

Targeting Filarial Abl-like Kinases: Orally Available, Food and Drug Administration–Approved Tyrosine Kinase Inhibitors Are Microfilaricidal and Macrofilaricidal

Elise M. O'Connell,¹ Sasisekhar Bennuru,¹ Cathy Steel,¹ Michael A. Dolan,² and Thomas B. Nutman¹

¹Laboratory of Parasitic Diseases, and ²Bioinformatics and Computational Biosciences Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

(See the editorial commentary by Geary and Mackenzie on pages 677–80.)

Background. Elimination of onchocerciasis and lymphatic filariasis is targeted for 2020. Given the coincident *Loa loa* infections in Central Africa and the potential for drug resistance development, the need for new microfilaricides and macrofilaricides has never been greater. With the genomes of *L. loa*, *Onchocerca volvulus*, *Wuchereria bancrofti*, and *Brugia malayi* available, new drug targets have been identified.

Methods. The effects of the tyrosine kinase inhibitors imatinib, nilotinib, and dasatinib on *B. malayi* adult males, adult females, L3 larvae, and microfilariae were assessed using a wide dose range (0–100 μ M) in vitro.

Results. For microfilariae, median inhibitory concentrations (IC₅₀ values) on day 6 were 6.06 μ M for imatinib, 3.72 μ M for dasatinib, and 81.35 μ M for nilotinib; for L3 larvae, 11.27 μ M, 13.64 μ M, and 70.98 μ M, respectively; for adult males, 41.6 μ M, 3.87 μ M, and 68.22 μ M, respectively; and for adult females, 42.89 μ M, 9.8 μ M, and >100 μ M, respectively. Three-dimensional modeling suggests how these tyrosine kinase inhibitors bind and inhibit filarial protein activity.

Conclusions. Given the safety of imatinib in humans, plans are underway for pilot clinical trials to assess its efficacy in patients with filarial infections.

Keywords. filaria; *Loa loa*; *Brugia malayi*; *Wuchereria bancrofti*; mass drug administration; microfilaricide; macrofilaricide; lymphatic filariasis; onchocerciasis.

In 2011, the World Health Organization released a roadmap for the global elimination of the most neglected tropical diseases [1]. Lymphatic filariasis, caused by the filarial parasites *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*, and onchocerciasis, caused by the filarial species *Onchocerca volvulus*, are slated for

elimination by 2020. Approximately 157 million individuals are infected with organisms that cause either lymphatic filariasis or onchocerciasis, which, together, are responsible for approximately 6.3 million disability-adjusted life-years [1].

The need for new drugs (particularly macrofilaricides) is crucial because the specter of ivermectin and/or albendazole resistance has been raised, especially for onchocerciasis, for which a single drug (ivermectin) is available [2–4]. Of enormous concern for the mass drug administration programs for lymphatic filariasis and onchocerciasis in Central and West Africa is coincident *Loa loa* infection, in which *Loa*-infected individuals with very high levels of microfilariae have had severe neurological adverse events (including encephalopathy and death) following ivermectin administration. This has led to a cessation of mass drug administration in areas where *L. loa* is present. Thus, new modalities of

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Correspondence: Elise M. O'Connell, MD, National Institute of Allergy and Infectious Diseases, Laboratory of Parasitic Diseases, 4 Center Dr, Bldg 4, Rm B1–05, Bethesda, MD 20892 (oconnellem@mail.nih.gov).

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treatment, not only for lymphatic filariasis and onchocerciasis, but also for loiasis, are critically needed.

Tyrosine kinase inhibitors (TKIs) have been suggested as potential treatment for a broad range of protozoan infections (due to *Trypanosoma* species, *Leishmania* species [5], and *Plasmodium falciparum* [6]), trematode infections (schistosomiasis), [7], and cestode infections due to *Echinococcus multilocularis* [8]. c-Abl is a ubiquitously expressed cytoplasmic protein tyrosine kinase that, following phosphorylation, plays an important role in cell proliferation and survival. Imatinib, which prevents the phosphorylation of c-Abl, has been demonstrated to profoundly alter both the morphology and the physiology of adult *Schistosoma mansoni* worms in vitro by influencing reproduction and nutrient acquisition [9, 10].

Imatinib has been used extensively and safely in humans for a variety of conditions but most notably for the treatment of chronic myelogenous leukemia [11], for which it works through inhibition of the oncoprotein Bcr-Abl. Since the Food and Drug Administration (FDA) approved imatinib in 2001, several next-generation TKIs, including dasatinib and nilotinib, have received FDA approval for treating imatinib-resistant chronic myelogenous leukemia. Following the genomic sequencing of the *L. loa* genome [12], it became clear that *L. loa*, as well as several other filariae, express an Abl-like protein, which may be susceptible to the tyrosine kinase inhibition that is seen with Bcr-Abl.

Thus, the purpose of this study was to assess the ability of imatinib, nilotinib, and dasatinib to kill each of the mammalian stages (adults, L3 larvae, and microfilariae) of the filarial parasite *B. malayi*, the only human filarial parasite for which each of these lifecycle stages is available for in vitro testing. We also assessed the universality of this activity against the other pathogenic filariae (*L. loa*, *W. bancrofti*, and *O. volvulus*) and used 3-dimensional modeling to understand the interactions between the TKIs and the filarial Abl-like proteins.

MATERIALS AND METHODS

Parasites and In Vitro Culture

Adult *B. malayi* females, adult males, L3 larvae, and microfilariae were obtained through a contract with the University of Georgia [11]. Prior to culture, worms were cleaned and processed as described previously [12]. Adults and microfilariae were cultured separately in serum-free Roswell Park Memorial Institute 1640 medium (Invitrogen, Carlsbad, Massachusetts) supplemented with 10 g/L glucose, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin. Approximately 1000 microfilariae per well were cultured in 200-µL aliquots in 96-well plates (Costar; Corning, New York). Adult male worms were cultured in 2 mL of medium at 2 worms/well or in 3 mL of medium at 3 worms/well, and adult females were cultured individually in 3 mL of medium in a 12-well plate (Costar; Corning,

New York). L3 larvae were cultured in a 96-well plate (Costar; Corning, New York) at a concentration of 10–15 L3 larvae/well in 200 µL in supplemented minimal essential medium- α , as previously described [12]. L3 larvae and adults were cultured for 24 hours to ensure viability prior to adding drug. Drug was added on day 0 at concentrations varying from 0 µM to 100 µM per well. All *B. malayi* stages were incubated at 37°C in 5% CO₂. Worms were observed daily by microscopy, with death defined as complete absence of movement upon visual inspection. Medium was not changed throughout the 6 days. Following initial experiments (n = 3–5) for each drug for each *B. malayi* life stage, repeat testing was performed to gather additional data at doses surrounding the filaricidal dose of each drug for each life stage. Each set of killing assays was replicated a minimum of 2 times at each tested concentration (range, 2–12 times), and the geometric mean percentage survival across each biologic replicate condition was generated.

