Use of modelling and simulation techniques to support decision making on the progression of PF-04878691, a TLR7 agonist being developed for hepatitis C

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- While several clinical studies have been published on toll-like receptor 7 (TLR7) agonists, there is no information on the quantitative link between 2',5'-oligoadenylate synthetase (OAS) and antiviral efficacy (viral load).

WHAT THIS STUDY ADDS

- This study provides the only quantitative dynamic clinical relationship between the pharmacokinetics (PK) of PF-04878691 and OAS and lymphocyte levels, together with the only published clinical relationship between markers of anti-viral pharmacology (OAS) and antiviral efficacy (viral load). This study highlights how modelling and simulation can be used to impact compound progression decisions in clinical development.

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AIM

To use non-linear mixed effects modelling and simulation techniques to predict whether PF-04878691, a toll-like receptor 7 (TLR7) agonist, would produce sufficient antiviral efficacy while maintaining an acceptable side effect profile in a 'proof of concept' (POC) study in chronic hepatitis C (HCV) patients.

METHODS

A population pharmacokinetic-pharmacodynamic (PKPD) model was developed using available 'proof of pharmacology' (POP) clinical data to describe PF-04878691 pharmacokinetics (PK) and its relationship to 2',5'-oligoadenylate synthetase (OAS; marker of pharmacology) and lymphocyte levels (marker of safety) following multiple doses in healthy subjects. A second model was developed to describe the relationship between change from baseline OAS expressed as fold change and HCV viral RNA concentrations using clinical data available in HCV patients for a separate compound, CPG-10101 (ACTILON™), a TLR9 agonist. Using these models the antiviral efficacy and safety profiles of PF-04878691 were predicted in HCV patients.

RESULTS

The population PKPD models described well the clinical data as assessed by visual inspection of diagnostic plots, visual predictive checks and precision of the parameter estimates. Using these relationships, PF-04878691 exposure and HCV viral RNA concentration was simulated in HCV patients receiving twice weekly administration for 4 weeks over a range of doses. The simulations indicated that significant reductions in HCV viral RNA concentrations would be expected at doses >6 mg. However at these doses grade ≥3 lymphopenia was also predicted.

CONCLUSIONS

The model simulations indicate that PF-04878691 is unlikely to achieve POC criteria and support the discontinuation of this compound for the treatment of HCV.

Introduction

Chronic hepatitis C (HCV) infection is the leading cause of liver disease, affecting over 180 million people worldwide [1, 2]. The prevalence of HCV infection in the United States between the years 1999 and 2002 was 1.6%, equating to about 4.1 million people [3]. Calculations indicate that mortality related to HCV infection will continue to increase over the next two decades [4].

The current standard of care (SoC) for HCV treatment is a combination of pegylated interferon-alpha (Peg IFN- α) and ribavirin (RBV). Complete removal of the virus occurs in more than 75% of individuals with genotype 2 and genotype 3 infections but less than 50% of individuals with genotype 1 infections [5]. Treatment is often poorly tolerated with many adverse effects being reported, e.g. flu-like symptoms, neuropsychiatric events and neutropenia which are often dose and treatment limiting [1]. To address these issues, a number of oral direct acting antiviral agents are being investigated as combination treatments with Peg IFN- α . The availability of an oral combination regimen could improve convenience of HCV therapy and subsequently compliance, improve response rates and reduce adverse events.

PF-04878691 is a novel, selective and potent agonist of toll-like receptor 7 (TLR7) being developed as an oral therapy for the treatment of HCV infection. TLRs are expressed by immune cells and recognize specific microbial molecular patterns that initiate and direct immune responses [6]. Successful host defence against HCV viral infections relies on the production of specific immunomodulatory cytokines and chemokines including interferons (IFN) [7]. Some of these cytokines, in particular IFN- α , can be activated by TLR7 agonists [8]. Much of the interferon produced comes from plasmacytoid dendritic cells [9]. As well as activating anti-inflammatory cytokines (e.g. IFN), TLR7 agonists also activate pro-inflammatory cytokines (e.g. IL6) which are thought to be responsible for the dose limiting side effects [10]. For any new HCV therapies a wider therapeutic window between the anti-inflammatory (reducing HCV infection) and inflammatory cytokines (side effects) would be required.

