Individually Tailored Immunosuppression: Is There a Role for Biomarkers?

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Therapeutic drug monitoring (TDM) of immunosuppressive drugs has evolved over the last few decades. The use of liquid chromatography–tandem mass spectrometry, for example, now provides robust and highly specific quantification in the clinical laboratory. Nevertheless, the measurement of immunosuppressive drug concentrations, even if carried out accurately and precisely, does not sufficiently reflect the effects of the applied drugs on immune cells, because of the considerable interindividual variation in the sensitivity to suppression of immune function. Irreversible chronic allograft rejection and the long-term side effects of immunosuppressive therapy are still major limiting factors in transplantation medicine. In this context, pharmacodynamic (PD) biomarkers are a potential key for further optimization of immunosuppressive therapy.

The PD effects of immunosuppressive drugs can be assessed with the aid of an increasing number of different biomarkers. For achieving an individually tailored immunosuppression, these biomarkers could be helpful for such aspects as the weaning or minimization of immunosuppressive therapy, the optimization of multidrug regimens that takes into account the synergistic and antagonistic effects of immunosuppressive drugs, or even the identification of operationally tolerant patients after solid-organ transplantation.

In this Q&A, 4 leading experts in the field of PD monitoring of immunosuppressive drugs share their thoughts on the analytical and clinical requirements, as well as the usefulness, of individual biomarkers.

From your perspective, what are the requirements for an ideal biomarker for individualization of immunosuppressive therapy, e.g., after solid-organ transplantation?

Merce` Brunet: In clinical practice, all biomarkers may have some limitations, and it would be difficult to achieve the status of ideal biomarker. In solid-organ transplantation, biomarkers should be useful tools for optimizing immunosuppressant therapy and identifying patients at risk of rejection. Biomarker monitoring combined with pharmacokinetics (PK) is a requirement to achieve personalized therapy. Some of these biomarkers could be strongly related to the mechanism of action of the drug and may reflect the personal response to the treatment, whereas other biomarkers are associated with graft injury and clinical outcome. In any case, assays for these biomarkers should be: (a) diagnostically accurate, reproducible, sensitive, and specific; (b) widely available, rapid, easy, and inexpensive; and (c) properly validated.

Richard Kowalski: Several drugs are used to generate a state of immunosuppression in an otherwise immune-competent individual. An ideal biomarker would quantitatively characterize the combinatorial influence of each drug on the ability to reduce an individual’s immune competence. Sampling would be noninvasive and not depend on the time from dosing. Additionally,
the biomarker should be relatively stable, easy to detect, and quantitative.

Alexander Vinks: In 2001, the Biomarker Definitions Working Group of the NIH defined a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.”

The term “biomarker” covers characteristics measured at baseline as well as those measured repeatedly over time, before, during, or after treatment. Clinical data, laboratory data, imaging data, and gene-expression and proteomic data can all be considered potential biomarkers. The requirements for an ideal biomarker are highly dependent upon the process or disease that one is investigating. The following characteristics are important for all biomarkers: (a) They should be non-invasive, easily measured, inexpensive, utilize standardized and reproducible laboratory platforms, and produce rapid results; (b) they should be from readily available sources, such as blood or urine; (c) they should have a high diagnostic sensitivity, allow early detection, and show no overlap in values between diseased patients and healthy controls; (d) they should have a high diagnostic specificity, being greatly up-regulated (or downregulated) specifically in the diseased samples and unaffected by comorbid conditions; (e) biomarker concentrations should vary rapidly in response to treatment; (f) biomarker concentrations should aid in risk stratification and possess prognostic value in terms of real outcomes; and (g) biomarkers should be biologically plausible and provide insight into the underlying disease mechanism.

Pierre Wallemacq: The ideal biomarker (or most likely a combination of biomarkers) should be predictive of the antidonor immune response. This response can trigger either rejection or tolerance. Early prediction of both ways would be most helpful for the individualization of immunosuppressive therapy. The ideal biomarker involved in the immune response prediction should be able to predict the occurrence of over-immunosuppression–related disorders (tumors, post-transplant lymphoproliferative disorders, infections, etc.). Other side effects, such as renal insufficiency and neurotoxicity, should be monitored by other biomarkers.

Which biomarkers would you propose as candidates for achieving an individually tailored immunosuppression?

