Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Curr Opin Organ Transplant. Author manuscript; available in PMC 2021 August 01.

Published in final edited form as:

Curr Opin Organ Transplant. 2020 August ; 25(4): 412–419. doi:10.1097/MOT.0000000000000772.

Precision Transplant Pathology

M.A. Wood-Trageser[1,2], **Qinyong Xu**[1,2], **A. Zeevi**[1,2], **P. Randhawa**[1,2], **D. Lesniak**[1,2], **A.J. Demetris**[1,2,3]

[1]Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA 15213 USA

[2]Division of Liver and Transplantation Pathology, Department of Pathology, University of Pittsburgh, PA 15213, USA;

Abstract

Purpose of review: Transplant pathology contributes substantially to personalized treatment of organ allograft recipients. Rapidly advancing next generation HLA sequencing and pathology are enhancing the abilities to improve donor/recipient matching and allograft monitoring.

Recent findings: This review summarizes the workflow of a prototypical patient through a pathology practice, highlighting histocompatibility assessment and pathological review of tissues as areas that are evolving to incorporate next generation technologies while emphasizing critical needs of the field.

Summary: Successful organ transplantation starts with the most precise donor-recipient histocompatibility matching. Next generation sequencing provides the highest resolution donor-recipient matching and enables eplet mismatch scores and more precise monitoring of specific DSAs that may arise after transplant. Multiplex labeling combined with hand-crafted machine learning is transforming traditional histopathology. The combination of traditional blood/body fluid laboratory tests, eplet and DSA analysis, traditional and next generation histopathology, and -omics-based platforms enables risk stratification and identification of early subclinical molecular based changes that precede a decline in allograft function. Needs include software integration of data derived from diverse platforms that can render the most accurate assessment of allograft health and needs for immunosuppression adjustments.

Keywords

Next Generation Sequencing; Precision Medicine; Next Generation Pathology; Image Analysis

INTRODUCTION

Precision medicine, defined by the Precision Medicine Initiative [1], is “an emerging approach for disease treatment and prevention that takes into account individual variability

[3]Corresponding author: A.J. Demetris, UPMC Montefiore, E741, 3459 Fifth Avenue, Pittsburgh, PA 15213; Phone: (412) 647-2072; demetrisaj@upmc.edu.

Conflict of interest:

The authors of this manuscript have conflicts of interest to disclose. Anthony J. Demetris: Receives research support from Q2 Solutions and is a member of an Adjudication Committee for Novartis. None of these conflicts are relevant to this article. The other authors have no conflicts of interest to disclose.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

in genes, environment, and lifestyle for each person [2, 3].” Transplant pathology epitomizes this initiative by generating a comprehensive data pool of patient information, driving downstream analyses to improve individualized patient management, especially in immunosuppression (IS) management. In this overview, various sections follow a prototypical patient through a pathology practice where critical data inputs are generated to enable individualized patient care: histocompatibility assessment; clinical laboratory testing, including routine blood chemistries and molecular profiling of blood and body fluids; tissue genomic testing; and tissue staining and analyses using automated morphometry and artificial intelligence (Figure 1).

Next Generation Sequencing in Histocompatibility Assessment

The major barrier to successful solid organ transplantation (and its solution) is known: solid organ allografts reject because of Human Leukocyte Antigen (HLA) genetic disparities between the donor and recipient. Better matching lessens the risk; HLA identity eliminates the risk. The HLA genomic locus (the most polymorphic human gene locus) is the driving force behind cellular and humoral immune responses leading to allograft rejection, which requires lifelong IS. Preservation methods for most organs make it impractical to mandate improved histocompatibility assessment. The combination of advances in organ preservation (e.g. extra-corporeal perfusion [4]) and a renaissance in effective and practical genetic manipulations (e.g. siRNA reagents [5]) should change this approach. Considerations of original disease, age, gender [6] and more precise molecular characterization of the donor-recipient differences can be used to individualize long-term IS management [7].

Traditional histocompatibility assessments for solid organ transplantation rely on low-level resolution typing such as reverse sequence-specific oligonucleotide probes (SSOP) that can distinguish the various HLA antigens (e.g. A2, A3) through DNA hybridization. This approach relies on analysis of small HLA gene segments that fail to distinguish among HLA alleles (e.g. HLA-A*02:01, HLA-A*02:03). Several reviews emphasize next generation sequencing (NGS) superiority in defining HLA mismatching in solid organ transplants based on precise molecular allele typing as compared to antigen-level typing [8, 9]; but application to a standard of care protocol remains debated [10].

Duquesnoy’s HLAMatchmaker algorithm is the most frequently used software for determining donor/recipient molecular mismatches [11]. It identifies small patches of polymorphic surface-exposed amino acids named “eplets” on each HLA allele and compares donor and recipient molecules to quantify the number of mismatched eplets [12]. Pre-transplantation, the level of HLA Class II antigens HLA-DR and HLA-DQ eplet mismatches, enables recipient risk stratification for development of de-novo, donor-specific HLA antibodies (DSA), antibody-mediated rejection (AMR) and graft failure [13, 14]. Post-transplantation, the degree of molecular HLA mismatch can guide IS management, including decisions on minimization [7].

Traditionally, anti-HLA specificity was based on families of HLA antigens. Antibodies to allele-specific epitopes, recognizing variations at the amino acid level, are prevalent in sensitized patients [10]. HLA antibodies are directed to epitopes that can be determined by

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

amino acid residues encoded by various HLA alleles. NGS-based histocompatibility assessment was helpful for risk assessment in 21-41% of transplant candidates and in 21-27% of cases of post-transplant DSA assessment [15].

