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Phase I dose-escalation study of F60008, a novel apoptosis inducer, in patients with advanced solid tumours

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ABSTRACT

Resistance of cancer cells to cytotoxic therapy can be caused by the activation of strong anti-apoptotic effectors, for example NF-κB. Therefore, compounds that inhibit NF-κB stimulation might overcome chemotherapy resistance. F60008, a semi-synthetic derivate of triptolide, is converted to triptolide in vivo and activates apoptosis in human tumour cells. We performed a phase I and pharmacological study of F60008 given intravenously as a weekly infusion for 2 weeks every 3 weeks in patients with advanced solid tumours. Twenty patients were enrolled, and a total of 35 cycles were administered. The most frequent haematological side-effect was mild grade 1–2 anaemia. Non-haematological toxicities included fatigue, nausea, vomiting, diarrhoea and constipation, all grade 1–2. Two lethal events were observed in which an increase in caspase-3 activity and overt apoptosis in monocytes and neutrophils could be seen. Pharmacokinetic studies showed high inter-individual variability and rendered F60008 a far from optimal derivate of triptolide.

1. Introduction

Apoptosis or programmed cell death (PCD) is an evolutionally conserved mechanism for the removal of unwanted or aberrant cells, involving a complex set of highly regulated pathways activated by diverse stimuli. In these pathways, p53 and NF-κB play an important role.

One of the mechanisms of resistance of cancer cells to cytotoxic therapy is the lack of response to apoptotic signals, either by defective PCD pathways (e.g. because of mutations) or by activation of anti-apoptotic stimuli. TNF, radiation therapy as well as cytotoxic therapy are known for their activation of NF-κB, a potent anti-apoptotic effector, which may play a role in preventing apoptosis in cancer cells.[1–6] Compounds that inhibit NF-κB stimulation might overcome chemotherapy resistance.

Triptolide (PG490) is a low molecular weight, diterpene triepoxide derived from Tripterygium wilfordii Hook f, a plant that has been used in traditional Chinese medicine for many years. Crude preparations of this herb have been used in the treatment of lupus erythematosus, rheumatoid arthritis and other autoimmune diseases.[7] The observed side-effects include anorexia, vomiting, abdominal pain, diarrhoea, oesophageal burning, leucopaenia, thrombocytopaenia, oral ulcers, skin pigmentation and amenorrhoea. The contribution of triptolide to these side-effects is, however, unclear because of the heterogenous nature of the preparations used.

F60008 is a semi-synthetic derivate of triptolide that is highly water soluble, and acts as a prodrug that is converted to triptolide in vivo following intravenous administration. Triptolide activates apoptosis in human tumour cells by a novel

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mechanism that appears to involve the modulation of p53 and associated proteins. Following exposure to triptolide, accumulation of p53 occurs by enhanced p53 translation as well as by the suppression of Mdm2, reducing the removal of p53 and p21, leading to the induction of the apoptotic response.[8–10] Triptolide has also been shown to induce apoptosis by inhibiting both the activation of NF-κB and the transcriptional activation of NF-κB-inducible genes, the activation of this transcription factor being one of the principal mechanisms of inducible tumour chemoresistance in response to chemotherapy.[10–13] Triptolide seems also able to inhibit the heat shock response rendering cells sensitive to stress-induced cell death.[14]

In vitro studies have shown that treatment with triptolide can result in significant inhibition of growth of a broad range of tumour cell lines, including NSCLC, colon cancer, prostate cancer and fibrosarcoma, and that treatment with F60008 exerts a strong antitumour effect against xenografts tumours derived from these cells.[15]

Toxicology studies in mice, dogs and monkeys demonstrated F60008 toxicity primarily in tissues with rapid proliferating cells, such as bone marrow, GI tract, lymphoid tissues and testicular tissue. At higher dose levels, vein irritation was seen in animals treated with multiple injections. In dose finding studies in which F60008 was administered daily for 7 d the NOAELs (no observed adverse effect levels) were 100 and 50 μg/kg in dogs and monkeys, respectively. No cardiovascular effects were observed in dogs, and no neurotoxicity was observed in mice. The pharmacokinetics (PKs) of F60008 have been studied in vitro in mouse, monkey and human plasma showing a large interspecies difference in conversion of F60008 to triptolide being rapid and complete in mice and incomplete in monkey plasma (approximately 5% at 24 h). However, in vivo, conversion of F60008 into triptolide in the plasma of 18 human volunteers showed a 2–3-fold variation between different plasma samples. At 24 h, 15–45% conversion had taken place and conversion had increased to 31–64% by 72 h.

The plasma half-life of F60008 in treated monkeys was less than 1 h with no evidence of drug accumulation over time of either F60008 or triptolide.

We performed a phase I and pharmacological study of F60008 given intravenously as a weekly infusion for 2 weeks every 3 weeks in patients with advanced solid tumours. The principal objectives of this study were to (a) determine the maximum-tolerated dose (MTD) and dose-limiting toxicity (DLT), (b) characterise safety and tolerability of F60008 including acute and chronic toxicities, (c) determine the pharmacological profile of F60008 and triptolide, (d) explore the pharmacodynamic activity of F60008 in peripheral blood cells and (e) seek preliminary evidence of antitumour activity.

