A Review on Various Analytical and Biological Methods for the Determination of Tepotinib

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Abstract: **Tepotinib is a MET tyrosine kinase inhibitor used to treat solid tumors that overexpress MET. There were several phases of clinical trials, and only a few analytical methods for Tepotinib were established using RP-HPLC technology. Chromatography makes use of the stationary phase. Ascentis 150 mm x 4.6 mm x 2.7 m, mobile phase Acetonitrile: 0.1% OPA in a 50:50 mixture, pumped at 1 ml/min, detection wavelength of 310 nm, column temperature of 30oC, and mobile phase as the diluent. Tepotinib (TPT), a fibroblast growth factor receptor inhibitor and anticancer medicine, has been approved by the FDA for use in the chemotherapy of urothelial carcinoma. HSA binding may affect the pharmacokinetics and pharmacodynamics of anticancer medicines. Tepotinib (TEPO), sotorasib (SOTO), and darolutamide (DARO) were tested as novel anticancer drugs utilizing a well-established, simple, rapid, and sensitive spectrofluorimetric approach. The spectrofluorimetric approach was based on the quantitative quenching of MER fluorescence at 538 nm after it had been activated at 350 nm with the prescribed drugs. Tepotinib, a highly selective MET inhibitor, was investigated in this Phase 1b/2 study in patients from the United States and Europe with advanced hepatocellular carcinoma (AHCC) who had previously received sorafenib treatment and showed MET overexpression.**

Key Words: Tepotinib, Tyrosine, MET, Fibroblast, Urothelial Carcinoma, Sotorasib, Darolutamide.

INTRODUCTION

Tepotinib is an oral tyrosine kinase inhibitor that targets the MET protein and is used to treat individuals with metastatic non-small cell lung cancer who have MET exon 14 skipping mutations. Tepotinib is a MET tyrosine kinase inhibitor used to treat various solid tumours that overexpress MET. Since its initial creation in 2009 as a collaboration between EMD Serono and the University of Texas M.D. Anderson Cancer Centre, it has been studied for the treatment of neuroblastoma, gastric malignancies, non-small cell lung cancer, and hepatocellular carcinoma. For the treatment of non-small cell lung cancers (NSCLC) with MET mutations, Tepotinib was initially licensed in Japan in March 2020. The US FDA then granted accelerated clearance of the drug in February 2021., MET exon 14 skipping mutations and adult patients with metastatic NSCLC are being treated with Tepmetko.7,9 The fact that it is the first oral MET-targeted tyrosine kinase inhibitor to support once-daily dosing9 may help reduce the pill burden frequently connected with chemotherapy regimens. Tepotinib was given the go-ahead to be used in Europe in February 2022. It appears that MET plays a crucial role in the growth and proliferation of tumours in which it is overexpressed and/or mutated, both directly and indirectly, making it a promising target in the therapy of several solid tumours.[1-2]

DRUG PROFILE

Category	Anti neo plastic agent
I.U.P.A.C Name	3-{1-[(3-{5-[(1-methylpiperidin-4-yl)methoxy]pyrimidin2-yl}phenyl)methyl]-6-oxo-1,6-dihydropyridazin3-yl}benzonitrile
Description	White powder.
Chemical Formula	$C_{29}H_{28}N_6O_2$
Molecular Mass	492.6 g/ mol.
Storage	Store at room temperature. Don't refrigerate.
Brand Name	TEPMETKO
Solubility	Freely soluble in aqueous hydrochloric acid (25% v/v) and soluble in DMSO slightly soluble in methanol, ethanol, very slightly soluble in 2-propanol, acetonitrile, water.

Table 1. DRUG PROFILE OF TEPOTINIB

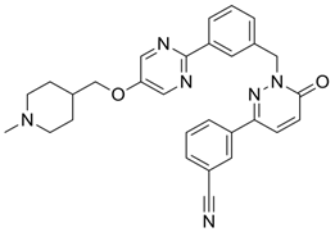

Figure 1. TEPOTINIB STRUCTURE

TITLE OF ARTICLE: Method Development and Validation for the Estimation of Tepotinib in Pharmaceutical Dosage Forms by RP-HPLC.

