



# T-cell-replete haploidentical stem cell transplantation is highly efficacious for relapsed and refractory childhood acute leukaemia

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## SUMMARY

**Background:** Despite improvements in first-line therapies, the outcomes of relapsed or refractory childhood acute leukaemia that has not achieved complete remission after relapse, has relapsed after stem cell transplantation (SCT), has primary induction failure and has relapsed with a very unfavourable cytogenetic risk profile, are dismal.

**Objectives and Methods:** We evaluated the feasibility and efficacy of T-cell-replete haploidentical peripheral blood stem cell transplantation (haplo-SCT) with low-dose anti-human thymocyte immunoglobulin (ATG), tacrolimus, methotrexate and prednisolone (PSL) in 14 paediatric patients with high-risk childhood acute leukaemia.

**Results:** All patients achieved complete engraftment. The median time to reaching an absolute neutrophil count of more than  $0.5 \times 10^9 \text{ L}^{-1}$  was 14 days. Acute graft-vs-host disease (aGVHD) of grades II–IV and III–IV developed in 10 (71%) and 2 (14%) patients, respectively. Treatment-related mortality and relapse occurred in one (7%) patient and six (43%) patients, respectively. Eleven patients were alive and seven of them were disease-free with a median follow-up of 36 months (range: 30–159 months). The probability of event-free survival after 2 years was 50%.

**Conclusion:** These findings indicate that T-cell-replete haplo-SCT, with low-dose ATG and PSL, provides sustained remission with an acceptable risk of GVHD in paediatric patients with advanced haematologic malignancies.

**Key words:** children, graft-vs-leukaemia effect, HLA-haploidentical stem cell transplantation, refractory leukaemia, T-cell-replete haploidentical stem cell transplantation.

## INTRODUCTION

The most common type of cancer in patients under the age of 18 is acute leukaemia (McNeil *et al.*, 2002). Although recent years have seen advances and improvements in effective therapies and outcomes, some cases remain persistently difficult to treat. Overall, the 5-year survival rates for patients with acute lymphoblastic leukaemia (ALL) and patients with acute myelogenous leukaemia (AML) are nearly 85% and 50–60%, respectively (Jemal *et al.*, 2008). Among children with relapsed acute leukaemia, 30–50% can be treated successfully with a combination of chemotherapy and allogeneic stem cell transplantation (SCT) (Hijiya *et al.*, 2004; Raetz *et al.*, 2008; Parker *et al.*, 2010; Tallen *et al.*, 2010). Despite these successes, children who fail to attain complete remission after relapse, relapse after an SCT, experience a primary induction failure, or relapse with a very unfavourable cytogenetic risk profile have very poor prognoses (Gaynon, 2005). With this in mind, it is crucial that new therapies and regimens be developed to meet the needs of this vulnerable population.

It is most challenging when a patient experiences a significant number of reinduction failures, particularly with an early recurrence of the disease. Furthermore, even when remission is achieved in these instances, it is less likely to persist. Gaynon *et al.* (2006) reported subsequent relapses in up to one-third of these patients within the median time to allogeneic SCT (allo-SCT). Longer-term event-free survival (EFS) rates for this population of high-risk acute leukaemia patients do not exceed 20% (Einsiedel *et al.*, 2005; Gaynon *et al.*, 2006), even with the use of intensive salvage strategies, including allo-SCT. Furthermore, patients with *fms*-like tyrosine kinase 3/internal tandem duplication (FLT3/ITD) positive or other NK-lineage leukaemia also have a very poor prognosis by conventional allo-SCT even if remission is achieved at transplantation (Suzuki *et al.*, 2006; Sengsayadeth *et al.*, 2012).

One form of immunotherapy that has proven to be highly efficacious for haematological malignancies that are primarily attributed to T-cell-mediated responses to human leukocyte

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antigen (HLA) disparities between donors and leukaemic cells is T-cell-replete haploidentical SCT (haplo-SCT) (Kolb, 2008).

