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A sensitive enzyme immunoassay for the quantitation of human CTLA4Ig fusion protein in mouse serum: pharmacokinetic application to optimizing cell line selection

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Abstract

A sensitive, accurate, and precise enzyme immunoassay (EIA) for the quantitation of human CTLA4Ig in mouse serum was validated. The EIA method employed a technique in which a monoclonal anti-CTLA4 antibody was adsorbed onto 96-well polystyrene microtiter plates and used to capture the CTLA4Ig in mouse serum samples. The captured CTLA4Ig was then detected using a goat anti-human IgG_{F_c} antiserum conjugated to the enzyme horseradish peroxidase. The validation included assessments of method accuracy and precision, range of reliable response, lower limit of quantitation (LLQ), inter-analyst robustness, storage stability in mouse serum and assay specificity. The results indicate that this validated assay is precise, accurate, and reproducible. This EIA has a range of reliable response in 10% mouse serum of 0.14–4.58 ng ml^{-1} resulting in a 100% serum equivalent curve of 1.4–45.8 ng ml^{-1}. Assessment of individual standard curve variations indicated a reproducible response with R^2 values of $\geqslant 0.995$. The LLQ was established at 1.4 ng ml^{-1}. The accuracy and precision estimates, based on the quality control values, were within 3.8% and 5.2% respectively, for CTLA4Ig. Stability of CTLA4Ig was established in mouse serum for 5 days at both 4°C and room temperature, for 2 months at −70°C and through five freeze–thaw cycles. This validated assay was successfully employed in the assessment of pharmacokinetic characteristics of CTLA4Ig in mice and to aid in the selection of an optimal CTLA4Ig-producing cell line.

Keywords: Biopharmaceutical; CTLA4Ig; Enzyme immunoassay; Fusion protein; Pharmacokinetics; Validation

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1. Introduction

A novel immunosuppressive agent, CTLA4Ig, is antigen-specific, can induce tolerance and has low in-vivo toxicity. This is in contrast to the currently used immunosuppressive drugs, such as cyclosporin, FK506 and rapamycin, which are generally considered to be non-specific, are not tolerogenic, and are often toxic [1]. CTLA4Ig, a soluble version of the T-cell transmembrane glycoprotein receptor CTLA-4, is a 91 kDa recombinant chimeric fusion protein consisting of the extracellular domain of human CTLA-4 and the FC region (hinge, CH2, and CH3 domains) of human IgG [2].

Antigen-specific T cell activation requires at least two signals for the optimum induction of IL-2 production and clonal expansion [3]. Antigen specificity is provided by the first signal which involves the engagement of the T-cell receptor/CD3 complex on T-cells with an antigenic peptide in association with a major histocompatibility complex (MHC) molecule on antigen presenting cells (APC) [4]. A critical second or co-stimulatory signal can be provided by the interaction of CD28 and CTLA4 on T-cells with B7 counter-receptors expressed on APC (activated B-cells, macrophage, and dendritic cells) [5,6]. CTLA4Ig binds to B7 counter-receptors on APC and functions as a competitive inhibitor of CD28/CTLA4 interactions [2,7–9]. This interaction results in the T cells entering a state of unresponsiveness, known as clonal anergy in vitro or tolerance in vivo [4,8].

Several in-vivo animal models of T-cell-dependent antibody response, autoimmunity, graft-versus-host disease (GvHD), and transplantation, have demonstrated that CTLA4Ig is a potent antigen-specific immunosuppressive agent [6,10–12]. T-cell-dependent antibody responses to sheep red blood cells (SRBCs) and keyhole limpet hemocyanin were suppressed with CTLA4Ig treatment in mice [13]. In autoimmune disease models, CTLA4Ig treatment suppressed lupus-like illness and prolonged the life in mice and provided partial protection against glomerulonephritis in rats [14,15]. The lethal effects in models of GvHD were ameliorated, following treatment in mice with CTLA4Ig [16,17]. Donor-specific transplantation tolerance was achieved following CTLA4Ig treatment of diabetic mice which had received human pancreatic islet cell xenografts [18]. Tolerance was also demonstrated in a vascularized murine cardiac allograft model [19] and, recently, CTLA4Ig has been used successfully to block skin allograft rejection in mice [20]. These studies provide further evidence that CTLA4Ig treatment does not result in a global immunosuppression, but rather is antigen-specific, unlike the currently used immunosuppressive drugs [1].