J. Nikhil et al. (2022) A simple, accurate procedure was used to determine the estimation of Tepotinib utilizing RP-HPLC technology. Chromatography makes use of the stationary phase. Ascentis 150 mm x 4.6 mm x 2.7 m, mobile phase Acetonitrile: 0.1% OPA in a 50:50 mixture, pumped at 1 ml/min, detection wavelength of 310 nm, column temperature of 30ºC, and mobile phase as the diluent. As the optimum line of action, conditions were established. The intermediate accuracy values for the method were determined to be 0.9 and 1.2. The respective LOQ and LOD are 0.85g/ml and 0.28g/ml. Using the aforementioned method, 99.15% of the marketing formulation passed the test. Investigations into the degradation of tepotinib were conducted; in every case, the purity threshold was higher than the impurity angle, and in appropriate range.[3]

TITLE OF ARTICLE: Development and Validation of Liquid Chromatography Method for Determination of Tepotinib in Bulk Drug and in Tablet Dosage Form.

Hameeda Begum et al. (2023) Tepotinib was simultaneously estimated in bulk drug and pharmaceutical fixed dose forms using an accurate, sensitive, precise, quick, and isocratic reversed-phase HPLC (RP-HPLC) approach that was developed and validated. With buffer: Acetonitrile [(40:60) (v/v)] in the isocratic mode of elution as mobile phase and a flow rate of 1 mL min1, the best separation was made on a 250 mm 4.6 mm i.e., 5-m particle size of x-tera RP-18. At 254 nm, UV detection was made. Tepotinib retention times were discovered to be 2.832 minutes. With a correlation coefficient of around 0.9988, response to Tepotinib was a linear function of concentration over the range of 45-270 mcg/m. Tepotinib % assay resulted in a finding of 99.37. Tepotinib was reported to have limits of detection (LOD) and quantitation (LOQ) of 0.0225 mcg/mL and 0.0675 mcg/mL, respectively. The formulation's excipients had no impact on the assay. Because of the method's ease of use, speed, accuracy, and precision, quality-control laboratories may make good use of it.[4]

TITLE OF ARTICLE: Development And Validation of Stability Indicating RP-HPLC Method For the Estimation of Tepotinib in Bulk and Formulation.

Monika M. Shirawar et al. (2023) The goal of the current work is to create a reverse-phase HPLC method that is simple, precise, quick, verified, and highly suited for estimating tepotinib in bulk and pharmaceutical formulations in accordance with ICH criteria. Method: A Phenomenox Kinetex XB-C18(1504.6 mm, 5) column was used to separate the components by chromatography. At 272 nm, the absorbance was measured together with the flow rate of. Ml min. The methods and capabilities of the approach were assessed in accordance with the ICH recommendations for linearity, precision, accuracy, system appropriateness, specificity, and robustness. Results: Tepotinib had a retention period of 3.4 minutes. The % mean recoveries for accuracy and precision of tepotinib were in the range (%Relative Standard Deviation 2), and the calibration plot was linear in the range 5-25 g/ml and (r2=.9993). It was established that the Limit of Quantification and Limit of Detection were, respectively, 0.429 g/ml and 1.3 g/ml.[5]

TITLE OF ARTICLE: Elucidation of binding dynamics of tyrosine kinase inhibitor tepotinib, to human serum albumin, using spectroscopic and computational approach.

