艾特吉
抗人T细胞猪免疫球蛋白
Anti-human T Lymphocyte Porcine Immunoglobulin

HSCT

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(版本：V1)

抗人 T 细胞猪免疫球蛋白（P-ATG)

武汉中生毓晋生物医药有限责任公司

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中华医学会血液学分会干细胞应用学组

The consensus of allogeneic hematopoietic transplantation for hematological diseases in China (2014)——indication, conditioning regimen and donor selection *Chinese Society of Hematology, Chinese Medical Association*

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异基因造血干细胞移植(allo-HSCT)已经广泛用于恶性血液病和非恶性血液病的治疗。在我国,仅参加HSCT登记的移植中心已经达到60个,但各个中心的移植指征、预处理方案和供者选择各不相同。为了规范我国allo-HSCT适应证和移植时机、为患者推荐合适的预处理方案和供者,我们在参考NCCN指南、欧洲骨髓移植协作组(EBMT)指南的基础上,制订了体现中国特色的专家共识。

一、allo-HSCT的适应证和移植时机

当具有某些特征的患者采用非移植疗法预期效果很差,或者已有资料显示该组患者接受移植的疗效优于非移植时,这类患者具有HSCT的指征。

(一)恶性血液病

1. 急性髓系白血病(AML):

(1)急性早幼粒细胞白血病(APL):APL患者一般不需要allo-HSCT,只在下列情况下有移植指征:

1)APL初始诱导失败;

2)首次复发的APL患者,包括分子生物学复发(巩固治疗结束后PML/RARα连续两次阳性按复发处理)、细胞遗传学复发或血液学复发,经再诱导治疗后无论是否达到第2次血液学完全缓解,只要PML/RARα仍阳性,具有allo-HSCT指征。

(2)AML(非APL):

1)年龄≤60岁:

①在CR1期具有allo-HSCT指征:

Ⅰ. 按照WHO分层标准处于预后良好组的患者,一般无须在CR1期进行allo-HSCT,可根据强化治疗后微小残留病(MRD)的变化决定是否移植,如2个疗程巩固强化后AML/ETO下降不足3 log或在强化治疗后由阴性转为阳性;

Ⅱ. 按照WHO分层标准处于预后中危组;

Ⅲ. 按照WHO分层标准处于预后高危组;

Ⅳ. 经过2个以上疗程达到CR1;

Ⅴ. 由骨髓增生异常综合征(MDS)转化的AML或治疗相关的AML。

②≥CR2期具有allo-HSCT指征:首次血液学复发的AML患者,经诱导治疗或挽救性治疗达到CR2后,争取尽早进行allo-HSCT;≥CR3期的任何类型AML患者具有移植指征。

③未获得CR的AML:难治及复发性各种类型AML,如果不能获得CR,可以进行挽救性allo-HSCT,均建议在有经验的单位尝试。

2)年龄>60岁:

如果患者疾病符合上述条件,身体状况也符合allo-HSCT的条件,建议在有经验的单位进行allo-HSCT治疗。

2. 急性淋巴细胞白血病(ALL):

(1)年龄>14岁:

1)在CR1期具有allo-HSCT指征:原则上推荐14~60岁所有ALL患者在CR1期进行allo-HSCT,尤其诱导缓解后8周MRD未转阴或具有预后不良临床特征的患者应尽早移植。对于部分青少年患者如果采用了儿童化疗方案,移植指征参考儿童部分。>60岁患者,身体状况符合allo-HSCT者,可以在有经验的单位尝试在CR1期移植治疗。

2)≥CR2患者均具有allo-HSCT指征。

3)挽救性移植:难治、复发后不能缓解患者,可尝试性进行allo-HSCT。

(2)年龄≤14岁:

1) CR1期患者的移植:推荐用于以下高危患者:

①33 d未达到血液学CR;

②达到CR但12周时微小残留病(MRD)仍≥10^{-3};

③伴有MLL基因重排阳性,年龄<6个月或起病时WBC>300×10^9/L;

④伴有Ph染色体阳性的患者,尤其对泼尼松早期反应不好或MRD未达到4周和12周均为阴性标准。

2)≥CR2期患者的移植:很早期复发及早期复发ALL患者(附件1),建议在CR2期进行HSCT;所有CR3以上患者均具有移植指征。

3)挽救性移植:对于难治、复发未缓解患者,可在有经验的单位尝试性进行allo-HSCT。

3. 慢性髓性白血病(CML):

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(1)新诊断的儿童和青年CML患者,具有配型相合的同胞供者时;如果有配型较好的其他供体,在家长完全知情和理解移植利弊的情况下,也可以进行移植;

(2)慢性期患者如果Sokal评分高危而EBMT风险积分≤2,且有HLA相合供者,可选择移植为一线治疗;

(3)对于伊马替尼治疗失败的慢性期患者,可根据患者的年龄和意愿考虑移植;

(4)在伊马替尼治疗中或任何时候出现BCR-ABL基因T315I突变的患者,首选allo-HSCT;

(5)对第二代酪氨酸激酶抑制剂(TKI)治疗反应欠佳、失败或不耐受的所有患者,可进行allo-HSCT;

(6)加速期或急变期患者建议进行allo-HSCT,移植前首选TKI治疗。

4. MDS:包括MDS及MDS/骨髓增殖性肿瘤(MPN)[慢性幼年型粒-单核细胞白血病(CMML)、不典型CML、幼年型粒-单核细胞白血病(JMML)、MDS/MPN未分类]。

(1)IPSS评分中危Ⅱ及高危患者应尽早接受移植治疗;

(2)IPSS低危或中危Ⅰ伴有严重中性粒细胞或血小板减少或输血依赖的患者;

(3)儿童JMML患者。

5. 骨髓纤维化(MF):中危Ⅱ和高危原发或继发性MF患者IPSS或动态IPSS(DIPSS)评分见附件2。

6. 多发性骨髓瘤(MM):allo-HSCT适用于具有根治愿望的年轻患者,尤其具有高危遗传学核型的患者,如t(4;14);t(14;16);17p-,或初次自体造血干细胞移植(auto-HSCT)后疾病进展需要挽救性治疗的患者。

7. 霍奇金淋巴瘤(HL):难治或auto-HSCT后复发患者。

8. 非霍奇金淋巴瘤(NHL):

(1)慢性淋巴细胞白血病/小淋巴细胞淋巴瘤(CLL/SLL):年轻患者在下列情况下具有auto-HSCT指征:

1)嘌呤类似物无效或获得疗效后12个月之内复发;

2)嘌呤类似物为基础的联合方案或auto-HSCT后获得疗效,但24个月内复发;

3)具有高危细胞核型或分子学特征,在获得疗效或复发时;

4)发生Richter转化。

(2)其他:滤泡淋巴瘤、弥漫大B细胞淋巴瘤(DLBCL)、套细胞淋巴瘤、淋巴母细胞淋巴瘤和Burkitt淋巴瘤、外周T细胞淋巴瘤、NK/T细胞淋巴瘤,在复发、难治或≥CR2患者具有allo-HSCT指征。成年套细胞淋巴瘤、淋巴母细胞淋巴瘤、外周T细胞淋巴瘤、NK/T细胞淋巴瘤患者,当配型相合的供者存在时,CR1期患者也可以考虑allo-HSCT。

(二)非恶性血液病

1. 再生障碍性贫血(AA):

(1)新诊断的重型再生障碍性贫血(SAA):患者年龄<50岁(包括儿童患者),病情为SAA或极重型SAA(vSAA),具有HLA相合的同胞供者;儿童SAA和vSAA患者,非血缘供者≥9/10相合,HSCT也可以作为一线选择;有经验的移植中心可以在患者及家属充分知情条件下尝试其他替代供者的移植。

(2)复发、难治SAA:

①经免疫抑制治疗(IST)失败或复发,<50岁的SAA或vSAA,有非血缘供者、单倍体相合供者具有移植指征,在有经验的单位,也可以尝试脐血移植;

②经IST治疗失败或复发,年龄50~60岁,体能评分≤2,病情为SAA或vSAA,有同胞相合供者或非血缘供者也可进行移植。

(3)输血依赖的非SAA患者,移植时机和适应证同SAA。

2. 阵发性睡眠性血红蛋白尿症(PNH):SAA/PNH移植参考SAA。

3. 地中海贫血:HSCT适用于依赖输血的重型地中海贫血,如重型地中海贫血、重型血红蛋白E复合地中海贫血、重型血红蛋白H病等。一般建议尽量在患儿(2~6岁)疾病进展到三级(附件3)前接受HSCT。

4. 范可尼贫血:在输血不多且并未转变为MDS或白血病时。

5. 其他:如重症联合免疫缺陷综合征(SCID)等先天性缺陷、黏多糖累积症等先天遗传代谢病等。

二、预处理方案

(一)恶性血液病

1. 白血病/MDS方案:

1)一般强度的预处理方案:清髓预处理方案(MAC)常用的有经典TBICy和BuCy方案及其改良方案,后者以北京大学血液病研究所的方案在国内应用最多(表1),其他如包含马法兰(Mel)的方案,因为药物来源受限国内很少应用。

抗胸腺细胞球蛋白(ATG)一般用于替代供者的移植,剂量不等,ATG(商品名即复宁)常用剂量为6~10 mg/kg,或费森尤斯生产的兔抗淋巴细胞球蛋白(ATG-F)应用剂量为20~40 mg/kg;为降低移植物抗宿主病(GVHD),更低剂量ATG也尝试用于配型相合的同胞HSCT中。

2)治疗白血病/MDS的减低强度预处理(RIC)方案:RIC方案有多种,主要为包括氟达拉滨的方案和(或)减少原有组合中细胞毒药物剂量增加了免疫抑制剂如ATG的方案(表2)。

3)加强的预处理方案:加强的预处理方案一般在经典方案基础上增加一些药物,常用Ara-C、依托泊苷(Vp16)、Mel、TBI或氟达拉滨、赛替哌等,常用于难治和复发的恶性血液病患者(表3)。

预处理方案的选择受患者疾病种类、疾病状态、身体状况、移植供者来源等因素的影响。55岁以下的患者一般选择常规剂量的预处理方案,年龄大于55岁或虽然不足55岁但重要脏器功能受损或移植指数大于3的患者,可以考虑选择RIC方案,而具有复发难治的年轻恶性血液病患者可以接受增加强度的预处理方案。增加强度的预处理在一定程度上降低了复发率,但可能带来移植相关死亡率增加,不一定

表1 经典和改良的清髓预处理方案

清髓预处理方案	药物名称	总剂量	应用时间(d)	移植类型
经典方案				
Cy/TBI	Cy	120 mg/kg	-6、-5	allo-HSCT
	分次TBI	12~14 Gy	-3~-1	
Bu/Cy	Bu	16 mg/kg(口服)或12.8 mg/kg(静脉滴注)	-7~-4	allo-HSCT
	Cy	120 mg/kg	-3、-2	
改良方案(北京大学人民医院方案)				
mBuCy	Hu	80 mg/kg,分2次	-10	同胞相合HSCT
	Ara-C	2 g/m^2	-9	
	Bu	9.6 mg/kg(静脉滴注)	-8~-6	
	Cy	3.6 g/m^2	-5、-4	
	MeCCNU	250 mg/m^2(口服)	-3	
mCy/TBI	单次TBI	770 cGy	-6	同胞相合HSCT
	Cy	3.6 g/m^2	-5、-4	
	MeCCNU	250 mg/m^2	-3	
mBuCy+ATG	Ara-C	4~8 g/m^2	-10、-9	URD、CBT、HID-HSCT
	Bu	9.6 mg/kg(静脉滴注)	-8~-6	
	Cy	3.6 g/m^2	-5、-4	
	ATG	10 mg/kg	-5~-2	
	或ATG-F	40 mg/kg	-5~-2	
mCy/TBI+ATG	TBI	770 cGy	-6	URD-HSCT、HID-HSCT
	Cy	3.6 g/m^2	-5、-4	
	MeCCNU	250 mg/m^2	-3	
	ATG	10 mg/kg	-5~-2	
	或ATG-F	40 mg/kg	-5~-2	

注:Cy:环磷酰胺;Bu:白消安;TBI:全身照射;Hu:羟基脲;Ara-C:阿糖胞苷;MeCCNU:甲环亚硝脲;ATG:抗胸腺细胞球蛋白,即复宁;ATG-F:费森尤斯生产的兔抗淋巴细胞球蛋白;allo-HSCT:异基因造血干细胞移植;URD:无关供者;CBT:脐血移植;HID-HSCT:单倍体相合造血干细胞移植

能带来存活的改善;而RIC方案提高了耐受性,需要通过免疫抑制剂和细胞治疗降低移植后疾病的复发率,有报道组合方案用于治疗复发难治的恶性血液病,如FLAMSA(氟达拉滨+安吖啶)续贯RIC。也可以采用常规预处理方案,移植后通过调节免疫抑制剂或细胞治疗加强移植物抗白血病(GVL)效应。

2. 其他恶性血液病:见表4。

也可以采用白血病的清髓预处理方案,如经典BuCy或TBICy方案,北京大学人民医院采用改良BuCy方案。

(二)非恶性血液病

1. SAA:同胞相合移植的预处理方案为Cy-ATG,非血缘供者移植推荐采用FluCy-ATG方案,单倍体相合的移植治疗SAA尚无统一的预处理方案(表5)。

2. 地中海贫血:采用与白血病相同的常规强度预处理方案疗效欠佳,国内一般采用加强的预处理方案(表6)。

3. 范可尼贫血:HSCT治疗范可尼贫血经常采用FluCyATG预处理(Flu 150 mg/m^2,Cy 5~10 mg·kg^{-1}·d^{-1},共4 d;兔抗人ATG 10 mg/kg)进行allo-HSCT,替代供者移植可以再增加低剂量TBI。

三、HLA配型及供者选择原则

(一)HLA配型:HLA相合的同胞是allo-HSCT的首选供者,次选供者为单倍体相合亲属、非血缘志愿供者和脐血。在没有相合的同胞供者时,供者的选择应结合患者情况(病情是否为复发高危、年龄、身体状况)、备选供者具体情况,及移植单位的经验综合考虑。

1. 单倍体相合供者移植特点:①绝大多数患者可以找到单倍体相合供者,而且单倍体供者往往不只1个,可以从中选优;②无需长时间等待,供者配型及查体一般2~3周,特别适于需要尽早移植的患者;③能够取到足够数量的细胞,对于高危复发患者,可以预存备用或再次采集;④可以根据需要获得骨髓和(或)外周造血干细胞;⑤对于高危的恶性血液病患者,移植后血液病复发率较非血缘移植低;⑥急性GVHD(aGVHD)发生率较非血缘移植略高,需要经验丰富的移植团队;⑦移植疗效与配型相合的同胞供者移植、非血缘供者移植疗效相似。在单倍体相合供者中,建议选择顺序为:子女、男性同胞、父亲、非遗传性母亲抗原(NIMA)不合的同胞、非遗传性父亲抗原(NIPA)不合的同胞、母亲及其他旁系亲属。

2. 非血缘供者移植特点:①查到供者的机会低,选择余地有限;②查询供者到移植需要等待的时间长,一般3~6个月;③对HLA配型相合的相合程度要求高,HLA-A、B、C、DRB1、DQ、高分辨中,最好的供者为高分辨9/10或10/10相

表2　治疗白血病/骨髓增生异常综合征的减低强度预处理(RIC)方案

预处理方案	药物名称	总剂量	应用时间(d)	移植类型
国际常用方案				
Flu//Mel	Flu	150 mg/m²	−7～−3	allo-HSCT
	Mel	140 mg/m²	−2、−1	
Flu/Bu	Flu	150 mg/m²	−9～−5	allo-HSCT
	Bu	8~10 mg/kg(口服)	−6～−4	
Flu/Cy	Flu	150 mg/m²	−7～−3	allo-HSCT
	Cy	140 mg/kg	−2、−1	
Flu/Bu/TT	Flu	150 mg/m²	−7～−5	allo-HSCT
	Bu	8 mg/kg(口服)	−6～−4	
	Thiotepa	5 mg/kg	−3	
TBI/Cy/ATG	TBI	4 Gy	−5	Flu+Ara-C+AMSA续
	Cy	120 mg/kg	−4、−3	贯, allo-HSCT
	ATG			
改良方案(北京大学人民医院方案)				
RIC-mBuCy	Hu	80 mg/kg(分2次)	−10	同胞相合HSCT
	Ara-C	2 g/m²(CI)	−9	
	Bu	4. 8 mg/kg(静脉滴注)	−10、−9	
	Cy	2.0 g/m²	−5、−4	
	MeCCNU	250 mg/m²(口服)	−3	
	ATG	10 mg/kg	−5～−2	
	或ATG-F	20~40 mg/kg	−5～−2	
RIC-BuFlu	Hu	80 mg/kg (分2次)	−10	同胞相合HSCT
	Ara-C	2 g/m²(CI)	−9	
	Bu	9.6 mg/kg(静脉滴注)	−8～−6	
	Flu	150 mg/m²	−6～−2	
	MeCCNU	250 mg/m²(口服)	−3	
RIC-mBuFluATG	Ara-C	8 g/m²(CI)	−10、−9	HID-HSCT
	Bu	9.6 mg/kg(静脉滴注)	−8～−6	
	Flu	150 mg/m²	−6～−2	
	MeCCNU	250 mg/m²(口服)	−3	
	ATG	10 mg/kg	−5～−2	
	或ATG-F	40 mg/kg	−5～−2	
RIC-mBuCyFlu+ATG	Ara-C	8 g/m²(CI)	−10、−9	HID-HSCT
	Bu	9.6 mg/kg(静脉滴注)	−8～−6	
	Flu	150 mg/m²	−6～−2	
	Cy	2.0 g/m²	−5、−4	
	MeCCNU	250 mg/m²(口服)	−3	
	ATG	10 mg/kg	−5～−2	
	或ATG-F	40 mg/kg	−5～−2	

注:Flu:氟达拉滨;Mel:马法兰;Cy:环磷酰胺;Bu:白消安;Thiotepa:塞替哌;TBI:全身照射;Hu:羟基脲;Ara-C:阿糖胞苷;MeCCNU:甲环亚硝脲;ATG:抗胸腺细胞球蛋白,即复宁;ATG-F:费森尤斯生产的兔抗淋巴细胞球蛋白;AMSA:安吖啶;allo-HSCT:异基因造血干细胞移植;URD:无关供者;CBT:脐血移植; HID-HSCT:单倍体相合造血干细胞移植

合，8/10相合同时满足A,B,DRB1中5/6相合时也可以考虑;④存在悔捐风险;⑤再次获取淋巴细胞或造血干细胞有一定难度;⑥非血缘移植后重度aGVHD发生率略低于单倍体移植,但在标危患者中复发率高于单倍体相合移植;⑦存活率和无病存活率与单倍体相合的供者移植相似。

3. 脐血移植的特点:①查询快、获得及时,无悔捐问题;②细胞数量受一定限制,CBT选择标准要结合配型、细胞数和病情综合考虑。对于恶性血液病,供受者HLA配型≥4/6位点相合,冷冻前TNC>(2.5～4.0)×10⁷/kg(受者体重),$CD34^+$细胞>(1.2～2.0)×10⁵/kg(受者体重);对于非恶性疾

表3 经常采用的加强预处理方案

预处理方案	药物名称	总剂量	应用时间(d)	移植类型
国际常用方案				
Cy /VP/TBI	Cy	120 mg/kg	−6、−5	allo-HSCT
	Vp16	30~60 mg/m^2	−4	
	fTBI	12.0~13.8 Gy	−3～−1	
TBI/TT/Cy	fTBI	13.8 Gy	−9～−6	allo-HSCT
	TT	10 mg/kg	−5、−4	
	Cy	120 mg/kg	−6、−5	
Bu/Cy/MEL	Bu	16 mg/kg(口服)	−7～−4	allo-HSCT
	Cy	120 mg/kg	−3、−2	
	Mel	140 mg/m^2	−1	
国内方案				
刘启发等	Flu	150 mg/m^2	−10～−6	allo-HSCT
	Ara-C	5~10 g/m^2	−10～−6	
	TBI	9 Gy	−5、−4	
	Cy	120 mg/kg	−3、−2	
	Vp16	30 mg/kg	−3、−2	

注:Cy:环磷酰胺;TT:Thiotepa,塞替哌;fTBI:分次全身照射;Flu:氟达拉滨;Bu:白消安;Mel:马法兰; Ara-C:阿糖胞苷; allo-HSCT:异基因造血干细胞移植

表4 多发性骨髓瘤(MM)、淋巴瘤的预处理方案

常用预处理方案	药物名称	总剂量	应用时间(d)	移植类型
BEAM	BCNU	300 mg/m^2	−6	淋巴瘤的allo-HSCT
	Vp16	800 mg/m^2	−5～−2	
	Ara-C	800 mg/kg	−5～−2	
	Mel	140 mg/m^2	−1	
Flu/Mel	Flu	150 mg/m^2	−7～−3	MM的allo-HSCT
	Mel	140 mg/m^2	−2、−1	
	硼替佐米			
Flu/Bu	Flu	150 mg/m^2	−9～−5	MM的allo-HSCT
	Bu	6.4~9.6 mg/kg(静脉滴注)	−6～−5/−4	

注:BCNU:卡氮芥;Vp16:依托泊苷;Ara-C:阿糖胞苷;Mel:马法兰;Flu:氟达拉滨;Bu:白消安;allo-HSCT:异基因造血干细胞移植

表5 重型再生障碍性贫血的预处理方案

预处理方案	药物名称	总剂量	应用时间(d)	移植类型
国际推荐方案				
Cy-ATG	Cy	200 mg/kg	−5～−2	同胞相合HSCT
	ATG	11.25~15.00 mg/kg	−5～−3,−2	
FluCy-ATG	Flu	120 mg/m^2	−5～−2	非同胞相合HSCT
	Cy	120 mg/kg	−5～−2	
	ATG	11.25~15.00 mg/kg	−5～−3,−2	
国内应用方案				
BuCyATG-SAA方案	Bu	6.4 mg/kg(静脉滴注)	−7、−6	单倍体相合HSCT
	Cy	200 mg/kg	−5～−2	
	ATG	10 mg/kg	−5～−2	
	或ATG-F	40 mg/kg	−5～−2	
BuCyFluATG方案	Bu	8 mg/kg(口服)	−7、−6	单倍体相合HSCT
	Flu	120 mg/m^2	−10~−7	
	Cy	200 mg/kg	−6～−3	
	ATG-F	20 mg/kg	−4～−1	
	或ATG	10 mg/kg	-4～−1	
FluCy-ATG方案	Flu	120 mg/m^2	−5～−2	单倍体相合HSCT
	Cy	90 mg/kg	−3、−2	
	ATG	10 mg/kg	−5～−2	

注:Cy:环磷酰胺;ATG:抗胸腺细胞球蛋白,即复宁;ATG-F:费森尤斯生产的兔抗淋巴细胞球蛋白;Flu:氟达拉滨;Bu:白消安;HSCT:造血干细胞移植

表6　地中海贫血的预处理方案

预处理方案	药物名称	总剂量	应用时间(d)	移植类型
常规强度方案				
与白血病预处理方案相同的方案	同白血病预处理	同白血病预处理		allo-HSCT
BuCy方案	Bu	14 mg/kg(口服)		allo-HSCT
	Cy	200 mg/kg		
加强的方案				
NF0-8-TM方案	Cy	110 mg/kg	-10、-9	HLA相合同胞移植
	Flu	200 mg/m^2	-8~-4	非血缘供者移植
	Thiotepa	10 mg/kg	-5	
	Bu	静脉滴注,QD(-8 d)	-8~-6	
		Css目标为300~600 ng/L		
	Azathioprine	3 mg/kg,QD	-45开始	
	Hu	30 mg/kg,QD	-45开始	
FluBuCyATG	Flu	150 mg/m^2	-12~-10	allo-HSCT
	Bu	12.8~16.0 mg/kg(静脉滴注)	-9~-6	
	Cy	200 mg/kg	-5~-2	
	ATG	10 mg/kg	-5~-2	
	Hu	20 mg/kg,QD	3个月前开始	

注:Bu:白消安;Cy:环磷酰胺;Flu:氟达拉滨;Thitepa:塞替哌;Azathioprine:硫唑嘌呤;ATG:抗胸腺细胞球蛋白;allo-HSCT:异基因造血干细胞移植;Css:稳态血浆药物浓度; Hu:羟基脲;QD:每日1次

病,HLA≥5/6位点相合,有核细胞计数(TNC)>3.5×10^7/kg(受者体重),CD34$^+$细胞>1.7×10^5/kg(受者体重);③GVHD发生率低且程度轻;④造血重建较慢,感染发生率较高;⑤不能再次获得造血细胞。需要移植经验丰富的团队;⑥治疗恶性血液病时可以达到与非血缘供者移植相似的疗效。

(二)供者选择的原则:当患者不具备同胞相合的供者时,高复发风险患者首选有血缘关系的供者以利于及时移植和移植后淋巴细胞输注,预计移植后不需要细胞治疗的标危患者可用选择非血缘供者,儿童患者可以选择脐血移植。

总之,移植的疗效受多个环节影响,与移植的预处理强度、供者选择和患者的病情、身体状况密切相关,对于群体的处理需要做到规范,对每例患者病情处理应该得到个体化。理想的状况是从诊断开始将患者进行危险度分层,为患者设计总体的治疗方案,有计划地使患者在最恰当的时机接受HSCT治疗。

(执笔:许兰平)

参加共识讨论的专家(以专家所在单位的首字母排序,同一单位多个专家按照姓氏首字母排序):安徽省立医院(孙自敏);北京大学第一医院(任汉云);北京大学人民医院、北京大学血液病研究所(黄晓军、刘代红、刘开彦、许兰平);第二军医大学附属长海医院(王健民);广西医科大学附属第一医院(赖永榕);哈尔滨市血液肿瘤研究所(马军);河南省肿瘤医院(宋永平);华中科技大学同济医学院附属协和医院(胡豫、邹萍);解放军第307医院(陈虎);南方医科大学南方医院(刘启发);上海新华医院儿科(陈静);四川大学华西医院(刘霆);苏州大学附属第一医院(吴德沛);天津医科大学总医院(邵宗鸿);新疆医科大学附属第一医院(江明);浙江大学医学院附属第一医院(黄河);中国医学科学院血液学研究所、血液病医院(韩明哲、王建祥);中国医学科学院北京协和医院(周道斌)

附件1:儿童ALL复发按时间分类标准

儿童急性淋巴细胞白血病复发:很早期复发指复发发生在诊断18个月内;早期复发指复发在诊断18个月以上,但停一线治疗6个月内;晚期复发指复发发生在一线治疗停药6个月及以上。

附件2:原发或继发骨髓纤维化的DIPSS分级

IPSS:年龄>65岁;症状;HGB<100 g/L;WBC>25×10^9/L;外周血原始细胞>1%。低危:没有上述危险因素;中危-Ⅰ:有上述一个危险因素;中危-Ⅱ:有上述2~3个危险因素;高危:危险因素≥4个。在病程的不同时间应用IPSS为动态IPSS(DIPSS)。

附件3:根据除铁情况的地中海贫血危险度分级

规则去铁定义为首次输血后18个月内开始去铁治疗,用去铁胺每周至少用5 d,每次至少皮下注射8~12 h。三个预后不良因素为肝大肋缘下2 cm、肝纤维化和不规则去铁,据此将地中海贫血患者分为3个危险等级:一级为无上述3种危险因素;二级有1~2种危险因素;三级有3种危险因素。

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中国异基因造血干细胞移植治疗血液系统疾病专家共识（Ⅲ）—急性移植物抗宿主病（2020年版）

中华医学会血液学分会干细胞应用学组

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Chinese consensus of allogeneic hematopoietic stem cell transplantation for hematological disease(III)—acute graft-versus-host disease (2020)

Stem Cell Application Group, Chinese Society of Hematology, Chinese Medical Association

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异基因造血干细胞移植（allo-HSCT）是治疗多种血液系统疾病的有效方法，单倍型造血干细胞移植（haplo-HSCT）的成功使我国造血干细胞移植病例数量快速增长[1-3]。据中国造血干细胞移植登记组报告，2019年我国140家单位实施allo-HSCT近万例，其中haplo-HSCT占60%。尽管allo-HSCT的疗效不断改善，移植物抗宿主病（GVHD）仍然是主要的合并症和死亡原因。目前急性GVHD的国际指南中的证据多来自HLA相合同胞供者（简称同胞相合）移植和非血缘供者移植，各种指南的推荐存在差异，推荐的疗效评判标准和严重程度分度标准也不尽相同[4-5]。与国外allo-HSCT以HLA相合移植和非血缘供者移植为主的模式明显不同，我国haplo-HSCT占第一位。鉴于我国的移植现状，加之各移植单位规模差别较大，急性GVHD的处理经验难免存在差别，所以有必要制定中国的专家共识。本共识在国际指南基础上纳入了中国医师在该领域的主要研究成果和临床经验，旨在形成适合中国情况的诊疗规范，为各移植单位提供指导性意见，并为移植中心之间的交流和合作奠定良好基础。共识由22位本领域的权威专家参与讨论制定。

一、急性GVHD的定义和发生率

（一）急性GVHD的定义

GVHD指由异基因供者细胞与受者组织发生反应导致的临床综合征。美国国立卫生研究院（NIH）的定义将急性GVHD分为经典急性GVHD和晚发急性GVHD：经典急性GVHD一般指发生在移植后100 d（+100 d）以内，且主要表现为皮肤、胃肠道和肝脏三个器官的炎性反应；晚发急性GVHD指具备经典急性GVHD的临床表现、但发生于+100 d后的GVHD。晚发急性GVHD包括以下几种情况：+100 d后新发生的急性GVHD、已获控制的经典急性GVHD在+100 d后再激活、经典急性GVHD延续至+100 d后。当急性GVHD表现和慢性GVHD同时存在时，诊断为重叠慢性GVHD。供者淋巴细胞输注（DLI）后急性GVHD诊断以DLI时间为计时

起点，其他与移植后急性 GVHD 诊断标准相同[5-7]。

（二）急性 GVHD 的发生率

国内资料显示，中度和重度急性 GVHD 发生率为13%~47%，发生率的差异主要与危险因素不同有关[8-25]。在同胞全相合移植中，II~IV、III/IV 度急性 GVHD 发生率分别为13%~35%、5.0%~7.7%[8-13]；在非血缘供者移植中，II~IV、III/IV 度急性 GVHD 发生率分别为12.5%~47.0%、6.6%~13.5%[14-16]；在 haplo-HSCT 中，II~IV、III/IV 度急性 GVHD 发生率分别为18.5%~43.9%、7.9%~13.8%[17-22]；在脐血移植中，II~IV、III/IV 度急性 GVHD 发生率分别为28.0%~30.6%、15.0%~19.4%[23-24]。在接受同胞全相合移植的再生障碍性贫血患者中，重度急性 GVHD 发生率最低。

二、急性 GVHD 的危险因素

在中国，haplo-HSCT 主要采用基于粒细胞集落刺激因子（G-CSF）和抗胸腺细胞球蛋白（ATG）的非体外去 T 细胞移植模式（北京方案）。haplo-HSCT 中急性 GVHD 的危险因素与同胞全相合移植和非血缘供者移植中的危险因素并不完全相同，而且通过改进急性 GVHD 的预防方案不同程度地削弱了这些危险因素的作用[2,12,25]。

（一）移植类型

既往认为急性GVHD与HLA不合的程度有关。近二十年来，大量资料表明，在同胞全相合移植、非血缘供者移植和haplo-HSCT三种移植类型中，重度急性GVHD发生率并无明显差别[8-9,11]。早期报告haplo-HSCT的II~IV度急性GVHD发生率高于同胞全相合移植，经优化供者选择及基于危险度的GVHD分层预防，II~IV度急性GVHD发生率在haplo-HSCT和非血缘供者移植中呈降低趋势[2,12,18-19]。

（二）供受者HLA不相合的位点数量

在非血缘供者移植中，急性GVHD发生率随着HLA不相合位点数量增加而增高[9,14-16]。在haplo-HSCT中，II~IV度急性GVHD和HLA不相合位点数量无关[2, 17, 20]。

（三）性别与年龄

在同胞全相合移植中，供者为女性（尤其是多次妊娠者）的患者具有较高的急性GVHD发生率，男性受者与女性供者、老年受者与老年供者均为GVHD的危险因素[12, 25]。而在haplo-HSCT中，母亲或非遗传性父体抗原（NIPA）不合同胞供者为急性GVHD的危险因素[2]。

（四）急性GVHD预防方案

急性GVHD预防方案与急性GVHD的发生密切相关，针对高危患者进行急性GVHD预防方案的改进可减弱危险因素的作用，如在环孢素A（CsA）+短程甲氨蝶呤（MTX）方案基础上增加霉酚酸酯（MMF）和（或）ATG等能够降低急性GVHD的发生率、减轻严重程度[10,22]。在母系或旁系供者haplo-HSCT中，移植后加入低剂量环磷酰胺可有效降低急性GVHD的发生率[19]。

三、急性 GVHD 的药物预防

药物能有效预防急性GVHD。

（一）同胞相合移植

1. CsA联合短程MTX[4-5]：①CsA：起始剂量1.5 mg/kg每12 h 1次，静脉输注，－1 d开始（也有主张－7 d或－9 d开始），有效谷浓度150~250 μg/L，消化道症状消失后改为口服。一般情况下恶性疾病移植后3个月CsA渐减，+6个月停用，但应根据复发风险和GVHD情况酌情缩短或延长CsA应用时间；良性疾病（如重型再生障碍

性贫血）移植后1年CsA减停，根据嵌合体和GVHD情况酌情缩短或延长CsA应用时间。当CsA不耐受时可更换为他克莫司，初始剂量0.02~0.03 mg • kg^{-1} • d^{-1}持续输注，一般有效血浓度为7~12 μg/L，消化道症状消失后改为口服给药，减量原则同CsA。②MTX：+1 d 15 mg/m^2，+3 d、+6 d 10 mg/m^2，静脉输注给药。回顾性研究结果提示在同胞全相合移植中+11 d是否应用MTX对急性GVHD没有影响[4,26]。每次MTX用药结束24 h后采用甲酰四氢叶酸钙解救。

2. MMF及其他：在上述基础预防方案基础上加用MMF或低剂量兔抗人胸腺细胞球蛋白（rATG）可进一步减低GVHD发生率[10,22]。MMF用法：成人或体重>35 kg儿童1.0 g/d，小儿一般30 mg • kg^{-1} • d^{-1}，分2~3次口服。MMF一般和CsA同时开始应用（或+1 d开始给药），植活或+30 d停用。

（二）haplo-HSCT和非血缘供者移植

1. CsA+短程MTX+MMF+ATG：CsA用法及用量同上。恶性血液系统疾病移植100 d后CsA渐减，移植后6~9个月停用（可根据复发风险和急性GVHD情况进行调整）；重型再生障碍性贫血等良性疾病移植后1年减停CsA。如CsA不耐受，可改为他克莫司，用法同上。

MTX用法同上，在重度口腔黏膜炎时+11 d MTX可不用。

MMF用量和开始时间同上，在非血缘供者移植和haplo-HSCT，通常在植入后减半，移植后2~3个月停药（可根据复发风险、GVHD和是否合并感染等情况进行调整），在无GVHD的高复发风险或病毒感染患者中酌情缩短MMF疗程。

ATG在国内最多应用的是rATG，推荐总剂量7.5~10 mg/kg，－5~－2 d分次输注[17-21]。

2. 其他：近年针对GVHD高危患者开展一些探索，如在haplo-HSCT中以生物标志（骨髓移植物的$CD4^+$细胞/$CD8^+$细胞比值）为指导分层短期应用低剂量糖皮质激素、在母系或旁系haplo-HSCT后加用低剂量环磷酰胺，均能有效降低急性GVHD发生率[18-19]。

（三）脐血移植

1. CsA联合MMF：CsA和MMF用法与同胞全相合移植相同。CsA也可采用持续静脉滴注方式给药，平均血药浓度200~300 μg/L。如恶性血液病无GVHD迹象，一般移植后2个月CsA开始逐渐减量，至少用至移植后6个月。

2. 关于ATG的应用：既往脐血移植多用ATG，近年来有学者认为不用ATG也是可行的。孙自敏等[24]采用清髓性预处理方案联合CsA+MMF预防GVHD进行非血缘脐血移植，对照组为清髓性预处理方案联合CsA+MMF+MTX或ATG-FreseniusS（7.5 mg • kg^{-1} • d^{-1}×3 d）预防GVHD进行的非血缘脐血移植，两组间II~IV、III/IV度急性GVHD的发生率差异均无统计学意义，而不含ATG组的植入率、无病存活率和总生存率明显优于使用ATG组，差异有统计学意义。

（四）DLI[27-28]

DLI一般输注G-CSF动员的淋巴细胞，也可以输注直接采集的供者淋巴细胞，其主要风险是GVHD发生率和致死率增高，不同类型DLI的GVHD预防方案有所不同。

1. 预防性DLI：一般情况下，在给予预防性DLI时CsA尚在应用中，DLI时将CsA调整至有效浓度。DLI后CsA的应用时间因移植类型（同胞全相合移植或haplo-HSCT）、DLI距移植的时间、输注细胞种类（G-CSF动员与否）而有所差异。

2. 干预性DLI：CsA在DLI前1 d开始应用并维持有效血药浓度。持续时间依不同移植类型而定，建议同胞

全相合移植患者DLI后4~6周减停，haplo-HSCT患者DLI后6~8周减停。干预性DLI后也可单用MTX预防GVHD，DLI后1、4、8 d各给药1次，以后10 mg每周1次共4~6次。

3. 治疗性DLI：一般采用CsA，用法与干预性DLI相同。黄晓军等认为治疗性DLI后短期应用免疫抑制剂可预防重症GVHD发生，而未影响移植物抗白血病（GVL）效应；在haplo-HSCT后血液学复发的患者中，DLI后采用MTX预防急性GVHD比用CsA可以更好保留GVL效应。MTX用法同干预性DLI[29]。也有人认为既往无重症GVHD病史的患者，同胞全相合移植的治疗性DLI后用可MTX或不用药物预防。

四、急性 GVHD 的鉴别诊断和严重程度分度标准

急性GVHD的诊断和分度主要依赖皮肤、胃肠道和肝脏的受累情况。

（一）急性 GVHD 临床表现[4,30-31]

1. 皮肤：皮肤是急性 GVHD 最多累及的靶器官，表现为斑丘疹，多始于头颈部、耳后、面部和肩部，多累及手掌、足心。患者常无明显不适或仅有轻度瘙痒、疼痛。

2. 胃肠道：胃肠道是急性GVHD第二位受累的靶器官，上消化道和下消化道均可累及。上消化道急性GVHD主要表现厌食消瘦、恶心呕吐，下消化道急性GVHD表现为水样腹泻、腹痛、便血和肠梗阻。下消化道急性GVHD与移植后非复发相关死亡密切相关。

3. 肝脏：肝脏急性GVHD表现为胆汁淤积导致的高胆红素血症、伴有或不伴有肝脏酶谱增高[32]。DLI后急性GVHD患者仅表现肝脏酶谱增高[33]，一般认为属于慢性GVHD，也有学者认为表现更似急性GVHD。

4. 其他表现：随着haplo-HSCT的广泛开展，除了上述三大器官典型急性GVHD表现之外，临床医师观察到疑似免疫原因导致发热和肺、中枢神经系统损伤的现象，有学者认为可能是急性GVHD的特殊表现，因为临床上鉴别诊断非常困难，这些表现是否归于急性GVHD尚有待进一步研究。

（二）诊断及鉴别

急性 GVHD 主要为临床诊断，需要注意排除其他可能情况，尤其在急性 GVHD 表现不典型或治疗效果欠佳时，鉴别诊断尤为重要[4-5]。

皮肤急性 GVHD 需要与导致皮疹发生的其他情况（预处理毒性、药疹或感染性皮疹等）进行鉴别；重度急性 GVHD 可以扩展至全身，表现为大疱甚至表皮剥脱，与 Stevens-Johnson 综合征或中毒性表皮坏死松解症进行鉴别。鉴别困难时可以考虑皮肤活检[4-5]。

当患者食欲不振、恶心和呕吐时，可能为上消化道急性 GVHD，仅表现上消化道症状时需要和念珠菌病、疱疹病毒感染和非特异性胃炎相鉴别[4-5]。上消化道急性 GVHD 的诊断：食欲不振伴体重下降、恶心持续至少3 d，或每天至少2次呕吐持续至少2 d。确诊需要胃或十二指肠活检病理结果[5-6]。

当腹泻为急性 GVHD 初始表现时，应注意与引起腹泻的其他原因相鉴别，包括感染（艰难梭菌、巨细胞病毒、EB 病毒、腺病毒、轮状病毒等）、药物不良反应、预处理毒性、血栓性微血管病、消化性溃疡等。近年有研究将生物标志物 ST2、REG3α 或 Elafin 等4因子组合应用于胃肠道急性 GVHD 的鉴别诊断及预后判断[34-35]，在常规应用于临床前尚需进一步研究。

当诊断肝脏急性 GVHD 时需与引起高胆红素血症的其他原因相鉴别，如预处理相关毒性、药物性肝损伤、肝窦阻塞综合征、脓毒症相关胆汁淤积和病毒性肝炎等。肝活检诊断急性 GVHD 应在权衡风险和获益后谨慎采用。

（三）急性 GVHD 的分度标准

急性 GVHD 的严重程度分度标准是根据急性 GVHD 对移植后非复发相关死亡的影响程度制定的，采用皮肤、胃肠道和肝脏急性 GVHD 分别积分后再形成总的分度。主要有三种分度标准，其中临床最常采用改良 Glucksberg 标准（表 1），近年来急性 GVHD 国际联盟（MAGIC）分级标准应用有增多趋势（表 2）[6,36]，此外还有 IBMTR 分级系统[37-38]。

表 1　改良的急性移植物抗宿主病（GVHD）Glucksberg 分级标准 [6,36]

项目	累及器官		
	皮肤	肝脏-胆红素血症	胃肠道
分级			
1级	皮疹面积<25%[a]	总胆红素2~3 mg/dl[b]	腹泻量>500 ml/d[c]或持续性恶心[d]
2级	皮疹面积25%~50%	总胆红素3.1~6 mg/dl	腹泻量>1 000 ml/d
3级	皮疹面积>50%，全身红斑	总胆红素6.1~15 mg/dl	腹泻量>1 500 ml/d
4级	全身红皮病伴大疱形成	总胆红素>15 mgdl	严重腹痛和（或）肠梗阻
分度[e]			
I度	1~2级		
II度	1~3级	1级	1级
III度		2~3级	2~4级
IV度[f]	4级	4级	

注：[a] 使用 9 分法或烧伤图表确定皮疹程度；[b] 以总胆红素表示范围（如果已经记录了导致总胆红素升高的其他原因，则将其降一级）；[c] 腹泻量适用于成人，儿童（≤14 岁）患者腹泻量应基于体表面积计算（如果记录了腹泻的另一个原因，则将其降一级）；[d] 持续恶心并有胃/十二指肠 GVHD 的组织学证据；[e] 作为授予该等级所需的最低器官受累程度的分级标准；[f]IV 度也可能包括较少的器官受累，但功能状态极度下降

表2　急性移植物抗宿主病（GVHD）国际联盟（MAGIC）分级标准[6]

分级	皮疹（仅活动性红斑）	肝脏	上消化道	下消化道（排便）
0级	无活动性（红斑）GVHD皮疹	总胆红素<2 mg/dl	无或间歇性恶心、呕吐或厌食	成人：<500 ml/d或<3次/d 儿童：<10 ml·kg^{-1}·d^{-1}或<4次/d
1级	<25%	总胆红素2~3 mg/dl	持续性恶心、呕吐或厌食	成人：500~999 ml/d或3~4次/d 儿童：10~19.9 ml·kg^{-1}·d^{-1}或4~6次/d
2级	25%~50%	总胆红素3.1~6 mg/dl		成人：1 000~1 500 ml/d或5~7次/d 儿童：20~30 ml·kg^{-1}·d^{-1}或7~10次/d
3级	>50%	总胆红素6.1~15 mg/dl		成人：>1 500 ml/d或>7次/d 儿童：>30 ml·kg^{-1}·d^{-1}或>10次/d
4级	全身红斑（>50%）伴水疱形成或表皮剥脱（>5%）	总胆红素>15 mg/dl		严重腹痛伴或不伴肠梗阻或便血（无论排便量如何）

注：整体临床分级（基于最严重的靶器官受累）：0 度：无任何器官 1~4 级；I 度：1~2 级皮肤，无肝脏、上消化道或下消化道受累；II 度：3 级皮疹和（或）1 级肝脏和（或）1 级上消化道和（或）1 级下消化道；III 度：2~3 级肝脏和（或）2~3 级下消化道，0-3 级皮肤和（或）0~1 级上消化道；IV 度：4 级皮肤、肝脏或下消化道受累，0~1 级上消化道受累。儿童：≤14 岁

五、疗效评估标准及糖皮质激素耐药急性 GVHD 的定义

急性GVHD开始治疗后每天评估疗效，及时识别糖皮质激素无效的患者。

（一）疗效评估标准

疗效评估通过各个靶器官的急性GVHD分级和整体分度与初始急性GVHD情况的比较获得。完全缓解（CR）指所有受累器官的急性GVHD表现完全消失；部分缓解（PR）指所有初始受累器官的急性GVHD改善（至少降

低一个级别）但未达到CR，无其他任何靶器官急性GVHD恶化；无反应（NR）指任何器官的急性GVHD严重程度无改善也没有恶化或患者死亡；进展（PD）指至少1个靶器官的急性GVHD加重（至少增加1个级别），伴或不伴其他器官急性GVHD的改善。PD和NR为治疗无效[4-6,38]。

（二）糖皮质激素耐药急性GVHD的定义

《Thomas' Hematopoietic Cell Transplantation: Stem Cell Transplantation》（第5版）将一线治疗3 d评估为PD、7 d评估为NR或14 d未达CR的情况定义为糖皮质激素耐药[30]。在2018年欧州骨髓移植学会-NIH-国际骨髓移植研究中心（EBMT-NIH-CIBMTR）的标准命名中，急性GVHD疗效评估时，将一线糖皮质激素开始治疗后3~5 d内疗效评估为PD或治疗5~7 d内疗效评估为NR或包括糖皮质激素在内的免疫抑制剂治疗28 d未达CR定义为糖皮质激素耐药。此外，将一线治疗糖皮质激素不能减量或减量过程中急性GVHD再激活定义为糖皮质激素依赖。糖皮质激素耐药和糖皮质激素依赖统称为糖皮质激素治疗失败[5-6]。

六、急性 GVHD 的治疗

原则上 I 度急性 GVHD 可以密切观察和局部治疗，II 度及以上急性 GVHD 诊断后应立即开始一线治疗，但在非血缘供者移植和 haplo-HSCT 中早期发生的急性 GVHD 往往进展较快，也应立即开始一线治疗。

（一）一线治疗

一线治疗药物为糖皮质激素，最常用甲泼尼龙，推荐起始剂量 1 mg • kg^{-1} • d^{-1} 或 2 mg • kg^{-1} • d^{-1}（分 2 次静脉注射），同时将 CsA 谷浓度调整至 150~250 μg/L 并及时评估糖皮质激素疗效[4,40-41]。

若疗效评估为有效，急性 GVHD 达 CR 后缓慢减少糖皮质激素用量，成年患者一般每 5~7 d 减量甲泼尼龙 10~20 mg/d（或等效剂量其他类型糖皮质激素），4 周减至初始量的 10%。儿童患者参照成人按比例缓慢减量。若判断为糖皮质激素耐药，需加用二线药物，并减停糖皮质激素；如判断为糖皮质激素依赖，二线药物起效后减停糖皮质激素。

（二）二线治疗

原则上在维持CsA有效浓度基础上加用二线药物，并及时评估疗效，当一种二线药物无效后再换用另一种二线药物。国际上尚无统一的二线药物选择流程，一般遵循各自中心的用药原则。鼓励患者参加临床试验。

1. 抗白细胞介素 2 受体抗体（IL-2RA）单抗（巴利昔单抗）：是迄今国内最多选用的急性 GVHD 二线药物。巴利昔单抗对成人糖皮质激素耐药急性 GVHD 患者的总有效率达 78.7%~86.8%，CR 率达 60.9%~69.8%；对儿童 haplo-HSCT 后糖皮质激素耐药急性 GVHD 的总有效率达 85%，CR 率为 74%[42-44]。巴利昔单抗推荐用法：成人及体重≥35 kg 儿童每次 20 mg、体重＜35 kg 儿童每次 10 mg，+1、+3、+8 d 各给药 1 次，以后每周 1 次，使用次数根据病情而定。

2. MTX：是由中国医师最先用于急性GVHD治疗的药物。黄晓军团队应用MTX联合低剂量甲泼尼龙（0.5 mg • kg^{-1} • d^{-1}）一线治疗急性GVHD，总有效率达81%，皮肤、胃肠道和肝脏急性GVHD的有效率分别为88%、75%、81%[45]。MTX二线治疗急性GVHD也取得很好疗效，治疗急性GVHD的有效率为94%，治疗DLI后GVHD的有效率为100%，对于皮肤、胃肠道、肝脏GVHD的有效率分别为100%、60%、71%[46]。推荐MTX用法：成人每次10 mg，+1、+3、+8 d各给药1次，以后每周1次，静脉或口服给药。儿童患者酌减。MTX的主要不良反应为血液毒性和口腔溃疡，适用于血象良好且没有口腔溃疡的患者。

3. 芦可替尼（Ruxolitinib）：刚被美国 FDA 批准用于糖皮质激素耐药急性 GVHD 的治疗[47-48]。推荐用法：

成人初始剂量为 10 mg/d（分 2 次口服），3 d 后若血液学参数稳定且未发生治疗相关不良反应可调整剂量至 20 mg/d。体重≥25 kg 的儿童患者，初始剂量为 10 mg/d（分 2 次口服）；体重<25 kg 的儿童患者，初始剂量为 5 mg/d（分 2 次口服）。主要不良反应是血液学毒性和增加感染风险（尤其是病毒感染）。芦可替尼国内应用经验有限，相关临床试验正在进行中。

4. 其他：可供选择的二线药物还有非吸收的糖皮质激素、霉酚酸（MPA）类药物、益赛普（Etanercept）、他克莫司（Tacrolimus）、西罗莫司（Sirolimus）等[4]。

（三）其他治疗

ATG、间充质干细胞（MSC）、粪菌移植等也有应用。此外，维多珠单抗（Vedolizumab）、托珠单抗（Tocilizumab）、英夫利昔单抗（Infliximab）、本妥昔单抗（Brentuximab）、抗CCR5单抗等均有进一步研究的潜力。

七、受累器官的局部管理和患者的整体管理

（一）强化受累器官的管理

1. 皮肤受累的急性 GVHD：加强局部护理，保持清洁，局部应用皮肤保护剂，减少渗出。

2. 胃肠道受累的急性 GVHD：应重视胃肠道休息、减少或停止经口摄入、部分或全部胃肠外营养补充热量，重视水电酸碱平衡，当不能除外肠道感染时给与经验性抗生素进行肠道除菌，不建议积极使用收敛剂对症处理，以免导致诊断评估的延误。便血患者加强输血支持。

3. 肝脏受累的急性 GVHD：慎用影响肝脏的药物，可以应用熊去氧胆酸。

（二）重视患者的整体管理

发生急性 GVHD 时，除了皮肤或黏膜屏障功能受损，免疫功能也受抑制，易于发生各种严重感染，所以治疗急性 GVHD 过程中注意感染的监测和预防，如预防疱疹病毒感染、真菌感染，常规监测巨细胞病毒、EB 病毒等。

八、总结

总之，在过去的十年中，尽管急性GVHD的防治获得了较大进展（尤其是haplo-HSCT），但急性GVHD仍然是allo-HSCT最常见的合并症和死亡原因之一，规范并优化急性GVHD防治对提高移植的成功率十分重要，期望未来可以实现预防和治疗GVHD方案的个体精准化。本共识将根据相关研究进展和临床实践而不断更新。

（执笔：许兰平、张晓辉）

参与共识制定和讨论的专家（以专家所在单位的首字母排序，同一单位多个专家按照姓氏首字母排序）：安徽省立医院（孙自敏）；北京大学第一医院（任汉云）；北京大学人民医院、北京大学血液病研究所（黄晓军、许兰平、张晓辉）；福建医科大学附属协和医院（胡建达）；广西医科大学附属第一医院（赖永榕）；河南省肿瘤医院（宋永平）；华中科技大学同济医学院附属协和医院（夏凌辉）；解放军总医院第一医学中心（刘代红）；解放军总医院第五医学中心（胡亮钉）；陆军军医大学附属第二医院（张曦）；南方医科大学南方医院（刘启发）；山东大学齐鲁医院（侯明）；上海交通大学医学院附属上海儿童医学中心（陈静）；上海交通大学医学院附属瑞金医院（胡炯）；苏州大学附属第一医院（唐晓文、吴德沛）；新疆医科大学附属第一医院（江明）；徐州医科大学附属医院（徐开林）；浙江大学医学院附属第一医院（黄河）；中国医学科学院血液病医院（韩明哲）

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·标准与讨论·

慢性移植物抗宿主病(cGVHD)诊断与治疗中国专家共识(2021年版)

中华医学会血液学分会造血干细胞应用学组 中国抗癌协会血液病转化委员会
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Chinese consensus on the diagnosis and management of chronic graft-versus-host disease (2021)

Hematopoietic Stem Cell Application Group, Chinese Society of Hematology, Chinese Medical Association; China Association for the Prevention of Hematology Diseases
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慢性移植物抗宿主病(cGVHD)指异基因造血干细胞移植(allo-HSCT)后,受者在重建供者免疫的过程中,来源于供者的淋巴细胞攻击受者脏器产生的临床病理综合征(包括经典型cGVHD和重叠综合征),是移植后主要并发症之一,发生率为30%~70%[1-2]。cGVHD发生机制复杂,临床表现多样,个体差异大,病程迁延持久,如不规范诊治,轻则影响患者生活质量,重则影响远期生存。随着移植技术体系的不断完善,患者对移植后生活质量的诉求越来越高,重视cGVHD的防治非常重要。在参考国内外该领域指南/共识和相关研究进展后制定本共识,旨在为cGVHD的规范化诊疗提供参考。

一、cGVHD的发生机制和诊断评估

(一)cGVHD的发生机制

cGVHD主要病理生理过程为免疫炎症反应,常见和特征性的病理改变是纤维化。基于基础和临床研究,将cGVHD的发生分为三个阶段:组织损伤引起的早期炎症(第一阶段),慢性炎症引起的胸腺损伤及B细胞和T细胞免疫失调(第二阶段),最终导致组织纤维化(第三阶段)[3-7]。应注意的是,cGVHD的三个阶段通常是连续性事件,但是第一阶段的患者也可以同时进入第二和第三阶段,三个阶段可共同致病[8]。

(二)cGVHD的诊断和临床评估

1. cGVHD的诊断:cGVHD的诊断主要依靠临床征象,类似于自身免疫性疾病,可累及全身任何一个或多个器官,最常累及的是皮肤、毛发、指甲、口腔、肝脏、眼睛、胃肠道、生殖器、关节筋膜或骨关节等[9-10](表1)。美国国家卫生研究院(NIH)共识将cGVHD的临床征象分为诊断性和区分性两种。诊断性征象包括:皮肤异色病/皮肤扁平苔藓样变/皮肤硬化样变/皮肤硬化性苔藓样变、口腔扁平苔藓样变、生殖器扁平苔藓样变/硬化性苔藓样变(女性阴道瘢痕或阴蒂/阴唇溃疡、男性包茎或尿道疤痕/狭

窄)、食管网格/食管中上三分之一狭窄、闭塞性细支气管炎(BOS)/肺活检诊断、筋膜炎/继发于筋膜炎或硬化的关节僵硬和挛缩;区分性征象指只见于cGVHD而不见于急性移植物抗宿主病(aGVHD)的临床表现,包括皮肤色素脱失/丘疹鳞状变、指甲萎缩/甲床分离/对称性脱落、新出现的斑秃/脱发、口腔干燥/黏液腺囊肿/黏膜萎缩/溃疡/假膜、眼结膜新发干燥/沙砾感/疼痛感/瘢痕性结膜炎/干眼症/点状角膜病、生殖道糜烂/龟裂/溃疡、经影像学诊断的空气潴留/支气管扩张、肌炎/多发性肌炎。虽然cGVHD主要以临床表现为诊断依据,但需排除感染、药物毒性、第二肿瘤等其他病因,必要时行组织活检明确诊断。allo-HSCT后患者出现至少1项cGVHD的诊断性征象,或至少1项cGVHD的区分性征象伴有同一或其他器官支持cGVHD的辅助检查(组织病理、实验室检查及肺功能实验等)阳性,可诊断为cGVHD[11-12]。

诊断需注意以下几点:①cGVHD临床表现本身错综复杂,可能并发感染、药物不良反应和其他疾病,应仔细鉴别,必要时专科会诊或多学科(MDT)综合诊治;②cGVHD早期征象不典型,应定期随访和密切观察。一旦出现晨僵、皮肤感觉异常、肌肉酸痛、不明原因低热、乏力或活动后喘息、不明原因消瘦、眼涩、口干、味觉异常、肝肾功能异常、感觉或运动轻度障碍、大便性状改变及生殖系统异常变化等征象均需高度重视,同时进行无创性筛查[13];③活检可以在难以明确诊断和安全的前提下安排;④诊断指标并不等同于评价严重性和治疗反应的指标[14]。

2. cGVHD的临床评估:cGVHD诊断明确后需要进行临床评估,以便对治疗指征和生存质量、预后进行判定,也是疗效评估的重要依据。

(1) cGVHD严重程度分级

根据八大受累器官(皮肤、口腔、眼、胃肠道、肝脏、肺部、关节和筋膜、生殖器)的严重程度进行划分:0分指无症状;1分指没有严重的功能受损,对日常活动没有影响;2分指对日常活动有明显影响但无残疾;3分指对日常活动有严重影响伴有严重残疾。综合各项积分将cGVHD分为轻、中、重三类,反映疾病的严重程度[12]。轻度包括:1~2个器官最高1分的患者(肺脏除外);中度为至少1个器官2~3分或多个器官1分,肺脏为1分直接归为中度;重度:至少1个器官3分以上,肺为2分时也归为重度(表2)[15,17-18]。

欧洲血液和骨髓移植学会(EBMT)通过研究分析接受同胞全相合、非血缘和脐血造血干细胞移植的白血病/MDS患者,确定了用于慢性GVHD预后评估的12项危险因素(表3)。根据得分将危险度分四组(Risk Group):RG1(0~3分),RG2(4~6分),RG3(7~9分),RG4(≥10分)。得分越高,预后越差[19-20]。

(2)cGVHD的预后危险分级

此外,生物标志物也可用于cGVHD的诊断、预后判断以及疗效评估。参与cGVHD发生发展的生物标志物包括白细胞介素(IL)、肿瘤坏死因子(TNF)、干扰素(IFN)、趋化因子家族(CXC)等。

表1　慢性移植物抗宿主病(cGVHD)的临床征象[15-16]

受累器官/部位	诊断性征象(诊断充分)	区分性征象(诊断不充分)	共同征象(aGVHD、cGVHD均可见)
皮肤	皮肤异色病、扁平苔藓样变、硬皮病	色素脱失	红斑、斑丘疹
指甲		病甲、甲软化、甲脱离	
头发和体毛		脱发,斑秃	
口腔	扁平苔藓样变、口腔活动受限	口干、黏液囊肿、溃疡、假膜[a]	齿龈炎、黏膜炎、红斑
眼		角膜结膜炎[a]、Sicca综合征(泪腺功能障碍)	
生殖系统	扁平苔藓样、阴道/尿道挛缩	糜烂、龟裂、溃疡[a]	
消化道	食管网格形成、狭窄或硬化		厌食、恶心、腹泻
肝脏			混合性肝炎
肺	活检证实的支气管闭塞	经肺功能或影像学诊断的支气管闭塞	
肌肉及筋膜	筋膜炎、关节挛缩	肌炎和多发性肌炎	
造血系统			血小板减少、嗜酸性粒细胞增多、低丙种球蛋白血症、高丙种球蛋白血症、自身抗体形成
其他			心包积液、胸腔积液、腹水

注:[a]必须排除感染、药物、肿瘤等因素;aGVHD:急性移植物抗宿主病

表2 慢性移植物抗宿主病(cGVHD)的分级评分系统[11-12]

	0分	1分	2分	3分
功能评分: □KPS □ECOG □LPS	□无症状,活动完全不受限(ECOG 0, KPS或LPS 100%)	□有症状,体力活动轻度受限(ECOG 1, KPS或LPS 80%~90%)	□有症状,可自理,<50%时间卧床(ECOG 2, KPS或LPS 60%~70%)	□有症状,生活自理受限,>50%时间卧床(ECOG 3~4, KPS或LPS <60%)
皮肤、毛发、指甲 □斑丘疹,扁平苔藓样变; □丘疹,鳞屑样病变或鳞癣; □色素沉着;□毛周角化; □红斑;□红皮病; □皮肤异色病;□硬化改变; □瘙痒症;□毛发受累; □指甲受累	□无体表受累 □皮肤无硬化病变	□<18%体表面积	□19%~50%体表面积 □皮肤浅层硬化,未绷紧,可捏动	□>50%体表面积 □皮肤深层硬化 □皮肤绷紧,不可捏 □皮肤活动受限 □皮肤溃疡
口腔 扁平苔藓样变 □有□无	□无症状	□轻度症状,摄入不受限	□中度症状,摄入轻度受限	□严重症状,摄入明显受限
眼 干燥性结膜炎 □有□无	□无症状	□轻度干眼症(需要滴眼液≤3次/d或无症状性干燥性角结膜炎)	□中度干眼症(滴眼液>3次/d),不伴视力受损	□严重干眼症,无法工作,视力丧失
胃肠道 □食管狭窄□吞咽困难□恶心□呕吐□腹痛腹泻□体重下降	□无症状	□有症状,三个月内体重减轻<5%	□中到重度症状,体重减轻5%~15%,或中度腹泻(不妨碍日常生活)	□体重减轻>15%,需要营养支持或食管扩张
肝脏	□总胆红素正常,ALT或碱性磷酸酶<3倍正常值上限	□总胆红素正常,ALT在正常值上限3~5倍,或碱性磷酸酶≥3倍正常值上限	□总胆红素升高,但≤3 mg/dl(51.3 μmol/L),或ALT>5倍上限	□总胆红素>3 mg/dl(51.3 μmol/L)
肺	□无症状 FEV1≥80%	□轻度症状(爬1楼气短) FEV1 60%~79%	□中度症状(平地活动气短),FEV1 40%~59%	□重度症状(静息气短,需吸氧),FEV1≤39%
关节和筋膜	□无症状	□肢体轻微僵直,不影响日常生活	□四肢至少1个关节僵硬,关节挛缩重度受限	□挛缩伴严重活动受限(不能系鞋带、系纽扣、穿衣等)
生殖系统	□无症状	□轻度症状,查体时无明显不适	□中度症状,检查时轻度不适	□严重症状
总体GVHD严重程度	□非GVHD	□轻度 1个或2个器官受累,得分不超过1分,肺0分	□中度 3个或多个器官受累,得分不超过1分;或者至少有1个器官(不包括肺)得分为2分;或者肺1分	□重度 至少有1个器官得分为3分,肺评分为2分或3分

注:KPS:Karnofsky功能状态评分;ECOG:美国东部肿瘤协作组评分;LPS:Lansky功能状态评分;FEV1:1秒用力呼气容积

以往研究显示,趋化因子CXCL9在皮肤cGVHD患者中表达水平增高,而肝脏cGVHD患者CCL17表达水平增高[4,21]。另外,ST2、sBAFF、TNF-α表达水平增高,IFN-γ、转化生长因子β(TGF-β)、IL-15表达水平降低等均有报道[8,17,22-25]。生物标志物的检测受感染、免疫抑制剂、标本采集时间、移植预处理、供者来源、疾病状态等多因素影响,有待进一步探索。

三、cGVHD的预防

表3　慢性移植物抗宿主病(cGVHD)预后危险评分系统

指标	分值
患者移植时年龄	
＜30岁	0
30~59岁	1
≥60岁	2
早期aGVHD	
无	0
有	1
cGVHD发生与移植间隔	
≥5个月	0
<5个月	1
cGVHD发生时血清胆红素	
＜2 mg/dl(34.2 μmol/L)	0
≥2 mg/dl(34.2 μmol/L)	2
cGVHD发生时KPS评分	
≥80分	0
<80分	1
cGVHD发生时外周血血小板计数	
≥100×10^9/L	0
<100×10^9/L	1
供者来源	
同胞间全相合/无关供者全相合或部分相合(1个位点不合)	0
其他相关/错配的无关供者(≥2个位点不合)	1
移植时疾病状态[a]	
早期	0
中期	1
晚期	2
性别错配(供者/受者)	
男/男,男/女,女/女	0
女/男	1
GVHD预防	
环孢素A+甲氨蝶呤+其他	0
他克莫司+甲氨蝶呤+其他/T细胞清除	1
cGVHD发生时外周血淋巴细胞计数	
≥1.0×10^9/L	0
<1.0×10^9/L	1
cGVHD发生时外周血嗜酸性粒细胞计数	
≥0.5×10^9/L	0
<0.5×10^9/L	1

注:aGVHD:急性移植物抗宿主病;KPS:Karnofsky功能状态评分;[a]移植时疾病状态分期:早期:急性白血病第1次完全缓解(AL-CR$_1$)、慢性髓系白血病慢性期(CML-CP)、骨髓增生异常综合征-难治性贫血(MDS-RA)、骨髓增生异常综合征-环形铁粒幼细胞性难治性贫血(MDS-RAS);中期:急性白血病第2次完全缓解(AL-CR$_2$)、慢性髓性白血病加速期(CML-AP);晚期:白血病复发或诱导失败、慢性髓性白血病急变期(CML-BC)、MDS伴原始细胞增多

GVHD的发生与供受者性别/年龄、HLA相合程度、预处理方案、造血干细胞来源等因素相关。在造血干细胞移植中,对于GVHD的预防往往是作为一个整体进行统筹,并不是专门针对cGVHD而设,涉及到的预防方法主要有:

(一)移植物来源选择

移植物来源是影响GVHD发生的重要因素。近年来,外周血干细胞成为主要移植物来源,外周血中含有较多成熟的供者T细胞,在减少复发风险的同时也增加了GVHD发生率[26]。单倍型供者、无关供者较同胞全相合供者更易引起GVHD[27];而脐血造血干细胞移植的GVHD较轻[28]。在选取移植物来源时,应充分评估患者的疾病状态、HLA相合情况来选择合适的供者。

(二)免疫抑制药物

免疫抑制剂是预防GVHD的主要手段,通过作用于T细胞增殖分化与激活的各个阶段,限制T细胞功能,抑制免疫反应。

临床常用免疫抑制剂组合包括:

1. 钙调磷酸酶抑制剂+甲氨蝶呤+霉酚酸酯/西罗莫司(CNI+MTX+MMF/SRL):这一组合是allo-HSCT的常用免疫抑制方案。其中,环孢素A(CsA)/他克莫司(FK506)应用3~6个月,CsA药物浓度控制在150~300 μg/L,他克莫司药物浓度控制在5~15 μg/L[29]。CNI在应用过程中应严格监测肝肾功能,避免肝肾毒性发生[30];MTX于+1 d 15 mg/m^2, +3 d、+6 d 10 mg/m^{2}应用;如果是非血缘HLA全相合移植或单倍型移植,在+11 d需要增加1剂MTX[31];MMF应用1~3个月,1.0 g/d(分2次给药)。国内有研究尝试小剂量MMF(0.5 g/d,−1 d用至+100 d)联合CsA、MTX预防HLA全相合非血缘allo-HSCT后GVHD,显示出较好的预防效果[32]。有条件的单位建议进行MMF血液浓度监测(完全AUC/简化AUC目标浓度30~60 mg·h/L),根据浓度调整MMF用量[33]。西罗莫司作为MTX/MMF的替代用药,一般从移植前3 d开始用药,持续用药3~6个月,维持血药浓度5~15 μg/L。应用西罗莫司后,cGVHD发生率有不同水平下降,但重度cGVHD发生率与传统预防方案无差别[34-35]。

2. 抗胸腺细胞球蛋白(ATG):去除移植物中T细胞是预防和控制GVHD最直接的手段。体外去T细胞虽然能预防GVHD,但降低了移植物抗白血病效应(GVL),增加复发风险[36],国内基本没有应用。目前,我国单倍型移植、非血缘供者移植及部

分HLA全相合同胞供者移植中，主要采用ATG进行T细胞体内去除[37]。兔抗人胸腺细胞免疫球蛋白用量为1.5～2.5 mg/kg，−5 d～−2 d使用[38]；抗人T细胞兔免疫球蛋白用量一般增加1倍；也有移植中心使用抗人T细胞猪免疫球蛋白，剂量为25～30 mg·kg^{-1}·d^{-1}，−5 d～−2 d。ATG的应用能显著降低GVHD发生率，改善患者生存，但随着ATG用量的增加，感染和复发相关死亡率显著增加[39]。有报道建议将兔抗人胸腺细胞免疫球蛋白总量从10 mg/kg降至7.5 mg/kg，两种剂量组aGVHD、cGVHD、复发率均相当，但7.5 mg/kg组病毒感染发生率较低[40]。对于供受者年龄均＞40岁的同胞全相合造血干细胞移植，可给予总量4.5 mg/kg，−3 d～−1 d应用，有助于降低cGVHD发生率[41]。

3. 移植后环磷酰胺（post-transplant cyclophosphamide, PT/CY）：对于HLA全相合移植患者，单独应用PT/CY预防方案（+3 d、+4 d静脉输注环磷酰胺50 mg/kg）而不使用MTX/CNI常规预防方案，cGVHD发生率仅为7%[42]。对于外周血造血干细胞移植患者，PT/CY预防cGVHD的效果也十分显著，发生率控制在20%以下[43]。对于HLA不全相合造血干细胞移植，可在PT/CY的基础上加用CsA、他克莫司、西罗莫司、ATG等药物，提高GVHD的预防效果[44]。PT/CY的主要不良反应为骨髓抑制，可引起白细胞下降，此外可出现膀胱刺激症、血尿、蛋白尿、肝功能损害、胃肠反应等[42-44]。

（三）间充质干细胞（MSC）

MSC是一群具有自我更新和多系分化的多功能细胞，独特的免疫调节作用使其在移植免疫方面有广泛应用前景。MSC防治cGVHD的机制尚待明确。研究表明，MSC可通过促进调节性T细胞（Treg细胞）增殖活化，调控Th1/Th2比例发挥免疫调节作用[45]；也可通过上调CD27$^+$记忆B细胞数量、降低血清B细胞激活因子（BAFF）水平和促进B细胞表面BAFF受体表达而诱导免疫耐受[46]。研究发现，经MSC治疗的aGVHD患者后期cGVHD发生率降低[47]。MSC输注的最佳剂量、最佳时间、最佳疗程仍需临床探索。有报道显示，在造血重建稳定后给予MSC输注（1×10^6/kg每月1次，共4次）可有效预防单倍型移植cGVHD的发生[45]。

四、cGVHD的治疗原则与一线治疗

（一）cGVHD的治疗原则和诊治流程

首先强调，不是所有的cGVHD患者确诊后都需要全身治疗。根据NIH的临床评估结果：轻度患者可观察或进行局部治疗，≥3个以上器官受累或单个器官受累2分以上（中、重度）患者应考虑进行全身治疗[12,48]。cGVHD的诊治流程见图1。

（二）cGVHD的一线治疗

糖皮质激素联合或不联合CNI是cGVHD一线治疗标准方案：如泼尼松±CsA/他克莫司。以泼尼松为例，剂量一般为1 mg·kg^{-1}·d^{-1}，单次服用；CsA（3～5 mg·kg^{-1}·d^{-1}分2次口服，血药浓度150～200 ng/ml）或他克莫司（0.1～0.3 mg·kg^{-1}·d^{-1}分两次口服，0.01～0.05 mg/kg持续静脉滴注，血药浓度5～15 ng/ml）一线治疗的有效率约为50%。

如果一线治疗有效，cGVHD症状得到有效控制，糖皮质激素应逐渐减量。糖皮质激素如何减量至今无统一方案，但需把握一个原则：缓慢减量、足够疗程，尽量使用足以控制GVHD症状的剂量。参考Paul J Martin教授等[49]采用每2周梯度递减上一剂量的20%～30%，具体遵照隔日减量法，如先减偶数天的服药剂量；糖皮质激素联合CsA等CNI治疗中，建议首先减糖皮质激素，其他免疫抑制剂每2～4周减量1次，3～9个月时间减停1种，免疫抑制剂治疗的中位时间应足够长，建议1～3年。

五、cGVHD的二线治疗

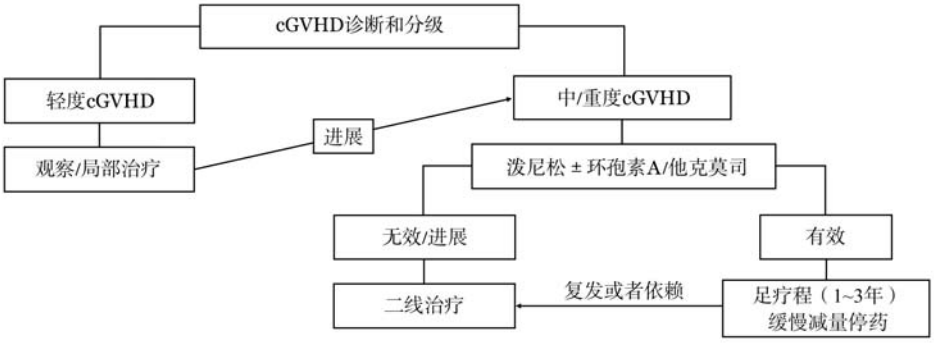

图1 慢性移植物抗宿主病（cGVHD）的诊治流程（二线治疗具体药物根据病情个体化选择）

临床出现以下情况需要考虑启动二线治疗：①既往累及的器官损伤加重；②出现新的器官受累；③正规用药1个月症状体征没有改善（如果单用糖皮质激素治疗，初始治疗2周有进展，6～8周无改善，考虑糖皮质激素耐药）；④2个月时，泼尼松不能减量到1.0 mg·kg^{-1}·d^{-1}以下[17]。符合上述情况可以启动二线治疗，但需要强调的是更换二线治疗药物后需要给予足够的观察期，这与aGVHD不同，不要急于短时间换药，一般需观察8～12周，除非4周内病情明显进展才能考虑再次更换其他二线药物。二线治疗目前尚无标准的优选治疗方案，各二线治疗药物可以互换，可依据个体化状况和靶器官特点尝试选择以下药物和措施[17]。

（一）MTX

MTX常用于allo-HSCT后GVHD的预防和aGVHD治疗，具有安全、有效的优点。北京大学人民医院应用小剂量MTX方案作为cGVHD的一线治疗和难治性cGVHD的挽救治疗，用量5～10 mg/m^{2}第1、3（或4）、8天给药，此后每周1次，直至GVHD症状缓解或不良反应不能耐受；如果患者白细胞计数低于2×10^9/L、血小板计数低于50×10^9/L，可减至5 mg/m^2。MTX治疗cGVHD的总缓解率为83%～94.7%，局限型和皮肤型cGVHD缓解率最高，不良反应可接受[50-51]。欧洲cGVHD诊断治疗指南已将此MTX方案列为糖皮质激素耐药或不耐受cGVHD重要的挽救治疗方案（5～10 mg/m^2，静脉给药，每周1次）[52]。

（二）芦可替尼（Ruxolitinib）

芦可替尼通过抑制JAK1/2信号转导，减少供者效应T细胞增殖、抑制针对同种异型抗原的促炎性细胞因子生成、介导抗原呈递细胞功能损伤，发挥治疗cGVHD作用[53]。芦可替尼治疗糖皮质激素耐药cGVHD的前瞻、对照、Ⅲ期临床研究显示，治疗总有效率为49.7%，起效时间比较短[54-56]，推荐剂量10 mg每日2次。部分单位尝试采用2.5～5 mg每日2次，也取得较好疗效[57]。如果长时间使用，要注意感染的发生，特别是病毒（如巨细胞病毒）再激活的问题[58-59]。贫血、血小板减少也是芦可替尼较常见的不良反应，发生率为21%～29%[60]。

（三）伊布替尼（Ibrutinib）

伊布替尼是一种布鲁顿氏酪氨酸激酶（BTK）抑制剂，主要通过抑制BTK信号通路抑制B和T淋巴细胞增殖、活化[61]。B细胞在cGVHD发病中占有重要地位，美国食品药品管理局（FDA）已经批准伊布替尼作为一线或多线系统治疗失败cGVHD的治疗选择，推荐剂量420 mg/d，治疗总缓解率为67%。与糖皮质激素合用，可显著减少糖皮质激素用量，延长缓解时间[62]。

（四）西罗莫司

西罗莫司是哺乳动物雷帕霉素靶蛋白（mTOR）抑制剂，通过与FKBP12蛋白结合发挥免疫抑制作用，最先用于实体器官移植后排斥预防及自身免疫性疾病[63]，后被用于难治性cGVHD的治疗[64]。具体用法：2 mg/d口服（首剂加倍）。体重小于40 kg者剂量为1 mg·m^{-2}·d^{-1}。有效治疗浓度为5～15 μg/L，维持治疗3～6个月或cGVHD症状明显缓解后减量停药。西罗莫司与CNI联用具有协同作用并降低肾毒性，1年总反应率（ORR）达59.3%，比较适合cGVHD长时间使用[65]。但浓度监测非常重要，和不良反应直接相关（口腔黏膜溃疡、高脂血症和骨髓毒性等）。除发挥免疫抑制作用外，西罗莫司尚具有抗纤维化、抗肿瘤及抗病毒活性（可用于预防巨细胞病毒、EB病毒感染）[66]。

（五）利妥昔单抗（Rituximab）

由于B细胞在cGVHD发病过程中起重要作用，故利妥昔单抗可能对治疗cGVHD有一定疗效。临床研究显示其治疗难治性cGVHD的疗效约为65%，对合并血小板减少症、硬皮病、皮肤病变、风湿性疾病的cGVHD患者疗效更佳，用量375 mg/m^{2}每周1次，连用4周[67]。利妥昔单抗联合MMF、他克莫司或西罗莫司三联疗法，总缓解率达88%，2年存活率为82%[68]，为替代糖皮质激素、减少不良反应、降低cGVHD治疗相关病死率开启新的治疗思路。

（六）伊马替尼（Imatinib）

伊马替尼可强效、持久阻断TGF-β和血小板源性生长因子受体（PDGF-R）通路，发挥抗纤维化和抗炎症作用，伊马替尼还可调节T细胞、B细胞而起到免疫抑制作用[69]。因此，伊马替尼可应用于合并纤维化cGVHD患者的治疗。起始剂量为100 mg/d，根据疗效和不良反应发生情况调整剂量，最大剂量400 mg/d；也可直接给予300 mg/d，后续调整药量[70]。治疗总缓解率为36%～79%，合并肝脏、肺、皮肤病变患者可获得更好的疗效[71]。伊马替尼的常见不良反应包括白细胞减少、血小板减少、水肿和皮疹，停药并对症处理可改善[72-73]。

（七）间充质干细胞（MSC）

MSC治疗难治性cGVHD的推荐剂量为1×10^6/kg每2周1次，共2～4次，缓解率达57.1%～73.7%，口

腔黏膜、胃肠道、肝脏和皮肤病变疗效最佳[10,74]。

（八）小剂量IL-2

Treg细胞功能缺失是cGVHD的重要特征，IL-2是促进Treg细胞增殖、迁移和发挥生物学功能的重要细胞因子[75]。我国已将小剂量IL-2作为糖皮质激素抵抗型cGVHD患者的二线治疗选择，推荐用量1×10^6 IU·m^{-2}·d^{-1}，疗程8～12周，治疗4周后起效，有效率为52%～61%，常见不良反应包括发热、乏力和骨关节疼痛，均为Ⅰ～Ⅱ级[76-77]。

（九）MMF

MMF主要与CsA、MTX、ATG联用以预防GVHD，也应用于难治性cGVHD的挽救治疗，总缓解率为69%～72%，不良反应可接受[78-79]。目前尚无充分临床证据显示MMF在cGVHD的一线三联疗法（糖皮质激素+CNI+ MMF）中发挥显著作用，但当糖皮质激素或CsA减量时适当增加MMF剂量，可减少cGVHD复发[80]。

（十）硫唑嘌呤（Azathioprine）

硫唑嘌呤一般与泼尼松联合用于治疗cGVHD。联合应用硫唑嘌呤可降低糖皮质激素治疗的失败率，近一半患者不会出现cGVHD复发[81]，但硫唑嘌呤与泼尼松联合应用可能引起间质性肺炎和严重全血细胞减少[82]，需要引起临床重视。目前尚无硫唑嘌呤二线治疗难治性cGVHD的大样本临床研究数据，因此，硫唑嘌呤并不作为难治性cGVHD的常规替代治疗推荐。

（十一）沙利度胺（Thalidomide）

沙利度胺治疗难治性cGVHD Ⅱ期临床研究的推荐治疗剂量为100～160 mg/d，总缓解率仅为50%[83]。联合泼尼松/CsA并不能提高疗效，且便秘、失眠、外周神经病变和血液学毒性等不良反应发生率较高，30%～90%的患者提前终止治疗[84]。所以，沙利度胺仅作为难治性cGVHD的备选和辅助治疗方案，并需要根据患者在治疗过程中的疗效和不良反应发生情况调整用量。

（十二）体外光分离置换疗法（extracorporeal photopheresis, ECP）：ECP是一种提取循环细胞后在体外进行长波紫外线照射的方法[85]，安全性高且不影响移植物抗白血病作用，推荐用于糖皮质激素抵抗型cGVHD[86]。治疗缓解率达80%，成人与儿童患者的缓解率无差异，尤其适用于皮肤、口腔、肝脏病变者[87]。目前国内暂时没有使用。

allo-HSCT后cGVHD的发生与移植类型、供受者HLA相合程度、患者年龄及一般情况等多种因素有关。cGVHD可累及全身任何器官，病理机制复杂，临床表现多样。以造血干细胞移植专科医生为主导、多学科协作的MDT诊治模式有利于cGVHD的诊断、受损器官评分、危险度分层和总体治疗方案的制定。另外，在cGVHD的诊治过程中，需和感染（细菌、真菌、巨细胞病毒、EB病毒等）相鉴别。感染和GVHD往往同时或相继发生、相互影响，形成恶性循环，给cGVHD的诊断和治疗带来一定难度。cGVHD的治疗是一个长期过程，应给予充分时间判断药物的疗效，避免频繁更换药物；同时，可作为慢病进行管理，定期进行cGVHD器官动态评分，判断治疗效果，配合cGVHD的综合治疗（如感染预防、营养支持、功能锻炼、心理干预等），逐步提高患者生活质量以达到治愈目的。

（执笔：张晓辉、张曦、高蕾、冯一梅、罗依、唐晓文、姜尔烈）

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部分作者在撰写论文时，只引用国外文献（或非中文语种的文献）。诚然，在医学的许多领域，国内的研究水平确实有待提高，有引用国外文献的必要。但是，不引用国内相关文献，将存在以下问题：①作者没有阅读国内文献，这样作者阅读的文献就不全面，作者所撰写的论文、综述等的科学性、先进性就值得商榷。②不引用国内文献，就不能准确、全面地反映国内的研究水平和进展，毕竟本刊发表的文章主要的阅读对象是中国医师。③有的作者虽然阅读了国内文献，但未引用。不引用国内文献的想法可能更为复杂，如轻视或忽略国内同行，或暗示首创权。除非是专门的国外医学文摘或国外文献综述，均应有国内文献的复习、引用和注解。本刊倡导在论文的撰写中应维护参考文献的科学性，鼓励作者在引用国外文献的同时检索并引用国内相关的文献。

本刊编辑部

·论著·

抗人T细胞猪免疫球蛋白联合重组人Ⅱ型肿瘤坏死因子受体-抗体融合蛋白治疗异基因造血干细胞移植后Ⅲ/Ⅳ度急性移植物抗宿主病35例临床研究

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【摘要】 目的 评估抗人T细胞猪免疫球蛋白(P-ATG)联合重组人Ⅱ型肿瘤坏死因子受体-抗体融合蛋白(益赛普)治疗Ⅲ/Ⅳ度急性移植物抗宿主病(aGVHD)的疗效及安全性。**方法** 对接受P-ATG(5 mg·kg^{-1}·d^{-1}×3~5 d,序贯5 mg/kg隔日1次~每周2次)联合益赛普(25 mg每周2次,儿童剂量减半)方案治疗的35例异基因造血干细胞移植(allo-HSCT)后合并Ⅲ/Ⅳ度aGVHD患者进行回顾性分析。**结果** ①35例Ⅲ/Ⅳ度aGVHD患者中,男21例,女14例,中位年龄10(3~54)岁。急性髓系白血病(AML)19例,急性淋巴细胞白血病(ALL)13例,重型再生障碍性贫血(SAA)、骨髓增生异常综合征(MDS)、混合表型急性白血病(MPAL)各1例。②治疗28 d疗效评估:完全缓解(CR)12例(34.3%),部分缓解(PR)18例(51.4%),总有效率为85.7%(30/35),Ⅲ度aGVHD组总有效率高于Ⅳ度aGVHD组[100%(19/19)对68.8%(11/16),P=0.004]。③治疗56 d疗效评估:CR 22例(62.9%),PR 5例(14.3%),总有效率为77.2%(27/35),Ⅲ度aGVHD组总有效率高于Ⅳ度aGVHD组[89.5%(17/19)对62.5%(10/16),P=0.009]。④不良反应:35例患者输注P-ATG过程中均为发生发热、寒战、皮疹等不良反应,亦无明显肝肾功能损害发生。巨细胞病毒、EB病毒再激活率分别为77.1%(27/35)、22.9%(8/35),细菌感染发生率为48.6%(17/35)。⑤中位随访时间为13(1~55)个月,移植后1、2年的总生存率分别为(68.1±8.0)%、(64.3±8.4)%,Ⅲ度aGVHD组移植后1年总生存率高于Ⅳ度aGVHD组[(84.2±8.4)%对(47.6±13.1)%,χ^2=3.38,P=0.05]。**结论** P-ATG联合重组人Ⅱ型肿瘤坏死因子受体-抗体融合蛋白治疗allo-HSCT后Ⅲ/Ⅳ度aGVHD有较好的疗效和安全性。

【关键词】 抗人T细胞猪免疫球蛋白; 重组人Ⅱ型肿瘤坏死因子受体-抗体融合蛋白; 移植物抗宿主病

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Clinical study of anti-human T cell porcine immunoglobulin with recombinant human tumor necrosis factor-α receptor II: IgG Fc in the treatment of 35 cases of grade III/IV acute graft-versus-host disease after allo-HSCT

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【Abstract】 Objective To evaluate the efficacy and safety of anti-human T lymphocyte porcine immunoglobulin (P-ATG) with recombinant human tumor necrosis factor-α receptor Ⅱ:IgG Fc fusion protein (rhTNFR:Fc, Etanercept) on grade Ⅲ/Ⅳ acute graft-versus-host disease (aGVHD) after allogenic hematopoietic stem cell transplantation (allo-HSCT). **Methods** Thirty-five patients with Grade Ⅲ/Ⅳ aGVHD who received P-ATG with etanercept therapy after allo-HSCT were retrospectively analyzed. P-ATGs (5 mg·kg^{-1}·d^{-1}) were administrated for 3 to 5 days, and then 5mg/kg was sequentially

administrated, QOD to BIW. Etanercepts were administrated 25 mg, twice a week (12.5 mg, BIW for pediatric patients). **Results** Among the 35 patients with grade Ⅲ/Ⅳ aGVHD, 21 were males and 14 females, with a median age of 10 (3 - 54) years. A total of 19 cases of acute myeloid leukemia, 13 of acute lymphoblastic leukemia, 1 of severe aplastic anemia, 1 of myelodysplastic syndrome, and 1 of mixed phenotypic acute leukemia were noted. The overall response (OR) rate of P-ATG with etanercept was 85.7% (30/35), with complete response (CR) and partial response (PR) rates of 34.3% (12/35) and 51.4% (18/35), respectively, on day 28. The OR rate of grade Ⅲ aGVHD group was higher than of grade IV aGVHD group [100% (19/19) *vs.* 68.8% (11/16), $P = 0.004$]. On day 56, the OR rate became 77.2% (27/35), with CR and PR rates of 62.9% (22/35) and 14.3% (5/35), respectively. The OR rate of grade Ⅲ aGVHD group was also higher than of grade Ⅳ aGVHD group [89.5% (17/19) *vs.* 62.5% (10/16), $P = 0.009$]. Thirty-five patients had no adverse effects such as fever, chills, and rash during the P-ATG infusion, and no obvious liver and kidney function damage was observed after treatment. The main treatment-related complication was infection. The reactivation rates of CMV and EBV were 77.1% (27/35) and 22.9% (8/35), respectively, and the bacterial infection rate was 48.6% (17/35). With a median follow-up time of 13 (1 - 55) months after HSCT, the 1-year and 2-year OS rates were (68.1±8.0)% and (64.3±8.4)%, respectively. The 1-year OS rate of grade Ⅲ aGVHD group was superior to grade Ⅳ aGVHD group [(84.2 ± 8.4)% *vs.* (47.6 ± 13.1)%, $\chi^2 = 3.38$, $P = 0.05$]. **Conclusion** This study demonstrated that P-ATG with etanercept was effective and safe in treating grade Ⅲ - Ⅳ aGVHD after allo-HSCT.

