UNIVERSITÀ DEGLI STUDI DI TORINO

This is an author version of the contribution published on:

Questa è la versione dell'autore dell'opera:

[*Expert Rev Hematol. 2016 Apr 18:1-12.*]

ovvero [Maffini E1,2, Giaccone L1,2, Festuccia M1,2, Brunello L1,2, Busca A1, Bruno B1,2.]

The definitive version is available at:

La versione definitiva è disponibile alla URL:

[http://www.tandfonline.com.offcampus.dam.unito.it/doi/abs/10.1080/17474086.2016.1174571?journalCode=ierr20]

Treatment of CMV Infection after

Allogeneic Hematopoietic Stem Cell Transplantation

Abstract: Despite a remarkable reduction in the past decades, cytomegalovirus (CMV) disease in allogeneic hematopoietic stem cell transplant (HSCT) recipients remains a feared complication, still associated with significant morbidity and mortality. Today first line treatment of CMV infection/reactivation is still based on dated antiviral compounds Ganciclovir (GCV), Foscarnet (FOS) and Cidofovir (CDF) with their burdensome weight of side effects. Maribavir (MBV), Letermovir (LMV) and Brincidofovir (BDF) are three new promising anti-CMV drugs without myelosuppressive properties or renal toxic effects that are under investigation in randomized phase II and III trials. Adoptive T-cell therapy (ATCT) in CMV infection possesses a strong rationale, demonstrated by several proof of concept studies; its feasibility is currently under investigation by clinical trials. ATCT from third-party and naïve donors could meet the needs of HSCT recipients of seronegative donors and cord blood grafts. In selected patients such as recipients of T-cell depleted grafts, ATCT, based on CMV-specific host T-cells reconstitution kinetics, would be of value in the prophylactic and/or preemptive CMV treatment. Vaccine-immunotherapy has the difficult task to reduce the incidence of CMV reactivation/infection in highly immunocompromised HSCT patients. Newer notions on CMV biology may represent the base to flush out the Troll of transplantation.

Key words: cytomegalovirus (CMV), immune response, CMV-vaccine, hematopoietic stem cell transplantation (HSCT), adoptive T-cell therapy (ATCT).

1.Introduction

Over thirty years ago, Henry Balfour [1] nicknamed cytomegalovirus (CMV) the troll of transplantation and since then it has remained a problem for clinicians and patients in the setting of solid organ transplantation (SOT) and hematopoietic stem cell transplantation (HSCT) [2]. Indeed, CMV specific viral load detecting methods and pre-emptive therapy with current antiviral drugs remarkably reduced the incidence of CMV disease in allogeneic HSCT recipients over the past twenty years. Estimated incidences of early CMV disease (before day 100 post-transplant) and late CMV disease (after day 100) in CMV-seropositive allogeneic recipients are currently around 5% and 15% [3]. Major risk factors for CMV infection in HSCT recipients, particularly refractory CMV reactivation, include a transplant from a CMV seronegative donor into a seropositive recipient [4]; the intensity of the immunosuppression; the degree of T-cell depletion including the use of T-cell depleting agents such as alemtuzumab, antithymocyte globulin [5], and mycophenolate mofetil [6]; a transplant from unrelated or human leucocyte antigen (HLA)-mismatched donors including haploidentical donors [7]; umbilical cord blood transplantation; acute and chronic graft versus host disease (GvHD) and its treatment [8]; low CD3+ content in the graft [9]. Both myeloablative and non-myeloablative conditionings are associated with relative risk of CMV infection [10, 11]. Conflicting results have been reported regarding the relative risk of CMV infection in bone marrow recipient as compared with peripheral blood stem cells (PBSC) recipients [12]. Interestingly, recipient hepatitis B virus serostatus has been associated with the development of CMV-DNAemia in a recent retrospective study on haploidentical HSCT [13]. The switch from prophylactic to pre-emptive therapy has substantially spared organ toxicities and costs [14]. Myelosuppression and nephrotoxicity are the most important side effects of the anti-CMV agents readily available today – ganciclovir (GCV), valganciclovir (VGC), foscarnet (FOS) and cidofovir (CDF). Antiviral resistance could be a difficult phenomenon to overcome and management options still have important limitations [15]. The need for newer agents with better toxicity profiles have led to develop several promising antiviral compounds [16, 17]. Most interesting agents are maribavir (MBV), letermovir (LMV) and brincidofovir (BDF). Phase II/III studies are in progress to evaluate their safety and efficacy in the clinical setting. As newer and deeper insights into immune reconstitution of transplant recipients and CMV biology are obtained, immunological strategies are drawing much more attention. Optimal reconstitution of CMV-specific cytotoxic T lymphocytes (CMV-CTL) after allogeneic HSCT is essential for immune control of

CMV infection. Its deficiency is a major factor for the development of CMV infection and disease and represents the major concept to set the base of adoptive T-cell therapy (ATCT) [18]. Moreover, vaccine immunotherapy appears to play a new role in CMV-prophylaxis [19].

2.1 The old drugs: Ganciclovir (GCV), Valganciclovir (VGC), Foscarnet (FOS), Cidofovir (CDF).

The drugs currently approved by the Food and Drug Administration (FDA) for the prophylaxis or treatment of systemic CMV infections are GCV and its oral prodrug VGC; FOS, and CDF. All of them target viral DNA polymerase.

2.1.1 Ganciclovir – the first choice is a myelosuppressive one.

It is still the first choice in CMV reactivation/infection and disease treatment [20]. GCV is phosphorylated intracellularly to ganciclovir monophosphate by a viral kinase encoded by the CMV gene UL97. It inhibits viral DNA polymerase. The most relevant side effect of GCV is bone marrow depression, particularly neutropenia with an absolute neutrophil count of less than 750/mL occurring in 30% of GCV recipients. The development of neutropenia has been associated with risk factors such as impaired renal function, high baseline viral load and low-level neutrophil counts prior to CMV-therapy [21]. As prophylaxis and pre-emptive therapy, GCV is usually infused at an induction dose of 5 mg/kg twice daily intravenously for two weeks, followed by 5 mg/kg/day for 7-14 days as maintenance. As mentioned above, a not negligible portion of transplanted patients cannot receive GCV because of persistent neutropenia after HSCT. A randomized study conducted on 68 HSCT recipients with documented CMV infection between standard dose (10 mg/kg/day) (n=32) versus low-dose (5 mg/kg/day) GCV (n=36) as pre-emptive therapy did not show any significant difference in CMV disease incidence [22]. Park et al. explored pre-emptive low-dose (5 mg/kg/day) versus conventional dose GCV in a prospective observational study on 97 allogeneic HSCT patients. By using a logistic regression model to reduce selection bias in the treatment assignment, the

Authors reported no significant differences in viral clearance, secondary episodes of CMV infection, CMV disease and overall mortality between the two groups [23]. In the setting of CMV disease, the addition of intravenous immunoglobulins, either CMV-specific or not, is not supported by clear efficacy in treatment outcomes. A recent retrospective study on over four hundred allogeneic HSCT patients with CMV-pneumonia clearly showed improved overall survival with both GCV and FOS, whereas the addition of intravenous pooled or CMV-specific immunoglobulins did not appear to improve overall or CMV-attributable mortality [24]. In case of progressive CMV disease despite first line therapy with GCV, it is mandatory to consider the presence of GCV-resistant CMV strains and look for alternative therapies including the increase of GCV dosage [25] or the use of other antiviral agents [26].

2.1.2 Valganciclovir – oral versus intravenous.

It is the valine ester of GCV and it is hydrolyzed to GCV after oral absorption. Since 85% of GCV delivered orally is excreted by the kidneys, VGC dosage should be recommended in the light of patient creatinine clearance (CrCl). Some Authors however suggest that the currently recommended renal adjustments based on their CrCl calculated using the ideal body weight may not be sufficient to prevent viral replication in overweight patients given the possible underestimation of the renal function in such patients [27, 28]. Large prospective randomized trials comparing VGC and GCV in HSCT patients are lacking. Though small, some interesting studies compared the efficacy of VGC therapy versus non-VGC therapy - primarily GCV - in the pre-emptive setting on allogeneic HSCT patients. In a pilot prospective randomized clinical trial conducted on 37 HSCT patients, VGC (n=19) was not inferior to intravenous GCV (n=18) as pre-emptive therapy with rates of viral clearance at 28 days after the start of therapy of 89.5% and 83% respectively. Similar toxicities were reported between the two arms [29]. A retrospective single center German study conducted on 118 allogeneic HSCT recipients demonstrated superiority of VGC (n=48) versus non-VGC (n=70) pre-emptive therapy in terms of viraemia clearance and mean duration of hospitalization, without neutropenia episodes during twice weekly neutrophil count monitoring [30]. Similar results emerged from a two-year prospective, comparative cohort study of CMV infection on 166 allogeneic HSCT recipients in which intravenous GCV,

FOS or oral VGC were given as first-line pre-emptive CMV treatment. VGC was as efficacious and safe as non-VGC treatment without requiring hospitalization [31]. To reduce myelotoxicity, a small retrospective study on 68 allogeneic HSCT recipients, comparing efficacy and safety of standard intravenous GCV (n=24) versus low-dose oral VGC (n=44) in pre-emptive treatment of CMV infection, demonstrated lower incidence of low-grade neutropenia and high-grade thrombocytopenia without any significant differences in viral titers between the two groups [32]. Conversely, Takahata et al. in a recent non-inferiority study on 38 HSCT patients, observed that by reducing the standard dosage of VGC from 900 mg twice daily (n=18) to 900 mg once a day (n=20) there was no statistically significant difference in myelotoxicity [33].

2.1.3 Foscarnet (FOS) – neutropenia-free but nephrotoxic.

Neutropenic patients prior engraftment or during GCV/VGC therapy or infected with UL-97- resistant CMV strains are commonly treated with FOS [20, 34]. A virustatic agent that inhibits viral DNA polymerase, FOS does not cause myelotoxicity [35] but primarily causes renal side effects with renal impairment and electrolytes imbalance. Seizures and local genital irritation/ulceration [36] are also occasionally seen. FOS has been compared to GCV for pre-emptive treatment of CMV infections in a randomized trial with satisfactory results in terms of control of antigenaemia and survival rates with remarkably less neutropenia episodes though renal toxicities were relevant [37]. Though rare, FOS resistance is mediated by mutation of the UL54 gene and it might also develop after a short drug exposure [38].

