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STUDIES ON POLYOMAVIRUSES IN HUMANS

In relation to haematopoietic stem cell transplantation and cancer

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"Every work of art is the child of its age and, in many cases, the mother of our emotions."[☯]

[☯] Wassily Kandinsky, M. T. Sadler (Translator), Adrian Glew (Editor). *Concerning the Spiritual in Art.* (New York: MFA Publications and London: Tate Publishing, 2001). 192pp. ISBN 0878467025

ABSTRACT

The simultaneous discovery of two polyomaviruses in humans in 1971, BK and JC viruses (BKV and JCV), initiated the research on polyomaviruses in relation to human diseases. This has now been intensified with the consecutive discoveries, the last three years, of three new family members, KI, WU and Merkel cell polyomaviruses (KIPyV, WUPyV and MCPyV). Notably, the frequent and reproductive presence of MCPyV in Merkel cell carcinoma, a rare skin cancer of the elderly, has opened new perspectives for polyomavirus research in humans. The ultimate aim of this thesis was to understand, prevent and cure tumour development and disease associated to polyomavirus infection in humans.

BKV is ubiquitous and infects humans in early childhood without any symptoms. In the context of allogeneic haematopoietic stem cell transplantation (HSCT), BKV can reactivate and has been associated to haemorrhagic cystitis complication (HC), usually occurring within three months after HSCT. Between 2002 and 2006, by following prospectively 175 allogeneic HSCT patients at the Karolinska University hospital Huddinge and collecting their urine samples weekly, we have been able to confirm the significant role of BKV in the risk of developing HC. In papers I, II and III we have also identified additional factors that contribute to the prediction of this complication. More specifically, full myeloablative conditioning and an unrelated donor graft significantly put the patients at risk for HC. A tendency to develop HC was also observed in patients receiving an HLA mismatched transplant. Acute graft-versus-host disease, could however not be confirmed as risk factors for HC. There was also no significant difference in the frequency of HC if the patients received stem cells from peripheral blood or bone marrow, while the risk for HC after using umbilical cord blood needs to be investigated further.

In this thesis, we also especially investigated the role of the new polyomaviruses in relation to some specific human cancers. In paper IV, we tested 38 mucosal melanomas for the presence of BKV, JCV, KIPyV, WUPyV and MCPyV DNA. Mucosal melanomas are malignant tumours of the skin areas that are not exposed to UV-light. Therefore, some aetiological factors, like viruses, remained to be uncovered. The absence of any polyomavirus tested suggests that these viruses do not play an aetiological role in these tumours.

In paper V we tested 31 neuroblastomas and 25 childhood CNS tumours for the presence of the recently discovered KIPyV, WUPyV and MCPyV. The absence of these viruses, despite highly sensitive methods and good DNA quality, suggests again that these viruses are not involved in these paediatric nervous system tumours. However, the results from the 25 CNS tumours remain preliminary since this material included a variety of diagnostic subsets. Furthermore, our results do not exclude the possibility that other so far not detected infectious agents could be involved in this disease.

LIST OF PUBLICATIONS

I. Bogdanovic, G., Priftakis, P., **Giraud, G.**, Kuzniar, M., Ferraldeschi, R., Kokhaei, P., Mellstedt, H., Remberger, M., Ljungman, P., Winiarski, J. and Dalianis, T. (2004) 'Association between a high BK virus load in urine samples of patients with graft-versus-host disease and development of hemorrhagic cystitis after hematopoietic stem cell transplantation.', *J Clin Microbiol*, 42(11), 5394-6.

II. **Giraud, G.**, Bogdanovic, G., Priftakis, P., Remberger, M., Svahn, B., Barkholt, L., Ringden, O., Winiarski, J., Ljungman, P. and Dalianis, T. (2006) 'The incidence of hemorrhagic cystitis and BK-viruria in allogeneic hematopoietic stem cell recipients according to intensity of the conditioning regimen.', *Haematologica*, 91(3), 401-4.

III. **Giraud, G.**, Priftakis, P., Bogdanovic, G., Remberger, M., Dubrulle, M., Hau, A., Gutmark, R., Mattson, J., Svahn, B., Ringden, O., Winiarski, J., Ljungman, P. and Dalianis, T. (2008) 'BK-viruria and haemorrhagic cystitis are more frequent in allogeneic haematopoietic stem cell transplant patients receiving full conditioning and unrelated-HLA-mismatched grafts.', *Bone Marrow Transplant*, 41(8), 737-42.

IV. **Giraud, G.**, Ramqvist, T., Ragnarsson-Olding, B. and Dalianis, T. (2008) 'DNA from BK Virus and JC Virus and from KI, WU, and MC Polyomaviruses as Well as from Simian Virus 40 Is Not Detected in Non-UV-Light-Associated Primary Malignant Melanomas of Mucous Membranes', *Journal of Clinical Microbiology*, 46(11), 3595-3598.

V. **Giraud, G.**, Ramqvist, T., Pastrana, D., Pavot, V., Lindau, C., Kogner, P., Orrego, A., Buck, C., Allander, T., Holm, S., Gustavsson, B. and Dalianis, T. (2009) 'DNA from KI, WU and Merkel cell polyomaviruses is not detected in childhood central nervous system tumours or neuroblastomas.', *PLoS One*, 4(12), e8239.

CONTENTS

LIST OF ABBREVIATIONS

aGVHD	Acute GVHD
AIDS	Acquired immunodeficiency syndrome
ATG	Anti-thymocyte globulins
BKV	BK-virus, *polyomavirus hominis 1*
BKVAN	BKV-associated nephropathy
BKVHC	BKV-associated haemorrhagic cystitis
BMT	Bone marrow transplantation
Bu	Busulfan
cGVHD	Chronic GVHD
CNS	Central nervous system
CMV	Cytomegalovirus
CTL	Cytotoxic T lymphocyte
Cy	Cyclophosphamide
DNA	Deoxyribonucleic acid
EBV	Epstein Barr virus
ELISA	Enzyme-linked immunoabsorbent assay
Flu	Fludarabin
fTBI	Fractioned-TBI
GVHD	Graft-versus-host disease
GVL	Graft-versus leukaemia
Gy	Gray
H	Histo-compatibility
HaPyV	Hamster polyomavirus
HC	Haemorrhagic cystitis
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigens
HSCT	Haematopoietic stem cell transplantation
HSV	Herpes simplex virus
IL-2	Interleukine-2
JCV	JC-virus, *polyomavirus hominis 2*
KIPyV	KI polyomavirus
LPV	Lymphotropic polyomavirus
LT	Large T-antigen
MC	Full myeloablative conditioning
MCC	Merkel cell carcinoma
MCPyV	Merkel cell polyomavirus
MHC	Major histo-compatibility complex
MPyV	Murine polyomavirus
miRNA	Micro ribonucleic acid
mRNA	Messenger ribonucleic acid
MS	Multiple sclerosis
MT	Middle T-antigen
NCCR	Non-coding control region

NK	Natural killer
ORF	Open reading frame
ORI	Origin of replication
PBSC	Peripheral blood stem cells
PCR	Polymerase chain reaction
PML	Progressive multifocal leukoencephalopathy
PTLD	Post-transplant lymphoproliferative disease
pRb	Retinoblastoma protein
RD	Related donor
RIC	Reduced intensity conditioning
siRNA	Silencer ribonucleic acid
ST	Small t-antigen
SV40	Simian virus 40
TBI	Total body irradiation
Th1	T-helper 1
TSTA	Tumour specific transplantation antigen
UCB	Umbilical cord blood
URD	Unrelated donor
UV-light	Ultraviolet light
VLP	Virus-like particle
VZV	Varicella zoster virus
WUPyV	WU polyomavirus

1 PREFACE

This past decade and the last three years in particular have been very exciting for polyomavirus research in general. The *Polyomaviridae* family has grown with many new members, three of them being recently described in humans. Notably, one polyomavirus was discovered in a human tumour, thereby opening a very special new area for polyomavirus research in humans.

Over half a century ago and for many decades, our knowledge of polyomaviruses was mainly extrapolated from research on murine polyomavirus (MPyV) and simian virus 40 (SV40), both being excellent and exciting laboratory models. In 1971 the first two polyomaviruses detected in humans, BK-virus (BKV) and JC-virus (JCV), were reported and the effects of these viruses in immunosuppressed patients have slowly been unravelled. In 2007-2008, as mentioned above three new polyomaviruses, KI, WU and Merkel cell polyomaviruses (KIPyV, WUPyV and MCPyV) were described in humans and have further enriched the field of polyomaviruses.

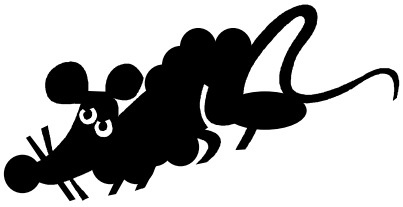
My thesis focuses on two different fields of polyomavirus infection in humans. One is the study of the pathogenesis of BKV in allogeneic haematopoietic stem cell transplanted (HSCT) patients with an attempt to increase the understanding of the association of BKV to hemorrhagic cystitis (HC) and which factors increase the risk to develop HC. The other has been to study the three newly reported polyomaviruses in the context of some human cancers.

2 POLYOMAVIRUSES

2.1 A SHORT HISTORY

2.1.1 The sunrise of viral oncology – Murine polyomavirus

In 1953, Ludwik Gross observed that mouse leukaemia extracts inoculated into newborn mice induced the development of salivary gland carcinomas in a large proportion of the animals[1]. This finding later led to the isolation of a small DNA tumour virus by Stewart and Eddy[2]. This virus was subsequently shown to induce a variety of tumours in newborn mice and revived the interest in tumour virology. The virus itself was named "poly" "oma" (Greek, for many tumours)[3] and today, it is commonly known as murine polyomavirus (MPyV) and *Polyomaviridae* is the name of the whole family.



2.1.2 The Polyomaviridae Golden History

Shortly after the description of MPyV, another important member of the polyomavirus family, SV40 was isolated from rhesus monkey kidney cell lines being used in the late 1950s to produce polio vaccines[4,5].

Consequently, during the following fifty years, work on MPyV and SV40 would lead to some of the most important paradigms in normal and tumour cell biology[4].

Once the structure and the genome of MPyV and SV40 had been well defined, the genome mapping studies with temperature sensitive mutants[6-8] showed that transformation defective mutants all mapped onto the early regions of MPyV and SV40. Thus this also indicated that the late region was not involved in tumour transformation. The viral genes required for cellular transformation of MPyV and SV40 were then described, large T and small t-antigens (LT and ST) and later also middle T-antigen (MT) for MPyV, and this led to the discovery of two major cellular tumour suppressors. More specifically, in the late 1970's, two independent groups reported that SV40 LT immunoprecipitated with a protein of a molecular weight of 53,000 Da[9,10]. Initially identified as the non-viral T-antigen, it is now commonly named p53. The nature of the interaction of LT with p53 was later revealed in the 1980's, when p53 was recognized as a tumour suppressor antigen, and its function was compromised by binding to LT. Similarly, the retinoblastoma gene product (pRb) (named Rb in figure 1), another tumour suppressor, was observed to bind to oncogenes of different DNA tumour viruses such as e.g. SV40 LT[11], adenovirus E1A[12] and HPV16 E7[13].

In parallel, in the field of tumour immunology, MPyV-induced tumours transplanted into fresh syngeneic hosts were rejected when the animals had been vaccinated with MPyV-

induced tumour lysate, or MPyV itself. This observation led to the definition of the tumour specific transplantation antigen (TSTA), the antigen inducing this rejection[14-16]. TSTA was only detectable in MPyV-transformed or infected cells, but it was not clear whether the antigen was virus-encoded or if it was a virus induced host antigen. Finally it was revealed to consist of different peptide epitopes derived from proteins of the early region (LT, MT and ST)[17-20]. Similar findings were found in parallel in the SV40 system[16,21-27].

2.1.3 The polyomaviruses identified so far – January 2010

Since the discovery of MPyV and SV40, the *Polyomaviridae* have expanded in numbers, due to improvement of molecular techniques. In the autumn of 2009, the family includes 22 members, 5 human and 17 non-human (14 mammalian and 3 avian)[28]. This thesis will deal specifically with the polyomaviruses described in humans: BKV, JCV, KIPyV, WUPyV and MCPyV.

2.2 ORGANISATION, STRUCTURE AND FUNCTION

Much of the current knowledge of polyomavirus molecular biology today is based on studies of MPyV and SV40 and will now be reviewed below.

2.2.1 A common genome organisation

Polyomaviridae are small DNA viruses. Their genome is constituted of a 5000 basepairs long and circular double-stranded DNA molecule that is coated by the host-cell histones H2A, H2B, H3 and H4 (forming the core nucleosome). The viral DNA together with 24-26 core nucleosomes constitute the minichromosome[29], which is packaged into a capsid of about 40-45 nm (see figure 2).

All polyomaviruses have a similar genome organization, arbitrarily divided into three functional regions. The two regions encoding the early and late proteins are defined as the early and late regions and are separated by a non-coding control region (NCCR) (named RR in figure 1) that contains the origin of replication (ORI), the viral early and late promoters, and regulatory proteins for early and late transcription. The early region consists of two overlapping open reading frames (ORFs) encoding two early messenger RNAs (mRNAs) by alternative splicing[30], which are translated into the non-structural proteins, LT and ST. MPyV and hamster polyomavirus (HaPyV) also produce a third alternatively spliced mRNA that codes for MT. The late region encodes the late mRNAs, also by alternative splicing, which are translated into the three capsid proteins, VP1, VP2 and VP3, and a fourth protein known as the agnoprotein (named LP1 in figure 1). The NCCR often shows multiple re-arrangements for polyomaviruses. This accounts also for human polyomaviruses, where in some cases the observed re-arrangements could be influenced or depend on the tissue source from which the virus was isolated.

2.2.2 Genomic organisation of the human polyomaviruses

The two human polyomaviruses BKV and JCV have six ORFs, encoding the early regulatory proteins LT and ST, and the late proteins, first the agnoprotein at the N-terminal end of the late region, followed by the capsid proteins VP1, VP2, VP3.

For BKV, the sequence designated as the "archetypal structure" is predominant in urine specimens from healthy individuals[31-33], and denoted BKV strain WW (GenBank: AB211371.1). Derivations from this archetypal sequence are considered to be rearranged, and concern variations in the NCCR. Another BKV classification based on sequence variation in the variable antigen-binding region of the major capsid protein (VP1), defines four serologic subtypes (see section 3.2).

Similarly JCV strain variations have also been described, all containing an archetypal regulatory sequence[34], like in the predominant European strain JCV Mad-1 (GenBank: J02226.1, Frisque *et al*. 1984). However, a single serotype is reported so far (see section 3.2).

KIPyV and WUPyV are closely related and group together, and share some significant homology with BKV and JCV with regard to genome organization and amino acid sequence of predicted viral proteins[35,36]. They also have an LT and a ST and VP1-3, but do not possess an agnoprotein.

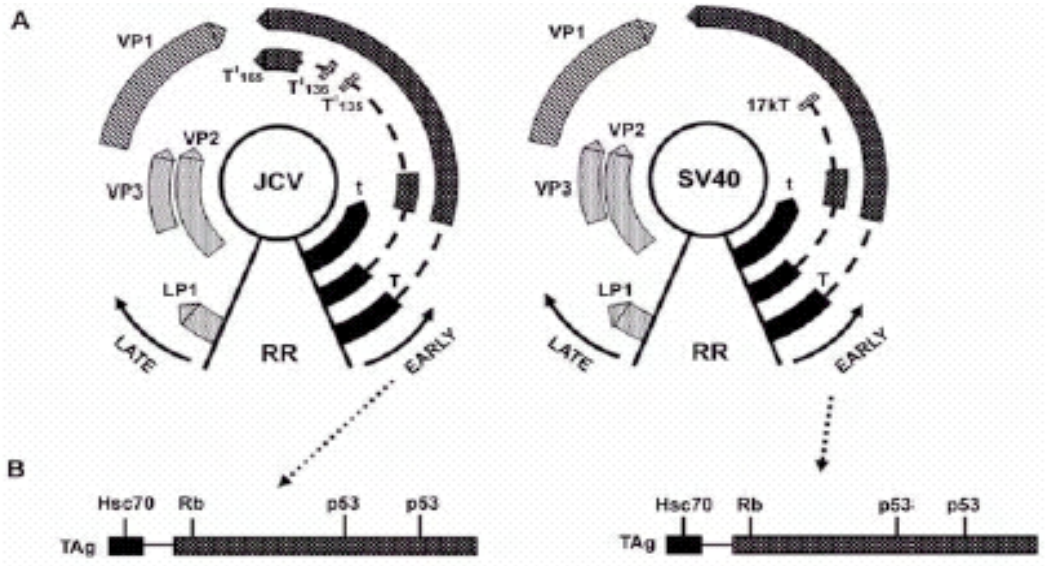


Figure 1.
JCV and SV40 genomes. A) The circular, double-stranded DNA genomes of JCV and SV40 are represented. The genomes are divided into early and late coding regions and a regulatory region (RR) containing replication and transcription signals. The single early precursor mRNA is alternatively spliced to yield mature transcripts encoding large T (T) and small t (t) antigens. The late region encodes three capsid proteins and a fourth protein, LP1 (also called Agnoprotein). B) The early coding region is expanded below each genome, and the positions of viral sequences binding to cellular Hsc70, Rb and p53 are shown.

MCPyV on the other hand, differs from the above four polyomaviruses and is more similar to the African green monkey lymphotropic polyomavirus (LPV)[37]. However, it has also a LT and a ST and VP1-3, and also lacks the agnoprotein.

2.2.3 Products of the early and late regions

2.2.3.1 Large T-antigen

As described above, early observations revealed that LT is required to establish and maintain transformation and for both viral DNA replication and stimulation of cellular DNA synthesis. The N-terminal region is generally highly conserved and mutations in this region abrogate transformation and the ability of the virus to make infectious virus particles[38]. Then I want to mention two critical domains, the pRb and the p53 binding sites, which are essential for transformation (see figure 1). On the one hand, the interaction of LT with the tumour suppressor proteins pRb and the related p107 and p130 [11,39,40], blocks the growth-suppressive functions of these proteins, resulting in improper control of the G1-S checkpoint leading to progression of the cell into the S phase as well as the activation of the p53 pathway. The subsequent transcription of p53-dependent genes would then result in cell cycle arrest and/or apoptosis. However, on the other hand, the interaction of LT with p53 blocks this response[41,42] Finally, in the C-terminal part, MPyV LT is often truncated without affecting the maintenance of transformation[43,44]. Similarly, studies on MCPyV reported frequent mutations leading to truncations in LT C-terminal end, in the helicase domain[43].

2.2.3.2 Middle T-antigen

MT is exclusively expressed by MPyV and HaPyV[45,46]. However, the also recently described MCPyV[37] has an ORF with similarities to the MT-encoding ORF in MPyV and HaPyV[28] although expression of MT mRNA has so far not been detected. MT is considered to be the major transforming protein for MPyV and HaPyV. However, MT also plays a role in regulation of replication and transcription[28].

2.2.3.3 Small t-antigen

ST is expressed by all polyomaviruses and its N-terminal part is common to both MT and LT (see figure 1). The unique functions of small t antigen are mainly mediated by interactions with PP2A[47]. By binding small t antigen, the enzymatic activity of PP2A is inhibited, and various signal transduction pathways are hyperactivated, involving MAPK, AKT, and PI3-K[48], thereby enhancing the oncogenic effects of LT.

2.2.3.4 VP1-3

VP1, VP2 and VP3 are the three structural proteins and build up the viral capsid structure detailed below. VP1, the major capsid protein, makes the outer layer of the capsid[49]. Most antibodies generated *in vivo* are therefore directed to VP1, whereas VP2 and VP3 (the minor capsid proteins) are assumed to be completely hidden inside the capsid and thus not accessible to antibodies[50] (see figure 2).

2.2.3.5 The agnoprotein

The agnoprotein is a small protein (8 kDa). Deficient mutants for this protein and also specific depletion of its expression by small interfering RNA (siRNA) revealed that BKV and JCV agnoproteins might be involved in virion assembly, but they are not absolutely required for viral multiplication[51].

2.2.3.6 Micro RNAs

Recently, micro RNAs (miRNAs), a type of siRNAs, have been identified in a variety of polyomaviruses and have further enriched the field of viral gene regulation.

RNA interference is an evolutionary conserved mechanism by which eukaryotic organisms use small double stranded RNA (or siRNAs) for sequence specific gene regulation. This mechanism is a natural biological response to protect the eukaryotic genome from parasitic DNA, and to defend the organism against viral infection.

The miRNAs are 21 to 23 nucleotides long non-coding single stranded RNA molecules produced from coding genes in the cellular or viral genome. The first miRNA whose target was identified was encoded by SV40[52]. SV40 miRNAs accumulate at late times during infection, are perfectly complementary to early viral mRNAs, and target these for cleavage. Thus, viral T antigen expression is reduced, but the yield of infectious virus is not altered by SV40 miRNAs.

Sullivan *et al.* have described miRNAs for BKV and JCV[53], MCPyV[54] and MPyV[55]. These miRNAs open the possibility for new targets in future therapeutics, e.g. by silencing mutated genes in specific tissues of whole organisms.

2.2.4 Structure

Polyomavirus virions are nonenveloped viruses and their outer shell is composed of 360 molecules of VP1, organised in 72 capsomeres, each comprised of a pentameric VP1 assembly, centred on the vertices of a T=7 icosahedral lattice[56,57]. Twelve capsomeres are pentavalent, and sixty are hexavalent. In addition, each of the 72 pentameres of VP1 is assembled on the inner side of the capsid with one molecule of VP2 or VP3.

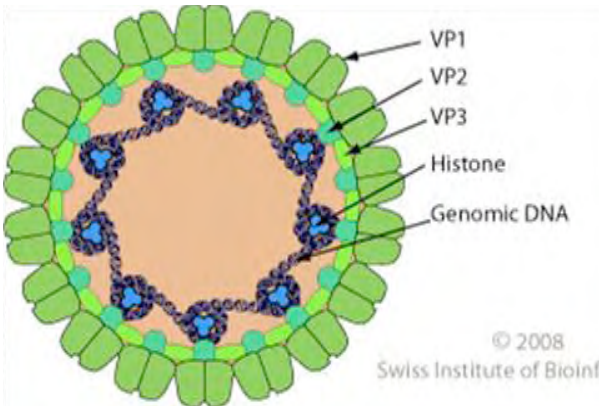


Figure 2. Schematic inner-view of a polyoma virion.

2.2.5 Attachment and entry into the cell

Polyomaviruses have the ability to bind to many types of cells depending on the various forms of carbohydrates they express on their surface as primary attachment receptors. These receptors determine the endocytic pathway the viruses will be taken up by. Early studies suggested that SV40 uses the major histocompatibility complex (MHC) class-I antigens to bind the cell[58], but finally expression of MHC class-I antigens on human kidney epithelial cells is not sufficient for SV40 infection[59]. Recent results indicate that SV40 utilizes the ganglioside, GM1, which is more consistent with the route of entry of the virus through caveolae[60]. BKV takes the same pathway[61]: it binds the gangliosides GD1b and GT1b[62], which then accumulate in the caveolae. A recent report suggests that MCPyV also binds a

ganglioside[63]. JCV preferentially utilizes α(2,6)-linked sialic acid attached to the 5-HT(2A) serotonin receptor[64] and is internalized by clathrin-dependent endocytosis[65].

2.2.6 Permissivity, assembly and release

Cell surface receptors for the polyomaviruses appear on many cell types and tissues. However *productive* or *non-productive* (transforming) infection may not occur, even with viral attachment and internalization. Thus, this reveals the intracellular control of viral processing in the cytoplasm or the nucleus[66]. Indeed very early studies on the lytic cycle and transformation by MPyV and SV40 have shown that not all cells support replication of the virus[4]. At the time the cells were therefore divided into "permissive" and "non" or "semi-permissive" cells. Permissive cells supported viral replication and were regarded to less often acquire the transformed phenotype, while transformation was suggested to be a characteristic of a failed lytic infection. Polyomaviruses most likely persist as infectious virus in semi-permissive cells, where a low virion output is minimally injurious to the host. However, changes in the microenvironment may convert these or neighbouring cells into a state fully permissive for productive viral infection[67].

In general transcription starts from the early region the first 24 hours, thereafter, viral replication and transcription from the late region takes place. Viral particles are then assembled with capsomeres of late viral proteins and incorporation of the DNA into the virion. By 48-72 hours post infection, the first viral particles can be released. The manner by which polyomaviruses leave the cell is still poorly understood. Some studies indicate that the virus causes lysis, while others indicate that virus can be shed from intact cells[66].

2.3 PATHOGENICITY OF POLYOMAVIRUSES

The immune system is extremely important for the control of the deleterious effects of polyomavirus infection. As mentioned above, under normal circumstances, the virus most likely stays latent in semi-permissive cells with the occasional outburst of viral shedding. However, when the immune system is suppressed polyomavirus infections may result in different diseases or tumour development.

MPyV has often been used in laboratory settings to study the mechanism of immunological control of persistent viral infections in the natural host of the virus. The outcome of MPyV infection in various immunodeficient adult mice has been reviewed by Swanson et al.[67]. The T-cell immune compartment as well as the presence of MHC class-I molecules allowing for the presentation of tumour specific antigens, i.e. peptides derived from T-antigens, seem to be key components to control MPyV infections and the development of tumours[17,68,69]. Moreover, it has also been shown that organs harbouring high levels of viral DNA are those that preferentially develop MPyV-induced tumours[70].

Nevertheless, with few exceptions, e.g. MPyV in newborn mice (with an immature immune system), HaPyV in lymphoma, and MCPyV in Merkel cell carcinoma, polyomaviruses do not naturally cause tumours in their hosts.

Similarly, clinical diseases associated to polyomaviruses seem to be observed exclusively in immune suppressed individuals. Avian polyomaviruses are the exception and cause life threatening diseases in their host. In humans, the most prevalent diseases associated to polyomaviruses will be reviewed below in section 3.3.

2.4 IMMUNITY TO POLYOMAVIRUSES

Both cellular and humoral immunity are involved in response to viral infections. In the polyomavirus family, much of the knowledge about immune responses to polyomavirus infection comes from experiments in mice with the MPyV, which I will shortly present below.

