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Functional Defects of NHL Patients' T-Cells after Different Chemotherapy Regimens Activated By CD19/CD3 Tetravalent Bispecific TandAb® AFM11

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Introduction

T-cell engaging immunotherapies such as bispecific T-cell recruiting antibodies or chimeric antigen receptor T-cells (CAR-T) have emerged as highly active therapeutics in patients with refractory or relapsed hematological malignancies such as ALL or NHL. However, a relevant number of heavily pretreated patients progress or do not respond to these novel therapies. The objective of this study was to determine whether patients´ treatment history impacts the T-cell engagement of novel immunotherapies. In the present study we analyzed the responsiveness of T-cells obtained from NHL patients after different chemotherapeutic regimens (R-Bendamustine, R-CHOP, HD-BEAM) towards AFM11[#1], a novel CD19/CD3-directed tandem diabody (TandAb®) construct that is currently in clinical Phase 1 development in patients with r/r NHL or ALL.

Methods

T-cells were isolated and enriched from 30 NHL patients 4-6 weeks after different therapeutic regimens and characterized side by side with T-cells enriched from healthy volunteers (n=13) by flow cytometry. All 9 patients who received prior R-CHOP treatment had DLBCL. The 12 patients that were treated with R-Bendamustine were diagnosed as having follicular lymphoma (4 patients), CLL (6 patients), marginal zone lymphoma (1 patient) and mantle cell lymphoma (1 patient). High- dose chemotherapy with the BEAM protocol followed by autologous stem cell transplantation was given to 4 relapsed DLBCL patients, 3 patients with mantle cell lymphoma as first line therapy and to 2 patients with indolent NHL.

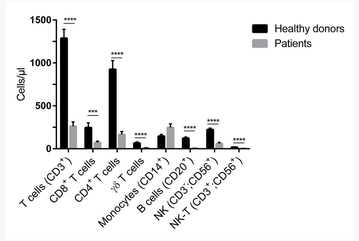
PBMC and enriched T-cells from patients and healthy volunteers were characterized for cell surface marker expression and relative numbers by flow cytometry.

The responsiveness of T-cells from NHL patients to AFM11 was compared with T-cells from healthy volunteers in proliferation and cytokine release assays. In addition, enriched T-cells were used as effector cells at limiting effector-to-target (E:T) ratios in heterologous cytotoxicity assays with Nalm-6 target cells in the presence of AFM11 or a HSA/CD3 TandAb as a negative control (cAb).

Patient characteristics

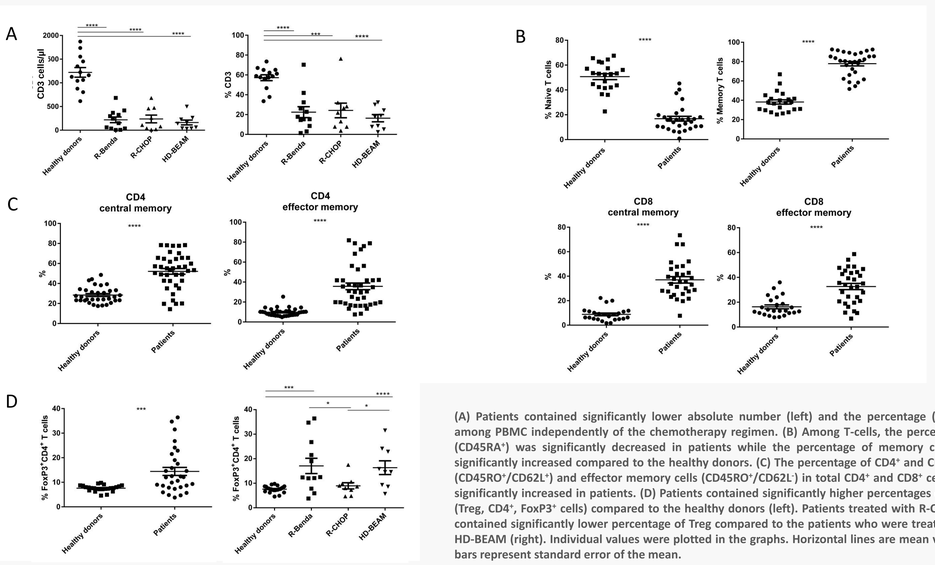
	All Patients N=30	R-CHOP N=9	R-Benda N=12	HD-BEAM N=9	Healthy N=13
Male, n (%)	21 (68%)	6 (67%)	9 (82%)	5 (50%)	8 (62%)
Median (range) age, years	46 (32-69)	44 (32-69)	43 (36-57)	52 (40-67)	54 (28-70)
ECOG					
0	0 (0%)	0 (0%)	0 (0%)	0 (0%)	n.a.
1	28 (93%)	8 (89%)	10 (80%)	9 (100%)	
2-3	2 (7%)	1 (11%)	2 (30%)	0 (0%)	
Prior chemotherapies	10 (30%)	0 (0%)	3 (33%)	7 (70%)	n.a.
Best response n (%)					
complete response	12 (40%)	5 (56%)	4 (33%)	2 (22%)	
partial response	16 (53%)	3 (33%)	8 (67%)	6 (67%)	n.a.
stable disease	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
progressive disease	2 (7%)	1 (11%)	0 (0%)	1 (11%)	