Mohd Amir et al. (2023) Tepotinib (TPT), a fibroblast growth factor receptor inhibitor and anticancer medication, has been given FDA approval for use in the chemotherapy of urothelial carcinoma. Anticancer medications' pharmacokinetics and pharmacodynamics may be impacted by I binding. The binding interaction between TPT and I was assessed using the absorption, fluorescence emission, circular dichroism, molecular docking, and simulation studies. The interaction of TPT with I was shown by the absorption spectra to have a hyperchromic impact. According to the Stern-Volmer and binding constant of the I-TPT complex, fluorescence quenching is caused by a static process rather than a dynamic one. Furthermore, it was discovered from the displacement experiments and molecular

docking studies that TPT preferred to bind to site III of I. TPT binding to I causes conformational changes and decreases -helical content, according to circular dichroism spectroscopy. The thermal CD spectra show that tepotinib increases protein stability in the 20 to 90 °C temperature range. The results of MDS investigations offer additional support for the stability of the I-TPT complex. The results of the current study thus paint a clear picture of how TPT affects I interaction. These interactions are hypothesized to increase the hydrophobicity of the milieu surrounding I compared to its natural condition.

TITLE OF ARTICLE; Approved spectrofluorimetric strategies for assurance of three modern antineoplastic drugs: tepotinib, sotorasib, and darolutamide in their dose forms and biological liquids utilizing mercurochrome.

Hesham Salem et al. (2022) Tepotinib (TEPO), sotorasib (SOTO), and darolutamide (DARO) were evaluated as novel antineoplastic medicines using an established, simple, quick, and delicate spectrofluorimetric method. The spectrofluorimetric method was based on the quantitative quenching of MER's fluorescence at 538 nm after it had been stimulated at 350 nm by the addition of the specified medicines in the presence of acetate buffer (pH 3.5). Within the concentration range of 0.5-10.0, 0.2-10, and 0.4-10.0 g ml1 for TEPO, SOTO, and DARO, respectively, the amount of fluorescence quenching was precisely proportional to the concentrations of the medications mentioned. For the medications under study, the mean and standard deviation (SD) were computed as follows: 99.9 0.87, 99.72 1.08, and 100.21 1.44 for TEPO, SOTO, and DARO, respectively. Limits of quantitation (LOQ) for TEPO, SOTO, and DARO were 0.5, 0.15, and 0.36 g ml1, respectively, whereas limits of detection (LOD) were 0.16, 0.05, and 0.11 g ml1. Greater insight was achieved through statistical comparison through certain tactics, and it was discovered that there were no notable differences in exactness and exactness amongst strategies. To analyse the measurement of various forms of the tested medications, the proposed technique was successfully applied. Additionally, the TEPO, SOTO, and DARO were examined in human plasma and urine tests using the suggested fluorometric approach.

TITLE OF ARTICLE: Translational pharmacokinetic-pharmacodynamic modeling of preclinical and clinical data of the oral MET inhibitor tepotinib to determine the recommended phase II dose.

Wenyuan Xiong et al. (2021) Tepotinib is a very effective and selective MET inhibitor that is being researched for the treatment of individuals with solid tumours. An efficacy-driven translational modelling technique was developed to determine the recommended phase II dose (RP2D) in light of the positive tolerability and safety profiles up to the maximum tested dose in the first-in-human (FIH) experiment. A subcutaneous KP-4 pancreatic cell-line xenograft model in mice sensitive to MET pathway inhibition was chosen as a surrogate tumour model to examine the link between in vivo pharmacokinetics (PKs), target inhibition, and tumour growth inhibition. The longitudinal PKs and target inhibition profiles from the mouse xenograft trial were combined with additional clinical PK and target inhibition data (derived from predose and postdose paired tumour samples) from a FIH investigation to create a translational PK/pharmacodynamic (PD) model. According to preclinical findings, tepotinib treatment caused tumours in KP-4 xenograft tumours to shrink, which equated to a 95% target inhibition. Therefore, we came to the conclusion that for tepotinib to be effective, it should target a PD criterion of sustained, nearly full (>95%) hosphor-MET inhibition in tumours. Tepotinib 500 mg once daily was chosen as the RP2D for simulations of dose-dependent target inhibition profiles in human tumours that exceeded the PD threshold in more than 90% of patients. The efficacy-driven justification for choosing the 500 mg once day tepotinib phase II dose is supported by this translational mathematical modelling technique. Tepotinib has been given regulatory permission to treat non-small cell lung cancer patients who have MET exon 14 skipping.[6]

TITLE OF ARTICLE; Phase 1b/2 trial of tepotinib in sorafenib pretreated advanced hepatocellular carcinoma with MET overexpression.

