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REVIEW

Virus-specific T-cell therapy in solid organ transplantation

Andy Roemhild[1,2] & Petra Reinke[1,2]

1 Department of Nephrology and Internal Intensive Care, Charité Universitätsmedizin Berlin, Berlin, Germany
2 Berlin-Brandenburg Center for Regenerative Therapy (BCRT), Charité Universitätsmedizin Berlin, Berlin, Germany

Correspondence
Prof. Dr. Petra Reinke, Department of Nephrology and Internal Intensive Care and Berlin-Brandenburg Center for Regenerative Therapy (BCRT), Charite Universitätsmedizin Berlin, Augustenburger Platz 1, Berlin 13353, Germany.
Tel.: +49(30) 450653490;
fax: +49(30) 450553904;
e-mail: petra.reinke@charite.de

SUMMARY

This article reviews the current state of T-cell therapy as therapeutic option for virus-associated diseases against the background of the most common viral complications and their standard treatment regimens after SOT. The available data of clinical T-cell trials in SOT are summarized. References to the hematopoietic stem cell transplantation are made if applicable data in SOT are not available and their content was considered likewise valid for cell therapy in SOT. Moreover, aspects of different manufacturing approaches including beneficial product characteristics and the importance of GMP compliance are addressed.

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Introduction

Solid organ transplantation (SOT) is often the only appropriate therapeutic option for patients with an end-stage organ disease. A successful transplantation needs a life-long immunosuppressive medication to prevent graft rejection. Virus infections are especially challenging due to the compromised T-cell compartment. They can either occur as de novo infection, via transmission from the transplanted donor organ or as consequence of reactivation from established latent virus infections. Unfortunately, these events are frequent in immunocompromised patients after SOT and can lead to a severe disease progression. Consequently, despite graft rejection, viral infections are affecting the long-term graft survival and remain a leading cause of morbidity and mortality in this patient population [1]. Besides less frequently reported complications with varicella-zoster or polyomavirus for example, the group of Herpes and Adenoviruses are major player in this respect [2–4].

Virus-associated complications after SOT

EBV

The Epstein–Barr virus (EBV) is a double-stranded DNA virus which belongs to the group of gamma herpes viruses and is also known as human herpes virus 4. More than 95% of the people worldwide are infected [5]. Usually, initial infection occurs asymptomatically in early childhood, but it can likewise cause infectious mononucleosis if infection is delayed to adolescence [6]. Following primary infection, the virus establishes life-long latency in B lymphocytes that is characterized by the expression of different antigen patterns. According to these patterns, latency can be grouped in phases 1–3

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commonly interrupted by episodes of virus reactivation into the lytic phase. In healthy individuals, the lytic replication cycle is controlled by EBV-specific T lymphocytes of the immune system [7]. However, in transplant patients, the administration of potent immunosuppressive drugs is hampering EBV-directed immune surveillance. Thereby an imbalance of the adaptive immunity can lead to potentially life-threatening conditions like post-transplant lymphoproliferative disease (PTLD) and tumor formation [4,8]. PTLD is the second most common malignancy after skin cancer in organ transplantation and leads to death of 40–60% of affected SOT recipients [9]. The incidence of PTLD is varying depending on the transplanted organ. It ranges from 1 to 3% for renal, heart, or liver and 7–33% for lung, intestine, or multi-organ recipients [10]. PTLD is typically of B-cell origin (>90%) and strongly associated with EBV, especially in pediatric patients [11]. Two major risk factors for the development of PTLD after SOT are a EBV sero-negative immune status before transplantation as well as an extensive and prolonged immunosuppressive treatment [8].

