Journal of Cancer Research and Clinical Oncology (2018) 144:587–592
https://doi.org/10.1007/s00432-018-2578-8

ORIGINAL ARTICLE – CLINICAL ONCOLOGY

CTLA-4 polymorphisms: influence on transplant-related mortality and survival in children undergoing allogeneic hematopoietic stem cell transplantation

Judith Hammrich[1] · Susan Wittig[1] · Thomas Ernst[2] · Bernd Gruhn[1]

Received: 18 November 2017 / Accepted: 6 January 2018 / Published online: 15 January 2018

Abstract

Purpose Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative approach for a variety of hematological diseases; however, it is still associated with substantial morbidity and mortality. Transplant-related mortality (TRM) after HSCT depends mainly on the toxicity of the conditioning regimen, infections, and graft-versus-host disease. The purpose of this study was to identify the association between CTLA-4 single nucleotide polymorphisms and TRM in children undergoing allogeneic HSCT.

Methods 153 donors and 153 children with acute lymphoblastic leukemia, acute myeloid leukemia or juvenile myelomonocytic leukemia who had undergone allogeneic HSCT were genotyped of CTLA-4 gene for rs3087243 (CT60G>A), rs231775 (+49 A>G) and rs4553808 using TaqMan real-time polymerase chain reaction.

Results We observed a significant association between the donor's CLTA-4 genotype of rs3087243 and TRM in children undergoing allogeneic HSCT. Genotype AG was found in 78 donors (51%), GG in 44 donors (29%) and 31 donors (20%) were homozygous for AA. 30 patients died as a result of transplant-related causes. Interestingly, we observed a significantly reduced TRM in children who were transplanted from a donor with the CTLA-4 genotype GG in comparison to genotype AG or AA (9 versus 19 versus 36%, $P=0.013$). In addition, we found significant differences of event-free survival (EFS) depending on the donor's genotype. The EFS was 64, 46 or 32% if the patient was transplanted from a donor with CTLA-4 genotype GG, AG or AA, respectively ($P=0.043$). In multivariate analysis, CTLA-4 genotype of rs3087243 was an independent risk factor for TRM ($P=0.011$) and EFS ($P=0.035$).

Conclusion This study provides first evidence that the CTLA-4 polymorphisms are significant risk factors for TRM and survival in children undergoing allogeneic HSCT and should be evaluated in further trials.

Keywords *CTLA-4* · Single nucleotide polymorphism · Allogeneic hematopoietic stem cell transplantation · Children · Transplant-related mortality

Introduction

Cancer is the second leading cause of death after accidents in children aged 1–14 years. The most common type of cancer in children is leukemia, which accounts for about one-third of all cases. Of these, acute lymphoblastic leukemia (ALL) is prevalent with about 75% followed by acute myeloid leukemia (AML) and juvenile myelomonocytic leukemia (JMML). However, thanks to significant improvement in treatment in recent decades, the 10-year survival of ALL has increased to over 90% (Pui et al. 2017). For most patients, chemotherapy is a sufficient treatment, although certain patients require a more aggressive treatment. Allogeneic hematopoietic stem cell transplantation (HSCT) has developed into an effective curative treatment for children with leukemia and poor risk features. The success of the allogeneic HSCT depends on several factors, of which the graft-versus-leukemia effect is very important for outcome (Hoffmann et al. 2015; Qin et al. 2016; Seggewiss and Einsele 2010).

✉ Bernd Gruhn
Bernd.Gruhn@med.uni-jena.de

[1] Department of Pediatrics, Jena University Hospital, Am Klinikum 1, 07747 Jena, Germany