High-resolution typing is essential for accurate assignment of antibody specificity, determination of allele-specific DSA, and facilitating pre-transplantation virtual crossmatch. High-resolution typing may be imputed from low-intermediate resolution typing, but this approach is limited to specific loci and various ethnic minority groups [15, 16]. United Network of Organ Sharing (UNOS) organ allocation relies on unacceptable antigens, and panel reactive antibody (cPRA) calculated with donor antigen frequencies. Patients with allele-specific antibodies are often under- or over-estimated for their true cPRA. An organ allocation system based on genomic alleles or specific HLA epitopes instead of whole protein-based antigens should be the future of precision medicine in transplantation [13].

Incorporating high-resolution NGS-based histocompatibility assessment facilitates implementation of precision medicine in solid organ transplantation, enables the best donor-recipient matches, and minimizes potential DSA development and organ rejection. NGS is approaching cost-neutrality with low-resolution typing methods, facilitating wide utilization in clinical practice to provide the most precise histocompatibility assessment for organ transplantation. However, the current three-day turnaround time, renders NGS only suitable for routine typing. A fast, high-resolution histocompatibility assessment method needs to be developed for deceased donors.

Allograft Monitoring: Body Fluid -Omics and Tissue Analytical Platforms and Analyses

In addition to “for cause” assays, our institution performs routine surveillance biopsies and follow-up DSA testing of heart, kidney, and liver allograft recipients at organ-appropriate intervals following transplantation. Biopsy-derived data are critical for personalizing patient care by capturing early subclinical signs of allograft injury (e.g. subclinical rejection) and for genotype-phenotype correlations with NGS donor-recipient matching.

We recently reviewed genomic, staining and imaging technologies used to monitor allograft rejection and health [17]. We divided these platforms into “non-invasive” and “invasive” transcriptomics platforms, on the basis of whether allograft tissue was required for the analyses. Non-invasive platforms (e.g. blood- and body fluid-based) have the advantages of safety, global organ assessment, minimizing sampling errors, and the ability to conduct serial measurements, albeit only indirectly monitoring the allograft [17]. Invasive non-histopathology platforms (e.g. mRNA based) tout objectivity, greater accuracy, precision, and mechanistic insights compared to traditional tissue pathology. However, thresholds for tissue-based diagnoses are arbitrary, not clinically validated, and confirmation of quantitative reproducibility is limited. It fails to analyze the broad spectrum of cases seen in a typical pathology practice. Both non-invasive and invasive non-histopathological tissue analytics have the major disadvantage of loss of spatial and inferred temporal context [17].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Nonetheless, non-invasive urine and blood-based -omics biomarkers have potential as screening tools, but blood mRNA profiles can reflect diet, the gut microbiome, endogenous metabolism, and renal clearance [18]. Urine specimens are subject to in-situ degradation of molecules within the urinary bladder before the specimen can be collected. Once a screening test becomes positive, a biopsy remains essential to: (a) confirm the diagnosis of intra-graft diseases (e.g. rejection); (b) rule out other inflammatory diseases; and (c) evaluate the extent of structural compromise by necrosis or fibrosis. Many diseases (e.g. glomerulonephritis, diabetic nephropathy, pyelonephritis, BK virus nephropathy, and vasculitis) can only be diagnosed by a biopsy. Biopsy-based histopathologic examination remains essential for evaluating allograft dysfunction.

Early diagnosis of subclinical rejection by protocol biopsies allows prompt intervention to maximize graft longevity. Conventional or traditional histopathology has limitations: (a) it is currently non-quantitative and subjective to interpretation with need for experienced pathologists which can result in significant inter-observer variability; (b) reliance on light microscopy, alone, fails to interrogate molecular changes that precede morphologic lesions; and (c) histopathologic lesions are not absolutely specific and resolution of the differential diagnosis requires additional laboratory studies, including immunohistochemistry, and possibly, ultrastructural evaluation of fine tissue structures (e.g. glomeruli).

Most tissue-based -omics studies used DNA microarray technology on fresh frozen tissue. Initial validation studies based on a full tissue core taken specifically for molecular analyses were promising, but attempts to implement the assays in a “real world” clinical setting unveiled limitations due to dependence on a small 3-4 mm tissue fragment taken from a longer core sent for routine histology [19]. While small fragments might be adequate to diagnose diffuse disease [e.g. acute tubular injury, well-developed acute T-cell mediated rejection (TCMR)], they do not perform well for focal lesions, seen in up to 37% of biopsy cores [20].

One study reported microarray-based diagnoses to have a sensitivity of 62% and a positive predictive value of 45% for a diagnosis of TCMR in biopsies that averaged 3-mm in length [21]. The authors concluded that the molecular data was more correct; an alternate explanation is that the tissue taken for molecular analysis did not have focal TCMR lesions. Data purporting to show reproducibility of the microarray measurements [22] must be interpreted knowing that tissue fragments, measuring only a few mm in length, cannot capture the variation seen in whole biopsy cores examined microscopically.

