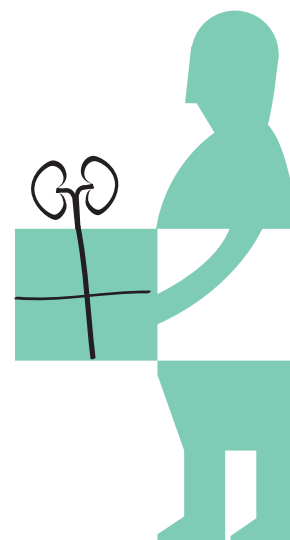


# The personalised touch



**Dr Minnie Sarwal** reveals the efforts of her lab in the pursuit of a novel approach to transplant medicine and renal diseases, spanning from basic research to application in patients

## Can you introduce the key research objectives of your lab, and the investigations that are currently underway?

I have a translational research lab that focuses on understanding the biological basis of kidney disease and organ transplant injury, with a view to developing improved diagnostics and finding novel targets for rational drug design. The ultimate objective of this work is to provide personalised and predictive medicine.

## Why are current biomarkers for organ transplantation – and kidney transplantation in particular – far from satisfactory?

Current measures of organ transplant dysfunction are based on a functional decline in serological markers that are non-specific for cause and reflect substantive – often irreversible – tissue injury. We need biomarkers that are minimally invasive, sensitive and predictive of tissue injury, and specific for the cause of that injury. This will allow for organ injury to be treated early and reversed, and it will also enable the extension of the life expectancy of the organ.

## How has your team addressed this situation through the development of new testing methods?

Our group has focused on the careful collection, annotation and archiving of biosamples and matched tissue biopsies from patients in a serial manner over the last 20 years. Highly selected samples with defined diagnoses were subjected to hypothesis-generating, unbiased, high-throughput genome-wide studies that resulted in the discovery of genes, proteins,

antibodies and metabolites that were correlative with disease. Robust, multicentre, prospective validation studies funded by the National Institutes of Health (NIH) supported our efforts to confirm the validity of specific biomarker sets for blood-based detection of organ transplant rejection and tolerance. Two robust assays have resulted from these efforts – the first of which is the kidney solid organ rejection test (kSORT), which defines the immune risk threshold for immune activation in the transplanted organ. The second is the kidney spontaneous operational tolerance test (kSPOT), which defines the immune risk threshold for graft acceptance and accommodation. The serial analysis of blood samples for kSORT and the kSPOT assays by qPCR every three months post-transplant can drive immunosuppression dosing customisation.

Understanding the biological basis of these conditions has also allowed us to develop new drug targets for transplant recipients by drug repositioning efforts.

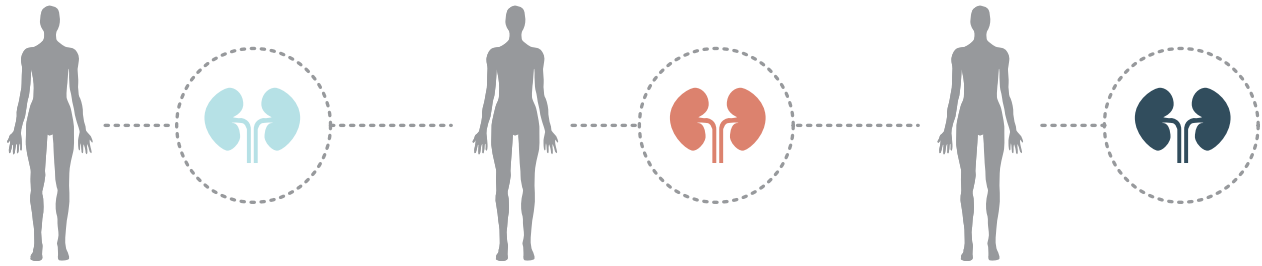
## What is the process by which you analyse potential biomarkers before applying them to patients?

