A Synergistic Interaction between Lapatinib and Chemotherapy Agents in a Panel of Cell Lines Is Due to the Inhibition of the Efflux Pump BCRP

Jackie Perry, Essam Ghazaly, Christiana Kitromilidou, Eva H. McGrowder, Simon Joel, and Thomas Powles

Abstract

Lapatinib is a specific HER1 and 2 targeted tyrosine kinase inhibitor now widely used in combination with chemotherapy in the clinical setting. In this work, we investigated the interactions between lapatinib and specific chemotherapy agents (cisplatin, SN-38, topotecan) in a panel of cell lines (breast (n = 2), lung (n = 2), testis (n = 4)). A high-sensitivity cell proliferation/cytotoxicity ATP assay and flow cytometry were used to determine cell viability, apoptosis, and the effect of the drugs on cell-cycle distribution. CalcuSyn analysis was employed to formally identify synergistic interactions between drugs. Intracellular concentrations of SN-38 were measured using a novel high-performance liquid chromatography (HPLC) technique. Flow cytometry and HPLC techniques were used to identify the effect of lapatinib on drug influx and efflux pumps, using specific substrates and inhibitors of these pumps. Results showed significant synergy between SN-38, and lapatinib in the majority of cell lines (combination index < 0.75), associated with increased apoptosis. This synergy was not universal but, when observed (Susa S/R, H1975, H358, and MDA-MB-231 cell lines), was related to SN-38 intracellular accumulation (2.2- to 4.8-fold increase, P < 0.05 for each), attributable to the inhibition of the breast cancer–related protein (BCRP) efflux pump by lapatinib. Flow cytometry analysis showed that lapatinib (10 μmol/L) inhibited the efflux of mitoxantrone, a specific substrate of the BCRP pump, in a manner similar to fumitremorgin C, a known BCRP inhibitor, confirming lapatinib as a BCRP inhibitor. This work shows that lapatinib has a direct inhibitory effect on BCRP accounting for the synergistic findings. The synergy is cell line dependent and related to the activity of specific efflux pumps. Mol Cancer Ther; 9(12); 3322–9. ©2010 AACR.

Introduction

Advances in molecular biology have resulted in new targets for cancer therapy. One of the more promising of these targets is the HER receptor family. HER1 targeted therapies have activity in both colorectal and lung cancers, whereas HER1 and 2 targeted therapies are widely used in breast cancer (1–3). Both monoclonal antibodies and tyrosine kinase inhibitors (TKI) have been successfully developed in this setting. As the HER1–4 receptors are dimerized, there is a potential advantage in targeting more than 1 of these receptors concurrently (4). Lapatinib, a 4-anilinoquinazoline, is one such agent that targets both HER1 and 2 receptors. Lapatinib has proven efficacy in both preclinical and clinical models (3, 5, 6). It has impressive single-agent activity and is being investigated in a range of malignancies (7). However, the most promising clinical data are seen when it is used in combination with chemotherapy, although the reasons for this are unclear. Preclinical data show that lapatinib downregulates nucleotide synthesis-related genes such as thymidylate synthase and that it may result in synergistic interactions with other chemotherapy agents (8). At the same time, it is apparent that HER targeted TKIs may affect drug efflux pumps (9, 10).

Drug efflux pumps are expressed in a broad spectrum of tissues, including the plasma membrane in stem cells, placenta, liver, small intestine, colon, lung, and kidney, suggesting their role in the protection/detoxification of xenobiotics (11, 12). They are also expressed in cancer tissues and are important in determining resistance to specific chemotherapy agents such as the taxanes and topoisomerase inhibitors (13). Drug efflux is largely mediated by the ATP-binding cassette (ABC) transporters p-glycoprotein (P-gp) ABCB1 and breast cancer–related
A Synergistic Interaction between Lapatinib and Chemotherapy

Table 1. EC50 values for drug compounds studied in breast, lung, and TGCT cell lines (fitted values from at least 3 separate experiments with 95% confidence interval)

