Molecular immunohaematology round table discussions at the AABB Annual Meeting, Orlando 2016

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Introduction

At an international meeting, we offered round table discussions on six topics of red cell genotyping: one donor, patient, technical, and scientific topic each, plus two health care topics. Views were discussed among professionals in the field of blood group serology and genetics, and opinions polled for six current questions: anti-CD38 therapy and red cell genotyping; ethics of next generation sequencing for blood donors; ABO genotyping outside of routine applications; dry-matching in the absence of red cell antibodies; centralised *vs* hospital-based red cell genotyping; and implications if red cell genotype were available upon admission. We summarise the participants' input to our questions and discuss the topics in view of current literature. Round table discussions can raise the understanding among experienced specialists whose perception will shape the adoption of molecular immunohaematology to benefit patients and enhance patient safety.

Organisation of the discussion rounds

An international group of transfusion medicine professionals gathered in a 1.5 hour workshop "Roundtable Discussions for Molecular Immunohematology Professionals", offered to any attendee of the AABB Annual Meeting & CTTXPO 2016. The format of this workshop was similar to that of the previous four years, 2012 to 2015 (see WebResources)[1]: participants at a table met sequentially with six chaperones for ten minutes each to discuss the six topics in the form of a question; group sizes were four to eight participants at each of 11 tables. The participants remained at the table discussing successive questions while the chaperones moved from table to table. The chaperones, selected prior to the workshop, listened to participant viewpoints, clarified questions, took notes regarding the points raised and kept the discussion on track. All chaperone pairs consisted of a US and an international expert in the field.

During registration for the annual meeting, 26 individuals signed up for the session; 92 logged in on site and attended the workshop, returning 38 evaluation forms after the event (41% reply rate) (Table I). The participants hailed from 10 Countries, 32% representing non-US attendees, and reported a wide range of experience with molecular testing and its application in transfusion medicine. They reported working at Hospital Transfusion Services, Blood Centres and commercial entities.

Round table results

The chaperone teams provided the following summaries of their round table discussions, representing only the participants' views. All participants had the opportunity to provide input to each of the six questions, which, as in every previous year since 2012 (see Online Supplementary Content), were not distributed before the session commenced. Hence, the chaperones noted the input from participants who had had no opportunity to prepare answers or join the session for a specific question of their interest. This year, we also presented six definitions that are widely used in the field (Table II).

Question 1: How can molecular immunohaematology contribute to the care of patients treated with anti-CD38?

Transfusion medicine specialists expressed frustration that diagnosis of multiple myeloma or treatment with anti-CD38 (daratumumab [DARA]; immunoglobulin G, subclass 1, κ light chain [IgG1κ]) was not regularly communicated to the laboratory when testing was requested. Only a few of those patients were found to have alloantibodies, limited to the KEL, FY and JK systems. One participant reported DARA to

Table I - Demographics of the participants.

Parameter and characteristics	Replies (n)	Percentage
Level of experience		
1 - 5 years	11	29%
6 - 10 years	4	11%
11 - 19 years	10	26%
20+ years	13	34%
Total	38	100%
Position*		
Director/Manager	9	25%
Scientist/Clinical Investigator	6	17%
Lead/Specialist	5	14%
Supervisor/Coordinator	5	14%
All other replies combined	11	30%
Areas of specialty†		
Patient laboratory testing	16	42%
Clinical practice/Patient care	9	24%
Molecular testing	8	21%
Education/Training	2	5%
All other replies combined	3	8%
Relevance of content		
Excellent	30	86%
Good	5	14%
All other (Fair/Poor)	0	0%

*Other replies: Physician (n=4), Technologist/Technician (n=3), Chief/Medical Director (n=2), CEO and Resident/Fellow (n=1 each). †Other replies: Research/Development, Supplier of Products/Services, Other (n=1 each). Multiple replies possible. Replies may not sum up to 38, because some fields were not answered on all forms. Recorded countries of origin: the Unites States of America, Canada, Mexico, Brazil, Denmark, the Netherlands, Spain, China, Thailand and New Zealand.

Table II - Glossary

Allele
One of a number of alternative forms of the same gene, which can possibly result in a different phenotype.