Thomas Decaens et al. (2021) Tepotinib, a highly selective MET inhibitor, was tested in this Phase 1b/2 research in US/European patients with advanced hepatocellular carcinoma (aHCC) that had previously received sorafenib treatment with MET overexpression.

Adults who met the criteria for inclusion had MET overexpression in Phase 2 only, progression after 4 weeks on sorafenib, and a diagnosis of HCC. In Phase 1b ('3 + 3' design), temozolomide was given once daily at doses of 300 or 500 mg, and in Phase 2, it was given at the Phase 2 dose that was advised (RP2D). the 12-week investigator-assessed progression-free survival (PFS; Phase 2) and dose-liming toxicities (DLTs; Phase 1b) objectives, respectively. The RP2D was verified to be 500 mg in Phase 1b (n = 17), where no DLTs happened. The primary goal for Phase 2 (n = 49) was achieved: 12-week PFS was 63.3% (90% CI: 50.5-74.7), which was significantly higher than the predetermined null hypothesis of 15% (one-sided binomial exact test: P 0.0001). It took 4 months on average for progression to occur. Peripheral oedema and lipase rise, both of which occurred in 6.1% of patients in Phase 2, were among the 28.6% of patients who experienced treatment-related Grade 3 adverse events. In aHCC with MET overexpression that had previously received sorafenib treatment, tepotinib was generally well tolerated, and the RP2D (500 mg) shown promising effectiveness, resulting in a favorable benefit-risk balance.[7]

TITLE OF ARTICLE; Tepotinib in Non–Small-Cell Lung Cancer with *MET* Exo14Skipping Mutations.

Paul k. paik et al. (2020) In 3–4% of people with non-small-cell lung cancer (NSCLC), a splice-site mutation results in the loss of exon 14 transcription in the oncogenic driver MET. We assessed tepotinib's effectiveness and security in this patient population as a highly selective MET inhibitor. In this open-label, phase 2 study, individuals with advanced or metastatic NSCLC and a verified MET exon 14 skipping mutation received tepotinib (at a dose of 500 mg once daily). The objective response by independent evaluation among patients who had undergone at least 9 months of follow-up was the main end point. Whether a MET exon 14 skipping mutation was found in the liquid biopsy or the tissue was another factor considered when analysing the response. Tepotinib had been administered to 152 individuals as of January 1, 2020, and 99 of those patients had been monitored for at least nine months. In the combined-biopsy group, the response rate by independent evaluation was 46% (95% confidence interval [CI], 36 to 57), with a median response length of 11.1 months (95% CI, 7.2 to could not be estimated). In the 66 patients in the liquid biopsy group, the response rate was 48% (95% CI, 36 to 61), and in the 60 patients in the tissue biopsy group, it was 50% (95% CI, 37 to 63); 27 patients achieved positive results using both techniques. Regardless of the prior therapy administered for advanced or metastatic cancer, the investigator-assessed response rate was 56% (95% CI, 45 to 66). Peripheral edoema was reported by 7% of the patients, while adverse events of grade 3 or higher that investigators believed to be attributable to tepotinib medication were recorded by 28% of the patients. 11% of patients had their tepotinib treatment permanently stopped due to adverse effects. Sixty-seven percent of the patients with matched liquid-biopsy samples showed a molecular response, as gauged by circulating free DNA, both at the start of the treatment and throughout. The usage of tepotinib was associated with a partial response in almost half of patients with advanced NSCLC who had a verified MET exon 14 skipping mutation. Grade 3 or higher toxicity was primarily manifested as peripheral edoema.

