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ARTICLE

Model-based dose selection to inform translational clinical oncology development of WNT974, a first-in-class Porcupine inhibitor

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Abstract

WNT974 is a potent, selective, and orally bioavailable first-in-class inhibitor of Porcupine, a membrane-bound O-acyltransferase required for Wnt secretion, currently under clinical development in oncology. A phase I clinical trial is being conducted in patients with advanced solid tumors. During the dose-escalation part, various dosing regimens, including once or twice daily continuous and intermittent dosing at a dose range of 5–45 mg WNT974 were studied, however, the protocol-defined maximum tolerated dose (MTD) was not established based on dose-limiting toxicity. To assist in the selection of the recommended dose for expansion (RDE), a model-based approach was utilized. It integrated population pharmacokinetic (PK) modeling and exposure–response analyses of a target-inhibition biomarker, skin *AXIN2* mRNA expression, and the occurrence of the adverse event, dysgeusia. The target exposure range of WNT974 that would provide a balance between target inhibition and tolerability was estimated based on exposure–response analyses. The dose that was predicted to yield an exposure within the target exposure range was selected as RDE. This model-based approach integrated PK, biomarker, and safety data to determine the RDE and represented an alternative as opposed to the conventional MTD approach for selecting an optimal biological dose. The strategy can be broadly applied to select doses in early oncology trials and inform translational clinical oncology drug development.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

WNT974 is a potent, selective, and orally bioavailable first-in-class inhibitor of Porcupine, a membrane-bound O-acyltransferase required for Wnt secretion, currently under clinical development in oncology. The conventional approach for dose selection in small-molecule oncology trials is based on the maximum tolerated dose (MTD).

WHAT QUESTION DID THIS STUDY ADDRESS?

How to inform the clinical development path and selection of the recommended dose for expansion (RDE) for a first-in-class oncology molecule.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

A model-based approach can be effectively used to integrate pharmacokinetic (PK), pharmacodynamic and safety data and inform RDE selection in oncology drug development.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

This model-based approach integrated population PK and exposure–response analyses of biomarker and safety to determine the RDE, rather than the conventional MTD approach. The strategy can be applied to support translational clinical oncology development, and dose selection in early oncology trials to inform later phase clinical development and study design.

INTRODUCTION

WNT974 is a potent, selective, and orally bioavailable first-in-class inhibitor of Porcupine, a membrane-bound O-acyltransferase enzyme required for Wnt secretion, currently under clinical development in oncology. The Wnt signaling pathway regulates cell proliferation, cell polarity, and cell fate determination during development and tissue homeostasis.[1] Aberrant activation of the canonical (β-catenin-dependent) Wnt pathway is also known to play a critical role in the pathogenesis of a variety of malignancies.[2–4] Inhibition of Porcupine by WNT974 inhibits the Wnt pathway by blocking palmitoylation and subsequent secretion of Wnt ligands.[5]

WNT974 exhibited antitumor activity in preclinical tumor models, including Wnt-dependent head and neck cancer and pancreatic cancer xenografts.[5–7] The antitumor effects of WNT974 are well-correlated with inhibition of proximal (phosphorylation of LRP6) and distal (transcription of *AXIN2*) Wnt signaling events. *AXIN2* is a transcriptional target of canonical signaling, and inhibiting Porcupine is expected to result in suppression of *AXIN2* expression.[5] WNT974 potently inhibited Wnt-dependent *AXIN2* mRNA expression in HN30 cells with an half-maximal inhibitory concentration (IC_{50}) of 0.3 nM.[5]

In the mouse, rat, and dog, WNT974 showed fast absorption after oral dosing, with the time to reach maximum plasma concentration (T_{max}) occurring between 0.25 and 1 h, and the oral bioavailability was high (60–100%). The elimination half-life ($t_{1/2}$) was short in all the animal species (0.6–2.2 h). WNT974 is extensively metabolized, primarily by CYP3A4, and is also a moderate reversible inhibitor of CYP3A in vitro. In both the xenograft and Wnt ligand-driven mouse tumor models, WNT974 exhibited potent antitumor activity and reduction of *AXIN2* mRNA expression in tumors.[5]

WNT974 has been studied in patients with advanced solid tumors as a single agent and in combination with spartalizumab (anti-PD-1 antibody) in a phase I first-in-human (FIH) study.[8–10] Following oral administration, WNT974 was rapidly absorbed (median T_{max} 1–3 h) and had a mean $t_{1/2}$ of ~5–8 h. WNT974 exposure was dose proportional over the dose range of 5–45 mg and interpatient exposure variability was generally moderate.[8,9]