Robust discovery and careful statistical analysis of clinical, demographic, racial and disease variables that may associate with biomarker drifts is important to limit false positives in the biomarker selection stage. It is then important to cross-validate using restricted sets of genes, proteins, metabolites or antibodies in independent datasets, and test real-life performance of the biomarkers in clinical trials with prospective, longitudinal analyses. In this manner, the biomarker can be

evaluated for its ability to predict disease well before it is clinically evident. The next step is to apply the biomarker in treatment intervention in a randomised and, ideally, blinded fashion to prove clinical and economic benefit. At this point, the data developed for the validated biomarker are also often considered for regulatory review. The biomarker is then ready for clinical deployment.

## The current focus of your lab is shifting towards finding novel drug targets for renal diseases. Could you offer some insights into this avenue of work?

Recently, we have used our discovery approaches to find novel biological bases for graft rejection and two proteinuric kidney diseases that result in rapid organ failure, cystinosis and focal segmental glomerulosclerosis (FSGS). The rejection studies suggested that using statins for treatment of hyperlipidaemia can also reduce inflammation in the graft and even treat rejection. The costimulatory axis of CD40 was found to be perturbed in FSGS, and blocking activation of CD40 in kidney cells actually reverted the injury that results from this condition. This has generated a lot of excitement, and we intend to work with our pharmaceutical partners in order to test new monoclonal antibodies for CD40 that can treat human FSGS. New drug discovery costs billions of dollars, and therefore we believe that our approach of repositioning US Food and Drug Administration (FDA)-approved drugs based on an improved biological understanding of human disease is a viable and exciting approach to drug design.



## A tale of two assays

A lab at the **University of California**, San Francisco, has made great strides in the targeted management of organ transplants, and its kSORT and kSPOT assays are poised to revolutionise the way in which recipients of donor kidneys are handled by medical practitioners

**FOR THOSE WITH** organ failure or severe disease, organ transplantation can offer a new lease of life that would otherwise be impossible. Kidneys are the most common candidate organs for transplantation in the US, but there are still a number of ways in which scientists are looking to improve the procedure.

One such scientist is Dr Minnie Sarwal, who has dedicated her career to developing a personalised approach to organ transplantation and overseeing the translation of these efforts into clinical practice. She leads a dedicated team of researchers at the University of California, San Francisco, where they are making use of high-throughput technologies to develop assays that will ultimately lead to reduced morbidity and improved quality of life.

One of the major issues to be overcome is the lack of suitable biomarkers for kidney transplant dysfunction. There have been notable increases in the success rate of kidney transplants in recent years, but complications are still fairly common, and early rejection of the transplanted organ still occurs in around 15 per cent of recipients. Current markers are not able to give early warning that dysfunction will take place, and often a biopsy is required in order to obtain an accurate diagnosis. Not only is this a very invasive procedure, it can also be unreliable in identifying issues with the organ.

### ALL THE OMICS

The research being undertaken in the Sarwal lab takes a complete end-to-end approach to transplant medicine, in order to achieve more personalised results. Their work begins by first seeking to understand the mechanisms behind a disease, and then investigating treatment and care options.

The high-throughput methods employed in this research range from studying DNA using sequencing, to genomics using microarray technology and proteomics using high-throughput LC MS/MS technology, as well as metabolomics and antiomics, for which the team uses protein array technology. "Rather than doing research where we have an

*a priori* hypothesis about specific molecules and pathways that we think we already know are deregulated in disease, we present a hypothesis generation approach," outlines Sarwal. "This way, we let the data speak for themselves and provide us with clues about differences between disease and non-disease."

### kSORT

The major focus of Sarwal's efforts, and indeed the crowning achievement of her 25-year research career, has been the development of two sensitive, specific assays for kidney transplant patients – the kidney solid organ rejection test (kSORT) and the kidney spontaneous operational tolerance test (kSPOT).

kSORT is a panel of 17 genes that can predict the onset of rejection three to four months earlier than current biomarkers. "It is also highly correlated with the risk of rejection when confirmed by biopsy, and more importantly it is picking up immune activation in the organ well before we can even detect it using any other available monitoring assay," Sarwal notes. Early diagnosis of immune injury means early treatment and organ preservation.