【Key words】 Anti-human T cell porcine immunoglobulin; Recombinant human tumor necrosis factor-α receptor Ⅱ: IgG Fc; Graft versus host disease

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急性移植物抗宿主病(aGVHD)是影响异基因造血干细胞移植(allo-HSCT)患者长期生存最重要的并发症,发生率为30%~50%,Ⅲ/Ⅳ度aGVHD发生率为5%~36%[1-5]。受累靶器官广泛且组织损伤程度重,约35~50%的患者对大剂量糖皮质激素一线治疗耐药,Ⅲ/Ⅳ度aGVHD尚缺乏指南性的二线方案[6]。虽然各种新开发的细胞因子类抗体、靶向药物、细胞治疗等提高了重度aGVHD治疗的反应率,但长期随访的生存率低于30%[7-9]。我们采用抗人T细胞猪免疫球蛋白(P-ATG)联合重组人Ⅱ型肿瘤坏死因子受体-抗体融合蛋白(商品名益赛普)治疗35例Ⅲ/Ⅳ度aGVHD患者,获得了较好的疗效。

病例与方法

1.病例:回顾性分析我院2015年11月12日至2020年4月30日期间接受P-ATG联合益赛普治疗的35例allo-HSCT后Ⅲ/Ⅳ度aGVHD患者。其中男21例,女14例,<16岁26例,≥16岁9例,中位年龄10(3~54)岁。原发疾病:急性髓系白血病(AML)19例,急性淋巴细胞白血病(ALL)13例,重型再生障碍性贫血(SAA)、骨髓增生异常综合征(MDS)、混合表型急性白血病(MPAL)各1例。急性白血病患者移植前疾病状态:第1次完全缓解(CR_1)22例,CR_2 7例,疾病进展4例。移植详细资料见表1。

表1 35例Ⅲ/Ⅳ度急性移植物抗宿主病(GVHD)患者的基本特征

病例特征	例数
供者类型	
同胞全相合	1
单倍型	34
供受者性别	
性别相合	17
性别不合	18
供、患者血型	
相合	18
主要不合	8
次要不合	6
主次均不合	3
预处理方案	
TBI/Cy为主	11
Bu/Cy为主	23
半量Bu+FAC	1
急性GVHD预防	
CsA+MMF+短疗程MTX	34
CsA+MMF	1
预处理ATG类型	
兔抗人胸腺细胞兔免疫球蛋白	27
抗人T细胞兔免疫球蛋白	7
抗人T细胞猪免疫球蛋白	1

注:TBI:全身照射;Cy:环磷酰胺;Bu:白消安;FAC:氟达拉滨+抗胸腺细胞球蛋白(ATG)+环磷酰胺;CsA:环孢素A;MMF:霉酚酸酯;MTX:甲氨蝶呤

2. 移植类型及预处理方案：35例患者中34例（97%）为单倍型造血干细胞移植，1例为同胞全相合造血干细胞移植。SAA单倍型移植预处理方案：半量白消安+氟达拉滨+环磷酰胺+抗胸腺细胞球蛋白（ATG）。白血病预处理方案：阿糖胞苷+全身照射（TBI）+环磷酰胺+ATG+司莫司汀（Me-CCNU）或阿糖胞苷+白消安+环磷酰胺+ATG+司莫司汀，进展期病例加用氟达拉滨/地西他滨/克拉曲滨/伊达吡星。ATG总量：兔抗人胸腺细胞免疫球蛋白（即复宁）7.5～10 mg/kg，抗人T细胞兔免疫球蛋白（ATG-F）20 mg/kg，P-ATG 120 mg/kg，均于−5～−2 d分次给药。

3. 移植物：移植物均为G-CSF动员的骨髓联合外周血造血干细胞（骨髓回输后第1天输注外周血干细胞）。单倍型移植患者于骨髓回输后第2天加用第三方细胞（非血缘脐血有核细胞数1×10^7/kg或单倍体相合骨髓1 ml/kg）输注及诱导免疫耐受。

4. 诊断标准：aGVHD的诊断及分度依据Glucksberg1994标准[10]。纤维结肠镜病理诊断依据Freiburg标准[11]。慢性GVHD诊断依据参照NIH标准[12]。

5. aGVHD治疗方案：所有病例确诊aGVHD后，在常规应用环孢素A或他克莫司、霉酚酸酯（MMF）基础上，予甲泼尼龙2 mg·kg^{-1}·d^{-1}。治疗3～7 d仍持续进展或无改善，加用益赛普25 mg每周2次（儿童剂量减半）和（或）巴利昔单抗（20 mg，第1、4、8、15天，儿童剂量减半）；Ⅲ/Ⅳ度患者给予P-ATG（5 mg·kg^{-1}·d^{-1}×3～5 d，序贯5 mg/kg隔日1次～每周2次）联合益赛普（25 mg每周2次，儿童剂量减半）治疗。抗aGVHD治疗期间，予两性霉素B 0.1～0.2 mg·kg^{-1}·d^{-1}预防真菌感染（如出现肌酐升高，则更换为泊沙康唑或伏立康唑）。予阿昔洛韦预防病毒感染，积极予抑酸、护胃、静脉营养等支持治疗。

6. 疗效判定标准及定义：P-ATG治疗28 d、56 d、进行疗效评估。完全缓解（CR）：受累器官无GVHD临床征象；部分缓解（PR）：至少1个受累器官aGVHD得到改善，其他受累器官无恶化；未缓解（NR）：受累靶器官aGVHD无变化或任一靶器官aGVHD进展。CR、PR定义为有效[13]。

7. 随访：随访资料来自门诊/住院病历及电话随访。随访截止日期为2020年4月30日。总生存（OS）时间定义为造血干细胞回输至随访截止或死亡时间。

8. 统计学处理：采用SPSS19软件进行统计学分析。非计量资料用百分数和率表示。组间总有效率比较采用Mann-Whitney和Kruskal-Wallis检验。生存分析采用Kaplan-Meier法，组间生存率比较采用Log-rank检验。使用Graphpad Prism5.0绘图。

结　果

1. aGVHD的发生情况：35例患者aGVHD的中位确诊时间为移植后21（6～110）d，Ⅲ/Ⅳ度aGVHD中位确诊时间为移植后28（17～132）d。Ⅲ度aGVHD 19例，Ⅳ度aGVHD 16例。单个器官受累10例（肠道9例，肝脏1例），2个器官受累15例（皮肤+肝脏1例，皮肤+肠道10例，肝脏+肠道4例），3个器官（皮肤+肝脏+肠道）受累10例。33例（94.3%）患者累及肠道，18例接受纤维结肠镜检查及病理活检，均符合aGVHD诊断，其中1例合并巨细胞病毒（CMV）感染。

2. P-ATG和益赛普剂量：P-ATG联合益赛普应用前，3例患者接受巴利昔单抗20 mg×2～7次治疗，6例患者接受益赛普（25 mg×1～5次）治疗，23例患者接受巴利昔单抗（20 mg×2～7次）联合益赛普（20 mg×2～7次）治疗，因aGVHD持续加重而纳入本研究。P-ATG联合益赛普治疗开始的中位时间为移植后29（17～250）d，P-ATG中位总剂量为70（25～300）mg/kg，益赛普中位总剂量为125（25～825）mg。

3. 疗效：①联合治疗28 d疗效：CR 12例（34.3%），PR 18例（51.4%），总有效率为85.7%（30/35）。Ⅲ度aGVHD组19例，CR10例，PR 9例；Ⅳ度aGVHD组16例，CR 2例，PR 9例，NR 5例。Ⅲ度aGVHD组总有效率高于Ⅳ度aGVHD组［100%（19/19）对68.8%（11/16），$P=0.004$］。单器官受累、2个器官受累、3个器官受累组的总有效率分别为90%、86.7%、80%（$P=0.527$）。②联合治疗56 d疗效：CR 22例（62.9%），PR 5例（14.3%），总有效率为77.2%（27/35）。Ⅲ度aGVHD组19例，其中CR 16例，PR 1例，NR 2例；Ⅳ度aGVHD组16例，其中CR 6例，PR 4例，NR 6例。Ⅲ度aGVHD组总有效率高于Ⅳ度aGVHD组［89.5%（17/19）对62.5%（10/16），$P=0.009$］。

4. 生存分析：随访截止日期为2020年4月30日，中位随访时间13（1～55）个月，移植后12个月OS率为（68.1±8.0）%，24个月OS率为（64.3±8.4）%。Ⅲ

度aGVHD组移植后1年OS率高于Ⅳ度aGVHD组[(84.2 ± 8.4)% 对 (47.6 ± 13.1)%，$\chi^2 = 3.38$，$P = 0.05$]。移植后1年cGVHD发生率为(66.8±10.1)%(广泛型7例，局限型10例)。

5. 治疗相关并发症：CMV、EB病毒(EBV)再激活发生率分别为77.1%(27/35)、22.9%(8/35)。CMV肠炎2例，均以更昔洛韦/膦甲酸钠、巨细胞免疫球蛋白、阿昔洛韦治愈。单纯疱疹病毒感染3例。出血性膀胱炎20例，1例儿童患者膀胱造瘘术后死于GVHD进展，19例经减量免疫抑制剂、碱化、水化、膀胱冲洗治愈。肺感染12例，急性胰腺炎2例，急性胆囊炎3例，肠梗阻5例。13例(37.1%)例死亡，死亡原因包括：aGVHD进展4例(其中3例合并血栓性微血管病)，感染5例，脑出血1例，胆囊穿刺术后出血1例，心脏性猝死1例，白血病复发1例。

讨　论

aGVHD的发生机制主要是患者组织抗原激活的以供者T细胞活化为核心的异源免疫反应，预防和治疗上以体内或体外耗竭T细胞为主[14]。ATG用于治疗aGVHD始于1974年[15]。Martin等[16]对1990年至2011年共计67篇糖皮质激素耐药aGVHD的文献进行分析，结果显示马ATG是应用频率最高的药物。此后多项研究显示，ATG治疗糖皮质激素耐药aGVHD的总有效率为30%～60%，导致死亡的最主要原因是GVHD进展和感染[17-20]。2001年Hsu等[21]研究显示，50%的移植中心应用马ATG(总剂量25～180 mg/kg)，24%的中心应用兔ATG(4～50 mg/kg)，多采用大剂量短期应用方案。Murata等[22]报道了兔ATG(即复宁)治疗99例糖皮质激素耐药aGVHD患者，有效率(CR+PR)为60%，CR+PR组、NR组移植后1年OS率分别为42%、5%。多因素分析显示不同剂量兔ATG组有效率差异无统计学意义，但高剂量与高非复发死亡率相关。不同动物来源ATG均可产生确切的淋巴细胞功能的抑制，但也显著增加了病毒、细菌及真菌感染发生率。于是，开始探索低剂量ATG单药或联合其他细胞因子抗体的应用并取得一定疗效。Tagliabue等[23]应用小剂量ATG治疗儿童Ⅲ/Ⅳ度aGVHD，6例患儿中5例获得CR，1例获得PR。刘静等[24]报道应用小剂量即复宁治疗Ⅱ～Ⅳ度aGVHD，6例患者中3例获得CR，1例获得PR。叶昌雄等[25]应用即复宁(总量4～7.5 mg/kg)联合抗CD25单抗治疗10例Ⅲ/Ⅳ度aGVHD，8例获得CR，2例获得PR，5例患者移植后存活超过2年。

P-ATG是国产多克隆抗淋巴细胞免疫球蛋白，治疗SAA的总有效率与兔ATG无明显差别，且不良反应及感染风险较低[26-27]。张湘兰[28]研究发现，SAA患者应用P-ATG联合免疫抑制剂治疗后，血清IL-2、IL-6、TNFα、INFγ、TGFβ1、IL-17水平均较治疗前显著下降。张晓辉等[29]研究显示，P-ATG在人体内维持有效血药浓度至少60 d。兔ATG在人体内有效血药浓度至少维持90 d[30]。杨楠等发现在SAA免疫抑制治疗(IST)中，兔ATG较P-ATG对淋巴细胞的清除程度更深、抑制时间更持久[31]。基于上述原因我们认为，与兔ATG相比，小剂量P-ATG治疗aGVHD可能具有更高的有效率和较低的机会性感染发生风险。考虑aGVHD靶器官组织及功能损害阶段以肿瘤坏死因子介导为主，我们采用小剂量P-ATG联合益赛普治疗35例Ⅲ/Ⅳ度aGVHD，治疗28 d的CR率为34.3%，但治疗进行至56 d时CR率达到62.9%，Ⅲ度aGVHD组总有效率及OS率均优于Ⅳ度aGVHD组。

重度aGVHD的低反应率及组织修复缓慢，原因可能包括：糖皮质激素耐药或抵抗，钙调磷酸酶抑制剂(CNI)不耐受或中断，机会性感染，消化道出血、梗阻和极度营养缺乏等。为减低感染风险，我们采取了以下措施：①判定糖皮质激素耐药后快速减停(每3～5 d减量0.2 mg·kg^{-1}·d^{-1}；②低剂量两性霉素B(0.1～0.2 mg·kg^{-1}·d^{-1})预防真菌感染，既能减少与CNI的相互作用，又有效降低了真菌感染风险。低剂量两性霉素B预防真菌可以追溯到20世纪90年代，Perfect等[32]报道预防性低剂量两性霉素B(0.1 mg·kg^{-1}·d^{-1})可有效减少口咽部酵母菌定植、降低真菌感染发生率。后续研究显示，在HSCT患者真菌预防中，0.2 mg·kg^{-1}·d^{-1}两性霉素B和氟康唑具有同样效果，但两性霉素B不良反应较为显著[33-34]。而脂质体两性霉素B具有较低的肾毒性，2020年Mendoza-Palomar等[35]将低剂量脂质体二性霉素B(1 mg·kg^{-1}·d^{-1})用于儿童HSCT，真菌突破感染率为7.7%，不良反应均为1级。③通过监测血浆sCD25、TNFα、IL-6、IL-10等细胞因子水平，对P-ATG和益赛普序贯治疗进行调整。经过上述多项措施，本组患者移植后24个月OS率为(64.3±8.4)%，移植后1年广泛型cGVHD发生率为(41.2±13.7)%，或许得益于ATG相对持久的免疫抑制效应。

本研究结果初步显示,P-ATG联合重组人Ⅱ型肿瘤坏死因子受体-抗体融合蛋白治疗allo-HSCT后Ⅲ/Ⅳ度aGVHD有较好的疗效和安全性。

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医学名词应使用全国科学技术名词审定委员会公布的名词。尚未通过审定的学科名词，可选用最新版《医学主题词表（MESH）》、《医学主题词注释字顺表》、《中医药主题词表》中的主题词。对于没有通用译名的名词术语，在文内第一次出现时应注明原词。中西药名以最新版《中华人民共和国药典》和《中国药品通用名称》（均由中国药典委员会编写）为准。英文药物名称则采用国际非专利药名。在题名及正文中，药名一般不得使用商品名，确需使用商品名时应先注明其通用名称。冠以外国人名的体征、疾病、试验、综合征等，人名可以用中译文，但人名后不加“氏”（单字名除外，例如福氏杆菌）；也可以用外文，但人名后不加“’s”。

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ORIGINAL ARTICLE

Outcomes of allogeneic haematopoietic stem cell transplantation for patients with severe aplastic anaemia using the porcine antilymphocyte globulin-containing conditioning regimen

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Abstract

Antithymocyte globulin (ATG) is widely used for allogeneic haematopoietic stem cell transplantation (allo-HSCT) in severe aplastic anaemia (SAA). Only rabbit-ATG (r-ATG) and porcine-antilymphocyte globulin (p-ALG) are available in China, but the p-ALG-containing conditioning regimen for allo-HSCT in SAA has seldom been reported. In this study, we retrospectively evaluated the outcomes of 41 SAA patients receiving allo-HSCT with a p-ALG-containing conditioning regimen in our transplantation centre. All patients engrafted, and no death during conditioning was observed. The actuarial 3-year overall survival (OS) rates were 95.1 ± 3.4%. The actuarial 3-year disease-free survival (DFS) rates were 85.0 ± 5.7%. Acute graft-versus-host disease (aGVHD) predicted inferior OS ($p < 0.05$). The interval from diagnosis to transplantation for more than 100 days predicted an inferior DFS rate ($p < 0.05$) and a higher graft rejection/poor graft function (GR/PGF) rate ($p < 0.01$). In conclusion, the p-ALG-containing regimen showed satisfactory effects and safety in allo-HSCT for SAA patients. P-ALG could be a potential alternative preparation for r-ATG in SAA allo-HSCT.

Keywords Porcine antilymphocyte globulin · Allogeneic haematopoietic stem cell transplantation · Severe aplastic anaemia · Conditioning regimen

Introduction

Severe aplastic anaemia (SAA) is a potential fatal haematologic disease characterized by pancytopenia and bone marrow aplasia or hypoplasia [1]. Allogeneic haematopoietic stem cell transplantation (allo-HSCT) offers the best chance for cure and is the preferred first-line treatment option for younger SAA patients with a fully matched 10/10 donor with long-term survival over 80% [2, 3]. An advantage of allo-HSCT over standard immunosuppressive therapy (IST) is a marked reduction both in the risk of relapse and the evolution of late clonal disorders such as myelodysplastic syndromes and paroxysmal nocturnal haemoglobinuria [4]. ATG reduces graft failure by killing recipient lymphocytes that mediate graft rejection, and the remaining ATG in the circulation after transplantation may also kill alloreactive donor T cells that mediate graft-versus-host disease (GVHD), so it improves the outcomes of patients and has been widely used for HSCT in SAA as well as in other haematological disorders [5–13]. There are three preparations available for clinical use as immunosuppressive agents: the historically first used preparation from horses, the subsequent approved preparation from rabbits, and the porcine preparation approved in China. Horse-ATG (h-ATG), however, is not available in China, and most Chinese clinical centres use r-ATG for HSCT and IST. Our previous data [14] showed that p-ALG had similar long-term efficacy and high overall survival (OS), as well as safety profiles for r-ATG in IST of acquired SAA, which was consistent with other recent studies [15, 16], but a systemic investigation of the conditioning regimen containing p-ALG for allo-HSCT in SAA was seldom reported. The incidence of SAA in China, as well as other countries in East Asia, is higher than that in Europe and North America [17]. Compared to r-ATG, which is not covered by any insurance,

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p-ALG is substantially cheaper, lowering the costs of therapy by at least 30% ($345 per bottle 25 mg vs. $245 per bottle 250 mg). Thus, we performed a retrospective study of conditioning including p-ALG for allo-HSCT in SAA patients.

Patients and methods

Ethics statements

The study was approved by the ethics committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology.

Subjects

We conducted a retrospective analysis of 41 consecutive SAA patients who received 10/10 HLA-matched allo-HSCT in our transplantation centre from May 2005 to June 2017. Aplastic anaemia diagnosis and severity classification were performed independently by at least three senior haematologists according to the complete blood count and multisite bone marrow (BM) examination. The diagnosis of SAA requires bone marrow cellularity less than 25% and at least two of the following three criteria from peripheral blood counts: corrected reticulocyte counts $\leq 1\%$ or $\leq 4 \times 109/L$, platelet counts $\leq 20 \times 109/L$, and neutrophil counts $\leq 0.5 \times 109/L$. Very severe aplastic anaemia was defined as neutrophil counts $\leq 0.2 \times 109/L$ [18]. Of the 41 patients, 3 were categorized as very severe aplastic anaemia.

Conditioning regimen

Thirty-seven patients received a conditioning regimen composed of fludarabine (30 mg/m^2/day i.v. on days − 9, − 8, − 7, − 6 and − 5), cyclophosphamide (60 mg/kg/day i.v. on days − 4 and − 3), and p-ALG® (Wuhan Institute of Biological Products, China, 30 mg/kg/day i.v. on days − 4, − 3, − 2, and − 1). Four patients received conditioning of cyclophosphamide (60 mg/kg/day i.v. on days − 4 and − 3) and p-ALG(30 mg/kg/day i.v. on days − 4, − 3, − 2, and − 1), two of whom received HLA-matched sibling(MSD) transplantation and two received HLA-matched unrelated transplantation.

GVHD prophylaxis

Patients received cyclosporine (3 mg/kg/day i.v. by continuous infusion from day − 1, then maintaining a trough concentration of 200–300 ng/ml with an oral form when a good oral intake was achieved, continued orally within 12 months after transplantation and gradually tapered off with careful follow-up of chimerism and hematologic parameters), methotrexate (15 mg/m2 i.v. on day 1, 10 mg/m^2 i.v. on days 3, 6 and 11), and mycophenolate mofetil (0.5 g bid p.o. from days − 1 to 30, 0.25 g bid p.o. from days 31 to 60).

Graft source

Thirty-five patients received HLA-matched sibling peripheral blood stem cell (PBSC) transplantation, three patients received HLA-matched sibling combinations of bone marrow and PBSCs, and three patients received PBSC transplantation from 10/10 HLA-matched unrelated donors. In addition to some donor's unwillingness to donate bone marrow, we used G-CSF mobilized PBSC instead of bone marrow to achieve a higher dose of infused CD34+ cells for engraftment.

Engraftment criterion

Engraftment was defined as neutrophil counts $\geq 0.5 \times 109/L$ for three consecutive days and platelet counts $\geq 20 \times 109/L$ without transfusion for 7 days.

Supportive care

All patients resided in a sterile laminar flow ward. Patients received prophylactic ganciclovir from the beginning of conditioning therapy until − 1d. Prophylactic acyclovir was administered orally from + 2d. The electrocardiogram was measured twice daily using cyclophosphamide. Mesna was given to prevent haemorrhagic cystitis. Methyltetrahydrofolate was used as a mouthwash from + 2d to + 16d. Patients routinely received G-CSF at a dose of 5 μg/kg per day from + 5d to neutrophil engraftment. A transfusion was given to keep the platelet level > 20 × 109/L and haemoglobin > 70 g/L. The blood routine was checked every day. The blood biochemistry and cyclosporine concentration were examined twice a week. Quantitative real-time polymerase chain reaction (PCR) assays for cytomegalovirus (CMV) DNA in plasma and Epstein-Barr virus (EBV) DNA in plasma and mononuclear cells in peripheral blood (PBMC) were performed once or twice per week until 180 days posttransplant or withdrawal of immunosuppressant.

Outcomes analysis standard

The last follow-up for all surviving patients was August 31, 2018. Diagnosing and grading of acute GVHD (aGVHD) was based on the Mount Sinai Acute GvHD International Consortium (MAGIC) criteria [19]. The 2014 National Institutes of Health consensus of chronic GVHD (cGVHD) [20] was used to diagnose and grade cGVHD. Patients who did not reach the engraftment criterion after transplantation were considered to have had primary graft rejection (GR) [21]. Patients who were initially engrafted, with recurrent

pancytopenia accompanied by mixed donor chimerism or autologous recovery and no moderate to severe aGVHD, were considered to have secondary GR [22]. Early GR was defined as GR occurrence within the first 100 days after transplantation, while late GR occurred later than 100 days after transplantation [23–25]. Poor graft function (PGF) was defined as severe cytopenia of at least two cell lines and/or transfusion requirement in the presence of hypoplastic/aplastic bone marrow with full donor chimerism and in the absence of severe GVHD or relapse [26, 27]. Chimerism analyses were routinely evaluated by PCR of short tandem repeat sequences and/or fluorescence in situ hybridization (FISH) analysis for the X and Y chromosomes (XY-FISH). Donor chimerism status was defined based on previous reports [28] as follows: full donor chimerism, ≥ 95% donor cells; mixed chimerism, 5–95% donor cells, and autologous recovery, ≤5 % donor cells. CMV viremia was defined as positive results of reverse transcriptase PCR (> 1 × 103 copies/ml) in blood. Invasive fungal disease was defined according to the revised EORTC/MSG criteria [29]. Severe bacterial infection was defined as bacteraemia or severe tissue infections. The probability of overall survival was calculated using the Kaplan-Meier estimator, death from any cause was considered an event, and surviving patients were censored at last follow-up. Disease-free survival (DFS) was measured from the date of HSCT, recurrence or clonal transformation or death was considered an event, and surviving patients were censored at last follow-up.

Statistical analysis

All data were analysed using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). The survival rate was analysed using the Kaplan-Meier method. Survival differences between groups were estimated by the log-rank test. Acute and chronic GVHD were calculated using the cumulative incidence function method in which death without the event was the competing event. Values are the mean ± standard deviation unless otherwise defined. The final model of significance attained ≤ 0.05.

Results

Pretransplant characteristics and treatment details

A total of 41 patients received HLA-matched 10/10 donor allogeneic HSCT. The overall characteristics of the patients are summarized in Table 1, whereas engraftment and conditioning regimens are shown in Table 2.

Conditioning-related toxicity

Common side effects of p-ALG were infusion-related hypersensitivity reactions and serum sickness, which were the same as other preparations and characterized by fever, mild rash, or joint pain [15], and these side effects could be controlled by non-steroidal anti-inflammatory drugs. During the conditioning regimen, 48.8% of the patients underwent a fever, and all returned to normal body temperature automatically or after non-steroidal anti-inflammatory drugs. Approximately 12.2% had mild anaphylactic reactions, mainly manifesting as skin reactions such as itching, wheal, and symptoms disappeared after anti-allergy treatment. Approximately 43.9% had mild liver damage, which was manifested by mildly elevated liver enzymes or bilirubin that did not exceed twice the upper limit of normal. No fatal serum sickness or anaphylactic shock was reported in this cohort, and no death during conditioning was observed.

Haematologic response

All patients engrafted, and none displayed primary GR. The median time of absolute neutrophil count (ANC) recovery was 11 days (range 7 to 17). The median time of platelet (PLT) recovery was 12 days (range 9 to 26), as shown in Table 3.

Table 1 Pretransplant characteristics

Characteristic	Number (%)
Gender	41 (100%)
Male	21 (51.2%)
Female	20 (48.8%)
Median age, year (range)	25 (15–66)
Median body weight, kg (range)	55 (37–81)
Median interval from diagnosis to transplantation, days (range)	31 (12–1630)
Failed treatment before HSCT	
No systemic treatment	21(51.2%)
Medication treatment, such as CsA and androgen	19(46.3%)
Rabbit-ATG therapy	1(2.5%)

HSCT haematopoietic stem cell transplantation, *CsA* cyclosporin A

Table 2 Treatment details

	Number (%)
Donor	41 (100%)
MSD	38 (92.7%)
URD	3 (7.3%)
Donor gender	
Male	25(61.0%)
Female	16 (39.0%)
GVHD prophylaxis	
CsA + MTX	34 (82.9%)
CsA + MTX + MMF	7 (17.1%)
Graft source	
PBSCs	38 (92.7%)
BM + PBSCs	3 (7.3%)
Median number of graft (range)	
PBSCs	
MNCs, × 108/kg	18.84 (7.01–32.42)
CD34+ cells, × 106/kg	4.60 (2.02–16.44)
BM + PBSCs	
MNCs, × 108/kg	15.21 (6.36–26.00)
CD34+ cells, × 106/kg	6.60 (4.00–11.00)

MSD HLA-matched sibling donor, *URD* 10/10 unrelated donor, *GVHD* graft-versus-host disease, *CsA* cyclosporin A, *MTX* methotrexate, *MMF* mycophenolate mofetil, *PBSC* peripheral blood stem cell, *BM* bone marrow, *MNC* mononuclear cell

Table 3 Outcomes of patients

Outcomes	Number (%)
Engraftment	
Median ANC, days (range)	11 (7–17)
Median PLT, days (range)	12 (9–26)
GVHD	
aGVHD	9 (22.0%)
Grades III–IV aGVHD	2 (4.9%)
Grades I–II aGVHD	7 (17.1%)
cGVHD	11 (26.8%)
Mild cGVHD	7 (17.1%)
Moderate-severe cGVHD	4 (9.7%)
5-year GR, %	5.3 ± 3.7
5-year PGF, %	4.9 ± 3.4
3-year OS, %	95.1 ± 3.4
3-year DFS,%	85.0 ± 5.7
Infection	
Severe bacterial infection	8 (19.5%)
IFD	7 (17.1%)
CMV viremia	8 (19.5%)

GR graft rejection, *PGF* poor graft function, *OS* overall survival, *DFS* disease-free survival, *IFD* invasive fungal disease

Graft-versus-host disease

Of the 41 patients, nine developed aGVHD (7 with grades I to II and 2 with grades III to IV). The day 100 cumulative incidence of grades III to IV aGVHD were 4.9 ± 3.4%. All patients responded well to the therapy consisting of glucocorticoids and immunosuppressive agents, and no patients died from aGVHD. The corresponding cGVHD was observed in eleven patients (seven with mild and four with moderate-severe). The 5-year cumulative incidence of moderate-severe cGVHD was 10.8 ± 5.1%. The data are shown in Fig. 1.

Infection

Of all the patients, bacteraemia occurred in 5 patients and 3 patients experienced maxillofacial bacterial infection. IFD occurred in 7 patients, including 1 case of intracranial infection. Pulmonary IFD occurred in 6 patients. The 1 case with intracranial IFD and 1 case with pulmonary IFD were not controlled by antifungal therapy, and the 2 patients eventually died. The other patients were successfully treated with antifungal therapy. CMV viremia occurred in 8 patients, and all patients were successfully treated with antiviral and globulin therapy.

Graft rejection/poor graft function

No patients displayed primary GR. Late GR was observed in three patients with 4-month, 20-month and 93-month follow-up (donor chimerism 23%, 82% and 68%, respectively). The 5-year probabilities of graft rejection were 5.3 ± 3.7%. Three patients showed poor graft function with 1-month, 1-month and 63-month follow-up after transplantation (donor chimerism > 97%). The 5-year cumulative incidence of PGF was 4.9 ± 3.4%, as shown in Fig. 2.

Deaths

As of August 31, 2018, 3(7.3%) patients died in the group, amongst whom median survival time was 4 months (range, 2 to 44). One patient died from myelitis with a follow-up of 2 months, one from intracranial IFD with a follow-up of 4 months, and one patient died of pulmonary IFD with a follow-up of 44 months undergoing poor graft function.

Survival

At a median follow-up of 62 months (range, 2 to 158), 38 patients were alive. The 3-year OS rate after transplantation was 95.1 ± 3.4%. The 3-year DFS rate after treatment was 85.0 ± 5.7%. The survival of patients is shown in Fig. 3. In univariate analysis of prognostic factors for overall survival, aGVHD predicted an inferior survival rate ($p < 0.05$).The

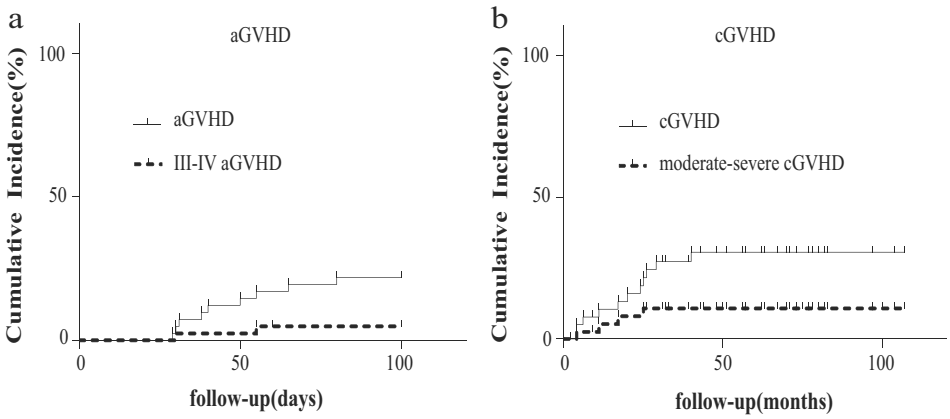

Fig. 1 Graft-versus-host disease cumulative incidence. **a** aGVHD cumulative incidence. **b** cGVHD cumulative incidence

interval from diagnosis to transplantation for more than 100 days predicted an inferior DFS rate ($p < 0.05$) and a higher GR/PGF rate ($p < 0.01$). In our study, different gender, age, donor gender and conditioning-related toxicity showed no difference in OS, DFS and GR/PGF rate (Fig. 4 and Table 4).

Discussion

Allogeneic haematopoietic stem cell transplantation is preferred as the first-line treatment option for younger SAA patients with 10/10 HLA-matched sibling donors with long-term survival over 80% [2, 3]. HSCT offers the best chance for cure, but its use is restricted by the relatively high morbidity and mortality, especially in older patients and those who lack an HLA-matched sibling donor [30]. The known optional conditioning regimen for allo-HSCT in SAA is based on the combination of cyclophosphamide and fludarabine plus ATG [31, 32]. The application of ATG improves patient prognosis after allo-HSCT. It is still controversial to identify the differences amongst mechanisms of ATG from different species. Some studies [6, 33] in vitro showed that r-ATG induced more severe and prolonged lymphopenia than h-ATG, mainly affecting the CD4+ T cell subset; absolute numbers of regulatory T cells were also lower in r-ATG-treated patients, both during and after treatment. A certain subset of CD4+ T cells may play an important role in haematopoietic recovery. Potent inhibition of CD4+ T cells by r-ATG may impede haematopoietic recovery. Interestingly, h-ATG can stimulate haematopoiesis while it inhibits immunity [15]. Ma et al. [16] evaluated absolute lymphocyte count as well as subsets of T cells until 6 months after IST in SAA patients, and r-ATG showed much stronger inhibition of lymphocytes compared with p-ALG. Although both r-ATG and p-ALG inhibited CD8+ cytotoxic T cells, r-ATG induced stronger and prolonged inhibition of CD4+ cells compared with p-ALG. However, the underlying mechanisms are still unknown. The results from other clinical centre [34] showed that the half-life of p-ALG was approximately 15 days, and an effective serum concentration of p-ALG was maintained for at least 60 days in vivo. In our study, the conditioning regimen was based on fludarabine plus cyclophosphamide with p-ALG, and no deaths during the conditioning regimen were observed. Study [35] showed that PBSCs offer advantages in terms of earlier neutrophilic engraftment and shorter hospitalization. Although bone marrow was the preferred graft source, PBSC may be an acceptable alternative in countries with limited resources when treating patients at high risk of graft

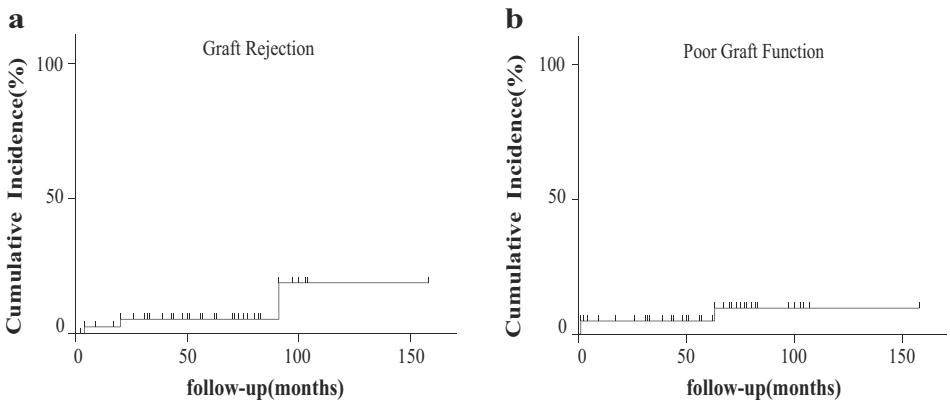

Fig. 2 Graft rejection/Poor graft function rate. **a** Graft rejection rate. **b** Poor graft function rate

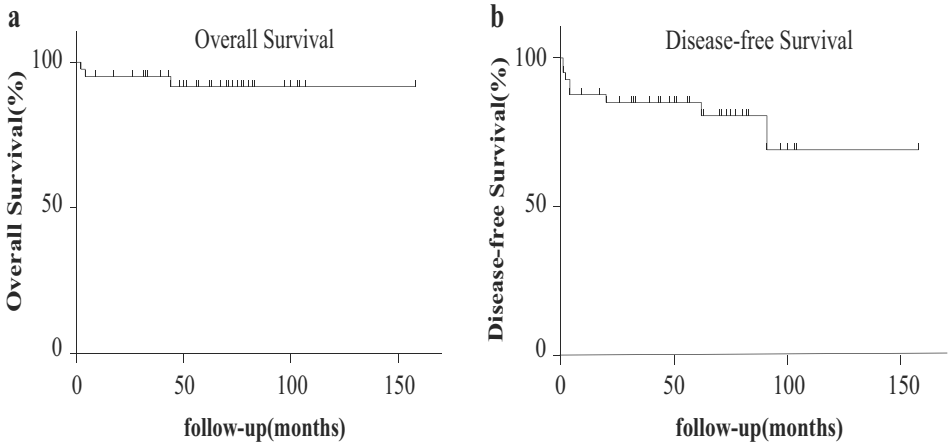

Fig. 3 Survival curve. **a** Overall survival. **b** Disease-free survival

failure or infective complications. Considering donor's willingness, risk of graft failure, earlier neutrophil recovery and shorter hospitalization, we used PBSC as graft source.

P-ALG was approved by the China Food and Drug Administration (CFDA) in 2004 and has been widely used in IST for acquired AA. Many systematic investigations [14, 15, 36, 37] of the use of IST for acquired AA have shown that p-ALG has at least comparable efficacy and safety to ATG raised in other species. P-ALG could be a suitable alternative for r-ATG with a lower medical expense. However, few studies have been performed on conditioning regimens. Data from our retrospective study showed a satisfactory survival rate after treatment using p-ALG-based regimens as well as a low incidence of severe GVHD. No patients died during the conditioning regimen. During the conditioning regimen, 48.8% of the patients developed fever, 12.2% with mild anaphylactic reaction and 43.9% with mild liver damage, and all the side effects were controlled on their own or after treatment with medication.

Anna Locasciull et al. [3] studied SAA patients receiving HSCT from HLA-matched sibling donors or alternative donors. The actuarial 10-year survival was 73%, and patients with younger age, a shorter diagnosis-transplant interval, and no irradiation had a better prognosis. In a recent study [38], SAA patients older than 30 years receiving MSD-HSCT showed a 5-year OS over 70%. Bacigalupo et al. [39] analysed SAA patients grafted in Europe between 1976 and 1998. The surviving proportions of patients grafted from an HLA-identical sibling donor were 66%. The major causes of failure were acute GVHD, infection, pneumonitis and rejection. Grade III–IV aGVHD was observed in 9% of the patients, and extensive cGVHD was observed in 10% of the patients. A recent retrospective study [34] analysed the outcomes of SAA patients in China after MSD-HSCT receiving either p-ALG or r-ATG as part of the conditioning regimen. P-ALG showed satisfactory efficacy and safety compared with r-ATG in the setting of MSD-HSCT for SAA patients. There were no significant differences between the r-ATG and p-ALG groups in terms of 3-year overall survival, grades III to IV acute GVHD, moderate to severe chronic GVHD or graft rejection. There was also no significant difference in the incidence of severe bacterial infection, invasive fungal disease or CMV viremia. Compared with previously reported results [3, 34, 38, 39], our data have indicated that p-ALG has at least a comparable or even better efficacy than ATG raised in other species.

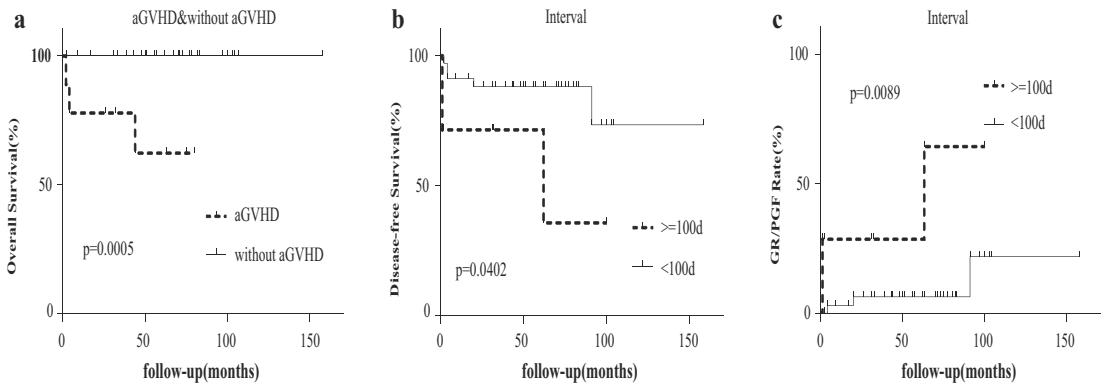

Fig. 4 Univariate analysis of prognostic factors. **a** Comparison of overall survival in relation to aGVHD. **b** Comparison of disease-free survival in relation to interval from diagnosis to HSCT. **c** Comparison of GR/PGF rate in relation to interval from diagnosis to HSCT

Table 4 Univariate analysis of prognostic factors for OS, DFS and GR/PGF rate

Factors	Subtype	*N* (%)	OS (*p* value)	DFS (*p* value)	GR/PGF rate (*p* value)
Gender	Male	21 (51.2)	0.4793	0.4453	0.0888
	Female	20 (48.8)			
Age, year	< 30	26 (63.4)	0.1515	0.2672	0.5413
	≥ 30	15 (36.6)			
Interval from diagnosis to HSCT	< 100	34 (82.9)	0.3485	0.0402	0.0089
	≥ 100	7 (17.1)			
Donor gender, days	Male	25 (61.0)	0.9899	0.8607	0.9821
	Female	16 (39.0)			
Conditioning-related toxicity	None	14 (34.1)	0.1973	0.9407	0.6047
	Existed	27 (65.9)			
aGVHD	None	32 (78.0)	0.0005	0.1146	0.9789
	Existed	9 (22.0)			
cGVHD	None	30 (73.2)	0.8703	0.2325	0.3657
	Existed	11 (26.8)			
Prior treatment	None	21 (51.2)	0.0731	0.4775	0.9163
	Existed	20 (48.8)			

GR graft rejection, *PGF* poor graft function

Graft failure is an uncommon complication post-allo-HSCT and is associated with poor outcomes [40]. Discrimination between graft rejection and poor graft function can be made by chimerism analysis. In GR, chimerism status is mixed, with the presence of recipient cells, or full recipient, whereas full donor chimerism is present in PGF. In our study, no primary graft rejection was observed, and three patients displayed secondary graft rejection. The 5-year cumulative incidence of GR was 5.3 ± 3.7%, which is consistent with the previous report [34]. Zehra et al. [41] indicated that GR incidence after HSCT ranges from 3.8 to 5.6%, and the cumulative incidence of GR was found to be significantly higher in nonmalignant disorders in comparison with malignant disorders. Poor graft function is also a life-threatening complication that occurs after HSCT in 5 to 27% of patients [42]. In our study, three patients showed poor graft function at the 1-month, 1-month and 63-month follow-up after transplantation. The 5-year cumulative incidence of PGF was 4.9 ± 3.4%. Graft failure remains a significant cause of treatment failure after allogeneic HSCT, especially for patients with an interval from diagnosis to transplantation for more than 100 days. No patients in our study showed primary graft rejection, but univariate analysis showed that these patients with an interval for more than 100 days had both inferior DFS rates ($p < 0.05$) and higher GR/PGF rates ($p < 0.01$). The 5-year DFS rate of patients who were transplanted no more than 100 days after diagnosis was 88.0%, while the 5-year DFS rate of patients who exceeded 100 days was 71.4%. The 5-year GR/PGF rate of patients with longer intervals was significantly higher (29.6% vs. 6.4%). When there is a suitable donor, we recommend SAA patients receiving the haematopoietic stem cell transplantation as soon as possible. In our study, aGVHD predicted an inferior overall survival rate ($p < 0.05$), while gender, age, side effect, cGVHD and prior treatment were not independent predictors of the overall survival rate ($p > 0.05$). It is well established that GVHD increases mortality after HSCT. The primary cause of death in patients with GVHD is infection. Faraci et al. [43] have shown that aGVHD, steroids administration, and secondary neutropenia are associated with the development of bacteremia or IFD, and both bacteremias and IFD may occur very late during the follow-up of patients receiving allogeneic HSCT, and these complications occur later in presence of severe aGVHD. Time to GVHD response might be an important predictor of outcome. Early intervention of GVHD is beneficial to the overall survival of patients.

In summary, p-ALG showed satisfactory effects and safety in our study compared with other published data using the r-ATG/h-ATG-containing conditioning regimen. P-ALG could be a potential alternative in allo-HSCT conditioning with similar effects and lower costs. Our results suggest that early intervention of GVHD and early transplantation have positive implications for patient prognosis. However, further larger-scale and prospective studies are needed to obtain more definitive conclusions.

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Compliance with ethical standards All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study.

Conflict of interest The authors declare that they have no conflict of interest.

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• 论著 •

国产猪抗人淋巴细胞免疫球蛋白在重型再生障碍性贫血异基因造血干细胞移植中的应用研究

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【摘要】 目的　探讨国产猪抗人淋巴细胞免疫球蛋白（p-ALG）在重型再生障碍性贫血（SAA）异基因造血干细胞移植（HSCT）中的疗效。**方法**　回顾性分析 2015 年 1 月至 2018 年 5 月 5 例异基因造血干细胞移植的 SAA 患者的临床资料。预处理方案为 p-ALG+环磷酰胺+氟达拉滨；异基因 HSCT 方式采用外周干细胞+骨髓血（采集骨髓血总量小于 400 ml，不更换穿刺点）。记录 p-ALG 相关并发症、移植后造血干细胞重建时间、疗效等。**结果**　发生 p-ALG 过敏反应 1 例，无血清病反应。5 例患者均获得造血重建，移植后中性粒细胞计数 > 0.5×10^9/L 时间为第 11 ~ 17 天，血小板计数 > 20×10^9/L 时间为第 11 ~ 15 天，短串重复序列聚合酶链反应检测均为完全供者嵌合体。3 例出现移植物抗宿主病，给予甲泼尼龙、他克莫司等治疗，均顺利控制。脱离输红细胞悬液时间为第 9 ~ 87 天。随访 1 ~ 37 个月，患者生活状况良好。**结论**　p-ALG 应用于 SAA 异基因 HSCT 中疗效肯定，费用明显降低，值得推广。

【关键词】 贫血，再生障碍性；　抗淋巴细胞血清；　造血干细胞移植；　治疗应用
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Application of domestic porcine antihuman lymphocyte immunoglobulin in allogeneic hemopoietic stem cell transplantation for severe aplastic anemia

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[Abstract] Objective　To investigate the efficacy of domestic porcine antihuman lymphocyte immunoglobulin (p-ALG) in the treatment of severe aplastic anemia (SAA) with allogeneic hemopoietic stem cell transplantation (HSCT). **Methods**　The clinical data of 5 SAA patients who received allogeneic HSCT from January 2015 to May 2018 were retrospectively analyzed. The conditioning regimen included p-ALG + cyclophosphamide + fludarabine. The method of peripheral stem cell and bone marrow blood was used in allogeneic HSCT (the total amount of bone marrow blood was less than 400 ml and the

puncture point was not replaced). The p-ALG related complications, post-transplantation hemopoietic stem cell reconstitution time and efficacy were recorded. **Results** Allergic reaction occurred in 1 patient when using p-ALG, and there was no serum reaction. Hemopoietic reconstitution was achieved in all the 5 patients. The time for neutrophilic granulocyte > 0.5×10^9/L was 11 to 17 d, and the time for platelet count > 20×10^9/L was 11 to 15 d after transplantation. The results of short-strand repeat polymerase chain reaction assays showed all complete donor chimera. Graft versus host disease occurred in 3 cases, and was successfully controlled by methylprednisolone and tacrolimus. The time for stopping red blood cell transfusion was 9 to 87 d. The patients were followed up for 1 to 37 months, and the patients all survived well. **Conclusions** The efficacy of p-ALG in SAA patients of allogeneic HSCT is affirmative, and the cost is obviously reduced. It is worthy of clinical use.

[Key words] Anemia, aplastic; Antilymphocyte serum; Hematopoietic stem cell transplantation; Therapeutic uses

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再生障碍性贫血（aplastic anemia，AA）是一种骨髓造血衰竭综合征，重型再生障碍性贫血（severe aplastic anemia，SAA）是 AA 中一种严重的类型，表现为严重的感染、贫血和出血，起病急，病情进展快，病死率高，以儿童及年轻患者为主要发病人群。SAA 一旦明确诊断均应尽快治疗，抗人胸腺细胞球蛋白（anti-human thymocyte globulin，ATG）/抗人淋巴细胞免疫球蛋白（antihuman lymphocyte immunoglobulin，ALG）联合环孢素的免疫抑制疗法（immunosuppressive therapy，IST）及造血干细胞移植（hemopoietic stem cell transplantation，HSCT）均为一线治疗方法，IST 治疗使 40%～60%的 SAA 患者生存率有了明显提高，HSCT 则有可能根治该病。ATG/ALG 在 IST 和 HSCT 治疗过程中作为最重要的药物之一起到了不可或缺的作用，目前国内有兔抗人胸腺细胞球蛋白（rabbit antihuman thymocyte globulin，r-ATG）和国产猪抗人淋巴细胞免疫球蛋白（porcine antihuman lymphocyte immunoglobulin，p-ALG）两种。在 p-ALG 上市后，杨楠等[1]比较了 p-ALG 和 r-ATG 在接受 IST 治疗的 SAA 患者情况，表明 p-ALG 在治疗效果、长期生存率、安全性上优于或相当于 r-ATG，不良反应较少，治疗费用明显降低，疗效肯定。因此，p-ALG 在国内非 HSCT 的 SAA 患者中应用越来越广泛。HSCT 治疗 SAA 预处理方案中 r-ATG 仍是大部分移植中心首选的药物，使用 p-ALG 的报道较少，为探讨 p-ALG 组成的预处理方案的疗效，我们将 p-ALG 应用于异基因 HSCT 的 5 例 SAA 患者，现将结果报道如下。

资料与方法

1. 一般资料：选择 2015 年 1 月至 2018 年 5 月在我科进行异基因 HSCT 的 5 例 SAA 患者，其中男 4 例，女 1 例，年龄 15～48 岁，中位年龄 21 岁。纳入标准：行血常规、骨髓细胞形态学、骨髓活检病理、染色体核型分析等检查，符合 SAA 诊断标准[2]；无 HSCT 绝对禁忌证，无严重并发症，年龄＜40 岁可行单倍体型 HSCT，年龄＞40 岁行人类白细胞抗原（HLA）全相合同胞供者 HSCT。排除标准：年龄＜13 岁的患者；年龄＞60 岁，体能评分≥2 分的患者；有活动性感染和出血患者；病史＞1 年，依赖于输血的 SAA-Ⅰ型患者；有重要脏器损害的患者；SAA-Ⅱ型患者。5 例患者均为 SAA-Ⅰ型，病程小于 1 年，病程中根据需要输注红细胞和血小板悬液。供者为同胞全相合或单倍体型，供受者的 A、B、C、DR、DQ 位点均进行高分辨分子生物学配型，其中 5/10 相合 1 例，6/10 相合 1 例，8/10 相合 1 例，10/10 相合 2 例。血型相合 3 例，血型主要不合或次要不合各 1 例。患者异基因 HSCT 前均使用环孢素治疗。供者干细胞动员、采集及移植后并发症的预防均采用我科传统方案[3]。移植前 5 例患者有不同程度的贫血，皮肤出血或者口腔、牙龈出血；2 例患者出现感染，其中 1 例患者出现反复严重感染，胸部 CT 提示真菌感染可能。经过抗细菌、抗真菌等治疗后活动性感染及出血控制；1 例患者上腹部不适。本研究已通过我院伦理委员会审核（批准号：2015/v1.6）。

2. 预处理方案和异基因 HSCT 方式：预处理方案采用 p-ALG（武汉生物制品研究所）+环磷酰胺+氟达拉滨方案，p-ALG 25 mg/（kg·d）连用 5 d（−5～−1 d），环磷酰胺 50 mg/（kg·d）连用 4 d（−5～−2 d），氟达拉滨 25～30 mg/（m^2·d）连用 4 d（−8～−5 d）。p-ALG 使用时给予甲泼尼龙 1～2 mg/kg 同时输注，维持 8 h 以上。1 例患者加用总量 2 Gy 的全身放疗方案（TBI）。异基因 HSCT 方式采用外周干

细胞 + 骨髓血（采集骨髓血总量小于 400 ml，不更换穿刺点）。

3. 疗效评价及植活证据检测[3]：HSCT 后每天或隔天检测血常规，中性粒细胞计数（ANC）> 0.5 × 10^9/L，连续 3 d，血小板计数 > 20 × 10^9/L，连续 7 d 不输注血小板，作为造血功能重建的标准。移植后 1、3、6、12 个月通过短串重复序列聚合酶链反应（STR-PCR）判断植入情况，STR-PCR 检测细胞在 95.0% ~ 100.0% 供者来源时表示完全供者嵌合体，患者体内造血完全是供者来源；供者细胞 ≥ 2.5% 但 < 95.0% 为混合嵌合体，患者体内造血既有供者来源又有患者来源；供者细胞 < 2.5% 为完全患者造血来源细胞，供者细胞被排斥或移植失败。血型不合者造血重建后每周进行血型分析至转为供者血型代表植入成功。

结　果

1. 异基因 HSCT 过程中患者情况：5 例患者预处理期间使用 p-ALG 安全性均较好，并发症可控，4 例出现轻度胃肠道反应；1 例女性患者出现较重度胃肠道反应，可能与联用其他预处理药物及胃肠道本身病变有关。1 例患者在使用 p-ALG 第 1 天时出现发热，畏寒反应，减慢输液速度，加强抗过敏治疗后好转；其余 4 例未发生过敏反应。5 例患者在异基因 HSCT 期间均未发生严重感染，无重要脏器功能严重异常，未发生血清病反应。

2. 疗效：5 例患者全部获得造血重建，干细胞植入顺利，STR-PCR 检测均为完全供者嵌合体。3 例患者移植后出现移植物抗宿主病（GVHD），给予甲泼尼龙、他克莫司等治疗，均顺利控制。随访至 2018 年 6 月，患者随访 1 ~ 37 个月，生活状况均良好。患者具体情况见表 1。

讨　论

SAA 患者的发病年龄多数 < 30 岁，对年龄 ≤ 35 岁、无活动性感染和出血患者治疗首选 HLA 相合同胞供者 HSCT[4-5]。对于年龄较大 HLA 配型不相合的患者，采用 ATG/ALG 联合环孢素的 IST 治疗[6]。SAA 病情非常凶险，骨髓呈重度造血衰竭，血常规表现为严重粒细胞缺乏及血小板减少，如采取的治疗方法不能快速起效，患者可能在明确诊断后较短时间内死于感染或出血等并发症[7]。我国独生子女较多，非血缘供者寻找困难，随着对疾病逐渐深入的研究及移植体系的不断完善，亲缘间单倍体 HSCT 治疗 SAA 越来越多，其成为挽救患者生命的一种重要方法[8-9]。HSCT 前患者评估较为重要，本研究的 5 例患者 HSCT 前均没有活动性出血，各脏器功能较好，虽然 1 例患者出现反复严重肺部感染，但是经过积极治疗感染已得到控制，体温正常。相对良好的身体状态决定了选择单倍体 HSCT 作为治疗手段，并且预后较好[10]。5 例患者中 3 例为单倍体移植，分别为父供子、母供子、姐供弟，平均年龄 18 岁，年轻患者更容易使干细胞植入，移植后 GVHD 等并发症的发生率更低，单倍体移植的 HLA 相合程度并不影响移植后干细胞植入，经过及时有效的监测及预防，可减轻 GVHD 的发生率及严重程度。本研究移植方式采集的骨髓血，对比传统采髓方法，采集量、所含单核细胞和 CD_{34}^+ 数量均较少，患者造血均成功恢复，表明 SAA 发病早期免疫及种子机制更加重要，土壤机制所占比例较低，患者的造血微环境尚未被严重损伤，我们采用了更少的骨髓血干细胞，并没有影响患者造血的恢复。

SAA 是由于各种原因引起患者体内 T 淋巴细胞异常活化，导致造血负调控因子大量释放，靶向攻击造血干细胞，破坏骨髓正常造血功能，表现为骨髓造血功能衰竭，外周血三系减少，患者不可避免地发生感染、贫血和出血[11-12]。针对发病机制，抑制功能异常的 T 淋巴细胞，恢复正常的骨髓造血功能成为治疗 SAA 的有效方案。ATG/ALG 是对异常活化的 T 淋巴细胞有清除作用，能抑制其功能亢进，调节患者免疫功能的多克隆抗淋巴细胞血清，主要有兔、马和猪三种种属来源。在比较不同种属来源 ATG/ALG 时，Feng 等[13]研究结果表明，马抗人胸腺细胞球蛋白（h-ATG）作为 SAA 的主要治疗方

表 1　5 例重型再生障碍性贫血患者行异基因造血干细胞移植治疗结果

患者	单核细胞计数（× 10^8/kg 体质量）	CD_{34}^+（× 10^6/kg 体质量）	中性粒细胞计数 > 0.5 × 10^9/L 时间	血小板计数 > 20 × 10^9/L 时间	移植物抗宿主病	肝静脉闭塞病	出血性膀胱炎	脱离输注红细胞悬液时间
1	9.9	2.10	第 11 天	第 15 天	Ⅲ级（皮肤）	无	无	第 87 天
2	11.0	2.87	第 17 天	第 11 天	无	无	无	第 9 天
3	6.3	4.97	第 11 天	第 10 天	无	无	无	第 17 天
4	12.9	5.69	第 13 天	第 13 天	Ⅱ级（皮肤）	无	无	第 13 天
5	9.1	9.39	第 11 天	第 11 天	Ⅰ级（肠道）	无	无	第 15 天

法优于 r-ATG。与 h-ATG 相比，血浆中可检测到 r-ATG 的时间更长，保留了与淋巴细胞结合长达 1 个月的能力，h-ATG 仅持续约 2 周。血清病与血液中 ATG 的清除和细胞因子的产生同时发生。这些差异可能与它们抑制免疫系统和恢复骨髓衰竭造血功效有关。Chen 等[14]回顾性分析了行 p-ALG 联合环孢素治疗的 102 例 SAA 患者的临床资料，中位年龄 29（12～72）岁，中位随访时间 59.6（0.2～176.8）个月，总应答率为 74.5%（完全缓解率为 42.1%，部分缓解率为 32.4%），5 年生存率为 81.8%。表明 p-ALG 治疗 SAA 长期疗效显著，总体存活率较高。所需费用较 r-ATG 明显降低，为性价比较高的 IST 主要药物之一，使得 p-ALG 越来越多地应用于临床，给无法承担高额治疗费用的患者多了一种选择方法。ATG/ALG 为异种球蛋白，马秀慧等[15]报道患者使用时过敏或血清病反应的发生率在 50%左右，两种药物比较，血清病反应无明显差别，r-ATG 较 p-ALG 更易引起过敏反应。本研究 5 例患者使用 p-ALG 前均常规给予甲泼尼龙 1～2 mg/kg 预防药物不良反应，其中有 1 例出现发热和畏寒反应，减慢输液速度，增加糖皮质激素用量和延长给药时间后症状控制，未出现血清病反应，移植期间未出现不可控制的感染。既往认为 r-ATG 有比 p-ALG 更强的免疫抑制作用，患者移植后 GVHD 发病率更低，但我们认为，更持久的免疫抑制带来更高的感染发生率，骨髓造血恢复时间延长，适当的免疫控制，更完善的预处理体系能更精准地治疗 SAA。

国产 p-ALG 用于 SAA 异基因 HSCT 的研究较少，我们把国产 p-ALG 应用于 SAA 患者的异基因 HSCT，利用 p-ALG 治疗 SAA 的优势，优化移植体系，使 5 例 SAA 患者获得治愈可能。5 例患者粒系和血小板植入的中位时间均为第 11 天，脱离输注红细胞悬液中位时间为第 15 天，治疗效果明显优于 IST，并且患者均对 p-ALG 耐受良好，无药物相关死亡发生，p-ALG 替代 r-ATG 的移植体系疗效较为满意，我们将扩大病例数，继续完善治疗方法，以期能推广应用，给患者带来更大的获益。

利益冲突　所有作者均声明不存在利益冲突

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·论著·

猪抗人淋巴细胞免疫球蛋白在替代供者移植治疗重型再生障碍性贫血患者中的疗效及安全性

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【摘要】 目的　比较预处理方案含猪抗人淋巴细胞免疫球蛋白(pALG)或兔抗人胸腺细胞免疫球蛋白(rATG)替代供者异基因造血干细胞移植(AD allo-HSCT)治疗重型再生障碍性贫血(SAA)患者的疗效及安全性。**方法**　回顾性分析2006年1月至2016年11月46例接受AD allo-HSCT SAA患者的临床资料，按预处理方案包含rATG或pALG分为两组，比较两组植入率、移植相关并发症发生率及转归。**结果**　rATG组30例患者均获得粒细胞植入，27例患者获得血小板植入。pALG组16例患者粒细胞及血小板均植入。两组患者在移植后急性移植物抗宿主病(aGVHD)($P=0.475$)、Ⅲ～Ⅳ度aGVHD($P=0.876$)、慢性移植物抗宿主病(cGVHD)($P=0.309$)、广泛型cGVHD($P=0.687$)、移植物排斥(GR)($P=0.928$)、血流感染($P=0.443$)、侵袭性真菌病($P=0.829$)、巨细胞病毒血症($P=0.095$)发生率方面差异均无统计学意义。rATG组中位随访14(2～102)个月，预期5年总生存率为(75.1±8.2)%；pALG组中位随访23(4～63)个月，预期5年总生存率为(53.6±13.3)%，差异无统计学意义($P=0.190$)。**结论**　SAA患者行AD allo-HSCT，预处理方案应用pALG可取得与rATG相近疗效，且并不增加GVHD、GR及感染等移植后并发症的发生率。

【关键词】 贫血，再生障碍性，重型；　造血干细胞移植；　替代供者；　猪抗人淋巴细胞免疫球蛋白

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Clinical efficacy and safety of porcine antihuman lymphocyte immunoglobulin in alternative donor allogeneic hematopoietic cell transplantation for severe aplastic anemia

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【Abstract】 Objective　To compare eficacy and safety of porcine antihuman lymphocyte immunoglobulin (pALG) and rabbit antithymocyte immunoglobulin (rATG) as a part of alternative donor allogeneic hematopoietic stem cell transplantation (AD allo-HSCT) for severe aplastic anemia (SAA). **Methods**　The clinical data of 46 SAA patients received AD allo-HSCT from January 2006 to November 2016 were retrospectively analyzed. The cohort of patients were divided into two groups based on rATG or pALG as a part of conditioning regimen to compare implantation rate, transplantation related complications and outcome. **Results**　In rATG group 30 patients achieved ANC reconstitution, 27 patients achieved PLT reconstitution. In pALG group all 16 patients achieved ANC and PLT reconstitutions. There were no significant differences between the two groups in terms of acute graft-versus-host disease (aGVHD) ($P=0.475$), Ⅲ-Ⅳ grade aGVHD ($P=0.876$), chronic GVHD (cGVHD) ($P=0.309$), extensive cGVHD ($P=0.687$), graft rejection (GR) ($P=0.928$), bloodstream infection ($P=0.443$), invasive fungal disease

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(P = 0.829), cytomegalovirus viremia (P = 0.095) respectively. Prospective 5-year overall survival (OS) in rATG and pALG groups were (75.1±8.2)% and (53.6±13.3)% with median follow-up of 14(2−102) and 23 (4−63) months, respectively (P = 0.190). **Conclusion** As a part of conditioning regimen, pALG could achieve similar efficacy as rATG, without increasing the incidences of transplantation complications such as GVHD, GR and infection, in the setting of AD allo-HSCT for SAA patients.