Sampling issues have led some investigators to use multiplexed color-coded “NanoString” probe-based gene expression on full-length cores of formalin fixed paraffin embedded kidney biopsies [23]. Limitations to this method include: (a) no gene amplification, (b) limited numbers of interrogated genes, and (c) technical problems inherent to working with mRNA. Proteins, the final effectors of disease pathogenesis, are more stable than mRNA and measured more accurately over a wide dynamic range, with inexpensive assays [24]. Human tissues show significant quantitative differences between mRNA and protein expression [25, 26]. Investment in the development of proteomics-based assays are important as a

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

complement to, or substitute for, mRNA-based assays. Mass spectrometric techniques are feasible for analysis of formalin fixed biopsies [27].

"Subjective" biopsy interpretation areas that might benefit from development of -omics based tests include biopsies with: (a) findings "indeterminate" for TCMR [28]; (b) extensive "non-specific" scarring where inflammation may be discounted; (c) concurrent pathology where light microscopy cannot determine whether or not the observed inflammation a response to allogeneic antigens (e.g. BK virus nephropathy, non-specific portal inflammation in liver allografts; < 2 R TCMR in cardiac allografts); and (d) findings suspicious for AMR, but where C4d stain is negative, and DSA testing has not been performed.

Image Analysis through Engagement of Machine Learning and Artificial Intelligence

Most anatomic pathology divisions focus on rapid turnaround of staining and interpretation of routine histochemical (e.g. H&E, PAS, trichrome, silver) and single colorimetric immunohistochemistry assays (e.g. C4d, CK7). Tissue pathologists assimilate multi-platform data extracted from electronic medical records (EMR) including (Figure 1): (a) demographic data, (b) transplant specific information (e.g. original disease, type of donor and transplant operation, and time since transplantation), (c) pharmacologic data (e.g. type and serum IS drug levels, patient compliance), (d) organ function tests (e.g. ALT, AST, GGTP, serum creatinine, platelet counts), (e) tests of donor humoral reactivity (e.g. DSA), and (f) radiological imaging studies of parenchymal findings and excretory conduit patency (e.g. organ ultrasounds, magnetic resonance cholangiopancreatography). Manual data compilation and integration into diagnoses requires attention to detail and is time-consuming. Efforts to streamline this process are hampered by cultures that re-enforce commercial EMR platform silos that lack data harvesting capabilities for integration with multiplatform analyses.

We have been adapting to criticisms of traditional histopathology practices. Histopathologist biopsy "scoring" is conducted using predefined templates where semi-quantitative assessments (e.g. mild, moderate, severe inflammation; Banff kidney and liver lesional scores; apoptotic counts in intestinal allografts) are translated into analyzable "digital" metrics by directing the pathologist toward specific findings (e.g. kidney scoring template [29, 30]). This has been employed in our clinical workflow for more than 25 years.

Our most recent adaptation is an "automated scoring sheet" for Banff categorization of kidney allografts biopsies (Figure 2). Pathologists evaluate kidney allograft biopsies using Banff lesional criteria (e.g. percent total inflammation, number of inflammatory cells in tubules, percent interstitial fibrosis) [29]. The software translates criteria findings into Banff lesional scores [glomerulitis (g), inflammation severity (i), tubulitis (t,), vasculitis (v), etc.; [31]], which are combined and further translated into diagnostic categories (normal; AMR; suspicious for and diagnostic of TCMR; interstitial fibrosis/tubular atrophy; and other, non-rejection-related findings). These automated scoring sheets minimize human error during biopsy scoring and diagnosis and improve inter-observer agreement.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Digital-based assessments of standard stains (e.g. steatosis quantification [32]) are proving to be more reliable in the clinical setting. Transplantation histopathology is evolving toward next generation pathology (NGP), defined as multiplex tissue staining, high-resolution digital imaging, and automated image analyses (reviewed by us [17] and others [33–37]). NGP enables the identification of sub-visual clues such as spatial and inferred temporal context, and extends the capabilities of pathologists to: a) augment natural conceptualization abilities of humans; and b) engage non-pathologist specialists to advance the field (e.g. image analysis experts, mathematicians, biostatisticians, system scientists; [17]).

The current state of digital transplantation histopathology was recently and comprehensively reviewed by Girolami et al [38], including use in donor evaluations and post-transplant monitoring [38]. Most studies highlighted the reliability between traditional light microscopy and digital microscopy to validate reviewing donor and recipient biopsies [38], thus supporting the use of digital pathology in the transplantation setting [38].

Image analytics are advancing through the use of deep learning and convolutional neural networks to recognize tissue compartments (e.g. glomeruli) and analyze tissue specimens [38, 39]. The machine learning field is reconsidering appropriate feature selection (the data points fed into the machine learning framework) and undergoing a shift from “unsupervised selection” to incorporate more “hand-crafted” features providing contextual accuracy while minimizing computational complexity [40]. The potential of clinical implementation is limited by available training data, but newer learning algorithms are emerging that incorporate data augmentation to maximize performance from smaller training data sets [41], promising improved performance without the frontloading cost. The regulatory approval environment/process will evolve, as challenges associated with evaluation, repeatability, and outcome consistency will need to be adapted to the pace at which new algorithms can be developed with machine learning support.

Presently, incorporation of multiplex labeling and automated image analysis platforms in the routine clinical workflow is rare, despite its ability to add considerable critical data (Figure 3). Comparatively, clinical tumor microenvironment evaluation [33–37] uses several single label immunohistochemistry stains to examine expression of immune checkpoint molecules on tumor cells and infiltrating lymphocytes to guide and predict therapeutic responses to specific expensive medications. Translational tumor microenvironment research studies rely on multiplex labeling and image analyses [33–37].

