Published in final edited form as:

Transplantation. 2015 February ; 99(2): 381–384. doi:10.1097/TP.0000000000000560.

Establishment of a Global Virtual Laboratory for Transplantation: A Symposium Report

Edward K. Geissler¹, Stefan G. Tullius², and Anita S. Chong³

¹University Hospital Regensburg, University of Regensburg, Department of Surgery, Section of Experimental Surgery, Regensburg, Germany

²Brigham and Women's Hospital, Division of Transplant Surgery and Transplant Surgery Research Laboratory, Harvard Medical School, Boston, MA, USA

³University of Chicago, Department of Surgery, Section of Transplantation, Chicago, IL, USA

Abstract

Advancing the field of transplantation by developing improved and novel treatment strategies will require a detailed understanding of changes and adaptations of alloimmunity, both over the short and long-term. Presently, we rely on traditional measures that may not optimally reflect benefits of new treatments. Thus, collecting reliable basic information about changes of the immune response along the entire post-transplantation course will improve our understanding of how immune mechanisms evolve and guide the introduction of novel clinical approaches. The gathering of good quality data from transplant recipients in various clinical trials will require immune monitoring that is reliable and comparable so that large sets of information from individual trials can be confidently analyzed to reach rigorous conclusions. A uniform standard of testing is thus a prerequisite towards this goal. Based on the assumption that the transplantation community will in general be supportive of this concept, this meeting proposed establishing a Global Virtual Laboratory (GVL) as a means of developing and disseminating detailed and rigorous protocols for the monitoring of alloimmune responses.

Introducing a Global Virtual Laboratory (GVL) Concept

A Transplantation Society (TTS)-sponsored symposium was held on July 26, 2014 in San Francisco to initiate steps aimed at making standardized testing methods and expertise more accessible to the global transplant research community conducting clinical trials. This concept was named “The Global Virtual Laboratory (GVL)” for Transplantation and the workshop served as an initial discussion on defining the need and structure of the GVL.

Addressing a fundamental problem

Clinical trials testing new treatment strategies in organ and cell transplantation are commonly evaluated by measuring traditional outcomes. For example, in solid organ

Contributions to manuscript:

Edward Geissler, Stefan G. Tullius, and Anita Chong participated in organizing the symposium and in writing of the paper.

Disclosure: The authors have no conflict of interest to declare.

transplantation the rate of biopsy proven acute rejections is considered the gold standard for assessing the success of any new immunosuppressive or tolerance-promoting treatment regimen. Other surrogates of treatment success are based on organ function measurements, such as serum creatinine levels or glomerular filtration rates, in the case of kidney transplants. Although novel functional assessments exist (1), they have not been widely adapted mostly due to a non-superiority compared to established approaches. Since early transplant outcomes with standard immunosuppressive regimens are already excellent according to the gold standard measure of biopsy proven acute rejection, the demonstration of significant benefits of new treatments is challenging and has spurred less innovation in transplantation. Therefore we reason that novel approaches providing more sensitive and accurate assessments of alloimmunity and predictions of graft function, especially in the early years post-transplantation, are desperately needed. With these tools, we will be able to determine if there were positive (or negative) effects that had been undetected with biopsies or standard measurements of donor-specific antibody. Moreover, these more sensitive assays may reduce the cost of clinical trials and predict a better (or worse) transplant outcome in addition to providing a rationale for an adaption or minimization of immunosuppression.

To implement novel transplantation strategies that can improve long-term outcomes (one of our current most challenging issues) (2,3), we need to develop sensitive measurements of alloimmunity while making these assays widely available to the transplant community. Because these assays can be technically intricate, it is critical that they be developed with a high degree of validation and with robust standard operating procedures (SOPs). This standardization will allow tests to be used reliably over time and between laboratories, thus providing a solid basis for the comparison between trials in order to learn more about the developing immune response in transplant recipients. For example, a particular new treatment strategy may not improve rejection rates early on, but may lead to a gradual amelioration of alloimmune responses that could eventually allow immunosuppression reduction or elimination. Indeed, development of tolerance appears to take several years to become established (4). This type of positive effect would likely be missed with our current means of evaluating novel treatments in clinical studies. Prospective clinical trials testing new strategies aimed at reducing conventional immunosuppression are precious and costly, and therefore, we as a transplantation community have an obligation to optimize the analysis from such studies, and to gain the ability to better compare results between different trials in different locations.

