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

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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-WT110-35, anti-hDEC205-WT191-138, anti-hDEC205-WT1223-273, and anti-hDEC205-WT1324-371 were obtained in good yields.

Since three of these fusion proteins contain most of the known immunogenic epitopes in their sequences, the anti-hDEC205-WT191-138, anti-hDEC205-WT1223-273, and anti-hDEC205-WT1324-371 were tested for their T-cell stimulatory capacities. Mature monocyte-derived DCs loaded with anti-hDEC205-WT191-138 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.

Introduction

Currently, patients with high risk acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) are treated with allogeneic hematopoietic stem cell transplantation (allo-HSCT) to benefit from a graft-versus-leukemia (GvL) effect and potential cure. However, a relapse rate of 20-70% after allo-HSCT [1] and an even poorer prognosis of patients who are not eligible for allo-HSCT urge alternative therapeutic approaches, e.g. vaccination to induce anti-leukemia immune responses.

For vaccination strategies in leukemia, Wilms tumor protein 1 (WT1), a tumor associated antigen (TAA) is prioritized as a vaccine candidate due to its therapeutic impact, tissue restricted expression and leukemogenic characteristics [2]. WT1-derived peptides are most extensively tested as peptide vaccines. However, a narrow clinical application due to the human leukocyte antigen (HLA) restriction and need for prior identification of immunogenic epitopes [3] are major drawbacks. Therefore, several strategies including transfection of dendritic cells (DCs) with WT1 DNA [4] or full mRNA [5] as well as treatment with long WT1 peptides [6] and polyvalent peptide [7,8] vaccines have been tested to overcome the drawbacks. As a transcription factor, WT1 protein shuttles between the cytoplasm and nucleus [9]. Because of its binding to nucleotide sequences [10] and its interaction with posttranscriptional regulators [11], the recombinant expression of WT1 as a full-length protein is assumed to be difficult [12]. So far, full-length human WT1 or its fragment has not yet been expressed for protein vaccine approaches.

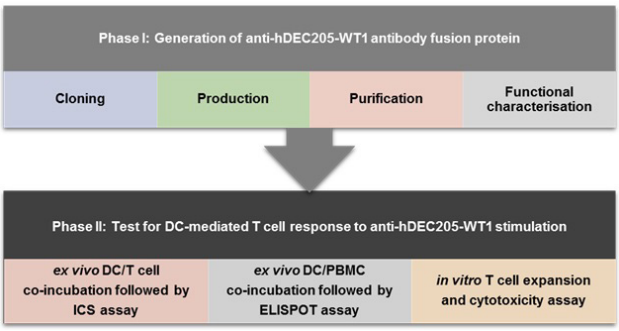
WT1-specific CD8+ T cells have a low frequency in healthy subjects and occur with elevated frequency in patients after allo-HSCT [13]. In healthy donors, the frequency of WT1-specific T cells ranges from 10-7 to 10-5 in the CD8+ T cell subsets and from 10-6 to 10-5 in the CD4+ T cell subsets [14]. In order to analyze such low frequencies of T cells without enrichment or prior expansion, an improvement of their responsiveness is necessary.

Since DCs initiate specific T cell immunity and harmonize innate and adaptive immune responses [15,16], it is worth testing the targeting of the WT1 protein to DCs either in situ or ex vivo. A potential experimental approach is the fusion of WT1 to an antibody specific to the endocytic receptor DEC205 on DCs. After binding to DEC205, the antibody fusion protein is taken up and processed intracellularly, resulting in the presentation of WT1-derived peptides on MHC molecules on the surface of DCs. Indeed, DC targeting of several other TAAs, including MAGE [17], NY-ESO1 [18], HER2/neu [19] via DEC205-specific antibodies improved immune responses to tumors expressing the aforementioned antigens in in vitro and in vivo models.

In this study, we generated 22 different anti-hDEC205-WT1 antibody fusion proteins by genetic fusion of the anti-hDEC205 antibody with full-length WT1 isoform D (WT1_Dfull) and various WT1 fragments. Although anti-hDEC205-WT1_Dfull could not be efficiently expressed, four different anti-hDEC205-WT1small fusion proteins covering the most potent antigenic epitopes of WT1 were expressed in good yields. They were further tested for their immunogenicity ex vivo and in vitro using blood samples of healthy and allo-HSC transplanted individuals.

Study design

This work was designed as a two phase sequential study as depicted below. 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.



