Advances in immunosuppressive regimens have led to longer allograft survival with the collateral emergence of long-term post-transplant complications. Malignancy is now a leading cause of death in thoracic transplantation, and in heart transplantation competes with cardiac allograft vasculopathy in determining late allograft loss. The notion that neoplasms, transplantation and immunosuppression are intricately intertwined has been documented for over 4 decades.1-4 Despite this recognition, malignancy in thoracic transplantation remains poorly understood and clinical investigations to guide improved allograft outcome remain sparse.5

General considerations: Lessons from solid-organ transplantation

In solid-organ transplantation, hematologic cancers occur early, whereas solid-organ malignancy, including skin cancers, typically appear in later time frames, exhibiting a bimodal appearance. In one of the largest studies, 175,732 solid-organ transplants were evaluated and the overall risk for 32 cancers was found to be elevated.5 Non-Hodgkin’s lymphoma peaked in incidence in the first year after transplant, whereas cancers of the lung were most frequent at later time-points (5 to 10 years) post-transplant. Interestingly, lung cancers were most common among recipients of lung allografts (6-fold), but also significantly increased among heart transplant patients (2.67-fold). In general, when one examines cancers in lung transplantation, the rates appear to be 60-fold greater than encountered in the general population.3 Comparative differences exist between various solid-organ transplant recipients in cancer rates. As an example, cardiac allograft recipients exhibit a 2-fold higher cancer
incidence than renal transplant patients. Indeed, visceral cancers are more prevalent by 6-fold, and incidence of lymphomas 4-fold higher in cardiac allograft recipients compared with their renal counterparts. Such high incidence of malignancies in the cardiac transplant population has been attributed to more aggressive immunosuppression and older age at transplant, although this remains somewhat speculative.

Similar patterns of cancer incidence noted in human immunodeficiency virus (HIV)-infected and solid-organ transplant populations point to immunosuppression as a common etiology. The etiology of malignancy involves impaired immunosurveillance, derangement of molecular signaling/DNA repair mechanisms, apoptosis and/or decreased anti-viral immune activity (Figure 1). In a pediatric study on patients with post-transplant lymphoproliferative disease (PTLD), natural killer (NK) cells downregulated the NK-cell receptors NKp46 and NKG2D, and significantly upregulated the programmed cell death protein 1 (PD-1), leading to NK-cell functional impairment and decreased cytotoxicity and suggesting a vital role for derangement of immunosurveillance in hematologic cancers.

Genetic, cellular, molecular and environmental factors all play significant roles. Mediterranean, Jewish, Arabic, Caribbean and African descent predisposes to post-transplant Kaposi sarcoma (KS) in kidney transplant patients. Genetic variation of the CYP3A4 A-to-G allele in different ethnic groups and homozygous GG genotype appear to predispose cardiac transplant patients to increased incidence of rejection. These genetic variations are also found among those with prostate and breast cancers as well as secondary leukemias. Tobacco use and radiation are important environmental factors that influence post-transplant malignancies. Tobacco use increases the risk of non-lymphoid malignancy in post–cardiac transplant patients and decreases long-term survival.

Radiation exposure before cardiac transplant has been shown in one small study to adversely affect early survival post-transplant with increased incidence of malignancies in long-term follow-up. Cardiac transplant patients have a 3.5-fold higher level of radiation exposure due to routine extensive surveillance testing. Use of fewer radiation-based tests is likely to change this trend. Large-scale studies are warranted to assess the effect of radiation on incidence of cancers in the post-transplant population.

**Skin cancers in thoracic transplantation**

Skin cancer is one of the most common malignancies noted in thoracic transplantation. Most data on non-melanoma skin cancers accrue from cardiac transplantation and only recently has the lung transplantation community begun to analyze this area intensively. This has largely been a consequence of the generally poorer lung transplantation survival compared with cardiac transplantation. Of all non-melanoma skin cancers, squamous cell carcinoma is most commonly encountered, with a 4-fold higher rate than basal cell carcinoma. In general, skin cancers in cardiac transplantation have also been linked with viral infections, attributable to the more aggressive immunosuppression used. A 2-fold higher incidence is seen in heart transplant patients as compared with renal allograft recipients, with an incidence of nearly 40% at 10 years. A review of earlier studies showed that male gender, excessive exposure to ultraviolet radiation and sunlight, aggressive immunosuppression, advanced age at transplant, human papilloma virus infection, decreased skin cancer risk awareness, and prior history of a non-melanoma skin cancer predispose toward developing cutaneous malignancies in cardiac transplant patients.

