Hematopoietic cells as site of first-pass catabolism after subcutaneous dosing and contributors to systemic clearance of a monoclonal antibody in mice

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ABSTRACT
The neonatal Fc receptor (FcRn) has been demonstrated to contribute to a high bioavailability of monoclonal antibodies (mAbs). In this study, we explored the cellular sites of FcRn-mediated protection after subcutaneous (SC) and intravenous (IV) administration. SC absorption and IV disposition kinetics of a mAb were studied in FcRn transgenic (Tg) bone marrow chimeric mice in which FcRn was restricted to radiosensitive hematopoietic cells or hematopoietic cells. SC bioavailabilities close to 90% were observed in FcRn Tg mice and chimeric mice with high SC bioavailability, whereas SC bioavailabilities were markedly lower when FcRn was missing in hematopoietic cells. Our study demonstrates: 1) FcRn in radiosensitive hematopoietic cells is required for high SC bioavailability, indicating first-pass catabolism after SC administration by hematopoietic cells; 2) FcRn-mediated transcytosis or recycling by radiosensitive cells is not required for high SC bioavailability; and 3) after IV administration hematopoietic and radiosensitive cells contribute about equally to clearance of the mAb. A pharmacokinetic model was devised to describe a mixed elimination via radiosensitive and hematopoietic cells from vascular and extravascular compartments, respectively. Overall, the study indicates a relevant role of hematopoietic cells for first-pass clearance of mAbs after SC administration and confirms their role in the overall clearance of mAbs.

Introduction
Numerous monoclonal antibodies (mAbs) are currently marketed as therapeutic agents for a multitude of diseases and disorders, and many additional mAbs are in various stages of clinical development.1 Subcutaneous (SC) administration, which is more convenient compared to intravenous (IV) administration, has been approved for the delivery of numerous mAbs, including adalimumab, canakinumab, efalizumab, golimumab, ustekinumab, tocilizumab, trastuzumab and rituximab.2 A drawback of SC administration is incomplete bioavailability. Compared to IV delivery, bioavailability for the mAbs mentioned above in humans typically ranges from 50 to 80%, and losses after SC administration range from 20–50%.3,4

The pH-dependent IgG-Fc receptor, FcRn, is centrally involved in the disposition of IgG regardless of the mode of administration. Its classical role in controlling IgG homeostasis within the circulatory system (the central compartment) is well described (see Ref. 6,7 for reviews). Vascular endothelial cells actively engage in fluid phase endocytosis that directs serum proteins to lysosomal degradation. Following pinocytic uptake by such cells, IgG binds via their Fc to FcRn in this slightly acidic endosomal environment (pH 5.8-6.0). FcRn redirects IgG from lysosomal degradation by recycling it to the cell surface, where at neutral physiological pH IgG is released. This process results in an extended serum half-life for IgG of 10–20 days, whereas serum proteins that are not rescued by IgG are rapidly eliminated (half-life of 1–2 days). Accordingly, IgG mAbs are more rapidly cleared from the circulation in FcRn knock-out (ko) as compared to wild-type (wt) mice,5,9 and mAb unable to bind FcRn exhibit similar rapid clearance in wt mice (88.1 vs. 24.2 mL/day/kg for FcRn non-binding and wt anti-body in wt mice).10 Importantly, serum albumin is a second ligand of FcRn.11 FcRn binds and traffics this most abundant serum protein by mechanisms that in many ways parallel those controlling IgG.12,13

It is increasingly clear, however, that FcRn-mediated recycling is not limited to the vascular endothelia. FcRn is also expressed by a variety of epithelial, stromal and parenchymal cells.14–18 FcRn is also expressed by hematopoietic cells derived from the myeloid lineage.14–21 Moreover, bone marrow (BM) chimera studies and conditional FcRn expression studies in mice indicate that such myeloid cells are functionally relevant in that they confer significant levels of FcRn-mediated protection to IgG.14,17,18

FcRn also has been shown in mouse models to enhance mAb bioavailability after SC administration.10,22 The SC bioavailability...
of the mAb 7E3 was found to be markedly lower in FcRn ko compared to wt mice (28.3 ± 6.9% vs. 82.5 ± 15.6%, P < 0.0001).22 Similarly, a mAb defective in mouse FcRn-binding showed a markedly lower SC bioavailability in wt mice compared to a chimeric mAb with mouse IgG2a constant regions that binds mouse FcRn (41.8% vs. 76.3%).10 Pre-systemic catabolism may occur in the local SC tissue or in the draining lymphatics through which mAb absorption after SC administration is generally assumed to occur.5 However, the cell types in which FcRn protects against pre-systemic catabolism are still poorly understood. FcRn-expressing vascular endothelial cells at the administration site may either salvage or transcytose administered mAbs, as has been demonstrated in cultured human endothelial cells.23 Additionally, FcRn-expressing myeloid cells, such as resident macrophages and dendritic cells in the local SC tissue or draining lymphatics, may confer FcRn-mediated protection.

