nature biotechnology

BRIEF COMMUNICATIONS

Enhanced antibody half-life improves *in vivo* activity

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Improved affinity for the neonatal Fc receptor (FcRn) is known to extend antibody half-life *in vivo*. However, this has never been linked with enhanced therapeutic efficacy. We tested whether antibodies with half-lives extended up to fivefold in human (h)FcRn transgenic mice and threefold in cynomolgus monkeys retain efficacy at longer dosing intervals. We observed that prolonged exposure due to FcRn-mediated enhancement of half-life improved antitumor activity of Fc-engineered antibodies in an hFcRn/Rag1$^{-/-}$ mouse model. This bridges the demand for dosing convenience with the clinical necessity of maintaining efficacy.

The well-established role of FcRn in IgG turnover has been the foundation for Fc engineering efforts aimed at improving the pharmacokinetic properties of therapeutic antibodies[1,2]. Despite contrary results about the relationship between FcRn affinity and half-life[3,4], several such efforts at pharmacokinetic engineering in nonhuman primates, whose FcRn is similar to that of humans, have demonstrated that engineered antibody variants have a prolonged half-life[5–8]. Yet, although the successful extension of half-life in pharmacokinetic experiments bodes well for the prospect of improving clinical dosing, a critical gap remains. For half-life extension technologies to be of practical use, efficacy of a biotherapeutic with longer half-life must be preserved at longer dosing intervals. Although the relationship between drug exposure and efficacy is well-established, this correlation has not thus far been established for antibodies engineered for longer half-life.

We coupled rational design methods with high-throughput protein screening to engineer a series of Fc variants with greater affinity for human FcRn. Variants were constructed in the context of the humanized anti-vascular endothelial growth factor (VEGF) IgG1 antibody bevacizumab[9] (Avastin), which is currently approved for the treatment of colorectal, lung, breast and renal cancers. A description of the construction, production and binding studies of the antibodies is provided in **Supplementary Methods**. As FcRn binds IgG at the lower pH of the early endosome (pH 6.0–6.5) but not at the higher pH of blood (pH 7.4), we used Biacore to screen antibodies for binding to human FcRn at pH 6.0. Our engineered variants demonstrated between 3- and 20-fold greater binding to FcRn at pH 6.0, with improvements due almost exclusively to slower off-rate (k_{off}) (**Supplementary Fig. 1** and **Supplementary Table 1**). A lead variant, M428L/N434S, subsequently selected principally based on its pharmacokinetic performance (see below), provided an 11-fold improvement in FcRn affinity at pH 6.0. We refer to this double substitution in the context of bevacizumab as Xtend-VEGF.

Details of a pharmacokinetic study in cynomolgus monkeys (*Macaca fascicularis*) to evaluate the capacity of the variants to improve serum half-life are provided in the **Supplementary Methods**. Binding improvements of the variants to monkey FcRn at pH 6.0 were comparable to improvements for human FcRn, and the rank order of the variants in FcRn affinity was the same (data not shown). When three monkeys per group were injected intravenously with 4 mg/kg variant or native IgG1 anti-VEGF antibody, we observed a large improvement in half-life for the variants relative to native IgG1 (**Supplementary Fig. 2a**). Fitted parameters for the full set of variants (**Supplementary Table 2**) indicated increases in β-phase half-life, area under the curve (AUC) measurements and the rate of antibody clearance from serum. The observed 9.7-d half-life for native IgG1 bevacizumab agrees with the published value (9.3 d) for a slightly lower (2 mg/kg) dose[10]. Among the engineered antibodies that were tested, the Xtend double variant performed best (**Fig. 1a**). It prolonged half-life from 9.7 to 31.1 d, a 3.2-fold improvement in serum half-life relative to native IgG1 (**Supplementary Table 2**). Simple allometric scaling extrapolations suggest that such improvement can potentially translate into human half-lives >50 d.

We then sought to further challenge the applicability of pharmacokinetic engineering by targeting an internalizing cell-surface antigen that potentially provides a competing sink for antibody clearance.

