An epitope-based approach to use of HLA matched platelets for transfusion: a non-inferiority randomised trial

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Abstract:
Platelet transfusion refractoriness results in adverse outcomes and increased healthcare costs. Managing refractoriness due to HLA alloimmunization necessitates the use of HLA antigen matched platelets, but requires a large platelet donor pool, and does not guarantee full matching. We report a first randomized, double-blind, non-inferiority, cross-over trial comparing HLA epitope-matched (HEM) platelets with HLA standard antigen-matched (HSM) platelet transfusions. Eligibility criteria were alloimmunized, platelet refractory, thrombocytopenic patients with aplastic anemia, myelodysplastic syndrome or acute myeloid leukemia. HEM platelets were selected using HLAMatchMaker epitope (specifically eplet) matching. Patients received up to eight prophylactic HEM and HSM transfusions provided in random order. Primary outcome was one-hour post transfusion platelet count increment (PCI). 49 patients were randomised at 14 UK hospitals. For intention-to-treat, number of evaluable transfusions was 107 and 112 for HEM and HSM, respectively. Unadjusted mean (SD) PCI for HEM and HSM was 23.9 (15) and 23.5 (14.1) respectively (adjusted mean difference -0.1, 95% CI -2.9, 2.8). As the lower limit of 95% CI was not greater than pre-defined non-inferiority limit, HEM was declared non-inferior to HSM. There were no differences in secondary outcomes of platelet counts, transfusion requirements and bleeding events. Adequate one-hour PCI was more frequently observed with a mean number of 3.2 of epitope mismatches compared to 5.5 epitope mismatches for inadequate 1 hour increments. For every additional one epitope mismatch, the likelihood of an adequate PCI decreased by 15%. Epitope matched platelets should be considered to support HLA alloimmunized patients. Funded by NHS Blood and Transplant, ISRCTN23996532

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An epitope-based approach of HLA matched platelets for transfusion: a noninferiority, cross-over, randomized trial

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KEY POINTS

- Management of platelet refractoriness due to HLA alloimmunization is a major and costly clinical problem
- A non-inferiority cross-over randomized trial supported a role for epitope matched platelets for HLA alloimmunized patients

ABSTRACT

Platelet transfusion refractoriness results in adverse outcomes and increased healthcare costs. Managing refractoriness due to HLA alloimmunization necessitates the use of HLA antigen matched platelets, but requires a large platelet donor pool, and does not guarantee full matching. We report the first ever randomized, double-blind, non-inferiority, cross-over trial comparing HLA epitope-matched (HEM) platelets with HLA standard antigen-matched (HSM) platelet transfusions. Eligibility criteria were alloimmunized, platelet refractory, thrombocytopenic patients with aplastic anemia, myelodysplastic syndrome or acute myeloid leukemia. HEM platelets were selected using HLAMatchMaker epitope (specifically eplet) matching. Patients received up to eight prophylactic HEM and HSM transfusions provided in random order. Primary outcome was one-hour post transfusion platelet count increment (PCI). 49 patients were randomized at 14 UK hospitals. For intention-to-treat, number of evaluable transfusions was 107 and 112 for HEM and HSM, respectively. Unadjusted mean (SD) PCI for HEM and HSM was 23.9 (15) and 23.5 (14.1) respectively (adjusted mean difference -0.1, 95% CI -2.9, 2.8). As the lower limit of 95% CI was not greater than pre-defined non-inferiority limit, HEM was declared non-inferior to HSM. There were no differences in secondary outcomes of platelet counts, transfusion requirements and bleeding events. Adequate one-hour PCI was more frequently observed with a mean number of 3.2 of epitope mismatches compared to 5.5 epitope mismatches for inadequate one-hour increments. For every additional one epitope mismatch, the likelihood of an adequate PCI decreased by 15%. Epitope matched platelets should be considered to support HLA alloimmunized patients. Funded by NHS Blood and Transplant, ISRCTN23996532.

BACKGROUND

Platelet refractoriness identifies patients with poorer outcomes and higher costs.[1-7] One prospective study of 245 hematology in-patients requiring platelet transfusions showed a significantly longer in-patient stay of a median of 35 days for patients with platelet refractoriness compared to 14.4 days in the absence of refractoriness, and higher in-patient costs of $103,956 vs $37,817, respectively.[8] Management of platelet refractoriness due to HLA alloimmunization, is based on provision of platelets from HLA-matched or HLA-compatible donors, in which platelets are selected that are negative for the HLA antigen(s) corresponding to the identified HLA antibodies. However, this strategy is labor intensive, and requires the maintenance of a large number of available HLA typed apheresis donors,[9-12] which in England is approximately 12,000 donors. Matching is performed at the antigen level, and there is no guarantee a full match will be identified especially if multi-specific HLA antibodies and/or rare HLA type are present.

Other strategies for platelet refractoriness include antigen negative platelets to avoid specific antibodies, or specifically platelets with low expression of specific antigens such as HLA-B8, -B12, -A25 despite HLA mismatches [13], platelet cross-matching [12] and acid-treated platelets [14].

Specific regions of polymorphism in HLA molecules determine the nature of public and private HLA epitopes. Matching at the epitope level, based around either the characterization of short sequences of amino acids from linear or discontinuous regions of the HLA molecules, may be more relevant for assessing HLA compatibility between patients and donors and the effect of donor-specific HLA antibodies. Further advantages to this approach of epitope matching might extend to more efficient matching for highly sensitized patients and may avoid some of the challenges of standard matching, without the need to maintain a large panel of HLA typed apheresis donors.[12, 15-19]

In a retrospective study, we reported the effectiveness of epitope matching in 37 aplastic anemia (AA) patients transfused with 1,579 HSM platelet units using the molecularly defined HLA eplet and triplet epitopes, and reported a correlation between the number of HLA epitope mismatches and the observed platelet count increment.[20] In other studies, HEM platelets have been reported to show improved platelet count increments (PCI),[21-24] but are limited by issues of design, all are retrospective, and lack clinical outcomes, and as highlighted in systematic reviews.[12,13,25] (Table S1 in Supplemental file).

