Citation: Clin Transl Sci (2020) 13, 807–817; doi:10.1111/cts.12777

ARTICLE

Prediction of Survival Benefit of Filgrastim in Adult and Pediatric Patients With Acute Radiation Syndrome

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Acute exposure to high doses of radiation leads to severe myelosuppression, but few treatments are currently available to treat hematopoietic syndrome of acute radiation syndrome. Granulocyte colony stimulating factors (e.g., filgrastim) stimulate proliferation of neutrophil precursors and enhance mature neutrophil function. Owing to ethical constraints on conducting clinical research in lethally irradiated humans, we developed a model-based strategy to integrate preclinical experience in irradiated nonhuman primates (NHPs) and other clinical myelosuppressive conditions to inform filgrastim dosing to treat hematopoietic syndrome of acute radiation syndrome. Models predicting neutrophil counts and overall survival based on drug exposures were calibrated and scaled from NHPs to adult and pediatric human subjects. Several scenarios were examined investigating variations in filgrastim doses, dose frequency, treatment initiation, and duration, as well as the effect of age and radiation dose rate. Model-based simulations and established safety profiles supported that a subcutaneous filgrastim dose of 10 μg/kg once daily provides a significant survival benefit (50%) over placebo in both adults and children, provided that the treatment is initiated within 1–14 days after radiation exposure and lasts 2–3 weeks. For treatment durations of longer than 3 weeks, filgrastim treatment is not expected to provide significantly greater benefit. This survival benefit is expected to hold for the wide range of radiation doses and dose rates (0.01–1,000 Gy/hours) examined.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ For conditions where human trials are unethical, the animal rule attempts to achieve equivalent exposures in humans as those in the most relevant preclinical species, where desired efficacy was observed.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ How can clinical and preclinical data be utilized to predict survival benefit of filgrastim in the acute radiation syndrome (ARS) setting?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ This study outlines how to use modeling and simulations to extrapolate findings between myelosuppressive disorders (chemotherapy-induced neutropenia to ARS in humans) based on available clinical and preclinical data in both conditions. The model characterized the major aspects of granulopoiesis, bone marrow injury, and filgrastim treatment effects in humans and preclinical species to reasonably predict survival benefit in humans with ARS.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ The methodology in this study demonstrates how more information can be derived from available preclinical and clinical data to provide further support for indications where it is either unethical or impractical to conduct clinical trials.

There is an urgent need to prepare for radiation and nuclear incidents and develop treatments for those exposed to ionizing radiation.[1] However, traditional clinical development paths are neither feasible nor ethical in the setting of acute myelosuppression from radiation exposure that results in hematopoietic syndrome of acute radiation syndrome (HS-ARS), a potentially fatal condition characterized by neutropenia, thrombocytopenia, and anemia.[2] Because of ethical constraints on clinical trials in lethally irradiated humans, approval of a medical countermeasure by the US Food and Drug Administration (FDA) in these cases may be granted under the requirements set forth by the "Animal Rule" (21 CFR 601.90, Subpart H).[3] Accordingly, the FDA relies on data from relevant animal species to provide evidence of treatment efficacy.

Granulocyte colony-stimulating factors (G-CSFs) stimulate the activation, proliferation, differentiation, maturation, and survival of neutrophil precursors in the bone marrow

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Received: September 5, 2019; accepted: January 22, 2020. doi:10.1111/cts.12777

and enhance mature neutrophil functions. Filgrastim and pegfilgrastim are exogenous hematopoietic growth factors with the biological activity of endogenous G-CSF and are used therapeutically for the treatment of chemotherapy-induced neutropenia (CIN) and other indications.[4,5] Several working groups reviewed management of acute exposures of humans and evaluated results of prospective, controlled studies of acutely irradiated animals treated with G-CSFs.[6,7]

G-CSFs bind to a cell surface receptor (G-CSFR) present on cells of the neutrophilic granulocyte lineage. The G-CSF/G-CSFR complex is subsequently internalized and degraded. Filgrastim and pegfilgrastim exhibit complex nonlinear pharmacokinetics (PKs) because they are largely cleared by binding to G-CSFRs and subsequent internalization/turnover on neutrophils. The turnover of receptor-expressing neutrophils contributes to clearance in addition to renal clearance in the case of filgrastim. Moreover, the increase in absolute neutrophil count (ANC) in response to treatment increases the G-CSFRs, amplifying target-mediated effects, suggesting the presence of pharmacodynamics-mediated disposition, which is characterized by nonlinear concentration and time-dependent disposition.[4-5,8-10]

In experiments in rhesus macaques, a well-characterized nonhuman primate (NHP) model of radiation-induced myelosuppression, filgrastim[6] and pegfilgrastim[11] improved survival. Based on this evidence, the expected effects of filgrastim and pegfilgrastim were investigated for the treatment of adults and children at risk of myelosuppression after radiological/nuclear incidents using model-based simulations. These studies have been conducted in accordance with the Animal Rule using data from the NHP model.[3,12]

A semimechanistic model was developed to characterize the time course of changes in ANC after exposure to acute radiation in the presence or absence of filgrastim treatment in the NHP model.[13] The model consists of two submodels: (1) a granulopoiesis model, and (2) an overall survival (OS) model. The relationship between ANC time course and OS in NHPs after radiation exposure in the presence and absence of G-CSF treatment was established, and this constituted the basis of the OS submodel. Additionally, the same granulopoiesis model was used to characterize the PK and ANC time courses in healthy adult humans, as well as adults and children with CIN receiving filgrastim.[14] Because CIN and HS-ARS are myelosuppressive disorders, components in the physiologically driven semimechanistic model can be extrapolated between the two conditions.

