The clinical perspective on malignancies in renal transplanted patients

VIVAN HELLESTRÖM
Abstract


Post-transplant malignancies cause significant morbidity and mortality. In this thesis we investigated malignancies in renal transplanted patients from a clinical viewpoint. The use of regional tumour registries considerably improved identification of pre- and post-transplant malignancies, which are generally underreported in transplant registries.

Despite previously adequate cancer treatments with favourable prognosis, patients with pre-transplant malignancies showed higher incidence of post-transplant cancer and reduced survival compared to a 1:3 ratio matched control group of patients without a previous cancer from the Collaborative Transplant Study in Europe. A careful oncological surveillance pre-transplant and post-transplant is recommended.

A multidisciplinary team evaluated the immunosuppressive and oncological treatment in a clinical prospective observational study of 120 renal transplanted patients with post-transplant malignancies. In two-thirds of the patients immunosuppression was possible to change to mTOR inhibitors with anti-tumour effects. Oncological treatment was adjusted in 50% of patients with solid or haematological tumours. MDT assessments are essential for optimizing treatment of post-transplant malignancies.

Number of previous cutaneous squamous cell carcinoma (SCC) posed the most significant risk variable in predicting subsequent SCCs during a two-years study of 73 transplanted patients with at least one SCC.

Incidence of transplant-derived tumours is 5 times higher than anticipated. Three of eleven cancers in urinary tract and two of four cancers in the transplants were transplant-derived. Five of eleven cancers of the urinary tract were BK-virus positive. Allograft immune response against these tumours offer new options for cancer treatment such as immunomodulatory or anti-viral treatment in combination with modified immunosuppression.

Keywords: renal transplantation, malignant tumours

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To My Family

Julia, Sofia, Maximilian, Ellen,
Natalie
and Anders
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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### Abbreviations

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<tr>
<td>6-MP</td>
<td>6-Mercaptopurine</td>
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<td>AK</td>
<td>Actinic keratosis</td>
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<td>ATG</td>
<td>Anti-thymocyte globulin</td>
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<td>BCC</td>
<td>Basal cell carcinoma</td>
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<td>BKV</td>
<td>Polyoma BK virus</td>
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<td>C</td>
<td>Complement</td>
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<td>CI</td>
<td>Confidence interval</td>
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<td>CMV</td>
<td>Cytomegalovirus</td>
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<td>CNI</td>
<td>Calcineurin inhibitor</td>
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<td>CT</td>
<td>Computed tomography</td>
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<td>CTS</td>
<td>Collaborative Transplant Study</td>
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<td>DAPI</td>
<td>4',6-Diamidino-2-phenylindole</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>EBV</td>
<td>Epstein-Barr virus</td>
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<td>FISH</td>
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<td>HBV</td>
<td>Hepatitis B virus</td>
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<td>HHV</td>
<td>Human herpes virus</td>
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<td>HIV</td>
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<td>HPV</td>
<td>Human papilloma virus</td>
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<td>HLA</td>
<td>Human leucocyte antigen</td>
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<td>HR</td>
<td>Hazard ratio</td>
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<td>ICD</td>
<td>International classification of diseases</td>
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<td>IL</td>
<td>Interleukin</td>
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<td>KM</td>
<td>Kaplan Meier</td>
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<td>KS</td>
<td>Kaposis sarcoma</td>
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<tr>
<td>MP</td>
<td>Mercaptopurine</td>
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<tr>
<td>mTOR</td>
<td>Mammalian target of rapamycin</td>
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<tr>
<td>mTORC1</td>
<td>Mammalian target of rapamycin complex 1</td>
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<td>NMSC</td>
<td>Nonmelanoma skin cancers</td>
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<td>Pap</td>
<td>Papanicolaou</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>PSA</td>
<td>Prostate specific antigen</td>
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<td>PTLD</td>
<td>Post-transplant lymphoproliferative disorder</td>
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<td>PV T-Ag</td>
<td>SV40 large T antigen</td>
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<td>RCC</td>
<td>Regional cancer center</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>SCC</td>
<td>Squamous cell carcinoma of the skin</td>
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<td>SD</td>
<td>Standard deviation</td>
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<td>SIR</td>
<td>Standardized incidence ratio</td>
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<td>SMR</td>
<td>Standardized mortality ratio</td>
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<td>SSP</td>
<td>Sequence-specific primers</td>
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<tr>
<td>TGF-β</td>
<td>Transforming growth factor beta</td>
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<tr>
<td>UV</td>
<td>Ultraviolet</td>
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<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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Introduction

Post-transplant malignancies occur at an increased rate, are considered more aggressive and are associated with worse outcome than malignancies in the general population (Ajithkumar et al. 2007, Webster et al. 2007, Miao et al. 2009, Lott et al. 2010). The original information on post-transplant tumours from 1971 has changed only slightly over the years. The essential life-long immunosuppression, which is a prerequisite for maintaining graft function, causes cancer by reducing immunologic surveillance. These contradictory effects of immunosuppression constitute the key problem in treating malignancies in solid organ transplanted patients.

In this thesis we approached cancer in renal transplanted patients from a clinical point of view. We intended to get clinically applicable data to, in the long run, improve outcome of these seriously ill patients.

Renal transplantation

History of renal transplantation

Renal transplantation was originally invented to save often young and otherwise healthy patients suffering from terminal uraemia. The first renal transplantation attempts were, however, unsuccessful due to limited knowledge of the immune system (Murray 1992). Cornerstones of modern transplantation immunology include host versus graft immune reactions, graft versus host immune reactions and acquired immunological tolerance, and these phenomena must be controlled to achieve a successful organ transplantation unless the donor and recipient share the same gene set (Groth et al. 2000). On December 23rd 1954 Dr Joseph E Murray successfully performed the first renal transplantation between identical twins without the need of immunosuppressive treatment (Murray et al. 2001).

Much effort was made to overcome the immunological barrier in the 1950s. Experimental treatments such as sublethal doses of total body irradiation followed by bone marrow transplantations resulted in occasionally functioning transplants, but the breakthrough appeared in 1959 when 6-mercaptopurine (6-MP) was developed. The imidazole derivate of 6-MP, named azathioprine, in combination with corticosteroids remarkably im-
proved graft survival and reduced the rejection frequency. As a result transplantation programmes expanded worldwide in the 1960s (Murray 1992).

The modern era of immunosuppressive treatment began with the discovery of cyclosporine in 1976, and it is one of the most important contributions to the field of transplantation. It reduced the allograft rejections and consequently prolonged graft survival. Combinations with newer immunosuppressants such as tacrolimus, rapamycin, mycophenolate mofetil and monoclonal antibodies that manipulate different targets of the immune response have resulted in even more improved patient and graft survival over time. Renal transplantation has been a success story and is now the first choice of treatment of end stage renal disease (Groth et al. 2000).

History of post-transplant tumours
As early as in 1968 the first report of the increased malignancy incidence in renal transplanted patients was published (Penn et al. 1971). As a consequence an informal, international tumour registry on post-transplant malignancies was established in Denver. In 1971 Dr I Penn pointed out an increased tumour risk of 10% compared to the general age matched population. The most common tumour types after organ transplantation were squamous and basal cell cancer of the skin, lymphomas, cervix cancer and lip cancer (Kauffman 2006, Ajithkumar et al. 2007). Post-transplant malignancies were associated with high morbidity and mortality (Vajdic and van Leeuwen 2009). Prognosis was good in patients conventionally treated for low-grade malignancies but was very poor for advanced cancer. Immunosuppression was stated to be oncogenic and suggested to be interrupted in patients with advanced cancers (Penn et al. 1971, Penn and Starzl 1972).

Immunosuppression and cancer
Introduction
Immunosuppression is divided into induction therapy, maintenance therapy and therapy of acute rejection. Induction therapy is administered at the time of transplantation. Maintenance immunosuppression is life-long medication, and most are administered once or twice daily. The assimilation of some drugs varies between individuals, and consequently serum drug concentrations must be determined and dosage adjusted at regular intervals. Immunosuppressive agents are given in combinations to affect different targets of the immune system and to reassure an adequate immunosuppression. The same agent can be used as maintenance and rejection therapy, e.g. corticosteroids, or induction and rejection therapy, e.g. anti-thymocyte globulin (ATG) (Tufveson and Johnsson 2002).
Induction therapy in renal transplant recipients consists of antibodies directed against T-lymphocytes such as ATG or the interleukin (IL)-2 receptor antagonist basiliximab in combination with high dose intravenous corticosteroids (Kalluri and Hardinger 2012). Alemtuzumab is a monoclonal antibody directed against CD52, which is present on all T- and B-lymphocytes, macrophages, natural killer cells and some granulocytes (Gundroo et al. 2015). Rituximab, an antibody against CD20 present on B-cells is used in AB0-incompatible renal transplantations (Hardinger and Brennan 2013).

Maintenance immunosuppressants are divided into corticosteroids, calcineurin inhibitors (CNI), i.e. cyclosporine and tacrolimus, anti-proliferative agents, e.g. mycophenolate mophetil and azathioprine, mammalian target of rapamycin (mTOR) inhibitors, e.g. sirolimus and everolimus and co-stimulation blockers, e.g. belatacept (Kalluri and Hardinger 2012, Hardinger and Brennan 2013).

Treatment options for acute cellular rejections comprise high-dose corticosteroids and ATG. Acute antibody-mediated rejections can be treated with plasmapheresis, ATG, anti-complement factor 5 (C5) antibodies e.g. ecuilsizumab and intravenous immunoglobulin (Ig) (Hardinger and Brennan 2013).

Immunosuppression and cancer
Malignancy incidence increases with the total amount and duration of immunosuppressive treatment (Alberu et al. 2011) and specific immunosuppressive regimens are associated with certain types of cancer induction. E.g. azathioprine is associated with elevated risk of SCC (Adami et al. 2003) while ATG is associated with an elevated risk of post-transplant lymphoproliferative disease (PTLD) (Webster et al. 2007, Zecher and Steiger 2012).

Interestingly mTOR inhibitors have both immunosuppressive and anti-tumour properties (Zecher and Steiger 2012, Gogia et al. 2013). Lower incidence rates of malignancies are documented in patients treated with mTOR inhibitors compared with patients on CNI based immunosuppression (Campbell et al. 2012, Metchnikoff et al. 2012). A switch from CNI to mTOR inhibitors is suggested to be favourable in patients with post-transplant tumours (Morath et al. 2004, Webster et al. 2008, Ulrich et al. 2009, Campistol et al. 2012, Bottomley and Harden 2013).

Calcineurin inhibitors may contribute to the development of malignant tumours by activating transforming growth factor beta (TGF-β) and vascular endothelial growth factor (VEGF) and thereby inducing cell proliferation and angiogenesis (Guba et al. 2004, Dantal and Soulillou 2005).

Another explanation might be the effect of CNI on telomeres. Telomeres stabilize the end of the chromosomes and telomere dysfunction may lead to genomic instability, which in turn promotes development of cancer (Heaphy and Meeker 2011). Shorter telomere lengths and telomerase markers may be
useful in predicting cancer progression (Shay 2013, Shay 2014). Telomere shortening is more pronounced in CNI treated cells than in mTOR inhibitor treated cells but no difference is observed between cells from older and younger patients (Welzl et al. 2014).

Reduction of immunosuppression may reverse some but not all types of cancer. The risk of Non-Hodgkin’s lymphoma, lip cancer and melanoma decreases when immunosuppression is interrupted in patients with renal graft failure. Cancers associated with renal failure such as kidney, urinary tract and thyroid cancers increase after return to dialysis. (van Leeuwen et al. 2010).

mTOR inhibitors and cancer

Mammalian target of rapamycin (mTOR) is a regulatory protein, a serine-threonine kinase, which is expressed in all cells. It has an effect on the mRNA translation of proteins regulating cell survival, cell proliferation and angiogenesis. Inhibiting the mTOR pathway results in reduced cell growth, cell proliferation and angiogenesis, and this explains their effects on tumour and endothelial cells (Tabernero et al. 2008, Tanaka et al. 2008). mTOR inhibitors also have immunosuppressive and anti-fungal properties (Mohindra et al. 2014).

The plasma concentration of everolimus is correlated to its effect on tumour tissue and skin (Tabernero et al. 2008). Lower doses of everolimus administered daily obtain the same anti-tumour effects as higher doses of everolimus administered weekly, but the full effect occurs a few days later (Tanaka et al. 2008). Consequently there is an anti-tumour effect even when everolimus is used as an immunosuppressant within the recommended concentration interval (3-10 ng/mL).

A lower incidence of malignancies has been reported in renal transplanted patients with mTOR based immunosuppressive protocols than in patients with calcineurin inhibitor (CNI) based immunosuppressive protocols (Campistol et al. 2006).

mTOR inhibitors have shown promising effects alone or in combination with other chemotherapeutics in many cancer types in non-transplanted patients. Everolimus is a registered drug in treatment of renal cancer, breast cancer, mantle cell lymphoma and pancreatic cancer in Sweden. They may also play a role in treatment of ovarian cancer, prostate cancer, lung cancer, colorectal cancer, bladder cancer, neuroendocrine tumours, Kaposis sarcoma (KS) and squamous cell carcinoma of the skin (SCC) (Itamochi and Kigawa 2012, Zagouri et al. 2012).

