Summary

The research described in this thesis aimed to study determinants of the course and outcome of treatment of herpesvirus infections in immunocompromised patients. Both viral factors, such as antiviral resistance, but also patient factors, including immunological parameters, were investigated. Techniques to study antiviral resistance were optimized for use in a clinical diagnostic setting. The aim of this research was to improve and facilitate management of herpesvirus infections in immunocompromised patients.

In **chapter two** the development and validation of a real-time pcr based phenotypical technique to study susceptibility of HSV-1 to antiviral drugs in a routine diagnostic setting is described. This assay was designed to be faster and less labor intensive than classical culture based phenotypical susceptibility tests for HSV-1, such as the plaque reduction assay.

The results from our DNA reduction assay (DRA) were in accordance with plaque reduction assay results and with sequence analysis. DRA appeared to have a better discriminative value for low-level resistance. Although the direct application of DRA in clinical samples appeared not possible, short pre-culture of 48 hours was sufficient and ensured results within a clinically relevant time frame of 5 days.

Oral ulcerations are frequent and debilitating complications after hematopoietic stem cell transplantation (HSCT). In **chapter three** the role of HSV-1, EBV and CMV in oral ulcerations in HSCT recipients was investigated. Insight on the precise role of herpesviruses in this setting may improve management.

In a prospective observational cohort study in 49 adult patients that underwent allogeneic HSCT, the occurrence and localization of oral ulcerations and the presence and quantity of HSV-1, EBV and CMV in oral washing samples were systematically documented. Persistent HSV-1 infection was defined as an infection that lasted at least 5 days despite antiviral treatment. Antiviral resistance was studied in all persistent HSV-1 infections by viral sequence analysis.

Having an HSV-1 or EBV DNA positive sample was found to be a significant predictor for ulceration of keratinized mucosa. HSV-1 was a significant predictor for ulcerations on non-keratinized mucosa as well. Furthermore, persistent HSV-1 infection occurred in 12 of 28 patients treated with antiviral medication and aciclovir resistant HSV-1 was found in 5 persistent infections. In conclusion, given the important role of HSV in oral ulcerations after HSCT, prophylaxis or rapid treatment of oral HSV-1 infection and timely resistance diagnosis are warranted after HSCT.

VZV infections are a relevant cause of morbidity and mortality in hematological patients and especially in HSCT recipients. However, little is known on the course and on the occurrence of antiviral resistance of VZV in this setting. In **chapter four** the course of VZV infections in hematological patients was studied including the role of antiviral resistance in persistent infections.

In a retrospective study including all 87 pediatric and adult hematological patients diagnosed with VZV in our laboratory between 2007 and 2010, the clinical and virological course of VZV infections was studied. Persistent infection was defined as an infection that lasted at least 7 days. Antiviral resistance was studied in all persistent infections by viral sequence analysis.

Persistent VZV was demonstrated in 59% of the 54 episodes with follow-up available. Complications occurred in 50% of the persistent episodes and possible resistance associated mutations were found in 27% of the patients with persistent VZV, including patients with treatment unresponsive dermatomal zoster that progressed to severe retinal or cerebral infection. Therefore, antiviral resistance of VZV needs to be investigated timely and in all affected body sites in persistent infections.

In **chapter five** the application of a novel technique using mass spectrometry-based comparative sequencing to detect ganciclovir resistance in CMV is addressed. Mass spectrometry-based comparative sequence analysis (MSCSA) might be advantageous for this purpose because of its suitability for semi-automation.

Comparison of results from MSCSA with conventional cycle sequencing showed 94.1% concordance. The threshold to detect mutant sequences in a mixture with wild-type material was 20% using either technique. Thus, MSCSA was found to be equally accurate compared to conventional cycle sequencing in the analysis of the UL97 gene of HCMV

Pre-emptive treatment of CMV infections does not always lead to a rapid viral response. The causes of this type of treatment failure can be diverse and include antiviral resistance, pharmacological aspects and immunological factors. In **chapter six** various determinants of the response to antiviral treatment of CMV infections in HSCT recipients were studied, including resistance to antivirals.

Consecutive adult recipients of allogeneic T-cell depleted SCT were studied retrospectively (n=92). Treatment failure was defined as a CMV DNA load of 1000 copies/ml or more after at least 2 weeks of treatment. Resistance was analyzed in all failure episodes by viral sequence analysis.

Treatment failure occurred in 45% of pre-emptive treatment episodes and occurred

more often during first treatment episodes and during the use of immunosuppressive medication. Antiviral resistance was found in only 1 patient with treatment failure. Hence this study showed that a slow response to pre-emptive antiviral treatment occurred frequently in this setting and that antiviral resistance played a minor role.

In **chapter seven** the response to treatment and the occurrence of antiviral resistance are compared between a preemptive and a sequential prophylactic-preemptive treatment regimen for CMV in D+R- renal transplant recipients.

Consecutive adult D+R- recipients of a renal transplant were studied retrospectively. Before 2006, a preemptive treatment regimen with valganciclovir was applied (42 patients). From 2006 onwards, patients first received prophylaxis with valganciclovir for 90 days, followed by a preemptive regimen (29 patients). Treatment failure was defined as a CMV DNA load of 1000 copies/ml or more after at least 2 weeks of treatment. Resistance was analyzed in all failure episodes by viral sequence analysis.

Treatment failure occurred less frequently in the prophylaxis cohort than in the preemptive cohort (14% vs. 71%). Resistant viral isolates were found during treatment in one patient in the prophylaxis cohort versus in three patients in the preemptive group. All CMV infections with resistant virus were cleared without switch of (val)ganciclovir treatment. In conclusion, treatment failure occurred not frequently in the sequential prophylaxis-preemptive cohort and antiviral resistance played a minor role in treatment failure.

The lectin pathway of complement activation, component of the innate immunity, is a crucial effector cascade of the innate immune response to pathogens. Because many effector proteins from this pathway are synthesized in the liver, after liver transplantation a unique situation exists in which a recipient acquires the donor derived genotype en phenotype. In **chapter eight** the role of gene polymorphisms influencing mannose-binding lectin (MBL2), ficolin-2 (FCN2) and MBL-associated serine protease (MASP2) on CMV infection after orthotopic liver transplantation was investigated.

Transplant recipients (n=295) and donors were genotyped for polymorphisms in MBL2, FCN2 and MASP2 genes. Combined analysis of independently associated variant MBL2 and wild-type FCN2 SNPs in the donor liver showed an increased risk of CMV infection for either and both risk genotypes, especially in D-R+ patients. A genetic donor–recipient mismatch for MBL2 and FCN2 increased the CMV risk independently, also combined, particularly in CMV D-/R+ patients. In conclusion, MBL2 and FCN2 risk alleles of donor liver and recipient constitute independent risk factors for CMV infection after OLT.

In the discussion, implications for management of herpesvirus infections in immunocompromised patients as well as suggestions for further research are described.