Imatinib mesylate is potent revolutionary anti-neoplastic medication of high specificity (1–3). It functions at the molecular level inhibiting activity of particular tyrosine kinase enzymes, namely bcr-abl kinase, platelet-derived growth factor (PDGF) receptor and receptor for stem cell factor (c-kit receptor) at submicromolar concentrations. In consequence, the signal transduction via ligand-stimulated receptor autophosphorylation, inositol phosphate formation and mitogen-activated protein kinase (MAP kinase) activation is selectively inhibited with resultant cell proliferation arrest (4, 5). This abnormal enzyme is associated with the Philadelphia chromosome (Ph+), which is a consequence of reciprocal translocation of genetic material between chromosomes 9 and 22 with generation of the chimeric BCR-ABL fusion gene. The highly elevated catalytic activity of the enzyme encoded by BCR-ABL fusion gene leads to a resistance to apoptosis, cell transformation and malignancy (including chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL)), and altered cell adhesion. Protein product of BCR-ABL fusion gene is present in 95% of CML and 10–25% of adult ALL cases. Imatinib mesylate competitively blocks the binding of ATP to the activation domain of bcr-abl, which modulates phosphorylation status of the kinase and diminishes its activity (3, 6). In consequence, imatinib mesylate suppresses the proliferation of bcr-abl+ cells (3). The efficacy of imatinib mesylate (STI571) in newly diagnosed chronic Ph+ CML was evaluated in an open-label, multicentre, international phase III study referred as International Randomized Study of Interferon and STI571 (IRIS)
Moreover, given the literature data, combining imatinib mesylate with conventional chemotherapy for the treatment of newly diagnosed or minimally treated Ph+ ALL may yield complete remission rates approaching 95%. The agent may be administered either concurrently or sequentially with chemotherapy (10).

Nowadays, it is known that certain other human tumors are considered to be associated with deregulation of c-kit receptor. These are gastrointestinal stromal tumors (GIST), germ cell tumors, mast cell tumors, small-cell lung cancers, melanoma, breast cancer, and neuroblastoma. Abnormal cell growth has been linked to mutation of c-kit, which leads to ligand-independent activation of the receptor (3, 5, 11–13). There are many studies from which it is well documented that imatinib mesylate has been shown to be highly effective in the treatment of unresectable and/or metastatic GIST, resulting in a substantial improvement in the survival rate. Imatinib has, therefore, become the standard of care in patients with advanced GIST (14–17).

Imatinib mesylate was also demonstrated to be effective in the treatment of adult patients with hypereosinophilic syndrome (HES) and/or chronic eosinophilic leukemia (CEL) who are Fip1-like1-platelet-derived growth factor receptor α chain (FIP1L1-PDGFRα) fusion kinase positive (18–21).

Imatinib mesylate has favorable pharmacokinetic profile. It is well absorbed from gastrointestinal tract, with bioavailability of almost 100% (22–24). At clinically relevant concentrations imatinib mesylate is highly bound to plasma protein (ca. 95%), primarily to α1-acid glycoprotein (22, 25). It undergoes a considerable metabolism in the liver, mainly via CYP3A4. The main metabolite, CGP 74588, is known to be active, and the elimination of this and other metabolites is more than 90% through the bile (22, 23). The elimination half-life of imatinib mesylate is approximately 18 h, with two- to three-fold accumulation at steady-state (22, 26).

The recommended dose of imatinib mesylate is 400 mg/day for adult patients in chronic phase CML and 100 mg/day with HES/CEL (27). The drug is a generally well tolerated drug, with chronic oral daily dosing.

The reference product in the present study was an already approved and commercially available Glivec (100, 400 mg), film-coated tablets (Novartis Pharma GmbH).

For the registration purposes, the efficacy and safety of this product has been proven already in clinical trials. This drug has therefore served as a reference and a basis for comparison to a imatinib mesylate test product: Imatynib-Biofarm (Imatenil).

It is known that imatinib mesylate exists in two polymorphic forms α and β, which differ in their physicochemical properties i.e., melting points (t_melt 226°C and 217°C, respectively) or enthalpy of thermal events (36). Moreover, the both polymorphs exhibit a tendency to generate amorphous form during some technological processes i.e., grinding (36).

There are some suggestions that differences in the apparent solubilities of the various polymorphic forms can affect the drug bioavailability and bioequivalence (37). Since imatinib mesylate in the Imatynib-Biofarm (Imatenil) exists in α form only (38), whereas in Glivec the β form is postulated (39), therefore the differences in their bioavailability cannot be excluded. So, the aim of the study was to investigate the pharmacokinetic properties and the bioequivalence of imatinib from a new preparation: Imatynib-Biofarm 100 and 400 mg film-coated tablets Imatenil (Biofarm Sp. z o.o.) compared with the reference formulation Glivec (100, 400 mg), film-coated tablets (Novartis Pharma GmbH) following a single oral dose administration of 100 or 400 mg under fed conditions. Moreover, its active metabolite derivative – desmethyl imatinib (CGP74588) pharmacokinetics was planned to be evaluated as supportive information. The secondary objective of the study was to compare tolerability of the products.