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Lapatinib, μmol/L</th>
<th>SN-38, nmol/L</th>
<th>Cisplatin, μmol/L</th>
<th>Topotecan, nmol/L</th>
<th>Paclitaxel, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7</td>
<td>7.0 (6.2–7.9)</td>
<td>20 (10–40)</td>
<td>N/A</td>
<td>20 (9–40)</td>
<td>0.013 (0.012–0.014)</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>6.4 (3.3–12.3)</td>
<td>30 (20–30)</td>
<td>N/A</td>
<td>30 (20–60)</td>
<td>N/A</td>
</tr>
<tr>
<td>H358</td>
<td>8.7 (5.4–13.9)</td>
<td>40 (10–100)</td>
<td>13.5 (9.8–18.5)</td>
<td>1,240 (1,150–1,340)</td>
<td>20 (10–30)</td>
</tr>
<tr>
<td>H1975</td>
<td>20 (12.6–31.7)</td>
<td>170 (90–340)</td>
<td>13.9 (11.0–17.6)</td>
<td>1,420 (1,040–1,920)</td>
<td>14 (10–19)</td>
</tr>
<tr>
<td>Susa S</td>
<td>4.0 (3.7–4.4)</td>
<td>9.8 (5.7–16.9)</td>
<td>0.50 (0.46–0.56)</td>
<td>N/A</td>
<td>5.1 (4.3–6.1)</td>
</tr>
<tr>
<td>Susa R</td>
<td>3.8 (2.9–4.8)</td>
<td>7.2 (5.5–9.5)</td>
<td>1.9 (1.6–2.3)</td>
<td>N/A</td>
<td>4.7 (3.8–5.8)</td>
</tr>
</tbody>
</table>

NOTE: Breast, lung, and TGCT cell lines were treated at 0 and 5 nonzero concentrations of each study drug for 72 h. Viability was then determined using a high-sensitivity cell proliferation/cytotoxicity kit (Vialight HS, Lonza). EC50 values with 95% confidence interval were then determined by Graphpad Prism, Version 3.03. Results presented are from a minimum of 3 separate experiments.

protein (BCRP) ABCG2. Although these efflux pumps can translocate unique compounds, there is an overlap with some drug substrates. Drugs transported by ABCB1 include hydrophobic compounds such as Vinca alkaloids, anthracyclines, taxanes, and epipodophyllotoxins. The spectrum of drugs transported by ABCG2 includes mitoxantrone (MXR), anthracyclines, topoisomerase I inhibitors, methotrexate, and flavopiridol. The situation is further complicated by the potential role of influx pumps in this setting, which results in a balance between influx and efflux. Relatively little is known about the effects of the TKIs on these efflux and influx pumps. Initial reports suggest that specific agents such as gefitinib and lapatinib may inhibit P-gp or BCRP (14, 15).

In the work presented here, we initially investigated potential interactions between lapatinib and a spectrum of chemotherapy agents, some of which are ABC transporter substrates. This was followed by experiments to specifically address the mechanism underlying the interactions observed, with a focus on the BCRP efflux pump. Initially, we used high-performance liquid chromatography (HPLC) to determine the effects of drugs on intracellular concentration of SN-38 and then used HPLC and flow cytometry to investigate the effects of drugs with substrates as well as inhibitors of these pumps.

SN-38 is the active metabolite of irinotecan (a topoisomerase I inhibitor). This family of drugs (irinotecan and topotecan) is widely used in the treatment of lung and ovarian cancer. SN-38 was chosen as the focus of the efflux pump experiments because a synergistic interaction was observed between SN-38 and lapatinib in our original combination experiments.

Material and Methods

Cell lines

The Susa cell line pair was obtained from University College Hospital (Prof. Masters, London, UK). All other cell lines were sourced from the American Type Tissue Collection. MCF-7 and MDA-MB-231 (breast) cancer cells were cultured in DMEM media. H1975, H358 (lung), Susa S (TGCT) and its cisplatin-resistant pair were all cultured in RPMI 1640. All media were supplemented with 10% FCS and 1% Pen/strep, and all cultures were maintained at 37°C, 5% CO2. Although the H358 cell line has only 1 mutation in EGFR (L858R), H1975 cells harbor both L858R and T790M EGFR mutations. The T790M substitution in EGFR was found to confer resistance to TKIs including lapatinib (16, 17). An overview of the cell lines can be found in Supplementary Data, Table 1. Cell lines were authenticated using DNA profile (short tandem repeat, STR) analysis and tested for mycoplasma (MycoAlert mycoplasma detection kit, Lonza) on a regular basis (this most recently occurred in May 2010).

