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REVIEW

Immune profiling and cancer post transplantation

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Abstract

Half of all long-term (> 10 year) australian kidney transplant recipients (KTR) will develop squamous cell carcinoma (SCC) or solid organ cancer (SOC), making cancer the leading cause of death with a functioning graft. At least 30% of KTR with a history of SCC or SOC will develop a subsequent SCC or SOC lesion. Pharmacological immunosuppression is a major contributor of the increased risk of cancer for KTR, with the cancer lesions themselves further adding to systemic immunosuppression and could explain, in part, these phenomena. Immune profiling includes; measuring immunosuppressive drug levels and pharmacokinetics, enumerating leucocytes and leucocyte subsets as well as testing leucocyte function in either an antigen specific or non-specific manner. Outputs can vary from assay to assay according to methods used. In this review we define the rationale behind post-transplant immune monitoring assays and focus on assays that associate and/or have the ability to predict cancer and rejection in the KTR. We find that immune monitoring can identify those KTR of developing multiple SCC lesions and provide evidence they may benefit from pharmacological immunosuppressive drug dose reductions. In these KTR risk of rejection needs to be assessed to determine if reduction of immunosuppression will not harm the graft.

Key words: Immune-profiling; Immunosuppression; Kidney; Malignancy; Transplantation

Core tip: Kidney transplant recipients (KTR) with cancer have different leukocyte compartmentalisations and immune cell functions than KTR with no cancer. These differences can be used to determine KTR at risk of developing cancer and identify those who do not mount a reaction to their graft. Indicating there is a group of KTR that may benefit from pharmacological immunosuppressive drug dose reductions.

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INTRODUCTION

Kidney Transplant Recipients (KTR) have a 3 to 12-fold increased risk of developing Non-Lymphoid or solid organ cancers (SOC) when compared to the general population[1-4]. Cancers in KTR have poorer prognoses for a given stage/grade than the general population, which leads to higher mortality[5-9]. In Australia, it is observed that 20% of KTR will develop SOC within 15 years post transplantation (the median graft survival). Over a 5 year period (2007-2011) 267 KTR (or 31%) of all KTR died with a functioning graft (ANZDATA, 2012).

Additionally, KTR have a 60 to 250-fold increased risk of developing a Non-Melanoma Skin Cancer (NMSC), which includes; squamous cell carcinoma (SCC), basal cell carcinoma, Kaposi's sarcoma, Merkel cell carcinoma, and adnexal tumours[1,7,10]. SCC is the most common cancer in KTR with 50% of KTR who are 15 years post transplantation developing an SCC[11]. The disease progression of SCC is much more aggressive than the general population and is exemplified by the development of multiple SCC lesions and metastatic potential, phenomena that rarely occur in the immune competent[5,6,12].

The cumulative risk of subsequent SCC tumours is 30%-32%, 60%-62% and 75%-80% over 1, 3 and 5 years after first tumour, respectively[13]. Compounded, this equates to approximately 10% of KTR having > 5 tumours within 5 years of their first tumour, with some individual KTR reaching 40 primary SCC tumours during recipient life[14]. A single SCC lesion is a risk factor for subsequent SCC development with 60%-80% of KTR with one or more tumours developing another tumour within 1-3 years[15]. SCC tumour characteristics that are risk factors of metastatic SCC and include: size[16], depth[16,17], thickness[17], diameter[18] and poor differentiation[17]. Depth > 2.8 mm has a three-fold greater risk of metastasizing in KTR than the general population[19].

Further evidence of tumour aggression is the invasive potential of SCC in KTR, with more perineural and lymphatic invasion that the general population[20]. Metastatic incidence increases by 5%-8% with every SCC tumour accrued in KTR[14]. Due to SCC lesions mainly located in ultra violet (UV) exposed areas, *e.g.*, the neck, face and scalp there is a possibility of invasion into subcutaneous cranial nerves in the perineural space, leading to extensive surgery and perhaps death[21]. Reports observed an incident mortality of 1%-18%[22,23]. Observational studies have showed a 37% incidence of SCC metastasizing[18] which leads to the median KTR survival after diagnosis being only 2 years[24]. Furthermore, it has been observed that a previous SCC is a risk factor for multiple SCC and even development of SOC[11,13,19]. This is probably due to the exposure of pro-carcinogenic agents as well as the compounding effects of cancer induced, and pharmacological administrated, immunosuppression.

Therefore there are various risk factors and clinical parameters that influence the development of post-transplant cancer. The next section will introduce some of these factors and the rationale behind why they are factors of risk.

IMMUNOSUPPRESSION TYPE

There are limited and conflicting data on the use of different types on immunosuppressive drugs and the associated cancer risks. The conflict mainly due to the multiple confounding factors associated to cancer, immunosuppressive drugs in particular have the dual capacity to suppress both anti-graft and anti-cancer immunity. The immunosuppressive drug types introduced in this section include; azathioprine (AZA), mycophenolate mofetil (MMF), calcineurin inhibitors (CNI), steroids and mammalian target of rapamycin inhibitors (mTORi). These immunosuppressants are rarely used in monotherapies and are therefore hard to compare one another; instead modes of action and evidence for cancer development are presented.

AZA

AZA is catabolised to 6-mercaptopurine, which directly affects the synthesis of purines and has the ability to incorporate into DNA[25,26]. Lymphocytes rely heavily on *de novo* purine synthesis making AZA an effective immunosuppressant. AZA was originally used as an anti-cancer therapy however some cancers intrinsically have, or gain, purine scavenging and are, or become, resistant to AZA treatment[27]. When incorporated, the metabolite and the DNA form a complex that can block DNA repair, is photosensitive and produces reactive oxygen species (ROS) under UV exposure[25,27]. These work synergistically to affect DNA repair which form lesions[26,27]. One case-controlled study identified that AZA increased risk of developing SCC by 5-fold. However, in the same study calcineurin inhibitors (CNI) and steroids were also identified as risk factors[28].

MYCOPHENOLATE

MMF is a pro-drug of mycophenolic acid (MPA), which directly affects purine synthesis and is classified as an anti-proliferative drug[29]. The reaction of MPA is reversible and does not interfere with the DNA structure as AZA does[29]. One study showed a decrease photosensitivity when a cohort was randomised onto a MMF from AZA suppression regimen[30]. In another study comparing MMF to AZA usage in organ transplant recipients showed that the MMF group had a 27% adjusted risk reduction[31]. Conversely, a 3 group randomised control trial of 133 KTR; 45 KTR randomised to AZA treatment, 44 KTR randomised to 3 g daily of MMF and 44 KTR randomised to 3 g daily of MMF with no differences in cancer incidences between all three groups[32].

CALCINEURIN INHIBITORS

Cyclosporine A (CsA) forms a complex with cyclophilin which inhibits calcineurin, making CsA and CNI[33]. Calcineurin de-phosphorylates nuclear factor of activated T cells (NFAT), which translocates to the nucleus. It is in the nucleus where NFAT activates pro-inflammatory cytokines such as interleukin 2 (IL-2)[34]. Therefore CsA indirectly affects pro-inflammatory cytokine IL-2 transcription. An isotype of cyclophilin is expressed in the mitochondria which releases apoptotic signals under oxidative stress. CsA blocks this signal transduction and allows cells to by-pass apoptosis when under oxidative stress, including ROS and UV-damage, contributing to carcinogenesis[35,36]. Other tumorigenic side effects of CsA are direct or in-direct suppression of p53, production of transforming growth factor beta (TGF-β) and vascular endothelial growth factor (VEGF)[37-39].

When investigating this in the clinic, a retrospective analysis of 1000 KTR showed that KTR on CsA based regimens had greater cumulative incidence of tumours than those on an AZA based regimens[40]. In another retrospective study any regimen with CsA had an Odd Ratio of approximately 4.5[41]. Inversely, A CsA based mono-therapy was shown to be less carcinogenic than a MMF and prednisone dual-therapy[42,43]. Another CNI, tacrolimus (TAC), inhibits calcineurin by forming a complex with FK506-binding protein 12 (FKBP12) and outcompetes calmodulin therefore still inhibiting IL-2 transcription. TAC does not target cyclophilin, so avoids all interference with the mitochondria that CsA has. In a retrospective study of 609 liver transplant patients, TAC had a higher incidence rate for *de novo* cancers than CsA[44]. However in most database analyses, TAC-based immunosuppressive regimens have either no significant difference or a reduced risk of cancer incidence and/or risk over CsA-based immunosuppression regimens[45-48].

CORTICOSTEROIDS

Corticosteriods are mainly utilised for treatment of auto-immunity, inflammatory disorders and transplantation rejection. Corticosteriods function by inhibiting transcription of IL-1, IL-2, IL-6, interferon (IFN)-γ and tumor necrosis factor (TNF)-α and transcription factors such as nuclear factor-κB[49-54]. Inhibition of these Th1 cytokines promotes a Th2 response, which provides another indirect immunosuppressive function[55]. Corticosteroids induce TGF-β and can increase the incidence of Kaposi's sarcoma cell proliferation[56,57].

MAMMALIAN TARGET OF RAPAMYCIN INHIBITORS

Both Sirolimus (SIR) and Everolimus (EVO), like TAC, bind to FKBP12. However the formed complex inhibits mTOR's *via* mTORC 1 subunit (Raptor) binding and are considered mTORi. mTORi can also be classified as anti-proliferatives as they induce apoptosis *via* p53 dependent and independent pathways. This and mTORi's ability to prevent IL-2 signalling cause it to have both anti-cancer and anti-rejection properties. Additionally, mTORi affect protein synthesis, including VEGF which inhibits metastatic potential in murine models[58,59]. SIR has been used to treat patients with renal cell carcinoma (RCC) and EVO has shown to benefit patients with metastatic RCC who do not response to mainstream treatment[60-62]. Sirolimus Conversion from CNI based regimens, is beneficial in Kaposi sarcoma and SCC involution[63-66] However it can often lead to increased adverse reactions and increases in rejection episodes if performed too early post-transplant[67,68].

ANTI-THYMOCYTE GLOBULIN INDUCTION THERAPY

Anti-thymocyte globulin (ATG) is either horse- or rabbit-derived antibodies directed against human T cells, given as an induction therapy of transplant recipients. The T cells that reconstitute have a regulatory phenotype and return much faster than other T cells[69]. There is an association with prolonged CD4 lymphopenia and ATG as well as CD4 lymphopenia and cancer[70]. Without knowing cause and effect it is speculative to say that ATG is associated with cancer.

Despite the various functions of immunosuppressive types each playing a role with cancer in KTR, overall immunosuppressive load or immunosuppressive dose can also have detrimental effects and promote cancer development.

IMMUNOSUPPRESSION DOSE

There is an association between immunosuppression dose and cancer incidence. KTR have 3-fold increased cancer risk compared to dialysis patients, in a retrospective registry based study[71]. Furthermore, heart transplant patients have higher levels of immunosuppression than KTR and also have corresponding increases in cancer (100% compared to 88% 5 year incidence, respectively[14]). Additionally, KTR randomised to a low dose CsA base regimen had reduced incidence of cancer following reduction, with the caveat that they had higher rejection rates[72].

IMMUNOSUPPRESSION DURATION

Maintained immunosuppression increases the risk of cancer over time which is evident in the steady increase in KTR that accrue cancer in the years post-transplant. Australian KTR SCC incidence is 20%, 50% and 80% at 5, 15 and 30 years post transplantation respectively[11,73]. Included in the duration of immunosuppression would be the age and aging of the KTR.

AGE AND GENDER

Age is a risk factor of cancer development, independent of immunosuppression duration[74]. This is exemplified in a retrospective study that showed both Age and male gender were risk factors[41]. When comparing KTR to the general population in an aged matched cohort of median age 39 years old, there was a 12-fold increased risk of developing non-skin cancers[4]. Age and gender can influence other parameters of cancer risk. This is particularly the case in Australia were certain, culturally male-orientated, jobs may involve higher exposure to UV radiation.

ULTRA-VIOLET RADIATION

It is evident that UV exposure increases the risk of skin cancer, including NMSC, by the observations recorded by clinicians of the locations of tumours. Cumulative sun exposure, including outdoor occupation, latitudinal residence and even childhood burning events all increase risk of post-transplantation cancer development[75-77]. These increases in carcinogenesis are in part to the aforementioned AZA-UV interactions but mainly *via* direct UV-related mutagenesis. Due to the structure of DNA, it absorbs of UV-A (315-400 nm) and UV-B light (280-315 nm), in doing so the DNA itself forms cyclobutane pyrimidine dimers in two adjacent pyrimidines of the same DNA strand, which alters the structure of DNA and restricts transcription[78,79]. A single point mutation can lead to transcriptional arrest[79]. A study found that invasive SCC contained mutations of the tumour suppressor gene P53[80]. An important conclusion from this study is that P53 mutation could have happened in childhood, as most UV exposure happens in childhood[81].

In addition to direct DNA mutagenesis, UV exposure can also have local and systemic effects on the immune system. It is thought that the local effect involves antigen presenting cells (APC)'s, including resident keratinocytes and Langerhans cells[82,83]. Whereas the systemic immunosuppression may come from splenic cells, migrated Langerhans cells, dendritic cells. Increased expression of IL-4, IL-10, prostaglandin E2, IL-1α and TNF-α with polarisation of immunity to a Th2 response also plays a role in systemic immunosuppression[83-85]. In combination with this, co-stimulation is effected on both APC and T cells[86]. Other cell types that are affected by UV irradiation are innate immune cells and suppressor cells[87-91]. Regulatory T cells (Tregs) that are induced by UV express lymph node homing molecule CD62L and may provide systemic immunosuppression[87,88].

