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See related article, "Inflammation Modifies the Paradoxical Association between Body Mass Index and Mortality in Hemodialysis Patients," on pages 1479–1486.

Cancer in ESRD: Clear on the Epidemiology, Hazy on the Mechanisms

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Cancer has long been recognized as a major barrier to long-term survival for patients with treated ESRD, particularly those sustained by kidney transplantation. Cumulative incidence data for cancer among Australian and New Zealand recipients of a first kidney transplant clearly demonstrates the inexorable growth of this problem with time after transplantation (Figure 1).

Our understanding of the incidence of cancer among patients with ESRD was significantly advanced nearly a decade ago by a major linkage project, whereby the authors linked the Australia and New Zealand Dialysis and Transplant registry, containing data for all Australians receiving RRT, with The

National Cancer Statistics Clearing House, which compiles data from all Australian population-based cancer registries. Through a series of reports, this group provided cumulative incidence rates of various types of cancer for patients with ESRD prior to RRT, during dialysis, and while transplanted.^{1,2} Most importantly, by comparing these to the expected rates of each type of cancer among the age-, sex-, and era-matched general population of Australia, standardized incidence ratios (SIRs) were calculated highlighting the differential effects of various states of ESRD on cancer incidence.¹

This data illustrated trends consistent with epidemiologic, biologic, and mechanistic concepts: (1) an increased incidence of cancer diagnoses after transplantation, including common cancers such as colon and lung cancer, consistent with the effects of immunosuppression; (2) a profound increase in virus-associated cancer after transplantation, consistent with the effects of immunosuppressive drugs in hindering antiviral defenses; (3) unaltered rates of endocrine-related cancers after transplant, including breast, prostate, and ovarian cancer; (4) modest, though significant, increases in cancer among patients with ESRD prior to the initiation of RRT (SIR, 1.16; 95% confidence interval [95% CI], 1.08 to 1.25) and in dialysis-dependent patients (SIR, 1.35; 95% CI, 1.27 to 1.45) as compared with the general population, with substantially increased incidence of cancers related to renal failure such as myeloma, kidney, renal tract, and thyroid cancers, and some virus-related cancers including Kaposi sarcoma and lymphoma.¹ This data, coupled with population-based reports from other countries,^{3,4} has been used to inform risk and guide practice in transplant recipient management.

In this edition of the *Journal of the American Society of Nephrology*, Yanik *et al.* confirm and significantly extend these findings through a much larger study conducted by linking the Scientific Registry of Transplant Recipients to various United States population-based cancer registries.⁵ In this study of over 200,000 kidney transplant recipients, the authors were powered to not only provide SIR estimates for various cancer types during periods of kidney transplant function and during time on dialysis, but also to track changes in SIRs between periods of transplant function and periods of dialysis among those who experienced graft failure, returned to dialysis, and were then retransplanted. Such sequential observations dramatically highlight the peaks in incidence of so-called virus- and immune-mediated cancers during transplantation, with corresponding falls in incidence following return to dialysis. Consistent with the Australian data,^{1,2} cancers linked to kidney failure, including kidney, urinary tract, and thyroid cancers, were increased maximally during times of dialysis and only modestly increased over the incidence in the general population during intervals of transplant function.

Several potentially important differences between the studies are noteworthy. The SIRs of cervical, liver, and colon cancers were significantly increased during dialysis and further increased after transplantation in the Australian study,^{1,2} though not in the United States study.⁵ Potential explanations include: (1) era

