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RESEARCH PAPER

Complex Pharmacokinetics of a Humanized Antibody Against Human Amyloid Beta Peptide, Anti-Abeta Ab2, in Nonclinical Species

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ABSTRACT

Purpose Anti-Aβ Ab2 (Ab2) is a humanized monoclonal antibody against amino acids 3–6 of primate (but not rodent) amyloid β (Aβ) and is being evaluated for the treatment of Alzheimer's disease (AD). This study was conducted to predict the human pharmacokinetics of Ab2.

Methods *In vivo* PK profile of Ab2 in preclinical species and *in vitro* mechanistic studies in preclinical and human systems were used for pharmacokinetic predictions.

Results In Tg2576 and PSAPP mice that have ~100-fold higher circulating levels of human Aβ compared to humans, elimination of Ab2 was target-mediated, such that exposure was 5–10 fold lower compared to wild-type rodents or to PDAPP mice that have human Aβ concentrations in plasma similar to humans. In cynomolgus monkeys, the $t_{1/2}$ of Ab2 was faster (<2.5 days) compared to that of the control antibody (~13 days). The fast elimination of Ab2 in cynomolgus monkeys was linked to off-target binding to cynomolgus monkey fibrinogen that was also causing incomplete recovery of Ab2 in cynomolgus monkey serum in blood partitioning experiments. Ab2 had significantly weaker to undetectable binding to human (and mouse) fibrinogen and had good recovery in human serum in blood partitioning experiments.

Conclusions These data predict that elimination of Ab2 in healthy or AD humans is expected to be slow, with $t_{1/2}$ similar to that observed for other humanized antibodies.

KEY WORDS Alzheimer's disease · anti-Abeta antibody · fibrinogen · human PK prediction · pharmacokinetics

INTRODUCTION

Alzheimer disease (AD) is a neurodegenerative disorder, characterized by the presence of an elevated burden of amyloid plaques in the brain. Amyloid beta (Aβ), a 40–42 amino acid peptide derived from human amyloid precursor protein (hAPP), is a major component of amyloid plaques and is thought to play a major role in pathogenesis of AD. For the treatment of AD, both passive Aβ immunization (i.e. peripheral administration of anti-Aβ antibodies) and active immunization with Aβ are being explored (1–5). The amino acid sequence of $Aβ_{1–42}$ is 100% conserved between humans and most primates; however, the N- terminal Aβ sequence is not conserved between primates and rodents. Therefore, transgenic mouse models of AD are used in the evaluation of the pharmacology and toxicology of anti-Aβ antibodies. Human APP transgenic mice (PSAPP Tg+, Tg2576, and PDAPP Tg+ mice) exhibit age-dependent changes that are similar to those seen in humans with AD (6–9). In mouse models of AD, administration of anti-Aβ antibodies or active immunization with Aβ improved cognitive function and reduced amyloid burden (3–5). Despite numerous preclinical studies with various anti-Aβ antibodies, there is no consensus on their mechanism of action. Proposed mechanisms of action of anti-Aβ antibodies in the central nervous system include Fc-receptor-mediated phagocyto-

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sis, disassembly of amyloid fibrils, as well as binding to soluble or oligomeric Aβ isoforms, preventing cytotoxic effects of Aβ oligomers (10–12). In addition, the peripheral mechanism of action has been proposed ("the peripheral sink hypothesis"), in which anti-Aβ antibodies bind soluble circulating Aβ, resulting in drainage of soluble brain Aβ into the blood. Several anti-Aβ antibodies are currently in development, with one in Phase III clinical trials (13). Characterization of pharmacokinetics (PK) and distribution of anti-Aβ antibodies in various murine models of AD and in nonhuman primates is important for a successful preclinical development of passive immunization approaches. Pharmacologically active regimens of anti-Aβ antibodies in hAPP-expressing mice have been reported to be dosing- regimen- and route-dependent, including reverse dose response (14,15). In this report, we describe preclinical PK profiles of anti-Aβ Ab2, which is a humanized antibody specific for amino acids 3–6 of human Aβ. We demonstrate that pharmacokinetics of anti-Aβ antibodies in preclinical species may be driven by both target- and off-target-mediated elimination mechanisms and are likely to be strain- and species-dependent. The *in vitro* mechanistic studies in animal and human systems, *in vivo* PK profile of anti-Aβ Ab2 in preclinical species, as well as target (Aβ) expression patterns in animals and humans were used to predict the PK profile of anti-Aβ Ab2 in humans.

MATERIALS AND METHODS

Test and Control Articles

Anti-Aβ Ab2 and control anti-$Aβ_{1–5}$ Ab humanized monoclonal IgG1 (k) antibodies against amino acids 3–6 and 1–5 of human Aβ, respectively, were generated at Pfizer (previously Wyeth Research) and produced using stable Chinese hamster ovary (CHO) cell lines. Formulation buffers were PBS (pH 7.2) or Histidine-Sucrose buffers (containing 5–10 mM histidine, 0–5% (w/v), 0–10 mM methionine (w/v), sucrose 0–4%mannitol (w/v), and/or 0.005% Polysorbate-80 (v/v); pH 6.0).

Animals

PSAPP Tg+ and transgene negative littermates (~1-year-old males and females) and Tg2576 mice (~3-month-old males) were from Pfizer (previously Wyeth Research, Princeton, NJ), PDAPP Tg+ mice (~1-year-old males and females) were from Janssen (previously Elan, Inc.; South San Francisco, CA), Sprague–Dawley rats (~2-month-old males with two jugular vein catheters) were from Taconic Farms (Germantown, NY), Wistar rats (~2–5-month-old males) were from Charles River, Inc. (Wilmington, MA). For *in vivo* PK studies, adult male cynomolgus monkeys (with no prior exposure to human-proteins) were not fasted and were housed at Pfizer (Pearl River, NY). For *in vitro* blood partitioning experiments, both male and female adult monkeys were used and were housed at Pfizer (Andover, MA).

Iodination Procedure and Preparation of [^{125}I]anti-Aβ Antibody Dosing Solution

For the *in vitro* blood partitioning studies, iodination was performed using the IODO-BEADS method according to manufacturer's instructions (Pierce, Rockford, IL) and labeled proteins were separated from free iodine as described previously (16). A spiking solution was prepared by mixing unlabeled test article (0, 6, or 20 μg/mL final concentration, as specified in Table I), a trace amount of ^{125}I-labeled test article, and a formulation buffer (PBS, pH 7.2). Specific activity ranged from 0.65–65 μCi/mg, and % free iodine, as determined by the trichloroacetic acid-precipitation (TCA) method, was ~1.0–6.5%.