Severe graft-vs-host disease (GVHD), rejection and a high risk of early death are related to major complications associated with haplo-SCT (Henslee-Downey *et al.*, 1997; Mehta *et al.*, 2004). The high risks of both graft failure and acute GVHD (aGVHD) associated with haplo-SCT have been mitigated in the past by infusing megadoses of purified *ex vivo* T-cell-depleted CD34<sup>+</sup> peripheral blood stem cells (PBSCs) (Aversa *et al.*, 1998, 2005). However, because the patients lost almost all of their T-cells, their immune reconstitution was slow. This led to high incidences of mainly viral and fungal infectious complications and high relapse rates. In recent studies, enabling haplo-SCT with a T-cell-replete graft using a vigorous pre- and post-transplantation pharmacologic GVHD prophylactic regimen has proven to be an encouraging alternative treatment for the patients (Huang, 2008; Chang & Huang, 2012). However, particularly among high-risk groups, the threat of relapse remains, and the treatment can fail.

Previously, we have reported on the safety profile of a GVHD prophylactic regimen consisting of anti-human thymocyte immunoglobulin (ATG), tacrolimus, methotrexate (MTX) and prednisolone (PSL) in T-cell-replete haplo-SCT and demonstrated that haplo-SCT with our GVHD prophylaxis regimen is as feasible as HLA matched unrelated SCT in terms of GVHD and treatment-related mortality (TRM) (Mochizuki *et al.*, 2011). Using a retrospective approach, we assessed this protocol and found that we were able to achieve an optimal graft-vs-leukaemia (GVL) effect on T-cells by performing T-cell-replete haplo-SCT using a myeloablative preconditioning regimen, accompanied by intensive GVHD prophylaxis, including low-dose ATG and steroids. Patients, even those at an advanced stage of the disease, responded to the treatment and relapse rates decreased.

## PATIENTS AND METHODS

Between August 2000 and April 2011, 14 consecutive patients with high-risk acute leukaemia underwent peripheral blood SCT from an HLA-haploidentical related donor at Fukushima Medical University Hospital. All patients were with a Karnofsky or Lansky performance score greater than or equal to 50 at transplantation. High-risk acute leukaemia was defined as primary refractory acute leukaemia not in remission or relapse. Those leukaemia are with poor prognostic features such as positivity for the Philadelphia chromosome, mixed lineage leukaemia gene rearrangements, FLT3/ITD and with early relapse ALL (<36 months from initial diagnosis). NK-lineage leukaemia is included because it has a very poor prognosis even after conventional SCT. The institutional review board approved the protocol, and written informed consent was obtained from the patients or their guardians and family donors. Data were analysed on 1 November 2013. The patient characteristics are shown in Table 1. Donors and patients shared one HLA haplotype, but differed in the other haplotype. For donor selection, there should only be at least three or four incompatible HLAs, and we

also preferred donors with the best possible health, who have the same blood type, and are not suffering from anaemia. Donors included fathers (nine), mothers (four) and siblings (one). HLA-A, HLA-B, HLA-C and HLA-DRB1 typing was performed by intermediate-resolution DNA typing (Genosearch HLA; Medical & Biological Laboratories Co., Ltd., Nagoya, Japan). HLA disparities in the graft-vs-host directions included four loci mismatches in 10 patients and three loci mismatches in four patients. The median patient age was 7.3 years (range: 0.8–17.9 years). Myeloablative conditioning was administered to 13 patients (total-body-irradiation-based, nine patients; Busulfan-based, four patients), and one patient with organ dysfunction received reduced intensity conditioning. The conditioning regimen of each patient is described in Table 2. Rabbit ATG (Genzyme Japan K. K., Tokyo, Japan) was used to treat 12 patients at a total dose of 2.5 mg kg<sup>-1</sup> body weight. GVHD prophylaxis was conducted using tacrolimus, MTX administered in a short term, and PSL. Tacrolimus was started on day -1, and was continuously administered intravenously. The concentration of tacrolimus in peripheral blood was adjusted to be between 7 and 15 ng mL<sup>-1</sup>. Three or four weeks after transplantation, the route of tacrolimus administration was changed to oral, with the target trough level within the range of 5–10 ng mL<sup>-1</sup>. MTX (10 mg m<sup>-2</sup>) was administered intravenously on day +1 and was reduced to 7 mg m<sup>-2</sup> on days +3 and +6 after transplantation. PSL was begun on day +0 at an initial dose of 1 mg kg<sup>-1</sup> body weight day<sup>-1</sup>. When there was no sign of aGVHD, from day +29, the PSL dose was tapered every week and was discontinued within 2 or 3 months after transplantation. All patients received granulocyte-colony-stimulating factor (G-CSF) intravenously on day +1 until sustained granulocyte recovery was achieved. Posttransplantation G-CSF was administered to all 14 patients.

