Short Communication

Conversion to sirolimus in kidney transplant recipients with squamous cell cancer and changes in immune phenotype

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Abstract

Background. Conversion to sirolimus from calcineurin inhibitor- (CNI), azathioprine- (AZA) and mycophenolate-based regimens reduces the risk of development of squamous cell carcinoma of the skin (SCC) in kidney transplant recipients (KTRs). Sirolimus conversion may also be protective by permitting beneficial changes in immune phenotype. It is not known how sirolimus will affect immune phenotype in KTRs with SCC.

Methods. Thirty-two KTRs with SCC were enrolled into this single-blinded randomized study and 13 KTRs randomized to sirolimus (4–10 mg/mL) and prednisolone 5 mg/day.

Results. Six-month post conversion to sirolimus FOXP3+; CD127low; CD25high; CD69−, the number of T cells (putative Treg) increased significantly (P = 0.008). Natural killer (NK) and CD56bright NK cells also increased significantly (P = 0.039 and 0.02). T-cell number only significantly increased in those KTRs where CNI was ceased as part of the conversion to mammalian target of rapamycin inhibitors (mTORi’s) (P = 0.031) implying CNI cessation rather than mTORi initiation induced an increase in T-cell number. Increases in the NK cell number was only significant in those KTRs where AZA was ceased (P = 0.040), implying AZA cessation rather than mTORi initiation caused the NK cell number to increase. At 6 months, sirolimus conversion reduces new SCC/year, rate ratio 0.49 (95%CI: 0.15–1.63), P = 0.276. On therapy analysis and intention-to-treat analysis over 24 months, the rate ratios were 0.84 and 0.87, respectively, and did not reach significance.

Conclusions. Conversion to mTORi from CNI may reveal a pre-existing high Treg phenotype by unmasking CNI inhibition of FOXP3 expression. Cessation of AZA leads to increased NK cell number. High FOXP3+ T-cell number on conversion to mTORi may predict those KTRs who continue to accrue SCC.

Keywords: immune phenotype; mTORi inhibitors; skin cancer; Treg

Introduction

Mammalian target of rapamycin inhibitors (mTORi’s) have been shown to slow or reduce the development of de novo cancer in kidney transplant recipients (KTRs) [1–3]. The effect of sirolimus on cell proliferation and neovascularization are potential mechanisms by which sirolimus prevents the development of malignancy [4–7].

Another possible mechanism is that sirolimus-based regimens are quantitatively less immunosuppressive, and it is known that reductions in immunosuppression reduce the risk of cancer [8] and metastasis [9]. One way of determining this is to define whether rejection rates are higher after sirolimus conversion or in comparison to calcineurin inhibitor (CNI)-based regimens. There were small, but statistically non-significant, increases in rejection after conversion to sirolimus in the CONVERT study and no rejection at all in long-term transplant with previous non-melanoma skin cancers (NMSCs) converted to sirolimus [3, 10, 11]. The increased rates of rejection in the SYMPHONY study may reflect sub-therapeutic dosing of sirolimus [12, 13]. Using acute rejection as a readout, there is no objective evidence that sirolimus regimens are quantitatively less immunosuppressive.

Another possible mechanism by which mTORi may have anti-cancer effects is via differential effects on immune cells in comparison to conventional immunosuppression. In KTR with a low CD4 count, high Treg numbers and low natural killer (NK) cell numbers, there is increased risk of cancer [14–16]. Importantly, these immune phenotypes were generated in KTR cohorts not treated with mTORi. mTORi preferentially allow the expansion and generation of Treg and CNI prevent the expression of FOXP3, the Treg master regulatory gene [17–19]. It is plausible therefore that induction of FOXP3+ T cells by mTORi initiation or CNI cessation may paradoxically oppose mTORi anti-cancer mechanisms.
Conversion to sirolimus in KTR

Up to 40% of KTR on mTORi still accrue SCC and/or solid-organ cancer and immune phenotype changes after conversion to mTORi may therefore predict KTR who still develop cancer on mTORi.

The RESCUE trial has shown that randomized conversion to mTORi and cessation of CNI, azathioprine (AZA) and mycophenolate in KTR with SCC skin was significantly associated with a reduced accrual of new SCC tumours in KTR with previous SCC. We therefore hypothesized that conversion would be associated with significant changes in immune phenotype of peripherally circulating lymphocytes.