For the *in vivo* study, iodination was performed using chloramine–T method and purified by size exclusion chromatography. Briefly, ~900 μg of test article was incubated for 1.5 min with 200 μg of 10 μg/mL chloramine–T (Fisher), ~9 mCi of ^{125}I (Amersham Pharmacia Biotech) and 0.1 mL of PBS, pH 7.2. The reaction was quenched by adding 20 μg of tyrosine, and free iodine was separated from [^{125}I]-labeled test article by size exclusion chromatography (NAP–10 column, Amersham Pharmacia Biotech). A dosing solution was prepared by mixing unlabeled test article (10 mg/mL final concentration), a trace amount of ^{125}I-labeled test article,

Table I Recovery of Anti-Aβ Ab2 in Plasma and Serum after *In Vitro* Spike into Whole Blood Obtained from Different Species

Species	Concentration (μg eq./mL)	Recovery in serum (%)	Recovery in plasma (%)
Cynomolgus monkeys ($n=6$)	20	50 ± 10^a	83 ± 11
	Tracer only	40 ± 1^a	75 ± 2
Human donors ($n=5$)	20	82 ± 4	82 ± 4
Tg2576 mice ($n=5$)	6	86 ± 5	90 ± 6
Wistar rats ($n=4$)	20	92 ± 3	91 ± 4

[a] Statistically significant difference between serum and plasma ($p < 0.001$), based on a paired Student *t*-test.

and a formulation buffer (PBS, pH 7.2). On the day of dosing, [^{125}I]anti-Aβ Ab2 dosing solution had ~11.7 μCi/mg specific activity, with 0.8% free iodine determined by the TCA-precipitation method and 2.4% free iodine determined by the ITLC (instant thin-layer chromatography) method.

PK Study Design

Test article was administered via IV or IP route at indicated dose levels (Table II and the text). For IV administration, the test article was administered as a single bolus dose into the tail vein, jugular vein via catheter, or saphenous vein for mice, rats, and monkeys, respectively. The dose was based on the most recent scheduled body weights, prior to dosing. The dosing volume was 4 mL/kg for mice, 1 mL/kg for rats and monkeys.

For the mouse studies, non-serial sampling design was used (n=3–6 per time point), with 1–3 blood draws per mouse (either by retro-orbital bleeding or by cardiac puncture from isoflurane-anesthetized animals). Serial sampling design was used for rat (n=6) and monkey studies (n=4 or n=3/group for radiolabeled or unlabeled test articles, respectively). Samples (serum for all studies and also plasma, with 3.2% sodium citrate as anticoagulant for money studies) for test article concentration analyses were collected at 5 min and then up to 6 weeks post dose. In the monkey study in which [^{125}I]anti-Aβ Ab2 was dosed, urine was collected daily during the 1 week period post dose.

All animal studies were performed in accordance with the National Institutes of Health guide for the care and use of laboratory animals, and Pfizer (previously Wyeth Research) Institutional Animal Care and Use Committee approved all aspects of these studies.

In Vitro Whole Blood Partitioning

Pre-weighted serum-separator or plasma (sodium citrate or EDTA as anticoagulant) tubes were spiked with [^{125}I]anti-Aβ antibody dosing solution or with unlabeled anti-Aβ antibody dosing solution (at specified concentrations) prior to blood collection. Blood samples from mice and rats were collected terminally by cardiac puncture. Blood samples from cynomolgus monkeys or human volunteers were drawn from the femoral and the median cubital vein, respectively. Collected blood was processed for serum or plasma by centrifugation within 1 h of collection.

Gamma-Count Analysis of Serum, Plasma, Whole Blood, and Urine Samples

The total and TCA-precipitable radioactivity in serum, plasma, whole blood, or urine samples (20–100 μL, in duplicates) was determined by gamma counting (Model 1480 WIZARD™, Wallac Inc., Gaithersburg, MD). Radioactive equivalent concentrations in serum, plasma, and whole blood samples were calculated as previously described (16). For the *in vitro* blood partitioning studies, the recovery of [^{125}I]test article in serum or plasma was calculated using the formula: [100% × plasma or serum concentration/[Dilution Factor (which was 2 for serum and 1.8 for

Table II PK Parameters of Anti-Aβ Ab2 in Mice, Rats, and Cynomolgus Monkeys

Strain species	Dose; Route (mg/kg)	C_{5min} or C_{max}[a] (μg/mL)	$AUC_{0-\infty}$ (μg*h/mL)	$AUC_{0-\infty}$/Dose (μg*h/mL)/(mg/kg)	CL (mL/h/kg)	Vd_{ss} (mL/kg)	$t_{1/2}$ (h)
PSAPP Tg+ mice	1; IV	14.4	333	333	3.01	139	49.9
	100; IV	2010	48641	486	2.06	78	58.5
PSAPP Tg- mice	1; IV	17.7	3165	3165	0.302	160	438
	100; IV	2501	150199	1502	0.663	248	368
Tg2576 Tg+ mice	1, IP	7.2	213	213	NA	NA	16
PDAPP Tg+ mice	20, IP	281	28707	1435	NA	NA	74.9
S-D Rats	2; IV	44.8 ± 4.6	6192 ± 781	3096 ± 391	0.328 ± 0.045	107 ± 9	254 ± 46
Cyno monkeys	2; IV	43.6 ± 7.45	2191 ± 463	1097 ± 232	0.941 ± 0.204	58 ± 8	52.6 ± 2.1
	10; IV	234 ± 22.0	11979 ± 775	1198 ± 77.5	0.837 ± 0.053	53 ± 3	54.7 ± 16
	10; IV (gamma-counting)[b]	218 ± 51.7	8908 ± 2197	891 ± 220	1.19 ± 0.36	67 ± 16	50.8 ± 9.9

[a] Concentration at 5 min (C5min) is shown for IV route and Cmax is shown for IP route. Tmax was 6 h for the two IP studies.

[b] [^{125}I]-labeled anti-Aβ Ab2 was dosed.

CL Total body clearance. Vd_{ss} Volume of distribution at steady-state. $t_{1/2}$ Elimination half-life. $AUC_{0-\infty}$ Area under the concentration-time curve from time 0 to infinity. *NA* Not applicable.

In mouse studies, PK parameters were determined based on mean serum concentrations (n = 3 per time point). Individual concentration data below the LOQ was treated as zero for calculations of mean and SD. In rats, PK parameters were determined based on individual animal serum concentrations, with n = 6. In monkeys, PK parameters were determined based on individual plasma concentrations, with n = 4 in the [^{125}I]anti-Aβ Ab2 study and n = 3/group in the other study.

plasma) × blood concentration)]. Cumulative radioactivity in the urine (cumulative excreted cpm, as % dose at day *t*) was defined as the sum of daily urinary excretions (excreted cpm adjusted for iodine decay, as % of dose) from day 0 to day *t*.

Immunoassay for Quantitation of anti-Aβ Antibodies in Serum or Plasma

Anti-Aβ Ab2 and control anti-$Aβ_{1–5}$ Ab concentrations were determined using enzyme-linked immunosorbent assays (ELISA). A test article was captured by a DAE-10 peptide (conjugated to either biotin or bovine serum albumin [BSA]) and was detected with a mouse anti-human IgG (Fc) horseradish peroxidase (HRP) conjugate. DAE-10 peptide contains the N-terminal 10 amino acids of the amyloid-β peptide (Aβ), followed by 14 amino acids that constitute a hydrophilic barrel (DAEFRHDSGYS-GENRSDQK). Tetramethylbenzidine (TMB) was used as the enzyme substrate for the colorimetric readout at 450 nm. Sample concentrations were determined by interpolation from a calibration curve that was fit using a four-parameter logistic equation (Softmax Pro, version 4.3.1, Molecular Devices and Watson LIMS, version 7.0.0.01, Thermo Electron Corporation). The lower limit of quantitation (LLOQ) ranged from ~33 to 100 ng/mL.

