Pharmacokinetics of paclitaxel and three major metabolites in patients with advanced breast carcinoma refractory to anthracycline therapy treated with a 3-hour paclitaxel infusion: A European Cancer Centre (ECC) trial


1Department of Medical Oncology, Free University Hospital; 2Department of Pharmacy, Slotervaart Hospital; 3Department of Medical Oncology, Antoni van Leeuwenhoek Hospital/Netherlands Cancer Institute, Amsterdam, The Netherlands

Summary

Background: Hepatic metabolism and biliary clearance play pivotal roles in the disposition of the anticancer drug paclitaxel. 6-α-hydroxypaclitaxel, 3′-p-hydroxypaclitaxel and 6-α,3′-p-dihydroxypaclitaxel were the major metabolite products of paclitaxel found in human bile. Recently, these metabolic products were detected in human plasma. The pharmacokinetics of paclitaxel and its metabolites were investigated in anthracycline-resistant breast cancer patients treated with high-dose paclitaxel and granulocyte colony-stimulating factor (G-CSF) support.

Patients and methods: Nine patients were entered into this study in which paclitaxel was administered at the relatively high dose of 250 mg/m² during a 3-hour infusion. G-CSF was administered daily subcutaneously (s.c.) on days 2 to 19 following chemotherapy. Analysis of paclitaxel and metabolite concentrations was performed by a new highly sensitive reversed-phase high performance liquid chromatographic (HPLC) assay.

Results: The dose-limiting toxicity in this study was cumulative neurotoxicity. One patient had a partial response and 2 patients had mixed responses of their skin metastases. Relatively low peak plasma concentration (Cmax), with mean values of 6.91 μmol/L (range 3.08 to 8.98) and area under the plasma concentration time curve (AUC), with mean values of 27.04 μmol·L·h (range 14.88 to 40.57), were observed. The total body clearance was 16.99 L/h (range, 10.25 to 27.39). The pharmacokinetic parameter for the prediction of leuko-neutropenia, the duration of the plasma concentration above the threshold of 0.1 μmol/L (T > 0.1 μM), was 19.72 h (range 10.54 to 26.31). The three major metabolites detected in human plasma were identified as 6-α-hydroxypaclitaxel, 3′-p-hydroxypaclitaxel and 6-α,3′-p-dihydroxypaclitaxel. Cmax and AUC values of these metabolites are reported.

Conclusions: The three major metabolic products of paclitaxel in human plasma are 6-α-hydroxypaclitaxel, 3′-p-hydroxypaclitaxel and the dihydroxy metabolite 6-α,3′-p-dihydroxy paclitaxel. Two patients with liver function disturbances showed a tendency to higher paclitaxel and 6-α-hydroxypaclitaxel AUC levels, with more pronounced neurotoxicity.

Key words: paclitaxel (Taxol®), hydroxylated metabolites, breast cancer, anthracycline, resistance, pharmacokinetics

Introduction

Paclitaxel (Taxol®, Fig. 1) is a novel antitumour agent which promotes the formation and stabilisation of microtubules [1]. In early clinical studies it displayed a broad spectrum of antitumour activity in solid tumours, including platinum-resistant ovarian cancer and metastatic breast cancer pretreated with anthracyclines [2]. The anthracyclines are still the cornerstone in the chemotherapeutic treatment of breast cancer. Disease progression, however, is mostly due to resistance of the tumour cells to anthracyclines, so identification of new drugs that lack cross-resistance is very much warranted. Although paclitaxel showed cross-resistance in multi-drug-resistant murine P388 leukaemia cells [3], there are indications in the clinic that the cross-resistance between paclitaxel and doxorubicin may be incomplete. Several clinical studies addressing a possible role of paclitaxel in the treatment of breast cancer patients resistant to an anthracycline have now been published [4–7].

Despite the use of the different dosages and infusion durations reported by several authors, the optimal dose and schedule of paclitaxel for the treatment of anthracycline-resistant breast cancer are still unknown [8].