EXPERIMENTAL

Subjects and Methods

Study products

The test products were: Imatynib-Biofarm 100 mg film-coated tablet (Imatenil) batch no: 030709 and Imatynib-Biofarm 400 mg film-coated tablet (Imatenil) batch no: 011210 manufactured by Biofarm Sp. z o.o. The reference product was Glivec (100 and 400 mg, film-coated tablets, batch no. S0041 and batch no. S0197, respectively, manufactured by Novartis Pharma GmbH).

Subjects

The study was performed as a single centre, open-labeled, randomized, two-period, 2-way crossover, single dose study under fed conditions with a washout period of 14 days between drug administrations in each treatment period. Subjects were selected according to the inclusion and exclusion criteria in order to obtain a low individual variability within the subject group. The demographic characteristics of the study population was shown in
Table 1. Healthy willing male and postmenopausal female subjects (male aged between 21 and 58 years and female aged between 42 and 58 years), able to communicate clearly with the study personnel and able to give written consent for participation in the study, who were non-smokers or no-users of tobacco products for at least ninety (90) days before screening, having body mass index (BMI) between 18.50 kg/m² and 26.99 kg/m² (the minimum body weight for males was not less than 60 kg, for females – not less than 50 kg), having no significant diseases (current or past), no clinically significant abnormal laboratory values or clinically significant abnormal results of 12-lead ECG, vital signs and chest X-ray, with physical examination without any clinically relevant abnormality were randomized and included to the study.

A single dose of test drug (Imatynib-Biofarm, 100 or 400 mg film-coated tablet) or a single dose of reference drug (Glivec, 100 or 400 mg film-coated tablet) were administered by the oral route for subsequent subjects in the morning on days 1 and 15 (treatment period I and II) in the sitting position, according to the randomization list and under open-label conditions. The tablet was administered, 30 min after standardized breakfast, with 240 mL of boiled water (at room temperature). No other food was allowed until 4 h after drug administration. No fluid intake apart from the fluid given at the time of drug intake was allowed from 2 h before until 2 h after dosing.

Study design
This study was prepared according to the Note for Guidance on the Investigation of Bioavailability and Bioequivalence, CPMP/EWP/QWP/1401/98 (28) and the Note for Guidance on the Investigation of Bioavailability and Bioequivalence, CPMP/EWP/QWP/1401/987/Rev 1 (29). The use of a generic preparation of a therapeutically well-established active drug principle has to be justified by an appropriate bioequivalence study, because the proof of bioequivalence of the test and reference products assures equal therapeutic efficacy. The study was conducted between January 2010 and June 2010 (for the dose of 100 mg) and December 2010 and April 2011 (for the dose of 400 mg) by Lambda Therapeutic Research Sp. z o.o., Centrum Badań Klinicznych NZOZ in Warszawa in compliance with the approved Protocols in adherence to Good Clinical Practices and Ethical Principles, as described in: ICH harmonized Tripartite Guidelines for Good Clinical Practice and World Medical Association Declaration of Helsinki and its amendments (30, 31). Ethical approval was received from the Independent Ethics Committee (IEC) in Warszawa. The clinical trial registration numbers of the study were KB/693/09 for Imatynib-Biofarm 100 mg film-coated tablet and KB/733/10 for Imatynib-Biofarm 400 mg film-coated tablet. The Ministry of Health approval was obtained on January 21, 2010 for Imatynib-Biofarm 100 mg film-coated tablets (approval number CEBK/0034/10) for Imatynib-Biofarm 400 mg film-coated tablet was obtained on January 11, 2011 (approval number CEBK/0007/11). The studies were registered in EudraCT and obtained the numbers: EudraCT 2009-016180-10 and 2010-021028-91 for the dose of 100 mg and 400 mg, respectively. All eligible subjects provided written informed consent to participate and were free to withdraw from the study at any time without any obligation.

Blood sample collection
Blood samples for determination of imatinib and its metabolite concentrations were collected up to 96 h after the drug administration in 19 time points in each treatment period.

