A phase I and pharmacokinetic study of lapatinib in combination with infusional 5-fluorouracil, leucovorin and irinotecan


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Background: This study determined the optimally tolerated regimen (OTR) of oral lapatinib administered in combination with infusional 5-fluorouracil (5-FU), leucovorin and irinotecan (FOLFIRI) and assessed the safety, tolerability and pharmacokinetics of the combination.

Patients and methods: Twenty-five patients were enrolled; 12 patients were treated at three dose levels to determine OTR; then 13 patients were treated at OTR to evaluate the pharmacokinetics of the combination.

Results: The 2-weekly OTR comprised lapatinib 1250 mg/day with irinotecan 108 mg/m² (day 1) and leucovorin 200 mg/m², 5-FU bolus 240 mg/m² and 5-FU infusion 360 mg/m² (days 1 and 2); doses of 5-FU and irinotecan represent a 40% reduction in dose compared to conventional FOLFIRI. Dose-limiting toxicities were grade 3 diarrhoea and grade 4 neutropenia. Co-administration of lapatinib increased the area under the plasma concentration-time curve of SN-38, the active metabolite of irinotecan, by an average of 41%; no other pharmacokinetic interactions were observed. Of 19 patients evaluable for disease response assessment, four patients had partial response and nine patients had stable disease.

Conclusion: The combination of lapatinib and FOLFIRI is safe and demonstrates clinical activity; the documented PK interaction can effectively be compensated by lowering the doses of 5-FU and irinotecan. This regime may be further tested in a phase II trial.

Key words: colorectal cancer, FOLFIRI chemotherapy, phase I, lapatinib

introduction

Lapatinib (Tykerb, Research Triangle Park, NC, USA) a member of the 4-anilinoquinazoline class of kinase inhibitors, has been shown to be a potent and selective dual inhibitor of substrate phosphorylation catalysed by the epidermal growth factor receptor (EGFR) and erbB2; it has demonstrated potent growth inhibition in models (both in vitro and in vivo) of colorectal tumours over-expressing EGFR and/or erbB2. Like other signal transduction agents, EGFR inhibitors, including lapatinib, are more active when administered in combination with other chemotherapeutic regimens. There is a growing literature on the role of the EGF receptor and its therapeutic inhibition in the management of colorectal cancer, particularly in combination with irinotecan [1].

Safety data from the first three studies of single-agent lapatinib indicated no clinically significant changes in biochemical or haematological indices or ECG parameters when daily doses of up to 1800 mg were administered. Common adverse events in these studies were mild headache, rash and diarrhoea. One of the standard regimes for therapy of metastatic colorectal cancer is a combination of 5-fluorouracil (5-FU), leucovorin (LV) and irinotecan, administered in the so-called FOLFIRI regimen, which has shown excellent activity in the first-line setting with a response rate of 57%, a median time to progression of 8.4 months and tolerable toxicity (grade 3 diarrhoea and neutropenia in 9% and 25% of patients respectively) [2].

Here we report a phase I study determining the safety, tolerability and pharmacokinetics of lapatinib when administered in combination with FOLFIRI in patients with a range of advanced cancers predominantly of gastrointestinal origin.

patients and methods

study design

This phase I study was an open-label, multiple-dose, dose-escalation study of lapatinib and intravenous irinotecan, 5-FU and LV (FOLFIRI regimen).
Subjects received irinotecan on day 1, and 5-FU (bolus and infusion) and LV on days 1 and 2 of 2-week cycles. Lapatinib was administered once daily for the duration of the study beginning on the morning of the first day of treatment in cycle 1. The study design involved a dose escalation phase to identify the optimally tolerated regimen (OTR), after which patients were treated at the OTR to characterize any pharmacokinetic interaction between lapatinib and the chemotherapy drugs.