We therefore undertook a randomized, double-blind, non-inferiority, cross-over trial comparing HEM with HSM platelet transfusions in alloimmunized thrombocytopenic patients with disorders of bone marrow failure. We hypothesized that HEM platelets would result in similar or improved platelet count increments, longer transfusion-free intervals, and reduced bleeding. A non-inferiority design of study was indicated as the objective was to establish that epitope matching was no worse than standard matching, with reduced costs and resource requirements compared to support for maintenance of a large panel of HLA typed apheresis donors. A secondary objective was to compare the two approaches to HLA matching of platelets, in the context of highly sensitized patients, and examine for HLA antibody and epitope specificities. The eplet version of the HLAMatchmaker programme was adapted to use high resolution (HR) typing and antibody screening of patients and donors.

METHODS

Eligibility criteria

Eligible patients aged 16 years or over, with a diagnosis of AA, myelodysplasia (MDS) or acute myeloid leukemia (AML), and refractory to random donor platelet transfusions with documented evidence of HLA antibodies, were eligible. Exclusion criteria were palpable splenomegaly, anti-thymocyte globulin treatment in the previous nine days, pregnant or lactating patients, or inability to give informed consent or comply with the trial protocol. The following factors were not exclusion criteria but were evaluated in a sensitivity analysis of primary outcome: use of antimicrobials within 6 hours prior to transfusion; anti-platelet or anti-coagulant medication or other medication known to affect platelet refractoriness within previous 7 days; sepsis or an invasive infection at time of transfusion. Refractoriness was defined as failure on two successive occasions to achieve a 10 min to one-hour post-transfusion platelet count increment of $>5x10^9$/L using ABO compatible fresh platelets <72 hour old [26,27].

Patients and donor HLA and HPA testing

All patients and donors were HLA and HPA typed using molecular techniques for HLA-ABC, and HPA 1,2,3,4,5 and 15. Luminex and ELISA methods were used to test for HLA and HPA specific antibodies respectively

HLA antigen and epitope matching

Standard HLA matching approach utilized first field (low resolution) HLA type and took into account donor specific antibodies detected in the patients' serum. Epitope matching was performed using a computer program that incorporates the HLAMatchmaker defined epitopes

into a 'search engine' to facilitate the process of HLA antigen and/or epitope-based platelet selection in platelet transfusion. This program was initially designed in Microsoft Access™, and later converted to a web-based system using the Microsoft SQL Server database and the C# programming language based on the Microsoft ASP.NET MVC™ application framework.[28] The program was designed to perform patient – donor compatibility testing by both standard HLA antigen and epitope matching at HLA-A and B loci, with an option to include HLA-C. The program allows patient HLA antibody definition at the standard HLA antigen as well as the epitope level and searches to find the most compatible platelet units for each patient, using either standard HLA antigen matching or epitope matching.[29] For characteristics of transfused platelets see Methods, Supplemental file.

Intervention

Each patient received up to eight prophylactic study transfusions, each of one platelet unit, four each of HSM and HEM units in random order (HSM/HEM platelets are defined in Supplemental file). Additional (non-trial) platelet transfusions were given to some patients, to treat bleeding or to cover an emergency invasive procedure as necessary (see Supplemental file Methods and Table 9). Figure 1 flow diagram shows the process of patient recruitment and progress after recruitment.

Outcomes

Primary outcome measure was PCI at one-hour post-transfusion defined as the difference between the platelet count at one-hour after transfusion (10-90 minutes) and the platelet count pre-transfusion (taken no more than 24 hours before the transfusion). An adequate 1-hour increment was defined as 10×10^9 /l. Secondary outcome measures were (1) PCI at 24 hours

(range 8-36 hours) post-transfusion (2) corrected count increment (CCI, definition in Supplemental file) at one-hour (10-90 minutes) and 24 hours (8-36 hours) post-transfusion (3) interval between trial platelet transfusions (4) patient bleeding events (5) grade of HSM matching (either grade A, B1, B2, B3 or B4) and number of epitope mismatches for HEM transfusions. Bleeding events were captured using a modified version of previously piloted self-assessment tools,[30,31] and reported as a score for 3 days post-transfusion, or until the next platelet transfusion, whichever was sooner.

Subgroup analysis of primary outcome assessed impact of HLA alloimmunization by calculated reaction frequency (for cRF definition see Supplemental file) (<40% vs ≥40% and separately as a post hoc analysis <85% and ≥85%), gender, diagnosis (AA vs MDS/AML), and post hoc donor-recipient blood group match/mismatch) on one-hour PCI, and an analysis of features of the highly sensitized patient group, in which we compared all the non-self eplets identified on the antigens targeted by the patient's serum.

Data collection and randomization

A daily bleeding assessment form was completed. Patients also completed clinical bleeding assessment forms for three days post-transfusion, or until next platelet transfusion, whichever was sooner. Grade of bleed was assigned centrally using a computer algorithm, which was validated previously by comparison to a manual assignment system, using a modified WHO definition of bleeding events.[31]

Randomization was managed by NHSBT Histocompatibility and Immunogenetics (H&I) laboratory once eligibility had been confirmed and consent obtained. Computer generated

randomization lists were created which indicated 8 prophylactic study transfusions randomly alternating between HSM or HEM, with 1:1 allocation, plus up to 22 additional transfusions in case any of the first 8 were not evaluable. These were created by the independent statistician and provided to staff at each platelet-issuing laboratory. These were the only individuals unblinded to treatment allocation during the trial.

Analyses: All primary and secondary outcomes were analyzed as intention to treat (ITT), which included all consented participants who were randomized as the primary analysis for the trial. A per-protocol analysis excluded participants randomized in error and any transfusions where the participant did not receive the treatment specified in the randomization list. No adjustment was made for any center nor H&I laboratory differences, as all laboratories followed the same national protocol. A trial transfusion was considered to be evaluable at one-hour (and 24 hours) if pre-transfusion and one-hour (24 hours) post-transfusion platelet counts were both recorded within the time windows: pre-transfusion within 24 hours prior to transfusion, and post transfusion within 10-90 minutes (8-36 hours).

Primary outcome was analyzed using a normal linear regression model adjusting for pre-transfusion platelet count, period effect and a random patient effect. Carry-over from the previous transfusion was not tested for in the model, as it was deemed there should be no carry-over effect.