2.4.1 B-cell response

Similar to other viral infections, MPyV induces a neutralizing antibody response. As mentioned above, already over 50 years ago, MPyV was shown to have the potential to induce tumour development [1,2]. Early reports by Eddy BE *et al.* also showed that neonatal mice born to MPyV infected mothers were resistant to tumour development induced by MPyV inoculation, due to the protective effect of maternal antibodies. Additionally, in neonatal mice free from MPyV, and with no maternal antibodies against MPyV, it was shown that MPyV-induced tumour development could be prevented by passive transfer of antival antibodies[71]. In addition, to prevent subsequent infection, MPyV-specific antibodies also play an important role in controlling ongoing infection by decreasing the viral load[72]. However B cell responses, alone, are not capable of preventing tumour development, instead here the T-cell response is essential[73] (see also below). Similarly, from studies on clinical diseases associated to BKV and JCV in immunosuppressed individuals, it appears that humoral immune responses to BKV or JCV neither protect against the development of the disease, nor clear the disease [74].

Antibody responses to MPyV are mainly directed against VP1, which is exposed on the outside of the capsid. Hence, it is also possible to achieve protection against MPyV infection in mice vaccinated with VP1 produced virus like-particles (VLPs). Notably while the antibodies recognizing the whole viral capsid (mainly VP1) persist once the immune system is activated, antibodies to polyomavirus T-antigens are relatively rare, although most individuals are infected with the virus, indicating that the latter antibodies are primarily linked to episodes of *productive infections* [75].

2.4.2 T-cell response

To protect against tumour development, the T-cell system is mandatory, and this was established also for MPyV and SV40 induced tumours several decades ago in experiments with thymectomized mice[76]. Furthermore, it was possible to inhibit MPyV induced tumour development after inoculation of MPyV into neonatal mice after passive transfer of immune T-cells[77].

Later, it was established that relevant and protective immune responses to inhibit tumour development or to induce tumour rejection required T-cell responses directed against viral

peptides[69,78]. Regarding virus persistence and tumour development, in single newborn CD4-/- , or CD8-/- or double CD4-/- 8-/- C57Bl/6 knockout mice, Berke *et al.* could establish that CD8+ T-cells were most likely slightly more essential than CD4+ cells for inhibiting tumour development[78,79]. Furthermore, only CD4-/- 8-/- double knockout adult mice developed tumours after inoculation with polyomavirus. In addition, for tumour rejection in already immunised mice, removal of both CD4+ and CD8+ T-cells was however necessary to abrogate the immune response after previous immunisation with polyomavirus[69].

More recently, a manifestation of JCV reactivation, the progressive multifocal leukoencephalopathy (PML), has been reported after treatment with natalizumab for multiple sclerosis (MS) and Crohn's disease[80]. The action of natalizumab, an antibody against α-4 integrins, which suppresses T-cell-mediated responses by blocking the binding of lymphocytes and monocytes to endothelial cells for trafficking of these cells across biological barriers, has also shed some light on T-cell responses to JCV. PML is the only opportunistic infection associated with natalizumab and therefore this drug seems to impair an aspect of the specific immune response to JCV. In fact, in MS patients treated with natalizumab, CD4/CD8 T-cell ratios in cerebrospinal fluid were reduced to levels similar to those observed in patients with acquired immunodeficiency syndrome (AIDS)[81], even though this study did not report the PML occurrence in these patients. Nevertheless, this suggests that the cytotoxic T lymphocyte (CTL) response, necessary for the control of PML[82,83], seems to require CD4 T cell help that may be impaired with natalizumab treatment[74].

Other studies on clinical diseases associated to polyomavirus in immunocompromised patients, have shown that a robust BKV-specific CTL response may not protect against viral reactivation within the allograft, because of the T cells being unable to recognize epitopes presented on HLA-mismatched allograft cells. In fact there is evidence for an increased incidence of BKV-associated nephropathy (BKVAN) in recipients of HLA-mismatched kidney allografts[84,85]. Similarly, I will present in this thesis our results on the effect of HLA mismatch in BKV-associated hemorrhagic cystitis (*Paper III*).

3 POLYOMAVIRUSES IN HUMANS

Five polyomaviruses have been described in humans so far and one polyomavirus (SV40) has been distributed to humans during the early era of polyomyelitis vaccination. BKV and JCV were isolated almost simultaneously and published side to side in the same issue of the Lancet in 1971. BKV was identified by cytology, electron microscopy and virus culture from the urine of a renal transplant patient[86], while JCV was cultivated and seen in electron-microscopic examination of sections from the brain of a patient with PML[87]. In 2007, KIPyV and WUPyV were reported consecutively, both detected from nasopharyngeal aspirates[35,36]. Finally, more recently in 2008, MCPyV was detected in Merkel cell carcinoma (MCC)[37].

3.1 ROUTE OF TRANSMISSION AND PERSISTENCE

BKV and JCV primary infections are unapparent and rarely cause clinical disease, although respiratory symptoms or urinary tract disease are sometimes found together with BKV infection[88-91]. Additionally, BKV and JCV can be detected in tonsillar tissue from both paediatric and adult donors[92,93]. It has been reported that JCV can replicate, not only in glial cells[94], but also in primary B-lymphocytes and tonsillar stromal cells[95], supporting the notion that the respiratory tract is the primary site of viral infection. After the initial infection, both BKV and JCV are suggested to disseminate and establish persistent infection in the urinary tract[96]; their occasional detection in lymphocytes suggested these cells to be carriers for these viruses to their specific sites[97-100]. In the case of JCV, persistent infection is also established in the brain [101].

Although many reports have confirmed the discovery of KIPyV and WUPyV in nasopharyngeal aspirates from patients suffering from acute respiratory diseases, so far the data do not suggest that KIPyV and WUPyV are aetiological agents for acute respiratory diseases [102-105]. However, their detection in the respiratory tract could still be concordant with a respiratory route of transmission[106,107].

There is limited information regarding the tropism of MCPyV, since the research conducted so far has focused on its presence in MCC (will be discussed further in section 3.3.3). Nonetheless the discovery in MCC indicates a tropism for neuroepithelial cells. However MCPyV was recently also found in nasopharyngeal aspirates and tonsillar tissues [106,108,109], possibly suggesting a similar route of transmission to that of the other four polyomaviruses. Moreover, MCPyV sequences have been found in other tumours of the skin [110-114].

3.2 GEOGRAPHIC REPARTITION AND AGE PREVALENCE

The diagnosis of a past polyomavirus infection is generally performed by serology, while for the detection of reactivations molecular techniques are necessary. Four serogroups are distinguishable for BKV: BKV prototype (I), BKV SB (II), BKV AS (III) and BKV IV [115], while JCV strains are all members of a single serotype, Mad-1[116]. Humans infected with BKV and JCV produce VP1-specific antibodies. Certain epitopes are associated with

hemagglutination and others with cellular binding. Hemagglutination inhibition assays are simple and the method of choice to measure antibody titres of BKV and JCV[117], although neutralization assays and immunoassays with VLPs are also used to measure antibody levels in human serum [118].

Serological data obtained over 40 years demonstrate that infection with BKV and JCV is established in early childhood and occurs globally with similar frequency in industrial and developing countries. BKV infects children at early age[119] with seropositivity reaching around 90% in children aged 5 to 9 [120,121]. JCV seropositivity increases more slowly to 50 % after the age of 10 [120,121] and reaches 72 % among the mothers >25 years of age[120]. After that, BKV and JCV establish a lifelong persistence, with a consistently high seropositivity, comparable through the different age groups [120,122,123]. Serology is therefore not routinely applied for the diagnosis of BKV/JCV reactivation-induced disease. However, as mentioned in section 2.4.1, it has been suggested that antibodies against LT might be an alternative for the diagnosis of BKV reactivation.

Also the three new polyomaviruses show high serological prevalence values, 55-90% for KIPyV, 69-98% for WUPyV [124-126], 46-88% for MCPyV strain 339 [124,126,127], while MCPyV variant 350 exhibited a lower prevalence (25%)[124,126]. The seroprevalences were similar in a healthy adult population as compared to a young population, suggesting again that, for all five polyomaviruses described in humans, the exposure may occur early in childhood[126].

Since infections with SV40 and LPV have occasionally been discussed in humans, I would like to mention also that a low seroprevalence for SV40 (9%) as well as LPV (15%) has been reported in humans[126].

3.3 REACTIVATION AND RELATIONSHIP TO DISEASE

3.3.1 Reactivation manifestations

None of the presently known polyomaviruses in humans cause any significant disease in immunocompetent individuals as far as we know. This is also the case for SV40 transmitted by poliomyelitis vaccines. However, in immunocompromised individuals, as discussed above, polyomaviruses can become pathogenic, through a reactivation from a persistent sub clinical state to a lytic infection resulting in viruria and viremia, potentially leading to severe or fatal diseases. If primary polyomavirus infection in immunosuppressed individuals causes similar diseases as after reactivation of the same viruses has not been studied extensively.

In healthy adults, BKV reactivation can occasionally manifest as BK-viruria although it is usually less common than JC-viruria [128]. However, in HSCT and renal transplantation, BKV reactivation is frequently observed, both in the urine and the serum of the patients. In some allogeneic HSCT patients BKV can induce hemorrhagic cystitis, while in some renal transplant patients it can cause a nephropathy (described in more details below). In addition, BKV reactivation has also been observed in other immunocompromised individuals e.g. in patients with autoimmune diseases such as systemic lupus erythematosus, solid organ transplants recipients, and AIDS patients [129-133].

In healthy adults, JCV reactivation often manifests as JC-viruria. However, JCV causes PML in immunosuppressed patients, e.g. HIV positive patients or patients with lymphoproliferative diseases, during transplantation and chemotherapy, as well as in inherited immunodeficiencies[134,135].

KIPyV, WUPyV and MCPyV have all been detected in nasopharyngeal aspirates (see above in section 3.1), but KIPyV and WUPyV have so far not been associated to human diseases. MCPyV was isolated from a MCC, a disease of elderly and immunosuppressed individuals, but we do not know if these new viruses are associated with any other diseases in the immunosuppressed. Moreover, we still do not know where they are latent and how they reactivate.

SV40 was inoculated to almost 100 million people during the early era of polio vaccination and has not induced a burst of SV40 induced tumours as originally could have been feared.

Below I will briefly describe some of the most prominent diseases associated with polyomaviruses in humans.

3.3.2 BKV-associated Hemorrhagic Cystitis

BKV-associated hemorrhagic cystitis (BKVHC) is a complication observed in immunosuppressed patients receiving allogeneic HSCT [136-142] and will be described in more detail in section 4.2.

3.3.3 BKV-associated Nephropathy

After renal transplantation, BKV-associated nephropathy (BKVAN) is an important cause of renal allograft dysfunction, which incidence is increased today due to the more efficient immunosuppressive drugs given after renal transplantation [143]. BKVAN is to be suspected in renal transplant patients showing a rise in serum creatinine levels during routine follow-up. The diagnosis is typically established by histological examination of kidney biopsies. However there is an increased interest in the use of less invasive diagnostic methods such as urine cytology or quantification of viral load in blood and urine[143].

The relation between the level of BKV replication and the development of BKVAN, and graft failure, is poorly understood. The determination of BKV replication in the urine has become the most pivotal test to exclude BKVAN. In patients with BK-viruria, plasma loads exceeding 10,000 copies/ml permit a presumptive diagnosis of BKVAN that needs to be confirmed subsequently by biopsy. In general, screening is recommended every three months during the first two years after transplantation[144].

3.3.4 JCV in Progressive multifocal leukoencephalopathy

PML is a demyelinating disease of the central nervous system caused by JCV lytic infection of oligodendrocytes [145,146]. PML is characterised by symptoms including hemiplegia, monoplegia, akinesia, visual disturbances like diplopia, and dementia. It usually develops only in individuals with a severely compromised immune system. With the emergence of Human immunodeficiency virus (HIV), PML is considered as an AIDS-defining disease

affecting around 5% of HIV-infected patients. However, the introduction of highly active antiretroviral therapy for HIV has induced a significant decrease in PML among HIV patients in industrialised countries the past decade [135], with one downside: the development of an immune reconstitution inflammatory syndrome in tandem with the recovering of the immune system [147-154].

3.3.5 MCPyV in Merkel cell carcinomas

MCPyV is observed in around 80% of all MCC, a rare disease of the elderly and immunosuppressed [37,111,112,155-163]. However, only a few studies report its integration in tumour cells [43,164]. Today there is still a debate regarding the causal relationship between MCPyV and MCC [165].

3.3.6 SV40 polio vaccination and malignant mesothelioma?

Early studies have shown that SV40 was able to induce tumours in hamsters and transform human cells in culture and hence was potentially oncogenic[166]. Later, simian cell lines were used to produce polio vaccine and around 98 million children and adults in the USA and world-wide were vaccinated with SV40 contaminated polio vaccine[167]. Luckily, an epidemic of SV40 induced tumours did not occur in the vaccinated subjects. However, several reports have indicated the presence of SV40 in malignant mesotheliomas, hence the role of SV40 in forming human malignancies is still not completely clear even today.

Our group examined the presence of SV40 in Swedish pleural malignant mesotheliomas from patients born 1893-1958 [168]. After that, Sweden produced SV40 free polio vaccines from 1958 [169].

4 ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

In order to better understand BKVHC as a complication after allogeneic stem cell transplantation, a short presentation of this context is necessary.

4.1 SHORT PRESENTATION

Haematopoietic stem cell transplantation (HSCT) is a therapeutic procedure, which has evolved explosively the recent fifty years. Today HSCT is used as a cure for haematological malignancies and non-malignant haematological disorders (e.g. aplastic anaemia, myelodysplastic syndrome, immunodeficiency syndromes, and inborn errors). It has also been attempted as therapy in patients with solid tumours (e.g. sarcomas, neuroblastomas, breast, testicular cancer) and in severe cases of autoimmune diseases.

HSCT can be divided into three groups: autologous, syngeneic and allogeneic. In *autologous* HSCT the patients receive their own stem cells that are harvested previous to transplantation. In *allogeneic* HSCT the patient receives stem cells from another non-related or related individual, while in *syngeneic* HSCT the stem cell donor is defined as genetically identical, i.e. a monozygotic twin. This thesis focuses on allogeneic HSCT.

The source of HSCT was initially limited to the *bone marrow* and the procedure therefore called bone marrow transplantation (BMT). Today the procedure is summarized as HSCT since the source of the hematopoetic stem cell can vary. Hematopoeitic stem cells can be mobilized in peripheral blood in higher amounts than in bone marrow after administration of G-CSF, thus named the *G-CSF-mobilized peripheral blood stem cells* (PBSC), which started to be used in 1993 [170,171]. *Umbilical cord blood* (UCB) is another source of peripheral blood stem cells, which started to be used in 1989 [172]. However, since the volume of cells collected in UCB is more limited than with PBSC, HSCT performed with UCB was originally only an option in children. Today, HSCT is often performed with more than one UCB donor to enhance engraftment and also to allow its use in adults.

4.1.1 MHC complex antigens and antigenic presentation

4.1.1.1 The first steps of transplantation immunology

By the end of the 1950s, the first allogeneic HSCTs were performed in the USA and in France [173,174], but it took another ten years for the first two successful cases of HSCT to be reported (see paragraph 4.1.1.2). In fact, the first 200 transplants reviewed by Mortimer M Bortin[175], described a generally poor outcome. Some patients with engraftment died of an immunological reaction characterized by skin lesions, weight loss and diarrhoea, today recognized as graft versus host disease (GVHD). The lack of knowledge regarding the immunological reactions involved and more specifically the MHC antigen system explains in part the low success rate of these early attempts. Hence, a major breakthrough for

transplantation immunology was the discovery of the antigens of the MHC system and the human leukocyte antigens (HLA), and their role for allograft transplantation [176-178].

The MHC includes a set of closely linked genes, present in the genome of all vertebrates. The human MHC, also called HLA, is localized on the short arm of the chromosome 6. No other known human loci are as polymorphic as some of the HLA loci, and the number of alleles known in January 2010 were 4556 (http://hla.alleles.org/alleles/index.html). It is divided into three regions, class-I, class-II and class-III. The class-I HLA-A, -B, -C antigens are highly polymorphic glycoproteins expressed on the surface of most nucleated cells in the body. The class-II HLA-DR, -DQ and –DP antigens are only found on a limited number of cell types. They are mainly expressed on the cells of the immune system called professional antigen presenting cells, for example B-cells, monocytes, macrophages and dendritic cells. The HLA class-III molecules are very diverse soluble molecules, including complement factors, heat shock proteins and certain cytokines.

The biological role of these MHC molecules, and especially their role for allograft transplantation was not fully understood until Zinkernagel and Doherty in 1974 showed that MHC class-I molecules act as a guiding system for T-cells in the immune defence[179]. The same findings were later expanded for MHC class-II and the general rule is that T-cells can only recognize antigens that are processed and presented on the cell surface. We call this co-recognition of peptide-MHC complex. This recognition is also MHC restricted, i.e. in general the T-cell will recognise a cell presenting a "foreign peptide" in the context of the same MHC antigen as itself. The peptide-MHC complex can be formed via two different pathways. Endogenous antigens, such as viral proteins that are produced within the cell, are degraded into small peptides in the cytosol and processed on MHC class-I molecules present in the endoplasmic reticulum membrane and then transported to the cell surface. Exogenous antigens (proteins) on the other hand, are internalized e.g. into lysosomes and are degraded into peptides that in the cells are processed and presented on MHC class-II molecules. Thus, HLA class-I molecules generally present intrinsic antigens to the CD8 molecules of cytotoxic T cells, which are important in controlling viral infections by killing infected cells. HLA class-II molecules, on the other hand present extrinsic antigens to the CD4 molecules of helper T cells, which aid B cells in generating antibody responses to extracellular protein antigens.

4.1.1.2 The first successful HSCT and HLA-typing today

Simultaneously in 1968, the first two successful cases of HSCT with HLA-identical sibling donors were reported in patients with immunological deficiencies [180,181]. From then, it became evident that HLA matching is important for successful transplantation, by significantly improving engraftment and decreasing the outcome of GVHD [182].

The first classic choice in the search for a stem-cell donor was as mentioned above therefore an HLA-identical sibling donor. However, in practice only 30% of the patients can benefit from an HLA-identical sibling donor [183]. Therefore the evaluation of alternative donor sources has been crucial for the development of HSCT.

Additionally, when DNA based typing methods started to replace HLA class-II serology in the end of the 1980s, the results after transplantation with grafts from unrelated donors started to improve. PCR with sequence-specific primers for HLA class-II typing was developed and used at Karolinska University Hospital Huddinge [184,185] and later applied for HLA class-I [186-188]. Genomic HLA-typing, together with the conditioning regimens used at this hospital, explains in part why the incidence of acute GVHD was reported to be similar for patients transplanted with grafts from HLA-identical siblings and HLA-matched unrelated donors identical for HLA-A, -B, and -DR[189]. In contrast during the same period, in many other studies from the 1990s, both GVHD and graft rejection were more common after MUD transplantations than after HSCT with matched siblings[190].

Today, thanks to the development of large donor registries, the chance for a patient to find a matched unrelated donor is about 80% (www.bmdw.org). For the remaining patients, grafts from HLA-haploidentical or partially mismatched related donors are potential possibilities for most patients, especially in children where parents may be motivated to serve as donors[191]. Finally all patients lacking an HLA-matched unrelated bone marrow donor are potentially eligible for cord blood transplants, because up to two HLA-antigen mismatches are acceptable[192].

4.1.1.3 Minor histocompatibility antigens and ABO antigens in HSCT

Despite the ideal setting of HLA matching of patient and donor, optimal patient conditioning and post-SCT GVHD prophylaxis (detailed below in paragraphs 4.1.2 and 4.1.3), a significant number of patients suffer from GVHD, quite a few relapse with their malignant disease, while fewer patients reject the SC graft (host-versus-graft disease).

Originally, histocompatibility (H) antigens were divided into major and minor antigens depending on skin graft rejection. Major histocompatibility antigens were later defined as being coded by genes located within the MHC. Minor antigens were early described as a number of distinct genetic loci outside of the major complex[193].

In the HLA-matched patient/donor combination, minor H antigens are considered as the key molecules responsible for the immune responses in both graft-versus-host and host-versus-graft directions[194]. Historically, minor H antigens were initially identified in the mid-1970s as antigens responsible for T-cell reactivation in the host-versus-graft direction after BMT[195,196]. Minor H antigens, as defined today, are peptides derived from self-proteins containing amino acid differences among the population and thus also possibly different between the HLA-matched patient and donor. Again also here, the patient most likely shares minor H-antigens more frequently with related donors as compared to non-related donors. Minor H antigens are encoded by autosomes and sex chromosomes; they can be inherited independently from the HLA molecules[194]. The minor H peptides generated from the minor H proteins are presented to the immune system in the binding grooves of HLA class-I and HLA class-II molecules. They can induce strong T-cell responses between HLA-matched minor-H-antigen-mismatched individuals.

Recently, minor H antigens related to gender have been shown to induce a beneficial graft-versus-leukaemia (GVL) response, when female grafts are transplanted into male

patients[197,198]. Interestingly, while GVHD is mainly mediated via broadly expressed minor H antigens, the GVL-reactivity is mediated by both broadly expressed and haematopoietic-system-restricted minor H antigens. This differential expression of broad and tissue/malignancy-restricted minor H antigens is the basis for targeting cancer with minor-H-antigen-specific immunotherapy with low risk of GVHD.

The ABO-histo-compatibility blood group antigens may also have an impact on the HSCT outcome. Haemolysis, delayed engraftment and GVHD can arise from an ABO-mismatch [199,200].

4.1.2 GVHD and GVL effect

GVHD is one of the most important complications in HSCT, and is associated to considerable morbidity and mortality. Hence, for allogeneic HSCT a subtle balance has to be found in order to prevent GVHD and at the same time preserve the GVL effect.

4.1.2.1 Graft-versus-host disease

GVHD can be classified as acute or chronic based on timing of onset and clinical features and is graded I-IV dependent on severity[201]. *Acute GVHD* (aGVHD) develops within the first 2 months of allogeneic HSCT and affects mainly the skin, the gastrointestinal tract, and the liver. Development of grade II to IV aGVHD is associated with decreased survival in patients with allogeneic HSCT [202,203]. *Chronic GVHD* (cGVHD) has a later onset than aGVHD and is often clinically distinct. Patients can manifest sclerodermatous skin changes, keratoconjunctivitis, sicca syndrome, lichenoid oral mucosal lesions, oesophageal and vaginal strictures, liver disease, and pulmonary insufficiency [204]. Although limited cGVHD often resolves spontaneously with minimal intervention, extensive cGVHD requires prolonged immunosuppressive treatment and is associated with significant morbidity and mortality.

4.1.2.2 Physiopathology, prevention and treatment of aGVHD

Acute GVHD is suggested to occur in 3 phases: (1) tissue damage from conditioning, (2) donor T-cell activation, and (3) inflammatory effectors. In the earliest phase, inflammatory cytokines are released from host tissue in response to damage by the pre-transplantation conditioning regimen [205]. In the second phase, donor T-helper 1 (Th1) cells are activated upon recognition of alloantigen and they secrete interleukine-2 (IL-2) and interferon-gamma, which in turn initiates recruitment of other T cells, such as CTLs, natural killer (NK) cells, monocytes, and macrophages[206]. In the last phase, mononuclear cells primed by Th1 cytokines secrete more tumour necrosis factor-alpha and interleukine-1, which induce cellular damage or apoptosis, and restart the cycle of inflammation.

Current approaches for the prevention and treatment of GVHD involve a wide range of approaches from a mild anti-inflammatory agent to direct blockade of T-cell function and the combination of different treatments. Milder treatments include e.g. corticoids. The selective down-regulation of T lymphocytes can be obtained by inhibiting cellular proliferation (methotrexate), inhibition of de novo purine synthesis (mycophenolate mofenil), suppression of IL-2 secretion by blocking calcineurin activity (cyclosporine, FK-506), interfering with

downstream growth signalling pathways (sirolimus), and reduction of T-cell responsiveness by blocking the IL-2 receptor (daclizumab).

One of the most effective means of GVHD prophylaxis however, is depletion of T cells from the donor inoculum [207]. However, from 1981 to 1986, hundreds of clinical trials with *in vitro* T cell depletion of the graft, avoiding GVHD, also revealed important negative effects e.g. an increased incidence of graft rejection, a delayed immune reconstitution, an increased incidence of Epstein-Barr virus-associated post transplantation lymphoproliferative disorders (EBV-PTLDs) and an increased rate of disease relapse[207]. The recent years have seen the development of *in vivo* prophylactic and therapeutic startegies using T-cell depletion. On the therapeutic front, many agents have been tested in steroid-refractory aGVHD, the most promising being the mesenchymal stem cells[208]. On the prophylactic front, *in-vivo* T-cell depletion methods, use horse- or rabbit-derived anti-thymocyte globulin (ATG) or alemtuzumab, as a complement to the pharmacologic prophylaxis listed above[209]

4.1.2.3 Realizing the Graft-versus-leukaemia effect

From the first transplantation attempts, the majority of patients suffered from an immunological reaction of donor origin against the recipient (GVHD). Evidence that the allo-immune reaction responsible for GVHD also involved a powerful immune response to leukaemia was put forward by Barnes and Loutit, who observed that mice given an experimental leukaemia and then transplanted with allogeneic marrow and spleen cells showed leukaemia regression at the time they succumbed to GVHD[210]. The first evidence of the GVL effect in man was shown by Weiden *et al.* who statistically associated acute and chronic GVHD with reduced relapse rate in leukaemia patients[211]. As mentioned above, in the mid 1980s, new techniques to deplete the T cells from the graft *in vitro* were developed to reduce the complications due to GVHD, but the high rate of relapse secondary to this treatment enlightened the importance of the anti-tumour effect of the donator T cells or GVL effect [212]. In a key study from the International Bone Marrow Transplant Registry, the relationship of GVL effect with allo-reactive transfused lymphocytes was confirmed in a large retrospective patient series, showing that relapse risk was higher in *in vitro* T-cell-depleted HSCT, and reduced in the presence of acute and chronic GVHD [202].

The GVL effect is mediated by donor-derived T lymphocytes, but possibly also other cells such as e.g. NK cells may be involved[213]. These cell types have distinct mechanisms of interaction with the malignant cell target. After transplantation the cytokine milieu is favourable for an early establishment of a GVL effect, but the need to prevent GVHD limits the full potential of this process. Interestingly, whereas NK cells may mediate GVL effects, they may not been implicated in GVHD[213].

The GVL effect, also called the *graft-versus-tumour* effect, is the base of HSCT used against solid tumours.

