

Molecular immunohaematology round table discussions at the AABB Annual Meeting, Boston 2012

Willy A. Flegel¹, Susan T. Johnson², Margaret A. Keller³, Ellen B. Klapper⁴, Hanh M. Khuu¹, Joann M. Moulds⁵, Axel W. Seltsam⁶, Gary Stack⁷, Maryse St-Louis⁸, Christopher A. Tormey⁷, Franz F. Wagner⁶, Christof Weinstock⁹, Mark H. Yazer¹⁰, Gregory A. Denomme²

¹Department of Transfusion Medicine, NIH Clinical Center, National Institutes of Health, Bethesda, MD; ²BloodCenter of Wisconsin, Milwaukee, WI; ³American Red Cross, Philadelphia, PA; ⁴Cedars Sinai Medical Center, Los Angeles, CA; ⁵Lifeshare Blood Centers, Shreveport, LA, United States of America; ⁶German Red Cross Blood Service NSTOB, Springe, Germany; ⁷Yale School of Medicine, New Haven CT, United States of America; ⁸Héma-Québec, Québec, Canada; ⁹German Red Cross Blood Service Baden-Württemberg - Hessen, Institute for Clinical Transfusion Medicine and Immunogenetics Ulm, and University of Ulm, Ulm Germany; ¹⁰Institute for Transfusion Medicine, Pittsburgh, PA, United States of America.

Introduction

Well trained, experienced serologists in transfusion medicine laboratories have been familiar with blood group serology for decades. With the advent of molecular immunohaematology, there is a need to adopt and embrace the clinical and diagnostic applications in order for patients to benefit from the advances that this technology offers¹⁻³. We organised an international forum to discuss molecular immunohaematology concepts that may be challenging even for some established professionals in the field of serology.

The objectives of the session were two-fold. First, the session allowed networking among immunohaematology professionals who have an interest in the application of molecular immuno-haematology and blood group genetics while meeting with leaders in the field of molecular immunohaematology. By giving input and asking questions, the participants could define their knowledge relative to the experience of the group as a whole. Second, the discussions and the input from experienced professionals were recorded. This approach allowed documentation of current knowledge, as well as acceptance and concerns of the participants. It is useful to gathering information in this field, because the perception at the level of the participants is critical for shaping the adoption of molecular immunohaematology.

We provide a summary report of the items discussed and issues raised by the participants. The results describe the status of molecular immunohaematology within this large group of experienced professionals in transfusion medicine laboratories and can guide targeted educational efforts.

Materials and methods

Organisation of the discussion rounds

Transfusion medicine professionals gathered in the 1.5-hour session "Speed Dating for Molecular Immunohematology Professionals" (n. 9212-TC) on

October 7, 2012 from 10.30 am to noon at the AABB Annual Meeting & CTTXPO 2012 in Boston, MA, USA. The session was offered to any attendee of the conference, organised to allow networking among immunohaematology professionals, who had an interest in the application of molecular immunohaematology and blood group genetics, and designed using our experience from a similar session held in 2011. Suggested participants were physicians, scientists, technologists, and managers/supervisors.

Small groups of participants met with one chaperone per table to discuss a thesis statement for 10 minutes, before the chaperone moved to another table. Each thesis statement was represented by two chaperones, who attended two different sets of six tables. At the end of the session, the chaperones presented a brief summary of their discussion rounds.

The primary role of the chaperones was to listen to what the participants had to say about the thesis statement. Chaperones were to try to clarify questions and to keep the discussion on track. Finally, they carefully recorded the participants' comments related to the thesis statement. Chaperones were not to lecture as an authority on the subject matter.

The six theses statements were designed by the organisers in a deliberately open or even controversial fashion. This approach was chosen to stimulate discussion and raise the interest of the participants who were welcome to challenge the theses. Forty-two evaluation forms were submitted after the event which enabled the demographics of the participants to be determined (Table I).

Approximately 80 transfusion medicine professionals gathered with the 12 chaperones and two session organisers. There were five to nine participants per group at the 12 tables. There was great variation in the participants' background, which was challenging as

Table I - Demographics of the participants and evaluation.

