Improvement of T-cell response against WT1-overexpressing leukemia by newly developed anti-hDEC205-WT1 antibody fusion proteins

Verbesserung der T-Zellantwort gegen WT1-überexprimierende Leukämie mit neu entwickelten anti-hDEC205-WT1-Antikörperfusionsproteinen

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This PhD project with two phases was accomplished in the Division of Molecular Internal Medicine (Prof. Dr. Harald Wajant) and Laboratory for Immunotherapy (PD. Dr. Götz-Ulrich Grigoleit), of the Department of Internal Medicine II (Prof. Dr. Hermann Einsele), the University Hospital of Würzburg from October 1st, 2012 to October 31st, 2015

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For my family and for Prof. Tserennadmid Choijiljav

Summary

Wilms tumor protein 1 (WT1) is a suitable target to develop an immunotherapeutic approach against high risk acute myeloid leukemia (AML), particularly their relapse after allogeneic hematopoietic stem cell transplantation (HSCT). As an intracellular protein traversing between nucleus and cytoplasm, recombinant expression of WT1 is difficult. Therefore, an induction of WT1-specific T-cell responses is mostly based on peptide vaccination as well as dendritic cell (DC) electroporation with mRNA encoding full-length protein to mount WT1-derived peptide variations presented to T cells. Alternatively, the WT1 peptide presentation could be broadened by forcing receptor-mediated endocytosis of DCs.

In this study, antibody fusion proteins consisting of an antibody specific to the human DEC205 endocytic receptor and various fragments of WT1 (anti-hDEC205-WT1) were generated for a potential DC-targeted recombinant WT1 vaccine. Anti-hDEC205-WT1 antibody fusion proteins containing full-length or major parts of WT1 were not efficiently expressed and secreted due to their poor solubility and secretory capacity. However, small fragment-containing variants: anti-hDEC205-$WT1_{10-35}$, anti-hDEC205-$WT1_{91-138}$, anti-hDEC205-$WT1_{223-273}$, and anti-hDEC205-$WT1_{324-371}$ were obtained in good yields.

Since three of these fusion proteins contain the most of the known immunogenic epitopes in their sequences, the anti-hDEC205-$WT1_{91-138}$, anti-hDEC205-$WT1_{223-273}$, and anti-hDEC205-$WT1_{324-371}$ were tested for their T-cell stimulatory capacities. Mature monocyte-derived DCs loaded with anti-hDEC205-$WT1_{91-138}$ could induce *ex vivo* T-cell responses in 12 of 16 blood samples collected from either healthy or HSC transplanted individuals compared to included controls ($P < 0.01$). Furthermore, these T cells could kill WT1-overexpressing THP-1 leukemia cells *in vitro* after expansion.

In conclusion, alongside proving the difficulty in expression and purification of intracellular WT1 as a vaccine protein, our results from this work introduce an alternative therapeutic vaccine approach to improve an anti-leukemia immune response in the context of allogeneic HSCT and potentially beyond.

Zusammenfassung

Für die Entwicklung eines immuntherapeutischen Ansatzes zur Behandlung von hoch Risikopatienten mit akuter myeloischer Leukämie (AML) und insbesondere zur Vorbeugung von Rezidiven nach allogener Stammzelltransplantation (SZT) stellt das Wilms-Tumor-Protein 1 (WT1) ein geeignetes Angriffsziel dar. Die rekombinante Expression von WT1, welches als Transkriptionsfaktor vom Zytosol in den Zellkern transloziert, gestaltet sich äußerst schwierig. WT1-spezifische T-Zellantworten werden daher hauptsächlich mittels Peptidvakzinierung oder Transfektion dendritischer Zellen (DC) mit mRNA, welche das vollständige WT1-Protein kodiert, herbeigeführt. Letzterer Ansatz bietet den Vorteil, dass passierenden T-Zellen eine größere Vielfalt an WT1-Peptidvarianten präsentiert werden kann. Eine verbesserte Peptidpräsentation kann außerdem über eine Optimierung der Rezeptor-vermittelten Endozytose der DCs erzielt werden.

Ziel der folgenden Arbeit war es, ein rekombinantes DC-gerichtetes WT1-Vakzin zu entwickeln. Dazu wurden anti-hDEC205-WT1-Fusionsproteine, bestehend aus einem Antikörper gegen den humanen DEC205-Endozytoserezeptor und verschiedenen WT1-Fragmenten, konstruiert. Während sich Fusionsproteine, die das vollständige WT1-Protein oder große Teile dessen beinhalteten, aufgrund ihrer schlechten Löslichkeit und schwachen Sekretion kaum exprimieren und aufreinigen ließen, lieferte die Produktion der Fusionsproteine mit kürzeren WT1-Fragmenten, anti-hDEC205-WT1$_{10\text{-}35}$, anti-hDEC205-WT1$_{91\text{-}138}$, anti-hDEC205-WT1$_{223\text{-}273}$ und anti-hDEC205-WT1$_{324\text{-}371}$, sehr gute Ausbeuten. Da letztere drei Proteine die meisten bislang bekannten immunogenen WT1-Peptide in ihrer Sequenz enthalten, wurde anschließend ihre Fähigkeit zur T-Zellstimulation untersucht. Dabei konnte in 12 von 16 Blutproben, die entweder von gesunden Spendern oder SZT-Patienten stammten, gezeigt werden, dass mit anti-hDEC205-WT1$_{91\text{-}138}$ beladene, reife, aus Monozyten generierte DCs *ex vivo* signifikant stärkere T-Zellantworten auslösen als die jeweils mitgeführten Kontrollen ($P < 0.01$).

Nach Expansion waren die so aktivierten WT1-spezifischen T-Zellen sogar in der Lage, die WT1-überexprimierende AML-Zelllinie THP-1 *in vitro* zu lysieren.

In der vorliegenden Arbeit konnten daher nicht nur die bereits bekannten Schwierigkeiten der WT1-Expression und Aufreinigung bestätigt werden, sondern

darüber hinaus konnte eine alternative therapeutische Vakzinierungsmethode zur Optimierung der anti-leukämischen Immunantwort im Rahmen einer allogenen SZT entwickelt werden.

Abbreviations

α-hDEC205	anti-hDEC205 antibody
aa	amino acid
Ab	antibody
allo-HSCT	allogeneic haematopoietic stem cell transplantation
AML	acute myeloid leukemia
ANOVA	analysis of varience
APCs	antigen presenting cells
ASTA	alanine, serine, threonine, alanine
ATP	adenosine three phosphate
BiTE	bispecific T cell engaging
bp	base pair
CAR	chimeric antigen receptor
cDNA	complementary DNA
C_{GpL}	concentration of scFv:hDEC205-GpL fusion protein
CHO	Chinese hamster ovary
CHO-hDEC205	hDEC205-stably expressing CHO cells
CMML	chronic myelo-monocytic leukemia
CMV	cytomegalovirus
CR	complete remission
CTL	cytotoxic T lymphocyte
DCs	dendritic cells
DFS	disease free survival
DNA	desoxyribonucleic acid
DTT	dithiothreitol
E : T	effector to target ratio
ELISA	enzyme-linked immunosorbent assay

ELISPOT	enzyme-linked immunospot
EU	endotoxin unit
FCS	fetal calf serum
FDA	Food and Drug Agency
GM-CSF	granulocyte-macrophage colony stimulating factor
GO	gemtuzumab ozogamicin
GpL	Gaussia princeps Luciferase
GvHD	graft-versus-host disease
GvL	graft-versus-leukemia
Gy	Gray (unit of ionizing radiation)
HC	heavy chain of immunoglobulun
hDEC205	human DEC205 endocytic receptor
HEK293	human embryonic kidney cells
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HSCs	haematopoietic stem cells
HSCT	haematopoietic stem cell transplantation
IC_{50}	50 % inhibitory concentration
ICS	intracellular cytokine staining
IFN	interferon
IL	interleukin
iNKT	invariant NKT cells
IQR	interquatile range
IRDye	infrared fluorescent dye
IU	international unit
K_d	dissociation constant
kDa	kiloDalton
K_i	dissociation constant of inhibitor
KTS	lysine, threonine, serine

LAL	Limulus amebocyte lysate
LAMP	lysosomal-associated membrane glycoprotein
LC	light chain of immunoglobulin
mAbs	monoclonal antibodies
MAPK	mitogen-activated protein kinases
mDCs	myeloid dendritic cells
MDS	myelodysplastic syndrome
MHC	major histocompatibility
miHC	minor histocompatibility
MMR	macrophage mannose receptor
moDCs	monocyte-derived dendritic cells
MRD	minimal residual disease
mRNA	messenger RNA
MUC-1	mucin 1
NBT/BCIP	Nitro blue tetrazolium/5-Bromo-4-chloro-3-indolyl phosphate
NCI	National Cancer Institute
NK	natural killer
OS	overall survival
PAP	prostate acetyl phophatase
PBMCs	peripheral blood mononuclear cells
PCR	polymerase chain reaction
pDCs	plasmacytoid dendritic cells
PEI	polyethylenimine
pep. pool	peptide pool
PHA-L	phytohaemagglutinin-L
pM	picomolar
PM	protein marker
PN	patient number

RHAMM	receptor for hyaluronan-mediated motility
RT-PCR	reverse transcriptase PCR
scFv	single chain variable fragment
SD	standard deviation
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	standard error of mean
SM	size marker of DNA
SQPA	serine, glutamine, proline, alanine
TAA	tumor-associated antigen
TBS	Tris-buffered saline
TCR	T cell receptor
TEMED	tetramethylethylendiamine
TET	ten-eleven translocation
TGF	transformation growth factor
T_H1 and T_H2	Helper T cells type 1 and 2
TLR	Toll-like receptor
TNF-α	tumor necrosis factor-alfa
Treg	regulatory T cells
unstimul.	unstimulated
UV	Ultraviolet
VH	variable heavy chain
VL	variable light chain
WT1	Wilms tumor protein 1
WT1_D	isoform D of WT1
$WT1_{full}$	WT1 protein in full-length
$WT1_{major}$	major fragments of WT1 protein
$WT1_{small}$	small fragments of WT1 protein

Section 1. Introduction

1.1. Background

Haematological malignant diseases are often difficult to be cured due to their heterogeneous and complex pathophysiology as well as progressive characters and systemic manifestation. On account of advanced treatment options including intensive chemotherapy, allogeneic haematopoietic stem cell transplantation (allo-HSCT) and molecular targeted therapy, the disease-free (DFS) and overall survival (OS) rates of patients suffering from these diseases have been remarkably ameliorated. For instance, acute myeloid leukemia (AML), one of the poor prognostic malignancies, is treated now with 60-80% of complete remission (CR) and 30-60% of long-term survival rates, not at least due to establishing allo-HSCT as a treatment option. However, 20-70% of the allo-HSC transplanted patients are affected by relapse and only 2-20% of them have a possibility to receive second transplantation (Savani et al. 2009). AML patients who are not candidate for allo-HSCT because of their age or comorbidities have even poorer prognosis. Thus, there is an urgent clinical need to improve the current treatment modalities by combining HSCT with other immunotherapy strategies (Barrett and Le Blanc 2010).

The most promising immunotherapeutic strategies for haematological malignant disorders are drug-conjugated monoclonal antibodies, T-cell engaging antibody constructs, adoptive transfer with chimeric antigen receptor (CAR) T cells, and dendritic cell (DC) vaccination (Lichtenegger et al. 2015). Plantinga *et al.* have recently reviewed that the latter strategy is an attractive and effective treatment modality to prime and/or stimulate tumor-specific cytotoxic T cells (CTLs) in early phase after allo-HSCT, in a setting of a better-predicted immune reconstitution. The selection of specific tumor-associated antigen (TAA) and manoeuver of antigen loading have a major impact on the

priming capacity of the DC (Plantinga et al. 2014). With this work, we contributed to the DC vaccination strategy to AML treatment in the context of allo-HSCT using Wilms tumor protein 1 (WT1).

1.2. State of current knowledge and contribution

1.2.1. AML, prognostic markers, and current treatment options

AML is a heterogeneous clonal disorder of haematopoietic progenitor cells. Approximately, four new cases of AML per 100 000 people are diagnosed each year. Various genetic alterations have been detected in patients with AML which result in a loss of normal regulation of growth, differentiation, and apoptosis of haematopoietic cells. This leads to an accumulation of cells (blasts) with uncontrolled proliferative capacity and ineffective function in peripheral blood and bone marrow. Consequently, normal haematopoiesis is displaced by the abnormal cell expansion. The survival rate is strongly dependent on age, comorbidities, disease type and advancement, and response to chemotherapy. Other adverse prognostic factors include central nervous system involvement with leukemia, systemic infection at diagnosis, elevated white blood cell count, therapy-induced AML, and history of MDS or other antecedent haematological disorders. The long-term survival rates in adult AML are inversely related to age (Table 1, Cancer research UK, statistics based on the data 2008-2012).

Table 1. The long-term survival rate in patients with AML.

Age	<14	15-24	25-64	65<
5-year survival	66%	60%	40%	20%

Cytogenetic analysis at the time of diagnosis provides the most important prognostic information, predicting outcome after induction chemotherapy, relapse rate, and OS (Estey and Dohner 2006). In 2010, recommendations from an international expert panel on the diagnosis and management of AML in adults were updated and proposed four different genetic groups (Table 2) to standardize reporting system of genetic abnormalities (Dohner et al. 2010). These genetic groups directly correspond to

clinical outcomes. Between 10% and 40% of the newly diagnosed patients do not achieve an complete remission with intensive induction therapy and are therefore categorized as primary refractory or resistant (Thol et al. 2015).

Table 2. Prognosis for AML patients on the basis of their genetic abnormalities (Dohner et al. 2010).

Genetic group	**Subsets**
Favorable	t(8;21)(q22;q22); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD (normal karyotype)
	Mutated *CEBPA* (normal karyotype)
Intermediate-I	Mutated *NPM1* and *FLT3*-ITD (normal karyotype)
	Wild-type *NPM1* and *FLT3*-ITD (normal karyotype)
	Wild-type *NPM1* without *FLT3*-ITD (normal karyotype)
Intermediate-II	t(9;11)(p22;q23); *MLLT3-MLL*
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
	t(6;9)(p23;q34); *DEK-NUP214*
	t(v;11)(v;q23); *MLL* rearranged
	−5 or del(5q); −7; abnl(17p); complex karyotype

Depending on age and other prognostic values, patients with AML are treated with standard chemotherapy regimens and HSCT as well as salvage therapies. The standard chemotherapy treatment is divided into two phases: a remission induction and a post remission. To achieve the complete remission, induction of a profound bone marrow aplasia is usually necessary with currently used combination chemotherapy regimens. The two most effective drugs used to induce remission in patients with AML are cytarabine and an anthracycline (mostly daunorubicin). Salvage chemotherapy regimens have been tested for different subtypes of the disease. Since the myelosuppression is an anticipated consequence of both leukemia and its treatment with chemotherapy, patients must be closely monitored during therapy. Antibacterial, antifungal prophylaxis or treatment, as well as haematopoietic growth factors, blood cell fractions (red blood cell and platelet), and other supportive care are needed.

The postremission therapy is to prolong the duration of the initial remission with induction chemotherapy or HSCT. In practice, most patients are treated with an intensive chemotherapy after remission is achieved, as only a small subset of the patients have a matched-family donor. The most commonly used regimen for postremission consolidation is high-dose cytarabine and it improves clinical outcome. Patients of the intermediate and adverse genetic groups are candidates for HSCT to benefit from the GvL effect and potential cure after significant reduction of leukemia burden as a result of prior induction/reinduction therapy. However, even with such advanced treatment options, the long-term survival rates of patients are still not satisfactory.

Thereupon huge research efforts and clinical trials have been carried out for refractory/relapsed AML patients since highly effective and standardized treatments for this situation are still not available. In this regard, immunotherapy is auspicious, indeed, various efforts to achieving to a good clinical outcome have been explored.

1.2.2. Immunotherapy to AML

The most prominent immunotherapy to fight AML is the HSCT. In addition, various immunotherapeutic approaches including cytokine therapy, monoclonal antibodies, therapeutic vaccines, chimeric antigen receptors and bispecific antibodies have been developed for AML treatment (Figure 1) (Grosso et al. 2015). IL-2 or IFN-α monotherapy was tested for stimulation of the immune system during the first remission period after induction chemotherapy. However, a significant impact of the cytokine therapy on OS and DFS was not reached, meaning that cytokine therapy alone is insufficient to the disease with complex mechanism of pathogenesis. Various types of monoclonal antibodies (mAbs) with or without conjugation of cytotoxic drugs and radioisotopes have been evaluated for AML treatment. Among them, Gemtuzumab ozogamicin (GO), a recombinant, humanized CD33 mAb has been reported to prolong survival of AML patients with favorable cytogenetics. The CD19-targeting bispecific T cell engaging (BiTE) antibody, Blinatumomab, was successful to initiate cytotoxic T cell response to acute lymphoblastic leukemia. Initial tests with the CD33 targeted BiTE AMG330 revealed furthermore that AMG330 is able to lyse primary leukemia cells (Krupka et al. 2014). Chimeric antigen receptor (CAR) T cells are thought to be promising to eliminate leukemia cells. CAR is antigen-specific variable heavy and light chain domains

constructed on the intracellular signaling domain of the T cell receptor (TCR). T cells expressing IL-3 receptor α chain (CD123)-specific CARs have been appreciated by preclinical studies and to be tested in phase I clinical trial (Mardiros et al. 2013).

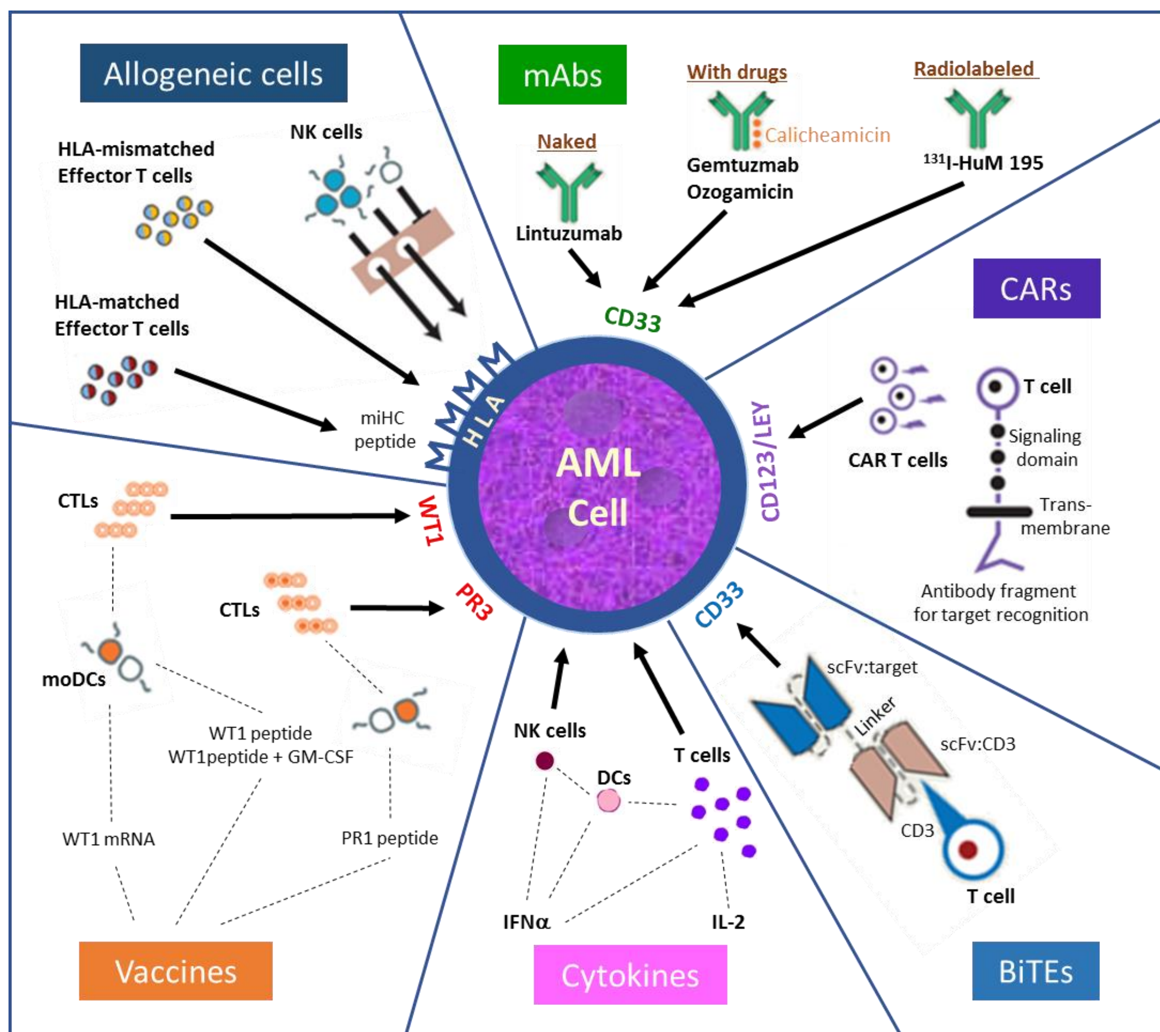

Figure 1. Current immunotherapeutic approaches to AML.

mAbs, monoclonal antibodies; ^{131}I-HuM195, iodine-131 labeled humanized monoclonal antibody 195; CAR, chimeric antigen receptor; LEY, Lewis antigen; BiTE, bispecific T-cell engager; scFv, single chain variable fragment; IFN, interferon; IL-2, interleukin-2; NK, natural killer; WT1, Wilms tumor 1; PR3, proteinase 3; CTL, cytotoxic lymphocytes; GM-CSF-granulocyte-macrophage-colony-stimulating factor; HLA, human leucocyte antigen; miHC, minor histocompatibility. (Adapted from Grosso, Hess et al. 2015)

1.2.2.1. *Haematopoietic stem cell transplantation (HSCT) and Graft-versus-Leukemia (GvL) effect*

It is exactly 60 years after the first human bone marrow grafting programme was began by E. Donnal Thomas and his colleagues. As long as it aged, HSCT, the most successful of the immune-based therapies, has developed to a standard therapy for AML and other haematological malignancies. HSCT is the transplantation of multipotent haematopoietic stem cells (HSCs), usually derived from bone marrow, peripheral blood, or umbilical cord blood. Depending on the source of HSCs, there are three types of the transplantation: autologous, allogeneic, and cord blood. Stem cells are defined as undifferentiated cells capable to divide for indefinite periods, to self-renew and to generate functional progeny of highly specialized cells. HSCs are the prototype of multipotent adult tissue stem cells. In a step-wise differentiation process, HSCs give rise to the committed oligopotent progeny of the lymphoid and myeloid lineages and, further downstream, to the lineage-restricted unipotent precursors of mature blood cells. It is thought that normal stem cells transform to malignant stem cells keeping the mechanism for self-renewal. Most leukemic cells have a limited capacity for proliferation and are continuously replenished by leukemic stem cells. Normal and malignant stem cells are quiescent, therefore insensitive to the chemotherapy which acts mostly on proliferating cells. Both normal and malignant stem cells repair DNA efficiently, resist apoptosis, and excrete toxic drugs with the help of ATP-binding transporters. Thus, although chemotherapy can destroy a tumor almost completely, the stem cells are remained, allowing the cancer to recur. Such cells can be eliminated by immunologically active donor cells in allogeneic grafts (Copelan 2006). In allo-HSCT, if recipient T cells recognize foreign donor antigens, graft rejection may occur, whereas donor T cells recognizing recipient antigens may induce graft-versus-host-disease (GvHD) and graft-versus-leukemia (GvL) effects.

The GvL effects are mediated by donor derived $CD8^{+}$ and $CD4^{+}$ T cells as well as by NK cells and macrophages. An important mechanism of the GvL effect is that donor immune cells are reactive to recipient major (MHC) and minor (miHC) histocompatibility antigens. MHC class I and II molecules on the recipient APCs can be directly recognized by allo-T cells. Alternatively, allo-T cells indirectly recognize recipient MHC class II-derived or other protein-derived peptides presented by donor APCs. Since GvHD and GvL

effect are strongly associated with each other, major histocompatibility molecules targeted by allo-T cells are able to induce both of them. Minor histocompatibility antigens are polymorphic and diversify recipient peptide-HLA complex presented to allo-T cells (Figure 2). HA-1, HA-2, HA-8, UGT2B17, HB-1, ACC-1, ACC-2, B8/H-Y, and LRH-1 miHC antigens are haematopoietic tissue-restricted, therefore, they are thought to be crucial to induce GvL effect against certain type of leukemia (Sprangers et al. 2007). If allo-T cells respond to miHC antigens expressed on haematopoietic and epithelial cells, rather a development of GvHD occurs. The allo-reactive cells to miHC antigens inhibit the growth of leukemic colonies, thus, leukemic stem cells can be eliminated by means of this mechanism. The third mechanism of allo-T cell mediated GvL effect is implemented by targeting of tumor associated antigens (TAAs).

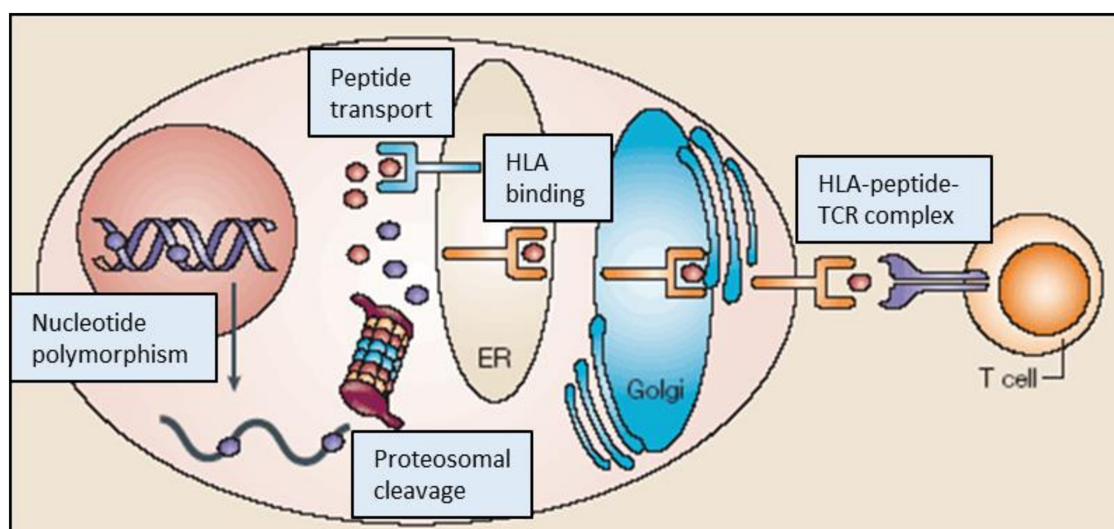

Figure 2. Generation of miHC antigens.

Minor histocompatibility antigens are generated by gene nucleotide polymorphisms that lead to differences in the amino-acid sequences of homologous proteins between donor and recipient cells. Polymorphic proteins are degraded within the proteasome. Peptides derived from the degraded protein are then transported to the endoplasmic reticulum, where they bind HLA glycoproteins. The peptide-HLA complexes travel through the Golgi apparatus to the cell surface, where the peptides are recognized as foreign by T cells. Adapted from (Bleakley and Riddell 2004).

TAAs are non-polymorphic and aberrantly expressed by tumor cells. WT1, proteinase 3, and survivin are known to eliciting anti-leukemia immune responses and T cells specific to those TAAs can inhibit leukemic, but not normal colony formation (Gao et al. 2000). Therefore, GvL effect can be strengthened by improving the TAA-specific T cell response.

1.2.2.2. AML vaccines

In 1977, a cancer vaccine trial with bacilli Calmette-Guerin (BCG) and irradiated autologous leukemia cells reported prolonged remission and survival in the vaccinated group (Powles et al. 1977). In 1990, the first donor lymphocyte infusion was performed for treatment of leukemic relapse after HSCT (Kolb et al. 1990). These important demonstrations of leukemia-specific T cell responses led to the development of an array of AML vaccines in order to support GvL effect in the setting of HSCT and control minimal residual disease (MRD) by stimulating anti-leukemic immune responses in high-risk patients after HSCT as well as non-HSCT candidates after cytotoxic therapy. There are two main directions in the development of AML vaccines: peptide vaccines derived from TAAs and DC vaccines.

1.2.2.2.1. Peptide vaccines:

Wilms tumor protein 1 (WT1), proteinase 3, mucin 1 (MUC-1) (Kuball et al. 2011) and receptor for hyaluranon-mediated motility (RHAMM) (Schmitt et al. 2008) have been evaluated as peptide vaccines in AML vaccine strategies. For various TAAs, WT1 was prioritized as a vaccine candidate due to its therapeutic impact, immunogenicity, tissue restricted expression, and leukemogenic characteristics (Cheever et al. 2009). $WT1_{126-134}$ (Keilholz et al. 2009), $WT1_{122-140}$, $WT1_{331-352}$, $WT1_{427-445}$ (Maslak et al. 2010), $WT1_{235-243}$ (Tsuboi et al. 2012) peptides were tested for their immunogenic capacities *in vitro* and *in vivo*. The $WT1_{126-134}$ peptide vaccine trial by Keilholz *et al.* showed that at week 18 after vaccination, the median frequency of WT1 tetramer positive T cells in the bone marrow and peripheral blood of patients increased from baseline frequencies of 0.18 % and 0.12 % to frequencies of 0.41 % and 0.28 %. Importantly, the increased frequency of WT1-specific T cells were functional and remained at stable levels for follow up period. Rezvani *et al.* reported that the median value of the absolute number of WT1-specific $CD3^{+}CD8^{+}$ T cells after vaccination were 4-fold higher than that before vaccination (328 vs 95 cells per mL). Entirely, WT1 peptide vaccine studies demonstrated immunologically as well as clinically positive and safe results.

Common limitations for peptide vaccines are the human leukocyte antigen (HLA) restriction and need for prior identification of immunogenic epitopes (Benteyn et al. 2013). To overcome these limitations, several approaches including transfection of dendritic cells (DCs) with WT1-encoding DNA (Chaise et al. 2008) or full mRNA (Van

Driessche et al. 2009) as well as treatment with long and polyvalent WT1 peptide (Maslak et al. 2010), (Weber et al. 2013), (Brayer et al. 2015) vaccines have been tested. Chaise *et al*. immunized HHD transgenic mice (HLA-A0201* expressing) with three different WT1 DNA vaccines each encoding one of the HLA-A0201* restricted WT1-derived peptides: WT1.37 (VLDFAPPGA), WT1.126 (RMFPNAPYL) and, WT1.235 (CMTWNQMNL). The mice received 50 μg of DNA by intramuscular injection for $CD8^+$ T cell priming at day 0, while at day 28, the same amount of DNA was applied by in vivo electroporation for boosting. The DNA vaccination induced functional T cells to the three clinically relevant HLA-A0201*-restricted epitopes, *in vivo*. By injection of the DNA encoding the three promising epitopes at different sites, tumor immune escape mechanism could be overcome, however, only HLA-A0201*-positive patients could benefit from this approach.

Modifying native peptides to more antigenic peptides by amino acid substitution in their sequences (heteroclitic peptides) is considered to be beneficial to improve immunity to self-proteins. Maslak et al. reported results of a pilot clinical study using polyvalent vaccine consisting of four heteroclitic WT1 peptides (one peptide for $CD8^+$, two long peptides for $CD4^+$, and one for both subsets) in nine AML patients at CR status. They showed that immune responses of both $CD4^+$ and $CD8^+$ T cell subsets to the peptides in seven patients, however, median DFS had not been reached. Notably, median OS of the patients were longer than estimated average (35+ months vs 9 months). Similarly, Brayer *et al*. assessed the safety, tolerability, and immunogenicity of vaccination with combination of WT1 peptides restricted to different HLA molecules. Three of the 14 vaccinated patients with AML or MDS showed prolonged relapse-free survival. Weber *et al*. generated multi-TAA-specific T cells from peripheral blood of healthy donors using peptide mix covering whole WT1 and other four TAAs. The generated pool of CTLs included different specificities and WT1-specific T cells were found within the pool. Overall, it is accepted that vaccination strategies using WT1 are safe and have a potential to improve immune responses against leukemia. Therefore, in WT1-based vaccination, alternatives have been searched to improve the feasibility and effectiveness of this approach.

1.2.2.2.2. DC vaccines:

DC vaccination is another icon to induce strong and sustained $CD4^+$ and $CD8^+$ T-cell responses in patients with AML. In the context of allo-HSCT, Fujii S. *et al.* were the

first to test DC vaccination. Four patients achieved tumor antigen-specific immunological response as a result of tumor cell pulsed HLA-matched allogeneic donor derived DC vaccination (Fujii et al. 2001). Subsequently, in this field, DCs loaded with tumor cell lysates or TAA-derived peptides, DCs electroporated with mRNA encoding full-length protein antigens, as well as tumor peptide pulsed DC-derived exosomes have been tested. DCs can be generated from AML patients in remission and be rendered more antigenic by exposure to AML lysates (Galea-Lauri et al. 2002) or fusion with AML blasts (Banat et al. 2004). Autologous moDCs transfected with mRNA encoding WT1 protein were able to induce a broad presentation on HLA class I and II molecules by DCs (Van Tendeloo et al. 2010). In detail, monocyte-derived DCs were electroporated with the mRNA encoding full-length WT1, then injected intradermally at biweekly intervals four times. As a result, an immunological response comprising NK and CD8^{+} T cell activation were induced. Moreover, five of ten vaccinated patients with AML achieved molecular remission and in the two of those five, incomplete remission converted to complete remission. The fact that the achieved effects were transient was probably explained by the lack of CD4^{+} T cell stimulation which is otherwise considered to be necessary for the maintenance of long-term CD8^{+} T cell memory. This approach is promising, but it seems to be laborious and the transfection efficiency of DCs is variable, thereby, the following effect could be varied.

Classical prophylactic vaccines are mostly injected intramuscularly or subcutaneously where they are captured by various types of APCs. Upon activation, these APCs migrate to the lymph nodes to activate T cells. Whereas the concept of direct targeting of DC subsets *in situ* (resident) may overcome the need for cell migration and facilitate the instant delivery of antigen to resident DC subsets in the spleen and the lymph nodes. To this end, intra- and extracellular receptors expressed by DCs have been extensively targeted (Kreutz et al. 2013). Importantly, targeting of antigen directly to DCs reduces the required dose of antigen remarkably.

The most common type of DCs used in DC vaccine studies are autologous moDCs, although isolation of mDCs or pDCs from peripheral blood as well as the use of the MUTZ-3 cell line has been reported. Recently, de Haar *et al.* reported that cord blood derived CD34^{+} DCs are capable of inducing a WT1-specific T cell response after their differentiation, maturation and electroporation with WT1 mRNA (de Haar et al. 2015). Important parameters to optimize DC vaccines after HSCT were listed in Table 3. In addition, intrinsic characteristics of DCs such as intracellular signaling, antigen processing, presentation, and expression of effector molecules seem to be crucial to

switch on their immunity-inducing capacity. Finally, interaction of DCs with other cellular and soluble factors may have an impact on induction of immune responses to leukemia cells, too. Nevertheless, DC vaccination is a virtue of immunotherapy against various tumors including AML.

Table 3. Parameters for optimization of DC vaccines (Plantinga et al. 2014).

Cell sources	Type of DCs	Maturation	Loading
Bone marrow	$CD14^+$ monocytes	Cytokine cocktails	Peptide
Cord blood	Adherent monocytes	TLR agonists	Protein
Mobilized HSCs in peripheral blood	CD34-derived	Interferons	Cell lysate
	Primary DCs	CD40L	Fusion
		TNF-α	RNA/DNA electroporation
		Others	Endocytic receptor-specific antibody conjugated protein

1.2.3. Antibody use for therapy and antibody engineering

Therapeutic antibodies inhibit a target that plays a major role in disease progression or cause the cytotoxic death of target cells. Alternatively, antibodies are used as carriers to target cytotoxic and imaging agents, such as radioisotopes, toxins, and drugs, to the site of a disease. The various clinical applications of antibodies lead to their modifications in terms of size, pharmacokinetics, immunogenicity, specificity, valency and effector functions. Antibodies have also been expressed as fusion proteins for the targeting of diverse cytokines, protein antigens, toxins, and enzymes for therapeutic applications (Jain et al. 2007). The first example of antibody engineering was the development of the single chain variable fragment (scFv) format of antibody, consisting of the variable heavy and light chains linked by a short peptide (Figure 3). The scFv format offers several advantages including better tissue penetrance, no binding to Fc receptors, avoidance of host antibody response, short serum half-life, and low cost. Another example of antibody engineering is the nanobody format, which is derived from the variable heavy domains of immunoglobulin, are the smallest antigen binding domains.

Multimerization of antibody fragments (miniabody, diabody, triabody) and incorporation of two specificities into a single antibody (bispecific antibody) have also been developed.

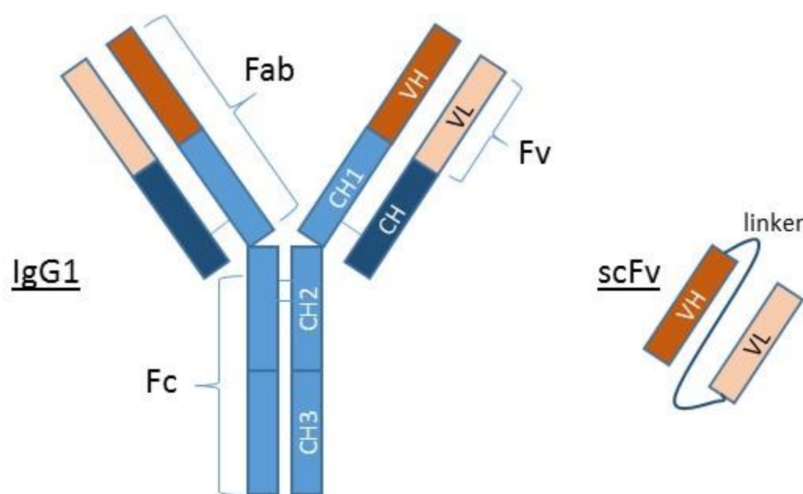

Figure 3. Structural scheme of therapeutic antibody (IgG1) and single chain variable fragment (scFv) constructs.

Fab, antigen binding fragment; Fv, variable fragment, Fc, constant fragment; VH, variable domain of the heavy and light chains.

Engineered antibodies have been used for cytokine carriers to tumor tissues (Kontermann 2012). For instance, IL-15, IL-2, GM-CSF, IFN-α, and death ligands (TNF, TRAIL, FasL) have been fused to antibodies with different specificities for various therapeutic applications. One of the latest developments in antibody engineering has been used for delivery of tumor antigen to APCs in order to facilitate accessibility of the antigen by the APCs (Idoyaga et al. 2011).

1.2.4. Wilms tumor protein 1 (WT1)

WT1 gene is located at chromosome 11q13 and encodes a Krüppel type zinc finger transcription factor that plays an essential role in cell growth and differentiation (Call et al. 1990), (Gessler et al. 1990). A continuous shuttling of WT1 protein between nucleus and cytoplasm (Niksic et al. 2004) may reflect its complex roles in different cell function during both embryogenesis and ontogenesis. In embryonic development, WT1 is mostly expressed in the endoderm and mesoderm originated organs: the highest in kidneys and gonads as well as heart, lungs, intestines, spleen, peritoneum, pleura, and pericardium.

Its expression is also detected in distinct regions of the brain, spinal cord, and eyes which are developed from ectoderm (Armstrong et al. 1993). Thus, the broad expression profile of WT1 indicates its essential and universal role in tissue generation and intrauterine development of human. Postnatally, the expression of WT1 is strongly restricted to a limited set of tissues, including the gonads, uterus, kidneys and mesothelium, and to progenitor cells in various types of tissues including haematopoietic stem cells (Park et al. 1993, Buckler et al. 1991).

