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Original Article

Development of a bioanalytical method for circulating human T cells in animals using *Arthrobacter luteus*-based quantitative polymerase chain reaction and its application in preclinical biodistribution studies

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ABSTRACT

Introduction: In the development of cell therapy products for human use, studies on the biodistribution of transplanted cells in animals are important for assessing the safety and efficacy of these products. Although a few reports have described the biodistribution of human cells in animals using *Arthrobacter luteus*-based-polymerase chain reaction (Alu-PCR), most have used genomic DNA or synthetic oligonucleotide as calibrators, as opposed to actual cells. In addition, bioanalytical variability in the quantification of cells with respect to specificity, selectivity, accuracy, and precision, has not been evaluated. Accordingly, in this study, we validated the utility of this bioanalytical method for human T cells in mice to establish assay performance using cells as a calibrator.

Methods: A standard curve was constructed for the addition of cell lysates to mouse tissues and blood, and DNA was extracted. Alu-PCR was applied for the quantification of human peripheral blood $CD8^+$ T cells in mice. To determine assay performance, we evaluated accuracy, precision, selectivity, specificity, and stability. *In vivo* cell kinetics and biodistribution were investigated based on intravenous administration of human T cells to mice.

Results: Alu-PCR enabled us to specifically detect human T cells in mouse blood and tissues. The lower detection limit of Alu-PCR was 10 cells/15 mg tissue (7.5 mg for spleen and lung) or cells/50 μL blood. Given that PCR threshold cycle (Cq) values among mouse samples (blood, liver spleen, lung, heart, and kidney) show slight variation, calibration curves should be generated using the same tissue as used for the assay. Most coefficients of variation in the assay were within 30%. The cell kinetics of administered human T cells in mice were successfully evaluated using the established Alu-qPCR.

Conclusions: The Alu-PCR technique developed in this study showed sufficient specificity and sensitivity in detecting human peripheral blood $CD8^+$ T cells in mice. This technique, which targets the primate-specific Alu gene, is applicable for quantifying transplanted human cells in animals without the necessity of cell labeling. The data presented herein will be useful for standardizing bioanalytical approaches in biodistribution studies of cell therapy products.

1. Introduction

In the development of cell therapy products, biodistribution studies are essential for preclinical safety and efficacy assessments [1–3]. In this regard, several technologies have been utilized to track transplanted human-derived cells in animals. As imaging modalities, positron emission tomography, single-photon emission computed tomography, bioluminescence imaging, and magnetic resonance imaging have been adopted [4–7]. Additional quantitative methods, such as radioactivity detection using radioisotopes and quantitative polymerase chain reaction (qPCR) using human-specific DNA sequences, have also been introduced [7–10].

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Among these methods, qPCR is the only method that does not require the labeling or modification of transplanted cells. Consequently, qPCR is considered to have multiple advantages with respect to biodistribution studies, given that the labeling and modification of cell products requires additional processes to determine appropriate conditions that have minimal impact on cell function.

Although certain specific and sensitive qPCR methods have been developed for biodistribution studies of cell therapy products [11–18], analysis of the cell quantification procedures and data validity of these studies indicates significant differences. One typical example is the unit of quantified output. Whereas some previous studies have presented results as cell numbers or percentages of cells per organ or dose level, others have presented results in terms of nanograms or picograms of human DNA per nanogram or microgram of host DNA [17]. In addition, with regards to calibration standards, some studies have used a mixture of human and host animal cells, whereas other have used a combination of human and host animal DNA. In addition, few studies have presented comprehensive data and information when validating data for parameters such as accuracy and precision. Given that these requirements are fundamental for interpreting the quantitative data of biodistribution studies, a consensus would be desirable.