The objectives of the present study were to validate an enzyme immunoassay (EIA) for quantitating CTLA4Ig in mouse serum and then use the assay to delineate the pharmacokinetics (PK) in mice after receiving a single intravenous bolus dose of CTLA4Ig. The results of the PK study were then used as criteria for selection of a CTLA4Ig-producing cell line.

2. Experimental

2.1. Reagents and Equipment

Bovine serum albumin, Fraction V (BSA), and Tween-20 were purchased from Sigma Chemical Co. (St. Louis, MO). Pooled mouse sera were obtained from Bioreclamation Inc. (East Meadow, NY) and stored at −30°C. The TMB Microwell Peroxidase Substrate System (TMB = 3,3′,5,5′-tetramethylbenzidine) was obtained from Kirkegaard and Perry Labs (Gaithersburg, MD) and stored at 4°C. Immulon IV 96-well flat bottomed EIA plates were purchased from Dynatech Laboratories Inc. (Alexandria, VA) and stored at room temperature (RT) in a sealed airtight bag. Assay plates were washed with a Titertek Plus microplate washer/stacker (Model No. 18000; ICN Biomedical, Costa Mesa, CA) and absorbance at 450 nm was determined using a Tecan microplate reader equipped with a 620 nm reference filter (Model NO. 340ATTC; Research Triangle Park, NC).

2.2. CTLA4Ig standards

CHO (Chinese hamster ovary)-cell-line-derived human CTLA4Ig, NSO (mouse myeloma)-cell-line-derived human CTLA4Ig and murine CTLA4Ig–murine Ig were prepared as previously described [2,21].

2.3. Antibodies

The anti-CTLA4Ig monoclonal antibody clone no. 7F8, prepared by Bristol-Myers Squibb, was obtained from a Cell-Pharm Micromouse hollow fiber cell culture system (Unisyn Technologies, San Diego, CA), purified by protein-G affinity chromatography, and stored in phosphate-buffered saline (PBS) containing 0.02% sodium azide at −70°C, as previously described [22]. Lyophilized goat anti-human IgG_{F_c} polyclonal antiserum conjugated to horseradish peroxidase (HRP) with minimal cross-reactivity to bovine, horse, and mouse serum proteins was purchased from Pierce (Rockford, IL) and stored at 4°C until opened. The antisera were reconstituted with high purity water (>18 MΩ, deionized and filtered through a 0.2 μm filter), aliquoted and stored at −30°C. A thawed aliquot was stored at 4°C and used within 24 h.

2.4. Preparation of the standard curve, quality control and study samples

The standard curve consisted of eight individually-prepared non-zero concentrations of CHO-cell-derived CTLA4Ig: 0.14, 0.29, 0.57, 1.14, 1.72, 2.29, 3.43, and 4.58 ng ml^{-1} in assay buffer. The assay buffer consisted of PBS (10 mM phosphate, 137 mM NaCl, 2.7 mM KCl, pH 7.3–7.5) containing 10% mouse serum. Both the standards and the assay buffer were prepared daily. The stock quality control (QC) samples were prepared to obtain final concentrations of 4.7, 18.6 and 37.2 ng ml^{-1} in 100% mouse serum, aliquoted and stored at −70°C. Daily, these stock QC samples were further diluted with PBS containing 1% BSA (PTB), resulting in final assay QC concentrations of 0.47, 1.86, and 3.72 ng ml^{-1} containing 10% mouse serum. These represent QC concentrations in the lower quartile, between the second and third quartiles, and in the upper quartile of the standard curve range respectively. Study samples were stored for up to 2 weeks at −70°C prior to analysis. Study samples were thawed at 4°C and diluted to 10% mouse serum with PTB. All subsequent dilutions, which ranged from 1:10 to $1:10^6$, were performed using assay buffer containing 10% mouse serum.

