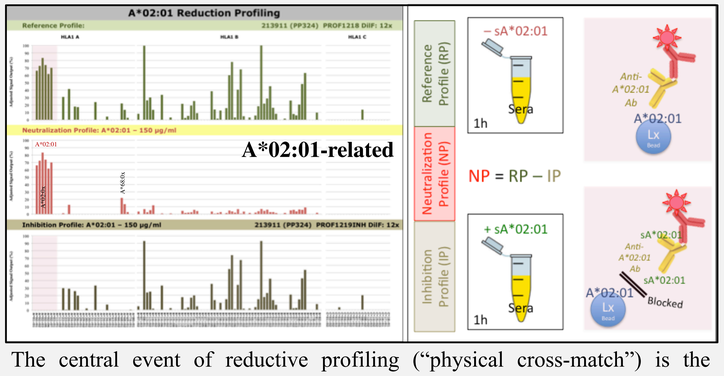
Determination of the Clinical Usefulness of HLA Neutralization Profiles

Rico Buchli[1], Arend Mulder[2], Annette Jackson[3], Anat R. Tambur[4], René J. Duquesnoy[5], Rebecca D. McAdams[1], Alyson Morris[3], Rick Eggers[6], Georgina Lopez Padilla[6], Daniel Zehnder[7], David P. Lowe[8], David C. Briggs[9], Robert Higgins[10], Frans H.J. Claas[11], Mike Hogan[6], William H. Hildebrand[12]

[1]Pure Protein LLC, Oklahoma City, OK; [2]Leiden University Medical Center, Leiden, Netherlands; [3]Johns Hopkins University, Baltimore, MD; [4]Feinberg School of Medicine, Northwestern University, Chicago, IL; [5]University of Pittsburgh Medical Center, Pittsburgh, PA; [6]gmsbiotech, Tucson, AZ; [7]The University of Warwick, Coventry, United Kingdom; [8]Royal Liverpool and Broadgreen University Hospitals NHS Trust, Liverpool, United Kingdom; [9]NHSBT Birmingham, Birmingham, United Kingdom; [10]University Hospitals of Coventry & Warwickshire NHS Trust, Coventry, United Kingdom; [11]Leiden University Medical Center, Leiden, Netherlands; [12]Oklahoma University Health Sciences Center, Oklahoma City, OK



The central event of reductive profiling ("physical cross-match") is the addition of a neutralizing sHLA molecule to the serological sample prior of measurement. Two wells, one loaded with untreated sera (reference), the other blocked with the sHLA of interest (inhibition), creates two distinguishable profiles that create once subtracted from each other a positive view of the blocking event (neutralization profile).

