesis (Thompson and Lyons 2005). The MAPK pathways transduce signals from various extracellular stimuli (growth factors, hormones, mitogens, cytokines, and environmental stress), leading to distinct intracellular responses (Chang and Karin 2001) as shown in Fig. 1. Mitogen-activated protein kinase or MAP2K or MAPKK are commonly known as MEK proteins. These MEK enzymes selectively phosphorylate serine/threonine and tyrosine residues within the activation loop of their specific MAP kinase substrates.

Trametinib (GSK1120212, JTP-74057) is a second-generation small molecule inhibitor of MEK kinase. It functions as allosteric, ATP non-competitive inhibitor with nanomolar activity against both MEK 1 and MEK 2 kinases with a half-maximal inhibitory concentration of 0.7–14.9 nmol/L for MEK1/MEK2 (Gilmartin et al. 2011; Yamaguchi et al. 2011). Inhibitors of MEK1/2 had been previously investigated as targeted therapies for tumors dependent on activating mutations in the MAPK pathway, but prior to trametinib, the success of MEK inhibitors with respect to clinical activity was limited due to the dependence of non-malignant cells on the MAPK pathway which precluded adequate dosing of the inhibitor (LoRusso et al. 2005; Yeh et al. 2007). When compared to other published MEK inhibitors, trametinib has a different pharmacokinetic profile, with a prolonged half-life, and small peak-to-trough ratios, which made it possible to overcome the narrow therapeutic index associated with MEK inhibition. Specificity of trametinib for MEK1/2 was confirmed against a panel of more than 180 kinases, including B-Raf, C-Raf, and MEK5 the closest kinase homolog (Yamaguchi et al. 2011), adjacent to the active site and defined on one side by the activation loop. The inhibitory effect of trametinib on cell growth was shown to be through inhibition of p-ERK 1/2. Therefore, most significant inhibition was achieved in tumor cell lines with mutant B-Raf or Ras (Yamaguchi et al. 2011). In vitro studies have demonstrated that trametinib decreases cell proliferation, causes G1 cell cycle arrest, and induces apoptosis. The structure and chemical characteristics of trametinib are shown in Fig. 2.

2 Preclinical Data

In the initial studies on trametinib, the proliferation across 94 cancer cell lines was evaluated systematically in vitro (Gilmartin et al. 2011; Yamaguchi et al. 2011). Among the different cell lines evaluated in the study, those with either $BRAF^{V600E}$

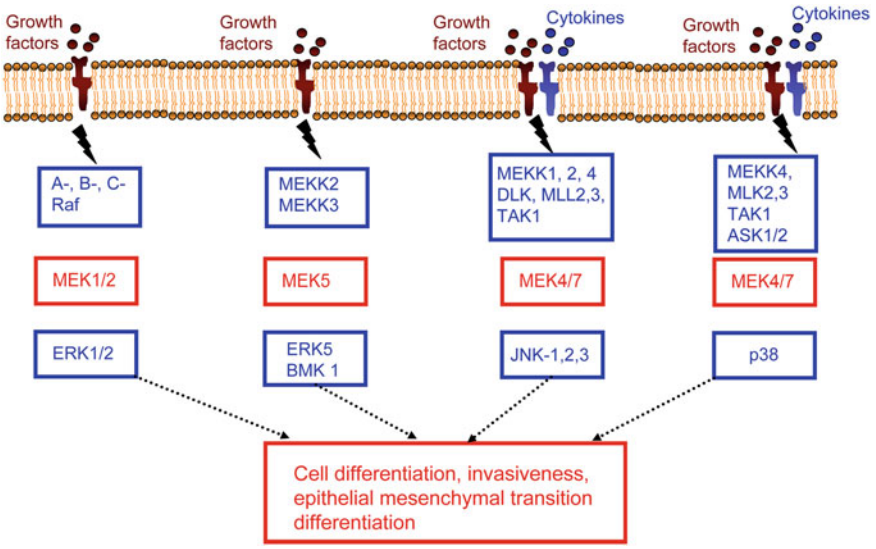

Fig. 1 The known MEK enzymes and their four signaling pathways. *Brown color* growth factor receptors, *blue color* cytokine receptors

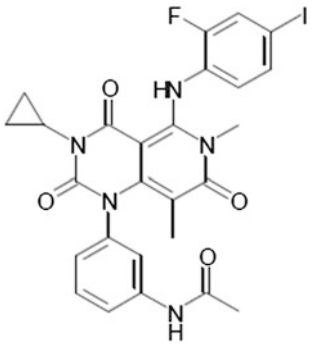

Fig. 2 The structure and chemical characteristics of trametinib. Mol. mass: 615.39 g/mol. Molecular formula: C26H23FIN5O4, chemical name: *N*-(3-{3-Cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido[4,3-d]pyrimidin-1(2*H*)-yl}phenyl)acetamide

