Supplementary Data Set

T-CELL THERAPY: OPTIONS FOR INFECTIOUS DISEASES

Shreemanta K Parida[1], Thomas Poiret[1, 2], Liu Zhenjiang[1], Qingda Meng[1], Jan Heyckendorf[3], Christoph Lange[3,4,5], Aditya S Ambati[1, 2, 5], Martin V Rao[1], Davide Valentini[1, 2], Giovanni Ferrara[5], Elena Rangelova[6], Ernest Dodoo[1,7], Alimuddin Zumla[8], Markus Maeurer[1, 2]

[1]Therapeutic Immunology Division, Dept of Laboratory Medicine, Karolinska Institutet, [2]Centre for Allogeneic Stem Cell Transplantation (CAST), Karolinska University Hospital, [3]Division of Clinical Infectious Diseases, German Center for Infection Research, Research Center Borstel, Germany, [4]International Health / Infectious Diseases, University of Lübeck, Germany, [5]Department of Medicine, Karolinska Institutet, [6]Pancreatic Surgery Unit, Division of Surgery, Department of Clinical Science, Intervention and Technology (CLINTEC), Stockholm, Sweden, [7]Dept of Neurosurgery, Karolinska University Hospital, Stockholm, Sweden, [8]Department of Infection, Division of Infection and Immunity, Centre for Clinical Microbiology, University College London, and NIHR Biomedical Research Centre, University College London Hospitals, London, United Kingdom.

Correspondence: Prof. Markus Maeurer, Therapeutic Immunology, F79, LabMed, Hälsovägen, Karolinska University Hospital, Huddinge, SE-14186 Huddinge, Sweden. Email: markus.maeurer@ki.se

Donor Lymphocyte Infusion

Adoptive transfer of CMV-specific CD8+ T-cells generated from CMV seropositive MHC identical bone marrow donors and expanded *in vitro* during 5-12 weeks showed a successful reconstitution of the CMV-specific immune responses in DLI-treated patients. *In vitro* selection/expansion and technologies turned the DLI into a more specific adoptive T-cell therapy. *Ex vivo* stimulation of peripheral blood mononuclear cells against target antigens to select specific T-cell population have been used to treat CMV [1] infections following HSCT. EBV-specific cytotoxic T-cells (CTL) lines were successfully established from donor leucocytes and infused into allograft recipients to treat patients with (EBV+) PTLD. The EBV-specific T-cells were prepared by stimulating the donor lymphocytes with EBV transformed B-cells from donors. After infusion, specific T-cells persisted for 10 weeks in this study [2]. The same group studied 114 patients receiving infusions of EBV-specific cytotoxic T lymphocytes to prevent or treat PTLD [3]. None of the patients (101) developed PTLD who received EBV specific T-cells in the prevention setting, whereas 11 of 13 patients treated with CTLs for biopsy-proven or probable PTLD achieved sustained complete remissions. The toxicity was described as minimal, consisting mainly of localized swelling at the sites of responsive disease and the study demonstrated the persistence of functional CTLs for up to 9 years. The same protocol for generation of the EBV-CTLs was used to treat the patients with locoregional (EBV+) nasopharyngeal carcinoma (NPC), 62% (5/8) relapsed patients remain disease free (at 17-75 months) [4]. The EBV-CTL lines in this study were predominantly CD3+ CD8+T-cells (mean, 83.2%). EBV-specific T-cell therapy has not shown similar efficiency in patients after solid organ transplant due to the lifelong immunosuppression. This may change since Ricciardelli and coworkers recently generated EBV-specific T-cell lines resistant to immunosuppressive treatment [5].