Cytotoxicity assay

During exponential growth phase, cells were trypsinized and harvested for single-agent and combination drug activity experiments. Cells were then seeded into flat-bottomed 96-well plates at a concentration of 5 x 10^4 cells/mL and placed in an incubator at 37°C, 5% CO2 overnight to adhere. Study drugs were then added to the plates in triplicate at 0 and 5 nonzero concentrations. Cells were cultured for a further 72 hours, after which drug effects were determined using a high-sensitivity cell proliferation/cytotoxicity assay based on the determination of intracellular ATP (Vialight HS kit, Lonza). Plates were read on a Polarstar Optima plate reader (BMG Labtech).

The EC50 values of each agent were determined using a sigmoidal EMAX method in Prism 5 (Graphpad Software) from which concentrations were established for use in combination experiments. Drugs were given simultaneously for 72 hours for the combination experiments. Combination data was then analyzed using CalcuSyn software (Version 2; Biosoft). Combinations with a combination index (CI) < 1 were considered synergistic and with CI < 0.5 highly synergistic.
Cell-cycle analysis by flow cytometry

Cells were seeded at 1 × 10^5 cells/mL in 6-well plates and allowed to adhere overnight. Single agents or drug combinations were then added directly to the culture media in these wells and incubated for a further 72 hours. Cells were then pelleted by centrifugation at 200 g for 6 minutes after trypsinization, washed in ice-cold Hank's buffer, fixed using 70% ethanol, and stored at −30°C. On the day of analysis, cells were pelleted by centrifugation (200 g for 6 minutes), washed in ice-cold Hank's buffer to remove the ethanol, resuspended in PI (50 μg/mL)/RNase (10 μg/mL) solution, and kept on ice in the dark until analysis. Samples were run on a FACSCalibur flow cytometer (Becton Dickinson), using CellQuest software, Version 3.3. The resulting data were then analyzed using winMDI, Version 2.8.

BCRP activity by flow cytometry

Cells were seeded at 1 × 10^5 cells/mL in 12-well plates and allowed to grow for 72 hours, after which the media was removed and replaced with 1 mL of fresh media for uptake and efflux assays. Mitoxantrone, 10 μmol/L, was used as a specific substrate for measuring BCRP activity. Cyclosporine A (CA), 10 μmol/L, and fumitremorgin C (FTC), 5 μmol/L, were used as known inhibitors of P-gp and BCRP, respectively, when investigating the effect of lapatinib, 10 μmol/L, on uptake and efflux. Intracellular MXR was determined using the FL-3 (670 nm) detector on a FACSCalibur flow cytometer. As the interactions seen were universal in all the cell lines, only representative cell lines with clinical relevance are presented. Experiments investigating the effect of MXR with both lapatinib and SN-38 together were not performed, as SN-38 is also a BCRP substrate and would result in both SN-38 and lapatinib competing with MXR for BCRP efflux.

Uptake Assays. Drugs/inhibitors were added directly to the fresh media in each well and incubated at 37°C for 2 hours to allow their uptake. Cells were then harvested by trypsinization, washed rapidly in ice-cold Hank’s buffer, and placed on ice until analysis.

Efflux Assays. After drug/inhibitor uptake, positive control samples (no efflux period) were trypsinized, washed in ice-cold Hank’s buffer, and placed on ice prior to the immediate determination of MXR. For other treatments, cells were washed in ice-cold Hank’s buffer and fresh media containing the various inhibitors to be investigated was then added. Samples were incubated for a further 2 hours to allow efflux to occur. After the efflux period, cells were harvested by trypsinization, washed in ice-cold Hank’s buffer, and placed on ice for immediate analysis by flow cytometry.