Drugs

Imatinib, nilotinib, and dasatinib were obtained from LC Laboratories (Woburn, Massachusetts). Imatinib is water soluble and was dissolved to a final concentration of 1 mM. Nilotinib was dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 50 mg/mL and then diluted with distilled water to a final concentration of 1 mM prior to adding to the parasites. Dasatinib was dissolved and diluted in DMSO to a final concentration of 1 mM prior to adding to *B. malayi*. Since DMSO could be toxic to the worms at high concentrations, each experimental condition with dasatinib had a control with DMSO alone at corresponding concentrations.

Abl and Abl-like Kinase Structures

Filarial homologues to the human c-Abl kinase (accession no. NP005148.2) were obtained using the protein blast database from the National Center for Biotechnology Information (NCBI) website (available at: <http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The sequence for *O. volvulus* Abl-like protein was obtained using the *O. volvulus* blast server from the Wellcome Trust Sanger Institute website (available at: http://www.sanger.ac.uk/cgi-bin/blast/submitblast/o_volvulus). The protein sequences for the Abl-like kinases of *W. bancrofti* (accession no. EJW86653), *B. malayi* (accession no. XP001897557), *L. loa* (accession no. XP003141453), and *O. volvulus* (accession no. OVOC3514) were downloaded into SeqBuilder (version 9.1; DNASTAR, Madison, Wisconsin) and aligned in MegAlign (version 11.0.0; DNASTAR), using the ClustalW method. Phylogenetic analysis was performed with bootstrapping (1000 bootstrap trials were performed). Putative conserved domains for Abl-kinase were obtained from the NCBI protein blast database.

Residues 216–496, corresponding to the kinase domain of the *L. loa* Abl-like sequence (accession no. EFO22616), were submitted to the homology modeling server I-TASSER 3.0

(available at: <http://zhanglab.ccmb.med.umich.edu/I-TASSER>) [13, 14] for protein structure prediction. The top model in terms of C-score was energy minimized using the SYBYL 7.0 program (Tripos International) and the AMBER99 force field with accompanying atom types and charges.

Inhibitor Docking

The docking of nilotinib, imatinib, and dasatinib to the *L. loa* Abl model was performed using SYBYL 7.0 by first superimposing the *L. loa* model onto a homolog with bound inhibitor (PDB ID 3CS9, 2HYY, and 2GQG, respectively; [Supplementary Materials](#)).

Electron Microscopy

Scanning and transmission electron microscopy were performed on imatinib-treated and untreated microfilariae on day 7 following exposure to 25 μ M of imatinib or to no drug. For transmission electron microscopy, samples were fixed in 2% paraformaldehyde/distilled water, 2.5% glutaraldehyde/distilled water, and 0.1 M phosphate buffer for 24 hours and then postfixed and processed as described elsewhere [15, 16]. For scanning electron microscopy, samples were fixed as described above, postfixed in 1% osmium, and processed as described previously [15].

Statistical Analyses

Unless otherwise stated, geometric means were used as a measure of central tendency. The log-rank (Mantel-Cox) test was performed to compare each drug-exposed filarial stage to respective negative controls (water for nilotinib and imatinib and DMSO for dasatinib). Median inhibitory concentrations (IC₅₀ values), survival curves, and statistical analyses were performed using GraphPad Prism 6.0c.

RNA Sequencing Information for Filarial Abl-like Protein

RNA sequencing information for the filarial Abl-like proteins was obtained from information in the public domain for *B. malayi* (GenBank accession no. XP001897557) [11] and for *O. volvulus* (sequence Ovoc3514, available at: <http://www.wormbase.org>).

RESULTS

Sequence Alignment and Phylogenetic Analyses

The sequences of the Abl-like protein for the 4 filariae were compared with that of the human c-Abl protein (Figure 1). All filariae showed significant homology (37.9%–38.9% identity) to the human oncogene protein sequence but even more so to each other. Indeed, the identity of the protein sequences between the filarial species ranged from 88% to 91% across the entire approximately 2400–amino acid molecule ([Supplementary Figure 1](#)). When the putative drug-binding site was examined specifically (Figure 1), the filarial Abl-like kinases each

demonstrated a significant degree of identity with the human c-Abl, which ranged from 70.6% to 71.0% depending on the particular filarial species. Phylogenetic analyses of these sequences showed that the Abl-like filarial sequences had significant relatedness to the human c-Abl protein sequence; as expected *B. malayi* and *W. bancrofti* were the most closely related among the filarial species.

TKI In Vitro Killing Assays

Brugia malayi microfilariae were most susceptible to imatinib and dasatinib, with lower drug concentrations being required to kill this stage, compared with the adults and L3 larvae. In addition, the death of microfilariae occurred with more-rapid kinetics. Against the microfilariae stage, dasatinib (Figure 2) at 20 μ M achieved 100% killing within 6 days, whereas imatinib did so only at 50 μ M. Compared with control (ie, no drug), the killing of microfilariae by imatinib was statistically significantly greater at all concentrations ranging from 5 to 50 μ M ($P < .0001$). Dasatinib also showed statistically significant killing against microfilariae over concentrations of 5–20 μ M ($P < .0001$). Nilotinib could achieve 100% killing only at high concentrations (100 μ M); below 50 μ M, it failed to significantly affect microfilariae viability, compared with control ($P = .083$).

When the effect of the TKIs was assessed against L3 larvae, larvicidal activity was achieved by day 3 with imatinib at 50 μ M ($P < .0001$), in contrast to dasatinib, which required 6 days for killing, but at a lower concentration (20 μ M; $P < .0001$). Nilotinib showed significantly less activity against L3 larvae, compared with imatinib and dasatinib.