Just before the first blood sampling (the morning of a day of drug administration), a cannula was introduced into a vein and blood samples were collected during the study by means of this cannula till pharmacokinetic blood sample at 72-h post dose in each treatment period. After the cannulation of the vein, a pre-dose PK sample of 7 mL was collected. Blood samples were obtained prior to dosing (baseline) and 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 5.0; 6.0; 8.0; 12.0; 16.0; 24.0; 36.0; 48.0; 72.0 and 96.0 h post-dose (19 samples per subject in each treatment period). Blood was collected from cubital vein or forearm vein or from veins into the tubes with anticoagulant. After collection, the blood samples were immediately cooled in an ice bath and then were centrifuged at 3000 ± 100 rcf or 4200 ± 100 rpm for 5 min below 10°C to separate plasma. The separated plasma in the study for Imatynib-Biofarm 100 mg was transferred to pre-labelled polypropylene tubes in 2 aliquots (around 0.7 mL in case of pre dose sample and rest of the volume in second lot) and stored upright at a temperature −55°C or colder for interim storage until shipment to bioanalytical laboratory, for analysis.

The separated plasma in the study for Imatynib-Biofarm 400 mg was transferred to pre-labelled polypropylene tubes in 2 aliquots (around 1.25 mL in first lot, 1.75 mL in case of pre dose sample and rest of the volume in second lot) and stored upright at a temperature −55°C or colder for
interim storage until shipment to bioanalytical laboratory, for analysis.

**Determination of imatinib and its metabolite N-desmethyl imatinib plasma concentrations**

Plasma samples of subjects were assayed for imatinib and its metabolite N-desmethyl imatinib (CGP74588) using a precise and accurate LC-MS/MS method (ACQUITY UPLC BSM binary solvent manager, Acquity UPLC SM sample manager with column heater and Waters Quattro Premier XE mass spectrometer) with imatinib-d₈ & N-desmethyl imatinib-d₈ as the internal standards (ISTD), which is validated according to the international guidelines at Lambda Therapeutic Ltd., UK. The method has been developed and validated for calibration curve ranging from 5.012 to 2999.700 ng/mL for imatinib and from 1.999 to 400.660 ng/mL for N-desmethyl imatinib. Briefly, the analytes and internal standards were extracted from plasma using liquid-liquid extraction method. It means that the frozen calibration curve standards, quality control samples and the study samples were thawed in a water bath maintained at room temperature and vortexed to ensure complete mixing of contents. Two hundred fifty microliters of each of the calibration curve standards, quality control samples and the study samples were aliquoted into pre-labeled tubes. Fifty microliters of the ISTD dilution mixture (about 200 ng/mL of imatinib-d₈ and about 375 ng/mL of N-desmethyl imatinib-d₈) was added to each tube except for standard blank, Subject blank and blank QC tubes and vortexed for 1 min. Thereafter, 100 µL of 100 mM ammonium formate buffer (pH 7.0) was added to each tube and vortexed for 1 min followed by the addition of 4 mL of extraction solution and vortexed for 10 min. The samples were centrifuged at 3345 ± 150 rcf for 5 min at 10°C using imatinib-d₈ and N-desmethyl imatinib-d₈ as the internal standards. The plasma layer was flash-frozen in alcohol freezing bath and organic layer was transferred into pre-labeled tubes. The contents were evaporated to dryness at room temperature under nitrogen stream and reconstituted with 250 µL of the reconstitution solution and vortexed. Next, the contents were transferred into appropriate autosampler vials for analysis. Ten microliters of each sample was chromatographed on Hypersil GOLD, 150 × 4.6 mm, 5 µm column maintained at 40°C using a binary mode of mobile phase system composed of 70% of acetonitrile and 30% of 2 mM ammonium formate buffer (pH 3.5). Imatinib and ISTD-1 and N-desmethyl imatinib and ISTD-2 were monitored in the positive ion mode using the MRM transitions (m/z 494.22 > 394.10 for imatinib, m/z 502.00 > 394.10 for ISTD-1, m/z 430.20 > 394.10 for N-desmethyl imatinib, m/z 488.44 > 394.15 for ISTD-2) and retention times (imatinib and ISTD-1 – 1.74 min; N-desmethyl imatinib and ISTD-2 – 1.70 min). MassLynx Software Version 4.1 was used for the evaluation of chromatographic data. A linear equation was judged to produce the best fit for the concentration vs. area response relationship. The regression type was 1/concentration and peak area ratio for an 8-point calibration curve was found to be linear from 5.012 (lower limit of quantification) to 2999.700 ng/mL for imatinib and from 1.999 (lower limit of quantification) to 400.660 ng/mL for N-desmethyl imatinib with correlation coefficient (r) greater than 0.99 for imatinib and N-desmethyl imatinib during the course of validation. For imatinib, the range of precision and accuracy of the back-calculated concentrations of the standard curve points was from

