Non metastasis protein 2 (NME2) is a potential target for leukemia-specific immunotherapy

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Abstract

Background: NME2 is an immunogenic overexpressed tumor-associated antigen in chronic myeloid leukemia (CML). We evaluated NME2's antigenicity in common HLA-backgrounds as a potential target for CML-specific immunotherapy.

Methods: NME2-specific cytotoxic T-lymphocytes (CTL) were generated by stimulating cultured PBMCs with artificial antigen presenting cells (aAPC) expressing NME2 and the appropriate HLA-A antigen. Cultured PBMCs were obtained from healthy donors or CML-patients and priming with aAPCs was repeated weekly. IFNγ production from CD8+CTLs was evaluated by ELISpot assays.

Results: NME2-specific CTLs were successfully generated both from healthy donors and from CML patients after hematopoietic stem cell transplantation. NME2-specific recognition by CTLs was HLA class I restricted (HLA-A 02, 03, 24) with no significant differences between the HLA-A backgrounds tested. The T315I TKI resistance mutation BCR::ABL1 did not affect susceptibility to CTL-mediated killing.

Conclusions: NME2-specific CTLs can eradicate CML progenitor cells remaining after TKI-therapy or HSCT. Immunotherapy targeting NME2 may complement existing therapies.

Introduction

Chronic myeloid leukemia (CML) is characterized by a reciprocal translocation t(9;22) (q34;q11) that fuses the *BCR* and *ABL1* genes on the Philadelphia chromosome (Ph^+). The resulting *BCR::ABL1* oncogene encodes a chimeric non-receptor tyrosine kinase with constitutive activity[1 2]. An appreciation of the molecular pathomechanisms in CML has enabled the development of specific BCR-ABL1 tyrosine kinase inhibitors (TKI), that have revolutionized the treatment of CML[3 4] and are now established as the gold standard of CML first-line treatment[5]. However, despite these impressive developments, there are still patients who do not achieve the desired response. The rates of major molecular response (MMR) after 1 year of 400 mg imatinib treatment ranged from 18–58%[6] and over the course of 8 years of follow-up 45% of the patients enrolled in the IRIS (International Randomized Study of Interferon and STI571) discontinued imatinib therapy[7]. Resistance to TKI often results from mutations in the *BCR::ABL1* gene, which are present in approximately 30% of patients who experience treatment failure in the chronic phase [8 9 10]. The adverse effects of imatinib treatment are mild. However, there is considerable interest in discontinuing therapy even in responding patients, either to permit pregnancy[11] or simply to avoid the high costs of long term TKI therapy[12]. Trials of imatinib discontinuation with sustained molecular remission have been published[13 14]. However, TKIs fail to eliminate residual leukemic cells in the short to medium term[15 16 17 18], and multicenter trials still claim that imatinib should be discontinued only in the context of clinical trials and only in patients having achieved and maintained for 2 or 3 years long in a sustained deep molecular response. Even in this patient group, an early molecular relapse occurs in over 50% of patients who stopped imatinib. For these reasons, allogenic hematopoietic stem cell

transplantation (HSCT) remains a relevant, curative therapy for a small but significant proportion of patients with CML.

HSCT in CML mainly exerts a graft-versus-leukemia (GVL) effect, mediated by leukemia reactive cytotoxic T-lymphocytes that target tumor antigens or minor histocompatibility antigens and thus have the potential to eradicate residual disease[19]. Donor lymphocyte infusion (DLI)[20 21] can be used to intensify this effect in clinical practice. This suggests that both TKI treatment and HSCT could be augmented by cancer vaccine strategies such as the administration of specific antibodies, antigenic peptides, peptide-loaded dendritic cells, Chimeric antigen receptor (CAR) T-cells, CAR-NK cells or killed tumor cells[22 23 24].

The efficacy of an antitumor immune response depends largely on the ability of cytotoxic T cells to recognize tumor associated antigens (TAA). In theory, such cells can be selectively activated and amplified by vaccination approaches and there are ongoing attempts to induce TAA-specific T cell responses to Wilms tumor protein 1 (WT1) and the PR1 epitope from proteinase 3 (PR3) in this way[25 26 27]. Targeting of recently identified TAAs in CML such as telomerase, hyaluronic acid mediated motility (RHAMM), CML-66, CML-28, CML-Ag165, PPP2R5C, ELA2, PRAME, MPP1, and Aur-A could be an additional strategy for CML therapy [28]. Trials with a BCR::ABL1 (e14a2)–derived peptide vaccine have shown some benefit[29 30] and a multicenter trial still awaits publication[31]. Given that a very high level of NME2 expression is a universal and specific feature of BCR::ABL1-positive cells in chronic-phase CML[32 33], and that the expression itself was originally identified via T cell-mediated recognition, it is of interest to determine the potential immunogenicity of NME2 in the context of CML.

To answer this question, we analyzed the immunogenicity of NME2 in the background of common HLA-A types by investigation of NME2-specific T cell-mediated killing of CML cells irrespective of TKI-resistance status and residual disease.

