A multicentre phase I and pharmacokinetic study of BN80915 (diflomotecan) administered daily as a 20-min intravenous infusion for 5 days every 3 weeks to patients with advanced solid tumours

L. Scott¹, O. Soepenberg², J. Verweij², M. J. A. de Jonge², A. S. Th Planting², D. McGovern⁴, P. Principe⁴, R. Obach⁵ & C. Twelves³*

¹Cancer Research UK, Centre for Oncology and Applied Pharmacology, University of Glasgow, Glasgow G61 1BD, UK; ²Department of Medical Oncology, Erasmus MC, Daniel den Hoed, PO Box 5201, 3008 AE Rotterdam, The Netherlands; ³University of Leeds and Bradford NHS Hospitals Trust, UK; ⁴Beaufour Ipsen R & D, Ipsen Ltd, 190 Bath Road, Slough, Berkshire, UK; ⁵Ipsen Pharma SA, carrera Laura Miro, 395, E-08980 San Felu de Llobregat, Spain

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Background: BN80915 (diflomotecan) is an E-ring modified camptothecin analogue, which possesses greater lactone stability in plasma compared with other topoisomerase I inhibitors. This phase I study was carried out using a daily times five administration schedule (d5) repeated three weekly. The primary objective was to determine the maximum tolerated dose (MTD) and recommended dose (RD) for phase II studies. Secondary objectives were to determine the safety and pharmacokinetic (PK) profile, and to make a preliminary assessment of antitumour activity.

Patients and methods: Diflomotecan was administered intravenously on days 1–5 every 3 weeks. Patients were treated in cohorts of three to six per dose level and the dose of diflomotecan was escalated according to modified Fibonacci schedule. Plasma concentrations of diflomotecan and its metabolite BN80942 were quantified.

Results: Thirty patients were assessable for toxicity. Dose levels explored were 0.05, 0.1, 0.125 and 0.15 mg/m²/day. The 0.15-mg/m² dose level was determined to be the MTD. Toxicity was acceptable at the 0.125-mg/m²/day dose level. PK analysis showed the principal parameters were neither time nor dose dependent. There was a wide interpatient variability in PK at all dose levels. One patient with colorectal cancer, previously treated with irinotecan, had a partial response. A further eight patients had disease stabilisation.

Conclusions: The MTD and RD of diflomotecan administered according to a d5 repeated three weekly are 0.15 and 0.125 mg/m²/day, respectively. In general, treatment was well tolerated; the principal toxicity was reversible myelosuppression. An objective response was seen in a patient previously treated with irinotecan.

Key words: BN80915, clinical trial, diflomotecan, phase I

Introduction

The fluorinated homocamptothecin diflomotecan (BN80915) (R)(5-ethyl 9,10-difluoro-4,5-dihydro-5 hydroxy-1H-oxepino[3',4':6,7]indolizino-1,2-b]quinoline-3,15[1H]dione) is a water insoluble topoisomerase I inhibitor [1, 2] (Figure 1). Homocamptothecins are camptothecin analogues bearing a 7-membered β-hydroxylactone ring, with enhanced lactone stability, in place of the naturally occurring 6-membered α-hydroxylactone. Since a 1-carbon ring expansion is chemically termed a homoligation, these new lactone- or E-ring modified compounds were named homocamptothecins. As a result of the enhanced stability of the active form, homocamptothecins were anticipated to exert greater topoisomerase I inhibition and antitumour efficacy than the currently approved analogues such as irinotecan and topotecan (Hycamtin; GlaxoSmithKline, Philadelphia, PA). In a variety of preclinical models the homocamptothecins do exhibit greater potency than either of these agents. Animal toxicology studies were consistent with previous findings from cytotoxic agents.

Topoisomerase I inhibitors exert their antiproliferative activity during S phase of the cell cycle where drug-induced cleavable complexes result in the formation of double-stranded DNA breaks in sensitive cell lines [3, 4]. The biology of this event was consistent with the results of preclinical models where diflomotecan was most active when administered as multiple fractions [5].