For subgroup analyses, an additional interaction term (between subgroup and treatment) was included. This model and adjustment were also used to analyze 24-hour PCI, CCI at one and 24 hours and participant bleeding self-assessment scores. Features of the highly sensitized patient group, in which all non-self eplets identified on antigens targeted by patient's serum were

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compared. <mark>(See Supplemental file, for details of secondary outcome, sensitivity analyses, and missing data).</mark>

Sample size calculation: Data were used from a retrospective cohort study, where mean platelet count increment was 29.1×10^9/L (95% CI 22.8-35.5) and the within and between patient standard deviations were both 18.5.[20] Non-inferiority margin was set to 5×10^9/L, as the maximum difference in favor of standard matching method (HSM) that would be clinically acceptable for the new HEM method to be implemented in practice. Such a difference was also compatible with the confidence interval around current estimated mean count increment for the standard method of around 30. Sample size was calculated using simulation (Stata version 12), with 2000 repetitions and one-sided significance level of 2.5%. Assuming a small amount of dropout (where 25% of patients received only 4 transfusions and the remaining 75% received all 8 transfusions), 40 patients would give 89% power to exclude a mean difference of 5, based on the lower bound of 95% confidence interval. It was prespecified that the HEM method would be declared non-inferior if the lower bound of 95% confidence interval was greater than a difference of 5×10^9/L in favor of the standard method. A pre-defined (blinded) interim analysis at 20 patients confirmed no changes were required to the sample size.

Oversight

This study was undertaken according to Declaration of Helsinki and Good Clinical Practice principles and ethics approval of the protocol was obtained. All patients gave written informed consent and safety was monitored by an independent data monitoring committee.

RESULTS

Screening, recruitment and follow up

Between October 2012 and November 2015, 135 adult patients, diagnosed with AA, MDS or AML, were assessed for eligibility at 14 UK hospitals. Figure 2, CONSORT diagram, summarizes participants' progression through the trial. Of these 135 patients, 86 (64%) were excluded and 49 randomized participants were included as per ITT. Seven participants did not receive any evaluable trial transfusions and two participants were randomized in error. There was balance in participant characteristics between the two treatment arms (Table 1).

Primary outcome

219 adequate transfusions were evaluated for the study (n=107 HEM; n=112 HSM). Results of primary outcome following transfusions with trial HSM and HEM platelets are presented in Table 2 for both ITT and PP analyzes (Figure S1 in Supplemental file). For ITT, there was no significant difference in platelet count increment at one-hour (10-90 mins) post transfusion between the two treatment arms (p = 0.9686; adjusted mean difference HEM vs HSM was -0.1 [95% CI -2.9, 2.8]). As the lower limit of 95% CI was greater than -5, HEM was declared non-inferior to HSM. Similar results were observed for the PP analysis (p-value = 0.7469; adjusted mean difference HEM vs HSM is -0.5 [95% CI -3.4, 2.4]).

The proportion of inadequate PCI was 16% in both arms (Table S2). The percentage of non-evaluable transfusions at one and 24 hours post transfusion was similar in both treatment arms (proportion of non-evaluable trial transfusions at one-hour post transfusion was 48% for HEM and 46% for HSM and at 24 hours post transfusion was 54% for HEM and 51% for HSM (Table S3). For both treatment arms, the most common reason for a non-evaluable one-hour trial

transfusion was missing one-hour post transfusion platelet count and time (34% in the HEM arm and 47% in the HSM arm).

Sensitivity analysis demonstrated no impact on primary outcome when considering use of antimicrobials within 6 hours prior to the transfusion; anti-platelet or anti-coagulant medication or other medication known to affect platelet refractoriness within previous 7 days; sepsis or an invasive infection at the time of the transfusion (Table S4).

When exploring subgroups, there was no interaction between treatment and gender or treatment and diagnosis (p=0.4978 and 0.1890, respectively;, Table 5 Supplemental File). For CRF subgroup analysis, using a cut off of ≥40 and <40, there were insufficient numbers to estimate a treatment effect (see Table 5, Supplemental File). Hence a post-hoc analysis of highly sensitized patients, as defined by CRF ≥85% was conducted and no significant interaction with PCI was found (p = 0.6601) (Table 6, Supplemental File). A further post-hoc analysis showed no evidence of an interaction between ABO blood group matching/mismatching on the primary outcome (Table 7, Supplemental File).

Secondary outcomes

For both ITT and PP analyses, there was no significant difference in secondary outcomes between the two treatment arms (Table 2). Reactions to trial platelet transfusions occurred in five patients, three after HSM and two after HEM platelets, with one SAE for each HSM and HEM (Table 8, Supplemental File). For both treatment arms, the most common reason for a non-evaluable 24-hour trial transfusion was missing 24 hours post transfusion platelet count and time (56% in the HEM arm and 61% in the HSM arm). Matching grades for HSM platelets transfused and the number of epitope mismatches for HEM platelets is shown in Table 3.

The impact of HEM mismatching and ABO matching on PCI was examined in a post hoc analysis. Adequate one-hour PCI was more frequently observed with a mean number of 3.2 (SD 3.6) of epitope mismatches compared to 5.5 (SD 5.3) for inadequate one-hour increments, p=0.0485 (Table 4). For every additional one-epitope mismatch, the chance of an adequate PCI decreased by 15%. There was no interaction between ABO blood group mismatching and treatment arm on one-hour PCI (Table 7, Supplemental File).

Reasons for non-trial platelet transfusions were most often due to non-availability of trial transfusions (n = 204; 54%) and requirement for a double dose of platelets (n = 159; 41%). A summary of non-trial transfusions is presented in Table 9 Supplemental file. There was no difference in the time interval between all platelet transfusions (trial and non-trial) between the two arms, with mean of 74.7 and 69.7 hours, p = 0.5929, for HEM and HSM transfusions, respectively.

HLA antibody specificity

HLA antibodies were more frequently directed against the more polymorphic HLA-B locus antigens compared to HLA -A and C. There was some evidence of 'epitope spreading' with

increased sensitization where there were more patients with antibodies directed against antigens from more than one locus in the highly sensitized group (Figure 3, panels A and B).

