2017-10

Red cell genotyping precision medicine: a conference summary

Denomme, GA

http://hdl.handle.net/10026.1/10282

Red Cell Genotyping 2015: Precision Medicine, Conference Summary

Gregory A Denomme[1], Waseem Q Anani[1], Neil D Avent[2], Gregor Bein[3], Lynne B Briggs, Razvan C Lapadat[1], Celina Montemayor[4], Maria Rios[5], Maryse St-Louis[6], Lynne Uhl[7], Silvano Wendel[8], Willy A Flegel[4]

From the [1]Diagnostic Laboratories and Medical Services Institute, BloodCenter of Wisconsin, Milwaukee, WI, [2]University of Plymouth, Plymouth, UK, [3]Justus-Liebig-University, Giessen, Germany, [4]Department of Transfusion Medicine, NIH Clinical Center, National Institutes of Health, Bethesda, MD, [5]Office of Blood Research and Review, CBER/FDA, Rockville, MD, [6]Héma-Québec, Québec, Canada, [7]Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, and [8]Hospital Sirio Libanês, São Paulo, Brazil

Address for correspondence: Gregory A Denomme, PhD, FCSMLS(D), Diagnostic Laboratories, BloodCenter of Wisconsin, 638 N 18th Street, PO Box 2178, Milwaukee, WI 53201-2178, phone: 414-937-6440, fax: 414-937-6404, email: greg.denomme@bcw.edu

Statement of Disclaimer: The views expressed do not necessarily represent the view of the National Institutes of Health (NIH), the Department of Health and Human Services (DHHS), or the U.S. Federal Government.

Authorship contribution: GAD and WAF organized the scientific content of the symposium and this review. The speakers provided summaries of their presentations. GAD compiled the report and WAF provided the edits.

Conflict of interest disclosure: GAD is the inventor of patents on red cell genotyping owned by the Canadian Blood Services and on the authorship and speaker's bureau of Grifols. NDA is a consultant for Grifols. GB is an inventor of a device patent and has contract research with BAG Health Care. WAF is inventor of patents owned by the German Red Cross Blood Service Baden-Württemberg-Hessen and holds intellectual property rights for *RHD* genotyping applications. The remaining authors declared no conflict of interests.

Running title: Precision Medicine by Molecular Immunohematology

Key Words: precision transfusion medicine; molecular immunohematology; red cell genotyping

Abstract word count: 118 words   **Word count:** 6108 words   **Tables:** 2   **Figures:** 3

Abstract

This review summarizes the salient points of the symposium "Red Cell Genotyping 2015: Precision Medicine" held on September 10, 2015 in the Masur Auditorium of the National Institutes of Health. The specific aims of this, the 6th annual symposium were to: 1) discuss how advances in molecular immunohematology are changing patient care, 2) exemplify patient care strategies by case reports (clinical vignettes), 3) review the basic molecular studies and their current implication in clinical practice, 4) identify red cell genotyping strategies to prevent alloimmunization, and 5) compare and contrast future options of red cell genotyping in precision transfusion medicine. This symposium summary captured the state-of-the-art of red cell genotyping and its contribution to the practice of precision medicine.

Introduction

Molecular immunohematology is gaining clinical application in transfusion medicine. To focus this field of immunohematology, a conference was initiated in 2011 entitled Red Cell Genotyping: At the Tipping Point. After that conference, BloodCenter of Wisconsin and Department of Transfusion Medicine, NIH Clinical Center have co-sponsored an annual red cell genotyping symposia since 2012. Several molecular immunohematology applications are now firmly established.

Personalized medicine is the use of diagnostic tests to determine which medical treatments will work best for an individual patient [1]. As personalized medicine is sometimes misinterpreted as implying that unique treatments can be designed for each individual, precision medicine refers to the tailoring of standard medical treatment to the patient taking individual variability into account [2]. It is not intended to mean the development of drugs or treatments for a specific patient, but rather applying the concept from the human variability obtained from information in biologic databases, such as genomics, and to use them to guide clinical practice. The US President initiated a Precision Medicine Initiative [3] and the National Institutes of Health has been supporting the concept through research on prevention and treatment strategies [2]. Hence, the Red Cell Genotyping 2015 symposium adopted the theme to exemplify how molecular immunohematology has been implementing precision medicine in clinical care to enhance patient safety.

The 2015 symposium reviewed applications in molecular immunohematology to benefit the patient. Clinical vignette presentations showcased transfusion recipient or obstetrical cases aimed to underscore challenging situations that were solved by applying molecular

immunohematology. Mass-scale genotyping of blood donors, the perspective on weak D testing, and quality assurance initiatives in the US and Europe, all provided a perspective on state-of-the-art testing and current developments. A summary of an international perspective on molecular immunohematology provided a timely conclusion to the program. This review summarizes the symposium proceedings documenting the state-of-the-art and the progress in the field of red cell genotyping.

Molecular immunohematology in the era of precision medicine: an overview (Lynne Uhl, Beth Israel Deaconess Medical Center and Harvard Medical School)

The concept of personalized medicine is not new; following President Obama's call out in his State of the Union address in 2015 supporting the scientific endeavors targeted toward improved health care delivery using a precision medicine approach [2], clinicians, researchers, and patients are re-energized in their efforts to further the application of novel technologies and therapeutics. Those of us practicing in the area of transfusion medicine recognize clearly the benefits of a more personalized approach to managing our patients. It goes beyond the 'simple' provision of ABO and D antigen compatible blood products for transfusion, with blood typing acknowledged by Collins and Varmus as one of the first applications of precision medicine [3].

The application of molecular technology has opened the doors to a new opportunity in transfusion medicine: red cell genomics for clinical application and improved healthcare and patient safety. The scope of application is large and varied; it ranges from molecular testing to better manage presumed Rh-incompatibility in the prenatal setting [4], to more specific red cell component selection for patients with hemoglobinopathies [5], and to explain the basis of complex serologic assessment of many patients. As the professional

organization of transfusion medicine whose mission is to advance the practice and standards of transfusion medicine and cellular therapies to optimize patient and donor care and safety, AABB actively supports red cell genomics research, development, and application in a number of ways. AABB spearheaded the first set of standards for molecular testing laboratories; initially published in 2008 with the third edition now available [6]. In addition, the organization has sponsored/co-sponsored workshops which brought together key thought-leaders and stakeholders affording an opportunity for robust, thoughtful discussion on the applications of this increasingly sophisticated technology. As important, AABB has tirelessly advocated for avenues for reimbursement of molecular testing related to transfusion management [7]. AABB will continue to be a voice in this arena, endeavoring to smooth the way for new innovation through its regulatory advocacy efforts; provide financial support for research endeavors through foundation support and industry relationships; and disseminate cutting edge findings through its educational initiatives and publications.

Clinical Vignette: Alloantibody formation to a high frequency antigen in the Rh blood group in the setting of hematopoietic progenitor cell transplantation for sickle cell disease (SCD) (Celina Montemayor, National Institutes of Health)

A clinical case was discussed involving a 19-year old male with SCD (HbS/HbS) treated by a 10/10 HLA-matched sibling hematopoietic progenitor cell (HPC) transplantation. Comprehensive serological and molecular data were presented demonstrating that both patient and donor carried a genotype comprising the *Weak D type 4.2.2–ceAR* and *(C)ce^s type 1* haplotypes. One day after transplantation, the patient developed an unexpected alloantibody to a high-prevalence Rh antigen. Evidence was presented that transfusion of

a crossmatch-compatible blood unit with the *(C)ce^s/(C)ce^s* genotype resulted in an acute hemolysis of the entire unit. Transfusion of a blood unit from the HPC donor was successful.