Here, we sought to evaluate the relative contributions of hematopoetic and radioresistant cells (inclusive of endothelial, parenchymal and stromal) in the physiological context of human (h) FcRn to the pharmacokinetic (PK) behavior of a humanized IgG1 mAb administered IV and SC. To do so, we take advantage of BM chimeric, hFcRn transgenic (Tg) mice to restrict the expression of hFcRn to the hematopoietic cells (HC) or radioresistant cells (RRC) compartments and compare the PK behaviors of a humanized IgG1 mAb administered SC and IV. We demonstrate that HC are significant sites of FcRn-mediated mAb protection following SC and IV administration, and they are involved in the first-pass catabolism of following SC administration. In addition, we estimate the contributions of RRC and HC to the clearances of a humanized mAb. Finally, we apply a semi-mechanistic PK model to compare the fractional mAb clearances in RRC and HC.

Results

Validation of bone marrow reconstituted mice

hFcRn Tg32 mice have been shown to approximate the tissue expression patterns of FcRn in normal humans.15,24-26 The PK of mAbs in Tg32 mice have been demonstrated to be predictive for the PK behavior of mAbs in humans.27,28 We used this model for BM reconstitution of recipients whose HC had been irreversibly damaged by lethal irradiation to create mice selectively expressing this hFcRn transgene in the HC or RRC compartments and compare the PK behaviors of a humanized IgG1 mAb administered SC and IV. We demonstrate that HC are significant sites of FcRn-mediated mAb protection following SC and IV administration, and they are involved in the first-pass catabolism of following SC administration. In addition, we estimate the contributions of RRC and HC to the clearances of a humanized mAb. Finally, we apply a semi-mechanistic PK model to compare the fractional mAb clearances in RRC and HC.

Table 1. Scheme for bone marrow (BM) reconstitution to generate mouse cohorts with differing expression of hFcRn.*

| Cohort No. | Recipient of BM graft | BM Donor | Expected hFcRn expression |
|-----------|-----------------------|---------|---------------------------|
| 1         | hFcRn Tg32            | hFcRn Tg32 | Radioresistant somatic/parenchymal cells and BM derived hematopoietic cells (RRC + HC) |
| 2         | hFcRn Tg32            | FcRn ko  | Radioresistant somatic/parenchymal cells (RRC) |
| 3         | FcRn ko               | hFcRn Tg32 | BM derived hematopoietic cells (HC) |
| 4         | FcRn ko               | FcRn ko  | None |

* hFcRn Tg32 homozygotes mice were used.

Plasma concentrations and non-compartmental PK analysis

We then used the reconstituted mice to investigate the extent to which the HC and RRC controlled the PK of a humanized IgG1 (mAb1) after IV and SC administrations. Plasma concentration-time curves of mAb1 after IV and SC administration of mAb1 are shown in Fig. 2 and the corresponding PK parameters from non-compartmental analysis (NCA) are presented in

Figure 1. Confirmation of BM reconstitution. A: FACS analysis of hFcRn expression by blood CD11b+ monocytes. % hFcRn+ cells detected by anti-hFcRn mAb ADM32 is shown. B: Endogenous serum albumin levels of mice cohorts 1–4 determined 12 wks after reconstitution. Errors bars indicate SEM of 16–18 mice per group. n.s., not significant; ***, p < 0.0001 by Tukey’s multicomparison ANOVA.
Tables 2 and 3. After IV administration, disposition of mAb1 was most rapid in cohort 4 animals (no FcRn) and slowest in cohort 1 (hFcRn in HC + RRC), while cohort 2 (RRC only) and 3 (HC only) were in between. Clearance in cohort 4 (no FcRn) was about 12-fold more rapid as compared to cohort 1, with clearances of cohorts 2 and 3 in between. The initial volumes of distribution (Vc) tended to be higher in mice based on FcRn ko mice (cohorts 3 and 4, with cohort 3 expressing hFcRn in HC). The volume of distribution at steady state (Vss) was similar in all cohorts, with values around 100 mL/kg. The different clearances across cohorts were associated with markedly different average terminal half-lives, ranging from 0.62 to 6.8 days in cohorts 4 (no hFcRn) and 1 (hFcRn in HC + RRC).

Following SC administration, mAb1 was detectable in plasma at the first sampling time (1 h) at similar levels (~7–9 μg/mL) in all cohorts, while at the 2 and 7 h time points plasma levels tended to be lower in mice lacking FcRn in HC (cohorts 2 and 4) (Fig. 2C). Maximum average plasma levels in cohorts 1–4 of 100, 29.8, 45.6, and 25.2 μg/mL, respectively, were reached after 24 h in cohort 1 or mostly at 7 h in cohorts 2, 3 and 4. Plasma levels then declined with half-lives similar to those observed after IV administration. SC bioavailability was estimated at 87.6, 39.5, 89.2, and 54.8% in cohorts 1, 2, 3, and 4, respectively. Thus, highest bioavailabilities were observed in cohorts with hFcRn in HC (cohorts 1 and 3).

