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Mechanisms and biomarkers of immune quiescence in kidney transplantation

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ABSTRACT

This review discusses the current understanding of biomarkers of immune quiescence based on reviews of published literature in kidney transplant operational tolerance and mechanistic studies based on a better characterization of the stable, well-functioning renal allograft.

1. Introduction

The concept of transplant tolerance encompasses the presence of a well-functioning graft, lacking histological signs of rejection, in the absence of any immunosuppressive (IS) drugs, in an immunocompetent host [1,2]. Most reports use a cut-off point of 1 year after IS withdrawal to see if stable (or metastable) tolerance has been achieved [1–3]. Spontaneous operational tolerance has incidentally been found in patients, who are either non-adherent or are under physician-directed IS minimization at the time of clinically evident over IS, such as in the context of malignancy and severe infections [3,4]. On the contrary, induction of deliberate tolerance has occasionally been observed in humans; for example, with induced mixed chimerism seen after adoptive transfer of tolerogenic regulatory cells [4–6]. Selecting which patient will achieve this state and when drugs should or can be withdrawn safely for deliberate tolerance induction, remains difficult, as no single tolerance specific biomarker has been validated sufficiently for clinical use [4]. Benefits from IS withdrawal are very attractive, such as less IS-related complications, lower drug costs, and resulting in a better quality of life [7]. Therefore, considerable interest has been garnered in the community for detection of marker "states" for kidney transplant tolerance, so as to identify the patient and the timing for IS withdrawal, rather than the current ad hoc, trial and error approach [8].

Stable transplant tolerance requires both a state of donor-specific hyporesponsiveness and active immune regulation [9], inclusive of suppression or apoptosis of donor-reactive inflammatory cells and expansion in the number/activation state of regulatory cells. Harnessing the pathophysiology and clinical definitions of transplant tolerance to develop diagnostic biomarkers of metastable tolerogenic states, as surrogate biomarkers of immune quiescence, has been one approach to better assess and detect a state of ongoing/active immune acceptance, that would be amenable to IS manipulation and minimization, without rebound graft rejection. The process for development of these diagnostic markers faces challenges of patient selection, clinical phenotyping, sample numbers, false discovery rates during unbiased approaches, and difficulty in obtaining replicate or equivalent validation and cross-validation cohorts (Fig. 1). Additionally, assays and clinical development processes cannot translate into clinical benefit without continued support from funding agencies and clinical collaborations. Finally, during the clinical development phase, multi-step trials are

Abbreviations: 5′AMP, 5′ adenosine monophosphate; ADO, adenosine; DSAs, anti-donor specific antibodies; APC, antigen-presenting cell; CXCL-9, chemokine (C-X-C motif) ligand 9; cAMP, cyclic adenosine monophosphate; CTLA4, cytotoxic T-lymphocyte associated protein 4; ELISAs, enzyme-linked immunosorbent assays; Fas/Fas-L, fas cell surface death receptor, fas cell surface death receptor-ligand; FoxP3+, forkhead box protein 3; GEO, gene expression omnibus; IS, immunosuppressive; IOT, indices of tolerance; IDO, indoleamine 2,3-dioxygenase; ICER, inducible cyclic adenosine monophosphate (cAMP) early repressor; iNOS, inducible nitric oxide synthase; IFN, interferon; IL-1β, interleukin -1β; IL-10, interleukin-10; IL-35, interleukin-35; IL-6, interleukin-6; kSORT, kidney solid organ response test; mRNA, messenger RNA; miRNA, microRNA; pDCs, plasmacytoid dendritic cells; PGE2, prostaglandin E2; qPCR, quantitative polymerase chain reaction; Bregs, regulatory B cells; Mregs, regulatory macrophages; Tregs, regulatory T cells; RE, reticuloendothelial; TIM3, T cell immunoglobulin and mucin domain-3; Helper T cells, Th; CLIA, the clinical laboratory improvement amendments; FDA, the food and drug administration; ITN, the immune tolerance network; TAIC-II, the transplant acceptance-inducing cell II trial; TRAIL, TNF-related apoptosis-inducing ligand; TGF-β, tumor growth factor-β; TNF, tumor necrosis factor; TRANCE, tumor necrosis factor (TNF)-related activation-induced cytokine

