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Enhanced antibody half-life improves in vivo activity

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Abstract

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 serum turnover has been the foundation for Fc engineering efforts aimed at improving the pharmacokinetic (PK) properties of antibodies[1, 2]. Despite contrary results about the relationship between FcRn affinity and half-life[3, 4], a number of such PK engineering studies in non-human primates, whose FcRn is similar to that of human, have demonstrated increased half-life by engineered antibody variants[5–8]. Yet while the successful extension of half-life in PK 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 Fc-engineered for longer half-life.

Rational design methods coupled with high-throughput protein screening were used to engineer a series of Fc variants with greater affinity for human FcRn. Variants were constructed in the context of the humanized anti-VEGF IgG1 antibody bevacizumab[9] (Avastin®, Genentech/Roche), 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 the Supplementary Methods. Antibodies were screened for binding to human FcRn at pH 6.0 using Biacore. Engineered variants provide 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, Supplementary Table 1). A lead variant M428L/N434S, subsequently selected principally based on its PK performance (see below), provided an 11-fold improvement in FcRn affinity at pH 6.0. This double substitution in the context of bevacizumab is referred to as Xtend™-VEGF.

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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. executed experiments, and J.Z., G.A.L., and J.R.D. wrote the manuscript.

COMPETING INTERESTS STATEMENT

J.Z., A.K.C., H.M.H., S.K., I.W.L.L., G.A.L., and J.R.D. are employees of, and have ownership interest in Xencor.

A PK study was carried out in cynomolgus monkeys (*macaca fascicularis*) in order to evaluate the capacity of the variants to improve serum half-life in monkeys. A description of these experiments is 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). Three monkeys per group were injected intravenously (i.v.) with 4 mg/kg variant or native IgG1 anti-VEGF antibody. The results showed 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, AUC, and the clearance of antibody from serum. The observed 9.7 day half-life for native IgG1 bevacizumab agrees with the published value (9.3 days) for a slightly lower (2 mg/kg) dose[10]. Among the engineered antibodies that were tested, the Xtend double variant performed best (Fig. 1a), extending half-life from 9.7 to 31.1 days, 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 exceeding 50 days.

We then sought to further challenge the applicability of PK engineering by targeting an internalizing cell-surface antigen that potentially provides a competing sink for antibody clearance. Antibodies to EGFR have well-established internalization behavior, and nonlinear dose-dependent clearance has been observed in monkeys and humans, leading to the hypothesis that receptor-dependent internalization is a significant clearance pathway for anti-EGFR antibodies11, 12. The M428L/N434S Xtend variant was constructed in a humanized version (huC225) of the anti-EGFR antibody cetuximab (C225)13 (Erbitux®, Imclone/Lilly), which is approved for the treatment of colorectal and head and neck cancers. This PK-enhanced anti-EGFR antibody is referred to as Xtend™-EGFR. The variant provided similar affinity improvement to human FcRn as for anti-VEGF, binding to human EGFR antigen was unperturbed, and both cetuximab and humanized cetuximab cross-react with cynomolgus EGFR14 (data not shown). The 7.5 mg/kg dose chosen for this study is in a range where the dose-clearance relationship is nonlinear14. In our hands cetuximab had a half-life of 1.5 days (Supplementary Table 2), similar to previously published data at the same dose (2.7–3.1 days) [14]. Consistent with the bevacizumab results, the Xtend variant anti-EGFR extended half-life to 4.7 days, reflecting a 3.1-fold improvement (Fig. 1b, Supplementary Table 2). We have thus demonstrated - for the first time - that Fc engineering of an internalizing antibody improves its PK, even when dosed within the nonlinear clearance regime.

We performed PK experiments in C57BL/6J (B6)-background mice that are homozygous for a knock-out 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 4-fold from approximately 3 to 12 days (Supplementary Table 2). In the anti-EGFR context, the Xtend variant improved half-life to 13.9 days relative to 2.9 days for cetuximab, resulting in an enhancement of 5-fold (Fig. 2b, Supplementary Table 2). The IgG1 version of huC225 also had a relatively short 2 day half-life (data not shown). Across two anti-VEGF and one anti-EGFR hFcRn PK studies, a general correlation was observed between antibody half-life and FcRn affinity at pH 6.0, and PK results for individual variants and native IgG1 were consistent and reproducible between the three studies (Supplementary Fig. 2b–c, Supplementary Table 2).

