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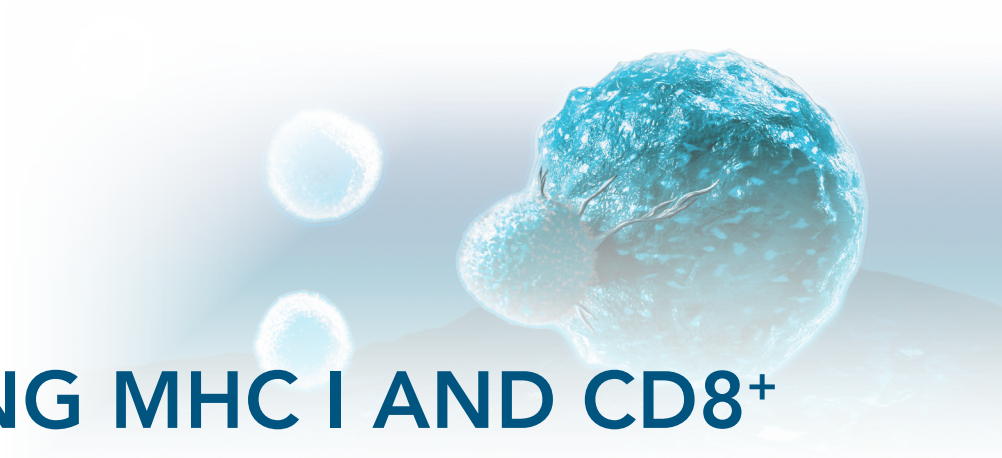
Application Note



COMBINING MHC I AND CD8+ FAB *STREP*TAMER® REAGENTS

Adoptive transfer of antigen-specific CD8+ cells has been successfully applied for the treatment of viral infections and tumors [1-3]. Most of these approaches were based on *in vitro* expansion of a small number antigen-specific T cells isolated from a donor. However, *in vitro* expanded cells have a compromised effector function when compared to the freshly isolated cells. In addition the cell expansion process under GMP conditions is costly and very time-consuming especially in the light of patients who usually have an urgent need for treatment. Therefore, the *Strep*tamer®-selected cells applied *ex in vivo* meaning direct adoptive transfer of the isolated cells without *in vitro* expansion offer an attractive alternative to *in vitro* cultured cell products. The problem of the *ex in vivo* approach is the accessibility of low frequency cell subsets such as tumor-specific T cells or adenovirus-specific T cells. The cell numbers of these T cell subsets are not sufficient to select enough cells for *ex in vivo* adoptive T cell therapy.

A solution to this problem is the tandem purification with a CD8 Fab *Strep*tamer® followed by an antigen-specific isolation step using MHC I *Strep*tamer® reagents . This sequential magnet-based isolation procedure is possible because the *Strep*tamer® reagents can be completely removed after each positive selection. The first CD8 Fab *Strep*tamer® isolation not only provide a fivefold enrichment of cytotoxic T cells and thus of the antigen-specific target T cells but also a very homogeneous cell population for the second antigen-specific isolation step. Therefore the subsequent MHC I *Strep*tamer® isolation of antigen-specific T cells out of the homogeneous, pre-enriched CD8+ cell fraction is greatly facilitated. This is of special importance when very low frequent cell subsets such as tumor-specific cytotoxic T cells should be selected with sufficient recovery and purity. The latter parameters are also very critical for isolated cell subsets intended for clinical use since maximal cell doses are calculated in many protocols on the basis of the purity of the cell product.