Published in 2009, the Minimum Information for Publication of Quantitative Real-Time PCR Experiments guidelines [19] focus primarily on the quantification of endogenous DNA and RNA for molecular biology research and address the principal criteria that determine the quality of qPCR data based on reference to a detailed checklist. Although these guidelines provide suitable recommendations for DNA and RNA quantification, they do not deal with the quantification of transplanted cells using qPCR and would be difficult to adapt to this technique. However, for bioanalysis in drug development, bioanalytical method validation (BMV) guidance/guidelines have been issued by regulatory authorities, such as the US Food and Drug Administration, the European Medicines Agency, and the Japan Ministry of Health Labour and Welfare [20–23]. The BMV guidance/guidelines focus on drug quantification using liquid chromatography–tandem mass spectrometry and ligand-binding assays, as these two methods are predominantly used for bioanalysis in drug development. Although the BMV guidance/guidelines do not include a description of the quantification of transplanted cells using qPCR, the basic concepts related to bioanalysis and method validation could be adapted to cell quantification using qPCR, given that both cells and drugs are administered to animals and their quantification in animal tissues has a common purpose.

Accordingly, in this study, we developed an *Arthrobacter luteus* (Alu)-qPCR method for the quantification of human cells in mouse tissues using cells as a calibrator. An Alu element is a short stretch of DNA originally characterized by the action of the Alu restriction endonuclease [24]. Alu elements are the most abundant transposable elements, containing over one million copies dispersed throughout the human genome, and therefore Alu-qPCR facilitates the highly sensitive detection of human cell-derived genomic DNA (gDNA) in animals [18,25]. We assessed the suitability of this method and the reliability of the data obtained by evaluating assay performance.

2. Materials and methods

2.1. Cells, reagents, and equipment

Cryopreserved human peripheral blood $CD8^+$ T cells were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). The cells were isolated from a healthy human male donor (Japanese, 22 years old). The cell number was determined using a FACSLyric flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) as previously described [26]. For control mouse samples, mouse blood and tissues (liver, spleen, kidney, heart, and lung) were obtained from severe combined immunodeficient (SCID) mice (male, 8 weeks old). For DNA extraction, we used a DNeasy 96 Blood and Tissue kit (Qiagen, Valencia, CA, USA). For qPCR, we used TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) and a QuantStudio 7 Flex Real Time PCR system equipped with SDS software (Thermo Fisher Scientific). PCR primers and a hydrolysis (TaqMan) probe were purchased from Greiner Bio-one (Kremsmünster, Austria). The sequences used for the detection of human Alu elements were as follows: primers, (forward) 5′-CATGGTGAAACCCCGTCTCTA-3′ and (reverse) 5′-GCCTCAGCCTCCCGAGTAG-3′; and TaqMan probe, 5′-FAM-ATTAGCCGGGCGTGGTGGCG-TAMRA-3′. These sequences and their efficacy have been reported previously [14,25]. All other chemicals and reagents were obtained from commercial sources.

2.2. Preparation of stock and working cell lysates

A stock cell lysate was prepared from specific numbers of human peripheral blood $CD8^+$ T cells by adding buffer ATL and proteinase K (9:1, v/v; Qiagen) and incubating at 56 °C for 2 h. The stock cell lysate was serially diluted with buffer ATL and proteinase K (9:1, v/v) to prepare appropriate concentrations of working cell lysates. The working cell lysates were spiked into control mouse tissue homogenates and control mouse blood samples to prepare calibration standards and other spiked samples.

2.3. Preparation of calibration standards and spiked samples

Mouse tissue homogenates were prepared by homogenizing whole tissues obtained from the liver, kidney, lung, heart, and spleen with buffer ATL and proteinase K (9:1, v/v) using a gentleMACS dissociator (Miltenyl Biotec, Bergisch Gladbach, Germany), and the homogenates were incubated at 56 °C overnight with continuous mixing. The liver, kidney, and heart homogenates were prepared at a final concentration of 15 mg tissue/150 μL homogenate, whereas for the spleen and lung, we prepared homogenates at a concentration of 7.5 mg tissue/150 μL homogenate, as these tissues tend to be characterized by a very high number of cells per tissue mass. All tissues were homogenized individually, and equal volumes of each homogenate were pooled. The calibration standards were prepared for tissue homogenates and blood samples by adding the working cell lysates. The calibration standard concentrations were 10, 10^2, 10^3, 10^4, and 10^5 cells/15 mg tissue (7.5 mg for spleen and lung) or cells/50 μL blood. The spiked samples were prepared in bulk by adding the working cell lysates to control mouse tissue homogenates and blood samples.