CMV

The human Cytomegalovirus (CMV) is an encapsulated double-stranded DNA virus. It is a member of the beta herpes viruses and is also known as human herpes virus 5. CMV is spreading worldwide and its epidemic level is varying between 30 and 90% depending on the living standards. Although the primary infection occurs usually silent, CMV reactivates constantly and stochastically in differentiating cells of the monocytic lineage. Therefore, the virus will never be cleared after infection. Instead, it enters a state of latency that is associated with a low level of viral load and a strong CMV-specific cellular immune response [12]. Thus, cellular immunity which is characterized by high frequencies of CMV-specific CD4+ and CD8+ T lymphocytes plays the major role in controlling CMV infection [13]. In the transplant setting, CMV is the most common opportunistic infection affecting SOT recipients [14]. Infection or reactivation are both causing substantial morbidity and mortality among this immunocompromised cohort [15]. Clinical symptoms of CMV comprise fever, pneumonia, hepatitis, gastroenteritis, leuco- and thrombocytopenia or chorioretinitis. Additionally, CMV infection has been linked to poor long-term graft function, acute rejection, chronic rejection and graft loss [16]. Major risk factors interrelated to CMV complications following SOT are the CMV sero-status of donor and recipients, age of donor and recipient, type and intensity of immunosuppression, antiviral drug resistances, time since transplantation, and the incidence of other co-infections [17]. Antiviral drug resistances have an incidence of up to 10% and are strongly depending on risk factors like prolonged antiviral prophylaxis, severe immunosuppressive regimen, high viral load, or donor–recipient constellation [18,19]. CMV-negative recipients receiving a CMV-positive graft in the absence of an effective preventive strategy develop CMV diseases with an incidence of up to 70% [14]. Patients receiving lung and small intestine transplants have the highest risk, while heart recipients have a lower one and renal transplant recipients have the lowest risk of CMV infection [20,21].

ADV

The adenovirus (ADV) comprises a group of nondeveloped, lytic double-stranded DNA viruses which are classified into seven subgroups (A-G) that can be further subdivided into more than 50 distinct serotypes [3]. ADV is ubiquitously distributed and can cause respiratory infections (conjunctivitis, pharyngitis, bronchiolitis, bronchitis, pneumonia) as well as acute gastrointestinal diseases that are mostly self-limited in immunocompetent patients. Thereby the different virus subtypes show serotype specificities toward the targeted organ [22]. ADV infection can either be acquired de novo or through different ways such as via transplanted organs, the respiratory route, or by person-to-person contact. The incidence of (severe) infection in adult SOT patients is rather low but appears to be more frequent in pediatric SOT patients, although most infections remain without symptoms even in SOT patients. ADV can cause severe prolonged diseases and affects morbidity, mortality, and graft survival, especially in children [3]. In liver-transplanted children, ADV-related hepatitis and pneumonia are reported to be associated with a high mortality rate of 43% and 75%, respectively [23]. Diseases can be localized to one organ, invasive (gastrointestinal or respiratory tract plus one other organ) or affecting more than two organs (disseminated). Despite age, the type of the transplanted organ and the intensity of the immunosuppressive regimens have been found to influence the risk of ADV infection [24]. An increased risk of infection is commonly associated with a deficient immunity to ADV [25]. ADV-specific CD4+ and CD8+ T cells are required for complete and sustained antiviral protection [26,27]. Therefore, immunosuppressive treatment usually has to be discontinued to

prevent fatal outcomes of ADV-related complications in SOT [22].

Standard treatment strategies

Viruses such as the descripted endogenous herpes viruses, for example, have evolved with its host for a long time. They have established immune escape mechanisms which enable them to persist latently in the infected host [28]. Once latency accomplished, healthy individuals commonly remain without clinical symptoms apart from occasional virus reactivations. Immunocompetence resembles a fine balanced state of viral load and cellular immune response. Unfortunately, in patients receiving immunosuppressive agents to avoid graft rejection after SOT, this equilibrium is disturbed due to a compromised immune functionality. Different (standard) treatment strategies established in this respect. All of them aim to rebalance the recipient's immune system in order to control the viral spread.