Reconstitution of PBMC after chemotherapy



Patients contained significantly lower absolute cell number of $CD3^+$ T-cells ($p=1.618x10^{-12}$), $CD8^+$ T-cells ($p=0.0002451$), $CD4^+$ T-cells ($p=3.199x10^{-11}$), γδ T-cells ($p=2.5x10^{-7}$), B-cells ($p=1.059x10^{-16}$), NK-cells ($p=1.003x10^{-7}$) and NK-T-cells ($p=8.141x10^{-7}$) than the healthy donors. The number of monocytes was insignificantly increased in patients ($p=0.116739$). Data are presented as mean values for 30 analyzed patients and 13 healthy donors. The error bars are standard errors of the mean (SEM).

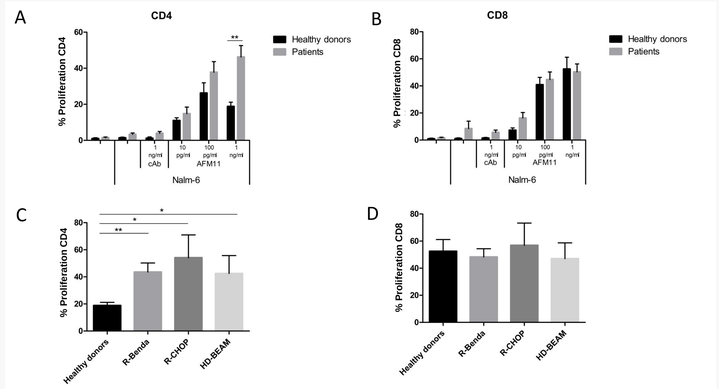
Comparison of T-cell subsets between healthy donors and NHL patients six weeks after the last chemotherapy cycle



(A) Patients contained significantly lower absolute number (left) and the percentage (right) of $CD3^+$ cells among PBMC independently of the chemotherapy regimen. (B) Among T-cells, the percentage of naïve cells ($CD45RA^+$) was significantly decreased in patients while the percentage of memory cells ($CD45RO^+$) was significantly increased compared to the healthy donors. (C) The percentage of $CD4^+$ and $CD8^+$ central memory ($CD45RO^+/CD62L^+$) and effector memory cells ($CD45RO^+/CD62L^-$) in total $CD4^+$ and $CD8^+$ cells respectively was significantly increased in patients. (D) Patients contained significantly higher percentages of regulatory T-cells (Treg, $CD4^+$, $FoxP3^+$ cells) compared to the healthy donors (left). Patients treated with R-CHOP chemotherapy contained significantly lower percentage of Treg compared to the patients who were treated with R-Benda or HD-BEAM (right). Individual values were plotted in the graphs. Horizontal lines are mean values and the error bars represent standard error of the mean.