Epitope analysis: identification of target epitopes

In serum from highly sensitized patients (Figure 3 panel C), the following eplets were identified more frequently: 166DG eplet epitopes found on HLA-A1, A23, A24, A80, B15:12; 62EE eplet epitopes found on HLA- A23, A24, A80, and 144KR eplet epitopes found on HLA- HLA-A1, A3, A11, A23, A24, A36, A80. An epitope found on HLA-A23 and A24 formed from amino acid residues at positions 56, 65,66,76 and 152 within 15A was shared between eplet 62EE and 166DG and these antibody reactive antigens were more frequent in patients with cRF>85% (48%) compared to patients with a cRF<85% (21%). There were no antibody reactive antigens bearing shared eplets that were specific for a highly sensitized patient. However, only 4 (14%) patients typed as HLA-A*23 or A*24 in the highly sensitized group, compared to 5 (35%) with cRF<85%. Allele frequency of HLA- A*23 and A*24 (14%) in the highly sensitized patients appeared similar to that found in the general population (16%). [32] The highly immunogenic HLA-A*02 and HLA-B*07 also had similar allele frequencies between the patients and normal population, but eplets found on these antigens were not recognized more frequently in the antisera of highly sensitized patients.

The following case study illustrates the potential value of HEM in specific patients who are highly sensitized:

Patient 20005 has an uncommon HLA type in the UK population and is highly sensitized. HLA typing shows A*66:01, A*69:01; B*41:02, B*55:01; C*01:02, C*17:01, and the cRF is 100%. This patient received no A or B1 grade matches only B2 to B4 mis-matches but all epitope

selected platelets had less than 12 eplet mismatches (mean 9.6 eplet mismatches). In contrast, patient 10002 has an HLA type found more frequently in the UK population and although highly sensitized received well matched donations. HLA typing shows A*03:01, B*07:02, B*35:01, C*04:01, C*07:02, and the cRF is 92.58%. All antigen matched platelets issued for this patient were A grade and all epitope selected platelets had zero eplet mismatches. Patient 20005 benefited from an HLA epitope matching strategy utilizing the same panel of apheresis donors as used for patient 10002. Therefore, it would not be necessary to expand the HLA typed apheresis panel to try and recruit donors with the less common HLA types seen in patient 20005 if an epitope matching strategy is used for transfusing patients with immunological platelet refractoriness.

DISCUSSION

Summary of findings

Our trial has established non-inferiority in the one-hour PCI using HEM compared to HSM platelets. We analyzed a large number of evaluable platelet transfusions in a group of patients with myeloid disorders treated with either immunosuppressive therapy or chemotherapy. Baseline characteristics showed that the two treatment groups were balanced, including exposure to ABO incompatible platelets, and infections. We used the HLAMatchMaker epitope matching program with high resolution HLA typing to provide matching at the eplet level. The robust methodology of our cross-over design may also be applicable to other clinical studies aimed at evaluating different products in transfusion medicine.[33] We did not demonstrate longer transfusion-free intervals or reduced bleeding using HEM compared to HSM, even though we

Implications

We consider our findings have implications for practice, including HLA laboratories supporting the provision of platelets to alloimmunized patients. Limitations of standard strategies based on use of HSM platelets are multiple, including less accuracy as matching is performed at the antigen level; no guarantee that a full match will be found, especially if there are multi-specific HLA antibodies and/or a rare HLA type; it is also expensive, time consuming and labor intensive; and requires a large number of available HLA typed donors. We showed that HEM platelets were available for sensitized patients who might not have been classed as matched using standard antigen matching.

Additionally, using an HEM approach, it is possible to identify matched products that would have been missed by searching for HSM platelets, especially important in highly alloimmunized patients. For these patients with a PRA greater than 80%, identification of HLA matched platelets has been reported to be especially difficult.[10] Knowledge of the restricted HLA antibody and epitope specificity could enable a more cost-effective future approach of specific 'epitope' avoidance without the alternative approach of further increasing the size of a platelet donor pool. We also explored the impact of degree of mismatching at the eplet level on PCI, showing that a median of 3.2 mismatches more often produced an adequate PCI compared to a median of 5.5. Furthermore, for every-one mismatch, the risk of an inadequate PCI increased by 15%. When analyzed in further detail, we showed that HLA antibodies were most often directed against HLA-B locus antigens compared to HLA-A and C. We also show that certain eplets (166DG,

144KR and 62EE) were recognized more frequently among highly sensitized patients, specifically an epitope found on HLA-A23 and A24.

A previous retrospective study reported the majority mapped to the HLA-A molecule compared to HLA-B, and certain epitopes were immunodominant.[34] Although we found no interaction of degree of sensitization on PCI, we were able to provide HEM platelets for heavily sensitized patients, in whom matched platelets would not have been otherwise available when using standard serological matching. This is illustrated by the case study above demonstrating that the chance of finding HLA compatible platelets depends on patient HLA type and level of sensitization.

Our findings extend results of earlier studies by providing a more robust evaluation of different approaches for selecting platelets for transfusion. In a small study in AA, low numbers of triplet mismatches were associated with a higher chance of successful transfusion outcome.[21] Brooks et al[22] reported that 9 or fewer triplet mismatches or 11 or fewer eplet mismatches was a threshold for 'successful' transfusion. Pai et al used HLAMatchmaker for prospective selection of platelets for alloimmunized patients in a study of 19 patients.[23] If no suitable platelets could be found by serological matching, patients were randomized to receive either CREG-matched platelets or HEM (eplet) platelets. There was no difference in 24-hour CCIs between grade A HSM platelets, CREG-matched or HEM platelets, but the major limitation of this study was the small number of patients evaluated prospectively with HEM platelets (n=9). In a study of 20 alloimmunized thrombocytopenic patients, higher PCI using eplet matched platelets have been reported after platelet transfusion, except for those patients receiving HEM with a high eplet mismatch score.[24]

Limitations of our study should be recognized. Although only 49 randomized patients were randomized and of these only 14 received the total of 8 transfusions, the trial objectives were comparisons not only between patients, but also within patients, for each transfusion given, resulting in 219 adequate transfusions evaluated for the study. The interim analysis confirmed that the number of patients was adequate. Additionally, the small numbers of highly sensitized patients and interpretation is limited by the relative frequency of different HLA types in the patients and platelet donor groups. Many patients received platelet transfusions on the day when the platelet count was > 10 x 10^9/l, reflecting prior established individual need for regular prophylactic platelets to prevent bleeding, and all patients fulfilled the entry criteria at time of randomization. Finally, disseminated intravascular coagulation, fever, infection were not exclusion criteria as we did not want to exclude such patients. We addressed multiple factors in the sensitivity analysis.