2. Patients and methods

2.1. Eligibility criteria

Patients with a cytologically or histologically confirmed diagnosis of an advanced solid malignancy for whom standard therapy options did not exist and who received no more than two prior combination chemotherapy regimens were eligible. Additional criteria included age between 18 and 80 years; WHO performance status $\leqslant 2$; a period > 4 weeks elapsed since the last chemotherapy or radiotherapy; an adequate bone marrow function (haemoglobin $\geqslant 8.5$ g/dl, absolute neutrophil count $\geqslant 1.5 \times 10^9\,l^{-1}$ and platelet count $\geqslant 100 \times 10^9\,l^{-1}$), liver function (bilirubin $\leqslant$ upper limit of normal (ULN), alanine aminotransferase and aspartate aminotransferase $\leqslant 3 \times$ ULN) and renal function (serum creatinine $\leqslant 1.5 \times$ ULN) and left ventricular ejection fraction (LVEF) $\geqslant 45\%$ measured by radionuclide angiography (MUGA scan). Specific exclusion criteria included primary central nervous system neoplasm or known brain or leptomeningeal metastases; prior high dose chemotherapy with haematopoietic stem cell rescue and prior extensive radiotherapy up to more than 25% of bone marrow reserve. This study was approved by the local ethics committees, and all patients gave written informed consent prior to study entry.

2.2. Study design

This was an open-label, dose escalation, single centre phase I study. F60008 was supplied by Pierre Fabre Medicament (Boulogne, France) as vials containing 2 mg of the active study drug. F60008 was stored at 2–8 °C until use. Once diluted, F60008 is chemically stable for up to 8 h at 2–8 °C. Each vial was reconstituted with 2 ml of sterile water to achieve a concentration of 1 mg/ml of F60008. The total daily dose was diluted to a final volume of 50 ml with normal saline and infused through a peripheral line over 30 min using a volumetric pump. Patients were treated in 3-week cycles consisting of 2 weekly infusions (days 1 and 8).

Dose escalation was based on an accelerated titration design,[16] with initial single patient cohorts. Once a patient experienced $\geqslant$ grade 2 toxicity, cohorts were expanded to 3–6 patients. The maximum-tolerated dose (MTD) was defined as the lowest dose at which 2 of 3 or 2 of 6 patients developed a dose-limiting toxicity (DLT) during the first cycle. The recommended dose (RD) was the next lower dose level below the MTD. After defining the MTD, the RD would be further confirmed by accrual of at least 6 new patients at that dose level.

DLT was based on the National Cancer Institute Common Toxicity Criteria version 2.0, and was defined as the occurrence during the first cycle of grade 4 neutropaenia $\geqslant 7$ days or grade $\geqslant 3$ neutropaenic fever, thrombocytopaenia $<25 \times 10^9\,l^{-1}$, grade $\geqslant 3$ non-haematological toxicity (excluding nausea, vomiting or diarrhoea responsive to optimal antiemetic or anti-diarrhoeal treatment) and grade 3 elevation of transaminases for $\geqslant 7$ d. DLT also included any other drug-related toxicity grade $\geqslant 3$, any drug-related adverse event during cycle 1 that required a dose modification or resulted in a missed dose and any drug-related adverse event that required a delay $\geqslant 2$ weeks in the administration of the first infusion of cycle 2. Patients who experienced a DLT were allowed to resume treatment once the toxicity resolved to baseline or grade $\leqslant 1$. Patients requiring more than 2 weeks to recover from a DLT were declared to have an unacceptable toxicity, and were withdrawn from the study. Intrapatient dose escalation was not allowed.

2.3. Pretreatment and follow-up studies

Before therapy, a complete medical history was taken and a physical examination was done. A complete blood cell count, including WBC differential and serum biochemistry, which included serum transaminases, alkaline phosphatase, total bilirubin, BUN, serum creatinine, glucose, calcium, sodium, potassium, magnesium, chloride, bicarbonate, total protein and albumin were done, as were urinalysis, electrocardiogram and chest X-ray. Also a Muga scan to assess the LVEF was performed prior to the start of therapy. During the first cycle, haematology assessments were performed twice weekly. Furthermore, weekly evaluations during the first cycle and on days 1 and 8 of each subsequent cycle included physical examination, toxicity assessment and complete blood count including WBC differential as well as serum biochemistry. Tumour measurements were performed every two cycles. Response was assessed using RECIST.[17] Patients were allowed to continue treatment in the absence of progressive disease or unacceptable toxicity. At the end of the study treatment, a second Muga scan was performed.