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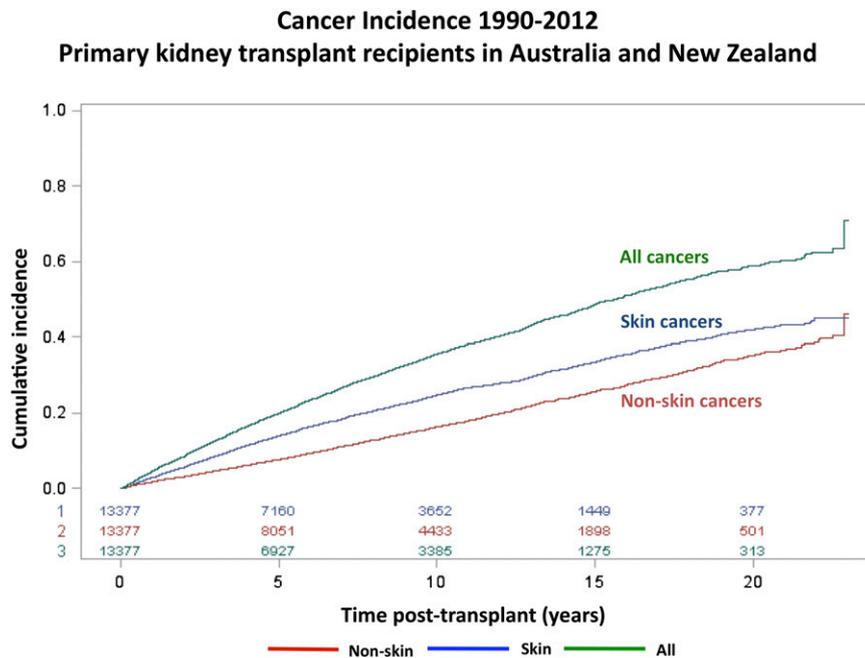


Figure 1. Cumulative incidence curves of NMSC (skin: blue line), other cancers (nonskin: red line) and any cancers (all, including NMSC and other cancers: green line) up to 20 years after transplantation of primary live and deceased donor kidney transplant recipients in Australia and New Zealand between 1990 and 2012 (data source: Australia and New Zealand Dialysis and Transplant Registry).

effects, as the 10-year difference between studies may have resulted in differences in transplant practices or trends in cancer epidemiology; (2) differences in cancer screening and reporting strategies, such as reimbursement of fecal occult blood testing among those over 50 years of age and Pap smear tests in Australia, which may create lead time bias; (3) geographic differences in viral infection patterns, such as a higher prevalence of the more carcinogenic Hepatitis B virus genotypes B and C in Australia⁶; (4) immunosuppression, with the use of cyclosporine, azathioprine, and steroids dominant in the Australian study,¹ versus tacrolimus and mycophenolate and common usage of T cell-depleting induction therapy in the current paper.⁷ Such differences may provide important clues regarding mechanisms.

Inherent limitations exist for all registry studies given their observational nature. Yanik *et al.* excluded 61% of the kidney transplant recipients reported by the Scientific Registry of Transplant Recipients due to the absence of linked data to cancer registries within many states. Information regarding the exposure of patients to immunosuppressive agents during periods of non-function was not available, although it is reasonable to assume that immunosuppressive medications are commonly significantly reduced or ceased after transplant failure. Total duration of ESRD was not available, which does impact cancer risk.⁵ Dialysis management and pretransplant assessment commonly entail screening tests, such as chest x-ray, parathyroid ultrasound, and imaging of the urinary tract, which may detect cancer and thereby increase cancer incidence during nontransplant

intervals through lead-time bias. Such limitations should be borne in mind in interpreting this data, as should the absence of data on the most common group of cancers seen among the post-transplant population: non-melanoma skin cancer (NMSC).

The epidemiologic insights provided by Yanik *et al.* may have important implications for the pathogenesis and mechanism of cancer development in patients treated for ESRD. The dramatic changes in SIRs between transplant-dependent and non-transplant periods for virus-related cancers, particularly non-Hodgkin lymphoma, and so called immune-mediated cancers, particularly melanoma, underscores the impact of immune suppression in the pathogenesis of these cancers and provides some rationale for cessation of immune suppression as a therapeutic option in their management.⁵

The effects of uremia, dialysis, and transplant immunosuppression on immune status are complex and incompletely understood.⁸ The varying associations between dialysis, transplantation, and the incidence of different cancers shows overarching themes,

such as increased virus-related cancer during transplant periods, but significant differences between individual cancers as shown by the strong increase in Epstein-Barr virus-related lymphomas, yet little or no increases in Human Papilloma Virus-associated cancers.^{1,5}

A second related paper in this edition of the *Journal of the American Society of Nephrology (JASN)* by Bottomley *et al.* explored the interesting hypothesis that a marker of T lymphocyte senescence, high-level cell surface expression of CD57 by CD8⁺ T cells as detected by flow cytometry, would identify kidney transplant recipients at increased risk of future development of NMSC.⁹ This is a key question both clinically and mechanistically as such cancers are very common (Figure 1), particularly among white recipients, and are a significant cause of morbidity and mortality. Early diagnosis requires frequent skin checks, which can potentially facilitate management and reduce tumor burden. Identifying recipients who are at high risk and therefore warrant intensive screening is a key aim, however currently available clinical algorithms are cumbersome and of relatively little practical value.