Diplotype
A pair of (two or more) linked SNPs. Used in pharmacogenomics for special cases of a genotype or a pair of haplotypes.

Genotype
A pair of alleles occurring at homologous sites on paired chromosomes. Generally *1/*1 is considered "common", "normal", or "wild-type".

Haplotype
Variants of a nucleotide sequence (or of a combination of alleles) located close together on the same chromosome and usually inherited together (linked).

Phenotype
Observable properties of an organism produced by the interaction of genotype and environment.

Single nucleotide polymorphism (SNP)
Precise position along a chromosome where DNA of different people may vary by a single nucleotide; for example, a thymidine substituting for a cytosine.

See additional supporting information in the Online Supplementary Content of this article.

interfere with platelet crossmatching. One blood centre sent their physicians to educate the laboratory staff of its hospitals' customers.

Testing

Some participants relied on serology if DARA was not yet administered. After DARA or recent transfusions, many reported using red cell genotyping. Some preferred to obtain both phenotype and genotype results. The extent of red cell genotyping varied and depended on the available testing platform. Reimbursement was a problem for some participants; one US-based participant secured reimbursement using a CPT® code[1], and a non-US participant was reimbursed for all costs by her national health system. The longer turnaround time for red cell genotyping caused several participants to reluctantly rely on phenotyping. Dithiothreitol (DTT) treatment was the preferred approach[2], either as an in-house test or as a send-out to the reference lab, one participant noting requests every three days for patients during DARA treatment. Cord blood cells (known to have extremely low to no CD38 on their outer membrane surface) were recognised as a tool[3], but not widely available for use. Participants were eager to learn if DTT-treated red blood cells (RBC) could be stored[4], as most were finding daily DTT-treatment labour-intensive.

Transfusions

Only one hospital provided extended antigen-matched blood (RH, KEL, JK, FY, S) to patients on DARA treatment. Some participants reported providing K-positive blood to patients receiving DARA if customers did not request or reimburse K-negative blood. However, many provided K-negative blood if genotyping results are not available in time. Some participants switched back to blood that was not typed for the K antigen once their patients stopped demonstrating DARA-associated reactivity, which they noted could be one week to one month after DARA discontinuation.

Pharmaceutical manufacturer

Participants from larger institutions in the US and Europe reported that the impact of anti-CD38 on blood group serology was communicated well in advance by the vendor, while smaller hospitals were surprised when they encountered their first patient with DARA treatment. The participants noted that a soluble CD38 protein (sCD38)[2,5], typically comprising the extracellular domain, inhibits anti-CD38 and stated that ideally sCD38 would be supplied by the drug company along with DARA.

The participants anticipated an increase in patients with DARA treatment and stressed the need for hospitals to have policies in place regarding how to manage patient

samples and communicate the diagnosis and DARA treatment. (Patient topic. Chaperones: CW and MAK).

Question 2: How should we characterise blood group genes and alleles so that patients will benefit most from Next Generation Sequencing (NGS) data?

Almost all participants (96%) raised ethical concerns with NGS-based typing of blood donors. Many acknowledged that targeted NGS, limited to blood group systems, can lower this concern close to that of SNP-based typing assays, which are widely

applied in blood donors today. Participants felt strongly that the donor should be properly consented about incidental genetic findings if these can present clinical implications for the donor. The donors should also be able to indicate their preferences for being notified or not about incidental findings. The participants listed factors important for this disclosure (Table III).

Most participants acknowledged that the ethical concerns regarding incidental findings in donor testing are similar and pertinent to other areas of molecular testing. However, only one out of 46 respondents had previously heard of the American College of Medical Genetics and Genomics (ACMG) guidelines for reporting incidental findings from NGS data[6]. The ACMG guidelines consider incidental findings as unrelated genetic findings, not part of the original indication for testing. Among RBC antigens, several molecular changes are themselves associated with pathogenic conditions (Table IV)[7-34], but are identified as a direct result of testing and not incidental; these results would not come under existing ACMG guidelines.

Table III - Factors considered important for the decision to disclose incidental genetic findings.

