Identifying potential pharmacokinetic profile modifiers to inform optimization of bispecific antibodies using an enhanced FcRn high-performance liquid chromatography, FcRn-pH-HPLC

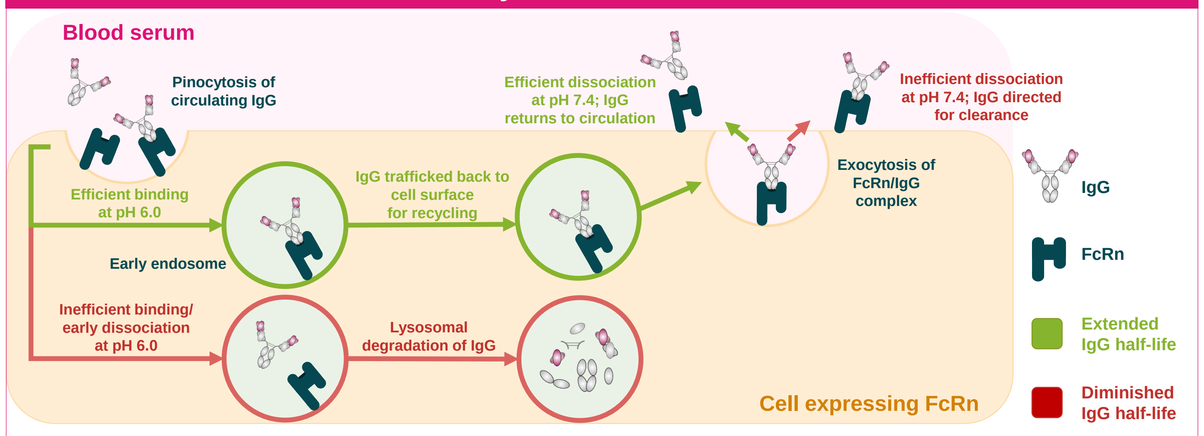
Carolin Tasser, Thomas Müller, Michael Tesar, Ivica Fucek, Ute Schniegler-Mattox, Joachim Koch, Kristina Ellwanger

Affimed GmbH, Mannheim, Germany

BACKGROUND

- There is a need for high-throughput, *in vitro* surrogate assays to identify candidates with optimal *in vivo* serum half-lives early in the discovery and design process for therapeutic antibodies[1]

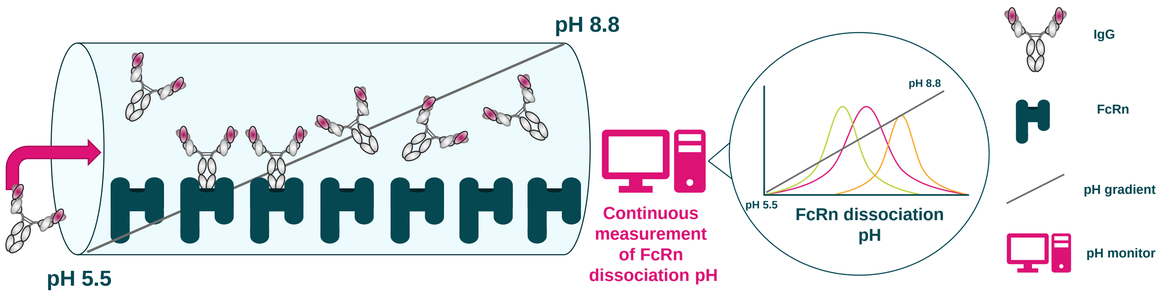
The pH values at which an antibody binds and dissociates from FcRn *in vivo* are key determinants of half-life[2,3]



- FcRn-high performance liquid chromatography (HPLC) was previously developed to estimate the serum half-lives of IgGs based on FcRn binding properties *in vitro*[1]
- This study presents a key optimization of this technique, FcRn-pH-HPLC, which incorporates a pH monitor to directly measure the pH of FcRn dissociation, allowing interpretation of results in a more physiological context
- Bispecific antibodies from the redirected optimized cell killing (ROCK®) platform, comprising many bi- and multispecific antibody modules and formats,[4] were subjected to FcRn-pH-HPLC to identify antibody features that could be altered to tailor half-lives of therapeutic antibodies

FcRn, neonatal Fc receptor; IgG, immunoglobulin G.