The FDA and different working groups based their recommendations for HS-ARS management on results from irradiated animals. This quantitative basis provided solid supportive evidence for selection of filgrastim doses and regimens in humans following acute irradiation exposure.[4,5] In this paper, we describe a model-based strategy that integrates the available nonclinical and clinical information to predict the survival benefit of G-CSF treatment at various doses and regimens in adults and children exposed to acute irradiation. We focus on filgrastim as a model G-CSF for the suggested methodologies. We adapted the granulopoiesis model to predict the time course of ANC in adults and children exposed to acute radiation in the presence and absence of filgrastim treatment. We used the previously developed survival model in NHPs to translate the effects of acute radiation to humans by calibrating radiation effects in NHPs to historical mortality data in humans exposed to myelosuppressive radiation doses. Finally, the HS-ARS full model (granulopoiesis and OS) in humans was used to project the survival benefit of filgrastim in adults and children exposed to acute radiation under different treatment scenarios. These results provided supportive evidence for the FDA approval of filgrastim to treat HS-ARS.[5,12]

1. METHODS

1.1. Simulation models

The development of a population model that integrates the available nonclinical and clinical information and predicts the survival benefit of filgrastim in adults and children exposed to acute radiation was based on three previously developed and validated components: (i) a population PK-ANC model in humans that described the pharmacodynamic-mediated disposition of filgrastim after s.c. administration and its stimulatory effects on granulopoiesis in the absence (adults) or presence (adults and children) of chemotherapy; (ii) an acute radiation effect model in NHPs that quantified the ANC response to lethal doses of radiation; and (iii) an OS model that linked the ANC time course to OS in NHPs. The details of the human PK-ANC in CIN and the PK-ANC-OS model in NHP have been previously described.[13,14] Briefly, the human PK-ANC model was informed by filgrastim dosing in healthy volunteers receiving doses between 75 and 750 μg and 5 μg/kg, adult chemotherapy patients receiving 5 μg/kg, and pediatric patients receiving 5, 10, or 15 μg/kg. NHPs in the pivotal filgrastim study of ARS received 10 μg/kg until recovery.

The model in **Figure 1**[13,14] was used to predict the survival benefit of filgrastim treatment in humans with HS-ARS. As described below, the portion of the model characterizing the effects of chemotherapy on the PK-ANC model developed in humans was replaced by the structural model of radiation effect and merged with the OS model developed in NHPs. To apply the OS model from NHPs to humans, the parameters related to radiation in NHPs were scaled to translate the effects of radiation on NHP granulopoiesis to humans.

1.2. Scaling radiation model parameters from NHPs to humans

The structural model of acute radiation effects developed in NHPs was assumed to describe the acute radiation effect in humans, provided the typical values of the radiation-specific model parameters obtained from NHPs ($k_{PD,e}$ (rate of elimination of the radiation effect), $k_{PD,kill}$ (rate of cell loss due to injury), and GAMMA (sensitivity to radiation)) were scaled to describe the effects of acute radiation on granulopoiesis and survival effects in humans.[13] Given the lack of human ANC data following acute radiation exposure, the acute radiation model parameters derived from NHPs ($k_{PD,e}$, $k_{PD,kill}$, and GAMMA) were calibrated to describe historical mortality data from humans exposed to acute radiation (in the absence of filgrastim) reported in the literature.[15] These data are reproduced with annotations in **Figure 2**.[15] In the first step of the scaling process, the $k_{PD,e}$ and $k_{PD,kill}$ values derived from NHPs exposed to the radiation dose that caused death in 50% of

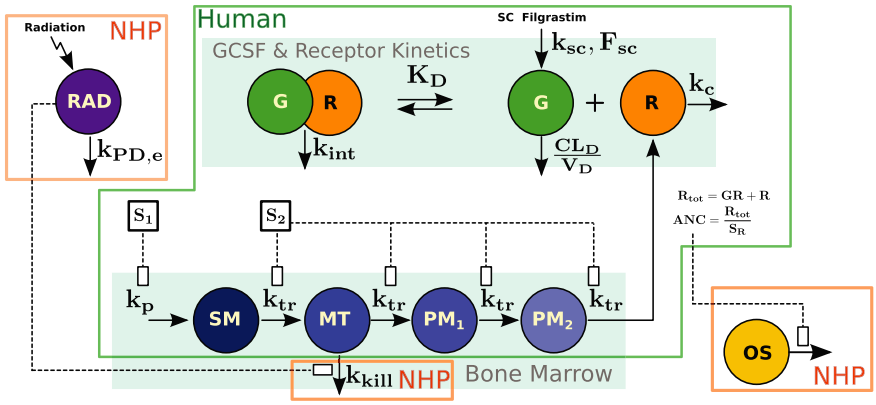

Figure 1 Proposed model for predicting survival in humans with hematopoietic syndrome of acute radiation syndrome. Green region, human model parameters[14]; orange region, parameters scaled from NHP models[13]; small white boxes, modulation of signals due to different interventions or injuries. ANC, absolute neutrophil count; CL_D, filgrastim clearance; F_{sc}, bioavailability; G, free filgrastim; G-CSF, granulocyte colony-stimulating factor; GR, filgrastim/G-CSFR complex; k_c, absolute neutrophil count (ANC) elimination rate; K_D, filgrastim/G-CSFR disassociation constant; k_{int}, filgrastim/G-CSFR complex internalization rate; k_{kill}, rate of cell loss due to injury; k_p, rate of progenitor cell production; $k_{PD,e}$, rate of elimination of the radiation effect; k_{sc}, subcutaneous rate of absorption; k_{tr}, maturation rate; MT, mitotic stem cells; NHP, nonhuman primate; OS, overall survival; PM_1 and PM_2, precursor cells; R, free G-CSF receptor (G-CSFR); R_{tot}, total G-CSFR concentration; S_i, stimulatory functions; SM, progenitor stem cells; S_R, ratio of G-CSFR to ANC values; V_D, filgrastim volume of distribution. .

the exposed population (median lethal dose (LD_{50}); 7.5 Gy at 48 Gy/hours) were adjusted to the human LD_{50} (3 Gy at 1 Gy/hours). A multiplicative factor that yielded mortality estimates of ~ 50% at day 60 in the absence of filgrastim was estimated. Because $k_{PD,e}$ and $k_{PD,kill}$ seemed to be highly correlated in NHPs, the same scaling factor was applied to both parameters. In the second step, GAMMA was empirically adjusted to describe the LD_{50} in humans as a function of radiation rates (1,000 to 0.01 Gy/hours).[15] The relationship between LD_{50} and radiation rate in humans was previously defined as:

$$LD_{50} = 3.0 + \frac{0.072}{DR} \quad (1)$$

where DR is the radiation dose rate in Gy/hours.[15]

Subsequently, the GAMMA value as a function of radiation rate (1,000 to 0.01 Gy/hours) was empirically described to obtain mortality estimates around 50% at day 60 in the placebo arm:

$$GAMMA = GAMMA_{max} \times \left(\frac{DR}{DR + 0.028} \right) \quad (2)$$

The radiation dose rates and corresponding LD_{50} doses used to establish the relationship with mortality were calculated from Eq. 1 (**Table 1**). These simulations were used to calibrate the radiation model parameters. Interindividual variability (IIV) on model parameters was not applied.