Wnt pathway is expressed in skin tissues, and *AXIN2* mRNA expression in skin is a robust and sensitive biomarker for the Wnt pathway. Therefore, skin *AXIN2* mRNA was utilized in the FIH study as a surrogate marker for the pharmacodynamic (PD) effect of WNT974.[8,9] Dysgeusia (change of taste) was the most common adverse event (AE) reported in ~50% of patients treated with WNT974 in the FIH study. Based on preclinical data, dysgeusia was believed to be an on-target effect of Wnt pathway inhibition.[11–13]

During the dose-escalation part of the phase I study, the maximum tolerated dose (MTD) was not established. The most common AE observed, dysgeusia, typically occurred outside of the first cycle (the dose limiting toxicity window) and also can be graded only to grade 2 criteria by Common Terminology Criteria for Adverse Events (CTCAE) scoring. To inform dose selection for the next part (phase Ib expansion), a model-based approach that integrated pharmacokinetic (PK), PD biomarker, and safety data was used. A population PK (PopPK) model was developed to characterize the PK and interpatient variability of WNT974 and evaluate the covariate effect in patients. Exposure–response (ER) analyses were conducted to determine the relationships of the exposure of WNT974 with the change in skin *AXIN2* mRNA expression from baseline and treatment-related AE dysgeusia. The exposure range specific to suppression of skin *AXIN2* expression and probability of dysgeusia was estimated and an optimal dosing regimen that balanced target inhibition and this side effect was determined. This dose regimen was used in the single-agent expansion part of the FIH study.[8]

METHODS

Clinical study

The phase I FIH study (NCT01351103) evaluated the safety and tolerability, PK, PD, and antitumor activity of WNT974 in patients with advanced solid tumors.[8] The primary objective was to determine the MTD and/or recommended dose for expansion (RDE) of WNT974. The study protocol was approved by an independent ethics committee/institutional review board for each center[10] and all patients provided written informed consent. The study was conducted according to the principles of the Declaration of Helsinki and was performed in compliance with Good Clinical Practice guidelines. This analysis used data from the dose-escalation phase of the study (data cutoff date: March 2, 2017).

In the dose-escalation part, the study included a 3-day PK run-in period prior to the initiation of once-daily (q.d.) oral dosing, where patients were administered a single oral dose of WNT974 on Cycle 1 Day 1 (C1D1), followed by oral administration of continuous q.d. dosing; the doses evaluated were 5, 10, 15, 20, 22.5, and 30 mg. Patients without a PK run-in period were dosed at 5, 7.5, 10, 15, 20, 22.5, or 30 mg continuous q.d. and 30 and 45 mg intermittent q.d. (4 days on followed by 3 days off treatment) or 5 mg continuous twice daily (b.i.d.).

PK and *AXIN2* sample collection and analysis

PK sample collection schedule for q.d. dosing was predose, 0.5, 1, 2, 3, 4, 6, 8, and 24 h on C1D1 and C1D15 and predose on C1D8, C1D22, C2D1, C3D1, C4D1, C5D1, and C6D1. PK sample collection schedule for b.i.d. dosing was predose, 0.5, 1, 2, 3, 4, 6, and 8 h post the first dose on C1D1, predose, 0.5, 1, 2, 3, 4, and 6 h post the first dose and predose, 1, 2, and 4 h post the second dose on C1D15, and predose on C1D8, C1D16, C1D22, C2D1, C3D1, C4D1, C5D1, and C6D1. Patients with PK run-in period did not have dose administration on C1D2 and C1D3 but had PK samples collected at 48 and 72 h after C1D1 dose. The plasma WNT974 concentrations were determined using a validated liquid chromatography–tandem mass spectrometry assay with a lower limit of quantification of 1.0 ng/ml. Pre- and on-treatment skin biopsies were collected to measure *AXIN2* mRNA expression levels. The postdose skin biopsy was collected at 5–10 h postdose between C1D5 and C1D28, following at last 5 consecutive days of treatment. For intermittent dosing schedules, biomarker samples were collected any time between Day 5 and Day 28 irrespective of consecutive dosing days. Total RNA was extracted from frozen skin by using the RNeasy Plus Mini Kit (Qiagen) and converted to cDNA using the High-Capacity cDNA Reverse Transcription Kit (ThermoFisher) according to the manufacturer's instructions. Quantitative polymerase chain reaction (PCR) was performed using TaqMan Universal PCR Master Mix (ThermoFisher) and the following gene expression assays (ThermoFisher): *AXIN2* (gene of interest), *GUSB* (reference gene), and *MRPL19* (reference gene). Thermo cycler conditions used were as follows: 20 s at 95°C, 40 cycles of 1 s at 95°C, and 20 s at 60°C.

