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Tumour Review

Trametinib in the treatment of multiple malignancies harboring MEK1 mutations

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ABSTRACT

The aberrant activation of RAS-derived mitogen-activated protein kinase (MAPK) signaling pathway plays a prominent role in tumorigenesis of an array of malignancies. The reasons are usually the upstream activated mutations including mitogen-activated protein kinase kinase 1/2 (MEK1/2). As oncogenic mutations, *MEK1* mutations have been observed in a variety of malignancies including melanoma, histiocytic neoplasms, colorectal cancer and lung cancer. Presently, the use of trametinib, a highly selective MEK1/2 inhibitor, was limited to *BRAF* mutations, according to the approvals of FDA. Therefore, we consider that this is a question worth studying that whether malignancies with *MEK1* mutations are sensitive to the treatment of trametinib. This review discussed the function of *MEK1* mutations, retrieved the frequency and distribution of *MEK1* mutations in various malignancies, and reviewed the basic experiments and clinical case reports on trametinib in the treatment of cell lines or patients with *MEK1* mutations. Most studies have demonstrated that trametinib was effective to cells or tumor patients harboring *MEK1* mutations, which suggest that the *MEK1* mutations might be potential indications of trametinib therapy. In addition, it was also reported that resistance was observed in the treatment of trametinib, suggesting that different *MEK1* mutations may have different response to trametinib, and further studies are necessary to distinguish that which *MEK1* mutations are appropriate for the treatment with trametinib and which are not.

Introduction

With the popularization of Next-generation sequencing technology, targeted cancer therapy based on gene mutations has attracted more and more attention. Aberrant activated mitogen-activated protein kinases (MAPK) pathway is considered to play a crucial role in the occurrence and development of various cancers [1]. Various mutations in pathway components including MEK1/2 can cause the aberrant activation of MAPK signaling pathway [2]. As oncogenic mutations, *MEK1* mutations were widely observed in many cancers [1,3,4], while trametinib, a MEK1/2 inhibitor, was limited to treat *BRAF*-mutant melanoma and non-small-cell lung cancer (NSCLC) according to the approval of FDA [2,5]. This review mainly focused on recent basic experiments and clinical case reports regarding efficacy of trametinib in malignancies with MEK1 mutants, described the MAPK signaling pathway and the role of MEK1, and summarized the frequency and genotypes of *MEK1* mutations in a variety of malignancies. These results may provide a novel therapeutic strategy for cancer patients with *MEK1* mutations.

Dual specificity mitogen-activated protein kinase kinase 1 (MEK1)

MEK1 in MAPK signaling pathway

The MAPK signaling pathway is thought to play a prominent role in nearly every cell process, and it regulates cell proliferation, differentiation, migration, survival and cell motility by activating or inactivating other kinase or transcription factors [6]. This pathway is a highly conserved pathway that transduces extracellular signals into intracellular regulatory effectors [6]. The core of this pathway is a three-layered protein kinase cascade that consist of RAF, MEK and ERK [7], which is activated by a variety of extracellular stimuli including growth and differentiation factors, cytokines, hormones and neuropeptides [3]. Activated receptor tyrosine kinases (RTKs) recruit adaptor

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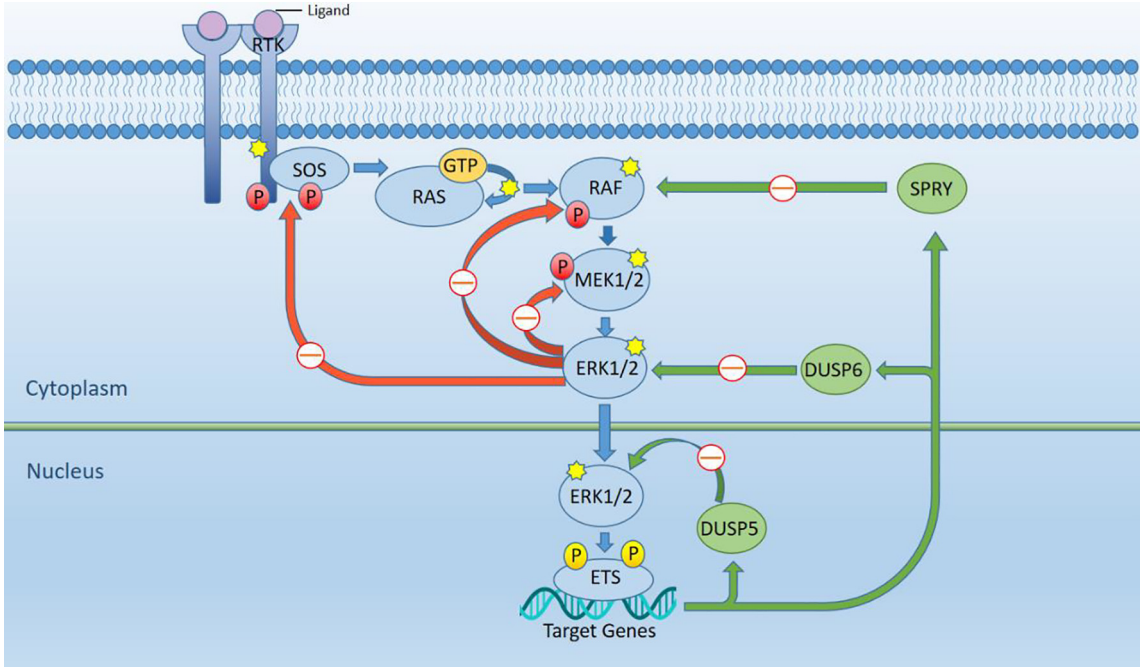

