Functional humanization of immunoglobulin heavy constant gamma 1 Fc domain human FCGRT transgenic mice

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ABSTRACT

A major asset of many monoclonal antibody (mAb)-based biologics is their persistence in circulation. The MHC class I family Fc receptor, FCGRT, is primarily responsible for this extended pharmacokinetic behavior. Engagement of FCGRT with the crystallizable fragment (Fc) domain protects IgG from catabolic elimination, thereby extending the persistence and bioavailability of IgG and related Fc-based biologics. There is a need for reliable in vivo models to facilitate the preclinical development of novel IgG-based biologics. FcRn-humanized mice have been widely accepted as translationally relevant surrogates for IgG-based biologics evaluations. Although such FCGRT-humanized mice, especially the mouse strain, B6.Cg-Fcgrtm1Dcr Tg(FCGRT)32Dcr (abbreviated Tg32), have been substantially validated for modeling humanized IgG-based biologics, there is a recognized caveat—they lack an endogenous source of human IgG that typifies the human competitive condition. Here, we used CRISPR/Cas9-mediated homology-directed repair to equip the hFCGRT Tg32 strain with a human IGHG1 Fc domain. This replacement now results in mice that produce human IgG1 Fc-mouse IgFab chimeric antibodies at physiologically relevant levels, which can be further heightened by immunization. This endogenous chimeric IgG1 significantly dampens the serum half-life of administered humanized mAbs in an hFCGRT-dependent manner. Thus, such IgG1-Fc humanized mice may provide a more physiologically relevant competitive hFCGRT-humanized mouse model for the preclinical development of human IgG-based biologics.

Introduction

Monoclonal antibody (mAb)-based biologics are among the fastest growing and most promising treatments for a broad range of human disorders, including cancer, autoimmune and inflammatory diseases, diabetes, respiratory diseases, and ophthalmologic diseases. A major asset of many mAb-based biologics is its persistence in circulation (~21-day serum half-life in humans). This extended pharmacokinetic (PK) behavior requires the crystallizable fragment (Fc) domain. For this reason, the majority of therapeutic mAbs, as well as many other protein-based biologics, include the human immunoglobulin G (IgG) Fc domain.

The Fc receptor of IgG was originally described as the neonatal Fc receptor (FcRn). This unusual MHC class I family Fc receptor is, however, used throughout life and is now named ‘Fc fragment of IgG receptor and transporter’ (FCGRT). It is primarily expressed in the early endosomes of cells within the circulatory system and is engaged in fluid-phase endocytosis. IgG-Fc binds the Fc of IgG at acidic pH, rescuing it from normal lysosomal catabolism by redirecting it to the plasma membrane, which, upon encountering a neutral pH, is released back into the circulation. This mechanism greatly slows IgG destruction and is the primary mechanism for the extended persistence and bioavailability of IgG (reviewed in Ref.2,3). Appreciation of FCGRT salvage has stimulated the redesign of many IgG-based biologics with Fcs engineered to enhance their persistence.4–6

However, the PK behavior of novel IgG-based biologics is still unpredictable, with modifications to either the Fab or Fc domains requiring extensive in vivo validation. Thus, the ability to relevantly and economically assess their PK in vivo is a critical aspect of the preclinical development of new IgG-based biologics. In vivo testing of mAb-based biologics in standard rodent models falls short in translational relevance owing to species differences between mouse and human FcRn.7–10 Non-human primates, e.g., cynomolgus monkeys, do provide a close evolutionary surrogate, but their use is encumbered by practical and ethical concerns. To overcome these, we have previously described FCGRT-humanized mouse models that serve for preclinical evaluations of IgG-based biologics in vivo. In particular, the mouse strain B6.Cg-Fcgrtm1Dcr Tg(FCGRT)32Dcr/DcrJ (abbreviated here to Tg32), which carries a null mutation for the mouse Fcgr a-chain gene and a transgene expressing the human FCGRT a-chain transgene under the control of its natural human promoter, has been rigorously validated and is regarded to be the most reliable non-primate surrogate for preclinical testing of IgG-based biologics.9–11–14