The cancer cells may develop resistance against mTOR inhibitors by activating different feedback loops in the mTOR pathway, especially in the mTOR complex 1 (mTORC1) (Mohindra et al. 2014). By genome sequencing two loss-of-function mutations in tumour cells have been identified: in
the tuberous sclerosis complex 1 (TSC1) gene and in the neurofibromatosis type 2 (NF2) gene. In some cancers the mutations of these genes are present from the beginning, in others they develop later (Iyer et al. 2012). These mutations may predict lack of sensitivity to mTOR inhibitors (Iyer et al. 2012, Mohindra et al. 2014).

Expression of phospho-mTOR, a molecule found in the mTOR signaling pathway has been used to measure the sensitivity of tumours to mTOR inhibitors (Karayannopoulou et al. 2013).

Oncogenic viruses

An oncogenic virus has some typical characteristics. It is able to a) interfere with the mechanisms controlling cell proliferation and b) escape detection of the host immune response to induce a tumour (Allison 1970, Morath et al. 2004).

The association between immune deficiency and cancer risk has been documented in organ transplanted patients and patients infected with immunodeficiency virus (HIV) (Grulich et al. 2007, Grulich and Vajdic 2015). More than 20 infection-related cancers occur at increased rates in these patients. The human herpes virus 8 (HHV8)-induced KS, Epstein Barr Virus (EBV)-induced non-Hodgkin lymphoma and human papilloma virus (HPV)-associated cutaneous SCC are all virus-induced and constitute the three most immunogenic cancer types in renal transplanted patients (Grulich and Vajdic 2015).

Other examples of oncogenic viruses are the Hepatitis B virus (HBV), hepatitis C virus (HCV) (Morath et al. 2004), polyoma BK virus (BKV) (Roberts IS et al. 2008) and Merkel cell polyoma virus.

HHV-8 correlates to all forms of KS, classic KS, AIDS-KS, endemic KS and transplant KS. The genome of the virus includes oncogenes that affect cell proliferation and apoptosis. Infection with the virus in combination with immunodeficiency is a prerequisite for development of KS. The heavier the immunosuppression the sooner the malignancy occurs (Clarke et al. 2015).

More than 90% of the adult population is seropositive to EBV. The virus remains latent in resting B-cells. It is associated with nasopharyngeal carcinoma, Burkitt’s lymphoma, Hodgkin lymphoma, some types of T-cell lymphoma and B-cell lymphoma, post-transplant lymphomas, and leiomyosarcomas in patients on immunosuppression (Young and Murray 2003).

HPV is associated with anogenital cancer, SCC in situ, invasive SCC as well as cancer of the head and neck (Reusser et al. 2015). HBV and HCV are correlated with hepatocellular carcinoma (Morath et al. 2004).

More than 90% of the normal population is BKV seropositive at the age of ten. This polyoma virus remains latent in the urothelial epithelium and about 30% of all immunocompromised patients are subjected to reactivation
of the virus. BKV has the characteristics of an oncogenic virus. It induces cell proliferation and inhibits apoptosis especially with the presence of immunosuppression (Alexiev et al. 2013). Its role in causing cancer in humans remains unverified; still different theories of BKV induced carcinogenesis has been proposed (Thamboo et al. 2007, Roberts IS et al. 2008, Abend et al. 2009, Alexiev et al. 2013, Kuppachi et al. 2013, Rinaldo et al. 2013).

A new polyoma virus, the Merkel cell polyomavirus, has been detected. It is associated with Merkel cell carcinoma, an aggressive neuroendocrine carcinoma of the skin (Ponticelli et al. 2014, Vaira et al. 2015).

Virus-induced tumours offer alternative treatment options such as antiviral treatment in combination with minimization of immunosuppression (Williams et al. 2005). Acyclovir has been used to treat EBV-positive B-cell lymphoma, and cidofovir has effects on HPV-related carcinomas (Baron et al. 2001, Reusser et al. 2015) Furthermore, vaccination against HPV might influence frequency and recurrence of anogenital cancers as well as to be used as cancer treatment (Avery and Michaels 2008, Mudry et al. 2011, Reusser et al. 2015). When immunosuppression is reduced or interrupted in solid organ transplant recipients or HIV patients receive adequate treatment, the risk of Kaposi sarcoma, non-Hodgkins lymphoma and SCC cancers is reversed (Grulich and Vajdic 2015). It is unclear whether other virus-induced cancers respond in the same way to reduction of immunosuppression.

Pre-transplant tumours

Between two and four per cent of all renal transplanted patients have experienced a malignancy prior to renal transplantation (Adami et al. 2003, Kauffman 2006, Viecelli et al. 2015). A previous diagnosis of a cancer in a potential renal allograft recipient brings the dual effects of immunosuppression to a head. The risk of recurrence or death due to cancer must be weighed against the risk of death due to renal failure. Risk assessments are made on individual basis and include tumour type, stage, prognosis as well as patient age, co-morbidity and long-term predicted outcome from renal failure without transplantation (Girndt and Kohler 2005, Campistol et al. 2012, Abramowicz et al. 2014). For practical purposes a time limit of two years between cancer treatment and renal transplantation has often been used because most malignancies relapse during the first two years after diagnosis (Penn 1993, Penn 1997, Girndt and Kohler 2005). Almost 60% of all post-transplant cancer recurrences occurred in patients with a cancer diagnosed within two years before transplantation, and more than 30% of post-transplant cancer recurrences occurred in patients with a cancer diagnosed between two to five years before transplantation. In addition, more than 10%
of all post-transplant recurrences occurred in patients treated for cancer more than five years before transplantation (Penn 1993).

Incidental renal cancers, cervix cancers in situ, thyroid cancers and some lymphomas have recurrence rates between 0 and 10%; cancer of the uterus, colorectal cancer, prostate cancer and breast cancer have recurrent rates of 11 to 25%; bladder cancer, sarcomas, malignant melanomas, multiple myelomas and non-melanoma skin cancers (NMSC) had recurrent rates of more 26% (Penn 1993).

Unfortunately, there are no uniform guidelines for the time to wait before renal transplantation, probably since most common types of pre- and post-transplant tumours differ throughout the world. (Batabyal et al. 2012). Even the types of pre-transplant cancer to accept for renal transplantation vary between different countries, and the cancer free surveillance periods for a distinct type of cancer also deviates. According to the European guidelines the recommended waiting time for renal cell carcinoma, bladder cancer, colorectal cancer, cervical in situ cancer, cancer of the uterine body and malignant melanoma is minimum of two years. The recommended tumour free period for breast cancer and invasive cervical cancer is five years. Corresponding guidelines in Canada comprise a tumour-free interval of two to five years for breast cancer, no waiting time for invasive cervical cancer, and five years for malignant melanoma. In the US the suggested time of tumour remission for in situ bladder cancer is null years, early stages of breast cancer is a minimum of two years and in situ cervical cancer is two to five years. In Australia advanced stages of colon cancer, breast cancer and multiple myeloma constitute contraindications for renal transplantation (Batabyal et al. 2012).

Over time the prognosis for many cancer types have improved and accordingly the eligibility criteria of renal transplantation have changed (Campistol et al. 2012). Consensus is that the 5-year patient survival rate after malignancy should exceed the 5-year life expectancy of the patient after renal transplantation (Kasiske et al. 2001, Fernandez-Vivancos et al. 2010, Campistol et al. 2012, Abramowicz et al. 2014).

In Sweden the overall and cancer specific mortality was increased in patients with a pre-transplant cancer. The risk was increased even for patients with a waiting time more than 5 years between cancer diagnosis and transplantation (Brattstrom et al. 2013).

Post-transplant tumours

Solid organ transplanted patients share conventional risk factors to cancer development with the general population. Additionally, underlying medical conditions and life-long immunosuppressive treatment are important risk factors leading to the 2 to 5 times elevated rates of cancer.
Figure 1. Cumulative incidence of post-transplant cancer in kidney transplanted patients and the general population. CTS-K-51109-0216.


Post-transplant tumours are considered more aggressive, often diagnosed at a more advanced stage and are associated with worse outcome than those of the general population (Grulich et al. 2007, Webster et al. 2007, Miao et al. 2009, Lott et al. 2010, Shiels et al. 2015).

Post-transplant malignant tumours after solid organ transplantation can be divided into three types according to origin: de novo tumours, recurrence of earlier malignancy and donor-related malignant tumours. From a practical point of view the post-transplant tumours are divided into skin-, solid- and haematological tumours.
De novo tumours

Skin tumours

The most frequently appearing post-transplant skin cancers SCC, basal cell carcinoma (BCC), actinic keratosis (AK) and malignant melanoma are ultraviolet (UV) radiation induced. Sun exposure enhances the oncogenic effect of immunosuppressants in the skin. It is the cumulative exposure of UV light, both earlier and present, that correlates to increased risk of future skin tumours. Accordingly these skin lesions most frequently appear in sun-exposed areas such as face, hands, trunk and neck (Ulrich et al. 2009). A high sun exposure is the main explanation why SCC occur in as much as 80% of all solid organ transplanted patients 10 years post-transplant in Australia. The corresponding number in Sweden is 5%. Thus, cumulative sun exposure seems to be an important risk factor to skin cancer and regular use of sun protection may reduce the risk of subsequent skin lesions (Moloney et al. 2005, Ulrich et al. 2008a).

Fitzpatrick’s classification was invented in 1975 to determine the effect of UV radiation on the skin in patients with different skin types (skin type I to VI). Skin type I includes people with fair skin and with blond or red hair, who never tan but always burn at sun exposure. Skin type VI includes people with black skin who never burn but get darker of sun exposure. Patients with skin type I are at an increased risk of developing skin cancer (Fitzpatrick 1988).

Squamous cell carcinoma (SCC)

SCC of the skin is the most common post-transplant tumour in renal transplanted recipients in Sweden. Twelve per cent of the renal transplanted population develop SCC in 20 years. Five years post transplantation the incidence of a first SCC is increased 52 fold and the incidence of all SCCs is increased 121 fold in renal transplant recipients compared with the general population (Krynitz et al. 2012). Half of the patients with a first SCC are at risk of multiple subsequent SCC and in the end metastatic SCC (Euvrard et al. 2006, Krynitz et al. 2012, Tessari and Girolomoni 2012).

The total amount and duration of immunosuppressants seem to have a greater impact on the development of SCC, than drug-specific oncogenic properties (Morath et al. 2004). Azathioprine is an exception; it is associated with increased rates of SCC, by inducing irreversible mutations in the deoxyribonucleic acid (DNA) (Ingvar et al. 2010). In contrast, mTOR inhibitors have documented anti-tumour effects and have been connected to reduced rates of a first as well as subsequent SCC (Campbell et al. 2012, Euvrard et al. 2012, Hoogendijk-van den Akker et al. 2013).

Risk factors contributing to the development of SCC include: skin type according to Fitzpatrick’s classification (Gogia et al. 2013), cumulative sun exposure (Moloney et al. 2005, Ulrich et al. 2008a), genetic factors (Laing

Furthermore, SCC behaves more aggressively in immunocompromised patients than in the general population (Lott et al. 2010). This might be explained by a different gene expressions in the tumours (Muehleisen et al. 2012), different patterns of expression of pro-oncogenic markers such as p53 and transforming growth factor beta (TGFβ) (Gutierrez-Dalmau et al. 2010), increased telomere lengths (Perrem et al. 2007, Perrem et al. 2008) and higher presence of high-risk human papillomavirus (HR-HPV) (Reuschenbach et al. 2011) in post-transplant SCC. Peritumoural infiltrates of T-cells, plasma cells and monocytes were also different in SCC of transplanted patients compared to SCC in non-transplanted patients (Krynitz et al. 2015b).

Mortality due to SCC of the skin is 10% in transplanted patients compared to 1% in the general population (Martinez et al. 2003). In a Swedish retrospective study from 2006, 7 of 201 SCC patients died due to metastases of SCC; all of the cancers were localized to the head and had metastasized to the parotic gland. The standardized mortality ratio (SMR) was 52, still this mortality rate was lower than in transplanted patients in other countries in Europe and USA (Lindelof et al. 2006).

**Basal cell carcinoma**

While the proportion of basal cell carcinomas (BCC) to cutaneous squamous cell carcinomas (SCC) is 4:1 in the non-transplanted population, it is 1:4 in the transplanted one (Euvrard et al. 2003, Jensen et al. 2010). The incidence of BCC in the transplanted population is higher in Australia and New Zealand (Euvrard et al. 2003) than in Sweden. The reporting of basal cell carcinoma is however not as accurate as the reporting of SCC. Based on the Swedish national basal cell carcinoma registry that started 2004, the standardized incidence ratio (SIR) of basal cell carcinoma is 7.2 among renal transplanted patients in Sweden. However, the median age of the investigated transplanted population was 53 years and median follow up time was only 3.4 years. The risk of a subsequent basal cell carcinoma was increased in patients with a previous one. Patients with previous SCC were more prone to develop basal cell carcinoma than patients without SCC (Krynitz et al. 2015a). mTOR inhibitors have not the same favourable effect on basal cell carcinomas as they have on SCCs. A higher expression of phospho-mTOR has been found in SCC than in BCC, which might explain the different effects of mTOR inhibitors on the two types of skin cancer (Karayannopoulou et al. 2013).
Malignant melanoma
Malignant melanomas occur at a 1.2- to 3.3-fold increased risk in transplant- ed patients compared to the general population (Vajdic et al. 2009, Dahlke et al. 2014). In Sweden the SIR for malignant melanomas is 2.3 among renal transplanted recipients (Krynitz et al. 2012). The corresponding SIR is 3.3 in Australia and New Zealand (Webster et al. 2007) and 2.4 in USA (Engels et al. 2011). Malignant melanomas were diagnosed with more advanced histopa-thological changes in transplant recipients (Vajdic et al. 2014). The risk of melanoma-induced mortality was 4 times higher in the transplanted population compared to the non-transplanted population (Vajdic et al. 2014, Krynitz et al. 2015b). Most post-transplant melanomas were located on the trunk and prophylactic excision of truncal naevi might therefore be suggest- ed (Krynitz et al. 2015b).