Research networks recognize the problem

Researchers in transplantation have long recognized the need to develop and validate assays that provide in-depth information on the immune response in allograft recipients. Large consortia in Europe (EU funded IOT: Indices of Tolerance, Riset: Reprogramming the Immune System for the Establishment of Tolerance) and North America (NIH funded ITN: Immune Tolerance Network, CTOT: Clinical Trials in Organ Transplantation) have recently recognized this challenge and taken steps towards improving immune monitoring (5–7). In addition, an ongoing European Union consortium (the *ONE Study* group) aimed at studying the potential of different regulatory cell products in renal transplant recipients compares immunological effects between closely related trials (8). Indeed, the unique setting of the

ONE Study where different cell products are being compared in parallel trials in different institutions has compelled investigators to design immune monitoring strategies ensuring that results from one trial can be accurately and fairly compared to those in other trials. In a larger sense, it seems logical that the transplantation research community should move towards a similar goal of standardizing immune monitoring testing so that results from any particular study can be discussed in the context of another similar study. For example, with the various types of cell therapy in the *ONE Study* some investigators are anticipating an increase in peripheral levels of regulatory T cells (Tregs); a standardized test is being used to make this measurement. If others trials in similar patient cohorts are also testing a therapy thought to enhance Treg levels peripherally (e.g. mTOR inhibitor, IL-2), and were to use the same standardized test, it would be possible to compare the effectiveness of these treatments. However, if each trial utilizes a different method of Treg measurement, the magnitude of change may be affected by how each assay is performed locally and therefore inter-trial efficiency comparison would be challenging. Therefore, to improve our ability to compare the immunological effects of new treatments in clinical trials, and to accurately observe fundamental changes in the immune system over the long term, we proposed the concept of a “Global Virtual Laboratory” for ascertaining standardized protocols for the monitoring of transplant recipients with the goal to disseminate these protocols to the global transplant community.

Shaping and defining a GVL

A GVL for immune monitoring should be instituted with the aim of providing training, protocols and advice to researchers conducting clinical trials. Implementing such a strategy raises important questions:

Which tests could be offered?

A great deal of discussion at the symposium surrounded which tests could potentially be offered by the GVL. A proposal from the *ONE Study* group is to offer standardized and validated flow cytometry phenotyping that has been developed within this consortium. At the meeting, Dr. Birgit Sawitzki from the central monitoring site in Berlin presented a number of advantages of the *ONE Study* system, since it involves utilization of fresh whole blood which ensures excellent sensitivity, especially when measuring infrequent cell populations. Other advantages include that seven sites within the *ONE Study* from Europe and the USA have been able to successfully adopt protocols and have shown highly comparable results using this flow cytometry phenotyping strategy. In addition, groups in the Netherlands and in Canada that are not part of the *ONE Study*, have been independently trained and have successfully adopted the same flow cytometry methods with excellent comparability results. Dr. Megan Levings presented impressive data from the Canadian National Transplant Research Program (CNTRP) that showed the practicality and robustness of this well-managed technology transfer. Therefore, experience from the *ONE Study* indicates that this type of peripheral blood cell phenotyping can be successfully transferred to multiple experienced laboratories with a focused effort. This EU-funded project is recognized as an important testing ground in terms of feasibility of the GVL concept.

From the presentation by Dr. Deborah Phippard, we recognize that experience from the ITN and CTOT projects will be valuable for those groups in the need of support for flow cytometry analysis of peripheral blood mononuclear cells (PBMCs), especially in trials where it will not be possible to analyze blood cells from fresh samples. Furthermore, in long-term studies, new discoveries in basic immunology would require retrospective analyses using archived patient samples. Thus, the ability to freeze and recover these samples reliably for years if not decades later will be essential. Finally, the issue of making flow cytometry data from different consortia available using their chosen methods “in the internet cloud” was proposed. This idea was well received since it could provide any investigator the opportunity to work with raw data generated through different flow cytometry phenotyping strategies. The conclusion of this discussion was that flow cytometry provides an excellent starting point for the GVL initiative.

