REVIEW

An analysis of the therapeutic benefits of genotyping in pediatric hematopoietic stem cell transplantation

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ABSTRACT Hematopoietic stem cell transplantation is a high-risk procedure that is offered, with curative intent, to patients with malignant and nonmalignant disease. The clinical benefits of personalization of therapy by genotyping have been demonstrated by the reduction in transplant related mortality from donor–recipient HLA matching. However, defining the relationship between genotype and transplant conditioning agents is yet to be translated into clinical practice. A number of the therapeutic agents used in stem cell transplant preparative regimens have pharmacokinetic parameters that predict benefit of incorporating pharmacogenomic data into dosing strategies. Busulfan, cyclophosphamide, thio-TEPA and etoposide have well-described drug metabolism pathways, however candidate gene studies have identified there is a gap in the identification of pharmacogenomic data that can be used to improve transplant outcomes. Incorporating pharmacogenomics into pharmacokinetic modeling may demonstrate the therapeutic benefits of genotyping in transplant preparative regimen agents.

The second diploid human genome to be sequenced and made publicly available and the first to be defined using next-generation sequencing belonged to James D Watson [1]. A biologist and a luminary of his generation, he was best known for his role in defining the structure of the DNA double helix. The publication of Watson's genome demonstrated that genomic technologies could be used to pursue personalized medicines theory. In his twilight years Watson required β-adrenergic antagonists to manage hypertension and described the sedative side effects experienced as leaving him "constantly falling asleep at inappropriate moments" [2].

β-adrenergic antagonists are metabolized by the hepatic micro-enzyme system and genetic variations in the gene that encodes for the CYP2D6 enzyme on chromosome 22 is associated with variability in drug response and in tolerability [3]. Watson's genome revealed he was homozygous for CYP2D6*10, that he had inherited two nonfunctional alleles which rendered him an intermediate metabolizer of this drug class. This insight into his own genetic code allowed Watson to reduce the β-blocker dosing frequency and ameliorate the malaise and drowsiness without compromising the antihypertensive effect [2].

Despite Watson's case, pharmacogenomic principles to guide dosage regimen design to an individual's genotype remain in the most instances theoretical. In cardiology, where β-blockers are prescribed most frequently, patients are not routinely genotyped and there is no wide spread genome led dosing for any of the other pharmacological classes used to manage hypertension [3].

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In the most part this is due to lack of sufficient evidence to demonstrate a clear link between genotype and phenotype, as well as a multitude of alternative therapeutic options for treating hypertension. This vast list of antihypertensives includes agents that vary mechanistically, have a wide therapeutic margin, have diverse side effect profiles and hence it is simply easier, less time consuming and more cost effective to adopt empirical approaches in dosage individualization. In cancer medicine however, dosing strategies exist at the edge of patient tolerability; most agents have a narrow therapeutic window and in some treatment options such as in hematopoietic stem cell transplantation, the therapeutic index is intentionally exceeded in order to achieve myeloablation and allow engraftment of the donor marrow in the hematopoietic niche. Consequently genotype-based dosage individualization provides an opportunity to reduce toxicity and improve transplant outcomes.

A short history of genotyping in stem cell transplant

The improvement in overall survival in pediatric malignancies can be described as one of modern medicines true success stories. The application of multimodality treatment is credited with the improvement in overall survival, as is the enrollment of children on clinical trials [4]. Advances in treatment and also in diagnostics have seen overall survival increase and in some cases, the toxicities reduced. In some high-risk malignancies however, relapse remains a real risk and stem cell transplant is the only potential curative therapy [5]. In stem cell transplant patients the treatment toxicity burden is high and although improving, relapse and nonrelapse mortality along with acute and long-term toxicity remains a significant cause of concern [6]. This makes the process of genotyping as a clinical approach in the management of drug-related toxicity an attractive proposition.

The allogeneic transplant journey is divided into a number of overlapping phases **(Figure 1)**. The first phase, the recipient donor search could be described as the poster child for the translation of genotyping into clinical practice [7]. High-resolution molecular typing of type I and type II HLA is employed in the donor-receipt search, and it has been demonstrated that there is a correlation between the number of mismatched alleles and the relative risk of mortality [8] with a fully matched donor recipient pair exhibiting

lower rates of engraftment failure, acute and chronic graft versus host disease (GVHD) and transplant-related mortality when compared with mismatched donors [7]. The tolerance of disparity for each additional mismatch demonstrates that genotyping of the MHC; the most densely populated portion of the human genome can be used to improve transplant outcomes [7].

In contrast to the evolution seen in tissue typing procedures, the introduction of genetic or genome-based therapeutic strategies have not been seen in the antineoplastic agents used in transplant preparative regimens. The therapeutic approach has remained largely the same as in early transplant protocols and when a myeloablative regimen intensity is employed, the doses and pharmacological agents utilized are similar to those developed from early canine models [9] and tested in subsequent human trials [10]. This level of empiricism in the therapeutics of transplantation is explained by the relative high-risk nature of the procedure and the small numbers of patients transplanted each year comparative to other treatment modalities [6,11–12]. Clinical trials that result in evidence-based guidelines are limited and despite the precedence set by HLA typing utilizing genetic information to guide donor choice, drug dosing strategies that utilize germline genotypes are rare in the transplant discipline.

The lack of uptake of pharmacogenomics is evidenced by the current regulatory status of pharmacogenomic guided labeling of therapeutic substances by the US FDA. There is limited pharmacogenomic detail in the labeling of therapeutic substances with 170 individual drug listings on the FDA Table of Pharmacogenomic Biomarkers in Drug Labeling [13]. Out of the 37 agents on the list routinely used in cancer medicine, eight are conventional cytotoxic antineoplastic agents. The agents and their molecular targets are listed in **Table 1.** In addition to conventional antineoplastic agents, there are 11 monoclonal antibodies and 18 small molecules and other inhibitory molecules on the list, however the genomic information for these agents is related to specific actions of the agent based on tumor biomarkers.

Out of the eight cytotoxic chemotherapeutic agents in **Table 1**, four contain pharmacogenomic dosing information that is related to dosing strategy; cisplatin, irinotecan, mercaptopurine and thioguanine. When the FDA genotype labels are investigated for pharmacotherapeutic application in stem cell transplant, only one
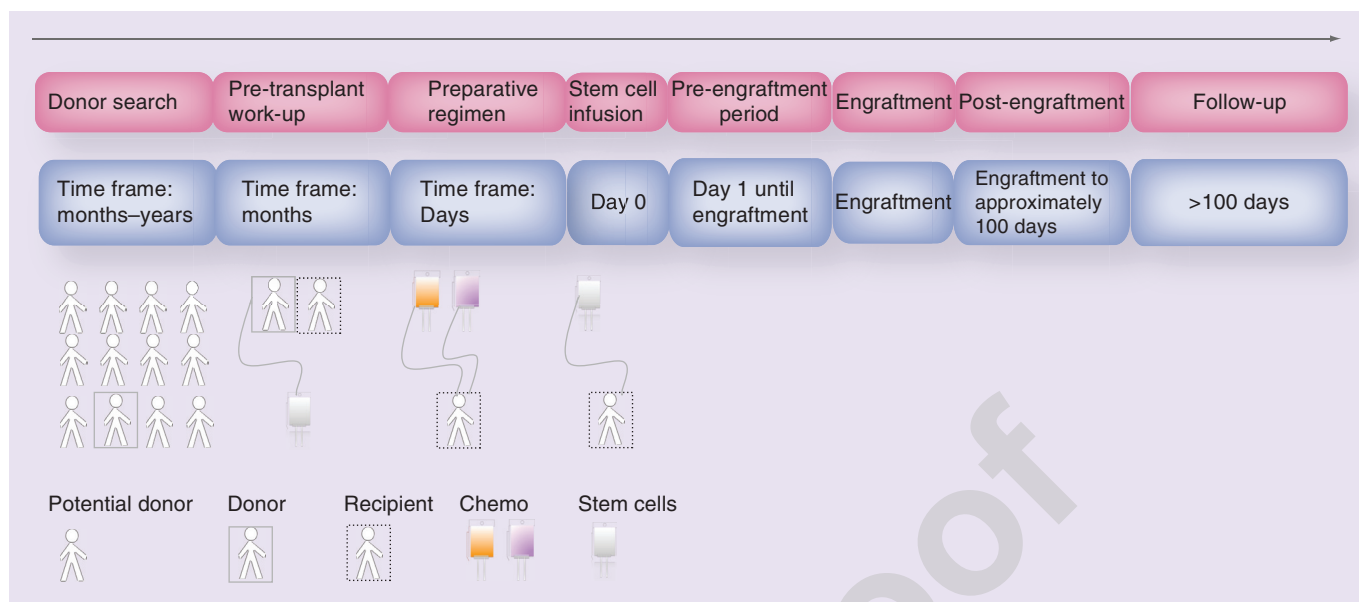
Figure 1. Allogeneic transplant journey stages.