Data collection

Plasma concentrations of WNT974 and actual sample collection timepoints were used in the PopPK modeling. C1D15 PK parameters used in the ER analysis (area under the curve during a dosing interval [AUC_{tau}]), maximum concentration during a dosing interval (C_{max}), and minimum concentration during a dosing interval (C_{min}) were derived using noncompartmental analysis (NCA) using Phoenix (version 6.4; Certara, Princeton, NJ).

The skin *AXIN2* variable used in the modeling was the relative quantity (RQ) of *AXIN2* gene in relation to two reference genes, *GUSB* and *MRPL19*. The percentage change from baseline of RQ (PRQ) was computed as (RQ_p-RQ_b)/RQ_b*100%, where RQ_b and RQ_p are the relative quantity of *AXIN2* at baseline and post-treatment, respectively. The equations to derive RQ_b and RQ_p are based on a published method[14] as provided below:

$$RQ\_b = 2^{[-(mean\_b\_goi - mean\_b\_ref)]}$$

where mean_b_goi is the mean of four measurements of b_ct_goi, and mean_b_ref is the mean of four measurements of b_ct_ref for the same subject (b_ct_goi is the baseline cycle threshold of gene of interest [goi] which is *AXIN2*, and b_ct_ref is the baseline cycle threshold of reference genes *GUSB* and *MRPL19*).

$$RQ\_p = 2^{[-(mean\_p\_goi - mean\_p\_ref)]}$$

where mean_p_goi is the mean of four measurements of p_ct_goi, and mean_p_ref is the mean of four measurements of p_ct_ref for the same subject (p_ct_goi is the post-treatment cycle threshold of goi, and b_ct_ref is the post-treatment cycle threshold of reference genes *GUSB* and *MRPL19*).

Patients in the study were monitored continuously for AEs, including dysgeusia, and its grade was assigned based on the CTCAE version 4.03. The AE records of treatment-emergent dysgeusia were included in the analysis.

Population PK modeling

The PK analysis was performed using a nonlinear mixed effects modeling approach, where the model has two components: a structural model which accounts for the systematic trends in the data and a random effects model, which accounts for both intersubject variability and residual variability.

WNT974 plasma concentration data were modeled by a two-compartment PK model with linear absorption and clearance (CL; Figure S1). The absorption kinetics were characterized by a first-order absorption rate constant (K_a) with delayed absorption time (T_{lag}). The disposition kinetics were modeled using a parameterization involving apparent central clearance (CL/F), apparent central volume (V_c/F), apparent distribution clearance (CL_d), and apparent peripheral volume of distribution (V_p). Covariates of the patients measured at baseline, including weight, race, age, sex, body mass index (BMI), total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, and Eastern Cooperative Oncology Group (ECOG) status, were evaluated and explored for correlation with PK parameters.

The concentration data were modeled in the log domain using the first-order conditional estimation method as implemented in NONMEM software (version VII, level 2.0; Icon Development Solutions, Ellicott City, MD) compiled with Intel Fortran Compiler (version 11.1) on the MODESIM high-performance computing environment diagnostic graphics and post-processing of NONMEM output were performed using the S-Plus software (version 8.1; TIBCO Software Inc., Palo Alto, CA).

Interindividual variability (IIV) was assumed to be log-normal and estimated using an exponential term, on the parameters KA (K_a), CL, V2 (V_c), Q (CL_d), V3 (V_p), and ALAG1 (T_{lag}). The IIV of CL and V2 was modeled using a two-dimensional Ω block accounting for potential correlations between them; for the IIV of all other parameters, diagonal Ω was estimated. The IIV was reported as coefficient of variation (%CV). The residual error was described using an additive model.

Exposure–response analysis for biomarker

The ER relationship of WNT974 exposure vs. skin *AXIN2* expression (RQ of *AXIN2* gene relative to the reference genes and the change of RQ from baseline) was described by a maximum effect (E_{max}) model using S-Plus (version 8.1; TIBCO Software Inc.). WNT974 exposure endpoints evaluated in the analysis included C1D15 AUC_{tau}, C_{max}, and C_{min}. The E_{max} model was used to fit the data using the following equation:

$$E_i = E_0 - E_{max} * C_{min,i} / \left(EC_{50} + C_{min,i}\right)$$

where E_i is the on-treatment RQ level of skin *AXIN2*, E_0 is the baseline RQ level of skin *AXIN2*, E_{max} is the maximal effect of WNT974 to inhibit skin *AXIN2*, and EC_{50} is WNT974 concentration at which half-maximal reduction in skin *AXIN2* is reached.

Exposure–response analysis for safety

ER analysis of WNT974 exposure vs. the probability of dysgeusia was conducted using a logistic regression model using R (version 3.0.2). Only data collected during the treatment period of the study and related to the study drug were included in the analysis.

