Supplementary Materials

Supplementary Methods

Pharmacokinetic (PK) samples were analysed for GSK2330811 concentrations in plasma and skin blister fluid using a validated analytical method based on sample dilution followed by chemiluminescent immunoassay analysis. Samples, 40 µL aliquot of plasma or a 10 µL aliquot of blister fluid, were diluted 25-fold with assay buffer (0.15 M sodium chloride, 0.05 M TRIS-HCL, 0.05% Tween 20, 0.1% BSA pH 7.6 (aq)). GSK2330811 was captured using an anti-idiotypic anti-GSK2330811 monoclonal antibody (GRITS51360, GSK) and detected with a HRP conjugated mouse anti-human immunoglobulin G1 (IgG1) antibody (A10648, Molecular Probes). Chemiluminescence was detected on a Perkin Elmer 2030 Workstation. The lower limit of quantification (LLQ) for GSK2330811 was 100 ng ml^{-1} with a higher limit of quantification (HLQ) of 5000 ng ml^{-1} for both plasma and blister fluid.

PD (free oncostatin M [OSM] and total OSM) samples were measured in serum and skin blister fluid using validated electrochemiluminescent ligand binding immunoassays. OSM was captured using either GSK2330811 (free OSM assay) or GSK315234 (total OSM assay) and detected using ruthenylated anti-OSM antibody AF295 (R&D Systems). Chemiluminescence was detected on a Sector Imager 6000 with the addition of T-read buffer (Meso Scale Discovery [MSD]). The LLQ for both free and total OSM (free and GSK2330811-bound) was 1.45 pg ml^{-1} with a HLQ of 2500 pg ml^{-1}.

A tiered testing approach of screening, confirmation and titration was used for the immunogenicity assays. For the screening assay the anti-GSK2330811 antibodies were detected in serum samples using a validated electrochemiluminescent immunoassay. Samples were mixed to generate a homogenous mixture containing 0.25 µg ml^{-1} biotinylated GSK2330811 and 0.5 µg ml^{-1} ruthenylated GSK2330811 and 2.5% serum sample in assay diluent (1% Casein in phosphate-buffered saline [PBS]). Samples were incubated for 1 hour on a streptavidin-coated plate (MSD), which was blocked overnight in 1% Casein in PBS. Chemiluminescence was detected on a MSD Sector Imager 6000 with the addition of T-read buffer (MSD). Normal human serum and anti-GSK2330811 antibody spiked into in normal human serum were used as negative and positive controls, respectively. The

screening assay cut point was generated by analysing an adequate number of normal human samples to provide a statistically valid assessment of both biological and assay variability. Using a risk-based approach, the screening cut point was determined at upper 95% confidence limit. A confirmatory assay by immunocompetition was used to determine whether a screening positive response was specific for the GSK2330811. Samples with potential anti-GSK2330811 antibodies were confirmed by exposing the samples to an excess concentration of free GSK2330811. The confirmation cut point was determined based on the percentage of signal inhibition due to addition of free GSK2330811 using the screening assay format.

The confirmed anti-GSK2330811 antibody positive samples were then tested at multiple dilutions to obtain the antibody titre. In this assay, the screening cut point was used as the titration cut point and the assay titre was the dilution factor interpolated at the cut point multiplied by the assay minimum required dilution.

One-compartment model

The model is described by the following system of equations:

$$TotOSM = y_3$$

$$FreeDrug = \frac{1}{2}(y_2 - KD - y_3) + \sqrt{(y_2 - KD - y_3)^2 + 4 \cdot y_2 \cdot KD}$$

$$Complex = y_3 \cdot \frac{FreeDrug}{FreeDrug+KD}$$

$$FreeOSM = TotOSM - Complex$$

$$\frac{dy_1}{dt} = -KAy_1$$

$$\frac{dy_2}{dt} = KA \cdot \frac{y_1}{V/F} - \frac{CL/F}{V/F} \cdot FreeDrug - KCX \cdot Complex$$

$$\frac{dy_3}{dt} = RSYN - KDEG \cdot FreeOSM - KCX \cdot Complex$$

with initial conditions:

$$y_1(0) = DOSE$$

$$y_2(0) = 0$$

$$y_3(0) = OSM_0$$

In the above equations, y_1 is the depot compartment, y_2 represents the total drug (free plus complex) in plasma and y_3 is total OSM (TotOSM; free plus complex) in plasma. KA (hr-1) is the drug absorption rate constant, V/F (L) is the apparent volume of the central compartment, CL/F (L/hr) is apparent clearance from the central compartment, KD (dissociation constant; nM) is the affinity between GSK2330811 and OSM, OSM_0 (nM) is total OSM at baseline (time=0), KDEG (1/h) is the free OSM degradation rate constant and KCX (1/h) is the complex degradation rate constant.