The presence of minimal residual disease (MRD) was analysed in bone marrow samples at diagnosis of ALL in patients by using sensitive quantitative real-time reverse transcriptase polymerase chain reaction (RT-qPCR) methods. A tumour specific primer could be obtained in three patients (Patients 5, 8, 10), and MRD was detected in all three patients using a cut-off point of 0.001% at haplo-SCT.

Each patient was isolated in a laminar air-flow room, and standard decontamination procedures were followed. Intravenous immunoglobulin was administered at a minimum dose of 100 mg kg<sup>-1</sup> body weight every week until day +100. TMP/SMX was administered for at least 1 year for prophylaxis against *Pneumocystis* infections. Acyclovir was administered at 10 mg kg<sup>-1</sup> body weight for 35 days after transplantation to prevent herpes simplex infections. CMV-pp65 antigen testing of peripheral blood was performed once weekly. After grafting, ganciclovir or foscarnet administration was initiated in patients with CMV antigenemia.

## RESULTS

All the patients received G-CSF-mobilised PBSCs. The patients received PBSCs containing a median of  $9.5 \times 10^6$  (range:

**Table 1.** Patient, donor and graft characteristics

Patient	Age (year/sex)	Diagnosis	Cytogenetics	Status at SCT (time point of relapse)	Donor	HLA disparity in GVH	Stem cell source	CD34 <sup>+</sup> cells ( $\times 10^6 \text{ kg}^{-1}$ )	CD3 <sup>+</sup> cells ( $\times 10^8 \text{ kg}^{-1}$ )
1	0.8/M	ALL	t(4;11)	CR2 (VER)	Mother	4/8	PB	11	NT
2	1.8/F	AML		Refractory relapse after CBT	Mother	4/8	PB	10.49	5.36
3	7.7/M	AML		Refractory relapse	Father	3/8	PB	8.43	3.79
4	12.0/M	ALL		Refractory relapse after BMT	Father	3/8	PB	13.2	4.36
5	11.9/F	ALL		Refractory relapse, MRD+ (ER)	Father	4/8	PB	11.5	5.25
6	6.8/M	ALL		CR2 (ER)	Father	4/8	PB	8.32	1.18
7	6.0/M	M/NK-AL		Primary refractory	Father	4/8	PB	13	3.86
8	5.0/M	ALL	t(9;22)	Relapse after BMT, MRD+	Mother	3/8	PB	10	5.51
9	13.9/M	ALL		Refractory relapse after BMT	Father	4/8	PB	7.14	2.74
10	9.8/M	ALL	t(9;22)	Relapse after BMT, MRD+	Father	4/8	PB	7.8	5.22
11	2.9/M	ALL		Refractory relapse (VER)	Mother	4/8	PB	5.65	5.01
12	13.8/M	AMoL	FLT3/ITD	Primary refractory	Father	4/8	PB	8.9	5.84
13	17.9/M	M/NK-AL	t(4;11)	Primary refractory	Sibling	4/8	PB	12.7	3.71
14	6.1/F	ALL	t(4;11)	CR2, relapse after BMT	Father	3/8	PB	6.3	1.83

AMoL, acute monocytic leukaemia; BMT, bone marrow transplantation; CR2, second complete remission; CBT, cord blood transplantation; ER, early relapse at least 18 months after diagnosis but less than 6 months after cessation of chemotherapy; F, female; M, male; M/NK-AL, myeloid NK precursor acute leukaemia; NT, not tested; PB, peripheral blood stem cells; VER, very early relapse within 18 months after diagnosis.

Patients are sorted by the day of transplantation.

