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Genetic manipulation and genetic-based dissection of tumor-specific immunity

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Chapter 4

Cdc25 Inhibitor Improves Transposon-Mediated Gene Modification of Human T Lymphocytes

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Vectors derived from integrating viruses currently form the gold standard for clinical T cell engineering. An interesting alternative with respect to cost and in particular versatility is represented by non-viral gene delivery systems, such as the Sleeping Beauty transposon-system. At present, the usage of this system is hampered by the high toxicity of the initial plasmid transfection step and lower gene modification efficiency, as compared to viral vector systems. In order to identify small molecule compounds that increase the performance of transposon-based gene modification systems, we conducted a high throughput screen of a library of compounds with defined pharmacological activity. Using this setup, we identified a Cdc25 inhibitor that both improves transfection efficiency and reduces cellular toxicity in human lymphocytes. Inclusion of Cdc25 inhibitor leads to a 60% increase in gene-modified T cells and a 46% increase in cell viability, while leaving phenotype and function of the resulting T cells unaltered. Our results demonstrate that limitations associated with transposon-mediated gene delivery can be pharmacologically mitigated, providing an interesting strategy to facilitate clinical application.

Introduction

Clinical and experimental evidences demonstrate that T cells have the potential to recognize and kill tumor cells. In tumors with a high mutational load, 'neo-antigens' that are formed as a consequence of DNA mutations can lead to a high affinity T cell response[1]. However, in many human tumors, the anti-tumor potential of the endogenous T cell pool is poor, both because the frequency of tumor reactive T cells is low, and because T cells directed against non-mutated tumor-associated self antigens can express low affinity receptors.

Over the past years, compelling preclinical and clinical evidence has been obtained indicating that the lack of antigenicity of such tumors can be overcome by *in vitro* gene modification of T cells with either chimeric antigen receptors or T cell receptors specific for tumor-associated antigens[2-8]. The most broadly used strategy for such T cell engineering relies on the use of integrating lentiviral and retroviral vectors. With now more than 10 years of clinical experience, the risk of adverse events, such as insertional mutagenesis, following viral modification of human T cells appears minimal, and the gene modification efficiencies that can be obtained are generally high. As a significant down side, the time and cost required for the production of clinical grade virus batches is significant, thereby making the flexibility of these systems low.

With the emerging evidence for the clinical activity of receptor-modified T cells[2-8], it has become important to assess the safety and efficacy of CARs and TCRs for a series of different antigens in the coming years. Furthermore, in order to exploit the patient-specific repertoire of tumor-specific TCRs for therapy (discussed in [9]), a highly flexible system for gene transfer will also be required. In this regard, transposon-based gene transfer systems represent an interesting alternative over viral vector systems, as their production is substantially less costly and time-consuming.

Transposon-based gene delivery systems rely on the activity of a transposase enzyme that is able to catalyze the genomic integration of the transposon, a gene expression cassette containing the gene of interest that is flanked by inverted regions (IR). Both the Sleeping Beauty transposon and PiggyBac transposon systems have successfully been used for human T cell modification[10,11] and recently the SB system has been used to generate chimeric antigen receptor-modified T cell products for patient treatment[12]. Furthermore, *in vitro* molecular evolution has led to the generation of hyperactive transposase variants, such as the SB transposase 100X, that yield higher efficiencies of gene transfer[13].

While conceptually appealing, widespread clinical application of transposon-based systems for T cell modification is hindered by two issues: First, introduction of DNA into human T cells leads to significant cell death, and while the required transposase activity may be obtained by transfection of encoding RNA[14], the delivery of the transposon itself in the form of DNA is obligatory. Second, and possibly related to this toxicity, the efficiency of transposon-mediated T cell modification is generally lower than that obtained with virus-based delivery systems.

As the toxicity that is seen upon DNA (but not RNA) transfection is likely to be mediated by the intracellular sensing of incoming DNA, we reasoned that pharmacological modulation may potentially reduce these issues. In order to evaluate this, we established a high throughput screening system in which we tested the effects of more than 1,200 compounds on T cell viability and gene modification efficiency upon SB100X-mediated gene transfer into human peripheral blood mononuclear cells (PBMC). This screen identified the irreversible Cdc25 inhibitor NSC 95397 as a compound that is able to increase both T cell viability and gene modification efficiency upon transposon-mediated gene transfer. Importantly, TCR gene-modified T cells generated in the presence of NSC 95397 show similar activity as compared to untreated cells in terms of specific cell killing and cytokine secretion. To our knowledge, this is the first study demonstrating that the efficiency of transposon-based gene delivery systems can be pharmacologically improved, providing a novel avenue to facilitate clinical application of these gene transfer systems.

Results

A high throughput screen to identify molecules that influence transposon-mediated gene transfer into human T cells

In order to evaluate whether pharmacological modulation can be used to enhance the efficacy of transposon-mediated gene modification, we set up a system to analyse the effects of a library of more than 1,200 compounds with described pharmacological activity (library of pharmaceutically active compound, LOPAC) in a high throughput fashion. To this purpose, T cells were transfected with the SB100X system and distributed over 384 well plates containing the individual compounds (Fig. 1A). 24 h later, T cells were activated and at day 5 following gene modification, cells were evaluated by flow cytometry for viability and transfection efficiency.