Parameter and characteristics	Replies (n.)	Percentage
Level of experience		
1-5 years	4	11%
6 -10 years	4	11%
11-19 years	11	31%
20+ years	17	47%
Total	36	100%
Areas of specialty*		
Patient laboratory testing	16	43%
Molecular testing	8	22%
Education/training	6	16%
Clinical practice/patient care	5	14%
All other replies combined	14	38%
Position†		
Director/manager	13	34%
Physician	7	18%
Technologist/technician	5	13%
All other replies combined	17	45%
Relevance of content		
Excellent	30	79%
Good	8	21%
Other (fair, poor)	0	0%

*Other replies: Research and development (n=3); Cellular therapy, Donor product testing, Quality/compliance, Supplier (n=2); Administration, Blood collection, Other (n=1). Multiple replies possible; †Other replies: Chief/medical director, Lead/specialist, Supervisor/coordinator (n=4); Other (n=3); Scientist/clinical investigator (n=2). Multiple replies possible. Replies do not sum up to 42, because some fields were not answered on all forms.

well as stimulating. All participants had the opportunity to give input on the six thesis statements, which were designed as a starting point for the deliberation of the groups and were not to be considered conclusive statements. The thesis statements covered the following areas: (i) use of red cell genotyping in haematopoietic progenitor cell transplantation; (ii) non-ABO haemolytic transfusion reactions; (iii) repetitive genotyping in donors; (iv) clinical guidance when red cell genotyping is appropriate; (v) molecular-based matching ("dry matching") by genotype without attempting to determine the blood group phenotype; and (vi) proficiency programmes to include molecular immunohaematology. The six teams of two chaperones each provided the following summaries of their discussion rounds, which represent only the views of the participants.

Thesis 1. Red blood cell genotyping has direct application in cord/peripheral blood stem cell transplantation.

The majority of participants were not familiar with cord/peripheral blood stem cell transplantation. There were two opinions amongst the participants;

some were very familiar with the information that can be obtained from genotyping and were enthusiastic about performing it on all stem cell recipients. Other participants were sceptical regarding the usefulness of red cell genotyping data; the acceptance of such data in general clinical practice was not evident. Where the technology was available, concerns were expressed regarding re-imburement.

Participants who were familiar with transplantation at their institutions and did perform red cell genotyping indicated that it was not routinely performed. They saw a utility for red cell genotyping data in (i) resolving a mixed field picture for antigens with serology after complex transfusion history or with multiple antibodies^{4,5}; (ii) patients who have received more than one allogeneic graft, such as dual cord transplants, haploidentical donor products and cord blood products; and (iii) patient management in the post transplant period, such as with delayed red blood cell engraftment.

Cord and peripheral blood stem cell transplantation are frequently performed across ABO barriers⁶⁻⁸. Therefore, other blood group incompatibilities may not surface or be considered until red blood cell engraftment becomes an issue.

Among participants who were familiar with genotyping, there was no widespread enthusiasm for providing extended antigen-matched red blood cells to stem cell recipients. It was considered a general advantage that donor and recipient DNA samples may be more readily available than red blood cells. Also, DNA is very stable and suitable for long term storage (Chaperones: MHY & HMK).

Thesis 2. The incidence of deaths due to non-ABO haemolytic transfusion reactions is under-reported to the Food and Drug Administration

The majority of participants expressed the belief that fatalities secondary to non-ABO antibodies are being under-reported to the Food and Drug Administration (FDA). Possible reasons mentioned for this under-reporting included: (i) lack of knowledge/education among clinicians regarding haemolytic transfusion reactions; (ii) the possibility that such reactions are obscured by underlying medical disorders (e.g. liver disease, massive trauma, sickle cell disease)⁹; and (iii) complexities in reporting (and a possible lack of understanding of the mechanisms of reporting) fatalities to the FDA and biovigilance agencies among blood bank and transfusion service personnel.

Many participants were surprised to learn that non-ABO antibodies are currently the second most common cause of transfusion-related mortality in the USA¹⁰. Proposed aetiologies for mortality due to non-ABO antibodies were: (i) an inability to detect blood group

antibodies adequately because of insensitive techniques or unique antibody phenomena (e.g. antibody evanescence); (ii) movement of alloimmunised patients from facility to facility without adequate communication of their blood group antibody history; (iii) loss of expertise in antibody identification among blood bank technical staff in smaller, community-based hospitals; and (iv) increasing numbers of transfused patients, some of whom may be receiving unnecessary red blood cell transfusions.