The DNA damage and immune suppression of UV can be reversed by IL-12 dependent induction of nucleotide excision repair protein[92]. Also immunity can be restored by the administration of IL-12[93], activating APC's, increasing IFN-γ and thus balancing Th1-Th2 polarisation[93,94].

Other clinical parameters are associated with cancer risk that are also orientated by human behaviour, apart from UV exposure, are communicable diseases such as oncogenic viral infections that remain latent in the immune competent.

VIRAL INFECTION

Human papillomavirus (HPV) is a group of more than 150 viruses with some types associating with anogenital, oropharyngeal and skin cancers[95,96]. It has been speculated that HPV infection may prevent UV light-induced apoptosis[97]. Between 65% and 90% of SCC lesions from transplant recipients are positive for HPV DNA[98].

Epstein barr virus (EBV) is associated with: sino-nasal angiocentric T-cell lymphoma, Hodgkin lymphoma and nasopharyngeal carcinoma[95]. There are data that EBV associates with mononucleosis, Burkitt lymphoma and post-transplant lymphoproliferative disorder in KTR[99,100].

Chronic Cytomegalovirus virus (CMV) infection can cause graft rejection, but with malignancy however it does have indirect associations with cancer[99]. A prospective study followed 63 KTR and retrospectively included 131 KTR, with convincing data that CMV positive KTR with increased $\gamma\delta$ T cell proportions, the $V\delta2^{neg}$ sub-population in particular, had decreased cancer incidence[101]. This case-control study compared 18 short-term KTR (median 3 years post Tx), who developed 12 skin and 6 solid tumours over the prospective period and compared to 45 KTR who did not develop cancer. The skin nor solid organ tumour types were not disclosed.

IMMUNE PHENOTYPING

The association with cellular markers and cancer has been previously studied. The identification of immune cell populations and sub-populations in patient blood is called immune phenotyping. Measurement of CD4 T cells in 150 KTR revealed that KTR with skin cancer had 330 $CD4^+$ cells/μL of blood in comparison to KTR with no cancer who had 565 $CD4^+$ cells/μL ($P < 0.01$). Additionally KTR with cancer had non-significant increases in CD8 and CD19 lymphocytes[102]. Another study involving 250 KTR over a 10 year period showed a mean of $CD4^+$ lymphocytes of < 600 $CD4^+$ T cells/μL for those with cancer and > 700 $CD4^+$ T cells/μL for those with no cancer, however there was no useful threshold found using receiver operator curve (ROC) analysis[103]. Additionally, $CD8^+$ T cells and $CD19^+$ B cells were also investigated in the same study; there was no difference between KTR with SCC when compared to KTR without SCC[104]. It was noted however, that immune phenotype was more pronounced in KTR with SOC compared to KTR with SCC: CD4 count: 234 cells/μL *vs* 543 cells/μL, $P < 0.001$; CD8: 328 cells/μL *vs* 640 cells/μL $P = 0.100$; CD19: 19 cells/μL *vs* 52 cells/μL, $P < 0.001$[104]. All these

studies showed an association with CD4 lymphopenia and cancer, however the majority of the cohorts underwent ATG induction therapy. However they did not define $CD4^+$ subsets or other lymphocytes that may be affected by cancer.

While these studies provide some evidence that cancer may influence the peripheral immune cells, there was no investigation into sub-types of these cells, primarily because multi-parameter flow was not common place. Recently, it was reported that high numbers of $CD4^+$ Regulatory T cells (Tregs, *i.e.*, $CD4^+FOXP3^+CD127^{Lo}CD25^{Hi}$) and low numbers of Natural Killer (NK cells, *i.e.*, $CD56^+CD16^+$), in peripheral blood associated with and predicted recurrent SCC in KTR[105]. This study also showed an increase in $CD8^+CD28^-$. These CD8 T cells co-localise with Tregs within cancer tissue and have been shown to be suppressive from patients with cancer, and therefore abbreviated to $CD8^+$ Tsupps[106]. Furthermore, there was a decrease in $CD8^+CD45RA^-CD62L^+$ CD8 central memory T cells ($CD8^+$ Tcm), which has been shown to decrease in KTR using the corticoid steroid prednisolone, despite cancer status[105]. This indicates that immunosuppression may affect immune phenotype and warrants investigation.

Operationally tolerant organ transplant recipients have increases in Regulatory T cells, B cells (particular naïve B cells), Vδ1 γδ T cells and decreases in $CD3^+$ proportions (B:T ratio), NK cells, Vδ2 γδ T cells within their peripheral blood[107]. Transplant patients have increased Regulatory T cells, B cells (memory B cells), $CD8^+$ γδ T cells and $CD8^+$ $CD27^-CD28^-$ T cells and decreases in CD4 counts, NK cells and $CD8^+$ Tcm[105,108].

REGULATORY T CELLS (TREGS)

Immune suppressor cell existence has been debated from the early 1970's through to the mid 1990's[109-112]. The pivotal paper adoptively transferred $CD4^+CD25^+$ T cells in CD25 depleted mice, which mitigated the autoimmune diseases that manifested[112]. However, CD25 is also expressed on activated lymphocytes with only the highest proportion being suppressive *in vitro via* competitive absorption of IL-2[112-115]. The discovery and transfection of the transcription factor forkhead box protein 3 (FOXP3) into naïve T cells helped identify FOXP3 and its function as the master regulatory gene[116,117] and CD127 inversed expression to FOXP3 expression has given Tregs the current phenotype $CD4^+FOXP3^+CD25^{hi}CD127^{lo}$[114].

Tregs are required in a healthy immune system to maintain self-tolerance and immune homoeostasis during immune reactions, pregnancy and disease. Uncontrolled immune reactions and organ failure result when mutations in FOXP3 occur, as observed in the scurfy mouse models and similarly Immunodysregulation, Polyendocrinopathy, enteropathy, and X-linked (IPEX) syndrome observed in humans[118-120]. Both IPEX and X-linked Autoimmunity-Allergic Dysregulation syndrome cause multi-organ failure due to mass lymphocyte proliferation of self-reactive effector cells [119].

$CD4^+$ TREG SUBSETS

The $CD4^+$ Treg in the periphery, defined by $FOXP3^+CD25^{hi}CD127^{lo}$, contain two subsets: those that originate from the thymus, known as natural Tregs (nTregs), and those that are induced in the periphery, known as induced Tregs[121]. The Ikaros family transcription factor, Helios is expressed in 100% of all $CD4^+FOXP3^+$ thymocytes of mice and approximately 70% of Tregs in the periphery of both mice and humans[122]. Though the premise that Helios only defines nTreg is currently under debate, nonetheless, it may provide evidence of *in vivo* activated Tregs[101,123]. Despite the debate it seems that KTR with cancer have similar Helios expression than KTR without cancer[108].

TREG MODES OF ACTION

Treg apoptosis induction requires cell contact with co-stimulatory molecule Cytotoxic T cell Late Antigen-4, Fas/Fas ligand interaction and release of Perforin and Granzyme B[124-126]. Indirectly, Tregs can down-regulate B7 Co-stimulation molecules CD80/CD86 on APC[127]. In addition, prostaglandin E2 (PGE2) excreted by Tregs, mediates expression of indoleamine 2,3-dioxygenase in APCs causing tryptophan starvation and leading to impaired lymphocyte proliferation[128]. Another form of suppression is the formation of localised adenosine by cleaving phosphate groups from ATP, ADP and AMP by ecto-NTPDase-1 (CD39) and ecto-5'-nucleotidase (CD73) cell surface enzymes[129]. Expression of CD39 and CD73 has been shown on murine and human Tregs[129]. Human Tregs also may work in concert with other CD73 expressing cells to elicit a regulatory response. Adenosine has been shown to act *via* Adenosine receptors (A1, A2a, A2b and/or A3), with A2a receptor being the dominate receptor on effector cells[130,131]. The adenosine formed by the hydrolysis of ATP can regulate lymphocyte proliferation in autoimmune disease, transplantation and cancers[132-134]. Additionally, it has been shown that adenosine and PGE2 in Tregs co-operate when regulating immune responses[133]. Other regulatory cells are $CD4^+$ helpers that have suppressive function are classified by the ability to secret of IL-10 (Tr1) and TGF-β (Th3) which they are also induced by, respectively.

TREGS IN VIRAL INFECTIONS

EBV antigen specific Tregs, mainly IL-10 secreting Tr1 and recruited nTregs, can inhibit the EBV-specific immunity permissive in tumour progression[100,135]. Thus reduction in Tregs may be beneficial in treatment of chronic viruses. Interestingly, Treg depletion in a herpes simplex virus (HSV) mouse model decreased paralysis onset, indicating that Tregs have an early role in protective immunity to HSV infection, similarly observed

in Lymphocytic Choriomenigitis virus mouse model, shown in the same study[136].

TREGS AND TRANSPLANTATION

In regards to transplantation, when isolated $CD4^+CD25^-$ cells are administered to BLABc *nu*/*nu* mice grafted with C57BL/6 skin there is a swifter rejection rate than administering untouched lymphocytes of the same source[112]. This indicates $CD4^+CD25^-$ T cell subpopulation has greater cytotoxicity when absent from $CD4^+CD25^+$ T cells and that $CD4^+CD25^+$ T cells are possible inducers of tolerance.

In KTR, Tregs can differ in accordance with the situation of the patient. Two different studies on clinically tolerant, chronic rejection, stable, minimally suppressed KTR and healthy controls, showed tolerant KTR and minimally suppressed KTR had similar $CD4^{+-}$ $CD25^+FOXP3^+$ and $CD4^+CD25^{hi}$ cells with similar FOXP3 transcription levels when compared to the healthy controls[137,138] and that chronically rejecting KTR had lower $CD4^+CD25^{hi}$ cells with low FOXP3 transcripts, indicating that Tregs may be protective or involved with tolerance[137,138]. An additional study supported this in liver transplant recipients which showed increased FOXP3 mRNA expression in $CD4^+CD25^{hi}$ T cells of tolerant patients compared to patients who had rejection episodes after cessation of immunosuppression[139]. Thus induction of Tregs for suppression of allograft cellular rejection episodes[140] and possible induction of tolerance[141] seem like an attractive substitute to immunosuppression. However Tregs that co-express CD25 and CD39 have been denoted as a memory subtype of Treg (mTreg) and are associated with cellular rejection episodes[142] in KTR. Increases in Tregs are also associated with cancer in the general population[143] and KTR[105].

TREGS IN CANCER AND IMMUNE SURVEILLANCE

It has been shown that the percentage of $CD4^+CD25^{high}$-$FOXP3^+$ Tregs and Tr1 cells are increased in Head and Neck Squamous Cell Carcinoma (HNSCC) patients in comparison to healthy controls[144,145]. Ectonucleotidase activity contributed by CD39 and CD73 is also increased on Tregs in this cohort[133]. CD39 has been shown to down-regulate IL-17 production, decreasing Th-17 cell linage. This particular Treg subtype, in the same study, has been shown to be down-regulated in autoimmune Multiple Sclerosis[132]. It has been shown that high levels of Treg in HNSCC patients from the general population associate with a poor prognosis[146-148].

Cancers and Tregs not only have commonalities between each other but they also promote each other. TGF-β and IL-10 secretions from tumours activate Th3 and Tr1 regulatory cells respectively, consequently regulating surrounding cancer cytotoxic lymphocytes[145]. Also tumour cells recruit Tregs with a series of chemokines such as C-X-C Ligand 12 and C-C motif 20 and 22 (CCL20/22)[100]. CD39 and CD73 have been shown to be expressed on Tr1 and tumour cells alike[129,149]. Cancer progresses by the tumours' ability to secrete these soluble factors into its microenvironment. PGE2 is a product of Cyclooxygenase 2 (COX-2) and is involved in aiding immune escape. COX-2 is expressed on Tr1 and over-expressed on cancer cells[145,150,151].

In a post-transplant cancer setting, it has been shown that Tregs ($CD4^+FOXP3^+CD25^{hi}CD127^{lo}$) in blood from KTR with a history of SCC can predict the risk of developing a subsequent SCC lesion[105]. Another study has shown that Tregs alone can predict cancer onset and associate to the severity of the cancer developed[108]. In this same study Hope *et al*[108] shows prospectively that Tregs increase in KTR when the cancer becomes apparent and then decreases post-resection of tumour tissue.

NK CELLS IN CANCER AND IMMUNE SURVEILLANCE

Carroll *et al*[152] revealed that NK cells, which have cytolytic ability to kill cancerous and pre-cancerous cells, are decreased in KTR with cancer. NK cells are a part of the innate immune system that identify abnormal cells and supply the signals to undergo apoptosis thus "killing" abnormal cells. The identification process involves Major Histo-incompatibility Complex (MHC) class I down regulation, which some viruses and cancerous cells adopted to avoid the adaptive immune system[153]. It is an important step in metastatic cells to successfully invade the host[154]. Once the cell has been identified the NK cell only activates if there is an imbalance of CD94: NKG2A and the killer-cell immunoglobulin-like receptors (KIR) family. Once activated internal granules locate to the synapse that is created between the NK cell and target cell[152]. During the effector stage the granules are released out of the NK cell and into the synapse and onto the target cell. These proteins include Perforin, granzyme A and B. It is these proteins that play their role in the killer phase of NK cells[155]. Perforin creates pores in the membrane that granzyme B can enter and activate the caspase kinase pathway and cause the target cell to undergo apoptosis[155]. This cytotoxic ability to kill cancer cells can be inhibited by Tregs but also cancer cells themselves[156,157]. This NK-Treg interaction is a TGF-β and cell-cell contact mechanism of down-regulation NKG2D and induction of apoptosis, respectively[158,159]. This leads to decreased NK cell numbers and function in the peripheral blood of cancer patients that have elevated TGF-β[160,161]. There are two other types of NK cells: those that express CD1-d restricted T cell receptor, NK T cells and those that lack Fc receptor CD16 and over express CD56, $CD56^{bright}$ NK cells[162-164]. Both these cells can interact with the adaptive immune system and enhance

anti-tumour ability by direct and indirect mechanism respectively[162,164].