2.4. DNA extraction

DNA was extracted according to the DNeasy Blood and Tissue Handbook (July 2006, Qiagen), with certain modifications designed to enhance recovery. Briefly, a 150-μL aliquot of the homogenate or 50-μL aliquot of blood was mixed with 70 μL of ATL buffer and proteinase K (9:1, v/v), whereas calibration standards were mixed with 70 μL of the working cell lysate prepared as described above. For blood samples, an additional 100 μL of buffer ATL and proteinase K (9:1, v/v) was added in order to obtain equal volumes of blood and tissue homogenate samples. To prepare the blood samples, 205 μL of buffer AL (Qiagen) was added, followed by incubation at 56 °C for 30 min, after which 205 μL of ethanol was added.

For tissue samples, 410 μL of buffer AL and ethanol (1:1, v/v) was added, followed by incubation at 56 °C for 15 min, and the samples were subsequently loaded onto a DNeasy 96 plate. The plate was washed twice with 500 μL of buffer AW1 (Qiagen) and then washed with 500 μL of buffer AW2 (Qiagen). Finally, DNA was eluted from each sample using 200 μL of buffer AE (Qiagen).

2.5. qPCR

qPCR was performed in a total reaction volume of 20 μL containing 2 μL of DNA extract, 10 μL of TaqMan Fast Advanced Master Mix, 2 μL of each forward and reverse primer solutions (9 μM), 2 μL of 2.5 μM TaqMan probe solution, and 2 μL of water. Reactions were conducted for 40 cycles (95 °C for 1 s/60 °C for 20 s) after initial incubation at 50 °C for 2 min and 95 °C for 20 s. PCR efficiency (E) was calculated using the formula: $E = 10^{(-1/\text{slope})} - 1$, and the slope was determined using SDS software (Applied Biosystems, Foster City, CA, USA). For each sample, reactions were performed in triplicate, and the mean values are presented.

2.6. Calculation of cell concentrations

Cell concentrations were calculated using equation $X = 10^{(Y - b)/a}$, where X is the calculated cell concentration [cells/15 mg tissue (7.5 mg for spleen and lung) or cells/50 μL blood], Y is the threshold cycle value (Cq) in the PCR of each sample, and a and b are the slope and y-intercept of the calibration curve, respectively.

2.7. Assessment of assay performance

The performance of the method was assessed by determining specificity, selectivity, assay linearity, precision, accuracy, and stability in accordance with the relevant BMV guidance/guidelines. The acceptance criteria described in the BMV guidance/guideline were not set in this study, as the assay performance data for the quantification of human cells using qPCR are still relatively limited and not suitable with respect to determining the appropriate criteria.

2.7.1. Specificity

To confirm that the method could specifically quantify human cells, even in the presence of mouse cells, we analyzed blank samples (no human cell samples) and samples spiked with the lower limit of quantification (LLOQ) level of the human cell lysate prepared with mouse tissues and blood.

2.7.2. Selectivity

To confirm whether the method could quantify human cells, even in the presence of other components, six individual mouse blood samples were used to prepare blank samples and spiked samples at the LLOQ level, and both sample types were analyzed.

2.7.3. Assay linearity

2Calibration standards from 10 to 10^5 cells/15 mg tissue (7.5 mg for the spleen and lung) or cells/50 μL blood were freshly prepared and analyzed on three different days (n = 1 each day). Calibration curves were generated from semi-log plots, with the x-axis representing the theoretical cell concentrations (log) and the y-axis representing the Cq values (linear) obtained using SDS software. The calibration curves were evaluated using the back-calculated concentration to the nominal concentration (%Accuracy), slope, y-intercept, coefficient of determination (R^2), and PCR efficiency.

2.7.4. Precision and accuracy

The precision and accuracy of the developed method were evaluated using spiked samples at four concentration levels: 10 (LLOQ), 30 (low), 1000 (medium), and 80,000 (high) cells/15 mg tissue (7.5 mg for the spleen and lung) or cells/50 μL blood. The spiked samples were analyzed in five replicates on three different days. Intra- and inter-run assay precision values were calculated as the coefficient of variation (%CV), and intra- and inter-run assay accuracy values were calculated as the error relative to the nominal concentration (%Accuracy).