Results

Generation of anti-hDEC205-WT1 antibody fusion proteins

As a basis of the anti-hDEC205-WT1 antibody fusion proteins, expression constructs encoding scFv:hDEC205-GpL and the heavy and light chain of the anti-hDEC205 antibody were cloned (Fig. 1a i-ii). WT1_D was previously reported as the dominant WT1 isoform in AML cases [23]. Consisting of 522 amino acids, it represents the longest isoform described so far, therefore WT1-derived peptide variations to be presented to T cells should be maximal. For these reasons, we intended to fuse the WT1_D to the anti-hDEC205 antibody. Due to the difficulty in amplifying a cDNA for full-length WT1_D from the indicated leukemia cell lines, we generated five different WT1_D amplicons covering the whole protein sequence. Then, with an exclusion of four amino acids, a full-length WT1_D was cloned by combining WT1_D1-227 with WT1_D217-522 using an internal Xho1 restriction site (Table 1). Each of the fragments as well as the full-length WT1_D were genetically fused to the C-terminus of the heavy chain of the anti-hDEC205 antibody (Fig. 1a iii). Western blot analysis of culture supernatants from cells transiently transfected with expression plasmids encoding the parental anti-hDEC205 and aforementioned anti-hDEC205-WT1_D fusion variants revealed good production of anti-hDEC205 but no 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 (Fig. 1b). Subsequently, we cloned N- and C-terminal as well as middle parts of WT1_D to the C-terminus of the light chain of anti-hDEC205 or to scFv:hDEC205 (Table 1) to test for better secretion. As shown in Figure 1c, these fusion proteins were again not secreted. Collectively, these results suggest that large fragments of WT1 interfere with efficient secretion. We also evaluated purification of the intracellularly trapped fusion protein anti-hDEC205-WT1_D1-227 by triton extraction and affinity chromatography on anti-Flag agarose. However, the obtained yields were not sufficient due to a poor solubility of the antibody fusion protein which led to precipitation during the extraction and purification.

Finally, we generated and screened a panel of anti-hDEC205 antibody fusion proteins containing different small fragments (26-51 aa) of WT1 for efficient secretion. This way, we identified four different anti-hDEC205-WT1small antibody fusion proteins yielding 2-4,4 µg/mL in supernatants of transiently transfected HEK293 cells. Three of them: anti-hDEC205-WT191-138, anti-hDEC205-WT1223-273 and anti-hDEC205-WT1324-371 were selected for their anti-leukemia vaccine potential, because several known antigenic epitopes are contained in these sequences of WT1. After purification, these anti-hDEC205-WT1small fusion proteins reached concentrations of 40-400 µg/mL with high purity and good integrity (Fig. 1d). Contained endotoxin levels in the anti-hDEC205-WT1small antibody fusion proteins were all below the threshold accepted for preclinical use of recombinant vaccines [24].

