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Molecular assessment of kidney transplant biopsies: reclassifying the disease states in kidney transplants

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Molecular assessment of kidney transplant biopsies: reclassifying the disease states in kidney transplants Philip F. Halloran[1] [1] University of Alberta, Edmonton, AB, Canada **Abstract** This review outlines the molecular rejection and injury states in kidney transplant biopsies as documented in the development of the Molecular Microscope® Diagnostic System (MMDx). The states include T cell-mediated rejection (TCMR), antibody-mediated rejection (ABMR), recent parenchymal injury, and irreversible atrophy-fibrosis. The MMDx project, initiated through a Genome Canada grant, is a collaboration involving many international centers, paralleling the Banff histology system. MMDx uses genome-wide microarrays to measure transcript expression, measuring expression of 19462 genes using 49495 probesets, and interpreting the results using ensembles of machine learning algorithms, and generating a report. Experimental studies in mouse models and cell lines were extensively used to annotate molecular features and interpret the biological mechanisms operating in the biopsy results. Current algorithms are derived from more than 5000 kidney biopsies. The machine learning algorithms identify T cell-mediated rejection (TCMR) and antibody-mediated rejection (ABMR) [1]. The molecular phenotype of all rejection I is dominated by IFNG-induced genes such as CXCL11 and genes shared by T cells and NK cells such as KLRD1[2]. Molecular TCMR is characterized by genes expressed in activated effector T cells (e.g. IFNG and LAG3) and activated macrophages (e.g. ADAMDEC1 and CXCL13)[3]. Molecular ABMR is dominated by NK cell genes (e.g. GNLY), IFNG-induced genes (e.g. IDO1), and genes induced in microcirculation endothelium e.g. ROBO4)[4]. TCMR has two classes: TCMR1, which is more intense and often accompanied by early-stage ABMR; and TCMR2, which is less intense but accompanied by fibrosis. ABMR is divided into early-stage (EABMR); fully-developed (FABMR); and late-stage (LABMR). Molecular rejection generally correlates with histologic rejection, but with many discrepancies, and a number of observations indicate that when there are discrepancies MMDX is more likely to be correct[5] [6]. MMDx also measures and classifies parenchymal injury, which is the principal determinant of dysfunction and risk of failure. Recent/ongoing injury (AKI") is indicated by expression of injury-induced molecules and macrophage infiltration[7,8] and correlates better with depression of GFR than histologic changes. There are two classes of AKI: AKI1 with minimal

inflammation and AKI1 with more inflammation and response to wounding[9,10]. Atrophy-fibrosis is associated with expression of immunoglobulin and mast cell transcripts[11,12]. All injury is accompanied by dedifferentiation: loss of the kidney transcripts associated with normal function, metabolism, and cellular respiration. Rejection-induced parenchymal injury: TCMR always induces parenchymal injury (visible as tubulitis) and atrophy-fibrosis, whereas ABMR initially spares the parenchymal but slowly induces atrophy-fibrosis. The parenchymal injury changes persist after rejection changes have been suppressed. All injury profoundly affects kidney survival. Prognosis is determined both by atrophy-fibrosis features and most strongly by AKI features, which are often present in progressing kidney transplants[13]. For example, prognosis in ABMR is determined by parenchymal injury, not by ABMR activity[14]. MMDx revealed unexpected aspects of the disease states e.g. ABMR is usually C4d-negative and often DSA-negative[15,16]. Subtle minor ABMR-like states are frequent and indicate that antibody injury may be more widespread than previously suspected. Parenchymal injury correlates with both reduced GFR and increased risk of graft loss and has considerable molecular diversity. Both TCMR and ABMR produce injury: TCMR induces severe nephron injury (visible as tubulitis in histology) and accelerates atrophy-fibrosis, and ABMR induces slowly progressive atrophy-fibrosis. The MMDx system has been used to document the relationships between plasma dd-cfDNA levels (the Prospera assay, Natera) and molecular processes in kidney biopsy. The dd-cfDNA levels correlate strongly with ABMR activity, as manifest by NK cell transcripts and IFNG-induced transcripts, and to a lesser extent with TCMR activity and recent injury[17]. The dd-cfDNA levels predict molecular rejection more strongly that histologic rejection[17,18]. The dd-cfDNA levels predict molecular ABMR is the biopsy better than DSA[16]. Molecular rejection correlated better with dd-cfDNA quantity than percent[19]. MMDx emerges as an objective diagnostic biopsy assessment system for kidney transplants that can be used to calibrate biomarkers, optimize histology interpretation, and guide clinical trials of new treatments. Similar assessments are in progress for heart transplant endomyocardial biopsies, lung transplant transbronchial biopsies and mucosal biopsies, and liver transplant biopsies. **References**

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