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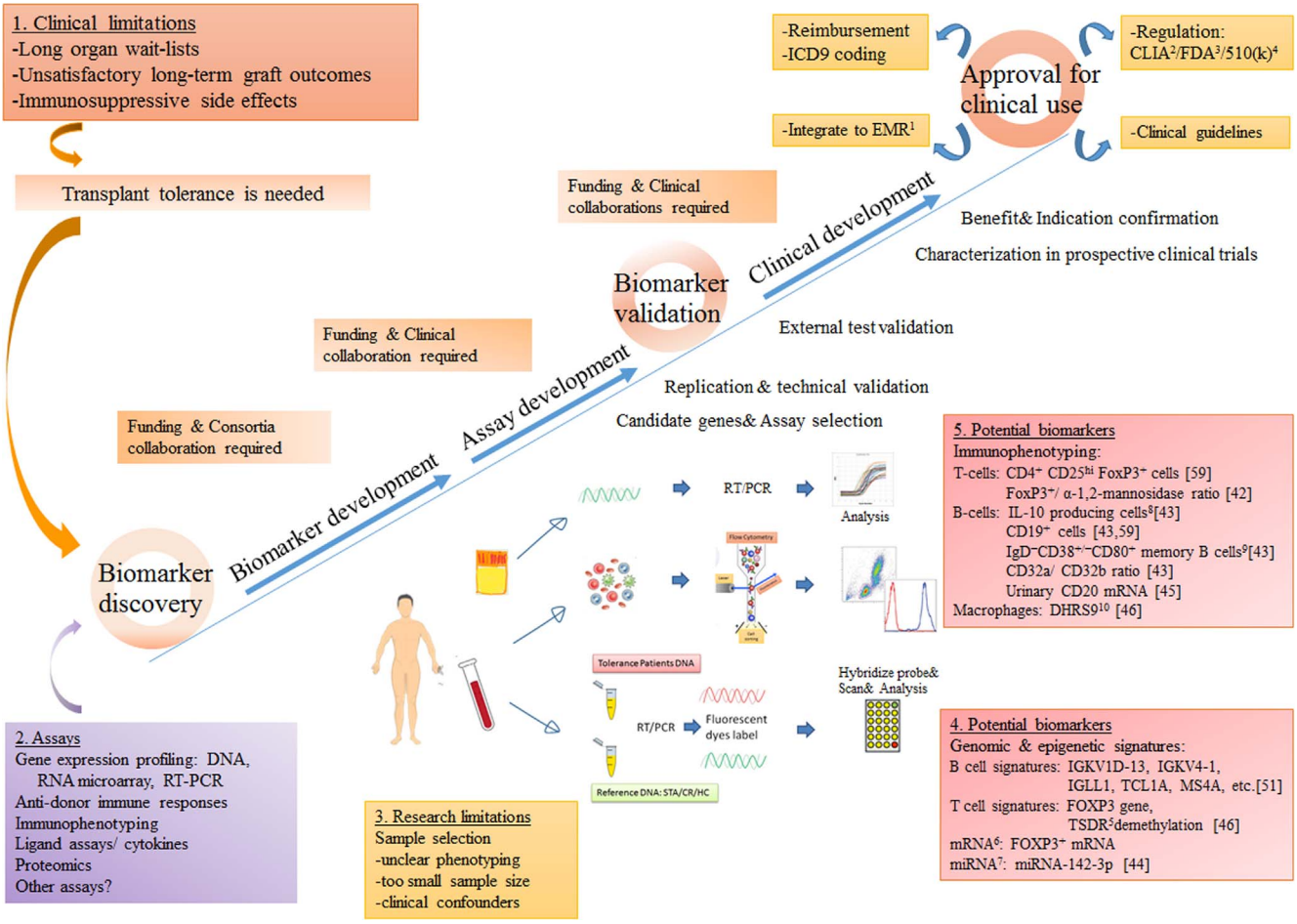

Fig. 1. A summary of different components of successful biomarker discovery and validation for transplant tolerance. Abbreviation: [1]EMR: electronic medical record, [2]CLIA: The Clinical Laboratory Improvement Amendments, [3]FDA: the Food and Drug Administration, [4]510(k): section of the Food, Drug and Cosmetic Act requires device manufacturer who must register, to notify FDA of their intent to market a medical device at least 90 days in advance., [5]TSDR: Regulatory T cells-specific demethylated region (TSDR), [6]mRNA: messenger ribonucleic acid, [7]miRNA: micro-ribonucleic acid, [8]IL-10: Interleukin-10, [9]IgD: immunoglobulin G, [10]DHRS9: dehydrogenase/reductase 9. References numbers: provided in the brackets.

needed to be approved by regulatory agencies before applying these discoveries back to the clinic, where they can be used to change practice guidelines, and support acquisition of reimbursement, and development of new or revised ICD-9 codes (Fig. 1).

2. How do we define immune quiescence?

An unanswered, yet important, a question is to re-evaluate our understanding of immune quiescence and its actual definition. A lack of coherence for this definition among clinical and research groups results in misleading results from different studies. The definition of immune quiescence, in the context of the kidney allograft, faces challenges from insensitive clinical diagnosis (with the redundancy of the serum creatinine for detecting early injury), the variability of tissue sampling by biopsy, the invasiveness of the biopsy, and the high inter-intraobserver variability in pathological diagnoses [10–12]. Our group and others have shown that normal "clinical" graft function cannot be quarantined from subclinical tissue injury and normal histology cannot entirely preclude patchy inflammatory molecular changes in the same kidney [13–16]. Thus, a clinical diagnosis of non-rejection is not necessarily a lack of inflammation; and stable graft function is not necessarily immune quiescence.

As the majority of genomic studies in kidney transplant tolerance have used a clinical diagnosis for stable graft function [17–23], it is likely that incorrect input phenotype diagnoses in those studies may be another reason why inconsistent gene signature patterns were found in different microarray analysis [17–23]. Before moving forward, the first hurdle to overcome is the lack of standardized molecular testing in order to discriminate stable graft function, or a control group, from a rejection group and other injuries. We would suggest that the *absence* of any of the validated biomarkers for graft injury and rejection from blood, such as donor-derived cell-free DNA, and the monocyte-specific 17 gene-set called the kidney solid organ response test, or kSORT, will support selection of stable transplant patients and more precise phenotyping of patients to be included in tolerance studies for finding the most sensitive and specific biomarkers for immune quiescence.

3. The kidney: resistant to tolerance induction

The kidney is vulnerable to immune injury from many events as seen in immune-mediated glomerular diseases, which are common causes of end-stage failure [24]. Even under IS therapy after transplantation, the kidney graft carries a high risk of immune injury which gates graft life expectancy. When compared with the liver graft, the most tolerogenic transplanted organ, with 20–42.6% being tolerant after deliberate IS withdrawal [25–30], the rates of operational tolerance observed in kidney transplantation are closer to 7% [8,31]. Studies also indicate that the kidney graft is more likely to be resistant to tolerance induction [32]. Some kidney transplant trials have found that T cell depletion results in the subsequent repopulation of activated memory T cells which are resistant to suppression by regulatory T cells [32,33].