Several TLR7 agonists have been evaluated in clinical studies and have been shown to reduce viral load [11–14]. This is believed to be mediated via stimulation of the IFN- α pathway, as measured by IFN- α and markers of IFN induction, e.g. 2',5'-oligoadenylate synthetase (OAS). Imiquimod, an imidazoquinoline with TLR7 agonist activity, is approved as a topical treatment for anogenital warts caused by human papilloma virus [11]. However, when administered orally little effect was observed in HIV-infected patients [12]. An intravenous isatoribine (TLR7 agonist) dose of 800 mg administered once a day for 1 week and an oral resiquimod dose (TLR7/8 agonist) of 0.02 mg kg⁻¹ administered twice a week for 4 weeks resulted in an increase in IFN- α concentrations and a transient decrease in HCV viral

load of 0.76 and 1–3 log₁₀, respectively. However, several patients reported adverse events consistent with elevated cytokine concentrations [13, 14].

PF-04878691 has been studied in preclinical and clinical studies sponsored by 3 M Pharmaceuticals and the Coley Pharmaceutical Group [15]. Several phase 1 and 2 studies were conducted in healthy volunteers and cancer patients. In order to determine its therapeutic index, we conducted in our laboratories *in vitro* human peripheral blood mononuclear cell stimulation experiments with PF-04878691, evaluating its antiviral efficacy in an HCV replicon assay. From these experiments, a separation of the antiviral response from the induction of pro-inflammatory cytokines was seen, supporting the hypothesis that repeated doses of PF-04878691 would activate the antiviral response *in vivo* without giving rise to significant side effects. Therefore we performed a 2 week 'proof of pharmacology' (POP) study with PF-04878691 in healthy volunteers to determine clinically whether sufficient pharmacology (as measured by OAS) could be achieved in the presence of a suitable side-effect profile. If successful, PF-04878691 would be taken forward to a randomized, double-blind, placebo-controlled 'proof of concept' (POC) study in HCV infected patients to investigate its pharmacodynamics (as measured by reduction in HCV RNA from baseline), pharmacokinetics (PK), safety and toleration following 4 weeks of monotherapy.

The aim of this work was to predict the likely outcome of PF-04878691 in a POC study in HCV patients in order to support further decision making around this compound. This was achieved by firstly developing a model using available POP clinical data to describe PF-04878691 PK and its relationship to OAS (marker of pharmacology) and lymphocyte levels (marker of safety) following multiple doses in healthy subjects. To date this compound has not been dosed in HCV patients. Therefore in order to predict possible HCV viral RNA profiles in patients, a model was developed to describe the relationship between change from baseline OAS expressed as fold change and HCV viral RNA concentrations using clinical data from HCV patients for a separate compound, CPG-10101 (ACTILON™), a TLR9 agonist [16]. Using these relationships and assuming that the relationship between OAS and HCV viral RNA is the same for TLR7 and TLR9 agonists, PF-04878691 exposure and HCV viral RNA concentrations were simulated in HCV patients receiving twice weekly administration for 4 weeks over a range of doses. The likelihood of a positive POC study (>1 log₁₀ decrease in HCV viral RNA concentrations) was assessed.

Methods

Clinical TLR7 study data

PK, OAS and lymphocyte data were obtained from a randomized, placebo controlled, blinded (third party open)

Figure 1

Schematic of modelling and simulation strategy

sequential multiple dose escalation study where male and female healthy volunteers were administered PF-04878691 orally twice a week for 2 weeks. The compound was dosed orally using an extemporaneously prepared solution. A total of 24 healthy volunteers participated in this study. The majority of the subjects were males (92%) with only two female subjects (8%). Median age and weight were 34 (range 21–55) years and 79 (range 57–97) kg. Volunteers were randomized to either PF-04878691 ($n = 6$) or placebo ($n = 2$) within each cohort and received doses of placebo, 3, 6 or 9 mg PF-04878691 on days 1, 4, 8 and 11.

