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Protein synthesis inhibitors of natural origin for CML therapy: semisynthetic homoharringtonine (Omacetaxine mepesuccinate)

Minireview

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Omacetaxine mepesuccinate is a drug approved in 2014 by FDA for the use in CML therapy in patients resistant to at least two thymidine kinase inhibitors (TKIs). It possesses unique mechanism of anticancer activity that is principally different from mechanism of activity of TKIs. Omacetaxine mepesuccinate inhibits protein translation through prevention of the initial elongation step of protein synthesis and its use benefits CML patients possessing the *BCR-ABL* oncogene. Because of the superior activity of Omacetaxine in patients who became resistant to therapy with TKIs, FDA decided on the accelerated approval of this drug taking its consideration not only its activity as such but also a favorable benefit-to-risk profile in patients included into clinical studies.

Key words: Omacetaxine mepesuccinate, homoharringtonine, analytical determination, anti-cancer properties, protein synthesis inhibition, CML

Harringtonine and homoharringtonine as natural compounds

Harringtonine (NSC 124147; molecular weight 531.59 D) and homoharringtonine (NSC 141633; molecular weight 545.62 D) are two alkaloids of rather complex structure. As alkaloids, they contain a nitrogen atom (see Figure 1). They are also classified as norditerpenes. This indicates the biochemical way of their synthesis in coniferous plants and partially the relationship of their chemical structure to diterpenes containing four isoprene units.

In nature, these two substances are being present in coniferous trees of *Cephalotaxus* genus belonging to *Cephalotaxaceae* family (Plum Yew or Cowtail Pine) includes 72 species according to the data in the Plant List 2013 [1]. *C. fortunei*, *C. griffithii*, *C. hainanensis*, *C. harringtonia*, *C. koreana*, *C. lanceolata*, *C. latifolia*, *C. mannii*, *C. oliveri*, *C. sinensis and C. wilsoniana* are the most known species. The plants are endemic to eastern Asia [1].

Analytical determination of harringtonine and homoharringtonine

Chemical determination of harringtonine and homoharringtonine in preclinical and clinical studies is usually performed using modern instrumental analytical methods, namely chromatography, mainly due to the complex structure of this molecule and because chemical properties of homoharringtonine and related compounds were not studied extensively before the discovery of its benefits in cancer patients. Mass spectrometry is a method of choice for detecting these alkaloids as analytes in analyzed samples of clinical or non-clinical origin. These methods were essential in developing homoharringtonine into the approved drug. The short overview of the methods used for analysis of both compounds (homoharringtonine and harringtonine) is in Table 1 [2-17] – the references are in the order from the most new to the oldest. The tremendous development in the area of instrumental analysis is obvious from the table when

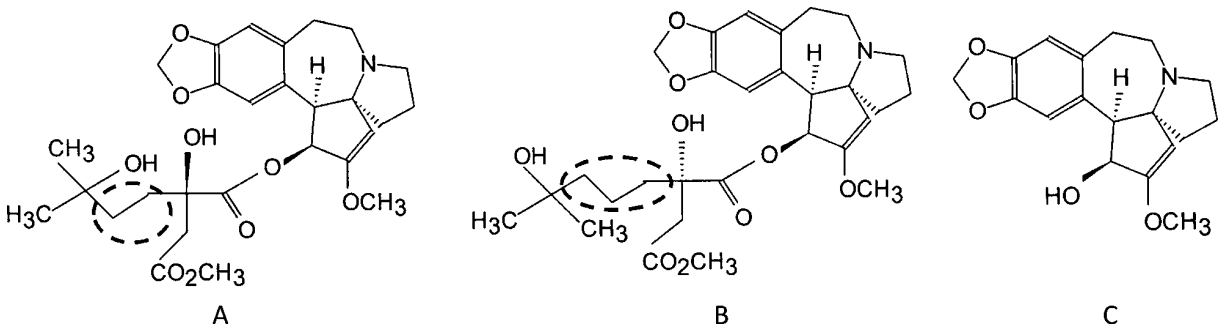

Figure 1. Chemical structures of A) harringtonine ($C_{28}H_{37}NO_9$), B) homoharringtonine (Ceflatonin, Omacetaxine mepesuccinate, Synribo; $C_{29}H_{39}NO_9$) and C) cephalotaxine . The marked areas in formulas A and B indicate the difference in harringtonine and homoharringtonine chemical structures.