Within a multi-centre setting under the auspices of the European Cancer Centre (ECC), a study with a 3-hour infusion of high-dose paclitaxel was conducted in anthracycline-resistant breast cancer patients. Short-term infusions of paclitaxel induces less bone marrow suppression, neutropenic fever and mucositis than do prolonged infusions. A second advantage may be the possibility of a dose intensification which does not appear to be feasible with the prolonged infusions with which the maximal tolerated dose was 36 mg/m²/d for 4 days [6].
Drug administration

Paclitaxel (Taxol\textsuperscript{\textregistered}, Bristol Myers Squibb, Syracuse, NY) for clinical use is provided as a concentrated sterile solution with 6 mg/ml in a 5 ml vial in a mixture of cremophor EL\textsuperscript{\textregistered} and dehydrated alcohol (1:1, vol/vol). This was diluted before use with 1000 ml 0.9% sodium chloride solution. The paclitaxel concentration in this solution did not exceed 0.6 mg/ml. The drug was administered as a 3-hour continuous intravenous infusion. Cremophor EL\textsuperscript{\textregistered} may leach plasticizers from solution bags containing polyvinyl chloride [13]; therefore, drug solutions were administered through an IVAC IV administration set with low sorbing tubing (IVAC Corp, San Diego, CA) or 2262 Gemini 20 IMED primary administration set for nitroglycerine and sat emulsion (IMED Corp, San Diego, CA). An IVEX-2 vented filterset (0.22 μm; Millipore, Molsheim, France) was inserted in the infusion line.

Patients were treated with a 3-hour infusion 250 mg/m\textsuperscript{2} of paclitaxel every 3 weeks. Premedication consisted of 20 mg of dexamethasone orally 6 and 12 hours prior to the infusion, clemastine 2 mg IV, and cimetidine 50 mg IV 30 minutes before paclitaxel infusion. All patients received G-CSF (Neupogen\textsuperscript{\textregistered}, Roche-Angen, Mijdrecht, The Netherlands) 5 μg/kg/d i.e. on days 2 through 19, or until the ANC recovery was ≥2000/μL. Following treatment, patients were monitored for myelosuppression on the basis of twice weekly complete blood cell counts.

Pharmacokinetics

Analytical techniques

Paclitaxel and metabolites were assayed in plasma using a sensitive HPLC assay with solid phase extraction as sample pretreatment procedure, previously described [9, 10]. The three major metabolites of paclitaxel (6-α-hydroxypaclitaxel, 3'-p-hydroxypaclitaxel and 6-α,3'-p-dihydroxypaclitaxel) were isolated from the faces of cancer patients treated with paclitaxel and chromatographically purified and structurally identified by mass spectrometry, as reported by Sparreboom et al. [11]. These compounds were used as reference standards for the quantitation of the metabolites in plasma. The procedures including sample pretreatment, for the analysis of the metabolites were identical to those for paclitaxel [9, 10].

The lower limit of quantification for all three metabolites was 10 ng/ml. The calibration curves were linear over the concentration ranges tested, i.e., 10–1000 ng/ml. The extraction recoveries of 6-α,3'-p-dihydroxypaclitaxel, 3'-p-hydroxypaclitaxel, and 6-α,3'-p-dihydroxypaclitaxel were 78, 91, 89 and 89%, respectively.

Pharmacokinetic sampling

Paclitaxel pharmacokinetic sampling was performed during the first cycle except in patient 2 (course 4) and patient 5 (course 2). Plasma samples were collected by IV sampling from the uninfused arm. Sampling times were prior to start, 1.5 hour after start, at the end of the infusion, and at 6, 18, 30 and 60 minutes and 2, 4, 8, 12, 24, 30 and 48 hours post-infusion.

The patients’ whole-blood samples (5 ml) were collected in EDTA tubes and immediately centrifuged (3000 rpm for 10 minutes), the plasma was removed, frozen at −20 °C and assayed within 4 weeks.