To date, the assay has been validated for rejection diagnosis in the presence of multiple comorbidities and across a wide demographic of patients, including almost 1,000 children and adults from the US, Spain, Sweden and Mexico. The sensitivity of the assay is at 85 per cent, and its specificity is over 90 per cent, whereas current monitoring by the serum creatinine has an assay specificity of only 20 per cent for acute rejection diagnosis. This assay performance makes kSORT a crucial tool at the disposal of medical practitioners for monitoring the health of kidney transplant patients. If this non-invasive test is conducted serially following transplantation, doctors will have a three to four month window into very early detection of incipient immune injury in the organ. This will enable them to act earlier and in a targeted manner for immunosuppression dose customisation to rejection risk, with significant downstream benefits for mitigating

### BIOMARKERS TO BEDSIDE

Having spent decades refining biomarker-based assays for organ transplantation, 2015 is the year in which Sarwal will finally see them translated into the clinic

- While working at Stanford University, Sarwal became the scientific founder of Organ-i – a company with which she endeavoured to take kSORT into commercial development. Following Organ-i's acquisition by Immucor, these goals have come to fruition and the assay will be available in the US for March 2015
- kSPOT has also been licensed out from Stanford by Immucor, and it is currently being developed with the intention of bringing it to market in 2016

chronic injury in the organ by intensification of immunosuppression for high immune risk. In addition, patient morbidity from the side effects of high dose immunosuppression will decrease as a result of reducing immunosuppression for patients at low immune risk.

### kSPOT

The second test, kSPOT, is a set of 21 genes that Sarwal hopes to reduce to three following further research and development. This assay complements the first by predicting the likelihood that a patient will develop some degree of graft accommodation (assessed by the study of natural human operational tolerance) to the new organ, minimising the need for harmful immunosuppressive drugs. The group came across such individuals by chance – people who had human leukocyte antigen-mismatched organs but had, for

**TRANSLATIONAL RESEARCH FOR PERSONALISED MEDICINE**

**OBJECTIVES**

- To develop biomarker-based assays for kidney transplant patients to ensure optimised, personalised management following transplant
- To broaden the scope of these assays so that they can be applied for other solid organ transplants
- To identify novel drug targets for renal diseases including focal segmental glomerulosclerosis and nephropathic cystinosis

**KEY COLLABORATORS**

**Dr Allan Kirk**, Duke University Medical Center, USA • **Dr Elaine Reed**, University of California, Los Angeles (UCLA), USA • **Dr Flavio Vincenti**, University of California, San Francisco (UCSF), USA • **Dr Adrianna Zeevi**, University of Pittsburgh Medical Center, USA • **Dr Ron Shapiro**, Mount Sinai School of Medicine, USA • **Dr Oriol Bestard**, Barcelona, Spain • **Dr Josefina Alberu**, Mexico

**PARTNERS**

**National Institutes of Health** (using kSORT and kSPOT in NIH-funded clinical trials on new drugs for suppression of immune activation and induction of tolerance); **Immucor** (clinical trials for kSORT in the US, EU, Mexico and Asia)

**FUNDING**

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

National Institute of Allergy and Infectious Diseases (NIAID)