This report was the second case that we observed [8] implying the need for genotype-identical, not just haplotype-compatible, transfusion in patients carrying an alloantibody to a high-prevalence Rh antigen (Figure 1). Interestingly, both reported cases involved a *(C)ce^s type 1* haplotype [9], an association of unknown significance at this time. In a large study of 226 patients with sickle cell disease, the 2 alleles comprising the *(C)ce^s type 1* haplotype, the *RHD* allele *DIIIa-CE(4-7)-D* and the *RHCE* allele *ce^s*, were identified 21 (5%) and 26 times (6%), respectively [10].

The major conclusion of this clinical vignette was that the molecular mechanism and antigenic determinants underlying the need for genotype matching in a subset of patients with certain alloantibodies to high-prevalence Rh antigens is intriguing and is not fully understood by current routine technologies. Further research is warranted to explore the benefit of a potential precision medicine application.

Clinical Vignette: Genotype dry matching (Razvan C Lapadat, BloodCenter of Wisconsin).

Increasing numbers of *RHCE* alleles have been identified. Many were detected because of weak or variable antigen expression, typically due to a decreased RhCE antigen density on the red blood cell (RBC) surface. Some patients can be mistyped by routine hemagglutination as negative for the c or e antigen. Individuals of African ancestry have a higher incidence of these *RH* variants, many encoding partial antigens leading to Rh alloimmunization due to transfusion of normal high-prevalence Rh antigens. *RH*

matching patients with SCD and selected African American donors can improve transfusion therapy. We describe an unusual *RHCE*ce48C* allele found in a 3-year old African male with SCD [11].

Although phenotyped as D+c+e+, he developed an anti-e after being transfused. Red cell genotyping revealed being homozygous for the *RHCE*ce48C* allele. Anemia and evidence of hemolysis were observed (hemoglobin concentration averaged 7.85 g/dL and total bilirubin 2.55 mg/dL). Besides the underlying SCD, these findings may be attributed to the anti-e driving hemolysis, because the transfused blood units were not matched for *RHCE*ce48C* [12]. Subsequently, blood units were transfused based on genotyping results, a process being termed dry matching [13], which is a type of precision medicine. Careful clinical follow-up in similar patients is needed to document the extent of the possible clinical impact.

The *RHCE*ce48C* allele, frequently associated with an R_0 phenotype (Dce), encodes a cysteine at amino acid position 16, but is not known to induce the presence or absence of an blood group antigen. Suitable antisera to detect the *RHCE*ce48C* allele are lacking, leaving red cell genotyping as the only resource to identify patients at risk and donors with matching blood.

Clinical Vignette: Crawford variants (Waseem Q. Anani, BloodCenter of Wisconsin).

Choosing antibody reagents for a blood bank laboratory can be a difficult task. By understanding the limitations associated with specific monoclonal antibodies, one can be aware of potential testing pitfalls. Discrepant serologic testing results are often difficult to interpret. The advent of red cell genotyping resolved many of these interpretation

difficulties. This case will illustrate the benefit of red cell genotyping in the context of a D typing discrepancy.

A 29-year old African American female (G2P1) arrived for her first obstetric visit at an outside hospital. Her pertinent history included no prior transfusions and no past RhIG administration. A blood sample was sent for a type and screen as a part of her routine prenatal care. Her forward and reverse type demonstrated that she was O D-positive, but her serum showed a clear anti-D. The outside blood bank suspected a D variant and applied a panel of 12 monoclonal anti-D (research use only) to detect D variants (Advanced Partial RhD Typing Kit, Alba Bioscience, Edinburgh, UK), and no agglutination occurred. By using 4 monoclonal anti-D reagents (FDA-licensed for clinical diagnostic use), agglutination was observed with 2 reagents (Gamma-clone: ImmucorGamma, Norcross, GA and Alba-clone: Alba Bioscience Limited, Edinburgh, United Kingdom). Recognizing a potentially rare phenotype, the outside blood bank referred the specimen to BloodCenter of Wisconsin Immunohematology Reference Laboratory for red cell genotyping. A partial *RHD* assay via gel electrophoresis, *RH* variant panel via polymerase chain reaction with sequence specific primers, and Sanger sequencing were performed. The most notable findings included the absence of an *RHD* allele and *ce697G and *ce773G single nucleotide substitutions in *RHCE*.

Although the RBCs were predicted to lack D antigen expression, agglutination was clearly observed with two monoclonal anti-D reagents. The incongruent results were first observed in 1980 when a reagent polyclonal anti-D was found to contain antibodies specific for the Crawford phenotype [14]. With molecular methodologies, it was later demonstrated that the Crawford phenotype was composed of a triad of variants in the

RHCE gene: 48G>C, 697C>G and 733C>G [15]. The result is a D-like epitope in the *RHCE* gene that is recognized by some FDA-approved monoclonal typing reagents.[15,16] The Gamma-clone has classically been observed to agglutinate at only immediate spin, because the IgM monoclonal antibody recognizes the D-like epitope. However, no agglutination is observed in the IAT or AHG phases, because the monoclonal IgG component of the reagent recognizes a different epitope.

Two clinical implications become apparent in the D-negative patient. Although the Crawford phenotype creates a D-like epitope, these patients are at risk of becoming alloimmunized when exposed to D-positive blood [15]. Only D-negative blood should be transfused to Crawford individuals that lack an *RHD* (D-negative individuals) and RhIG administration in pregnant women is recommended. Second, the use of the Gamma-clone, while FDA approved, can incorrectly type patients with the D-like epitope present in Crawford patients [15]. The rarity of the phenotype has limited epidemiologic studies, but the incidence this phenotype is estimated to be present in less than 0.1% of African Americans [17]. Although uncommon, any blood bank that transfuses a significant number of African American patients or performs prenatal type and screens should consider discontinuing the Gamma-clone for D typing in these settings.

The molecular methodologies resolved a result that had been previously discrepant by serology alone and guided the management of the patient through the use of D-negative products, if necessary, and RhIG administration to reduce the risk of anti-D alloimmunization; another example of an inexpensive precision medicine application.

Weak D: the English perspective (Neil D. Avent, University of Plymouth)

We are lucky to work in transfusion medicine at an age where the molecular characterization of most blood group active genes has been completed, and the major clinically significant antigens have been described at the DNA level. However, the pathway of how this was achieved sometimes included controversy and friendly competition between a number of research groups in Europe and North America. In some European healthcare systems, it is routine to be able to prenatally define fetal *RHD* status using maternal plasma [18]. DNA analysis of D variants is routine and the next phase, including complete gene sequencing is underway using the new techniques of next generation sequencing [19].

Having worked on the purification of Rh proteins and their eventual cloning in the late 1980s [20,21], the next phase of work inevitably turned to the characterisation of D variants, including the mysterious and legendary partial D phenotype DVI and of course the strange phenomena of the weak D phenotype. Key contributions to the body of knowledge come from the groups of Jean-Pierre Cartron [22,23], Neil Avent [24,25], Bill Flegel [26,27], Ellen van der Schoot [28], Cathy Hyland [29], Greg Denomme [30], Connie Westhoff and Marion Reid [31], and several others.