This initial LOPAC screen revealed a series of compounds that had a beneficial effect on either cell viability or transgene expression relative to controls (Fig. 1B). To confirm and narrow down the optimal concentration, the effects of the ten compounds

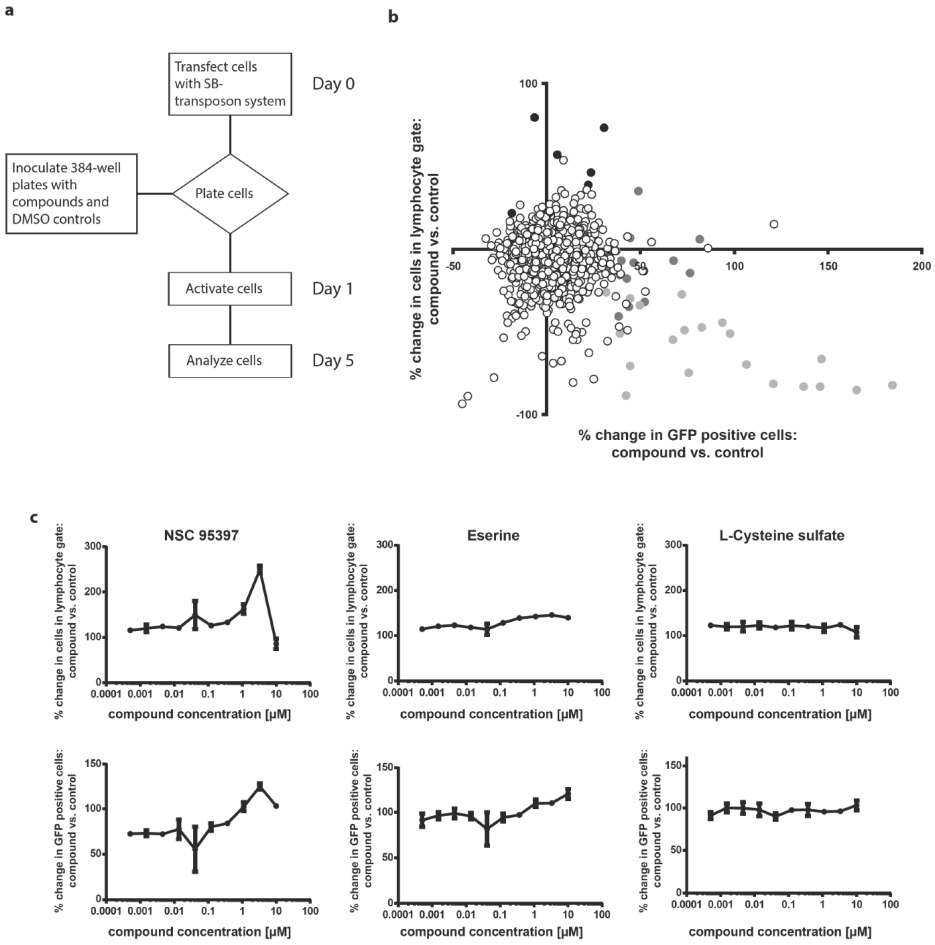

Fig. 1. LOPAC screen and hit validation. a). Experimental scheme of the high throughput compound screen. Frozen PBMCs were thawed, 24h later, cells were electroporated and directly transferred to wells containing individual compounds (1 µM). 24h after electroporation, T cells were stimulated using phytohemagglutinin. Cell viability and transfection efficiency were analyzed by flow cytometry at day 5 after transfection. b). Compound effects on transfection efficiency and cell viability. Dots indicate the effects of individual compounds on viability and transfection efficiency, as compared to untreated transfected cells. Black: significant increase in survival, no significant effect on transfection efficiency. Dark grey significant increase in transfection efficiency, no significant effect on survival. Light grey significant increase in transfection efficiency, significant decrease in survival. c). Compound effect validation. Effect of three hits identified within the primary LOPAC screen on survival (upper panels) and transgene expression (lower panel) of SB100X transfected cells at the indicated concentrations. Of the primary hits, only NSC 95397 showed a statistically significant effect in confirmation experiments.

possessing the highest effect on at least one of the two assessed parameters were evaluated at a series of concentrations (Fig. 1C and data not shown). Of these ten compounds, the irreversible Cdc25 inhibitor NSC 95397 showed the most pronounced effects, with optimal activity at concentrations between 1 – 5 µM. Inhibition of Cdc25 by NSC 95397 has previously been shown to induce cell cycle arrest at the G2/M transition in various cell types, but its effect on T cell expansion has to our knowledge not been reported (see below). The positive effect of NSC 95397 on T cell modification was not restricted to the conditions used for high throughput screening, but was also observed in three independent donors under the standard (larger scale) conditions for SB transposon-mediated gene transfer (Fig. 2). Increased cell viability was both observed when assessed by morphology (fsc/ssc, as performed in the initial screen), and when assessed by propidium iodide staining (data not shown). Specifically, at day six, the fraction of viable cells in samples treated with 5 µM NSC 95397 was on average 34% higher than in non-treated controls (Fig. 2A-B). Treatment with NSC 95397 also had a beneficial effect on the frequency of stable gene modification assessed thirteen days after transfection (Fig. 2C, average increase of 38% at 5 µM).