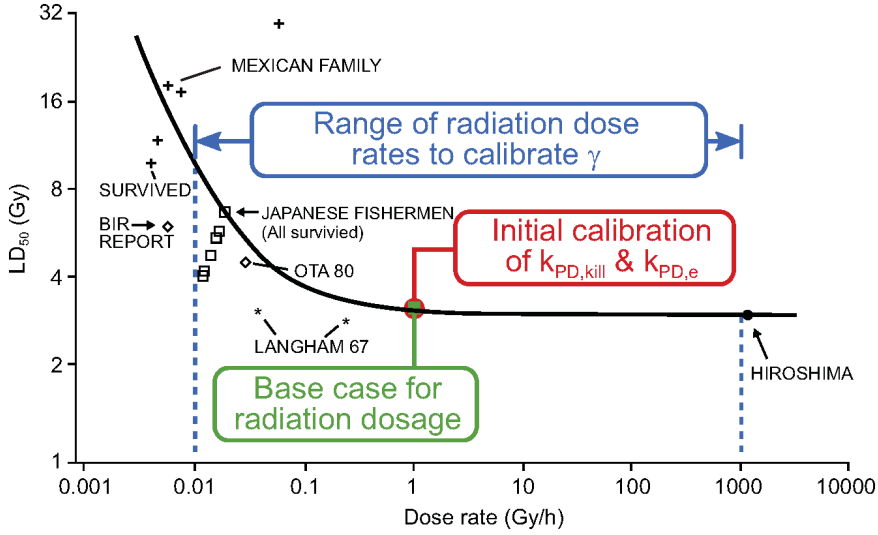

Figure 2 Relationship between radiation LD_{50} and dose rate. BIR Report, British Working Party, 1982; $k_{PD,kill}$, rate of cell loss due to injury; $k_{PD,e}$, rate of elimination of the radiation effect; γ, radiation sensitivity parameter; LD_{50}, median lethal dose; OTA 80, Office of Technology assessment, 1980. Adapted from Scott *et al.*[15] with permission.

Table 1 LD_{50}, radiation duration, GAMMA, and survival in the placebo and filgrastim arms (5 μg/kg q.d. starting 1 day after radiation and lasting for 28 days) for different dose rates of radiation

Dose rate, Gy/hours	LD_{50}[a], Gy	Duration, hours	GAMMA[b]	Survival		RSB
				Placebo	Filgrastim	
1,000	3.00	0.00300	2.20	0.50	0.77	1.52
100	3.00	0.0300	2.20	0.50	0.77	1.52
10	3.01	0.301	2.19	0.50	0.77	1.52
1	3.07	3.07	2.14	0.51	0.77	1.51
0.1	3.72	37.2	1.72	0.54	0.78	1.46
0.075	3.96	52.8	1.60	0.54	0.78	1.45
0.05	4.44	88.8	1.41	0.54	0.79	1.45
0.03	5.40	180.0	1.14	0.52	0.79	1.52
0.025	5.88	235.2	1.04	0.49	0.79	1.59
0.024	6.00	250.0	1.02	0.49	0.78	1.62
0.02	6.60	330.0	0.92	0.45	0.78	1.72
0.015	7.80	520.0	0.77	0.47	0.77	1.64
0.01	10.2	1020	0.58	0.52	0.68	1.30

LD_{50}, dose required to kill 50% of a population; RSB, relative survival benefit.
[a]$LD_{50} = 3.0 + \left(\frac{0.072}{\text{dose rate}}\right)$ Equation per Scott BR, Dillehay LE. *Br. J. Radiol.* 63, 862–870 (1990).
[b]$\text{GAMMA} = \frac{2.2 \times \text{dose rate}}{0.028 + \text{dose rate}}$.

1.3. Simulated treatment scenarios

A base scenario was simulated as a reference to compare different treatment regimens with respect to treatment initiation and duration relative to the radiation exposure, and to explore the effects of changing the filgrastim dose and radiation dose rates on predicted OS in humans. In the base scenario, a radiation dose of 3.07 Gy at 1 Gy/hours occurred on day 0, then one virtual study arm received s.c. filgrastim daily (q.d.) at 5 μg/kg for 28 days starting 1 day after radiation. The other virtual study arm received matching placebo. In each arm, 1,000 virtual adults (weight range, 45–125 kg) were simulated with IIV.

Other relevant scenarios were simulated and compared with the base scenario in adults. The effect of filgrastim on OS with respect to the key elements of the study design were jointly evaluated using filgrastim treatment durations of 1, 2, 3, 4 (base scenario), and 5 weeks; and treatment initiation at 1 (base scenario), 2, 3, 4, 7, 10, 14, 17, and 21 days after irradiation. Additional elements were evaluated: (i) filgrastim s.c. daily dose amounts of 5 (base scenario), 7.5, 10, and 15 μg/kg; (ii) radiation dose rate at LD_{50} of 1 Gy/hours for 3 hours (3.07 Gy; base scenario) vs. 0.024 Gy/hours for 250 hours (6 Gy); and (iii) populations consisting of adults (base scenario) or children in three age groups (1 to < 6, 6 to < 12, and 12 to < 16 years of age). Because weight was a determinant of filgrastim clearance, volume of distribution, and dosing, the body weight corresponding with a specific age was calculated as:

$$\text{Body weight} = (3 \times \text{Age}) + 7 \quad (3)$$

where body weight is in kg, and age is in years.[16]

Serum filgrastim concentrations, ANC, ANC at the effect compartment (ANCe), and survival were simulated daily for 60 days. IIV was applied as described in the population PK-ANC and radiation models. However, during exploratory simulations, numerical difficulties were encountered owing to the high variability in $k_{PD,kill}$ (416%), which may have resulted from very sparse NHP data. Thus, we reduced variability by 50% (208%), which is closer to the IIV in CIN.[14] No residual variability was applied to minimize random noise. To trigger death, the predicted individual probability to survive at each observation time was compared with an individual random value sampled at the start of the study from a uniform distribution (0–1). According to this approach, death occurred when individual random value > predicted individual probability to survive. In addition, no dropouts were allowed in the study (no censoring before day 60).

