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DRUG EVALUATION

An evaluation of masitinib for treating systemic mastocytosis

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ABSTRACT

Introduction: Systemic Mastocytosis (SM) is a complex family of rare diseases, against which pharmacological therapies are still very few. It is a c-kit driven disease, whose disregulation leads to uncontrolled activation and proliferation of mast cells (MCs) with consequent release of effector molecules which are responsible for its clinical manifestations.

Areas covered: Masitinib is a relatively new potential drug against SM and its chemical structure strictly derives from imatinib, the first tyrosine kinase inhibitor which entered the pharmaceutical market about 15 years ago. In this review, the authors present masitinib in all its properties, from chemistry to pharmacology and toxicity to its potential clinical application in SM, focusing the discussion on the few clinical trials in which it has been involved, with a particular attention on the still open challenge to determine how to measure the response to therapy.

Expert opinion: In spite of their similarity in chemistry and biological activity against submolecular targets, masitinib is much more selective towards c-kit receptors than other tyrosine kinases, such as Bcl-Abl. Furthermore, its ability to inhibit degranulation, cytokine production and MCs migration from bone marrow gives it a great chance to become an important therapeutic option for selected SM patients.

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KEYWORDS
C-kit receptor; clinical trials; masitinib; mast cells; tyrosine kinase inhibitors

1. Introduction

Systemic mastocytosis (SM) is worldwide recognized as a family of rare diseases, caused by an abnormal proliferation of mast cells (MCs) [1] in several body tissues, bone marrow and with or without cutaneous involvement (Figure 1). SM is an heterogeneous group of malignant blood disorders, whose world prevalence is estimated between 1/20,000 and 1/40,000 [2], in particular in caucasic over 60 years-old population. The principal distinction among all pathologic forms, identifies benign indolent (ISM) and malignant systemic mastocytosis, which commonly includes different subtypes of diseases, progressively debilitating [3]:

a. Aggressive Systemic Mastocytosis (ASM), characterized by a median survival of 41 months;
b. Systemic Mastocytosis associated with hematologic non-mast cell lineage disease (SM-AHNMD) with median survival of 24 months;
c. Mast Cell Leukemia (MCL) with a median survival of only 2 months.

General clinical evidences of the disease depend on MCs' mediator release, such as histamine, leukotrienes, prostaglandins, heparin, causing frequent syncopes, headache, hot flashes, till anaphylaxis shock. The proliferation of MCs in the bone marrow arises peripheral blood disorders, such as anemia, cytopenia and pancytopenia. The diffusion of abundant MCs [4] can involve gastrointestinal district (esophagus, stomach, intestine, liver) with abdominal pain, diarrhea, nausea and vomiting; to this regard, in the liver, it causes ascites, portal hypertension, hepatomegaly often evolving in portal fibrosis and cirrhosis. MCs can migrate to cutaneous district giving urticaria pigmentosa, to the spleen with asymptomatic splenomegalia, and to the skeleton with bone pain, arthralgia and osteolysis, osteosclerosis or osteoporosis at the diagnostic images.

SM is usually sporadic, nevertheless rare familiar cases have been reported [5] and actually the choice of a pharmacologic treatment is limited to a few authorized therapeutic options, imatinib and midostaurine, two target therapies belonging to the class of tyrosine kinase inhibitors (TKIs), with some unmet needs to overcome for treating SM, in particular, side effects and differently genetic pattern subgroup of patients, respectively. Other TKIs, avapritinib and ripretinib are new investigational active compounds in ongoing clinical trials for SM. Two other unlabeled drugs are interferon-α and cladribine, whose effect is quite unspecific and are both referred more to a symptomatic treatment than to an intent to eradicate the cause.

From the physio-pathologic point of view, mastocytosis is a disregulation of the c-kit receptor activity, a 976 amino acid protein with 145 kDa molecular weight, which is responsible of a permanent signal of autoproliferation and autoactivation of MCs and, in general, of hematopoietic stem cells. The protoncogene c-kit, also known as mast/stem cell factor receptor (SCFR) and CD117, is a receptor tyrosine kinase protein type III, encoded by the KIT gene [6–8]. KIT gene was identified for the

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Article highlights

- A disregulation of c-kit receptor causes abnormal proliferation of mast cells and systemic mastocytosis.
- Masitinib is a tyrosine kinase inhibitor which acts on some mutated c-kit driven forms of systemic mastocytosis.
- Masitinib is able to inhibit mast cells' degranulation, cytokine production and migration from bone marrow.
- Masitinib is less toxic than imatinib, because it is more selective towards tyrosine kinase targets.
- As a rare disease, there are not standardized response criteria for evaluating the trend of the pharmacologic treatments for systemic mastocytosis.

This box summarizes key points contained in the article.

first time in 1987 by the German biochemist Ullrich, during his studies on the feline sarcoma viral oncogene v-kit, its cellular homolog. In humans, KIT gene is localized in chromosome 4, band 4q12, from 54,657,918 to 54,740,715 base pairs (bp) for a total of 21 exons. C-kit is a membrane receptor (Figure 2) binding to SCFR (or c-kit ligand); this link starts a dimerization which activates its tyrosine kinase function stimulating the phosphorylation of cellular substrates which transmit to the nucleus the information leading to MCs proliferation and differentiation [9,10].

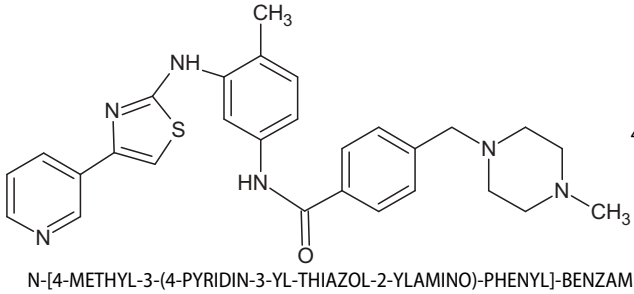
Tyrosine kinase inhibitors (TKIs) are potent and targeted drugs which are able both to inhibit c-kit activity and, to some extends, to deplete human MCs. TKIs have revolutionized not only anticancer therapy, but also are conditioning the way to face those diseases characterized and driven by abnormal MCs proliferation, such as mastocytosis. Apart from well-known TKIs (Figure 3) approved in therapy for chronic myeloid leukemia (imatinib, dasatinib, nilotinib) and gastrointestinal stromal tumour or GIST (imatinib, sunitinib), masitinib (AB1010) is a relatively novel molecule which has a peculiar mechanism of action with multiple effects and a certain selectivity towards specific biological functions (Box 1).

The aim of this review is to shed light on the activity and potentiality of masitinib towards MCs disorders, starting from its safety and activity profile in terms of pharmacodynamic and pharmacokinetic properties and to discuss the results of the clinical trials in human SM, after its approval in veterinary oncology in unresectable grade II/III canine mastocytomas with mutated c-kit.