Advances in adoptive T-cell technology

The last 10 years witnessed new approaches in establishing clinically relevant T-cell products for therapy. For instance, an Ad5f35pp65 vector was successfully used to create T-cells specific for CMV, EBV and several serotypes of adenovirus from a single cell culture [6]. Feasible use of banked third-party virus-specific T-cell (VST) lines was demonstrated by the same group to treat severe viral infections after HSCT. The use of anti-viral directed T-cell products showed 74% complete or partial responses [7]. Following *in vitro* stimulation and expansion, the isolation of virus specific T-cell population can be assessed by class I or II multimers coupled with magnetic beads: The first trial using multimers in adoptive therapy was demonstrated 10 years ago [8]: Patients received purified CMV-specific CD8+ T-cells using HLA class I-peptide tetramers. All patients had a reduction of their CMV viral load and some patients experienced clearance of CMV infection. Similarly, streptamer [9]-selected pp65-specific CD8+ effector T-cells were infused into patients with CMV-drug resistant strains [10]. CMV was cleared after a single infusion of a pp65 specific cells dose <1 million/kg. HLA-A2 multimers have also been used in the context of PTLD [11] and adenovirus (HAdV)-specific CD8+ T-cell targets [12]. Therefore multimers have shown successful results, yet the technology is limited by the HLA-restriction, GMP (Good-manufacturing practice)-related issues, and the availability of potential donors with reactive T-cells against the virus in sufficient numbers.

Unlike multimers, IFN-γ capture T-cell technology and magnetic bead-mediated selection does not have HLA-restriction. It has first proven its efficiency by adoptive transfer of HAdV specific T-cells [13-15]. The same technology has shown its efficiency to select EBV specific T-

cells using EBNA-1 antigen in order to treat PTLD patients after HSCT [16], it was also used for the transfer of CMV-pp65-specific T-cells in a prevention setting. [17].Low numbers of (differentiated effector-memory) CMV-specific T-cells predominantly CD4+ (81% of the products) showed a clinically relevant effect, which was independently confirmed in a different study [18]. In both studies, patients were treated preemptively with antiviral drugs (ganciclovir, foscarnet and valganciclovir); adoptive T-cell therapy is not yet able to be used as single therapy and acts effectively as adjunct treatment in anti-viral treatment.

A different way to transfer anti-specific T-cells against infectious agents was the *ex vivo* enrichment of viral specific T-cells. Expansion of virus specific CD8+ cytotoxic T-cells was challenging with the use of virally infected MHC compatible cells as antigen presenting cells or repeated stimulation with antigen and IL-2 in limiting dilution and maintenance of (CMV specific) T-cell clones with anti-CD3 and anti-CD28 monoclonal antibodies [19]. Expansion of antigen-specific T-cells can be achieved using different techniques, *i.e.* Artificial Antigen-Presenting Cells (aAPC) [20] and second generation aAPC with engineered capabilities of effective antigen presentation, cytokine production, expression of adhesion molecules and micropinocytosis [21]. A different approach to expand antigen-specific T-cells is the use of overlapping synthetic peptides [22]. However, the isolation of these T-cells may take too much time (from expansion to effective transfer), it may not be GMP-conform and it may very well be that T-cell precursors are simply not present in PBMCs from patients. Therefore, a 'hard-copy' of the antigen-specific TCR has been isolated and can be transferred using a retroviral or lentiviral system. This has been successfully achieved for a range of cancer-related targets [23], as well as for infectious pathogens, e.g. HPV [24] or Hepatitis B [25] or EBV [5]. The transfer of antigen-specific TCRs is dependent on the match for the MHC class I or class II-genetic background of the recipient. Of note, cross-reactive T-cells may also cause

harm and target non-pathogen related structures. The clinical relevance of such cross-reactive T-cell responses has recently been demonstrated by the unexpected cross-reactivity of the adoptive transfer of an MAGE-3 directed T-cell product that resulted in a cytokine storm and death of the patient [26].

γδ T-cells in therapy of infectious diseases and cancer

These Vδ2neg γδ T-cell populations were observed to represent an average of 13.4% in CMV+ patients. Ten years later, a similar pattern was observed in the context of HSCT [27] Vδ2neg γδ T-cells have been demonstrated to play a role in the immune response to CMV reactivation exhibiting activity against CMV-infected cells. In addition to the anti-CMV activity, Vδ2neg γδ T-cells have been shown to be implicated in tumor defense by cytotoxic activity [28] or directly infiltrating the tumor [29]. The Vδ2neg γδ T-cell population also showed a cross-recognition activity between CMV and cancer [30, 31]; co-incubation of CMV-reactive Vδ2neg γδ T-cells clone with hematological tumor cell lines resulted in CMV-specific T-cell populations that cross-recognized residual leukemia blasts.

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