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
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<td></td>
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<td>n = 37</td>
<td>n = 42*</td>
<td>n = 37</td>
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<tr>
<td></td>
<td>(Subjects who were enrolled into the study)</td>
<td>(Subjects who completed all phases of the study)</td>
<td>(Subjects who were enrolled into the study)</td>
<td>(Subjects who completed all phases of the study)</td>
</tr>
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<td>Age (years)</td>
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<td>32.4 ± 9.84</td>
<td>33.9 ± 11.78</td>
<td>34.1 ± 11.59</td>
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<td>Height (cm)</td>
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<td>177.4 ± 7.12</td>
<td>177.1 ± 8.70</td>
<td>177.4 ± 9.11</td>
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<tr>
<td>Weight (kg)</td>
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<td>75.0 ± 7.63</td>
<td>75.6 ± 7.28</td>
<td>75.6 ± 7.16</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>23.63 ± 1.89</td>
<td>23.83 ± 1.84</td>
<td>24.2 ± 1.79</td>
<td>24.1 ± 1.86</td>
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Arithmetic mean ± SD. *– 4 females; *– 4 females

<table>
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<tr>
<th>Parameter (units)</th>
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<th>Mean ± SD</th>
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</thead>
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<tr>
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<td>n = 43*</td>
<td>n = 37</td>
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<tr>
<td></td>
<td>(Subjects who were enrolled into the study)</td>
<td>(Subjects who completed all phases of the study)</td>
</tr>
<tr>
<td>Age (years)</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>75.0 ± 7.63</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.63 ± 1.89</td>
<td>23.83 ± 1.84</td>
</tr>
</tbody>
</table>

Arithmetic mean ± SD. *– 4 females
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1.0 to 5.5% and from 97.0 to 102.7%, respectively, whereas for N-desmethyl imatinib these parameters were from 1.9 to 4.2% and from 96.5 to 102.2%, respectively. Precision and accuracy of imatinib and N-desmethyl imatinib was determined for limit of quantification, low, medium and high concentrations of quality control samples in the biological matrix, based on the expected range. Accuracy (% nominal) for inter-day and intra-day was within 85–115% of the nominal value for all quality control samples except for LOQ QC, which was within 80–120%. For precision, the % CV was ≥ 15% for all quality control samples, except for LOQ QC, which was ≥ 20%.

**Pharmacokinetic and statistical analyses**

The pharmacokinetic parameters for imatinib and its metabolite N-desmethyl imatinib were determined from the plasma concentration vs. time curve with the aid of the WinNonlin Professional Software version 5.3. The parameters selected as primary endpoints of the study were: the area under the plasma concentration vs. time curve (AUC_{0-\infty}), and the maximum plasma concentration of the drug (C_{max}). The time to reach maximum plasma concentration of the drug (T_{max}), the elimination half-life (t_{1/2}) and ln as a first order rate constant associated with the terminal (log-linear) portion of the curve. C_{max} and T_{max} were obtained directly from the experimental data. The elimination rate constant (ln) was estimated by linear least squares regression analysis using at least last three or more non-zero plasma concentration values. The t_{1/2} was calculated as ln 2/ln. The AUC_{0-\infty} was calculated as the area under the plasma concentration versus time curve from time zero to the last measurable concentration as calculated by linear trapezoidal method. The AUC_{0-\infty} was calculated where AUC_{0-\infty} = AUC_{0-t} + C/t_{1/2}, where C is the last measurable concentration and z is the terminal elimination rate constant. For metabolite N-desmethyl imatinib: AUC_{t_{max}} = AUC_{0-96} (truncated at 96 h). The residual area in percentage (AUC_%Extrap_obs) was determined by the formula \[(AUC_{0-\infty} - AUC_{0-t})/ AUC_{0-\infty}\] ◊ 100. The statistical calculations were performed using the SAS (Version 9.2 or higher) software. The tests for normality of ln-transformed pharmacokinetic parameters were performed with proper tests. The analysis of variance (ANOVA) was performed on the ln-transformed data. The statistical significance of effects was determined on basis of the calculated p-values with value larger than 0.05 meaning no statistical significance.

Based on the ANOVA results, 90% confidence interval (CI) for the µT/µR (ratio of geometric means for the test and the reference product) of the analyzed pharmacokinetic parameters was constructed. Bioequivalence was assumed when 90%

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**Table 2. Plasma pharmacokinetic parameters of imatinib after a single dose administration of the Imatynib-Biofarm 100 mg (test product) and the Glivec 100 mg (reference product).**