2. KIT mutations and mastocytosis

Abnormal expression genes and their expression products caused by c-kit mutations are key reasons for different malignancies [11], in particular, c-kit activating mutant forms are associated to some types of malignancies (GIST, testicular seminoma, melanoma, acute myeloid leukemia) and to mastocytosis. In detail, an exon 11 mutation, involving c-kit intracellular juxtamembrane domain is responsible for 65% GIST which respond to imatinib, while exon 9, 13, 17 mutations, encoding for extracellular juxtamembrane, tyrosine kinase and phosphotransferase domains, respectively, account for 10% GIST cases and are less sensible or insensible to imatinib [12]; 5–7% GIST cases present platelet-derived growth factor receptor alpha (PDGFRα) gene mutation but not c-kit one [13,14] and another 10–15% are wild-type for both c-kit and PDGFRα [15]. Exon 17 mutation is also involved in seminomas and leukemias

Box 1. Drug summary box.

Drug name	Masitinib Mesylate
Phase	III
Indication	Systemic mastocytosis
Pharmacology description	Masitinib's main pharmacological target is the c-kit receptor expressed on mast cells, whose interaction provokes their depletion/ablation. The molecular interactions between masitinib and c-kit receptor occur in the ATP-binding site in its inactive conformation. It acts as a mixed inhibitor in some mutated c-kit forms, in particular on exon 11 mutations, i.e. V560G, while it doesn't target the most frequent exon 17 D816V one. Masitinib is also active on PDGRFα, FIP1L1-PDGRFα protein and has a moderate action on cFms and LynB proteins, being inactive on important receptors like Bcr-Abl, Flt3, VEGFR1, VEGFR2, EGFR. Oral bioavailability of masitinib is not linear and its administration is safer for two equal doses twice daily than only higher one, to break down the risk of an increased systemic exposure. Adverse Events are generally mild to moderate and occur early after initiation of masitinib treatment. Among the most frequent AEs are diarrhea, urticaria, rash, asthenia, peripheral edema, pruritus and neutropenia, occurring during the first 6 months.
Route of administration	Oral
Chemical structure	 4-(4-METHYL-PIPERAZIN-1-YLMETHYL)-N-[4-METHYL-3-(4-PYRIDIN-3-YL-THIAZOL-2-YLAMINO)-PHENYL]-BENZAMIDE METHANESULFONATE
Pivotal trial(s)	[60,62]

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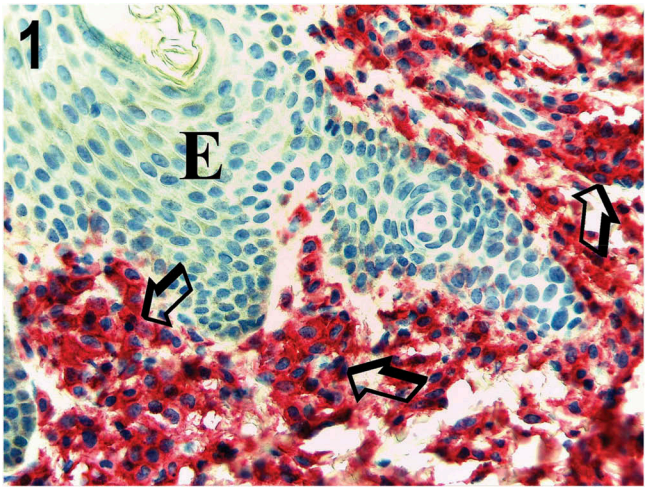

Figure 1. Cutaneous involvement from SM. Immunohistochemistry method, employing a primary anti-tryptase monoclonal antibody, arrows indicate clusters of neoplastic MC red immunostained with blue-dark nucleus. 'E' indicates epidermis. x400 magnification light microscopy.

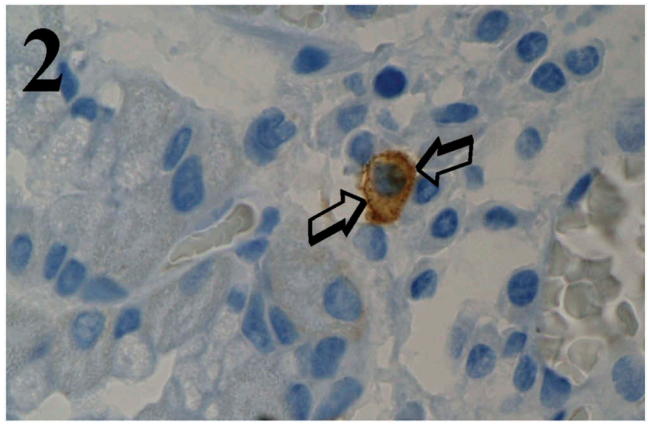

Figure 2. Tissue section processed with immunohistochemistry method employing a primary anti-c-kit receptor (CD-117) monoclonal antibody. Arrows indicate a single mast cell with a classical filiform brown membrane staining. The central MC blue nucleus is evident. x1000 magnification, in oil, light microscopy.

(sensible to dasatinib and nilotinib, but not to imatinib) and exon 9, 11, 13, 17, 18 mutations, also as coupled 9–17 and 9–13 mutations, recently demonstrated to be expressed in oral mucosal melanoma (OMM) [16], which rarely harbors Braf mutations [17–19], unlike cutaneous melanoma that boasts a larger arsenal of active targeted drugs.

The most common c-kit mutation in patients affected by SM presents a point substitution aspartic acid to valine at residue 816 (D816V) [3] on the activation loop domain in exon 17; this mutation causes a conformational change in the enzymatic pocket of c-kit receptor which activates, with a ligand-independent constitutive pathway, increasing cell proliferation and apoptosis reduction [20,21]. All kit-activating mutations involved in mastocytosis onset are represented in Table 1, together with the subtypes of disease associated to the mutation [22,23].

3. Masitinib in veterinary oncology and in human medicine

Dogs have been the perfect spontaneous model to start the studies on masitinib's activity in humans [24–26]. Dog mast cell tumors (MCTs) are very common as compared with human MCTs and from a biological point of view they share important similarities with SM [27]. In agree with the last assessment, a lot of dog MCTs are driven by c-kit aberrations [28] and because of the high incidence of MCTs in dog, they represent a unique spontaneous model to study MCs disorders [29]. In particular, MCTs are classified as follow: i) well differentiated with no metastatic properties; ii) with intermediate differentiation that are often able to spread metastases; iii) poor differentiated that are very malignant and aggressive tumors with the higher metastatic capacity [30]. On these basis, an open-label phase II study in 13 dogs with grade II/III mastocytomas was first conducted in 2006 [31], giving the cue for a randomized, double-blind, placebo-controlled phase III clinical trial in more than 200 dogs in the same clinical and pathological conditions [25]. In this enlarged study, even if the overall response rate was not different between placebo- and masitinib-treated dogs (15% vs. 16%, respectively), the time to progression between the two groups (75 vs. 118 days) opened a new scenario of clinical research to discover why and how masitinib acts on MCs disorders. Moreover, in the sub-study involving disease's causes, dogs with c-kit mutations did not show significantly greater response to masitinib compared to those placebo-treated (20% versus 10%), nevertheless kit-mutated dogs experienced a substantial longer time to progression [32]. After 2 years, an exploratory study by Dubreuil [33] investigated and found that masitinib exerted a sensitizing effect in various canine cell lines to chemotherapy, in particular to doxorubicin, vinblastine (in histiocytic sarcoma) and gemcitabine (in osteosarcoma and mammary carcinoma). Hence, a series of studies testing the effect and the safety of masitinib in animals have been conducted [34–36]

Though the uncertain differences, the very promising results obtained by masitinib in canine models brought to a great interest towards a possible clinical activity in man and towards how it effectively acts in MCs disorders.