2.4. Pharmacokinetic sampling and data analysis

For pharmacokinetic analyses, blood samples (13 ml for the pre-dosing sample and 3 ml for all other samples) were collected using an indwelling intravenous (i.v.) canula, in the opposite arm of infusion, before dosing and 15, 30, 35 and 50 min, and 1.5, 2.5, 4, 6, 9, 24 and 48 h after the start of the infusion of the drug on days 1 and 8 of the first cycle. A subsequent blood sample was taken just before the administration of the drug on day 1 of cycle 2. Blood samples were collected in precooled NaF-EDTA containing tubes, and plasma was separated by centrifugation at 4 °C during 10 min at 2800 rpm as soon as possible after collection. Plasma samples were stored at –80 °C until analysis. Pharmacokinetic analysis included the maximum plasma concentration (C_{max}), the time to C_{max} (T_{max}), the observed area under the plasma concentration–time curve (AUC_{last}), the area under the plasma concentration–time curve extrapolated to infinity (AUC_{inf}), the terminal half-life ($T_{1/2z}$), total body clearance (Cl_{tot}), terminal volume of distribution (V_d) and volume of distribution at a steady state (V_{ss}). Plasma concentrations were quantified by a validated LC-MC/MS method with a LLOQ at 1.0 ng/ml and 0.1 ng/ml for 60008 and triptolide, respectively.

2.5. Pharmacodynamic sampling and analysis

For pharmacodynamic analyses, blood samples were collected pre-dosing, at 6 and at 48 h after infusion of F60008 on days 1 and 8. In these samples, the absolute numbers of lymphocyte subsets, the proportion of leukocyte subsets displaying apoptosis and the immune functions of lymphocytes and monocytes were assessed.

The absolute numbers of lymphocyte subsets in blood samples were assessed by flow cytometry as described.[18] The percentage of leukocyte subsets prone for apoptosis was monitored by flow cytometry by assessing the level of activation of caspase-3 during the incubation of blood at 37 °C versus incubation at 4 °C for 18 h. The surface membrane staining, in order to identify the leukocyte subsets, using CD3, CD14 and CD66e in combination with CD45 for lymphocytes, monocytes and granulocytes, respectively, was followed by intracellular staining of activated caspase-3.

The potential immunosuppressive effects of F60008 on blood leukocytes were studied by (1) the assessment of the level of induction of the early activation antigen CD69 on blood lymphocytes following activation by the co-mitogenic mAbs CD2/CD2R,[19] (2) the induction of IFN production by blood T lymphocytes following activation by PMA + ionomycin[20] and (3) the induction of IL-8 and TNF production by blood monocytes following activation by LPS.[20]

3. Results

A total of 20 patients were enrolled into 9 dose cohorts. Patient's characteristics are listed in Table 1. All patients were eligible. A total of 35 cycles were administered with a median of 2 cycles per patient, range 1–4. One female patient never started therapy because of rapid deterioration of her clinical condition due to the underlying illness. Another patient treated at the 12 mg/m^2 dose level did not receive on day 8 of cycle 1. He was admitted on day 3 for one day with grade 3 diarrhoea, rapidly responding to treatment with loperamide and therefore not qualifying as DLT. However, on patient's request further administrations were halted. Although this patient was not evaluable for pharmacokinetic and pharmacodynamic analyses and was therefore replaced, he was included in the toxicity analyses. Dose levels studied ranged from 0.5 to 18 mg/m^2 (Table 2).

In the absence of grade $\geqslant$ 2 toxicity, the dose of F60008 was escalated in single patient cohorts to 18 mg/m^2. At the dose level of 18 mg/m^2, a 59 years old male with a diagnosis of stage IV oesophageal carcinoma with metastases to the liver and mediastinal lymph nodes was included. He had previously received 6 courses of oxaliplatin and capecitabine, followed at disease progression by 2 courses of irinotecan. After further disease progression, he participated in an

Table 1 – Patient characteristics.

Patient characteristics	Number of patients
Number entered	20
Number started	19
Number assessable for toxicity	19
Number assessable for pharmacokinetics/-dynamics	16
Male/female	16/3
Age (years)	
Median	62
Range	41–73
WHO performance status	
Median	1
Range	0–1
Tumour type	
Colorectal carcinoma	5
Oesophageal carcinoma	4
GIST	2
Miscellaneous	8

Table 2 – Dose-escalation scheme.

Dose level (mg/m^2)	Number of patients	Total number of cycles	Number of patients with DLT
0.5	1	1	0
1	1	2	0
1.5	1	4	0
2.2	1	2	0
3.4	1	2	0
5	3	6	0
7.5	3	6	0
12	7	11	1
18	1	1	1

experimental phase I study with an anti-VEGF compound. His medical history revealed a myocardial infarction 9 years prior to the inclusion in the present study with currently no cardiac symptoms. At study entry WHO performance score was 0, ECG normal and LVEF 58%. Twenty-four hours after receiving the study drug he complained of nausea and vomiting grade I and he developed fever (38.5 °C), shivers and nasal congestion. Physical examination did not show any signs of infection. During that day the temperature increased to 39.4 °C with a pulse-rate of 102 min^{-1}. Laboratory tests showed WBC 2.3×10^9 l^{-1}, ANC 1.47×10^9 l^{-1}, Hb 8 mmol/l, platelets 172×10^9 l^{-1} and a negative CRP. Blood cultures were taken and remained negative. The patient was treated with i.v. antibiotics, initially improving symptoms. However, after another 4 h there was a further and rapid clinical deterioration with dyspnoea, epigastric pain and vomiting. A chest X-ray showed bilateral pulmonary oedema. Subsequently, grade 4 metabolic acidosis, with hypoxia and hypotension developed, and despite extensive supportive care the patient died within 45 min. Autopsy showed pulmonary oedema with pre-existing extensive emphysema, but no embolism or pneumonia. There was also liver congestion and fibrosis with slight steatosis as well as spleen fibrosis. All cultures remained negative. There were no other relevant findings, and a clear cause of death could not be established.