Severity of disease
Treatability of the disease
Likelihood of developing disease
Implications for offspring
Culture differences

Table IV - Blood group systems known to be associated with pathogenicity.

Blood group system*				Pathogenicity or benign clinical phenotype		References
Symbol	Name	Number	Gene	Antigen or phenotype	Pathology or clinical phenotype	
ABO	ABO	001	*ABO*	A, B, O	Association with thrombosis, haemorrhage, infections, cancer, coronary heart disease, and preeclampsia. Resistance to severe malaria	7-9 10 11
MNS	MNS	002	*GYPA, GYPB, GYPE*	S−s−	Relative resistance to *Plasmodium falciparum* malaria	8,9
RH	Rh	004	*RHD, RHCE*	Rh_{null}	Haemolytic anaemia, often compensated	12
LE	Lewis	007	*FUT3*	Le(b+)	*Helicobacter pylori* receptor involved in gastric ulcers	7,13
FY	Duffy	008	*ACKR1*	Fy(a−b−) on RBC	Relative resistance to *P. vivax* and *P. knowlesi* malaria	8,14
JK	Kidd	009	*SLC14A1*	Jk(a−b−)	Reduced urea transport and urine concentration capacity	15
DI	Diego	010	*SLC4A1*	Di(a−b+)	Southeast Asian ovalocytosis	16,17
CO	Colton	015	*AQP1*	Co(a−b−)	Reduced water transport and urine concentration capacity	18-20
CH/RG	Chido-Rodgers	017	*C4A*	Ch(a−)	Association with systematic lupus erythematosus	21
XK	Kx	019	*XK*	Kx−	McLeod phenotype, acanthocytosis, McLeod syndrome	22-24
GE	Gerbich	020	*GYPC, GYPD*	Leach-type, Ge-	Elliptocytosis, ovalocytosis, reduced risk of severe malaria	8,25,26
RAPH	Raph	025	*MER2*	MER2 positive	Human Papillomavirus receptor	9
I	I	027	*GCNT2*	I− (adult i)	Congenital cataracts in some alleles	27
GLOB	Globosid	028	*B3GALT3*	P−	Parvovirus B19 receptor	28
RHAG	RHAG	030	*RHAG*	Rh_{mod}	Haemolytic anaemia, often compensated	29
JR	Jr	032	*ABCG2*	Jr(a−) = JR_{null}	Increased risk of gout in some variant alleles	30
LAN	LAN	033	*ABCB6*	Lan positive	Coloboma (eye development defect) in some alleles	31
CD59	CD59	035	*CD59*	CD59null	Neuronal defects, paroxysmal nocturnal haemoglobinuria	32
AUG	Augustine	036	*SLC29A1*	At_{null} and At(a−)	Ectopic calcification	33
Total		19 systems				

*The remaining blood group systems of the currently defined 36 systems are not known to be associated with pathology or clinical phenotype. The rarity of null phenotypes in some of them may still hint to a biologic relevance[34] and to unrecognised clinical consequences. Updated and modified from Flegel[34].

Participants were split as to how to develop guidelines for NGS in donor testing, with 60% saying they should be developed at the regional level (e.g. FDA, AABB, EU) and 40% wanting international consensus. The main reasons for developing the guidelines regionally were logistics and diverse cultural attitudes about genetic testing. (Donor topic. Chaperones: FP and WJL).

Question 3: What are current indications for *ABO* genotyping outside of routine transfusion practice?

Slightly more than half of the participants indicated there is a use for *ABO* genotyping (Table V), most commonly to evaluate serologic ABO typing discrepancies in routine practice for hospital patients or blood donors. Blood centres benefited if a serologic discrepancy was resolved and the donor could be retained despite a discrepancy. Most participants sent their *ABO* genotyping to specialised US or European laboratories, and few noted that such assays were available at their facilities. Participants who had never employed *ABO* genotyping cited lack of knowledge about where testing could be obtained and concerns about the complexity of result interpretation. Many participants from hospitals ultimately side-stepped ABO discrepancies by simply transfusing group O red cell units (Table V), although such approaches failed to resolve the discrepancy and tapped into precious supply more often than publically recognised. Some participants also noted that ABO

discrepancies were quite rare in their regions and populations, thereby limiting the need for extensive ABO-related serologic testing or red cell genotyping.