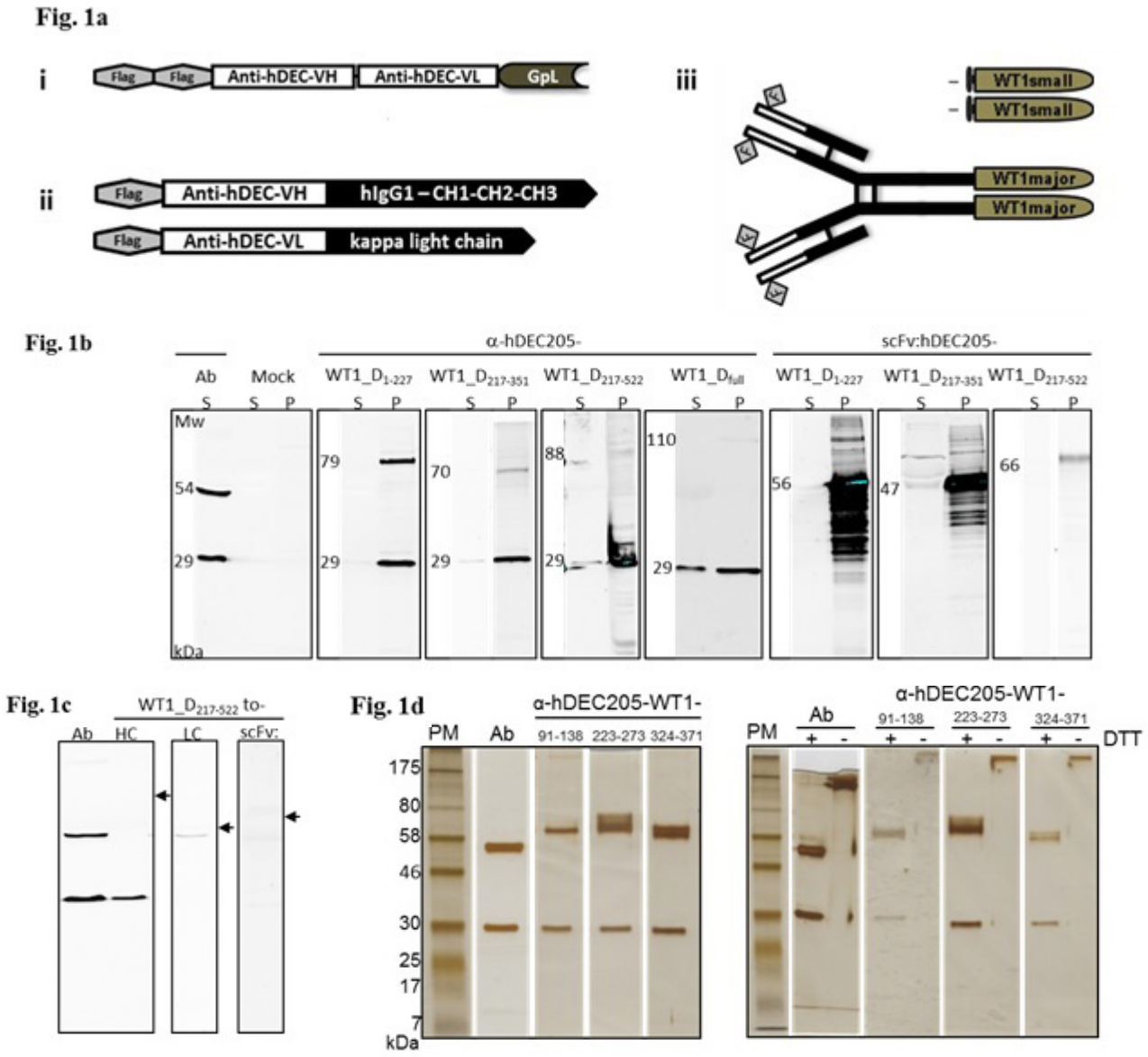


Fig. 1. Structure and production of anti-hDEC205-WT1 antibody fusion proteins.
a Scheme of the anti-hDEC205 antibody fusion proteins.

(i) The scFv:hDEC205-GpL single chain fragment variable 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. (ii) Primary structure of anti-hDEC205 antibody. The heavy and light chains of antibody were constructed by inserting respective variable heavy and light chains into the N-terminus of the corresponding constant domains of human IgG1 isotype's heavy chain (hIgG1-CH1-CH2-CH3) and kappa light chain. Each chain of the antibody was Flag-tagged N-terminally. (iii) Tertiary structure of anti-hDEC205-WT1 antibody fusion proteins. One of the major fragments of WT1D (WT1major) was cloned to the C-terminus of the anti-hDEC205 heavy full chain. The anti-hDEC205-WT1small fusion proteins were constructed by C-terminal linking of the respective WT1small fragments to the anti-hDEC205 heavy chain through a flexible spacer of four amino acids. b 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. c Western blot analysis of three different anti-hDEC205-WT1_D217-522 fusion constructs. The WT1_D217-522 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. d Purity and integrity of anti-hDEC205-WT1small fusion proteins by SDS-PAGE and silver staining. PM, protein marker, molecular weights in kDa, + reducing, - non-reducing condition, DTT dithiothreitol.

Binding capacity of anti-hDEC205-WT1small antibody fusion proteins

Initially, we evaluated hDEC205 binding of anti-hDEC205, anti-hDEC205-WT191-138, anti-hDEC205-WT1223-273, and anti-hDEC205-WT1324-371 by flow cytometry analysis of parental and hDEC205 expressing CHO cells (Fig. 2a). To quantify binding of anti-hDEC205 to hDEC205, we performed equilibrium binding studies with scFv:hDEC205-GpL, a highly traceable luciferase fusion protein of a single chain variable fragment variant of the hDEC205 antibody. There was nearly no non-specific binding to parental CHO cells (Fig. 2b). The mean Kd of scFv:□-hDEC205-GpL was found to be 1445 pM (average of 7 experiments). Furthermore, by heterologous competition assay analyses with 660 pM scFv:hDEC205-GpL, we obtained an IC50-value of the conventional anti-hDEC205 antibody of 1175 pM on average. This allowed the Ki calculation of the anti-hDEC205 antibody which resulted

in a value of 810±310 pM. The obtained Ki value of the antibody corresponds to an affinity of 8.1 x 10-10 M. In equilibrium binding studies, the specific binding of scFv:hDEC205-GpL was comparably blocked by each of the anti-hDEC205-WT1small antibody fusion proteins and the parental anti-hDEC205 IgG1 leading to the conclusion that these fusion molecules bind to hDEC205 with a similar efficacy (Fig. 2c). Since binding of the anti-hDEC205 antibody variants was not significantly altered by C-terminal extension of the heavy chain with the various WT1small fragments, we proved binding to endogenously expressed DEC205 only for the parental antibody using human immature moDCs (Fig. 2d).