Fig. 1. A simplified representation of the MAPK signaling pathway. The MAPK signaling pathway. A simplified representation of the RAS-regulated RAF–MEK1/2–ERK1/2 signaling cascade. After RTKs (such as epidermal growth factor receptor (EGFR)) is activated, RAS, RAF, MEK and ERK are activated in turn (showed in blue, the yellow seven horns stars represent activation). Activated ERK1/2 regulates the expression of target genes through the phosphorylation and activation of transcription factors, including the ETS family, to modulate many critical processes of cell physiology. In addition, the MAPK signaling pathway is also regulated by an extensive range of negative feedback systems including rapid, transient mechanism and delayed, long term mechanism. The former is the ERK1/2-catalysed inhibitory phosphorylation of upstream pathway components such as BRAF, CRAF, MEK1, SOS and some RTKs (shown in red). The second mechanism induces the de novo expression of negative regulators including MAP Kinase Phosphatases (MKPs or DUSPs) and sprouty proteins (shown in green). The ERK1/2 pathway is frequently de-regulated in cancer due to mutations in components such as BRAF, RAS, NF1, MEK or certain RTKs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

proteins and guanine nucleotide exchange factors such as Son-of-Sevenless (SOS), which can activate RAS through driving RAS to combine with GTP [3]. GTP-bound RAS (RAS-GTP) catalyzes the formation of high-activity dimers of the RAF that include three subtypes: ARAF, BRAF and CRAF. These three RAF family members directly phosphorylate and activate MEK1/2 (Fig. 1). RAF and MEK can form the heterodimers, which promotes MEK activation [4]. In addition, MEK has been observed to form face-to-face homodimers where the activation loop of one protomer aligns with the catalytic site of the other, which suggests the potential occurrence of intradimer cross-phosphorylation [4]. MEK1/2 are dual-specificity kinases that activate the terminal kinases in the cascade, ERK1 and ERK2, by phosphorylating the T-E-Y motif in their activation loop (Fig. 1). Activated ERK1/2 promotes the expression of many transcription factors (such as FOS, FRA1, EGR1) which in turn regulate the expression of genes and cell cycle progression (such as CCND1, p21CIP1, p16INK4A) and control of cell survival (such as MCL1, BCL2, BCL-XL) [1].

As the core components of MAPK signaling pathway, MEK1/2 are the only activators of ERK1/2 and regulated critically by negative feedback control from ERK1/2. This regulation is considered to include two major mechanisms: the directly inhibitory phosphorylation to upstream components and increased expression of the dual-specificity phosphatases (DUSPs, also known as MAP kinase phosphatases or MKPs) and sprouty proteins (SPRY) [1] (Fig. 1). Activated ERK1/2 not only directly phosphorylate the activation loop of MEK1 to inhibit its activity, but also phosphorylate BRAF, CRAF, SOS and some RTKs to repress the activation of the downstream pathway including MEK1 [3]. These are rapid and transient negative feedback mechanisms. In addition to direct phosphorylation to other components of signaling pathway, ERK1/2 can also combine with and phosphorylate transcription factors, including the ETS family, to increase new proteins expression (Fig. 1). These are delayed, long term feedback mechanisms including the *denovo* expression of the DUSPs and the SPRY proteins [3]. The DUSPs can inhibit the activation of ERK1/2 by dephosphorylating the T-E-Y motif. The SPRY proteins inactivate RTKs, SOS and interfere with the RAF catalytic domain to inhibit the activation of MEK1 and MEK2 [8,9]. In addition to regulating the activation loop of MEK1, helix A, a region at the N-terminus, has also been shown to negatively regulate MEK1 activity. Partial activation of MEK1 is caused by either N-terminus deletion or phosphomimetic substitutions in the activation loop [10].

Structure and function of MEK1

Human MEK1 consists of 393 amino acids including trifunctional N-terminal sequence, a kinase catalytic domain and a C-terminal sequence [11]. The trifunctional N-terminal sequence includes a domain bound by ERK1/2 (EBD), the nuclear export sequence (NES) and the negative regulatory region (NRR) domain (Fig. 2). The C-terminal sequence mainly contains the MAP kinase kinase kinase (MAP3K) docking domain (domain of versatile docking (DVD)) [1]. The kinase catalytic domain of MEK1 involves the activation loop (AL), the proline-rich segment (PRS) and a short carboxy-terminal sequence (Fig. 2). RAF and other MAP3Ks activate MEK1 by phosphorylate Ser218 and Ser222 within the MEK1 activation loop. Simultaneously, Ser218 is also action site of trametinib that inhibit the activation of MEK1 by preventing the phosphorylation of Ser218 (Fig. 2). In the proline-rich domain, Thr292 is a site of negative feedback phosphorylation by ERK1 and ERK2, and Ser298 can be phosphorylated by p21-activated kinase (PAK) (Fig. 2) [1].