In summary, this first-ever randomized trial supports the use of epitope matched platelets for HLA alloimmunized patients with platelet refractoriness, and may have benefits in terms of more efficient use of resources. Our design of study provides a more robust assessment of different platelet products for HLA alloimmunized patients. Management of heavily sensitized patients represents a major clinical challenge as they remain at high risk of serious bleeding since serologically matched HLA compatible platelets are less often available. A secondary objective of our study was to explore HLA matching of platelets in the context of highly sensitized patients or those cases where discrepancies were identified between results for identification of compatible platelet component between HSM and HEM platelets. We show that HEM platelet selection can help overcome this problem, and we demonstrate the importance of degree of mismatching in selection of platelet units. Review of our national data from the platelet

laboratory for NHSBT indicates that from 01/04/2019 to 31/03/2020 18,552 HLA selected platelets were issued. There were 908 (4.9%) platelets issued in patients with more than 2 mismatches. and it is these mismatched platelets where an epitope-based approach is being used at present to support more efficient matching (C Brown, personal communication). The alternative approach based on standard matching may require further increase in the platelet donor panel, with associated costs.

Authors' contributions

All authors critically reviewed the manuscript, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Specific contributions: Conception and design of the study, interpretation of the data, preparation of the manuscript (JM, SS, CN, CB), study management (CL, AD, ASM), acquisition of data (CP, JB, EL, AG, KH), data analysis (CC, LP) , data management (RH), data interpretation (CB, AM, GM). As corresponding author, JM had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

None of the authors have any conflicts of interest to declare.

Data sharing statement

Individual participant data that underlie all published HLA Epitope trial results will be available upon request from the National Health Service Blood and Transplant (NHSBT) Clinical Trials Unit after de-identification (text, tables, figures and appendices). Beginning 9 months and ending 5 years following article publication, data will be shared with investigators whose use of the data has been assessed and approved by an NHSBT review committee as a methodologically sound proposal. Data will be shared to achieve the aims in the approved proposal.

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Tables and Figures

Table 1 – Baseline characteristics of the study patients according to treatment group

Table legend

Pt=patient. Missing Data: There were no missing data.

<mark>At registration some participants did not require an immediate platelet transfusion but they met the eligibility criteria for the trial and it was known that their platelet count would drop</mark>.

Table 2: Primary and secondary outcomes

Table legend: Trfns = transfusions; [1] Unadjusted; [2]Adjusted for pre-transfusion platelet count, period effect and random participant effect; [3]Excludes two transfusions where pre-transfusion platelet count not reported; [4]The mean interval for each participant/treatment type was first calculated, and using these, an overall mean for each treatment type is presented. Only intervals less than or equal to 14 days in duration were included; [5] Adjusted for random participant effect; [6] Excludes four transfusions where pre-transfusion platelet count was not reported; [7] Adjusted ratio of geometric means of interval from HSM over HEM and corresponding 95% CI are reported.

Table 3: Number of epitope mismatches for HEM platelets and match grades for HSM platelets

Table legend

[1]Column percentages are shown. Mismatch was not reported for two evaluable and two non-evaluable HEM transfusions and grade was not reported for two evaluable and one non-evaluable HSM transfusion.

Table 4: Impact of HEM epitope mismatching on one-hour PCI

Table legend: Trfns=transfusion; [1] Adjusted for pre-transfusion platelet count, period effect and random participant effect; [2] Excludes 2 transfusions where number of mismatches was not reported.

Figure 1: Flow diagram showing process of patient recruitment and progress during trial

Figure 2 - CONSORT Diagram

Figure legend : Evaluable here means evaluable at one-hour post transfusion. Treatments are as randomized.

Figure 3: HLA antibody specification and target epitope identification

Figure legend: Panel A shows frequency of patient antibody specificities and degree of HLA sensitisation as defined by cRF of >80% versus <85%. Panel B lists the percentage of patients with antibodies directed against specific and groups of HLA antigens and according to the degree of HLA sensitisation. Panel C shows the amino acid composition of eplet epitopes more frequently found in highly sensitised patients.

Table 1 – Baseline characteristics of the study patients according to treatment group

Baseline characteristics									
Characteristic				Evaluable transfusions presented by randomised treatment					
		Patients		HEM			HSM		
		N	%	N	Median per pt	IQR per pt	N	Median per pt	IQR per pt
Total		**49**	**100**	**107**	**3**	**1-4**	**112**	**3**	**2-4**
Sex	Male	15	31	25	2.5	1-4	34	3	1.5-4
	Female	34	69	82	3	1-4	78	3	2-4
Age (years)	18-30	6	12	9	3	1-5	13	2	2-4
	31-50	13	27	27	2	1-4	26	2	2-4
	51-70	22	45	48	3.5	1.5-4	47	3	2-4
	≥71	8	16	23	4	3-4	26	4	3-4
Ethnicity	Caucasian	40	82	83	3	1-4	92	3	2-4
	Black	3	6	2	2	2-2	4	2	2-2
	Hispanic	1	2	6	6	6-6	4	4	4-4
	Asian	4	8	15	4.5	2.5-5	12	4	4-4
	Other	1	2	1	1	1-1	0	-	-
Diagnosis	Aplastic Anaemia (AA)	14	29	26	3.5	1.5-4.5	31	3.5	2-4
	Myelodysplastic syndrome (MDS)	9	18	21	4	1-4	22	4	4-4
	Acute myeloid leukaemia (AML)	26	53	60	3	1-4	59	3	2-4
Height (cm)	≤150	3	6	7	3.5	3-4	7	3.5	3-4
	151-160	17	35	49	4	2-4	47	4	2-4
	161-170	15	31	31	2	1-4	30	3	2-4
	171-180	9	18	15	3	1-3	17	2	2-4
	≥181	5	10	5	2	1-2	11	2.5	1-4.5
Weight (kg)	<50.0	2	4	6	3	1-5	5	2.5	1-4
	50.0 - <60.0	10	20	22	2.5	1.5-4	20	3	3-4
	60.0 - <70.0	9	18	16	3	2-4	18	2.5	2-4
	70.0 - <80.0	11	22	22	3	1-4	21	2	2-4
	80.0 - <90.0	13	27	34	4	2-4	41	4	3-4
	≥90.0	4	8	7	3.5	3-4	7	2	1-4
Platelet FBC result at trial registration (prior to first	0-10	12	24	20	2	1-4	27	2.5	2-4
	11-20	13	27	41	4	3-4	36	4	3-4
	21-30	11	22	19	2	1-4	19	4	4-5