Assessment of macrofilaricidal activity indicated a statistically significant killing effect of dasatinib across a wide range of concentrations (50 μ M, $P < .0001$; 10 μ M, $P < .0001$; and 5 μ M, $P = .003$) among adult males at 4 days (Figure 2). Surprisingly, despite relatively reduced efficacy against the other parasite stages, nilotinib was able to kill >90% of adult males by day 5 at 100 μ M ($P < .0001$), with statistically significant killing also achieved at concentrations of 50 μ M ($P = .0005$) and 10 μ M ($P = .0156$). Imatinib was able to achieve >90% filaricidal activity by day 5 at 100 μ M ($P < .0001$) and less, but still significant, killing at 50 μ M ($P = .0072$).

The effects of the TKIs on adult females were also assessed. As can be seen in Figure 2, dasatinib was the most efficacious against adult females among the TKIs tested, with >80% killing at 50 μ M ($P = .039$), as well as significant killing at concentrations as low as 10 μ M ($P = .0087$). Imatinib was able to achieve >90% killing of adult females at 100 μ M ($P < .0001$) and >50% killing at 50 μ M ($P = .0003$). Nilotinib had the least effect on adult females, with only 25% killing occurring at 100 μ M ($P = .013$) and 20% killing at 50 μ M ($P = .023$). At lower concentrations (eg, 25 μ M), imatinib caused the extrusion of early embryo stages from the adult females (data not shown).

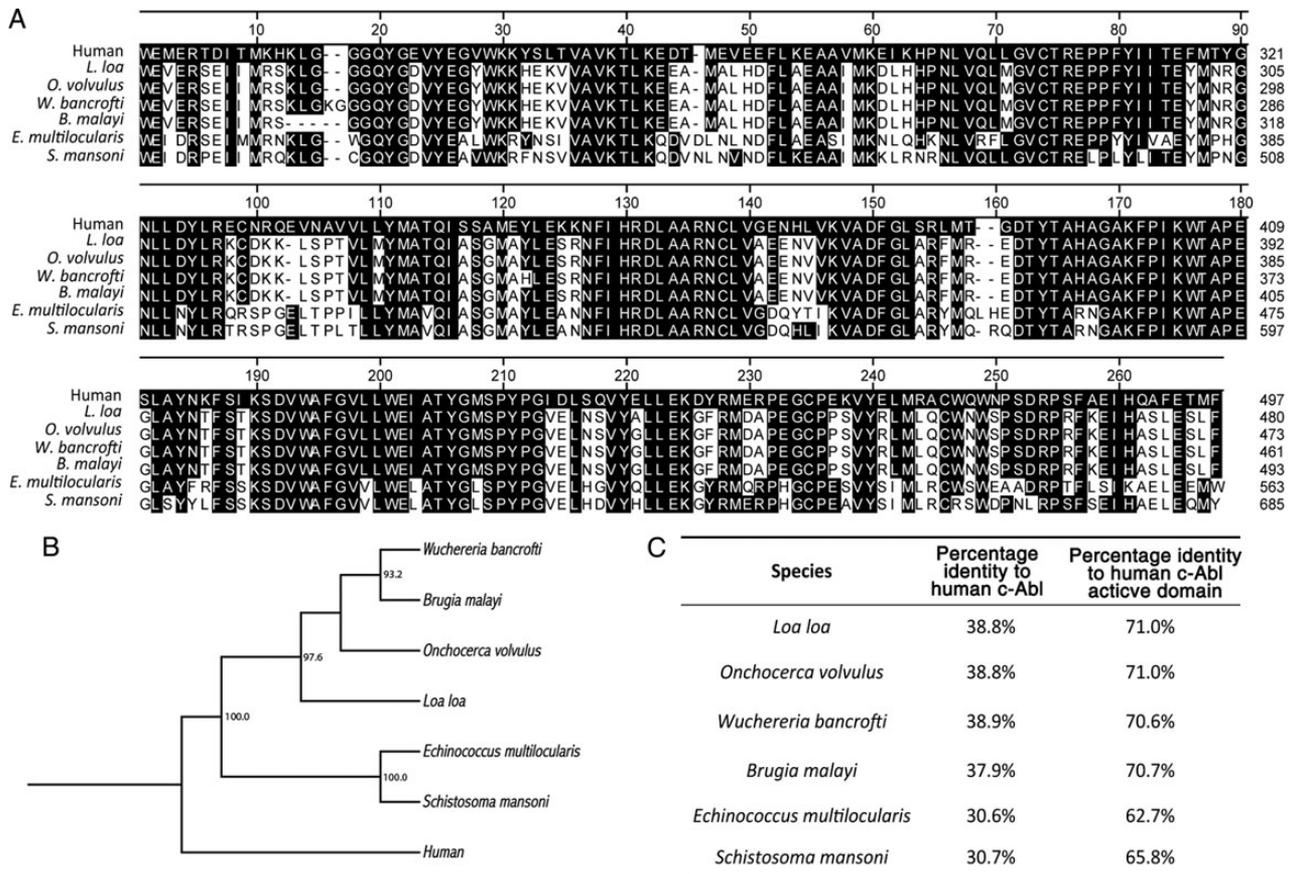


Figure 1. Phylogenetic interrelationships between parasite and human Abl-like kinases. **A**, Multiple sequence alignment of the catalytic domain of human c-Abl, to which imatinib, nilotinib, and dasatinib bind, compared with the sequences of Abl-like proteins found in *Wuchereria bancrofti*, *Brugia malayi*, *Loa loa*, *Onchocerca volvulus*, *Schistosoma mansoni*, and *Echinococcus multilocularis*, with black shading denoting amino acids that are identical to those found in the human protein. **B**, Phylogenetic tree of the human Abl and parasite Abl-like proteins. Bootstrap values are depicted. **C**, Percentage identities of the filarial c-Abl-like protein sequence to the entire human protein sequence and of the filarial drug-binding pocket to the human active domain.

IC₅₀ values were calculated for each drug against each life stage of *B. malayi* (Figure 3). As indicated by the representative curve for imatinib against microfilariae, the IC₅₀ was 6.06 μM. When these curves were generated for each of the 3 drugs for each of the life cycle stages and IC₅₀ values were calculated, microfilariae and L3 larvae were most susceptible to TK inhibition by imatinib and dasatinib, but killing could be achieved in all stages. Moreover, dasatinib had the lowest IC₅₀ among microfilariae, adult males, and adult females.

