T cell therapy targeting a public neoantigen in microsatellite instable colon cancer reduces in vivo tumor growth

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ABSTRACT

T-cell receptor (TCR) transfer is an attractive strategy to increase the number of cancer-specific T cells in adoptive cell therapy. However, recent clinical and pre-clinical findings indicate that careful consideration of the target antigen is required to limit the risk of off-target toxicity. Directing T cells against mutated proteins such as frequently occurring frameshift mutations may thus be a safer alternative to tumor-associated self-antigens. Furthermore, such frameshift mutations result in novel polypeptides allowing selection of TCRs from the non-tolerant T-cell repertoire circumventing the problem of low affinity TCRs due to central tolerance. The transforming growth factor-β Receptor II frameshift mutation (TGFβRIImut) is found in Lynch syndrome cancer patients and in approximately 15% of sporadic colorectal and gastric cancers displaying microsatellite instability (MSI). The -1A mutation within a stretch of 10 adenine bases (nucleotides 709–718) of the TGFβRII gene gives rise to immunogenic peptides previously used for vaccination of MSI+ colorectal cancer patients in a Phase I clinical trial. From a clinically responding patient, we isolated a cytotoxic T lymphocyte (CTL) clone showing a restriction for HLA-A2 in complex with TGFβRIImut peptide. Its TCR was identified and shown to redirect T cells against colon carcinoma cell lines harboring the frameshift mutation. Finally, T cells transduced with the HLA-A2-restricted TGFβRIImut-specific TCR were demonstrated to significantly reduce the growth of colorectal cancer and enhance survival in a NOD/SCID xenograft mouse model.

Introduction

Colorectal carcinoma (CRC) is the third most common cancer in men and the second most common in women worldwide, with the highest rates in the Western world.1 Virtually all hereditary non-polyposis colorectal cancers (HNPPC), and a subset of sporadic cancers, including colorectal and gastric cancers, display microsatellite instability (MSI). MSI+ colon cancers are considered to be more immunogenic than microsatellite stable (MSS) cancers due to the generation of neoantigens caused by frameshift mutations. Transforming growth factor-β Receptor II (TGFβRII) is a common target for inactivating mutations occurring in approximately 90% of MSI2,3 and 15% of MSS colon cancers. Interestingly, Lynch syndrome (or HNPPC) and sporadic colon cancer patients with the MSI+ subtype have an improved prognosis compared with other sporadic colorectal cancer patients. This might be due to the presence of certain frameshift mutations in MSI+ colon cancers, which correlates with the increased density of tumor-infiltrating lymphocytes (TILs).6,7 Accordingly, an improved survival of MSI+ compared with non-MSI+ colorectal cancer patients is seen.8,9,11 These observations suggest that some patients with MSI+ CRC may benefit from immunotherapy targeting public neoantigens such as mutated TGFβRII. Recent clinical trials of adoptive transfer of TCR redirected T-cells targeting cancer germline antigens have, however, shown that immunotherapy can be associated with severe toxicity emphasizing the need for careful consideration of the choice of antigen. In one study, three out of nine cancer patients treated with autologous anti-MAGE-A3 TCR-engineered T cells experienced severe neurologic toxicity, being lethal in two cases, due to cross-reactivity of the TCR.12 A second study targeting MAGE-A3 in myeloma and melanoma patients with a HLA-A*01-restricted TCR demonstrated lethal cross-reactivity with myocardial damage.13,14 An alternative strategy is to exploit the panel of private neoantigens, isolate the corresponding TCR and reinfuse redirected T cells.15 Although attractive, this personalized approach will still need further development before being proposed as a realistic therapy. Therefore, true tumor-specific neoantigens that are more frequent may

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represent ideal targets for TCR therapy achieving tumor eradication in the absence of normal tissue destruction.\textsuperscript{15,16} Several T-cell epitopes have been identified within these frameshift peptides,\textsuperscript{17,18} including CD4\textsuperscript{+} and CD8\textsuperscript{+} T-cell epitopes in TGFβRII (1A).\textsuperscript{19-21} The present study describes the isolation of an HLA-A2-restricted TGFβRII frameshift mutation-specific T-cell clone. We demonstrate that its TCR is efficiently and functionally expressed in redirected CD8\textsuperscript{+} and CD4\textsuperscript{+} T cells, resulting in production of IFNγ and TNF-α upon specific stimulation. TCR-transfected CD8\textsuperscript{+} T cells were also capable of target cell lysis. Finally, a murine model of colon cancer used to test the in vivo potency of the TCR-redirected T cells showed a significant reduction in tumor growth and an enhanced survival of the study animals. We conclude that this TCR is a potential candidate for immunotherapy. The present study might pave the way for the exploitation of TCRs isolated from successfully vaccinated patients in development of clinical cancer therapy.

Results

Isolation of a TGFβRII frameshift mutation-specific T-cell clone

An MSI\textsuperscript{+} colon cancer patient had been vaccinated with a 23-mer TGFβRII frameshift peptide and showed a long survival (> 10 yr, manuscript in preparation). We therefore detected an immune response against the vaccine. A TGFβRII frameshift mutation-reactive HLA-A2-restricted CTL was previously isolated from his blood. The CTL was isolated and shown to be CD8\textsuperscript{+}CD4\textsuperscript{−}CD56\textsuperscript{+}, suggesting it may be Natural Killer (NK) cell-like (Fig. 1A). The CTL expressed TCR Vβ3 (or TRBV 28, IMGT nomenclature) chain.\textsuperscript{22} The clone (TC 30) showed specific lysis of the colon cancer cell lines HCT 116 and SW 480 (HLA-A2\textsuperscript{+}, frameshift\textsuperscript{+}). As expected, the effector:target (E:T) ratio required for lysis of cell lines with endogenous peptide was higher than if cell lines were loaded exogenously with TGFβRII frameshift peptide (p573). As a control, another colon cancer cell line, LS174T (HLA-A2\textsuperscript{−}, frameshift\textsuperscript{−}) was not killed (Fig. 1B). Importantly, despite the expression of CD56 on the T-cell clone (Fig. 1A); the HLA-A2 negative LS174T cell line was not killed, indicating that the killing was not mediated by NK-cell like activity, but by specific recognition of MHC molecules loaded with the correct peptide.

