Mutation-specific control of BCR-ABL T315I positive leukemia with a recombinant yeast-based therapeutic vaccine in a murine model

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ABSTRACT

Chromosomal translocations generating the *BCR-ABL* oncogene cause chronic myeloid leukemia (CML) and a subset of acute lymphoblastic leukemia. The BCR-ABL[T315I] mutation confers drug resistance to FDA-approved targeted therapeutics imatinib mesylate, dasatinib, and nilotinib. We tested the ability of a recombinant yeast-based vaccine expressing the T315I-mutated BCR-ABL antigen to stimulate an anti-BCR-ABL[T315I] immune response. The yeast-based immunotherapy significantly reduced or eliminated BCR-ABL[T315I] leukemia cells from the peripheral blood of immunized animals and extended leukemia-free survival in a murine model of BCR-ABL+ leukemia compared to animals receiving sham injection or yeast expressing ovalbumin. With immunization, leukemic cells harboring BCR-ABL[T315I] were selectively eliminated after challenge with a mixed population of BCR-ABL and BCR-ABL[T315I] leukemias. In summary, yeast-based immunotherapy represents a novel approach against the emergence of cancer drug resistance by the pre-emptive targeted ablation of tumor escape mutants.

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1. Introduction

The *BCR-ABL* oncogene results from a chromosomal translocation at position t(9;22)(q34;q11) [1,2]. *BCR-ABL* is causal for chronic myelogenous leukemia (CML) and present in 20–40% of adults with acute lymphoblastic leukemia (ALL) [3–6]. CML and ALL result in a massive accumulation of clonal myeloid or lymphoid progenitor cells respectively. Untreated CML results in death at a median of 3 years after diagnosis, while the more aggressive ALL results in death at a median of 11 months after diagnosis, even with chemotherapy [6,7]. The targeted BCR-ABL kinase inhibitor, imatinib mesylate (IM), achieves a complete cytogenetic response at 18 months in 76% of patients with CML [8–11]. However, after 3.5 years, ~16% of chronic phase CML patients relapse despite IM therapy. Most of these relapses are due to BCR-ABL point mutations that prevent IM binding and restore aberrant kinase activity [12,13]. The T315I mutation is a particularly relevant target, as this "gatekeeper" mutation occurs with the highest incidence (16%, [14–16]) and is the only point mutation causing cross-resistance to both IM and second generation kinase inhibitors [17–19].

Investigational approaches for drug-resistant leukemia treatment involve small molecule inhibitors effective against BCR-ABL[T315I] and other drug escape mutations, inhibitors targeting effectors downstream of BCR-ABL, or drugs with epigenetic targets [20,21]. An attractive alternative would be to activate T cell immunity in CML or ALL patients against tumor cells expressing leukemia-specific or leukemia-associated antigens [22]. The mutated BCR-ABL[T315I] protein is a tumor specific antigen, and thus is not subject to central self-tolerance mechanisms [23,24]. The activation of T cell immunity against IM escape mutants in combination with IM treatment could suppress or eliminate the emergence of resistant variants.

Immunotherapy with whole heat-killed recombinant Tarmogen® yeast delivering the target antigen activates a potent innate response to the yeast vector, plus an adaptive T cell immune response to the target antigen. Thus, immune recognition of the target antigen is directly coupled to the innate danger response against the yeast vector and uptake by phagocytic dendritic cells (DC), macrophages and neutrophils. Another advantage of this immunotherapy approach is the delivery of target polypeptides whose processing by host DCs affords a full spectrum of epitopes derived from the polypeptide, facilitating presentation of and response to epitopes relevant for each person's HLA (MHC-I and MHC-II) receptor repertoire.

Immunization with GI-4000 Tarmogen® yeast elicits mutation-selective killing of tumors harboring the mutated Ras protein in a carcinogen-induced murine lung tumor model [25,26]. GI-4000

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Tarmogen® yeast delivering mutated Ras polypeptides are being tested in clinical trials in patients with pancreatic cancer, non-small cell lung cancer, and colorectal cancer. In those trials, the tumors are genotyped to establish the presence of *ras* mutations and to administer the GI-4000 Tarmogen® yeast product that would activate T cell immunity against the matched mutant Ras protein (e.g. ClinicalTrials.gov Identifier: NCT00300950). Given the immune activation against mutated Ras proteins using Tarmogen® yeast immunotherapy, we tested whether this approach would elicit T cell responses against the T315I mutation in BCR-ABL, for the purpose of preventing or limiting BCR-ABL[T315I] IM drug-resistance.

2. Materials and methods

2.1. MHC-binding peptide prediction algorithms and REVEAL assay

Eighty-one overlapping 8–10 amino acid peptides spanning BCR-ABL[T315I] and two other BCR-ABL IM point mutations, E255K and M351T, were analyzed for potential CD8^{+} T cell epitopes. The MHC-binding peptide prediction algorithms, Arbmatrix [27], libscore [28], smm [29], arb [27], bimas [30], SYFPEITHI [31] and ann [32], were used to predict binding to the C57BL/6 and BALB/c MHC class I alleles; H-2D[b], H-2K[b], H-2D[d], H-2K[d] and H-2L[d]. Prediction software algorithms with accuracy scores estimated to be higher than 66.5% for any given allele were used for these analyses (Supplementary Fig. 1). The 62 highest scoring peptides were synthesized. These were evaluated using the REVEAL® *in vitro* H-2D[b] and H-2K[b] MHC class I-binding assays performed by Proimmune.