Intracellular SN-38 accumulation

Intracellular SN-38 concentration was measured using a novel HPLC assay. Cells were trypsinized and reconstituted in cell culture media at a concentration of 2 × 10^5/mL. Drugs were then added to 3 mL of the reconstituted cells at the following concentrations: SN-38 (0.1 μmol/L), lapatinib (0 μmol/L), disodium 4,4’-diisothiocyanato stilbene-2,2’-disulfonate (DIDS, organic anion transport inhibitor, 100 μmol/L), CA (10 μmol/L), and FTC (5 μmol/L). Cells were incubated at 37°C, 5% CO2, for 2 hours, washed twice with ice-cold Hank’s buffer, and reconstituted in 1 mL of ice-cold Hank’s buffer. After centrifugation (900 g, 4°C for 5 minutes), the supernatant was removed and 300 μL of cell extraction solution [ice-cold methanol containing 0.1 mol/L of HCl and 2 μmol/L of the internal standard camptothecin (CPT)] was added to the cell pellet. The extraction solution and cell pellet were vortex mixed for 1 minute, kept on ice for a further 10 minutes, and then centrifuged at 20,000 g at 4°C for 10 minutes. Two hundred fifty microliters of the resulting supernatant was removed and evaporated to dryness under a gentle air stream. Dried extracts were reconstituted in 250 μL of 50-mmol/L NaH2PO4 (pH = 3.1). The solution was vortex mixed and transferred to HPLC vials. Separation of SN-38 and CPT was carried out using a Luna ODS column (3 μmol/L, 150 × 4.6-mm ID; Phenomenex) and an isocratic mobile phase (50 mmol/L of NaH2PO4, pH = 3.1, containing 10 mmol/L of sodium dodecyl sulfate 65%:acetonitrile 35%) at a flow rate of 1 mL/min with fluorescence detection (excitation, 380 nm; emission, 540 nm). As the interactions seen were universal in all the cell lines, representative cell lines with clinical relevance are presented in the article.

Results

Activity of lapatinib alone and in combination with chemotherapy drugs

The activity of lapatinib and the cytotoxic drugs studied was summarized to an EC50 value, as shown in Table 1. These values varied markedly and were cell line and drug dependent. Of particular note was the lack of cross-resistance to lapatinib in the TGCT cell line with cisplatin-sensitive and -resistant sublines.

The effect of the chemotherapy drugs in combination with lapatinib was then investigated. CalcuSyn plots and CI values for some of the combinations are presented in Tables 2 and 3 and Supplementary Data A. Clear synergistic interaction was seen between SN-38 and lapatinib in the majority of cell lines (CI < 0.75), with strong synergy (CI < 0.5) in most (Table 2). The exception to this was MCF-7 cells in which the effects were additive (Table 2, Supplementary Data Fig. A) or antagonistic. No clear or consistent synergistic interaction was seen between cisplatin or paclitaxel and lapatinib (Supplementary Data and Table 2).

To investigate whether the interaction with SN-38 and lapatinib was a class effect, the experiments were repeated with topotecan (also a topoisomerase I inhibitor). Marked synergy was also identified (CI < 0.5 at most concentrations; Supplementary Data Fig. A). Therefore, the interaction was a class effect.
Effect of lapatinib alone or in combination on cell-cycle distribution

Flow cytometry data for the individual chemotherapy agents showed a concentration-dependent increase in apoptosis, with a G2/M-phase-arrested population with SN-38. Lapatinib had little or no effect on cell-cycle distribution, apart from a small increase in S-phase cells in some cell lines.

When given in combination, the synergistic combinations were associated with a marked increased in apoptosis (P < 0.05; Supplementary Data Fig. B). In MCF-7 cells, the combination of lapatinib and SN-38, which was antagonistic in cell viability assays, resulted in a significant decrease in the apoptotic fraction with an increased G2/M-phase-arrested population (Supplementary Data Fig. B).

Effect of lapatinib on the intracellular accumulation of SN-38

Cells that were pretreated with lapatinib showed a significant increase in intracellular SN-38 concentration compared with cells with SN-38 alone (Table 3, 2.2- to 4.8-fold increase, P < 0.05 for each; and Supplementary Fig. C). The exception was the MCF-7 cell line, in which no significant change was observed (Table 3). As the MCF-7 cell line did not exhibit synergy between SN-38 and lapatinib, increased SN-38 accumulation in the presence of lapatinib is a potential mechanism underlying the synergistic interactions observed.