The Need for Integrative Analyses

Significant advances are needed in two areas: 1) integration of simple, non-arcane, task-specific, image analysis toolkits that can easily be accessed via the web; and 2) software that enables integration of currently-available multiple data inputs for translational research analytics, similar to cancer immune microenvironment studies [42–44]. It should also present user interfaces tailored to domain-specific experts such as histocompatibility professionals, clinicians, pathologists, and researchers. Both commercial and open-source pathology data integration and business analytics tools are emerging, however the tradeoff of implementation complexity vs application flexibility remains a major constraint (reviewed in

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

[45]). As whole slide scanners and image management systems gain approval for use in primary molecular diagnostics, this provides an entry point for integration of artificial intelligence into the pathology workflow. Conceptually, pathologist slide review would be carried out in parallel with the quantitative data generated from computational modeling, enabling a hybrid tissue review that could yield additional insights.

CONCLUSIONS

Solid organ allograft recipients experience rejection because they are mismatched with the donor. Currently, the field, including funding agencies, devote less than deserved resources toward improving matching or altering HLA gene expression in transplanted organs. The penultimate data contribution from precision transplant pathology quantifies donor-recipient histocompatibility through NGS and the various enabled downstream analyses (e.g. HLAMatchMaker). It is likely that other genomic loci (e.g. cytokine and chemokine, drug absorption and metabolism polymorphisms) significantly interact with eplet matching and require additional attention. NGS significantly impacts all subsequent steps including DSA analysis, tissue and body fluid -omics analytical platforms and analyses, and tissue biopsy staining and image analytics.

Precision transplant pathology is just starting to penetrate routine clinical care, mostly in large, academic transplant centers. Currently, this is accomplished by humans using “brute force” to examine and integrate diverse data inputs that assist in testing algorithms and biopsy interpretations. Development of NGP panel stains, web-based automated image analytics, and artificial intelligence platforms will facilitate the integration of various genetic characteristics with other recipient and donor attributes for advancing precision medicine.

Acknowledgements:

Financial Support and Sponsorship:

The authors’ research is supported by funding from: NIH [NIAID, Immune Tolerance Network (1UM1AI109565)], 1U19 AI131453, 4U01AI104347; NIDDK, 1 RO1 DK114180; Institutional Support [UPMC, ITTC initiative; Thomas E. Starzl Professor of Pathology Endowment]; Corporate Sponsors (TransMedics; Q2 Solutions).

Abbreviations:

ALT	alanine aminotransferase
AMR	antibody mediated rejection
AST	aspartate aminotransferase
cPRA	calculated panel reactive antibody
DSA	donor specific antibodies
EMR	electronic medical records
IS	immunosuppression
HLA	human leukocyte antigen

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

NGP	next generation pathology
NGS	next generation sequencing
mRNA	messenger ribonucleic acids
miRNA	micro-ribonucleic acids
siRNA	small interfering RNA
TCMR	T-cell mediated rejection
UNOS	United Network of Organ Sharing

REFERENCES

[1]. Holst L The Precision Medicine Initiative: Data-Driven Treatments as Unique as Your Own Body [Internet]. The White House: President Barak Obama Available at: https://obamawhitehouse.archives.gov/blog/2015/01/30/precWoSon-medicine-initiative-data-driven-treatments-unique-your-own-body: 2015.

[2]. Collins FS, Varmus H. A new initiative on precision medicine. N Engl J Med 2015; 372:793–795. [PubMed: 25635347]

[3]. Lister Hill National Center for Biomedical Communications. Help Me Understand Genetics Precision Medicine [Internet]. Genetics Home Reference. U.S. National Library of Medicine, Available at: https://ghr.nlm.nih.gov/primer: National Institutes of Health, Department of Health and Human Services; 2020.

[4]. Nasralla D, Coussios CC, Mergental H et al. A randomized trial of normothermic preservation in liver transplantation. Nature 2018; 557:50–56. [PubMed: 29670285]

[5]. Rüger J, Ioannou S, Castanotto D, Stein CA. Oligonucleotides to the (Gene) Rescue: FDA Approvals 2017-2019. Trends Pharmacol Sci 2020; 41:27–41. [PubMed: 31836192]

[6]. Naderi G, Azadfar A, Yahyazadeh SR et al. Impact of the donor-recipient gender matching on the graft survival from live donors. BMC Nephrol 2020; 21:5. [PubMed: 31906885]

[7]. Wiebe C, Rush DN, Nevins TE et al. Class II Eplet Mismatch Modulates Tacrolimus Trough Levels Required to Prevent Donor-Specific Antibody Development. J Am Soc Nephrol 2017; 28:3353–3362. [PubMed: 28729289]

[8]. Wiebe C, Nickerson PW. Human leukocyte antigen molecular mismatch to risk stratify kidney transplant recipients. Curr Opin Organ Transplant 2020; 25:8–14. [PubMed: 31789952]

[9]. Wiebe C, Nickerson P. Human leukocyte antigen mismatch and precision medicine in transplantation. Curr Opin Organ Transplant 2018; 23:500–505. [PubMed: 29787417]

[10]. Duquesnoy RJ, Gebel HM, Woodle ES et al. High-Resolution HLA Typing for Sensitized Patients: Advances in Medicine and Science Require Us to Challenge Existing Paradigms. Am J Transplant 2015; 15:2780–2781. [PubMed: 26177785]

[11]. Duquesnoy R HLAMatchmaker [Internet]. Available at: http://www.epitopes.net/index.html: 2019.