Potential solutions to decrease the risks associated with non-ABO antibodies were discussed. These solutions included: (i) increasing educational efforts for clinicians and patients regarding the significance of non-ABO antibodies and haemolytic transfusion reactions; (ii) creation of local or regional non-ABO alloantibody registries; and (iii) extended serological phenotyping or molecular genotyping to avoid common, clinically-significant alloantibodies prior to transfusion¹¹.

Some drawbacks or obstacles associated with alloantibody registries included concerns about patient privacy, lack of regional facilities willing to coordinate these efforts and their associated costs, competing blood donor centres in close proximity that might be unwilling to share data with non-customers, and whether to rely on data generated by other facilities.

A major concern about the proposal of extended serological or molecular typing was choosing the populations of patients who may benefit from extended phenotyping or genotyping¹¹. Participants debated whether to match all patients or just those chronically transfused. Timing was also discussed as participants wondered whether blood banks should wait until a patient forms an antibody or rather match antigens prophylactically before alloimmunisation. One participant suggested that extended testing should be performed for all patients with delayed haemolytic transfusion reactions. There was little consensus on any of the above points. Other concerns included (i) which antigens to match for; (ii) costs associated with extended testing; and (iii) lack of adequate mechanisms to bill for these activities.

It was also unclear where molecular testing would be done (donor centre versus hospital transfusion service) if this technique were to be performed for large numbers of transfusion recipients. Many participants felt that there was a lack of molecular diagnostic knowledge among most blood bank technical staff in the community. Moreover, large-volume genotype send-out testing to donor centres did not seem feasible at present (Chaperones: CAT & EBK).

Thesis 3. Should all blood donations be genotyped each time, or are two genotypes on a donor sufficient?

Participants from European countries, where the technology is approved (CE-labelled), are using

molecular testing and are seeing benefit¹²⁻¹⁴. Some of the participants from the USA noted that current genotyping platforms are not approved in their country and, thus, blood cannot be labelled based on these methods; many favoured a resolution of this regulatory issue for easier access to molecular technology for donor as well as patient testing.

The majority of participants felt that a genotype performed twice on two different donations was sufficient for labelling products. Many participants pointed out the need to prove donor identity and felt that current methods were insufficient to allow for a single genotype. Hence, genotyping twice would allow for detection of sample identification errors or sample mix-ups. Genotyping more than twice was considered too expensive with little value added. The majority of the participants felt it was unnecessary to use two different genotyping platforms, while some suggested this might be appropriate for rare donors or when DNA and serology were discordant. The issues of how to label a unit when the two methods were discordant could not be addressed within the short time frame.

All groups put forth an alternative plan: one serological and one (concordant) genotype on two separate donations would be acceptable, which seemed to represent a wide consensus at this time.

There was discussion: (i) that only targeted donor groups as determined by the blood centre need to be genotyped rather than all donors; and (ii) that there is a need for a national genotyped donor database. Time did not permit exploration of the factors determining the characteristics of such donors or the feasibility of donor databases involving different blood donor services (Chaperones: JMM & STJ).

Thesis 4. A clinical guidance document should be developed to identify when testing for red blood cell genotypes is appropriate in the transfusion medicine setting

The development of a clinical guidance document was favoured by the vast majority of participants. Polling of individual participants revealed that 91% (67/74) supported guideline development, 5% (4/74) were opposed, and 4% (3/74) were undecided. The four participants opposed to guidelines were staff members of reference laboratories, who felt they already knew the indications. However, this sentiment was not universal among participants who worked at reference laboratories that currently perform red cell genotyping. A few participants, including those who opposed guideline development, expressed concerns about possible limitations and premature restrictions imposed by guidance at this time. In several situations such concerns were allayed by comments from other

participants indicating that guidelines by their nature are not meant to be mandatory or inflexible.

The indications for red cell genotyping most frequently cited as appropriate for inclusion in a forthcoming guidance were: (i) chronically-transfused patients, such as those with sickle cell disease, thalassaemia, myelodysplastic syndrome, or aplastic anaemia; (ii) multiply-alloimmunised patients; (iii) patients with autoimmune haemolytic anaemia; and (iv) rare donor search. A full list of appropriate, possible, and inappropriate indications for red cell genotyping proposed by the participants was tallied (Table II). Buccal swaps, often considered useful in this context, were not mentioned during the discussions. In only a few situations, such as trauma and surgery, was red cell genotyping felt to be inappropriate. Nevertheless, the consensus was that red cell genotyping of all patients who receive red blood cell transfusions was not indicated at this time.