2.7.5. Stability

The stability of human cell-derived gDNA in the sample matrix was assessed using mouse blood and tissue homogenates spiked with working cell lysates at four concentration levels: 10 (LLOQ), 30 (low), 1000 (medium), and 80,000 (high) cells/15 mg tissue (7.5 mg for the spleen and lung) or cells/50 μL blood. The spiked blood and tissue homogenates were stored for 1 month, subjected to three freeze–thaw cycles at −80 °C, and then analyzed.

2.8. In vivo cell kinetics and biodistribution study

Male C.B-17 SCID mice (8 weeks old) were housed under controlled temperature and humidity conditions and a 12/12-h light/dark cycle. The animals were provided with free access to laboratory chow (CE-2; Clea Japan, Inc., Tokyo, Japan) and water. Blood, liver, kidney, lung, heart, and spleen samples were collected at 1 and 24 h (three animals per time point) after a single intravenous administration of human peripheral blood CD8+ T cells (1×10^6 cells/animal). Collected samples were immediately frozen on dry ice and stored at −80 °C. All samples were homogenized, and DNA was extracted on the day of qPCR analysis. The number of cells in each organ was expressed as cells/organ weight, and the number of cells in the blood was expressed as cells/milliliter of blood. All animal protocols were approved by the Institutional Animal Care and Use Committee of the Shonan Research Center, Takeda Pharmaceutical Company Ltd. (Kanagawa, Japan, approval no. AU-00020703).

3. Results and discussion

In this study, we successfully developed a bioanalytical method based on Alu-qPCR, in which cells were used for calibration. By using cells as a calibrator, the quantified value could be interpreted as cell number per volume or weight of tissues. After evaluation of assay performance, the method was applied in an *in vivo* biodistribution study to further confirm its practical utility.

3.1. Method development

Among several qPCR applications for quantifying transplanted human cells in mice, a method using 300-nt-long primate-specific Alu sequences with a copy number of more than 10^6/genome is widely used [13–18,25]. In the present study, we also utilized Alu sequences, as they are expected to be highly sensitive and specific, owing to the high copy number and lack of cross-reactivity with rodent DNA. The PCR primers and probe were prepared using the same design as used in previously published studies [14,25]. Given that the selected Alu sequences showed sufficient sensitivity and specificity when using these primers and probe, we did not assess the applicability of other sequences; however, there may be some flexibility with regards to primer and probe design [25].

A summary of the human cell quantification procedures is shown in Fig. 1. The major differences between this method and conventional qPCR analyses [18,25] are as follows. First, the