Materials And Methods

Patient samples

The presence of peripheral blood T cells able to respond to appropriately presented NME2 peptides was assessed both in healthy donors (n = 5) and HSC transplanted CML patients (n = 10) by stimulation of peripheral blood MNC using aAPC expressing full length NME2 and a matching HLA-A protein. Peripheral blood mononuclear cells (PBMCs) expressing the common HLA-A alleles 02, 03 or 24, were obtained following approval by the local ethics committee (University of Leipzig, approval-number 323-08) and informed consent from 5 healthy donors (age > 18 years) and from 10 CML-patients after hematopoietic stem cell transplantation (characteristics shown in supplemental material Table S1). PBMC were purified by density gradient centrifugation over LSM 1077 Lymphocyte Separation Medium (PAA Laboratories, Pasching, Austria). The selection of CD14 + cells was performed using a mini-magnetic activated cell sorting (MACS)-positive selection system (Miltenyi Biotech, Bergisch Gladbach, Germany) as described by the manufacturer.

Generation of T-cell lines

NME2-specific CTLs from healthy donors and CML-patients were generated by priming and weekly restimulation of PBMCs with irradiated artificial antigen presenting cells, (aAPC) expressing NME2 and the appropriate HLA-A antigen, in a responder-to-stimulator-ratio of 10:1. PBMCs (1 x 10 per well) were cultured on 24 well-plates in 2 ml AIM-V medium (Gibco BRL, Gaithersburg, MD, USA), containing 10% AB-human serum (Sigma-Aldrich, St Louis, MO, USA) with the inclusion of 250U/ml recombinant IL-2 (Proleukin, Chiron/Novartis, Nürnberg, Germany) from day 4 onwards. On day 14, isolation of CD8 + T-cells was performed, using a mini-magnetic activated cell sorting (MACS)-positive selection system (Miltenyi Biotech, Bergisch Gladbach, Germany) as described by the manufacturer. To investigate CD8-expression, T-cell lines were stained with an allophytocyanin (APC)-conjugated CD8 monoclonal antibody (Beckton Dickinson, Heidelberg, Germany) and subject to flow cytometry (FACS Calibur, Becton Dickinson, New Jersey, USA) and analysis using Cell Quest software (Becton Dickinson). The purity of CD8 + T-cells after isolation was always > 95%.

Generation of artificial antigen presenting cells (aAPCs)

293T cells were cultured in RPMI 1640 (Biochrom AG, Berlin, Germany), containing 10% fetal calf serum, 12 mM glutamine and 100 U/ml penicillin/streptomycin (all from Invitrogen Carlsbad, CA, USA). Plasmid DNA was introduced using non-liposomal FuGENE® HD Transfection Reagent according to the manufacturer's recommendations (Promega, Mannheim, Germany). Briefly, 293T cells were resuspended in RPMI to a concentration of 2 x 10 cells/ml (1 ml per well) and were plated in 24-well plates. 400 ng/well NME2 DNA, 200 ng/well HLA-A DNA and 3 µl/well FuGENE® HD Transfection Reagent were added to the cells. The transfected 293T cells were kept for 48 hours at 37°C, and 5% CO_2. Following irradiation with 100 Gy, aAPCs were applied to stimulate T cell lines. Western blotting was used to test transfection efficiency. To test MHC I restriction, aAPC transfected with both HLA-A and NME2 were pre-incubated with an MHC I (major histocompatibility complex class I) neutralising monoclonal antibody (W6/32, Sigma-Aldrich, St. Louis, USA).

Generation of artificial antigen presenting cells (aAPCs)

Artificial antibody presenting cells (aAPC) were generated from human embryonic kidney 293T cells (ATCC, Rockville, USA) co-transfected with plasmids pcDNA3.1 (+/-) (Invitrogen, Carlsbad CA, USA) expressing NME2 and the appropriate HLA-A antigen. Culture and transfection of 293T cells is described in detail in the supplemental material.

Generation of T-cell lines

NME2-specific CTLs from healthy donors and CML-patients were generated by priming and weekly restimulation of PBMCs with irradiated artificial antigen presenting cells (aAPC) expressing NME2 and the appropriate HLA-A antigen (detailed information in the supplemental material).

IFNγ Enzyme-linked immunosorbent spot-forming cell assay (ELISpot assay)

NME2-specific IFNγ release from CD8 + T-cells was assessed by ELISpot assay on day 14, 21 and 28 and is described in detail in the supplemental material. Spot counting was carried out using an ELISpot reader system and software (Autoimmun Diagnostika GmbH, Straßberg, Germany). The average ratio of specific recognition of triplicates was calculated as follows: [(test counts − spontaneous counts) / (maximum counts − spontaneous counts)] × 100.

Ba/F3 cells

Cells were grown in RPMI 1640 medium (Biochrom AG, Berlin, Germany) supplemented with penicillin, streptomycin, L-glutamine, and 10% fetal bovine serum (FBS) with 10% WEHI conditioned medium as a source of murine IL-3. Expression of BCR-ABL1 in these cells abrogates their IL-3 dependence. Stable transfected Ba/F3 cell lines expressing full length wildtype (wt) BCR::ABL1 or BCR::ABL1 with point mutations T315I in the kinase domain have been previously described[34] (additional information in the supplemental material).

Colony-forming inhibition assay of leukemic and normal hematopoietic progenitors

CD34 + cells were isolated and purified by MACS (Miltenyi Biotech) from the bone marrow of 3 HLA-A02 or A03^{+} healthy donors, from peripheral blood of 5 HLA-A02 or A03^{+} and 2 HLA-A02/A03^{-} CML patients at diagnosis. CD34 + cells (3x10^{5}) were then co-incubated with NME2-CTLs at an effector:target ratio of 20:1 or 50:1 in 1 ml of complete medium at 37°C for 6 hours. The cells were then plated into methylcellulose medium supplemented with recombinant cytokines (MethoCult, StemCell Technologies, Köln, Germany) and incubated at 37°C. After 2 weeks of culture, colonies (> 50 cells) were counted using a high-quality inverted microscope. All experiments were performed in triplicate. In selected experiments, colonies were recovered out of the methylcellulose and RNA extracted for RT-PCR detection of *BCR::ABL1* mRNA as described elsewhere. [33, 35]

RT-PCR

The extraction and the in vitro quantification of *BCR::ABL1* mRNA of CML cells by quantitative real time PCR were performed as described previously[33].