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Arithmetic mean</th>
<th>Median</th>
<th>Geometric mean</th>
<th>SD</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Imatynib-Biofarm 100 mg (test product)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>2.9</td>
<td>2.5</td>
<td>2.5</td>
<td>1.5</td>
<td>51.7</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>427</td>
<td>428</td>
<td>405</td>
<td>149</td>
<td>35.0</td>
</tr>
<tr>
<td>AUC_{0-t} (ng ◊ h/mL)</td>
<td>6582</td>
<td>6510</td>
<td>6252</td>
<td>2169</td>
<td>32.9</td>
</tr>
<tr>
<td>AUC_{0-\infty} (ng ◊ h/mL)</td>
<td>67009</td>
<td>6558</td>
<td>6365</td>
<td>2244</td>
<td>33.4</td>
</tr>
<tr>
<td>λz (1/h)</td>
<td>0.041</td>
<td>0.040</td>
<td>0.041</td>
<td>0.0083</td>
<td>20.1</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>17.4</td>
<td>17.5</td>
<td>17.066</td>
<td>3.2</td>
<td>18.7</td>
</tr>
<tr>
<td>AUC_%Extrap_obs (%)</td>
<td>2.036</td>
<td>1.690</td>
<td>1.831</td>
<td>1.04</td>
<td>51.1</td>
</tr>
<tr>
<td><strong>Glivec 100 mg (reference product)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>3.2</td>
<td>3.0</td>
<td>2.8</td>
<td>1.5</td>
<td>47.8</td>
</tr>
<tr>
<td>Cmax (ng / mL)</td>
<td>410</td>
<td>370</td>
<td>388</td>
<td>146</td>
<td>35.6</td>
</tr>
<tr>
<td>AUC_{0-t} (ng ◊ h / mL)</td>
<td>6418</td>
<td>5999</td>
<td>6012</td>
<td>2344</td>
<td>36.5</td>
</tr>
<tr>
<td>AUC_{0-\infty} (ng ◊ h / mL)</td>
<td>6554</td>
<td>6104</td>
<td>6126</td>
<td>2440</td>
<td>37.2</td>
</tr>
<tr>
<td>λz (1 / h)</td>
<td>0.041</td>
<td>0.041</td>
<td>0.040</td>
<td>0.0088</td>
<td>21.3</td>
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<tr>
<td>t_{1/2} (h)</td>
<td>17.5</td>
<td>16.9</td>
<td>17.2</td>
<td>3.8</td>
<td>22.0</td>
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<tr>
<td>AUC_%Extrap_obs (%)</td>
<td>2.141</td>
<td>1.715</td>
<td>1.871</td>
<td>1.45</td>
<td>67.7</td>
</tr>
</tbody>
</table>
Table 3. Plasma pharmacokinetic parameters of N-desmethyl imatinib after a single dose administration of the Imatynib-Biofarm 100 mg (test product) and the Glivec 100 mg (reference product).

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Imatynib-Biofarm 100 mg (test product)</th>
<th>Glivec 100 mg (reference product)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arithmetic mean</td>
<td>Median</td>
</tr>
<tr>
<td>T_max (h)</td>
<td>3.2</td>
<td>3.0</td>
</tr>
<tr>
<td>C_max (ng/mL)</td>
<td>40.6</td>
<td>37.1</td>
</tr>
<tr>
<td>AUC_{0-t} (ng ◊ h/mL)</td>
<td>937</td>
<td>861</td>
</tr>
<tr>
<td>AUC_{0-∞} (ng ◊ h/mL)</td>
<td>1136</td>
<td>1016</td>
</tr>
<tr>
<td>λz (1/h)</td>
<td>0.017</td>
<td>0.017</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>17.3</td>
<td>16.0</td>
</tr>
<tr>
<td>AUC_%,Extrap_obs (%)</td>
<td>17.3</td>
<td>16.0</td>
</tr>
</tbody>
</table>

CI of the point estimate (test over reference products) for AUC_{0-t}, AUC_{0-∞} and for C_max falls within the 80.00–125.00% range and when the Schuirmann’s TOST test (two one-sided t-test) was complied (p < 0.05) (28, 29, 32, 33). The statistical analysis for T_max was performed on the untransformed data using the non-parametric Wilcoxon test.

**Tolerability/safety analysis**

In order to prevent the occurrence of an adverse events during the study, the following measures have been taken: the drug administration was limited to a single oral dose of 100 or 400 mg/study period; only healthy adult volunteers with no history of hypersensitivity reactions to the drug or other related molecules were enrolled; the investigator has checked each volunteer’s well being prior to his/her discharge from the clinic. Tolerability and safety were determined by monitoring vital signs (blood pressure, heart rate, body temperature) at baseline and at the end of each period. Laboratory results (hematology, urinalysis, blood biochemistry) collected before and after the study of all the subjects were also considered. The participants were interviewed by the physician as well as nonspecific questioning. All the subjects were advised to report any adverse event or undesirable sign or symptom at any time during the study period.