3-Dimensional Modeling

When restricted to the residues only within 3 Å of the respective inhibitors, the C-alpha atoms from the Abl-like *Loa* model had the most homology to the conformation of dasatinib bound with human c-Abl (root mean square deviation, 0.74 Å), followed by imatinib (1.29 Å), and nilotinib (1.45 Å). Figure 4 demonstrates the 3-dimensional *Loa* protein structure bound with the respective inhibitors overlaid with human c-Abl protein

bound with the TKIs. Amino acids within 3 Å of the binding drug and residues that, based on human x-ray crystallography data [13, 14], were found to interact are listed next to the equivalent residue on the *Loa* model.

Electron Microscopy

To assess the functional and anatomical consequences of targeting Abl-like kinases in filarial parasites, scanning electron microscopy and transmission electron microscopy were performed on microfilariae following exposure to 25 μM of imatinib and on untreated microfilariae. Figure 5 demonstrates that imatinib-exposed microfilariae had a shriveled, thin sheath and disrupted hypodermal nuclei, compared with imatinib-untreated microfilariae.

RNA Sequencing of Filarial Life Cycle Stages

RNA sequencing for Abl-like messenger RNA across the life cycle stages demonstrated expression in each of these life cycle stages (Table 1).

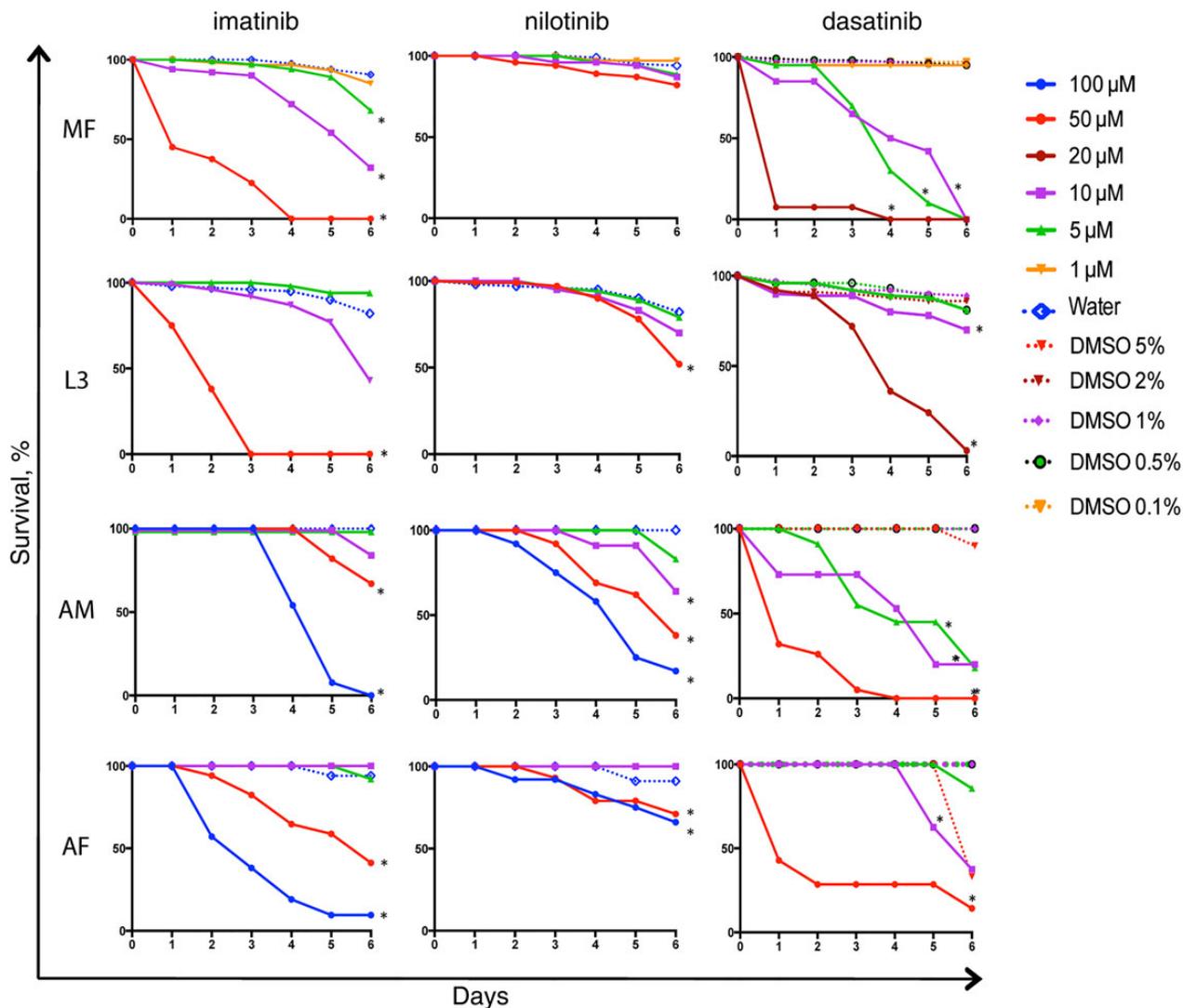


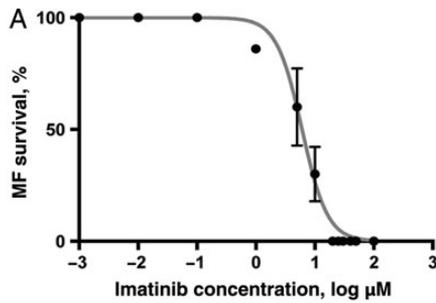
Figure 2. Survival curves of *Brugia malayi* microfilariae (MF), L3 larvae, adult males (AM), and adult females (AF) for imatinib, nilotinib, and dasatinib. Shown is the percentage survival of each stage following exposure to varying concentrations of imatinib, nilotinib, or dasatinib and of negative controls (water for imatinib and nilotinib and dimethyl sulfoxide [DMSO] for dasatinib) as a function of time in days following drug administration. * $P < .05$, by the log-rank test.