For each scenario, visual inspections of representative graphical outputs were performed on serum filgrastim concentrations, ANC, ANCe, survival, or other relevant parameters to check validity of outputs. Results were compared between the simulated filgrastim and placebo treatment arms under the same conditions. The following were also summarized or graphically presented: OS, hazard ratio associated with filgrastim treatment relative to placebo, and relative survival benefit (RSB) of filgrastim, defined as the fraction of filgrastim-treated patients surviving relative to placebo at day 60.

1.4. Software

Simulations were performed with Simulo version 5.3.2 (SGS Exprimo NV, Mechelen, Belgium), a JAVA-based software that creates and runs R scripts (version 2.14.2).[17] Simulation outputs were evaluated with R (version 3.1.0; CRAN.R-project.org) running under RStudio (version 0.98.0501) or Microsoft Excel 2013. The Simulo model input is provided in **Table S2**.

2. RESULTS

2.1. Scaling radiation model parameters from NHPs to humans

Considering radiation exposure at the LD_{50} of 3 Gy at 1 Gy/hours in humans, different values of the radiation-specific

parameters, $k_{PD,e}$ and $k_{PD,kill}$, that could lead to a prediction of 50% mortality were tested. A scaling factor of 0.72 for both parameters reasonably predicted 50% mortality in the placebo arm at day 60 and was applied to further simulations. Several empirical relationships between GAMMA and the radiation dose were tested to describe the LD_{50} in humans as a function of radiation rates ranging from 1,000 to 0.01 Gy/hours. Ultimately, the algebraic relationship in Eq. 2 accurately predicted the parameters related to LD_{50} based on the radiation rates.

Table 2 The population pharmacokinetics-absolute neutrophil count,[14] radiation, and overall survival[13] model parameters used for the simulations in adults

Parameter, unit	Mean, SE	95% CI	Description
FSC_{FIL}	1	–	Relative bioavailability after s.c. administration of filgrastim
KSC_{FIL}, hours^{-1}	0.123 (0.0036)	0.116–0.130	Absorption rate after subcutaneous administration of filgrastim
VD_{FIL}, L	3.12 (0.13)	2.87–3.37	Volume of distribution of filgrastim
$\beta VD_{(WT/70)}$	0.943 (0.10)	0.747–1.14	The exponent of the power relationship between normalized weight and drug volume of distribution
CLD_{FIL}, L/hours	0.833 (0.031)	0.772–0.894	Clearance of filgrastim
$\beta CLD_{(WT/70)}$	0.641 (0.10)	0.445–0.837	The exponent of the power relationship between normalized weight and drug clearance
K_P, nM/hours	0.0276 (0.00041)	0.0268–0.0284	Production rate of receptors
K_{TR}, hours^{-1}	0.0330	–	Transit rate between the receptor compartments in the bone marrow
K_C, hours^{-1}	0.120	–	Elimination rate of neutrophils from the blood into the tissues
KD_{FIL}, nM	0.0237 (0.0018)	0.0202–0.0272	Dissociation constant of the filgrastim-receptor complex
STM1	7.53 (0.16)	7.22–7.84	Stimulation of the receptor production rate
$STM2_{PT}$	3.89 (0.064)	3.76–4.02	Stimulation of the transit rate between the receptor compartments in patients with cancer
S_R, recep. 6×10^3/cell	0.0590	–	Scaling factor between receptors and ANC
$KINT_{PT}$, hours^{-1}	0.113 (0.0041)	0.1050–0.121	Rate constant of internalization in patients with cancer
BSLD, nM	0.00299 (9.70×10^{-5})	0.00280–0.00318	Baseline endogenous G-CSF concentration
$K_{PD,e}$[a], hours^{-1} KPD^{-1}	0.0141 (0.00053)[a]	0.0130–0.0153	Rate of elimination of the radiation effect
$K_{PD,kill}$[a], hours^{-1} KPD^{-1}	0.425 (0.11)[a]	0.218–0.879	Rate of cell loss due to injury
GAMMA[c]	2.17 (0.13)[c]	1.910–2.400	Exponent of sensitivity to radiation injury
λ_{ANC}	–2.14 (0.60)	–3.32 to –0.962	Slope relating the hazard to a Box-Cox transformation of the delayed ANC (ANC_e)
k_{e0}, hours^{-1}	0.0278 (0.0016)	0.0247–0.0310	Equilibration rate constant for the ANC effect compartment
λ_{BC}	–0.347 (0.14)	–0.616 to –0.0785	Power parameter of the Box-Cox transformation
Random effects			
Ω_{FSC}	0.440 (0.021)	0.399–0.481	SD of the log-normal interindividual variability in FSC parameter
Ω_{KSC}	0.225 (0.011)	0.203–0.247	SD of the log-normal interindividual variability in KSC parameter
Ω_{VD}	0.282 (0.020)	0.243–0.321	SD of the log-normal interindividual variability in VD parameter
Ω_{CLD}	0.370 (0.021)	0.329–0.411	SD of the log-normal interindividual variability in CLD parameter
Ω_{KP}	0.265 (0.012)	0.241–0.289	SD of the log-normal interindividual variability in KP parameter
Ω_{KD}	0.726 (0.039)	0.650–0.802	SD of the log-normal interindividual variability in KD parameter
Ω_{STM1}	0.315 (0.017)	0.282–0.348	SD of the log-normal interindividual variability in STM1 parameter
Ω_{STM2}	0.273 (0.013)	0.248–0.298	SD of the log-normal interindividual variability in STM2 parameter
Ω_{KINT}	0.570 (0.027)	0.517–0.623	SD of the log-normal interindividual variability in KINT parameter
Ω_{BSLD}	0.260 (0.031)	0.199–0.321	SD of the log-normal interindividual variability in BSLD parameter
$\Omega_{KPD,e}$	0.314 (0.035)	0.246–0.386	SD of the log-normal interindividual variability in $K_{PD,e}$ parameter
$\Omega_{k\ PD,kill}$	4.16[b] (0.58)	3.02–4.97	SD of the log-normal interindividual variability in $K_{PD,kill}$ parameter
$corr_{(KPD,e,\ KPD,kill)}$	0.910	–	Correlation coefficient between the SD in $K_{PD,e}$ and $K_{PD,kill}$ parameters
Exponential residual error model			
$a_{1\ (PK)}$	0.537 (0.0057)	0.526–0.548	Residual error for predicted PK concentration in the log domain
$a_{2\ (PD)}$	0.298 (0.0029)	0.292–0.304	Residual error for the ANC prediction in the log domain

ANC, absolute neutrophil count; CI, confidence interval; G-CSF, granulocyte colony-stimulating factor; PK, pharmacokinetic.
[a]$k_{PD,e}$ and $k_{PD,kill}$ were multiplied by 0.72.
[b]$\Omega_{k\ PD,kill}$ was divided by 2.
[c]GAMMA was adjusted to the dose rate: $GAMMA = \frac{2.2 \times dose\ rate}{0.028 + dose\ rate}$.