[2] Department of Internal Medicine II, Jena University Hospital, Jena, Germany

Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a crucial inhibitory immune checkpoint molecule (Alatrash et al. 2016). The CTLA-4 gene is located on chromosome 2q33 and consists of 4 exons. Exons 1 and 2 code for the extracellular domain, exon 3 encodes the transmembrane domain and exon 4 encodes the cytoplasmic part. In humans, there are two main isoforms: the isoform full length CTLA-4 (flCTLA-4) that contains 4 exons and the isoform soluble CTLA-4 (sCTLA-4) which is generated by alternative splicing and lacks the transmembrane part (Mossallam and Samra 2013; Teft et al. 2006; Zhang et al. 2016). In resting T cells, flCTLA-4 is mainly localized in intracellular compartments (Linsley et al. 1996; Salama and Hodi 2011). The translocation to the cell surface is provoked by T cell receptor contact combined with further costimulatory signals (Egen and Allison 2002; Linsley et al. 1996; Salama and Hodi 2011). Subsequently, CTLA-4 binds CD80 and CD86 on antigen-presenting cells with an affinity 20 times higher than the competing CD28 indicating that the inhibitory signal predominates. The downregulation of the T cell-mediated immune response is particularly implemented by the inhibition of the cell cycle, decrease of transcription factors NFκB, NF-AT and AP-1 and reduced production of cytokines such as IL-2 (Karabon et al. 2015; Krummel and Allison 1995; Linsley et al. 1992; Sansom and Walker 2006; Teft et al. 2006; Valk et al. 2008).These mechanisms lead to modulation of the immune response, influence on T cell homeostasis, differentiation, and affection of central and peripheral tolerance (Krummel and Allison 1995; Laurent et al. 2010; Olsson et al. 1999; Sellami et al. 2011; Valk et al. 2008; Zhang et al. 2016). The significance of CTLA-4 was verified by studies with monoclonal antibodies and CTLA-4 deficient mice which developed lymphoproliferative diseases and died within 4 weeks (Fife et al. 2006; Tivol et al. 1995; Waterhouse et al. 1995). To ensure the required balance sCLTA-4, constitutively expressed by regulatory T cells, amplifies the immune response (Magistrelli et al. 1999; Purohit et al. 2005). Genetic polymorphisms within the CTLA-4 gene affect the ratio of the two isoforms and, therefore, the extent of the T cell activity (Karabon et al. 2015; Mossallam and Samra 2013; Perez-Garcia et al. 2007; Ueda et al. 2003). The single nucleotide polymorphism (SNP) CT60 has gained increasing attention in the latest research, because a large number of studies described CT60 as a possible predictor of transplantation outcome (Bosch-Vizcaya et al. 2012; Jagasia et al. 2012; Karabon et al. 2015; Mossallam and Samra 2013; Orrù et al. 2012; Qin et al. 2016; Sellami et al. 2011; Metaxas et al. 2012). Our aim was to examine the association between certain CTLA-4 polymorphisms and the outcome after allogeneic HSCT in children.

Patients and methods

Patients

We retrospectively analyzed 153 patients and their donors transplanted at the Department of Pediatrics, Jena University Hospital, Jena, Germany. We included all children with ALL, AML, and JMML who received allogeneic hematopoietic stem cell transplantation. Further characteristics of the patients are presented in Table 1.

Genotyping of CTLA-4 polymorphism

DNA was isolated from blood, cord blood, or bone marrow aspirates using High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. The concentration of the purified

Table 1 Characteristics of patients and donors ($n = 153$)

Characteristics	Total, no. (%)
Median age of the patients (y)	11
Sex of the patients	
Male	88 (57.5)
Female	65 (42.5)
Disease	
ALL	90 (58.8)
AML	58 (38.0)
JMML	5 (3.2)
Remission (complete)	
First	49 (32.0)
Second	40 (26.2)
Third	15 (9.8)
Not in remission	49 (32.0)
Conditioning regimen (based on)	
Total body irradiation	79 (51.6)
Busulfan	68 (44.4)
GVHD prophylaxis	
Cyclosporine A/methotrexate	98 (64.1)
Cyclosporine A	38 (24.8)
None	12 (7.8)
Others	5 (3.3)
Donor type	
HLA-matched unrelated	71 (46.6)
HLA-mismatched unrelated	20 (13.1)
HLA-identical related	47 (30.7)
HLA-haploidentical related	15 (9.8)
Cell type	
Bone marrow	105 (68.6)
Peripheral blood stem cells	46 (30.1)
Cord blood	2 (1.3)

template DNA was quantified at 260 and 280 nm using the BioPhotometer plus (Eppendorf, Wesseling-Berzdorf, Germany). We generated a mix containing 1 µL (10 ng/µL) DNA, 10 µL Genotyping Mastermix, 9.5 µL sterile aqua and 0.5 µL primer-probe mix (TaqMan Genotyping Assays by Applied Biosystems). The samples and minimum five negative controls were transferred into 96-well optical Reaction Plates with Barcode by pipettes. Using 7900HT Fast Real-Time PCR System by Applied Biosystems, the DNA underwent an absolute quantification process with two diverse detectors. Therefore, the samples were heated 10 min to 95 °C for activation, followed by 40 cycles each of 15 s at 92 °C for denaturation and closing with 60 s at 60 °C for annealing and extension. Afterwards, the particular SNP was analyzed during an allelic discrimination post-read run. Overall we explored three different SNPs: rs3087243 (C_3296043_10), rs231775 (C_2415786_20) and rs4553808 (C_27916481_10).