**eligibility**

Male and female patients 18 years or older with advanced solid tumours, Karnofsky Performance Status greater than or equal to 70 and a life expectancy of more than 12 weeks were eligible for inclusion in this trial. Patients with significant haematological or biochemical dysfunction were excluded, as were patients who had received prior treatment with combination irinotecan, 5-FU and LV, or prior radiation that either involved greater than or equal to 25% of bone marrow or encompassed the pelvis. Other exclusion criteria included evidence of uncontrolled brain metastases, other serious illness, psychiatric disorder or active infection, known contraindications to the use of irinotecan, 5-FU or LV, pregnancy, lactation and diseases that could affect the absorption of lapatinib.

The study was conducted in accordance with the principles of the International Conference of Good Clinical Practice (ICH-GCP) guidelines and the Declaration of Helsinki. Local ethics committees approved the study protocol, and all patients gave their written informed consent.

**treatment and dose escalation**

Lapatinib was supplied as 250 mg tablets by GlaxoSmithKline (Research Triangle Park, NC, USA) and irinotecan, 5-FU and LV were obtained from stock purchased by each centre. The starting dose of lapatinib was 1250 mg daily, with irinotecan 150 mg/m² (day 1) and 5-FU 320 mg/m² (bolus); 5-FU 480 mg/m² (22-h infusion) and LV 200 mg/m² repeated on days 1 and 2. The starting dose of irinotecan and 5-FU represented an approximate 20% reduction of the standard FOLFIRI schedule. Leucovorin was given at the same standard dose throughout the study.

During the OTR-defining phase, three patients were to be treated at each dose level and monitored for toxicity. Patients were not entered at a higher dose level until all those in the previous cohort had been followed for at least 14 days after dosing. Dose-limiting toxicity (DLT) was based on toxicities observed in cycle 1, although persistent grade 2 or higher non-haematological toxicities could be considered dose-limiting at the discretion of the study team. In the absence of DLT, a further three patients were to be entered at the next higher dose level and so on until DLT was observed or the maximum planned dose level was reached in the absence of DLT. In the event of DLT at the first dose level, a decrease in the doses of the cytotoxic drugs was permitted for subsequent cohorts. Planned doses of lapatinib ranged between 750 mg/day and 1500 mg/day and planned doses of irinotecan and 5-FU ranged from a 40% dose reduction of standard FOLFIRI to the full dose regimen. Where one of three patients experienced a DLT at a particular dose level an additional three were entered at that level. The OTR was defined as the dose of lapatinib and the level of irinotecan, 5-FU and LV at which no more than two of six patients experienced a DLT.

Where more than one-third of patients experienced DLT at a given dose level (e.g. three of six patients), a lower dose level could be explored.

Once the OTR was determined, at least nine additional patients were to be treated to characterize pharmacokinetic interactions. Blood sampling occurred over a 24-hour period on each of 3 days in the first three treatment cycles so as to assess the pharmacokinetics of lapatinib alone and in combination with the FOLFIRI regimen, and the pharmacokinetics of irinotecan, its metabolite SN-38 and 5-FU administered with and without lapatinib.

**evaluation criteria and procedures**

The pre-treatment evaluation included a complete medical history and physical examination, full blood count, biochemical profile, urinalysis, assessment of cardiac function and ECG. Females with child-bearing potential underwent a pregnancy test. Appropriate radiology to evaluate tumour sites was performed. Safety and toxicity were evaluated at least weekly during the study period and toxicities were graded according to the National Cancer Institute Common Toxicity Criteria Version 2. Safety was assessed by physical examination, observation and questioning of patients regarding adverse experiences, and monitoring of clinical biochemistry and haematology. A treatment delay of up to 2 weeks was allowed for resolution of drug-related toxicity. Dose modification of study drugs by one or two dose levels was permitted in the event of DLT in the preceding cycle. Patients who continued to fulful the original eligibility criteria could remain on study until occurrence of unacceptable toxicities, disease progression, withdrawal of consent or if treatment was delayed for more than 2 weeks.