Pharmacokinetic and pharmacodynamic analysis

The post-infusion plasma disappearance curves were modelled by using the Kinet drug program (MWPharm, Mediware BV, Groningen, The Netherlands) [13]. This non-linear least squares, iterative regression program determines slopes and intercepts of the logarithmically plotted curves of multi-exponential functions and provides a correlation coefficient of the fitted curve. The post-
infusion paclitaxel kinetics are best described by the use of a triexponential function (n = 3):
\[ C(t) = \sum_{i=1}^{N} C_i \times e^{(-\lambda_i \times t)} \]

where \( \lambda_i \) is the exponent of the i-th exponential term, \( C_i \) is the initial concentration of the i-th component of the curve and \( C(t) \) is the paclitaxel concentration at time t. Curve fitting with this model yields the parameters \( C_1, C_2, \lambda_1, \lambda_2, \) and \( \lambda_3 \). The half-lives are calculated from the equations \( \lambda_1(a) = 0.693/\lambda_1, \lambda_2(b) = 0.693/\lambda_2, \) and \( \lambda_3(c) = 0.693/\lambda_3 \). Other pharmacokinetic parameters were calculated by a non-compartmental analysis. The total area under the curve (AUC_{total}) was calculated using the linear trapezoidal method with extrapolation of the terminal phase to infinity (C_{t=\infty/\lambda_3}), where C_{t=\infty} is the last measured concentration. The area under the moment curve (AUC_{0-\infty}) was also calculated by the trapezoidal rule with extrapolation to infinity ((C_{t=0} \times t_{\text{test}})/\lambda_3 + C_{t=\infty}/\lambda_3), with t_{\text{test}} as the last measured time point with C_{t=\infty}. The mean residence time (MRT) was calculated by dividing the AUC by the AUMC.

Total body clearance (CL_B) was calculated by dividing the Dose (D_N) by the AUC_{total}. Volume at steady state (V_ss) was calculated with the equation [14]:
\[ V_{ss} = \frac{D_N \times AUMC}{AUC^2} \]

where T is the infusion time.

The peak plasma concentrations (C_{max}) were the highest measured values. The time spent above the threshold concentration of 0.1 \( \mu \)M (T > 0.1 \( \mu \)M) was derived graphically from the pharmacokinetic curves. The AUCs of the metabolite products were determined using the trapezoidal rule without extrapolation to infinity. The C_{max} (and the T_{max}) for the three major metabolites are the highest measured values.

Relationships between paclitaxel pharmacokinetic parameters and metabolic pharmacokinetic parameters were evaluated by linear regression. Likewise, relationships between alkaline phosphatase (AP) and the paclitaxel AUC and T > 0.1 \( \mu \)M were investigated by linear regression. AUC and T > 0.1 \( \mu \)M for patients with liver involvement or neurotoxicity were also compared with those of patients with no liver involvement or neurotoxicity by the Students' t-test. Arbitrarily, we use the terms significant and non-significant according to whether the p value is less or greater than 0.05, respectively.

Results

A total of 9 patients were entered in the pharmacokinetic part of this trial. All patients had metastatic breast cancer and were pretreated with either doxorubicin or epirubicin. Eight patients were eligible for this trial. In principle, one patient (no. 3) was not eligible due to progressive disease 3 months after the last anthracycline infusion. All patients were considered assessable for toxicity and response evaluation. The median age of the population was 53 years (range 33 to 63) with a median ECOG performance status of 1 (range 0 to 1). A total of 35 courses of paclitaxel was administered to the patients who participated in this trial; the median number was 4 (range 1 to 8). The reason for discontinuation of therapy was disease progression in all cases. Clinical data of this study will be presented elsewhere.

Table 1. Toxicity data according to the WHO criteria.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Myelosuppression</th>
<th>Neutropenia</th>
<th>Myalgia</th>
<th>Arthralgia</th>
<th>Diarrhoea</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0/0</td>
<td>II</td>
<td>I</td>
<td>-</td>
<td>I</td>
</tr>
<tr>
<td>2</td>
<td>0/0</td>
<td>II</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>0/0</td>
<td>I</td>
<td>-</td>
<td>I</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>0/0</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>5</td>
<td>0/0</td>
<td>-</td>
<td>-</td>
<td>III</td>
<td>I</td>
</tr>
<tr>
<td>6</td>
<td>0/0</td>
<td>-</td>
<td>II</td>
<td>I</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>0/0</td>
<td>III/II</td>
<td>-</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>8</td>
<td>0/0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>I</td>
</tr>
<tr>
<td>9</td>
<td>0/0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\[ a \] Pharmacokinetic monitoring during course 4.

\[ b \] Pharmacokinetic monitoring during course 2, patient had itching in both legs.

\[ c \] Not available for toxicity follow-up.