Baseline characteristics									
Characteristic				Evaluable transfusions presented by randomised treatment					
		Patients		HEM			HSM		
		N	%	N	Median per pt	IQR per pt	N	Median per pt	IQR per pt
transfusion in the trial) (x10^9/L)	31-100	9	18	19	3	1-4	22	4	2-4
	≥101	4	8	8	3	1-4	8	2	1.5-2.5
Hb FBC results at baseline (prior to first transfusion in the trial) (g/dL)	7.0 - <8.0	5	10	15	4	1-4	16	4	4-4
	8.0 - <9.0	15	31	32	3	1-4	32	3	2-4
	9.0 - <10.0	18	37	40	3	2-4	39	3	2-4
	10.0 - <11.0	6	12	13	2	2-3	18	2.5	2-5
	≥11.0	5	10	7	3.5	1-6	7	2	1-4

Table 2: Primary and secondary outcomes

Primary and secondary outcomes				
	Intention-to-treat		Per-protocol	
Outcome	HEM	HSM	HEM	HSM
Platelet count increment at one-hour (10-90mins) post transfusion				
No. of evaluable transfusions	107	112	105	108
Mean (SD)[1]	23.9 (15)	23.5 (14.1)	23.8 (15.2)	24.0 (14.1)
Mean difference (95% CI)[2]	-0.1 (95% CI -2.9, 2.8)		-0.5 (95% CI -3.4, 2.4)	
p-value[2]	0.9686		0.7469	
Platelet count increment at 24 hrs (8-36hrs) post transfusion				
No. of evaluable transfusions	94	101	94	97
Mean (SD)[1]	12.2 (11.9)	12 (12.6)	12.2 (11.9)	12.2 (12.8)
Mean difference (95% CI)[2]	0.1 (95% CI -2.3, 2.5)		0.0 (95% CI -2.4, 2.4)	
p-value[2]	0.9193		0.9872	
Corrected count increment at one-hour (10-90mins) post transfusion				
No. of evaluable transfusions	107	112	105	108
Mean (SD)[1]	17.7 (11.0)	17.5 (10.2)	17.7 (11.1)	17.8 (10.2)
Mean difference (95% CI)[2]	0.1 (95% CI -2.0, 2.1)		-0.3 (95% CI -2.3, 1.8)	
p-value[2]	0.9468		0.8014	
Corrected count increment at 24 hrs (8-36hrs) post transfusion				
No. of evaluable transfusions	94	101	94	97
Mean (SD)[1]	9.1 (8.7)	9.0 (9.4)	9.1 (8.7)	9.2 (9.5)
Mean difference (95% CI)[2]	0 (95% CI -1.8, 1.7)		-0.1 (95% CI -1.9, 1.6)	
p-value[2]	0.9804		0.8850	

Clinically assessed bleeding scores pre-transfusion				
Total number of trfns with a score	151	149	148	146
Number trfns with score 0	99	85	96	83
Number trfns with score 1	29	37	29	36
Number trfns with score 2	23	26	23	26
Number trfns with score 3	0	1	0	1
Number trfns with score 4	0	0	0	0
Number of trfns with score >0	52	64	52	63
Odds ratio of a bleeding score of >0 (95% CI)[2,6]	0.74 (95% CI 0.42, 1.30)	1	0.79 (95% CI 0.45, 1.41)	1
p-value[2,6]	0.2920		0.4245	
Participant self-assessed scores 3 days post transfusion, or until the next platelet transfusion, whichever is sooner				
Number of participants with at least one score	34	31	34	30
Total number of transfusions with at least one score	93	106	91	103
Number trfns with max score 0	64	71	64	68
Number trfns with max score 1	11	19	10	19
Number trfns with max score 2	17	16	16	16
Number trfns with max score 3	1	0	1	0
Number trfns with max score 4	0	0	0	0
Number trfns with max score >0	29	35	27	35
Odds ratio of a bleeding score of > 0 (95% CI)[2,3]	0.94 (95% CI 0.47, 1.90)	1	0.84 (95% CI 0.42, 1.71)	1
p-value[2,3]	0.8653		0.6355	
Interval between trial platelet transfusions during treatment period				
Number of participants with at least 1 interval, less than or equal to 14 days in duration	41	39	40	38
Overall mean (SD) in hours[1,4]	94.0 (62.8)	97.3 (58.0)	96.3 (62.3)	99.8 (57.7)
Treatment effect (95% CI)[5,7]	0.95 (95% CI 0.78, 1.15)		0.96 (95% CI 0.78, 1.17)	
p-value[5]	0.5782		0.6634	

Table 3: Number of epitope mismatches for HEM platelets and match grades for HSM platelets

Number of epitope mismatches for HEM platelets and match grades for HSM platelets						
HEM			HSM			
Epitope mismatches	Transfusions		Grade	Number (%[1]) of transfusions		
	Evaluable trial (n=107)	Non-evaluable trial (n=97)		Evaluable trial (n=112)	Non-evaluable trial (n=95)	
N	105	95	A	57 (52%)	37 (39%)	
Median	3	2	B1	36 (33%)	34 (36%)	
Q1-Q3	0-6	0-6	B2	13 (12%)	19 (20%)	
Min-Max	0-15	0-16	B3	3 (3%)	3 (3%)	
Mean (SD)	3.6 (4)	3.7 (4.5)	B4	1 (1%)	1 (1%)	