4.1.3 Conditioning and Immunosuppression

Conditioning is necessary before HSCT and has three main objectives[214]:

- To eliminate leukaemia cells
- To provide adequate immunosuppression to prevent graft rejection
- To eliminate the recipient haematopoietic stem cells to make place for the graft in the bone marrow.

Conditioning regimens differ depending on the diagnosis of the patient and on the compatibility between the donor graft and the recipient. In the case of a malignant disease the main goal is to eradicate as many malignant cells as possible to diminish the tumour load and prevent relapse. Patients with malignant disease gain from mild graft versus host disease, since GVHD is correlated to the GVL effect. Therefore, immunosuppressive treatment is discontinued as soon as possible, since prolonged immunosuppression increases the risk of relapse. In non-malignant diseases, the main purpose of the conditioning is to avoid graft rejection. A harsher regimen in that case may still be motivated if there is a high degree of incompatibility with the graft. Conditioning regimens can be classified in two major principles, full myeloablative conditioning (MC) and reduced intensity conditioning (RIC).

4.1.3.1 Myeloablative conditioning

Historically, a standard or myeloablative regimen was used, since it was assumed that increased conditioning intensity was associated to an increased anti-leukaemic effect. The most commonly used MC was developed by the Seattle group in the early 1970s, and consisted of total body irradiation (TBI) of 10 Grays (Gy) preceded or followed by Cyclophosphamide (Cy)[215]. In the following years other drugs were tested Etoposid, Melphalan, Ara-C, which are less commonly used nowadays.

TBI was initially given in one fraction with varying dosage, leading to early and late secondary effects, e.g. severe cognitive sequelae in young children. Nowadays, fractioning of the dose maintains the same anti-leukaemic and immunosuppressive effect, but irradiation toxicity is reduced. For example, at the Karolinska University hospital Huddinge, twelve Gy fractioned-TBI (fTBI) is given as 3 Gy per day during four days.

Cyclophosphamide is not regarded as myeloablative, but has a strong immunosuppressive effect. To prevent HC, Cy is infused together with MESNA/Uromitexan under hyper hydration to protect the walls of the urinary tract against the drug metabolites.

Busulfan (Bu) combined with Cy can replace the Cy/TBI regimen. No preventive measures are uesd to prevent HC under the period where Bu is given alone, even though Bu has been reported associated to HC[140,216-219].The Bu/Cy combination is used especially in children, because they are more sensitive to the toxic effects of irradiation. However, compared to TBI/Cy, Bu/Cy increases the risk for veno-occlusive disease and occasionally irreversible alopecia, but it has fewer severe late effects on general development, on CNS and a lower risk of cataract formation.

MC can lead to a major toxicity, thus limiting its use in elderly patients.. The drugs have secondary effects on different organs e.g. the heart, the lungs, the liver or the central nervous system. In addition, the intensity of the conditioning is associated to a higher risk for GVHD[220], since the conditioning regimen is both immunosuppressive and cytotoxic, through cell damage and increase of cytokine levels, therefore enhancing the activation of GvHD. Moreover, following a standard myeloablative regimen, a complete aplasia is obtained earlier, thus prolonging the period where the patient is extremely sensitive to infections or bleedings. These complications are the reason why reduced intensity conditioning started to be used.

4.1.3.2 Reduced Intensity Conditioning

The primary objectives of a reduced intensity conditioning (RIC) are to reduce the incidence and the severity of acute GVHD, to reduce the infectious complications, as well as to reduce the toxic mortality due to the high doses of chemotherapy and radiotherapy. The aplasia following RIC usually less pronounced (but it depends on the regimen), and presents a prolonged period of mixed chimerism (simultaneous presence of haematopoietic stem cell from the donor and the recipient) over 1 to 3 months. Furthermore, the reduced toxicity of this regimen expanded the clinical use of allogeneic HSCT to patients over 55 years old and those with co-morbidities.

This new strategy focuses on immunosuppression rather than on myeloablation. In fact the role of the graft immune effectors in the allogeneic antitumour effect mechanisms started to be understood, and it was assumed that, in RIC, the transplanted cells would gradually eradicate the lymphohematopoeitic system of the host, as well as the remaining malignant cells.

In 2001, RIC was provided to patients for the first time in a clinical trial [221]. This regimen typically includes a purine analogue, such as Fludarabin, an alkylating agent, or low-dose TBI (2 Gy alone or with chemotherapy) [222]. Hence RIC should not be used for highly proliferative malignant disorders because of the prolonged mixed chimerism, and because a rapid complete chimerism is related to an early GVL effect. At Karolinska University Hospital Huddinge, when comparing MC versus RIC, a higher graft failure was observed for patients receiving RIC [223].

4.1.4 Immune reconstitution

Secondary to the conditioning and the transplantation, the immune system is dysfunctional. Both the innate and the adaptive immunity have to recover for the transplantation to be successful. Following the myeloablative therapy and stem cell transplantation a severe neutropenia phase occurs with a major risk for bacterial, viral and fungal infections.

4.1.4.1 Recovery of innate immunity

The innate immunity includes granulocytes, antigen presenting cells and NK-cells and complement.

An expansion of donor *granulocytes* after MC occurs within 14-21 days after HSCT and reduces the risk for infection with bacteria and fungi. It is defined by the first day with a minimal absolute neutrophil count of 0.5 $x10^9$/l [224]. The *duration of granulopenia* depends on the nature of the transplant. More stem cells can be collected with PBSC transplantation, thus speeding up the engraftment. UCB is associated with slower engraftment and an increased risk of all types of infections, counterbalanced by a lower incidence of severe graft-versus-host disease[192]. Commonly, the time observed between receiving the stem cell transplantation and the engraftment is 12-13 days after PBSC, 16-18 days after BMT, and 21 days or longer for UCB. RIC reduces the period and intensity of neutropenia and mucositis, but the overall risk of infectious complications due to opportunists during the first year after HSCT is not decreased since the early reduction in infections is counterbalanced by an increased risk for infections occurring later when complete chimerism is achieved.

NK cells are the first lymphogenous cells to repopulate after engraftment. Donor-versus-recipient NK cell alloreactivity derives from a mismatch between inhibitory receptors for self-MHC class I molecules on donor NK clones and the MHC class I ligands on recipient cells. In haploidentical transplantation, where they seem to be mainly studied, alloreactive NK cells of donor origin were detectable from 1 to 3 months up until at least 12 months post-transplantation. They seem to be important for the antiviral immunity, GVL effect and graft rejection [225].

Antigen-presenting cells numbers may be only half of the normal level by 1 year[209].

4.1.4.2 Adaptive immunity recovery

Cell mediated immunity includes T and B lymphocytes, and is mainly donor-derived after HSCT.

The *T lymphocyte* population includes CD8+ and CD4+ T lymphocytes. *CD8+ T lymphocytes* recover fairly rapidly by 3 months post-HSCT, and normalize within 1 year. *CD4+ T lymphocyte* reconstitution is generally slower, and can take up to 20 months, depending on the activity of the thymus. The speed of T lymphocyte recovery is associated to the size of the T lymphocyte inoculum, which is higher in G-CSF-mobilized PBSC than in bone marrow or in cord blood. Moreover, even if recipients have normal total lymphocyte counts within two months after HSCT, they have abnormal CD4/CD8 ratios, reflecting their decreased CD4 and increased CD8 cell counts. The number of naïve CD4+ T cells in the peripheral blood of patients after HSCT is very low, since the graft contains only mature memory cells with restricted diversity of T cell receptors[209].

The *B lymphocyte* population is restored approximately 6 months after HSCT, but its ability to produce antibodies is frequently decreased due to lack of T cell help. Serum isotypes develop in the order they develop in children, IgM followed by IgG within one year after HSCT, although levels of some IgG (IgG2 and IgG4) and IgA can remain low for years post-transplantation[209] especially in patients suffering from chronic GVHD. Moreover, re-vaccination is sometimes necessary.

4.1.5 Immune reconstitution syndrome

Little is known about immune response to polyomaviruses in humans, although polyomaviruses in humans have been mainly studied in different contexts of immunosuppression (described above in section 3.3).

However, as early as in 2000, just before I started with my studies in this thesis, Isabelle Binet *et al.* suggested that the pathogenesis of late-onset hemorrhagic cystitis complicating allogeneic HSCT corresponds to a sequence of events reminiscent of an immune reconstitution syndrome. The authors assumed that "a sequence of events might be required such as urothelial injury by the conditioning procedures including Bu, Cy, acrolein, and TBI, followed by high-level BKV replication during the immunodeficient phase and subsequently an exacerbated inflammatory response with reconstitution of the cellular immunity, which is consistent with the late onset hemorrhagic cystitis". Moreover, this model can easily be accommodated with other contributing or substituting antigens such as cytomegalovirus and adenovirus[226].

4.1.6 Infectious complications of HSCT

Infections are among the most important causes of morbidity and mortality after HSCT. According to Marty and Rubin[227] three factors should be considered in the context of transplantation: Exposure, Darwinian competition at a specific site, and the status of immune suppression. Exposure can be environmental (community or hospital exposure) or endogenous (previous colonization, central and peripheral venous lines, disruption of mucosal membranes). The presence of particular organisms at a given site is the result of a selection process among different microbial species, so called the Darwinian competition. Additionally, in the specific context of HSCT two major determinants are involved: the rate at which bone marrow and immune reconstitution occur, and whether or not significant GVHD is occurring. In fact, the use of intensive GVHD prophylaxis (*in vitro* or *in vivo* T-cell depletion or pharmacological prophylaxis) complicates the situation because it is associated with an impaired post-transplant immune reconstitution, which increases the risk of infections, as well as the reduces the GVL effect leading to increased relapse rates[209]. In this paragraph, I will detail the dynamics of engraftment before describing some viral complications after HSCT

4.1.6.1 The different engraftment phases

The timetable of infection for HSCT patients receiving MC can be divided into 3 phases (see also figure 3) the immediate post-transplant period, the intermediate post-transplant phase, and the late transplant phase. For patients receiving RIC these periods are more blurred.

The *immediate post-transplant period* (pre-engraftment phase) defined as days 0 to +30, coincides or overlaps with the agranulocytic phase. Common infections during this period are e.g. those with gram negative Escherichia Coli, Klebsiella pneumoniae and Pseudomonas aeruginosa, and gram positive Coagulase negative staphylococci, Staphylococcus aureus and streptococci, as well as other aerobic bacteria[228,229]. Notably, to prevent infections, prophylactic fluoroquinolones and systemic antifungals are administred.

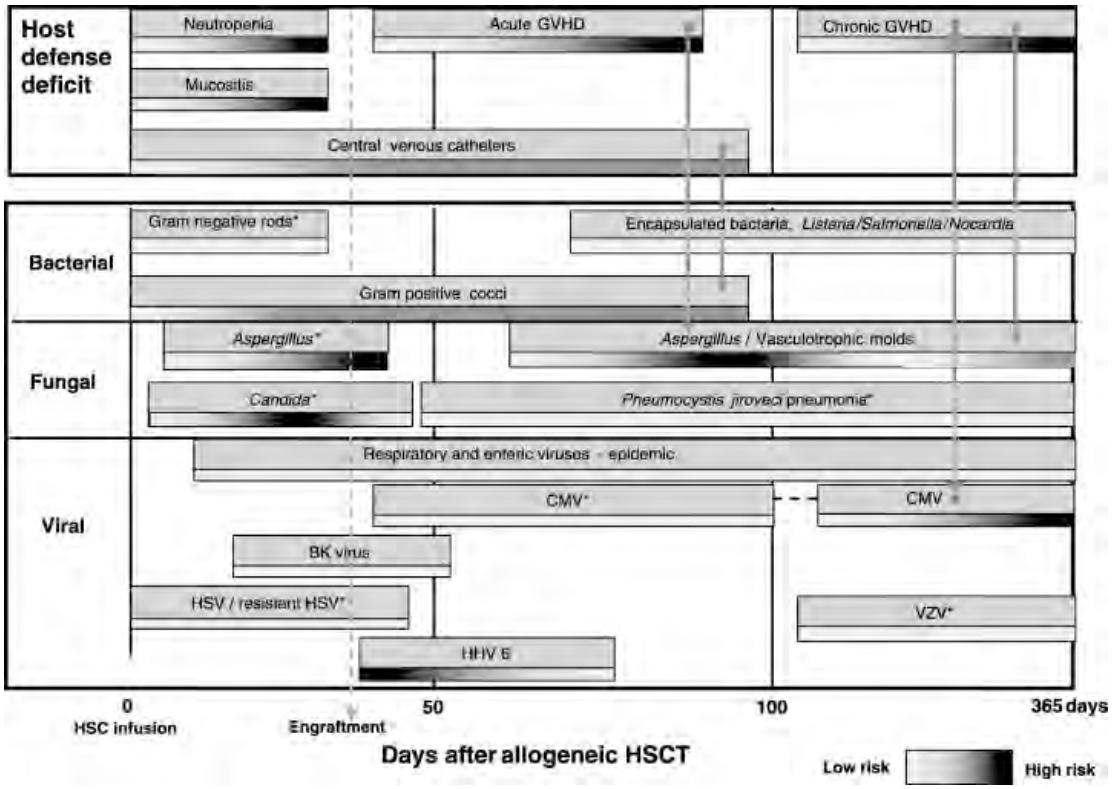


Figure 3.

Timetable of infection after HSCT. Infections are graphed in relation to evolving underlying host defects during the transplantation process. The risk density is represented by the bar underlying a specific pathogen(s). *Highlights microorganisms for which an established antimicrobial strategy is commonly used in clinical practice. HHV6, human herpesvirus-6.

Later, during the *intermediate post-transplant phase* (post-engraftment phase), from day +30 up to approximately three months after HSCT, there is a lower risk for bacterial and fungal infections. This risk may however persist in the presence of GVHD, due to additional suppression of immune function. Nevertheless, during the intermediate post-transplant phase, T cell function is still poor and associated with high risk of viral infections such as cytomegalovirus and adenovirus, as well as BKV, and Aspergillus or other moulds[230] . Again, the situation is worsened by GVHD[231], EBV-reactivation, and EBV-PTLDs. In addition, all through the aplastic period, fungal infections (mainly caused by Aspergillus and Candida species) and reactivation of herpes simplex virus (HSV) in seropositive patients might also occur.

During the *late post-transplant phase*, after day +100, patients are at low risk of serious infection, unless they have ongoing cGVHD [232]. Infections are more often due to varicella zoster virus (VZV), community-acquired infections, or in patients with cGVHD, recurrent bacterial infections especially pneumococci and other bacteria having a polysaccharide capsule[233-235].

4.1.6.2 Viral infections after HSCT

In principal, all latent virus infections may potentially be reactivated after HSCT. They may be life threatening, especially when affecting lungs, liver or CNS. Examples of viral reactivations and infections under HSCT are e.g. those with Herpes viruses: CMV especially,

but also HSV, VZV, HHV-6, and EBV; Infections with viruses of the respiratory system: adenoviruses, Influenza A and B, Parainfluenza, Respiratory syncytial virus, Metapneumovirus, and enteroviruses; BKV reactivation is also common, and it is possible that also reactivation of other polyomavirus and papillomavirus infections occur.

CMV primary infection occurs in 80% of the general population and gives rise to a cell mediated immune defence. While CMV is relatively non-pathogenic in healthy individuals, it is a major cause of opportunistic disease in humans with impaired cellular immunity. In these hosts, CMV can cause multi-organ disease including pneumonitis, chorioretinitis, hepatitis, gastrointestinal disease, myocarditis, meningoencephalitis, endocrinal impairments, as well as cutaneous manifestations.
In the context of allogeneic HSCT, the period between engraftment to day +100 after SCT, characterized by deficiency of the cellular immunity, is the classical phase for reactivation of CMV. Since almost all patients will reactivate CMV after HSCT and up to one third develop symptomatic infection, CMV disease causes high morbidity and mortality in HSCT, and is one of the most important obstacles in successful transplantation[236-238]. Especially, CMV seronegative patients receiving a CMV+ donor can, if left untreated, develop a life-threatening disease.
Therefore the intensive attempts to find effective treatment against CMV disease were made from the very early successful HSCTs. Today, the recommendations are the use of chemoprophylaxis, pre-emptive therapy (early treatment of CMV infection before the occurrence of CMV disease) or treatment of symptomatic CMV infection, when the risk of development of CMV disease is high, in immunosuppressed patients[239]. More specifically, it is recommended that a seronegative patient should not receive a graft from a CMV+ donor if possible, but there is no clear evidence that a seropositive patient would benefit more from a seronegative donor than from a seropositive one. Pre-emptive treatment strategies, based on PCR-surveillance of CMV, have greatly reduced the risk of fatal disease. Most currently used antiviral agents active against CMV are nucleoside analogues (Acyclovir, Valacyclovir, Ganciclovir, Foscarnet and Cidofovir). Recently, the generation of CMV-specific T cells or CMV-primed dendritic cells can be potential alternatives to antiviral treatment strategy[239].

EBV is latent in B lymphocytes, in the blood and the marrow of most adults. Most children in the developing world get an asymptomatic primary infection within the first 3 years of life, whereas in the Western world, half of the population acquires the virus in young adulthood with an infectious mononucleosis[66].
In the context of immunosuppression, EBV-reactivation occurs, in organ transplantation, but also in HSCT, especially with T-cell depletion[240-242]. When T-cell recovery is delayed, the T-cell surveillance is absent and EBV has the propensity to drive B-cell into uncontrolled proliferation, which is called the post-transplant lymphoproliferative disease (PTLD) and is a life threatening condition[240]. The disease presents with fever, adenopathy or non-specific symptoms and, occasionally, with acute systemic disease. Generally most PTLDs arise within one year after transplantation[243]. PTLD incidence is generally less than 1% for allogeneic HSCT recipients but this can rise dramatically among patients receiving T-cell depleted grafts (*in vitro* depletion) after SCT[244]. The depletion of the graft B cells in some protocols have markedly reduced the risk of PTLD[245]. Another therapeutic approach is to restore EBV immunity by adoptive transfer of EBV-specific cytotoxic T-cells[246].

BKV reactivation is observed as viruria and viremia mostly in immunosuppressed patients, but it can, as mentioned above, also be observed in the urine of healthy individuals (see section 3.3.1). In the context of HSCT, BKV reactivation is frequent, since the life long persistence is 72-98% (paragraph 3.2) and seen the first 100 days after HSCT as viruria in patients, both with and without HC. Clinical manifestations of BKV are almost exclusively late hemorrhagic cystitis (see paragraph 4.2), but in some sporadic cases transient hepatic dysfunction[141,247], pneumonia [248], and meningo-encephalitis [249], have been reported.

Adenovirus reactivation has also been reported after HSCT and represents a well-known cause of disease in these patients. It is associated with gastroenteritis, hepatitis as well as in disseminated disease, especially in children[250]. Adenovirus disseminated disease is most commonly seen following conditioning regimens that include *in-vivo* or *ex-vivo* T-cell depletion[209]. Strict isolation of patients with viral gastroenteritis is mandatory nowadays within HSCT units in order to decrease the risk of epidemic diffusion. In addition, adenovirus has been associated with HC (see section 4.2.4)

4.2 HEMORRHAGIC CYSTITIS

4.2.1 Definition

Hemorrhagic cystitis (HC) was originally observed as a self-limited disease occasionally seen in children as well as adults, characterized by sudden onset of haematuria, dysuria, frequency and supra-pubic pain. It must be differentiated from serious renal disorders, as well as from urinary tract infections, which are characterized by bacteriuria.

However, in different contexts like e.g. HSCT, HC seems to differentiate into distinct entities. Today, it is generally recognized that toxic HC (also called *early onset* HC) occurs within 24 to 48 hours after treatment with Cy, or other immunosuppressive agents with bladder toxicity such as Bu, ifosfamide, etc. A different entity is *late onset* HC, occurring from one week to 3 months after HSCT, and assumed to have an infectious origin.

Clinically, HC can be graded from I to IV, from microscopic (grade I) /macroscopic hematuria (grade II) - to painful blood transfusion depending cystitis – to severe painful cystitis with clotting (grade III) – to obstruction of the urethra due to clotting and urosepsis (grade IV) with high mortality[251].

4.2.2 From idiopathic to viral origin

Historically, the aetiology of HC remained vague until the end of the 1960s, when virus isolation procedures became more widely used in the study of urinary-tract disease.

In 1968, Numazaki *et al.* reported for the first time the presence of a viral agent in the urine of eleven immunocompetent (non HSCT) children suffering from HC[252]. These results were confirmed, with adenovirus 11 and 21 [253-255].

It was in 1976 that Hashida *et al.* first raised the issue that another virus could cause HC, after the observation by electron microscopy of *papovavirus-like particles* in the nuclei of cells from the urine of an immunocompetent boy with HC [91]. At that time, papoviruses had just recently been identified [86,256-258] but never associated with any clinical manifestations. Therefore in their report Hashida *et al.* could not eliminate the idea that the viral particles came from a passenger virus with no etiologic significance for HC.

It was not until 1982 that Mininberg *et al.* described the first cytopathic effect in the nucleus of cells typical for polyomaviruses in the urine of a 3-year-old boy with non-hemorrhagic cystitis[89]. Padgett *et al.* inoculated the urine onto primary human foetal glial cells, known to support the multiplication of polyomaviruses, adenoviruses and cytomegalovirus, all with distinguishable cytopathic effects, and could identify BKV[88].

4.2.3 Drug-associated Hemorrhagic cystitis

HC has also been observed after treatment with some drugs. HC has particularly been well described after treatment with *cyclophosphamide,* a cytotoxic alkylating agent, developed by Arnold and Bourseaux in 1957[259] and which has been in use for treatment of neoplastic disease since 1958. The bladder damage due to this drug has been recorded in man since 1960. The usual picture is the development of haematuria, dysuria within 24 to 48 hours of

large intravenous infusions of the drug, and these side effects last four to five days and subside spontaneously. Cases of severe and prolonged bleeding are rare, and therefore the serious and even lethal potentiality of this complication is not generally appreciated. Nevertheless, they do occur[260-263] with some reports of fatal cases[264-267]. Interestingly, Hutter *et al.* reported that severe and prolonged HC developed in only a few patients on long-term Cy therapy, suggested the possibility of alternative factors predisposing patients for the development of HC in the discussion[264].

In 1969 a case of severe hemorrhagic cystitis following Cy administration was reported, with the detection of CMV inclusions in the bladder[268]. Although the presence of CMV appeared to be incidental, the authors raised the possibility that CMV could enhance the susceptibility of the bladder to the effects of the drug. In fact the outcome of "sterile" hemorrhagic cystitis was a common finding with the wider use of Cy, in 2 to 40 % of the patients.

4.2.4 Hemorrhagic cystitis in the context of HSCT

After having covered this extensive but rather necessary background on allogeneic HSCT as well as some historical data about HC, in general, I would like to go more in detail in the section below regarding the association of BKV with the HC occurring during the intermediate post-transplantation phase.

4.2.4.1 Different viral etiologies

As I discussed earlier, the definition of HC has been deployed over time with the discovery of a variety of viral agents in the urine of the patients presenting the so-called "sterile or bacteria free" hemorrhagic cystitis.

With more broad use of allogeneic HSCT, CMV infection was observed as one of the major complications and clinicians started to follow the shedding of the virus in the urine of all patients post-transplant as a routine screening. At the Royal Postgraduate Medical School, all urine specimens were inoculated into human embryo lung fibroblasts[269]. The observation by Rice and Bishop of an unusual cytopathic effect in four cases led to the identification of BK virus, with the detection of a large number of viral particles in electron-microscopy. This clarified an earlier report by O'Reilly who observed papovavirus particles in the urine of bone marrow recipients[247]. The same year, Arthur *et al.*, confirmed these results in urine using DNA hybridization for detection of BKV viral DNA and a double-antibody indirect enzyme-linked immunoabsorbent assay (ELISA) for detection of BKV viral capsid protein[270].

The association of BK virus with HC in the context of BMT was first studied in more detail by Arthur *et al.*[142]: in a prospective study, they cultured urine of BMT patients with the aim to follow viruria, i.e. with the possibility to isolate CMV, adenovirus as well as BKV. BKV was detected in at least one urine specimen from 25/53 (47%) recipients of BMT. Among the 38 recipients of allogenic BMT, HC was observed in 71% of the 21 patients excreting BKV, versus 24% of the 17 patients with BKV negative urine (the urines were examined for the presence of BKV by ELISA and DNA hybridization assays). BKV was hence associated to HC in 16/18 cases where the disease was adequately characterized. One HC case was linked to adenovirus 11, and 3/6 patients with CMV in their urine also had HC. Today, it is known

that 50-100% of all allogeneic HSCT patients show reactivation of BKV as BK-viruria between 14 days and three months after HSCT, and 6-40% of these patients develop HC [136-141]. However, it is not easy to predict which patients will develop HC and if HC will take a mild or serious course.

Since *adenovirus* is the most common cause of viral acute HC in childhood, it could also be a major cause of HC after BMT. As reported earlier, outside of the context of BMT, the finding of adenovirus in the urine of patients with acute HC was more common in Japan compared with reports from USA (see paragraph 4.2.2). Indeed, also in the context of BMT, Japanese authors[271,272] found adenovirus in urine more often than others[273-275]. Notably, Childs *et al.* could show an increased rate of adenovirus associated HC in a population of T cell-depleted allogeneic BMT recipients[276].

Cytomegalovirus induced HC is very uncommon although CMV is a major cause of morbidity and mortality after HSCT and has various other clinical presentations (see paragraph 4.1.6.2). In the literature, one can read occasional case reports[277,278] and a single cohort study [279], which unfortunately did not follow BKV reactivation.

5 POLYOMAVIRUS IN CANCER

In 1976, Padgett and Walker wrote: "[Since] BKV and JCV [are] viruses that infect children at early age, infect a large portion of the human population, infect deep organs and tissues, and are representatives of a tumour-producing family of viruses, it is necessary to investigate the possible role of these viruses in the aetiology of human tumours". Today similar features are described, step-by-step, for the newly discovered KIPyV, WUPyV and MCPyV, and therefore it is, for us, equally important to investigate the possible role of these viruses in the aetiology of human tumours. This causal relationship will now be discussed in the second part of this thesis.

5.1 TUMOURS OF THE CENTRAL AND PERIPHERAL NERVOUS SYSTEMS

5.1.1 Childhood brain tumours overview

5.1.1.1 Epidemiology

Central Nervous System (CNS) tumours are the most common solid tumours in children and account for 27% of all childhood malignancies, second only to leukaemia[280]. More than 50% of all childhood CNS tumours are gliomas and, in contrast to adults, most childhood gliomas are classified as low grade. Notably, childhood high-grade gliomas differ biologically from those occurring in adulthood[281].