FcRn-pH-HPLC directly measures FcRn dissociation pH; results can be interpreted in a physiological context



FcRn-pH-HPLC: Fc containing antibodies are analyzed on human FcRn molecules immobilized on beads in a HPLC column. Antibodies are injected at low pH, 5.5, and dissociate from FcRn after applying a linear pH gradient up to pH 8.8. By continuously measuring the pH, the FcRn dissociation pH of antibodies was reliably determined, allowing a direct comparison to the optimal FcRn dissociation pH *in vivo*, pH 7.4, thus providing a potential surrogate assay for estimating PK profiles.

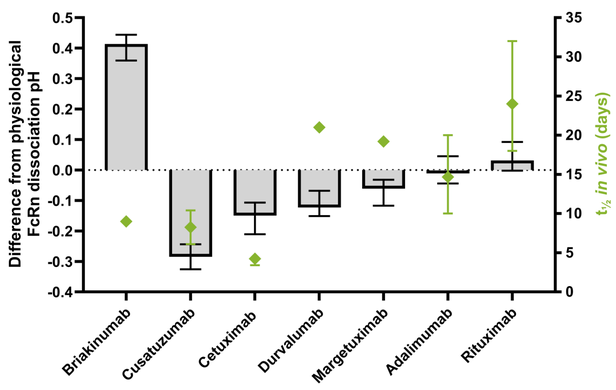
FcRn, neonatal Fc receptor; HPLC, high performance liquid chromatography; IgG, immunoglobulin G; PK, pharmacokinetic.

OBJECTIVES

To establish FcRn-pH-HPLC as a method of predicting potential serum half-lives of candidate therapeutic antibodies *in vitro*, and using the ROCK® platform to identify features of antibodies that act as PK modifiers

RESULTS

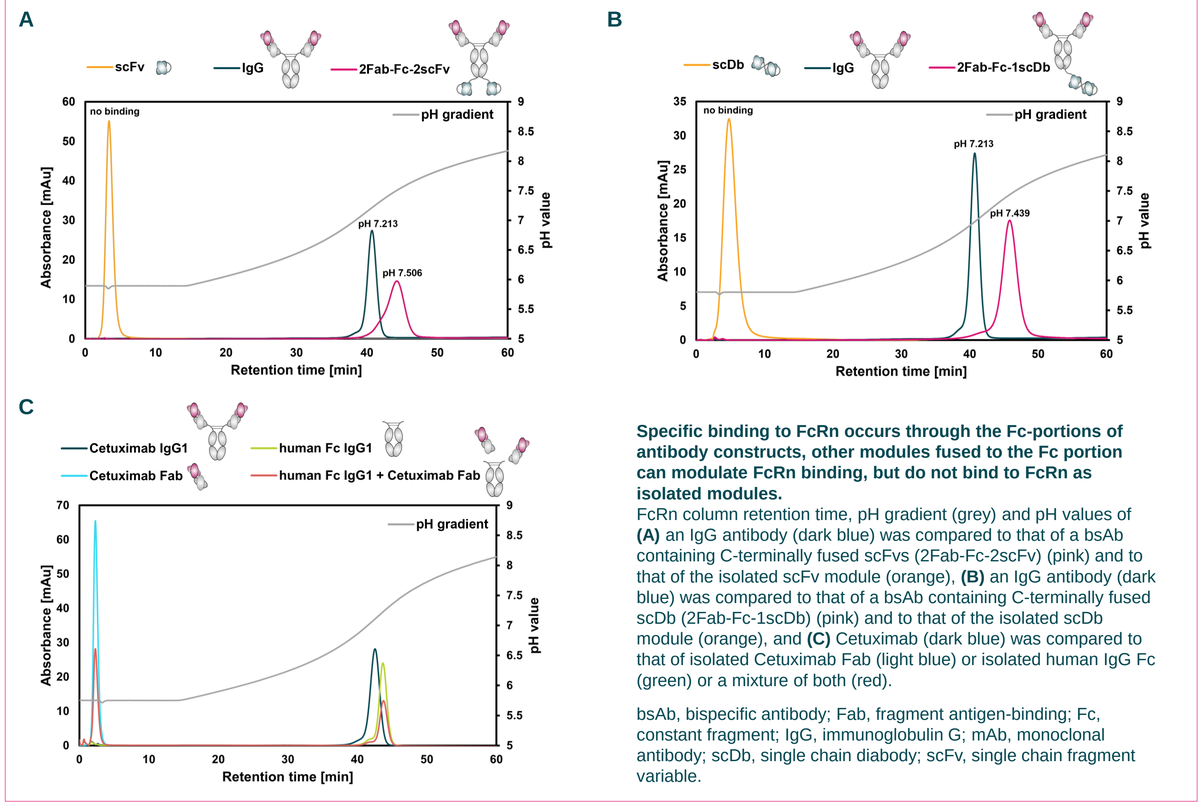
FcRn dissociation pH measured by FcRn-pH-HPLC correlates with previously established half-lives of mAbs in humans