Because a relationship with the study drug could not be ruled out further dose escalation was halted. The protocol was amended, and the former dose levels starting at 5 mg/m^2, followed by 7.5 and 12 mg/m^2, were expanded to 3–6 patients. Toxicities observed are displayed in Tables 3 and 4. During expansion of the cohort to 3 patients at 5 and 7.5 mg/m^2, no DLTs were observed. The first 4 additional patients at 12 mg/m^2 did not encounter any DLTs as well. The sixth patient in this cohort was a 60 years old male with a metastatic rectal carcinoma. He had previously been treated with the combination of oxaliplatin and capecitabine, followed at disease progression by irinotecan. Shortly after his first administration he experienced nausea and vomiting as well as a sudden feeling of fatigue, all grade 2. Twenty-four hours after the administration he felt well enough to go home. Just prior to discharge neutrophils had dropped to a grade 4. The second day after the administration he experienced abdominal cramping, sweating and agitation. There were no signs of dyspnoea, or fever. Vital signs remained normal. Diclofenac and omeprazol were prescribed by the GP to control the abdominal pain. Ten hours thereafter the GP noted abdominal bruising of the skin and absent bowel sounds. There was no fever or dyspnoea. All of a sudden hypotension and bradycardia occurred, and the patient died shortly thereafter, prior to enabling transportation to the hospital. CPR was not successful. Autopsy was performed and revealed multiple colorectal metastases as well as some lymph node metastases in the anterior mediastinum. Pulmonary oedema was observed, and both lungs contained bacterial conglomerates. Culturing these conglomerates revealed Streptococcus Pneumoniae. It was concluded that the patient's most likely cause of death was neutropaenic sepsis. In view of this second possibly drug-related death, patient accrual was stopped.

3.1. *Toxicity*

Apart from the 2 lethal events toxicities were mild. The most frequent haematological side-effect was mild grade 1–2 anaemia (Table 3). The most common non-haematological toxicities (Table 4) were fatigue, nausea, vomiting, diarrhoea and constipation, all grade 1–2.

No renal or liver toxicities were seen.

3.2. *Pharmacokinetics*

A total of 19 patients were treated of which all patients were evaluable for PK assessment on day 1 and 16 on day 8.

Pharmacokinetics of F60008 can be described by a bi-exponential decay, and plasma concentrations reached their maximum at the end of the infusion where after a rapid decline

Table 3 – Worst haematological toxicity per patient per dose level.

Dose level (mg/m^2)	Number of pts.	Hb		WBC		ANC	
		Grade I/II	Grade III/IV	Grade I/II	Grade III/IV	Grade I/II	Grade III/IV
0.5	1	–	–	–	–	–	–
1	1	–	–	–	–	–	–
1.5	1	1	–	–	–	–	–
2.2	1	1	–	–	–	–	–
3.4	1	–	–	–	–	–	–
5	3	2	–	1	–	–	–
7.5	3	3	–	–	–	–	–
12	6	4	–	–	1	–	1
18	1	–	–	1	–	1	–

Table 4 – Worst non-haematological toxicity per patient per dose level.

Dose level (mg/m^2)	Number of pts.	Nausea		Vomiting		Diarrhoea		Constipation		Fatigue	
		Grades I/II	Grades III/IV	Grades I/II	Grades III/IV	Grades I/II	Grades III/IV	Grades I/II	Grades III/IV	Grades I/II	Grades III/IV
0.5	1	–	–	–	–	–	–	–	–	–	–
1	1	–	–	–	–	–	–	–	–	–	–
1.5	1	–	–	–	–	–	–	–	–	1	–
2.2	1	1	–	–	–	1	–	–	–	1	–
3.4	1	1	–	–	–	1	–	–	–	1	–
5	3	2	–	1	–	–	–	2	–	2	–
7.5	3	–	–	–	–	1	–	1	–	2	–
12	6	3	–	2	–	2	1	–	–	4	–
18	1	1	–	1	–	–	–	–	–	–	–

within the first hour after infusion was observed (Fig. 1). Plasma concentrations were quantifiable up to 9 h. For all dose levels, plasma concentrations were comparable between days 1 and 8 (data not shown), and plasma concentrations showed no signs of accumulation between days or between cycles.

Table 5 shows the main pharmacokinetic parameters of F60008 on day 1 of cycle 1. The elimination was rapid with a $T_{1/2z}$ around 3 h. AUC_{inf} increased with dose level and presented a moderate inter-individual variability.

Triptolide, the active metabolite of F60008, plasma concentrations reached their maximum values (C_{max}) between 0.5 and 2.5 h post-administration of F60008. After that, concentrations decreased sharply according to a mono-exponential decay (Fig. 2), and were quantifiable up to 24 h at dose level 5 mg/m^2 or 48 h post-dosing for levels 7.5 and 12 mg/m^2.