[12]. Duquesnoy RJ, Marrari M. HLAMatchmaker-based definition of structural human leukocyte antigen epitopes detected by alloantibodies. Curr Opin Organ Transplant 2009; 14:403–409. [PubMed: 19417654]

[13]. Duquesnoy RJ. Are We Ready for Epitope-Based HLA Matching in Clinical Organ Transplantation? Transplantation 2017; 101:1755–1765. [PubMed: 28207632] *This is a comprehensive review regarding the concept of epitope/eplet, and its applications in organ transplantation and beyond. The literature supports the superiority of HLA matching on epitopes vs. antigens, in organ allocation and immunological risk stratification.

[14]. Wiebe C, Kosmoliaptsis V, Pochinco D et al. HLA-DR/DQ molecular mismatch: A prognostic biomarker for primary alloimmunity. Am J Transplant 2019; 19:1708–1719. [PubMed:

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

30414349] *In this single-center cohort study of the kidney transplant recipients, the investigators found HLA DR/DQ eplet mismatch predicted rejection (both TCMR and AMR), de novo DSA development, and graft survival. DR/DQ eplet mismatch was proposed to FDA as a biomarker.

[15]. Huang Y, Dinh A, Heron S et al. Assessing the utilization of high-resolution 2-field HLA typing in solid organ transplantation. American Journal of Transplantation 2019.

[16]. Madbouly A, Gragert L, Freeman J et al. Validation of statistical imputation of allele-level multilocus phased genotypes from ambiguous HLA assignments. Tissue antigens 2014; 84:285–292. [PubMed: 25040134]

[17]. Wood-Trageser MA, Lesniak AJ, Demetris AJ. Enhancing the Value of Histopathological Assessment of Allograft Biopsy Monitoring. Transplantation 2019; 103:1306–1322. [PubMed: 30768568] *This is a comprehensive overview of next generation pathology which discusses new genomic, staining and imaging technologies used to monitor allograft rejection and health, dividing platforms into “non-invasive” and “invasive” platforms, on the basis of whether allograft tissue was required for the analyses.

[18]. Kriss M, Verna EC, Rosen HR, Lozupone CA. Functional Microbiomics in Liver Transplantation: Identifying Novel Targets for Improving Allograft Outcomes. Transplantation 2019; 103:668–678. [PubMed: 30507741]

[19]. Halloran PF, Reeve J, Akalin E et al. Real Time Central Assessment of Kidney Transplant Indication Biopsies by Microarrays: The INTERCOMEX Study. Am J Transplant 2017; 17:2851–2862. [PubMed: 28449409]

[20]. Drachenberg CB, Papadimitriou JC, Hirsch HH et al. Histological patterns of polyomavirus nephropathy: correlation with graft outcome and viral load. Am J Transplant 2004; 4:2082–2092. [PubMed: 15575913]

[21]. Reeve J, Sellarés J, Mengel M et al. Molecular diagnosis of T cell-mediated rejection in human kidney transplant biopsies. Am J Transplant 2013; 13:645–655. [PubMed: 23356949]

[22]. Madill-Thomsen KS, Wiggins RC, Eskandary F et al. The Effect of Cortex/Medulla Proportions on Molecular Diagnoses in Kidney Transplant Biopsies: Rejection and Injury Can Be Assessed in Medulla. Am J Transplant 2017; 17:2117–2128. [PubMed: 28226404]

[23]. Beechem JM. High-Plex Spatially Resolved RNA and Protein Detection Using Digital Spatial Profiling: A Technology Designed for Immuno-oncology Biomarker Discovery and Translational Research. Methods Mol Biol 2020; 2055:563–583. [PubMed: 31502169]

[24]. Ruelcke JE, Loo D, Hill MM. Reducing the cost of semi-automated in-gel tryptic digestion and GeLC sample preparation for high-throughput proteomics. J Proteomics 2016; 149:3–6. [PubMed: 27084685]

[25]. Wegler C, Olander M, Wisniewski JR et al. Global variability analysis of mRNA and protein concentrations across and within human tissues. NAR Genomics and Bioinformatic 2020; 2.

[26]. Edfors F, Danielsson F, Hallström BM et al. Gene-specific correlation of RNA and protein levels in human cells and tissues. Mol Syst Biol 2016; 12:883. [PubMed: 27951527]

[27]. Song L, Fang F, Liu P et al. Quantitative Proteomics for Monitoring Renal Transplant Injury. Proteomics - Clinical Applications 2020; in press.

[28]. Wehmeier C, Amico P, Hirt-Minkowski P et al. Acute Rejection Phenotypes in the Current Era of Immunosuppression: A Single-Center Analysis. Transplant Direct 2017; 3:e136. [PubMed: 28361120]

[29]. Nalesnik MA, Harper T, McHugh J et al. Kidney: Specimen Adequacy and Lesion Scoring (Banff components as of 2018) [Internet]. Transplant Pathology Internet Services, UPMC Available at: https://tpis.upmc.com/changebody.cfm?url=/tpis/schema/KNCode2018.cfm: 2019.

[30]. Nalesnik MA, Harper T, McHugh J et al. Allograft Kidney Biopsy Template. Transplant Pathology Internet Services, UPMC Available at: https://tpis.upmc.com/changebody.cfm?url=/tpis/schema/AlloKidney2018.cfm: 2018.