Toxicity

Table 1 lists the toxicity observed after the courses with pharmacokinetic monitoring. G-CSF was discontinued at an early stage (mean range 2–10 days) in all patients, as the absolute neutrophil count (ANC) was \( \geq 2,000/\mu L \). A transient leuko-neutropenia occurred in one patient (patient no. 8). Fever occurred in three patients (patients nos. 1, 3 and 4) with no signs of infection, and leukocyte counts were between 8,000 and 10,000 \( \mu L \). In one patient there was a documented infection which required antibiotic treatment. Mild thrombocytopenia occurred in 3 patients. Paresthesias of fingers and toes occurred frequently and was cumulative. Only mild paresthesias occurred after the first cycle. In one patient (patient no. 5) severe arthralgia occurred in combination with itching of the legs, and diarrhoea.

One patient (no. 9) was not eligible for toxicity follow-up. Four days after paclitaxel infusion intrathecal administration of morphine and lidocaine was needed because of pain intensification.

Chromatography

A total of 11 (I–XI) peaks (putative metabolites) were seen in the chromatograms of all patients. The major metabolites were compounds I, II, V. These three products were identified as 6-\( \alpha \)-hydroxy paclitaxel (V), 3'-\( \beta \)-hydroxy paclitaxel (II) and 6-\( \alpha \),3'-\( \beta \)-dihydroxy paclitaxel (I) with relative retention times (retention time of metabolite divided by the retention time of paclitaxel) of 0.70, 0.49 and 0.38, respectively.

Pharmacokinetic analysis

The pharmacokinetic parameters of paclitaxel in the 9 patients treated with 250 mg/m\(^2\) during a 3-hour infusion are listed in Table 2.

Pharmacokinetic analysis of the drug concentration versus time curves for each patient revealed wide interindividual differences in \( C_{\text{max}}, \) AUC, T > 0.1 \( \mu \)M, Cl_T
Table 2. Pharmacokinetic parameters of paclitaxel (250 mg/m²) in patients treated with a 3-hour infusion.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>C_{max} (µmol/L)</th>
<th>AUC (µmol/L·h)</th>
<th>Cl_{f} (L/h)</th>
<th>t_{1/2} hours</th>
<th>V_{ss} (L/m²)</th>
<th>MRT (hours)</th>
<th>T &gt; 0.1 µM (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.87</td>
<td>37.78</td>
<td>12.4</td>
<td>0.49</td>
<td>1.89</td>
<td>10.77</td>
<td>38.06</td>
</tr>
<tr>
<td>2</td>
<td>8.02</td>
<td>34.27</td>
<td>15.03</td>
<td>-</td>
<td>0.96</td>
<td>11.74</td>
<td>42.03</td>
</tr>
<tr>
<td>3</td>
<td>3.08</td>
<td>15.09</td>
<td>27.03</td>
<td>0.31</td>
<td>2.18</td>
<td>12.89</td>
<td>92.61</td>
</tr>
<tr>
<td>4</td>
<td>6.54</td>
<td>23.34</td>
<td>22.32</td>
<td>-</td>
<td>0.73</td>
<td>13.22</td>
<td>45.80</td>
</tr>
<tr>
<td>5</td>
<td>6.13</td>
<td>21.72</td>
<td>13.48</td>
<td>0.21</td>
<td>1.16</td>
<td>7.85</td>
<td>29.35</td>
</tr>
<tr>
<td>6</td>
<td>6.36</td>
<td>21.22</td>
<td>13.60</td>
<td>0.17</td>
<td>1.79</td>
<td>14.24</td>
<td>40.70</td>
</tr>
<tr>
<td>7</td>
<td>8.96</td>
<td>27.47</td>
<td>19.18</td>
<td>0.17</td>
<td>1.63</td>
<td>15.49</td>
<td>56.39</td>
</tr>
<tr>
<td>8</td>
<td>6.39</td>
<td>22.15</td>
<td>19.04</td>
<td>0.22</td>
<td>1.41</td>
<td>10.34</td>
<td>59.06</td>
</tr>
<tr>
<td>9</td>
<td>7.85</td>
<td>40.57</td>
<td>10.25</td>
<td>0.49</td>
<td>3.69</td>
<td>33.50</td>
<td>43.82</td>
</tr>
</tbody>
</table>