Although such FCGRT-humanized mice have been substantially validated for modeling of humanized IgG-based biologics, they lack an endogenous source of human IgG that typifies the human competitive condition. Of particular relevance here is that FCGRT trafficking is saturable. It operates with high efficiency when serum IgG concentrations are low, but declines asymptotically when serum IgG is in excess. This
phenomenon is referred to as the concentration–catabolism effect in which the persistence of therapeutic mAbs is inversely correlated with the endogenous levels of competing endogenous IgG. Preexisting FcRn-humanized models have very low levels of endogenous mouse IgG due to the inability of FcRn to effectively bind and recycle mouse IgG. Thus, PK assessments of human IgG-based biologics in such FcRn-humanized mice lack competing human IgG required for evoking the concentration–catabolism effect. While preloading mice by using intravenous immunoglobulin (IVIg) has been employed as a means to provide a bolus of competing human IgG, an endogenous source of humanized IgG will provide a more appropriate physiological context.

To address this need, we used CRISPR/Cas9-mediated homology-directed repair (HDR) to further engineer the hFCGRT Tg32 strain, replacing the mouse hinge and Ighg1 Fc domain with the equivalent human IgG1 domains. The replacement resulted in the mouse strain, B6.Cg-Fcgr1tm1Dcr Ighg1tm2(IGHG1)Mvw Tg(FCGRT)32Dcr/Mvw (abbreviated to Tg32-hFc) that produce IgG1 Fc-mouse IgG Fab2 chimeric antibodies in serum concentrations at physiologically relevant levels, which can be further heightened by immunization. We show that the endogenous production of the chimeric IgG1 significantly dampens serum half-life of administered humanized mAbs in an FCGRT-dependent manner consistent with an appreciable concentration-competition effect. Thus, such IgG1-humanized, FCGRT humanized mice provide a more physiologically appropriate mouse model for the preclinical assessment of human IgG-based biologics.

Results

Construction of Tg32-hFc model

To provide a more relevant humanized IgG recycling environment, we genetically modified the Tg32 mouse strain to produce human Fc-mouse Fab2 chimeric IgG1 antibodies. The modification strategy outlined in Figure 1 uses CRISPR/Cas9-mediated HDR leading to a switching out of the mouse Ighg1 locus hinge (Exon 2), CH2 (exon 3) and CH3 (exon 4) regions replacing it with the human equivalent regions while preserving splice junctions (Figure 1a). From 95 micro-injected embryos, six survived to term (3 F, 3 M). Long-range PCRs extending beyond the homology arms identified two females as having transgene replacement. These were backcrossed to the parental Tg32 strain. While both candidates faithfully transmitted the transgenic allele, a single strain was ultimately established: B6.Cg-Fcgr1tm1Dcr Ighg1tm2(IGHG1)Mvw Tg(FCGRT)32Dcr/Mvw, abbreviated here to Tg32-hFc. This strain was brought to homozygosity and characterized in depth, including sequence on the modified region; see Supplemental Figure 1.

To confirm that the modified gene is appropriately transcribed and spliced, RNA was isolated from Tg32-hFc spleen and subjected to RT-PCR and Sanger sequencing. Analysis of the resulting sequence products demonstrated that the chimeric mouse-human in-frame mRNA spliced as predicted (Figure 1b). Two isoforms were detected, with and without sequences encoding the transmembrane and cytoplasmic tail domains; see Supplemental Figure 2. These data demonstrate that the Ighg1 locus has undergone a functional modification and now expresses an Ighg1 chimeric mRNA predicted to give rise to intact human Fc and/mouse (Fab2) IgG1 antibodies.