Statistical analysis

Our primary aim was to examine a potential correlation between CTLA-4 polymorphism and event-free survival (EFS), overall survival (OS), transplant-related mortality (TRM), and relapse rate (RR). EFS and OS were calculated by the Kaplan–Meier method. Differences were compared using the log-rank test. OS was defined as the time between stem cell transplantation and death of any cause. EFS describes the time between stem cell transplantation and relapse, secondary malignancy, or death. RR and TRM were analyzed using survival calculation with competing risks. Differences between the curves were evaluated with the Gray test (Gray 1988). RR describes the cumulative incidence of relapse. TRM was defined as death without a former sign of progression or relapse. All calculations were made using IBM SPSS Statistics 23 and R Foundation for Statistical Computing 3.2.5. A P value of <0.05 was considered statistically significant. Multivariate analyses were performed to identify possible confounding variables such as disease risk, gender match, and age at time of diagnosis. Disease risk was considered low if the patients were transplanted in complete first or second remission and high if the transplantation was performed in more than second remission or in relapse.

Results

Frequency of polymorphisms

We analyzed 153 pairs of patients and their donors. The heterozygote genotype AG of CTLA-4 SNP rs3087243 was found in 78 donors (51.0%), GG in 44 donors (28.8%) and AA in 31 donors (20.3%). The distribution of CTLA-4 SNPs did not differ from results of a publication with 536 donors (Perez-Garcia et al. 2007).

Event-free survival

Three years post-transplantation, 74 of 153 patients (48.4%) survived without a relapse. Interestingly, we found a significant association between the donor's genotype and the event-free survival (EFS). The EFS was 63.6, 46.2 or 32.3% if the patient was transplanted from a donor with CTLA-4 genotype GG, AG, or AA, respectively ($P=0.043$) (Fig. 1). This difference was also detected when analyzing only patients who were in first or second remission at time of transplantation ($P=0.020$). The EFS was also better for patients transplanted from donors with CTLA-4 genotype GG, when analyzing only patients with ALL ($P=0.038$).

Transplant-related mortality

Seventy patients died within 3 years post-transplantation, 30 deaths of which were transplant related. Ten patients (33.3%) died of infection in which seven patients (23.3%) suffered from viral infection and three patients (10.0%) from invasive fungal disease. Three of the ten patients were additionally diagnosed with graft-versus-host disease (GVHD). Further five patients (16.7%) died of acute GVHD, two patients (6.7%) of chronic GVHD and three patients (10.0%) of hepatic sinusoidal obstruction syndrome. Further ten patients (33.3%) died of multi-organ failure with different leading focuses. In four cases (13.3%), the patient's

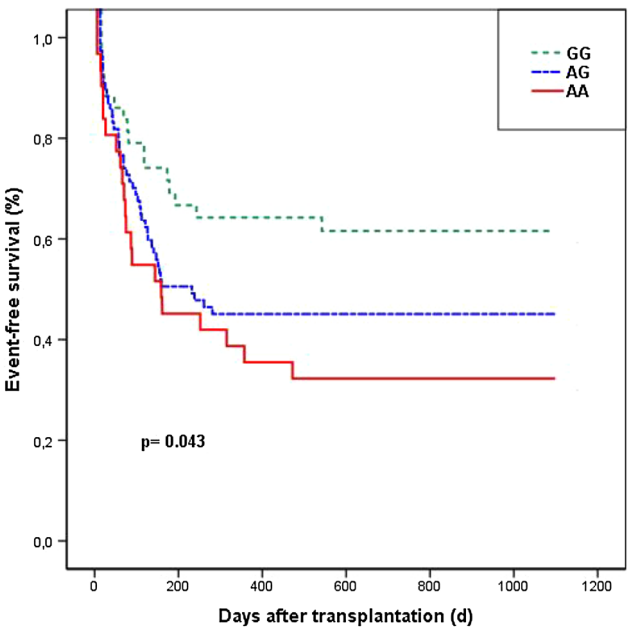

Fig. 1 Event-free survival according to donor's SNP rs3087243 status

condition was primarily impaired by pulmonary failure, in three cases (10.0%) by sepsis without an identified pathogen, in one case (3.3%) by cardiomyopathy, in one case (3.3%) by capillary leak syndrome and in one case (3.3%) by intracranial hemorrhage. We observed a significantly reduced TRM in children who were transplanted from a donor with the CTLA-4 genotype GG in comparison to genotype AG or AA (9.1 versus 19.2 versus 35.5%, $P=0.013$) (Fig. 2).