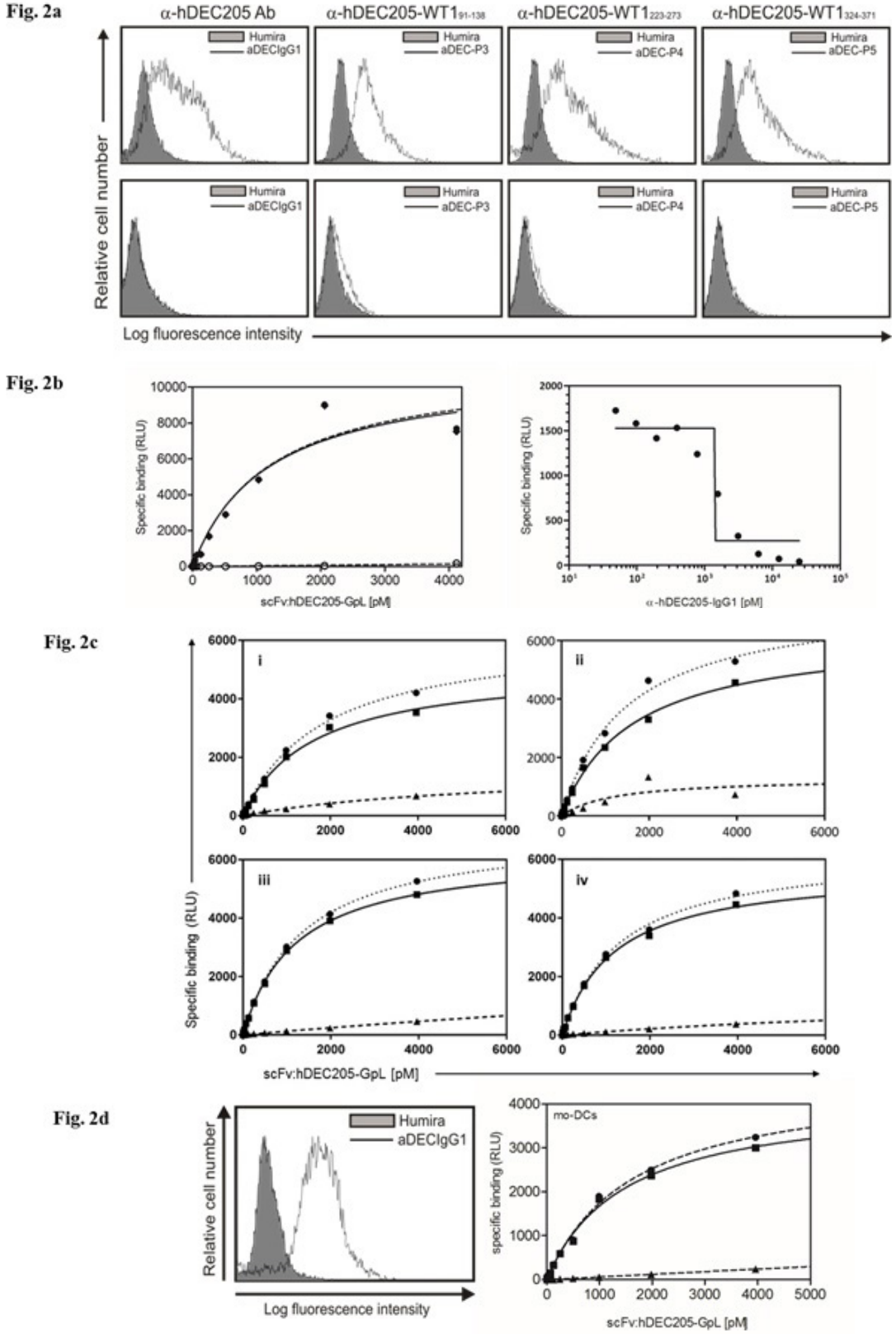


Fig. 2. Functional evaluation of anti-hDEC205 and anti-hDEC205-WT1small antibody fusion proteins.

a 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-□ antibody served as a negative control. b Binding studies for functional verification of anti-hDEC205 antibody. Left:

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Equilibrium binding study of the scFv:hDEC205-GpL. Filled circles total binding values, empty circles nonspecific binding values, and filled rombs specific binding values. Right: Heterologous competition binding assay. RLU, relative light unit; pM, picoMolar. The affinity constant (KD) of the scFv:hDEC205-GpL interaction with hDEC205 and IC50 of the anti-hDEC205 antibody were calculated by non-linear regression using the GraphPad Prism 5.0 software. c Equilibrium binding study confirming the binding capability of the anti-hDEC205-WT1small 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-WT1small fusion proteins: anti-hDEC205 antibody (i), anti-hDEC205-WT191-138 (ii), anti-hDEC205-WT1223-273 (iii), anti-hDEC205-WT1324-371 (iv). d Specific binding of anti-hDEC205 antibody to DEC205 on immature human monocyte-derived DCs. Left: FACS analysis, Right: equilibrium binding study.

WT1-specific T-cells are better detected via anti-hDEC205-targeted delivery of WT1 than non-targeted delivery of WT1 peptide pool

To investigate T cell response mediated by anti-hDEC205-WT1 protein loaded mature moDCs, we measured the frequency of IFN-γ+ T cells by flow cytometry. Of the three antibody fusion proteins tested, only the anti-hDEC205-WT191-138 showed a significant T cell stimulatory effect ex vivo (Fig. 3a). The anti-hDEC205-WT191-138 loaded mature moDCs were able to activate T cells while treatment of mature moDCs with the parental a-hDEC205 antibody or with a WT1 15-mer peptide pool showed no effect (Fig. 3b). Of note the IFN-γ producing cells induced by anti-hDEC205-WT191-138 loaded mature moDCs were mostly CD8+ T cells. It was interesting that the frequency of T cells activated by the targeted self-antigen WT191-138 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.

Immunogenicity of the anti-hDEC205-WT191-138 was tested with CD3+ T cells derived from 10 patients (Table 3). 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-WT191-138 loaded moDCs were 0.44%, 0.45% and 0.05% (Fig. 3c). 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 WT191-138-derived peptides can be presented by several HLA alleles. In contrast, only one patient showed an IFN-□+ T cell response to mature moDCs loaded with 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 donors to evaluate whether DEC205 targeted WT191-138 is able to stimulate WT1-specific T cells in healthy individuals (Fig. 3d). A T cell response was observed in five of six healthy donors with lower intensities than that of the patient derived cells. Two of the five healthy donors were HLA A02 negative. This suggested again that resultant peptides from the WT191-138 fragment are presented on different HLA alleles. Nevertheless, without prior expansion or enrichment, the median frequency of CD3+IFN-γ+ and CD8+IFN-γ+ cells obtained after co-incubation with anti-hDEC205-WT191-138 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-γ+ 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.