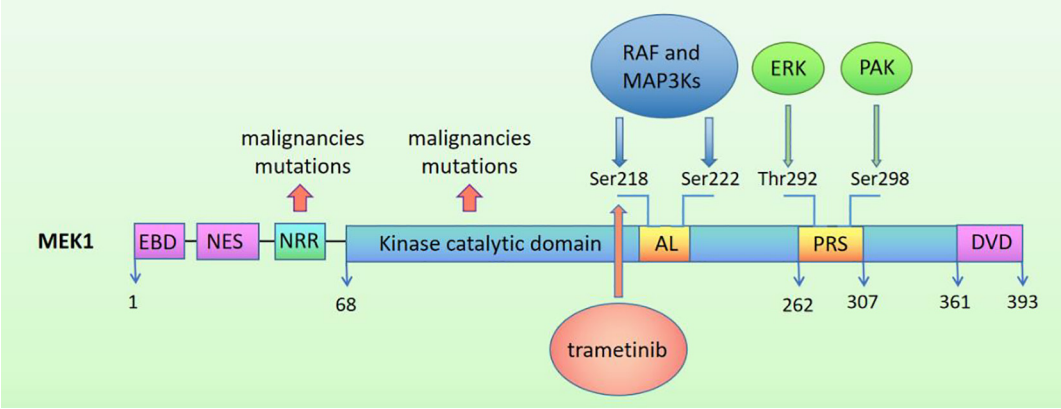

Fig. 2. Linear representation of the key structure of the human MEK1.

MEK1 mutations in malignancies

Frequency of MEK1 alterations in malignancies

Activating mutations in MEK1 have been observed in various tumors including melanoma, colorectal cancer, lung cancer and histiocytic neoplasms [3,4,12]. To research and characterize the presence of *MEK1* alterations in various malignancies, we retrieved The Cancer Genome Atlas (TCGA), the cBioportal for Cancer Genomics database and International Cancer Genome Consortium (ICGC) database. The frequency of *MEK1* alterations in various malignancies was summarized in Table 1.

Above results showed that mutations accounted for a large proportion in all kinds of alterations, followed by amplifications. Incidence of *MEK1* mutations in hematological malignancies was greatly higher than that in other malignancies, in which the highest incidence occurred in histiocytic neoplasms. Among all malignancies, the top three of *MEK1* mutations incidences were histiocytic neoplasms, Melanoma and DLBCL, respectively. In addition, *MEK1* mutations were generally detected in solid malignancies such as lung cancer, gastric cancer,

Table 1
The general picture of *MEK1* alterations in malignancies.

Cancer Type	Mutations	Amplification	Fusion	Deep deletion	Source
Histiocytosis	11.76% (6 out of 51)	–	–	–	MSK, Nature 2019
SKCM	6.31% (28 of 444)	0.68% (3 of 444)	–	–	TCGA
Metastatic Melanoma	5.26% (2 of 38)	–	–	–	UCLA, Cell 2016
DLBCL	4.44% (6 of 135)	–	–	–	DFCI, Nat Med 2018
ACyC	4%(1 of 25)	–	–	–	JHU, Cancer Prev Res 2016
NHL	3.77% (2 of 53)	–	–	–	BCGSC, Nature 2011
Mesothelioma	–	3.45% (3 of 87)	–	–	TCGA
LGG	3.28% (2 of 61)	–	–	–	UCSF, Science 2014
Uterine	2.65% (14 of 529)	0.38% (2 of 529)	–	0.19% (1 of 529)	TCGA
Ampullary Carcinoma	3.13% (5 of 160)	–	–	–	Baylor College of Medicine, Cell Reports 2016
Cholangiocarcinoma	2.78% (1 of 36)	–	–	–	TCGA
Pancreatic Cancer	–	2.75% (3 of 109)	–	–	UTSW, Nat Commun 2015
NEPC	0.88% (1 of 114)	1.75% (2 of 114)	–	–	Multi-Institute, Nat Med 2016
mPC	0.68% (3 of 444)	1.13% (5 of 444)	–	0.45% (2 of 444)	SU2C/PCF Dream Team, PNAS 2019
Esophagus	1.65% (3 of 182)	–	–	0.55% (1 of 182)	TCGA
Colorectal	2.19% (13 of 549)	–	–	–	TCGA
Stomach	1.59% (7 of 440)	0.45%(2 of 440)	–	–	TCGA
Cervical	1.68% (5 of 297)	–	0.34% (1 of 297)	–	TCGA
Lung adeno	2% (2 of 100)	–	–	–	TCGA
Kidney Chromophobe	–	1.54% (1 of 65)	–	–	TCGA
Lung squ	1.03% (5 of 487)	0.21% (1of 487)	–	–	TCGA
Sarcoma	–	0.78% (2 of 255)	–	0.39% (1 of 255)	TCGA
Head & neck	0.96% (5 of 523)	–	–	–	TCGA
Breast Invasive Carcinoma	0.28% (3 of 1048)	0.37%(4of 1084)	–	–	TCGA
Thymoma	0.81% (1 of 123)	–	–	–	TCGA
Bladder	0.49% (2 of 411)	–	–	–	TCGA
Ovarian	0.17% (1 of 584)	0.34% (2 of 584)	0.17% (1 of 584)	–	TCGA
PCPG	0.56% (1 of 178)	–	–	–	TCGA
Liver	0.27% (1 of 372)	0.27% (1 of 372)	–	–	TCGA
ccRCC	–	–	0.2% (1 of 511)	–	TCGA
PC	–	0.2%(1 of 494)	–	–	TCGA