Multivariate analysis

Multivariate analysis was used to test whether the association between donor's CTLA-4 polymorphism and TRM and EFS was affected by other factors. The results are presented in Table 2 and indicate that the rs3087243 CTLA-4 SNP is an independent risk factor for EFS and TRM.

The donor's CTLA-4 SNP CT60 was not significantly associated with either acute GVHD ($P=0.477$) or chronic GVHD ($P=0.55$). There was no significant correlation between CT60 SNP and RR ($P=0.748$). The recipient's genotype had no significant effect on the surveyed clinical outcomes. Furthermore, no significant correlation was observed between the CTLA-4 polymorphisms rs231775 or rs4553808 and acute GVHD, chronic GVHD, OS or TRM.

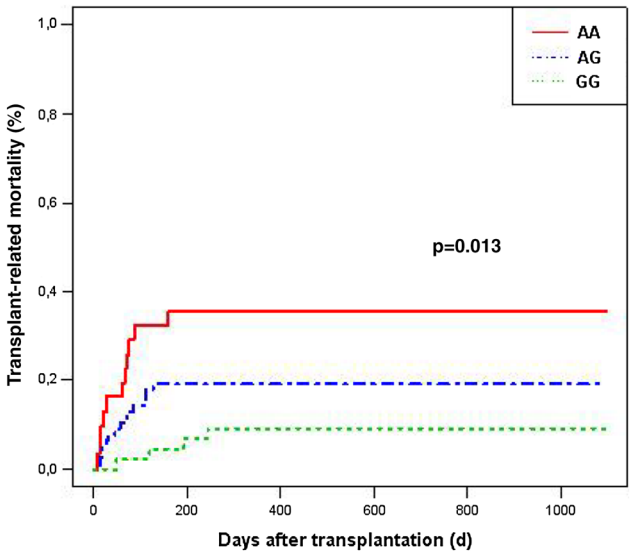

Fig. 2 Transplant-related mortality according to donor's SNP rs3087243 status

Discussion

In this paper, we would like to point out that the donor's CTLA-4 polymorphism CT60 has a significant influence on TRM ($P=0.011$) and EFS ($P=0.035$). CT60 is located in the 3'untranslated region of the CTLA-4 gene (Purohit et al. 2005). Many studies have evaluated the correlation between the exchange of guanine and adenine in CT60 and the outcome after allogeneic HSCT: Perez Garcia et al. (2007) postulated in their study that donor's CT60G>A [AA] increased the risk of acute GVHD II-IV but with a reduced relapse rate and better 5-year overall survival. Similarly, Qin et al. (2016) found an association between CT60G>A [AA] or [AG] and the higher risk of developing an extensive chronic GVHD compared to CT60G>A [GG] in patients diagnosed with ALL. However, results of other studies conflict with the conclusions above. Karabon et al. (2015) described a lower frequency, that was not statistically significant, of acute GVHD if the donor had the genotype CT60G>A [AA] compared to CT60G>A [AG] or [GG]. Correspondingly, the study of Xiao et al. (2012) indicated that patients receiving hematopoietic stem cells from donors with CT60G>A [AA] had a lower risk of developing acute GVHD II-IV. This incoherency may be explained by different types of transplantation, different conditioning protocols, and GVHD prophylaxis or different ethnic constellations. For example, Karabon et al. (2015) and Xiao et al. (2012) included cases with both related and unrelated donors. Whereas Perez Garcia et al. (2007) and Qin et al. (2016) used more homogenous groups with either HLA-identical siblings or HLA-haploidentical donors. In addition, our patients are children while all other studies analyzed adults (median age 11 years versus 23–34 years). Further pediatric studies are needed to evaluate the significance of this difference.