Table 4: Impact of HEM epitope mismatching on one-hour PCI

Adequate one-hour post transfusion increment and number of epitope mismatches		
Outcome	Intention-to-treat HEM	Per-protocol HEM
No. of evaluable transfusions	107	105
Adequate one-hour increment		
Number of trfns (% of total number of evaluable trfns)	90 (84%)	88 (84%)
Mean (SD) no. of epitope mismatches	3.2 (3.6) [2]	3.2 (3.6)
Inadequate one-hour increment		
Number of trfns (% of total number of evaluable trfns)	17 (16%)	17 (16%)
Mean (SD) no. of epitope mismatches	5.5 (5.3) [2]	5.5 (5.3)
Odds ratio of adequate increment per epitope mismatch (95% CI)[1]	0.85 (95% CI [0.73,1.00]) [2]	0.85 (95% CI [0.73,1.00])
p-value[1]	0.0485[2]	0.0485

FIGURES

Figure 1: Flow diagram showing process of patient recruitment and progress during trial

Flow diagram of patient recruitment
Patient with diagnosis of AA or MDS or AML identified as potentially or already known to be refractory to platelets by PI or H&I Lab staff
Patient approached to participate in the trial
Patient consents
Blood collected for HLA type, HLA & HPA Ab screen
H&I Lab staff check eligibility criteria and assign randomisation schedule and patient number
Consent form sent to H&I Lab with request for HLA matched platelets and screening samples
Complete Bleeding Assessment pre-transfusion (day 0)
Patient receives HSM or HEM platelets according to randomisation schedule (day 0) - *except for emergency bleeding or invasive procedures*

After each transfusion

Patient given diary card to record any bleeding for the next 3 days (days 1-3)

After each transfusion

Blood for 1 and 24 hour platelet count
Patient completes 8 transfusions (4 x HSM and 4 x HEM)
Patient reverts to receive HSM platelets only
6 and 12 months follow up HLA and HPA antibody screen
Patient completes trial

Figure 2: CONSORT Diagram


```
                    ┌─────────────────────────────────────┐
                    │ Assessed for eligibility (n=135)     │
                    └─────────────────────────────────────┘
                                    │
                                    │──────────────► ┌──────────────────────────────────────┐
                                    │                │ Excluded (n=86):                     │
                                    │                │ - Not transfusion dependent (n=24)   │
                                    │                │ - Transfused locally (n=20)          │
                                    │                │ - Patient died/palliative/end of life│
                                    │                │   (n=9)                              │
                                    ▼                │ - Not alloimunised (n=9)             │
                    ┌───────────────────────┐        │ - Refused or unable to consent or    │
                    │ Consented and         │        │   comply (n=7)                       │
                    │ Randomised (n=49)     │        │ - Patient did not have eligible      │
                    └───────────────────────┘        │   disease (n=6)                      │
                                                     │ - Unknown (n=5)                      │
                                                     │ - Palpable splenomegaly (n=4)        │
                                                     │ - Receiving ATG treatment (n=1)      │
                                                     │ - Patient due to undergo a bone      │
                                                     │   marrow transplant (n=1)            │
                                                     └──────────────────────────────────────┘
```
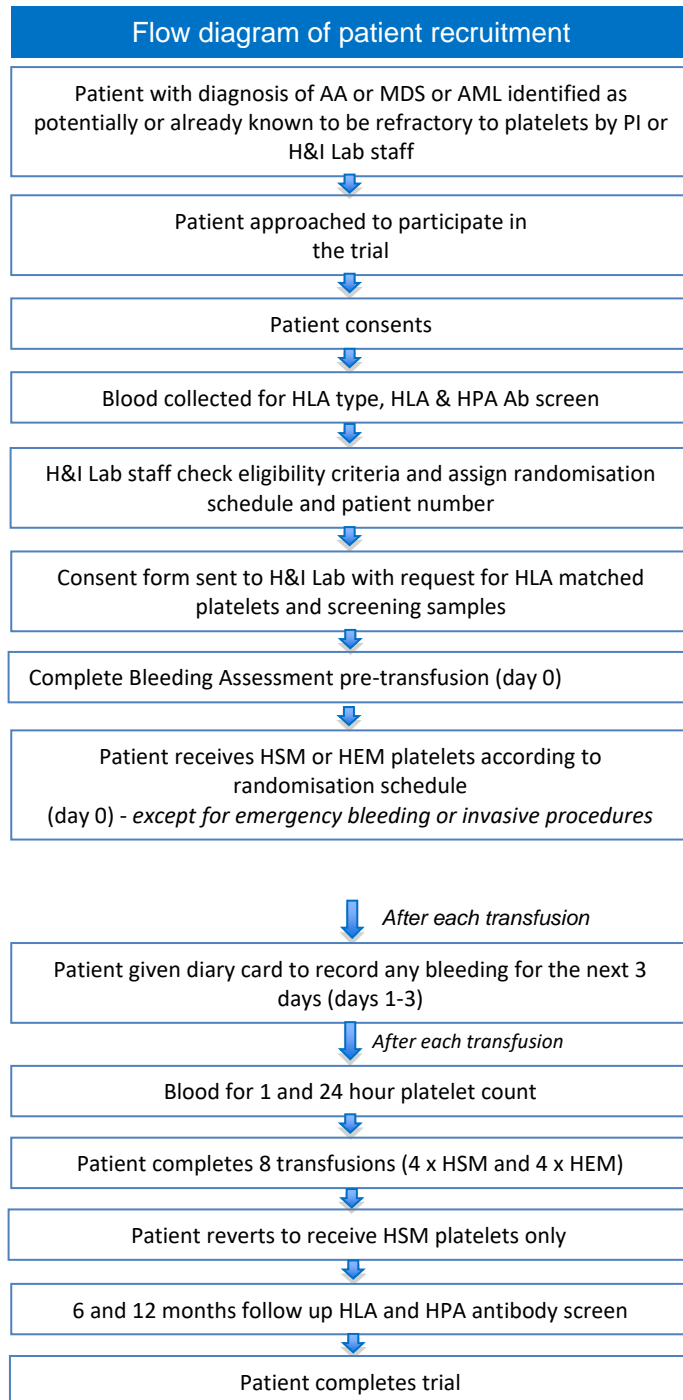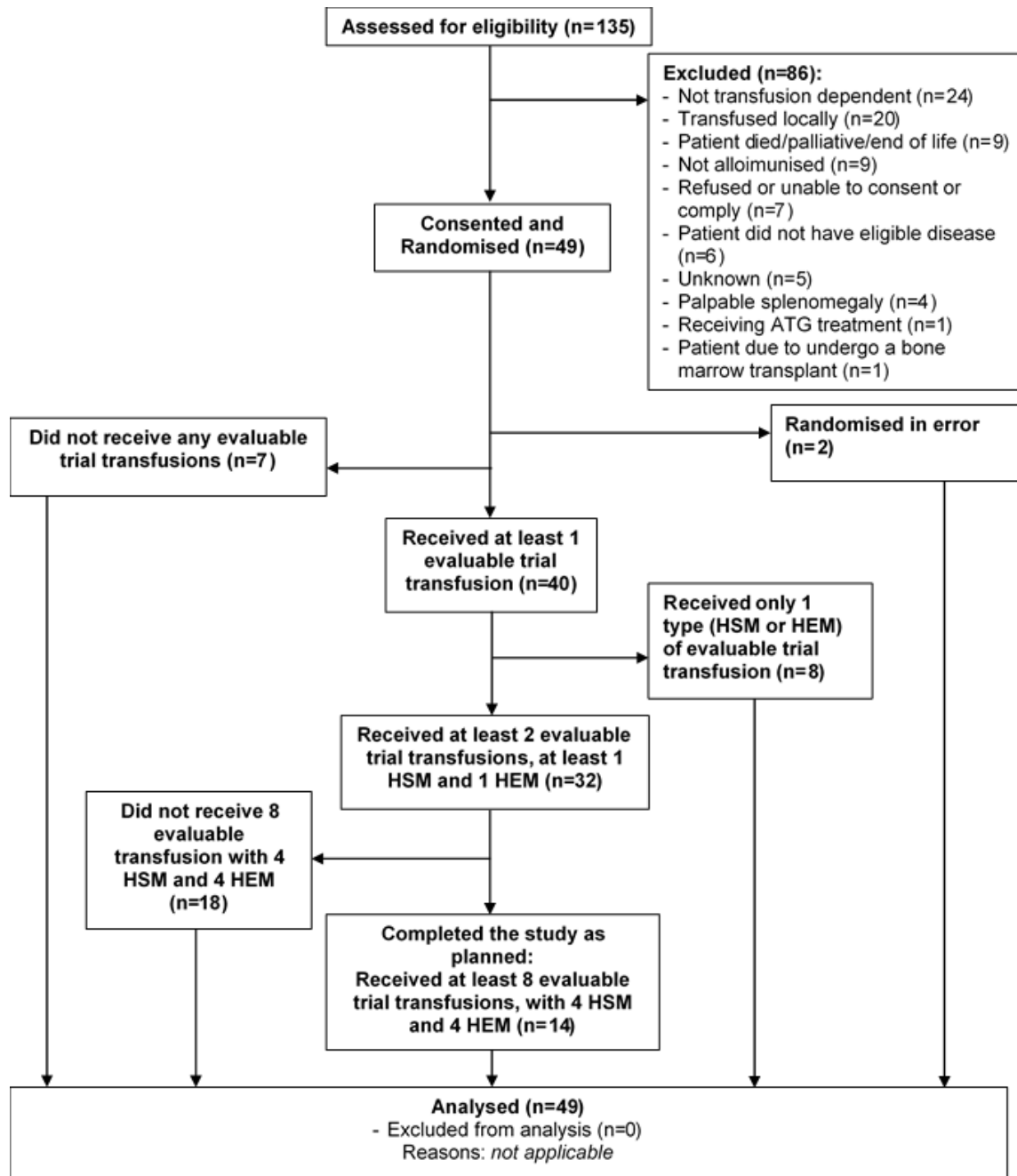
Note: Evaluable here means evaluable at 1 post hour transfusion. Treatments are as randomised.