DISCUSSION

We demonstrate that FDA-approved, orally available TKIs were able to kill microfilariae, L3 larvae, and adult filarial males and females in vitro. Pharmacokinetic studies in humans have shown that imatinib can achieve a maximum concentration of 4.3 μM in the blood following a single 400-mg dose [17] and 13.3 μM (mean maximum concentration [$\pm\text{SD}$], $7.83 \pm 3.8 \mu\text{g}/\text{mL}$) following a single 600-mg oral dose [18]; the latter was higher than the IC_{50} for imatinib against the blood- and skin-transiting stages of the parasite, microfilariae (6.06 μM) and L3 larvae (11.27 μM). Clinically, doses as high as 800 mg/day are FDA approved, and doses even higher are used safely. However,

what levels are achievable where the adults reside—the lymphatics (for *W. bancrofti* and *Brugia* spp.) or subcutaneous tissues (for *L. loa* and *O. volvulus*)—makes it difficult to extrapolate these in vitro findings to the in vivo state [18].

We observed that high doses of imatinib (100 μM) caused significant morbidity in both adult males and females within 24 hours (Supplementary Materials). The most striking results were the ability of imatinib to significantly damage the sheath and internal structures of the microfilariae (Figure 5), as well as the low concentration of imatinib (6.06 μM) required to achieve an IC_{50} at this life stage. Dasatinib also demonstrated statistically significant activity against microfilariae, L3 larvae, and adult *B. malayi* and, for most parasite stages, killed at



B

Stage of <i>B. malayi</i>	IC ₅₀ , μM (95% CI)		
	Imatinib	Dasatinib	Nilotinib
Microfilariae	6.06 (5.19–7.07)	3.72 (2.85–4.86)	81.35 (53.63–123.4)
L3	11.27 (9.87–12.87)	13.64 (8.4–22.17)	70.98 (21.9–230)
Adult males	41.6 (31.55–54.85)	3.87 (1.83–8.1)	68.22 (45.8–101.7)
Adult females	42.89 (34.6–53.2)	9.8 (8.03–11.98)	>100

Figure 3. Median inhibitory concentrations (IC₅₀ values) for imatinib, dasatinib, and nilotinib against each of the *Brugia malayi* life stages. *A*, Representative IC₅₀ curve for imatinib against microfilariiae (MF). *B*, IC₅₀ values and 95% confidence intervals (CIs) for *B. malayi* MF, L3 larvae, and adult males and females for imatinib, dasatinib, and nilotinib at day 6 following drug administration.

much lower concentrations than those of the other drugs tested, although lower levels are physiologically possible [19]. Dasatinib likely also binds the filarial homologue to human SRC (unpublished data). Interestingly, although nilotinib binds to the same conserved site on c-Abl as its sister drugs, it performed less well than either imatinib or dasatinib. This may be due to its low solubility or to the subtle differences in the way nilotinib binds the catalytic pocket of c-Abl, given that it had the highest deviation of alpha carbons within 3 Å of the drug (1.45 Å), compared with imatinib (1.29 Å) and dasatinib (0.74 Å).

The success of the global programs to eliminate filarial infections depends greatly on the continued ability of the drugs used in mass drug administration to be effective. There have been major efforts to find new macrofilaricides for lymphatic filariasis and onchocerciasis, both to combat potential resistance to the current drugs and for use in regions where *L. loa* infection is coendemic. The ideal drug would have a different mechanism of action than the drugs that are currently used and would not elicit posttreatment reactions [20–23].

Imatinib has been safely used in humans for many years for a variety of conditions [24–26]. It is nearly 100% bioavailable orally [27], is partially metabolized into a biologically active metabolite, and has a half-life of approximately 13 hours [17] with an excellent safety profile. Although non-dose-limiting cytopenias are sometimes seen in patients with chronic myelogenous leukemia after weeks to months of drug administration, this is thought to be a reflection of a defective underlying bone marrow in these patients, and it is reversible with a short

drug holiday [28–30]. Single-dose-administration trials in healthy subjects have been instructive, as nausea is the most common side effect in less than half the participants, with no severe adverse events [17, 31].

In normal human cells, the c-Abl protein is in a state of inhibition under normal circumstances. In chronic myelogenous leukemia, the autoinhibitory component is removed and c-Abl becomes the constitutively active Bcr-Abl oncoprotein [32]. In filariae and *S. mansoni*, this Abl-kinase and other tyrosine kinases are very highly expressed under homeostatic conditions, most notably in the worm's reproductive tract [9]. As shown in Table 1, the Abl-like kinase is expressed in all mammalian stages of *B. malayi* [11] and *O. volvulus*. In *B. malayi*, the lowest levels of expression were in the L3 larvae and adult males (reads per kilobase transcript per million mapped reads [RPKM], 68 and 63, respectively), while the microfilariae had relatively higher levels of expression (RPKM, 125) and adult females the highest (RPKM, 246). The relative differences in effects on different stages of the filarial parasites are largely not explained by differences in kinase expression. This may reflect the differing solubility and relative ability of the various drugs to penetrate each life stage, as well as differing roles the kinase plays in various life stages. For instance, electron microscopy revealed that low-dose imatinib (10 μM) significantly affects embryogenesis in females (unpublished data). While this dose does not achieve complete macrofilaricidal activity in vitro, it may have a sterilizing effect.

Studies in schistosomes have demonstrated that imatinib causes significant structural and transcriptional changes in pathways involved in reproduction and mobility [9, 10]. However, an *S. mansoni* mouse model failed to demonstrate parasite death in the presence of imatinib [33]. These results are not necessarily discouraging since, compared with the sequence of the schistosome SmAbl1, the sequences for the Abl-like proteins of *L. loa*, *B. malayi*, *W. bancrofti*, and *O. volvulus* all have significantly higher homology to the human protein than do those from *S. mansoni* or from cestodes (Figure 1).

Our data suggest that imatinib impairs the ability of the microfilariae to maintain their protective sheath (Figure 5A and 5B) and may alter embryogenesis in adult females. The importance of rendering the adult worm infertile as a key component to eradication efforts is underscored by the fact that onchocerciasis is still not eradicated in northern Cameroon despite 17 years of treatment with the microfilaricide ivermectin [34]. Antifilarial therapy with safe medications that are both macrofilaricidal and microfilaricidal, such as the TKIs, would shorten the course of treatment, thereby lessening the chance for resistance developing and saving time and cost. Given the widespread use and over a decade of safety data, moving directly to human studies to assess the effects of imatinib (and dasatinib) in filarial infections is warranted, particularly because there are no good permissive animal models for the pathogenic *O. volvulus*, *W. bancrofti*, or *L. loa*.