**RESULTS**

**Study population**

The study was conducted in 43 (Imatinib-Biofarm 100 mg, film coated tablet) and in 42 (Imatinib-Biofarm 400 mg, film coated tablet) Caucasian non-smoking healthy male and female subjects. In each studies 37 subjects completed all phases of the study. The results of the physical examination for all subjects during the pre-study visit and post-study visit were found to be normal. No subject abandoned the study for any reason. The summary of the demographic data of the population is presented in Table 1. The clinical part of the study with 100 mg of Imatinib-Biofarm was completed without deaths, serious adverse events and suspected unexpected serious adverse reactions. During the study, a total number of adverse events was 35. During the whole study period, 35 non-serious adverse events were reported in 18 subjects. No deaths or serious adverse events were reported during the study. Twelve adverse events occurred in treatment period I, 1 adverse event in wash-out period, 15 in treatment period II and 7 adverse events occurred during the follow-up examination. Twenty adverse events were of mild intensity and 15 of moderate. There was 1 adverse event classified as significant adverse event — allergic reaction. Moreover, there were 6 adverse
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events in post-study laboratory values estimated as clinically relevant abnormalities. One adverse event was related to the study drug, 20 adverse events were possible related and 14 were not related to the study drug. All adverse events were resolved.

In the study for Imatynib-Biofarm 400 mg dose, the 43 non-serious adverse events were reported in 21 subjects for both product (test and reference). No death or serious adverse events were reported during the study. Twenty three adverse events occurred in treatment period I, 2 adverse events in wash-out period, 8 in treatment period II and 10 adverse events occurred during the follow-up examination. Thirty one adverse events were of mild nature, 11 moderate and 1 severe. Twenty nine adverse events were assessed as possibly related to the study drug and 14 as not related to the study drug. All adverse events were resolved. The adverse events were: ALT increased (4 events), vascular access complication (4 events), blood glucose increased (3 events), blood triglycerides increased (3 events), constipation (3 events), AST increased (2 events), CRF increased (2 events), weakness (2 events), abnormal body temperature (1 event), back pain (1 event), bad feeling (1 event), blood cholesterol increased (1 event), blood pressure increased (1 event), cough (1 event), diarrhea (1 event), dry skin (1 event), faint (1 event), headache (1 event), loose stools (1 event), muscle pain (1 event), nausea (1 event), pain of orchis (1 event), proteinuria (1 event), rhinitis (1 event), sleep disturbances (1 event), WBC abnormal (1 event), wind (1 event) and vertigo (1 event).

Figure 1. Linear plot of mean plasma concentration of imatinib versus time curves after administration of reference product- R (Glivec) and test product-T (Imatynib-Biofarm) under fed conditions in healthy volunteers. A: Glivec – 100 mg, Imatynib-Biofarm – 100 mg; B: Glivec – 400 mg, Imatynib-Biofarm – 400 mg
Pharmacokinetics and bioequivalence analysis

The mean plasma concentrations vs. time profiles after a single oral administration of both products with imatinib in two doses are shown in Figure 1, whereas its metabolite, N-desmethyl imatinib, in Figure 2. The descriptive statistics of pharmacokinetic parameters is shown in Tables 2, 4 for imatinib and in Tables 3, 5 for metabolite – N-desmethyl imatinib, respectively. It appeared that one cannot reject the hypothesis on the ln-normal distribution of the AUC_{0-t}, AUC_{0-∞}, AUC_{%Extrap \_obs}, C_{max}, t_{1/2} and Iz for both test and reference products at the significance level \( \alpha = 0.05 \). The \( T_{max(s)} \) distribution values were significantly different from the normal distribution; therefore, in the subsequent analysis non-parametric tests were used for the evaluation. All primary pharmacokinetic parameters, i.e., AUC_{0-∞}, AUC_{0-∞} and C_{max}, met the bioequivalence regulatory criteria and there were no statistical differences between proper values of the pharmacokinetic parameters (Tables 6, 7). The descriptive statistics for \( \lambda_{z} \) and \( t_{1/2} \) were similar for the test and reference products and there were no statistical differences between proper values of these pharmacokinetic parameters (Tables 2–5). The descriptive statistics of \( T_{max} \) of imatinib were similar for the test and reference products (Tables 2, 4), but lower variability in \( T_{max} \) was observed for the test product (CV = 47.8\% (100 mg) and 39.6\% (400 mg)) than for the reference product (CV = 51.7\% (100 mg) and 58.6\% (400 mg)), respectively. However, since p-value for Wilcoxon-Signed-Rank Test was \( p > 0.05 \), therefore...
the null hypothesis was not rejected and led to conclude that the difference between the two treatments R and T (both for 100 and 400 mg) with respect to pharmacokinetic parameter $T_{\text{max}}$ was not significant. Similarly, there were not significant differences between $T_{\text{max}}$ of N-desmethyl imatinib for test and reference products both in the dose of 100 and 400 mg (Tables 3, 5).