【**Key words**】 Anemia, aplastic, severe; Hematopoietic stem cell transplantation; Donor, alternative; Porcine antihuman lymphocyte immunoglobulin

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重型再生障碍性贫血(SAA)是严重危及患者生命的骨髓衰竭性疾病,其主要治疗手段为免疫抑制治疗(IST)及异基因造血干细胞移植(allo-HSCT)。抗胸腺/淋巴细胞免疫球蛋白(ATG/ALG)在IST及allo-HSCT中均占有重要地位。猪源ALG(pALG)联合环孢素A(CsA)治疗SAA的疗效已得到肯定,可替代兔源ATG(rATG)进行IST[1-2]。但目前pALG应用于allo-HSCT,尤其是在替代供者(无关供者、脐带血、单倍体相合供者)异基因造血干细胞移植(AD allo-HSCT)治疗SAA患者中疗效的研究甚少。因此,我们比较了应用pALG与rATG作为预处理方案进行AD allo-HSCT治疗SAA患者疗效的差异,现报道如下。

病例与方法

1. 病例:回顾性分析2006年1月至2016年11月中国医学科学院血液病医院造血干细胞移植中心进行AD allo-HSCT的46例SAA患者临床资料,为连续性资料,按预处理方案含rATG或pALG分为两组。其中rATG组患者30例,均无既往rATG/pALG应用史,pALG组患者16例,6例患者有rATG治疗无效史(P = 0.001),两组移植前其余临床特征差异无统计学意义(表1)。

2. 供者来源及移植情况:两组患者均接受以环磷酰胺(Cy)+rATG/pALG+氟达拉滨(Flu)为基础预处理方案:Cy 50 mg·kg^{-1}·d^{-1},−4~−2 d;rATG(美国Genzyme公司产品)2.5 mg·kg^{-1}·d^{-1},−9~−5 d或pALG(武汉生物制品研究所产品)20 mg·kg^{-1}·d^{-1},−9~−5 d,Flu 30 mg·m^{-2}·d^{-1},−9~−5 d;11例患者(rATG组5例,pALG组6例)联合白消安(Bu)3.2 mg·kg^{-1}·d^{-1},−9~−8 d。两组患者供者来源、移植方式、移植物抗宿主病(GVHD)预防方案及移植物输注量见表2,供受者特征及移植情况差异无统计学意义。按我科常规方案采集供者骨髓,动员和采集供者外周血造血干细胞[3]。

3. 支持治疗:所有移植均在百级层流病房进行,移植前予复方磺胺甲恶唑1.0 g每日2次连用7 d预防卡氏肺孢菌肺部感染,予更昔洛韦10 mg·kg^{-1}·d^{-1}静脉滴注7 d预防巨细胞病毒(CMV)感染;对于移植前无真菌感染患者,移植后给予氟康唑预防真菌感染。

4. 疗效评定:移植后ANC连续3 d > 0.5×10^9/L时的首日为粒细胞植入时间,脱离血小板输注的情况下PLT > 20×10^9/L连续7 d的首日为血小板植入时间。以DNA可变数目串联重复序列(VNTR)、短串联重复序列(STR)、性染色体荧光原位杂交、ABO血型检测等判定植入情况。

5. 随访:随访截至2016年12月30日,30例

表1 预处理方案含rATG与含pALG重型再生障碍性贫血(SAA)患者替代供者异基因造血干细胞移植前临床特征比较

组别	例数	性别(例,男/女)	年龄[岁,M(范围)]	儿童[例(%)]	诊断至移植时间[d,M(范围)]	疾病亚型[例(%)]			VSAA[例(%)]	移植前感染[例(%)]		
						SAA-Ⅰ	SAA-Ⅱ	HAAA		血流感染	其他严重细菌感染	IFD
rATG组	30	16/14	11(3~42)	14(46.7)	86(30~8 577)	21(70.0)	5(16.7)	4(13.3)	18(60.0)	2(6.7)	2(6.7)	9(30.0)[a]
pALG组	16	10/6	16(6~37)	8(50.0)	196(30~1 800)	8(50.0)	4(25.0)	4(25.0)	8(50.0)	2(12.5)	0(0)	6(37.5)[b]
统计量		0.357	−0.727	0.046	−1.233		2.007		0.425		1.297	
P值		0.550	0.467	0.829	0.217		0.447		0.515		0.626	

注:rATG:兔抗人胸腺细胞免疫球蛋白;pALG:猪抗人淋巴细胞免疫球蛋白;儿童:年龄<14岁;HAAA:肝炎相关性再生障碍性贫血;VSAA:极重型再生障碍性贫血;IFD:侵袭性真菌病。[a]1例患者移植前IFD未控制;[b]2例患者移植前IFD未控制

表2 预处理方案含rATG与含pALG重型再生障碍性贫血(SAA)患者替代供者异基因造血干细胞移植情况比较

移植情况	rATG组(30例)	pALG组(16例)	统计量	*P*值
供者来源[例(%)]			1.850	0.174
无关供者	6(20.0)	7(43.7)		
亲缘半相合供者	24(80.0)	9(56.3)		
供受者HLA匹配程度[例(%)]			8.997	0.109
全相合	5(16.7)	5(31.2)		
1个位点不合	5(16.7)	6(37.5)		
2个位点不合	3(10.0)	3(18.8)		
3个位点不合	5(16.7)	1(6.3)		
4个位点不合	5(16.7)	1(6.3)		
5个位点不合	7(23.3)	0(0)		
供受者性别[例(%)]			0.289	0.865
相同	12(40.0)	6(37.5)		
男供女	9(30.0)	6(37.5)		
女供男	9(30.0)	4(25.0)		
供受者血型[例(%)]			2.387	0.496
相合	16(53.3)	9(56.3)		
主要不合	5(16.7)	1(6.3)		
次要不合	7(23.3)	3(18.8)		
主次均不合	2(6.7)	3(18.8)		
GVHD预防[例(%)]			0.003	0.956
CsA+MTX+MMF	19(63.3)	10(62.5)		
FK506+MTX+MMF	11(36.7)	6(37.5)		
干细胞类型[例(%)]			0.545	0.761
骨髓	4(13.3)	3(18.8)		
外周血	20(66.7)	11(68.7)		
骨髓+外周血	6(20.0)	2(12.5)		
NC/MNC输注量[$\times10^8$/kg, *M*(范围)]			−1.802	0.072
骨髓	4.78(3.65～6.12)	7.56(5.94～8.11)		
外周血	8.00(5.17～16.00)	7.00(3.81～8.07)		
骨髓+外周血	7.50(6.79～9.74)	5.62(5.08～6.16)		
$CD34^+$细胞输注量[$\times10^6$/kg, *M*(范围)]			−0.542	0.588
骨髓	3.18(2.20～4.38)	3.12(2.11～3.37)		
外周血	3.35(1.87～8.32)	3.59(2.02～6.70)		
骨髓+外周血	4.38(3.37～5.25)	3.84(3.59～4.08)		

注:rATG:兔抗人胸腺细胞免疫球蛋白;pALG:猪抗人淋巴细胞免疫球蛋白;GVHD:移植物抗宿主病;CsA:环孢素A;MTX:甲氨蝶呤;MMF:霉酚酸酯;FK506:他克莫司;NC:有核细胞;MNC:单个核细胞

rATG组患者中位随访14(2～102)个月,16例pALG组患者中位随访23(4～63)个月。无失访病例。主要观察两组患者植入率、移植相关并发症的发生率、总生存(OS)率等指标。OS定义为移植当天至患者死亡或随访截止的时间。

6. 统计学处理:采用SPSS 16.0软件进行统计学分析。连续变量以中位数(范围)进行表示,分类变量以例数(构成比)进行表示。采用Kaplan-Meier法进行生存分析,组间比较采用Log-rank检验。两组间各因素采用卡方检验或秩和检验进行比较。$P<0.05$为差异有统计学意义。

结　果

一、造血重建

rATG组全部30例患者均获得粒细胞植入,中位植入时间为移植后14(10～23)d;27例移植后早

期获得血小板植入，中位植入时间为移植后15(10～26)d。3例患者移植后3个月内血小板未获重建，其中1例患者移植后45 d输注冻存供者外周血及骨髓干细胞，于移植后5个月脱离血小板输注，另2例始终未脱离血小板输注，1例死于肺部感染，1例死于Ⅳ度急性GVHD(aGVHD)。pALG组全部16例患者粒细胞及血小板均获得植入。粒细胞中位植入时间为移植后15(11～24)d，血小板中位植入时间为移植后19(9～39)d。两组患者粒细胞(P=0.113)及血小板(P=0.394)植入率差异均无统计学意义。

二、移植相关并发症

1. GVHD：rATG组30例患者中20例发生aGVHD，发生率为(66.7±8.6)%，其中5例发生Ⅲ～Ⅳ度aGVHD，发生率为(16.7±6.8)%。给予甲泼尼龙、MMF、英夫利昔单抗、抗CD25单抗和(或)间充质干细胞等治疗，1例患者死于Ⅳ度肝脏合并肠道aGVHD，其余患者aGVHD均得到控制。8例患者发生慢性GVHD(cGVHD)，发生率为(30.2±9.0)%，其中5例发生广泛型cGVHD，发生率为(19.1±7.7)%。予甲泼尼龙、MMF、Cy和(或)西罗莫司等治疗，cGVHD症状均得到控制。

16例pALG组患者移植后，12例发生aGVHD，发生率为(75.0±10.8)%，其中3例发生Ⅲ～Ⅳ度aGVHD，发生率为(18.8±9.8)%。给予甲泼尼龙、MMF、英夫利昔单抗、抗CD25单抗和(或)间充质干细胞等治疗，1例患者死于Ⅳ度肝脏合并肠道aGVHD，其余患者aGVHD均得到控制。6例患者发生cGVHD，发生率为(49.4±14.8)%，其中3例发生广泛型cGVHD，发生率为(22.7±11.7)%。予甲泼尼龙、MMF、Cy和(或)西罗莫司等治疗，1例死于广泛型cGVHD，其余患者cGVHD症状均得到控制。

rATG及pALG两组患者aGVHD(P=0.475)、Ⅲ～Ⅳ度aGVHD(P=0.876)、cGVHD(P=0.309)、广泛型cGVHD(P=0.687)发生率差异均无统计学意义。

2. 移植物排斥(GR)：30例rATG组患者移植后2例发生GR，发生率为(6.7±4.6)%，中位发生时间为移植后35(33～37)d，给予供者骨髓或外周血干细胞输注后，1例获得造血恢复，1例死于颅内出血。16例pALG患者移植后，1例于移植后61 d发生GR，发生率为(6.2±6.1)%，给予供者外周血干细胞输注后获得造血恢复。两组患者GR发生率差异无统计学意义(P=0.928)。

3. 移植后感染：rATG组患者移植后5例发生血流感染，大肠埃希菌3例，肺炎克雷伯菌、铜绿假单胞菌各1例，予抗细菌治疗后均治愈。移植前2例其他严重细菌感染患者(阑尾炎、面颊蜂窝组织炎各1例)，移植后未再发生原部位感染；16例患者移植后发生侵袭性真菌病(IFD)，其中7例为移植前IFD的延续；新发IFD 9例，其中肺IFD 8例，肝脏IFD 1例，予抗真菌治疗后，3例肺IFD患者治疗无效死亡，其余患者感染得到控制。17例患者发生CMV血症，抗病毒治疗后CMV均转阴。

pALG组患者移植后5例发生血流感染，肺炎克雷伯菌、铜绿假单胞菌、豚鼠气单胞菌、玫瑰色库克菌、人葡萄球菌各1例，除1例豚鼠气单胞菌感染患者最终死于感染性休克，其余患者均治愈。8例患者发生IFD，其中3例为移植前IFD的延续；新发IFD 5例，均为肺IFD，予抗真菌治疗后，2例肺IFD患者治疗无效死亡，其余患者感染得到控制。13例患者发生CMV血症，予抗病毒治疗后12例CMV转阴，1例死于CMV肺炎。

两组患者血流感染(P=0.443)、IFD(P=0.829)、CMV血症(P=0.095)的发生率差异均无统计学意义。

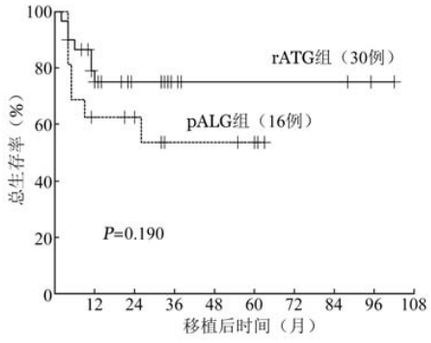
4. 移植相关死亡：rATG组患者7例死亡，死因分别为肺IFD 3例，麻疹性肺炎、暴发性肝炎、Ⅳ度aGVHD、GR后合并颅内出血各1例。pALG组患者7例死亡，死因分别为肺IFD 2例，卡氏肺孢菌肺部感染、CMV肺炎、豚鼠气单胞菌感染性休克、Ⅳ度aGVHD、广泛型cGVHD各1例。6例IST无效后的移植患者中，1例患者死于广泛型cGVHD，其余5例移植后均存活。

三、转归

30例rATG组患者移植后23例存活，预期5年OS率为(75.1±8.2)%，16例pALG组患者移植后9例存活，预期5年OS率为(53.6±13.3)%，差异无统计学意义(P=0.190)(图1)。

讨　论

HLA同胞全相合移植治疗SAA疗效显著，尤其在年轻患者中OS率可高达90%[4-5]，但仅有30%的患者有合适的供者。随着分子生物学配型技术的发展、预处理方案和支持治疗的改善，AD allo-HSCT的疗效也得到显著提高[6-9]。Xu等[10]进行了一项多中心研究，分析2012年6月至2015年



rATG：兔抗人胸腺细胞免疫球蛋白；pALG：猪抗人淋巴细胞免疫球蛋白

图1 预处理方案含rATG与含pALG重型再生障碍性贫血患者替代供者异基因造血干细胞移植后生存分析

9月移植治疗的158例获得性SAA患者疗效，其中89例患者行单倍体相合供者（HID）移植，69例行HLA匹配同胞供者（MRD）移植，结果示髓系植入时间HID组为12（9～20）d，MRD组为11（8～19）d，植入率分别为97.8%和97.1%（$P=0.528$）；HID组患者Ⅲ～Ⅳ度aGVHD发生率为10.1%，明显高于MRD组的1.5%（$P=0.026$），但两组患者广泛型cGVHD发生率差异无统计学意义（3.4%对0，$P=0.426$），3年OS率差异亦无统计学意义（86.1%对91.3%，$P=0.358$）。Choi等[11]研究显示，AD allo-HSCT组患儿无病生存率显著高于IST组，且首次进行移植患儿的无病生存率也显著高于IST无效后进行挽救移植治疗的患儿。Kang等[12]进行了一项Ⅱ期前瞻性临床研究，应用Cy 60 mg·kg^{-1}·d^{-1}、−8～−7 d，Flu 40 mg·m^{-2}·d^{-1}、−6～−2 d联合马源ATG 2.5 mg·kg^{-1}·d^{-1}、−3～−1 d的预处理方案对SAA患者行MSD-HSCT，相比于Cy 50 mg·kg^{-1}·d^{-1}，−9～−6 d，Flu 40 mg·m^{-2}·d^{-1}、−5～−2 d联合马源ATG 2.5 mg·kg^{-1}·d^{-1}、−3～−1 d的预处理方案，移植后OS率明显提高（96.7%对67.9%，$P=0.004$）。本组46例无HLA匹配同胞供者的SAA患者，虽部分患者移植年份较早，支持治疗尚不够完善，移植前部分患者存在活动性感染，部分患者移植前有IST无效史，HLA相合位点数偏低，但以Cy 50 mg·kg^{-1}·d^{-1}，−4～−2 d，Flu 30 mg·m^{-2}·d^{-1}，−9～−5 d联合rATG 2.5 mg·kg^{-1}·d^{-1}，−9～−5 d或pALG 20 mg·kg^{-1}·d^{-1}，−9～−5 d为基础预处理方案行AD allo-HSCT，结果仍显示，两组患者粒细胞均植入，血小板植入率分别为（90.0±5.5）%及100.0%；Ⅲ～Ⅳ度aGVHD的发生率分别为（16.7±6.8）%及（18.8±9.8）%；广泛型cGVHD发生率分别为（19.1±7.7）%及（22.7±11.7）%，5年OS率分别为（75.1±8.2）%及（53.6±13.3）%。因此，在无HLA匹配同胞供者的年轻SAA患者中，AD allo-HSCT不失为一种有效的替代治疗。

rATG在SAA患者allo-HSCT预处理中的疗效已得到肯定[13-15]。pALG是我国自主研发的一种新型免疫抑制剂，相关研究显示pALG联合CsA治疗SAA患者疗效显著[1-2,16-17]。而近期也有研究者应用pALG替代rATG作为AD allo-HSCT的预处理方案治疗SAA患者取得不错的疗效。Zhang等[18]报道了8例SAA患者进行单倍体相合移植，应用Cy 50 mg·kg^{-1}·d^{-1}，−5～−2 d，Flu 25～30 mg·m^{-2}·d^{-1}，−8～−5 d，pALG 25 mg·kg^{-1}·d^{-1}、−5～−1 d，2例患者此基础上还加用全身照射作为预处理方案，结果显示，粒细胞中位植入时间为14.8 d，血小板中位植入时间为15 d；7例患者发生aGVHD，其中Ⅰ～Ⅱ度5例，Ⅲ度、Ⅳ度各1例；2例患者发生cGVHD，局限型及广泛型各1例；中位随访8.5（2～18）个月，无一例患者死亡。本组患者rATG组与pALG组粒细胞植入率（$P=0.113$）、血小板植入率（$P=0.394$）差异无统计学意义。两组患者GVHD、GR及感染等移植相关并发症发生率差异均无统计学意义，预期5年OS率[（75.1±8.2）%对（53.6±13.3）%，$P=0.190$]差异亦无统计学意义。pALG组患者OS率低于rATG组患者，考虑与本组16例pALG组患者移植前6例IST无效有关。Kobayashi等[19]研究了66例儿童SAA患者骨髓移植疗效，多因素分析发现，移植前曾接受rATG联合CsA治疗是GR的独立危险因素（$RR=16.6$，95%CI 1.9～146.7，$P=0.001$），影响患儿的无病生存[（69.7±8.0）%对（87.9±6.2）%，$P=0.044$]。

本研究结果显示，SAA患者行AD allo-HSCT，预处理方案应用pALG可取得与rATG近似疗效，且并不增加GVHD、GR及感染等移植相关并发症的发生率。但由于pALG组患者例数偏少，有待进一步增加病例验证其疗效。

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Effect of Antithymocyte Globulin Source on Outcomes of HLA-Matched Sibling Allogeneic Hematopoietic Stem Cell Transplantation for Patients with Severe Aplastic Anemia

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A B S T R A C T

We wanted to evaluate efficacy of porcine antithymocyte globulin (ATG) in HLA-matched sibling donor allogeneic hematopoietic stem cell transplantation (MSD-HSCT) for patients with severe aplastic anemia (SAA). The clinical data of 113 SAA patients who received MSD-HSCT from January 2005 to November 2016 were analyzed retrospectively. Of these, 58 patients received rabbit ATG as a part of conditioning regimen (R-ATG group), whereas the other 55 patients received porcine ATG (P-ATG group). Patient baseline characteristics and donor conditions of the 2 groups were similar, except patients were older and more received peripheral blood stem cell transplantation in the P-ATG group. All patients engrafted in 2 groups. There were significant differences in the incidence of acute (20.7% ± 5.3% versus 43.4% ± 7.0%, $P = .015$) and chronic graft-versus- host disease (GVHD; 20.1% ± 5.8% versus 46.0% ± 7.9%, $P = .003$) between the R-ATG and P-ATG groups. However, there were no significant differences in terms of 3-year overall survival (93.1% ± 3.3% versus 84.4% ± 5.7%, $P = .235$), grades III to IV acute GVHD (3.4% ± 2.4% versus 12.3% ± 4.7%, $P = .098$), moderate to severe chronic GVHD (12.6% ± 4.9% versus 11.5% ± 4.9%, $P = .905$), or graft rejection (7.4% ± 3.6% versus 5.5% ± 3.1%, $P = .852$). There was also no significant difference with regard to the incidence of severe bacterial infection ($P = .075$), invasive fungal disease ($P = .701$), or cytomeglovirus viremia ($P = .770$). P-ATG showed satisfactory efficacy and safety compared with R-ATG in the setting of MSD-HSCT for SAA patients. P-ATG could be a potential alternative preparation for R-ATG, further offering the advantage of lower costs.

INTRODUCTION

Severe aplastic anemia (SAA) is a rare disorder leading to bone marrow (BM) failure that if untreated is invariably fatal [1]. The current standard therapy includes allogeneic hematopoietic stem cell transplantation (HSCT) or immunosuppressive therapy with antithymocyte globulin (ATG) and cyclosporine [2]. HSCT for SAA from an HLA-identical sibling donor has improved considerably over the years, with a 75% to 80% chance of long-term cure. Therefore, it is recommended as first-line treatment if the disease is severe or very severe and if the patient is younger than age 50 years [3].

In patients with SAA the conditioning regimens vary among different centers: cyclophosphamide (CY) alone, CY and ATG, and CY plus fludarabine (Flu) and ATG. The addition of ATG (usually rabbit ATG [R-ATG]) to the conditioning regimen improves engraftment and reduces the risk of graft-versus-host disease (GVHD). ATG improves engraftment by killing recipient lymphocytes that mediate graft rejection (GR) and may also remain in the circulation at the time of the transplant, killing alloreactive donor T cells that mediate GVHD [4-12]. Porcine ATG (P-ATG) is another preparation that is available in China for clinical use as an immunosuppressive agent. Both drugs have been used in our center as allogeneic HSCT conditioning agents for patients with SAA. Thus, we conducted a retrospective study to determine the effect(s) of the 2 ATG preparations in the setting of SAA patients receiving allogeneic HSCT.

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METHODS

Patients

We conducted a retrospective analysis of the clinical data of 113 SAA patients who received HLA-matched sibling donor allogeneic HSCT (MSD-HSCT) from January 2005 to November 2016 in our transplantation center. As a part of the conditioning regimen, 58 patients received R-ATG (R-ATG group) and 55 patients who failed R-ATG before or had a poor economic condition received P-ATG (P-ATG group). The study was approved by the ethics committees of the Institute of Hematology, Chinese Academy of Medical Science & Peking Union Medical College according to the guidelines of the Declaration of Helsinki.

In the R-ATG group 33 patients were male and 25 female, with a median age of 21 years (range, 4 to 49). The median time from diagnosis to HSCT was 61 days (range, 23 to 6935). Of the 58 patients, 29 cases were categorized as very SAA, 4 cases were identified as hepatitis-associated aplastic anemia, and 1 patient failed ATG before HSCT. In the P-ATG group 28 patients were male and 27 female, with a median age of 25 years (range, 7 to 46). The median time from diagnosis to HSCT was 86 days (range, 23 to 2920). Of the 55 patients, 29 cases were categorized as very SAA, 2 cases were identified as hepatitis-associated aplastic anemia, and 2 patients failed ATG before HSCT.

Some patients experienced infectious events before HSCT. In the R-ATG group 3 and 9 patients developed bacteremia and invasive fungal disease (IFD), respectively. Bacteremia had not been controlled in 2 cases before HSCT. In the P-ATG group 4 and 12 patients developed bacteremia and IFD pretransplant, respectively. Pulmonary IFD had not been clinically controlled in 2 cases before HSCT.

Donor

All 113 patients received HLA-A, -B, -C, -DR, and -DQ matched MSD-HSCT. Also, 40 patients in the R-ATG group and 37 patients in the P-ATG group had the same RBC type as their donors.

Conditioning Regimen

All patients received conditioning based on Flu 30 mg/m^2/day × 5 days, CY 50 mg/kg/day × 3 days, R-ATG (2 to 2.5 mg/kg/day × 4 to 5 days), or P-ATG (20 to 25 mg/kg/day × 4 to 5 days), with or without busulfan (Bu) 3.2 mg/kg/day × 2 days. Bu was used as a part of the conditioning regimen for highly transfused patients (interval time between diagnosis to HSCT was longer). In the R-ATG group 8 patients received Bu, whereas in the P-ATG group 7 patients received Bu.

Post-Transplant Immunosuppression

GVHD prophylaxis regimen consisted of short-course methotrexate at a dose of 15 mg/m^2 i.v. on day 1 followed by 10 mg/m^2 i.v. on days 3, 6, and 11, and cyclosporine 2 mg/kg/day or tacrolimus .03 mg/kg/day i.v. beginning day −1 in both groups.

Graft Source

In the R-ATG group 19 patients underwent BM transplantation. The median numbers of BM mononuclear cells (MNCs) and CD34$^+$ cells were 4.51 × 10^8/kg (range, 3.45 to 9.62) and 3.40 × 10^6/kg (recipient body weight) (range, 2.39 to 4.80), respectively. Another 37 patients received peripheral blood stem cell transplantation (PBSCT), and the median numbers of MNCs and CD34$^+$ cells were 7.33 × 10^8/kg (range, 4.35 to 12.40) and 2.67 × 10^6/kg (range, 1.09 to 5.98), respectively. Source combinations of BM and PBSCs were used in 2 patients, and the median numbers of MNCs and CD34$^+$ cells were 6.58 × 10^8/kg (range, 6.18 to 6.97) and 3.28 × 10^6/kg (range, 2.02 to 4.54), respectively.

In the P-ATG group 5 patients received BM transplantation, and the median numbers of MNCs and CD34$^+$ cells were 4.01 × 10^8/kg (range, 3.11 to 5.00) and 2.61 × 10^6/kg (range, 2.12 to 3.95), respectively. Forty-seven patients underwent PBSCT, with median numbers of MNCs and CD34$^+$ cells 8.18 × 10^8/kg (range, 2.75 to 19.82) and 2.66 × 10^6/kg (range, 1.97 to 5.73), respectively. Source combinations of BM and PBSC were used in 3 patients, and the median numbers of MNCs and CD34$^+$ cells were 6.62 × 10^8/kg (range, 5.79 to 10.43) and 5.44 (range, 4.20 to 6.43), respectively.

Patient Routine Supportive Care

All patients resided in a class 100 laminar flow ward, receiving Paediatric Compound Sulfamethoxazole Tablets o 1 g twice daily for 1 week to prevent *Pneumocystis carinii* pneumonia and ganciclovir 10 mg/kg/day i.v. for 1 week to prevent cytomegalovirus (CMV) infection before transplantation. Prevention of fungal infections in the patients who had not been diagnosed IFD before transplantation was applied by fluconazole until 3 months after transplantation. The other patients who had IFD before transplantation received itraconazole, voriconazole, micafungin, or caspofungin according to their individual pretransplant situations.

Engraftment Standard

Neutrophil (absolute neutrophil count [ANC]) recovery was defined as achieving an ANC ≥ .5 × 10^9/L for 3 consecutive days and platelet (PLT) recovery as achieving a PLT count ≥ 20 × 10^9/L, unsupported by transfusion for 7 days. Chimerism analysis was done using short tandem repeat.

Outcomes Analysis Standard

Diagnosing and grading of acute GVHD (aGVHD) were based on the Seattle diagnostic criteria. The 2014 National Institutes of Health consensus of chronic GVHD (cGVHD) [13] was used to diagnose and grade cGVHD. According to short tandem repeat PCR detection, cells > 95% to 100% represented complete chimerism of donors, namely the hematopoiesis in the body of patients was completely from donors. The donor cells > 2.5% to 95% represented mixed chimerism, namely the hematopoiesis in the body of patients was from both donors and patients. The donor cells within 0 to 2.5% represented the hematopoiesis in the body of patients was completely from patients and donor cells were rejected or grafted unsuccessfully [14]. Patients who did not reach ANC > .5 × 10^9/L for 3 consecutive days after transplantation were considered to have had primary GR. Patients with initial engraftment in whom recurrent pancytopenia with obviously hypocellular BM and without moderate to severe aGVHD were considered to have had secondary GR [15]. Early GR was defined as GR occurrence within the first 100 days after transplantation. Late GR was defined as GR occurrence later than 100 days after transplantation [16-18]. CMV viremia was defined as positive results of reverse transcriptase PCR (1 × 10^3 copies/mL) in blood. We defined IFD according to the revised EORTC/MSG criteria [19]. Severe bacterial infections was defined as bacteremia and severe tissue infections. Overall survival (OS) was defined as the time from the date of HSCT to death from any cause or last follow-up.

Survival Analysis

The last follow-up for all surviving patients was December 31, 2016. SPSS 16.0 software (Tianjin Pacific Pharmaceutical Co., Ltd, China) was used to analyze the survival rate and the survival curve by the Kaplan-Meier method. Survival differences between groups were estimated by the log-rank test. Patient-, disease-, and transplant-related characteristics were compared using the chi-square and rank sum tests. The final model of significance attained $P \le .05$.

RESULTS

Pretransplant Clinical Characteristics and Treatment Regimens

Pretransplant clinical characteristics of the 2 groups are shown in Table 1, whereas treatment regimens of the 2 groups are shown in Table 2.

Table 1
Patient Pretransplant Clinical Characteristics

Characteristic	R-ATG Group (n = 58)	P-ATG Group (n = 55)	*P*
Gender			.523
Male	33 (57)	28 (51)	
Female	25 (43)	27 (49)	
Media age at treatment, yr (range)	21 (4-49)	25 (7-46)	.052
Median interval from diagnosis to treatment, days (range)	61 (23-6935)	86 (23-2920)	.420
Diagnosis			.772
SAA	29 (50)	26 (47)	
VSAA	29 (50)	29 (53)	
Failed ATG treatment before HSCT	1 (2)	2 (4)	.612
Infection before HSCT			
Bacteremia	3 (5)	4 (7)	.712
IFD	9 (16)	12 (22)	.389
Viruses	0	0	
Uncontrolled infection before HSCT			
Bacteremia	2 (3)	0	
IFD	0	2 (4)	

Values are n (%) unless otherwise defined. VSAA indicates very SAA.

Table 2
Treatment Details

	R-ATG Group (n = 58)	P-ATG Group (n = 55)	*P*
Donor	MSD	MSD	
Conditioning regimens	Flu, CY, R-ATG ± Bu	Flu, CY, P-ATG ± Bu	
n (%)			
GVHD prophylaxis			.646
CSA, MTX	41 (71)	41 (75)	
FK506, MTX	17 (29)	14 (25)	
Graft source			.004
BM	19 (33)	5 (9)	
PBSCs	37 (64)	47 (85.5)	
BM + PBSCs	2 (3)	3 (5.5)	
	median (range)		
No. of s.c. infusions			
BM			
MNC, $\times 10^8$/kg	4.51 (3.45-9.62)	4.01 (3.11-5.00)	.135
$CD34^+$ cells, $\times 10^6$/kg	3.40 (2.39-4.80)	2.61 (2.12-3.95)	.303
PBSCs			
MNC, $\times 10^8$/kg	7.33 (4.35-12.40)	8.18 (2.75-19.82)	.067
$CD34^+$ cells, $\times 10^6$/kg	2.67 (1.09-5.98)	2.66 (1.97-5.73)	.889
BM + PBSCs			
MNC, $\times 10^8$/kg	6.58 (6.18-6.97)	6.62 (5.79-10.43)	.990
$CD34^+$ cells, $\times 10^6$/kg	3.28 (2.02-4.54)	5.44 (4.20-6.43)	.248

Table 3
Outcomes of Groups

Outcomes	R-ATG Group (n = 58)	P-ATG Group (n = 55)	*P*
Engraftment			
Median ANC, days (range)	12 (9-23)	12 (9-22)	.771
Median PLT, days (range)	16 (8-38)	15 (7-37)	.714
GVHD			
aGVHD, %	20.7 ± 5.3	43.4 ± 7.0	.015
Grades III-IV aGVHD, %	3.4 ± 2.4	12.3 ± 4.7	.098
cGVHD, %	20.1 ± 5.8	46.0 ± 7.9	.003
Moderate-severe cGVHD, %	12.6 ± 4.9	11.5 ± 4.9	.905
GR, %	7.4 ± 3.6	5.5 ± 3.1	.852
OS, %	93.1 ± 3.3	84.4 ± 5.7	.235
Infection, n (%)			
Severe bacterial infection	8 (14)	15 (27)	.075
IFD	15 (26)	16 (29)	.701
CMV viremia	14 (24)	12 (22)	.770

Values are mean ± standard deviation unless otherwise defined.

Engraftment

All patients engrafted, and none displayed primary GR in either group. In the R-ATG group the median times to ANC and PLT recovery were 12 days (range, 9 to 23) and 16 days (range, 8 to 38), respectively. In the P-ATG group the median durations to ANC and PLT recovery were 12 days (range, 9 to 22) and 15 days (range, 7 to 37), respectively.

Graft-versus-Host Disease

In the R-ATG group 12 patients developed aGVHD (10 with grades I to II and 2 with grade III), and all patients responded to a combination of immunosuppressive agents and steroids. In the P-ATG group 22 patients developed aGVHD (16 with grades I to II and 6 with grades III to IV), and 2 patients died of intestinal grade IV aGVHD. Significant differences in aGVHD were found between the 2 groups (20.7% ± 5.3% versus 43.4% ± 7.0%, $P = .015$).

In the R-ATG group 10 patients developed cGVHD (4 with mild and 6 with moderate). cGVHD was controlled in all patients by immunosuppressive agents. In the P-ATG group 20 patients developed cGVHD (15 patients with mild, 4 with moderate, and 1 with severe cGVHD, who died from the disease). There were significant differences in cGVHD between the 2 groups (20.1% ± 5.8% versus 46.0% ± 7.9%, $P = .003$). There were no significant differences in terms of grades III to IV aGVHD or moderate to severe cGVHD between the 2 groups (3.4% ± 2.4% versus 12.3% ± 4.7% [$P = .098$] and 12.6% ± 4.9% versus 11.5% ± 4.9% [$P = .905$], respectively).

Graft Rejection

In the R-ATG group 2 patients developed early GR and 2 patients developed late GR (the occurrence time was 8 and 14 months, respectively). In the P-ATG group 3 patients developed early GR and none developed late GR. There was no significant difference between the 2 groups (7.4% ± 3.6% versus 5.5% ± 3.1%, $P = .852$), and none died of GR.

Infection

In the R-ATG group bacteremia occurred in 7 patients and frontal parietal lobe bacterial infection in 1 patient. These 8 patients recovered after the use of antibacterial therapy. IFD occurred in 15 patients, including 1 case of liver and 1 case of nasal infection separately. Pulmonary IFD occurred in 13 patients. The pulmonary IFD was not successfully controlled by antifungal therapy in 3 patients, and they finally died. The other patients with IFD were successfully treated with antifungal therapy. CMV viremia occurred in 14 patients, and all cases were controlled by antiviral and globulin therapy.

In the P-ATG group bacteremia occurred in 14 patients, and 1 patient experienced maxillofacial bacterial infection, which was not successfully treated with antibacterial therapy, and finally died. IFD occurred in 16 patients, including 1 case in liver and 1 case in intracranial infection. Pulmonary IFD occurred in 14 patients. The 1 case with pulmonary and 1 case with intracranial IFD were not controlled by antifungal therapy, and the 2 patients finally died. The other patients with IFD were successfully treated with antifungal therapy. CMV viremia occurred in 12 patients, and all patients were successfully treated with antiviral and globulin therapy. There were no significant differences in terms of incidence of severe bacterial infections ($P = .075$), IFD ($P = .701$), or CMV viremia ($P = .770$) between the 2 groups.

Deaths

Four patients died in the R-ATG group, 3 with pulmonary IFD and 1 with intracranial hemorrhage. Seven patients died in the P-ATG group, 3 with GVHD and 4 with intracranial IFD, maxillofacial infection, pulmonary IFD, and suicide, respectively.

Survival

In the R-ATG group, at a median follow-up of 67 months (range, 3 to 143), 54 patients were alive and the 3-year OS was 93.1% ± 3.3%. In the P-ATG group, at a median follow-up of 18 months (range, 1 to 85), 48 patients were alive and the 3-year OS was 84.4% ± 5.7%. There was no significant difference in terms of the 3-year OS between the 2 groups ($P = .235$). The outcomes of the 2 groups are shown in Table 3 and Figure 1.

DISCUSSION

Both immunosuppressive therapy and allogeneic HSCT represent definite therapies for patients with SAA [11,12,20,21]. MSD-HSCT is the preferred first-line treatment option for younger (≤50 years) SAA patients [22]. GVHD and GR, both of which are critically influenced by the composition of the

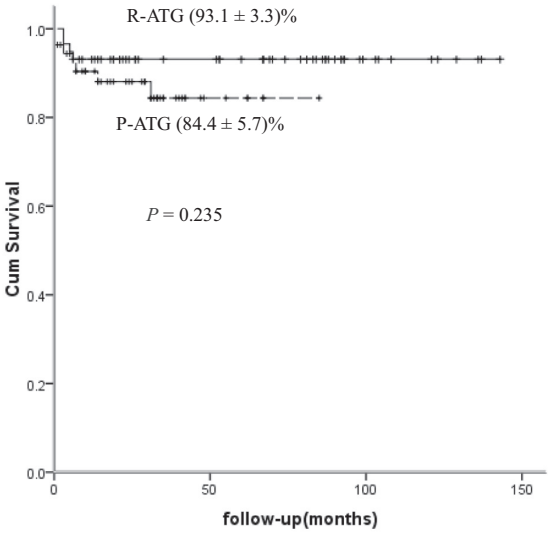

Figure 1. Comparison of 3-year survival rates of the 2 groups of patients.

allograft and the agents used in the preparative regimen, remain obstacles to the success of HSCT [1]. Many investigators [12,23-26] have explored the optimal conditioning regimen for SAA patients who receive MSD-HSCT to achieve sustained engraftment with minimal fatal complications. The addition of ATG to CY for conditioning resulted in infrequent GVHD and GR as well as improved OS [11,12]. Data reported to the Center for International Blood and Marrow Transplant Research in recent years showed that 75% of MSD-HSCT include ATG in the transplant conditioning regimen [27]. Bacigalupo et al. [28] showed that no ATG in the conditioning was 1 of the strongest negative predictors of survival for SAA patients who received HSCT. European Group for Blood and Marrow Transplantation data [29] showed that a Flu-based conditioning regimen may reduce the negative impact of age in older patients with SAA receiving MSD-HSCT. Current guidelines from the European Group for Blood and Marrow Transplantation [30] and the British Society for Standards in Haematology [31] called for a combination of Flu-CY with ATG or alemtuzumab (CAMPATH) for SAA patients over age 30 years receiving MSD-HSCT. Our conditioning regimens were based on Flu-CY with ATG, and no patient died from conditioning regimen–related toxicity.

A retrospective, nonrandomized study compared horse ATG with R-ATG in addition to CY as conditioning for allogeneic HSCT patients with SAA [32]. Conditioning with R-ATG was more protective against aGVHD and cGVHD than conditioning with horse ATG. Also, R-ATG was associated with a higher incidence of stable mixed chimerism. Recipients of R-ATG were also observed to have a higher incidence of viral and fungal infections. Kekre et al. [27] analyzed 546 SAA transplants between 2008 and 2013 using MSD who received ATG as part of their conditioning regimen and BM transplantation. aGVHD (17% versus 6%, $P < .001$) was higher with horse compared with R-ATG. There was no difference in 3-year OS (87% and 92%, $P = .76$). The above studies seemed to show that the immunosuppressive effects of R-ATG were stronger than that of horse ATG.

P-ATG is another preparation, available in China, for clinical use as an immunosuppressive agent, and it is cheaper than R-ATG. One study showed that the drug metabolism curve of P-ATG had an advantage over R-ATG in immunosuppressive therapy; however, the underlying mechanisms were unknown [33]. Results from our hospital (F.K. Zhang et al., unpublished data) showed that the half-life of P-ATG was about 15 days and an effective serum concentration of P-ATG was maintained for at least 60 days in vivo. Zhang et al. [34] demonstrated that the half-life of R-ATG was 29.7 ± 2.60 days and the effective serum concentration of R-ATG was maintained for at least 90 days in vivo. Ma et al. [35] compared the safety and efficacy of P-ATG with R-ATG in SAA and found the lymphocyte count decreased in both groups, whereas the R-ATG group had a longer time of lymphocyte decrease and lower lymphocyte counts. They then further compared the changes in subsets of T cell until 6 months after the treatment and found the inhibitory effects on the $CD8^+$ T cells were not significantly different between the 2 groups, whereas the inhibitory effects on $CD4^+$ T cells were significantly stronger in the R-ATG group than in the P-ATG group. Therefore, they concluded that R-ATG demonstrated stronger immunosuppressive effects than P-ATG, and the effects were mainly on $CD4^+$ T cells.

Some studies [33,36] indicated that P-ATG showed similar efficacy and safety profiles to R-ATG in immunosuppressive therapy of acquired SAA. P-ATG could be a suitable alternative preparation for R-ATG with the advantage of lower medical expense. However, few studies have been done on conditioning regimens. We conducted this retrospective analysis to compare the efficacy of P-ATG with R-ATG in MSD-HSCT for SAA. In our study patient baseline characteristics and donor conditions of the 2 groups were similar, except that patients were older and more received PBSCT in the P-ATG group. The results showed that all patients engrafted in both groups. There was no significant difference in terms of 3-year OS (93.1% ± 3.3% versus 84.4% ± 5.7%, $P = .235$), grades III to IV aGVHD (3.4% ± 2.4% versus 12.3% ± 4.7%, $P = .098$), moderate to severe cGVHD (12.6% ± 4.9% versus 11.5% ± 4.9%, $P = .905$), or GR (7.4% ± 3.6% versus[5.5% ± 3.1%, $P = .852$). There were also no significant differences in the incidences of severe bacterial infections ($P = .075$), IFD ($P = .701$), or CMV viremia ($P = .770$) between the 2 groups.

Gupta et al. [37] analyzed 1307 patients with SAA after MSD-HSCT. The risks of grades II to IV aGVHD in patients younger than 20 years was 11%, with higher risk in patients aged 20 to 40 years (17%) and in those over 40 years (27%, $P < .001$). Bacigalupo et al. [38] analyzed 1886 patients with SAA who received MSD-HSCT between 1999 and 2009 with either BM (n = 1163) or PB (n = 723) as the source of stem cells. Grades II to IV aGVHD developed in 11% and 17% of patients receiving BM and PB grafts, respectively ($P = .001$), whereas the corresponding percentages for grades III to IV aGVHD were 4% and 7%, respectively ($P = .005$). aGVHD was more frequent in PBSCT. In our study these 2 characteristics were the only differences between the 2 groups. Patients were older ($P = .052$) and more who received PBSCT ($P = .004$) in the P-ATG group. There were significant differences in the incidences of aGVHD (20.7% ± 5.3% versus 43.4% ± 7.0%, $P = .015$) and cGVHD (20.1% ± 5.8% versus 46.0% ± 7.9%, $P = .003$) between the 2 groups. Although there was no statistically significant difference, the incidence of grades III to IV aGVHD was slightly higher in the P-ATG group, which may explain the inferior rates of OS in the P-ATG group.

In summary, although the groups were not homogenous regarding cell source and ages, overall GVHD incidence seemed to be different, and survival was a little inferior in the P-ATG

group, P-ATG still showed satisfactory efficacy and safety compared with R-ATG in our study of SAA patients receiving MSD-HSCT. P-ATG could be a potential alternative preparation for R-ATG and has the advantage of lower costs. However, because this report was a retrospective analysis and the sample size of each group was relatively small, our results should be considered preliminary. Further studies with larger cohorts and longer follow-up periods are needed to derive more conclusive findings.

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Clinical applications of haploidentical hematopoietic stem cell transplantation in severe aplastic anemia

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Abstract. – OBJECTIVE: The purpose of this study was to investigate the efficacy and safety of haploidentical allogeneic hemopoietic stem cell transplantation (allo-HSCT) in severe aplastic anemia (SAA) and prophylaxis of complications involved.

PATIENTS AND METHODS: 8 patients with clinically diagnosed SAA (5 cases of SAA-I and 3 cases of SAA-II) were recruited, with the parents as the donors of hemopoietic stem cells. The conditioning regimen before HSCT included cyclophosphamide, fludarabine, pig anti-human lymphocyte immune globulin (p-ALG) and/or total body irradiation (TBI). The recipients received short-term methotrexate (MTX), mycophenolate mofetil (MMF), and cyclosporin A (CsA) for graft *versus* host disease (GVHD) prophylaxis. Subsequent to successful allo-HSCT, the hematopoietic reconstitution was observed, coupled with periodical surveillance of the chimerism rate, the occurrence, and severity of postoperative complications as infection, GVHD, veno-occlusive disease (VOD), hemorrhagic cystitis (HC), cytomegalovirus (CMV) as well as the long-term survival rate, etc.

RESULTS: We found that hematopoietic reconstruction was achieved in all of the 8 patients with the average time of 14.8d for absolute neutrophil count (ANC) > 0.5×10^9/L, and the average time of 15.0d for platelet count was more than 20×10^9/L. Follow-up for 1 month later revealed that DNA chimeric rate of donor cells was 95%-100%. The complications were aGVHD in 7 cases including 5 cases of grade I-II (62.5%), 1 case of grade III (12.5%) and 1 case of grade IV (12.5%), as well as chronic graft versus host disease (cGVHD) in 2 patients, including 1 case (12.5%) localized in the oral cavity and 1 case (12.5%) with extensive type cGVHD in the whole body skin. No VOD or HC was observed, and no transplantation-related death occurred at median following-up of 8.5 months (2 to 18 months).

CONCLUSIONS: Allo-HSCT is safe and effective in patients with SAA and has great clinical perspective.

Key Words:
Severe aplastic anemia, Hematopoietic stem cell transplantation, Haploidentical hematopoietic stem cell transplantation.

Introduction

The severe aplastic anemia (SAA) is a kind of disease involving bone marrow stem cells injury and hematopoietic microenvironment abnormality resulting from physical, chemical and biological factors and unknown causes followed by the replacement of blood-forming marrow by adipose tissues and cells decrease. The SAA can happen at widely different ages mainly among children and young patients, and the conventional immunotherapy approaches based on cyclosporin A (CsA) adopted by the majority of domestic hospitals in the past are characteristic of slow effect, low efficiency and poor prognosis. Although the immunosuppressive therapy based on antithymocyte globulin (ATG) and cyclosporin A greatly improves the survival rate of SAA patients, only 40%-60% of patients can have their hemogram recovered, and the efficacy of the said therapy approach can only be evaluated after about 6 months, and the effect of treatment still needs improving. Compared with the immunosuppressive therapy (IST), allogeneic hemopoietic stem cell transplantation allo-HSCT is characteristic of rapid hematopoietic reconstitution, low recurrence rate, high failure free survival rate, and less secondary malignant tumors. Because the majority of families have only one child in China, only 30% of patients can find a HLA-matched donor, and such percentage is even lower among adolescent patients and children patients. It is more challenging to find unrelated donors; thus, the majority of

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patients fail to be effectively treated due to time and sources restrictions. Haplo-HSCT can search for unrelated donors without the need to wait for the donors from bone marrow bank, and almost all patients can rapidly find haplotype-matched related donors, and also the preparation time is only 1-2 weeks, thus, greatly reducing the costs spent for bone marrow bank search. Therefore, our department will apply Haplo-HSCT to the clinical treatment of SAA patients and will make a primary research on its efficacy.

Patients and Methods

Patients

The haploidentical allogeneic hemopoietic stem cell transplantation was applied to 8 SAA patients in the Hematology Department of Xuzhou Central Hospital from June 2014 to December 2015. Among those cases, there were 5 male patients and 3 female patients, and the age range of which was within 5-26 years old, and the median age of patients was 14 years old. The medical history of 8 patients lasted for 2 months-6 years. Among the 8 patients, there were 5 patients with SAA-I and 3 patients with SAA-II. The patient with the longest medical history was injected with erythrocyte and platelet concentrate suspended for more than 10 times. All patients were checked in terms of blood routine, bone marrow morphology, bone marrow biopsy pathology, and chromosomal karyotype analysis in accordance with the diagnostic criteria of SAA as specified in the third edition of *Diagnosis and Therapeutic Effect Criterion of Blood Disease*[1]. All donors were parents of patients with age range within 25-52 years old and the median age of 37 years old. The patients were given physical examination and found qualified for donating hemopoietic stem cells. The locus of A, B, C, DR, DQ of donors were given high-resolution molecular biology matching, among which there were 4 donors of 5/10 match, 3 patients of 6/10 match and 1 patient of 7/10 match. There were 5 blood-compatible patients, 1 major incompatible patient and 2 minor incompatible patients (Table I).

Stem Cell Mobilization, Collection and Transplantation Type

5-10 μg/(kg.d) of recombinant human granulocyte colony-stimulating factor (rhG-CSF) is applied to donors to carry out stem cells mobilization, and total dosage of which was divided into 2 times a day, and the hypodermic injection was implemented for 5-6 days. In the fifth day, the COBESpectra (produced by Gambro BCT Company, Lockwood, CO, USA) was adopted to separate peripheral blood stem cells, and the circulating blood volume was 8-12L for each time. In the sixth day, 300-800 ml bone marrow stem cells were collected from posterior superior spine after the local anesthesia was given to patients in a bioclean environment, and CD34+ was counted and transported back to the patients by adopting the parallel flow cell sorter so as to ensure mean neutrophil count (MNC) >5.0×108/kg, CD34+cell population >2.0×10^6/kg. The hydroxyethyl starch was adopted to sediment red cells as for the major compatibility of ABO blood type, and the blood plasma was removed as for the minor compatibility of ABO blood type. All patients were treated with mixed transplantation of peripheral blood stem cells and bone marrow.

Pretreatment Scheme

6 patients were treated with the regimen of cyclophosphamide (CTX)+fludarabine (Flu)+ antilymphocyte globulin (ALG, pig): Cy 50 mg.kg-1.d-1×4d (-5d/-2d), Flu 25-30 mg. kg-1.d-1×4d (-8d/-5d), ALG 25 mg. kg-1.d-1×5d (-5d/-1d). 2 patients were treated with Cy+Flu+ALG+total body irradiation (TBI). The total body irradiation was applied to patients based on the above-mentioned dosage, and the total dosage was 2-3Gy, and the eyes and testes of patients were covered upon irradiation.

Prevention and Supportive Treatment of Transplantation Complications

Patients started to be treated in class 100 laminar ward and to have disinfection diet from pretreatment. The fluconazole or itraconazole was adopted to prevent fungal infections. The ursodeoxycholic acid + prostaglandin E and low molecular heparin calcium were adopted to prevent veno-occlusive disease (VOD), and the patients were stop having low molecular heparin calcium until the blood platelet count ≤20×10^9/L. The ganciclovir or acyclovir failed to be used in 4 patients to prevent cytomegalovirus (CMV) infection, and CMV-DNA and Epstein-Barr virus (EBV)-DNA were tested weekly after the transplantation. ALG + CsA + mycophenolate mofetil (MMF) + short course methotrexate (MTX) prevention aGVHD was adopted as below. The CsA 2.5 mg/(kg.d) was applied to patients in -7 days, and CsA blood concentration was tested weekly, and patients were given the medicines orally until the intestinal function was recovered, and

Table I. Clinical characteristics of patients and donors.

Case No	Age (year)	Sex	Diagnosis Recipient/Donor	Relationship Recipient/Donor	Blood group	HLA-matched
1	Six years and nine months old	Male	SAA-II	son/father	O/O	5/10
2	Eight years and three months old	Male	SAA-II	son/mother	A/A	6/10
3	Fourteen years old	Female	SAA-I	daughter/mother	AB/B	7/10
4	Twenty six year old	Female	SAA-I	daughter/father	A/A	5/10
5	Twenty one year old	Male	SAA-I	son/father	B/O	5/10
6	Fifteen years old	Male	SAA-I	son/mother	O/O	6/10
7	Four year and eight months old	Female	SAA-I	daughter/mother	O/A	5/10
8	Sixteen years old	Male	SAA-II	son/mother	A/A	6/10

the blood concentration was maintained at the level of 150-200 μg/L generally, and MTX (+1, +3, +6, +11d) was applied to short treatment course together with MMF. The compound sulfamethoxazole tablets were adopted to prevent *Pneumocystis carinii pneumonia*. The donors were given medicines of sodium bicarbonate, dexamethasone, and promethazine before being injected with stem cells. If the patients suffer from concurrent infection during the transplantation for lack of granule cell, the broad-spectrum antibiotics combined with antifungal agents was adopted, and the patients were actively injected with blood products going through 25Gy irradiation by γ radial according to the value of blood routine so as to maintain the hemoglobin >80 g/L, blood platelet count $<20\times10^9$/L.

Therapeutic Efficacy Evaluation and Test of Engraftment Evidence

The blood was tested every day or every other day after the transplantation, and absolute neutrophil count (ANC) $>0.5\times10^9$/L, and the blood platelet count (Plt) $\geq20\times10^9$/L for consecutive 3 days, and the patients were not injected with blood platelet for consecutive 7 days as the index for hematopoietic reconstruction. The myelogram was rechecked 1, 3, 6 or 12 months after the transplantation, and the physicians made the judgment on transplantation condition according to the short tandem repeat polymerase chain reaction (STR-PCR). According to STR-PCR detection, cells >95%-100.0% represented complete chimerism of donors, namely the hematopoiesis in the body of patients was completely from donors. The donor cells >2.5%-95.0% represented mixed chimerism, namely the hematopoiesis in the body of patients were both from donors and patients. The donor cells within 0-2.5% represented the hematopoiesis in the body of patients were completely from patients and donor cells are rejected or grated unsuccessfully. The person with blood group incompatibility were analyzed weekly after hematopoietic reconstitution until the blood type turns to the blood type of donors, which means that the transplantation was successful.

Diagnosis and Grading of GVHD

The diagnosis, analysis, and treatment of acute GVHD (aGVHD) and chronic (cGVHD) were conducted in accordance with BSBMT guidebook[2]. aGVHD usually broke out upon the recovery of hematopoiesis after the transplantation, and the early occurrence of aGVHD suggested that the prognosis was poor. The target organs involved in aGVHD usually included the skin, intestinal tract, liver and sometimes may include joints, eyes, etc. The physicians conducted a clinical observation on pruritus, skin rash, diarrhea, abdominal pain, the color and property of the stool, photophobia of eyes, conjunctival hemorrhage, and tested the liver function to intervene the aGVHD in the early period. cGVHD is the main complication and cause of death except for recurrence. The skin, oral cavity, liver and eyes were the parts which were easy to get infected. The local treatment measures were usually adopted to treat with local cGVHD. The generalize cGVHD was impossible to be relieved by itself, so the physicians took comprehensive treatment measures including MMF and FK506 to treat with the generalized cGVHD.

Statistical Analysis

SPSS13.0 software (SPSS Inc., Chicago, IL, USA) was adopted to carry out statistical anal-

Table II. Result of haplo-HSCT in 8 cases of severe aplastic anemia.

Case No	MNC cell ($\times10^8$/kg)	CD34+ cell ($\times10^6$/kg)	Time of ANC > 0.5×10^9/L	Time of Plt > 20×10^9/L	aGVHD	VOD	HC	CMV-DNA	Survival time (month)
1	15.27	6.69	+17d	+21d	II	none	none	(+)	15
2	8.62	3.46	+11d	+13d	II	none	none	(+)	13
3	7.65	2.24	+13d	+18d	II	none	none	(+)	18
4	5.14	2.21	+16d	+16d	0	none	none	(+)	7
5	9.90	2.10	+11d	+15d	III	none	none	(+)	7
6	11.01	2.87	+17d	+11d	I	none	none	(–)	4
7	13.20	5.66	+13d	+11d	IV	none	none	(+)	2
8	7.64	8.96	+20d	+15d	II	none	none	(+)	2

MNC: mononuclear cells; ANC: absolute neutrophil count; Plt: platelets

ysis; the means comparison was tested by the independent sample t, the comparison of sample rate was tested by χ^2, and $p<0.05$ shows that the difference has statistical significance. The Kaplan-Meier method was adopted to carry out survival analysis and single factor analysis, and the follow-up visits were conducted until December 31, 2015. $p<0.05$ was considered statistically significant.

Results

Complications During the Pretreatment

During the pretreatment period, all patients suffered from the mild gastrointestinal reactions, including nausea, emesis, etc., and there were 3 patients with different degrees of infection, and all patients improve markedly after being given active symptomatic and supportive treatment. There were no patients suffering from organ function lesions. The two total body irradiation (TBI) patients did not have any post-radiation complications including swelling parotid gland, pains etc.

Reconstruction of Hemopoietic Stem Cells

8 patients have had their hemopoietic stem cells reconstructed, and mononuclear cells (MNC) median of injected donor stem cells was 9.80 (5.14-15.27) $\times10^8$/kg, and the median of CD34+ was 4.27 (2.10-8.96)$\times10^6$/kg. The median time for the engraftment of transplanted granulocyte was 14.8 (11-20) days, and the median time for the engraftment of blood platelet was 15.0 (11-21) days respectively. The chimerism conditions of patients were rechecked 1 month after the transplantation, and DNA chimerism rate of donor cells was within 95%-100%. Where the blood type of donors was different from that of patients, their blood type was transformed into the blood type of donors after the transplantation. The transplants in 3 patients were rejected 3 months after the transplantation, and the chimerism rate reduced as low as 72% as well as the peripheral blood cell. The chimerism rate rebounded to 85%-100%, and the blood routine returned to the normal level after the patients were injected with donors' bone marrow blood (Table II).

Occurrence of GVHD

There were 7 patients suffer from a Graft *Versus* Host Disease (GVHD), among which 5 patients (62.5%) suffered from aGVHD of I-II degree, 1 patient (12.5%) suffered from aGVHD of III degree and 1 patient (12.5%) suffered from aGVHD of IV degree. There were 3 patients (37.5%) who had their skin involved and 4 patients (50.0%) who had their intestinal tracts involved. There were 1 patient (12.5%) suffer from oral cavity-located cGVHD, and the main symptoms may include oral pain, oral lichenoid lesions and oral ulcer difficult to be healed. There were 1 patient (12.5%) suffer from generalized cGVHD, and the main symptoms may include pruritus and lichenoid lesions. Upon the occurrence of aGVHD, the patients shall be injected with 1-2 mg/(kg.d) methylprednisolone (MP) in the veins, and the dosage of methylprednisolone was reduced after it takes effect until the patients stop taking the given medicine. If the medicine did not take any effect, CSA was replaced by FK506 treatment or added with CD25 monoclonal antibody treatment. cGVHD was usually treated by the method of monotherapy or combination

therapy, including local treatment, methylprednisolone, cyclosporine or FK506 and other medicines, FK506 was taken by patients orally as per 0.1 mg/(kg.d), and the blood concentration was maintained at the level of 5-10 μg/L, and GVHD occurred to patients was controlled to different extents after active treatment (Table II).

Other Post-transplantation Complications and Follow-up Visits

After the transplantation, all patients suffered from no hepatic vein occlusion syndromes, hemorrhagic cystitis, and interstitial pneumonia. 8 patients suffered from different degrees of infection and fever, and body temperature of patients began to recover to the normal level after being treated with broad-spectrum antibiotic, vancomycin and antifungal therapy. There were 7 patients suffer from cytomegalovirus (cytomegalovirus, CMV) and EB viremia (Epstein-Barr virus, EBV) among 8 patients. For the 7 patients with transplanted their lungs, intestinal tracts and other organs, etc., blood CMW or EBV was tested to be within the normal level after the patients were treated with ganciclovir or acyclovir. There were 2 patients suffer from oral mucositis of II-III degree, and the symptoms began to improve after the patients were treated with comprehensive treatment methods, including oral nursing, physiotherapy, kangfuxin lotion, rhGM-CSF mouthwash, recombinant human epidermal growth factor, etc. Two patients suffered from epilepsy during the process to take FK506, and patients improve after replacing FK506 with CsA. The median follow-up time was 8.5 months (2-18 months), during which there was no transplantation-related fatality (Table II).

Discussion

SAA, a serious failure of bone marrow haematopoietic function caused by varied reasons is characterized by acute occurrence, serious conditions, poor treatment response, and high mortality rate[3]. At the present stage, ATG together with CsA (an immunosuppressive) and HSCT are the main measures to treat SAA[4]. However, the immunosuppressive therapy has a slow onset, and most of the patients in the treatment process can only be out of the blood transfusion temporarily and easy to cause serious infection[5], it increases more economic expenditure. HSCT can provide hematopoietic stem cells and mesenchymal stem cells for patients with SAA and correct the disorder of the immune system for providing efficient treatment for SAA[6]. Based on China's national conditions and the characteristics of the development of the disease, in only a small number of cases the sibling matched donors were found on time for the treatment[7]. Chinese bone marrow bank needs a long time to look for an unrelated donor, resulting in large transplantation risk comparatively. To expand the source of the donor, Haplo-HSCT is becoming a hot spot of research in recent years[8]. Haplo-HSCT's main risk lies in the difficult transplantation, after transplantation the incidence of infection and GVHD are high, and the immune function recovery delays, etc. All of these restrict the clinical application of the SAA treatment[9].

To overcome the difficulty of transplantation, several studies have been conducted. As reported by Wang et al[10], haplotype hematopoietic stem cell transplantation has been carried out on 17 children and adolescents with SAA. The median time to implant the neutrophil insertion in place was 16 days, the time of platelet 22 days. The prevalence rate of aGVHD in the second to fourth degree in more than 100 days was 30.53 ± 11.12%, and the third to fourth-degree aGVHD only occurred to one patient. The occurrence rate of cGVHD was 21.25 ± 13.31%. The overall survival rate was 71.60 ± 17% in the median follow-up of 362 days (36 to 1321 days), indicating that haplotype hematopoietic stem cell transplantation is feasible and can be a salvage therapy for children and adolescents with SAA deficiency. As the domestic transplant center reports[11], in the treatment of blood haploidentical transplantation for the SAA patients, there were 15 survival cases among 21 patients with the survival rate of 71.4% in the median follow-up 16 (3-46) months. It confirmed that HLA the blood haploidentical stem cell transplantation for treatment of SAA is safe and effective. It can be seen that the blood haploidentical HSCT solves the problem of donor source, the implantation success rate is satisfactory, and the complications after transplantation can be well controlled.

Through the patients' previous follow-up observation and statistical analysis, we found that the blood haploidentical HSCT has certain advantages in the treatment of patients with the malignant hematologic disease[12]. Based on the successful treatment of haploidentical HSCT of leukemia and other malignant hemopathy

patients, since from 2014 we explored treatment gradually by using haploid HSCT for our SAA patients. In donor selection, if there was no matched donor blood cell, the relative haploid transplantation was selected which can win the opportunity to find a complete cure for the disease and the donor stem cell source was convenient with no risk of regretting donation. If the transplanted stem cells were transplanted into the patient imperfectly, the bone marrow stem cells were taken out more times in a timely manner to the patients so the implantation rate can be improved. Based on the classical CTX+ALG, preprocessing scheme added the use of fludarabine and TBI, which can not only increase the intensity of immunosuppressive, but also help promote implantation and alleviate GVHD. The transplantation methods were determined to be mixed transplantation of G-CSF mobilized bone marrow and peripheral blood stem cell, which contained a large number of hematopoietic stem cells and a certain amount of bone marrow stroma cells to accelerate hematopoietic reconstitution and significantly reduce the incidence of severe GVHD. To save the cost of patients, we implemented local anesthesia to the donor with family difficulties under the sterile and monitoring environment and repeat extraction of bone marrow from posterior superior iliac crest with less puncture point. The results showed that the donor's pain was small, and appropriate reduction of the donor's bone marrow collection did not affect the patients' smooth hematopoietic reconstruction, which explored a new way of bone marrow collection for the economically underdeveloped areas. Before transplantation, given an intramuscular injection of long-acting riboflavin, only two cases had oral mucositis inflammation of II-III degree. After active and symptomatic treatment, the rapid recovery showed that the prevention method was effective. The department adopted Alprostadil Injection + ursodeoxycholic acid + low molecular weight heparin calcium (PLT $<20\times10^9$/L disabled) to prevent VOD. All 8 patients did not show VOD, suggesting that the application of alprostadil injection 10 μg once daily was sufficient and ursodeoxycholic acid had a more important role for VOD prophylaxis. There was no HC in all 8 cases after patients got adequate hydration, alkalization, mesna application and forced diuresis. Among 8 cases of patients, 7 appear blood CMV-DNA and EBV-DNA copy number increased after transplantation. Considering that in some cases preventing the effects of drugs on blood cells, the use of ganciclovir prophylaxis was not adopted. Other explanation could be that hospital laboratory detection methods were too sensitive, with ganciclovir and acyclovir antiviral therapy, none of the patients had appeared CMV disease. For the prevention of GVHD, we adopted traditional CSA+ small dose MTX+ALG, by using CSA 7 days in advance. After giving sufficient CSA and detecting plus maintaining the concentration of CSA, most patients did not have severe GVHD. If GVHD does appear, enough methylprednisolone and FK506 should be applied, and refractory of intestinal GVHD should be treated by CD25 monoclonal antibody in a timely manner. The patients were improved, and pretreatment with pig ALG instead of rabbit ATG the effect was acceptable, which were successfully implanted and severe GVHD does not occur after transplantation, with expenditure reduced significantly. The EBMT-SAA working group through more than 100 cases demonstrated that the patients have a higher long-term survival rate with a low dose of total body irradiation for SAA patients[13]. So there were 2 patients to whom we have taken the TBI pretreatment methods, and achieved good results. After transplantation through of STR-PCR and blood routine test, when the chimeric rate, white blood cell count and platelet count were found to decline, 2-3 ml/kg unused donors' bone marrow blood was infused once timely, 1-2 times a week, for 2-3 times consecutively, which of great help to promote stable stem cell implantation. In the 19 cases treatment of refractory SAA by Haplo-HSCT therapy exercised by Xu et al[14], all patients were successfully implanted, and the occurrence of aGVHD, the control, and long-term survival rate were more satisfying showing the reliability of the haploid transplantation.

Conclusions

Due to the current use of foreign the blood haploidentical HSCT for SAA is still less, the study group only explored a smaller number of cases preliminary, and patients were followed up for a short time. But the completed follow-up observation of HSCT for SAA patients showed that without HLA All-matched donor, it was safe, effective and worth promoting for the treatment of SAA patients.

Conflict of interest

The authors declare no conflicts of interest.

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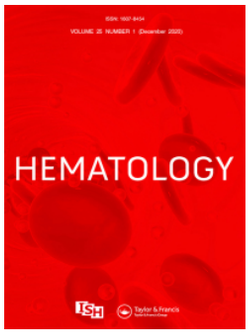

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A comparative study of porcine antihuman lymphocyte globulin versus antithymocyte globulin-fresenius in an allogeneic hematopoietic cell transplantation conditioning regimen for severe aplastic anemia

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ABSTRACT

Objectives: To compare the outcomes of antihuman T lymphocyte globulin (ATG-F) and porcine antihuman lymphocyte globulin (p-ALG) as part of a conditioning regimen in hematopoietic stem cell transplantation (HSCT) for severe aplastic anemia (SAA).

Methods: we performed a retrospective analysis, evaluating the outcome of patients with SAA who received ATG-F based conditioning ($n = 26$) with those receiving p-ALG conditioning ($n = 34$).

Results: The median time to neutrophil engraftment was 11 days (range, 8 – 38) and 11 days (range, 9 – 24) in the p-ALG and ATG-F groups ($P = 0.857$); the median platelet engraftment time was 15 (range, 9 – 330) days and 13 (range, 10 – 56) days ($P = 0.155$). There were no significant differences in grades II – IV acute graft-versus-host disease (aGVHD), grades III – IV aGVHD, chronic GVHD (cGVHD), and the moderate-severe cGVHD between the ATG-F and p-ALG groups (P>0.05).

Discussion: Patients in the ATG-F group functioned significantly better on role-physical ($P = 0.006$), general health ($P = 0.029$), and physical component summary ($P = 0.009$). The estimated overall survival and failure free survival rates at 5 years were 88.5% ± 6.3% vs. 82.4% ± 6.5% ($P = 0.515$), 84.6% ± 7.1% vs. 79.4% ± 6.9%, respectively ($P = 0.579$). The infection rates were 61.53% and 47.05%, respectively ($P = 0.265$).

Conclusion: As part of the conditioning regimen, p-ALG achieved a similar efficacy as ATG-F without increasing the incidence of transplantation complications in SAA patients.

KEYWORDS
Severe aplastic anemia; hematopoietic stem cell transplantation; porcine antihuman lymphocyte globulin; antithymocyte globulin

Introduction

In most cases, severe aplastic anemia (SAA) is an autoimmune disorder resulting from autoreactive cytotoxic T lymphocyte attack of the hematopoietic component of the bone marrow. The disease results in fatal complications, such as infection and/or hemorrhage [1,2]. Allogeneic haematopoietic stem cell transplantation (allo-HSCT) from a matched-related donor (MRD) is the initial treatment of choice for newly diagnosed SAA or very SAA (vSAA) patients who are less than 35 years of age [1]. According to the current therapeutic algorithms, immunosuppressive therapy (IST) with a combination of horse antithymocyte globulin (ATG) and cyclosporin A (CsA) is the preferred first-line treatment for patients without an MRD and older patients [3]. Although patients respond to IST, the long-term risks of relapse, CsA dependence, and clonal evolution are high; those unresponsive to initial IST or experiencing clonal evolution will be considered for transplantation using an alternative donor [4]. Alternative HSCTs from sources of stem cells, from groups including HLA-matched unrelated donors (URDs), mismatched unrelated donors, haploidentical family donors, and unrelated umbilical cord blood (UCB) are options for individuals with no suitable MRD [4–9]. URD haematopoietic stem cell transplantation (URD-HSCT) has even been considered as a first-line treatment for SAA in children and young patients by some studies [2,10–13]. Haploidentical haematopoietic stem cell transplantation (haplo-HSCT) as a treatment for SAA has greatly improved [6,14–18]. Patients who underwent haplo-HSCT as an initial therapy had primary engraftment and survival outcomes similar to SAA patients who received MRD-HSCT [19–21]. In China, haplo-HSCT is recommended for newly diagnosed young SAA patients without an MRD [22].

In previous studies, improved survival after transplantation was shown for both MRD and alternative donors in children and adults. Improved outcomes

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may have been a consequence of changes in graft-versus-host disease (GVHD) prophylaxis, changes in conditioning regimens, better donor selection, and a larger use of ATG. Conditioning regimens for HSCT in SAA with ATG led to better survival associated with a lower risk of grade II – IV acute GVHD (aGVHD) [23,24]. There are different species of animals utilized to produce ATG, and the available sources currently include horse ATG (h-ATG) and rabbit ATG (r-ATG). Two different r-ATG formulations, thymoglobulin-ATG (ATG-T) and fresenius-ATG (ATG-F) (Fresenius Biotech GmbH, Germany), are usually employed as prophylaxis for GVHD [25]. ATG-T use resulted in fewer adverse effects compared with ATG-F, and similar clinical outcomes were observed in patients undergoing HSCT, suggesting that ATG-T has stronger immunosuppressive activity than ATG-F [26–29]. On the other hand, HSCT with ATG remain to be a heavy financial burden for SAA patients even in Western countries. Anti-human T lymphocyte porcine immunoglobulin (p-ALG) (Wuhan Institute of Biological Products, China, State Medical Permit No. S10830001) was approved by the Sino Food and Drug Administration (SFDA) in 2004. It is commonly used in China to treat people with SAA due to its similar efficacy and safety as rabbit ATG; because of its lower cost (p-ALG costs only about 40% of the cost of rabbit ATG), it is widely used as a first-line therapy against acquired SAA in China [30,31].

As part of a component of the conditioning regimen, whether p-ALG achieves a similar efficacy as ATG-F without increasing the incidence of transplantation complications, such as GVHD, graft failure (GF), and infection in SAA patients have not been evaluated. These mechanistic differences between ATG-F and p-ALG may not generate the same results following HSCT, and the effects of p-ALG versus ATG-F in an allo-HSCT conditioning regimen for SAA are unknown. Therefore, we retrospectively analyzed the outcome of 60 SAA patients who underwent HSCT with ATG-F or p-ALG in the conditioning regimen at our centers from September 2011 to November 2017.

Materials and methods

Patients

Between September 2011 and November 2017, 60 SAA patients who underwent HSCT with ATG-F or p-ALG as a conditioning regimen at our transplantation unit were enrolled in this study. Patients met the following criteria: diagnosis and management of SAA or vSAA, as defined by the guidelines [1], no response to previous immune-suppressive therapy (IST) (CsA or r-ATG/p-ALG plus CsA) or HSCT as an initial treatment, transfusion dependence; received p-ALG or ATG-F as a conditioning regimen, voluntary participation in HSCT, and absence of severe liver, renal, lung, and heart diseases. Iron chelation therapy was administered when the serum ferritin was > 1000 μg/L, to decrease the level to less than 1000 μg/L before transplantation. All of the patients and donors provided written informed consent for this protocol. This study was approved by the hospital Ethics Committee.

HLA typing and donor selection

The HLA-A, -B, -C, DRB1 and -DQB1 in the recipients were typed. Those with appropriate HLA type, age, gender, health status, and willingness were considered as qualified donors. MRD was most preferred, followed by URD, haploidentical donors or UCB units.

Conditioning regimen

The time sequences in the transplant were differentially named. For instance, the days before transplantation were marked with '−', and those after the last stem cell infusion with '+'. A therapy based on fludarabine (FLU)/cyclophosphamide (CY) was used to deal with patients with an MRD or UCB. Protocols were as follows. FLU:30 mg/m2/day (i.v.) on days −7 and −2; CY: 50 mg/kg/day i.v. on days −4 to −3; and ATG-F (Zetbulin, ATG-F, Fresenius, Munich, Germany): 5 mg/kg/day i.v. or p-ALG (porcine, Wuhan Institute of Biological Products): 20 mg/kg/day i.v. on days −5 to −2. For those receiving haploidentical donors, a therapy was designed with busulfan (BU)/CY. The protocols were as follows. BU: 3.2 mg/kg/day intravenously (i.v.) on days −7 and −6; CY: 50 mg/kg/day i.v. on days −5 to −2; and ATG-F: 5 mg/kg/day i.v. or p-ALG: 20 mg/kg/day i.v. on days −5 to −2. For those receiving URD, FLU/CY-based or BU/CY-based regimens were introduced. Protocols were as follows: FLU:30 mg/m2/day (i.v.) on days −7 and −2; CY: 50 mg/kg/day i.v. on days −4 to −3; a therapy was designed with busulfan (BU)/CY: BU: 3.2 mg/kg/day intravenously (i.v.) on days −7 and −6; CY: 50 mg/kg/day i.v. on days −5 to −2.

Graft collection and infusion

As preparation for stem cell mobilization of haploidentical donors, granulocyte colony-stimulating factor (G-CSF) (10 mg/kg/day) was subcutaneously injected from day −4. 'day 01' referred to the first day of stem cell injection. On day 01, BM aspiration were employed to prepare BM grafts in the surgery room. As the mononuclear cell (MNC) count rose to $2 - 4 \times 10^8$/kg of recipient weight, the peripheral blood stem cells (PBSCs) were harvested on day 02 through apheresis accomplished by a COBESPECTRA device (Gambro BCT, Lakewood, CO, USA). In the total cells from the BM and peripheral blood (PB), the MNC count was allowed to

grow to $6-8\times10^8$/kg of the recipient weight. If the count was below this level, PBSCs were supplied the next day. BM and PBSCs, once collected, were translated into the recipient.

GVHD prophylaxis and treatment strategy

The patients who were assigned to FLU/CY-based regimen received CsA for aGVHD prophylaxis. Infusion of CsA (3 mg/kg/day) sustained for over 24 h, from day −4 to the day when the infusion was replaced with oral intake (PO). The requested whole-blood trough level was kept at 200 – 300 ng/mL for 12 months after HSCT. CsA was gradually reduce till withdrawn during the next 2−3 months. In patients assigned to BU/CY-based regimen, CsA (from day −7), mycophenolate mofetil (MMF), and short-term methotrexate (MTX) were administered for aGVHD prophylaxis. MMF (1.0 g, or 0.5 g for children, PO, twice daily) was given from day −7 to +30, then gradually reduced until day +60. MTX doses were set as 15 mg/m^2/day on day +1, and at 10 mg/m^2/day on days +3, +6 and +11. As CsA dropped, CsA was continued and raised to the therapeutic dose in the case of GVHD. Acute GVHD was resolved with methylprednisolone (1–2 mg/kg, once daily). Steroid refractory aGVHD was copedwith second-line immunosuppressive therapy based on including tacrolimus (FK506), CD25 monoclonal antibody, MMF and MTX.

Supportive care and post-transplantation surveillance

Once preparative regimen initiated, all patients were restricted inside sterile rooms for reverse isolation. Prior to conditioning 3 days, the gut was selectively decontaminated with fluconazole (200 mg twice daily), albendazole (200 mg once daily, 3 days) and levofloxacin (200 mg twice daily, gentamycin for children). During the conditioning and immunosuppressive period, prophylactic antibiotics, antifungal and antiviral therapies were administered. Trimethoprim/sulfamethoxazole (four tablets per day, twice weekly) was adopted for preventing Pneumocystis jiroveci infection. G-CSF (5 μg/kg/day) was given on day +7, until the confirmation of myeloid recovery [absolute neutrophil count (ANC) ≥ 2 × 109/L for 3 consecutive days]. Heparin and prostaglandin E1 were taken to prevent veno-occlusive disease. Prophylactic i.v. immunoglobulin (400 mg/kg, once weekly) was used in the first month after HSCT. Irradiated and leukodepleted blood products were given to maintain a haemoglobin (Hb) level > 60 g/L and a platelet count > 20×109/L.

After neutrophil recovery, the chimerism of donor cells was evaluated through weekly multiplex fluorescent analysis on short tandem repeat (STR) in PB for 1 year. Cytomegalovirus (CMV) and Epstein–Barr virus (EBV) viremia was confirmed using real-time PCR weekly. The case of positive CMV was treated with pre-emptive therapy based on ganciclovir or foscarnet.

Assessment of health-related quality of life (HRQoL)

As a tool for measuring HRQoL among adults, the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) consists of eight subscales pertaining to physical functioning, role-physical functioning, bodily pain, general health, vitality, social functioning, role-emotional functioning, and mental health. The scores of eight subscales are summarized into the physical component summary (PCS) and the mental component summary (MCS) [32,33].

All the eligible patients were informed of the objective of the study. A consent form, questionnaires, and a self-addressed stamped envelope were mailed to those volunteers. Their written informed consent and questionnaires were returned as early as possible. Their clinical information was put into a medical database.

Post-transplantation evaluations

ANC > 0.5×10^9/L during three consecutive days indicated neutrophil engraftment. Platelet count > 20×10^9/L without transfusion support for seven consecutive days indicated platelet engraftment. Other deifications were as follows. Primary GF: neutrophil engraftment failure after HSCT at day +28. Secondary GF: recurrent ANC < 0.5×10^9/L after initial engraftment by loss of donor cells. Platelet recovery delay: platelet engraftment after > 30 days. Early death: death within 60 days after HSCT. Transplantation-related mortality (TRM): death without disease progression. Failure-free survival (FFS): survival without treatment failure. Death, primary or secondary GF, and relapse suggested treatment failures. Acute GVHD was scored by the 1994 Consensus Conference on Acute GVHD Grading [34], and chronic GVHD (cGVHD) by the NIH Consensus Development Project on the Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: Diagnosis and Staging Working Report [27].

After HSCT, recipient BM was sampled monthly during the first 3 months and every 3 – 6 months during 1 – 2 years of follow-up.

Statistical methods

The chi-square or Fisher's exact test was used to compare categorical variables, and the Mann – Whitney nonparametric test was used for continuous

variables and HRQoL. Overall Survival (OS) and FFS were calculated using the Kaplan-Meier method and compared between different patient groups using the log-rank test. The cumulative incidence function was used to calculate the incidence of TRM, aGVHD and cGVHD. SPSS 22.0 software (SPSS, Chicago, IL, USA) was used for statistical analysis. All of the *P* values are two-sided, and the results were considered to be statistically significant when $P < 0.05$.

Table 1. Characteristics of SAA patients undergoing HSCT.