2.1.4 Cidofovir (CDF) – the last resort.

CDF is a nucleotide analogue that does not require viral phosphorylation for its activation with a favorable pharmacokinetic profile. It is considered a third line drug, because of its important nephrotoxicity, gastrointestinal and ocular morbidities such as uveitis, retinal detachment and chronic ocular hypotension. To decrease nephrotoxicity, CDF is usually infused with Probenecid (PBC), an inhibitor of organic anion transport. Administered orally with each dose of CDF, PBC blocks active renal tubular secretion of CDF. Side effects like nausea and vomiting are generally mild; Hypersensitivity reactions can also occur. The Infectious

Disease Working Party of the European Society for Bone and Marrow Transplantation reported the largest recipient series of allogeneic HSCT treated with CDF. Mostly given at 5 mg/kg/week in both primary or secondary pre-emptive therapy for CMV infection with response rates of 50% for CMV disease and 62% to 66% for CMV infection, significant renal toxicities were reported [39]. Several applications of CDF against different viral pathogens in pediatric patients [40] have been reported in recent years, in particular against adenovirus [41], BK virus-associated hemorrhagic cystitis [42, 43] and poxvirus [44].

2.2 Antiviral drug resistance

CMV antiviral drug resistance among allogeneic HSCT recipients is an uncommon but feared complication because it is associated with poor clinical outcomes and potential graft-loss. Its incidence is estimated around 2-8% of allograft recipients [45] and it is generally associated with prior prolonged exposure, at least six weeks, to the antiviral drug and persistent reactivation episodes which usually lead to the identification of resistant strains [46]. Antiviral drug resistance should be suspected in the presence of poor clinical response and progressive rise in viral load for more than fourteen days or with stable serum CMV-DNA levels despite antiviral therapy. In this case, genotypic testing for sequence analysis of UL97 – phosphotransferase - and UL54 - polymerase - genes and switch to an alternative antiviral drug should be recommended. UL97 kinase mutations, that alter the phosphorylation process, are the most represented cause of CMV-antiviral resistance, Seven most common UL97 mutations account for over 80% of GCV-resistant CMV strains whereas UL54 mutations are generally associated with FOS and CDF- resistance though cross-resistance to GCV may be also observed [47]. Newer technologies such as next-generation sequencing are being explored for the detection of genotypic resistance [48]. CMV multidrug-resistance with mutations in both UL97 and UL54 genes usually results from UL54 cross-resistance [49, 50]. At present, there are no standardized therapeutic approaches and new antiviral drugs targeting alternative CMV key structures are needed [51].

2.3 The others: Artesunate and Leflunomide

2.3.1 Leflunomide (LFN) – not only for rheumatic arthritis.

An FDA-approved drug for rheumatoid arthritis, LFN is an oral compound with good bioavailability immunosuppressive properties (its active metabolite, teriflunomide, is a strong lymphocyte proliferation inhibitor) and antiviral activity against a number of viruses [52]. Its mechanism of action resides in the ability to interfere with the assembly of the virionic capside without cross-resistance activity with current anti-CMV compounds. Its side effects includes gastrointestinal toxicity with diarrhea, liver toxicity and polyneuropathy. Given the relatively slow onset of complete antiviral action, the drug is not however ideal for infections with rapidly increasing viral loads [53]. Its proper dosage, timing and duration of treatment for CMV need further investigations in future randomized studies [54].

2.3.2 Artesunate – the antimalarial.

It is an artemisinin-derived monomer able to inhibit *in vitro* CMV replication in human foreskin fibroblasts at micromolar concentrations through cell cycle modulation with an early arrest in G1 phase [55]. Cell cycle modulation via cyclin-dependent kinases and retinoblastoma protein appears to play an important role in artemisinins activities [56]. Unfortunately, there is not yet a thorough evaluation of its efficacy but only sporadic reports of its *in vivo* anti CMV activity with contrasting results [57]. Interesting insights come from artemisinin-derived dimers, novel compounds with more potent *in vitro* anti-CMV effects [58, 59] the efficacy of which remains to be addressed.

2.4 The new drugs: Maribavir (MBV) , Letermovir (LMV) and Brincidofovir (BDF).

Three multicenter, randomized, placebo-controlled phase II, proof of concept studies [60, 61, 62] led to the identification of three new compounds with anti-CMV activity (Table I).

2.4.1 Maribavir – a false start.

A benzimidazole riboside compound that, unlike GCV, is a direct inhibitor of the UL97 protein kinase. It is active against GCV- and CDF-resistant CMV strains. Its bioavailability is greater than that of oral GCV, but less than that of VGC. It is not associated with hematological or renal toxicities. Its most relevant side effects are dysgeusia and nausea. Strengthened by encouraging results from a phase II study [60] and granted fast track status drug, MBV was tested in a subsequent multicenter, randomized, double blind, phase III study, conducted on 681 HSCT patients. Rather surprisingly, it failed to demonstrate superior efficacy to prevent CMV disease as compared with to placebo [63]. Explanations may partly be due to the choice to adopt CMV end organ disease as the primary study endpoint and the exclusions of parameters such as viral load or start of pre-emptive therapy, the exclusion of high risk patients, the possibly too low dose of MBV (100 mg twice daily) employed [64, 65]. Not conclusive results were reported in a randomized, multicenter, double-blind study on a cohort of 303 CMV-seropositive liver transplant recipients with CMV-seropositive donors where prophylaxis with MBV at 100 mg twice daily was compared to oral GCV. The non-inferiority endpoint of MBV in preventing CMV disease was not reached. Moreover, significantly fewer CMV events – both as overt disease or increased viral load – were registered in the GCV arm both at day +100 and at six months post transplant [66]. Opposite results came from a recent French report on a cohort of 12 transplant recipients – including three HSCT – with resistant CMV strains. Treatment with MBV at a minimum daily dose of 800 mg was successful in seven patients without significant toxicities [67]. To address the issue as whether there is still a role today for MBV in CMV disease prevention and treatment in HSC and SOT patients, a phase II (NCT01611974), double blind, randomized, dose-ranging study (with high dose MBV at 400 mg BID, 800 mg BID and 1200 mg BID) was recently completed and final results are eagerly awaited. Furthermore, warnings on a rapid rise of CMV strains resistant to high MBV doses have recently been published [68]. Different isoforms of kinase pUL97 greatly affect susceptibility of CMV to MBV efficacy [69]. There is generally no overlap between the kinase ATP binding site mutations and the UL97 mutations, that respectively confer MBV and GCV resistance, with the exception of a single p-loop

mutation (F342S) involved in dual resistance to both drugs, even though it has not yet been observed *in vivo* [70, 71].

2.4.2 Letermovir - the terminaSEtor.

Member of the new antiviral class of quinazolines, it acts after viral DNA synthesis by inhibiting the subunit protein pUL56 that, together with pUL89, is a key element of the enzyme complex named terminase,[72, 73] directly involved in the cleavage and package of viral DNA chains in the virionic capside [74, 75]. Because of its distinct mechanism of action, it does not show cross-resistance with other antiviral drugs and there are reports of clinically relevant activity against GCV-, FOS- and CDF-resistant CMV strains [76, 77]. Given its virus-specific, human-cell sparing mechanism of action, it has not been associated with any clinical significant side effects. LMV was studied as CMV prophylaxis in a randomized, placebo-controlled phase II study on 131 CMV-seropositive allogeneic HSCT recipients, across various US and German transplant centers, at three different dosages - 60, 120 or 240 mg once a day. It showed higher efficacy in reducing plasma CMV-DNA levels as compared with placebo, with the greatest anti-CMV effect at the 240 mg dose. Failures were recorded only in 21%, 19% and 6% of patients, at the 60, the 120 and the 240 mg dose, respectively, as compared with 36% in the placebo arm. Of note, the tolerability profile of the drug was reported to be excellent, with no hematological or renal toxicity [61]. Efficacy and safety of this compound were similarly investigated in a recent phase II study performed on 27 kidney transplant recipients, with LMV at 40 mg BID or 80 mg once daily in comparison with the current standard of care (SOC). Viral clearance was reached in 50% of treated patients as compared with 29% in those receiving SOC [78]. A phase III randomized, placebo-controlled, multicenter study conducted by Merck (that acquired the rights to develop and market the drug in 2012) on the prevention of CMV infection and CMV disease in allogeneic HSCT recipients, is currently recruiting patients. Primary endpoint of this study is to compare the CMV infection rate between the two arms through week 24 after transplant. The study is expected to

complete the accrual in January 2017 for primary outcome (NCT02137772). Overall, *in vitro* LMV resistant CMV strains have been reported [79, 80, 81].

2.4.3 Brincidofovir (CMX001) – the fatty one.

Originally developed as an agent against the hypothetical biological warfare with smallpox virus, this drug has potent *in vitro* activity against a wide range of double-stranded viruses, including CMV. This is a lipid pro-drug of CDF, which is intracellularly converted into CDF diphosphate and inhibits DNA polymerase of adenoviruses, polyomaviruses, orthopoxviruses and herpesviruses. Given its high oral bioavailability and long half-life, it can be administered twice weekly by oral route. Its lipophilic nature, obtained with the addition of a lipidic side chain to the parent compound, allows the absorption through plasma membranes, reducing the amount of circulating drug and avoiding damage to renal tubules [82]. In a multicenter double blind, placebo-controlled, phase II dose-escalation study on a cohort of 230 allogeneic HSCT patients, BDF reduced incidence of CMV infection and CMV disease in those patients who received BDF at doses of 100 mg weekly or higher as compared with those who received placebo [62]. Of 15 patients in the study cohort who developed GvHD and required systemic steroid treatment, only one developed CMV reactivation. Overall, at 100 mg twice weekly, the drug was well tolerated and efficacious, without increasing myelosuppression or nephrotoxicity. Its principal side effect was significant diarrhea in over 50% of the patients treated with 100 mg twice weekly which became dose-limiting at 200 mg twice weekly. This toxicity was likely determined by an excessive concentration of CDF in enterocytes as reported in animal models [83]. The SUPPRESS trial, a phase III randomized multicenter, placebo-controlled (ratio 2:1), on 450 CMV seropositive allogeneic HSCT recipients treated with BDF at a dose of 100 mg twice weekly has recently stopped recruiting patients. Results are expected in 2016 (NCT01769170). BDF is also being evaluated in two randomized, double-blind, multicenter, phase III registered clinical trials for the prevention of CMV-disease in CMV seropositive (SURPASS trial - NCT02439957) and seronegative (SUSTAIN trial - NCT02439970) kidney transplant recipients. Furthermore, BDF is under investigation for its potential

antiviral activity against other types of viral pathogens such as adenovirus and herpes simplex virus [84], BK polyomavirus in immunocompromised, mostly transplant, patients [85]. Preliminary data from transplant patients who received BDF for adenovirus infections in an ongoing multicenter open-label phase III trial are encouraging in terms of efficacy and safety (NCT02087306).