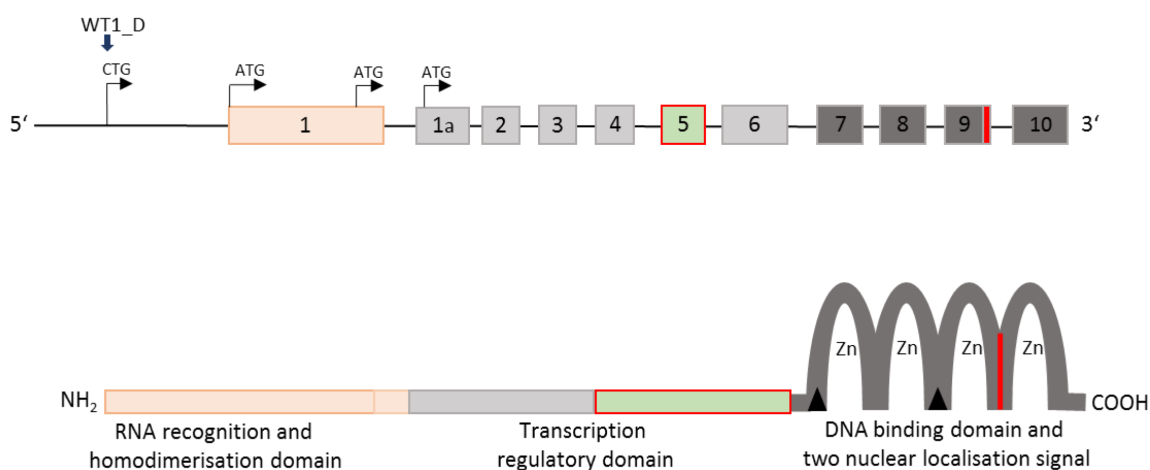

Figure 4. Scheme structure of WT1 gene (top) and WT1_D protein (bottom).

Red frames, alternative splices; black triangles, nuclear localization signals; Zn, zinc ion in the DNA-binding zinc finger domain.

The *WT1* gene consists of ten exons flanking huge introns and there are two different alternative splices of WT1; exon 5 encoding 17 amino acids and 9 nucleotides encoding terminal three amino acids (KTS) of exon 9 (Figure 4) (Haber et al. 1991) (Gessler et al. 1992). Due to alternative transcription initiation, alternative pre-mRNA splicing, RNA editing and alternative translation initiation, at least 36 different isoforms are translated from the same DNA template (Kramarzova et al. 2012). An isoform (P19544-1, UniProtKB) consisting of 449 amino acids, has been chosen as canonical sequence. It is translated from the first AUG site in exon 1 of the gene and the two splices are present. Depending on presence of the two splices, there are four major isoforms of WT1: WT1_A (exon5-/KTS-), WT1_B (exon5+/KTS-), WT1_C (exon5-/KTS+), and WT1_D (exon5+/KTS+). Consisting of 522 amino acids, WT1_D (P19544-7, UniProtKB) is the longest isoform described so far, because its translation is initiated from non-AUG site at upstream of the gene.

Three functional domains have been defined within WT1 protein: RNA recognition and homodimerisation, transcriptional regulatory, and DNA binding (zinc finger) domains. The zinc finger domains of WT1 bind to GC-rich sequences, or $(TCC)_n$ motif of thereby regulating the expression of downstream target genes (Tatsumi et al. 2015). An array of genes involved in MAPK, Wnt, and TET signaling, epithelial-mesenchymal transition etc. is supposed to be potential WT1 targets. Moreover, WT1 function is complicated by its ability to bind RNA (Larsson et al. 1995). In general, several opposing functions of WT1: as an activator or repressor in transcriptional processes, a role for WT1 in RNA metabolism, and posttranscriptional regulation has been described (Hohenstein and Hastie 2006).

WT1 plays significant roles in human diseases. The WT1 gene was originally defined as a tumor suppressor gene in Wilms tumor, a pediatric kidney cancer of embryonic origin. Virtually, 10-20 % of Wilms tumor cases have a mutated *WT1* gene and over 50 % of cases have decreased expression of wild type WT1 (Huff 1998). A mutation or deletion affecting *WT1* gene induces rare genetic disorders presenting symptoms of nephropathy, genitourinary anomaly, Wilms tumor, mental and physical retardation (WAGR, Denys-Drash syndromes) in humans. In these diseases, WT1 serves as a tumor suppressor gene. In contrast, *WT1* gene is able to act as an oncogene in leukemogenesis (Sugiyama 2005) (Osaka et al. 1997) (Menke et al. 1998). It is highly expressed in most cases of acute myeloid and lymphoid leukemia (Inoue et al. 1997), advanced myelodysplastic syndrome (Tamaki et al. 1999), chronic myelogenous leukemia (Inoue et al. 1994), and multiple myeloma (Hatta et al. 2005). Sugiyama *et al*. conceptualized that WT1-expressing progenitor cells can differentiate into tissue-specific cells by down-regulation of WT1 expression, but if this down-regulation is impaired, the WT1-expressing progenitor cells continue to proliferate and transform as a result of occurrence of secondary, tertiary or further genetic alterations (Sugiyama 2010). Consistent with the cell survival and oncogenic roles of the WT1 protein, increased WT1 levels have prognostic significance and are associated with a poor response to therapy (Barragan et al. 2004, Casalegno-Garduno et al. 2015). For AML cases, WT1_D is the most predominant from the four major isoforms (Kramarzova et al. 2012, Siehl et al. 2004).

Due to the supporting evidences of its oncogenic function, WT1 has been extensively targeted for immunotherapy to treat cancer. WT1-derived peptide specific cytotoxic CD8+ T cells are induced by stimulation with WT1 peptide pools and efficiently kill leukemic cells (Oka et al. 2000), (Gao et al. 2000), (Tsuboi et al. 2002), (Azuma et al. 2002). In patients with haematological malignancies, serum titers of IgM and IgG, specific

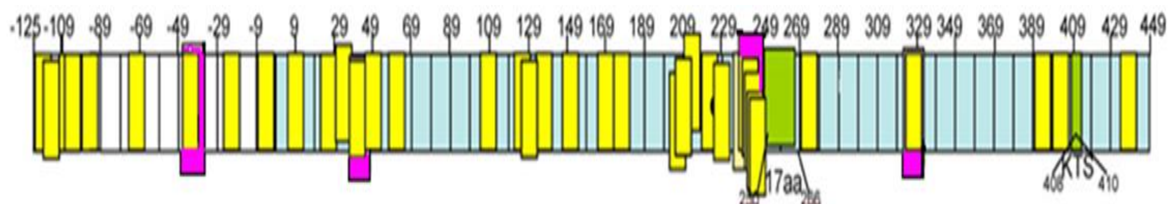

Figure 5. Potential antigenic epitopes spread in WT1 protein sequence.

White, N-terminal extension of WT1_D; blue, canonical sequence of WT1; green, two alternative splices; yellow, potential epitopes restricted to HLA class I; pink, epitopes restricted to HLA class II. Adapted from (Doubrovina et al. 2012).

for WT1 have been detected at higher levels than those in healthy donors (Elisseeva et al. 2002). Importantly, in previous vaccination trials using WT1-peptide loaded or WT1 mRNA transfected DCs, there were no reports about autoimmunity induced by the vaccine (Oka et al. 2004), (Kitawaki et al. 2008), (Van Tendeloo et al. 2010). Van Driessche *et al.* have reviewed early clinical trials targeting WT1 protein for patients with haematological malignancies and solid tumors (Van Driessche et al. 2012). Based on the WT1 antigen source, the clinical trials can be divided into four groups: (a) human leukocyte antigen (HLA)-restricted peptide vaccines, (b) non-HLA restricted long peptide vaccines, (c) dendritic cell (DC) vaccines loaded with HLA-restricted peptide and (d) DC vaccines loaded with mRNA encoding full-length WT1. These vaccines could elicit WT1-specific immune responses and exhibited objective clinical responses in up to 64% of evaluable vaccinated patients with haematological malignancies. Although several antigenic epitopes are known for induction of T cell response, it is still needed to detect more antigenic epitopes from WT1. Contributing to this, Doubrovina *et al.* identified clusters of antigenic epitopes in WT1 protein sequence and these epitopes are spread mainly in N-terminal and middle part of WT1 (Figure 5) (Doubrovina et al. 2012).

Because of the strong binding to nucleotide sequences (Hamilton et al. 1995) and its complex interactions with posttranscriptional regulators (Ladomery et al. 1999), WT1

is assumed to be hardly expressed as a full-length protein (Geng and Carstens 2006). So far, using different soluble tags and buffer conditions, full-length human WT1 and its zinc finger domain has been expressed and purified for functional studies (Nurmemmedov and Thunnissen 2006) (Fagerlund et al. 2012), but not yet for protein vaccine studies.

1.2.5. DCs, DEC205 and DC targeting approach

Dendritic cells (DCs) are present in all tissues and involved in the initiation of immune responses. They are part of the myeloid lineage of haematopoietic cells and arise from a precursor that can also differentiate into monocytes but nor granulocytes. Maturation of DCs is dependent on a cytokine called Flt3 ligand, which binds to the Flt3 tyrosine kinase receptor on the precursor cells. All subsets of human DCs are defined by high levels of MHC class II (HLA-DR) and lack of lineage markers ($CD11c^{+}HLA\text{-}DR^{+}lin^{-}$ cells). Those cells are divided into three classes: myeloid (mDCs), plasmocytoid (pDCs) and monocyte derived (moDCs) DCs (Collin et al. 2013). There are also very specialized DC subsets in the skin (Langerhans cells) and the parenchyma of the brain (microglia).

Immature DCs in extra-lymphoid tissues endocytose foreign proteins. If they are activated or matured by various co-stimuli, DCs migrate to the draining lymph nodes and present antigenic determinants via HLA class I and II molecules to T cells. By virtue of especial cytokines, different types of DCs are able to prime distinct helper T cell subsets (Table 4). In the presence of immunostimulatory cytokines IL-12 and IFN-γ, mature DCs prime T_H1 cells which provide help in priming $CD8^{+}$ effector T cells. T_H2 cells that drive humoral responses are primed with mature DCs secreting IL-4, IL-6, and IL-10. Whereas DCs which are either unable to secrete cytokines, or secrete immunosuppressive cytokines IL-10, TGF-β can be responsible for regulatory T cells (Treg) (Mays and Wilson 2011). In the absence of activation signals, immature DCs elicit immunological tolerance toward the antigen (Bonifaz et al. 2002). However, in combination with activation signals such as CD40 agonists, protective antigen-specific $CD4^{+}$ and $CD8^{+}$ T cell responses can be stimulated (Bonifaz et al. 2004) (Gurer et al. 2008).

Usually, antigens derived from intracellular origins are presented by the HLA class I presentation system while extracellular antigens are preferentially presented on the HLA class II molecules. However, DCs have a unique ability to cross present antigens: extracellular antigens on the HLA class I molecules (mediated by cytosolic processing of

antigens escaped from endosome) whereas intracellular antigens on the HLA class II molecules (mediated by autophagy) (Romao et al. 2013). Antigen cross-presentation by DCs is of key importance for the induction of anti-tumor immunity thereby $CD8^+$ cytotoxic T cells specific to tumor derived antigens are primed. Furthermore, it could be inevitable to sustain the anti-tumor immunity because tumor cell autophagy-derived endogenous antigen presentation on MHC class II molecules by tumor cells activate $CD4^+$ T cells which in turn endorse the effector function of cytotoxic T cells.

Table 4. Cytokines and helper T cell priming of DCs.

Maturation state of DCs	DC cytokines (Signal 3)	Primed helper T cells	Signature cytokines	Response
Mature	IL-12, IFN-γ	T_H1	IFN-g, TNF-a, IL-2	Cytotoxic T cell response
Mature	IL-4, IL-6, IL-10, low IL-12	T_H2	IL-4, IL-6, IL-10, IL-13	B cells and antibody
Mature or semi-mature	TGF-β, IL-10	T_{reg}	IL-10, TGF-b	Immune suppression
Immature	Cytokine negative			Ignorance, Anergy/deletion

The type of receptors on the surface of DCs and their interaction with cognitive antigen are involved in the maturation and cytokine secretion of different subsets of either immunogenic or tolerogenic DCs. DCs express various C-type lectin receptors such as macrophage mannose receptor (MMR), DC-SIGN, DEC205 (Figure 6) which all function as endocytic receptors (van Kooyk 2008). The extra-cellular domain of those molecules contains a cysteine-rich domain (CysR), a fibronectin type II domain (FNII), and multiple C-type lectin-like domains (CTLD). The cytoplasmic domains contain motifs for tyrosine (Tyr-) and/or dihydrophobic amino acid-based endocytosis.

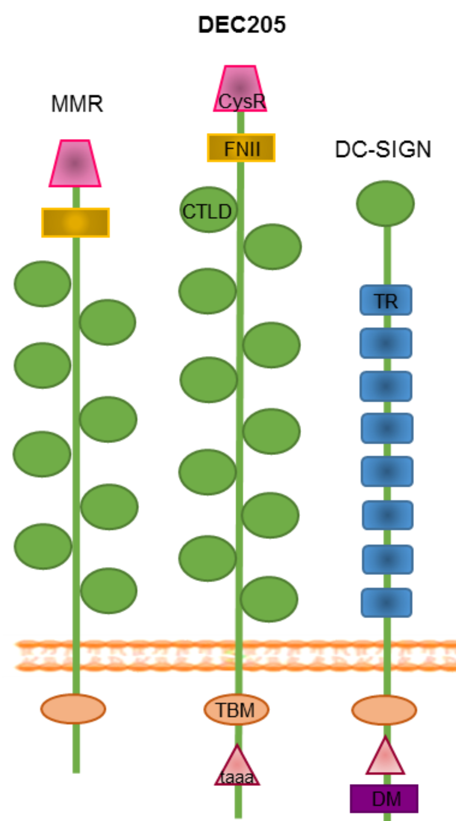

Figure 6. Structural scheme of C-type lectin receptors on the surface of DCs.

MMR, macrophage mannose receptor; CysR, Cysteine rich domain; FNII, Fibronectin type II repeat; CTLD, C-type lectin (carbohydrate recognition) domains; TBM, Tyrosine-based motif; taaa, triad of acidic amino acids; DM, Di-leucine motif; TR, tandem repeat. (Adapted from Figdor et al. 2002)

The DEC205 (CD205, Ly75, 205kDa) receptor was first identified in mice (Jiang et al. 1995). Its expression in human was mainly identified on DCs and thymic epithelial cells. Park *et al.* reported that DEC205 is the only endocytic receptor that has been visualized on most DCs in the T cell areas of lymph nodes in humans (Park et al. 2012). Similar to other members of C-type lectin receptors, the DEC205 consists of CysR, FNII and ten CTLDs. Depending on environmental pH, the 12 extracellular domains undergo conformational changes (Figure 7), i.e., form a compact double ring-shaped conformation at acidic pH and become linear at basic or physiological pH (~7.4). This pH-dependent conformational change of the DEC205 may result in ligand binding and release. The DEC205 only binds to apoptotic and necrotic cells at acidic pH, whereas live cells cannot be recognized by DEC205 at either acidic or basic conditions (Cao et al. 2015). These results suggest that DEC205 is an immune receptor that recognizes apoptotic and necrotic cells specifically through a pH-dependent mechanism.

The DEC205 has been supposed to be an attractive target for therapeutic antigen delivery in the areas of autoimmunity, as well as vaccination strategies for infections or tumors (Erbacher et al. 2009). When antigens (for instance, HIV gag-p24 protein) are targeted to the DEC205 *in vivo* (mice and rhesus macaques) by conjugation to a monoclonal antibody directed against the DEC205, antigen presentation is significantly, 100-fold enhanced (Bonifaz et al. 2004). Recently DEC205-targeting has been evaluated

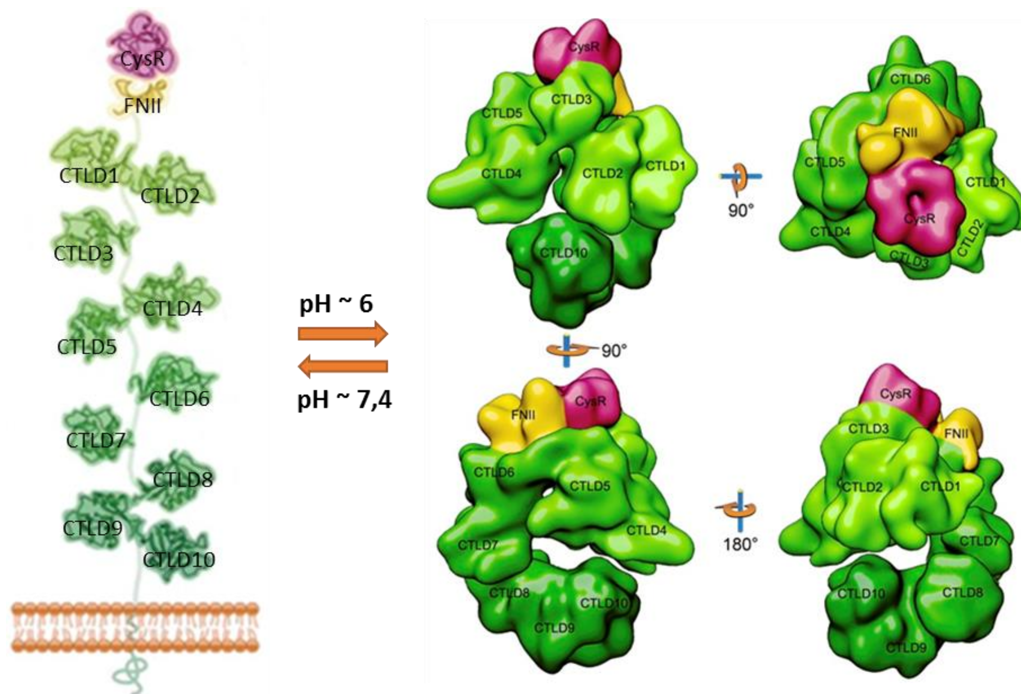

Figure 7. pH-dependent conformational change of the DEC205 receptor.

Left: linear "steady"conformation; CysR, cysteine rich domain; FNII, fibronectin II domain, CTLD, C-type lectin domain. Right: ring-shaped "active" conformation. (Adapted from Cao, Shi et al. 2015).

for its efficiency of HLA class I and II antigen presentation (Reuter et al. 2015). As they determined, nearly 80% of surface DEC205 was internalized by both human BDCA-1^{+} and BDCA-3^{+} DCs within 90 min, with the time to maximum internalization being 30 min. A considerably less internalization of antigens targeted to DEC205 ensued under conditions of *in vivo* inflammation, but the efficiency of antigen presentation on the molecules of MHC class I and MHC II was not impaired, *i.e.* "DEC205 continues to elicit efficient MHC I and MHC II antigen presentation outcomes *in vivo*". This finding is important because activation of DCs using inflammatory cytokines is necessary for induction of immunity by tumor vaccines.

Since DCs initiate specific T cell immunity and harmonize innate and adaptive immune response (Banchereau and Steinman 1998, Steinman and Banchereau 2007), it is important to test targeting of WT1 protein to DCs either *ex vivo* or *in vivo*. Indeed the targeting of several other tumor associated antigens such as MAGE (Birkholz et al. 2010), NY-ESO1 (Tsuji et al. 2011), HER2/neu (Wang et al. 2012) to DCs via antibodies specific for the endocytic receptor DEC205 has been studied using *in vitro* and *in vivo* models and appreciated to induce an improved immune response to tumors expressing these antigens. The targeted, matured DCs trigger the specific $CD8^+$ and $CD4^+$ T cell response against the antigen processed and presented by the DCs. To initiate stronger anti-tumor immune response to haematological malignancies, a highly effective antigen delivery system to APCs is important, especially it is essential for protein antigens which are usually weak immunogenic.

1.2.6. T cell response against WT1-overexpressing leukemia

T cell responses to tumors are initiated when their antigens are ingested by host antigen presenting cells (APCs), particularly DCs. The ingested tumor derived protein antigens are processed inside the DCs and peptides from these antigens are then displayed bound to MHC class I molecules for recognition by $CD8^+$ T cells (cross priming/presentation). DCs express co-stimulators that provide the signals needed for differentiation of $CD8^+$ T cells into anti-tumor cytotoxic T lymphocytes (CTLs). Thus, the predominant mechanism of adaptive immunity to tumor is stimulation of the killing function of $CD8^+$ T cells that is orchestrated by DCs. Also, MHC class II molecules on the surface of DCs may present internalized tumor antigens and activate $CD4^+$ helper T cells. As a result of T_{H1} polarization, helper T cells play a crucial role in establishment of sustained anti-tumor immunity by providing cytokines for effective CTL development (Figure 8). In addition, helper T cells specific for tumor antigens may secrete cytokines, such as TNF and IFN-γ, that can increase the expression of MHC class I molecules by tumor cells. Cytolytic $CD4^+$ T cells can exert direct functions against malignancies by lysing HLA class II expressing targets, therefore, vaccine strategies should aim to induce this subset of cells (Brown 2010). For a sustained T-cell protection, the presence of both $CD4^+$ and $CD8^+$ cells has been shown to be essential (Feuchtinger et al. 2010).

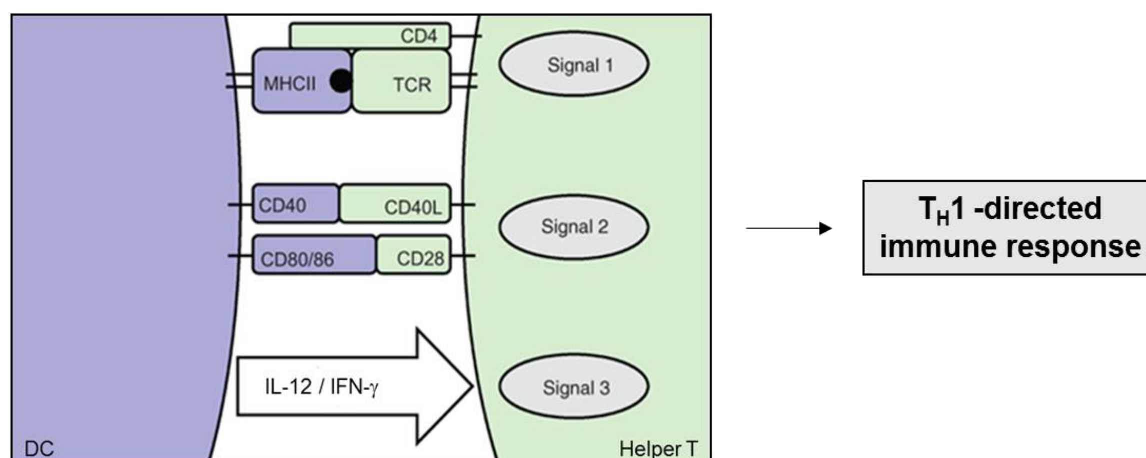

Figure 8. T cell activation mediated by DCs.

Signal 1 is the antigen-specific signal that is mediated through T cell receptor triggering by MHC class I- or II-associated peptides processed from tumor antigens after internalization through endocytosis or pattern recognition receptors. Signal 2 is the co-stimulatory signal, mainly mediated by triggering of CD28 by CD80 and CD86 that are expressed by mature DCs. Signal 3 is the polarizing signal that is mediated by various soluble or membrane-bound factors, such as IL-12, that promote the development of T_H1 cells. Adapted from (Mays and Wilson 2011).

T cells reactive to self-proteins, e.g. to WT1 are deleted by the mechanism of central and peripheral tolerances. However, WT1-specific $CD8^+$ T cells exist at low frequency in healthy donors (Rezvani et al. 2003) and occur with elevated frequency in allo-HSC transplanted patients with leukemia (Rezvani et al. 2005). In healthy donors, the frequency of WT1-specific T cells ranges from 10^{-7} to 10^{-5} in the $CD8^+$ T cell subset and from 10^{-6} to 10^{-5} in the $CD4^+$ T cell subset in the natural repertoire (Schmied et al. 2015). An interesting phenomenon is that during early trimesters of pregnancy, there were increased frequencies of WT1-specific T cells to decline after delivery (Lutz et al. 2015). Due to the rare number in whole T cell repertoire, analyses of WT1-specific T cells are performed after enrichment or expansion of the specific T cells.

With regard to phenotype of WT1-specific T cells and their functional capacity, a couple of studies have been performed. Rezvani *et al.* showed that central ($CD45RO^+CD27^+CD57^-$) or effector memory ($CD45RO^-CD27^-CD57^+$) T cells constitute the WT1-specific T cell population in leukemia patients received HSCT. Whereas Schmied *et al.* demonstrated that two distinct phenotypes of WT1-specific T cells are present in healthy donors: one is $CD4^+$ T cells in the memory pool, the other is $CD8^+$ T cells in the naïve population. An early report on the functional capacity of WT1-specific T cells was that these T cells recognize leukemic $CD34^+$ cells expressing WT1 and exert anti-leukemia immune response (Gao et al. 2000). After vaccination with a combination of WT1

($WT1_{126-134}$ and $WT1_{235-243}$ in Montanide adjuvant) peptides, WT1-specific T cells were emerged and WT1 mRNA level was declined in responding patients. However, the raise of WT1-specific T cells was temporary and did not correlated with disease response. A deletion of high avidity T cells by self-tolerance mechanism and short life expectance of T cells induced by single peptide stimulation could be an explanation (Uttenthal et al. 2014). Nevertheless, WT1-specific T cells can be detected in both peripheral blood and bone marrow of patients with leukemia and they are supposed to be functional in terms of interferon-γ and granzyme B production (Casalegno-Garduno et al. 2015). Casalegno-Garduno *et al.* could also show that WT1-specific T cell presence corresponds to the clinical outcome.

Taken together, very low frequencies of T cells specific to WT1 protein are present in both healthy donors and patients. Those T cells are functional, but may be short-lived. To analyze the low frequency of WT1-specific T cells without prior enrichment or expansion, an improvement of responsiveness of those cells is necessary.

Section 2. Central hypothesis, aim, and design of the study

2.1. Central hypothesis:

WT1 involvement in leukemogenesis, overexpression along with disease progress and proved GvL effect mediated by WT1-specfic T cells after HSCT render WT1 a promising target for immunotherapy. Due to its intracellular localization as well as close interaction with nucleotides and proteins, WT1 protein is thought to be not easily expressed as s recombinant protein. Therefore, full-length human WT1 has not yet been expressed for vaccination studies although its expression for functional studies were optimized. The current WT1-based immunotherapies are mainly based on peptide vaccination. However, WT1 peptides presented on various HLA alleles are not completely defined yet. In addition, a short-lived T cell response to the peptide stimulations hampers the effectiveness of WT1 peptide vaccination. It seemed that targeting of WT1 to dendritic cells could be a feasible approach to improve DC-mediated WT1-specific T cell response either *ex vivo* or *in vivo.* A potential experimental approach was the fusion of WT1 to an antibody specific to the endocytic receptor DEC205 expressed on DCs. After binding to DEC205, the antibody fusion protein is taken up by the DC and processed intracellularly, resulting in the better and broader presentation of WT1-derived peptides on MHC molecules on the surface of the DCs (Figure 9). This way, an efficiency of WT1 protein expression and peptide variety of WT1 to be loaded on HLA class I and II molecules might be better and broader. Thereby, T-cell immune responses to WT1-positive tumors could be effective and sustainable.

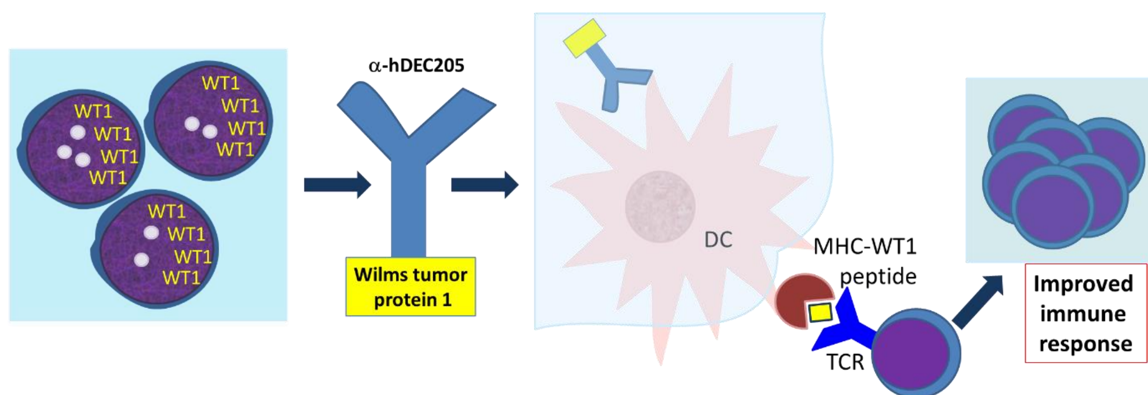

Figure 9. Targeting WT1 protein to DCs.

2.2. Aim

The prior aim of this work was the generation and production of an anti-hDEC205-WT1 antibody fusion protein as a recombinant vaccine candidate for AML immunotherapy. The second aim was the directly *ex vivo* detection of the anti-hDEC205-WT1 loaded mature moDC-mediated T cell response. Moreover, we wanted to check WT1-specific cytotoxicity function of T cells stimulated with this approach by *in vitro* experiments.

2.3. Study design

This work was designed as a two phase sequential study as depicted in Figure 10. The first package of the work included molecular cloning of the antibody fusion protein and production, purification as well as functional tests of the anti-hDEC205-WT1 antibody fusion proteins. In the second phase of the work, the immune stimulatory capacity of the anti-hDEC205-WT1 were tested *ex vivo* and *in vitro* corresponding to the aims of the study. Healthy donors and HSC transplanted patients (divided into two blocks: healthy and diseased) with AML were randomly selected and blood samples were collected for the planned investigations.

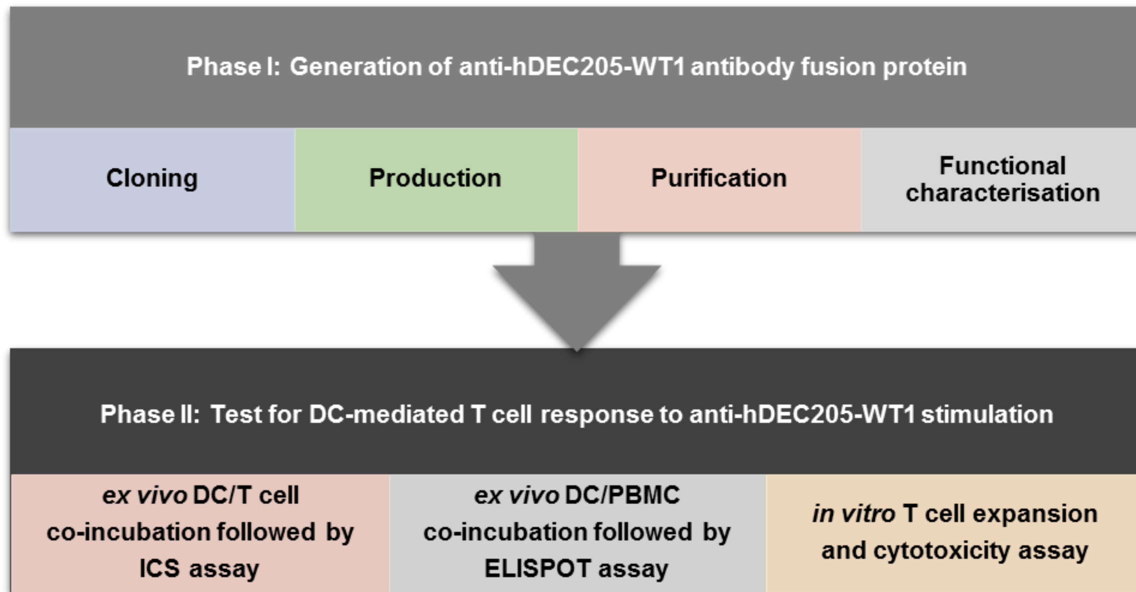

Figure 10. Study design

Section 3. Materials and methods

3.1. Materials

3.1.1. Cells

3.1.1.1. Human primary cells

Sample types: Leukoreduction system chambers

Buffy coats

Peripheral venous blood samples

Cell types: PBMCs

Monocyte derived dendritic cells (moDCs)

CD3+ T cells

3.1.1.2. Cell lines

The human cancer cell lines used for this work were available in the Division of Molecular Internal Medicine, and Laboratory for Immunotherapy, University Hospital of Würzburg. CHO-hDEC205 cells were kindly provided by Professor Dudziak D. (Erlangen, Germany). Adherent cells were cultured in a culture flask supplied with RPMI 1640/10 % FCS/1 % Penicillin and Streptomycin until full confluency and were harvested from the flasks by incubation of cells with 1 % trypsin at 37°, 5 % CO_2 for one min. Suspension cells were cultured in a culture flask supplied with RPMI 1640 GlutaMax/10 % FCS and 50µg/mL gentamycin at density of 1 x 10^6 cells per mL. All cells were split at 1:10 or 1:5 when cell confluency had reached 90-100 %.

Table 5. Cell lines

Cell line	Species and cell types
CHO	Chinese hamster ovary
CHO-hDEC205	h-DEC205 stably expressing CHO
DG75	Human Burkitt lymphoma
HEK293	Human embryonic kidney
HeLa	Human cervical carcinoma
ML2	Human myelomonocytic leukemia
NALM-6	Human B cell precursor leukemia
THP1	Human acute monocytic leukemia

3.1.1.3. Prokaryotic cells

Chemically competent cells were transformed for cloning of the expression plasmids.

Table 6. Prokaryotic cells.

Bacterial competent cells	Strain	Manufacturer, Country
E. Coli	K12 (NEB 5α low competent)	New England Biolabs, Frankfurt, Germany
E. Coli	K12 (NEB 5α high competent)	New England Biolabs, Frankfurt, Germany

3.1.2. Plasmids

The expression plasmids for each construct used in this work were derived from pCR3 expression plasmid (Invitrogen, Darmstadt, Germany). A scFv:hDEC205 construct was encoded in pMA-T plasmid (Life Technologies, Darmstadt, Germany).

3.1.3. Cell culture media and cytokines

Cell culture media were supplemented with 2-10 % FCS and appropriate antibiotics prior to use. Cytokines were solubilized and aliquoted in accordance with manufacturers' protocol.

Table 7. Culture media and cytokines.

Culture medium, cytokine	Manufacturer, Country
RPMI 1640	Sigma-Aldrich, Deisenhofen, Germany
RPMI 1640 GlutaMax	Life Technologies, Darmstadt, Germany
Granulocyte-macrophage colony-stimulating factor (GM-CSF)	Miltenyi Biotec, Bergisch Gladbach, Germany
Interleukin-4 (IL-4)	R&D Systems, Wiesbaden, Germany
Interleukin-2 (IL-2, Proleukin)	Novartis, Nürnberg, Germany
Interleukin-7 (IL-7)	Peprotech, Hamburg, Germany
Interleukin-15 (IL-15)	Peprotech, Hamburg, Germany
TNFα	R&D Systems, Wiesbaden, Germany

3.1.4. Enzymes

Table 8. Enzymes.

Enzyme	Manufacturer, Country
T4-Ligase	Fermentas, St. Leon-Rot, Germany
BamHI	New England Biolabs, Frankfurt, Germany
BglII	New England Biolabs, Frankfurt, Germany
EcoRI	New England Biolabs, Frankfurt, Germany
HindIII	New England Biolabs, Frankfurt, Germany
Mfe	New England Biolabs, Frankfurt, Germany
SalI	New England Biolabs, Frankfurt, Germany
Calf Intestinal alkaline Phosphatase (SIP)	New England Biolabs, Frankfurt, Germany
XbaI	New England Biolabs, Frankfurt, Germany
XhoI	New England Biolabs, Frankfurt, Germany

3.1.5. Antibodies

Table 9. Antibodies.

Antibody	Source	Manufacturer, Country
Anti-CD3-FITC	Mouse anti-human CD3 Clone SK7	BD Biosciences, Heidelberg, Germany
Anti-CD4-PerCP	Mouse anti-human CD4 Clone SK3	BD Biosciences, Heidelberg, Germany
Anti-CD8-APC	Mouse anti-human CD4 Clone SK1	BD Biosciences, Heidelberg, Germany
Anti-CD137-PE or -APC	Mouse anti-human CD137	BD Biosciences, Heidelberg, Germany
Anti-Interferon-γ-PE	Mouse anti-human IFN-γ	Beckman Coulter, Krefeld, Germany
Anti-CD14-APC	Mouse anti-human CD14 Clone HCD14	BioLegend, Fell, Germany
Anti-CD86-PE	Mouse anti-human CD86	BD Biosciences, Heidelberg, Germany
Anti-CD80-PE	Mouse anti-human CD80	BD Biosciences, Heidelberg, Germany
Anti-CD40-APC	Mouse anti-human CD40	BD Biosciences, Heidelberg, Germany
Anti-HLA-ABC-PE	Mouse anti-human HLA-ABC	BD Biosciences, Heidelberg, Germany
Anti-CCR7-APC	Mouse anti-human CCR7 IgG2A	R&D Systems, Wiesbaden, Germany
Anti-HLA-DR-PerCP	Mouse anti-human HLA-DR	BD Biosciences, Heidelberg, Germany
Anti-CD1a-APC	Mouse anti-human CD1a	BD Biosciences, Heidelberg, Germany
Anti-CD205-PE	Mouse anti-human CD205	Miltenyi Biotec, Bergisch Gladbach, Germany

Anti-Flag mAb M2	Mouse IgG1 monoclonal	Sigma-Aldrich, Deisenhofen, Germany
Anti-mouse IRDye 800	Goat, polyclonal	LI-COR Bioscience, Bad Homburg, Germany
Anti-mouse-HRP	Goat, polyclonal	Dako-Cytomation, Glostrup, Denmark
Anti-CD16-PE	Mouse anti-human CD16 (Leu 11c)	BD Biosciences, Heidelberg, Germany
Anti-iNKT-PE	Mouse anti-human iNKT	BD Biosciences, Heidelberg, Germany
Anti-CD56-APC	Mouse anti-human CD56 Clone HCD56	BioLegend, Fell, Germany
Anti-CD107a-APC	Mouse anti-human CD107a	BD Biosciences, Heidelberg, Germany
Anti-HLA-A2-PE	Mouse anti-human HLA-A2	BD Biosciences, Heidelberg, Germany
Anti-human IFN-γ capture antibody	Mouse anti-human IFN-γ	BD Biosciences, Heidelberg, Germany
Anti-human IFN-γ detection antibody	Mouse anti-human IFN-γ	BD Biosciences, Heidelberg, Germany
Mouse IgG1 isotype conjugated FITC, APC, PE, or PerCP		BD Biosciences, Heidelberg, Germany
Mouse IgG2A-APC		R&D Systems, Wiesbaden, Germany

3.1.6. Peptides and recombinant proteins

Table 10. Peptide and recombinant protein.

Peptide	Manufacturer, Country
WT1 peptivator 15-mer peptide pool	Miltenyi Biotec, Bergisch Gladbach, Germany
WT1 A24 100018 single peptide	University of Tübingen, Germany
WT1 A02 3181 single peptide	University of Tübingen, Germany
CMV pp65 recombinant protein	Miltenyi Biotec, Bergisch Gladbach, Germany

3.1.7. Chemicals and reagents

Table 11. Chemicals and reagents.