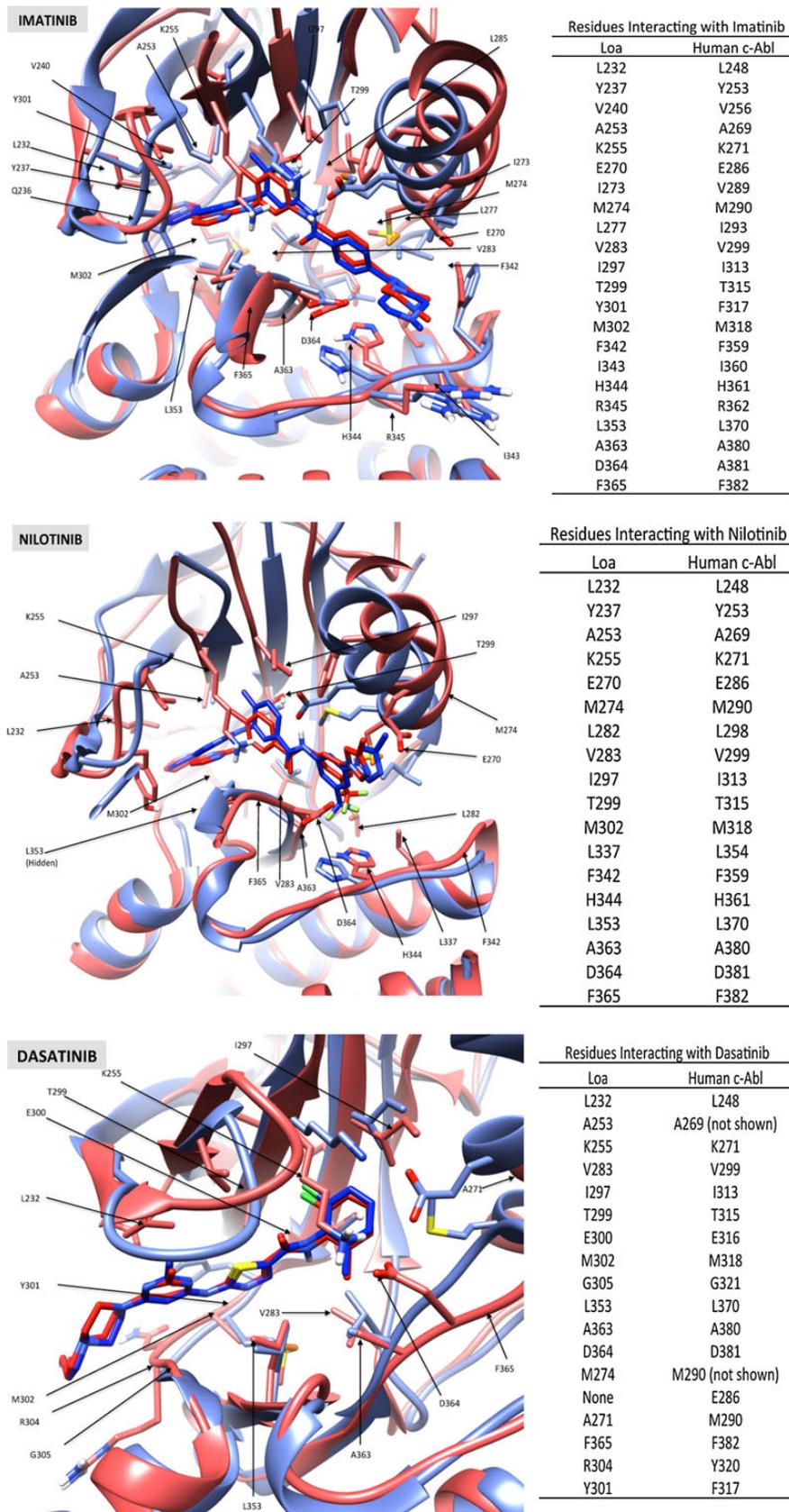


Figure 4. Projected model of *Loa loa* Abl-like protein interacting with imatinib, nilotinib, and dasatinib. *Loa* protein model (red; projected) is superimposed on human (blue; based on x-ray crystallography data: PDB ID 3CS9 [nilotinib], 2GQG [dasatinib], and 2HYY [imatinib]). All residues within 3Å of the inhibitor and known binding sites for the human protein are listed next to the corresponding residue on the *Loa* model.