Dose-limiting toxicities were defined as the following: grade 3 or 4 clinically significant non-haematological toxicity (nausea, vomiting or diarrhoea only if grade 3 or 4 in the presence of maximal support); grade 4 granulocytopenia lasting 25 days with or without fever; thrombocytopenia 525 × 10³/µl (25000/mm³); inability to begin next course of treatment within 2 weeks of scheduled dosing due to unresolved toxicity; and persistent grade 2 toxicity that in the judgment of the study team was dose-limiting.

Anti-tumour effects were assessed using the Response Evaluation Criteria in Solid Tumours (RECIST) guidelines, with assessments being made after every four treatment cycles.

**pharmacokinetic sampling and methods**

Blood samples for determination of lapatinib plasma concentration were taken prior to, and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12 and 24 hours after the lapatinib dose. Blood samples for determination of irinotecan and SN-38 plasma concentrations were taken prior to, and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12 and 24 hours after the start of the irinotecan infusion. Blood samples for determination of 5-FU plasma concentration were taken prior to, and 4, 5, 6, 8, 10 and 12 hours after the start of the 5-FU infusion.

Plasma lapatinib was determined by the method described by Hsieh [3]. Irinotecan (1–1000 ng/ml) and SN-38 (0.2–200 ng/ml) were extracted along with internal standard (camptothecin) from 100 µl plasma, and 5-FU (5–3000 ng/ml) with 15N₂-5-fluourouracil internal standard from 200 µl plasma, both after protein precipitation with acetonitrile. Extracts were analysed by LCMS/MS using Turbo IonSpray with multiple reaction monitoring in the positive ion mode.

Plasma concentration vs time data was analysed by standard noncompartmental methods, using WinNonlin Professional software (Version 4.1, Pharsight Corporation, Mountain View, CA). Pharmacokinetic parameters were compared between treatments by analysis of variance using PROC MIXED within the SAS/STAT module version 8.02 of the SAS system (SAS Institute, Cary, NC, USA).

**results**

**patient demographics and dose-finding results**

The characteristics of the 25 patients entered in the study are summarized in Table 1. All but one of the patients was Caucasian; 56% were male and 44% were female; the mean age was 58 years (range: 34 to 78 years); median Karnofsky Performance Status was 90 prior to starting treatment.
The most common primary was colorectal cancer (9 patients, 36%). All of the patients were assessable for toxicity, although one patient received only lapatinib before withdrawing from the study. Nineteen had measurable disease and were assessed for tumour response. Twelve of the 13 patients recruited to the pharmacokinetics cohort completed the sampling protocol.

The dose levels of lapatinib and FOLFIRI studied are summarized in Table 2. Twelve patients were treated in the dose-finding phase of the study. The initial dose level of lapatinib 1250 mg/day given in combination with FOLFIRI with a 20% reduction in standard doses (irinotecan 150 mg/m², 5-FU bolus 320 mg/m², 5-FU infusion 480 mg/m²) was not tolerated (n=7). A reduction in lapatinib (to 1000 mg) with the same doses of irinotecan and 5-FU (20% dose reduction) yielded dose-limiting toxicity (febrile neutropenia) in one of two patients. No further patients were entered at this dose level as it seemed more appropriate to maintain the dose intensity of the test drug, lapatinib. Therefore, the third dose level comprised lapatinib 1250 mg/day given in combination with FOLFIRI (40% dose reduction; irinotecan 108 mg/m², 5-FU bolus 240 mg/m², 5-FU infusion 360 mg/m²). This dose combination was well tolerated (Table 3) and designated the OTR, with a further 13 patients being treated at this level within the pharmacokinetic cohort of the study.

safety
The most frequently occurring adverse events are described in Table 3. Dose-limiting toxicities were grade 3 diarrhoea and grade 4 neutropenia and febrile neutropenia. At the OTR (combination of lapatinib 1250 mg/day and the 40% reduction in the standard FOLFIRI doses of irinotecan and 5-FU) the regimen was generally well tolerated in this phase I population. Grade 3 diarrhoea, nausea, vomiting and neutropenia were each seen in only 2 patients (13%) and no patients suffered grade 4 toxicity.

