CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number:	BLA 761166
Regulatory Pathway:	351(a)
Supporting document/s:	0001
Applicant's letter date:	March 6th 2020
CDER stamp date:	March 13th 2020
Product:	Ropeginterferon alfa-2b (Besremi)
Indication:	Adult Patients with Polycythemia Vera (PV) without Symptomatic Splenomegaly
Applicant:	PharmaEssentia
Review Division:	DPT/OCHEN
Clinical Division:	DNH/OCHEN
Reviewer:	Jeffrey Quinn, PhD
Supervisor/Team Leader:	Todd Bourcier, PhD
Division Director:	Ann Farrell, MD
Project Manager:	Charlene Wheeler
Review completion date:	January 10th , 2021

1 Executive Summary

Introduction

PharmaEssentia submitted a Biologics License Application (BLA 761166; 351a) for ropeginterferon alfa-2b (P1101) Injection (0.5 mg/1 mL in a pre-filled syringe), a mono-pegylated proline-interferon α-2b analog for the treatment of adult patients with polycythemia vera (PV) without symptomatic splenomegaly in the United States.

Interferons and pegylated interferons have been used extensively for the treatment of polycythemia vera; however, the mechanism(s) driving its action remain unclear. Nonclinical studies indicate that the therapeutic effects of IFNs are most likely related to stimulation of the immune system and depletion and/or elimination of mutant hematopoietic stem cell lineages.

Pegylation of IFN reduces the elimination of the molecule and facilitates a prolongation of the plasma half-life. Conventional PEGylated IFNs currently approved in the United States are administered once per week, compared to 3-times per week for unmodified interferon alfa. Ropeginterferon alfa-2b (P1101) is a third-generation mono-pegylated IFN that intends to provide a more convenient treatment schedule (every 2-weeks) that the sponsor claims will provide lower toxicity and higher hematologic and molecular response rates in adult patients with polycythemia vera.

The toxicological evaluation of P1101 was limited to studies of ≤ 1 month in duration due to the production of neutralizing anti-drug antibodies (ADAs) in cynomolgus monkeys (4-Weeks) and the lack of biologic activity in Sprague Dawley rats (14-Days).

PEG accumulation and vacuolation of cells in the nervous system were not observed during the pivotal 1-month monkey toxicity study of P1101; however, the results were confounded by the presence of ADAs and the short study duration. Mononuclear perivascular infiltrates in the brain and an increase in the incidence and severity of atrophy of the thymus and mild necrosis of the epithelium in the stomach were observed in a 14-Day monkey bridging study (Study 44103-08-300); however, the reviewer considers these findings attributable to the animal source (China), as similar results were not observed in monkey studies (2-weeks and 4-weeks) that employed US-sourced monkeys. Based on the comparable toxicology (vs conventionally PEGylated IFNs) and clinical experience a nonclinical assessment of cytokine release was not deemed necessary.

Drug-related effects noted during the pivotal 4-Week monkey toxicity study (US sourced) were consistent with the known effects of administration of human IFN to cynomolgus monkeys. P1101 caused significant thrombocytopenia at the HD in monkeys and is consistent with the known effects of PEGylated IFN use in human subjects. Hence, the MD is considered the most clinically relevant NOAEL and is associated with Day 1 (due to ADAs) exposure margins (sex-averaged) of 533x (C_{max}) and 2790x (AUC_{0-t}).

Comparison of Exposure at the NOAEL (MD) for the Pivotal 4-Week Cynomolgus Monkey Toxicity Study with Clinical Data at the MRHD

Time point (NOAEL) Sex	**C_{max} [ng/mL]**	**MoE[a]**	**AUC_{0-t} [ng.hr/mL][a]**	**MoE[a]**
Day 1 (2 mg/kg) Males / Females	28697 / 23128	590x / 475x	1687204 / 1396471	3053x / 2527x
Sex-Averaged	25913	533x	1541837	2790x

[a] Multiple of Exposure (MoE) was calculated as ratio of serum PK parameters at the reviewer determined NOAEL in the pivotal toxicity study (2 mg/kg) and at the clinical dose of 450 µg that resulted in the highest measured exposure in humans (C_{max} = 48.64 ng/mL and AUC_{0-t} = 552.57 ng.hr/mL).