Statistical analysis

The t-test was used to assess statistical differences between samples. P values < .05 were taken to indicate significance. The data in all Figures are shown as mean and standard deviations. P value limits are shown as follow: * $P \leq 0.05$; ** $P \leq 0.005$; *** $P \leq 0.0005$.

Results

NME2 specific CTLs can be generated from healthy donors and from patients with CML after HSCT

After a total of 4 rounds of weekly stimulation (d 28), CTLs expanded from healthy donors (n = 5) had expanded mean 23-fold (range 5.7–68), while those generated from patients with CML after HSCT (n = 10) were more active, with a mean 30-fold expansion (range 0.2–99). The NME2-specific response of the CTLs as tested by Elispot assay for IFNγ production is shown in Fig. 1. CTL populations derived by stimulation with NME2-expressing aAPC contained a significantly higher frequency of spot forming cells than those expanded in response to the control aAPC expressing the HLA-A protein only. The frequency of spot forming cells was consistently, though not significantly, higher in CTLs derived from transplanted CML patients, than from those derived from healthy control marrow. Pre-incubation of the transfected aAPC with an anti-MHC I antibody before adding the CTLs led to a sharp reduction in IFNγ spot formation, confirming MHC I restriction.

Generation of NME2-specific T cells is possible in the context of various and frequently occurring HLA alleles.

15 NME2-specific CTLs were expanded from HLA-A02 (n = 4), HLA-A03 (n = 6) and HLA–A24 (n = 5) positive healthy donors and patients with CML after HSCT. PBMCs were stimulated with aAPCs expressing NME2 and partially matched HLA-A according to the protocol described above.

The mean expansion after 4 weeks of culture was 17-fold (range 7.6–26.6), 33-fold (range 5.7–98.75) and 29-fold (range 0.18–85) for HLA-A02, HLA-A03 and HLA-A24, respectively, with no significant differences between the groups. The generation of CTLs was successful in all HLA-A contexts tested. With the exception of one test, these CTLs showed reactivity against NME2 (4/4 HLA-A02 CTL, 5/6 HLA-A03 CTL, and 5/5 HLA-A24 CTL) in IFNγ ELISpot assays as illustrated in Fig. 2. For NME2 in the context of HLA-A02, mean spot formation over background was 90 ± 60 IFNγ SFCs / 1 x 10^4 T cells, for NME2 restricted by HLA-A03 the corresponding number was 97 ± 148 and for NME2 restricted by HLA-A24 113 ± 45, respectively. However, these differences were not statistically significant.

NME2-specific CTLs target primary CML-cells

As shown in Fig. 3a, NME2-specific CTLs expanded over two to four stimulation cycles show a significantly stronger response to HLA-A matched CML-cells (168 ± 49 SFCs / 1 x 10^4 T-cells) than to CD14 + cells from healthy donors (61 ± 30 SFCs / 1 x 10^4 T-cells, p = 0.037). Primary CML-cells did not elicit a response from unspecific CTLs (55 ± 7 SFCs / 1 x 10^4 T-cells). Pre-incubation of the CML-cells with an anti-HLA class I antibody (W6/32, Sigma-Aldrich, St. Louis, USA), resulted in a significant reduction in the frequency of IFNγ-releasing T cells down to 66 ± 18 SFCs/ 1 x 10^4 T-cells (p = 0.04).

As shown in Fig. 3b, there is a positive correlation between the *Bcr::Abl / Abl* expression ratio of CD14 + primary CML samples and the CTL IFNγ response that they elicit.

The response of NME2-specific CTLs to BCR::ABL1 expressing cells is unaffected by TKI resistance mutation

BaF/3 cells stably expressing either wild-type BCR::ABL1 (BCR::ABL1^{wt} Ba/F3) or T315I mutated BCR::ABL1 (BCR::ABL1^{T315I} Ba/F3) were used as stimulator cells in Elispot assays of CTLs generated from 6 HLA-A02 or HLA-A03 CML-patients. The *Bcr::Abl* expressing BaF/3 lines, which are known to also express high levels of NME2[36], were transfected in each case with the appropriate HLA-A expression plasmids. As shown in Fig. 4, the IFNγ release from NME2-specific CTLs was similar after stimulation with either BCR::ABL1^{wt} Ba/F3 (mean 33 ± 21 SFCs/ 10^4 T-cells) or BCR::ABL1^{T315I} Ba/F3 (mean 29 ± 19 SFCs/ 1 x 10^4 T-cells). MHC I dependence was confirmed by the much poorer response ($p = 0.02$) to untransfected BCR::ABL1^{wt} Ba/F3 (mean 12 ± 8 SFCs/ 1 x 10^4 T-cells) and BCR::ABL1^{T315I} Ba/F3 (mean 12 ± 8 SFC/ 1 x 10^4 T-cells) as target-cells.