Kaposis sarcoma
Kaposis sarcoma is a vascular, often multifocal malignancy, which mostly is diagnosed between one and three years after transplantation. The lesions appear initially on the extremities. The spread to mucocutaneous or visceral organs is mainly seen in immunosuppressed patients (Clarke et al. 2015). KS responds well to mTOR inhibitor treatment as well as to reduction of immunsuppression (Grulich and Vajdic 2015). The risk of Kaposis sarcoma is 29 times higher in transplanted patients than in the general Swedish population (Krynitz et al. 2012).

Merkel cell carcinoma
Merkel cell carcinoma is a rarely occurring, aggressive neuroendocrine tu-mour of the skin (Rockville Merkel Cell Carcinoma 2009). A new polyoma- virus, Merkel cell polyomavirus, was identified in 2008 (Feng et al. 2008). It is found in 80% of the cases with Merkel cell carcinoma (Vaira et al. 2015). The virus is associated to tumour progress due to its ability to produce oncoproteins (Ponticelli et al. 2014). Renal transplant recipients have a particular bad prognosis with decreased tumour free interval, early metastases and decreased survival (Clarke et al. 2015).

Solid tumours
Five years after renal transplantation the cumulative incidence for a first solid malignancy is 1.8%, at 10 years post-transplant it is 5% and at 20 years post-transplant it is 12% (Krynitz et al. 2012). This results in an SIR of 2.3 for all solid tumours after renal transplantation compared to the general pop- ulation in Sweden.

Among transplanted patients SIR is increased more than 5 times for cancer types such as Kaposis sarcoma, vaginal cancer, non-Hodgkins lymphoma and kidney cancer compared to the general population. SIR is increased up
to 5 times for common cancers such as cancers of the lung, colon, cervix, liver and stomach in the transplanted population. Cancer of oro-pharynx, oesophagus, bladder and leukaemia also occur up to 5 times more often than in the general population; however these types of cancers occur rarely in the normal population. In contrast, breast, prostate and rectal cancers are not increased in the transplanted population.

Cancers of kidney, urinary tract and thyroid gland as well as myeloma have been correlated to end stage renal disease and occur more frequently in patients on dialysis and in renal transplanted patients (Stewart et al. 2009).

**Haematological tumours**
The risk of haematological malignancies among renal transplanted patients is almost 4 times higher than in the Swedish general population (Kryniitz et al. 2012). Post-transplant lymphoid disorders (PTLD) comprise a heterogeneous group of uncontrolled proliferations originating from plasma cells or lymphocytes in immunocompromised patients. About 60 to 70% of all PTLD are EBV induced (Kinch et al. 2014a, Maksten et al. 2016). They are classified into early lesions, polymorphic PTLD, monomorphic PTLD, and classical Hodgkin lymphoma-type PTLD (Swerdlow et al. 2008). Most lesions originate from B-cell lymphocytes and the most common PTLD type is non-Hodgkin lymphoma, followed by Hodgkin lymphoma and other haematological malignancies. The incidence of PTLD has a bimodal curve with the first peak one year post transplantation and the second peak ten years post transplantation. In a Danish study 3.2% of all renal transplanted patients developed PTLD, almost 60% showed a monomorphic pattern and about 20% comprised early lesions. Patient survival, but not graft survival was reduced in these patients (Kinch et al. 2014a, Maksten et al. 2016).

**Donor related tumours**
Donor-related tumours are divided into donor-transmitted tumours and donor tissue-derived (donor-derived) tumours (Gandhi and Strong 2007). Donor-transmitted tumours exist in the donor at the time of transplantation, while donor-derived tumours are malignancies that develop from donor tissue with no known pre-existing malignancy in the donor. These tumours occur rarely, having an incidence estimated to 0.01-0.05%. Most probably these tumours are significantly underreported (Kauffman et al. 2002, Ison and Nalesnik 2011, Xiao et al. 2013, Desai et al. 2014).

**Donor-transmitted tumours**
Undiagnosed malignancies occur in 1.3% of the donors (Birkeland and Storm 2002). The general guidelines for organ procurement in Europe and USA aim to reduce the risk of donor-transmitted tumours (Europe 2013). Donor-transmitted tumours grow in the recipient and all recipients of the
grafts from the same donor may be affected. Heart, lung or liver transplants are lifesaving and cannot be removed in contrast to kidney transplants. The risk of donor-transmitted cancer is however small and must be weighed against the increased risk of mortality for the individual patient remaining on dialysis. Renal cell carcinomas comprise over 40% of such malignancies in USA (Desai et al. 2014). Undiagnosed prostate adenocarcinomas in elderly deceased donors are probably common, still donor-transmitted prostate cancer is seldom reported (Ison and Nalesnik 2011). Patients with a previous history of adequately treated small malignancies with favourable prognosis such as skin cancer or renal cancers have been accepted as donors, if the follow up has been long enough to regard the patient as cured. Donor-transmitted malignancies may, however, cause significant morbidity with a fatal outcome. An aggressive metastatic ovarian cancer developed in a male recipient (Lipshutz et al. 2009), small cell lung cancer was diagnosed in two renal transplant recipients from the same donor (Morath et al. 2005), neuroendocrine carcinomas were diagnosed in all recipients with grafts from the same donor (Foltys et al. 2009) and malignant melanoma was diagnosed in all graft recipients except the recipient of the heart graft (Milton et al. 2006). Renal grafts are often removed from patients with donor-transmitted malignancies.

**Donor-derived tumours**
Different types of donor-derived tumours have previously been described such as renal cancer (Barama et al. 2005), PTLD (Olagne et al. 2011), KS (Barozzi et al. 2003) and hepatocellular carcinoma (Vernadakis et al. 2010). They generally develop many years post-transplantation.

Donor-derived tumours are managed by reduction or cessation of immunosuppression to allow the recipient’s own immune system to be activated and reject tumour cells. Most patients need conventional therapy such as surgery, chemotherapy or radiation in addition to cessation of immunosuppression (Morath et al. 2005). In addition to allograft immune responses these tumours may offer new options for cancer treatment such as immunomodulatory treatment options.

**Tumour epidemiology in renal transplant recipients**
Renal graft survival, especially short-time survival, has dramatically improved over time mainly due to advances in immunosuppressive treatment. Better treatment options of cardiovascular complications and infections have also contributed. Since patient survival after kidney transplantation has increased, long-term complications such as malignancies have become more frequent. The transplanted community are nowadays older than decades ago.
Post-transplant malignancies are now the second most common cause of death among renal transplant recipients. More than 90% of the different tumours post transplantation occurred at increased rates (Grulich et al. 2007, Engels et al. 2011), especially those of known or suspected viral origin (Grulich et al. 2007, Vajdic and van Leeuwen 2009, Krynitz et al. 2012). The post-transplant cancer incidence is higher among liver, heart and lung transplant recipients than among kidney transplant recipients Heart and lung transplanted patients are in need of heavier immunosuppression than renal transplanted patients and liver transplantations can be performed due to cancer in the liver (Engels et al. 2011, Krynitz et al. 2012). Renal transplanted recipients have a 2 to 5 fold overall increase of malignant tumours worldwide compared to the general population (Villeneuve et al. 2007, Engels et al. 2011, Krynitz et al. 2012).

The risk of solid organ and haematological cancers remains stable over time, while the risk of SCC is accelerating and has trebled in the last 20 years (Krynitz et al. 2012). This is due to a small subgroup of SCC patients who frequently develop subsequent SCCs.

![Graph showing calculated half-life of renal grafts from diseased donors between 1986 and 2014. CTS- K-14103-0216.](image-url)

**Figure 2.** Calculated half-life of renal grafts from diseased donors between 1986 and 2014. CTS- K-14103-0216.
Geographic variations

The incidence and type of cancer, both in the general population and in the renal transplanted population, vary widely in different geographic areas. This is mainly caused by environmental and genetic factors but also by common viral infections in distinct populations. The post-transplant cancer incidence is however increased compared with the general population throughout the world (Cheung et al. 2012).

Geographical variations occur regarding the most common tumour type, e.g. SCC is the most common post-transplant cancer in Australia, USA and Western Europe. Non-Hodgkin lymphoma, kidney and bladder have the highest SIR in Hong Kong (Cheung et al. 2012). Renal cancer, gastric cancer and Non-Hodgkin lymphoma are most common in Japan (Hoshida et al. 1997). Urothelial cancer, hepatic cancer, gastrointestinal cancers and lymphomas are most common in China (Zhang J et al. 2014).

Figure 3. Cumulative incidence of post-transplant cancer at different age of transplantation. CTS- K-51209-0216.
There were however differences in cancer incidence and cancer types between Northern and Southern China; In Northern China the most common cancer types are urothelial cancer, hepatocellular cancer, renal cancer and gastrointestinal cancer, while urothelial cancer, gastrointestinal cancer, hepatocellular cancer and lymphomas are most common in Southern China (Zhang J et al. 2014). Renal cancer, thyroid cancer, lymphoma and uterine cancer occur most frequently in Japan (Hoshida et al. 1997) (Table 1 and 2).

**Mortality**

Patient survival has dramatically improved since the early days of renal transplantation. Long-term graft and patient survival are influenced by recipient factors (age, ethnicity, co-morbidity, smoking, time on dialysis, recipient/donor weight ratio, immunization), donor factors (age, co-morbidity, living/deceased donor, cause of death) and peri- and postoperative factors (donor specific antibodies, cold ischaemic time, delayed graft function, rejection, kidney function and proteinuria from 3 months post-transplant).

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**Table 1. Geographic variations of post-transplant cancer incidence after renal transplantation. Ranking of the most common types of cancer in each country. Skin cancers excluded.**

<table>
<thead>
<tr>
<th>Ca type</th>
<th>Canada</th>
<th>UK</th>
<th>Sweden</th>
<th>HK</th>
<th>Japan</th>
<th>Korea</th>
<th>Australia</th>
</tr>
</thead>
<tbody>
<tr>
<td>All ca</td>
<td>2.4†</td>
<td>2.3†</td>
<td>2.9</td>
<td>2.8</td>
<td>1.9</td>
<td>3.3†</td>
<td></td>
</tr>
<tr>
<td>Lip</td>
<td>31.3 (4)</td>
<td>65</td>
<td>10.0 (5)</td>
<td>2.8</td>
<td>1.4 (2)</td>
<td>0.9 (2)</td>
<td>47.0 (1)</td>
</tr>
<tr>
<td>Stomach</td>
<td>2.1</td>
<td>2.0</td>
<td>1.8</td>
<td>2.8</td>
<td>1.4 (2)</td>
<td>0.9 (2)</td>
<td>1.8</td>
</tr>
<tr>
<td>Colon</td>
<td>1.4 (5)</td>
<td>1.8 (2)</td>
<td>2.3</td>
<td>1.7 (3)</td>
<td>1.0</td>
<td>2.4 (4)</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1.8</td>
<td>2.4</td>
<td>2.7</td>
<td>2.5 (2)</td>
<td>1.36</td>
<td>1.1 (3)</td>
<td>3.1</td>
</tr>
<tr>
<td>Lung</td>
<td>2.1 (2)</td>
<td>1.4 (3)</td>
<td>1.7</td>
<td>1.7 (4)</td>
<td>0.7</td>
<td>2.4 (3)</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>1.3</td>
<td>1.0 (5)</td>
<td>1.2</td>
<td>1.6</td>
<td>1.53</td>
<td>2.4 (3)</td>
<td>1.0 (5)</td>
</tr>
<tr>
<td>Gyn ca</td>
<td>1.6</td>
<td>2.3</td>
<td>2.4 (3)</td>
<td>7.2</td>
<td>4.1 (4)</td>
<td>1.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Prostate</td>
<td>0.9</td>
<td>1.1</td>
<td>1.1 (1)</td>
<td>0.9</td>
<td>1.5</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>7.3 (3)</td>
<td>7.9 (4)</td>
<td>6.2 (4)</td>
<td>12.5 (5)</td>
<td>79.9 (1)</td>
<td>6.9 (3)</td>
<td>7.3</td>
</tr>
<tr>
<td>Bladder</td>
<td>2.0</td>
<td>2.4</td>
<td>2.0</td>
<td>8.2</td>
<td>6.6</td>
<td>5.1</td>
<td>3.3</td>
</tr>
<tr>
<td>Kaposi</td>
<td>17.1</td>
<td>40</td>
<td>4.3</td>
<td></td>
<td></td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>4.6</td>
<td>7.0</td>
<td>4.1</td>
<td></td>
<td>12.4 (5)</td>
<td>2.5 (1)</td>
<td>6.9</td>
</tr>
<tr>
<td>Non-Hodgkin 3</td>
<td>8.8 (1)</td>
<td>12.5 (1)</td>
<td>4.8 (2)</td>
<td>15.8 (1)</td>
<td>11.1 (3)</td>
<td>11.3 (1)</td>
<td>9.9 (2)</td>
</tr>
</tbody>
</table>

† NMCS excluded, ² CIN III included in Sweden, Gyn ca = gynecological cancer i.e. cervix cancer for all countries except Japan where uterus cancer was more common, ³ Non Hodgkin lymphoma. HK = Hong Kong, ca = cancer.
Table 2. Number of renal transplanted patients and post-transplant cancers in studies from different countries.