simple TLC (thin layer chromatography) was used [14] to application of sophisticated MS/MS mass spectrometry/mass spectrometry) methods [3] and microemulsion electrokinetic chromatography coupled with field-amplified sample injection [2]. HPLC (high-pressure liquid chromatography) is obviously a relatively simple and reliable method of analysis of harringtonine and homoharringtonine [4-8, 10-12, 16]. So are the counter-current chromatography [9] and originally used gas chromatography [17].

The use of MS/MS detection in its current state of art is advantageous as it allows direct detection and determination of the original drug and also of its metabolites, mainly the product of hydrolysis of the ester bond by various esterases [18] resulting in the formation of 4'-demethylharringtonine (also called homoharringtonine acid) [19]. The mentioned instrumental methods were used for harringtonine/Omacetaxine mepesuccinate determination during its preclinical development and clinical applications where omacetaxine mepesuccinate was determined in complex biological samples originating from patients. All of these methods are suitable for routine determination of homoharringtonine and harringtonine and provide clinicians with invaluable therapy-related information.

Omacetaxine mepesuccinate

To avoid any confusion, it is important to clarify that homoharringtonine is an alkaloid of a natural origin present in trees of *Cephalotaxus* genus. However, after discovering its strong anticancer activity, it was clear that these trees can only serve as a source for limited amount of homoharringtonine. The semisynthetic homoharringtonine that is for some reason of higher purity and stronger myelosuppression [20] is distinguished from the homoharringtonine from natural sources and is known and commercially available as Omacetaxine mepesuccinate.

Various syntheses of homoharringtonine and related harringtonine were attempted and reported in scientific literature. The first synthesis of harringtonine and its derivative by partial esterification of cephalotaxine (NSC 128487; 245454 and 245455; Figure 1) was reported in 1982 [21]. The semi-synthesis of enantiopure homoharringtonine *via* anhydrohomoharringtonine was reported in 1999 [22]. This synthesis was based on a direct esterification of cephalotaxine by the activated substituted tetrahydropyrancarboxilic acid (that was entered into synthesis in the form of methyl ester). Selective ring opening of the resulting anhydrohomoharringtonine resulted in enantiopure homoharringtonine (Figure 2) [22].

Reasons for semi-synthetic approach in chemical preparation is based on complexity of harringtonine chemical structure and on a relatively high availability of the natural alkaloid cephalotaxine in *Cephalotaxus* conifers. Additionally, cephalotaxine esterification used in homoharringtonine synthesis and resulting in an ester – homoharringtonine is based only on a few synthetic and purification steps/procedures. The semi-synthetic homoharringtonine in well known as omacetaxine mepesuccinate.

Various syntheses of cephalotaxine were reported as well [i.e. 23, 24], however they did not reach practical application. Moreover, chemical modification/esterification of cephalotaxine probably yields a substance that is superior in purity to homoharringtonine obtained from natural source, as it was observed that omacetaxine has been more myelosuppressive than homoharringtonine [20].

Mechanism of activity

Homoharringtonine (or omacetaxine) mechanism of action is based on inhibition of protein translation through prevention of the initial elongation step of protein synthesis. Omacetaxine interacts with A-site of the ribosome and disturbs the correct positioning of amino acid side chains of incoming aminoacyl-tRNAs (Figure 3). It acts on the first step of protein translation only and does not inhibit further steps of protein synthesis from mRNAs when the translation have already proceeded [25]. Probably, the site of homoharringtonine binding on 80S ribosomes overlaps or is the same as the acceptor site of the ribosome peptidyl transferase center [26, 27]. The detailed mechanism of homoharringtonine binding to ribosomes needs additional investigations elucidating details of this interaction.