Serological analysis of naive and immunized Tg32 and Tg32-hFc mice

To determine if the targeting strategy resulted in chimeric IgG1, we determined the levels of chimeric IgG1 plasma in naïve and immunized Tg32 and Tg32-hFc mice. Naïve Tg32-hFc mice yielded chimeric IgG1 levels that ranged from 280 to 1,320 μg/ml (mean 600 μg/ml) and following immunization of Tg32-hFc mice with 2.4-dinitrophenyl-keyhole limpet hemocyanin (DNP-KLH), chimeric IgG1 was significantly increased (mean value 4,200 μg/ml) (Figure 2a). Consistent with the targeting strategy, chimeric IgG1 was not detected in Tg32

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**Figure 1.** (a) Targeting strategy to create Tg32-hFc mouse strain. Two sgRNAs 1652 and 1630 were combined with Cas9 in the presence of human donor DNA to mediate HDR replacement of mouse Igh1 exons 2-4 with the human equivalent, whilst maintaining splice acceptor and donor sites; see Supplemental Figure 1. Mouse segments in blue, human in green. Correct integration and expression are predicted to give two chimeric mRNAs isoforms. CH1, constant heavy chain 1; CH2, constant heavy chain 2; CH3, constant heavy chain 3; Fc, fragment crystallizable region. (b) Shows predicted mRNAs from the region which were verified by RT-PCR, amplicons generated from the cDNA template used a common forward primer targeting the mouse Ighg1 and a reverse primer specific to the 3’ UTR of each variant; note, both chimeric isoforms were found and their correct splicing and predicted sequence confirmed; see Supplemental Figure 2. Mouse regions shown in blue, human in green. (c) Schematic of the predicted chimeric IgG1 antibody, with the human hinge and Fc (CH2 and CH3) domains (in green).
mice (Figure 2a). We additionally assessed the antigen specificity of chimeric IgG developed by the immunized Tg32-hFc mice. Robust activity was evident even at a 1/25,000 dilution, indicating that the human genetic substitution did not impede the ability of the locus to elicit a strong antigen-specific chimeric IgG1 response after immunization (Figure 2b).

We also determined the impact of the modified Ighg1 locus on the production of mouse FCGRT-dependent (IgG1, IgG2a, IgG2b) and mouse FCGRT-independent (IgM and IgA) immunoglobulins from the same mice as well as Fcgrt KO mice. As expected for the targeted genetic modification, we did not detect fully mouse IgG1 in naïve or immunized Tg32-hFc mice, while significant but low levels of mouse IgG1 (averaging 17 μg/ml) were detected in naïve Tg32 mice (Figure 2c). Furthermore, immunization of Tg32-hFc mice leads to significant increases in mouse IgM, IgM IgG2a, IgG2b and IgA, indicating that the modified Ighg1 locus did not negatively impact the overall antibody response (Figure 2d). The data indicated that the hFc knock-in strategy resulted in Tg32 mice that undergo class switch, affinity maturation and produce antibodies that include chimeric IgG1. Moreover, the appreciable plasma concentrations of chimeric IgG1 found in naïve and immunized Tg32-hFc mice, ~600 μg/ml and ~4,200 μg/ml, respectively, provide strong evidence that chimeric IgG1 is actively recycled by human FCGRT.

Pharmacokinetics of humanized mAbs in Tg32 models

Having confirmed that the Tg32-hFc mice have significant levels of chimeric IgG1 antibodies, we addressed if this constitutive endogenous production significantly dampens the half-lives of administered humanized mAbs in a human FCGRT-dependent manner. We tested two different mAbs: 1) HuLys11, a humanized mAb containing the CH1-3 region of human IgG1 that was raised against hen egg-white lysozyme,17 and 2) trastuzumab, a humanized mAb that binds the extracellular domain of the HER2 receptor and is in clinical use18 (Figure 3). The experimental cohorts included the naïve and immunized Tg32-hFc and naïve Tg32 mice whose plasma were analyzed above, as well as similarly aged and sex-matched Fcgrt knockout (KO) mice. As preloading with human IgG is known to enhance the clearance of administered humanized mAbs,9 the study also included naïve Tg32 mice injected 2 days earlier with purified human IgG (IVlg); their human IgG levels in plasma averaged 2,300 μg/ml on day 0 of the experiment. Each cohort was injected intravenously with an admixture of trastuzumab and HuLys11 (10 mg/kg each) and their blood was monitored serially. As is evident by the plasma concentration-time courses (Figure 3; see Table 1 for detailed PK analyses), naïve Tg32-hFc mice cleared HuLys11 significantly faster than naïve Tg32 mice, with β-phase T1/2 of 8.7 days and 17.4 days, respectively (p = .01) (Figure 3b). Previously immunized Tg32-hFc mice also increased clearance (T1/2 8.2) but did not differ significantly from naïve Tg32-hFc mice. IVlg-preloaded Tg32 mice showed the most rapid clearance (T1/2 5.4 days); see Table 1 for details. Dependence on FCGRT was evident by the inability to detect HuLys11 in Fcgrt KO mice.