The maximum GAMMA was 2.20 and the dose rate that gave 50% of maximum GAMMA was 0.028 Gy/hours. The individual dose rates from 1,000 to 0.01 Gy/hours, corresponding LD_{50} radiation dose rates, and duration (calibration inputs) are listed in **Table 1**. The calculated GAMMA values and predicted survival in the placebo and treated arms were also provided. The scaling function developed for GAMMA resulted in predicted mortality rates in the placebo arm consistent with the LD_{50} (45–54%) over the range of radiation rates investigated. Results also indicated that s.c. filgrastim at 5 μg/kg q.d. starting 1 day postradiation and lasting for 28 days would increase the fraction of patients surviving from 48–54% to 77–79%. The aforementioned scaling factor and function were applied to additional simulations, including IIV in PK-ANC parameters.

2.2. Simulation model parameters

The parameter values used in the simulations for adults are summarized in **Table 2**.[13,14] Despite the limited data in children,[14] PK and pharmacodynamic parameters specific to children were used to predict the benefit of filgrastim treatment.

2.3. Simulated treatment scenarios

Base scenario simulations were used to predict outcomes in cohorts treated with filgrastim or placebo after radiation exposure (**Figure S1**). The rate of cellular loss over time was similar for both cohorts (**Figure S1a**). The difference in the ANC response indicates that treated patients may have a slightly lower nadir, but faster ANC recovery compared with placebo (**Figure S1b**). This limits the duration of severe neutropenia and expected susceptibility to infection. This can be seen by comparing the absolute hazard of death (λ) over time (**Figure S1c**) and hazard as a function of ANC (**Figure S1d**) for the two treatment cohorts. Notably, filgrastim treatment was associated with similar or lower hazard for a given time and ANC value. The hazard was driven by the ANCe, which collapses the hysteresis between the ANC and the hazard. Peak hazard associated with ANCe for the placebo group was predicted to be higher than that for filgrastim-treated subjects (**Figure S1e**). All these features are reflected in the differences in predicted survival between the placebo-treated and filgrastim-treated cohorts (**Figure S1f**). **Figure 3** shows the typical (with uncertainty) Kaplan-Meier survival curves for the placebo and filgrastim treatment arms for the base scenario. The results demonstrate that for this scenario, OS at day 60 is ~ 50% in the placebo arm and 77% in the treatment arm.

The effect of daily s.c. filgrastim on OS of adults receiving a LD_{50} radiation for the different simulated scenarios is presented in **Table 3**, **Figure 4a**, and **Figure S2**. They are presented as a function of filgrastim treatment initiation and duration, filgrastim dose, and radiation dose rate. These results indicate that if filgrastim treatment were limited to 1 week, the greatest benefit would be achieved by administration from days 14–21 postexposure. Moreover, no relevant differences in hazard ratio and RSB were observed among filgrastim doses. However, a slightly higher effect on OS is expected for the dose rate of 0.024 Gy/hours compared with 1 Gy/hours (hazard ratio of 0.318 vs. 0.389; RSB of 1.79 vs. 1.51; **Table 3**). If filgrastim treatment is initiated within the first 4 days postradiation, treatment duration of 2 weeks would result in better survival (**Figure 4a**). However, treatment for 2 weeks might be slightly suboptimal because RSB is slightly lower relative to ≥ 3 weeks.

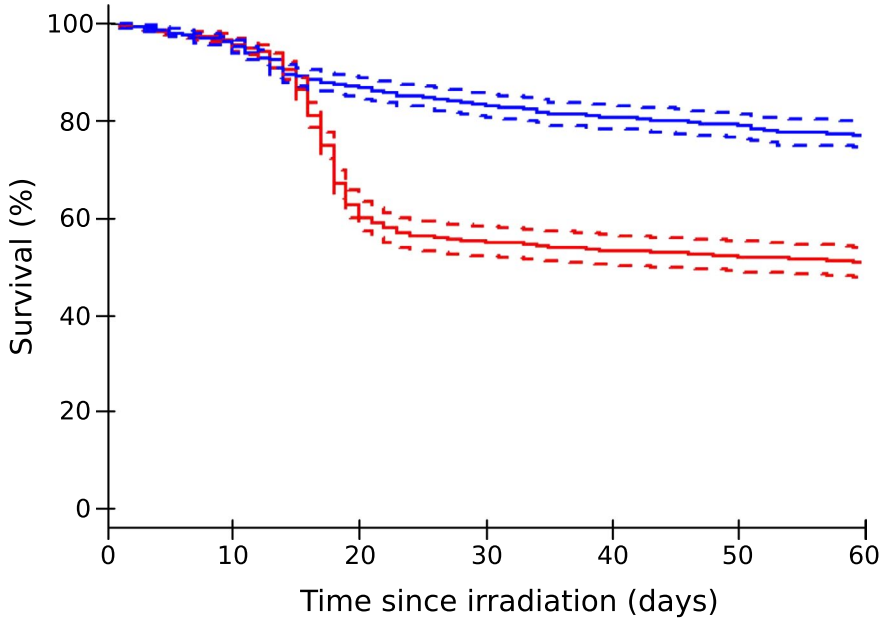

Figure 3 Predicted typical Kaplan-Meier survival curve from a base scenario simulation. Predicted survival of 1,000 human subjects treated with placebo (red line) or filgrastim 5 μg/kg q.d. (blue line) for 28 days starting 1 day after radiation exposure of 3 Gy (1 Gy/hours). Dotted lines, 95% prediction intervals.