NME2 specific CTLs target CML progenitors

Bone marrow CD34 + cells were obtained from 3 HLA-A02 or A03$^+$ healthy donors and from 5 HLA-A02 or A03$^+$ and 2 HLA-A02/A03$^-$ patients with CML at diagnosis. As shown in Fig. 5a, NME2-specific CTLs significantly reduced the median number of colonies generated by leukemic progenitors in a dose-dependent manner (E:T ratio 20:1, 76% ± 15% reduction of CFU, $p = 0.01$; at an E:T ratio of 50:1 59% ± 19% reduction of CFU, $p = 0.004$). Also depicted in this figure is a tendency towards stronger reduction by a higher effector:target ratio. There is no reduction in colony formation by CD34 + cells derived from healthy donors (E:T ratio 20:1, 100% ± 5% CFU; E:T ratio 50:1, 100% ± 4% CFU) or from HLA mis-matched patients CML cells (E:T ratio 20:1, 102% ± 7% CFU; E:T ratio 50:1, 100% ± 5% CFU).

Finally, RT-PCR analysis of picked single colonies showed that pre-incubation with HLA-matched CTLs (E:T 20:1 or 50:1) not only reduces the overall number of colonies generated from a CML sample by 30–40%, but also reduces the Bcr::Abl ratio in the emerging colonies themselves (Fig. 5b), suggesting activation of Bcr::Abl negative progenitors.

Discussion

Here we demonstrate the antigenicity of NME2 on the background of common HLA-A antigens and imply a role for NME2-specific CTLs in eradication of residual leukemic cells, irrespective of TKI-resistance mutations in the *BCR::ABL1* tyrosine kinase.

The ultimate goal in CML therapy is the attainment of complete and durable remission, even after discontinuation of treatment. The introduction of TKI therapies has resulted in an impressive improvement in overall survival and the currently management of CML is focused increasingly on maintaining the quality of life and reducing of the economic burden of TKI therapy. Nonetheless, a significant proportion of patients is refractory or develops resistance to TKIs. Furthermore, younger and female CML patients in particular can experience an inferior health-related quality of life due to various

side effects of inhibitor treatment [36]. Children, who may face decades of therapy, commonly suffer from growth deceleration resulting from impairment of the growth hormone/IGF-1 axis by TKIs[37], while women of child-bearing age who wish to conceive face critical challenges while on TKI[38].

For these reasons, there is much interest in enhancing treatment modalities to overcome TKI resistance and to avoid molecular recurrence after cessation of therapy. In addition to the pharmacological targeting of pathways contributing to leukemic stem cell activity, strategies that recruit the immune system to eliminate otherwise refractory leukemic cells appear to be highly promising. Adoptive cell therapy (ACT), involving the application of (increasingly manipulated) immune cells with activity against cancer cells is able to improve the outcome of established cancer therapies[39]. Nevertheless, such procedures rely heavily on the identification of tumor antigen targets that are sufficiently specific to enable elimination of cancer without serious damage to normal cells.

We have previously verified the presence of NME2-reactive T-cells after HSCT in a Ph and HLA-A32 CML-patient and identified NME2 as an immunogenic CML associated antigen[32]. Subsequently, we demonstrated the overexpression of NME2 protein in 30/30 patients with CML at diagnosis, in the absence of increased level of NME2 mRNA. The level of NME2 protein was closely linked to the BCR::ABL1 tyrosine kinase activity, implying the post-transcriptional up-regulation or stabilization of NME2 protein in CML cells[33].

Since classic T-cell activation pathways require the presentation of antigenic epitopes by HLA proteins, the ultimate potential of CML-specific immunotherapy approaches directed against NME2 will depend on the level and specificity of activity attainable in HLA-backgrounds most common in the target population. Our demonstration of comparable T-cell responses to NME2 presented in HLA-A02, 03 and 24 backgrounds, each of which is present in 40–44% of the human population[40], suggests a capacity for the induction of NME2-mediated leukemia specific immune responses in the majority of the human population with no clear allele-specific preferences.

Furthermore, both the very high levels of NME2 protein[33] and the CTL response appear to be unaffected by mutations in BCR::ABL1 that are held responsible for around half of all cases of TKI resistance, meaning that these patients should be equally susceptible to NME2-mediated approaches. It remains unclear whether or not NME2 levels are maintained in CML cells that become resistant to TKIs through means other than acquired mutations in the BCR::ABL1 kinase domain. However, the close association between NME2 levels and BCR::ABL activity[36] suggests that any cell that is reliant on BCR::ABL1 activity is likely to express abnormally high levels of NME2 that mark it as a potential target for NME2-mediated immunotherapy.

This raises the question of whether an NME2 targeted immunotherapy has the potential to be effective against the CML stem cells that are responsible for disease persistence even in patients who are TKI sensitive. We have not yet investigated the very rare population of $CD34^+$ $CD38^-$ CML stem cells to address the decisive issue of whether quiescent cells may be susceptible. However, it is encouraging that

pre-incubation with NME2-specific CTLs reduces the activity of colony-forming cells from CML patients, but not from normal donors.

NME2 is a highly conserved protein expressed in a wide range of tissues, and although Nme2 knockout mice develop normally, double knockout of Nme2 and the closely related Nme1 results in perinatal death, with severe anemia (DOI: 10.1007/s00210-011-0649-3). This clearly raises questions concerning the potentially detrimental effects on non-CML cells in the context of immunotherapy directed against NME2. Here, it is encouraging to note that the successful derivation of functional, NME2-specific CTLs from healthy donor and CML bone marrow suggests that there may be sufficient differences between normal and pathological NME2 protein/antigen expression to enable selective targeting.