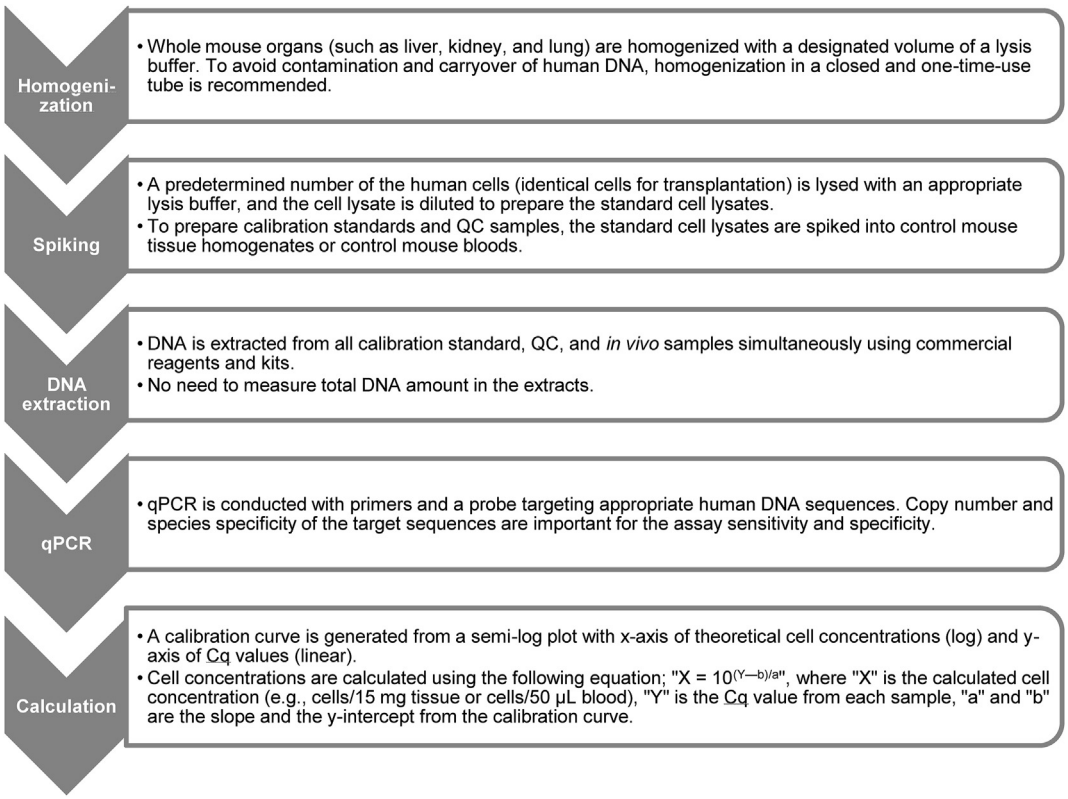

Fig. 1. Proposed procedures for human cell quantification using quantitative polymerase chain reaction (qPCR) for biodistribution studies.

calibration standards and spiked samples, including quality control (QC) samples, were prepared by spiking the standard cell lysates into each control matrix. This enabled us to construct calibration curves of Cq values versus cell concentrations, and we were thus able to obtain the absolute cell concentration of samples directly from the calibration curves. Second, the unit of the results obtained using the proposed qPCR method was "cells/organ or mL blood." Our method enabled the expression of results in terms of volume or weight-based units, expressed as units of "cells/mg tissue" or "cells/mL blood". On the basis of these weights and volumes, we were able to calculate cell concentrations, expressed in terms of units of "cells/organ or mL blood". In conventional qPCR analyses, calibration curves are typically generated using Cq values versus DNA concentrations or the copy numbers of target sequences and use "copies/μg DNA" and "μg human DNA/μg host DNA" as units. The objective of performing biodistribution studies on cell therapy products is to clarify the distribution patterns and tissue concentrations of transplanted cells in the body. Consequently, the units used in the present study would be more appropriate from the perspective of interpreting results. Having developed the assay procedure, we proceeded to assay performance based on the criteria of specificity, selectivity, assay linearity, precision, accuracy, and stability.

3.2. Specificity

For assessment of specificity, we analyzed blank and spiked samples (with and without human cells prepared with mouse tissues and blood, respectively) (Table 1). Analysis of non-human cell samples (Blank) from mouse blood and tissues revealed a weak signal (0.779–1.95 cells), which can be attributed to intrinsic signals in the Alu-PCR [25]. In contrast, analysis of human cell-spiked samples at the LLOQ level showed corresponding signals (10 cells/sample as a nominal concertation). This observation accordingly indicated that the developed method can be used to specifically detect human cells in mouse blood and tissues. Moreover, we also assessed the specificity of the Alu-qPC in other species, including rats, dogs, and monkeys, given that the Alu element is known to be a gene specific to primates. The developed Alu-qPCR method was confirmed to show specificity in dogs but not in monkeys (Supplemental Figure S1).

3.3. Selectivity

For the assessment of assay selectivity, six individual mouse blood samples (n = 6 each) were used to prepare blanks and spiked samples at the LLOQ (Table 2). We found that the maximum response for the blank samples was 1.70 cells, which is slightly less than one-fifth of the LLOQ (10 cells). Moreover, precision (%CV) and accuracy (%) at the LLOQ were 11.9% and 114.4%, respectively. These results thus confirmed the selectively of the assay using six blood samples, thereby indicating that the developed Alu-qPCR method

Table 1
Concentrations of human cells in human T cell-spiked samples of mouse blood and tissues (n = 1) in assay specificity assessment.

Nominal concentration (cells/50 μL blood or 15 mg tissue)	Blank	LLOQ
	0	10
Blood	1.48	10.2
Liver	1.95	11.3
Heart	0.828	10.9
Kidney	0.779	10.0
Lung[a]	1.10	10.7
Spleen[a]	0.913	11.4

LLOQ: lower limit of quantification.
[a] Cells/7.5 mg tissue.