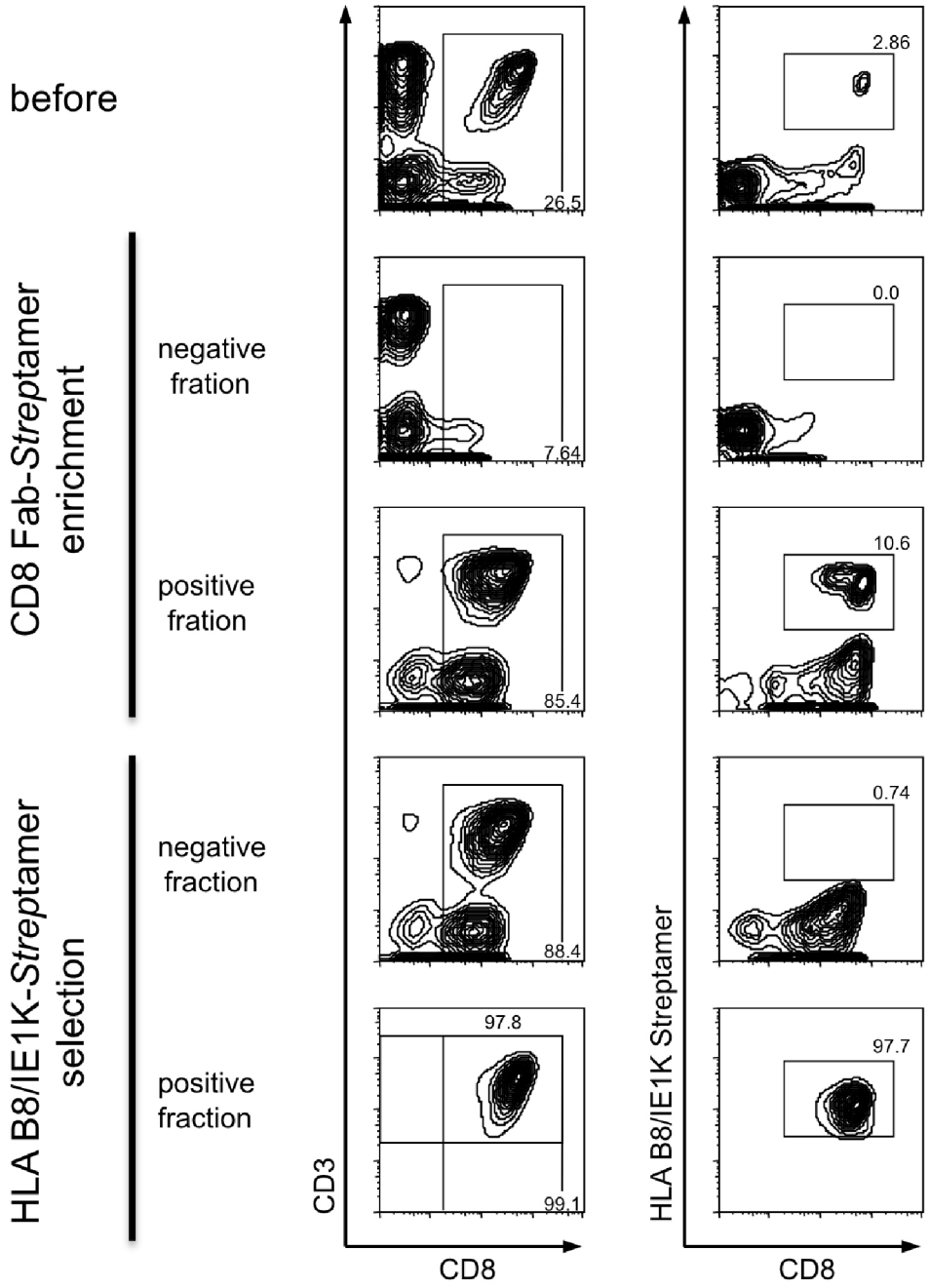


Figure 1: Magnetic enrichment of HLA-B8/IE1K-specific CD8+ T cells from fresh PBMCs.
Cells were first incubated with (reversible) CD8 magnetic Fab *Strep*tamer® reagents in order to pre-select CD8+ cells. The resulting positive fraction was then further processed by D-biotin treatment and subsequent washing to remove all CD8 selection reagents. In a second step, the target antigen-specific T cell population (specific for HLA-B8/IE1K) was then highly enriched from the pre-selected CD8+ cell pool via the respective magnetic MHC I *Strep*tamer® reagents . Live lymphocytes for the respective positive and negative fractions of both selection steps are shown.