[31]. Nalesnik MA, Harper T, McHugh J et al. Kidney: 2017 Update of Banff Diagnostic Categories for Renal Allograft Biopsies [Internet]. Transplant Pathology Internet Services, UPMC Available at: https://tpis.upmc.com/changebody.cfm?url=/tpis/schema/KDxCat2017update.cfm: 2018.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

[32]. Abudhaise H, Luong TV, Watkins J et al. Reliability and Accuracy of Clinical Assessment and Digital Image Analysis for Steatosis Evaluation in Discarded Human Livers. Transplant Proc 2019; 51:1679–1683. [PubMed: 31301860]

[33]. Patel SS, Rodig SJ. Overview of Tissue Imaging Methods. Methods Mol Biol 2020; 2055:455–465. [PubMed: 31502165]

[34]. Francisco-Cruz A, Parra ER, Tetzlaff MT, Wistuba II. Multiplex Immunofluorescence Assays. Methods Mol Biol 2020; 2055:467–495. [PubMed: 31502166]

[35]. Akturk G, Sweeney R, Remark R et al. Multiplexed Immunohistochemical Consecutive Staining on Single Slide (MICSSS): Multiplexed Chromogenic IHC Assay for High-Dimensional Tissue Analysis. Methods Mol Biol 2020; 2055:497–519. [PubMed: 31502167]

[36]. Eng J, Thibault G, Luoh SW et al. Cyclic Multiplexed-Immunofluorescence (cmIF), a Highly Multiplexed Method for Single-Cell Analysis. Methods Mol Biol 2020; 2055:521–562. [PubMed: 31502168]

[37]. Manesse M, Patel KK, Bobrow M, Downing SR. The InSituPlex. Methods Mol Biol 2020; 2055:585–592. [PubMed: 31502170]

[38]. Girolami I, Parwani A, Barresi V et al. The Landscape of Digital Pathology in Transplantation: From the Beginning to the Virtual E-Slide. J Pathol Inform 2019; 10:21. [PubMed: 31367473]

[39]. Hermsen M, de Bel T, den Boer M et al. Deep Learning-Based Histopathologic Assessment of Kidney Tissue. J Am Soc Nephrol 2019; 30:1968–1979. [PubMed: 31488607]

[40]. Hosseini S, Lee SH, Cho NI. Feeding Hand-Crafted Features for Enhancing the Performance of Convolutional Neural Networks In: Computer Vision and Pattern Recognition (cs.CV). Cornell University: 2018 p. 1801.07848v07841 [cs.CV].

[41]. Falk T, Mai D, Bensch R et al. U-Net: deep learning for cell counting, detection, and morphometry. Nat Methods 2019; 16:67–70. [PubMed: 30559429] *This manuscript highlights the practical application of a newer, deep-learning framework called U-Net towards automated cell segmentation. The benefits of this architecture include improved adaptability and rapid integration of training data accuracy for a strong demonstration of deep-learning capabilities.

[42]. Ziv E Systems Biology for Multiplatform Data Integration: An Overview. Methods Mol Biol 2020; 2055:641–647. [PubMed: 31502172]

[43]. Palmisano A, Krushkal J, Li MC et al. Bioinformatics Tools and Resources for Cancer Immunotherapy Study. Methods Mol Biol 2020; 2055:649–678. [PubMed: 31502173]

[44]. Thorsson V Multiplatform Integrative Analysis of Immunogenomic Data for Biomarker Discovery. Methods Mol Biol 2020; 2055:679–698. [PubMed: 31502174]

[45]. Aeffner F, Zarella MD, Buchbinder N et al. Introduction to Digital Image Analysis in Whole-slide Imaging: A White Paper from the Digital Pathology Association. J Pathol Inform 2019; 10:9. [PubMed: 30984469]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Key points:

- Precision transplantation pathology serves as the foundation of precision transplant medicine by providing critical data inputs used to individualize patient care.
- Critical data inputs from precision transplant pathology include: (a) NGS-based donor-recipient matching; (b) DSA analyses; (c) clinical chemistry evaluation of organ allograft function; (d) various blood, body fluid, and tissue -omics assays, and (e) tissue biopsy analyses using traditional and next generation pathology.
- Machine learning and artificial intelligence are beginning to penetrate the analytical platforms that contribute NGP data.
- Data integration software with unique “theme-based” user interfaces are needed to assist laboratory professionals in consumption and delivery of domain-specific information.
- More facile software platforms that enable integration of multiple data inputs are needed to translational research to further advance personalized care.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