2.5. EIA procedure

All steps were performed at room temperature. Immulon IV assay plates were prepared by adding 100 μl of anti-CTLA4Ig capture antibody (7F8; 1 μg ml^{-1}) in PBS to each well. The plates were covered with a plate sealer and incubated at 4°C for a minimum of 16 h. For the remainder of the assay, the plates were incubated uncovered. The antibody solution was aspirated and the remaining sites were blocked by the addition of 200 μl of PTB for 1–2 h. This was followed by five 300 μl washes with PBS containing 0.05% Tween 20 (PBST). Then, 100 μl of the standards, QC samples, and study samples, containing 10% mouse serum, were added to the plate. Following a 2 h incubation the plates were washed with PBST as before and 100 μl of HRP-conjugated goat anti-human IgG_{F_c} antisera (1:15 000 in PTB) was added and incubated for 1 h. The plates were then washed with PBST again and 100 μl of TMB peroxidase substrate solution was added and incubated for 15 min. Lastly, the color development was stopped by the addition of 100 μl of 1 M H_3PO_4 and the absorbance was measured at 450 nm using a 620 nm reference filter. A schematic representation of this assay is given in Fig. 1.

2.6. Validation procedure

2.6.1. Range of reliable response and lower limit of quantitation

This validation used a four-parameter logistic regression model of the form $Y = \max + ((\min - \max)/(1 + \text{conc}/ED_{50})^B)$ to describe the relationship between the absorbance readings and nominal concentrations of CTLA4Ig standards on

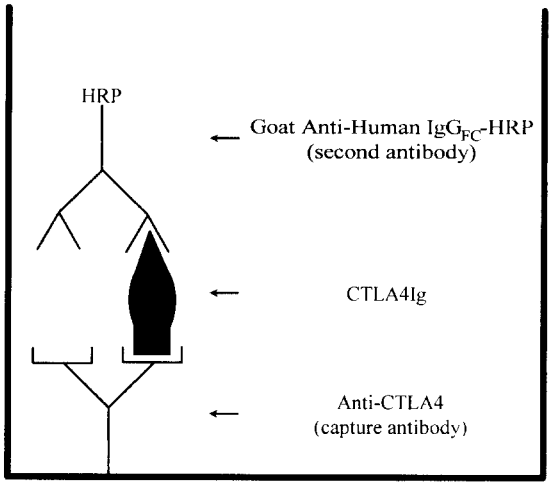

Fig. 1. A schematic representation of the double antibody sandwich CTLA4Ig EIA. Clone 7F8 was used as the anti-CTLA4Ig capture antibody. The plates were blocked with PBS containing 1% BSA prior to the addition of the CTLA4Ig.

each plate, where max is the estimated maximum of the function, min is the estimated minimum of the function, conc is the values of the nominal concentrations of the standard curve range, ED_{50} is the estimated midpoint of the regression line, and B is the slope of the apparent linear region of the curve. Evaluation of potential outliers in the individual plate standard curves was performed as

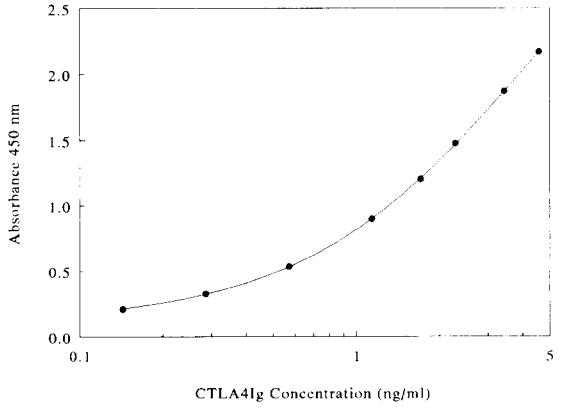

Fig. 2. A representative standard curve of the CTLA4Ig EIA in 10% mouse serum (mean ± SD, $n = 3$). The error bars which represent the standard deviation are less than the size of the symbols.