Substance	Manufacturer, Country
1kb DNA-ladder	Fermentas, St. Leon-Rot, Germany
Acetic acid	J. T. Baker, Leipzig, Germany
Acrylamide (30 %)	Carl Roth, Karlsruhe, Germany
Agar	Carl Roth, Karlsruhe, Germany
Agarose	Carl Roth, Karlsruhe, Germany
Ammonium persulfate (APS)	AppliChem, Darmstadt, Germany
Ampicillin	Carl Roth, Karlsruhe, Germany
Anti-Flag M2 agarose beads	Sigma-Aldrich, Deisenhofen, Germany
BCIP/NBT liquid substrate system	Sigma-Aldrich, Deisenhofen, Germany
Bovine serum albumin (BSA)	Sigma-Aldrich, Deisenhofen, Germany
Crystal violet (CV) powder	Carl Roth, Karlsruhe, Germany
Dimethyl sulfoxide (DMSO)	Carl Roth, Karlsruhe, Germany
Ethanol	J. T. Baker, Leipzig, Germany
Ethylenediaminetetraacetic acid (EDTA)	Carl Roth, Karlsruhe, Germany
Fetal calf serum (FCS)	Life Technologies, Darmstadt, Germany
Flag peptide	Sigma-Aldrich, Deisenhofen, Germany
Geneticin disulfate (G418-Sulfate)	Carl Roth, Karlsruhe, Germany
Gentamycin	Life Technologies, Darmstadt, Germany
Lymphocyte separation medium	PAA, Pasching, Austria
Methanol	J. T. Baker, Leibzig, Germany
Midori Green Advance DNA stain	Intas Science Imaging, Göttingen , Germany
Nonfat dried milk powder	Sigma-Aldrich, Deisenhofen, Germany
Paraformaldehyde	Carl Roth, Karlsruhe, Germany
Penicillin-Streptomycin (100 x)	PAA, Pasching, Austria
Peptone	Carl Roth, Karlsruhe, Germany
Phosphatase inhibitor II	Sigma-Aldrich, Deisenhofen, Germany
Phosphate buffered saline (PBS)	PAA, Pasching, Austria
DPBS	Life Technologies, Darmstadt, Germany

Polyethylenimine (PEI)	Sigma-Aldrich, Deisenhofen, Germany
Prestained protein marker (broad range)	New England Biolabs, Frankfurt, Germany
Protease inhibitor cocktail	Roche, Mannheim, Germany
Silver gel marker (low molecular weight)	GE Healthcare, Garching, Dassel, Germany
Sodium dodecyl sulfate (SDS)	Carl Roth, Karlsruhe Garching, Germany
Streptavidin-AP (Alkaline Phosphatase)	Southern Biotech, Eching, Germany
Tetramethylethylenediamine (TEMED)	Sigma-Aldrich, Deisenhofen, Germany
Tris	Carl Roth, Karlsruhe, Germany
Triton X-100	Sigma-Aldrich, Deisenhofen, Germany
Trypsin-EDTA solution (10X)	PAA, Pasching, Austria
Tween-20	Carl Roth, Karlsruhe, Germany
Yeast extract	Carl Roth, Karlsruhe, Germany
ß-Mercaptoethanol	Sigma-Aldrich, Deisenhofen, Germany

3.1.8. Kits

Table 12. Kits.

Kit	**Manufacturer, Country**
CD3+ pan T cell untouched selection kit	Miltenyi Biotec, Bergisch Gladbach, Germany
CD137-PE isolation kit	Miltenyi Biotec, Bergisch Gladbach, Germany
Gaussia Luciferase Assay kit	New England Biolabs, Frankfurt, Germany
Pierce® Silver Stain kit	Fermentas, St. Leon-Rot, Germany
Pure Yield Plasmid Miniprep/Midiprep System	Promega, Mannheim, Germany
Kod Hot Start DNA polymerase kit	Merckmillipore, Darmstadt, Germany
PCR clean-up, Gel extraction kit	Macherey-Nagel, Düren, Germany
RNeasy mini kit	Qiagen, Hilden, Germany
QuantiTect Rev. Transcription Kit	Qiagen, Hilden, Germany

Rapid DNA ligation kit	Fermentas, St. Leon-Rot, Germany

3.1.9. Equipments and disposable materials

Table 13. Equipments and disposable materials.

Instrument or material/equipment	**Manufacturer, Country**
96-well ELISA plates (high binding)	Greiner, Frickenhausen, Germany
Black 96-well ELISA plates	Greiner, Frickenhausen, Germany
96-well ELISPOT plates	Merckmillipore, Darmstadt, Germany
Casting chambers for SDS-PAGE	PeqLab, Erlangen, Germany
Cell culture flasks	Greiner, Frickenhausen, Germany
Cell culture petri dishes	Greiner, Frickenhausen, Germany
Cell culture plates	Greiner, Frickenhausen, Germany
Centrifuge Rotana 460R	Hettich, Tuttlingen, Germany
CO2 incubator Heraeus	Cell Safe Heraeus, Hanau, Germany
Dialysing tubes, Viking, MWCO 15kDa	Carl Roth, Karlsruhe, Germany
Cryotubes	Greiner, Frickenhausen, Germany
ELISPOT reader/C.T.L	USA
Eppendorf tubes, 1,5 ml und 2 ml	Eppendorf, Hamburg, Germany
Equibio EasyjecT Plus electroporator	PeqLab, Erlangen, Germany
Flow cytometer FACS Calibur	BD Biosciences, Heidelberg, Germany
Flow cytometry tubes	Falcon, Heidelberg, Germany
Heat block	PeqLab, Erlangen, Germany
LI-COR Odyssey® Infrared Imager	LI-COR Biosciences, Lincoln, USA
Lucy 2 luminometer/ELISA-reader	Anthos Labtec, Krefeld, Germany
MACS LS and MS columns	Miltenyi Biotec, Bergisch Gladbach, Germany
MACS multistand	Miltenyi Biotec, Bergisch Gladbach, Germany
Microcentrifuge 5417C	Eppendorf, Hamburg, Germany
Nitrocellulose membranes, 0,2 µM pore size	Whatman, Dassel, Germany

PCR-Thermocycle	Primus MWG Biotech, Ebersberg, Germany
Pipetus	Hirschmann Laborgeräte, Eberstadt, Germany
Polyallomer tubes	Seton, Los Gatos, CA, USA
Polypropylene tubes	Greiner, Frickenhausen, Germany
Sterile filters (0,2µm)	Sarstedt, Nümbrecht, Germany
Sterile plastic Pasteur pipettes	Hartenstein, Würzburg, Germany
Ultracentrifuge OPTIMA-L70	Beckman Coulter, Krefeld, Germany
UV-transilluminator	PeqLab, Erlangen, Germany
Well plates for cell culture	Greiner, Frickenhausen, Germany
Wet/tank blotting system	PeqLab, Erlangen, Germany
Whatman papers	Hartenstein, Würzburg, Germany

3.1.10. Preparations and buffers

Table 14. Preparations and buffers.

Preparation	Prescription
Assay diluent	1 x PBS 10 % (v/v) FCS
Blot buffer 10x	0,025 M Tris 0,192 M glycin 20 % (v/v) methanol pH 8,3
Laemmli lysis buffer (SDS-PAGE, 4 x)	8 % (w/v) SDS 10 % ß-Mercaptoethanol 40 % gycerol 0,2 M Tris 0,04 % bromphenol blue pH 8
LB medium	10 g peptone 5 g yeast extract

	10 g/l NaCl
Lysis buffer for immunoprecipitation (IP)	1 M Tris-HCl pH 7.4 2M NaCl 100 % glycerol 100 % triton volume adjusted to 1 L with distilled water
MACS buffer	1 x PBS 1 % (w/v) FCS 2 mM EDTA
PBST	1 x PBS 0,05 % (v/v) tween-20
Milk in PBST	1 x PBS 0,05 % (v/v) tween-20 5 % (w/v) nonfat dried milk powder
Running buffer 10x (SDS-PAGE)	0,05 M Tris 0,38 M glycin 0,004 M SDS pH 8,3
Separating gel buffer (SDS-PAGE)	1,5 M Tris 0,015 M SDS pH 8,8
Stacking gel buffer (SDS-PAGE)	0,5 M Tris 0,015 M SDS pH 6,8
TAE buffer	2 M Tris 1 M acetic acid 0,1 M EDTA pH 8,3
TBS	0,02 M Tris 8 % (w/v) NaCl pH 7,6
ELISPOT detection buffer	RPMI 1640 10 FCS

3.2. Methods

3.2.1. Cloning

3.2.1.1. Cloning of single chain variable fragment of anti-hDEC205 fused to GpL (scFv:hDEC205-GpL) and anti-hDEC205 antibody

To clone scFv:hDEC205-GpL, IgG1 isotype (accession number P01857 (heavy constant domain), AAA59000 (kappa constant domain)) of anti-hDEC205 antibody, the following expression constructs were digested with restriction endonucleases listed in the Table 15.

Table 15. Vectors and inserts for expression constructs encoding scFv:hDEC205-GpL and anti-hDEC205 antibody.

Construct	Vector	Vector digestion	Insert	Insert digestion
scFv:hDEC205-GpL	Fn14(ed)-2xFlag-GpL-pCR3	HindIII BamHI	anti-DEC205(h)-Mg38-3-pMA-T	HindIII BamHI
Anti-hDEC205-VH-heavy const chain-IgG1-full	18D1-VH-heavy-full(hIgG1)-pCR3	EcoR1 BamH1	VH chain of anti-DEC205(h)-Mg38-3-pMA-T	EcoRI BglII
Anti-hDEC205-VL-light-const chain-full	18D1-VL-light chain full-pCR3	EcoR1 BamH1	VL chain of anti-DEC205(h)-Mg38-3-pMA-T	EcoRI BglII

The digested and dephosphorylated vectors were ligated with the corresponding insert DNAs with help of T4 DNA ligase. The ligation mix were then transformed in NEB 5α high competent cells by heat shock at 42°C and plated onto LB agar plates. After overnight incubation at 37°C, positive colonies were selected. To replicate the expression constructs, the selected colonies were cultured in midi preps overnight. Subsequently, the expression constructs were isolated from the microbial host via PureYield Plasmid Midiprep system according to the manufacturer's protocol. The concentration of the plasmid DNA yields were measured by spectrophotometer at 260 nm and purities were calculated on the basis of A_{260}/A_{280} ratio. The DNA sequences were proved by an external DNA sequencing provider (GATC Biotech., Köln, Germany).

3.2.1.2. Cloning of anti-hDEC205-WT1$_{major}$ and anti-hDEC205-WT_D$_{full}$ constructs

Of the cDNAs obtained from the indicated leukemia cell lines, four different DNA sequences encoding major fragments of WT1 isoform D (WT1_D), so named WT1_D$_{1-227}$, WT1_D$_{217-351}$, WT1_D$_{217-487}$, WT1_D$_{217-522}$ were amplified using corresponding primer pairs (Table 18). With exclusion of four amino acids, cDNA encoding the full-length WT1_D was cloned by combining WT1_D$_{1-227}$ with WT1_D$_{217-522}$ using its internal Xho1 site. Each of WT1_D major fragments were cloned C-terminally to the heavy chain of the anti-hDEC205 antibody to obtain anti-hDEC205-WT1_D$_{1-227}$, anti-hDEC205-WT1_D$_{217-351}$, anti-hDEC205-WT1_D$_{217-487}$, and anti-hDEC205-WT1_D$_{217-522}$ expression constructs. In addition, the WT1_D major fragments were cloned to the single chain variable fragment of anti-hDEC205 (scFv:hDEC205), as well as to the C-terminus of light chain of the anti-hDEC205 antibody to identifying better producibility for the antibody fusion proteins (Table 16).

Table 16. Overview of cloning of the anti-hDEC205-WT1_D$_{major}$ expression constructs.

Antibody format	Construct	Vector	Vector digestion	Insert	Insert digestion
Single chain variable fragment of anti-hDEC205 (scFv:hDEC205)	scFv:2xFlag-anti-hDEC205-WT1_D$_{217-487}$	scFv:2xFlag-anti-hDEC205-GpL-pCR3	XhoI XbaI	WT1_ D$_{217-487}$	XhoI XbaI
	scFv:2xFlag-anti-hDEC205-WT1_D$_{217-522}$	scFv:2xFlag-anti-hDEC205-GpL-pCR3	XhoI XbaI	WT1_D$_{217-522}$	XhoI XbaI
	scFv:2xFlag-anti-hDEC205-WT1_D$_{1-227}$	scFv:2xFlag-anti-hDEC205-GpL-pCR3	XhoI XbaI	WT1_ D$_{1-227}$	SalI XbaI
	scFv:2xFlag-anti-hDEC205-WT1_D$_{217-351}$	scFv:2xFlag-anti-hDEC205-GpL-pCR3	XhoI XbaI	WT1_ D$_{217-351}$	XhoI XbaI
	scFv:2xFlag-anti-hDEC205-WT1_D$_{full}$	scFv:2xFlag-anti-hDEC205-WT1_D$_{1-222}$-pCR3	XhoI XbaI	WT1_D$_{217-522}$	XhoI XbaI
Anti-hDEC205-VH-heavy const IgG1 chain full	Flag-anti-hDEC205-VH-heavy const full-WT1_ D$_{217-487}$	scFv:2xFlag-anti-hDEC205-WT1_D$_{212-482}$-pCR3	HindIII XhoI	anti-hDEC205-VH-heavy const full	HindIII XhoI
	Flag-anti-hDEC205-VH-heavy const full-WT1_D$_{217-522}$	scFv:2xFlag-anti-hDEC205-WT1_D$_{212-517}$-pCR3	HindIII XhoI	anti-hDEC205-VH-heavy const full	HindIII XhoI
	Flag-anti-hDEC205-VH-heavy const full-	Flag-anti-hDEC205-VH-heavy const full-WT1_D$_{212-517}$-	XhoI XbaI	WT1_ D$_{1-227}$	SalI XbaI

	WT1_ D_{1-227}	pCR3			
	Flag-anti-hDEC205-VH-heavy const full-WT1_ $D_{217-351}$	Flag-anti-hDEC205-VH-heavy const full-WT1_ $D_{212-517}$-pCR3	XhoI XbaI	WT1_ $D_{217-351}$	XhoI XbaI
	Flag-anti-hDEC205-VH-heavy const full-WT1_ $D_{347-522}$	Flag-anti-hDEC205-VH-heavy const full-WT1_ $D_{212-517}$-pCR3	XhoI XbaI	WT1_ $D_{347-522}$	XhoI XbaI
	Flag-anti-hDEC205-VH-heavy const full-WT1_ D_{full}	Flag-anti-hDEC205-VH-heavy const full-WT1_ D_{1-222}-pCR3	XhoI XbaI	WT1_ $D_{217-522}$	XhoI XbaI
Anti-hDEC205-VL-light kappa const chain full	Flag-anti-hDEC205-VL-light const full-WT1_ $D_{217-351}$	Flag-anti-hDEC205-VH-heavy const full-WT1_ $D_{212-346}$-pCR3	EcoRI XhoI	anti-hDEC205-VL-light const chain full	EcoRI XhoI
	Flag-anti-hDEC205-VL-light const full-WT1_ $D_{217-522}$	Flag-anti-hDEC205-VH-heavy const full- WT1_ $D_{217-522}$-pCR3	EcoRI XhoI	anti-hDEC205-VL-light const chain full	EcoRI XhoI
	Flag-anti-hDEC205-VL-light const full-WT1_ D_{1-227}	Flag-anti-hDEC205-VL-light const full-WT1_ $D_{217-351}$-pCR3	XhoI XbaI	WT1_ D_{1-227}	SalI XbaI

3.2.1.3. Cloning of anti-hDEC205-WT1$_{small}$ constructs:

Different primer pairs (Table 18) were designed for various DNA sequences encoding small fragments of the WT1_D protein. Those primers encoded additionally a flexible spacer consisting of four amino acids (ASTA). In total, eight different small DNAs were amplified and cloned to the carboxyl terminus of the heavy chain of anti-hDEC205 (Table 17).

Table 17. Overview of cloning of the anti-hDEC205-WT1_D$_{small}$ expression constructs.

Construct	Vector	Vector digestion	Insert	Insert digestion
Flag-anti-hDEC205-VH-heavy const full-WT1$_{(-73)-53}$	Flag-anti-hDEC205-VH-heavy const full-TNC-CD70ed-pCR3	XhoI XbaI	WT1$_{(-73)-53}$	SalI XbaI
Flag-anti-hDEC205-VH-heavy const full-WT1$_{10-35}$	Flag-anti-hDEC205-VH-heavy const full-TNC-CD70ed-pCR3	XhoI XbaI	WT1$_{10-35}$	XhoI XbaI
Flag-anti-hDEC205-VH-heavy const full-WT1$_{91-138}$	Flag-anti-hDEC205-VH-heavy const full-TNC-CD70ed-pCR3	XhoI XbaI	WT1$_{91-138}$	XhoI XbaI
Flag-anti-hDEC205-VH-heavy const full-	Flag-anti-hDEC205-VH-heavy const full-	XhoI XbaI	WT1$_{223-273}$	XhoI XbaI

$WT1_{223-273}$	TNC-CD70ed-pCR3			
Flag-anti-hDEC205-VH-heavy const full-$WT1_{324-371}$	Flag-anti-hDEC205-VH-heavy const full-TNC-CD70ed-pCR3	XhoI XbaI	$WT1_{324-371}$	XhoI XbaI
Flag-anti-hDEC205-VH-heavy const full-$WT1_{10-53}$	Flag-anti-hDEC205-VH-heavy const full-TNC-CD70ed-pCR3	XhoI XbaI	$WT1_{10-53}$	XhoI XbaI
Flag-anti-hDEC205-VH-heavy const full-$WT1_{144-273}$	Flag-anti-hDEC205-VH-heavy const full-TNC-CD70ed-pCR3	XhoI XbaI	$WT1_{144-273}$	XhoI XbaI
Flag-anti-hDEC205-VH-heavy const full-$WT1_{223-371}$	Flag-anti-hDEC205-VH-heavy const full-TNC-CD70ed-pCR3	XhoI XbaI	$WT1_{223-371}$	XhoI XbaI

3.2.2. RNA extraction, RT-PCR and PCR

First, mRNAs were extracted from three different leukemia cell lines (THP-1, ML-2, NALM-6) by RNA easy extraction kit (Qiagen, Hilden, Germany) according to instructions of the manufacturer. The extracted mRNAs were then reversibly transcribed to cDNAs by RT-PCR using QuantiTect Rev. Transcription Kit (Qiagen, Hilden, Germany). PCR was performed using Kod Hot Start DNA polymerase kit (Merckmillipore, Darmstadt, Germany) according to the manufacturer's instructions. An array of primer pairs were designed for various sequences encoding WT1 protein. The used primer pairs and templates for the PCR were listed in Table 18.

Table 18. Sequences of the designed primer pairs and templates for PCR.

Template	Forward primers 5'-3'	Reverse primers 3'-5'	PCR product
Human cDNA (ML2, THP-1, NALM-6 cell lines)	ATTCGCAATCAGGGTTACAGCA	TCAGAAGGGCTTTTCACTTGTTTTACC	$WT1_D_{217-487}$
	ATTCGCAATCAGGGTTACAGCA	TTAAAGCGCCAGCTGGAGTTTGGTCAT	$WT1_D_{217-522}$
Human cDNA (ML2 cell line)	TTCCTCTTGCTGCAGGACCCGG	TTAGAAGGTGACCGTGCTGTAACC	$WT1_D_{1-227}$
scFv:2xFlag-anti-hDEC205-$WT1_D_{212-517}$	ATTCGCAATCAGGGTTACAGCA	TTACGTTGTGTGGTTATCGCTCTCG	$WT1_D_{217-351}$
	GATAACCACACAACGCCCATCC	TTAAAGCGCCAGCTGGAGTTTGGTCAT	$WT1_D_{347-522}$
scFv:2xFlag-anti-hDEC205-$WT1_D_{1-222}$	TTCCTCTTGCTGCAGGACCCGG	TTAGCCGCCCAACGACCCGTAAGCC	$WT1_{(-73)-53}$
	GCGCTGCTGCCCGCCGTCC	TTACGCCCACTGCGCCGCGCCGCTC	$WT1_{10-35}$
	GCCTTCACTGTCCACTTTT	TTAGAGGCAGCTGGGCAGGTAGGGC	$WT1_{91-138}$
Flag-anti-hDEC205-VH-heavy const full-$WT1_D_{212-517}$	AGTGACAATTTATACCAAA	TTAGCTCTCGTACCCTGTGCTGTGG	$WT1_{223-273}$
	ATGTGTGCTTACCCAGGCT	TTATTTGAGCTGGTCTGAACGAGAA	$WT1_{324-371}$
scFv:2xFlag-anti-hDEC205-$WT1_D_{full}$	GCGCTGCTGCCCGCCGTCC	TTAGCCGCCCAACGACCCGTAAGCC	$WT1_{10-53}$
Flag-anti-hDEC205-VH-heavy const full-$WT1_D_{full}$	GCCTTCACTGTCCACTTTT	TTAGCTCTCGTACCCTGTGCTGTGG	$WT1_{144-273}$
Flag-anti-hDEC205-VH-heavy const full-$WT1_D_{212-517}$	AGTGACAATTTATACCAAA	TTATTTGAGCTGGTCTGAACGAGAA	$WT1_{223-373}$
anti-DEC205(h)-Mg38-3-pMA-T	GAGGTGCAGCTGCAGCAGTCTG	GCTAGACACTGTCAGGGTTGTGCCC	anti-hDEC205-VH
	CAGGCTGTCGTGACCCAGGAAA	CAGCACGGTCACTTTGGTGCCG	anti-hDEC205-VL
anti-hDEC205-VH-heavy const chain-IgG1-full	AAGCTTCAAAACATGAACTTCGGCTTCA	CTTGCCGGGGCTCAGGCTCAGGGA	anti-hDEC205-VH-heavy const full
Anti-hDEC205-VL-light-const chain-full	CAGGCTGTCGTGACCCAGGAAA	GCACTCGCCCCGGTTGAAGCTCTTGGTC	anti-hDEC205-VL-light const full

3.2.3. Gel electrophoresis and DNA purification

Gel electrophoresis was performed for qualification of the expression vectors and insert DNAs as well as for DNA purification. Depending on the size of DNAs, 1.2-1.8% agarose gels were used in this study. To prepare 1.2% agarose gel, 0.7 g of dry agarose was mixed with 60 mL of 1x TAE buffer in Erlenmeyer flask. Then the agarose was completely dissolved by cooking and swirling the flask. After short cooling down, 4 μL of Midori Green Advance DNA stain (Intas, Göttingen, Germany) was added into the agarose gel to visualize DNAs under ultraviolet (UV) light. The agarose gel was poured into a gel tray placed well combs in and incubated at room temperature until its complete solidification. Then the gel was placed in an electrophoresis unit filled with 1x TAE buffer. DNA samples mixed with 2 μL of the loading buffer and DNA ladder (10μL) were loaded into wells of the gel and the gel was run at 80-150V until DNA separation was completed. DNA bands were then visualized using UV-transilluminator (PeqLab, Erlangen, Germany). In cases of DNA purification, the appropriate DNA band was sliced under a short exposure of the UV light and melted at 52°C. DNA was purified from the melted gel using PCR clean-up, Gel extraction kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions.

3.2.4. Protein production

For protein production two transient transfection methods were used: electroporation and polyethylenimine (PEI) transfection.

3.2.4.1. Electroporation:

Electroporation is a highly efficient method for the introduction of foreign nucleic acids into many cell types, including bacteria and mammalian cells. By applying an electrical pulse, cell membranes are temporarily permeabilized resulting in forming tiny pores through which DNA can enter into cells (Rols 2008). Approximately $5x10^7$ HEK293 cells were harvested from the bottom of 175 cm^2 cell culture flask by trypsinization. Cells were pelleted by centrifugation at 1200 rpm, room temperature (RT) for 4 min and resuspended in 1 mL of culture media (RPMI 1640 supplied with 10% FCS and 1% penicillin-streptomycin). Then cells were transferred to 4 millimetre electroporation

cuvette. After adding 40 µg of expression plasmid (in case of antibody, two plasmids encoding light and heavy chains were mixed at 1:1 ratio) to the cuvette, cells were electrically pulsed by Easyject Plus equipment with adjustment of 250 V, 1800 µF, maximum resistance. The electroporated cells were immediately transferred to 58 cm^2 cell culture dish with 17 mL of serum reduced culture media (2 % FCS) and cultured at 37°C, 5 % CO_2 for 6-7 days for production.

3.2.4.2. PEI transfection:

Polyethylenimine (PEI) is a stable cationic polymer and condenses DNA into positively charged particles, that bind to anionic cell surface. Consequently, the DNA:PEI complex is endocytosed by the cells and the DNA released into the cytoplasm (Longo et al. 2013). HEK293 cells were cultured in 58 cm^2 cell culture dish at 80-100% confluency. Then culture media of cells were replaced by 17 mL of serum-free media (only RPMI 1640). All reagents, expression plasmids were at RT. Expression plasmid and PEI at 1:3 ratio (DNA : PEI = 12 µg : 36 µg) were added to 2 mL of RPMI 1640 in Eppendorf tubes and mixed well by vortex. After 10 min of incubation at RT, the DNA and PEI mixtures were added drop-wise to the cells and left cells at 37°C, 5 % CO_2 for overnight. The next day, culture media were changed by 17 mL of fresh serum reduced culture media (2 % FCS) and the transfected cells were cultured at 37°C, 5 % CO_2 for 6-7 days for production.

After production period, cell culture supernatants were collected into 50 mL conical tube and removed cell debris by centrifugation at 4600rpm, RT for 10 min. The culture supernatants were analyzed for their antibody content and kept at -20°C in the dark until protein purification.

3.2.5. Protein purification

The antibody fusion proteins were purified from culture supernatants of the transfected HEK293 cells by column affinity chromatography using anti-Flag M2 affinity gel. The anti-Flag M2 affinity gel (Sigma-Aldrich, Deisenhofen, Germany) is a glycerol suspension containing a purified murine IgG1 monoclonal antibody covalently attached to agarose by hydrazide linkage. Anti-Flag M2 antibody binds to Flag peptide in a calcium independent manner. To purify proteins, first, a clean chromatography column and column reservoir were assembled. Then 3 mL of anti-Flag M2 agarose beads were

transferred to the column and allowed beads to form a bed on the bottom by running glycerol through the column. After washing the agarose bed twice with 5 column volumes of sterile 1% TBS, 200-400 mL of culture supernatants were loaded onto the assembled column without disturbing the agarose bed. A gravitational flow rate was adjusted to allow the Flag-tagged antibody fusion proteins in culture supernatants are bound to the anti-Flag M2. The column was left at 4°C until whole volume of culture supernatants were passed through the agarose bed. Afterwards, unbound proteins within the agarose bed were removed by washing with 10 column volumes of 1% TBS. Flag-tagged antibody fusion proteins bound to the anti-Flag M2 were eluted by 2-3 column volumes of TBS containing 100 μg/mL Flag peptide. The eluted proteins were dialysed against 1% PBS at 4°C overnight. The next day, the antibody fusion proteins were sterile filtered (200μm) and stored at -20°C for further usage. The purification quality was evaluated by checking contents of antibody fusion proteins in culture supernatant, flow through, elutes, and washes before and after elution using SDS-PAGE and western blotting. The used agarose beads were extensively washed with 1% TBS and stored in 50% glycerol/TBS buffer containing 0.02% sodium azide at -20°C. Protein elutes were pooled depending on determined concentration on silver stained gel and stored at -20°C until their use.

3.2.6. SDS-PAGE and Western Blot

SDS-PAGE and Western blotting were used for detection and determination of concentration of the antibody fusion proteins after production and purification.

3.2.6.1. SDS-PAGE: The proteins were separated by their size and molecular mass using SDS-PAGE. First, 6% stacking gel and 12% resolving gel were prepared and solidified in the assembled rack. The gel was placed in electrophoresis unit filled with 1x running buffer. All antibody fusion protein samples and standards (Flag-TNF60 fusion protein, 3 μg/mL) were boiled at 96°C for 3 min in presence of 8 μL of 4x Laemmli lysis buffer to be converted from complex to primary structures of proteins. After centrifugation at RT 14000 rpm for 1 min, the samples, standards and broad range protein marker were applied into corresponding wells of stacking gel layered on the top of resolving gel. Then the electrophoresis was run at 90-120V and 400mA for 105 min.

3.2.6.2. Blotting: Proteins separated by SDS-PAGE were transferred to solid support, a nitrocellulose membrane using wet blotting and electrophoresis system. The

membrane was carefully placed together with the gel between anode and cathode in a sandwich consisting of fiber sponge at each outer side, and filter papers protecting the gel and blotting membrane. Afterwards, the blotting sandwich was placed inside the blotting electrophorator filled with transfer buffer. The proteins were electrically (90V, 400mA) transferred onto the nitrocellulose membrane for 180 min.

3.2.6.3. Blocking: Blotted membranes were incubated in 5% nonfat milk in 1x PBST for 45 min to block a nonspecific binding of detection antibodies to the membrane. Then membranes were washed three times with 1x PBST for 5-10 min.

3.2.6.4. Protein detection: Membranes were incubated with anti-Flag M2 antibody (primary antibody) diluted 1:2000 in PBST at 4°C overnight. The next day the membranes were washed three times with PBST for 10 min. Then IRDye 800CW-conjugated goat anti-mouse IgG (secondary antibody) diluted 1:10000 in PBST was added to the membranes and incubated at room temperature for one hour. After three times washing with PBST, the proteins were detected in the 800 nm channel of the LI-COR Odyssey Imager.

3.2.7. Silver staining

Silver staining is the most sensitive method for detection of proteins in polyacrylamide gels. Proteins bind silver ions, which are reduced into metallic silver under appropriate conditions. This phenomenon develops a visible image in the gel. In this study, silver staining was used for quantification of the antibody fusion proteins as well as for analysis of purity and integrity of their light and heavy chains. The silver staining was performed using Pierce® Silver Stain kit (Fermentas, St. Leon-Rot, Germany) according to the manufacturer's protocol. Briefly, all purified proteins were sampled as 100 ng protein in each calculated volume and boiled at 96°C for 3 min with 5 μL of loading buffer either presence of DTT or not. The protein samples and 10 μL of 1x silver gel marker were loaded into wells of the gel and separated by SDS-PAGE as described in western blot technique. Then gels were fixed with 30% ethanol and 10% acetic acid mix for 30 min to remove background signal. Following the sensitization, gels were incubated with silver stain to impregnate silver ions to the gel. The stained gels were developed until the protein bands clearly visualized. The development was stopped by addition of 5 % acetic acid solution. By comparing the intensity of the protein band to

the silver gel marker band which has known mass, concentrations of the proteins were determined.

3.2.8. Endotoxin screening in purified proteins

To check if the purified antibody fusion proteins were contaminated with endotoxin of gram-negative bacteria, each batch of the purification were tested for endotoxin content using Pierce® LAL Chromogenic Endotoxin Quantitation Kit (Thermo Scientific) according to instructions of the manufacturer.

3.2.9. Binding studies

Parental and hDEC205-stably expressing CHO cells (CHO-hDEC205, kindly provided by Prof. D. Dudziak, Erlangen, Germany) were seeded into wells of a 24-well plate at a density of 2x10^{5} cells per well and cultured overnight at 37°C.

3.2.9.1. Equilibrium binding studies

For equilibrium binding studies, a GpL fusion protein of a single chain variable fragment (scFv) variant of the anti-hDEC205 antibody (scFv:hDEC205-GpL) was used. Supernatants of parental CHO and CHO-hDEC205 cells were pairwise replaced by a medium containing increasing concentrations of the scFv:hDEC205-GpL and incubated at 37°C for one hour. Unbound scFv:hDEC205-GpL molecules were removed by washing the cells 10 times with ice-cold PBS. Cells with bound GpL fusion protein molecules were scraped into a 96-well black plates in 50 µL medium (RPMI/0.5%FCS) and cell-bound GpL activity was measured using the Gaussia Luciferase assay kit (NEB) and a Lucy2 Luminometer according to manufacturers' instructions. Specific binding values of scFv:hDEC205-GpL to hDEC205 were obtained by subtracting parental CHO-derived values (non-specific binding) from the corresponding values derived of the CHO-hDEC205 cells (total binding). In another series of equilibrium binding studies, CHO-hDEC205 cells were blocked with a constant concentration (2 µg/mL) of the various anti-hDEC205-WT1 antibody fusion proteins at 37°C for one hour prior to incubation with

increasing concentrations of scFv:hDEC205-GpL for an additional hour to obtain total and nonspecific binding to calculate the K_d values.

3.2.9.2. Heterologous competition binding studies

For the heterologous competition binding assay, CHO-hDEC205 cells were incubated with mixtures of a constant concentration (50 ng/mL (660 pM, C_{GpL})) of the scFv:hDEC205-GpL and increasing concentrations of the conventional anti-hDEC205 IgG1 antibody at 37 °C for 1.5 hours. The K_i (K_d) of the antibody was calculated by help of the K_d-value of scFv:hDEC205-GpL and IC_{50} concentration of the anti-hDEC205 antibody using following formula: $K_i = IC_{50} / (1 + C_{GpL} / K_d)$.

3.2.10. Collection and isolation of human PBMCs

3.2.10.1. Blood samples

Blood samples from healthy donors were obtained as leukoconcentrate in leukoreduction system chamber or buffy coats in blood collection bag after aphaeresis procedures from the Department of Clinical Transfusion Medicine and Haemotherapy of the University Hospital of Würzburg. Peripheral venous blood samples of haematopoietic stem cell transplanted patients with acute myeloid leukemia or myelodysplastic syndrome in EDTA-Vacutainers were obtained from the Outpatient Clinic of HSCT unit of the University Hospital of Würzburg. All blood samples were collected after written informed consent approved by the Institutional Ethical Board of the University of Würzburg.

3.2.10.2. Isolation of the peripheral blood mononuclear cells (PBMCs)

The obtained blood samples were transferred into 50 mL collection tube. Leukoconcentrates and buffy coats were diluted with 10-20 mL of D-PBS. Then the blood samples were carefully layered onto the 15 mL of Ficoll gradient solution in 50 mL tube and centrifuged at 1800 rpm room temperature for 20 minutes to separate blood components by their density gradient. PBMCs between plasma and Ficoll solution were

isolated by careful pipetting into the 10mL pipette and transferred to 50 mL tube. By washing twice with 50 mL of D-PBS and centrifugation at 1500 rpm 4°C for 10 min, platelets were removed. The isolated PBMCs were resuspended in 10-20 mL of culture media (RPMI 1640 GlutaMax/10 % FCS and 50 µg/mL gentamycin) and counted on light microscope with haemocytometer using 0.04 % trypan blue dye. The cell numbers in one mL volume were then calculated using a formula:

Viable cells counted at two diagonal large squares/2 x 10 (dilution factor) x 10^4 *(volume of one large square)*

3.2.10.3. Freezing and thawing of PBMCs

After isolation, PBMCs were used directly or frozen as 1.2-1.8x10^7 cell aliquots in 1.5 ml freezing medium at -80°C using Mr. Frosty. The frozen cells were stored at -196°C in liquid nitrogen tank unless they were used within 1-2 days. When needed, the cryopreserved PBMCs were taken from the liquid nitrogen and quickly thawed in 37°C water bath. Surface of the cryovial containing partially thawed PBMCs were desinfected with 70% ethanol before opening them under safety cabinet. The half thawed PBMCs were transferred into 50 ml conical tube with 15 ml prewarmed media. The PBMCs were then centrifuged at 1500 rpm room temperature for 10 min. The cell pellets were resuspended in 10 ml of media and rested at 37°C 5% CO_2 overnight prior to further usage. Avoiding osmotic cell death, the freezing and thawing procedures were carried out in cold chain condition as quick as possible.

3.2.11. $CD14^+$ cell (monocyte) isolation and DC generation

3.2.11.1. $CD14^+$ cell isolation

$CD14^+$ cells were separated from freshly isolated PBMCs using $CD14^+$ positive selection kit according to the manufacturer's protocol (Miltenyi Biotec). The determined number of PBMCs was resuspended in 20 mL MACS buffer (PBS /2mM EDTA/ 0.5 % FCS) and centrifuged at 1500 rpm, 4°C for 10 min. After the washing, cells were incubated with 80 µL MACS buffer and 20 µL CD14 magnetic microbeads per 10^7 cells at 4°C in the dark

for 15 min to magnetically label $CD14^+$ cells. After washing with 20 mL of MACS buffer as described above, cells were resuspended in 500 µL MACS buffer and loaded onto LS column which was placed in the magnetic field of MACS separator. During gravitational flow of the cell suspension, the $CD14^+$ labeled cells were retained in the column. Then the column was three times washed with 3 ml of MACS buffer and removed from the magnetic field. The positively selected $CD14^+$ cells were eluted in 2 ml MACS buffer and cell number were determined.

3.2.11.2. Generation of monocyte derived DCs (moDCs)

Monocytes ($CD14^+$ cells) were suspended in culture medium (RPMI1640 GlutaMax with 10 % FCS and 50µg/mL gentamycin) supplemented with 100 ng/mL GM-CSF, 20 ng/mL IL-4 and seeded into a 6-well culture plate at $3x10^6$ cells per well. On day two and four, one third of the media was changed with fresh media supplemented with GM-CSF and IL-4. Maturation of monocyte-derived DCs (moDCs) was triggered by adding 10 ng/mL TNF-α on day four and six. On day six, prior to cytokine supplementation, semi-mature moDCs dedicated for loading with the antibody fusion proteins were harvested and seeded into the wells of 96- or 48-well plates. Maturation of moDCs were monitored by flow cytometry using following antibody staining: anti-CD1a-APC, anti-CD86-PE, anti-CD14-APC, anti-CD80-PE, anti-CD40-APC, anti-HLA-ABC-PE, anti-CD83-APC, anti-HLA-DR-PerCP (BD Biosciences), anti-CCR7-APC, and anti-CD205-PE (Myltenyi Biotec).

3.2.12. $CD3^+$ T cell selection

$CD3^+$ T cells were selected by depletion of mononuclear cells including monocytes, B cells, stem cells, dendritic cells, NK cells and residual granulocytes, erythroid cells using flow through fraction of the $CD14^+$ cell selection from PBMCs. First, cell number in the flow through was determined and centrifuged at 1500 rpm for 10 min. After decanting supernatant, cells were mixed well with 40 µL of MACS buffer and 10 µL of Pan T Cell Biotin-Antibody cocktail per 10^7 cells and incubated at 4°C in the dark for 5 min. Then the cells were along incubated with 30 µL of the buffer and 20 µL of Pan T Cell Microbead Cocktail per 10^7 cells for 10 min. MACS separator system was prepared as described in 9.1. Cells were loaded onto LS column and the column were washed once with 3 ml of MACS buffer. Finally, unlabeled $CD3^+$ T cells that passed through the column were

collected and counted. The CD3⁺ T cells were directly cryopreserved after the selection until necessary experiments.

3.2.13. WT1-specific T cell expansion

On day six, $7.5x10^5$ semi-mature moDCs were incubated with 2 µg/mL of the anti-hDEC205-$WT1_{91-138}$ antibody fusion protein at 37°C for 1.5 hours to allow internalization. Then GM-CSF, IL-4 and TNF-α were added and cells were further incubated for 20-22 hours. On day seven, the loaded, fully matured moDCs were washed twice with 2 mL RPMI1640 GlutaMax and resuspended in 1 mL media. Parallel to this, autologous $CD3^+$ T cells were counted and resuspended at a density of $3x10^6$/mL in media. Then moDCs and T cells were mixed in one well of a 24-well culture plate and co-cultured at 37°C. After two days co-cultures were supplemented with 5 ng/mL IL-7 and IL-15 (Peprotech). Cytokines were then added every 2-3 days when half of the media was replenished. On day 12-13, cells were restimulated with 2 µg/mL of the antibody fusion protein for 24 hours, then CD137 frequencies were assessed by flow cytometry.

3.2.14. CD137 selection and expansion of $CD137^+$ WT1-specific T cells

When frequencies of CD137-expressing cells reached more than 3 % as determined by flow cytometry, activated T cells were selected using the CD137 selection kit in accordance with the manufacturer's protocol (Miltenyi Biotec). Parallel to, allogeneic PBMC pool prepared from buffy coats of ten different healthy donors were thawed and cell division was inhibited by irradiation (35Gy) prior to add them as feeder cells to the $CD137^+$ T cells. The $CD137^+$ T cells and allogeneic irradiated feeder cells were seeded into the wells of a 48-well plate at a ratio of 1:10 and supplemented with 1 µg/mL PHA-L and 50 IU/mL IL-2. Specific T cells were expanded further for 14-83 days by supplementation of IL-2, IL-7 and IL-15 every 2-3 days, together with a half media change, and restimulation of autologous moDCs loaded with the antibody fusion protein every 14 days.