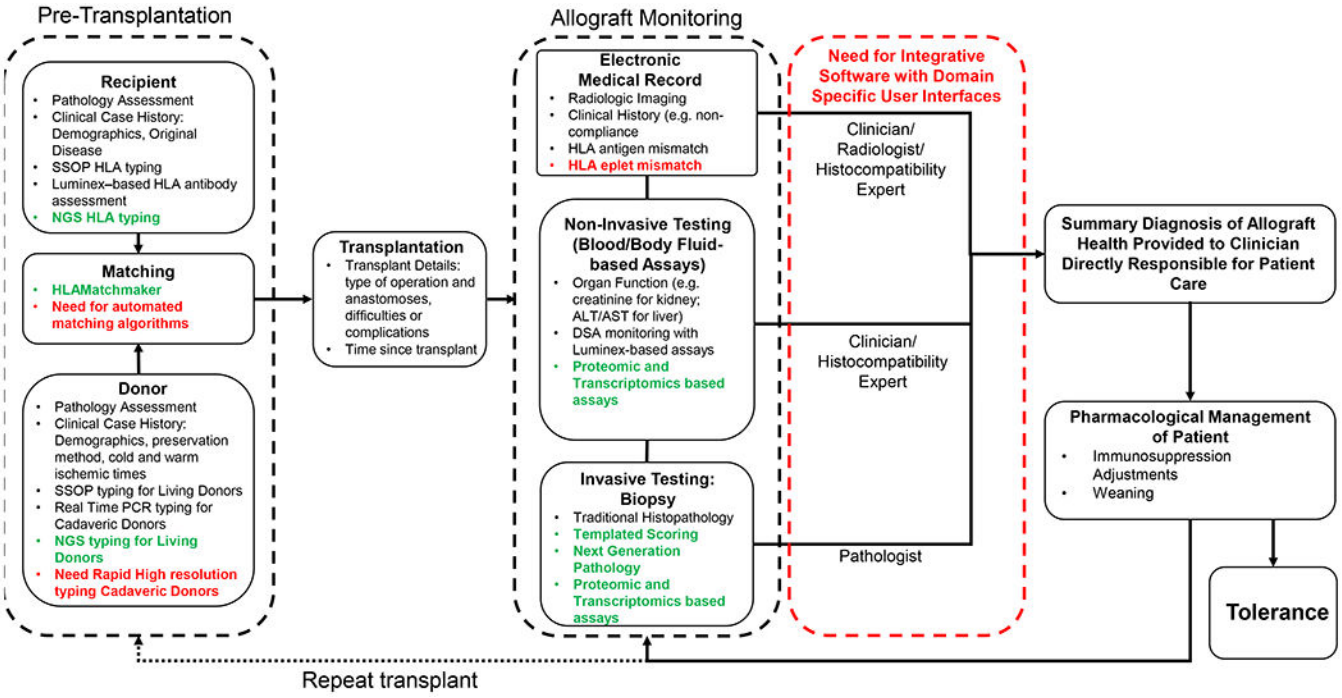


Figure 1. Overall workflow for precision transplant pathology.

For standard of care (in black), donors and recipients must undergo pre-transplantation assessment for histocompatibility, pathology, and clinical case histories. A suitably matched pair will proceed to transplant and subsequent standard of care monitoring (in black). Post-transplantation, standard monitoring includes electronic medical record review, blood and body fluid assessment for organ function and formation of *de novo* donor-specific antibody (DSA), and protocol biopsies. A series of domain experts must then contribute their findings to the clinician who ultimately manages the patient care decisions. Workflow improvements (in green) are slowly being implemented in the areas of next generation sequencing (NGS) for HLA typing, proteomic and transcriptomic based assays for early/subclinical molecular changes indicative of disease/rejection progression, templated scoring, and next generation pathology (NGP). The greatest needs for improvement (in red) include algorithms for automated eplet-matching, rapid high-resolution typing for cadaveric donors, reliable reporting of HLA eplet mismatches, and penultimate integrative software that can render a summation of overall allograft health based on multi-platform inputs from experts of various disciplines. Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; RT-PCR, reverse transcription polymerase chain reaction; SSOP = sequence-specific oligonucleotide probes.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