Serial blood samples were collected following multiple dose administration (pre dose and at specified times up to 312 h post last dose). Plasma samples were analysed by Covance Bioanalytical Services, LLC (Indianapolis, IN, USA) to determine PF-04878691 concentrations using high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). The lower limit of quantification was 0.1 ng mL^{-1} and the inter- and intra-assay variability was $<4.9\%$. The bioanalytical procedure for PF-04878691 has been previously reported and was used without further modification [17].

Plasma samples were also analysed to determine levels of OAS, lymphocyte and various other biomarkers at a reduced number of time points. The bioanalytical procedure for determination of OAS RNA expression level has been described previously [18]. OAS fold change was determined by the maximal observed median change from baseline at a specific time point using an internal control housekeeping gene as a control. Absolute count of lymphocytes was measured by immunophenotyping.

Written informed consent was obtained from each volunteer. The study was approved by the institutional review board of the research centre and was conducted in compliance with the principles derived from the Declaration of Helsinki including all International Conference on Har-

monization Good Clinical Practice guidelines and local regulatory requirements. The study is registered on Clinicaltrials.gov with identifier NCT00810758. Further details of the study are disclosed in [19].

Clinical TLR9 study data

Rich OAS and HCV viral RNA concentrations were available from a previously published phase 1 study in patients with chronic HCV (CPG-10101) [16]. Sixty HCV patients were randomized to either placebo ($n = 13$) or subcutaneous (s.c.) CPG-10101 at 0.25, 1, 4, 10 or 20 mg twice weekly for 4 weeks or at 0.5 or 0.75 mg kg^{-1} once weekly for 4 weeks. Serial blood samples were collected for determination of OAS and HCV viral RNA concentrations pre dose and up to 50 days post dose following multiple dose administration at days 1, 4, 22 and 25. Serum biological markers of immune stimulation were determined by enzyme-linked immunosorbent assay (ELISA).

Modelling strategy

The analysis was performed using nonlinear mixed effects population modelling methodology as implemented in the NONMEM software system, version VI level 1.2 (Icon Development Solutions, Ellicott City, Maryland, USA, 2006), the NM-TRAN subroutines version III level 1.2. The PK and response parameter estimations were performed sequentially using the subroutine ADVAN6. The first order conditional estimation (FOCE) method with INTERACTION was used to estimate all the parameters [20].

The modelling process is shown as a schematic in Figure 1 and consisted of:

- 1 Development of a PK structural model to describe the population and individual exposure over the 2 week period. Subsequent generation of empirical Bayesian

- estimate (EBE) PK parameters for these subjects. Assessment of model adequacy by diagnostic plots and a visual predictive check (VPC).
- 2 Using the EBE PK estimates, development of exposure response models to describe the increase in OAS and the decrease in total lymphocyte levels for the population and individuals. Estimation of model parameters for these subjects. Assessment of model adequacy by diagnostic plots and VPC.
 - 3 Development of a PD model to describe the relationship between HCV viral RNA concentrations and OAS fold change with the available data from study CPG-10101–002. Population PD parameters and their inter-individual variability (IIV) were subsequently used for clinical trial simulation to predict the antiviral activity of PF-04878691 with its PK.
 - 4 Use of population parameter estimates with IIV from PK and response models to simulate response profiles in HCV patients receiving twice weekly administration of PF-04878691 using a range of doses for 4 weeks.

Population PKPD analysis

A two compartment model with first order absorption was fitted to the PF-04878691 plasma concentration data. The disposition kinetics were modelled using a parameterization involving apparent oral clearance (CL), apparent central volume (V_c), apparent inter-compartment clearance (Q), and apparent peripheral volume (V_p). A first order absorption rate constant (k_a) was used to characterize the absorption process.

An indirect response model was used to describe the OAS response over time, where PF-04878691 stimulates the production of OAS as defined below.

$$dOAS/dt = k_{in} \times (1 + (E_{max} \times C^\gamma / (EC_{50}^\gamma + C^\gamma))) - k_{out} \times OAS \quad (1)$$

where k_{in} is the production rate of OAS, k_{out} is the elimination rate of OAS, E_{max} is the maximal response, EC_{50} is the PF-04878691 concentration resulting in 50% of E_{max} and γ is the sigmoidicity factor describing the steepness of the concentration–OAS relationship. Baseline levels are defined as k_{in}/k_{out} and the EBE PK parameter estimates were used to estimate exposure.