3.2.15. Intracellular Cytokine Staining (ICS) and CD107a degranulation assay

ICS assay was used to detect interferon-γ producing T cells induced by moDCs loaded with the anti-hDEC205-WT1 antibody fusion proteins. ICS assay was performed as described previously (Lamoreaux et al. 2006) with modifications. Briefly, $1x10^5$ semi-mature moDCs per well of a 96-well plate were left unstimulated or loaded with 2 µg/mL anti-hDEC205 antibody (negative control), 2 µg/mL anti-hDEC205-$WT1_{small}$ antibody fusion proteins, 5 µg/mL WT1 peptide pool, or 1 µg/mL CMV pp65 recombinant protein (positive control) for 1.5 hours prior to supplementation with GM-CSF, IL-4 and TNF-α. After 20-22 hours of incubation at 37°C, cells were washed twice with 200 µL RPMI1640 and resuspended in 100 µL medium. Then, $4x10^5$ autologous $CD3^+$ T cells were added to the corresponding wells. The mixtures of moDCs and T cells were incubated at 37°C for 1 hour, before addition of 10 µg/mL Brefeldin A (Sigma-Aldrich). At the same time, 0.5 µg/mL PMA and 1 µg/mL Ionomycin were added into a well for the assay control. For CD107a degranulation assay, 1 µL monensin and 2 µL anti-CD107a-APC (both BD Biosciences) were added. After 16 hours of co-incubation, cells were harvested and stained with the following antibodies: anti-CD3-FITC or -PerCP, anti-CD8-FITC or -PerCP or -APC, anti-CD4-APC or -PerCP (BD Biosciences). After surface staining, cells were permeabilized with FACS Permeabilizing Solution 2 (BD Biosciences) and stained with anti-IFN-γ-PE (Beckman Coulter). Cells were analysed with the FACS Calibur and CellQuest software (both from BD Biosciences).

3.2.16. ELISPOT assay

Semi-mature moDCs (5 x 10^4 per well) unloaded or loaded with 2 µg/mL anti-hDEC205-$WT1_{91\text{-}138}$ or control anti-hDEC205 or 5 µg/mL WT1 peptide pool (Miltenyi Biotec) were co-incubated with PBMCs (2 x 10^5 per well) in a 96-well ELISPOT plate (Millipore) precoated with anti-IFN-γ capture antibody (BD Biosciences) for 16 hours. After co-incubation, cells were removed from the wells and the biotinylated anti-IFN-γ detection antibody (BD Biosciences) was added prior to visualization with Streptavidin-AP (Southern BioTech) and NBT/BCIP liquid substrate system (Sigma-Aldrich). Spots were determined on Immunospot S5 ELISPOT reader (C.T.L).

3.2.17. Vital-FR assay

VITAL-FarRed assays were performed as described elsewhere (Stanke et al. 2010) with modifications. Briefly, $5x10^6$ THP-1 cells were labelled with 5 µM CFSE and the same number of DG-75 cells were labelled with 5 µM FarRed (Invitrogen) for 5 min at 37°C. Both cell lines were HLA A02 positive. Specific T cells derived from HLA A02 positive patients were serially diluted and each dilution of T cells was mixed with $5x10^4$ $CFSE^+$ target and $5x10^4$ $FarRed^+$ control cells, resulting in graded effector : target (E:T) ratios. After 22 hours of incubation at 37°C in 5 % CO_2, the cytotoxicity was assessed on the basis of specific lysis of the target cells compared to that of the control cells by flow cytometry using previously described method (Stanke et al. 2010).

3.2.18. Statistics

Data are shown as median with IQR, mean ± SEM, or mean ± SD. Statistical significance was analyzed with GraphPad Prism 5.0 (GraphPad Software Inc.) using unpaired *t* test or one-and two-way analysis of variance (ANOVA) followed by Tukey's and Bonferroni's post hoc test as indicated. $p \leq 0.05$ was regarded to be significant.

Section 4. Results and observations

4.1. Generation of anti-hDEC205-WT1 antibody fusion proteins

The anti-hDEC205-WT1 antibody fusion protein encoding plasmids were cloned in three steps using standard techniques (Figure 11). The final constructs also encoded a Flag-tag to facilitate purification and detection of the various recombinant proteins.

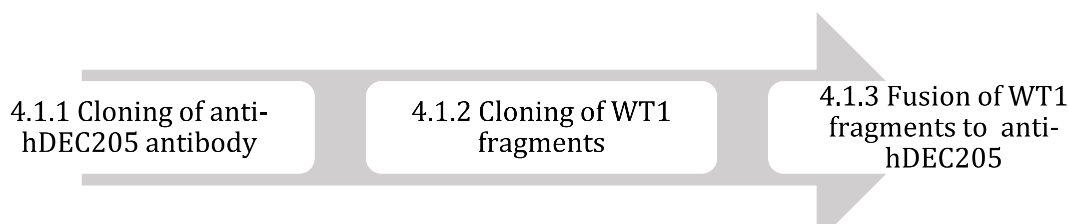

Figure 11. Cloning workflow of anti-hDEC205-WT1 antibody fusion proteins.

4.1.1. Construction of scFv:hDEC205-GpL and anti-hDEC205 antibody

Therapeutic antibodies can be expressed as single chain variable fragments (scFvs) or as whole antibodies. As a basis of the anti-hDEC205-WT1 antibody fusion proteins, scFv:hDEC205 consisting of the variable heavy and variable light chain domains of the anti-hDEC205 clone MG-38.3 (Birkholz et al. 2010) connected by $(GGGGS)_3$-linker were designed. The cDNA encoding scFv:hDEC205 was synthesized by GeneArt AG (Life Tecnologies Co. Ltd.). Then the scFv:hDEC205 DNA fragment was inserted 5' into a pCR3-based (Invitrogen) expression vector encoding *Gaussia princeps* luciferase (GpL), a highly traceable molecule, to obtain a scFv:hDEC205-GpL fusion construct (Figure 12).

Simultaneously, DNA fragments encoding the variable heavy and light chain domains of anti-hDEC205 were separately amplified by PCR using primers with

restriction sites for later cloning. To construct the heavy and light full-length chain encoding expression plasmids, the amplicons were then inserted into another pCR3-derived expression vectors encoding the human IgG1 constant heavy or kappa light chain domains (Figure 12). Concentration and purity of the cloned expression constructs were measured at 260nm by spectrophotometer while sequences of the expression constructs were proved by DNA sequencing (GATC Biotech, a worldwide provider of DNA sequencing). The expression vectors passed all these quality tests were left at -20°C until transient transfection.

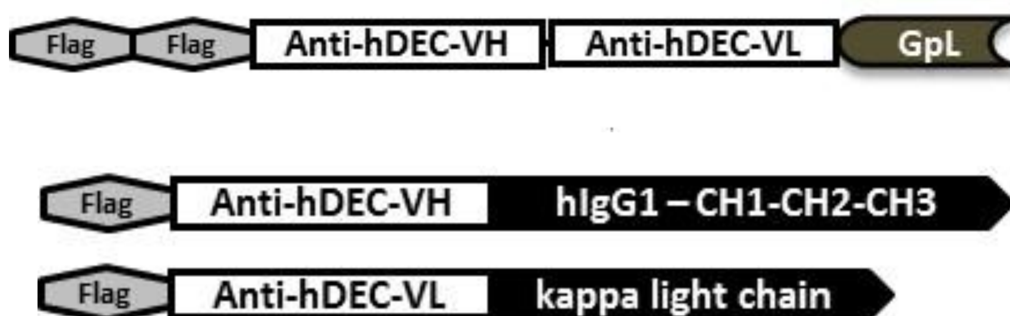

Figure 12. Scheme of anti-hDEC205 antibody constructs.

Top: The scFv:hDEC205-GpL single chain variable fragment of anti-hDEC205 consisting of the variable heavy and light chains (anti-hDEC-VH and anti-hDEC-VL) was C-terminally fused to Gaussia princeps Luciferase and tagged N-terminally by a double Flag epitope. Bottom: Primary structure of anti-hDEC205 antibody. The heavy and light chains of the antibody were constructed by inserting respective variable heavy and light chain domain into the N-terminus of the corresponding constant domains of the human IgG1 isotype. Each chain of the antibody was Flag-tagged N-terminally.

4.1.2. Cloning of WT1 protein

WT1_D was reported as the dominant WT1 isoform in AML cases (Kramarzova et al. 2012). Since it represents the longest isoform, WT1-derived peptide variations to be presented to T cells should be maximized with this isoform. For this reason, we intended to fuse WT1_D to the anti-hDEC205 antibody. Due to the difficulty in amplifying a cDNA for full-length WT1_D from various leukemia cell lines, we generated five different WT1_D amplicons covering the whole protein sequence (Figure 13). Then, with an exclusion of four amino acids, a full-length WT1_D was cloned by combining WT1_D$_{1-227}$ with WT1_D$_{217-522}$ using an internal Xho1 restriction site (Table 19).

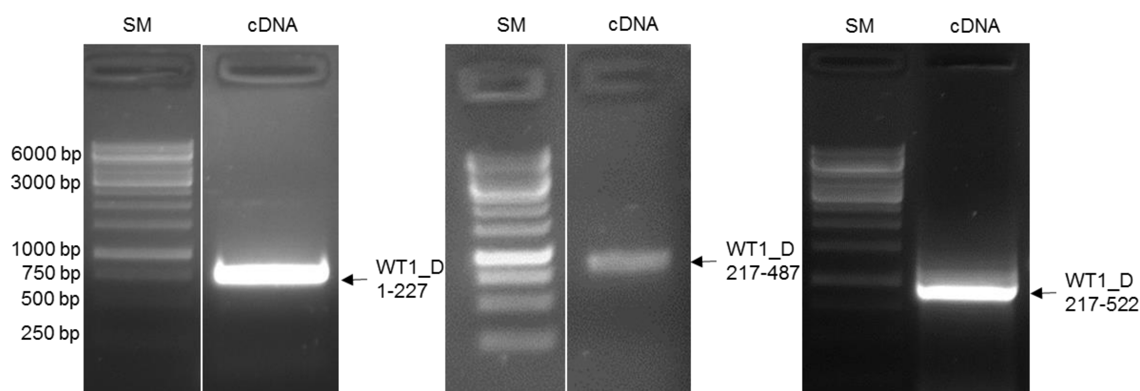

Figure 13. PCR amplification of cDNA encoding three major fragments of WT1_D of the five obtained amplicons.

SM, size marker for DNA; bp, base pairs; cDNA obtained from leukemia cell lines (THP-1, ML-2, NALM-6) by RT-PCR.

4.1.3. Generation of anti-hDEC205-WT1_D$_{major}$ and anti-hDEC205-WT1_D$_{full}$ antibody fusion proteins

Each WT1_D$_{major}$ fragment as well as the full-length WT1_D was genetically fused to the C-terminus of the heavy chain of anti-hDEC205 antibody to obtain various anti-hDEC205-WT1 antibody fusion proteins (Figure 14) and expressed by transient transfection of HEK293 cells.

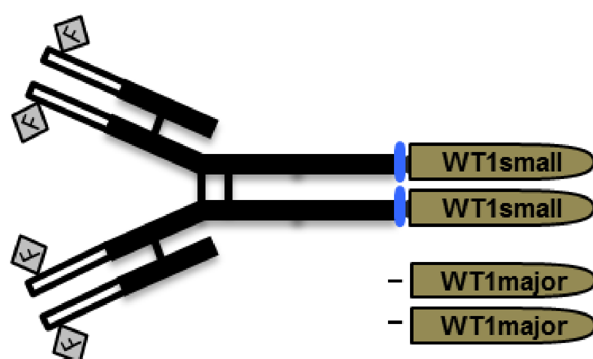

Figure 14. Scheme of anti-hDEC205-WT1 antibody fusion proteins.

The anti-hDEC205-WT1$_{major}$ and -WT1$_{small}$ fusion proteins were constructed by C-terminal linking of the respective WT1 fragments to the anti-hDEC205 heavy chain. Small fragments of WT1 were fused through a flexible spacer of four amino acids (blue).

Western blot analyses of culture supernatants from cells transiently transfected with expression plasmids encoding the parental anti-hDEC205 and the aforementioned

anti-hDEC205-WT1_D fusion variants revealed good production of anti-hDEC205 but no relevant production of the anti-hDEC205-WT1_D variants. To figure out whether the anti-hDEC205-WT1_D fusion proteins were not expressed at all or simply not secreted, transfected cells were pelleted and analyzed in parallel with the supernatants. In lanes loaded with cell lysates there were significant amounts of the WT1 antibody fusion proteins, indicating that the fusion proteins were produced but not secreted (Figure 15).

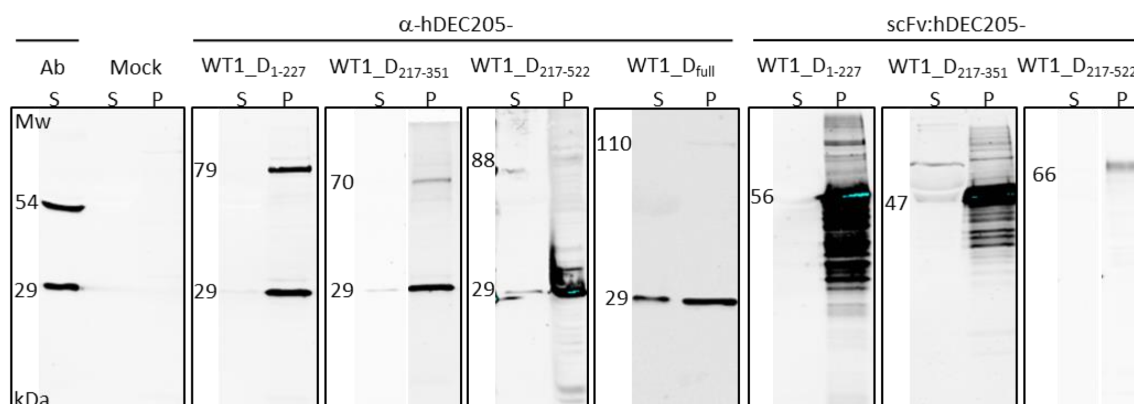

Figure 15. Production and secretion characteristics of anti-hDEC205-WT1_D antibody fusion proteins by SDS-PAGE and Western blot analysis.

Proteins were detected with mouse anti-Flag M2 IgG (primary antibody) and goat anti-mouse IgG-IRDye 800CW (secondary antibody). S culture supernatant collected from transfected HEK293 cells, P lysate of pelleted HEK293 cells mock transfected or transfected with different anti-hDEC205-WT1_D antibody fusion proteins.

Cloning to different sites or proteins was expected to be a possible solution to the poor secretion of the anti-hDEC205-WT1_D fusion constructs. Thus, we cloned N- and C-terminal as well as middle parts of WT1_D to the either C-terminus of the light chain of anti-hDEC205 or scFv:hDEC205 (Table 19) to test our expectation. As shown in *Figure 16*, these fusion proteins were again not secreted. Collectively, these results suggest that large fragments of WT1 interfere with efficient secretion.

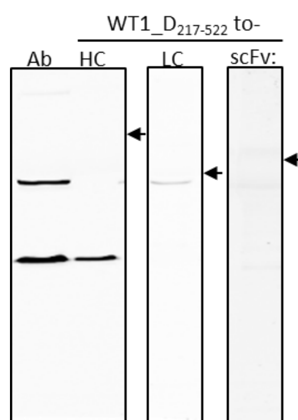

Figure 16. Western blot analysis of three different anti-hDEC205-WT1_$D_{217-522}$ fusion constructs.

The WT1_$D_{217-522}$ fragment was cloned to three different cloning sites as indicated HC heavy chain, LC light chain, and scFv single chain variable fragment of anti-hDEC205 antibody. Ab parental anti-hDEC205 antibody. Black arrow heads indicate expected fusion chains of the anti-hDEC205 antibody.

To overcome the insufficient yields due to the poor secretion, we also evaluated purification of the intracellularly trapped fusion protein anti-hDEC205-WT1_D_{1-227} by triton extraction and affinity chromatography on anti-Flag agarose. In principle, purification of intracellular anti-hDEC205-WT1_D_{1-227} was possible, but the obtained yields were not sufficient for extensive work due to the poor solubility of the antibody fusion protein (*Figure 17*). However, 3 μg of the anti-hDEC205-WT1_D_{1-227} antibody fusion protein (at 1μg/mL of concentration) could be purified from pellets of 20 x 10^7 HEK293 cells.

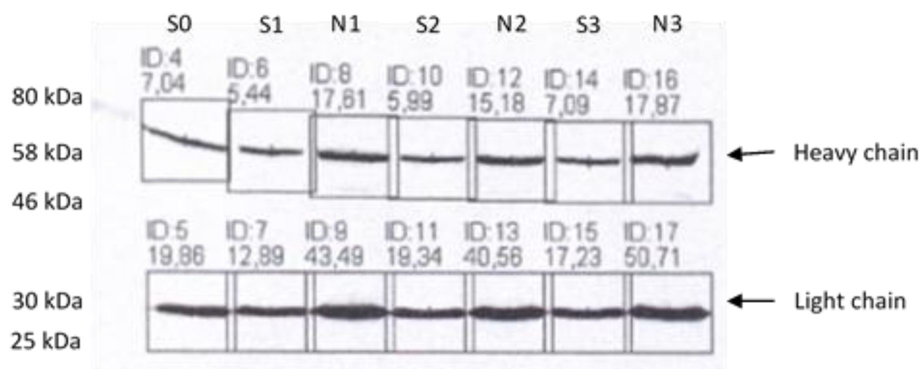

Figure 17. Extraction of the intracellularly retained anti-hDEC205-WT1_D_{1-227} antibody fusion protein by triton-100 lysis followed by sonication.

S0 initial yields of protein extract, S1 S2 S3 protein yields in supernatant after each centrifugation, N1 N2 N3 Non-soluble pelleted proteins. Boxes and numbers were used for calculation of protein concentrations.

Table 19. Summary of all cloned anti-hDEC205-WT1 antibody fusion proteins containing different fragments of WT1.

aa amino acids; VH-CH heavy chain and VL-CL light chain of anti-hDEC205 antibody; scFv:single chain fragment variable of anti-hDEC205; + cloned, - not cloned; ┼ four amino acids (aa213-216:SQPA) were excluded; ***Bold****: WT1 protein sequence with good secretory capacity. Note that there is a difference for numbering of amino acid sequences between isoform D and canonical isoform of WT1.*

No	WT1 isoform D (522 aa)	WT1 canonical (449 aa)	VH-CH	VL-CL	scFv:
1	WT1_D$_{1-227}$	WT1$_{(-73)-154}$	+	+	+
2	WT1_D$_{217-351}$	WT1$_{144-278}$	+	+	+
3	WT1_D$_{217-487}$	WT1$_{144-414}$	+	-	+
4	WT1_D$_{217-522}$	WT1$_{144-449}$	+	+	+
5	WT1_D$_{347-522}$	WT1$_{274-414}$	+	-	-
6	WT1_D$_{1-522}$┼	WT1$_{(-73)-449}$	+	-	+
7	WT1_D$_{1-126}$	WT1$_{(-73)-53}$	+	-	-
8	**WT1_D$_{83-108}$**	**WT1$_{10-35}$**	+	-	-
9	**WT1_D$_{164-211}$**	**WT1$_{91-138}$**	+	-	-
10	**WT1_D$_{296-346}$**	**WT1$_{223-273}$**	+	-	-
11	**WT1_D$_{397-444}$**	**WT1$_{324-371}$**	+	-	-
12	WT1_D$_{83-126}$	WT1$_{10-53}$	+	-	-
13	WT_D$_{217-346}$	WT1$_{144-273}$	+	-	-
14	WT1_D$_{296-444}$	WT1$_{223-371}$	+	-	-

4.1.4. Generation of anti-hDEC205-WT1$_{small}$ antibody fusion proteins

Since the large parts of WT1 protein showed poor secretion, we generated and screened a panel of anti-hDEC205 antibody fusion proteins containing different small fragments (26-51 aa) of WT1 for efficient secretion. First, small fragments of WT1 cDNA were selected on the basis of the encoded immunogenic epitopes that were previously published elsewhere (Rezvani et al. 2008), (Kobayashi et al. 2006), (Doubrovina et al. 2012). Then by primer combinations, more fragments were propagated. Finally, eight different anti-hDEC205-WT1$_{small}$ antibody fusion protein constructs were successfully generated. This way, we identified four different anti-hDEC205-WT1$_{small}$ (Table 20) antibody fusion proteins yielding 2-4,4 μg/mL in supernatants of transiently transfected HEK293 cells. Out of the four, anti-hDEC205-WT1$_{91-138}$, anti-hDEC205-WT1$_{223-273}$, and anti-hDEC205-WT1$_{324-371}$ were purified for further analyses. After purification, the anti-hDEC205-WT1$_{small}$ fusion proteins reached concentrations of 40-400 μg/mL with high purity and good integrity (Figure 18).

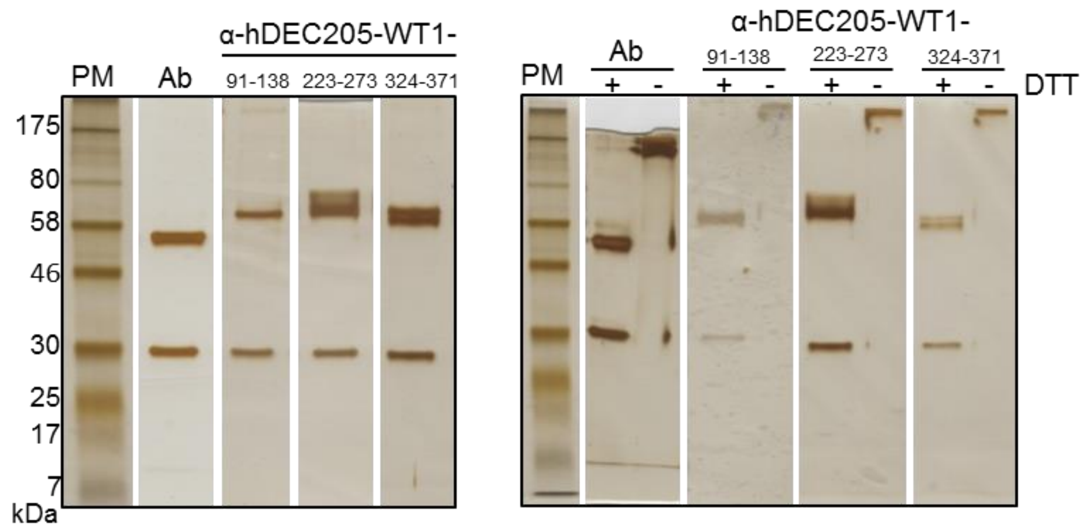

Figure 18. Purity and integrity of anti-hDEC205-WT1$_{small}$ fusion proteins by SDS-PAGE and silver staining.

PM, protein marker; molecular weights in kDa; +, reducing; -, non-reducing condition; DTT, dithiothreitol.

Table 20. Protein sequence of WT1 small fragments for four secreted anti-hDEC205-WT1 antibody fusion proteins. ǂnot published

Antibody fusion protein	Protein sequence of the WT1 cloned to the C-terminus of the a-hDEC205	Sequence of known epitopes
Anti-hDEC205-WT1$_{10-35}$	ALLPAVPSLGGGGGCALPVSGAAQWA	GGCALPVSGA
Anti-hDEC205-WT1$_{91-138}$	AFTVHFSGQFTGTAGACRYGPFGPPPPSQASSGQARMFPNAPYLPSCL	RMFPNAPYL QARMFPNAPYLPSCL
Anti-hDEC205-WT1$_{223-273}$	SDNLYQMTSQLECMTWNQMNLGATLKGVAAGSSSSVKWTEGQSNHSTGYES	WNQMNLGAT CMTWNQMNLGATLKG KGVAAGSSSSVKWTE
Anti-hDEC205-WT1$_{324-371}$	MCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSRSDQLK	DFKDCERRF ǂ KRYFKLSHLQMHSRKH

4.2. Binding capacity of anti-hDEC205-WT1$_{small}$ antibody fusion proteins

4.2.1. Detection of cell-bound DEC205 with anti-hDEC205-WT1 antibody fusion proteins

To confirm the DEC205 receptor-specific binding capacity of the various anti-hDEC205-WT1 antibody fusion proteins, parental and human DEC205-stably expressing CHO cells were incubated with the anti- hDEC205-WT1$_{small}$ proteins.

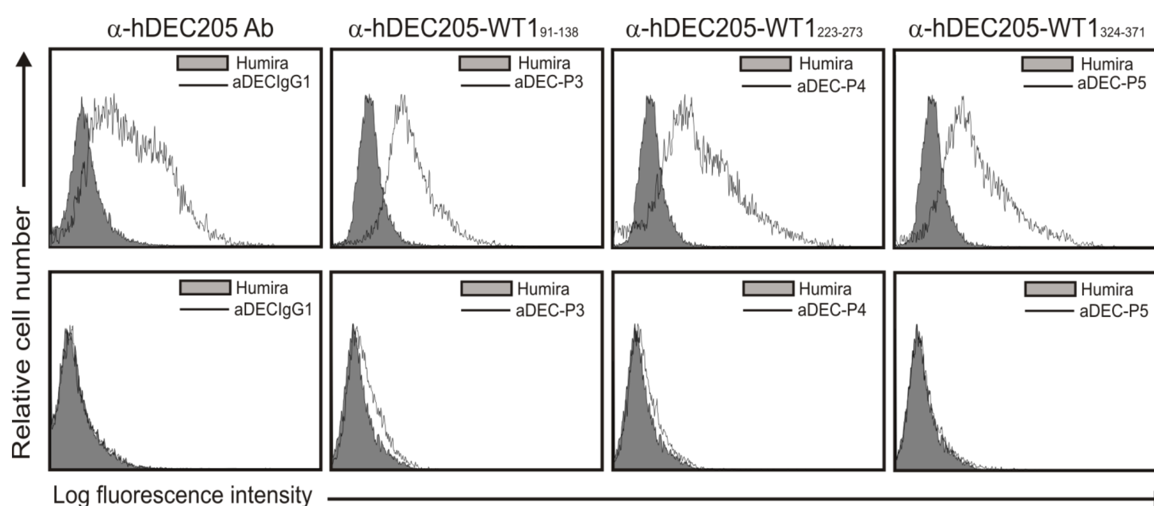

Figure 19. FACS analysis to assess binding capacities of the antibody fusion proteins to human DEC205.

Top: CHO-hDEC205 cells. Bottom: parental CHO cells. Humira, anti-hTNF-α antibody served as a negative control.

Then the antibody fusion proteins bound to DEC205 were detected with anti-Flag M2 and PE-conjugated anti-mouse IgG antibodies by flow cytometry. The parental anti-hDEC205 antibody as well as anti-hDEC205-WT1$_{91-138}$, anti-hDEC205-WT1$_{223-273}$, and anti-hDEC205-WT1$_{324-371}$ bound to human DEC205-expressing CHO cells but not to parental CHO cells (Figure 19).

4.1.2. Binding studies

4.1.2.1. DEC205 binding specificity of scFv:hDEC205-GpL

To quantify binding of anti-hDEC205 to cell surface expressed hDEC205, we performed equilibrium binding studies with scFv:hDEC205-GpL, a highly traceable luciferase fusion protein of the single chain variable fragment variant of the hDEC205-specific antibody clone MG-38.3. CHO-hDEC205 and parental CHO cells were incubated with increasing concentrations of the scFv:hDEC205-GpL protein to allow binding of the antibody variable fragment luciferase fusion protein to cell expressed hDEC205. After 1 hour incubation, non-bound scFv:hDEC205-GpL was removed by 10 times washing with PBS and GpL activity of the remaining cell-bound molecules was measured with a Luminometer. The specific binding of the scFv:hDEC205-GpL was determined by subtracting non-specific binding to parental CHO cells from the total binding to CHO-hDEC205 cells. On the base of the concentration dependent binding data, a non-linear regression curve was fitted and the dissociation constant (K_d) was calculated by help of the GraphPad Prism 5 software. There was nearly no non-specific binding to parental CHO cells (Figure 20). The mean K_d of scFv:hDEC205-GpL was found to be 1445 pM (average of 7 experiments).

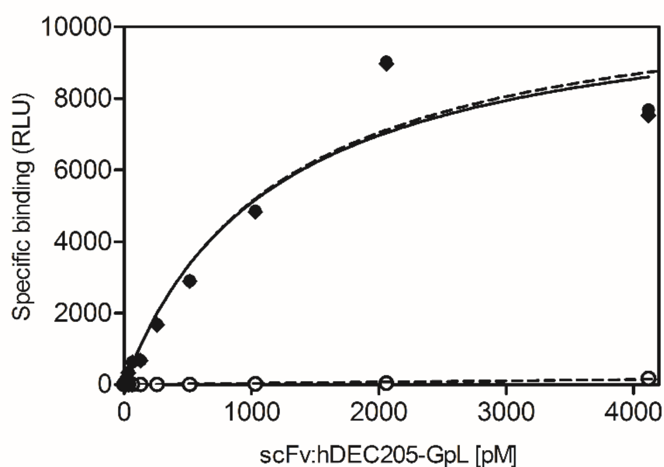

Figure 20. Equilibrium binding study of the scFv:hDEC205-GpL to the cell expressed hDEC205.

Filled circles total binding values, empty circles nonspecific binding values, and filled rombs specific binding values.

4.1.2.2. Binding of anti-hDEC205 antibody

The binding of the scFv:hDEC205-GpL to DEC205 on the cell surface can be blocked by heterologous inhibitor molecules such as the parental antibody or the various anti-hDEC205-WT1 antibody fusion proteins. The rate of the inhibition capacity of inhibitors is concentration dependent. The binding capacity of the inhibitor can be calculated on the basis of the constant concentration and the K_d value of the scFv:hDEC205-GpL molecule and the IC_{50} of the inhibitor using the formula: $K_i = IC_{50} / (1 + C_{GpL} / K_d)$. By such heterologous competition assay analyses with 660 pM scFv:hDEC205-GpL, we obtained an IC_{50}-value of the conventional anti-hDEC205 antibody of 1175 pM on average (Figure 21). This allowed the K_i calculation of the parental anti-hDEC205 antibody which resulted in a value of 810±310 pM. The obtained K_i value of the antibody corresponds to an affinity of 8.1 x 10^{-10} M.

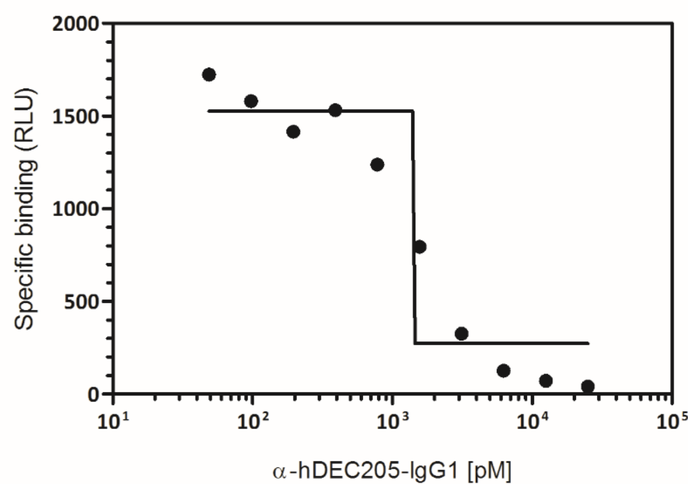

Figure 21. Heterologous competition binding assay.

RLU, relative light unit; pM, picoMolar. The binding value (K_d) of the scFv:hDEC205-GpL interaction with hDEC205 and IC_{50} of the anti-hDEC205 antibody were calculated by non-linear regression using the GraphPad Prism 5.0 software.

4.1.2.3. Binding of the anti-hDEC205-WT1$_{small}$ antibody fusion proteins

In equilibrium binding studies, the specific binding of scFv:hDEC205-GpL was comparably well blocked by each of the anti-hDEC205-WT1$_{small}$ antibody fusion proteins and the parental anti-hDEC205 IgG1 leading to the conclusion that these fusion molecules bind to hDEC205 with a similar efficacy (Figure 22).

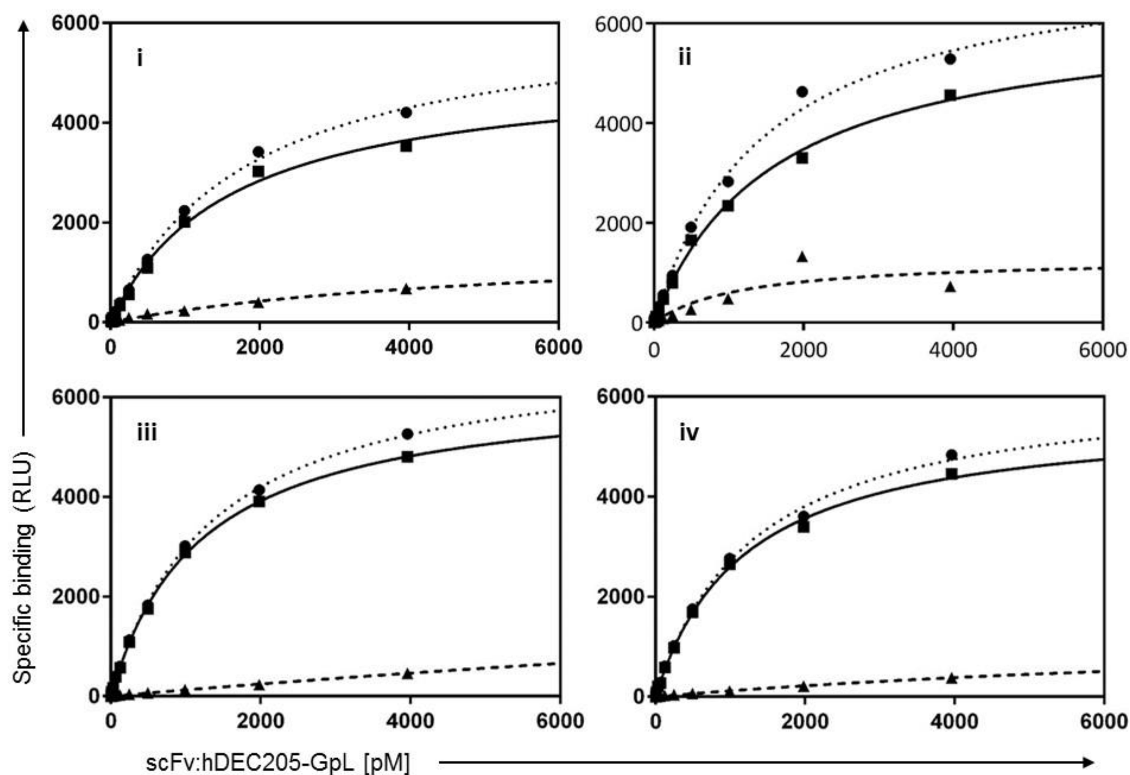

Figure 22. Equilibrium binding study confirming the binding capability of the anti-hDEC205-WT1$_{small}$ antibody fusion proteins to hDEC205.

·●· total binding, ━■━ specific binding, -▲- non-specific binding of the scFv:hDEC205-GpL by blocking with the parental antibody or the respective anti-hDEC205-WT1$_{small}$ fusion proteins: anti-hDEC205 antibody (i), anti-hDEC205-WT1$_{91-138}$ (ii), anti-hDEC205-WT1$_{223-273}$ (iii), anti-hDEC205-WT1$_{324-371}$ (iv).

Since binding of the anti-hDEC205 antibody variants was not significantly altered by C-terminal extension of the heavy chain with the various WT1$_{small}$ fragments, we proved binding to endogenously expressed DEC205 only for the parental antibody using human immature moDCs (Figure 23).

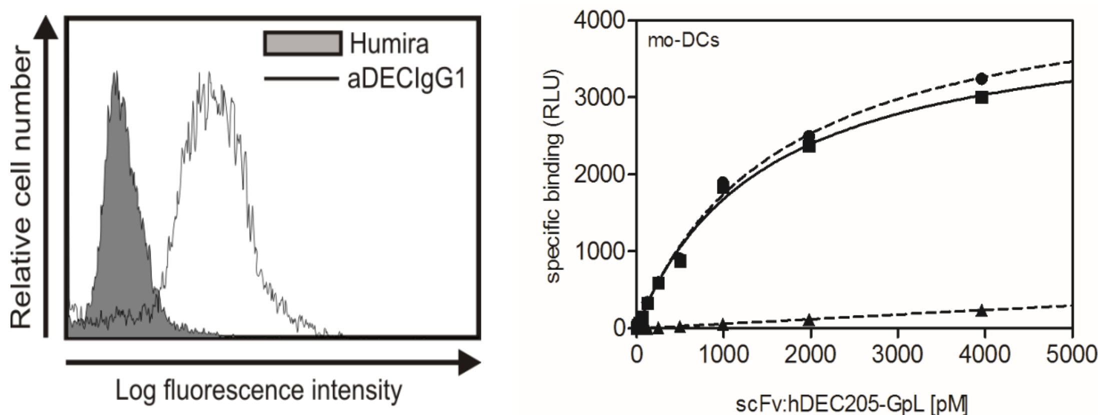

Figure 23. Specific binding of anti-hDEC205 antibody to DEC205 on immature human monocyte-derived DCs.

Left: FACS analysis, Right: equilibrium binding study.

4.3. Screening of endotoxin content in the purified anti-hDEC205-WT1 antibody fusion proteins

Endotoxins are complex lipopolysacharides found in outer cell membrane of gram-negative bacteria and have many biological impacts such as triggering of TLR4 activation (Gorbet and Sefton 2005). Cell growth and function are both affected by endotoxin effects *in vitro* and *in vivo*. Therefore, all purified antibody fusion proteins were tested for their endotoxin level using the Limulus Amebocyte Lysate (LAL) chromogenic assay. This assay is based on the fact that bacterial endotoxin activates a proenzyme in the modified LAL. The activated proenzyme leads to release of *p-nitroaniline* from the colorless substrate, *Ac-Ile-Glu-Ala-Arg-p-nitroaniline*; the activation rate is proportional to the endotoxin concentration of the sample considered. The released *p-nitroaniline* is photometrically measured at 405-410nm. The correlation between photo absorbance and endotoxin concentration is linear in the 0.1-1.0 endotoxin unit (EU) per mL range. The developed color intensity is proportional to the amount of endotoxin present in the sample and can be calculated using a standard curve. As showed in Table 1, besides the parental antibody, levels of endotoxin in all antibody fusion protein samples were below the threshold accepted for preclinical use of recombinant vaccines (Brito and Singh 2011). The estimated endotoxin levels in cell cultures were ranged between 0.004-1.9 EU/mL (Table 21).

Table 21. Endotoxin levels of the purified antibody fusion proteins and cell cultures.

Antibody fusion protein	Concentration of stock solution	Endotoxin concentration in stock solution	Endotoxin concentration in cell culture
a-hDEC205 Ab	130 µg/mL	127,02EU/mL	1,9 EU/mL
a-hDEC205-WT1$_{91-138}$	40 µg/mL	12 EU/mL	0,6 EU/mL
a-hDEC205-WT1$_{223-273}$	400 µg/mL	0,8 EU/mL	0,004 EU/mL
a-hDEC205-WT1$_{324-371}$	200 µg/mL	0,9 EU/mL	0,009 EU/mL

4.4. Frequency of DEC205-expressing DCs in PBMCs was not sufficient to induce DC-dependent T-cell response *ex vivo*

At the outset, we stimulated human PBMCs with the anti-hDEC205-WT1$_{91-138}$, anti-hDEC205-WT1$_{223-273}$, anti-hDEC205-WT1$_{324-371}$ fusion proteins and anti-hDEC205 antibody alone. However, we could not detect any T cell response *ex vivo*, which resulted from the fact that DEC205-expressing DCs were barely detectable in PBMCs (Figure 24). Therefore, priming/activating of T cells *ex vivo* was performed with moDCs in the following experiments. Moreover, the maturation of moDCs was not negatively affected by DEC205-targeting (Figure 27).