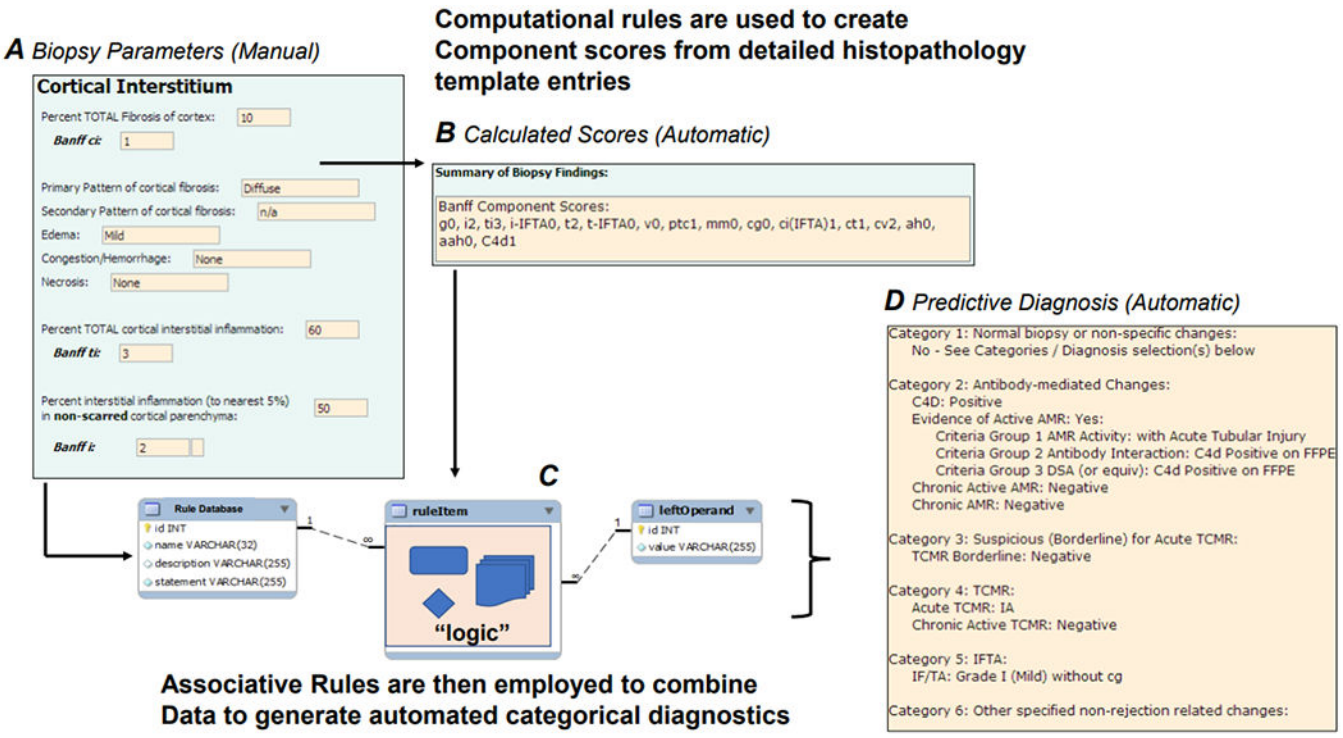


Figure 2. Standardized Template for Biopsy Scoring.

(A) Automated software-based Banff kidney classification via application of computational rules and decision tree engine(s). This workflow utilizes a "smart" template that collects key biopsy related parameters (e.g. glomeruli counts, fibrotic area percentages) and (B) translates the morphological data into Banff-component sub-scores via published guidelines. (C) An additional "decision tree", or inference engine is then layered into the software using plain language to describe the combination of variables that result in a given categorical diagnosis (D). This database-driven inference engine can be combined, layered as needed to model the complexity of the decision. Additionally, AI machine learning can be combined in this step for predictive diagnostic output.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

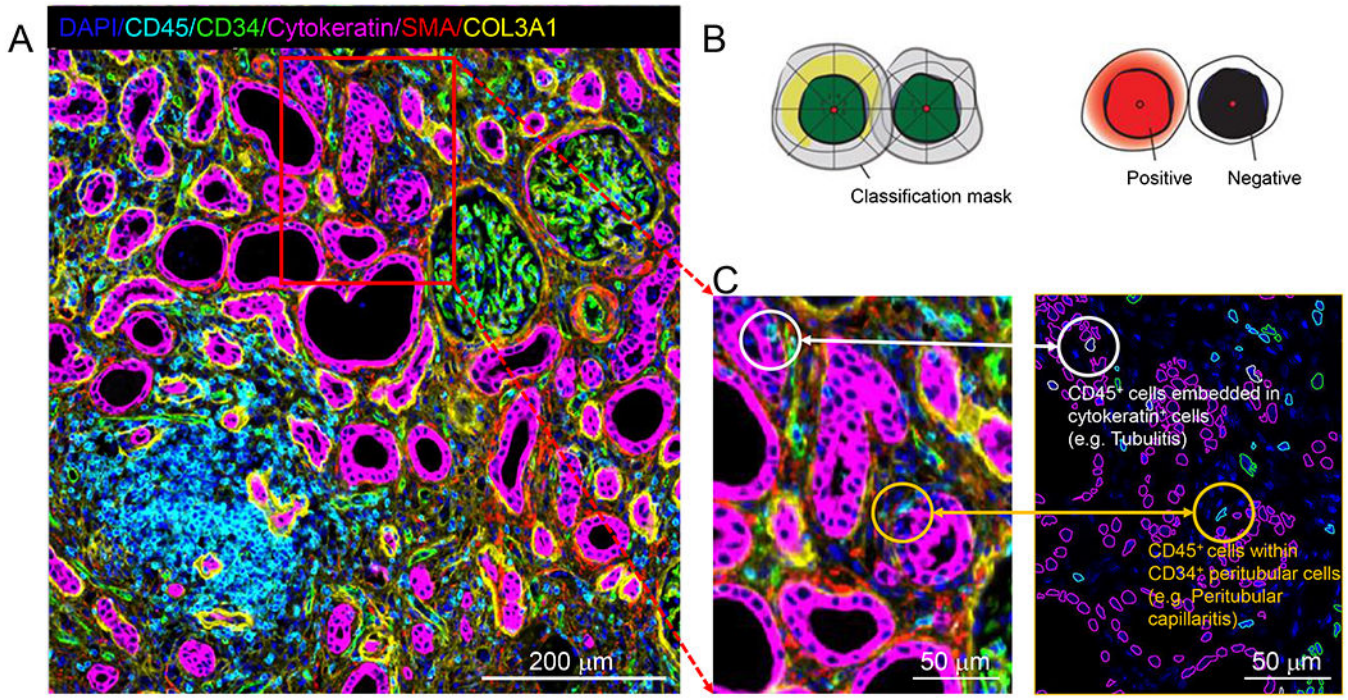


Figure 3. Next Generation Pathology.

(A) A multiplex labeled kidney section demonstrating quantitative scoring by "tissue-tethered" cytometry. The formalin fixed paraffin embedded specimen stained for DAPI (blue), CD45 (teal), CD34 (green), cytokeratin (magenta), smooth muscle actin (SMA; red) and type III collagen (COL3A1; yellow). (B) Machine vision techniques can identify individual cells and their phenotypic characteristics based on surrounding/overlapping analyte expression (classification mask), which can be used to objectively report the total number of each cell type per mm^2 of biopsy area or in certain tissue areas (e.g. peri-tubular expression). (C) We localize nuclear (blue), endothelium ($CD34^+$, green) and pan-leukocyte ($CD45^+$, teal) analytes in possible identified cytoplasmic regions per segmented nucleus, to determine inflammatory cell populations that comprise positive tubulitis and peri-tubular capillaritis expression.