**Semi-mechanistic pharmacokinetic modeling of mAb1**

The conventional approach for PK analysis by NCA or compartmental PK analysis is based on clearance from the central vascular compartment. The volume of the central compartment for mAbs is usually equal to plasma volume, i.e., the fluid-fraction of vascular space. PK analysis of mAb1 administered IV by NCA indicated similar and additive contributions of HC and RRC to FcRn protection and clearance (for additional details, see Discussion). To further analyze the PK of mAb1 administered IV, we implemented a compartmentalized semi-mechanistic model (Fig. 3). In this model, the clearance via RRC and via HC are organized into central and peripheral compartments, respectively. The best fit (based on Akaike information criteria (AIC)) was

### Table 2. Pharmacokinetic parameters of mAb1 following IV administration of 10 mg/kg mAb1 to mice (from non-compartmental pharmacokinetic analysis; n = 6–8; mean ± SD).

| Parameter | Unit | Cohort 1: Tg32 BM in Tg32 mice | Cohort 2: ko BM in Tg32 mice | Cohort 3: Tg32 BM in ko mice | Cohort 4: ko BM in ko mice |
|-----------|------|-------------------------------|-----------------------------|----------------------------|----------------------------|
| CL        | [mL/day/kg] | 12.9 ± 2.5a | 67.4 ± 18.1b | 98.8 ± 17.0c | 159 ± 24 |
| Vc        | [μg/mL] | 19.4 ± 3.7 | 20.1 ± 5.1 | 30.9 ± 10.2 | 50.5 ± 7.5 |
| Vss       | [μg/mL] | 112 ± 23 | 78.2 ± 19.9 | 102 ± 23 | 103 ± 22 |
| t1/2      | [day] | 6.8 ± 1.1a | 1.2 ± 0.2b | 1.0 ± 0.3c | 0.62 ± 0.10 |
| AUC(0-inf)| [μg h/mL] | 19300 ± 3860 | 3750 ± 840 | 2490 ± 425 | 1530 ± 224 |
| Cmax      | [μg/mL] | 510 ± 104 | 437 ± 72 | 334 ± 111 | 192 ± 29 |

*n = 6; CL: clearance; Vc: central volume of distribution; Vss: volume of distribution at steady state; t1/2: apparent terminal half-life; AUC: area under the plasma concentration-time curve; Cmax: maximum plasma concentration. Statistical comparisons by one-way ANOVA followed by Tukey’s multiple comparison test:

- *p < 0.0001 versus cohorts 2, 3 and 4;
- #p < 0.01 versus cohort 3, p < 0.001 versus cohort 4;
- $p < 0.001 versus cohort 4;
- non-significant (ns) versus cohort 3, p < 0.01 versus cohort 4;
- *p < 0.01 versus cohort 4.
obtained when the volume of distribution of the central compartment was allowed to vary according to the type of chimeric mice. Parameter estimates are presented in Table 4.

As found in non-compartmental PK analysis, the central volume was larger in cohorts lacking FcRn in HC (cohort 3 and 4). Elimination was a slow process, at least 3-fold slower compared to tissue distribution or endocytosis/recycling processes. The apparent endocytosis and recycling rates were in the same order of magnitude for both RRC and HC. A new overlay of observed PK profiles with model simulations is presented at Fig. 4. Overall, all observed profiles are within 5 to 95 percentile of the model projection. In line with this, model-derived areas under the plasma concentration-time curve (AUC) are in good agreement with NCA-derived parameter values (Table 5).

Discussion

The goal of our study was to obtain mechanistic insights into the role of hFcRn in the disposition of a humanized mAb after SC and IV administrations, including its role in first-pass catabolism after SC dosing. We addressed this issue in the physiologically relevant context of human hFcRn rather than mouse FcRn by use of hFcRn Tg32 mice in which hFcRn expression is controlled by its endogenous human regulatory elements. This mouse model has been shown to express hFcRn in a cellular pattern similar to that of humans and to reliably model the PK of therapeutic mAbs in humans.

We utilized BM chimeric Tg32 mice to discriminate the relative contributions of hFcRn in HC and RRC. RRC expressing FcRn, and potentially relevant to mAb clearance, include vascular endothelial cells, liver sinusoidal cells, hepatocytes, kidney podocytes, and proximal epithelial cells. HC expressing FcRn include a variety of myeloid derivatives such as macrophages, dendritic cells, Kupffer cells, monocytes, and neutrophils, all of which express FcRn.