A previous phase I trial was carried out using an oral formulation of diflomotecan given once daily for 5 days every 3 weeks. As there was not a suitable oral formulation, the i.v. solution of diflomotecan was taken by mouth in that trial.
Toxicity was acceptable at the 0.27-mg/day oral dose level with only two out of 11 patients experiencing dose-limiting toxicity (DLT) (grade 4 neutropenia >7 days). Other toxic effects observed included anaemia, grade 3 thrombocytopenia, mucositis and fatigue [6]. It was not practical to further develop the i.v. drug for oral administration, in part because of its taste. It was therefore, necessary to investigate additional i.v. schedules of diflomotecan until an acceptable oral formulation became available. As a result we carried out this phase I and pharmacokinetic (PK) study of diflomotecan employing a daily and mean oral bioavailability of 65% (range 7.6% to 100%). This is equivalent to a mean i.v. dose of 0.19 mg/day at the RD. Adjusting for body surface area, this equates to a mean daily dose of 0.11 mg/m².

The starting dose of diflomotecan was 0.05 mg/m²/day given as a 20-min infusion once daily for five consecutive days every 3 weeks. This was based on preclinical data (being less than one-tenth the MTD of 1.475 mg/m²/day in rats), and experience in a previous dose escalation and bioavailability trial of oral diflomotecan using a 5-day oral administration schedule. This trial established an RD of 0.27 mg/day [6], and mean oral bioavailability of 65% (range 7.6% to 100%). This is dependent on the nature and severity of toxicity at the prior dose level. Dose escalation ceased once the MTD had been identified. Up to 12 additional patients were then treated at the previous lower dose to confirm its tolerability as an RD for further studies. At least three patients were entered in all dose levels. If one of the three patients experienced DLT, three additional patients were entered at that dose level.

Dose-limiting toxic effects were identified during the first cycle only. These were defined as one of the following:

1. Grade 4 neutropenia lasting for ≥7 days.
2. Febrile neutropenia or neutropenic infection.
3. Thrombocytopenia <25 × 10⁹/l, or <50 × 10⁹/l with haemorrhage.
4. Any grade 3 non-haematological toxic effects attributable to the study drug (except untreated nausea, vomiting or diarrhoea).
5. Inability to complete repeated infusions of diflomotecan during cycle 1 as a consequence of drug-related toxicity.

The MTD was defined as that dose which induced DLT in no more than one-third of patients during their first cycle of treatment. The dose recommended for further development, or RD, was that immediately below the MTD. Toxic effects were defined according to the National Cancer Institute Common Toxicity Criteria (Version 2.0 1999) and judged dose limiting only if attributable to the study drug. Intrapatient dose escalation was not allowed.

Treatment was repeated every 3 weeks, provided haematological and serum chemistry parameters had recovered to within the limits specified at study entry, and any other treatment-related toxic effects (with the exception of alopecia) had resolved to baseline (grade 2) or levels permitted at study entry. If a patient experienced a DLT or other severe toxicity during treatment the dose for subsequent cycles was reduced to the previous dose level investigated. If treatment was delayed for >2 weeks the patient was withdrawn from the study.
treatment assessment
Before initiating therapy, a full medical history was taken and physical examination carried out. A complete blood cell (CBC) count, including haemoglobin, white blood cell (WBC) with differential count, platelets, red blood cell and serum biochemistry (including sodium, potassium, chloride, calcium, creatinine or creatinine clearance, glucose, total bilirubin, total protein, serum albumin, alkaline phosphatase, lactic dehydrogenase, AST, ALT and gluatryl transpeptidase), were carried out, as were tumour markers (if appropriate), urine analysis by dipstick, pregnancy test (in all females with reproductive potential), ECG and chest X-ray.