Since the use of PBMCs is less laborious and time-saving, compared to purified CD3+ T cells, we tested if PBMCs could be activated by this approach. For this, anti-hDEC205-WT191-138 loaded autologous mature moDCs and PBMCs from two patients were co-incubated to measure IFN-□ by ELISPOT assay. The average number of IFN-γ spots induced by anti-hDEC205-WT191-138, was more than 4-fold higher (239.3) than that of the negative controls (unstimulated 52.3; parental antibody 49.3; Fig. 3e). Thus, both T cells and PBMCs can be activated by mature moDCs loaded with anti-hDEC205-WT191-138.

Fig. 3a

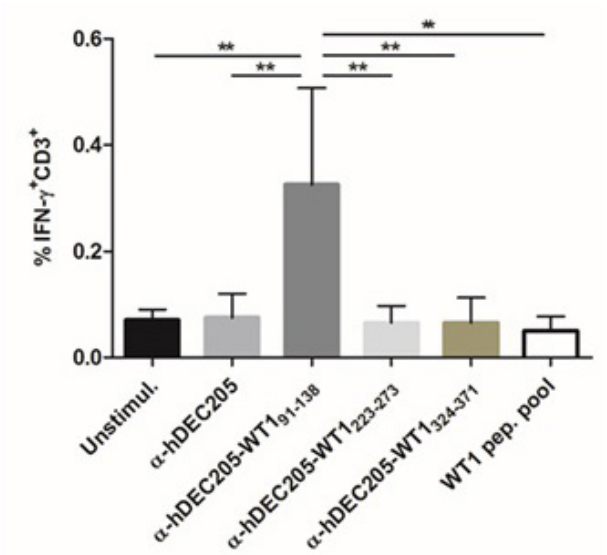


Fig. 3b

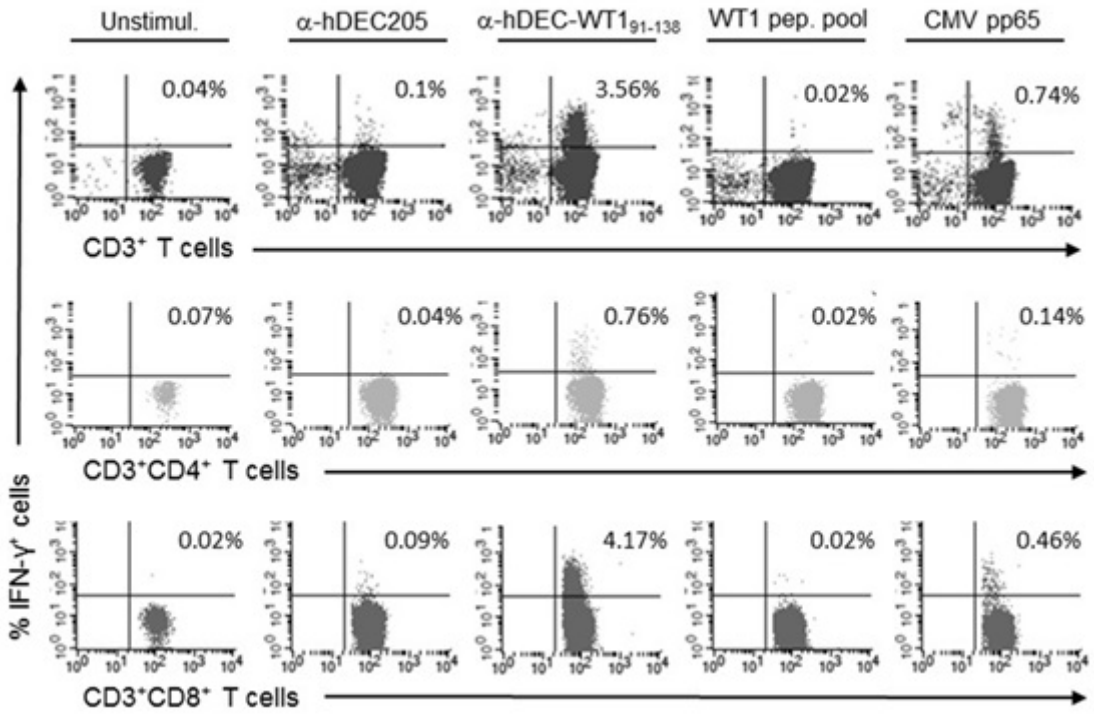


Fig. 3c

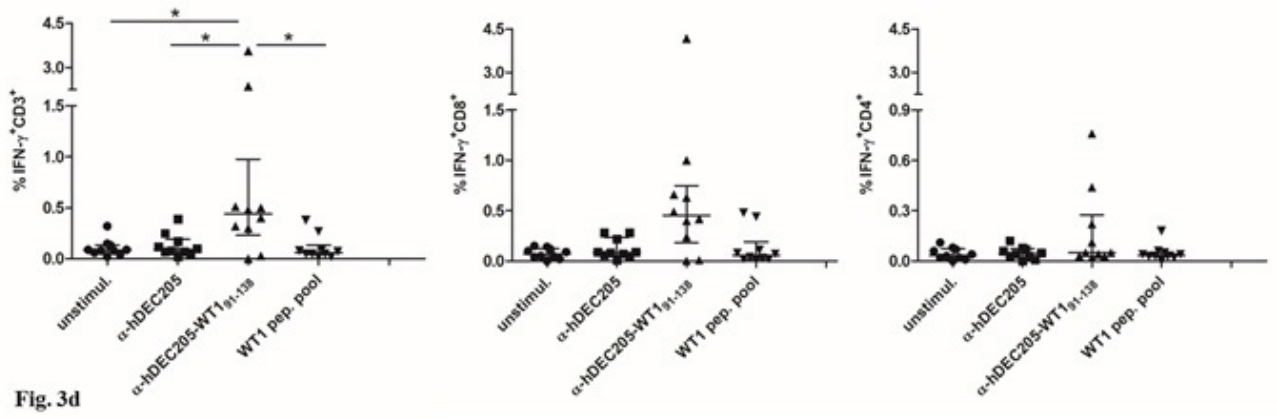
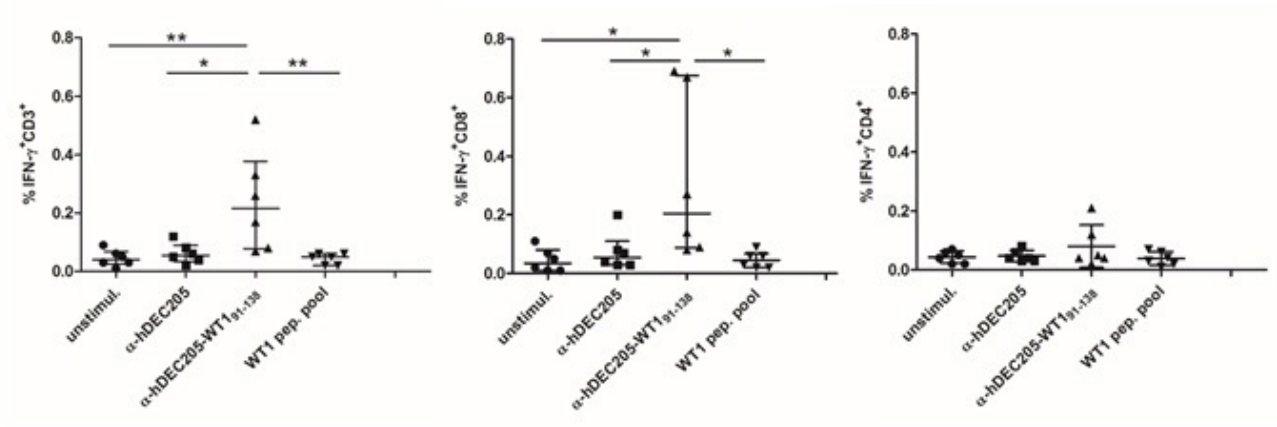


Fig. 3d



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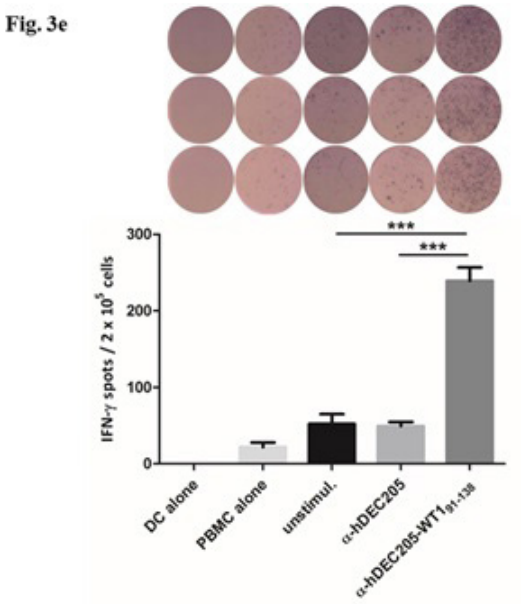


Fig. 3. DC-mediated T cell response to anti-hDEC205-WT1small antibody fusion proteins.

a 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. b IFN-γICS and FACS analysis of cells from one of the patients (PN2). Top: CD3+IFN-γ+ T cell frequencies gated on lymphocytes, Middle and Bottom: frequencies of CD3+-gated CD3+CD4+IFN-γ+ (middle) and CD3+CD8+IFN-γ+ T cells (bottom). T cells were co-incubated with unloaded moDCs (Unstimul.), with moDCs loaded with α-hDEC205-antibody, α-hDEC205-WT191-138 antibody fusion protein, WT1 peptide pool, or CMV pp65 protein. c and d Summary of IFN-γ ICS results from all patients (n = 10) and healthy individuals (n = 6). Plots show frequencies of IFN-γ+CD3+ (left), IFN-γ+CD8+ (middle) and IFN-γ+CD4+ (right) T cells. Each data point represents one individual, bars indicate median frequencies with IQR, Tukey's test *p < 0.05; **p < 0.01. e 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.