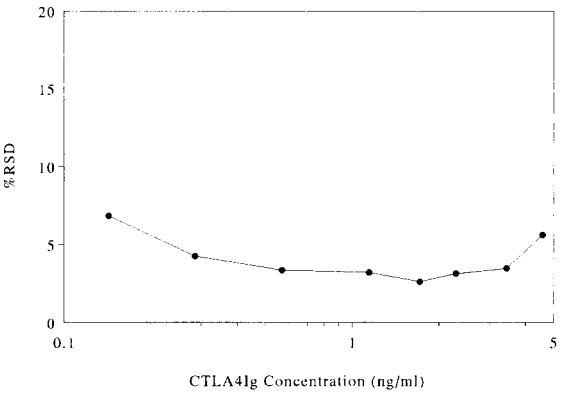

Fig. 3. Intra-assay precision profile of the CTLA4Ig EIA obtained from nine consecutive runs ($n = 27$). The 20% RSD corresponds to the maximum acceptable value for the lowest standard (15% RSD for all other standards).

previously described [23]. An eight-point standard curve ranging from 0.14–4.58 ng ml^{-1} of CTLA4Ig in 10% mouse serum was assayed in triplicate. This results in a 100% serum equivalent curve of 1.4–45.8 ng ml^{-1}. Individual curves were examined for appropriateness of model fit and reproducibility [24]. The lower limit of quantitation (LLQ) of CTLA4Ig was determined experimentally using 10 different pooled lots of mouse serum. Ten individually-prepared concentrations containing 1.4 ng ml^{-1} CTLA4Ig in 100% mouse serum were assayed by diluting 10-fold with PTB, resulting in 0.14 ng ml^{-1} CTLA4Ig in 10% mouse serum. Accuracy at the LLQ was calculated as the deviation of the predicted concentrations from the

Table 1
CTLA4Ig EIA LLQ determination in mouse serum

Nominal conc. (ng ml^{-1})	Predicted conc. (ng ml^{-1})	%DEV
1.4	1.5	7.1
	1.5	7.1
	1.9	35.7
	1.5	7.1
	1.6	14.3
	2.0	42.9
	1.5	7.1
	1.3	−7.1
	1.6	14.3
	1.4	0.0

Table 2
Evaluation of accuracy and precision of CTLA4Ig EIA in mouse serum

Analyst	Parameter	Nominal concentration (ng/ml^{-1})		
		4.7	18.6	37.2
1	Mean observed concentration ($n = 36$)	4.7	18.5	36.6
	%DEV	0.0	−0.5	−1.6
	Inter-assay precision (%RSD)	3.8	3.9	3.5
	Intra-assay precision (%RSD)	5.2	2.8	3.9
2	Mean observed concentration ($n = 36$)	4.8	18.8	38.6
	%DEV	2.1	1.1	3.8
	Inter-assay precision (%RSD)	3.2	0.0[a]	0.0[a]
	Intra-assay precision (%RSD)	3.9	3.3	2.8

[a] No significant additional variation was observed as a result of performing the assay in different runs.

nominal concentration, expressed as a percentage of the nominal concentration (%DEV). For the concentration to be accepted as the LLQ, at least 80% of the samples assayed have to be within 20% of nominal (the 15%DEV deemed acceptable for the QC samples plus 5%) [25]. The predicted (observed) concentration refers to that value determined based on the inverse prediction from the standard regression curve.

2.6.2. Accuracy, precision and robustness

Assessment of accuracy and precision involved using predicted QC concentrations calculated from individual standard curves. Prior to sample analysis the three stock QC concentrations of 4.7, 18.6, and 37.2 ng ml^{-1} prepared in 100% mouse serum were analyzed over 3 days. In each analytical run, three replicates of each standard concentration were assayed, along with three replicates of each diluted QC concentraiton, all in 10% mouse serum. Robustness of the assay was estimated by having two analysts perform the same assay. To determine assay precision, the predicted QC concentrations were evaluated using an ANOVA model. Variance component analysis was utilized to calculate an estimate of inter-assay and intra-assay precision from the factors in the ANOVA model [26]. Estimates of precision were expressed as a percent relative standard deviation (%RSD) relative to the overall mean predicted concentration for all analytical runs at the concentration level. Estimates of accuracy were expressed as a %DEV of the overall mean predicted concentrations from the corresponding nominal concentration.