Abbr eviations: SKCM: Skin Cutaneous Melanoma; **DLBCL**: Diffuse Large B cell Lymphoma; **ACyC**: Adenoid Cystic Carcinoma; **NHL**: Non-Hodgkin Lymphoma; **LGG:** Low-Grade Gliomas; **Uterine:** Uterine Carcinosarcoma; **NEPC:** Neuroendocrine Prostate Cancer; **mPC:** Metastatic Prostate Adenocarcinoma; **Esophagus:** Esophageal Adenocarcinoma; **Colorectal:** Colorectal Adenocarcinoma; **Stomach:** Stomach Adenocarcinoma; **Cervical:** Cervical Squamous Cell Carcinoma; **Lung adeno:** Lung Adenocarcinoma; **Lung squ:** Lung Squamous Cell Carcinoma; **Head & neck:** Head and Neck Squamous Cell Carcinoma; **Breast:** Breast Invasive Carcinoma; **Bladder:** Bladder Urothelial Carcinoma; **Ovarian:** Ovarian Serous Cystadenocarcinoma; **PCPG:** Pheochromocytoma and Paraganglioma; **Liver:** Liver Hepatocellular Carcinoma; **Thyroid:** Thyroid Carcinoma; **ccRCC:** Kidney Renal Clear Cell Carcinoma; **PC:** Prostate Adenocarcinoma. **Note:** "–" means that related alteration was not observed in this kind of malignancy.

Table 2
Mutation genotype of MEK1 in malignancies.

Cancer type	Mutations (putative driver)
SKCM	P124S (13), P124L (4), K57N (2), E203K (2), F53I (1), Q56P (1), E203V (1)
Colorectal	G128D (2), D67N (2), F53L (1), R49H (1), P124S (1), F53V (1), Q56P (1)
Lung adeno	G128V (1), K57T (1), C121S (1), F53L (1), K57N (1), G128D (1), E102_I103del (1)
Stomach	L177M (2), C121S (1), E203K (1), G128V (1)
Cervical	R47Q (1), R49H (1), I103N (1)
Head & Neck	K57N (1), E102_I103del (1)
Cholangiocarcinoma	G128R (1)
Uterine	C121R (1)
Esophagus	D67Y (1)
Lung Squ	G128D (1)
Thymoma	F53L (1)
PCPG	F53_Q58delinsL (1)
Breast	E203K (1)
Liver	Y130C (1)
Thyroid	E102_I103del (1)

Note: The abbreviation of Table 2 is same as Table 1.

colorectal cancer and bladder cancer. Most *MEK1* mutations were found in the NRR or the N-terminal lobe of the kinase domain [1,13]. These mutations in MEK1 may break down the negative regulatory region of the enzyme, making it more easily activated, even without the phosphorylation of BRAF [10]. A study showed that *MEK1* mutations could be a marker to define a distinct subset of smoking-related NSCLC [14]. *MEK1* mutations occur mutually exclusively with other oncogenic mutations in NSCLC, among which the most common mutations were *K57N* followed by *Q56P*, suggesting *MEK1* mutations are only drivers of aberrant activation of MAPK pathway in these cases [14].

Genotype of MEK1 mutations in malignancies

According to results of TCGA database, we summarized *MEK1* mutations genotype in various malignancies (Table 2). Notably, *MEK1 P124S* mutation accounts for a large proportion in SKCM while the distribution of genotype is relatively even in other malignancies. Some mutations are observed in multiple malignancies, for example, MEK1 K57N is observed in head and neck cancer, SKCM and lung cancer; E203k in SKCM, stomach cancer and breast cancer; E102_I103del in lung cancer, head and neck cancer and thyroid cancer. By contrast, the presence of other genotype is more limited.

MEK1 mutations in histiocytic neoplasms

Some studies showed that *MEK1* mutations play a crucial role in histiocytic neoplasms, particularly in BRAF wild-type (WT) cases [12,15,16]. Histiocytic neoplasms are rare hematological malignancies with heterogeneous clinical presentations thought to arise from the dendritic or monocytic lineages and comprise less than 1% of neoplasms of the soft tissue and lymph nodes [12]. They encompass disorders including Langerhans cell histiocytosis (LCH) and non-Langerhans cell histiocytosis (non-LCH), and non-LCH are also classified as Erdheim-Chester disease (ECD), juvenile xanthogranuloma (JXG), Rosai-Dorfman disease (RDD), histiocytic sarcoma (HS), indeterminate cell histiocytosis (ICH), follicular dendritic cell sarcoma (FDCS), and interdigitating dendritic cell sarcoma (IDCS) [12]. We further queried related literature about the frequency of MEK1 mutations in LCH and non-LCH, reviewed related results [15–22] in Table 3.