Pharmacokinetic Calculations

In rat and monkey studies, PK parameters were determined for each individual animal. In mouse studies, PK parameters were determined based on the mean concentration profiles. Non-compartmental analysis module (Model 200 for IP route and Model 201 for IV route) of the pharmacokinetic software package WinNonlin, ver. 5.1 (Pharsight, Mountain View, CA) was used for PK calculations.

Immunoprecipitation and Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Biotinylated anti-Aβ Ab2 or control anti-Aβ Ab (10 μg for mass spectrometry (MS) analysis and 0.5 μg for Western blotting (WB)) were incubated with 20 mL of pooled cynomolgus monkey (Bioreclamation) or human (Rockland) plasma or serum (20 mL for MS and 0.5 mL for WB), and bound proteins were precipitated with strepatavidin-coated magnetic beads (1 mg for MS and 50 μg for WB; Invitrogen). The samples obtained by immunoprecipitation or original plasma or serum were loaded onto a 10% Bis-Tris NuPage gel. Electrophoresis was carried out in MOPS buffer (Invitrogen), and gel was stained with Coomassie Blue for mass spectrometry (MS) analysis or transferred to a PVDF membrane (Immobilon-P; Millipore) for WB analysis.

Mass Spectrometry and Western Blot Analyses

In-gel trypsin digestion was carried out on excised gel slices of the protein bands of interest using the Abimed DigestPro automated digestor (Abimed, Langenfeld, Germany) according to the manufacturer's protocol. After volume reduction, half of each sample (10 μL) was applied to a 11 cm×75 μm C18 reverse-phase column packed with Magic C18AQ (Michrom Bioresources Inc.) in a Picofrit needle that was in line with an LTQ mass spectrometer (Thermo). Liquid chromatography was carried out using an Agilent 1100 nanopump, and the peptides were eluted with a gradient of 10–50% of Buffer B (98% acetonitrile and 0.1% formic acid). The peptide masses were recorded by scanning an m/z range from 375 to 1200. The fragment ion spectra (MS/MS) were acquired in a data-dependent manner in which each MS scan was followed by consecutive MS/MS scans on the first four most intense ions from the MS scan. The resulting MS/MS data were searched against the NCBI non-redundant database consisting of human and cynomolgus monkey sequences using the Sequest program. Results were summarized using Sequest-On-Oracle software developed in-house.

For Western blot analysis, each membrane was probed with a goat anti-fibrinogen-β antibody (Santa Cruz Biotechnology, 1.0 μg/mL) diluted in Tris-Buffered Saline with Tween (TBST) with 5% Blotto (Santa Cruz Biotechnology). The membranes were washed with TBST and then incubated with HRP-conjugated anti-goat IgG (Rockland, 1.0 μg/mL). The blot was developed using Super Signal West Pico chemilumenescent substrate (Pierce), and the resulting signal was captured using a Kodak image station.

RESULTS

Recovery of [^{125}I]anti-Aβ Ab2 in Serum and Plasma After *In Vitro* Spike into Whole Blood

To select the sampling matrix for PK studies, we tested recovery of [^{125}I]anti-Aβ Ab2 after *in vitro* spike into whole blood samples obtained from mice, rats, cynomolgus monkeys, and normal human volunteers. [^{125}I]anti-Aβ Ab2 spiking solution was prepared by mixing unlabeled anti-Aβ Ab2 (at 6 to 20 μg eq./mL final concentration) and [^{125}I]anti-Aβ Ab2 tracer. This spiking solution was added to fresh whole blood samples, which then were partitioned into serum and plasma and analyzed for [^{125}I]anti-Aβ Ab2 radioactive equivalent concentrations by gamma counting. [^{125}I]anti-Aβ Ab2 had similar (approximately 80% to100%) recovery in serum and plasma after *in vitro* addition to blood from hAPP transgene-expressing Tg2576 mice, Sprague-Dawley rats, and human volunteers (Table I). In contrast to

the other species tested, approximately 50% of [^{125}I]anti-Aβ Ab2 was recovered in cynomolgus monkey serum obtained from whole blood, while recovery in monkey plasma was approximately 83% and similar to that seen in plasma of other species. Another anti-Aβ antibody (binding to Aβ1-5 (anti-$Aβ_{1–5}$ Ab)) had good recovery in cynomolgus monkey serum and plasma (~75–95%) in the similar blood partitioning experiment (data not shown). These blood partitioning data suggested that [^{125}I]anti-Aβ Ab2 is not completely recovered upon *ex vivo* processing of cynomolgus monkey blood for serum. For cynomolgus monkey samples, blood partitioning experiments were also performed with the unlabeled anti-Aβ Ab2 (with plasma and serum concentration measurements performed by the ELISA), and the results were similar to those obtained with the iodinated test article (data not shown). Therefore, both plasma and serum were collected in PK studies of anti-Aβ Ab2 in cynomolgus monkeys.

Pharmacokinetics of Anti-Aβ Ab2 in Mice and Rats

Wild-type or human APP-expressing transgenic (PSAPP Tg+, Tg2576, and PDAPP Tg+) mice and wild-type (Sprague-Dawley) rats were given a single IV or IP dose of anti-Aβ Ab2. Serum concentrations of anti-Aβ Ab2 were measured by specific immunoassays, and PK parameters were calculated by non-compartmental analysis. Aged ~12-month-old PSAPP and PDAPP mice and ~3-month-old Tg2576 mice were used for PK studies to match the respective age range employed for the pharmacology and toxicology studies. It should be noted that different dose levels and routes of administration were used for different strains, since the primary goal of PK assessments was to support parallel pharmacology or toxicology studies, which utilized different routes and dosing regimens. In addition, in lieu of non-serial sampling design in mouse PK studies and the potential for anti-product antibody response against human antibodies in rodents, the significant variability in the estimated $t_{1/2}$ was expected. Therefore, all PK parameters need to be examined together when interpreting potential inter-strain differences in PK profiles.

After a single IV dose to wild-type (PSAPP Tg-) mice and rats, anti-Aβ Ab2 was cleared slowly from the circulation, as evidenced by a low total body clearance (CL of ~0.3–0.6 mL/h/kg), long elimination half-life ($t_{1/2}$ of ~10–18 days), and high dose-normalized exposure ($AUC_{0–\infty}$/Dose) (Fig. 1 and Table II).