Table 6 shows the main pharmacokinetic parameters of triptolide on day 1 of cycle 1. For dose levels from 0.5 to 5 mg/m^2, most PK parameters were not calculated: triptolide

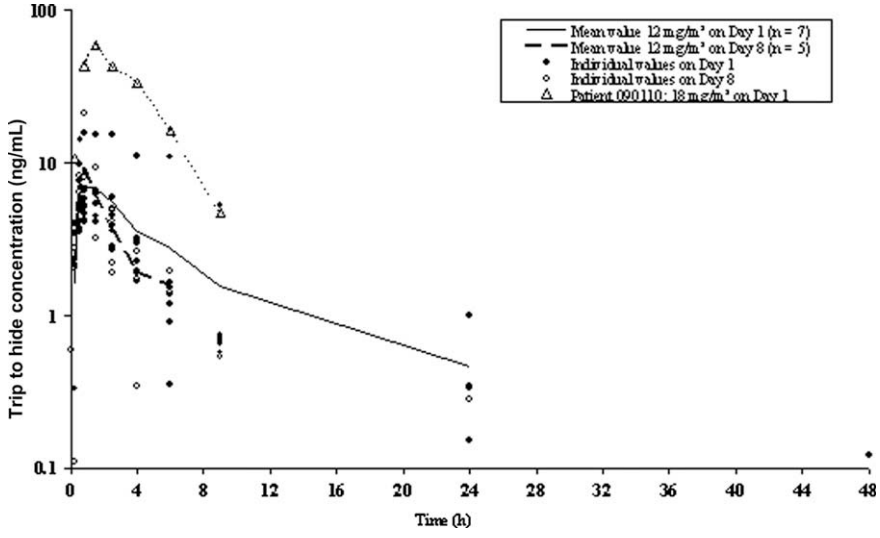

Fig. 1 – Concentration versus time profiles of F60008 on day 1 and 8 (dose level 12 mg/m^2).

Table 5 – Main pharmacokinetic parameters of F60008 on day 1 of cycle 1.

Dose level (mg/m^2)	n	C_{max} (ng/ml) Median [range]	T_{max} (h) Median [range]	AUC_{inf} [(ng/ml) h] Median [range]	$T_{1/2z}$ (h) Mean (s.d.)	Cl_{tot} (L/h) Mean (s.d.)
0.5–3.4[a]	5	[20.5–224]	[0.417–0.583]	[128–361][b]	[1.36–2.93][b]	[18.8–21.8][b]
5	3	268 [218–469]	0.467 [0.467–0.6]	456 [436–642]	3.28 (1.21)	20.3 (3.16)
7.5	3	270 [193–480]	0.467 [0.467–0.833]	518 [354–673]	3.51 (2.86)	34.0 (9.87)
12	7	508 [249–887]	0.467 [0.25–0.617]	701 [494–1553]	2.42 (1.15)	32.1 (12.3)
18	1	1361	0.5	2229	1.76	15.7

a No statistical analysis was performed since one patient was included by dose level. Only ranges are presented.
b $n = 2$. Extrapolated part of AUC > 15% for the 3 other patients.

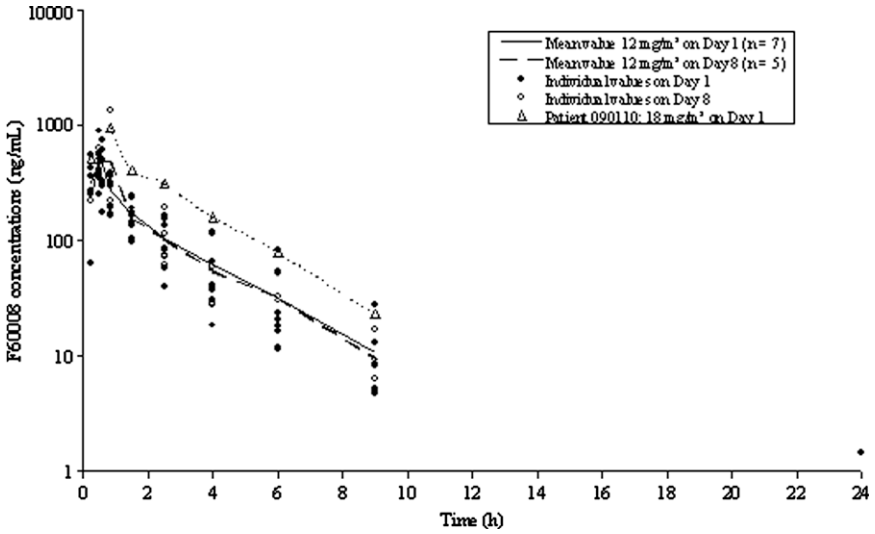

Fig. 2 – Concentrations versus time profiles of triptolide on day 1 and 8 (dose level 12 mg/m^2).

Table 6 – Main pharmacokinetic parameters of triptolide per dose level on day 1 of cycle 1.