Reduction in immunosuppression

The reduction in immunosuppression is an accepted first-line treatment, which aims to restore the natural virus-specific immune surveillance. This option is more commonly used in EBV-associated complications such as PTLD even if the clinical benefit in adults is poor as demonstrated by Swinnen [29] and only successful if a long-term viral replication can be observed, particularly in children [30]. In contrast, for SOT patients suffering from rare severe ADV-related infections, the reduction in immunosuppression is recommended and could help to reconstitute the antiviral immune response. SOT patients with severe leuco- or thrombopenia or life-threatening CMV disease profit from reduction in IS. Immune recovery due to the reduction in immunosuppression is reported to reconstitute cellular immune responses by T lymphocytes in general which can lead to a successful disease clearance [30,31]. Nevertheless, lowering immune suppression increases the risk of graft rejection due to a rise in alloreactive immunity. Moreover, even the lowest tolerable dose of immunosuppression might be too high to allow the restoration of an effective virus-specific cellular immunity. To manage this problem, it is of importance to have a deep knowledge about the virus–host balance. This can be analyzed by viral load and virus-specific T cells as helpful clinical biomarker. Furthermore, PTLD-associated tumors may become refractory to the withdrawal of immunosuppression [4].

Antiviral & chemotherapy

The aim of antiviral medication is to prevent the replication and dissemination of the respective viruses. Different agents are available. (Val) ganciclovir and (val) acyclovir are often used to treat and prevent CMV infections, respectively, and in special situation EBV infections also, while cidofovir and ribavirin are active on ADV. The administration of antiviral drugs such as (val) acyclovir and (val) ganciclovir is expected to inhibit the lytic viral replication, although both are ineffective against established latent viral infections [4]. Antiviral treatment is commonly used to inhibit CMV infection and/or reactivation following SOT to prevent CMV disease. This can be performed either in a prophylactic or preemptive approach [14,32]. Prophylaxis starts soon after transplantation and has a predetermined duration usually between three and 6 months. This overlaps the time frame in which CMV infection is most likely to occur [14]. The preemptive approach relies on a frequent monitoring for viral replication measured by quantitative PCR in the blood. The antiviral treatment is initiated when the viral load rises, indicating a rising risk of CMV [33]. Both approaches have been tested, compared, and meta-analyzed, with various results, but prophylaxis seems to impart a superior outcome particularly in high-risk recipients [34]. This has to be weighed against (serious) side effects such as toxicity, impaired immune reconstitution, fungal infections, and bacterial sepsis [5,31,33]. Furthermore, viral resistances are known to occur which than renders these drugs ineffective [14]. In addition, the reduction in early onset CMV disease due to respective strategies is at the expense of an increase in late onset CMV disease, which is most probably related to missing cellular immune responses [35,36].

Chemotherapy and anti-CD20 regimen

Chemotherapy is recommended for EBV-related diseases such as PTLD. Hence, high levels of toxicity are required, which compromises the graft stability and increases recipients risk of opportunistic infections [4]. Accordingly, different chemotherapy schemes have been developed and demonstrated improved remission rates [37]. These schemes are commonly composed of the drugs cyclophosphamide, hydroxydaunorubicin, oncovin and predniso(lo)ne and known as CHOP regimen even though modified regimens are existing. Nevertheless, CHOP chemotherapy is associated with substantial toxicity and mortality [38].

The CD20 protein is expressed on almost all stages of B-cell development. It is used as target of rituximab, which is an anti-CD20 humanized chimeric monoclonal antibody that is employed as B lymphocyte-depleting agent, for example. PTLD tumoral cells are frequently of B-cell lineage and express CD20. The binding of rituximab to the CD20 receptor induces apoptosis in B cells [39]. Although encouraging results are reported, rituximab is often used in combination with other treatment strategies, which makes it difficult to estimate the benefit of a monotherapy. Moreover, Zimmermann and Trappe suggested the agent to be suboptimal for intermediate- and high-risk patients in the PTLD setting [38].

In case CHOP and rituximab are combined in a treatment scheme known as R-CHOP, toxicity seems to be reduced compared with CHOP monotherapy, while the rate of complete remission in PTLD is higher compared with rituximab monotherapy [38].

Virus-specific T-cell therapy

The standard treatment strategies for virus-related diseases in SOT are primarily targeting the associated symptoms such as elevated viral load levels, for example. However, in case of virus infections, it is broadly accepted that the restoration of an effective cellular immunity is necessary. The transfer of virus-specific T-cell products is currently the only therapeutic option, which directly supports the reconstitution of this part of the adaptive immunity. In contrast to HSCT, SOT recipients are usually not in lymphopenic conditions. This strongly influences the proliferative niches for the infused cellular products.