After a single IV or IP dose of anti-Aβ Ab2 to PSAPP Tg+ and Tg2576 mice with plasma concentrations of human Aβ of at least 100-fold higher than those in humans (8,17–19), the elimination of anti-Aβ Ab2 was faster when compared with wild-type mice and rats, evidenced by increased CL, shorter $t_{1/2}$ values, and lower dose-normalized $AUC_{0–\infty}$/Dose (Fig. 1 and Table II). Specifically, after IV administration of anti-Aβ Ab2 to PSAPP Tg+ mice, $t_{1/2}$ values were ~2.0–2.5 days, CL values were ~3-2 mL/h/kg, and $AUC_{0–\infty}$/Dose values were 333–486 μg*h/mL, which corresponded to ~5–10-fold difference when compared to those PK parameter in wild-type mice or rats. After single IP administration of anti-Aβ Ab2 to Tg2576 mice, the $t_{1/2}$ and $AUC_{0–\infty}$/Dose were ~16 h and 213 μg*h/mL, respectively. The decrease in apparent $t_{1/2}$ (as well as in dose normalized $AUC_{0–\infty}$) after a single IP administration to Tg2576 mice compared to wild-type mice was also observed for a control anti-$Aβ_{1–5}$ Ab (data not shown). Thus, the mechanism of the faster CL and shorter $t_{1/2}$ of anti-Aβ Ab2 in PSAPP Tg+ and Tg2576 human APP-expressing mice appeared to be antigen (Aβ) dependent.

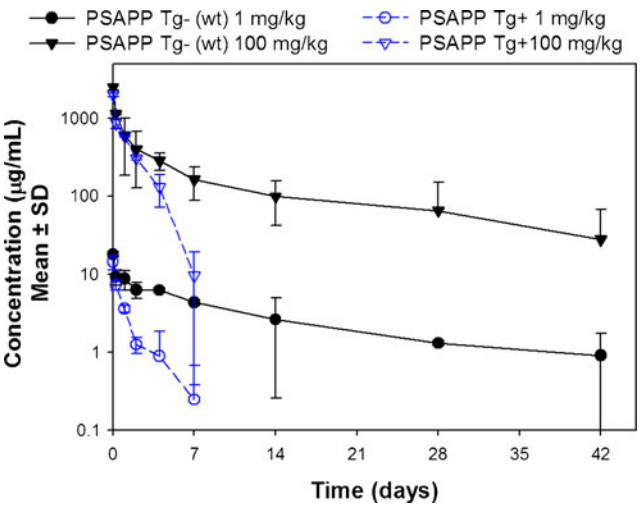

Fig. 1 Mean serum concentrations of anti-Aβ Ab2 following a single IV dose to PSAPP Tg+ and wild-type control (Tg- littermate) mice. Approximately one-year-old PSAPP Tg+ (open symbols, *dashed line*) or wild-type (PSAPP Tg-) littermates (closed symbols, *solid line*) were given a single 1 mg/kg (*circles*) or 100 mg/kg (*triangles*) IV dose of anti-Aβ Ab2. Serum concentrations were monitored by ELISA over the 42-day period. Individual animal concentration values that were less than the limit of quantitation (LOQ of 80 ng/mL) were treated as zero for calculations of the mean and standard deviation ($n = 3$ per time point per strain).

PK of anti-Aβ Ab2 was also examined in another strain of human APP expressing mice, PDAPP, following a single 20 mg/kg IP dose. PDAPP mice have hAPP expression pattern similar to those in humans, with ~100-fold lower plasma Aβ concentrations compared to those in Tg2576 or PSAPP mice (8,17–19). After a single 20 mg/kg IP dose of anti-Aβ Ab2 to PDAPP mice, the $AUC_{0–\infty}$/Dose was similar to that observed after a single 100 mg/kg IV dose to PSAPP Tg- mice and 3–7-fold higher compared to that in PSAPP Tg+ or Tg2576 mice (Table II). The estimated $t_{1/2}$ of anti-Aβ Ab2 after IP administration to PDAPP mice was ~3.1 days, which was longer than that in PSAPP Tg + Tg2576 mice, but appeared to be not as long as that after IV administration to PSAPP Tg- mice, possibly due to study-to-study variability (Table II). Thus, the exposure

($AUC_{0-\infty}$/Dose) of anti-Aβ Ab2 was similar in wild-type and PDAPP mice. It should also be noted that the exposure of anti-$A\beta_{1-5}$ Ab was similar after IP administration to PDAPP *versus* wild-type mice (data not shown).

Thus, the overall PK profile of anti-Aβ Ab2 in mice suggested that anti-Aβ Ab2 was eliminated faster after a single dose to Tg2576 and aged PSAPP Tg+ mice, compared to wild-type aged PSAPP Tg- mice or to aged PDAPP mice (or to wild-type rats). Similarly, a control anti-$A\beta_{1-5}$ Ab appeared to be eliminated faster after a single IP dose to Tg2576 mice, compared to wild-type or PDAPP mice.

Pharmacokinetics of Anti-Aβ Ab2 in Cynomolgus Monkeys

Two studies were conducted to assess PK of anti-Aβ Ab2 in cynomolgus monkeys. In the first study, 2 and 10 mg/kg single IV doses were used (n=3/group), and anti-Aβ Ab2 concentrations in plasma (and serum for comparison) were determined by immunoassays. In the second study, 4 cynomolgus monkeys were administered a single 10 mg/kg IV dose of [^{125}I]-labeled anti-Aβ Ab2, and [^{125}I]anti-Aβ Ab2 radioactive equivalent concentrations in plasma (and serum) were determined by gamma counting. In addition, excretion of ^{125}I in urine of [^{125}I]anti-Aβ Ab2-dosed cynomolgus monkeys was also assessed.

The elimination of anti-Aβ Ab2 in cynomolgus monkey was faster compared to another anti-$A\beta_{1-5}$ control antibody (Fig. 2a) or compared to anti-Aβ Ab2 elimination in wild-type mice and rats (Table II). Specifically, in cynomolgus monkeys, CL of anti-Aβ Ab2 determined using plasma concentrations was 0.84–1.2 mL/h/kg, and $t_{1/2}$ of anti-Aβ Ab2 was 2.1–2.3 days (Table II), while the $t_{1/2}$ of the control anti-$A\beta_{1-5}$ Ab was ~13 days. The average volume of distribution at steady-state (Vd_{ss}) in plasma of anti-Aβ Ab2 was low (<60 mL/kg), suggesting that anti-Aβ Ab2 was mainly distributed in the vascular space and to limited extracellular fluids after IV administration.

In serum, the apparent concentration of anti-Aβ Ab2 at 5 min (C_{5min}), was 3–4-fold lower, and apparent exposure ($AUC_{0-\infty}$) was 6–7-fold lower as compared to that in plasma (Fig. 2b and data not shown). Since the decline in serum concentrations was parallel to that in plasma, $t_{1/2}$ values of anti-Aβ Ab2 in serum and plasma were comparable. The serum concentration profiles obtained after *in vivo* administration of anti-Aβ Ab2 to cynomolgus monkeys were consistent with poor recovery of [^{125}I]anti-Aβ Ab2 in serum of cynomolgus monkeys observed in the *in vitro* blood partitioning experiments (Table I). Thus, plasma, rather than serum, concentration data were considered relevant for calculations of final PK parameters of anti-Aβ Ab2 in cynomolgus monkeys (Table II).