To evaluate whether the results obtained with these three donors were significant over a large set of experiments, we analysed the effects of NSC 95397 inclusion in a set of technical replicates. The average viability of cells treated with NSC 95397 relative to non-treated controls six days after transfection (n=52) was 146% (Fig. 2E), and the average percentage of cells showing stable transgene expression (n=19) was 160% relative to matched controls (Fig. 2F and table S1). Non-parametric tests comparing viability and stable GFP transgene expression for paired samples in this data set revealed that the effects of NSC 95397 on both viability and transgene expression were highly significant (P-values of 0.0003 and <0.0001, respectively).

As during transposon-mediated gene transfer, both the introduction of foreign DNA into the cells and the subsequent DNA integration event may conceivably lead to a cellular stress response, we explored whether the positive effects of cdc25 inhibiton requires transposase-mediated DNA integration. To assess whether NSC 95397 acts by preventing a DNA damage stress response caused by the transposase-mediated integration event, we determined the effect of NSC 95397 addition when using a catalytically inactive form of the SB transposase (SB-D3). Also in this setting, the improvement in viability at day six relative to control was substantial (Fig. 3A, long term transgene expression not assessed for obvious reasons). This indicates that the positive effect of NSC 95397 is not based on preventing a fatal DNA damage stress response caused by the transposition event, but rather is likely to dampen the adverse effects of DNA introduction.

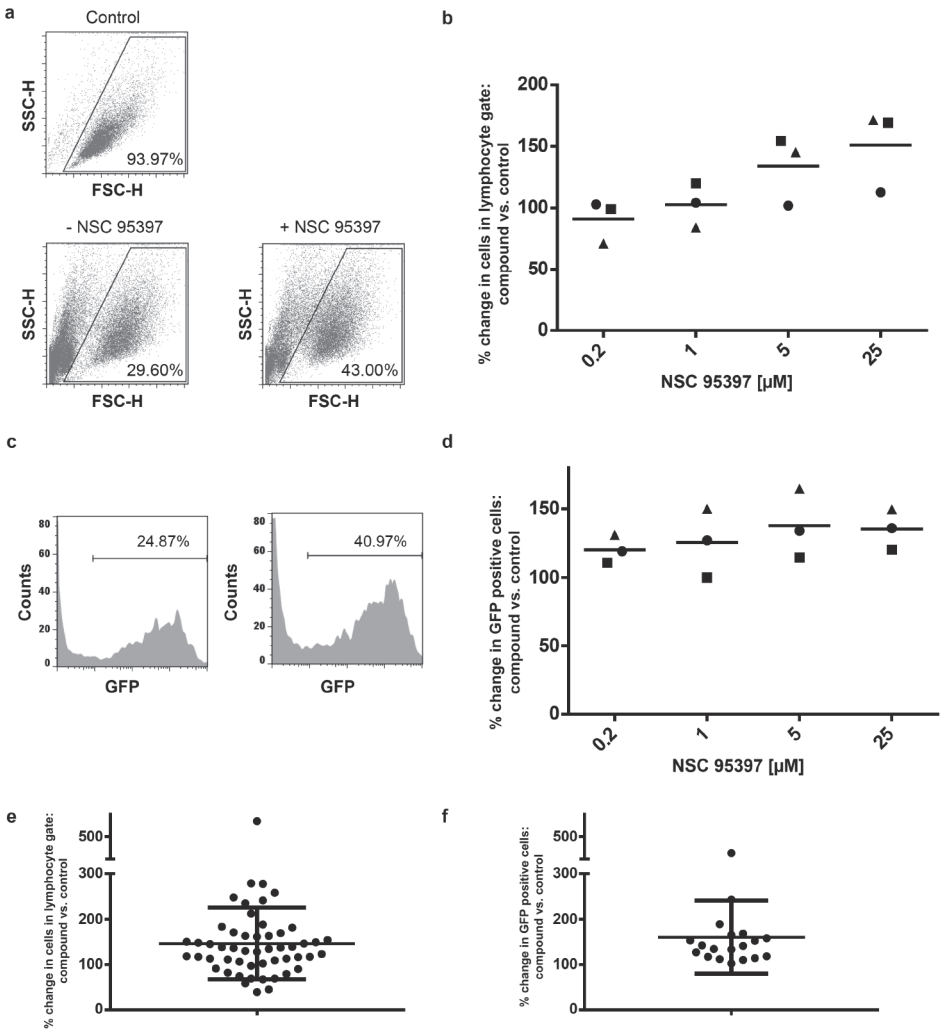

Fig. 2. Effect of NSC 95397 on T cell survival and transfection efficiency. a,b). Effect of the indicated NSC 95397 concentrations on T cell survival upon SB100X mediated T cell modification under standard (large scale) conditions. c,d). Effect of the indicated NSC 95397 concentrations on transfection efficiency upon SB100X mediated T cell modification under standard (large scale) conditions. e,f). Effect of NSC 95397 (5 µM) on T cell survival and transfection upon SB100X mediated T cell modification in a large series of experiments. Effects on both survival (e) and efficiency (f) are significant ($p = 0.0003$ and $p < 0.0001$, respectively).

Effect of NSC 95397 on the PiggyBac (PB) transposon system

To address whether the effects of NSC 95397 are unique to the SB100X transposon system or can be extended to other non-viral gene transfer systems, we compared the efficiency of PiggyBac (PB) transposon-mediated gene transfer in the presence or absence of this compound. In three donors tested, treatment with NSC 95397 increased the fraction of viable cells on day six after transfection on average by approximately 50% (Fig. 3A). Furthermore, while the frequency of stable gene modification using the PB transposon-system is lower than observed for the SB100X-system, the relative improvement obtained by NSC 95397 addition was similar for both systems (Fig. 3A-B).