Merce Brunet: There are different kinds of biomarkers that could be considered.

Pharmacodynamics: Focus on the measurement of the immunomodulatory drug effect: (a) Target inhibition [calcineurin activity, inosine-monophosphate dehydrogenase (IMPDH), mammalian target of rapamycin (mTOR), etc.]; (b) use functional assays for cellular markers, e.g., lymphocyte proliferation [proliferating cell nuclear antigen (PCNA) measurement], expression of specific T- and B-cell surface antigens, and intracellular markers (such as cytokine expression, cytokine mRNA expression, and drug concentration); (c) measure soluble cytokines; (d) measure interferon-γ (IFN-γ) alloreactivity [enzyme-linked immunosorbent spot (ELISPOT)]; and (e) balance T-effector cells/T-regulatory cells (Tregs) (alloreactivity vs tolerance).

Pharmacogenetics: Many different single-nucleotide polymorphisms (SNPs) and their associations with individual responses to drugs and clinical outcomes have been tested [IMPDH] (inosine 5’-monophosphate dehydrogenase 1), CYP3A5 (cytochrome P450, family 3, subfamily A, polypeptide 5), UGT1A9 (UDP glucuronosyltransferase 1 family, polypeptide A9), ABCB1 (ATP-binding cassette, sub-family B (MDR/TAP), member 1; formerly MDR1), IL10 (interleukin 10), TGFBI (transforming growth factor, beta 1).

Pharmacokinetics: Measurement of drug exposure in whole blood, intracellularly, and in tissue.

Richard Kowalski: An algorithm based on the mechanistic activity of drug targets (e.g., calcineurin, mTOR, and IMPDH) is an attractive candidate. However, secondary effects of these compounds may substantially impact immune function. For instance, calcineurin inhibitors (CNIs) also inhibit the activity of proline isomerases involved with protein folding. Biomarkers that assess lymphocyte response to nonsilent antigens

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8 Human genes: IMPDH, inosine 5’-monophosphate dehydrogenase 1; CYP3A5, cytochrome P450, family 3, subfamily A, polypeptide 5; UGT1A9, UDP glucuronosyltransferase 1 family, polypeptide A9; ABCB1, ATP-binding cassette, sub-family B (MDR/TAP), member 1 (formerly MDR1); IL10, interleukin 10; TGFBI, transforming growth factor, beta 1; CYP3A4, cytochrome P450, family 3, subfamily A, polypeptide 4; ABCC2, ATP-binding cassette, sub-family C (CFTR/MRP), member 2 (formerly known as MRP2); TPMT; thiopurine S-methyltransferase.
would be useful, the caveat being that today’s immunosuppressants are not specific to alloantigens. Markers such as the ImmuKnow® assay (which measures T-helper activity to nonspecific stimulation), ELISPOT assays that detect the production of specific cytokines, HLA/DSA (donor-specific HLA antibody) antibody measurements, and gene profiling to predict rejection (cardiac), are useful in this setting.

Alexander Vinks: From a clinical pharmacology perspective, the most suited candidates for individualized immunosuppression are biomarkers that describe the PD response(s) to immunosuppressive drugs and their variability. This would allow dose individualization based on approaches that integrate PK and PD, for instance, by applying PK/PD models as part of a Bayesian feedback algorithm. First-tier biomarkers include the mechanistic drug target enzymes calcineurin, IMPDH, and mTOR.

Cyclosporine exerts its effect through binding to cyclophilin, an intracellular protein of the immunophilin family, forming a complex that subsequently inhibits calcineurin. Tacrolimus binds to another immunophilin, FK506-binding protein 12 (FKBP12), to create a complex that inhibits calcineurin with greater potency than does cyclosporine. The first papers on calcineurin monitoring appeared in the mid-nineties. More recently, other groups have started to investigate the applicability of calcineurin inhibition as a PD biomarker of immunosuppression, and although the field is considered to still be in its infancy, several encouraging clinical applications have been reported. A promising example of the application of biomarker data was in the development of a chemically modified cyclosporine, voclosporine (ISA247), which relied on extensive PK/PD biomarker data modeling.