Absorption and disposition after SC administration

To assess the roles of RRC and HC in first-pass catabolism after SC administration, we determined the relative contributions of FcRn in HC and RRC to SC absorption of mAb1 by comparing

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**Table 3. Pharmacokinetic parameters of mAb1 following SC administration of 10 mg/kg mAb1 to mice (from non-compartmental pharmacokinetic analysis; n = 8; mean ± SD).**

| Parameter | Unit        | Cohort 1: Tg32 BM in Tg32 mice | Cohort 2: ko BM in Tg32 mice | Cohort 3: Tg32 BM in ko mice | Cohort 4: ko BM in ko mice |
|-----------|-------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Cmax      | [µg/mL]     | 100 ± 22                      | 29.8 ± 7.5                   | 46.2 ± 18.7                  | 25.2 ± 7.24                  |
| tmax      | [h]         | 24.0 ± 0.0                    | 7.0 ± 0.0                    | 13.4 ± 8.8                   | 7.0 ± 0.0                    |
| t1/2      | [day]       | 4.3 ± 1.5                     | 0.91 ± 0.10                  | 0.78 ± 0.08                  | 0.56 ± 0.4                   |
| AUC(0-inf)| [µg-h/mL]   | 16900 ± 4820                  | 1480 ± 278                   | 2220 ± 790                   | 839 ± 158                    |
| CL/F      | [mL/day/kg]| 15.1 ± 3.6                    | 167 ± 30                     | 119 ± 37                     | 295 ± 53                     |
| F         | [%]         | 87.0 ± 25.0                   | 39.4 ± 7.45                  | 89.0 ± 31.7                  | 54.9 ± 10.4                  |

**Figure 3.** Semi-mechanistic PK model after IV administration of mAb1 in mice.
the bioavailabilities of mAb1 across BM chimeric cohorts. High SC bioavailabilities approaching 90% were found in mice with hFcRn expressed in their HC (cohorts 1 and 3). By contrast, SC bioavailabilities in cohorts lacking FcRn in HC were markedly reduced, with values of 39.5 and 54.8% in cohorts 2 and 4, respectively. These differences are reflected in plasma concentration-time curves during the initial absorption phase, with plasma levels in cohorts 1 and 3 being higher than in cohorts 2 and 4 (Fig. 2C, Table 3). Thus, FcRn in HC of the SC tissue and/or in the draining lymphatics is required for a high SC bioavailability.

The limited contribution of hFcRn in RRC to SC bioavailability argues against the involvement of FcRn-mediated salvage or transcytosis through blood vessel endothelial cells. Paracellular absorption into blood is unlikely because subcutaneous blood capillaries typically have a continuous endothelial structure with tight interendothelial junctions and an uninterrupted base membrane that hampers the transport of large proteins.32 The high bioavailability in mice lacking hFcRn in RRC is also inconsistent with conclusions from modeling of SC absorption of rituximab in standard mice, where a contribution from receptor-medi-

### Table 4. Parameter estimates in final PK model following IV administration of mAb1 to mice (from compartmental analysis).

| Parameter | Units | Fixed Effect | %CV | \( \alpha^2 \) | %CV |
|-----------|-------|--------------|-----|-------------|-----|
| \( V_{V1} \) in Cohort 1 mice | ml.kg\(^{-1}\) | 33.7 | 5 | 0.134 | 33 |
| \( V_{V1} \) in Cohort 2 mice | ml.kg\(^{-1}\) | 50.7 | 6 | 0.161 | 30 |
| \( V_{V1} \) in Cohort 3 mice | ml.kg\(^{-1}\) | 36.8 | 6 | 0.154 | 31 |
| \( V_{V1} \) in Cohort 4 mice | ml.kg\(^{-1}\) | 65.2 | 12 | 0.322 | 26 |
| \( K_{V/Ext} \) | d\(^{-1}\) | 17 | 7 | 0.302 | 17 |
| \( K_{V/Ext/V} \) | d\(^{-1}\) | 13.6 | 6 | 0.07 | x |
| \( K_{V} (End.V) \) | d\(^{-1}\) | 1.68 | 7 | 0.272 | 18 |
| \( K_{V} (End.V) \) | d\(^{-1}\) | 1.48 | 17 | 0.478 | 28 |
| \( K_{V} (Hem.Ext) \) | d\(^{-1}\) | 1.2 | 7 | 0.04 | x |
| \( K_{V} (Hem.Ext) \) | d\(^{-1}\) | 1.48 | 10 | 0.04 | x |
| \( K_{d} \) | d\(^{-1}\) | 0.361 | 14 | 0.53 | 18 |

\( \alpha^2 \): parameter fixed as discussed in Methods

Ext and Dep refers to extravascular and depot compartments.

\( K_{V/Ext} \) and \( K_{Ext/V} \): Distribution rate constant between vascular \( V \) and extravascular Ext compartments.