To test the relative avidity of the T cell clone, TAP-deficient T2 cells were loaded with titrated amounts of peptide (0.01–1.0 μM). We observed that the killing activity was following the peptide concentration and that the addition of HLA-blocking antibodies specifically reduced the killing (Fig. 1C). Similar observations were made when autologous Epstein–Barr virus-transformed lymphoblastoid cell lines (EBV-LCLs) were used as APC. Taken together, our data show that, although the TGFβRII\textsuperscript{mut}-specific T-cell clone was co-receptor negative, the clone was still peptide-specific and HLA class I restricted.

Radium-1 TCR redirects both CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells

The TCR α and β chains from the TGFβRII\textsuperscript{mut}- reactive T-cell clone were identified and referred to hereafter as Radium-1 TCR. A 2A construct was designed and sub-cloned into an
mRNA expression vector for equimolar expression of the TCR α and -β chains (see Materials and Methods). T cells expanded from PBMCs were electroporated with Radium-1 encoding mRNA to assess their ability to specifically recognize their targets. Transferred TCR expression was measured by anti-Vβ3 antibody surface staining and showed that between 60 and 70% of transfected T cells expressed the Vβ3 chain in both CD4+ and CD8+ cells, whereas less than 5% of the cells naturally expressed Vβ3 (Fig. 2A and Fig. S3).

We then monitored the activity of Radium-1-transfected T cells by intracellular cytokine staining upon co-incubation with the colon cancer cell lines SW 480 and LS174T for 15 h. We chose a long incubation to get maximal activation of CD4+ as well as CD8+ T cells. SW 480 cells were recognized by both CD8+ and CD4+ T cells in the absence and presence of exogenously loaded peptide. The T cells produced TNF-α and IFNγ (Fig. 2B). As expected the colon cancer cell line LS174T was not recognized. These data confirmed the HLA-peptide restriction of Radium-1; furthermore, it suggests that this TCR is very potent as it was able to efficiently redirect both CD4+ and CD8+ T cells. (Gating strategy for intracellular staining is shown in Fig. S5).

**In vitro cytotoxicity of Radium-1 redirected T cells**

To determine the cytotoxic potential of Radium-1 TCR-transfected CD8+ T cells, mRNA-electroporated T cells were co-incubated with colon cancer cell lines for only 6 h and stained with antibodies against the degranulation marker CD107a and IFNγ (Fig. 2C). Surprisingly, very low levels of IFNγ and
CD107a were detected in the absence of exogenously loaded peptide. Importantly, this was also the case for the original clone, suggesting that both co-receptor independent, Radium-1TCR signal strength in response to cell lines presenting endogenous TGFβRII frameshift peptide may be due to low stability of pMHC complexes. The increased T-cell activation seen upon longer incubation times could be a result of increased level of cumulative TCR stimulation over several hours. Upon the addition of peptide (p573), both Radium-1-re-directed T cells and the original T-cell clone were strongly activated after 6-h incubation. Interestingly, TCR-transfected T cells were more efficient IFNγ producers and also displayed higher levels of degranulation than the original T-cell clone. Since mock-transfected T cells were not activated, this result suggests that the CD8+ co-receptor, although not present in the original clone, could still improve Radium-1 signaling. We further tested the influence of the co-receptor by using HEK 293 cells (HLA-A2 negative) transfected with either HLA-A2 wild type (wt) or a mutant defective in CD8 binding 23 and loaded with peptide to stimulate Radium-1-transfected T cells. The fraction of stimulated redirected T cells was slightly but reproducibly decreased when the mutant was presenting (CD107a+ 36% in the WT versus 26% in the mutant, Fig. S1). Since the original clone was double negative (DN), we investigated if Radium-1-transfected T cells were capable of target cell lysis. We tested Radium-1 reactivity against colon cancer cell lines in 6-h Cr-release assays (Fig. 2D). Radium-1-transfected T cells lysed HCT 116 cells at levels comparable to the original patient T-cell clone. As expected, lysis was further increased when exogenous p573 was added. The lysis of the HLA-A2 negative cell line LS174T was similar to that of mock-transfected T cells demonstrating low background lysis of HCT 116 is likely due to TRAIL-R expression on the target cells (Fig. S2). 24 To validate that the frameshift-mutated TGFβRII T-cell epitope is processed and presented by HLA- A2+ target cells, we also tested Radium-1 TCR reactivity against non-transfected target cells and target cells transfected with mRNA encoding full-length frameshift-mutated TGFβRII (Fig. 2E). As anticipated, the Radium-1 TCR-transfected T cells recognized only the target cells transfected with FL mRNA and not the non-transfected cells (NT). Taken together these data suggest that the Radium-1 is unique in the sense that it is a TCR with a strong co-receptor independency and recognizes a processed and presented T-cell epitope. The TCR signal strength in response to peptide endogenously processed and presented may require cumulative TCR stimulation as longer incubation times are required for full activation of TCR-bearing T cells. One may speculate that this is due to low stability of the pMHC complex. However, due to the ability of Radium-1 to transfer both cytotoxic activity and cytokine release in redirected T cells this prompted us to test its efficacy in vivo.

**Radium-1 TCR-transfected T cells are effective in vitro and in vivo**

We established a xenograft mouse model of colon cancer by intraperitoneal (i.p.) injection of HCT 116 cells modified to express luciferase. Radium-1 2A construct was transferred into a retroviral construct (see Materials and Methods). T cells were transduced with Radium-1 and DMF5 TCR (negative control, 25) and TCR expression was shown to be around 60% for both constructs (Fig. 3A). Prior to injection, T cells were confirmed to be functional against HLA-A2+ EBV-LCLs loaded or not with specific peptides (Fig. 3B). NSG mice were injected i.p. with 10⁶ HCT 116 cells on day 0 (d0) and on d2, d5 and d10 mice were injected with 8×10⁶ and 20×10⁶ redirected T cells, respectively (Fig. 4A). Control mice were treated with T cells from the same donor expressing DMF5. In vivo live imaging of the mice showed that the tumor load was significantly lower (p = 0.043) in mice that received the treatment with Radium-1 T cells compared with the MART-1-specific control T cells (Fig. 4B and Fig. S4). The mice receiving Radium-1 T cells also had enhanced survival compared with control mice (p = 0.0035, Fig. 4C). Tumors from mice that had to be killed due to high tumor load were dissected and single-cell suspension stained with anti-human CD3 and anti-Vβ3 or MART-1 multimer. This revealed that the percentage of TCR expressing T cells in the tumor was significantly higher in Radium-1-treated mice despite a similar transduction efficiency (p = 0.0038, Fig. 4D) indicating that the Radium-1 T cells were either recruited to the tumor more efficiently and/or proliferated in...
vivo due to antigenic stimulation. Taken together, these data demonstrate the pre-clinical potency of Radium-1 TCR in vivo.