3. Adoptive T-cell therapy

In the HSCT setting, several factors weaken the host immunologic defense. The intensity of the conditioning regimens, prevention/treatment of GvHD, often with high dose steroids, and the long process of immune-reconstitution are all associated with the risk of viral infections. *In vivo* expansion and persistence of CMV-CTL is crucial for an appropriate immune response both in the early and late post-transplant phases until the establishment of stem cell-derived immunity. Soon after transplant, most CMV-CTLs are of donor origin, while newly "educated" endogenous T cells from thymic output appear only later [86]. In haploidentical HSCT, early T-cell recovery is primarily based on peripheral expansion of naïve T cells and it appears delayed when compared with that of HSCT from HLA-identical siblings [87-90] Long-term immune reconstitution, however, mostly thymus-dependent, appears appropriate to maintain an adequate naïve T cell pool [91-94]. Adoptively transferred CMV-CTL can be detected long after HSCT and up to 2 years after infusion [95]. There are two main ways to obtain virus-specific T cells (VSTCs): a) *in vitro* expansion and b) *ex vivo* separation and *in vivo* expansion (Fig.1).

3.1 Ex vivo culture

The first is a culture-based technique with many amplification procedures to increase the specificity of T cells after repeated *in vitro* expansions. It is a time-consuming and costly procedure but with the advantage of generating a large number of T cells (> 10^7/kg) with the desired antiviral specificity regardless of the

host immunity. Given the long *in vitro* process*,* the expansion should be carried out prior to the transplant in selected all high-risk patients as only a few of these patients will eventually need this treatment.

One of the most significative experience on HSCT patients who received donor CMV-VSTCs and compared with a control group was reported in 2013 [96]. The endpoint was to evaluate if prophylaxis with CMV-specific T cells could provide short- and long-term protection against CMV infection. VSTCs were generated through dendritic cells genetically modified with an adenoviral vector encoding the full pp65 antigen or pulsed with a HLA2-restricted immunodominant peptide from the CMV pp65 antigen. One major limitation was however the risk of escape mutants among the VSTCs. Overall, there was a reduction in the percentage of patients who required CMV antiviral therapy and in a number the duration was shorter. Importantly, there was not an increased rate of acute or chronic GvHD attributable to VSTCs as compared to the control arm - two cases of acute grade III-IV GvHD , and an overall incidence of chronic GvHD of 42%.

3.2 Direct ex vivo selection techniques.

Techniques for direct selection of VSTCs include the use of peptide pools derived from viral antigens to expand T cells with multiple antigen specificities [97], the selection of VSTCs based upon the secretion of interferon-gamma (IFN-gamma) [98, 99] or the binding to class I HLA-multimers [100] or immunomagnetic beads [101]. The multimer selection method requires HLA-specific elements for every viral epitope and is actually restricted to CD8+ T-cells, while the IFN-gamma secretion technique is based upon a HLA-unrestricted selection of CD4+ and CD8+. Both techniques require a considerable volume of donor blood and imply the fact that only viruses with a high frequency of circulating T-cell precursors can be targeted. The number of recovered cells is usually small. Multimer selection technique was first exploited by Cobbold [102] with a direct selection technique by using a panel of CMV IE1 and pp65 tetramers to select specific T cells from HSCT donors. Later, Uhlin and colleagues [101] described a separation technique based upon positive selection with HLA-pentamers and magnetic beads that bind to CD8+T-cells reactive to CMV, EBV and adenovirus from donor lymphocyte infusion and peripheral blood of haploidentical donors. A total of 8

patients with infections caused by different viruses were treated. Six out of 8 showed a decrease in viral titers within two weeks post-VSTCs infusion. Overall, the study emphasized the need for preemptive rather than therapeutic use of immune therapy after allogeneic HSCT. Concerns have been raised about the potential clonal exhaustion of the multimer-bound T cells after a prolonged HLA-peptide/T-cell receptor interaction. In an attempt to overcome this problem, Schmitt and colleagues [103] utilized streptamers that could be dissociated from T cells by the addition of a competitor molecule and injected CD8+T-cells isolated with this technique into two allogeneic HSCT patients with CMV refractory disease. In both cases there was an increase in reactive cells and CMV viral load clearance, without GvHD. Another method to select VSTCs is based upon the ability of memory T cells to secrete IFN-gamma in an antigen-dependent manner. Feuchtinger first reported this method in severe adenovirus infections in pediatric patients and later in CMV refractory infection and disease which included two cases of CMV-encephalitis, in T-cell depleted allogeneic HSCT [98]. In 2011, in a phase I/II study, Peggs and colleagues described the use of IFN-gamma captured CMV pp65-specific T cells as prophylaxis and preemptive CMV-treatment with increase of both CD4+ and CD8+ T-cells. However, 8 cases of acute GvHD in the 18 patients treated and 3 cases of chronic extensive GvHD [99] that posed relevant safety issues were reported. IFN-gamma selection technique was also used in ATCT for clinically relevant adenovirus infection in allogeneic HSCT patients, with promising results [104]. Two randomized studies, recently completed, exploited direct selection of VSTCs through the streptamer and IFN-gamma CMV-CTL selection methods in T-cell depleted HSCT from CMV-seropositive donors: the IMPACT (NCT01077908) and the ASPECT trials (NCT01220895). The first is a multicenter, prospective, controlled, open-label phase III study of CMV-prophylaxis in T cell depleted HSCT from sibling donors with VSTCs selected by both multimer and IFN-gamma selection. T-cells were administered on day 27 post transplant and primary objectives of the study were the number of CMV reactivation episodes and GvHD incidence. The ASPECT trial is a randomized, multicenter open label phase II study of pre-emptive adoptive CMV cellular therapy where T cells were collected by multimer selection technique in recipients of unrelated donor transplants. In this study, the VSTCs selection occurred during the stem cell collection procedures. Primary end point was to establish the efficacy of pre-emptive VSTCs with CMV-specific T cells and their post infusion *in vivo* expansion. Results of both studies are expected early in 2016.

3.3 Recent developments: Third-party VSTCs and naïve donors T-cells.

The ability to isolate and expand VSTCs from seronegative donors or cord blood units may represent a clinical issue. Potential solution in this scenario is the so called "third party bio-banks" of VSTCs where they can be selected by HLA haplotypes [105]. The first multicenter trial of ATCT with cells obtained from banked third-party VSTCs for the treatment of refractory viral infections after HSCT was reported by Leen et al. in 2013 [106]. The Authors developed a bio-bank of 32 virus-specific lines from volunteers with common HLA polymorphisms against Epstein-Barr virus, CMV, or adenovirus. Eighteen lines were administered to 50 HSCT patients with refractory viral disease. Cumulative incidence of complete or partial responses was overall 74%: 73.9% for CMV (n = 23), 77.8% for adenovirus (n = 18) and 66.7% for EBV (n = 9). Only four responders had recurrence or progression. GvHD developed in eight patients (two of them showed *de novo* GvHD, and in only one of grade 3) confirming previous observations [107, 108]. Most interestingly, the VSTCs used in this study were "off-the-shelf" and only partially HLA-matched. If the low rate of GvHD is confirmed in further studies, this treatment option will have the potential to be widely employed given its rapid action and immediate efficacy. Nevertheless, it is important to point out that the results of this trial must be validated also in more compromised patients with active acute GvHD; or treated with T-cell–depleting monoclonal agents such as anti-thymocyte globulin or alemtuzumab, or more than 0.5 mg/kg/day of prednisone. T-cells restricted by specific HLA alleles exhibited different clinical activity and certain HLA alleles were more capable of inducing clearance of CMV disease/infection as recently reported by O'Reilly et al. [109]. Notably, the same group also proposed the use of artificial antigen-presenting cells (AAPCs) to more easily generate VSTCs for the treatment of infections in HSCT recipients. AAPCs consist of genetically modified murine cells, expressing human molecules required for T-cell stimulation such as ICAM-1 and LFA-3. VSTCs sensitized with AAPCs not only recognize well known immunogenic HLA epitopes but also subdominant epitopes, generally not recognized by autologous APCs [109]. There are two interesting phase II studies currently recruiting - one conducted by Prockop and colleagues at Memorial Sloan Kettering Cancer Center, the other by Betul and colleagues at MD Anderson Cancer Center (NCT02136797 and

NCT02210078) - on the role of third party donor derived T-cells in CMV disease/infection in allogeneic HSCT recipients. These studies are expected to be completed in 2017- 2018. T-cell products able to recognize unusual epitopes of different pathogens would potentially be a valid tool to avoid immune escape when donor and recipient T-cells are not fully HLA matched. The naïve donor T-cell compartment may be of interest in this setting given its propensity to generate a broad spectrum of immune control over several pathogens [110]. In 2011, a study made by Jedema first described a method of *in vitro* generation of antigen-specific CD8+ T cells obtained from a naïve T-cell donor repertoire. This method implied the depletion of CD45R0+ T cells that resulted in increased antigen-specific naïve T cells, but its poor reproducibility hampered its wide application [111]. In 2012 Hanley et al. demonstrated that multivirus-specific T-cells (against Adenovirus, EBV and CMV) from naïve T-cell populations, from both cord blood and peripheral blood of seronegative donors, are protective *in vivo* despite their unusual atypical epitope repertoire. VSTCs, mostly CD8+, were obtained with the use of a protocol based on both professional antigen presenting cells and activating Th1-polarizing cytokines [112, 113]. A recently published phase I feasibility study demonstrated the feasibility of generating CMV pp65-specific T-cells from CMV-negative individuals and cord blood units, and the ability of naïve-origin T-cells to recognize atypical epitopes of pp65. Given its phase I design, definitive conclusions cannot be drawn. The preliminary results are however encouraging and should be confirmed in larger studies [114]. Preliminary results of the currently ongoing clinical trial MUSTAT (Multivirus-Specific Cytotoxic T-Lymphocytes for the Prophylaxis and Treatment of EBV, CMV, and Adenovirus Infections post Allogeneic Stem Cell Transplant; NCT01945814) that compares clinical efficacy of CTLs derived from CMV-seropositive versus CMV-naïve donors are eagerly awaited.