The effect of drug uptake and efflux inhibitors on intracellular SN-38 concentration was further investigated in the H1975 and H358 cell line pair. Lapatinib (10 μmol/L) resulted in a 4.8- and 2.6-fold increase in SN-38 in H1975 and H358 cells, respectively. The influx inhibitor DIDS (100 μmol/L), which inhibits multispecific

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**Table 2.** Combination index values for the combination of lapatinib with SN-38 and cisplatin (CDDP) in a panel of cell lines

<table>
<thead>
<tr>
<th>Lapatinib, μmol/L, with SN-38, μmol/L</th>
<th>Sussa S</th>
<th>Sussa R</th>
<th>H1975</th>
<th>H358</th>
<th>MDA-MB-231</th>
<th>MCF-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lap/SN-38 CI</td>
<td>0.43</td>
<td>0.39</td>
<td>0.11</td>
<td>0.20</td>
<td>0.38</td>
<td>1.296</td>
</tr>
<tr>
<td>0.5/0.001</td>
<td>0.5/0.001</td>
<td>0.5/0.01</td>
<td>0.1/0.001</td>
<td>0.2/0.001</td>
<td>0.5/0.005</td>
<td>1.296</td>
</tr>
<tr>
<td>1/0.005</td>
<td>0.26</td>
<td>0.19</td>
<td>0.11</td>
<td>0.20</td>
<td>0.38</td>
<td>0.48</td>
</tr>
<tr>
<td>2/0.01</td>
<td>0.31</td>
<td>0.28</td>
<td>0.32</td>
<td>0.18</td>
<td>0.18</td>
<td>1.53</td>
</tr>
<tr>
<td>4/0.05</td>
<td>0.30</td>
<td>0.75</td>
<td>0.67</td>
<td>0.16</td>
<td>0.16</td>
<td>2.03</td>
</tr>
<tr>
<td>8/0.1</td>
<td>0.15</td>
<td>0.85</td>
<td>0.96</td>
<td>0.32</td>
<td>0.32</td>
<td>3.58</td>
</tr>
</tbody>
</table>

**Table 3.** Effect of lapatinib (10 μmol/L) on the accumulation of SN-38 in a panel of cell lines as measured by a novel HPLC method

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Intracellular SN-38 concentration, nmol/L</th>
<th>SN-38 alone</th>
<th>SN-38 + Lap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sussa S</td>
<td>5.7 ± 0.7</td>
<td>14.6 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Sussa R</td>
<td>4.8 ± 0.4</td>
<td>11.0 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>H1975</td>
<td>7.2 ± 1.9</td>
<td>34.4 ± 7.2</td>
<td></td>
</tr>
<tr>
<td>H358</td>
<td>9.1 ± 2.3</td>
<td>23.8 ± 5.9</td>
<td></td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>9.2 ± 4.0</td>
<td>18.5 ± 7.1</td>
<td></td>
</tr>
<tr>
<td>MCF-7</td>
<td>21.2 ± 1.3</td>
<td>19.2 ± 1.2</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Breast, lung, and TGCT cell lines were preloaded with SN-38 (0.1 μmol/L) for 2 h, at 37°C, 5% CO2, in the presence or absence of lapatinib (10 μmol/L). Data shown are the mean ± SD of 3 separate experiments.

Abbreviations: CI, combination index; Lap, lapatinib.
organic anion transport, inhibited the influx of SN-38 by 36%. DIDS had a similar effect on SN-38 concentration in the presence of lapatinib, suggesting that lapatinib did not have a significant effect on influx pumps (P > 0.05). (Table 4). FTC, an established BCRP inhibitor, had an effect on SN-38 accumulation similar to that seen with lapatinib (4.3- and 2.7-fold accumulation of SN-38 in H1975 and H358 cells, respectively, compared with 4.8- and 2.6-fold increase with lapatinib), suggesting lapatinib may result in SN-38, and other, drug accumulation via BCRP inhibition.

Cyclosporine A (CA, 10 μmol/L), a P-gp inhibitor, had a significant effect on SN-38 concentration in only one of the cell lines (H1975). However, the addition of CA to FTC had no increased effect compared with FTC alone (P > 0.05; Table 4), suggesting that SN-38 accumulation with lapatinib is due to BCRP inhibition in these cell lines.

**Analysis of the effects of lapatinib on BCRP function by flow cytometry**

After establishing that lapatinib resulted in the accumulation of SN-38, most likely by inhibiting BCRP-mediated drug efflux, we used flow cytometry in 3 cell lines (H1975, H358, and MDA-MB-231) to confirm these findings, using the fluorescent BCRP substrate MXR.

Flow cytometry data showed a reduction in the fluorescent MXR signal after 2 hours of incubation at 37°C, representing MXR efflux (Figs. 1 and 2). Lapatinib inhibited this MXR efflux to the same extent as FTC, confirming lapatinib as a BCRP inhibitor. The effect of CA (an inhibitor of P-gp) was typically between that of no inhibitor (efflux) and lapatinib or FTC, in all 3 lines. This supported the HPLC data that showed only a modest effect of CA (Table 4).