Many different techniques for the manufacturing of virus-specific T-cell products have been developed since the pioneering days of adoptive T-cell therapy in the 1990s (Table 2). Until the beginning of this century, T-cell lines were commonly manufactured with the help of specially generated antigen-presenting cells (APCs). These approaches comprise the use of virally infected fibroblasts, EBV-infected B cells (lymphoblastoid cell lines, LCL), peptide-pulsed dendritic cells (DCs) as well as genetically modified or artificial APCs for the induction of T-cell lines with specificities for EBV and CMV for example [40–47]. Many of these methods are depending on further repetitive antigen stimulation cycles during the manufacturing process. Virus particles and gene modification offer the possibility to generate cell products, which finally cover a broad spectrum of the naturally occurring antigens, although their translation into GMP-compliant processes is at least very challenging, if possible at all due to safety issues. Moreover, the need for an initial APC production step and recurrent restimulation is time-consuming, labor intensive, and therefore expensive.

The technical progress enabled the development of advanced manufacturing processes based on direct labeling of the desired cell population. One method directly selecting virus-specific T-cell populations makes use of peptide MHC multimers (pMHC), which are binding to the T-cell receptor. Nonetheless, the knowledge of immunodominant HLA-restricted peptide epitopes for the loading of these multimers is prerequisite [32,48]. This technology allows the isolation of the corresponding cells to high purities by magnetic beads or FACS and needs no prior activation step of the cells [49]. Another advantage comes along with the improvement of reversibly binding pMHC multimers, which offers the possibility to select almost untouched cells. Nonetheless, this regulatory benefit is only significant if the cells are not manipulated further on, like during *in vitro* culture. Accordingly, large amounts of peripheral blood mononuclear cells (PBMCs) are needed to isolate substantial amount of specific cells for the subsequent treatment of patients. Maybe the most important disadvantage besides the HLA restriction is the still existing limitation in available pMHC class II multimers for the isolation of CD4+ T lymphocytes [32,48].

Another technique employs chemical synthesized pools of overlapping peptide fragments (15 mers e.g.) to stimulate cytokine secretion in the targeted cell population of PBMCs, for example [50–53]. The cell fraction is consecutively labeled with magnetic particles and can thereby be isolated in a GMP-compliant closed selection process. This approach is of advantage as it is HLA-type independent and the design of the peptide pools supports the stimulation of CD8+ and CD4+ T cells in the same way. The method can easily be adapted to any antigen if immune-dominant targets are known and memory T cells are present in adequate quantities. It is also possible to combine different antigen sequences to generate bi- or multispecific T-cell products like successfully demonstrated by Gerdemann and colleagues [54]. Even very small amounts of specific cells can be expanded to therapeutically relevant numbers without any restimulation needs [52]. This allows starting the manufacturing process with <50 ml peripheral blood, which is of importance in the SOT setting.

Another aspect of the manufacturing process concerns the source of the starting material. Recently, the use of allogeneic T-cell preparations has gained attention. Haque and coworkers used partially HLA-matched allogeneic T-cell preparations for the treatment of PTLD

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[55,56]. They could achieve an overall response (complete or partial remission) in about 52% of the cohort at 6 months after treatment [56]. Unfortunately, the reinfused cells showed a poor persistence *in vivo,* which raises efficacy concerns [55,57]. Although this approach is currently presumably the most promising one, future trials will have to compare and rate this number to other (e.g. autologous) therapy strategies. According to the allogeneic administration, the cell products have to be cryo-banked. Therefore, regulatory issues such as the demonstration of (long-term) stability and questions regarding repeated applications of potentially different batches will have to be satisfied.

Beneficial product characteristics

Virus-related diseases as they occur in SOT recipients are controlled by cellular immune responses. Upcoming results from basic research and first clinical data constantly increase the knowledge about T-cell product characteristics that might be associated with therapy benefit. However, the early manufacturing strategies were focused on CD8+-dominated T-cell products. Nonetheless, it has become obvious that CD4+ T cells support the functionality and survival of CD8+ T cells *in vivo* [58–61]. It is suggested that clearing of ADV infections as well as frequency, function, and therapeutic benefit of EBV-specific and CMV-specific CD8+ T cells is depending on CD4+ lymphocytes [62,63].

