Blocking programmed cell death 1 in combination with adoptive cytotoxic T-cell transfer eradicates chronic myelogenous leukemia stem cells

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In chronic myelogenous leukemia (CML), quiescent, self-renewing leukemia stem cells (LSCs) are resistant against chemotherapy and specific tyrosine kinase inhibitors (TKIs).\(^1\,2\) During treatment, LSCs remain in the bone marrow (BM) and cause disease relapse after drug discontinuation.\(^3\,4\) Moreover, persisting LSCs may acquire BCR-ABL1 mutations that interfere with TKI binding or accumulate genetic alterations that lead to blast crisis. T-cell-based immunotherapy has been shown to be efficacious in solid tumors and in leukemia\(^5\) and allogeneic stem cell transplantation and donor lymphocyte infusions (DLIs) are the only curative treatments in CML.\(^6\) However, LSCs evolved several mechanisms to escape cytotoxic CD8\(^+\) T cells (CTLs)-mediated immune attack.\(^3\) We recently demonstrated that interferon gamma (IFN-\(\gamma\)) secreted by adoptively transferred leukemia-specific CTLs in a situation with high leukemia load even increased the numbers of LSCs while more differentiated leukemia cells were efficiently eliminated.\(^7\) Why LSCs are selectively resistant against elimination by CTLs is unknown. One major immune escape mechanism of tumor cells is the dysregulation of T-cell inhibitory pathways, so-called immune checkpoints.\(^1\) Indeed, CML cells express T-cell inhibitory ligands such as programmed cell death ligand 1 (PD-L1), light and herpes virus entry mediator and high expression of PD-L1 on CD34\(^+\) CML cells is a negative prognostic factor.\(^8\,9\) Here, we addressed the expression of PD-L1 in the differentiation hierarchy of BM CML stem/progenitor cell subsets.\(^4\,10\) CML was induced by retroviral transduction of BM from 5-fluorouracil-treated H8 transgenic mice\(^11\) with BCR-ABL1-GFP, followed by transfer to sublethally irradiated C57BL/6 (BL/6) recipient mice (H8 CML). In this setting, BCR-ABL1-GFP\(^+\) leukemia cells express the glycoprotein epitope gp33 of lymphocytic choriomeningitis virus (LCMV) under the control of a major histocompatibility complex-I (MHC-I) promoter as model leukemia antigen. Nineteen days after leukemia induction, H8 CML mice with comparable leukemia burden (109\(\pm\)13 BCR-ABL1-GFP\(^+\)Gr-1\(^+\) granulocytes/\(\mu\)l blood) were randomized to control treatment with vehicle (Veh), p14 CTLs (p14), a blocking PD-1 monoclonal antibody (mAb; αPD-1, clone RMP1-14), treatment with PD-1-deficient p14 CTLs (p14xPD-1\(^−/−\)) or p14 CTLs in combination with αPD-1 (αPD-1/p14), p14xPD-1\(^−/−\) and αPD-1/p14 treatments reduced leukemia burden as indicated by smaller spleen size and lower numbers of leukemia progenitors in the BM compared with Veh, αPD-1 and p14 CTL-treated groups (Figures 1e–g). As reported previously,\(^7\) p14 CTL transfer increased LSC numbers (Figures 1h and i). Importantly, PD-1 blocking during adTf of p14 CTLs significantly reduced LSCs and resulted in fewer BCR-ABL1-GFP\(^+\) methylcellulose colonies from Lin\(^−\) BM cells (Figures 1h and i). In CML, only LT-LSCs are able to propagate the disease in vivo, whereas other LSC subsets and more differentiated progenitors are able to form colonies in vitro.\(^1\,2\) To investigate if the reduced numbers of LSCs as defined immunophenotypically by FACS or functionally in vitro actually accounted for reduced numbers of leukemia-initiating cells in vivo, we secondarily transplanted BM cells from H8 CML mice of all treatment groups into lethally irradiated BL/6 mice. Animals transplanted with BM from p14 CTL-treated CML mice succumbed significantly faster to the disease than mice after transplantation of Veh-treated control CML BM.\(^7\) In contrast, mice transplanted with BM from αPD-1-treated H8 CML mice survived significantly longer than controls, but the prolongation of survival was marginal. Importantly, only PD-1 blockade during transfer of p14 CTLs in primary CML mice substantially prolonged the survival of secondary recipient mice, indicating that this treatment indeed targets leukemia-initiating cells (Figure 1j). However, LSCs were not eliminated completely within 2 days after treatment (Figures 1h–j).