Dose level (mg/m^2)	*n*	C_{max} (ng/ml) Median [range]	T_{max} (h) Median [range]	AUC_{0-9h} [(ng/ml) h] Median [range]	AUC_{inf} [(ng/ml) h] Median [range]	$T_{1/2z}$ (h) Mean (s.d.)
1–3.4[a]	4	[1.15–4.02]	[0.467–2.72]	[0.257–9.41]	n.c.	n.c.
5	3	9.82 [5.1–10.3]	0.833 [0.467–2.53]	34.7 [27.1–45.3]	n.c.	n.c.
7.5	3	7.31 [6.35–9.3]	1.50 [0.467–1.5]	32.9 [21.7–41.1]	[37.9-80.6] [b]	[2.92-13.1] [b]
12	7	6.78 [4.45–15.6]	0.833 [0.467–1.52]	22.2 [13.4–97.2]	33.3[c] [19.2–144]	5.26[c] (3.26)
18	1	58.5	1.5	233	248	2.00

a No statistical analysis was performed since only 1 patient per dose level was included. For Patient 090101 (dose level 0.5 mg/m^2), triptolide concentrations were not quantifiable.
b *n* = 2. No statistical analysis was performed, only ranges are presented.
c *n* = 6. For Patient No. 090109, extrapolated part of AUC > 15%. n.c.: not calculated – all extrapolated parts of AUC > 15%.

was either not quantifiable in plasma samples, or the extrapolated part of AUC was higher than 15%.

C_{max} and AUC_{0-9h} did not increase with dose levels but the inter-individual variability calculated on AUC_{0-9h}/dose was 83%. The elimination half-life of triptolide was 3–13 h, and accumulation of triptolide was negligible; the accumulation ratio was close to 1, irrespective of dose level. For all dose levels, plasma concentrations were similar between day 1 and 8 (data not shown). Triptolide concentrations of the patient who received 18 mg/m^2 and died on day 2 were 10 times higher than those observed at 12 mg/m^2, but had a similar pharmacokinetic profile (Fig. 2). When linear regression between doses of F60008 and Triptolide was calculated, the coefficient of determination (R^2) was 0.34. High inter-individual variability could explain the lack of pharmacokinetic linearity on AUC_{0-9h}/dose.

Exposure parameters on day 1 of F60008 (AUC_{inf}) and triptolide (AUC_{0-9h}) of patients who experienced DLTs or drug-related SAEs revealed that the adverse events of the patients who died in dose level 12 mg/m^2 and 18 mg/m^2 were due to overexposure (AUC_{inf} and AUC_{0-9h} were 1553 and 97.2 (ng ml)h and 2229 and 233 (ng ml)h, respectively). However, PK parameters of the patient in dose level 12 mg/m^2 who experienced grade 3 diarrhoea were comparable with those of other patients without adverse events.

3.3. *Pharmacodynamics*

Potential effects of F60008 on peripheral blood leukocytes were assessed by flow cytometric enumeration of peripheral blood lymphocyte subsets and by flow cytometric enumeration of intracellular cytokine production in lymphocytes and monocytes using several techniques as described in the patients and methods section. At dose levels 0.5–7.5 mg/m^2, no statistically significant difference could be found in peripheral blood lymphocyte subsets and intracellular cytokine production in lymphocytes between pre-administration and post-administration time-points (data not shown).

Fig. 3 shows the absolute numbers of leukocytes and lymphocyte subsets prior to and after 48 h of F60008 administration in the 12 and 18 mg/m^2 dose levels.

These analyses showed, when comparing day 3 observations versus pre-treatment values, that infusion of F60008 induced a decrease of blood leukocytes, but not of lymphocyte subsets. In addition, F60008 infusion induced slightly lower activation induction potency, as shown by lower induction of the activation marker CD69. Furthermore, it induced the production of cytokines by blood lymphocytes and monocytes, as well as a decreased proportion of apoptotic monocytes (not lymphocytes and granulocytes). Our data confirm