Very similar patterns were found for trastuzumab, despite its lower overall persistence (8.5 days in Tg32 mice; see Figure 3c,d). Compared with naïve Tg32 counterparts, naïve and immunized Tg32-hFc mice increased clearance of
trastuzumab to a similar extent ($T_{1/2}$ 7.4 and 6.5 days, respectively), while Tg32 mice preloaded with IVIG exhibited a more appreciable reduction ($T_{1/2}$ 5.3 days). Again, dependence on FCGRT in all cases was evident by the very short half-lives found for mice lacking FCGRT ($T_{1/2}$ 0.02 days); see Table 1 for details. These combined data strongly support the contention that the endogenous production of chimeric IgG1 in Tg32-hFc mice, both naïve and immunized,
significantly dampens the half-lives of administered humanized mAbs in a human FCGRT-dependent manner, but not to the extent found in bolus IVIg-preloaded mice.

**Discussion**

FCGRT-humanized mice have been substantially validated for modeling of humanized IgG-based biologics, but lack an endogenous condition of competing human IgG that typifies the human condition. Here, we used gene editing to seamlessly humanize the mouse IgG1 Fc and hinge domains. Our targeting strategy resulted in Tg32 mice, Tg32-hFc, that develop fully mouse antibodies of the expected classes, subclasses, and abundance, but with the IgG1 gene carrying a humanized Fc domain, leading to its substantial plasma elevation.

Tg32 mice are hypergammaglobulinemic because of the failure of human FCGRT to bind and recycle mouse IgGs. In keeping with this, plasma concentrations of IgG1 in naïve Tg32 mice averaged 17 μg/ml, whereas that of naïve Tg32-hFc mice averaged 600 μg/ml. This 35-fold elevation of circulating IgG1 is almost certainly due to active hFCGRT-specific recycling, extending the IgG half-life and thus its plasma concentrations. The level of chimeric IgG1 now observed is comparable to that of native IgG1 reported in wildtype C57BL/6 J mice (~280 μg/ml mg/ml). Furthermore, after immunization with DNP-KLH, we found that the Tg32-hFc mice mount a robust antibody response, with chimeric IgG1 plasma levels increasing substantially. The elevated plasma elevations of chimeric IgG1 in comparison to mouse IgG2a and IgG2b provide further confirmation that the chimeric IgG1 is uniquely protected by hFCGRT in Tg32-hFc mice. Moreover, the detection of all immunoglobulin classes in plasma of naïve Tg32-hFc mice and its elevation post immunization indicates that the Fc substitution did not impair the overall capacity and capability of B cells to respond, class switch and differentiate to plasma cells. Combined, these data provide functional evidence that the chimeric IgG1 is appropriately regulated and uniquely protected by hFCGRT in the Tg32-hFc model.

Our key prediction is that the chimeric IgG1 in Tg32-hFc mice would compete for hFCGRT recycling with administered humanized mAbs. In support of this, we evaluated two humanized mAbs, trastuzumab and HuLys11. Our data show reduced β-phase half-lives when these mAbs are administered to Tg32-hFc mice compared with the noncompetitive environment of standard Tg32 mice. This more rapid clearance clearly documents the ability of chimeric IgG, endogenously produced and maintained homeostatically by human FCGRT-mediated recycling, to diminish the persistence of administered therapeutic mAbs. Although these data cannot be directly equated to cynomolgus monkeys or humans, Tg32-hFc mice do provide a more physiologically relevant competitive FCGRT-humanized rodent model for assessing the behavior of Fc-based therapeutics and human FCGRT biology.