1.1 Recommendations

1.1.1 Approvability

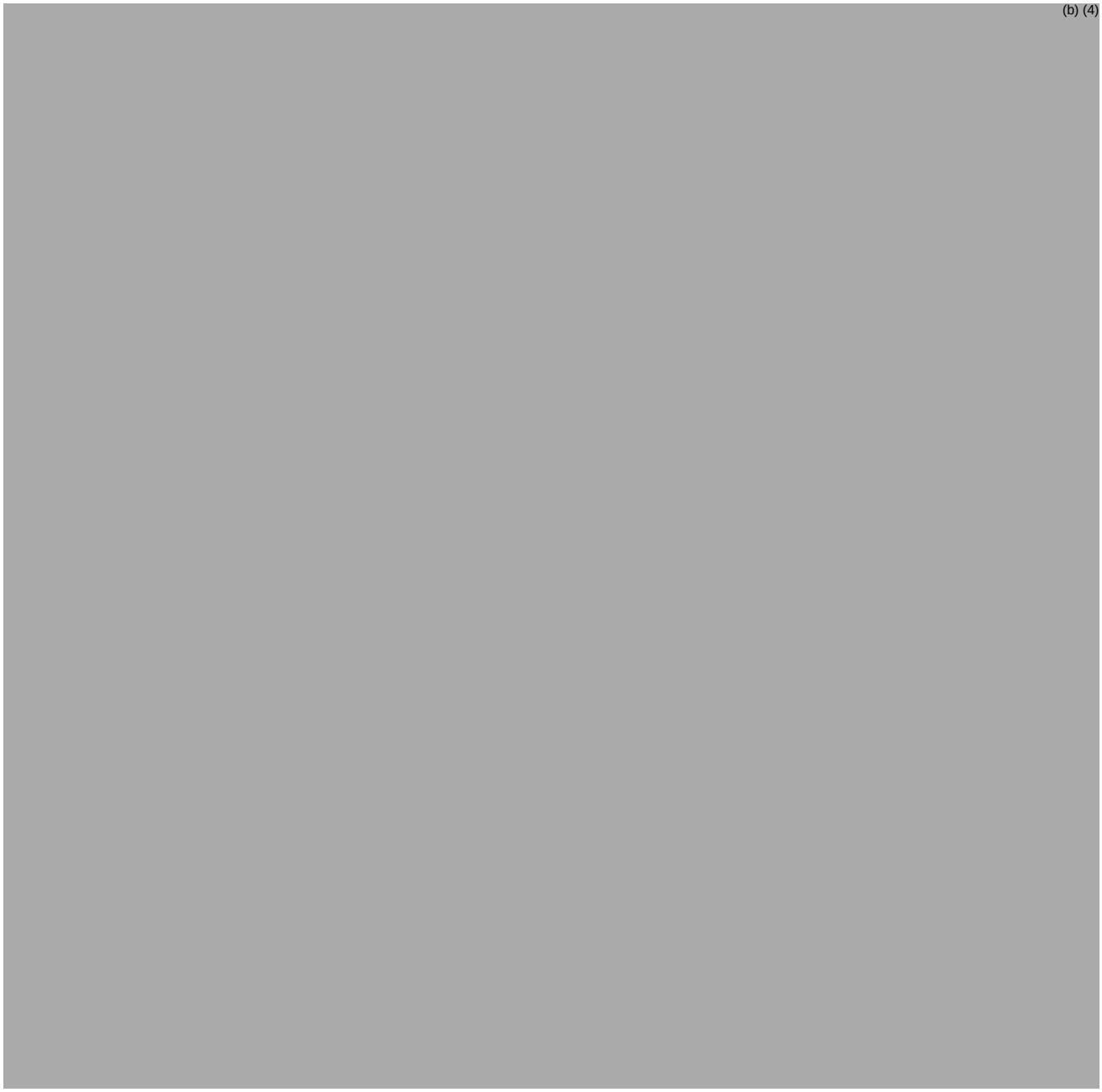
There are no deficiencies in the nonclinical data that would preclude approval of BLA 761166**.**

1.1.2 Additional Nonclinical Recommendations

No additional nonclinical studies are recommended.

1.1.3 Labeling Recommendations

(b) (4)



(b) (4)

1.2 Brief Discussion of Nonclinical Findings

Toxicology data used to support approval of ropeginterferon alfa-2b (P1101) was derived predominantly from the pivotal one-month cynomolgus monkey study as animal studies exceeding 1 month in duration were not deemed feasible and would not provide additional information with regards to the safety assessment of the drug product.

- The pharmacokinetics, pharmacodynamics and biological activity of P1101 are comparable to those of marketed pegylated IFN alfa products.
- P1101 is a weak inhibitor of hERG channels in vitro (↓49.9% at 2.5 μM - HD) and in vivo correlates (i.e., QT prolongation or ECG morphology changes) were not observed in monkey studies. The mean serum C_{max} (49 ng/mL) of P1101 achieved in polycythemia vera patients at the MRHD (0.5 mg) represents a concentration that is 1000-fold lower than the approximated hERG IC_{50} (2.5 μM = 50 μg/mL).
- P1101 exhibited time-dependent inhibition of CYP2A6 at a concentration equivalent to the observed C_{max} at the MRHD in polycythemia vera patients. Drugs with a narrow therapeutic index metabolized by CYP2A6 (letrozole, tegafur, coumarin, valproic acid, methoxyflurane, artesunate, disulfiram, halothane and fadrozole) should be used with caution when co-administered with P1101. This is of particular relevance to patients undergoing cytoreductive therapy who may be administered Warfarin (coumarin).
- P1101 was found to be negative for inducing chromosomal aberrations in vitro without metabolic activation, but equivocal with metabolic activation at concentrations where increased cytotoxicity was observed. P1101 is produced by covalent attachment of PEG to the N-terminal proline residue of recombinant proline-interferon alfa-2b (b) (4) No separate chemical linker is employed (direct conjugation). Considering the reactive linker molecule does not represent a toxicological concern and P1101 will be metabolized by proteases (not CYPs) the genotoxicity assessment is likely uninformative with regards to assessing the safety of P1101.
- Reproductive and developmental toxicity studies were not conducted with P1101 as IFNs are known to be abortifacient in primates[1,2]. Long term carcinogenicity studies were not conducted due to the lack of pharmacologic activity of P1101 in rodents.