6.3–13.2  $\times 10^6$  CD34<sup>+</sup> cells  $\text{kg}^{-1}$  body weight without T cell depletion. Consequently, a median of 4.36  $\times 10^8$  (range: 1.18–5.84  $\times 10^8$ ) CD3<sup>+</sup> cells  $\text{kg}^{-1}$  body weight were transfused. All the patients were engrafted with a median time of 14 days (range: 11–15 days) for neutrophil recovery (absolute neutrophil count  $>0.5 \times 10^9 \text{ L}^{-1}$ ). The platelet recovery to more than  $20 \times 10^9 \text{ L}^{-1}$  was achieved in 13 patients in a median time of 28 days (range: 18–93 days). All the patients who began in non-remission achieved complete remission on day +30 and showed complete donor chimerism by day +30. Acute GVHD occurred in 12 of the 14 patients. Acute GVHD was grade I in two patients, grade II in eight patients and grade III in two patients. Of the two patients with grade III GVHD, one did not receive ATG. The other patient, who had received ATG, improved symptoms of gut GVHD by temporary augmentation with PSL and oral administration of beclomethasone dipropionate (BDP). Chronic GVHD (cGVHD) developed in 11 of 12 evaluable patients. Relapse occurred in six patients (43%) on days +45, +117, +159, +405, +600 and +670. Among the six patients who relapsed, one (Patient 6) received additional chemotherapy, one (Patient 11) received a second haplo-SCT from another haploidentical donor and one (Patient 13) received donor lymphocyte infusion for his bone marrow (BM) relapse. Another one (Patient 10) received radiation therapy for his local bone relapse. All four of these patients achieved complete remission and survived. TRM occurred in one patient (7%) as a result of Epstein–Barr virus (EBV)-associated lymphoproliferative disorder (Patient 4). Seven patients survived and were free of disease between 30 and 159 months after transplantation (Table 2). With a median follow up of 36 months, the probability of 2-year EFS and the overall survival (OS) rate were 50% and 79%, respectively (Fig. 1).

## DISCUSSION

Seeking to achieve an optimal GVL effect on T-cells, in this study we investigated the technique of T-cell-replete haplo-SCT using a myeloablative preconditioning regimen accompanied by intensive GVHD prophylaxis in high-risk paediatric patients. It is important to focus on these patients because this patient population has a longer-term EFS of no more than 20%, despite our conventional allo-SCT and our chemotherapy protocols known to be efficacious (Einsiedel *et al.*, 2005; Gaynon *et al.*, 2006). The transplant regimen detailed in this study decreased the incidence of relapse, despite the patients' advanced-stage leukaemia.

The outcome for patients who undergo haplo-SCT after myeloablative conditioning and standard GVHD prophylaxis is particularly dismal because they have the highest risk of graft rejection, aGVHD and, therefore, TRM (Powles *et al.*, 1983; Beatty *et al.*, 1985). Among BM transplants from partially HLA-matched donors, the incidence of primary graft failure is 12.3%. For recipients from an HLA-identical sibling, the failure rate is only 2.0% (Anasetti *et al.*, 1989). Several reports (Chang & Huang, 2012; Huang, 2008) detailed procedures for patients undergoing a haploidentical transplant with a more intensive *in vivo* GVHD prophylaxis. In patients receiving a T-cell-depleted graft, the rate of graft failure remains in the range of 0–17%, and the rate ranges of grades II–IV aGVHD, cGVHD and TRM are 8–48%, 0–35% and 28–65%, respectively. The graft failure rate among those receiving T-cell-replete grafts is in the range of 0–17%, and the incidence ranges of grades II–IV aGVHD, cGVHD and TRM are 16–66%, 13–53% and 9–35%, respectively.