The participants had several interesting and thought-provoking ideas for *ABO* genotyping outside of 'normal' or routine requests. Indications that cannot be addressed by serology would expand the diagnostic possibilities and benefit the affected patients (Table VI). Use of *ABO* genotyping to establish foetal ABO types in mothers with a history of severe haemolytic disease of the foetus and newborn attributable to ABO antibodies could easily mimic what is currently done for non-ABO antigens using free foetal DNA in the mother's plasma, and become an important application of *ABO* genotyping in the future. Transfusion medicine specialists have encountered issues with compatibility testing for therapies such as DARA (see Question 1); with the continued development of drugs targeting cell surface antigens, it is conceivable that ABO antigens could eventually be impacted and *ABO* genotyping become a useful tool in such settings. In cellular therapies, *ABO* genotyping was potentially seen as useful for ABO typing of donors and recipients in complex cases, particularly cord blood units. One participant had used *ABO* genotyping to resolve an ABO typing discrepancy in the setting of organ transplantation for a donor who had been recently transfused with massive amounts of blood products. (Technical topic. Chaperones: CAT and MSL).

Table V - Commonly cited reasons for or against using *ABO* genotyping.

Used in practice?	Participants n.	Commonly cited reasons
Yes (at least once)	25	*For the use* - To resolve ABO type after massive transfusions - Discrepancies in ABO typing for and blood donors - ABO subgroup analysis in donors and recipients
No (never)	21	*Against the use* - Not widely available - Can always provide group O red blood cells - High complexity testing to perform and interpret - Regulatory hurdles
Total	46	

Table VI - Potential applications of *ABO* genotyping outside of routine practice.

Field	Potential applications
Transfusion medicine	Prediction of foetal ABO type in mothers with history of severe haemolytic disease of the foetus and newborn due to ABO antibodies Drug interferences with standard serologic ABO front and/or back typing
Cell therapy and transplantation	ABO typing in massively transfused organ donors ABO typing in complex stem cell transplantation cases Chimerism analysis in post-stem cell transplantation in complex cases
Solid organ transplantation	Rapid ABO typing in potential organ donors after massive transfusions
Pathology and medicine: other	Paternity testing in unusual circumstances Forensic testing in unusual circumstances

Question 4: Are there scientific reasons to consider dry-matching of antigens as beneficial for patients even in the absence of any detectable antibody to the cognate antigen or of any antibody at all?

We learned that the participants did not have much to say about how dry-matching could prevent antibody-independent, cell-mediated red cell lysis by non-ABO blood group antigens. We noted the comment that "most of us blood bankers are practical, cost-conscious people, who are less interested in speculation." Dry-matching describes the process of matching the blood group antigens of a RBC unit with a transfusion recipient using red cell genotypes only[35].

The clear consensus, voiced by about 80% of participants, was that dry-matching of non-ABO blood group antigens is beneficial even in the absence of detectable antibody for patients who receive chronic transfusions and are at high risk of alloimmunisation, such as those with sickle cell disease (SCD), thalassaemia, and myelodysplastic syndrome (Table VII). Virtually all participants believed that patients with autoimmune haemolytic anaemia (AIHA) also benefit, because of the difficulty that autoantibodies pose to serologic work-ups. Some also advocated for prophylactic dry-matching for transfusions given to females of childbearing age for antigens that are not routinely typed by serology.

Virtually no participant advocated dry-matching to avoid delayed haemolytic transfusion reactions (DHTRs) in sporadically transfused patients, although the scientific validity of the approach was acknowledged (Table VII). Some participants indicated that the alloimmunisation rates are too low to justify the effort and expense, and that the supply of antigen-negative RBC units would be limiting. One participant advised that resources should instead be devoted to improving RBC antibody data sharing between institutions (see Question 6 of the 2015 session[1]). Many felt that extensive genotype matching was impractical, because most donor databases could not support it. Some cautioned that RBCs with rare genotypes or phenotypes should be reserved for patients with the cognate antibodies.