chemotherapeutic agent is represented, busulfan. However, the specific details refer to a superseded oral tablet formulation label from 2003 that indicated the drug might be less effective in patients who expressed the Philadelphia chromosome [13]. This leaves no preparative regimen agents on the FDA list with pharmacogenomic information in the label.

There are a further three agents on the FDA list that would be considered part of the transplant formulary. Denileukin diftitox is used in steroid refractory GVHD; however, it would not routinely be used in a first- or second-line setting [15]. The remaining two, trimethoprim/sulfamethoxazole and voriconazole are used routinely as part of the prophylactic strategy for supportive care [16]. The information for trimethoprim/sulfamethoxazole refers to glucose-6-phosphate dehydrogenase deficiency leaving only voriconazole as the only pharmaceutical agent used routinely in the transplant formulary with pharmacogenomic dosing information included on the label.

Preparative regimen agent pharmacogenomics

Due to highly toxic treatment regimens, the transplant community were early adopters of a personalized approach to care delivery in each phase of the transplant course **(Figure 1)**. However more recent approaches to personalize therapeutics including pharmacogenomics have not been integrated, evidenced by the lack

of pharmacogenomic data in FDA labeling and by gaps in the current published literature that tie pharmacogenomic information to improvement in transplant outcomes. The translational impact of pharmacogenomics has great potential and so far candidate gene studies have shown that genotype can be studied in the context of stem cell transplant preparative regimens; however, clinical relevance is yet to be elucidated.

Preparative regimen agents that cause full or partial myeloablation along with pharmacological immunosuppression are important causes of morbidities such as delayed engraftment and end-organ damage including acute kidney injury or hepatic dysfunction [17]. Pharmacogenomic principles examine the relationships among polymorphisms in drug metabolizing enzymes, drug pharmacokinetics and tumor response and treatment toxicity. There are a number of genes that encode for enzymes that hinder metabolism of preparative agents demonstrating that individual differences in drug efficacy or toxicity can be influenced by genetic factors [18].

There are a number of approaches used to identify which genes are responsible for variations in drug behavior [19]. Although it is expected that single nucleotide polymorphism (SNP) studies and genome-wide SNP arrays will provide further details, to date, in stem cell transplant the methodology used in the majority of studies are candidate gene in nature. The candidate gene approach has identified a number of transplant end points that correlate with

Table 1. List of antineoplastic agents with pharmacogenomic dosing information on the US FDA-approved label.			
Drug	**HUGO Symbol**	**Referenced subgroup**	**Action based on genotype**
Arsenic trioxide	*PML/RARA*	*PML/RARα* (t[15;17]) gene expression positive	Clinical indication
Busulfan	Ph chromosome	Ph chromosome negative	Refers to previous noncurrent label for tablet formulation (2003)
Capecitabine	*DPYD*	DPD deficient	Contraindication
Cisplatin	*TPMT*	TPMT intermediate or poor metabolizers	Increased toxicity with *TPMT*3B* and *TPMT*3C*
Fluorouracil	*DPYD*	DPD deficient	Contraindication
Irinotecan	*UGT1A1*	*UGT1A1*28* allele carriers	*UGT1A1* 7/7 genotype consider dose reduction. Increased risk of neutropenia
Mercaptopurine	*TPMT*	TPMT intermediate or poor metabolizers	Upfront dose reduction for homozygous-TPMT deficiency
Thioguanine	*TPMT*	TPMT poor metabolizer	Upfront dose reduction for homozygous-TPMT deficiency

Ph: Philadelphia; PML: Promyelocytic leukemia.
Data taken from FDA Table of Pharmacogenomic Biomarkers in Drug Labeling [13] and HUGO gene nomenclature committee [14].

genotype. A correlation between suspected toxicities and individual agents may, in time, identify those at risk of adverse outcomes [17].

The hematopoietic stem cell transplant preparative or conditioning regimen consists of chemotherapeutic and immunological agents administered in adjunct with total body irradiation in certain patient cohorts. The regimen serves three main functions. To produce a profound immunosuppressive effect on the recipient to facilitate engraftment of donor marrow, to make space in the hematopoietic niche and, if the transplant is for a malignant indication, the combination of therapies aggressively treat the malignant disease [5].

The hallmark of antineoplastic therapy in stem cell transplantation is that the patients are treated beyond the limit of acceptable toxicity then rescued with hematopoietic stem cell reinfusion. The therapeutic index of the conditioning agents is intentionally exceeded in order to allow engraftment of recipient marrow and the resulting toxicity of these agents is thus a significant burden. Unlike many antineoplastic protocols where multiple agents are given over many months or even years the transplant preparative regimen sees all active agents given in a short space of time, generally in under 1 week. In addition much higher doses are generally applied. This potentially alters both the pharmacokinetic and pharmacodynamic properties of the agents and generates a high level of toxicity in the transplant recipient [5].

There is evidence at the receptor level that polymorphisms alter pharmacodynamic response and that variation in the genes that encode intracellular enzymes, cell surface receptors, transmembrane ion channels and drug transporters

result in both expected adverse reactions, off-target toxicities and idiosyncratic effects [20]. In the stem cell transplant setting, there are a number of candidate gene studies that have attempted to correlate genotype with pharmacokinetic parameters. There are also a small number of studies that have investigated the association of common functional and nonfunctional alleles with transplant outcomes by preparative regimen agent. Busulfan and cyclophosphamide are the workhorses of the transplant preparative regimen formulary and hence their use is the most extensively studied.

Busulfan

Busulfan is a bifunctional alkylating agent [20] that is used as a preparative regimen agent in malignant and nonmalignant stem cell transplantation [21]. Its use has become more prevalent with the introduction in the late part of last century of an intravenous formulation, which offers a favorable and more predictable pharmacokinetic profile when compared with oral administration [22–24]. In addition the shift from oral to intravenous drug administration has been embraced due to the large number of tablets required when using the oral formulation.

For busulfan, like many other alkylating agents with steep dose-response curves, exposure predicts efficacy. Due to this dose–response relationship and inconsistent interpatient pharmacokinetic profiles, the majority of transplant centers employ therapeutic drug monitoring [25] with either area under concentration time curve (AUC) or concentration steady state measured with the first-administered dose. Subtherapeutic exposure on first dose pharmacokinetics either by AUC or concentration steady state predicts

graft rejection and disease recurrence while supratherapeutic levels result in toxicity [21,25–36]. There is a link between high first dose exposure levels and endothelial damage resulting in transplant-related microangiopathy, sinusoidal obstructive syndrome or veno-occlusive disease (VOD) and capillary leak syndrome [26].

Current dosing strategies of pharmacokinetic guided and weight-adjusted dosing leave some patients outside the target therapeutic range during the first phase of the preparative regimen [27] and limitations in logistics prevent follow-up pharmacokinetic monitoring due to lag time in returning results [37]. This situation has resulted in a number of novel candidate gene studies being undertaken with the aim to better predict starting dosing requirements [25,27,30–34,38]. These studies, their gene targets and correlation between genotype and either pharmacokinetic or outcome data are presented in **Table 2**.