These results showed that the frequency of MEK1 mutations in *BRAF V600E* wild-type histiocytic neoplasms was relatively higher, suggesting *MEK1* mutation may act as a crucial driver and lead to activation of MAPK pathway in *BRAF* mutation-negative histiocytic neoplasms patients. In addition, the incidence of *BRAF V600E* and *MEK1* mutations was higher in children, compared with that in adult patients [16,21]. In terms of the differentiation-related markers, BRAF/MEK1-mutant cases expressed CD14 but rarely expressed CD83 or CD86, whereas MEK1 and BRAF wild-type cases were opposite. This indicates BRAF/MEK1-mutant histiocytic neoplasms are more immature [16]. Some studies indicated that the presence of *BRAF* mutations and *MEK1* mutations are mutually exclusive in LCH [16,22–25]. Therefore, it is of great significance for *BRAF* mutation-negative patients to study the therapy targeting *MEK1* mutations.

MEK1 mutations in virus-associated liver cancer in Asian

We found that the *MEK1* mutations frequency of virus-associated liver cancer in Asian was significantly higher than the above frequency through retrieving the ICGC database. Among 402 HBV-associated hepatocellular carcinoma Chinese patients, 74 *MEK1* mutations were detected in 50 cases (12.44%) and mainly included intron (37), exon (11) and missense (10) mutations. Missense mutations included *MEK1 G128V, G17V, D315Y, Q34H, D139Y, V66L, V242L, V173L, G49C, G225C, R234I, R58I, A171S, A347S, S209G* and *S385G*. In a study of virus-associated hepatocellular carcinoma Japanese patients, 95 *MEK1* mutations were detected in 71 of 258 (27.52%), including 81 intron mutations. These results suggest that the driver mutations in virus-associated liver cancers may be different from that in other liver cancers (such as secondary to alcohol and adiposity), and *MEK1* mutations may play a more important role in virus-associated liver cancers. This might provide a new therapeutic strategy for patients with virus-associated liver cancer.

The use of trametinib targeting *MEK1* mutations

Trametinib

Trametinib (also known as GSK1120212) is an oral, reversible and highly selective inhibitor of MEK1/2, and it was firstly approved for the treatment of unresectable or metastatic melanoma harboring *BRAF V600E/K* mutations, both as a single-agent and in combination with dabrafenib [5]. After this, a combination of trametinib and dabrafenib was approved on the basis of two phase III studies (COMBI-v and COMBI-d) [26,27]. In 2017, trametinib in combination with dabrafenib was approved by FDA for the treatment of *BRAF V600E*-mutant metastatic NSCLC [5]. Trametinib could cause G1 cell cycle arrest and accelerate cells apoptosis [2] through interfering with the conformation of the activation loop sites of MEK 1/2, so that they are no longer effectively phosphorylated by RAF. Therefore, although the use of trametinib also promote activation of upstream components of pathway, there is little or no increase the rebound of MEK1/2 phosphorylation or ERK1/2 activity, which results into more persistent inhibition with better efficacy in preclinical trials [1]. A report indicated that trametinib can prevent the phosphorylation of MEK1 at Ser218, but cannot at Ser222, and this monophosphate form of MEK1 has severely limited kinase activity [1]. The common adverse effects of trametinib therapy are rash, dermatitis, diarrhea, and fatigue. Compared with other inhibitors, trametinib has good pharmacokinetic characteristics, long half-life, low peak-trough ratio, limited toxicity and low likelihood of interaction with other drugs [2].

Trametinib in basic experiments

Trametinib in NIH3T3 cells expressing mutant MEK1

NIH3T3 cells were transfected with multiple *MEK1* mutations respectively, including *D67N, P124L, P124S, C121S, F53L, Q56P, K57N, ΔL98-I103, ΔI99-K104* and *ΔE102-I103*. After the treatment with trametinib, the cells with *D67N, P124L, P124S, C121S, F53L, Q56P, K57N* were sensitive to trametinib while that with *ΔL98-I103, ΔI99-K104* and *ΔE102-I103* were insensitive to trametinib [28]. This study showed that

Table 3
Frequency of MEK1 mutations in Histiocytic neoplasms.

Reference	Clinical type	MEK1 Mutations	Genotype (case)
Diamond et al. [15]	LCH	40% (4 of 10)	Q58_E62del (3), F53_Q58del
	ECD	14% (2 of 14)	K57N, F68L,
	ECD*	50% (9 of 18)	–
	RDD*	27% (3 of 11)	–
	JXG*	Wild-type (0 of 8)	–
Zeng et al. [16]	LCH	17.5% (17 of 97)	Q56P(3), C121S(2), E38K(3), R47Q, R47G(2), R49C(2), P105S (2), A106T, G128V.
Mcginnis et al. [17]	LCH	8.6% (5 of 58)	V82M (2), L115P, Q58_E62del, E102_I103del
Xerri et al. [18]	LCH	21% (4 of 19)	–
Alayed et al. [19]	LCH*	46% (6 of 13)	Gln58X, Ala76Val, Met94Ile, G61_D65del, L63_D67del, I103_K104del
Nelson et al. [20]	LCH*	15% (3 of 20)	C121S, C121S/G128D, 56_61QKQKVG > R
Garces et al. [21]	RDD*	14.3% (3 of 21)	F53V, P124R, G128D
Chakraborty et al. [22]	LCH*	33% (7 of 21)	E102_I103del (2), Q58_E62del (3), F53_Q58delinsL, Q56P