Table 1. Analytical determination of homoharringtonine and harringtonine.

Method	Analytes	Matrix	Publ.	Ref.
MEEKC (microemulsion electrokinetic chromatography) coupling with field-amplified sample injection (FASI)	HHT and 4 other isoquinoline alkaloids	urine	2009	2
HPLC-electrospray tandem MS (HPLC-MS/MS)	HT HHT deoxy HT iso HT & 5 other alkaloids	*C. harringtonia* leaves - various extracts	2003	3
HPLC; NMR; MS	HT HHT iso HT ethyl HHT	pharmaceutical preparations	2000	4
HPLC (detection at 290 nm)	HHT	serum	1999	5
HPLC (detection at 288 nm)	HHT	injection solutions	1999	6
HPLC; MS	HT HHT cephalotaxine	*C. harringtonia* callus and roots (methanol and chloroform extract)	1996	7
HPLC (detection at 280 nm)	HHT	urine, plasma and cell homogenate	1995	8
Counter-current chromatography; TLC; MS	HT HHT iso HT	*C. fortunii -a crude extract*	1992	9
HPLC (detection at 290 nm)	HT HHT iso HT cephalotaxine	*Cephalotaxus* bark extracted in aqueous NH3 solution	1991	10
HPLC with amperometric detection; IS – harringtonine	HHT	plasma, serum	1989	11
RP-HPLC	HT	tissue samples, extracted by 0.2M HCl ;	1989	12
spectrophotometry (detection at286 and 282 nm)	HT	liposomes	1984	13
TLC; detection by Dragendorff's reagent & scanner	HHT (epimers)	natural homoharringtonine	1984	14
HPLC (preparative)	HT and diastereo isomers		1983	15
HPLC	HT HHT	plasma, serum	1982	16
GC-MS	HT HHT	serum	1982	17

HHT – homoharringtonine; HT – harringtonine; NMR – nucleic magnetic resonance; IS – internal standard; GC – gas chromatography

It was shown for related harringtonine [28] that it may cause decrease in intracellular levels of centromere proteins. Possibly, this is caused by the inhibition of mRNA expression of corresponding genes, especially of CenpB and other centromere protein genes, as the expression of these genes is related to apoptosis induction by harringtonine. Additionally, it was shown that TGF-beta, TNF, FAS, p38MAPK and p53 apoptosis signaling pathways are activated during homoharringtonine-induced apoptosis in QGY-7703 cells [29]. When tested in myeloma cells, homoharringtonine significantly reduced Mcl-1 – an essential protein for myeloma cell survival [30]. Additionally, homoharringtonine causes dual downregulatiton of c-FLIP(L/S), protein characterized by its anti-apoptotic properties. Consequently, dual c-FLIP(L/S) downregulation sensitizes cells to induced apoptosis [31]. It is documented that apoptosis induced by homoharringtonine is mediated via both intrinsic and extrinsic apoptosis pathways [30].

Interesting results were obtained when a pro-apoptotic ligand from the TNF-alpha family -TNF-related apoptosis-