Table 2. Stem cell transplantation and clinical outcome

Patient	Conditioning regimen	Engraftment Neut	Plt	Acute GVHD grade and stage (skin, liver, gut)	Chronic GVHD (affected organ)	Complications within 100 days	Outcome	Survival after SCT (days)	Cause of death	PS (%)
1	TBI + CA + Mel	15	33	III (2, 0, 2)	Moderate (skin, lung, gut)	TMA	CR	4783+	-	90
2	TBI + CA + Mel	15	21	I (1, 0, 0)	Mild (skin)	<i>Aspergillus</i>	CR	3964+	-	90
3	TBI + CY + CA* + ATG	15	28	II (3, 0, 1)	Mild (gut)	CMV antigenemia, <i>candida</i> sepsis	CR	1457+	-	90
4	Bu2 + Flu + Mel + ATG	15	34	0	Moderate (skin, mouth, gut)	NO	Death	439	EBV-LPD	-
5	TBI + VP16 + CY + ATG	12	22	II (3, 0, 0)	Mild (gut)	CMV antigenemia	CR	1393+	-	90
6	TBI + VP16 + CY + ATG	11	93	II (3, 0, 0)	Mild (skin, gut)	CMV antigenemia	Relapse on day 670	1198+ (CR survival)	-	90
7	TBI + VP16 + CY + ATG	14	28	II (3, 0, 0)	Mild (gut)	CMV antigenemia, <i>candida</i> sepsis	CR	1134+	-	90
8	Bu4 + Mel + ATG	13	18	I (1, 0, 0)	Mild (mucosa)	CMV antigenemia, BKV-HC, zoster	CR	1120+	-	90
9	Bu4 + CA + Mel + ATG	13	35	II (3, 0, 0)	NE	NO	Relapse on day 117	549	Relapse	-
10	Bu4 + Flu + Mel + ATG	12	21	II (3, 0, 0)	NO	HC (RRT)	Relapse on day 600	1050+ (CR survival)	-	100
11	TBI + VP16 + CY + ATG	15	27	II (3, 0, 0)	Mild (eye)	EBV-LPD, PRES, HHV6	Relapse on day 405	993+ (CR survival)	-	90
12	TBI + CY + CA* + ATG	15	NE	0	NE	NO	Relapse on day 45	132	Relapse	-
13	TBI + VP16 + CY + ATG	13	25	II (3, 0, 0)	Mild (gut)	<i>Klebsiella</i> sepsis, CMV antigenemia	Relapse on day 159	952+ (CR survival)	-	90
14	Bu4 + Flu + Mel + ATG	11	75	III (2, 0, 3)	Mild (skin)	BKV-HC, EBV-LPD, zoster	CR	913+	-	90

ATG, anti-human thymocyte immunoglobulin at 2.5 mg kg<sup>-1</sup>; Bu2, busulfan at 8 mg kg<sup>-1</sup>; Bu4, busulfan at 12–16 mg kg<sup>-1</sup>; CA, cytarabine at 12 g m<sup>-2</sup>; CA\*, cytarabine at 12 g m<sup>-2</sup> combined with G-CSF; CR, complete remission; CMV, cytomegalovirus; CY, cyclophosphamide at 120 mg kg<sup>-1</sup>; Flu, fludarabine at 150 mg m<sup>-2</sup>; HC, hemorrhagic cystitis; Mel, melphalan at 140 mg m<sup>-2</sup>; Neut, days to reach neutrophil count >0.5 × 10<sup>9</sup> μL<sup>-1</sup>; NO, not observed; NE, not evaluated; PRES, posterior reversible encephalopathy syndrome; PS, performance status; Plt, days to reach platelet count >20 × 10<sup>9</sup> μL<sup>-1</sup>; RRT, regimen-related toxicity; TBI, total-body-irradiation (12 Gy); TMA; thrombotic microangiopathy; VP16, VP16 at 1800 mg m<sup>-2</sup>.