The metabolism of busulfan lends itself to both pharmacokinetic and pharmacogenomic directed dosing. From a pharmacogenomic perspective there is a high level of polymorphism in the main isoform in the enzyme family responsible for busulfan metabolism and there is also a relationship between enzyme regulation and plasma concentrations of busulfan [21,28]. The alkylating moieties in busulfan undergo metabolic electrophilic biotransformation solely via glutathione conjugation [26] and this conjugation pathway is primarily catalysed by glutathione-S-transferase-α (GST-A) [27,29]. Once conjugated, the alkylating capacity of busulfan is diminished.

In the hematopoietic stem cell transplant procedure busulfan produces dose-dependent myeloablation [5]. The hematopoietic stem cells in the bone marrow lack the gene to code for the GST-A enzyme and hence the alkylating capacity of busulfan is not reduced by the presence of glutathione-S-transferase. Like hematopoietic stem cells, endothelial cells either lack, or have reduced levels of the isoenzymes of GST and in particular sinusoidal cells lack the GST-A isoform [26]. On exposure to busulfan, reduced intracellular capacity for glutathione production in sinusoidal epithelial cells compared with hepatocytes results in extended busulfan activity and subsequent sinusoidal obstructive damage [27].

Polymorphisms in glutathione-S-transferase

The glutathione-S-transferase family are a group of enzymes involved in the phase II detoxification of a substantial number of therapeutic agents [18] and the cystolic isoenzymes in humans include GST-A, GST-M, GST-P and GST-T. GST-A has the strongest relationship with busulfan biotransformation where GST-A1–1 is the predominant isoform catalysing the conjugation of busulfan with glutathione [27]. Like the HLA genes that code for cell surface proteins used to match donor to recipient there is a high degree of allelic polymorphism in the glutathione family [29]. There is also an interdependence that exists in the gene expression and the subsequent effect on pharmacokinetic behavior. This makes the glutathione-S-transferase family an *a priori* candidate for pharmacogenetic studies of busulfan in stem cell transplant patients.

Pharmacokinetics, transplant outcomes & glutathione-S-transferase isoenzyme function

• GST-A1

There are a number of studies that have evaluated the relationship between GST-A1 polymorphisms and busulfan pharmacokinetics in pediatrics [25,31], in adult transplant populations [33,38] and in both age cohorts [32] as well as GST-A1 polymorphisms and clinical outcomes using pharmacokinetic modeling in pediatrics [27,29–30] and in adults [34,36].

In the pediatric studies that looked purely at pharmacokinetics, two studies independently demonstrated that there was a relationship between GST-A1 mediated drug clearance and patient genotype. Johnson showed that compared with GST-A1 *A/*A wild-type, intravenous busulfan clearance was decreased by 30% for patients who were either hetero or homozygous variants A1 *A/*B or *B/*B [25]. A relationship between enzyme expression and gene or promoter region mutations in linkage disequilibrium and clearance was also found [25]. Where mutations in the promoter regions of the GST-A1 gene (–631T>G, –567T>G, –69 C>T and –52G>A) were associated with reduced clearance [25]. A second study analyzing the pharmacokinetics of intravenous busulfan in patients with β-thalassemia major demonstrated a 10% reduction in clearance for GST-A1*B genotype patients compared with wild-type [31]. This was in contrast to an earlier study using oral busulfan in thalassemia patients where GST-A1 was associated with a lower C_{max} and higher clearance [39]. It is unknown if the pharmacogenomic

Table 2. Candidate gene studies for busulfan containing preparative regimens.

Study (year)	Cohort	n	Diagnosis	Preparative regimen busulfan details	Genetic variant studied	Comparator	Association	Ref.
Srivastava (2004)	Pediatric	114	β-Thal	Route: oral Dose: 16 mg/kg or 600 mg/m²PK guided: no With: Cy200 ± Atg Seizure Px: phenytoin	GSTM1*0 null allele GSTT1	Clinical VOD	GSTM1*0 null genotype compared with GSTM1*0 positive had significant increase in VOD GSTM1*0 null genotype had higher busulfan clearance and lower plasma concentration GSTA1 expression increased in GSTM1 null genotype No relationship between GSTT1 genotype and VOD	[39]
Kusama (2006)	Adult	12	AML CML MDS NHL Pancreatic	Route: oral Dose: 16 mg/kg or 8 mg/kg PK guided: no With: Cy, Flu or Flu + Gem Seizure Px: phenytoin or VPA	GSTA1 (-69C/T: GSTA1*A, GSTA1*B)	PK clearance Cmean	GSTA1*A/*B genotype lower busulfan clearance, higher busulfan concentration on dose 5 compared with GSTA1*A/*A Promoter variant of GSTA1 (GSTA1*A/*B) resulted in slower elimination of busulfan compared with common GSTA1*A/*A genotype	[38]
Johnson (2008)	Pediatric	29	Malignant (n = 10) Nonmalignant (n = 19)	Route: iv. Dose: age-dependant ≤12 kg 1.1 mg/kg × 16 doses >12 kg 0.8 mg/kg × 16 doses PK guided: first dose With: Cy or Flu No TBI Seizure Px: phenytoin	GST A1 (-69C/T: GSTA1*A, GSTA1*B) GSTP1 GSTM1 (GST M1*0)	PK AUC Css	GSTA1*B (hetero or homozygous *A/*B or *B/*B) had 2.6 × AUC and Css compared with noncarriers. Reduced clearance by 30% GST P1*2 or GSTP1*3 had no effect on busulfan exposure GST M1*0 (null) no effect on busulfan exposure GSTP and GSTM do not contribute substantially to busulfan	[25]
Zwaveling (2008)	Pediatric	77	Malignant (n = 35) Nonmalignant (n = 42)	Route: iv. Dose: q.i.d (n = 31), daily (n = 46) ≤12 kg 1.1 mg/kg × 16 doses >12 kg 0.8 mg/kg × 16 doses or 80 or 120 mg/m² × 4 doses PK guided: first dose With: Cy, Mel, Flu, Etop Seizure Px: not stated	GSTA1 (-69C/T: GST A1*A, GSTA1*B) (null allele) GSTP1 (313A/G) GSTP1 SNP rs947894 GSTT1 (null allele)	VOD PK AUC weight	Only body weight was important covariate for VOD None of the studied polymorphisms in the genes encoding GSTA1, M1, P1 or T1 nor combinations of genotypes were significant co-variates	[27]

ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; Atg: Antithymocyte globulin; AUC: Area under the concentration time curve; BZD: Benzodiazepine; β-Thal: β-thalassemia major; BZD: Benzodiazepine; C_{max}: Concentration maximum; Cmean: Mean concentration; CML: Chronic myeloid leukemia; Css: Concentration steady state; Cy: Cyclophosphamide; Cy200: Cyclophosphamide 50 mg/kg × days; Etop: Etoposide; Flu: Fludarabine; Gem: Gemcitabine; GST: Glutathione-S-transferase; GVHD: Graft versus host disease; iv.: Intravenous; Mel: Melphalan; MDS: Myelodysplastic syndrome; MM: Multiple myeloma; MPD: Myeloproliferative disease; NHL: Non-Hodgkin's lymphoma; OS: Overall survival; PK: Pharmacokinetics; PNH: Paroxysmal nocturnal hemoglobinuria; Px: Prophylaxis; q.i.d.: Four-times daily; SNP: Single nucleotide polymorphism; TRM: Transplant related mortality; VPA: Valproic acid; VOD: Veno-occlusive disease.

Table 2. Candidate gene studies for busulfan containing preparative regimens (cont.).