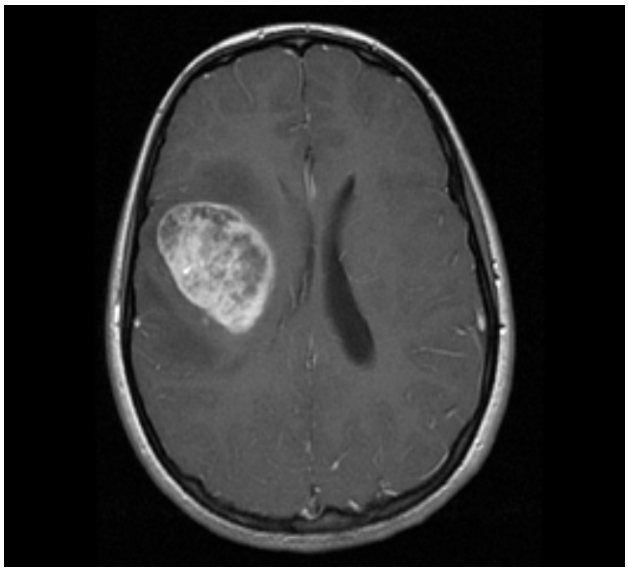


Figure 4. Glioblastoma multiforme, the most malignant paediatric CNS tumour.

They vary in type, location and growth rate, and cause therefore different symptoms that also depend on the age of the child. The acute symptoms often reveal a hydrocephalus. Thus, a specific diagnosis is difficult. The exact localisation and some important diagnostic features of CNS tumours are established through imaging of the brain e.g. by a computed

tomographic examination or magnetic resonance imaging, but the tumour type is usually established by histopathology even though the pathologist might be wrong since he is only looking at a very small area of the tumour.

Treatment depends on the size, location, expected growth rate of the tumour and the age of the child, and consists mainly of surgery, radiation therapy and chemotherapy. A few experimental therapies may be offered in the context of clinical trials at large hospitals. The survival of children with CNS tumours varies depending on the diagnosis, but also on the therapeutic tools available. Relative survival probability is the lowest for patients who have high-grade gliomas (including, in children, glioblastoma multiforme and anaplastic astrocytoma).

Nonetheless, the cause of CNS tumours in children is virtually unknown, the only established risk factors remain exposure to therapeutic ionizing radiation, rare mutations of penetrant genes (including the tuberous sclerosis complex, neurofibromatosis types 1 and 2, and familial history)[282]. If viruses contribute to the development of some tumours types, vaccination could eliminate some of these tumours in the future and immunotherapy could be an option for treatment.

5.1.1.2 International Classification and dominant types

CNS tumours are classified, on the basis of histopathology, into the following major six groups: Astrocytomas, Ependymomas, Medulloblastomas/PNET, Other Gliomas, Other specified neoplasms, and Unspecified intracranial and intraspinal neoplasms. In children, medulloblastoma/PNET is the most common malignant CNS tumour, followed by pilocytic astrocytoma, a low-grade astrocytoma. I will only detail below the most common types.

Table 1. Relative frequencies of CNS tumours in Sweden between 1984 and 2005

CNS tumours Diagnostic groups*	**VCTB 1984-2005****
Ependymoma	10.5%
Astrocytoma	44.6%
Medulloblastoma/PNET	18.8%
Other Gliomas	10%
Others specified neoplasms	13.5%
Unspecified intracranial/intraspinal neopl.	2.5%

* according to the "classification scheme for childhood cancer" (Birch and Marsden[283])

** from the Swedish Childhood CNS Tumour Working Group (VCTB) (Lannering *et al.*[284])

Ependymomas are mostly tumours from the posterior fossa and account for 10% of childhood CNS tumours[285]. In therapy, gross total resection is crucial with a significant impact on survival. Therefore second-look surgery can be needed and is warranted. As a complement, focal radiation to primary tumour bed is part of the "standard of care". Chemotherapy can be useful in very young children to avoid radiation. Unfortunately, half of the patients diagnosed will experience relapse, and surgical resectability is also for them the first modality to be explored.

Medulloblastomas/PNET represent the most common malignant brain tumour in children (15-20% of all childhood brain tumours) and reaches a peak incidence between 5 and 7 years of age[286]. Medulloblastomas are midline tumours that arise from one of the cerebellum's two germinal zones –either the ventricular zone or the external granular layer[287]. Primitive Neuro-Ectodermal Tumours (PNET) are similar tumours occurring in all other locations. Treatment for medulloblastoma and PNET consists of total resection by surgery, usually followed by craniospinal radiation therapy associated with chemotherapy during and after radiation.

Gliomas are the largest group of childhood CNS tumours and are classified into low-grade and high-grade gliomas. Additionally, brainstem gliomas are much more frequent in children and may represent a distinct biological entity[288]. ***Low-grade gliomas*** cover the majority of childhood CNS tumours (35-40%) and are often found in the posterior fossa, but they can also be supratentorial and located in the optic tract or hypothalamus. The predominant type is pilocytic astrocytoma from the posterior fossa (25% of childhood CNS tumours). Notably, the optic/chiasmatic gliomas can be associated with Neurofibromatosis while subependymal Giant cell astrocytomas are part of the Tuberous Sclerosis syndrome. The treatment starts with surgery if possible, and chemotherapy or radiotherapy depending on the age. ***High-grade gliomas*** comprise, in children, anaplastic astrocytomas and glioblastomas, and represent 8-10% of all paediatric CNS tumours. They are mainly supratentorial, and aggressive, with a poor prognosis. Therapeutic strategies combine surgery, chemotherapy and radiation. ***Brain stem gliomas*** (included in the Other gliomas group) arise in the central area of the brain and account for 10-20% of childhood CNS tumours. The majority are diffuse pontine gliomas with a median survival time of shorter than one year. They are difficult to treat since surgical removal is not possible, and conventional radiotherapy is therefore the standard treatment. The combination with anti-angiogenic therapy might be used in experimental protocols.

5.1.2 Neuroblastoma overview

Neuroblastoma is a malignant embryonic tumour of the neural crest cells, most common among children less than 1 year of age worldwide. Each year, approximately 1500 cases occur in Europe and 700 in the USA and Canada[289], accounting for about 28% of all cancers diagnosed in European and US infants, and for 5,5% of all childhood cancers, being the most common extra cranial solid tumour in childhood after lymphomas[280].

The neural crest cells arise in the third to fourth week of embryonic development and some of these cells differentiate and migrate to create the sympathetic nervous system. Neuroblastomas may arise anywhere along the sympathetic nervous system, but are found

most frequently in the adrenal glands (approximately 40% of tumours) or elsewhere in the abdomen, chest, or pelvis[289].

The diagnosis of neuroblastoma is based on the tumour biopsy that is useful for determining genetic aberrations, which have to be taken into consideration for the therapeutic decision. For low risk tumours surgical resection may be curative and for some tumours even observation alone could be the best approach. Children with high-risk disease need a multimodal therapy with intensive induction chemotherapy followed by an attempt to radical surgery and irradiation of the primary tumour to achieve local control, even though they have a poor prognosis[289].

5.2 TUMOURS OF THE SKIN

The skin is the outside barrier of the body to the environment and the first organ to come in contact with infectious agents. Some of these infectious agents, such as e.g. some types of human papillomaviruses and herpesvirus 6/8 are associated to different types of cancer in the skin, e.g. non-melanoma skin cancer in *Epidermodysplasia Verucciformis* and Kaposi sarcoma [290-292]. Recently, Yuan Chang and Patrick Moore and their team, who discovered HHV-8 in Kaposi sarcoma in 1994, identified MCPyV in MCC, a skin carcinoma of neuroepithelial origin[37].

Today it is known that the skin is the main site of latency for many more types of papillomaviruses[293] and since this family is closely related to the polyomavirus family, it is possible that we, with the increase of the sensitivity of the molecular techniques, may detect more polyomaviruses in the skin. The known neurotropism of MCPyV in MCC has oriented my interest in studying a possible relationship between mucosal melanoma, which are not primarily caused by Ultraviolet (UV)-light exposure, and polyomaviruses.

5.2.1 Merkel cell carcinomas

Two well known factors are important for the development of MCC, an uncommon and aggressive skin cancer: UV-light exposure and age, with 90% of the patients being over the age of 50[294]. Moreover there is a striking epidemiologic association between immunosuppression and MCC, chronically immunosuppressed individuals have 15 times the risk of age-matched controls to develop this cancer[294]. Interestingly there are several reports of MCC regression following restoration of immune function[295,296].

Very recently the discovery of a new polyomavirus in MCC[37] has opened up new avenues for polyomavirus research and also new therapeutic possibilities for MCC like anti tumour-vaccines. In fact the incidence of MCC has tripled over the past 20 years because of improved detection and a rise in the number of elderly and immunosuppressed individuals who are at risk[297].

Today, significant observations suggesting that MCPyV contributes to the development of MCC (detailed below in paragraph 5.3) coexist with arguments against an essential role for MCPyV in MCC oncogenesis[165,298-300].

5.2.2 Mucosal melanomas

The single most important factor for the development of malignant melanomas of the skin is UV-light radiations. In addition, numerous other risk factors or cofactors have been demonstrated, such as, e.g., hereditary/familial predisposition, a large number of nevi, the skin phenotype, and the hair and eye colours[301]. However, UV radiation presumably cannot be responsible for some subgroups of cutaneous melanomas on body surfaces more or less sheltered from the sun, such as the acral lentiginous melanomas of the palms, soles, and sub-ungual areas. Moreover, melanomas can appear on body surfaces completely sheltered from the sun, e.g., mucosal membranes of the sinonasal cavity, the anus-rectum, the vulva-vagina, and the penis. Some other interesting features that raised our interest are their questioning density and their age-standardized incidence. These melanomas, sometimes named extracutaneous melanomas, are rare in absolute numbers compared to the cutaneous melanomas, but are similar to them if density (i.e., the average number of tumours per square unit of body surface area) is taken into account[302]. Moreover, the density of ano-rectal melanomas density has been shown to be higher than that of cutaneous melanomas on average[303]. They also have a high age-standardized incidence, with age peaking at 75-84 years in both genders in primary ano-rectal malignant melanomas[303], and with 75% of the patients with vulvar melanoma and 73% with vaginal melanoma being older than 60 years of age[304], thus being a pathology of the elderly. Finally, melanocytes are embryologically derived from the neural crest and migrated to the basal layer of the epidermis, and this neuroepithelial origin is of particular interest in relation to the neurotropism of polyomavirus in humans.

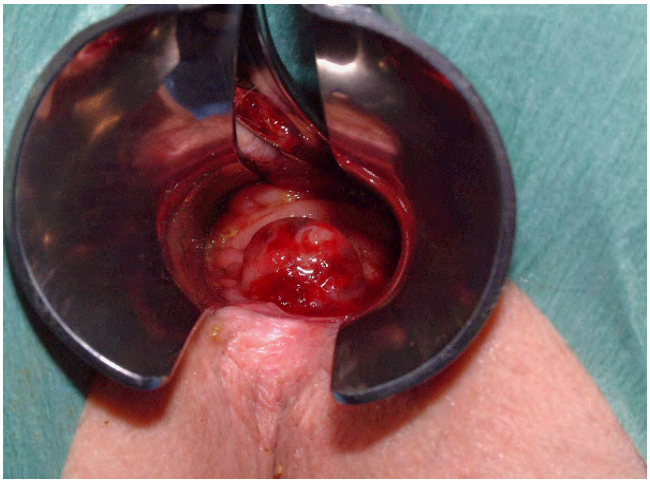


Figure 5. Primary ano-rectal malignant melanoma. *Courtesy of Dr PJ Nilsson*

Additionally, there are biological differences between cutaneous and extracutaneous melanomas and it is possible that by comparing extracutaneous melanomas with cutaneous melanomas, we may find non-UV-light-associated factors or cofactors in melanoma genesis. Viruses could be such factors. It is possible that DNA viruses are associated with melanoma genesis, even if this is so far has been extremely difficult to demonstrate. In previous reports, we have investigated whether HPV or any of the eight viruses of the human herpesvirus group were present in more than 40 extracutaneous melanomas. No HPV DNA-positive

melanoma was detected, although different general primers and several type-specific primers were used, and only two herpesvirus-positive melanomas were detected, indicating that neither HPV nor viruses of the herpesvirus family are major etiological agents for extracutaneous melanomas[305,306]. In this thesis we have analysed for the presence of all 5 human polyomaviruses in extracutaneous melanomas.

5.3 *IN VITRO* AND *IN VIVO* EVIDENCE

SV40, BKV and JCV can cause tumour transformation in experimental animals. Furthermore, in all these cases, there is evidence of viral gene expression[307], suggesting that the viral products are of importance for the transformed state. However, integration of the virus is not always necessary for transformation.

JCV has a unique tropism for replication in glial cells and its replication in humans causes a fatal demyelinating disease of the central nervous system, PML, where the death of oligodendrocytes leads to development of focal areas of progressive demyelination[145]. Three decades of research demonstrated the ability of JCV to transform cells in culture, particularly cells of glial origin including human fetal glial cells and primary hamster brain cells[308]. In laboratory animals, many studies have established the highly oncogenic potential of JCV. Moreover, JCV is the only human polyomavirus that induces solid tumours in non-human primates. JCV inoculation intracerebrally, subcutaneously, or intravenously, induces astrocytomas, glioblastomas and neuroblastomas in owl and squirrel monkeys[309-311], as well as glioblastomas, medulloblastomas, pineocytomas, and other neuroectodermal tumours when inoculated intracerebrally, intraperitoneally or subcutaneously into newborn Syrian hamsters [312-315]. In transgenic mice, JCV T antigen expression induces adrenal neuroblastomas[316], neuroectodermal tumours[317], pituitary gland tumours[318].

In humans JCV has also been found associated with CNS tumours in patients without PML. More specifically, the DNA sequence of JCV as well as expression of JC viral oncoprotein T antigen, LT, was demonstrated in the brain of an immunocompetent patient with oligoastrocytoma[319], and in the brain of children with medulloblastomas[320]. However, there are numerous reports, where JCV has not been found in tumours of the CNS, including a recent comprehensive investigation by Rollison *et al.*[321], and so far, the evidence is not sufficient to conclude that there is an increased ability of the virus to enter and/or replicate in pathological brain tissue, or that JCV indeed contributes to the process of transformation or neoplastic progression in humans[308].

BKV is also highly oncogenic in young or newborn mice, rats and hamsters, inducing ependymoma, neuroblastoma and pineal gland tumours [308]. In humans, there are conflicting reports about the role for BKV in the aetiology of brain tumours, where the use of PCR to enhance assay sensitivity must lead to extreme care in interpretation[322]. Rollison *et al.* addressed this question by a parallel investigation of 225 brain tumours in two different laboratories: one laboratory found 3 BKV positive samples with PCR followed by Southern blot analysis, while the other reported no BKV-positive samples by real-time PCR indicating that BKV does not play a major role in human brain tumours[321]. In a similar way, a possible

role for BKV in the aetiology of embryonal neuroblastomas of the sympathetic nervous system was suggested[323] but could not be confirmed[324].

With the discovery of *MCPyV* in 2008, for the first time a strong association between a human cancer of neuroepithelial origin and a polyomavirus was demonstrated and later confirmed by several groups[37,111,112,155-163]. The presence of MCPyV in MCC, its integration[37,164] and its clonal mutation in the C-terminal region of the LT[43] have been demonstrated. The presence of MCPyV, thus merits further investigation both on the epidemiological and *in vitro* level in order to conclude a direct oncogenic role of this polyomavirus.

So far *KIPyV and WUPyV* have not been associated to human diseases. As members of the polyomavirus family, all three viruses can however be potentially oncogenic and this compelled us to investigate for their presence in human tumours.

6 AIMS

Our ultimate aim is to understand, prevent and cure tumour development and disease associated to polyomavirus infection in humans.

For this purpose we have worked in two directions, on the one hand on the role of BKV in the risk for the development of HC in allogeneic HSCT patients, and on the other hand on the human polyomaviruses detection in cancer, with focus on the following aims.

- To identify factors that, together with BK virus infection increase the risk for development of hemorrhagic cystitis in allogeneic HSCT patients, in order to target patients at risk who could receive early treatment to prevent the disease (Papers I, II and III).

- To examine if the newly discovered members of the *Polyomaviridae* in humans are associated with tumour development (Papers IV and V).

7 PATIENTS, MATERIAL AND METHODOLOGICAL CONSIDERATIONS

7.1 ALLOGENEIC HSCT PATIENTS

In this thesis, 175/269 (65%) successive allogeneic HSCT patients, undergoing 179 HSCT events, accepted to participate in this study*. The prospective patient collection started in May 2002 and ended in December 2006 at the Karolinska University Hospital Huddinge.

During this study, in order to collect data regarding the diagnosis of HC, other infections post-transplantation and GVHD, I went through the patient files of all 175 HSCT patients.

Donor and Stem cell sources

Haematopoietic stem cells were obtained from bone marrow, peripheral blood or umbilical cord blood, either from related donors (RD) or unrelated donors (URD) registered in national or international donor centres. Of the 179 transplantation events, 66 (37%) were performed with grafts received from a sibling donor (RD), while 113 (63%) were performed with grafts from unrelated donors (URD), and among them 9 received unrelated cord blood transplants (UCB). In addition to these nine UCB transplantations, 39/179 (22%) were performed using bone marrow stem cells (BM) and 131/179 (73%) using peripheral blood stem cells (PBSC).

Conditioning regimen specificities for this cohort

A *reduced intensity regimen* was given in 108/179 (60%) HSCT events; The most common protocols were Flu combined with Bu (Slavin protocol) given to patients with myeloid malignancies, Flu in combination with Cy to patients with solid tumours or CLL, Flu with 2 Gy of TBI given to patients with multiple myeloma, Flu and treosulphan for patients with non-malignant disorders, and Flu, Cy, and 6 Gy TBI given to patients with lymphoid malignancies. Notably, all 21 patients in the solid tumour group received RIC.

Myeloablative conditioning (MC) was given in the remaining 71 (40%) HSCT events; 12 Gy f-TBI with Cy, or Bu and Cy, or BEAM.

Moreover, at the time of the study, all patients receiving a URD graft or a mismatched sibling donor graft were treated with *in vivo* T-cell depletion with ATG. Additionally, some patients transplanted for a myelodysplasic symdrome received alemtuzumab (Campath®).

During the time of this study, Fluoroquinolones (Ciproxin®) were systematically used for all patients as a preventive therapy against bacterial infection. Adults received 500 mg 1x2, children over 40kg received 250 mg 1x2, and small children received 100 mg 1x2.

40

* conducted according to permissions 357/01 and 2007/1673-31 from the human research ethics committee of Karolinska Institutet

Definition of late onset BKV-associated HC cases

All cases of haematuria, even microscopic haematuria, reported by the patients/paramedical or medical staff, were recorded on a computerized patient files system (Take Care®), making it possible to perform a retrospective identification of the HC cases. Thirty-three patients were initially categorized as having HC. We first eliminated, based on the patients files, all the other possible causes of acute HC, due to conditioning toxicity, thrombocytopenia, bacterial infections, adenovirus and CMV cystitis. Twenty-seven patients with HC remained defined as *late onset* HC (i.e. > 1 week after HSCT in our study).

Definition of GVHD

GVHD was graded from I to IV as defined previously in section 4.1.2.1.

Urine samples

Over eight hundred urine samples (1-28 samples/patient) were collected prospectively during hospitalisation and follow up, from the week of conditioning up to one year after transplantation.

7.2 MUCOSAL MELANOMAS

Thirty-eight adult patients with primary mucosal melanomas resected at the Karolinska University Hospital were included in this thesis project[**]. The tumours were paraffin-embedded and had been previously tested for the presence of papillomaviruses and herpes viruses[305,306].

In all, 38 samples were tested with the nested PCR to detect BKV, JCV and SV40, but due to lack of material only 37 samples could be further tested with the KI- and WUPyV PCR, and only 36 samples were tested with the MCPyV PCR.

7.3 NEUROBLASTOMAS

Thirty children aged 0 to 11.5 years old, diagnosed between 1991 and 2008 with neuroblastoma operated at Karolinska University Hospital were included in this thesis project[***]. Thirty-one fresh frozen neuroblastomas were collected prospectively. More specifically, one patient had two biopsies from separate manifestations of multifocal disease[325].

Six tumours had been previously tested for BKV DNA detection[323]. During this study, the tumors were tested with the KI- and WUPyV PCR, and the MCPyV PCRs.

[**] conducted according to permissions 03-463 and 2006/604-32 from the human research ethics committee of the Karolinska Institutet.

[***] conducted according to permissions 03-736 and 2007/1253-31/3 from the human research ethics committee of the Karolinska Institutet

7.4 CENTRAL NERVOUS SYSTEM TUMOURS AND PATIENT SERA

In all, 25 patients aged 0 to 18 years old with CNS tumours operated between March 2008-September 2009 at Karolinska University Hospital were included in this thesis project, which was conducted according to permission 2007/1253-31/3 from the human research ethics committee of the Karolinska Institutet.

Twenty-five fresh frozen CNS tumours were collected prospectively. In the operation room a biopsy measuring in average 2x2 mm could be collected from each tumour (a larger material was sometimes available). Additionally, in 14 cases, pieces from the ultrasound aspirate could be obtained at the end of the operation. For 18/25 patients blood samples could be collected at the operation room, or within 1 week after operation, from which sera was extracted and stored at -20^0 C.

During this study, in order to follow and collect data, with regard to diagnosis, disease and patient history, I went through the patient files of all 25 patients with CNS tumours. In parallel, the tumors were tested with the KI- and WUPyV PCR, and the MCPyV PCRs.

7.5 PCR DETECTION AND SENSITIVITY

BKV and JCV detection

A *nested PCR* was designed to detect BKV and JCV (papers I-IV) [326], which inner primers have been previously described [327]. This PCR is also able to detect SV40, and all three detected viruses give amplicons of similar size. Therefore, in order to differentiate the three viruses, we performed a cleavage of the amplicons by Hinf I restriction enzyme, as described previously[328].

P1, P2 and P4 primers had 100% homology with BKV strains MM (V01109), Dunlop (accession no) and AS (accession no), as well as JCV strain Mad-1 (NC_001699). Notably, some mismatches for P3 were present with JCV strain Mad-1 (NC_001699) and BKV strains MM (V01109). This PCR could detect 30 copies of JCV and 40 copies of BKV in recent tests (data not shown). All primers were mismatched with SV40 (see table 1), even though we still could detect this virus (data not shown).

Additionally a *real time quantitative PCR* for BKV was used in the ABI prism 7700 system, which could detect 10^2 to 10^8 copies[329].

We tested all urine specimens from the HSCT patients without preliminary DNA extraction, in both PCR assays in Papers I and II. In Paper III, the urine samples of the additional patients were only tested qualitatively with the Nested PCR. Notably, the Nested PCR inhibition by urine components was prevailed with the use of two different volumes of template in each PCR reaction, 2,5 and 5 μl[326]. Similarly, in order to avoid the real time PCR inhibition by urine components, we diluted the urine specimens up to 1:500 before running them, as suggested by Biel *et al.*[330].

Although we have detected dual infection in some samples, we cannot exclude the eventual competition between BKV and JCV in this assay(data not shown).

KIPyV and WUPyV detection

The PCR used for the simultaneous detection of the related KI and WU polyomaviruses could detect as few as 10 copies of a KIPyV VP1 gene containing plasmid corresponding to a detection limit of <0.001 viral DNA copies/cell genome.

MCPyV detection

For detection of MCPyV we performed a previously established PCR assay using primers situated outside of the helicase domain and generating an amplicon of 138 bp. With this assay, 1000 copies of a plasmid, pUC57MC1, containing the corresponding region of LT could be detected corresponding to a detection limit of <0.1 viral DNA copies/ cell genome.

In addition, we also used a previously established *real time quantitative PCR for MCPyV*[109], where a plasmid control with 2 copies/reaction was reproducibly positive, corresponding to <0.0002 copies/cell genome.

Table 2. Sequence of primers used in PCR for detection of BKV, JCV and SV40, KIPyV and WUPyV, and MCPyV.

Name[116]	Sequence 5'-3'	Virus	Region	Position (bp)	Genbank accession no.
Nested outer primers[1]					
P-3	GTATACACAGCAAAGGAAGC[5]	BKV	LT	2475-2494[2]	V01109 (BKV MM strain)
	GTATACACAGCAAAGGAAGC[5]	JCV	LT	4179-4198[3]	NC_001699 (JCV Mad-1 strain
	GTATACACAGCAAAGGAAGC[5]	SV40	LT	4327-4346[4]	AF316139 (SV40 776 strain)
P-4	GCTCATCAGCCTGATTTTGG	BKV	LT	2827-2846	
	GCTCATCAGCCTGATTTTGG	JCV	LT	4790-4809	
	GCTCATCAGCCTGATTTTGG[5]	SV40	LT	4940-4959	
Nested inner primers[2]					
P-1	AGTCTTTAGGGTCTTCTACC	BKV	LT	2551-2570[2]	V01109 (BKV MM strain)
	AGTCTTTAGGGTCTTCTACC	JCV	LT	4255-4274[3]	NC_001699 (JCV Mad-1 strain
	AGTCTTTAGGGTCTTCTACC[5]	SV40	LT	4406-4425[4]	AF316139 (SV40 776 strain)
P-2	GGTGCCAACCTATGGAACAG	BKV	LT	2707-2726	
	GGTGCCAACCTATGGAACAG	JCV	LT	4408-4427	
	GGTGCCAACCTATGGAACAG[5]	SV40	LT	4453-4572	
BKV Q-PCR[3]					
BK-S	GGGGCGACGAGGAT AAAATGAAGA	BKV	LT	4994–5017	V01108 (BKV Dunlop strain
BK-AS	GCAATCTATCCAAAC CAAGGGCTCTT			4746–4771	
BK-probe	6-FamTTTTTGGAACAAATAGGCCATT CCTTGCAG-TAMRA			4834–4872	
KIPyV2263.F	TTGGATGAAAATGGCATTGG	KIPyV	VP1	2263-2282[6]	EF127906
		WUPyV	VP1	2411-2430[7]	EF444550
KIPyV2404.R	TAACCCTTCTTTGTCTAAAATGTAGCC[5]	KIPyV	VP1	2404-2378	
		WUPyV	VP1	2552-2526	
MCPyLT1709.F	CAGGCATGCCTGTGAATTAGGATG	MCPyV	LT	1709-1732[8]	EU375803
MCPyLT1846.R	TCAGGCATCTTATTCACTCC	MCPyV	LT	1846-1827	
MCPyV Q-PCR[4]					
LT.1F	CCACAGCCAGAGCTCTTCCT	MCPyV	LT	1034-1053	EU375803
LT.1R	TGGTGGTCTCCTCTCTGCTACTG			1179-1157	
LT probe	FAM-TCCTTCTCAGCGTCCCAGGCTT CA-TAMRA			1065-1086	

[1]First described in Bogdanovic et al.[326]

[2]First described in Arthur et al.[327]

[3] First described in Priftakis et al.[329]

[4] First described in Goh et al.[109]

[5]Site of mismatch with the corresponding virus sequence

8 RESULTS

8.1 PAPERS I, II AND III

Paper I: Association between a high BK virus load in urine samples of patients with graft versus host disease and development of hemorrhagic cystitis after hematopoetic stem cell transplantation.