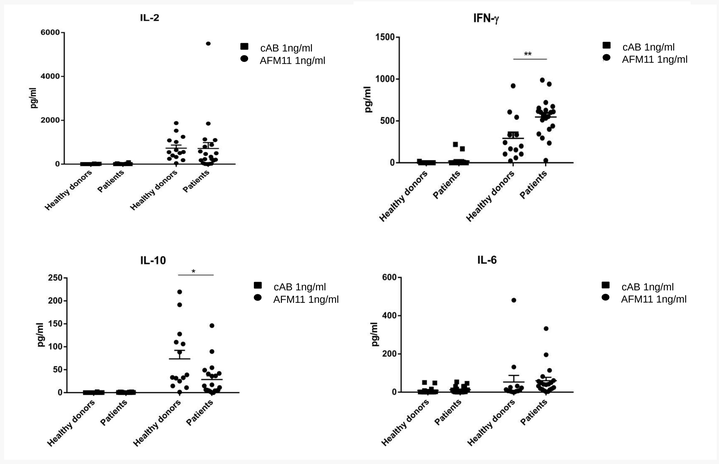
In vitro activity of AFM11 with T-cells from NHL patients and healthy volunteers

AFM11 induced proliferation of $CD4^+$ and $CD8^+$ T-cells from HD-BEAM-, R-Benda-, and R-CHOP-treated patients



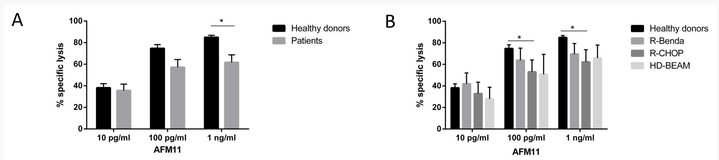
(A, B) CFSE-stained T-cells from patients and healthy donors were incubated with or without Nalm-6 target cells and increasing concentrations of AFM11 as indicated (10 pg/mL, 100 pg/mL, 1 ng/mL). As a negative control, T-cells and Nalm-6 cells were cultured either alone or with 1 ng/mL of an irrelevant HSA/CD3 control TandAb antibody (cAb). After four days the cells were stained with CD4 and CD8 markers and the data are presented separately for these two cell types. $CD4^+$ T-cells of patients proliferated more than those of healthy donors for all concentrations of AFM11, and the difference was statistically significant in the presence of 1 ng/mL AFM11 ($p=0.00730289$). In case of $CD8^+$ T-cells there was no significant difference in proliferation capacity between cells isolated from patients and healthy donors. (C, D) Comparison of the healthy donors and patients $CD4^+$ and $CD8^+$ T-cell proliferation capacity in response to 1 ng/mL AFM11. AFM11-activated $CD4^+$ T-cells of patients treated with R-Bendamustine, R-CHOP or HD-BEAM proliferated significantly more than $CD4^+$ T-cells of healthy donors ($p=0.0034$, $p=0.0204$, and $p=0.0434$, respectively) (C). There was no statistically significant difference in proliferation capacity between $CD8^+$ T-cells of healthy donors and patients independently of chemotherapy regimen (D).

Stronger IFN-γ and lower IL-10 secretion by patient T-cells in response to AFM11



T-cells were incubated with Nalm-6 target cells and 1 ng/mL AFM11 or irrelevant control TandAb (cAb). After 24 hours the indicated cytokines were quantified in the cell culture supernatants. The production of IL-2 was similar between the patient and healthy donor T-cells. Patient T-cells produced significantly higher amounts of IFN-γ ($p=0.025$) and significantly lower amounts of IL-10 ($p=0.0067$) than the T-cells of healthy donors when treated with AFM11.