\( K_{V}, K_{d} \) and \( K_{r} \): Endocytosis, elimination and recycling transfer rate constant.

End and Hem refers to parenchymal/endothelial and hematopoietic cells respectively.

### Figure 4. Observed (mean ± SD) versus predicted (5, 50 and 95 percentile prediction) plasma concentration after IV administration of mAb1 at 10 mg/kg to the different mouse cohorts.

### Table 5. Observed vs. model predicted AUC (mean ± SD).

| Parameter | Unit | Cohort 1 mice: Tg32 BM in Tg32 mice | Cohort 2 mice: ko BM in Tg32 mice | Cohort 3 mice: Tg32 BM in ko mice | Cohort 4 mice: ko BM in ko mice |
|-----------|------|------------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Observed  | AUC(0-inf) IV | \( \mu g.d.mL^{-1} \) | 804 ± 161 | 156 ± 35 | 104 ± 17.7 | 63.8 ± 9.33 |
| Model predicted | AUC(0-inf) IV | \( \mu g.d.mL^{-1} \) | 580 ± 376 | 141 ± 40.8 | 117 ± 37.4 | 52.9 ± 21.8 |
ated transport, i.e., transcytosis presumably via FcRn, was postulated.\textsuperscript{33,34} Moreover, reported data in mice suggest only a very low lymphatic absorption of the SC administered bevacizumab.\textsuperscript{35} Reconciliation of these studies with our findings in support of FcRn-dependent first-pass catabolism by HC might be explained by differences in experimental paradigms, including antibodies tested, human vs. mouse FcRn, or sites of administration.

The high FcRn-dependent SC bioavailability conferred by HC is the summation of HC localized to the SC injection site and/or its lymphatic drainage. HC are present both in SC tissue and the lymphatic system. Macrophages are a major cellular component of the SC tissue\textsuperscript{36} and account for a quarter of the cells in the SC connective tissue of rats.\textsuperscript{36} Dermal and epidermal resident dendritic cells may also migrate to the SC site upon administration.\textsuperscript{37} In addition, loosely fenestrated collecting lymph vessels may permit mAbs to interact with HC in surrounding fatty tissues.\textsuperscript{36} Finally, lymphatic flow is interrupted by lymph nodes that are abundant in HC prior to entering the bloodstream. The extent to which FcRn at each HC site contributes to overall bioavailability remains to be determined.

Our data indicate that the SC first-pass catabolism occurs in HC. HC are not only involved in the local catabolism after SC dosing, but also in the systemic mAb catabolism/clearance after IV administration (see also below). This may explain the observed inverse correlation of SC bioavailability and systemic clearance across several mAbs in the absence of relevant target-mediated disposition in both minipigs and humans, with mAbs having lower systemic clearance exhibiting also lower first-pass catabolism, i.e., a higher SC bioavailability.\textsuperscript{39,40}

There is circumstantial evidence that our observations on the role of HC in SC first-pass catabolism can be translated to humans. The overall and cellular patterns of hFcRn expression in hFcRn Tg32 mice, including skin, blood vessels and lymphatics, parallels those described in humans.\textsuperscript{15} Macrophages are abundant in subcutaneous adipose tissue, accounting for 5–10% of total cells in both lean mice and humans.\textsuperscript{41} The endogenous levels of IgG in hFcRn Tg32 mice do differ from humans (ca. 0.02 mg/mL vs. ca. 10 mg/mL).\textsuperscript{42} That difference does not appear to impinge on the overall clearances of therapeutic mAbs in hFcRn Tg32 mice that correlate well with that found for humans.\textsuperscript{27,28,30,43} Moreover, given these similarities, the clearances at both HC and RRC sites should be affected in a similar manner in both species.

**mAb Disposition after IV administration**

Another goal was to obtain insights into the disposition of mAb1 after IV administration. The disposition kinetics of mAb1 reflects the differences in hFcRn expression across mouse cohorts. In cohort 1 with hFcRn expressed both by HC and RRC, the average clearance was 12.9 mL/day/kg, which is in the range reported for other mAbs in hFcRn Tg32 mice.\textsuperscript{42} The clearance in FcRn ko mice lacking hFcRn on both HC and RRC (cohort 4) was about 12-fold higher compared to cohort 1 expressing hFcRn ubiquitously, inferring that mAb1 undergoes 11 FcRn-mediated recyclings before being cleared.