Aim. *To examine the temporal relationship between HC and BK viruria, viral load, GVHD, and conditioning regimen, in a pilot study.*

In this first study (*Paper I*), a pilot study with 31 patients, we collected 170 urine samples between 1-360 days after HSCT and analysed for BK-viruria and viral load. BKV viral load was followed at different time points after HSCT. Furthermore, we separately examined patient files to identify which patients developed HC and which factors were associated to the development of HC. HC patients and grading of HC were defined as described above (see section 7.1) and GVHD was assessed and graded as described above (see sections 7.1 and 4.1.2.1).

Of the 31 patients, 17 (10/18 children and 7/13 adults) excreted BKV in their urine (55%), of which 6/17 (3/10 children and 3/7 adults) developed HC. In addition, we could show that BKV DNA and particularly $>10^6$ BKV copies/µl of urine occurred prior or adjacent to HC and was predictive for HC (p=0.01). In addition, the presence of BKV together with GVHD prior to HC has a predictive ability for HC (p=0.02).

We concluded from the above data that a high viral load together with GVHD prior or at the time of HC could be indicative of a risk for development of HC.

Paper II: The incidence of hemorrhagic cystitis and BK-viruria in allogeneic hematopoietic stem cell transplanted patients according to the intensity of the conditioning regimen.

Aim. *To confirm the results from the pilot study above, and examine for additional risk factors in an extended study.*

To confirm our data and also examine the influence of conditioning and donor source the study was later expanded to include 90 patients (*Paper II*), counting the 31 patients described previously. In total 300 urine samples, including the 170 samples described previously, were collected and analysed for BK-viruria and viral load. The results were correlated to patients' data, including the conditioning regimen, the donor source and patient history of GVHD and HC. HC patients and GVHD were defined as in Paper I.

Forty-seven (52%) patients received MC and 43 (48%) RIC. Fifty-eight (64%) patients had an URD and 32 (36%) had a RD. HC developed in 15 (17%) patients and 39 (43%) patients excreted BKV in their urine. We confirmed that BK-viruria and $>10^6$ BKV copies/µl urine were more common in HC patients, than in non-HC patients (p=0.008 and p = 0.001, respectively).

Despite our previous findings (*Paper I*), there was no difference in the frequency of acute GVHD in patients with HC and without HC, or in patients receiving RIC and MC, or in patients receiving RD and URD graft. Furthermore, there was no difference in the risk for having a viral load $>10^6$ BKV copies/µl urine for patients receiving RIC and MC or in patients transplanted with RD and URD. However, we noticed that none of the 16 patients transplanted for the treatment of non-hematopoietic solid tumours developed HC, and none of them had received a MC, bringing our attention to conditioning as a risk factor for HC. Indeed, BK-viruria and HC were less common in patients receiving RIC compared to patients receiving MC (p = 0.01 and p=0.004 respectively). In addition HC was less common in patients receiving RD as compared to patients with URD grafts (p=0.02).

We thus concluded that patients presenting BK viruria, receiving a transplant from an URD or receiving MC had an increased risk for developing HC.

Paper III: Haemorrhagic cystitis and BK-viruria are more frequent in allogeneic haematopoietic stem cell transplanted patients receiving full conditioning regimen and unrelated HLA mismatched grafts.

Aim. *To examine the impact of recipient-donor HLA matching on HC development.*

In continuation, together with the influence of conditioning regimen and the donor background, we also wanted to examine the impact of HLA matching in HC development (*Paper III*). For this purpose we followed 175 allogeneic HSCT patients, including most of the 90 patients from *Paper II*, undergoing 179 HSCT events. In this study altogether 71 (40%) HSCTs were performed with MC and 108 (60%) with RIC. Sixty-six (37%) HSCTs were performed with RD grafts and 113 (63%) with URD grafts, of which 9 were UCB.

Since this paper describes all HSCT patients included in this thesis a more detailed description of our results is presented here.

HC events

In all, 27 patients (15%) developed HC between 9-158 days after HSCT, of which 26 cases occurred during a first HSCT event, and one during the second HSCT. Moreover, most patients (70%) had severe HC grades (III or IV), while moderate HC (grade II) was documented in 30% of the patients.

BK-viruria

BK-viruria was observed after 83/179 HSCT and occurred primarily during the first 6 months after HSCT. BK-viruria was more common after MC as compared to using RIC (p < 0.001)

and when having a HLA-mismatched donor ($p < 0.01$), but there was no difference between HSCT performed with RD and URD grafts.

HC and BK-viruria and other factors such as conditioning, donor source and HLA

BK-viruria was determined in 23/27 HC events and BK-viruria during the first 6 months after HSCT was more common in HC patients than in non-HC patients ($p<0.0001$). Moreover, most HC patients, i.e. 89% (24/27) of all, and 90% (21/23) of those with BK-viruria, developed HC within the first 2 months after HSCT.

HC was more frequent during HSCT performed with MC than with RIC ($p<0.0001$). And having a URD rather than an RD graft was more common ($p<0.05$) in the HC patients group.

In addition, having a HLA-mismatched donor was also more frequent in patients with HC than in patients without HC ($p<0.05$). There were too few cases to make any additional correlations, but some tendencies could be observed. More specifically, a graft with a major HLA (HLA-A*, -B*, or DRB1*) mismatch was more common in HC cases (7/27, 26%) than in HSCT events without HC (15/152, 10%) ($p=0.054$), whereas having a minor HLA (HLA-C*, -DQA*, -DQB*) mismatch did not make any difference between the two groups. Furthermore, neither HLA class-I mismatches (HLA-A*, -B* and –C*) nor HLA class-II mismatches (HLA-DRB1*, -DQA*, -DQB*) were significantly associated to the occurrence of HC (data not shown).

Summarizing the above, in a multivariate logistical regression analysis, we found that significant independent risk factors for HC were BKV (OR 6.7; 95% CI 2.0-21.7; $p = 0.001$), MC (OR 6.0; 95% CI 2.1-17.3; $p < 0.001$) and URD (OR 3.4; 95% CI 1.1-10.6; $p = 0.03$), while male gender again had a borderline effect (OR 2.8; 95% CI 0.95-8.4; $p > 0.06$).
Since the use of an URD graft was a risk factor for HC, we also constructed multivariate models separately for patients receiving RD or URD grafts. In patients receiving URD grafts, BKV (OR 5.5; 95%CI 1.6-18.3) and MC (OR 4.4; 95% CI 1.4-13.2; $p = 0.009$) increased the risk for HC in patients, while no factor was identified increasing the risk for HC in patients receiving RD grafts.

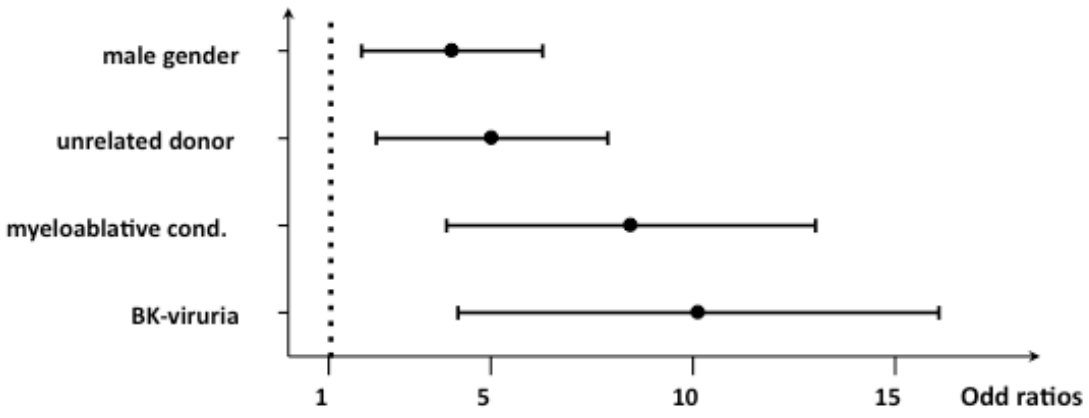


Figure 6. Independent risk factors for Hemorrhagic Cystitis

To conclude, BK-viruria was correlated to HC, and an increased risk for HC was confirmed for patients receiving MC or an URD graft. Furthermore, HLA disparity was found to be a significant risk factor for BK-viruria ($p < 0.01$) and also important for HC ($p<0.05$).

In a multivariate logistical regression analysis, BK-viruria, MC and URD graft remained *independent* risk factors for HC development. Furthermore, when analysing patients receiving RD or URD grafts separately, BKV and MC remained *independent* risk factors for patients with URD grafts, but not with RD grafts.

8.2 PAPER IV

DNA from BK virus and JC virus and from KI, WU, and MC polyomaviruses as well as from simian virus 40 is not detected in non-UV-light-associated primary malignant melanomas of mucous membranes.

Aim. *To examine the presence of the known human polyomaviruses in extracutaneous melanomas, a skin cancer of the sun sheltered body surfaces, where UV light cannot explain carcinogenesis.*

Thirty-eight mucosal melanomas were analysed for the presence of BKV, JCV, SV40, KI, WU and MCPyV DNA. After DNA extraction, to test if the DNA was amplifiable by PCR, a PCR (S14PCR) of a household gene was run. Thereafter the presence of polyomavirus DNA was examined by nested PCR for BKV, JCV, and SV40, a newly designed PCR for KIPyV and WUPyV, and a newly designed PCR for MCPyV. None of the tumours exhibited the presence of polyoma DNA in any of the samples.

Our conclusion was therefore that the so far known human polyomaviruses do not play any role in the development of the 38 tested extracutaneous melanomas.

8.3 PAPER V

DNA from KI, WU and Merkel Cell Polyomaviruses is not Detected in Childhood Central Nervous System Tumours or Neuroblastomas.

Aim. *To examine the existence of the newly discovered polyomaviruses in neuroblastomas and CNS tumours of children, where efforts to confirm the presence of JCV, BKV or SV40 have been inconclusive.*

Thirty-one neuroblastomas and 25 childhood CNS tumours were collected. After DNA extraction, to test the quality of the DNA a S14 PCR was run as in paper IV.

Polyomavirus DNA was then tested for, but not detected when using a PCR detecting KIPyV and WUPyV, and a newly designed PCR for MCPyV complemented by a real-time PCR

detecting MCPyV LT. Hence, none of the tumours exhibited the presence of polyoma DNA in any of the samples.

Additionally, available sera from 18/25 CNS tumour patients were analysed for MCPyV-specific antibody responses, and 56% of the sera were completely negative in a highly sensitive neutralization assay, which very accurately excludes false negative results and has been described previously [127]. Of the MCPyV antibody-positive sera, all but one had antibody titres within two fold of the geometric mean titre (2700) calculated from an adult population (between 47 and 75 years old) tested earlier[127]. The remaining patient had an antibody titre of 98,740, which, while greater than the titres of other subjects in this study, was still below the geometric mean titre (160,000) reported for patients suffering from MCPyV-associated MCC[127].

In conclusion, in this pilot study MCPyV, KIPyV and WUPyV have so far not been shown to be causative of childhood CNS tumours or neuroblastomas.

9 DISCUSSION

STUDIES ON THE RISK TO DEVELOP BKV-ASSOCIATED HEMORRHAGIC CYSTITIS (PAPERS I, II, III)

In this thesis the significance of BKV and possible co-factors for the development of HC in allogeneic HSCT patients was studied. Our studies confirmed similar to previous studies that the presence of BK-viruria was correlated to a higher risk of developing HC as compared to the absence of BK-viruria. In a pilot study (*Paper I*), the presence of aGVHD was, together with BK-viruria, associated to HC development, but this correlation was not confirmed later in an extended material. However, with a larger material (*Papers II and III*), we could show that the use of MC and a URD were correlated to the development of HC. Finally, in a multivariate analysis (*Paper III*), BK-viruria, MC and URD graft remained independent risk factors for HC development, and when analysing patients receiving RD or URD grafts separately, BKV and MC remained risk factors for patients with URD grafts, but not with RD grafts.

We concluded that BKV-viruria was correlated to the development of HC and although a high BK-viral load was indicative of, it was not diagnostic for HC. In our prospective study with 175 patients we were able to confirm the association of BK-viruria and the occurrence of HC, first reported by Arthur *et al.*[142], and confirmed later by others [136-141]. However, BKV reactivation in the urine was not exclusively found in HC patients. We also attempted to find if BK-viral load determined in urine samples could be indicative of HC. We could show that BK-viruria and viral load $>10^6$ BKV copies/µl urine were more common in HC than in non-HC patients (*Paper I and II*), as previously reported by others[329-332]. However, the latter suggestion must be cautiously interpreted, since a statistically significant difference in BKV load was not noted between RIC and full conditioning. Moreover, it is obvious that different individuals produce different quantities of urine thus potentially also influencing BKV load. We therefore excluded the quantification of BKV viral load in the last study (*Paper III*).

We also concluded that acute GVHD is not correlated to the development of HC. In the pilot study in paper I, aGVHD grade II to IV was more common in patients with HC (83%) than in patients without HC (20%) (p=0.008) and in non-HC patients with BK-viruria (30%) (p=0.014). However, the findings in the pilot study could not be confirmed in paper II or III. In paper III, aGVHD was more common in patients with HC (44%) or with BKVHC (48%) than during HSCT events without HC (28%), but this difference was not statistically significant. In addition, when correcting for donor type and HLA-mismatch, there was no influence of aGVHD on the risk for HC in patients receiving either RD or URD grafts.

Moreover, in our population of the 27 patients with HC, 14 did not develop aGVHD grade II or more. In fact, 4/14 never developed any symptoms of GVHD, despite that they had MC and had moderate to severe HC (one grade II, two grade III and one grade IV), developing between day 18 to 72. For these four cases, the severity of the HC argues against the possibility that this would be an aGVHD disease strictly isolated to the bladder. In previous

reports, as in paper I, aGVHD has been suggested to be associated or be part of the development of HC[138,217,273,333,334], but we (*Paper II*) and others [136,140,335] have not been able to confirm these observations.

We could not conclude that the stem cell source influenced the risk of developing HC in our studies. We did not observe that obtaining a BMT graft or PBSCT was a risk for HC and the number of UCBs was too low to draw any definite conclusions. In paper II, 4 patients received a UCB graft and 2/4 developed HC. In paper III, 9 patients received a UCB graft and 3/9 developed HC. The limited amount of UCB transplants in our study did not allow further conclusions. Notably, in the last study, an important proportion of these nine patients had a constitutional immune deficiency leading to impaired immune response to viral infection.

In this thesis study we concluded that patients receiving an URD had an increased risk for HC. We were able to show that a URD presented a risk for the development of HC (*Paper II*) and this was in agreement to the report by El-Zimaity *et al.*[336] published just prior to ours and this confirmed previous suggestive reports[137,335]. However BK-viruria was not more common in patients receiving a URD graft as compared to patients receiving a RD graft.

We were first to conclude that patients receiving MC have an increased risk for BKVHC as compared to those receiving RIC, and this was simultaneously reported by others[337]. In fact, the observation that none of the 21 patients transplanted for the treatment of non-hematopoietic solid tumours developed HC, whereas BK-viruria was detected in 80% of them, was enigmatic. All these 21 patients had received RIC. Moreover, they received a more aggressive anti-GVHD prophylaxis possibly leading to a probable delayed chimerism. The latter is tolerated in patients with non-haematopoietic cancers and thereby we speculate that these patients could have enough autologous BKV- specific cytotoxic T cells to protect them against BKVHC.

In our study we observed that receiving a graft with a major HLA-mismatch may play a role in the development of BKVHC. Having a HLA-mismatch was an independent risk factor for BK-viruria ($p < 0.01$), but not for HC. However, a HLA-mismatch donor was more common in HC patients ($p<0.05$), but the study was too small to pinpoint the risk of developing HC to a certain HLA antigen. However a larger study is necessary to confirm this tendency. In fact apart from the mismatch itself, making an immune response less accurate, a second effect of HLA-mismatch on BK-viruria may be explained by the more frequent use of anti-thymocyte globulin treatment for recipients with HLA-mismatch grafts. Hence, BK-viruria could be enhanced by the T-cell depletion rather than by the HLA-mismatch immunological effect. The latter, could instead contribute to the tissue damage to the bladder, when a specific anti-BKV cytotoxic T-cell response from the graft is induced, due to a possible subtle imprecise recognition of the BKV infected cells by the engrafted CD8+ T cells. In some contexts, this phenomenon has been described as immune reconstitution disease[226,338-341].

Specific use of Ciprofloxacin during the aplastic phase for this cohort. During the time of this study, Fluoroquinolones (Ciproxin®) were systematically used for all patients in the immediate post-transplant phase, as a preventive therapy against bacterial infection (Jonas

Mattsson personal communication). Ciprofloxacin has been reported to significantly decrease BKV viruria, but has not been shown to reduce the incidence of HC[342]. In our study, this could explain the absence of BKV in some urine samples taken during the aplastic phase, from patients presenting an authentic HC later on with a “delayed” BK viruria. We do not support that BKV primary infections play a major role for HC in these patients however, since we have shown previously that primo-infections are unlikely to occur after HSCT[119]. Furthermore, in the case of a primo-infection, one would expect a severe HC in this context of immunosuppression, but this was not the case for our patients with delayed BKV viruria.

POLYOMAVIRUS IN RELATION TO HUMAN CANCER (PAPERS IV, V)

In this thesis we were not able to detect polyomavirus DNA from JCV, BKV, KI, WU or MCPyV in mucosal melanomas (*Paper IV*). In addition, we were not able to detect the presence of KI, WU or MCPyV DNA in neuroblastomas or CNS tumours in children (*Paper V*).

The methods used for the analysis have been robust and have the ability to detect between 2-1000 copies/ PCR reaction. In each PCR reaction we have included at least 100ng DNA corresponding to about 15 000 cells. Hence, the sensitivity of the reaction has been high and should detect between 0.00013 and 0.06 viral DNA copies /cell genome. Hence the negative results are not due to a low sensitivity of our assay. The fact that MCPyV is difficult to detect has, to some extent, also been circumvented by the use of two different technologies. Indeed it has been demonstrated [37,113,155-157,160-163,343] that tumours positive for MCPyV do not always show positivity for all primer pairs. Whether this is due to a low sensitivity or different viral strains may still have to be resolved.

There are conflicting reports showing the presence of MCPyV in other non-MCC skin cancers[110-114]. However none of these studies analysed the expression of MCPyV genes and the presence of MCPyV was not very common, e.g. MCPyV was reported in 2/15 squamous cell carcinomas (SCC)[112], and in basal cell carcinomas (BCC), MCPyV was detected in 3/24 tumours[111].

Nonetheless, the fact that JCV, BKV, KI, WU and MCPyV DNA could by using these methods still not be detected in mucosal melanomas suggests that these viruses do not play an aetiological role for these tumours. Hence, the aetiology of these tumours still remains to be clarified, whether it is another virus or a factor of entirely different character and not connected to infectious agents.

We have also demonstrated the absence of the newly discovered polyomaviruses KI, WU and MCPyV in paediatric CNS tumours and neuroblastomas. Here, we did not analyse for the presence of JCV and BKV, since several previous scientific reports have performed such studies and the presence of JCV and BKV as aetiological agents in both neuroblastoma and childhood CNS tumours have so far not been confirmed[319-321,323,324].

Again as mentioned above the sensitivity of our assays was good and should detect down to 0.06 viral DNA copies /cell genome. Moreover, in this study, the use of a real-time combined with a standard PCR (*paper V*) increased the sensitivity for MCPyV detection, and finally we could detect less than 0.0002 copies/cell genome of MCPyV or one copy among 5000 cells. Moreover we chose, both in the standard and the real-time PCR, a primer pair situated before the critical helicase region, where deletions have been reported [43].

Thus the data suggest that KI, WU and MCPyV are not involved in the aetiology of neuroblastomas. Our findings do however not in any way exclude the possibility that other so far not detected infectious agents could be involved in this disease.

The fact that we could not detect DNA of polyomaviruses KI, WU and MCPyV in childhood CNS tumours should however be taken with more caution, since our analysis includes a limited number of samples from a variety of diagnostic subsets, although the proportion of each tumour group was fairly similar to that of the Swedish registry (table 2 in paper V). Moreover it should be noted that 6/25 (24%) patients had a genetic cancer syndrome known to play a role for CNS tumour development, a proportion above the average population.

10 CONCLUSIONS

- BK-viruria, full myeloablative conditioning (MC) and an unrelated donor (URD) graft increase the risk for the development of HC after HSCT, even though MC, but not an URD graft, was associated to BK-viruria.

- Using a recipient-donor with a HLA mismatch may present an increase in the risk for developing BKVHC, but this tendency remains to be confirmed in a larger study.

- aGVHD or a specific stem cell source (BMT or PBSCT) are not significantly associated to BKVHC in our studies, while if there is an increased risk for HC after receiving UCB needs to be further investigated.

- JCV, BKV, KIPyV, WUPyV and MCPyV DNA are not detected in 38 mucosal melanomas and this suggests that these viruses do not play an aetiological role in these tumours. Hence, the aetiology of these tumours still remains to be clarified, whether it is another virus or something of entirely different character and not connected to infectious agents.

- KI, WU and MCPyV are not involved in the aetiology of neuroblastomas. Our findings do however not in any way exclude the possibility that other so far not detected infectious agents could be involved in this disease.

- We have been first to report the absence of KI, WU and MCPyV DNA in childhood CNS tumours. However, these are preliminary results that remain to be confirmed, since our analysis includes a limited number of samples from a variety of diagnostic subsets.

11 FUTURE PERSPECTIVES

Today, some more efforts remain to be done to identify additional factors predicting the risk for development of BKVHC in allogeneic HSCT patients. Further down, I will briefly mention some recent reports that could orientate further research more specifically, and the actual treatments that could be tested in order to prevent the disease.

With regard to human polyomaviruses and cancer, I have only looked at a few viruses and a few tumour types and the work on the brain tumours of children should be continued. Moreover, in the future, there could be additional members of the human polyomavirus family and then of course there will be many tumours that could be examined for the presence of these viruses. I will not discuss this further here.

BK-viremia in relation to HC

BK-viremia has recently been shown to correlate with post-engraftment HC [344,345], but these reports did not detect BK-viruria. However we have shown in a previous study on limited material that no BK-viremia could be detected in some cases of BKVHC[346], it is still of great interest to investigate for the presence of BK-viremia, in addition to BK-viruria, since detection of BK-viruria and/or BK-viremia has recently, in other reports, been shown to have a predictive value for BKVHC[136].

Specificities of a subpopulation of unrelated donor recipients: the cord blood transplanted patients

UCB was more common in patients with BKV-associated HC 3/23 (13%) as compared to all HC patients 3/27 (11%) or non-HC patients 6/131 (4%). However, our material was limited and includes patients with constitutive immune deficiencies, therefore we could not make any further conclusions. Nevertheless, before us, Zimaty *et al.* reported a significant association of UCB HSCT and HC outcome [336]. Furthermore, in a study focusing on cord blood transplants Rocha et al. could show an association with the delayed engraftment and an increased risk of all types of infections[192]. Additionally, a better HLA-matching and a higher cell dose have been associated to better engraftment and survival in this specific type of unrelated-donor recipients[347]. These clinicians noticed the particular severity of HC after UCB transplantation and speculated that this could be explained by the combination of a delayed immune reconstitution associated with the presence of the BKV (Vanderson Rocha personal communication). However no study on this specific problem has been published so far. Interestingly, at the University of Tokyo, the only large study focusing on HC after URD UBC HSCT showed a questioning high cumulative incidence of HC in this cohort, within one year after transplantation (41.8 %). Unfortunately, these authors did not study the presence of BKV and focused instead on adenovirus, known to be especially associated to HC in Japan

(see section 4.2.4), which incidence in the urine was notably low in this study (3%). Whether a high prevalence of HC is observed after UBC HSCT merits further investigation.

When to prevent or treat HC?

The final goal for the HSCT patients is to find patients at risk and try to prevent the occurrence of BKVHC. Today several drugs are available when treatment is necessary, but they have several side effects and can therefore not always be used in the allogeneic HSCT setting. Carefully balanced reduction of immunosuppression plays an important role in BKVAN treatment. From the therapeutic studies on BKVAN, we have learned the anti-viral efficacy of some drugs on BKV: Cidofovir [348-354], and Leflunomide, which is particularly appealing for both its immunosuppressive and anti-viral activities[355,356]. In BKVHC, the use of Cidofovir has been studied[348,357,358]. Fluoroquinolones have also been reported to reduce BK-viral load, but no effect on the outcome of HC was observed in this limited cohort[342]. In our cohort, patients systematically received fluoroquinolones, and therefore an effect could not be studied. However a prospective study on the early use of Cidofovir in patients at risk for HC after allogeneic stem cell transplantation remains to be done. By better defining the patients at risk to develop HC, we have hopefully contributed to defining which patient groups these therapeutic trials should target.