The ER relationship of probability of event versus WNT974 exposure (C1D15 AUC_{tau}, C_{max}, or C_{min}) was evaluated using a logistic regression model as follows:

$$\log\frac{P_i}{1-P_i} = \beta_0 + \beta_1 \cdot C_i$$

where P_i is the probability of dysgeusia event, C_i is the exposure end point, β_0 is the intercept, and β_1 is the coefficient for exposure.

Model evaluation

Model evaluation was based on successful convergence, change in objective function values, goodness-of-fit plots, and visual predictive check (VPC). The VPC was performed with 1000 simulated subjects obtained from the final model by Monte Carlo simulation. The 5th, 50th, and 95th percentiles across the 1000 simulations were estimated and overlaid with the observed data and were plotted.

Model application

Based on the ER analyses of skin *AXIN2* expression and dysgeusia, the target exposure range of WNT974 that balances the two responses was identified. Steady-state WNT974 PK profiles on C1D15 were simulated using the PopPK model in 200–500 hypothetical patients at different continuous dosing scenarios. The concentration profiles at each WNT974 dose were graphically presented, along with the target range of exposure determined. The RDE was selected based on the simulated PK profiles and the estimated target exposure range.

RESULTS

Population PK modeling

PK data for WNT974 used in the modeling contains 1098 observations arising from 66 patients across 10 dose regimens (q.d. continuous: 5, 7.5, 10, 15, 20, 22.5, and 30 mg; q.d. 4 days on/3 days off: 30 and 45 mg; and b.i.d. continuous: 5 mg). Patient characteristics are summarized in Table 1.

A two-compartment model with delayed first-order absorption and linear CL adequately described WNT974 plasma concentration profiles. As shown in the goodness-of-fit plots (Figure S2), observed versus population prediction (PRED) and observed versus individual prediction demonstrated random scatter around the line of unity, showing good agreement. Residual plots of conditional weighted residuals (CWRES) versus PRED and CWRES versus time after previous dose demonstrated random scatter around the line of zero. The percentage of the relative standard error of parameter estimates showed good precision and WNT974 showed moderate intersubject variability (Table 2A). VPCs for both single (Figure S3) and repeat (Figure S4) dosing showed that the observed data fell within or near the corresponding 90% prediction intervals, indicating the model provides an adequate predictive performance.

Graphical exploration showed no apparent relationship of the covariate examined with CL/F or V_c/F, and, hence, further covariate analysis was not conducted.

TABLE 1 Summary statistics of patient baseline characteristics

Covariate, unit	Summary statistics		
	N	Mean (SD)	Median (range)
Age, years	68	57 (11.3)	59 (28–75)
Weight, kg	66	76 (18)	72 (47–124)
BMI, kg/m^2	64	26 (5.5)	25 (17.4–44.6)
BSA, m^2	64	2 (0.3)	1.8 (1.4–2.4)
AST, U/L	68	36 (19.7)	30 (9–100)
ALT, U/L	68	35 (21.6)	30 (6–119)
TBIL, U/L	68	10 (6.0)	8 (2–31)
ALB, g/L	68	38 (5.2)	38 (25–50)
CRCL, ml/min	66	105 (36.4)	104 (47.1–219)
Sex			
Male	29	–	–
Female	39	–	–
Race			
White	58	–	–
Black	5	–	–
Asian	1	–	–
Other	4	–	–
ECOG			
0	15	–	–
1	45	–	–
2	6	–	–
3	1	–	–
Missing	1	–	–

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BSA, body surface area; CRCL, creatinine clearance; ECOG, Eastern Cooperative Oncology Group; SD, standard deviation; TBIL, total bilirubin.

Exposure–response analysis for biomarker

Skin *AXIN2* mRNA level is best related to C_{min} but not AUC_{tau} or C_{max} based on visual inspection. An E_{max} model adequately described the relationship and the parameter estimates are summarized in Table 2B. VPCs showed that the observed data generally fell within or near the corresponding 90% prediction intervals, indicating the model provides an adequate predictive performance (Figure 1).

Exposure–response analysis for safety

Logistic regression analysis was conducted for C1D15 exposure (AUC_{tau}, C_{max}, and C_{min}) and treatment-emergent dysgeusia. It showed positive correlation between probability of dysgeusia and C1D15 AUC_{tau} or C_{max} (Figure 2). At the C_{max} < 118 ng/ml or AUC from zero to 24 h (AUC_{24h}) < 762 ng h/ml, the median probability of Grade ≥2 dysgeusia was estimated to be <25%.