The type of immunosuppressant used appears to influence the risk for basal cell carcinomas. A retrospective review showed an increased risk of basal cell carcinoma in patients taking mycophenolate mofetil as compared with those taking azathioprine. Tacrolimus and sirolimus showed a statistically non-significant decrease in the risk of basal skin cancers. Molecular/cellular basis of post-transplant malignancies.

**Figure 1** Molecular/cellular basis of post-transplant malignancies.
cell and squamous-cell carcinomas, respectively. Cutaneous malignancies were found to develop between Years 2 and 3 after cardiac transplantation in patients with significant exposure to sunlight/ultraviolet radiation, particularly with certain skin types classified according to degrees of fairness. In an Australian study of 619 recipients of cardiothoracic transplants, 66 (10.7%) of the patients had major malignancies. Of these, 40% developed aggressive cutaneous malignancies by 2-year follow-up, suggesting that a subset of post-transplant patients may be more susceptible.

In a more recent study it was found that transplant-related squamous-cell carcinoma had a higher percentage of interleukin-22 (IL-22)–producing CD8(+) T cells and higher expression of IL-22 and its receptor by immunohistochemistry compared with non-transplant squamous-cell carcinoma. Increased Tc22 and T-regulatory (Treg)/CD8 cell ratio contributes to aggressive growth of transplant-associated squamous-cell carcinoma, as IL-22 is a keratinocyte proliferator. This is suggestive of specific molecular mechanisms that warrant further investigation to help develop targeted diagnostics and therapeutics.

Adjunct prophylactic therapies also play a role in predisposition to skin cancer, as recently exemplified by observations in lung transplant recipients. Duration of voriconazole treatment and increased exposure to sunlight influenced incidence of squamous-cell carcinoma. Long-term voriconazole use in lung transplant patients was linked to development of aggressive cutaneous squamous-cell carcinoma.

**Role of induction and immunosuppression**

Although not of great clinical relevance currently, the best observations of malignancy and induction therapy come from data with muromonab-CD3 (Orthoclone OKT3), which has been shown to carry a higher risk for post-transplant malignancies compared with IL-2 receptor (IL-2R) antibodies, rabbit anti-thymocyte globulin (rATG) or other anti-lymphocyte agents. The observation that OKT3 is associated with lymphoproliferative disorders, particularly in a dose-dependent manner, has been described. On the contrary, skin cancers are noted to be the most prevalent malignancy with rATG use, but in this context the cancers appear early and are generally more aggressive. Induction therapy other than that with IL-2R blockers generally increased the risk of neoplasia except when acyclovir was administered prophylactically during the first 3 months after heart transplant. No significant differences in PTLD or other cancers were noted with use of these agents in cardiac transplant recipients. So far, the use of alemtuzumab for induction in heart transplant patients has not yielded any clear-cut benefit in the context of post-transplant malignancies.

**Calcineurin inhibitors and post-transplant malignancies**

Calcineurin inhibitors (CNIs) exhibit pro-carcinogenic potential via inhibition of DNA repair mechanisms and apoptosis. Cyclosporine (CsA)-treated cells show induction of pseudopodia, increased cell motility and invasive growth, possibly mediated by transforming growth factor-beta 1 (TGF-β1). TGF-β1 has a dual role in induction and progression of carcinogenesis. TGF-β enhances survival, progression and metastasis of established tumors and inhibits DNA repair facilitating accumulation of mutations. Tacrolimus has a higher immunosuppressive potency and a lower incidence of de novo solid tumors compared with CsA.