Note: "*" means this study was performed in *BRAF V600E* wild-type samples.

the MEK1 mutants can be classified as three classes, RAF-dependent, RAF-regulated and RAF-independent, according to the manner activating ERK signaling [28]. RAF-dependent and RAF-regulated MEK1 mutants are sensitive to allosteric MEK inhibitors including trametinib that do not compete with ATP for binding and preferentially inhibit the inactive conformation of the enzyme [28]. On the contrary, RAF-independent MEK1 mutants are insensitive to such inhibitors. These results suggest that the efficacy of trametinib to different *MEK1* mutations is distinct, and developing therapeutic strategies based on genotype is helpful to the clinics.

Trametinib in lung adenocarcinoma cells

NCI-H1437, a kind of lung adenocarcinoma cell lines with *MEK1 Q56P* mutation while without other oncogenic mutations of MAPK signaling pathway, was confirmed to be sensitive to the treatment of trametinib, which decreased the phosphorylated ERK level and strongly suppressed the growth of NCI-H1437 [29]. The estimated IC_{50} value of this cell line was 1000 times lower than that of similarly treated MEK1 wild-type cell lines. *In vivo*, the mice bearing xenografts derived from NCI-H1437 or A549, a lung cancer cell line harboring an activated *KRAS G12S* mutation, were treated with 0.3 mg/kg trametinib in compared with those treated with DMSO. The tumors induced by NCI-H1437 in mice treated with trametinib showed little or no growth while those treated with DMSO presented rapid growth of tumors during the experiment. In comparison, the growth of A549-induced tumors was not affected by trametinib and DMSO [29].

Trametinib in colorectal and gastric cancer cells

SNU-C1, a colorectal cancer cell line with *MEK1 F53L* mutation as sole oncogenic mutation of MAPK signaling pathway, revealed strong sensitivity to trametinib compared with other colorectal cancer cell lines with *KRAS* or *BRAF* mutations [29]. In addition, SW48, a colorectal cancer cell line harboring three detected mutations in *MEK1* (*Q56P, H119Y,* and *D351G*) and an activated mutation in *EGFR* (*G719S*), was observed to have higher sensitivity to trametinib compared with the control group, but not to the same degree of NCI-H1437 [29]. Since activated *MEK1* mutations are almost always mutually exclusive with other mutations in the MAPK signaling pathway, an atypical situation existed in SW48 cells where activated EGFR signaling may counteract partial inhibition of trametinib. Furthermore, like SNU-C1, OCUM-1, a gastric cell line, also harbors only *MEK1* mutation (*Q56P*) as oncogenic mutation in MAPK signaling pathway. It was sensitive to picomolar concentrations of trametinib, similar to that observed in the NCI-H1437 and SNU-C1 cells [29].

In another study, OCUM-1 and Okajima, a gastric cancer cell line harboring *MEK1 S72G* mutation, were hypersensitive to trametinib [30]. The focus formation assay and tumorigenicity assay of nude mice showed that *MEK1 Q56P* and *S72G* mutations presented transformational abilities and improved the tumorigenicity. After the treatment of trametinib, a significant decrease was observed in phosphorylation levels of ERK1/2 in the OCUM-1 and Okajima, compared with the wild-type. Moreover, in the xenograft study, the tumors from the Okajima cells were dramatically reduced by treatment with trametinib and the phosphorylation of ERK1/2 in the tumors was also significantly inhibited, compared with controls from SNU-16 cell line (MEK1 wild-type) [30].

Trametinib in NIH/3T3 cells transfected with MEK1 mutations from LCH patients

NIH/3T3 cells, a mouse embryonic fibroblast, transduced with various mutations in *MEK1* (*F53_Q58 > L*, *Q58_E62del* and *C121S/G128V*) were sensitive to trametinib [23]. The *MEK1* mutations from LCH patients were transfected into NIH/3T3 cells. After the treatment of trametinib, MEK1 mutant-induced ERK aberrant activity decreased in cells, while significant change was not observed in the control group treated with BRAF inhibitor vemurafenib. These results indicated that trametinib can effectively decrease aberrant ERK1/2 phosphorylation caused by *MEK1 F53_Q58 > L, MEK1 Q58_E62del*, and *MEK1 C121S/G128V* [23].

In another kinase assays, the HEK-293 T cells transfected with *MEK1 C121S/G128D* and *56_61QKQKVG > R* variants, a deletion in the N-terminal regulatory domain, were resistant to trametinib *in vitro* [20]. These *MEK1* mutations were detected in 30 LCH patients. The IC_{50} of the cells with *MEK1 G128D, C121S* single variants and wild-type MEK1 were comparable at 1–10 nM, while the cells with compound C121S/G128D showed resistance to trametinib, with an IC_{50} between 10 and 100 nM. The cell with 56_61QKQKVG > R were observed to have slightly stronger resistance to trametinib [20].