4. CMV vaccines

Vaccine-induced immunity with safe and immunogenic compounds represents a feasible way to reduce the rate of CMV reactivation/disease in high risk patients. However, the precarious state of immuno-

competence of HSCT recipients with altered lymphocyte repertoires and antigen-presenting cells remains a barrier that hampers the efficacy of this strategy [115]. It is clear that robust protection against CMV relies on both cellular and humoral immunity and the ideal vaccine should be able to elicit a strong stimulation of both the adaptive and the natural immunity compartments [116]. Interesting clinical experiences came from two studies. In a randomized, placebo-controlled phase II clinical trial on SOT - 140 kidney or liver transplant patients - a subunit vaccine made up of purified glycoprotein B protein coupled with MF59 adjuvant led to a significantly shorter duration of viraemia, defined as viral loads higher than 200 genome/mL of blood, and a shorter duration of anti-viral therapy as compared with the placebo group. A strong antibody activity was seen without, however, a T cell-immunity involvement that determined a short durability of the immune response [117]. A second study employed a vectored vaccine, also known as ASP0013 or TransVax, with plasmids encoding CMV glycoprotein B and phosphoprotein pp65. This vaccine is employed in the only approved phase III study of vaccine-immunotherapy against CMV in the setting of hematopoietic stem cell transplantation. This trial is currently in progress and its completion is expected in 2017. A previous phase II, multicenter, randomized, placebo-controlled, double-blind study, was developed to test efficacy, safety and immunogenicity of ASP0113. Patients enrolled in the study were CMV-seropositive recipients of a myeloablative or a reduced-intensity HSCT for hematological malignancies. They were randomly assigned with a 1:1 ratio to receive CMV therapeutic vaccine (n=48) or placebo (n=46) on a four times schedule injections – the first before, the other three after the transplant. The vaccine was safe and only one patient discontinued it because of a minor allergic reaction. Though the primary endpoint of the study, the reduction of CMV viraemia requiring CMV-specific antiviral therapy, was not reached, there was a reduction in CMV viraemia episodes, defined as CMV copies >500/mL in the blood, in the vaccine arm. The immunogenicity analysis showed a statistically not significant increased rate of pp65, IFN-gamma producing T-cells in the vaccine cohort without a clear involvement of the B-cell compartment [118, 119]. An interesting, currently on going, multicenter phase II randomized, double-blind, placebo-controlled study in HSCT recipients has been designed to evaluate efficacy of another vectored vaccine, PepVax, containing plasmids encoding for pp65 combined with a toll-like receptor 9-agonist (NCT02396134). Primary endpoint of the study is the incidence rate of CMV reactivation/infection or CMV disease up to day 100 after HSCT.

The estimated completion date is 2019. Further progress in the scientific knowledge on CMV cell cycle and its biology currently offers potential novel approaches in vaccine-immunotherapy. CMV enters the host cells through two different pathways: CMV enters fibroblast by employing glycoproteins gB and gH/gL, whereas to enter epithelial and endothelial cells an additional five-member protein complex, composed of gH, gL, UL128, UL130, and UL131A, referred to as the gH/gL-pentamer complex, is required. Neutralizing antibodies that prevent gH/gL-pentamer complex mediated CMV entry into epithelial cells (AbNEIs) are putative candidates for an *in vivo* protective role against CMV infection [120, 121]. Recently, Gimenez et al. addressed the potential role of CMV-specific AbNEIs in CMV infection control in allogeneic HSCT patients by using a neutralization assay. The results did not confirm the protective role of AbNEIs both in prevention and clearance of CMV-DNAemia. AbNEIs levels did not correlate with CMV-DNAemia nor with viral load kinetics. The observation that patients with high baseline and peak AbNEIs levels were more likely to develop CMV-DNAemia was of interest. Possible explanations include a major role played by memory B cells of donor and recipient origins [122]. The gH/gL-pentamer complex represents the platform for prophylactic Pentamer-based vaccines of absolute interest [123, 124].

5. Expert commentary

The development of new tools against CMV infection/disease in HSCT recipients has been very active in recent years. Clinical trials now in progress have been designed to define the role of new antiviral compounds and to replace the currently broadly employed antiviral drugs associated with important toxicities and growing inefficacy due to mechanisms of drug-resistance. New anti-viral drugs such as BDF and LMV showed promising results in phase II studies and are being evaluated in phase III clinical trials. ATCT may play a role in the future because of its promising mechanism of action, able to sunder specific antiviral T-cell response from significant alloreactivity. Different techniques for isolation and expansion of CMV-specific T-cells are emerging as the knowledge on immune-reconstitution post allogeneic HSCT and CMV biology is expanding. The ability to determine and monitor CMV-CTL levels in the blood of HSCT

recipients would be a useful tool to identify patients with poor CMV-specific immune reconstitution at higher risk of developing CMV infection/reactivation and overt disease [125, 126]. In selected patients such as recipients of T-cell depleted grafts, ATCT, based on CMV-CTL reconstitution kinetics, would be of value in the prophylactic and/or preemptive setting. Third party and pathogen-naïve donor CTLs could represent an alternative option for patients who undergo cord blood transplants or receive grafts from CMV-seronegative donor [127]. Vaccine immunotherapy may soon become a clinical reality although concerns about its real efficacy in highly immuno-compromised patients such as HSCT recipients raise legitimate doubts.

6. Five-year view

A future paradigm may include first line antiviral therapy with one of the current investigated agents (LMV, BDF or MBV) and, in case of treatment failure and/or progressive CMV disease, ATCT may play a role as salvage strategy. ATCT may allow shorter courses of antiviral drug prophylaxis to stimulate cellular immune response. The creation of "bio-banks" with HLA-typed T-cells from peripheral blood of healthy donors specifically "armed" against different viruses that could readily be used in HSCT recipients without seropositive donors appear a promising approach. The use of VSTCs in HSCT patients with GvHD is currently under investigation with some promising reports. Menger recently elaborated a proof of concept study [128] based upon the genetic disruption of steroid cellular receptor in streptamer-selected CMV-specific CD8+ T cells, adopting the technique of transcription activator-like effectors nuclease (TALEN) messenger RNA, through electropermeabilization. The modified VSTCs showed resistance to steroid-induced apoptosis whereas they preserved their CMV-specific killing ability. However, there are concerns about the possibility of conferring steroid-resistance to CD8+ T-cells responsible of GvHD. The possibility of inserting suicide genes capable to interrupt VSTCs activity by inducing apoptosis has not been explored. In another recently published, phase I study in the setting of haploidentical HSCT, Zhou X et al. [129] employed haploidentical T-cells with inducible human caspase 9 (iC9) suicide gene to delete the alloreactive T-cells responsible of

GvHD, sparing the virus-reactive T cells even during GvHD treatment. The authors demonstrated that alloreplete iC9-T cells provide protection against EBV, CMV, human herpesvirus-6, Varicella zoster virus and BKV infections, with a possible role played by the recovery of endogenous T cells. This hallmark study may highly contribute to the future management of viral infections/disease in HSCT patients with life-threatening GvHD.

7. Key Issues

- The use of current antiviral drugs for CMV is hampered by potentially severe side effects and by the growing issue of drug-resistance.

- New antiviral compounds, currently under investigation, appear promising for their efficacy against CMV-resistant strains and for their low toxicity profile both in the prophylactic/pre-emptive and therapeutic settings.

- In the next future, ATCT, possibly combined with shorter courses of new antiviral compounds in the prophylactic/pre-emptive setting will be likely to play an important role especially in high-risk patients.

- Third-party virus-specific T-cells, possibly with the creation of VSTCs "bio-banks", will be crucial for the treatment of overt CMV disease in critically ill patients.

- CMV-vaccine strategies may become key factors to reduce incidence rate of CMV reactivation/infection in immuno-compromised patients such as HSCT recipients.

Bibliography

Papers of special note have been highlighted as

either of interest (*) or of considerable interest

(**) to readers.

1. Balfour H. Cytomegalovirus The Troll of Transplantation. Arch Intern Med. 1979;139(3):279-280

2. Pollack M, Heugel J, Xie H, Leisenring W, Storek J, Young JA, Kukreja M, Gress R, Tomblyn M, Boeckh M. An international comparison of current strategies to prevent herpesvirus and fungal infections in hematopoietic cell transplant recipients. Biol Blood Marrow Transplant. 2011;17:664-673

3. Boeckh M. Complications, diagnosis, management, and prevention of CMV infections: current and future. Hematology Am Soc Hematol Educ Program. 2011;2011:305-309

4. Buyck HC, Griffiths PD, Emery VC. Human cytomegalovirus (HCMV) replication kinetics in stem cell transplant recipients following anti-HCMV therapy. J Clin Virol. 2010;49(1):32-36

5. Schmidt-Hieber M, Schwarck S, Stroux A, Ganepola S, Reinke P, Thiel E, Uharek L, Blau IW. Immune reconstitution and cytomegalovirus infection after allogeneic stem cell transplantation: the important impact of in vivo T cell depletion. Int J Hematol. 2010;91(5):877-885

6. Hambach L, Stadler M, Dammann E, Ganser A, Hertenstein B. Increased risk of complicated CMV infection with the use of mycophenolate mofetil in allogeneic stem cell transplantation. Bone Marrow Transplant. 2002;29(11):903-906

7. Mead AJ, Thomson KJ, Morris EC, Mohamedbhai S, Denovan S, Orti G, Fielding AK, Kottaridis PD, Hough R, Chakraverty R, Linch DC, Mackinnon S, Peggs KS. HLA-mismatched unrelated donors are a viable alternate graft source for allogeneic transplantation following alemtuzumab-based reduced-intensity conditioning. Blood. 2010;24;115(25):5147-5153

8. Lilleri D, Gerna G, Zelini P, Chiesa A, Rognoni V, Mastronuzzi A, Giorgiani G, Zecca M, Locatelli F. Monitoring of human cytomegalovirus and virus-specific T-cell response in young patients receiving allogeneic hematopoietic stem cell transplantation. PLoS One. 2012;7(7):e41648