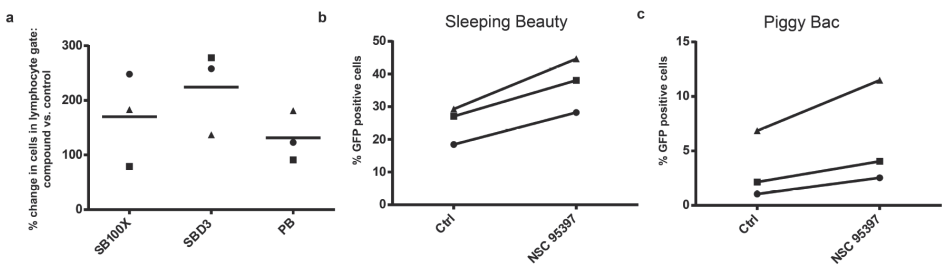

Fig. 3. Effect of NSC95397 addition on different transposon-based gene transfer systems. a). Improvement in human T cell survival upon transfection of PBMCs with SB100X, catalytically inactive SB (SBD3), or PiggyBac by NSC95397 addition. b). Improvement in SB100X mediated gene transfer in human T cells by NSC95397 addition. c). Improvement in PiggyBac-mediated gene transfer in human T cells by NSC95397 addition.

Effect of NSC 95397 on phenotype and functionality of human T cells

To determine whether NSC 95397 treatment influences various aspects of T cell behaviour, transposon modified T cells were generated from three donors. In these experiments, T cells were genetically engineered to express the HLA-A2 restricted, MART-1 specific 1D3 T cell receptor as a clinically relevant transgene [15]. In this small data set, the percentage of transgene expressing cells was not significantly improved by NSC 95397 treatment (data not shown). However, data from a larger series of experiments (n=12) indicates that NSC 95397 also mildly increases the percentage of cells with stable TCR transgene expression (20% increase, $p<0.01$ Fig. S1).

Analysis of the expression of a number of cell surface markers that reflect cell differentiation state (CD62L, CD45RO, CD45RA, CD27) after 13 days of culturing revealed that the differentiation state of T cells exposed to NSC 95793 was comparable to that of untreated transposon-modified T cells (Fig. 4A-B,D). Specifically, in both cases, the majority of T cells consisted of a mixture of cells with effector memory and central memory phenotypes, as based on CD45RA and CD45RO expression. Likewise, the fraction of CD8 positive and negative cells was unaltered by NSC 95793 addition (Fig. 4C).

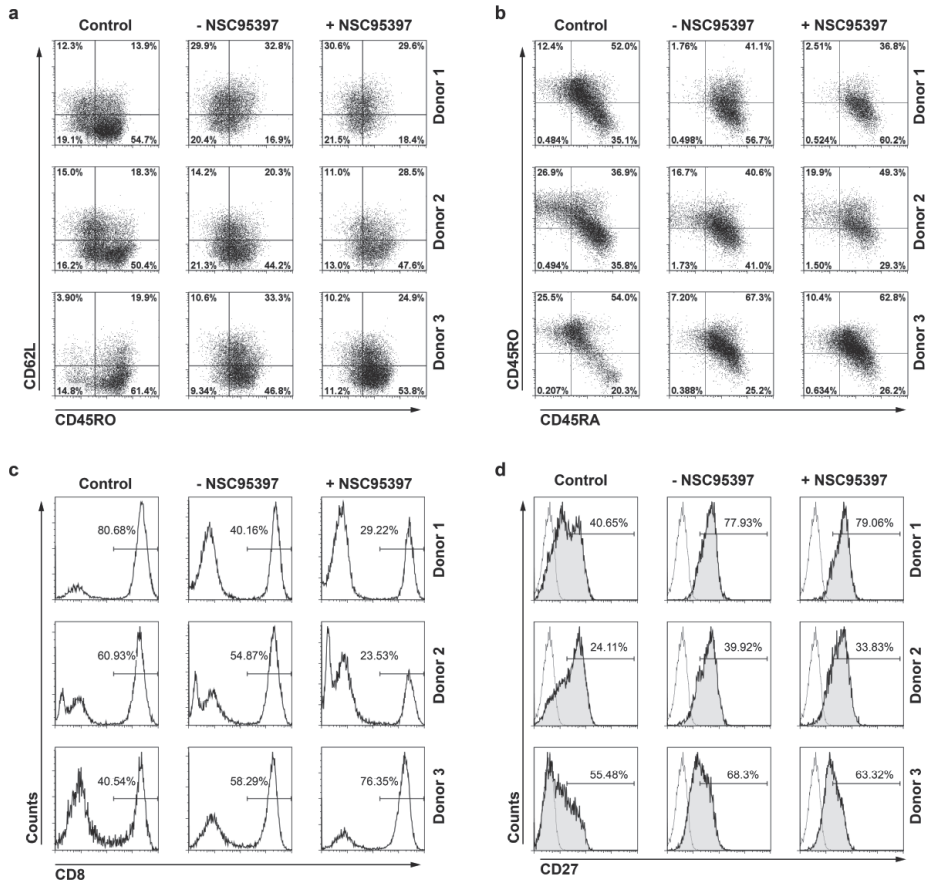

Fig. 4. Unaltered phenoptype of NSC 95397 exposed T cells. Phenotypic characteristics of transfected cells that were either untreated or treated with NSC 95397. Expression of CD45RO and CD62L (a), CD45RO and CD45RA (b), CD8 (c), or CD27 (d) is depicted for NSC 95397 treated and untreated cells, and for untransfected control cells.