FcRn-pH-HPLC is capable of reliably assessing the FcRn dissociation pH of mAbs, which can be correlated with half-life established *in vivo* in humans.
Mean difference in measured FcRn dissociation pH of the indicated therapeutic mAbs from the optimal FcRn dissociation pH *in vivo* (pH 7.4) as established by FcRn-pH-HPLC.
Values were calculated from three independent experiments using the following: mean FcRn dissociation pH of the indicated antibody - optimal FcRn dissociation pH 7.4
Mean ± SD are shown for each antibody. Data points/ranges (in green): *In vivo* half-lives in human of the indicated therapeutic mAbs, as established previously in clinical studies, and presented in their respective FDA prescribing information or a representative clinical study.[5–11]

FDA, US food and drug administration; mAb, monoclonal antibody; SD, standard deviation; $t_{½}$, half-life.

Non-Fc containing antibody modules do not interact with FcRn but modulate FcRn interaction when fused to Fc-portions

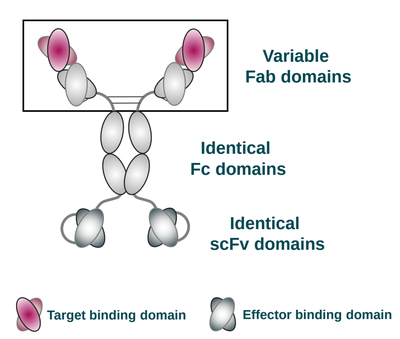


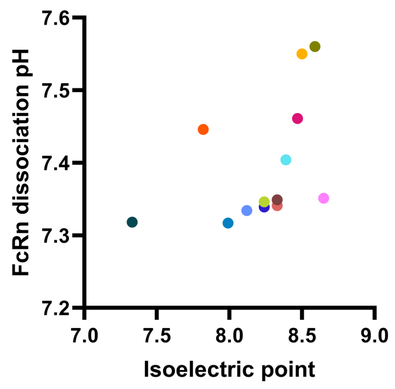
Specific binding to FcRn occurs through the Fc-portions of antibody constructs, other modules fused to the Fc portion can modulate FcRn binding, but do not bind to FcRn as isolated modules.
FcRn column retention time, pH gradient (grey) and pH values of **(A)** an IgG antibody (dark blue) was compared to that of a bsAb containing C-terminally fused scFvs (2Fab-Fc-2scFv) (pink) and to that of the isolated scFv module (orange), **(B)** an IgG antibody (dark blue) was compared to that of a bsAb containing C-terminally fused scDb (2Fab-Fc-1scDb) (pink) and to that of the isolated scDb module (orange), and **(C)** Cetuximab (dark blue) was compared to that of isolated Cetuximab Fab (light blue) or isolated human IgG Fc (green) or a mixture of both (red).

bsAb, bispecific antibody; Fab, fragment antigen-binding; Fc, constant fragment; IgG, immunoglobulin G; mAb, monoclonal antibody; scDb, single chain diabody; scFv, single chain fragment variable.