Table 3 Hazard ratio and relative survival benefit for different simulation scenarios of adult subjects exposed to lethal radiation and treated with placebo or filgrastim

	Radiation			Filgrastim Treatment					Survival		
Scenario	Dose, Gy	Rate, Gy/hours	Duration, hours	Dose, μg/kg	First dose[a]	Last dose[a]	Duration, weeks	HR	Placebo	Filgrastim	RSB
Base – high rate – 4 weeks	3.07	1	3.07	5	1	28	4	0.389	0.508	0.769	1.51
Base – high rate – 4 weeks	3.07	1	3.07	7.5	1	28	4	0.355	0.508	0.789	1.55
Base – high rate – 4 weeks	3.07	1	3.07	10	1	28	4	0.404	0.508	0.761	1.50
Base – high rate – 4 weeks	3.07	1	3.07	15	1	28	4	0.397	0.508	0.766	1.51
Base – low rate – 4 weeks	6	0.024	250	5	1	28	4	0.318	0.426	0.761	1.79
Base – high rate – 5 weeks	3.07	1	3.07	5	1	35	5	0.389	0.508	0.770	1.52
Base – high rate – 3 weeks	3.07	1	3.07	5	1	21	3	0.421	0.508	0.754	1.48
Base – high rate – 2 weeks	3.07	1	3.07	5	1	14	2	0.485	0.508	0.715	1.41
Base – high rate – 1 week	3.07	1	3.07	5	1	7	1	1.632	0.508	0.361	0.71
Base – high rate – 4 weeks – day 1	3.07	1	3.07	5	1	35	4	0.389	0.508	0.770	1.52
Base – high rate – 4 weeks – day 2	3.07	1	3.07	5	2	36	4	0.400	0.508	0.763	1.50
Base – high rate – 4 weeks – day 3	3.07	1	3.07	5	3	37	4	0.371	0.508	0.774	1.52
Base – high rate – 4 weeks – day 5	3.07	1	3.07	5	4	38	4	0.350	0.508	0.788	1.55
Base – high rate – 4 weeks – day 7	3.07	1	3.07	5	7	41	4	0.297	0.508	0.818	1.61
Base – high rate – 4 weeks – day 10	3.07	1	3.07	5	10	44	4	0.303	0.508	0.815	1.60
Base – high rate – 4 weeks – day 14	3.07	1	3.07	5	14	48	4	0.400	0.508	0.768	1.51
Base – high rate – 4 weeks – day 17	3.07	1	3.07	5	17	51	4	0.666	0.508	0.647	1.27
Base – high rate – 4 weeks – day 21	3.07	1	3.07	5	21	55	4	0.869	0.508	0.560	1.10

HR, hazard ratio; RSB, relative survival benefit.
[a]Day after irradiation.

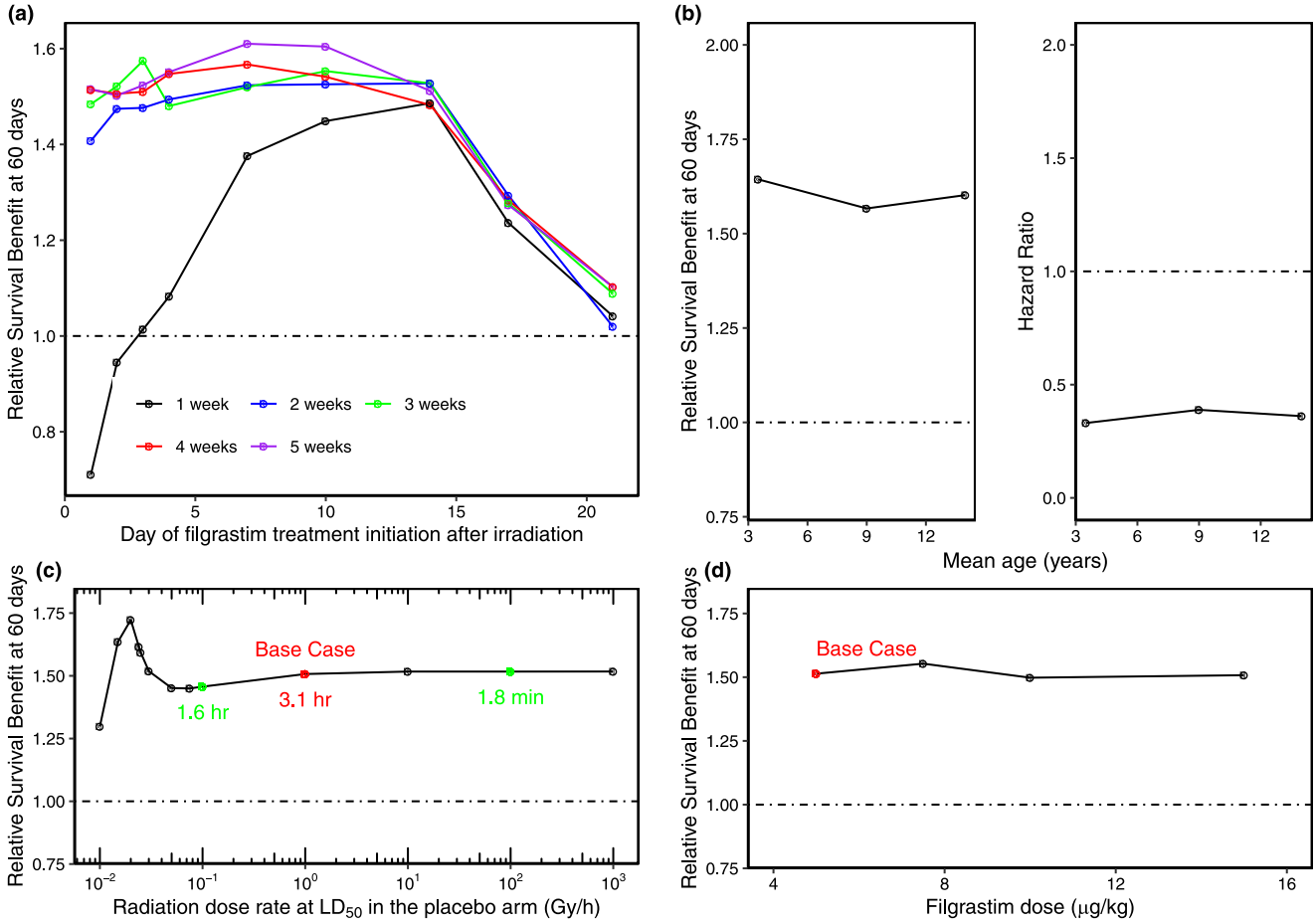

Figure 4 Filgrastim survival benefit. (**a**) Filgrastim relative survival benefit at 60 days after radiation exposure. Survival after a simulated exposure to 3 Gy of radiation at 1 Gy/hours (LD_{50}) as a function of filgrastim dose (5 μg/kg q.d. for 28 days), and treatment initiation (1–21 days after exposure) and duration (1–5 weeks). (**b**) Filgrastim relative survival benefit and survival hazard ratio in pediatric subjects at 60 days after radiation exposure. Simulated benefits of filgrastim 5 μg/kg q.d. for 28 days) in pediatric subjects 60 days after exposure to 3 Gy of radiation at 1 Gy/hours (LD_{50}) as a function of age group (1–6, 6–12, and 12–16 years); relative survival benefit (left) and survival hazard ratio (right). (**c**) Filgrastim relative survival benefit at 60 days after LD_{50} dose of radiation at different radiation dose rates treated with filgrastim (5 μg/kg q.d. for 28 days starting 1 day after radiation). (**d**) Filgrastim relative survival benefit of different filgrastim doses QD for 28 days at 60 days after 3 Gy of radiation at 1 Gy/hours (LD_{50}). LD_{50}, median lethal dose.