In our study, we found no statistically significant correlation between CT60G>A polymorphisms and acute or chronic GVHD. The lack of association was also reported by Mossallam et al. (2013) and Murase et al. (2011). However, we postulate that allele A is associated with a higher risk of TRM referring to an explanatory model, which is

Table 2 Multivariate analysis. Event-free survival (EFS) and transplant-related mortality (TRM)

Variable	EFS		TRM	
	HR (95% CI)	*P*	HR (97.5% CI)	*P*
Donor CTLA-4 (rs3087243)	0.704 (0.509–0.975)	0.035	0.507 (0.301–0.853)	0.011
Disease risk	0.413 (0.259–0.658)	<0.001	3.029 (1.407–6.519)	0.005
Gender match	1.398 (0.870–2.248)	0.166	1.570 (0.744–3.312)	0.240
Age at time of diagnosis	0.937 (0.589–1.491)	0.782	2.041 (0.959–4.345)	0.064

supported by the assumptions of Perez et al. (2007) and Qin et al. (2016): CTLA-4 exists in two isoforms. The isoform flCTLA-4 inhibits the T-cell activation by negative signaling and leads to T-cell anergy by B7 sequestration (Schwartz 2003; Krummel and Allison 1995; Jagasia et al. 2012). The isoform sCTLA-4 antagonizes this process to keep the immune response in balance. The A allele leads to a higher expression of sCTLA-4 and consequently the T cells stay more active (Perez-Garcia et al. 2007; Qin et al. 2016; Ueda et al. 2003). This explains the higher incidence of GVHD if patients received stem cells from donors with CT60G>A [AA]. However, this also means that the graft-versus-leukemia effect is intensified. This consideration matches with the fact that in other CTLA-4 polymorphisms, a correlation between A allele and decreased risk of relapse was shown because the more active T cells destroy residual leukemic cells (Jagasia et al. 2012).

We found a significant correlation between the children receiving CT60 [GG] T-cells from their donor and a better EFS (63.6 versus 46.2 and 32.3%). For an explanation, several studies focused only on one complication, the GVHD. In our research, the transplant-related mortality was predominated by infections (10 cases) and multi-organ failure (10 cases), whereas acute and chronic GVHD caused death in seven cases. We assume that CT60 [GG] optimizes the activity of the T-cells, so that the balance between suppression of the immune response and an effective defense is well adjusted. Precisely, patients are protected from an excessive T-cell activity that can cause multi-organ failure, but the level of activity stays high enough to fulfill an accurate defense of infections. This hypothesis matches with the results of Bosch et al. (2012) who found a non-significant trend of increased TRM, when the donor had genotype CT60 [AA]. Knowing the level of T-cell activity, determined by CTLA-4 polymorphisms, could improve the risk assessment and promote the selection of the most suitable donor and medication.

In conclusion, this study provides first evidence that the CTLA-4 polymorphisms could be significant risk factors for TRM and survival in children undergoing allogeneic HSCT. Thus, further pediatric multicenter studies with a larger number of patients are necessary to properly evaluate our findings.

Compliance with ethical standards

Conflict of interest The authors have no potential conflicts of interest to declare.

Ethical standard All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study has been approved by the Jena University Hospital Ethics Committee (5154-05/17). Informed consent was obtained from all individual participants or the responsible persons included in the study.

References

Alatrash G, Daver N, Mittendorf EA (2016) Targeting immune checkpoints in hematologic malignancies. Pharmacol Rev 68:1014–1025

Bosch-Vizcaya A, Pérez-García A, Brunet S, Solano C, Buño I, Guillem V, Martínez-Laperche C, Sanz G, Barrenetxea C, Martínez C, Tuset E, Lloveras N, Coll R, Guardia R, González Y, Roncero JM, Bustins A, Gardella S, Fernández C, Buch J, Gallardo D (2012) Donor CTLA-4 genotype influences clinical outcome after T cell-depleted allogeneic hematopoietic stem cell transplantation from HLA-identical sibling donors. Biol Blood Marrow Transpl 18:100–105

Egen JG, Allison JP (2002) Cytotoxic T Lymphocyte Antigen-4 accumulation in the immunological synapse is regulated by TCR signal strength. Immunity 16:23–35

Fife BT, Griffin MD, Abbas AK, Locksley RM, Bluestone JA (2006) Inhibition of T cell activation and autoimmune diabetes using a B cell surface–linked CTLA-4 agonist. J Clin Investig 116:2252–2261