Due to the current lack of evidence, no participant believed that dry-matching was indicated to potentially avoid clearance of antigen-mismatched, transfused RBCs by cell-mediated (Table VIII)[36-42], as opposed to antibody-mediated[2,42], mechanisms. (Scientific topic. Chaperones: GS and LC).

Table VII - Consensus among participants regarding dry matching in the absence of red cell antibodies.

Consensus	Clinical setting	Scientifically established benefit
Indicated	Chronic transfusion	Reduce alloimmunisation, acute and delayed haemolytic transfusion reaction, or hyperhaemolysis[60,61]; avoid costs to provide compatible RBC units[62]
Indicated	Warm autoimmune haemolytic anaemia	Reduce alloimmunisation and acute and delayed haemolytic transfusion reaction[35]
Possibly indicated	Females of childbearing potential	Reduce alloimmunisation and haemolytic disease of the foetus and newborn[35]
Not indicated*	Recipient of previous sporadic transfusion	Reduce delayed haemolytic transfusion reaction (antibody may have evanesced)[63,64]
Not indicated†	Recipient of transfusion	Prevent, in theory, antibody-independent, cellular cytotoxicity and red cell lysis[65]

*Cost-effectiveness questioned[64]; †Lack of scientific evidence[41]; RBC: red blood cell.

Table VIII - Likelihood of antibody-independent, cell-mediated haemolysis due to mismatched blood group antigens.

Cell type*	Antibody-independent, cell-mediated haemolysis			Other features	
	Likelihood	*Requirement for target cell*	*Reason*	*Somatically-rearranged antigen receptor†*	*Immunologic memory ‡*
CD8+ cytotoxic T cell[36]	Questionable	Must express HLA class I	RBCs have very low expression of HLA class I[37], except Bga (HLA-B7), Bgb (HLA-B17), and Bgc (HLA-A28)	Yes	Yes
CD4+ cytotoxic T cell[38]	Not possible	Must express HLA class II	RBCs lack HLA class II	Yes	Yes
Natural killer cell[39]	Highly unlikely	Typically virus-infected or tumour-derived	Cannot generate receptor diversity necessary to recognise non-self blood group antigens; no evidence of direct binding to blood group antigens; ADCC required	No	Limited[40]
Monocytes - macrophages[41]	Not possible	Typically carries antibody or complement	Cannot generate receptor diversity necessary to recognise non-self-blood group antigens; no evidence of direct binding to blood group antigens; ADCC required	No	No

*Other potential effector cells are granulocytes and dendritic cells[42]. †The presence of recombination activating genes along with some other enzymes is required for the recombination events that permit the generation of large diversity in T-cell receptors, which in turn allows T cells to recognise virtually any foreign peptide. ‡Immunologic memory is necessary for secondary (anamnestic) immune responses, such as those that mediate delayed haemolytic transfusion reactions. RBCs: red blood cells; ADCC: antibody-dependent cellular cytotoxicity.

Question 5: Should red cell genotyping for patients be centralised or should the technology be advanced such that any hospital transfusion laboratory could perform the tests?

A majority of participants felt such testing should be centralised, while a few believed it could be decentralised. Several participants expected red cell genotyping to be performed in every hospital, depending on assay availability and costs. At least one-third of the participants had the technology available in their laboratories and a few were looking to establish it. The majority were not going to establish the technology and sent their samples instead to reference laboratories. Work on patient cases in hospitals invariably started with serology and, as necessary, later reflexed to red cell genotyping: some hospitals required physician approval for that and others did not.

Regional blood centres increased their test volumes by adding donor samples to patient testing runs. Hospital-based laboratories without this option limited testing runs to one or two days per week. Several countries including Canada, Spain, Denmark and Brazil have centralised their genotyping to a few laboratories in collection centres or large regional hospitals. (Health care topic. Chaperones: DAW and QC).

Question 6: What would be the implications for a health care system (or your hospital) if red cell genotyping is always available at the time an antibody identification is ordered?