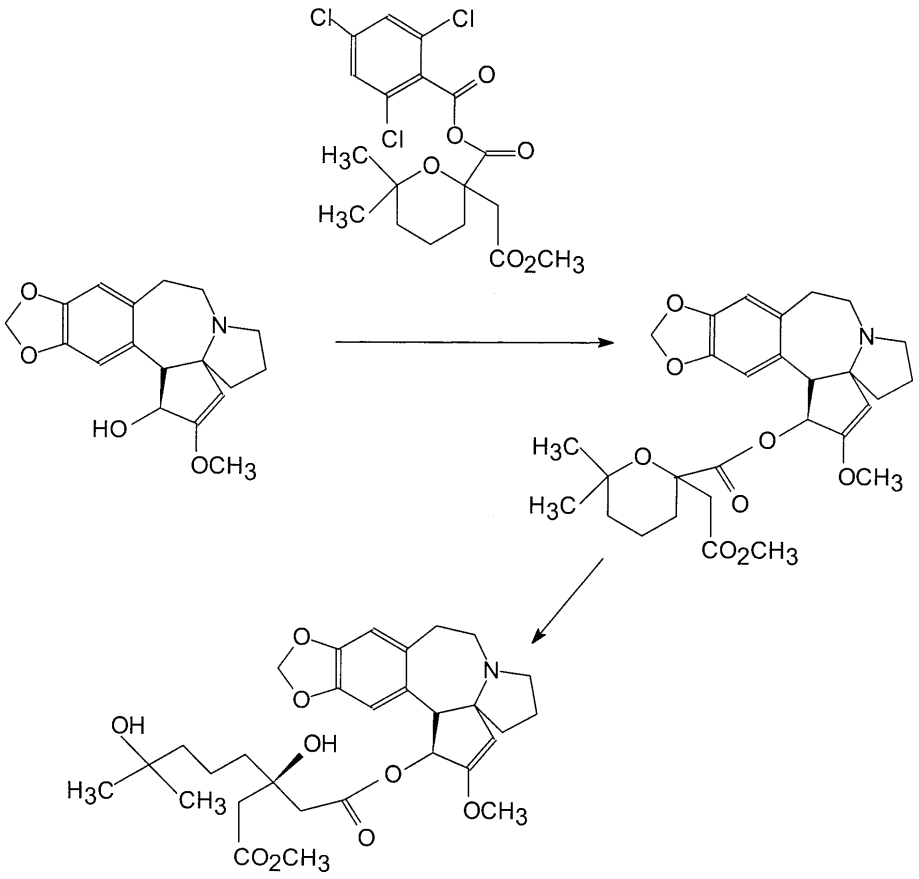

Figure 2. The semi-synthesis of enantiopure homoharringtonine according Robin J-P et al. [22]

inducing ligand (TRAIL) was applied to resistant cell lines together with homoharringtonine that demonstrated to be a very efficient enhancer of TRAIL-induced apoptosis. The application of very low quantities of homoharringtonine resulted in apoptosis induction. This was achieved through down regulation of the expression of the anti-apoptotic proteins Mcl-1 and cFLIP. Additionally, TRAIL-triggered activation of JNK and p38 kinases was observed [32].