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To assess the therapeutic role of PD-1 blockade during adTf in more detail, H8 CML mice (161 ± 16 BCR-ABL1-GFP+Gr-1+ granulocytes/μl blood) were treated as described and disease progression and survival were monitored. Transfer of p14 CTLs or treatment with αPD-1 mAb alone did not improve survival. In contrast, PD-1 blockade during CTL transfer led to long-term survival of all H8 CML mice (Figure 1k). These data suggest that LSCs were either eliminated or effectively controlled by this treatment.

Therefore, we next analyzed if the surviving mice had eliminated LSCs 73 days after adTf of CTLs. Some mice harbored residual disease as indicated by low percentages of BCR-ABL1-GFP+Gr-1+ granulocytes in peripheral blood (Figure 1l). In addition, Lin+ BM cells from surviving animals still formed few BCR-ABL1-GFP+ colonies (Figure 1m). To functionally investigate whether leukemia-initiating cells had persisted, we transferred BM cells from surviving mice into lethally irradiated secondary BL/6 recipients. Only 3 out of 10 secondary recipients developed a CML, whereas the other 7 survived long term without signs of leukemia, as analyzed by FACS of peripheral blood (Figure 1n and data not shown). These results indicate that one single transfer of leukemia-specific effector CTLs in combination with genetic deletion or mAb blocking of PD-1 eliminated the disease-initiating LSCs in a majority of animals treated at a late stage of the disease.

LCMV-gp33 was used in the first experiments as a model leukemia antigen that allows an in-depth characterization of T-cell responses. To analyze whether our findings are of relevance in a more physiological setting, we made use of an MHC-I mismatch model (Balb/c × BL/6 F1-generation, CB/6) that has been used to analyze the effect of human leukemia antigen mismatched DLIs.15 CB/6 in BL/6 mice consists of H2-Kb/Kd15; Balb/c mice express H2-Kb/Dd. CB/6 mice consequently express H2-Kb/Dd, whereas the other 7 survived long term without signs of leukemia, as analyzed by FACS of peripheral blood (Figure 1n and data not shown). These results indicate that one single transfer of leukemia-specific effector CTLs in combination with genetic deletion or mAb blocking of PD-1 eliminated the disease-initiating LSCs in a majority of animals treated at a late stage of the disease.

