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Developing therapeutic “arrows” with the precision of William Tell: the time has come for targeted therapies in kidney disease

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Abstract

Purpose of review—A core mission for modern medicine is the development of precision therapeutics. Cancer therapies have been at the leading edge of this effort, while nephrology has lagged on the path to precision medicine. Breaking the stalemate, recent work revealed CD80 (B7-1) as a candidate for targeted therapy in treatment of resistant nephrotic syndrome. This review aims to summarize the current state of our understanding of podocyte CD80 biology, its therapeutic implications, and the challenges that lie ahead in essential future validation studies.

Recent findings—The CD80 targeting agent abatacept (CTLA4-Ig), approved to treat rheumatoid arthritis, was shown to induce remission of nephrotic range proteinuria in four patients with recurrence of disease post-transplant, and one patient with primary, treatment resistant nephrotic syndrome. The concept of “CD80-positive” proteinuric kidney disease due to podocyte CD80 staining in patient kidney biopsies was introduced as a molecular biomarker to define disease and guide treatment. The mechanism of action of CTLA4-Ig in podocytes was shown to center on $\beta 1$ integrin activation in a T-cell-independent fashion. Subsequent work revealed a putative role for podocyte CD80 in diabetic kidney disease.

Summary—These studies have direct implications for patient care, and intense interest has focused on validating these findings in upcoming clinical trials.

Keywords

CD80/B7-1; Integrin Inactivation; Nephrotic Syndrome; Precision Medicine; Proteinuria

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Introduction

“Tonight, I’m launching a new Precision Medicine Initiative [...]” — President Barack Obama, State of the Union Address, January 20, 2015. In their *New England Journal of Medicine* perspective [1], Drs. Francis Collins and Harold Varmus quoted President Obama’s words from his 2015 State of the Union address, where he presented his vision for precision medicine, that is, treatment given to patients taking into account their individual variability. Rather than defining diseases based on symptoms, we can now use modern tools to provide molecular definitions for diseases, which can in turn guide our therapies, hoping to avoid unnecessary toxicities and complications. As Drs. Collins and Varmus also noted, indeed “the time is right for this visionary initiative”[1]. Is there a place for kidney disease therapeutics in this space? And if so, what is required to make strides toward the concept of “precision nephrology?” This perspective aims to address these questions using recent work in CD80-positive proteinuric kidney disease [2, 3] as an example for the large body of work that lies ahead if we are to take aim on kidney disease targets with therapeutic “arrows” as precise as these of the famous archer William Tell.

Current diagnostic limitations

To date, kidney disease diagnoses have largely relied on kidney biopsy findings. And yet, it is often with frustration that clinicians encounter terms such as “global” or “focal and segmental sclerosis,” which do not reveal much about the specific molecular pathologic mechanisms that led to these histologic abnormalities in individual patients. Genetic mutations, exposure to toxins, or immune dysregulation can lead to the same histologic pattern we often call “focal and segmental glomerulosclerosis” or FSGS. We sometimes mistakenly teach our residents and students that this is a specific disease entity, forgetting perhaps that “FSGS” is simply a histologic description in a kidney biopsy, similar to other generic histologic terms such as “fibrosis,” which carries no specific meaning as to how, in molecular terms, the tissue came to be fibrotic. Nevertheless, the past decade has seen a growing number of studies aimed at understanding the molecular underpinnings of glomerular pathology, and indeed important insights have been gained. One essential and fundamental understanding is that proteinuria, one of the earliest and perhaps most reliable hallmarks of progressive kidney disease, is the result of either direct or indirect injury to essential glomerular cells, the podocytes [4].

Podocyte injury causes proteinuria

The kidney glomerulus is a highly specialized structure that ensures selective ultrafiltration of plasma, so that essential proteins are retained in the blood. Glomerular podocytes, with their foot processes and interposed slit diaphragms, serve as a final barrier to urinary protein loss. Disrupted podocyte function damages the kidney filter, leading to proteinuria and nephrotic syndrome [4]. Clinically, proteinuria is the common denominator of a heterogeneous group of histologic abnormalities, such as minimal change disease (MCD) and FSGS, or diseases such as membranous nephropathy (MN), lupus nephritis and diabetic kidney disease, conditions that affect millions of patients worldwide often leading to end stage kidney disease (ESKD) [4]. In particular, primary FSGS, and its recurrence after

kidney transplantation, remain largely untreatable diseases associated with kidney failure, need for dialysis and allograft loss [3, 5].