Study (year)	Cohort	n	Diagnosis	Preparative regimen busulfan details	Genetic variant studied	Comparator	Association	Ref.
Ansari (2010)	Pediatric	28	Malignant (n = 18) Nonmalignant (n = 10)	Route: iv. Dose: age-dependent ≥3 months to 1 year: 0.8 mg/kg × 16 ≥1 to <4years: 1 mg/kg × 16 ≥4years: 0.8 mg/kg × 16 PK guided: first dose With: Cy200 Seizure Px: BZD or phenytoin	GSTA1 (C-69T, A-513G, G-631T, C-1142G) GSTM1 (null allele) GSTP1 (A1578G, C2293T)	VOD GVHD Css AUC Clearance Cumulative dose age phenytoin	GSTM1 null had higher drug exposure and lower clearance GSTM1 genotype best predictor of first dose pk variability other than drug dose and age GSTM1 null also had lower cumulative busulfan doses No relationship between GSTA1 or GSTP1 variants	[29]
Elhasid (2010)	Pediatric	18	B-Thal (n = 15) Sickle cell (n = 2) Sickle trait (n = 1)	Route: oral Dose: 1 mg/kg × 16 doses PK guided: no With: Cy Seizure Px: phenytoin	GSTA1 –52 (C > T; rs3957356) GSTP1 SNP rs1695 (A > G) GSTM1 (null allele) GSTT1 (null allele)	GVHD VOD OS PK C_{max} AUC clearance	GSTA1 and GSTP1 genotype are independent predictors of busulfan C_{max}, AUC and clearance Busulfan C_{max} lower in GSTA1*A/*B heterozygous compared with wild-type GSTA1*A/*A and GSTA1*B/*B homozygous Busulfan C_{max} 15% lower in GSTP1 homozygous compared with GSTP1 variant Lower busulfan AUC in GSTM1 null compared with GSTM1 positive GSTM1 null may predict GVHD	[30]
Gaziev (2010)	Pediatric	71	β-Thal (n = 68) Sickle cell (n = 3)	Route: iv. Dose: weight dependent 9 to <16 kg: 1.2 mg/kg × 16 16 to <23 kg: 1.1 mg/kg × 16 23 to <34 kg: 0.95 mg/kg × 16 >34 kg: 0.8 mg/kg × 16 PK guided: first dose With: Cy, +/-TT, +/-Atg, +/-preconditioning Seizure Px: VPA	GSTA1 (GSTA1*A, GSTA1*B) GSTP1 (GSTP1*A, GSTP1*B, GSTP1*C) GSTM1 (null allele) GSTT1 (null allele)	GST as covariate in PK model	GSTA1B 10% lower clearance than wild-type	[31]
Abbassi (2011)	Adult and pediatric	152	Malignant and nonmalignant	Route: iv. (n = 57), oral (n = 95) Dose: q.i.d. (n = 18), daily (n = 39) starting dose q.i.d.: 0.92 mg/kg starting dose daily: 3.2 mg/kg PK guided: first dose With: Cy, Atg, Etop, Flu, Mel Seizure Px: phenytoin	GSTA1 (GSTA1*A, GSTA1*B -613T/G, -567T, -69C, -52G, -631G, -567G, -69T, -52A) GSTM1 (null allele)	Clearance	GSTA1 or GSTM1 genotypes were not associated with iv. clearance Oral busulfan clearance was associated with GSTA1 genotype Germline GSTA1*A*A or *A*B haplotype had higher oral busulfan clearance than GSTA1*B*B haplotype	[32]

ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; Atg: Antithymocyte globulin; AUC: Area under the curve; BZD: Benzodiazepine; β-Thal: β-thalassemia major; BZD: Benzodiazepine; C_{max}: Concentration maximum; Cmean: Mean concentration; CML: Chronic myeloid leukemia; Css: Concentration steady state; Cy: Cyclophosphamide; Cy200: Cyclophosphamide 50 mg/kg × days; Etop: Etoposide; Flu: Fludarabine; Gem: Gemcitabine; GST: Glutathione-S-transferase; GVHD: Graft versus host disease; iv.: Intravenous; Mel: Melphalan; MDS: Myelodysplastic syndrome; MM: Multiple myeloma; MPD: Myeloproliferative disease; NHL: Non-Hodgkin's lymphoma; OS: Overall survival; PK: Pharmacokinetics; PNH: Paroxysmal nocturnal hemoglobinuria; Px: Prophylaxis; q.i.d.: Four-times daily; SNP: Single nucleotide polymorphism; TRM: Transplant related mortality; VPA: Valproic acid; VOD: Veno-occlusive disease.

Table 2. Candidate gene studies for busulfan containing preparative regimens (cont.).

Study (year)	Cohort	n	Diagnosis	Preparative regimen busulfan details	Genetic variant studied	Comparator	Association	Ref.
Kim (2011)	Adult	58	AML ALL CML MDS MPD PNH	Route: iv. Dose: randomized daily or q.i.d. 3.2 mg/kg × 4 0.8 mg/kg × 16 PK guided: first dose With: Cy or (Flu +/-Atg) Seizure Px: phenytoin	GST1A GSTM1 GSTT1	Clearance	GSTA1*B, GSTM1/GSTT1 double-null genotype and actual body weight were associated with lower clearance of busulfan. Clearance of iv. busulfan was similar between patients with GST1A**A and GSTM1/GSTT1 double-null genotype and those with GST1*A/*B and GSTM1/GSTP1 double-positive genotype	[33]
Ten-Brink (2012)	Adult	66	Malignant (n = 88) Nonmalignant (n = 12)	Route: iv. Dose: variable myeloablative 0.8 mg/kg × 16 nonmyeloablative 0.8 mg/kg × 8 PK guided: first dose With: Cy and Alem or Flu and Atg Seizure Px: phenytoin	GSTA1 (-69C/T) GSTM1 (null allele) GSTP1 (313A/G rs1695)	VOD GVHD Engraftment Clearance AUC	14% reduction in busulfan clearance was seen for GSTA1*B allele and an increase in busulfan exposure was found. No relationship between GSTM1 or GSTP1 and busulfan PK	[34]
Bonifazi (2014)	Adult	185	AML ALL MDS MM NHL CML	Route: iv. Dose: 0.8 mg/kg × 16 nonmyeloablative dose not stated PK guided: first dose With: Cy or Flu Seizure Px: not stated	40 polymorphisms at 27 loci	VOD GVHD OS TRM clearance AUC bilirubin	GSTA2 S112T serine allele homozygosity was an independent risk factor for poorer survival, increased any time and 100 day mortality. The genotype also predicts a wider busulfan AUC and higher bilirubin level. GSTA2 did not impact GVHD or relapse. GSTA2 serine allele does not impact TRM in cohort not receiving busulfan	[36]

ALL.: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; Atg: Antithymocyte globulin; AUC: Area under the concentration time curve; AUC: Area under the curve; BZD: Benzodiazepine; β-Thal: β-thalassemia major; BZD: Benzodiazepine; C$_{max}$: Concentration maximum; Cmean: Mean concentration; CML: Chronic myeloid leukemia; Css: Concentration steady state; Cy: Cyclophosphamide; Cy200: Cyclophosphamide 50 mg/kg × days; Etop: Etoposide; Flu: Fludarabine; Gem: Gemcitabine; GST: Glutathione-S-transferase; GVHD: Graft versus host disease; iv.: Intravenous; Mel: Melphalan; MDS: Myelodysplastic syndrome; MM: Multiple myeloma; MPD: Myeloproliferative disease; NHL: Non-Hodgkin's lymphoma; OS: Overall survival; PK: Pharmacokinetics; PNH: Paroxysmal nocturnal hemoglobinuria; Px: Prophylaxis; q.i.d.: Four-times daily; SNP: Single nucleotide polymorphism; TRM: Transplant related mortality; VPA: Valproic acid; VOD: Veno-occlusive disease.

profiles and genotype correlation are similar for intravenous and oral busulfan preparations particularly in thalassemia patients who present for transplant with a high incidence iron overload and hepatic dysfunction [30].