mutation or activating mutations in KRAS or NRAS were the most sensitive. In all cancer cell lines evaluated in this study, trametinib inhibited the MEK1/2-dependent activating dual phosphorylation of ERK1/2 on both T202 and Y204 (Gilmartin et al. 2011). The observation that trametinib did not directly inhibit either C-Raf or B-Raf activity in enzymatic assays led to the conclusion that the drug must bind to MEK such that it specifically blocks the accessibility of S217 to Raf kinases. Tumor cell proliferation assay curves for 94 cancer cell lines evaluating sensitivity to

trametinib demonstrated that among cell lines with activating KRAS mutation, 16 of 25 (64 %) had a partial response to the drug and 19 of 25 (76 %) cell lines had cytotoxic or cytostatic responses (Gilmartin et al. 2011). These findings were then further studied in vivo in nude mice in which the tumor cells were implanted and grown as tumor xenografts (Gilmartin et al. 2011). The treatment with trametinib was initiated when the tumors had reached a volume of 150 mm^3 to mimic the clinical situation. Trametinib or vehicle was administered by oral garvage, and the maximum tolerated dose was defined as the highest dose that produced less than 20 % mortality and less than 20 % weight loss. The activity of trametinib against the tumor was defined according to clinical criteria including growth inhibition, partial remission (PR), or complete remission (CR). The preclinical studies showed efficient inhibition of p-ERK 1/2 which correlated with potent cell growth inhibition in tumor lines with mutant B-Raf or Ras (Gilmartin et al. 2011; Yamaguchi et al. 2011). In xenograft models of HT-29 and COLO205 colorectal tumor cell lines, trametinib demonstrated robust anticancer activity when administered daily for 14 days (Gilmartin et al. 2011; Yamaguchi et al. 2011). Pharmacokinetic profiling in mice indicated a mean effective half-life ($t_{1/2}$) of 33 h, with a low peak/trough ratio of around 1.6:2.8 after single or repeat dosing of trametinib (Gilmartin et al. 2011). This is compatible with other studies showing that pharmacological MEK inhibition completely abrogated tumor growth in BRAF mutant xenografts, whereas RAS mutant tumors were only partially inhibited (Solit et al. 2006). These preclinical findings provided robust evidence that MEK inhibition has in vivo antitumor activity and led to clinical trials.

3 Clinical Data

The rationale for clinical studies was build on the strong in vitro and mouse model data and on the finding in humans that mutated oncogenic forms of RAS are found in approximately 15 % of all cancers (Davies et al. 2002) with a variable prevalence of RAS mutations depending on the tumor types. KRAS mutations which predispose for sensitivity to trametinib responsiveness are frequently found in colorectal, lung, pancreatic, and cervical cancer (Schubbert et al. 2007), and activating BRAF mutations have been reported in approximately 60 % of cutaneous melanoma, approximately 50 % of papillary thyroid, 5–20 % of colorectal, approximately 30 % of ovarian, and approximately 26 % of germ cell tumors (Wellbrock et al. 2004; Honecker et al. 2009). Based on these findings, an early phase I dose increase trial of trametinib was performed, which enrolled 206 patients with different advanced solid tumors. This clinical trial determined that dose-limiting toxic effects of trametinib were rash, diarrhea, and central serous retinopathy (Infante et al. 2012). While these dose-limiting toxic effects grade 3–4 were infrequent (<8 %), common treatment-related adverse events were dermatitis acneiform (80 %) and diarrhea (42 %). The authors described the effective half-life of trametinib with about 4 days. Based on the results of this study, the recommended dose for the following phase II study was 2 mg per day. While the overall objective response rate in the different solid tumor types

was 10 %, B-Raf mutant melanoma had a higher response rate of 33 % (Infante et al. 2012). These encouraging results led to several phase II/III clinical trials of trametinib alone or in combination with other agents including NCT01553851, NCT01682083, NCT01362296, NCT01619774, and NCT01245062 (details are available on clinicaltrials.gov). In the first published phase III trial of trametinib (METRIC trial), 322 patients with advanced melanoma previously treated with interferon or chemotherapy with a proven V600E or V600K B-Raf mutation were randomly assigned in a 2:1 ratio to receive oral trametinib or intravenous chemotherapy consisting of either dacarbazine or paclitaxel, every 3 weeks (Flaherty et al. 2012a, b). The median progression-free survival (PFS) of patients who received trametinib was significantly longer than that of patients who received chemotherapy (4.8 vs. 1.5 months, respectively) and at 6 months, the rate of overall survival (OS) was 81 % in the trametinib group versus 67 % in the chemotherapy group. These findings indicated that trametinib, as compared to chemotherapy, improved rates of PFS and OS among patients who had metastatic melanoma. The response rate was higher in trametinib-treated patients when compared with other MEK inhibitors such as selumetinib which had an response rate of 10 % in BRAF mutant melanoma (Kirkwood et al. 2012), and PD0325901 was poorly tolerated (Haura et al. 2010; LoRusso et al. 2010). Since in vitro studies and analyses of predose and postprogression tumor biopsies in clinical trials have shown both MEK-dependent and MEK-independent resistance following exposure to a BRAF inhibitor (Montagut et al. 2008; Johannessen et al. 2010; Nazarian et al. 2010; Villanueva et al. 2010; Fedorenko et al. 2011) a combination of trametinib with a BRAF inhibitor was a logical next step. In a more recent study dabrafenib and trametinib were combined and compared with trametinib monotherapy. The rate of pyrexia was increased with combination therapy, whereas the rate of proliferative skin lesions was nonsignificantly reduced. Progression-free survival was significantly improved in the combination therapy compared to monotherapy (Flaherty et al. 2012a, b). Based on the data from the clinical trials, the FDA has approved Mekinist (trametinib) as a single-agent oral treatment for unresectable or metastatic melanoma in adult patients with BRAF V600E or V600K mutations. This result was confirmed by a later study in which significant clinical activity was observed in BRAF inhibitor-naive melanoma patients while almost no clinical activity was observed as sequential therapy in patients previously treated with a BRAF inhibitor (Kim et al. 2013). These data lead to the conclusion that BRAF inhibitor resistance mechanisms likely confer resistance to MEK inhibitor monotherapy. Consequently, tramtinib was not approved for the indication melanoma in patients who have received a prior BRAF inhibitor therapy.