An indirect response model used to describe the lymphocyte levels over time, where PF-04878691 stimulates the re-distribution of the lymphocytes as defined below.

$$dlymphocytes/dt = k_{in} - k_{out} \times (1 + (E_{max} \times C^\gamma / (EC_{50}^\gamma + C^\gamma))) \times lymphocytes \quad (2)$$

where k_{in} is the production rate of lymphocytes, k_{out} is the elimination rate of lymphocytes, E_{max} is the maximal response, EC_{50} is the PF-04878691 concentration resulting in 50% of E_{max} and γ is the sigmoidicity factor describing the steepness of the concentration–lymphocyte relationship. Baseline levels are defined as k_{in}/k_{out} and the EBE PK parameter estimates were used to estimate exposure.

Using the CPG-10101 data, an inhibitory sigmoid E_{max} model was used to describe the relationship between $\log_{10}$ transformed HCV viral RNA concentrations and change from baseline OAS levels expressed as fold change.

$$VL = BASE + I_{max} \cdot OAS^\gamma / (VO_{50}^\gamma + OAS^\gamma) \quad (3)$$

where BASE is the baseline HCV viral RNA concentration in units of $\log_{10}$ copies ml^{-1} , I_{max} is the maximum drop in HCV viral RNA concentration, VO_{50} is the OAS at which 50% of I_{max} is produced and γ is the sigmoidicity parameter which describes the steepness of the relationship between OAS and HCV viral RNA concentrations.

Covariates were not included in the modelling. All doses were however normalized for body weight to ensure that any IIV estimated was purely due to variability in the parameter rather than dose.

IIV in the PK and response parameters was modelled using multiplicative exponential random effects and expressed as the approximate percent coefficient of variation (%CV).

Intra-individual (residual) variability was generally described using a proportional error model except for the TLR9 OAS-viral load model where residual error was described by an additive model in the $\log_{10}$ domain.

Goodness of fit of different models to the data was evaluated using the following criteria: change in the objective function, visual inspection of different diagnostic plots, precision of the parameter estimates, as well as decreases in both IIV and residual variability. The performance of the final model was evaluated by conducting a VPC. One thousand data sets with identical design to the original data set were simulated using the final parameter estimates including IIV and residual variability.

Using the final models, the PK, OAS, lymphocyte and HCV viral RNA concentrations were simulated in a population of 1000 subjects for a range of doses over a 4 week period.

Results

Overview of study results

OAS levels increased in a dose dependent manner in all subjects (except for one non-responder in the 9 mg group). The majority of subjects (18/24) experienced treatment-related adverse events (AEs), the majority being mild. Dose- and time-dependent decreases in total lymphocyte count were observed in all subjects dosed with PF-04878691.

Two subjects in the 9 mg dose group experienced treatment related serious adverse events (SAEs). For the first subject, these SAEs included chills, body aches and lower back pain as well as a temperature increase after the fourth dose of PF-04878691. The second subject developed

hypotension and mild pyrexia as well as nausea and abdominal cramps after the first dose of PF-04878691, which worsened after the second dose with additional symptoms of lightheadedness, headaches, backache, decreased appetite, fatigue and chills. As a result of these SAEs, the study was prematurely terminated. This resulted in the withdrawal of the last four subjects during their active treatment phase (following the second dose), one subject being in the placebo group, and three subjects in the PF-04878691 group.

Population PK model

The exposure of PF-04878691 increased over time with the C_{max} on day 11 up to three times higher than the C_{max} on day 1. This was inconsistent with the reasonably short terminal half-life of 12–16 h. A linear time-invariant two compartmental model therefore produced poor estimates of oral exposure, by over estimating C_{max} on day 1 and under estimating exposure on day 11. The PK model was adapted to incorporate an increase in exposure over time. Several approaches were investigated. These included an increase in k_a and bioavailability over time and a reduction in V_c and CL over time. The model that best described the data incorporated a changing CL over time. The change in CL was modelled as follows:

$$CL = CLF + (CL0 \times \exp(-DEG \times TAFD)) \quad (4)$$

where CLF is the final clearance, CL0 is the initial clearance, DEG is the degradation rate constant for clearance and TAFD is the time after first dose.