**Pazopanib (a VEGF TKI) and SN-38**

To establish whether the synergistic interactions seen with lapatinib and SN-38 were not exclusive to HER targeted TKIs, we investigated whether the same effects were seen with pazopanib (a VEGF TKI). These were investigated in 2 paired testis cancer cell lines (Susa S and Susa R). No synergistic interactions were observed (CI > 1 for each).

**Discussion**

TKIs are increasingly being used in combination with chemotherapy agents with the aim of improving the survival of cancer patients. Lapatinib is a prominent HER1 and HER2 TKI (Fig. 3) that has been successfully used with a spectrum of agents (3). The *in vitro* work presented here identified a synergistic interaction between SN-38 and lapatinib. This interaction was not

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**Table 4. Effect of lapatinib and influx/efflux pump inhibitors on the accumulation of SN-38 in H1975 and H358 cell lines**

<table>
<thead>
<tr>
<th>Drug treatments</th>
<th>SN-38 concentration in H1975 cells, nmol/L</th>
<th>SN-38 concentration in H358 cells, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN-38 (0.1 μmol/L)</td>
<td>7.2 ± 1.9</td>
<td>9.1 ± 2.3</td>
</tr>
<tr>
<td>SN-38 + lapatinib (10 μmol/L)</td>
<td>34.4 ± 7.2</td>
<td>23.8 ± 5.9</td>
</tr>
<tr>
<td>SN-38 + DIDS (100 μmol/L)</td>
<td>5.0 ± 0.4</td>
<td>6.0 ± 1.3</td>
</tr>
<tr>
<td>SN-38 + lapatinib (10 μmol/L) plus; DIDS (100 μmol/L)</td>
<td>26.6 ± 1.9</td>
<td>14.8 ± 0.9</td>
</tr>
<tr>
<td>SN-38 + CA (10 μmol/L)</td>
<td>14.2 ± 3.7</td>
<td>12.3 ± 3.6</td>
</tr>
<tr>
<td>SN-38 + FTC (5 μmol/L)</td>
<td>31.2 ± 7.3</td>
<td>24.6 ± 4.2</td>
</tr>
<tr>
<td>SN-38 + CA + FTC</td>
<td>35.9 ± 8.3</td>
<td>23.2 ± 7.2</td>
</tr>
</tbody>
</table>

NOTE: Cells were preloaded with SN-38 (0.1 μmol/L) for 2 h, at 37°C, 5% CO2, in the presence or absence of lapatinib (10 μmol/L) or various other known uptake/efflux pump inhibitors. SN-38 concentration was then measured by HPLC analysis. Data shown are the mean ± SD from 3 separate experiments. Abbreviations: CA, cyclosporine A; DIDS, disodium 4,4′-diisothiocyanatostilbene-2,2′-disulfonate; FTC, fumitremorgin C.
exclusive to SN-38, occurring with another topoisomerase I inhibitor (topotecan), suggesting a class effect. The synergy was seen across a range of concentrations. Cell-cycle data showed that these effects were associated with an increased sub-G1 (apoptotic) population. However, these synergistic interactions were not universal in all cell lines, with a lack of synergy in the MCF-7 cell line. In addition, the interaction was TKI specific, in that a synergistic interaction was not seen with pazopanib (a VEGF TKI) and SN-38.

In view of previous reports suggesting an effect of lapatinib on drug efflux pumps, particularly BCRP (14, 18), we developed a novel HPLC method to determine the effect of lapatinib on intracellular SN-38 concentrations. These results confirmed an increase in intracellular SN-38 in the presence of lapatinib, further confirmed by a lapatinib-mediated increase in MXR by using flow cytometry. This suggests that inhibition of the BCRP efflux pump by lapatinib was responsible for the synergistic interaction in the H358 and H1975 lung cancer cell lines and in the breast cancer cell line MDA-MB-231. BCRP has previously been reported to be involved in the efflux of topoisomerase I inhibitors (19), but the intracellular SN-38 concentration data presented here are the strongest evidence to date for this direct pharmacokinetic effect of lapatinib and have not been described previously.