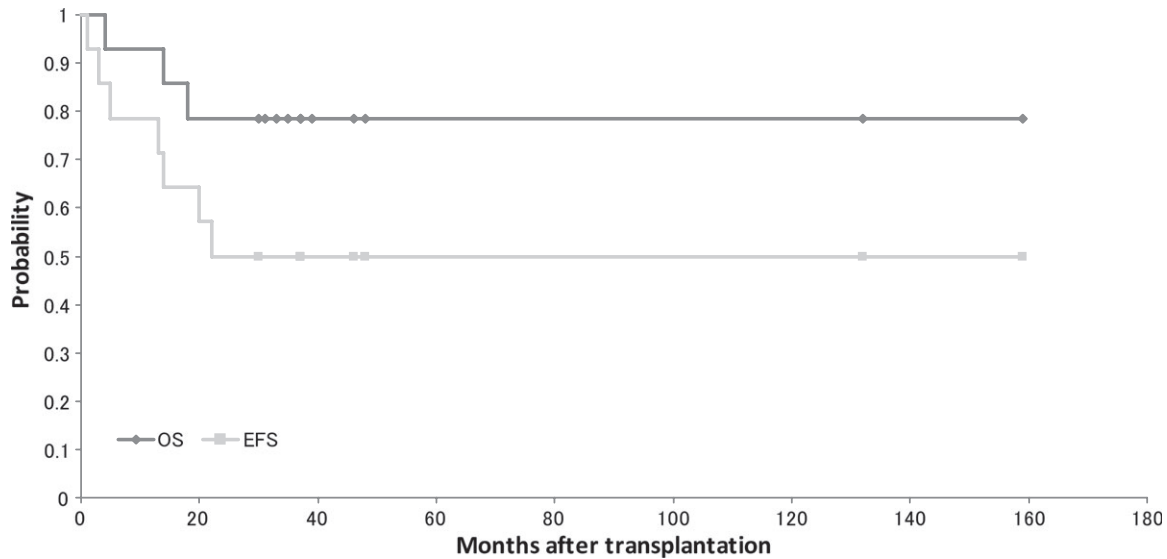


Fig. 1. Probability of OS and EFS. Kaplan–Meier estimates of OS and EFS in 14 patients who underwent T-cell-replete HLA-haploidentical SCT for advanced haematologic malignancies.

The conditioning treatment outlined in this present study, which includes low-dose ATG, demonstrated a satisfactory immunosuppressive effect. All the recipients achieved donor-type engraftments. On the basis of the data from the 14 patients we studied, we consider that the use of PBSCs containing a large number of T-cells (median:  $4.36 \times 10^8$  CD3<sup>+</sup> cells kg<sup>-1</sup> body weight) is also beneficial to obtain a high engraftment rate. In our study, 7% of patients (one patient) experienced TRM; this was associated with a viral infection. This risk seems to be acceptable, given the patient's poor condition. However, severe immunosuppression resulted in the high risk of infectious complications. We treated these complications through early detection and treatment of infection, including pre-emptive treatment for cytomegalovirus viraemia. We can decrease these infectious complications by using oral administration of BDP for gut GVHD patients instead of increasing general steroid administration. Frequent monitoring of EBV viral load by PCR and pre-emptive rituximab therapy may also reduce the risk of EBV-related lymphoproliferative disease (LPD) (Peric *et al.*, 2011).

The first two patients we treated did not receive ATG, and one of them developed grade III GVHD. All subsequent patients received ATG, and one of them developed grade III GVHD. Neudorf *et al.* (2004) reported on the beneficial effects of grades I–II aGVHD, but also noted the detrimental effects of grades III–IV aGVHD in children with AML. The role of aGVHD in ALL has been less clearly defined than the generally accepted notion that the GVL effect plays an important role in the treatment of AML. Many recent publications (Zecca *et al.*, 2002; Gustafsson Jernberg *et al.*, 2003; Nordlander *et al.*, 2004) have reported the effects of GVHD and GVL on remission rates and OS for acute leukaemic relapse after SCT in both adult and paediatric

populations. This indicates that the GVL effect may also improve the ALL survival rate.

To achieve a partial T-cell-depletion of the graft, we used low-dose ATG (2.5 mg kg<sup>-1</sup> body weight). Although this low-dose ATG can result in high incidences of grade II aGVHD and cGVHD, these GVHDs are manageable with steroids. Temporary augmentation with PSL and the oral administration of BDP are effective treatments. This may be one of the possible reasons for our favourable outcomes.

Haploidentical transplantation is now possible with high sustained engraftment rates, low early post-transplantation mortality rates and low rates of severe GVHD, owing to recent advances in effective T-cell depletion techniques. Despite these successes, poor immune recovery and associated infectious complications leading to mortality and relapse remain major obstacles to overcome. Participants in our study had generally very poor prognoses, yet, with this new treatment protocol, the estimated probability of EFS at 2 years is about 50%. Despite the relatively high incidences of acute and chronic GVHD, our data are encouraging in terms of survival and TRM.

In conclusion, we have shown that T-cell-replete myeloablative haplo-SCT using a GVHD prophylactic regimen consisting of low-dose ATG, tacrolimus, MTX and PSL can reconstitute long-term haematopoiesis with acceptable incidences of treatment-resistant GVHD while preserving GVL effects in high-risk paediatric patients. A large-scale study, however, is needed to confirm these results more definitively.

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H. S., K. M., M. A., S. K. T. W. and Y. O. collected the data; A. K. and S. K. analysed the data, interpreted the results and wrote the manuscript; M. H. and H. O. co-designed the experiments and discussed analyses and interpretation. All the authors discussed the results and commented on the manuscript.

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## CONFLICT OF INTEREST

The authors declare that there are no competing financial interests regarding this article.