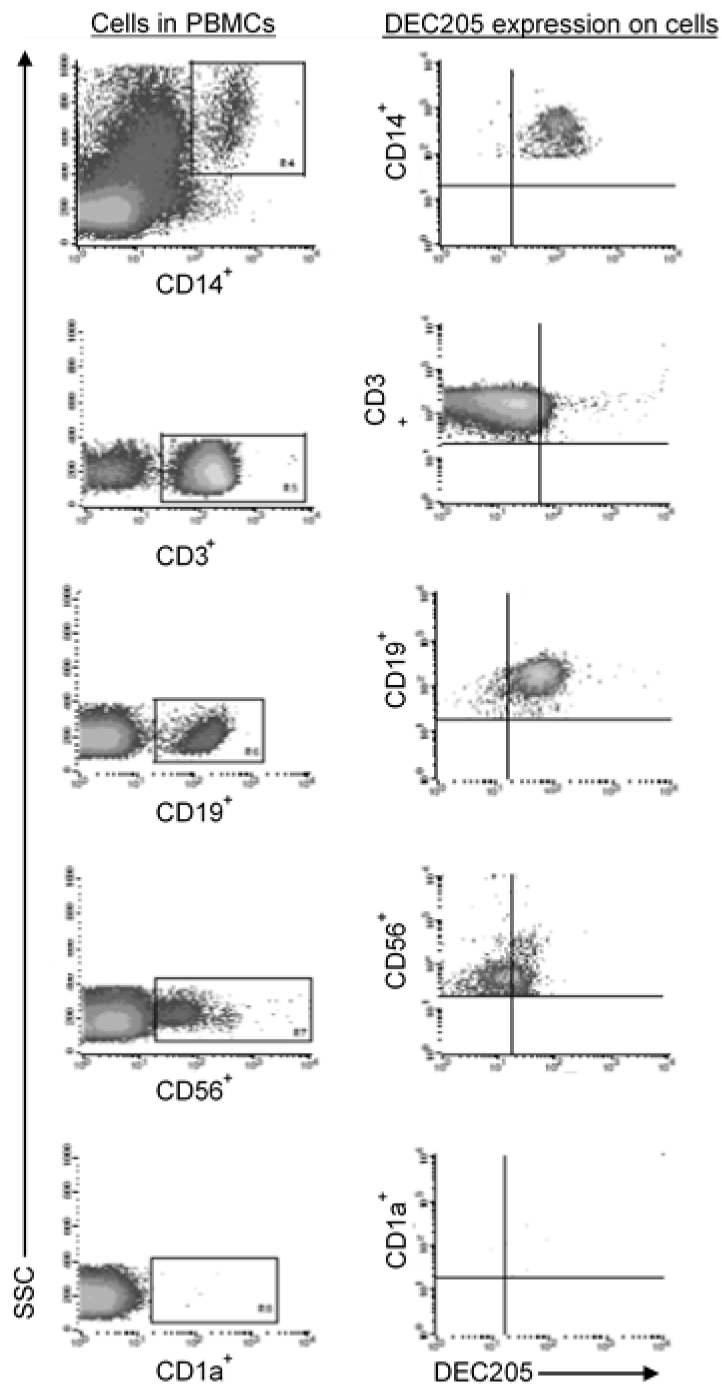

Figure 24. Frequency of DEC205+ cells in human PBMCs.

SSC side scatter; Left: frequency of peripheral blood mononuclear cell subsets: CD14+ monocytes;, CD3+ T cells, CD19+ B lymphocytes, CD56+ NK cells, CD1a+ DCs; Right: DEC205 expression on the surface of the respective cells.

4.5. Evaluation of monocyte derived dendritic cells (moDCs)

On day seven of DC generation from $CD14^{+}$ cells, a maturation state of moDCs were checked morphologically on the phase-contrast inverted microscope and phenotypically by flow cytometry using anti-CD80, anti-CD83, anti-CD86, anti-CD1a, anti-CD14, anti-CD40, anti-CD205, anti-HLA-ABC, anti-HLA-DR, anti-CCR7 antibodies conjugated with APC or PE fluorescence molecules. On the phase-contrast microscope, the protein loaded moDCs showed irregular shapes with cytoplasmic projection (Figure 26).

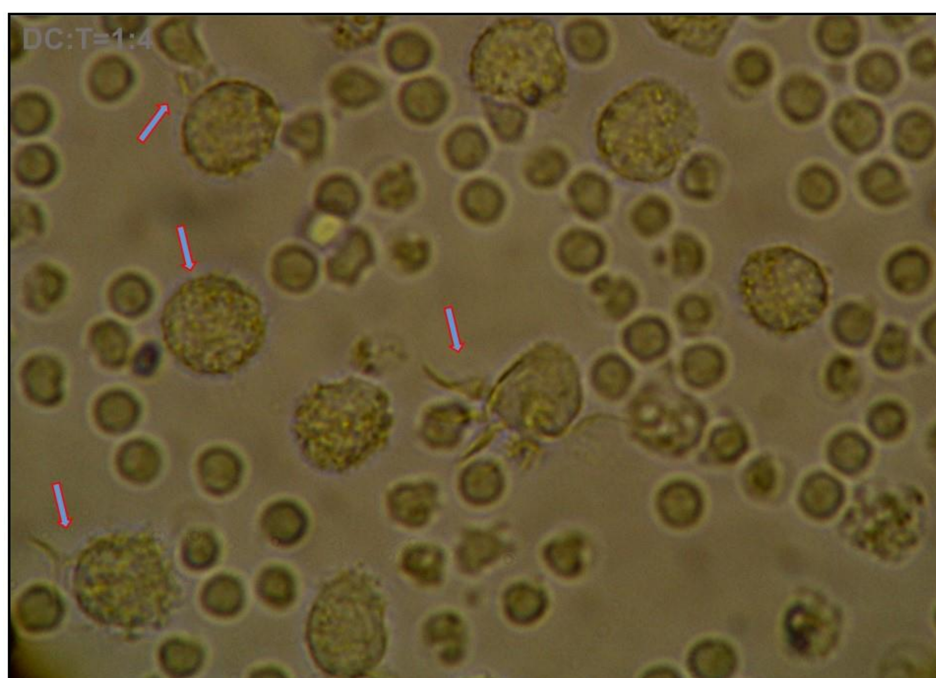

Figure 26. Mature moDCs at the beginning of DC-T cell co-culture.

Cells with large size and more extended dendrites projecting from the cell body are moDCs, round small cells are $CD3^{+}$ T cells.

By immunophenotyping, the strong expression of CD80, CD86, CD83, CD1a, HLA-ABC, HLA-DR molecules were detected on the cell surface. The expression of CD40 and CCR7 was also upregulated (Figure 27).

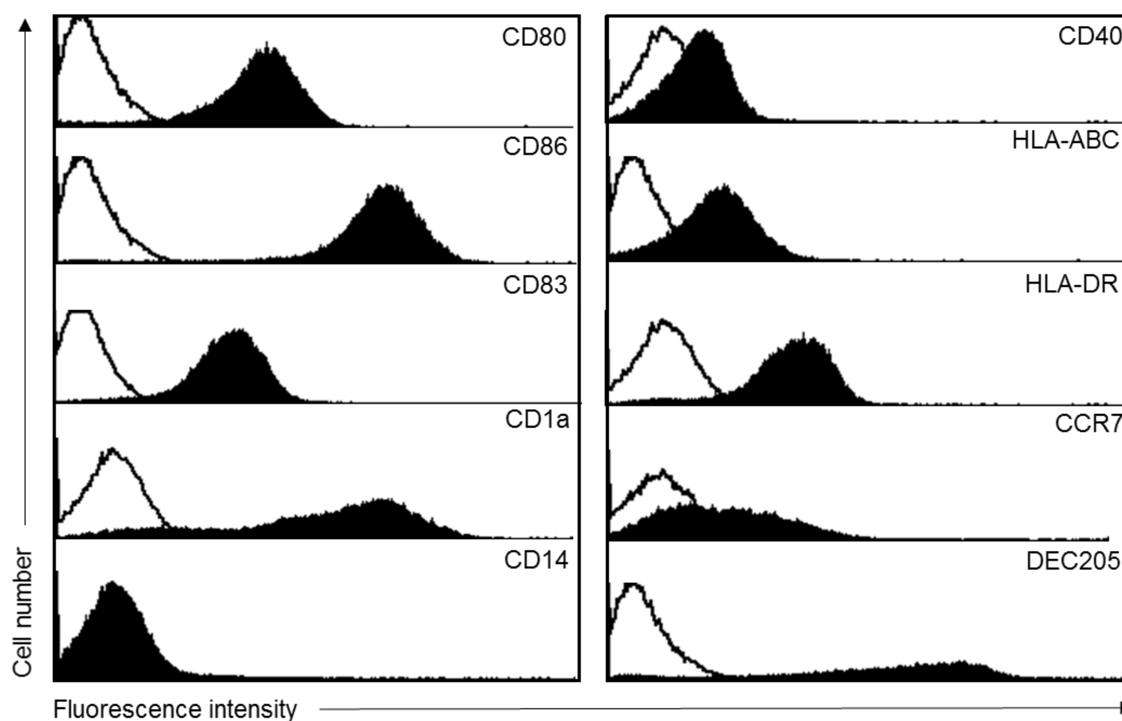

Figure 27. The expression of maturation markers on moDCs after the anti-hDEC205-WT1 antibody fusion protein loading (day seven).

Empty histograms are isotype controls, filled histograms are the expression of the indicated markers on moDCs.

4.6. Evaluation of quality of CD3$^+$ T cells

To accurately check T cell response to the anti-hDEC205-WT1$_{small}$ antibody fusion proteins, it is essential to monitor T cell quality. Therefore, CD3$^+$ T cells were monitored for their purity after the pan-T cell selection procedure (Figure 28). Specifically, T cells were stained with anti-CD3-FITC, anti-CD8-APC, anti-CD4-PerCP, anti-iNKT-PE, anti-CD56-APC, and anti-CD16-FITC and detected by flow cytometry. As shown in Figure 27, the purity of CD3$^+$ T cells in average was 95.8 ± 4.1 %. NK cells were detected at frequencies of 0.8-3.3 % in the CD3$^+$ T cell preparation .

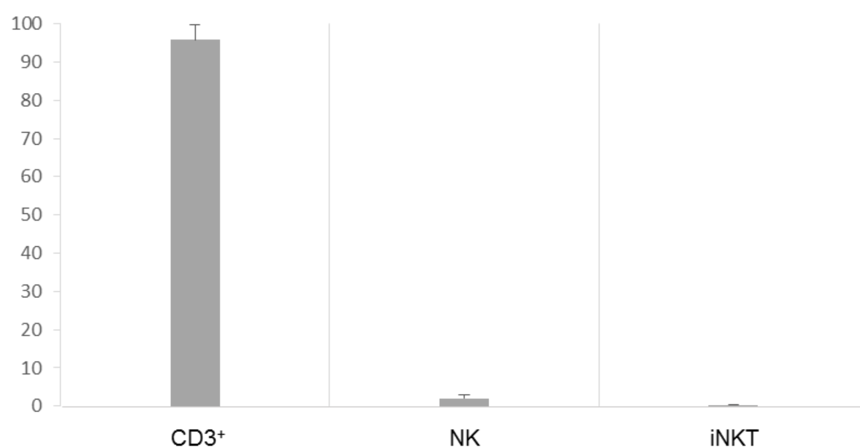

Figure 28. The purity of CD3+ T cells after pan T cell selection.

The columns are the mean ± SD obtained from flow cytometry analyses of six different CD3+ T cell preparations.

4.7. Anti-hDEC205-WT1_D1-227 is able to activate T cells

To test the immunogenic capacity of anti-hDEC205-WT1_D1-227, purified from HEK293 cell lysates, we performed interferon-γ ELISPOT assay. The anti-hDEC205-WT1_D1-227 antibody fusion protein was used at a concentration of 0.5 μg/mL to load

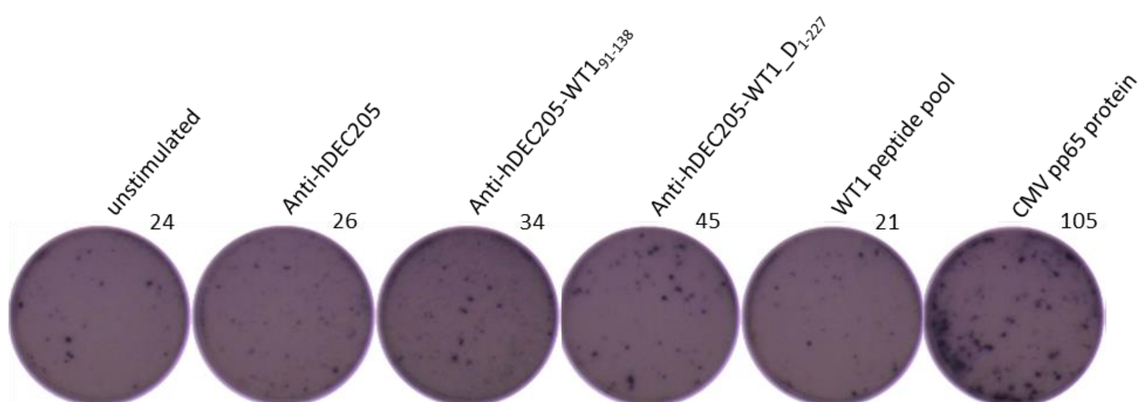

Figure 29. IFN-γ response of DC/T cell co-incubation from one patient assessed by IFN-γ ELISPOT assay.

Actual numbers of IFN-γ spots are shown at the top right.

mature moDCs, however, the greatest number of IFN-γ spots were counted (Figure 29). This result indicated that the anti-hDEC205-WT1_D1-227 protein might have stronger T

cell stimulatory capacity compared to the smaller fragments contained in anti-hDEC205-WT1$_{small}$ antibody fusion proteins. Unfortunately, the major fragment bearing antibody fusion protein could not be further evaluated due to the insufficient yields after inadequate secretion and unsatisfactory purification from the whole cell compartment (see 4.1.3.).

4.8. WT1-specific T-cells are better detected via anti-hDEC205-targeted delivery of WT1$_{91-138}$ than non-targeted WT1 peptide pool

To investigate T cell responses triggered by anti-hDEC205-WT1 protein loaded mature moDCs, we measured the frequency of IFN-γ^+ T cells after stimulation by flow cytometry. Unloaded or anti-hDEC205 parental antibody loaded DCs were used as negative controls, while 15-mer WT1 peptide pool loaded DCs were used as positive and comparison controls. We also considered CMV status of the study subjects, because CMV pp65 recombinant protein was used to determine general kinetics of protein uptake and

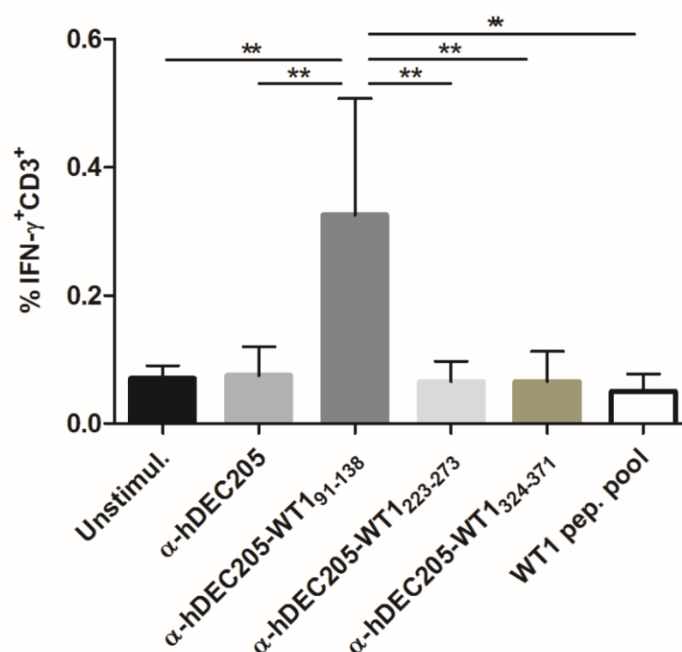

Figure 30. DC-mediated T cell response to anti-hDEC205-WT1$_{small}$ antibody fusion proteins.

*Compiled data from all individuals (n = 16) analyzed for IFN-γ^+ T cell responses by ICS. Shown are median frequencies with interquartile range (IQR), one-way ANOVA, Tukey's test **$p < 0.01$.*

processing by moDCs. As well as the CMV status was helpful to control the quality of the performed assays. Of the three antibody fusion proteins tested, only the anti-hDEC205-$WT1_{91\text{-}138}$ showed a significant T cell stimulatory effect *ex vivo* (Figure 30).

The anti-hDEC205-$WT1_{91\text{-}138}$ loaded mature moDCs were able to activate freshly isolated T cells while treatment of mature moDCs with the parental a-hDEC205 antibody or with a WT1 15-mer peptide pool showed no effect (Figure 31). Of note the IFN-γ producing cells induced by anti-hDEC205-$WT1_{91\text{-}138}$ loaded mature moDCs were mostly $CD8^+$ T cells. It was interesting that the frequency of T cells activated by the targeted self-antigen $WT1_{91\text{-}138}$ stimulation was higher than that of T cells activated by the non-targeted viral protein CMVpp65 in the shown case. However, the mean frequency of the WT1-specific T cells was 2.3-fold lower than the CMVpp65-specific counterparts in total number of patients.

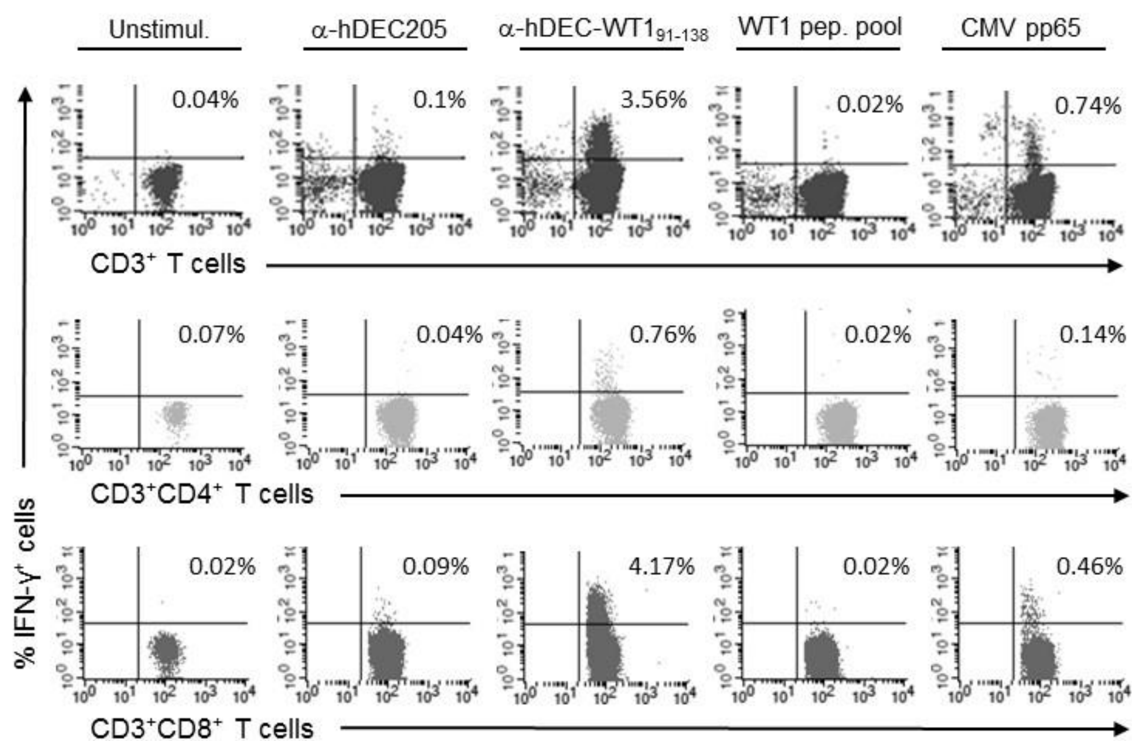

Figure 31. IFN-γ ICS and FACS analysis of cells from one of the patients (PN2).

Top: $CD3^+IFN\text{-}\gamma^+$ T cell frequencies gated on lymphocytes, Middle and Bottom: frequencies of $CD3^+$-gated $CD3^+CD4^+IFN\text{-}\gamma^+$ (middle) and $CD3^+CD8^+IFN\text{-}\gamma^+$ T cells (bottom). T cells were co-incubated with unloaded moDCs (Unstimul.), with moDCs loaded with anti-hDEC205-antibody, anti-hDEC205-$WT1_{91\text{-}138}$ antibody fusion protein, WT1 peptide pool, or CMV pp65 protein.

Table 22. Patient details.

R recipient, D donor, AML acute myeloid leukemia, CMML chronic myelomonocytic leukemia, MDS myelodysplastic syndrome, MRD minimal residual disease, neg negative, pos positive.

PN	Age/sex	Diagnosis, disease status	HLA type	Post Transplant time (Months)	CMV status R/D
1	73/F	Secondary AML	A*0201,3201, B*0801,2705 DRB1*0401,1101 DQB1*0301	74	neg/pos
2	58/F	CMML, AML	A*0201, B*4001,5201 DRB1*1301,1502 DQB1*0601,0603	54	pos/pos
3	57/F	AML M5	A*0201,24 B*1801,3501 DRB1*0101,0301 DQB1*0201,0501	86	pos/pos
4	43/F	AML M4	A*0201, B*2702,4901 DRB*1101,1201 DQB*0301	25	neg/neg
5	62/F	MDS	A*0101,0201, B*1517,3701 DRB1*0701,1302 DQB1*0202,0604	83	pos/pos
6	64/M	AML M1/M2	A*0201,0301 B*0702,5701 DRB1*0407,0701 DQB1*0301,0303	10	pos/pos
7	56/F	AML MRD+	A*0101,2601 B*2705,3801 DRB1*0101,1301 DQB1*0501,0603	13	pos/pos
8	62/F	AML	A*0301, 3101 B*0801,5001 DRB1*0701,1501 DQB1*0202,0602	15	neg/neg
9	56/M	AML	A*0201, 2901 B*1501,4403 DRB1*0401,0701 DQB1*0202,0301	6	neg/neg
10	68/M	CMML	A*0201,0301 B*0701 DRB1*0701 DQB1*0202	24	pos/neg

Immunogenicity of the anti-hDEC205-WT1$_{91-138}$ was tested with $CD3^+$ T cells derived from 10 patients (Table 22). In eight cases, we detected T cell responses against the fusion protein mainly in the $CD8^+$ subset. The median frequencies of $CD3^+$IFN-γ^+, $CD8^+$IFN-γ^+ and $CD4^+$IFN-γ^+ cells upon overnight stimulation with anti-hDEC205-WT1$_{91-138}$ loaded moDCs were 0.44%, 0.45% and 0.05% (Figure 32).

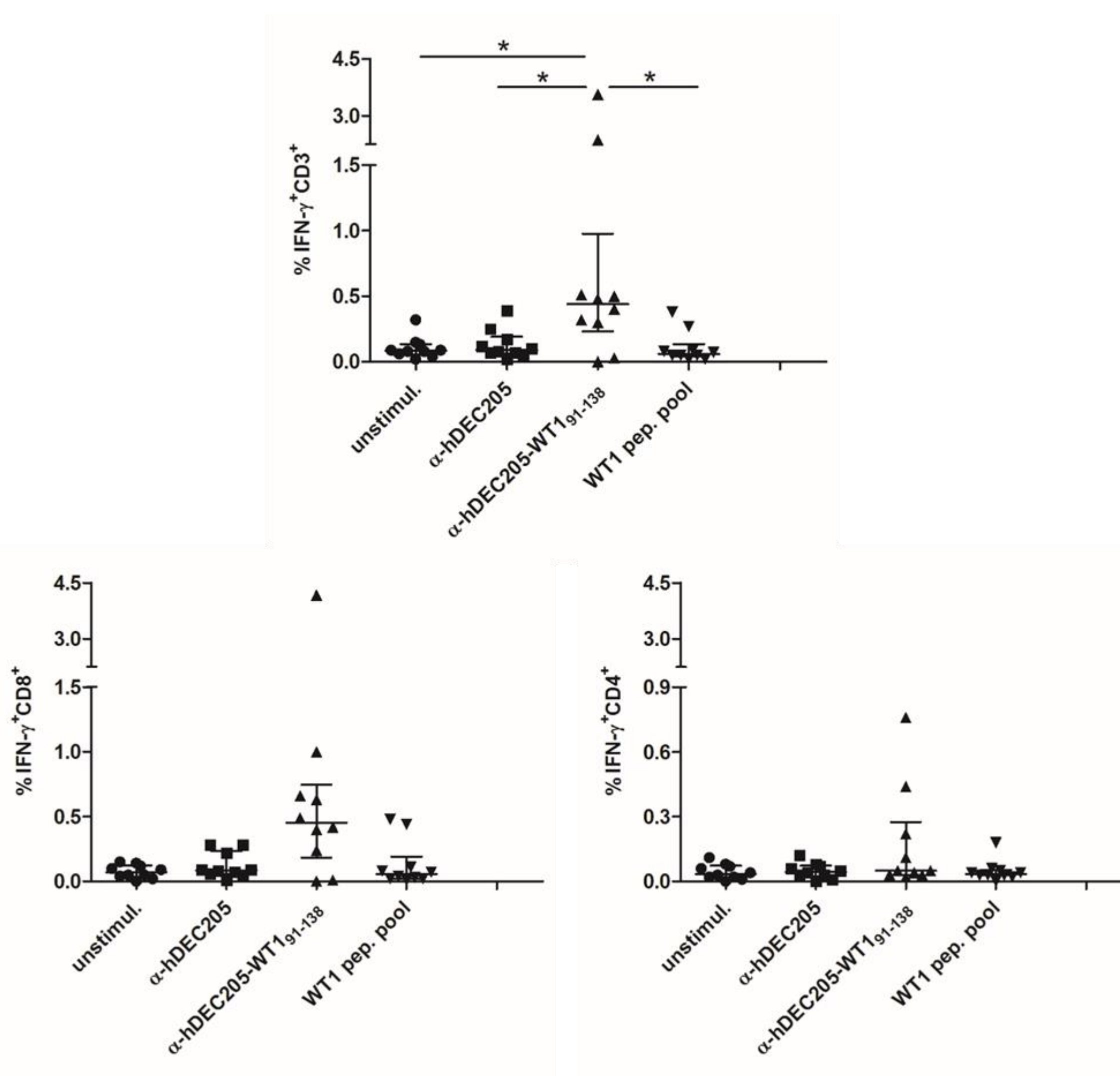

Figure 32. Summary of IFN-γ ICS results from all patients (n = 10).

*Plots show frequencies of IFN-$\gamma^+$$CD3^+$ (top), IFN-$\gamma^+$$CD8^+$ (left bottom) and IFN-$\gamma^+$$CD4^+$ (right botom) T cells. Each data point represents one individual, bars indicate median frequencies with IQR, Tukey's test *$p < 0.05$.*

The majority of the responders was determined to be HLA A02. However, one patient (PN8) was HLA A03 positive but HLA A02 negative, suggesting that WT1$_{91-138}$-derived peptides can be presented by several HLA alleles. In contrast, only one patient showed an IFN-γ^+ T cell response to mature moDCs loaded with a WT1 peptide pool.

It is broadly accepted that GvL effects after allo-HSCT are mediated by donor derived T cells. Therefore, we tested blood samples of healthy individuals as potential haematopoietic stem cell donors to evaluate whether DEC205 targeted $WT1_{91\text{-}138}$ is able to stimulate WT1-specific T cells in healthy donors (Figure 33). A T cell response was observed in five of six healthy donors with lower intensities than that of the patient derived cells.

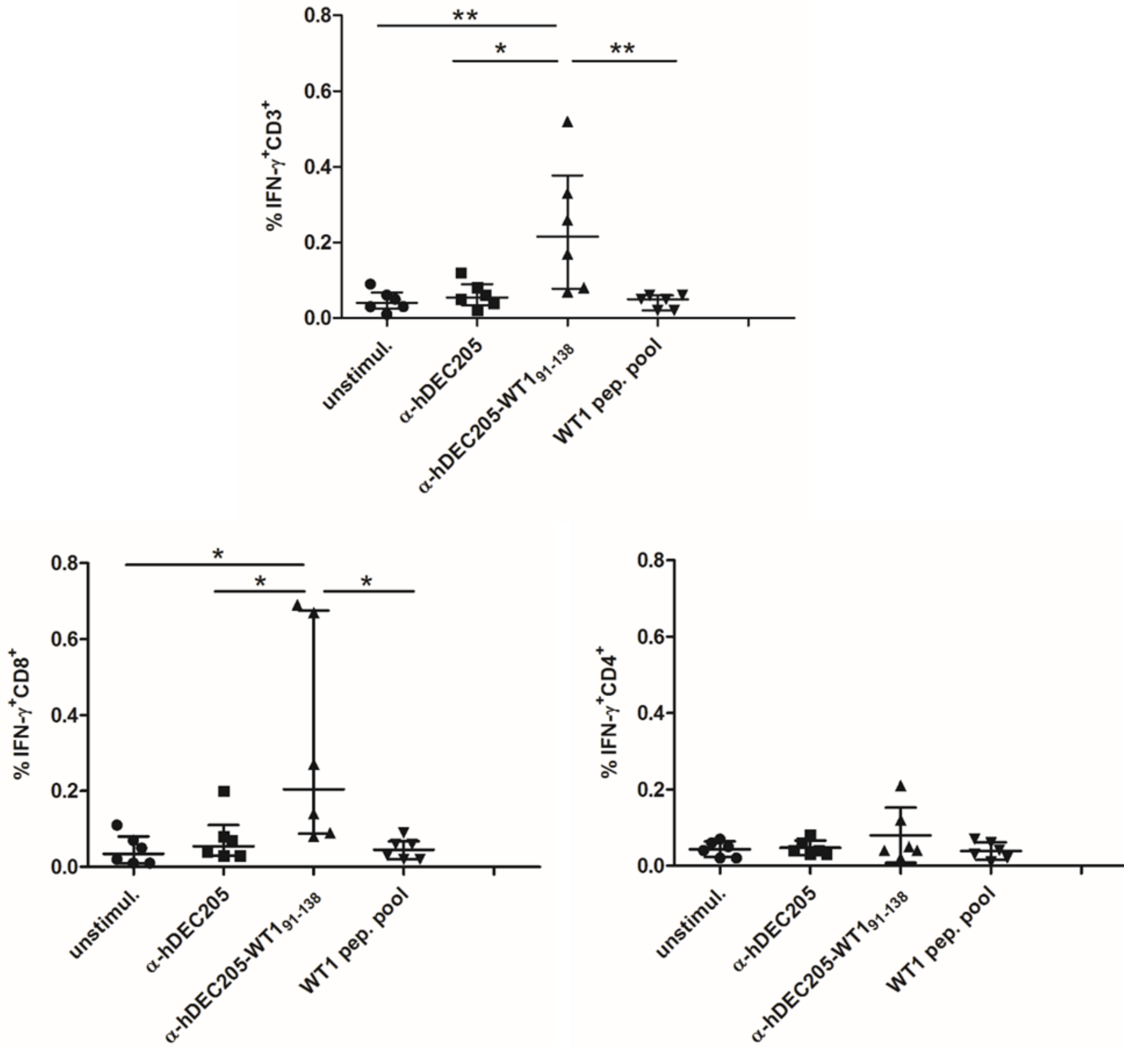

Figure 33. Summary of IFN-γ ICS results from all healthy individuals (n = 6).

*Plots show frequencies of IFN-γ⁺CD3⁺ (top), IFN-γ⁺CD8⁺ (left bottom) and IFN-γ⁺CD4⁺ (right bottom) T cells. Each data point represents one individual, bars indicate median frequencies with IQR, Tukey's test *$p < 0.05$; **$p < 0.01$.*

intensities than that of the patient derived cells. Three of the six healthy donors were HLA A02 negative. This suggested again that processed peptides from the $WT1_{91\text{-}138}$ fragment

are presented on different HLA alleles. Nevertheless, without prior expansion or enrichment, the median frequency of $CD3^{+}IFN\text{-}\gamma^{+}$ and $CD8^{+}IFN\text{-}\gamma^{+}$ cells obtained after co-incubation with anti-hDEC205-$WT1_{91\text{-}138}$ loaded moDCs was significantly higher than after co-incubation with moDCs treated with the parental antibody or a non-targeted WT1 peptide pool in both patients and donors. With regard to $CD4^{+}IFN\text{-}\gamma^{+}$ cells, there seemed to be a minor but not significant shift. Notably, we did not use co-stimulating agents such as anti-CD40 for $CD4^{+}$ T cell activation.

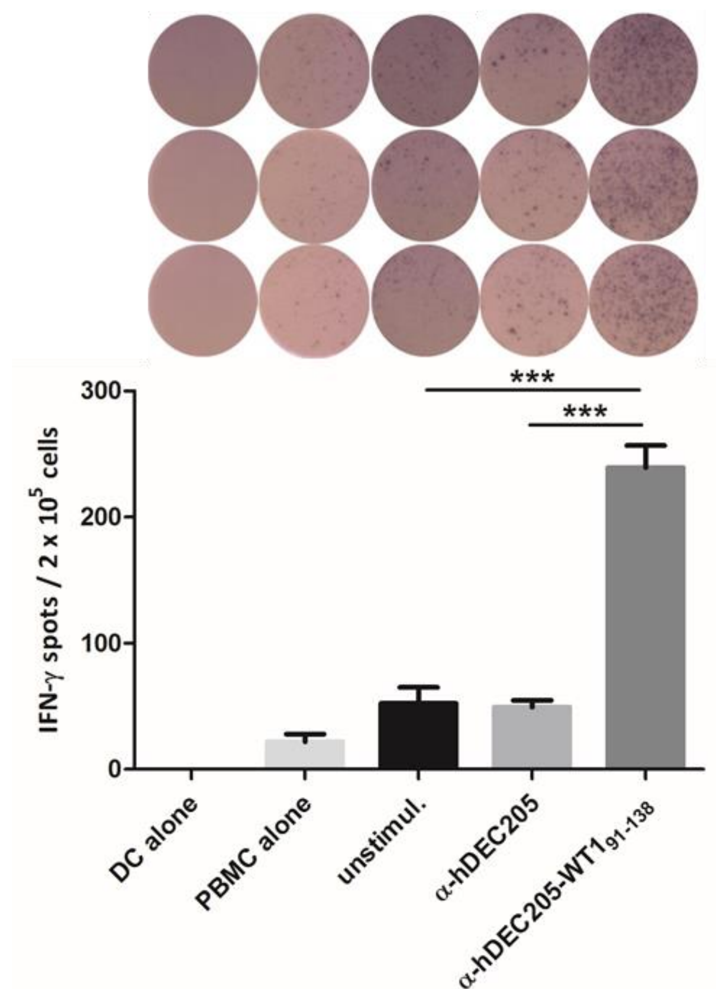

Figure 34. IFN-γ response of PBMCs from one patient assessed by IFN-γ ELISPOT assay.

*Top: triplicate of one representative IFN-γ ELISPOT assay. Bottom: graphical analysis of the IFN-γ ELISPOT assay shown above. Shown are means with SEM, unpaired Student T test ***p < 0.001.*

4.9. Activation of T cells in PBMCs by anti-hDEC205-WT1$_{91-138}$-loaded moDCs

Since the use of PBMCs is less laborious and more time-saving, compared to the use of purified $CD3^+$ T cells, we tested if T cells could be activated without selection from the whole PBMCs by this approach. For this, anti-hDEC205-WT1$_{91-138}$ loaded autologous mature moDCs and PBMCs from two patients were co-incubated to measure IFN-γ by ELISPOT assay (Figure 34). The average number of IFN-γ spots induced by anti-hDEC205-WT1$_{91-138}$, was more than 4-fold higher (239.3) than that of the negative controls (unstimulated 52.3; parental antibody 49.3). Thus, both purified T cells and unselected T cells in PBMCs can be activated by mature moDCs loaded with anti-hDEC205-WT1$_{91-138}$.

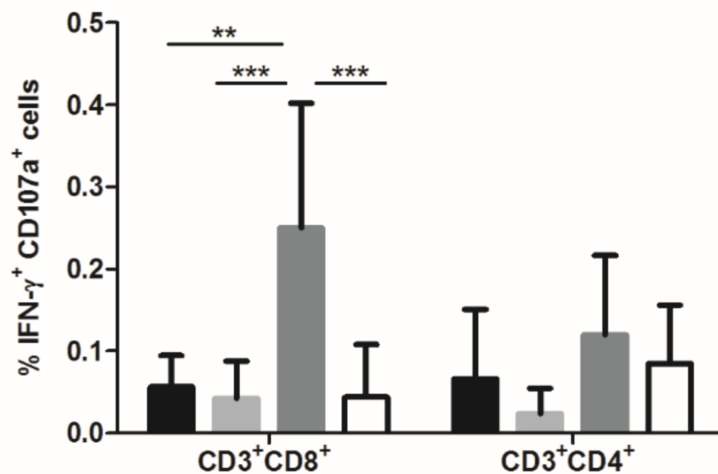

Figure 35. Cytotoxicity of T cells ex vivo by IFN-γ ICS combined with CD107a degranulation assay.

unstimulated, anti-hDEC205 Ab, anti-hDEC205-WT1$_{91-138}$, WT1 peptide pool stimulation. Shown are means ± SD from five patients including PN 2, 3, 4.

4.10. Cytotoxic activity of T cells activated by moDC-targeted WT1$_{91-138}$ protein fragment

4.10.1. Evaluation of T cell cytotoxicity by directly *ex vivo* stimulation with anti-hDEC205-WT1$_{91-138}$ loaded moDCs

The main function of CD8$^+$ CTLs is direct cell-mediated killing of target cells presenting cognate antigens which are recognized specifically by the CTL T cell receptors. Two distinct mechanisms of killing by effector T cells are known. The first mechanism is production of TNF-α, Fas ligand (FasL) or TRAIL. These ligands induce multimerization of their corresponding receptors on the surface of target cells what leads to apoptotic death of the target cells (Peter and Krammer 2003). The second mechanism is the activation of diverse lytic pathways in target cells resulting from the release of cytotoxic granules comprising a pore-forming toxin, perforin, and granzymes, pro-apoptotic serine proteases from cytotoxic effector cells into the immunological synapse assembled between effector and target cells (Lieberman 2003). The lipid bilayer surrounding cytotoxic granules contains exclusively lysosomal-associated membrane glycoproteins

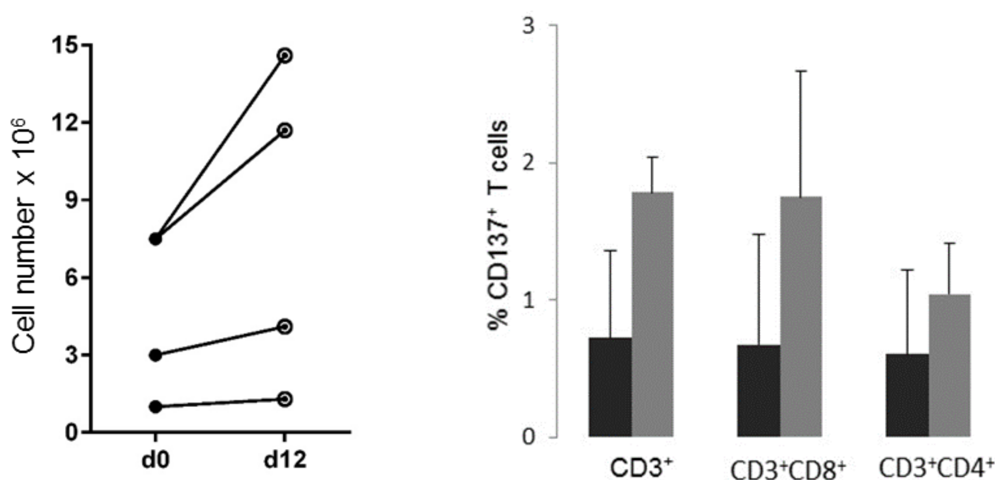

Figure 36. In vitro T cell expansion by co-culturing with mature moDCs loaded with anti-hDEC205-WT1$_{91-138}$ antibody fusion protein.