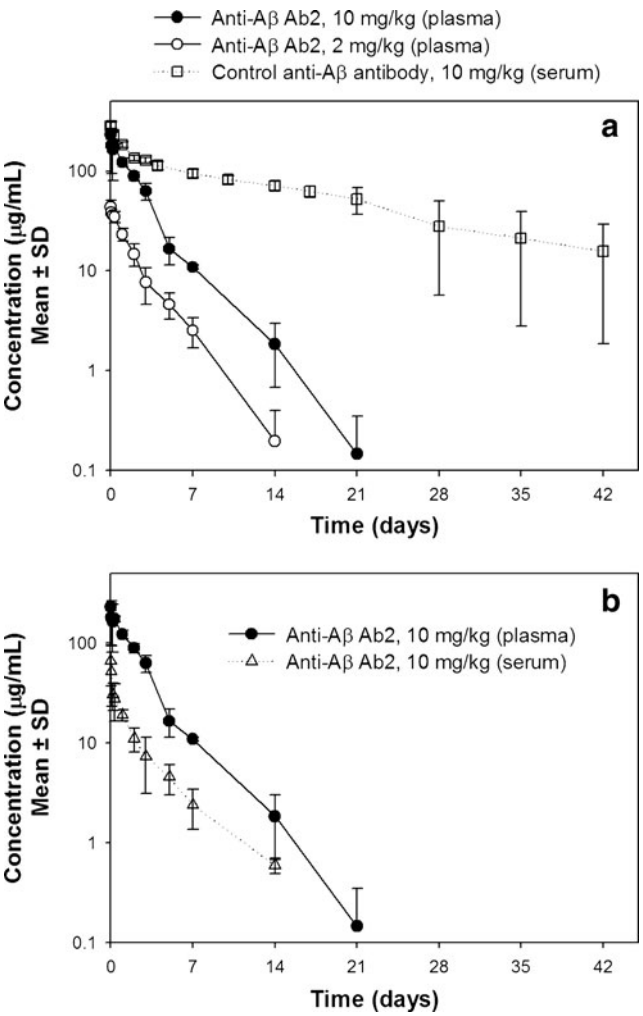

Fig. 2 Mean plasma and serum concentrations of anti-Aβ Ab2 and the control anti-Aβ Ab following a single IV dose to cynomolgus monkeys. (**a**) Protein-naïve male adult cynomolgus monkeys were given a single 2 (*open circle*) or 10 mg/kg IV dose (*closed circle*) of anti-Aβ Ab2 or a single 10 mg/kg IV dose of the control anti-Aβ Ab (*open square*). Concentrations of anti-Aβ Ab2 and control anti-Aβ Ab were monitored by ELISA over the 42-day period in plasma and serum, respectively. Individual animal concentration values that were less than the limit of quantitation (LOQ of 50 ng/mL) were treated as zero for calculations of the mean and standard deviation (n = 3 per group). (**b**) Following a single 10 mg/kg IV dose to cynomolgus monkeys, anti-Aβ Ab2 concentrations were lower in serum (*open triangles*) compared to those in plasma (*closed circles*) for all time points tested.

It should be noted that following 10 mg/kg IV administration to cynomolgus monkeys, the PK profile of [^{125}I]-labeled anti-Aβ Ab2 assessed by gamma counting were similar to the PK profile of unlabeled anti-Aβ Ab2 assessed by ELISA (Table II). In accordance with the relatively short $t_{1/2}$ (<2.5 days) of [^{125}I]anti-Aβ Ab2 in plasma of cynomolgus monkeys after a single IV dosage, approximately 96% of the total administered radioactivity was excreted in urine 1 week after dosing, mostly as free iodine. This result suggested that there was no noteworthy

uptake of anti-Aβ Ab2 in the cynomolgus monkey tissues and that the short $t_{1/2}$ of anti-Aβ Ab2 was most likely caused by metabolism/catabolism of anti-Aβ Ab2.

Plasma Protein Interactions with Anti-Aβ Ab2 in Mice and Primates

To investigate incomplete recovery of [^{125}I]anti-Aβ Ab2 in serum (but not in plasma) in whole blood partitioning experiments, the interactions of anti-Aβ Ab2 with plasma proteins from multiple primate species (cynomolgus and rhesus monkeys, baboons, marmosets and humans), as well as mice, were evaluated.

In the initial experiments, anti-Aβ Ab2-biotin was added to cynomolgus monkey or human plasma or serum, and bound proteins were captured by streptavidin-coated magnetic beads. These specifically reactive proteins were visualized with either Coomassie Blue or silver staining of one-dimensional polyacrylamide gel and identified using mass spectrometry. While there were common bands observed in most samples, there were predominant bands only observed in anti-Aβ Ab2-spiked cynomolgus monkey plasma. SEQUEST search results of the mass spectra of peptides derived from the predominant protein bands yielded the identification of the three fibrinogen subunits. Specifically, upon filtering the SEQUEST search results for those peptides with a Xcorr >= 2 and a rank of one, the predominant proteins identified from the indicated bands in Fig. 3a were as follows: Fibrinogen beta, 153 total peptides (53 unique) with a total protein coverage of 67%; fibrinogen gamma, 98 total peptides (21 unique) with a total protein coverage of 31%; and fibrinogen alpha, 78 total peptides (16 unique) with a total protein coverage of 51%. These fibrinogen-related proteins specifically immunoprecipitated with anti-Aβ Ab2 (but not with the control anti-$Aβ_{1-5}$ Ab) in cynomolgus monkey plasma (Fig. 3a). The mass spectrometry results were confirmed by immunoprecipitation of anti-Aβ Ab2 from plasma samples and Western blot analysis with anti-fibrinogen β antibody. Similar amounts of fibrinogen were immunoprecipitated from cynomolgus and rhesus monkey plasma, slightly less immunoprecipitated from baboon plasma, and markedly less to undetectable (with some inter-donor variability) from human and marmoset plasma (Fig. 3b and c and data not shown). In addition, in contrast to cynomolgus monkey and rhesus plasma, no detectable amounts of fibrinogen immunoprecipitated with anti-Aβ Ab2 in mouse plasma (data not shown).

DISCUSSION

In this report, we evaluated pharmacokinetics of anti-Aβ Ab2, a humanized antibody specific for amino acids 3–6 of human and non-human primate (but not rodent) Aβ, in wild-type and human APP-expressing mice, wild-type rats, and cynomolgus monkeys. Our data indicated that pharmacokinetics of anti-Aβ Ab2 were strain- and species-dependent, likely due to differential contribution of target (Aβ)-mediated clearance and off-target (fibrinogen)-dependent disposition in various nonclinical species and strains. The *in vitro* mechanistic studies in animal and human systems, the *in vivo* PK profiles of anti-Aβ Ab2 in preclinical species, as well as Aβ expression patterns in various species