Trametinib in HEK-293 T cells harboring Y130C mutant and Q56P mutant

In addition, the HEK-293 T cells expressing the Y130C-mutant and Q56P-mutant MEK1 were demonstrated to be sensitive to trametinib. Significant down-regulation of MAPK signaling pathway activity was observed in HEK-293 T cells with MEK1 mutations after the treatment of trametinib, compared with cells with wild-type MKE1 [31].

Trametinib in clinical case reports

Trametinib in non-Langerhans cell histiocytosis

A patient with histiocytic sarcoma harboring an activating *MEK1 F53L* mutation was treated with trametinib, and images showed a rapid and durable complete response that continued for more than 2 years [32]. This patient is a 62-year-old man with a history of untreated grade 1 or grade 2 follicular lymphoma (BCL2 rearranged). Combinations of rituximab and bendamustine were ineffective. Three days after treatment of trametinib (2 mg daily), all of B symptoms were resolved, and leukocytosis, thrombocytosis, and anemia were reduced [32]. Two weeks after trametinib, images showed interval resolution of

splenomegaly and a decrease in the number, size, and metabolism of lesions in the multiple organs. Four months after treatment, images show further response to treatment, with resolution of multiple lesions. MIP and PET-CT after 2 years of treatment show a complete response to treatment, with no evidence of FDG neoplastic disease [32].

In addition, a 53-year-old refractory ECD patient with *MEK1 K57N* mutation had inflammatory ascites and renal failure after the treatment with both interferon-alpha and anakinra. Trametinib was recommended to use, resulting in quick cessation of ascitic accumulation and normalization of creatinine. The patient continued to receive trametinib monotherapy with clinical response lasting for more than 180 days [15].

Trametinib in Langerhans cell histiocytosis

The complete remission was also reported in a LCH patient with an *MEK1 E102-I103del* mutation treated with trametinib [33]. This patient was a 46-year-old man with limited cutaneous lesions without the involvement of other organs. Multiple treatments were ineffective, and grade 2 liver toxicity and involvement of pituitary gland were detected. Next-generation sequencing showed an activating mutation in MEK1 (*E102_I103delEI*), and trametinib was started at a dose of 2 mg once daily. After 9 weeks of treatment, trametinib was interrupted due to enteritis. PET-CT and MRI performed in week 12 of treatment showed a slight increase of the pituitary glandin size. No new or recurrent skin lesions were found. The dose of desmopressin was reduced during the treatment of trametinib. After LCH relapsed, the reuse of trametinib make recurring lesions resolve rapidly. No disease progression was observed at the last follow-up in week 46 [33].

On the contrary, it was reported that a patient with LCH harboring *MEK1* mutation (*MEK1 p.L98_K104 > Q* deletion) presented clinical resistance to trametinib [34]. This patient was a 16-year-old male diagnosed with multifocal LCH primarily, and LCH-III therapy were efficacious. One year later, first recurrence of LCH was treated with afuresertib, and the patient responded to this therapy. After seven months, the patient relapsed again and cytarabine was ineffective. Then he was lost to follow-up. Three years after his initial diagnosis, the patient relapsed again, and a *MEK1* mutation (*MEK1 p.L98_K104 > Q* deletion) was tested, which has been shown to be an activated mutation [34]. Therefore, he was recommended to start trametinib therapy (2 mg daily). Eight weeks later, PET-CT showed disease progression, and the therapy with trametinib was discontinued [34]. These results suggest that different mutations in MEK1 could lead to different sensitivities to trametinib.

Trametinib in variant hairy cell leukemia

Trametinib was reported to have great anticancer activity in a variant hairy cell leukemia (vHCL) patient with a *MEK1 K57N* mutation [35]. Activated mutations in *MEK1* were detected in more than half of vHCL cases [36]. This patient is a 52-year-old man who was initially diagnosed with BRAF wild-type vHCL. He has received various treatments previously including allogeneic transplantation and not obtained great effects. Next-generation sequencing shown *MEK1 K57N* mutation. Therefore, he was treated with trametinib 2 mg daily [35]. Within a week, his skin nodules had markedly diminished and generalized rash had resolved. Simultaneously, serum markers have also significantly decreased. Skin nodules behind each ear were near completely resolved and visible skin rash disappeared after cycle 2 of therapy. Repeat skin biopsies showed diminished lymphocyte involvement and decreasing phosphorylated ERK, which is consistent with trametinib effects. The adverse events caused by trametinib therapy were not serious and can be improved by doxycycline and sun avoidance. Thus, this therapy has been well tolerated, which did not require dose reduction [35].

Trametinib in clinical trials

We searched the ClinicalTrials.gov (https://www.clinicaltrials.gov), and found only one clinical trial (ClinicalTrials.gov Identifier: NCT01362296) about trametinib in which patients with *MEK1* mutations were included. This is a phase II, open-label, multicenter, randomized study and the main purpose of this trial is to evaluate the efficacy and safety of trametinib compared with docetaxel in the second line setting for subjects with locally advanced or metastatic (Stage IV) NSCLC harboring a *KRAS* mutation who have failed one platinum-containing chemotherapy regimen. A small subset of NSCLC subjects harboring BRAF, *NRAS*, or *MEK1* mutations will be randomized in addition to the primary *KRAS* population, for exploratory purposes. According to the above results, the clinical trials of trametinib on patients harboring *MEK1* mutations are very rare.