At the moment, masitinib is commercially available only in veterinary medicine with the branded AB Science Masivet®. A second formulation in tablets, named Alsitek® for human administration has been used since 2016 as orphan drug in Amyotrophic Lateral Sclerosis (ALS). It is thought to act by reducing the activity of microglia, the main immune defense cells of the brain, and MC, both involved in the inflammation and damage to nerves in ALS. By reducing their activity, the drug was expected to reduce inflammation and damage to nerves, thereby slowing down the worsening of the patient's symptoms.

Nevertheless, in April 2018, the Committee for Medicinal Products for Human Use (CHMP) adopted a negative opinion, recommending the refusal of its marketing authorisation, being uneffective at slowing down the progression of the disease in spite of a positive effect on symptoms when the disease worsened at a normal rate (but not when it worsened rapidly). Being the rate of progression an arbitrary way of classifying patients and showing the study some deficiencies in form, the drug did not provide reliable evidence of its benefits.

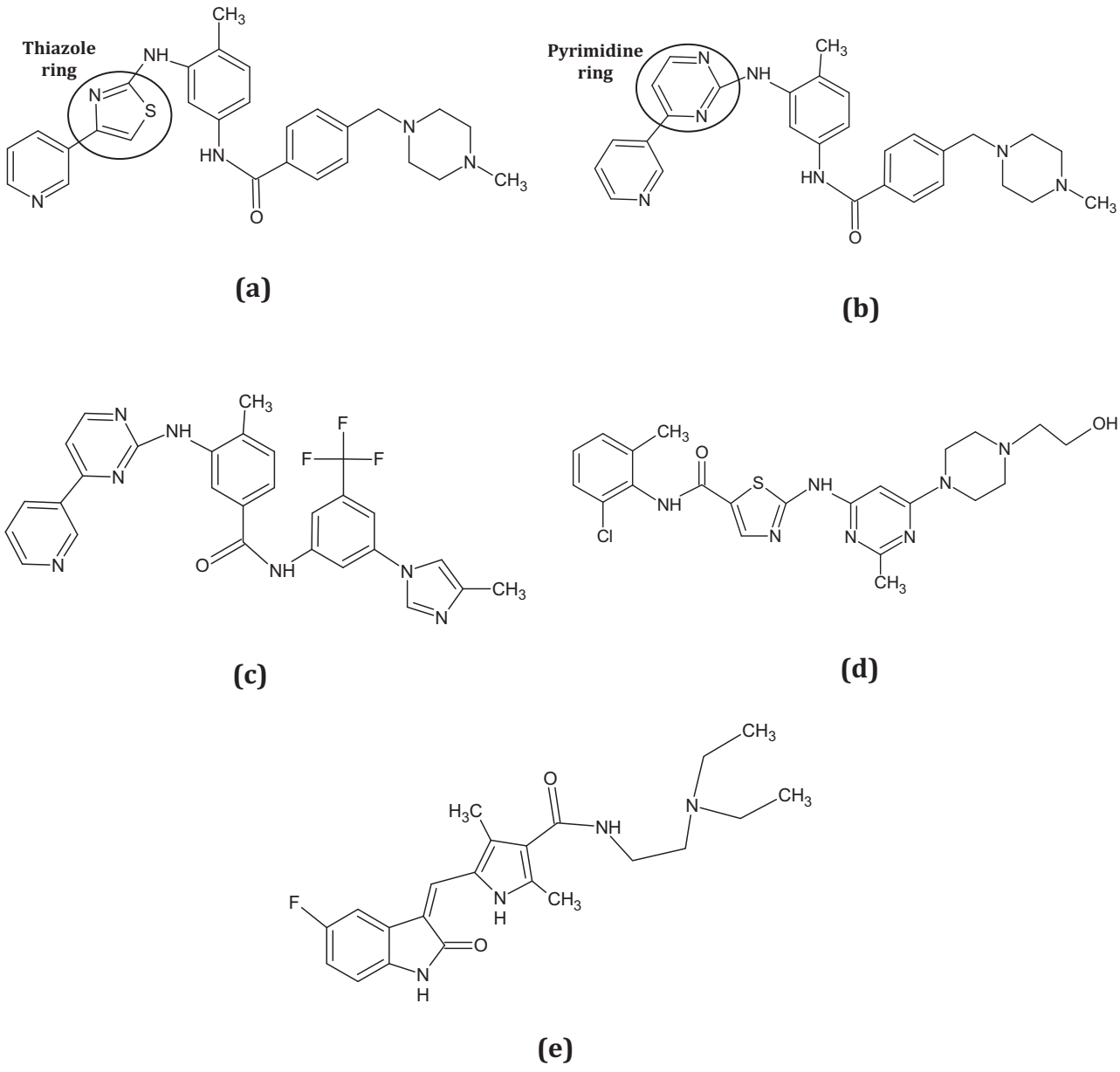

Figure 3. Chemical structure of some tyrosine kinase inhibitors (TKIs): a) Masitinib b) Imatinib c) Nilotinib d) Dasatinib e) Sunitinib.

Besides SM and ALS, the European Clinical Trials Register currently displays clinical trials testing masitinib in melanoma, GIST, in pancreatic, breast, prostate, ovarian, metastatic colorectal cancers, esophagogastric adenocarcinoma, peripheral T-cell lymphoma, multiple myeloma, hepatocellular carcinoma, but also in Alzheimer's and Parkinson's diseases, mood disorders with major depression, severe chronic obstructive pulmonary disease, Crohn's disease, Rheumatoid Arthritis and acute ischemic stroke, but no clinical results are still available.