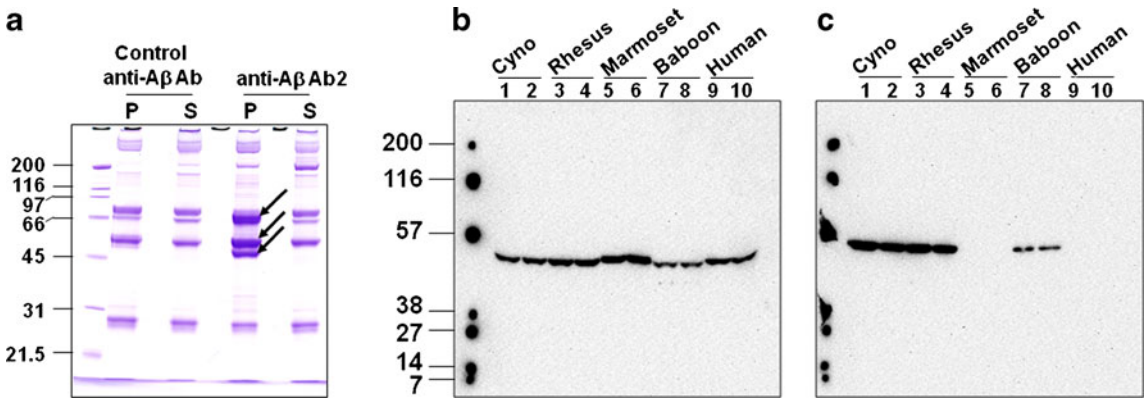

Fig. 3 Analysis of the interaction between anti-Aβ Ab2 and fibrinogen-β across five primate species. Either anti-Aβ Ab2-biotin or control anti-Aβ Ab-biotin (as indicated) was used to capture interacting proteins from primate plasma (P) or serum (S) by streptavidin coated magnetic beads. (**a**) For mass spectrometry analysis, immunoprecitpitated samples from cynomolgus monkeys ("cyno") were subjected to gel electrophoresis, and proteins were visualized by Coomassie Blue staining. The bands of interest that were identified as α (top), β (middle), and γ (bottom) subunits of fibrinogen are indicated with arrows. (**b** and **c**) For Western blot analysis, plasma from each indicated primate species was loaded (in duplicate) either directly (**b**, 1 μL/lane) or after immunoprecipitation with anti-Aβ Ab2-biotin (**c**) on to the SDS-polyacrylamide gel, transferred to a PVDF membrane, and probed with goat anti-fibrinogen-β. Molecular weight markers are indicated on the left of each panel.

predicted that elimination of anti-Aβ Ab2 in normal or AD humans is expected to be slow, with CL and $t_{1/2}$ similar to that for a control anti-$A\beta_{1-5}$ Ab.

For monkey PK studies, PK studies in PSAPP mice (16), and *in vitro* blood partitioning studies, two different assay formats were used: gamma counting upon dosing with the ^{125}I-labeled test article and ELISA, which uses antigen containing peptide as a capture and anti-human Fc as a detector. Both assay formats gave comparable results, suggesting that ELISA was able to detect most of the forms of circulating test article (including anti-Aβ antibody in complex with Aβ or fibrinogen) and that iodination had no significant impact on Aβ or fibrinogen binding.

The PK profile of anti-Aβ Ab2 in mice suggested that anti-Aβ Ab2 was eliminated faster after a single dose to Tg2576 and PSAPP Tg+ compared to wild-type rodents or to PDAPP mice with little or no change in Vd_{SS} (particularly at the low dose), as evidence by a 3–10-fold decrease in dose-normalized exposure, faster CL, and shorter half-life. Similarly, a control anti-$A\beta_{1-5}$ Ab appeared to be eliminated faster after a single dose to Tg2576 mice, compared to wild-type or PDAPP mice (data not shown).

PSAPP and Tg2576 mice express the same human APP (hAPP) gene, use the same promoter for expression of the hAPP gene, and have less restricted tissue expression of Aβ relative to PDAPP mice or humans, which express hAPP primarily in the brain (8). In Tg2576 (and PSAPP) mice, plasma Aβ concentrations are over 100-fold higher than in PDAPP mice or in humans (8,17–19, and Pfizer, unpublished observations). In accordance, elimination of anti-Aβ antibodies in strains that have high Aβ levels was faster, suggesting that antigen (Aβ)-dependent mechanism accounted for fast clearance of anti-Aβ antibodies from Tg2575 or PSAPP mice.

Differences in ADME profile of a monoclonal antibody in target-expressing *versus* control animals, as well as effects of soluble target-antibody complexes of antibody disposition, have been reported previously (20,21), with the general observation/assumption that these types of phenomena are saturable with the increasing doses of a test article. Since Aβ turnover rate in plasma is relatively high (~3 min (22)) and baseline Aβ levels are high compared to other mouse strains (suggesting a relatively high rate of synthesis), it is anticipated that relatively high doses of anti-Aβ antibodies would be needed to saturate target-mediated disposition in PSAPP mice. However, there was only a small decrease in CL of anti-Aβ Ab2 at a very high dose of 100 mg/kg, which was unexpected even if the high turnover/synthesis rates of the target are taken into account. Therefore, additional studies are needed to characterize the disposition of anti-Aβ antibodies in PSAPP mice, including studies on saturation of the observed phenomenon.

It should be noted that due to differences in the target expression pattern and levels in PSAPP mice *versus* humans, the pharmacological and toxicological (as well as pharmacokinetic) data in PSAPP mouse model should be interpreted with caution. Specifically, since humans (healthy as well as AD patients) and non-human primates (NHPs) have significantly lower circulating levels of Aβ with restricted expression pattern (8,17–19, and Pfizer, unpublished observations), and it is considered unlikely that antigen (Aβ)-dependent mechanism would have significant contribution to elimination of anti-Aβ antibodies from humans or NHPs. In fact, a control anti-$A\beta_{1-5}$ Ab was eliminated slowly from cynomolgus monkeys, with $t_{1/2}$ of ~13 days.

PK profile of anti-Aβ Ab2 in cynomolgus monkeys appeared to be affected by off-target binding. There was reduced (~50%) recovery of anti-Aβ Ab2 (but not of a control anti-$A\beta_{1-5}$ antibody) in cynomolgus monkey serum, upon processing of whole blood that was either spiked *in vitro* with anti-Aβ Ab2 or obtained from anti-Aβ Ab2-dosed monkeys. The elimination of anti-Aβ Ab2 in cynomolgus monkeys was faster than expected ($t_{1/2}$ <2.5 days), when compared with that for a control anti-$A\beta_{1-5}$ Ab ($t_{1/2}$ of ~13 days). The *in vitro* co-immunoprecipitation and mass spectrometry data indicated that anti-Aβ Ab2 (but not a control anti-$A\beta_{1-5}$ Ab) interacted with fibrinogen present in cynomolgus and rhesus monkey plasma, as well as baboon plasma. Binding of anti-Aβ Ab2 to marmoset, human, and mouse fibrinogen appeared to be significantly weaker to undetectable. The interaction of anti-Aβ Ab2 with cynomolgus monkey fibrinogen, a major component of the blood clot, suggested that the incomplete recovery of anti-Aβ Ab2 in serum of cynomolgus monkeys was likely due its localization to the blood clot.