The first partial Ds to be deciphered at the molecular level were the D category VI variants, which were found to be formed by gene conversions between *RHCE* and *RHD,* causing the expression of hybrid RhD-RhCcEe proteins [22,32]. Initial analysis of the weak D phenotype had incorrectly suggested that quantitative variation in *RHD* mRNA levels were responsible [23], which could not be corroborated [33]. It was somewhat a surprise to learn at the 1998 ISBT congress that an unknown group from the German Red Cross from Ulm in Germany had methodically analysed vast numbers of weak D

phenotypes. The work was later published in 1999 [27] and is indeed a landmark in molecular biology of the Rh system. Single nucleotide polymorphisms in the *RHD* gene were known prior to the 1999 paper to occur in several partial D phenotypes [24,34], but the scale of mutations in *RHD* causing weak D was certainly not appreciated at the time, however with the benefit of hindsight was rather obvious. There are now over 150 defined weak D type alleles [35] and gaining detailed knowledge of their clinical significance [36], some of which are capable of eliciting anti-D when individuals are exposed to D-positive blood, thus giving some credence to the opinion that the terms weak and partial D should be considered D variants [36,37].

During the mid-2000's the definition of the crystal structure of AmtB allowed the prediction of the structure of the Rh core complex trimer, comprising two Rh associated glycoproteins (RhAG) and one Rh protein monomer. This information gave critical insight into the approach of the affected amino acids in partial and weak D phenotypes. Unsurprisingly, those amino acid exchanges causing partial D phenotypes occur on exofacial loops, although the extent by which these loops penetrate the membrane bilayer was not appreciated until these structural models appeared [38]. The concept of the D *antigenic vestibule* is now fully appreciated. However, weak D mutations appear to affect the assembly of the Rh core complex trimer, and some indeed occur within G-XXX-G motifs, critical for inter membrane protein interactions, namely that of RhD with RhAG [39].

The focus on Rh molecular biology has been for the past decade predominantly at the level of the gene. The implementation of *RH* genotyping has seen rapid uptake in implementation and, in particular within the US, this information is invaluable in the

management of highly transfused patients for example those with SCD. The next decade of Rh research will be an interesting phase, and will certainly embrace molecular diagnostics even more strongly contributing to precision medicine procedures. Personally, the unknown function and protein chemistry of the RhD and RhCE proteins have been somewhat neglected, and the speaker looks forward to a new generation of Rh researchers to pick up the mantle of the pioneering work done over the past 25 years.

Integration of red cell genotyping into the blood supply chain (Gregory A. Denomme, BloodCenter of Wisconsin)

One import role of a blood center is to provide antigen-negative blood units for the alloimmunized transfusion recipient [40]. Presently, the identification of such blood units can be prohibitively time-consuming and expensive by phenotyping with serologic reagents. The molecular basis of blood group antigen expression has been confirmed for many more antigens than can be phenotyped using existing phenotyping techniques. Mass-scale red cell genotyping of blood donors provides the opportunity to create and maintain large databases that can serve the transfusion recipient with specific antigen-negative requirements beyond ABO and D. In 2010, a database of over 25,000 blood donors was created within six months at the BloodCenter of Wisconsin [41]. The database included all blood donors who self-declared their ethnicity as African American and Hispanic regardless of their frequency of donation to capture rare blood types found amongst these donor populations. The largest set of donors had a history of repeat donation following a 3:3:1:1 rule; three donations within the last three years of which one donation was within the last year.

Mass-scale genotyping was performed on 32 nucleotide polymorphism linked to the expression for 42 blood group antigens. Additional blood units were identified for 14 blood group antigens using phenotype techniques with unlicensed antisera. Within 3 years, 43,066 donors donated a total of 202,275 blood units that had a red cell genotype on file. Antigen-negative requests were filled for 99.8% of 5672 patient encounters, with an 'encounter' defined as one order for blood units regardless of the number of units requested. The database was comprised of 1.67 million genotypes that were assembled in 4 years. Contrasting that with 320,000 phenotypes accumulated over the past 30 years, red cell genotyping was able to assemble 5 times the serologic data in $1/7^{th}$ of the time. Red cell genotyping met the demand for antigen-negative blood for 94.1% of all requests, with a total of 333 requests filled with phenotype data alone. It was important to note that individual donor records contained no less than 28 genotypes, whereas 95% of the phenotype data comprised no more than 15 antigens. In addition, the cumulative mass-scale data resulted in 30% of blood units had a genotype on any given day in the final year of the program. The database could be maintained annually by genotyping 4000 repeat donors each year, which reflected the year-over-year donor loss-rate.

The construction of a large central database with nearly $1/3^{rd}$ of the blood units having red historical red cell genotype information affords the opportunity to provide the information throughout the supply chain [42]. The BloodCenter of Wisconsin gave seven hospitals access to historical genotype and phenotype via a web-based antigen query portal. The hospital would scan the ISBT128 code available on blood units into a query form, which is submitted electronically to the blood center along with the number of units and the antigen-negative attributes desired among the common blood group antigens: C, c

E, e, M, N, S, s, K, k, Fy(a/b) and Jk(a/b). The result returned to the hospital identified the oldest blood units that meet the requirements. If the query is not successful, additional blood units could be scanned or the order can be 'pushed' to the blood center to be filled by the reference laboratory. This online approach helped hospitals, up to 200 miles away from the blood center, locate antigen-negative blood units in their inventories. The 8-month pilot program identified 71 blood units from 52 antigen-negative requests. By the end of 2016, a total of 14 hospitals use the portal to access the available antigen-negative information within their inventories. The program is poised to use two historical phenotypes or genotypes to enhance the ability to provide antigen-negative blood for patients who need such blood units, and enables a wide cost-effective implementation of precision medicine in the blood supply chain (Figure 2).

Finding a needle in a haystack: technology enabling visibility of rare and uncommon blood units (Lynne B. Briggs, Vice President and Chief Information Officer BloodCenter of Wisconsin/Versiti)

Information technology (IT) requirements to support a clinical need often move from the innovation pipeline to an industry-standard practice. The drivers of such innovation are a composite of 'what is possible?' with 'what is desirable?', from a customer perspective, to 'what will be viable?' in the marketplace. The development of an IT solution for a need in blood banking and transfusion medicine should have the goal of improved patient outcome. For red cell genotyping data, the goal was to provide a fast and cost-effective means of identifying donors and their associated red cell genotypes that match the needs of a patient, while at the same time reduce or where possible eliminate data handling. The first step to translating mass-scale red cell genotype data in a meaningful and sustainable

way to the benefit of the patient was to identify the current gap with handling such large amounts of data. The BloodCenter of Wisconsin recognized the gaps to be: data transmission, the link between a genotype and phenotype, storage of historical results, and the labeling of donor units.

For transmission, the output of data from an instrument is handled on the basis of the file type protocol and the format. Transfer of data is not new to blood centers given the volume of tests and the need to add new tests, often on short notice, as deemed by regulatory entities. To address the link between a phenotype and a genotype, the BloodCenter of Wisconsin took the approach of maintaining the source of the results, be it a genotype or a phenotype, and to create a meaningful link between the two using a 'Rosetta Stone' governed by a rules engine [43]. From the linked phenotype and genotype data, exception files were designed to report phenotype-genotype discordant results or when duplicate data was entered. Those solutions were built keeping in mind the eventual need to comply with ICCBBA ISBT Standards for the labeling of blood units with historical results [44].