2.6.3. Stability of CTLA4Ig in mouse serum

Long and short-term storage stability of CTLA4Ig prepared in mouse serum was assessed. Long-term storage stability of CTLA4Ig was assessed at −70°C. Short-term storage stability was evaluated at 4°C and room temperature for 5 days. Freeze–thaw stability was assessed over five repetitive freeze (−70°C) and thaw (4°C) cycles.

2.6.4. Assay specificity

To ensure that the anti-CTLA4Ig antibody did not cross-react with other similar moieties, CTLA4Ig (human CTLA4–human Ig) QC samples were assayed in the presence of up to a 1000-fold molar excess of mCTLA4Ig (murine CTLA4–murine Ig). The predicted QC concentrations of CTLA4Ig in the presence and absence of mCTLA4Ig were then compared.

2.7. Pharmacokinetic application

2.7.1. Animal protocol

Six BDF1 female mice (Charleston River Labs., Wilmington, MA) between 7 and 9 weeks of age were used in the study to obtain preliminary pharmacokinetic data on CTLA4Ig obtained from two different cell lines. Prior to dosing, all mice were acclimatized with appropriate temperature and humidity control. On study day, each mouse received a single dose of 0.2 mg (i.e. 0.2 ml

Table 3
Evaluation of CTLA4Ig long-term storage stability in mouse serum at −70°C

Control (Day 0) 4.58 ng ml^{-1}			17.55 ng ml^{-1}		34.77 ng ml^{-1}	
Assay date	Predicted	%DEV	Predicted	%DEV	Predicted	%DEV
Day 1	4.62	0.87	18.05	2.85	36.53	5.06
Day 6	5.00	9.17	19.17	9.23	37.13	6.79
Day 19	4.72	3.06	19.10	8.83	37.87	8.92
2 Months	4.71	2.84	19.45	10.83	38.37	10.35

of 1 mg ml^{-1} solution) of CTLA4Ig intravenously, administered into the tail vein by a bolus injection. A 27-gauge needle and a 1 cm^3 tuberculin syringe were used to administer the drug. Serial blood samples, approximately 100 μl, were collected under anesthesia by retro-orbital bleeding at 3 min, and at 4, 24, 48, 72 and 144 h post-dose. Standard procedures were employed to perform retro-orbital bleeding and to harvest serum [27]. Blood samples were allowed to clot for no more than 1 h at room temperature, and were spun in a refrigerated centrifuge for the separation of the serum. The serum samples were stored in labeled storage tubes at or below −70°C until analysis.

2.7.2. Pharmacokinetic calculations

Intravenous data were subjected to noncompartmental pharmacokinetic analysis [28,29] and the following parameters were calculated: area under the time curve from time = 0 to time = infinity (AUC_{inf}); elimination half-life ($T_{1/2}$); total systematic clearance (CL_T); and volume of distribution at steady state (V_{ss}). The peak serum concentration (C_{max}) was tabulated using the raw data.

3. Results

3.1. Validation of the EIA

The 0.14 ng ml^{-1} and 4.58 ng ml^{-1} standards served as boundaries for the concentrations corresponding to the maximum and minimum absorbance readings which could be calculated by the four-parameter logistic regression function as illustrated in Fig. 2. The absorbance attributed to non-specific binding was less than 0.100. The associated R^2 value of each curve was ≥ 0.995. An intra-assay precision profile based on nine consecutive runs is shown in Fig. 3. As illustrated in Fig. 3, the RSDs ($n = 27$) ranged from 2.6–6.8% for the predicted concentrations of the standards. The results for the LLQ determination at 1.4 ng ml^{-1} diluted to 0.14 ng ml^{-1} in 10% mouse serum are given in Table 1. Eight of the 10 individually-prepared LLQ samples predicted within the established criterion of 20% of nominal. The overall %DEV and %RSD values for the 10 individually-prepared LLQ samples were 10.5 and 13.6 respectively, well within the established 20% criteria. Based on these results, the LLQ for this assay was established at 1.4 ng ml^{-1} CTLA4Ig in 100% serum.

Accuracy and precision were based on the prediction of 36 QC concentration determinations at each QC level as summarized in Table 2. Inter- and intra-assay precision, for both analysts, calculated from the predicted QC values, were within 3.9% and 5.2% respectively. The overall accuracy across concentrations was within 3.8%.