9. Ciáurriz M, Zabalza A, Beloki L, Mansilla C, Pérez-Valderrama E, Lachén M, Bandrés E, Olavarría E, Ramírez N. The immune response to cytomegalovirus in allogeneic hematopoietic stem cell transplant recipients. Cell Mol Life Sci. 2015;72(21):4049-4062

10. Cohen L, Yeshurun M, Shpilberg O, Ram R. Risk factors and prognostic scale for cytomegalovirus (CMV) infection in CMV-seropositive patients after allogeneic hematopoietic cell transplantation. Transpl Infect Dis. 2015;17(4):510-517

11. Piñana JL, Martino R, Barba P, Margall N, Roig MC, Valcárcel D, Sierra J, Rabella N. Cytomegalovirus infection and disease after reduced intensity conditioning allogeneic stem cell transplantation: single-centre experience. Bone Marrow Transplant. 2010;45(3):534-542

12. Guerrero A, Riddell SR, Storek J, Stevens-Ayers T, Storer B, Zaia JA, Forman S, Negrin RS, Chauncey T, Bensinger W, Boeckh M. Cytomegalovirus viral load and virus-specific immune reconstitution after peripheral blood stem cell versus bone marrow transplantation. Biol Blood Marrow Transplant. 2012;18(1):66-75

13. Chen Y, Xu LP, Liu KY, Chen H, Chen YH, Zhang XH, Wang Y, Wang FR, Han W, Wang JZ, Yan CH, Huang XJ. Risk factors for cytomegalovirus DNAemia following haploidentical stem cell transplantation and its association with host hepatitis B virus serostatus. J Clin Virol. 2016;75:10-15

14. Emery V, Zuckerman M, Jackson G, et al. Management of cytomegalovirus infection in haemopoietic stem cell transplantation. Br J Haematol. 2013;162:25-39

15. Sellar RS, Peggs KS. Management of multidrug-resistant viruses in the immunocompromised host. Br J Haematol. 2012;156:559-572 * clinical recommendations about, among the others, CMV-multidrug resistant, especially in HSCT setting.

16. Griffiths P, Lumley S. Cytomegalovirus. Curr Opin Infect Dis. 2014;27(6):554-559

17. Sellar RS, Peggs KS. Therapeutic strategies for cytomegalovirus infection in haematopoietic transplant recipients: a focused update. Expert Opin Biol Ther. 2014;14(8):1121-1126

18. Rooney C, Leen A. Moving successful virus-specific t-cell therapy for hematopoietic stem cell recipients to late phase clinical trials. Mol Ther Nucleic Acids. 2012;1:e55

19. Griffiths P, Plotkin S, Mocarski E, Pass R, Schleiss M, Krause P, Bialek S. Desirability and feasibility of a vaccine against cytomegalovirus. Vaccine. 2013;18;31 Suppl 2:B197-203 * a glance to CMV vaccine-immunotherapy.

20. Ljungman P, de la Camara R, Cordonnier C, Einsele H, Engelhard D, Reusser P, Styczynski J, Ward K. Management of CMV, HHV-6, HHV-7 and Kaposi-sarcoma herpesvirus (HHV-8) infections in patients with hematological malignancies and after SCT. Bone Marrow Transplant. 2008;42(4):227-240

21. Venton G, Crocchiolo R, Fürst S, Granata A, Oudin C, Faucher C, Coso D, Bouabdallah R, Berger P, Vey N, Ladaique P, Chabannon C, le Merlin M, Blaise D,El-Cheikh J. Risk factors of Ganciclovir-related neutropenia after allogeneic stem cell transplantation: a retrospective monocentre study on 547 patients. Clin Microbiol Infect. 2014;20(2):160-166

22. Kim ST, Lee MH, Kim SY, Kim SJ, Kim DH, Jang JH, Kim K, Kim WS, Jung CW. A randomized trial of preemptive therapy for prevention of cytomegalovirus disease after allogeneic hematopoietic stem cell transplantation. Int J Hematol. 2010;91:886–891

23. Park SY, Lee SO, Choi SH, Kim YS, Woo JH, Baek S, Sung H, Kim MN, Kim DY, Lee JH, Lee KH and Kim SH. Efficacy and safety of low-dose ganciclovir preemptive therapy in allogeneic haematopoietic stem cell transplant recipients compared with conventional-dose ganciclovir: a prospective observational study. J. Antimicrob. Chemother. 2012;67(6):1486-1492

24. Erard V, Guthrie KA, Seo S, Smith J, Huang M, Chien J, Flowers ME, Corey L, Boeckh M. Reduced Mortality of Cytomegalovirus Pneumonia After Hematopoietic Cell Transplantation Due to Antiviral Therapy and Changes in Transplantation Practices. Clin Infect Dis. 2015;61(1):31-39 * *elegant overview about recent progress in the management of CMV-related pneumonia.*

25. Gracia-Ahufinger I, Gutiérrez-Aroca J, Cordero E, Vidal E, Cantisán S, del Castillo D, Martín-Gandul C, Rivero A, Torre-Cisneros J. Use of high-dose ganciclovir for the treatment of cytomegalovirus replication in solid organ transplant patients with ganciclovir resistance-inducing mutations. Transplantation. 2013;95(8):1015-1020

26. Stuehler C, Stüssi G, Halter J, Nowakowska J, Schibli A, Battegay M, Dirks J, Passweg J, Heim D, Rovo A, Kalberer C, Bucher C, Weisser M, Dumoulin A, Hirsch HH, Khanna N. Combination therapy for multidrug-resistant cytomegalovirus disease. Transpl Infect Dis. 2015;17(5):751-755

27. Posadas Salas MA, Taber DJ, Chua E, Pilch N, Chavin K, Thomas B. Critical analysis of valganciclovir dosing and renal function on the development of cytomegalovirus infection in kidney transplantation. Transpl Infect Dis. 2013;15(6):551-558

28. Winter M, Guhr K, Berg G. Impact of various body weights and serum creatinine concentrations on the bias and accuracy of the Cockcroft-Gault equation. Pharmacotherapy. 2012;32(7):604–612

29. Chawla JS, Ghobadi A, Mosley J 3rd, Verkuyse L, Trinkaus K, Abboud CN, Cashen AF, Stockerl-Goldstein KE, Uy GL, Westervelt P, DiPersio JF, Vii R. Oral Valganciclovir versus ganciclovir as delayed pre-emptive therapy for patients after allogeneic hematopoietic stem cell transplant: a pilot trial (04-0274) and review of the literature. Transpl Infect Dis. 2012;14(3):259-267

30. Barkam C, Kamal H, Dammann E, Diedrich H, Bucholz S, Eder M, Krauter J, Ganser A, Stadler M. Improving safety of pre-emptive therapy with oral valganciclovir for cytomegalovirus infection after allogeneic hematopoietic stem cell transplantation. Bone Marrow Res. 2012;2012:ID874601

31. Ruiz-Camps I, Len O, de la Cámara R, Gurguí M, Martino R, Jarque I, Barrenetxea C, Díaz de Heredia C, Batlle M, Rovira M, de la Torre J, Torres A, Aguilar M, Espigado I, Martín-Dávila P, Bou G, Borrell N, Aguado JM, Pahissa A. Valganciclovir as pre-emptive therapy for cytomegalovirus infection in allogeneic haematopoietic stem cell transplant recipients. Antivir Ther. 2011;16(7):951-957

32. Kaynar L, Metan G, Gökahmetoğlu S, Kurnaz F, Mumcuoğlu H, Öztürk A, Şıvgın S, Pala C, Yıldız O, Eser B, Ünal A, Çetin M. Can low-dose preemptive valganciclovir replace standard intravenous ganciclovir treatment in recipients of allogeneic stem cell transplantation? J Chemother. 2013;25(5):286-291

33. Takahata M, Hashino S, Nishio M, Sugita J, Shigematsu A, Onozawa M, Fujimoto K, Endo T, Kondo T, Tanaka J, Imamura M, Teshima T. Occurrence of adverse events caused by valganciclovir as preemptive therapy for cytomegalovirus infection after allogeneic stem cell transplantation is reduced by low-dose administration. Transpl Infect Dis. 2015;17(6):810-815

34. Bacigalupo A, Boyd A, Slipper J, Curtis J, Clissold S. Foscarnet in the management of cytomegalovirus infections in hematopoietic stem cell transplant patients. Expert Rev Anti Infect Ther. 2012;10(11):1249-1264

35. Ishiyama K, Katagiri T, Ohata K, Hosokawa K, Kondo Y, Yamazaki H, Takami A, Nakao S. Safety of pre-engraftment prophylactic foscarnet administration after allogeneic stem cell transplantation. Transpl Infect Dis. 2012;14(1):33-39

36. Asakura M, Ikegame K, Yoshihara S, Taniguchi S, Mori T, Etoh T, Takami A, Yoshida T, Fukuda T, Hatanaka K, Kanamori H, Yujiri T, Atsuta Y, Sakamaki H,Suzuki R, Ogawa H. Use of foscarnet for cytomegalovirus infection after allogeneic hematopoietic stem cell transplantation from a related donor. Int J Hematol. 2010;92(2):351-359

37. Reusser P, Einsele H, Lee J, Volin L, Rovira M, Engelhard D, Finke J, Cordonnier C, Link H, Ljungman, P. Randomized multicenter trial of foscarnet versus ganciclovir for preemptive therapy of cytomegalovirus infection after allogeneic stem cell transplantation. Blood. 2002;99:1159–1164

38. Gregg K, Hakki M, Kaul DR. UL54 foscarnet mutation in an hematopoietic stem cell transplant recipient with cytomegalovirus disease. Transpl Infect disease. 2014;16(2):320-323

39. Ljungman P, Deliliers GL, Platzbecker U, Matthes-Martin S, Bacigalupo A, Einsele H, et al. Cidofovir for cytomegalovirus infection and disease in allogeneic stem cell transplant recipients. The Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. Blood. 2001;15;97(2):388-392

40. Caruso Brown AE, Cohen MN, Tong S, Braverman RS, Rooney JF, Giller R, Levin MJ. Pharmacokinetics and safety of intravenous cidofovir for life-threatening viral infections in pediatric hematopoietic stem cell transplant recipients. Antimicrob Agents Chemother. 2015;59(7):3718-3725 * *a new spotlight for CDF in HSCT theater.*