CD8 SUBSETS IN CANCER AND IMMUNE SURVEILLANCE

Another cell type with anti-tumour properties is $CD8^+$ cytotoxic T lymphocytes (CTL). $CD8^+$ CTL are in the effector arm of the adaptive immune system. CTLs use the ability to lyse tumour cells using Fas-Fas ligand as well as perforin-IFN-γ granules similar to NK cells[165]. It has been shown that antigen specific CTL are defective in cancer patients and that removal of Tregs can restore cytolytic function[166-168].

CD4 and CD8 T cells follow an immunogenic pathway to immune senescence. T cells exiting the thymus are naïve since they express both CD27 and CD28 co-stimulation molecules and home to the lymphoid organs[169,170]. When antigen is presented they become CTL, clear the threat, and the majority apoptose with the minority homing to lymphoid organs as central memory T cells or extra-lympoid sites as effector memory T cells[169,170]. Upon subsequent exposures the cells become exhausted and lose expression of co-stimulation molecules and are termed T effector memory CD45RA+ or TemRA cells[169,171]. These cells are loosely phenotyped as $CD8^+CD28^-$ and shown to be regulatory in cancer patients and may associate with poor prognosis[106]. Tumours themselves may induce this loss of CD28[106,172] and they are also expanded in patients with CMV infection[173]. It has been shown that Memory T cells and (NK cells have anti-tumorigenic properties and that Tregs regulate both of these lymphocyte subsets[158,174]. Thus, an excess of Tregs is associated with poor prognosis in cancer and is thought to aid cancer cells evade this immune surveillance.

IMMUNE CELL FUNCTIONS

Kidney transplant recipients (KTR) with cancer have increased numbers and proportions of Regulatory T cells (Tregs) and decreased numbers and proportions of NK cells[105,108]. However, the immune system's effectiveness cannot be gauged by cell numbers and proportions alone; this chapter investigates the immune function of KTR with cancer.

It has been shown that Tregs isolated from tumour tissue and the peripheral blood of KTR with cancer have higher suppressive function than Tregs from the blood of normal donors[145,175,176]. Importantly, the stage and grade of HNSCC are associated with greater numbers and greater suppression capacity of the Tregs on a cell-per-cell basis than healthy controls[177] and, as such, also associate with poor cancer prognosis in the general population[176].

In the Transplant population it is known that CNI regimens are associated with reduced numbers and proportions of Tregs and how mTORi maintain these Treg parameters[178,179]. Furthermore, Tregs numbers and proportions are increased by mTORi usage in KTR with no cancer and CNI usage decreases Tregs in KTR with cancer. A proposed mechanism is CNI's ability to reduce Nuclear Factor of Activated T cells (NFAT), decreasing production of IL-2 which is vital for function and homeostasis, in mice[180]. Molecular interactions between NFAT and FOXP3 show that NFAT acts as a molecular switch between immune stimulator and immune regulator, thus down regulation decreases FOXP3 expression and FOXP3's ability to form these regulatory complexes[178,181]. Additionally, FOXP3 mRNA transcription was decreased in CNI treated peripheral blood mononuclear cells compared to Rapamycin in an allo-stimulated mixed lymphocyte reaction[182]. There is also an inverse correlation to CNI level and Treg function[183].

Tregs promote cancer survival whereas NK cells have anti-cancer abilities. The function or dysfunction of NK cells plays an important role in the apoptosis of pre-cancer and cancerous cells. Patients with genetically (*MCM4* or *GATA2* mutations) related NK cell deficiencies in either number or function, have increased risk of infections, in particular: Herpes viruses, HPV, CMV and EBV (reviewed elsewhere[184]).

NK cells are large granular lymphocytes that lack the CD3 T cell complex. They function by identifying and spontaneously causing apoptosis in cancerous and infected cells without prior antigen presentation[152,185]. The identification process requires abnormal cells to display stress signals such as down-regulation of "self" surface proteins: Major Histo-incompatibility Complex (MHC) class Ⅰ and regulatory KIR[154,155,186]. The down regulation of MHC-Ⅰ, reduces the effectiveness of cytotoxic CD8+ T cells and adaptive immune responses but makes the cells more sensitive to NK and innate immune responses[187]. Once an NK cell identifies this down-regulation, it binds and activates, expressing a type Ⅱ transmembrane glycoprotein CD69 and other surface markers of activation[188]. Internal granules locate to the immune synapse that is created between the NK cell and the target cell and the effector molecules (perforin, TNF-α, granzymes and interferons) are released into the synapse and onto the target cell. Upon degranulation, Lysosome-Associated Membrane Protein 1 (CD107a) is exposed on the surface of the NK cell[155]. The released perforin creates pores in the target cell membrane through which granzyme B can enter the target cell and initiate apoptosis *via* the caspase kinase pathway. Therefore there are several ways to measure NK cell activity including: CD69 up-regulation in the activation stage, CD107a in the effector stage, release of cytokines (perforin, granzyme B, IFN-γ) in the killing stage, and total cytolysis of the target cells.

Cancer cells have greater metabolic demands than normal cells[189], utilising glycolysis and lactate pathways, *via* Lactate Dehydrogenase (LDH), causing an 18-fold increase in glucose utilisation, even under aerobic conditions[190]. This LDH can be measured as a cytotoxic assay (first described in 1988[191]). Additionally, in *in*

vitro assays, NK cells undergo apoptosis when they are exhausted from their last kill. Recently, it has been shown that the loss of NK cells from an *in vitro* assay with a set number of NK cells, can relate to the amount of target cells killed. This loss has been termed "target induced NK cell loss" (TINKL). These two assays have been chosen for clinical application. LDH is a single platform, self-contained, non-radioactive, sensitive assay that can be used in any laboratory. TINKL is a flow-based assay that can be readily implemented in clinical flow laboratories.

It is widely accepted that NK cell function is decreased in cancer patients however it is not reported if KTR with cancer have further reduced NK cell function. The effect immunosuppression has on NK cells have been investigated both *in vitro* and *in vivo*[192,193]. Immunosuppressive drugs: AZA, MMF, CNI, and prednisolone all have individual effects. These effects depend on the how the NK cells are stimulated and how NK function is measured. One particular study showed only a decrease in NK function in short term KTR compared to healthy controls, which was not observed in long term KTR[192]. Both IFN-γ and CD107a expression have been shown to decrease when NK cells were co-cultured in the presence of clinically relevant concentrations of a variety of immunosuppressive drugs[193].

TREATMENT OPTIONS FOR KTR WITH CANCER

The aforementioned assays give clinicians the ability to objectively identify patients that may develop premetastatic cancer with relatively high sensitivity and specificity. However they do not inform clinicians if KTR will benefit from cancer prevention therapy.

A randomised control trial randomised pre-transplant KTR to a standard level CNI regimen and a CNI sparing regimen[72], thus investigating the benefit of reduced immunosuppression as primary cancer prevention. However, those with reduced CNI had increases in rejection episodes[72]. Other studies investigated converting CNI based regimens to mTORi based regimens as secondary prevention therapy, as mTORi are used as anti-cancer therapies[194,195]. There was a benefit, however not all conversions were successful (30%) and an additional 30% did not tolerate the mTORi side effects[14,196,197]. Furthermore, immune phenotype has revealed that those who maintain high levels of Tregs after mTORi conversion (> 20 Tregs/μL) do not benefit from conversion and may benefit from immunosuppressive drug reduction. To perform immunosuppressive drug reduction as secondary cancer prevention, risk of graft rejection will need to be measurable.

Pre-transplant anti-Human Leukocyte Antigen (HLA) and IFN-γ ELISPOT associate post-transplant with antibody and cellular mediated rejection episodes[198-200]. Monitoring HLA molecules and Donor Specific Antibodies (DSA) routinely has decreased antibody mediated rejection episodes dramatically[201,202]. IFN-γ ELISPOT has been used to predict 6-mo graft function and rejection episodes[200]. Additionally it has been used pre-transplant to categorise patients into CNI or mTORi maintenance therapy[203]. These studies are limited in clinical application as donor specific cells were used to stimulate the mixed lymphocyte reactions, requiring use of precious or non-existent deceased donor material. This restricts the utility of ELISPOT to live recipient/donor pairs. An IFN-γ ELISPOT assay has been developed that utilises a variety of unrelated HLA disparate material to measure total allo-response and is termed "Panel of Reactive T cells"[204]. This assay has been shown to have potential to determine post-transplant risk of rejection when measured pre-transplant. However there are no current studies utilising IFN-γ post-transplant as a form of rejection prediction in long-term KTR.

The IFN-γ ELISPOT may be extended to guide immunosuppression reductions[205,206]. There are a few studies utilising a viral peptide stimulated IFN-γ ELISPOT to discriminate KTR who may benefit from reduced immunosuppressive drugs as a form of treatment[205,206]. KTR with unresolved BK pathogenesis also had a non-significant decrease in EBV peptide and phytohaemagglutinin mitogenic IFN-γ ELISPOT responses[205,207]. This may share a link with development of malignancy as they are both considered manifestations of over-immunosuppression.

When KTR have a cancerous lesion, surgical resection is the recommended treatment. There are no randomised control trials investigating the effect of tumour resection and minimal evidence of benefit in KTR when reducing immunosuppression. However, treatment in the general population is associated with a decrease in Tregs. Failure of Tregs to fall after tumour excision, chemo or immunotherapy is due to incomplete resection or predicted relapse of disease[208,209].

When switching or reducing immunosuppression, adequate precautions must be used. Currently there are no assays that reliably determine cancer risk although there is an immune phenotype that can predict time to next tumour in KTR with a history of SCC[105]. CNI avoidance or reduction results in increases of rejection; one way to potentially avoid these rejection episodes is to identify those KTR with cancer who have evidence of a potential alloresponse and exclude them from dose reduction. In order to reduce immunosuppression safely, both the cellular and humoral alloresponses need to be assessed.

PRE-TREATMENT ALLORESPONSE MEASURES

Assessment of allo-responses would be needed to assess risk of rejection episodes for it to be possible to reduce immunosuppression. Currently cytokines and HLA antibodies can be measured by Enzyme Linked Immuno SPOT (ELISPOT) and Luminex technologies respectively[198,210]. Inflammatory cytokines such as IFN-γ are secreted by Th1 effector T cells and are a predictor of

acute rejection and infection[200,204]. A National Institute of Health funded Clinical Trials in Organ Transplant consortium approved ELISPOT has been able to detect 6-mo post-transplant acute rejection in pre-transplant patients[211,212]. Additionally a similar assay has been used to run CNI avoidance maintenance therapy with a 3-fold reduction in acute rejection as shown in literature[203]. The humoral aspect of the immune system is already routinely assessed in most transplant programmes by solid phase alloantibody detection systems[202]. HLA DSA are clinically relevant and observed DSA presence has informed clinicians to alter immunosuppression regime of patients[199,201]. However both these techniques have not been measured in long-term kidney transplant recipients with a history of cancer.

CONCLUSION

Long-term immunosuppression increases the risk of cancer development. The dose of immunosuppression can be increased by closely monitoring graft function and survival. In this review we present that there are several emerging immune monitoring tools that are available to potentially help reduce immunosuppression. Future studies may be undertaken to determine if these assays can help identify those at risk of cancer development and if reduction of immunosuppression is of benefit.