Mycophenolate mofetil is a prodrug that is rapidly converted into mycophenolic acid (MPA). MPA inhibits IMPDH, a key enzyme in purine synthesis. Mycophenolates have largely replaced azathioprine and are now widely used in combination with other immunosuppressive agents. IMPDH activity in peripheral blood mononuclear cells or CD4+ cells has been extensively studied to assess MPA PD. An important clinical finding is that transplant patients with high IMPDH activity and who received a dose reduction had the highest incidence of acute rejection. On the basis of these findings, pretransplant IMPDH activity is now being considered as helpful to guide the required level of MPA immunosuppression. However, the optimal PD target range has not been fully characterized.

Sirolimus (rapamycin) and everolimus bind to FKBP12 to form complexes that bind and inhibit mTOR-mediated signal-transduction pathways. Inhibition of mTOR eventually leads to suppression of new protein synthesis and arrest at the G1-S phase of the cell cycle. One of the well-characterized downstream mTOR effectors is p70 ribosomal protein S6 kinase 1 (S6K1). Several assays have been developed, and preliminary studies suggest that the phosphorylation status of S6K1 could be a useful PD biomarker to provide clinically relevant information on the level of immunosuppression in individual patients. ELISA-based technology is currently being tested, given its relative ease for quantifying the phosphorylation status of S6K1 as a function of mTOR exposure, including other downstream effectors of the mTOR-induced pathways. As the relationship between drug concentration and phosphorylation status typically is nonlinear, full interpretation of the data would require a PK/PD modeling approach.

Interestingly, corticosteroids are being used for shorter or longer periods in almost all protocols, yet there are no well-developed monitoring strategies. In addition, downstream effects can be measured by changes in cytokine levels, markers of lymphocyte proliferation or activation, markers of immune cell response, and potential predictors of tolerance.

From a clinical standpoint, biomarkers are also needed to monitor for immunosuppressive-drug toxicities. One example of this approach is the use of kidney injury biomarkers, such as urinary neutrophil gelatinase–associated lipocalin (NGAL), for the early prediction of cyclosporine-induced nephrotoxicity.

Pierre Wallenacq: The list below is not exhaustive but just suggests some potential candidates from the current literature, including soluble and intracellular interleukins and antigens: (a) lymphocyte proliferation (PCNA); (b) expression of surface antigens of T cells (flow cytometry) (CD3, CD4, CD8, CD25, CD28, CD38, CD69, CD95, CD154); (c) IFN-γ ELISPOT assay; (d) quantification of intracellular interleukin-2 (IL-2) in CD8+ T cells, which appears to be a useful PD marker in liver transplantation to predict organ rejection (P = 0.003), better than calcineurin activity; (e) measurement of ATP production from stimulated T cells (Cylex ImmuKnow assay); (f) the activities of specific enzymes (IMPDH, calcineurin, and so on); (g) Tregs, CD4/CD25high/FOXP3 expression, CD4/CD25/CD45RO/CD127low/high expression, and tolerizing cytokines (e.g., IL-10, transforming growth factor-β).

Different fields of application have been emphasized for individual biomarkers, e.g., identification of operationally tolerant patients, as well as risk stratification for either infection or rejection. In which of these fields will biomarkers have a chance to make an impact?

Mercé Brunet: With reference to the identification of operationally tolerant patients, biomarkers from
Biomarkers predictive of the risk of rejection or infection may have a tremendous impact on the recipient’s clinical-outcome improvement, on pediatric and adult patients, in the period early after transplantation, and in the maintenance period of immunosuppressive treatment.

Richard Kowalski: In the setting of operational tolerance, risk stratification would not be beneficial since immunosuppressive therapy is not needed. The risk is defining a nontolerant patient as tolerant. Only a small percentage of solid-organ transplant recipients demonstrate operational tolerance, and expectations are that a significant proportion of transplant patients will require lifelong immunosuppression. This larger group of patients would certainly benefit from biomarker(s) that assist with balancing the amount of immunosuppression to avoid risks of infection or rejection. Ultimately, the goal of both approaches is to minimize the amount of needed immunosuppression.