Weekly evaluations included history, physical examination, toxicity assessment, haematology and biochemistry tests. Haematology tests were determined on day 1, twice weekly of each cycle, and daily if febrile neutropenia occurred. Serum biochemistry was determined weekly. Radiological tumour evaluation was assessed after every two cycles and the best response to diflomotecan expressed according to RECIST criteria [7]. All patients who received at least one infusion of diflomotecan were analysed for toxicity, and those who had at least one on-treatment tumour measurement were assessed for response.

sample collection and drug analysis
For PK analysis, a total of 23 blood samples were collected on days 1 and 4 of the first cycle. Samples were taken immediately after the administration of diflomotecan, at 10, 20, 30 and 45 min after administration, then 1.5, 3.0, 4.5, 6.0, 8.5, 10.0, 24, 48, 72, 72.17, 72.3, 72.75, 73.5, 76.5, 78, 80, 82 and 96 h after treatment. All blood specimens were immediately put in an ice water bath (4°C) until centrifuged at 2000 rpm for 15 min at 4°C. For each blood sample, two 1.2 ml aliquots of plasma were transferred to polypropylene tubes (Eppendorf) and frozen at –80°C until analysis.

The plasma concentrations of diflomotecan and its metabolite BN80942 were quantified by high-performance liquid chromatography-tandem mass spectrometry-MS-MS after extraction from the matrix with acid diethyl ether as described previously [6].

PK and pharmacodynamic data analysis
PK parameters were calculated by standard noncompartmental methods using WinNonlin software (Scientific Consulting Inc.). The parameters determined for BN80915 and BN80942 included peak plasma concentration (Cmax), elimination rate constant (λz) estimated by linear regression of the terminal phase of the semilogarithmic plasma levels curve when this was clearly defined and elimination half-life (t1/2,λz) using the equation: t1/2,λz = ln 2/λz. The area under the plasma concentration time curve (AUC) from time zero to the last experimental time point (AUC0–t) was estimated by the linear-log trapezoidal rule and AUC(0–t) was calculated using the equation: AUC(0–t) = AUC(0–t) + (Ct/λz), where Ct is the predicted concentration at the last sampling time plasma clearance was calculated using the equation: CL = D (dose)/AUC0–t. The area under the first moment curve from time zero to the last experimental time point (t) (AUMC0–t) was estimated by the linear-log trapezoidal rule and the area under the first moment curve from time zero to infinity (AUMC0–∞) was estimated by adding the AUMC(0–t) value to the expression of the equation: AUMC0–∞ = [∫(t>Ct)/λz] + [Ct/λz]. Furthermore, the mean residence time (MRT) of the infusion (MRT0–t) was calculated by using the equation: MRT0–t = AUMC0–t/AUC0–t. The MRT was determined using the equation: MRT = MRT0–t – (T/2), T being the infusion duration. The volume of distribution (Vd) was calculated according to the equation: Vd = [D/AUC0–t/λz], and at steady state using the equation: Vss = CL·MRT.

The relationship between AUC and the resulting myelosuppression expressed as a percentage of maximum inhibition from baseline levels of haematological parameters obtained during the first cycle of administration was explored using the pharmacodynamic models simple Emax and sigmoid Emax according to the following mathematical equations, respectively: E = Emax·C/C0 + EC50 and E = Emax·C/C0 + EC50 where E = effect, Emax = maximum effect and fixed at 100; EC50 = AUC required to produce 50% of the drug-induced maximal effect (percentage of maximum inhibition for the haematological parameters obtained during the first cycle of administration), C = AUC value, and γ = shape parameter.

statistical analysis
The analysis of variance (ANOVA) of repeated measures was used to estimate residual variances in some PK parameters as well as to compare PK parameters for which full PK data were available at both day 1 and day 4 of administration. The intrapatient variability was expressed as the coefficient of variation (CV) in percentage terms and calculated by means of the expression CV (%) = 100 (eγ−1)1/γ, where VR is the residual variance obtained using ANOVA.