We wished to test whether the slower clearance of our PK-engineered antibodies results in improved exposure-related pharmacology. We therefore developed an hFcRn transgenic, $Rag1^{-/-}$ immunodeficient mouse strain to enable the development of tumor models for both VEGF and EGFR systems in mice expressing human FcRn. 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 days. This dosing schedule approximated the half-life of the Xtend variant, but was 3–4 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 PK 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 the important relationship between PK 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 there is potential for efficacy loss when the dosing frequency is not justified by the PK of the drug, and thus far the critical issue of whether slower antibody clearance through Fc engineering leads to superior exposure-dependent efficacy had remained to be demonstrated. Our results indicate that for at least some therapies efficacy can be preserved with extended dosing intervals enabled by PK engineering. This work thus paves the way for second generation antibody therapies and biosuperior versions of approved antibody drugs that deliver finer control over dosing while providing greater convenience to patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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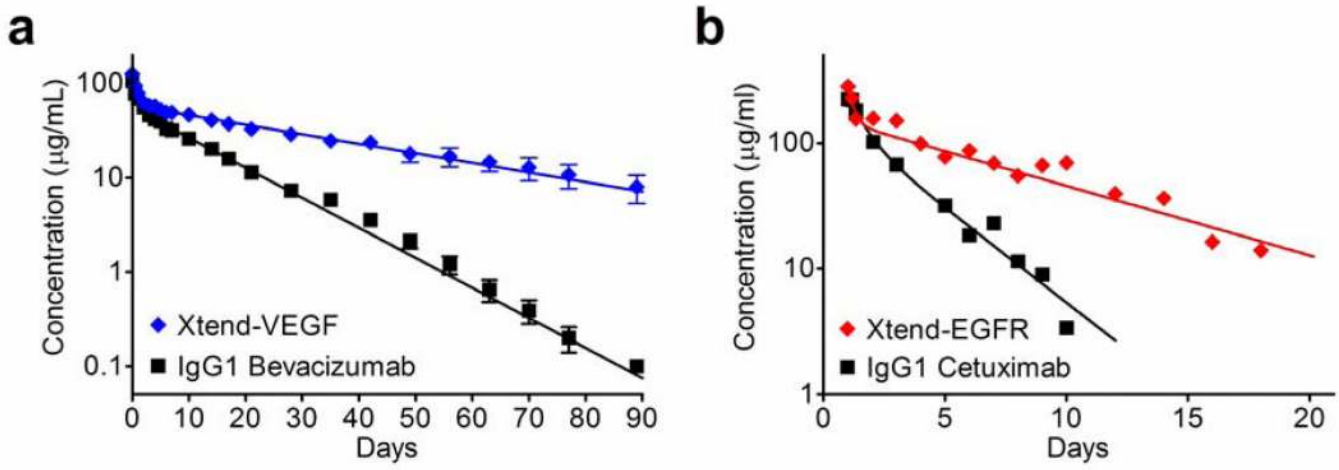

Figure 1. Increasing antibody affinity to FcRn promotes half-life extension in cynomolgus monkeys

(a) Log-linear serum concentration versus time profiles of anti-VEGF (bevacizumab) antibodies in cynomolgus monkeys. All antibodies were administered via single 60 minute i.v. infusion at 4 mg/kg and serum antibody concentrations were determined using a VEGF antigen-down immunoassay. Results are shown as mean ± standard error (N = 2 for bevacizumab and N = 3 for variants). **(b)** Log-linear serum concentration versus time profiles of anti-EGFR antibodies in cynomolgus monkeys. Monoclonal antibodies were administered via single 30 minute i.v. infusion at 7.5 mg/kg and serum antibody concentrations were determined using an EGFR antigen-down immunoassay. Results are shown as mean of N = 2 animals per test article.