**Effect of purine synthesis inhibitors**

6-Thioguanine, a derivative of azathioprine, acts synergistically with ultraviolet light in mutagenesis. However, a systematic correlation of azathioprine use with malignancy in transplant recipients has not been established. Mycophenolate mofetil has potent immunosuppressive/anti-proliferative properties and inhibits endothelial-cell proliferation in vitro, and blocks tumor-induced angiogenesis in vivo. This drug interacts with adhesion molecules to prevent integrin-dependent tumor dissemination/metastasis. Mycophenolate mofetil (MMF) induces alterations of the β1-integrin profile to block tumor cell adhesion to vascular endothelium. MMF is also involved in the induction of tumor cell differentiation. MMF in standard immunosuppressive regimens is associated with a significantly lower risk of malignancy and may offer a more favorable profile in transplantation medicine.

**Role of mammalian target-of-rapamycin (mTOR) inhibition and signal transduction**

mTOR, a regulatory serine-threonine kinase, activated via the phosphatidylinositol-3-kinase–AKT pathway has been implicated in progression of malignancies. Sirolimus inhibits the (PI3K) signaling pathway contributing to the regulation of cell proliferation and angiogenesis. Sirolimus also inhibits transcription activator 3 (STAT3). STAT3 mediates gene expression in cell growth and apoptosis and remains unregulated in many tumor types. Elevated expression levels correlate with poor prognosis. mTOR inhibition can be used in targeting STAT3 in cancer therapy.

mTOR inhibitors exert potent anti-angiogenic activity in vitro and in vivo in primary and metastatic tumors via inhibition of vascular endothelial growth factor (VEGF) production, possibly contributing to the regression of KS. In organ transplantation, mTOR inhibitors may exert a dual role by preventing immunosuppression and anti-cancer activity. mTOR inhibitors may avert growth of Epstein–Barr virus (EBV)-transformed B lymphocytes in conjunction with anti-CD20 antibodies. No randomized, controlled studies have been done in this area; however, promising early observations suggest a potential cancer-modifying effect. Clinical studies suggest that mTOR inhibitors, such as sirolimus and everolimus, may reduce cutaneous cancers in transplant recipients. Interestingly, the anti-tumor effects are most evident in squamous-cell cancers, whereas basal-cell tumors appear to be less responsive. As it turns out, squamous-cell cancers express cytoplasmic phospho-mTOR immune reactivity, yet most basal-cell cancers do not. Unfortunately, in a 2-year randomized, controlled trial of stable renal transplant patients with cutaneous squamous-cell...
tumors, the conversion to sirolimus failed to show a benefit.\textsuperscript{47} In thoracic transplantation, anecdotal cases depicting such benefit do exist, but the evidence is not compelling at this time.

**Possible cancer prevention strategies**

T- and B-cell modulation for induction of tolerance T cells could serve as primary targets of immunotherapy. However, B cells play an equally important role because of their capacity to present antigens. Induction of tolerance therefore needs both T- and B-cell populations to be appropriately modulated.

Achieving developmental balance between Tregs and T-helper 17 (Th17) cells may lead to tolerance. In a mouse reporter system (Foxp3-GFP), Tregs and Th17 cells could be derived from the same precursors by altering the cytokine environment. Predominance of TGF-β influenced the development of Tregs, whereas IL-6 channeled them into the Th17-cell pathway.\textsuperscript{48} One way to achieve tolerance would be to eliminate IL-6 and IL-17, which would inhibit the toll-like receptor-9 (TLR-9) agonist CpG from preventing induction of tolerance, as shown in the murine system.\textsuperscript{49} TLR agonists are involved in peri-operative blockade of induction of transplantation tolerance.\textsuperscript{50,51} Although generation of alloreactive Tregs may be the clue to transplantation tolerance, sustaining it may also require targeting the potential effector cells.\textsuperscript{52}

Long-term depletion of memory B cells and increase in immature B cells by induction agents appears to drive graft survival. Alemtuzumab depletes B cells, followed by rapid repopulation with naive and transitional B cells, suggesting a role for such depletion agents.\textsuperscript{53} Predisposing cells toward tolerance at transplantation may eliminate alloreactive molecules from the recipient repertoire. Future investigations should provide more answers.