There are three adult studies that are in concordance with these pediatric results. In an early study in 2006 Kusama showed that in 12 adult patients conditioned with oral busulfan without pharmacokinetic guided dosing, there was reduced clearance on the fifth dose in patients with the mutant allele GST-A1*A/*B genotype compared with *A/*A wild-type. It was demonstrated again, like in the Johnson study that it was the variant in the promoter region of GST-A1 (GST-A1*A/*B) that was responsible for the increased terminal elimination half-life of busulfan compared with GST-A1*A/*A genotype [38]. Similarly a 14% reduction in busulfan clearance was seen for patients with the GST-A1*B allele and an increase in busulfan exposure was found in an adult study of 66 patients transplanted for malignant and nonmalignant disease [34].

In a sequence of studies Kim et al., first developed a multiplex PCR for seven genetic polymorphisms of GST [40] to allow high-throughput screening of GST alleles. This method was subsequently used in a clinical study, which demonstrated that GST gene polymorphisms significantly affected the pharmacokinetics of intravenous busulfan [33]. This group did not find a correlation for each individual isoform GST genotype but on multivariate analysis demonstrated that actual body weight and the combination of GST-A1*B and the GST-M1/GST-T1 double-null genotype was associated with lower clearance of busulfan [33].

For studies that assessed both clinical transplant outcomes and pharmacokinetic parameters in pediatrics, there was no relationship found between GST-A1 genotype [27,29–30]. There were correlations in transplant outcome data for another isoenzyme genotype, GST-M1-null however [29]. Regardless of the GST-A1 genotype, busulfan toxicity is increased in cells that have reduced intracellular production of GST; hence any allelic polymorphism that results in reduced enzyme function can theoretically lead to increased busulfan toxicity [26]. This may explain the delayed clearance, higher C_{max} and AUC however the pharmacokinetic correlation doesn't translate to measurable clinical outcomes hence GST-A1 genotyping currently has an unknown role in clinical practice.

• GST-P1

The P1 isoform is the most abundant glutathione-S-transferase in pulmonary tissue and it has been demonstrated that SNPs in the polymorphic genes that encode the expression of enzyme results in reduced enzyme activity of GST-P1 [25]. However, this did not result in a correlation between genotype with pharmacokinetic or outcome end points in the majority of studies [25,27,29,33–34] although pulmonary dysfunction was not explicitly addressed as an assessed outcome factor. Only one study demonstrated a relationship between GST-P1 genotype and pharmacokinetic parameters for oral busulfan where C_{max} was 15% lower in GST-P1 homozygous for the ancestral gene compared with GST-P1 variant SNP rs1695 [30]. It would be worthwhile to complete candidate gene studies that assessed the correlation between GST-P1 expression and interstitial pulmonary fibrosis associated with busulfan and pulmonary presentation of VOD.

• GST-M1 & T1

Although the A1 isoform is the predominant contributor to busulfan biotransformation, GST-M1 and GST-T1 also contribute at half and a fifth of the activity of GST-A1 [27] where the contribution of GST-M1 is higher when GST-A1 activity is reduced [39]. Like the GST-A1 isoenzyme, GST-M1 and GST-T1 are highly polymorphic however unlike the A1 variant where polymorphism results in altered enzyme capacity, for the M1 and T1 isoforms polymorphisms results in a lack of protein product production and enzyme activity [29]. This results in a null genotype, GST-M1*0 or GST-T1*0 for individuals with homozygous deletion of the gene [25]. For the M1 isoform there are a number of studies that indicate that there is no relationship between pharmacokinetic parameters, clinical end points and the null genotype [25,27,32,34]. However, there are three studies that showed a relationship between the M1 null genotype and VOD [39], GVHD [30] and pharmacokinetic end points [29].

The two studies that demonstrated a relationship for transplant outcomes used oral busulfan without pharmacokinetic guided dosing in patients with thalassemia major or congenital hemoglobinopathies. In the first, 114 pediatric patients with thalassemia major receiving myeloablative doses of busulfan combined with cyclophosphamide were genotyped from

peripheral blood prior to transplant [39]. Patients with homozygous deletion of *GST-M1*, the *GST-M1*0*-null genotype, had higher busulfan clearance and lower plasma concentration and an increase in incidence and severity of VOD compared with *GST-M1*0* positive patients [39]. In the second oral study, 18 pediatric patients transplanted for thalassemia major, sickle cell or presence of sickle cell trait demonstrated that in patients with a *GST-M1*0*-null genotype had a lower busulfan AUC compared with positive genotype and that the presence of *GST-M1*-null may predict GVHD [30]. The results of these oral studies are confounded by the formulation of busulfan administered, lack of pharmacokinetic guided dosing and the presence of liver dysfunction in the patients at the time of presentation for transplant [30]. In the study that used intravenous busulfan with first dose pharmacokinetic guided dosing no relationship with clinical outcomes was found [29]. However, from a pharmacokinetic perspective the *GST-M1* null genotype was associated with higher drug exposure, lower clearance and a lower cumulative dose. This study also found that other than drug dose and age, *GST-M1* genotype was predictor of variability in first dose pharmacokinetic results [29].

GST-T1 does not contribute on a significant scale to the electrophilic biotransformation of busulfan [30,32,35]. Not surprisingly, *GST-T1* has not been shown to be an independent variable in busulfan pharmacokinetic behavior in stem cell transplant patients. Its contribution to the overall pharmacokinetic profile is however demonstrated in a single study where presence of the *GST-T1* null genotype in combination with the *GST-M1* null genotype in patients who also exhibit *GST-A1*B/*B* results in a lower clearance of intravenous busulfan in adult patients transplanted for malignant and nonmalignant diseases [33].

• *GST* genotype conclusions

Glutathione-S-transferase is the enzyme responsible for biotransformation of busulfan and glutathione conjugation by the *GST* isoenzyme family and is the sole mechanism by which busulfan undergoes this transformation [27]. Polymorphisms in the genes that encode the *GST* isoenzyme family result in differing levels or absence of expression and subsequently affect drug metabolism. In isolation it would be expected that activity of this isoenzyme family would be predictive of measurable

pharmacokinetic parameters. However there is no clear relationship shown from multiple patient cohorts between hepatic enzyme gene expression, pharmacokinetic parameters and transplant outcomes based on *GST* genotype.

In addition, it must be remembered that no agent in transplant is administered in isolation and the presence of other agents in the preparative regimens as well as the supportive care used to prevent transplant related complications may influence the relationship between drug behavior and genotype. Busulfan lowers the seizure threshold and without prophylaxis tonic-clonic seizure can occur [41]. The majority of studies that assess the pharmacokinetics of busulfan use phenytoin as seizure prophylaxis. As phenytoin is a potent inhibitor of *GST-A1* expression [28] in preparative regimens containing busulfan the role of the agent used for seizure prophylaxis must be considered. Ansari *et al.* included the effect of phenytoin in their analysis [29] and demonstrated that while co-administration of phenytoin was not significantly associated with busulfan pharmacokinetics or on multivariate analysis, with patient genotype, it would be prudent to include the presence of phenytoin in any future studies.

There is currently insufficient evidence to support the use of *GST* genotyping to guide busulfan dosing and predict pharmacokinetic parameters in hematopoietic stem cell transplantation. There is also insufficient evidence to implement pre-emptive supportive care based on *GST* genotype to prevent transplant related complications of GVHD and VOD.