To address whether NSC 95397 treatment influences T cell functionality, lymphocytes from those three donors were analyzed for antigen-induced cytokine production and target cell lysis. For T cells from all three donors, the fraction of T cells producing either IL-2, INF-γ, or both were comparable between the two groups over the entire peptide concentration range tested (Fig 5A). To determine lytic capacity, engineered T cells were co-cultured with HLA-A2 positive or negative tumor cell lines expressing MART-1 (in this experiment, T cell receptor expression within the CD8 compartment was near equal for all groups, thereby allowing intergroup comparison). Specific lysis observed upon encounter of relevant tumor cell lines was near identical between the two groups and background activity against an HLA-A2 negative tumor line was equally low (Fig 5B), indicating that also by this criterion, functional activity of NSC 95397 treated cells is fully maintained.

Effect of NSC 95397 on the expansion rate of transposon-modified T cells

In order to generate T cell products for adoptive cell therapy, a shorter *ex vivo* expansion period is considered preferable, as prolonged proliferation biases T cells towards terminal differentiation [16]. To determine whether the enhanced viability of NSC 95397 treated cells translates into an increased expansion capacity, growth curves of transposon-modified T cells from the three donors were generated (Fig. 6A). For all three donors, the fold expansion achieved at day 13 post-transfection was at least three to four times higher for cells treated with NSC 95397 as compared to controls. Importantly the percentage of 1D3 TCR expressing cells was maintained at the same level after 13 days of culturing, showing that there is no growth bias for non-modified T cells in the NSC 95397 treated group (Fig. 6B).

Discussion

At present, retroviral and lentiviral vector systems represent the gold standard for genetic redirection of T cells for adoptive T cell therapy[17]. However, to allow the evaluation of large series of antigen-specific TCRs and CARs, and to assess the potential of personalized TCR gene therapy, alternative systems that allow rapid and cost effective clinical testing of series of receptors would be of value.

Transposon-based systems have significant potential in this respect, as the required GMP grade DNA production is low-cost and rapid, and can easily be performed for multiple transgenes in parallel, without any loss of performances in T cells[18]. At present, the largest obstacle for widespread use of transposon-based systems for human T cell modification is formed by the high cell loss during transfection[19] and