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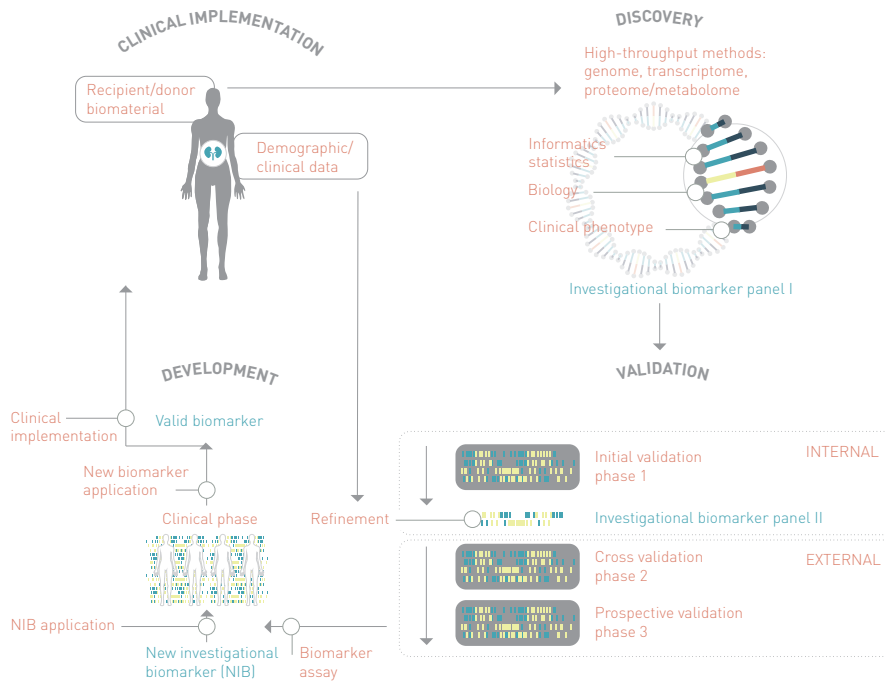
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**MINNIE SARWAL** holds a PhD in Molecular Genetics from the University of Cambridge, UK; a Diploma in Child Health from London, UK; Membership of the Royal College of Physicians and is an elected Fellow of the Royal College of Physicians, UK. She has previously held the Professor of Surgery/Immunology/Peds and Medical Director position in the Peds Kidney Transplant Program at Stanford University. She is on the US Food and Drug Administration Science Board, is a Councillor for The Transplantation Society (the international society for organ transplantation) and is also Adjunct Professor of Surgery at the University of Southern Denmark, Odense. She has received numerous awards, including the Roche Outstanding Achievement Award in Clinical Science and the Cuneo Richardson National Kidney Foundation Award for Scientific Excellence.



The stages of development for kSORT and kSPOT: At the discovery stage, high throughput technologies are used to identify genomic (epigenetics, SNPs), transcriptomic (gene regulation), proteomic or metabolomics (gain/loss of function) biomarkers. Any potential targets are then subjected to rigorous validation. The initial, internal validation consists of meta-analyses using independent samples from public databases; elimination of any bias using confounder analyses; pathway analyses and gene-set enrichment. Further external validation includes more independent samples, MA back validation and testing in clinical settings. The development stages comprise a sensitive, specific and reliable assay establishment phase; clinical trials; and FDA approval and regulation. This brings the kSORT or kSPOT biomarker panels to the clinical implementation stage, wherein they can be used in patients to provide more personalised treatments following kidney transplant. Data can also be collected from these patients in order to refine the biomarkers for future use.

unknown reasons, stopped taking their immunosuppressive medication and did not experience acute rejection. Sarwal and her colleagues set out to understand the genetic basis for this tolerance, and in doing so began to develop the kSPOT assay. High scores of the kSPOT assay, generated by modelling gene expression data from three of the 21 genes, strongly correlate with human operational kidney transplant tolerance.

The idea is that these assays will work together to monitor kidney transplant patients via a blood test every three months, and establish the optimum treatment regimen for each patient. "The use of the kSORT and the kSPOT could provide the 'ying and yang' of the immune risk threshold value – the upper end of immune risk is read by kSORT, and the lower end of immune risk is read by a negative kSORT and a positive kSPOT assay," Sarwal enthuses.

**BRANCHING OUT**

While these tests were originally designed for use on kidney transplant patients, Sarwal has found that the same panel of genes used in kSORT is also applicable for testing other solid organs. Therefore, she has expanded her research and has already used the test

in hearts, with an eye towards applying it in the lungs, pancreas and intestine in the near future. However, the genes used in kSPOT have turned out to be very different across organs, meaning the researchers have had to identify a separate set of genes when developing new tolerance assays. Further research is underway in this respect to ensure a comprehensive set of assays for all solid organs, not just the kidneys.

Understanding the mechanisms of immune rejection and accommodation in organ transplantation has also allowed Sarwal to discover new drug targets for organ transplant patients. Recent studies by her group have focused on either repositioning US Food and Drug Administration (FDA)-approved drugs for non-transplant indications into organ transplantation, based on the mechanistic genomic approaches that have unraveled new pathways in organ injury, or discovering new drug targets that Sarwal will be developing in partnership with pharmaceutical partners. All of this work will be a major step in revolutionising how healthcare professionals manage organ transplant patients, ensuring a personalised approach that ultimately benefits everyone involved.