Potential applications of sequential CD8+ Fab *Strep*tamer® and MHC I *Strep*tamer® selections

1. Selection of virus-specific T cells for adoptive T cell transfer

Adoptive T cell transfer of virus-specific CD8+ cells has been successfully applied in the clinic to prevent or treat opportunistic virus infections in allogeneic hematopoietic stem cell transplant (HCT) recipients. Reactivation of latent cytomegalovirus (CMV) in allogeneic HCT recipients remains a significant cause of morbidity and mortality despite antiviral drug therapy[4]. The positive correlation of progressive CMV infection with deficient CMV-specific CD8+ and CD4+ T cell responses suggested that the adoptive transfer of CMV-specific T cells isolated from the immunocompetent donor might be effective to control viremia in the recipient. The initial studies of adoptive immunotherapy for CMV infused CD8+ T cell clones or polyclonal T cell lines that were derived from the donor and selected for recognition of CMV infected cells and lack of cross reactivity with recipient alloantigens. These studies demonstrated that CD8+ and CD4+ CMV-specific T cells could be adoptively transferred to patients early after HCT with minimal toxicity, and that the transferred cells not only persist and function *in vivo*, but control infection[5,6,7].

*Strep*tamer®-selected CMV-specific T cells have been already used to successfully treat CMV infected patients after HCT. *Strep*tamer®-selected T cells were directly transferred from the donor to the recipient without *in vitro* expansion. This approach resulted in a long lasting T cell response and a complete clearance of the virus[8]. The sequential selection of CMV-specific T cells using CD8+ Fab *Strep*tamer® reagents and MHC I *Strep*tamer® reagents would extent the applicability of direct adoptive T cell transfer since donors with very low T cell frequencies would become amenable for donation of T cells. In addition the purities of the cell products from the tandem cell isolation are usually higher enabling the application of higher cell doses to the patient without the risk of GVHD.

Rooney and colleagues have isolated polyclonal EBV-specific T cells containing variable numbers of CD8+ and CD4+ T cells from the blood of HCT donors by repeated *in vitro* stimulation with EBV transformed B cell lines that express the EBNA proteins, and administered these T cells to the respective recipients with EBV-LPD or at high risk for EBVLPD. Adoptive T cell therapy targeting EBV was highly effective, both for promoting tumor regression in patients with established EBV-LPD and preventing the development of LPD when used as prophylaxis[4,9].

2. Selection of tumor-specific T cells for adoptive T cell transfer

Adoptive T cell therapy for human malignancy has been so far significantly less effective than for opportunistic viral infections. This reflects several severe problems of this treatment, such as the difficulty isolating the rare highly avid T cells that are specific for tumor cells from most cancer patients; the requirement that transferred tumor-reactive T cells persist *in vivo*, traffic to tumor sites and function in an immunosuppressive tumor microenvironment; and the potential for escape tumor cells variants which are not recognized by the immune system[10–13]. Longest persistence of adoptively transferred T cells was observed in lymphopenic HCT patients. This effect is thought to be mainly due to the homeostatic situation and the lack of regulatory T cells and other suppressive cells in lymphopenic patients[14,15]. Direct evidence for the correlation of lymphopenia and efficacy of adoptively transferred anti tumor T cells was shown by impressive clinical results in the treatment of advanced metastatic melanoma[16-18]. In the majority of patients transferred T cells underwent massive *in vivo* expansion, persisted long term, infiltrated into tumors and promoted tumor regression.

The sequential positive selection of CMV-specific T cells using CD8+ Fab *Strep*tamer® reagents and MHC I *Strep*tamer® reagents for tumor-specific CD8+ T cells could enable to isolate rare populations of avide, tumor-specific T cells. Especially the combination of this selection process with additional markers such as CD62L, a marker for central memory T cells, may deliver T cell subsets effective against certain types of tumors.

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