Objectives

At the end of this session the learner will be able to:

1. Discuss advantages of molecular genotyping vs. Serologic phenotyping
2. Identify patients that may be candidates for molecular genotyping
3. Use case studies to illustrate how molecular genotyping can be used in selection of red blood cells for transfusion
Carolinas Medical Center

- Flagship hospital of Atrium Health
- Blood bank serves both the adult acute care hospital and Levine Children's Hospital
- Level 1 Trauma Center
- Transplant Program – Heart, kidney, pancreas, liver
- Blood Bank is staffed 24/7/365 by a team of dedicated blood bankers
- 2018 Statistics
  - Transfusions – 56,769 blood products
  - 523 Total RBC exchanges performed for patients with Sickle Cell Disease
- Serves as a reference laboratory for 11 additional hospitals within Atrium Health
- Standard for lab – Automated Solid Phase Testing
- Backup methods
  - Manual Solid Phase
  - Tube testing with PeG, LISS, or saline
- Specialized reference techniques utilized in antibody identification and work up:
  - Serologic phenotyping
  - Autologous cell separation
  - Elutions
  - EGA treatment
  - Minimal use of adsorptions
- Molecular genotyping is sent out to IRL
Importance of Patient History

Questions crucial to antibody identification:

1. Has the patient ever been transfused?
2. Has the patient ever been pregnant?
3. What is the patient’s diagnosis?
4. What medications is/has the patient been taking?
• Often “misses” patient phenotypes that are genetic variants, particularly those in the Rh family
• Relies on accurate transfusion history from patient
• Is made more difficult with recent history of transfusion
• Not all antigens can be tested using commercially prepared antisera
Molecular Genotyping

- May help distinguish allo vs auto antibody
- Accurately detects genotypic variants
  - Rh and duffy
- Allows for accurate phenotype in recently transfused patients
  - Avoids time consuming cell separation techniques that are often unsuccessful
- Allows for accurate antigen typing in patients with red cells coated with IgG
  - Avoids time consuming chemical treatments to red cells
Who should have genotyping?

- Patients who have been recently transfused
  - Particularly those that are not producing reticulocytes
- Patients with IgG coated red cells
- Patients with Sickle Cell Disease
- Patients who appear positive serologically for antigen that they have made antibody to
  - Rule out allo vs auto antibody
  - Genetic variants
Case # 1

• 3 month old male
• Hgb = 3.8 G/dL
• Orders for type and cross for 35 mLs of PRBC’s
• Mother has negative antibody screen

• Results:
  • B pos,
  • panagglutination in antibody screen
  • DAT = Polyspecific pos
    C3 pos
Case #1 cont’d

- Screen & panel using Solid Phase was positive in all cells with similar reactivity

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<th>Special Type</th>
<th>Donor</th>
<th>Rh-Hr</th>
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Case #1 cont’d

- Tube testing was all positive using PeG, Liss, & saline.
- Auto control was positive in tube.
- Modified cold panel was performed.
- All positive at RT and 4 deg C.
- Non-specific cold auto antibody was identified.
- Pre-warm screen was negative.

![Blood test results](image-url)
• Lack of specimen did not allow for complete phenotype on a pretransfusion specimen
• Autologous cell separation techniques were not attempted due to sample size
• Using prewarm technique, must give PRBC’s negative to patient’s corresponding Kidd (Jka, Jkb) type
• How do we determine patient’s phenotype?
Genotyping!!
Genotype results

- Patient specimen was sent for genotyping using HEA technology
- Neg for E, K, Fya, N & s
- Pos for C, c, e, Jka, Jkb, M, S
- Do not have to worry about giving units negative for Jka or Jkb
Case #2

- 66-year-old African American Female
- New diagnosis of pancreatic cancer
- Type & Screen performed

Results:
  - AB Neg
  - Pos antibody screen
  - DAT & auto control neg
# Case #2 Screen

Screen performed using Solid Phase

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What’s so strange about this?

- Patient ABO/Rh type tested as AB neg
- Weak D was performed
- Patient tested 3+ weak D positive
  - ABO/Rh is actually AB pos
What’s next?

- D variant kit test used as a screening tool
- D variant investigation kit show DVI specificity
What does genotyping prove?