4. Masitinib's pharmacodynamics compared to imatinib

The main Masitinib's pharmacological target is c-kit receptor expressed on MCs, towards which it is able to provoke depletion/ablation. MCs were discovered by Erlich more than 130 years ago [37] and one of their multiple functions is their ability to secrete a wide range of effector molecules (cytokines, prostaglandins and chemokines [38]), some of which are involved in tumor growth, angiogenesis and immune response [39]. A great part of mature MC is formed by secretory granules [40] containing complexes of proteases (carboxypeptidase A3, granzyme B, cathepsin G, MC proteases mMCP1→10) ionically bound to proteoglycans [41]. Among these proteases, tryptase is the most abundant constituent and is a specific tissue marker of MCs (Figure 4), it is degranulated from MCs and is a serological marker of SM; finally, tryptase is also a strong pro-angiogenic factor [42]. Other pro-angiogenic factors contained and released from MCs are heparanase [43], vascular endothelial growth factor (VEGF), angiopoietin 1, heparin, TNF, FGF-2, but also molecules with possible antitumor activity [44]. MC development, survival and

Table 1. List of c-kit mutations in chromosome 4 involved in SM.

Kit mutation	Exon	Region of mutation	Mastocytosis subtype
A533D	10	Transmembrane domain	Familial cutaneous mastocytosis
C443Y	9	Extracellular domain	Cutaneous pediatric mastocytosis
D419Y	9	Extracellular domain	Cutaneous pediatric mastocytosis
D572A	11	Juxtamembrane domain	Cutaneous pediatric mastocytosis
D816F	17	Kinase domain (activation loop)	Systemic mastocytosis
D816H	17	Kinase domain (activation loop)	Systemic mastocytosis
D816I	17	Kinase domain (activation loop)	Cutaneous pediatric mastocytosis
D816V	17	Kinase domain (activation loop)	- Systemic mastocytosis - Cutaneous pediatric mastocytosis - Familial cutaneous mastocytosis - Aggressive systemic mastocytosis
D816Y	17	Kinase domain (activation loop)	Systemic mastocytosis
D820G	17	Kinase domain (activation loop)	Aggressive systemic mastocytosis (ASM)
Del419	9	Extracellular domain	Familial cutaneous mastocytosis
Dup(501–502)	9	Extracellular domain	Mast cell leukemia
E839K	17	Kinase domain (activation loop)	Urticaria pigmentosa
F522C	10	Transmembrane domain	Well-differentiated systemic mastocytosis (WDSM)
I817V	17	Kinase domain (activation loop)	Well-differentiated systemic mastocytosis (WDSM)
InsFF419	9	Extracellular domain	Cutaneous pediatric mastocytosis
InsV815-I816	17	Kinase domain (activation loop)	Systemic mastocytosis
K509I	9	Extracellular domain	Familial systemic mastocytosis
N822I	17	Kinase domain (activation loop)	Familial cutaneous mastocytosis
N822K	17	Kinase domain (activation loop)	Systemic mastocytosis
R815K	17	Kinase domain (activation loop)	Pediatric urticaria pigmentosa
T417Y	9	Extracellular domain	Pediatric mastocytosis
V560G	11	Juxtamembrane domain	- Systemic mastocytosis - Familial cutaneous mastocytosis
V559I	11	Juxtamembrane domain	Aggressive systemic mastocytosis (ASM)
V654A	11	Juxtamembrane domain	Systemic mastocytosis
Y418Y	9	Extracellular domain	Cutaneous pediatric mastocytosis

activity are regulated by c-kit in such a strictly dependent way that the absolute absence of MCs have been demonstrated in c-kit deficient mice.

Masitinib has a slightly different mechanism of action with respect to its parent imatinib, towards which it has been compared [45]. Imatinib was the first TKI which entered pharmaceutical market for the treatment of kit-positive GISTs, Philadelphia-positive myeloid chronic and acute linfoblastic leukemias (Bcr-Abl positive), myelodysplastic/myeloproliferative diseases with PDGFR gene rearrangements, advanced or chronic hypereosinophilia leukemia with FIP1L1 and PDGFR mutations and dermatofibrosarcoma protuberans at oral doses of 400 or 600 mg once a day up to 800 mg (400 mg twice a day) [46]. Summing up, imatinib inhibits Bcr-Abl, PDGFR, c-kit, c-Fms, Syk. Adverse reactions associated to imatinib administration include neutropenia, thrombocytopenia, nausea, vomiting, diarrhea, abdominal pain, asthenia, fatigue, myalgia, muscle cramps, rashes, periorbital and lower limb edemas to solve with diuretic therapy, but the potential most important adverse reaction occurs in the cardiovascular system with hemorrhagia and cardiotoxicity, due to the inhibition of Abl and VEGF receptor (VEGFR).

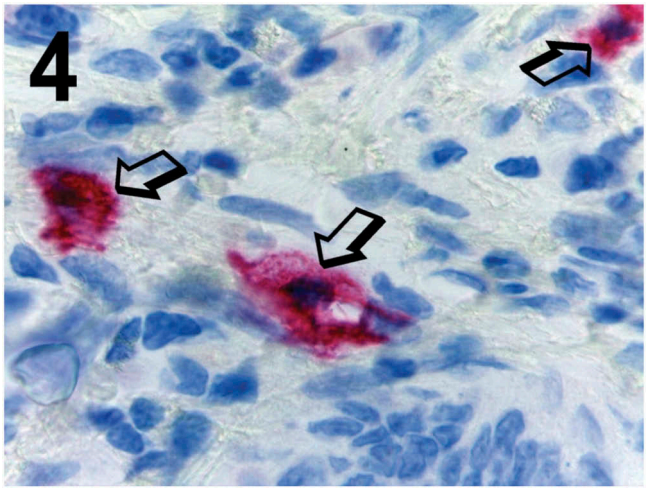

Figure 4. Tissue section processed with immunohistochemistry method employing a primary anti-tryptase monoclonal antibody. Arrows indicate single MC red immunostained with blue-dark nucleus. x1000 magnification, in oil, light microscopy.

The molecular interactions between masitinib and c-kit receptor occur in the ATP-binding site in its inactive conformation; the strength of these interactions, and consequently the duration of the link drug-receptor, are strictly dependent on its chemical structure and the possibility to form weak (hydrogen bonds) or stronger (covalent bonds or hydrophobic interactions) links in the active pocket. The substantial difference in structure between masitinib and imatinib lies on the presence of the aminothiazole group in the former, substituted by a pyrimidine ring in the latter, whereas the other portions of the two molecules (methylpiperazine, pyridine and amide groups) are almost stackable. The molecular modeling of masitinib binding to c-kit has been reported in previous reports [45]: in the X-ray structure of c-kit/masitinib and c-kit/imatinib complexes, it is evident that masitinib's thiazole ring is more hydrophobic than imatinib's pyrimidine ring and cannot mediate a hydrogen bond with a co-crystallized water molecule, as occurs with imatinib. Consequently, masitinib molecule focuses to interact more with c-kit than with intracellular fluid, as imatinib is supposed to do.