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13 REFERENCES

1. Gross L. A filterable agent, recovered from Ak leukemic extracts, causing salivary gland carcinomas in C3H mice. Proc Soc Exp Biol Med 1953;83(2):414-421.
2. Stewart S, Eddy B, Borgese N. Neoplasms in mice inoculated with a tumor agent carried in tissue culture. J Natl Cancer Inst 1958;20(6):1223-1243.
3. Eddy B, Stewart S, Berkeley W. Cytopathogenicity in tissue culture by a tumor virus from mice. Proc Soc Exp Biol Med 1958;98(4):848-851.
4. Atkin S, Griffin B, Dilworth S. Polyoma virus and simian virus 40 as cancer models: history and perspectives. Semin Cancer Biol 2009;19(4):211-217.
5. Sweet B, Hilleman M. The vacuolating virus, S.V. 40. Proc Soc Exp Biol Med 1960;105:420-427.
6. Eckhart W. Cell transformation by polyoma virus and SV40. Nature 1969;224(5224):1069-1071.
7. Di Mayorca G, Callender J, Marin G, Giordano R. Temperature-sensitive mutants of polyoma virus. Virology 1969;38(1):126-133.
8. Fried M. Cell-transforming ability of a temperature-sensitive mutant of polyoma virus. Proc Natl Acad Sci U S A 1965;53:486-491.
9. Linzer D, Levine A. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. Cell 1979;17(1):43-52.
10. Lane D, Crawford L. T antigen is bound to a host protein in SV40-transformed cells. Nature 1979;278(5701):261-263.
11. DeCaprio J, Ludlow J, Figge J, Shew J, Huang C, Lee W, Marsilio E, Paucha E, Livingston D. SV40 large tumor antigen forms a specific complex with the product of the retinoblastoma susceptibility gene. Cell 1988;54(2):275-283.
12. Whyte P, Buchkovich K, Horowitz J, Friend S, Raybuck M, Weinberg R, Harlow E. Association between an oncogene and an anti-oncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product. Nature 1988;334(6178):124-129.
13. Dyson N, Howley P, Münger K, Harlow E. The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science 1989;243(4893):934-937.
14. Hellström I, Sjögren H. Demonstration of H-2 isoantigens and polyoma specific tumor antigens by measuring colony formation in vitro. Exp Cell Res 1965;40(1):212-215.
15. Habel K. Specific complement-fixing antigens in polyoma tumors and transformed cells. Virology 1965;25:55-61.
16. Koch M, Sabin A. Specificity of virus-induced resistance to transplantation of polyoma and SV 40 tumors in adult hamsters. Proc Soc Exp Biol Med 1963;113:4-12.
17. Berke Z, Palmer S, Bergman T, Wester D, Svedmyr J, Linder S, Jornvall H, Dalianis T. A short peptide eluted from the H-2Kb molecule of a polyomavirus-positive tumor corresponds to polyomavirus large T antigen peptide at amino acids 578 to 585 and induces polyomavirus-specific immunity. J Virol 1996;70(5):3093-3097.
18. Ramqvist T, Reinholdsson G, Carlquist M, Bergman T, Dalianis T. A single peptide derived from the sequence common to polyoma small and middle T-antigen induces immunity against polyoma tumors. Virology 1989;172(1):359-362.
19. Ramqvist T, Pallas D, DeAnda J, Ahrlund-Richter L, Reinholdsson G, Roberts T, Schaffhausen B, Dalianis T. Immunization against the polyoma tumor-specific transplantation antigen (TSTA) with polyoma T-antigens. Int J Cancer 1988;42(1):123-128.
20. Ramqvist T, Reinholdsson G, Szigeti R, Klein G, Dalianis T. Studies on the polyoma tumor-specific transplantation antigen (TSTA): selection and

characterization of TSTA-negative segregants from somatic hybrids. Int J Cancer 1987;40(1):74-80.
21. Campbell A, Foley F, Tevethia S. Demonstration of multiple antigenic sites of the SV40 transplantation rejection antigen by using cytotoxic T lymphocyte clones. J Immunol 1983;130(1):490-492.
22. Flyer D, Pretell J, Campbell A, Liao W, Tevethia M, Taylor J, Tevethia S. Biology of simian virus 40 (SV40) transplantation antigen (TrAg). X. Tumorigenic potential of mouse cells transformed by SV40 in high responder C57BL/6 mice and correlation with the persistence of SV40 TrAg, early proteins and sequences. Virology 1983;131(1):207-220.
23. Chang C, Simmons D, Martin M, Mora P. Identification and partial characterization of new antigens from simian virus 40-transformed mouse cells. J Virol 1979;31(2):463-471.
24. Habel K, Jensen F, Pagano J, Koprowski H. Specific complement-fixing tumor antigen in SV40-transformed human cells. Proc Soc Exp Biol Med 1965;118:4-9.
25. Black P, Rowe W, Turner H, Huebner R. A specific complement-fixing antigen present in SV40 tumor and transformed cells. Proc Natl Acad Sci U S A 1963;50:1148-1156.
26. Khera K, Ashkenazi A, Rapp F, Melnick J. Immunity in hamsters to cells transformed in vitro and in vivo by SV40. Tests for antigenic relationship among the papovaviruses. J Immunol 1963;91:604-613.
27. Defendi V. Effect of SV 40 virus immunization on growth of transplantable SV 40 and polyoma virus tumors in hamsters. Proc Soc Exp Biol Med 1963;113:12-16.
28. Andreasson K, *Doctoral thesis,* Institutionen för Onkologi-Patologi. Vaccination against Her2/neu-expressing cancer using chimeric virus-like particles. Karolinska Institutet, Stockholm; 2009.
29. Müller U, Zentgraf H, Eicken I, Keller W. Higher order structure of simian virus 40 chromatin. Science 1978;201(4354):406-415.
30. Treisman R, Cowie A, Favaloro J, Jat P, Kamen R. The structures of the spliced mRNAs encoding polyoma virus early region proteins. J Mol Appl Genet 1981;1(2):83-92.
31. Gosert R, Rinaldo C, Funk G, Egli A, Ramos E, Drachenberg C, Hirsch H. Polyomavirus BK with rearranged noncoding control region emerge in vivo in renal transplant patients and increase viral replication and cytopathology. J Exp Med 2008;205(4):841-852.
32. Flaegstad T, Sundsfjord A, Arthur R, Pedersen M, Traavik T, Subramani S. Amplification and sequencing of the control regions of BK and JC virus from human urine by polymerase chain reaction. Virology 1991;180(2):553-560.
33. Negrini M, Sabbioni S, Arthur R, Castagnoli A, Barbanti-Brodano G. Prevalence of the archetypal regulatory region and sequence polymorphisms in nonpassaged BK virus variants. J Virol 1991;65(9):5092-5095.
34. Yogo Y, Kitamura T, Sugimoto C, Ueki T, Aso Y, Hara K, Taguchi F. Isolation of a possible archetypal JC virus DNA sequence from nonimmunocompromised individuals. J Virol 1990;64(6):3139-3143.
35. Gaynor AM, Nissen MD, Whiley DM, Mackay IM, Lambert SB, Wu G, Brennan DC, Storch GA, Sloots TP, Wang D. Identification of a novel polyomavirus from patients with acute respiratory tract infections. Plos Pathogens 2007;3(5):595-604.
36. Allander T, Andreasson K, Gupta S, Bjerkner A, Bogdanovic G, Persson MAA, Dalianis T, Ramqvist T, Andersson B. Identification of a third human polyomavirus. Journal of Virology 2007;81(8):4130-4136.
37. Feng HC, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science 2008;319(5866):1096-1100.
38. Peden K, Spence S, Tack L, Cartwright C, Srinivasan A, Pipas J. A DNA replication-positive mutant of simian virus 40 that is defective for transformation and the production of infectious virions. J Virol 1990;64(6):2912-2921.

39. Zalvide J, Stubdal H, DeCaprio J. The J domain of simian virus 40 large T antigen is required to functionally inactivate RB family proteins. Mol Cell Biol 1998;18(3):1408-1415.
40. Ewen M, Ludlow J, Marsilio E, DeCaprio J, Millikan R, Cheng S, Paucha E, Livingston D. An N-terminal transformation-governing sequence of SV40 large T antigen contributes to the binding of both p110Rb and a second cellular protein, p120. Cell 1989;58(2):257-267.
41. Schmieg F, Simmons D. Characterization of the in vitro interaction between SV40 T antigen and p53: mapping the p53 binding site. Virology 1988;164(1):132-140.
42. Kierstead T, Tevethia M. Association of p53 binding and immortalization of primary C57BL/6 mouse embryo fibroblasts by using simian virus 40 T-antigen mutants bearing internal overlapping deletion mutations. J Virol 1993;67(4):1817-1829.
43. Shuda M, Feng HC, Kwun HJ, Rosen ST, Gjoerup O, Moore PS, Chang Y. T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus. Proceedings of the National Academy of Sciences of the United States of America 2008;105(42):16272-16277.
44. Ito Y, Spurr N. Polyoma virus T antigens expressed in transformed cells: significance of middle T antigen in transformation. Cold Spring Harb Symp Quant Biol 1980;44 Pt 1,:149-157.
45. Delmas V, Bastien C, Scherneck S, Feunteun J. A new member of the polyomavirus family: the hamster papovavirus. Complete nucleotide sequence and transformation properties. EMBO J 1985;4(5):1279-1286.
46. Courtneidge S, Goutebroze L, Cartwright A, Heber A, Scherneck S, Feunteun J. Identification and characterization of the hamster polyomavirus middle T antigen. J Virol 1991;65(6):3301-3308.
47. Yang S, Lickteig R, Estes R, Rundell K, Walter G, Mumby M. Control of protein phosphatase 2A by simian virus 40 small-t antigen. Mol Cell Biol 1991;11(4):1988-1995.
48. Skoczylas C, Fahrbach K, Rundell K. Cellular targets of the SV40 small-t antigen in human cell transformation. Cell Cycle 2004;3(5):606-610.
49. Finch J. The surface structure of polyoma virus. J Gen Virol 1974;24(2):359-364.
50. Jiang M, Abend J, Tsai B, Imperiale M. Early events during BK virus entry and disassembly. J Virol 2009;83(3):1350-1358.
51. Moens U, Van Ghelue M, Johannessen M. Oncogenic potentials of the human polyomavirus regulatory proteins. Cell Mol Life Sci 2007;64(13):1656-1678.
52. Sullivan C, Grundhoff A, Tevethia S, Pipas J, Ganem D. SV40-encoded microRNAs regulate viral gene expression and reduce susceptibility to cytotoxic T cells. Nature 2005;435(7042):682-686.
53. Seo G, Fink L, O'Hara B, Atwood W, Sullivan C. Evolutionarily conserved function of a viral microRNA. J Virol 2008;82(20):9823-9828.
54. Seo G, Chen C, Sullivan C. Merkel cell polyomavirus encodes a microRNA with the ability to autoregulate viral gene expression. Virology 2009;383(2):183-187.
55. Sullivan C, Sung C, Pack C, Grundhoff A, Lukacher A, Benjamin T, Ganem D. Murine Polyomavirus encodes a microRNA that cleaves early RNA transcripts but is not essential for experimental infection. Virology 2009;387(1):157-167.
56. Stehle T, Gamblin S, Yan Y, Harrison S. The structure of simian virus 40 refined at 3.1 A resolution. Structure 1996;4(2):165-182.
57. Liddington R, Yan Y, Moulai J, Sahli R, Benjamin T, Harrison S. Structure of simian virus 40 at 3.8-A resolution. Nature 1991;354(6351):278-284.
58. Breau W, Atwood W, Norkin L. Class I major histocompatibility proteins are an essential component of the simian virus 40 receptor. J Virol 1992;66(4):2037-2045.
59. Low J, Humes H, Szczypka M, Imperiale M. BKV and SV40 infection of human kidney tubular epithelial cells in vitro. Virology 2004;323(2):182-188.
60. Tsai B, Gilbert J, Stehle T, Lencer W, Benjamin T, Rapoport T. Gangliosides are receptors for murine polyoma virus and SV40. EMBO J 2003;22(17):4346-4355.

61. Eash S, Querbes W, Atwood W. Infection of vero cells by BK virus is dependent on caveolae. J Virol 2004;78(21):11583-11590.
62. Low J, Magnuson B, Tsai B, Imperiale M. Identification of gangliosides GD1b and GT1b as receptors for BK virus. J Virol 2006;80(3):1361-1366.
63. Erickson K, Garcea R, Tsai B. Ganglioside GT1b is a putative host cell receptor for the Merkel cell polyomavirus. J Virol 2009;83(19):10275-10279.
64. Elphick G, Querbes W, Jordan J, Gee G, Eash S, Manley K, Dugan A, Stanifer M, Bhatnagar A, Kroeze W, Roth B, Atwood W. The human polyomavirus, JCV, uses serotonin receptors to infect cells. Science 2004;306(5700):1380-1383.
65. Pho M, Ashok A, Atwood W. JC virus enters human glial cells by clathrin-dependent receptor-mediated endocytosis. J Virol 2000;74(5):2288-2292.
66. Fields BN, Knipe DM, Howley PM, Griffin DE. Fields virology. Philadelphia: Lippincott Williams & Wilkins; 2007. S. 1551-3091 p.
67. Swanson Pn, Lukacher A, Szomolanyi-Tsuda E. Immunity to polyomavirus infection: the polyomavirus-mouse model. Semin Cancer Biol 2009;19(4):244-251.
68. Drake Dr, Lukacher A. Beta 2-microglobulin knockout mice are highly susceptible to polyoma virus tumorigenesis. Virology 1998;252(1):275-284.
69. Liunggren G, Liunggren H, Dalianis T. T cell subsets involved in immunity against polyoma virus-induced tumors. Virology 1994;198(2):714-716.
70. Wirth J, Martin L, Fluck M. Oncogenesis of mammary glands, skin, and bones by polyomavirus correlates with viral persistence and prolonged genome replication potential. J Virol 1997;71(2):1072-1078.
71. Allison A. Interactions of antibodies, complement components and various cell types in immunity against viruses and pyogenic bacteria. Transplant Rev 1974;19(0):3-55.
72. Berke Z, Mellin H, Heidari S, Wen T, Berglöf A, Klein G, Dalianis T. Adult X-linked immunodeficiency (XID) mice, IGM-/- single knockout and IGM-/- CD8-/- double knockout mice do not clear polyomavirus infection. In Vivo 1998;12(2):143-148.
73. Szomolanyi-Tsuda E, Le QP, Garcea RL, Welsh RM. T-Cell-independent immunoglobulin G responses in vivo are elicited by live-virus infection but not by immunization with viral proteins or virus-like particles. J Virol 1998;72(8):6665-6670.
74. Lacey SF, Zhou W. Immune responses to BK and JC viruses in immunocompromised patients. Future Virology 2007;2(3):255-266.
75. Rekvig O, Moens U, Sundsfjord A, Bredholt G, Osei A, Haaheim H, Traavik T, Arnesen E, Haga H. Experimental expression in mice and spontaneous expression in human SLE of polyomavirus T-antigen. A molecular basis for induction of antibodies to DNA and eukaryotic transcription factors. J Clin Invest 1997;99(8):2045-2054.
76. Allison A, Taylor R. Observations on thymectomy and carcinogenesis. Cancer Res 1967;27(4):703-707.
77. Law L, Ting R, Leckband E. Prevention of virus-induced neoplasms in mice through passive transfer of immunity by sensitized syngeneic lymphoid cells. Proc Natl Acad Sci U S A 1967;57(4):1068-1075.
78. Berke Z, Wen T, Klein G, Dalianis T. Polyoma tumor development in neonatally polyoma-virus-infected CD4-/- and CD8-/- single knockout and CD4-/-8-/- double knockout mice. Int J Cancer 1996;67(3):405-408.
79. Berke Z, Wen T, Jin S, Klein G, Dalianis T. Polyomavirus persists in CD4/8 double-knockout, but not in CD4 or CD8 single-knockout mice. Virology 1995;212(1):268-271.
80. Berger J, Koralnik I. Progressive multifocal leukoencephalopathy and natalizumab--unforeseen consequences. N Engl J Med 2005;353(4):414-416.
81. Stüve O, Marra C, Bar-Or A, Niino M, Cravens P, Cepok S, Frohman E, Phillips J, Arendt G, Jerome K, Cook L, Grand'Maison F, Hemmer B, Monson N, Racke M. Altered CD4+/CD8+ T-cell ratios in cerebrospinal fluid of natalizumab-treated patients with multiple sclerosis. Arch Neurol 2006;63(10):1383-1387.

82. Du Pasquier R, Kuroda M, Zheng Y, Jean-Jacques J, Letvin N, Koralnik I. A prospective study demonstrates an association between JC virus-specific cytotoxic T lymphocytes and the early control of progressive multifocal leukoencephalopathy. Brain 2004;127(Pt 9):1970-1978.
83. Koralnik I, Du Pasquier R, Kuroda M, Schmitz J, Dang X, Zheng Y, Lifton M, Letvin N. Association of prolonged survival in HLA-A2+ progressive multifocal leukoencephalopathy patients with a CTL response specific for a commonly recognized JC virus epitope. J Immunol 2002;168(1):499-504.
84. Awadalla Y, Randhawa P, Ruppert K, Zeevi A, Duquesnoy R. HLA mismatching increases the risk of BK virus nephropathy in renal transplant recipients. Am J Transplant 2004;4(10):1691-1696.
85. Hirsch H, Knowles W, Dickenmann M, Passweg J, Klimkait T, Mihatsch M, Steiger J. Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients. N Engl J Med 2002;347(7):488-496.
86. Gardner SD, Field AM, Coleman DV, Hulme B. New Human Papovavirus (BK) Isolated form Urine after Renal Transplantation. Lancet 1971;1(7712):1253-&.
87. Padgett BL, Walker DL, Zurhein GM, Eckroade RJ, Dessel BH. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. Lancet 1971;1(7712):1257-&.
88. Padgett B, Walker D, Desquitado M, Kim D. BK virus and non-haemorrhagic cystitis in a child. Lancet 1983;1(8327):770.
89. Mininberg D, Watson C, Desquitado M. Viral cystitis with transient secondary vesicoureteral reflux. J Urol 1982;127(5):983-985.
90. Goudsmit J, Baak M, Sleterus K, Van der Noordaa J. Human papovavirus isolated from urine of a child with acute tonsillitis. Br Med J (Clin Res Ed) 1981;283(6303):1363-1364.
91. Hashida Y, Gaffney P, Yunis E. Acute hemorrhagic cystitis of childhood and papovavirus-like particles. J Pediatr 1976;89(1):85-87.
92. Monaco M, Jensen P, Hou J, Durham L, Major E. Detection of JC virus DNA in human tonsil tissue: evidence for site of initial viral infection. J Virol 1998;72(12):9918-9923.
93. Goudsmit J, Wertheim-van Dillen P, van Strien A, van der Noordaa J. The role of BK virus in acute respiratory tract disease and the presence of BKV DNA in tonsils. J Med Virol 1982;10(2):91-99.
94. Major E, Miller A, Mourrain P, Traub R, de Widt E, Sever J. Establishment of a line of human fetal glial cells that supports JC virus multiplication. Proc Natl Acad Sci U S A 1985;82(4):1257-1261.
95. Monaco M, Atwood W, Gravell M, Tornatore C, Major E. JC virus infection of hematopoietic progenitor cells, primary B lymphocytes, and tonsillar stromal cells: implications for viral latency. J Virol 1996;70(10):7004-7012.
96. Chesters PM, Heritage J, McCance DJ. Persistence of DNA-sequences of BK virus and JC virus in normal human-tissues and in diseased tissues. Journal of Infectious Diseases 1983;147(4):676-684.
97. Monaco M, Shin J, Major E. JC virus infection in cells from lymphoid tissue. Dev Biol Stand 1998;94:115-122.
98. Dubois V, Dutronc H, Lafon M, Poinsot V, Pellegrin J, Ragnaud J, Ferrer A, Fleury H. Latency and reactivation of JC virus in peripheral blood of human immunodeficiency virus type 1-infected patients. J Clin Microbiol 1997;35(9):2288-2292.
99. Azzi A, De Santis R, Ciappi S, Leoncini F, Sterrantino G, Marino N, Mazzotta F, Laszlo D, Fanci R, Bosi A. Human polyomaviruses DNA detection in peripheral blood leukocytes from immunocompetent and immunocompromised individuals. J Neurovirol 1996;2(6):411-416.
100. Portolani M, Piani M, Gazzanelli G, Borgatti M, Bartoletti A, Grossi M, Corallini A, Barbanti-Brodano G. Restricted replication of BK virus in human lymphocytes. Microbiologica 1985;8(1):59-66.
101. Imperiale M. The human polyomaviruses, BKV and JCV: molecular pathogenesis of acute disease and potential role in cancer. Virology 2000;267(1):1-7.

102. Norja P, Ubillos I, Templeton K, Simmonds P. No evidence for an association between infections with WU and KI polyomaviruses and respiratory disease. Journal of Clinical Virology 2007;40(4):307-311.
103. Han TH, Chung JY, Koo JW, Kim SW, Hwang ES. WU polyomavirus in children with acute lower respiratory tract infections, South Korea. Emerging Infectious Diseases 2007;13(11):1766-1768.
104. Abed Y, Wang D, Boivin G. WU polyomavirus in children, Canada. Emerging Infectious Diseases 2007;13(12):1939-1941.
105. Jiang MX, Abend JR, Johnson SF, Imperiale MJ. The role of polyomaviruses in human disease. Virology 2009;384(2):266-273.
106. Kantola K, Sadeghi M, Lahtinen A, Koskenvuo M, Aaltonen LM, Mottonen M, Rahiala J, Saarinen-Pihkala U, Riikonen P, Jartti T, Ruuskanen O, Soderlund-Venermo M, Hedman K. Merkel cell polyomavirus DNA in tumor-free tonsillar tissues and upper respiratory tract samples: Implications for respiratory transmission and latency. Journal of Clinical Virology 2009;45(4):292-295.
107. Bialasiewicz S, Whiley D, Lambert S, Nissen M, Sloots T. Detection of BK, JC, WU, or KI polyomaviruses in faecal, urine, blood, cerebrospinal fluid and respiratory samples. J Clin Virol 2009;45(3):249-254.
108. Bialasiewicz S, Lambert SB, Whiley DM, Nissen MD, Sloots TP. Merkel Cell Polyomavirus DNA in Respiratory Specimens from Children and Adults. Emerging Infectious Diseases 2009;15(3):492-494.
109. Goh S, Lindau C, Tiveljung-Lindell A, Allander T. Merkel Cell Polyomavirus in Respiratory Tract Secretions. Emerging Infectious Diseases 2009;15(3):489-491.
110. Loyo M, Guerrero-Preston R, Brait M, Hoque M, Chuang A, Kim M, Sharma R, Liégeois N, Koch W, Califano J, Westra W, Sidransky D. Quantitative detection of merkel cell virus in human tissues and possible mode of transmission. Int J Cancer 2009.
111. Becker J, Houben R, Ugurel S, Trefzer U, Pföhler C, Schrama D. MC polyomavirus is frequently present in Merkel cell carcinoma of European patients. J Invest Dermatol 2009;129(1):248-250.
112. Garneski K, Warcola A, Feng Q, Kiviat N, Leonard J, Nghiem P. Merkel cell polyomavirus is more frequently present in North American than Australian Merkel cell carcinoma tumors. J Invest Dermatol 2009;129(1):246-248.
113. Kassem A, Technau K, Kurz AK, Pantulu D, Loning M, Kayser G, Stickeler E, Weyers W, Diaz C, Werner M, Nashan D, Hausen AZ. Merkel cell polyomavirus sequences are frequently detected in nonmelanoma skin cancer of immunosuppressed patients. International Journal of Cancer 2009;125(2):356-361.
114. Dworkin A, Tseng S, Allain D, Iwenofu O, Peters S, Toland A. Merkel Cell Polyomavirus in Cutaneous Squamous Cell Carcinoma of Immunocompetent Individuals. J Invest Dermatol 2009.
115. Knowles W, Gibson P, Gardner S. Serological typing scheme for BK-like isolates of human polyomavirus. J Med Virol 1989;28(2):118-123.
116. Jensen P, Major E. A classification scheme for human polyomavirus JCV variants based on the nucleotide sequence of the noncoding regulatory region. J Neurovirol 2001;7(4):280-287.
117. Hamilton R, Gravell M, Major E. Comparison of antibody titers determined by hemagglutination inhibition and enzyme immunoassay for JC virus and BK virus. J Clin Microbiol 2000;38(1):105-109.
118. Viscidi R, Clayman B. Serological cross reactivity between polyomavirus capsids. Adv Exp Med Biol 2006;577:73-84.
119. Bogdanovic G, Priftakis P, Taemmeraes B, Gustafsson A, Flaegstad T, Winiarski J, Dalianis T. Primary BK virus (BKV) infection due to possible BKV transmission during bone marrow transplantation is not the major cause of hemorrhagic cystitis in transplanted children. Pediatr Transplant 1998;2(4):288-293.
120. Stolt A, Sasnauskas K, Koskela P, Lehtinen M, Dillner J. Seroepidemiology of the human polyomaviruses. J Gen Virol 2003;84(Pt 6):1499-1504.