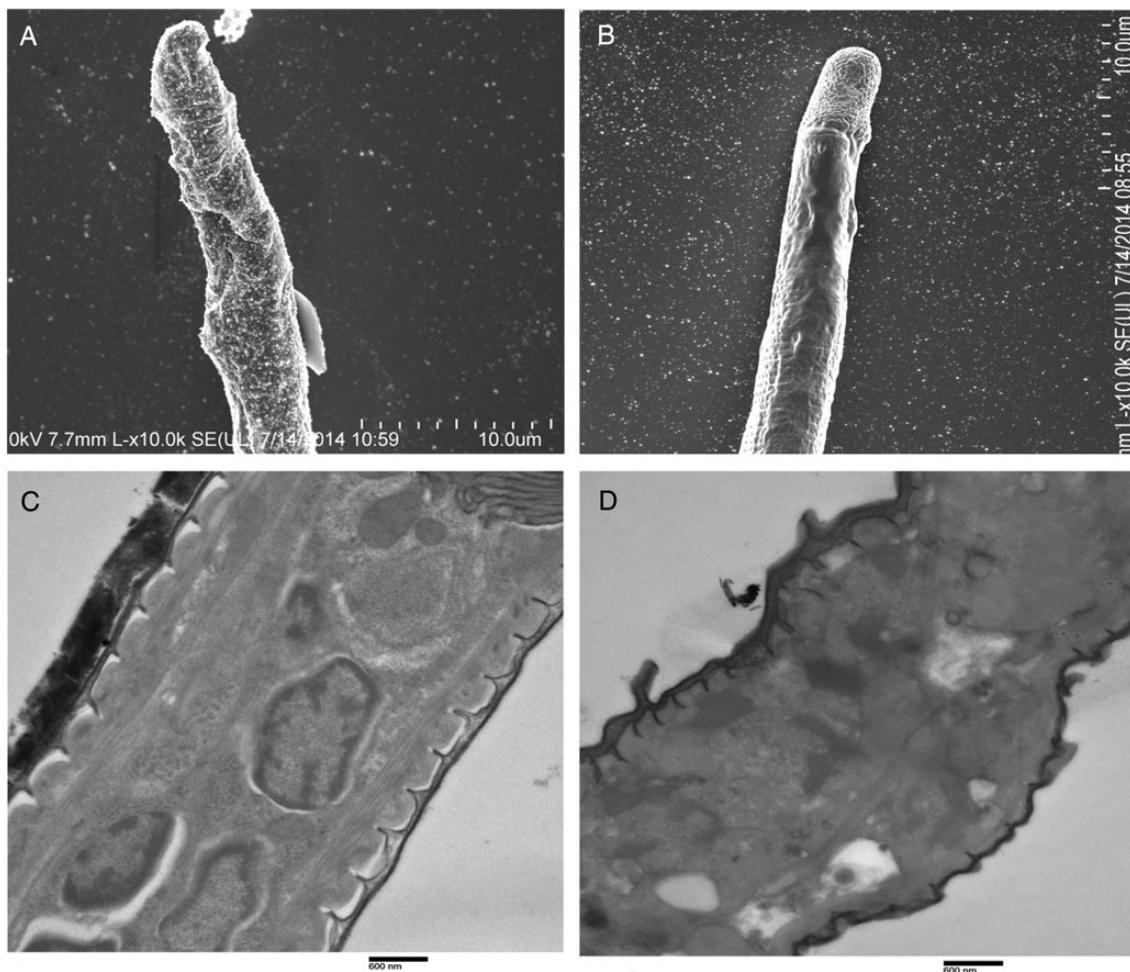


Figure 5. Electron microscopy of microfilariae (MF) following imatinib administration. *A* and *B*, Scanning electron micrograph (10 000× original magnification) of MF on day 7 following no drug (*A*) or exposure to 25 μM imatinib (*B*). *C* and *D*, Transmission electron microscopy (7000× original magnification) of MF on day 7 following no drug (*C*) or exposure to 25 μM imatinib (*D*).

Given their microfilaricidal activity, the safety of TKIs in patients with high levels of microfilariae is still of concern. If, however, dosing can be titrated so that there is less rapid microfilariae killing, it is possible that the adverse events that are clearly microfilariae directed [20] can be mitigated. Additionally, although diethylcarbamazine and ivermectin clearly possess microfilaricidal effects in vivo, neither show activity in vitro at therapeutic doses and therefore likely require a component of the immune system to cause filarial death. We demonstrate

Table 1. Relative Expression, Based on RNA Sequencing of Abl-like Proteins From Various Life Cycle Stages of *Brugia malayi* and *Onchocerca volvulus*

Organism	Microfilariae	L3 Larvae	Adult Male	Adult Female
<i>B. malayi</i>	125	68	63	246
<i>O. volvulus</i>	1.07	58.6	5.5	14.2

Data are reads per kilobase transcript per million mapped reads.

here that, at therapeutic doses, imatinib can kill *B. malayi* microfilariae directly. Therefore, it is possible that, unlike ivermectin and diethylcarbamazine treatment, reactions following TKI administration may be minimized, as well.

In this study, the *L. loa*, *O. volvulus*, *B. malayi*, and *W. bancrofti* Abl-kinase sequences were shown to be quite homologous (>60%) to that of the human oncogenic Bcr-Abl, with the most highly conserved region of the protein being the active sites, including the important competitive binding region for TKIs. The *L. loa* Abl-like protein model has nearly identical residues interacting with imatinib, nilotinib, and dasatinib, as does human c-Abl (Figure 4). Given the limited expression in normal human cells [35], the TKIs would appear to be a safe and effective choice as novel antifilarial agents.

Our data indicate that TKIs, particularly imatinib, which has the most extensive clinical safety data, deserve further study in human filarial infection. While these in vitro data are a starting point, it will be up to in vivo studies to determine the dose

required in humans, including whether several doses are needed to have macrofilaricidal effects and to determine safety in this setting. If imatinib (or other related TKIs) proves to be a safe, highly effective microfilaricide and macrofilaricide, it is possible that the significant advantages afforded by these drugs would outweigh some of the challenges, such as drug cost and availability, some of which could be overcome, for instance, through drug donation.