REFERENCES

1 **Dantal J**, Pohanka E. Malignancies in renal transplantation: an unmet medical need. *Nephrol Dial Transplant* 2007; **22** Suppl 1: i4-i10 [PMID: 17456618 DOI: 10.1093/ndt/gfm085]

2 **Kasiske BL**, Snyder JJ, Gilbertson DT, Wang C. Cancer after kidney transplantation in the United States. *Am J Transplant* 2004; **4**: 905-913 [PMID: 15147424 DOI: 10.1111/j.1600-6143.2004.00450.x]

3 **Miao Y**, Everly JJ, Gross TG, Tevar AD, First MR, Alloway RR, Woodle ES. De novo cancers arising in organ transplant recipients are associated with adverse outcomes compared with the general population. *Transplantation* 2009; **87**: 1347-1359 [PMID: 19424035 DOI: 10.1097/TP.0b013e3181a238f6]

4 **Apel H**, Walschburger-Zorn K, Häberle L, Wach S, Engehausen DG, Wullich B. De novo malignancies in renal transplant recipients: experience at a single center with 1882 transplant patients over 39 yr. *Clin Transplant* 2013; **27**: E30-E36 [PMID: 23278453 DOI: 10.1111/ctr.12050]

5 **Karagas MR**, Stukel TA, Greenberg ER, Baron JA, Mott LA, Stern RS. Risk of subsequent basal cell carcinoma and squamous cell carcinoma of the skin among patients with prior skin cancer. Skin Cancer Prevention Study Group. *JAMA* 1992; **267**: 3305-3310 [PMID: 1597912]

6 **Barksdale SK**, O'Connor N, Barnhill R. Prognostic factors for cutaneous squamous cell and basal cell carcinoma. Determinants of risk of recurrence, metastasis, and development of subsequent skin cancers. *Surg Oncol Clin N Am* 1997; **6**: 625-638 [PMID: 9210358]

7 **Hartevelt MM**, Bavinck JN, Kootte AM, Vermeer BJ, Vandenbroucke JP. Incidence of skin cancer after renal transplantation in The Netherlands. *Transplantation* 1990; **49**: 506-509 [PMID: 2316011]

8 **Wisgerhof HC**, van der Geest LG, de Fijter JW, Haasnoot GW, Claas FH, le Cessie S, Willemze R, Bouwes Bavinck JN. Incidence of cancer in kidney-transplant recipients: a long-term cohort study in a single center. *Cancer Epidemiol* 2011; **35**: 105-111 [PMID: 20674538 DOI: 10.1016/j.canep.2010.07.002]

9 **Martinez JC**, Otley CC, Stasko T, Euvrard S, Brown C, Schanbacher CF, Weaver AL. Defining the clinical course of metastatic skin cancer in organ transplant recipients: a multicenter collaborative study. *Arch Dermatol* 2003; **139**: 301-306 [PMID: 12622621]

10 **Penn I**. Skin disorders in organ transplant recipients. External anogenital lesions. *Arch Dermatol* 1997; **133**: 221-223 [PMID: 9041837]

11 **Carroll RP**, Ramsay HM, Fryer AA, Hawley CM, Nicol DL, Harden PN. Incidence and prediction of nonmelanoma skin cancer post-renal transplantation: a prospective study in Queensland, Australia. *Am J Kidney Dis* 2003; **41**: 676-683 [PMID: 12612993 DOI: 10.1053/ajkd.2003.50130]

12 **Cantwell MM**, Murray LJ, Catney D, Donnelly D, Autier P, Boniol M, Fox C, Middleton RJ, Dolan OM, Gavin AT. Second primary cancers in patients with skin cancer: a population-based study in Northern Ireland. *Br J Cancer* 2009; **100**: 174-177 [PMID: 19127269 DOI: 10.1038/sj.bjc.6604842]

13 **Wisgerhof HC**, Edelbroek JR, de Fijter JW, Haasnoot GW, Claas FH, Willemze R, Bavinck JN. Subsequent squamous- and basal-cell carcinomas in kidney-transplant recipients after the first skin cancer: cumulative incidence and risk factors. *Transplantation* 2010; **89**: 1231-1238 [PMID: 20410852 DOI: 10.1097/TP.0b013e3181d84cdc]

14 **Euvrard S**, Kanitakis J, Decullier E, Butnaru AC, Lefrançois N, Boissonnat P, Sebbag L, Garnier JL, Pouteil-Noble C, Cahen R, Morelon E, Touraine JL, Claudy A, Chapuis F. Subsequent skin cancers in kidney and heart transplant recipients after the first squamous cell carcinoma. *Transplantation* 2006; **81**: 1093-1100 [PMID: 16641592 DOI: 10.1097/01.tp.0000209921.60305.d9]

15 **Lindelöf B**, Sigurgeirsson B, Gäbel H, Stern RS. Incidence of skin cancer in 5356 patients following organ transplantation. *Br J Dermatol* 2000; **143**: 513-519 [PMID: 10971322]

16 **Johnson TM**, Rowe DE, Nelson BR, Swanson NA. Squamous cell carcinoma of the skin (excluding lip and oral mucosa). *J Am Acad Dermatol* 1992; **26**: 467-484 [PMID: 1564155]

17 **Rowe DE**, Carroll RJ, Day CL. Prognostic factors for local recurrence, metastasis, and survival rates in squamous cell carcinoma of the skin, ear, and lip. Implications for treatment modality selection. *J Am Acad Dermatol* 1992; **26**: 976-990 [PMID: 1607418]

18 **Peat B**, Insull P, Ayers R. Risk stratification for metastasis from cutaneous squamous cell carcinoma of the head and neck. *ANZ J Surg* 2012; **82**: 230-233 [PMID: 22510179 DOI: 10.1111/j.1445-2197.2011.05994.x]

19 **Brantsch KD**, Meisner C, Schönfisch B, Trilling B, Wehner-Caroli J, Röcken M, Breuninger H. Analysis of risk factors determining prognosis of cutaneous squamous-cell carcinoma: a prospective study. *Lancet Oncol* 2008; **9**: 713-720 [PMID: 18617440 DOI: 10.1016/S1470-2045(08)70178-5]

20 **Lott DG**, Manz R, Koch C, Lorenz RR. Aggressive behavior of nonmelanotic skin cancers in solid organ transplant recipients. *Transplantation* 2010; **90**: 683-687 [PMID: 20808266 DOI: 10.1097/TP.0b013e3181ec7228]

21 **Streams BN**, Eaton JS, Zelac DE. Perineural spread of squamous cell carcinoma involving the spinal accessory nerve in an immunocompromised organ transplant recipient. *Dermatol Surg* 2005; **31**: 599-601 [PMID: 15962752]

22 **Buell JF**, Hanaway MJ, Thomas M, Alloway RR, Woodle ES. Skin cancer following transplantation: the Israel Penn International Transplant Tumor Registry experience. *Transplant Proc* 2005; **37**: 962-963 [PMID: 15848591 DOI: 10.1016/j.transproceed.2004.12.062]

23 **Mackenzie KA**, Wells JE, Lynn KL, Simcock JW, Robinson BA, Roake JA, Currie MJ. First and subsequent nonmelanoma skin cancers: incidence and predictors in a population of New Zealand renal transplant recipients. *Nephrol Dial Transplant* 2010; **25**: 300-306 [PMID: 19783601 DOI: 10.1093/ndt/gfp482]

24 **Moloney FJ**, Kelly PO, Kay EW, Conlon P, Murphy GM. Maintenance versus reduction of immunosuppression in renal transplant recipients with aggressive squamous cell carcinoma. *Dermatol Surg* 2004; **30**: 674-678 [PMID: 15061854 DOI: 10.1111/j.1524-4725.2004.00155.x]

25 **O'Donovan P**, Perrett CM, Zhang X, Montaner B, Xu YZ, Harwood CA, McGregor JM, Walker SL, Hanaoka F, Karran P.

Azathioprine and UVA light generate mutagenic oxidative DNA damage. *Science* 2005; **309**: 1871-1874 [PMID: 16166520]
26 **Zhang X**, Jeffs G, Ren X, O'Donovan P, Montaner B, Perrett CM, Karran P, Xu YZ. Novel DNA lesions generated by the interaction between therapeutic thiopurines and UVA light. *DNA Repair* (Amst) 2007; **6**: 344-354 [PMID: 17188583 DOI: 10.1016/j.dnarep.2006.11.003]
27 **Gueranger Q**, Kia A, Frith D, Karran P. Crosslinking of DNA repair and replication proteins to DNA in cells treated with 6-thioguanine and UVA. *Nucleic Acids Res* 2011; **39**: 5057-5066 [PMID: 21398635 DOI: 10.1093/nar/gkr112]
28 **Ingvar A**, Smedby KE, Lindelöf B, Fernberg P, Bellocco R, Tufveson G, Höglund P, Adami J. Immunosuppressive treatment after solid organ transplantation and risk of post-transplant cutaneous squamous cell carcinoma. *Nephrol Dial Transplant* 2010; **25**: 2764-2771 [PMID: 19729465 DOI: 10.1093/ndt/gfp425]
29 **Allison AC**, Eugui EM. Mechanisms of action of mycophenolate mofetil in preventing acute and chronic allograft rejection. *Transplantation* 2005; **80**: S181-S190 [PMID: 16251851]
30 **Hofbauer GF**, Attard NR, Harwood CA, McGregor JM, Dziunycz P, Iotzova-Weiss G, Straub G, Meyer R, Kamenisch Y, Berneburg M, French LE, Wüthrich RP, Karran P, Serra AL. Reversal of UVA skin photosensitivity and DNA damage in kidney transplant recipients by replacing azathioprine. *Am J Transplant* 2012; **12**: 218-225 [PMID: 21943390 DOI: 10.1111/j.1600-6143.2011.03751.x]
31 **O'Neill JO**, Edwards LB, Taylor DO. Mycophenolate mofetil and risk of developing malignancy after orthotopic heart transplantation: analysis of the transplant registry of the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant* 2006; **25**: 1186-1191 [PMID: 17045930 DOI: 10.1016/j.healun.2006.06.010]
32 **Clayton PA**, McDonald SP, Chapman JR, Chadban SJ. Mycophenolate versus azathioprine for kidney transplantation: a 15-year follow-up of a randomized trial. *Transplantation* 2012; **94**: 152-158 [PMID: 22728292 DOI: 10.1097/TP.0b013e31825475a3]
33 **Walsh CT**, Zydowsky LD, McKeon FD. Cyclosporin A, the cyclophilin class of peptidylprolyl isomerases, and blockade of T cell signal transduction. *J Biol Chem* 1992; **267**: 13115-13118 [PMID: 1618811]
34 **Stepkowski SM**. Molecular targets for existing and novel immunosuppressive drugs. *Expert Rev Mol Med* 2000; **2**: 1-23 [PMID: 14585137 DOI: 10.1017/S1462399400001769]
35 **Lemasters JJ**, Nieminen AL, Qian T, Trost LC, Elmore SP, Nishimura Y, Crowe RA, Cascio WE, Bradham CA, Brenner DA, Herman B. The mitochondrial permeability transition in cell death: a common mechanism in necrosis, apoptosis and autophagy. *Biochim Biophys Acta* 1998; **1366**: 177-196 [PMID: 9714796]
36 **Zamzami N**, Larochette N, Kroemer G. Mitochondrial permeability transition in apoptosis and necrosis. *Cell Death Differ* 2005; **12** Suppl 2: 1478-1480 [PMID: 16247494 DOI: 10.1038/sj.cdd.4401682]
37 **Hojo M**, Morimoto T, Maluccio M, Asano T, Morimoto K, Lagman M, Shimbo T, Suthanthiran M. Cyclosporine induces cancer progression by a cell-autonomous mechanism. *Nature* 1999; **397**: 530-534 [PMID: 10028970]
38 **Guba M**, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, Bruns CJ, Zuelke C, Farkas S, Anthuber M, Jauch KW, Geissler EK. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. *Nat Med* 2002; **8**: 128-135 [PMID: 11821896 DOI: 10.1038/nm0202-128]
39 **Wu X**, Nguyen BC, Dziunycz P, Chang S, Brooks Y, Lefort K, Hofbauer GF, Dotto GP. Opposing roles for calcineurin and ATF3 in squamous skin cancer. *Nature* 2010; **465**: 368-372 [PMID: 20485437 DOI: 10.1038/nature08996]
40 **McGeown MG**, Douglas JF, Middleton D. One thousand renal transplants at Belfast City Hospital: post-graft neoplasia 1968-1999, comparing azathioprine only with cyclosporin-based regimes in a single centre. *Clin Transpl* 2000: 193-202 [PMID: 11512313]
41 **Marcén R**, Pascual J, Tato AM, Teruel JL, Villafruela JJ, Fernández M, Tenorio M, Burgos FJ, Ortuño J. Influence of immunosuppression on the prevalence of cancer after kidney transplantation. *Transplant Proc* 2003; **35**: 1714-1716 [PMID: 12962768]
42 **Abou Ayache R**, Thierry A, Bridoux F, Bauwens M, Belmouaz M, Desport E, Touchard G. Long-term maintenance of calcineurin inhibitor monotherapy reduces the risk for squamous cell carcinomas after kidney transplantation compared with bi- or tritherapy. *Transplant Proc* 2007; **39**: 2592-2594 [PMID: 17954185 DOI: 10.1016/j.transproceed.2007.08.016]
43 **Giese T**, Sommerer C, Zeier M, Meuer S. Monitoring immunosuppression with measures of NFAT decreases cancer incidence. *Clin Immunol* 2009; **132**: 305-311 [PMID: 19398376 DOI: 10.1016/j.clim.2009.03.520]
44 **Wimmer CD**, Angele MK, Schwarz B, Pratschke S, Rentsch M, Khandoga A, Guba M, Jauch KW, Bruns C, Graeb C. Impact of cyclosporine versus tacrolimus on the incidence of de novo malignancy following liver transplantation: a single center experience with 609 patients. *Transpl Int* 2013; **26**: 999-1006 [PMID: 23952102 DOI: 10.1111/tri.12165]
45 **Opelz G**, Döhler B. Lymphomas after solid organ transplantation: a collaborative transplant study report. *Am J Transplant* 2004; **4**: 222-230 [PMID: 14974943]
46 **Mayer AD**, Dmitrewski J, Squifflet JP, Besse T, Grabensee B, Klein B, Eigler FW, Heemann U, Pichlmayr R, Behrend M, Vanrenterghem Y, Donck J, van Hooff J, Christiaans M, Morales JM, Andres A, Johnson RW, Short C, Buchholz B, Rehmert N, Land W, Schleibner S, Forsythe JL, Talbot D, Pohanka E. Multicenter randomized trial comparing tacrolimus (FK506) and cyclosporine in the prevention of renal allograft rejection: a report of the European Tacrolimus Multicenter Renal Study Group. *Transplantation* 1997; **64**: 436-443 [PMID: 9275110]
47 **Pirsch JD**, Miller J, Deierhoi MH, Vincenti F, Filo RS. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression after cadaveric renal transplantation. FK506 Kidney Transplant Study Group. *Transplantation* 1997; **63**: 977-983 [PMID: 9112351]
48 **Wiesner RH**. A long-term comparison of tacrolimus (FK506) versus cyclosporine in liver transplantation: a report of the United States FK506 Study Group. *Transplantation* 1998; **66**: 493-499 [PMID: 9734494]
49 **Kleinert H**, Euchenhofer C, Ihrig-Biedert I, Förstermann U. Glucocorticoids inhibit the induction of nitric oxide synthase II by down-regulating cytokine-induced activity of transcription factor nuclear factor-kappa B. *Mol Pharmacol* 1996; **49**: 15-21 [PMID: 8569701]
50 **Lee SW**, Tsou AP, Chan H, Thomas J, Petrie K, Eugui EM, Allison AC. Glucocorticoids selectively inhibit the transcription of the interleukin 1 beta gene and decrease the stability of interleukin 1 beta mRNA. *Proc Natl Acad Sci USA* 1988; **85**: 1204-1208 [PMID: 3257575]
51 **Almawi WY**, Hess DA, Rieder MJ. Multiplicity of glucocorticoid action in inhibiting allograft rejection. *Cell Transplant* 1998; **7**: 511-523 [PMID: 9853580]
52 **Zanker B**, Walz G, Wieder KJ, Strom TB. Evidence that glucocorticosteroids block expression of the human interleukin-6 gene by accessory cells. *Transplantation* 1990; **49**: 183-185 [PMID: 2301010]
53 **Arya SK**, Wong-Staal F, Gallo RC. Dexamethasone-mediated inhibition of human T cell growth factor and gamma-interferon messenger RNA. *J Immunol* 1984; **133**: 273-276 [PMID: 6427338]
54 **Vacca A**, Felli MP, Farina AR, Martinotti S, Maroder M, Screpanti I, Meco D, Petrangeli E, Frati L, Gulino A. Glucocorticoid receptor-mediated suppression of the interleukin 2 gene expression through impairment of the cooperativity between nuclear factor of activated T cells and AP-1 enhancer elements. *J Exp Med* 1992; **175**: 637-646 [PMID: 1740658]
55 **McFarland HF**. Complexities in the treatment of autoimmune disease. *Science* 1996; **274**: 2037-2038 [PMID: 8984662]
56 **Cai J**, Zheng T, Lotz M, Zhang Y, Masood R, Gill P. Glucocorticoids induce Kaposi's sarcoma cell proliferation through the regulation of transforming growth factor-beta. *Blood* 1997; **89**: 1491-1500 [PMID: 9057628]