Comparison of clearance values across cohorts reveals the degree to which hFcRn in HC and RRC affect the clearance of mAb1. The clearance differences between cohort 2 (hFcRn in RRC only) and cohort 3 (hFcRn in HC only) versus cohort 1 reflect the additional clearance from the lack of hFcRn in HC and RRC (54.5 and 85.9 mL/day/kg, respectively). The additional clearances from the lack of hFcRn in HC and RRC, as well as the residual clearance when hFcRn is ubiquitous (cohort 1), add up to a clearance value of 153 mL/day/kg, which is very similar to the clearance in FcRn ko mice (cohort 4) of 159 mL/day/kg (see also Table 7). The similar values indicate that hFcRn protection in HC and RRC are additive. Comparison of the additional clearances from lack of hFcRn in HC and RRC suggests that HC and RRC contribute around 39 and 61%, respectively, to the overall hFcRn protection of mAb1. If we assume the same FcRn recycling efficiency in both cell types and FcRn salvage in all cells involved in IgG clearance, the above contributions to FcRn protection also reflect the combined contributions of HC and RRC to overall IgG clearance.

To compare the findings found here in the hFcRn Tg32 model with BM reconstitution studies in which a mouse IgG1 anti-trinitrophenol (TNP) mAb was administered to mouse FcRn intact wt and FcRn ko BM chimeric mice, we re-analyzed data reported by Akilesh et al.\textsuperscript{14} (Fig. 5). Following intraperitoneal administration, the apparent clearance values (CL/F) were 14.6, 66.5, 60.1, and 100 mL/day/kg in mice with FcRn expression in both HC and RRC, RRC only, HC only, and in neither, (cohorts 1, 2, 3 and 4, respectively) (Table 6). The clearance differences between cohort 1 (mFcRn in both RRC and HC) and cohort 2 (mFcRn in RRC) or cohort 3 (mFcRn in HC), reflect the additional clearance when mFcRn is missing in HC or RRC (51.9 and 45.5 mL/day/kg, respectively). The additional clearances from lack of mFcRn in HC and RRC, as well as the residual clearance when FcRn is ubiquitously expressed (cohort 1), add up to a clearance value of 112 mL/day/kg, which is very similar to the clearance in FcRn ko mice (cohort 4) of 100 mL/day/kg (Table 7). Thus, the partial clearances are roughly additive to match the clearance value in mFcRn ko mice. Comparison of the additional clearances when mFcRn is absent in HC and RRC shows that HC and RRC contribute 53 and 47% respectively, to the overall mFcRn-mediated protection of mouse IgG1 mAb. While it is unclear whether the trend towards a higher contribution from RRC in hFcRn Tg mouse reflects a true animal model difference or rather the experimental differences in dosing route or test compounds, the results for both hFcRn Tg mice and wt mice suggest that HC and RRC contribute about equally to FcRn-mediated protection.

![Figure 5](image-url)
By estimating the quantitative contributions of both HC and RRC to humanized mAb clearance in the hFcRn Tg model, our study expands earlier results comparing FcRn wt and ko mice.14,17,18 HC are present in circulation (e.g., monocytes), in lymphatic and extravascular spaces (e.g., macrophages), but their relative contributions to IgG clearance are unknown. A role for macrophages is supported by studies in which clodronate treatment reduces clearance of a bispecific antibody in cynomolgus monkeys by 30%.44 Standard PK evaluations by compartmental and non-compartmental methods assume elimination from the central compartment and do not consider extravascular elimination. The repercussions of this limitation on volume of distribution estimates have already been discussed elsewhere.55,56 The data from our study support the importance of "normal" catabolic elimination of mAbs exterior to the central vascular compartment.

In a further step, analysis of the experimental data was refined using a semi-mechanistic PK model, linking the PK to interactions of mAb1 with FcRn on HC and RRC. This model connects the above results to a rapidly growing body of mathematical models capturing the IgG-FcRn interaction and the IgG dispositions of mAb1 with FcRn on HC and RRC. This model connects using a semi-mechanistic PK model, linking the PK to interactions between HC and RRC.

### Materials and methods

#### Materials

mAb1 was produced at Roche Diagnostics GmbH (Penzberg, Germany). It was produced in Chinese hamster ovary cells and purified by standard methods. The test substance was formulated as an aqueous buffer solution at a concentration of 25.6 mg/ml. Purity was > 99.2% as determined by size exclusion chromatography and SDS-PAGE. This formulation was used as such for IV bolus and SC administration to mice. MAb1 is a humanized IgG1 antibody that recognizes the human insulin-like growth factor 1 receptor (IGF-1R), but does not cross-react with the murine IGF-1R (F. Hoffmann-La Roche, data on file).