and V_{ss}. The average C_{max} value of paclitaxel was 6.92 ± 1.81 µmol/L (range 3.08 to 8.98). After cessation of the infusion the plasma concentration declined in a tri-exponential fashion with a final elimination half-life ranging from 7.85 to 33.50 hours. The mean AUC values of paclitaxel were 27.07 ± 8.61 µmol/L·h (range 15.09 to 40.57). The mean distribution volume at steady stage (V_{ss}) was 49.75 ± 19 L/m².

Four patients (Nos. 1, 2, 7 and 9) suffered from either liver metastasis or mild liver function disturbances (slightly elevated serum AP, low albumin or increased SGOT). The median AUC of these patients with liver involvement was 30.3 µmol/L·h (range 21.72 to 37.78), while in patients with no signs of liver metastasis or liver function impairment it was 20.45 (range 15.09 to 23.34) (p=0.049). The mean T > 0.1 µM values were 22.62 ± 4.07 (range 10.54 to 20.14) for patients with liver involvement, and 16.04 ± 3.22 (range 19.35 to 26.31) for those without liver involvement (p=0.044). Patients with neurotoxicity grade II (patient 1 and 2) had significantly (p<0.05) higher AUC values (median: 36.03 µmol/L·h) than the patients (n=6) with grade 0 and 1 neurotoxicity (21.83 µmol/L·h).

Because of the irregularity of the plasma concentration versus time curves of the metabolites, it was not possible to fit them with exponential terms (Fig. 2).

The AUCs of paclitaxel were strongly correlated with the AUCs of 6-α-hydroxyplactaxel (p=0.006) and 3'-p-hydroxyplactaxel (p=0.005).

The AUCs of 6-α-hydroxyplactaxel were also positively correlated with the AP (p=0.002). The mean C_{max} values were 0.53 ± 0.24 µmol/L (range 0.24 to 0.86), 0.22 ± 0.14 µmol/L (range 0.12 to 0.52) and 0.20 ± 0.10 µmol/L (range 0.07 to 0.41) for 6-α-hydroxyplactaxel, 3-p-hydroxyplactaxel and 6-α,3'-p-dihydroxyplactaxel, respectively (Table 3). The peak plasma concentration ratio of paclitaxel and the major metabolite 6-α-hydroxyplactaxel is approximately 14.3. For the other two metabolites it is about 33. All 3 metabolites were detected in plasma for only a limited period of time (Table 3). One patient (no 9) exhibited a different pharmacokinetic profile. A high AUC was observed for 3'-p-hydroxyplactaxel, the major metabolite rather than the 6-α-hydroxyplactaxel found in the other patients. The AUC ratio in this case was 13.1 and 21.5 and the duration of circulation time of detectable levels of all three metabolites was more than 10 hours. Paclitaxel concentrations were detectable for more than 60 hours (t_{1/2}, γ = 33.5 h) in the plasma of this patient.

**Discussion**

The detection of metabolic products of paclitaxel in human bile (6-α-hydroxyplactaxel, 3'-p-hydroxyplactaxel and 6-α,3'-p-dihydroxyplactaxel) [15] and putative metabolic products in human plasma indicate that hepatic metabolism and biliary excretion probably play major roles in the plasma disposition of paclitaxel and metabolites [10, 16].

Recently, the main metabolic product in human plasma was identified and quantified as 6-α-hydroxyplactaxel [16]. Before then the quantification of metabolites in human plasma was not reported. Development of a quantitative assay for the metabolites
was hampered by the lack of sufficient amounts of paclitaxel metabolites as reference material for the validation. We used a relatively simple isolation procedure to obtain sufficient paclitaxel metabolites from faeces of patients treated with paclitaxel and, after purification and structural identification, they were applied as reference standards for the HPLC assay [11].