Alexander Vinks: Several studies have attempted to analyze series of biological traits in peripheral blood cells of operationally tolerant transplant recipients in an attempt to define a multiparameter “fingerprint” of tolerance. A promising approach that merits further study was presented in a recent report on the use of biomarkers to support the implementation of personalized therapeutics in transplantation. These investigators described a comprehensive 3-pronged approach using biomarkers and functional immunological assays that includes (a) evaluation of pretransplant risk evaluation and risk stratification, (b) prediction of risk of rejection episodes and long-term graft outcome, and (c) identification of operationally tolerant patients. The summary of signatures includes genes encoding γδ T-cell and natural killer–cell receptors, proteins involved in cell proliferation arrest, and numbers of circulating Treg subsets (e.g., CD4+CD25+FoxP3+ Tregs). The integrated evaluation of these assays will provide novel mechanistic insights into immunological risk factors and what constitutes allograft operational tolerance. This constitutes a first step in the search for noninvasive diagnostic panels for guiding therapeutic intensity and predicting tolerance as part of drug-weaning strategies. Alternatively, the efficacy of more-generalized monitoring of cellular immune function by measuring increases of intracellular ATP in CD4+ T cells after activation by mitogenic stimulation has been reported in numerous studies. Data from studies of the only immune-monitoring assay cleared by the US Food and Drug Administration (ImmunoKnow, Cylex) suggest clinical utility to predict relative risk for infection or acute rejection.

Richard Kowalski: No single strategy is likely to work for all patients. With that said, use of a “tolerance” screen to categorize patients as those needing immunosuppression and those who do not, followed by risk assessments, may provide an overall strategy for minimizing immunosuppressive therapy. Depending on the specificity of the tolerance screen, both groups would benefit from routine risk assessments to avoid adverse events associated with over- or under-immunosuppression.

Alexander Vinks: Immunosuppressive drugs can be completely withdrawn in some transplant recipients, commonly referred to as “operationally” tolerant. Predicting the level of over-immunosuppression with an increased risk of infections and malignancies remains a major clinical challenge. Immunologic characterization of these patients, however, has not been performed in detail. Despite the fact that drug concentration is an established biomarker for exposure, which is associated with clinical effect and drug-related toxicity, acute rejection and drug-related adverse events do occur de-
spite apparently “therapeutic” drug concentrations. This highlights the continued need to better establish each individual patient’s dose concentration–response relationship. The integration of PK guidance (not TDM numbers only) with mechanism-based PD biomarkers, as outlined before in relation with the immunologic indices, will be a good first step towards immunosuppressive minimization.

Pierre Wallemacq: The synthesis of clinical signs, biological and histological parameters, and potential biomarkers would together allow minimization of immunosuppressive therapy.

**Will analytical optimization of conventional TDM further improve clinical outcome?**

Mercé Brunet: Analytical optimization of TDM may play a role in the improvement of clinical outcome. New approaches based on the measurement of immunosuppressive drugs in tissues and within lymphocytes have shown a better relationship between drug concentration and biological effect. On the other hand, we have to be aware that PK combined with PD (reflecting the individual response) is the way to achieve personalized therapies.

Richard Kowalski: Development of an algorithm, as described previously, that measures the combinatorial effects of the various immunosuppressant drugs on immune competence could improve clinical outcome. There are many difficulties with developing this type of algorithm, including the well-documented PK/PD differences between individuals. In addition, next-generation immunosuppressants, such as belatacept, are not “conventional” therapeutic agents. The blood/plasma concentrations of these agents are not directly monitored after dosing.

Alexander Vinks: Given that we currently do not have predictive biomarkers of outcome, we will continue to treat transplant patients according to their drug concentrations, as has been done very successfully in the past. Conventional TDM has contributed tremendously to the way we dose patients today. More importantly, increasing numbers of prospective studies show that PK-guided dosing strategies can further improve clinical outcomes by optimizing the target attainment of drug exposure for each drug in the regimen. Areas for novel analytical expansion include the monitoring of intracellular CNI concentrations in target cells and tissues, determination of CNI metabolites (including metabolomics), and the use of free drug concentrations (e.g., for mycophenolates). It will allow us to better differentiate between each individual patient’s needs and the rather broadly defined “therapeutic range.”

The generation of therapeutic targets for new drug combinations and in the different transplant populations will be useful additions to the conventional TDM armamentarium.

Pierre Wallemacq: Most likely, yes, but moderately. The expected major progress should come from a better interpretation and use of conventional TDM, independently of analytical optimization. Such progress may be achieved by improvement of AUC (area under the curve) prediction from conventional TDM (based on limited sampling strategies, population PK with Bayesian estimators, etc.) and from prediction of drug concentrations in target cells (lymphocytes).