The inclusion of IIV on CLF and k_a resulted in the best model fit in terms of objective function, goodness of fit plots and VPC. This final PK model provided a good description of the population and individual PF-04878691 plasma concentration-time profiles. The parameter estimates for this model are given in Table 1. Reasonable %CV were obtained for all fixed and random effect parameters (<40%), with the exception of VC, Q and CL0 which had %CV values >40%. However, when considered with other

predictive checks and diagnostic techniques this represented the best model. The VPC plot stratified for dose and diagnostic plots are shown in Figures 2 and 3 respectively. As can be seen from these plots, the parameter estimates and IIV estimates adequately describe the observed data.

Population PK-OAS and PK-lymphocyte models

As a result of the limited number of doses, the typical E_{max} indirect response model could not adequately identify the model parameters. Linear and power functions were therefore investigated. The replacement of the E_{max} function with a power function ($SLP \times C^p$) best described both the OAS and lymphocyte data, where SLP is the slope describing the increase in effect on k_{in} for OAS and k_{out} for lymphocytes and p is the power function.

The inclusion of IIV on baseline and k_{out} (for the OAS model) and SLP, baseline and k_{out} (for the lymphocyte model) resulted in the best model fits in terms of objective function, goodness of fit plots and VPC. The final OAS and lymphocyte models provided a good description of the population and individual OAS- and lymphocyte-time profiles. The parameter estimates for these models are given in Tables 2 and 3 for OAS and lymphocytes respectively. Reasonable %CV were obtained for all fixed and random effect parameters (<40%), except for the IIV on k_{out} and BASE (>40%) for the OAS model and IIV on k_{out} and slope (>40%) for the lymphocyte model. However when considered with other predictive checks and diagnostic techniques these represented the best models. The VPC plots stratified for dose and diagnostic plots are shown in Figures 4 and 5 for OAS and Figures 6 and 7 for lymphocytes respectively. As can be seen from these plots, the parameter estimates adequately describe the observed data. There appeared to be an over-prediction in IIV particularly for the OAS baseline model. However the central tendency was well predicted. There was one non-responder at the 9 mg dose who was not captured.

Population OAS-viral load model

A total of 39 HCV patients contributed 314 viral load observations to the population OAS-viral load analysis. An inhibitory sigmoid E_{max} model provided a good description of the observed relationship between $\log_{10}$ HCV viral RNA and change from baseline OAS levels expressed as fold change. IIV was modelled as a variance covariance matrix for I_{max} and γ . The parameter estimates for the final OAS-viral load model are given in Table 4. The CV% for all parameters were reasonable (<40%) except for IIV of γ , 64.3%. The final OAS-viral load model provides a good description of the population and individual relationship between OAS and HCV viral RNA concentrations. The VPC plots stratified for dose and diagnostic plots are shown in Figures 8 and 9 respectively. In general, the observations were within the 90% degenerative tolerance intervals for all treatment arms. Given the highly variable OAS and HCV RNA concentrations, the VPC suggests the ability of the final OAS-viral

Table 1

Parameter estimates (SE, %CV) for the final PF-04878691 PK model

Model	Final estimate	SE	%CV
TH1 – CLF (l h ⁻¹ kg ⁻¹)	1.7	0.12	6.8
TH2 – V_c (l kg ⁻¹)	3.3	1.6	47
TH3 – k_a (h ⁻¹)	0.078	0.017	22
TH4 – Q (l h ⁻¹ kg ⁻¹)	0.74	0.38	51
TH5 – V_p (l kg ⁻¹)	21	3.4	17
TH6 – CL0 (l h ⁻¹ kg ⁻¹)	3.5	1.5	42
TH7 – DEG (h ⁻¹)	0.24	0.083	35
OM1 – IIV CLF	0.067	0.018	28
OM3 – IIV k_a	0.19	0.069	36
SIG1 – residual error	0.046	0.0082	18

Figure 2

Visual predictive check from the final PF-04878691 PK model stratified by dose. Open circles represent observed concentrations; the lines represent the VPC 5, 50 and 95 quantiles from 1000 simulations

load model to describe the relationship between the pooled OAS and HCV RNA concentrations.