<table>
<thead>
<tr>
<th>Country</th>
<th>Tx(n)</th>
<th>Posttx(n)</th>
<th>Tx Reg</th>
<th>Ca Reg</th>
<th>Nr ca</th>
<th>Source</th>
<th>Timespan</th>
<th>D(y)</th>
<th>Qua(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>102 654</td>
<td>10 656骚</td>
<td>OPTN</td>
<td>SRTR</td>
<td>All</td>
<td>Engels 2013</td>
<td>1987-2008</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>11 155</td>
<td>778骚</td>
<td>CORR</td>
<td>CCR</td>
<td>1</td>
<td>Villeneuve 07</td>
<td>1981-1998</td>
<td>17</td>
<td>95</td>
</tr>
<tr>
<td>Sweden</td>
<td>7952</td>
<td>2774</td>
<td>SNPR</td>
<td>SCR</td>
<td>1</td>
<td>Krynitz 2014</td>
<td>1970-2008</td>
<td>7.9骚</td>
<td>97</td>
</tr>
<tr>
<td>HK</td>
<td>4674</td>
<td>299</td>
<td>RR HK</td>
<td>RR HK</td>
<td>All</td>
<td>Cheung 2012</td>
<td>1972-2011</td>
<td>8.2骚</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Australia, NZ</td>
<td>10 180</td>
<td>1236骚</td>
<td>ANZ-DATA</td>
<td>NCSCH</td>
<td>Vajdic 2012</td>
<td>1982-2003</td>
<td>8.5骚</td>
<td>&gt;95</td>
<td></td>
</tr>
</tbody>
</table>

Tx = transplant(ed); ca = cancer; n = number; Reg = Registry; Nr = first (1) or all cancers; D = duration; Qua = quality, completeness. HK = Hong Kong; NZ = New Zealand. 1 Non melanoma skin cancers excluded; 2 Median; 3 Mean; 4 From USA all solid organ recipients included.

OPTN=US Organ Procurement and Transplantation network; SRTR=Scientific Registry of Transplant; CORR=Canadian Organ Replacement Register database; CCR=Canada Cancer Registry; UK TR=United Kingdom Transplant Registry; CR=Cancer Registry in United Kingdom; SNP=Swedish National Patient Register; SCR=Swedish Cancer Registry; RR HK=Renal Registry of the Hospital Authority of Hong Kong and Medical Records; CR K=Annual Report on Cancer Registry of Korea; TR MR=Transplant Registry and Medical Records in Seoul; LH=Department of pathology of the Local Hospitals.

Death with a functioning graft (DWFG) accounts for 47% of all transplant losses 10 years post-transplant (Bottomley and Harden 2013) and cardiovascular diseases constitute the most common cause of mortality (40-50%) and malignant diseases constitute the second most common cause of death (27%) (Morath et al. 2004). Mortality from cardiovascular diseases occurs 20 times more often than in the general population; still this risk is smaller than in the patients with renal failure on dialysis. While atherosclerosis is the main feature of cardiovascular diseases in the general population, medial calcification of the arteries and left ventricular hypertrophy are the most dominant features in the renal transplanted population (Bottomley and Harden 2013).
Cancer specific mortality

Mortality due to cancer is 2.8 times higher in liver, heart and lung transplant recipients than in the matched general population in Australia. Median follow up was 5.2 years. De novo cancer was the leading cause of death among heart and lung transplanted patients (Na et al. 2012).

Mortality due to cancer is 2.3 times higher in renal transplanted recipients than in the general population in Hong Kong with the corresponding malignancy, (3.4 times higher for women and 1.7 times higher for men). More than half of the deaths occurred within the first year after the cancer diagnosis, 72% of them were due to cancer and 17% were due to sepsis. The SMR was 18.2 for Non-Hodgkin lymphoma, 4.4 for kidney cancer and 4.7 for bladder cancer compared to the normal population (Cheung et al. 2012).

Cancer specific death rates in renal transplant recipients were compared by indirect standardization to cancer specific death rates in the general population in a register study from USA. Overall SMR was 0.96, lower in the transplanted population than in the general population, the exception was younger age groups. Median follow up was 5 years. 5.3% of all deaths were cancer induced, SMR was 0.85 when with patients with graft loss were excluded. The type of cancer leading to death was not known. Hypothesis was that SMR was not increased due to competing risks of death such as infections and cardiovascular diseases (Kiberd et al. 2009). In a recently published article from Canada the cancer specific mortality is elevated in all solid organ recipients, even when patients with pre-transplant malignancies were excluded from the analyses (Acuna et al. 2016). It was also increased in patients with pre-transplant cancer compared with transplanted patients without a previous cancer (Brattstrom et al. 2013).

Registries

Transplant registries

The Collaborative Transplant Study (CTS)
The Collaborative Transplant Study Registry in Heidelberg (CTS) started in 1982 and is one of the largest transplant registries collaborating with more than 300 transplant centres worldwide. It collects information on renal, liver, pancreas, heart and lung transplant recipients. At one year post-transplant the clinical follow up records are complete for 97% of the patients and at 10 years post-transplant for 92% of the patients (Opelz et al. 2013). Human leucocyte antigen (HLA) types of the donor and recipients, graft function, graft failure, death, de novo malignancies, type of immunosuppression, rejection episodes, blood pressure, anti-hypertensive medication, de novo diabetes, fractures, cataract and laboratory values are registered. The risk of
underreporting malignancies has been managed by excluding patients from CTS cancer analysis if the annual confirmation regarding malignancies from the participating centres was missing (Opelz et al. 2013). The Department of Transplantation Surgery at Uppsala University Hospital has reported information of all kidney and simultaneous pancreas and kidney transplanted patients for approximately 2700 patients to the registry since 1982.

The Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry

The ANZDATA was founded in 1982 and it contains data of all patients with renal failure (receiving haemodialysis or peritoneal dialysis) and of renal transplanted patients as well as data on heart- lung- and pancreas-transplanted recipients in Australia and New Zealand. Information on donors, recipients, underlying renal disease, co-morbidity, rejections, cancer, graft function, graft failure and death is recorded. Approximately 27,000 renal transplanted patients are included in the registry (www.anzdata.org.au). The Australian National Cancer Statistics Clearing House also started in 1982 and contains information on all cancers except SCC in the general population. Information on cancer incidence among transplanted patients has been obtained by linking these registries together (McDonald and Russ 2013).

US Organ Procurement and Transplantation Network (OPTN)

OPTN was established in 1984 and it is mandatory for US transplant programmes to be members of OPTN (optn.transplant.hrsa.gov). It collects information from transplant centres from 6 months after transplantation and then yearly. The data are further provided to the Scientific Registry of Transplant Recipients (SRTR), founded in 1987, which contains data of all US solid organ transplant recipients. Since 1987 information of more than 200,000 solid organ recipients have been collected. About half of them received a renal transplant. By cross-matching SRTR with 13 US population-based cancer registries information of post-transplant cancers was obtained on cancer incidence in the solid organ transplanted population. Mortality is obtained by linking SRTR with the US Social Security Death master File (Engels et al. 2011).

Svenska Njur Registrat (SNR)/ Swedish Renal Registry (SRR)

SRR is a national quality registry comprising information of all patients with renal failure, patients on haemo- or peritoneal dialysis and patients with renal graft(s) in Sweden. It follows the individual patient from progress of renal failure to follow up after renal transplantation. It aims to form guidelines for general care and treatment in patients with renal failure, to follow the progression of renal failure, to identify risk factors or the effect of different treatment options on the progression of renal failure and to improve the
treatment of these patients in the future. Information such as underlying renal disease, number of transplant graft function, anti-hypertensive medication graft function, graft failure, immunosuppression, malignancies, cardiovascular diseases, causes of death are collected. At the end of 2014, 810 patients were on peritoneal dialysis, 3049 patients received haemodialysis and 5361 patients had a functioning renal graft in Sweden (www.medscinet.net/snr).

**Uppsala University Hospital Transplantation Database**

Information of all kidney and simultaneous pancreas and kidney transplanted patients have been collected at the Uppsala University Hospital Transplantation Database since 1982. In all approximately 2700 patients are included in the registry. About 90 patients have been lost to follow up. Information on e.g. underlying disease, transplant information, graft function, immunosuppression, co-morbidity, laboratory values, social status, rejections, treatment of rejection, induction therapy, maintenance immunosuppression are collected annually until 5 years after transplantation and then at 5-year intervals.

**Cancer registries**

**The Israel Penn International Transplant Tumour Registry**

Dr Israel Penn was the first doctor, in 1967, to recognize the elevated risk of post-transplant malignancies. He founded the Denver Transplant Tumour Registry, later renamed the Cincinnati Tumour Registry and finally the Israel Penn International Transplant Tumour Registry (IPITTR). He collected information on 15,000 post-transplant malignancies during his lifetime. This registry is the largest and most comprehensive transplant tumour registry in the world (https://ipittr.uc.edu). IPITTR collects data from the USA as well as from all over the world. It provides information on a consultancy basis regarding tumours in transplanted patients and aims to provide data concerning post-transplant malignancies. Patient demographics, transplant information, rejection episodes, immunosuppression adjustments, malignancies, chemotherapy regimens, immunotherapy regimens, radiation treatments, surgical procedures, and patients update status are continuously recorded (Witherow et al. 2003). There is a known underreporting of malignancies to this registry because reporting is voluntary in the United States.

**The Swedish Cancer Registry**

The Swedish Cancer Registry was founded in 1958 and is a national registry covering the entire Swedish population. It is divided into six regional cancer registries, and the regional tumour registry of the Uppsala Örebro region (RTR) covers the region of Uppsala University Hospital. It is by law mandatory to report all incident malignant tumours to the registry. More than 98%
of all malignancies are reported and 97% of the histological verification can be found in the registry (Barlow et al. 2009). Patient data such as personal identity number and place of residence, and cancer information such as date of cancer diagnosis, histological code, morphological code, base of diagnosis, hospital at which the cancer was diagnosed, pathological identification number of the cancer, stage of the tumour, date of death and cancer induced death is found in the registry. The diagnoses of the tumours follow different versions of International Classification of Diseases (ICD) according to the time the tumour was diagnosed. Cancer types are divided into 15 main groups and 97 subgroups depending on localization of the tumour (www.cancercentrum.se, www.icd.nu).

Screening of cancer

Screening is defined as examination of a group usually consisting of asymptomatic individuals to detect those with a high probability of having or developing a given disease. The benefit of screening is supposed to outweigh harm-harm and harm-benefit analyses and the screening is supposed to be cost effective.

Which types of cancer should be screened for in the transplanted population? Should transplanted patients be screened in a different way than the normal population? Are screening methods valid in transplanted patients? And who is responsible for screening of the transplanted population? According to the Clinical Practice Guidelines for Kidney Disease (KDIGO) the transplanted population should attend the national general screening program for breast, cervix, prostate and colon cancer (Kidney Disease: Improving Global Outcomes Transplant Work 2009, Ad-hoc working group of et al. 2012). In USA it is advisable to expand the general screening recommendations to include breast, colon and prostate cancer for renal transplant recipients (Kiberd et al. 2003). The number of patients needed to screen to save one life is increased in the transplanted population due to the decreased life expectancy. Screening is therefore less cost effective in transplanted than in non-transplanted patients (Kiberd et al. 2003). Among transplanted patients screening with Papanicolau (Pap) smear for cervix cancer is recommended (Courtney et al. 2009, Meeuwis et al. 2010). It is also probably cost effective to screen for colon cancer but not for breast, prostate (Kiberd et al. 2003) or renal cancer (Wong et al. 2011).

The guidelines for prostate cancer screening have recently changed in the general population (Cooperberg 2016). Transplanted men should be screened with prostate specific antigen (PSA) when symptoms appear according to the recommendations of the Nordic Group on Post-transplant Malignancies. The screening of colon cancer should be carried out in accordance with local practice. Campistol et al suggest faecal occult blood test
annually, sigmoideoscopy every five years and colonoscopy every 10 years (Campistol et al. 2012).

A dermatologist should evaluate all renal transplant recipients for risk stratification within two years post-transplantation and thereafter see the patients at regular intervals, depending on the risk stratification. All organ recipients should receive information regarding sun exposure and adequate sun protection.

A recommendation of the Nordic Group on Post-transplant Malignancies is that transplanted women should participate in the general breast screening programs. They should also undergo HPV or Pap smear tests annually as a part of cervix cancer screening. HPV tests are increasingly used instead of Pap smears for cervix cancer screening because cervical dysplasia and cancer are HPV induced (Mbatani et al. 2016). Unfortunately only 10 to 20% of all eligible renal transplanted women participate in the screening programs. Still 20% of all Pap smears in transplanted patients show abnormalities compared to 7% in the general population. A speculation why not only transplanted women but all transplanted patients are less prone to participate in screening programs is that they get tired of all medical follow-ups or that they have not been adequately informed of the increased cancer risk (Courtney et al. 2009, Meeuwis et al. 2010).

The Nordic Group on Post-transplant Malignancies suggests that the nephrologists stress the importance of screening because they regularly see all renal transplanted patients.