**How do you assess the chances for pharmacogenetic or gene-expression profiling for an individualized immunosuppression?**

Mercé Brunet: It is well known that gene polymorphisms may be predictive of PK and PD outcomes after transplantation. Previous studies have shown that some polymorphisms in genes encoding proteins implicated in immunosuppressive-drug transport and metabolism may play a role in the exposure and effect of these agents. Genetic polymorphisms in CYP3A5, CYP3A4 (cytochrome P450, family 3, subfamily A, polypeptide 4), MDR1, UGT1A9, ABCC2 [ATP-binding cassette, sub-family C (CFTR/MRP), member 2; formerly known as MRP2], and TPMT (thiopurine S-methyltransferase) have an impact on tacrolimus, MPA, and azathioprine metabolism and transport, respectively. The exact contribution to patient care has been evaluated in both multicenter clinical trials and single-center experiences with the aim to study their impact on drug exposure and the incidence of rejection and adverse events. The obtained results, mainly in kidney transplantation, suggest that there are currently no data supporting systematic analysis of these polymorphisms in all patients to be treated, so the request for some specific SNPs should be based on clinical reasons for the treated patients.

With reference to gene-expression profiling first, results demonstrate that gene-expression profiling may be a useful tool for identifying patients at high risk of rejection, patients with an operational-tolerance profile, or candidates for minimization or immunosuppression withdrawal. Further studies of solid-organ transplant recipients are needed for evaluating the clinical impact and the routine application of such genetic and proteomic analyses.

Richard Kowalski: Not enough is known about the various nuances of immunosuppressive therapy to rely solely on an individual’s pharmacogenetic/gene-expression profile for adjusting/minimizing therapy.
However, these tools are useful for estimating the metabolic clearance of certain drugs, determining whether an individual is predisposed to known drug toxicities, and assessing the risk of rejection (cardiac) in solid-organ transplantation. Continued research including the use of proteomic data increases the chances of these technologies being employed for individualizing immunosuppressive therapies.

**Alexander Vinks:** The “low-hanging fruit” currently are the PK-related drug-metabolizing enzymes and drug transporter SNPs and haplotypes that are predictive of between-patient differences in metabolic capacity [e.g., CYP3A5 and P-gp (P-glycoprotein) genotype for a priori dose stratification of tacrolimus and sirolimus; UGT1A9 and MRP2 genotype to predict drug exposure and the likelihood of adverse events]. Exciting new PD-related genotypic differences, such as recent IMPDH activity profiling, will allow a priori identification of patients less likely to respond to standard therapy and who require more-tailored treatment.

There have been many publications describing genetic variability in molecules affecting innate and adaptive immunity. The expression of nuclear factor of activated T cells (NFAT)-regulated genes (encoding IL-2, granulocyte-macrophage colony stimulating factor, IFN-γ) is a biomarker for a higher degree of functional immunosuppression and is an example of a genomics technology that may prove helpful in predicting the level of CNI-induced immunosuppression. Another recent application of genotype–phenotype association is the impact of the Toll-like receptor (TLR) system on early and late kidney transplantation outcome. It may represent a promising target in future therapeutic strategies. Finally, high-throughput microarray technology can provide unbiased, simultaneous global expression patterns across many different experiments, thereby offering a means to study diseasespecific transcriptional changes in tissue biopsies, peripheral blood, and other biofluids. Yet, the translation of genomic data and genomewide association-study data [including epigenetics (DNA methylation and histone modifications), gene-expression data, and transcriptomics (including microRNA and small interfering RNA studies)] from the laboratory to the clinic has proved not to be an easy task.

**Pierre Wallemacq:** Pharmacogenetics clearly proved its role in explaining variations in blood concentrations depending on the expression of CYP3A activity. This knowledge before transplantation might allow a more rapid choice of optimal dosage and allow the steady state to be reached faster. It is unclear if such advantages produce a better clinical outcome, because TDM would detect inappropriate blood levels anyway. Furthermore, blood concentrations provide approximate information about patient exposure (useful for detecting important under- and overexposure) but cannot establish a clear relationship with clinical outcome. The role of pharmacogenetics in the prediction of intracellular drug concentrations might be more promising (kidney tissue for side effects, lymphocytes for pharmacological activity, etc.) because it is more directly related to clinical outcome.

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