Most participants felt that red cell genotyping can replace serologic typing in most circumstances. They would gladly use historical genotyping data without the need to retype a patient or a red cell unit, as long as fully validated methods and record keeping were applied. All participants felt it was acceptable to use red cell genotyping results to confirm or refute the identity of an alloantibody. Genotyping patient samples would help guide work-up for potential antibodies, and be used to provide matched blood to prevent alloimmunisation in patients likely to require long-term transfusion support. These patients would include those with sickle cell disease, myelodysplastic syndrome and patients receiving DARA (see Question 1).

Participants from some immunohaematology reference laboratories genotyped all samples referred for work-up and supplied such information to their clients. Participants from hospitals performed or requested red cell genotyping only on selected cases due to cost concerns. Hence, should the cost for genotyping be reduced, universal genotyping might become accepted practice. All participants agreed that information management systems need to catch up with the increased demands necessitated by red cell genotyping. This includes not only the increased amount of information gathered for a "blood type", but also the requirements that systems are available to share information and thus avoid the expense of repeat testing.

Red cell genotyping was widely preferred in certain circumstances, if not uniformly performed: samples with a positive direct antiglobulin test, presence of warm autoantibodies, recent transfusion[43], difficult to interpret serologic reactions, and patients with haemoglobinopathies. There was a general consensus that in these circumstances genotyping is very cost-effective, as more information is obtained with less labour and cost than by applying serologic methods. In addition, the increased cost would be justified if genotyping could lead to provision of higher quality blood for transfusion, particularly for patients with sickle cell disease or thalassaemia. (Health care topic. Chaperones: EBK and SW).

Discussion

Participants came from 10 Countries and worked in Hospitals, Blood Centres, and Industry; their responses may have varied based on these demographics (Table I). We noted an example in which the outcome[44] from one of our previous round tables has adjusted the direction of research[45]. Discussions among experienced specialists, as facilitated by the current workshop, may soon shape the adoption of molecular immunohaematology to benefit patients and enhance patient safety.

Topic 1. Anti-CD38 therapy and red cell genotyping

DARA is a monoclonal antibody approved by the Food & Drug Administration (FDA)[46] in the US and the European Medicines Agency (EMA) in the EU as a second-line drug for multiple myeloma treatment. The antibody binds CD38, highly expressed on myeloma cells. As RBCs also carry some CD38, antibody screening and crossmatching in patients receiving DARA may show positive reactivity[2,5]. This interference can be circumvented by use of DTT, DTE (dithioerythritol) and AET (2-amino-ethyl-thio-isouronium bromide hydrobromide) to reduce, or trypsin to cleave, CD38 on test cells[2],cord blood cells[3], anti-idiotype antibody[2,5], and sCD38[2,5]. Or the cost-efficient[47] transfusion of antigen- or dry-matched units can be utilised[47,48]. Despite a broad discussion covering more than one year[5,48-50] of how to communicate pertinent patient information to laboratories, improving this communication between the patient care team and the hospital laboratory still remains the key element for expediting patient testing in the interest of safety and cost containment. Recommendations on preparation and storage of DTT-treated[4] and cord blood cells[3] have been published.

Topic 2. Ethics of next generation sequencing for blood donors

Next generation sequencing (NSG) is a widely applied method in research and clinical settings, repeatedly sequencing DNA stretches of several hundred base pairs, to yield precise results over large segments of the genome[51-54]. Starting in 2011[55], NGS has been successfully applied to blood group genes[56,57] and viewed as a promising development[52,54]; red cell antigen prediction is feasible using whole genome sequencing (WGS) data derived by NGS[58]. Identifying 'rare' donors has been considered a legitimate donor motivation, retention and recruitment tool (see Question 5 and Table VI of the 2014 session[44]), and NGS could massively contribute to this goal.

The participants were interested to learn of the ACMG guidelines for reporting incidental findings from NGS data[6] and considered how to apply its recommendations to blood donors. Incidental (secondary or "off target") findings are typically unrelated to the original indication for genotyping, for example, performing whole exome sequencing for a metabolic syndrome and finding a non-metabolic gene variant associated with another disease[6]. In contrast, RBC results will always be "on target", as long as we test blood group genes only. Although our diagnostic indication (blood group typing) may reveal RBC antigen changes that are themselves associated with pathogenic conditions (Table IV)[7,8], 17 of the currently defined 36 systems are associated neither with clinical symptoms nor with known advantageous traits[34], and some "unanticipated findings" may have positive clinical aspects, such as an incidental Gerbich null phenotype (Ge:-2,-3,-4) conferring resistance to severe malaria (Table IV)[25].