IgG recycling by FCGRT is a logarithmic function of serum IgG concentrations. It operates at high efficiency when IgG is limiting and diminishes when IgG is in excess. Thus, the persistence of IgG is predicted to be inversely related to its concentration in circulation. Our findings that the half-lives of the test therapeutic mAbs were longest in Tg32 mice (lacking Fc-humanized IgG1), naïve Tg32-hFc mice (having ~600 μg/ml chimeric IgG1) being intermediate, and with IVIg-preloaded Tg32 mice (having 2,300 μg/ml IgG at Day 0 of the experiment) showing the greatest reduction were consistent with this inverse relationship. However, our finding that immunized Tg32-hFc mice (having ~4,200 μg/ml chimeric IgG1) showed only an intermediate reduction is a conspicuous exception not readily explained by the standard concentration–catabolism model.

It is well established that FCGRT is a saturable Fc receptor. The FCGRT-dependent concentration–catabolism effect incorporates two variables: FCGRT availability and IgG concentrations in circulation. While modeling of this phenomenon usually assumes that FCGRT availability is invariant, it is well established in transgenic models that hIgG concentrations correlate positively with the transgenic hFCGRT copy number. Increases in FCGRT thus increase serum concentrations of IgG. The reticuloendothelial system is a major repository of functionally competent FCGRT expression and IgG recycling. Immunization protocols that include a strong adjuvant, such as the Freund’s adjuvant used in our study, are highly immunostimulatory to this organ system, with a substantial expansion of myeloid derivatives, their activation state, and upregulated levels of FCGRT expression. We thus posit that the relatively anemic half-life reductions of HuLys11 and trastuzumab in immunized Tg32-hFc mice occurred because immunization increased the overall capacity of hFCGRT to recycle IgG.

Overall, the development of these Tg32-hFc mice provides a relevant and economic model to rapidly evaluate novel engineered Fc-based biologics within a more competitive environment, and could provide a more reliable preclinical PK analyses model. This Tg32-hFc model also provides a convenient vehicle for the rapid generation of Fc-humanized IgG1 monoclonal antibodies.

**Materials and methods**

**Generation of Tg32-hFc mice**

Mice used and created in this study are listed with genotype, full and abbreviated names in Table 2. An overview of the targeting strategy is shown in Figure 1a. Fertilized oocytes isolated from the Tg32 strain were microinjected with two sgRNAs (#1630, #1652) targeting the sequence encoding the beginning of the *Ighg1* hinge domain and the end of the CH3 domain, respectively. The sgRNAs were designed as TRU-guides with 18nt of target-specific sequence. sgRNA

| Short Name | Full Strain Name | JAX Stock Ref # | Genotype |
|------------|------------------|----------------|----------|
| Fcgrt KO   | B6.129X1-Fcgrt<sup>−/−</sup>/Dcr1 | 003982 | /- null wt |
| Tg32       | B6.Cg-Fcgrt<sup>−/−</sup>/Dcr1Tg32(Dcr1<i>hFcgrt</i>) | 014565 | Tg/Tg wt |
| Tg32-hFc   | B6.Cg-Fcgrt<sup>−/−</sup>/Dcr1Ighg1<sup>em2(295G1)</sup>Tg(Fcgrt)32Dcr/Mvw | 029686 | Tg/Tg Tg/ |
#1630 required pre-pending with a non-homologous 5’ G to facilitate in vitro transcription. Guide target sequences are shown with the PAM in parentheses: #1630: 5’ gcacaatc CCTGGGACTG(ttg) and #1652: 5’ GAGCCCTTCCTC ACTCTCC(ttg); see Figure 1a. Each sgRNA was microinjected at 30 ng/µl, with Cas9 mRNA (Trilink) at 60 ng/µl, and plasmid donor at 20 ng/µl. The plasmid donor (partly synthesized by Genscript) consisted of a 902 bp human sequence (hinge (Exon 2), CH2 (exon 3) and CH3 (exon 4)) flanked by ~5kb homology arms. A total of 95 embryos derived from Tg32 were microinjected and transferred into five pseudopregnant females. Three transfers failed to deliver any liveborn, the remaining two females delivered 6 mice (3 F/3 M) from 38 embryos implanted, two of which were identified as candidates for the transgene replacement by PCR. Following backcrossing to Tg32 mice, both strains transmitted the transgenic allele. Ultimately, ahomozogous single strain was established and characterized; B6.Cg-Fegrt tm1Dcr Ighg1em2(IghG1)Mvw Tg(FCGRT)32Dcr/Mvw, JAX # 029686, abbreviated here to Tg32-hFc.