Although basic blood cell phenotyping by flow cytometry is an excellent springboard to introduce a GVL concept, other tests should also be considered. Dr. Manikkam Suthanthiran provided an overview on recent advances in the analysis of urinary cell mRNA profiling for the identification of kidney transplant rejection (9), and indicated that SOPs have been developed with CTOT for the preparation of urine specimens for mRNA extraction, as well as protocols for reverse transcription and quantitative polymerase chain reaction performance. These protocols have resulted in consistent results in multiple laboratories not only for urine but also for blood. Because gene expression profiling of urine can potentially be the best non-invasive monitor of kidney graft function, there is strong interest in incorporating these protocols into the GVL. Finally, there was also considerable interest in the transcriptional profiling of peripheral blood to generate biomarkers predictive of rejection and tolerance. Issues regarding methodologies for the detection of active genes through microarray or RNA-sequencing, analyzing the huge amount of data that such approaches generate, and testing the reproducibility of data were discussed by Dr. Dan Salomon (10). While much of the analyses can be performed in “designated core” laboratories, the critical aspects for standardizing these approaches are in the sample collection and RNA isolation and storage, with flow cytometry of the cellular components in the peripheral blood being a critical component in the final interpretation of the genetic data. Ultimately the challenge of such “omics” approaches is the identification of a limited number of genes that robustly and selectively predict graft acceptance, tolerance and rejection, and then converting these into easy to use assays for the clinic.

How could GVL training be conducted?

In the case of flow cytometry, proper training on the use of standardized protocols and instrumentation will be key to generating comparable phenotyping data in clinical trials. The GVL could potentially offer at least two forms of training assistance. First, it was discussed that experienced core research centers can be identified to provide personal on-site training to set up an immune phenotyping laboratory. This is an approach that has already been used by the *ONE Study* consortium to instruct their own participating centers and to help international non-*ONE Study* sites successfully adopting this robust phenotyping strategy. Second, it was also discussed that core centers could be funded to produce detailed training videos and seminars meant to instruct sites that want to use the standardized methods. In

addition, these educational websites could incorporate blog sites to provide real-time interactive problem identification and solving. This type of approach could potentially also be useful for the transfer of other immune monitoring tests such as the IFN γ ELISPOT, sampling handling and storage, RNA isolation and qPCR. More resources would be required to support the former approach to training within a GVL, but would provide data that can be directly comparable between trials; the latter slightly less interactive method would be less costly and labor intensive, and may be ideal for smaller pilot trials with less financial resources.

The third option is to simply provide detailed SOPs for validated methods. However, while this approach represents the most simple means of spreading and transferring knowledge on immune monitoring, it is also the least likely to ensure that procedures are actually being done correctly at a high enough standard allowing the comparison of results from different trials. While the symposium discussion group fully recognized the potential of such an approach in terms of rapid dissemination, others raised serious concerns that a level of confidence could not be reached in terms of accurately comparing results between clinical trials without proper training.

The GVL workshop concluded that providing training to ensure that procedures are transferred under optimal conditions will be critical to safeguarding that reliable data will be generated for comparison of ongoing and future trials.

Challenges

Many challenges lay ahead, both organizational and scientific. What is not clear is the type of organizational structure that will be instituted. Whilst the ONE Study investigators have taken a lead in this area regarding the use of fresh blood for flow cytometry, other methodologies such as functional assays and genomics and proteomics will need to be incorporated and agreed standards developed. How these new assays will be incorporated and who will establish the appropriate standards, will depend on the GVL organizational structure and available financial support. Another technical issue that will need to be addressed is the methodology for transferring an established technology from a “central” laboratory into a robust technology that is truly “global”; although initial discussions surround the use of direct hands-on training, video-based training or simple SOP distribution, only experience with these methods will teach us which is best under different circumstances. We will need to learn from systems where global standardization has been successfully implemented, such as the Banff Conferences for the standardization of biopsy diagnosis and tissue typing through tissue exchange.

Support needed to build a GVL

Establishing a GVL will best be realized through a collaborative effort from different organizations. Partners in the *ONE Study* consortium have already begun sharing their standardized antibody panels and procedures on immunophenotyping with research groups in North America, Australia and countries within Europe, and have committed to continue this initiative. Detailed *ONE Study* operating procedures have been made available in a recent publication (8), however experience from the *ONE Study* group indicates that on-site

training is necessary to reliably transfer the knowledge. The overarching goal of the GVL in maximizing the comparability of data collected from various global clinical trials is at the heart of TTS' mission. Indeed, the Transplantation Science Committee of TTS had taken the lead by sponsoring this recent GVL symposium in San Francisco. With a vision of improving cross-comparison of clinical trial monitoring of the immune system, we call for additional support from other stakeholders that share our interest in spreading the use of standardized immune and genetic testing as predictors of graft outcomes.

Conclusions, next steps

A follow-up meeting to develop details of the next specific steps has been scheduled for later this year, with plans to introduce the GVL concept to larger general audiences and move towards implementation of the first offering to investigators planning or ready to conduct clinical trials. The GVL organizers would be pleased to get feedback about the concept, and appreciate any support that the leaders of various stakeholders might be able to provide. The complexities associated with such an initiative are recognized, but we believe that a GVL could help strengthen immune monitoring at experienced sites, and offer basic guidance to emerging laboratories conducting critical investigator-initiated clinical trials on novel concepts in transplantation.