The statistical analysis was carried out by means of the NCSS 2001 software.

dose escalation
Patient characteristics are shown in Table 1. A total of 30 patients entered the study from July 2001 to May 2003 and received at least one cycle of i.v. diflomotecan. One patient was consented for the study, but did not receive any treatment due to a decline in performance status. The dose levels...
explored were 0.05, 0.1, 0.125 and 0.15 mg/m²/day for 5 days every 3 weeks. The total number of treatment cycles was 89 and the median number of cycles per patient was two (range 1–7).

Of the three patients treated at the initial dose level of 0.05 mg/m², none experienced DLTs, and the dose was doubled to 0.1 mg/m²/day. Three patients were initially entered at this level. Only mild, grade 2 drug-related toxic effects were observed, which allowed for a further dose escalation to 0.15 mg/m²/day. Of the first three patients entered at this level, one experienced a DLT (grade 4 neutropenia >7 days); a further three patients were entered at this dose level and another DLT was observed (grade 4 neutropenic infection, grade 4 diarrhoea, grade 3 dehydration, grade 3 bilirubin and grade 3 stomatitis). As a result, an additional three patients were treated at the lower 0.1 mg/m²/day dose level. None of these patients experienced DLTs, so a further three patients were treated at the intermediate dose level of 0.125 mg/m²/day in the next cohort. One of the three patients at the 0.125-mg/m²/day dose level had their dose capped due to obesity and received a dose equivalent to 0.105 mg/m²/day, but there were no DLTs, so the investigators and sponsor elected to investigate the 0.15-mg/m² dose level again. Two additional patients were entered at this level, one of whom had a DLT (grade 4 neutropenia >7 days). The 0.15-mg/m²/day dose level having caused DLT in three of eight patients (two of six patients when this dose was first investigated and one of two patients when it was investigated for the second time) was confirmed as the MTD by the investigators and sponsor. An additional 10 patients were treated at the 0.125-mg/m²/day dose level. In total three of the 13 patients treated at 0.125-mg/m²/day dose level, experienced DLT and this dose level was confirmed as the RD.

Table 2 summarises the DLTs experienced at the MTD and RD. Four patients required dose reductions after experiencing DLT (one patient experienced a DLT at both 0.15 mg/m² and 0.125 mg/m² per day and required two dose reductions to 0.1 mg/m²) and two patients were taken off study.

**Table 2. Summary of DLTs experienced at the MTD and RD**

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>Neutropenia grade 4 &gt;7 days</th>
<th>Neutropenic infection (grade)</th>
<th>Febrile neutropenia (grade)</th>
<th>Bilirubin (grade)</th>
<th>Diarrhoea (grade)</th>
<th>Rash (grade)</th>
<th>Stomatitis (grade)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.125 a (RD; n = 13)</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.15 a (MTD; n = 8)</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Three patients experienced DLTs in each dose level.
DLT, dose-limiting toxicity; MTD, maximum tolerated dose; RD, recommended dose.

**Table 3. NCI-CTC grade 3/4 related haematological toxicity of intravenous diflomotecan (all cycles)**

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>No. of patients/No. of cycles</th>
<th>Leucocytopenia (grade)</th>
<th>Neutropenia (grade)</th>
<th>Thrombocytopenia (grade)</th>
<th>Anaemia (grade)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>3/7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.10</td>
<td>6/22</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.125</td>
<td>13/37</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>0.15</td>
<td>8/23</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Patients had grade I anaemia at baseline.
NCI-CTC, National Cancer Institute Common Toxicity Criteria.

**haematologic toxicity**

Reversible neutropenia was the principal DLT. The severity of haematologic toxicity was dose dependent (Table 3). In total, three of the 13 patients treated at 0.125 mg/m² dose level, experienced dose-limiting grade 4 neutropenia and this was defined as the RD. All of these three patients were heavily pretreated, having received three or more lines of prior chemotherapy.