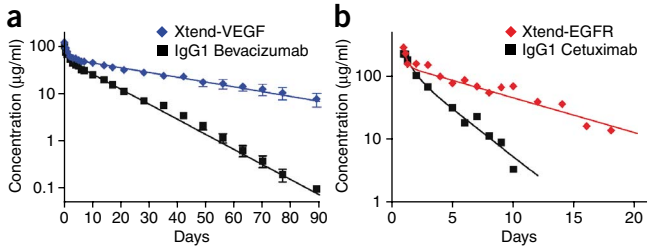

Figure 1 Increasing antibody affinity to FcRn promotes half-life extension in cynomolgus monkeys. (**a**) Log-linear changes in serum concentrations for anti-VEGF (bevacizumab) antibodies in cynomolgus monkeys. All antibodies were administered by single 60-min intravenous infusion at 4 mg/kg and serum antibody concentrations were determined using a VEGF antigen-down immunoassay. Results are means ± s.e.m. ($n = 2$ for bevacizumab and $n = 3$ for variants). (**b**) Log-linear changes in serum concentrations for anti-EGFR antibodies in cynomolgus monkeys. Monoclonal antibodies were administered by single 30-min intravenous infusion at 7.5 mg/kg and serum antibody concentrations were determined using an EGFR antigen-down immunoassay. Results are means ($n = 2$ animals per test article).

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Received 20 October 2009; accepted 14 December 2009; published online 17 January 2010; doi:10.1038/nbt.1601

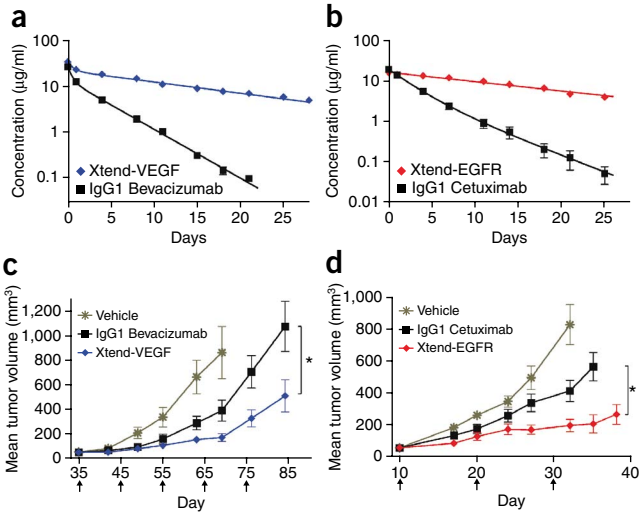

Figure 2 Improved antibody half-life translates to greater *in vivo* efficacy. (**a**) Log-linear changes in serum concentrations of anti-VEGF antibodies in hFcRn mice. All antibodies were administered via single intravenous bolus at 2 mg/kg, and serum antibody concentrations were determined using a human immunoglobulin recognition immunoassay. Results are means ± standard errors ($n = 6$). For some data points, errors are smaller than can be indicated. (**b**) Log-linear changes in serum concentrations of anti-EGFR antibodies in hFcRn mice. The study design was identical to that described in **a**, except that serum concentrations were measured with an EGFR antigen-down immunoassay. (**c**) Xenograft study in hFcRn/Rag1$^{-/-}$ mice comparing activity of native IgG1 and Xtend variant versions of bevacizumab against established SKOV-3 tumors. Tumor volume is plotted against day after tumor cell injection. Antibodies were dosed at 5 mg/kg every 10 d starting on day 35 (indicated by the arrows). $n = 8$ mice/group. *, $P = 0.028$ at 84 d. (**d**) Xenograft study in hFcRn/Rag1$^{-/-}$ mice comparing activity of anti-EGFR antibodies against established A431 tumors. Tumor volume is plotted against day after tumor cell injection. Antibodies were dosed 5 mg/kg every 10 d starting on day 10 (indicated by the arrows). $n = 9$ mice/group. *, $P = 0.005$ at 35 d.

Several studies have demonstrated that antibodies to epidermal growth factor receptor (EGFR) are internalized. Moreover, nonlinear dose-dependent clearance has been observed in monkeys and humans, leading to the hypothesis that receptor-dependent internalization makes a major contribution to clearance of anti-EGFR antibodies[11,12]. The M428L/N434S Xtend variant was constructed in a humanized version (huC225) of the anti-EGFR antibody cetuximab (C225)[13] (Erbitux), which is approved for the treatment of colorectal and head and neck cancers. We refer to this pharmacokinetically enhanced anti-EGFR antibody as Xtend-EGFR. The improvement in affinity for human FcRn resembled that observed for anti-VEGF; binding to human EGFR antigen was unperturbed, and both cetuximab and humanized cetuximab cross-react with cynomolgus EGFR[14] (data not shown). The 7.5 mg/kg dose chosen for this study is in a range where the dose-clearance relationship is nonlinear[14]. In our hands cetuximab had a half-life of 1.5 d (**Supplementary Table 2**), similar to previously published data at the same dose (2.7–3.1 d)[14]. Consistent with the bevacizumab results, the Xtend variant anti-EGFR increased half-life to 4.7 d, reflecting a 3.1-fold improvement (**Fig. 1b** and **Supplementary Table 2**). We have thus demonstrated pharmacokinetic improvements conferred by Fc engineering of an internalizing antibody, even when it is dosed within the nonlinear clearance regime.