RESULTS

Altering target specificity in bsAbs induces large variance in FcRn dissociation pH





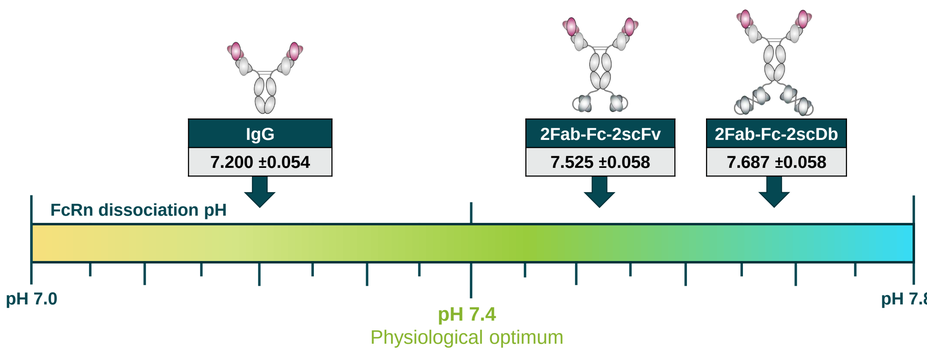
Origin of Fab domain	Antigen target
IDEC-C2B8	CD20
MOR208	CD19
NIST RM8761	RSV
XmAb14045	CD123
CSL-362	CD123
h7G3ec	CD123
MCLA-117	CLL1
SC02-357	CLL1
6E7L4Hle	CLL1
HRS3	CD30
GM102	AMHRII
PF-06747143	CXCR4
HA1–4.117	PSCA

Incorporation of different target domain sequences specific to different antigens within N-terminal Fab modules in bsAbs influences FcRn dissociation pH. FcRn dissociation pH of bsAbs containing different N-terminal target domain sequences in Fab modules, plotted against the isolelectric point of the whole molecules.

bsAb, bispecific antibody; DSB, stabilizing disulfide bond; Fab, fragment antigen-binding; Fc, constant fragment; scFv, single chain fragment variable.

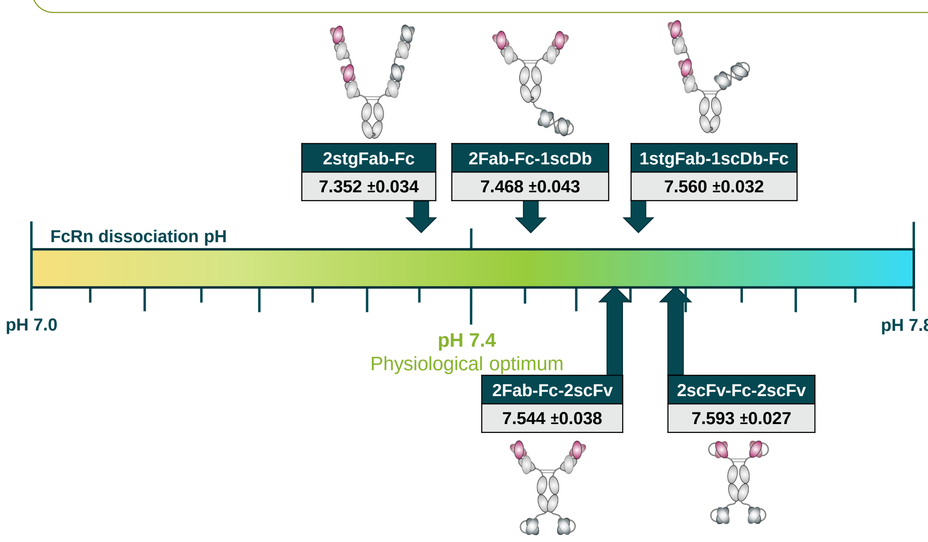
The number, module of presentation, and orientation of antigen-binding domains influence FcRn dissociation pH