**Discussion**

From a long-term surviving patient with MSI+ colorectal cancer vaccinated with a long synthetic TGFβRII(-1A) peptide, we isolated a CD4+ CD8− T-cell clone specific for a CTL epitope of the peptide presented on HLA-A2. The clone was able to lyse HLA-A2+ colon cancer cell lines harboring the specific TGFβRII frameshift mutation in a co-receptor-independent and dose-dependent manner. Since CD8-independence is generally seen as a feature associated with TCRs of high affinity, we therefore hypothesized that the TCR from this clone was a promising candidate for TCR transfer in adoptive cell therapy. We isolated and sub-cloned Radium-1 and used an mRNA transfection protocol to redirect T cells in vitro. Our results demonstrated that the redirected T cells were poly-functional cytokine producers and after overnight stimulation, TNF-α and IFNγ production was observed in both CD8+ and CD4+ T cells. However, after shorter incubation (6 h) with target cells, cytokine production in both CD4+ and CD8+ T cells and the accumulation of CD107a in CD8+ T cells could be detected only in the presence of exogenously loaded peptide. The cognate peptide for Radium-1 is predicted to be a good HLA-A2 binder (IEDB analysis resource Consensus tool), but our preliminary data suggest that the stability of the pMHC complex may be low. It is therefore tempting to speculate that this peptide has a fast K_{OFF} rate and therefore does not stay bound to the HLA-A2 molecule for very long. Supporting this, we were never able to obtain pMHC multimers despite the attempted production by several companies. Cumulative TCR stimulation with increased co-incubation times may compensate for this and could also be important in vivo (reviewed in Ref.28). The difference in activation seen in CD4+ vs. CD8+ TCR-transfected T cells could be due to different activation thresholds, differential requirements for co-stimulatory molecules, and distinct mechanisms for effector cell development which exist in CD4+ and CD8+ T cells.29 The requirement for CD28 co-stimulation in CD4+ T cells can be compensated for by higher concentrations of antigen or longer durations of exposure to antigen, which explains the reduced difference between CD8+ and CD4+ T-cell activation seen after overnight stimulation compared with 6 h. In T cells transduced with a CD8-independent tyrosinase-specific TCR, an important difference in CD4+ T-cell tumor reactivity was observed in vitro.31 Although CD56+, the same kinetics were observed in the original clones, suggesting that the TCR was per se the only killing force. This was finally supported by in vivo experiments: Our xenograft mouse model of colon cancer showed that redirected T cells reduced tumor growth and enhanced survival compared with control T cells expressing a MART-1-specific TCR (DMF5). There are several limitations with the use of such xenograft models. The HCT 116 used is a very fast growing cancer cell line and we therefore have to start treatment as soon as we can detect the tumor engraftment (2 d later). The i.p. injection of this cell line provided a more physiologic model replicating human disease. The disadvantage of this in a therapeutic model is that the tumor cell line tends to grow faster than in subcutaneous models. Infusing human T cells into immunodeficient mice where T-cell xenoreactivity is reported
to be seen from day 30–40 limits the possibility for observation of long lived T-cell responses. We chose not to use systemic dosing of exogenous human IL-2 in this model based on results from other xenograft studies of adoptive T-cell therapy, but still observed live TCR-transduced T cells in excised tumors 20 d post-infusion. In summary, we have demonstrated the ability to confer reactivity against frameshift-mutated TGF/βRII to human T cells. Interestingly, the level of peptide-MHC (pMHC) complexes did not make a difference in cytokine production after 24 h. It is well established that both the MHC class I and antigen expression levels influence CTL recognition. An important difference in these assays was the E:T cell ratio which was 1:2 for intracellular cytokine staining assays, but was titrated down from 100:1 down to 12.5:1 for the 51Cr-release assays. Although the specific lysis was clearly dose-dependent, the pMHC density did not strongly influence the lysis efficacy at high E:T ratios, whereas there is a striking difference between the specific target-cell lysis in the absence and presence of exogenously loaded peptide at lower E:T ratios.

The Radium-1 target antigen, a particular TGF/βRII frameshift mutation (-1A), has been reported to be present in 76% of MSI+ colorectal cancer. Being strictly tumor-specific, redirecting T-cells against this antigen in adoptive cell therapy would reduce the risk of on-target off-tumor toxicity, which was observed in clinical trials using TCRs and CARs targeting overexpressed antigens. Furthermore, several studies have shown that metastatic MSI+ colon cancers have reduced sensitivity to chemotherapy, leaving these patients with few treatment options. Radium-1 may therefore have potential for adoptive T-cell therapy of patients with MSI+ colon cancers, and, in particular, HNPC. Recent clinical results have shown that anti-PD-1 treatment is effective in patients with MSI+ colon cancers. Our observation that HCT 116 cells express high levels of PD-L1 (data not shown) indicate that more robust clinical responses may be obtained by combining anti-PD1/anti-PDL-1 treatment with adoptive T cell therapy using Radium-1 retargeted T cells.