We performed pharmacokinetic experiments in C57BL/6J (B6)-background mice that are homozygous for a knockout allele of murine FcRn and heterozygous for a human FcRn transgene (mFcRn$^{-/-}$, hFcRn^{+})[15], referred to here as hFcRn mice. A description of these experiments is provided in the **Supplementary Methods**. Serum concentration data for native IgG1 and Xtend anti-VEGF antibodies showed a dramatic enhancement in half-life for the variant relative to native IgG1 (**Fig. 2a**), improving half-life fourfold from ~3–12 d (**Supplementary Table 2**). In the anti-EGFR context, the Xtend variant improved half-life to 13.9 d relative to 2.9 d for cetuximab, resulting in an enhancement of about fivefold (**Fig. 2b** and **Supplementary Table 2**). The IgG1 version of huC225 also had a relatively short half-life of 2 d (data not shown). We observed a general correlation between antibody half-life and FcRn affinity at pH 6.0 across two anti-VEGF studies and one anti-EGFR hFcRn pharmacokinetic study. The pharmacokinetic results for individual variants and native IgG1 were consistent and reproducible between the three studies (**Supplementary Fig. 2b–c** and **Supplementary Table 2**).

To test whether the slower clearance of our pharmacokinetic-engineered antibodies results in improved exposure-related pharmacology, we developed an hFcRn transgenic, Rag1$^{-/-}$ immunodeficient mouse strain (**Supplementary Methods** and **Supplementary Fig. 3**). For VEGF, SKOV-3 tumors were established to 25–60 mm^3 and then treated with either vehicle or 5 mg/kg native IgG1 or Xtend variant bevacizumab every 10 d. This dosing schedule approximated the half-life of the Xtend variant, but was three to four half-lives longer than the half-life of the native IgG1 version (**Supplementary Table 2**). A statistically greater level of tumor reduction ($P = 0.028$ at study termination) was observed for the Xtend variant relative to the native IgG1 version (**Fig. 2c**). A similar study in hFcRn/Rag1$^{-/-}$ mice comparing Xtend-EGFR to a native IgG1 version showed similar improvements in tumor reduction ($P = 0.005$) against established A431 epidermoid carcinoma tumors (**Fig. 2d**). Consistent with the pharmacokinetic results in hFcRn mice (**Fig. 2a–b**), the variants reduced clearance in the hFcRn/Rag1$^{-/-}$ mice (**Supplementary Fig. 3a–b**), demonstrating an inverse correlation between tumor volume and serum concentration of antibody at study termination. These results indicate that the slower clearance of the variant antibodies leads to higher drug exposure and consequently superior tumor-suppressing pharmacology. Additional studies comparing various dosing intervals of the Xtend variants and parent antibodies will be necessary to precisely define dosing regimens for optimal clinical benefit. However, the results described here firmly establish a positive correlation between pharmacokinetic enhancement and *in vivo* efficacy.

Despite the reasonably long half-lives of monoclonal antibodies, market pressures for higher patient convenience and compliance continue to drive antibody drug programs toward less frequent dosing schedules. Yet, because of the potential loss in efficacy when the dosing frequency is not justified by the pharmacokinetics of the drug, the critical issue of whether slower antibody clearance through Fc engineering leads to superior exposure-dependent efficacy has remained unresolved. Our results indicate that, for at least some therapies, efficacy can be preserved with extended dosing intervals enabled by pharmacokinetic engineering. This work thus paves the way for a new generation of antibody therapies and biologically superior versions of approved antibody drugs that deliver finer control over dosing while providing greater convenience to patients.

Note: Supplementary information is available on the Nature Biotechnology website.

ACKNOWLEDGMENTS
We thank The Jackson Laboratory JAX West and SNBL USA for carrying out pharmacokinetic experiments, B. Dahiyat for helpful discussions, and A. Eivazi, D.-H.T. Nguyen, H. Herman, J.M. Jacinto and U.S. Muchhal for technical contributions.

AUTHOR CONTRIBUTIONS
J.Z., A.K.C., H.M.H., G.A.L., D.C.R. and J.R.D. designed the research, J.Z., A.K.C., H.M.H., S.K., I.W.L.L. and T.J.S. carried out experiments, and J.Z., G.A.L. and J.R.D. wrote the manuscript.

COMPETING INTERESTS STATEMENT
The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturebiotechnology/.

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