The major paclitaxel metabolite found in human plasma was 6-α-hydroxypaclitaxel. It reaches its maximal concentration several minutes after the end of paclitaxel infusion and is rapidly eliminated from the plasma. On this dosage regimen this metabolite circulates for about 6 hours in the central plasma compartment. The most active metabolic product in vitro, 3'-p-hydroxypaclitaxel, [11] appears later in the circulation than 6-α-hydroxypaclitaxel and reaches its maximum concentration after approximately 0.1 to 0.5 hours after the end of the infusion. Apparently hydroxylation at the C6 position is very much favoured in humans. The dihydroxy derivative appears about 15 minutes later in the circulation than the mono-hydroxylated products.

Several studies reported a stronger correlation between neuro-muscular toxicity and paclitaxel AUC levels than with the administered dose [17]. Our study is in concordance with this observation, marked neurotoxicity (grade II) was observed in two patients who achieved the highest AUC values.

Wilson et al., recently reported a significant association between liver metastasis and paclitaxel clearance and a correlation between paclitaxel Cmax above 0.07 μM and clinical toxicity [17]. Our study indicates that the higher paclitaxel AUC levels and a prolonged duration of T > 0.1 μM were associated with liver disease and higher AP levels (liver metastasis or cholestasis).

In our previous study we described a positive correlation between the T > 0.1 μM and bone marrow suppression according to a sigmoidal Emax model [10]. In this study no correlation was seen between the myelosuppression and the T > 0.1 μM as no leucopenia occurred. This might be explained by the relatively short T > 0.1 μM in this study. However, patients with liver disease showed higher paclitaxel AUC (30.3 μM · h vs. 20.45 μM · h) levels and a prolonged T > 0.1 μM (22.62 h vs. 16 h) in comparison with patients with no liver disease. We observed an increase in neurotoxicity in these patients but not in myelosuppression, although it should be noted that the number of patients is small (n = 2).

Another interesting feature in this study is that in patients with liver function impairment and/or low albumin levels (<35 g/L) the kinetics of the metabolites were different. In this patient group the metabolites circulated over a longer period and at higher concentrations, indicating that the elimination of the two main mono-hydroxylated metabolites is also saturable.

One of the patients (no. 9) with a deviant metabolic profile received daily doses of diazepam (3 dd 10 mg). It is known from the literature that drug-drug competition and/or enzymatic induction/inhibition can alter the metabolic profile in vitro [18]. Diazepam is a strong inhibitor of the cytochrome P450 iso-enzyme C3 [19]. Normally the metabolite 6-α-hydroxypaclitaxel is the most abundant one in plasma of the patients treated in this study [10,16]. In patient 9, however, relatively low 6-α-hydroxypaclitaxel metabolite concentrations were observed with relatively high levels of the more active 3'-p-hydroxypaclitaxel. Competition between diazepam and paclitaxel for this enzyme, and leaving more of the drug available for hydroxylation at the para-position of 3'-phenyl moiety, could explain this.

Earlier pharmacokinetic studies showed that there is a disproportionate increase in both Cmax and AUC with higher doses during a 3-hour paclitaxel infusion, indicating that saturation occurs on both elimination pathways and tissue distribution [10,16,17]. The inverse non-linear relationship between Vc and steady-state concentration may indicate saturable tissue binding or saturable cellular membrane transport [17]. Our results are in agreement with this observation, in that the distribution volume at steady state (50 L/m²) was smaller than that observed in a previous study (100 L/m²) in which a lower dosage was administered [10].

Although our data are limited there is an indication that both altered plasma protein levels and impaired
hepatic metabolism may have a pronounced effects on serum levels and metabolic clearance of paclitaxel, resulting in higher paclitaxel AUC levels and a prolonged duration of a supra-threshold concentration.

Acknowledgements

The authors thank the research nurses of the Antoni van Leeuwenhoekhuis and the nurses of units 4-mid and 7-east of the Free University Hospital for excellent support of the study.

References


Received 9 January 1995; accepted 11 April 1995.

Correspondence to:
M. T. Huizing, M.D.
Dept. of Medical Oncology and Pharmacy
Louwesweg 6
1066 EC Amsterdam
The Netherlands