Persistent plasma cells are the mainstay of autoimmunity. Inhibition of pathways leading to the activation of plasma cells could lead to tolerance. Atacicept, a recombinant fusion protein, binds to B-lymphocyte stimulator (BLyS) and a proliferation-inducing ligand (APRIL) with the constant region of immunoglobin. It selectively blocks activation of mature B cells and plasma cells by the tumor necrosis factor receptor superfamily member 13B (TACI). Addition of mepoluzimab to block IL-5 on eosinophils may also reduce the availability of APRIL. Bortezomib (26S proteasome inhibitor) reduces plasma-cell alloantibody production. Blocking receptor activator of nuclear factor-kappaB ligand (RANKL) on persistent plasma cells by adding denosumab would provide further inhibition.\textsuperscript{54,55} A multi-pronged approach to inhibiting plasma cells appears attractive, although the pathways described merit further study.

**Role of biomarkers in pharmacodynamic monitoring**

Pharmacodynamic monitoring using lymphocyte function assays to achieve therapeutic levels would prevent over-immunosuppression and toxicity leading to undesirable effects, including cancer. Biomarkers may be identified by genomics, proteomics or metabolomics. In the peri-operative period, the immune systems of the graft and recipient are activated by surgical stress and ischemia/reperfusion injury to the graft. Many candidate surface markers (receptors, costimulatory molecules, adhesion proteins and major histocompatibility complex [MHC] Class II molecules) are upregulated in proliferating lymphocytes,\textsuperscript{56} which can be followed by flow cytometry. A specific assay to measure the pharmacodynamic effects of mTOR inhibitors on phosphorylated S6 ribosomal protein (p-S6RP), a downstream target of mTOR, has been validated recently. Such an approach opens the way for biomarkers to be utilized for better clinical outcomes.\textsuperscript{57}

**Regulatory B cell as a marker of tolerance**

Depletion of B regulatory cells appears to aggravate autoimmune diseases in murine models.\textsuperscript{58} Identification of surface markers and use of intracellular staining for IL-10 to localize these cells could be used to monitor tolerance in transplantation. Recent studies have identified biomarkers of tolerance in liver and kidney transplants.\textsuperscript{59} Such studies need to be extended to thoracic transplantation.

**Farnesyl inhibitors in cancer prevention**

Farnesylation of Ras proteins by the enzyme protein: farnesyltransferase (PFT) is crucial for signal transduction in cell growth and differentiation. PFT inhibition prevents Ras from maturing into its biologically active form, and is

<table>
<thead>
<tr>
<th>Table 1A</th>
<th>Potential Algorithm for Prevention and Management of Post-transplant Malignancies (Evidence-based)</th>
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<tbody>
<tr>
<td><strong>I. Risk factor modification</strong></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Tobacco use—smoking cessation.\textsuperscript{13,14}</td>
</tr>
<tr>
<td>b.</td>
<td>Viral infections (CMV, EBV, HHSV8)—routine testing (PCR for viral titers) and prophylaxis.\textsuperscript{66}</td>
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<tr>
<td>c.</td>
<td>Exposure to sunlight—education and routine use of sunscreen (≥ SPF 30).\textsuperscript{65}</td>
</tr>
<tr>
<td>d.</td>
<td>Use of anti-viral prophylaxis with acyclovir in first 3 months post-transplant.\textsuperscript{28,63}</td>
</tr>
<tr>
<td>e.</td>
<td>Avoid voriconazole use to reduce cancer risk.\textsuperscript{21,22}</td>
</tr>
<tr>
<td><strong>II. Cancer surveillance</strong></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Annual dermatological examination.\textsuperscript{67}</td>
</tr>
<tr>
<td>b.</td>
<td>Increased frequency of colonoscopy.\textsuperscript{68}</td>
</tr>
<tr>
<td>c.</td>
<td>Annual pap smear and gynecological examinations.\textsuperscript{69}</td>
</tr>
<tr>
<td>d.</td>
<td>Annual screening for prostate cancer.\textsuperscript{59}</td>
</tr>
<tr>
<td>e.</td>
<td>Annual screening for breast cancer in females.\textsuperscript{59}</td>
</tr>
<tr>
<td><strong>III. Modification of immunosuppression status after diagnosis of malignancies</strong></td>
<td></td>
</tr>
<tr>
<td>Add statins in addition to mTOR inhibitors, especially if initiating chemotherapy.\textsuperscript{70}</td>
<td></td>
</tr>
<tr>
<td><strong>IV. Non-immune therapies</strong></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Initiate statin therapy post-transplant.\textsuperscript{64}</td>
</tr>
<tr>
<td>b.</td>
<td>Initiate anti-viral therapy post-transplant.\textsuperscript{28,63,66,71}</td>
</tr>
<tr>
<td>c.</td>
<td>Vaccination against hepatitis A and B.\textsuperscript{72}</td>
</tr>
<tr>
<td>d.</td>
<td>Vaccination against human papilloma virus.\textsuperscript{72}</td>
</tr>
</tbody>
</table>