Left: Numbers of T cells obtained from four patients after 12 days of DC/T cell co-culture. Each line represents one patient. Right: Summarized data of CD137 expression in co-cultured T cells. Shown are means ± SD of data from all four patients. Black, unstimulated; gray, restimulated cells.

(LAMPs) including CD107a (LAMP-1), CD107b (LAMP-2), and CD63 (LAMP-3). During the degranulation process, lysosomal and cellular membranes are fused, as a consequence, LAMPs are exposed to the cell surface. This process is assessed by flow cytometry-based CD107a staining (Zaritskaya et al. 2010). Therefore, we used IFN-γ ICS combined with a CD107a degranulation assay for the ex vivo assessment of the cytotoxic potential of T cells activated by anti-hDEC205-$WT1_{91-138}$ loaded moDCs. The results of five independent assays are summarized in Figure 34. While $CD4^{+}$ T cells treated with anti-hDEC205-$WT1_{91-138}$ showed a slight CD107a degranulation parallel to IFN-γ production, $CD8^{+}$ T cells displayed a significant cytotoxic profiles directly *ex vivo* in comparison to the included controls (Figure 35).

4.10.2. Expansion of WT1-specific T cells

Since CD107a degranulation in conjunction with IFN-γ production was superior to $CD8^{+}$ T cells treated with the anti-hDEC205-$WT1_{91-138}$, it was assumed that they would have a potential to lyse WT1 endogenously expressing leukemic cells. To validate this assumption T cells of four AML patients that received allo-HSCT were *in vitro* expanded. Autologous $CD3^{+}$ T cells and the fusion protein loaded moDCs were co-cultured in presence of IL-7 and IL-15. In order to avoid an early T cell terminal differentiation, the co-cultures were not supplemented by IL-2 until CD137 selection. T cells dominated by $CD8^{+}$ subsets were expanded 1.3 to 2 fold in numbers within 10-14 days (Figure 36). Frequencies of $CD137^{+}$ T cells of the patients on this time point were ranged between 1.6-10 % (Figure 37). In addition to the $CD3^{+}CD8^{+}$, $CD3^{+}CD4^{+}$ subset was also able to express CD137 in the cases that they were expanded. Then WT1-specific T cells selected based on the CD137 expression were further expanded until number of the specific T cells was sufficient to perform cytotoxicity assays. Thus, it seemed to be that the expanded WT1-specific T cells were composed of the both subsets of T cells. However, unfortunately, the expanded T cells were not identified in terms of their subset and phenotype in these experiments.

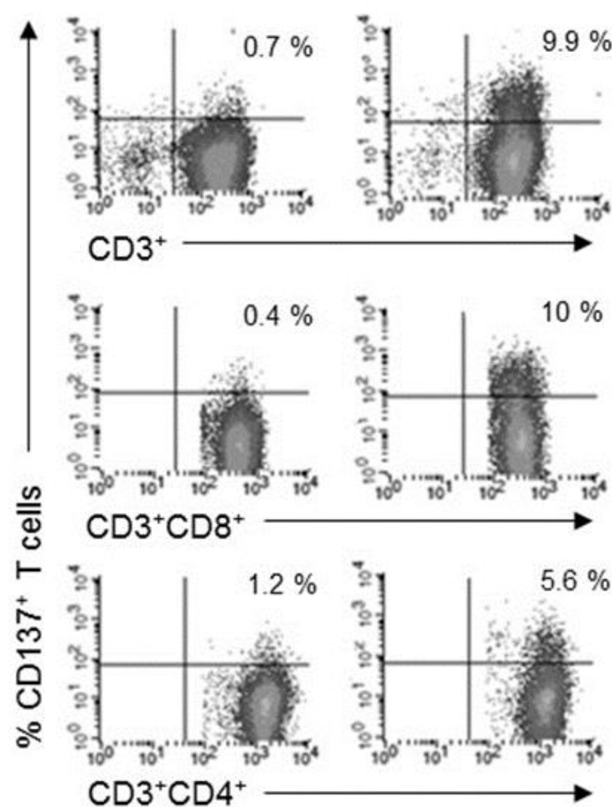

Figure 37. Representative FACS analysis of CD137 expression on T cells.

After 12 days of co-culture, T cells were left unstimulated (left) or restimulated (right) with anti-hDEC205-WT1$_{91-138}$ antibody fusion protein.

4.10.3. T cells activated by DC-targeted WT1$_{91-138}$ lyse WT1-overexpressing leukemia cell line

The VITAL-FR, a classical cytotoxicity assay, is ideally suited for monitoring T cell-mediated cytotoxicity for vaccination studies in scientific and diagnostic applications. Thus, to test specific lysis of WT1 positive leukemia cells via T cells stimulated with our antibody fusion protein, the VITAL-FR assay was performed using expanded CD137^{+} T cells derived from three HLA A02 positive patients. There was HLA A02-restricted lysis of THP-1 cells at relatively low effector to target (E : T) ratios indicating that the WT1$_{91-138}$ specific T-cells possess a strong cytotoxic activity (Figure 38). It is worth mentioning that one of three T cell donors in this kind of experiment was patient 4 who showed no T-cell response directly *ex vivo* (Figure 39). Interestingly, expanded T cells from patient 2 showed an inferior cytotoxicity, although their IFN-γ^{+} T cell response was the strongest in the *ex vivo* experiments.

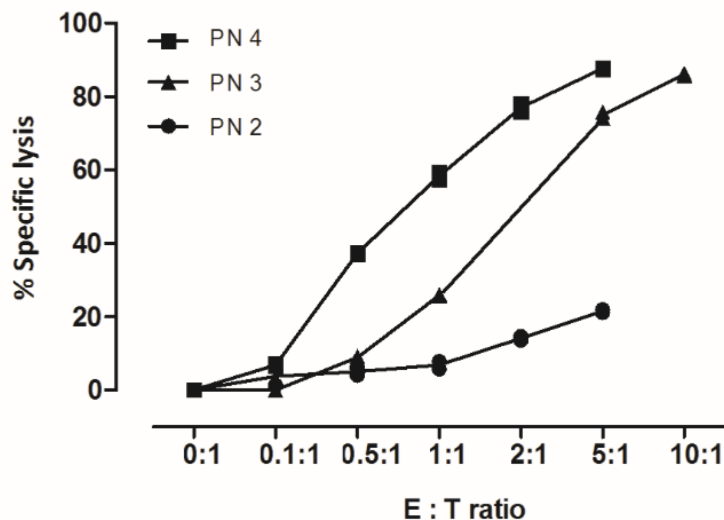

Figure 38. Cytotoxic effector function of in vitro expanded WT1-specific T cells induced by anti-hDEC205-WT1$_{91-138}$ loaded mature moDCs.

VITAL-FarRed cytotoxicity assay. Each experiment was duplicated and each replicate was shown from three different patients (PN 2, 3, 4).

Taken together, the *ex vivo* detected cytotoxic capacity of T cells by IFN-γ ICS combined with CD107a degranulation assay (Figure 35) could be confirmed by the VITAL-FR cytotoxicity test. Specifically, when targeted to DEC205 on mature moDCs, the WT1$_{91-138}$ fragment is able to activate cytotoxic T cells to lyse WT1-overexpressing THP-1 leukemia cells.

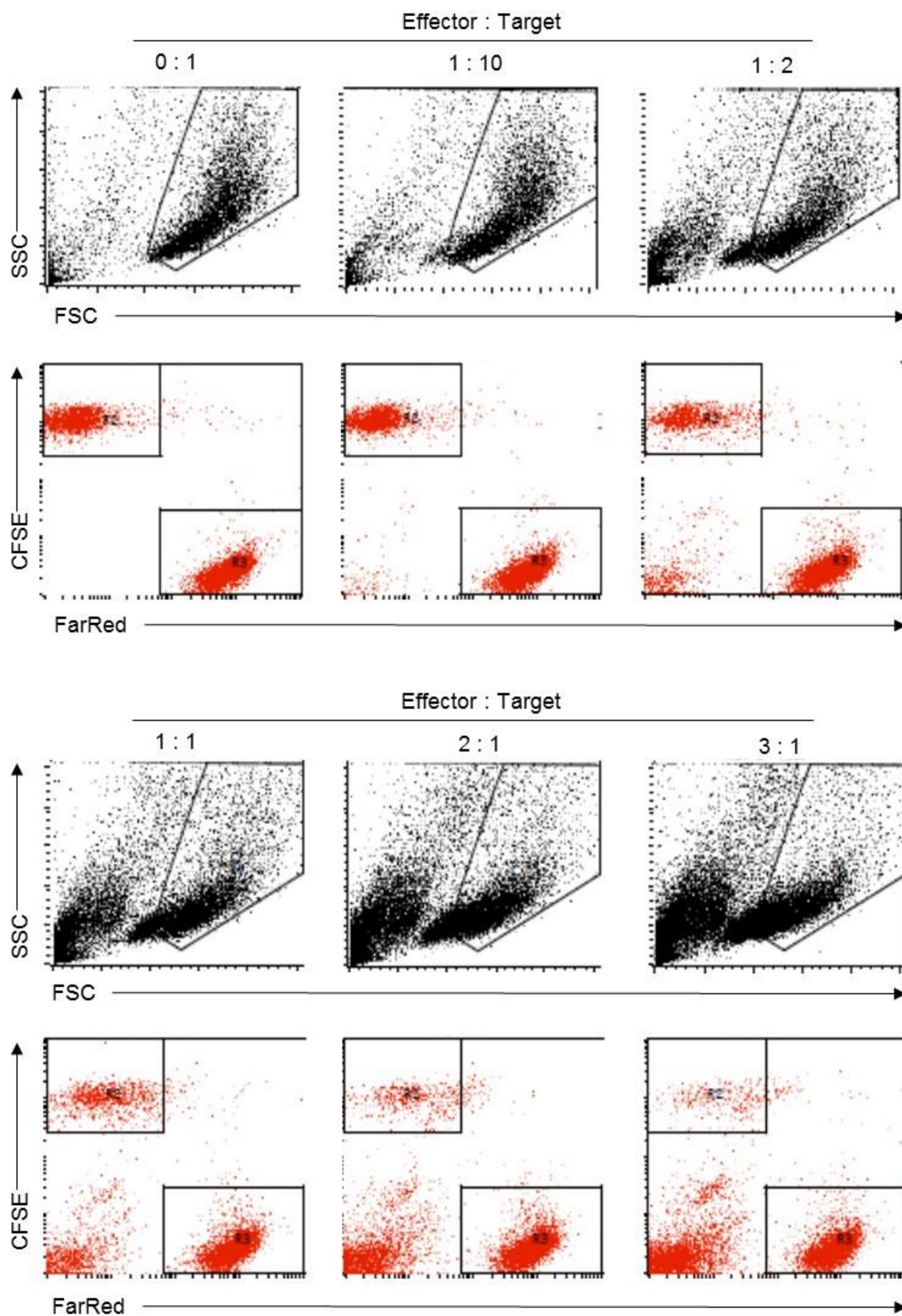

Figure 39. Representative FACS-plots of the flow cytometry-based VITAL-FR assays (PN4).

CFSE+ THP-1 (target) and FarRed+ DG-75 (control) cells were gated in R1. The R2-gated target cell frequencies were detected compared with constant frequencies of the control cells gated through R1 and R3. At each E : T ratio, specific lysis was calculated as described by Stanke et al. (2010).

Section 5. Discussion

5.1. Actual challenges in AML therapy

Acute myeloid leukemia is one of the haematological malignancies which have a poor prognosis after conventional therapy. An older age (60<), high numbers of white blood cells, existence of prior MDS and previous cytotoxic therapy to treat other diseases strongly contribute to the poor prognosis. Only one fourth of the adult AML patients survive more than 5 years (NCI 2015). Genetic alterations that play a key role in disease development indicate the prognosis and clinical outcome for both, remission and post-remission therapy. Intermediate and adverse genetic groups have a high risk of therapy resistance and relapse. To date, allo-HSCT in the first or second complete remission phase is the only curative treatment option for patients with high risk AML. Unfortunately, not all patients achieve complete remission prior to HSCT (Pfrepper et al. 2015), which in turn, remarkably increases relapse risk. Correspondingly, relapse remains as a major cause of death after HSCT. The therapeutic effect of allo-HSCT is mainly based on GvL effect, but partially opposed by treatment-related mortality and loss of quality of life caused by acute and chronic GvHD (van Besien 2013). Therefore, strengthening GvL effects without provoking GvHD is the major challenge to augment the therapeutic efficiency of HSCT. HSCT itself is a complex procedure which starts from finding a suitable donor who matches the recipient's HLA profile, and conditions recipients by an aggressive myelo- and immune suppressive therapy etc. Thus, disease course of patients is further negatively affected by circumstances like a treatment delay, lack of a suitable donor, exclusion from treatment due to an older age or severe comorbidity can occur in patients during their disease course. Overall, the aforementioned aspects call for an improvement of the current treatment modalities as well as for alternative strategies in the field of AML therapy.

As immune cells are able to detect and destroy malignant cells, various immunotherapeutic strategies have been developed either to promote immune responses against tumors or to counteract tumor immune escape mechanisms. Vaccination to induce T cell responses against leukemia, one of the immunotherapeutic approaches, seems to be promising to strengthening the GvL effect after HSCT. Moreover, effective therapeutic vaccines could be alternative options in an autologous setting for patients who are not eligible to HSCT. DCs and TAAs have been thoroughly investigated for the vaccination strategies. Within the TAAs, WT1 is prioritized for the therapeutic vaccine against cancer due to its oncogenic function, immunogenic capability, tissue restricted expression, and progress-dependent abundance in leukemic cells (Cheever et al. 2009). Although various WT1 peptide vaccines have been established, their therapeutic use is generally limited due to the high diversity of individual immune responses. The known WT1-derived peptides are restricted mostly to the HLA A0201 and A2401 which reduces their wide clinical application. To expand the clinical application, there is a need for prior identification of immunogenic epitopes (Benteyn et al. 2013) in the protein sequence. Furthermore, the peptide-elicited immune responses can be short-lived and non-functional (Kuball et al. 2011) due to the tolerance mechanisms to WT1 as a self-protein. To overcome the aforementioned limitations, a proper optimization of WT1-specific T cell responses in terms of induction, amplitude, and duration is essential.

The most crucial prerequisites to achieve more effective and sustained T-cell responses are variety and immunogenicity of the presented peptides as well as co-stimulatory signaling, provision of cytokines and direct cell contact by the antigen presenting DCs . Thus, the aim of this study was to generate DC-targeted anti-hDEC205-WT1 antibody fusion proteins as an anti-leukemia vaccine and explore their immunogenicity by directly *ex vivo* and *in vitro* assays. The main hypothesis was that by triggering DEC205 receptor-mediated endocytosis through anti-hDEC205-WT1 antibody fusion protein, DCs are stimulated to uptake the WT1. The directly delivered WT1 whole protein or longer protein fragments to DCs for intracellular processing should lead to MHC class I- and II-mediated presentation of a great diversity of WT1-derived peptides. This approach leads to improved WT1-specific T-cell response and gives an opportunity for immunotherapy to the broad patient collective independently from ethnic background and HLA type.

5.2. Generation and evaluation of the anti-hDEC205-WT1 antibody fusion proteins

A Flag-tagged anti-hDEC205 IgG1 antibody was correctly constructed and effectively produced. Production and purification did not affect antibody function. Functional characteristics were verified by binding to hDEC205-expressing CHO cells and human immature moDCs using flow cytometry. The affinity of the anti-hDEC205 was determined by binding studies and found to be high. A N-terminally fused Flag tag exerted minimal effects on the structure and function of the anti-hDEC205 antibody as described elsewhere (Hopp 1988).

Since a delivery of the longest sequence of WT1 to DCs was the initial aim of this study, obtaining cDNA encoding full-length WT1_D was necessary. As described in the introduction (see 1.2.4), more than 36 different isoforms of WT1 are detected in cells as a result of alternative transcription, translation initiations, RNA editing, and splice events. In particular, a discovery of WT1 longer isoforms including WT1_D by Bruening *et al*. demonstrated that WT1 gene expression is more complex than anticipated, with a non-AUG translational initiation event producing additional protein isoforms of 54 –56 kDa in addition to the known isoforms of 47-49 kDa (Bruening and Pelletier 1996). Due to the above mentioned complexities, a cDNA encoding full-length WT1_D could not be cloned in one step, hence, we cloned two cDNAs, each encoding the respective other part of WT1_D protein. Linkage of the two cDNAs through an internal XhoI restriction site resulted in a cDNA encoding the full-length WT1_D protein. After obtaining the WT1-encoding cDNAs, it was easy to construct anti-hDEC205-WT1 antibody fusion proteins.

Expression and purification of the full-length canonical isoform of murine and human WT1 and its zinc finger domains for functional studies were performed by a couple of groups beforehand (Geng and Carstens 2006, Nurmemmedov and Thunnissen 2006, Fagerlund et al. 2012). To improve the stability and solubility of WT1, which are major obstacles in intracellular protein expression, in these studies, various expression systems, and buffers as well as soluble tags have been tested. By means of the huge efforts, sufficient amounts of WT1 protein were purified from inclusion bodies of *E. Coli* or eukaryotic HEK293T cell lysates. By contrast, we aimed to yield the anti-hDEC205-WT1 antibody fusion proteins from cell culture supernatant in a soluble and secreted form. Proving the aforementioned difficulties, there were certain hindrances in

production and purification of our antibody fusion proteins. First, anti-hDEC205 antibody fusion proteins containing full-length and major fragments of WT1_D were produced with different efficiencies. The production of the anti-hDEC205-WT1_D_{1-227} was the most superior while the anti-hDEC205-WT1_D_{full} showed the less efficiency of expression. Second, all constructed anti-hDEC205-WT1_D_{major} antibody fusion proteins possessed poor secretory characteristics, namely, these proteins were left largely cell-associated instead they were secreted into culture supernatants. Next, in principle, purification of anti-hDEC205-WT1_D_{1-227} variant from triton extract of HEK293 cell lysates was possible, however, the final yields were reduced significantly due to the precipitation/aggregation of the antibody fusion protein, confirming results from Fagerlund *et al.* (Fagerlund et al. 2012).

Of note, the purified anti-hDEC205-WT1_D_{1-227} fusion protein was once evaluated by IFN-γ ELISPOT assay. The relatively high number of IFN-γ spots shown by this assay indicated that the DEC205-targeted WT1_D_{1-227} major fragment could be more potent to induce WT1-specific T-cell responses compared to smaller fragments of WT1. Although it could not be reproduced by more experiments due to insufficient protein yields, it is meaningful to demonstrate a general feasibility of DEC205 targeting approach for WT1.

In view of the poor secretion of WT1 major fragment containing hDEC205-specific antibody fusion proteins, smaller fragments encoding immunogenic epitopes that were previously published elsewhere (Rezvani et al. 2008), (Kobayashi et al. 2006), (Doubrovina et al. 2012) were screened for efficient expression and secretion. It seemed that anti-hDEC205 antibody bearing WT1 fragments of more than 50 amino acids are not able to excrete through transfected cells. All four antibody fusion proteins identified with sufficient production and secretion compose 26 to 51 amino acids. This could probably be explained by the fact that biochemical properties authorizing the intracellular nature of the protein may be lost in smaller sequences of WT1 within the antibody fusion protein. However, despite the relative shortness of the found $WT1_{small}$ fragments, more than ten of variable peptides are possibly be generated by the antigen processing machinery of DCs. The finally identified four different fragments of WT1 targeted to DCs through anti-hDEC205 antibody are obtainable in sufficient yields by routine protein expression and purification system. This would be an advantage for WT1 vaccination strategy. Furthermore, anti-hDEC205-$WT1_{91-138}$, anti-hDEC205-$WT1_{223-273}$, and anti-hDEC205-$WT1_{324-371}$ were analyzed for their directly *ex vivo* T cell stimulatory capacity.

It is of importance that all three anti-hDEC205-WT1$_{small}$ antibody fusion proteins were functional with respect to antibody integrity and antigen binding.

The direct stimulation of PBMCs with each of the three antibody fusion proteins did not induce IFN-γ responses in T cells directly *ex vivo*, probably due to the low frequencies of DCs in PBMCs. Therefore, the uptaken amount of antibody fusion proteins and the resulting amount of processed and presented peptides in the given time might be insufficient to result in an optimal T-cell activation. Moreover, instead of immunity a possible tolerance could have been induced, as different APC subtypes in PBMCs undergo distinct maturation processes resulting in different functional profiles. Thus, fully matured and protein loaded moDCs were used to fairly investigate the T-cell stimulatory capacity of the DC-targeted anti-hDEC205-WT1 antibody fusion proteins.

An effective adaptive immune response to a tumor is mainly triggered/mediated by tumor antigen specific cytotoxic CD3^{+} T cells (Rosenberg et al. 1988, Celluzzi et al. 1996). To evaluate CD3^{+} T-cell responses to anti-hDEC205-WT1, we selected CD3^{+} T cells from PBMCs. Purity of the CD3^{+} T cells tested prior to the antigenic stimulation via mature moDCs was 95 % in average, thereby ensuring low variability of the performed ICS and ELISPOT assays. Of importance, the results from PBMC/moDC co-incubation experiments showed that T cells in PBMCs were able to respond to anti-hDEC205-WT1$_{91-138}$ when the loaded moDCs triggered the strong enough stimulation. This indicates an optimized priming and/or activation is a crucial factor to induce WT1-specific T cell response towards the antigen-expressing cells.

Patients' blood samples were drawn between six and 192 months post-transplantation meaning that patients were in different phases of immune reconstitution. However, WT1-specific T cells were detected in freshly isolated T cells from PBMCs of most patients indicating a possibility of T cell repopulation driven by encountered tumor associated peptides in allo-HSC transplanted patients (Goldrath and Bevan 1999). In this context, it was important to investigate T-cell responses of the participants with diverse HLA patterns to ensure a variability of moDC-generated and presented peptides derived from the WT1 protein fragments.

Anti-hDEC205-WT1$_{91-138}$ was the only one that improved directly *ex vivo* T cell responses in healthy donors and allo-HSC transplanted AML patients. The contained WT1$_{91-138}$ fragment comprises five previously determined epitopes (Doubrovina et al.

2012, Kobayashi et al. 2006) with immunogenic potential including the well documented $WT1_{126-134}$ peptide (Oka et al. 2000, Rezvani et al. 2008). Since responders to anti-hDEC205-$WT1_{91-138}$ stimulation were not exclusively HLA A0201 positive, the good immunogenicity of anti-hDEC205-$WT1_{91-138}$ might be explained by relatively broad epitope variety presented on different HLA molecules of efficiently targeted DCs.

It is known that DEC205-targeting protein vaccines strongly enhance cross presentation (Bonifaz et al. 2004, Cheong et al. 2010). Accordingly, the strongest T cell response was observed for $CD8^+$ T cells, but there was also a tendency for activation of $CD4^+$ helper T cells after DEC205-targeted $WT1_{91-138}$ stimulation. This finding confirms previous studies showing that a combination of HLA class I and II restricted peptides induces both, cytotoxic and helper T-cell responses (Maslak et al. 2010, Koido et al. 2014). Due to natural processing by DCs, peptides derived from the endocytosed protein could be presented via HLA class I and II molecules resulting in improved T cell responses.

An important aim that we reached in the study was the detection of an upgraded and robust WT1-specific T-cell response directly *ex vivo*. T cells responded substantially stronger to DEC205-targeted $WT1_{91-138}$ than to a 15-mer WT1 peptide pool, demonstrating a successful targeting of the moDCs by the antibody fusion protein. However, it is not clear why only one of the three anti-hDEC205-$WT1_{small}$ constructs induced a decent response. Previous studies showed that prior T cell enrichment (Schmied et al. 2015) or expansion (Krishnadas et al. 2011) using WT1 peptide pool is necessary to enhance detection of low frequency of WT1-specific T cells in healthy donors. Our results indicate that such rare T cells could be better monitored by targeting of the $WT1_{91-138}$ to DCs at least in the context of allo-HSCT.

Although anti-hDEC205-$WT1_{223-273}$ and anti-hDEC205-$WT1_{324-371}$ did not induce detectable *ex vivo* T cell responses, their stimulatory capacity should be further tested by *in vitro* T cell expansion prior to detection. Of note, sequences of the other two antibody fusion proteins: anti-hDEC205-$WT1_{223-273}$, and anti-hDEC205-$WT1_{324-371}$ also contain previously identified HLA class I- and II- restricted epitopes (Oka et al. 2000) (Fujiki et al. 2007). Repeated stimulations of T cells by moDCs loaded with these WT1 fusion proteins might be an option to increase low or undetectable frequencies of specific T cells. Specifically, supplementation of various epitopes may enhance T-cell responses even though the single epitopes are only of subdominant character.

To augment the therapeutic efficacy of HSCT, strengthening GvL effect independently of GvHD progression is essential. The targeting of these small fragments of WT1 protein to DEC205 on DCs should be further explored *in vivo* for its potential to support the GvL effect through WT1-specific T cells within an allo-HSCT setting and its ability to induce an effective immunity against the underlying disease. Generally, anti-hDEC205-WT1$_{small}$ antibody fusion proteins may directly reach to DCs *in vivo* and show their immune stimulatory effect in presence of an adjuvant that is indispensable to maturing DCs. However, in individuals with insufficient frequency or function of DCs, an improvement of the immune response could possibly be achieved by donor-derived moDC vaccination loaded with these proteins. This strategy may allow an efficient in vivo T cell stimulation even early after allo-HSCT. Of importance, using CMV-derived peptide loaded moDCs, Grigoleit *et al.* previously showed that DC vaccination can be performed safely in allo-HSCT setting (Grigoleit et al. 2007).

The first FDA-approved therapeutic vaccine, Sipuleucel-T (PROVENGE), demonstrated that *ex vivo* activated and matured APCs are able to prime T cells both *ex vivo* and *in vivo*. Prostate cancer antigen so called prostate acetyl phosphatase (PAP)-specific T cells then recognize and kill the PAP-positive cancer cells (Cheever and Higano 2011). Resembling the Sipuleucel-T effect, in this study, the anti-hDEC205-WT1$_{91-138}$-loaded moDCs were capable to prime and/or activate T cells which were freshly isolated from patients with AML. Therefore, it seems likely that such DCs have enough potential to prime and activate T cells *in vivo* as well. Thus, our earliest results confirming WT1-specific T-cell responses induced by the DC-targeting anti-hDEC205-WT1$_{91-138}$ approach could be a basis for a therapeutic vaccine against WT1-expressing malignancies and furthermore, be applicable for induction of a WT1-specific immune response to solid tumors since WT1 is overexpressed in various cancers (Sugiyama 2010).

In conclusion, a transplantation-vaccine or -adoptive transfer of leukemia-specific T cells to boost GvL effect and reduce relapse after HSCT has been considered as a highly effective strategies for the control of high-risk leukemia (Rezvani 2011). WT1 has been a real basis for the both strategies to evoke antileukemia T-cell immune responses. However, intracellular nature of this TAA is a development barrier to advance such promising strategies. With this regard, DC-targeting approaches offer whole potential for intracellular antigen-targeted immunotherapy compared to direct T-cell manipulating approaches. Despite WT1 mRNA-electroporated DC vaccine, there are no crucial efforts

to overcome the barrier in WT1-based vaccine approaches. Raising this issue, we developed hDEC205-targeted WT1 antibody fusion proteins as anti-leukemia vaccines and explored their immune stimulatory capacity by studies *in vitro* and directly *ex vivo*. Our approach contributes to overcoming the challenge of poor expression of the intracellular tumor associated antigen WT1 and introduces an alternative that could be easily translated into clinical practice to improve antileukemia immune response. Furthermore, because anti-hDEC205-$WT1_{small}$ antibody fusion proteins are able to directly target DCs *in vivo*, their potential clinical application would need a lower costs and less *ex vivo* manipulation (Sehgal et al. 2014).

5.3. Future outlook

T cells recognizing peptides derived from TAAs are generally short-lived and of low avidity, as most of those antigens are self-proteins. The known explanation is that maturing TAA-specific T cells with high avidity are depleted in the thymus by negative selection due to self-tolerance mechanisms. A possible approach to sustain or reestablish the usually "undesired" immunity, a frequent vaccination mode could be an option. In this case, recombinant therapeutic vaccines with appropriate therapeutic efficacy as well as a simple administration and low costs of production are inevitable. The anti-hDEC205-$WT1_{91-138}$ could be an example of such a recombinant vaccine.

In addition, the anti-hDEC205-$WT1_{223-273}$ and anti-hDEC205-$WT1_{324-371}$ antibody fusion proteins could be potential "team players" for improvement of T cell responses against relapse of the underlying malignancies. Specifically, leukemia cells that are ignored by particular T cells specific for $WT1_{91-138}$ -derived peptides can potentially be recognized by T cells specific for epitopes from the other two WT1 protein fragments. Thus, the latter two antibody fusion proteins need to be further investigated to reveal their immune stimulatory capacity. Furthermore, *in vivo* tests of anti-hDEC205-WT1 antibody fusion proteins should be initiated to provide evidence for the immunogenicity of these vaccine constructs. Another perspective is the generation of more anti-hDEC205-TAA antibody fusion proteins using other TAAs (e.g. Bcl-2, MUC-1) to induce multi-specific T cells against leukemia.

Ultimately, two areas of data drive WT1-based vaccination strategies to be intensely improved and combined with other treatment options: first, the growing data of clinical studies demonstrating that vaccination using WT1-derived peptides is safe and feasible for patients with advanced MDS/AML (Di Stasi et al. 2015), and; second, distinct data confirming the significant correlation between WT1 overexpression and worse clinical outcomes in either hematological (Woehlecke et al. 2015, Yi-Ning et al. 2015) or solid tumor patients (Qi et al. 2015). In light of these data, our alternative approach offers an attractive perspective to be translated into clinical practice.

Section 6. End matter

6.1. Bibliography

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6.2. Sequences of antibody fusion constructs

6.2.1. Anti-hDEC205-VH-heavy constant chain full

	Nucleotide	*Amino acid*
Leader	1-78	1-26
Flag tag	85-109	29-36
Variable heavy chain domain	115-463	39-154
Constant heavy chain domain of IgG1	469-1453	159-489

```
   1 ATGAACTTCGGCTTTCGCCTGATCTTCCTGGTGCTGGTGCTGAAGGGCGTGCAGTGCGAAGTGAAG
   1  M  N  F  G  F  R  L  I  F  L  V  L  V  L  K  G  V  Q  C  E  V  K
  67 CTGGTGCCCCGGCAATTGGACTACAAGGACGACGACGACAAAGAATTCGAGGTGCAGCTGCAGCAG
  23  L  V  P  R  Q  L  D  Y  K  D  D  D  D  K  E  F  E  V  Q  L  Q  Q
 133 TCTGGCCCCGTGCTCGTGAAACCTGGCGCCTCCGTGAAGATGAGCTGCAAGGCCAGCGGCAACACC
  45  S  G  P  V  L  V  K  P  G  A  S  V  K  M  S  C  K  A  S  G  N  T
 199 TTCACCGACAGCTTCATGCACTGGATGAAGCAGAGCCACGGCAAGAGCCTGGAATGGATCGGCATC
  67  F  T  D  S  F  M  H  W  M  K  Q  S  H  G  K  S  L  E  W  I  G  I
 265 ATCAACCCCTACAACGGCGGCACCTCCTACAACCAGAAGTTCAAGGGCAAGGCCACCCTGACCGTG
  89  I  N  P  Y  N  G  G  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T  V
 331 GACAAGAGCAGCAGCACCGCCTACATGGAACTGAACAGCCTGACCAGCGAGGACAGCGCCGTGTAC
 111  D  K  S  S  S  T  A  Y  M  E  L  N  S  L  T  S  E  D  S  A  V  Y
 397 TACTGCGCCAGAAACGGCGTGCGGTACTACTTCGACTACTGGGGCCAGGGCACAACCCTGACAGTG
 133  Y  C  A  R  N  G  V  R  Y  Y  F  D  Y  W  G  Q  G  T  T  L  T  V
 463 TCTAGCAGATCCTCTAGCGCCAGCACAAAGGGCCCCAGCGTGTTCCCTCTGGCCCCTAGCAGCAAG
 155  S  S  R  S  S  S  A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K
 529 AGCACATCTGGCGGAACAGCCGCCCTGGGCTGCCTCGTGAAGGACTACTTTCCCGAGCCCGTGACA
 177  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T
 595 GTGTCCTGGAACTCTGGCGCCCTGACAAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGCAGC
 199  V  S  W  N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S
 661 GGCCTGTACTCTCTGAGCAGCGTCGTGACTGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATC
 221  G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I
 727 TGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGCGAC
 243  C  N  V  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D
 793 AAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAACTGCTGGGCGGACCTTCCGTGTTCCTGTTC
 265  K  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F
 859 CCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAT
 287  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D
 925 GTGTCCCACGAGGACCCTGAAGTGAAGTTTAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCC
 309  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A
 991 AAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACAGTGCTG
 331  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L
1057 CACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCC
 353  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P
1123 ATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAACCCCAGGTGTACACACTGCCTCCC
 375  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P
1189 AGCAGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTCGTGAAAGGCTTCTACCCCTCC
 397  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S
1255 GATATCGCCGTGGAATGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTG
 419  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V
1321 CTGGACAGCGACGGCTCATTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAG
 441  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q
1387 GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCAAGTCCCTGAGC
 463  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  K  S  L  S
1453 CTGAGCCCCGGCAAGTAA
 485  L  S  P  G  K  *
```

6.2.2. Anti-hDEC205-VL-light constant chain full

	Nucleotide	*Amino acid*
Leader	1-78	1-26
Flag tag	85-109	29-36
Variable light chain domain	115-441	39-147
Constant light kappa chain domain	463-777	155-259

```
  1 ATGAACTTCGGCTTTCGCCTGATCTTCCTGGTGCTGGTGCTGAAGGGCGTGCAGTGCGAAGTGAAG
  1  M  N  F  G  F  R  L  I  F  L  V  L  V  L  K  G  V  Q  C  E  V  K
 67 CTGGTGCCCCGGCAATTGGACTACAAGGACGACGACGACAAAGAATTCCAGGCTGTCGTGACCCAG
 23  L  V  P  R  Q  L  D  Y  K  D  D  D  D  K  E  F  Q  A  V  V  T  Q
133 GAAAGCGCCCTGACAACCAGCCCTGGCGAGACAGTGACCCTGACCTGCAGATCCTCTACAGGCGCC
 45  E  S  A  L  T  T  S  P  G  E  T  V  T  L  T  C  R  S  S  T  G  A
199 GTGACCATCAGCAACTACGCCAACTGGGTGCAGGAAAAGCCCGACCACCTGTTCACCGGCCTGATC
 67  V  T  I  S  N  Y  A  N  W  V  Q  E  K  P  D  H  L  F  T  G  L  I
265 GGCGGCACAAACAACAGAGCACCTGGCGTGCCCGCCAGATTCAGCGGCTCTCTGATCGGAGATAAG
 89  G  G  T  N  N  R  A  P  G  V  P  A  R  F  S  G  S  L  I  G  D  K
331 GCCGCACTGACCATCACAGGCGCCCAGACCGAGGACGAGGCCATCTACTTTTGCGCCCTGTGGTAC
111  A  A  L  T  I  T  G  A  Q  T  E  D  E  A  I  Y  F  C  A  L  W  Y
397 AACAACCAGTTCATCTTCGGCAGCGGCACCAAAGTGACCGTGCTGAGATCCGAAATCAAGCGTACG
133  N  N  Q  F  I  F  G  S  G  T  K  V  T  V  L  R  S  E  I  K  R  T
463 GTGGCCGCTCCCAGCGTGTTCATCTTCCCACCTAGCGACGAGCAGCTGAAGTCCGGCACAGCCTCT
155  V  A  A  P  S  V  F  I  F  P  P  S  D  E  Q  L  K  S  G  T  A  S
529 GTCGTGTGCCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTGGACAATGCC
177  V  V  C  L  L  N  N  F  Y  P  R  E  A  K  V  Q  W  K  V  D  N  A
595 CTGCAGAGCGGCAACAGCCAGGAAAGCGTGACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTG
199  L  Q  S  G  N  S  Q  E  S  V  T  E  Q  D  S  K  D  S  T  Y  S  L
661 AGCAGCACCCTGACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAAGTGACC
221  S  S  T  L  T  L  S  K  A  D  Y  E  K  H  K  V  Y  A  C  E  V  T
727 CACCAGGGCCTGTCTAGCCCCGTGACCAAGAGCTTCAACCGGGGCGAGTGCTAA
243  H  Q  G  L  S  S  P  V  T  K  S  F  N  R  G  E  C  *
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6.2.3. scFv:hDEC205-GpL

	Nucleotide	*Amino acid*
Leader	1-78	1-26
Variable heavy chain domain	85-438	29-146
Linker	439-474	147-158
Variable light chain domain	475-801	159-267
Flag tag 2x	808-861	270-287
GpL	868-1380	290-460

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  1 ATGAACTTCGGCTTTCGCCTGATCTTCCTGGTGCTGGTGCTGAAGGGCGTGCAGTGCGAAGTGAAG
  1  M  N  F  G  F  R  L  I  F  L  V  L  V  L  K  G  V  Q  C  E  V  K
 67 CTGGTGCCCCGGCAATTGGAGGTGCAGCTGCAGCAGTCTGGCCCCGTGCTCGTGAAACCTGGCGCC
 23  L  V  P  R  Q  L  E  V  Q  L  Q  Q  S  G  P  V  L  V  K  P  G  A
133 TCCGTGAAGATGAGCTGCAAGGCCAGCGGCAACACCTTCACCGACAGCTTCATGCACTGGATGAAG
 45  S  V  K  M  S  C  K  A  S  G  N  T  F  T  D  S  F  M  H  W  M  K
199 CAGAGCCACGGCAAGAGCCTGGAATGGATCGGCATCATCAACCCCTACAACGGCGGCACCTCCTAC
 67  Q  S  H  G  K  S  L  E  W  I  G  I  I  N  P  Y  N  G  G  T  S  Y
265 AACCAGAAGTTCAAGGGCAAGGCCACCCTGACCGTGGACAAGAGCAGCAGCACCGCCTACATGGAA
 89  N  Q  K  F  K  G  K  A  T  L  T  V  D  K  S  S  S  T  A  Y  M  E
331 CTGAACAGCCTGACCAGCGAGGACAGCGCCGTGTACTACTGCGCCAGAAACGGCGTGCGGTACTAC
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 111  L  N  S  L  T  S  E  D  S  A  V  Y  Y  C  A  R  N  G  V  R  Y  Y
 397 TTCGACTACTGGGGCCAGGGCACAACCCTGACAGTGTCTAGCGGCGGAGGAAGCGGAGGCGGATCT
 133  F  D  Y  W  G  Q  G  T  T  L  T  V  S  S  G  G  G  S  G  G  G  S
 463 GGCGGAGGATCTCAGGCTGTCGTGACCCAGGAAAGCGCCCTGACAACCAGCCCTGGCGAGACAGTG
 155  G  G  G  S  Q  A  V  V  T  Q  E  S  A  L  T  T  S  P  G  E  T  V
 529 ACCCTGACCTGCAGATCCTCTACAGGCGCCGTGACCATCAGCAACTACGCCAACTGGGTGCAGGAA
 177  T  L  T  C  R  S  S  T  G  A  V  T  I  S  N  Y  A  N  W  V  Q  E
 595 AAGCCCGACCACCTGTTCACCGGCCTGATCGGCGGCACAAACAACAGAGCACCTGGCGTGCCCGCC
 199  K  P  D  H  L  F  T  G  L  I  G  G  T  N  N  R  A  P  G  V  P  A
 661 AGATTCAGCGGCTCTCTGATCGGAGATAAGGCCGCACTGACCATCACAGGCGCCCAGACCGAGGAC
 221  R  F  S  G  S  L  I  G  D  K  A  A  L  T  I  T  G  A  Q  T  E  D
 727 GAGGCCATCTACTTTTGCGCCCTGTGGTACAACAACCAGTTCATCTTCGGCAGCGGCACCAAAGTG
 243  E  A  I  Y  F  C  A  L  W  Y  N  N  Q  F  I  F  G  S  G  T  K  V
 793 ACCGTGCTGGGATCCGACTACAAGGACGACGACGACAAAGAATTCGACTACAAGGACGACGACGAC
 265  T  V  L  G  S  D  Y  K  D  D  D  D  K  E  F  D  Y  K  D  D  D  D
 859 AAACTCGAGAAACCAACCGAGAATAATGAGGATTTCAACATCGTGGCTGTGGCATCCAATTTTGCT
 287  K  L  E  K  P  T  E  N  N  E  D  F  N  I  V  A  V  A  S  N  F  A
 925 ACCACCGACCTCGATGCCGATCGGGGAAAACTGCCTGGCAAAAAACTGCCCCTGGAAGTGCTGAAA
 309  T  T  D  L  D  A  D  R  G  K  L  P  G  K  K  L  P  L  E  V  L  K
 991 GAGATGGAGGCCAACGCTAGAAAAGCTGGCTGTACTAGAGGATGTCTCATCTGCCTGTCCCACATC
 331  E  M  E  A  N  A  R  K  A  G  C  T  R  G  C  L  I  C  L  S  H  I
1057 AAGTGTACCCCAAAAATGAAAAAATTCATCCCTGGCCGGTGTCACACATACGAGGGCGACAAGGAA
 353  K  C  T  P  K  M  K  K  F  I  P  G  R  C  H  T  Y  E  G  D  K  E
1123 TCTGCTCAGGGCGGAATCGGAGAGGCTATTGTGGATATTCCTGAAATTCCTGGATTCAAGGACCTG
 375  S  A  Q  G  G  I  G  E  A  I  V  D  I  P  E  I  P  G  F  K  D  L
1189 GAGCCTATGGAACAGTTTATCGCCCAGGTGGACCTCTGTGTCGATTGTACAACTGGCTGCCTGAAA
 397  E  P  M  E  Q  F  I  A  Q  V  D  L  C  V  D  C  T  T  G  C  L  K
1255 GGGCTGGCCAATGTCCAGTGTAGTGACCTGCTGAAAAAATGGCTGCCCCAGAGATGTGCCACTTTC
 419  G  L  A  N  V  Q  C  S  D  L  L  K  K  W  L  P  Q  R  C  A  T  F
1321 GCCTCTAAAATTCAGGGCCAGGTCGACAAAATCAAAGGCGCTGGAGGAGACTCTGGAGCTTAA
 441  A  S  K  I  Q  G  Q  V  D  K  I  K  G  A  G  G  D  S  G  A  *
```