Clinical studies reporting severe toxicities when using engineered T cells targeting MAGE-A3 used high affinity TCRs. While enhancing tumor antigen recognition, such modifications may also cause the receptors to recognize additional and unrelated peptides expressed by normal tissues. Reports have demonstrated that T-cell function reaches a plateau which cannot be further enhanced above a certain TCR-pMHC affinity threshold. Whereas affinity matured TCRs have been shown to enhance the speed of T-cell activation, they may require higher densities of pMHC complexes for the initiation of T-cell responses. The close relationship between T-cell antitumor activity and autoimmunity requires tight regulation of high avidity T-cell responses and therefore TCRs with affinities above the natural range may be inhibited by negative feedback mechanisms. We speculated that since Radium-1 might have been part of the patient beneficial response as is, we would use it as a non-modified TCR. Furthermore, any modification of the protein sequence could result in unpredictable effects for the receiver. This was supported by our assays with a modified version of Radium-1 where extra cysteines were added to the constant part of the TCR and although this improved activity in vitro, it brought conflicting results in vivo (data not shown).

Many tumor antigens targeted with T-cell therapy are non-modified self-antigens. The affinity of TCRs specific for these antigens is limited by central tolerance during T-cell development in the thymus, resulting in decreased antitumor efficacy. Common approaches to overcome this are to generate high-affinity TCRs through mutation of TCR genes from naturally occurring T cells or to generate these TCRs by vaccination of HLA transgenic mice. The first report on TCR therapy in colon cancer was targeting carcinoembryonic antigen (CEA) where some evidence of clinical response was seen, but the T-cell function may have been inhibited due to the necessity to resolve the severe colitis which occurred. This demonstrates not only the feasibility of T-cell therapy in metastatic colon cancer, but also the limitations of targeting CEA as an antigen. Another approach to circumvent central tolerance is to isolate self-antigen-specific TCRs from allogeneic HLA-mismatched TCR repertoires. This could generate TCRs unable to distinguish between cancer cells expressing high levels of the self-antigen and healthy cells expressing low levels of the antigen. TCRs generated in an allogenic setting can also have a high potential for cross-reactivity as shown by Arber et al.

Recent pre-clinical and clinical results support the use of private neoantigens as targets, fitting well with the use of TCRs to target frequent frameshift mutations.

Materials and methods

Ethical approval

The study was approved by the Regional Committee for Medical Research Ethics (Oslo, Norway). All mouse experiments were conducted under an Institutional Animal Care and Use Committee-approved protocol.

Cell lines, media and reagents

A TGF/βRII frameshift mutation-reactive, HLA-A2-restricted CTL clone was isolated from the blood of a MSI+ colon cancer patient and cloned by limiting dilution. The patient had been vaccinated with a 23-mer TGF/βRII (-1A) frameshift peptide. The clinical trial was approved by the Norwegian Medicines Agency, the Committee for Medical Research Ethics Region South and the Hospital Review Board. The treatment was performed in compliance with the World Medical Association Declaration of Helsinki. Informed consent was obtained from the patient. The autologous Epstein–Barr–Virus-transformed lymphoblastoid cell line (EBV-LCLs) was generated by transformation of B cells from the donor. The antigen processing-deficient T2 cell line was used as a T-cell target in flow cytometry and cytotoxicity assays. Colon cancer cell lines HCT 116, SW 480 and LS174T as well as Human Embryonic Kidney (HEK) 293 cells were obtained from ATCC (Rockville, MD, USA). All cell lines were passaged for fewer than 6 mo after their purchase. Human cell line identities were verified using short tandem repeat profiling. Cell lines were tested for mycoplasma contamination using a PCR-based detection kit (Venor®GeM, Minerva Biolabs).

Hek-Phoenix (Hek-P, our collection) were grown in DMEM (PAA Laboratories) supplemented with 10% HyClone FCS (GE...
Healthcare) and 1% antibiotic-antimicrobial (penicillin/streptomycin, p/s, PAA). Where nothing else is indicated, cells were cultured in RPMI-1640 (PAA Laboratories) supplemented with gentamicin, 10% heat-inactivated FCS (PAA Laboratories). Colon cancer cell lines were treated with 500 U/mL IFN-α (AstraZeneca AS), 0.01 M HEPES (Life Technologies, Norway) gentamicin 0.05 mg/mL (Garamycin, Schering-Plough Europe), denoted complete medium hereafter, and otherwise stated.

Generation of T-cell lines and clones specific for TGFβRII frameshift peptides

PBMCs collected pre- and post-vaccination were analyzed. The PBMCs had been isolated and frozen as described previously.50 Thawed PBMCs were stimulated one round in vitro with peptide for 10–12 days and then tested in triplicates in T-cell proliferation assays (3H-Thymidine) using autologous peptide divided by proliferation without peptide and an SI. The stimulation Index (SI) was defined as proliferation with peptide for 10 days and then tested in triplicates.

All T cells were grown in CellGro DC medium (CellGenix GmbH) supplemented with 5% heat-inactivated human serum (Trina Bioreactives AG), 10 mM N-acetylcysteine (Mucomyst 200 mg/mL, AstraZeneca AS), 0.01 M HEPES (Life Technologies, Norway) gentamicin 0.05 mg/mL (Garamycin, Schering-Plough Europe), denoted complete medium hereafter, and otherwise stated.

PBMCs were generated using TGFβRII frameshift peptides. This included peptide 573 (p573), RLSSCVVPVA (amino acid sequence 131–139) and 621 (p621), KSLVRLSSCVPVALMSAMT (amino acid sequence 127–145) from a TGFβRII frameshift protein resulting from a 1 bp-deletion (-1A) in an adenosine stretch (A10) from base number 709–718 of TGFβRII. The GenBank sequence for wild-type human TGFβRII: NM 003242. hTERT peptide I540 (ILAKFLHWL) was used as a negative control. Both peptides were provided by Norsk Hydro ASA.

The MART-1 peptide EAAGIGILTV (amino acid sequence 131–139) and 621 (p621), KSLVRLSSCVPVALMSAMT (amino acid sequence 127–145) from a TGFβRII frameshift protein resulting from a 1 bp-deletion (-1A) in an adenosine stretch (A10) from base number 709–718 of TGFβRII. (The GenBank sequence for wild-type human TGFβRII: NM 003242. hTERT peptide I540 (ILAKFLHWL) was used as a negative control. Both peptides were provided by Norsk Hydro ASA).