In 2009, the first important and well-built research study on *in vitro* and *in vivo* activity of masitinib has been conducted

[45]; as far as *in vitro* investigations are concerned, often in comparison with the parent imatinib, the drug was tested for its ability to inhibit:

a. recombinant human wild-type kit protein, in terms of IC50;
b. human and murine kit in intact Ba/F3 cells, in terms of IC50;
c. kit gain-of-function mutants, in terms of IC50;
d. human MC degranulation, in terms of inhibition percentage of β-hexosaminidase release;
e. human MC cytokine production, in terms of inhibition percentage of TNFα release;
f. bone marrow MC migration, in terms of migration percentage.

In experiment a), researchers have demonstrated that masitinib has two dose-dependent mechanisms of inhibition towards the intracellular domain (amino acids 567–976), normally activated by ATP (with a Km = 9.0 ± 2.0 μM), by using as a substrate of phosphorylation a poly(Glu, Tyr 4:1), which imitates the tyrosine kinase area. At concentration ≤500 nM, masitinib acts as a competitive inhibitor against ATP, while at higher concentrations >1 mM it has a mixed competitive/noncompetitive mechanism of phosphorylation inhibition. Imatinib, on the contrary, is a strictly competitive inhibitor versus ATP at any concentration. A competitive inhibitor links the active site of the protein in a reversible way and is structurally similar to the natural substrate (ATP in the specific case), while a non-competitive inhibitor links the protein in an area of its quaternary structure different from the active site. A mixed inhibitor, on the contrary, binds both the free protein and the substrate/protein complex. Lineweaver–Burk plots present differently on the basis of these mechanisms, with all lines of increasing tested drug concentrations intersecting on the Y-axis for a competitive process, or to the left of the Y-axis for the mixed competitive/noncompetitive one (in pure noncompetitive inhibition lines never intersect Y-axis). Moreover, masitinib showed a half inhibitory concentration IC50 = 200 ± 40 nM towards wild-type c-kit versus the corresponding imatinib's IC50 = 470 ± 120 nM, more than two-fold quantity.

This evident difference in IC50 reduces in experiment b), conducted in intact cells, in particular on IL3-dependent murine hematopoietic Ba/F3 cell line expressing wild-type c-kit, with a comparable IC50 of about 150 nM in the case of SCF-induced cell proliferation and a higher value >5 μM in the case of IL3-stimulated proliferation for both drugs.

Ba/F3 cells were also used in experiment c), testing masitinib's activity in the presence of kit gain-of-function mutants [23], in detail V559D mutant in exon 11 commonly associated to GIST onset, a Δ27 mouse kit mutant with the deletion of 547–555 codons in exon 11 considered to cause constitutive activation and ligand-independent cell proliferation [47], and D816V and murine D814V kit mutants in exon 17, known to be involved in adult mastocytosis, myeloproliferative disorders and acute myeloid leukemia. In V559D and Δ27 kit mutants, the very low IC50 values of masitinib's inhibition, 3.0 ± 0.1 nM and 5.0 ± 0.3 nM, respectively, together with the results on wild-type c-kit in experiment b), confirm the same inhibition profile of imatinib, whose IC50 for V559D was 11.0 nM. This deduction is also enforced by masitinib's weak inhibition on D816V and D814V kit mutants, also resistant to imatinib as all tested 17 exon mutations [48]; in particular, for D816V masitinib showed an IC50 = 5.0 ± 2.0 μM versus imatinib's IC50 = 10.6 μM [49], while for Δ27 and D814V kit mutants imatinib's IC50 values have not been found in literature.

Interestingly, analogous experiments on various murine mastocytoma cell lines were performed, testing HMC-1α155 and FMA3 cells having kit mutations in exon 11, too [50]. These cells show kit constitutive activation, similar to a point mutation in well-known tumors of mast cell lines HMC-1, P-815 and RBL-2H3 [50]. Masitinib's IC50 values in HMC-1α155 and FMA3 cells were 10 ± 1 nM and 30 ± 1.5 nM, respectively.

The most important discovery in all performed experiment, with the exception of D816V and D814V mutants, was masitinib's ability to reduce kit autophosphorylation, evidenced by an immunoprecipitation-western blotting experiment; to this regard, imatinib is known to have a weak activity on c-kit autophosphorylation (strong for Bcl-Abl) only in a few kit mutants [51].

Masitinib's activity on MC functions was also tested in terms of inhibition of degranulation, cytokine production and migration from bone marrow (experiments d) e) f). In particular, bone marrow mast cell (BMMC) migration is stimulated by SCF; in presence of SCF and 1.0 μM masitinib, BMMCs migration underwent an evident reduction of 79.6% relative to unstimulated cells, while the corresponding value for imatinib was 58.1%.

Masitinib was also tested towards wild-type c-kit expressed both in SCF-stimulated and IL3-stimulated MC: in the first case, IC50 was 200 ± 50 nM, in the second case it was >10 μM, suggesting that masitinib could influence SCF control on various biological functions, such as erythropoiesis and lymphopoiesis, but also gametogenesis and melanogenesis.

Studies on masitinib versus imatinib in terms of IC50 for their inhibitory action on other tyrosine kinase proteins have also been conducted [45]. Using Ba/F3 cells expressing wild-type PDGFRα and Bcr-Abl, masitinib showed an IC50 = 300 ± 5 nM and 2800 ± 800 nM, respectively, versus an IC50 = 0.1 μM and 0.6 μM of imatinib: these data showed that masitinib is active on PDGRFα but very little on Bcr-Abl, which is imatinib's preferred target. In the case of recombinant PDGRFα, PDGRFβ and Bcr-Abl expressed once more in Ba/F3 cell, masitinib showed more favorable IC50 values, even if in the case of PDGRFα the difference between the two drugs is little (540 ± 60 nM for masitinib versus 400 nM for imatinib).

Masitinib is also inactive towards other tyrosine kinase receptors (Flt3, VEGFR1, VEGFR2, epidermal growth factor, fibroblast growth factor 1 and 2, insulin-like growth factor-1 receptor, cMET, TRKB, cRET) and non-receptor (focal adhesion kinase, Src, HCK, Jak 1,2,3, TVK 2, Btk, Bmx, Syk and recombinant serine/threonine kinases Ca, Akt1, Pim-1) [45]; on the contrary, imatinib has a certain effect of inhibition towards Src, VEGFR, Flt3. Like imatinib, masitinib also exerts a strong inhibition towards FIP1L1-PDGRFα protein, expressed in hypereosinophilic tumor cells associated to chronic eosinophil leukemia (IC50 = 0.2 ± 0.1 nM) and a moderate action on cFms and LynB proteins.