Model application

Based on the ER analysis of skin *AXIN2* expression and dysgeusia, the WNT974 exposure to achieve the target inhibition response, and clinically acceptable probability of dysgeusia were estimated and the target exposure range was determined (Table 3). Steady-state C_{min} > 2.6 ng/ml was estimated to achieve 95% probability of >50% maximal inhibition and was used as the target exposure for Wnt pathway inhibition. Steady-state C_{max} < 118 ng/ml and steady-state AUC_{24h} < 762 ng h/ml were estimated to provide 50% probability that <25% of patients have Grade ≥2 dysgeusia, which was considered clinically manageable.

PK simulation based on the PopPK model showed that the PK profile at 10 mg q.d. mostly fell within the estimated target exposure range based on PD response (suppression of *AXIN2* expression) and tolerability (dysgeusia;

TABLE 2 (A) Model-estimated population pharmacokinetic parameters of WNT974. (B) Model-estimated parameters for exposure–response analysis of WNT974 exposure and skin *AXIN2* reduction

Parameter description	Population estimate (%RSE)	Interpatient variability (%RSE)[a]
(A)		
Absorption rate constant (K_a), 1/h	1.60 (17.0)	100% (30.0)
Absorption lag time (T_{lag}), h	0.412 (3.2)	45.7% (12.5)
Apparent central clearance (CL/F), L/h	20.7 (3.9)	38.6% (16.2)
Central volume of distribution (V_c), L	138 (2.0)	44.1% (25.7)
Peripheral volume of distribution (V_p), L	260 (9.4)	78.5% (43.0)
Distribution clearance (CLd), L/h	7.11 (9.4)	60.5% (36.9)
Residual error, proportional	0.39 (3.1%)	

Parameter description	Unit	Estimated mean (90% prediction interval)	%SEE
(B)			
Baseline effect (E_0)		0.84 (0.66–1.0)	12.4
Maximal effect (E_{max})	%	0.65 (0.46–0.83)	17.8
WNT974 concentration resulting in 50% E_{max} (EC_{50})	ng/ml	0.45 (0.09–2.62)	129

Abbreviation: RSE, relative standard error (=standard error of estimate/population mean estimate ×100%); SEE, standard error of estimation.

[a]Reported as coefficient of variation (%CV).

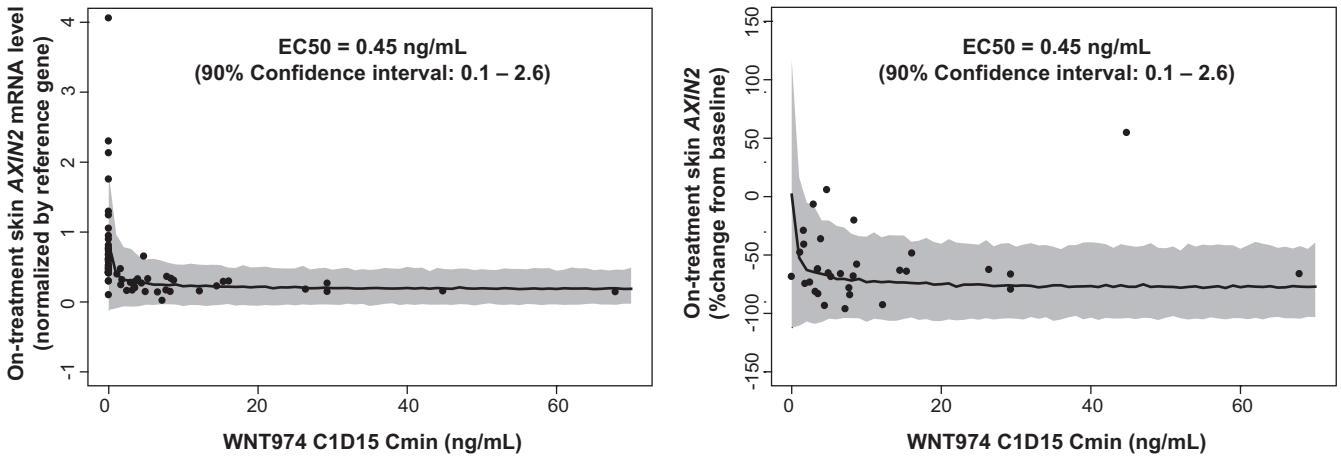

FIGURE 1 Exposure–response relationship for WNT974 exposure and skin *AXIN2* expression (left: absolute level normalized by reference gene; right: %change from baseline). Symbols represent observed data; curve and shaded area represent the predicted median and 90% prediction interval, respectively. C1D15, Cycle 1 Day 15; C_{min}, minimum plasma concentration; EC_{50}, WNT974 concentration resulting in 50% maximum effect

Figure 3). The PK parameters also showed that 10 mg q.d. is predicted to be the dose that yields the population exposure mostly within the estimated target exposure range (Table 4). Based on this analysis, 10 mg q.d. was selected as the RDE.