Given that we have demonstrated that imatinib is able to affect all life stages of *B. malayi* at concentrations likely achievable in vivo and have shown the highly conserved nature of the TKI binding sites, one would expect imatinib to be successful in treating lymphatic filariasis, loiasis, and onchocerciasis. Plans for clinical testing of imatinib are under consideration, and, if successful, imatinib therapy could be the final stage in the campaign to eliminate these important human filarial pathogens.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Fenwick A. The global burden of neglected tropical diseases. *Public Health* **2012**; 126:233–6.
2. World Health Organization. Global programme to eliminate lymphatic filariasis: progress report on mass drug administration, 2010. *Wkly Epidemiol Rec* **2011**; 86:377–88.
3. Bockarie MJ, Taylor MJ, Gyaopong JO. Current practices in the management of lymphatic filariasis. *Expert Rev Anti Infect Ther* **2009**; 7: 595–605.
4. Gyaopong JO, Kumaraswami V, Ottesesen E. Treatment strategies underpinning the global programme to eliminate lymphatic filariasis. *Expert Opin Pharmacother* **2005**; 6:179–200.
5. Naula C, Parsons M, Mottram JC. Protein kinases as drug targets in trypanosomes and Leishmania. *Biochim Biophys Acta* **2005**; 1754:151–9.
6. Ward P, Equinet L, Packer J, Doerig C. Protein kinases of the human malaria parasite *Plasmodium falciparum*: the kinome of a divergent eukaryote. *BMC Genomics* **2004**; 5:79.
7. Dissous C, Ahier A, Khayath N. Protein tyrosine kinases as new potential targets against human schistosomiasis. *Bioessays* **2007**; 29:1281–8.
8. Hemer S, Brehm K. In vitro efficacy of the anticancer drug imatinib on *Echinococcus multilocularis* larvae. *Int J Antimicrob Agents* **2012**; 40:458–62.
9. Beckmann S, Grevelding CG. Imatinib has a fatal impact on morphology, pairing stability and survival of adult *Schistosoma mansoni* in vitro. *Int J Parasitol* **2010**; 40:521–6.
10. Buro C, Beckmann S, Oliveira KC, et al. Imatinib treatment causes substantial transcriptional changes in adult *Schistosoma mansoni* in vitro exhibiting pleiotropic effects. *PLoS Negl Trop Dis* **2014**; 8:e2923.
11. Choi YJ, Ghedin E, Berriman M, et al. A deep sequencing approach to comparatively analyze the transcriptome of lifecycle stages of the filarial worm, *Brugia malayi*. *PLoS Negl Trop Dis* **2011**; 5:e1409.
12. Bennuru S, Semnani R, Meng Z, Ribeiro JM, Veenstra TD, Nutman TB. *Brugia malayi* excreted/secreted proteins at the host/parasite interface: stage- and gender-specific proteomic profiling. *PLoS Negl Trop Dis* **2009**; 3:e410.
13. Tokarski JS, Newitt JA, Chang CY, et al. The structure of Dasatinib (BMS-354825) bound to activated ABL kinase domain elucidates its inhibitory activity against imatinib-resistant ABL mutants. *Cancer Res* **2006**; 66:5790–7.
14. Cowan-Jacob SW, Fendrich G, Floersheimer A, et al. Structural biology contributions to the discovery of drugs to treat chronic myelogenous leukaemia. *Acta Crystallogr D Biol Crystallogr* **2007**; 63:80–93.
15. Beare PA, Howe D, Cockrell DC, Omsland A, Hansen B, Heinzen RA. Characterization of a *Coxiella burnetii* ftsZ mutant generated by HimarI transposon mutagenesis. *J Bacteriol* **2009**; 191:1369–81.
16. Coleman SA, Fischer ER, Howe D, Mead DJ, Heinzen RA. Temporal analysis of *Coxiella burnetii* morphological differentiation. *J Bacteriol* **2004**; 186:7344–52.
17. Parrillo-Campiglia S, Ercoli MC, Umpierrez O, et al. Bioequivalence of two film-coated tablets of imatinib mesylate 400 mg: a randomized, open-label, single-dose, fasting, two-period, two-sequence crossover comparison in healthy male South American volunteers. *Clin Ther* **2009**; 31:2224–32.
18. Gambacorti-Passerini C, Zucchetti M, Russo D, et al. Alpha1 acid glycoprotein binds to imatinib (STI571) and substantially alters its pharmacokinetics in chronic myeloid leukemia patients. *Clin Cancer Res* **2003**; 9:625–32.
19. Demetri GD, Lo Russo P, MacPherson IRJ, et al. Phase I dose-escalation and pharmacokinetic study of dasatinib in patients with advanced solid tumors. *Clin Cancer Res* **2009**; 15:6232–40.
20. Francis H, Awadzi K, Ottesen EA. The mazzotti reaction following treatment of onchocerciasis with diethylcarbamazine: clinical severity as a function of infection intensity. *Am J Trop Med Hyg* **1985**; 34: 529–36.
21. Moreno Y, Nabhan JF, Solomon J, Mackenzie CD, Geary TG. Ivermectin disrupts the function of the excretory-secretory apparatus in microfilariae of *Brugia malayi*. *Proc Natl Acad Sci U S A* **2010**; 107:20120–5.
22. Verissimo CJ, Niciura SC, Alberti AL, et al. Multidrug and multispecies resistance in sheep flocks from Sao Paulo state, Brazil. *Vet Parasitol* **2012**; 187:209–16.
23. Osei-Atweneboana MY, Eng JKL, Boakye DA, Gyaopong JO, Prichard RK. Prevalence and intensity of *Onchocerca volvulus* infection and efficacy of ivermectin in endemic communities in Ghana: a two-phase epidemiological study. *Lancet* **2007**; 369:2021–9.
24. Gambacorti-Passerini C, Antolini L, Mahon FX, et al. Multicenter independent assessment of outcomes in chronic myeloid leukemia patients treated with imatinib. *J Natl Cancer Inst* **2011**; 103:553–61.
25. Helbig G, Kyrz-Krzemien S. Diagnostic and therapeutic management in patients with hypereosinophilic syndromes. *Pol Arch Med Wewn* **2011**; 121:44–52.
26. Pisters PW, Patel SR. Gastrointestinal stromal tumors: current management. *J Surg Oncol* **2010**; 102:530–8.
27. Peng B, Hayes M, Resta D, et al. Pharmacokinetics and pharmacodynamics of imatinib in a phase I trial with chronic myeloid leukemia patients. *J Clin Oncol* **2004**; 22:935–42.
28. Breccia M, Alimena G. Occurrence and current management of side effects in chronic myeloid leukemia patients treated frontline with tyrosine kinase inhibitors. *Leuk Res* **2013**; 37:713–20.

29. van Deventer HW, Hall MD, Orlowski RZ, et al. Clinical course of thrombocytopenia in patients treated with imatinib mesylate for accelerated phase chronic myelogenous leukemia. *Am J Hematol* **2002**; 71: 184–90.
30. Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABK tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* **2001**; 344:1031–7.
31. Kim KA, Park SJ, Kim C, Park JY. Single-dose, randomized crossover comparisons of different-strength imatinib mesylate formulations in healthy Korean male subjects. *Clin Ther* **2013**; 35: 1595–602.
32. Hantschel O, Nagar B, Guettler S, et al. A myristoyl/phosphotyrosine switch regulates c-Abl. *Cell* **2003**; 112:845–57.
33. Katz N, Couto FF, Araujo N. Imatinib activity on *Schistosoma mansoni*. *Mem Inst Oswaldo Cruz* **2013**; 108:850–3.
34. Katarawa MN, Eyamba A, Nwane P, et al. Seventeen years of annual distribution of ivermectin has not interrupted onchocerciasis transmission in North Region, Cameroon. *Am J Trop Med Hyg* **2011**; 85:1041–9.
35. Wetzler M, Talpaz M, Van Etten RA, Hirsh-Ginsberg C, Beran M, Kurzrock R. Subcellular localization of Bcr, Abl, and Bcr-Abl proteins in normal and leukemic cells and correlation of expression with myeloid differentiation. *J Clin Invest* **1993**; 92:1925–39.