Figure 3: HLA antibody specification and target epitope identification

A

Patient Antibody Specificities and HLA Sensitisation

B

Antibodies directed against HLA	Patient cRF<85%	Patient cRF>85%
HLA-A locus antigens	8%	9%
HLA-B locus antigens	31%	9%
HLA-A+B locus antigens	46%	57%
HLA-A+B+C locus antigens	15%	26%

C Amino acid residues within 15 Angstroms for eplets 166DG, 62EE and 144KR

		56	65	66	76	152	163	166	167
A*23:01	62EE	G	G	K	E	V	T	D	G
A*23:02	62EE	G	G	K	E	V	T	D	G
A*24:02	62EE	G	G	K	E	V	T	D	G
A*24:03	62EE	G	G	K	E	V	T	E	W
A*80:01	62EE	E	R	N	A	R	E	D	G

		56	62	65	66	70	105	109	158	163
A*01:01	166DG	G	Q	R	N	H	P	F	V	R
A*23:01	166DG	G	E	G	K	H	S	F	A	T
A*23:02	166DG	G	E	G	K	H	S	F	A	T
A*24:02	166DG	G	E	G	K	H	S	F	A	T
A*80:01	166DG	E	E	R	N	H	S	F	A	E
B*15:12	166DG	G	R	Q	I	N	P	L	A	L

		76	77	79	80	81	82	83	127	150	151	152	156	158
A*01:01	144KR	A	N	G	T	L	R	G	N	V	H	A	R	V
A*03:01	144KR	V	D	G	T	L	R	G	N	A	H	E	L	A
A*11:01	144KR	V	D	G	T	L	R	G	N	A	H	A	Q	A
A*11:02	144KR	V	D	G	T	L	R	G	N	A	H	A	Q	A
A*24:02	144KR	E	N	R	I	A	L	R	K	A	H	V	Q	A
A*24:03	144KR	E	N	R	I	A	L	R	K	A	H	V	Q	A
A*36:01	144KR	A	N	G	T	L	R	G	N	V	H	A	A	V
A*80:01	144KR	A	N	G	T	L	R	G	N	A	R	R	L	A