Abatacept in CD80 positive proteinuric kidney disease

Podocyte injury is associated with the development of proteinuria and the induction of podocyte CD80 expression in human lupus nephritis, murine lupus nephritis, $\alpha 3$ integrin ($\alpha 3^{-/-}$) knockout mice, nephrin knockout mice, and murine LPS induced proteinuria [6]. More recently, we reported induction of podocyte CD80 in biopsies of patients with nephrotic syndrome, including primary and recurrent FSGS [2]. Podocyte CD80 staining may thus serve as a biomarker to facilitate the diagnosis and targeted treatment of a subset of “CD80-positive” proteinuric kidney diseases. Importantly, treatment with Abatacept, a CD80 blocker currently approved for the treatment of rheumatoid arthritis and juvenile idiopathic arthritis [7], was successful in inducing remission of proteinuria in four patients with rituximab-resistant recurrent FSGS in renal allografts and one patient with steroid-resistant FSGS in native kidneys [2]. Extending these findings, Fiorina and colleagues showed in genetic and epidemiologic studies the association of two single nucleotide polymorphisms at the CD80 gene with diabetic nephropathy [8]. The functional relevance of these polymorphisms is presently unknown and future studies will be needed to address this interesting question. Furthermore, increased levels of the soluble isoform of the CD80 ligand CD28 correlated with ESKD in patients with type 2 diabetes. *In vivo* podocyte expression of CD80 was induced in two mouse models of diabetic nephropathy, and treatment with CTLA4-Ig prevented increases in proteinuria, and improved kidney pathology in these animals [8]. Therefore, this study identified CD80 inhibition as a potential therapeutic strategy for the prevention or treatment of diabetic kidney disease.

Current limitations in CD80 kidney biopsy staining

Is podocyte CD80 induction common to all proteinuric kidney diseases, representing a final common pattern of injury? Or is it a specific marker for a subset of patients, perhaps patients with specific genetic susceptibility as shown by Fiorina and colleagues [8]? The answer to this question is largely limited by the specificity and sensitivity of CD80 staining in human kidney biopsies, a procedure which has turned out to be technically difficult and often misinterpreted. Firstly, antibodies to CD80 have been notoriously difficult to work with since the early days of its discovery in co-stimulation [9]. Secondly, the abundance of CD80 in podocytes, even after injury and stress, is modest, compounding the lack of a workable dynamic range for immune-detection methods [10]. Finally, the epitope for CD80, detectable only in fresh frozen tissue, appears to readily degrade over time, leading once again to false negatives [10]. These technical considerations beg the question: are we underestimating, due to technical limitations leading to false negative results, the number of patients with treatment resistant proteinuria in whom podocyte CD80 positivity may render them candidates for abatacept treatment? The answer may be yes, but ongoing studies using novel antibodies, specific staining protocols and advanced imaging techniques will define the proportion of “CD80 positive” proteinuric kidney disease, which was originally thought to represent around 30% of all kidney biopsies for refractory proteinuria (across different histologic classifications), based on analysis of archival fresh frozen tissue from Brigham

and Women's Hospital Pathology Department [2]. Furthermore, while recent efforts at urinary detection of CD80 may be difficult to interpret [11, 12], this approach, once optimized further, may be complementary to the podocyte-specific detection of CD80 in kidney biopsy material. Most importantly, the reliable detection of CD80 induction in the setting of treatment-resistant proteinuric diseases promises to serve as the foundational example of biomarker-driven, targeted therapy in nephrology, opening the possibility for future work in "precision nephrology."

CD80 targeted therapy for proteinuria: clinical experience

Subsequent to the publication of five cases of successful remission of proteinuria with abatacept in the *New England Journal of Medicine* [2], a small number of additional cases emerged. Firstly, a small series of patients with a variety of underlying diseases, all post-transplant, were treated with a close analogue of abatacept called belatacept, with disappointing results [13]. However, the lack of consistent underlying diagnoses, the late onset of treatment with a CD80 targeted agent, the fact that belatacept was used as a replacement for calcineurin inhibitors, and the choice itself of a different agent (belatacept versus abatacept) make it impossible to draw any firm conclusions from this series [10]. Furthermore, a single case of successful discontinuation of plasmapheresis after abatacept treatment in a patient with recurrent FSGS was published as an abstract at ASN Kidney Week 2014 [14]. Finally, two patients with treatment resistant nephrotic syndrome and unknown CD80 status on kidney biopsy were treated with abatacept, with one achieving a rapid remission and the other not responding [15]. Once again, it is impossible to draw valid conclusions from these few reported cases. Yet, these data, and the many communications in the lay press and on patient advocacy websites, collectively speak to the great unmet need for patients with treatment resistant nephrotic syndrome, and thus the urgent need for validation studies done with appropriate controls, in a randomized controlled fashion. We hope that such a randomized controlled trial testing the effect of abatacept in treatment resistant nephrotic syndrome will begin enrollment soon.

How does CD80 targeted therapy work in the podocyte?