T cells activated by DC-targeted WT191-138 lyse WT1-overexpressing leukemia cell line

To validate the cytotoxic function of T cells activated by anti-hDEC205-WT191-138 loaded mature moDCs, T cells were expanded for 10-14 days resulting in a 1.3-2 fold increase of WT1-specific T cells (Fig. 4a) to frequencies of 1.6-10.2 % CD137+ cells (Fig. 4b) that were dominated by CD8+ T cells (Fig. 4c)

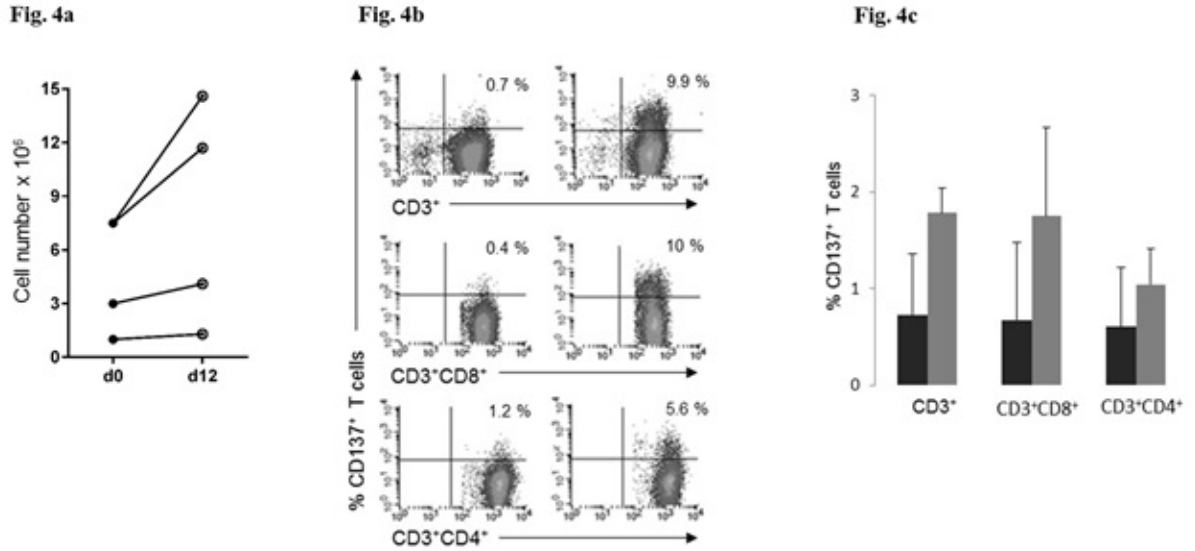


Fig. 4. In vitro T cell expansion by co-culturing with mature moDCs loaded with anti-hDEC205-WT191-138 antibody fusion protein.

a T cell number of four patients after 12 days of DC/T cell co-culture. Each line represents one patient. b Representative FACS analysis of CD137 expression on T cells. After 12 days of co-culture, T cells were left unstimulated (left) or restimulated with anti-hDEC205-WT191-138 antibody fusion protein (right). c Summarized data of CD137 expression in co-cultured T cells. Shown are means ± SD of data from all four patients.

Following the CD137+ T cell expansion, a VITAL-FR cytotoxicity assay was performed with T cells derived from three HLA A02 positive patients. There was HLA A02-restricted lysis of THP-1 cells at relatively low E:T ratios indicating that the WT191-138 specific T-cells possess a strong cytotoxic activity (Fig. 5a and Supplementary Fig. 4). It is worth mentioning that one of three T cell donors in this experiment was patient 4 who showed no T-cell response ex vivo. Interestingly, expanded T cells from patient 2 showed an inferior cytotoxicity, although

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their IFN-□+ T cell response was the strongest in the ex vivo experiments. A shown cytotoxic capacity of T cells in the ex vivo IFN-□ ICS combined with CD107a degranulation assay (Fig. 5b) could be confirmed by the VITAL-FR cytotoxicity assay. Thus, when targeted to DEC205 on mature moDCs, WT191-138 is able to activate cytotoxic T cells to kill WT1-overexpressing THP-1 leukemia cells.

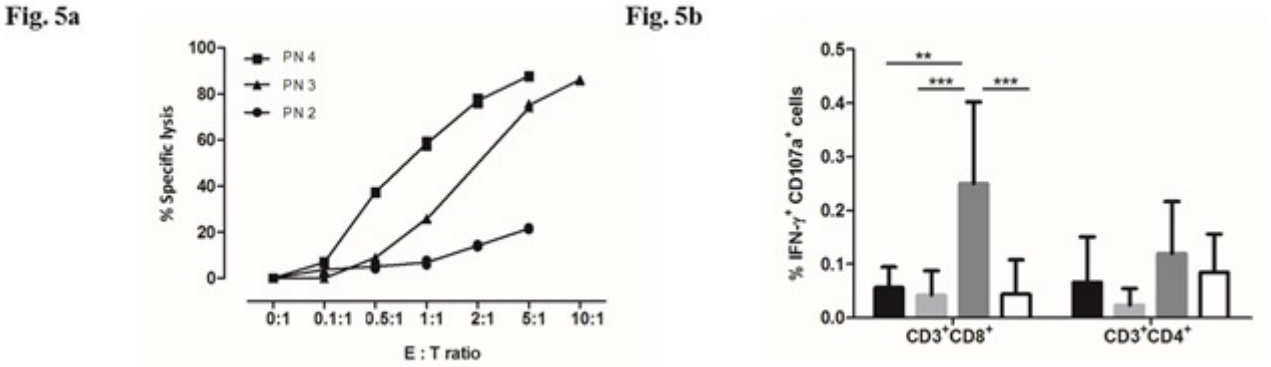


Fig. 5. Cytotoxic effector function of in vitro expanded WT1-specific T cells induced by anti-hDEC205-WT191-138 loaded mature moDCs.