To determine the stability of CTLA4Ig, a criterion that the predicted concentration of CTLA4Ig had to be within 15% of the Day 0 concentration was established. Based on the criterion, CTLA4Ig was stable in mouse serum for at least 2 months at −70°C (Table 3), and for 5 days at 4°C and room temperature (Table 4). CTLA4Ig was also stable after five repeated freeze–thaw cycles (Table 4).

The prediction of the CTLA4Ig QCs was unaffected by the presence of a 1000-fold molar excess of mCTLA4Ig, indicating that the assay is specific

Table 4
Evaluation of CTLA4Ig short-term storage stability in mouse serum

Control (Day 0)	4.80 ng ml^{-1}		19.70 ng ml^{-1}		40.23 ng ml^{-1}	
Assay conduction	Predicted	%DEV	Predicted	%DEV	Predicted	%DEV
4°C						
3 Days	4.70	−2.08	20.50	4.06	40.17	−0.15
5 Days	4.51	−6.04	18.94	−3.86	36.49	−9.30
Room temperature						
3 Days	4.23	−11.88	18.51	−6.04	38.00	−5.54
5 Days	4.84	0.83	17.85	−9.39	36.80	−8.53
Freeze–Thaw cycles						
3 Days	4.63	−3.54	19.89	0.96	38.47	−4.37
5 Days	4.53	−5.62	18.17	−7.77	35.03	−12.93

for human CTLA4Ig (Table 5) and is unaffected by murine CTLA4 or murine Ig.

3.2. Pharmacokinetic application in cell line selection

The mean serum concentration–time profiles of CTLA4Ig in mice receiving the drug obtained from two different cell lines are depicted in Fig. 4. The individual and mean pharmacokinetic parameters are presented in Table 6. As evident from the serum concentration–time profile, as well as from the derived pharmacokinetic parameters, the CTLA4Ig molecules obtained from the two different cell lines exhibited considerable differences with respect to their pharmacokinetic disposition in mice.

Table 5
Evaluation of assay specificity

Nominal concentration	13.00 ng ml^{-1}	
Molar ratio of human CTLA4Ig: murine CTLA4Ig	Predicted	%DEV
1:0	12.04	−7.38
1:0.1	11.56	−11.08
1:1	11.70	−10.00
1:10	12.27	−5.02
1:100	12.55	−3.46
1:1000	13.16	1.23

C_{max}, obtained from the first sample 3 min after dosing, showed an approximately two-fold difference between the CHO-cell-line-(235 μg ml^{-1}) and NSO-cell-line-(121 μg ml^{-1}) derived CTLA4Ig, suggesting differences between the cell lines in the initial distribution phase immediately following intravenous administration. It was clear that with progress of time, differences in the serum levels of CTLA4Ig between the lots became more pronounced due to a faster clearance of CTLA4Ig derived from NSO cell line than CHO cell line (Fig. 4). This observation was unequivo-

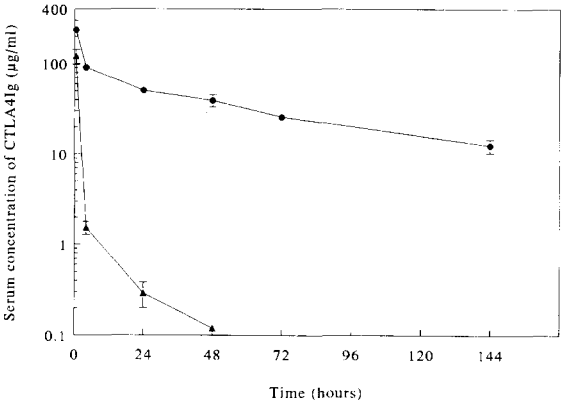

Fig. 4. Serum concentration versus time profile for CTLA4Ig in mice following administration of a single 0.2 mg intravenous dose of CHO-(●) or NSO-(▲) derived CTLA4Ig. Each data point represents the mean ±SD ($n = 3$). For data points with no error bars, the error bars are less than the size of the symbol.