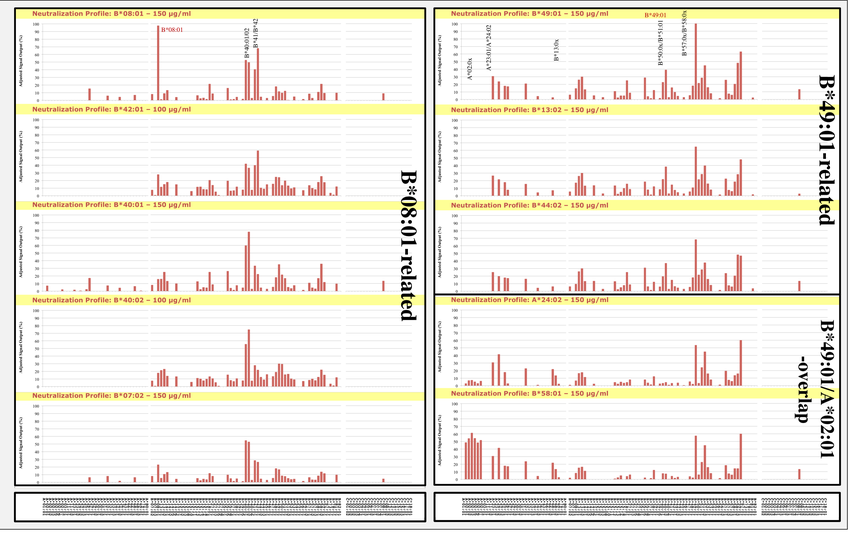
The Workup

Assembling various **neutralizer profiles** subtracting the inhibitory profile from the reference profile is most helpful for better analytical assessment of signal patterns, delivering a positive view of the blocking event. In order to demonstrate the variation and complexity within a single sera, extensive data collection was accumulated and resulted in three major relationships in this case study.

In Box 1 (left), A*02:0x alleles are the dominant reactions. Surprisingly, no effective response was directed against A*02:0x/B*57:0x sharing epitopes common in A2 immunized patient's. In this case, B*57:0x/B*58:0x responses seem to be originated from B*49:01.

In Box 2 (right), the most dominant Ab reaction appears to be directed against a specific B*08:01-epitope not shared by other alleles within this group. In addition, cluster analysis showed low variability and a strong relationship of additional epitope-recognition between B*40:0x and B*41:01/B*42:01.

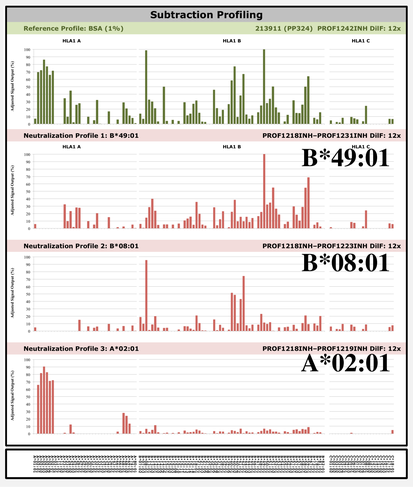
Box 3 (right) shows a very broad immune reactivity against multiple epitopes triggered by B*49:01. Two main categories can be distinguished the ones cross-reacting with A*02:0x (bottom) the other ones not (top). Different nuances in pattern suggest more than one epitope involved in both categories. Overall, this patient seem to trigger a much broader response against B* alleles than A* alleles. Not considering the A*02:0x cluster, all A*-related responses seemed to have originated from a shared epitope on B*49:01.