Gray RJ (1988) A class of K-sample tests for comparing the cumulative incidence of a competing risk. Ann Statist 16:1141–1154

Hoffmann GF, Lentze MJ, Spranger J, Zepp F (2015) Pädiatrie: Grundlagen und Praxis. Springer, New York

Jagasia M, Clark WB, Brown-Gentry KD, Crawford DC, Fan KH, Chen H, Kassim A, Greer JP, Engelhardt BG, Savani BN (2012) Genetic variation in donor CTLA-4 regulatory region is a strong predictor of outcome after allogeneic hematopoietic cell transplantation for hematologic malignancies. Biol Blood Marrow Transpl 18:1069–1075

Karabon L, Markiewicz M, Partyka A, Pawlak-Adamska E, Tomkiewicz A, Dzierzak-Mietla M, Kyrcz-Krzemien S, Frydecka I (2015) A CT60G>A polymorphism in the CTLA-4 gene of the recipient may confer susceptibility to acute graft versus host disease after allogeneic hematopoietic stem cell transplantation. Immunogenetics 67: 295–304

Krummel MF, Allison JP (1995) CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med 182: 459–465

Laurent S, Carrega P, Saverino D, Piccioli P, Camoriano M, Morabito A, Dozin B, Fontana V, Simone R, Mortara L, Mingari MC, Ferlazzo G, Pistillo MP (2010) CTLA-4 is expressed by human monocyte—derived dendritic cells and regulates their functions. Hum Immunol 71: 934–941

Linsley PS, Wallace PM, Johnson J, Gibson MG, Greene JL, Ledbetter JA, Singh C, Tepper MA (1992) Immunosuppression in vivo by a soluble form of the CTLA-4 T cell activation molecule. Science 257: 792–795

Linsley PS, Bradshaw J, Greene JA, Peach R, Bennett KL, RS Mittler (1996) Intracellular trafficking of CTLA-4 and focal localization towards sites of TCR engagement. Immunity 4: 535–543

Magistrelli G, Jeannin P, Herbault N, Coignac ABde, Gauchat J-F, Bonnefoy J-Y, Delneste Y (1999) A soluble form of CTLA-4 generated by alternative splicing is expressed by nonstimulated human T cells. Eur J Immunol, 29: 3596–3602

Metaxas Y, Bertz H, Spyridonidis A, Spyroupoulou-Vlachou M, Porzelius C, Finke J (2012) CT60 single-nucleotide polymorphism as a surrogate marker for donor lymphocyte infusion outcome after allogeneic cell transplantation for acute leukemia. Bone Marrow Transpl 47: 411–415

Mossallam GI, Samra MA (2013) CTLA-4 polymorphism and clinical outcome post allogeneic hematopoietic stem cell transplantation. Hum Immunol 74:1643–1648

Murase M, Nishida T, Onizuka M, Inamoto Y, Sugimoto K, Imahashi N, Murata M, Miyamura K, Kodera Y, Inoko H, Naoe T (2011) Cytotoxic T-lymphocyte antigen 4 haplotype correlates with relapse and survival after allogeneic hematopoietic SCT. Bone Marrow Transpl 46:1444–1449

Olsson C, Riebeck K, Dohlsten M, Michaëlsson E (1999) CTLA-4 ligation suppresses CD28-induced NF-κB and AP-1 activity in mouse T cell blasts. J Biol Chem 274:14400–14405

Orrù S, Orrù N, Manolakos E, Littera R, Caocci G, Giorgiani G, Bertaina A, Pagliara D, Giardini C, Nesci S (2012) Recipient CTLA-4* CT60-AA genotype is a prognostic factor for acute graft-versus-host disease in hematopoietic stem cell transplantation for thalassemia. Hum Immunol 73: 282–286

Perez-Garcia A, De la Camara R, Roman-Gomez J, Jimenez-Velasco A, Encuentra M, Nieto JB, de la Rubia J, Urbano-Ispizua A, Brunet S, Iriondo A, Gonzalez M, Serrano D, Espigado I, Solano C, Ribera JM, Pujal JM, Hoyos M, Gallardo D, G. VHD/Immunotherapy Committee of the Spanish Group of Hematopoietic Stem Cell Transplantation (2007) CTLA-4 polymorphisms and clinical outcome after allogeneic stem cell transplantation from HLA-identical sibling donors. Blood 110: 461–467