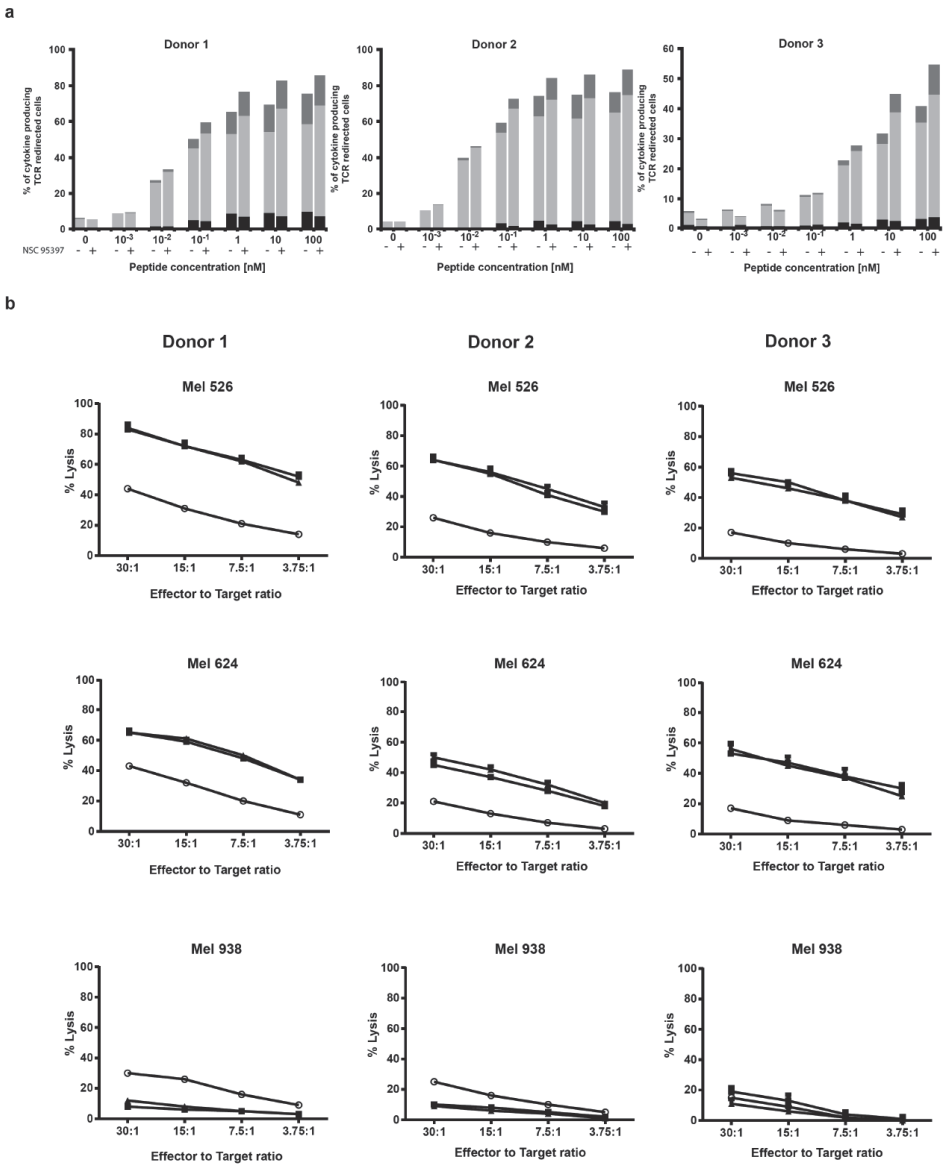

Fig. 5. Cytokine secretion and cytolytic capacity of SB TCR gene modified T cells is maintained upon NSC 95397 exposure. a). PBMC of three different donors were modified with the 1D3 TCR by SB100X-mediated gene transfer. Following cell expansion, the ability of T cells to secrete cytokine upon exposure to T2 cells pulsed with the indicated concentrations of peptide was evaluated. For every peptide concentration, the left and right bar represent untreated samples and NSC 95397 treated samples, respectively. b). PBMC of three different donors were modified with the 1D3 TCR by SB100X-mediated gene transfer (Squares: NSC 95397 treated and transfected; triangles untreated and transfected; open circles: untransfected). Following cell expansion, the lytic capacity of the resulting T cells was evaluated by incubation with relevant (Mel 526 and Mel 624) or control (Mel 938) melanoma lines.

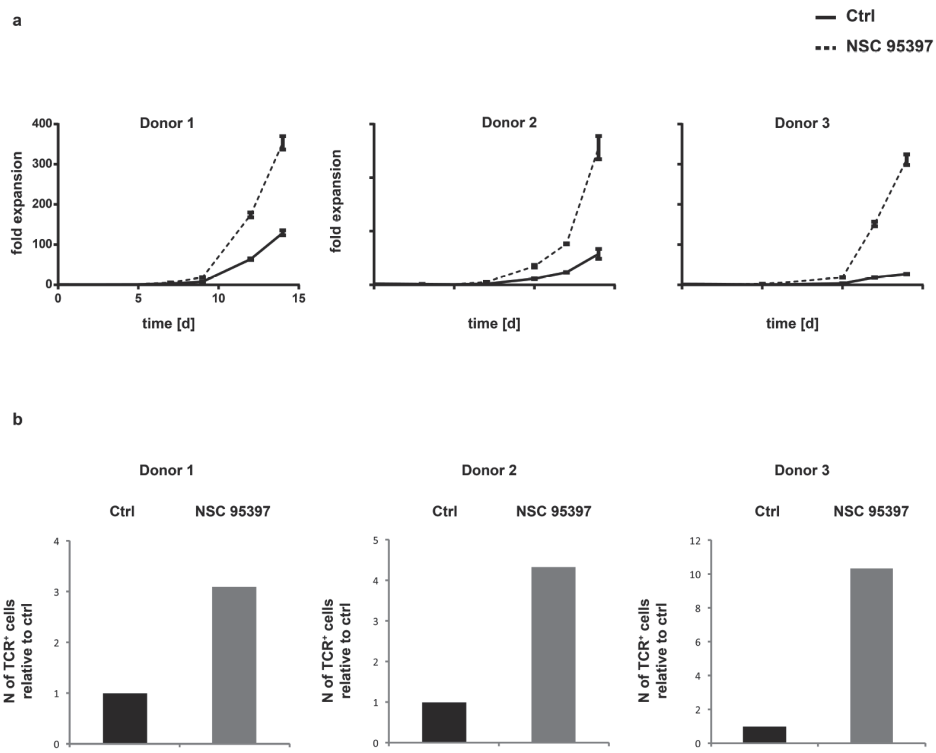

Fig. 6. Improved expansion of NSC 95397 exposed T cells. a) Growth kinetics of NSC 95397 and untreated transfected T cells from three different donors. Cell counts at the indicated time points from the day after transfection show that cells treated with NSC 95397 recover faster from transfection than untreated counterparts. b) Relative fraction of 1D3+ T cells in NSC 95379 treated and untreated transfected T cells.

– possibly because of this – the suboptimal frequency of T cells that stably express the transgene used. In order to mitigate these issues, we here screened a library of 1,280 pharmacologically active compounds for their capacity to improve transposon-mediated gene delivery.

The most significant hit obtained in this screen was the irreversible Cdc25 inhibitor NSC 95397. Under optimized conditions, NSC 95397 addition increased T cell survival at day six after transfection by on average 40% relative to control, and this survival advantage at early time points translated to a very substantial increase in the number of T cells obtained during subsequent culture. The addition of NSC 95397 also led to a significant increase in the percentage of T cells showing stable transgene expression (average increase of 60% relative to control for GFP gene-modified T cells). A possible

explanation for the observation that NSC 95397 addition increases both cell survival and gene modification efficiency is that those cells that take up large amounts of DNA experience higher amount of stress. As such, the cells that are most likely to integrate the transposon are also most likely to benefit from NSC 95397 inclusion. Others have observed that alteration in the cell cycle can impact on Sleeping Beauty transposition[20].