[1] Ihara T, et al. An embryotoxic/teratogenic potential and abortifacient effect study of interferon alfacon1 via subcutaneous administration to rhesus monkeys. 1999. Cong Anom 39;223-242.
[2] Buckley et al. 2008; International Journal of Toxicology, 27: 303-312

2 Drug Information

2.1 Drug: BESREMI (Ropeginterferon alfa-2b) P1101

Chemical Name

Poly (oxy-1,2-ethanediyl), α-hydro-ω-methoxy-,1,1-diester with interferon α-2b [1-[1-[3,7-bis (carboxyamino) heptyl] proline]] (synthetic human)

Molecular Formula/Molecular Weight

$C_{876}H_{1376}N_{232}O_{260}S_9$▪$(C_2H_4O)n$ (where n = 864 to 1044)/~60 kDa

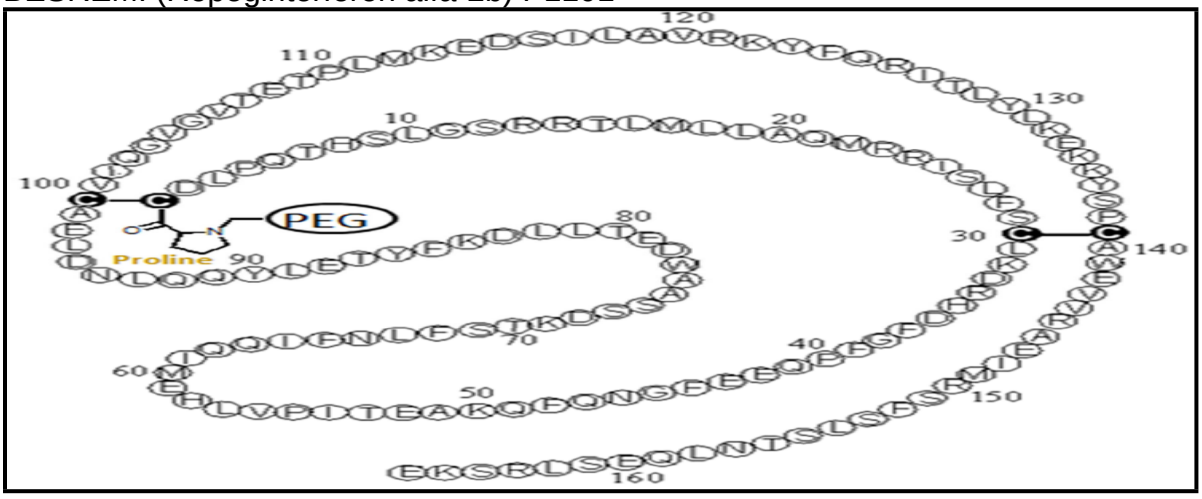
CAS

1335098-50-4

Structure or Biochemical Description

Ropeginterferon alfa-2b (DP) is produced by covalent attachment of a 40kDa PEG molecule directly to the N-terminal proline residue of recombinant proline-interferon alfa-2b (b) (4) .

BESREMI (Ropeginterferon alfa-2b) P1101



Pharmacologic Class

Ropeginterferon alfa-2b is an interferon alfa-2b analog.

Ropeginterferon alfa-2b is comprised of recombinant interferon alfa-2b conjugated with a two-arm methoxypolyethylene glycol (mPEG) (b) (4) and is considered a long-acting mono-pegylated interferon alfa-2b analog.

Ropeginterferon alfa-2b (P1101) was granted Orphan Drug Designation for the treatment of polycythemia vera on April 2, 2012 (#12-3670) by the FDA.

Planned Clinical Route of Administration

Subcutaneous Injection

2.2 Relevant INDs, NDAs, BLAs and DMFs

The European Commission granted marketing authorization for ropeginterferon alfa-2b (BESREMI) for the treatment of adults with polycythemia vera without symptomatic splenomegaly on February 15 h, 2019.

INDs: 119047 (P1101, Polycythemia Vera, DNH). (b) (4)
(b) (4)

BLAs: 103145 (Non-Pegylated IFNα-2a, ROFERON-A, Roche, Approved 1986, Hepatitis and Hairy Cell Leukemia, Status – Withdrawn/Revoked 2014)
103964 (Pegylated IFNα-2a, PEGASYS, Roche, Approved 2002, Hepatitis)
103132 (Non-Pegylated IFNα-2b, Intron A, Schering, Approved 1986, Leukemia)
103949 (Pegylated IFNα-2b, PEGINTRON, Schering, Approved 1/2011, Hepatitis and SYLATRON, Approved 3/2011 (Discontinued 2019), Melanoma)

2.3 Drug Formulation

The drug product contains (b) (4) ropeginterferon alfa-2b solution, the formulation excipients, and prefilled syringe (PFS) container and closure system. All excipients are well known and comply with the requirements and specifications of the relevant compendial monographs. There are no novel constituents used in this formulation.

Composition of the Ropeginterferon alfa-2b in Prefilled Syringe

Name of Ingredient	Function	Compliance	Quantity per mL	Quantity per PFS (b) (4)
Ropeginterferon alfa-2b	API	PEC specification	0.5 mg	
Sodium Chloride	(b) (4)	Ph. Eur., USP, JP, BP	8.0 mg	
Sodium Acetate, (b) (4)		USP	1.58 mg	
Acetic Acid, Glacial		Ph. Eur., USP	0.05 mg	
Benzyl Alcohol		Ph. Eur., USP, JP, BP	10.0 mg	
Polysorbate 80		Ph. Eur., USP, JP, BP	0.05 mg	
Water for Injection		Ph. Eur., USP, JP	q.s. to 1 mL	

2.4 Comments on Novel Excipients

There are no novel excipients used in the production of ropeginterferon alfa-2b.

2.5 Comments on Impurities/Degradants of Concern

Product related impurity and process related impurity issues have been addressed. Levels of (b) (4) have been assessed. The levels of product related impurities/degradants represented in the final drug product are considered acceptable.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population is adult patients with polycythemia vera without symptomatic splenomegaly in the United States.

The ropeginterferon alfa-2b (P1101) dose is titrated individually with a recommended starting dose of 0.1 mg (or 0.05 mg in patients under another cytoreductive therapy) and is proposed to be administered subcutaneously, one injection every 2 weeks.