**nonhaematologic toxicity**

There were no grade 4 non-haematological toxic effects or DLTs (Table 4). Gastrointestinal toxicity was generally mild and diarrhoea uncommon. Only one patient experienced grade 3 diarrhoea at the 0.15-mg/m²/day dose level, which lasted <24 h. Twelve patients experienced mucositis, mainly grade 1 or 2 in intensity. The patient who had grade 3 diarrhoea also experienced grade 3 mucositis. Fatigue was frequently observed, but usually only grade 1 or 2 and often related to disease progression or anaemia rather than treatment with diflomotecan; four patients experienced grade 3 fatigue associated with disease progression. Alopecia was seen in 14 of the 30 patients. A transient grade 3 rise in γ-glutamyltransferase (GGT) was observed in four patients but resolved without intervention; subsequent cycles of treatment were administered and increases in GGT were again observed, but were not clinically significant. One patient treated at the 0.125-mg/m²/day dose level experienced a grade 3 erythematous, itchy, maculopapular skin rash over the torso and limbs judged secondary to diflomotecan, which resolved over a 2-week period with the use of emollients and mild topical steroid creams. The patient had, however, clinical disease progression with worsening liver biochemistry tests and subsequently withdrew from the study.

**antitumour activity**

A partial response was observed in one colorectal cancer patient with liver metastases who had previously received treatment with irinotecan/5-fluorouracil (5-FU)/folinic acid combination (the patient previously
had a partial response in liver metastases after six cycles of the FOLFIRI regimen. This was achieved after four cycles of treatment and confirmed by radiological reassessment after the sixth cycle of treatment. The patient requested to withdraw from the study at that point and remained well with no evidence of disease progression for a further 8 months. An additional eight patients had disease stabilisation for a median of 15 weeks, (range 7–24 weeks).

**PKs and pharmacodynamics**

PKs was evaluated in 29 patients; one patient at the 0.125-mg/m²/day level was not included due to difficulties in blood sampling. The mean [± standard deviation (SD)] PK parameters after the first and fourth dose administration in cycle 1 for all dose levels are shown in Table 5. Figure 2 shows the mean (± SD) diflomotecan plasma concentration levels following i.v. administration for day 1 and day 4 of cycle 1 at all dose levels.

When the ANOVA of repeated measures test was carried out to compare the most relevant PK parameters, using data from patients obtained on both day 1 and day 4 of administration at different doses, no statistical differences could be evidenced in Cmax/D, AUC/D, CL, t1/2,z, MRT, Vz and Vss attributable to the administration day (day 1 or day 4) providing no indication of either time-dependent or dose-dependent kinetics. There was, however, wide variability obtained in the main PK parameters such as AUC and CL, with CV (%) ranging from 17.1% to 76.1% and 15.1% to 100%, respectively, across all dose levels including data at day 1 and day 4.

In order to explore the relationship between myelosuppression and diflomotecan exposure, the percentage maximum inhibition of neutrophil and platelet counts was correlated with AUC after i.v. administration of diflomotecan following the first cycle in all patients. A summary of mean ± SD data is given in Table 6. At the RD 0.125 mg/m²/day, mean percentages of maximum inhibition for WBC, neutrophils and platelets were 57.1%, 63.1% and 46.0%, respectively (Table 6).

The relationship between AUC and the percentage maximum inhibition for the haematological parameters obtained during the first cycle of administration was explored using simple Emax and sigmoid Emax models. The results are summarised in Table 7 in which the observed AUC values required to produce 50% of the drug-induced maximal effect using a sigmoid Emax model were 27.88, 24.00 and 37.73 ng/h/ml for WBC, neutrophils and platelets, respectively. These results are consistent with those obtained from another phase I trial [6] in which diflomotecan was given as a solution orally daily for 5 days every 3 weeks where these values were 36.63, 29.58 and 52.47 ng/h/ml, respectively.