#### Animals

The study was conducted using female mice lacking the mFcRn α-chain (B6.129 × 1-Fcgrt<sup>tm1Dcr</sup>/Dcr); abbreviated mFcRn ko).

| Parameter | Unit | Cohort 1 mice: Thy1.1 BM in Thy1.1 mice | Cohort 2 mice: ko BM in Thy1.1 mice | Cohort 3 mice: Thy1.1 BM in ko mice | Cohort 4 mice: ko BM in ko mice |
|-----------|------|---------------------------------------|-------------------------------------|-------------------------------------|--------------------------------|
| CL/F | [mL/day/kg] | 14.6 | 66.5 | 60.1 | 100 |
| Vz/F | [mL/kg] | 174 | 274 | 225 | 200 |
| t1/2 | [day] | 8.26 | 2.86 | 2.59 | 1.39 |
| AUC(0-inf) | [(μg-h)/mL] | 6580 | 1440 | 1600 | 960 |
| Cmax | [μg/mL] | 29.3 | 14.4 | 18.0 | 17.3 |

Table 6. Pharmacokinetic parameters of anti-TNP-specific IgG (mAb 187.11) following intraperitoneal administration at a dose of 4 mg/kg to B6.PL-Thy1a/CyJ (Thy1.1), and C57BL/6J FcRn knock-out (ko) mice as well as chimera thereof (from non-compartmental pharmacokinetic analysis of average concentration-time profiles of 10 to 12 mice/dose group) (Data from14).

Table 7. Additivity of FcRn protection in hematopoietic and radioresistant cells after IV administration: For all test compounds clearance in FcRn ko mice resembles the sum of clearance with full FcRn expression and the additional clearances from the lack of FcRn in hematopoietic and radioresistant endothelial/somatic cells.

| Mouse model | Test compound | Average clearance in test system with full FcRn (mL/day/kg) | Average additional clearance (mL/day/kg)* from lack of FcRn in Hematopoietic cells<sup>1</sup> | Average additional clearance (mL/day/kg)* from lack of FcRn in Radioresistant cells<sup>1</sup> | Sum of clearance with full FcRn and additional clearances from lack of FcRn (mL/day/kg) | Average clearance in FcRn ko mice (mL/day/kg) |
|-------------|--------------|----------------------------------------------------------|-----------------------------------------------------------|-----------------------------------------------------------|-----------------------------------------------------------|---------------------------------------------|
| hFcRn Tg32  | mAb1         | 12.9                                                     | 54.5                                                      | 85.9                                                      | 153                                                       | 159                                         |
| C57BL/6    | anti-TNP mAb | 14.6                                                     | 51.9                                                      | 45.5                                                      | 112                                                       | 100                                         |

*estimated from differences of clearance in cohort 2 and cohort 3 vs. cohort 1.
and female B6.Cg-Fcgrt$^{tm1Dcr}$ Tg(FCGRT)32Dcr/DcrJ mice (abbreviated hFcRn Tg32). Tg32 mice carry a ko allele of the FcRn α-chain and are homozygous for a human FcRn α-chain genomic transgene under control of its human promoter, as described. All mice were bred at the Jackson Laboratory (Bar Harbor, USA) and are available from The Jackson Laboratory (strain numbers 003982 and 014565). Experiments were performed under Protocol 01022 approved by the Institutional Animal Care and Use Committee of The Jackson Laboratory.

**Methods**

**Bone marrow transplantation**

Groups of mFcRn ko and hFcRn Tg32 homozygous mice were given two equal doses of irradiation for a total lethal dose of 12 Gy (1200R) to eliminate HC. Within 3 h, the irradiated mice were then given IV injections of $5 \times 10^6$ bone marrow (BM) cells from either FcRn/-/- and hFcRn Tg32 mice according to the scheme shown in Table 1. Twelve weeks after BM transfer, expression of hFcRn was determined by FACS analysis on blood CD11b$^+$ leukocytes of recipient mice using the human FcRn-specific mAb ADM32 to monitor the extent of chimerism. MSA quantification was performed on plasma from retroorbital blood by a CAS clinical chemistry AU680 analyzer.

**Pharmacokinetic study with mAB1**

mAb1 was administered by IV or SC bolus injections at 10 mg/kg (each) in dose volumes of 10 ml/kg to mouse cohorts 1–4 16 weeks after BM transfer (8 mice of ca. 20 g body weight per dose group). IV administration was by tail vein injection and SC administration was by tail vein injection and SC administration. In IV administration, the mice were bled after BM transfer (8 mice of ca. 20 g body weight per dose group). SC administration, expression of hFcRn was determined by FACS analysis on blood CD11b$^+$ leukocytes of recipient mice using the human FcRn-specific mAb ADM32 to monitor the extent of chimerism.56 MSA quantification was performed on plasma from retroorbital blood by a CAS clinical chemistry AU680 analyzer.

**Data analysis**

**Non-compartmental PK analysis**

The plasma mAb concentration-time data following IV or SC administration of mAb1 were analyzed by standard NCA using ToxKin$^\text{®}$ (version 3.5, Entimo, Berlin, Germany). PK data were calculated for each mouse using individual plasma concentration-time data. After SC administration in cohort 1, some mice plasma concentration-time data later than 336 or 504 h were markedly lower than projected from the log-linear decline of plasma levels, thus reflecting an accelerated clearance probably due to formation of anti-drug antibodies (not measured). These time points were excluded from PK evaluation. SC bioavailabilities of individual mice were calculated using average AUC (0-inf) values for each cohort after IV administration.