Treatment

Multidisciplinary conferences (MDT)

Multidisciplinary cancer conferences (MDT) contribute to improved clinical decision making, clinical outcome and patient experience (Taylor et al. 2010). However 10 to 20% of the evaluated cancer patients at MDT do not get adequate oncological treatment due to co-morbidity (Kastner et al. 2006). Renal dysfunction and renal transplantation is regarded as co-morbidity; besides many transplanted patients also suffer from diabetes or cardiovascular diseases. Consequently they are regarded as high-risk patients and they have many significant negative prognostic factors for long-term cancer survival (Kastner et al. 2006). Decisions regarding oncological treatment at MDT are not taken, do not follow clinical guidelines or are less likely to be implemented (Sarfati et al. 2009, Lamb et al. 2013). Accordingly these patients get a more conservative treatment, despite the fact that an active treatment, most of the time, probably would be tolerated (Gross et al. 2007, Stairmand et al. 2015).
Oncological treatment in renal transplanted patients

**Solid organ tumours**

Most transplanted patients with malignancies need conventional oncological therapy such as surgery, chemotherapy or radiation (Campistol et al. 2012).

Treatments for virus-induced tumours may include anti-viral treatment in combination with minimization of immunosuppression (Baron et al. 2001, Reusser et al. 2015).

Donor tissue-derived tumours are managed by reduction or cessation of immunosuppression to activate the recipient’s own immune system. The tumours can be rejected merely by host immune surveillance.

Immunotherapy, e.g. using antibody-mediated blockade of cytotoxic T cell antigen 4 (CTLA4) or CD40 ligand have shown promising results in non-transplanted cancer patients (Honeychurch et al. 2015) with urothelial cancer (Malmstrom et al. 2010) and malignant melanoma (Zhang M et al. 2016) and could be usable in patients with donor-derived tumours. They may stimulate anti-tumour or anti-viral responses or both like they do in other cancer patients with autologous tumours, and they may amplify the allogeneic reaction against the donor-derived tumour. They increase, however, the risk for acute rejection (Loskog A and Totterman 2007, Li et al. 2008, Loskog AS and Eliopoulos 2009, Sandin et al. 2014).

**Skin tumours**

*Sun protection*

Renal transplant recipients are advised to avoid sun exposure and to use broad-spectrum sunscreens. Sun protective clothing and UV protective sunglasses are recommended. Patients are encouraged to avoid active sun bathing and staying out during the peak hours of 11 am to 3 pm (Ritchie et al. 2012). Regular use of broad-spectrum sunscreens with a sun protective factor (SPF) >50, high UV absorption and application of 2 mg/cm² significantly reduced development of de novo actinic keratosis, SCC in situ, invasive SCC and partly BCC during a follow up of 2 years (Ulrich et al. 2009).

Self-examination of the skin, dermatological follow up, patient education and multidisciplinary care are needed to prevent skin cancers.

*Dermatological treatment*

Heredity for skin cancer, sun damage, skin type according to Fitzpatrick’s classification, history of cutaneous malignancies, precancerous lesions, number, histology, staging and clinical features of the skin cancers are considered at the dermatological evaluation. Treatment strategies and follow-up intervals are based on this information (Ulrich et al. 2008b). The three main categories of treatment are primary, preventative and adjuvant treatment (Ritchie et al. 2012). Treatment options of low risk SCC comprise surgical therapy, cyclical topical therapy, photodynamic therapy and systemic retin-
oid therapy as chemoprevention. Treatment options of high risk SCC include aggressive surgical therapy, sentinel lymph node biopsy, adjuvant radiation therapy, reduction of immunosuppression and chemotherapy (Zwald and Brown 2011).

Immunosuppressive treatment in renal transplanted patients with malignancies

In patients with post-transplant malignancies the immunosuppression is generally reduced to prevent further growth and spread of malignant cells (Ajithkumar et al. 2007). Furthermore, the immunosuppressive effects of chemotherapy occasionally render immunosuppression unnecessary. A switch from CNI to mTOR inhibitors could be considered (Kauffman 2006, Ajithkumar et al. 2007, Alberu et al. 2011, Campbell et al. 2012, Budde et al. 2015). There are also known interactions between immunosuppressants and many chemotherapeutic agents, which explain why doctors might be reluctant to change the therapy. Adequate renal transplant function is often also a prerequisite for chemotherapy.
Aims

I To assess whether previous malignancies pose an increased risk of post-transplant cancer and reduced patient survival.

II To add an mTOR inhibitor to the immunosuppressive regimen in transplanted patients with post-transplant malignancies, to improve identification of post-transplant malignancies and to reassure adequate oncological treatment of these patients by implementing MDT evaluations of oncological and immunosuppressive treatments.

III To investigate which risk factors or combination of risk factors predict a subsequent SCC among kidney or simultaneous pancreas and kidney transplanted patients with a history at least one previous SCC.

IV To investigate recipient or donor origin and presence of BKV in urological tumours in kidney transplanted patients.
Materials and Methods

Patient registries (Paper I-IV)
In the Uppsala-Örebro region of Sweden with a population of two million inhabitants, all kidney and simultaneous pancreas and kidney transplantations are performed at Uppsala University Hospital. Information, including data on malignancies, is annually registered in the Uppsala University Hospital Transplantation Database.
Information on all transplanted patients has been reported to the CTS registry since 1982. From 2009 onwards information on post-transplant malignancies has been retrieved by linking the Uppsala University Hospital Transplantation Database with RTR. This allowed for more than 95% detection of post-transplant malignancies. Information on causes of death was obtained from medical records or the Swedish Renal Registry.

Patients (Paper I-IV)
Between June 1969 and December 2014, 2835 kidney or simultaneous pancreas and kidney transplantations were performed in 2437 patients at the Uppsala University Hospital in Sweden. Ninety-two patients were lost to follow up. A total of 651 patients were diagnosed with malignancies until August 2015; 120 were diagnosed with pre-transplant malignancies and 531 patients with post-transplant malignancies.

Maintenance immunosuppression in Uppsala consisted of azathioprine and corticosteroids until 1982. After that it consisted of CNI in combination with corticosteroids with or without azathioprine. Between 1995 and 2005 MMF was prescribed for one year to most patients, but since 2005 MMF has been administered continuously up to 10 years post-transplant. Since 2000 basiliximab has been used as induction therapy for most patients while ATG has been used as induction therapy only in highly immunized patients.
Figure 4. CONSORT flow chart of all kidney and simultaneous pancreas and kidney transplanted patients included in Studies I-IV.
Patients (Paper I)

**Study group**

From January 1982 to December 2013, 471 of 2085 renal transplanted patients with pre- and post-transplant cancers were identified in the Regional Tumour Registry. In total 104 were diagnosed with a malignant tumour prior to first kidney transplantation. Additionally, 12 patients with a pre-transplant cancer were found in the Uppsala University Hospital Transplantation Database and CTS. Hence, in total pre-transplant cancers were found in 116 patients. Patients with the following criteria were excluded from the study: undiagnosed malignancies at the time of transplantation (i.e. diagnosis < 2 months post-transplant, \(n=5\)), death within a week after transplantation (\(n=3\)) and first transplantation at another hospital (\(n=6\)). Seven patients were excluded because the malignancy diagnosis could not be verified in the medical records.

In total 95 patients with documented pre-transplant cancer were included in the study (Figure 4). The follow-up period was at least one year.

Clinical data were retrieved from medical records to confirm the diagnosis, treatment and recurrent disease. Cause of death was obtained from medical records or Swedish Renal Registry.

**Control groups**

The study group was compared with two different control groups, one nested case control group that comprised renal transplanted patients with no pre-transplant cancer in Europe and one group being all transplanted patients in Uppsala without pre-transplant cancer. CTS matched the case control groups according to age and year of transplantation, gender and number of kidney transplantations (first or retransplantation) in a 1:3 ratio from a total of 189,322 patients in Europe (Sweden excluded). Ninety-five matched pairs were found in the 1:3 matched control group from Europe. The study group was also compared to the entire renal transplanted population in Uppsala (\(n=1990\), transplanted after 1981).

Patients (Paper II-III)

All patients who had encountered a malignancy or recurrence of malignancy from September 2006 or at most one year before inclusion were eligible to be included in this study (\(n=230\)). The inclusion criteria were adult patients (> 18 years old) willing and capable of giving written informed consent of participation in the project and patients with a previous diagnosed or presently diagnosed or recurrence of a malignancy (other than basal cell carcinoma). All participants signed a written informed consent. One hundred and twenty study patients were included between September 2007 and December 2012 in the “Follow up of malignant tumours in renal transplanted patients”
Patients (Paper IV)

In total 531 patients were diagnosed with post-transplant malignancies between 1969 and August 2015. Forty-three patients were diagnosed with urological malignancies, 13 patients with malignancy of the urinary tract and 30 patients with malignancy of the kidney. Of the 30 patients with malignancy in the kidney, 26 patients had tumour in the native kidney(s) and 4 patients had tumour in the kidney transplant (Figure 4).

Seventeen patients were included in this partly retrospective study; all patients with malignancy of the urinary tract (n=13) as well as all patients with malignancy in the renal transplant (n=4). Clinical data including survival of the patients were collected from the medical records. Information regarding sex, age and HLA genotype of donor and recipient were retrieved from the Uppsala University Hospital Transplantation Database.

Archival paraffin blocks of urological tumour tissue were obtained from the respective regional hospitals of the patients. In one patient there was not enough tumour tissue for analysis and in another patient tumour tissue was accidently not correctly coded. These tumours of the urinary tract (n=2) were excluded from the analyses. Hence the final number of included patients in the study was n=15.

Study design (Paper I)

The study was designed as a retrospective nested case-control study. De novo cancers, recurrent cancers, patient survival, mortality due to cancer and graft survival were analysed in patients with pre-transplant cancers and in the control groups.

Study design (Paper II)

The study was designed as a prospective, observational one-armed study. A multidisciplinary team consisting of transplant surgeons, nephrologists, oncologists and dermatologists evaluated the patients regarding the oncological and immunosuppressive treatment. This included the aim to add or to convert to mTOR inhibitors. In this study everolimus was used as mTOR inhibitor. Patients with a GFR > 20mL/min, haemoglobin count > 80 g/dl, platelet count > 50x10^9/L, white blood cell count > 2.5x10^9/L, total cholesterol < 9
mmol/L, triglycerides < 6 mmol/L, spot urinary albumin/creatinine ratio < 70 mg/mmol, transaminases or bilirubin within normal ranges, low or moderate immunological risk profile (<30% panel reactive antibodies) and without contraindications to mTOR inhibitors were eligible for an addition of or switch to everolimus. Everolimus was given in combination with low dose CNI in patients at higher immunological risk defined as immunised patients (>30% panel reactive antibodies), patients with previous episodes of acute rejections or retransplanted patients, and in patients with adverse events on full dose of mTOR inhibitors.

Changes in immunosuppression, adjustment of the oncological treatment, tumour progression, regression or recurrence of the diagnosed malignancy, de novo diagnosed malignant tumours, renal function, graft and patient survival and immune response were followed. The trough levels of different immunosuppressants after adding everolimus to the treatment regimen were 3-6 ng/mL for everolimus, 30-80 ng/mL for cyclosporine A and 1.5-4 ng/mL for tacrolimus.

All patients were examined at the first visit, 2 to 3 months after inclusion, and once yearly until 3 years after inclusion.

Follow-up information about patients who were unable to appear at regular visits was collected from the regional hospitals.

Study design (Paper III)

The patients were assessed clinically regarding cutaneous sun damage and recorded risk factors of SCC and type of immunosuppressive treatment. Baseline was identified as the date of inclusion. The information was noted in the medical records at the Department of Transplantation Surgery and transcribed to the general study chart. Primary end point comprised the first de novo SCC appearing < 2 years after inclusion. We investigated if the occurrence of de novo SCCs correlated to clinical assessment of sun damage. Secondary endpoint was to identify the most important risk factor or combination of risk factors to develop de novo SCC.

Clinical assessment of sun damage (Paper III)

To get an overall impression of the skin, a senior specialist in dermatology on an empirical basis evaluated the skin of 68 of 73 SCC patients. The remaining five patients, who did not meet the dermatologist, were excluded from this analysis. The examined patients were divided into three groups depending on the extent of cutaneous sun damage i.e. patients with mild, moderate or severe sun damage. The clinical assessment included skin type, history of sun exposition, and concomitant skin lesions.
Risk factors for developing cutaneous SCC (Paper III)

The potential risk factors of de novo SCC were: age at first renal transplantation, age at first and current SCC diagnosis, time span between first renal transplantation and first respective most recent SCC, skin type according to Fitzpatrick’s classification, sun exposure, skin status, histology of the current SCC lesion, number of previous SCC, type of immunosuppression and gender.

Skin type
All patients were Caucasians except one who was an Asian. Skin types were distributed as skin type I (n=23), skin type II (n=17), skin type III (n=27) and skin type IV (n=6) according to Fitzpatrick (Roberts WE 2009).

Sun exposure
Sun exposure was assessed based on anamnestic information and divided in groups; low exposure for patients avoiding the sun (n=13), medium exposure (n=23) and high exposure for patients actively sun bathing in the past (before transplantation) (n=30) or continuously (n=7). Patients with low or medium exposure were classified as group 1 (low risk) and patients with high exposure were classified as group 2 (high risk).