41. Lugthart G, Oomen MA, Jol-van der Zijde CM, Ball LM, Bresters D, Kollen WJ, Smiers FJ, Vermont CL, Bredius RG, Schilham MW, van Tol MJ, Lankester AC. The effect of cidofovir on adenovirus plasma

DNA levels in stem cell transplantation recipients without T cell reconstitution. Biol Blood Marrow Transplant. 2015;21(2):293-299

42. Kwon HJ, Kang JH, Lee JW, Chung NG, Kim HK, Cho B. Treatment of BK virus-associated hemorrhagic cystitis in pediatric hematopoietic stem celltransplant recipients with cidofovir: a single-center experience. Transpl Infect Dis. 2013;15(6):569-574

43. Rascon J, Verkauskas G, Pasauliene R, Zubka V, Bilius V, Rageliene L. Intravesical cidofovir to treat BK virus-associated hemorrhagic cystitis in children afterhematopoietic stem cell transplantation. Pediatr Transplant. 2015;19(4):E111-4

44. Andrei G, Snoeck R. Cidofovir Activity against Poxvirus Infections. Viruses. 2010;2(12):2803-2830

45. Gohring K, Hamprecht K, Jahn G. Antiviral drug- and Multidrug Resistance in Cytomegalovirus Infected SCT patients. Comput Struct Biotechnol J. 2015;13:153-159

46. Hakki M, Chou S. The biology of cytomegalovirus drug resistance. Curr Opin Infect Dis. 2011;24(6):605-611

47. Komatsu TE, Pikis A, Naeger LK, Harrington PR. Resistance of human cytomegalovirus to ganciclovir/valganciclovir: a comprehensive review of putative resistance pathways. Antiviral Res. 2014;101:12-25.

48. Sahoo MK, Lefterova MI, Yamamoto F, Waggoner JJ, Chou S, Holmes SP, Anderson MW, Pinsky BA. Detection of cytomegalovirus drug resistance mutations by next-generation sequencing. J Clin Microbiol. 2013;51(11):3700-3710.

49. Göhring K, Wolf D, Bethge W, Mikeler E, Faul C, Vogel W, Vöhringer MC, Jahn G, Hamprecht K. Dynamics of coexisting HCMV-UL97 and UL54 drug-resistance associated mutations in patients after haematopoietic cell transplantation. J Clin Virol. 2013;57(1):43-49

50. Schnepf N, Dhédin N, Mercier-Delarue S, Andreoli A, Mamez AC, Ferry C, Deback C, Ribaud P, Robin M, Socié G, Simon F, Mazeron MC. Dynamics of cytomegalovirus populations harbouring mutations in genes UL54 and UL97 in a haematopoietic stem cell transplant recipient. J Clin Virol. 2013;58(4):733-736

51. Hanson KE, Swaminathan S. Cytomegalovirus antiviral drug resistance: future prospects for prevention, detection and management. Future Microbiol. 2015;10:1545-1554

52. Lu CH, Tsai JH, Wu MZ, Yu CL, Hsieh SC. Can leflunomide play a role in cytomegalovirus . disease prophylaxis besides its antirheumatic effects? Antivir Ther. 2015;20(1):93-96 * intriguing potential new tool against CMV.

53. Avery RK, Mossad SB, Poggio E, Lard M, Budev M, Bolwell B, Waldman WJ, Braun W, Mawhorter SD, Fatica R, Krishnamurthi V, Young JB, Shrestha R,Stephany B, Lurain N, Yen-Lieberman B. Utility of leflunomide in the treatment of complex cytomegalovirus syndromes. Transplantation. 2010;90(4):419-426

54. Chacko B, John GT. Leflunomide for cytomegalovirus: bench to bedside. Transpl Infect Dis. 2012;14(2):111-120

55. Roy S, He R, Kapoor A, Forman M, Mazzone JR, Posner GH, Arav-Boger R. Inhibition of human cytomegalovirus replication by artemisinins: effects mediated through cell cycle modulation. Antimicrob Agents Chemother. 2015;59(7):3870-3879

56. Wolf DG, Shimoni A, Resnick IB, Stamminger T, Neumann AU, Chou S, Efferth T, Caplan O, Rose J, Nagler A, Marschall M. Human cytomegalovirus kinetics following institution of artesunate after hematopoietic stem cell transplantation. Antiviral Res. 2011;90:183-186

57. Germi R, Mariette C, Alain S, Lupo J, Thiebaut A, Brion JP, Epaulard O, Saint Raymond C, Malvezzi P, Morand P. Success and failure of artesunate treatment in five transplant recipients with disease caused by drug-resistant cytomegalovirus. Antiviral Res. 2014;101:57-61.

58. Arav-Boger R, He R, Chiou CJ, Liu J, Woodard L, Rosenthal A, Jones-Brando L, Forman M, Posner G. Artemisinin-derived dimers have greatly improved anti-cytomegalovirus activity compared to artemisinin monomers. PLoS One. 2010;28;5(4):e10370

59. Lai HC, Singh NP, Sasaki T. Development of artemisinin compounds for cancer treatment. Invest New Drugs. 2013;31(1):230-246 * *novel insights into artemisinines and their putative role against cancer*.

60. Winston DJ, Young JA, Pullarkat V, Papanicolau GA, Vij R, Vance E, Alangaden, Chemaly RF, Petersen F, Chao N, Klein J, Sprague K, Villano SA, Boeckh M. Maribavir prophylaxis for prevention of cytomegalovirus infection in allogeneic stem-cell transplanted recipients: a multicenter randomized, double-blind, placebo-controlled, dose.ranging study. Blood. 2008;111(11):5403-5410

61. Chemaly RF, Ullmann A, Stoelben S, Richard MP, Bornhäuser M, Groth C, Einsele H, Silverman M, Mullane K, Brown J, Nowak H, Kölling K, Stobernack HP, Lischka P, Zimmermann H, Rübsamen-Schaeff H, Champlin RE and Ehninger G, for the AIC246 Study Team. Letermovir for Cytomegalovirus Prophylaxis in Hematopoietic-Cell Transplantation. N Engl J Med. 2014;370:1781-1789

62. Marty FM, Winston DJ, Rowley SD, Vance E, Papanicolaou GA, Mullane KM,. Brundage TM, Robertson AT, Godkin S, Momméja-Marin H, Boeckh M, for the CMX001-201 Clinical Study Group. CMX001 to prevent cytomegalovirus disease in hematopoietic cell transplantation. N Engl J Med. 2013;369(13):1227-1236

63. Marty FM, Ljungman P, Papanicolau GA, Winston DJ, Chemaly RF, Strasfeld L, Young JA, Rodriguez T, Maertens J, Schmitt M, Einsele H, Ferrant A, Lipton JH, Villano SA, Chen H, Boeckh M. Maribavir prophylaxis for prevention of cytomegalovirus disease in recipients of allogeneic stem-cell transplant: a phase 3, double blind, placebo controlled, randomized trial. Lancet Infect Dis. 2011;11(4):284-292

64. Marty FM, Boeckh M. Maribavir and human cytomegalovirus- what happened in the clinical trial and why might the drug have failed? Curr Opin Virol. 2011;1(6):555-562

65. Griffiths PD. Of London buses and the treatment of cytomegalovirus infection. Rev Med Virol. 2014;24:221-222 * *mordacious and extremely effective opinion.*

66. Winston DJ, Salibab F, Blumberg E, Abouljoud M, Garcia-Diaze JB, Gossf JA, Clough L, Avery R, Limaye AP, Ericzon BG, Navasa M, Troisi RI, Chen H, Villano SA and Uknis ME. Efficacy and Safety of Maribavir Dosed at 100 mg Orally Twice Daily for the Prevention of Cytomegalovirus Disease in Liver Transplant Recipients: A Randomized, Double-Blind, Multicenter Controlled Trial. American Journal of Transplantation. 2012;12:3021–3030

67. Alain S, Revest M, Veyer D, Essig M, Rerolles JP, Rawlinson W, et al. Maribavir use in practice for cytomegalovirus infection in French transplantation centers. Transplantation Proceedings. 2013;45:1603-1607 * *a work that contributes to requalify MBV in clinical practice.*

68. Schubert A, Elhert K, Schuler-Luettmann S, Gentner E, Mertens T, Michel D. Fast selection of maribavir resistant cytomegalovirus in a bone marrow transplant recipient. BMC Infect Dis. 2013;13:330

69. Webel R, Hakki M, Prichard MN, Rawlinson WD, Marschall M, Chou S. Differential properties of cytomegalovirus pUL97 kinase isoforms affect viral replication and maribavir susceptibility. J Virol. 2014;88(9):4776-4778

70. Lurain NS, Chou S. Antiviral drug resistance of human cytomegalovirus. Clin Microbiol Rev. 2010;23(4):689-712

71. Chou S, Ercolani RJ, Marousek G, Bowlin TL. Cytomegalovirus UL97 kinase catalytic domain mutations that confer multidrug resistance. Antimicrob Agents Chemoter. 2013;57(7):3375-3379

72. Griffiths PD, Emery VC. Taming the transplantation troll by targeting terminase. N Engl J Med. 2014;370(19):1844-1846 * *excellent explanation of CMV-terminase charcteristics.*

73. Borst EM, Kleine-Albers J, Gabaev I, Babic M, Wagner K, Binz A, Degenhardt I, Kalesse M, Jonjic S, Bauerfeind R, Messerle M. The human cytomegalovirus UL51 protein is essential for viral genome cleavage-packaging and interacts with the terminase subunits pUL56 and pUL89. J Virol. 2013;87(3):1720-1732 * *another fascinating overview of CMV-terminase biology.*

74. Goldner T, Hewlett G, Ettischer N, Ruebsamen-Schaeff H, Zimmermann H, Lischka P. The novel anticytomegalovirus compound AIC246 (Letermovir) inhibits human cytomegalovirus replication through a specific antiviral mechanism that involves the viral terminase. J Virol. 2011;85(20):10884-10893

75. Pilorgé L, Burrel S, Aït-Arkoub Z, Agut H, Boutolleau D. Human cytomegalovirus (CMV) susceptibility to currently approved antiviral drugs does not impact on CMV terminase complex polymorphism. Antiviral Res. 2014;111:8-12