Variable	ATG-F ($n = 26$)	*p*-ALG ($n = 34$)	*P*
Patient age, years			0.653
Median age, years (range)	27 (3-52)	25.5 (7-54)	
≤ 20 years, no. (%)	10 (38.46)	10 (29.41)	
21–40 years, no. (%)	12 (46.15)	16 (47.06)	
? 40 years, no. (%)	4 (15.38)	8 (23.53)	
Gender (male/female)	13/13	17/17	1.000
Disease and status at transplantation, no. (%)			0.489
SAA	15 (57.69)	19 (55.88)	
VSAA	10 (38.46)	15 (44.12)	
SAA with PNH clone	1 (3.85)	0 (0.00)	
Median time from diagnosis to transplantation, months (range)	2.0 (1.0-180.0)	4.0 (0.8-240.0)	0.914
Previous treatment, no. (%)			0.280
CsA + andriol	4 (15.38)	3 (8.82)	
CsA + ATG/ALG + andriol	2 (7.69)	5 (14.71)	
Courses of ATG/*p*-ALG, no. (%)			0.499
1	2 (7.69)	4 (11.76)	
2	0 (0.00)	1 (2.94)	
Donor median age, years (range)	42 (25-56)	38 (13-52)	0.096
Donor–recipient sex match, no. (%)			0.566
Male–male	8 (32.00)	6 (11.65)	
Male–female	8 (32.00)	10 (29.41)	
Female–male	5 (20.00)	10 (29.41)	
Female–female	4 (16.00)	3 (8.82)	
Donor–recipient relationship, no. (%)			0.011
URD	1 (3.85)	5 (14.71)	
MRD	7 (26.92)	16 (47.06)	
Haplo-HSCT	17 (65.38)	8 (23.53)	
UCBT	1 (3.85)	5 (14.71)	
Blood types of donor to recipient, no. (%)			0.444
Matched	18 (69.22)	17 (50.00)	
Major mismatched	4 (15.38)	8 (23.53)	
Minor mismatched	4 (15.38)	8 (23.53)	
Major and minor mismatched	0 (0.00)	1 (2.94)	
Source of graft, no. (%)			0.055
BM	2 (7.69)	3 (8.82)	
PB	3 (11.54)	11 (32.35)	
BM + PB	20 (76.92)	15 (44.12)	
CBT	1 (3.85)	5 (14.71)	
Time from diagnosis to transplant			0.006
?6 months	8 (30.77)	18 (52.94)	
≥6 months	18 (69.23)	16 (47.06)	

SAA indicated severe aplastic anemia; VSAA, very severe aplastic anemia; PNH, paroxysmal nocturnal hemoglobinuria; CsA, cyclosporine A; ATG-F, antithymocyte immunoglobulin fresenius; *p*-ALG, porcine antihuman lymphocyte globulin; HSCT, hematopoietic stem cell transplantation; RBC, red blood cell; BM, bone marrow; PB, peripheral blood; MRD, matched related donor; URD, unrelated donor; haplo-HSCT, haploidentical hematopoietic stem cell transplantation; UCBT, unrelated cord blood transplantation.

Results

Patient, disease, and transplant characteristics

A total of 60 patients were enrolled in this study. The characteristics of the patients and donors prior to transplantation are shown in Table 1. The p-ALG group included 17 male patients and 17 female patients, the median age was 25.5 years (range: 7 – 54 years). Data from 19 vSAA patients were included, six patients with failure of IST who underwent salvage therapy, and 28 patients underwent transplantation as first-line therapy. The median interval from SAA diagnosis to HSCT was 4.0 months (range 0.8 – 240.0 months). The ATG-F groups included 13 male patients and 13 female patients, the median age was 27 years (range: 3 – 52 years). Data from 15 vSAA patients were included, one patient with paroxysmal nocturnal haemoglobinuria (PNH) clones, two patients with failure of IST that underwent salvage therapy, and 24 patients underwent transplantation as first-line therapy. The median interval from SAA diagnosis to HSCT was 2.0 months (range 1.0 – 180.0 months). Details of the graft subtypes are also presented in Table 1. The ATG-F group had the highest incidence of haplo-HSCT, followed by all, MRD, URD and UCBT. The p-ALG group had the highest incidence of MRD transplantation, followed by haplo-HSCT, URD, UCBT.

Hematopoietic recovery

The median values of absolute mononuclear cells (MNCs) were 10.36×10^8/kg (range $0.41 – 22.39 \times 10^8$/kg) and 13.06×10^8/kg (range $0.62 – 24.00 \times 10^8$/kg) in the p-ALG group and ATG-F groups, respectively ($P = 0.142$). The median values of absolute $CD34^+$ cells were 4.23×10^6/kg (range $0.15 – 5.94 \times 10^6$/kg) and 4.15×10^6/kg (range $0.86 – 5.95 \times 10^6$/kg), respectively ($P = 1.000$). The median time to neutrophil engraftment was 11 days (range, 8 – 38) and 11 days (range, 9 – 24; $P = 0.857$) and the median time to platelet engraftment was 15 days (range, 9 – 330) and 13 days (range, 10 – 56) in the p-ALG group and ATG-F groups, respectively ($P = 0.155$).

In the ATG-F group, all of the patients achieved successful donor myeloid engraftment, no patient experienced primary GF, and one patient experienced secondary GF. One patient demonstrated delayed platelet recovery and one patient demonstrated platelet GF. In the p-ALG group, two patients experienced early mortality and among the evaluable 32 patients, two UCBT patients experienced hematopoietic function spontaneous recovery. The other 30 patients achieved successful donor myeloid engraftment, and no patient experienced primary or secondary GF. Four patients demonstrated delayed platelet recovery and one patient demonstrated platelet GF (Table 2).

Infections and transplantation-related toxicities

The infection rate was 61.53% in the ATG-F group and 47.05% in the p-ALG group ($P = 0.265$) within the first 100 days after transplantation (Table 2). The infections included febrile neutropenia (19.23% vs. 5.88%, $P = 0.234$), pulmonary infection (15.38% vs. 20.59%, $P = 1.000$), septicaemia (15.38% vs. 11.76%, $P = 1.000$), urinary infection (0.00% vs. 2.94%, $P = 1.000$), mucositis/stomatitis (11.53% vs. 8.82%, $P = 0.728$), viremia (0.00% vs. 2.94%, $P = 0.378$), upper airway (0.00% vs. 5.88%, $P = 0.208$), and others (3.85% vs. 8.23%, $P = 0.720$). No patients died due to lethal organ toxicities during the 40 days after HSCT.

GVHD incidence and severity

The cumulative incidence of grades II to IV aGVHD on day +100 was 23.08% ± 8.26% and 38.24% ± 8.33% in the ATG-F and p-ALG groups, respectively ($P = 0.208$; Figure 1A). The cumulative incidence of grades III to IV aGVHD on day +100 was 15.39% ± 7.08% and 23.53% ± 7.28% ($P = 0.442$; Figure 1B), and the cumulative incidence of cGVHD was 21.05% ± 8.38% and 35.97% ± 8.71%, respectively ($P = 0.255$; Figure 1C). The cumulative incidence of moderate-severe cGVHD was 8.33% ± 5.64% and 9.68% ± 5.31%, respectively ($P = 0.872$; Figure 1D).

Table 2. Clinical outcomes after HSCT.

Variable	ATG-F ($n = 26$)	p-ALG ($n = 34$)	P
Median MNC, $\times 10^8$/kg (range)	13.06 (0.62–24.00)	10.36 (0.41–22.39)	0.142
Median CD34$^+$ cells, $\times 10^6$/kg (range)	4.15 (0.86–5.95)	4.23 (0.15–5.94)	1.000
Median neutrophil recovery, days (range)	11 (9–24)	11 (8–38)	0.857
Median platelet recovery, days (range)	13 (10–56)	15 (9–330)	0.155
Hematopoietic function spontaneous recovery	0 (0.00)	2 (5.88)	0.501
Secondary graft failure, no. (%)	1 (3.85)	0 (0.00)	0.433
Delayed platelet recovery, no. (%)	1 (3.85)	4 (11.76)	0.530
Platelet graft failure, no. (%)	1 (3.85)	1 (2.94)	1.000
Infection, no. (%)			
Febrile neutropenia	5 (19.23)	2 (5.88)	0.234
Pulmonary infections	4 (15.38)	7 (20.59)	1.000
Septicemia	4 (15.38)	4 (11.76)	1.000
Urinary infection	0 (0.00)	1 (2.94)	1.000
Mucositis/ stomatitis	3 (11.53)	2 (5.88)	0.728
Viremia	0 (0.00)	1 (2.94)	0.378
Upper airway	0 (0.00)	2 (5.88)	0.208
Others	1 (3.85)	3 (8.23)	0.720
Early mortality	0 (0.00)	2 (5.88)	0.208
Causes of death, no. (%)			
GVHD	1 (3.85)	2 (5.88)	0.720
Infection	2 (7.69)	2 (5.88)	0.781
Thrombotic microangiopathy	0 (0.00)	1 (2.94)	0.378
Median follow-up time among living patients, months (range)	44 (38-77)	92 (28–102)	0.001

HSCT, haematopoietic stem cell transplantation; ATG-F: fresenius-antithymocyte globulin; p-ALG, anti-human T lymphocyte porcine immunoglobulin; BM, bone marrow; PB, peripheral blood; MNC, mononuclear cell; TRM, transplantation related mortality; GVHD, graft-versus-host disease.

Health-related quality of life

As shown in Table 3, patients in the ATG-F group functioned comparably or better than the p-ALG group on HRQoL, which was indicated by comparable or higher scores in PCS, physical functioning, role-physical, general health, MCS, vitality, social functioning and mental health. Patients in the ATG-F group scored better in role-physical ($P = 0.006$), general health ($P = 0.029$) and PCS ($P = 0.009$) compared with the p-ALG group.

Relapse, treatment-related mortality, and survival

The median follow-up time among living patients was 44 months (range, 38 – 77) and 92 months (range, 28 – 102) in the ATG-F and p-ALG groups, respectively. No patients relapsed during the follow-up period in the two groups. The TRM rate was 11.54% in the ATG-F group and 15.15% in the p-ALG group ($P = 0.703$; Figure 2). The causes of TRM included GVHD in one case and infection in two cases; GVHD in two cases, infection in two cases, and thrombotic microangiopathy in one case, respectively. The estimated overall survival (OS) at 5 years was 88.5% ± 6.3% in the ATG-F group and 82.4% ± 6.5% in the p-ALG group ($P = 0.515$; Figure 3A). The estimated FFS at 5 years was 84.6% ± 7.1% in the ATG-F group and 79.4% ± 6.9% in the p-ALG group ($P = 0.579$; Figure 3B).

Discussion

MRD-HSCT for young and adult patients remains the treatment of first choice for SAA [1]. Alternative donor types should be considered in the absence of a MRD in cases where patients fail to respond to IST. URD, haplo-HSCT, or UCBT are generally regarded as a salvage treatment option for SAA due to the high rate of GF and refractory GVHD [6,7,14,19,35,36]. For recipients of MRD-HSCT, Flu/Cy/ATG or Cy/ATG led to the best survival and are considered optimal transplant conditioning regimens. In recipients of URD, there were no differences in survival between regimens. Rabbit-derived ATG was associated with a lower risk of grade II to IV aGVHD but not cGVHD [11]. The conditioning regimen was a combination of BU/Cy/ATG or Flu/Cy/TBI/ATG for haplo-identical transplantation [6,14].

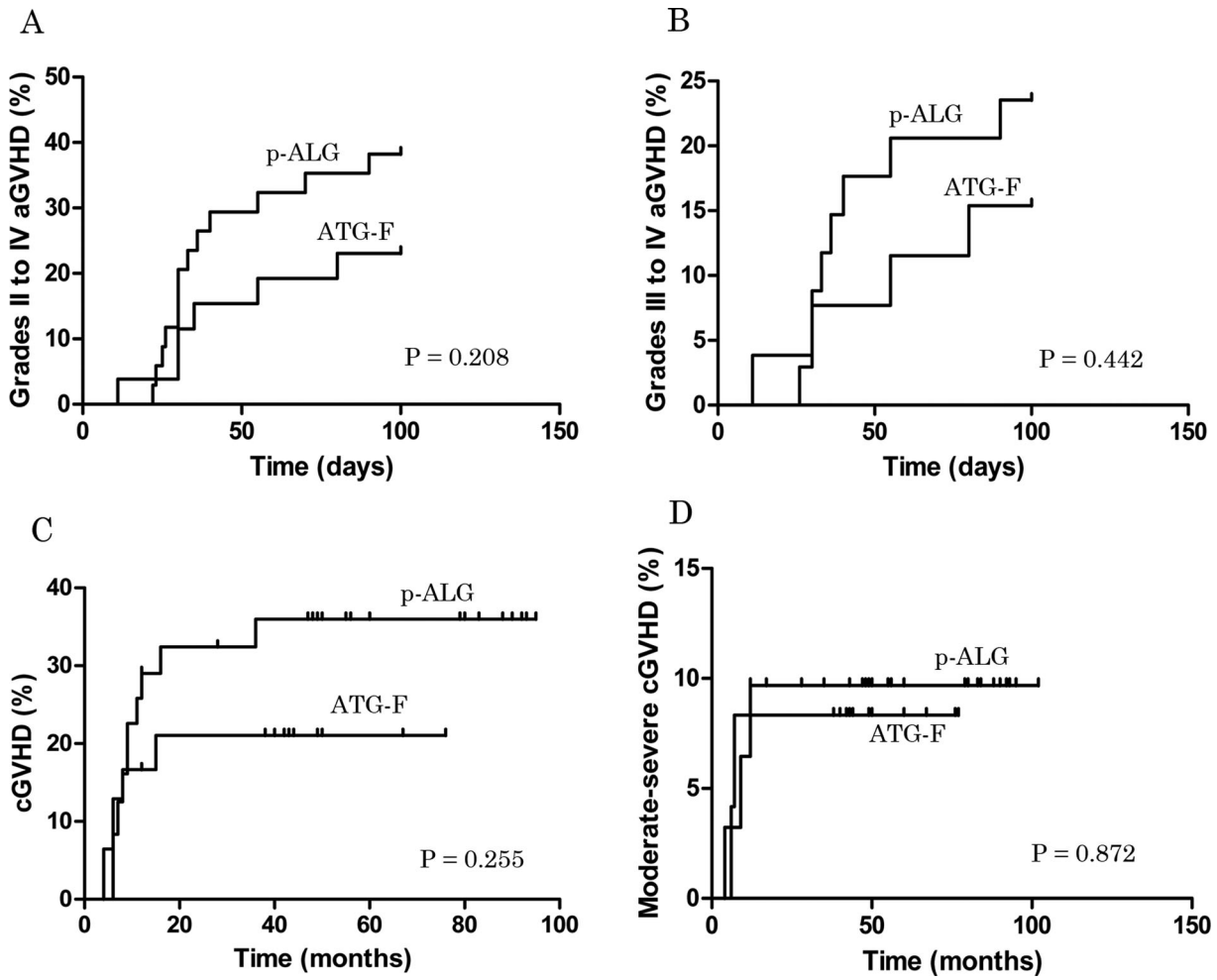

Figure 1. The cumulative incidences of GVHD. (A) The cumulative incidence of grades II to IV aGVHD on day +100 was 23.08% ± 8.26% and 38.24% ± 8.33% in the ATG-F and p-ALG groups, respectively ($P = 0.208$). (B) The cumulative incidence of grades III to IV aGVHD on day +100 was 15.39% ± 7.08% and 23.53% ± 7.28% in the ATG-F and p-ALG groups, respectively ($P = 0.442$). (C) The cumulative incidence of cGVHD was 21.05% ± 8.38% and 35.97% ± 8.71% in the ATG-F and p-ALG groups, respectively ($P = 0.255$). (D) The cumulative incidence of moderate-severe cGVHD was 8.33% ± 5.64% and 9.68% ± 5.31% in the ATG-F and p-ALG groups, respectively ($P = 0.872$).

ATG is an important component of conditioning regimens for preventing GVHD and prevent GF in SAA patients undergoing HSCT.

In this study, we assessed the differences between p-ALG and ATG-F formulations in the conditioning regimen for HSCT in SAA. We analyzed the efficacy, survival, and safety profiles in the two groups. The hematopoietic recovery was similar in the ATG-F group and the p-ALG group. In the ATG-F group, all of the patients achieved successful donor myeloid engraftment, one patient experienced secondary GF, one patient demonstrated delayed platelet recovery, and one patient demonstrated platelet GF. In the p-ALG group, two patients experienced early mortality,

Table 3. SF-36 scores for survivors by therapy (median, range).

HQoL measure	ATG-F	*p*-ALG	*P*
Total participators, n	20	19	
Physical			
Physical component summary	83.19 (77.25–91.75)	73.00 (61.25–81.75)	0.009
Physical functioning	90.00 (85.00–95.00)	85.00 (80.00–95.00)	0.093
Role-physical	75.00 (75.00–100.00)	50.00 (25.00–75.00)	0.006
Bodily pain	100.00 (95.50–100.00)	100.00 (81.50–100.00)	0.631
General health	67.00 (54.50–77.00)	57.00 (40.00–67.00)	0.029
Psychological			
Mental component summary	92.81 (82.75–95.69)	87.88 (69.88–91.13)	0.103
Vitality	87.50 (76.25–93.75)	80.00 (70.00–85.00)	0.125
Social functioning	93.75 (87.50–109.38)	87.50 (75.00–87.50)	0.053
Role-emotional	100.00 (100.00–100.00)	100 (66.67–100.00)	0.517
Mental health	86.00 (72.00–95.00)	84.00 (72.00–92.00)	0.755

HRQoL, health-related quality of life; ATG-F, fresenius-antithymocyte globulin; *p*-ALG, porcine antihuman lymphocyte globulin.

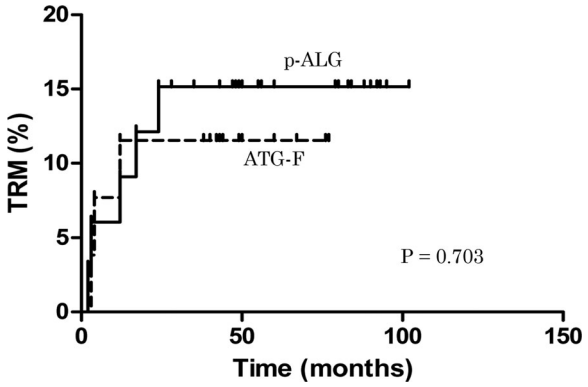

Figure 2. The TRM rate during follow-up. The TRM rate was 11.54% in the ATG-F group and 15.15% in the p-ALG group ($P = 0.703$).

and among the evaluable 32 patients, two UCBT patients experienced hematopoietic function spontaneous recovery. The other 30 patients achieved successful donor myeloid engraftment. Four patients demonstrated delayed platelet recovery and one patient demonstrated platelet GF.

HSCT for SAA has made significant progress over the past decade; this is true especially for alternative donor transplants including URD, CBT, and haplo-identical grafts [4,8,9,37]. Although antithymocyte globulin had been widely used in HSCT to its ability to prevent acute and chronic GVHD. Most previous studies have focused on two rabbit ATG products: ATG-T and ATG-F. They exert immunomodulatory functions primarily via in *vivo* depletion of T-lymphocytes [38]. Paiano S et al. compared two rabbit ATG products in allo-HSCT after reduced intensity conditioning (RIC) for hematologic malignancies. There were no differences in OS, disease-free survival (DFS), relapse incidence, GF, infectious complications, immune reconstitution, and acute or chronic GVHD [39]. Nicola Polverelli et al. compared the two different ATG formulations in a cohort of 77 allo-HSCT patients transplanted from URD. Their results suggest a different immunological activity for the different ATG, with ATG-F ensuring a more extensive and long-lasting effect on more severe forms of cGVHD [27]. Huang et al. did a retrospective analysis of patients who underwent HLA-mismatched allogeneic peripheral blood stem cell transplantation from unrelated donors (UR-PBSCT) and received pre-transplant r-ATG or ATG-F. This was the first comparison of two commonly used ATG preparations administered at fixed doses in HLA-mismatched UR-PBSCT patients, and results suggested that ATG-F is as effective as r-ATG and had fewer adverse effects [26]. In our findings, a trend was observed showing that ATG-F was associated with less GVHD but more infections. The 5-year OS was 88.5% ± 6.3% and 82.4% ± 6.5% in the ATG-F and p-ALG groups, respectively ($P = 0.515$). The infection rate was 61.53% in the ATG-F group and 47.05% in the p-ALG group ($P = 0.265$). The overall mortality rate was not different between the ATG-F and p-ALG groups; infection-related deaths were 7.69% vs. 5.88%, ($P = 0.781$) and GVHD related deaths were 3.85% vs. 5.88%, respectively ($P = 0.720$). Others have noted that the timing and dose of ATG are important to outcomes of engraftment, infection rate, and survival post HSCT. Some studies have shown that higher doses of ATG are associated with lower rates of GVHD but higher rates of infection, including post-transplant lymphoproliferative disorder [25,40]. In addition, there are likely other unmeasured or unknown factors that may have affected GVHD rates

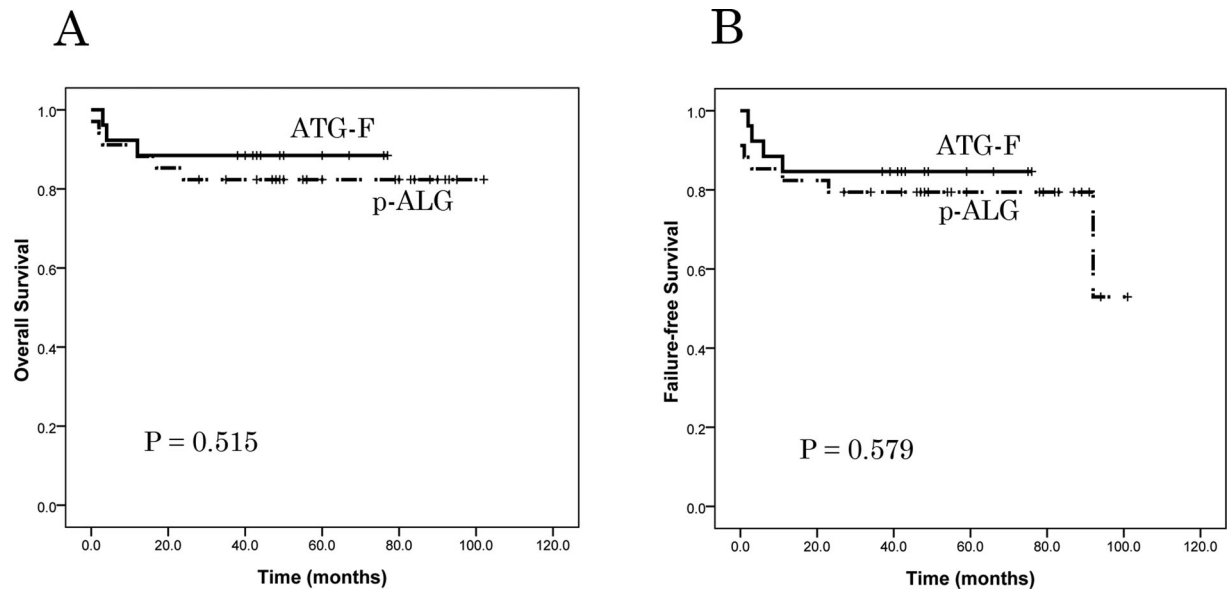

Figure 3. Patient overall survival (OS) and failure-free survival (FFS), as assessed using Kaplan-Meier analysis. (A) The probability of 5-year OS was 88.5% ± 6.3% in the ATG-F group and 82.4% ± 6.5% in the p-ALG group ($P = 0.515$). (B) The probability of 5-year GFFS was 84.6% ± 7.1% in the ATG-F group and 79.4% ± 6.9% in the p-ALG group ($P = 0.579$).

and survival [41]. This should be considered when designing clinical protocols.

For SAA patients, OS is no longer the only parameter used to determine the optimal treatment. Survival rates after HSCT have improved considerably during the past 30 years. There is a growing population of survivors, particularly in individuals who received HSCT as children. To date, no assessments on HRQoL in long-term SCT survivors for SAA have been reported. Issues of the long-term side-effects of intensive therapies, return to prior activities, and reconstitution of normal levels of functioning are of tremendous importance for patients and their relatives during the decision-making process leading to admittance for or refusal of SCT. SAA patients experience poor quality of life, including severe fatigue, poor global health status, impaired functioning, pain, and dyspnea. Although many studies have evaluated the HRQoL of patients after HSCT, to the best of our knowledge, this study was the first comparative trial focused on HRQoL in patients receiving p-ALG or ATG-F as the conditioning regimen for allo-HSCT. There are a few studies focused exclusively on recipients of HSCT for SAA; however, the instruments used to measure HRQoL were heterogeneous, making comparisons difficult. Many studies have suggested that 25%−93%, 63%−77%, and 84% of those who received MRD, URD, or HSCT, respectively, exhibited strong recovery in terms of general health, physical health, and mental health [42]. Patients receiving HLA-haploidentical/partially matched related allo-HSCT can achieve desirable HQoL comparable with those receiving HLA-identical sibling allo-HSCT [43]. Measured by multivariate analysis, cGVHD (especially extensive cGVHD) was the most common adverse factor affecting HRQoL, while male gender status, lower age when receiving allo-HSCT, and returning to work or school were associated with positive impacts on at least one subscale. Also, long-term survivors exhibit better HRQoL [43–45]. Comparing the two groups, patients in the ATG-F group had higher scores on role-physical ($P = 0.006$), general health ($P = 0.029$), and PCS ($P = 0.009$) than the p-ALG group.

In conclusion, these results suggest that ATG-F and p-ALG in an allo-HSCT conditioning regimen for SAA had similar outcomes in engraftment, infection, TRM, GVHD, OS, and FFS. However, ATG-F group survivors exhibited better HRQoL than those in the p-ALG group. Our study is limited by its retrospective nature and relatively few patients. Therefore, further large-scale multi-centre cooperative studies should be conducted to completely evaluate our results.

Authors' contributions

Depei Wu designed the research; Yanming Zhang, Limin Liu, Yejun Si analyzed the data and wrote the paper; and all authors provided patient data and gave final approval for the paper.

Data availability statement

The datasets generated during and/or analyzed during current study are available from the corresponding author on reasonable request.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Ethics approval

This study was approved by the hospital Ethics Committee, and this study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent

All of the patients and donors provided written informed consent for this protocol.

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Full Length Article
Haploidentical

Haploidentical Transplantation with Modified Post-transplantation Cyclophosphamide for Patients with Primary Aplastic Anemia: A Multicenter Experience

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A B S T R A C T

Aplastic anemia (AA) is a life-threatening hematological disorder that can be cured by hematopoietic stem cell transplantation. Haploidentical transplantation becomes an alternative choice for patients in the absence of a matched sibling donor. We used a retrospective study aimed to confirm the feasibility of busulfan-based modified post-transplantation cyclophosphamide (PTCY) strategy in haploidentical hematopoietic stem cell transplantation for AA patients. We analyzed the outcomes of 27 patients from 3 clinical centers who had undergone haploidentical transplantation between October 2018 and July 2020. The modified condition regimen consisted of anti-thymoglobulin/antilymphocyte globulin, fludarabine, busulfan and low-dose cyclophosphamide, and high-dose cyclophosphamide, mycophenolate mofetil (MMF) and tacrolimus were administered as graft versus host disease (GVHD) prophylaxis after transplantation. The median follow-up time was 370 (range 65-721) days. One patient developed primary graft failure, and successful engraftment was observed in 96.29% (95% confidence interval [CI], 93.45%-97.91%) of patients. The median times for neutrophil and platelet engraftment were 13 (range 11-18) days and 13 (range 11-28) days, respectively. The most common regimen-related toxicity was bladder toxicity, followed by stomatitis and gastrointestinal toxicity. The cumulative incidence of grade II-IV aGVHD was 25.93% (95% CI, 5.84%-52.64%), whereas the cumulative incidence of grade III-IV aGVHD was 7.4% (95% CI, 0%-52.16%). Chronic GVHD was observed in 3 patients by the end of follow-up. All 27 patients are alive, with a failure-free survival rate of 96.30% (95% CI, 6.49%-99.47%) and GVHD relapse-free survival rate of 88.89% (95% CI, 69.39%-96.28%). Virus reactivation was comparable, with rates of 53.54% for cytomegalovirus (CMV) reactivation and 41.57% for Epstein-Barr virus, but the CMV diseases and post-transplantation lymphoproliferative disorder were rare. Our study using haploidentical transplantation with modified PTCY demonstrated an encouraging result with prolonged survival and reduced complications for aplastic anemia patients.

Aplastic anemia (AA) is a clinical syndrome characterized by bone marrow failure and hematopoietic stem cell (HSC) deficiency. Although some patients with AA can exhibit an improvement or even be cured by immunosuppressive therapy (IST), including cyclosporine A (CsA) or anti-thymoglobulin (ATG), the possibility of disease relapse or the risk of malignant transformation persists [1]. Therefore allogeneic hematopoietic stem cell transplantation (allo-HSCT) is recommended as the first-line treatment for patients with aplastic anemia, particularly for those with severe aplastic anemia (SAA), for whom a matched sibling donor (MSD) is available [2,3].

Data from the Center for International Blood and Marrow Transplant Research showed that the 3-year survival of AA patients receiving MSD allogeneic HSC transplantation was 93% (<18 years old) or 79% (≥18 years old) [4]. However, only 25% to 30% of patients with matched sibling donors could benefit from HSCT. For those lacking an MSD, haploidentical-SCT (haplo-SCT) has been increasingly proposed as an alternative therapy [5]. However, low engraftment, severe infectious complication, and high graft-versus-host disease (GVHD) showed

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unsatisfactory results compared with MSD-HSCT [6]. In the past 2 decades, studies have proposed various haplo-SCT protocols to improve outcomes, including ex vivo graft T-cell depletion, immune tolerance induced by granulocyte colony-stimulating factor (G-CSF) [7] and post-transplantation cyclophosphamide (PTCY) [8].

At the beginning of this century, Chang et al. [9] proposed a haplo-SCT protocol on the basis of G-CSF–induced immune tolerance combined with ATG as a conditioning regimen, which was widely applied in the clinic to treat various hematology disorders and revealed a similar efficacy to MSD-SCT [10-13]. A result of a multicenter clinical trial from Xu et al. [14] reported that the overall survival (OS) rate of haplo-SCT based on G-CSF combined with ATG as GVHD prophylaxis for SAA was up to 89%, whereas the incidence rates of grade II-IV aGVHD were 33.7% and 22.4% of the chronic GVHD (cGVHD) rates, respectively, indicating the success of haplo-SCT. However, we expect a lower incidence of GVHD, which we do not want to observe in nonmalignant diseases.

Recently, several studies revealed the mechanisms by which PTCY inhibits GVHD, including the selective inhibition of activated alloreactive T cells and regulation of Treg [15,16]. In 2008, Luznik et al. [17] used PTCY as GVHD prophylaxis for haplo-SCT, showing a relatively low incidence of aGVHD compared with a previous protocol, without affecting the engraftment of donor HSC and reconstitution of a donor-derived immune system in the recipient.

However, the attempt of the PTCY regimen in haplo-HSCT for nonmalignant diseases such as AA is limited. In 2017, DeZern et al. [18,19] used high-dose cyclophosphamide at 50 mg/kg/d on days 3 and 4 combined with a nonmyeloablative conditioning regimen comprising ATG, fludarabine, low-dose cyclophosphamide, and total body irradiation (TBI) named the Baltimore regimen to expand the donor pool in AA patients with a successful result. Data from EBMT presented a better OS in patients conditioned with the Baltimore regimen than in those with non-Baltimore regimens [20]. On the basis of the above studies, we hypothesized that a modified regimen using busulfan to replace TBI could improve the haplo-HSCT outcomes for patients with AA. Therefore we retrospectively investigated AA patients who had received allo-HSCT at 3 clinical centers and were undergoing a G-CSF–based fludarabine (Fu)/ busulfan (Bu)/ATG conditioning regimen combined with high-dose PTCY, followed by tacrolimus and MMF as GVHD prophylaxis. We aimed to determine the efficacy and safety of this modified PTCY regimen for haplo-HSCT in AA patients.

METHODS

Patients

We retrospectively analyzed 27 patients diagnosed with SAA or transfusion-dependent NSAA who had received allo-HSCT at the Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology (n = 21), Wuhan No. 1 Hospital (n = 4), and The Central People's Hospital of Yichang (n = 2) from 2018 to 2020. All the patients received a modified PTCY regimen as described below. The AA diagnosis and severity classification were determined according to the International Aplastic Anemia Study Group [21]. All the patients and donors had undergone the donor-specific antibody assay before transplantation. The study was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, in accordance with the Declaration of Helsinki and was granted a waiver to require informed consent from the study participants.

Transplantation Protocol

The transplantation procedure included the conditioning regimen, GVHD prophylaxis, graft collection, and supportive care. The conditioning regimen was the modified Baltimore protocol comprising the following: porcine-antilymphocyte globulin (ALG) 90 mg/kg on days −11 to −8 or ATG 6 mg/kg intravenously on days −11 to −10, low-dose cyclophosphamide at 14.5 mg/kg intravenously on days −9 to −8, fludarabine at 30 mg/m^2 intravenously on days −7 to −3 and busulfan at 3.2 mg/kg intravenously on days −4 to −3.

All transplantation recipients received a combination of PTCY, mycophenolate mofetil (MMF) and tacrolimus as GVHD prophylaxis. High-dose cyclophosphamide was administered from day 3 to day 4 at 50 mg/kg. Tacrolimus was administered from day −1 (the target concentration was adjusted to 8-10 ng/mL), with oral administration for up to 1 year after transplantation and then tapered and discontinued over the following 2 to 3 months. MMF was administered orally at 0.5 to 1 g every 12 hours from day 1 to day 30 after transplantation. The entire protocol is presented in Figure 1.

Donors were treated with subcutaneous G-CSF at a dosage of 10 μg/kg at day −4 before graft collection. Bone marrow stem cells were collected at day 0, and peripheral blood stem cells (PBSCs) were collected on the next day.

All the patients were hospitalized in sterile laminar flow wards. Oral levofloxacin, ganciclovir, acyclovir, and sulfamethoxazole were applied for infection prophylaxis. Mesna was applied to prevent hemorrhagic cystitis because of the high dose of cyclophosphamide. Routine blood tests were performed every day, and G-CSF was administered at 5 μg/kg until neutrophil recovery. Transfusion was performed if the platelet level was $<20 \times 10^9$/L or hemoglobin level was <60 g/L to prevent bleeding or hypoxia, respectively, until engraftment.

Definition and Assessments

Neutrophil engraftment was defined when the absolute neutrophil count exceeds 0.5×10^9/L for the first 3 consecutive days, and platelet engraftment was considered as the first 7 consecutive days with a platelet count of at least 20×10^9/L without transfusion. Acute GVHD (aGVHD) and cGVHD were defined and graded according to standard international criteria (Glucksberg-Seattle criteria) [22]. Patients were considered as having primary graft failure when the neutrophil count did not exceed 0.5×10^9/L for 3 consecutive days by 28 days after HSCT with low donor chimerism. Secondary graft failure was

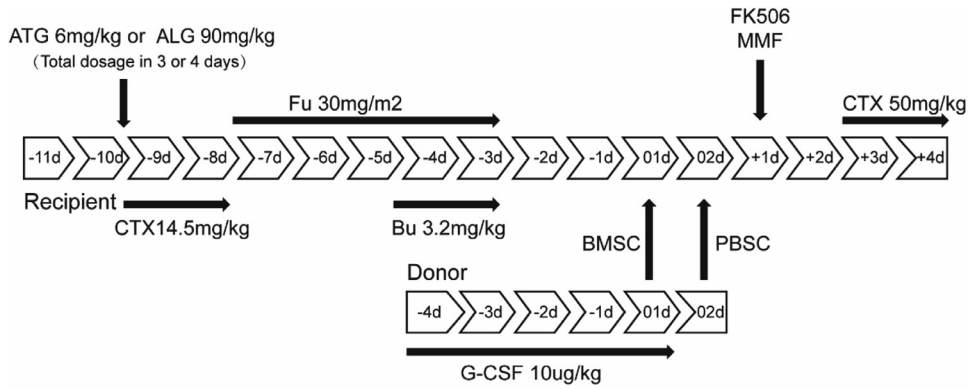

Figure 1. Conditioning regimen: The total dosage of porcine-ALG at 90 mg/kg or ATG at 6 mg/kg was administered intravenously on days −11 to −8, followed by low-dose cyclophosphamide at 14.5 mg/kg intravenously on days −9 to −8, fludarabine at 30 mg/m^2 intravenously on days −7 to −3 and busulfan at 3.2 mg/kg intravenously on days −4 to −3. High-dose cyclophosphamide was administered from day 3 to day 4 at 50 mg/kg. FK506 and MMF were administered as GVHD prophylaxis from day 1.

defined as neutropenia with low or absent donor chimerism in patients with a history of engraftment. Poor graft function was defined as neutropenia but with complete donor chimerism [23].

Chimerism analyses were evaluated by chromosomal fluorescence in situ hybridization for sex-mismatched patients and multiplex short tandem repeat polymerase chain reaction for sex-matched patients. Full donor chimerism was considered as the presence of >95% donor cells. OS was defined as the date of HSCT to death or the last follow-up for any cause. GVHD relapsed-free survival was defined as the date of HSCT to the date of events of grade III-IV acute GVHD, extensive chronic GVHD, relapse, or death. Failure-free survival (FFS) was defined as survival without treatment failure, such as death, graft failure, or relapse. Regimen-related toxicity was defined and graded according to the Bearman criteria [24].

Virus Monitoring

Cytomegalovirus (CMV) in plasma and Epstein-Barr virus (EBV) in plasma and mononuclear cells in peripheral blood (PBMC) were monitored twice a week until 180 days after HSCT by quantitative real-time polymerase chain reaction assays. Virus activation was defined as the CMV DNA or EBV DNA copy number exceeding 500 copies/mL in plasma or PBMCs.

Statistical Analysis

Continuous variables that did not conform to the normal distribution were described by medians (range) and classified variables by frequencies. Survival curves were plotted using the Kaplan-Meier method. All the data were statistically analyzed using SPSS 22.0 software.

RESULTS

Patient Characteristics

Twenty-seven patients from 3 clinical centers received allo-HSCT using the modified PTCY regimen. The patient characteristics are summarized in Table 1. The median follow-up time was 370 days (range 65-721 days). Twenty-one patients were diagnosed with SAA, and the other 6 patients were diagnosed with transfusion-dependent NSAA. The median age of all the patients was 25 years (range 5-52 years). Seventeen patients (62.9%) had undergone at least 1 previous line of treatment including IST. The graft sources of 24 patients (88.9%) were bone marrow stem cells and PBSCs, and those of the other 3 patients were PBSCs only. The blood types of 12 (44.4%) patients were matched between the donors and recipients, where as those of 15 patients were mismatched.

Engraftment

In this study, the median number of CD34+ cells in the graft was 6.75×10^6/kg (range $1.82\text{-}17.07 \times 10^6$/kg). Successful engraftment was observed in 96.29% (95% CI, 93.45%-97.91%) of patients. Despite the development of primary graft failure with 13% donor chimerism within 28 days after transplantation in 1 patient who had received secondary transplantation then, the other recipients achieved myeloid engraftment within 20 days and the median time of neutrophil engraftment was 13 days (95% CI, 12.4-13.6 days). Similarly, except for one patient with primary graft failure, the remaining patients achieved platelet engraftment within a median time of 13 days (95% CI, 11.8-14.1 days). The cumulative incidence of engraftment is shown in Figure 2.

Regimen-Related Toxicity

All the recipients received the modified PTCY conditioning regimen as mentioned above. Most of the conditioning-related toxicities were mild or moderate. In total, 16 (59.2%) patients exhibited varying degrees of toxicity (Table 2). The most common toxicity was stomatitis, with 8 (29.6%) patients affected, followed by bladder toxicity and gastrointestinal toxicity. Cardiac, hepatic, and renal toxicities were rare and were only observed in 1 patient whose Eastern Cooperative Oncology Group score was 2 before HSCT. However, ALG-related serum disease manifested by rash, fever, and joint pain was observed

Table 1
Patients Characteristics

Characteristics	Total (%)
Gender	27
Male	17 (63)
Female	10 (37)
Age, year (range)	25 (5-52)
Median time from diagnosis to transplantation, day (range)	55 (21-3941)
Treatment before Transplantation	1 (0-3)
Diagnosis	
SAA	21 (77.8)
NSAA	6 (22.2)
Blood counts before Transplantation	
WBC	1.5 (0.01-6.49)
ANC	0.28 (0.01-2.89)
HGB	69 (46-92)
PLT	21 (5-62)
Donor gender	
Male	25 (92.6)
Female	2 (7.4)
Matched HLA	
5/10	22 (81.5)
6/10	4 (14.8)
8/10	1 (3.7)
Blood type	
Matched	12 (44.4)
Major mismatched	9 (33.3)
Minor mismatched	6 (22.2)
Graft source	
PBSC	3 (11.1)
BMSC+PBSC	24 (88.9)
DSA	
Negative	18 (66.7)
Positive	2 (7.4)
NA	7 (25.9)
CD34+cells $\times 10^6$/kg (range)	5.49 (1.1-13.48)
Neutrophil engraftment (range)	13 (11-18)
Platelets engraftment (range)	13 (11-28)
Follow-up time, day (range)	370 (65-721)

WBC indicates white blood cells; ANC, absolute neutrophil count; HGB, hemoglobin; PLT, platelet; BMSC, bone marrow stem cells; DSA, donor-specific antibody; NA, not available.

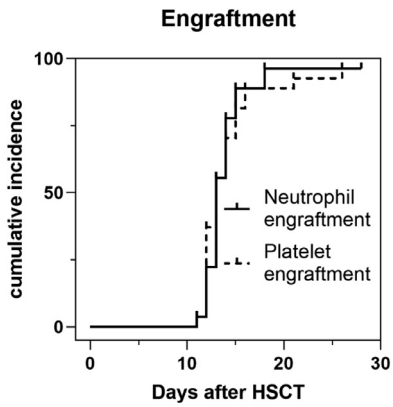

Figure 2. Engraftment: 26/27 (96.3%) recipients were successfully engrafted within 28 days after HSCT. The median time of neutrophil engraftment was 13 days (95% CI, 12.4-13.6 days), and the median time of platelet engraftment was 13 days (95% CI, 11.8-14.1 days).

Table 2
Regimen-Related Toxicity

	Grade 1	Grade 2	Grade 3	Total
GI toxicity	7 (25.9)	0 (0)	0 (0)	7
Bladder toxicity	0 (0)	7 (25.9)	0 (0)	7
Stomatitis	8 (29.6)	0 (0)	0 (0)	8
CNS toxicity	1 (3.7)	0 (0)	0 (0)	1
Pulmonary, cardiac, hepatic and renal toxicity	1 (3.7)	0 (0)	0 (0)	0
Total	17	7	0	24

Regimen-related toxicity was defined and graded according to the Bearman criteria as described above in Methods section.
GI indicates gastrointestinal; CNS, central nervous system.

in 7 patients after ALG administration. These toxicities could be controlled by symptomatic treatment. No death was observed during the conditioning regimen.

GVHD and Other Post-Transplantation Complications

Of all 27 patients, 13 developed aGVHD, 7 (25.9%) had grade II-IV aGVHD, and only 2 (7.4%) developed grade III aGVHD. The cumulative incidence of grade II-IV aGVHD within 100 days after HSCT was 25.93% (95% CI, 5.84%-52.64%), and that for grade III-IV aGVHD was 7.4% (95% CI, 0%-52.16%). However, no patient died because of aGVHD during the follow-up. All the patients developed aGVHD that could be controlled by steroids. Three patients developed limited cGVHD. The data are shown in Figure 3.

Additionally, thrombotic microangiopathy (TMA) occurred in 2 patients and could be controlled by treatment. Engraftment syndrome, characterized by fever and rash, was observed in 1 patient. Posterior reversible encephalopathy syndrome, characterized by epileptic seizures and headaches, was a rare complication that occurred in 1 patient. No patient died because of these post-transplantation complications.

Infection and Virus Activation

Of all 27 patients, 11 (40.7%) developed infection of different degrees. The most common infection site was the lung (4 patients), followed by upper respiratory infection. Only 1 patient developed bacteremia as a result of *Staphylococcus hominis* without septic shock and was controlled by antibiotic administration.

In this cohort, approximately 61.53% (95% CI, 45.43%-74.93%) of patients developed virus activation, including CMV and EBV reactivation, within 1 year after transplantation. The median time of virus activation was 46 days after HSCT. CMV reactivation was more frequent, with a cumulative incidence of 53.54%, than EBV reactivation, with a cumulative incidence of 41.57%. Among the 27 patients, 18 patients received ALG, and the other nine patients received ATG. The 1-year cumulative incidence of CMV reactivation was 57.2% in the ALG group and 48.1% in the ATG group. There was no significant difference between the 2 groups ($P = .584$). Similarly, no significant difference was observed in EBV reactivation between the two groups ($P = .965$). The cumulative incidence of virus reactivation in 2 groups is showed in Supplementary Figure S1. However, only 1 patient developed CMV colitis according to the high number of CMV-DNA copies in stools and was cured by systematic antiviral therapy, and the other 14 patients developed only CMV viremia. The median EBV viral loads in PBMCs are 1.16×10^4 copies/mL (range 1.1×10^3-5.03×10^6 copies/mL). Fortunately, no patient progressed to post-transplantation lymphoproliferative disorder although EBV reactivation was relatively high compared with that in other studies. The cumulative incidence rates of EBV and CMV reactivation are shown in Figure 4.

Survival Outcomes

The median follow-up time was 370 days (range 65-721 days) in our study, and all the patients were alive. The estimated 1-year OS was 100%, and the estimated 1-year GVHD relapse-free survival was 88.89% (95% CI, 69.39%-96.28%). The 1 year FFS was 96.296% (95% CI, 76.49%-99.47%). The survival data of the patients are shown in Figure 5.

DISCUSSION

Allo-HSCT has significantly increased the prognosis of AA patients, with a long-term survival rate greater than 80%. Additionally, recent studies have shown that haploidentical transplantation in the treatment of AA provides an alternative strategy for those without MSDs or matched unrelated donors. However, GVHD, graft failure and other post-transplantation complications limit the application of haplo-HSCT in AA patients.

Recent studies have demonstrated that PTCY is a very attractive option because of its effectiveness in reducing GVHD in haplo-HSCT for hematology malignancies. However, few data have been reported concerning nonmalignant disorders, particularly AA. Notably, relapse was nonnegligible in some hematology malignancies by applying PTCY, likely because of the mitigation of the graft-versus-leukemia effect, although this concern is ignorable in nonmalignant diseases [25]. The first attempt of the PTCY regimen in AA was reported in 2011. Dezern et al. [26] used MSDs and myeloablative conditioning,

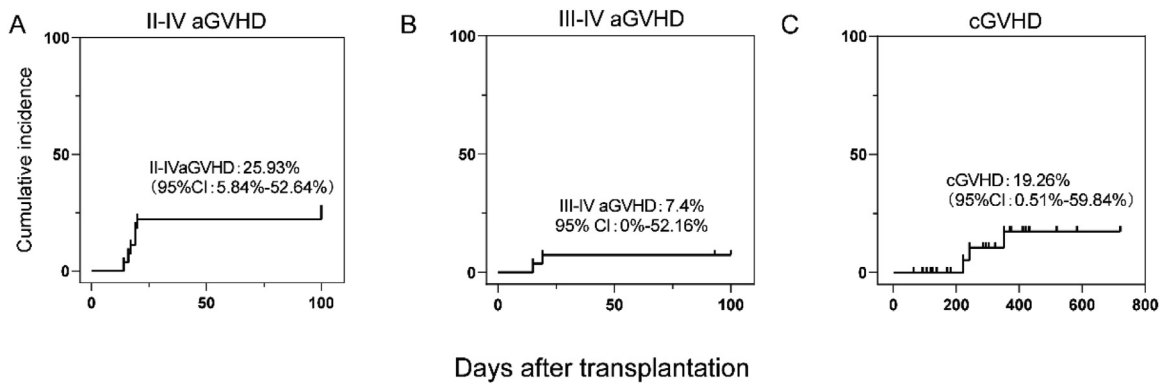

Figure 3. Cumulative incidence of GVHD. (A) Grade II-IV aGVHD. (B) Grade III-IV aGVHD. (C) cGVHD.

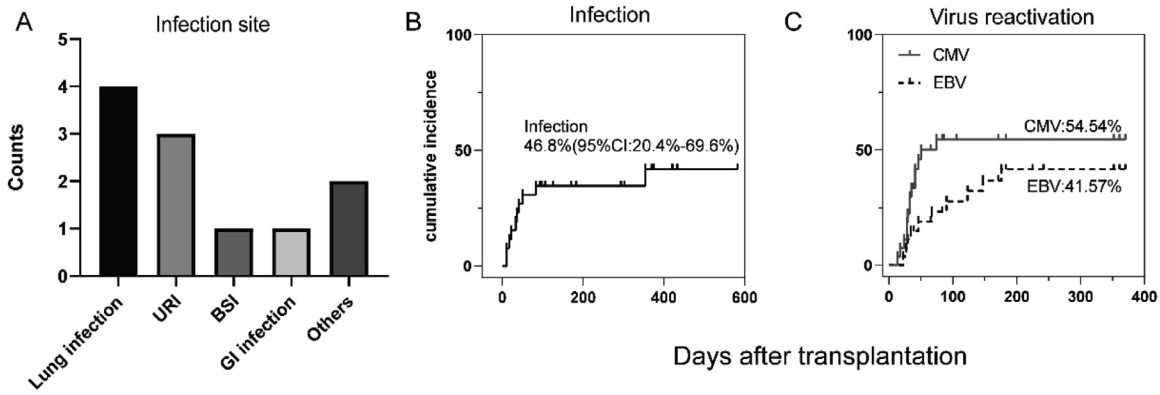

Figure 4. Infection complications: Various infections were observed in 11 of 27 (40.7%) patients. (A) Infection sites: URI indicates upper respiratory infection; BSI, bloodstream infection; GI, gastrointestinal. (B) Cumulative incidence of infection. (C) Cumulative incidence of CMV and EBV reactivation.

with both patients engrafted without GVHD. In 2014 and 2015, researchers from the United Kingdom and Brazil, respectively, applied the PTCY regimen in AA patients, with satisfactory efficacy results [27,28]. A meta-analysis of the European Group for Blood and Marrow Transplantation compared the efficacy of the PTCY regimen in haplo-HSCT for AA patients at different centers and emphasized that the Baltimore regimen, which was proposed by scholars at Johns Hopkins University in 2017 [18], was superior to other PTCY regimens in OS specifically [20].

However, one of the major problems for haploidentical transplantation in AA patients is graft failure, especially in relapsed and refractory patients. Although the traditional TBI-based PTCY regimen significantly reduces the incidence of GVHD, the concerns of delayed myeloid and reduction of engraftment remain. Clay et al. [27] showed that 6/8 patients had neutrophil engraftment successfully while the rate of platelet engraftment was only 62.5%. Similarly, Esteves et al. [28] used the high-dose PTCY regimen and reported the successful platelet engraftment of 75%, which is relatively low in AA patients. Research from EBMT also indicated that the TBI-based regimen had a relatively lower engraftment rate (97.7% versus 91.7%), whereas the Bu-based regimen showed a more rapid and stable engraftment, as well as a lower incidence of graft failure [29]. In China, the Bu-based regimen is used for haploidentical transplantation in AA patients in some clinical centers and results in encouraging engraftments ranging from 94.1% to 98%, but the incidence of GVHD, respectively, is higher than that of the PTCY regimen [30-34]. In addition, we hope that patients undergoing transplantation could have better quality long-term survival, especially for patients with nonmalignant hematology disorders. Among them, secondary tumor after transplantation is an important factor. TBI conditioning has been identified as a risk factor for post-transplantation malignancies in patients with severe AA, especially for pediatric patients [35]. Besides, the long-term effects of radiation exposure on children include growth impairment, cataracts, hypothyroidism, and cognitive impairment. Busulfan, as an alternative to TBI, avoids these radiation sequelae [35]. From the above, the efforts of removing radiation from the conditioning regimen may benefit AA patients. Hence, we performed this study using a modified high-dose PTCY regimen based on FU/BU/CY/ATG conditioning combined with MMF and FK506 as GVHD prophylaxis to explore its feasibility in haplo-HSCT in nonmalignant disorders such as AA.

In our study, the rate of graft failure (GF) the engraftment was superior than traditional PTCY regimen. Only 1 patient developed primary GF and then received secondary transplantation immediately with a successful engraftment. Secondary GF was not observed in all 27 patients. Meanwhile, the neutrophil and platelet engraftment appears more rapid and stable than previous PTCY regimens. As we stated previously, these improvements may be explained by the fact that we used the Bu-based conditioning regimen. Furthermore, we followed the Beijing protocol using G-CSF mobilized bone marrow (GM-BM) and G-CSF mobilized peripheral blood (GM-PB) as the graft sources [7]. Although a higher percentage of CD3+ T cells in GM-PB, the CD34+ cells were more abundant, promoting stable and rapid engraftment [36]. Moreover, for recipients with a higher weight, stem cells derived from the BM may be insufficient while GM-PB was a safe and painless supplement. However, considering the infusion-related CRS associated with

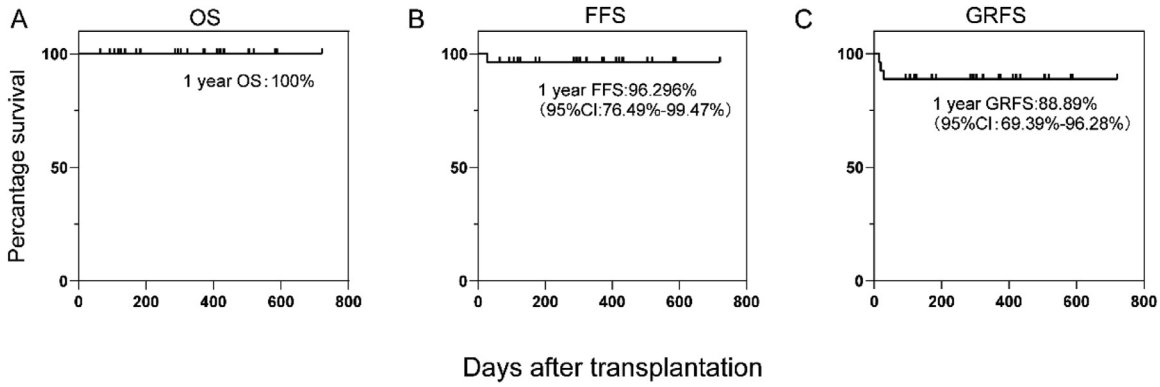

Figure 5. Survival curves. (A) Overall survival. (B) Failure-free survival. (C) GVHD relapse-free survival.

BM-PB that significantly delayed neutrophil engraftment and was related to worse outcomes, we advanced tacrolimus and MMF to +1 day to alleviate and control CRS [37].

Another concern for allo-HSCT in the treatment of AA is the development of severe GVHD. Although the incidence of GVHD in haplo-HSCT occurs more frequently than MSD-HSCT, significant changes were observed in several cases recently. Recent studies on haplo-HSCT using G-CSF/ATG–based conditioning regimen reported the incidence rates of grade II-IV aGVHD of 20%-43.9% and chronic GVHD rates of 15% to -39.3% in AA patients [30-34,38]. Moreover, several small-sample studies, such as those described above, suggested a significant decrease in severe GVHD using the PTCY regimen. Only 13% of patients developed II-IV aGVHD in Esteves' research and in 1 of 8 patients in the studies by Clay et al. [27] and Dezern et al. [28], indicating that the PTCY regimen has an obvious advantage in reducing GVHD compared with Beijing protocol. In our study, the incidence of grade II-IV aGVHD was 25.93%; the trend of reducing GVHD was observed compared with other GVHD prophylaxis strategies. Notably, only two patients developed grade III aGVHD among all 27 patients and achieved remission by systemic treatment. No patient died because of GVHD. Regarding cGVHD, 3 of 27 patients developed limited skin, oral, and ocular cGVHD that could be controlled well. However, because of the relatively short follow-up in some patients, the improvement of cGVHD by our modified PTCY regime requires prolonged follow-up for verification.

Additionally, the high incidence of viral infections is another major concern for allo-HSCT because of the delayed immune reconstitution, particularly CMV, with the incidence of reactivation ranging from 30% to 70% at different centers. Previous studies have demonstrated that the rates of CMV reactivation in haplo-HSCT and MSD patients were 65% and 39%, respectively [39]. Xu et al. [14] also showed a similar result with rates of 68.3% and 39.6%, respectively. A recent study on haplo-HSCT in China reported a rate of CMV reactivation of 51.7% and a rate of EBV reactivation of 28.1% [38]. However, data from 2 independent clinical centers indicated that the use of PTCY was associated with a higher rate of CMV viremia [40]. In our study, the rate of CMV reactivation was 54.54%, which was comparable with other studies, while the rate of EBV viremia was 41.57%. However, few cases of fatal CMV disease occurred during the follow-up period because of more frequent virus monitoring and pre-emptive antiviral therapy. Similarly, EBV-related lymphoproliferative diseases such as post-transplantation lymphoproliferative disorder were not observed during the follow-up period, although the rate of EBV reactivation was relatively high. Some cases could be explained by the baseline of EBV infection in the Chinese population being relatively higher than in the European and American populations. Overall, our data on virus reactivation were acceptable and comparable to those of previous studies and other clinical centers.

The OS and FFS rates in our cohort were greater than 90%, superior to that in previous studies. Data from the Center for International Blood and Marrow Transplant Research predicted an OS rate of 70% in adult AA patients [4]. Xu et al. [38] reported a 3-year estimated OS rate of 86.1% and an FFS rate of up to 85% in AA patients receiving haplo-HSCT. Additionally, a study on EBMT revealed a 2-year OS rate of 78% in AA patients receiving haplo-HSCT based on the PTCY regimen; surprisingly, the 2-year OS rate was higher among patients receiving the Baltimore regimen, up to 93%, than among those receiving other PTCY regimens [20]. Overall, because of the reduced graft failure rate, the improvement of GVHD and monitoring of other post-transplantation complications, the OS of the patients was significantly prolonged. However, although fewer side events were observed within the short median follow-up time, further continuous follow-up is necessary to confirm the efficacy of our protocol.

However, our study has several limitations. First, as a retrospective study, selection bias and recall bias are ineluctable. Besides, we did not separate the patients according to age. Although several pediatric recipients were included in our study, longer follow-up is necessary to clarify the prospect of this modified regimen in pediatric patients. Additionally, the low number of patients and short follow-up period prevented the achievement of sufficient statistical power.

In conclusion, this study demonstrated encouraging outcomes that the modified PTCY regimen based on FU/BU/CY/ATG and combined with FK506 and MMF can be used as prophylaxis in patients with AA and is associated with low rates of aGVHD and prolonged survival. Meanwhile the faster hematopoietic reconstitution and more stable engraftment are the advantages of our regimen. However, viral infections are still a major concern. Further follow-up and large-sample prospective trials are necessary to confirm this modified PTCY regimen.

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Authorship statement: YCZ designed this research; YL analyzed the data and wrote the manuscript; LL collected the data and prepared the tables; NW, YC, JHX, JW, LFH, LLW, LZ, HYW and YX provided the patients' data; JW and YCZ revised the manuscript; all authors approved the final manuscript.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2021.01.018.

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自体外周血干细胞移植治疗系统性红斑狼疮的临床研究

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摘要：目的　探讨自体外周血干细胞移植(autologous peripheral blood stem cell transplantation,APBSCT)治疗难治性系统性红斑狼疮(systemic lupus erythematosus，SLE)的疗效和方法。方法　对13例住院的难治性SLE患者进行APBSCT治疗，动员方案为环磷酰胺4g/m²·d，当白细胞降低到最低值时，应用粒细胞集落刺激因子(G-CSF)5 μg/kg·d，预处理方案为环磷酰胺(50mg/kg·d，-2d，-1d，+1d，+2d)，猪抗人淋巴细胞球蛋白(ALG)(20mg/kg·d，-2d，-1d，+1d，+2d)。结果　随访时间3~15个月，13例患者造血干细胞均成功植活。植活时间：WBC14d(11~16d)；PLT13d(9~18d)。SLEDAI评分(系统性红斑狼疮疾病活动指数)平均降低6分。显效10例(治愈7例，基本治愈3例)，进步2例，死亡1例。常见并发症为发热、呕吐、脱发等。出现4例真菌感染，2例巨细胞病毒感染，6例出现心血管并发症，表现为急性左心衰竭，心律失常等。结论　自体外周血干细胞移植治疗系统性红斑狼疮有良好的近期疗效，但应注意移植过程中各种感染以及心血管并发症。

关键词：系统性红斑狼疮；造血干细胞；移植
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Clinical Research of Autologous Peripheral Blood Stem Cell Transplantation for Systemic Lupus Erythematosus

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Abstract:Objective To evaluate the therapeutic effect and methods of autologous peripheral blood stem cell transplantation (APBSCT) in patients with systemic lupus erythematosus(SLE).**Methods** Thirteen patients with refractory SLE received APBSCT.The mobilization program was cyclophosphamide (CTX) 4g/m²·d.When leukocyte decreased to the minimum value,G-CSF5μg/kg·d is applicable.The condition program was CTX (50mg/kg·d)for 4 days,ATG 20mg/kg·d for 4 days,the hematopoietic stem cells were infused to the patients after conditioning.**Results** The follow-up time is 3 to 15 months.SLEDAI scores have averagely decreased by six points.The time of engrafement:WBC 14d(11~16d);PLT13d(9~18d).Among these patients,10 patients achieved remarkably excellent effects(7 patients arrived at elementary curement and 3 patients acquired good effects),2 patients improved,1 patient died.All cases had been successfully engrafted.The transplantation related mortality was 0%.The frequent complications during the transplantation were fever,vomiting,heart failure,arrhythmia and so on.Among these patients,there are four cases of fungous infection,two cases of cytomegalovirus infection,and six cases of cardiovascular complications that mainly manifested as acute left ventricular failure and arrhythmia.**Conclusions** The APBSCT for SLE has got remarkable therapeutic effect in short -term observation,at the same time,we should pay attention to various infections and cardiovascular complication in this process.

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Key wrds: systemic lupus erythematosus; hematopoietic stem cell; transplantation

系统性红斑狼疮(systemic lupus erythematosus，SLE)是一组异常干/祖细胞增殖分化的多克隆T、B淋巴细胞疾病，主要损害肾脏，皮肤，关节，血液系统等。常规治疗为应用糖皮质激素，细胞毒药物等免疫抑制剂，但少数患者对于免疫抑制剂的治疗反应差。而且患者常需终身免疫抑制治疗，一些患者死于疾病本身、药物治疗的不良反应、感染等，临床处理相当棘手。应用自体外周血干细胞移植(autologous peripheral blood stem cell transptantation，APBSCT)重建正常免疫体系，可能是根治本病的方法。我们对13例SLE患者进行自体外周血干细胞移植术，取得令人满意的临床效果，移植过程安全，未发生移植相关严重并发症。

1 材料与方法

1.1 一般资料

本院血液科2005年9月至2007年8月共实施自体外周血干细胞移植术病例13例，女性12例，男性1例，年龄14~49岁，平均26岁，病程0.5~10年，平均4年(见表1)。诊断符合美国风湿学会(ACR)1997年修订的诊断标准，SLE疾病活动性指数为SLEDAI评分10~33分，全部因病情控制不理想，不能耐受常规治疗等原因，患者及家属要求做自体外周血干细胞移植术。

1.2 方法

1.2.1 造血干细胞的动员：于移植前1~3个月进行，

表1 13例SLE病例资料

序号	年龄(岁)	病史(年)	SLEDAIScore(分)	重要脏器损伤	MNC采集干细胞(10^8/kg)
1	23	3	23	肾脏	2.87
2	21	10	22	关节	2.91
3	23	2	13	肾脏,脑病	3.92
4	20	5	27	心脏,脑病	3.72
5	38	6	33	肾脏,脑病	4.28
6	34	1	30	脑病	2.67
7	22	1	10	——	2.90
8	24	7	10	肾脏	2.76
9	25	5	24	心,肾,脑	3.83
10	49	2	13	关节,肾	2.97
11	18	0.5	27	肾脏,	3.74
12	24	12	29	肾脏,脑病	3.56
13	14	3	20	肾脏,脑病	2.85

环磷酰胺4g/m^2·d静脉滴注，同时予以水化，碱化，每天检查血象，当白细胞降低到最低值时，粒细胞集落刺激因子(G-CSF)5 μg/kg·d，皮下注射。

1.2.2 造血干细胞的采集 当患者单核细胞和淋巴细胞总数达到确定相应的参数值(外周单个核细胞记数大于3×10^9/L，或者外周血白细胞总数大于20×10^9/L)，使用血细胞分离机采集外周血干细胞，平均采集单个核细胞数为3.24×10^9/kg，采集的细胞置于-80℃冻存。

1.2.3 预处理方案 环磷酰胺(50mg/kg·d，-2d，-1d，+1d，+2d)静脉滴注，充分水化，碱化，并静脉注射美司钠预防出血性膀胱炎。猪抗人淋巴细胞球蛋白(ALG)(20mg/kg·d，-2d，-1d，+1d，+2d)静脉滴注，为预防其副作用，用甲泼尼龙(500mg/d，-2d，-1d，+1d，+2d)。静脉注射前列腺素E120 μg/d(-5d～+20d)，预防肝静脉闭塞病。另外保护心，肝，肾等脏器的功能。

1.2.4 感染的预防 患者经过肠道消毒，全身药浴后进入无菌层流病房，予以无菌护理，进无菌饮食。预处理后开始口服氟哌酸、黄连素清洁肠道，氟康唑预防真菌感染，丽科平预防病毒感染。移植后5d开始使用G-CSF 5 μg/kg·d至造血重建。

1.2.5 干细胞回输 预处理后，将冻存的造血干细胞放入42℃水浴箱快速解冻，复苏，然后经锁骨下静脉插管快速回输。白细胞>0.5×10^9/L，血小板>20×10^9/L时，表示移植成功。

2 结果

2.1 移植过程中的血象变化 WBC最低值(中位数)0.2×10^9/L(0.02~0.5)，持续5~8d；PLT最低值4×10^9/L(1~10)，持续6~10d。造血恢复时间：WBC>2.0

×10⁹/L，14d(11~16d)；PLT>20×10⁹/L，13d(9~18d)；粒细胞>1.5×10⁹/L，14d(11~18d)；有核细胞>1.0×10⁹/L，12d(9~15d)；淋巴细胞/NK细胞>0.8×10⁹/L，23d(20~29d)。移植过程中输注红细胞8U(2~14U)，输注血小板2.8U(0~5U)。

2.2 临床症状和体征 患者移植过程均安全，动员和预处理相关死亡率为0%，大部分患者移植后3个月内除蛋白尿外其他症状明显好转，3个月以后完全消失，蛋白尿也开始减少。其中一例横贯性脊髓炎患者未再出现大小便失禁现象，生活恢复自理。狼疮脑病患者未再出现。体格检查也同步好转。所有患者糖皮质激素用量明显减少(<12.5/d)。SLEDAI评分明显下降，平均降低6分(见图1)。

2.3 实验室检查 10例患者移植前自身抗体升高，移植后所有患者抗核抗体(ANA)滴度下降，但仅有1例转阴；6例移植前抗dsDNA阳性的患者在移植后转阴。8例患者C3、C4补体下降的患者有6例在3个月后恢复正常，另外2例接近正常。9例患者移植前24小时尿蛋白升高，移植后6例转为正常，另3例好转。10例患者移植前血沉升高，移植后3个月8例恢复正常。

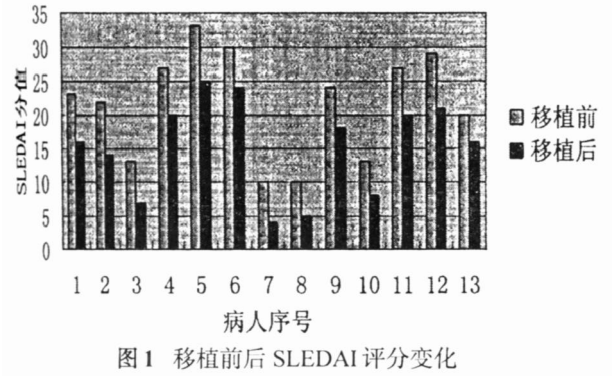

图1 移植前后SLEDAI评分变化

2.4 移植相关并发症

2.4.1 发热 有12例患者均出现发热，持续天数平均5.3d，予以物理降温或者解热药处理。其中有4例患者为真菌感染，予以两性酶素B或伊曲康唑治疗后好转；有2例血培养出大肠埃希菌，予以美罗培南治疗有效；有2例患者在移植后3~4周出现巨细胞病毒感染，予以更昔洛韦抗病毒治疗有效。

2.4.2 心血管并发症 有6例患者出现不同程度的心血管并发症，有3例出现急性左心衰竭，予以强心、利尿等处理后好转；有1例发生室性早搏，口服贝他洛克24h后消失；有2例为房颤，予以扩张冠状血管和应用西地兰后转为窦性。

2.4.3 消化系统并发症 所有患者均出现恶心，呕吐，予以止吐药对症处理后好转。所有患者均有不同程度的食欲不振，予以高营养食物，少食多餐，必要时静脉补充营养。

2.4.4 肝肾等功能变化 所有患者未出现肝肾功能损害，所有患者未出现出血性膀胱炎。

3 讨论

近年来，自体外周血干细胞移植(APBSCT)开始应用于治疗自身免疫性疾病，这无疑是一种极有希望的治疗方法。近10年来，大剂量免疫抑制剂联合自体造血干细胞移植治疗SLE的研究工作已经取得许多重大的进展，不少文献报告了自体外周血干细胞移植在治疗恶性疾病的同时，治愈了患者合并的自身免疫性疾病，并且在自身免疫性疾病的动物模型中得到证实[1]。

APBSCT治疗SLE的确切机制尚不清楚，但可以肯定的是，通过摧毁病态免疫，重建正常免疫细胞体系，原来的产生自身抗体的细胞克隆受到摧毁或者诱导产生对自身抗原的免疫耐受，这是产生治疗效果的主要原因[2]。符粤文等[3]学者研究表明SLE患者经过自体外周血造血干细胞移植后，移植后患者免疫功能抑制可达1年之久，植入的淋巴细胞各个亚克隆的比例发生变化，CD4细胞比例降低，CD4/CD8比例倒置，造成SLE发病的一些寡克隆增生的T淋巴细胞不再是优势细胞群。鉴于此，我们在传统的方法上加用了猪抗人淋巴细胞球蛋白(ALG)(20mg/kg·d，-2d，-1d，+1d，+2d)静脉滴注，其中移植前两天应用ALG的目的是进一步清除患者体内剩余的淋巴细胞，这样彻底清除了体内致病性自身反应性T淋巴细胞，进而降低了移植后患者复发率。移植后两天应用ALG的目的是进一步清除采集的干细胞残存的淋巴细胞，同样起到降低了移植后患者复发率的作用。但是，需要注意的是应用ALG降低了患者的免疫力，更容易发生感染。在我们移植的13例患者中，有2例在移植后1~2d患者出现发热，予以停用ALG，并加强抗感染和支持治疗后得到了有效控制。

自体外周血干细胞移植对于拯救重症SLE和提高患者的生活质量，其短期和长期疗效均得到了肯定[4]。同时，其安全性较好，孙凌云学者和Snowden JA学者估计在我国，动员相关死亡率为1%~2%，SLE移植相关死亡率为6%左右[5,6]。本组13例进行APBSCT的SLE患者均为强的松治疗及CTX冲击治疗无效的病例，候选标准参照了学者Burt RK的选择标准[7]。随访3~15个月，移植后显效10例(76.9%)，进步2例(15.4%)，死亡1例(7.7%)。2例患者减用

强的松，其余由治疗剂量减为维持剂量(<10mg/d)，以补充长期激素治疗所造成的肾上腺功能不足。13例患者移植后造血功能全部正常恢复，未发生预处理相关的不可逆的脏器功能损伤。

因为SLE患者严重的自身免疫失调所致单个或多个器官功能衰竭，尤其是接受干细胞移植者多为病史长，复发及治疗失败者，干细胞移植过程中预处理脏器损害较明显，随着SLE病态免疫被纠正，脏器功能在移植后3月较移植前明显恢复。13例SLE患者在进行APSCT治疗过程中，未发生移植相关死亡，在预处理过程中也未发生致死性的并发症，其中最凶险的1例患者在预处理过程中出现心力衰竭，予以大剂量的利尿剂、吸氧、强心、护心等处理后度过危险期。另外，在移植后出现的真菌、细菌、病毒感染的几例患者经过规范的抗真菌、抗感染、抗病毒治疗后感染得到有效的控制。死亡的1例患者，血象已经恢复正常，凝血象亦正常，其死亡原因为原有的基础疾病—高血压导致的脑卒中。这说明即使患者的脏器损伤比较严重，在合理治疗下仍然可以平安的度过移植难关。

总之我们的研究初步证实自体外周血干细胞移植治疗SLE的治疗方法从总体上来说是安全有效的，但也存在着以下的一些风险：①心血管并发症。 难治性SLE患者一般都有严重的血管炎和心脏损害(如心包炎，心肌炎等)，肾功能也严重受损，患者有严重的低蛋白血症，加上预处理时的大量输液，这些都是在移植过程中发生心血管并发症的诱发因素。所以我们应该积极预防，如补充蛋白纠正低蛋白血症、在预处理时适当控制输液量、适当利尿以减轻心脏负荷等。②各种感染。患者长期使用大量的免疫抑制剂，长期蛋白尿造成的低蛋白血症，预处理时我们使用ALG，这些都是在移植过程中发生感染的危险因素。所以在移植过程中应积极预防真菌、细菌、病毒、原虫感染。③基础疾病造成的其他风险。例如在我们移植组中死亡的一例患者，由于存在着基础疾病高血压，加上长期严重血管炎形成的血管瘤，最后形成脑卒中而导致患者死亡。④复发。由于采用的是自体外周血干细胞移植，患者的基因并未改变，从理论上来说仍然有复发的可能[8]。因此在移植前应该对患者进行全面的评估衡量可能的收益和所面临的风险，这需要我们深入研究评估SLE疗效的方法。从理论上来讲，如果患者在移植后避免了红斑狼疮的诱发因素，使其致病的淋巴细胞无法成为克隆优势，患者就可以长期无病生存，这也是我们期盼的目标。

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·论 著·

双份脐带血移植治疗黏多糖贮积症临床疗效分析

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【摘要】目的 了解黏多糖贮积症患儿非血缘异基因双份脐带血干细胞移植的疗效及特点。方法 回顾性分析 13 例黏多糖贮积症患儿接受双份非血缘异基因脐带血干细胞移植的临床资料，包括受者情况、供者选取情况、移植后粒细胞及血小板植入时间、酶学指标、移植嵌合度及移植并发症情况。结果 13 例患儿脐带血干细胞均顺利植活，其中单份植入 10 例，双份植入 3 例，此 3 例监测半年到一年，最终转为单份优势脐带血完全植入。移植后酶学检查均恢复正常，所有患儿均存活，发生Ⅰ度 GVHD 2 例，无慢性 GVHD，4 例发生巨细胞病毒血症，2 例出现了 BK 病毒性膀胱炎，1 例患儿发生了肾病综合征。结论 双份脐带血干细胞移植是治疗大体重或单份脐血干细胞数量不足黏多糖贮积症患儿的有效治疗方法。

【关键词】 黏多糖贮积症； 非血缘异基因造血干细胞移植； 双份脐带血干细胞移植

Clinical analysis of double umbilical cord blood transplantation in the treatment of mucopolysaccharidosis

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【**Abstract**】 **Objective** To analyze the effect and characteristics of unrelated allogeneic double umbilical cord blood stem cell transplantation in children with mucopolysaccharidosis. **Methods** Clinical data including the recipient situation, donor selection, time of granulocyte and platelet implantation after transplantation, enzyme indexes, graft chimerism and transplant complications of 13 children with mucopolysaccharidosis receiving unrelated allogeneic double umbilical cord blood stem cells were analyzed retrospectively. **Results** All 13 patients were successfully transplanted umbilical cord blood stem cells, including 10 cases of single implantation and 3 cases of double implantation. The monitoring lasted from half a year to one year, and finally the single dominant umbilical cord blood was completely implanted. After transplantation, the enzyme examination returned to normal. All patients survived. Grade I graft versus host reaction (GVHD) occurred in 2 cases, no chronic GVHD occurred, cytomegalovirus disease occurred in 4 cases, BK virus cystitis occurred in 2 cases, and nephrotic syndrome occurred in 1 case. **Conclusions** Double umbilical cord blood stem cell transplantation is an effective treatment for pediatric mucopolysaccharidosis with large body weight or insufficient number of single umbilical cord blood stem cells.