**Tolerability/safety**

Both products in two doses were well tolerated. Thirty seven subjects completed the study without any significant serious adverse events. No clinically significant abnormalities on physical examination including vital signs measurement, ECG recordings and laboratory results were observed.

**DISCUSSION**

The aim of the study was to evaluate the bioequivalence between the test (Imatynib-Biofarm manufactured by Biofarm Sp. z o.o.) and the reference (Glivec manufactured by Novartis Pharma GmbH) products. The clinical part of the study was designed in compliance with the respective EMA guidelines (28, 29). Based on the imatinib elimination half-life of 18 h (22, 26), and taking into account imatinib metabolite long half-life (about 40 h) (34) and that to avoid carryover effect in second study period washout period should be at least 5 times of the half-life long, wash-out period of 14 days was chosen in that study. For all profiles, the AUC$_{\text{r}}$ was at least 80% of the AUC$_{\text{t},s}$, which confirmed the proper duration of sampling. The current study demonstrated comparable bioavailability of Imatynib-Biofarm 100 and 400 mg film-coated tablets with Glivec 100 and 400 mg film-coated tablets, respectively. The pharmacokinetic values ($C_{\text{max}}$, $T_{\text{max}}$, and AUC) for N-desmethyl imatinib were also found to be nearly identical for both the test and reference dose forms of imatinib. If the 90% confidence interval of two one-sided tests for ratio of geometric means (test formulation Imatynib-Biofarm over reference formulation Glivec) for both $C_{\text{max}}$ and AUC$_{\text{r},s}$ of imatinib were included entirely in the acceptance range of 80.00–125.00%, the test formulation (Imatynib-Biofarm) was assumed as bioequivalent to the reference formulation (Glivec). The similar pharmacokinetic property of the metabolite further supports the bioequivalence of the tablet product. This study indicated relatively rapid absorption of the 100 and 400 mg tablet (median $T_{\text{max}}$ 2.5 and 2.5 h, respectively) after oral administration that was comparable with that of Glivec tablets ($T_{\text{max}}$ 3.0 and 4.0, respectively). The coefficient for varia-

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Arithmetic mean</th>
<th>Median</th>
<th>Geometric mean</th>
<th>SD</th>
<th>CV [%]</th>
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</thead>
<tbody>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>2.9</td>
<td>2.5</td>
<td>2.5</td>
<td>1.7</td>
<td>58.6</td>
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<td>$C_{\text{max}}$ (ng/mL)</td>
<td>15477</td>
<td>1549</td>
<td>1452</td>
<td>579</td>
<td>37.4</td>
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<td>AUC$_{\text{r},s}$ (ng ◊ h/mL)</td>
<td>24705</td>
<td>22562</td>
<td>23295</td>
<td>8902</td>
<td>36.0</td>
</tr>
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<td>AUC$_{\text{r},s}$ (ng ◊ h/mL)</td>
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<td>22808</td>
<td>23640</td>
<td>9005</td>
<td>35.9</td>
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<tr>
<td>$\lambda$ (1/h)</td>
<td>0.043</td>
<td>0.043</td>
<td>0.043</td>
<td>0.0065</td>
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<td>$t_{1/2}$ (h)</td>
<td>16.4</td>
<td>16.0</td>
<td>16.2</td>
<td>2.6</td>
<td>15.9</td>
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<td>AUC$_{%\text{Extrap}\text{obs}}$ (%)</td>
<td>1.45</td>
<td>1.14</td>
<td>1.27</td>
<td>0.86</td>
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<th>Geometric mean</th>
<th>SD</th>
<th>CV [%]</th>
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<td>$T_{\text{max}}$ (h)</td>
<td>3.5</td>
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<td>3.2</td>
<td>1.38</td>
<td>39.6</td>
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<td>$C_{\text{max}}$ (ng / mL)</td>
<td>1506</td>
<td>1439</td>
<td>1443</td>
<td>459</td>
<td>30.5</td>
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<tr>
<td>AUC$_{\text{r},s}$ (ng ◊ h / mL)</td>
<td>24545</td>
<td>24149</td>
<td>23437</td>
<td>778</td>
<td>31.7</td>
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<tr>
<td>AUC$_{\text{r},s}$ (ng ◊ h / mL)</td>
<td>24913</td>
<td>24304</td>
<td>23772</td>
<td>7957</td>
<td>31.9</td>
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<tr>
<td>$\lambda$ (1 / h)</td>
<td>0.044</td>
<td>0.044</td>
<td>0.044</td>
<td>0.007</td>
<td>15.6</td>
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<td>$t_{1/2}$ (h)</td>
<td>15.9</td>
<td>15.9</td>
<td>15.8</td>
<td>2.44</td>
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<td>AUC$_{%\text{Extrap}\text{obs}}$ (%)</td>
<td>1.41</td>
<td>1.20</td>
<td>1.19</td>
<td>0.88</td>
<td>62.7</td>
</tr>
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</table>
tion for $T_{\text{max}}$, $C_{\text{max}}$ and AUCs showed considerable intersubject variability (up to 15.7%). Nikolova et al. (34) reported that the intra subject variability for $C_{\text{max}}$ was on the level of 20%. Although the cause of this was not clear, it is below observed in the above mentioned study and may be attributed to intersubject differences in plasma proteins binding to the parent compound or to variations in CYP3A4, the major CYP isoenzyme involved in the microsomal metabolism of imatinib. It is known that variability in CYP3A activity between individuals is large (35) and may in part have contributed to the large intersubject variability.