76. Melendez DP, Razonable RR. Letermovir and inhibitors of the terminase complex: a promising new class of investigational antiviral drugs against human cytomegalovirus. Infect Drug Resist. 2015;5;8:269-277

77. Kaul DR, Stoelben S, Cober E, Ojo T, Sandusky E, Lischka P, Zimmermann H, Rubsamen-Schaeff H. First report of successful treatment of multidrug-resistant cytomegalovirus disease with the novel anti-CMV compound AIC246. Am J Transplant. 2011;11(5):1079-1084

78. Stoelben S, Arns W, Renders L, Hummel J, Mühlfeld A, Stangl M, Fischereder M, Gwinner W, Suwelack B, Witzke O, Dürr M, Beelen DW, Michel D, Lischka P, Zimmermann H, Rübsamen-Schaeff H, Budde K. Preemptive treatment of Cytomegalovirus infection in kidney transplant recipients with letermovir: results of a Phase 2a study. Transpl Int. 2014;27(1):77-86

79. Goldner T, Zimmermann H, Lischka P. Phenotypic characterization of two naturally occurring human Cytomegalovirus sequence polymorphisms located in a distinct region of ORF UL56 known to be involved in in vitro resistance to letermovir. Antiviral Res. 2015;116:48-50

80. Chou S. Rapid In Vitro Evolution of Human Cytomegalovirus UL56 Mutations That Confer Letermovir Resistance. Antimicrob Agents Chemother. 2015;59(10):6588-6593

81. Goldner T, Hempel C, Ruebsamen-Schaeff H, Zimmermann H, Lischka P. Geno –and phenotypic characteritazion of human cytomegalovirus mutants selected in vitro after letermovir (AIC246) exposure. Antimicrob Agents Chemother 2014;58(1):610-613

82. Painter W, Robertson A, Trost LC, Godkin S,Lampert B, Painter G. First pharmacokinetic and safety study in humans of the novel lipid antiviral conjugate CMX001, a broad-spectrum oral drug active against double-stranded DNA viruses. Antimicrob Agents Chemother. 2012;56:2726-2734

83. Quenelle DC, Lampert B, Collins DJ, Rice TL,Painter GR, Kern ER. Efficacy of CMX001 against herpes simplex virus infections in mice and correlations with drug distribution studies. J Infect Dis 2010;202:1492-1499

84. Florescu DF, Pergam SA, Neely MN, Qiu F, Johnston C, Way S, Sande J, Lewinsohn DA, Guzman-Cottrill JA, Graham ML, Papanicolaou G, Kurtzberg J, Rigdon J, Painter W, Mommeja-Marin H, Lanier R, Anderson M, van der Horst C. Safety and efficacy of CMX001 as salvage therapy for severe adenovirus infections in immunocompromised patients. Biol Blood Marrow Transplant. 2012;18(5):731-738

85. Papanicolaou GA, Lee YJ, Young JW, Seshan SV, Boruchov AM, Chittick G, Momméja-Marin H, Glezerman IG. Brincidofovir for polyomavirus-associated nephropathy after allogeneic hematopoietic stem cell transplantation. Am J Kidney Dis. 2015;65(5):780-784

86. Leen AM, Heslop HE, Brenner MK. Antiviral T-cell therapy. Immunol Rev. 2014;258(1):12-29

87. Lilleri D, Gerna G, Fornara C, Chiesa A, Comolli G, Zecca M, Locatelli F. Human cytomegalovirus-specific T cell reconstitution in young patients receiving T cell-depleted, allogeneic hematopoietic stem cell transplantation. Journal of Infectious Diseases. 2009;199(6)829–836

88. Federmann B, Hägele M, Pfeiffer M, Wirths S, Schumm M, Faul C, Vogel W, Handgretinger R, Kanz L, Bethge WA. Immune reconstitution after haploidentical hematopoietic cell transplantation: impact of

reduced intensity conditioning and CD3/CD19 depleted grafts. Leukemia. 2011;25(1),121–129

89. Bader P, Soerensen J, Jarisch A, Ponstingl E, Krenn T, Faber J, Durken M, Reinhardt H, Willasch A, Esser R, Bonig H, Koehl U, Klingebiel T. Rapid immune recovery and low TRM in haploidentical stem cell transplantation in children and adolescence using CD3/CD19-depleted stem cells. Best Practice and Research: Clinical Haematology. 2011;24(3)331–337

90. Chang YJ, Zhao XY, Huo MR, Xu LP, Liu DH, Liu KY, Huang XJ. Immune reconstitution following unmanipulated HLA-mismatched/haploidentical transplantation compared with HLA-identical sibling transplantation. Journal of Clinical Immunology, 2012;32(2)268–280

91. Azevedo RI, Soares MV, Albuquerque AV, Tendeiro R, Soares RS, Martins M, Ligeiro D, Victorino R, Lacerda JF, Sousa AF. Long-term immune reconstitution of naive and memory T cell pools after haploidentical hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2013;19(5)703–712

92. Kato R, Tamaki H, Ikegame K, Yoshihara S, Kaida K, Taniguchi K, Inoue T, Ishii S, Nakata J, Fujioka T, Eguchi R, Soma T, Okada M, Ogawa H. Early detection of cytomegalovirus-specific cytotoxic T lymphocytes against cytomegalovirus antigenemia in human leukocyte antigen haploidentical hematopoietic stem cell transplantation. Ann Hematol. 2015;94(10):1707-1715

93. Luo XH, Chang YJ, Huang XJ. Improving cytomegalovirus-specific T cell reconstitution after haploidentical stem cell transplantation. J Immunol Res. 2014;2014:631951

94. Luo XH, Huang XJ, Li D, Liu KY, Xu LP, Liu DH. Immune reconstitution to cytomegalovirus following partially matched-related donor transplantation: impact of in vivo T-cell depletion and granulocyte colony-stimulating factor-primed peripheral blood/bone marrow mixed grafts. Transpl Infect Dis. 2013;15(1):22-33

95. Koehne G, Hasa A, Doubrovina E, Prockop S, Tyler E, Wasilewski G, O'Reilly RJ. Immunotherapy with Donor T Cells Sensitized with Overlapping Pentadecapeptides for Treatment of Persistent Cytomegalovirus Infection or Viremia. Biol Blood Marrow Transplant. 2015;21:1663-1678

96. Blyth E, Clancy L, Simms R, Ma CK, Burgess J, Deo S, Byth K, Dubosq MC, Shaw PJ, Micklethwaite KP, Gottlieb DJ. Donor-derived CMV-specific T cells reduce the requirement for CMV-directed pharmacotherapy after allogeneic stem cell transplantation. Blood. 2013;121:3745–3758.

97. Gerdemann U, Keirnan JM, Katari UL, Yanagisawa R, Christin AS, Huye LE, Perna SK, Ennamuri S, Gottschalk S, Brenner MK, Heslop HE, Rooney CM, Leen AM. Rapidly generated multivirus-specific cytotoxic T lymphocytes for the prophylaxis and treatment of viral infections. Molecular therapy : the journal of the American Society of Gene Therapy. 2012;20:1622–1632

98. Feuchtinger T, Opherk K, Bethge WA, Topp MS, Schuster FR, Weissinger EM, Mohty M, Or R, Maschan M, Schumm M, Hamprecht K, Handgretinger R, Lang P, Einsele H. Adoptive transfer of pp65-specific T cells for the treatment of chemorefractory cytomegalovirus disease or reactivation after haploidentical and matched unrelated stem cell transplantation. Blood. 2010;18;116(20):4360-4367

99. Peggs KS, Thomson K, Samuel E, Dyer G, Armoogum J, Chakraverty R, Pang K, Mackinnon S, Lowdell MW. Directly selected cytomegalovirus-reactive donor T cells confer rapid and safe systemic reconstitution of virus-specific immunity following stem cell transplantation. Clin Infect Dis. 2011;52(1):49-57

100. Casalegno-Garduno R., Schmitt A., Yao J., Wang X., Xu X., Freund M., et al. Multimer technologies for detection and adoptive transfer of antigen-specific T cells. Cancer Immunol. Immunother. 2010;59:195–202

101. Uhlin M, Gertow J, Uzunel M, Okas M, Berglund S, Watz E, Brune E, Ljungman P, Maeurer M, JMattsson. Rapid Salvage Treatment With Virus-Specific T Cells for Therapy-Resistant Disease. Clinical Infectious Diseases. 2012;55(8):1064–1073

102. Cobbold M, Khan N, Pourgheysari B, Tauro S, McDonald D, Osman H, Assenmacher M, Billingham L, Steward C, Crawley C, Olavarria E, Goldman J,Chakraverty R, Mahendra P, Craddock C, Moss PA.

Adoptive transfer of cytomegalovirus-specific CTL to stem cell transplant patients after selection by HLA-peptide tetramers. J Exp Med. 2005;202(3):379-386

103. Schmitt A, Tonn T, Busch DH, Grigoleit GU, Einsele H, Odendahl M, Germeroth L, Ringhoffer M, Ringhoffer S, Wiesneth M, Greiner J, Michel D, Mertens T ,Rojewski M, Marx M, von Harsdorf S, Döhner H, Seifried E, Bunjes D, Schmitt M. Adoptive transfer and selective reconstitution of streptamer-selected cytomegalovirus-specific CD8+ T cells leads to virus clearance in patients after allogeneic peripheral blood stem cell transplantation. Transfusion. 2011;51(3):591-599

104. Qasim W, Gilmour K, Zhan H, Derniame S, McNicol AM, Ip W, Hirwarkar P, Veys P, Gaspar HB. Interferon-g capture T cell therapy for persistent adenoviraemia following allogeneic haematopoietic stem cell transplantation. Br J Haematol. 2013;161:449-452

105. Doubrovina E, Oflaz-Sozmen B, Prockop SE, Kernan NA, Abramson S, Teruya-Feldstein J, Hedvat C, Chou JF, Heller G, Barker JN, Boulad F, Castro-Malaspina H, George D, Jakubowski A, Koehne G, Papadopoulos EB, Scaradavou A, Small TN, Khalaf R, Young JW, O'Reilly RJ. Adoptive immunotherapy with unselected or EBV-specific T cells for biopsy-proven EBV+ lymphomas after allogeneic hematopoietic cell transplantation. Blood. 2012;119(11):2644–2656

106. Leen AM, Bollard CM, Mendizabal AM, Shpall EJ, Szabolcs P, Antin JH, Kapoor N, Pai SY, Rowley SD, Kebriaei P, Dey BR, Grilley BJ, Gee AP, Brenner MK, Rooney CM, Heslop HE Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. Blood. 2013;121(26):5113-5123

107. Melenhorst JJ, Leen AM, Bollard CM, Quigley MF, Price DA, Rooney CM, Brenner MK, Barrett AJ, Heslop HE. Allogeneic virus-specific T cells with HLA alloreactivity do not produce GVHD in human subjects. Blood. 2010;116(22):4700-4702

108. Melenhorst JJ, Castillo P, Hanley PJ, Keller MD, Krance RA, Margolin J, Leen AM, Heslop HE, Barret AJ, Rooney C and Bollard CM. Graft versus leukemia response without graft-versus-host disease elicited by adoptively transferred multivirus-specific T-cells. Molecular Therapy. 2015;23(1):179–183 * *very promising area of study in the adoptive T-cell therapy focusing on the GvL effect.*

109. Hasan AN, Prockop, SE, Koehne G, Doubrovina E, O'Reilly RJ. Banked, GMP Grade Third Party T-Cell Lines Specific for CMVpp65 Epitopes Presented By Certain Prevalent HLA Alleles More Consistently Clear CMV Infections in a Genetically Heterogeneous Population of HSCT Recipients. Blood. 2014;124(21):309.