3 Studies Submitted

Safety Pharmacology

- PEG-IFNα 2b (P1101): Electrocardiographic investigations on single-lead ECG's using radio-telemetry in the conscious cynomolgus monkey following intravenous administration (Study 2872-001)
- PEG-IFNα 2b (P1101): Effects on General Activity, Behavior and Body Temperature in the Rat (Irwin study) Following Subcutaneous Administration (Study 2872-002)
- PEG-IFNα 2b (P1101): Measurement of Respiratory Parameters in the Freely Moving Conscious Rat Using Whole Body Plethysmography (Study 2872-004)
- Effects of PEG-IFNα 2b (P1101) on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (Study 7975-106)

ADME/Pharmacokinetics/Pharmacodynamics

- A Pharmacokinetic and Pharmacodynamic Study of P1101 versus Pegasys in Male Cynomolgus Monkeys (44104-08-242)
- In Vitro Study for Evaluating Potential of TA-19-172 (Ropeginterferon alfa-2b (P1101)) on CYP450 Induction using human primary hepatocytes (RD-S1909-12)
- In Vitro Study for Evaluating Direct Inhibitory Potential of P1101 on Cytochrome P450 Enzymes in Human Liver Microsomes (Study 8417-482)
- In Vitro Study for Evaluating Time-Dependent Inhibitory Potential of P1101 on Cytochrome P450 Enzymes in Human Liver Microsomes (Study 8417-483)

Toxicology

- Single and Multiple-Dose Subcutaneous Injection Pilot Toxicity Study with PEG-IFNα 2b in Rats (Study 7975-100, non-GLP)
- Escalating Dose Range-Finding Study and a 14-Day Repeat Dose Toxicity Study with PEG-IFNα 2b in Cynomolgus Monkeys (Study 7975-102, non-GLP)
- Pivotal 4-Week Subcutaneous Toxicity and Toxicokinetic Study with PEG-IFNα 2b in Cynomolgus Monkeys with a 4-Week Recovery Period (Study 7975-101, GLP)
- 2-Week Subcutaneous Administration Toxicity, Toxicokinetics and Immunogenicity Bridging Study of Two Lots of P1101 in Cynomolgus Monkeys (Study 7975-107, GLP with GLP inadequacies)
- A 14-Day Repeat-Dose Subcutaneous Toxicity Study with Toxicokinetics and Immunogenicity of P1101 in Cynomolgus Monkeys (Study 44103-08-300, GLP excluding ADA analysis)
- Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay (Study 7975-104, GLP)
- Effect of PEG-IFNα 2b on Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells (Study 7975-105, GLP)

4 Pharmacology

4.1 Primary Pharmacology

Drug Activity Related to Proposed Indication

Interferons and pegylated interferons have been used off-label for the treatment of polycythemia vera for over 30 years. Despite the extensive use of IFNs for the treatment of polycythemia vera, the mechanism(s) driving its action remain undetermined.

It has been proposed that the therapeutic effects of IFNs are mediated by a broad range of biological activities, such as stimulation of the immune system and suppression of hematopoietic cell proliferation, but also direct effects on reducing the mutant hematopoietic stem cell population (Kiladjian, Giraudier, and Cassinat 2016).

Studies employing JAK2V617F-driven mouse models of polycythemia vera have suggested that IFNα may promote cell cycle entry of the mutant long-term hematopoietic stem cells and enhance terminal erythroid differentiation of the aberrant lineage (Mullally et al. 2013). The combined effect of IFNα on the proliferation and differentiation of mutant hematopoietic stem cells may promote depletion of the aberrant stem cell pool. This model may also explain the reduced mutant allele burden and persistent molecular and hematological responses observed in some PV patients even after cessation of interferon treatment (Kiladjian et al. 2008).

Additionally, it was speculated that interferons may delay disease progression in a murine model of polycythemia vera by nullifying the proliferative advantage of the JAKV617F mutant hematopoietic stem cells over the normal cells via sensitization of the mutant cells to apoptosis and induction of cell cycling in the normal stem cells (Hasan et al. 2013).

The biologic activity of P1101 (b) (4) was evaluated in a cytopathic effect (CPE) assay conducted in Madin-Darby bovine kidney (MDBK) cells. The CPE assay measures the ability of a compound to protect cells from virus-induced CPE. MDBK cells were incubated with P1101 (IFNα-2b), Roferon (IFNα-2b) or NIBSC IFNα-2b (WHO standard) and then exposed to vesicular stomatitis virus (VSV). The cell viability was determined and the EC_{50} for P1040 was found to be slightly lower than the IFN comparators.

Cytopathic Effect Assay in Bovine Kidney Cells

Compound	EC_{50} (ng/mL)*
P1040 (Proline-IFNα-2b)	0.0275
Roferon (IFNα-2b)	0.0269
NIBSC IFNα-2b (WHO standard)	0.0374

* The specific activity was calculated as the reciprocal of the sample concentration that yields 50% protection of cells against vesicular stomatitis virus–induced CPE.

An in vivo combined PD/PK study was performed with the aim to demonstrate the biological activity of P1101 in cynomolgus monkeys and compare it to Pegasys (Study 44104-08-242). P1101 elicited PD effects (induced serum activity of 2',5'-oligoadenylate synthetase (OAS), an enzyme induced by interferons involved in antiviral response) in cynomolgus monkeys consistent with the administration of interferons, that were similar in magnitude but of longer duration in comparison to Pegasys. Pegylation of the N-terminally introduced proline did not have a negative impact on biological activity of the IFNα-2b protein.

4.3 Safety Pharmacology

(Neurological and Pulmonary)

Ropeginterferon alfa-2b (P1101) did not elicit neurological (Study 2872-002, ≤ 20 mg/kg) or pulmonary effects (Study 2872-004, ≤ 10 mg/kg) when administered as single doses to rats. However, these data are of limited relevance to the safety assessment of P1101 as human IFNα-2b lacks biological activity in rodents. Of more relevance, no clinical signs indicating effects on the central nervous system or respiratory system were observed during toxicity studies conducted in cynomolgus monkeys that assessed subcutaneous doses of P1101 at ≤ 6.75 mg/kg.