T-cell clones were grown in CellGro DC medium (CellGenix GmbH) and resuspended in RPMI-1640 (PAA Laboratories) supplemented with gentamicin, p/s, PAA. Where nothing else is indicated, cells were cultured in RPMI-1640 (PAA Laboratories) supplemented with gentamicin, 10% heat-inactivated FCS (PAA Laboratories). Colon cancer cell lines were treated with 500 U/mL IFN-α (AstraZeneca AS), 0.01 M HEPES (Life Technologies, Norway) gentamicin 0.05 mg/mL (Garamycin, Schering-Plough Europe), denoted complete medium hereafter, and otherwise stated.

In vitro mRNA transcription

The in vitro mRNA synthesis was performed essentially as described previously. Anti-Reverse Cap Analog (Trilink Biotechnologies Inc.) was used to cap the RNA. The mRNA was assessed by agarose gel electrophoresis and Nanodrop (Thermo Fisher Scientific).

In vitro expansion of human T cells

T cells from healthy donors were expanded using a protocol adapted for GMP production of T cells using Dynabeads CD3/CD28 essentially as described previously. Anti-Reverse Cap Analog (Trilink Biotechnologies Inc.) was used to cap the RNA. The mRNA was assessed by agarose gel electrophoresis and Nanodrop (Thermo Fisher Scientific).

Electroporation of expanded T cells

Expanded T cells were washed twice and resuspended in CellGro DC medium (CellGenix GmbH) and resuspended to 70 × 10^6 cells/mL. The mRNA was mixed with the cell suspension at 100 μg/mL, and electroporated in a 4-mm gap cuvette at 500 V and 2 ms using a BTX 830 Square Wave Electroporator (BTX Technologies Inc., Hawthorne, NY, USA). Immediately after transfection, T cells were transferred to complete culture medium at 37°C in 5% CO2 overnight to allow TCR expression.

Antibodies and flow cytometry

T cells were washed in staining buffer (SB) consisting of phosphate-buffered saline (PBS) containing 0.1% human serum albumin (HSA) and 0.1% sodium azide before staining for 20 min at RT. The cells were then washed in SB and fixed in SB containing 1% paraformaldehyde. For intracellular staining, T cells were stained for 6 h or overnight with APCs, loaded or not with p573, at a T-cell to target ratio of 1:2 and in the presence of BD GolgiPlug and BD Golgistop at 1/1,000 dilution.

The HLA-A*0201pC1p102 was cloned as described previously.53 This construct was used as a template to generate a CD8+ binding-deficient mutant by targeting the residues D227 and T228 and replacing them with K and A, respectively, as described by Xu et al.24 A standard site-direct mutagenesis was performed using the following primers: 5′-GAGGACCAGACCCCAGAGCGAGCTCGTGGAGAC-3′ and 5′-GTCTCCAGAGCTCCGGCTTCTG GGTCTGTCTCTC-3′. HEK 293 cells were transfected with these constructs using FuGENE-6 (Roche) following the manufacturer’s protocol.

The sequence of TGFβRII frameshift mutant was ordered as codon optimized to Eurofins MWG Operon (Ebersberg, Germany) and subcloned into pCIpA102 for mRNA production (see next section).
Cells were stained both extracellular and intracellular using the PerFix-nc kit according to the manufacturer’s instructions (Beckman Coulter Inc., USA). The following antibodies were used: Vβ3–FITC (Beckman Coulter-Immunotech SAS, France), CD3-εfluor450, CD4-εfluor 450, CD4-PE-Cy7, CD8-APC, CD8-εfluor 450, CD8-PE-Cy7, CD56-PE-Cy5.5 (BD Biosciences, USA) and CD107a-PE-Cy5 (BD Biosciences, USA). MART-1 (aa 26–35)-specific TCR was detected with dextramer staining (Immudex) following the manufacturer’s recommendations. All antibodies were purchased from eBioscience, except where noted. Cells were acquired on a BD LSR II flow cytometer and the data analyzed using FlowJo software (Treestar Inc.).

**51Cr-release assays**

51Cr-release cytotoxicity assay was performed by labeling of 2 × 10⁶ target cells in 0.5 mL FCS with Na₅¹⁸CrO₄ (7.5 MBq) (Perkin Elmer), for 1 h with gentle mixing every 15 min. Cells were washed three times in cold RPMI-1640 and seeded at 2 × 10⁵ target cells in 96-well, U-bottomed microtitre plates. Autologous EBV-LCL, T2 target cells or colon cancer cell lines HCT 116, SW 480, and LS174T, were pulsed with 10 μM 5-IPTG or 51-p540 for 1 h at 37°C. The original T-cell clone, TCR-transfected T cells, or mock-transfected T cells were added at the effector-to-target (E:T) ratios indicated and the plate was left for 4–6 h at 37°C as indicated. The maximum and spontaneous 51Cr release of target cells was measured after incubation with 5% Triton X-100 (Sigma-Aldrich) or medium, respectively. Supernatants were harvested onto Luma Plates (Packard, Meriden, USA) and transduced twice with retroviral supernatant. Spinoculation of PBMC was performed with I Volume of retroviral supernatant in a 12-well culture non-treated plate (Nunc A/S) pre-coated with reticulin (20 μg/mL, Takara Bio, Inc.). After 2 days, cells were harvested with PBS-EDTA (0.5 mM). Transduced T cells were further expanded using Dynabeads CD3/CD28 as described above.

**Retroviral transduction**

PBMCs isolated from healthy donors were cultured and activated in CellGro DC medium (CellGenix GmbH) supplemented with 5% human serum (HS) and 100 U/mL IL2 (Proleukin, Novartis Healthcare) for 48 h in a 24-well plate pre-coated with anti-CD3 (OKT-3) and anti-CD28 antibodies (BD Biosciences). After 2 d of culture PBMCs were harvested and transduced twice with retroviral supernatant. Spinoculation of PBMC was performed with 1 Volume of retroviral supernatant in a 12-well culture non-treated plate (Nunc A/S) pre-coated with reticulin (20 μg/mL, Takara Bio, Inc.). After 2 days, cells were harvested with PBS-EDTA (0.5 mM). Transduced T cells were further expanded using Dynabeads CD3/CD28 as described above.