- Sent for molecular RH D variant testing
- Results:
  - Patient was confirmed to be a DVI
  - DVI is a partial variant of D
  - Can make allo Anti-D
- Transfuse with AB neg (or any Rh neg) PRBC’s
Case #3

- 44 year old African American female
- History of Sickle Cell Disease
- Previous history of Anti-Jkb & weak warm auto
- Historical serologic phenotype: C-E-c+e+, K-, Fy(a-b-), Jk(a+b-), S- s+

- Results:
  - AB pos
  - Pos screen using saline tube technique
  - DAT neg
  - Auto control 1+ using saline tube technique
• Antibody screen previously neg 1 month earlier using saline
• Patient received RBC exchange transfusion
  • 11 Units were matched to patient’s serologic phenotype
  • All were compatible
• 2 Units transfused 2 weeks after exchange – only partially matched
Screen results using saline

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• Completed serologic phenotype for M, N, Le typings
  • Results: M+ N+ Le(a-b-)
• Elution Studies were performed

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5 days later

- Screen results show increase in strength

- DAT and auto control are both negative with this specimen
- Specimens sent to IRL for identification and genotyping
IRL Results

- IRL identified probable anti-Goa and HTLA like antibody with probable white cell antibodies
- Genotyping showed patient to have 2 RHCE variants and possible hrB-e variant testing showed patient to be at risk for formation of allo Anti-e and Anti-f. Unclear on ability to form Anti-hrB.
  - Serologic phenotype showed patient to be e pos
1 month later

• Specimens demonstrated pos DAT with IgG and C3
• More work by IRL showed that patient has Anti-C, Anti-S, and a probable Anti-Ytb
• Patient now receives genotypically matched PRBC’s to prevent antibody formation
  • Not a candidate for autologous donation due to sickle cell disease
Hypothetical Case #3

- 50-Year-old Caucasian male
- History of multiple myeloma
- Previously negative antibody screen
- No phenotype
- Recently transfused

Results:
- O pos
- Positive screen using saline tube technique
- DAT negative
• Patient is currently on Daratumamab (anti-CD38)
• Process is to perform baseline serologic phenotype on patients before starting Dara
• Did not happen in this instance
• Autologous cell separation techniques generally are unsuccessful on these patients due to lack of reticulocytes
• What to do?
Genotyping!!
Case #3 cont’d

- Patient was sent for genotyping
- Used results to determine which antigens needed to be matched and transfused accordingly
Case #4

- 73-year-old African American Female
- GI Bleed - Hgb 6g/dL
- History of Anti-C, Anti-E, warm auto, nonspecific cold auto
- Serologic phenotype: C-c+E-e+, Fya-Fyb-, K-, Jka+Jkb+, S+s-

Results
- A pos
- Positive screen
- Negative DAT & auto control
## Screen result

### Table 1: Rh-Hr, Kell, Duffy, Kidd, Lewis, P, MN, Lutheran, Xg Antigens

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### Table 2: Rh-Hr, Kell, Duffy, Kidd, Lewis, P, MN, Lutheran, Xg Antigens

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### Notes
- Indicates those antigens whose presence or absence may have been determined using only a single example of a specific antibody.
- Indicates those antigens that may or may not agree with all examples of the corresponding antibody.
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- 1 negative reaction out of all cells tested
What Next?

- Negative DAT and auto control
- Reactions with varying strength
- Began to suspect presence of an antibody to a high frequency antigen
- Patient samples sent to IRL for further investigation
- Preliminary testing indicated presence of a probable anti-hrB
- Molecular testing performed to confirm
Molecular Testing Results

• Predicted molecular Rh phenotype: Partial D+ C- E- partial c+ partial e+ hrS-

• Molecular testing confirmed the presence of RHD*DAR allele
  • Partial D (D variant) at risk for production of allo anti-D

• Presence of 2 partial RHCE*ce alleles
  • Associated with hrS- phenotype
  • At risk for production of allo anti-e and/or anti-hrS

• IRL confirmed presence of anti-hrS and anti-c
  • Patient has history of anti-C
  • Serologically c+
How do you transfuse?

- Rh null or genotypically matched units
- Autologous donation
Conclusions

• Molecular genotyping is a valuable tool for the transfusion service
• Considerations must be made
  • Cost
  • Time
  • Space
  • Staffing

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Questions?