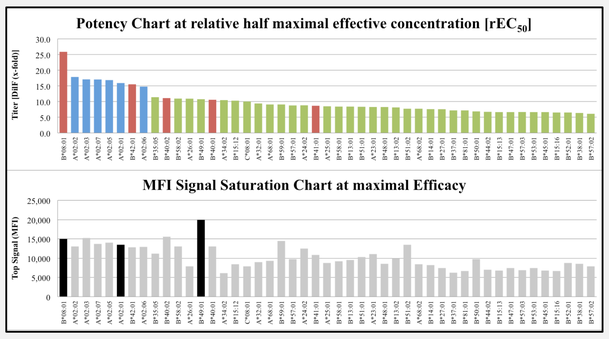


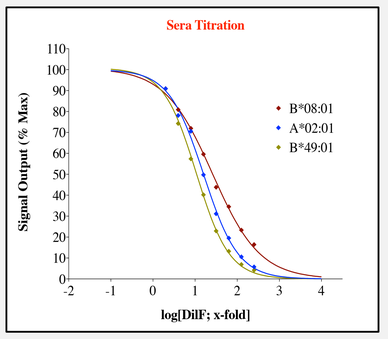
The Immunizing Events

HLA alloimmunization is caused by various sensitization events, such as transfusion, pregnancy, or previous organ transplantation. However, experimental identification of particular HLA allele(s) triggering the sensitization event has not been directly investigated to date. Utilizing sHLA molecules as neutralizing agents, we were able not only to identify specific alleles as immune triggers but also assign cross-reactive patterns and potency values to the event. In the case study presented here, we found three alleles making up the entire reference profile (Box 1). B*08:01, A*02:01 and B*49:01 together are capable of completely eliminating all response signals of the bead assay. Potency analysis (Box 2), a quantitative measure of antibody titers revealing the half maximal effective concentrations (EC_{50}), showed that B*08:01 had the strongest impact on the response profile followed by A*02:01 and B*49:01. It is not surprising to see highest efficacy levels (MFI) with these immunizer alleles as they provide the best-fit epitopes for the sera Ab pool. As immunizing molecules have the highest potential for strong Ab/allele interactions, they always should be considered "high risk" and excluded when matching a potential organ.

Important, potency analysis charts are created by individual, single-allele titration curves (Box 3) to establish optimal performance range and adequate dilution factors for the system. By identifying the linear range of antibody-bead interactions, semi-quantitative observations are possible where titer comparisons can be easily made and saturation issues such as "prozone effects" are eliminated. Keep in mind: "Potency values are a composition of concentration (titer) and antibody strength (affinity)".





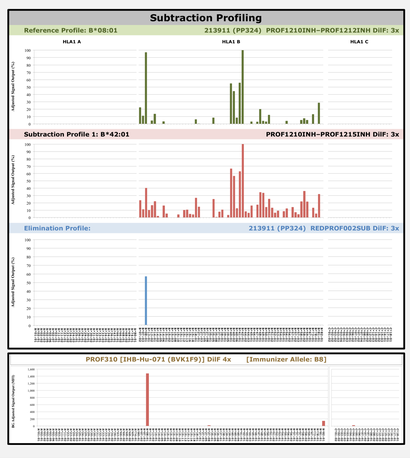


Introduction

About 30% of transplant patients are sensitized. This means that they have harmful antibodies which will attack foreign tissue. These antibodies develop through previous exposures to foreign tissue, such as pregnancy, previous transplants, or blood transfusions. Sensitized patients may wait 3-4 times longer than unsensitized patients for a compatible deceased donor organ. In order to test and characterize a recipient for these antibodies, we investigated a new technology of a "physical crossmatch". A sera sample is mixed with a sample of a potential donor allele in form of a sHLA molecule (neutralizer) and run on a bead assay. A neutralizer profile is obtained by subtracting the inhibitory profile from the reference profile delivering a unique view of all Ab/Allele interactions unique to a single allele in question. The technique is thought to allow a better risk assessment in Ab-related graft rejection.

The Dominant Species

"Subtraction profiling" is a new algorithm which uses an undefined single HLA pattern generated by polyclonal Abs that recognizes multiple epitopes on a single HLA allele [Immunologic Profile (iProf)] and subtracts the iProf pattern derived from a second neutralizing experiment. As shown, the idea was to eliminate shared epitope reactivities between B*08:01 and B*42:01. In this case, the result was the identification of a single antibody species recognizing a defined single HLA epitope. The existence of such a B*08:01 profile was supported by matching a previously described human B*08:01 monoclonal Ab (IHB-Hu-071/BVK1F9) to our subtraction profile. Overall, observing highest potency of B*08:01 and only modest values for B*08:01 group-related alleles suggest that this Ab is the dominant player for this patient and has to be considered "extremely high risk".



Conclusion

Policies, implemented by the OPTN, are in place to regulate how organs are allocated to those on the waiting list. To support better match-making and minimizing the possibility of antibody-mediated hyperacute rejection, our result indicate that the "physical crossmatch" goes way beyond test results being just positive or negative. They focus on specific Ab/allele-interactions eliminating non-related patterns, directly reveal the complex involvement of the antibody response, make measurements on strength, and provide a rank order of high risk alleles. In addition, applying sHLA as neutralizers greatly enhances our understanding of epitope-associations and increases our ability to confirm/reject matched epitopes for transplant. The knowledge gained about the apparent complexity of specific immune patterns will help us to reach the next chapter of patient care and contribute to a better risk management in Ab-related graft rejection.