In summary, we demonstrate here that NME2 has potentially attractive characteristics for eradicating residual leukemogenic progenitor cells and more differentiated CML cells via vaccination or adoptive T-cell therapy (ACT) approaches. The combination of TKI or HSCT with a complementary immune therapy targeting NME2 may have the potential to further improve anti-leukemic efficacy and thus reduce the cumulative TKI dosage and/or the duration of treatment.

Declarations

Individual authors contribution:

TD performed laboratory work, validated the data generated and wrote the first draft.

ST supported the laboratory work and assisted with manuscript writing.

MC assisted with manuscript writing.

DN planned the study, supervised all steps of the progress, and assisted with manuscript writing.

All authors approved the final version of the text. This manuscript represents part of the medical doctoral thesis of TD.

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Declaration of conflict of interests:

Tobias Daebritz received honoraria from Novartis.

All other authors declare no conflicts of interest.

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Figures

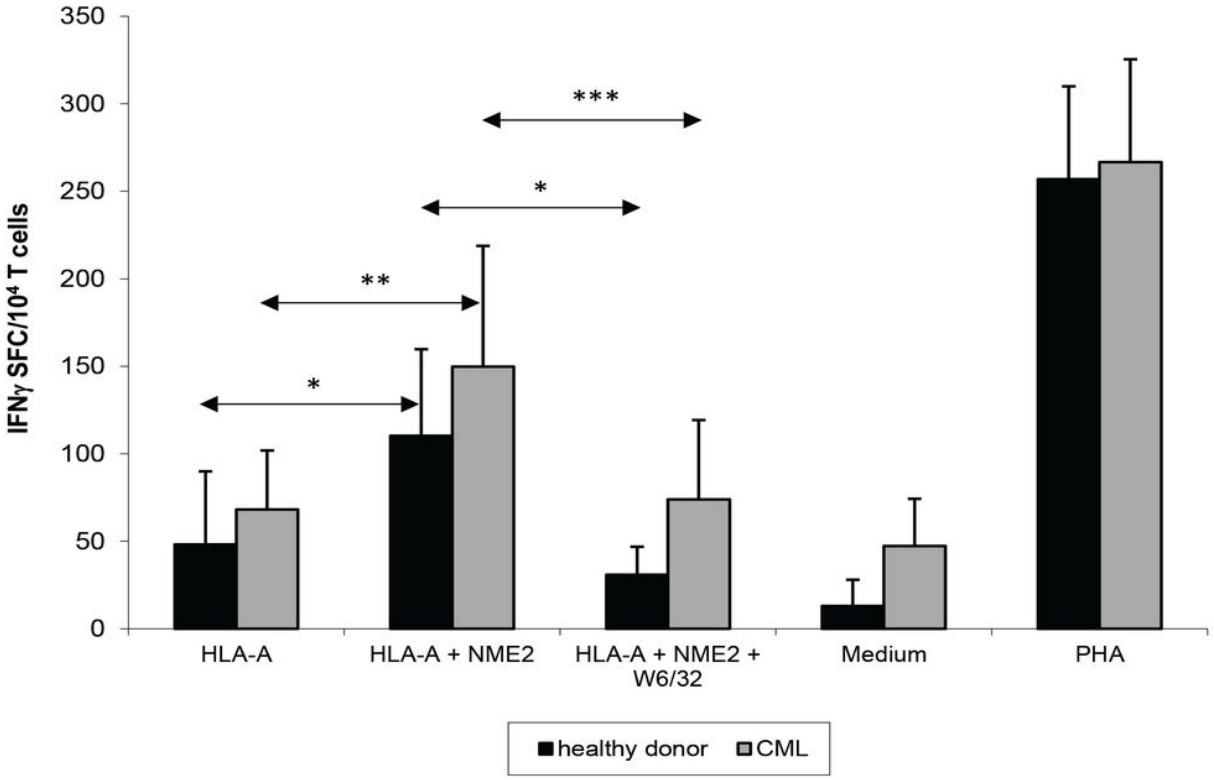

Figure 1

Elispot analysis of CTL populations derived from PBMCs by stimulation with NME2 Elipsot analysis was used to determine the frequency of interferon-y releasing spot-forming cells in T-cell lines generated from 5 healthy donors and from 10 CML-patients (HLA-A02, HLA-A03 or HLA-A24 positive) after HSCT. P value limits: * $P \leq 0.05$; ** $P \leq 0.005$; *** $P \leq 0.0005$.