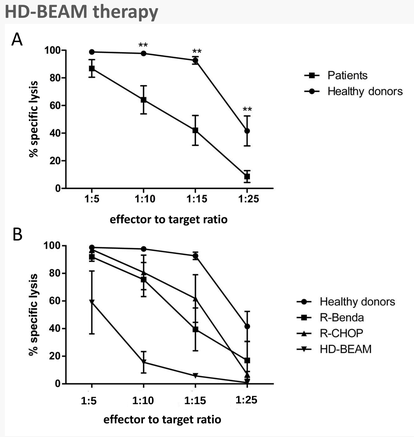
Efficacious and dose-dependent AFM11-mediated target cell lysis by T-cells from NHL patients and healthy volunteers



$1x10^5$ T-cells as effectors were cultured with $0.5x10^5$ CFSE-labeled Nalm-6 cells and AFM11 (10 pg/mL, 100 pg/mL, or 1 ng/mL). After 22 hours co-cultured cells were analyzed with FACS. (A) T-cells isolated from patients had reduced cytotoxic activity for all three concentrations of AFM11. This difference was statistically significant for 1 ng/mL AFM11 ($p=0.006$). (B) There was no statistically significant difference in cytotoxic activity of T-cells isolated from patients treated with different chemotherapy regimens. T-cells of patients treated with R-CHOP had significantly lower cytotoxic activity than T-cells of healthy donors in the presence of 100 pg/mL ($p=0.03$) and 1 ng/mL AFM11 ($p=0.01$). The columns show mean values and error bars are standard error of the mean (SEM).

AFM11-mediated serial killing of target cells

Reduced AFM11-mediated cytotoxic activity of T-cells from patients after HD-BEAM therapy



Nalm-6 target cells and effector T-cells were incubated at the indicated E:T ratios in the presence of AFM11. After 96 h incubation specific lysis was calculated. (A) The specific lysis mediated by patient T-cells (n=13) and T-cells from healthy donors (n=11). AFM11-activated T-cells from patients had significantly reduced serial cytotoxic activity than AFM11-activated T-cells from healthy donors at E:T ratio 1:10 ($p=0.01$), 1:15 ($p=0.002$), and 1:25 ($p=0.005$). (B) Specific lysis mediated by T cells of healthy donors and T cells of patients after different chemotherapy regimens. The serial cytotoxicity potential was analyzed for patients treated with R-Bendamustine (n=4), R-CHOP (n=6), or HD-BEAM (n=3).

Key results

1. Selected NHL patients were found to have less $CD3^+$ T-cells, less NKT- and NK-cells, and no B-cells, but higher numbers of Tregs in the peripheral blood when compared with PBMC from healthy donors.
2. AFM11 induced in the presence of target cells the proliferation of both $CD4^+$ and $CD8^+$ T-cells from patients and healthy donors, with significantly higher proliferative response in $CD4^+$ T-cells from NHL patients relative to $CD4^+$ T-cells from healthy donors.
3. T-cells from patients produced significantly higher levels of IFN-γ, but lower levels of IL-10 in response to AFM11 when compared to T-cells from healthy donors.
4. At high E:T ratios (2:1) AFM11-mediated cytotoxicity with T-cells from patients was only slightly lower relative to T-cells from healthy donors, but there was no significant difference between patient T-cells after different chemotherapeutic regimens.
5. At lower E:T ratios (<1:5) significant differences between different chemotherapeutic regimens were observed in AFM11-mediated target cell lysis by patient T-cells: T-cells from patients after HD-BEAM treatment exhibited substantial lower cytotoxic efficacy in AFM11-mediated target cell lysis relative to T-cells from patients after R-Benda or R-CHOP treatment.

References

- [#1]Reusch, U. *et al.* A tetravalent bispecific TandAb (CD19/CD3), AFM11, efficiently recruits T cells for potent lysis of $CD19^+$ tumor cells. Mabs 7(3): 584-604 (2015)

Disclosures

- H.E.: Novartis: Consultancy, Honoraria; Merck: Membership on an entity's Board of Directors or advisory committees, Research Funding; Amgen: Consultancy, Honoraria, Speakers Bureau; Celgene: Consultancy, Honoraria, Speakers Bureau; Janssen: Consultancy, Honoraria, Speakers Bureau; Chimerix: Consultancy, Honoraria.
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