Genotyping of the hFc allele used PCR primers flanking the humanized region (1731 F, 5’ GAAATGGATCT CAGCCCCAGAG and 1732 R, 5’ AGCTAGAGAAGGCTG GTAAG), generating a 1,484 bp amplicon. Sanger sequencing of the PCR product was used to determine the zygosity of the chimeric allele. Partial verification of the replacement event used long-range PCR primers 1838 F, 5’ CAGCCGGAGAA CAACTCAAA and 1839 R, 5’ AACAGTCCCGGGCTA AATG generating a 5,825 bp amplicon, one located outside of the homology arm and the other located within the humanized portion of the chimeric allele followed by sequencing. Additionally, Long Read Sequencing (PacBio) was used to verify the complete sequence across both homology arms as base-perfect; see Supplemental Figure 1. This sequence was generated using genomic DNA isolated from the liver of a mouse homozygous for the humanized Ighg1 allele. Long-Range PCR (LongAmp° Taq, New England Biolabs) was performed with primers (1839 R, 5’ AACAGTCCCGAGCTTAATG; and 1840 F, 5’GACAGGTGAGGTGT GTTGA) to generate an 11,665 bp amplicon for Long Read Sequencing (PacBio). Alignment of the LR Sequence to the predicted allele confirmed that no unintended mutations were incorporated into the chimeric allele.

**Verification of the Tg32-hFc humanized immunoglobulin heavy constant gamma 1 transcript and splicing**

Total spleen RNA from a 12-week-old homozygous male Tg32-hFc mouse was isolated and used to make poly-T primed cDNA (Invitrogen 18080–044; First Strand cDNA Synthesis Kit). This cDNA was subject to PCR followed by Sanger Sequencing verifying the predicted splice variants (Ighg1-v1 and Ighg1-v2), and that splicing of the human sequence occurred as predicted. Amplicons generated from the cDNA template used a common forward primer targeting the mouse Ighg1 exon 1 (F1, 5’ AGTGAACCTGGAACATTG), and a reverse primer specific to the 3’ UTR of each variant: either Rv1 (5’ CAGTGCTGGGTGCTTTATTAC) or Rv2 (5’ CTGTA GACGAGAAGGAGTCTA); see Figure 1b. The resulting product was sequenced; see Supplemental Figure 2.

**Immunoglobulin quantification**

Chimeric IgG1 was quantified from the plasma of Tg32-hFc mice by capture with mouse anti-human IgG1 (clone G17-1, BD Biosciences) and detection with mouse anti-human kappa-HRP (clone SB81a, Southern Biotech). Human IgG1 antibodies purified from healthy donors were used as a standard (Enzo Life Sciences). Some mice were primed with 100 µg of DNP-KLH in complete Freund’s adjuvant, challenged with DNP-KLH in incomplete Freund’s adjuvant, and bled 2 weeks later. Anti-DNP quantification of chimeric IgG1 was determined by capture with DNP-albumin conjugate (Sigma-Aldrich) coated at 5 µg/ml phosphate-buffered saline (PBS), and detection with mouse anti-human IgG1-HRP (clone G17-1, BD Biosciences). Mouse Ig isotypes were quantified using the Meso Scale Discovery mouse isotyping panel per manufacturer’s instructions with the exception of mouse IgG1. Mouse IgG1 was quantified by capture with goat anti-mouse IgG1 (Southern Biotech) at 5 µg/ml PBS, and detection with goat anti-mouse kappa-AP (Southern Biotech). A mouse IgG1 isotype control (Southern Biotech) was used as a standard. Statistical analysis of antibody concentrations was determined with PRISM software by a 2-sided T-test with Welch’s correction or ANOVA using the Turkey’s multiple sample comparison test.