Some divergence in opinion was expressed with regard to which blood donors should be genotyped for the purpose of identifying rare donors. One viewpoint was that only documented repeat donors (e.g. donors who have already donated two or three times) should be genotyped, since genotyping costs might be wasted on one-time donors. An alternative opinion was that all donors should be genotyped, since this increases the chance of finding rare donors and special efforts can be focused on recruiting such individuals to become regular, repeat donors. Other proposed approaches included red cell genotyping for group O donors only, since they would have widest applicability, or donors of specific ethnic origin, since that would increase the probability of finding particular rare genotypes. Time did not permit exploration of the factors that might affect which of these varied approaches a blood donor centre should take (Chaperones: FFW & GAS).

Thesis 5. The path to routine use of molecular "dry" matched transfusions will lie in the acceptance of genotyping as a result rather than its use to predict phenotypes

Genotyping of donors was generally considered to be feasible. Opinions varied regarding the extent of genotyping for molecular-based matching ("dry matching") purposes^{1,11}: requests spanned from "RH, KEL, FY, JK, and MNS" to "all blood group systems" and "sequence-based typing of known polymorphisms". The participants expected a psychological barrier for some serologists who are working with phenotypes in blood group serology for a long time and who may not easily accept dry matching as equal and safe.

Many participants were convinced that dry matching benefits patients with warm reactive autoantibodies or with a positive serological cross-match for other reasons: "Dry matched units" were considered superior to units that are "least incompatible" by serology. Except ABO, matching by serology and dry matching were considered similar regarding the risk of adverse events, such as immunisation or haemolytic reactions caused by mismatched transfusions due to failures in serology or molecular testing.

Comments regarding the use of molecular dry matching included: (i) safety issues may arise if unknown null alleles occur; (ii) the exceptional clinical relevance of the ABO blood group for transfusion and the existence of many null alleles in this system may increase the risk of severe haemolytic transfusion reactions if dry matched transfusions are performed; (iii) most participants felt uncomfortable with dry matching without prior antibody screening; and (iv) costs of genotyping at present were considered as a drawback.

Some participants feared that molecular dry matching may reduce the availability of compatible red blood cell units for some patients supported by conventional serologic matching, and advocated that dry matching should be reserved for chronically transfused patients.

Table II - Indications for red cell genotyping.

Appropriate	Possible	Not feasible at this time
Chronically-transfused patient*	Haematology-oncology patient	Surgical patient
Patient with multiple alloantibodies	Oncology patient (non-haematological)	Trauma patient at presentation†
Patient with autoimmune haemolytic anaemia	Patient with a single blood group alloantibody	All transfused patients
Patient with an alloantibody to a high prevalence antigen	Donor and recipient in hematopoietic progenitor cell transplantation	
Foetus in a mother with an alloantibody‡	Patient with advanced renal failure	
Search for donors with rare phenotypes	Patient with advanced hepatic failure	
Sample with a phenotyping discrepancy or difficulty		
Reagent red blood cell development and testing		

*For example, one with sickle cell disease, thalassaemia, myelodysplastic syndrome, or aplastic anaemia; †: consider buccal swaps in some situations; ‡: intrauterine foetal transfusions matched to the mother's antigens to prevent further alloimmunisation.

It was noted that different blood group genotypes will be compatible with each other at the phenotype level. Hence, there remains a need to translate genotypes into phenotypes or to sort genotypes into compatibility groups (Chaperones: AWS & CW).

Thesis 6. All red blood cell genotypes should be routinely tested in molecular testing proficiency programmes each year

All participants agreed that proficiency programmes should be mandatory and be routinely evaluated in molecular testing proficiency programmes each year. Specific questions that arose were how frequently the programmes be performed, on which types of samples, whether all analytes should be tested and who should be the overseer.

With regards to opinions on the frequency of the programmes, these ranged from once a year to four times a year. Most participants felt that several samples throughout the year that cover the most important antigens would be reasonable.

Which type of samples? The attendees involved in serological proficiency programmes categorised samples as representing "common" or "not rare" phenotypes. Some participants expressed the feeling that a genotyping proficiency programme should use samples of similar level of difficulty, while others felt that laboratories could get a "routine" sample with the option of requesting a "challenge sample". This could include antigens that are difficult to type serologically, such as the Dombrock antigens.