a VITAL-FarRed cytotoxicity assay. Each experiment was duplicated and each replicate was shown from three different patients (PN 2, 3, 4). b Cytotoxicity of T cells ex vivo by IFN-γ ICS combined with CD107a degranulation assay. unstimulated, anti-hDEC205 Ab, anti-hDEC205-WT191-138, WT1 peptide pool stimulation. Shown are means ± SD from five patients including PN 2, 3, 4.

Discussion

Among vaccination strategies to induce anti-leukemia immune responses, DC vaccination after allo-HSCT holds a strong promise in immunotherapy for relapsed hematological malignancies [25]. To contribute to this field, we generated hDEC205-targeted WT1 antibody fusion proteins as an anti-leukemia vaccine and explored their immunogenicity by ex vivo and in vitro studies.

The anti-hDEC205 antibody fusion proteins containing major parts of WT1 were not satisfactorily secreted. Expression and purification of the full-length canonical isoform of murine and human WT1 (449 amino acids) and its zinc finger domains for protein functional studies were performed by a couple of groups [12,26,27] beforehand. 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 efforts, sufficient amounts of WT1 were purified from inclusion bodies of E. Coli or eukaryotic HEK293T cell lysates. Our study goal was different, because we wanted a secretable anti-hDEC205-WT1 antibody fusion protein. However, we also faced the preceding obstacles, which in our term, was poor secretion. To overcome this problem, we tested purification of the cell-associated anti-hDEC205-WT1_D1-227 from HEK293 cell lysates. However, the final yields were reduced significantly due to the precipitation of the antibody fusion protein, confirming results from Fagerlund et al [27].

In view of the poor secretion of WT1major-containing antibody fusion proteins, we tested secretion of anti-hDEC205 fusion proteins containing small fragments of WT1 and identified four different antibody fusion proteins showing sufficient production and secretion. Out of the four, three candidates were further analyzed. Anti-hDEC205-WT191-138 was the only one that induced ex vivo T cell responses in T cell/mature moDC co-incubations of healthy donors and allo-HSC transplanted AML patients. The WT191-138 fragment contained in this antibody fusion protein comprises five previously determined epitopes [28,29] with antigenic potential including the well documented WT1126-134 peptide [30,31]. Since responders to the anti-hDEC205-WT191-138 loaded mature moDC stimulation were not exclusively HLA A0201 positive, the good immunogenicity of anti-hDEC205-WT191-138 might be explained by relatively broad epitope variety presented on different HLA molecules through efficiently targeted DCs. Although anti-hDEC205-WT1223-273 and anti-hDEC205-WT1324-371 did not induce ex vivo T cell responses, their stimulatory capacity should be further tested by in vitro T cell expansion prior to detection.

An important finding from this study was an upgraded and robust detection of WT1-specific T cells ex vivo. T cells responded better to DEC205-targeted WT191-138 than to a 15-mer WT1 peptide pool. This could be explained by the targeting approach; however, it is not clear why only one of the three anti-hDEC205-WT1small constructs induced a decent response. Previous studies showed that prior T cell enrichment [14] or expansion [32] 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 WT191-138 to DCs at least in the context of allo-HSCT. This could also be applicable for the detection of a WT1-specific immune response to solid tumors since WT1 is overexpressed in various cancers [33]. 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.

It is known that DEC205-targeting protein vaccines strongly enhance cross presentation [34,35]. Thus, 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 WT191-138 stimulation. This finding is supported by the fact that combination of HLA class I and II restricted peptides induce both

cytotoxic and helper T cell responses [6,36]. Due to natural processing by DCs, the endocytosed protein-derived peptides could be presented via HLA class I and II molecules resulting in improved T cell responses. Our study highlights the value of a DC-targeting protein vaccine approach to induce a sustained and strong cytotoxic immune response against WT1-overexpressing leukemia. Furthermore, anti-hDEC205-WT1small antibody fusion proteins may not require in vitro loading of DCs since they are able to directly target DCs in situ. Thereby, their potential clinical application would need a lower cost and less labor [37]. 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 [38], and; second, distinct data confirming the significant correlation between WT1 overexpression and worse clinical outcomes in either hematological [39,40] or solid tumor patients [41]. In light of these data, our alternative approach offers an attractive perspective to be translated into clinical practice.

(Please find the listed references from the original paper)

The original paper:

Dagvadorj, N., Deuretzbacher, A., Weisenberger, D. et al. Targeting of the WT191–138 fragment to human dendritic cells improves leukemia-specific T-cell responses providing an alternative approach to WT1-based vaccination. Cancer Immunol Immunother 66, 319–332 (2017). https://doi.org/10.1007/s00262-016-1938-y