While the effect of NSC 95397 on cell survival was equally pronounced when introducing either a GFP or TCR transgene, the effect on gene modification frequency was more profound for the GFP-encoding than for the TCR-encoding transposon (60%, $p< 0.0001$ relative to control condition versus 20%, $p< 0.01$ relative to control condition). In general, stable gene modification frequencies achieved upon TCR introduction without NSC 95397 addition were higher than for GFP (table S1-2), and also cell recovery was faster. Conceivably, the effect of NSC 95397 addition on gene modification frequency is most profound when utilizing transgenes that are more stressful to cells. This effect may be due to specific motifs within the transgenic DNA sequence or because of the protein encoded. In theory, cellular stress may also be influenced by transgene size. However, as the two transgene cassettes have a similar size (2.7 kb for the GFP vector versus 3.0 kb for the TCR vector), this is excluded as a potential factor here.

Importantly, although the effect of NSC 95397 addition on transfection efficiency is moderate, the combined effect of increased transfection efficiency and faster recovery of cells after transfection allow the production of a clinical grade cell product from at least 5 times less starting material. Furthermore, activity is not restricted to the SB system, but is also observed for the PiggyBac transposon system, making it unlikely that the compound directly interacts with the transposase.

The current data underline the value of unbiased screening, as *a priori*, it would have been difficult to predict the survival benefit of an anti-mitotic drug such as NSC 95397. The data presented here indicate that pharmacological optimization of transposon-mediated gene delivery is feasible, and it is interesting to note that another Cdc25 inhibitor, ARQ 501, has previously been tested in clinical trials. While further mechanistic data are required, the available data suggest that NSC 95397 may increase cell modification efficiency by inhibiting a cell cycle stage dependent pathway that 'senses' incoming DNA. We speculate that with our increased understanding of the molecules that detect incoming DNA[21-23], additional interventions that inactivate this cellular pathway may be developed within the coming years.

Material and Methods

Vectors, transposon-mediated gene modification and cell culture: The transposon vector pT2-CAGGS-GFP, encoding GFP under control of the chicken albumin promoter, and the vectors pCMV-SB100X and pCMV-SB-D3, encoding the hyper active SB100X and inactive variant of the Sleeping Beauty transposase under the control of the CMV promoter, respectively, were generously provided by Z. Izsvak (Max Delbrück Institute) and have been described previously[13]. The transposon vector encoding the codon optimized MART-1 specific T cell receptor 1D3[15] under the control of the MP71[242] promotor was generated by cloning the corresponding expression cassette[25] between the EcoRI and NotI sites of pT2-HB[26]. For experiments involving the PiggyBac transposon-system, the transposase was expressed from a CMV expression plasmid, and GFP was expressed under the control of the chicken albumin promoter from the PiggyBac vector pXL-BacII-GFP, both a kind gift of M. H. Wilson (Baylor College of Medicine).

Melanoma cell lines Mel 526 (HLA-A2$^+$, MART-1$^+$), Mel 624 (HLA-A2$^+$, MART-1$^+$) and Mel 938 (HLA-A2$^-$, MART-1$^+$), and the TAP-deficient T·B cell hybrid T2-A1 (HLA-A1$^+$/A2$^+$) have been described previously[27,28]. All cell lines were maintained in RPMI (GIBCO, Invitrogen, Carlsbad, CA), in the presence of 10% FCS. Peripheral blood mononuclear cells (PBMCs), derived from buffy coats from anonymous healthy donors (Sanquin, Amsterdam, The Netherlands) were isolated by Ficoll-Isopaque density centrifugation and cryopreserved in liquid nitrogen.

For gene transfer, cells were transfected using the Amaxa Nucleofector 4D system (Lonza, Basel, Switzerland), according to the manufacturer's protocol. In brief, PBMCs were thawed one day prior to nucleofection and cultured in RPMI, supplemented with 10% human serum, 50 IU/ml IL-2 (Novartis, Basel, Switzerland) and 10 ng/ml IL-15 (Peprotech, Rocky Hill, NJ). On the day of transfection, cells were harvested and washed once with PBS/ 0.5% BSA. For each transfection reaction, 8 x 10^6 cells were resuspended in 100µl nucleofection reagent P3 and transferred into a cuvette together with 5 µg of transposase and 10 µg of transposon DNA, respectively. For all transfections, program E0-115 was used. Following transfection, prewarmed medium was added to the cells and cells were transferred into a 24-well plate either without compounds, or in the presence of indicated compounds. One day after transfection, T cells were activated using anti-CD3/anti-CD28 Human T-Activator beads, at 1 bead/ cell (Invitrogen, Carlsbad, CA), and maintained in culture as described above. The compound was kept in the culture medium until day 5 post transfection, when it was diluted out by passaging the cells.