Additionally, if filgrastim treatment is initiated within 3 days after the acute radiation exposure, treatment should last ≥ 3 weeks. However, if treatment starts 4–14 days after acute radiation exposure, then it should last ≥ 2 weeks. When filgrastim is initiated 1 day after radiation, treatment > 3 weeks would provide limited additional RSB relative to the 3-week regimen in adults (**Figure 4a** and **Figure S2**). Additionally, initiating filgrastim treatment 15 to 21 days after acute radiation is associated with lower RSB than earlier treatment, as long as the treatment duration is ≥ 2 weeks. Initiating treatment > 21 days after exposure is not associated with an appreciable increase in RSB. As discussed later, to maximize potential benefit, it is recommended to administer 10 μg/kg q.d. as soon as possible following radiation exposure for 2–3 weeks.

The base scenario was also simulated for children in different age categories (**Table S1**; **Figure 4b**). These results indicated that no substantial differences in hazard ratio or RSB are expected among these age categories, relative to adults under the same scenario. Assuming weight is a surrogate for age in children and that no age-specific toxicities would limit dosing, the optimal dosing treatment for filgrastim in adults also applies to children and is dependent on weight, not age. The RSB for varying radiation rates (and corresponding LD_{50} radiation dose) and filgrastim doses relative to the base scenario are shown in **Figure 4c** (**Table 1**), and **Figure 4d** (**Table 3**), respectively. No relevant differences were predicted in the hazard ratio and RSB among the filgrastim doses investigated. However, a slightly higher filgrastim effect on OS could be expected for 0.024 Gy/hours compared with 1 Gy/hours (hazard ratio of 0.318 vs. 0.389; RSB of 1.79 vs. 1.51).

3. DISCUSSION

Over the past 2 decades, information from several international conferences on treatment of acute radiation injury,[18-25] together with preclinical data,[26-30] have provided

valuable information regarding treating patients with ARS. Because of the ethical constraints on prospective, controlled clinical trials in humans with acute radiation injury, several working groups reviewed management strategies for acute exposures of humans and evaluated the results of prospective, controlled studies in acutely irradiated animals. However, for radiologic terrorism events, definitive studies are required in animals to demonstrate impact on mortality and other clinical end points according to requirements for licensure under the FDA's Animal Rule.3

Previously published data suggested that the mean lethal dose of whole-body radiation required to kill 50% of humans at 60 days (LD_{50}/60) without supportive care is 3.5–4 Gy.31 This agrees with the estimated LD_{50} of ~ 3 Gy at 1 Gy/hours identified during parameter calibration based on Scott *et al*.[15] Clinical components of ARS include hematopoietic, gastrointestinal, and cerebrovascular syndromes. The time course and severity of clinical symptoms for these components at different dose ranges have been described previously.[32] Hematopoietic changes peak in untreated patients within 2–3 weeks following exposure to radiation (3–4 Gy). This agrees with our model predictions in the untreated group, where the ANC nadir occurred within 1 week and lasted 2–3 weeks. As stated previously, treated patients may have a slightly lower nadir, but faster ANC recovery relative to the placebo. This limits the duration of severe neutropenia and susceptibility to infections, thus improving survival. The model calibrated for LD_{50} predicted that filgrastim treatment could lead to 50% improvement in OS. The simulated placebo response after parameter calibration suggested ~ 50% OS at the LD_{50} at 60 days, whereas treatment with 5 μg/kg filgrastim q.d. starting 1 day after radiation led to a 50% improvement. These results are consistent with previously reported survival in NHPs[13] and with the known effects of severe neutropenia to increase the risk of life-threatening infection.[33-35] These results support the scaling of radiation model parameters and are in agreement with the well-established mechanism of action of G-CSFs.[36]

The incidence of infection is inversely related to the ANC, and the risk of death is lowest after neutropenia is resolved.[37,38] In NHPs, it was shown that the estimated proportion of the filgrastim treatment effect on OS that may be explained by ANC was 76% (95% confidence interval 41–97%).[13] Starting treatment 1 day after radiation injury and lasting for 3–4 weeks was predicted to coincide with the return of ANC to ~ 2.0×10^9 cells/L in most individuals. Thus, the predicted enhancement of ANC rate of recovery and functionality are expected to result in significant RSB.

The results also suggest that delaying the initiation of filgrastim for ≤ 2 weeks would result in comparable RSB to that from immediately initiating filgrastim following radiation. This prediction is consistent with reports indicating that neutrophil recovery times are similar for both early and delayed treatment with G-CSF or analogues after transplantation.[39-41] Hematopoietic reconstitution has been shown to be possible with partial-body radiation exposure < 2 Gy. Recovery may result from proliferation and differentiation of non-cycling radioresistant stem cells that were spared.[42] These cells may play an important role in hematopoietic recovery a few weeks after exposure to low-to-moderate radiation.[43,44] Accordingly, treatment with filgrastim for < 2 weeks, even when initiated early, would not capitalize on the availability of these recovering cells.

Additionally, for all practical radiation rates resulting in a dose of ~ 3 Gy, no added RSB was predicted by increasing the filgrastim dose. The model predicts full saturation of the G-CSF receptors at 5 μg/kg q.d. and consequently no improvement in ANC recovery at higher doses (**Figure S3**). This is consistent with previous results in both humans and animals demonstrating that the acute effects of radiation on lymphocytes were determined by the cumulative radiation dose, with little contribution from the dose rate.[45] Moreover, simulations suggested that increasing the dose of filgrastim ≤ 15 μg/kg q.d. was associated with marginal improvements in RSB. This is supported by previous findings from chemotherapy in patients with breast cancer, where increasing the daily doses of filgrastim from 5 to 10 μg/kg did not shorten the duration of severe or moderate neutropenia.[46] No differences were observed between the two dose groups in the incidence or duration of hospitalization for toxicities.