Red cell genotyping technology, along with the ability to genotype thousands of donors, makes finding rare blood units a true 'needle in a haystack'. IT and informatics changes are required to fully operationalize the use of red cell genotyping data throughout the supply change that includes the inventory in hospital transfusion services. The BloodCenter of Wisconsin included the development of an IT solution that makes available red cell genotype and phenotype data to hospitals for blood units in their inventory (Figure 3). In this way, antigen-negative blood units can be identified throughout the supply chain to the benefit of the patient [42].

PCR-free red cell genotyping using a nanobiosensor (Maryse St-Louis, Héma-Québec)

For decades serology was the sole technique used in immunohematology. It depends on reliable sera, is labor-intensive at times and could present major limitations. In the molecular world, actual single nucleotide detection (SNP) depends on different techniques most using enzymatic amplification, such as PCR-RFLP, PCR-SSP, modular and multiplex PCR [45]. However, enzymatic-based amplification presents many drawbacks: prone to contamination and enzyme inhibition, time-consuming, costly and the economy of scale is met only for batches. We explored a PCR-free red cell genotyping technique using a nanobiosensor [46].

The goal of this study was to amplify the signal generated by the recognition of hybridization events between oligonucleotide probes and perfectly complementary targets rather than amplifying the number of targets by PCR. The challenge was to maintain a good sensitivity and specificity. Nanomaterials were selected based on their multiple properties: color changes, fluorescent signal, or luminescent signal. Nanoscopic properties are nothing new. Several examples can be found throughout history. The Lycurgus cup held at the British Museum contains nanoparticles made of silver and gold causing light transmission and scattering. The Damascus blade was made of carbone nanotubes steel. Nanoparticles can be described as antenna because of the metal-enhanced fluorescence (MEF). When a fluorophore is in close proximity to a metal, after excitation, the electron in the metal creates an electron cloud called surface plasmon increasing the fluorescent signal. The distance between the fluorophore and the metal has to be 5-50 nm apart for this to happen.

The strategy was to make silver nanoparticles (d=50 nm) with a silica shell containing eosin (d=10 nm) to which were grafted *DO*01* and *DO*02* single stranded probes. These nanoparticles tended to aggregate in presence of a cationic polymer. Once the genomic DNA sample was added, the polymer changed its conformation and emitted light. The MEF amplified the light signal enough to be detected by cytometry.

DNA hybridization was performed at 58.5°C although the optimal temperature for *DO*02* probe was higher. Nine samples were assayed, three of each genotype: *DO*01/DO*01*, *DO*01/DO*02*, *DO*02/DO*02*. Results were compared to classic PCR and were concordant. The turnaround time for the nanobiosensor scheme was less than 30 minutes once the DNA was extracted. The hybridization time could be further reduced.

Results were reliable, even for homozygous and heterozygous fluorescent signals sometimes difficult to differentiate with other techniques. These nanobiosensors present advantages over PCR techniques by being fast, low-cost and specific, but are limited to known SNPs.

Red cell genotyping assays: FDA regulations and development of reference reagents
(Maria Rios, Food and Drug Administration)

Molecular testing of donors has proven useful in identifying blood units that are more likely to match alloimmunized patients, and these genotyping assays have been used to assist patient care. Due to the common chemistry involved, genotyping may be carried out in high-throughput platforms as multiplex assays. However, the use of red cell genotyping in clinical settings presents some challenges.

Regulatory considerations for devices. Red cell genotyping platforms are classified as high-risk Class III devices, for which the pathway to market requires an investigational device exemption (IDE) to perform clinical studies and a premarket approval (PMA) submission to demonstrate reasonable assurance of safety and effectiveness based on scientific evidence. A PMA has a 180-day review, which may be extended up to 320 days if the submission is sent to an advisory committee.

Well-characterized specimens are needed as raw material to prepare reference reagents for use in the development, validation, and manufacturing of genotyping tests, and for the optimal use of Class III devices in routine practice. Currently, there is paucity of well-characterized material for use as reference reagents for extensive red cell genotyping. In order to demonstrate efficacy of assay performance and to fulfill regulatory requirements, OBRR is developing DNA reference reagents covering genetic variants for antigens from the 17 blood group systems recommended by AABB. These reference reagents consist of well-characterized genomic DNA from human cells containing several red cell genetic variants that may not be readily available during clinical trials. This material will be made available to facilitate analytical evaluation during assay development, for proficiency testing and for QC.

Development of reference reagents. OBRR has supported a project for the development of comprehensive DNA reference panels for red cell genotyping (Table 1). This project was initiated in 2012, led by Dr. Jason Liu with intramural funding by OBRR, CBER and the FDA Office of Minority Health, with the overall goal of producing reference reagents to support development and validation of new assays and to fulfill regulatory requirements for the application submission and approval process.

The specimen acquisition and processing has been achieved through recruitment of donors with the desired historical phenotype and the genotype characteristics of the homozygous and heterozygous genotypes for each allele. An IRB approved project was initiated that provided de-identified donor samples under contract with the BloodCenter of Wisconsin. Whole blood units have been collected from 53 specifically-recruited donors with desired genetic variants. The whole blood units were shipped to CBER for processing and preparation of frozen RBCs, plasma, and buffy coat components. Genomic DNA from each donor was isolated from leukocytes and characterized with molecular typing assays developed in the laboratory to determine/confirm blood genotypes associated with 56 blood group antigens. Laboratory methods include TaqMan allelic discrimination assays, PCR-SSP and PCR-RFLP assays, Sanger bi-directional sequencing, and next generation sequencing. The samples have been characterized for 39 alleles associated with 56 antigens from 17 blood group systems by TaqMan assays, and the genotypes confirmed by Sanger sequencing. Immortalized cell lines were generated as renewable source of DNA using PBMC obtained from buffy coats by transformation with EBV. To date, 51 cell lines have been produced and stored in liquid nitrogen tanks. The verification of the integrity of target genes after transformation is ongoing by testing the DNA from the cell lines by PCR and TaqMan. We have selected 19 of these 51 established cell lines that cover all genetic variants collected to date. Cell line expansion and bulk genomic DNA has been extracted for 14 out of these 19 samples. The effects of EBV transformation on allelic discrimination assay results were also assessed. The most appropriate formulation for freeze-drying has been chosen after pilot lyophilization runs, and the resultant materials were assessed for reproducibility of previously performed

assays by testing target genes by PCR and/or TaqMan to verify performance of reagents after reconstitution. To date, of the 123 allele combinations proposed, blood samples covering 88 variants have been acquired and cell lines established (Table 2) [47].

The next steps will be formulation of the panel and initiation of stability and accelerated degradation studies. In parallel, external evaluations will be performed for final characterization, which will include identifying appropriate laboratories to participate in the study, setting up agreements with collaborating laboratories, shipping panel material, developing testing protocols and reporting worksheets and establishing deadlines for participation. At the time of this writing, we are collecting and analyzing the results to formally publish this collaborative study [47].