5. Masitinib's pharmacokinetics

As to our knowledge, still only a few studies on masitinib's pharmacokinetics in man have been conducted [24,52,53], opposite to corresponding studies on animals, cats in particular [35,36,54,55]. In cats, masitinib reaches its peak concentration 1–2 h after administration [35] with oral bioavailability of about 83%, with a high volume of distribution, a rapid elimination, evidenced by the trace amounts detectable 24-h post-dose and its 93% of plasma proteins binding [56]. The excretion is predominately intestinal (90%) and the intact drug accounts for nearly 50% of the excreted. Besides, starting from the process of metabolism by N-dealkylation [57], 8 and 12 metabolites are identified and detected in feces and in urine, respectively [57]. In feces, the principal active metabolites are a N-desmethyl derivative, formed by gut microflora and a sulfate conjugate of mono-hydroxy-masitinib; in urine, a carboxylic acid metabolite derived from the hydrolysis of the central amid linkage has been recognized [56,58]. Masitinib concentration increases with increasing dose-levels in a nearly dose-proportional manner, but there is no evidence of saturation or a time-dependent effect of absorption.

Interestingly, in rats the time to maximal concentration (Tmax) value is in the range 2–7 h after dose repeated administrations for 4, 13 and 26 weeks, with a gender-dependent effect leading to a two-fold higher exposure of females (also observed in dogs after single-dose administration); moreover, terminal half-life is slightly shorter for masitinib (range 2.72–3.16 h) than for its major metabolite AB3280 (range 3.55–4.23 h), without any variation related to gender or over time after repeated doses for 14 days [56,58]. Studies about its tissue distribution, in progressing time after oral single daily administration, reveal an enrichment of masitinib in adrenals, kidneys, spleen and intestine (0.006–6.43% of the dose after 24 h) [58,59]. Finally, both in rats and in dogs, masitinib mean half-life (t½) after single administration is about 5 h.

In humans, after repeated-dose administration, t½ values are about 13 h, increasing up to 33 h with escalating doses. Moreover, as far as potential drug–drug interactions are concerned, besides its high protein binding, masitinib (but not AB3280) inhibits reversibly CYP3A4, 2C9 and 2D6 with IC50 values of 14 μM, 20 μM and >30 μM, respectively, but any action of induction on different cytochrome P450 isoforms have been observed.

In another pharmacokinetic study by Soria [52], parameters were calculated from plasma and urine concentration of masitinib's free salt AB1003 and AB3280, in patients affected by solid tumors, a larger cohort of patients than those affected by only MCs diseases [52]. After 14 days of treatment at escalating doses of masitinib from 3 to 12 mg/kg/day, the free salt peak concentration Cmax reaches a fourfold increase with respect to the active metabolite and a median Tmax 3.2 h (range 1.7–4.7 h), an average half-life of 24 h (range 18–36 h) and high apparent clearance and volume of distribution. Weight-adjusted doses and coefficients of correlation for AUC and Cmax were necessary for better correlations, nevertheless it is clear that oral bioavailability of masitinib is not linear and its administration is safer for two equal doses twice daily than only higher one, to break down the risk of an increased systemic exposure.

6. Clinical trials

Actuality, there is only one large-randomized phase III study by Lortholary in Lancet 2017 about masitinib in patients with symptomatic indolent systemic mastocytosis leading to a conclusion that masitinib is effective in reducing MC activation syndrome. However, there are very little data on masitinib's effectiveness as disease-modifying agent, as there are no pathologic response data provided. This is clearly the 'Achilles heel' of this agent and it needs to be placed in a proper context with other agents currently being developed for both systemic and indolent systemic mastocytosis.

Paul et al. in a phase 2 multicenter study [60] evaluated masitinib in 25 patients with systemic and cutaneous mastocytosis with related handicap (i.e. disabilities associated with flushes, depression, pruritus and quality-of-life) and at least two organs confirmed with MCs infiltration, one or both of which had to show no detectable mutations in c-kit. This condition was intended to help ensure that any beneficial treatment-response was measurable in this proof-of-concept study, because masitinib and other TKIs typically exhibit a poor activity against the D816V mutation.

Response was based upon change of clinical symptoms associated with patient handicap at week 12 relative to baseline, regardless of disease subtype. As compared with baseline, improvement was observed in all primary endpoints at week 12, including a reduction of flushes, Hamilton rating for depression and pruritus by 64% (p = 0.0005), 43% (p = 0.0049) and 36% (p = 0.0077), respectively. At week 12, a clinical response (i.e. improvement of ≥50% in baseline Hamilton rating, flushes or pruritus) was observed in 56% of patients without a detectable D816V mutation from at least one affected organ. Individually, these handicaps showed clinical response rates of 60%, 50% and 25%, respectively. Therapeutic effect was observed since week 4 in all clinical symptoms associated with mastocytosis with handicap, indicating a rapid onset of action.

Georgin-Lavialle et al. described a case of 66-year-old woman with MCL, characterized by 42% of circulating MCs, treated with masitinib at dose of 6.5 mg⁄ kg ⁄day [61]. The cutaneous biopsy was normal, serum tryptase was very high and the bone marrow (BM) smear showed 70% massive infiltration with immature MCs bearing c-kit, evidenced by flow cytometry analysis. BM biopsy showed an infiltration by 90% of dysmorphic spindled MCs, as highlighted by c-kit immunohistochemical staining. The absence of D816Vc-Kit mutation was confirmed by sequencing after a RT-PCR, associated with a previously unidentified c-kit mutation dup(501–502) in exon 9 and in adult mastocytosis. After 3 months of treatment, symptoms of flush and diarrhea disappeared and circulating MCs, serum histamine, tryptase level and MCs infiltration on the BM decreased. Moreover, no adverse events were reported.

Lortholary et al. in a placebo-controlled phase 3 study [62] assessed safety and efficacy of masitinib in 135 severely symptomatic patients who were unresponsive to standard symptomatic treatments. By 24 weeks, masitinib was associated with a cumulative response, in at least one of four severe baseline symptoms of MC mediator release (pruritus, flushes, depression,

Table 2. Clinical trials that evaluated masitinib in patients with SM.

Reference	Phase of study	N° patients	Previous treatments	c-kit status	Dose	Other drug associated	ORR
Lortholary et al. Lancet 2017	3	135	Yes (not with TKI)	wyld-type/ mutated	6 mg/kg/day	None	n.e.
Georgin-Lavialle et al. Eur J Haematol. 2012	case report	1	None	mutated	6,5 mg/kg/day	None	100%
Paul et al. Am J Hematol 2010	2a	25	Yes	wyld-type/ mutated	3–6 mg/kg/ day	None	56%

TKI: Tyrosine Kinase Inhibitor; n.e.: not evaluated.

or asthenia), of 19% compared with 7% for placebo (p = 0.0076). At week 24, the mean change of tryptase level from baseline in the modified ITT population was a decrease of 18% in the masitinib arm versus an increase of 2% in the placebo arm (p < 0.0001). The response to masitinib of urticaria pigmentosa lesions differed when compared with placebo (p = 0.0210) as evidenced by a decrease in average body surface area of 12% for masitinib versus an increase of 16% for placebo. The response to masitinib included one c-kit D816V-positive patient who had a complete response at week 24 (from baseline body surface area of 18%). This observation was also supported by the disappearance of Darier's sign in 19% of patients treated with masitinib versus 3% treated with placebo (p = 0.0187).