Table 6
Individual and mean (SD) pharmacokinetic parameters of CTLA4Ig following intravenous administration to mice

Mouse No.	C_{max} (μg ml^{-1})	AUC_{inf} (h μg ml^{-1})	$T_{1/2}$[a] (h)	CL_T (ml h^{-1})	V_{ss} (ml)
CHO-cell-derived					
1	221	5946	54	0.03	2.3
2	226	6018	61	0.03	2.6
3	257	6901	61	0.03	2.4
Mean	235	6288	59	0.03	2.4
(SD)	(20)	(532)	(3.9)	(0.0)	(0.1)
NSO-cell-derived					
4	135	298	11	0.67	1.2
5	134	290	12	0.69	1.0
6		212	13	0.94	2.3
Mean	121	267	12	0.77	1.5
(SD)	(23)	(47)	(1.2)	(0.1)	(0.7)

[a] Apparent value since blood sampling interval was slightly greater than two half-life values.

cally supported by approximate 25-fold differences in AUC_{inf} and CL_T parameters between the CHO cell line (6288 h μg ml^{-1} and 0.03 ml h^{-1} respectively) and the NSO cell line (267 h μg ml^{-1} and 0.77 ml^{-1} respectively). The mean V_{ss} value of 1.51–2.4 ml obtained in this study suggested that the distribution volume of CTLA4Ig was within 1.5 times the blood volume in a mouse [30]. The $T_{1/2}$ value for CTLA4Ig from the CHO cell line (59 h) was approximately five times greater than that from the NSO cell line (12 h). $T_{1/2}$ values obtained from the CHO cell line may not be definitive since the blood sampling was only slightly greater than two half-life values.

4. Discussion

In the present study a sensitive, accurate, and reproducible EIA has been developed and validated which permits the quantitation of CTLA4Ig in mouse serum. The assay uses an anti-CTLA4 monoclonal capture antibody in conjunction with a polyclonal anti-human-Ig-G_{F_c}-specific detection antiserum. Quantitation therefore requires that both CTLA4 and Ig regions of the fusion protein be present. The selection of the anti-human IgG_{F_c} specific antiserum was not a trivial aspect of developing this assay. Among the 12 different antisera evaluated, the selected antiserum was the only antiserum which did not cross-react with the capture antibody.

The validation results demonstrate that the assay variation is low and well within the established assay criteria of ±15% DEV (±20% for LLQ) from nominal and ±15% RSD (±20% for LLQ) for standards and QCs. Furthermore, these results provide evidence that the assay is robust, easily transferable, and maintains low assay variation across analysts. The sensitivity of the assay (1.4 ng ml^{-1} in 100% serum) is appropriate to support in-vivo studies [13,20]. The assay is specific for human CTLA4–human Ig and is unaffected by murine CTLA4 or murine Ig. The epitopes recognized by the antibodies used in this assay are stable in mouse serum when stored for 5 days at 4°C and room temperature, for 2 months at −70°C as well as through five freeze–thaw cycles.

A common problem of quantitating analytes in biological matrices by EIA is the undesired serum matrix effects. To minimize this problem while maintaining equivalent serum influences throughout the assay, a constant concentration of 10% mouse serum is included in all samples. This

includes standards, QCs, and study samples. Thus, a sample which is diluted ten-fold has the same serum matrix effect contribution as a sample diluted 10 million-fold.

In conclusion, an accurate, precise, and robust EIA was validated and applied to quantitate CTLA4Ig in serum samples from pharmacokinetic studies in mice. This EIA has a range of reliable response in 10% mouse serum of 0.14–4.58 ng ml^{-1}, resulting in a 100% serum equivalent curve of 1.4–45.8 ng ml^{-1} with an LLQ of 1.4 ng ml^{-1}. CTLA4Ig is stable in mouse serum for at least 2 months at −70°C, 5 days at 4°C and room temperature, and through five freeze–thaw cycles. With the aid of the assay, pharmacokinetics of CTLA4Ig were obtained for selection of cell line, optimization of cell culture conditions, and prior to large-scale production. The information obtained from the use of a short pharmacokinetic model precludes the need for extensive biochemical profiling as the cell line selection criterion. This allowed for the selection of a cell line which produces CTLA4Ig with a desirable in-vivo pharmacokinetic profile, including a long serum half-life.

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