Table 2
Concentrations of human cells in human T-cell spiked samples from six individual mouse blood samples in assay selectivity assessment.

Nominal concentration (cells/50 μL blood)	Blank	LLOQ
	0	10
Animal no. 1	1.10	9.08
Animal no. 2	1.70	12.2
Animal no. 3	1.38	13.1
Animal no. 4	1.20	11.8
Animal no. 5	1.42	12.0
Animal no. 6	1.38	11.9
Mean (cells/50 μL blood)	1.17	11.4
Precision (%CV)	17.8	11.9
Accuracy (%)	–	114.4

LLOQ: lower limit of quantification, CV: coefficient of variation.

quantified human cells even in the presence of other mouse matrix components.

3.4. Assay linearity

Fig. 2 shows a representative amplification plot for mouse blood and calibration curves generated for mouse blood, liver, and heart tissues spiked with human CD8+ T cell lysates, which show a clear proportional response between Cq values and the number of cells used to spike samples. Notably, the calibration curve should be generated using the same sample type as used for the assay, given that there are slight differences in the Y-intercepts of Cq values for each sample type (Fig. 2b), which can significantly alter the quantified values. We presume that observed differences in the Y-intercept for Cq values can be attributed to the variability in DNA extraction efficiency. Table 3 shows representative data of the calibration curve for mouse blood, liver, and heart samples (Supplemental Table S1 shows the data for other tissues). For all evaluated matrices, the calibration curves were linear from 10 to 10^5 cells, and the associated R^2 values were greater than 0.995. Moreover, PCR efficiencies were greater than 92.5%, whereas the overall accuracy was between 77.8% and 118.8%. These results accordingly revealed that the current assay enabled quantification of human cells at cell numbers from 10 to 10^5 cells/15 mg tissue (7.5 mg for spleen and lung) or cells/50 μL blood.

3.5. Precision and accuracy

When used to analyze mouse blood, the overall precision (CV) and accuracy of the assay were 2.6%–17.3% and 74.4%–111.0%, respectively (Table 4). Evaluation of the overall assay variability for different matrices revealed that, with the exception of lung and liver samples, most CV values were within 30%, which can presumably be explained by the relatively large variability in the efficiency of extracting DNA from the lung and liver (Table 4 and Supplemental Table S2). When using the conventional Alu-qPCR method, DNA extraction efficiency may vary between wells of 96-well plates, thereby resulting in problems in interpretating the quantified values. In the newly developed method, all samples, including the calibration standard, QC, and *in vivo* samples, were simultaneously processed using the same 96-well plate. Although this reduced variability in the extraction efficiency, there could still be inherent differences between wells, and we suspect that a proportion of the variability observed in the precision and accuracy results could be associated with this phenomenon. In this regard, an internal standard could be used to correct the variability, such as

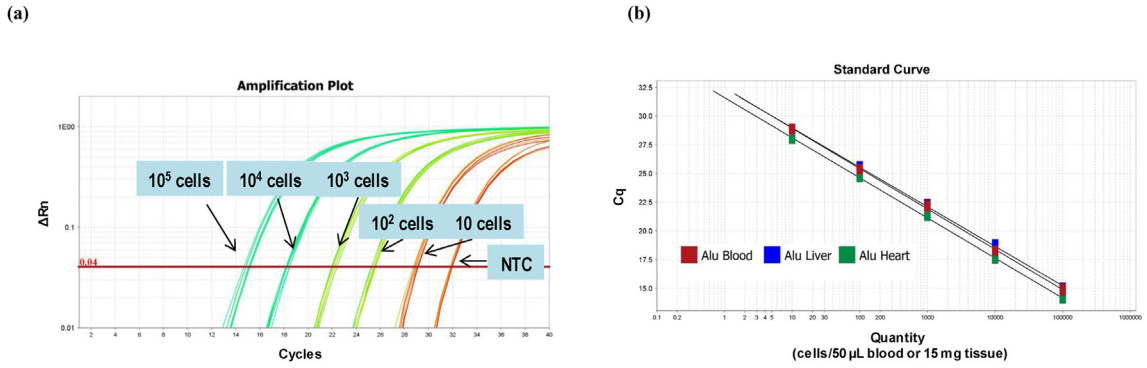

Fig. 2. (a) Amplification plot of human cell-spiked mouse blood (n = 3), and (b) calibration curves for human cell-spiked mouse blood, liver, and heart samples (n = 3 for each sample type) assayed using the developed *Arthrobacter luteus* (Alu)-based qPCR method.