Binding of anti-Aβ Ab2 to cynomolgus monkey fibrinogen *in vivo* was considered to be the likely cause of the relatively short plasma $t_{1/2}$ of anti-Aβ Ab2 observed in cynomolgus monkeys. Further studies are needed to elucidate the mechanistic relation between the fibrinogen binding and accelerated clearance of anti-Aβ Ab2 in cynomolgus monkeys. Compared to unbound anti-Aβ Ab2, anti-Aβ Ab2/fibrinogen complexes may be cleared faster from circulation because of the accelerated elimination of immune complexes by the reticuloendothelial system. In addition, potential of anti-Aβ Ab2 to localize to the fibrin clots may lead to accelerated degradation of this antibody. While the mechanism of fibrinogen clearance remains to be fully elucidated, it is believed that most of the fibrinogen is catabolized through the clotting mechanisms (23). Under physiological condition, fibrinogen is believed to be protected from degradation by fibrinolytic inhibitors. Fibrinogen is continuously clotted to fibrin, and since the inhibitors do not bind to fibrin, the formed fibrin is catabolized. The observed apparent elimination half-life of anti-Aβ Ab2 in cynomolgus monkeys in the single dose PK studies (~2–2.5 days) is in the range for that reported

for fibrinogen in primate species (1.2–2 days in rhesus monkeys; 3–5 days for humans) (24–27), in line with the hypothesis that anti-Aβ Ab2 and fibrinogen share common elimination mechanisms in primate species in which anti-Aβ Ab2/fibrinogen binding is observed. The elimination half-life of cynomolgus monkey fibrinogen has not been reported; however, it is likely to be in the range observed for other primates. Since fibrinogen plasma levels are relatively high in primates (~2–2.5 mg/mL in cynomolgus monkeys (28)), a significant fraction of administered anti-Aβ Ab2 may be bound to cynomolgus monkey fibrinogen *in vivo* and be cleared by fibrinogen-dependent mechanisms.

Further studies are needed to elucidate the mechanistic relation between the fibrinogen binding and accelerated clearance of anti-Aβ Ab2 in cynomolgus monkeys, including studies on saturation of the observed phenomena in the *ex vivo* and *in vivo* setting. In addition, the molecular basis of interaction between the anti-Aβ Ab2 and cynomolgus monkey fibrinogen remains to be understood. It is likely that anti-Aβ recognizes a conformation-dependent or non-linear conformational epitope on fibrinogen, because anti-Aβ Ab2 does not bind to denatured fibrinogen on Western blots (data not shown). If this conformational epitope is indeed present on cynomolgus and rhesus monkey and baboon fibrinogen, it is likely to encompass Aβ sequence outside the 1–5 region (since a control anti-$Aβ_{1–5}$ Ab has no detectable binding to cynomolgus monkey fibrinogen) and is not expected to be completely conserved between the fibrinogens of various primate species. The interaction of other anti-Aβ antibodies with human fibrinogen has been reported previously ((29) and beta Amyloid antibody, clone 3G5 http://www.abcam.com/beta-Amyloid-antibody-3G5-ab17905.html). Furthermore, there is a reciprocal cross-reaction between anti-human fibrinogen antibodies and Aβ peptide (29). These cross-reacting antibodies were generated by immunization with $Aβ_{1–28}$ protein, and the exact epitopes of the resulting monoclonal antibodies were not mapped. These interactions were ascribed to conformational, rather than linear homologies between the Aβ peptide and fibrinogen. In general, there are many parallels between Aβ and fibrin assembly, aggregation, and cross-linking. The presence of common conformational epitope on Aβ and fibrinogen may be a molecular basis for ability of Aβ to modulate fibrin assembly and polymerization (30). In fact, fibrin clots formed in the presence of Aβ are structurally abnormal and resistant to degradation and suggested that one important contribution of Aβ to the pathogenesis of AD is via its effects on fibrin clots (31).

It should be noted that both fibrinogen and Aβ bind the α2β3 integrins through RGD and RHDS (corresponding to amino acids 5 to 8 of Aβ) motifs, respectively (32,33). Since anti-Aβ Ab2 binds to similar region of Aβ as α2β3 integrins, it is possible that anti-Aβ Ab2 interacts with fibrinogen through the same RGD region. We have aligned the published sequences of human and rhesus monkey fibrinogen (cynomolgus monkey fibrinogen sequence is not available) to examine possible differences in the RGD-intergin recognition region. The alignment analysis indicated that rhesus monkeys have RGDIVSS sequence, while humans have RGDFSSA sequence in the region of interest on fibrinogen. These sequence differences may contribute to a different recognition of human *versus* rhesus monkey fibrinogen by anti-Aβ Ab2, as well as differences in overall folding of the fibrinogen molecules across the different primate species. Additional studies are needed to determine the binding epitope of anti-Aβ Ab2 on rhesus and cynomolgus monkey fibrinogen. Regardless of the epitope, our *in vitro* binding and blood partitioning data indicate that, in contrast to rhesus or cynomolgus monkeys, interaction of anti-Aβ Ab2 with human fibrinogen is very weak to undetectable and unlikely to substantially affect PK profile of anti-Aβ Ab2 in humans.

In summary, PK profile of anti-Aβ Ab2 in nonclinical species was complex, due to species and strain differences in target (Aβ) and off-target (fibrinogen)-mediated disposition. In Tg2576 (and PSAPP) mice, plasma Aβ concentrations are over 100-fold higher than in PDAPP mice and humans. In accordance, elimination of anti-Aβ Ab2 (as well as of a control anti-$Aβ_{1–5}$ Ab) in PSAPP and Tg2576 mice was markedly faster compared to wild-type mice or rats or PDAPP mice, suggesting that antigen (Aβ)-dependent mechanism accounted for fast clearance of anti-Aβ antibodies from Tg2575 or PSAPP mice. Since humans (healthy or AD patients) have significantly lower circulating levels of Aβ with restricted tissue expression pattern, it is considered unlikely that antigen (Aβ)-dependent mechanism would have significant contribution to elimination of anti-Aβ antibodies in humans. In cynomolgus monkeys, the elimination of anti-Aβ Ab2 was faster than expected ($t_{1/2}$ <2.5 days), when compared with that for a control anti-$Aβ_{1–5}$ Ab ($t_{1/2}$ of ~13 days). While not entirely proven, the fast elimination of anti-Aβ Ab2 in cynomolgus monkeys is likely related to off-target binding to cynomolgus monkey fibrinogen, which has $t_{1/2}$ similar to that of anti-Aβ Ab2 in monkeys. Additional studies are needed to determine the binding epitope of anti-Aβ Ab2 on cynomolgus monkey fibrinogen and provide a mechanistic link between the fibrinogen binding and faster elimination; however, the presence of the common conformational epitope on fibrinogen and Aβ has been reported previously. *In vitro* binding and blood partitioning data indicated that in contrast to rhesus or cynomolgus monkeys, interaction of anti-Aβ Ab2 with human fibrinogen is very weak to undetectable and unlikely to substantially affect PK profile of anti-Aβ Ab2 in humans. The *in vitro* mechanistic studies in rodent, NHP, and human systems, *in vivo* PK profile of anti-Aβ Ab2 in preclinical species, as well as Aβ

expression patterns in various species were used to predict that elimination of anti-Aβ Ab2 in normal or AD humans is expected to be slow, with CL and $t_{1/2}$ similar to that for a typical IgG1 antibody.