Application of external quality assessment in the clinical immunohematology laboratory (Gregor Bein, Justus-Liebig-University)

Genotyping for RBC, platelet (PLT), and granulocyte antigens is a new tool in the diagnostic evaluation of immunohematological disorders and typing of blood donors and recipients. Proficiency in laboratory tests can be established by external quality assessments (EQAs), which are required for clinical application in many health care systems and to maintain accreditation. There are few EQAs for molecular immunohematology [48]. One of the first workshops on molecular immunohematology was originally supported by a grant from the German Federal Ministry of health in 1998 [49]. This funding jumpstarted an EQA program organized by the German Society for Transfusion Medicine and Immunohematology, which was transitioned to a nonprofit provider in 2006 (INSTAND, Dusseldorf, Germany) [50]. Other international EQA programs include workshops that are organized by the College of American Pathologists

(CAP) [51], the International Society of Blood Transfusion (ISBT) [52], and the Consortium for Blood Group Genes [53]. The INSTAND EQA offers proficiency sets twice a year. Each set is always composed of four specimens of genomic DNA. Participants are requested to test all four specimens, even if only a single blood group system and a single proficiency certificate are of interest. A list of pre-defined mandatory alleles per blood group system guarantees maximum transparency [50]. The number of participants is steadily increasing and now encompasses 96 participants from 18 countries mainly from central Europe (October 2015). Almost all institutions participate in RBC, 60% in the PLT, and 25% in the granulocyte proficiencies. A recent summary of the workshop results revealed that the overall pass rates per system exceed 93% $\pm$ 4% (mean $\pm$ SD). However, low pass rates were obtained for *RHD* (82% $\pm$ 10%) and ABO (93% $\pm$ 4%). Frequent errors include failures of zygosity testing and identification of rare alleles (e.g. weak D types). High pass rates are obtained for blood group systems that are characterized by single nucleotide polymorphisms (e.g. JK, HPA) that can be genotyped without determination of extended haplotypes and/or haplotype phasing. The passing rates did not correlate with the experience of the participants with this EQA.

In summary, *ABO* and *RHD* systems seem to pose challenges for genotyping, possibly due to the large number of alleles caused by recombination. We conclude that intrinsically more robust technologies, such as sequencing platforms that allow for long-distance haplotype phasing may be needed for reliable genotyping for diagnostic purposes at least in some rare allele combinations. Future perspectives include the establishment of larger numbers of B-lymphoblastoid cell lines as a source of reference

DNA, the inclusion of additional blood group systems (e.g. LU, DI, YT, HPA-2), and EQA for the non-invasive prenatal diagnosis of fetal blood groups [47,49].

Molecular Immunohematology and precision medicine: international perspective

(Silvano Wendel, Hospital Sirio Libanês)

Molecular biology has considerably developed in the immunohematology field, not only confirming previous serological data, but also elucidating unsolved puzzles. The aim of this presentation was to compare what can be readily applicable for molecular immunohematology tests (MIT) taking into account the previous experience already acquired with molecular methods developed for screening of transfusion-transmitted diseases (TTID), which have now over a decade of international use.

General issues. Most of TTID molecular tests adopted for blood donor screening are used as universal testing (HIV, HCV and HBV). Selective testing is applied mainly for West Nile Virus (WNV), according to seasonal periods. This renders a tremendous impact as far as the annual number of tests, given that more than 10 million donations are tested only in the US. Thus, one can expect a great interest from pharmaceutical industries not only to develop, validate and register commercial tests, but also to pursue a continuous improvement in the test´s performance and related hardware.

One the other hand, MITs targeted at genetic markers (RBCs, leukocytes and platelets) from healthy donors or their recipients. Since the genetic code is permanent, one does not need to apply the same MIT to the same donor every time there is a blood donation; actually it is very likely that whenever a given donor is tested once (or twice to be certain), results will be considered as permanent in the donor's file. Whatever is the adopted strategy, blood centers usually face a restricted budget for this activity, rendering

the final number of MIT intended to guarantee a stable wide-tested donor panel much lower than those annually tested for infectious diseases.

The situation concerning recipients must be looked by a different perspective. Although most recipients will not have their genetic code changed throughout their lifetime, the increasing number of transplants (bone marrow, cord blood and solid organs) poses a challenge in the follow-up of patients. In this respect, there are clinical situations where MITs could be quite useful (e.g. chronic transfusion recipients, pregnancies) [54,55].

Finally, one cannot neglect the power that the public perception or medical agencies exert over authorities to facilitate the introduction of a new test in order to improve blood safety. For example, the World Health Organization (WHO) Aide Memoire for Ministries of Health [56] defines in its section on clinical transfusion in patient management that there should be "Systems, processes and procedures for compatibility testing and issue of blood; the rational use of blood and blood products; safe transfusion practice at the bedside and patient monitoring and follow-up" without mentioning which kind of testing should be performed. In addition, another important WHO document, the 2015 Global Reference List of 100 Core Health Indicators [57] when dealing with service utilization is quite vague, once there is only mention to health service access, hospital bed density and availability of essential medicines and commodities. Thus, it is important that blood centers advocate for the benefits of MIT in order to raise the public awareness. Nevertheless, despite the relative imbalance between infectious diseases and MIT, one can consider that the use of MIT will become a current practice [42]. Actually, an ever increasing expansion in the use of MITs is expected in this decade.

MIT in the global economic crisis. Even when applied in large scale, MIT are not a cheap procedure at this time, involving not only complex procedures, but also expensive equipment and trained professionals. Thus, its immediate application requires a substantial strategy in the budget of blood centers, which are affected, at least partially by the economic situation of a given country. Naturally, the higher health expenditure per capita (e.g. US$) or the relative percentage of the national gross domestic product (GDP) provided by a country, the higher chance that this problem could be overcome by blood services authorities. Detailed differences concerning health expenditures of all countries (low, medium and high income) can be found in the annual World Bank reports [58,59].

Current applications of MITs. Despite the numerous papers available in the literature dealing with highly specific antigens, large scale testing of regular blood donors is a reality [40,41,60-64]. The Ebola outbreak of 2014/2015 has also raised some unique proposals for the use of molecular methods whenever Ebola-infected patients require a transfusion [65].

The dramatic development of molecular biology has facilitated its current application in a number of routine blood services procedures, enhancing the blood safety worldwide. Technology is now available and might be used for several applications. However, due to its high costs MIT will be used as a selective way for most of immunohematological cases [66], as opposed to universal adoption for some TTIDs. On the other hand, the recent economic reports from several countries show a progressive recovery, which facilitates its adoption in a foreseeable future. Despite the lack of public perception and support, its use cannot be obstructed, requiring an active role of professionals engaged in the field in order to show to the public their real benefits. Naturally, every new blood

policy must be implemented based on judicious criteria, with balanced views, public acceptance, and good sense being key points.

Acknowledgements

References

[1] Personalize Medicine Coalition.

http://personalizedmedicinecoalition.org/Educaton/The_Basics#sthash.Ay19Auqi.dpuf

[2] Collins FS, Varmus H. A new initiative on precision medicine. NEJM 2015;372:793-5.

[3] FACT SHEET: President Obama's Precision Medicine Initiative www.whitehouse.gov/the-press-office/2015/01/30/fact-sheet-president-obama-s-precision-medicine-initiative (accessed 11.23.2016)

[4] Sandler SG, Flegel WA, Westhoff CM, et al. It's time to phase in RHD genotyping for patients with a serologic weak D phenotype. Transfusion 2015;55:680-9.

[5] Noizat-Pirenne F, Lee K, Pennec PY, Simon P, Kazup P, Bachir D, Rouzaud AM, Roussel M, Juszczak G, Ménanteau C, Rouger P, Kotb R, Cartron JP, Ansart-Pirenne H. Rare RHCE phenotypes in black individuals of Afro-Caribbean origin: identification and transfusion safety. Blood 2002;100:4223-31.