DISCUSSION

Identifying the optimal dose of a novel oncology therapeutic is critical for successful drug development and is a major objective in translational clinical oncology studies. Aiming to best balance toxicity and antitumor activity, selecting the right dose and schedule is essential to accomplishing successful pivotal clinical trials and achieving the greatest benefit for patients. It is increasingly acknowledged that the conventional MTD approach used to select the recommended dose may not be ideal, and as such there is a growing movement to identify novel approaches for selecting optimal treatment regimens.[15–19] In addition, during early oncology clinical development, evidence of efficacy of a molecule may be limited or inconclusive

due to limitations in sample size and heterogeneity of the patient population. Hence, initial efficacy may not effectively inform expansion/phase II dose selection and thus a relevant biomarker may be a more useful surrogate to help identify an optimal biological dose (OBD). Recently, the US Food and Drug Administration (FDA) launched Project Optimus aiming to reform the dose optimization and dose selection paradigm in oncology drug development.[20]

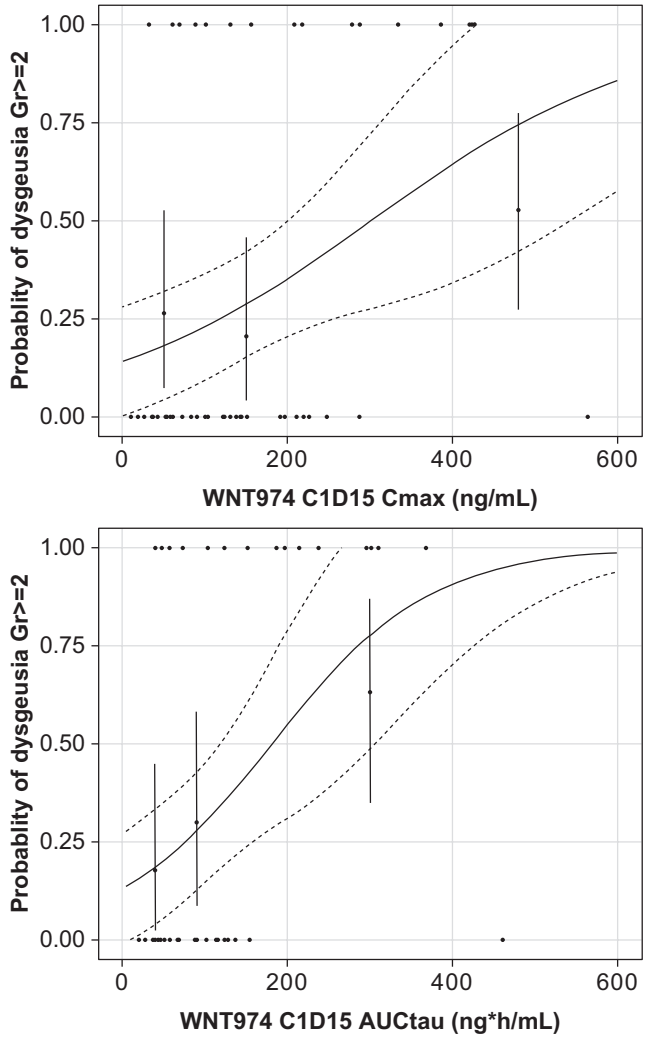

FIGURE 2 Exposure–response relationship for WNT974 exposure and probability of dysgeusia. Solid circles and vertical bars represent observed data. Curves represent model-predicted median (solid line) and 95% prediction interval (dash line). AUC_{tau}, area under the plasma concentration–time curve during a dosing interval; C1D15, Cycle 1 Day 15; C_{max}, maximum plasma concentration

Due to high medical needs, developing novel medicines, especially first-in-class molecules, is demanding in oncology. It is critical to balance the agility and speed of clinical development, the challenge of limited data in early development, and adequacy of the data to make well-informed decisions. Here, we present a case study of a model-based approach, which integrated PK, biomarker, and safety data available during the phase I escalation study of WNT974, an oncology molecule, and was able to quantify the target exposure to balance biological response and safety management and identify the OBD. The approach allowed timely data-driven decision for dose selection that supported the path forward to the phase I expansion and phase II clinical development of the first-in-class molecule.

The phase I FIH dose-escalation study of WNT974 was designed to identify the MTD and/or RDE. Due to the nature of the most common on-target AE of Wnt inhibition, dysgeusia, which was initially low in grade and had a delayed onset, it was not captured by traditional dose-limiting toxicity criteria. As a result, the protocol-defined MTD was not identified. Given the nature of the dysgeusia, which was common, and, despite its low grade, often resulted in treatment cessation, it was deemed important to consider it in the selection of the RDE but in itself insufficient to optimize the dose for expansion using MTD approach. Alternative dose regimens than continuous q.d. dosing, intermittent dosing and b.i.d. dosing were studied during the dose-escalation part, with the attempt to reduce dysgeusia.