One patient died whilst on study, but this was ascribed to progression of their disease and was not related to lapatinib or the chemotherapy. Across all grades of toxicity, the majority of patients experienced drug-related diarrhoea (76%) or nausea (72%). Other common drug-related side effects, affecting over a quarter of the patients, were vomiting and fatigue.

Nine patients (36%) suffered toxicities that led to premature withdrawal from the study. These included chest pain, diarrhoea, fatigue, febrile neutropenia, palpitations, blurred vision and vomiting. Thirteen patients (52%) had at least one study drug administration delay and/or a dose reduction due to toxicity. Lapatinib dose was reduced in two instances, once for diarrhoea and once for neutropenia, and doses of irinotecan and/or 5-FU were reduced in four cases due to diarrhoea or at the investigator’s discretion. Treatment delays were associated with diarrhoea, vomiting, stomatitis, neutropenia, febrile neutropenia or disordered coagulation function. Two patients had asymptomatic LVEF decreases of greater than 20%. In one case this was detected at the end of study scan, and in the other the patient continued on treatment and the ejection fraction showed an improvement on subsequent scans.

pharmacokinetic results
Pharmacokinetic parameters for the 12 patients who completed all the scheduled assessments are shown in Table 4. No clinically significant differences were observed in the pharmacokinetics of lapatinib, irinotecan or 5-FU when used in combination. Treatment ratios for the combination relative to separate administration were within 14% for lapatinib, 10% for irinotecan, and 6% for 5-FU. However, significant increases in the area under the plasma

**Table 1.** Patient and tumour characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>25</td>
</tr>
<tr>
<td>Male/female</td>
<td>14/11</td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>58 (34–78)</td>
</tr>
<tr>
<td>Performance status (Karnofsky)</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>8</td>
</tr>
<tr>
<td>90</td>
<td>12</td>
</tr>
<tr>
<td>80</td>
<td>4</td>
</tr>
<tr>
<td>70</td>
<td>1</td>
</tr>
<tr>
<td>Primary tumour site</td>
<td></td>
</tr>
<tr>
<td>Colorectal</td>
<td>9</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>3</td>
</tr>
<tr>
<td>Adenocarcinoma, unknown</td>
<td>3</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>2</td>
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<tr>
<td>Pancreas</td>
<td>2</td>
</tr>
<tr>
<td>Others</td>
<td>6*</td>
</tr>
<tr>
<td>Prior chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Median number of prior regimens</td>
<td>2 (1–11)</td>
</tr>
</tbody>
</table>

*One each liver, cholangiocarcinoma, bladder, kidney, sarcoma and pelvis.

**Table 2.** Dose levels of lapatinib and FOLFIRI

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Lapatinib PO (mg/day)</th>
<th>FOLFIRI IV (% full dose)</th>
<th>Number of patients assigned dose level</th>
<th>Number of patients assessable for response</th>
<th>Total number of weeks on treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1250</td>
<td>80</td>
<td>7</td>
<td>3</td>
<td>55</td>
</tr>
<tr>
<td>–1</td>
<td>1000</td>
<td>80</td>
<td>2</td>
<td>1</td>
<td>40</td>
</tr>
<tr>
<td>–2</td>
<td>1250</td>
<td>60</td>
<td>3</td>
<td>3</td>
<td>42</td>
</tr>
<tr>
<td>PK</td>
<td>1250</td>
<td>60</td>
<td>13</td>
<td>12</td>
<td>132</td>
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</tbody>
</table>

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combination with lapatinib. SN-38 by 32% from 22.6 ng/ml (95% CI 17.1–29.9) to 28.7 ng/ml (95% CI 21.1–39.2) were observed when administered in the presence of lapatinib. SN-38 undergoes glucuronidation and possibly CYP3A4-mediated oxidation [5], hepatic extraction mediated by OATP1B1 [4] and then action of carboxylesterase. Circulating SN-38 undergoes glucuronidation and possibly CYP3A4-mediated oxidation [5], hepatic extraction mediated by OATP1B1 [4] and then action of carboxylesterase.