4 Toxicity

In individuals with advanced solid tumors, a dose increase study was performed to define the maximum tolerated dose (Infante et al. 2012). The most common treatment-related adverse events were rash or dermatitis acneiform (80 %) and diarrhea (42 %), most of which were grade 1 and 2. Dose-limiting toxicities

included rash, central retinopathy, and diarrhea (Infante et al. 2012). Based on this study, the dose of 2 mg/day was chosen for further studies. In patients treated with trametinib for malignant melanoma, most common adverse events observed were rash, diarrhea, peripheral edema, fatigue, and dermatitis acneiform (Flaherty et al. 2012a, b). Among the patients with rash, less than 8 % had grade 3 or 4 rash. A decreased ejection fraction or ventricular dysfunction was observed in 14 patients (7 %) in the trametinib group; of these, patients 11 had a decreased ejection fraction and 3 had left ventricular dysfunction (Flaherty et al. 2012a, b). In the light of the reported toxicity data, the dose of 2 mg trametinib once a day was shown to be tolerable and the side effects were manageable. Administration of trametinib in combination with standard gemcitabine dosing (1,000 mg/m2 IV Days 1, 8, and 15 every 28 days) was shown to be feasible (Infante et al. 2013). Though most toxicities were manageable, the addition of trametinib was shown to lead to slightly higher gemcitabine-associated myelosuppression.

5 Drug Interactions

The low C_{max} in plasma with the 2 mg a day dose suggests that trametinib is low risk for drug interactions [unpublished data, mentioned in (Infante et al. 2012)]. In vitro and in vivo data suggest that GSK1120212 is unlikely to affect the PK of other drugs.

6 Biomarkers

Biomarkers are essential to identify patients with a better chance to respond to targeted therapies. In order to find predictive biomarkers for the sensitivity to trametinib, a recent study had profiled 218 solid tumor cell lines and 81 hematologic malignancy cell lines (Jing et al. 2012). The authors found that *RAF* and *RAS* mutations were a strong predictor of sensitivity to MEK inhibition by trametinib in solid tumor cells. By using transcriptomics analysis in *KRAS* mutant cell lines, the authors identified cell lines with a gene signature indicative of epithelial-to-mesenchymal transition (EMT) to be less sensitive to trametinib (Jing et al. 2012). Also, the gene *DUSP6* was identified to predict for trametinib sensitivity while a lack of expression was associated with resistance to the drug irrelevant of the *RAF/RAS* mutation status. When colon cancer cells had both *RAF/RAS* mutations and *PIK3CA*/*PTEN* mutations, this was predictive for a cytostatic response instead of a cytotoxic response (Jing et al. 2012). The evaluation of trametinib sensitivity within hematological malignancies demonstrated that acute myeloid leukemia and chronic myeloid leukemia cell lines were more sensitive than other entities (Jing et al. 2012). Overall, the different studies for trametinib sensitivity identified multiple biomarkers including mutant *RAF*, *RAS*, *PIK3CA*/*PTEN*, and *DUSP6* in solid tumors and thereby will help in the future to identify patients who could benefit from trametinib treatment.

7 Summary and Perspectives

Targeted therapies with small molecular inhibitors for solid tumors and hematological malignancies are moving rapidly from bench to bedside. The MEK inhibitor a trametinib has shown promising clinical efficacy and is being evaluated by the FDA for the treatment of metastatic melanoma. Since targeted therapies attempt to inactivate a mutated oncogenic pathway, critical to survival of cancer cells while sparing normal cells, which do not carry the mutation and are not similarly addicted to the pathway, informative biomarkers for trametinib have been identified including *RAF*, *RAS*, *PIK3CA*/*PTEN,* and *DUSP6*. Trametinib holds promise to overcome paradoxical MEK activation seen in different solid tumors such as melanoma that become resistant to BRAF inhibition and thereby contribute to the solution of a major clinical problem. This concept will not only apply to BRAF and MEK inhibitors, and combination of targeting agents against different signaling pathways may provide additional benefits and warrant further clinical studies.

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