Empirical assessment of the skin
One experienced dermatologist (YE) classified the skin status of all patients into risk-groups based on dermatological evaluation and information in the dermatological records. Patients classified as group 1 (low risk) had normal skin status according to age (n=14) or minor skin lesions such as aged skin, angiectasies, elastosis, and some solar lentignes (n=7). Patients classified into group 2 (high risk) suffered from actinic keratosis, plenty of solar lentignes (n=20) or more advanced lesions like numerous actinic keratosis and field cancerization (n=32).

The evaluation was indirectly validated by independent blinded classification of all available data by another experienced dermatologists (FN). The classification into risk groups was consistent for 67 patients (92%). In six patients different result was achieved and the higher risk group was chosen for these patients.

Histology of current SCC
Patients with SCC in situ (group 1, n=37) were compared to patients with invasive SCC (group 2, well differentiated (n=13), moderately differentiated (n=16) or poorly differentiated (n=7) SCC).
**Number of previous SCC**

Patients with no earlier SCC (n=25) were classified as group 1, patients with 1 earlier SCC (n=14) were classified as group 2 and patients with more than 1 previous SCC (n=34) were classified as group 3.

**Immunosuppression**

All patients except one had CNI based maintenance immunosuppression, and all patients except n=4 (5%) had corticosteroids in combination with CNI at inclusion. Four patients (5%) had an SCC diagnosed before the first renal transplantation.

Previous azathioprine treatment (n=26) or no previous azathioprine treatment (n=47) were recorded. Ongoing MMF medication (n=30) as well as no medication (n=29) or interrupted MMF medication (n=14) were noted. Ongoing everolimus medication i.e more than 21 months (n=19), no previous (n=36) as well as less than 3 months use of everolimus (n=18) were registered.

The risk factors were compared between patients developing and not developing de novo SCC.

**Study design (Paper IV)**

In this study we assessed the donor or recipient origin and presence of BKV in urological tumours in renal transplanted patients.

**Histology (Paper IV)**

An experienced pathologist re-evaluated the histology of the tumour tissue in the obtained archival paraffin blocks. Representative tumour material from transplantectomy specimens and biopsies from the urinary tract and renal transplants had been fixed in 4% phosphate buffered formaldehyde and embedded in paraffin. Tissue sections (4 to 5 µm thick) had been processed for routine histology and stained with haematoxylin-eosin, and Sirius red for collagen.

**Analysis of donor or recipient origin of tumours by HLA genotyping (Paper IV)**

HLA genotyping of the tumour was performed when the donor and recipient were HLA-A, HLA-B or HLA-DRB1 incompatible. Genomic DNA was extracted from manually dissected tumour tissue by using Recover All Total Nucleic Acid Isolation kit (Ambion, Austin, TX, USA) following the protocol of the manufacturer. The HLA region was amplified by polymerase
chain reaction (PCR) using sequence-specific primers (SSP; Olerup, Stockholm, Sweden) according to standard protocol (Kinch et al. 2014b). HLA-A, HLA-B and HLA-DRB1 were routinely tested (Mazzi et al. 2008). The HLA type in the tumour was compared to the known HLA type of the recipient and the donor.

Fluorescence in situ hybridization (FISH) analysis of different genders in tumours (Paper IV)

FISH was used to determine donor or recipient origin of tumours in cases where the donor and recipient were HLA identical but had different gender. Representative tumour specimens, 4 µm thick, formalin-fixed and paraffin-embedded were analysed. First the tumour specimen was deparaffinised and incubated in hydrochloric acid (0.2M). After 20 minutes at room temperature the tumour specimen was rinsed and kept in VP2000 Pretreatment Reagent. In order to separate the DNA strands a protease was added (Abbott Molecular Inc, Abbott Park, IL, USA). DNA probes with fluorescent label, AneuVysion probe kit (Vysis CEP 18, X, Y-alpha satellite probe Abbott Molecular Inc), caused hybridization to the centromeres of the X- and Y-chromosomes. Vectashield mounting medium with 4',6-diamidino-2-phenylindole (DAPI; Vector Laboratories Inc, Burlingame, CA, USA) was used to mount the chromosomes. To identify the XX or XY karyotype a fluorescence microscope (Zeiss, Oberkochen, Germany) was used. The analyses of the images were performed with the ISIS software (MetaSystems, Altlußheim, Germany).

Analysis of BKV in tumours by immunohistochemistry

The immunohistochemical stainings were performed according to standard methods (Sternberger 1979). Briefly, 3- to 4-µm sections were prepared from the paraffin blocks and attached to positively charged glass slides (Superfrost Plus; Menzel Gläser, Braunschweig, Germany). A monoclonal antibody against SV40 large T antigen (PV T-Ag) (clone pAb416; Calbiochem, Merck Millipore, Darmstadt, Germany) was used to detect BKV in tumours.

Immunostainings were done on a Dako Autostainer (Dako, Stockholm, Sweden) according to established protocols. The slides were pretreated in DAKO target retrieval solution at pH 9. The primary antibody was incubated for 20 min at room temperature. The staining result was visualised using the DAKO Envision flex polymer kit. Slides were counterstained, mounted and examined microscopically for nuclear staining.
Immunomodulatory treatment (Paper IV)

**GMP grade AdCD40L**

The clinical grade vector batch used to treat one of the patients was manufactured by the Center for Cell and Gene Therapy (Baylor College of Medicine, Houston, TX, USA).

Anti-viral treatment (Paper IV)

Cidofovir, (Vistide® produced by Pfizer and Gilead) was administered in the dose of 0.5mg/kg as an intravenous infusion once a week in two patients with BKV positive cancer of the urinary tract.

Ethics (Paper I-IV)

The study of pre-transplant tumours (Paper I) was approved by the Regional Ethical Review Board in Uppsala, Sweden (Dnr 2014/038) and registered at ClinicalTrials.gov (NCT02491580).

The clinical studies of post-transplant malignancies and risk factors for de novo SCC (Paper II and III) were approved by the Regional Ethical Review Board in Uppsala (Dnr 2007/032). The MALTX study was registered at ClinicalTrials.gov (NCT02241564).

Paper IV was approved by the Regional Ethical Review Board in Uppsala, Sweden (Dnr 2007/032 amendment 5) and approved by the Uppsala Biobank, Sweden (ID: BbA-827-2015-081). The use of the vector was approved as compassionate treatment by the Swedish Medical Products Agency (EudraCT 2006-000985-34).

Statistical methods (Paper I-III)

**Paper I**

Post-transplant cancer incidence, graft and patient survival were determined by using the Kaplan-Meier (KM) method and entered with ± standard error. The Log-Rank (Mantel-Cox) test was used to determine whether the KM survival curves differed between the groups. Propensity score matching was performed to account for the influence of the confounding factors year of transplant, transplant number, recipient age and gender. The univariate Kaplan-Meier results were confirmed by hazard ratios (HR) and the corresponding 95% confidence intervals (CI) of multivariable Cox regression analysis with the confounders transplant year, transplant number, recipient age and gender, donor age, original disease leading to transplantation, HLA-
A+B+DR mismatches and pretransplant antibodies. The software IBM SPSS Statistics version 22 (SPSS Inc, IBM Corporation, Somers, NY, USA) was used for survival analyses, including the extension package “Propensity Score Matching Version 3.0.2” of F Thoemmes (Thoemmes, F. an SPSS R Menu for Propensity Score matching. 2011, arxiv.org). Differences in characteristics and cancer between women and men were analysed using the non-parametric Mann Whitney U-test. Data are presented as medians with ranges. A P-value less than 0.05 was considered significant.

Paper II

The post-transplant tumour incidence, between the renal transplanted population in Uppsala and the renal transplanted population in CTS before and after the linkage to RTR, was determined by using the KM analysis, described earlier. The significance levels were adjusted for multiple testing by Bonferroni’s corrections. SPSS (IBM) was used for all KM analyses. The tumour incidence was presented as SIR.

Serum creatinine levels were presented as mean ± standard deviation (SD). The non-parametric Kruskal-Wallis test was used to evaluate the difference in serum creatinine levels from the time of inclusion until the three-year follow-up. A p-value less than 0.05 was considered significant.

Paper III

Statistical analyses were performed using software R version 3.1.1 (The R Foundation for Statistical Computing, Vienna, Austria). Occurrence of de novo SCC was determined by using Cox regression. Log-Rank (Mantel-Cox) test was used within a 95% confidence interval to determine whether the Kaplan Meyer curves were statistically different between the clinical risk groups.

Continuous variables were presented as first quartile, median and third quartile values. Nominal variables were presented as absolute counts and percentages. The sample size was relatively small compared to the number of potential explanatory variables and it was unlikely a priori that statistical significance alone would be able to identify all interesting prognostic covariates. Therefore, in addition to a multivariate analysis of all candidate variables (the full model), we removed covariates one at the time to see which could be discarded with the smallest deterioration in predictive value, as measured with R2 (compared to the full model). In each step such a covariate was removed until we reached one of two levels of overall R2 reduction, 95% (model 1) and 90% (model 2). Thinking of the full model as the best we can achieve, the reduced model hints at what set of variables are more important.
Wilcoxon test was used for analyses of numerical data; age at transplantation, age at first SCC, time between first transplantation and first SCC, age at current SCC and time between transplantation and current SCC. Fischer’s exact test were used for analyses of categorical data; skin type, sun exposure, clinical assessment of the skin, histology of current SCC, type of immunosuppression (Azathioprine, mTOR inhibitors or MMF), number of previous SCC and gender.
Results

Paper I
A total of 36 of 95 patients (38%) in the study group experienced a malignancy during post-transplant follow up (median 5 years, range 0.2 to 26 years). Recurrent cancer occurred in 18 of 95 (19%) patients. De novo cancer occurred in 24 of 95 (25%) patients. In total 13 patients faced three or more cancers. The median follow up time from pre-transplant cancer was 13 years (range 1 to 42 years).

Cancer incidence
The post-transplant cancer incidence was significantly higher in patients with a previous malignancy than in patients without a previous malignancy.

Figure 5. Cumulative cancer incidence in patients with pre-transplant (+Pre-TX) cancer and in patients without pre-transplant (-Pre-TX) cancer.
in the 1:1 matched population in Uppsala ($P=0.002$), the 1:3 matched population in Europe ($P<0.001$) (Figure 4) as well as the entire renal transplanted population in Uppsala ($P<0.001$).

Even when patients with recurrent cancers were excluded, the incidence of de novo cancers in the study group remained significantly increased compared to the entire population in Uppsala 10 years post-transplantation ($P<0.001$).

Patient survival

Patient survival rate was lower in patients with a pre-transplant cancer than in patients without a pre-transplant cancer in the 1:3 matched population in Europe ($P=0.021$) (Figure 5) as well as in the entire renal transplanted population in Uppsala 10 years after transplantation ($P=0.004$).

![Patient survival curve](image)

*Figure 6. Patient survival in patients with pre-transplant (+Pre-TX) cancer and in patients without pre-transplant (-Pre-TX) cancer.*

Cancer induced mortality

Death due to malignancy was more common in patients with a previous malignant tumour than in patients without a previous malignant tumour in
the 1:3 matched population in Uppsala and the entire renal transplanted population in Uppsala 10 years after transplantation ($P<0.001$).

**Graft survival**

Death censored graft survival was unaffected in patients with a previous malignancy in comparison with the 1:3 matched transplanted patients in Europe ($P=0.81$) and all renal transplanted patients in Uppsala 10 years post-transplantation ($P=0.73$).

**Paper II**

**Patient identification and incidence of malignancies post transplantation**

When the project started, the cumulative incidence of post-transplant malignancies in Uppsala was equal to other European countries reporting to CTS with an SIR of 1.86 vs. 1.85 for solid organ and haematological malignancies (Figure 7A) ($p=0.55$) and an SIR of 20.7 vs. 20.1 for skin malignancies (Figure 7C) ($p=0.37$). From January 2009, when linkage of the transplanted patients with RTR was started, the cumulative incidence of all malignancies increased compared to other European countries with an SIR of 2.35 vs. 2.03 for solid organ and haematological malignancies (Figure 7B) ($p=0.015$) and an SIR of 33.3 vs. 18.5 for skin malignancies (Figure 7D) ($p<0.001$). The SIR for gynaecological malignancies was 0.8 in Uppsala and 1.85 in CTS before 2009 (Figure 7E). The SIR increased to 4.81 in Uppsala compared with 1.99 in CTS after the linkage (Figure 7F).

**Change in immunosuppression**

In total, the immunosuppression was converted to everolimus either by a complete switch ($n=44$) or as an addition to low dose CNI ($n=32$) for 76 of 120 patients (63%). The total immunosuppression was interrupted in 7 patients (6%), reduced in 3 patients (2%) and was unchanged in 35 of 120 patients (29%).

Within one year 28 of 76 patients (37%) with mTOR inhibitors interrupted the treatment due to adverse effects.
Figure 7. SIR of (A) solid and hematological cancer for renal transplanted patients in Uppsala and CTS before 2009 and SIR of (B) solid and hematological cancer for renal transplanted patients in Uppsala and CTS after 2009. SIR of (C) NMSC for renal transplanted patients in Uppsala and CTS before 2009 and SIR of (D) NMSC for renal transplanted patients in Uppsala and CTS after 2009. SIR of (E) gynecological cancer for renal transplanted patients in Uppsala and CTS before 2009 and SIR of (F) gynecological cancer for renal transplanted patients in Uppsala and CTS after 2009.