Clinical trials evaluating the treatment with masitinib in patients with SM are summarized in Table 2.

7. Masitinib's toxicity

Adverse Events (AEs) were generally mild to moderate and occurred early after initiation of masitinib treatment.

In Paul et al. trial, the most common (10%) AEs were nausea/vomiting (52%), edema (44%), muscle spasms (28%), and rash (28%), prevalently from mild to moderate severity with a significant decline in frequency observed after 12 weeks of treatment. One patient experienced a serious AE of reversible agranulocytosis at a dose of 3 mg/kg/day. Thus, masitinib is a promising treatment for indolent forms of mastocytosis with handicap and indicates acceptable tolerability for long-term treatment regimens. At the cut-off date, 84% of patients reported at least one suspected masitinib-related AE during the initial 12-week phase, but all severe AEs recovered spontaneously or with symptomatic treatments. After experiencing AEs from mild to moderate intensity, 12% of patients discontinued treatment due to AEs, whereas no deaths occurred during this study. A decrease in the occurrence and severity of AEs was evident for patients entering the extension phase; in detail, no incidence of skin rash was reported after week 12 and a reduction in the incidence of nausea/vomiting (52% vs. 18%), edema (44% vs. 6%) and nausea (44% vs. 12%) were observed between the initial and extension phases, respectively.

According to Lortholary et al. trial, the most frequently occurred severe AEs in the treatment with masitinib were diarrhea (11%), rash (6%), asthenia (6%), peripheral edema (3%), pruritus (4%), and neutropenia (4%). The most frequent serious AEs (SAEs) were diarrhea (4%) and urticaria (3%). No deaths were reported in the masitinib group.

Overall, more AEs occurred during the first 6 months.

Table 3. All grade toxicity from clinical trials that evaluated masitinib in patients with SM.

Toxicity (%)	Lortholary et al. Lancet 2017	Paul et al. Am J Hematol 2010
Nausea/Vomiting	n.r.	52
Nausea	n.r.	44
Edema	n.r.	44
Muscle spasms	n.r.	28
Rash	6	28
Asthenia	6	24
Vomiting	n.r.	20
Headache	n.r.	20
Abdominal pain	n.r.	16
Diarrhea	11	12
Eructation	n.r.	12
Dyspnea	n.r.	12
Blepharitis	n.r.	5,9
Aphthous stomatitis	n.r.	5.9
Gingivitis	n.r.	5.9
Cytolytic hepatitis	n.r.	5.9
γ-glutamyltransferase increased	n.r.	5.9
Arthralgia	n.r.	5.9
Dermatitis psoriasiform	n.r.	5.9
Eczema	3	5.9
Pruritis	4	n.r.
Edema	3	5.9
Neutropenia	4	n.r.

n.r.:, not reported.

All grade toxicities from clinical trials evaluating masitinib in patients with SM are summarized in Table 3.

8. Conclusions

Mast cell disorders, systemic mastocytosis in particular, can be pursued acting with the inhibition of MC degranulation, of their cytokine production and their migration from bone marrow to the inflammatory areas.

The *in vitro* and *in vivo* studies reported in this review look at masitinib in a good perspective in the next future for diseases caused by abnormal presence of MCs, in particular SM, giving surprising and good results in the inhibition of MCs functions. As evidenced while describing the experiments, this activity towards MC occurs at very high concentration of masitinib, but considering its limited toxicity with respect to other TKIs, this aspect could be taken into account in a personalized approach to doses and, above all in the forms of SM without important handicaps at the starting of the disease's discovery. Unfortunately, clinical trials testing masitinib in the treatment of SM are still few and to this regard, it would be useful a fast screening in the identification of the disease, often recognized when symptomatic, being

mastocytosis a rare disease with very few patients. Hence, the difficulty to have patients eligible to treatment.

Another aspect to consider is that researchers have not elaborated neither consensus response criteria nor unanimous approach towards the different subtypes of SM. For instance, against Valent's opinion on the higher similarity of MCL to ASM than to leukemia [63,64], the experts of Mayo Clinic also justify their choice to apply the response criteria for SM-AHNMD to the treatment response criteria for AHNMD (not for SM) and that MCL treatment response criteria should follow the response criteria for acute leukemia [65].

In 2016 and 2018 consensus guidelines for cutaneous manifestations in mastocytosis have been published [66,67], on the contrary the non-standardized response criteria (also in comparison with other TKIs), the few clinical trials and, above all, the small patient populations do not permit to have a totally complete panorama on these diseases in terms of drug efficacy and benefits with respect to other available therapeutic agents. Therefore, an important topic for future research would be consensus response criteria for patients in treatment with TKIs for advanced SM in its different forms.

9. Expert opinion

Masitinib mesylate is a relatively new TKI, as mesylate salt, whose mechanism of action, involves the degranulation and activity of MS. It is structurally the direct prosecutor of imatinib mesylate, acting both drugs by inhibiting c-kit receptor, involved in important biological functions and submolecular pathways of all cells' life. The substitution of the pyrimidine with a thiazole ring increases the hydrophobic properties of masitinib which evidently interacts with c-kit through a more long-lasting link with respect to imatinib. For this reason, in according to the reported clinical data, a multiple daily administration rather than a unique daily dose, reduces the risk of prolonged exposure and toxic accumulation. Masitinib is a mixed inhibitor of c-kit at a certain concentration and this mechanism of inhibition could be the key element for the subcellular differences with the strictly competitive inhibitor imatinib. As a mixed inhibitor, masitinib is able to bind both free c-kit and c-kit/ATP substrate complex in a reversible way; this would explain why masitinib succeeds in blocking autophosphorylation more than imatinib, known to have a limited activity to this regard. In spite of this difference, masitinib's IC50 value towards wild-type and mutant kit is similar to imatinib and this justifies the low interest to abandon the already authorized drug for the pathologies strictly kit-driven in which MCs are only partially involved, cancer in particular. Besides all, imatinib is in commerce also as generic formulation, playing an important role for rationalizing the costs of public health associated to therapy.

On the contrary, the potential role of masitinib in MCs disorders and SM, for which therapeutic options are only a few, could be of great interest, considering that exon 11 mutations are more sensitive to masitinib (i.e. for V560G, IC50 is 50 nM) with respect to wild-type receptor (150 nM) [68,69]. Unfortunately, exon 17 mutant c-kit, i.e. D816V, responsible for a great part of SM forms (about 85–90%), is still a tough contender, even if recently two new investigational TKIs, avapritinib (BLU-285) and ripretinib (DCC-2618) have shown promising clinical results, with significant pathologic responses in patients with SM [70,71], with IC50 of 0,5 nM and 14 nM on D816V c-kit mutation, respectively.