【**Key words**】 Mucopolysaccharidosis; Unrelated allogeneic hematopoietic stem cell transplantation; Double umbilical cord blood stem cell transplantation

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黏多糖贮积症(Mucopolysaccharidosis, MPS)属于溶酶体病中最常见的一种类型,出生时因基因异常引起溶酶体酶结构及活性异常,从而导致氨基葡聚糖底物在心、肝、脑、肾等脏器累积,致使重要脏器受损。黏多糖贮积症分为Ⅰ、Ⅱ、Ⅲ、Ⅳ、Ⅵ、Ⅶ、Ⅸ等7种类型,各型致病基因和临床表现有差异[1]。MPS的治疗包括对症支持治疗、酶替代治疗、造血干细胞移植、基因治疗等方法[2-4]。部分类型可用酶替代治疗,但价格昂贵,基因治疗尚在探索阶段,国内目前更多患者采用造血干细胞移植治疗本病。我中心开展了非血缘异基因脐带血干细胞移植治疗MPS,对于大体重或单份脐带血干细胞数量不足的患儿,选用双份非血缘异基因脐带血干细胞作为移植物。现将我院2019年6月—2020年7月双份脐带血干细胞移植治疗的13例MPS患儿临床疗效进行回顾性分析。

资料与方法

1 一般资料 选择2019年6月—2020年7月河北中西医结合儿童医院诊治的13例MPS患儿为研究对象,男9例,女4例,年龄2-9岁。详见表1。

2 纳入标准 患儿经临床表现、基因及酶学检查符合MPS诊断标准,同时体重偏大或者单份脐带血干细胞数量不足。单份脐血不满足指:(1)冷冻前脐带血CD34+细胞数 $< 1.7 \times 10^5$/kg(受者体重);(2)供受者HLA配型6/6位点相合时,冷冻前TNC $< 3.0 \times 10^7$/kg(受者体重);供受者HLA配型5/6位点相合时,冷冻前TNC $< 4.0 \times 10^7$/kg(受者体重);供受者HLA配型4/6位点相合时,冷冻前TNC $< 5.0 \times 10^7$/kg(受者体重)[5-6]。

3 方法

3.1 供体来源及HLA相合情况 本研究供体均来源于国家卫健委批准的国家脐带血公共库(北京库、山东库和广东库)。HLA配型情况为4/6、5/6、6/6,或8/10、9/10、10/10。

3.2 预处理方案 抗人T细胞猪免疫球蛋白(ALG)总量(100~125)mg/kg,分4 d静脉滴注(-10d~-7d);白消安(Bu)(0.8~1.2)mg/(kg·次),q6h静脉滴注3 d(-8d~-6d),根据受者体重按欧洲白舒非儿童给药剂量标准;氟达拉滨(Flu)40mg/m^{2}静脉滴注5 d(-7d~-3d);环磷酰胺(CY)40mg/(kg·d)静脉滴注4 d(-6d~-3d)。

3.3 移植物抗宿主病预防方案 为预防急性移植物抗宿主病(GVHD),-9d开始给予环孢素或他克莫司,CsA(2~3)mg/(kg·次),维持2 h,q12h,监测药物浓度,谷浓度达(100~200)ng/mL,FK506(0.03~0.05)mg/kg,持续24 h泵入,平均浓度为(5~10)ng/mL。吗替麦考酚酯(MMF)300mg/(m^2·次),q12h,口服,+1d至+30d;甲氨蝶呤15mg/m^2,d1,10mg/m^2,d3,d5。

表1 13例研究对象资料及移植结果

序号	性别	年龄(岁)	体重(kg)	类型	移植前酶学检测	HLA相合情况	回输TNC计数($\times 10^7$/kg)	回输CD34+计数($\times 10^5$/kg)	粒细胞植入时间(天)	血小板植入时间(天)	移植并发症	1个月时嵌合率(%)
1	男	5	24.5	Ⅱ	0.1[a]	6/6;5/6	6.2; 3.9	2.2; 2.5	19	28	Ⅰ度GVHD	96.5
2	女	8	25	Ⅰ	0.2[a]	5/6;4/6	3.46;6.16	4.23;2.04	15	22	无	90.74
3	男	2	12.5	Ⅰ	0.1[a]	10/10;8/10	7.17;15.97	1.15;2.71	15	25	无	99.08
4	男	7	23	Ⅰ	0.1[a]	5/6;5/6	4.0; 7.73	3.27;5.26	15	22	CMV血症	100
5	男	3	17	Ⅳ	1.6[b]	8/10;7/10	7.8; 6.0	2.0; 3.6	14	23	CMV血症	90.8
6	女	4	14.4	Ⅵ	11.1[c]	5/6;4/6	13.2;5.0	2.17;2.5	13	20	BK病毒感染	96.15
7	男	8	23	Ⅵ	4[c]	6/6;5/6	4.01;6.59	1.4; 3.56	16	24	肾病	25.1/74.9
8	女	5	19	Ⅳ	2.5[b]	8/10;7/10	8.5; 7.5	2.98;1.45	17	42	BK病毒感染	100
9	男	8	24	Ⅱ	0[a]	5/6;5/6	3.14;4.63	0.6; 1.57	20	48	Ⅰ度GVHD CMV血症	100
10	男	7	25	Ⅱ	0.3[a]	10/10;6/6	5.38;4.15	1.78;1.58	15	19	无	32.07/67.93
11	男	7	17.5	Ⅰ	0.46[a]	8/10;7/10	7.1; 4.2	2.8; 2.7	16	24	CMV血症	97.2
12	男	3	25.5	Ⅱ	0.1[a]	10/10;8/10	4.6; 5.7	1.3; 2.2	15	17	无	88.59/11.41
13	女	2	16	Ⅰ	0[a]	8/10;4/6	7.0; 7.87	1.05;4.65	22	41	无	100

注:a:nmol/(g·min),b:nmol/(g·h),c:nmol/(mg·h)

3.4 植入情况 患儿 +6d 开始给予 G-CSF 5μg/(kg·d) 静脉滴注,TPO 300u/(kg·d) 皮下注射。中性粒细胞及血小板植活标准: 中性粒细胞绝对值(ANC) $>0.5\times10^9$/L,持续 3 d 以上,血小板 $>20\times10^9$/L,持续 1 周以上[7]。采用短串联重复序列方法监测植入状态,当供者细胞比例≥95% 为完全嵌合,混合嵌合指双份脐带血各占一定比例或者受者与供者细胞各占一定比例[8]。原发性植入失败定义为 +28d 中性粒细胞仍未植入[9]。

3.5 病毒监测 所有患儿在移植前、后采用逆转录聚合酶链反应监测 CMV、EBV、BKV 及 JC 病毒拷贝数,移植后前 3 个月约每周 1 次。

3.6 酶学监测 酶活力检查送检北京中科医学检验实验室,移植后 1 个月常规进行酶活力检查。

3.7 随访 对所有患儿进行常规随访,终点为 2020 年 11 月 10 日,中位随访时间 12(4~17) 个月,随访内容包括血常规、生化功能、淋巴细胞亚群、酶活力检查、嵌合度、GVHD、症状改善等情况。

结 果

1 临床特点 13 例 MPS 患儿移植前临床资料见表 1。中位移植年龄为 5(2~8) 岁,分型Ⅰ型 5 例,Ⅱ型 4 例,Ⅳ型 2 例,Ⅵ型 2 例。主要临床表现为蒙古斑、面容粗糙、智力低下、骨骼发育异常、脐疝、腹股沟疝等。

2 移植结果 回输脐带血 TNC 细胞中位数为 12.8(7.77~23.14) $\times10^7$/kg,CD34+ 细胞中位数为 4.86(2.17~8.53) $\times10^5$/kg,中性粒细胞植活中位时间 16(13~22) d,血小板植活中位时间 27(17~48) d。患者移植后 1 个月时查嵌合度,8 例为完全嵌合;3 例为两份移植物混合嵌合(总体为完全嵌合),随访半年到一年,逐渐转为单份完全嵌合状态;2 例嵌合未达到 95% 以上,后期随访逐渐转为完全嵌合。移植后 1 个月进行常规酶学检测,1 例稍低于正常,第 2 个月复查时已恢复正常,其它患者酶学检测均恢复正常。每份脐带血细胞数量、植活天数、嵌合度、酶值等详见表 1。

3 随访及并发症情况 13 例患儿上呼吸道阻塞、听力、语言水平、角膜混浊、面容、关节僵硬、运动能力等方面在移植后改善较快,多在 3~6 个月有显著改善;心脏瓣膜疾病、身高、智力水平、骨骼畸形、疝气等改善较缓慢,常在 1 年后有所改善。有 6 例患儿在移植后出现病毒激活,其中 2 例为 BK 病毒,表现为出血性膀胱炎Ⅱ度,4 例为 CMV 病毒血症,经免疫抑制剂减量、抗病毒药物及静注人免疫球蛋白后均症状消失、病毒转阴或复制量下降。所有患儿均未观察到真菌感染。1 例患儿移植后 5 个月出现肾病综合征,加量免疫抑制剂治疗后恢复正常。2 例患儿出现Ⅰ度皮肤 GVHD,表现为少量皮疹,加用芦可替尼口服后好转。其它患儿均未出现急性及慢性 GVHD。

讨 论

MPS 是儿童罕见的遗传病,除Ⅱ型为 X 连锁隐性遗传、男孩发病外,其它类型男女均有发病。目前 MPS 的主要治疗方法为酶替代治疗(ERT) 和异基因造血干细胞移植(allo-HSCT)。ERT 治疗能够有效改善部分 MPS 患者的临床症状,同时降低相关并发症。但是,由于酶无法透过血脑屏障,对患者中枢神经系统症状改善作用有限; 另外 ERT 价格昂贵,需要终生维持给药,应用受限。Allo-HSCT 可使 MPS 患者获得永久产酶能力,同时源于健康供者的巨噬细胞可透过血脑屏障,并转化为脑小胶质细胞,部分纠正中枢神经系统的产酶能力,从而达到改善患者中枢神经系统症状的作用。随着 HSCT 技术的进步,其风险显著降低,费用也为多数患者家庭可承受[10]。

脐带血干细胞具有储存、应用方便,细胞活性强,排异率低等优点,但 TNC 及 CD34+ 细胞数相对偏少是其缺点。欧洲移植协作组和美国国际移植协作组数据显示,脐血移植物获得完全植入和正常酶活性的比例优于其它移植物: 92% 和 98% *VS* 69% 和 59%[11]。国际多中心研究显示,疾病基因携带供者移植后酶活性水平和远期预后存在一定缺陷[12]。国内外对于双份脐带血干细胞移植均有报道。2000 年陆道培院士就开始应用双份脐带血移植治疗大体重血液病患者,一例为 32 岁 95kg 的男性 ALL,回输的 2 份脐血所含 MNC 分别为 1.8×10^7 和 0.8×10^7/kg (受者体重); 另一例为 27 岁 60kg 的女性 CML,回输的脐血 MNC 分别为 1.54×10^7 和 1.21×10^7/kg (受者体重),两例患者均获得完全造血重建[13]。国内对于双份脐带血干细胞移植的选择标准[14]为: (1) 每份脐带血冻融前的 TNC $\geq1.5\times10^7$/kg; (2) 双份脐带血冻融前 TNC 总数至少达到单份脐带血移植标准; (3) 脐带血与受者的 HLA 配型至少为 4/6 位点相合。我中心对于患儿体重偏大,TNC 细胞数 $<3\times10^7$/kg,CD34+ 细胞数 $<1.7\times10^5$/kg 者,一般选用双份脐带血干细胞移植,具体参考指标: (1) HLA 相合度情况,采用 5/6、6/6、8/10、9/10、10/10 位点中一份搭配另外较低位点一份,常搭配 4/6、7/10,每个大位点不能都错配;

(2) 细胞数方面,TNC 及 CD34 + 细胞数均远远大于最低参考指标,即双份脐带血总 TNC > 3.0×10^7/kg (受者体重),总 CD34 + > 1.7×10^5/kg。脐带血细胞数少是其劣势,因其相对于外周血干细胞移植来看,TNC 及 CD34 + 细胞数都降了一个对数级,所以困扰脐带血移植的主要问题是植活问题。为保证植活,所以我们选择两份脐带血移植。因脐带血移植 GVHD 发生率低,所以细胞数选择是越多越优;(3) 血型方面,双份脐带血血型必须相同,供者与受者没有特别要求;(4) 性别方面,首选同性别;(5) 脐血年份方面,优先选择近 5 年内脐带血。

脐带血干细胞移植后,患儿酶学指标多在 1 ~ 2 个月即恢复正常,个别患儿第 1 个月酶学指标偏低于正常参考值,但较移植前已明显上升,考虑移植后 1 个月时外周血白细胞不稳定,而且可能部分酶去参与了黏多糖的代谢,往往第 2 或 3 个月检查时,酶学指标恢复正常。部分患儿出现了 CMV 血症及 BK 病毒相关性膀胱炎,但总体症状轻微,加之无明显排异出现,所以可以尽快减量免疫抑制剂,同时予静注人免疫球蛋白及抗病毒药物应用,指标及症状均很快好转。各型黏多糖贮积症患儿移植后症状改善来看,表现不同、改善时间也有差异,MPS Ⅰ型患者多无智力损伤,骨骼畸形较Ⅳ型、Ⅵ型轻,总体疗效较好。MPS Ⅱ型多伴有神经系统损伤、智力低下,既往对于移植疗效存在疑义[15],但近些年研究对比发现,HSCT 仍具有积极的改善[10]。MPS Ⅳ型、Ⅵ型骨骼畸形尤为明显,HSCT 后骨关节病改善缓慢,然而近些年长期随访发现 HSCT 联合手术治疗可使患者行走功能、骨骼变形、骨质疏松等均有改善[16]。此外,随访发现,呼吸功能、肝脾肿大、关节伸展、面容、中耳炎等症状体征改善较快,考虑可能与这些组织器官血流丰富,使更多的带酶白细胞进入相关;而神经系统有血脑屏障、骨骼系统血流相对较少,所以症状改善缓慢。

综上所述,脐带血干细胞移植治疗黏多糖贮积症疗效确切,移植排异率较低;双份脐带血干细胞移植作为互补性治疗方案克服了细胞数量少、植入失败的风险,前期做好预防,不增加相关并发症,后期逐渐转为单份优势脐带血完全植入。所以,对于大体重或单份脐带血干细胞数量偏少的黏多糖贮积症患儿,可以选择双份脐带血干细胞移植。

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RESEARCH

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Haploidentical haematopoietic stem cell transplantation for malignant infantile osteopetrosis and intermediate osteopetrosis: a retrospective analysis of a single centre

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Abstract

Objective: To evaluate the clinical efficacy of haploidentical haematopoietic stem cell transplantation (haplo-HSCT) for the treatment of malignant infantile osteopetrosis (MIOP) and intermediate osteopetrosis.

Methods: Children with MIOP and IOP who underwent haplo-HSCT in Beijing Children's Hospital, Capital Medical University, from January 2010 to May 2018 were retrospectively analysed. Data relating to the clinical manifestations, engraftment, and prognosis of the children were extracted from medical records.

Results: Twenty-seven patients, including 18 males and 9 females, with an onset age of 12 (0.04–72) months were enrolled in this study. The median time from diagnosis to transplantation was 4 (1–23) months. All patients received haplo-HSCT with a myeloablative conditioning regimen (including fludarabine, busulfan, and cyclophosphamide). Graft versus host disease (GVHD) prophylaxis was based on anti-human T lymphocyte porcine immunoglobulin/anti-human thymus globulin, methotrexate, and mycophenolate mofetil. The median observation time was 55.2 (0.3–126.2) months. By the end of follow-up, twenty patients survived and seven patients died. The 5 year overall survival rate was 73.9%. Stage I-II acute GVHD was observed in 20 patients, stage III GVHD in 1 patient and no patients had stage IV disease. Chronic GVHD was observed in 11 patients (40.7%) and was controlled by anti-GVHD therapy.

Conclusions: Haplo-HSCT was an effective treatment for MIOP and IOP, with a high survival rate and significantly improved clinical symptoms. For patients with a vision impairment before HSCT, the improvement was slow after transplantation. The incidence of GVHD was high but mild and was effectively controlled by appropriate treatment. These data indicated that haplo-HSCT was a feasible treatment for MIOP and IOP.

Keywords: Malignant infantile osteopetrosis, Intermediate osteopetrosis, Haploidentical haematopoietic stem cell transplantation, Prognosis, Graft versus host disease

Introduction

Osteopetrosis, which is also called marble bone disease, refers to a heterogeneous group of rare inherited skeletal dysplasias. Inheritance is divided into autosomal recessive, autosomal dominant or X-linked patterns [1]. The most severe cases are almost always autosomal recessive and are termed malignant infantile osteopetrosis

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(MIOP). This rare inherited disease has an incidence of 1:200,000 to 1:300,000, but higher rates are reported in Russia and the Middle East [2, 3]. It is characterized by quantitative or qualitative osteoclast defects that lead to increased bone mass and density. These children present with dysplasia, hydrocephalus, fracture, hypocalcaemia, progressive bone marrow failure, neurological disorders and other conditions in the first year of life.

The prognosis of patients with MIOP is very poor, and death in the first decade is common without appropriate therapy. Haematopoietic stem cell transplantation (HSCT)is the only effective treatment for MIOP [4, 5]. Intermediate osteopetrosis (IOP) is mostly caused by the autosomal dominant inheritance of an abnormal CLCN7 gene. Some patients have earlier onset ages and may also show manifestations such as MIOP. No clear guidelines are available for transplantation in these patients [6, 7]. Here, we report the long-term survival of 27 patients who received haploid haematopoietic stem cell transplantation (haplo-HSCT) to treat MIOP and IOP in Beijing Children's Hospital affiliated with Capital Medical University to further explore the safety and feasibility of haplo-HSCT as a treatment for osteopetrosis.

Patients and methods

Patients

This study employed a retrospective observational design. Children suffering from MIOP/IOP who underwent haplo-HSCT between January 2010 and May 2018 were enrolled in this study. None of the patients had an HLA-matched sibling or unrelated donors in the China Bone Marrow Bank. Data were retrospectively reviewed for the source of haematopoietic stem cells, conditioning regimen, adverse effects, and prognosis. This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (IRB) of Beijing Children's Hospital, Capital Medical University. All patients' parents or guardians signed informed consent forms.

Conditioning regimen

All patients received transplants from unmatched related donors and myeloablative conditioning regimens. Fludarabine (Flu) was administered at a dose of 120–150 mg/m^2, busulfan (Bu) was administered at 16–19.2 mg/kg (weight adapted, due to our technology, most children did not undergo PK testing) and cyclophosphamide (Cy) was administered at 200 mg/kg to the 26 patients. One patient received TBI (12 Gy) and Cy 120 mg/kg as a conditioning regimen. An individualized dose of treatment was used according to the patient's condition (Fig. 1). Compatibility was defined by HLA-A/B/C/DR/DQ serotypes and high-resolution molecular HLA typing. All donors were injected subcutaneously with granulocyte colony-stimulating factor (5 μg/kg

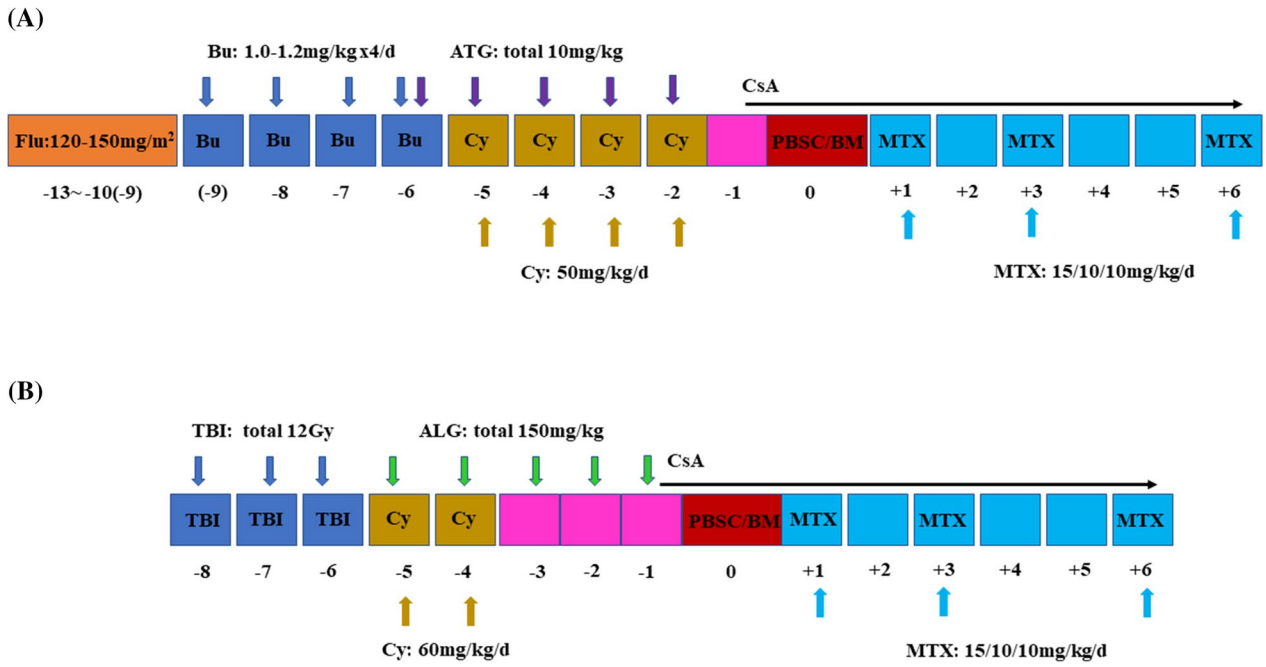

Fig. 1 Conditioning regimen for patients with malignant infantile osteopetrosis and intermediate osteopetrosis

twice daily for 5 days). Mobilized PBSCs and bone marrow were collected on days 5 and 6.

GVHD prophylaxis and treatment

Graft versus host disease (GVHD) prophylaxis: Acute and chronic graft versus host disease (GVHD) were diagnosed and graded by physicians according to defined criteria [8, 9]. The diagnosis of GVHD mainly depends on the signs and symptoms of patients. All patients received cyclosporine A (CsA) at 5 mg/kg per day from d-1; mycophenolate mofetil (MMF) at 15 mg/m^2 on d1 and 10 mg/m^2 on d3, 6, and 11; methotrexate (MTX) and anti-human thymocyte globulin (ATG) at 10 mg/kg or anti-human T lymphocyte porcine immunoglobulin (ALG) at 150 mg/kg to prevent the occurrence of GVHD.

Engraftment and chimaerism

The evidence of engraftment included increases in peripheral white blood cell and monocyte counts and the presence of mature granulocytes 2–4 weeks after transplantation, which were confirmed by a chimaerism analysis. Chimaerism was detected using routine karyotyping, and DNA fingerprinting of short tandem repeats was conducted on whole blood samples at one month, three months, six months, and twelve months after transplantation. Neutrophil engraftment was defined as the first day of an absolute neutrophil count $> 0.5 \times 10^9$/L for 3 consecutive days. Platelet engraftment was defined as a platelet count $> 50 \times 10^9$/L for at least 7 days without transfusion support. Primary graft failure was defined as the absence of donor-derived myeloid cells on day + 30 or reconstitution with autologous cells, and secondary graft failure was defined as the loss of a previously functioning graft, resulting in cytopenia involving at least 2 blood cell lineages and confirmed by the chimaerism analysis when the technique was available [10].

Supportive care

Thrombotic microangiopathy (TMA) was diagnosed by physicians according to defined overall TMA (O-TMA) criteria reported by Cho et al [11]. Patients were diagnosed with hepatic veno-occlusive disease (HVOD) based on the Seattle criteria, depending on the signs/symptoms of patients and the result of a liver ultrasound [12]. Ursodeoxycholic acid was administered at a dose of 5–7 mg/kg per day po (from d-14) and low molecular weight heparin was administered at a dose of 100 IU/kg per day by subcutaneous injection (from d-10) to prevent the occurrence of HVOD. Phenytoin sodium was administered at a dose of 5 mg/kg per day po (from d 14), followed by gradual tapering for the next 1.5 weeks to prevent epilepsy. G-CSF was subcutaneously injected at a dose of 5 μg/kg per day (from d + 5) to stimulate haematopoiesis. Acyclovir was iv injected at a dose of 30 mg/kg per day (from d0) to prevent viral infection. Viral PCR screening and quantification were performed weekly for cytomegalovirus, Epstein-Barr virus, and adenovirus. All patients were administered antimicrobial prophylaxis for fungi. HSCT recipients were treated in single rooms with laminar airflow systems.

End points

The primary end point was survival. Death from any cause was considered an event, and surviving patients were censored at the last follow-up. After the transplant, patients were required to return to the hospital once a month for posttransplant evaluations after the first discharge, including routine blood tests, biochemical tests, imaging, and chimaerism analyses. The time of last follow-up was defined as the number of days between the date of transplantation and the last clinic visit.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 24 software (IBM, USA). Data with a skewed distribution are presented as medians (interquartile ranges). The log-rank test was used to verify overall survival and to compare the survival rates between different groups. $P < 0.05$ indicated a significant difference.

Results

General patient information

Twenty-seven patients with osteopetrosis were enrolled in this study, including 19 males and 8 females. The ratio of males to females was 2.375:1. Among the patients, 23 were diagnosed with MIOP and 4 were diagnosed with IOP. The median age of disease onset was 12 (0.04–72) months. The median age of diagnosis was 8 (0.1–84) months. Twenty-two patients (81.5%) were less than 1 year old at disease onset. The onset age of MIOP was 2 (0.04–29) months and that of IOP was 33 (21–72) months. The median time from diagnosis to transplantation was 4 (1–23) months.

All the children who underwent a bone X-ray examination showed increased density with a reduced/disappeared bone marrow cavity. Ten patients had skull deformities, including two with square heads and one with lost teeth. Two patients had deformed X-shaped legs. Twenty-six patients (96.3%) presented optic nerve damage, and imaging indicated optic canal stenosis. Eighteen patients experienced clinical manifestations, including double nystagmus in 11 patients, abnormal ocular pursuit in 4 patients, exotropia in 2 patients, and blurred vision in 8 patients. Five patients (18.5%) had a hearing impairment. Splenomegaly was observed in 23 children, and hepatomegaly was observed in 26

children. The laboratory examination showed that all patients had anaemia 80 (49–115) g/L (reference range 120–190 g/L), and 21 patients had thrombocytopenia 55 (7–174) $\times 10^9$/L (reference range 100–400 $\times 10^9$/L). Eleven patients had liver damage with high levels of ALT and AST, and 26 patients had myocardial damage with high levels of CK-MB 82 (24-1061) U/L (reference range 0–25 U/L). Ten patients had an infection history, and two patients had a bone fracture history.

CLCN7 gene mutations were identified in 6 patients, including 2 compound heterozygous and 4 heterozygous mutations. TCIRG1 gene mutations were detected in 18 patients. The remaining 3 patients did not undergo a genetic analysis (Table 1).

Engraftment and chimaerism

One patient failed to receive the same matched unrelated donor transplantation twice and then underwent unmatched related donor transplantation from her father. The remaining 26 patients underwent unmatched related donor transplantation. The median infused mononuclear cell (MNC) count was 23.12 (10.04–51.90) $\times 10^8$ cells/kg, and the median infused $CD34^+$ cell count was 10.22 (5.96–24.88) $\times 10^6$ cells/kg. The graft source and conditioning regimen are shown in Table 2.

Twenty-six patients experienced successful neutrophil engraftment, and the median time of engraftment was 25 (10–37) days. Twenty-three patients experienced successful platelet engraftment, and the median time of engraftment was 43 (10–155) days. Donor chimaerism was 92.6% at day 30 posttransplantation. Two patients (A and B) presented a mixed donor type in the early stage after transplantation, and the lowest chimaerism rates were 45.2% and 85.1%, respectively. This type changed to the full donor type after donor $CD34^+$ cells were reinfused. However, three patients (C, D and E) exhibited a mixed donor type 6 months after transplantation that changed to the full donor type again after donor $CD34^+$ cells were reinfused or a donor lymphocyte transfusion (Fig. 2).

Table 1 General information

Total patients	27
Sex (%)	
Male	18 (66.7)
Female	9 (33.3)
Age at transplant (median)	12 months (range: 4–107 months)
Gene (%)	
TCIRG1 compound heterozygous mutation	18 (66.7)
CLCN7 compound heterozygous mutation	2 (7.4)
CLCN7 heterozygous mutation	4 (14.8)
Visual impairment	26/27
Hearing impairment	5/27
Splenomegaly	23/27
Abnormal haemogram	27/27

Table 2 Engraftment and GVHD

Overall survival (%)	73.9%
Engraftment source	
Father	19 (70.4)
Mother	8 (29.6)
HLA-matched	
5/10 (3/6)	18 (66.7)
6/10	2 (7.4)
7/10	4 (14.8)
5/6	2 (7.4)
9/10	1 (3.7)
Conditioning regimen	
TBI (12) + Cy (120)	1
Flu (120–150) + Bu (16–19.2) + Cy (200)	26
Blood type	
Matched	12
Mismatched	15
Stem cells infused	
MNC $\times 10^8$/kg	23.12 (10.04–51.90)
$CD34^+ \times 10^6$/kg	10.22 (5.96–24.88)
aGVHD (%)	21/27 (77.8)
Stage I–II	20
Stage III	1
Stage IV	0
cGVHD (%)	11/27 (40.7)
Infection	
CMV infection (dead/alive)	0/15
EBV infection, reactivated	10
Bacterial	12
Haemorrhagic cystitis	3
HVOD (%)	5 (18.5)
Engraftment failure	0

TBI, total body irradiation; Cy, cyclophosphamide; Flu, fludarabine; Bu, busulfan; GVHD, graft versus host disease; HVOD, hepatic veno-occlusive disease

GVHD and transplant-related morbidity

In our study, 21 (77.8%) patients suffered acute GVHD (aGVHD), twenty of whom were graded as stage I-III and one of whom was graded as stage III. The most common location of aGVHD was the skin (n = 19), with a red haemorrhagic rash predominating and without sclerosis. The remaining 3 aGVHD cases involved the gastrointestinal tract. Chronic GVHD (cGVHD) was observed in 11 patients (40.7%). Among them, ten presented skin

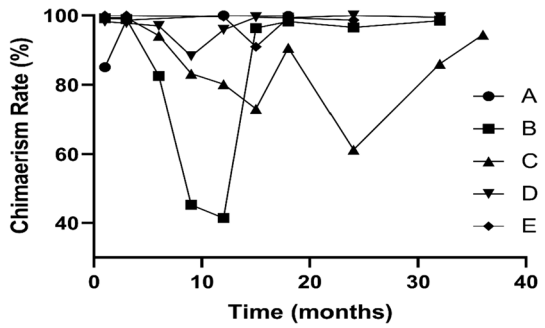

Fig. 2 The donor chimaerism rate of five patients with a mixed donor type. Two patients (**A** and **B**) presented a mixed donor type in the early stage after transplantation, and the lowest chimaerism rates were 45.2% and 85.1%, respectively. The type changed to the full donor type after donor $CD34^+$ cells were reinfused. However, three patients (**C**–**E**) exhibited the mixed donor type 6 months after transplantation that changed to the full donor type after donor $CD34^+$ cells were reinfused or a donor lymphocyte transfusion

involvement, including 3 cases that were widely distributed throughout the body, whereas the remaining cases were locally limited. The other patient presented with gastrointestinal tract involvement along with diarrhoea. After treatment with corticosteroids and tacrolimus, the GVHD of the children improved, drugs were withdrawn from some patients, and no child died of GVHD.

In terms of transplant-related morbidity, 15 patients (55.6%) had a CMV infection, including one case of CMV pneumonia. All patients' plasma CMV-DNA tests became negative after antiviral therapy. Ten patients had an EBV infection or reactivation. Twelve patients had a bacterial infection, including 10 cases of pneumonia and 2 cases of septicaemia. Hypercalcaemia occurred in 4 patients with a median serum calcium level of 2.95 (2.85–2.99) mmol/L, which was improved after proper treatment. Four patients experienced autoimmune haemolytic anaemia, and 3 patients experienced haemorrhagic cystitis. Five patients had HVOD, and 1 patient had transplantation-related thrombotic microangiopathy.

Follow-up and survival

The last follow-up was November 1, 2020, and the median follow-up time was 55.2 (0.3–126.2) months. No patients were lost to follow-up. Among the enrolled patients, 20 patients survived and 7 patients died. All deaths occurred within 2 months after the transplant. Causes of death were pulmonary haemorrhage (42.8%), severe pneumonia after transplant (28.6%), heart failure secondary to autoimmune haemolytic anaemia (14.3%), and gastrointestinal bleeding and multiple organ failure secondary to HVOD (14.3%). Among all survivors, no patients experienced graft failure or rejection. The disease was controlled, and the haematopoietic function was restored [WBC 6.60 (5.24–22.32) $\times 10^9$/L, Hb 129 (122–146) g/L, and PLT 210 (126–308) $\times 10^9$/L]. The myocardial damage in most patients also recovered. The size of the liver and spleen returned to normal (Fig. 3). Sixteen patients were continuously monitored for changes in CD subclasses in our hospital, and the results are shown in Fig. 4. The bone X-ray examination at 6 months after transplantation showed that the bone mineral density was lower than before surgery, and the bone marrow cavity gradually formed 9–12 months after transplantation, suggesting bone remodelling (Fig. 5). The visual acuity of 17 children was monitored continuously in our hospital. The eye condition was assessed using a fundus examination, electroretinogram, visual evoked potential, flash visual evoked potential and head CT. Among them, one patient returned to normal visual acuity, three patients exhibited improved optic canal stenosis and optic nerve compression. The visual acuity of the other 3 cases was not significantly altered. Hearing was monitored using pure tone audiometry and auditory brainstem response tests. The hearing condition of the five patients who had a hearing impairment improved but did not return to normal.

The 5-year overall survival (OS) was 73.9% in our cohort of 27 patients who underwent haplo-HSCT. The 5-year OS of all patients with osteopetrosis who underwent HSCT (including matched unrelated donors, matched related donors and other donors) was 76.7% in the same period. A statistically significant difference was not observed between patients with MIOP and IOP (69.6% vs. 100.0%, $P=0.2444$) (Fig. 6). According to the source of haematopoietic stem cells, we divided the patients into stem cells from the father and mother, and no statistically significant difference was observed in 5-year OS between these groups (68.4% vs. 87.5%, $P=0.273$). Patients were divided into two groups according to whether the donor and patients had the same blood type, and no statistically significant difference in 5-year OS was observed between groups (66.7% vs. 79.4%, $P=0.373$). Based on the log-rank analysis, statistically significant associations between the age at onset, age at transplantation and infused $CD34^+$ cell count and 5-year OS were not observed.

Discussion

Osteopetrosis is a disease caused by the differentiation, development, or dysfunction of osteoclasts. The balance between osteoclasts and osteoblasts is very important for maintaining bone homeostasis. When osteoclasts are dysfunctional, a series of clinical manifestations may arise, such as short stature, fractures, osteomyelitis, hypocalcaemia, convulsions, and neurological lesions [13]. Defective osteoclast activity can

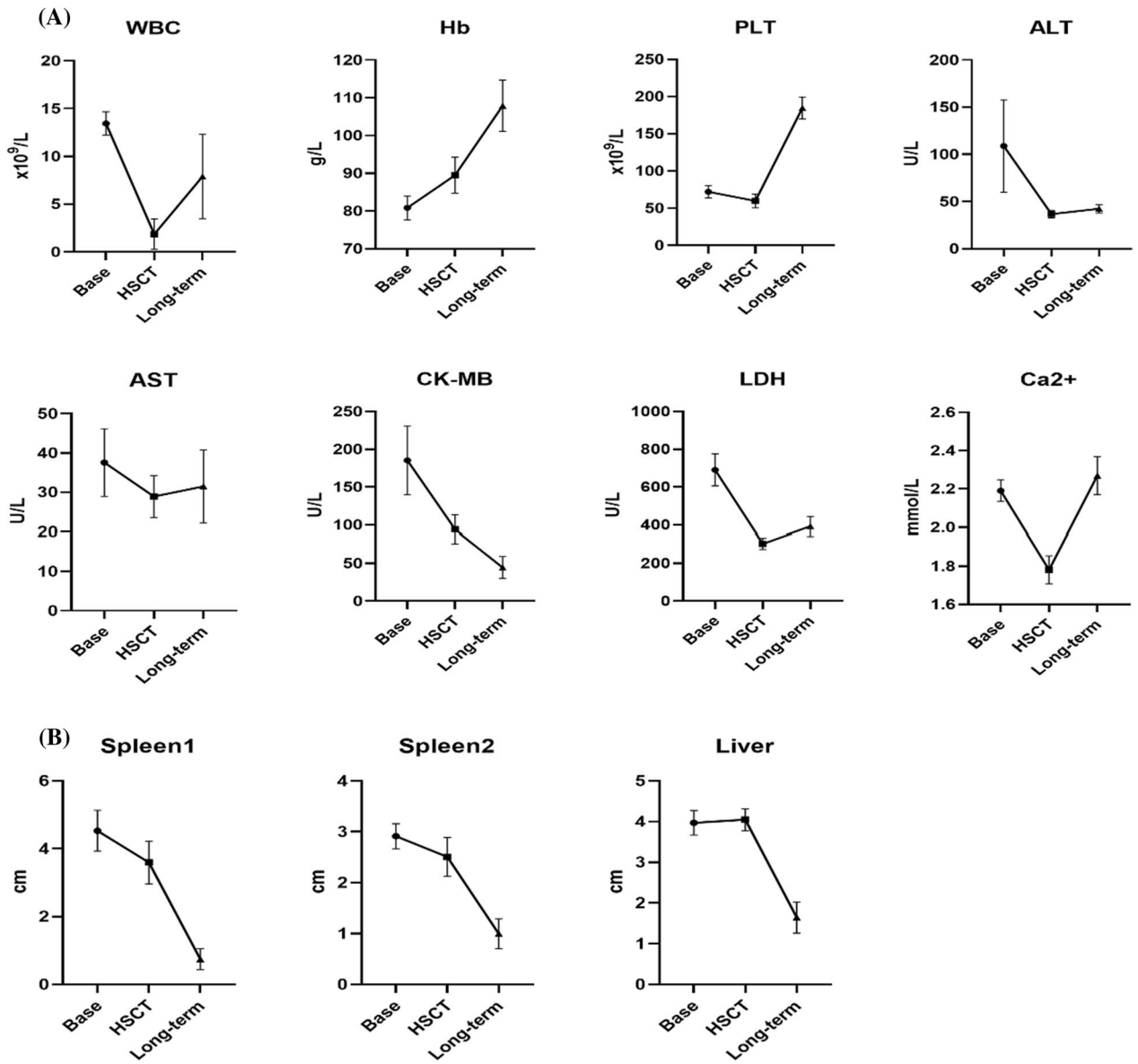

Fig. 3 Clinical characteristics of children with MIOP/IOP and progression after treatment (**A**, **B**), "Base" indicates detection at diagnosis; "HSCT" indicates detection at HSCT; "Long-term" indicates detection at the end of follow-up. "Spleen 1" represents the lower margin of the spleen under the subcostal margin, which was measured using abdominal ultrasound. "Spleen 2" represents the thickness of the spleen, which was measured using abdominal ultrasound. "Liver" represents the lower margin of the liver under the subcostal margin, which was measured using abdominal ultrasound. WBC: white blood cell; Hb: haemoglobin; PLT: platelet; AST aspartate aminotransferase; ALT alanine aminotransferase; CK-MB: creatine kinase-MB; LDH lactic dehydrogenase; Ca^{2+}: calcium

be caused by mutations in genes affecting osteoclast development (such as RANK and RANKL) and function (such as TCIRG1, SNX10, CLCN7, and OSTM1). Biallelic mutations in these genes will lead to malignant infantile osteopetrosis (MIOP). This subtype has severe clinical manifestations and a poor prognosis. The onset age is early, generally in the first year after birth. Autosomal dominant osteopetrosis is caused by monoallelic mutations in the CLCN7 gene, which is called IOP. The onset age of this type is relatively late, and the progression of the disease is slow [14]. In the present study, 22 patients (81.5%) were less than 1 year old at the time of onset, the age of onset of MIOP was 2 (0.04–29) months, and the age of onset of IOP was 33 (21–72) months.

Because osteoclasts are myeloid cells and allogeneic haematopoietic stem cell transplantation can provide

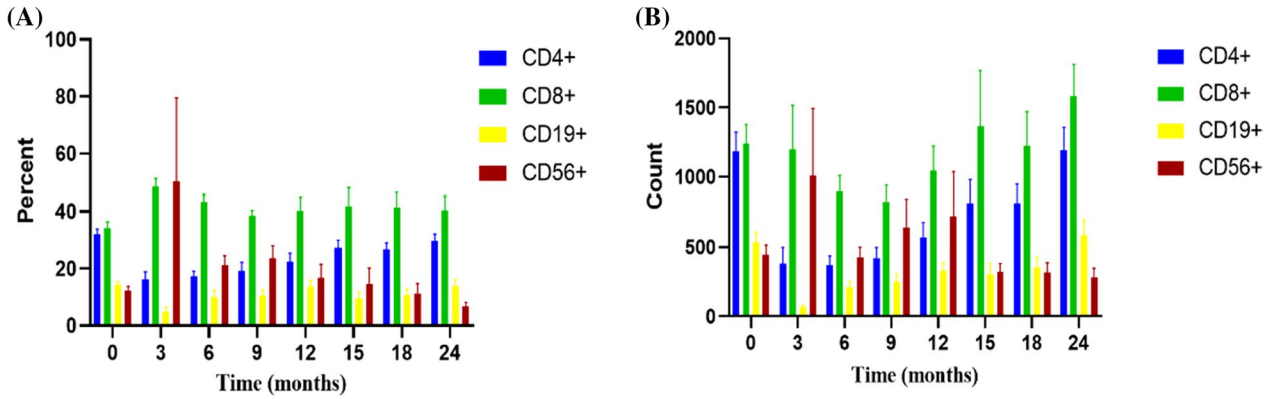

Fig. 4 Changes in CD subtypes after HSCT. (**A**) Percent and (**B**) absolute count

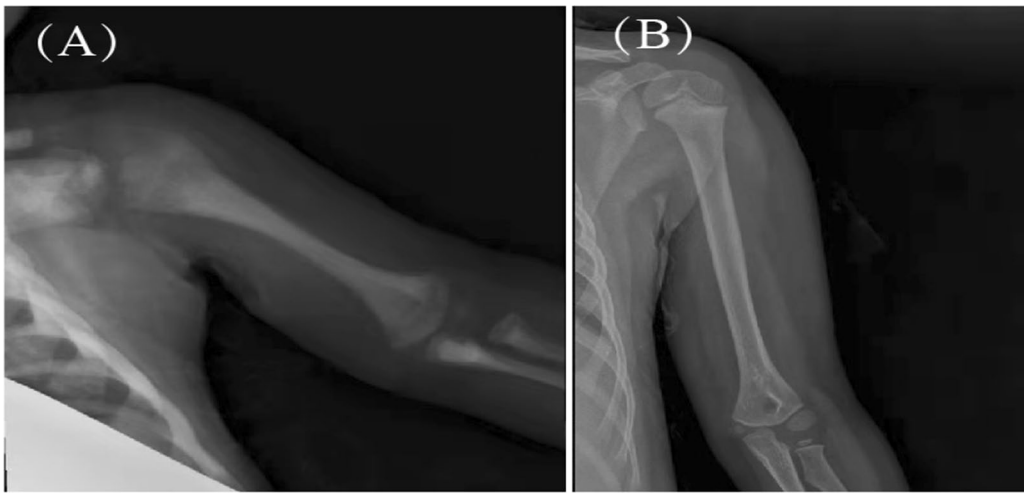

Fig. 5 The bone density of the left humerus increased unevenly, and the bone cortex thickened before transplantation (**A**). Four years after transplantation, the bone mineral density decreased, and the bone marrow cavity was clear (**B**)

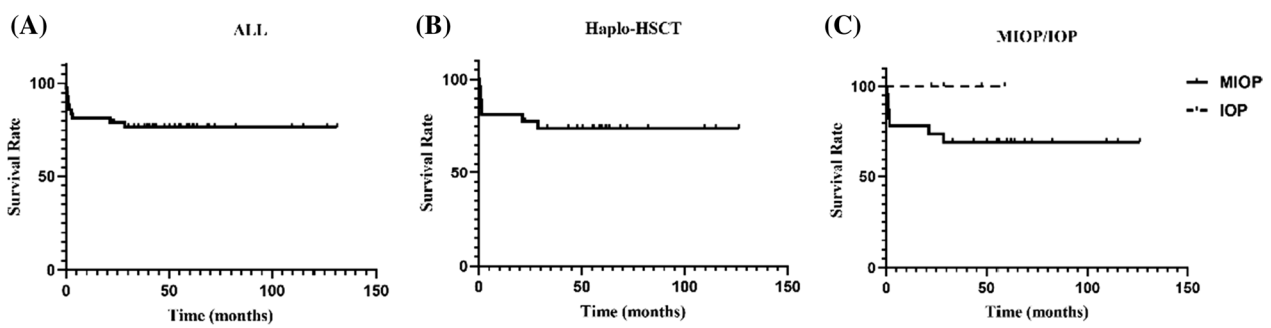

Fig. 6 The 5-year overall survival (OS) rates of all patients with osteopetrosis who underwent HSCT was 76.7% (**A**). The 5-year overall survival (OS) rate was 73.9% in patients who underwent haplo-HSCT (**B**). A comparison of patients with MIOP and IOP did not reveal a statistically significant difference (69.6% vs. 100.0%, P = 0.2444) (**C**). ALL: all patients

osteoclasts for patients, allogeneic HSCT is the only effective treatment for MIOP at present [15]. Driessen et al. [5] reported 122 patients who underwent allogeneic HSCT treatment for MIOP and found that the overall successful engraftment rate was 77%. The higher failure rate of transplantation was mainly related to dysfunction of bone haematopoietic capacity, spleen retention or destruction of stem cells and HLA mismatch. This article also found that the 5-year EFS of HLA-matched patients was significantly higher than that of HLA-mismatched patients (73% vs. 24%). Natsheh et al. [16] reported 38 patients who underwent HSCT for MIOP, including 36 HLA-matched patients, 1 haploidentical transplant recipient and 1 T cell-depleted (TCD) in vitro transplant recipient. The 5-year OS was 84%. Orchard et al. [17] reported the long-term survival of 193 patients with MIOP after transplantation. Eighty-nine patients underwent transplantation using grafts from HLA-matched siblings and 104 patients received transplants from HLA-mismatched donors (including 25 patients with transplants from mismatched sibling donors and 79 patients with transplants from mismatched unrelated donors). The conditioning regimen was Bu and Cy. The 5- and 10-year probabilities of survival were 62% and 62% after HLA-matched sibling transplantation and 42% and 39% after mismatched donor transplantation. Graft failure was the most common cause of death. Bahr et al. [18] reported 3 patients with MIOP who were treated with Haplo-HSCT. With the posttransplantation cyclophosphamide regimen, only 1 patient survived. Stepensky et al. [14] reported that 6 of 7 children with IOP survived after receiving HSCT, and 1 died of CMV infection and pulmonary hypertension after haplo-HSCT. In summary, previous research indicated that the first choice for MIOP treatment is an HLA-matched sibling donor for HSCT, and that haplo-HSCT is not a suitable treatment for MIOP and IOP. Graft failure was the main factor affecting the treatment of MIOP and IOP with haplo-HSCT.

However, in China, HLA-matched sibling donors are difficult to obtain for HSCT. The time for seeking matched unrelated donors is long, and the success rate is not high. Parents who are carriers can become potential donors, which can reduce the waiting time and complications. Torres et al. [10] reported 2 patients who were treated with in vitro TCD (purified $CD34^+$ stem cells) and Bu, Flu combined with ATG as a conditioning regimen. One patient survived, and 1 patient died of graft failure. Driessen et al. [5] reported 37 patients with MIOP who were treated with TCD in vitro, with an overall survival rate of 34%, and the main cause of death was severe infection. In contrast to previous research, in this study, 27 patients received haplo-HSCT treatment. Twenty patients survived, and the 5-year OS rate was 73.9%. This retrospective observational study showed that the in vivo T removal regimen achieved a more successful engraftment rate than the in vitro T removal regimen, which was approximately or even higher than previous reports of HLA-matched HSCT. In terms of the conditioning regimen, previous research found that the 5-year OS in the fludarabine group was higher than that in the group treated without fludarabine (96% vs. 58%). Our research indicated that the myeloablative conditioning regimen was beneficial for stem cell engraftment. In the present study, the 30-day neutrophil engraftment rate was greater than 95%. Although the platelet engraftment time was later, the 120-day engraftment rate was more than 60%. Faster stem cell engraftment was beneficial to haematopoietic recovery, and it was more convenient for a donor lymphocyte transfusion due to poor engraftment. In addition, compared with umbilical cord blood HSCT, haplo-HSCT had another advantage. A previous report described 51 patients who received umbilical cord blood HSCT for MIOP, and the 6-year OS rate was 43%. Most of the patients died of engraftment failure [19]. Compared with cord blood HSCT, mobilized bone marrow and peripheral blood stem cells may provide more $CD34^+$ cells for patients, which would promote engraftment.

Our study adopted the "Beijing Protocol", which included the myeloablative conditioning regimen (Flu + Bu + Cy), the in vivo TCD regimen (ATG/ALG), and stem cells collected from mobilized bone marrow and peripheral blood [20]. All these measures worked together to reduce the occurrence of severe GVHD. Acute GVHD stage I-II was observed in 20 patients, stage III disease occurred in 1 patient and no patients had stage IV GVHD. Chronic GVHD was observed in 11 patients, ten of whom had skin involvement, and 7 of whom had a locally limited disease. The condition of GVHD was improved after using hormones and other anti-GVHD drugs, such as tacrolimus, a CD25 monoclonal antibody and ruxolitinib. The data indicated that the incidence of GVHD was high but mild and was effectively controlled by the appropriate treatment. GVHD would not affect the survival of patients. Kapelushnik et al. [21] found that patients who died of acute respiratory distress syndrome or pulmonary haemorrhage after transplantation may have pulmonary arterial hypertension. According to another study, ATP6i (TCIRG1) mutations lead to pulmonary arterial hypertension [22]. In our study, 3 children who died of pulmonary haemorrhage all had TCIRG1 mutations, but pulmonary haemorrhage and pulmonary arterial hypertension may also be related to the aggravation of pulmonary symptoms caused by high-dose chemotherapy and massive fluid infusion. Patients who experienced these severe toxicities all developed

pneumonia before transplantation. Therefore, the poor condition before HSCT might be related to severe toxicities. The myeloablative conditioning regimen led to a higher engraftment rate, but the toxicity was also relatively high, especially the incidence of HVOD [23]. In our study, the early addition of ursodeoxycholic acid, low molecular weight heparin calcium and defibrinylate significantly reduced the HVOD death rate. Hypercalcaemia was also a serious complication after transplantation, mainly because of the short-term release of large amounts of intraosseous calcium from bone into the blood [24]. In the present study, only 4 patients experienced hypercalcaemia, and the elevated serum calcium levels were not significant. This finding might be because the donor was also a carrier, and the function of osteoclasts was lower than normal. A large amount of calcium is unable to easily enter the blood in a short period and be compensated by the body.

This retrospective observational study also observed significant bone remodelling after haplo-HSCT, and the curative effect was lasting and stable. However, other complications, especially blindness and deafness caused by nervous system involvement, were not significantly improved after transplantation. A previous study showed that only 7% of eyesight was restored, and vision problems still progressed in 25% of patients. In our study, only one patient's visual acuity returned to normal, and the visual acuity of 13 patients was improved, which may be related to the older age at the time of diagnosis and the involvement of the nervous system before HSCT. Although our study did not find a relationship between the age at diagnosis, age at transplantation and prognosis, early diagnosis and transplantation are very to improve the quality of life and reduce the rate of disability.

In this study, 66.7% of the patients had TCIRG1 gene mutations (recessive inheritance), and 22.2% had CLCN7 gene mutations (recessive and dominant inheritance). Currently, controversy still exists regarding which patients are suitable for HSCT. For patients with TCIRG1 mutations, once diagnosed, they should undergo HSCT as soon as possible. However, for some patients with CLCN7 and OSTM1 gene mutations, previous articles showed that central involvement was irreversible and might be further aggravated after HSCT, and thus transplantation was not recommended [6, 25]. However, 6 patients with CLCN7 gene mutations were included in this group, including 2 patients with compound heterozygous mutations and 4 patients with heterozygous mutations (without neurodegeneration). In the present study, the clinical symptoms were significantly improved and no aggravation of nervous system involvement was observed after transplantation. Therefore, we postulate that these patients should still undergo HSCT if the disease occurs early with typical clinical manifestations [13, 26]. Furthermore, if patients have RANKL gene mutations, HSCT is not recommended because abnormalities in the gene may impede osteoclast maturation [27].

Conclusions

Based on the results described above, haplo-HSCT is an effective and feasible treatment for MIOP and IOP. The incidence of GVHD was high but mild and was effectively controlled after appropriate treatment. However, the symptoms of nervous system involvement did not readily recover, and thus the early identification and diagnosis of MIOP and IOP are the key to improving the prognosis. If no sibling and unrelated matched donors are available, a haploidentical related donor is also an important choice. The number of patients in this study was small, and the follow-up time must be increased to expand the analysis of the prognosis of patients with MIOP and IOP, especially the growth and intellectual development of children. The mortality rate of pulmonary haemorrhage and severe pneumonia in patients was high, which might be related to the intensity of the conditioning regimen, and thus further adjustments to the dose of the conditioning regimen are needed. The occurrence of aGVHD might be related to the larger number of infused cells, which also requires further adjustment.

Abbreviations

MIOP: Malignant infantile osteopetrosis; IOP: Intermediate osteopetrosis; haplo-HSCT: Haploidentical allogeneic haematopoietic stem cell transplantation; Flu: Fludarabine; Bu: Busulfan; Cy: Cyclophosphamide; GVHD: Graft versus host disease; CsA: Cyclosporin A; MMF: Mycophenolate mofetil; ALG: Anti-human T lymphocyte porcine immunoglobulin; ATG: Anti-human thymus globulin; HVOD: Hepatic veno-occlusive disease; OS: Overall survival; TCD: T cell-depleted; PTC: Posttransplantation cyclophosphamide.

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Authors contribution

GZ, AW: Writing—original draft. BW, JY, and YY: Writing—review and editing. YL, KW, and CJ: Data curation. SL and XZ: Formal analysis. TW, HZ, and MQ: Project administration. All authors read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author.

Declarations

Ethical approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (IRB) of Beijing Children's Hospital, Capital Medical University.

Consent for publication
All authors have read and approved the final manuscript. All parents signed informed consent forms and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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Publisher's Note

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Comparison of porcine ALG and rabbit ATG on outcomes of HLA-haploidentical hematopoietic stem cell transplantation for patients with acquired aplastic anemia

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Abstract

Objective: To evaluate the efficacy and safety of P-ALG (porcine anti-lymphocyte globulin) and R-ATG (rabbit anti-thymocyte globulin) in the conditioning regime for patients with acquired aplastic anemia who underwent HLA-haploidentical hematopoietic stem cell transplantation (halpo-HSCT).

Methods: A total of 91 patients with acquired aplastic anemia who received haplo-HSCT at our center between January 2014 and December 2020 were retrospectively reviewed. Twenty-eight patients were in the P-ALG group while sixty-three patients were in the R-ATG group.

Results: The median time was 11 versus 13 days (P = 0.294) for myeloid engraftment and 12.5 versus 15 days (P = 0.465) for platelet engraftment in the P-ALG and R-ATG groups, respectively. There were no significant difference in 5-year overall survival (74.83% ± 8.24% vs 72.29% ± 6.26%, P = 0.830), GVHD-free, failure-free survival (71.05% ± 8.65% vs 62.71% ± 6.22%, P = 0.662), failure-free survival (74.83% ± 8.24% vs 66.09% ± 5.84%, P = 0.647) and transplantation-related mortality (25.17% ± 8.24% vs 26.29% ± 6.22%, P = 0.708) between the two groups. The incidence of aGVHD (acute graft versus host disease) (65.39% ± 9.33% vs 62.71% ± 6.30%, P = 0.653), II–IV aGVHD (38.46% ± 9.54% vs 35.64% ± 6.24%, P = 0.695), III–IV aGVHD (19.23% ± 7.73% vs 10.53% ± 4.07%, P = 0.291), cGVHD (chronic graft versus host disease) (22.22% ± 12.25% vs 22.31% ± 6.30%, P = 0.915), and moderate to severe cGVHD (5.56% ± 5.40% vs 9.28% ± 4.46%, P = 0.993) were not significantly different. Similar outcomes were observed between the P-ALG and R-ATG groups for severe bacterial infection (17.9% vs 25.4%, P = 0.431), invasive fungal diseases (3.6% vs 9.5%, P = 0.577) and graft rejection (0% vs 9.5%, P = 0.218). However, the incidence of cytomegalovirus infection and Epstein-Barr virus infection was significantly lower in the P-ALG group (46.4% vs 71.4%, P = 0.022; 3.6% vs 25.4%, P = 0.014).

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Conclusion: The efficacy and safety of P-ALG were similar with R-ATG in the setting of haplo-HSCT for patients with acquired aplastic anemia patients. P-ALG could be an alternative for R-ATG.

Keywords: Anti-lymphocyte immunoglobulin, Anti-thymocyte immunoglobulin, Efficacy and safety, Acquired aplastic anemia, Haploidentical hematopoietic stem cell transplantation

Introduction

Acquired aplastic anemia (AA) is a rare bone marrow failure syndrome characterized by pancytopenia and hypocellular bone marrow which is clinically manifested by anemia, hemorrhage, and infection [1]. Currently, immunosuppressive therapy (IST) comprising antithymocyte globulin (ATG) with cyclosporine (CsA) and hematopoietic stem cell transplantation (HSCT) are the recommended treatment for severe AA/very severe AA (SAA/VSAA) and transfusion-dependent non-severe AA (NSAA). IST is the first line therapy for NSAA patients requiring transfusion support, SAA or VSAA patients without a matched sibling donor (MSD), and patients with older age [2]. For young patients (< 40 years), MSD HSCT should be the first-line therapy [3, 4]. However, only a small proportion of patients has a MSD, while almost every patient has a haploidentical donor. In recent years, more and more centers tried haplo-HSCT as the first-line therapy for young patients and demonstrated similar or better survival outcomes and acceptable complications compared with MSD-HSCT and IST [5–8]. ATG is a key drug in treatment of AA, whether in the IST or conditioning regime of HSCT. Rabbit ATG (R-ATG) and horse ATG (H-ATG) are widely used in different area of the world and proved to have good efficacy. However, horse ATG is not available in China, while a new porcine anti-lymphocyte globulin (P-ALG) is available. Several studies have compared the efficacy and safety of R-ATG and P-ALG in IST and suggested that P-ALG was similar to or even better than R-ATG [9–11]. In addition, P-ALG containing conditioning regime before HSCT in the setting of MSD-HSCT and MUD-HSCT have also demonstrated good efficacy and tolerance [12, 13]. However, P-ALG-containing conditioning regimen for haplo-HSCT has seldom been reported. Since haplo-HSCT has become an increasingly important treatment for AA patients, we aimed to evaluate the efficacy and safety of P-ALG comparing with R-ATG in the setting of haplo-HSCT in the paper.

Patients and methods

Patients and definitions

We retrospectively analyzed the data of 91 consecutive AA patients who received haplo-HSCT in stem cell transplantation center of the Institute of Hematology, Chinese Academy of Medical Science & Peking Union Medical College from January 2014 and December 2020. Institution and years for the patients who were treated with haplo-HSCT were listed in Additional file 1. For patients without events, the final date of follow-up was April 30, 2021. Of the 91 patients, 28 were categorized as SAA, 51 were VSAA and 12 were transfusion-dependent NSAA according to the diagnosis criteria [2]. Patients enrolled in the study did not have available MSD and voluntarily underwent haplo-HSCT. The exclusion criteria were as followings: patients with severe liver, kidney, heart, lung and other immunological diseases, patients who were pregnant, and whose bone marrow analysis were positive for myelodysplastic syndrome.

All patients and donors provided written informed consent for this protocol. For patients younger than 18 years old in the cohort, the consent was carried out by their patients. This study was approved by the Ethics Review Committee of the Institute of Hematology, Chinese Academy of Medical Science & Peking Union Medical College and was in compliance with the Declaration of Helsinki.

Conditioning regime

All patients undergoing haplo-HSCT received conditioning based on Flu (fludarabine) 30 mg/m^2/day × 5 days, Cy (cyclophosphamide) 50 mg/kg/day × 3 days, R-ATG (2.5 mg/kg/day × 5 days), or P-ALG (20–25 mg/kg/day × 5 days), with or without Bu (busulfan) 3.2 mg/kg/day × 2 days. Sixty-three patients received R-ATG, while twenty-eight patients received P-ALG as part of conditioning regime.

GVHD prophylaxis

For GVHD prophylaxis, all transplant recipients received FK506 or CSA (cyclosporine A), short-term methotrexate, in addition to MMF (mycophenolate mofetil) or not.

Supportive care

All patients resided in a class 100 laminar flow ward until neutrophil recovery. They routinely received antibiotic prophylaxis before transplantation: Compound Sulfamethoxazole Tablets 1.0 g twice a day for 1 week to prevent Pneumocystis carinii pneumonia and ganciclovir 10 mg/kg per day i.v. for 1 week to prevent CMV infection. For patients without history of invasive fungal disease (IFD) before transplantation, fluconazole

was applied for prophylaxis of IFD until 3 months after transplantation. Patients who had IFD before transplantation received itraconazole, voriconazole, micafungin or caspofungin according to their pretransplant situations.

Patients received platelet transfusion when their platelet levels were below 20×10^9/L or red blood cell transfusion if their hemoglobin levels were below 70 g/L. All patients received G-CSF (5 μg/kg once daily) from day + 6 until myeloid recovery.

CMV and EBV monitorization was done three times a week after transplantation when patients were in the hospital, and when they were out of hospital it was tested weekly until six months post transplantation.

Criteria of outcomes

Engraftment was defined as ANC (absolute neutrophil counts) $\geq 0.5 \times 10^9$/L for three consecutive days and platelet counts $\geq 20 \times 10^9$/L without transfusion for 7 consecutive days.

The Mount Sinai Acute GVHD International Consortium (MAGIC) criteria was used to diagnose and grade acute GVHD (aGVHD) [14], while diagnosis and classification of chronic GVHD (cGVHD) was according to the 2014 National Institutes of Health consensus of cGVHD [15].

Graft rejection was defined as not reach the engraftment criterion of ANC $\geq 0.5 \times 10^9$/L after transplantation or lose initial engraftment with minimal (< 5%) chimerism or entire recipient chimerism [16]. Chimerism status was evaluated by PCR of short tandem repeat sequences.

CMV (cytomegalovirus)—DNA was detected by plasma sample using real-time PCR and CMV viremia was defined as > 1000 copies/mL, and so was EBV (Epstein-Barr virus) viremia.

Invasive fungal disease (IFD) was defined according to the revised EORTC/MSG criteria [17]. Severe bacterial infection referred to bacteraemia or severe tissue infections. Regimen-related toxicity (RRT) was assessed according to Seattle Toxicity Criteria [18]. Overall survival (OS) was calculated from HSCT to death of any cause or last follow-up. GVHD-free, failure-free survival (GFFS), failure-free survival (FFS), transplantation-related mortality (TRM) were defined according to previous studies [6, 19]. GFFS (GVHD-free, failure-free survival) was defined as survival without grades III-IV acute GVHD, extensive chronic GVHD, and treatment failures. Treatment failures included death, relapse and primary or secondary graft failure. Failure-free survival (FFS) was defined as survival with response. Transplantation-related mortality (TRM) referred to death without relapse.

Statistical analysis

The data were analyzed by the software GraphPad Prism 8, IBM SPSS statistics 25. The descriptive statistics for continuous variables and Chi-square test and Fisher's exact test for categorical variables were used to compare incidence in univariate analysis. The Kaplan–Meier method was used to estimate the cumulative survival/incidence and differences were compared by the log-rank test. A two-sided $P < 0.05$ was considered as statistically significant.

Results

Characteristics of patients

A total of 91 patients were enrolled in the study. Twenty-eight and sixty-three patients were in the P-ALG group and R-ATG group, respectively. The baseline characteristics of patients in the two groups were listed in Table 1.

Transplantation details

Transplantation associated details including graft source, donor/recipient match (bloodtype, gender, HLA), GVHD prophylaxis and donor age between the two groups were similar (Table 2). The median dose of infused MNC and $CD34^+$ cell in the P-ALG group were 10.66×10^8/kg (range 5.08–22.68) and 3.44×10^6/kg (range 2.02–8.60), which is not significantly different from the R-ATG group [MNC: 9.37×10^8/kg (range 6.00–25.49) and $CD34^+$ cell: 3.25×10^6/kg (range 1.59–7.35)].

Table 1 Characteristics of patients

Characteristics	P-ALG (N = 28)	R-ATG (N = 63)	P value
Gender			
Male	13 (46.4%)	40 (63.5%)	0.128
Female	15 (53.6%)	23 (36.5%)	
Age (years)			
Median (range)	23 (6–55)	16 (4–52)	0.140
Diagnosis			
SAA	7 (25.0%)	21 (33.3%)	0.277
VSAA	15 (53.6%)	36 (57.1%)	
NSAA	6 (21.4%)	6 (9.6%)	
ATG before HSCT			
Yes	4 (14.3%)	9 (14.3%)	1.000
No	24 (85.7%)	54 (85.7%)	
Infection before transplantation			
Yes	3 (10.7%)	13 (20.6%)	0.396
No	25 (89.3%)	50 (79.4%)	
Interval from diagnosis to HSCT (months)			
Median (range)	4 (1–247)	3 (1–214)	0.248

Table 2 Transplantation details

Conditioning regime	P-ALG (N = 28) Flu, CY, P-ALG ± Bu	R-ATG (N = 63) Flu, CY, R-ATG ± Bu	P value
Graft source			
PBSC	24 (85.7%)	47 (74.6%)	0.237
PBSC + BM	4 (14.3%)	16 (25.4%)	
Donor-recipient bloodtype match			
Matched	14 (50.0%)	34 (54.0%)	0.627
Major mismatched	7 (25.0%)	9 (14.3%)	
Minor mismatched	4 (14.3%)	13 (20.6%)	
Bidirectional mismatch	3 (10.7%)	7 (11.1%)	
Donor-recipient gender match			
Female to female	3 (10.7%)	9 (14.3%)	0.256
Female to male	5 (17.9%)	15 (23.8%)	
Male to female	12 (42.9%)	14 (22.2%)	
Male to male	8 (28.6%)	25 (39.7%)	
Donor-recipient HLA match			
5/10	15 (53.6%)	32 (50.8%)	0.108
6/10	3 (10.7%)	14 (22.2%)	
7/10	1 (3.6%)	10 (15.9%)	
8/10	4 (14.3%)	4 (6.3%)	
9/10	3 (10.7%)	2 (3.2%)	
10/10	2 (7.1%)	1 (1.6%)	
GVHD prophylaxis			
CSA + MTX	22 (78.6%)	45 (71.4%)	0.475
FK506 + MTX	6 (21.4%)	18 (28.6%)	
Donor-recipient CMV serostatus			
D^+/R^+	21 (95.5%)	54 (98.2%)	0.492
D^-/R^+	1 (4.5%)	1 (1.8%)	
D^+/R^-	0	0	
D^-/R^-	0	0	
Donor age (years)			
Median (range)	34 (9–54)	36 (8–62)	0.447
Dose of MNC ($\times 10^8$/kg)			
Median (range)	10.66 (5.08–22.68)	9.37(6.00–25.49)	0.411
Dose of $CD34^+$ cells($\times 10^6$/kg)			
Median (range)	3.44 (2.02–8.60)	3.25 (1.59–7.35)	0.827

Engraftment

All patients except three patients (1 in the P-ALG group and 2 in the R-ATG group) with early death (< 14 days) had ANC engraftment, whereas 24 patients (85.7%) in the P-ALG group and 52 patients (82.5%) in the R-ATG group had platelet engraftment in 100 days post transplantation. The median time of ANC recovery in the P-ALG group and R-ATG group was 11 days (range 10–22) and 13 days (range 10–23), respectively. For platelet recovery, the median time was 12.5 days (range 8–95) and 15 days (range 10–83), respectively.

No patient in the P-ALG group experienced GR, while 6 patients (9.5%) developed GR in the R-ATG group.

Infection

One patient in the P-ALG group and six patients in the R-ATG developed IFD (3.6% vs 9.5%, P = 0.577). Five patients in the P-ALG and sixteen patients in the R-ATG group developed severe bacterial infection (17.9% vs 25.4%, P = 0.431). The percentage of patients developing CMV viremia and EBV viremia in the P-ALG group were 46.4% (13/28) and 3.6% (1/28), respectively. While in the

R-ATG group, the incidence of CMV viremia and EBV viremia was 71.4% (45/63) and 25.4% (16/63), respectively. The incidence of CMV viremia and EBV viremia was significantly lower in the P-ALG group than the R-ATG group (P = 0.022 and P = 0.014, respectively).

GVHD

In the P-ALG group, 17 patients developed aGVHD, and 5 of whom were grade III-IV aGVHD. In the R-ATG group, 37 patients developed aGVHD, and 6 of whom were grade III-IV aGVHD. There was no significant difference of aGVHD between the two groups. The incidence of cGVHD or moderate to severe cGVHD of P-ALG group and R-ATG group were also similar (22.22% ± 12.25% vs 22.31% ± 6.30%, P = 0.915, 5.56% ± 5.40% vs 9.28% ± 4.46%, P = 0.993, respectively) (Table 3, Fig. 2).

Table 3 Outcomes of patients

Outcomes	P-ALG (N = 28)	R-ATG (N = 63)	P value
Time of engraftment			
ANC, days (range)	11 (10–22)	13 (10–23)	0.294
PLT, days (range)	12.5 (8–95)	15 (10–83)	0.465
Infection			
CMV			
Yes	13 (46.4%)	45 (71.4%)	0.022
No	15 (53.6%)	18 (28.6%)	
EBV			
Yes	1 (3.6%)	16 (25.4%)	0.014
No	27 (96.4%)	47 (74.6%)	
IFD			
Yes	1 (3.6%)	6 (9.5%)	0.577
No	27 (96.4%)	57 (90.5%)	
Severe bacterial infection			
Yes	5 (17.9%)	16 (25.4%)	0.431
No	23 (82.1%)	47 (74.6%)	
Graft rejection			
Yes	0 (0%)	6 (9.5%)	0.218
No	28 (100%)	57 (90.5%)	
GVHD			
aGVHD, %	65.39 ± 9.33	62.71 ± 6.30	0.653
II–IV aGVHD, %	38.46 ± 9.54	35.64 ± 6.24	0.695
III–IV aGVHD, %	19.23 ± 7.73	10.53 ± 4.07	0.291
cGVHD, %	22.22 ± 12.25	22.31 ± 6.30	0.915
Moderate-severe cGVHD, %	5.56 ± 5.40	9.28 ± 4.46	0.993
5-year OS, %	74.83 ± 8.24	72.29 ± 6.26	0.830
5-year GFFS, %	71.05 ± 8.65	62.71 ± 6.22	0.662
5-year FFS, %	74.83 ± 8.24	66.09 ± 5.84	0.647
5-year TRM, %	25.17 ± 8.24	26.29 ± 6.22	0.708

Deaths and survival

Seven patients died in the P-ALG group, 5 with aGVHD and 2 with infection. Sixteen patients died in the R-ATG group, 4 with aGVHD, 3 with cGVHD, 6 with infection, 2 with graft rejection, and 1 with intracranial hemorrhage, respectively. In the P-ALG group, at a median follow-up of 212 days (range from 10 to 2557), 21 patients survived and the 5-year OS was 74.83% ± 8.24%. In the R-ATG group, at a median follow up of 729 days (range from 6 to 2648), 47 patients survived and the 5-year OS was 72.29% ± 6.26%. There was no significant difference in terms of the 5-year OS between the 2 groups (P = 0.830), and so were the 5-year GFFS, 5-year FFS and 5-year TRM (Table 3, Figs. 1, 2).

Discussion

Immunosuppressive therapy and MSD-HSCT are the frontline therapy for AA patients. Since a matched sibling donor is not available for every patient, haplo-HSCT has increasingly become a therapeutic option for patients with AA due to optimal conditioning regimens and improved supportive care. In recent years, a series of studies [5–8, 20] suggested that haplo-HSCT showed an overall efficacy comparable to those with IST and MSD-HSCT.

Horse or rabbit ATG is commonly used worldwide, while horse ATG is not available in China. Porcine ALG is a product developed in China and was approved by the Sino Food and Drug Administration in 2004. A series of retrospective studies evaluating the efficacy of P-ALG in treatment of AA were conducted [8–13, 21–24]. P-ALG was reported to have similar or superior overall response at 6 months compared to R-ATG (64.0–79.4% vs 48.1–64.7%) in the IST treatment for AA patients [25]. Liu et al. [21] retrospectively analyzed SAA patients treated with either P-ALG (n = 43) or R-ATG (n = 32) plus CSA, and suggested there were no significant difference in 2-year OS between the 2 groups (87.4% ± 6.2% vs 83.2% ± 7.8%, P = 0.493). Other studies [10, 11] comparing the efficacy of P-ALG and R-ATG in the IST treatment showed similar results.

Moreover, some studies have compared the efficacy of P-ALG and R-ATG in the conditioning regime before transplantation. We have previously compared the efficacy of P-ALG and R-ATG in MSD-HSCT for patients with SAA, including 55 patients in the P-ALG group and 58 patients in the R-ATG group [12]. There was also no significant difference in 3-year overall survival

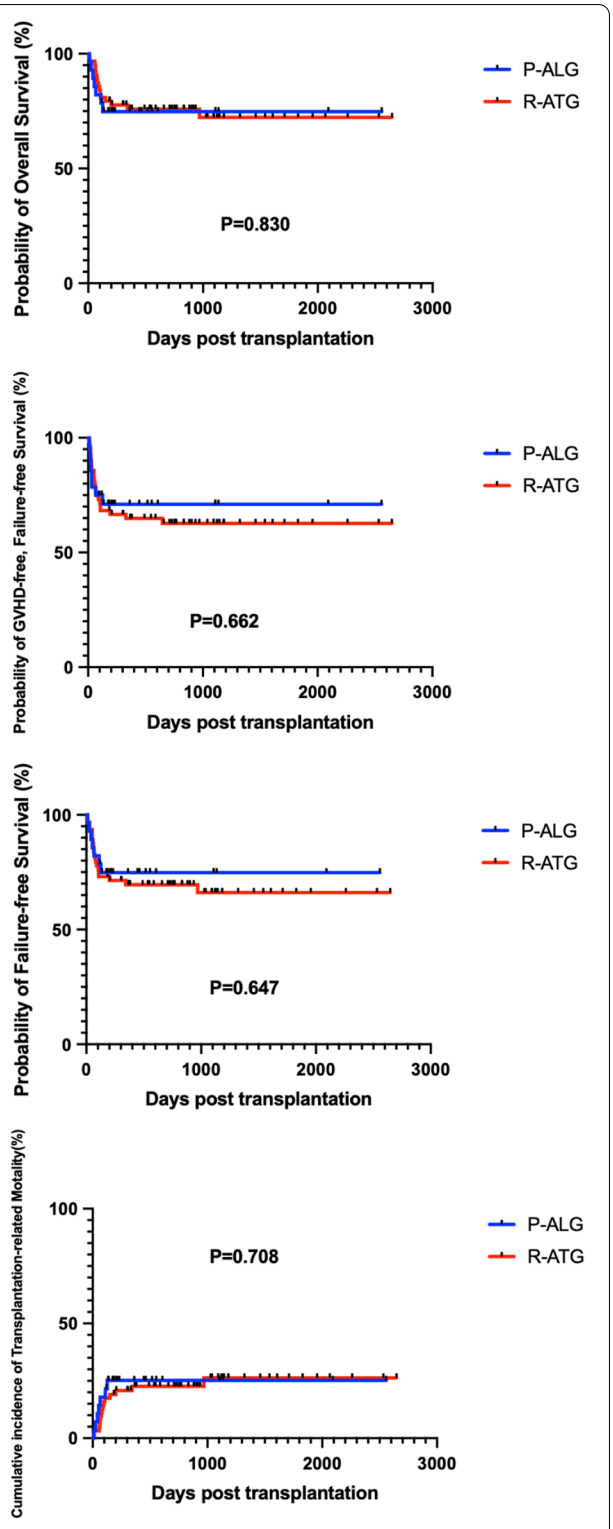

Fig. 1 Survival curves

(84.4% ± 5.7% in the P-ALG group vs 93.1% ± 3.3% in the R-ATG group, P = 0.235), whereas the incidence of aGVHD and cGVHD was higher in P-ALG group (20.7% ± 5.3% vs 43.4% ± 7.0%, P = 0.015; 20.1% ± 5.8% vs 46.0% ± 7.9%, P = 0.003). The higher incidence of aGVHD and cGVHD might be related to older age and more PBSC as graft source in the P-ALG group. Recently, Li et al. [13] evaluated the outcomes of 41 SAA patients receiving a P-ALG-containing conditioning regimen before MSD-HSCT and matched-unrelated HSCT (URD-HSCT). The actuarial 3-year OS and disease-free survival (DFS) were 95.1% ± 3.4% and 85.0% ± 5.7%, respectively. The cumulative incidence of grades III to IV aGVHD and 5-year cumulative incidence of moderate-severe cGVHD was 4.9% ± 3.4% and 10.8% ± 5.1% , respectively. These studies suggested the P-ALG-containing regimen has satisfactory effects and safety in MSD-HSCT and URD-HSCT for SAA patients. However, the efficacy and safety of P-ALG in haplo-HSCT has seldom been reported.

In this study, we wanted to compare the efficacy and safety of P-ALG and R-ATG in the setting of haplo-HSCT. The baseline characteristics and transplantation details of patients of the two groups were similar (Tables 1, 2).

The incidence of aGVHD, II-IV aGVHD and III-IV aGVHD in the P-ALG group and R-ATG group were comparable and so were the incidence of cGVHD and moderate to severe cGVHD (Table 3). And the incidence rate of aGVHD and cGVHD were similar to previous reported in the setting of haplo-HSCT [6].

There were no significant difference of IFD (P = 0.577) and severe bacterial infection (P = 0.431), graft rejection (P = 0.218) and engraft time (Table 3).