6.2.4. Anti-hDEC205-heavy-WT1_D1-227

	Nucleotide	*Amino acid*
Leader	1-78	1-26
Flag tag	85-109	29-36
Heavy chain variable domain	116-468	39-156
Heavy chain constant domain of IgG1	475-1468	159-489
WT1_D1-227	1469-2148 At 1812: silent mutation C ➔ T	490-716

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   1 ATGAACTTCGGCTTTCGCCTGATCTTCCTGGTGCTGGTGCTGAAGGGCGTGCAGTGCGAAGTGAAG
   1  M  N  F  G  F  R  L  I  F  L  V  L  V  L  K  G  V  Q  C  E  V  K
  67 CTGGTGCCCCGGCAATTGGACTACAAGGACGACGACGACAAAGAATTCGAGGTGCAGCTGCAGCAG
  23  L  V  P  R  Q  L  D  Y  K  D  D  D  D  K  E  F  E  V  Q  L  Q  Q
 133 TCTGGCCCCGTGCTCGTGAAACCTGGCGCCTCCGTGAAGATGAGCTGCAAGGCCAGCGGCAACACC
  45  S  G  P  V  L  V  K  P  G  A  S  V  K  M  S  C  K  A  S  G  N  T
 199 TTCACCGACAGCTTCATGCACTGGATGAAGCAGAGCCACGGCAAGAGCCTGGAATGGATCGGCATC
  67  F  T  D  S  F  M  H  W  M  K  Q  S  H  G  K  S  L  E  W  I  G  I
 265 ATCAACCCCTACAACGGCGGCACCTCCTACAACCAGAAGTTCAAGGGCAAGGCCACCCTGACCGTG
  89  I  N  P  Y  N  G  G  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T  V
 331 GACAAGAGCAGCAGCACCGCCTACATGGAACTGAACAGCCTGACCAGCGAGGACAGCGCCGTGTAC
 111  D  K  S  S  S  T  A  Y  M  E  L  N  S  L  T  S  E  D  S  A  V  Y
 397 TACTGCGCCAGAAACGGCGTGCGGTACTACTTCGACTACTGGGGCCAGGGCACAACCCTGACAGTG
 133  Y  C  A  R  N  G  V  R  Y  Y  F  D  Y  W  G  Q  G  T  T  L  T  V
 463 TCTAGCAGATCCTCTAGCGCCAGCACAAAGGGCCCCAGCGTGTTCCCTCTGGCCCCTAGCAGCAAG
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 529 AGCACATCTGGCGGAACAGCCGCCCTGGGCTGCCTCGTGAAGGACTACTTTCCCGAGCCCGTGACA
 177  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T
 595 GTGTCCTGGAACTCTGGCGCCCTGACAAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGCAGC
 199  V  S  W  N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S
 661 GGCCTGTACTCTCTGAGCAGCGTCGTGACTGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATC
 221  G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I
 727 TGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGCGAC
 243  C  N  V  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D
 793 AAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAACTGCTGGGCGGACCTTCCGTGTTCCTGTTC
 265  K  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F
 859 CCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAT
 287  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D
 925 GTGTCCCACGAGGACCCTGAAGTGAAGTTTAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCC
 309  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A
 991 AAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACAGTGCTG
 331  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L
1057 CACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCC
 353  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P
1123 ATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAACCCCAGGTGTACACACTGCCTCCC
 375  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P
1189 AGCAGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTCGTGAAAGGCTTCTACCCCTCC
 397  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S
1255 GATATCGCCGTGGAATGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTG
 419  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V
1321 CTGGACAGCGACGGCTCATTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAG
 441  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q
1387 GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCAAGTCCCTGAGC
 463  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  K  S  L  S
1453 CTGAGCCCCGGCAAGCTCGACTTCCTCTTGCTGCAGGACCCGGCTTCCACGTGTGTCCCGGAGCCG
 485  L  S  P  G  K  L  D  F  L  L  L  Q  D  P  A  S  T  C  V  P  E  P
1519 GCGTCTCAGCACACGCTCCGCTCCGGGCCTGGGTGCCTACAGCAGCCAGAGCAGCAGGGAGTCCGG
 507  A  S  Q  H  T  L  R  S  G  P  G  C  L  Q  Q  P  E  Q  Q  G  V  R
1585 GACCCGGGCGGCATCTGGGCCAAGTTAGGCGCCGCCGAGGCCAGCGCTGAACGTCTCCAGGGCCGG
 529  D  P  G  G  I  W  A  K  L  G  A  A  E  A  S  A  E  R  L  Q  G  R
1651 AGGAGCCGCGGGGCGTCCGGGTCTGAGCCGCAGCAAATGGGCTCCGACGTGCGGGACCTGAACGCG
 551  R  S  R  G  A  S  G  S  E  P  Q  Q  M  G  S  D  V  R  D  L  N  A
1717 CTGCTGCCCGCCGTCCCCTCCCTGGGTGGCGGCGGCGGCTGTGCCCTGCCTGTGAGCGGCGCGGCG
 573  L  L  P  A  V  P  S  L  G  G  G  G  G  C  A  L  P  V  S  G  A  A
1783 CAGTGGGCGCCGGTGCTGGACTTTGCGCCTCCGGGCGCTTCGGCTTACGGGTCGTTGGGCGGCCCC
 595  Q  W  A  P  V  L  D  F  A  P  P  G  A  S  A  Y  G  S  L  G  G  P
1849 GCGCCGCCACCGGCTCCGCCGCCACCCCCGCCGCCGCCGCCTCACTCCTTCATCAAACAGGAGCCG
 617  A  P  P  P  A  P  P  P  P  P  P  P  P  P  H  S  F  I  K  Q  E  P
1915 AGCTGGGGCGGCGCGGAGCCGCACGAGGAGCAGTGCCTGAGCGCCTTCACTGTCCACTTTTCCGGC
 639  S  W  G  G  A  E  P  H  E  E  Q  C  L  S  A  F  T  V  H  F  S  G
1981 CAGTTCACTGGCACAGCCGGAGCCTGTCGCTACGGGCCCTTCGGTCCTCCTCCGCCCAGCCAGGCG
 661  Q  F  T  G  T  A  G  A  C  R  Y  G  P  F  G  P  P  P  P  S  Q  A
2047 TCATCCGGCCAGGCCAGGATGTTTCCTAACGCGCCCTACCTGCCCAGCTGCCTCGAGAGCCAGCCC
 683  S  S  G  Q  A  R  M  F  P  N  A  P  Y  L  P  S  C  L  E  S  Q  P
2113 GCTATTCGCAATCAGGGTTACAGCACGGTCACCTTCTAA
 705  A  I  R  N  Q  G  Y  S  T  V  T  F  *
```

6.2.5. Anti-hDEC205-heavy-WT1_D217-522

	Nucleotide	*Amino acid*
Leader	1-78	1-26
Flag tag	85-109	29-36
Heavy chain variable domain	116-468	39-156
Heavy chain constant domain of IgG1	475-1468	159-489
WT1_D217-522	1475-2391	492-797

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   1 ATGAACTTCGGCTTTCGCCTGATCTTCCTGGTGCTGGTGCTGAAGGGCGTGCAGTGCGAAGTGAAG
   1  M  N  F  G  F  R  L  I  F  L  V  L  V  L  K  G  V  Q  C  E  V  K
  67 CTGGTGCCCCGGCAATTGGACTACAAGGACGACGACGACAAAGAATTCGAGGTGCAGCTGCAGCAG
  23  L  V  P  R  Q  L  D  Y  K  D  D  D  D  K  E  F  E  V  Q  L  Q  Q
 133 TCTGGCCCCGTGCTCGTGAAACCTGGCGCCTCCGTGAAGATGAGCTGCAAGGCCAGCGGCAACACC
  45  S  G  P  V  L  V  K  P  G  A  S  V  K  M  S  C  K  A  S  G  N  T
 199 TTCACCGACAGCTTCATGCACTGGATGAAGCAGAGCCACGGCAAGAGCCTGGAATGGATCGGCATC
  67  F  T  D  S  F  M  H  W  M  K  Q  S  H  G  K  S  L  E  W  I  G  I
 265 ATCAACCCCTACAACGGCGGCACCTCCTACAACCAGAAGTTCAAGGGCAAGGCCACCCTGACCGTG
  89  I  N  P  Y  N  G  G  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T  V
 331 GACAAGAGCAGCAGCACCGCCTACATGGAACTGAACAGCCTGACCAGCGAGGACAGCGCCGTGTAC
 111  D  K  S  S  S  T  A  Y  M  E  L  N  S  L  T  S  E  D  S  A  V  Y
 397 TACTGCGCCAGAAACGGCGTGCGGTACTACTTCGACTACTGGGGCCAGGGCACAACCCTGACAGTG
 133  Y  C  A  R  N  G  V  R  Y  Y  F  D  Y  W  G  Q  G  T  T  L  T  V
 463 TCTAGCAGATCCTCTAGCGCCAGCACAAAGGGCCCCAGCGTGTTCCCTCTGGCCCCTAGCAGCAAG
 155  S  S  R  S  S  S  A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K
 529 AGCACATCTGGCGGAACAGCCGCCCTGGGCTGCCTCGTGAAGGACTACTTTCCCGAGCCCGTGACA
 177  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T
 595 GTGTCCTGGAACTCTGGCGCCCTGACAAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGCAGC
 199  V  S  W  N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S
 661 GGCCTGTACTCTCTGAGCAGCGTCGTGACTGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATC
 221  G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I
 727 TGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGCGAC
 243  C  N  V  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D
 793 AAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAACTGCTGGGCGGACCTTCCGTGTTCCTGTTC
 265  K  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F
 859 CCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAT
 287  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D
 925 GTGTCCCACGAGGACCCTGAAGTGAAGTTTAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCC
 309  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A
 991 AAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACAGTGCTG
 331  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L
1057 CACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCC
 353  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P
1123 ATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAACCCCAGGTGTACACACTGCCTCCC
 375  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P
1189 AGCAGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTCGTGAAAGGCTTCTACCCCTCC
 397  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S
1255 GATATCGCCGTGGAATGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTG
 419  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V
1321 CTGGACAGCGACGGCTCATTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAG
 441  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q
1387 GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCAAGTCCCTGAGC
 463  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  K  S  L  S
1453 CTGAGCCCCGGCAAGCTCGAGATTCGCAATCAGGGTTACAGCACGGTCACCTTCGACGGGACGCCC
 485  L  S  P  G  K  L  E  I  R  N  Q  G  Y  S  T  V  T  F  D  G  T  P
1519 AGCTACGGTCACACGCCCTCGCACCATGCGGCGCAGTTCCCCAACCACTCATTCAAGCATGAGGAT
 507  S  Y  G  H  T  P  S  H  H  A  A  Q  F  P  N  H  S  F  K  H  E  D
1585 CCCATGGGCCAGCAGGGCTCGCTGGGTGAGCAGCAGTACTCGGTGCCGCCCCCGGTCTATGGCTGC
 529  P  M  G  Q  Q  G  S  L  G  E  Q  Q  Y  S  V  P  P  P  V  Y  G  C
1651 CACACCCCCACCGACAGCTGCACCGGCAGCCAGGCTTTGCTGCTGAGGACGCCCTACAGCAGTGAC
 551  H  T  P  T  D  S  C  T  G  S  Q  A  L  L  L  R  T  P  Y  S  S  D
1717 AATTTATACCAAATGACATCCCAGCTTGAATGCATGACCTGGAATCAGATGAACTTAGGAGCCACC
 573  N  L  Y  Q  M  T  S  Q  L  E  C  M  T  W  N  Q  M  N  L  G  A  T
1783 TTAAAGGGAGTTGCTGCTGGGAGCTCCAGCTCAGTGAAATGGACAGAAGGGCAGAGCAACCACAGC
 595  L  K  G  V  A  A  G  S  S  S  S  V  K  W  T  E  G  Q  S  N  H  S
1849 ACAGGGTACGAGAGCGATAACCACACAACGCCCATCCTCTGCGGAGCCCAATACAGAATACACACG
 617  T  G  Y  E  S  D  N  H  T  T  P  I  L  C  G  A  Q  Y  R  I  H  T
1915 CACGGTGTCTTCAGAGGCATTCAGGATGTGCGACGTGTGCCTGGAGTAGCCCCGACTCTTGTACGG
 639  H  G  V  F  R  G  I  Q  D  V  R  R  V  P  G  V  A  P  T  L  V  R
1981 TCGGCATCTGAGACCAGTGAGAAACGCCCCTTCATGTGTGCTTACCCAGGCTGCAATAAGAGATAT
 661  S  A  S  E  T  S  E  K  R  P  F  M  C  A  Y  P  G  C  N  K  R  Y
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2047 TTTAAGCTGTCCCACTTACAGATGCACAGCAGGAAGCACACTGGTGAGAAACCATACCAGTGTGAC
 683  F  K  L  S  H  L  Q  M  H  S  R  K  H  T  G  E  K  P  Y  Q  C  D
2113 TTCAAGGACTGTGAACGAAGGTTTTCTCGTTCAGACCAGCTCAAAAGACACCAAAGGAGACATACA
 705  F  K  D  C  E  R  R  F  S  R  S  D  Q  L  K  R  H  Q  R  R  H  T
2179 GGTGTGAAACCATTCCAGTGTAAAACTTGTCAGCGAAAGTTCTCCCGGTCCGACCACCTGAAGACC
 727  G  V  K  P  F  Q  C  K  T  C  Q  R  K  F  S  R  S  D  H  L  K  T
2245 CACACCAGGACTCATACAGGTAAAACAAGTGAAAAGCCCTTCAGCTGTCGGTGGCCAAGTTGTCAG
 749  H  T  R  T  H  T  G  K  T  S  E  K  P  F  S  C  R  W  P  S  C  Q
2311 AAAAGTTTGCCCGGTCAGATGAATTAGTCCGCCATCACAACATGCATCAGAGAAACATGACCAAA
 771  K  K  F  A  R  S  D  E  L  V  R  H  H  N  M  H  Q  R  N  M  T  K
2377 CTCCAGCTGGCGCTTTGA
 793  L  Q  L  A  L  *
```

6.2.6. Anti-hDEC205-heavy-WT1_D$_{217-351}$

	Nucleotide	*Amino acid*
Leader	1-78	1-26
Flag tag	85-109	29-36
Heavy chain variable domain	116-468	39-156
Heavy chain constant domain of IgG1	472-1468	159-489
WT1_D$_{217-351}$	1475-1881	492-626

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   1 ATGAACTTCGGCTTTCGCCTGATCTTCCTGGTGCTGGTGCTGAAGGGCGTGCAGTGCGAAGTGAAG
   1  M  N  F  G  F  R  L  I  F  L  V  L  V  L  K  G  V  Q  C  E  V  K
  67 CTGGTGCCCCGGCAATTGGACTACAAGGACGACGACGACAAAGAATTCGAGGTGCAGCTGCAGCAG
  23  L  V  P  R  Q  L  D  Y  K  D  D  D  D  K  E  F  E  V  Q  L  Q  Q
 133 TCTGGCCCCGTGCTCGTGAAACCTGGCGCCTCCGTGAAGATGAGCTGCAAGGCCAGCGGCAACACC
  45  S  G  P  V  L  V  K  P  G  A  S  V  K  M  S  C  K  A  S  G  N  T
 199 TTCACCGACAGCTTCATGCACTGGATGAAGCAGAGCCACGGCAAGAGCCTGGAATGGATCGGCATC
  67  F  T  D  S  F  M  H  W  M  K  Q  S  H  G  K  S  L  E  W  I  G  I
 265 ATCAACCCCTACAACGGCGGCACCTCCTACAACCAGAAGTTCAAGGGCAAGGCCACCCTGACCGTG
  89  I  N  P  Y  N  G  G  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T  V
 331 GACAAGAGCAGCAGCACCGCCTACATGGAACTGAACAGCCTGACCAGCGAGGACAGCGCCGTGTAC
 111  D  K  S  S  S  T  A  Y  M  E  L  N  S  L  T  S  E  D  S  A  V  Y
 397 TACTGCGCCAGAAACGGCGTGCGGTACTACTTCGACTACTGGGGCCAGGGCACAACCCTGACAGTG
 133  Y  C  A  R  N  G  V  R  Y  Y  F  D  Y  W  G  Q  G  T  T  L  T  V
 463 TCTAGCAGATCCTCTAGCGCCAGCACAAAGGGCCCCAGCGTGTTCCCTCTGGCCCCTAGCAGCAAG
 155  S  S  R  S  S  S  A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K
 529 AGCACATCTGGCGGAACAGCCGCCCTGGGCTGCCTCGTGAAGGACTACTTTCCCGAGCCCGTGACA
 177  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T
 595 GTGTCCTGGAACTCTGGCGCCCTGACAAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGCAGC
 199  V  S  W  N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S
 661 GGCCTGTACTCTCTGAGCAGCGTCGTGACTGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATC
 221  G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I
 727 TGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGCGAC
 243  C  N  V  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D
 793 AAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAACTGCTGGGCGGACCTTCCGTGTTCCTGTTC
 265  K  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F
 859 CCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAT
 287  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D
 925 GTGTCCCACGAGGACCCTGAAGTGAAGTTTAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCC
 309  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A
 991 AAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACAGTGCTG
 331  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L
1057 CACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCC
 353  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P
1123 ATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAACCCCAGGTGTACACACTGCCTCCC
 375  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P
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1189 AGCAGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTCGTGAAAGGCTTCTACCCCTCC
 397  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S
1255 GATATCGCCGTGGAATGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTG
 419  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V
1321 CTGGACAGCGACGGCTCATTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAG
 441  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q
1387 GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCAAGTCCCTGAGC
 463  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  K  S  L  S
1453 CTGAGCCCCGGCAAGCTCGAGATTCGCAATCAGGGTTACAGCACGGTCACCTTCGACGGGACGCCC
 485  L  S  P  G  K  L  E  I  R  N  Q  G  Y  S  T  V  T  F  D  G  T  P
1519 AGCTACGGTCACACGCCCTCGCACCATGCGGCGCAGTTCCCCAACCACTCATTCAAGCATGAGGAT
 507  S  Y  G  H  T  P  S  H  H  A  A  Q  F  P  N  H  S  F  K  H  E  D
1585 CCCATGGGCCAGCAGGGCTCGCTGGGTGAGCAGCAGTACTCGGTGCCGCCCCCGGTCTATGGCTGC
 529  P  M  G  Q  Q  G  S  L  G  E  Q  Q  Y  S  V  P  P  P  V  Y  G  C
1651 CACACCCCCACCGACAGCTGCACCGGCAGCCAGGCTTTGCTGCTGAGGACGCCCTACAGCAGTGAC
 551  H  T  P  T  D  S  C  T  G  S  Q  A  L  L  L  R  T  P  Y  S  S  D
1717 AATTTATACCAAATGACATCCCAGCTTGAATGCATGACCTGGAATCAGATGAACTTAGGAGCCACC
 573  N  L  Y  Q  M  T  S  Q  L  E  C  M  T  W  N  Q  M  N  L  G  A  T
1783 TTAAAGGGAGTTGCTGCTGGGAGCTCCAGCTCAGTGAAATGGACAGAAGGGCAGAGCAACCACAGC
 595  L  K  G  V  A  A  G  S  S  S  S  V  K  W  T  E  G  Q  S  N  H  S
1849 ACAGGGTACGAGAGCGATAACCACACAACGTAA
 617  T  G  Y  E  S  D  N  H  T  T  *
```

6.2.7. Anti-hDEC205-heavy-WT1_D347-522

	Nucleotide	*Amino acid*
Leader	1-78	1-26
Flag tag	85-109	29-36
Heavy chain variable domain	116-468	39-156
Heavy chain constant domain of IgG1	475-1468	159-489
WT1_D347-522	1475-2044	492-682

```
   1 ATGAACTTCGGCTTTCGCCTGATCTTCCTGGTGCTGGTGCTGAAGGGCGTGCAGTGCGAAGTGAAG
   1  M  N  F  G  F  R  L  I  F  L  V  L  V  L  K  G  V  Q  C  E  V  K
  67 CTGGTGCCCCGGCAATTGGACTACAAGGACGACGACGACAAAGAATTCGAGGTGCAGCTGCAGCAG
  23  L  V  P  R  Q  L  D  Y  K  D  D  D  D  K  E  F  E  V  Q  L  Q  Q
 133 TCTGGCCCCGTGCTCGTGAAACCTGGCGCCTCCGTGAAGATGAGCTGCAAGGCCAGCGGCAACACC
  45  S  G  P  V  L  V  K  P  G  A  S  V  K  M  S  C  K  A  S  G  N  T
 199 TTCACCGACAGCTTCATGCACTGGATGAAGCAGAGCCACGGCAAGAGCCTGGAATGGATCGGCATC
  67  F  T  D  S  F  M  H  W  M  K  Q  S  H  G  K  S  L  E  W  I  G  I
 265 ATCAACCCCTACAACGGCGGCACCTCCTACAACCAGAAGTTCAAGGGCAAGGCCACCCTGACCGTG
  89  I  N  P  Y  N  G  G  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T  V
 331 GACAAGAGCAGCAGCACCGCCTACATGGAACTGAACAGCCTGACCAGCGAGGACAGCGCCGTGTAC
 111  D  K  S  S  S  T  A  Y  M  E  L  N  S  L  T  S  E  D  S  A  V  Y
 397 TACTGCGCCAGAAACGGCGTGCGGTACTACTTCGACTACTGGGGCCAGGGCACAACCCTGACAGTG
 133  Y  C  A  R  N  G  V  R  Y  Y  F  D  Y  W  G  Q  G  T  T  L  T  V
 463 TCTAGCAGATCCTCTAGCGCCAGCACAAAGGGCCCCAGCGTGTTCCCTCTGGCCCCTAGCAGCAAG
 155  S  S  R  S  S  S  A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K
 529 AGCACATCTGGCGGAACAGCCGCCCTGGGCTGCCTCGTGAAGGACTACTTTCCCGAGCCCGTGACA
 177  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T
 595 GTGTCCTGGAACTCTGGCGCCCTGACAAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGCAGC
 199  V  S  W  N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S
 661 GGCCTGTACTCTCTGAGCAGCGTCGTGACTGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATC
 221  G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I
 727 TGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGCGAC
 243  C  N  V  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D
 793 AAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAACTGCTGGGCGGACCTTCCGTGTTCCTGTTC
 265  K  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F
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 859 CCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAT
 287  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D
 925 GTGTCCCACGAGGACCCTGAAGTGAAGTTTAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCC
 309  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A
 991 AAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACAGTGCTG
 331  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L
1057 CACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCC
 353  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P
1123 ATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAACCCCAGGTGTACACACTGCCTCCC
 375  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P
1189 AGCAGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTCGTGAAAGGCTTCTACCCCTCC
 397  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S
1255 GATATCGCCGTGGAATGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTG
 419  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V
1321 CTGGACAGCGACGGCTCATTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAG
 441  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q
1387 GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCAAGTCCCTGAGC
 463  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  K  S  L  S
1453 CTGAGCCCCGGCAAGCTCGAGCACTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAGCTCGAG
 485  L  S  P  G  K  L  E  H  Y  T  Q  K  S  L  S  L  S  P  G  K  L  E
1519 GATAACCACACAACGCCCATCCTCTGCGGAGCCCAATACAGAATACACACGCACGGTGTCTTCAGA
 507  D  N  H  T  T  P  I  L  C  G  A  Q  Y  R  I  H  T  H  G  V  F  R
1585 GGCATTCAGGATGTGCGACGTGTGCCTGGAGTAGCCCCGACTCTTGTACGGTCGGCATCTGAGACC
 529  G  I  Q  D  V  R  R  V  P  G  V  A  P  T  L  V  R  S  A  S  E  T
1651 AGTGAGAAACGCCCCTTCATGTGTGCTTACCCAGGCTGCAATAAGAGATATTTTAAGCTGTCCCAC
 551  S  E  K  R  P  F  M  C  A  Y  P  G  C  N  K  R  Y  F  K  L  S  H
1717 TTACAGATGCACAGCAGGAAGCACACTGGTGAGAAACCATACCAGTGTGACTTCAAGGACTGTGAA
 573  L  Q  M  H  S  R  K  H  T  G  E  K  P  Y  Q  C  D  F  K  D  C  E
1783 CGAAGGTTTTCTCGTTCAGACCAGCTCAAAAGACACCAAAGGAGACATACAGGTGTGAAACCATTC
 595  R  R  F  S  R  S  D  Q  L  K  R  H  Q  R  R  H  T  G  V  K  P  F
1849 CAGTGTAAAACTTGTCAGCGAAAGTTCTCCCGGTCCGACCACCTGAAGACCCACACCAGGACTCAT
 617  Q  C  K  T  C  Q  R  K  F  S  R  S  D  H  L  K  T  H  T  R  T  H
1915 ACAGGTAAAACAAGTGAAAAGCCCTTCAGCTGTCGGTGGCCAAGTTGTCAGAAAAAGTTTGCCCGG
 639  T  G  K  T  S  E  K  P  F  S  C  R  W  P  S  C  Q  K  K  F  A  R
1981 TCAGATGAATTAGTCCGCCATCACAACATGCATCAGAGAAACATGACCAAACTCCAGCTGGCGCTT
 661  S  D  E  L  V  R  H  H  N  M  H  Q  R  N  M  T  K  L  Q  L  A  L
2047 TAA
 683  *
```

6.2.8. Anti-hDEC205-heavy-WT1_D_{full}

	Nucleotide	*Amino acid*
Leader	1-78	1-26
Flag tag	85-109	29-36
Heavy chain variable domain	116-468	39-156
Heavy chain constant domain of IgG1	475-1468	159-489
WT1_D_{full}	1469-3021	490-1007

```
   1 ATGAACTTCGGCTTTCGCCTGATCTTCCTGGTGCTGGTGCTGAAGGGCGTGCAGTGCGAAGTGAAG
   1  M  N  F  G  F  R  L  I  F  L  V  L  V  L  K  G  V  Q  C  E  V  K
  67 CTGGTGCCCCGGCAATTGGACTACAAGGACGACGACGACAAAGAATTCGAGGTGCAGCTGCAGCAG
  23  L  V  P  R  Q  L  D  Y  K  D  D  D  D  K  E  F  E  V  Q  L  Q  Q
 133 TCTGGCCCCGTGCTCGTGAAACCTGGCGCCTCCGTGAAGATGAGCTGCAAGGCCAGCGGCAACACC
  45  S  G  P  V  L  V  K  P  G  A  S  V  K  M  S  C  K  A  S  G  N  T
 199 TTCACCGACAGCTTCATGCACTGGATGAAGCAGAGCCACGGCAAGAGCCTGGAATGGATCGGCATC
  67  F  T  D  S  F  M  H  W  M  K  Q  S  H  G  K  S  L  E  W  I  G  I
 265 ATCAACCCCTACAACGGCGGCACCTCCTACAACCAGAAGTTCAAGGGCAAGGCCACCCTGACCGTG
  89  I  N  P  Y  N  G  G  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T  V
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 331 GACAAGAGCAGCAGCACCGCCTACATGGAACTGAACAGCCTGACCAGCGAGGACAGCGCCGTGTAC
 111  D  K  S  S  S  T  A  Y  M  E  L  N  S  L  T  S  E  D  S  A  V  Y
 397 TACTGCGCCAGAAACGGCGTGCGGTACTACTTCGACTACTGGGGCCAGGGCACAACCCTGACAGTG
 133  Y  C  A  R  N  G  V  R  Y  Y  F  D  Y  W  G  Q  G  T  T  L  T  V
 463 TCTAGCAGATCCTCTAGCGCCAGCACAAAGGGCCCCAGCGTGTTCCCTCTGGCCCCTAGCAGCAAG
 155  S  S  R  S  S  S  A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K
 529 AGCACATCTGGCGGAACAGCCGCCCTGGGCTGCCTCGTGAAGGACTACTTTCCCGAGCCCGTGACA
 177  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T
 595 GTGTCCTGGAACTCTGGCGCCCTGACAAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGCAGC
 199  V  S  W  N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S
 661 GGCCTGTACTCTCTGAGCAGCGTCGTGACTGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATC
 221  G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I
 727 TGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGCGAC
 243  C  N  V  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D
 793 AAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAACTGCTGGGCGGACCTTCCGTGTTCCTGTTC
 265  K  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F
 859 CCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAT
 287  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D
 925 GTGTCCCACGAGGACCCTGAAGTGAAGTTTAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCC
 309  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A
 991 AAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACAGTGCTG
 331  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L
1057 CACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCC
 353  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P
1123 ATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAACCCCAGGTGTACACACTGCCTCCC
 375  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P
1189 AGCAGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTCGTGAAAGGCTTCTACCCCTCC
 397  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S
1255 GATATCGCCGTGGAATGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTG
 419  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V
1321 CTGGACAGCGACGGCTCATTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAG
 441  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q
1387 GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCAAGTCCCTGAGC
 463  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  K  S  L  S
1453 CTGAGCCCCGGCAAGCTCGACTTCCTCTTGCTGCAGGACCCGGCTTCCACGTGTGTCCCGGAGCCG
 485  L  S  P  G  K  L  D  F  L  L  L  Q  D  P  A  S  T  C  V  P  E  P
1519 GCGTCTCAGCACACGCTCCGCTCCGGGCCTGGGTGCCTACAGCAGCCAGAGCAGCAGGGAGTCCGG
 507  A  S  Q  H  T  L  R  S  G  P  G  C  L  Q  Q  P  E  Q  Q  G  V  R
1585 GACCCGGGCGGCATCTGGGCCAAGTTAGGCGCCGCCGAGGCCAGCGCTGAACGTCTCCAGGGCCGG
 529  D  P  G  G  I  W  A  K  L  G  A  A  E  A  S  A  E  R  L  Q  G  R
1651 AGGAGCCGCGGGGCGTCCGGGTCTGAGCCGCAGCAAATGGGCTCCGACGTGCGGGACCTGAACGCG
 551  R  S  R  G  A  S  G  S  E  P  Q  Q  M  G  S  D  V  R  D  L  N  A
1717 CTGCTGCCCGCCGTCCCCTCCCTGGGTGGCGGCGGCGGCTGTGCCCTGCCTGTGAGCGGCGCGGCG
 573  L  L  P  A  V  P  S  L  G  G  G  G  G  C  A  L  P  V  S  G  A  A
1783 CAGTGGGCGCCGGTGCTGGACTTTGCGCCTCCGGGCGCTTCGGCTTACGGGTCGTTGGGCGGCCCC
 595  Q  W  A  P  V  L  D  F  A  P  P  G  A  S  A  Y  G  S  L  G  G  P
1849 GCGCCGCCACCGGCTCCGCCGCCACCCCCGCCGCCGCCGCCTCACTCCTTCATCAAACAGGAGCCG
 617  A  P  P  P  A  P  P  P  P  P  P  P  P  P  H  S  F  I  K  Q  E  P
1915 AGCTGGGGCGGCGCGGAGCCGCACGAGGAGCAGTGCCTGAGCGCCTTCACTGTCCACTTTTCCGGC
 639  S  W  G  G  A  E  P  H  E  E  Q  C  L  S  A  F  T  V  H  F  S  G
1981 CAGTTCACTGGCACAGCCGGAGCCTGTCGCTACGGGCCCTTCGGTCCTCCTCCGCCCAGCCAGGCG
 661  Q  F  T  G  T  A  G  A  C  R  Y  G  P  F  G  P  P  P  P  S  Q  A
2047 TCATCCGGCCAGGCCAGGATGTTTCCTAACGCGCCCTACCTGCCCAGCTGCCTCGAGATTCGCAAT
 683  S  S  G  Q  A  R  M  F  P  N  A  P  Y  L  P  S  C  L  E  I  R  N
2113 CAGGGTTACAGCACGGTCACCTTCGACGGGACGCCCAGCTACGGTCACACGCCCTCGCACCATGCG
 705  Q  G  Y  S  T  V  T  F  D  G  T  P  S  Y  G  H  T  P  S  H  H  A
2179 GCGCAGTTCCCCAACCACTCATTCAAGCATGAGGATCCCATGGGCCAGCAGGGCTCGCTGGGTGAG
 727  A  Q  F  P  N  H  S  F  K  H  E  D  P  M  G  Q  Q  G  S  L  G  E
2245 CAGCAGTACTCGGTGCCGCCCCCGGTCTATGGCTGCCACACCCCCACCGACAGCTGCACCGGCAGC
 749  Q  Q  Y  S  V  P  P  P  V  Y  G  C  H  T  P  T  D  S  C  T  G  S
2311 CAGGCTTTGCTGCTGAGGACGCCCTACAGCAGTGACAATTTATACCAAATGACATCCCAGCTTGAA
 771  Q  A  L  L  L  R  T  P  Y  S  S  D  N  L  Y  Q  M  T  S  Q  L  E
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2377 TGCATGACCTGGAATCAGATGAACTTAGGAGCCACCTTAAAGGGAGTTGCTGCTGGGAGCTCCAGC
 793  C  M  T  W  N  Q  M  N  L  G  A  T  L  K  G  V  A  A  G  S  S  S
2443 TCAGTGAAATGGACAGAAGGGCAGAGCAACCACAGCACAGGGTACGAGAGCGATAACCACACAACG
 815  S  V  K  W  T  E  G  Q  S  N  H  S  T  G  Y  E  S  D  N  H  T  T
2509 CCCATCCTCTGCGGAGCCCAATACAGAATACACACGCACGGTGTCTTCAGAGGCATTCAGGATGTG
 837  P  I  L  C  G  A  Q  Y  R  I  H  T  H  G  V  F  R  G  I  Q  D  V
2575 CGACGTGTGCCTGGAGTAGCCCCGACTCTTGTACGGTCGGCATCTGAGACCAGTGAGAAACGCCCC
 859  R  R  V  P  G  V  A  P  T  L  V  R  S  A  S  E  T  S  E  K  R  P
2641 TTCATGTGTGCTTACCCAGGCTGCAATAAGAGATATTTTAAGCTGTCCCACTTACAGATGCACAGC
 881  F  M  C  A  Y  P  G  C  N  K  R  Y  F  K  L  S  H  L  Q  M  H  S
2707 AGGAAGCACACTGGTGAGAAACCATACCAGTGTGACTTCAAGGACTGTGAACGAAGGTTTTCTCGT
 903  R  K  H  T  G  E  K  P  Y  Q  C  D  F  K  D  C  E  R  R  F  S  R
2773 TCAGACCAGCTCAAAAGACACCAAAGGAGACATACAGGTGTGAAACCATTCCAGTGTAAAACTTGT
 925  S  D  Q  L  K  R  H  Q  R  R  H  T  G  V  K  P  F  Q  C  K  T  C
2839 CAGCGAAAGTTCTCCCGGTCCGACCACCTGAAGACCCACACCAGGACTCATACAGGTAAAACAAGT
 947  Q  R  K  F  S  R  S  D  H  L  K  T  H  T  R  T  H  T  G  K  T  S
2905 GAAAAGCCCTTCAGCTGTCGGTGGCCAAGTTGTCAGAAAAAGTTTGCCCGGTCAGATGAATTAGTC
 969  E  K  P  F  S  C  R  W  P  S  C  Q  K  K  F  A  R  S  D  E  L  V
2971 CGCCATCACAACATGCATCAGAGAAACATGACCAAACTCCAGCTGGCGCTTTAA
 991  R  H  H  N  M  H  Q  R  N  M  T  K  L  Q  L  A  L  *
```

6.2.9. scFv:a-hDEC205-WT1_$D_{217\text{-}522}$

	Nucleotide	*Amino acid*
Leader	1-78	1-26
Variable heavy chain domain	85-438	29-146
Linker	439-474	147-158
Variable light chain domain	475-801	159-267
Flag tag 2x	808-861	270-287
WT1_$D_{217\text{-}522}$	869-1785	290-595