Long-lasting protection and efficacy is often biased by missing longevity and persistence of the adoptively transferred T-cell products *in vivo* [64,65]. Antigenic stimulation drives the differentiation of central memory T cell (Tcm)-derived clones and effector memory T cells (Tem) into effective, but short-lived effector T cells (Teff). *In vivo* Tcm-derived clones were able to migrate to lymph nodes or bone marrow, convert into Tcm and Tem, and most notably showed long-term persistence in the circulation [66]. On the contrary, Tem-derived clones did not persist and were not detectable in lymph nodes or bone marrow, which underlines the differentiation status of T-cell products as important parameter for sustained therapeutical benefit [61].

The functional capacity of T-cell preparations, as determined, for example, by cytotoxicity or cytokine production, is of importance to trigger therapeutically relevant cellular immune responses after their transfer. Besides specific cytotoxicity, also substantial secretion of cytokines such as interferon gamma, tumor necrosis factor alpha, or interleukin 2 after antigen-specific challenge supports antiviral effects via the induction of pro-inflammatory conditions [4]. In this context, T lymphocytes that are capable of secreting multiple cytokines in parallel were correlated with increased therapeutic success in HIV-infected patients [67]. In contrast, cytokine production might be hampered if T cells reach a state of exhaustion/ senescence (e.g. due to a prolonged *in vitro* expansion period) that is characterized by an upregulation of markers like programmed death receptor 1 or pro-apoptotic molecules like CD95 [68–70]. This state can also be associated with a reduced telomere length and an impaired engraftment or persistence of adoptively transferred T lymphocytes [64,71]. Moreover, cytokine secretion and clinical benefit might be improved if regulatory T cells are depleted before cell expansion [72].

Clinical data

The currently available clinical data of adoptively transferred virus-specific T-cell products in SOT are summarized in Table 1 to our best knowledge. The intensive characterization of the aforementioned phenotypic and functional parameter of the applied products would be desirable but was rarely done in the past. Accordingly, only the more recent investigations are addressing these issues [53,56]. In 2007 Haque and colleagues firstly reported a positive correlation of the CD4+ T-cell number and treatment outcome [56]. Unfortunately, most of the EBV-specific T-cell products are CD8 dominated most probably as a result of the expansion phase and the need for repetitive stimulation circles with the LCL-based generation method.

Initially, T-cell approaches were solely used in an autologous manner [73–75]. Nonetheless, limitations due to the complicated and time-consuming standard generation process (using EBV-infected LCLs) were identified straightaway and allogeneic applications were investigated in parallel with acknowledged success [55,56,76,77].

The cell dosage (in total) as well as the number of cell applications is heterogeneous, and no general conclusion can be drawn. This probably reflects the "personalized medicine" character of adoptive immunotherapy. However, the dosage as well as the number of applications is increased in the allogeneic treatment. Surprisingly, no correlation of HLA match between cell product and recipient and clinical benefit was found with an allogeneic application [55].

Besides clinical outcome, all T-cell products were analyzed for cytotoxicity and/or phenotype before the application. However, predetermined *in vitro* cytotoxic-

Table 1. Overview of studies applying virus-specific T-cell products in SOT recipients.