121. Knowles WA. Discovery and epidemiology of the human polyomaviruses BK virus (BKV) and JC virus (JCV). Polyomaviruses and Human Diseases. Volume 577, Advances in Experimental Medicine and Biology; 2006. p 19-45.
122. Egli A, Infanti L, Dumoulin A, Buser A, Samaridis J, Stebler C, Gosert R, Hirsch H. Prevalence of polyomavirus BK and JC infection and replication in 400 healthy blood donors. J Infect Dis 2009;199(6):837-846.
123. Dörries K, Sbiera S, Drews K, Arendt G, Eggers C, Dörries R. Association of human polyomavirus JC with peripheral blood of immunoimpaired and healthy individuals. J Neurovirol 2003;9 Suppl 1:81-87.
124. Carter J, Paulson K, Wipf G, Miranda D, Madeleine M, Johnson L, Lemos B, Lee S, Warcola A, Iyer J, Nghiem P, Galloway D. Association of Merkel cell polyomavirus-specific antibodies with Merkel cell carcinoma. J Natl Cancer Inst 2009;101(21):1510-1522.
125. Nguyen N, Le B, Wang D. Serologic evidence of frequent human infection with WU and KI polyomaviruses. Emerg Infect Dis 2009;15(8):1199-1205.
126. Kean JM, Rao S, Wang M, Garcea RL. Seroepidemiology of Human Polyomaviruses. Plos Pathogens 2009;5(3).
127. Pastrana D, Tolstov Y, Becker J, Moore P, Chang Y, Buck C. Quantitation of human seroresponsiveness to Merkel cell polyomavirus. PLoS Pathog 2009;5(9):e1000578.
128. Zhong S, Zheng H, Suzuki M, Chen Q, Ikegaya H, Aoki N, Usuku S, Kobayashi N, Nukuzuma S, Yasuda Y, Kuniyoshi N, Yogo Y, Kitamura T. Age-related urinary excretion of BK polyomavirus by nonimmunocompromised individuals. J Clin Microbiol 2007;45(1):193-198.
129. Vidal J, Fink M, Cedeno-Laurent F, Delbue S, Ferrante P, Dauar R, Filho F, Nogueira R, Calore E, Pannuti C, Trujillo J, de Oliveira A. BK virus associated meningoencephalitis in an AIDS patient treated with HAART. AIDS Res Ther 2007;4:13.
130. Cubukcu-Dimopulo O, Greco A, Kumar A, Karluk D, Mittal K, Jagirdar J. BK virus infection in AIDS. Am J Surg Pathol 2000;24(1):145-149.
131. Muñoz P, Fogeda M, Bouza E, Verde E, Palomo J, Bañares R. Prevalence of BK virus replication among recipients of solid organ transplants. Clin Infect Dis 2005;41(12):1720-1725.
132. Sundsfjord A, Osei A, Rosenqvist H, Van Ghelue M, Silsand Y, Haga H, Rekvig O, Moens U. BK and JC viruses in patients with systemic lupus erythematosus: prevalent and persistent BK viruria, sequence stability of the viral regulatory regions, and nondetectable viremia. J Infect Dis 1999;180(1):1-9.
133. Chang D, Tsai R, Wang M, Ou W. Different genotypes of human polyomaviruses found in patients with autoimmune diseases in Taiwan. J Med Virol 1996;48(2):204-209.
134. Brooks B, Walker D. Progressive multifocal leukoencephalopathy. Neurol Clin 1984;2(2):299-313.
135. Berger J. Progressive multifocal leukoencephalopathy. Curr Neurol Neurosci Rep 2007;7(6):461-469.
136. Cesaro S, Facchin C, Tridello G, Messina C, Calore E, Biasolo M, Pillon M, Varotto S, Brugiolo A, Mengoli C, Palù G. A prospective study of BK-virus-associated haemorrhagic cystitis in paediatric patients undergoing allogeneic haematopoietic stem cell transplantation. Bone Marrow Transplant 2008;41(4):363-370.
137. Chakrabarti S, Osman H, Collingham K, Milligan D. Polyoma viruria following T-cell-depleted allogeneic transplants using Campath-1H: incidence and outcome in relation to graft manipulation, donor type and conditioning. Bone Marrow Transplant 2003;31(5):379-386.
138. Leung A, Mak R, Lie A, Yuen K, Cheng V, Liang R, Kwong Y. Clinicopathological features and risk factors of clinically overt haemorrhagic cystitis complicating bone marrow transplantation. Bone Marrow Transplant 2002;29(6):509-513.

139. Vögeli T, Peinemann F, Burdach S, Ackermann R. Urological treatment and clinical course of BK polyomavirus-associated hemorrhagic cystitis in children after bone marrow transplantation. Eur Urol 1999;36(3):252-257.
140. Bedi A, Miller C, Hanson J, Goodman S, Ambinder R, Charache P, Arthur R, Jones R. Association of BK virus with failure of prophylaxis against hemorrhagic cystitis following bone marrow transplantation. J Clin Oncol 1995;13(5):1103-1109.
141. Apperley J, Rice S, Bishop J, Chia Y, Krausz T, Gardner S, Goldman J. Late-onset hemorrhagic cystitis associated with urinary excretion of polyomaviruses after bone marrow transplantation. Transplantation 1987;43(1):108-112.
142. Arthur RR, Shah KV, Baust SJ, Santos GW, Saral R. Association of BK viruria with hemorragic cystitis in recipients of bone-marrow transplants. New England Journal of Medicine 1986;315(4):230-234.
143. Hirsch H, Brennan D, Drachenberg C, Ginevri F, Gordon J, Limaye A, Mihatsch M, Nickeleit V, Ramos E, Randhawa P, Shapiro R, Steiger J, Suthanthiran M, Trofe J. Polyomavirus-associated nephropathy in renal transplantation: interdisciplinary analyses and recommendations. Transplantation 2005;79(10):1277-1286.
144. Egli A, Binggeli S, Bodaghi S, Dumoulin A, Funk G, Khanna N, Leuenberger D, Gosert R, Hirsch H. Cytomegalovirus and polyomavirus BK posttransplant. Nephrol Dial Transplant 2007;22 Suppl 8:viii72-viii82.
145. Richardson EJ, Webster H. Progressive multifocal leukoencephalopathy: its pathological features. Prog Clin Biol Res 1983;105:191-203.
146. Astrom K, Mancall E, Richardson EJ. Progressive multifocal leuko-encephalopathy; a hitherto unrecognized complication of chronic lymphatic leukaemia and Hodgkin's disease. Brain 1958;81(1):93-111.
147. Manabe Y, Campbell J, Sydnor E, Moore R. Immune reconstitution inflammatory syndrome: risk factors and treatment implications. J Acquir Immune Defic Syndr 2007;46(4):456-462.
148. Thurnher M, Post M, Rieger A, Kleibl-Popov C, Loewe C, Schindler E. Initial and follow-up MR imaging findings in AIDS-related progressive multifocal leukoencephalopathy treated with highly active antiretroviral therapy. AJNR Am J Neuroradiol 2001;22(5):977-984.
149. Miralles P, Berenguer J, Lacruz C, Cosín J, López J, Padilla B, Muñoz L, García-de-Viedma D. Inflammatory reactions in progressive multifocal leukoencephalopathy after highly active antiretroviral therapy. AIDS 2001;15(14):1900-1902.
150. Cinque P, Bossolasco S, Brambilla A, Boschini A, Mussini C, Pierotti C, Campi A, Casari S, Bertelli D, Mena M, Lazzarin A. The effect of highly active antiretroviral therapy-induced immune reconstitution on development and outcome of progressive multifocal leukoencephalopathy: study of 43 cases with review of the literature. J Neurovirol 2003;9 Suppl 1:73-80.
151. Safdar A, Rubocki R, Horvath J, Narayan K, Waldron R. Fatal immune restoration disease in human immunodeficiency virus type 1-infected patients with progressive multifocal leukoencephalopathy: impact of antiretroviral therapy-associated immune reconstitution. Clin Infect Dis 2002;35(10):1250-1257.
152. Du Pasquier R, Koralnik I. Inflammatory reaction in progressive multifocal leukoencephalopathy: harmful or beneficial? J Neurovirol 2003;9 Suppl 1:25-31.
153. French M, Lenzo N, John M, Mallal S, McKinnon E, James I, Price P, Flexman J, Tay-Kearney M. Immune restoration disease after the treatment of immunodeficient HIV-infected patients with highly active antiretroviral therapy. HIV Med 2000;1(2):107-115.
154. Stone S, Price P, Brochier J, French M. Plasma bioavailable interleukin-6 is elevated in human immunodeficiency virus-infected patients who experience herpesvirus-associated immune restoration disease after start of highly active antiretroviral therapy. J Infect Dis 2001;184(8):1073-1077.
155. Bhatia K, Goedert J, Modali R, Preiss L, Ayers L. Merkel cell carcinoma subgroups by merkel cell polyomavirus DNA relative abundance and oncogene expression. Int J Cancer 2009.

156. Sihto H, Kukko H, Koljonen V, Sankila R, Böhling T, Joensuu H. Clinical factors associated with Merkel cell polyomavirus infection in Merkel cell carcinoma. J Natl Cancer Inst 2009;101(13):938-945.
157. Nakajima H, Takaishi M, Yamamoto M, Kamijima R, Kodama H, Tarutani M, Sano S. Screening of the specific polyoma virus as diagnostic and prognostic tools for Merkel cell carcinoma. J Dermatol Sci 2009;56(3):211-213.
158. Andres C, Belloni B, Puchta U, Sander C, Flaig M. Prevalence of MCPyV in Merkel cell carcinoma and non-MCC tumors. J Cutan Pathol 2009.
159. Touzé A, Gaitan J, Maruani A, Le Bidre E, Doussinaud A, Clavel C, Durlach A, Aubin F, Guyétant S, Lorette G, Coursaget P. Merkel cell polyomavirus strains in patients with merkel cell carcinoma. Emerg Infect Dis 2009;15(6):960-962.
160. Varga E, Kiss M, Szabó K, Kemény L. Detection of Merkel cell polyomavirus DNA in Merkel cell carcinomas. Br J Dermatol 2009;161(4):930-932.
161. Duncavage E, Zehnbauer B, Pfeifer J. Prevalence of Merkel cell polyomavirus in Merkel cell carcinoma. Mod Pathol 2009;22(4):516-521.
162. Foulongne V, Kluger N, Dereure O, Brieu N, Guillot B, Segondy M. Merkel cell polyomavirus and Merkel cell carcinoma, France. Emerg Infect Dis 2008;14(9):1491-1493.
163. Kassem A, Schöpflin A, Diaz C, Weyers W, Stickeler E, Werner M, Zur Hausen A. Frequent detection of Merkel cell polyomavirus in human Merkel cell carcinomas and identification of a unique deletion in the VP1 gene. Cancer Res 2008;68(13):5009-5013.
164. Sastre-Garau X, Peter M, Avril MF, Laude H, Couturier J, Rozenberg F, Almeida A, Boitier F, Carlotti A, Couturaud B, Dupin N. Merkel cell carcinoma of the skin: pathological and molecular evidence for a causative role of MCV in oncogenesis. Journal of Pathology 2009;218(1):48-56.
165. Garneski K, DeCaprio J, Nghiem P. Does a new polyomavirus contribute to Merkel cell carcinoma? Genome Biol 2008;9(6):228.
166. Shein H, Enders J. Transformation induced by simian virus 40 in human renal cell cultures. I. Morphology and growth characteristics. Proc Natl Acad Sci U S A 1962;48:1164-1172.
167. Eddy B, Borman G, Grubbs G, Young R. Identification of the oncogenic substance in rhesus monkey kidney cell culture as simian virus 40. Virology 1962;17:65-75.
168. Priftakis P, Bogdanovic G, Hjerpe A, Dalianis T. Presence of simian virus 40 (SV40) is not frequent in Swedish malignant mesotheliomas. Anticancer Res 2002;22(3):1357-1360.
169. Olin P, Giesecke J. Potential exposure to SV40 in polio vaccines used in Sweden during 1957: no impact on cancer incidence rates 1960 to 1993. Dev Biol Stand 1998;94:227-233.
170. Russell N, Hunter A, Rogers S, Hanley J, Anderson D. Peripheral blood stem cells as an alternative to marrow for allogeneic transplantation. Lancet 1993;341(8858):1482.
171. Dreger P, Suttorp M, Haferlach T, Löffler H, Schmitz N, Schroyens W. Allogeneic granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells for treatment of engraftment failure after bone marrow transplantation. Blood 1993;81(5):1404-1407.
172. Gluckman E, Broxmeyer H, Auerbach A, Friedman H, Douglas G, Devergie A, Esperou H, Thierry D, Socie G, Lehn P. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. N Engl J Med 1989;321(17):1174-1178.
173. Mathe G, Jammet H, Pendic B, Schwarzenberg L, Duplan J, Maupin B, Latarjet R, Larrieu M, Kalic D, Djukic Z. [Transfusions and grafts of homologous bone marrow in humans after accidental high dosage irradiation.]. Rev Fr Etud Clin Biol 1959;4(3):226-238.
174. Thomas E, Lochte HJ, Lu W, Ferrebee J. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. N Engl J Med 1957;257(11):491-496.

175. Bortin M. A compendium of reported human bone marrow transplants. Transplantation 1970;9(6):571-587.
176. Van Rood J, Eernisse J, Van Leeuwen A. Leucocyte antibodies in sera from pregnant women. Nature 1958;181(4625):1735-1736.
177. Dausset J. [Demonstration of the Rh (D) antigen on leukocytes and platelets from humans.]. C R Hebd Seances Acad Sci 1958;246(14):2171-2173.
178. Snell G. Methods for the study of histocompatibility genes. J Genet 1948;49(2):87-108.
179. Zinkernagel R, Doherty P. Immunological surveillance against altered self components by sensitised T lymphocytes in lymphocytic choriomeningitis. Nature 1974;251(5475):547-548.
180. Gatti R, Meuwissen H, Allen H, Hong R, Good R. Immunological reconstitution of sex-linked lymphopenic immunological deficiency. Lancet 1968;2(7583):1366-1369.
181. Bach F, Albertini R, Joo P, Anderson J, Bortin M. Bone-marrow transplantation in a patient with the Wiskott-Aldrich syndrome. Lancet 1968;2(7583):1364-1366.
182. Storb R, Thomas E, Buckner C, Clift R, Johnson F, Fefer A, Glucksberg H, Giblett E, Lerner K, Neiman P. Allogeneic marrow grafting for treatment of aplastic anemia. Blood 1974;43(2):157-180.
183. Bortin M, Horowitz M, Rimm A. Increasing utilization of allogeneic bone marrow transplantation. Results of the 1988-1990 survey. Ann Intern Med 1992;116(6):505-512.
184. Olerup O, Zetterquist H. HLA-DRB1*01 subtyping by allele-specific PCR amplification: a sensitive, specific and rapid technique. Tissue Antigens 1991;37(5):197-204.
185. Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens 1992;39(5):225-235.
186. Olerup O, Daniels T, Baxter-Lowe L. Correct sequence of the A*3001 allele obtained by PCR-SSP typing and automated nucleotide sequencing. Tissue Antigens 1994;44(4):265-267.
187. Olerup O. HLA-B27 typing by a group-specific PCR amplification. Tissue Antigens 1994;43(4):253-256.
188. Krausa P, Moses J, Bodmer W, Bodmer J, Browning M. HLA-A locus alleles identified by sequence specific PCR. Lancet 1993;341(8837):121-122.
189. Ringdén O, Remberger M, Persson U, Ljungman P, Aldener A, Andström E, Aschan J, Bolme P, Dahllöf G, Dalianis T. Similar incidence of graft-versus-host disease using HLA-A, -B and -DR identical unrelated bone marrow donors as with HLA-identical siblings. Bone Marrow Transplant 1995;15(4):619-625.
190. Davies S, Kollman C, Anasetti C, Antin J, Gajewski J, Casper J, Nademanee A, Noreen H, King R, Confer D, Kernan N. Engraftment and survival after unrelated-donor bone marrow transplantation: a report from the national marrow donor program. Blood 2000;96(13):4096-4102.
191. Mattsson J, Ringdén O, Storb R. Graft Failure after Allogeneic Hematopoietic Cell Transplantation. Biol Blood Marrow Transplant 2008;14(Supplement 1):165-170.
192. Rocha V, Gluckman E. Clinical use of umbilical cord blood hematopoietic stem cells. Biol Blood Marrow Transplant 2006;12(1 Suppl 1):34-41.
193. Callahan G, Dewitt C. Rat cell surface antigens. II. Isolation of a minor histocompatibility antigen. J Immunol 1975;114(2 pt 2):779-781.
194. Hambach L, Spierings E, Goulmy E. Risk assessment in haematopoietic stem cell transplantation: minor histocompatibility antigens. Best Pract Res Clin Haematol 2007;20(2):171-187.
195. Goulmy E, Termijtelen A, Bradley B, van Rood J. Alloimmunity to human H-Y. Lancet 1976;2(7996):1206.
196. Goulmy E, Termijtelen A, Bradley B, van Rood J. Y-antigen killing by T cells of women is restricted by HLA. Nature 1977;266(5602):544-545.

197. Takami A, Sugimori C, Feng X, Yachie A, Kondo Y, Nishimura R, Kuzushima K, Kotani T, Asakura H, Shiobara S, Nakao S. Expansion and activation of minor histocompatibility antigen HY-specific T cells associated with graft-versus-leukemia response. Bone Marrow Transplant 2004;34(8):703-709.
198. Randolph S, Gooley T, Warren E, Appelbaum F, Riddell S. Female donors contribute to a selective graft-versus-leukemia effect in male recipients of HLA-matched, related hematopoietic stem cell transplants. Blood 2004;103(1):347-352.
199. Remberger M, Watz E, Ringdén O, Mattsson J, Shanwell A, Wikman A. Major ABO blood group mismatch increases the risk for graft failure after unrelated donor hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2007;13(6):675-682.
200. Stussi G, Halter J, Schanz U, Seebach J. ABO-histo blood group incompatibility in hematopoietic stem cell and solid organ transplantation. Transfus Apher Sci 2006;35(1):59-69.
201. Glucksberg H, Storb R, Fefer A, Buckner C, Neiman P, Clift R, Lerner K, Thomas E. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation 1974;18(4):295-304.
202. Horowitz M, Gale R, Sondel P, Goldman J, Kersey J, Kolb H, Rimm A, Ringdén O, Rozman C, Speck B. Graft-versus-leukemia reactions after bone marrow transplantation. Blood 1990;75(3):555-562.
203. Nash R, Pepe M, Storb R, Longton G, Pettinger M, Anasetti C, Appelbaum F, Bowden R, Deeg H, Doney K. Acute graft-versus-host disease: analysis of risk factors after allogeneic marrow transplantation and prophylaxis with cyclosporine and methotrexate. Blood 1992;80(7):1838-1845.
204. Sullivan K, Shulman H, Storb R, Weiden P, Witherspoon R, McDonald G, Schubert M, Atkinson K, Thomas E. Chronic graft-versus-host disease in 52 patients: adverse natural course and successful treatment with combination immunosuppression. Blood 1981;57(2):267-276.
205. Ferrara J, Levy R, Chao N. Pathophysiologic mechanisms of acute graft-vs.-host disease. Biol Blood Marrow Transplant 1999;5(6):347-356.
206. Via C, Finkelman F. Critical role of interleukin-2 in the development of acute graft-versus-host disease. Int Immunol 1993;5(6):565-572.
207. Ho V, Soiffer R. The history and future of T-cell depletion as graft-versus-host disease prophylaxis for allogeneic hematopoietic stem cell transplantation. Blood 2001;98(12):3192-3204.
208. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, Lanino E, Sundberg B, Bernardo M, Remberger M, Dini G, Egeler R, Bacigalupo A, Fibbe W, Ringdén O. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet 2008;371(9624):1579-1586.
209. Wingard J. Hematopoietic stem cell transplantation: a handbook for clinicians. Wingard J, Gastineau D, Leather H, Snyder E, Szczepiorkowski Z, editors. Bethesda, Maryland: AABB; 2009.
210. Barnes D, Corp M, Loutit J, Neal F. Treatment of murine leukaemia with X rays and homologous bone marrow; preliminary communication. Br Med J 1956;2(4993):626-627.
211. Weiden P, Flournoy N, Thomas E, Prentice R, Fefer A, Buckner C, Storb R. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. N Engl J Med 1979;300(19):1068-1073.
212. Maraninchi D, Gluckman E, Blaise D, Guyotat D, Rio B, Pico J, Leblond V, Michallet M, Dreyfus F, Ifrah N. Impact of T-cell depletion on outcome of allogeneic bone-marrow transplantation for standard-risk leukaemias. Lancet 1987;2(8552):175-178.
213. Ljunggren H, Malmberg K. Prospects for the use of NK cells in immunotherapy of human cancer. Nat Rev Immunol 2007;7(5):329-339.

214. Thomas E, Storb R, Clift R, Fefer A, Johnson L, Neiman P, Lerner K, Glucksberg H, Buckner C. Bone-marrow transplantation (second of two parts). N Engl J Med 1975;292(17):895-902.
215. Thomas E, Buckner C, Clift R, Fefer A, Johnson F, Neiman P, Sale G, Sanders J, Singer J, Shulman H, Storb R, Weiden P. Marrow transplantation for acute nonlymphoblastic leukemia in first remission. N Engl J Med 1979;301(11):597-599.
216. Yamamoto R, Kusumi E, Kami M, Yuji K, Hamaki T, Saito A, Murasgihe N, Hori A, Kim S, Makimoto A, Ueyama J, Tanosaki R, Miyakoshi S, Mori S, Morinaga S, Heike Y, Taniguchi S, Masuo S, Takaue Y, Mutou Y. Late hemorrhagic cystitis after reduced-intensity hematopoietic stem cell transplantation (RIST). Bone Marrow Transplant 2003;32(11):1089-1095.
217. Trotman J, Nivison-Smith I, Dodds A. Haemorrhagic cystitis: incidence and risk factors in a transplant population using hyperhydration. Bone Marrow Transplant 1999;23(8):797-801.
218. Ringdén O, Labopin M, Tura S, Arcese W, Iriondo A, Zittoun R, Sierra J, Gorin N. A comparison of busulphan versus total body irradiation combined with cyclophosphamide as conditioning for autograft or allograft bone marrow transplantation in patients with acute leukaemia. Acute Leukaemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). Br J Haematol 1996;93(3):637-645.
219. Morgan M, Dodds A, Atkinson K, Szer J, Downs K, Biggs J. The toxicity of busulphan and cyclophosphamide as the preparative regimen for bone marrow transplantation. Br J Haematol 1991;77(4):529-534.
220. Ferrara J, Abhyankar S, Gilliland D. Cytokine storm of graft-versus-host disease: a critical effector role for interleukin-1. Transplant Proc 1993;25(1 Pt 2):1216-1217.
221. McSweeney P, Niederwieser D, Shizuru J, Sandmaier B, Molina A, Maloney D, Chauncey T, Gooley T, Hegenbart U, Nash R, Radich J, Wagner J, Minor S, Appelbaum F, Bensinger W, Bryant E, Flowers M, Georges G, Grumet F, Kiem H, Torok-Storb B, Yu C, Blume K, Storb R. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. Blood 2001;97(11):3390-3400.
222. Sandmaier B, Mackinnon S, Childs R. Reduced intensity conditioning for allogeneic hematopoietic cell transplantation: current perspectives. Biol Blood Marrow Transplant 2007;13(1 Suppl 1):87-97.
223. Le Blanc K, Remberger M, Uzunel M, Mattsson J, Barkholt L, Ringdén O. A comparison of nonmyeloablative and reduced-intensity conditioning for allogeneic stem-cell transplantation. Transplantation 2004;78(7):1014-1020.
224. Ali M, Oyama Y, Monreal J, Winter J, Tallman M, Gordon L, Williams S, Singhal S, Mehta J. Reassessing the definition of myeloid engraftment after autotransplantation: it is not necessary to see 0.5 x 10(9)/l neutrophils on 3 consecutive days to define myeloid recovery. Bone Marrow Transplant 2002;30(11):749-752.
225. Gill S, Olson J, Negrin R. Natural killer cells in allogeneic transplantation: effect on engraftment, graft- versus-tumor, and graft-versus-host responses. Biol Blood Marrow Transplant 2009;15(7):765-776.
226. Hirsch H. BK virus: opportunity makes a pathogen. Clin Infect Dis 2005;41(3):354-360.
227. Marty F, Rubin R. The prevention of infection post-transplant: the role of prophylaxis, preemptive and empiric therapy. Transpl Int 2006;19(1):2-11.
228. Ninin E, Milpied N, Moreau P, André-Richet B, Morineau N, Mahé B, Vigier M, Imbert B, Morin O, Harousseau J, Richet H. Longitudinal study of bacterial, viral, and fungal infections in adult recipients of bone marrow transplants. Clin Infect Dis 2001;33(1):41-47.
229. Bochud P, Calandra T, Francioli P. Bacteremia due to viridans streptococci in neutropenic patients: a review. Am J Med 1994;97(3):256-264.

230. Williamson E, Millar M, Steward C, Cornish J, Foot A, Oakhill A, Pamphilon D, Reeves B, Caul E, Warnock D, Marks D. Infections in adults undergoing unrelated donor bone marrow transplantation. Br J Haematol 1999;104(3):560-568.
231. Aschan J. Allogeneic haematopoietic stem cell transplantation: current status and future outlook. Br Med Bull 2006;77-78:23-36.
232. Kulkarni S, Powles R, Treleaven J, Riley U, Singhal S, Horton C, Sirohi B, Bhagwati N, Meller S, Saso R, Mehta J. Chronic graft versus host disease is associated with long-term risk for pneumococcal infections in recipients of bone marrow transplants. Blood 2000;95(12):3683-3686.
233. Einsele H, Bertz H, Beyer J, Kiehl M, Runde V, Kolb H, Holler E, Beck R, Schwerdfeger R, Schumacher U, Hebart H, Martin H, Kienast J, Ullmann A, Maschmeyer G, Krüger W, Niederwieser D, Link H, Schmidt C, Oettle H, Klingebiel T. Infectious complications after allogeneic stem cell transplantation: epidemiology and interventional therapy strategies--guidelines of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Oncology (DGHO). Ann Hematol 2003;82 Suppl 2:S175-185.
234. Engelhard D, Cordonnier C, Shaw P, Parkalli T, Guenther C, Martino R, Dekker A, Prentice H, Gustavsson A, Nurnberger W, Ljungman P. Early and late invasive pneumococcal infection following stem cell transplantation: a European Bone Marrow Transplantation survey. Br J Haematol 2002;117(2):444-450.
235. Ochs L, Shu X, Miller J, Enright H, Wagner J, Filipovich A, Miller W, Weisdorf D. Late infections after allogeneic bone marrow transplantations: comparison of incidence in related and unrelated donor transplant recipients. Blood 1995;86(10):3979-3986.
236. Nichols W, Boeckh M. Recent advances in the therapy and prevention of CMV infections. J Clin Virol 2000;16(1):25-40.
237. Ljungman P, Aschan J, Lewensohn-Fuchs I, Carlens S, Larsson K, Lönnqvist B, Mattsson J, Sparrelid E, Winiarski J, Ringdén O. Results of different strategies for reducing cytomegalovirus-associated mortality in allogeneic stem cell transplant recipients. Transplantation 1998;66(10):1330-1334.
238. Meyers J, Ljungman P, Fisher L. Cytomegalovirus excretion as a predictor of cytomegalovirus disease after marrow transplantation: importance of cytomegalovirus viremia. J Infect Dis 1990;162(2):373-380.
239. Ljungman P. CMV infections after hematopoietic stem cell transplantation. Bone Marrow Transplant 2008;42 Suppl 1:S70-S72.
240. Orazi A, Hromas R, Neiman R, Greiner T, Lee C, Rubin L, Haskins S, Heerema N, Gharpure V, Abonour R, Srour E, Cornetta K. Posttransplantation lymphoproliferative disorders in bone marrow transplant recipients are aggressive diseases with a high incidence of adverse histologic and immunobiologic features. Am J Clin Pathol 1997;107(4):419-429.
241. Nalesnik M. Clinical and pathological features of post-transplant lymphoproliferative disorders (PTLD). Springer Semin Immunopathol 1998;20(3-4):325-342.
242. Knowles D, Cesarman E, Chadburn A, Frizzera G, Chen J, Rose E, Michler R. Correlative morphologic and molecular genetic analysis demonstrates three distinct categories of posttransplantation lymphoproliferative disorders. Blood 1995;85(2):552-565.
243. Meijer E, Cornelissen J. Epstein-Barr virus-associated lymphoproliferative disease after allogeneic haematopoietic stem cell transplantation: molecular monitoring and early treatment of high-risk patients. Curr Opin Hematol 2008;15(6):576-585.
244. Capello D, Rossi D, Gaidano G. Post-transplant lymphoproliferative disorders: molecular basis of disease histogenesis and pathogenesis. Hematol Oncol 2005;23(2):61-67.
245. Hale G, Waldmann H. Risks of developing Epstein-Barr virus-related lymphoproliferative disorders after T-cell-depleted marrow transplants. CAMPATH Users. Blood 1998;91(8):3079-3083.