**Statistical evaluation of PK parameters**

One-way ANOVA of PK parameter logarithms followed by Tukey’s multiple comparison test was performed using significance at a p value $<0.05$. For reasons of brevity, only statistical information is presented for clearances and half-lives after IV administration and for Cmax and bioavailabilities after SC administration.

**Semi mechanistic PK model**

A semi-physiologic PK model presented in Fig. 3 was used to describe observed plasma PK data. This model assumes linear processes and was originally developed for compounds without relevant target binding. In the following equations, “A” is amount of compound and indices refer to compartment as described in Fig. 3. Transfer rates are all first order process. The following equations describe compound amount change in each compartment after IV administration when FcRn is present:

$$
\frac{dA_1}{dt} = -\left(K_{V/Ext} + K_e (End, V) \right) \times A_1
+ K_{Ext/V} \times A_2 + K_r (Ext, V) \times A_3 + iv(t) A_1(0) = 0
$$

$$
\frac{dA_2}{dt} = K_{V/Ext} \times A_1 + K_3 \times A_3 + \left(K_{Ext/V} + K_e (Hem, Ext) \right) \times A_2
+ K_r (Hem, Ext) \times A_4 A_2(0) = 0
$$

$$
\frac{dA_3}{dt} = K_e (End, V) \times A_1 - \left(K_d + K_r (End, V) \right) \times A_3 A_3(0) = 0
$$

$$
\frac{dA_4}{dt} = K_e (Hem, Ext) \times A_2 - \left(K_d + K_r (Hem, Ext) \right) \times A_4 A_4(0) = 0
$$

Following IV administration, the compound was delivered into the vascular compartment ($A_1$) as defined elsewhere. iv(t) is the input function after IV administration. It distributes to extravascular compartment ($A_2$) with rate constant $K_{V/Ext}$ and $K_{Ext/V}$. Endosomal elimination is assumed to occur in Endothelial ($A_3$) and Hematopoietic ($A_4$) cells in the vascular and extravascular compartments, respectively. Endocytosis, elimination and recycling transfer rates are $K_e$, $K_d$ and $K_r$ parameters, respectively. Elimination rate transfer is assumed identical whatever cell type and location. Endocytosis and recycling transfer rates were differentiated according to cell type (End and Hem for endothelial and hematopoietic cells respectively) and location (Ext for extravascular compartments). Recycling is assumed to not occur in FcRn ko cells (no $K_r$ parameter estimated).
The observed plasma concentrations were fitted to a $\frac{V_t}{V_c}$ ratio where $V_t$ is volume in the vascular compartment. Type of mice was considered as a covariate of the vascular volume.

For data analysis, PK parameters were estimated simultaneously in each experimental cohort based on their plasma exposure using a population approach in Monolix® v.433s.59 Parameters were assumed to be log-normally distributed with fixed effect and random effect parameters representing population typical value and inter-individual variability, respectively. An exponential model described the log-normal distribution of random effect parameters with mean zero and variance $\sigma^2$. Residual error model was assumed to be proportional with mean zero and variance $\sigma^2$. Parameter estimates were considered as acceptable when the coefficient of variation was below 40% for typical values, and any random effect above this cut-off value is fixed to the estimated value. Model performance was assessed via visual inspection of diagnostic plots (Residuals, Observed versus Predicted and Visual Predictive Check plots) on population predictions. The AIC was used to discriminate covariate model on vascular volume.

Simulated profiles were generated in Matlab v.2013b60 and 5, 50 and 95 percentiles were extracted from 1000 random sample drawn from population analysis and using the Matlab statistical toolbox.

### Abbreviations

- **AIC**: Akaike information criteria
- **AUC**: area under the plasma concentration-time curve
- **BM**: bone marrow
- **CL/F**: apparent clearance
- **FACs**: fluorescence-activated cell sorting
- **FcRn**: neonatal Fc receptor
- **HC**: hematopoietic cells
- **IGF-1R**: insulin-like growth factor 1 receptor
- **IV**: intravenous
- **mAb**: monoclonal antibody
- **MSA**: mouse serum albumin
- **NCA**: non-compartmental analysis
- **PK**: pharmacokinetic
- **RRC**: radioresistant cells
- **SC**: subcutaneous
- **Tg**: transgenic
- **TNP**: trinitrophenol
- **Vc**: initial volume of distribution
- **Vss**: volume of distribution at steady state
- **wt**: wild-type

### Disclosure of interest

WFR, NF and HPG are employees of F. Hoffmann-La Roche Ltd; DCR, GJC and GP are or were employees of the Jackson Laboratory. DCR is an inventor on certain patents related to hFcRn transgenic mice. Other authors report no conflict of interest.

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