Nonbusulfan preparative regimen agents

To carry out the main functions of the preparative regimen, immunosuppression, making space in the hematopoietic niche and treating the underlying malignancy, combination therapy is required. Alone, each agent would not produce the appropriate amount of ablation of marrow or prevent rejection in the transplant recipient. A wide range of therapeutic agents with nonoverlapping toxicities with or without total body irradiation are employed in the transplant preparative formulary resulting in a regimen of varying intensity and toxicity. The combination used is chosen by underlying disease, performance scores and donor source [5]. While the majority of published data attempting to correlate genotype with clinical outcome has been for busulfan, there are some key studies in other

preparative regimen agents that include promising results for pharmacogenetic guided dosing. These studies are summarized in **Table 3.**

Cyclophosphamide

Like busulfan and other alkylators, cyclophosphamide has a profound dose-response curve [20]. It is most frequently used in a high dose in myeloablative regimens [18,35,42]. It has also garnered recent attention as re-emergence of its role post stem cell infusion to prevent GVHD [46]. The metabolism of cyclophosphamide is complex and produces active metabolites that facilitate engraftment and inactive metabolites that are implicated in transplant regimen toxicity [18]. Cyclophosphamide is a prodrug that is activated via hydroxylation in the liver predominantly by CYP2B6 although other members of the hepatic microenyzme family contribute with CYP2C19, CYP2C9 and CYP3A4/5 also involved in the metabolic process. The product of hydroxylation is 4-hydroxycyclophosphamide and this compound exists in tautomeric equilibrium with aldophosphamide [47,48]. When in the aldophosphamide form the compound decomposes to phosphoramide mustard, the active alkylating agent and acrolein, the metabolite responsible for endothelial toxicity. Minor pathways simultaneously generate metabolites, for example, chloroacetaldehyde the metabolite implicated in neurotoxicity is produced via CYP3A4/5 mediated dechloroacetylation [48]. Cyclophosphamide is a component of most hepatotoxic myeloablative regimens and it is its activity on sinusoidal endothelial cells that results in liver microcirculation damage. Interpatient variability in levels of each metabolite are implicated in the extent of end organ damage post-transplant [47]. Due to the heavy dependency on hepatic hydroxylation, a large interpatient variability in the metabolic pathway and its contribution to transplant-related toxicity cyclophosphamide is a good candidate for pharmacogenomic studies.

There are two key studies of cyclophosphamide use and patient genotype in hematopoietic stem cell transplant patients. In the first, Kim *et al.*, studied 21 adult patients with thalassemia major who received cyclophosphamide and busulfan preparative regimen underwent genomic DNA analysis on pretransplantation peripheral blood [42]. Four SNPs in the drug transport enzymes, ATP-binding cassette subfamily B and C as well as eight SNPs and two null alleles in the hepatic micro enzyme family and glutathione-S-transferase were analysed. Inducible and noninducible clearance of cyclophosphamide and its relationship to the presence of the SNPs or null alleles was calculated using a hypothetical enzyme compartment model. *ABCC2* or multidrug resistance-associated protein 2 1249 genotype affected both noninduced and induced clearance of cyclophosphamide. In patients with the *ABCC2* 1249GA heterozygous genotype, noninduced clearance of cyclophosphamide was reduced by 51% compared with 1249GG wild-type. No other allelic variation was associated with clearance [42].

Rocha *et al.* examined adult and pediatric patients undergoing HLA-identical sibling transplant for *de novo* acute or chronic leukemia between 1994 and 1999. Although the study did not specifically look at cyclophosphamide, the drug was a component of the majority of preparative regimens (88/107). A correlation between a number of transplant outcomes and genotype was found. The findings included an association between toxicity and recipient genotype, with oral mucositis being associated with CYP2B6*4, hemorrhagic cystitis associated with recipient CYP2B6*2A, GVHD associated with recipient methylenetetrahydrofolate reductase (*MTHFR*) c677T and higher mortality and lower overall survival for recipients with vitamin D receptor TaqI SNP. There were also some associations for donor genotype with chronic GVHD associated with donor *GST-P1* and VOD associated with donor CYP2B6*6. There was no association of *GST-P1*, *T1* or *M1* polymorphisms with liver toxicity of VOD [18].

There are a number of studies that have determined a correlation between cyclophosphamide and genotype in the published literature. The majority are in medical oncology, with breast cancer the most common pathology represented when the literature is reviewed. The literature in hematopoietic stem cell transplantation is more limited however large-scale studies using genomic array analysis are required to further understand the relationship between cyclophosphamide, donor and recipient genotype in the stem cell transplant discipline.

Thio-TEPA

Triethylenethiophosphoramide or thio-TEPA is a polyfunctional alkylating agent that rapidly undergoes CYP2B6 and 3A4 mediated oxidative desulfuration to TEPA [45]. Both thio-TEPA and TEPA are active alkylators [20] crosslinking

Table 3. Candidate gene studies for nonbusulfan containing preparative regimens.

Study (year)	Cohort	n	Dx	Preparative regimen details	Genetic variant studied	Comparator	Association. Inclusion in table requires drug component association	Ref.
Kim (2013)	Adult	21	β-Thal	Cyclophosphamide 60 mg/kg × 2 days busulfan 0.8 mgkg/dose q.i.d. × 4 days phenytoin seizure prophylaxis	ABCB1 2677G>T/A, 3435C>T ABCC2 -24C>T, 1249G>A GSTA1 G-52A GSTM1 null allele GSTP1 GSTP1*B GSTT1 null allele CYP2B6*2, *3 CYP2C19 *2, *3CYP3A5	VOD clearance	ABCC2 1249 genotype significantly affected noninduced and induced clearance of cyclophosphamide. Noninduced clearance of cyclophosphamide was decreased by 51% in ABCC2 1249GA heterozygous genotype compared with 1249GG wild-type	[42]
Rocha (2009)	Adult and pediatric	107	Acute leukemia (39) chronic leukemia (68)	TBI cyclophosphamide 15 TBI melphalan 17 busulfan and cyclophosphamide 73 busulfan and other 2	CYP 2B6 (*2(C64T), *3(C777A) *4(A785G), *5(C1459T) *6(G516T) GSTM1 (null) GSTT1 (null) GSTP1 (A313G) MDR1 (C3435T) MTHFR (C677T, A1298C) VDR (ApaI, TaqI, BsmI) RFLP	Oral mucositis hemorrhagic cystitis VOD GVHD TRM OS	Oral mucositis associated with recipient CYP2B6*4. Hemorrhagic cystitis associated with recipient CYP2B6*2A. VOD associated with donor CYP2B6*6 aGVHD associated with recipient MTHFR c677T cGVHD associated with GSTP1. Higher TRM and lower OS recipient VDR TaqI. No association of GSTP1, T1 or M1 polymorphisms with liver toxicity of VOD	[18]
Gruhn (2012)	Pediatric	166	AML (72) ALL/NHL (38) MDS (21) CML (12) SAA (10) other (13)	TBI (77) busulfan (78) cyclophosphamide (7) thio-TEPA (4)	TLR4 SNPs Asp 299Gly (rs4986790) Thr399Ile (rs4986791)	Hemorrhagic cystitis	TLR4 Asp299Gy variant is associated with reduced risk of hemorrhagic cystitis post-transplant. Absence of Asp299Gly risk factor for hemorrhagic cystitis post-transplant. Absence of Thr399Ile also trend but not significant	[43]
Kuhne (2008)	Adult	84	Myeloma	Melphalan autograft	GSTM1 GSTT1 GSTP1	Adverse events	GSTP1 codon may be relevant for mucositis. GSTT1 deletion may predict diarrhoea. More related to dose than genotype	[44]
Ekhart (2009)	Adult	124	Breast (93) ovarian (5) germ cell (26)	Thio-TEPA, carboplatin and cyclophosphamide autograft full dose n = 67 tiny dose (67% intensity) n = 57	CYP 2B6 CYP 3A4 CYP 3A5 GSTA1 GSTP1	Thio-TEPA clearance tepa clearance	Noninducible Thio-TEPA clearance increased by CYP3A5*3 and GSTP1 C341T and decreased by GSTA1 (C-69T, C-52A). Inducible thio-TEPA clearance was decreased by CYP2B6 C1459T variant and decreased by CYP 3A4*1B. Cyp2B6 C1459T significantly decreased clearance of tepa. GSTP1 C341T decreased clearance of tepa	[45]

ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; β-Thal: β-thalassemia major; CML: Chronic myeloid leukemia; MDS: Myelodysplastic syndrome; NHL: Non-Hodgkin's lymphoma; RFLP: Restriction fragment length polymorphism; SAA: Severe aplastic anemia; SNP: Single nucleotide polymorphism; TBI: Total body irradiation; VDR: Vitamin D receptor.