246. Gustafsson A, Levitsky V, Zou J, Frisan T, Dalianis T, Ljungman P, Ringden O, Winiarski J, Ernberg I, Masucci M. Epstein-Barr virus (EBV) load in bone marrow transplant recipients at risk to develop posttransplant lymphoproliferative disease: prophylactic infusion of EBV-specific cytotoxic T cells. Blood 2000;95(3):807-814.
247. O'Reilly R, Lee F, Grossbard E, Kapoor N, Kirkpatrick D, Dinsmore R, Stutzer C, Shah K, Nahmias A. Papovavirus excretion following marrow transplantation: incidence and association with hepatic dysfunction. Transplant Proc 1981;13(1 Pt 1):262-266.
248. Sandler E, Aquino V, Goss-Shohet E, Hinrichs S, Krisher K. BK papova virus pneumonia following hematopoietic stem cell transplantation. Bone Marrow Transplant 1997;20(2):163-165.
249. Behzad-Behbahani A, Klapper P, Vallely P, Cleator G, Bonington A. BKV-DNA and JCV-DNA in CSF of patients with suspected meningitis or encephalitis. Infection 2003;31(6):374-378.
250. Boeckh M, Erard V, Zerr D, Englund J. Emerging viral infections after hematopoietic cell transplantation. Pediatr Transplant 2005;9 Suppl 7:48-54.
251. Droller M, Saral R, Santos G. Prevention of cyclophosphamide-induced hemorrhagic cystitis. Urology 1982;20(3):256-258.
252. Numazaki Y, Shigeta S, Kumasaka T, Miyazawa T, Yamanaka M, Yano N, Takai S, Ishida N. Acute hemorrhagic cystitis in children - isolation of adenovirus type 11. New England Journal of Medicine 1968;278(13):700-&.
253. Numazaki Y, Kumasaka T, Yano N, Yamanaka M, Miyazawa T, Takai S, Ishida N. Further study on acute hemorrhagic cystitis due to adenovirus type-11. New England Journal of Medicine 1973;289(7):344-347.
254. Mufson MA, Zollar LM, Mankad VN, Manalo D. Adenovirus infection in acute hemorrhagic cystitis - study in 25 children. American Journal of Diseases of Children 1971;121(4):281-&.
255. Mufson MA, Belshe RB, Horrigan TJ, Zollar LM. Cause of acute hemorrhagic cystitis in children. American Journal of Diseases of Children 1973;126(5):605-609.
256. Takemoto K, Rabson A, Mullarkey M, Blaese R, Garon C, Nelson D. Isolation of papovavirus from brain tumor and urine of a patient with Wiskott-Aldrich syndrome. J Natl Cancer Inst 1974;53(5):1205-1207.
257. Dougherty R, DiStefano H. Isolation and characterization of a papovavirus from human urine. Proc Soc Exp Biol Med 1974;146(2):481-487.
258. Lecatsas G, Prozesky O, van Wyk J, Els H. Papova virus in urine after renal transplantation. Nature 1973;241(5388):343-344.
259. Arnold H, Bourseaux F, Brock N. Chemotherapeutic action of a cyclic nitrogen mustard phosphamide ester (B 518-ASTA) in experimental tumours of the rat. Nature 1958;181(4613):931.
260. Riggenbach R, Barrett OJ, Shown T. Hemorrhagic cystitis due to cyclophosphamide: report of two cases. South Med J 1968;61(2):139-141.
261. Anderson E, Cobb O, Glenn J. Cyclophosphamide hemorrhagic cystitis. J Urol 1967;97(5):857-858.
262. Rubin J, Rubin R. Cyclophosphamide hemorrhagic cystitis. J Urol 1966;96(3):313-316.
263. Spiers ASD. Haemorrhagic cystitis and cyclophosphamide. Lancet 1963;2(732):1282-&.
264. Hutter AJ, Bauman A, Frank I. Cyclophosphamide and severe hemorrhagic cystitis. N Y State J Med 1969;69(2):305-309.
265. Starke O. Nitrogen mustard phosphamide ester and urinary bladder damage. Report on prolonged therapy of a case of gamma-plasmocytoma. Med Welt 1964;44:2378-2384.
266. Pearlman C. Cystitis due to cytoxan: case report. J Urol 1966;95(5):713-715.
267. George P. Haemorrhagic cystitis and cyclophosphamide. Lancet 1963;2(731):942-&.

268. Goldman R, Warner N. Hemorrhagic cystitis and cytomegalic inclusions in the bladder associated with cyclophosphamide therapy. Cancer 1970;25(1):7-11.
269. Rice SJ, Bishop JA, Apperley J, Gardner SD. BK virus as a cause of hemorrhagic cystitis after bone-marrow transplantation. Lancet 1985;2(8459):844-845.
270. Arthur RR, Beckmann AM, Li CC, Saral R, Shah KV. Direct detection of the human papovavirus BK in urine of bone-marrow transplant recipients-comparison of DNA hybridization with ELISA. Journal of Medical Virology 1985;16(1):29-36.
271. Akiyama H, Kurosu T, Sakashita C, Inoue T, Mori Si, Ohashi K, Tanikawa S, Sakamaki H, Onozawa Y, Chen Q, Zheng H, Kitamura T. Adenovirus is a key pathogen in hemorrhagic cystitis associated with bone marrow transplantation. Clin Infect Dis 2001;32(9):1325-1330.
272. Miyamura K, Takeyama K, Kojima S, Minami S, Matsuyama K, Morishima Y, Kodera Y. Hemorrhagic cystitis associated with urinary excretion of adenovirus type 11 following allogeneic bone marrow transplantation. Bone Marrow Transplant 1989;4(5):533-535.
273. Seber A, Shu X, Defor T, Sencer S, Ramsay N. Risk factors for severe hemorrhagic cystitis following BMT. Bone Marrow Transplant 1999;23(1):35-40.
274. Shields A, Hackman R, Fife K, Corey L, Meyers J. Adenovirus infections in patients undergoing bone-marrow transplantation. N Engl J Med 1985;312(9):529-533.
275. Ambinder R, Burns W, Forman M, Charache P, Arthur R, Beschorner W, Santos G, Saral R. Hemorrhagic cystitis associated with adenovirus infection in bone marrow transplantation. Arch Intern Med 1986;146(7):1400-1401.
276. Childs R, Sanchez C, Engler H, Preuss J, Rosenfeld S, Dunbar C, van Rhee F, Plante M, Phang S, Barrett A. High incidence of adeno- and polyomavirus-induced hemorrhagic cystitis in bone marrow allotransplantation for hematological malignancy following T cell depletion and cyclosporine. Bone Marrow Transplant 1998;22(9):889-893.
277. Tutuncuoglu S, Yanovich S, Ozdemirli M. CMV-induced hemorrhagic cystitis as a complication of peripheral blood stem cell transplantation: case report. Bone Marrow Transplant 2005;36(3):265-266.
278. Spach D, Bauwens J, Myerson D, Mustafa M, Bowden R. Cytomegalovirus-induced hemorrhagic cystitis following bone marrow transplantation. Clin Infect Dis 1993;16(1):142-144.
279. Xu L, Zhang H, Huang X, Liu K, Liu D, Han W, Chen H, Chen Y, Gao Z, Zhang Y, Lu D. Hemorrhagic cystitis following hematopoietic stem cell transplantation: incidence, risk factors and association with CMV reactivation and graft-versus-host disease. Chin Med J (Engl) 2007;120(19):1666-1671.
280. Institute NC. www.seer.cancer.gov., National Cancer Institute, Division of Cancer Prevention and Control, Surveillance Program, Cancer Statistics Branch, registry data 1975–2006; Accessed September 14, 2009.
281. Packer R. Childhood brain tumors: accomplishments and ongoing challenges. J Child Neurol 2008;23(10):1122-1127.
282. Fisher J, Schwartzbaum J, Wrensch M, Wiemels J. Epidemiology of brain tumors. Neurol Clin 2007;25(4):867-890, vii.
283. Birch JM, Marsden HB. A classification scheme for childhood-cancer. International Journal of Cancer 1987;40(5):620-624.
284. Lannering B, Sandström P, Holm S, Lundgren J, Pfeifer S, Samuelsson U, Strömberg B, Gustafsson G. Classification, incidence and survival analyses of children with CNS tumours diagnosed in Sweden 1984-2005. Acta Paediatr 2009.
285. Zacharoulis S, Moreno L. Ependymoma: an update. J Child Neurol 2009;24(11):1431-1438.
286. Dhall G. Medulloblastoma. J Child Neurol 2009;24(11):1418-1430.
287. Read TA, Hegedus B, Wechsler-Reya R, Gutmann DH. The neurobiology of neurooncology. Ann Neurol 2006;60(1):3-11.
288. Hargrave D. Paediatric high and low grade glioma: the impact of tumour biology on current and future therapy. Br J Neurosurg 2009;23(4):351-363.

289. Träger C, *Doctoral thesis,* Institutionen för Kvinnors och Barns Hälsa. Neuroblastoma incidence, biology and outcome with special emphasis on quantitative analysis of tyrosine hydroxylase mRNA in blood and bone marrow. Karolinska Institutet, Stockholm; 2009.
290. Chang Y, Cesarman E, Pessin M, Lee F, Culpepper J, Knowles D, Moore P. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 1994;266(5192):1865-1869.
291. Majewski S, Jabłońska S. Epidermodysplasia verruciformis as a model of human papillomavirus-induced genetic cancer of the skin. Arch Dermatol 1995;131(11):1312-1318.
292. Orth G, Jablonska S, Favre M, Croissant O, Jarzabek-Chorzelska M, Rzesa G. Characterization of two types of human papillomaviruses in lesions of epidermodysplasia verruciformis. Proc Natl Acad Sci U S A 1978;75(3):1537-1541.
293. Ekström J, Forslund O, Dillner J. Three novel papillomaviruses (HPV109, HPV112 and HPV114) and their presence in cutaneous and mucosal samples. Virology 2009.
294. Heath M, Jaimes N, Lemos B, Mostaghimi A, Wang L, Peñas P, Nghiem P. Clinical characteristics of Merkel cell carcinoma at diagnosis in 195 patients: the AEIOU features. J Am Acad Dermatol 2008;58(3):375-381.
295. Burack J, Altschuler E. Sustained remission of metastatic Merkel cell carcinoma with treatment of HIV infection. J R Soc Med 2003;96(5):238-239.
296. Friedlaender M, Rubinger D, Rosenbaum E, Amir G, Siguencia E. Temporary regression of Merkel cell carcinoma metastases after cessation of cyclosporine. Transplantation 2002;73(11):1849-1850.
297. Hodgson N. Merkel cell carcinoma: changing incidence trends. J Surg Oncol 2005;89(1):1-4.
298. Buck C, Lowy D. Getting stronger: the relationship between a newly identified virus and Merkel cell carcinoma. J Invest Dermatol 2009;129(1):9-11.
299. Laude H, Lebon P. Polyomavirus newly discovered. Pathologie Biologie 2009;57(2):184-187.
300. zur Hausen H. A specific signature of Merkel cell polyomavirus persistence in human cancer cells. Proc Natl Acad Sci U S A 2008;105(42):16063-16064.
301. Holman C, Armstrong B. Pigmentary traits, ethnic origin, benign nevi, and family history as risk factors for cutaneous malignant melanoma. J Natl Cancer Inst 1984;72(2):257-266.
302. Ragnarsson-Olding B, Kanter-Lewensohn L, Lagerlöf B, Nilsson B, Ringborg U. Malignant melanoma of the vulva in a nationwide, 25-year study of 219 Swedish females: clinical observations and histopathologic features. Cancer 1999;86(7):1273-1284.
303. Ragnarsson-Olding B, Nilsson P, Olding L, Nilsson B. Primary ano-rectal malignant melanomas within a population-based national patient series in Sweden during 40 years. Acta Oncol 2009;48(1):125-131.
304. Ragnarsson-Olding B, Johansson H, Rutqvist L, Ringborg U. Malignant melanoma of the vulva and vagina. Trends in incidence, age distribution, and long-term survival among 245 consecutive cases in Sweden 1960-1984. Cancer 1993;71(5):1893-1897.
305. Dahlgren L, Schedvins K, Kanter-Lewensohn L, Dalianis T, Ragnarsson-Olding B. Human papilloma virus (HPV) is rarely detected in malignant melanomas of sun sheltered mucosal membranes. Acta Oncol 2005;44(7):694-699.
306. Lundberg R, Brytting M, Dahlgren L, Kanter-Lewensohn L, Schloss L, Dalianis T, Ragnarsson-Olding B. Human herpes virus DNA is rarely detected in non-UV light-associated primary malignant melanomas of mucous membranes. Anticancer Res;26(5B):3627-3631.
307. Benjamin T. Virus-specific RNA in cells productively infected or transformed by polyoma virus. J Mol Biol 1966;16(2):359-373.

308. White MK, Gordon J, Reiss K, Del Valle L, Croul S, Giordano A, Darbinyan A, Khalili K. Human polyomaviruses and brain tumors. Brain Research Reviews 2005;50(1):69-85.
309. Major E, Vacante D, Traub R, London W, Sever J. Owl monkey astrocytoma cells in culture spontaneously produce infectious JC virus which demonstrates altered biological properties. J Virol 1987;61(5):1435-1441.
310. Miller N, McKeever P, London W, Padgett B, Walker D, Wallen W. Brain tumors of owl monkeys inoculated with JC virus contain the JC virus genome. J Virol 1984;49(3):848-856.
311. London W, Houff S, Madden D, Fuccillo D, Gravell M, Wallen W, Palmer A, Sever J, Padgett B, Walker D, ZuRhein G, Ohashi T. Brain tumors in owl monkeys inoculated with a human polyomavirus (JC virus). Science 1978;201(4362):1246-1249.
312. Zu Rhein G, Varakis J. Perinatal induction of medulloblastomas in Syrian golden hamsters by a human polyoma virus (JC). Natl Cancer Inst Monogr 1979(51):205-208.
313. Varakis J, ZuRhein G, Padgett B, Walker D. Induction of peripheral neuroblastomas in Syrian hamsters after injection as neonates with JC virus, a human polyoma virus. Cancer Res 1978;38(6):1718-1722.
314. Padgett B, Walker D, ZuRhein G, Varakis J. Differential neurooncogenicity of strains of JC virus, a human polyoma virus, in newborn Syrian hamsters. Cancer Res 1977;37(3):718-720.
315. Walker D, Padgett B, ZuRhein G, Albert A, Marsh R. Human papovavirus (JC): induction of brain tumors in hamsters. Science 1973;181(100):674-676.
316. Small J, Khoury G, Jay G, Howley P, Scangos G. Early regions of JC virus and BK virus induce distinct and tissue-specific tumors in transgenic mice. Proc Natl Acad Sci U S A 1986;83(21):8288-8292.
317. Franks R, Rencic A, Gordon J, Zoltick P, Curtis M, Knobler R, Khalili K. Formation of undifferentiated mesenteric tumors in transgenic mice expressing human neurotropic polymavirus early protein. Oncogene 1996;12(12):2573-2578.
318. Gordon J, Del Valle L, Otte J, Khalili K. Pituitary neoplasia induced by expression of human neurotropic polyomavirus, JCV, early genome in transgenic mice. Oncogene 2000;19(42):4840-4846.
319. Rencic A, Gordon J, Otte J, Curtis M, Kovatich A, Zoltick P, Khalili K, Andrews D. Detection of JC virus DNA sequence and expression of the viral oncoprotein, tumor antigen, in brain of immunocompetent patient with oligoastrocytoma. Proceedings of the National Academy of Sciences of the United States of America 1996;93(14):7352-7357.
320. Krynska B, Del Valle L, Croul S, Gordon J, Katsetos CD, Carbone M, Giordano A, Khalili K. Detection of human neurotropic JC virus DNA sequence and expression of the viral oncogenic protein in pediatric medulloblastomas. Proceedings of the National Academy of Sciences of the United States of America 1999;96(20):11519-11524.
321. Rollison DEM, Utaipat U, Ryschkewitsch C, Hou J, Goldthwaite P, Daniel R, Helzlsouer KJ, Burger PC, Shah KV, Major EO. Investigation of human brain tumors for the presence of polyomavirus genome sequences by two independent laboratories. International Journal of Cancer 2005;113(5):769-774.
322. Abend JR, Jiang MX, Imperiale MJ. BK virus and human cancer: Innocent until proven guilty. Seminars in Cancer Biology 2009;19(4):252-260.
323. Flaegstad T, Andresen PA, Johnsen JI, Asomani SK, Jorgensen GE, Vignarajan S, Kjuul A, Kogner P, Traavik T. A possible contributory role of BK virus infection in neuroblastoma development. Cancer Research 1999;59(5):1160-1163.
324. Stolt A, Kjellin M, Sasnauskas K, Luostarinen T, Koskela P, Lehtinen M, Dillner J. Maternal human polyomavirus infection and risk of neuroblastoma in the child. International Journal of Cancer 2005;113(3):393-396.
325. Krona C, Carén H, Sjöberg R, Sandstedt B, Laureys G, Kogner P, Martinsson T. Analysis of neuroblastoma tumour progression; loss of PHOX2B on 4p13 and

17q gain are early events in neuroblastoma tumourigenesis. Int J Oncol 2008;32(3):575-583.
326. Bogdanovic G, Brytting M, Cinque P, Grandien M, Fridell E, Ljungman P, Lönnqvist B, Hammarin A. Nested PCR for detection of BK virus and JC virus DNA. Clin Diagn Virol 1994;2(3):211-220.
327. Arthur R, Dagostin S, Shah K. Detection of BK virus and JC virus in urine and brain tissue by the polymerase chain reaction. J Clin Microbiol 1989;27(6):1174-1179.
328. Hammarin A, Bogdanovic G, Svedhem V, Pirskanen R, Morfeldt L, Grandien M. Analysis of PCR as a tool for detection of JC virus DNA in cerebrospinal fluid for diagnosis of progressive multifocal leukoencephalopathy. J Clin Microbiol 1996;34(12):2929-2932.
329. Priftakis P, Bogdanovic G, Kokhaei P, Mellstedt H, Dalianis T. BK virus (BKV) quantification in urine samples of bone marrow transplanted patients is helpful for diagnosis of hemorrhagic cystitis, although wide individual variations exist. J Clin Virol 2003;26(1):71-77.
330. Biel S, Held T, Landt O, Niedrig M, Gelderblom H, Siegert W, Nitsche A. Rapid quantification and differentiation of human polyomavirus DNA in undiluted urine from patients after bone marrow transplantation. J Clin Microbiol 2000;38(10):3689-3695.
331. Leung A, Suen C, Lie A, Liang R, Yuen K, Kwong Y. Quantification of polyoma BK viruria in hemorrhagic cystitis complicating bone marrow transplantation. Blood 2001;98(6):1971-1978.
332. Azzi A, Cesaro S, Laszlo D, Zakrzewska K, Ciappi S, De Santis R, Fanci R, Pesavento G, Calore E, Bosi A. Human polyomavirus BK (BKV) load and haemorrhagic cystitis in bone marrow transplantation patients. J Clin Virol 1999;14(2):79-86.
333. Russell S, Vowels M, Vale T. Haemorrhagic cystitis in paediatric bone marrow transplant patients: an association with infective agents, GVHD and prior cyclophosphamide. Bone Marrow Transplant 1994;13(5):533-539.
334. Ost L, Lönnqvist B, Eriksson L, Ljungman P, Ringdén O. Hemorrhagic cystitis--a manifestation of graft versus host disease? Bone Marrow Transplant 1987;2(1):19-25.
335. Hale G, Rochester R, Heslop H, Krance R, Gingrich J, Benaim E, Horwitz E, Cunningham J, Tong X, Srivastava D, Leung W, Woodard P, Bowman L, Handgretinger R. Hemorrhagic cystitis after allogeneic bone marrow transplantation in children: clinical characteristics and outcome. Biol Blood Marrow Transplant 2003;9(11):698-705.
336. El-Zimaity M, Saliba R, Chan K, Shahjahan M, Carrasco A, Khorshid O, Caldera H, Couriel D, Giralt S, Khouri I, Ippoliti C, Champlin R, de Lima M. Hemorrhagic cystitis after allogeneic hematopoietic stem cell transplantation: donor type matters. Blood 2004;103(12):4674-4680.
337. Gorczynska E, Turkiewicz D, Rybka K, Toporski J, Kalwak K, Dyla A, Szczyra Z, Chybicka A. Incidence, clinical outcome, and management of virus-induced hemorrhagic cystitis in children and adolescents after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant 2005;11(10):797-804.
338. Hirsch H, Kaufmann G, Sendi P, Battegay M. Immune reconstitution in HIV-infected patients. Clin Infect Dis 2004;38(8):1159-1166.
339. Leung A, Yuen K, Kwong Y. Polyoma BK virus and haemorrhagic cystitis in haematopoietic stem cell transplantation: a changing paradigm. Bone Marrow Transplant 2005;36(11):929-937.
340. Bogdanovic G, Priftakis P, Giraud G, Dalianis T. A related donor and reduced intensity conditioning reduces the risk of development of BK virus-positive haemorrhagic cystitis in allogeneic haematopoetic stem cell-transplanted patients. Anticancer Res 2006;26(2B):1311-1318.
341. Comoli P, Hirsch H, Ginevri F. Cellular immune responses to BK virus. Curr Opin Organ Transplant 2008;13(6):569-574.

342. Leung A, Chan M, Yuen K, Cheng V, Chan K, Wong C, Liang R, Lie A, Kwong Y. Ciprofloxacin decreased polyoma BK virus load in patients who underwent allogeneic hematopoietic stem cell transplantation. Clin Infect Dis 2005;40(4):528-537.
343. Katano H, Ito H, Suzuki Y, Nakamura T, Sato Y, Tsuji T, Matsuo K, Nakagawa H, Sata T. Detection of Merkel cell polyomavirus in Merkel cell carcinoma and Kaposi's sarcoma. J Med Virol 2009;81(11):1951-1958.
344. Erard V, Storer B, Corey L, Nollkamper J, Huang M, Limaye A, Boeckh M. BK virus infection in hematopoietic stem cell transplant recipients: frequency, risk factors, and association with postengraftment hemorrhagic cystitis. Clin Infect Dis 2004;39(12):1861-1865.
345. Erard V, Kim H, Corey L, Limaye A, Huang M, Myerson D, Davis C, Boeckh M. BK DNA viral load in plasma: evidence for an association with hemorrhagic cystitis in allogeneic hematopoietic cell transplant recipients. Blood 2005;106(3):1130-1132.
346. Bogdanovic G, Ljungman P, Wang F, Dalianis T. Presence of human polyomavirus DNA in the peripheral circulation of bone marrow transplant patients with and without hemorrhagic cystitis. Bone Marrow Transplant 1996;17(4):573-576.
347. Rocha V, Gluckman E. Improving outcomes of cord blood transplantation: HLA matching, cell dose and other graft- and transplantation-related factors. Br J Haematol 2009;147(2):262-274.
348. Rinaldo C, Hirsch H. Antivirals for the treatment of polyomavirus BK replication. Expert Rev Anti Infect Ther 2007;5(1):105-115.
349. Kuypers D, Vandooren A, Lerut E, Evenepoel P, Claes K, Snoeck R, Naesens L, Vanrenterghem Y. Adjuvant low-dose cidofovir therapy for BK polyomavirus interstitial nephritis in renal transplant recipients. Am J Transplant 2005;5(8):1997-2004.
350. Bjorang O, Tveitan H, Midtvedt K, Broch L, Scott H, Andresen P. Treatment of polyomavirus infection with cidofovir in a renal-transplant recipient. Nephrol Dial Transplant 2002;17(11):2023-2025.
351. Kuypers D, Bammens B, Claes K, Evenepoel P, Lerut E, Vanrenterghem Y. A single-centre study of adjuvant cidofovir therapy for BK virus interstitial nephritis (BKVIN) in renal allograft recipients. J Antimicrob Chemother 2009;63(2):417-419.
352. Cabello V, Margarit N, Díaz Pedrero M, Bernal G, Pereira P, Gentil M. Treatment of BK virus-associated nephropathy with Cidofovir in renal transplantation. Transplant Proc 2008;40(9):2930-2932.
353. Araya C, Lew J, Fennell R, Neiberger R, Dharnidharka V. Intermediate dose cidofovir does not cause additive nephrotoxicity in BK virus allograft nephropathy. Pediatr Transplant 2008;12(7):790-795.
354. Akioka K, Okamoto M, Ushigome H, Nobori S, Kaihara S, Yoshimura N. BK virus-associated nephropathy in a kidney transplant recipient successfully treated with cidofovir, the first case in Japan. Int J Urol 2008;15(4):369-371.
355. Bernhoff E, Tylden G, Kjerpeseth L, Gutteberg T, Hirsch H, Rinaldo C. How Leflunomide inhibits BK virus replication in renal tubular epithelial cells. J Virol 2009.
356. Wu J, Harris M. Use of leflunomide in the treatment of polyomavirus BK-associated nephropathy. Ann Pharmacother 2008;42(11):1679-1685.
357. Faraci M, Cuzzubbo D, Lanino E, Di Marco E, Cirillo C, Dallorso S, Morreale G, Moroni C, Castagnola E. Low dosage cidofovir without probenecid as treatment for BK virus hamorrhagic cystitis after hemopoietic stem cell transplant. Pediatr Infect Dis J 2009;28(1):55-57.
358. Cesaro S, Hirsch H, Faraci M, Owoc-Lempach J, Beltrame A, Tendas A, Baltadakis I, Dalle J, Koc Y, Toporski J, Styczynski J, Yesilipek M, Heinz W, Caniglia M, Rascon J, Fauser A, Michallet M, Lopez-Corral L, Neuburger S, Tridello G, Einsele H. Cidofovir for BK virus-associated hemorrhagic cystitis: a retrospective study. Clin Infect Dis 2009;49(2):233-240.