**Mouse xenograft studies**

NOD.Cg-Prkdcsid Il2rgtm1Wjl/SzJ (NSG) mice were bred in-house under an approved institutional animal care protocol and maintained under pathogen-free conditions. 6–8 week old mice were injected in intra-peritoneally (i.p.) with 1–1.5 × 10⁶ HCT 116 tumor cells. The HCT 116 cells were engineered with a retroviral vector (provided by Dr Rainer Löw, EUFETS AG) to express firefly luciferase and EGFP. Tumor growth was monitored by bioluminescent imaging using the Xenogen Spectrum system and Living Image v3.2 software. Anaesthetized mice were injected i.p. with 150 mg/kg body weight of D-luciferin (Caliper Life Sciences). Animals were imaged 10 min after luciferin injection.

**Statistical analysis**

Continuous data were described with median, mean and range. The Mann–Whitney test was used for analysis of tumor load, while survival was calculated using the Kaplan–Meier method with the unpaired t-test used for comparison of survival between groups. All p-values given are 2-tailed values. A p-value below 0.05 was considered significant. All statistical analyses were performed using GraphPad Prism® (GraphPad Software, Inc.).

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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**References**

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127:2893–917; PMID:21351269; http://dx.doi.org/10.1002/ijc.25516

2. Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B et al. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. Science 1995; 268:1336–8; PMID:7761852; http://dx.doi.org/10.1126/science.7761852

3. Parsons R, Myeroff LL, Liu B, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B. Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colorectal cancer. Cancer Res 1995; 55:5548–50; PMID:7585632
4. Grady WM, Myeroff LL, Swinler SE, Rajput A, Thiyagalingam S, Lutterbaugh JD, Neumann A, Brattain MG, Chang J, Kim SJ et al. Mutational inactivation of transforming growth factor beta receptor type II in microsatellite stable colon cancers. Cancer Res 1999; 59:320-4; PMID:9927040

5. Tougeron D, Fauquembergue E, Rouquette A, Le Pessot F, Seboue R, Laurent M, Berthet P, Maullon J, Di Fiore F, Sabourin JC et al. Tumor-infiltrating lymphocytes in colorectal cancers with microsatellite instability are correlated with the number and spectrum of frameshift mutations. Mod Pathol 2009; 22:1186-95; PMID:19503063; http://dx.doi.org/10.1038/modpathol.2009.80

6. Di Giorgio A, Botticelli J, Menegotti P, Flammia M. The influence of tumor lymphocytic infiltration on long term survival of surgically treated colorectal cancer patients. Int Surg 1992; 77:256-60; PMID:1478805

7. Smyrk TC, Watson P, Kaul K, Lynch HT. Tumor-infiltrating lymphocytes are a marker for microsatellite instability in colorectal carcinoma. Cancer 2001; 91:2417-22; PMID:11413553; http://dx.doi.org/10.1002/1097-0142(20010615)91:2.3.CO;2-U

8. Banerji A, Bustin SA, Dorudi S. The immunogenicity of colorectal cancers with high-degree microsatellite instability. World J Surg Oncol 2005; 3:26; PMID:15890075; http://dx.doi.org/10.1186/1477-7819-3-26

9. Watt AG, House AK. Colon cancer: a quantitative assessment of lymphocyte infiltration at the periphery of colonic tumors related to prognosis. Cancer 1987; 41:279-82; PMID:626936; http://dx.doi.org/10.1002/1097-0142(197801)1:1.3.CO;2-A

10. Jass JR. Lymphocyte infiltration and survival in rectal cancer. J Clin Pathol 1986; 39:585-9; PMID:3722412; http://dx.doi.org/10.1136/jcp.39.6.3585

11. Drescher KM, Sharma P, Watson P, Gatalica Z, Thibodeau SN, Lynch HT. Lymphocyte recruitment into the tumor site is altered in patients with MSI-H colon cancer. Fam Cancer 2009; 8:231-9; PMID:19166525; http://dx.doi.org/10.1007/s10689-009-9233-0

12. Morgan RA, Chinnasamy N, Abate-Daga D, Gros A, Robbins PF, Zheng Z, Dudley ME, Feldman SA, Yang JC, Sherry RM et al. Cancer progression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. J Immunother 2013; 36:133-51; PMID:23377668; http://dx.doi.org/10.1097/JIT.0b013e3182829903

13. Linette GP, Stadmayer EA, Maus MV, Rapport AP, Levine BL, Emery L, Litzky L, Bagg A, Carreno BM, Cinmino PJ et al. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. Blood 2013; 122:863-71; PMID:23770775; http://dx.doi.org/10.1182/blood-2013-03-490565

14. Cameron BJ, Gerry AB, Dukes J, Harper JV, Kannan V, Bianchi FC, Linette GP, Stadtmauer EA, Maus MV, Rapoport AP, Levine BL, Arnold H, MacDonald ME, Albertson P et al. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced chemokine release in both TRAIL-resistant and TRAIL-sensitive cells via nuclear factor kappa B. J Exp Med 2007; 206:581-93; PMID:17192405; http://dx.doi.org/10.1084/jem.20061185

15. Jorissen RN, Deschepper E, Luyckx B, Depondt C, Dessen P, van den Berghe P, Heirman C, De Vos G et al. Correction for standardizing microarray gene expression data: a critical assessment of methods. Am J Pathol 1986; 39:585-9; PMID:3722412; http://dx.doi.org/10.1136/jcp.39.6.3585

16. Grady WM, Myeroff LL, Swinler SE, Rajput A, Thiyagalingam S, Lutterbaugh JD, Neumann A, Brattain MG, Chang J, Kim SJ et al. Mutational inactivation of transforming growth factor beta receptor type II in microsatellite stable colon cancers. Cancer Res 1999; 59:320-4; PMID:9927040

17. Tougeron D, Fauquembergue E, Rouquette A, Le Pessot F, Seboue R, Laurent M, Berthet P, Maullon J, Di Fiore F, Sabourin JC et al. Tumor-infiltrating lymphocytes in colorectal cancers with microsatellite instability are correlated with the number and spectrum of frameshift mutations. Mod Pathol 2009; 22:1186-95; PMID:19503063; http://dx.doi.org/10.1038/modpathol.2009.80