LOPAC screen: Compounds that influenced gene modification efficiency and/ or cell viability were identified in 4 consecutive screens, with each screen covering one quarter of the Library of Pharmaceutically Active Compounds (LOPAC^{1280}) (Sigma Aldrich, St Louis, MO). Screens were performed in duplicate, on two identically prepared 348-well plates. 11 x 10^7 cells were transfected with the combination of SB100X transposase vector and the GFP encoding transposon pT2-CAGGS-GFP, as described above. Cells were pooled and subsequently plated into 348-well plates (40µl per well), pre-filled with individual compounds or solvent controls (40µl per well), at a final concentration of 1 µM for each compound. After 24h, T cells were activated by addition of phytohaemagglutinin-L (Biochrom, Berlin, Germany) at 2 µg/ml. Four days after activation, cells were analyzed for viability and GFP expression on a FACSCalibur equipped with a high throughput sampling device (Becton Dickinson, Franklin Lakes, NJ). Compound effects were defined as significant when for both duplicates the change relative to control in either viability or gene expression was larger than twice the standard deviation measured for the solvent controls (n= 32) included in both plates.

Cytokine production and chromium release assays: T2 cells were pulsed with peptides for 1-2 hours at 37°C. Subsequently, 0.5×10^6 TCR-modified PBMCs were incubated with 0.5×10^6 peptide-pulsed T2 cells in RPMI containing 10% FCS and 1µL/mL Golgiplug (BD Biosciences, Basel, Switzerland). After a 4- to 5-hour incubation at 37°C, cells were washed and stained with FITC-labeled anti-CD3 antibody and PerCP-Cy5 labeled anti-CD8 antibody, and analyzed for IFN-γ and IL-2 production by intracellular cytokine staining (all antibodies from BD Biosciences, Basel, Switzerland). For every group, cytokine production was normalized to the percentage of TCR-modified T cells. In order to measure cytotoxic activity of TCR-modified T cells, target cells were labeled with 100 µCi (3.7 MBq) ^{51}Cr (GE healthcare, Little Chalfront, UK) for 1 hour at 37°C. Labeled target cells were incubated with effector cells at the indicated E/T ratios for 4 hours at 37°C in 200 µL RPMI containing 10% FCS. Subsequently, 50 µl of supernatant was transferred onto LumaPlates (Perkin Elmer, Waltham, MO) and analyzed on a TopCount NXT (Perkin Elmer, Waltham, MO).

Flow cytometric analysis: TCR cell surface expression on gene-modified PBMCs was measured by staining with MHC multimers generated through UV-induced peptide exchange [29,30], in combination with allophycocyanin (APC) labeled anti-CD8 (Becton Dickinson, Franklin Lakes, NJ) staining. Phenotypic properties of cells were assessed by staining with FITC-labeled anti-CD62L, PE-labeled anti-CD45RO, APC-labeled anti-CD45RO, and FITC-labeled anti-CD27 antibody (all Becton Dickinson, Franklin Lakes, NJ). For analysis of intracellular cytokine production, cells were stained with PE-

labeled anti-IL2, and APC-labeled anti-INFγ antibody (both Becton Dickinson, Franklin Lakes, NJ). Cells were analyzed using a FACSCalibur (Becton Dickinson, Franklin Lakes, NJ) and data were analyzed with FlowJo 7.6.

Cell expansion analysis: Cells were counted in triplicates at the indicated time points using the CASY cell counting system (Innovatis, Zurich, Switzerland). At each time point, 10^6 cells were placed back into a 24-well plate and kept under standard culture conditions. For each pair of consecutive time points, fold expansion was calculated as (cell count at time point n / cell count at time point n-1), and expansion curves were created by multiplication of consecutive expansion values.

Statistical analysis: Wilcoxon signed-rank tests were used to assess statistical significance of differences in absolute cell numbers and gene modification efficiencies between the indicated groups. Statistical analyses were performed using Prism V6.01.

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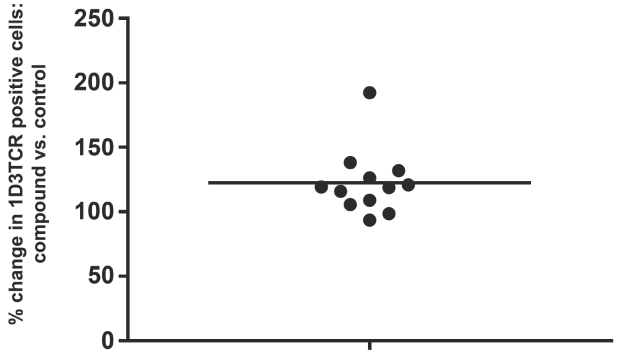
Table S1: Gene transfer efficiency in GFP gene transfer experiments. Percentages of stably gene modified cells for each experiment are shown.

Untreated	NSC 95397
6,73	31,17
16,44	19,35
15,67	22,22
24,56	28,82
21,86	24,46
26,65	33,77
11,32	17,83
17,99	18,4
25,69	34,43
18,29	20,92
24,87	40,97
37,39	41,31
18,42	28,21
1,04	2,53
27,05	38,06
2,14	4,04
29,29	44,65
6,85	11,48
29,94	39,93

Table S2: Gene transfer efficiency in TCR gene transfer experiments. Percentages of stably gene modified cells for each experiment are shown.

Untreated	NSC 95397
11,09	10,36
5,98	11,5
20,54	24,46
19,36	26,73
36,17	39,36
26,04	32,85
23,72	28,66
24,45	28,29
49,7	65,44
44,42	46,84
23,05	27,37
24,61	24,2



Supplementary Figure 1. Effect of NSC95397 on TCR gene transfer efficiency. Effect of NSC 95397 (5 μM) on SB100X mediated 1D3 TCR modification of human T cells in a large series of experiments ($p<0.01$).