Should all analytes be tested? There was no consensus on whether or not every genotype should be tested. Some commented that any genotypes reported should be tested. Initially some participants said all analytes should be tested, however, after more discussion, the groups agreed that this would be unrealistic and unnecessary. Participants pointed out that HLA laboratories cannot be tested on their ability to detect every allele (as there are thousands). It was remarked that proficiency programmes are not meant to evaluate the methods (as this is the manufacturer's responsibility). Synthetic samples, such as plasmid DNA or oligonucleotides spiked into genomic DNA, could be used to cover a wider range of genotypes; however, participants pointed out that this does not simulate a "real-life" sample, which is the goal of a proficiency programme. Thus, synthetic samples might be better suited to assay development, controls and/or training material.

For laboratories using automated systems, it is easier to test for every genotype available. There was an opinion that proficiency participants should share information among users of the same technology, for instance array-based platforms. Most participants felt

that the most (clinically relevant) common systems and prevalent alleles should be tested including *RHD*, *RHCE*C*, *RHCE*c*, *RHCE*E*, *RHCE*e*, *KEL*01*, *KEL*02*, *FY*01*, *FY*02*, *FY GATA-1*, *JK*01*, *JK*02*, *MNS*03*, and *MNS*04*.

With regards to who should oversee the proficiency programmes, many participants preferred that samples and summary reports would be provided by an external professional organisation, for example AABB and CAP. The group was aware that no vendor proficiency test is available, but some participants expressed an interest in participating in one. If a sample comes from a vendor, one participant pointed out, the sample likely has proven to perform well on that platform previously. The participant commented that laboratories sometimes use proficiency summary reports to assess the failure rate when looking to purchase and that proficiency programmes involving vendors may not be an unbiased assessment of instruments or platforms (Chaperones: MAK & MSL).

Discussion

A forum in which the participants openly share their thoughts with their colleagues is an excellent environment for learning. Such networking can also forge professional relationships and collaborations. Molecular immunohaematology is a relatively new field for many immunohaematologists, and there is great interest in the clinical applications of this technology in the treatment of transfusion recipients. The evaluations indicated that we reached experienced professionals, who valued the exercise as very informative (Table I).

Several key clinical points emerged from this round table exercise, which we discuss here by bringing in the authors' views. First, a national registry of alloimmunised patients was proposed as one solution to address delayed haemolytic transfusion reactions^{15,16}. This proposal created concerns regarding patients' privacy and compliance with the Health Insurance Portability and Accountability Act (HIPAA). If we are to provide better care to transfusion recipients, our field needs to integrate these and related regulatory requirements¹⁶, which offer options for a shared national donor genotype registry beyond rare donors. Second, the development of a clinical guidance document that recommends which transfusion recipient population(s) to genotype was widely accepted (91%) as helpful and necessary. We tabulated the current indications considered by the group (Table II), which were rather extensive when compared to a similar list of indications from an expert group in 2000¹⁷. There were gaps in the understanding what the logistics of patient and donor genotyping could look like and which patient populations would benefit most. Is it valuable in patients

undergoing hematopoietic progenitor cell transplantation and, if so, what are the other groups of patients also likely to benefit? Third, the concept of one serological and one molecular typing on blood donors emerged as a powerful tool in the identification of donor antigens, as was the recommendation to perform the test(s) on two donations. Phenotyping donors at one donation and genotyping at the next seemed to provide a level of comfort to many participants. This concept was discussed in the context of labelling units with genotypes and the assurance that historical data are accurate. Improvements to donor identification came out as a theme to support such practice. There is a need to genotype first time donors and to identify rare units, which can be used for alloimmunised patients requiring matched red blood cell transfusions. Fourth, proficiency testing in molecular immunohaematology is lagging behind the need. As in the field of human leucocyte antigens, proficiency testing of every genotype every year is unnecessary. Proficiency programmes circulated twice a year seemed to be acceptable to the majority of participants, who also liked to share array samples among those who use platforms interrogating multiple targets simultaneously. The use of synthetic samples as proficiency samples was not encouraged because their design use does not reflect practice. Yet, the distribution of DNA samples does not challenge laboratories for a key part of their practice: the isolation of DNA from whole blood.