Table 3
Calibration curves for mouse blood, liver, and heart samples.

Nominal concentrations	Blood		Liver		Heart	
	Back-calculated concentrations (cells/50 μL blood)	Accuracy (%)	Back-calculated concentrations (cells/15 mg tissue)	Accuracy (%)	Back-calculated concentrations (cells/15 mg tissue)	Accuracy (%)
100,000	99,300	99.3	113,000	113.0	104,000	104.0
10,000	11,000	110.0	9500	95.0	10,300	103.0
1000	884	88.4	867	86.7	914	91.4
100	103	103.0	95.9	95.9	94.5	94.5
10	10.2	102.0	11.3	113.0	10.9	109.0
0	1.48	–	1.95	–	0.828	–
Slope	−3.52		−3.43		−3.49	
Y-Intercept	32.5		32.4		31.6	
R^2	0.999		0.998		0.999	
PCR efficiency (%)	92.5		95.5		93.4	

Table 4
Assay precision and accuracy using mouse blood.

Run	Nominal Concentration (cells/50 μL blood)	Blood			
		LLOQ	Low	Medium	High
		10	30	1000	80,000
1	Mean (cells/50 μL blood)	11.1	28.5	992	66,400
	Precision (%CV)	7.2	7.4	5.2	17.3
	Accuracy (%)	111.0	94.9	99.2	83.0
	n	5	5	5	5
2	Mean (cells/50 μL blood)	9.45	24.5	861	70,600
	Precision (%CV)	5.9	2.6	7.8	10.9
	Accuracy (%)	94.5	81.7	86.1	88.3
	n	5	5	5	5
3	Mean (cells/50 μL blood)	8.43	23.0	744	59,600
	Precision (%CV)	6.4	5.2	9.5	7.3
	Accuracy (%)	84.3	76.8	74.4	74.5
	n	5	5	5	5
Inter-assay	Mean (cells/50 μL blood)	9.66	25.3	866	65,500
	Precision (%CV)	13.3	10.7	13.9	13.8
	Accuracy (%)	96.6	84.4	86.6	81.9
	n	15	15	15	15