Author (year)	Age	Number of Patients/ Sex	Transplant type	Dosage/ source	Number of applications/ application	Indication	Premedication	Manufacturing approach/ product specificity	Results
Haque *et al.* (1998) [73]	29–54	3 (male)	Liver & kidney	5–20 × 10e7; autologous	3/ Prophylaxis	EBV	Immunosuppr.	LCL/ EBV	No adverse effects; iovstc: 30–1100×; survival: n.a.
Khanna *et al.* (1999) [74]	40	1 (male)	Lung	0.35–6 × 10e7; autologous	4/ Treatment	EBV/ PTLD	Immunosuppr.	LCL/ EBV	No signs of PTLD after 2 infusions; patient relapsed and showed evidence of vascular invasion with necrosis after death (ca. 12 weeks after first infusion); iovstc: 20–40×
Comoli *et al.* (2002) [75]	4–60	7 (2 female & 5 male)	Heart, liver or kidney	2 × 10e7/m^2; autologous	1-5/ Prophylaxis	EBV/ PTLD	Immunosuppr.	LCL/ EBV	No toxicity or evidence of rejection o bserved, all patients alive up to 46 months after infusion; iovstc: 2–30×
Sherritt *et al.* (2003) [78]	57	1 (female)	Heart	2 × 10e7; autologous	6/ Treatment	EBV/ PTLD	Immunosuppr.	LCL/ EBV	First three dosages showed minimal side effects; CR after dose 6 is ongoing; iovstc: n.a.
Comoli *et al.* (2005) [79]	2–14	6 (1 female & 5 male)	Kidney	2 × 10e7/m^2; autologous	2–5/ Treatment combination	EBV/ PTLD	Immunosuppr.	LCL/ EBV	No toxicity or evidence of rejection observed, all patients alive with good renal function up to 31 months after infusion; iovstc: 3–7×
Savoldo *et al.* (2006) [80]	0.6–40	12 (6 female & 6 male)	Liver or heart	2–10 × 10e7/m^2; autologous	1–4/ Treatment	EBV	Immunosuppr.	LCL/ EBV	No toxicity observed, despite patient 3; all patients alive for more than 1 year; iovstc: 1.5–4.8×
Sun *et al.* (2002) [76]	n.a.	4 (1 female & 3 male)	Kidney, l iver or no Transplantation	0.5 × 10e7/kg; allogeneic/ HLA-matched sibling	3/ Treatment	EBV-related Lymphoma	Diverse	LCL/ EBV	No complications following infusion; 2 patients with CR, 1 patient with disease progression, 1 patient with PR; iovstc: 2–4×
Haque *et al.* (2002) [55]	1.5–60	8 (1 female & 7 male)	Liver, kidney or HSCT	0.1 × 10e7/kg; allogeneic	1–6/ Treatment	EBV/ PTLD	Reduction in or withdrawal of immunosuppr.	LCL/ EBV	No toxicity or GvHD; 3 patients with CR, 1 patient with PR, 4 patients did not respond; iovstc: 40–120×; survival: 4 dead / 4 alive
Haque *et al.* (2007) [56]	1–76	33 (14 female & 19 male)	Liver, heart, kidney, lung or HSCT	0.2 × 10e7/ kg; allogeneic	1–8/ Treatment	EBV	Reduction in or withdrawal of immunosuppr. (except three patients)	LCL/ EBV	No toxicity or evidence of rejection observed after infusion; CR or PR in 21 patients 5 weeks and 17 patients 6 months after infusion, 12 patients did not respond; iovstc: n.a.; survival 7 dead
Gandhi *et al.* (2007) [77]	18–58	3 / female	Heart, lung or kidney	0.2 × 10e7/ kg; allogeneic	1–8/ Treatment	EBV/ PTLD	Rituximab + chemo	n.a./ EBV	Negligible immediate toxicity after infusion; patient 1 CR ongoing; patient 2 died, but autopsy confirmed CR; patient 3 died due to respiratory/ renal failure; iovstc: n.a.

Table 1. Continued.

Author (year)	Age	Number of Patients/ Sex	Transplant type	Dosage/ source	Number of applications/ application	Indication	Premedication	Manufacturing approach/ product specificity	Results
Brestrich *et al.* (2009) [53]	47	1 (male)	Lung	1 × 10e7/ m^2; autologous	2/ Treatment	CMV pneumonia	Immunosuppr.	PBMC stimulation with CMV peptide pools	No side effects after first infusion; patient relapsed and died from graft failure; iovstc: 2×
Macesic *et al.* (2015) [81]	61	1 (male)	Kidney	1.6 × 10e7/ m^2; allogeneic	1/ Treatment	CMV	Immunosuppr.	Monocyte-derived dendritic cells with CMV peptides	Mild fever after infusion; after 1 year CMV DNA viral load decreased from 1.8 × 10e6 to 73 copies/ ml; patient is alive

EBV, Epstein–Barr virus; CMV, Cytomegalovirus; Immunosuppr., Immunosuppression; PTLD, post-transplant lympho-proliferative disorder; CR, complete remission; PR, partial remission; GvHD, graft versus host disease; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; iovstc, increase in virus-specific T cells.

ity failed to certainly predict therapeutic benefit *in vivo* irrespective of the treatment strategy (autologous or allogeneic).