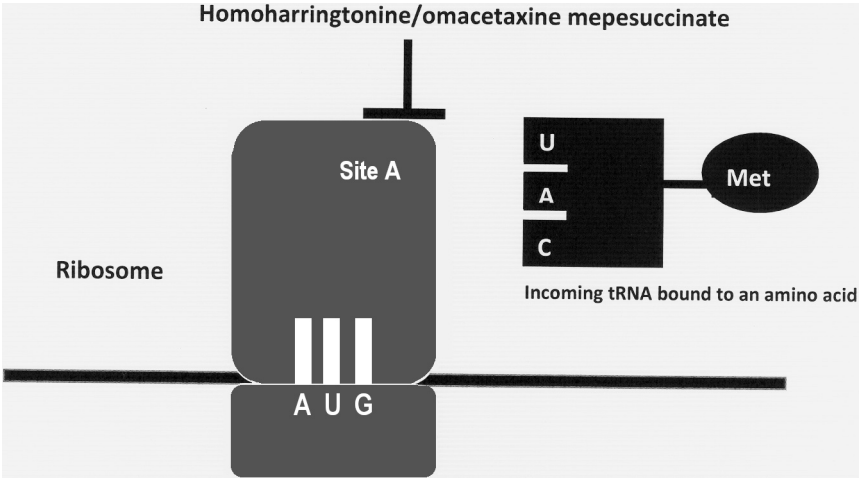

Figure 3. Homoharringtonine (omacetaxine mepesuccinate) mechanism of action

Sensitivity of tyrosine kinase inhibitors-resistant cells to omacetaxine

The success of Omacetaxine mepesuccinate in patients with chronic myeloid leukemia (with the Philadelphia chromosome positive stem cells; CML) is based on the fact that its mechanism of action differs from that of tyrosine kinase inhibitors competing with ATP for binding site at ABL of the BCR-ABL fusion gene. In general, while Omacetaxine mepesuccinate inhibits the synthesis of BCR-ABL tyrosine kinase. On the other hand, clinically used TKIs compete with already synthesized BCR-ABL tyrosine kinase thus inhibiting its action. The product of BCR-ABL gene is active tyrosine kinase promoting leukemogenesis via downstream signaling. The result of the TKIs action is inhibition of the BCR-ABL-produced tyrosine kinase. This leads to the block of cytokine-independent cell cycle unresponsive to apoptotic signaling [33, 34].

Omacetaxine mepesuccinate, on the other hand, does not bind to BCR-ABL and its action is not affected by the mutation of kinase domain erasing from CML chemotherapy by TKIs [35]. This drug was shown to be effective even in patients with a T315I ABL mutation that is generally a poor prognosis population [36]. Additionally, it was shown to block synthesis of other proteins that help to stabilize the ABL protein, i.e. heat shock protein 90 (HSP90) [37]. As Omacetaxine mepesuccinate initiates a rapid disappearance of T315I-mutated TKIs-resistant transcripts in the half of CML patients. The exact mechanism of this process is not elucidated yet but it allows for re-introduction or 'rechallenge' of TKIs therapy in previously TKIs resistant chronic-phase CML patients [38].

Pharmacokinetics of Omacetaxine mepesuccinate

Pharmacokinetic parameters are essential for administration of any substance employed as a therapeutic agent. Early in homoharringtonine studies, the only metabolite of this substance in human (and murine) plasma was identified as 2'-hydroxy-2'-(alpha-acetic acid)-6'-hydroxy-6'-methylheptanoyl cephalotaxine [19]. The metabolism proceeds due to plasma esterases' [19]. Urine excretion represented 29% of homoharringtonine and 20% as its metabolite 24 hours after homoharringtonine administration. Later, 72 hours after homoharringtonine administration, urinary elimination of this substance increased to 40% of the dose, while only 22% metabolized [19]. Approximately 14% of homoharringtonine are excreted through biliary tract [39].

Therapeutic use of Omacetaxine mepesuccinate as replacement of tyrosine-kinase inhibitors in resistant leukemia patients

Omacetaxine mepesuccinate use benefits CML patients possessing the *BCR-ABL* oncogene. Cytogenetic diagnosis of CML is based on the determination of Philadelphia chromosome (Ph) resulting in formation of *BCR-ABL*. *BCR-ABL* oncogene, the production of which is directly related to the presence of Ph chromosome, deregulates activity of tyrosine kinase. This leads to generation of different BCR-ABL proteins and, consequently, to the CML advancement [40]. Many reports on the use of the natural alkaloid homoharringtonine and the same molecule of the semisynthetic commercial product Omacetaxine mepesuccinate in CML appeared in scientific literature from 1990. Only the some of the latest studies will be mentioned as the use of this substance in CML therapy got much improved, justified and explained, and directed to the CML patients resistant to TKIs during the years.

The use of Omacetaxine mepesuccinate represents a significant addition to the progressive CML management after multi-TKI resistance develops – 'third-line management of CML' using non-TKI drugs. Additionally, Omacetaxine mepesuccinate (or ponatinib) should be used in patients with T315I mutation at any CML phase [41]. Results of the analysis of 24 months follow-up of patients on Omacetaxine mepesuccinate shown that a major cytogenetic response was registered in 18% of patients in chronic phase CML (median duration 12.5 months). The median overall survival was 40.3 months (23.8 months in patients not responsive to the therapy) [42]. A major hematologic response, but not a major cytogenetic response, was registered in 14% of patients in acute phase CML with median duration 4.7 months (3.6 months in patients not responsive to the therapy with Omacetaxine mepesuccinate). All the data of the analysis of this 24 months follow-up indicate that the long-term use of Omacetaxine mepesuccinate is possible and that it significantly benefits some patients [41]. Similar conclusions were drawn by other authors confirming that Omacetaxine mepesuccinate is beneficial even for some poor prognosis TKI-resistant CML patients [i.e. 43-45].