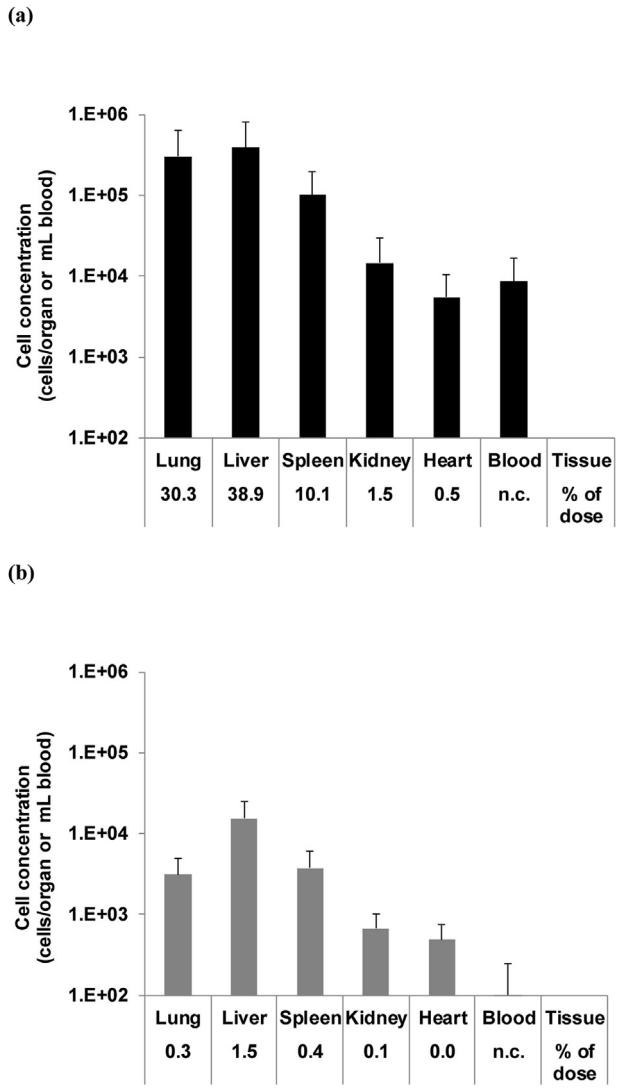

Fig. 3. Tissue and blood concentrations of human CD8$^+$ T cells after a single intravenous administration to mice at (a) 1 h and (b) 24 h. Bars represents means ± standard deviations (SDs, n = 3). Percentage dose (1×10^6 cells/animal) for each tissue is indicated (n.c. = not calculated).

spiking samples with cells of non-rodent and non-primate species, such as those of dogs and pigs, prior to DNA extraction. Indeed, a similar approach has been reported previously by Hong et al. [10].

3.6. Stability

We also assessed the stability of the newly developed Alu-qPCR method by analyzing spiked blood and tissue homogenates that had been stored for 1 month and subjected to three freeze–thaw cycles at −80 °C. The stability in each matrix was evaluated with respect to the accuracy of cell concentration against the spiked nominal value. The accuracies of stored and freeze–thawed human T cell-spiked blood samples and tissue homogenates were as follows: blood (−4.8%), liver (−21.7%), heart (−16.9%), kidney (−17.1%), and lung (23.6%). These values were within the inter-assay accuracy limit, which confirmed the stability of the Alu element-derived from human T cells for 1 month and after three freeze–thaw cycles at −80 °C.

3.7. In vivo cell kinetics and biodistribution study

When we used the developed Alu-qPCR method to analyzed tissue and blood concentrations of human CD8$^+$ T cells in SCID mice after a single intravenous administration (Fig. 3a and b), we found that most T cells were detected in the lung (30.3%), liver (38.9%), and spleen (10.1%) 1 h after intravenous administration and a few cells were also detected in the kidney, heart, and blood. However, we found that whereas T cells were rapidly eliminated from the blood and lungs within 24 h, they tended to be retained in the spleen and liver. A similar cell biodistribution has previously been reported for ^{51}Cr-labeled mouse splenocytes (>85% were CD8+ T cells) [27]. Furthermore, the blood concentrations of human CD8$^+$ T cells measured in the present study were found to be comparable to those detected previously using flow cytometry [26]. These results thus indicate that Alu-qPCR could be used to accurate quantify human cells and to elucidate their cellular kinetics in the body in the absence of any specific labeling.

4. Conclusion

In this study, we developed an Alu-qPCR assay that could be used to express the results in terms of cell number per volume or weight. Having developed the assay, we proceeded to perform bioanalytical method validation to assess assay specificity, selectivity, and variability. Alu-qPCR facilitated the specific detection of human T cells in mouse blood and tissue samples. The LLOQ of the Alu-qPCR was 10 cells/50 μL blood or 15 mg tissue (7.5 mg for the spleen and lung), and the corresponding standard curves were linear from 10 to 10^5 cells/50 μL blood or 15 mg tissue (7.5 mg for the spleen and lung). Our assessment of assay variability revealed that most CV (%) values of the assay were within 30%. The cell kinetics of administered human T cells in mice were successfully evaluated using the established Alu-qPCR, which exhibited sufficient specificity and sensitivity to detect human peripheral blood CD8+ T cells in mice. Our findings thus indicate that Alu-qPCR, which targets the primate-specific *Alu* gene, is a useful technique for quantifying transplanted human cells in animals that does not necessitate any specific cell labeling. From the perspectives of assay performance and criteria decisions, accumulation of assay performance data, as described in the current study, is necessary for future discussions on human cell quantification using qPCR. Furthermore, the data presented herein may contribute to standardizing bioanalytical approaches in the biodistribution studies of other cell therapy products.

Declaration of competing interest

None.

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Wrote or contributed to writing of the manuscript: H. Shimizu, Y. Kuze, T. Higuchi, S. Matsumoto, S. Yamamoto, A. Goto, Y. Moriya, and H. Hirabayashi.

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Appendix A. Supplementary data

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