Simulations

Using the final models, the PK, OAS, lymphocyte and viral load levels were simulated for a range of doses over a 4 week period in HCV patients. The results of these simulations are shown in Figure 10 for viable doses. The simulations indicate that significant reductions in viral load would be expected at doses >6 mg. However at these doses grade ≥ 3 lymphopenia was also predicted.

Discussion

A multiple dose escalation study where male and female healthy volunteers were administered PF-04878691, a TLR7 agonist, orally twice a week for 2 weeks has been performed. Population PK, OAS and lymphocyte models were developed that adequately described the observed clinical data from this study. In addition a model was developed using the individual level data following administration of a TLR9 agonist, CPG-10101 (ACTILON™) subcutaneously once/twice weekly for 4 weeks to describe the relationship between OAS and HCV viral RNA concentrations. These

Figure 3

Diagnostic plots from the final PF-04878691 PK model. (A) individual predicted vs. observed, (B) population predicted vs. observed, (C) time vs. weighted residuals and (D) population predicted vs. weighted residuals

Table 2

Parameter estimates (SE, %CV) for the final OAS model

Model	Final estimate	SE	%CV
TH1 – k_{out} (h ⁻¹)	0.034	0.012	35
TH2 – SLP	3.5	0.63	18
TH3 – BASE (fold change)	0.96	0.064	6.7
TH4 – gamma	1.6	0.17	10
OM1 – IIV k_{out}	1.7	1.6	97
OM3 – IIV BASE	0.18	0.076	43
SIG1 – residual error	0.19	0.032	17

Table 3

Parameter estimates (SE, %CV) for the final lymphocyte model

Model	Final estimate	SE	%CV
TH1 – k_{out} (h ⁻¹)	0.044	0.0089	20
TH2 – SLP	0.44	0.064	15
TH3 – BASE (pg ml ⁻¹)	1890	88	4.7
TH4 – gamma	2.2	0.37	17
OM1 – IIV k_{out}	0.19	0.16	83
OM2 – IIV slope	0.20	0.11	53
OM3 – IIV BASE	0.051	0.015	29
SIG1 – residual error	0.021	0.0044	21

models were used to predict the likely outcome of PF-04878691 in HCV patients in order to support further decision making around this compound.

Observed PF-04878691 plasma exposure increased over time in a manner that was not consistent with

the clearance of the compound. A standard two-compartmental model over-predicted exposure on day 1 (the first dose) and under-predicted exposure on day 11 (the fourth dose). An empirical model was used to describe the time dependent increase in exposure and provided an

Figure 4

Visual predictive check from the final OAS model stratified by dose. Open circles represent observed data; the lines represent the VPC 5, 50 and 95 quantiles from 1000 simulations

adequate description of PF-04878691 plasma concentrations. *In vitro* data suggest that PF-04878691 is partially metabolized by CYP1A2. Drug–drug interactions have been reported between theophylline (CYP1A2 substrate) and IFN, where IFN increases the exposure of theophylline [21]. The production of IFN over time as a result of TLR7 agonism, could result in the inhibition of CYP1A2 and consequently the time dependent increase in exposure of PF-04878691. Another IFN inducer, tilorone hydrochloride, has been shown to reduce drug metabolizing activity in rat liver [22].

The OAS response observed in the clinical study at doses at and above 6 mg is in a similar range to those

observed with other TLR7 agonists and with IFN treatment in the clinic where therapeutic reductions in HCV viral load were obtained [13–15, 23–29]. Data from IFN treatment indicate that a maximum increase in OAS of approximately 8-fold can be expected at efficacious doses [23–29]. Isatorabine showed a 7.6 fold increase in OAS from baseline with moderate changes in antiviral activity (0.75 log₁₀ reduction from baseline) [13]. In our multidose study with PF-04878691, OAS increases of 8-fold or more from baseline were seen in 3/6 individuals at 3 mg and 6/6 at 6 mg and 5/6 at 9 mg (a non-responder was identified in the 9 mg cohort). Two healthy volunteers who received 9 mg of PF-04878691 developed serious adverse events consis-