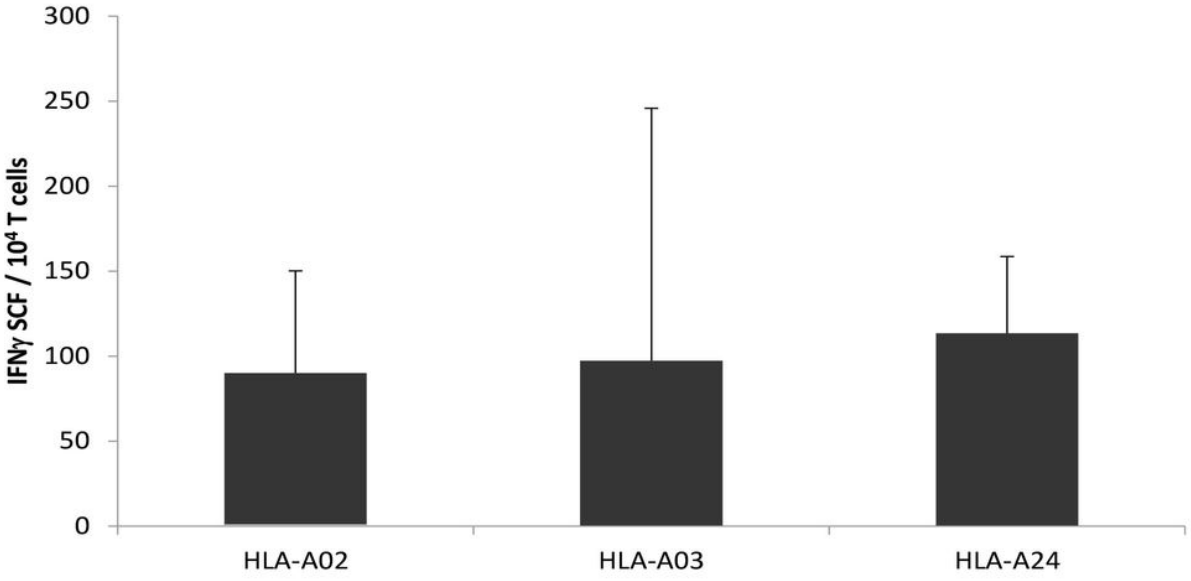

Figure 2

Influence of HLA-A alleles on the derivation of NME2-specific CTLs. CTL populations derived from HLA-A02 (n=4), HLA-A03 (n=6) or HLA-A023 (n=5). PBMNC samples were tested for NME2-specific reactivity in an Elispot assay challenged with aAPC expressing NME2 together with the appropriate HLA-A protein. The data shown are mean and standard deviation above background. There is no significant difference.