57 **Trattner A**, Hodak E, David M, Sandbank M. The appearance of Kaposi sarcoma during corticosteroid therapy. *Cancer* 1993; **72**: 1779-1783 [PMID: 8348508]

58 **Bansbach CC**, Wancio D, Sehgal SN. Sirolimus (rapamycin) inhibits mitogen-induced stimulation of protein synthesis in primary lymphocytes. *Inflamm Res* 1995; **44** Suppl 2: S179-S180 [PMID: 8548386]

59 **Luan FL**, Ding R, Sharma VK, Chon WJ, Lagman M, Suthanthiran M. Rapamycin is an effective inhibitor of human renal cancer metastasis. *Kidney Int* 2003; **63**: 917-926 [PMID: 12631072 DOI: 10.1046/j.1523-1755.2003.00805.x]

60 **Hudes GR**. Targeting mTOR in renal cell carcinoma. *Cancer* 2009; **115**: 2313-2320 [PMID: 19402072]

61 **Calvo E**, Escudier B, Motzer RJ, Oudard S, Hutson TE, Porta C, Bracarda S, Grünwald V, Thompson JA, Ravaud A, Kim D, Panneerselvam A, Anak O, Figlin RA. Everolimus in metastatic renal cell carcinoma: Subgroup analysis of patients with 1 or 2 previous vascular endothelial growth factor receptor-tyrosine kinase inhibitor therapies enrolled in the phase III RECORD-1 study. *Eur J Cancer* 2012; **48**: 333-339 [PMID: 22209391 DOI: 10.1016/j.ejca.2011.11.027]

62 **van den Eertwegh AJ**, Karakiewicz P, Bavbek S, Rha SY, Bracarda S, Bahl A, Ou YC, Kim D, Panneerselvam A, Anak O, Grünwald V. Safety of everolimus by treatment duration in patients with advanced renal cell cancer in an expanded access program. *Urology* 2013; **81**: 143-149 [PMID: 23273080 DOI: 10.1016/j.urology.2012.09.019]

63 **Stallone G**, Schena A, Infante B, Di Paolo S, Loverre A, Maggio G, Ranieri E, Gesualdo L, Schena FP, Grandaliano G. Sirolimus for Kaposi's sarcoma in renal-transplant recipients. *N Engl J Med* 2005; **352**: 1317-1323 [PMID: 15800227 DOI: 10.1056/NEJMoa042831]

64 **Alberú J**, Pascoe MD, Campistol JM, Schena FP, Rial Mdel C, Polinsky M, Neylan JF, Korth-Bradley J, Goldberg-Alberts R, Maller ES. Lower malignancy rates in renal allograft recipients converted to sirolimus-based, calcineurin inhibitor-free immunotherapy: 24-month results from the CONVERT trial. *Transplantation* 2011; **92**: 303-310 [PMID: 21792049 DOI: 10.1097/TP.0b013e3182247ae2]

65 **Campistol JM**, Eris J, Oberbauer R, Friend P, Hutchison B, Morales JM, Claesson K, Stallone G, Russ G, Rostaing L, Kreis H, Burke JT, Brault Y, Scarola JA, Neylan JF. Sirolimus therapy after early cyclosporine withdrawal reduces the risk for cancer in adult renal transplantation. *J Am Soc Nephrol* 2006; **17**: 581-589 [PMID: 16434506 DOI: 10.1681/ASN.2005090993]

66 **Mathew T**, Kreis H, Friend P. Two-year incidence of malignancy in sirolimus-treated renal transplant recipients: results from five multicenter studies. *Clin Transplant* 2004; **18**: 446-449 [PMID: 15233824 DOI: 10.1111/j.1399-0012.2004.00188.x]

67 **Cibrik D**, Silva HT, Vathsala A, Lackova E, Cornu-Artis C, Walker RG, Wang Z, Zibari GB, Shihab F, Kim YS. Randomized trial of everolimus-facilitated calcineurin inhibitor minimization over 24 months in renal transplantation. *Transplantation* 2013; **95**: 933-942 [PMID: 23422495 DOI: 10.1097/TP.0b013e3182848e03]

68 **Mjörnstedt L**, Sørensen SS, von Zur Mühlen B, Jespersen B, Hansen JM, Bistrup C, Andersson H, Gustafsson B, Undset LH, Fagertun H, Solbu D, Holdaas H. Improved renal function after early conversion from a calcineurin inhibitor to everolimus: a randomized trial in kidney transplantation. *Am J Transplant* 2012; **12**: 2744-2753 [PMID: 22812414 DOI: 10.1111/j.1600-6143.2012.04162.x]

69 **Gurkan S**, Luan Y, Dhillon N, Allam SR, Montague T, Bromberg JS, Ames S, Lerner S, Ebcioglu Z, Nair V, Dinavahi R, Sehgal V, Heeger P, Schroppel B, Murphy B. Immune reconstitution following rabbit antithymocyte globulin. *Am J Transplant* 2010; **10**: 2132-2141 [PMID: 20883548 DOI: 10.1111/j.1600-6143.2010.03210.x]

70 **Ducloux D**, Bamoulid J, Courivaud C, Gaugler B, Rebibou JM, Ferrand C, Chalopin JM, Borg C, Tiberghien P, Saas P. Thymic function, anti-thymocytes globulins, and cancer after renal transplantation. *Transpl Immunol* 2011; **25**: 56-60 [PMID: 21620972 DOI: 10.1016/j.trim.2011.05.003]

71 **Vajdic CM**, McDonald SP, McCredie MR, van Leeuwen MT, Stewart JH, Law M, Chapman JR, Webster AC, Kaldor JM, Grulich AE. Cancer incidence before and after kidney transplantation. *JAMA* 2006; **296**: 2823-2831 [PMID: 17179459 DOI: 10.1001/jama.296.23.2823]

72 **Dantal J**, Hourmant M, Cantarovich D, Giral M, Blancho G, Dreno B, Soulillou JP. Effect of long-term immunosuppression in kidney-graft recipients on cancer incidence: randomised comparison of two cyclosporin regimens. *Lancet* 1998; **351**: 623-628 [PMID: 9500317 DOI: 10.1016/S0140-6736(97)08496-1]

73 **Ramsay HM**, Fryer AA, Hawley CM, Smith AG, Harden PN. Non-melanoma skin cancer risk in the Queensland renal transplant population. *Br J Dermatol* 2002; **147**: 950-956 [PMID: 12410706]

74 **Kessler M**, Jay N, Molle R, Guillemin F. Excess risk of cancer in renal transplant patients. *Transpl Int* 2006; **19**: 908-914 [PMID: 17018126 DOI: 10.1111/j.1432-2277.2006.00383.x]

75 **Ramsay HM**, Fryer AA, Reece S, Smith AG, Harden PN. Clinical risk factors associated with nonmelanoma skin cancer in renal transplant recipients. *Am J Kidney Dis* 2000; **36**: 167-176 [PMID: 10873887 DOI: 10.1053/ajkd.2000.8290]

76 **Urwin HR**, Jones PW, Harden PN, Ramsay HM, Hawley CM, Nicol DL, Fryer AA. Predicting risk of nonmelanoma skin cancer and premalignant skin lesions in renal transplant recipients. *Transplantation* 2009; **87**: 1667-1671 [PMID: 19502958 DOI: 10.1097/TP.0b013e3181a5ce2e]

77 **Ramsay HM**, Fryer AA, Hawley CM, Smith AG, Nicol DL, Harden PN. Factors associated with nonmelanoma skin cancer following renal transplantation in Queensland, Australia. *J Am Acad Dermatol* 2003; **49**: 397-406 [PMID: 12963901]

78 **Kim JK**, Patel D, Choi BS. Contrasting structural impacts induced by cis-syn cyclobutane dimer and (6-4) adduct in DNA duplex decamers: implication in mutagenesis and repair activity. *Photochem Photobiol* 1995; **62**: 44-50 [PMID: 7638271]

79 **Donahue BA**, Yin S, Taylor JS, Reines D, Hanawalt PC. Transcript cleavage by RNA polymerase II arrested by a cyclobutane pyrimidine dimer in the DNA template. *Proc Natl Acad Sci USA* 1994; **91**: 8502-8506 [PMID: 8078911]

80 **Brash DE**, Rudolph JA, Simon JA, Lin A, McKenna GJ, Baden HP, Halperin AJ, Pontén J. A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proc Natl Acad Sci USA* 1991; **88**: 10124-10128 [PMID: 1946433]

81 **Marks R**, Jolley D, Lectsas S, Foley P. The role of childhood exposure to sunlight in the development of solar keratoses and non-melanocytic skin cancer. *Med J Aust* 1990; **152**: 62-66 [PMID: 2296232]

82 **el-Ghorr AA**, Norval M. A monoclonal antibody to cis-urocanic acid prevents the ultraviolet-induced changes in Langerhans cells and delayed hypersensitivity responses in mice, although not preventing dendritic cell accumulation in lymph nodes draining the site of irradiation and contact hypersensitivity responses. *J Invest Dermatol* 1995; **105**: 264-268 [PMID: 7636311]

83 **Rivas JM**, Ullrich SE. The role of IL-4, IL-10, and TNF-alpha in the immune suppression induced by ultraviolet radiation. *J Leukoc Biol* 1994; **56**: 769-775 [PMID: 7996051]

84 **Chung HT**, Burnham DK, Robertson B, Roberts LK, Daynes RA. Involvement of prostaglandins in the immune alterations caused by the exposure of mice to ultraviolet radiation. *J Immunol* 1986; **137**: 2478-2484 [PMID: 3463622]

85 **Gurish MF**, Lynch DH, Daynes RA. Changes in antigen-presenting cell function in the spleen and lymph nodes of ultraviolet-irradiated mice. *Transplantation* 1982; **33**: 280-284 [PMID: 6977903]

86 **Ullrich SE**. Modulation of immunity by ultraviolet radiation: key effects on antigen presentation. *J Invest Dermatol* 1995; **105**: 30S-36S [PMID: 7615994]

87 **Schwarz A**, Maeda A, Wild MK, Kernebeck K, Gross N, Aragane Y, Beissert S, Vestweber D, Schwarz T. Ultraviolet radiation-induced regulatory T cells not only inhibit the induction but can suppress the effector phase of contact hypersensitivity. *J Immunol* 2004; **172**: 1036-1043 [PMID: 14707077]

88 **Schwarz A**, Navid F, Sparwasser T, Clausen BE, Schwarz T. In vivo reprogramming of UV radiation-induced regulatory T-cell migration

to inhibit the elicitation of contact hypersensitivity. *J Allergy Clin Immunol* 2011; **128**: 826-833 [PMID: 21762977 DOI: 10.1016/j.jaci.2011.06.005]

89 **Simon JC**, Hara H, Denfeld RW, Martin S. UVB-irradiated dendritic cells induce nonproliferating, regulatory type T cells. *Skin Pharmacol Appl Skin Physiol* 2002; **15**: 330-334 [PMID: 12239427]