Results of the meta-analysis of 21 Chinese studies were published this year [46]. The included studies analyzed information on 1310 patients who were given Omacetaxine mepesuccinate with several anticancer agents, i.e. daunorubicin, idarubicin, cytarabine, aclacinomycin, mitoxantrone or granulocyte colony-stimulating factor. Observed rate of complete remissions was 65.2% in regimens involving Omacetaxine mepesuccinate. This was lower compared to complete remissions in regiments without this drug in randomized trials – 69.1%. The achieved complete remission in retrospective studies were achieved in 62.8% of treated patients [46]. A complete remission rate was significantly decreased in elderly patients: only 47.5% in elderly patients compared to 65.2% in the mixed age patients' cohorts [46]. This may indicate a higher benefit from the use of Omacetaxine mepesuccinate in younger patients.

Omacetaxine mepesuccinate is being used in combinations with low dose cytarabine [47], IFN [48] or both [49] and also with imatinib [50]. This was recently well summarized in a published review [51].

Adverse side-effects of Omacetaxine therapy

It was clearly demonstrated that Omacetaxine is active and has an acceptable safety profile in pretreated patients with advanced CML, irrespective of mutational status through promotion of apoptosis via inhibition of oncoproteins production [52]. The addition of Omacetaxine to the therapeutic armamentarium of chronic myeloid leukemia was significantly improved, especially for patients resistant to both first- and second-generation tyrosine-kinase inhibitors with developed BCR-ABL kinase domain mutations, against which TKIs have extremely low cross-activity [53]. Accumulated experience shows that a treatment of leukemia patients with Omacetaxine is accompanied only with generally mild non-hematologic toxicity, possibly with higher prevalence in elderly patients [54].

Data were published on the ability of homoharringtonine (a commercial product Omacetaxine mepesuccinate) to induce changes in the gastrointestinal tract, i.e. diarrhea and nausea/vomiting [55]. Reasons behind these side effects were not elucidated. Recently, the results of the study [55] indicated that homoharringtonine changes the epithelial permeability of intestinal Caco-2 cell monolayers through reduction of the trans-epithelial electrical resistance of these cells in a time- and dose-dependent manner via modulation of the expression and localization of protein claudin isoforms. However, the changes in the epithelial permeability were reversible and free of cytotoxicity [55].

The dose-related myelosuppression represents a principal adverse side effect of omacetaxine mepesuccinate therapy. The granulocytopenia was observed in 27% – 39% of patients and 13% – 25% of patients suffered from thrombocytopenia [56, 57].

The following non-hematologic adverse effects on omacetaxine mepesuccinate are the most prominent: fatigue, diarrhea, pyrexia, nausea, headache, asthenia, anorexia, hyperglycemia, injection-site erythema and tachycardia or chest pain [57-60]. The non-hematological adverse effects are not common [61]. The important fact is that results of safety analysis of the use of subcutaneous omacetaxine mepesuccinate in tyrosine kinase inhibitors-resistant patients confirmed an acceptable safety profile in all CML patients with the concern remaining regarding the potential adverse effects resulting from long-term use of this drug [61].

More recent studies found the occurrence of grade 3/4 hematologic toxicities to be 54% [62] or 76% [63] for thrombocytopenia, 48% [62] or 44% [63] for neutropenia and 33% [62] or 39% [63] anemia, respectively. Observed non-hematological effects were usually of grade 1 or 2. They included diarrhea (44% [62] or 40% [63]), nausea (30% [62] or 34 % [63]), fatigue (24%), pyrexia (20%), headache (20%), asthenia (20%) and infection (42%) [62]. These adverse effects of Omacetaxine mepesuccinate therapy in CML patients were not significant enough to prevent granting of an accelerated approval of the drug (Synribo; Teva Pharmaceuticals USA, Inc., North Wales, PA, http://www.tevausa.com) for CML with resistance and/or intolerance to two or more tyrosine kinase inhibitors (TKIs) in October 2012 by the U.S. Food and Drug Administration (FDA). The accelerated approval was granted based on the therapeutic "activity and a favorable benefit-to-risk profile for the studied population of adult patients" [61]. It was approved by FDA for the use in such patients in 2014 [64] as it has demonstrated activity and a favorable benefit-to-risk profile in the studied populations of adult patients with both phases CML.