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PD-1 treatment alone was largely ineffective. This may be because of the fact that at later stages of disease, the majority of leukemia-specific T cells have been exhausted and deleted.8 Numerous leukemia-associated antigens (LAAs) are expressed by human CML cells that may be used for adoptive immunotherapy.16 However, not all of these antigens are expressed by LSCs. Stem-cell enriched cells from CML patients preferentially express the LAAs Wilms tumor protein (WT-1) and PRAME, but not proteinase 3, survivin or human telomerase (hTert).16 Therefore, WT-1 and PRAME may represent promising therapeutic targets for CTL-based immunotherapy in combination with αPD-1-treatment to eliminate LSCs.
Figure 2. (a–g) CB/6 CML mice \((n = 4\) mice per group) were treated either with rat-IgG (Veh, 200 μg intraperitoneally every third day), \(8 \times 10^6\) MACS-purified CD8+ T cells from BL/6 mice (CD8), αPD-1 mAb (αPD-1, 200 μg intraperitoneally every third day) alone or in combination with BL/6 CD8+ T cells (αPD-1/CD8) starting 11 days after CML induction. Ten days later, lin−BCR-ABL1-GFP+ BM was analyzed for (a) LSCs (c-kit+Scal+), (b) LT-LSCs (c-kit+Scal−CD135−CD48−CD150+), (c) ST-LSCs (c-kit+Scal−CD135−CD48−CD150−), (d) leukemia MPP1s (c-kit+CD135+CD48+CD150−) and (e) leukemia MPP2s (c-kit+CD135−CD48−CD150−). (f) Equal numbers of total lin− cells were plated in methylcellulose and BCR-ABL1-GFP+ colonies were enumerated 7 days later by inverted fluorescence microscopy. (g) Primary CB/6 CML mice were treated as described in a starting 11 days after transplantation. Ten days later, \(5 \times 10^6\) BM cells were transplanted into lethally irradiated (2 × 6.5 Gy) recipient mice \((n = 4\) mice per group) and survival was monitored. (h) Primary CB/6 CML mice were treated as described in a starting 11 days after transplantation \((n = 5\) per group). (h) Numbers of BCR-ABL1-GFP+ granulocytes/μl blood and (i) Kaplan–Meier survival curves \((n = 5\) mice per group). (j) BCR-ABL1-GFP+ colonies in lin− BM and (k) survival of lethally irradiated (2 × 6.5 Gy) secondary recipients \((n = 4\) mice) that received \(1 \times 10^7\) BM cells from αPD-1/CD8-treated primary CML mice that were alive 90 days after CML induction. (i, k) Numbers of mice that succumbed to CML of total transplanted mice are indicated. Data are displayed as mean ± s.e.m. Statistics: (a–f) one-way analysis of variance (ANOVA), (g, i) log-rank test, (h) two-way ANOVA. *\(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\).
CONFICT OF INTEREST
The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS
CR designed and performed experiments, analyzed data and wrote the manuscript; TG, A-LH and CMS performed experiments and analyzed data; AFO designed experiments, analyzed data and wrote the manuscript. All authors revised the manuscript and approved the final version.

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Cellular origin of prognostic chromosomal aberrations in AML patients

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Acute myeloid leukemia (AML) represents an aggressive cancer entity, whose malignant cells respond abnormally to regulatory stimuli and have lost the ability to differentiate and become fully mature blood cells.1,2 AML evolves through accumulation of independent genetic aberrations, including chromosomal structural rearrangements and single nucleotide variants (SNVs). Conventional AML diagnostics and recent seminal next-generation sequencing (NGS) studies have identified more than 200 recurrent genetic aberrations, presenting in various combinations in individual patients.1,3–7 Significantly, many of these aberrations occur in normal hematopoietic stem and progenitor cells (HSCs/HPCs) before definitive leukemic transformation through additional acquisition of a few (that is, mostly 1 or 2) leukemia-promoting driver aberrations.5 NGS studies on sorted bone marrow (BM) populations of AML patients with a normal karyotype have demonstrated the presence of prognostic driver aberrations (that is, NPM1, FLT3-ITD and FLT3-TKD) in committed HPCs but not in multipotent HSCs.8–10 However, the HSC populations lacking the prognostic driver aberrations contained preleukemic clones harboring a series of recurrent molecular aberrations that were present in the fully transformed committed HPCs together with the prognostic driver aberration.8,9,10,11 Adding to this vast heterogeneity and complexity of AML genomes and their clonal evolution, a recent study of a murine AML model demonstrated that (9;11) AML originating from HSCs responded poorly to in vivo chemotherapy treatment as compared with (9;11) AML originating from HPCs.12 Hence, recent advances in genetics support that AML is initiated and sustained by a few aberrations that, in concert with their cellular origin, define the leukemic phenotype of AML patients and ultimately clinical outcome following conventional chemotherapy.

In the present study we examined the variegation among AML patients with prognostic chromosomal driver aberrations with respect to (i) the cellular composition of their HSC/HPC compartment, (ii) the cellular origin of their ‘driver’ aberrations and (iii) the expression of aberrant transcriptional programs as compared with normal.

For this, we applied an integrative experimental strategy combining flow cytometry-based immunophenotyping, as well as

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