This global initiative is timely as it will improve the impact and relevance of center-driven trials under the current conditions of challenging financial support.

Acknowledgments

Meeting Funding:

The Transplantation Science Committee (TSC) of The Transplantation Society

This meeting was sponsored by Transplantation Science Committee of The Transplantation Society. We are grateful to the speakers, Drs. Birgit Sawitski (ONE Study), Megan Levings (CNTRP/ONE Study), Deborah Phippard (ITN), Manikkam Suthanthiran (CTOT/AST) and Daniel Salomon (ITN/AST) for sharing their experience and their unpublished results. We would also like to thank the various *ONE Study* (Grant 260687) participating institutions that produced flow cytometry data for the centralized immune phenotyping model.

We especially thank the meeting participants, as well as their organizations: TTS Council: Francis Delmonico, Randy Morris, Elmi Muller, Philip O'Connell, Stefan G. Tullius (SGT); TSC of TTS: Anita S. Chong (ASC), Edward K. Geissler (EKG), Shane Grey, Maria Hernandez Fuentes (MHF), Fadi Issa (FI), Olle Korsgren, Nancy Kwan Man (NKM), Megan Levings, Xian Li, Olivia Martinez, Hideki Ohdan, Irene Norhona, (also SGT); ONE Study: Birgit Sawitski, Kathryn Wood (KW), Larry Turka (LT) (also EKG, FI, MHF, LT); ITN/CTOT/AST: Robert Fairchild, Jonathan Maltzman, Debora Phippard, Dan Salomon, Manikkam Suthanthiran, (also KW, LT); Transplantation Journal: Carla Baan, Jonathan Bromberg (also SGT, EKG, AC, NKM); Others: Stephen Alexander, Medhat Askar, and Lori West.

Abbreviations

AST	American Society of Transplantation
CNTRP	Canadian National Transplant Research Program
CTOT	Clinical Trials in Organ Transplantation
ELISPOT	enzyme-linked ImmunoSpot assay

GVL	global virtual laboratory
IOT	Indices of Tolerance
ITN	Immune Tolerance Network
PBMC	peripheral blood mononuclear cells
RISSET	Reprogramming the Immune System for the Establishment of Tolerance
SOP	standard operating procedure
TSC	Transplantation Science Committee
TTS	The Transplantation Society
WTC	World Transplant Congress 2014

References

1. Lo DJ, Kaplan B, Kirk AD. Biomarkers for kidney transplant rejection. *Nat Rev Nephrol.* 2014; 10:215–225. [PubMed: 24445740]
2. Kirk AD, Knechtle SJ, Larsen CP, Newell KA, Pearson TC. Miles to go. *Am J Transplant.* 2011; 11:1119–1120. [PubMed: 21645248]
3. Stegall MD, Gaston RS, Cosio FG, Matas A. Through a glass darkly: Seeking clarity in preventing late kidney transplant failure. *J Am Soc Nephrol.* 2014:26.
4. Londono MC, Rimola A, O'Grady J, Sanchez-Fueyo A. Immunosuppression minimization vs complete drug withdrawal in liver transplantation. *J Hepatol.* 2013; 59:872–879. [PubMed: 23578883]
5. Hernandez-Fuentes MP, Lechler RI. A 'biomarker signature' for tolerance in transplantation. *Nat Rev Nephrol.* 2010; 6:606–613. [PubMed: 20717098]
6. Sagoo P, Perucha E, Sawitzki B, et al. Development of a cross-platform biomarker signature to detect renal transplant tolerance in humans. *J Clin Invest.* 2010; 120:1848–1861. [PubMed: 20501943]
7. Newell KA, Asare A, Kirk AD, et al. Identification of a B cell signature associated with renal transplant tolerance in humans. *J Clin Invest.* 2010; 120:1836–1847. [PubMed: 20501946]
8. Streitz M, Miloud T, Kapinsky M, et al. Standardization of whole blood immune phenotype monitoring for clinical trials: panels and methods from the ONE study. *Transplant Res.* 2013; 2:17. [PubMed: 24160259]
9. Suthanthiran M, Schwartz JE, Ding R, et al. Urinary-cell mRNA profile and acute cellular rejection in kidney allografts. *N Engl J Med.* 2013; 369:20–31. [PubMed: 23822777]
10. Salomon DR. Challenges to doing genetic association studies in transplantation. *Am J Transplant.* 2012; 12:3173–3175. [PubMed: 23198786]