DNA strands by reacting with phosphate groups to inhibit DNA and RNA synthesis. In addition when combined with total body irradiation, thio-TEPA has a radiomimetic action causing further disruption the bonds of DNA. There is a wide range of interpatient variability in the production of TEPA from thio-TEPA as well an inducible metabolic process in the presence of other drugs including cyclophosphamide [26]. This leads to an unpredictable efficacy and adverse effect profile [45]. In addition to the hepatic P450 isoenzyme desulfuration thio-TEPA and TEPA are conjugated to glutathione catalysed by *GST A1-1* and *P1-1* [45] thus mechanistically the drug is a candidate for pharmacogenomic guided dosing.

There is a single study that investigated the relationship between genotype and pharmacokinetic parameters in autograft patients receiving high-dose cyclophosphamide, thio-TEPA and carboplatin for breast cancer, ovarian and germ cell tumors [45]. Patients were genotyped for SNPs with expected association with thio-TEPA metabolism and those with an allelic frequency of greater than 4% were included [45]. It was demonstrated that noninducible thio-TEPA clearance was increased in patients with the CYP3A5*3 and *GST-P1* C341T genotypes and decreased in *GST-A1* (C-69T, C-52A). Inducible thio-TEPA clearance was decreased by CYP2B6 C1459T variant and in those expressing the CYP 3A4*1B genotype. For the TEPA metabolite, the presence of either CYP 2B6 C1459T SNP or *GSP-P1* C341T SNP was associated with a decreased clearance of TEPA when compared with wild-type. CYP 2B6, CYP 3A4, CYP 3A5, *GST-A1* and *GST-P1* polymorphisms may contribute to clinical efficacy of thio-TEPA [45]. Genotyping patients receiving thio-TEPA is a promising area of research for stem cell transplant patients and warrants further candidate gene and genome array studies to determine if the autograft data can be translated into the allograft population.

Etoposide

There are no specific studies outlining the influence of genotype on etoposide efficacy or toxicity in hematopoietic stem cell transplant however as the agent is widely used in nontransplant medicine and is a substrate for multidrug resistance protein 1, CYP 3A4 and 5 and GST [49] there is thought that genotype may play a role in future dosing strategies. There are two key genotype studies that may guide future transplant trials. In the first study, genome wide methodology identified 63 SNPs associated with gene expression and etoposide-induced toxicity [50]. In the second, a nonparametric whole genome linkage analysis of five phenotypes, 22 SNPs on four genes were identified in playing a role in etoposide mediated topoisomerase inhibition and gene expression [51]. There are no studies investigating SNPs and clinical outcomes and to date the genome wide association studies have not translated into clinical practice. The role of genotyping patients receiving etoposide as part of their preparative regimen is still to be defined.

Melphalan

Melphalan is an alkylating agent that produces dose-dependent myelosuppression. Used frequently in the autologous and allogeneic stem cell transplant setting, the toxicities are known to be related to dose administered. There is one adult study published that analysed the relationship between *GST-M1*, *GST-T1* and *GST-P1* and melphalan adverse events in patients receiving autograft for multiple myeloma [44]. The findings included that the *GST-P1* codon may be relevant for mucositis and that *GST-T1* deletion may predict diarrhea. However, adverse events were more like to be associated with dose than genotype [44] and no specific model for predicting adverse effects was generated.

There are no pediatric specific studies that analyse genotype with melphalan efficacy or toxicity in either the autograft or allograft setting. There were a small number of patients (17) in the Rocha study that received busulfan-melphalan regimen however there is no breakdown of patient characteristics by preparative regimen nor is it known many pediatric patients were in the busulfan-melphalan cohort.

Fludarabine

Fludarabine is a nucleoside analog that primarily provides dose-dependent immunosuppression in the preparative regimen. Widely applied in both myeloablative and nonmyeloablative regimens, the drug is characterized by its dose-dependent renal clearance and its role in neurotoxicity in the transplant recipient [5]. There are no studies regarding genotype and fludarabine in the transplant population however there is some promising data for the use of other nucleoside analogs in other specialities. In patients receiving nucleoside reverse transcriptase inhibitors for

treatment for HIV, mitochondrial DNA polymerase polymorphisms have been indicated in the toxicity of these agents. As mitochondrial DNA polymerase gamma is the sole polymerase responsible for replication of the mitochondrial genome, polymorphisms may explain the wide range of toxicity and tolerability exhibited by these agents [52]. There is also evidence in chronic leukemia patients that fludarabine drug efficacy is related to variants gene expression [53]. There is promise that genotyping may help explain the variable pharmacokinetic and pharmacodynamic profile of fludarabine in transplant patients. Further candidate gene or SNP array studies are required to determine the significance of mitochondrial genotype and fludarabine efficacy in hematopoietic stem cell recipients.

Pharmacological T-cell depletion

Alemtuzumab and antithymocyte globulin are both used for pharmacological *in vivo* T-cell depletion in nonmatched sibling donor transplants [5]. Currently there are no pharmacogenomic studies that have investigated any correlation between genotype, pharmacokinetic parameters and transplant outcomes. The dissolution, metabolism and excretion pathways of antithymocyte globulins have not been the subject of pharmacogenetic analysis. There is a complex relationship between drug exposure and lymphocyte binding that is dependent on the individual's ability to metabolize foreign immunoglobulins. There is a potential that genotype may play a role in the T-cell depletion however this is yet to be elucidated.

For alemtuzumab the picture is less clear again, there is wide range of interpatient variability, the drug exhibits nonlinear time and white cell count dependent pharmacokinetics [54] however as the majority of pharmacokinetic studies come from its use in chronic lymphocytic leukemia it is difficult to extrapolate these results to the transplant setting. There is some work on individualizing immunosuppression including alemtuzumab in transplant patients however this has focused more on calcineurin and mammalian target of rapamicin inhibitors post stem cell infusion than preparative regimen T-cell suppression.

GVHD prophylaxis & treatment

There is a small but significant set of publications regarding the relationship between genotype and efficacy and toxicity of post transplantation immunosuppression in the allogeneic setting. High-resolution molecular HLA typing does not completely ameliorate GVHD and it remains the leading cause of mortality in many transplant types [17] hence improvements would be welcomed. The pathophysiology of GVHD is complex and incompletely understood and although there is evidence that there is a high degree of allelic polymorphism in the immunomodulatory genes that govern GVHD there is no clear link between genotype and outcome.

Pharmacological immunosuppression post-transplant is one of the most difficult to categorize due to pharmacodynamic noise of dose adjustment based on drug levels. For cyclosporin [55] and mycophenolate mofetil [56] there are some key studies that associate genotype to GVHD incidence however there are no good models to support genotype led dosing.

There is literature surrounding the key polymorphisms in methotrexate metabolism and its application in GVHD prophylaxis. Short course methotrexate plus calcineurin inhibitor is the most widely used GVHD prophylaxis regimen in T-cell replete grafts and there have been a number of population pharmacokinetic models that have demonstrated a link between gene expression and methotrexate clearance [57–59]. In one study, Kim utilized a two-compartment model to demonstrate that *ABC-B1* C3435T SNP is associated with methotrexate clearance [60]. In patients with the CC or CT genotype clearance was increased by 21% compared with the TT genotype [60].