Potent inhibition of the Wnt pathway was evidenced by suppression of *AXIN2* mRNA expression in skin at most dose levels without a clear increase in suppression at higher doses. Consistently, the mean unbound C_{min} of WNT974 on C1D15 following a continuous q.d. dose of 5–30 mg (1.1–7.6 nM) was above the in vitro cellular IC_{50} of WNT974 for Porcupine (0.4 nM).[8] However, WNT974 showed limited antitumor activity in the dose-escalation

TABLE 3 Estimated target exposure for WNT974

Endpoint	Criteria	Steady-state exposure threshold
Skin *AXIN2*	95% probability to achieve >50% maximal inhibition of mean *AXIN2*	$C_{min} > 2.6$ ng/ml
Dysgeusia	50% probability that < 25% of patients have Grade ≥ 2	$C_{max} < 118$ ng/ml
Dysgeusia	50% probability that < 25% of patients have Grade ≥ 2	$AUC_{24h} < 762$ ng h/ml

Abbreviations: AUC_{24h}, area under the plasma concentration–time curve from time zero to 24 h; C_{max}, maximum plasma concentration; C_{min}, minimum concentration during a dosing interval.

study, which, despite recognition that most sensitive target population may not be represented in the dose escalation, showed uncertainty that lower doses provided sufficient exposure of the first-in-class drug. A model-based approach to choose the RDE was therefore taken, which permitted a more systematic and comprehensive approach to integrate the available data from the dose escalation part to provide the most confidence to select a dose for expansion that optimized pathway suppression with clinical tolerability.

Suppression of tumor *AXIN2* mRNA expression was also observed in patients. Due to the more homogeneous nature of skin, skin AXIN2 assay was less variable than tumor AXIN2 assay, providing a more consistent and reliable assessment of the Wnt pathway. Disparity was observed in some cases between *AXIN2* mRNA biomarker suppression in skin and tumor samples. Sequencing information available for one patient illustrated how tumor-specific genetic alterations in β-catenin, which is downstream of Porcupine in the Wnt pathway, could prevent *AXIN2* downregulation even when Porcupine is inhibited.[8] The observed disparity may also result from tumor heterogeneity, differing biopsy sites of paired samples, and/or mutations in downstream components of the canonical of Wnt pathway (e.g., adenomatous polyposis coli or ß-catenin).

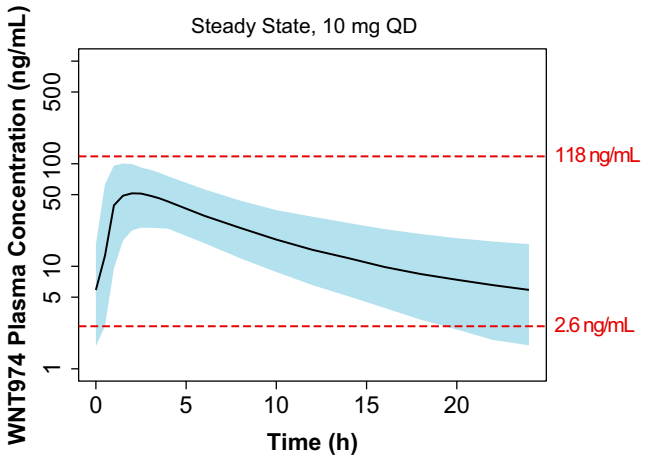

FIGURE 3 Predicted steady-state WNT974 pharmacokinetic profile at 10 mg once daily and estimated target exposure range based on *AXIN2* response and dysgeusia. Curve and shaded area represent model-predicted median and 90% prediction interval, respectively. Red dashed lines represent estimated target exposure range. The two horizontal dashed lines represent 2.6 and 118 ng/ml of WNT974 concentration

The PopPK analysis showed that a two-compartment model with delayed first-order absorption and linear CL described WNT974 plasma concentration profiles across the dose range studied (5–45 mg). Estimated IIV for PK parameters ranged from 30% to 80% except for K_a, suggesting a moderate interpatient PK variability. These results are consistent with dose proportionality and moderate variability of WNT974 PK based on NCA.[8] Considering the primary focus at the early development stage (estimating the PopPK parameters and IIV) and smaller sample size compared to late-phase clinical studies, covariate effects were only assessed graphically. No covariate was identified to correlate with CL or V_p. Graphical examination revealed no apparent impact of age, body weight, BMI, albumin, bilirubin, ALT, AST, sex, or ECOG on WNT974 PK. The active metabolite LHA333 was also measured in the study, however, LHA333 is considered to have minimal contribution to the pharmacological response.[8]