One of the many important questions that remain revolves around CD80 versus CD86 expression in podocytes, and the possible relationship of these molecules to the choice of abatacept versus belatacept. Indeed, CD86 is not detectable in podocytes neither at baseline, nor under stress conditions. Importantly, belatacept, approved for maintenance immunosuppression in renal transplantation as a replacement for calcineurin inhibitors, has enhanced potency over abatacept as an immunosuppressant because of its increased affinity for CD86 [16]. Since podocytes do not express CD86 [2], and one would favor the *absence* of a strong immunosuppressive profile for a candidate anti-proteinuric, podocyte-targeted drug, abatacept emerges as a better choice for "CD80 positive" treatment resistant nephrotic syndrome. However, future studies may and should address the potential for belatacept as a treatment for proteinuric kidney disease, especially in the post-transplant setting.

It is also important to remember that it is not the extracellular portion of CD80 that disrupts podocytes, but rather, the binding of its intracellular tail to $\beta 1$ integrin (Figure 1). This is in

contrast to our understanding of classical CD80 and CD86 biology in co-stimulation, where the extracellular domains of these molecules comport important roles for them in immune regulation [17]. The idea that a conformational change induced by the specific CTLA4-Ig called abatacept may result in a specific conformational change that disrupts binding to $\beta 1$ integrin, while speculative at this point, may hold the key as to why abatacept, and not belatacept, whose corresponding CTLA4-Ig is structurally different by two amino acids [16], is more effective in our limited experience with patients treated thus far. More studies are needed at the structural/functional level to elucidate this testable hypothesis.

Due to their privileged “geographic” location as an integral component of the kidney filter barrier, where they are exposed to all antigens, including bacteria, toxins and viruses circulating in the bloodstream, podocytes may have indeed evolved mechanisms similar to antigen presenting cells, including a repertoire of inducible molecules related to the innate immune response, such as Toll-like receptors, CD80, and type I interferon signaling. Gurkan and colleagues recently showed that podocytes, which receive signals from the vascular space, including circulating antigens, constitutively express TLR1–6 and TLR8 [18]. They additionally found that podocytes can respond to TLR ligands including staphylococcal enterotoxin, poly I:C, or lipopolysaccharide with pro-inflammatory cytokine release and activation of type I interferon signaling [18]. This induced podocyte CD80 expression, and transient proteinuria, which could be blocked by a type I interferon receptor-blocking antibody [18]. This study significantly extended our understanding of podocyte responses to immune stimuli and revealed a novel mechanism for infection- or inflammation-induced proteinuria in a CD80-dependent, T-cell independent manner.

The road ahead: clinical trials

Podocyte CD80 and its targeted inhibition may be one of hopefully many more precision medicine strategies in nephrology in the upcoming years. Using this as an example, and summarizing what we know to date, should serve as a reminder that all new approaches must be validated in randomized controlled trials, which we should strive to pursue collectively in our field. Important questions remain, and their answers, or the hypotheses they generate, will inform critical aspects of trial design. For example, what is the optimal window for abatacept dosing in patients with treatment resistant primary nephrotic syndrome? When may we give abatacept in relation to other treatments, and should we tolerate concurrent use of other immunomodulatory agents? How many doses should be given, and should this therapy continue indefinitely, or can it be safely stopped after remission is achieved? Are there different induction versus maintenance dosing regimens we can develop? What should be done for patients with treatment resistant disease who are CD80 negative, but may have no other therapeutic options? Is there a role for pre-emptive abatacept dosing prior to transplantation in high-risk cases? These are some of the many questions that remain, a subset of which will be hopefully addressed in upcoming randomized controlled trials.

Conclusion

In conclusion, like William Tell of the famous fable, we must redefine kidney disease in molecular terms and then work hard to develop the precise therapeutic “arrows” which will allow us in nephrology to join our colleagues from other fields in fulfilling a timely call for precision medicine.

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Key points

- Nephrology must join other fields in developing precision therapeutics
- Podocyte CD80 targeted therapy may offer a foundation for future precision medicine efforts in nephrology
- Clinical trials and further validation work are necessary to bolster the clinical efficacy of CD80 targeted therapy for proteinuric kidney diseases

Figure 1. Protection of β_1 integrin activation in podocytes is the putative mechanism underlying the anti-proteinuric action of abatacept

In the absence of talin binding to the cytoplasmic tail of β_1 integrin, $\alpha_3\beta_1$ integrin is in an inactive state. Talin binding to the cytoplasmic domain in β_1 integrin disrupts the salt bridge between the α/β integrin tails, leading to tail separation and conformation changes that result in integrin activation. B7-1 blocks $\alpha_3\beta_1$ integrin activation by disrupting the binding of talin to β_1 integrin. PM: plasma membrane.