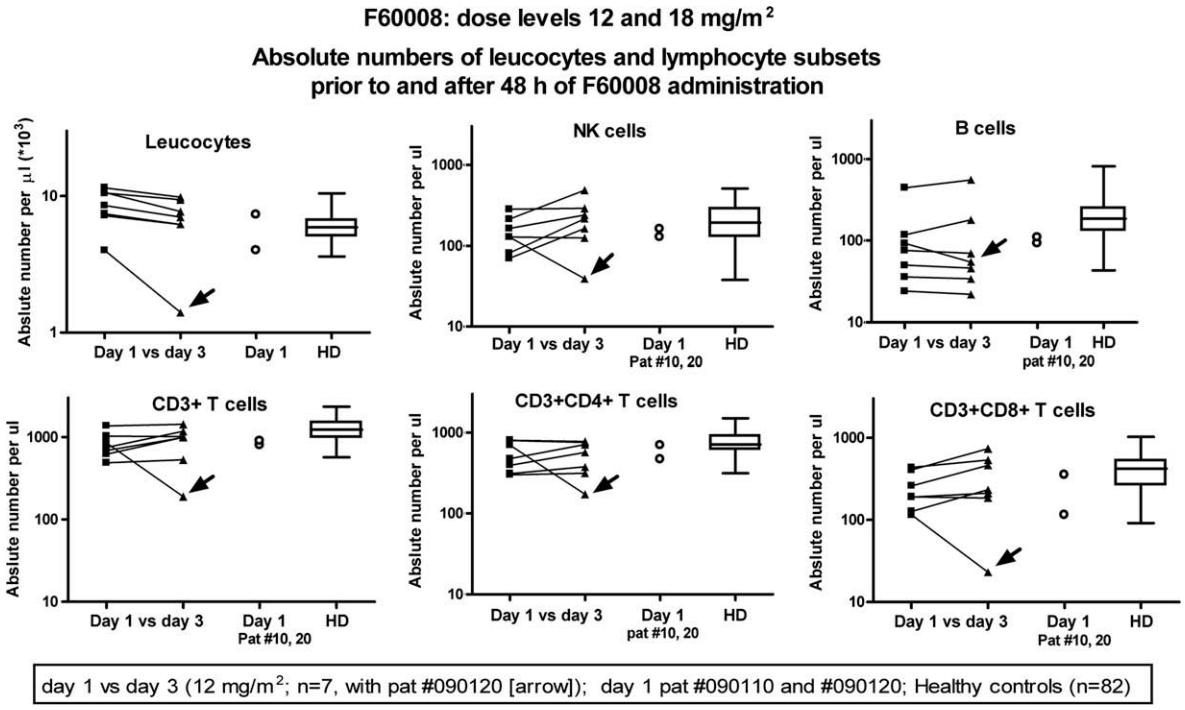

Fig. 3 – In the left part of the figures the levels of specific subsets can be seen on day 1 and 3. In the middle part the specific levels of the patient who died in the dose level of 18 mg/m² (number 10) and the patient who died in the dose level of 12 mg/m² (number 20) are shown. On the right side of the figures the healthy donor controls are shown in Box-Whisker plots. The horizontal line depicts the median observation, the box depicts the interquartile range 25–75% and the whiskers depict the 5–95% confidence interval. The arrow corresponds to patient number 20.

the observation that blood lymphocyte(s) (subsets) in cancer patients are lower than in healthy controls.

The two patients who experienced DLT showed, in addition to above mentioned observations, a decrease of all lymphocyte subsets, and at 6 h post-infusion increase of apoptotic monocytes, and granulocytes (not lymphocytes). In addition, in the blood of the patient dosed with 18 mg/m² also blood cytokine levels were determined showing an increase of the 'trauma'-related cytokines VEGF, sTNFRI and IL-8 (Fig. 4).

Fig. 5 shows that the caspase-3 activity in lymphocytes is within the range of healthy donors prior to and 6 h after therapy. Forty-eight hours after therapy, however, all patients showed a slight decrease of caspase-3 activity (not significant)

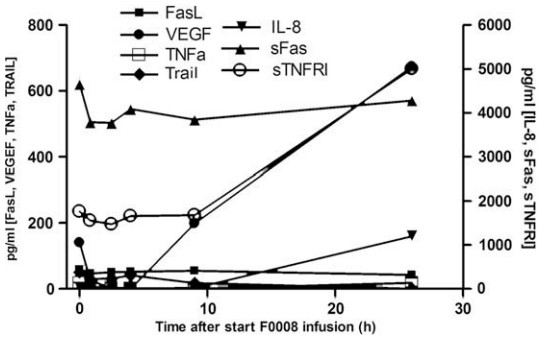

Fig. 4 – Levels of different cytokines in the patient treated at dose level 18 mg/m².

except patient number 20 (dose level 12 mg/m²) who showed an increase (arrow). The caspase-3 activity in monocytes was also within the normal range prior to F60008 therapy. Six hours after administration of F60008, however, the level of caspase-3 activity strongly increased in the 2 patients who died and were treated in dose levels 12 and 18 mg/m², whereas the other 5 patients showed a decrease. Caspase-3 activity in granulocytes was slightly lower than in healthy donors, prior to therapy ($P = 0.025$). Six hours after administration the caspase-3 levels increased to match the levels in healthy donors.

3.4. *Discussion*

Apoptosis or programmed cell death (PCD) is an evolutionally conserved mechanism for the removal of unwanted or aberrant cells. Resistance of cancer cells to cytotoxic therapy can at least partly be explained by the lack of response to apoptotic stimuli and hence defective apoptosis. Understanding the complex mechanisms involved in apoptosis is therefore of major clinical importance.

There are two main routes to apoptosis: one involving stimulation of death receptors (e.g. the tumour necrosis factor receptor, TNFR family) by external ligands such as TNF or TRAIL (TNF-α-related apoptosis inducing ligand), and the other involving the mitochondria. The latter pathway is activated in case of DNA damage or in case of disruption of the delicate balance between pro- and anti-apoptotic stimuli (e.g. pro-apoptotic and anti-apoptotic members of the Bcl-2