(Cardiovascular)

In Vitro

Drug-related inhibition of hERG channels was detected in vitro (Study 7975-106), however the signal was considered weak (49.9% Inhibition at 2.5 μM - HD) and occurred in the absence of in vivo correlates (i.e., QT prolongation or ECG morphology changes). For the purpose of assessing torsadogenic potential, a concentration 2.5 μM was used as the hERG IC_{50}. Mean patient serum C_{max} (49 ng/mL, unbound + bound drug fractions) at the MRHD is 1000-fold lower than the hERG IC_{50} (2.5 μM = 50 μg/mL) and a comparison at the lower concentration assessed in the hERG assay 0.25 μM (5 μg/mL) at which 11% inhibition of hERG current was observed yielded a 103-fold exposure margin to the patient exposure at the MRHD (0.5 mg). It should be noted that hERG is generally not considered a suitable assay for the assessment of biologics as the large size of biologics limit the ability of the molecule to enter the ion channel.

In Vivo

Drug-related increases in body temperature and heart rate were accompanied by decreased QT and QTcf intervals in telemetered monkeys (Study 2872-001) and are similar to those observed with Pegasys (PEG-IFNα-2a). Changes in heart rate, QT, and QTcf were not observed in repeat-dose toxicity studies and are not considered to be adverse.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

In the single combined PK/PD study (Study 44104-08-242), subcutaneous (SC) administration of 30 μg/kg P1101 to male cynomolgus monkeys yielded a similar PK/PD profile as 30 μg/kg of Pegasys. Increasing the P1101 dose to 300 μg/kg improved systemic exposure but not the PD response. Relative bioavailability of 30 μg/kg was 80%.

(Metabolism/Excretion)

During the pre-BLA meeting (September 2019), the FDA informed the Sponsor that they would need to provide data describing the metabolism and elimination of ropeginterferon alfa-2b or provide a detailed justification for why metabolism and elimination studies were not conducted and included in the BLA submission. The sponsor submitted and the reviewer agrees with the justification that biotransformation studies of ropeginterferon alfa-2b would be uninformative based on the understanding that metabolism of a biotechnology-derived pharmaceutical is, in

general, degradation to smaller peptides and/or individual amino acids. The metabolic pathways for peptides are generally understood and do not require the classical biotransformation studies that are typically performed for pharmaceuticals as discussed in ICH guidances directed at small molecules.

The half-life ($t_{1/2}$) of ropeginterferon alfa-2b in monkeys was 66 hrs (SC). Histologically, there was no evidence of cellular vacuolization observed during the pivotal 4-Week monkey study (a known consequence of the metabolism of pegylated pharmaceuticals, Achanzar, et al 2019).

Excretion studies are, in general, not considered appropriate to assess the mass balance of biotechnology-derived pharmaceuticals. An evaluation of the elimination/excretion routes of ropeginterferon alfa-2b would not likely provide additional information with regards to the safety assessment of the P1101.

(Drug-Drug Interactions)

Pharmacodynamic studies investigating the potential for interactions between concomitantly administered drugs were not performed in vivo. The drug-drug interaction (DDI) potential of ropeginterferon alfa-2b was investigated by an in vitro experimental approach using pooled human liver microsomes (Study 8417-482), human cryopreserved hepatocytes (Study 8417-843) or primary human hepatocytes (RD-S1909-12).

Ropeginterferon alfa-2b exhibited no inhibitory or inductive potential on CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 in human liver microsomes and (excluding CYP2A6, see below) is expected to have no clinically meaningful effect on the pharmacokinetics of concomitantly administered medications that are metabolized by these CYP enzymes.

CYP Enzymes and IC_{50} Values of Control Inhibitors and Ropeginterferon Alfa 2b (Study 8417-482)

CYP450	Substrate (µM)	Control Inhibitor		Ropeginterferon Alfa-2b
		Name	IC50 (µM)	IC50 (ng/mL)
1A2	Phenacetin (10)	α-Naphthoflavone	0.0101	> 500
2A6	Coumarin (5)	8-Methoxypsoralen	0.406	> 500
2B6	Bupropion (50)	Ticlopidine	0.0725	> 500
2C8	Amodiaquine (0.5)	Montelukast	0.208	> 500
2C9	Diclofenac (10)	Sulfaphenazole	0.630	> 500
2C19	Omeprazole (0.5)	Tranylcypromine	8.70	> 500
2D6	Dextromethorphan (5)	Quinidine	0.170	> 500
2E1	Chlorzoxazone (50)	Disulfiram	7.78	> 500
3A4	Midazolam (5)	Ketoconazole	0.129	> 500
	Testosterone (100)	Ketoconazole	0.0801	> 500

Source: Covance 8417-482 Report Table 5 and Table 6
Abbreviations: µM – micromolar; CYP450 – cytochrome P450; IC_{50} – half maximally inhibitory concentration; ng/mL – nanogram per milliliter

Ropeginterferon alfa-2b exhibited a time-dependent inhibitory potential on CYP2A6 (37% loss of activity at 50 ng/mL, which is equivalent to the observed C_{max} at the MRHD in PV patients). Drugs with a narrow therapeutic index metabolized by CYP2A6 (e.g. Warfarin) should be administered with caution when co-administered with P1101.