90 **Schwarz A**, Beissert S, Grosse-Heitmeyer K, Gunzer M, Bluestone JA, Grabbe S, Schwarz T. Evidence for functional relevance of CTLA-4 in ultraviolet-radiation-induced tolerance. *J Immunol* 2000; **165**: 1824-1831 [PMID: 10925260]

91 **Moodycliffe AM**, Nghiem D, Clydesdale G, Ullrich SE. Immune suppression and skin cancer development: regulation by NKT cells. *Nat Immunol* 2000; **1**: 521-525 [PMID: 11101875 DOI: 10.1038/82782]

92 **Schwarz A**, Maeda A, Kernebeck K, van Steeg H, Beissert S, Schwarz T. Prevention of UV radiation-induced immunosuppression by IL-12 is dependent on DNA repair. *J Exp Med* 2005; **201**: 173-179 [PMID: 15657287 DOI: 10.1084/jem.20041212]

93 **Ando O**, Suemoto Y, Kurimoto M, Horikawa T, Ichihashi M. Deficient Th1-type immune responses via impaired CD28 signaling in ultraviolet B-induced systemic immunosuppression and the restorative effect of IL-12. *J Dermatol Sci* 2000; **24**: 190-202 [PMID: 11084301]

94 **Suemoto Y**, Ando O, Kurimoto M, Horikawa T, Ichihashi M. IL-12 promotes the accessory cell function of epidermal Langerhans cells. *J Dermatol Sci* 1998; **18**: 98-108 [PMID: 9833976]

95 **Parkin DM**. The global health burden of infection-associated cancers in the year 2002. *Int J Cancer* 2006; **118**: 3030-3044 [PMID: 16404738 DOI: 10.1002/ijc.21731]

96 **Bouwes Bavinck JN**, Feltkamp M, Struijk L, ter Schegget J. Human papillomavirus infection and skin cancer risk in organ transplant recipients. *J Investig Dermatol Symp Proc* 2001; **6**: 207-211 [PMID: 11924829 DOI: 10.1046/j.0022-202x.2001.00048.x]

97 **Storey A**, Thomas M, Kalita A, Harwood C, Gardiol D, Mantovani F, Breuer J, Leigh IM, Matlashewski G, Banks L. Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. *Nature* 1998; **393**: 229-234 [PMID: 9607760 DOI: 10.1038/30400]

98 **Harwood CA**, Surentheran T, McGregor JM, Spink PJ, Leigh IM, Breuer J, Proby CM. Human papillomavirus infection and non-melanoma skin cancer in immunosuppressed and immunocompetent individuals. *J Med Virol* 2000; **61**: 289-297 [PMID: 10861635]

99 **van Zanten J**, de Leij L, Prop J, Harmsen MC, The TH. Human cytomegalovirus: a viral complication in transplantation. *Clin Transplant* 1998; **12**: 145-158 [PMID: 9642503]

100 **Baumforth KR**, Birgersdotter A, Reynolds GM, Wei W, Kapatai G, Flavell JR, Kalk E, Piper K, Lee S, Machado L, Hadley K, Sundblad A, Sjoberg J, Bjorkholm M, Porwit AA, Yap LF, Teo S, Grundy RG, Young LS, Ernberg I, Woodman CB, Murray PG. Expression of the Epstein-Barr virus-encoded Epstein-Barr virus nuclear antigen 1 in Hodgkin's lymphoma cells mediates Up-regulation of CCL20 and the migration of regulatory T cells. *Am J Pathol* 2008; **173**: 195-204 [PMID: 18502823 DOI: 10.2353/ajpath.2008.070845]

101 **Couzi L**, Levaillant Y, Jamai A, Pitard V, Lassalle R, Martin K, Garrigue I, Hawchar O, Siberchicot F, Moore N, Moreau JF, Dechanet-Merville J, Merville P. Cytomegalovirus-induced gammadelta T cells associate with reduced cancer risk after kidney transplantation. *J Am Soc Nephrol* 2010; **21**: 181-188 [PMID: 19713314 DOI: 10.1681/ASN.2008101072]

102 **Ducloux D**, Carron PL, Rebibou JM, Aubin F, Fournier V, Bresson-Vautrin C, Blanc D, Humbert P, Chalopin JM. CD4 lymphocytopenia as a risk factor for skin cancers in renal transplant recipients. *Transplantation* 1998; **65**: 1270-1272 [PMID: 9603180]

103 **Thibaudin D**, Alamartine E, Mariat C, Absi L, Berthoux F. Long-term kinetic of T-lymphocyte subsets in kidney-transplant recipients: influence of anti-T-cell antibodies and association with posttransplant malignancies. *Transplantation* 2005; **80**: 1514-1517 [PMID: 16340799]

104 **Ducloux D**, Carron PL, Motte G, Ab A, Rebibou JM, Bresson-Vautrin C, Tiberghien P, Saint-Hillier Y, Chalopin JM. Lymphocyte subsets and assessment of cancer risk in renal transplant recipients. *Transpl Int* 2002; **15**: 393-396 [PMID: 12221457 DOI: 10.1007/s00147-002-0410-4]

105 **Carroll RP**, Segundo DS, Hollowood K, Marafioti T, Clark TG, Harden PN, Wood KJ. Immune phenotype predicts risk for posttransplantation squamous cell carcinoma. *J Am Soc Nephrol* 2010; **21**: 713-722 [PMID: 20110382 DOI: 10.1681/ASN.2009060669]

106 **Filaci G**, Fenoglio D, Fravega M, Ansaldo G, Borgonovo G, Traverso P, Villaggio B, Ferrera A, Kunkl A, Rizzi M, Ferrera F, Balestra P, Ghio M, Contini P, Setti M, Olive D, Azzarone B, Carmignani G, Ravetti JL, Torre G, Indiveri F. CD8+ CD28- T regulatory lymphocytes inhibiting T cell proliferative and cytotoxic functions infiltrate human cancers. *J Immunol* 2007; **179**: 4323-4334 [PMID: 17878327]

107 **Puig-Pey I**, Bohne F, Benítez C, López M, Martínez-Llordella M, Oppenheimer F, Lozano JJ, González-Abraldes J, Tisone G, Rimola A, Sánchez-Fueyo A. Characterization of γδ T cell subsets in organ transplantation. *Transpl Int* 2010; **23**: 1045-1055 [PMID: 20477999 DOI: 10.1111/j.1432-2277.2010.01095.x]

108 **Hope CM**, Grace BS, Pilkington KR, Coates PT, Bergmann IP, Carroll RP. The immune phenotype may relate to cancer development in kidney transplant recipients. *Kidney Int* 2014; **86**: 175-183 [PMID: 24429406 DOI: 10.1038/ki.2013.538]

109 **Gershon RK**, Cohen P, Hencin R, Liebhaber SA. Suppressor T cells. *J Immunol* 1972; **108**: 586-590 [PMID: 4401006]

110 **Kronenberg M**, Steinmetz M, Kobori J, Kraig E, Kapp JA, Pierce CW, Sorensen CM, Suzuki G, Tada T, Hood L. RNA transcripts for I-J polypeptides are apparently not encoded between the I-A and I-E subregions of the murine major histocompatibility complex. *Proc Natl Acad Sci USA* 1983; **80**: 5704-5708 [PMID: 6193520]

111 **Green DR**, Webb DR. Saying the 'S' word in public. *Immunol Today* 1993; **14**: 523-525 [PMID: 8274193 DOI: 10.1016/0167-5699(93)90180-S]

112 **Sakaguchi S**, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995; **155**: 1151-1164 [PMID: 7636184]

113 **Baecher-Allan C**, Brown JA, Freeman GJ, Hafler DA. CD4+-CD25high regulatory cells in human peripheral blood. *J Immunol* 2001; **167**: 1245-1253 [PMID: 11466340]

114 **Liu W**, Putnam AL, Xu-Yu Z, Szot GL, Lee MR, Zhu S, Gottlieb PA, Kapranov P, Gingeras TR, Fazekas de St Groth B, Clayberger C, Soper DM, Ziegler SF, Bluestone JA. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. *J Exp Med* 2006; **203**: 1701-1711 [PMID: 16818678 DOI: 10.1084/jem.20060772]

115 **O'Garra A**, Vieira P. Regulatory T cells and mechanisms of immune system control. *Nat Med* 2004; **10**: 801-805 [PMID: 15286781 DOI: 10.1038/nm0804-801]

116 **Zhang L**, Zhao Y. The regulation of Foxp3 expression in regulatory CD4(+)CD25(+)T cells: multiple pathways on the road. *J Cell Physiol* 2007; **211**: 590-597 [PMID: 17311282 DOI: 10.1002/jcp.21001]

117 **Hori S**, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003; **299**: 1057-1061 [PMID: 12522256 DOI: 10.1126/science.1079490]

118 **Gambineri E**, Torgerson TR, Ochs HD. Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. *Curr Opin Rheumatol* 2003; **15**: 430-435 [PMID: 12819471]

119 **Chatila TA**, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C, Bowcock AM. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. *J Clin Invest* 2000; **106**: R75-R81 [PMID: 11120765 DOI: 10.1172/JCI11679]

120 **Wildin RS**, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N, Levy-Lahad E, Mazzella M, Goulet O, Perroni L, Bricarelli FD,

Byrne G, McEuen M, Proll S, Appleby M, Brunkow ME. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat Genet* 2001; **27**: 18-20 [PMID: 11137992 DOI: 10.1038/83707]

121 **Valmori D**, Merlo A, Souleimanian NE, Hesdorffer CS, Ayyoub M. A peripheral circulating compartment of natural naive CD4 Tregs. *J Clin Invest* 2005; **115**: 1953-1962 [PMID: 16007258 DOI: 10.1172/JCI23963]

122 **Schena F**, Volpi S, Faliti CE, Penco F, Santi S, Proietti M, Schenk U, Damonte G, Salis A, Bellotti M, Fais F, Tenca C, Gattorno M, Eibel H, Rizzi M, Warnatz K, Idzko M, Ayata CK, Rakhmanov M, Galli T, Martini A, Canossa M, Grassi F, Traggiai E. Dependence of immunoglobulin class switch recombination in B cells on vesicular release of ATP and CD73 ectonucleotidase activity. *Cell Rep* 2013; **3**: 1824-1831 [PMID: 23770243 DOI: 10.1016/j.celrep.2013.05.022]

123 **Zabransky DJ**, Nirschl CJ, Durham NM, Park BV, Ceccato CM, Bruno TC, Tam AJ, Getnet D, Drake CG. Phenotypic and functional properties of Helios+ regulatory T cells. *PLoS One* 2012; **7**: e34547 [PMID: 22479644 DOI: 10.1371/journal.pone.0034547]

124 **Huang CT**, Workman CJ, Flies D, Pan X, Marson AL, Zhou G, Hipkiss EL, Ravi S, Kowalski J, Levitsky HI, Powell JD, Pardoll DM, Drake CG, Vignali DA. Role of LAG-3 in regulatory T cells. *Immunity* 2004; **21**: 503-513 [PMID: 15485628 DOI: 10.1016/j.immuni.2004.08.010]

125 **Mantel PY**, Ouaked N, Rückert B, Karagiannidis C, Welz R, Blaser K, Schmidt-Weber CB. Molecular mechanisms underlying FOXP3 induction in human T cells. *J Immunol* 2006; **176**: 3593-3602 [PMID: 16517728]

126 **Sakaguchi S**, Setoguchi R, Yagi H, Nomura T. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in self-tolerance and autoimmune disease. *Curr Top Microbiol Immunol* 2006; **305**: 51-66 [PMID: 16724800]

127 **Cederbom L**, Hall H, Ivars F. CD4+CD25+ regulatory T cells down-regulate co-stimulatory molecules on antigen-presenting cells. *Eur J Immunol* 2000; **30**: 1538-1543 [PMID: 10898488 DOI: 10.1002/1521-4141(200006)30]

128 **Jung ID**, Jeong YI, Lee CM, Noh KT, Jeong SK, Chun SH, Choi OH, Park WS, Han J, Shin YK, Kim HW, Yun CH, Park YM. COX-2 and PGE2 signaling is essential for the regulation of IDO expression by curcumin in murine bone marrow-derived dendritic cells. *Int Immunopharmacol* 2010; **10**: 760-768 [PMID: 20399909 DOI: 10.1016/j.intimp.2010.04.006]

129 **Mandapathil M**, Hilldorfer B, Szczepanski MJ, Czystowska M, Szajnik M, Ren J, Lang S, Jackson EK, Gorelik E, Whiteside TL. Generation and accumulation of immunosuppressive adenosine by human CD4+CD25highFOXP3+ regulatory T cells. *J Biol Chem* 2010; **285**: 7176-7186 [PMID: 19858205 DOI: 10.1074/jbc.M109.047423]

130 **Ohta A**, Sitkovsky M. Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. *Nature* 2001; **414**: 916-920 [PMID: 11780065 DOI: 10.1038/414916a]

131 **Raskovalova T**, Huang X, Sitkovsky M, Zacharia LC, Jackson EK, Gorelik E. Gs protein-coupled adenosine receptor signaling and lytic function of activated NK cells. *J Immunol* 2005; **175**: 4383-4391 [PMID: 16177079]

132 **Fletcher JM**, Lonergan R, Costelloe L, Kinsella K, Moran B, O' Farrelly C, Tubridy N, Mills KH. CD39+Foxp3+ regulatory T Cells suppress pathogenic Th17 cells and are impaired in multiple sclerosis. *J Immunol* 2009; **183**: 7602-7610 [PMID: 19917691 DOI: 10.4049/jimmunol.0901881]