CMV, cytomegalovirus; EBV, EpsteinBarr virus; HHSV8, human herpesvirus 8; PCR, polymerase chain reaction; SPF, sun protection factor.
Table 1B  NonEvidence-based Strategies for Clinical Management of Post-transplant Malignancies

I. Risk factor modification
Pharmacologic agents-prudent use of induction agents-recommend IL-2 inhibitors and restrict use in high-risk/renal insufficiency patients.

II. Cancer surveillance
Dental examination for oral hygiene and oral cancer screening every 3-6 months (www.nidcr.nih.gov-organtransplantation and oral health). Men >40 years of age should undergo yearly rectal examination and prostate specific antigen assay every year.

III. Modification of immunosuppression
(a) Reduction of incidence
1. Add mTOR inhibitors if tolerated to CNI.
2. Continue MMF/wean steroids off by 6 months post-transplant.
4. Add farnesyl- and geranylgeranyl inhibitors in addition to statins.
(b) Modification of immunosuppression status after diagnosis of malignancies.
1. Reduce CNI dosage to 50% and continue MMF.
2. Reduce doses of all immunosuppressants in patients with lymphoid malignancies to 50%.

IV. Non-immune therapies
Reduce craving in tobacco users with medications (bupropion or varenicline).

CNI, calcineurin inhibitor; IL, interleukin; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin.

therefore of interest as a potential therapeutic target. Its role in preventing angiogenesis is of particular interest. In combination with geranylgeranyl transferase inhibitors there is a synergistic action of these compounds. Future studies on the use of these compounds in prevention and treatment of post-transplant malignancies are warranted.60-62

Role of anti-viral propylaxis
Induction therapy, other than with IL-2R blockers, generally increases the risk of cancers, except when acyclovir is administered prophylactically during the first 3 months after heart transplant. Prophylactic dosing of acyclovir reduces the risk of lymphoma by half, regardless of other therapies. Administration of mycophenolate during this time also reduces the incidence of skin malignancies.28,63

Role of statins in prevention of post-transplant malignancies
Statins inhibit 3-hydroxy-3-methyl-glutaryl–coenzyme A (HMG-CoA) reductase and downregulate products such as dolichol, ubiquinol, farnesyl and geranyl pyrophosphates, which impact cell transformation/proliferation and angiogenesis. Statins induce apoptosis via caspase and mitochondrial pathways and downregulate expression of adhesion molecules, metalloproteinases and chemokines/receptors in a geranyl-dependent mechanism. Statins are associated with increased survival and reduction in malignancies after heart transplantation.24 Genetic polymorphisms appear to drive efficacy of statins in lipid lowering and cancer prevention.65 Statins may be used as adjunct therapy in cancer prevention post-transplant. Larger studies in this area are needed to further validate this concept.

Summary of potential management strategies
We have proposed a potential 4-pronged approach (Tables 1A and 1B) consisting of risk factor modification, cancer surveillance, modification of immunosuppression, and addition of adjunct non-immune therapies prophylactically. Future research should define strategies for: tolerance induction and immune modulation; post-transplant malignancy prevention; memory B-cell reduction; and biomarker utilization for predicting tolerance.

Disclosure statement
The authors have no conflicts of interest to disclose.

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