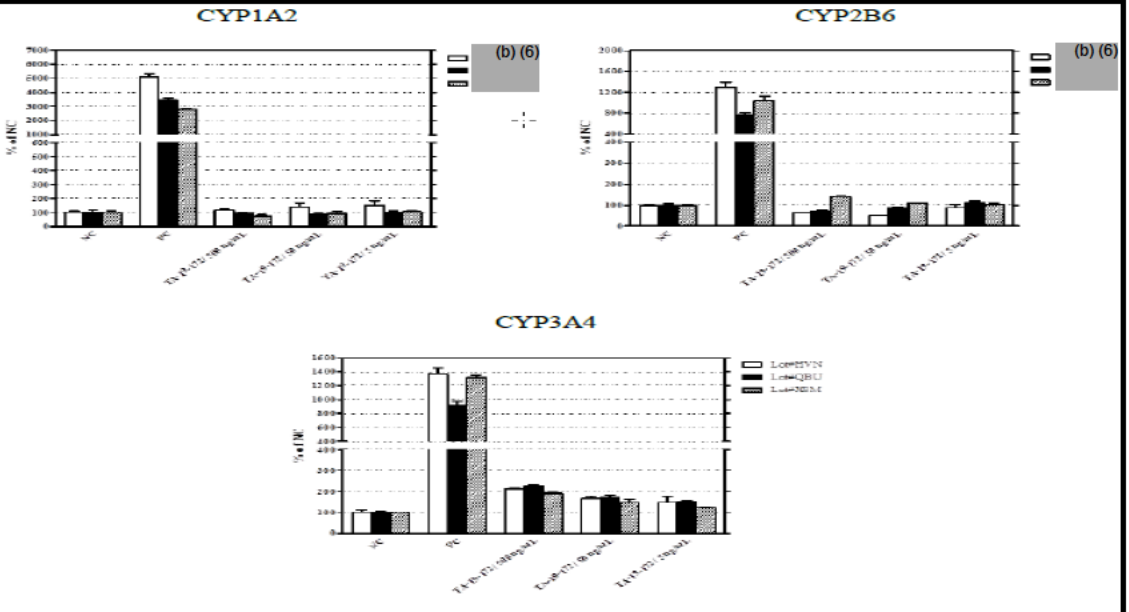
Reduction in CYP Activity in the Presence of Inhibitors and Ropeginterferon Alfa 2b (Study 8417-483)

CYP450	Substrate (µM)	Control Inhibitor (µM)		Ropeginterferon Alfa-2b
		Name	Loss of Activity (%)	Loss of Activity (%) at 50 ng/mL
1A2	Phenacetin (10)	Furafylline (1)	58.2	0.00
2A6	Coumarin (5)	8-Methoxypsoralen (0.075)	63.4	37.3
2B6	Bupropion (50)	Ticlopidine (0.06)	51.1	6.37
2C8	Amodiaquine (0.5)	Gemfibrozil 1-O-β-glucuronide (1)	57.2	0.00
2C9	Diclofenac (10)	Tienilic acid (0.25)	56.1	1.66
2C19	Omeprazole (0.5)	Ticlopidine (0.15)	75.3	0.00
2D6	Dextromethorphan (5)	Paroxetine (0.5)	32.8	0.00
2E1	Chlorzoxazone (50)	Disulfiram (1.5)	28.0	0.00
3A4	Testosterone (100)	Mifepristone (1)	74.2	0.00

Source: Covance 8417-483 Report Table 6 and Table 7
NOTE: Loss of activity (%) was assumed to be equal to zero if the calculated value was negative.
Abbreviations: % - percent; µM – micromolar; CYP450 – cytochrome P450; ng/mL – nanogram per milliliter

Ropeginterferon alfa-2b was not cytotoxic to hepatocytes at concentrations of 5 and 50 ng/mL as the cell viability was ≥ 75% (Study RD-S1909-12). However, ropeginterferon alfa-2b was toxic to hepatocytes at the highest concentration of 500 ng/mL where cell viability was < 75% in 2 of 3 donors (71.6% and 67.3%); cell viability of the 3rd donor was 89.9%. Therefore, the induction potential at 500 ng/mL was considered to be inconclusive in this study although results were included at the HD for completeness. Ropeginterferon alfa-2b (TA-19-172/P01101) demonstrated no induction potential on both enzyme activity and mRNA expression of CYP1A2, CYP2B6 and CYP3A4 at concentrations of ≤ 500 ng/mL in human primary hepatocyte cultures derived from 3 separate donors. The 500 ng/mL concentration of ropeginterferon alfa-2b is approximately 11-fold higher than the observed C_{max} in PV patients (MRHD). Note: The enzyme activity and mRNA expression of CYP2A6 (See Potential DDI, above) were not assessed.

Effects of Ropeginterferon Alfa 2b on Enzyme Activities of CYP1A2, CYP2B6, and CYP3A4 in Primary Hepatocytes (Study RD-S1909-12)



Source: RILD RD-S1909-12 Report Figure 1, Figure 2, and Figure 3
NOTE: (b) (6) represent the 3 human donors.
Abbreviations: % – percent; NC – negative control; ng/mL – nanogram per milliliter; PC – positive control; TA-19-172 – ropeginterferon alfa-2b

6 General Toxicology

Toxicology studies were limited to ≤ 1 month in duration due to the production of ADAs (neutralizing) in monkeys (1 month) and the lack of biologic activity in rats (14 days). (b) (4) .

The toxicity of P1101 was evaluated in cynomolgus monkeys (Pivotal 4-Week, Study 7975-101) and Sprague Dawley (SD) rats (2-Week, non-GLP, Study 7975-100). In addition to the pivotal 4-Week study in monkeys (BIW), two 2-Week bridging studies (Study 7975-107 and Study 44103-08-300, GLP) were conducted to compare different lots of P1101. A non-pivotal dose range-finding study and 14-Day repeat-dose toxicity study was also conducted with PEG-IFNα-2b in cynomolgus monkeys (Study 7975-102, non-GLP).