Oncological treatment of solid or haematological tumors

At the MDT conferences the planned chemotherapy was modified in 19 of 44 patients (43%), the planned radiation therapy was adjusted in 10 of 44 patients (23%) and/or the planned surgical treatment was altered in 4 of 44 patients (11%). In total, the oncological treatment was changed in 23 of 44 patients (52%). As a result of the MDT decisions 36 of 44 patients (82%) received adequate oncological treatment in line with the national guidelines,
despite renal dysfunction and immunosuppression. The other 8 patients (18%) were not treated in compliance with the guidelines. For these patients the oncological therapy was reduced or changed due to renal dysfunction, poor general condition or the patient’s choice. In conclusion, the MDT conferences modified the oncological and/or immunosuppressive treatment for 43 of 44 patients (97%) with solid or haematological malignancies.

Renal function, patient survival and graft survival
Serum creatinine was mainly unchanged during the follow up for all patients with a functioning graft at inclusion (P=0.644). The overall one year patient survival was 90% and the overall three year patient survival was 70%. The overall one-year death censored graft survival was 89% and the overall three-year death censored graft survival was 74%.

Paper III
During follow up 31 patients (42%) developed de novo SCC, whereof 15 (49%) were in situ SCC and 16 (51%) were invasive SCC.

Extent of cutaneous sun damage and development of subsequent SCC
De novo SCC did not occur more frequently in patients with severe sun damage than in patients with moderate or mild sun damage (p=0.216) after 2 years of follow up.

Registered risk factors and development of subsequent SCC
Patients with more than one previous cutaneous SCC had a 5 (1-21) times increased risk (R²) of a subsequent skin SCC, and patients with more than two cutaneous SCC had a 14 (3.1-63) times increased risk (R²), compared with patients without a previous SCC (p<0.001). Lower age at baseline SCC contributed more than the remaining risk factors to de novo SCC. Histology of the baseline SCC did not correlate to the histology of the subsequent SCC (p=1).

Paper IV
Eight of all 22 renal allografts originated from living donators and 8 renal allografts originated from deceased donators. The median age at transplantation was 43 years (range 22 to 64 years). Median duration from first transplantation to cancer diagnosis was 14 years (range 2 to 23 years).

Four of 6 examined patients were diagnosed with BK viraemia, defined as >10,000 copies/mL before the cancer diagnosis. Two patients had BKV
nephritis in the kidney allograft. Nine patients were not screened for BKV infection in blood.

Figure 6. BKV positive urothelial call carcinoma stained with PV-T-Ag. The surrounding tissue does not stain positive for BKV.

Donor or recipient origin of tumour
The results of the HLA genotyping and FISH analyses showed that 3 of 10 tumours in the urinary tract and 2 of 4 tumours in the renal grafts were of donor origin.

BKV occurrence in tumours
The immunostaining with PV T-Ag showed that 5 of 15 tumours were positive for BKV. BKV occurred in none of the four malignancies in the renal allografts but in five of eleven patients with malignancy in the urinary tract.

Treatment
Withdrawal of immunosuppression positively affected the first patient. The cancer was cured and he is alive 12 years after the cancer diagnosis. Immunostimulating therapy such as AdCD40L was an interesting option for the transplant-derived tumour in the urinary tract of the second patient. He expe-
rienced a dramatic improvement of the general condition and he lived nine months after the cancer diagnosis. Minimized immunosuppression combined with antiviral treatment was an option for the BK virus positive urothelial cancer of donor origin in the third patient. The tumour went into remission for almost a year and she lived 22 months after the cancer diagnosis.
Discussion

The main novel findings of this thesis with substantial clinical relevance are:

- Patients with a cancer prior to kidney transplantation showed a higher post-transplant cancer incidence and reduced survival compared with patients without a pre-transplant cancer than expected (Paper I).
- Post-transplant malignancies are heavily underreported in transplant registries (Paper II).
- In this first study of MDT evaluations focusing on malignancies in patients with a defined co-morbidity, i.e. renal transplantation, it was possible to optimize the immunosuppressive and oncological treatments for two-thirds of the patients (Paper II).
- Renal transplanted patients with post-transplant SCC are fewer than anticipated.
- Patients with one and especially two previous SCCs were at highest risk of developing subsequent SCCs. Younger age at diagnosis of current SCC comprised the most significant individual risk factor of de novo SCC (Paper III).
- The incidence of transplant-derived tumours is at least 5 times higher than earlier anticipated. We found that 50% of the cancers in renal transplants and 25% of the cancers in the urinary tract were donor-derived. 45% of the latter were BKV positive (Paper IV).

In this thesis we identified the study populations by using registries, i.e. the Uppsala University Hospital Transplantation Database, RTR and CTS. We verified the cancer diagnosis in the medical records and in the histological records. Hence, we used the most efficient and objective way to verify the validity of the registries. We also found patients with malignancies in the CTS and Uppsala University Hospital Transplantation Database, who due to timeliness were not yet processed or never reported to RTR. Consequently, even if the number of patients in each study is limited, the quality of the results can be regarded reliable.

With the rapidly expanding global knowledge, registries provide possibilities to assemble and analyse information and trends otherwise difficult to obtain. This is especially true for small patient populations such as solid organ transplant recipients. Linkage between transplanted population registries and cancer registries has resulted in mainly epidemiological information such as cancer incidence, cancer induced mortality, risk groups, need
for screening etc. This represents most of what is known about post-transplant malignancies. Information retrieved from registries is essential for finding relevant focus in clinical studies.

The value of cancer registry research is proportional to the quality of data and the quality control procedures. Comparability, completeness, validity and timeliness are essential for meaningful interpretation (Bray and Parkin 2009, Parkin and Bray 2009).

The criteria of comparing results from different registry studies are seldom fulfilled as Table 1 and 2 in the introduction describe. In smaller local studies it is possible to confirm the cancer diagnosis in the medical records, which is not possible in larger studies on a national level. Sometimes only the first malignancy is included, whereas in other studies all malignancies are taken into account. The median time of follow up is 5 years in some studies (Yoosabai et al. 2015); still median duration from transplantation to a first post-transplant tumour is more than 5 years. Some studies comprise all solid organ recipients, other focus on recipients of a particular transplanted organ, transplanted kidneys, livers or hearts and lung transplants. The cancer incidence is known to be highest in heart and lung recipients probably due to a higher level of immunosuppressants (Collett et al. 2010, Engels et al. 2011, Krynitz et al. 2012). In some countries it is by law mandatory to report the cancer to national cancer registries but in other countries it is voluntary. The choice of control groups differs, etc. A US author who performed a register study (based on voluntary reporting) on cancer-induced mortality found a lower cancer-induced mortality rate in patients with post-transplant cancer than in the general population (Kiberd et al. 2009) and than studies from other countries indicated (Acuna et al. 2016). The same US author also published screening recommendations (Kiberd et al. 2003). How far-reaching conclusions can then be drawn from the results?

We knew that the cancer incidence is elevated in the transplanted population compared to the general population, but we were not aware of the degree of underreporting post-transplant malignancies to the transplantation registries, nor of the increased post-transplant cancer risk in patients with a previous cancer.

The phenomenon of underreporting malignancies to transplantation registries has been observed in unpublished studies from the Nordic countries and the United Kingdom. Initially, 80% of the cancers of the female genital tract, 75% of the skin cancers, 30% of the gastrointestinal cancers, 50% of urological cancers and 50% of the haematological cancers were missing in the our local transplant registry.

Why are malignancies not reported to the transplant registries as transplanted patients in general are well taken care of and other information is collected more methodically? This may be due to lacking communication between different medical specialities. It may also be unclear which medical speciality has the overall responsibility for a patient suffering from different
medical conditions. Cancers with a good prognosis such as skin cancers can be regarded as a minor problem compared with the other clinical problems in a patient. Further, information of for example cancers of the gynaecological tract is not obtained if it is not asked for. And finally, very aggressive cancers diagnosed at an advanced stage with just a few weeks of patient survival seem to be the ones that are not reported even to the national cancer registries.

After linking the transplanted patients to RTR more than 90% of the eligible patients were included in the studies. Thus, it is essential to develop a structure to actively look for these patients in order to identify them.

Paper I

We investigated post-transplant cancer risk and patient survival between renal transplanted patients with pre-transplant cancer and renal transplanted patients without pre-transplant cancer. We found a higher post-transplant cancer incidence and a lower patient survival cancer than the other studies have found.

Viecelli et al compared post-transplant cancer survival between other categories of patients i.e. renal transplanted patients with pre-transplant cancer who developed recurrence or a de novo cancer post-transplant and patients without a previous cancer who developed a primary post-transplant cancer in Australia (Viecelli et al. 2015). Penn et al focused only on post-transplant recurrent cancers but excluded de novo cancers in renal transplanted patients in the USA (Penn 1997). Yoosabai et al studied post-transplant cancer among heart-transplanted patients in the USA (Yoosabai et al. 2015). The median age at transplantation was significantly lower in the Australian cohort than in our cohort (44 years versus 60 years) and nonmelanoma skin cancers were excluded from the Australian study (Viecelli et al. 2015). These two differences may explain the diverse outcome of the studies (Collett et al. 2010, Krynitz et al. 2012). Additionally the time eras between the studies varied and the majority of our patients received the renal grafts from deceased donors and may consequently pose an elevated risk of post-transplant cancer (Ma et al. 2014). However, both the Australian and our study are based on national cancer registries with a known high accuracy of cancer information.

Our results indicate that the increased cancer incidence and reduced patient survival in patients with a previous cancer is of considerable concern. No correlations were found between type of pre-transplant cancer and type of post-transplant de novo malignancy. Nor was any specific type of pre-transplant malignancy bound to recur more often. These results might reflect a genetic predisposition to develop cancer, which is accentuated by the im-
munosuppressive treatment. They also illustrate the importance of immunological surveillance for many cancer types pre-transplant.

Should we then abstain from transplanting patients with previous malignancies? My answer to that question is no, because still more than half of the patients survive 10 years or more after organ transplantation. But a MDT with experience of handling post-transplant malignancies is recommended to also evaluate patients with pre-transplant malignancies, as this problem is more complex than we anticipated. Reintroduction of a tumour free interval of at least two years, in some cases five years, is recommended. Further, the malignancy surveillance is proposed to continue at least five years post-transplantation irrespectively of malignancy type. And finally, we should inform the patients that despite adequately treated cancer with favourable prognosis almost 50% of them are at risk of a cancer post-transplantation.

**Paper II**

This is the first study to systematically clinically evaluate patients with a malignancy in combination with a defined comorbidity, i.e. kidney transplanted, at MDT conferences. In the general population the recommended oncological treatment of different types of cancer is not implemented mainly due to comorbidity in 5 to 20% of the cases (Stairmand et al. 2015). Still adjuvant therapy results in significant prolonged survival even in patients with comorbidity (Sarfati et al. 2009).

Overall, the oncological treatment was modified in 52% of the transplant ed patients with solid or haematological malignancies, and 82% of the patients received oncological treatment according to national guidelines in our study. When a change to or addition of mTOR inhibitors as chemotherapeutic agent was added to the adjustments 97% of the patients with solid or haematological malignancies received altered oncological or immunosuppressive treatment.

The timing of introducing mTOR inhibitors to the patients demanded special attention because mTOR inhibitors prolong postoperative wound healing, enhance radiation therapy effect with 10% and interact with chemotherapy. The timing depended on the specific type of cancer the patient suffered from and the originally planned treatment. Even if one-third of the patients interrupted the treatment of mTOR inhibitors, the anti-tumour tissue effects might have been achieved, because most patients used the mTOR inhibitors for two months.

To sum up, the composition of doctors with experience in handling transplanted patients at the MDT conferences was a prerequisite to optimize the evaluations. This study illustrated the potential to improve the immunosuppressive and oncological treatment. Hopefully, such treatment adjustments
will be implemented in clinical practice and hopefully they will prolong the survival for transplanted patients developing cancers in the long run.

**Paper III**

We observed that the magnitude of the problem with post-transplant SCC in our renal transplanted patients was smaller than we estimated. The proportion of these patients was on the same level in Uppsala as in all of Sweden. Five per cent of the patients are diagnosed with an SCC 10 years post-transplantation. Still almost half of them do not develop a subsequent skin SCC in ten years. Thus 95% of all renal transplanted patients in Sweden are not at risk of numerous subsequent and in the end metastasizing SCCs (Krynitz et al. 2012). In an international perspective the occurrence of post-transplant SCC is much more frequent in Australia (Euvrard et al. 2003), mainly due to the different climate. However we are unable to explain why post-transplant SCC:s in renal transplanted patients are diagnosed at higher rates in the UK (Hoogendijk-van den Akker et al. 2013) and Germany (Ulrich et al. 2008b) than in our population.

We found that patients with one and especially two previous SCCs were at highest risk of developing subsequent SCCs, which is in concordance with earlier studies (Tessari et al. 2010, Euvrard et al. 2012). Lower age at diagnosis of current SCC comprised the most significant individual risk factors of a de novo SCC. In contrast, histology of current SCC and degree of sun damage did not pose an increased risk of developing subsequent SCC.

We faced two special aspects in studying post-transplant SCCs. First dermatology is a descriptive field of science. The dermatological examination comprises a subjective and personal impression of the dermatologist. Some dermatologists take biopsies of skin lesions before the lesions are surgically removed, whereas others do not perform biopsies. Correlation between the subjective impression of a given skin lesion and the objective final histopathological report deviated for many patients in our study. The first but not necessarily all subsequent SCC:s are reported to the national cancer registry. Consequently, the risk of underreporting skin malignancies is apparent. The facts that details and characteristics of the SCC:s varied between different pathological reports and that not until recently a basal cell carcinoma registry was founded further illustrates the difficulties of systematizing the dermatological evaluation.