The quasi-steady state (QSS) approximation was adopted, [1] with the free drug, the target, and the complex assumed to be in a QSS, where the binding rate is balanced by the sum of the dissociation and internalization rates on the scale of the other processes:

$$k_{on} \cdot FreeDrug \cdot FreeOSM - \left(k_{int} + k_{off}\right) \cdot Complex = 0$$

$$\frac{FreeDrug \cdot FreeOSM}{Complex} = \frac{(k_{int}+k_{off})}{k_{on}} = K_D$$

The schematic representation of the one-compartment model is shown in Figure 1, left panel.

Minimal physiology-based pharmacokinetic **(mPBPK) model**

The model is described by the following system of equations:

$$TotOSM_{pl} = y_4$$

$$FreeDrug_{pl} = \frac{1}{2}(y_2 - KD - y_4) + \sqrt{(y_2 - KD - y_4)^2 + 4 \cdot y_2 \cdot KD}$$

$$Complex_{pl} = y_4 \cdot \frac{FreeDrug_{pl}}{FreeDrug_{pl}+KD}$$

$$FreeOSM_{pl} = TotOSM_{pl} - Complex_{pl}$$

$$TotOSM_{bf} = y_5$$

$$FreeDrug_{bf} = \frac{1}{2}(y_3 - KD - y_5) + \sqrt{(y_3 - KD - y_5)^2 + 4 \cdot y_3 \cdot KD}$$

$$Complex_{bf} = y_5 \cdot \frac{FreeDrug_{bf}}{FreeDrug_{bf}+KD}$$

$$FreeOSM_{bf} = TotOSM_{bf} - Complex_{bf}$$

$$\frac{dy_1}{dt} = -KAy_1$$

$$\frac{dy_2}{dt} = F \cdot KA \cdot \frac{y_1}{VP} + \frac{LR \cdot y_7 - LR1 \cdot (1-\sigma_1) \cdot FreeDrug_{pl} - LR2\ (1-\sigma_2) \cdot FreeDrug_{pl} - CL \cdot FreeDrug_{pl}}{VP} - KCX \cdot Complex_{pl}$$

$$\frac{dy_3}{dt} = \frac{LR2 \cdot (1-\sigma_2) \cdot FreeDrug_{pl} - LR2 \cdot (1-\sigma_L) \cdot FreeDrug_{bl}}{V_{Le}} - KCX \cdot Complex_{bf}$$

$$\frac{dy_4}{dt} = RSYN - KDEG_{pl} \cdot FreeOSM_{pl} - KCX \cdot Complex_{pl}$$

$$\frac{dy_5}{dt} = RSYN - KDEG_{bf} \cdot FreeOSM_{bf} - KCX \cdot Complex_{bf}$$

$$\frac{dy_6}{dt} = \frac{LR1 \cdot (1-\sigma_1) \cdot FreeDrug - LR1 \cdot (1-\sigma_L) \cdot y_6}{V_{Ti}}$$

$$\frac{dy_7}{dt} = \frac{LR1 \cdot (1-\sigma_L) \cdot y_6 + LR2 \cdot (1-\sigma_L) \cdot FreeDrug_{bl} - LR \cdot y_7}{V_{Ly}}$$

with initial conditions:

$$y_1(0) = DOSE$$

$$y_2(0) = 0$$

$$y_3(0) = 0$$

$$y_4(0) = OSM_0$$

$$y_5(0) = OSM_0$$

$$y_6(0) = 0$$

$$y_7(0) = 0$$

Where y_1 is the depot compartment, y_2 represents the total drug (free plus complex) in plasma, y_3 is the compartment representing the total drug (free plus complex) in blister fluid and corresponds to the compartment associated with the leaky tissue in the mPBPK model, y_4 is total OSM (free plus complex) in plasma, y_5 is total OSM (free plus complex) in blister fluid, y_6 represents tight tissues and y_7 is the lymph compartment. Parameters have the same meaning as the parameters described for the one-compartment model.

As for the one-compartment model, the QSS approximation was adopted [1] with the free drug, the target, and the complex assumed to be in a QSS in plasma and in leaky tissue.

Figure 1, right panel, shows the schematic representation of the mPBPK model.

Supplementary Reference

1. Gibiansky L, Gibiansky E, Kakkar T, Ma P. Approximations of the target-mediated drug disposition model and identifiability of model parameters. J Pharmacokinet Pharmacodyn 2008; 35: 573-91.