Specific toxicity and grade	1250 mg lapatinib/–20% FOLFIRI (n = 7)	1250 mg lapatinib/–40% FOLFIRI (n = 15)*

Diarrhoea	7 (100%) 14 (93%) 21 (95%)
1	0	4 (27%) 4 (180)
2	2 (29%) 8 (53%) 10 (45%)
3	5 (71%) 2 (13%) 7 (32%)
Nausea	6 (86%) 14 (93%) 20 (91%)
1	1 (14%) 6 (40%) 7 (32%)
2	4 (57%) 6 (40%) 10 (45%)
3	1 (14%) 2 (13%) 3 (14%)
Vomiting	4 (57%) 11 (73%) 15 (68%)
1	1 (14%) 4 (27%) 5 (23%)
2	2 (29%) 5 (33%) 7 (32%)
3	1 (14%) 2 (13%) 3 (14%)
Fatigue	4 (57%) 10 (67%) 14 (64%)
1	0	4 (27%) 4 (180)
2	4 (57%) 5 (33%) 9 (41%)
3	0	1 (7%) 1 (5%)
Alopecia	2 (29%) 8 (53%) 10 (45%)
1	0	4 (27%) 4 (180)
2	2 (29%) 4 (27%) 6 (278)
Mucositis	2 (29%) 8 (53%) 10 (45%)
1	1 (14%) 3 (20%) 4 (180)
2	1 (14%) 4 (27%) 5 (238)
3	0	1 (7%) 1 (5%)
Neutropenia	5 (71%) 3 (20%) 8 (36%)
2	2 (29%) 1 (7%) 3 (14%)
3	0	2 (13%) 2 (9%)
4	3 (43%) 0	3 (14%)
Fever	1 (14%) 0	1 (5%)

*Included are the patients who received chemotherapy at the first dose level (1250 mg/–20% FOLFIRI) and at the final OTR doses (1250 mg/–40% FOLFIRI).

Table 4. Pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Analyte Alone*</th>
<th>In combination† (95% CI)</th>
<th>Ratio‡ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lapatinib	AUC$_{24}$ (h*ng/ml)</td>
<td>40.0 (29.3–54.7) 42.2 (31.2–57.0)</td>
<td>1.02 (0.80–1.32)</td>
</tr>
<tr>
<td>C$_{max}$ (mg/ml)</td>
<td>2.83 (2.15–3.73) 3.24 (2.60–4.05)</td>
<td>1.14 (0.92–1.41)</td>
</tr>
<tr>
<td>5-FU	C$_{ss}$ (ng/ml)</td>
<td>117 (106–129) 121 (99–147)</td>
<td>1.04 (0.87–1.24)</td>
</tr>
<tr>
<td>Irinotecan	AUC$_{ss}$ (h*ng/ml)</td>
<td>9.38 (7.33–12.0) 10.2 (7.95–13.0)</td>
<td>1.10 (0.98–1.23)</td>
</tr>
<tr>
<td>CL (l/h)</td>
<td>24.1 (18.0–32.1) 22.0 (16.5–28.7)</td>
<td>0.91 (0.81–1.02)</td>
</tr>
<tr>
<td>V$_{ss}$ (l)</td>
<td>167 (138–201) 161 (129–201)</td>
<td>0.96 (0.82–1.11)</td>
</tr>
<tr>
<td>SN-38	C$_{max}$ (ng/ml)</td>
<td>22.6 (17.1–29.9) 28.7 (21.1–39.2)</td>
<td>1.32 (1.17–1.50)</td>
</tr>
<tr>
<td>AUC$_{24}$ (h*ng/ml)</td>
<td>165 (119–231) 239 (182–313)</td>
<td>1.41 (1.24–1.60)</td>
</tr>
</tbody>
</table>

*Geometric mean.
†Ratio is ratio of that observed in combination compared to that seen when the drug was administered alone.
‡Ratio is ratio of that observed in combination compared to that seen when the drug was administered alone.

adenocarcinoma of unknown primary, one oesophageal cancer and one kidney cancer. Six patients had progressive disease despite therapy.