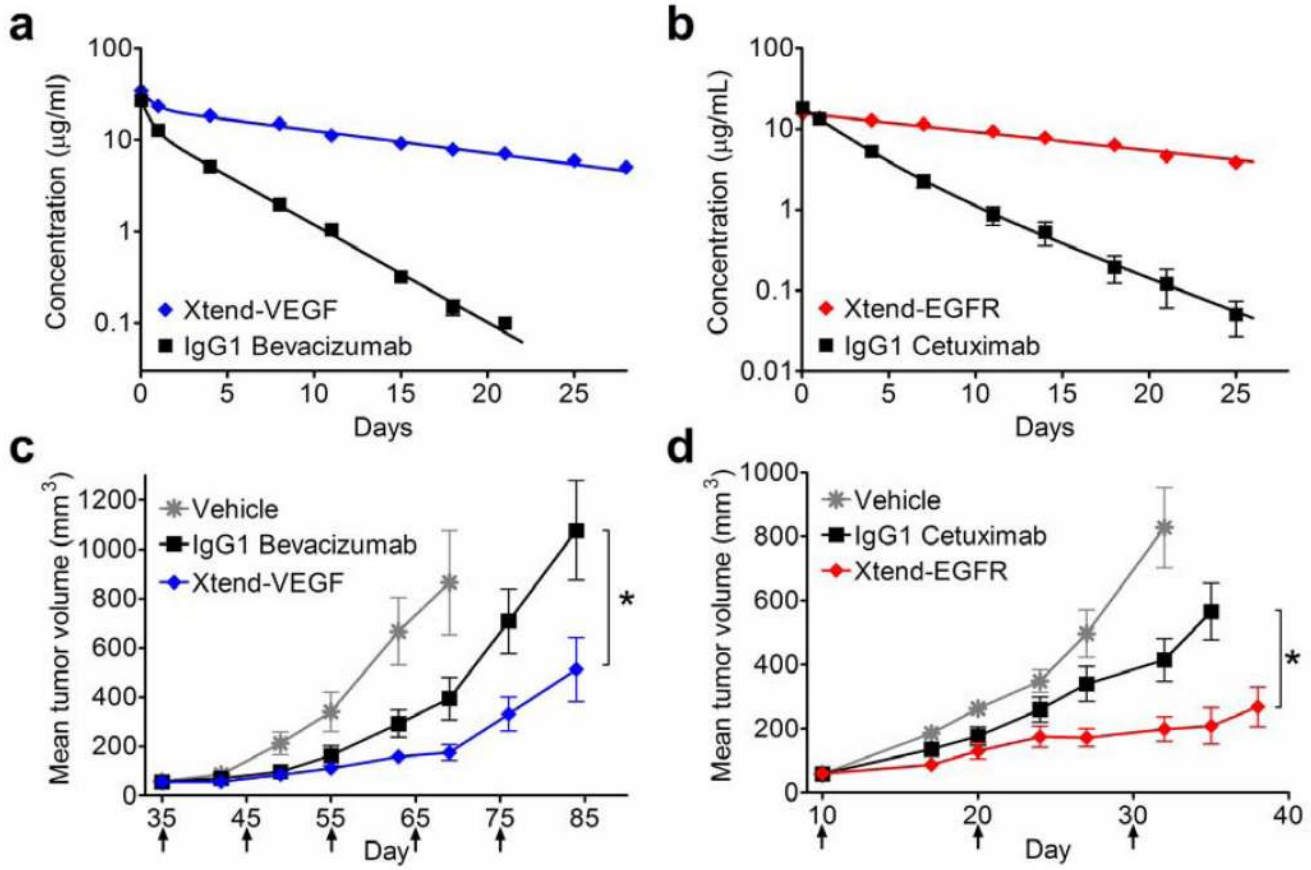

Figure 2. Improved half-life translates into greater in vivo efficacy

(a) Log-linear serum concentration versus time profiles of anti-VEGF antibodies in hFcRn mice. All antibodies were administered via single i.v. bolus at 2 mg/kg, and serum antibody concentrations were determined using a human immunoglobulin recognition immunoassay. Results are plotted as mean ± standard error (N = 6). **(b)** Log-linear serum concentration versus time profiles of anti-EGFR antibodies in hFcRn mice. The study design was identical to that described in panel (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 versus day post tumor cell injection. Antibodies were dosed 5 mg/kg every 10 days starting on day 35 (indicated by the arrows). N=8 mice/group. * p= 0.028 at 84 days. **(d)** Xenograft study in hFcRn/Rag1$^{-/-}$ mice comparing activity of anti-EGFR antibodies against established A431 tumors. Tumor volume is plotted versus day post tumor cell injection. Antibodies were dosed 5 mg/kg every 10 days starting on day 10 (indicated by the arrows). N=9 mice/group. * p= 0.005 at 35 days.