110. Hinrichs CS, Borman ZA, Gattinoni L, Yu Z, Burns WR, Huang J, Klebanoff CA, Johnson LA, Kerkar SP, Yang S, Muranski P, Palmer DC, Scott CD, Morgan RA, Robbins PF, Rosenberg SA, Restifo NP. Human effector CD8+ T cells derived from naive rather than memory subsets possess superior traits for adoptive immunotherapy. Blood. 2011;117:808–814

111. Jedema I, van de Meent M, Pots J, Kester MG, van der Beek MT, Falkenburg JH. Successful generation of primary virus-specific and anti-tumor T-cell responses from the naive donor T-cell repertoire is determined by the balance between antigen-specific precursor T cells and regulatory T cells. Haematologica. 2011;96(8):1204-1212

112. Hanley PJ, Lam S, Shpall EJ, Bollard CM. Expanding cytotoxic T lymphocytes from umbilical cord blood that target cytomegalovirus, Epstein-Barr virus, and adenovirus. J Vis Exp. 2012;7(63):e362

113. Hanley PJ, Cruz CR, Shpall EJ, Bollard CM. Improving clinical outcomes using adoptively transferred immune cells from umbilical cord blood. Cytotherapy. 2010;12:713–720

114. Hanley PJ, Melenhorst JJ, Nikiforow S, Scheinberg P, Blaney JW, Demmler-Harrison G, Cruz CR, Lam S, Krance RA, Leung KS, Martinez CA, Liu H, Douek DC, Heslop HE, Rooney CM, Shpall EJ, Barrett AJ, Rodgers JR, Bollard CM. CMV-specific T cells generated from naïve T cells recognize atypical epitopes and may be protective in vivo. Sci Transl Med. 2015;7(285):285 * an overview to a new potential source of CMV-specific T-cells.

115. Six A, Bellier B, Thomas-Vaslin V, Klatzmann D. System biology in vaccine design. Microb Biothecnol. 2012;5(2):295-230

116. Lilja AE, Mason PW. The next generation recombinant human cytomegalovirus vaccine candidates- beyond gB. Vaccine. 2012;30(49):6980-6990

117. Griffiths PD, Stanton A, McCarrell E, Smith C, Osman M, Harber M, et al. Cytomegalovirus glycoprotein-B vaccine with MF59 adjuvant in transplant recipients: a phase 2 randomised placebo-controlled trial. Lancet. 2011;377:1256–1263

118. Kharfan-Dabaja MA, Boeckh M, Wilck MB, Langston AA, Chu AH, Wloch MK, Guterwill DF, Smith LR, Rolland AP, Kenney RT. A novel therapeutic cytomegalovirus DNA vaccine in allogeneic haemopoietic stem-cell transplantation: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Infect. Dis. 2012;12(4):290-9

119. Kharfan-Dabaja, M.A.; Boeckh, M.; Wilck, M.B.; Langston, A.A.; Chu, A.H.; Wloch, M.K.; Smith, L.R.; Rolland, A.P.; Kenney, R.T. Reanalysis of TransVax immunogenicity. Lancet Infect. Dis. 2013;13:18

120. Fouts AE, Chan P, Stephan JP, Vandlen R, Feierbach B. Antibodies against the gH/gL/UL128/UL130/UL131 complex comprise the majority of the anti- CMV neutralizing antibody response in CMV-HIG. J Virol. 2012;86:7444–7447

121. Lilleri D., Kabanova A., Lanzavecchia A., Gerna G. Antibodies against neutralization epitopes of human cytomegalovirus gH/gL/pUL128-130-131 complex and virus spreading may correlate with virus control in vivo. J Clin Immunol. 2012;32:1324–1331

122. Giménez E, Blanco-Lobo P, Muñoz-Cobo B, Solano C, Amat P, Pérez-Romero P, Navarro D. Role of cytomegalovirus (CMV)-specific polyfunctional CD8+ T-cells and antibodies neutralizing virus epithelial infection in the control of CMV infection in an allogeneic stem-cell transplantation setting. J Gen Virol. 2015;96(9):2822-2831 * novel insights into CMV-specific AbNEIs in CMV infection control in allogeneic HSCT.

123. Wussow, F., Chiuppesi, F., Martinez, J., Campo, J., Johnson, E., Flechsig, Diamond, D. J. Human Cytomegalovirus Vaccine Based on the Envelope gH/gL Pentamer Complex. PLoS Pathog. 2014;10(11):e1004524

124. Ciferri C, Chandramouli S, Leitner A, Donnarumma D, Cianfrocco MA, Gerrein R, Friedrich K, Aggarwal Y, Palladino G, Aebersold R, Norais N, Settembre EC, Carfi A. Antigenic Characterization of the HCMV gH/gL/gO and Pentamer Cell Entry Complexes Reveals Binding Sites for Potently Neutralizing Human Antibodies. PLoS Pathog. 2015;11(10)

125. Gratama JW, Boeckh M, Nakamura R, Cornelissen JJ, Brooimans RA, Zaia JA, Forman SJ, Gaal K, Bray KR, Gasior GH, Boyce CS, Sullivan LA, Southwick PC. Immune monitoring with iTAg MHC Tetramers for prediction of recurrent or persistent cytomegalovirus infection or disease in allogeneic hematopoietic stem cell transplant recipients: a prospective multicenter study. Blood. 2010;116(10):1655–1662

126. Borchers S, Luther S, Lips U, Hahn N, Kontsendorn J, Stadler M, Bucholz S, Diedrich H, Eder M, Koehl U, Ganser A, Mischak-Weissinger E. Tetramer monitoring to assess risk factors for recurrent cytomegalovirus reactivation and reconstitution of antiviral immunity post allogeneic hematopoietic stem cell transplantation. Transpl Infect Dis. 2011;13(3):222–236

127. Hanley PJ, Cruiz RY, Melenhorst J, Scheinberg P, Blaney J, Savoldo B, Dotti G, Heslop H, Rooney C, Shpall EJ, Barrett JA, Rodgers J, Bollard CM. Naïve T-cell-derived CTL recognize atypical epitopes of CMVpp65 with higher avidity than CMV-seropositive donor-derived CTL – a basis for treatment of post-transplant viral infection by adoptive transfer of T-cells from virus-naïve donors. Cytotherapy. 2013;15(4):S9.

128. Menger L, Gouble A, Marzolini M, Pachnio A, Bergerhoff K, Henry JY, Smith J, Pule M, Moss P, Riddell, Quezada S, Peggs K. TALEN-mediated genetic inactivation of the glucocorticoid receptor in cytomegalovirus-specific T cells. Blood. 2015;126(26):2781-2789 * *a proof of concept study raising the usefulness of CMV-specific T-cells in GVHD affected patients with viral infections/disease*.

129. Zhou X, Dotti G, Krance R, Martinez C, Naik S, Kamble R, Durett A, Dakhova O, Savoldo B, Di Stasi A, Spencer D, Lin YF, Liu H, Grilley B, Gee A, Rooney C, Heslop H, Brenner M. Inducible caspase-9 suicide gene controls adverse effects from alloreplete T cells after haploidentical stem cell transplantation. Blood. 2015;125(26):4103-4113

Appendix

2. Table I. Principal characteristics of the newer anti-CMV compounds.

Drug	Route of administration	Mechanism of action	Side efffects	Dosing	Main publications
Maribavir	Per os	Inhibition of CMV protein-kinase UL97	Gastrointestinal: dysgeusia and nausea, vomiting.	From 400 mg to 1200 mg twice daily	.Winston et al. Blood. 2008 (60) .Marty et al. Lancet Infect Dis. 2011 (63) .Winston et al. American Journal of transpl. (66) .clinicaltrialsgov: NCT01611974
Letermovir	Per os	Inhibition of terminase complex subunit UL56	Gastroenteritis, nasopharyngitis, dyspnea, and elevation in serum creatinine	120 mg or 240 mg once-daily	. Chemaly et al. NEJM. 2014 (61) .clinicaltrialsgov NCT02137772)
Brincidofovir	Per os	Inhibition of DNA polymerase	Gastrointestinal: diarrhea (dose limiting at 200 mg twice weekly)	100 mg twice weekly	. Marty et al. NEJM. 2013 (62) .clinicaltrialsgov NCT02137772

Figure 1.

Notes: A) Ex-vivo expansion. In the classic process antigen presenting cells (APC) are transduced with viral vectors or plasmids encoding antigens of interest. APCs are then combined with T cells to stimulate them until a sufficient number have been expanded. B) Direct selection techniques: multimer selection. In this case T cells are incubated with HLA multimers (tetramers, pentamers or, more recently, streptamers) that resembles the peptide binding HLA-mediated. The complex multimer-T cells is then isolated with magnetic beads or cell sorting. C) T cells are stimulated utilizing virus-derived overlapping peptides. Activated T cells secrete interferon gamma (IFN-gamma). Virus-specific T-cells are then immune-magnetically selected.