This single-center cohort study of mostly white kidney transplant recipients, half of whom were selected because of prior history of NMSC, were followed for 14–20 months to determine the association between baseline CD57 expression on CD8⁺ lymphocytes isolated from peripheral blood, dichotomized into CD57^{hi} (indicating immunosenescence) or CD57^{low}, and subsequent incidence of squamous cell carcinoma (SCC) of the skin. Patient age, dialysis duration prior to transplant, history of previous SCC, higher number of $\gamma\delta$ T cells and CD8⁺CD57^{hi}

phenotype were predictive of SCC development, of which history of previous SCC, number of $\gamma\delta$ T cells and $CD8^+CD57^{hi}$ phenotype remained independently predictive on multivariate modeling. Published clinical risk prediction scores were modestly predictive, age-dependent, and less discriminatory than $CD8^+CD57^{hi}$ phenotype.⁹

Mechanistically, this study builds on earlier findings from this group of collaborators which suggest that an imbalance between immune regulation and competence underpin the development of SCC post-transplantation.^{9,10} Consistent themes of an imbalance between an excess of regulatory T cells (resulting in potential inhibition of antitumor response),¹⁰ reduction in the proportion or functional deficiency of tumor-surveillant cells that are known to conditionally promote antitumor responses (e.g., low number or functional impairment of dendritic cells, natural killer cells, and effector T cells),^{9–11} in addition to an excess of $CD57^{hi}$ immunosenescent $CD8^+$ T cells (associated with impaired protective immunity to viral or tumor antigens),⁹ may help to build a theoretical construct in the pathogenesis of carcinogenesis after transplantation. The role of $\gamma\delta$ T cells in tumor immune surveillance remains unclear, with these cells exhibiting both antitumor and potential tumorigenic effects.¹¹ The finding of a direct association between the number of $\gamma\delta$ T cells and SCC incidence requires further examination to determine whether it is simply the total number, the ratio of $\gamma\delta$ T cells compared with other immune-surveillant and suppressive cell types, or the presence of regulatory $\gamma\delta$ T cells that drives risk. This is one of several issues requiring clarification, as indicated by the variability of associations between specific measures of immune phenotype and SCC development across different studies from this group.^{9,10}

The authors suggest patient stratification according $CD8^+CD57^{hi}$ phenotype prior to immunosuppression may facilitate a tailored approach to post-transplant care by enhancing risk prediction of cancer and rejection.⁹ While a measure of immune-competence for clinical use has been long awaited, much remains unknown as to the external validity of these findings in the prediction of SCC and other nonskin cancers before measurement of $CD8^+CD57^{hi}$ phenotype could be considered for this role. The current study was restricted to a largely white population enriched for risk of SCC, most of whom were maintained on azathioprine, cyclosporine, and prednisolone, which is not the routine maintenance immunosuppressive regimen in the current era. There is an urgent need to demonstrate that immune phenotype can be reliably reproduced at different laboratories and centers, and whether it is predictive of SCC among broader cohorts of transplant recipients who are at standard risk of SCC and are maintained on the current standard immunosuppressive medications of tacrolimus and mycophenolate. The impact of contributing factors that are known to affect immune responses such as viral infection, treatment for acute rejection, and switch to mammalian target of rapamycin inhibitors, and whether the test is practical and affordable are just some of the questions that will need to be addressed in future studies.

Our current status in the clinic is clear: cancer is a major barrier to achieving improvements in patient survival on dialysis,

particularly after transplantation. Our understanding of the epidemiology of cancer among patients treated for ESRD has been greatly clarified by recent registry analyses of large cohorts of ESRD and kidney transplant recipients.^{1,5} The same analyses demonstrate clear themes regarding carcinogenesis in this patient group, but at the same time indicates significant differences in the prevalence of specific cancers across the spectrum of ESRD and kidney transplantation. To better understand mechanism, further studies such as that by Bottomley *et al.*⁹ in this edition of the *JASN* are required to provide clarity and ultimately guide clinical practice.