[6] Standards for molecular testing for red cell, platelet and neutrophil antigens, 3rd edition, AABB press, Bethesda MD. 2016.

[7] AABB endorsement of CPT Tier 1 Code for blood group genotyping.

http://www.aabb.org/advocacy/reimbursementinitiatives/Documents/AABB-Endorsement-of-CPT-Tier-1-Code-for-Blood-Group-Genotyping.pdf (accessed 11.23.2016).

[8] Fasano RM, Monaco A, Meier ER, et al. RH genotyping in a sickle cell disease patient contributing to hematopoietic stem cell transplantation donor selection and management. Blood 2010;116:2836-8.

[9] Flegel WA, Wagner FF. Two molecular polymorphisms to detect the (C)ce(s) type 1 haplotype. Blood Transfus 2014;12:136-7.

[10] Chou ST, Jackson T, Vege S, Smith-Whitley K, et al. High prevalence of red blood cell alloimmunization in sickle cell disease despite transfusion from Rh-matched minority donors. Blood 2013;122:1062-71.

[11] Lapadat R, Meyer E, Josephson C, Stadele S, Gottschall J, Annene KM, Denomme GA. Exon sequencing improves red cblood cell phenotype matching in a patient homozygous for the RHCE*ce48C allele. Transfusion 2015;55(S3):152A.

[12] Vidler JB, Gardner K, Amenyah K, Mijovic A, Thein SL. Delayed haemolytic transfusion reaction in adults with sickle cell disease: a 5-year experience. Br J Haematol 2015;169:746-53.

[13] Denomme GA, Flegel WA. Applying molecular immunohematology discoveries to standards of practice in blood banks: now is the time. Transfusion 2008;48:2461-75.

[14] Cobb M. Crawford: Investigation of a new low frequency red cell antigen [abstract]. Transfusion 1980;20:631.

[15] Flegel WA, Wagner FF, Chen Q, Schlanser G, Frame T, Westhoff CM, Moulds MK. The RHCE allele ceCF: the molecular basis of Crawford (RH43). Transfusion 2006;46:1334-42.

[16] John Roback BG, Teresa Harris, Christopher Hillyer. Technical Manual. 17 ed: AABB, 2011.

[17] MK Moulds GS, T Frame, CN Adamson, S MacNeill. Reactivity of monoclonal anti-D reagents with 27 samples shown to have the Crawford (Rh43) low-incidence antigen and/or a novel RHCE variant Rhce allele. Transfusion 2003;43(S):7A.

[18] Damkjaer MB, Perslev A, Clausen FB, Dziegiel MH, Jørgensen FS. Study of compliance with a new, targeted antenatal D immunization prevention programme in Denmark. Vox Sang 2012;103:145-9.

[19] Flegel WA, Castilho L, Heaton WA, Keller MA, Klapper EB, Lane WJ, Pirenne F, Shehata N, Stack G, St-Louis M, Tormey CA, Wagner FF, Waxman DA, Denomme GA. Molecular immunohaematology round table discussions at the AABB Annual Meeting, Anaheim 2015. Blood Transfus 2016;14:557-565.

[20] Avent N.D., Ridgwell K., Mawby W.J., Tanner M.J.A., Anstee D.J., Kumpel B. (1988) Protein sequence studies on Rh-related polypeptides suggest the presence of two groups of proteins which associate in the human red cell membrane. Biochem J 256:1043-1046.

[21] Avent N.D., Ridgwell K., Tanner M.J.A., Anstee D.J. (1990) cDNA cloning of a 30kDa erythrocyte membrane protein associated with Rh (Rhesus) blood group antigen expression. Biochem J 271:821-825.

[22] Mouro I, Le Van Kim C, Rouillac C, van Rhenen DJ, Le Pennec PY, Bailly P, Cartron JP, Colin Y. Rearrangements of the blood group RhD gene associated with the DVI category phenotype. Blood 1994;83:1129-35.

[23] Rouillac C, Gane P, Cartron J, Le Pennec PY, Cartron JP, Colin Y. Molecular basis of the altered antigenic expression of RhD in weak D(Du) and RhC/e in RN phenotypes. Blood 1996;87:4853-61.

[24] Avent ND, Finning KM, Liu W, Scott ML. Molecular biology of partial D phenotypes. Transfus Clin Biol 1996;3:511-6.

[25] Avent ND, Martin PG, Armstrong-Fisher SS, Liu W, Finning KM, Maddocks D, Urbaniak SJ. Evidence of genetic diversity underlying Rh D-, weak D (Du), and partial D phenotypes as determined by multiplex polymerase chain reaction analysis of the RHD gene. Blood 1997;89:2568-77.

[26] Wagner, F. F., et al. (1998). Three molecular structures cause Rhesus D category VI phenotypes with distinct immunohematologic features. Blood 91:2157-2168.

[27] Wagner FF, Gassner C, Müller TH, Schönitzer D, Schunter F, Flegel WA. Molecular basis of weak D phenotypes. Blood 1999;93:385-93.

[28] Maaskant-van Wijk, P. A., et al. (1998). Genotyping of RHD by multiplex polymerase chain reaction analysis of six *RHD*-specific exons. Transfusion 38:1015-1021.

[29] Cowley NM, Saul A, Hyland CA. RHD gene mutations and the weak D phenotype: an Australian blood donor study. Vox Sang 2000;79:251-2.

[30] Denomme GA, Wagner FF, Fernandes BJ, Li W, Flegel WA. Partial D, weak D types, and novel RHD alleles among 33,864 multiethnic patients: implications for anti-D alloimmunization and prevention. Transfusion. 2005;45(:1554-60.

[31] Murdock A, Assip D, Hue-Roye K, Lomas-Francis C, Hu Z, Vege S, Westhoff CM, Reid ME. RHD deletion in a patient with chronic myeloid leukemia. Immunohematology 2008;24:160-4.

[32] Avent, N.D., Liu, W., Jones, J.W., Scott, M.L., Voak, D., Pisacka, M., Watt, J., Fletcher, A. (1997) Molecular analysis of Rh transcripts and polypeptides from individuals expressing the D^{VI} variant phenotype: A *RHD* gene deletion event does not generate all D^{VI}ccEe phenotypes. Blood 89:1779-1786.

[33] Beckers EA, Faas BH, Ligthart P, Overbeeke MA, von dem Borne AE, van der Schoot CE, van Rhenen DJ. Lower antigen site density and weak D immunogenicity cannot be explained by structural genomic abnormalities or regulatory defects of the RHD gene. Transfusion 1997;37:616-23.

[34] Legler, T. J., et al. (1998). *RHD* genotyping in weak D phenotypes by multiple polymerase chain reactions. Transfusion 38:434-440.

[35] Wagner FF, Flegel WA. The Rhesus Site. Transfus Med Hemother 2014;41:357-63.

[36] Sandler SG, Flegel WA, Westhoff CM, Denomme GA, Delaney M, Keller MA, Johnson ST, Katz L, Queenan JT, Vassallo RR, Simon CD; College of American Pathologists Transfusion Medicine Resource Committee Work Group.. It's time to phase in RHD genotyping for patients with a serologic weak D phenotype. College of American Pathologists Transfusion Medicine Resource Committee Work Group. Transfusion 2015;55:680-9.

[37] Wagner FF, Frohmajer A, Ladewig B, Eicher NI, Lonicer CB, Müller TH, Siegel MH, Flegel WA. Weak D alleles express distinct phenotypes. Blood 2000;95:2699-708.