Materials and methods

Patient characteristics are presented in Table 1. All patients provided informed consent, and the study was approved by a multicentre ethics committee and performed according to STROBE guidelines [20]. The study group consisted of 32 of the 155 white KTR with a functioning transplant and at least one histologically diagnosed SCC recruited to the RESCUE study. Patients from centres in Birmingham, Cardiff, London and Oxfordshire were invited to consent to immune cell monitoring.

After a baseline dermatological assessment, patients were randomized to stay on current therapy or switch to 5 mg of prednisolone and sirolimus (tough levels 5–10 ng/mL). Every 3 months, until 2 years of follow-up, patients were assessed by a dermatologist and any suspicious lesions biopsied and/or resected. The dermatologist was blinded to the randomization status of the patient. At baseline, 3, 6 and 24 months, these patients were also immune phenotyped with the laboratory technician blinded to the randomization status.

Immune phenotyping was performed on peripheral blood within 2-h venesection as described previously [16]. In brief, peripheral blood was collected from KTR during trough levels of CNI and mTORi. Peripheral blood mononuclear cells were separated from whole blood by standard centrifugation and Ficoll techniques. Lymphocytes were stained with multiple monoclonal antibodies conjugated to fluorochromes to elucidate FOXP3+CD127lowCD25highCD69− tumours in KTR with previous SCC. We therefore hypothesized that conversion would be associated with a reduced accrual of new SCC tumours in KTR with previous SCC.

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at 6 months, $P = 0.008$ and $0.039$, respectively. Figures 3 and 4 represent the changes in immune phenotype in those who did or did not cease CNI as part of conversion to sirolimus (Figure 3) and those who did or did not cease AZA as part of the conversion to mTORi (Figure 4). Only those KTR where AZA was ceased as part of conversion to sirolimus had a significant increase in NK cell numbers.

Discussion

We have shown that there are significant and sustained changes in immune phenotype which occur when KTR are converted to mTORi from other immunosuppressive drug regimens (Figures 1 and 2). These changes are slow in development, taking 6 months to develop. Although an increasing disparity in FOXP3$^+$ T cell and NK cell numbers was observed at 24 months, the data were not analysed statistically due to the small numbers of KTR remaining on mTORi. This prolonged time-to-effect may be due to the prolonged effects of AZA on bone marrow suppression after decades of usage, in particular given that the median duration of immunosuppression in this group approached 20 years.

Sub-group analysis revealed that cessation of CNI and AZA are most likely the cause of these changes rather than initiation of mTORi (Figures 3 and 4). mTORi initiation/CNI-AZA cessation increased both FOXP3$^+$ T cells and NK cells. The increases in FOXP3$^+$ T would be expected to increase the risk of subsequent SCC development and the increase in NK cell number would be expected to be protective for SCC development. Therefore,
there are potential opposing changes in immune phenotype in this cohort, although statistically the changes in FOXP3+ T cells were more pronounced, and this finding may inform subsequent SCC development.

In the RESCUE study total population (n = 155), the RR for SCC for conversion was 0.43 and reached statistical significance. The sub-study reported here was never intended to be powered to detect differences in tumour accrual between drug regimens but did show RR of 0.49–0.89 depending on the duration of follow-up, though not reaching significance.

A plausible explanation therefore is that if FOXP3+ T-cell numbers increase with cessation with CNI and/or conversion to sirolimus, this may define a population of KTR who continue to accrue SCC in spite of the anti-proliferative or other proposed anti-cancer effects of sirolimus. Indeed, when tumour accrual for the entire group (n = 32) is assessed, irrespective of drug regimen, the median number of FOXP3+ T cells were higher in KTRs who developed SCC but did not reach statistical significance (data not shown).

In summary, it would seem that conversion to sirolimus and cessation of CNI’s and AZA is associated with significant changes in immune phenotype that are slow in time course to develop but are prolonged. Particular changes in immune phenotype may define those at risk of new SCC development and may define those who may still accrue SCC on sirolimus.

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Conflict of interest statement. None declared.

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