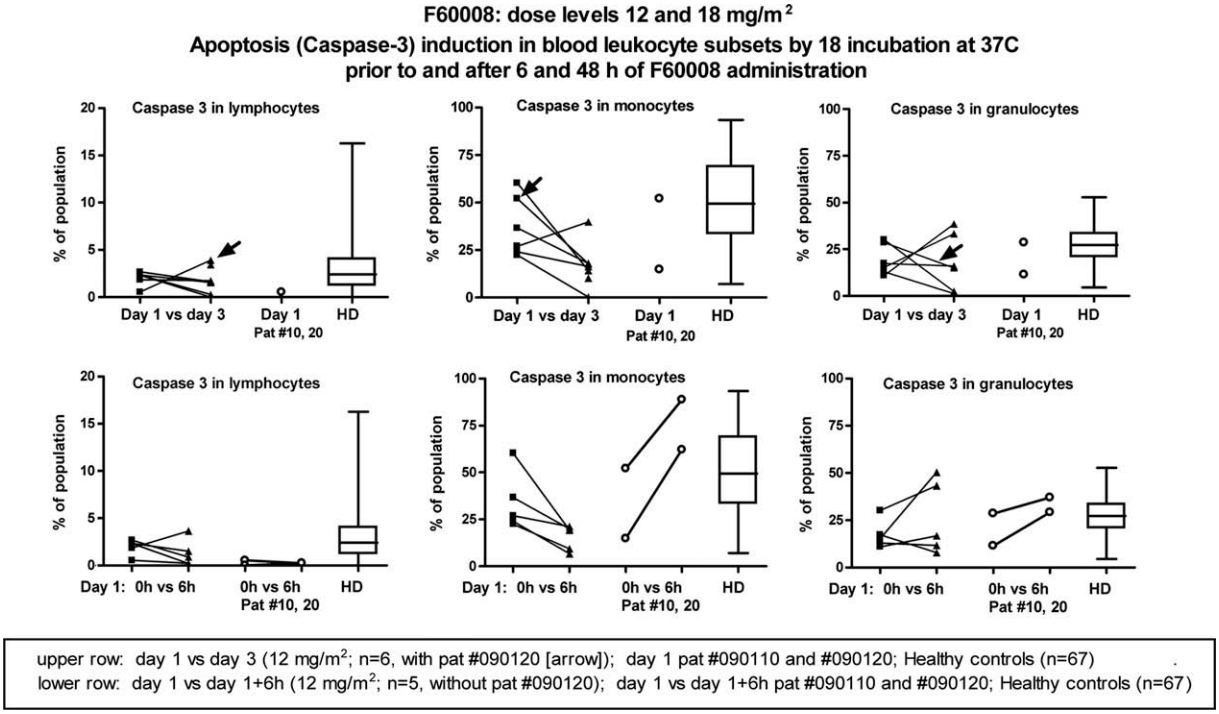

Fig. 5 – In the left part of the figures the levels of specific subsets can be seen on day 1 and 3 (upper row) and at 0 and 6 hours (lower row). In the middle part the specific levels of the two patients who died (number 10 and 20) are shown. On the right side of the figures the healthy donor controls are shown in Box-Whisker plots. The arrow corresponds to patient number 20.

family). NF-κB can interfere with apoptotic signals at various levels of both the TNFR-related and the mitochondrial-related pathway. Activation of NF-κB induces transcription of genes coding for anti-apoptotic proteins such as c-IAP-1 and c-IAP-2 (IAP, inhibitor of apoptosis protein). Induction of NF-κB also results in the inhibition of caspase activity in several ways. In the mitochondrial pathway, stimulation of NF-κB results in the expression of genes coding for the anti-apoptotic family members of the Bcl-2 family, hereby preventing cytochrome *c* release and thus preventing caspase activation and ultimately preventing apoptosis. It follows that NF-κB is a key molecule in the apoptosis cascade and that the development of drugs interacting with NF-κB could promise to be a hopeful new pathway in increasing drug effectiveness in cancer.[21–23]

We performed a phase I study on F60008 a semi-synthetic derivative of triptolide, a first in class inhibitor of NF-κB activation. Although toxicity was manageable in most patients, two patients with lethal side-effects were encountered. One patient at 12 mg/m^2 died of neutropaenic sepsis and one patient at 18 mg/m^2 died of a complex clinical syndrome which might be caused by cytokine release. Both patients were outliers in the extent of exposure to the active drug. F60008 is converted to triptolide by plasma esterases. However, it is not known presently which esterases are responsible for the conversion. Further knowledge could provide the opportunity to determine the influence of pharmacogenomics on the conversion of the drug to its active compound and might enable us to regulate exposure and toxicity.

Pharmacodynamic data derived from the patients who experienced lethal side-effects showed at 6 h after administration of the drug both an increase in caspase-3 activity and overt apoptosis in monocytes and neutrophils, which might be indicative of a drug effect. In the patient treated at 18 mg/m^2 and with the highest exposure to triptolide, we were able to document increases in VEGF, sTNFRI and IL-8, which could be related to the mechanism of action of the drug or could be secondary to the observed toxic effects.

Taking into account all PK parameters, F60008 cannot be considered the optimal derivative of triptolide. As discussed the extent of conversion was unpredictable, probably caused by inter-patient variability in plasma esterase activity. Furthermore, the conversion was slow and incomplete. Presently, second generation compounds are under investigation further exploring the inhibition of the NF-κB pathway in cancer therapy.

Conflict of interest statement

M. Brandely and Ch. Puozzo are both employed by the Institut de Recherche Pierre Fabre. No other conflicts of interest exist.

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