TITLE OF ARTICLE; Phase I trial of the MET inhibitor tepotinib in Japanese patients with solid tumors.

Kohei Shitara et al. (2020) Tepotinib (MSC2156119J) is an oral, powerful, and highly selective small molecule mesenchymal-epithelial transition factor (MET) inhibitor with a Phase II dose of 500 mg once daily based on a first-in-man trial done in the United States and Europe. To assess the required Phase II dose in Japanese patients with solid tumours, we conducted a multicenter Phase I trial using a conventional '3 + 3' design (NCT01832506).

Tepotinib at 215, 300, or 500 mg once day in a 21-day cycle was given to patients aged 20 years with advanced solid tumours (refractory to standard therapy or for whom no viable standard therapy was available). Dose-limiting toxicities occur when Cycle 1 was performed to identify the maximum tolerated dose. To support the dose determination, efficacy, safety, and pharmacokinetics were also assessed.

Twelve patients were treated. Tepotinib was generally well tolerated, with no dose-limiting toxicities detected; treatment-related side events were mostly grade 1-2. Tepotinib's tolerability profile was similar to that reported in non-Japanese populations. Pharmacokinetics in Japanese and Western individuals were comparable. One patient with stomach cancer and one with urachal

carcinoma had stable illness for 12 weeks. The reported safety profile and pharmacokinetics are comparable to those observed in individuals from the United States and Europe, and the recommended Phase II dose of tepotinib in Japanese patients was verified to be 500 mg once day.

These findings, which include preliminary anticancer activity signals, justify the continued development of tepotinib in Japanese cancer patients.[8]

TITLE OF ARTICLE Open-label, single-center, phase I trial to investigate the mass balance and absolute bioavailability of the highly selective oral MET inhibitor tepotinib in healthy volunteers.

Andreas Johne et al. (2020) Tepotinib (MSC2156119J) is an oral MET inhibitor that is both powerful and highly selective. This open-label, phase I trial (EudraCT 2013-003226-86) in healthy volunteers assessed its mass balance (part A) and absolute bioavailability (part B). Tepotinib (498 mg spiked with 2.67 MBq [14C]-tepotinib) was given orally to six subjects in Part A. Up to day 25, blood, plasma, urine, and faeces were collected until radioactivity excretion was 1% of the supplied dose. Six participants were administered 500 mg tepotinib orally as a film-coated tablet, followed by an intravenous [14C]-tepotinib tracer dosage (53-54 kBq) four hours later in part B. Blood samples were taken up until the 14th day. In Part A, a median of 92.5% (range, 87.1-96.9%) of the [14C]-tepotinib dose was retrieved in excreta. Radioactivity was primarily eliminated in the faeces (median, 78.7%; range, 69.4-82.5%). Urinary excretion was a modest mode of elimination (median, 14.4% [8.8-17.7%]). The parent chemical was the most abundant ingredient in excreta (45% [faeces] and 7% [urine] of the radioactive dosage). M506 was the sole significant metabolite. After oral administration of 500 mg tablets (the dose and formulation used in phase II trials), absolute bioavailability was 72% (range, 62-81%). Finally, tepotinib and its metabolites are primarily excreted in the faeces; the parent drug is the most heavily excreted ingredient. Tepotinib has a good oral bioavailability, bolstering the use of the present tablet formulation in clinical trials. In this trial of healthy individuals, tepotinib was well tolerated.[10]

CONCLUSION

According to this review there were different clinical trials were performed and only few analytical methods were developed. one RP-HPLC method is available in single dosage form (TEPOTINIB) and one spectroscopy method is available in combined dosage form (tepotinib, sotorasib, and darolutamide) and other reported methods are mostly biological methods were developed for the determination of tepotinib in human serum and plasma the activity of drug again MET is performed based on various clinical trials. since there were only few analytical methods were reported for Tepotinib.

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