Chemically speaking, avapritinib has got an internal piperazine ring, closed and protected by two steric burdens, one of which is a derived complexed Fluorobenzene and the other a double heterocyclic-azotated ring, while in ripretinib the piperazine ring is lost, having in the central structure a polihalogenated benzene. From this point of view, the comparison with masitinib and imatinib is not immediate and, in fact, they do not share the same target mutant c-kit. Avapritinib is a very potent kit mutant D816V inhibitor, but it is also active towards N822K mutation, both located on exon 17; on the contrary, ripretinib has affinity both for wild type (IC50 = 4 nM) and for exon 17 mutated D816V c-kit, but also for exon 11 V654A mutation (IC50 = 8 nM) [72], a mutation which is principally responsible for pharmacological resistance to imatinib and TKIs [73]; finally, midostaurine is active against D816V c-kit mutant, too. In contrast, masitinib could have an important role in the case of exon 11 c-kit mutated forms, in particular in V559D (IC50 = 3 nM) and V560G (IC50 = 50 nM) mutations. For a better comprehension of these different subcellular events, a recent study in 2013 [74] has identified, through Western Blot analyses, the different signaling molecular pathways for some c-kit mutations; in particular, V560G prefers the Janus kinase 3/signal transducer (JAK3/STAT) and D816V prefers the mechanistic target of rapamycin complex 1/4E binding protein 1 (mTORC1/4E-BP1).

In the light of these considerations and, above all, after still lacking assessed phase III clinical trial results, masitinib could become in some years a possible therapeutic chance in that group of patients with SM expressing exon 11 c-kit mutations, i.e. V560G, independently from the most frequent exon 17 D816V one. Moreover, considering its inactivity towards other kinase target, it could be used in SM patients with comorbidities and cardiovascular pain. In these patients, it could be used as first or second-line therapy and prescribed and dispensed to patients in specialized hospital centers for daily oral administration at home.

Masitinib is also characterized by a ten-fold higher selectivity for c-kit versus Abl and is inactive against Flt3, VEGFR, Epidermal Growth Factor Receptor, Src, justifying the absence of cardiotoxicity (left ventricular dysfunction or congestive heart failure) with respect to other TKIs [68,75].

In Paul's study, the rates of response to masitinib were similar in patients with c-kit mutation in one infiltrated organ and patients with no c-kit mutations in any infiltrated organs, suggesting that masitinib may have clinical activity in patients harboring c-kit mutations [60]. Surprisingly, the confirmed presence of the D816V mutation does not adversely affect masitinib treatment of mastocytosis with handicap, on the contrary, it proved effective also with this clinical condition. A possible explanation is that masitinib's inhibitory action on Lyn/Fyn also plays a significant role in controlling MC degranulation and hence handicap, independent of the c-kit signaling pathway and survival of MCs. Consistent with this report,

data from Georgin-Lavialle demonstrated that a patient with SM who developed the kit mutation dup(501–503) prior to therapy, had clinical improvement with masitinib, with disappearance of circulating MCs and decreased serum histamine and tryptase levels. The c-kit mutation dup(501–503) represents a new mechanism of c-kit autoactivation, and this discovery underlines the importance of sequencing the entire c-kit gene when searching for atypical mutations in adults when D816V c-kit is not found [61].

With special regard to its mechanism of action, consistent with clinical observations that type and severity of symptoms are kit D816V-independent, this common mutation might not activate MCs to release pro-inflammatory mediators. Hence, the inactivity of masitinib against this target is not necessarily a limitation [76–79]. The treatment effect is hypothesized to be a result of masitinib targeting wild-type MCs, leading to a reduction in MC burden, an effect seen in long-term treatment of chronic myeloid leukemia with the wild-type kit-inhibitor imatinib [80,81] or by reducing activation of Kit D816V MCs. The latter-proposed mechanism is mediated through dual inhibition of Lyn and Fyn, which contribute to modulation of MC degranulation in a Kit D816V-independent manner [45].

The evident decrease in mean tryptase levels in 85% of masitinib-treated patients in Lortholary's study is consistent with both supposed effects. However, unknown factors could also contribute to these effects, as evidenced by the non-universal patient susceptibility to masitinib, with identification of predictive markers for patient treatment selection remaining a goal for future research.

As far as toxicity is concerned, according to Paul's study, although occurrence of Aes was relatively high (84%) over the first 12 weeks, the majority were from mild to moderate severity and, in general, occurred early during the course of treatment, which is consistent with the known safety profile of TKIs [82]. This trend, albeit deriving from a relatively small population size, is evident when comparing safety data from the initial to the extension phases. Thus, though masitinib is not completely free from side-effects, the majority of these are manageable and can be overcome with appropriate symptomatic treatments and with good tolerance experienced after week 12 and during any long-term treatment regimen. Moreover, one patient-experienced agranulocytosis, which resolved upon drug withdrawal with positive rechallenge. The initial dose randomization undertaken in Paul's study was conducted with the aim to determine optimal dosing of masitinib in indolent mastocytosis with handicap. Based upon analyses of dose at time of first response and frequency of Aes according to dose, an initial dose of 6 mg/kg/day administered in two daily intakes confirmed the acceptable balance between therapeutic benefit and risk.

In Lortholary's phase 3 study, long-term safety over the extension period was assessed according to incidence per patient months of exposure; this parameter is more appropriate than frequency of Aes, as some patients had been exposed to masitinib for over 2 years. This analysis revealed a comparable incidence of severe Aes between masitinib and placebo.

In both studies, toxicities were predominantly gastrointestinal or cutaneous, easily managed by dose reduction. The phase 3 study confirmed data by Paul's study about safety profile of masitinib: a substantial improvement in tolerance of masitinib occurs after the initial 12-week treatment period. Aes could be mitigated via implementation of a dose-escalation scheme, i.e. initial dose of 3 or 4.5 mg/kg per day with increments of 1.5 mg/kg per day every 4 weeks depending on absence of toxicity until reaching the target dose of 6 mg/kg/day.

In the light of these considerations, another aspect the physician has to evaluate during pharmacologic treatment with TKIs is the response criteria to therapy for patients with different forms of SM. In 2003, Valent et al. [83] developed standard response criteria for measuring response in patients treated for ASM, defined C-findings, which are still widely applied. These response measurements include standard infiltration of MC in organs, tryptase levels, organomegaly. A major response has 1 C-finding completely resolved, with no progression in other C-findings, a disappearance or decrease of MC infiltration in organs and organomegaly and a decrease of serum tryptase levels to 50% (good partial response) or decreases by 50% (minor response) and no increase in any other C-findings. No response has no change or an increase in C-findings [83]. Nevertheless, these criteria do not satisfy required specifications, such as the minimum duration of an improvement needed to qualify as a response or the overall duration of response to treatment. For this reason in 2010, the Mayo Clinic published new recommendations to make response criteria more intuitive, standard, objective, and reproducible for practicing physicians [65], but the topic is still an open question.

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