Omacetaxine mepesuccinate in non-CML patients

Omacetaxine mepesuccinate approval by FDA for the use in CML refractory to TKIs indicate where this drug is the most successful. However, other indication of the use of omacetaxine mepesuccinate were investigated. These reports are just few but may be important for future development. The omacetaxine mepesuccinate activity in treatment of acute myeloid leukemia was summarized by a meta-analysis in 2015 [46] with the conclusion that this drug may serve as an active agent in the management of acute myeloid leukemia.

In China, this substance is used not only for the therapy of CML and acute myeloid leukemia (AML) but also in patients with myelodysplastic syndrome (MDS). Eleven homoharringtonine-based therapy clinical trials were summarized [64] with complete remissions achieved ranging from 71 to 86% of patients and with 3-years complete survival ranging from 45 to 55%. Complete remissions achieved in patients with MDS ranged from 47 to 58% [64]. A phase II open-label study of the intravenous administration of homoharringtonine in the MDS therapy of 9 patients performed in the USA [65] reported omacetaxine mepesuccinate to produce complete remission in one patient. The other eight enrolled-in-the-trial patients did not respond to this therapy. Consequently, some patients may benefit from inclusion of omacetaxine mepesuccinate into their therapeutic regime but more clarifications and clinical trials are necessary for full understanding of various aspects of therapeutic activity of this substance.

Based on available literature, it seems safe to conclude that omacetaxine mepesuccinate will be evaluated extensively on its usefulness in patients with AML and MDS in near future.

Historical note on the development of Omacetaxine mepesuccinate

Development of this drug is an interesting example of the influence of the geographical location of the source of the active natural ingredient homoharringtonine on the drug development. As the source of the alkaloid homoharringtonine is endemic to eastern Asia [1]. Consequently, the significant attention to the biologically active compound was paid in China. Later, when the issue of limited source of homoharringtonine was overcome by preparing it semisynthetically from another more available alkaloid cephalotaxine under the name Omacetaxine mepesuccinate [i.e. 22], clinical investigations were no longer limited by availability of the therapeutic substance. Twenty five clinical trials were performed according to PubMed

during the last 10 years. Out of these trials, 17 were performed in China and 7 in the USA. Only one clinical trial was performed in France in 2006 at early stages of the omacetaxine mepesuccinate development [18]. However, omacetaxine mepesuccinate clinical trials shifted to the USA during the last five years when 8 of clinical trials were performed in China, 6 in the USA and none in another country (PubMed: http://www.ncbi.nlm.nih.gov/pubmed). It is possible that with the FDA approval, some clinical trials would be performed outside of the USA and China or some trial would be performed in collaboration.

Conclusion

Approval of omacetaxine mepesuccinate for the use in CML therapy in 2014 brought new options to CML patients. As it targets protein translation, this drug possesses a new mechanism of action. However, additional information on omacetaxine mepesuccinate and its pharmacokinetic and pharmacodynamic behavior is still needed as better elucidation of properties and biological activities of this drug will help in its optimal use. Also, improved understanding of the omacetaxine mepesuccinate action in various patients' populations (age, gender, stage of the disease) need to be elucidated and understand together with the most successful combination of omacetaxine mepesuccinate with other anticancer agents, including TKIs. The primary success of omacetaxine mepesuccinate in CML patients resistant to multiple TKIs may be broadened by finding alternatives for its inclusion in other cancers therapies or finding more efficient combinations for therapy of present indications. However, at the present time, the use of omacetaxine mepesuccinate and investigation of its benefits for patients concentrates on CML.

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