There were no serious adverse events reported during the conduct of the trials. Only 1 adverse event was assessed as significant. Based on the clinical results for the parent drug, the study clearly demonstrated that new formulations of imatinib (Imatynib-Biofarm 100 and 400 mg film-coated tablet) were tolerated in the same way as reference drug (Glivec 100 and 400 mg film-coated tablet).

Table 5. Plasma pharmacokinetic parameters of N-desmethyl imatinib after a single dose administration of the Imatynib-Biofarm 400 mg (test product) and the Glivec 400 mg (reference product).

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Imatynib-Biofarm 400 mg (test product)</th>
<th>Glivec 400 mg (reference product)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arithmetic mean</td>
<td>Median</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>2.6</td>
<td>2.0</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>168</td>
<td>151</td>
</tr>
<tr>
<td>$AUC_{\text{bio}}$ (ng × h/mL)</td>
<td>3698</td>
<td>3477</td>
</tr>
<tr>
<td>$AUC_{\infty}$ (ng × h/mL)</td>
<td>4561</td>
<td>4318</td>
</tr>
<tr>
<td>$\lambda z$ (1/h)</td>
<td>0.017</td>
<td>0.017</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>43.9</td>
<td>40.8</td>
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<tr>
<td>$AUC_{%\text{Extrap}}$</td>
<td>18.4</td>
<td>16.7</td>
</tr>
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</table>

Table 6. The 90% confidence intervals based on the Schuirmann’s TOST test and using mean square error estimated from ANOVA analysis of pharmacokinetic parameters for imatinib.

<table>
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<tr>
<th>Pharmacokinetic parameter</th>
<th>100 mg</th>
<th>400 mg</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Point estimate [%]</td>
<td>90% confidence interval [%]</td>
</tr>
<tr>
<td>$AUC_{\text{bio}}$</td>
<td>104.2</td>
<td>99.3–109.2</td>
</tr>
<tr>
<td>$AUC_{\infty}$</td>
<td>104.1</td>
<td>99.2–109.1</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>104.6</td>
<td>98.4–111.2</td>
</tr>
<tr>
<td>$AUC_{\text{bio}}$</td>
<td>99.6</td>
<td>94.8–104.7</td>
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<tr>
<td>$AUC_{\infty}$</td>
<td>99.7</td>
<td>94.8–104.8</td>
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<tr>
<td>$C_{\text{max}}$</td>
<td>100.7</td>
<td>94.8–107.0</td>
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</table>
CONCLUSION

The results of this single-dose study in healthy white volunteers indicated that Imatynib-Biofarm 100 and 400 mg film-coated tablets manufactured by Biofarm Sp. z o.o. (test products) are bioequivalent to Glivec 100 and 400 mg film-coated tablets manufactured by (Novartis Pharma GmbH) (reference products), even though the imatinib mesylate as the active substance in these preparations differs in polymorphic forms, as it was mentioned earlier. Both products were well tolerated. It should be noted that the results for AUC (for imatinib and N-desmethyl imatinib in the study for 100 mg and 400 mg) are generally fitting in the limited acceptance interval for drug in narrow therapeutic index 90.00–111.11%. It is an important premise allowing to conclude the high quality of tested products in term of bioequivalence.

Acknowledgments

This study was supported by Biofarm Sp. z o.o. We thank Lambda Therapeutic Research for the imatinib and N-desmethyl imatinib assays in blood of volunteers and the raw data analysis.

REFERENCES


Table 7. The 90% confidence intervals based on the Schuirmann’s TOST test and using mean square error estimated from ANOVA analysis of pharmacokinetic parameters for N-desmethyl imatinib.

<table>
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<th>Pharmacokinetic parameter</th>
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<tr>
<td></td>
<td>Point estimate [%]</td>
<td>90% confidence interval [%]</td>
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<tr>
<td>AUC&lt;sub&gt;0-t&lt;/sub&gt;</td>
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<td>96.6-104.7</td>
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