Figure 3a

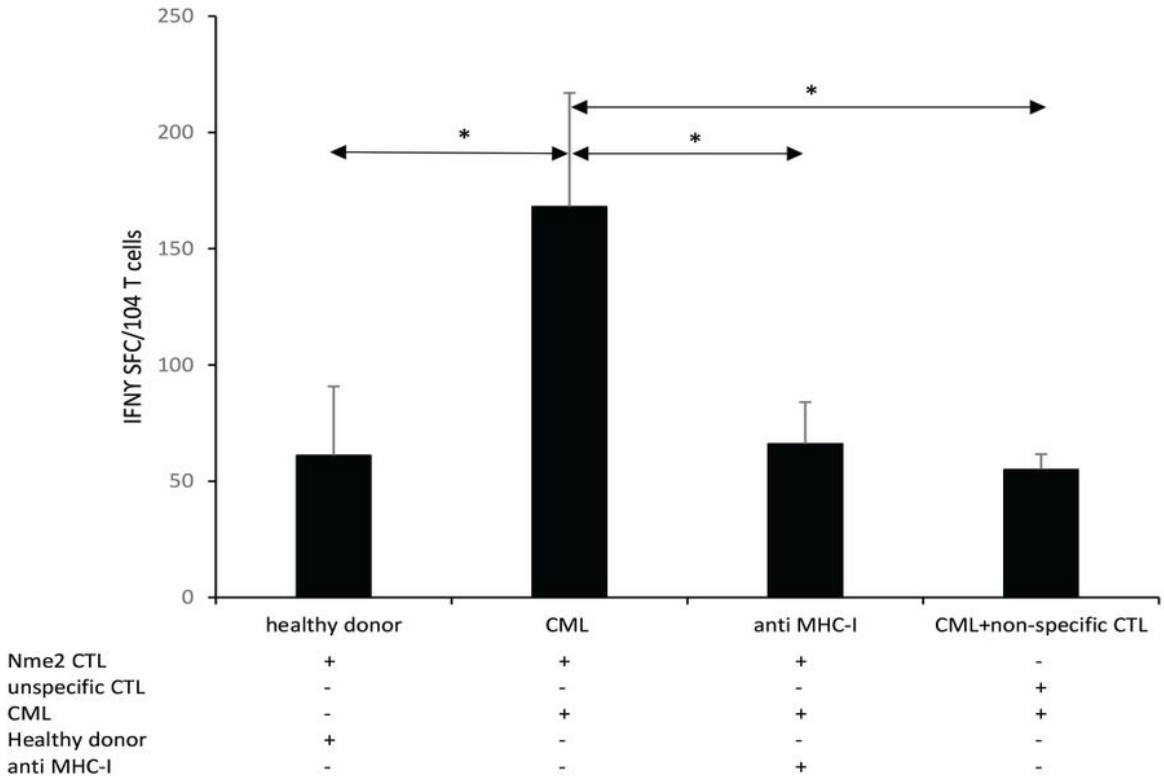

Figure 3b

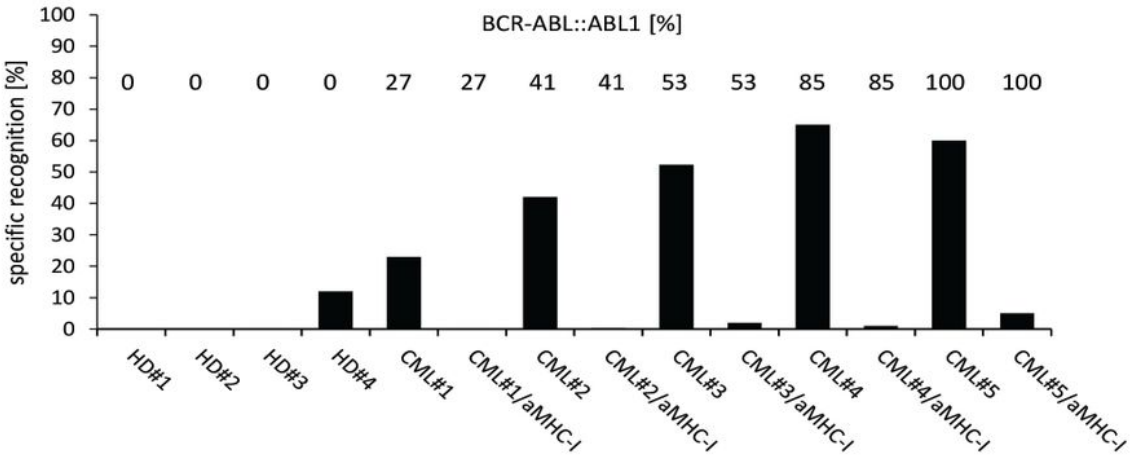

Figure 3

NME2-specific CTLs detect primary HLA-A matched CML-cells (A) Specificity, assessed by IFNγ ELISpot assay, of NME2-specific CTLs against HLA-matched primary CML-cells, primary CML-cells after preincubation with anti-HLA-class I antibody, CD14+ healthy donor cells and unspecific CTLs against primary CML cells **(B)** Specific recognition of 5 different primary HLA matched CML samples without (CML#1-5) and with HLA-class I antibody CML#1-5/aMCH-I) and four CD14+ healthy donor cells (HD#1-

4) by NME2-specific CTLs in correlation to the ratio BCR-ABL1::ABL1. P value limits: * P ≤ 0.05; ** P ≤ 0.005; *** P ≤ 0.0005.

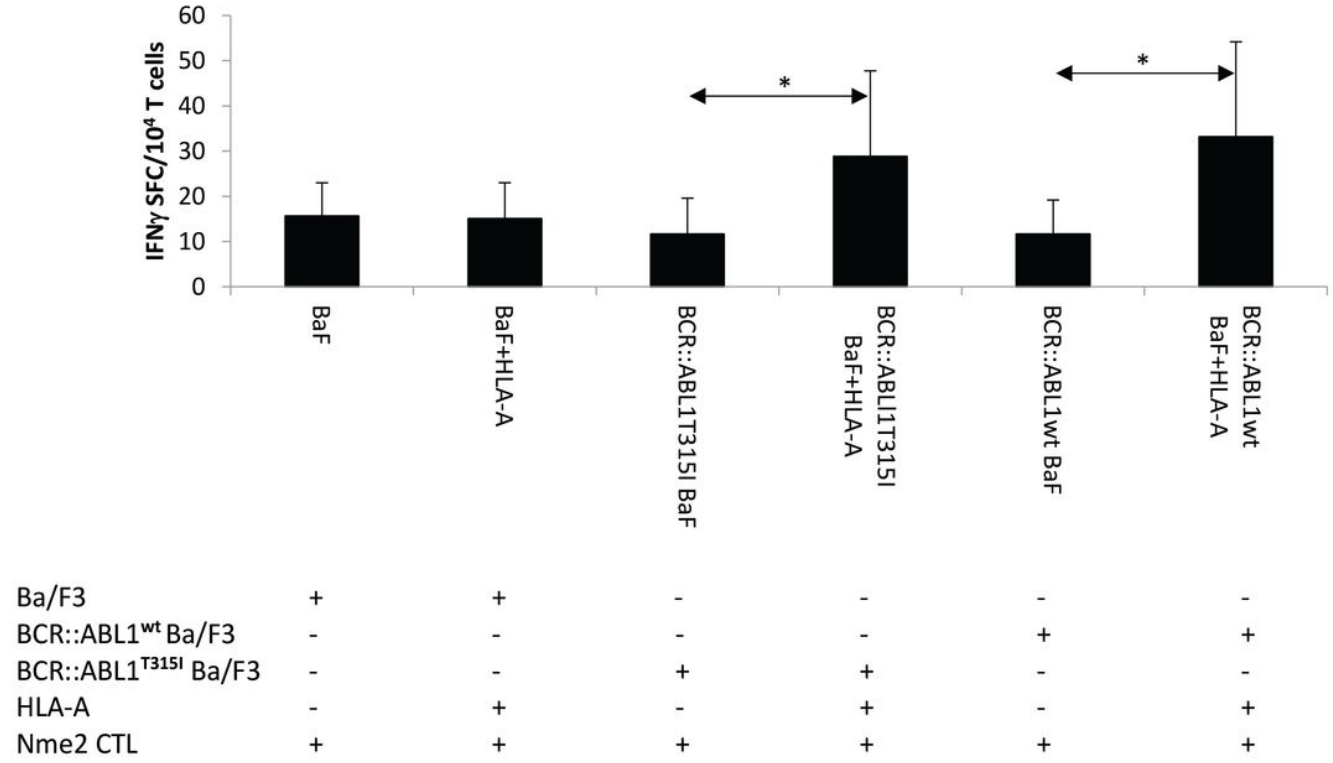

Figure 4

Interferon-γ ELISpot-assay for specificity of NME2 recognition irrespective of T315I mutation in the BCR::ABL1 tyrosine kinase. The spot counts of NME2 specific Figure 4 **Interferon-γ ELISpot-assay for specificity of NME2 recognition irrespective of T315I mutation in the BCR::ABL1 tyrosine kinase.** The spot counts of NME2 specific CTLs derived from HLA-A02 and HLA-A03 PBMCs of CML-patients after HSCT, in response to HLA-A transfected and non-transfected Ba/F3-cells, expressing wild type BCR::ABL1, the T315I mutation of BCR::ABL1 and no BCR::ABL1. P value limits: * P ≤ 0.05; ** P ≤ 0.005; *** P ≤ 0.0005.