In all pediatric age categories exposed to myelosuppressive radiation and treated with 5 μg/kg q.d., simulations predicted slightly better RSB, but were essentially comparable to adults receiving the same dose. This is in agreement with the dosing recommendation for children in nuclear events by the Strategic National Stockpile Radiation Working Group.[32] However, in pediatric patients on chemotherapy, mean time to recovery from neutropenia nadir was 6.6–8.2 days in patients receiving a total of 10 μg/kg q.d. vs. 10.4–10.6 days when treated with 5 μg/kg q.d.[47]

The gravity of injuries associated with HS-ARS necessitates efforts to maximize beneficial effects of G-CSF treatment. In a condition with such serious possible outcomes, marginal benefits from increasing doses in both adults and pediatrics may be life-saving. In bone marrow transplant recipients, the approved dose of filgrastim 10 μg/kg q.d. is associated with an acceptable safety profile.[5] Additionally, per guidance on the Animal Rule,[3] the selection of a human dose usually aims at doses providing filgrastim exposures that exceed those observed in animal efficacy studies. The 10 μg/kg q.d. dose is predicted to provide human filgrastim exposures that are expected to exceed the exposures associated with the same dose in NHPs in the HS-ARS setting.[6] Therefore, to maximize potential benefit of filgrastim treatment in HS-ARS, 10 μg/kg q.d. administered as soon as possible following radiation exposure and lasting for 2–3 weeks (average time to return to healthy ANC levels of 1.0×10^9 to 2.0×10^9 cell/L) is recommended.

Although this semimechanistic analysis derives predictions based on available radiation injury, driving survival based on ANC response to radiation and filgrastim treatment dose represents a simplification. Multiple prognostic factors contribute to OS prediction in different conditions. As related to bone marrow-driven cell lineage changes in many conditions, general leukopenia and/or other white blood cells contribute to prediction of survival (e.g., absolute lymphocyte and monocyte counts) besides predictive value derived from ANCs.[48-50] In addition, data on the rate of fatal infections and other causes of death in subjects who experience bone marrow suppression, especially due to high doses of radiation,

may help support the linkage between different leukocyte changes and OS. The model structure and predictive utility may be modified when/if such data become available.

A population PK/ANC/OS model has been developed to predict the filgrastim survival benefit in adults and children exposed to acute radiation. This expanded model captures the human survival data from Scott *et al*.[15] Based on the assumption that the ANC time course is driving OS after irradiation, simulations suggested that implementing the approved filgrastim clinical dosing regimen for CIN (5 μg/kg s.c. q.d.) would provide a substantial RSB over placebo (> 50%) in both adults and children, provided treatment is initiated ≤ 14 days after radiation exposure and lasts 2–3 weeks. This substantial RSB is predicted to hold for the wide range of radiation dose rates examined. Treatment durations > 3 weeks or higher daily filgrastim doses were not expected to provide additional OS benefit. However, higher doses of 10 μg/kg q.d. have been proven as safe to use in other indications associated with neutropenia. In this study, our model-based simulation methodology along with established safety profiles indicates that an s.c. filgrastim dose of 10 μg/kg daily provides a significant survival benefit (50%) over placebo in both adults and children when it is initiated within 1–14 days after radiation exposure and lasts 2–3 weeks.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

Acknowledgments. Medical writing support was provided by James Balwit, MS, CMPP, Miranda Tradewell, PhD (both Complete Healthcare Communications, LLC, North Wales, PA), whose work was funded by Amgen Inc., and Micah Robinson, PhD (Amgen).

Funding. This work was funded by Amgen Inc.

Conflict of Interest. At the time of analysis, M.M., J.J.P.R, and A.C. were employees of Amgen Inc. J.H. is an employee of Amgen Inc. and owns Amgen stocks. P.O.G. has received consulting fees from Amgen; the current affiliation for P.O.G. is POG Pharmacometrics, Hampshire, United Kingdom. J.J.P.R. was an employee of Amgen Inc. at the time this work was conducted and owns Amgen stock; the current affiliation for J.J.P.R. is Janssen Research & Development, Valencia, Spain. I.D. has received consulting fees from MnS SPRL. A.C. was an employee of Amgen Inc. at the time this work was conducted, owns Amgen stock, and has Amgen stock options; the current affiliation for A.C. is Rigel Pharmaceuticals Inc., South San Francisco, CA. P.J. has received consulting fees from MnS SPRL. M.M. was an employee of Amgen Inc. at the time this work was conducted and owns Amgen stock; the current affiliation for M.M. is Vertex Pharmaceuticals, Boston, MA.

Data Availability Statement. There is a plan to share data. This may include de-identified individual patient data for variables necessary to address the specific research question in an approved data-sharing request; also, related data dictionaries, study protocol, statistical analysis plan, informed consent form, and/or clinical study report. Data sharing requests relating to data in this manuscript will be considered after the publication date and (i) this product and indication (or other new use) have been granted marketing authorization in both the United States and Europe, or (ii) clinical development discontinues and the data will not be submitted to regulatory authorities. There is no end date for eligibility to submit a data sharing request for these data. Qualified researchers may submit a request containing the research objectives, the Amgen product(s) and Amgen study/studies in scope, end points/outcomes of interest, statistical analysis plan, data requirements, publication plan, and qualifications of the researcher(s). In general, Amgen does not grant external requests for individual patient data for the purpose of re-evaluating safety and efficacy issues already addressed in the product labeling. A committee of internal advisors review requests. If not approved, requests may be further arbitrated by a Data Sharing Independent Review Panel. Requests that pose a potential conflict of interest or an actual or potential competitive risk may be declined at Amgen's sole discretion and without further arbitration. Upon approval, information necessary to address the research question will be provided under the terms of a data sharing agreement. This may include anonymized individual patient data and/or available supporting documents, containing fragments of analysis code where provided in analysis specifications. Further details are available at the following: http://www.amgen.com/datasharing

Author Contributions. All authors wrote the manuscript. M.M. and J.H. designed the research. J.H., M.M., A.C., and J.J.P.R. performed the research. J.H., M.M., A.C., and J.J.P.R. analyzed the data.

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