The blood group systems no. 001, 002, 004, 007 to 009, and 015, often covered by today's red cell genotyping platforms, do not cause pathogenicity or their incidental findings express clinical phenotypes that are common[7,8,13], desirable[8,11], mild[12,30] and of low, incomplete penetration[7,8]. The tabulation of pathogenicity (Table IV) convincingly documents no ethical concern for any of these 7 systems which are also not encountered by using common serologic techniques (of course, permitted under current donor consents). Time constraints did not allow us to explore why an additional donor consent should be required for applying targeted NGS typing to these 7 blood group systems.

Many of the remaining 29 blood group systems (typically not covered by today's platforms) do pose ethical concerns (Table IV). One example was the McLeod phenotype of blood group system 19[24]. The most extreme exception of substantial ethical concern is the newly recognised blood group system 35 (CD59)[32] associated with untreatable severe neurologic defects and early death. Autonomy will be preserved if the donors (or patients) are informed which incidental findings might result with NGS; they then must be offered the right to decline if, in their view, possible findings may outweigh the benefits of blood donation or testing.

The ACMG guidelines address mostly autosomal dominant traits with severe clinical consequences. Most blood group variants (Table IV) are autosomal recessive traits, and findings may often be more relevant to the offspring than the donors. These ethical concerns are rather special to our field and are predicted to affect an increasing number of donors. We have to respect and honour the concerns (see Question 2 and Table II of the 2015 session[1]) and work with our peers and the donors to resolve the concern and find ethically sound solutions. New NGS-based blood group and blood donor typing guidelines would need to either be created or modified from the existing ACMG guideline[6].

Topic 3. *ABO* genotyping outside of routine applications

Only one-third of the participants had previously reported using *ABO* genotyping (see Question 3 of the 2015 session[1]) compared to more than half in 2016 (Table V). Overall, participants were very enthusiastic about potential applications of *ABO* genotyping for both routine and novel indications. Limitations to the widespread adoption were nonetheless persistently perceived and mentioned at almost every table, including the cost-effectiveness when compared to routine serologic methods; the fact that *ABO* genotyping is not widely available, as it is highly complex to perform and interpret; and the significant regulatory hurdles to performing the testing routinely at hospitals or blood donor centres (Table V).

The participants generated several ideas for important applications of *ABO* genotyping, if it were widely available, many that are not routine today (Table VI). Nearly all participants agreed that a rapid turn-around time[45] was the most critical step (see Question 4 of the 2014 session[44]) toward a widespread adoption of *ABO* genotyping in clinical practice. There are applications of *ABO* genotyping in potential organ donors after massive transfusion with group O blood, and confirmation of A1 status in organ donors with ambiguous A1 lectin typing. *ABO* genotyping could be utilised as a screening tool, without much regulatory burden if not used as a test of record.

Topic 4. Dry-matching in the absence of red cell antibodies

Identifying donors with rare phenotypes or rare combinations of a large number of antigen-negative types using red cell genotyping is now routine at large blood centres and been shown to be economically

preferred[59]. According to most participants, dry-matching provides relevant clinical benefit in certain clinical settings, such as chronic transfusion, by reducing the incidence of alloimmunisation (Table VII). For example, patients with SCD and alloimmunisation have decreased survival compared to non-alloimmunised SCD patients[60]; in a recent case series[61], adverse and fatal outcomes were associated with alloimmunisation, including DHTRs. Red cell genotyping aids in finding compatible RBCs[62]. Clinical evidence indicates that we miss the majority of immunisation events[63,64] involving antibodies that cannot be detected by current crossmatching methods due to the limited sensitivity of antiglobulin techniques. Participants did not believe the scientific benefit of dry-matching for sporadically-transfused patients was compelling, despite evidence that most alloimmunisation goes undetected[63,64].