Research progresses of other MEK1 inhibitors

Researches on other MEK1 inhibitors are also performing, such as selumetinib, binimetinib and cobimetinib. Recently, a research showed cobimetinib was greatly efficacious for patients with histiocytic neoplasms containing various MAPK mutations. These patients were observed to harbor *ARAF, BRAF, RAF1, NRAS, KRAS, MEK1* and *MEK2* mutations. After cobimetinib treatment, overall response rate was 89%, 72% of the patients had a complete response, including all of patients containing *MEK1 (P124L, P124Q, Q56P, P105_107del)* and *MEK2 (Y134H)* mutations. Responses occurred at all sites of disease, including the central nervous system. No patient had progressive disease. The median time to best response was 3.2 months. At one year, 100% of responses were ongoing [37]. Similarly, a refractory ECD patient with *MEK1 Q56P* mutation was treated with cobimetinib, achieving multiple-site response including kidney, aorta and maxillary sinus evaluated by PET [15].

Previous researches reported that some *MEK1* mutations, such as *MEK1 P124S, P129L* and *Q56P*, acquire resistance to the other MEK inhibitors [38]. However, in this study, no acquired resistance to cobimetinib was observed, including previously reported mutations led to resistance to MEK inhibitors [37]. These results suggested that histiocytic neoplasms might lack the adaptability to MEK inhibition and further demonstrated that a common feature of histiocytic neoplasms was dependence on MAPK signaling pathway. Therefore, MEK inhibitors would have a great efficacy to histiocytic neoplasms [37].

In addition, A study suggested that *MEK1* mutations with resistance to trametinib are sensitive to MAP588, an ATP-competitive MEK inhibitor [28]. These results may be a novel therapeutic strategy for tumors driven by MEK1 mutants.

Conclusion

At present, the function mechanism of *MEK1* mutations has not been fully understood. It was reported that *MEK1* mutations can be divided into two categories: those that alleviate inhibitory interactions with the helix A region and those that are in-frame deletions of the β3-αC loop, which promote MEK1 homodimerization [4]. The former, helix A-associated mutants, are inhibited by traditional MEK inhibitors, while the latter can result in differential resistance to MEK inhibitors [4]. The loop-deletion mutant-related increase of homodimerization enhanced cross-phosphorylation of the activation loop [4]. MEK1 dimerization played a crucial role in both its activation by the kinase RAF and for its catalytic activity toward the kinase ERK, which helps us further understand the role and function of *MEK1* mutations.

In most cell experiments and clinical case reports, the cells and patients with various MEK1 mutations were sensitive to trametinib, suggesting MEK1 mutations might be a potential indicator of trametinib therapy. However, it was also reported in a few studies that MEK1 mutations led to resistance to trametinib. For examples, in LCH patients, the patient with an MEK1 p.E102-I103del mutation received complete remission by the treatment of trametinib, while the patient with MEK1 p.L98_K104 > Q deletion presented clinical resistance to

trametinib therapy [33,34]. A similar situation was also observed in cell experiments, the cells harboring F53_Q58 > L, Q58_E62del and C121S/G128V were sensitive to trametinib, while the cells with C121S/G128D and 56_61QKQKVG > R were resistant to trametinib [20,23]. A study analyzing 17 tumor-associated *MEK1* mutations found that they activated ERK signaling in a RAF-independent (ΔL98-I103, ΔI99-K104, ΔE102-I103, ΔI103-K104), RAF-regulated (ΔE51-Q58, ΔF53-Q58, E203K, L177M, C121S, F53L, K57E, Q56P, K57N) or RAF-dependent (D67N, P124L, P124S, L177V) manner. The latter two are sensitive to feedback inhibition of RAF, which limits their functional output and often co-occur with RAS or RAF mutations [28]. By contrast, another class of *MEK1* mutations delete a unrecognized negative regulatory segment of MEK1, and is RAF- and phosphorylation-independent, unaffected by feedback inhibition of upstream signaling [28]. They drive high ERK output and transformation in the absence of RAF activity. Moreover, these RAF-independent mutants are insensitive to allosteric MEK inhibitors including trametinib which bind to the inactivated form of MEK1 [28]. All the mutants were sensitive to MAP588, an ATP-competitive MEK inhibitor [28]. A study also indicated that activating *MEK1 F129L* mutation is a mechanism of acquired resistance to MEK inhibition in cancer carrying *BRAF V600E* mutation, which strengthened binding to c-Raf, suggesting an underlying mechanism of higher intrinsic kinase activity [39]. These results show that different MEK1 mutations may have different response to trametinib, which needs further studies to distinguish which MEK1 mutations are appropriate for the treatment with trametinib and which are not. The present clinical case reports showed trametinib has great anticancer activity in the patients with malignancies harboring MEK1 mutations, but the large-scale clinical trials are lacking. Hence, further studies are necessary, which might provide a new therapeutic strategy and choice for tumor patients with MEK1 mutations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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