Interestingly, in our study, the incidence of CMV viremia and EBV viremia was significantly lower in the P-ALG group than the R-ATG group (46.4% vs 71.4%, P = 0.022 and 3.6% vs 25.4%, P = 0.014). Previous studies of ATG/ALG in IST indicated more clearance of peripheral blood lymphocytes by R-ATG than by P-ALG [9, 10, 25]. Patients in R-ATG group had a significantly lower minimum number of lymphocytes than the P-ALG group and remained significantly lower after 1, 3 and 6 months of treatment initiation and recovered to equivalent levels after 12 months [10]. Furtherly, when comparing the changes of subsets in T cells, the inhibitory effects on $CD4^+$ T cells were significantly higher in the R-ATG group than in the P-ALG group, while the inhibitory effects on the $CD8^+$ T cells were not different between

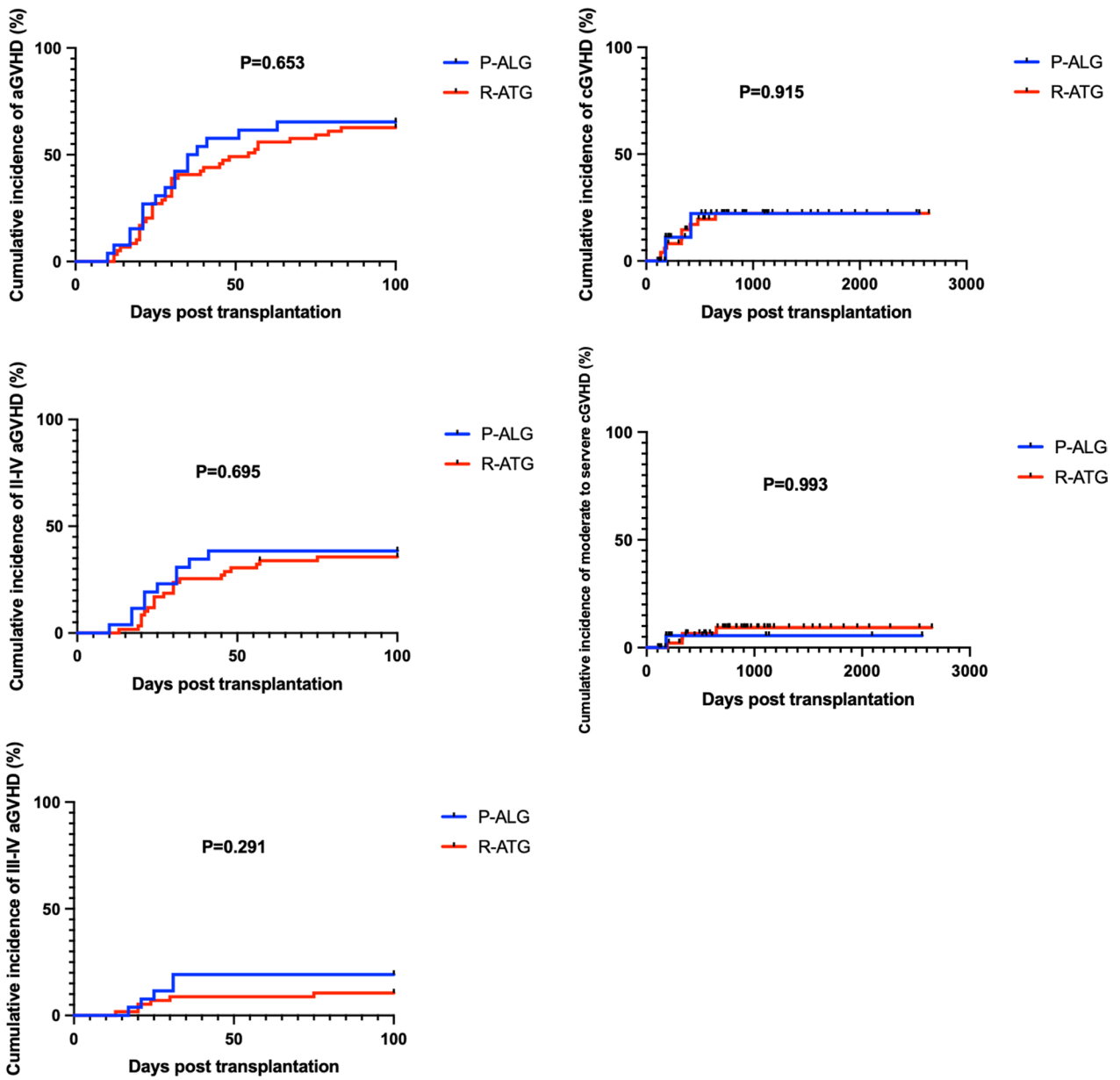

Fig. 2 Incidence of aGVHD and cGVHD

the two groups. Thus, the stronger immunosuppressive effects of R-ATG than P-ALG, mainly on $CD4^+$ T cells, may possibly account for the higher incidence of CMV and EBV infection in R-ATG group than the P-ALG group.

Until the date of last follow-up, 21 patients and 47 patients were alive in the P-ALG group and R-ATG group, respectively. The 5-year OS, GFFS and FFS and TRM were similar in the two groups (P = 0.830, P = 0.662, P = 0.647, P = 0.708).

In conclusion, P-ALG achieved similar survival and safety profiles compared with R-ATG. The present study provided evidence in clinical application of P-ALG in the conditioning regime of haplo-HSCT. P-ALG could be a potential alternative preparation for R-ATG. However, due to the retrospective origin and small sample size, further prospective, large-scaled clinical trials are needed to investigate the effects and explore the underlying molecular mechanisms for ATG/ALG.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12935-021-02410-z.

Additional file 1. Institution and years for the patients who were treated with haplo-HSCT

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Authors' contributions
SZF, JXZ, JC and YFZ conceived and designed the study. JC and YFZ analysed the data and drafted the manuscript. SZF secured financing of the study. XC, AMP, YQZ, LL, RZM, JLW, YH, DLY, RLZ, WHZ, QLM, ELJ and MZH contributed to the review and editing. All authors read and approved the final manuscript.

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Availability of data and materials
The data and materials can be obtained from the first author and corresponding author.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Review Committee of the Institute of Hematology, Chinese Academy of Medical Science & Peking Union Medical College. Written informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

Consent for publication
Written informed consent for publication was obtained from all participants.

Competing interests
The authors declare that they have no competing interests.

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Comparison of Hematopoietic Stem Cell Transplantation Outcomes Using Matched Sibling Donors, Haploidentical Donors, and Immunosuppressive Therapy for Patients With Acquired Aplastic Anemia

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We retrospectively compared the outcomes of 387 consecutive patients with acquired aplastic anemia (AA) who underwent hematopoietic stem cell transplantation (HSCT) with a fludarabine-based conditioning regimen from matched sibling donors (MSD) (n = 108) or haploidentical donors (HID) (n = 91) and immunosuppressive therapy (IST) (n = 188) from 2014 to 2020 at our hospital. Compared with HID-HSCT, MSD-HSCT had a lower incidence of graft failure (1% *vs*. 7%, $p = 0.062$), grade II–IV acute graft versus host disease (aGvHD) (16% vs. 35%, $p = 0.001$), and mild to severe chronic GvHD (cGvHD) (8% vs. 23%, $p = 0.007$), but an equivalent incidence of grade III–IV aGvHD (8% vs. 12%, $p = 0.237$) and moderate to severe cGvHD (3% vs. 9%, $p = 0.076$). HSCT had superior blood count recovery at 3, 6, and 12 months compared with IST ($p < 0.001$). The estimated 5-year overall survival (OS) of the MSD, HID, and IST groups were 86%, 72%, and 79% ($p = 0.02$), respectively; accordingly, the failure-free survival (FFS) rates were 85%, 68%, and 56%, respectively ($p < 0.001$). For patients aged ≤40 years, the OS rate was still significantly superior for MSD-HSCT receipients compared to HID-HSCT receipients (89% vs. 76%, $p = 0.024$) while the HID-HSCT recipients showed similar OS (76% vs. 78%, $p = 0.166$) but superior FFS ($p = 0.047$) when follow-up was longer

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than 14.5 months in contrast to IST. In a multivariate analysis, HID-HSCT and a conditioning regimen that included busulfan were adversely related to OS among patients who received allografts. In conclusion, MSD-HSCT was the frontline choice for patients with severe AA aged ≤40 years, while HID-HSCT was as effective as IST for patients without an MSD.

Keywords: aplastic anemia, transplantation, matched sibling donor, haploidentical donor, immunosuppressive therapy

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) from HLA-matched sibling donors (MSD) is recommended for young and adult patients with severe aplastic anemia (SAA) (1). For those patients without an MSD but with comorbidities or an older age, immunosuppressive therapy (IST) should be considered. However, the efficacy of IST is limited due to treatment non-response, relapse, and clonal evolution (2). Outcomes following alternative donor HSCT, such as haploidentical donor HSCT (HID-HSCT), have improved dramatically over the last decade due to the reduced incidences of graft failure (GF) and graft versus host disease (GvHD). In China, the addition of busulfan (BU) to high-dose cyclophosphamide (CY) (200 mg/kg) combined with rabbit antithymocyte globulin (rATG) 10 mg/kg was an effective conditioning regimen for HID-HSCT and resulted in a similar overall survival (OS) to MSD-HSCT (3) and a superior failure-free survival (FFS) than IST (4, 5). Importantly, for young patients, fertility is a critical issue that may be helped by modifying the conditioning regimen. There is currently no comprehensive comparison of the feasibility and safety of MSD-HSCT, HID-HSCT, and IST for patients with SAA. Further, we used a FAC-conditioning regimen consisting of fludarabine (FLU)+ATG+CY for patients with SAA, with the addition of BU (BFAC) for those with a high risk of GF, such as those with a long disease history, heavy transfusion, and transfusion-dependent non-severe AA (NSAA). We therefore performed a retrospective study to compare the outcomes of MSD-HSCT, HID-HSCT, and IST for patients with AA at our hospital over the same time period.

PATIENTS AND METHODS

Patients

From 2014 and 2020, a total of 387 consecutively patients with acquired AA who received MSD-HSCT (108), HID-HSCT (91), or IST (188) were enrolled into this study. Enrollment criteria included SAA, severe SAA, or transfusion-dependent NSAA as defined by clinical guidelines (6); voluntary use of HSCT or IST; the absence of severe organ dysfunction; and age ≤60 years for HSCT (no age limitation for IST). Excluded were patients with underlying inherited marrow failure disorders such as Fanconi anemia; myelodysplastic syndrome (MDS); pregnant patients; and those with severe organ impairment or an uncontrolled active infection. Patients with paroxysmal nocturnal hemoglobinuria (PNH) clones were also included in this analysis. All written informed consent was attained from the patients or their relatives. This study was approved by the Ethics Committee at our hospital (IIT2021011-EC-1).

Transplantation and IST Procedures

Transplantation: the FAC conditioning regimen was composed of FLU 150 mg/m^2 i.v. in divided doses on days -6 to -2, CY 120 or 150 mg/kg i.v. in a divided dose on days -5 to -2, and rATG (Thymoglobulin®, Genzyme, Cambridge, MA) 12.5 mg/kg or porcine antilymphocyte globulin (pALG) (Anti-lymphocyte Immunoglobulin®, Wuhan Institute of Biological Products Co., Ltd., China) 100 or 125 mg/kg i.v. in divided doses on days -5 to -2. The BFAC conditioning regimen was composed of BU 6.4 mg/kg i.v. in divided doses on days -7 to -6, FLU 150 mg/m^2 i.v. in divided doses on days -6 to -2, CY 80 to 150 mg/kg i.v. in divided doses on days -5 to -2, and rATG 12.5 mg/kg, pALG 100, or 125 mg/kg i.v. in divided doses on days -5 to -2. We have demonstrated the similar efficacy between rATG and pATG among the MSD-HSCT recipients (7). Since then, selection of ATG was according to the intention of patients and doctors while rATG was mostly used before. Since 2016, the donor-specific anti-HLA antibody (DSA) test was routinely performed during donor selection of HID-HSCT and donors with a negative DSA were chosen. Otherwise, in the absence of an alternative suitable donor, it is recommended that measures against DSAs should be taken (8). Totally, 68 HID-HSCT recipients were identified DSAs and 1 patient was positive. GvHD prophylaxis, infection prevention, and surveillance were in accordance with our previous report (7) and the experience of HID-HSCT performed at other centers in China (4, 5).

IST: The patients in the IST group were treated with rATG at a total dose of 17.5 mg/kg i.v. in divided doses for 9 consecutive days, in line with our previous report (9), or pALG 125 mg/kg i.v. in divided doses for 5 consecutive days. Oral cyclosporine-A (3–5 mg/kg/day) was started 2 weeks after the last day of the rATG/pALG treatment and was administered for at least 2 years (with dose adjustments to achieve a whole blood trough level of 100–200 ng/ml for adults and 100–150 ng/ml for children).

Definitions

Blood count after therapy was classified as complete response (CR), partial response (PR), and no response (6), and anti-infection response was classified as complete remission, partial remission, stable disease (SD), and progressive disease (10). Days of neutrophil and platelet engraftment (5), acute GvHD (aGvHD) (11), chronic GvHD (cGvHD) (12), and GF (13) were defined according to previously reported criteria. Therapy-related mortality (TRM) was

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defined as death without relapse. Treatment failures from IST included death, non-response at 6 months and beyond, disease progression requiring intervention, relapse, and clonal evolution (14). Treatment failures after HSCT were defined as death, and primary or secondary GF, whichever came first. FFS was defined as survival without treatment failure. GvHD-free, failure-free survival (GFFS) was defined as survival without grade III–IV aGvHD, moderate to severe cGvHD, or treatment failure (15). OS was defined as the time from treatment start to death or last follow-up.

Statistical Analysis

The primary objective of this study was to compare the OS of AA patients who received different procedures. Other major outcomes included infection, engraftment, aGvHD, cGvHD, TRM, GFFS, and FFS.

All patients involved had an outpatient department or telephone follow-up. The final date of follow-up was April 30, 2021. Continuous and categorical variables were compared using the Mann–Whitney U, chi-square test, or Fisher exact test, respectively. The cumulative incidence of GvHD and TRM was estimated using a competing risk model and compared with the Gray's test. Death was considered a competing event for GvHD. The probabilities of OS and FFS were estimated using the Kaplan–Meier method and compared between the different groups of patients using the log-rank test. A landmark analysis was performed when the curves crossed (16). Variables with p values ≤0.05 in the univariate analysis were entered into multivariate analyses using a Cox proportional hazards model to identify factors impacting OS, FFS, and GFFS of transplant patients. According to the results of previous studies (17, 18), variables including patient age, interval from diagnosis to transplantation, RBC transfusions before transplantation, graft source, conditioning regimen, and ATG source were used as covariates in propensity score matching. Patients in the MSD groups were matched to those in the HID group using 1:1 nearest neighbor matching with a caliper width of 0.2. Statistical analyses were performed with the R software package (R 4.0.5), GraphPad Prism 5, and SPSS 20.0 statistical software. GraphPad Prism 5 was also used to generate figures. All p values were two-sided, and the results were considered statistically significant when $p < 0.05$.

RESULTS

Patient Characteristics

The basic characteristics of enrolled patients and their donors are summarized in **Table 1** and **Supplementary Table 1**. The median ages of patients in the MSD, HID, and IST groups were 26 years (range, 4–54 years), 19 years (range, 4–55 years), and 28 years (range, 7–65 years) ($p < 0.001$), respectively. The IST group had a higher proportion of patients who were older than 40 years (26%) than the HSCT groups ($p < 0.001$ in both cases). The median time from diagnosis to therapy start was 5 months (range, 1–248 months) in the HID group, which was significantly longer than in the MSD (3 months, range, 1–205 months) and IST (2 months, range, 0.4–59 months) ($p < 0.001$) groups. There were no differences between three groups in terms of patient gender and the presence of PNH clones. Details of patients and their donors are provided in **Supplementary Table 1**. The MSD group had a lower rate of male donors (47% vs. 65%, $p = 0.019$) but higher rates of infection (complete remission, partial remission, and stable disease) before HSCT (29% vs. 19%, $p = 0.009$), and younger donors (median age, 26 years vs. 36 years, $p < 0.001$) compared with the HID group. There were also significant differences

TABLE 1 | Characteristics and outcomes of patients with acquired aplastic anemia.

Variables	MSD group (108)	HID group (91)	IST group(188)	*p* value
Patient age, years, median (range)	26 (4–54)	19 (4–55)	28 (7–65)	<0.001
Patient age group (years), no. (%)				<0.001
≤20	41 (38)	49 (54)	57 (30)	
>20 ≤ 40	54 (50)	34 (37)	83 (44)	
>40	13 (12)	8 (9)	48 (26)	
Patient sex (male), no. (%)	68 (63)	53 (58)	105 (56)	0.489
Diagnosis, no. (%)				<0.001
Severe aplastic anemia	60 (56)	51 (56)	97 (52)	
Very severe aplastic anemia	40 (37)	29 (32)	90 (48)	
Non-severe aplastic anemia	8 (7)	11 (12)	1 (1)	
Presence of PNH clones	19 (18)	18 (20)	36 (19)	0.917
Interval from diagnosis to therapy, moths, median (range)	3 (1–205)	5 (1–248)	2 (0.4–59)	<0.001
28-day death, no. (%)	2 (2)	4 (4)	2 (1)	0.208
60-day death, no. (%)	3 (3)	7 (8)	6 (3)	0.184
3-month complete remission of CBC, no. (%)	32 (35)[a]	25 (32)[a]	9 (5)[a]	<0.001
6-month complete remission of CBC, no. (%)	51 (60)[b]	46 (67)[b]	26 (15)[b]	<0.001
12-month complete remission of CBC, no. (%)	46 (78)[c]	46 (82)[c]	43 (31)[c]	<0.001
MDS/AML transformation, no. (%)	0	0	11 (6)	0.002
Follow-up of alive patients, moths, median (range)	37 (4–87)	25 (4–87)	23 (3–87)	0.011

[a]*91, 77, and 182 evaluable patients.*

[b]*85, 69, and 173 evaluable patients.*

[c]*59, 56, and 140 evaluable patients.*

MSD, matched sibling donor; HID, haploidentical donor; IST, immunosuppressive therapy; no., number of patients; PNH, paroxysmal nocturnal hemoglobinuria; CBC, complete blood count; MDS, myelodysplastic syndrome; AML, acute myelocytic leukemia.

between the MSD and HID groups in terms of ATG source and the number of mononuclear, $CD34^+$, $CD3^+$, $CD4^+$, and $CD8^+$ cells in the graft (**Supplementary Table 1**).

Transplantation Outcomes

Twenty-eight days post-HSCT, 106 of 108 and 87 of 91 patients survived in the MSD and HID groups, respectively. All surviving patients achieved neutrophil engraftment, while patients in the MSD group had a higher rate of 28-day platelet engraftment (94% vs. 76%, $p < 0.001$). The rates of primary graft failure were not significantly different (0 vs. 2%, $p = 0.202$). The median days until neutrophil and platelet engraftment were 12 days (range, 8–19 days) and 13 days (range, 7–37 days) in the MSD group compared with 12 days (range, 10–23 days) ($p = 0.039$) and 14 days (range, 8–95 days) ($p = 0.023$) in the HID group, respectively. MSD patients had a lower incidence of GF compared with HID patients (1% vs. 7%, $p = 0.062$), and one HID-HSCT recipient was diagnosed as primary GF with a median fluorescent intensity of DSA 3735 which was previously reported in another paper (8).

Only patients who survived beyond 28 and 100 days after transplantation were evaluated for aGvHD (1 patient in the HID group who experienced grade III aGvHD on day 17 and died on day 26 was included) and cGvHD, respectively. aGvHD occurred in 25 of 106 patients (24%) in the MSD group vs. 59 of 88 patients (67%) in the HID group ($p < 0.001$). In the MSD group, 5, 11, 6, and 3 patients developed grade I, grade II, grade III, and grade IV aGvHD, respectively, while in the HID group, 27, 21, 9, and 2 patients developed grade I, grade II, grade III, and grade IV aGvHD, respectively. As shown in **Figure 1**, the cumulative incidences of grade II–IV aGvHD at day 100 ($p = 0.001$) and mild to severe cGvHD at 5 years ($p = 0.007$) were significantly lower following MSD grafts vs. HID grafts. However, the cumulative incidences of grade III–IV aGvHD at day 100 ($p = 0.237$) and that of moderate to severe cGvHD at 5 years were similar ($p = 0.076$). MSD patients had a lower incidence of CMV (26% vs. 58%, $p < 0.001$) and EBV viremia (2% vs. 21%, $p < 0.001$) compared with HID patients. However, incidences of bloodstream infection (BSI) before neutrophil engraftment between the two groups were similar ($p = 0.668$).

By 28 days post-HSCT, 2 and 4 patients died in the MSD and HID groups, respectively. All 6 patients had infections prior to transplantation. In detail, 1 patient had mucormycosis confirmed with a bronchoscopy pre-HSCT and had a perianal swab before transplantation that grew carbapenem-resistant enterobacteriaceae (CRE); 1 patient had *Pseudomonas aeruginosa* (PA)-BSI at diagnosis, a nasal swab grew carbapenem-resistant *Klebsiella pneumoniae* (CRKP) colonization before transplantation, and nasopharyngeal and perianal swabs screened positive for CRPA and CRE colonization during conditioning; 1 patient had a disseminated infection of their lung and spleen, which was defined as SD and CRKP colonization pre-HSCT; 1 patient had CRPA-BSI at diagnosis and possibly an invasive pulmonary fungal disease defined as SD at transplantation; and 2 patients had prior

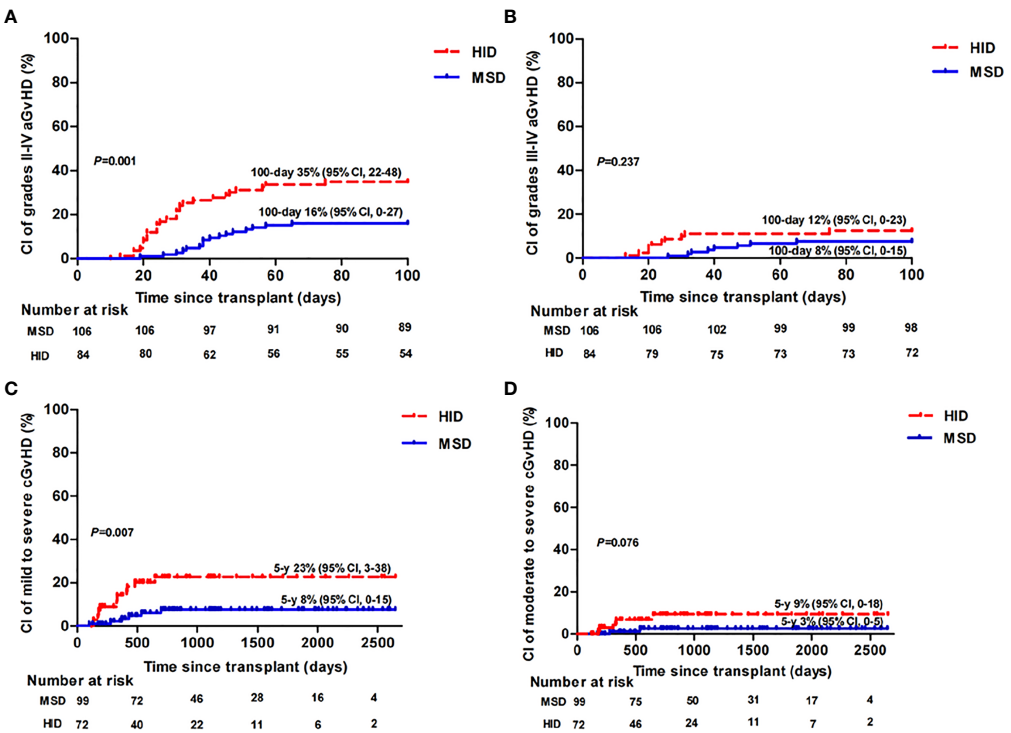

FIGURE 1 | Cumulative incidence (CI) of grade II–IV aGvHD **(A),** grade III–IV aGvHD **(B)**, mild to severe cGvHD **(C),** and moderate to severe cGvHD **(D)** in MSD-HSCT and HID-HSCT patients.

long-term courses of broad-spectrum antibiotics pre-HSCT due to multiple sites of infection in partial remission at the time of transplantation. Five out of the 6 patients died of multidrug-resistant organism (MDRO)-BSI (n = 4) or progressive pulmonary infection (n = 1). GF occurred in 7 patients, of whom 3 received a secondary transplantation with 1 expired due to aGvHD 2 patients recovered with partial autologous blood recovery, 1 patient experienced blood recovery from a frozen donor stem cell infusion and supportive care, and 1 patient died from infection. With a median follow-up of 37 months (range, 4–87 months) and 25 months (range, 4–87 months), 14 and 23 patients died in the MSD and HID groups, respectively. As shown in **Table 2**, the leading causes of death were aGvHD (n = 8 vs. n = 9) followed by infection (n = 5 vs. n = 8) in the MSD and HID groups, respectively.

IST Outcomes

Three months after IST, 6 patients were not evaluable due to death. In total, 182 of 188 patients were contacted. Of these, only 9 (5%) were in CR and 75 (41%) patients were in PR. A total of 173 patients were evaluable at 6 months, of whom 26 (15%) and 79 (46%) achieved CR and PR, respectively. One year after IST, a total of 18 were not evaluated for a response due to death (n = 17) or transplantation (n = 1). Of the 140 patients accessed, 43 (31%) were in CR and 60 (43%) were in PR. At the last follow-up on April 30, 2021, of the 157 evaluable patients, 79 (50%) were in CR and 46 (29%) were in PR. Compared with transplantation, IST had a significantly lower rate of complete blood count recovery at these timepoints (**Table 1**, $p < 0.001$). Nine patients underwent HID-HSCT due to treatment failure, 7 patients after non-response at 6 months, 1 patient with acute myelocytic leukemia (AML) transformation at 11 months, and 1 patient with MDS transformation at 12 months after initial IST and received HID-HSCT.

With a median follow-up of 23 months (range, 3–87 months), a total of 31 patients died. The causes of death included infection in 22 patients, gastrointestinal hemorrhage in 1 patient, intracranial hemorrhage in 6 patients, and MDS/AML evolution in 2 patients.

Survival, TRM, and Clone Evolution

The estimated 5-year OS rates were 86% [95% confidence interval (CI), 81–95], 72% (95% CI, 64–84), and 79% (95% CI, 76–89) ($p = 0.02$) (**Figure 2B**) among patients in the MSD, HID, and IST groups, respectively; accordingly, the estimated 5-year FFS rates were 85% (95% CI, 80–94), 68% (95% CI, 59–80), and 56% (95% CI, 47–65), respectively ($p < 0.001$) (**Figure 2C**). Both MSD-HSCT and IST had statistically superior OS rates over HID-HSCT ($p = 0.014$; $p = 0.032$). However, there was no difference in OS rate between the MSD and IST groups at a landmark point of 13 months after treatment ($p = 0.178$). There was a significant difference in FFS between the MSD and HID groups ($p = 0.003$) and the MSD and IST groups ($p < 0.001$). Landmark analysis was performed to compare the FFS of HID and IST patients. The differences were not statistically significant both before ($p = 0.097$) and after ($p = 0.228$) the landmark point of 15 months following therapy (**Figure 2C**). The estimated 5-year GFFS rate of MSD patients was 81% (95% CI, 77–91) vs. 65% (95% CI, 55–76) of HID patients ($p = 0.002$) (**Figure 2D**).

In the subgroup analysis, when comparing patients aged ≤20 years to 20–40 years, the estimated 5-year OS rate of the MSD group was 95% (95% CI, 91–100) vs. 84% (95% CI, 76–98) ($p = 0.144$); the estimated 5-year OS rate of the HID group was 79% (95% CI, 70–94) vs. 79% (95% CI, 69–97) ($p = 0.879$); and the estimated 5-year OS rate of the IST group was 85% (95% CI, 77–99) vs. 74% (95% CI, 64–98) ($p = 0.144$), respectively. Thereafter, for patients aged ≤40 years, the estimated 5-year OS rate was 89% (95% CI, 83–98) following an MSD graft, which was statistically higher than following an HID graft (76% [95% CI, 67–88], p = 0.024), but not statistically higher than following IST when landmark analysis at 12 months was performed (78% [95% CI, 71–88], p =0.105). There was also no difference in OS between the HID and IST groups (p = 0.166) (**Figure 3A**). However, the estimated 5-year FFS rate was 88% (95% CI, 82–97) following an MSD graft (p = 0.001), which was significantly higher than following an HID graft (71% [95% CI, 62–84], p = 0.005) and IST (58% [95% CI, 48–68], p < 0.001) (**Figure 3B**). We performed a landmark analysis to compare the FFS before and after a landmark point of 14.5 months of HID and IST patients. HID patients had superior FFS compared with IST patients after that point ($p = 0.047$). For patients aged >40 years, owing to the small sample sizes of MSD (n = 13) and HID (n = 8) patients, we did not perform any further statistical analysis. The 5-year OS rate was 81% (95% CI, 72–97) among the IST older patients, which was equivalent to that of patients aged ≤20 or 20–40 years ($p = 0.977$).

The cumulative incidence of TRM 1 year after MSD, HID, and IST was 11% (95% CI, 0–21), 24% (95% CI, 8–36), and 11% (95% CI, 0–15) ($p = 0.029$), respectively (**Figure 2A**). Eleven patients (6%) in the IST group experienced MDS/AML evolution compared with none of patients in the transplant groups ($p = 0.002$). As expected, during follow-up, we noticed that young women (≤40 years) receiving a FAC conditioning regimen were more likely to restore menstruation after transplantation (9 out of 10) compared with those receiving a BFAC conditioning regimen (3 out of 10 patients) ($p = 0.02$).

TABLE 2 | Primary causes of death (COD) among patients who received allografts.

COD	MSD group (14)	HID group (23)
aGvHD	8	9
Infection	5	8
cGvHD	–	3
Graft failure	–	2
Suicide	1	–
Intracranial hemorrhage	–	1

MSD, matched sibling donor; HID, haploidentical donor; BFAC, conditioning regimen consisting of busulfan/fludarabine/antithymocyte globulin/cyclophosphamide; FAC, conditioning regimen consisting of fludarabine/antithymocyte globulin/cyclophosphamide; aGvHD, acute graft versus host disease; cGvHD, chronic graft versus host disease.

Multivariate Analysis

We performed univariate and multivariate analyses to identify risk factors associated with allograft survival (**Supplementary Table 2**). HID and a BFAC conditioning regimen were adverse

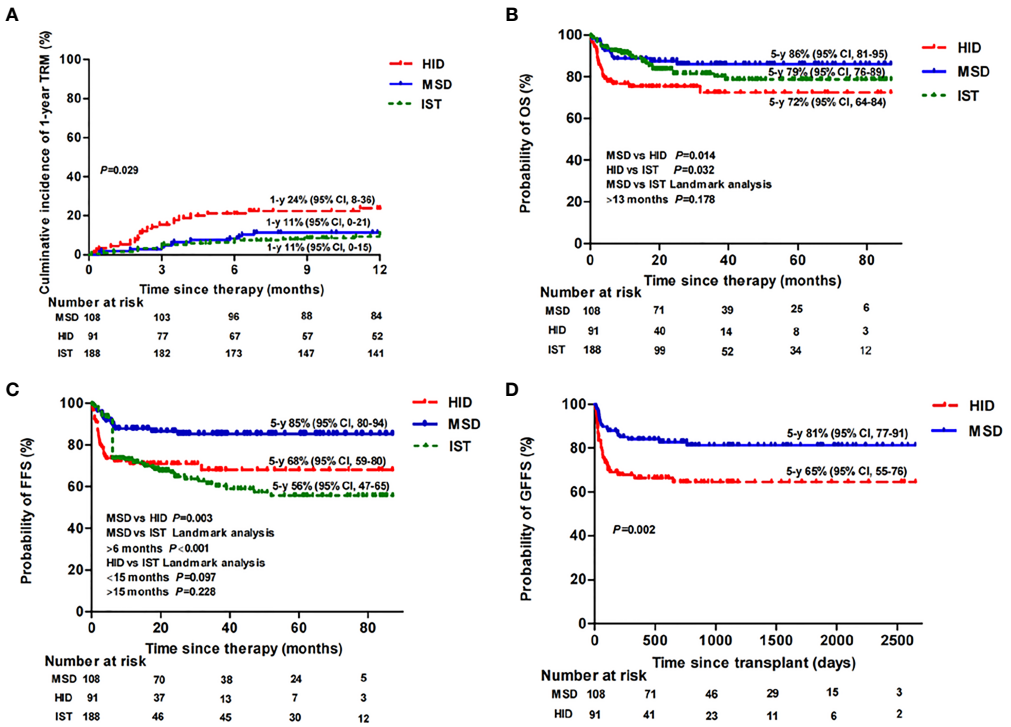

FIGURE 2 | Therapy-related mortality (TRM) **(A)**, overall survival (OS) **(B)**, failure-free survival (FFS) **(C),** and GvHD-free, failure-free survival (GFFS) **(D)** of MSD-HSCT, HID-HSCT, and IST patients.

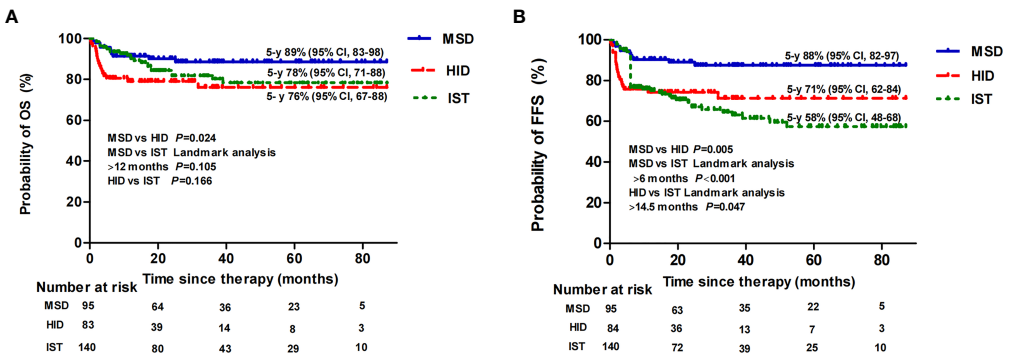

FIGURE 3 | Overall survival (OS) **(A)** and failure-free survival (FFS) **(B)** following MSD-HSCT, HID-HSCT, and IST for patients aged ≤40 years.

factors for OS while HID and MDRO colonization were the only risk factors for FFS and GFFS, respectively. For patients aged ≤40 years undergoing transplantation, the P value of HID for OS in multivariate analyses was 0.05 with marginal significance, a BFAC conditioning regimen was the only risk factor for OS and FFS, and no risk factor for GFFS was identified. However, the addition of BU had an adverse effect on young MSD patients but not on young HID patients. In detail, the estimated OS at 5 years of young MSD patients receiving a BFAC conditioning regimen was 67% (95% CI, 51–97) vs. 96% for those receiving a FAC conditioning regimen (95% CI, 93–100) ($p < 0.001$), (**Figure 4A**), while the estimated OS rate at 5 years among young HID patients receiving a BFAC conditioning regimen was 70% (95% CI, 56–89) vs. 81% (95% CI, 72–96) for those receiving a FAC conditioning regimen ($p = 0.115$), (**Figure 4B**).

Propensity Score Matching

We also performed propensity score matching to minimize effects of confounding donor factors (56 patients in the MSD and HID groups, respectively). The matched patient and donor baseline characteristics were not significantly different between the MSD and HID groups ($p < 0.05$) (**Supplementary Table 3**).

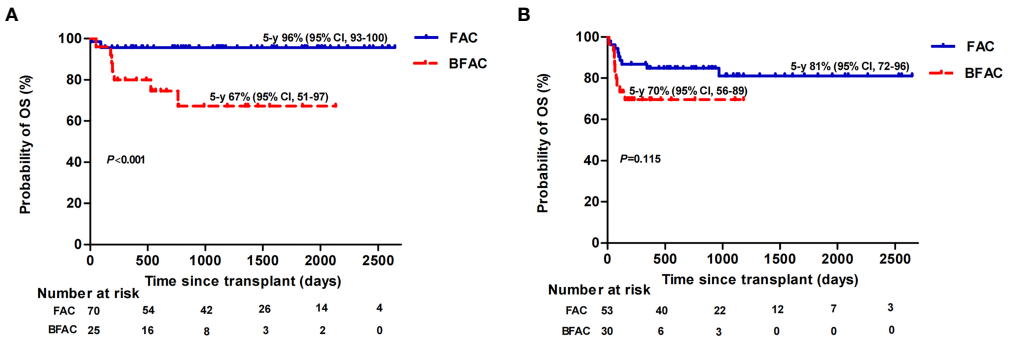

FIGURE 4 | Overall survival (OS) of patients aged ≤40 years stratified by conditioning regimen in MSD **(A)** and HID **(B)** patients.

The standardized mean differences were less than 0.2 (**Supplementary Image 1**). After matching, MSD-HSCT still exhibited significant superiority over HID-HSCT in terms of survival. In detail, the estimated 5-year OS, FFS, and GFFS rates of MSD patients were 92% (95% CI, 88–100), 92% (95% CI, 88–100), and 87% (95% CI, 80–100) vs. 77% (95% CI, 67–90) (p = 0.012), 75% (95% CI, 65–88) (p = 0.006), and 69% (95% CI, 58–84) (p = 0.026) of HID patients (**Supplementary Image 2**), respectively. However, none of the evaluated factors was recognized as an independent risk factor of OS in multivariate analysis.

DISCUSSION AND CONCLUSION

SAA is usually a life-threatening disease with a high mortality rate due to bleeding and infections. An effective therapy is therefore critical. For candidates with SAA, MSD-HSCT is the first-line therapy, IST is the recommended choice for those without an MSD, and HSCT from an alternative donor could be considered if IST fails (6). However, treatment failures after IST are high, especially for long-term survivors (19). Of 386 children with SAA, even though a 10-year OS rate of 88% was achieved, FFS rate was only 56% (20). As finding a matched unrelated donor is time-consuming and has limitations, HID-HSCT emerged mostly as a salvage after IST failures but has shown to be equivalent to MSD (3) and has a superior FFS to IST (4, 5) as a first-line strategy in China. The present work was the first to our knowledge that compared these three procedures together, concluding similar as well as different findings compared to prior studies.

Patient age is still an independent negative factor for survival following HSCT. However, the survival gap between age groups may be narrowing, especially for those aged ≤40 years, which is ascribed to improvements in supportive care as well as the transplant procedure itself, such as modifications to the conditioning regimen and effective management of complications post-HSCT over the last decade. This is evidenced by the findings of a prior work on EBMT that reported superior survival of patients younger than 20 years (17). We did not observe differences in the survival of MSD-HSCT or HID-HSCT patients younger than 40 years (21).

Consistent with previous reports (20, 22, 23), our study showed that MSD-HSCT achieved superior outcomes in terms of OS and FFS compared with IST. Compared with MSD-HSCT, our study found that HID-HSCT had a significantly higher incidence of GF, TRM, aGvHD, viremia, cGvHD, and eventually a lower survival. Due to the heterogeneous effects of variables such as patient age on survival following HSCT and IST, we only performed univariate and multivariate analyses between the allografts. In multivariate analysis, HID-HSCT was also identified as an independent risk factor of OS and FFS. Xu et al. observed that the survival of HID-HSCT was compared to that of MSD-HSCT (3). This is different from our results. We observed that the HID-HSCT recipients showed a longer interval from diagnosis to HSCT, and more heavily red blood cell transfusions than the cohort reported by Xu et al., which may contribute to the relative inferior outcomes of our HID-HSCT recipients. In addition, we enrolled 8 (9%) patients who were older than 40 years in the HID-HSCT group. In those who were equal to and younger than 40 years, we observed that the OS rate was still significantly superior for MSD-HSCT receipients compared to HID-HSCT receipients (89% vs. 76%, p = 0.024). However, a prospective study could help to further identify the efficacy of our conditioning regimen in old patients. In addition, our study showed similar estimated 5-year OS among HID-HSCT patients in contrast to another study of China (72% vs. 74.8%) (24).

The benefits of transplantation declined with increasing age, especially among HID-HSCT patients (24). We therefore compared the survival following HID and IST in those patients younger than 40 years. There was no difference in OS between HID and IST patients younger than 40 years, whereas those after HID had a higher rate of FFS when follow-up was longer than 14.5 months and a rapid blood recovery compared with IST patients. These findings were consistent with reports from other centers in China (4, 5). In accordance with other studies (5, 20, 25–27), we noticed that more patients experienced MDS/AML evolution in the IST group than in the HSCT group (6% vs. 0, p = 0.002). This may ascribe to an advantage of HSCT over IST in eradicating potential clonal hematopoiesis at risk of evolving to AML/MDS, especially during long-term follow-up. Taking quality of life into

consideration, cGvHD was the major concern of HID-HSCT compared to IST. However, in line with other studies in China (4, 5, 28), our work reported a culminative incidence of estimated 5-year moderate to severe cGvHD after HID-HSCT of 9% (95% CI, 0–18) vs. 3% (95% CI, 0–5) ($p = 0.076$) after MSD-HSCT, and no patients had severe cGvHD in the HID group.

Cardiac toxicity related to high-dose CY is of great concern to patients with SAA who are elderly or have poor cardiac function due to anemia with lethal cardiotoxicity up to 2.3% (29). The FAC regimen showed evidence of reduced toxicity and high engraftment rates (21, 30), but secondary GF was still as high as 12.7% with CY 100 mg/kg and R-ATG 10 mg/kg for MSD patients in another Korean study (21). As a result, in our work most (72%) MSD patients received a CY dose of 150 mg/kg plus a moderate dose of ATG (R-ATG 12.5 mg/kg or P-ATG 100–125 mg/kg), and BU was added to the regimens of those at a high risk of graft failures, such as patients who required heavy transfusions and had a long interval from diagnosis to transplantation and NSAA. Of note, only 1 patient was diagnosed with secondary GF in the MSD group. This was encouraging as we applied this reduced-intensity conditioning to SAA patients receiving MSD-HSCT independent of age without impairing engraftment but reducing TRM, which was similar to a previous report (30). However, it appeared that the addition of BU did not translate into a survival benefit for those MSD patients who had longer intervals from diagnosis to transplantation and required heavily additional RBC transfusions. As a comparison, another study of 26 patients who required heavy transfusions (median 54 units) and had long-time intervals (median 26 months) reported successful engraftment using a FAC regimen without a primary GF (31). Of note, we observed the fertility maintenance effect of a FAC over a BAFC conditioning regimen ($p = 0.02$).

In contrast, the effects of a BFAC conditioning regimen on HID-HSCT may be different from MSD-HSCT. With experience learned from alternative donors (32), HLA disparity is a major obstacle of GF of HID-HSCT. Another Chinese study reported that 3 out of 26 patients (12%) undergoing HID-HSCT had a graft failure based on the FAC conditioning regimen (33). Likewise, in our study there were 6 graft failures in the HID group but only 1 patient with a positive DSA received a BFAC conditioning regimen and OS was equivalent between the two conditioning regimens for younger patients. The rate of GF among HID patients in the present work was higher than reported by other studies based on BU and CY (200 mg/kg) (3, 5, 34). The addition of BU to a FAC conditioning regimen may reduce the rate of GF. However, TRM was still high mainly due to infections. A strategy to improve outcomes further may be *via* a reduced dose of CY, according to another Chinese study that reported the same conditioning regimen with a dose of CY 2,000 mg/m^2 for enrolled patients (median age, 11 years) undergoing HID-HSCT. None of the enrolled patients had a primary or secondary GF in their study, and the 3-year OS rate was 80.3 ± 5.1% (35). Another widely used model of HID-HSCT is post-transplant cyclophosphamide (PTCY) for GvHD prophylaxis. However, the engraftment rates of PTCY may be relatively lower than methotrexate-containing regimens, although prospective data are not available (36, 37).

Our study had several limitations. First, it was a retrospective study with imbalanced basic characteristics between groups and choice of therapy based on clinical physician or patient preference. Although final conclusions therefore cannot be drawn from our study, our comparative data are from a single large transplant center over the same study period with relatively consistent protocols and showed similar survival rates compared with other studies. We also performed stratification, regression analysis, and propensity score matching to reduce these confounders, and similar conclusions were achieved. Second, few patients diagnosed with NSAA or SAA evolving from NSAA received a FAC conditioning regimen, making a sound comparison of conditioning regimens between these patients difficult. Third, data on the rates of full immune reconstitution after transplantation between different groups were unavailable. Fourth, the follow-up of our study was limited and the fertility maintenance of a FAC conditioning regimen needs to be confirmed in the future.

In conclusion, with respect to OS and FFS, our study showed that MSD is still the preferred first-line therapy for patients with SAA younger than 40 years. Meanwhile, for younger patients (≤40 years) without an MSD, HID-HSCT was a valid choice comparable with IST. A FAC conditioning regimen was feasible for MSD-HSCT, whereas the addition of busulfan to the FAC regimen was harmful to MSD patients but might be beneficial for HID patients. Follow-up prospective, large sample size multicenter studies are needed to identify the optimal alternative therapy and conditioning regimen for SAA patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

SF and YzZ contributed to study design and manuscript reviewing. YfZ and JH contributed to the data collection, analysis, and manuscript composition. LL, YS, JC, and TZ contributed to the data collection and interpretation. XC, AP, DY, RZ, QM, WZ, YH, JW, EJ, and MH contributed to the treatment of the disease and data collection. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.837335/full#supplementary-material

Supplementary Figure 1 | Propensity score matching of patients undergoing transplantation.

Supplementary Figure 2 | The estimated 5-year overall survival (OS) (A), failure-free survival (FFS) (B), and GVHD-free, failure-free survival (GFFS) (C) rates of MSD patients and HID patients after matching.

Supplementary Table 1 | Characteristics and outcomes of patients with acquired aplastic anemia and their donors in the transplant groups.

Supplementary Table 2 | Matched patient and donor baseline characteristics of MSD and HID patients.

Supplementary Table 3 | Univariate analysis and multivariate analysis of all patients undergoing transplantation and only those aged≤40 years.

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· 论著 ·

猪抗人淋巴细胞免疫球蛋白联合 CD25 单抗用于恶性血液病单倍体移植中急、慢性移植物抗宿主病预防的临床分析

刘敏　雷荟融　成延娟　黄知平

[摘要]　目的　探讨猪抗人淋巴细胞免疫球蛋白(ALG)联合 CD25 单抗在恶性血液病单倍体移植中预防急、慢性移植物抗宿主病(GVHD)的疗效和安全性。方法　回顾性分析我院 2018 年 5 月～2020 年 6 月首次单倍体移植中行 ALG 联合 CD25 单抗预防 GVHD 的恶性血液病患者 18 例,记录预防期间的不良反应、干细胞植入时间、急性移植物抗宿主病(aGVHD)和慢性移植物抗宿主病(cGVHD)的发生率、累及器官及治疗方法、巨细胞病毒(CMV)和 EB 病毒(EBV)激活率、感染发生率、嵌合状态、总生存(OS)率及无排异无复发生存(GRFS)率。结果　纳入患者使用 ALG 期间的不良反应包括皮疹、心慌、呼吸困难等,通过激素及抗过敏治疗后均能有效缓解。所有患者均成功植入,白细胞植入中位时间为 11(9,13)天,血小板植入中位时间为 12(10,16)天。aGVHD 发生率为 33.3%,其中 2 例为Ⅲ～Ⅳ度(11.1%),10 例发生 cGVHD(55.6%),其中 3 例为广泛性(16.7%),7 例为局限性(38.9%)。6 例患者(33.3%)出现 EBV 血症,7 例患者(38.9%)出现 CMV 血症,5 例(27.8%)患者发生细菌感染,其中深部真菌感染发生 2 例(11.1%)。所有患者在 +1、+3、+6、+9、+12、+18 月行供受者嵌合度检测,均为完全供者型。中位随访时间 384(219,530)天,死亡 2 例,中位生存时间未达到,总生存(OS)率 85.23%。结论　ALG 联合 CD25 单抗在恶性血液病单倍体移植中预防 GVHD 是有效和安全的。

[关键词]　猪抗人淋巴细胞免疫球蛋白;　单倍体移植;　移植物抗宿主病;　总生存
[中图分类号]　R459.9　　[文献标识码]　A

异基因造血干细胞移植(allo-HSCT)是目前恶性血液系统疾病[如急性白血病、骨髓增生异常综合征(MDS)等]可能的治愈手段之一,通过移植治疗,可以将患者的 5 年总生存率提升至 55%以上[1],同时降低高危患者的复发风险。近几年单倍体移植的开展解决了供者来源的问题,但是急、慢性移植物抗宿主病(GVHD)的发生也随之增加,严重影响了患者的生存质量。临床上一般使用抗人胸腺细胞免疫球蛋白(ATG)或后置环磷酰胺(PTCy)联合钙调磷酸酶抑制剂、甲氨蝶呤(MTX)等作为 GVHD 预防的标准方案,国内多选择前者,其中又以进口药物兔抗人胸腺细胞免疫球蛋白(r-ATG)为最常用,国产 ATG 即猪抗人淋巴细胞免疫球蛋白(p-ATG 或 ALG)在恶性血液病单倍体移植中行 GVHD 预防的研究鲜有报道。我科从 2018 年开始使用 ALG(武汉生物)联合 CD25 单抗(沈阳三生,商品名健尼哌)用于单倍体移植患者 GVHD 的预防,本研究通过探讨 ALG 预防 GVHD 的疗效和安全性,为单倍体移植患者 GVHD 预防药物的选择提供临床依据。

对象与方法

1. 对象:回顾分析我院 2018 年 5 月～2020 年 6 月首次单倍体移植中行 ALG 联合 CD25 单抗预防 GVHD 的恶性血液病患者 18 例,其中男 10 例,女 8 例,中位年龄 38.5(21.0,55.0)岁。18 例 GVHD 患者的原发疾病包括急性髓系白血病(AML)、急性淋巴细胞白血病(ALL)、MDS,见表 1。本研究经我院伦理委员会审核批准,移植前所有患者均获得完全缓解,且在移植前行术前讨论及签署知情同意书。

2. 方法

(1)预处理方案:采用以白消安(Bu)/环磷酰胺(Cy)为主体的清髓预处理。AML 为 BuCy + 阿糖胞苷(Ara-C);ALL 为 BuCy + 依托泊苷(VP-16);MDS 为地西他滨 + BuCy。

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表 1 18 例使用 ALG 联合 CD25 单抗预防 GVHD 的单倍体移植患者基本情况

编号	性别	年龄(岁)	诊断	干细胞来源	HLA 配型
1	男	47	AML	PB	7/10
2	女	48	AML	PB	5/10
3	男	55	AML	PB	5/10
4	男	50	AML	PB	6/10
5	女	31	ALL	PB	7/10
6	男	52	AML	PB	6/10
7	男	28	MDS-EB-Ⅱ	PB + BM	5/10
8	男	55	AML	PB + BM	5/10
9	男	23	ALL	PB	7/10
10	女	31	AML	PB + BM	5/10
11	男	25	ALL	PB + BM	6/10
12	女	53	ALL	PB	7/10
13	女	28	ALL	PB	6/10
14	女	29	AML	PB	7/10
15	男	47	AML	PB	5/10
16	女	28	AML	PB	8/10
17	女	21	MDS-EB-Ⅱ	PB	7/10
18	男	46	MDS-EB-Ⅱ	PB	8/10

(2) 预防 GVHD 的方法: -4 d ~ -1 d 输注 ALG (30 mg·kg^{-1}·d^{-1}),0 d、+4 d 分别予以输注 CD25 单抗(50 mg/d) 2 剂,第 1 剂在输注干细胞前 2 h 执行,每剂输注时间 30 min。-1 d 开始服用他克莫司胶囊。+1 d、+3 d、+6 d、+11 d 予以甲氨蝶呤静脉滴注。+7 d 服用霉酚酸酯,1 个月后停用。

(3) 随访指标: 随访时间由输注干细胞当天截至 2020 年 6 月,随访内容包括预防期间的不良反应、干细胞植入时间、急性移植物抗宿主病(aGVHD) 和慢性移植物抗宿主病(cGVHD) 的发生率、累及器官及治疗方法、巨细胞病毒(CMV) 和 EB 病毒(EBV) 激活率、感染发生率、嵌合状态、总生存(OS) 率及无排异无复发生存(GRFS) 率。

3. 统计学分析: 应用 SPSS 22.0 软件进行统计分析。不符合正态分布的计量资料以 $M(P_{25},P_{75})$ 表示,计数资料以例数和百分比表示。采用 *Kaplan-Meier* 法绘制生存曲线。

结 果

1. 预防期间不良反应发生情况: 使用 ALG 预防 GVHD 期间,3 例患者出现躯干部红色皮疹伴瘙痒,1 例出现呼吸困难,1 例出现血压增高,4 例出现畏寒、发热,体温最高至 39.7 ℃,行吸氧、静滴激素及钙剂等抗过敏处理后均快速缓解。

2. 使用 ALG 联合 CD25 单抗预防 GVHD 的单倍体移植患者干细胞植入及生存时间: 18 例患者的白细胞植入中位时间为 11(9,13) 天,血小板植入中位时间为 12(10,16) 天。见表 2。

表 2 使用 ALG 联合 CD25 单抗预防 GVHD 的单倍体移植患者植入及生存时间

编号	单个核细胞 ($\times10^8$/kg)	CD34 ($\times10^6$/kg)	白细胞植入时间(d)	血小板植入时间(d)	生存时间(d)
1	4.18	5.90	12	13	722
2	9.50	9.60	22	106	681
3	5.00	4.70	8	9	562
4	13.00	13.00	11	11	547
5	15.40	9.30	9	10	524
6	6.47	6.60	9	10	506
7	15.85	9.30	12	30	435
8	7.70	5.50	9	10	403
9	5.11	6.36	13	15	398
10	13.90	6.30	15	16	370
11	5.53	5.12	11	20	353
12	6.24	5.38	12	17	349
13	10.06	7.62	10	10	287
14	11.30	10.20	9	11	221
15	8.70	7.80	9	10	213
16	15.70	11.40	10	14	188
17	10.00	10.20	13	11	174
18	15.21	11.70	15	14	140

3. GVHD 的发生率、累及器官及治疗方法: 18 例患者中,6 例发生 aGVHD(33.3%),其中 2 例为Ⅲ~Ⅳ度(11.1%);10 例发生 cGVHD(55.6%),其中 3 例为广泛性(16.7%),7 例为局限性(38.9%)。所有患者治疗后均缓解,具体治疗方法见表 3。

表 3 发生 GVHD 患者的临床特征及治疗方法

类型	例数	累及器官	治疗方法
aGVHD			
Ⅰ~Ⅱ度	3	皮肤	甲强龙
Ⅲ~Ⅳ度	2	皮肤 + 肠道	1 例为甲强龙 + CD25 单抗; 1 例激素耐药,为 MTX + 霉酚酸酯 + CD25 单抗
cGVHD			
局限性	7	皮肤	甲强龙
广泛性	3	皮肤 + 口腔 + 肝脏/眼部/肺部	甲强龙 + 他克莫司

4. 病毒激活率: 6 例(33.3%) 患者出现 EBV 血症,7 例(38.9%) 患者出现 CMV 血症。

5. 感染发生率: 5 例(27.8%) 患者发生细菌感染,其中深部真菌感染 2 例(11.1%)。

6. 嵌合状态: 所有患者在移植后 +1、+3、+6、+9、+12、+18 月行供受者嵌合度检测,均为完全供者型。

7. 随访情况: 随访至 2020 年 6 月,18 例患者的中位随访时间为 384(219,530) 天,中位生存时间未达到,OS 率为 85.23%(95% *CI* 51.89% ~96.18%)。死亡 2 例,分别为中枢神经系统感染及移植相关血栓性微血管病。2 年的 GRFS 率为 41.91%(95% *CI* 18.78% ~63.62%)。

讨　论

在过去 50 年里，超过 100 万患者接受了 HSCT，虽然供体移植物抗白血病效应和大剂量放化疗为移植成功提供保障，但是 GVHD 作为造血干细胞移植主要的并发症之一，始终是 HSCT 需要解决的热点问题[2]。相关文献报道 aGVHD 的发生率约 30% ~80%，主要累及皮肤、肝脏及胃肠道，导致红疹、转氨酶增高、黄疸、腹泻等，cGVHD 发生率约 20% ~60%，累及皮肤、眼、口腔、肺、筋膜、生殖器等，严重影响患者的生存质量[3-5]。CIBMTR 登记的 2018 ~2019 年美国行 HSCT 的患者中，aGVHD 作为致患者 100 天内死亡的重要因素之一，在各类型移植患者中分别占 13%（同胞全合）、8%（单倍体）、16%（无关供者）。在 GVHD 预防方案里，ATG 的加入可以降低急、慢性 GVHD 发生率。在一项纳入了 356 例 AML 或 MDS 患者的回顾性分析研究中，139 例采用粒细胞集落刺激因子（G-CSF）动员后的外周血干细胞（PBSC）作为移植物，47 例使用 ATG 预防，92 例未使用，结果显示 ATG 组Ⅲ ~ Ⅳ度 aGVHD 及 cGVHD 发生率均低于对照组，同时两组复发及感染相关死亡率比较差异无明显统计学意义[6]。ATG 为人淋巴细胞免疫动物后从其血清中提取的多克隆性免疫球蛋白，通过结合淋巴细胞表面抗原来激活补体介导的细胞毒性作用，从而直接杀伤 T 淋巴细胞，ATG 还可以使调节性 T 细胞（Treg）数量扩增，诱导 NK/T 细胞、促进 B 细胞凋亡，与树突状细胞（DC）表面抗原 CD38、CD11c、CD123、CD83、CD36、CD40 及 HLA-DR 紧密结合后影响 DC 的细胞功能，介导白细胞-内皮相互作用的细胞表面分子，更快速、更完全地下调 LFA-1（CD11a），阻止排异及缺血灌注损伤反应的启动，减轻 GVHD 及排异反应，达到免疫调节的目的[7-8]。ATG 的类型根据免疫动物的不同分为 3 种：马 ATG、兔 ATG 和猪 ATG，后者因为使用的是人胸导管淋巴细胞免疫猪之后获得，所以也称为 ALG。ALG 比 ATG（后文均表示兔 ATG）的免疫抑制强度弱，王筱啸等[9]在体外淋巴毒试验中发现，ATG 和 ALG 对健康志愿者外周血中淋巴细胞的杀伤作用随稀释滴度的增加逐渐下降，相同浓度下，ATG 比 ALG 的效价高 2 倍，具有更强的淋巴细胞清除作用。“北京方案”的核心为 G-CSF + ATG，在中国异基因移植专家共识里面也是推荐兔 ATG[10] 或具有相似清除 T 淋巴细胞效力的 ATG-F[11] 用于 GVHD 预防，AML、MDS 等的单倍体移植文献中更是鲜有使用 ALG 行 GVHD 预防，在 PubMed 数据库中以关键词“Thymoglobulin”、“antithymocyte globulin”、“Rabbit Anti-Thymocyte Globulin”、“porcine antilymphocyte globulin”、“allogeneic Stem Cell transplantation”、“GVHD”进行检索，通过标题和摘要对检索的文献进行筛查并分类，绝大部分 HSCT 文献（约 489 篇）使用的是 ATG，ALG 用于单倍体 HSCT 中 GVHD 预防的仅有针对重度再生障碍性贫血的文献，且数量极少，约 7 篇，没有白血病、骨髓增生异常综合征等移植的相关证据。在本研究中，我们使用了 ALG 进行 GVHD 预防，因担心 ALG 的免疫抑制强度不够，出现植入失败或严重的 GVHD，遂联合了 CD25 单克隆抗体。CD25 单抗为白细胞介素 2 受体拮抗剂，能够清除活化的 T 淋巴细胞。Chen 等[12] 在 13 例高危白血病患者单倍体移植中使用 CD25 单抗组合环孢素、甲氨蝶呤、霉酚酸酯用于 GVHD 预防，结果显示没有患者发生Ⅱ ~ Ⅳ度 aGVHD，且存活超过 12 个月未复发的患者表现出有限的慢性皮肤 GVHD，可见 CD25 可有效降低单倍体移植中严重的 GVHD，考虑原因为 CD25 单抗选择性地消除或减少同种异体反应性 T 细胞的数量，从而预防 GVHD 或减轻 GVHD 的严重程度。Zhang 等[13] 的研究中纳入了 110 例高危白血病患者行单倍体移植，予 ATG 联合 CD25 单抗预防 GVHD，结果显示Ⅱ ~ Ⅳ、Ⅲ ~ Ⅳ aGVHD 的累积发生率分别为 28.6% 和 14.3%，而有限和广泛 cGVHD 的累积发生率为 19.4% 和 13.8%。所有结果与对照组中同胞全合移植的结果相同，可见 ATG 联合 CD25 单抗可以进一步降低 GVHD 的发生率。本研究中，2 年 aGVHD 发生率比上述文献数据稍低，但 cGVHD 发生率明显增加，考虑可能为 ALG 半衰期（11 d）短于 ATG（30 d），T 淋巴细胞恢复更快。

然而，淋巴细胞清除效果越强，其移植后免疫重建时间就越长，复发风险、感染风险、EBV、CMV 等激活风险及移植后淋巴增殖性疾病（PTLD）发生率也会越高。余国攀等[14] 研究发现 allo-HSCT 中 ATG 预防 GVHD 组 EBV 感染率为 57.6%，PTLD 发生率为 16.2%，而未使用 ATG 组 EBV 感染率为 10.3%，PTLD 发生率为 8.0%，两组数据有明显统计学差异，提示 ATG 预防是 allo-HSCT 后患者 EBV 感染的危险因素之一，其感染率与 ATG 预防剂量呈正相关。本研究在恶性血液病的单倍体移植中使用 ALG 替代 ATG，再联合 CD25 单抗预防 GVHD，并与国内文献分析数据对比，结果显示感染发生率特别是病毒感染发生率比使用 ATG 更低，25 个月 OS 率达 85.2%，2 年 GRFS 率为 41.91%，提示此组合方案在恶性血液病中行 GVHD 预防安全有效。但本研究为单臂试验，且样本量稍小，后期还需增加样本量通过单中心随机临床研究来对比兔 ATG 预防 GVHD 数据。

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· 论著 ·

非布司他与别嘌醇治疗慢性肾脏病合并高尿酸血症的疗效及安全性比较

苏晓娟 李芳 宋颖 袁颖

[摘要] 目的 比较非布司他与别嘌醇在治疗慢性肾脏病(CKD)患者合并高尿酸血症(HUA)的临床疗效及安全性。方法 回顾性分析 2016 年 1 月~2019 年 1 月我院收治的 CKD 合并 HUA 患者 104 例,随机分为观察组(52 例)及对照组(52 例)。观察组患者使用非布司他治疗,剂量为 20~40 mg/d,对照组患者使用别嘌醇治疗,剂量为 100~200 mg/d,均治疗 24 周。分析比较两组患者治疗前后肾功能相关指标[血尿酸(SUA)、血肌酐(SCr)、估算的肾小球滤过率(eGFR)、胱抑素 C(Cys-C)]、炎症相关指标[C 反应蛋白(CRP)、红细胞沉降率(ESR)、白细胞介素 6(IL-6)]、心功能相关指标[心肌钙蛋白 T(cTnT)、N 末端 B 型利钠肽前体(NT-proBNP)]水平、临床疗效及药物不良反应情况。结果 治疗后观察组患者 UA、SCr、Cys-C、CRP、ESR、NT-proBNP 水平均低于同组治疗前,eGFR 高于同组治疗前;对照组患者 UA、Cys-C、CRP、ESR、NT-proBNP 水平均低于同组治疗前($P<0.05$)。治疗后观察组患者 UA 水平低于同期对照组($P<0.05$)。观察组治疗总有效率明显高于对照组($P<0.05$)。两组均未出现明显的心血管不良事件及药物不良反应。结论 与别嘌醇相比,非布司他降尿酸的作用更强,同时能保护肾功能及改善炎症状态。

[关键词] 非布司他; 别嘌醇; 慢性肾脏病; 高尿酸血症
[中图分类号] R589.7 [文献标识码] A

随着生活水平的提高及检测手段的进步,高尿酸血症(HUA)的发生率呈逐年增高趋势,已成为继高血压、高血糖、高血脂之后的“第四高”。有研究显示血尿酸(SUA)和黄嘌呤氧化酶(XO)可以通过促进炎症、损害内皮功能、加速氧化应激和激活肾素-血管紧张素系统(RAS)促进肾脏纤维化[1-2]。循证医学的证据表明通过降 SUA 水平能够有效减少肾脏疾病的发病率,并且能够延缓肾脏疾病的进展,因此慢性肾脏病

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Retrospective Comparison of Efficacy and Safety of Rabbit Anti-Thymocyte Globulin and Porcine Anti-Lymphocyte Globulin in Patients With Acquired Aplastic Anemia Undergoing Hematopoietic Stem Cell Transplantation From Matched Sibling Donors

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We compared the efficacy and safety of porcine anti-lymphocyte globulin (pALG) (n=140) and rabbit anti-thymocyte globulin (rATG) (n=86) in patients with acquired aplastic anemia (AA) receiving hematopoietic stem cell transplantation (HSCT) from matched sibling donors (MSD) in two transplantation centers in China ranging from 2005 to 2020. The groups had similar baseline characteristics except for a higher number of infused mononuclear cells (P<0.001) and a higher proportion of peripheral blood stem cells as graft sources (P=0.003) in the pALG group. The rates of neutrophil engraftment at day 28 (P=1), platelet engraftment at day 28 (P=0.228), bloodstream infection before engraftment (P=0.867), invasive fungal diseases (P=0.362), cytomegalovirus viremia (P=0.667), and graft rejection (P=0.147) were similar in the two groups. A higher cumulative incidence of grades II-IV acute graft versus host disease (aGvHD) at 100 days occurred in the pALG group (19% vs. 8%, P=0.035) while no significant differences in grades III-IV aGvHD (P=0.572), mild to severe chronic GvHD (cGvHD) (P=0.181), and moderate to severe cGvHD (P=0.586) were observed. The actuarial 5-year overall survival (OS), failure-free survival (FFS), and GvHD-free, FFS rates of the pALG group were 87% (95% confidence interval [CI], 82-93), 85% (95% CI, 80-92), and 78% (95% CI, 72-92)

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versus 91% (95% CI, 86-99) (*P*=0.33), 88% (95% CI, 82-97) (*P*=0.428), and 79% (95% CI, 72-90) (*P*=0.824) in the rATG group, respectively. A busulfan-containing conditioning regimen was the only adverse risk factor for OS and FFS in multivariate analysis. In conclusion, pALG is an alternative to rATG in patients with severe AA receiving MSD-HSCT. A prospective, large-sample study is needed to explore this therapy further.

Keywords: rabbit, porcine, aplastic anemia, stem cell transplantation, matched sibling donors

INTRODUCTION

Severe aplastic anemia (SAA) is a disease with a high mortality rate, mainly due to infections or bleeding caused by persistent pancytopenia. Hematopoietic stem cell transplantation (HSCT) is preferred for patients with matched sibling donors (MSD). Many studies have shown significant superiority of MSD-HSCT over immunosuppressive therapy (IST) in terms of overall survival (OS) and failure-free survival (FFS) (1–5). Over the last two decades, further dramatic progress has been made on several fronts to tackle this disease. The incorporation of anti-thymocyte globulin (ATG) into the conditioning regimen was first investigated. Since 1994, the efficacy of ATG in the conditioning regimen of HSCT from MSD for patients with SAA has been confirmed (6). Storb et al. showed that the actual survival rate at three years was 92%, which was higher than the 72% (historical) survival rate, in 39 patients. In addition, a fludarabine (FLU)-based conditioning regimen also showed reduced toxicity and similar survival compared to ATG plus cyclophosphamide (CTX), especially for older patients (7–9). However, among these studies, rabbit-ATG (rATG) was most commonly used. In China, porcine anti-lymphocyte globulin (pALG) is also available, and studies have reported similar efficacy to rATG among patients receiving IST (10–13). The effect and safety of pALG in patients with SAA receiving HSCT have been previously reported in our centers, with limited sample sizes (14, 15). Therefore, we designed this extended retrospective study to evaluate and compare the efficacy and safety of rATG and pALG in patients with acquired SAA undergoing MSD-HSCT in two transplant centers in China.

PATIENTS AND METHODS

Patients

From 2005 and 2020, a total of 226 patients with acquired AA who consecutively received MSD-HSCT from Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College and Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology were enrolled in this study. Of these patients evaluated, 140 patients received pALG while 86 patients received rATG. Enrollment criteria included SAA, very SAA or transfusion-dependent non-severe AA defined by guideline (16); voluntary participation in HSCT; absence of severe organs dysfunction. Excluding criteria included underlying inherited marrow failure disorders such as Fanconi anemia; myelodysplastic syndrome; patients with pregnancy, severe organs impairment, or uncontrolled active infection. Patients with paroxysmal nocturnal hemoglobinuria clones were also included in this study. All written informed consent was attained from patients or their relatives. This study was approved by the Ethics Committees of Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College and Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, respectively.

Procedures of Transplantation

The conditioning regimen, graft-versus-host disease (GvHD) prophylaxis, infection prevention, and surveillance followed those in our previous report (14, 15). In particular, pALG was prepared using thymic cells (as antigens) introduced in swine and separating anti-lymphocyte serum from the swine (Wuhan Institute of Biological Products Co., Ltd.) (11, 17).

Definitions

Neutrophil and platelet engraftment (18), acute GvHD (aGvHD) (19), and chronic GvHD (cGvHD) (20) were defined according to previously reported criteria. Graft rejection (GR) was defined as less than 5% T-cells of donor origin (21). Primary GR was defined as the failure to achieve neutrophil engraftment after HSCT until day +28. Secondary GR was defined as the absence of graft function after achieving initial full engraftment (22). Transplantation-related mortality (TRM) was defined as death without rejection. Treatment failure after HSCT was defined as death or primary or secondary GR, whichever came first. The FFS was defined as survival without treatment failure. GvHD-free and failure-free survival (GFFS) was defined as survival without grades III-IV aGvHD, moderate to severe cGvHD, or treatment failure (23). OS was defined as the time from treatment to death or the last follow-up.

Statistical Analysis

The objective of this study was to compare major outcomes, including engraftment, infection, GvHD, TRM, and survival, among different ATG groups in patients with AA.

Each patient involved had an electronic-database, outpatient-department, or telephone follow-up. The final follow-up was November 31, 2021. Continuous and categorical variables were compared using the Mann–Whitney U test, chi-square test, or Fisher's exact test. Median follow-up was calculated using the reverse Kaplan–Meier method. Cumulative incidences of

GvHD were compared with the Gray's test. Death and GR were considered as competing events for GvHD. The probabilities of OS and FFS were estimated using the Kaplan–Meier method and compared between different groups of patients using the log-rank test. Variables with P values ≤ 0.2 in the univariate analysis were entered in multivariate analyses using a Cox proportional hazards model to identify factors impacting OS, FFS, and GFFS of transplant patients. Statistical analyses were performed using the R software packages (R 4.1.2), GraphPad Prism 5, and SPSS 20.0. GraphPad Prism 5 was used to generate figures. All P values were two-sided, and the results were considered statistically significant at $P<0.05$.

RESULTS

Characteristic of Patients and Donors

As shown in **Table 1**, 140 and 86 patients were enrolled in the pALG and rATG groups, respectively. There were no significant differences in terms of patient age (P=0.15), patient sex (P=0.857), donor sex (P=0.797), diagnosis (P=0.396), interval from diagnosis to transplantation (P=0.375), conditioning regimen (P=0.415), and dose of $CD34^+$ cells infused (P=0.161) between the two groups, while the pALG group had a higher proportion of peripheral blood stem cells (PBSCs) as a graft source (85% vs. 67.44%, P=0.003), and a higher median dose of infused mononuclear cells (10×10^8/kg vs. 8×10^8/kg, $P<0.001$).

Hematopoietic Recovery

Only patients who survived for >28 days were analyzed for engraftment. There were two early deaths due to respiratory failure and septic shock at day 11 and 15 in the pALG group, while none of the patients in the rATG group suffered early deaths. The neutrophil engraftment rate was 100% at day 28 in the pALG group versus 100% at day 28 in the rATG group; accordingly, the platelet engraftment rate was 96.65% versus 90.7%, respectively (P=0.228). Patients in the pALG group had a faster engraftment of neutrophils and platelets. The median days of neutrophil and platelet engraftment were 12 (range, 7-22) and 12 (range, 7-30) days for patients in the pALG group and

TABLE 1 | Characteristics and outcomes of patients with acquired aplastic anemia.

Variables	pALG group (140)	rATG group (86)	*P* value
Patient age, years, median (range)	26 (7-66)	24 (4-54)	0.15
Patient gender (male), no. (%)	80 (57.14)	51 (59.30)	0.857
Donor gender (male), no. (%)	64 (45.71)	37 (43.02)	0.797
Diagnosis, no. (%)			0.396
severe aplastic anemia	90 (64.29)	49 (56.98)	
very severe aplastic anemia	40 (28.57)	32 (37.21)	
non-severe aplastic anemia	10 (7.14)	5 (5.81)	
Interval from diagnosis to transplant, moths, median (range)	2 (0.4-204)	2 (0.5-231)	0.375
Conditioning regimen			0.415
ATG+CTX ± FLU	115 (82.14)	66 (76.74)	
BU+FLU+ATG ± CTX	25 (17.86)	20 (23.26)	
Graft source, no. (%)			0.003
Peripheral blood	119 (85.00)	58 (67.44)	
Bone marrow ± peripheral blood	21 (15.00)	28 (32.56)	
Mononuclear cells, $\times10^8$/kg, median (range)	10 (2.8-47)	8 (2.8-38)	<0.001
$CD3^+$ cells, $\times10^6$/kg, median (range)	127.3 (13.7-384.1)[&]	115.8 (6.7-292.5)[&]	0.377
$CD34^+$ cells, $\times10^6$/kg, median (range)	3 (1.5-17)	3 (0.75-10)	0.161
Neutrophil engraftment, days, median (range)	12 (7-22)	12 (9-23)	0.004
Platelet engraftment, days, median (range)	12 (7-30)	14 (8-34)	0.001
28-day neutrophil engraftment, no. (%)	138 (100)	86 (100)	1
28-day platelet engraftment, no. (%)	132 (95.65)	78 (90.70)	0.228
Graft rejection			0.147
Primary graft rejection, no. (%)	1 (0.71)	0 (0)	
Secondary graft rejection, no. (%)	3 (2.14)	6 (6.98)	
Bloodstream infection before engraftment, no. (%)	22 (15.71)	12 (13.95)	0.867
Invasive fungal diseases, no. (%)	10 (7.14)	10 (11.63)	0.362
Cytomegalovirus viremia, no. (%)	31 (22.14)	22 (25.58)	0.667
100-day aGvHD grades I-IV, no. (%)	33 (24.26)*	20 (23.26)*	0.992
100-day aGvHD grades II-IV, no. (%)	26 (19.12)*	7 (8.14)*	0.041
100-day aGvHD grades III-IV, no. (%)	11 (8.09)*	5 (5.81)*	0.71
Mild to severe cGvHD, no. (%)	29 (22.31)[#]	9 (11.11)[#]	0.061
Moderate to severe cGvHD, no. (%)	6 (4.76)[#]	6 (7.69)[#]	0.577
Overall deaths, no. (%)	19 (13.57)	8 (9.30)	0.454
Follow-up of alive patients, moths, median (range)	62 (7-190)	79 (3-169)	0.087

**Among enrolled patients, 136 and 86 patients were evaluable; [#]among enrolled patients, 130 and 81 patients were evaluable; [&]among enrolled patients, 74 and 33 patients were evaluable.*

pALG, porcine anti-lymphocyte globulin; rATG, rabbit anti-thymocyte globulin; no., number of patients; CTX, cyclophosphamide; FLU, fludarabine; BU, busulfan; aGvHD, acute graft versus host disease; cGvHD, chronic graft versus host disease.

12 (range, 9-23) (P=0.004) and 14 (range, 8-34) days (P=0.001) for patients in the rATG group, respectively (**Table 1**).

Graft Rejection

Ten patients experienced GR after transplantation (one primary and nine secondary). There was no difference in GR rates between the groups (P=0.147). The median time of secondary GR was 4 months, ranging from 1.2 months to 107 months. Among these patients, all were treated with CTX (50 mg/kg × 2 days) with or without FLU followed by an infusion of frozen PBSCs from the original donor. Eight patients acquired complete blood recovery with donor origin, while two patients received autologous blood recovery.

aGvHD and cGvHD

With regard to aGvHD and cGvHD, although patients in the pALG group mostly received PBSCs as the graft source, there was only a marginally significant difference in the rate of grades II to IV aGvHD between the two groups (P=0.041), whereas rates of grades I to IV aGvHD, grades III to IV aGvHD, mild to severe cGvHD, and moderate to severe cGvHD were similar (**Table 1**). **Figure 1** shows that the cumulative incidences of grades II to IV aGvHD and III to IV aGvHD at 100 days were 19% (95% confidence interval [CI], 6–30) and 8% (95% CI, 0–15) in the pALG group compared to 8% (95% CI, 0–16) (P=0.035) and 6% (95% CI, 0–12) (P=0.572), respectively, in the rATG group. The cumulative incidence of mild-to-severe and moderate-to-severe cGvHD at 5 years was 24% (95% CI, 9–36) and 5% (95% CI, 0–11) in the pALG group versus 13% (95% CI, 0–25) (P=0.181) and 8% (95% CI, 0–16) (P=0.586) in the rATG group.

Infections

There were no statistical differences in bloodstream infections before engraftment (P=0.867), invasive fungal diseases (P=0.362), or cytomegalovirus viremia (P=0.667) between the two groups (**Table 1**).

Deaths

With a median follow-up of 62 months (range, 7-190 months) and 79 months (range, 3-169 months), 19 and 8 deaths occurred in the pALG and rATG groups (P=0.454), respectively. The primary causes of death (COD) are listed in **Table 2**. The leading COD was infection (n=13), followed by aGvHD (n=8). Remarkably, four patients died from invasive fungal diseases of the lung (n=3) or brain (n=1) before 2010. Secondary COD followed by aGvHD included infections (n=4), organ failure (n=3), and gastrointestinal bleeding (n=1). More patients receiving busulfan (BU)-containing regimens suffered COD owing to aGvHD (11.1% vs. 1.7%, P=0.009), whereas COD caused by infection between the two conditioning groups was similar (6.1% vs. 2.2%, P=0.468).

Survival

The actuarial 5-year OS, FFS, and GFFS rates of the pALG group were 87% (95% CI, 82-93), 85% (95% CI, 80-92), and 78% (95% CI, 72-92) compared to 91% (95% CI, 86-99) (P=0.33),

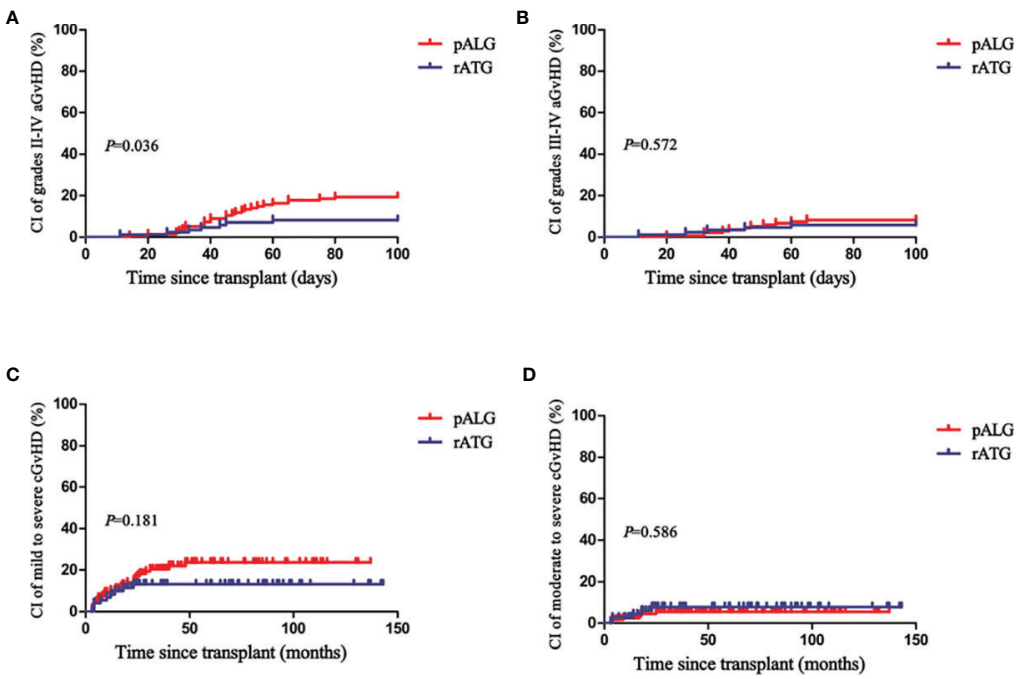

FIGURE 1 | Cumulative incidences (CI) of grades II-IV aGvHD **(A)**, grades III-IV aGvHD **(B)**, mild to severe cGvHD **(C)**, and moderate to severe cGvHD **(D)** in the pALG and rATG groups.