ER analyses for skin *AXIN2* expression and dysgeusia showed that both the reduction of skin *AXIN2* expression from baseline and the probability of dysgeusia were related to WNT974 exposure. The probability of Grade ≥2 dysgeusia increased with increasing WNT974 exposure over the dose range investigated, and skin *AXIN2* reduction also increased with WNT974 exposure but approached a plateau at higher exposures in the dose range studied. The upper limit of the target exposure range, the maximal probability of dysgeusia for the study to move forward (50% probability that <25% of patients have Grade ≥2 dysgeusia), was selected based on clinical experience. The ER analysis of skin *AXIN2* showed that maintaining steady-state $C_{min} > 2.6$ ng/ml would be required to achieve 95% probability of >50% maximal inhibition of *AXIN2*, whereas ER analysis of dysgeusia showed that maintaining steady-state $C_{max} < 118$ ng/ml and $AUC_{24h} < 762$ ng h/ml would provide 50% probability that <25%

TABLE 4 Predicted steady-state WNT974 pharmacokinetic parameters

Parameters (mean and 90% confidence interval)			
Dose	**C_{min} (ng/ml)**	**C_{max} (ng/ml)**	**AUC_{24h} (ng h/ml)**
5 mg q.d.	2.83 (0.835–8.02)	28.1 (13.7–59.1)	233 (133–445)
10 mg q.d.	5.78 (1.66–16.3)	55.7 (26.1–109)	468 (254–880)
15 mg q.d.	8.21 (2.60–24.3)	80.6 (41.7–166)	699 (394–1294)

Abbreviations: AUC_{24h}, area under the plasma concentration–time curve from time zero to 24 h; C_{max}, maximum plasma concentration; C_{min}, minimum concentration during a dosing interval; q.d., once daily.

of patients have Grade ≥2 dysgeusia. Based on these data, the exposure range was established and was anticipated to provide a balance between maximizing target inhibition and minimizing AE.

Simulation using the PopPK model was conducted for different dose levels to estimate the OBD. It revealed that 10 mg q.d. would yield an exposure mostly within the target exposure range from the population standpoint based on the 90% confidence interval (CI) of the estimated exposure. At the dose of 15 mg, the upper bound of 90% CI is ~70 and 40% above the target threshold for AUC and C_{max}, respectively, which could put the patients at risk for dysgeusia; whereas at the dose of 10 mg, the upper bound of 90% CI is within the threshold for C_{max} or only 15% over the threshold for AUC. On the other hand, the dose of 5 mg would not maximize the probability for target inhibition. Therefore, 10 mg was predicted to provide the best balance for safety and target inhibition, and thus was selected as the RDE for the dose-expansion part of the single-agent study. The dose regimen was demonstrated to be safe and tolerable in the dose-expansion part of the study, with the observed WNT974 concentrations consistent with the predicted data by PopPK modeling.[8]

In Wnt ligand-driven rodent tumor models, tumor *AXIN2* mRNA expression was suppressed and reached maximal reduction between 5 and 10 h postdose.[5] To balance the expected time of PD effect while providing some flexibility on the needed clinical scheduling, postdose skin and tumor biopsies for *AXIN2* were collected at one timepoint at 5–10 h postdose between days 5 and 28 in the phase I FIH study. The dynamics of *AXIN2* mRNA expression in patients is therefore unknown, but may provide insight to understand the PK/PD relationship. Despite potent suppression of *AXIN2* expression, the best response in the dose-expansion part of the single-agent study was that 16% of patients had stable disease (median duration 19.9 weeks). The limited antitumor activity may be attributable to tumor characteristics, such as oncogenic co-mutations that limit the dependence of tumors on Wnt signaling alone.[8]

In conclusion, this first PopPK and ER analyses for the first-in-class Porcupine inhibitor WNT974 guided dose selection for the phase I expansion part and supported early oncology clinical development of WNT974. Presenting a quantitative and holistic understanding of the clinical data, the model-based approach integrated PK, target-inhibition marker, and safety data during early phase I study to determine the RDE, rather than the conventional MTD approach. As demonstrated by our case study, this model-based approach effectively informed RDE selection and supported translational clinical oncology development and can be applied to dose selection in early oncology trials to inform later-phase clinical development.

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CONFLICT OF INTEREST

All authors are employees of Novartis. Y.J., P.H.H., and A.M. also hold stocks with Novartis.

AUTHOR CONTRIBUTIONS

Y.J. wrote the manuscript. Y.J. and A.M. designed the research. Y.J. developed the concept and methodology and analyzed the data. P.H.H. and S.W. prepared the data. All authors acquired and/or interpreted the data, and reviewed and revised the manuscript.

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