Other studies have reviewed the role of *MTHFR* the key regulatory enzyme in folate metabolism and methotrexate exposure and efficacy. In patients with GVHD two nonsynonymous SNPs in *MTHFR* (C677T and A1298C) influenced *MTHFR* activity [17] and resulted in an altered risk of GVHD. It is postulated that there is an interrelationship between *MTHFR* C677T and thymidylate synthase resulting in a reduced risk of GVHD due to greater methotrexate exposure associated with these alleles. It was also found that the *MTHFR* C667T SNP had a role in GVHD outcomes [58,59] where a reduced risk GVHD was seen possibly due to greater sensitivity to methotrexate. The role of genotyping patients in patients planned to receive short course methotrexate warrants further investigation.

Supportive care

Currently donor and recipient serology and the presence of pretransplant infection determines anti-infective supportive care prophylaxis, however there are some novel studies that have analyzed the relationship between genotype and post-transplant infective complications [8]. There is a relationship between SNP haplotype and the incidence of gram-negative bacteria with a mutation in the promoter region of the lipopolysaccharide gene resulting in unchecked replication in patients with a rare homozygous variant [8]. There is also an association between SNP polymorphisms in toll-like receptor 4 and the presence of hemorrhagic cystitis where the incidence was 0% in pediatric transplant patients with a certain SNP haplotype [43]. As the pathogenesis of hemorrhagic cystitis is often related to common post transplant viral infections due to BK virus or adenovirus [43] as well as the acrolein metabolite of cyclophosphamide [18] this is certainly an area that warrants further investigation.

Also of note in the supportive care setting is the relationship between genotype and incidence of fungal infection and the efficacy of azole anti-fungals used to treat and provide prophylaxis against invasive infections [8]. It has been demonstrated that non-HLA polymorphisms are involved in pulmonary infections and that polymorphisms again in Toll-like receptor 4 result in an increase in the risk of invasive aspergillus infection after allogeneic stem cell transplantation [8]. In addition, polymorphisms in genes that encode the enzymes in common drug metabolism pathways, for example, GST and in hepatic isoenzyme P450 families result in variation in the measured pharmacokinetic parameters of azole antifungals [16]. As commercial availability of genotyping for these CYPs is available and the FDA includes guidance on allelic polymorphisms on the voriconazole label, this is perhaps the closest representation of pharmacogenomic guided dosing in clinical practice in hematopoietic stem cell transplantation.

Conclusion

Despite advances in chemotherapy and the advent of molecular targeted agents, allogeneic hematopoietic stem cell transplantation remains the only curative regimen for many high-risk hematological malignancies [5]. Overall survival has increased [5,11], due to a number of factors notably the use of high-resolution molecular typing to match the donor to the recipient as well as other factors such as earlier patient referral and improvements in supportive and psychosocial care [4–5,17].

A range of studies, all of candidate gene design, have been published for busulfan and initial links between pharmacokinetic parameters, transplant related outcomes and overall survival are evident. The first exploratory studies, building on the candidate gene approach, have been published for busulfan. [61] The exploratory methodology clinical application did not yield identifiable targets for busulfan and requires larger cohort studies to determine the clinical application. For cyclophosphamide the majority of evidence for pharmacogenomic behavior comes from nontransplant disciplines however there is one key study that has elucidated a relationship between cytochrome P450 polymorphisms and drug tolerability. The extension of autograft data shows promise in Thio-TEPA containing regimens and etoposide, has some preclinical data in laboratory cell lines but no clinical studies in transplant populations. There

Table 4. Transplant preparative agents with correlation between pharmacokinetics and candidate gene studies.

Drug	Pharmacokinetic model correlation with genomic profile
Alemtuzumab	No model. Complex elimination kinetics dependent on white cell surface CD52 receptor expression prevents correlation between genotype and pharmacokinetics
Atg-equine	No model. Genomic profile unable to predict pharmacokinetic parameters due to lymphocyte binding
Atg-rabbit	No model. Genomic profile unable to predict pharmacokinetic parameters due to lymphocyte binding
Busulfan	Yes
Cyclophosphamide	Yes
Etoposide	Yes
Fludarabine	Yes
Melphalan	No model. Pharmacogenomic results explained by dose variance due to weight-based dosing
Thio-TEPA	Yes
Treosulfan	Insufficient data

Table 5. Future targets for preparative regimen agents where pharmacokinetic parameters correlate with genomic profile.	
Drug	**Candidate gene identified**
Busulfan	*GST* family
Cyclophosphamide	CYP2B6, *GST-P1*
Etoposide	*MRP1*, CYP3A4/5, GST family
Fludarabine	Mitochondrial DNA polymerase polymorphisms
Thio-TEPA	CYP2B6, CYP3A4/5, *GST-A1*, *GST-P1*
GST: Glutathione-S-transferase.	

are limited data in other agents used frequently in preparative regimens including pharmacological T-cell depletion with antithymocyte globulin or alemtuzumab. Other notable exclusions from literature include fludarabine, melphalan outside the myeloma population and treosulfan.

Many therapeutic agents used in hematopoietic stem cell preparative regimens have pharmacokinetic and pharmacodynamic profiles that predict a theoretical benefit from genomic-based dosing. Despite this, and the extensive availability of genetic data and well-described drug metabolism pathways, genotype lead dosing has not been widely adapted. Current studies are limited to a candidate gene single conditioning agent structure and do not adequately explain the effect of other conditioning agents, post-transplant immunosuppression and the effect of the underlying disease or even the graft itself.

Future perspective

To date, in hematopoietic stem cell transplantation, pharmacogenomics have not had widespread translation to the bedside nor the clinic. Population genetics and pharmacokinetic modeling do not explain drug efficacy or toxicity primarily due to a lack of correlation between genotype and phenotype and the influence of environmental and epigenetic factors. The genotype-phenotype conundrum and the unknown effect of epigenetic factors currently prevents the widespread therapeutic application of genotype guided dosing in the hematopoietic stem cell transplant discipline.

In order to translate current pharmacogenomic knowledge into clinical practice, future research requires focus to be not only on the collection of genomic data for both patients and healthy controls but also on bioinformatics and the development of biostatical and pharmacokinetic models. There are a limited number of known correlations between pharmacokinetic and pharmacogenomic parameters for common preparative regimen agents **(Table 4)**. Further exploration of the relationship between pharmacokinetic behavior and genotype for busulfan, cyclophosphamide, etoposide, fludarabine and thio-TEPA is warranted and may yield a therapeutic benefit for transplant patients. Potential targets for pharmacokinetic modeling at an individual patient or population level that include genomic data for these agents are shown in **Table 5**.

Although analysis of the genotype–kinetic relationship in order to develop dosing algorithms may allow a patient's pharmacogenomic profile to act as an adjunct to weight-based dosing and therapeutic drug monitoring, it must also be considered that non-HLA genotyping may have no role in predicting doses for hematopoietic stem cell transplant patients and that weight, surface area and where appropriate pharmacokinetic guided dosing are adequate to define dose.

Individualized dosing strategies in the high-risk procedure of hematopoietic stem cell transplantation are potentially of great significance to the transplant community. Genome wide or other novel methodologies that expand the knowledge of pharmacogenomics based on the candidate gene approach will assist in determining the future role of non-HLA genotyping in hematopoietic stem cell discipline. Future research identifying the relationship between pharmacokinetic behavior, pharmacodynamic response, genotype and phenotype will allow the clinical significance of non-HLA genotyping to be elucidated in the allogeneic transplant setting.

EXECUTIVE SUMMARY

- The hematopoietic stem cell transplant discipline was an early adopter of the principles of personalized medicine utilizing HLA genotyping to identify suitable transplant donors.

- High resolution molecular typing of HLA genotype has led to a significant improvement in overall survival and transplant-related outcomes.

- Overall survival, transplant-related mortality and drug toxicity from preparative regimen agents may have a relationship with genotype for some preparative regimen agents.

- Expansion of candidate gene methodology and studies that are able to correlate genotype and phenotype will define the future of the therapeutic benefits of genotyping in the hematopoietic stem cell transplant procedure.

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