Figure 5

Diagnostic plots from the final OAS model. (A) individual predicted vs. observed, (B) population predicted vs. observed, (C) time vs. weighted residuals and (D) population predicted vs. weighted residuals

tent with IFN and cytokine production, following the second and fourth doses of the compound, respectively. A less severe but similar event was observed in one volunteer following the fourth dose at 6 mg. Transient dose and time dependent decreases in lymphocyte counts were observed in the 6 and 9 mg dose groups. The adverse effects were similar to what was seen in a single dose healthy volunteer study with PF-04878691, evaluating oral doses of 2, 10, 15 and 20 mg, where transient, dose-dependent decreases in mean absolute lymphocyte count were observed [17]. The only dose therefore with a safety/tolerability profile similar to SoC (IFN- α /RBV) was the 3 mg dose. There was significant overlap in exposure between the 3 and 6 mg doses, resulting in an extremely small safety window (<2-fold). Some of the side effects such as flu-like symptoms, are associated with, the current SoC for the treatment of HCV [30, 31]. The degree of lymphopenia observed in the 6 mg and 9 mg cohorts of PF-04878691 was more severe than is normally seen with IFN- α /RBV treatment [19].

In order to determine whether other dosing regimens could be explored to provide an adequate OAS fold increase while maintaining adequate safety and tolerability, a model was developed to describe the OAS and lymphocyte level data. As a result of the limited number of doses studied with PF-04878691, the typical $E_{\max}$ style indirect response model could not adequately identify the model parameters and a power function was used to describe the relationship between PF-04878691 and OAS as well as total lymphocyte levels. This was deemed appropriate for fitting and further simulations as simulations were to be performed within the dose range already studied.

To date PF-04878691 has not been dosed in HCV patients. Therefore in order to predict possible HCV viral RNA profiles in patients, a model was developed to describe the relationship between change from baseline OAS expressed as fold change and HCV viral RNA concentrations using clinical data available in patients for a separate compound, CPG-10101 (ACTILON™), a TLR9 agonist

Figure 6

Visual predictive check from the final lymphocyte model stratified by dose. Open circles represent observed concentrations; the lines represent the VPC 5, 50 and 95 quantiles from 1000 simulations

[16]. The OAS data reported by McHutchison *et al.* [16] were measured using an activity assay, whereas OAS data in our study were measured using a gene expression assay [18]. Based on literature evidence, the relationship between OAS expressed as fold change and HCV viral RNA concentrations seems largely independent of the OAS assay type [13–15, 23–29].

Traditionally the correlation between PK (dose), HCV RNA level and OAS or other biomarkers, such as IFN and IP-10, has been determined using the maximum change from baseline approach [13, 16]. This approach only uses the maximum biomarker level and correlates it with the maximum change from baseline HCV RNA concentration.

The biggest disadvantage to this is that the variation of biomarker levels with time, as well as within-subject and between-subject variabilities are ignored. In the current analysis, an attempt to model the relationship between OAS and HCV RNA concentration was made.

There are several challenges when attempting to determine the relationship between OAS and HCV RNA concentrations. The first is the precision and sensitivity of the assay used to determine the OAS expression. The second is the heterogeneity of the HCV patient population in terms of disease status and baseline HCV RNA concentrations. The third is the highly variable HCV RNA levels even within an individual. All these could be the key factors for explaining

Figure 7

Diagnostic plots from the final lymphocyte model. (A) individual predicted vs. observed, (B) population predicted vs. observed, (C) time vs. weighted residuals and (D) population predicted vs. weighted residuals