[38] Conroy, M.J., Bullough, P.A., Merrick, M., Avent, N.D. (2005) Modelling the human rhesus proteins: Implications for Structure and function. Br J Haematol 131:543-551.

[39] Avent N.D. (2007) New insight into the Rh system: structure and function. Vox Sanguinis ISBT Science Series 2:35-43.

[40] Denomme GA. Prospects for the provision of genotyped blood for transfusion. Br J Haematol 2013;163:3-9.

[41] Flegel WA, Gottschall JL, Denomme GA. Implementing mass-scale red cell genotyping at a blood center. Transfusion 2015;55:2610-5.

[42] Flegel WA, Gottschall JL, Denomme GA. Integration of red cell genotyping into the blood supply chain: a population-based study. Lancet Haematol 2015;2:e282-9.

[43] Bhagat A, Briggs L, Denomme G, Johnson ST. Genotyping: Creating a Rosetta Stone. *AABB News* 2011;13:4-8

[44] ICCBBA guidance document for the use and printing of historical red cell antigen data https://www.iccbba.org/tech-library/iccbba-documents/guidance-documents

[45] Flegel, W. A., et al. (1998). Rh phenotype prediction by DNA typing and its application to practice. Transfus Med 8:281-302.

[46] Brouard D, Ratelle O, Perreault J, Boudreau D, St-Louis M. PCR-free blood group genotyping using a nanobiosensor. Vox Sang 2015;108:197-204.

[47] Vokkova E, Liu M, Liu Z, Mercado T, Illoh O, Rios M. Renewable DNA reference panels for blood group genotyping. Transfusion 2016;56(S4):152A.

[48] Delaney M. Proficiency testing for blood group genotyping. Transfusion 2013;53(S2):2847–2849.

[49] Kroll H, Carl B, Santoso S, Bux J, Bein G. Workshop report on the genotyping of blood cell alloantigens. Transfus Med 2001;11:211–219.

[50] Flegel WA, Chiosea I, Sachs UJ, Bein G. External quality assessment in molecular immunohematology: the INSTAND proficiency test program. Transfusion 2013;53(S2):2850–2858.

[51] Allen TC, Hammond MEH, Robboy SJ. Quality and the College of American Pathologists. Arch Pathol Lab Med 2011;135:1441.

[52] Daniels G, van der Schoot, C E, Olsson ML. Report of the fourth International Workshop on molecular blood group genotyping. Vox sanguinis 2011;10:327–332.

[53] Denomme GA, Westhoff CM, Castilho LM, St-Louis M, Castro V, Reid ME. Consortium for Blood Group Genes (CBGG): 2009 report. Immunohematology 2010;26:47–50.

[54] Legler TJ, Eber SW, Lakomek M, Lynen R, Maas JH, Pekrun A, Repas-Humpe M, Schröter W, Köhler M. Application of RHD and RHCE genotyping for correct blood group determination in chronically transfused patients. Transfusion 1999;39:852-5.

[55] Rujirojindakul P, Flegel WA. Applying molecular immunohaematology to regularly transfused thalassaemic patients in Thailand. Blood Transfus. 2014;12:28-35.

[56] Afesa World Health Organization (WHO) - Aide-Mémoire for Ministries of Health, available at http://www.who.int/bloodsafety/publications/am_developing_a_national_blood_system.pdf?ua=1 (last seen, November 2015)

[57] World Health Organization (WHO) - 100 Core Health Indicators, available at http://www.who.int/healthinfo/indicators/2015/en

[58] World Bank - Health expenditure per capita (US$), available at

http://data.worldbank.org/indicator/SH.XPD.PCAP/countries?display=map

[59] World Bank - Health expenditure, total (% of GDP), available at

http://data.worldbank.org/indicator/SH.XPD.TOTL.ZS/countries/1W?display=map

[60] Manfroi S, Pagliaro P. Comment on "Applying molecular immunohaematology to regularly transfused

thalassaemic patients in Thailand." Blood Transfus 2015;13:164-5.

[61] Tanaka M, Kamada I, Takahashi J, et al. Evaluation of a blood group genotyping platform

(BLOODchip ® Reference) in Japanese samples. Transfus Med 2014;24:39-44.

[62] Latini FR, Gazito D, Arnoni CP, et al. A new strategy to identify rare blood donors: single polymerase

chain reaction multiplex SNaPshot reaction for detection of 16 blood group alleles. Blood Transfus

2014;12(S1):s256-63.

[63] St-Louis M. Molecular blood grouping of donors. Transfus Apher Sci 2014;50:175-82.

[64] Haer-Wigman L, Ji Y, Loden M, et al. Comprehensive genotyping for 18 blood group systems using a

multiplex ligation dependent probe amplification assay shows a high degree of accuracy. Transfusion

2013;53(S2):2899-909.

[65] Flegel WA, Castilho SL, Keller MA, Klapper EB, Moulds JM, Noizat-Pirenne F, Shehata N, Stack G,

St-Louis M, Tormey CA, Waxman DA, Weinstock C, Wendel S, Denomme GA. Molecular

immunohematology round table discussions at the AABB Annual Meeting, Philadelphia 2014. Blood

Transfus 2016;14:425-33.

[66] Delaney M. What is the value of a blood type? (Editorial). Transfusion 2015;55;2057–2059.

Table 1. Reference reagent development project at CBER since 2012: specific aims

1. Acquire samples from donors with desired alleles

2. Generate cell lines as renewable source of DNA

3. Prepare DNA stocks and verify quality and integrity of target genes

4. Produce prototype panels of lyophilized material

5. Perform collaborative studies to characterize the material for use as reference reagents

6. Analyze results and assign specifications to the Reference Panel

Table 2. EBV-transformed cell lines for renewable DNA for reference purposes

Blood group	Total alleles combination	Acquired	Missing
ABO	18	13	5
Cartwright	3	3	0
Colton	3	3	0
Cromer	3	2	1
Diego	3	2	1
Dombrock	9	6	3
Duffy	9	8	1
Indian	3	1	2
Kell	9	8	1
Kidd	3	3	0
Knops	9	7	2
Landsteiner-Weiner	3	1	2
Lutheran	3	3	0
MNS	12	8	4
OK	3	1	2
RHD	27	16	11
Scianna	3	2	1