(Monkey – Repeat-Dose Toxicity)

Common drug-related effects observed in monkeys administered P1101 at doses ≤ 6.75 mg/kg (BIW) included abnormal feces, slightly decreased body weight/body weight gain, decreased food consumption, hematological changes (decreased erythrocytic parameters, platelet reductions and increased APTT), ketonuria and injection site lesions. With the exception of a decrease in ALP at the high dose, clinical chemistry changes were observed at all dose levels and included decreases in total protein, albumin, calcium, triglycerides and phosphorus. These findings are consistent with the administration of human IFN to monkeys.

Platelet counts were notably reduced (↓31% to ↓41%) at the HD (6.75 mg/kg) and correlated with aPTT prolongation in the 4-Week monkey study. As thrombocytopenia is a known adverse effect of IFNα treatment in human subjects, the reduction in platelet counts observed at the HD was considered adverse and related to the administration of P1101.

Results of toxicokinetic (TK) evaluations demonstrated an increase in systemic exposure and C_{max} values for P1101 after the first dose, followed by a general decline as dosing progressed. The TK data is consistent with the presence of anti-drug antibodies (ADAs) demonstrated in the immunogenicity evaluation. Drug-related changes were considered minor, reversible and monitorable and the NOAEL for the 4-Week monkey study was deemed the LD (0.675 mg/kg).

The Sponsor contends that the NOAEL for the pivotal 4-Week monkey study is the HD (6.75 mg/kg) and the NOAEL for the 2-Week non-pivotal monkey study is the MD (2 mg/kg). The 2- and 4-Week monkey studies employed the same doses and dosing schedule and the difference in the NOAEL determination likely stems from the fact that the observations in the pivotal toxicity study were evaluated in the context of their reversibility following the 4-Week recovery period.

Following independent reviews of the pivotal 4-Week toxicity study in monkeys, the nonclinical review teams (DAP and DNH) determined that the NOAEL for this study was best represented by the LD (0.675 mg/kg) based on the findings observed at the MD/HD. Based on the severity of thrombocytopenia at the HD, the Reviewer considers the MD the most clinically relevant NOAEL as this condition is a known adverse effect of PEGylated IFN use in human subjects.

It should be noted that the Sponsor originally considered the (b) (4) but at some point, prior to submitting IND 119047 in 2014, decided to amend the NOAEL to the HD (6.75 mg/mL).

(Monkey - Bridging Studies)

Minor (qualitative/quantitative) differences were observed between the two lots of P1101 (toxicity lot versus clinical lot) when compared in a 2-week monkey bridging study conducted by (b) (4) (7975-107). There were no significant differences detected between the two lots of P1101 when compared in a 2-week monkey bridging study by (b) (4) (44103-08-300).

The observations in the bridging studies were comparable to the pivotal toxicity study, with a few exceptions that occurred exclusively in the (b) (4) (44103-08-300). Administration of 4 subcutaneous doses of P1101 at 6.75 mg/kg led to a decrease in blood pressure and body temperature. Increases in ALT, AST, BUN and BUN/C and decreased total protein, albumin and globulin indicated that P1101 may be affecting liver and kidney function, however these changes occurred in the absence correlative histological findings. Thymic atrophy correlating with gross observations and decreased organ weight was also observed at the HD (6.75 mg/kg).

Additional pathological lesions were noted in the brain, lung, uterus, and stomach from monkeys of Chinese origin that were employed in the (b) (4) Study (44103-08-300). These findings were considered of uncertain relationship to the administration of P1101 and were not observed in the US sourced monkeys used during the pivotal 4-Week toxicity study (appear to be unique to the Chinese source).

(Rat – Repeat Dose Toxicity)

Limited data was collected during the 2-Week (BIW) rat study and based on the absence of adverse effects, the NOAEL for this study was deemed the HD (20 mg/kg). No additional toxicology assessments were conducted in rats due to the lack of biologic activity and this justified the use of a single non-rodent species (Cynomolgus Monkeys) for the toxicological assessment of P1101. The 2-Week rat study supports the absence impurity-related toxicities.

7 Genetic Toxicology

P1101 was negative for mutagenicity in an Ames assay (Study 7975-104). P1101 was found to be negative for inducing chromosomal aberrations in cultured CHO cells without metabolic activation, but equivocal with metabolic activation at concentrations ≥ 795 µg/mL where increased cytotoxicity was noted (Study 7975-105). Considering the reactive linker molecule does not represent a toxicological concern and P1101 will be metabolized by proteases (not CYPs) these assays are considered inappropriate for biologics as these products are not expected to react with genetic material.

8 Carcinogenicity

Long term carcinogenicity studies with P1101 were not conducted due to the lack of pharmacologic activity in rodents. During the pre-BLA meeting (September 2019), the Office of Hematology and Oncology Products communicated to the Sponsor that the completed nonclinical studies (that exclude the completion of a carcinogenicity assessment) would provide the necessary information to support the submission of a BLA.

9 Reproductive and Developmental Toxicology

The Agency agreed during IND development that embryo-fetal and pre- and post-natal developmental studies are not needed since interferons are known to be abortifacient; for example, see Buckley et al. 2008; International Journal of Toxicology, 27: 303-312. Patients administered P1101 will be advised to use effective methods of contraception.

10 Special Toxicology Studies

The Sponsor did not conduct a tissue cross reactivity assay or a cytokine release assay for P1101. Both rats and monkeys have been used in the toxicology studies for IFNα and related compounds (e.g., Pegasys). Based on the comparable toxicology data of P1101 and Pegasys, and the clinical experience of Pegasys and P1101, the nonclinical reviewer (Dr. Shwu-Luan Lee, DHOT) determined that further nonclinical assessment of cytokine release for P1101 would not provide added information for clinical monitoring in PV patients treated with P1101.