Table 4

Parameter estimates (SE, %CV) for the final OAS-viral load model

Model	Final estimate	SE	%CV
TH1 – BASE (log ₁₀ copies ml ⁻¹)	7.3	0.10	1.4
TH2 – I _{max} (log ₁₀ copies ml ⁻¹)	-2.7	0.40	15
TH3 – VO ₅₀ (fold change)	3.6	0.40	11
TH4 – gamma	0.68	0.17	25
TH5 – additive residual error	0.41	0.026	6.3
OM2 – IIV I _{max}	0.29	0.11	36
OM3 – IIV VO ₅₀	0.25	0.16	64

the relatively high IIV and %CV for parameters such as I_{max} and VO₅₀. In general the VPC plots suggested that the random effects parameters were over predicted for all the models used in this study. However the 50% percentile indicates the models captured the central tendency cor-

rectly. Among the 60 HCV patients in study CPG-10101, there were two prior treatment naive patients, 10 patients prior treatment intolerant, nine partial responders (≥ 1 log₁₀ decrease in viral titre reported on prior treatment, but did not clear virus), 30 treatment relapsers (cleared virus on previous treatment, but relapsed), and nine uncharacterized responders (≥ 1 log₁₀ decrease in viral titre reported on prior treatment, uncertain if ever cleared virus, but viral positive on screening before study) [16]. Though there is a wide range of baseline HCV RNA concentration across the HCV patients (4.73–7.73 log₁₀ copies ml⁻¹) the model based estimate appears to be relatively precise, 7.26 log₁₀ copies ml⁻¹ with a CV% of 1.38%.

For the current translational modelling approach, across mechanisms (TLR7 and TLR9) and across populations (healthy volunteers and HCV patients), the following key assumptions were necessary. Firstly, the subsequent intracellular signalling pathways are similar after binding of both TLR7 and TLR9 agonists to their receptors; thus the

Figure 8

Visual predictive check for the final OAS-viral load model stratified by dose. Open circles represent observed concentrations; the lines represent the VPC 5, 50 and 95 quantiles from 1000 simulations

Figure 9

Diagnostic plots from the final OAS-viral load model. (A) individual predicted vs. observed, (B) population predicted vs. observed, (C) OAS fold change vs. weighted residuals and (D) population predicted vs. weighted residuals

magnitude of OAS and IFN response required for an anti-viral effect by each of the pathways would be comparable. As both TLR7 and TLR9 work through the same pathway, this is a reasonable assumption [32]. Secondly, there is no PK or biomarker difference between the healthy volunteer and HCV patient populations. There is some evidence, however, to suggest that biomarker level production could be reduced in HCV patients compared with healthy subjects [33]. The simulations made could therefore represent a 'best case' scenario in terms of pharmacological activation.

Using these relationships, PF-04878691 exposure and clinical outcome was simulated in HCV patients receiving twice weekly administration of PF-04878691 using a range of doses for 4 weeks. The likelihood of achieving adequate HCV viral RNA concentration reduction (>1 log₁₀ decrease in HCV viral RNA concentrations) at a dose/dosing regimen that was considered safe was assessed. Results indicate that significant reductions (1–2 log drop) in viral load

would be expected only at doses >6 mg. However at these doses, grade 3 lymphopenia would also be predicted (Figure 10). POC criteria were agreed as demonstration of a mean reduction of >1 log₁₀ in HCV RNA following 4 weeks of dosing with a dose that has a safety profile that is no worse than SoC. Population models to describe the PK and PD response of PF-04878691 indicate that this compound is unlikely to achieve POC criteria. The models developed during this exercise support the discontinuation of this compound. This, together with subsequent data indicating that *in vitro* activity against HCV was only observed at doses where mechanism-related adverse events were seen, raises concerns regarding the therapeutic window and potential utility of this compound class for the treatment of HCV. A robust approach for translation of anti- and pro-inflammatory responses from *ex vivo* to *in vivo* could also have aided in this decision process. However the translation of such responses from *ex vivo* data has not been widely studied.

Figure 10

Simulation of viral load and lymphocyte profiles for 4 weeks. 3 mg two times a week for 2 weeks followed by 4.5 mg two times a week for 2 weeks, (A) viral load and (B) lymphocytes; 6 mg two times a week for 4 weeks, (C) viral load and (D) lymphocytes. Solid line represents the median prediction and the shaded area represents the prediction intervals (5 and 95 percentile lines) from 1000 simulations. Dashed line represents 1-fold log drop in viral load, dot-dash line represents grade 2 lymphopenia level and dot-dot-dash line represents grade 3 lymphopenia

Competing Interests

All authors are currently Pfizer employees, owning shares in this company. All authors declare no further conflict of interest.

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