Increasing the number of effector binding domains from 0 (standard IgG) to 2 (2× C-terminal scFv) to 4 (2× C-terminal scDb) significantly increases the pH at which bsAbs dissociate from FcRn



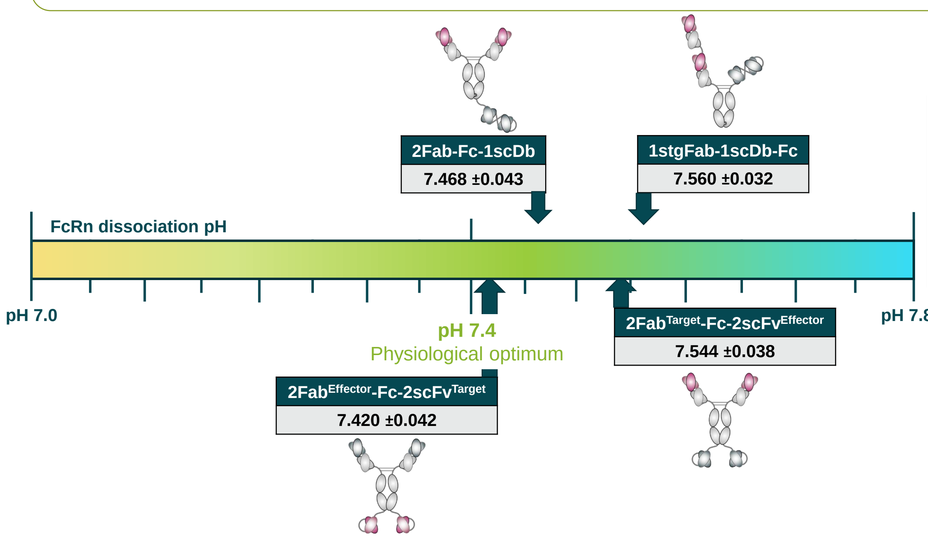
Comparison	P value
	<0.01
	<0.001
	<0.05

Presenting antigen-binding domains in a stgFab format vs scDb format on the N-terminus significantly affects FcRn dissociation pH; other tested module variations have a minimal effect



Comparison	P value
	ns
	ns
	<0.01

Presenting the same antigen binding domains in different positions and orientations in a bsAb significantly affects FcRn dissociation pH



Comparison	P value
	<0.05
	<0.05
	<0.05

Increasing the number of anti-CD16A effector domains, altering the module of presentation for an antigen-binding domain, and altering the position of an antigen-binding module within a bsAb significantly influences FcRn dissociation pH. The FcRn dissociation pHs of the indicated antibodies were determined by FcRn-pH-HPLC and comparisons performed as depicted in the tables to the right. pH scale schematics indicate the mean ± SD pH at which an antibody dissociated from FcRn. Statistical significance was determined by unpaired t-test following three independent experiments.

bsAb, bispecific antibody; Fab, fragment antigen-binding; Fc, constant fragment; mAb, monoclonal antibody; scDb, single chain diabody; scFv, single chain fragment variable; SD, standard deviation; stgFab, staggered fragment antigen-binding.

CONCLUSIONS

- FcRn-pH-HPLC directly measures the FcRn dissociation pH of therapeutic antibodies *in vitro*, allowing a direct comparison with FcRn dissociation pH *in vivo,* thus providing a surrogate assay for estimating serum half-life early in the development process
- The number, sequence, structure, and orientation of modules within bsAbs significantly affect FcRn dissociation pH and may, therefore, modify serum half-life
- This study may inform the generation of mono-, bi- and multispecific antibodies with tailored PK properties for optimal developability based on FcRn binding *in vitro*

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