Local Tolerance

The local tolerance of P1101 was evaluated within the GLP repeat-dose toxicity studies and were performed with the formulation and route of administration intended for marketing. Hemorrhage (focal/multifocal); infiltrate, mononuclear cell (focal/multifocal); inflammation, granulomatous (focal) were noted at the injection site of monkeys dosed twice a week for 2 weeks and fibrosis/fibroplasia, hemorrhage and degeneration/necrosis (collagen) were observed at the injection site of monkeys dosed twice a week for 4 weeks with P1101.

Infiltrates tended to resolve or diminished and were present with only minimal severity in two HD monkeys following the 4-Week recovery period. The injection site findings observed in monkeys are consistent with the subcutaneous administration of a foreign protein.

11 Integrated Summary and Safety Evaluation

Toxicology studies were limited to ≤ 1 month in duration due to the production of neutralizing ADAs in cynomolgus monkeys (4-Weeks) and the lack of biologic activity in Sprague Dawley rats (14-Days). Drug-related effects observed in the pivotal 4-Week monkey toxicity study were consistent with the known effects of IFN (abnormal feces, slightly decreased body weight/body weight gain, decreased food consumption, hematology (decreased erythrocytic parameters, platelets and increased APTT), clinical chemistry, ketonuria and injection site lesions).

Following independent reviews of the pivotal 4-Week monkeys toxicity study, the nonclinical review teams (DAP and DNH) determined that the NOAEL for this study was best represented by the LD (0.675 mg/kg) based on the findings noted in MD/HD monkeys that were predominantly related to the exaggerated pharmacological effects of IFN. Multiples of exposure (MoE) at the LD compared to the MRHD are provided below.

Comparison of Exposure at the Low Dose (LD) for the Pivotal 4-Week Cynomolgus Monkey Toxicity Study with Clinical Data at the MRHD

Time point (Dose) Sex	C_{max} [ng/mL]	MoE[a]	AUC_{0-t} [ng.hr/mL][a]	MoE[a]
Day 1 (0.675 mg/kg) Males / Females	6488 / 8093	133x / 166x	379403 / 491057	687x / 889x
Sex-Averaged	7291	150x	435230	788x
Day 24 (0.675 mg/kg) Males / Females	1618 / 105	33x / 2x	80138 / 3433	145x / 6x
Sex-Averaged	862	18x	41786	76x

[a] Multiple of Exposure (MoE) was calculated as ratio of serum PK parameters at the low dose (LD) in the pivotal toxicity study (0.675 mg/kg) and at the clinical dose of 450 µg that resulted in the highest measured exposure in humans (C_{max} = 48.64 ng/mL and AUC_{0-t} = 552.57 ng.hr/mL).

Ropeginterferon alfa-2b (P1101) elicited a reduction in platelet counts and a prolongation of aPTT that were considered adverse in HD monkeys. Hence, the MD is considered the most clinically relevant NOAEL as thrombocytopenia is a known adverse effect of PEGylated IFN use in human subjects.

Comparison of Exposure at the Reviewer Derived NOAEL (MD) for the Pivotal 4-Week Cynomolgus Monkey Toxicity Study with Clinical Data at the MRHD

Time point (NOAEL) Sex	C_{max} [ng/mL]	MoE[a]	AUC_{0-t} [ng.hr/mL][a]	MoE[a]
Day 1 (2 mg/kg) Males / Females	28697 / 23128	590x / 475x	1687204 / 1396471	3053x / 2527x
Sex-Averaged	25913	533x	1541837	2790x
Day 24 (2 mg/kg) Males / Females	1670 / 694	34x / 14x	37300 / 17558	68x / 32x
Sex-Averaged	1182	24x	27429	49x

[a] Multiple of Exposure (MoE) was calculated as ratio of serum PK parameters at the Reviewer determined NOAEL (MD) in the pivotal toxicity study (2 mg/kg) and at the clinical dose of 450 µg that resulted in the highest measured exposure in humans (C_{max} = 48.64 ng/mL and AUC_{0-t} = 552.57 ng.hr/mL).

While the Sponsor agreed with the initial nonclinical assessment of the NOAEL (LD) (b) (4) the Sponsor now contends under IND 119047/BLA 761166 (polycythemia vera indication) that the HD represents the NOAEL based on, "the non-adverse and reversible character of the effects observed in monkeys" at all doses. While the Reviewer does not agree with the Sponsors' assessment of the NOAEL the Sponsor provided multiples of exposure (MoE) at the HD are depicted below.

Comparison of Exposure at Sponsor Derived NOAEL (HD) for the Pivotal 4-Week Cynomolgus Monkey Toxicity Study with Clinical Data at the MRHD

Time point (NOAEL) Sex	Cmax [ng•mL]	MoE[a]	AUC_{0-t} [h•ng/mL][a]	MoE[a]
Day 1 (6.75 mg/kg) Males / Females	82713 / 81030	1701 / 1666	4517087 / 4716740	8175 / 8536
Day 24 (6.75 mg/kg) Males / Females	31296 / 44561	643 / 916	789175 / 2549419	1428 / 4614

*Multiple of Exposure (MoE) was calculated as ratio of serum PK parameters at the Sponsor determined NOAEL (HD) in the pivotal toxicity study (6.75 mg/kg) and at the clinical dose of 450 µg that resulted in the highest measured exposure in humans (C_{max} = 48.64 ng/mL and AUC_{0-t} = 552.57 ng.hr/mL).

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/s/

JEFFREY A QUINN
02/01/2021 12:54:31 PM

TODD M BOURCIER
02/02/2021 12:15:07 PM
I concur