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DISCLOSURES

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Immune Response against Autoantigen PLA2R Is not Gambling: Implications for Pathophysiology, Prognosis, and Therapy

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Membranous nephropathy (MN) is an organ-specific autoimmune disease which targets the kidney glomerulus, resulting in the formation of immune deposits along the glomerular basement membrane, activation of complement, and proteinuria. Although spontaneous remission occurs in up to 40% of patients, 30%–40% of patients will progress to ESRD in 5–15 years, requiring RRT with increased patient comorbidity and substantial economic health care burden.¹ The first major target autoantigen in adult primary MN was identified as the M-type phospholipase A2 receptor (PLA2R), a protein that belongs to the C-type lectin family. PLA2R is a transmembrane glycoprotein composed of a large extracellular portion consisting of an N-cysteine-rich region (CysR), a fibronectin 2 type domain, and a tandem repeat of eight C-type lectin-like domains (CTLDS).² The majority of circulating antibodies detected in 75%–80% of patients bind to a conformational, discontinuous epitope stabilized by disulfide bonds.²

PLA2R discovery was translated very quickly into clinical practice. Simple serologic assays such as the indirect immunofluorescence test and ELISA developed during the past few years provide specific, sensitive, and quantitative measurements

of circulating anti-PLA2R antibodies. Anti-PLA2R antibodies are highly specific for MN, not being detected in other nephropathies, autoimmune diseases, or healthy individuals.³ A number of recent studies further showed that levels of circulating anti-PLA2R antibodies were good prognostic biomarkers and enabled precise monitoring of the response to immunosuppressive treatment.⁴ Despite these major advances, there are still many unresolved questions regarding the mechanisms involved in triggering immune response, progression and remission of the disease, and response to therapy. It is still not known why the rate of stable remission does not exceed 70%, irrespective of immunosuppressive treatment. Further molecular insights based on the identification of B and T cell epitopes on PLA2R are required to design more targeted and less toxic therapies and to deliver specific markers of disease initiation and progression.

Recently, a first step in this direction has been taken. In 2015, two independent groups identified an immunodominant epitope region in the PLA2R protein using two different technical approaches. The first epitope region was identified in the three most N-terminal domains of PLA2R by Kao *et al.*⁵ The reduction-sensitive conformational epitope was formed by regions from the CysR and CTLD1 domains brought into contact by the fibronectin 2 type domain. In parallel, Fresquet *et al.* found that the dominant epitope was exclusively localized to disulfide-bonded peptide within the CysR domain.⁶ However, this epitope was maintained only under nondenaturing conditions and the CTLD3 domain was needed for preserving it under denaturing conditions. In both studies, the epitope region in the N-terminal portion of PLA2R explained most of the reactivity of anti-PLA2R antibodies. Surprisingly, at variance with observations made in most autoimmune disorders,^{7–9} epitope spreading was not observed, possibly because studies were performed on a small number of patients or pooled sera.

This discrepancy has been resolved by Seitz-Polski and colleagues in the current issue of the *Journal of the American Society of Nephrology*.¹⁰ Using nine PLA2R mutants generated by successive deletion of the extracellular domains of the receptor, they confirmed that the CysR region contains the primary dominant epitope, but in addition, they first demonstrated epitope spreading toward the CTLD1 and CTLD7 domains. By using this new approach, they also showed that CysR and CTLD1 are two independent domains recognized by distinct anti-PLA2R autoantibodies.

Seitz-Polski *et al.*¹⁰ have defined epitope regions of PLA2R autoantigen using recombinant truncated molecules containing approximately 140 amino acid residues. However, such studies do not provide clues on actual contact amino acid residues for antibody paratopes which, in the case of PLA2R, engage conformational structures stabilized by disulfide bonds. Most antibodies produced during immune responses react with conformational epitopes formed from assembled topographic determinants made up of amino acid residues brought into contact on the surface of the molecule during protein folding. Such conformational epitopes can encompass 20–30 amino acids on the surface of the antigenic protein, but

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