```
   1 ATGAACTTCGGCTTTCGCCTGATCTTCCTGGTGCTGGTGCTGAAGGGCGTGCAGTGCGAAGTGAAG
   1  M  N  F  G  F  R  L  I  F  L  V  L  V  L  K  G  V  Q  C  E  V  K
  67 CTGGTGCCCCGGCAATTGGAGGTGCAGCTGCAGCAGTCTGGCCCCGTGCTCGTGAAACCTGGCGCC
  23  L  V  P  R  Q  L  E  V  Q  L  Q  Q  S  G  P  V  L  V  K  P  G  A
 133 TCCGTGAAGATGAGCTGCAAGGCCAGCGGCAACACCTTCACCGACAGCTTCATGCACTGGATGAAG
  45  S  V  K  M  S  C  K  A  S  G  N  T  F  T  D  S  F  M  H  W  M  K
 199 CAGAGCCACGGCAAGAGCCTGGAATGGATCGGCATCATCAACCCCTACAACGGCGGCACCTCCTAC
  67  Q  S  H  G  K  S  L  E  W  I  G  I  I  N  P  Y  N  G  G  T  S  Y
 265 AACCAGAAGTTCAAGGGCAAGGCCACCCTGACCGTGGACAAGAGCAGCAGCACCGCCTACATGGAA
  89  N  Q  K  F  K  G  K  A  T  L  T  V  D  K  S  S  S  T  A  Y  M  E
 331 CTGAACAGCCTGACCAGCGAGGACAGCGCCGTGTACTACTGCGCCAGAAACGGCGTGCGGTACTAC
 111  L  N  S  L  T  S  E  D  S  A  V  Y  Y  C  A  R  N  G  V  R  Y  Y
 397 TTCGACTACTGGGGCCAGGGCACAACCCTGACAGTGTCTAGCGGCGGAGGAAGCGGAGGCGGATCT
 133  F  D  Y  W  G  Q  G  T  T  L  T  V  S  S  G  G  G  S  G  G  G  S
 463 GGCGGAGGATCTCAGGCTGTCGTGACCCAGGAAAGCGCCCTGACAACCAGCCCTGGCGAGACAGTG
 155  G  G  G  S  Q  A  V  V  T  Q  E  S  A  L  T  T  S  P  G  E  T  V
 529 ACCCTGACCTGCAGATCCTCTACAGGCGCCGTGACCATCAGCAACTACGCCAACTGGGTGCAGGAA
 177  T  L  T  C  R  S  S  T  G  A  V  T  I  S  N  Y  A  N  W  V  Q  E
 595 AAGCCCGACCACCTGTTCACCGGCCTGATCGGCGGCACAAACAACAGAGCACCTGGCGTGCCCGCC
 199  K  P  D  H  L  F  T  G  L  I  G  G  T  N  N  R  A  P  G  V  P  A
 661 AGATTCAGCGGCTCTCTGATCGGAGATAAGGCCGCACTGACCATCACAGGCGCCCAGACCGAGGAC
 221  R  F  S  G  S  L  I  G  D  K  A  A  L  T  I  T  G  A  Q  T  E  D
 727 GAGGCCATCTACTTTTGCGCCCTGTGGTACAACAACCAGTTCATCTTCGGCAGCGGCACCAAAGTG
 243  E  A  I  Y  F  C  A  L  W  Y  N  N  Q  F  I  F  G  S  G  T  K  V
 793 ACCGTGCTGGGATCCGACTACAAGGACGACGACGACAAAGAATTCGACTACAAGGACGACGACGAC
 265  T  V  L  G  S  D  Y  K  D  D  D  D  K  E  F  D  Y  K  D  D  D  D
 859 AAACTCGAGATTCGCAATCAGGGTTACAGCACGGTCACCTTCGACGGGACGCCCAGCTACGGTCAC
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 287  K  L  E  I  R  N  Q  G  Y  S  T  V  T  F  D  G  T  P  S  Y  G  H
 925 ACGCCCTCGCACCATGCGGCGCAGTTCCCCAACCACTCATTCAAGCATGAGGATCCCATGGGCCAG
 309  T  P  S  H  H  A  A  Q  F  P  N  H  S  F  K  H  E  D  P  M  G  Q
 991 CAGGGCTCGCTGGGTGAGCAGCAGTACTCGGTGCCGCCCCCGGTCTATGGCTGCCACACCCCCACC
 331  Q  G  S  L  G  E  Q  Q  Y  S  V  P  P  P  V  Y  G  C  H  T  P  T
1057 GACAGCTGCACCGGCAGCCAGGCTTTGCTGCTGAGGACGCCCTACAGCAGTGACAATTTATACCAA
 353  D  S  C  T  G  S  Q  A  L  L  L  R  T  P  Y  S  S  D  N  L  Y  Q
1123 ATGACATCCCAGCTTGAATGCATGACCTGGAATCAGATGAACTTAGGAGCCACCTTAAAGGGAGTT
 375  M  T  S  Q  L  E  C  M  T  W  N  Q  M  N  L  G  A  T  L  K  G  V
1189 GCTGCTGGGAGCTCCAGCTCAGTGAAATGGACAGAAGGGCAGAGCAACCACAGCACAGGGTACGAG
 397  A  A  G  S  S  S  S  V  K  W  T  E  G  Q  S  N  H  S  T  G  Y  E
1255 AGCGATAACCACACAACGCCCATCCTCTGCGGAGCCCAATACAGAATACACACGCACGGTGTCTTC
 419  S  D  N  H  T  T  P  I  L  C  G  A  Q  Y  R  I  H  T  H  G  V  F
1321 AGAGGCATTCAGGATGTGCGACGTGTGCCTGGAGTAGCCCCGACTCTTGTACGGTCGGCATCTGAG
 441  R  G  I  Q  D  V  R  R  V  P  G  V  A  P  T  L  V  R  S  A  S  E
1387 ACCAGTGAGAAACGCCCCTTCATGTGTGCTTACCCAGGCTGCAATAAGAGATATTTTAAGCTGTCC
 463  T  S  E  K  R  P  F  M  C  A  Y  P  G  C  N  K  R  Y  F  K  L  S
1453 CACTTACAGATGCACAGCAGGAAGCACACTGGTGAGAAACCATACCAGTGTGACTTCAAGGACTGT
 485  H  L  Q  M  H  S  R  K  H  T  G  E  K  P  Y  Q  C  D  F  K  D  C
1519 GAACGAAGGTTTTCTCGTTCAGACCAGCTCAAAAGACACCAAAGGAGACATACAGGTGTGAAACCA
 507  E  R  R  F  S  R  S  D  Q  L  K  R  H  Q  R  R  H  T  G  V  K  P
1585 TTCCAGTGTAAAACTTGTCAGCGAAAGTTCTCCCGGTCCGACCACCTGAAGACCCACACCAGGACT
 529  F  Q  C  K  T  C  Q  R  K  F  S  R  S  D  H  L  K  T  H  T  R  T
1651 CATACAGGTAAAACAAGTGAAAAGCCCTTCAGCTGTCGGTGGCCAAGTTGTCAGAAAAAGTTTGCC
 551  H  T  G  K  T  S  E  K  P  F  S  C  R  W  P  S  C  Q  K  K  F  A
1717 CGGTCAGATGAATTAGTCCGCCATCACAACATGCATCAGAGAAACATGACCAAACTCCAGCTGGCG
 573  R  S  D  E  L  V  R  H  H  N  M  H  Q  R  N  M  T  K  L  Q  L  A
1783 CTTTGA
 595  L  *
```

6.2.10. Anti-hDEC205-VH-heavy const. full-WT1$_{10-35}$

	Nucleotide	*Amino acid*
Leader	1-78	1-26
Flag tag	85-109	29-36
Heavy chain variable domain	116-468	139-156
Heavy chain constant domain of IgG1	472-1471	156-490
WT1_D$_{10-35}$	1492-1571	497-522

```
   1 ATGAACTTCGGCTTCAGCCTGATCTTCCTGGTGCTGGTGCTGAAGGGCGTGCAGTGCGAAGTGAAG
   1  M  N  F  G  F  S  L  I  F  L  V  L  V  L  K  G  V  Q  C  E  V  K
  67 CTGGTGCCCCGGCAATTGGACTACAAGGACGACGACGACAAAGAATTCGAGGTGCAGCTGCAGCAG
  23  L  V  P  R  Q  L  D  Y  K  D  D  D  D  K  E  F  E  V  Q  L  Q  Q
 133 TCTGGCCCCGTGCTCGTGAAACCTGGCGCCTCCGTGAAGATGAGCTGCAAGGCCAGCGGCAACACC
  45  S  G  P  V  L  V  K  P  G  A  S  V  K  M  S  C  K  A  S  G  N  T
 199 TTCACCGACAGCTTCATGCACTGGATGAAGCAGAGCCACGGCAAGAGCCTGGAATGGATCGGCATC
  67  F  T  D  S  F  M  H  W  M  K  Q  S  H  G  K  S  L  E  W  I  G  I
 265 ATCAACCCCTACAACGGCGGCACCTCCTACAACCAGAAGTTCAAGGGCAAGGCCACCCTGACCGTG
  89  I  N  P  Y  N  G  G  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T  V
 331 GACAAGAGCAGCAGCACCGCCTACATGGAACTGAACAGCCTGACCAGCGAGGACAGCGCCGTGTAC
 111  D  K  S  S  S  T  A  Y  M  E  L  N  S  L  T  S  E  D  S  A  V  Y
 397 TACTGCGCCAGAAACGGCGTGCGGTACTACTTCGACTACTGGGGCCAGGGCACAACCCTGACAGTG
 133  Y  C  A  R  N  G  V  R  Y  Y  F  D  Y  W  G  Q  G  T  T  L  T  V
 463 TCTAGCAGATCCTCTAGCGCCAGCACAAAGGGCCCCAGCGTGTTCCCTCTGGCCCCTAGCAGCAAG
 155  S  S  R  S  S  S  A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K
 529 AGCACATCTGGCGGAACAGCCGCCCTGGGCTGCCTCGTGAAGGACTACTTTCCCGAGCCCGTGACA
 177  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T
 595 GTGTCCTGGAACTCTGGCGCCCTGACAAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGCAGC
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 199  V  S  W  N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S
 661 GGCCTGTACTCTCTGAGCAGCGTCGTGACTGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATC
 221  G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I
 727 TGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGCGAC
 243  C  N  V  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D
 793 AAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAACTGCTGGGCGGACCTTCCGTGTTCCTGTTC
 265  K  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F
 859 CCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAT
 287  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D
 925 GTGTCCCACGAGGACCCTGAAGTGAAGTTTAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCC
 309  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A
 991 AAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACAGTGCTG
 331  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L
1057 CACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCC
 353  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P
1123 ATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAACCCCAGGTGTACACACTGCCTCCC
 375  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P
1189 AGCAGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTCGTGAAAGGCTTCTACCCCTCC
 397  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S
1255 GATATCGCCGTGGAATGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTG
 419  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V
1321 CTGGACAGCGACGGCTCATTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAG
 441  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q
1387 GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTG
 463  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L
1453 AGCCTGAGCCCCGGCAAGCTCGAGGCTAGTACAGCTGCGCTGCTGCCCGCCGTCCCCTCCCTGGGT
 485  S  L  S  P  G  K  L  E  A  S  T  A  A  L  L  P  A  V  P  S  L  G
1519 GGCGGCGGCGGCTGTGCCCTGCCTGTGAGCGGCGCGGCGCAGTGGGCGTAA
 507  G  G  G  G  C  A  L  P  V  S  G  A  A  Q  W  A  *
```

6.2.11. Anti-hDEC205-VH-heavy const. full-WT1$_{91-138}$

	Nucleotide	*Amino acid*
Leader	1-78	1-26
Flag tag	85-109	29-36
Heavy chain variable domain	116-468	139-156
Heavy chain constant domain of IgG1	472-1471	156-490
WT1_D$_{91-138}$	1492-1634	497-544

```
   1 ATGAACTTCGGCTTCAGCCTGATCTTCCTGGTGCTGGTGCTGAAGGGCGTGCAGTGCGAAGTGAAG
   1  M  N  F  G  F  S  L  I  F  L  V  L  V  L  K  G  V  Q  C  E  V  K
  67 CTGGTGCCCCGGCAATTGGACTACAAGGACGACGACGACAAAGAATTCGAGGTGCAGCTGCAGCAG
  23  L  V  P  R  Q  L  D  Y  K  D  D  D  D  K  E  F  E  V  Q  L  Q  Q
 133 TCTGGCCCCGTGCTCGTGAAACCTGGCGCCTCCGTGAAGATGAGCTGCAAGGCCAGCGGCAACACC
  45  S  G  P  V  L  V  K  P  G  A  S  V  K  M  S  C  K  A  S  G  N  T
 199 TTCACCGACAGCTTCATGCACTGGATGAAGCAGAGCCACGGCAAGAGCCTGGAATGGATCGGCATC
  67  F  T  D  S  F  M  H  W  M  K  Q  S  H  G  K  S  L  E  W  I  G  I
 265 ATCAACCCCTACAACGGCGGCACCTCCTACAACCAGAAGTTCAAGGGCAAGGCCACCCTGACCGTG
  89  I  N  P  Y  N  G  G  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T  V
 331 GACAAGAGCAGCAGCACCGCCTACATGGAACTGAACAGCCTGACCAGCGAGGACAGCGCCGTGTAC
 111  D  K  S  S  S  T  A  Y  M  E  L  N  S  L  T  S  E  D  S  A  V  Y
 397 TACTGCGCCAGAAACGGCGTGCGGTACTACTTCGACTACTGGGGCCAGGGCACAACCCTGACAGTG
 133  Y  C  A  R  N  G  V  R  Y  Y  F  D  Y  W  G  Q  G  T  T  L  T  V
 463 TCTAGCAGATCCTCTAGCGCCAGCACAAAGGGCCCCAGCGTGTTCCCTCTGGCCCCTAGCAGCAAG
 155  S  S  R  S  S  S  A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K
 529 AGCACATCTGGCGGAACAGCCGCCCTGGGCTGCCTCGTGAAGGACTACTTTCCCGAGCCCGTGACA
 177  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T
 595 GTGTCCTGGAACTCTGGCGCCCTGACAAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGCAGC
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 199 V  S  W  N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S
 661 GGCCTGTACTCTCTGAGCAGCGTCGTGACTGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATC
 221 G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I
 727 TGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGCGAC
 243 C  N  V  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D
 793 AAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAACTGCTGGGCGGACCTTCCGTGTTCCTGTTC
 265 K  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F
 859 CCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAT
 287 P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D
 925 GTGTCCCACGAGGACCCTGAAGTGAAGTTTAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCC
 309 V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A
 991 AAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACAGTGCTG
 331 K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L
1057 CACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCC
 353 H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P
1123 ATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAACCCCAGGTGTACACACTGCCTCCC
 375 I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P
1189 AGCAGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTCGTGAAAGGCTTCTACCCCTCC
 397 S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S
1255 GATATCGCCGTGGAATGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTG
 419 D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V
1321 CTGGACAGCGACGGCTCATTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAG
 441 L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q
1387 GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTG
 463 G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L
1453 AGCCTGAGCCCCGGCAAGCTCGAGGCTAGTACAGCTGCCTTCACTGTCCACTTTTCCGGCCAGTTC
 485 S  L  S  P  G  K  L  E  A  S  T  A  A  F  T  V  H  F  S  G  Q  F
1519 ACTGGCACAGCCGGAGCCTGTCGCTACGGGCCCTTCGGTCCTCCTCCGCCCAGCCAGGCGTCATCC
 507 T  G  T  A  G  A  C  R  Y  G  P  F  G  P  P  P  P  S  Q  A  S  S
1585 GGCCAGGCCAGGATGTTTCCTAACGCGCCCTACCTGCCCAGCTGCCTCTAA
 529 G  Q  A  R  M  F  P  N  A  P  Y  L  P  S  C  L  *
```

6.2.12. Anti-hDEC205-VH-heavy const. full-WT1$_{223-273}$

	Nucleotide	*Amino acid*
Leader	1-78	1-26
Flag tag	85-109	29-36
Heavy chain variable domain	116-468	139-156
Heavy chain constant domain of IgG1	472-1471	156-490
WT1_D$_{223-273}$	1492-1634	497-547

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   1 ATGAACTTCGGCTTCAGCCTGATCTTCCTGGTGCTGGTGCTGAAGGGCGTGCAGTGCGAAGTGAAG
   1 M  N  F  G  F  S  L  I  F  L  V  L  V  L  K  G  V  Q  C  E  V  K
  67 CTGGTGCCCCGGCAATTGGACTACAAGGACGACGACGACAAAGAATTCGAGGTGCAGCTGCAGCAG
  23 L  V  P  R  Q  L  D  Y  K  D  D  D  D  K  E  F  E  V  Q  L  Q  Q
 133 TCTGGCCCCGTGCTCGTGAAACCTGGCGCCTCCGTGAAGATGAGCTGCAAGGCCAGCGGCAACACC
  45 S  G  P  V  L  V  K  P  G  A  S  V  K  M  S  C  K  A  S  G  N  T
 199 TTCACCGACAGCTTCATGCACTGGATGAAGCAGAGCCACGGCAAGAGCCTGGAATGGATCGGCATC
  67 F  T  D  S  F  M  H  W  M  K  Q  S  H  G  K  S  L  E  W  I  G  I
 265 ATCAACCCCTACAACGGCGGCACCTCCTACAACCAGAAGTTCAAGGGCAAGGCCACCCTGACCGTG
  89 I  N  P  Y  N  G  G  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T  V
 331 GACAAGAGCAGCAGCACCGCCTACATGGAACTGAACAGCCTGACCAGCGAGGACAGCGCCGTGTAC
 111 D  K  S  S  S  T  A  Y  M  E  L  N  S  L  T  S  E  D  S  A  V  Y
 397 TACTGCGCCAGAAACGGCGTGCGGTACTACTTCGACTACTGGGGCCAGGGCACAACCCTGACAGTG
 133 Y  C  A  R  N  G  V  R  Y  Y  F  D  Y  W  G  Q  G  T  T  L  T  V
 463 TCTAGCAGATCCTCTAGCGCCAGCACAAAGGGCCCCAGCGTGTTCCCTCTGGCCCCTAGCAGCAAG
 155 S  S  R  S  S  S  A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K
 529 AGCACATCTGGCGGAACAGCCGCCCTGGGCTGCCTCGTGAAGGACTACTTTCCCGAGCCCGTGACA
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 177  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T
 595 GTGTCCTGGAACTCTGGCGCCCTGACAAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGCAGC
 199  V  S  W  N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S
 661 GGCCTGTACTCTCTGAGCAGCGTCGTGACTGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATC
 221  G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I
 727 TGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGCGAC
 243  C  N  V  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D
 793 AAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAACTGCTGGGCGGACCTTCCGTGTTCCTGTTC
 265  K  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F
 859 CCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAT
 287  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D
 925 GTGTCCCACGAGGACCCTGAAGTGAAGTTTAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCC
 309  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A
 991 AAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACAGTGCTG
 331  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L
1057 CACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCC
 353  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P
1123 ATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAACCCCAGGTGTACACACTGCCTCCC
 375  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P
1189 AGCAGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTCGTGAAAGGCTTCTACCCCTCC
 397  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S
1255 GATATCGCCGTGGAATGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTG
 419  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V
1321 CTGGACAGCGACGGCTCATTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAG
 441  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q
1387 GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTG
 463  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L
1453 AGCCTGAGCCCCGGCAAGCTCGAGGCTAGTACAGCTAGTGACAATTTATACCAAATGACATCCCAG
 485  S  L  S  P  G  K  L  E  A  S  T  A  S  D  N  L  Y  Q  M  T  S  Q
1519 CTTGAATGCATGACCTGGAATCAGATGAACTTAGGAGCCACCTTAAAGGGAGTTGCTGCTGGGAGC
 507  L  E  C  M  T  W  N  Q  M  N  L  G  A  T  L  K  G  V  A  A  G  S
1585 TCCAGCTCAGTGAAATGGACAGAAGGGCAGAGCAACCACAGCACAGGGTACGAGAGCTAA
 529  S  S  S  V  K  W  T  E  G  Q  S  N  H  S  T  G  Y  E  S  *
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6.2.13. Anti-hDEC205-VH-heavy const. full-WT1$_{324-371}$

	Nucleotide	*Amino acid*
Leader	1-78	1-26
Flag tag	85-109	29-36
Heavy chain variable domain	116-468	139-156
Heavy chain constant domain of IgG1	472-1471	156-490
WT1_D$_{324-371}$	1492-1634	497-544

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   1 ATGAACTTCGGCTTCAGCCTGATCTTCCTGGTGCTGGTGCTGAAGGGCGTGCAGTGCGAAGTGAAG
   1  M  N  F  G  F  S  L  I  F  L  V  L  V  L  K  G  V  Q  C  E  V  K
  67 CTGGTGCCCCGGCAATTGGACTACAAGGACGACGACGACAAAGAATTCGAGGTGCAGCTGCAGCAG
  23  L  V  P  R  Q  L  D  Y  K  D  D  D  D  K  E  F  E  V  Q  L  Q  Q
 133 TCTGGCCCCGTGCTCGTGAAACCTGGCGCCTCCGTGAAGATGAGCTGCAAGGCCAGCGGCAACACC
  45  S  G  P  V  L  V  K  P  G  A  S  V  K  M  S  C  K  A  S  G  N  T
 199 TTCACCGACAGCTTCATGCACTGGATGAAGCAGAGCCACGGCAAGAGCCTGGAATGGATCGGCATC
  67  F  T  D  S  F  M  H  W  M  K  Q  S  H  G  K  S  L  E  W  I  G  I
 265 ATCAACCCCTACAACGGCGGCACCTCCTACAACCAGAAGTTCAAGGGCAAGGCCACCCTGACCGTG
  89  I  N  P  Y  N  G  G  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T  V
 331 GACAAGAGCAGCAGCACCGCCTACATGGAACTGAACAGCCTGACCAGCGAGGACAGCGCCGTGTAC
 111  D  K  S  S  S  T  A  Y  M  E  L  N  S  L  T  S  E  D  S  A  V  Y
 397 TACTGCGCCAGAAACGGCGTGCGGTACTACTTCGACTACTGGGGCCAGGGCACAACCCTGACAGTG
 133  Y  C  A  R  N  G  V  R  Y  Y  F  D  Y  W  G  Q  G  T  T  L  T  V
 463 TCTAGCAGATCCTCTAGCGCCAGCACAAAGGGCCCCAGCGTGTTCCCTCTGGCCCCTAGCAGCAAG
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 155  S  S  R  S  S  S  A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K
 529 AGCACATCTGGCGGAACAGCCGCCCTGGGCTGCCTCGTGAAGGACTACTTTCCCGAGCCCGTGACA
 177  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T
 595 GTGTCCTGGAACTCTGGCGCCCTGACAAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGCAGC
 199  V  S  W  N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S
 661 GGCCTGTACTCTCTGAGCAGCGTCGTGACTGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATC
 221  G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I
 727 TGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGCGAC
 243  C  N  V  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D
 793 AAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAACTGCTGGGCGGACCTTCCGTGTTCCTGTTC
 265  K  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F
 859 CCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAT
 287  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D
 925 GTGTCCCACGAGGACCCTGAAGTGAAGTTTAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCC
 309  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A
 991 AAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACAGTGCTG
 331  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L
1057 CACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCC
 353  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P
1123 ATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAACCCCAGGTGTACACACTGCCTCCC
 375  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P
1189 AGCAGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTCGTGAAAGGCTTCTACCCCTCC
 397  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S
1255 GATATCGCCGTGGAATGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTG
 419  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V
1321 CTGGACAGCGACGGCTCATTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAG
 441  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q
1387 GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTG
 463  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L
1453 AGCCTGAGCCCCGGCAAGCTCGAGGCTAGTACAGCTATGTGTGCTTACCCAGGCTGCAATAAGAGA
 485  S  L  S  P  G  K  L  E  A  S  T  A  M  C  A  Y  P  G  C  N  K  R
1519 TATTTTAAGCTGTCCCACTTACAGATGCACAGCAGGAAGCACACTGGTGAGAAACCATACCAGTGT
 507  Y  F  K  L  S  H  L  Q  M  H  S  R  K  H  T  G  E  K  P  Y  Q  C
1585 GACTTCAAGGACTGTGAACGAAGGTTTTCTCGTTCAGACCAGCTCAAATAA
 529  D  F  K  D  C  E  R  R  F  S  R  S  D  Q  L  K  *
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6.2.14. Anti-hDEC205-VH-heavy const. full-WT1$_{144-273}$

	Nucleotide	*Amino acid*
Leader	1-78	1-26
Flag tag	85-109	29-36
Heavy chain variable domain	116-468	139-156
Heavy chain constant domain of IgG1	472-1471	156-490
WT1_D$_{144-273}$	1479-1868	493-622

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   1 ATGAACTTCGGCTTCAGCCTGATCTTCCTGGTGCTGGTGCTGAAGGGCGTGCAGTGCGAAGTGAAG
   1  M  N  F  G  F  S  L  I  F  L  V  L  V  L  K  G  V  Q  C  E  V  K
  67 CTGGTGCCCCGGCAATTGGACTACAAGGACGACGACGACAAAGAATTCGAGGTGCAGCTGCAGCAG
  23  L  V  P  R  Q  L  D  Y  K  D  D  D  D  K  E  F  E  V  Q  L  Q  Q
 133 TCTGGCCCCGTGCTCGTGAAACCTGGCGCCTCCGTGAAGATGAGCTGCAAGGCCAGCGGCAACACC
  45  S  G  P  V  L  V  K  P  G  A  S  V  K  M  S  C  K  A  S  G  N  T
 199 TTCACCGACAGCTTCATGCACTGGATGAAGCAGAGCCACGGCAAGAGCCTGGAATGGATCGGCATC
  67  F  T  D  S  F  M  H  W  M  K  Q  S  H  G  K  S  L  E  W  I  G  I
 265 ATCAACCCCTACAACGGCGGCACCTCCTACAACCAGAAGTTCAAGGGCAAGGCCACCCTGACCGTG
  89  I  N  P  Y  N  G  G  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T  V
 331 GACAAGAGCAGCAGCACCGCCTACATGGAACTGAACAGCCTGACCAGCGAGGACAGCGCCGTGTAC
 111  D  K  S  S  S  T  A  Y  M  E  L  N  S  L  T  S  E  D  S  A  V  Y
 397 TACTGCGCCAGAAACGGCGTGCGGTACTACTTCGACTACTGGGGCCAGGGCACAACCCTGACAGTG
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 133  Y  C  A  R  N  G  V  R  Y  Y  F  D  Y  W  G  Q  G  T  T  L  T  V
 463 TCTAGCAGATCCTCTAGCGCCAGCACAAAGGGCCCCAGCGTGTTCCCTCTGGCCCCTAGCAGCAAG
 155  S  S  R  S  S  S  A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K
 529 AGCACATCTGGCGGAACAGCCGCCCTGGGCTGCCTCGTGAAGGACTACTTTCCCGAGCCCGTGACA
 177  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T
 595 GTGTCCTGGAACTCTGGCGCCCTGACAAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGCAGC
 199  V  S  W  N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S
 661 GGCCTGTACTCTCTGAGCAGCGTCGTGACTGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATC
 221  G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I
 727 TGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGCGAC
 243  C  N  V  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D
 793 AAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAACTGCTGGGCGGACCTTCCGTGTTCCTGTTC
 265  K  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F
 859 CCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAT
 287  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D
 925 GTGTCCCACGAGGACCCTGAAGTGAAGTTTAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCC
 309  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A
 991 AAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACAGTGCTG
 331  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L
1057 CACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCC
 353  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P
1123 ATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAACCCCAGGTGTACACACTGCCTCCC
 375  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P
1189 AGCAGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTCGTGAAAGGCTTCTACCCCTCC
 397  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S
1255 GATATCGCCGTGGAATGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTG
 419  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V
1321 CTGGACAGCGACGGCTCATTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAG
 441  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q
1387 GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTG
 463  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L
1453 AGCCTGAGCCCCGGCAAGCTCGAGATTCGCAATCAGGGTTACAGCACGGTCACCTTCGACGGGACG
 485  S  L  S  P  G  K  L  E  I  R  N  Q  G  Y  S  T  V  T  F  D  G  T
1519 CCCAGCTACGGTCACACGCCCTCGCACCATGCGGCGCAGTTCCCCAACCACTCATTCAAGCATGAG
 507  P  S  Y  G  H  T  P  S  H  H  A  A  Q  F  P  N  H  S  F  K  H  E
1585 GATCCCATGGGCCAGCAGGGCTCGCTGGGTGAGCAGCAGTACTCGGTGCCGCCCCCGGTCTATGGC
 529  D  P  M  G  Q  Q  G  S  L  G  E  Q  Q  Y  S  V  P  P  P  V  Y  G
1651 TGCCACACCCCCACCGACAGCTGCACCGGCAGCCAGGCTTTGCTGCTGAGGACGCCCTACAGCAGT
 551  C  H  T  P  T  D  S  C  T  G  S  Q  A  L  L  L  R  T  P  Y  S  S
1717 GACAATTTATACCAAATGACATCCCAGCTTGAATGCATGACCTGGAATCAGATGAACTTAGGAGCC
 573  D  N  L  Y  Q  M  T  S  Q  L  E  C  M  T  W  N  Q  M  N  L  G  A
1783 ACCTTAAAGGGAGTTGCTGCTGGGAGCTCCAGCTCAGTGAAATGGACAGAAGGGCAGAGCAACCAC
 595  T  L  K  G  V  A  A  G  S  S  S  S  V  K  W  T  E  G  Q  S  N  H
1849 AGCACAGGGTACGAGAGCTAA
 617  S  T  G  Y  E  S  *
```

6.2.15. Anti-hDEC205-VH-heavy const. full-WT1$_{223\text{-}371}$

	Nucleotide	*Amino acid*
Leader	1-78	1-26
Flag tag	85-109	29-36
Heavy chain variable domain	116-468	139-156
Heavy chain constant domain of IgG1	472-1471	156-490
WT1_D$_{223\text{-}371}$	1492-1935	497-645

```
   1 ATGAACTTCGGCTTCAGCCTGATCTTCCTGGTGCTGGTGCTGAAGGGCGTGCAGTGCGAAGTGAAG
   1  M  N  F  G  F  S  L  I  F  L  V  L  V  L  K  G  V  Q  C  E  V  K
  67 CTGGTGCCCCGGCAATTGGACTACAAGGACGACGACGACAAAGAATTCGAGGTGCAGCTGCAGCAG
```

```
  23 L  V  P  R  Q  L  D  Y  K  D  D  D  D  K  E  F  E  V  Q  L  Q  Q
 133 TCTGGCCCCGTGCTCGTGAAACCTGGCGCCTCCGTGAAGATGAGCTGCAAGGCCAGCGGCAACACC
  45 S  G  P  V  L  V  K  P  G  A  S  V  K  M  S  C  K  A  S  G  N  T
 199 TTCACCGACAGCTTCATGCACTGGATGAAGCAGAGCCACGGCAAGAGCCTGGAATGGATCGGCATC
  67 F  T  D  S  F  M  H  W  M  K  Q  S  H  G  K  S  L  E  W  I  G  I
 265 ATCAACCCCTACAACGGCGGCACCTCCTACAACCAGAAGTTCAAGGGCAAGGCCACCCTGACCGTG
  89 I  N  P  Y  N  G  G  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T  V
 331 GACAAGAGCAGCAGCACCGCCTACATGGAACTGAACAGCCTGACCAGCGAGGACAGCGCCGTGTAC
 111 D  K  S  S  S  T  A  Y  M  E  L  N  S  L  T  S  E  D  S  A  V  Y
 397 TACTGCGCCAGAAACGGCGTGCGGTACTACTTCGACTACTGGGGCCAGGGCACAACCCTGACAGTG
 133 Y  C  A  R  N  G  V  R  Y  Y  F  D  Y  W  G  Q  G  T  T  L  T  V
 463 TCTAGCAGATCCTCTAGCGCCAGCACAAAGGGCCCCAGCGTGTTCCCTCTGGCCCCTAGCAGCAAG
 155 S  S  R  S  S  S  A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K
 529 AGCACATCTGGCGGAACAGCCGCCCTGGGCTGCCTCGTGAAGGACTACTTTCCCGAGCCCGTGACA
 177 S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T
 595 GTGTCCTGGAACTCTGGCGCCCTGACAAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGCAGC
 199 V  S  W  N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S
 661 GGCCTGTACTCTCTGAGCAGCGTCGTGACTGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATC
 221 G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I
 727 TGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGCGAC
 243 C  N  V  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D
 793 AAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAACTGCTGGGCGGACCTTCCGTGTTCCTGTTC
 265 K  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F
 859 CCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAT
 287 P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D
 925 GTGTCCCACGAGGACCCTGAAGTGAAGTTTAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCC
 309 V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A
 991 AAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACAGTGCTG
 331 K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L
1057 CACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCC
 353 H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P
1123 ATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAACCCCAGGTGTACACACTGCCTCCC
 375 I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P
1189 AGCAGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTCGTGAAAGGCTTCTACCCCTCC
 397 S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S
1255 GATATCGCCGTGGAATGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTG
 419 D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V
1321 CTGGACAGCGACGGCTCATTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAG
 441 L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q
1387 GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTG
 463 G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L
1453 AGCCTGAGCCCCGGCAAGCTCGAGGCTAGTACAGCTAGTGACAATTTATACCAAATGACATCCCAG
 485 S  L  S  P  G  K  L  E  A  S  T  A  S  D  N  L  Y  Q  M  T  S  Q
1519 CTTGAATGCATGACCTGGAATCAGATGAACTTAGGAGCCACCTTAAAGGGAGTTGCTGCTGGGAGC
 507 L  E  C  M  T  W  N  Q  M  N  L  G  A  T  L  K  G  V  A  A  G  S
1585 TCCAGCTCAGTGAAATGGACAGAAGGGCAGAGCAACCACAGCACAGGGTACGAGAGCGATAACCAC
 529 S  S  S  V  K  W  T  E  G  Q  S  N  H  S  T  G  Y  E  S  D  N  H
1651 ACAACGCCCATCCTCTGCGGAGCCCAATACAGAATACACACGCACGGTGTCTTCAGAGGCATTCAG
 551 T  T  P  I  L  C  G  A  Q  Y  R  I  H  T  H  G  V  F  R  G  I  Q
1717 GATGTGCGACGTGTGCCTGGAGTAGCCCCGACTCTTGTACGGTCGGCATCTGAGACCAGTGAGAAA
 573 D  V  R  R  V  P  G  V  A  P  T  L  V  R  S  A  S  E  T  S  E  K
1783 CGCCCCTTCATGTGTGCTTACCCAGGCTGCAATAAGAGATATTTTAAGCTGTCCCACTTACAGATG
 595 R  P  F  M  C  A  Y  P  G  C  N  K  R  Y  F  K  L  S  H  L  Q  M
1849 CACAGCAGGAAGCACACTGGTGAGAAACCATACCAGTGTGACTTCAAGGACTGTGAACGAAGGTTT
 617 H  S  R  K  H  T  G  E  K  P  Y  Q  C  D  F  K  D  C  E  R  R  F
1915 TCTCGTTCAGACCAGCTCAAATAA
 639 S  R  S  D  Q  L  K  *
```

6.2.16. Anti-hDEC205-VH-heavy const. full-WT1$_{10\text{-}53}$

	Nucleotide	*Amino acid*
Leader	1-78	1-26
Flag tag	85-109	29-36
Heavy chain variable domain	116-468	139-156
Heavy chain constant domain of IgG1	472-1471	156-490
WT1_D$_{10\text{-}53}$	1492-1621	497-540

```
   1 ATGAACTTCGGCTTCAGCCTGATCTTCCTGGTGCTGGTGCTGAAGGGCGTGCAGTGCGAAGTGAAG
   1  M  N  F  G  F  S  L  I  F  L  V  L  V  L  K  G  V  Q  C  E  V  K
  67 CTGGTGCCCCGGCAATTGGACTACAAGGACGACGACGACAAAGAATTCGAGGTGCAGCTGCAGCAG
  23  L  V  P  R  Q  L  D  Y  K  D  D  D  D  K  E  F  E  V  Q  L  Q  Q
 133 TCTGGCCCCGTGCTCGTGAAACCTGGCGCCTCCGTGAAGATGAGCTGCAAGGCCAGCGGCAACACC
  45  S  G  P  V  L  V  K  P  G  A  S  V  K  M  S  C  K  A  S  G  N  T
 199 TTCACCGACAGCTTCATGCACTGGATGAAGCAGAGCCACGGCAAGAGCCTGGAATGGATCGGCATC
  67  F  T  D  S  F  M  H  W  M  K  Q  S  H  G  K  S  L  E  W  I  G  I
 265 ATCAACCCCTACAACGGCGGCACCTCCTACAACCAGAAGTTCAAGGGCAAGGCCACCCTGACCGTG
  89  I  N  P  Y  N  G  G  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T  V
 331 GACAAGAGCAGCAGCACCGCCTACATGGAACTGAACAGCCTGACCAGCGAGGACAGCGCCGTGTAC
 111  D  K  S  S  S  T  A  Y  M  E  L  N  S  L  T  S  E  D  S  A  V  Y
 397 TACTGCGCCAGAAACGGCGTGCGGTACTACTTCGACTACTGGGGCCAGGGCACAACCCTGACAGTG
 133  Y  C  A  R  N  G  V  R  Y  Y  F  D  Y  W  G  Q  G  T  T  L  T  V
 463 TCTAGCAGATCCTCTAGCGCCAGCACAAAGGGCCCCAGCGTGTTCCCTCTGGCCCCTAGCAGCAAG
 155  S  S  R  S  S  S  A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K
 529 AGCACATCTGGCGGAACAGCCGCCCTGGGCTGCCTCGTGAAGGACTACTTTCCCGAGCCCGTGACA
 177  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T
 595 GTGTCCTGGAACTCTGGCGCCCTGACAAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGCAGC
 199  V  S  W  N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S
 661 GGCCTGTACTCTCTGAGCAGCGTCGTGACTGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATC
 221  G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I
 727 TGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGAGCTGCGAC
 243  C  N  V  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D
 793 AAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAACTGCTGGGCGGACCTTCCGTGTTCCTGTTC
 265  K  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F
 859 CCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAT
 287  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D
 925 GTGTCCCACGAGGACCCTGAAGTGAAGTTTAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCC
 309  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A
 991 AAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACAGTGCTG
 331  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L
1057 CACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCC
 353  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P
1123 ATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAACCCCAGGTGTACACACTGCCTCCC
 375  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P
1189 AGCAGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTCGTGAAAGGCTTCTACCCCTCC
 397  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S
1255 GATATCGCCGTGGAATGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTG
 419  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V
1321 CTGGACAGCGACGGCTCATTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAG
 441  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q
1387 GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTG
 463  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L
1453 AGCCTGAGCCCCGGCAAGCTCGAGGCTAGTACAGCTGCGCTGCTGCCCGCCGTCCCCTCCCTGGGT
 485  S  L  S  P  G  K  L  E  A  S  T  A  A  L  L  P  A  V  P  S  L  G
1519 GGCGGCGGCGGCTGTGCCCTGCCTGTGAGCGGCGCGGCGCAGTGGGCGCCGGTGCTGGACTTTGCG
 507  G  G  G  G  C  A  L  P  V  S  G  A  A  Q  W  A  P  V  L  D  F  A
1585 CCTCCGGGCGCTTCGGCTTACGGGTCGTTGGGCGGCTAA
 529  P  P  G  A  S  A  Y  G  S  L  G  G  *
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6.3. Publications

10 February 2016, (in revision, submitted to the Journal of Cancer Immunology, Immunotherapy) **Nergui Dagvadorj[1]**, Anne Deuretzbacher[1], Daniela Weisenberger[2], Elke Baumeister[1], Johannes Trebing[2], Isabell Lang[2], Carolin Köchel[1], Kerstin Kapp[1], Markus Kapp[1], Andreas Beilhack[3], Thomas Hünig[4], Hermann Einsele[1], Harald Wajant[2], and Götz-Ulrich Grigoleit[1] “An alternative approach to WT1-based vaccination: targeting of $WT1_{91-138}$ to dendritic cells improves leukemia-specific T cell responses”

Posters and oral presentations

29-30 October 2015, “Dendritic cell-targeted WT1 protein residues induce cytotoxic T cell response against WT1-expressing leukemia” poster, Translational Immunology: from target to therapy Symposium, Else-Kröner Forschungskolleg Würzburg, Würzburg, Germany

05-07 June 2015 “Generation of anti-humanDEC205-WT1 antibody fusion proteins to improve DC-dependent T-cell response against WT1-positive leukemia” poster, Session G, The 4th European MDPhD conference 2015, Groningen, Netherlands

14-15 October 2014 “Targeting of antigenic epitopes of Wilms tumor protein 1 and CD70 to dendritic cells using DEC205-specific antibody-fusion proteins” Poster session, Eureka, the 9th International PhD students symposium 2014, GSLS, Würzburg, Germany

23-25 June 2014 “Modulation of cellular immunity against haematological malignancies using DEC205-targeted tumor associated antigens” Oral presentation, SessionVI: Cancer Immunotherapy, The 9th annual meeting-immunology training network Erlangen, Tuebingen and Würzburg, Kloster Schöntal, Germany

6.4. Curriculum vitae