Generally, T-cell therapy in SOT targeting EBV is far more frequently used compared with CMV, although CMV is the most common opportunistic infection in SOT recipients [14]. This might be related to existing efficient medication or missing knowledge about straightforward manufacturing strategies like combined peptide pool and cytokine capture-based techniques. Likewise, no information about the use of ADV-specific T cells in clinical SOT trials was available, although first data in allogeneic stem cell transplantation were promising [63].

The central insight of all adoptive T-cell trials is the excellent tolerability of the applied products regardless of the treatment strategy (autologous or allogeneic; single or multiple infusion), the dosage, the specificity (EBV or CMV) and the indication (SOT or HSCT). The potential of this alternative therapy is further underlined if the patient's cohort is considered. The majority of these patients failed to standard treatment regimen and frequently received medication. This at least did not support subsequent T-cell treatment. Nevertheless, almost no signs of infusion-related toxicity, graft impairment, or rejection were reported in all studies (Table 2).

Regulations and good manufacturing practice

Cell-based therapies in general and adoptive T-cell therapy in particular compete with established treatment standards that are usually based on small molecules. Nonetheless, both have to comply with given laws and quality standards that should ensure the patients safety. These aspects are increasingly important issues concerning the manufacture of T-cell products. Therefore, their implementation and general points to consider are outlined according to the European Medicine Agency (EMA) and Food and Drug Administration (FDA) standards. Both agencies are major opinion leader and decision-maker in this field.

The manufacturing process of virus-specific T cells mostly relies on substantial manipulation of the product. Cells that have been stimulated or expanded, for example, are considered substantially manipulated. They are categorized as advanced therapy medicinal product (ATMP) in Europe [86] or somatic cell therapy product (SCTP) in the United States (U.S.), for example, and both are regulated under public health and pharmaceutical legislations. Although EMA and FDA regulations differ, both require compliance with the rules of good manufacturing practice (GMP; for further details please

Table 2. Overview of generation methods for virus-specific T cells.

Specificity	Starting material	Stimulation method	Isolation technique	Cultivation approach	Comment	Protocol details/references
EBV; CMV; ADV	PBMCs (peripheral blood or leukapheresis)	Overlapping peptide pools	IFNg catch	Feeder cell layer (irradiated PBMCs), cytokines, no further re-stimulation needed	No HLA restriction; modular technique expandable by exchanging or combining different peptide pools	Brestrich *et al.* [52]
EBV	PBMCs and LCLs	Repetitive stimulation with irradiated LCL	No isolation; co-culture	Feeder cell layer (irradiated LCL), cytokines, repetitive re-stimulation needed	EBV-infected B-cell line (LCL) must be established in advance; lengthy process; handling of infectious material needed	Rooney *et al.* [82]
CMV	PBMCs (peripheral blood or leukapheresis)	CMV lysate; irradiated feeder cells	No isolation; co-culture	Feeder cell layer (irradiated PBMCs), cytokines; further re-stimulation	Several restimulations and handling of infectious material needed	Einsele *et al.* [83]
CMV	PBMCs (peripheral blood or leukapheresis)	Peptide-pulsed dendritic cells	No isolation; co-culture	Re-stimulation with B cells or LCL, cytokines	DCs and LCL must be established in advance; lengthy process; handling of infectious material needed	Szamnia *et al.* [41]
ADV	PBMCs (peripheral blood or leukapheresis)	ADV lysate from ADV-infected cells	IFNg catch	Feeder cell layer (irradiated PBMCs), cytokines, addition of fresh feeder cells	Handling of ADV lysate	Feuchtinger *et al.* [84]
CMV	PBMCs (peripheral blood)	No stimulation needed	MHC class I tetramers	No cultivation done	Tetramers must be established in advance; immunodominant epitopes must be known; MHC class I restricted	Keenan *et al.* [49], Cobbold *et al.* [85]

EBV, Epstein–Barr virus; CMV, Cytomegalovirus; ADV, Adenovirus; HLA, human leukocyte antigen; MHC, Major Histocompatibility Complex; IFNg, Interferon gamma; PBMCs, peripheral blood mononuclear cells; LCLs, lymphoblastoid cell line.