133 **Mandapathil M**, Szczepanski MJ, Szajnik M, Ren J, Jackson EK, Johnson JT, Gorelik E, Lang S, Whiteside TL. Adenosine and prostaglandin E2 cooperate in the suppression of immune responses mediated by adaptive regulatory T cells. *J Biol Chem* 2010; **285**: 27571-27580 [PMID: 20558731 DOI: 10.1074/jbc.M110.127100]

134 **Sitkovsky M**, Lukashev D, Deaglio S, Dwyer K, Robson SC, Ohta A. Adenosine A2A receptor antagonists: blockade of adenosinergic effects and T regulatory cells. *Br J Pharmacol* 2008; **153** Suppl 1: S457-S464 [PMID: 18311159 DOI: 10.1038/bjp.2008.23]

135 **Marshall NA**, Vickers MA, Barker RN. Regulatory T cells secreting IL-10 dominate the immune response to EBV latent membrane protein 1. *J Immunol* 2003; **170**: 6183-6189 [PMID: 12794149]

136 **Lund JM**, Hsing L, Pham TT, Rudensky AY. Coordination of early protective immunity to viral infection by regulatory T cells. *Science* 2008; **320**: 1220-1224 [PMID: 18436744 DOI: 10.1126/science.1155209]

137 **Louis S**, Braudeau C, Giral M, Dupont A, Moizant F, Robillard N, Moreau A, Soulillou JP, Brouard S. Contrasting CD25hiCD4+T cells/FOXP3 patterns in chronic rejection and operational drug-free tolerance. *Transplantation* 2006; **81**: 398-407 [PMID: 16477227 DOI: 10.1097/01.tp.0000203166.44968.86]

138 **Moraes-Vieira PM**, Silva HM, Takenaka MC, Monteiro SM, Lemos F, Saitovitch D, Kalil J, Coelho V. Differential monocyte STAT6 activation and CD4(+)CD25(+)Foxp3(+) T cells in kidney operational tolerance transplanted individuals. *Hum Immunol* 2010; **71**: 442-450 [PMID: 20122976 DOI: 10.1016/j.humimm.2010.01.022]

139 **Pons JA**, Revilla-Nuin B, Baroja-Mazo A, Ramírez P, Martínez-Alarcón L, Sánchez-Bueno F, Robles R, Rios A, Aparicio P, Parrilla P. FoxP3 in peripheral blood is associated with operational tolerance in liver transplant patients during immunosuppression withdrawal. *Transplantation* 2008; **86**: 1370-1378 [PMID: 19034005 DOI: 10.1097/TP.0b013e318188d3e6]

140 **Baan CC**, Velthuis JH, van Gurp EA, Mol WM, Klepper M, Ijzermans JN, Weimar W. Functional CD25(bright+) alloresponsive T cells in fully immunosuppressed renal allograft recipients. *Clin Transplant* 2007; **21**: 63-71 [PMID: 17302593]

141 **Yoshizawa A**, Ito A, Li Y, Koshiba T, Sakaguchi S, Wood KJ, Tanaka K. The roles of CD25+CD4+ regulatory T cells in operational tolerance after living donor liver transplantation. *Transplant Proc* 2005; **37**: 37-39 [PMID: 15808539]

142 **Dwyer KM**, Hanidziar D, Putheti P, Hill PA, Pommey S, McRae JL, Winterhalter A, Doherty G, Deaglio S, Koulmanda M, Gao W, Robson SC, Strom TB. Expression of CD39 by human peripheral blood CD4+ CD25+ T cells denotes a regulatory memory phenotype. *Am J Transplant* 2010; **10**: 2410-2420 [PMID: 20977632 DOI: 10.1111/j.1600-6143.2010.03291.x]

143 **Roederer M**. Spectral compensation for flow cytometry: visualization artifacts, limitations, and caveats. *Cytometry* 2001; **45**: 194-205 [PMID: 11746088]

144 **Strauss L**, Bergmann C, Gooding W, Johnson JT, Whiteside TL. The frequency and suppressor function of CD4+CD25highFoxp3+ T cells in the circulation of patients with squamous cell carcinoma of the head and neck. *Clin Cancer Res* 2007; **13**: 6301-6311 [PMID: 17975141 DOI: 10.1158/1078-0432.CCR-07-1403]

145 **Bergmann C**, Strauss L, Zeidler R, Lang S, Whiteside TL. Expansion of human T regulatory type 1 cells in the microenvironment of cyclooxygenase 2 overexpressing head and neck squamous cell carcinoma. *Cancer Res* 2007; **67**: 8865-8873 [PMID: 17875728 DOI: 10.1158/0008-5472.CAN-07-0767]

146 **Bates GJ**, Fox SB, Han C, Leek RD, Garcia JF, Harris AL, Banham AH. Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse. *J Clin Oncol* 2006; **24**: 5373-5380 [PMID: 17135638 DOI: 10.1200/JCO.2006.05.9584]

147 **Ling KL**, Pratap SE, Bates GJ, Singh B, Mortensen NJ, George BD, Warren BF, Piris J, Roncador G, Fox SB, Banham AH, Cerundolo V. Increased frequency of regulatory T cells in peripheral blood and tumour infiltrating lymphocytes in colorectal cancer patients. *Cancer Immun* 2007; **7**: 7 [PMID: 17388261]

148 **Fox SB**, Launchbury R, Bates GJ, Han C, Shaida N, Malone PR, Harris AL, Banham AH. The number of regulatory T cells in prostate cancer is associated with the androgen receptor and hypoxia-inducible factor (HIF)-2alpha but not HIF-1alpha. *Prostate* 2007; **67**: 623-629 [PMID: 17328069 DOI: 10.1002/pros.20538]

149 **Stagg J**, Divisekera U, McLaughlin N, Sharkey J, Pommey S, Denoyer D, Dwyer KM, Smyth MJ. Anti-CD73 antibody therapy inhibits breast tumor growth and metastasis. *Proc Natl Acad Sci USA* 2010; **107**:

1547-1552 [PMID: 20080644 DOI: 10.1073/pnas.0908801107]
150 **Denkert C**, Winzer KJ, Hauptmann S. Prognostic impact of cyclooxygenase-2 in breast cancer. *Clin Breast Cancer* 2004; **4**: 428-433 [PMID: 15023244]
151 **Mrena J**, Wiksten JP, Thiel A, Kokkola A, Pohjola L, Lundin J, Nordling S, Ristimäki A, Haglund C. Cyclooxygenase-2 is an independent prognostic factor in gastric cancer and its expression is regulated by the messenger RNA stability factor HuR. *Clin Cancer Res* 2005; **11**: 7362-7368 [PMID: 16243808 DOI: 10.1158/1078-0432.CCR-05-0764]
152 **Biron CA**, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu Rev Immunol* 1999; **17**: 189-220 [PMID: 10358757 DOI: 10.1146/annurev.immunol.17.1.189]
153 **Kärre K**, Ljunggren HG, Piontek G, Kiessling R. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. *Nature* 1986; **319**: 675-678 [PMID: 3951539 DOI: 10.1038/319675a0]
154 **Algarra I**, Cabrera T, Garrido F. The HLA crossroad in tumor immunology. *Hum Immunol* 2000; **61**: 65-73 [PMID: 10658979]
155 **Trapani JA**, Smyth MJ. Functional significance of the perforin/granzyme cell death pathway. *Nat Rev Immunol* 2002; **2**: 735-747 [PMID: 12360212 DOI: 10.1038/nri911]
156 **Wolf AM**, Wolf D, Steurer M, Gastl G, Gunsilius E, Grubeck-Loebenstein B. Increase of regulatory T cells in the peripheral blood of cancer patients. *Clin Cancer Res* 2003; **9**: 606-612 [PMID: 12576425]
157 **Smyth MJ**, Teng MW, Swann J, Kyparissoudis K, Godfrey DI, Hayakawa Y. CD4+CD25+ T regulatory cells suppress NK cell-mediated immunotherapy of cancer. *J Immunol* 2006; **176**: 1582-1587 [PMID: 16424187]
158 **Trzonkowski P**, Szmit E, Myśliwska J, Dobyszuk A, Myśliwski A. CD4+CD25+ T regulatory cells inhibit cytotoxic activity of T CD8+ and NK lymphocytes in the direct cell-to-cell interaction. *Clin Immunol* 2004; **112**: 258-267 [PMID: 15308119 DOI: 10.1016/j.clim.2004.04.003]
159 **Friese MA**, Wischhusen J, Wick W, Weiler M, Eisele G, Steinle A, Weller M. RNA interference targeting transforming growth factor-beta enhances NKG2D-mediated antiglioma immune response, inhibits glioma cell migration and invasiveness, and abrogates tumorigenicity in vivo. *Cancer Res* 2004; **64**: 7596-7603 [PMID: 15492287 DOI: 10.1158/0008-5472.CAN-04-1627]
160 **Yoon SJ**, Heo DS, Kang SH, Lee KH, Kim WS, Kim GP, Lee JA, Lee KS, Bang YJ, Kim NK. Natural killer cell activity depression in peripheral blood and ascites from gastric cancer patients with high TGF-beta 1 expression. *Anticancer Res* 1998; **18**: 1591-1596 [PMID: 9673375]
161 **Lee JC**, Lee KM, Kim DW, Heo DS. Elevated TGF-beta1 secretion and down-modulation of NKG2D underlies impaired NK cytotoxicity in cancer patients. *J Immunol* 2004; **172**: 7335-7340 [PMID: 15187109]
162 **Terabe M**, Swann J, Ambrosino E, Sinha P, Takaku S, Hayakawa Y, Godfrey DI, Ostrand-Rosenberg S, Smyth MJ, Berzofsky JA. A nonclassical non-Valpha14Jalpha18 CD1d-restricted (type II) NKT cell is sufficient for down-regulation of tumor immunosurveillance. *J Exp Med* 2005; **202**: 1627-1633 [PMID: 16365146 DOI: 10.1084/jem.20051381]
163 **Cooper MA**, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. *Trends Immunol* 2001; **22**: 633-640 [PMID: 11698225]
164 **Cooper MA**, Fehniger TA, Turner SC, Chen KS, Ghaheri BA, Ghayur T, Carson WE, Caligiuri MA. Human natural killer cells: a unique innate immunoregulatory role for the CD56(bright) subset. *Blood* 2001; **97**: 3146-3151 [PMID: 11342442]
165 **Qin Z**, Schwartzkopff J, Pradera F, Kammertoens T, Seliger B, Pircher H, Blankenstein T. A critical requirement of interferon gamma-mediated angiostasis for tumor rejection by CD8+ T cells. *Cancer Res* 2003; **63**: 4095-4100 [PMID: 12874012]
166 **Colombo MP**, Piconese S. Regulatory-T-cell inhibition versus depletion: the right choice in cancer immunotherapy. *Nat Rev Cancer* 2007; **7**: 880-887 [PMID: 17957190 DOI: 10.1038/nrc2250]
167 **Lee PP**, Yee C, Savage PA, Fong L, Brockstedt D, Weber JS, Johnson D, Swetter S, Thompson J, Greenberg PD, Roederer M, Davis MM. Characterization of circulating T cells specific for tumor-associated antigens in melanoma patients. *Nat Med* 1999; **5**: 677-685 [PMID: 10371507 DOI: 10.1038/9525]
168 **Sutmuller RP**, van Duivenvoorde LM, van Elsas A, Schumacher TN, Wildenberg ME, Allison JP, Toes RE, Offringa R, Melief CJ. Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of CD25(+) regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. *J Exp Med* 2001; **194**: 823-832 [PMID: 11560997]
169 **Campbell JJ**, Bowman EP, Murphy K, Youngman KR, Siani MA, Thompson DA, Wu L, Zlotnik A, Butcher EC. 6-C-kine (SLC), a lymphocyte adhesion-triggering chemokine expressed by high endothelium, is an agonist for the MIP-3beta receptor CCR7. *J Cell Biol* 1998; **141**: 1053-1059 [PMID: 9585422]
170 **Förster R**, Schubel A, Breitfeld D, Kremmer E, Renner-Müller I, Wolf E, Lipp M. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* 1999; **99**: 23-33 [PMID: 10520991]
171 **Gupta S**, Su H, Bi R, Agrawal S, Gollapudi S. Life and death of lymphocytes: a role in immunesenescence. *Immun Ageing* 2005; **2**: 12 [PMID: 16115325 DOI: 10.1186/1742-4933-2-12]
172 **Tsukishiro T**, Donnenberg AD, Whiteside TL. Rapid turnover of the CD8(+)CD28(-) T-cell subset of effector cells in the circulation of patients with head and neck cancer. *Cancer Immunol Immunother* 2003; **52**: 599-607 [PMID: 12827303 DOI: 10.1007/s00262-003-0395-6]
173 **Gamadia LE**, Rentenaar RJ, Baars PA, Remmerswaal EB, Surachno S, Weel JF, Toebes M, Schumacher TN, ten Berge IJ, van Lier RA. Differentiation of cytomegalovirus-specific CD8(+) T cells in healthy and immunosuppressed virus carriers. *Blood* 2001; **98**: 754-761 [PMID: 11468176]
174 **Valmori D**, Scheibenbogen C, Dutoit V, Nagorsen D, Asemissen AM, Rubio-Godoy V, Rimoldi D, Guillaume P, Romero P, Schadendorf D, Lipp M, Dietrich PY, Thiel E, Cerottini JC, Liénard D, Keilholz U. Circulating Tumor-reactive CD8(+) T cells in melanoma patients contain a CD45RA(+)CCR7(-) effector subset exerting ex vivo tumor-specific cytolytic activity. *Cancer Res* 2002; **62**: 1743-1750 [PMID: 11912149]
175 **Strauss L**, Bergmann C, Szczepanski M, Gooding W, Johnson JT, Whiteside TL. A unique subset of CD4+CD25highFoxp3+ T cells secreting interleukin-10 and transforming growth factor-beta1 mediates suppression in the tumor microenvironment. *Clin Cancer Res* 2007; **13**: 4345-4354 [PMID: 17671115 DOI: 10.1158/1078-0432.CCR-07-0472]
176 **Mandapathil M**, Szczepanski MJ, Szajnik M, Ren J, Lenzner DE, Jackson EK, Gorelik E, Lang S, Johnson JT, Whiteside TL. Increased ectonucleotidase expression and activity in regulatory T cells of patients with head and neck cancer. *Clin Cancer Res* 2009; **15**: 6348-6357 [PMID: 19825957 DOI: 10.1158/1078-0432.CCR-09-1143]
177 **Bergmann C**, Strauss L, Wang Y, Szczepanski MJ, Lang S, Johnson JT, Whiteside TL. T regulatory type 1 cells in squamous cell carcinoma of the head and neck: mechanisms of suppression and expansion in advanced disease. *Clin Cancer Res* 2008; **14**: 3706-3715 [PMID: 18559587 DOI: 10.1158/1078-0432.CCR-07-5126]
178 **Akimova T**, Kamath BM, Goebel JW, Meyers KE, Rand EB, Hawkins A, Levine MH, Bucuvalas JC, Hancock WW. Differing effects of rapamycin or calcineurin inhibitor on T-regulatory cells in pediatric liver and kidney transplant recipients. *Am J Transplant* 2012; **12**: 3449-3461 [PMID: 22994804 DOI: 10.1111/j.1600-6143.2012.04269.x]
179 **Segundo DS**, Ruiz JC, Izquierdo M, Fernández-Fresnedo G, Gómez-Alamillo C, Merino R, Benito MJ, Cacho E, Rodrigo E, Palomar R, López-Hoyos M, Arias M. Calcineurin inhibitors, but not rapamycin, reduce percentages of CD4+CD25+FOXP3+ regulatory T cells in renal transplant recipients. *Transplantation* 2006; **82**: 550-557 [PMID:

16926600 DOI: 10.1097/01.tp.0000229473.95202.50]
180 **Furtado GC**, Curotto de Lafaille MA, Kutchukhidze N, Lafaille JJ. Interleukin 2 signaling is required for CD4(+) regulatory T cell function. *J Exp Med* 2002; **196**: 851-857 [PMID: 12235217]
181 **Wu Y**, Borde M, Heissmeyer V, Feuerer M, Lapan AD, Stroud JC, Bates DL, Guo L, Han A, Ziegler SF, Mathis D, Benoist C, Chen L, Rao A. FOXP3 controls regulatory T cell function through cooperation with NFAT. *Cell* 2006; **126**: 375-387 [PMID: 16873067 DOI: 10.1016/j.cell.2006.05.042]
182 **Baan CC**, van der Mast BJ, Klepper M, Mol WM, Peeters AM, Korevaar SS, Balk AH, Weimar W. Differential effect of calcineurin inhibitors, anti-CD25 antibodies and rapamycin on the induction of FOXP3 in human T cells. *Transplantation* 2005; **80**: 110-117 [PMID: 16003241]
183 **San Segundo D**, Fábrega E, López-Hoyos M, Pons F. Reduced numbers of blood natural regulatory T cells in stable liver transplant recipients with high levels of calcineurin inhibitors. *Transplant Proc* 2007; **39**: 2290-2292 [PMID: 17889166 DOI: 10.1016/j.transproceed.2007.07.076]
184 **Musarò A**, McCullagh KJ, Naya FJ, Olson EN, Rosenthal N. IGF-1 induces skeletal myocyte hypertrophy through calcineurin in association with GATA-2 and NF-ATc1. *Nature* 1999; **400**: 581-585 [PMID: 10448862 DOI: 10.1038/23060]
185 **Trinchieri G**. Biology of natural killer cells. *Adv Immunol* 1989; **47**: 187-376 [PMID: 2683611]
186 **Kärre K**, Ljunggren HG, Piontek G, Kiessling R. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. 1986. *J Immunol* 2005; **174**: 6566-6569 [PMID: 15905492]
187 **O'Leary JG**, Goodarzi M, Drayton DL, von Andrian UH. T cell- and B cell-independent adaptive immunity mediated by natural killer cells. *Nat Immunol* 2006; **7**: 507-516 [PMID: 16617337 DOI: 10.1038/ni1332]
188 **Lanier LL**, Buck DW, Rhodes L, Ding A, Evans E, Barney C, Phillips JH. Interleukin 2 activation of natural killer cells rapidly induces the expression and phosphorylation of the Leu-23 activation antigen. *J Exp Med* 1988; **167**: 1572-1585 [PMID: 3259252]
189 **Shim H**, Chun YS, Lewis BC, Dang CV. A unique glucose-dependent apoptotic pathway induced by c-Myc. *Proc Natl Acad Sci USA* 1998; **95**: 1511-1516 [PMID: 9465046]
190 **Greiner EF**, Guppy M, Brand K. Glucose is essential for proliferation and the glycolytic enzyme induction that provokes a transition to glycolytic energy production. *J Biol Chem* 1994; **269**: 31484-31490 [PMID: 7989314]
191 **Decker T**, Lohmann-Matthes ML. A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. *J Immunol Methods* 1988; **115**: 61-69 [PMID: 3192948]
192 **Morteau O**, Blundell S, Chakera A, Bennett S, Christou CM, Mason PD, Cornall RJ, O'Callaghan CA. Renal transplant immunosuppression impairs natural killer cell function in vitro and in vivo. *PLoS One* 2010; **5**: e13294 [PMID: 20967261 DOI: 10.1371/journal.pone.0013294]
193 **Meehan AC**, Mifsud NA, Nguyen TH, Levvey BJ, Snell GI, Kotsimbos TC, Westall GP. Impact of commonly used transplant immunosuppressive drugs on human NK cell function is dependent upon stimulation condition. *PLoS One* 2013; **8**: e60144 [PMID: 23555904 DOI: 10.1371/journal.pone.0060144]
194 **Sánchez-Fructuoso A**, Conesa J, Perez Flores I, Ridao N, Calvo N, Prats D, Rodríguez A, Barrientos A. Conversion to sirolimus in renal transplant patients with tumors. *Transplant Proc* 2006; **38**: 2451-2452 [PMID: 17097964 DOI: 10.1016/j.transproceed.2006.08.063]
195 **Yelken B**, Caliskan Y, Ozkan O, Gorgulu N, Yazici H, Turkmen A, Sever MS. Conversion to sirolimus in renal transplant recipients: a single-center experience. *Artif Organs* 2010; **34**: E230-E237 [PMID: 20618227 DOI: 10.1111/j.1525-1594.2010.01022.x]
196 **Hoogendijk-van den Akker JM**, Harden PN, Hoitsma AJ, Proby CM, Wolterbeek R, Bouwes Bavinck JN, de Fijter JW. Two-year randomized controlled prospective trial converting treatment of stable renal transplant recipients with cutaneous invasive squamous cell carcinomas to sirolimus. *J Clin Oncol* 2013; **31**: 1317-1323 [PMID: 23358973 DOI: 10.1200/JCO.2012.45.6376]
197 **Carroll RP**, Hester J, Wood KJ, Harden PN. Conversion to sirolimus in kidney transplant recipients with squamous cell cancer and changes in immune phenotype. *Nephrol Dial Transplant* 2013; **28**: 462-465 [PMID: 23223314 DOI: 10.1093/ndt/gfs474]
198 **Terasaki PI**, Ozawa M. Predicting kidney graft failure by HLA antibodies: a prospective trial. *Am J Transplant* 2004; **4**: 438-443 [PMID: 14961999]
199 **Riethmüller S**, Ferrari-Lacraz S, Müller MK, Raptis DA, Hadaya K, Rüsi B, Laube G, Schneiter G, Fehr T, Villard J. Donor-specific antibody levels and three generations of crossmatches to predict antibody-mediated rejection in kidney transplantation. *Transplantation* 2010; **90**: 160-167 [PMID: 20658760]
200 **Heeger PS**, Greenspan NS, Kuhlenschmidt S, Dejelo C, Hricik DE, Schulak JA, Tary-Lehmann M. Pretransplant frequency of donor-specific, IFN-gamma-producing lymphocytes is a manifestation of immunologic memory and correlates with the risk of posttransplant rejection episodes. *J Immunol* 1999; **163**: 2267-2275 [PMID: 10438971]
201 **Amico P**, Hönger G, Mayr M, Steiger J, Hopfer H, Schaub S. Clinical relevance of pretransplant donor-specific HLA antibodies detected by single-antigen flow-beads. *Transplantation* 2009; **87**: 1681-1688 [PMID: 19502960 DOI: 10.1097/TP.0b013e3181a5e034]
202 **Eng HS**, Bennett G, Tsiopelas E, Lake M, Humphreys I, Chang SH, Coates PT, Russ GR. Anti-HLA donor-specific antibodies detected in positive B-cell crossmatches by Luminex predict late graft loss. *Am J Transplant* 2008; **8**: 2335-2342 [PMID: 18782289 DOI: 10.1111/j.1600-6143.2008.02387.x]
203 **Bestard O**, Cruzado JM, Lucia M, Crespo E, Casis L, Sawitzki B, Vogt K, Cantarell C, Torras J, Melilli E, Mast R, Martinez-Castelao A, Gomà M, Reinke P, Volk HD, Grinyó JM. Prospective assessment of antidonor cellular alloreactivity is a tool for guidance of immunosuppression in kidney transplantation. *Kidney Int* 2013; **84**: 1226-1236 [PMID: 23783240 DOI: 10.1038/ki.2013.236]
204 **Poggio ED**, Clemente M, Hricik DE, Heeger PS. Panel of reactive T cells as a measurement of primed cellular alloimmunity in kidney transplant candidates. *J Am Soc Nephrol* 2006; **17**: 564-572 [PMID: 16382020 DOI: 10.1681/ASN.2005030293]
205 **Chakera A**, Bennett S, Lawrence S, Morteau O, Mason PD, O' Callaghan CA, Cornall RJ. Antigen-specific T cell responses to BK polyomavirus antigens identify functional anti-viral immunity and may help to guide immunosuppression following renal transplantation. *Clin Exp Immunol* 2011; **165**: 401-409 [PMID: 21671906 DOI: 10.1111/j.1365-2249.2011.04429.x]
206 **Binggeli S**, Egli A, Schaub S, Binet I, Mayr M, Steiger J, Hirsch HH. Polyomavirus BK-specific cellular immune response to VP1 and large T-antigen in kidney transplant recipients. *Am J Transplant* 2007; **7**: 1131-1139 [PMID: 17359507 DOI: 10.1111/j.1600-6143.2007.01754.x]
207 **Schaub S**, Hirsch HH, Dickenmann M, Steiger J, Mihatsch MJ, Hopfer H, Mayr M. Reducing immunosuppression preserves allograft function in presumptive and definitive polyomavirus-associated nephropathy. *Am J Transplant* 2010; **10**: 2615-2623 [PMID: 21114642 DOI: 10.1111/j.1600-6143.2010.03310.x]
208 **Kono K**, Kawaida H, Takahashi A, Sugai H, Mimura K, Miyagawa N, Omata H, Fujii H. CD4(+)CD25high regulatory T cells increase with tumor stage in patients with gastric and esophageal cancers. *Cancer Immunol Immunother* 2006; **55**: 1064-1071 [PMID: 16328385 DOI: 10.1007/s00262-005-0092-8]
209 **Perez SA**, Karamouzis MV, Skarlos DV, Ardavanis A, Sotiriadou NN, Iliopoulou EG, Salagianni ML, Orphanos G, Baxevanis CN, Rigatos G, Papamichail M. CD4+CD25+ regulatory T-cell frequency in HER-2/neu (HER)-positive and HER-negative advanced-stage breast cancer patients. *Clin Cancer Res* 2007; **13**: 2714-2721 [PMID: 17473204 DOI: 10.1158/1078-0432.CCR-06-2347]
210 **Zhang W**, Caspell R, Karulin AY, Ahmad M, Haicheur N,

Abdelsalam A, Johannesen K, Vignard V, Dudzik P, Georgakopoulou K, Mihaylova A, Silina K, Aptsiauri N, Adams V, Lehmann PV, McArdle S. ELISPOT assays provide reproducible results among different laboratories for T-cell immune monitoring--even in hands of ELISPOT-inexperienced investigators. *J Immunotoxicol* 2009; **6**: 227-234 [PMID: 19908941 DOI: 10.3109/15476910903317546]

211 **Hricik DE**, Rodriguez V, Riley J, Bryan K, Tary-Lehmann M, Greenspan N, Dejelo C, Schulak JA, Heeger PS. Enzyme linked immunosorbent spot (ELISPOT) assay for interferon-gamma independently predict0s renal function in kidney transplant recipients. *Am J Transplant* 2003; **3**: 878-884 [PMID: 12814480]

212 **Gebauer BS**, Hricik DE, Atallah A, Bryan K, Riley J, Tary-Lehmann M, Greenspan NS, Dejelo C, Boehm BO, Hering BJ, Heeger PS. Evolution of the enzyme-linked immunosorbent spot assay for post-transplant alloreactivity as a potentially useful immune monitoring tool. *Am J Transplant* 2002; **2**: 857-866 [PMID: 12392292]

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