Surgeons performing colonoscopies are nowadays trained and a score of how many adenocarcinomas each one find in 1000 colonoscopies is used. Maybe a corresponding score of how many SCC:s a dermatologist find in 1000 biopsied skin lesions will be used in the future.

The majority of the skin patients especially those with advanced sun damage reported a general improvement of the skin within a few weeks after
introduction of mTOR inhibitors. How to describe this subjective, only occasionally objective improvement of the skin in a scientific way? Further, mTOR inhibitors are associated with reduced number of subsequent SCCs and prolonged interval between current and subsequent SCC (Campbell et al. 2012, Euvrard et al. 2012, Hoogendijk-van den Akker et al. 2013). However, this effect is not always obtained in studies because subsequent SCCs often are used as primary endpoint.

The other aspect of dermatology is that uniform classifications of high-risk SCC patients or high-risk SCCs do not exist (Urwin et al. 2009, Metchnikoff et al. 2012, Parikh et al. 2014). The only common golden standard is that transplanted patients with a first SCC belong to the highest risk group. This stratification seems however to work. We intended to find a more detailed description of patients at risk of developing subsequent SCC:s, mainly based on skin status, but we did not fully reach this goal. Still we know that all investigated distinct risk factors contribute to development of SCC (Ulrich et al. 2008a, Urwin et al. 2009, Gogia et al. 2013). Maybe the development of subsequent SCC:s reflects a natural course which accelerates with time and which gets resistant to external influence after a critical point that has still not been identified.

A clinical evaluation of the skin is advisable before and shortly after renal transplantation (Zwald and Brown 2011, Tessari and Girolomoni 2012). It is essential to identify all patients with SCC because SCC can lead to metastasized disease in immunocompromised patients, SCC belong to the most immunogenic types of cancers, the risk of subsequent SCCs decrease dramatically when immunosuppression is interrupted (Grulich and Vajdic 2015), mTOR inhibitors probably have an effect on SCCs (Campistol et al. 2012) and SCC is the only tumour type where different features of the cancers have been found in the transplanted population compared with the general population (Perrem et al. 2008, Gutierrez-Dalmau et al. 2010, Muehleisen et al. 2012, Krynitz et al. 2015b). It is also important that pre-malignant lesions are methodically treated in all renal transplanted patients to prevent development of SCC.

Given that from a clinical and resource-saving perspective it is essential to make an early identification of patients most prone to develop de novo SCC, which patients should be focussed on? Based on this study we can only recommend that renal transplanted patients with a first SCC are followed up more frequently than patients without a previous SCC.

Paper IV

The incidence of donor-derived tumours was at least 5 times higher than we expected earlier, and still donor-transmitted tumours were not included. Donor-derived tumours compose a unique immunological feature because they
occur as a consequence of transplantation and the associated immunosuppression. This offers new treatment opportunities because the host allograft response may be directed against the cancer. Further, due to the immunosuppressive status, opportunistic viruses that cause or support cancer progress may thrive in tumours developed in those patients, which makes them accessible to anti-viral treatment (Reusser et al. 2015).

We showed that it was possible to prolong the lives of three patients with advanced metastasized urothelial cancer of donor origin, two of which were BKV positive. The prognosis of advanced urothelial cancer is pessimistic even in the general population, so these alternative treatment options are appreciated.

The treatment of the three patients with cancer in the urinary tract varied due to different times at which the tumours appeared. Withdrawal of immunosuppression positively affected the first patient. Immunostimulating therapy such as AdCD40L is an interesting option for donor-derived tumours, and minimized immunosuppression combined with anti-viral treatment may be an option for BK virus positive urothelial cancer of donor origin.

To our surprise two of four cancers in the renal transplants were of recipient and not donor origin, which shows that we still have much to learn about oncogenesis in transplanted patients. Consequently, we recommend that all cancers in renal transplants and the urinary tract are investigated for donor or recipient origin and presence of BKV.
Conclusions

I  The increased cancer incidence in patients with a previous cancer is of considerable concern. Despite previously adequate cancer treatments and favourable prognoses the risk of post-transplant cancer in patients with a previous cancer is nearly 4 fold compared to transplanted patients without a previous cancer. Still these findings do not justify abstaining from transplanting all patients with previous malignancies, because more than 50% of the patients survive more than 10 years post-transplantation. Reintroduction of the tumour free duration of at least two years pre-transplant is recommended in clinical practice. The malignancy surveillance is proposed to continue at least five years post-transplantation irrespective of malignancy type.

II  Paper II is the first study to systematically evaluate patients with a malignancy in combination with a defined comorbidity, i.e. kidney transplantation, at MDT evaluations. We suggest that all transplanted patients with a diagnosed post-transplantation malignancy should be referred to a MDT that addresses and individually tailors the oncological and immunosuppressive treatment. The study indicates that malignant diseases generally are underreported in international transplant registries. We recommend linkage between transplant databases and national tumour registries to improve identification of post-transplant malignancies.

III  It is advisable that patients with more than one or two SCCs especially in combination lower age at current one are more intensively followed up. The findings can be of importance in resource allocation for follow up of transplanted patients in regards to SCC in the skin.

IV  Paper IV suggests that donor-derived tumours may occur more frequently than previously thought. We suggest that urologic malignancies in renal transplant recipients are investigated for donor origin as well as presence of BKV. Donor-derived and BKV positive tumours are unique from an immunological point of view, and this offers new options for cancer treatment by anti-viral treatment and immunomodulatory drugs in addition to reduced immunosuppression.
Patients with post-transplant malignancies constitute a very heterogeneous population in terms of graft function and type and stage of the malignancy. Even if the ratio of post-transplant malignancies is elevated, the number of patients with a defined cancer diagnosis is limited due to the high diversity of cancer types. This is the main reason why few clinical studies have been conducted on post-transplant malignancies.

International cooperation between many transplant centres is a prerequisite for meaningful clinical studies on post-transplant malignancies. A Nordic cooperation on post-transplant malignancies has been initiated. The Nordic screening recommendations are a result of that. Further, a study on risk factors and post-transplant cancer incidence in the Nordic countries is proceeding. A Nordic study on treatment differences between the transplanted population and the general population is planned. Even common European guidelines on how to treat individual patients with post-transplant malignancies would be useful and ought to be possible to accomplish.

Post-transplant cancers occur at increased rates, have a more aggressive behaviour and have a worse prognosis than cancers in the general population. Is it the impaired immunosurveillance that plays a key role or do distinct features develop in cancers of immunocompromised patients? Post-transplant cancers offer interesting and unique opportunities to study the mechanisms of carcinogenesis, especially the protective role of the immune system. Comparisons on a molecular and genetic level between cancers in the transplanted and general population will generate exciting information on carcinogenesis and, in the long run, alternative more directive treatments.
Bakgrund


Antalet patienter med en tidigare genomgången cancer har ökat som följd av att kriterierna för att acceptera patienter på väntelistan för njurtransplantation har utvidgats. Hur den ökade risken för tumörer efter transplantation påverkas av en tidigare genomgången cancer är hittills okänt.

Njurtomater med maligna tumörer är medicinsk komplexa – på ena sidan behöver de immunpressiva behandling för att njurartransplantaten skall fungera, på andra sidan är den immunpressiva behandlingen utvecklingen av cancer. En sort av immunpressiva läkemedel kallas mammalian target of rapamycin (mTOR) hämmare. De har både immundämpande och cancerhämmande egenskaper och man rekommenderar byte till mTOR-hämmare hos alla transplanterade patienter med posttransplant tumörer. En förutsättning för att ge patienterna en optimal behandling av såväl cancer som transplantatet är att man etablerar ett multidisciplinärt samarbete mellan onkologer, dermatologer, njurmedicinare och transplantationskirurger.


Tumörer som uppstår efter transplantation kan antingen härstamma från donator eller mottagaren. Fram tills nu har man trott att tumörer av donatoronsursprung är väldigt sällsynt förekommande. Dessa tumörer är immunolo-
giskt mycket intressanta eftersom immunsystemet kan stöta bort dem om man avslutar behandlingen med immunsuppressiva läkemedel. En del tumörer är sannolikt också virusorsakade, vilket öppnar för möjligheten till alternativa behandlingar riktade direkt mot viruset.

Syfte och frågeställningar

I. Att ta reda på om njurtransplanterade patienter med tidigare genomgången cancer har högre risk för maligniteter efter transplantation jämfört med njurtransplanterade patienter utan tidigare tumörer.

II. Att förbättra identifieringen av njurtransplanterade patienter med postransplant tumörer samt bedöma i hur stor grad det är möjligt att byta immunsuppressiv behandlingen till mTOR-hämmare hos dessa patienter. Syftet var även att optima den immunsuppressiva och den onkologiska behandlingen genom individuell bedömning vid multidisciplinära konferenser.

III. Att analysera vilken riskfaktor eller kombination av riskfaktorer som bäst förutsäger nya SCC hos njurtransplanterade patienter med tidigare SCC.

IV. Att identifiera hur ofta tumörer i njurtransplantat eller urinvägar hos njurtransplanterade patienter härrör från donatorsceller samt i vilken utsträckning dessa tumörer innehåller BK-virus.

Metoder

Samtliga fyra studier var godkända av regionala etikprövningsnämnden i Uppsala. Tumörer hos alla njur- och pankreastransplanterade patienter som transplanterats vid Akademiska sjukhuset i Uppsala identifierades genom samkörning av ett lokalt transplantationsregistret och kvalitetsregistret vid Regionalt Cancer Centrum (RCC) i Uppsala och Örebro i alla delarbeten.

I den första studien jämfördes incidensen av posttransplant cancer hos 95 patienter med pretransplant cancer i Uppsala med en matchad europeisk kontrollgrupp i förhållande 1:3 i en retrospektiv registerstudie. Våra patienter hämtades från vårt eget transplantationsregister medan den europeiska kontrollgruppen var hämtad från Collaborative Transplant Study (CTS), ett av världens största transplantationsregister. Cancerincidens och patientöverlevnad analyserades genom Kaplan Meier-analysen.

I den andra studien möttes ett multidisciplinärt team bestående av onkologer, dermatologer, njurmedicinare och transplantationskirurger som tillsammans bedömde alla patienter individuellt gällande diagnos, cancerbehandling och immunsuppressiv behandling. Det inkluderade bland annat
ett ställningstagande till eventuellt byte till mTOR-hämmare. Alla 120 patienter följdes upp en gång per år i tre års tid. Studien genomfördes som en prospektiv klinisk observationsstudie. Teamets behandlingsförslag noterades och jämfördes med tidigare planerad onkologisk och immunosuppressiv behandling.

I den tredje studien gjorde en dermatolog en klinisk bedömning av graden av solskadad hud och delade in alla 73 patienter med SCC i tre riskgrupper. Riskgrupperna samt andra potentiella riskfaktorer hos de 73 patienterna med SCC analyserades om de korrelerade med utvecklingen av nybildade SCC under en uppföljningstid av 2,5 år. Studien genomfördes som en prospektiv klinisk studie och analyserna genomfördes med Cox regression och Kaplan Meier.


Sammanfattning av resultaten

I den första studien framkom att patienter med pretransplant cancer hade signifikant högre incidens av posttransplant cancer och signifikant kortare överlevnad än kontrollgruppen. Andra studien visade att identifieringen av tumörer hos transplanterade patienter ökade signifikant efter samkörningen med RCC. Hos 76 av 120 patienter (63%) byttes immunsuppressiva behandlingen till mTOR-hämmare. Den ursprungligen planerade onkologiska behandlingen justerades hos 23 av 44 patienter med solid cancer. I den tredje studien visades att antal tidigare SCC och lägre ålder vid aktuell SCC korrelerade till utvecklingen av nya SCC. I sista arbetet konstaterades att tre av cancernna i urinblåsan och två av cancernna i njurtransplantaten var av donatorsursprung. Fem av cancernna i urinblåsa var BK virus positive, medan ingen av cancernna i njurtransplantaten var det.

Betydelse

Det mesta man känner till om posttransplant tumörer baseras på registerstudier. I den här avhandlingen har vi undersökt posttransplant tumörer hos
njurtransplanterade patienter ur ett kliniskt perspektiv med målet att kunna implementera erfarenheterna i den kliniska vardagen.

Baserat på studierna föreslår vi att canceruppföljningen av patienter med pretransplant cancer bör fortsätta åtminstone två och i visa fall fem år före njurtransplantation. Vi föreslår även att samkörning med RCC är nödvändig för att identifiera alla njurtransplanterade patienter som drabbas av cancer. Bedömningen av ett multidisciplinärt team som inkluderar exporter inom transplantation är nödvändig för att patienter med posttransplant tumörer skall få adekvat onkologisk och immunosuppressiv behandling. Dermatologiska resurser avseende uppföljning av hudkostymen hos njurtransplanterade bör i första hand fokuseras på de transplanterade patienter som tidigare haft minst en SCC. Vi föreslår också att alla cancralar i njurtransplantat och urinvägar bör undersökas avseende donators- eller mottagarursprung samt BK-virusförekomst eftersom den kunskapen kan möjliggöra alternativa cancerbehandlingar för dessa patienter som generellt sett har en dålig prognos.
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