**Discussion**

Topoisomerase I inhibitors are an increasingly important class of anticancer drugs, with irinotecan widely used as a single agent or in combination with a fluoropyrimidine in metastatic colorectal cancer and topotecan used in the second-line management of ovarian cancer. Both of these compounds, however, have limitations in terms of activity and toxicity. The active lactone form of the camptothecin derivative is unstable and this may explain the sometimes limited antitumour activity in humans. Diflomotecan is more potent in several in vitro models than either irinotecan or topotecan and this may, in part, be explained by the increased stability of its active lactone form over the topoisomerase I inhibitors currently in use.

This study shows that i.v. administration of diflomotecan over 5 days, repeated every 3 weeks is feasible in adults with advanced solid malignancies. In general the regimen was well tolerated, with myelosuppression being the main toxicity. At the RD of 0.125 mg/m²/day, four out of the 13 patients treated experienced grade 4 neutropenia at the first cycle, three of which constituted a DLT. Only one episode of febrile neutropenia was observed at this dose level.

As in the previous phase I study of oral diflomotecan, and in contrast to some other topoisomerase I inhibitors, gastrointestinal toxicity was mild. Diarrhoea was surprisingly uncommon and generally not clinically serious with only grade 2 diarrhoea, nausea and vomiting observed at the RD. This is in contrast to irinotecan where grade 3 or 4 diarrhoea may occur in up to 25% of patients [8]. Fatigue was frequently observed, but felt in many cases to be related to anaemia or tumour progression. Alopecia was seen in 14 out of the 30 patients treated. Transient grade 3 rises in GGT were frequent but settled without intervention, did not delay subsequent cycles of treatment and were not clinically significant. One patient at the RD experienced a grade 3 skin rash.

PK analysis showed that the most relevant PK parameters were neither time nor dose dependent. There was, however, a wide interpatient variability in the main PK parameters such as AUC and CL at all dose levels. This high interpatient variability in PKs has also been observed with other topoisomerase I inhibitors, irinotecan [9] and topotecan. Analysis of the relationship between the CBC and diflomotecan exposure demonstrated that at the RD, the mean percentages of maximum inhibition for WBC, neutrophils and platelets were 57.5%, 63.1% and 46.0%, respectively (Table 6).

Antitumour activity was not the primary end point of this study and the patient population was, in general, heavily pretreated with over half the patients having cancers not predicted to respond to a topoisomerase I inhibitor. One of
Mean standard deviation BN80915 pharmacokinetic parameters after BN80915 first and last administration (all doses)

<table>
<thead>
<tr>
<th>Parameter Dose BN80915 (mg/m²)</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 1</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/ml)</td>
<td>0.05 (n = 3)</td>
<td>0.10 (n = 6)</td>
<td>0.05 (n = 3)</td>
<td>0.10 (n = 6)</td>
</tr>
<tr>
<td>AUC (ng/ml·h)</td>
<td>0.125 (n = 6)</td>
<td>0.125 (n = 6)</td>
<td>0.125 (n = 6)</td>
<td>0.125 (n = 6)</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>0.125 (n = 6)</td>
<td>0.125 (n = 6)</td>
<td>0.125 (n = 6)</td>
<td>0.125 (n = 6)</td>
</tr>
</tbody>
</table>

PK, pharmacokinetic; PD, pharmacodynamic.

The patients with metastatic colorectal cancer (who previously received combination treatment with irinotecan and 5-FU), however, achieved a partial response and a further 11 patients had disease stabilisation for between two and six cycles of treatment.

In conclusion, the MTD and the RD of diflomotecan administered according to d×5 repeated three weekly is 0.15
and 0.125 mg/m²/day, respectively. Although interpatient PK variability was wide, in general treatment was well tolerated, the principal toxicity being reversible myelosuppression. Further studies of diflomotecan (BN80915), using this i.v. daily × 5 schedule, are warranted.

references