Theoretically, dry-matching could prevent antibody-independent, cell-mediated red cell lysis by non-ABO blood group antigens. While there is little evidence for this, non-antibody mediated, mechanism to lyse RBCs, sensitised CD8+ cytotoxic T cells, for example, can clear HLA-mismatched platelets in mice in the absence of antibodies[65]. Similar cell-mediated clearance of RBCs by CD8+ T cells seems unlikely, because RBCs express very low levels of HLA class I antigens[8] (Table VIII). CD4+ cytotoxic T cells, natural killer (NK) cells or macrophages seem even less likely to mediate antibody-independent immune clearance of transfused RBCs. The lack of enthusiasm expressed by participants for viewing dry-matching as a scientific benefit to reduce antibody-independent, cell-mediated clearance of RBCs is, therefore, actually supported by current knowledge.

The effect and its clinical benefits cannot, however, be ruled out, offering an intriguing and clinically relevant research topic for *in vitro* and pragmatic clinical trials. We noted that the possibility of antibody-independent, cell-mediated red cell lysis has not received much attention in the past, at least as a discussion point among transfusion specialists.

Topic 5. Centralised *vs* hospital-based red cell genotyping

The sentiment "molecular genotyping of patients must be centralised to reduce costs" published by Manfroi and Pagliaro[66] was in fact shared by the majority of the participants and implemented in their laboratory setups. Obstacles to adopting red cell genotyping included the test volume to sustain the platform, personnel to perform the testing and, more importantly, knowledge to interpret the results. The setup costs for additional equipment and training were also mentioned. One participant named turnaround time as a critical factor. There was concern for space, but the need for a clean room or designated space for pre-PCR steps was not actively raised. As technology advances, the current preferences for utilising centralised[66] and reference laboratories[67,68] may evolve into point-of-care testing in every hospital[45], which in urgent situations[44] is the only way to serve a patient in need of this critical technology.

Topic 6. Implications if red cell genotype were available upon admission

This topic generated a very lively discussion. We noted a general consensus that in circumstances such as sickle cell disease, genotyping was considered cost-effective, while published calculations implied a lack of cost-effectiveness[69]. The reuse of previously obtained red cell genotyping data for patients was commonplace, where data integrity was assured. The previously accepted reuse of donor genotype data (see Thesis 3 of the 2012 session[70]) was now also widely applied to patient genotype data. When phenotyping is impossible due to a positive direct antiglobulin test or recent transfusion[43], red cell genotyping was widely, if not uniformly, performed and seemed to replace the requirement for phenotyping. While organisational structures to reduce costs are important considerations[66], most participants would justify incurring higher costs if genotyping could lead to provision of higher quality transfusion products. Red cell genotyping is a potent tool to ensure a better quality and effectiveness of transfusion therapy[66] over the use of blood group serology alone.

Authorship contributions

WAF directed and GAD moderated the workshop; both designed the topics and questions. The six teams of two chaperones, who volunteered to participate, each wrote the summaries of their discussion rounds. WAF compiled and wrote the report.

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 US Federal Government. In the U.S., the first molecular immunohaematology kit was approved for clinical application in 2014[71]. Special considerations and disclaimers are required in the U.S. and elsewhere for off label use of approved red cell genotyping kits and for using "research use only (RUO)" kits in clinical applications[1].

Disclosure of conflicts of interest

EBK receives research support from Immucor. GAD is on the speakers' bureau of Grifols. WAF holds intellectual property rights for RHD *genotyping applications. WJL receives support from Wiley Publishing for educational activities related to the Transfusion News Question of the Day[1]. The remaining Authors have no competing financial interests relevant to this article to declare.*

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Web Resources
Molecular Immunohematology Roundtable Discussions 2012 to 2015. Available at: http://go.usa.gov/x89sd/. Accessed on 11/11/2016. Transfusion News Question of the Day:1. Available at: http://transfusionnews.com/path-questions/?q=HBwqUjm3. Accessed on 7/7/2017.

Supporting information
Additional supporting information may be found in the Online Supplementary Content of this article: Panel. 9313-TC Roundtable AABB 2016 Definitions.

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