18. Di Giorgio A, Botticelli J, Menegotti P, Flammia M. The influence of tumor lymphocytic infiltration on long term survival of surgically treated colorectal cancer patients. Int Surg 1992; 77:256-60; PMID:1478805

19. Smyrk TC, Watson P, Kaul K, Lynch HT. Tumor-infiltrating lymphocytes are a marker for microsatellite instability in colorectal carcinoma. Cancer 2001; 91:2417-22; PMID:11413553; http://dx.doi.org/10.1002/1097-0142(20010615)91:2.3.CO;2-U

20. Jass JR. Lymphocyte infiltration and survival in rectal cancer. J Clin Pathol 1986; 39:585-9; PMID:3722412; http://dx.doi.org/10.1136/jcp.39.6.3585

21. Drescher KM, Sharma P, Watson P, Gatalica Z, Thibodeau SN, Lynch HT. Lymphocyte recruitment into the tumor site is altered in patients with MSI-H colon cancer. Fam Cancer 2009; 8:231-9; PMID:19166525; http://dx.doi.org/10.1007/s10689-009-9233-0

22. Morgan RA, Chinnasamy N, Abate-Daga D, Gros A, Robbins PF, Zheng Z, Dudley ME, Feldman SA, Yang JC, Sherry RM et al. Cancer progression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. J Immunother 2013; 36:133-51; PMID:23377668; http://dx.doi.org/10.1097/JIT.0b013e3182829903

23. Linette GP, Stadmayer EA, Maus MV, Rapport AP, Levine BL, Emery L, Litzky L, Bagg A, Carreno BM, Cinmino PJ et al. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. Blood 2013; 122:863-71; PMID:23770775; http://dx.doi.org/10.1182/blood-2013-03-490565

24. Cameron BJ, Gerry AB, Dukes J, Harper JV, Kannan V, Bianchi FC, Grand F, Brewer JE, Gupta M, Plesa G et al. Identification of a Titin-derived HLA-A1-presented peptide as a cross-reactive target for engineered MAGE-A3-directed T cells. Sci Transl Med 2013; 5:197ra03; PMID:23926201; http://dx.doi.org/10.1126/scitranslmed.3006034

25. Lu YC, Robbins PF. Cancer immunotherapy targeting neoantigens. Semin Immunol 2016; 28:22-7; PMID:26653770; http://dx.doi.org/10.1016/j.smim.2015.11.002

26. Offringa R. Antigen choice in adoptive T-cell therapy of cancer. Curr Opin Immunol 2009; 21:190-9; PMID:19297140; http://dx.doi.org/10.1016/j.coim.2009.02.006

27. Schwitalla Y, Linnebacher M, Ripberger E, Gebert J, von Knebel Doeberitz M. Immunogenic peptides generated by frameshift mutations in DNA mismatch repair-deficient cancer cells. Cancer Immun 2004; 4:14; PMID:15563124

28. Schwitalla Y, Kloos M, Eiermann S, Linnebacher M, Kienle P, Knebel HP, Tarverdian M, Benner A, von Knebel Doeberitz M. Immune response against frameshift-induced neoepitopes in HNPPC patients and healthy HNPPC mutation carriers. Gastroenterology 2008; 134:988-97; PMID:18395080; http://dx.doi.org/10.1053/j.gastro.2008.01.015

29. Saetertdal I, Gjertsen MK, Straten P, Eriksen JA, Gaudernack G. A TGF betaRII frameshift-mutation-derived CTL epitope recognised by HLA-A2-restricted CD8+ T cells. Cancer Immunol Immunother 2001; 50:469-76; PMID:11761441; http://dx.doi.org/10.1007/s002620010022

30. Saetertdal I, Bjørheim J, Lisslerud K, Gjertsen MK, Bukholm IK, Olsen OC, Nesland JM, Eriksen JA, Möller M, Lindblom A et al. Frameshift-mutation-derived peptides as tumor-specific antigens in inherited and spontaneous colorectal cancer. Proc Natl Acad Sci U S A 2001; 98:13255-60; PMID:11687624; http://dx.doi.org/10.1073/pnas.231326898
35. Cormier JN, Panelli MC, Hackett JA, Bettinotti MP, Mixon A, Wunderlich J, Parker LL, Restifo NP, Ferrone S, Marincola FM. Natural variation of the expression of HLA and endogenous antigen modulates CTL recognition in an in vitro melanoma model. Int J Cancer 1999; 80:781-90; PMID:10048982; http://dx.doi.org/10.1002/(SICI)1097-0215(19990301)80:5%3C781::AID-IJC2%3E3.0.CO;2-A

36. Marincola FM, Hijazi YM, Fetsch P, Salgaller ML, Rivoltini L, Cormier J, Simons TB, Duray PH, Herlyn M, Kawakami Y et al. Analysis of expression of the melanoma-associated antigens MART-1 and gp100 in metastatic melanoma cell lines and in situ lesions. J Immunother Emphasis Tumor Immunol 1996; 19:192-205; PMID:8811494; http://dx.doi.org/10.1097/00002371-199605000-00004

37. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Marincola FM, Hijazi YM, Fetsch P, Salgaller ML, Rivoltini L, Cormier J, Panelli MC, Hackett JA, Bettinotti MP, Mixon A, Wunderlich J, Parker LL, Restifo NP, Ferrone S, Marincola FM. Natural variation of the expression of HLA and endogenous antigen modulates CTL recognition in an in vitro melanoma model. Int J Cancer 1999; 80:781-90; PMID:10048982; http://dx.doi.org/10.1002/(SICI)1097-0215(19990301)80:5%3C781::AID-IJC2%3E3.0.CO;2-A

38. Bracht K, Nicholls AM, Liu Y, Bodmer WF. 5-Fluorouracil resistance in a large panel of colorectal cancer cell lines is associated with mismatch repair deficiency. Br J Cancer 2010; 103:340-6; PMID:20660684; http://dx.doi.org/10.1038/sj.bjc.6605780

39. Carethers JM, Smith EJ, Behling CA, Nguyen L, Tajima A, Doctolero RT, Cabrera BL, Goel A, Arnold CA, Miyai K et al. Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer. Gastroenterology 2004; 126:394-401; PMID:14762775; http://dx.doi.org/10.1053/gastro.2003.12.023

40. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D et al. PD-1 blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med 2015; 372:2509-20; PMID:26028253; http://dx.doi.org/10.1056/NEJMoai1500596

41. John LB, Devaud C, Duong CP, Yong CS, Beavis PA, Haynes NM, Chow MT, Smyth MJ, Kershaw MH, Darcy PK. Anti-PD-1 antibody therapy potently enhances the eradication of established tumors by gene-modified T cells. Clin Cancer Res 2013; 19:5636-46; PMID:23873688; http://dx.doi.org/10.1158/1078-0432.CCR-13-0458

42. Schmid DA, Irving MB, Posevitz V, Hebeisen M, Posevitz-Fejfar A, John LB, Devaud C, Duong CP, Yong CS, Beavis PA, Haynes NM, Chow MT, Smyth MJ, Kershaw MH, Darcy PK. Anti-PD-1 antibody therapy potently enhances the eradication of established tumors by gene-modified T cells. Clin Cancer Res 2013; 19:5636-46; PMID:23873688; http://dx.doi.org/10.1158/1078-0432.CCR-13-0458

43. Thomas S, Xue SA, Bangham CR, Jakobsen BK, Morris EC, Stauss HJ. Human T cells expressing affinity-matured TCR display accelerated responses but fail to recognize low density of MHC-peptide antigen. Blood 2011; 118:319-29; PMID:21606483; http://dx.doi.org/10.1182/blood-2010-12-326736

44. Zhong S, Malecek K, Johnson LA, Yu Z, Vega-Saenz de Miera E, Darvishian F, McGary K, Huang K, Boyer J, Corse E et al. T-cell receptor affinity and avidity defines antitumor response and autoimmunity in T-cell immunotherapy. Proc Natl Acad Sci U S A 2013; 110:6973-8; PMID:23576742; http://dx.doi.org/10.1073/pnas.1221609110

45. Cohen CJ, Li YF, El-Gamil M, Robbins PF, Rosenberg SA, Morgan RA. Enhanced antitumor activity of T cells engineered to express T-cell receptors with a second disulfide bond. Cancer Res 2007; 67:3898-903; PMID:17440104; http://dx.doi.org/10.1158/0008-5472.CAN-06-3986

46. Parkhurst MR, Yang JC, Langan RC, Dudley ME, Nathan DA, Feldman SA, Davis JL, Morgan RA, Merino MJ, Sherry RM et al. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. Mol Ther 2011; 19:620-6; PMID:21157437; http://dx.doi.org/10.1038/mt.2010.272

47. Xue S, Gao L, Gillimore R, Bendle G, Holler A, Downs AM, Tsallios A, Ramirez F, Ghani Y, Hart D et al. WT1-targeted immunotherapy of leukaemia. Blood Cells Mol Dis 2004; 33:288-90; PMID:15281486; http://dx.doi.org/10.1016/j.bcmd.2004.08.018

48. Arber C, Feng X, Abhyankar H, Romero E, Wu MF, Heslop HE, Barth P, Dotti G, Savoldo B. Survivin-specific T cell receptor targets tumor but not T cells. J Clin Invest 2015; 125:157-68; PMID:25415440; http://dx.doi.org/10.1172/JCI75876

49. Tran E, Turcotte S, Gros A, Robbins PF, Lu YC, Dudley ME, Wunderlich JR, Somerville RP, Hogan K, Hinrichs CS et al. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. Science 2014; 344:641-5; PMID:24812403; http://dx.doi.org/10.1126/science.1251102

50. Brunsvig PF, Aamdal S, Gjertsen MK, Kvalheim G, Markowski-Grimsmrud CJ, Sve I, Dyrhaug M, Trachsel S, Møller M, Erikson JA et al. Telomerase peptide vaccination: a phase I/II study in patients with non-small cell lung cancer. Cancer Immunol Immunother 2006; 55:1553-64; PMID:16491401; http://dx.doi.org/10.1007/s00262-006-0145-7

51. Saebø-Larssen S, Fossberg E, Gaudernack G. mRNA-based electrotansfection of human dendritic cells and induction of cytotoxic T lymphocyte responses against the telomerase catalytic subunit (hTERT). J Immunol Methods 2002; 259:191-203; PMID:11730854; http://dx.doi.org/10.1016/S0022-1759(01)00506-3

52. Walchli S, Loset GA, Kumari S, Johansen JN, Yang W, Sandlie I, Oweeus J. A practical approach to T-cell receptor cloning and expression. PloS One 2011; 6:e27930; PMID:22132171; http://dx.doi.org/10.1371/journal.pone.0027930

53. Brunsvig PF, Aamdal S, Gjertsen MK, Kvalheim G, Markowski-Grimsmrud CJ, Sve I, Dyrhaug M, Trachsel S, Møller M, Erikson JA et al. Telomerase peptide vaccination: a phase I/II study in patients with non-small cell lung cancer. Cancer Immunol Immunother 2006; 55:1553-64; PMID:16491401; http://dx.doi.org/10.1007/s00262-006-0145-7

54. Ericsson J, Liljeström P, Johansson SE, Ringden O, Nilsson S, Sjöstrand A, Sjoback F, Kallberg M, Nilsson A, Eklund A et al. The immunogenicity of a T-cell receptor targets tumor but not T cells. J Clin Invest 2015; 125:157-68; PMID:25415440; http://dx.doi.org/10.1172/JCI75876

55. Tran E, Turcotte S, Gros A, Robbins PF, Lu YC, Dudley ME, Wunderlich JR, Somerville RP, Hogan K, Hinrichs CS et al. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. Science 2014; 344:641-5; PMID:24812403; http://dx.doi.org/10.1126/science.1251102

56. Brunsvig PF, Aamdal S, Gjertsen MK, Kvalheim G, Markowski-Grimsmrud CJ, Sve I, Dyrhaug M, Trachsel S, Møller M, Erikson JA et al. Telomerase peptide vaccination: a phase I/II study in patients with non-small cell lung cancer. Cancer Immunol Immunother 2006; 55:1553-64; PMID:16491401; http://dx.doi.org/10.1007/s00262-006-0145-7