In the round table discussions, two cost-related themes became evident, which may be an underlying impediment to the implementation of molecular immunohaematology. First, participants raised concerns regarding reimbursement for molecular testing in Thesis groups 1 and 3 to 5. Molecular immunohaematology testing can be reimbursed through molecular Current Procedural Terminology (CPT) codes, when "research use only (RUO)" tests are applied to patients' care as "tests of high complexity" under the Clinical Laboratory Improvement Amendments (CLIA). How those codes are exactly applied to molecular immunohaematology was not evident to all participants, and guidelines in this regard would be welcome. Second, the cost of molecular immunohaematology testing seems to be paramount in the minds of some professionals, given widespread pressure to address healthcare costs and, specifically, to reduce laboratory costs.

What was lacking is an understanding and consensus that costs must be seen in light of the benefit to the patients. Many of the opinions expressed clearly indicated the benefit that molecular immunohaematology offers to a patient and yet sentiments were cautiously pessimistic on the issue of cost. How can we address obvious benefits to the patient, when they may be associated with a higher price tag in the laboratory?

It is an important consideration that none of the molecular immunohematology kits or platforms discussed in the session has been cleared or approved by the FDA. Any use of such devices for clinical purposes is, therefore, restricted in the USA, and the results are not intended as the sole means for clinical diagnosis or decisions regarding patients' management. Some of the devices have had the Conformité Européenne (CE)-label for many years, which implies that it is technically and legally possible, within the specifications of the CE-certification process for in vitro diagnostic devices in the European Union, to replace several blood group serology tasks by red cell genotyping. No information was communicated on the regulatory status outside the USA and European Union. Resolving regulatory issues would facilitate the implementation of the technology to improve patient care in the USA to a level that cannot be reached by any available serology alone.

On the topic of dry matching, the concept has gained theoretical understanding since participants expressed a concern that the practice would be equal to and as safe as serology. The concerns are valid and reminiscent of the time when the electronic cross-matching was first proposed. Solutions to the concerns can be provided by well-designed observational studies and clinical trials that document patient-donor genotype matching, in the absence of serological phenotyping, is at least as safe as current practice. Certainly, some participants stated that they use "dry matched blood" based on the concept that it is superior to commonly used "least incompatible blood". Concerns with the presence of null phenotypes seemed to recur in the adoption of dry matching in the near future. These concerns apply to the patient rather than the donor population and are addressed by continuously improving red cell genotyping.

An open forum discussion is a method of education and a way of learning new topics. This information-sharing approach allows professionals to understand other points of view and provides opportunities to connect with colleagues who otherwise may not seek their advice. Molecular immunohaematology has been adopted in reference laboratories and tertiary care transfusion services. Its widespread adoption needs careful attention to accepted clinical practice and assurance of safety. We should appreciate that increasing the benefit for transfusion recipients does not typically come free of cost. Continuing discussions among experienced professionals and peers on these issues are an important part of the implementation of molecular immunohaematology in the routine laboratory.

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Authorship contribution

Willy A. Flegel and Gregory A. Denomme designed the thesis statements; organised and moderated the session; and compiled the report. The six teams of two chaperones, who volunteered to participate, each wrote the summaries of their discussion rounds.

Statement of Disclaimer

The views expressed do not necessarily represent the view of the National Institutes of Health, the Department of Health and Human Services, or the U.S. Federal Government.

None of the molecular immunohaematology kits and platforms discussed in this forum has been cleared or approved by the Food and Drug Administration (FDA).

Conflict of interest disclosure

Franz F. Wagner and Willy A. Flegel receive royalties for RHD genotyping patents. Willy A. Flegel holds intellectual property rights for RHD genotyping and serves on the Scientific Advisory Board of Immucor (non-remunerated). Joann M. Moulds is principal investigator for a research agreement between LifeShare and Immucor/BioArray Solutions, serves as a consultant for Hologic/Genprobe and Novartis/Progenika and is on the Transfusion Medicine Advisory Board of Grifols. Gregory A. Denomme is a beneficiary of blood group genotyping patents in European countries. The remaining authors declare no competing interests relevant to this article.

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Correspondence: Willy A Flegel
 Department of Transfusion Medicine
 Clinical Center, National Institutes of Health
 Bethesda MD 20892, USA
 e-mail: bill.flegel@nih.gov