**PK analysis**

We essentially followed the approach of Petkova et al. In brief, groups of naïve Tg32, naïve Tg32-hFc, Tg32-hFc mice previously immunized with DNP-KLH, naïve Tg32, preloaded with 250 mg/kg human IV Ig, and Fegrt KO mice were pre-bled at day 0, and intravenously co-injected with the humanized mAbs trastuzumab18 and HuLys1117 (10 mg/kg each). The mice were then bled serially (25 µl samples) into K₂EDTA at 1, 3, 5, 7, 13, 17, 21, and 28 days, and the blood processed to plasma. The plasma was diluted 1 to 10 in 50% glycerol in PBS and stored at −20°C until analysis. HuLys11 was quantified by capturing with lysozyme purified from chicken egg white (Sigma-Aldrich) coated at 5 µg/ml in carbonate buffer (pH 9.6) and detected with mouse anti-human IgG1–HRP (clone G17-1, BD Biosciences). Trastuzumab was quantified in the plasma samples by capturing activity on ELISA plates with anti-idiotypic antibody AbD18018 (Bio-Rad) coated at 5 µg/ml PBS, detecting with mouse anti-human IgG Fc CH2 domain with HRP conjugation (Bio-Rad) at 2 µg/ml. HuLys11 was quantified by capturing with lysozyme purified from chicken egg white (Sigma-Aldrich) coated at 5 µg/ml in carbonate buffer (pH 9.6) and detected with mouse anti-human IgG1–HRP (clone G17-1, BD Biosciences). Trastuzumab and HuLys11 plasma concentrations were analyzed using PK Solutions Software (Summit Research Services). Human IgG1 was quantified from the plasma of Tg32-hFc mice by capture with mouse anti-human IgG1 (clone G17-1, BD Biosciences), detection with mouse anti-human kappa-HRP (clone SB81a, Southern Biotech), with human IgG1 isotype control purified from healthy donors (Enzo Life Sciences) used as a standard. Anti-DNP activity resulting from the DNP-KLH immunization was quantified by capture with DNP-albumin conjugate...
Mouse kappa-AP Discovery PCR | PBS mRNA MHC mAbs IV IGHG1 IgA HDR FcRn FCGRT Fab DNP-KLH 2,4-dinitrophenyl-keyhole limpet hemocyanin Fc fragment of IgG, receptor, transporter, alpha - human FcgRT Fc fragment of IgG, receptor, transporter, alpha - mouse FcRn Fragment crystallizable receptor neonate (old name for Fcgrt) G Guanine HDR Homology directed repair IgA Immunoglobulin A IgG Immunoglobulin G IGHG1 Immunoglobulin heavy constant gamma 1 IgM Immunoglobulin M IV Intravenous K3EDTA Tripotassium ethylenediaminetetraacetic acid mAbs Monoclonal antibody mg/kg Milligram per kilogram MHC Major histocompatibility complex mRNA Massager ribonucleic acid PBS Phosphate-buffered saline PCR Polymerase chain reaction pH Potential of hydrogen PK Pharmacokinetic RNA Ribonucleic acid T Thymine T Time UTR Untranslated region μg/ml Microgram per milliliter

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

Funding
This work was supported by the National Institutes of Health Grant OD011190 (M.V.W. and B.E.L.) and The Jackson Laboratory.

Ethics statement
All animals experiments are done under The Jackson Laboratory Animal Care and Use Committee (IACUC), those specific to MVW used IACUC summary # 11006. Animals were euthanized by cervical dislocation.

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