TABLE 2 | Primary causes of death (COD) among patients.

COD	pALG group (n=19) (%)	rATG group (n=8) (%)	*P* value
aGvHD	7 (5)	1 (1.2)	0.16
Infection	10 (7.1)	3 (3.5)	0.379
cGvHD	1 (0.7)	–	1
Accident	1 (0.7)	1 (1.2)	1
Intracranial hemorrhage	–	3 (3.5)	0.054

pALG, porcine anti-lymphocyte globulin; rATG, rabbit anti-thymocyte globulin; aGvHD, acute graft versus host disease; cGvHD, chronic graft versus host disease.

88% (95% CI, 82-97) (*P*=0.428), and 79% (95% CI, 72-90) (*P*=0.824) of the rATG group, respectively (**Figure 2**). In the subgroup analysis, the actuarial 5-year OS rates of patients aged<20 years, 20-40 years, and >40 years were 91% (95% CI, 86-100), 88% (95% CI, 83-95), and 83% (95% CI, 72-100) (*P*=0.42) (**Figure 3**), respectively.

As shown in **Table 3**, in univariate and multivariate analysis, a BU-containing regimen was the only adverse risk factor of OS and FFS.

DISCUSSION AND CONCLUSION

MSD-HSCT, which promotes effective and fast recovery of blood counts, is the preferred treatment for young patients with SAA. As SAA is a nonmalignant disease, it is recommended to sustain engraftment and minimize GvHD by modifying the conditioning regimen. High-dose CTX plus ATG is the standard conditioning regimen for SAA patients undergoing MSD-HSCT (24, 25). The addition of ATG to CTX reduces GR and GvHD rates (6). In different multivariate analyses, a conditioning regimen without ATG was a negative risk factor for survival in patients with SAA who received HSCT (26–28).

However, the mechanisms of ATG in conditioning are not well understood. It plays a role in suppressing recipient T cells to promote engraftment, as well as donor-activating T cells to reduce GvHD (29). There are three types of ATG worldwide. *In vivo* studies have demonstrated that the immunosuppressive effect of rATG was stronger than that of horse ATG (hATG) in SAA (30, 31); on the other hand, more infections and lower rates of aGvHD were related to rATG for patients with SAA receiving HSCT (32, 33). In China, no hATG or pALG has been approved by the China Food and Drug Administration as a drug in the conditioning regimen for transplantation. Several studies have demonstrated comparable outcomes between pALG and rATG as IST in patients with SAA (10–13). Previously, another study in IST has demonstrated that compared to pALG, r-ATG exhibited a stronger and prolonged inhibition effect on the $CD4^+$ T cell subset while a subset of $CD4^+$ T cells played a role in hematopoietic recovery (12).

Consistent with our previous study (14), we found no differences in the rates of neutrophil engraftment (*P*=1), platelet engraftment (*P*=0.228), bloodstream infections (*P*=0.867), invasive fungal diseases (*P*=0.362), cytomegalovirus viremia (*P*=0.667), or GR (*P*=0.147) between the two groups. Patients in the pALG group experienced faster recovery of neutrophils (*P*=0.004) and platelet (*P*=0.001). Meanwhile, a higher cumulative incidence of grades II-IV aGvHD at 100 days occurred in the pALG group (19% vs. 8%, *P*=0.036), while no differences were observed in the cumulative incidence of grades III-IV aGvHD (*P*=0.572), mild to severe cGvHD (*P*=0.181), and moderate to severe cGvHD (*P*=0.586). Schrezenmeier et al. reported the median days of neutrophil and platelet engraftment were 13 and 19 days in 134 PB recipients compared to 19 and 25 days in 558 bone marrow (BM)recipients of MSD-HSCT for SAA (34). Bacigalupo et al. have compared the efficacy of PB (n=723) with BM (n=1138) as graft sources for patients with AA receiving MSD-HSCT. They demonstrated that the median days of neutrophil and platelet engraftment in PB patients were 15 (5-68) and 15 (5-68) days versus 20 (3-156) and 27 (4-305) days in BM patients. Grades II to IV aGvHD of PB patients was higher than that of BM patients (17% vs. 11%, *P*=0.001) (26). Therefore, we should notice that a higher proportion of PB (*P*=0.003) as a graft source and a higher amount of infused MNC (*P*<0.001) in the pALG group may lead to faster recovery in the WBC and PLT engraftment as well as a

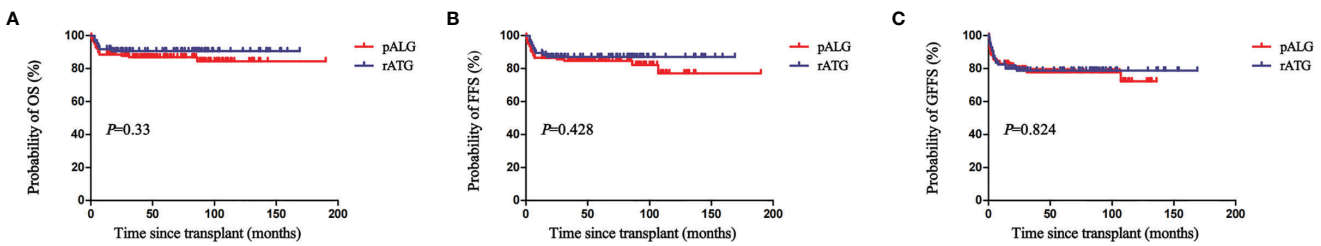

FIGURE 2 | The actuarial 5-year overall survival (OS) **(A)**, failure-free survival (FFS) **(B)**, and GvHD-free, failure-free survival (GFFS) **(C)** rates of patients in the pALG and rATG groups.

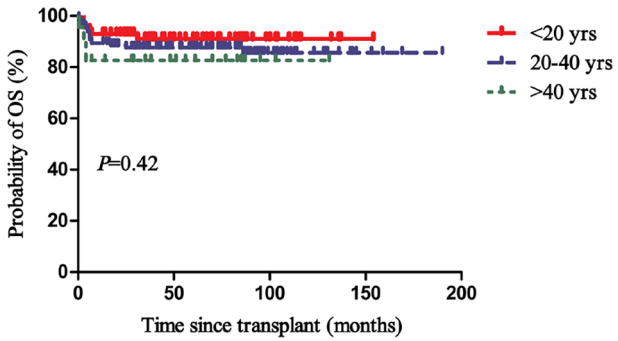

FIGURE 3 | Overall survival (OS) of different age groups.

higher rate of grades II to IV aGvHD. Even so, the actuarial 5-year OS, FFS, and GFFS rates between our two groups were similar.

In our study, we applied a non-myeloablative conditioning regimen consisting of FLU, a reduced dose of CTX, and ATG in patients with acquired AA. Several studies have reported similar efficacy of FLU-based conditioning regimens for SAA compared with a standard dose of CTX plus ATG conditioning regimen, especially for patients older than 30 years (7–9). Usually, a dose of BU 6.4 mg/kg was added to patients with a high risk of graft failure, for instance, patients with long intervals from diagnosis to transplantation or heavy blood cell transfusion. Based on the intensity of conditioning (35), this is defined as reduced-intensity conditioning. Only one patient in our study experienced primary GR. Although we demonstrated that a BU-containing conditioning regimen was an adverse predictor of OS, and FFS, these results should be interpreted with critical caution. As we know, the interval from diagnosis to transplantation and heavy transfusions before transplantation are associated with poor outcomes in patients with SAA, which may impact these results as well (26, 27, 36). Meanwhile, enhancing the intensity of the conditioning regimen may improve engraftment at the cost of more toxicity, as revealed by a meta-analysis (37). In our study, we found that more patients receiving a BU-containing conditioning regimen died of aGvHD (P=0.009). Notably, none of our patients with GR died, and most of them were successfully salvaged by the original donors' PBSC infusion. In the subgroup analysis, there was no difference in OS among patient age groups, which indicated that this regimen may be applied to older patients (8). Therefore, these results indicate that a fludarabine-based conditioning regimen was effective for patients with SAA undergoing MSD-HSCT, independent of age.

Our study had several limitations. First, it was a retrospective study with unavoidable bias. Notably, our enrolled patients had relatively similar basic characteristics to minimize the effect of potential bias. Second, our data on the rates of full immune reconstitution at different times between the two groups was incomplete. In the future, we could use this as a useful secondary endpoint in prospective studies. Third, longer follow-up is necessary as the significant difference in cGvHD rate after PB and BM allografts was most obvious with follow-ups of more than 6 to 7 years (34).

TABLE 3 | Univariate and multivariate analysis of survival.

Variables	Comparison	Overall survival				Failure-free survival			
		Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
		HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*P* value
Patient gender	Female vs male	0.44 (0.19-1.05)	0.065	0.47 (0.17-1.29)	0.144	0.53 (0.25-1.1)	0.088	0.96 (0.3-1.32)	0.221
Donor gender	Female vs male	1.42 (0.65-3.11)	0.377			1.19 (0.6-2.35)	0.62		
Patient age	Continuous variable	1.02 (0.99-1.05)	0.269			1.01 (0.98-1.04)	0.503		
Diagnosis (VSAA)	VSAA vs SAA	1.71 (0.77-3.82)	0.19	2.05 (0.81-5.14)	0.128	1.84 (0.89-3.77)	0.097	1.94 (0.92-4.07)	0.082
Diagnosis (NSAA)	NSAA vs SAA	2.13 (0.61-7.47)	0.238	1 (0.21-4.77)	1	2.45 (0.82-7.34)	0.109	1.74 (0.56-5.4)	0.337
Treatment	ATG vs no ATG	1.45 (0.2-10.73)	0.715			1.14 (0.16-8.36)	0.898		
ATG source	rATG vs pALG	0.67 (0.29-1.52)	0.336			0.75 (0.36-1.54)	0.43		
Conditioning regimen	Bu vs non-Bu	3 (1.39-6.49)	0.005	3.4 (1.35-8.56)	0.009	2.07 (1.01-4.27)	0.048	0.35 (0.1-1.15)	0.031
Interval from D to T	Continuous variable	1 (0.99-1.01)	0.395			1 (0.99-1.01)	0.617		
Graft source	BM ± PB vs PB	0.43 (0.13-1.42)	0.167	0.65 (0.15-2.86)	0.566	0.31 (0.1-1.03)	0.056	2.35 (1.08-5.1)	0.084
Amount of MNC	Continuous variable	0.99 (0.93-1.05)	0.66			0.98 (0.93-1.04)	0.462		
Amount of $CD34^+$ cells	Continuous variable	0.99 (0.84-1.16)	0.871			0.97 (0.83-1.12)	0.651		

HR, hazard ratio; CI, confidence interval; VSAA, very severe aplastic anemia; NSAA, non-severe aplastic anemia; SAA, severe aplastic anemia; ATG, anti-thymocyte globulin; rATG, rabbit ATG; pALG, porcine anti-lymphocyte globulin; D, diagnosis; T, transplantation; Bu, busulfan; BM, bone marrow; PB, peripheral blood; MNC, mononuclear cells.

In summary, our study showed that pALG is an alternative treatment for patients with SAA undergoing HSCT from an MSD. Its safety and efficacy were similar to those of rATG. A prospective, large-sample study is needed to validate our findings.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committees of Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College and Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

SF and YcZ contributed to the study design and manuscript review. YfZ, XC, LinL, YL, LiL, GY, and YN contributed to data collection and analysis. YfZ wrote the manuscript, and YfZ, XC, and LinL performed statistical analyses. XC, AP, DY, RZ, QM, WZ, YH, JW, EJ, and MH contributed to disease treatment and data collection. All authors have contributed to the manuscript and approved the submitted version.

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Full Length Article
Haploidentical

Comparisons of Modified Post-Transplantation Cyclophosphamide and Granulocyte Colony-Stimulating Factor/Antithymocyte Globulin Regimens for Haploidentical Stem Cell Transplantation in Patients with Aplastic Anemia

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A B S T R A C T

Haploidentical stem cell transplantation (HSCT) has become an alternative treatment option for patients with aplastic anemia (AA) without matched sibling donors or matched unrelated donors. Recently, post-transplantation cyclophosphamide (PTCy) and granulocyte colony–stimulating factor (G-CSF)/antithymocyte globulin (ATG) regimens have become the most common protocols used worldwide. In this retrospective study, we retrospectively reviewed and analyzed the clinical data of 130 AA patients who underwent haploidentical HSCT and received the modified PTCy (mPTCy) regimen (n = 55) or G-CSF/ATG regimen (n = 75) between January 2013 and June 2021 across 7 transplantation centers. Neutrophil engraftment was successful in all patients within 30 days in the G-CSF/ATG group. The cumulative neutrophil engraftment rate in the mPTCy group was 96.36% (95% confidence interval [CI], 94.57 to 97.57; $P = .010$). The median time to neutrophil engraftment in the G-CSF/ATG group was 10 days (range, 7 to 28 days), which was more rapid than that observed in the mPTCy group ($P < .001$). There was no significant difference in the incidence of graft-versus-host disease (GVHD) between the 2 groups. The cumulative incidence of grade II-IV acute GVHD was 18.40% (95% CI, 4.27% to 40.31%) in the mPTCy group and 19.32% (95% CI, 5.86% to 38.58%) in the G-CSF/ATG group, whereas the cumulative incidence of grade III-IV acute GVHD was 7.31% (95% CI, .09% to 37.48%) in the mPTCy group and 7.57% (95% CI, .20 to 34.19) in the G-CSF/ATG group. Similarly, there were no significant between-group differences in overall survival (OS), failure-free survival (FFS), and GVHD-free relapse-free survival (GRFS). The 2-year OS, FFS, and GRFS rates were 95.91% (95% CI, 84.59% to 98.96%), 92.25% (95% CI, 80.59% to 97.03%), and 86.68% (95% CI, 73.98% to 93.44%), respectively, in the mPTCy group and 86.67% (95% CI, 76.64% to 92.59%), 81.28% (95% CI, 70.45% to 88.46%), and 77.20% (95% CI, 65.89% to 85.16%), respectively, in the G-CSF/ATG group. Transplantation-related mortality (TRM) was significantly higher in the G-CSG/ATG group than in the mPTCy group (13.33% versus 1.96%; $P = .022$). In multivariate analysis, the use of a female donor, a higher Hematopoietic Cell Transplantation Comorbidity Index, and grade III-IV acute GVHD were associated with worse survival outcomes. The mPTCy and G-CSF/ATG regimens led to similar outcomes in AA patients, but quicker engraftment was observed with the ATG/G-CSF regimen, and a lower incidence of TRM was observed with the mPTCy regimen.

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INTRODUCTION

Severe aplastic anemia (SAA) is a life-threatening bone marrow (BM) failure syndrome associated with high mortality in the absence of specific treatment. The timely administration of immunosuppressive therapy (IST) plays a crucial role in improving the overall survival (OS) of AA patients, yet failure-free survival (FFS) remains suboptimal [1]. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has been recognized as the front-line treatment for patients with matched sibling donors (MSDs); however, <30% of AA patients have an HLA-matched donor [2]. For patients who lack an MSD, IST is traditionally favored before unrelated donors or haploidentical donors are considered. However, recent studies have reported lower FFS in patients who received IST compared with those who underwent HSCT [3–5]. Recent studies have indicated that haploidentical HSCT (haplo-HSCT) is associated with similar OS and FFS outcomes as MSD allo-HSCT with advancements in conditioning regimens and graft-versus-host disease (GVHD) prophylaxis [6,7]. In addition, in China the current consensus recommends that haplo-HSCT be considered as first-line treatment for patients age <50 years with severe AA [8].

Currently, 2 haplo-HCT protocols are widely used worldwide. Xi and Huang [9] proposed a granulocyte colony–stimulating factor (G-CSF)/antithymocyte globulin (ATG) regimen known as the "Beijing protocol" consisting of a conditioning regimen including busulfan (Bu), cyclophosphamide (Cy), ATG, and a combination of cyclosporin A (CsA), mycophenolate mofetil (MMF), and methotrexate (MTX) as GVHD prophylaxis. The graft is derived from G-CSF-primed BM and peripheral blood (PB). The other model is the post-transplantation Cy (PTCy) regimen proposed by Johns Hopkins University, also called the "Baltimore" regimen, comprising nonmyeloablative total body irradiation (TBI), fludarabine (Flu), and a combination of ATG and low-dose Cy comprise the conditioning regimen. GVHD prophylaxis consists of high-dose PTCy, calcineurin inhibitors (CNIs), and MMF [10].

These 2 regimens have exhibited satisfactory clinical outcomes. The PTCy regimen significantly reduced the rate of GVHD, but graft failure (GF) and delayed engraftment remained, resulting in problems related to infection and virus reactivation [11]. Although the rate of GVHD is higher with the G-CSF/ATG regimen compared with the PTCy regimen, the faster engraftment and lower rate of GF have certain advantages [12].

We recently evaluated a total body irradiation (TBI)-free modified PTCy (mPTCy) regimen for AA patients undergoing haplo-HSCT, and the results were encouraging [13]. However, there has been little research comparing the mPTCy and G-CSF/ATG regimens and exploring the implications for clinical prognosis. Thus, on behalf of the Aplastic Anemia Group of Hubei Province, we designed this multicenter retrospective study based on real-world data to evaluate the clinical outcomes of the mPTCy and G-CSF/ATG regimens in AA patients undergoing haplo-HSCT.

METHODS

Patients

This real-world retrospective study enrolled a total of 130 patients diagnosed with AA and undergoing haplo-HCT between January 2013 and June 2021. These patients were drawn from 7 clinical centers across Hubei Province: Tongji Hospital, Union Hospital, Wuhan No.1 Hospital, Yichang Central People's Hospital, Jinzhou Central Hospital, Xiangyang Central Hospital, and the First People's Hospital of Jinzhou. Diagnostic and classification criteria were from the International Aplastic Anemia Study Group [14]. We compared the clinical outcomes of patients in the 2 groups according to conditioning regimen and GVHD prophylaxis regimen. One group received the mPTCy regimen, and the other group received the G-CSF/ATG regimen. This study was approved by the Ethics Committees of Tongji Hospital, Tongji Medical College, and Huazhong University of Science and Technology and was performed in accordance with the Declaration of Helsinki (TJ-IRB20210948).

Conditioning Regimen

Details of the mPTCy protocol have been published previously [13]. In brief, patients received a Flu/Bu/Cy/ATG conditioning regimen including 100 mg/kg porcine antilymphocyte globulin or 10 mg/kg ATG i.v. on days -11 to -8, low-dose Cy 14.5 mg/kg/day i.v. on days -9 to -8, Flu 30 mg/m^2/day i.v. on days -7 to -3, and Bu 3.2 mg/kg/day i.v. on days -4 to -3. Patients in the G-CSF/ATG group were conditioned with a Bu/Cy/ATG regimen consisting of Bu 3.2 mg/kg/day i.v. on days -7 to -6, Cy 50 mg/kg/day i.v. on days -5 to -2, and ATG 2.5 mg/kg/day i.v. on days -5 to -2 [8]. These 2 protocols are detailed in Supplementary Figure S1. Patients who developed primary GF underwent a second HSCT with the Bu/Flu/Cy/ATG-F regimen, which consisted of Bu 3.2 mg/kg i.v. on day -6, Flu 30 mg/m^2/day i.v. from day -5 to day -2, Cy 500 mg/m^2/day i.v. on days -5 to -2, and ATG-F 5 mg/kg/day i.v. on days -5 to -2.

Donor, Mobilization, and Graft

HLA-haplo donors received G-CSF (Filgrastim; Hangzhou Jiuyuan Gene Engineering, Hangzhou, Zhejiang Province, China) at a dose of 10 μg/kg/day s.c. for 5 days before transplantation. The graft source was mostly G-CSF-mobilized BM and PB. BM was collected on day 0, and PB was harvested the next day. Mononuclear cells (MNCs) were counted, and CD34$^+$ cells were determined by FACS analysis. Mobilization was considered successful at a CD34$^+$ cell count >2 $\times$10^6/kg or an MNC count >6 $\times$ 10^8/kg.

GVHD Prophylaxis

GVHD prophylaxis in the mPTCy group consisted of high-dose Cy, tacrolimus, and MMF. High-dose Cy was administered at 50 mg/kg on days +3 and +4 post-transplantation during the years 2018 to 2019, with a changed to days +3 and +5 in 2020 to 2021 based on a previous study [15]. Tacrolimus was administered orally starting on the day of transplantation and continuing for 1 year, with a target concentration of 8 to 10 ng/mL. MMF was administered every 12 hours at a dose of .5 to 1 g from day +1 to day +40.

The G-CSF/ATG group received a combination of CsA, MMF, and MTX as GVHD prophylaxis. CsA was administered i.v. at a dose of 1.5 mg/kg every 12 hours, followed by oral maintenance when the patient's bowel function recovered. The concentration of CsA was monitored and adjusted between 200 and 250 ng/mL for 1 year after transplantation. The MTX dosage was determined based on a previous study [16], and the MMF dosage was the same as that in the mPTCy group.

Supportive Care and Virus Monitoring

All patients were admitted to a laminar flow ward and received G-CSF s.c. at a dose of 5 μg/kg/day until hematopoietic reconstruction was verified. Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) infection were monitored by real-time PCR performed twice weekly. Virus reactivation was defined as either a CMV DNA copy number >400 copies/mL or an EBV DNA copy number >500 copies/mL on 2 consecutive measurements. Preemptive therapy with ganciclovir was initiated if CMV reactivation occurred. Acyclovir was administered as prophylaxis for EBV viremia. Once EBV-DNA was >100,000 copies/mL or showed a 100-fold increase up to 50,000 copies/mL within 1 week, rituximab at a once-weekly dose of 375 mg/m^2 was administrated as preemptive therapy until EBV-DNA negativity occurred, with reference to the Sixth European Conference on Infections in Leukemia guideline [17]. Other infection prevention, transfusion, and supportive care measures have been described in detail previously [13].

Definition and Assessment

Neutrophil engraftment was defined as an absolute neutrophil count >.5 $\times$ 10^9/L for 3 consecutive days. Platelet engraftment was defined as an absolute platelet count >20 $\times$ 10^9/L for 3 consecutive days without transfusion. Chimerism analyses were performed as described in our previous study [18]. Patients who did not exhibit engraftment by day +28 with low donor chimerism were considered to have primary GF. The prognostic assessment included evaluation of OS, FFS, GVHD-free relapse-free survival (GRFS), and transplantation-related mortality (TRM). FFS was defined as survival without treatment failure, including death, GF, and relapse. TRM was defined as death without relapse. The diagnostic and classification criteria for acute GVHD (aGVHD) and chronic GVHD (cGVHD) were defined according to the Glucksberg-Seattle criteria [19].

Table 1
Patient Characteristics

Characteristic	mPTCy Group	G-CSF/ATG Group	*P* Value
Number	55	75	
Sex, n (%)			
Male	32 (58.2)	47 (62.7)	.605
Female	23 (41.8)	28 (37.3)	
Age, yr, median (range)	22 (5-58)	22 (7-49)	.74
Disease classification, n (%)			**.002**
SAA	36 (65.5)	62 (82.7)	
NSAA	9 (16.4)	9 (12)	
VSAA	10 (18.2)	1 (1.3)	
SAA-PNH	0 (0)	3 (4)	
Previous IST, n (%)	4 (7.3)	6 (8.0)	1.000
HCT-CI, n (%)			**.001**
0	32 (58.2)	66 (88)	
1	19 (34.5)	9 (12)	
2	3 (5.5)	0 (0)	
≥3	1 (1.8)	0 (0)	
Donor sex, n (%)			**.003**
Male	48 (87.3)	48 (64)	
Female	7 (12.7)	27 (36)	
Donor type, n (%)			**.006**
Parent to child	36 (65.5)	47 (62.7)	
Sibling to sibling	10 (18.2)	26 (34.7)	
Child to parent	9 (16.4)	2 (2.7)	
ABO mismatch, n (%)			.07
Matched	33 (60)	46 (61.3)	
Major mismatch	14 (25.5)	11 (14.7)	
Minor mismatch	8 (14.5)	11 (14.7)	
Different	0 (0)	7 (9.3)	
Blood values before HSCT, median (range)			
WBC	1.59 (.01-6.49)	1.38 (0-8.51)	.066
Hemoglobin	69 (41-110)	62 (28-92)	**.001**
Platelets	21 (3-85)	26 (1-186)	.068
Graft source, n (%)			.093
BM and PB	51 (92.7)	62 (82.7)	
PB	4 (7.8)	10 (17.3)	
$CD34^+$ cell count, $\times 10^6$/kg, median (range)	6.95 (1.1-13.48)	6.15 (1-17.39)	.982
$CD34^+$ cell count $\geq 8 \times 10^6$/kg, n (%)	19 (36.5)	21 (28.4)	.333
Follow-up, mo, median (range)	12.7 (1.1-38.3)	31.5 (0-65.3)	<**.001**

Significant *P* values are in bold type.
NSAA, nonsevere aplastic anemia; *PNH*, paroxysmal nocturnal hemoglobinuria; *VSAA*, very severe aplastic anemia.

Statistical Analysis

The data were analyzed using SPSS version 22.0 (IBM, Armonk, NY) and Prism version 8.0 (GraphPad Software, San Diego, CA). Continuous variables not conforming to a normal distribution were expressed as the median (range) and compared using the rank-sum test, and categorical variables were expressed as frequency and compared using the chi-square test. Survival was analyzed by Kaplan-Meier survival analysis. A log-rank test was applied to compare the survival curves. Given the heterogeneity in patient characteristics, the Cochran-Mantel-Haenszel test was applied to analyze the categorical variables, and the Breslow-Day test was used to assess heterogeneity. For survival data, univariate analysis and multivariate Cox analysis were performed to evaluate the risk factors for prognosis. Variables with a *P* value <.1 in univariate analysis were selected for the stepwise analysis and input into the multivariate Cox regression model. A *P* value <.05 was considered statistically significant.

RESULTS

Patient Characteristics

A total of 130 patients from 7 clinical centers in Hubei Province were analyzed. The patients were assigned to a conditioning regimen and GVHD prophylaxis regimen based on their clinical data. Fifty-five patients received the mPTCy regimen, and the other 75 patients received the G-CSF/ATG regimen. Baseline patient characteristics for both groups are summarized in Table 1. The median duration of follow-up was 12.7 months (range, 1.1 to 38.3 months) in the mPTCy group and 31.5 months (range, 0 to 65.3 months) in the G-CSF/ATG group. The age distribution and graft sources were similar in the 2 groups. Four patients (7.3%) in the mPTCy group and 6 patients (8.0%) in the G-CSF/ATG group received IST before haplo-HSCT (*P* = 1.000). In the G-CSF/ATG group, 27 patients (36%) received grafts from female donors, a higher proportion than that in the mPTCy group (*P* = .006). The Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI) was higher in the mPTCy group (*P* = .001). The results of heterogeneity tests are presented in Supplementary Table S1.

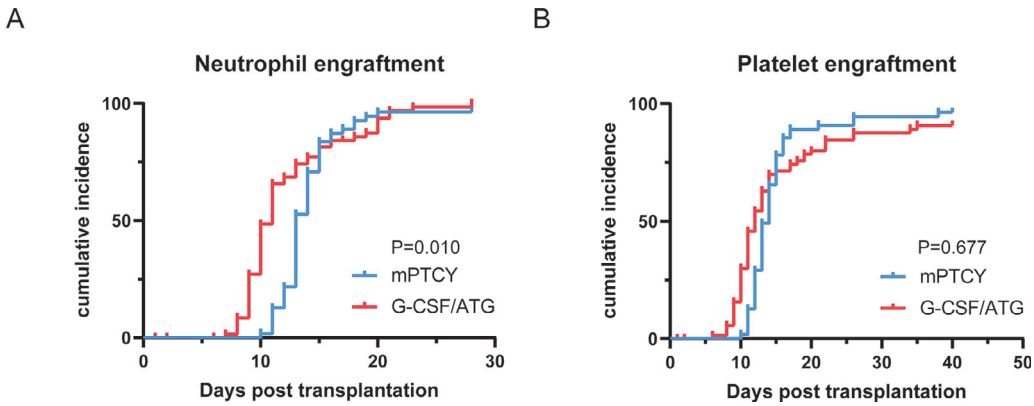

Figure 1. The cumulative incidence of engraftment after transplantation in the mPTCy and G-CSF/ATG groups. (A) Neutrophil engraftment. (B) Platelet engraftment.

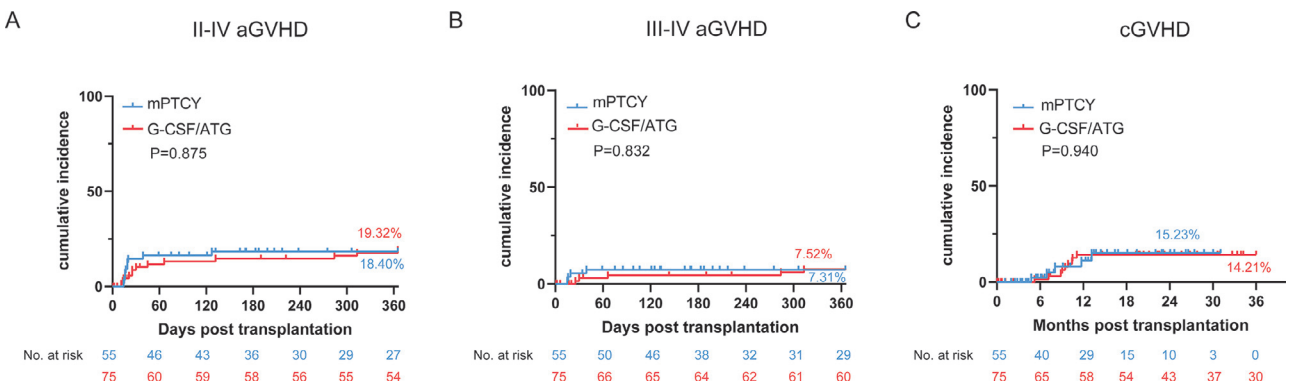

Figure 2. The cumulative incidence of GVHD after transplantation in the mPTCy and G-CSF/ATG* groups. (A) Grade II-IV aGVHD. (B) Grade III-IV aGVHD. (C) cGVHD.

Engraftment

The median CD34$^+$ cell count in grafts was similar in the 2 groups at 6.95 $\times$ 10^6/kg (range, 1.1 to 13.48 $\times$ 10^6/kg) in the mPTCy group and 6.15 $\times$ 10^6/kg (range, 1 to 17.39 $\times$ 10^6/kg) in the G-CSF/ATG group. Two patients were diagnosed with primary GF; both underwent a second transplantation with a Bu/Flu/Cy/ATG-F conditioning regimen and experienced successful engraftment. Unfortunately, one of these patients died from relapse at 4.5 months post-transplantation. The cumulative rate of neutrophil engraftment in the mPTCy group was 96.36% (95% CI, 94.57% to 97.57%), which was lower than that in the G-CSF/ATG group (P = .010) (Figure 1A). Engraftment was successful in all patients in the G-CSF/ATG group, and 6 patients died due to infection and heart failure before hematopoietic reconstruction. No cases of secondary GF were observed in the 2 groups. The median time to neutrophil engraftment was faster in the G-CSF/ATG group compared with the mPTCy group (10 days [range, 7 to 28 days] versus 13 days [range, 10 to 28 days]; P < .001). The cumulative rate of platelet engraftment was 96.36% (95% CI, 94.57% to 97.57%) in the mPTCy group and 90.77% (95% CI, 87.56% to 93.18%) in the G-CSF/ATG group (Figure 1B); the difference was not statistically significant.

GVHD

We analyzed the incidences of aGVHD and cGVHD in patients who survived until the end of follow-up. The 1-year cumulative rate of grade II-IV aGVHD was similar in the 2 groups (mPTCy group: 18.40% [95% CI, 4.27% to 40.31%]; G-CSF/ATG group: 19.32% [95% CI, 5.86% to -38.58%]; P = .875) (Figure 2A). The cumulative rate of grade III-IV aGVHD was 7.31% (95% CI, .09% to 37.48%) in the mPTCy group and 7.57% (95% CI, .20% to 34.19%) in the G-CSF/ATG group (P = .832) (Figure 2B). The 2-year cumulative rate of cGVHD was 15.24% (95% CI, .97% to 46.56%) in the mPTCy group and 9.45% (95% CI, .52% to 34.68%) in the G-CSF/ATG group (P = .940) (Figure 2C).

Infection

We analyzed the infection and virus reactivation status of all patients after transplantation. Twenty-one patients (38.2%) in the mPTCy group and 38 (50.7%) in the G-CSF/ATG group experienced infection (P = .158) (Table 2). The sites of infection were also similar in the 2 groups. Pulmonary infection was the most frequent infection in both groups, followed by bloodstream infection.

CMV viremia was observed in 25 patients in the mPTCy group, and the cumulative incidence of CMV reactivation was similar in the 2 groups, at 50.76% (95% CI, 36.82% to 63.12%) in the mPTCy group versus 52.82% (95% CI, 40.98% to 63.34%) in the G-CSF/ATG group (Figure 3A). Notably, 2 patients in the mPTCy group were diagnosed with CMV-related disease (1 with CMV pneumoniae and 1 with CMV colitis), which was controlled well by antiviral treatment, and no patient died from CMV disease. In addition, the cumulative rate of EBV reactivation was similar in the 2 groups, at 34.08% (95% CI, 15.79% to 53.38%) in the mPTCy group and 22.57% (95% CI, 8.43% to 40.83%) in the G-CSF/ATG group (Figure 3B). Post-transplantation lymphoproliferative disorder and EBV-related cytopenia were not observed in the patients experiencing EBV reactivation as a result of preemptive therapy.

Table 2
Clinical Outcomes

Outcome	mPTCy Group	G-CSF/ATG Group	*P* Value
Number	55	75	
Time to neutrophil engraftment, d, median (range)	13 (10-28)	10 (7-28)	**<.001**
Time to platelet engraftment, d, median (range)	14 (10-38)	12 (6-123)	**.004**
GF, n (%)	2 (3.6)	5 (6.7)	.792
aGVHD grade, n (%)			
I-IV	25 (49)	26 (34.7)	.213
II-IV	10 (18.2)	13 (17.3)	.900
III-IV	4 (7.3)	5 (6.7)	1.000
Infection, n (%)	21 (38.2)	38 (50.7)	.158
Pulmonary infection	9 (16.4)	25 (33.3)	**.030**
Gastrointestinal infection	2 (3.6)	3 (4)	1.000
Bloodstream infection	4 (7.3)	7 (9.3)	.677
Upper respiratory infection	3(5.5)	2 (2.7)	.723
Urinary system infection	4 (7.3)	2 (2.7)	.416
Soft tissue infection	0 (0)	2 (2.7)	.514
NA	1 (1.8)	4 (5.3)	.570
Virus reactivation, n (%)			
CMV	28 (50.9)	36 (48)	.743
EBV	16 (29.1)	15 (20)	.229

Significant *P* values are in bold type.
NA, not available.

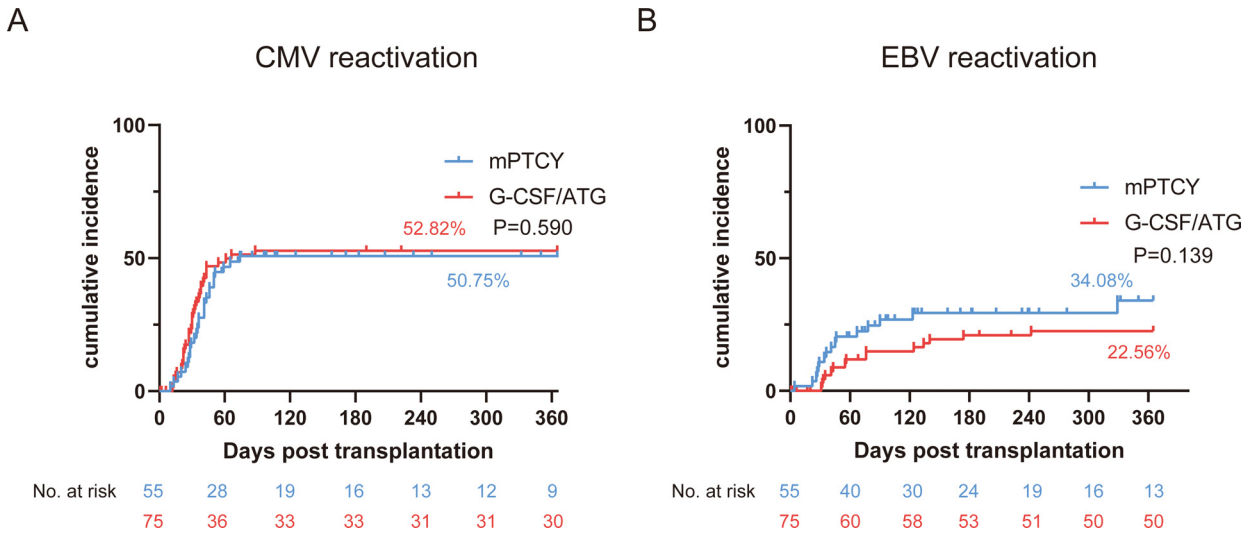

Figure 3. The cumulative incidence of virus reactivation within 1 year post-HSCT in the mPTCy and G-CSF/ATG groups. (A) CMV reactivation. (B) EBV reactivation.

Survival Outcomes

The 2-year OS was 95.91% (95% CI, 84.59% to 98.96%) in the mPTCy group and 86.67% (95% CI, 76.64% to 92.59%) in the G-CSF/ATG group. A suggestive but not significant benefit in OS was seen in the mPTCy group (P = .066) (Figure 4A). The 2-year FFS was 92.25% (95% CI, 80.59% to 97.03%) in the mPTCy group and 81.28% (95% CI, 70.45% to 88.46%) in the G-CSF/ATG group (P = .117) (Figure 4B), and the 2-year GRFS in the 2 groups was 86.68% (95% CI, 73.98% to 93.44%) and 77.20% (95% CI, 65.89% to 85.16%), respectively (P = .218) (Figure 4C).

Because we found that most deaths occurred early after transplantation, we next analyzed the TRM post-transplantation. The cumulative incidence of TRM was significantly higher in the G-CSF/ATG group compared with the mPTCy group (13.33% [95% CI, 2.35% to 33.84%] versus 1.96% [95% CI, 0 to 57.47%]; P = .022) (Figure 5). One patient in the mPTCy group died from multiple organ failure (with an HCT-CI of 4 before transplantation), and the other patient died from relapse after a second transplantation. Ten deaths occurred in the G-CSF/ATG group, from severe aGVHD in 1 patient, heart failure in 3 patients, infection or hemorrhage in 5 patients, and thrombotic microangiopathy (TMA) in 1 patient.

Multivariate Analysis

We conducted univariate and multivariate Cox regression analyses to identify prognostic factors for our patients undergoing haplo-HSCT for AA. The results, presented in Table 3, show that the 2 transplantation protocols were not independent prognostic factors in AA. We identified higher HCT-CI, female donor, and grade III-IV aGVHD as independent risk factors for OS (hazard ratios, 46.10 [95% CI, 4.15 to 512.35;

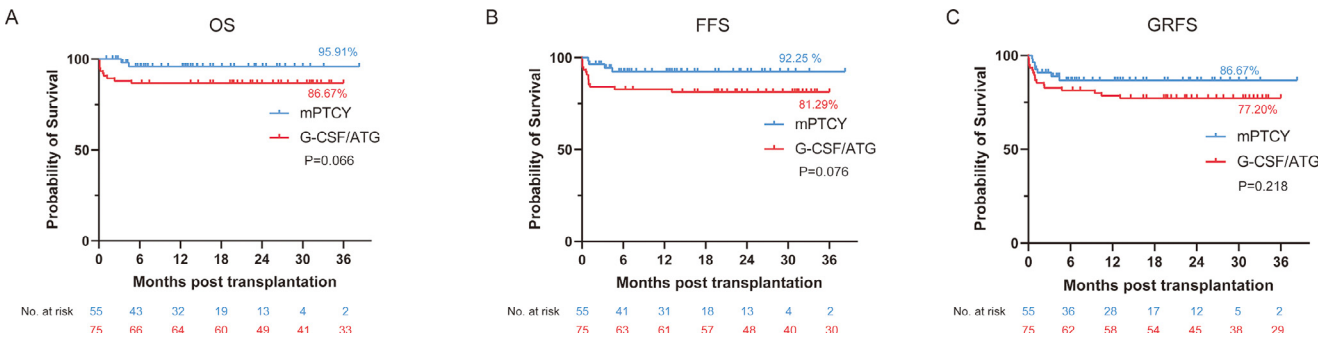

Figure 4. Survival outcomes in the mPTCy and G-CSF/ATG groups. (A) OS. (B) FFS. (C) GFRS.

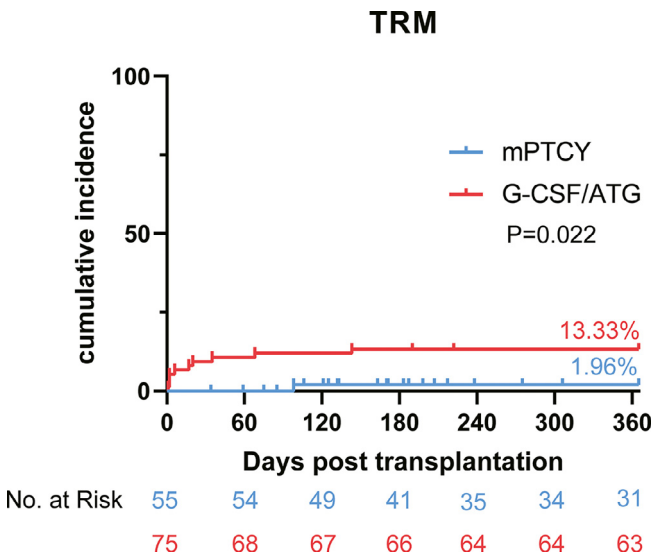

Figure 5. The cumulative incidence of TRM after HSCT in the mPTCy and G-CSF/ATG groups.

Table 3
Multivariate Analysis

Outcome	Variate	HR (95% CI)	*P* Value
OS			
	Female donor	9.69 (2.013-46.679)	**.005**
	HCT-CI ≥3	46.096 (4.147-512.351)	**.002**
	Grade III-IV aGVHD	9.712 (1.368-68.965)	**.026**
FFS			
	Female donor	3.561 (1.373-9.233)	**.005**
	HCT-CI ≥3	13.016 (1.616-104.855)	**.01**
GRFS			
	HCT-CI ≥3	18.064 (2.138-152.652)	**.002**
	Grade III-IV aGVHD	23.908 (8.299-69.29)	**<.001**
	Female donor	3.782 (1.312-10.902)	**.014**
Grade II-IV aGVHD			
	$CD34^+$ cell count ≥8×10^6/kg	3.721 (1.528-9.063)	**.002**
	PB graft	5.706 (2.28-14.277)	**<.001**
	HCT-CI ≥3	17.221 (2.048-144.8377)	**.009**
Grade III-IV aGVHD			
	PB graft	4.382 (1.024-18.764)	**.035**

Significant *P* values are in bold type.

P = .002], 9.69 [95% CI, 2.01 to 46.68; *P* = .005], and 9.71 [95% CI, 1.37 to 68.97; *P* = .026], respectively) and higher CD34+ cell counts (≥8×10^6/kg) and use of PB grafts as independent risk factors for grade II-IV aGVHD.

DISCUSSION

Allo-HSCT remains the primary treatment for AA patients with an MSD, but unfortunately, <30% of candidates have an available MSD. However, recent studies have suggested that

better outcomes can be achieved with haplo-HSCT compared with IST, because some patients do not respond to IST [20]. In addition, IST is associated with lower FFS and higher clonal evolution [21]. Owing to the limitations of IST and the wider availability of haploidentical donors, haplo-HSCT may be a better option for those who lack an MSD or matched unrelated donor. Furthermore, based on recent evidence, haplo-HSCT has been recommended by a Chinese consensus as front-line treatment for patients age <50 years with AA, and in China more than one-half of patients with severe AA undergo haplo-HSCT [8].

Xu et al. [6] used a G-CSF/ATG-based haplo-HSCT regimen in AA patients for the first time in 2016 and reported 100% neutrophil engraftment and a 21.1% cumulative incidence of grade II-IV aGVHD, which was confirmed by a series of subsequent large-sample studies reported grade II-IV aGVHD rates of 20.0% to 30.3% [7]. In 2014 Clay et al. [22] used a PTCy regimen for AA patients undergoing haplo-HSCT and observed no severe aGVHD and a GF rate of 25% [22]. These results are consistent with the study reported by Esteves et al. [23] in 2015. To improve engraftment, DeZern et al. [24,25] added ATG to the traditional PTCy regimen and reported a better engraftment. We recently evaluated a TBI-free mPTCy regimen and found satisfactory results [13]. We hope that the present retrospective study systematically comparing engraftment, survival, GVHD, and infection between the G-CSF/ATG and mPTCy regimens in patients with AA will help clinicians improve outcomes in patients with AA.

GF is one of the most severe complications in AA patients undergoing haplo-HSCT. Studies based on the G-CSF/ATG regimen have reported lower rates of GF, ranging from approximately 0% to 7% [6,7,26]. In their multicenter prospective study, Xu et al. [6] reported no GF in their 101 patients and a median time to neutrophil engraftment of 12 days (range, 9 to 25 days). Although Zhang et al. [26] reported a 7% rate of GF, the median time to neutrophil engraftment remained rapid at 14 days. In contrast, an early PTCy regimen was associated with a higher rate of GF and slower engraftment. Clay et al. [22] reported on 8 AA patients undergoing haplo-HSCT who received the PTCy regimen. Six of these 8 patients had neutrophil engraftment and 5 had platelet engraftment, and the median time to neutrophil engraftment was 18.5 days [22]. In the study of DeZern et al. [25], the median time to neutrophil engraftment was 17 days (range, 14 to 88 days), and 3 of 7 treatment-naïve patients experienced GF after low-dose (200 cGy) TBI. Research from the European Society for Blood and Marrow Transplantation has also shown a lower engraftment rate with PTCy regimens compared with other haplo-HSCT protocols [11].

Although our mPTCy regimen was associated with a lower rate of GF compared with traditional PTCy regimens, the rate was higher compared with the G-CSF/ATG regimen in this study, which is consistent with our previous findings. This result may be related to the mechanism of PTCy. High-dose PTCy selectively suppresses alloreactive T cells, allowing the recipient's memory T cells to escape, resulting in delayed engraftment [27,28]. However, we did not observe a higher incidence of infection in the mPTCy group, even though neutrophil engraftment was delayed for 3 days. Nevertheless, prolonged neutropenia results in prolonged hospital length of stay, particularly in the laminar unit, with attendant higher costs.

Despite significant advances in haplo-HSCT, GVHD remains a fatal complication and an important cause of early mortality. According to previous studies, the incidence of GVHD has decreased significantly with the PTCy regimen, to 11% to 23% for grade II-IV aGVHD and <10% for grade III-IV aGVHD. Conversely, the incidence of aGVHD is higher with the G-CSF/ATG regimen compared with the PTCy regimen. Xu et al. [6,7,12] reported rates of grade II-IV aGVHD and grade III-IV aGVHD of 20% to 30.3% and 6% to 10.1%, respectively. In more recent studies, the incidence of aGVHD was comparable in the G-CSF/ATG and PTCy regimens. In a study by Wang et al. [26], the incidence of grade II-IV aGVHD was 18.9%, and that of grade III-IV aGVHD was 10% [26]. In this study, the incidence of GVHD in the mPTCy group was comparable to the incidence that we reported recently; however, there was no significant difference in the incidence of GVHD between the mPTCy group and the G-CSF/ATG group. In our multivariate Cox regression model, we identified higher $CD34^+$ cell count and use of PB grafts as independent risk factors for severe aGVHD. Owing to the limitation of leukapheresis, the alloreactive T cell counts were increased, accompanied by higher $CD34^+$ cell counts in grafts, particularly in PB grafts [29]. A previous multicenter study demonstrated that haplo-HSCT with mixed BM + PB grafts was associated with longer disease-free survival compared with haplo-HSCT PB grafts alone [30]. In another study, although a higher percentage of $CD3^+$ T cells was seen in G-CSF-mobilized peripheral blood (GM-PB), $CD34^+$ cells were more abundant, promoting stable and rapid engraftment [31]. Our present results show that both the mPTCy regimen and G-CSF/ATG regimen significantly mitigated severe aGVHD, indicating that haplo-HSCT is an ideal option for patients with AA compared with MSD or MUD HSCT.

In previous studies, the OS of AA patients who underwent haplo-HSCT was 67% to 94% [9]. In our present study, OS was 95.91% in the mPTCy group and 88.67% in the G-CSF/ATG group. Although this is a suggestive but not significant benefit in OS, statistical significance possibly could be found with increased follow-up or by increased sample sizes. Meanwhile, the FFS and GRFS in the 2 groups were 92.25% versus 82.28% and 86.68% versus 77.20%, respectively, with no significant between-group differences. However, these 2 haplo-HSCT protocols were not independent risk factors for survival in the multivariate analysis, as was the case for higher HCT-CI, female donors, and grade III-IV aGVHD. Recent studies have shown that higher HCT-CI is associated with a poorer prognosis [32,33]. In the present study, a patient with HCT-CI ≥3 before transplantation received the mPTCy regimen and died from severe infection and multiple organ failure. Thus, the intensity of the conditioning regimen should be considered and supportive care emphasized for patients with a high HCT-CI who need salvage transplantation. However, although more patients with very severe AA received the mPTCy regimen, no deaths or GFs were reported in those patients, indicating that the mPTCy regimen may benefit patients with very severe AA. In addition, Xu et al. [34] analyzed the impact of grafts from different donors and demonstrated that younger and noninherited maternal antigen-male (NIMA-male) donors were better sources, in agreement with our findings. The use of more female donors may be the reason for the relatively lower survival in the G-CSF/ATG group, as reported above.

Because most deaths occurred in the early weeks post-transplantation, we analyzed TRM after transplantation. Surprisingly, TRM was significantly higher in the G-CSF/ATG group compared with the mPTCy group. Causes of death included infection in 5 patients and heart failure in 3 patients, which may be correlated with the high Cy dose in the conditioning regimen. The total Cy dose in the G-CSF/ATG group reached 200 mg/kg, so toxicity cannot be neglected, especially in patients receiving multiple transfusions and those with

impaired cardiac function. A previous study reported a cumulative incidence of 5.6% for severe cardiotoxicity in patients with severe AA undergoing haplo-HSCT with a 200 mg/kg Cy-based conditioning regimen [35]. Thus, some transplantation centers using a G-CSF/ATG regimen have attempted to adjust the Cy dose to 30 mg/kg/day for 4 days to attenuate the cardiotoxicity of Cy. However, further studies are needed to confirm the plausibility of adjusting the Cy dose in the G-CSF/ATG regimen. Similarly, to address this issue, Cy has been administered on days -3 and -5 in the mPTCy regimen, resulting in minor toxicities similar to those reported previously [15,36]. In recent years, more patients have received the mPTCy regimen at our center, and we will refine the mPTCy regimen through continuous exploration to further improve the clinical outcomes of patients with severe AA. Overall, the mPTCy regimen has shown better safety and a lower rate of TRM than the G-CSF/ATG regimen.

Of course, the present study has some limitations. First, as a real-world retrospective study, the potential for recall bias cannot be avoided. In addition, the sample size in the mPTCy group was relatively small and follow-up was relatively short, because application of the mPTCy regimen remains exploratory. These limitations may be the source of the clinical heterogeneity noted across subgroups. A prospective study is ongoing in our center (ChiCTR2100043831) to further explore the feasibility of the mPTCy regimen. Further prospective studies with large sample sizes are needed, especially for PTCy regimens based on the current evidence.

In conclusion, in this retrospective study comparing the differences in survival and efficacy between the G-CSF/ATG and mPTCy regimens in AA patients undergoing haplo-HSCT has shown low incidences of GVHD and similar survival outcomes in the 2 regimens, with individual advantages. The G-CSF/ATG regimen allows for faster hematopoietic reconstruction and a higher rate of engraftment, whereas the mPTCy regimen was associated with a lower incidence of TRM. Further prospective randomized controlled studies with large sample sizes are still needed to confirm these outcomes.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jtct.2022.04.021.

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CORRESPONDENCE

Modified umbilical cord-blood transplantation for pediatric patients with mucopolysaccharidosis

Bone Marrow Transplantation; https://doi.org/10.1038/s41409-022-01858-5

TO THE EDITOR:

Mucopolysaccharidoses (MPSs) are a group of inherited errors of metabolism caused by gene encoding mutation that affects the degradation of mucopolysaccharides or glycosaminoglycans (GAGs), resulting in the accumulation of glycosaminoglycans in various organs and tissues of patients with MPS leads to multi-systemic clinical picture with a broad range of clinical signs and symptoms [1]. Although the advent of enzyme replacement therapy (ERT) has paved the way for MPS treatment, the blood-brain barrier (BBB) has prevented patients with central nervous system manifestations from benefiting from ERT [2]. Therefore, allogenic bone marrow transplantation (HSCT) is still the only effective treatment option for patients with MPS [3, 4].

This retrospective study included 60 patients with MPS who underwent a new conditioning regimen based on a slightly modified position of ATG or the first use of ALG in patients with MPS from December 2018 to February 2022 treated at the Hospital of Hebei Pediatrics of Integrated Traditional Chinese and Western Medicine ($n = 56$) and the Hospital of Beijing Jingdu Pediatrics ($n = 4$). The study was approved by the institutional review board of the two centers. Written informed consent was obtained from the parents or legal guardians of the patients.

All patients received a bulsufan (Bu), cyclophosphamide (Cy) combined with fludarabine (Flu) based conditioning regimen. At the Hospital of Hebei Pediatrics of Integrated Traditional Chinese and Western Medicine, conditioning ($n = 56$) consisted of Bu (4 mg/kg/day x 3 days) from day −8 to −6, Cy (40 mg/kg/day x 4 days) from day −6 to −3, and Flu (40 mg/m^2/day x 5 days) from day −8 to −4, ALG (Wuhan Biological Products Research institute Co., Ltd. 25 mg/kg/d × 4 days) from day −10 to −7($n = 56$), while at the Hospital of Beijing Jingdu Pediatrics, rabbit anti-human thymocyte immunoglobulin (ATG 7.5 mg/kg), divided into four days, Sanofi Pharmaceuticals Co., Ltd, Cambridge, MA ($n = 4$) from day −10 to −7 as graft-vs.-host disease (GvHD) prophylaxis. Flu (40 mg/m^2/day x 4 days), Tacrolimus (0.01 to 0.05 mg/kg/day) or cyclosporine (3 mg/kg/day) as commenced on day -6 and was tapered off by day +90 if there was no GvHD, and mycophenolate mofetil (MMF) were used on day -6 and tapped off by day +28. All patients received methotrexate (10 mg/m^2/day) on days +1, +3, +6, +11. The details are shown in Fig. 1a.

The cord blood selection criteria based on reported data [5], was cell dose (>5.0 × 10^7 total nucleated cells [TNC]/kg) and CD34+/kg ≥ 1.5 × 10^5 to find the most suitable HLA match. High-resolution typing for HLA -A, -B, -C, and -DRB1 of patients and UCB units excluded UCB units with >2 HLA mismatches and HLA-C mismatches. If the criterion for the minimum number of cells in a single UCB unit is not achieved, a double UCBT should be considered. D-UCBT: TNC/kg: ≥1.5 × 10^7 for each unit and/or CD34+/kg ≥1 × 10^5 for each unit [6].

All patients' organ function was evaluated before and after HSCT. The diagnostic criteria included genetic testing, significant decreased level of lysosomal enzymes, positive urine GAGs, and the exclusion of multiple sulfatase deficiency and GM1 ganglioside disease. Statistical analysis was performed using GraphPad Prism software version 5.01 or R version 3.5.2. Categorical variables were compared using the chi-square test or Fisher's exact test as appropriate. Continuous variables were compared using the non-parametric Mann-Whitney U or Wilcoxon test when appropriate. Survival analysis was evaluated utilizing the Kaplan-Meier method. OS was calculated from transplantation to death or last follow-up. A two-sided P value < 0.05 was considered to indicate a statistical significance.

The median age at HSCT transplantation was three years (range, 1–13 years). Twenty-six patients (42%) were between the ages of 0 and 2 years at the time of transplantation. Forty-three (72%) received S-UCB, and 17 (28%) received D-UCB. All baseline characteristics are shown in Supplementary Table 1. The estimated 3-year overall survival (OS) was 96.5%, while median OS was not reached in our cohort, as two patients with MPS died during long-term follow-up. Details are shown in Fig. 1b and Supplementary Table 2. The results summarized in Supplementary Table 2 shows that sixty (100%) patients achieved full donor chimerism, all of them achieved normal enzyme after HSCT. Early toxicity in the form of mucositis was not observed; no patient developed VOD and transplant-related thrombosis (TA-TMA). The incidence of grades I to II acute GvHD (aGvHD) was 6.7%, only one (1.7%) patient developed grade IV aGvHD and died of multifunction organ failure 4 months post-transplant. No patient developed cGvHD. Eleven patients (18%) displayed cytomegalovirus (CMV) activity after transplantation, one patient displayed Epstein-Barr virus (EBV) activity, and one patient had an activity rate of BKV-related hemorrhage cystitis (6.7%). According to the comparative analysis of the incidence of virus activation, there was no statistical difference between the D-UCB and S-UCB groups (0.294 vs. 0.186, $P = 0.570$). Furthermore, based on the recovery analysis after transplantation, we found that neither D-UCB nor S-UCB affected the recovery days of neutrophil and platelet ($P = 0.313$, $P = 0.209$), respectively (Fig. 1c).

All patients achieved full donor chimerism, with a median follow-up of three years, two patients died. Organ function outcome was only evaluated in 58 patients. Before transplantation, the most common clinical manifestations were lower activity of daily living (ADL), developmental quotient (DQ)/intelligence quotient (IQ), multiple bone dysplasia, hepatosplenomegaly, upper-airway obstruction, mental retardation, valvular heart disease, hearing loss, and corneal clouding. After transplantation,

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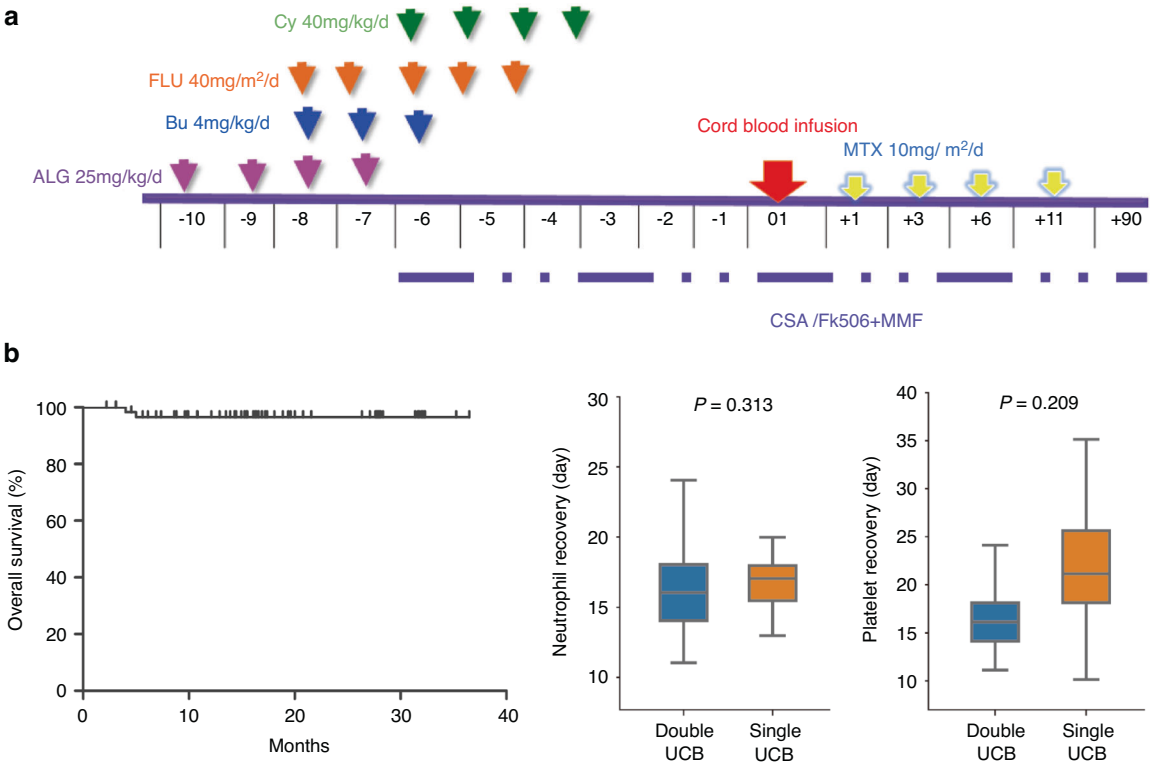

Fig. 1 Conditioning regimens and overall survival after transplantation for patients with MPS. a Sequential conditioning regimens for patients with MPS. Abbreviations: Bu busulfan, ALG porcine anti-human lymphocyte immunoglobulin, BM bone marrow, PBSC peripheral blood stem cell, Flu flurabine, Cy cyclophosphamide, MMF mycophenolate mofetil; MTX methotrexate, CSA clclosporin A, Fk506 tacrolimus, MMF mycophenolate mofetil. Note: Fifty-six patients received this conditioning regimen, another four patients received rabbit Anti-human thymocyte immunoglobulin as GvHD prophylaxis. **b** Overall Survival of patients with MPS after transplantation. Survival analysis showed the 3-year OS was 96.5% and medium OS was not reached in our cohort, as two patients with MPS were dead during long-term follow-up. **c** Comparation between double UCB and single UCB on recovery after transplantation according to Neutrophil and Platelet, respectively. Based on the analysis of the recovery after transplantation, we found that neither double UCB nor single UCB affected the recovery days of neutrophil and platelet ($P = 0.313$, $P = 0.209$), respectively.

Table 1. Organ function for patients with MPS after transplantation.

Patient type	MPS type I-Hurler $N = 17$		MPS type II-Hunter $N = 32$			MPS type III-Sanfilippo $N = 1$		VIa-Morquio $N = 10$	
	Pre-HSCT	Post-HSCT	Pre-HSCT	Post-HSCT		Pre-HSCT	Post-HSCT	Pre-HSCT	Post-HSCT
	P (*n* %)	E (*n* %)	P (*n* %)	E (*n* %)	M N = 2	P (*n* %)	E (*n* %)	P (*n* %)	E (*n* %)
Upper-airway obstruction	3 (17.6%)	2/3 (66.7%)	15/32 (46.7%)	12/15 (80%)	2#	0/1 (0%)		6/10 (60%)	5/6 (83.3%)
Valvular Heart disease	15 (88.2%)	3/15 (20%)	30/32 (93.8%)	4/30 (13.3%)		0/1 (0%)		5/10 (50%)	3/5 (60%)
Decreased vision	10 (58.8%)	5/10 (50%)	2/32 (6.3%)	1/2 (50%)		0/1 (0%)		3/10 (30%)	1/3 (33.3%)
Recurrent otitis	6 (35.3%)	6/6 (100%)	10/32 (31.2%)	4/10 (40%)		0/1 (0%)		3/10 (30%)	2/3 (66.7%)
Hearing loss	4 (100%)	3/4 (75%)	7/32 (21.8%)	4/7 (57.1%)		0/1 (0%)		2/10 (20%)	1/2 (50%)
Bone dysplasia	17 (100%)	16/17 (94.1%)	32/32 (100%)	30/32 (93.8%)	2#	1/1 (100%)	1/1 (100%)	10/10 (100%)	10/10 (100%)
Hepatos-Plenomegaly	15 (88.2%)	15/15 (100%)	30/32 (93.8%)	28/30 (93.3%)	2#	1/1 (100%)	1/1 (100%)	1/10 (10%)	1/1 (100%)
Mental retardation	7 (47.1%)	6/7 (85.7%)	14/32 (43.75%)	7/14 (50%)	1#	1/1 (100%)	0/1 (0%)	0/10 (0%)	0/10 (0%)
Lower DQ/IQ	10 (58.8%)	9/10 (90%)	15/32 (46.9%)	6/15 (40%)	1	1/1 (100%)	0/1 (0%)	1/10 (10%)	1/1 (100%)
Lower ADL	14 (82.4%)	10/14 (71.4%)	16/32 (50%)	10/16 (62.5%)		1/1 (100%)	0/1 (0%)	1/10 (10%)	1/1 (100%)

P present, *N* the number of died patient, *M* mortality, After after hematopoietic cell transplantation, *E* effective and organ function improvement post-transplant, *DQ* developmental quotient (using Gesell Developmental Schedules), *IQ* intelligence quotient (using Wechsler Preschool and Primary Scale), *ADL* activity of daily living (using the Barthel index of ADL); #, After HSCT, two patients died.

patient' organ function, ADL, and DQ/IQ was improved to a certain extent. details are in Table 1.

In this retrospective study, our high-rate OS verified the safety and efficacy of the conditioning regimen. compared with recent results observed in similar patient cohorts [7] these results are encouraging. HSCT has proven efficacy in correcting the disease course in patients with MPS I, MPS II, MPS VI, especially when the procedure is done at an early stage [8, 9]. Our results have shown

that most cases of hepatosplenomegaly and upper-airway obstruction resolved within several months after transplantation; recurrent otitis, corneal clouding, and joint stiffness were also improved in most cases. Hearing and visual acuity improved in some patients. Outcomes of cardiac involvement, orthopedic complications, and some neurodevelopment impairment improved. Our results are similar with recent reports [9, 10]. Since the median follow-up of our patients was 3 years, a longer period for organ evaluation is needed.

Intravenous enzyme replacement therapy (ERT) is available for MPSs I, II, IVA, VI, and VII. ERT has limited ability to cross the blood-brain barrier (BBB) and does not modify the neurological phenotype, the use of traditional ERT in combination with other treatments will help in improving prognosis of MPS patients over the next years so some scientists have used IV administration to direct intraparenchymal (IP), intrathecal (IT), or intracerebroventricular (ICV) administration. Although delivered centrally, the ability to deliver ERT uniformly to diffuse cerebral regions following the IT or ICV routes of administration is not fully clarified and requires further evaluation of efficacy [11].

In summary, our results showed that patients with MPS without suitable donor could benefit from our modified UCBT protocol as UCB's important positive effect on the neurological development compared to ERT. Although the recent study is encouraging, follow-up time was limited, therefore further investigation is needed.

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DATA AVAILABILITY

The data for this article are not publicly available. Requests to access the data should be directed to the corresponding authors.

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AUTHOR CONTRIBUTIONS

YY, GQ, YS, and XS designed, wrote, and revised the manuscript. YY, GQ, ZL, YZ, YS, XZ, XQ, FJ, SF, JC collected data and provided clinical care. YY, GQ, and JQ analyzed the clinical data. All authors approved the final manuscript for publication.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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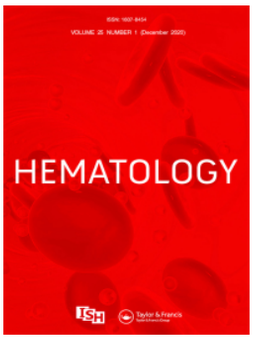

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Application prospect of low-dose porcine anti-thymocyte globulin in HLA-matched sibling donor transplantation

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RESEARCH ARTICLE

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Application prospect of low-dose porcine anti-thymocyte globulin in HLA-matched sibling donor transplantation

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ABSTRACT

Objective: To investigate the preventive effect of low-dose porcine anti-thymocyte globulin (P-ATG) on graft versus host disease (GVHD) in patients' donors over 40 years old or female donors undergoing HLA-matched sibling donor hematopoietic stem cell transplantation (MSD-HSCT).

Methods: The clinical data of 30 patients received Low-dose Porcine antithymocyte globulin (P-ATG) as a part of the conditioning regimen (the P-ATG group), while the other 30 patients didn't receive ATG (the Non-ATG group).

Results: There was a significant difference in the incidence of aGVHD ([23.3 (10.1–39.7) %] vs [50.0 (30.8–66.5) %], $P = 0.028$), grade II–IV aGVHD ([16.7 (5.94–32.1) %] vs [40.0 (22.4–57.0) %], $P = 0.049$) and chronic GVHD (cGVHD) ([22.4 (6.03–45.1) %] vs [69.0 (43.4–84.8) %], $P = 0.001$) between two groups. But there was no significant difference in terms of moderate-severe cGVHD ($P = 0.129$), 1-year relapse rate ($P = 0.742$), non-relapse mortality ($P = 0.237$), or overall survival ($P = 0.441$).

Conclusion: The application of low-dose P-ATG in patients/donors over 40 years old or female donors undergoing MSD-HSCT for hematological malignancy can significantly reduce the incidence of aGVHD, grade II–IV aGVHD and cGVHD, doesn't increase the risk of relapse.

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Porcine anti-thymocyte globulin; MSD-HSCT; aGVHD

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is an important therapeutic option and a potentially curative procedure for a variety of hematological malignancies. Graft versus host disease (GVHD) is the most frequent and serious complication following allogeneic HSCT (allo-HSCT), which remarkably impacts a patient's survival and quality of life [1]. Even among recipients of recipient human leukocyte antigen (HLA)-matched sibling donor HSCT (MSD-HSCT), incidence rates of acute GVHD (aGVHD) and chronic GVHD (cGVHD) reach 40–50% and 30–70%, respectively [2,3]. Especially in patients/donors over 40 years old and female donors with malignant hematological diseases have an increased GVHD risk in MSD-HSCT [4–6]. It is reported that anti-thymocyte globulin (ATG) can effectively decrease the incidence of aGVHD in MSD-HSCT [7]. Porcine ATG (P-ATG) is another ATG preparation that is available in China for clinical use as an immunosuppressive agent. However, there are a few reports that it are used in the conditioning regimen of MSD-HSCT. To address the concern, we investigated the effect of P-ATG on the risk of GVHD in MSD-HSCT.

Methods

Patients

We conducted a retrospective analysis of the clinical data of 60 patients with hematological malignancies who received MSD-HSCT from July 2020 to October 2021 in our transplantation center. These patients/donors were over 40 years old or the donors were female. Of these, 30 patients received low-dose P-ATG as a part of the conditioning regimen (the P-ATG group), while the other 30 patients didn't receive ATG (the Non-ATG group). The study was approved by the Ethics Committees of the Institute of Hematology, CAMS & PUMC according to the Guidelines of the Declaration of Helsinki.

In the P-ATG group, 16 patients were male, the rest 14 patients female, with a median age of 47 (33–61) years old. All 30 patients had malignant hematological diseases, 21 patients reached bone marrow complete remission (CR) before transplantation, and the other 9 patients did not reach CR. In the Non-ATG group, 16 patients were male, the rest 14 patients female, with a median age of 35 (18–53) years old. All 30 patients also suffered from malignant hematological

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diseases, 26 patients reached CR before transplantation, and the other 4 patients did not reach CR.

Some patients experienced infectious events before HSCT. In the P-ATG group, 8, 4 and 4 patients developed severe bacterial infection, invasive fungal disease (IFD) and viral infection, respectively. In the Non-ATG group, 7, 7 and 2 patients developed severe bacterial infection, IFD and viral infection, respectively. The infection was controlled in both groups before HSCT.

Donor

All 60 patients received HLA-A, B, C, DR and DQ-matched MSD-HSCT. In the P-ATG group, 16 donors were male, the rest 14 donors female, with a median age of 47 (34–58) years old. In the Non-ATG group, 11 donors were male, the rest 19 donors female, with a median age of 35 (17–58) years old.

Conditioning regimen

All patients received a myeloablative conditioning regimen. In the P-ATG group, P-ATG 20 mg/kg/day was used as a part of the conditioning regimen in -3, -2, -1 day. In the Non-ATG group, no ATG preparation was used in the conditioning regimen.

Post-transplant immunosuppression

GVHD prophylaxis regimen consisted of a short course of methotrexate (MTX) at a dose of 15 mg/m^2 intravenously on day 1 followed by 10 mg/m^2 intravenously on days 3 and 6, and CSA 2 mg/kg/day or tacrolimus (FK506) 0.03 mg/kg/day intravenously beginning day -1 in both groups.

Table 1. Patients' pre-transplant clinical characteristics.

	P-ATG	Non-ATG	*P*-value
Number of patients	30	30	
Gender			1.000
Male (%)	16 (53.3)	16 (53.3)	
Female (%)	14 (46.7)	14 (46.7)	
Age at treatment	47 (33–61)	35 (18–53)	<0.001
Years, median (range)			
Diagnosis, n. of patients (%)			0.590
ALL	7 (23.3)	6 (20.0)	
AML	16 (53.3)	13 (43.3)	
MDS	6 (20.0)	7 (23.3)	
Other	1 (3.3)	4 (13.3)	
Pre-transplant remission, n. of patients (%)			0.209
CR	21 (70.0)	26 (86.7)	
NR	9 (30.0)	4 (13.3)	
Infection before HSCT, n. of patients (%)			
Bacteremia	8 (26.7)	7 (23.3)	0.766
IFD	6 (20.0)	7 (23.3)	0.754
Viruses	4 (13.3)	2 (6.7)	0.671

Abbreviation: ATG, antithymocyte globulin; P-ATG, porcine ATG; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CR, complete remission; NR, no remission; HSCT, hematopoietic stem cell transplantation; IFD, invasive fungal disease.

Graft source

All patients underwent peripheral blood stem cell transplantation (PBSCT). In the P-ATG group, the median numbers of MNC and CD34$^+$ cells were 11.1 (8.0–21.7) $\times 10^8$/kg, 2.4 (1.4–4.6) $\times 10^6$/kg, respectively. In the Non-ATG group, the median numbers of MNC and CD34$^+$ cells were 10.1 (6.4–27.7) $\times 10^8$/kg, 2.5 (1.7–5.7) $\times 10^6$/kg, respectively.

Patient routine supportive care

All patients resided in class 100 laminar flow ward, receiving SMZco 1 g twice daily for one week to prevent pneumocystis carinii pneumonia, and ganciclovir 10 mg/kg/day intravenously for one week to prevent cytomegalovirus (CMV) infection before transplantation. Prevention of fungal infections in the patients who hadn't been diagnosed with IFD before transplantation was applied by voriconazole or posaconazole until 3 months after transplantation. The other patients who had IFD before transplantation received voriconazole, posaconazole or caspofungin according to their individual pre-transplant situations.

Engraftment standard

Neutrophil (ANC) recovery was defined as achieving an absolute ANC count of $\geq 0.5 \times 10^9$/L for 3 consecutive days and platelet (PLT) recovery as achieving a PLT count $\geq 20 \times 10^9$/L, unsupported by transfusion for 7 days. Chimerism analysis was done using STR.

Outcome analysis standard

Diagnosing and grading of aGVHD were based on the Seattle diagnostic criteria. The 2014 NIH consensus of cGVHD [8] was used to diagnose and grade cGVHD. Recurrence after HSCT is defined as the proportion of primitive or immature cells in bone marrow greater than 5%. CMV viremia was defined as positive results of RT-PCR (1×10^3copies/mL) in the blood. We defined IFD according to the revised EORTC/MSG criteria [9]. Severe bacterial infections were defined as bacteremia and severe tissue infections.

Survival analysis

The last follow-up for all surviving patients was on 28 February 2022. SAS 9.4 software was used to analyze the survival rate and the survival curve by the Kaplan–Meier method. Survival differences between groups were compared by the Log-Rank test. Patient, disease and transplant-related characteristics were compared using the Chi-square test and Rank sum test. The final model of significance attained a *P*-value of ≤ 0.05.

Results

Pre-transplant clinical characteristics

The pre-transplant clinical characteristics of the two groups are shown in Table 1.

Treatment regimens

The treatment regimens of the two groups are shown in Table 2.

Engraftment

All patients were engrafted in two groups. In the P-ATG group, the median periods for ANC and PLT recovery were 12 (9–16) and 15 (8–38) days, respectively. In the Non-ATG group, the median durations for ANC and PLT recovery were 12 (11–20) and 13 (10–23) days, respectively.

Graft versus host disease

In the P-ATG group, 7 patients developed aGVHD, 2 out of 7 patients had grade I aGVHD, and 5 patients had grade II–IV aGVHD. In the Non-ATG group, 15 patients developed aGVHD, 3 out of 15 patients had grade I aGVHD, and 12 patients had grade II–IV aGVHD. All patients responded to a combination of immunosuppressive agents and steroids, no one died of severe aGVHD. There was significant difference in aGVHD ([23.3 (10.1–39.7)] % vs [50.0 (30.8–66.5)] %, $P = 0.028$) and grade II–IV aGVHD ([16.7 (5.94–32.1)] % vs [40.0 (22.4–57.0)] %, $P = 0.049$) between the two groups, respectively.

In the P-ATG group, 4 patients developed cGVHD, and 1 patient had mild-moderate cGVHD. In the Non-ATG group, 16 patients developed cGVHD, and 6 patients had mild-moderate cGVHD. CGVHD was controlled in all patients by immunosuppressive agents. There was significant difference in cGVHD ([22.4 (6.03–45.1)] % vs [69.0 (43.4–84.8)] %, $P = 0.001$) between the two groups. There was no significant difference in terms of moderate-severe cGVHD between the two groups ([12.5 (1.67–34.8)] % vs [25.9 (9.82–45.6)] %, $P = 0.129$).

The incidence of GVHD in the two groups is shown in Figure 1.