This synergistic interaction was not observed in all cell lines studied. Indeed, no synergy was seen between SN-38 and lapatinib in the MCF-7 cell line. This cell line overexpresses P-gp rather than BCRP, which is less important for the efflux of topoisomerase I inhibitors (14, 20–22). The relative expression and activity of different ABC efflux transporters may, therefore, be important in determining the degree of synergistic interaction between cytotoxic agents and lapatinib and potentially between TKIs (10, 23). Our experiments measured pump activity rather than protein level, which is theoretically advantageous in terms of understanding the mechanism of the interactions seen. However, the mechanism by which lapatinib inhibits BCRP remains unknown and requires further investigation.

**Figure 2.** Representative flow cytometry histograms illustrating the effect of lapatinib (Lap) and fumitremorgin C (FTC) on mitoxantrone (MXR) efflux through BCRP. Lung (A and B) and breast cancer (C) cell lines were preloaded with MXR (a specific substrate of BCRP) for 30 minutes at 37°C, 5% CO2 (MXR uptake), after which efflux was allowed to occur in control cells (efflux), or the presence or absence of lapatinib (10 µmol/L) or FTC (5 µmol/L, a specific inhibitor of BCRP). In the left-most peak in each panel, *-ve Control* refers to negative control.

**Figure 3.** Chemical structure of lapatinib.
Efﬂux pumps are expressed in nonmalignant tissue such as the kidneys as well as in tumors. Therefore, these results have implication in terms of drug toxicity as well as efﬁcacy (24).

Other groups have investigated potential interactions between lapatinib and cytotoxic chemotherapy agents. Coley and colleagues demonstrated that cisplatin and lapatinib were synergic in an ovarian cancer cell line (9). This is in contrast to our data, which show no synergistic interaction. Indeed, cisplatin is not excreted from the cells by either P-gp or BCRP, which is why cisplatin was chosen as a negative control for our experiments. Cisplatin is not associated with excretion via an efﬂux pump, and no speciﬁc mechanism was identiﬁed in this work. Moreover, McHugh and colleagues also investigated the combination of cisplatin and lapatinib with a number of sequencing experiments, demonstrating antagonism when the drugs were given together but some evidence of synergy with certain sequences of exposure to each agent (25). The reasons for this are unclear and require further investigation. However, this emphasizes the time-dependent nature of potential interactions between lapatinib and other agents and suggests that other mechanisms may be implicated that are independent of drug efﬂux pumps.

The concentrations of lapatinib required to achieve these synergistic interactions are clinically relevant (10). This is evident from phase I clinical data investigating lapatinib and irinotecan (SN-38 is the active metabolite of irinotecan) in combination, which showed that lapatinib signiﬁcantly altered the pharmacokinetics of SN-38, with an increase in the area under the plasma concentration–time curve of SN-38 for the combination (26). The data presented here therefore have clinical implications, particularly as drug combinations are being given in large randomized studies, whereas unacceptable toxicity has been noted with other combinations (27, 28).

The data presented here are somewhat dependent on the speciﬁcity of established substrates and inhibitors of efﬂux pumps. Although the optimal agents available were used, it is conceivable that cross activity with other efﬂux pumps occurs. This was particularly true in the 2 lung cancer cell lines for which the experiments suggested that MXR had some efﬂux through P-gp or that CA shows some cross-selectivity for both P-gp and BCRP.

The effect of TKIs on efﬂux pumps is established in hematologic malignancies (29). Our work showed that lapatinib did not increase the inﬂux of SN-38 via organic anion transporters, as coincubation with DIDS had little, if any, effect on SN-38 concentration.

SN-38 is the active metabolite of irinotecan, which is widely used in cancer medicine. This interaction with lapatinib has clinical implications in tumor types, such as lung cancer, where both HER1 and HER2 targeted therapy and topoisoeramse I inhibitors are given concurrently.

In summary, lapatinib has an inhibitory effect on BCRP resulting in a synergistic interaction with topoisoeramse I inhibitors mediated by increased intracellular drug concentration. This effect is cell-line dependent and results in speciﬁc synergistic interactions. These data have clinical signiﬁcance in the further development of lapatinib combinations.

Disclosure of Potential Conflicts of Interest

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References

A Synergistic Interaction between Lapatinib and Chemotherapy

A Synergistic Interaction between Lapatinib and Chemotherapy Agents in a Panel of Cell Lines Is Due to the Inhibition of the Efflux Pump BCRP

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