The table summarizes, to our best knowledge, the most common manufacturing approaches for virus-specific T cells. Nevertheless, different, modified, varied or partial combinations of these methods (particularly regarding stimulation, isolation and cultivation techniques) are existing.

visit the respective websites: http://www.ema.europa.eu and http://www.fda.gov). In December 2008, the EMA put the ATMP Regulation in force to mandate the marketing authorization (supported by the Committee for Advanced Therapies; CAT) for ATMPs [87]. Nonetheless, each member state is still responsible for the authorization of national clinical trials and can autonomously decide to regulate products manufactured on a nonroutine basis via the hospital exemption. In the United States, SCTPs are regulated by the Center for Biologics Evaluation and Research under title 21 of the Code of Federal regulations Part 1271 and must be approved by the FDA. Additionally, the FDA is in charge to regulate all aspects of SCTPs, comprising clinical trial authorization as well as GMP compliance.

Currently, the research, development, and translation of T-cell products into the clinics are driven by academic institutions, hospitals, and charities with different levels of expertise and experience in the regulatory field [88]. Therefore, GMP compliance is often underestimated as it requires investments in infrastructure and personnel [89]. Furthermore, the infrastructure causes substantial running costs due to electricity for the ventilation system and maintenance of the facility or equipment, for example, as outlined by Abou-El-Enein *et al.* [90]. On the other hand, personnel are needed not only for the manufacturing but also for qualification, validation, quality management, quality control, and regulatory affairs. Moreover, a strong scientific background is needed to develop and validate specifications and test methods for cell identity, potency, viability, purity, adventitious agents, and impurities for instance. These points are issued in directives like the GMP guidelines, the Guidance for Industry or quality guidelines suggested by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human (ICH). Although guidelines are generally not legally binding, they are recommended by authorities and should be considered if GMP compliance is envisioned.

The leading role of academic GMP facilities in conducting phase I/II trials was already outlined. In Europe, these facilities are subject to inspections and a manufacturing authorization is mandatory for products like virus-specific T cells. Additionally, the compliance with release criteria and GMP must be verified by a qualified person for each batch. In contrast to Europe, these requirements are not known in the United States, which facilitates and accelerates the work of academia in this field.

Outlook

The adoptive transfer of virus-specific T-cell products in SOT recipients is promising due to remarkable clinical data and its good tolerability. This role as therapeutic alternative to standard medication regimens might even be extended by the identification of further therapy targets and via a specified selection of an appropriate patient cohort.

Besides single viral diseases, complications in SOT can be related to multiviral problems or primary infections with less widespread viruses. Therefore, the priming of T lymphocytes from naïve donors and the implementation of multivirus-specific products, like already demonstrated in HSCT [91,92], would be promising. Currently, the knowledge about culture conditions, supplements, and their impact on desired product characteristics like enhanced Tcm differentiation and multifunctionality is evolving fast [61]. Further improvements in this direction and progress in isolation techniques will help to shorten the production process and enhance treatment benefit. Presently, this bottleneck is bridged by allogeneic product applications with at least curtailed in vivo detectability/ survival compared with autologous approaches. However, as T-cell products are moving toward market authorization and clinical routine, rising demands are predictable. Therefore, the need for large-scale productions and process automation will grow. Both are challenging improvements in process management and the development of GMP-compliant closed culture systems to increase the output and to decrease the time-intensive handling during the manufacture of these products.

Additionally, the harmonization of the legislation and guidelines must be continued to achieve equal conditions and comprehensive patient safety. Accordingly, the discussion with regulatory authorities on the shaping and implementation of the framework has to be continued intensively and interactively.

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