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REFERENCES

1. Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, *et al.* Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. Nat Med. 2000;6:916–9.
2. Games D, Bard F, Grajeda H, Guido T, Khan K, Soriano F, *et al.* Prevention and reduction of AD-type pathology in PDAPP mice immunized with A beta 1–42. Ann N Y Acad Sci. 2000;920:274–84.
3. Lemere CA, Maier M, Jiang L, Peng Y, Seabrook TJ. Amyloid-beta immunotherapy for the prevention and treatment of Alzheimer disease: lessons from mice, monkeys, and humans. Rejuvenation Res. 2006;9:77–84.
4. Jicha GA. Is passive immunization for Alzheimer's disease 'alive and well' or 'dead and buried'? Expert Opin Biol Ther. 2009;9:481–91.
5. Wilcockand DM, Colton CA. Anti-amyloid-beta immunotherapy in Alzheimer's disease: relevance of transgenic mouse studies to clinical trials. J Alzheimers Dis. 2008;15:555–69.
6. Holcomb L, Gordon MN, McGowan E, Yu X, Benkovic S, Jantzen P, *et al.* Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. Nat Med. 1998;4:97–100.
7. Irizarry MC, Soriano F, McNamara M, Page KJ, Schenk D, Games D, *et al.* Abeta deposition is associated with neuropil changes, but not with overt neuronal loss in the human amyloid precursor protein V717F (PDAPP) transgenic mouse. J Neurosci. 1997;17:7053–9.
8. Kawarabayashi T, Younkin LH, Saido TC, Shoji M, Ashe KH, Younkin SG. Age-dependent changes in brain, CSF, and plasma amyloid (beta) protein in the Tg2576 transgenic mouse model of Alzheimer's disease. J Neurosci. 2001;21:372–81.
9. Jacobsen JS, Wu CC, Redwine JM, Comery TA, Arias R, Bowlby M, *et al.* Early-onset behavioral and synaptic deficits in a mouse model of Alzheimer's disease. Proc Natl Acad Sci U S A. 2006;103:5161–6.
10. Golde TE, Das P, Levites Y. Quantitative and mechanistic studies of abeta immunotherapy. CNS Neurol Disord Drug Targets. 2009;8:31–49.
11. Levites Y, Smithson LA, Price RW, Dakin RS, Yuan B, Sierks MR, *et al.* Insights into the mechanisms of action of anti-Abeta antibodies in Alzheimer's disease mouse models. Faseb J. 2006;20:2576–8.
12. Dodel RC, Hampel H, Du Y. Immunotherapy for Alzheimer's disease. Lancet Neurol. 2003;2:215–20.
13. Brody DL, Holtzman DM. Active and passive immunotherapy for neurodegenerative disorders. Annu Rev Neurosci. 2008;31:175–93.
14. Gaugler MN, Tracy J, Kuhnle K, Crameri A, Nitsch RM, Mohajeri MH. Modulation of Alzheimer's pathology by cerebroventricular grafting of hybridoma cells expressing antibodies against Abeta *in vivo*. FEBS Lett. 2005;579:753–6.
15. Karlnoski RA, Rosenthal A, Alamed J, Ronan V, Gordon MN, Gottschall PE, *et al.* Deglycosylated anti-Abeta antibody dose-response effects on pathology and memory in APP transgenic mice. J Neuroimmune Pharmacol. 2008;3:187–97.
16. Vugmeyster Y, DeFranco D, Szklut P, Wang Q, Xu X. Biodistribution of [125I]-labeled therapeutic proteins: application in protein drug development beyond oncology. J Pharm Sci. 99:1028–45.
17. Janus C, Westaway D. Transgenic mouse models of Alzheimer's disease. Physiol Behav. 2001;73:873–86.
18. Lichtlen P, Mohajeri MH. Antibody-based approaches in Alzheimer's research: safety, pharmacokinetics, metabolism, and analytical tools. J Neurochem. 2008;104:859–74.
19. Yu P, Oberto G. Alzheimer's disease: transgenic mouse models and drug assessment. Pharmacol Res. 2000;42:107–14.
20. Davis CB, Garver EM, Kwok DC, Urbanski JJ. Disposition of metabolically radiolabeled CE9.1–a macaque-human chimeric anti-human CD4 monoclonal antibody–in transgenic mice bearing human CD4. Drug metabolism and disposition: the biological fate of chemicals. 1996;24:1032–7.
21. Putnam WS, Li J, Haggstrom J, Ng C, Kadkhodayan-Fischer S, Cheu M, *et al.* Use of quantitative pharmacology in the development of HAE1, a high-affinity anti-IgE monoclonal antibody. The AAPS Journal. 2008;10:425–30.
22. Ghiso J, Shayo M, Calero M, Ng D, Tomidokoro Y, Gandy S, *et al.* Systemic catabolism of Alzheimer's Abeta40 and Abeta42. J Biol Chem. 2004;279:45897–908.
23. Amris A, Amris CJ. Turnover and distribution of 131-iodine-labelled human fibrinogen. Thromb Diath Haemorrh. 1964;11:404–22.
24. Areekul S, Devakul K, Chongsuphajaisiddhi T, Vivatanasesth P, Kanakakorn K, Kasemsuth R. Metabolism of 131 I-labelled fibrinogen in monkeys infected with Plasmodium coatneyi. Southeast Asian J Trop Med Public Health. 1971;2:455–61.
25. Moza AK, Sapru RP. Turnover of radio-iodinated and biosynthetically labelled fibrinogen in rhesus monkeys. Indian J Med Res. 1982;76:609–17.
26. Negrier C, Rothschild C, Goudemand J, Borg JY, Claeyssens S, Alessi MC, *et al.* Pharmacokinetics and pharmacodynamics of a new highly secured fibrinogen concentrate. J Thromb Haemost. 2008;6:1494–9.
27. Tytgat GN, Collen D, Verstraete M. Metabolism of fibrinogen in cirrhosis of the liver. J Clin Invest. 1971;50:1690–701.
28. Bonfanti U, Lamparelli D, Colombo P, Bernardi C. Hematology and serum chemistry parameters in juvenile cynomolgus monkeys (Macaca fascicularis) of Mauritius origin: comparison between purpose-bred and captured animals. J Med Primatol. 2009.
29. Stern RA, Trojanowski JQ, Lee VM. Antibodies to the beta-amyloid peptide cross-react with conformational epitopes in human fibrinogen subunits from peripheral blood. FEBS Lett. 1990;264:43–7.
30. Merkle DL, Cheng CH, Castellino FJ, Chibber BA. Modulation of fibrin assembly and polymerization by the beta-amyloid of Alzheimer's disease. Blood Coagul Fibrinolysis. 1996;7:650–8.

31. Cortes-Canteli M, Paul J, Norris EH, Bronstein R, Ahn HJ, Zamolodchikov D et al. Fibrinogen and beta-amyloid association alters thrombosis and fibrinolysis: a possible contributing factor to Alzheimer's disease. Neuron. 66:695–709.
32. Ghiso J, Rostagno A, Gardella JE, Liem L, Gorevic PD, Frangione B. A 109-amino-acid C-terminal fragment of Alzheimer's-disease amyloid precursor protein contains a sequence, -RHDS-, that promotes cell adhesion. Biochem J. 1992;288(Pt 3):1053–9.
33. Springer TA, Zhu J, Xiao T. Structural basis for distinctive recognition of fibrinogen gamma C peptide by the platelet integrin alphaIIbbeta3. J Cell Biol. 2008;182:791–800.