Figure 5a

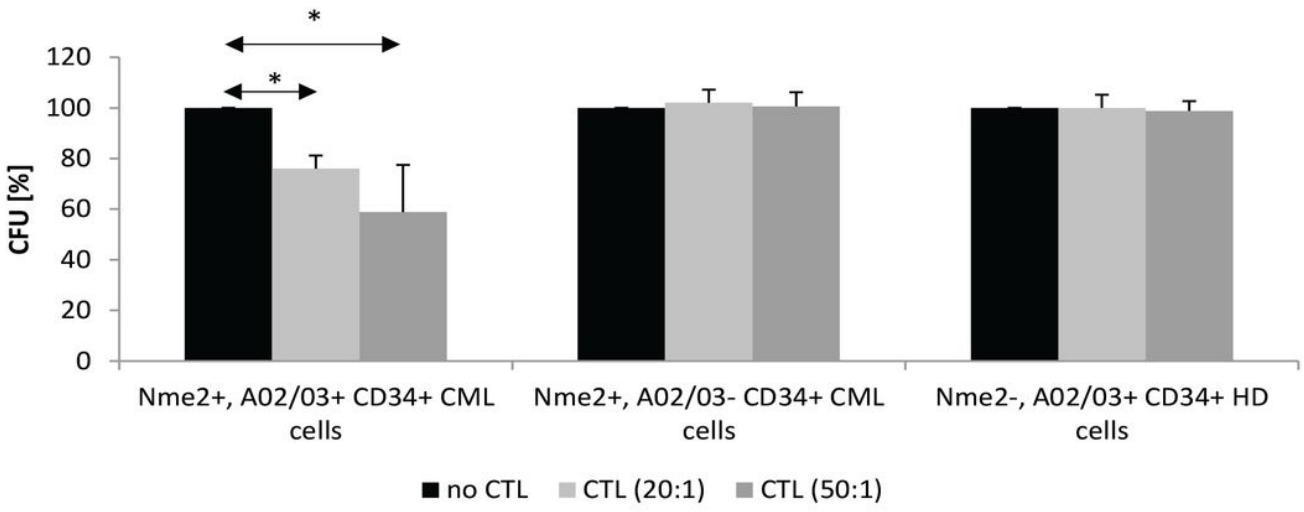

Figure 5b

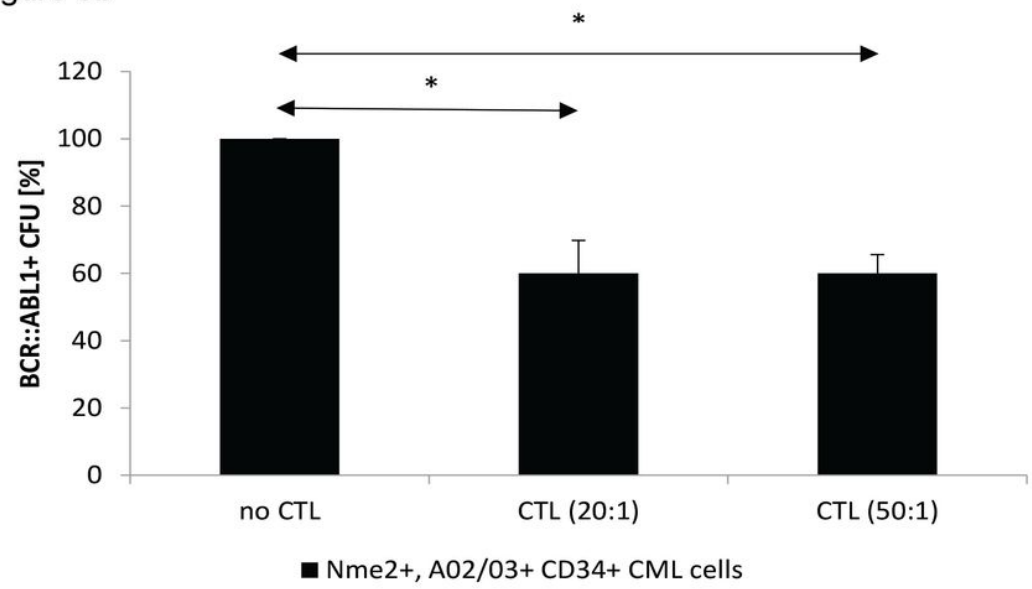

Figure 5

NME2-specific CTLs diminished the count of CML progenitors in Colony forming inhibition assay. (a) Bone marrow derived CD34+ of 3 HLA-A02 or A03^{+} healthy donors, 5 HLA-A02 or A03^{+} and 2 HLA-A02/A03^{-} patients with CML at diagnosis were co-incubated with NME2-specific CTLs in an effector:target ratio of 20:1 and 50:1. NME2-specific CTLs had significantly antileukemic effects against leukemic precursor cells, when HLA matched CML precursors were co-incubated with NME2-specific CTLs (b) BCR::ABL1 positive CFU were significantly diminished by NME2-specific CTLs. P value limits: * $P \leq 0.05$; ** $P \leq 0.005$; *** $P \leq 0.0005$.

Supplementary Files

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