Office of Blood Research and Review (CBER/FDA) has identified 41 target alleles across 17 blood group systems among 53 individuals. A reference panel of DNA from 19 cell lines, ensuring a renewable DNA supply, will be made available to manufacturers for developing and calibrating red cell genotyping kits and to laboratories for validating and monitoring the performance of such kits [47].
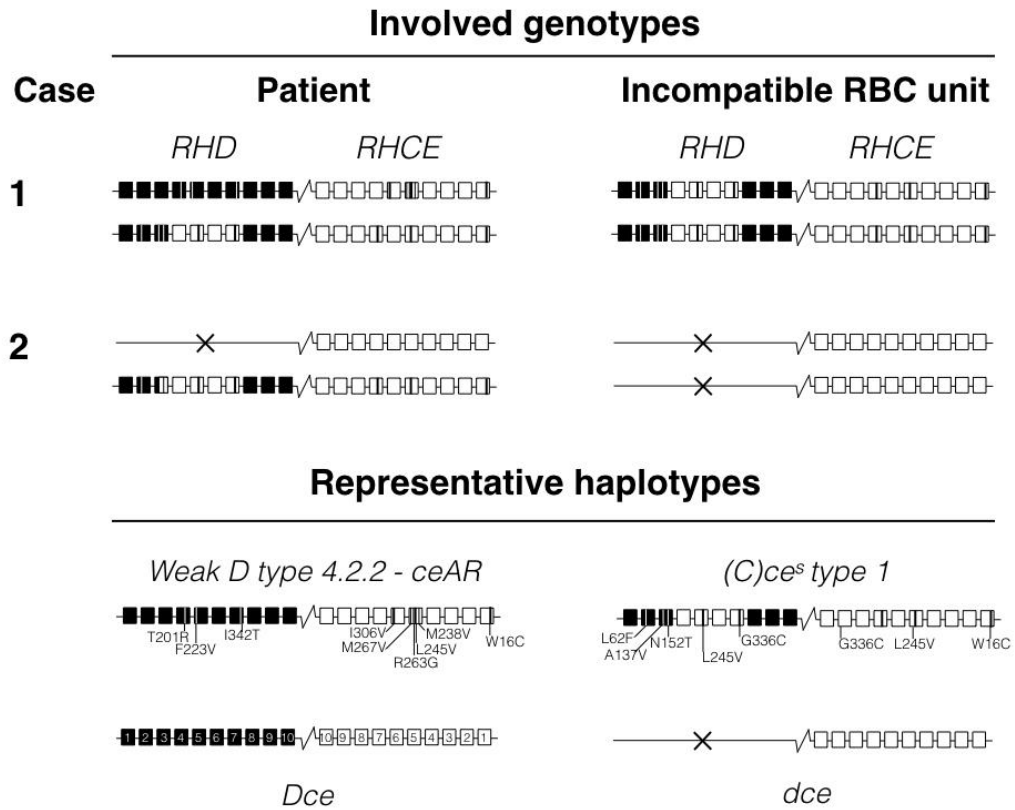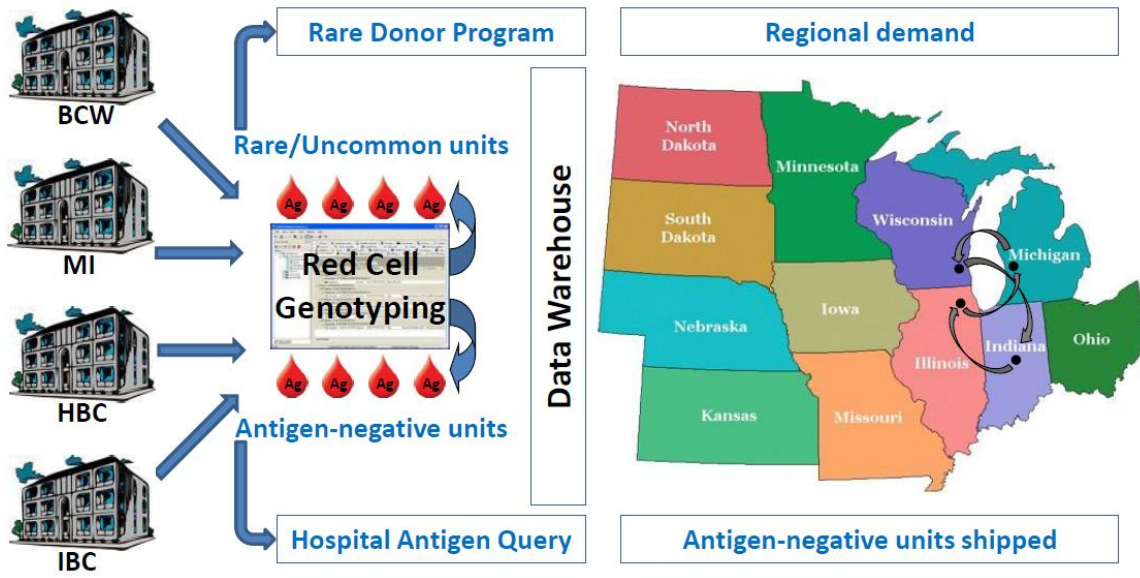
Figure 1.

Involved genotypes

Case	Patient		Incompatible RBC unit	
	RHD	*RHCE*	*RHD*	*RHCE*

Representative haplotypes

Weak D type 4.2.2 - ceAR

T201R F223V I342T   I306V M267V M238V L245V R263G W16C

(C)ce^s type 1

L62F A137V N152T L245V   G336C G336C L245V W16C

Dce

dce

Figure 2.

Figure 3.

Antigen Query – Unit Type Selection – VA MEDICAL CENTER

Patient Name   [Contact IRL]

Last * **Doe**   First * **John**   Mi **A**

DOB * **01/01/1900**   Gender * ● M ○ F Patient's ABO/Rh * **A Pos** ▼

MRN * **11223344**   Reason * **Known antibody(ies)** ▼

Notes
Anti-Fya, anti-Jkb; antigen-match for C, E, K

* Please select at least one

☑ O pos	☑ A pos	☐ B pos	☐ AB pos	☐ O neg	☑ A neg	☐ B neg	☐ AB neg	⊙CMV	ⓘIRR

* Please select at least one antigen negative checkbox

Rh	Kell	MNS	Kidd	Duffy
☑ C	☑ K	☐ M	☐ Jka	☑ Fya
☐ c̄		☐ N	☑ Jkb	☐ Fyb
☑ E		☐ S		
☐ ē		☐ s̄		
☐ Cw				

* Please enter number of units to find
Units To Find **2**

[Scan / Enter Units]   [Clear - Start Over]   [Place Order]

Figure 1. Schematic comparison of 2 patients' *RH* genotypes with the genotype of the respective incompatible RBC units. Case 1 represents the patient reported at the conference (upper panel); case 2 has been published previously by Fasano et al. [8]. Four representative haplotypes carrying distinct amino acid substitutions in the *RHD* and *RHCE* exons are shown for comparison (lower panel). A conventional *Dce* haplotype is depicted for reference; each square corresponds to an exon that is numbered in the *Dce* haplotype. The variant amino acid substitutions and their location are indicated by bars (black or white lines in the squares of the representative haplotypes).

Figure 2. Red cell genotyping across Versiti blood centers. Four blood centers participate in mass-scale red cell genotyping of donor units (left side) [41]. Rare units are identified and diverted to rare donor programs worldwide. Antigen-negative information is shared throughout the supply chain via a web-based antigen query portal in the cloud [42]. A data warehouse is accessible online and offers real-time web-based red cell genotype information on blood units. A blood center queries the data warehouse to fill orders that cannot be met from their current inventory. Units can be shipped between blood centers in a large region and across state lines to meet antigen-negative demands (right side). Versiti is comprises of the BloodCenter of Wisconsin (BCW), Michigan Blood (MI), Heartland Blood Centers (HBC) and Indiana Blood Center (IBC).

Figure 3. The web-based solution for red cell genotype and phenotype data.
Hospitals can access such data in the cloud for all blood units in their inventory. The hospital enters patient information (top portion), and the blood type and antigen-negative

attributes (middle portion), and number of units required (lower left). Blood unit ISBT

128 identification numbers are scanned (not shown) and then the electronic query is sent

to the blood center (lower center-left). The query returns the ISBT identification number

of the oldest unit(s) with the required attributes (not shown). If the required units are not

found, the process can be started again (lower center) by scanning additional inventory,

or the hosptial can place the order with the blood center (lower center-right).