Pui C-H, Pei D, Raimondi SC, Coustan-Smith E, Jeha S, Cheng C, Bowman WP, Sandlund JT, Ribeiro RC, Rubnitz JE, Inaba H, Gruber TA, Leung WH, Yang JJ, Downing JR, Evans WE, Relling MV, Campana D (2017) Clinical impact of minimal residual disease in children with different subtypes of acute lymphoblastic leukemia treated with response-adapted therapy. Leukemia 31: 333–339

Purohit S, Podolsky R, Collins C, Zheng W, Schatz D, Muir A, Hopkins D, Huang Y-H, She J-X (2005) Lack of correlation between the levels of soluble cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) and the CT-60 genotypes. J Autoimmun Dis 2:8–8

Qin XY, Wang Y, Li GX, Qin YZ, Wang FR, Xu LP, Chen H, Han W, Wang JZ, Zhang XH, Chang YJ, Liu KY, Jiang ZF, Huang XJ (2016) CTLA-4 polymorphisms and haplotype correlate with survival in ALL after allogeneic stem cell transplantation from related HLA-haplotype-mismatched donor. J Transl Med 14:100

Salama AKS, Stephen Hodi F (2011) Cytotoxic T-lymphocyte–associated antigen-4. Clin Cancer Res 17:4622–4628

Sansom DM, LSK Walker (2006) The role of CD28 and cytotoxic T-lymphocyte antigen-4 (CTLA-4) in regulatory T-cell biology. Immunol Rev 212: 131–148

Schwartz RH (2003) T cell anergy. Annu Rev Immunol 21: 305–34

Seggewiss R, Einsele H (2010) Immune reconstitution after allogeneic transplantation and expanding options for immunomodulation: an update. Blood 115:3861–3868

Sellami MH, Bani M, Torjemane L, Kaabi H, Ladeb S, Othmane TB, Hmida S (2011) Effect of donor CTLA-4 alleles and haplotypes on graft-versus-host disease occurrence in Tunisian patients receiving a human leukocyte antigen–identical sibling hematopoietic stem cell transplant. Hum Immunol 72: 139–143

Teft WA, Kirchhof MG, Madrenas J (2006) A molecular perspective of CTLA-4 function. Annu Rev Immunol 24:65–97

Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH (1995) Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity 3: 541–447

Ueda H, Howson JMM, Esposito L, Heward J, Snook H, Chamberlain G, Rainbow DB, Hunter KMD, Smith AN, Genova GD, Herr MH, Dahlman I, Payne F, Smyth D, Lowe C, Twells RCJ, Howlett S, Healy B, Nutland S, Rance HE, Everett V, Smink LJ, Lam AC, Cordell HJ, Walker NM, Bordin C, Hulme J, Motzo C, Cucca F, Hess JF, Metzker ML, Rogers J, Gregory S, Allahabadia A, Nithiyananthan R, Tuomilehto-Wolf E, Tuomilehto J, Bingley P, Gillespie KM, Undlien DE, Ronningen KS, Guja C, Ionescu-Tirgoviste C, Savage DA, Maxwell AP, Carson DJ, Patterson CC, Franklyn JA, Clayton DG, Peterson LB, Wicker LS, Todd JA, Gough SCL (2003) Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature 423: 506–511

Valk E, Rudd CE, Schneider H (2008) CTLA-4 trafficking and surface expression. Trends Immunol 29: 272–279

Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP, Thompson CB, Griesser H, Mak TW (1995) Lymphoproliferative disorders with early lethality in mice deficient in *CTLA-4*. Science 270: 985–988

Xiao H, Luo Y, Lai X, Fu S, Shi J, Tan Y, He J, Xie W, Zheng W, Wang L-M, Zhang L, Liu L, Ye X, Yu X, Cai Z, Lin M, Huang H (2012) Genetic variations in T-cell activation and effector pathways modulate alloimmune responses after allogeneic hematopoietic stem cell transplantation in patients with hematologic malignancies. Haematologica 97:1804–1812

Zhang C, Hou W-H, Ding X-X, Wang X, Zhao H, Han X-W, Wang W-J (2016) Association of cytotoxic T-lymphocyte antigen-4 polymorphisms with malignant bone tumors risk—a meta-analysis. Asian Pac J Cancer Prev 17:3785–3791