Table 2. Treatment details.

	P-ATG	Non-ATG	*P*-value
Number of patients	30	30	
Donor	MSD	MSD	
Donor gender			0.195
Male (%)	16 (53.3)	11 (36.7)	
Female (%)	14 (46.7)	19 (63.3)	
Donor age	47 (34-58)	35 (17-58)	<0.001
Years, median (range)			
Numbers of infusion PBSC MNC	11.1 (8.0-21.7)	10.1 (6.4-27.7)	0.490
median (range) $\times 10^8$/kg $CD34^+$ cells median (range) $\times 10^6$/kg	2.4 (1.4-4.6)	2.5 (1.7, 5.7)	0.375

Abbreviations: ATG, anti-thymocyte globulin; P-ATG, porcine ATG; MSD, HLA-matched siblings; PBSC, peripheral blood stem cell; MNC, mononuclear cells.

Relapse

In the P-ATG group, 3 patients developed relapse, including 2 patients of extramedullary relapse and 1 patient of bone marrow relapse. In the non-ATG group 4 patients developed relapse, including 2 patients of extramedullary relapse and 2 patients of bone marrow relapse. There was no significant difference in relapse rate (RR) between the two groups ([13.6 (2.73–33.2)] % vs [15.2 (4.40–32.1)] %, $P = 0.742$). Except for 1 patient in the P-ATG group 2 patients in the Non-ATG group gave up treatment and died after relapse, the other patients did not die of relapse directly.

Infection

In the P-ATG group, bacteremia occurred in 3 patients, and pulmonary bacterial infection occurred in 3 patients. These 6 patients recovered after the use of antibacterial therapy. IFD occurred in 7 patients, all of whom had pulmonary infections. The pulmonary IFD was not successfully controlled by antifungal therapy in 3 patients, and they died. The other patients with IFD were successfully treated with antifungal therapy. There were 7 patients with CMV infection. CMV viremia occurred in 5 patients, including 2 patients with CMV enteritis and 1 patient with CMV encephalitis, and the patient with CMV encephalitis died, the other patients with CMV viremia were controlled by antiviral and globulin therapy. Another 2 patients had simple CMV enteritis, 1 died after treatment and 1 survived. Five patients in the P-ATG group developed CMV disease, of which four ultimately died.

In the Non-ATG group, bacteremia occurred in 1 patient, 1 patient soft tissue bacterial infection, and 1 patient intestinal bacterial infection. The patient's intestinal bacterial infection was not successfully treated with antibacterial therapy and finally died. IFD occurred in 6 patients, all of whom had pulmonary infections. All patients with IFD were successfully treated with antifungal therapy. CMV viremia occurred in 4 patients, no patients with CMV disease, and all patients were successfully treated with antiviral and globulin therapy.

There was no significant difference in terms of incidence of severe bacterial infections ($P = 0.472$), IFD ($P = 1.000$) or CMV infection ($P = 0.298$) between the two groups.

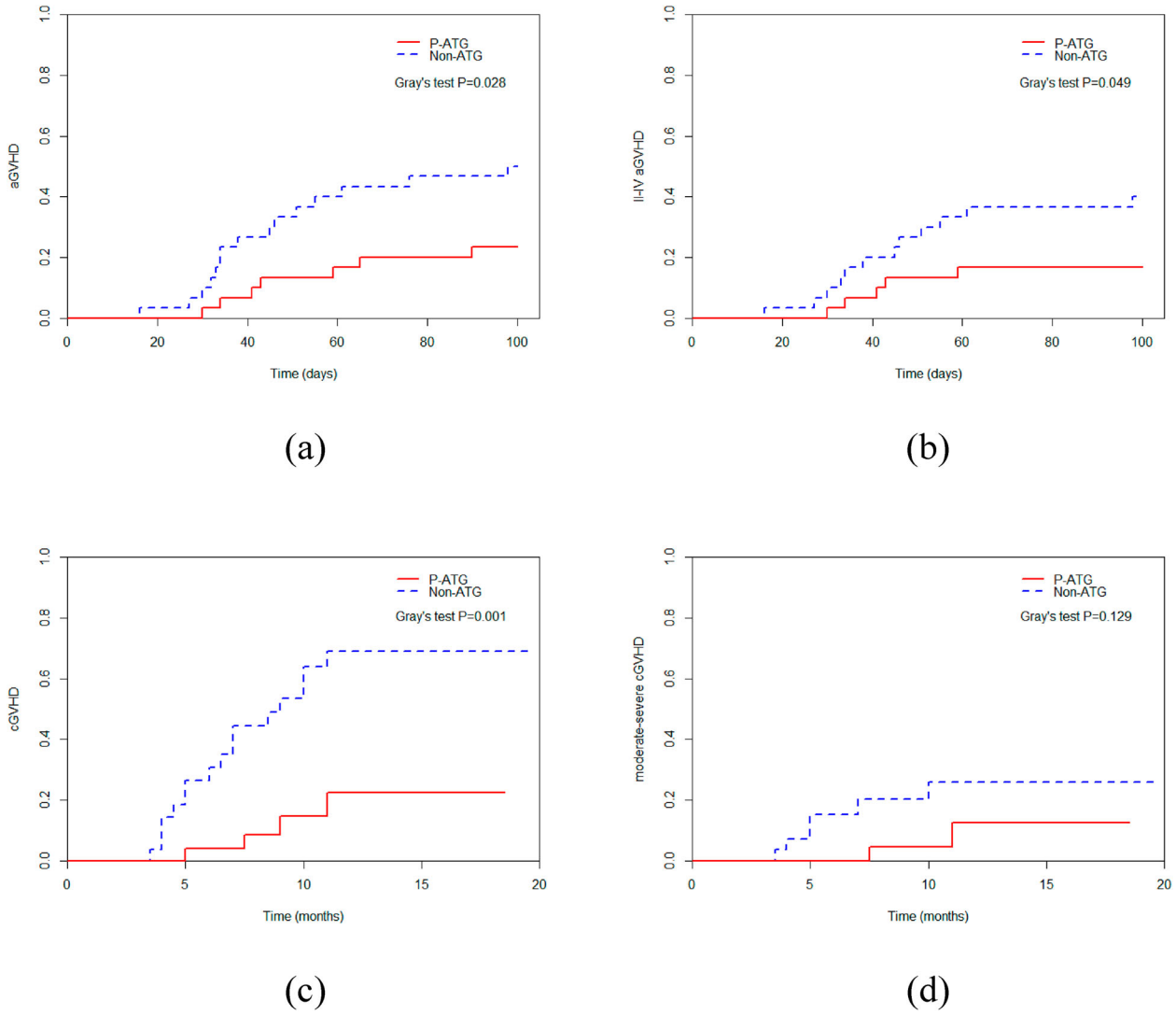

Figure 1. Incidence of GVHD in the two groups (a) aGVHD. (b) III–IV aGVHD. (c) cGVHD. (d) moderate-severe cGVHD.

Deaths

In all, 6 patients died in the P-ATG group, 3 with pulmonary IFD, the others account for giving up treatment after relapse, CMV encephalitis, and gastrointestinal bleeding caused by CMV enteritis. A total of 4 patients died in the Non-ATG group, 2 with relapse, and the other 2 patients with intestinal infection perforation, and intracranial hemorrhage, respectively. 80% (4/5) of patients in the P-ATG group died of a secondary infection after grade II–IV aGVHD, and 16.7% (2/12) in the Non-ATG group.

There was no significant difference in terms of the 1-year non-relapse mortality (NRM) between the two groups ([16.7 (5.95–32.1) %] vs [6.67 (1.14–19.5) %], $P = 0.237$).

Survival

In the P-ATG group, at a median follow-up of 10 (2–18.5) months, 24 patients were alive and the 1-year overall survival (OS) was 79.9 (60.5–90.4) %. In the Non-ATG group, at a median follow-up of 12.5 (2–20) months, 26 patients were alive and the 1-year OS was 84.0 (61.2–94.0) %. There was no significant difference in terms of the 1-year OS between the two groups ($P = 0.441$).

The outcomes of the two groups patients are shown in Table 3 and Figure 2.

Discussion

AGVHD and cGVHD are the leading causes of non-relapse mortality following allo-HSCT. The most consistently reported factors significantly associated with an increased risk of aGVHD were recipient HLA mismatching with the donor, alloimmunization of the donor, the use of a female donor for male recipients, and older patient age. For cGVHD, the most consistently reported risk factors include prior aGVHD, grafting with growth factor–mobilized blood cells, the use of a female donor for male recipients, older patient age, and mismatched and unrelated donors [10–12]. In vivo T-cell depletion using ATG-Fresenius (ATG-F), and rabbit ATG (R-ATG) has been shown to reduce

Table 3. The outcomes of the two groups' patients.

	P-ATG	Non-ATG	*P*-value
Number of patients	30	30	
Engraftment			
ANC, Days, median (range)	12 (9–16)	12 (11–20)	0.530
PLT, Days, median (range)	15 (8–38)	13 (10–23)	0.127
GVHD			
aGVHD (%)	23.3 (10.1–39.7)	50.0 (30.8–66.5)	0.028
Grade II–IV aGVHD (%)	16.7 (5.94–32.1)	40.0 (22.4–57.0)	0.049
cGVHD (%)	22.4 (6.03–45.1)	69.0 (43.4–84.8)	0.001
Moderate–severe cGVHD (%)	12.5 (1.67–34.8)	25.9 (9.82–45.6)	0.129
RR (%)	13.6 (2.73–33.2)	15.2 (4.40–32.1)	0.742
NRM (%)	16.7 (5.95–32.1)	6.67 (1.14–19.5)	0.237
OS (%)	79.9 (60.5–90.4)	84.0 (61.2–94.0)	0.441
Infection			
n. of patients (%)	6 (20.0)	3 (10.0)	0.472
Severe Bacterial infection	7 (23.3)	6 (20.0)	1.000
IFD	7(23.3)	4 (13.3)	0.298
CMV infection			

Abbreviations: ATG, anti-thymocyte globulin; P-ATG, porcine ATG; ANC, Neutrophil; PLT, platelet; GVHD, graft versus host disease; aGVHD, acute GVHD; cGVHD, chronic GVHD; RR, relapse rate; NRM, non-relapse mortality; DFS, disease-free survival; OS, overall survival; IFD, invasive fungal disease; CMV, cytomegalovirus.

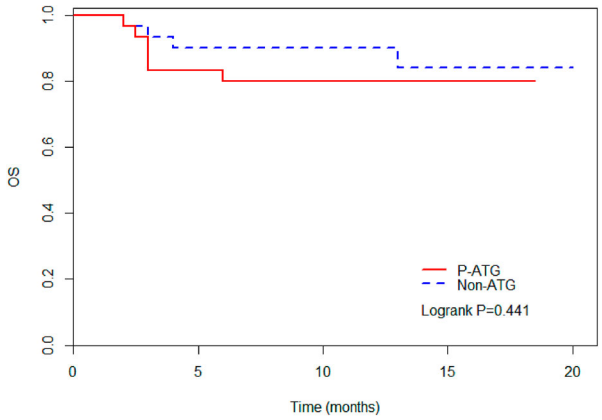

Figure 2. Comparison of 1-year survival rate of the two groups' patients.

the risks of aGVHD and cGVHD in unrelated HSCT [13,14]. Some researchers tried to add ATG preparations to a conditioning regimen in MSD-HSCT to reduce the incidence of GVHD: D-Y Shin et al. [15] demonstrated that a high dose (⩾4.5 mg/kg) of R-ATG reduces the risk of aGVHD following MSD-HSCT using busulfan (Bu) Fludarabine (Flu) conditioning with no significant effect on relapse-free survival (RFS) and OS. Kroger et al. [16] investigated the use of ATG-F at a dose of 10 mg/kg on 3, 2, and 1 days before the transplantation of MSD-HSCT in patients with acute leukemia (AML). The rate of grades II–IV aGVHD was 10.8%, the 2-year cumulative incidence of cGVHD was 32.2%, and the 2-year cumulative incidence of clinical extensive cGVHD was 7.6%. Liping Dou et al. [17] used low-dose R-ATG for GVHD prophylaxis in patients or donors aged ≥40 years with hematological malignancies receiving MSD-HSCT. R-ATG was administered to 40 patients at an intravenous dose of 5 mg/kg divided over day 5 and day 4 before graft infusion. The cumulative incidence of grades II–IV and grades III–IV aGVHD at day +100 was 30.0% and 2.6%, respectively. The 2-year cumulative incidence of extensive cGVHD and severe cGVHD was 11.4% and 14.7%. The 2-year cumulative incidence of transplant-related mortality (TRM) and relapse was 14.0% and 22.6%, respectively. The cumulative incidence of CMV reactivation, Epstein–Barr virus reactivation, and fungal infection was 22.3%, 12.9%, and 12.5%, respectively. Kaplan–Meier estimates for OS, disease-free survival (DFS), and GVHD-free and relapse-free survival 3 years after transplantation were 68.9%, 68.9%, and 54.0%, respectively. Thus, consensus-based recommendations by an international expert panel agreed that ATG should be recommended before MSD-HSCT to reduce the occurrence of GVHD [18].

P-ATG is another ATG preparation that is a new product developed in China. It is first applied to immunosuppressive therapy (IST) of severe aplastic anemia (SAA). Several studies have shown that P-ATG exhibited good therapeutic effects in SAA for IST [19]. Zhang FK et al. (data not yet published) showed that the half-life of P-ATG was about 15 days and effective serum concentration of P-ATG was maintained for at least 60 days in vivo. The drug metabolism curve of P-ATG had an advantage over R-ATG in IST, P-ATG therapy combined with cyclosporine A had significant long-term efficacy and high OS in SAA [20,21]. Subsequently, P-ATG was gradually applied in the conditioning regimens for SAA. The clinical data of 113 SAA patients who received MSD-HSCT from January 2005 to November 2016 in our center showed [22]: 58 patients received R-ATG as a part of the conditioning regimen (R-ATG group), whereas the other 55 patients received P-ATG (the P-ATG group). There was a significant difference in the incidence of aGVHD (20.7% ± 5.3% versus 43.4% ± 7.0%, $P = 0.015$) and cGVHD (20.1% ± 5.8% versus 46.0% ± 7.9%, $P = 0.003$) between the R-ATG and P-ATG groups. However, there was no significant difference in terms of 3-year OS (93.1% ± 3.3% versus 84.4% ± 5.7%, $P = 0.235$), grades III–IV aGVHD (3.4% ± 2.4% versus 12.3% ± 4.7%, $P = 0$ .098), moderate-severe cGVHD (12.6% ± 4.9% versus 11.5% ± 4.9%, $P = 0.905$), or graft rejection (GR) (7.4% ± 3.6% versus 5.5% ± 3.1%, $P = 0.852$). There was also no significant difference with regard to the incidence of severe bacterial infection ($P = 0.075$), IFD ($P = 0.701$), or CMV viremia ($P = 0.770$). P-ATG showed satisfactory efficacy and safety compared with R-ATG in the setting of MSD-HSCT for SAA patients. A total of 91 patients with SAA who received haploid HSCT (haplo-HSCT) in our center

between January 2014 and December 2020 were retrospectively reviewed [23]. 28 patients were in the P-ALG group while 63 patients were in the R-ATG group. There was no significant difference in 5-year OS (74.83% ± 8.24% vs 72.29% ± 6.26%, $P = 0.830$), GVHD-free, failure-free survival (71.05% ± 8.65% vs 62.71% ± 6.22%, $P = 0.662$), failure-free survival (74.83% ± 8.24% vs 66.09% ± 5.84%, $P = 0.647$) and TRM (25.17% ± 8.24% vs 26.29% ± 6.22%, $P = 0.708$) between the two groups. The incidence of aGVHD (65.39% ± 9.33% vs 62.71% ± 6.30%, $P = 0.653$), II–IV aGVHD (38.46% ± 9.54% vs 35.64% ± 6.24%, $P = 0.695$), III–IV aGVHD (19.23% ± 7.73% vs 10.53% ± 4.07%, $P = 0.291$), cGVHD (chronic graft versus host disease) (22.22% ± 12.25% vs 22.31% ± 6.30%, $P = 0.915$), and moderate-severe cGVHD (5.56% ± 5.40% vs 9.28% ± 4.46%, $P = 0.993$) was not significantly different. Similar outcomes were observed between the P-ALG and R-ATG groups for severe bacterial infection (17.9% vs 25.4%, $P = 0.431$), IFD (3.6% vs 9.5%, $P = 0.577$) and GR (0% vs 9.5%, $P = 0.218$). However, the incidence of CMV infection and Epstein–Barr virus infection was significantly lower in the P-ALG group (46.4% vs 71.4%, $P = 0.022$; 3.6% vs 25.4%, $P = 0.014$).

However, a few studies about P-ATG had been done on conditioning regimens for hematological malignancy. We conducted this retrospective analysis to compare the incidence of GVHD in the P-ATG group and the Non-ATG group in patients/donors over 40 years old or female donors undergoing MSD-HSCT for hematological malignancy. In our study, in addition to the fact that the ages of the patients and donors were older in the P-ATG group, all patients' other baseline characteristics and donor conditions of the two groups were similar. The results showed that all patients were engrafted in two groups. There was significant difference in aGVHD ([23.3 (10.1–39.7)] % vs [50.0 (30.8–66.5)] %, $P = 0.028$), grade II–IV aGVHD ([16.7 (5.94–32.1)] % vs [40.0 (22.4–57.0)] %, $P = 0.049$), cGVHD ([22.4 (6.03–45.1)] % vs [69.0 (43.4–84.8)] %, $P = 0.001$). There was no significant difference in terms of moderate-severe cGVHD ([12.5 (1.67–34.8)] % vs [25.9 (9.82–45.6)] %, $P = 0.129$), 1-year RR ([13.6 (2.73–33.2)] % vs [15.2 (4.40–32.1)] %, $P = 0.742$), NRM ([16.7 (5.95–32.1)] % vs [6.67 (1.14–19.5)] %, $P = 0.237$), or OS ([79.9 (60.5–90.4)] % vs [84.0 (61.2–94.0)] %, $P = 0.441$). There was also no significant difference with regard to the incidence of severe bacterial infections ($P = 0.472$), IFD ($P = 1.000$) or CMV infection ($P = 0.298$) between the two groups. In our study, the ages of the patients were older ($P < 0.001$) in the P-ATG group. 80% (4/5) of patients in the P-ATG group died of a secondary infection after grade II–IV aGVHD, and 16.7% (2/12) in the Non-ATG group. The risk of death was significantly higher caused by a secondary infection after grade II–IV aGVHD. In addition, Jhong-Lin Wu et al. As reported while P-ATG reduced the incidence of GVHD, its long half-life may cause delayed immune reconstitution, particularly delayed cluster of differentiation (CD)4+ T-cell reconstitution, which increases the risk of CMV infection [24]. Although there was no significant difference in terms of incidence of CMV infection between the two groups ($P = 0.298$) in our research, the incidence of CMV disease with poor prognosis was significantly increased in the P-ATG group. Five patients in the P-ATG group developed CMV disease, of which four ultimately died, while no one in the Non-ATG group developed CMV disease. So it may be the reason for a little high NRM and slightly inferior OS in the P-ATG group. It also indicates that older patients with II–IV aGVHD after MSD-HSCT need strict monitoring of infection and early prevention of CMV to reduce the mortality of subsequent secondary infection and occurrence of severe CMV disease. Subsequently, it is also necessary to expand the sample size to clarify whether there are differences in NRM and OS between the two groups.

In summary, the application of low-dose P-ATG in patients/donors over 40 years old or female donors undergoing MSD-HSCT for hematological malignancy can significantly reduce the incidence of aGVHD, grade II–IV aGVHD and cGVHD, doesn't increase the risk of relapse. However, this report was a retrospective analysis and the sample size of each group was relatively small, our results should be considered preliminary. Further studies with larger cohorts and longer follow-up periods are needed to derive more conclusive findings.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

Data are available on request from the authors.

Author contributions

Xin Chen performed the data analyses and wrote the manuscript; Erlie Jiang guided article ideas and writing; Rongli Zhang, Weihua Zhai, Qiaoling Ma, Aiming Pang, Donglin Yang, Jialin Wei, Yi He collected data;Yueshen Ma, Zhen Song carried out statistical analysis; Sizhou Feng, Mingzhe Han helped perform the analysis with constructive discussions.

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ORIGINAL PAPER

Transplantation

Effectiveness of in vivo T-cell-depleted regimen containing porcine anti-lymphocyte globulin or rabbit anti-thymocyte globulin in preventing acute graft-versus-host disease after haploidentical haematopoietic stem cell transplantation

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Summary

To compare the clinical efficacy of porcine anti-lymphocyte globulin (p-ALG) and rabbit anti-thymocyte globulin (r-ATG) in the treatment of haematological malignancies using haploidentical haematopoietic stem cell transplantation (haplo-HSCT), this study was conducted. The incidences of neutrophil and platelet engraftment, respectively, were 100%, 93.6% and 94.4%; 100%, 93.6% and 90.3% in p-ALG 75 mg/kg ($n = 57$), p-ALG 90 mg/kg ($n = 49$), and r-ATG 7.5 mg/kg ($n = 72$). The median time to neutrophil engraftment and platelet engraftment were 11, 12 and 12 days ($p = 0.032$); 13, 14 and 13 days ($p = 0.013$), respectively. The incidence of grades II–IV acute graft-versus-host disease and cumulative incidence of chronic graft-versus-host disease were 16.7% versus 12.5% versus 13.3% ($p = 0.817$) and 14.7% versus 12.1% versus 19.5% in p-ALG 75 mg/kg, p-ALG 90 mg/kg and r-ATG groups. Notably, the cytomegalovirus infection rate in the p-ALG 75 mg/kg group was significantly lower than the other two groups. The cumulative incidence of 2-year relapse and 2-year overall survival rates were similar ($p = 0.901$, $p = 0.497$). The lower dose of p-ALG (75 mg/kg) had a similar efficacy and safety profile compared with r-ATG (7.5 mg/kg) in the setting of haplo-HSCT. Therefore, p-ALG (75 mg/kg) may be an appropriate alternative to r-ATG in the conditioning regimen of haplo-HSCT.

KEYWORDS
graft-versus-host disease, haploidentical haematopoietic cell transplantation, infection, porcine anti-lymphocyte globulin, rabbit anti-thymocyte globulin

INTRODUCTION

Haploidentical haematopoietic stem cell transplantation (haplo-HSCT) is a potential treatment for patients with haematological malignancies who do not have a suitable HLA-matched donor.[1] In recent decades, haplo-HSCT has achieved vigorous development and become the conventional therapy.[2,3] 'Beijing Protocol' and 'Baltimore Protocol' are the major regimens used in haplo-HSCT.[4–6] In Asian countries, the 'Beijing Protocol' is widely utilized for haplo-HSCT with myeloablative conditioning (MAC) regimens. It involves the use of granulocyte colony-stimulating factor (G-CSF) mobilized bone marrow or peripheral blood stem cells (PBSCs), as well as anti-thymocyte globulin (ATG) to deplete T cells in vivo.[4,5,7]

ATG plays a critical role in the 'Beijing protocol' for preventing graft-versus-host disease (GVHD) and promoting engraftment.[8] ATG is associated with delayed immune reconstitution and a higher incidence of infections, particularly viral diseases, when administered at high doses.[8]

However, the potential impact of ATG dosage on relapse remains unclear. Finding the right balance between risk and benefit and determining the optimal dosage of ATG is currently an urgent problem that needs to be solved.

The rabbit anti-thymocyte immunoglobulin (r-ATG; Sanofi, Paris, France) and the anti-human T lymphocyte porcine immunoglobulin (p-ALG; Wuhan Institute of Biological Products, Wuhan, China) are available in China. p-ALG was mainly used in combination with ciclosporin as a standard immunosuppressive regimen for severe aplastic anaemia (SAA), but it was rarely reported in haplo-HSCT.

In this study, we performed a retrospective study to evaluate and compare the effectiveness of two different dosages of p-ALG (90 or 75 mg/kg) and r-ATG (7.5 mg/kg) in a relatively homogenous population undergoing haplo-HSCT with T cell–deplete in vivo conditions. All patients received MAC, were treated with the same GVHD prophylactic strategy and received grafts from the same source.

PATIENTS AND METHODS

Study design

From January 2020 to December 2022, a retrospective analysis was conducted on the clinical data of 187 patients who underwent T-cell deplete in vivo, related-donor haplo-HSCT at the Union Hospital of Huazhong University of Science and Technology. Nine patients were excluded due to insufficient follow-up. All patients received p-ALG ($n = 106$) or r-ATG ($n = 72$) as GVHD prophylaxis, in addition to a MAC based on busulfan and cyclophosphamide (Cy). The study was approved by the institutional review board at the Union Hospital of Huazhong University of Science and Technology, and all patients or their guardians submitted consent forms before transplantation. The World Health Organization's classification of haematological malignancies was applied.[9] Patients with severe comorbidities or active infections were not eligible for transplantation. Patients who had undergone previous allogeneic transplantation were excluded. Demographic and disease-specific characteristics were summarized in Table 1. Follow-up for all patients went through 1 July 2023.

Transplant procedure

All patients in this study received PBSCs as the sole source of stem cells. Donor PBSCs were collected using standard mobilization protocols. The donors were administrated subcutaneously with recombinant human G-CSF at doses of 8–10 μg/kg/day for five consecutive days. PBSCs were harvested on Days 4 and 5 and infused without manipulation through a central venous catheter on the same day. The median number of $CD34^+$ cells infused was 7.32×10^6/kg, ranging from 1.81 to 20.46×10^6/kg, and the median number of nucleated cells infused was 14.59×10^8/kg, ranging from 6.49 to 31.26×10^8/kg.

Conditioning regimens

All the patients underwent a MAC, which was divided into two categories according to the disease type. Patients with acute myeloid leukaemia (AML) or myelodysplastic syndrome received the following conditioning regimen: an intravenous injection of cytarabine (Ara-c) at a dosage of 4 g/m^2 on Day −7, followed by an intravenous injection of busulfan (BU) at a dosage of 3.2 mg/kg per day from Days −6 to −4 and an intravenous injection of Cy at a dosage of 1.8 g/m^2 per day from Days −3 to −2. Additional intravenous injections of decitabine (20 mg/m^2 daily on Days −11 to −7) were given to relapsed and refractory AML patients. The conditioning regimen for acute lymphoblastic leukaemia (ALL) patients were as follows: BU (3.2 mg/kg daily, on Days −9 to −7), Cy (1.8 g/m^2 once daily, on Days −3 to −2) and etoposide (VP-16, 5 mg/kg daily, on Days −7 to −5). Hyperhydration and alkalization were simultaneously administered to prevent haemorrhagic cystitis from Days −3 until the completion of the Cy infusion.

GVHD prophylaxis

All patients received uniform GVHD prophylaxis with tacrolimus, short-term methotrexate (MTX), mycophenolate mofetil, anti-CD25 MoAb (basiliximab) and p-ALG or ATG. p-ALG was administered intravenously at a dose of 30 25 mg/kg daily from Days −4 to −2. r-ATG was intravenously at a dose of 7.5 mg/kg from Days −4 to −2. The recommended oral dose of tacrolimus is 0.05 mg/kg administered twice daily, starting from Day −1. The blood levels of tacrolimus should be maintained between 5 and 10 ng/mL until Day +60, followed by a gradual reduction of 5% per week. MTX was administered intravenously at dosages of 20 mg/m^2 on Day +1 and 15 mg/m^2 on Days +3, +6 and +11. Mycophenolate mofetil (1000 mg/day, orally twice a day) was started on Day +7 and was discontinued around Day +60 if the patient was engrafted and free of acute GVHD (aGVHD). Basiliximab was administered intravenously at a dose of 20 mg on Days 0 and +4. aGVHD and chronic GVHD (cGVHD) were evaluated according to the consensus criteria.[10,11]

Support care

To prevent epilepsy, phenytoin (100 mg) was administered orally three times daily from the beginning to the end of the conditioning regimen. All the patients received prophylactic antibiotics: ganciclovir 5 mg/kg twice daily from Day −10 to −2, moxifloxacin 400 mg per day and oral fluconazole from Day −10 until engraftment, as previously reported.[12] Cotrimoxazole was used twice daily for pneumocystis prophylaxis until 6 months post-transplantation. Monitoring of cytomegalovirus (CMV) and Epstein–Barr virus (EBV) DNA by qPCR in blood was performed twice weekly, starting from neutrophil recovery and continuing

TABLE 1 Clinical characteristics of the patients in the three groups.

Characteristic	p-ALG75 mg/kg ($n=57$)	p-ALG 90 mg/kg ($n=49$)	r-ATG 7.5 mg/kg ($n=72$)	p
Age, year, median (range)	33 (15–61)	32 (13–58)	34 (14–56)	0.513
Man gender, *n* (%)	32 (56.1)	26 (53.1)	37 (51.4)	0.864
Remission at transplantation, *n* (%)				0.529
Complete remission	52 (91.2)	43 (87.8)	61 (84.7)	
Incomplete remission	5 (8.8)	6 (12.2)	11 (15.3)	
Diagnosis, *n* (%)				0.869
AML	34 (59.6)	31 (63.3)	43 (59.7)	
ALL	19 (33.3)	15 (30.6)	21 (29.2)	
Others	4 (7.0)	3 (6.1)	8 (11.1)	
Conditioning regime, *n* (%)				0.778
Ara-c + BUCY	24 (36.4)	17 (34.7)	25 (34.7)	
Dec + Ara-c + BUCY	18 (31.6)	21 (42.9)	27 (37.5)	
VP-16 + BUCY	15 (26.3)	11 (22.4)	20 (27.8)	
DRI, *n* (%)				0.674
High + very high	41 (71.9)	35 (71.4)	47 (65.3)	
Low + intermediate	16 (28.1)	14 (28.6)	25 (34.7)	
Time from diagnosis to HCT, *n* (%)				0.477
<6 Months	26 (45.6)	21 (42.8)	28 (38.9)	
6–12 Months	23 (40.4)	19 (38.8)	33 (45.8)	
>12 Months	8 (14.0)	9 (18.4)	11 (15.3)	
Sex of donors, *n* (%)				0.909
Male	41 (71.9)	37 (75.5)	53 (73.6)	
Female	16 (28.1)	12 (24.5)	19 (26.4)	
Cytomegalovirus donor/recipient, *n* (%)				0.984
D^-/R^-	8 (14.0)	6 (12.2)	8 (11.1)	
D^-/R^+	14 (24.6)	10 (20.4)	14 (19.4)	
D^+/R^-	9 (15.8)	9 (18.4)	12 (16.7)	
D^+/R^+	26 (45.6)	24 (49.0)	38 (52.8)	
Donor/recipient sex match, *n* (%)				0.317
Match	28 (49.1)	19 (38.8)	34 (47.2)	
Mismatch	29 (50.9)	30 (61.2)	38 (52.8)	
ABO mismatch, *n* (%)				0.353
Match	33 (57.9)	29 (59.2)	34 (47.2)	
Mismatch	24 (42.1)	20 (40.8)	38 (52.8)	
Median $CD^{34+} \times 10^6$/kg (range)	7.40 (2.07–15.50)	6.70 (2.28–20.46)	7.40 (2.07–15.50)	0.138
Median TNC $\times 10^8$/kg (range)	13.69 (6.49–27.30)	14.06 (6.80–24.50)	13.69 (6.49–27.30)	0.327

Abbreviations: ALL, acute lymphoid leukaemia; AML, acute myeloid leukaemia; Ara-c, cytarabine; BUCY, busulfan, cyclophosphamide; Dec, decitabine; DRI, disease risk index; p-ALG, porcine anti-lymphocyte globulin; r-ATG, rabbit anti-thymocyte globulin; VP-16, etoposide.

until Day +100. CMV reactivation was defined by positive CMV DNA detection consecutively in two blood samples.[13] Patients with CMV reactivation were treated with pre-emptive ganciclovir or foscarnet. EBV reactivation was defined as EBV DNA copies $\geq 1 \times 10^4$/mL or $\geq 1 \times 10^3$/mL in two consecutive tests.[14] Invasive fungal diseases (IFD) were confirmed by the classification proposed by the Infectious Disease Society of America 2016 update.[15] Cytokine release syndrome (CRS) was defined and graded using the criteria described by Lee et al.[16]

Engraftment and chimerism assessment

Neutrophil engraftment was defined as the first occurrence of three consecutive days with an absolute neutrophil count

(ANC) $\geq 0.5 \times 10^9$/L. Similarly, platelet engraftment was defined as the first day with a platelet count $\geq 20 \times 10^9$/L for seven consecutive days without transfusion. Graft failure was defined as failure to attain neutrophil recovery within 28 days of the graft infusion. Graft rejection was defined when ANC cannot reach the engraftment criteria after transplantation or loses initial engraftment with minimal (<5%) chimerism or complete recipient chimerism. In order to assess chimerism, fluorescein in situ hybridization was used in sex-mismatched donor–recipient pairs every month for 6 months post haplo-HSCT. When the patient and donor were sex-matched, chimerism assessment was analysed by PCR-based analyses of polymorphic minisatellite or microsatellite regions (short tandem repeats). Complete donor chimerism was determined when only the haematopoietic cells of the donor type were present after transplantation.

Statistical analysis

Patients' baseline characteristics were summarized using frequencies with percentages for categorical variables and medians with ranges for continuous outcomes. Continuous variables were tested using the Mann–Whitney *U*-test, and categorical data were tested using the chi-square test or Fisher exact test. The disease risk index was defined and calculated based on established definitions.[17] Overall survival (OS) was defined as the time interval from transplantation until death from any cause, and surviving patients were censored at the last contact. Relapse was a competing event, defined by haematological, cytogenetic or molecular criteria. Non-relapse mortality (NRM) was defined as death without previous evidence of disease relapse. Disease-free survival (DFS) was defined as the time from transplantation to either death or disease relapse. Patients alive and relapse-free were censored at the last follow-up. For GVHD, death without the event was considered a competing event. Gray's test was used to analyse engraftment, infection, GVHD and NRM in the three groups. DFS and OS were calculated according to the Kaplan–Meier method, and cohorts were compared by log-rank test. All *p*-values were two-sided, and $p < 0.05$ was considered statistically significant. Statistical analyses were performed with SPSS v26.0 and R v 3.5.2.

RESULTS

Patients characteristics

A total of 178 patients were enrolled in the study with a median age of 30 (range, 14–61) years, including 95 men and 83 women. One hundred and six patients (59.6%) received p-ALG, while 72 patients (40.4%) received ATG in total. The clinical characteristics of the three groups were described in Table 1. Among the patients, 87.6% were in remission and 12.4% had active disease. AML was the most common diagnosis, accounting for 60.7% of cases, followed by ALL (30.9%) and others (8.4%).

Engraftment

The incidences of neutrophil and platelet engraftment, respectively, were 100%, 93.6% and 94.4% ($p = 0.133$); 100%, 93.6% and 90.3% ($p = 0.033$) in p-ALG 75 mg/kg, p-ALG 90 mg/kg and r-ATG 7.5 mg/kg (Table 2). The median time to neutrophil engraftment was 11 days for the p-ALG 75 mg/kg group, 12 days for the p-ALG 90 mg/kg group and 12 days for the r-ATG 7.5 mg/kg group ($p = 0.032$), showed in Figure 1A. The median time to platelet engraftment was 13 days for the p-ALG 75 mg/kg group, 14 days for the p-ALG 90 mg/kg group and 13 days for the r-ATG 7.5 mg/kg group ($p = 0.013$), showed in Figure 1B.

Early fever

Within 14 days after transplantation, fever occurred in 16 patients in the p-ALG 75 mg/kg group, 24 patients in the p-ALG 90 mg/kg group and 35 patients in the r-ATG group (Table 2). The main cause of early fever in all patients was CRS. In the p-ALG 90 mg/kg group and the r-ATG group, 13.2% and 20.8% of patients, respectively, suffered from infection within 14 days after transplantation. In addition, there were two cases of engraftment syndrome in both the p-ALG 90 mg/kg group and the r-ATG group.

aGVHD and cGVHD

The results of Figure 2A indicated that the cumulative incidence of aGVHD on Day 100 was parallel among the groups receiving p-ALG at 75 mg/kg, p-ALG at 90 mg/kg and r-ATG at 7.5 mg/kg, with percentages of 29.8%, 26.5% and 32.4% respectively ($p = 0.874$). The incidence of grades II–IV aGVHD and grades III–IV aGVHD, respectively, were 16.7%, 12.5% and 13.3% ($p = 0.817$); 6.0%, 4.4% and 5.9% ($p = 0.939$) in the p-ALG 75 mg/kg, p-ALG 90 mg/kg and r-ATG groups. The cumulative incidence of cGVHD in the p-ALG 75 mg/kg, p-ALG 90 mg/kg and r-ATG 7.5 mg/kg groups were 14.7%, 12.1% and 19.5% respectively.

Infections

Within 180 days after transplantation, 105 patients experienced infections. The group that received p-ALG at a dose of 75 mg/kg had the lowest infection rate, while the group that received p-ALG at a dose of 90 mg/kg had the highest infection rate, showed in Table 2. Among all infections, CMV reactivation was the most common. The rates of CMV reactivation in the p-ALG 75 mg/kg group, p-ALG 90 mg/kg group

TABLE 2 Clinical outcomes in the three groups.

Characteristic	p-ALG 75 mg/kg (*n* = 57)	p-ALG 90 mg/kg (*n* = 49)	r-ATG 7.5 mg/kg (*n* = 72)	*p*
Neutrophil	57 (100)	46 (93.6)	68 (94.4)	0.133
Platelet	57 (100)	46 (93.6)	65 (90.3)	0.033
ANC, median (range), days	11 (7–21)	12 (8–19)	12 (8–19)	0.032
Platelet, median (range), days	13 (7–31)	14 (7–43)	13 (9–38)	0.013
Early fever	16 (28.1)	24 (49.0)	35 (48.6)	0.18
CRS	10 (17.5)	14 (28.6)	18 (25.0)	
Infections	6 (10.5)	8 (16.3)	15 (20.8)	
Engraftment syndrome	0	2 (4.2)	2 (2.8)	
Outcomes				0.5
Alive, *n* (%)	45 (78.9)	35 (71.4)	56 (77.8)	
Dead, *n* (%)	12 (21.1)	14 (28.6)	16 (22.2)	
Cause of death				0.975
Relapse, *n* (%)	6 (10.5)	6 (12.2)	8 (11.1)	
TRM, *n* (%)	6 (10.5)	7 (14.3)	8 (11.1)	
aGVHD, *n* (%)	17 (29.8)	13 (26.5)	23 (32.4)	0.874
Grades II–IV, *n* (%)	9 (16.7)	6 (12.5)	9 (13.3)	0.817
Grades III–IV, *n* (%)	3 (6.0)	2 (4.4)	4 (5.9)	0.939
cGVHD, *n* (%)	8 (14.7)	5 (12.1)	11 (19.5)	0.393
Relapse, *n* (%)	18 (31.6)	13 (26.5)	17 (23.6)	0.901
AML, *n* (%)	9 (15.8)	8 (16.3)	13 (18.1)	
ALL, *n* (%)	8 (14.0)	5 (10.2)	4 (5.6)	
Others, *n* (%)	1 (1.8)	0	0	
Infection, *n* (%)				
EBV	25 (43.9)	35 (71.4)	45 (62.5)	0.012
CMV	11 (19.3)	11 (22.4)	15 (20.8)	0.972
Bacteria	17 (29.8)	28 (57.1)	35 (48.6)	0.013
Fungi	7 (12.3)	16 (32.7)	18 (25.0)	0.039

Abbreviations: aGVHD, acute graft-versus-host disease; ALL, acute lymphoid leukaemia; AML, acute myeloid leukaemia; ANC, absolute neutrophil count; cGVHD, chronic graft-versus-host disease; CRS, cytokine release syndrome; p-ALG, porcine anti-lymphocyte globulin; r-ATG, rabbit anti-thymocyte globulin; RM, transplantation-related mortality.

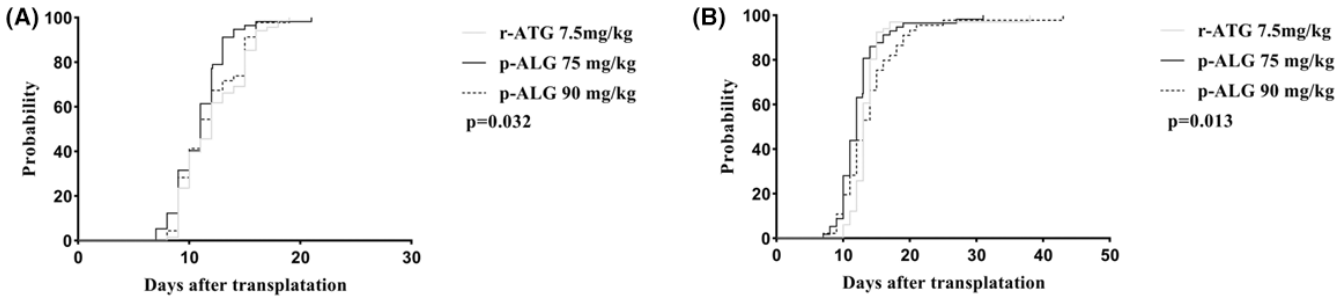

FIGURE 1 Cumulative incidence of neutrophil and platelet engraftment (A) neutrophil. (B) Platelet.

and r-ATG 7.5 mg/kg group were 29.8%, 57.1% and 48.6% respectively ($p = 0.013$). Additionally, 11 patients (19.3%) in the p-ALG 75 mg/kg group, 11 patients (22.4%) in the p-ALG 90 mg/kg group and 15 patients (20.8%) in the r-ATG 7.5 mg/kg group had EBV infections.

Survival and relapse mortality

Out of 178 patients, 41 died within a median follow-up time of 20.1 months after transplantation (range, 1.7–36.9 months). The OS rates were similar in the three groups ($p = 0.497$)

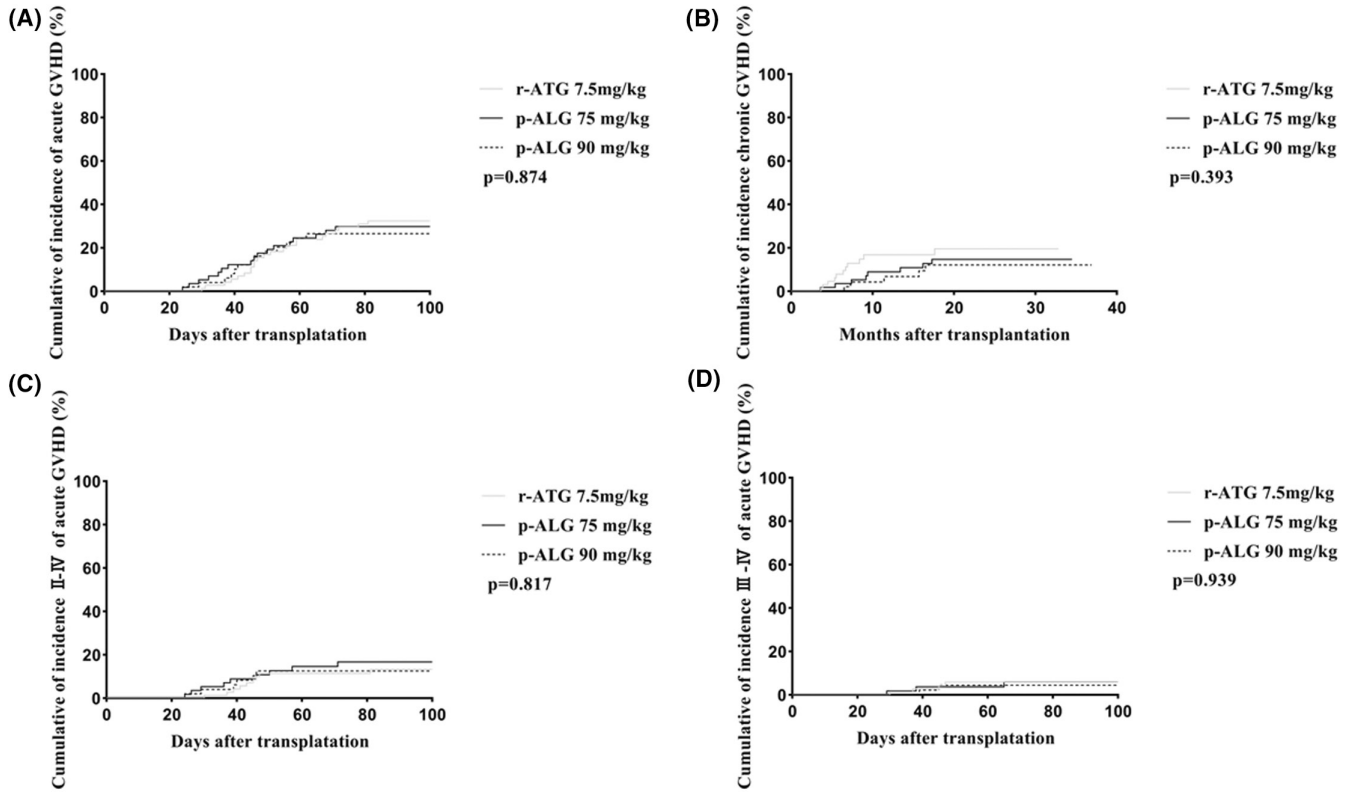

FIGURE 2 Cumulative incidence of graft-versus-host disease (GVHD). (A) Acute GVHD. (B) Chronic GVHD. (C) Grades II–IV acute GVHD. (D) Grades III–IV acute GVHD.

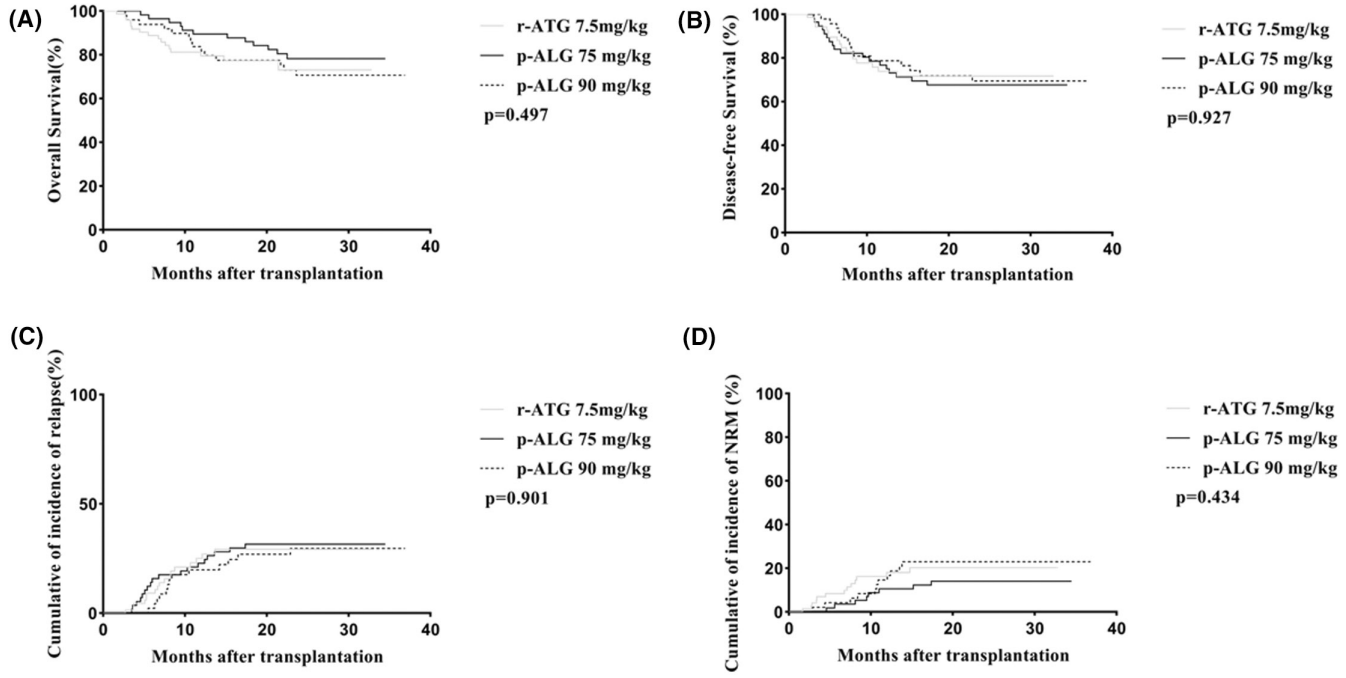

FIGURE 3 Kaplan–Meier curves (A) Overall survival. (B) Disease-free survival. (C) Relapse. (D) Non-relapse mortality (NRM).

(Figure 3A). Specifically, the 2-year OS rates were 78.2%, 70.6% and 73.0% in the p-ALG 75 mg/kg, p-ALG 90 mg/kg and r-ATG 7.5 mg/kg groups respectively. Disease relapse was observed in 18 patients in the p-ALG 75 mg/kg group, 13 patients in the p-ALG 90 mg/kg group and 17 patients in the r-ATG 7.5 mg/kg group during the final follow-up (Table 2).

TABLE 3 The cause of death between patients with p-ALG and r-ATG.

Patients	Total	Cause of death (*n*, %)				
		Relapse	Rejection	Infection	GVHD	Others
p-ALG 75 mg/kg	57	6 (10.5)	0	2 (3.5)	3 (5.3)	1 (1.8)
p-ALG 90 mg/kg	49	6 (12.2)	3 (6.1)	3 (6.1)	1 (2.0)	0
r-ATG 7.5 mg/kg	72	8 (11.1)	4 (5.6)	2 (2.8)	2 (2.8)	0

Abbreviations: GVHD, graft-versus-host disease; p-ALG, porcine anti-lymphocyte globulin; r-ATG, rabbit anti-thymocyte globulin.

Among the relapsed patients, 30 (62.5%) were diagnosed with AML, while 17 (35.4%) had ALL. The cumulative incidence of 2-year relapse rate was 31.6%, 29.6% and 29.2% in the p-ALG 75 mg/kg, p-ALG 90 mg/kg and r-ATG 7.5 mg/kg groups respectively ($p = 0.901$) (Figure 3C). The proportion of patients who died from recurrence or transplant-related causes was similar (Table 3). In the p-ALG 75 mg/kg group, six patients died of relapse and NRM, while eight patients died of relapse and NRM in the r-ATG 7.5 mg/kg group. In the p-ALG 90 mg/kg group, six patients died of relapse and seven died of NRM. The overall cumulative incidence of a 2-year NRM was 14.0%, 20.2% and 22.9% in the p-ALG 75 mg/kg, p-ALG 90 mg/kg and r-ATG 7.5 mg/kg groups respectively ($p = 0.434$). The 2-year DFS was 67.6%, 69.4% and 71.7% for the p-ALG 75 mg/kg, p-ALG 90 mg/kg and r-ATG 7.5 mg/kg groups respectively ($p = 0.927$).

DISCUSSION

ATG is commonly used to prevent GVHD in patients who undergo related haploidentical donor allogeneic stem cell transplantation. Numerous retrospective or clinical trials studies have reported that r-ATG is associated with a reduced risk of GVHD and cGVHD.[18–20] ATG not only depletes donor T cells in vivo but also possesses pleiotropic functions on the immune system, which have been correlated with the pathogenesis of GVHD.[21,22] However, it is important to note that r-ATG and p-ALG, the two ATG forms currently used for the prophylaxis of GVHD after haplo-HSCT in our centre, present different pharmacokinetic and pharmacodynamic characteristics due to the discrepancies in their manufacture processes. r-ATG is a polyclonal immunoglobulin produced by immunizing pathogen-free rabbits with fresh human thymocytes.[23] On the other hand, p-ALG is derived from pigs that have been immunized with human T lymphocytes. The immunized plasma was pretreated with human plasma, human placental tissue and human red blood cells to remove unwanted histotoxic anti-human antibodies.[24] When combined with ciclosporin A, p-ALG has been proven to be an effective and well-tolerated treatment for patients with SAA.[24] Chen et al. reported that the efficacy and safety of p-ALG were comparable with r-ATG for SAA patients receiving haplo-HSCT.[25] Nevertheless, the comparable dosages between different forms of ATG have not been identified yet and depend on the physician's decision.

In this retrospective study, we collected and compared the outcomes in patients with haematological malignancies who received haplo-HSCT using either r-ATG or p-ALG. The conditioning regimens, GVHD prophylaxis, graft sources and support care strategies were relatively homogenous among all the patients. In our study of 178 patients, we did not find any significant differences between the three groups regarding the cumulative incidence of aGVHD and cGVHD, the cumulative incidence of 2-year relapse and 2-year OS. However, there were significant differences observed in engraftment and infection rates among the three groups.

Chen et al. reported that the incidence of aGVHD and cGVHD was higher in the p-ALG group than in the r-ATG group for SAA patients who underwent matched sibling donor HSCT.[26] However, we found no significant difference in the incidence of aGVHD and cGVHD among patients with different doses of p-ALG or r-ATG.

Until the date of last follow-up, 12, 13 and 16 patients died in the p-ALG 75 mg/kg, p-ALG 90 mg/kg and r-ATG groups. The 2-year OS, DFS, NRM and cumulative incidence of 2-year relapse were similar in the three groups ($p = 0.497$, $p = 0.927$, $p = 0.434$ and $p = 0.901$).

The median time to neutrophil engraftment was 11 days for the p-ALG 75 mg/kg group and 12 days for the r-ATG 7.5 mg/kg group ($p = 0.010$). In addition, the incidence of platelet engraftment in the p-ALG 75 mg/kg group was significantly higher than that in the r-ATG 7.5 mg/kg group (100% vs. 90.3%, $p = 0.033$). Three patients in the p-ALG 90 mg/kg group and four patients in the r-ATG group failed to receive neutrophil engraftment due to primary graft failures and subsequently died due to infections. The previous study indicated that patients in the p-ALG group had a lower incidence of CMV viraemia than the r-ATG group after haplo-HSCT.[25] In our study, patients in the group receiving a higher dose of p-ALG (90 mg/kg) had the highest rates of infection. In addition, the incidence of CMV reactivation in the p-ALG 75 mg/kg group was significantly lower than that in the r-ATG group ($p = 0.046$). There were no significant differences in the rates of IFD ($p = 0.087$) and EBV viraemia ($p = 0.972$) among the three groups.

According to the data we collected, 49% of patients in the p-ALG 90 mg/kg group and 48.6% of patients in the r-ATG group developed fever within 14 days after transplantation. However, fever occurred in only 28.1% of patients in the p-ALG 75 mg/kg group. The most common reason for early fever was CRS, which needs to be brought to the attention of clinicians.

Our study has several limitations, including being a retrospective single-centre study with a limited number of patients. Therefore, prospective and multicentre studies are needed to validate our findings in the future. In addition, the reconstitution of T cells and B cells was not monitored. In the future, we will regularly test patients for immune reconstitution, which might provide the cellular evidence for virus reactivation.

In conclusion, a lower dose of p-ALG (75 mg/kg) had a similar efficacy and safety profile compared with r-ATG (7.5 mg/kg) in the setting of haplo-HSCT. Furthermore, the risk of infection was lower in the p-ALG 75 mg/kg group than in the r-ATG group. Therefore, p-ALG (75 mg/kg) may be an appropriate alternative to r-ATG in the conditioning regimen of haplo-HSCT.

AUTHOR CONTRIBUTIONS

Huafang Wang conceived the study, secured funding, supervised the work and contributed to being the corresponding author. Xi Zhou, Xiaoyan Zhao and Xuan Lu contributed with data collection; Ziwei Xu analysed the data and wrote the paper.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The study was approved by the institutional review board at the Union Hospital of Huazhong University of Science and Technology. This study was performed in line with the principles of the Declaration of Helsinki.

PATIENT CONSENT STATEMENT

All patients gave written informed consent before participating in this study.

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· 论著——研究报告 ·

抗人胸腺淋巴细胞球蛋白/抗淋巴细胞免疫球蛋白在异基因干细胞移植治疗血液病的临床观察

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［摘要］ **目的**：比较抗人胸腺淋巴细胞球蛋白(anti-thymocyte globulin，ATG)/抗淋巴细胞免疫球蛋白(anti-lymphocyte globulin，ALG)在异基因干细胞移植应用中的临床疗效及不良反应。**方法**：回顾性分析 2015 年 1 月至 2021 年 4 月我院 36 例接受异基因干细胞移植的恶性血液病患者的临床资料，包括基础疾病、年龄、性别、供者类别、HLA 配型相合度及回输的单个核细胞计数、CD34 细胞计数、预处理方案等。按照预处理方案选择 ATG 或 ALG 将患者进行分组，ATG 组 11 例，ALG 组 25 例。观察 2 组患者中性粒细胞及血小板植入情况，急性及慢性移植物抗宿主病发生情况、患者生存情况及移植后病毒感染率。**结果**：中位随访时间 26.4(2.0～71.3)个月，ATG 组和 ALG 组患者中位生存期分别为 32.9(9.0～41.0)个月和 38.0(5.0～71.3)个月，差异无统计学意义($P=0.44$)。截至随访结束，ALG 组和 ATG 组患者中性粒细胞植入的中位时间分别为 13.4 d 和 13.3 d，差异无统计学意义($P=0.30$)。ATG 组和 ALG 组患者死亡率分别为 27.3%和 36.0%，差异无统计学意义($P=0.47$)。ALG 组和 ATG 组移植后病毒感染率分别为 12.0%和 18.2%，差异无统计学意义($P=0.63$)。ALG 组和 ATG 组急性移植物抗宿主病发生率分别为 44.0%和 45.5%，差异无统计学意义($P=1.00$)；慢性移植物抗宿主病发生率分别为 44.0%和 18.2%，差异亦无统计学意义($P=0.26$)。**结论**：ALG 在异基因干细胞移植的疗效与 ATG 相当，虽然 ALG 组慢性移植物抗宿病发生率高于 ATG 组，但差异无统计学意义。

［关键词］ 抗人胸腺淋巴细胞球蛋白/抗淋巴细胞免疫球蛋白；异基因造血干细胞移植；急性移植物抗宿主病；慢性移植物抗宿主病；恶性血液病

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Clinical observation of anti-thymocyte globulin/anti-lymphocyte globulin in allogeneic stem cell transplantation

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Abstract **Objective**: To observe the clinical efficacy and adverse events of anti-thymocyte globulin(ATG)/anti-lymphocyte globulin(ALG) in allogeneic stem cell transplantation. **Methods**: The clinical data of 36 patients with hematologic malignancies who received allogeneic stem cell transplantation in our hospital from January 2015 to April 2021 were retrospectively analyzed. The clinical data of patients included age, basic disease, sex, donor type, match degree, mononuclear cell counts, CD34 counts and preparative regimen. According to the pretreatment scheme, patients were divided into ATG group and ALG group, with 11 cases in the ATG group and 25 cases in the ALG group. The implantation of granulocytes and platelets, the occurrence of acute and chronic graft versus host disease(GVHD), the survival of patients and the virus infection after transplantation in the two groups were observed. **Results**: The median follow-up time was 26.4(range 2.0 to 71.3) months. The median survival time of ATG group and ALG group was 32.9(range 9.0 to 41.0) months and 38.0(range 5.0 to 71.3) months, respectively, and there was no significant difference($P=0.44$). By the end of follow-up, the duration of neutrophils implantation in ALG group and ATG group was 13.4 days and 13.3 days, respectively, and there was no significant difference($P=0.30$). The mortality in ATG group and ALG group was 27.3% and 36.0%, respectively, and there was no significant difference($P=0.47$). The virus infection rates of ALG group and ATG group were 12.0% and 18.2%, respectively, and there was no significant difference($P=0.63$). The rates of acute GVHD in ALG group and ATG group were 44.0% and 45.5%, respectively, and there was no significant difference($P=$

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1.00). The rates of chronic GVHD in ALG group and ATG group were 44.0% and 18.2%, respectively, and there was no significant difference($P=0.26$). **Conclusion**: The efficacy of ALG in allogeneic stem cell transplantation is similar to that of ATG. However, the incidence of chronic GVHD in ALG group is higher than that of ATG group, but there is no significant difference.

Key words anti-thymocyte globulin/anti-lymphocyte globulin; allogeneic hematopoietic stem cell transplantation; acute graft versus host disease; chronic graft versus host disease; hematological malignancy

目前异基因造血干细胞移植(allogeneic hematopoietic stem cell transplantation，allo-HSCT)被认为是治疗恶性血液病患者的唯一方法[1]。研究指出移植相关的移植物抗宿主病(graft versus host disease，GVHD)对预后造成不良影响，是影响该技术广泛应用的障碍[2-5]。临床上，根据发生GVHD的时间分为急性和慢性GVHD(acute graft versus host disease，aGVHD/chronic graft versus host disease，cGVHD)，aGVHD发生在移植100 d内，cGVHD发生于移植100 d后；而目前对于GVHD的病理诊断比较困难，对其的识别及临床诊断主要依据发生时间和受累靶器官的症状和严重程度而定。GVHD的影响因素主要包括供、受体间HLA配型相合度、有无血缘关系、性别、发病年龄、合并基础疾病、预处理方案及GVHD的预防等，进一步降低移植相关GVHD是我们追求的目标。多项研究报道[6-9]证实抗人胸腺细胞球蛋白(anti-thymocyte globulin，ATG)在异基因干细胞移植包括减低预处理剂量、非血缘及单倍体移植中可有效降低GVHD的发生。在抗人淋巴细胞免疫球蛋白(anti-lymphocyte globulin，ALG)上市后，杨楠等[10]通过比较猪ALG和兔ATG在接受免疫抑制治疗的再生障碍性贫血患者的情况，表明猪ALG在治疗效果、长期生存率、安全性及降低GVHD方面与兔ATG相当，但其治疗相关费用明显降低，疗效肯定。目前关于ALG/ATG在异基因干细胞移植中GVHD的发生率，尤其是在替代供者(无关供者、单倍体相合供者)的研究不多。本研究回顾性分析我院2015年至2021年36例接受异基因干细胞移植过程中使用ATG/ALG预防GVHD的血液病患者，收集患者的临床相关资料，比较截至随访时2组患者aGVHD及cGVHD的发生情况及病毒感染情况。

1　资料与方法

1.1　资料

纳入标准：①诊断为恶性血液病需要行异基因干细胞移植并已完善骨髓细胞学、骨髓活检、免疫分型、基因等相关检查明确诊断的患者；②有干细胞移植的适应证，并且有合适的干细胞供者；③在预处理方案中加入ATG或ALG预防GVHD；④移植后存活超过30 d；⑤无异基因干细胞移植的禁忌证。排除标准：①合并严重心、肝、肾等重要脏器功能异常；②无合适干细胞供者；③移植后存活少于30 d；④预处理方案中无ATG或ALG。

回顾性分析我院2015年1月至2021年4月在异基因干细胞移植预处理方案中使用ATG或ALG的36例患者，收集整理患者的临床相关资料，包括年龄、供者类别、性别、配型相合度及回输的单个核细胞计数及CD34细胞计数。本研究经徐州市中心医院伦理委员会批准(批准文号：XZXY-LK—20211118-04)，所有患者签署知情同意书。

1.2　预处理方案

19例患者采用预处理方案为氟达拉滨＋环磷酰胺＋ALG/ATG(FLu＋CTX＋ALG/ATG)，具体为Flu 25～30 mg/m^2/d －8～－5 d；CTX 50 mg/d －5～－2 d；ALG 25 mg/kg/d －5～－1 d(ATG 2.5 mg/kg/d －5～－1 d)；17例患者采用白消安＋环磷酰胺＋ALG/ATG(BU＋CTX＋ALG/ATG)，具体为BU 0.8 mg/kg q6 h －7～－4 d；CTX 60 mg/kg qd －3～－2 d；ALG 25 mg/kg/d －5～－1 d(ATG 2.5 mg/kg/d －5～－1 d)。

1.3　GVHD的防治

采用环孢素A或他克莫司联合短疗程甲氨蝶呤、ATG或ALG，包含或不包含霉酚酸酯预防GVHD。对于非血缘全相合异基因干细胞移植采用环孢素(－9 d 2.5 mg/kg/d，持续24 h静脉输注，根据环孢素浓度调整用量，造血重建后可耐受再改为口服5 mg/kg/d分2次服用)＋甲氨蝶呤(15 mg/m^2＋1 d；10 mg/m^2＋3、＋6、＋11 d)＋ALG 25 mg/kg/d －5～－1 d(ATG 2.5 mg/kg/d －5～－1 d)预防GVHD；半相合异基因干细胞移植除环孢素及甲氨蝶呤、ATG或ALG，增加吗替麦考酚脂(0.25 g bid －4 d开始约28 d)预防GVHD；而GVHD的一线治疗选用糖皮质激素，二线治疗选择包括间充质干细胞、抗CD25单抗、芦可替尼等。

1.4　感染及肝静脉闭塞病的预防

预处理前－9 d给予阿昔洛韦预防病毒感染，头孢类抗菌素(三代)预防细菌感染及盐酸小檗碱、诺氟沙星行肠道准备。移植后－3 d开始口服泊沙康唑预防真菌感染。每周2天口服复方磺胺甲恶唑片预防卡氏肺囊虫感染直至停用免疫抑制剂。移植前－9 d行坐浴及漱口预防局部感染；预防肝静脉闭塞病：移植前－9 d开始应用前列地尔剂量

为 10 μg/d，根据血小板情况调整，口服熊去氧胆酸 0.25 g bid 至移植后 30 d。

1.5 随访

通过电话回访、患者门诊记录及住院病历资料进行随访。随访截至 2021 年 4 月 1 日，观察 2 组患者中性粒细胞及血小板植入情况、急慢性 GVHD 发生率、病毒感染和总生存时间。

1.6 造血重建评定

中性粒细胞计数≥0.5×10^9/L 持续 3 d 的第 1 天为粒系植入时间，无血小板输注的情况下血小板计数≥20×10^9/L 持续 7 d 的第 1 天为巨核系植入时间[11]。急慢性 GVHD 定义参照文献[12]，具体为 aGVHD 发生在移植后 100 d 内，cGVHD 发生于移植 100 d 后；总生存期：患者造血干细胞回输后第 1 天至随访截止或患者死亡时间。

1.7 统计学处理

应用 SPSS 22.0 软件对数据进行统计分析。计数资料以例(%)表示，组间比较采用 χ^2 检验；符合正态分布的计量资料以 $\overline{X}\pm S$ 表示，组间比较采用独立样本 t 检验；不符合正态分布的计量资料以 $M(P_{25},P_{75})$ 表示，组间比较采用秩和检验；生存分析采用 Kaplan-Meier 法及 log-rank 检验。以 $P<0.05$ 为差异有统计学意义。

2 结果

2.1 一般资料

2015 年 1 月至 2021 年 4 月共收集 36 例接受 ATG/ALG 预处理的 allo-HSCT 的血液病患者，主要疾病类型为再生障碍性贫血、急性髓系白血病、急性淋巴细胞白血病、骨髓增生异常综合征(高危组)及慢性髓系白血病，供者大多数为亲缘供者(34 例)，HLA 配型大多数为半相合供者(25 例)。根据预处理方案中选择 ATG 或 ALG 进行分组，其中 ATG 组 11 例，ALG 组 25 例。ALG 组和 ATG 组外周血干细胞计数：单个核细胞计数的中位数分别为 8.26(5.74，9.61)×10^8/kg 和 10.31(5.90，13.02)×10^8/kg；CD34 细胞计数的中位数分别为 5.15(3.58，7.26)×10^6/kg 和 3.90(3.29，6.09)×10^6/kg。ALG 组和 ATG 组患者中位年龄分别为 31.5(15～49)岁和 31.8(9～65)岁。2 组患者性别、疾病类型、HLA 配型、预处理方案、供者类型、单个核细胞计数、CD34 细胞计数等基本资料比较，差异均无统计学意义，见表 1。

表 1 ATG 组和 ALG 组基本资料比较

	ALG 组(25 例)	ATG 组(11 例)	统计值	P
性别/例(%)			$\chi^2=1.178$	0.471
男	14(56.0)	4(36.4)		
女	11(44.0)	7(63.6)		
疾病类型/例(%)			$\chi^2=1.100$	0.820
再生障碍性贫血	14(56.0)	5(45.5)		
急性淋巴细胞白血病	4(16.0)	2(18.2)		
急性髓系白血病	5(20.0)	3(27.3)		
骨髓增生异常综合征	1(4.0)	1(9.1)		
慢性髓系白血病	1(4.0)	0		
年龄/岁	31.96±13.63	30.64±21.34	$t=0.225$	0.210
HLA 配型/例(%)			$\chi^2=1.140$	0.439
半相合	16(64.0)	9(81.8)		
全相合	9(36.0)	2(18.2)		
预处理方案/例(%)			$\chi^2=0.340$	0.720
FLu+CTX+ALG/ATG	14(56.0)	5(45.5)		
BU+CTX+ALG/ATG	11(44.0)	6(54.5)		
供者类型/例(%)			$\chi^2=1.286$	0.936
父亲	8(32.0)	3(27.3)		
兄弟	7(28.0)	2(18.2)		
姐妹	5(20.0)	2(18.2)		
子女	3(12.0)	2(18.2)		
母亲	1(4.0)	1(9.1)		
非血缘	1(4.0)	1(9.1)		
单个核细胞计数/(10^8/kg)	8.26(5.74,9.61)	10.31(5.90,13.02)	$Z=-1.570$	0.114
CD34 细胞计数/(10^6/kg)	5.15(3.58,7.26)	3.90(3.29,6.09)	$Z=-0.704$	0.481

2.2　造血重建

ALG 组和 ATG 组患者中性粒细胞植入的中位时间分别为 13.4(9～19)d 和 13.3(11～15) d，差异无统计学意义(P=0.30)。ATG 组 1 例骨髓增生异常综合征患者在异基因干细胞移植后 6 个月血小板植入。

2.3　移植并发症

ATG 组和 ALG 组患者移植后病毒感染主要为 EB 病毒和巨细胞病毒感染，分别为 18.2%(2/11)和 12.0%(3/25)，差异无统计学意义(P=0.63)。ATG 组在干细胞移植后发生 1 例 EB 病毒感染，1 例巨细胞病毒感染；ALG 组发生 2 例 EB 病毒感染，1 例巨细胞病毒感染。同时在预处理过程中，ATG 组发生过敏反应 5 例(45.5%)，ALG 组发生过敏反应 10 例(40.0%)。ATG 组在干细胞移植后 5 例(45.5%)发生 aGVHD，2 例(18.2%)发生 cGVHD；ALG 组 11 例(44.0%)发生 aGVHD，主要是皮肤型；11 例(44.0%)发生 cGVHD，分别为 5 例肠道 cGVHD，1 例肝脏 cGVHD，4 例肺 cGVHD，1 例眼睛及皮肤 cGVHD；2 组 aGVHD 和 cGVHD 发生率比较，差异均无统计学意义(P=1.00、0.26)。ATG 组和 ALG 组患者移植后发生总排斥率分别为 63.6%(7/11)和 64.0%(16/25)，差异无统计学意义(P=0.72)。

2.4　生存情况

截至随访时间，ATG 组和 ALG 组患者中位生存期分别为 32.9(9.0～41.0)个月和 38.0(5.0～71.3)个月，差异无统计学意义(P=0.44)，见图 1。ATG 和 ALG 组患者异基因干细胞移植后死亡率分别为 27.3%(3/11)和 36.0%(9/25)，ALG 组死亡率高于 ATG 组，但差异无统计学意义(P=0.47)。ATG 组 11 例患者中移植后死亡 3 例，主要死亡原因为肠道 cGVHD；ALG 组 25 例患中移植后死亡 9 例，主要死亡原因为 4 例肠道 cGVHD，1 例肝脏 cGVHD，4 例肺 cGVHD。

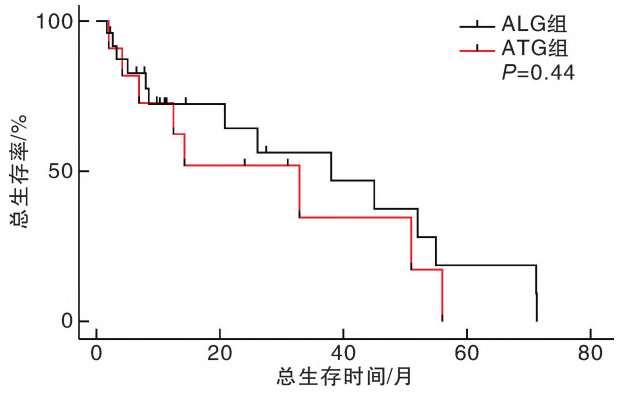

图 1　ATG 组与 ALG 组总生存率比较

3　讨论

目前对于血液恶性肿瘤患者，尤其是预后差且复发率高的患者，异基因干细胞移植有望成为根治的有效治疗方法。任瑞瑞等[13]在 82 例恶性血液病患者分别接受异基因干细胞移植半相合和同胞全相合移植的疗效比较发现，移植方式对患者生存时间无影响，但移植前缓解状态和移植后重度 aGVHD 对患者的长期生存具有重要意义。研究证实 ATG 能够降低急慢性 GVHD 的发生率[6]。Chen 等[14]研究报道在移植预处理过程中 ALG 总应答率高于 ATG，且治疗费用减少，同样在一项[15]回顾性 41 例重型再生障碍性贫血的研究中发现，ALG 在异基因干细胞移植的预处理方案应用中安全性良好，可作为 ALG 的替代药物。因此越来越多的临床医生将 ALG 作为异基因干细胞移植预防 GVHD 的首选。张璞等[16]研究观察国产 ALG 在我院初治重度再生障碍性贫血异基因干细胞的疗效发现，其预防 GVHD 与 ATG 相当，不良反应可耐受，且治疗费用降低。本研究通过回顾性分析 2015 年 1 月至 2021 年 4 月在我院接受异基因干细胞移植的 36 例恶性血液病患者，2 组患者基本临床信息包括性别、年龄、供者类别、预处理方案、单个核细胞计数、CD34 细胞计数等差异无统计学意义，但移植后发现使用 ALG 较 ATG 恢复快，中性粒细胞植入较早，与既往文献[10,16]报道一致。截至随访时间，ATG 组和 ALG 组患者中位生存期分别为 32.9(9.0～41.0)个月和 38.0(5.0～71.3)个月，2 组患者总生存率差异无统计学意义。

马秀慧等[17]研究发现患者使用免疫抑制剂(ATG/ALG)发生血清病或过敏反应率约为 50%，但 ATG 较 ALG 更易引起过敏反应。我院在预处理过程中应用 ATG/ALG 时，ATG 组发生过敏反应 5 例(45.5%)，ALG 组发生过敏反应 10 例(40.0%)，与既往文献报道一致。另外，ATG 组发生 1 例关节痛，不排除血清性关节病。

移植相关 GVHD 的发病机制包括 T、B 淋巴细胞的免疫性效应，炎症细胞因子释放，预处理的组织损伤等最终导致宿主靶器官的损害。目前研究发现可通过减轻预处理毒性及改良免疫抑制药物、提高供-受体 HLA 匹配度等预防 GVHD 的发生。刘硕等[18]在异基因干细胞治疗急性白血病中发现过多的淋巴细胞在一定程度上有增加 GVHD 发生的趋势，由于 ATG 有更强的淋巴细胞清除率，可能会降低 GVHD。陈欣等[19]通过回顾性分析 46 例再生障碍性贫血患者接受异基因干细胞移植发现，ALG 组与 ATG 组患者治疗后 2 组总生存率相近，但前者并不增加 GVHD 及感染等移植后并发症的发生率。本文回顾性分析我院 36 例接受异基因干细胞移植的恶性血液病患者，发现 ALG

组与 ATG 组患者移植后发生总排斥率及急性 GVHD 差异无统计学意义。关于 cGVHD 发生率，本研究发现 ALG 组高于 ATG 组，分别为 44.0%和 18.2%，但差异无统计学意义。根据 ATG 在体内代谢特点，研究表明若在回输干细胞前患者体内 ATG 较高，可有效降低 cGVHD 及中重度 cGVHD 的发生，并且患者干细胞植入的失败率也较低[20]。由于研究入组患者较少，还需进一步验证。另外，本研究发现 ALG 组 1 例再生障碍性贫血患者在移植后 3 年出现Ⅳ度皮肤型 cGVHD，表现为全身皮疹伴苔藓样改变，给予激素联合环孢素、他克莫司后效果不佳，给予间充质干细胞联合他克莫司软膏、甲氨蝶呤等抗排斥治疗后，患者苔藓样皮肤逐渐消退，恢复正常皮肤，但具体机制不明。Whangbo 等[21]研究发现调节性 T 细胞可抑制自身和同种异体免疫耐受，猜测可能为间充质干细胞在骨髓中可分化为各种非造血细胞的祖细胞及免疫调节细胞，进而减轻 cGVHD[22]。另外有文献报道在异基因干细胞移植后预防性使用间充质干细胞，可降低急慢性 GVHD 的发生率[23]。

本研究发现 ATG 组和 ALG 组患者死亡率分别为 27.3%和 36.0%，ALG 组死亡率高于 ATG 组，但差异无统计学意义。原因可能是 ATG 早期对淋巴细胞尤其对调节性 T 细胞的杀伤力较 ALG 强，回输干细胞后体内高 ATG 浓度可减慢 $CD4^+$ T 细胞重建，减少移植相关死亡的发生。另外有研究[24]报道 ATG 可减少慢性失功能肺病和 cGVHD 的发生，从而降低晚期移植相关死亡率。陈欣等[19]研究报道在异基因干细胞移植预处理过程应用 ATG/ALG 后常合并感染，致死率较高。上官思雨等[25]研究发现在异基因干细胞移植后巨细胞病毒感染因素由于供受者免疫细胞被激活，供受者之间发生不同组织相容性抗原，供受者之间相互攻击，使受体发生多脏器损伤，从而增加巨细胞病毒感染风险。本研究发现 2 组患者移植后病毒感染主要为 EB 病毒及巨细胞病毒感染，发生率分别为 18.2%和 12.0%，差异亦无统计学意义。

本文因收集病例数有限，对于在异基因干细胞移植预处理过程中选择 ALG 是否增加 cGVHD，还需扩大病例数进一步验证；同时在同病种间进一步比较，可能临床意义更大。为深入研究 GVHD 的发病机制，明确其对受者的影响，寻找更有效的防治 GVHD 而不影响抗白血病的新方法，将为造血干细胞移植的应用打开更广阔的天地。

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