**Discussion**

We have evaluated three combination dose levels of FOLFIRI and lapatinib. Grade 3 diarrhoea and grade 4 febrile neutropenia at the starting doses necessitated dose reductions rather than the planned escalations. The optimally tolerated regime was determined to be the combination of lapatinib 1250 mg/day with FOLFIRI at 60% of the standard doses (i.e. irinotecan 108 mg/m² [day 1], 5-FU bolus 240 mg/m² [days 1 and 2], 5-FU infusion 360 mg/m² [days 1 and 2] and 200 mg/m² LV [days 1 and 2]). At these doses the combination was generally well tolerated in this phase I population (28% of patients with ≥2 prior cancer chemotherapy regimens), with a 13% rate of grade 3 diarrhoea, nausea, vomiting and neutropenia and no grade 4 toxicity. These are comparable to previous published toxicities of FOLFIRI in the second-line setting of colorectal cancer (grade 3/4 diarrhoea 7%/1% and grade 3 neutropenia 21%) [2].

Concomitant administration of lapatinib with the FOLFIRI regimen had no clinically significant effect on the pharmacokinetics of lapatinib, 5-FU or irinotecan. However, plasma concentrations of SN-38 (the active metabolite of irinotecan) was increased by approximately 40% when lapatinib was co-administered. SN-38, which accounts for only 3–4% of the total irinotecan dose, is formed by the action of carboxylesterase. Circulating SN-38 undergoes hepatic extraction mediated by OATP1B1 [4] and then glucuronidation and possibly CYP3A4-mediated oxidation [5], and lapatinib/–20% FOLFIRI and lapatinib/–40% FOLFIRI.

Table 3. Most commonly occurring adverse eventsa
as well as being secreted into bile through the action of efflux transporters such as MRP, Pgp, and BCRP [6]. Lapatinib has been shown to inhibit OATP1B1, CYP3A4 and Pgp with an IC₅₀ of 4 μM, and BCRP with an IC₅₀ of 1.85 μM in vitro, both of which were exceeded by the peak plasma concentrations of lapatinib achieved in this study. The increase in SN-38 exposure observed in this study could be explained in this way; however, further investigation is needed to determine whether one or all of these mechanisms is responsible for the observed in vivo interaction.

In contrast to our findings, Suegel-Lakhai et al. have studied the combination of 5-FU/oxaliplatin (FOLFOX) and lapatinib in a phase I trial including patients with solid tumours and have found that the OTR consists of lapatinib 1500 mg/day combined with full-dose FOLFOX (infusional 5-FU/oxaliplatin) regimen [7], suggesting no pharmacokinetic interaction.

In conclusion, the maximum tolerated dose of lapatinib in combination with the FOLFIRI regime (−40%) is 1250 mg/day. The OTR defined here appears feasible and safe, with relatively low rates of grade 3/4 toxicity. In terms of clinical activity, this is difficult to assess because of the heterogeneity of the tumours of the patients enrolled. However, the disease control rate (PR + SD) in the nine patients with colorectal cancer was 55% (5 of 9 patients). Although these are small numbers and scans were not centrally reviewed, this compares favourably with historical response rates for 5-FU/irinotecan combination regimens in the second-line treatment of advanced colorectal cancer (disease control rate 34% [2]), suggesting that this combination warrants further investigation in a phase II trial. Certainly these results are superior to the single-agent lapatinib response rates; in a phase II study these were documented as partial response rate and overall disease control rate of 1% and 6% respectively [8]. The fact that there was a pharmacokinetic interaction between lapatinib and the active metabolite of irinotecan in our trial is reflected in the requirement for a dose reduction in the 5-FU/irinotecan compared to the standard FOLFIRI regime.

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references