2.2. Computer modeling

Crystal structures were used for generating the H-2K[b]/nanomeric peptide model (pdb 1VAC [33]) and the H-2K[b]/octameric peptide model (2VAB [34]) by using the swiss pdb viewer program [35]. The initial amino acid conformations of the peptides were manually adjusted to avoid clashes. Energy minimization refinement was performed on the peptides in CNS [36]. The H-2K[b] molecules were fixed in the refinement. The final models were inspected in O [37] on Silicon Graphics OCTANE2 Workstations.

2.3. Animals

Wild-type BALB/c and C57BL/6 mice were purchased from the National Cancer Institute or bred at the University of Colorado Denver School of Medicine. The Institutional Animal Care and Use Committee approved all experiments using mice.

2.4. Primary leukemia generation

Murine stem cell viruses (MSCV) were prepared by transient transfection of Pheonix-E cells together with pCL-Eco (encodes Gag, Pol and Env), and titered on fibroblasts using GFP or RFP as the marker BCR-ABL expression. Bone marrow cells were harvested from C57BL/6 or BALB/c mice and enriched for CD117^{+} cells by MACS® (Miltenyi Biotec, Bad Gladback, Germany). The cells were first cultured overnight in DMEM plus 10% defined fetal bovine serum with the cytokines hSCF (gift from Dr. Chris Hogan, UC Denver), hIL-11, mIL-3, and hIL-6 (PeproTech, Rocky Hill, New Jersey, USA). The cells were infected for 6 h with 6 μL/mL Polybrene (Sigma) and supernatants containing MSCV-ires-GFP or MSCV-ires-RFP viruses encoding p210 *BCR-ABL* or p210 *BCR-ABL[T315I]* (the drug-resistant mutant was a gift from Drs. Michael Deininger and Brian Druker, Oregon Health Sciences University). The cells were then transplanted intravenously into sublethally

irradiated (3 Gy) mice using an X-ray source (RadSource RS2000 irradiator). Infection efficiencies were determined after culturing aliquots of transduced cells under HSC conditions for 48–72 h [38]. Primary leukemias expressing BCR-ABL or BCR-ABL[T315I] developed with similar kinetics between 3 and 8 weeks. Transplanted mice were monitored for disease development, as judged by increasing percentages of GFP or RFP expressing cells with blast morphology in peripheral blood of transplanted animals, as well as symptoms, such as reduced mobility, hunching, and labored breathing. Moribund animals were sacrificed and leukemias were characterized and stored at −80 °C. Automated hematocrit analysis of peripheral blood was performed using a Cell-Dyn 1700 System (Abbott, Abbott Park, IL, USA).

2.5. Yeast vaccine

Immunotherapy constructs were made by subcloning the *BCR-ABL* junction, SH3, SH2 and kinase domains by blunt end ligation into the pBlueScript SKII-® vector (Fermentas) and introducing point mutations using sequential QuikChange Site Directed Mutagenesis Kit® (Stratagene). To avoid triggering a xenogeneic immune response of the human Abl protein by the murine immune system, two amino acids were altered to match the mouse *ABL* sequence ortholog (Fig. 1a). The altered *ABL* sequence spanning just downstream of the junction epitope through the region encoding the three escape mutant epitopes was cloned into the Tarmogen® expression plasmid under control of a strong constitutive promoter (Fig. 1a). A C-terminal hexahistidine tag was added for detection and quantification. The cloned DNA inserts were bidirectionally sequenced and the plasmid was transfected into *S. cerevisiae* yeast (W303alpha) using the EZ yeast transfection kit (Zymo research). Transfectants were selected on solid medium lacking uracil and growth of cultures was in medium containing 20 g/L glucose, 6.7 g/L yeast nitrogen base, and 2 mg/mL each of adenine, histidine, tryptophan, and leucine. Liquid yeast cultures were grown to mid-exponential phase, harvested by centrifugation, washed once in PBS, and heat inactivated at 56 °C for 1 h. The yeast were washed three times in PBS and re-suspended in PBS at 20 YU/mL (1 YU = 10[7] Tarmogen® yeast cells). Tarmogen® yeast (5 YU) were injected subcutaneously at 2 sites per immunization. Dosing was administered 10, 8, 6 and 1 weeks prior to leukemia challenge. The parental yeast vector expressing an irrelevant antigen, chicken ovalbumin (yeast[OVA]) was used as a control immunogen [39].

2.6. Detection of Abl T315I protein by western blotting

The expression of Abl protein by yeast[T315I] was quantified by western blotting using an anti-(His)[6] tag monoclonal antibody. Additional positive identification of the expressed protein was conducted by immunoblotting with a monoclonal antibody recognizing the SH2 domain of the Abl protein (Mouse Anti-ABL Monoclonal Antibody 554148, BD Pharmingen).

2.7. Challenge with primary leukemias

Primary leukemia cells (1.7×10^5 GFP[+] or RFP^{+} cells) suspended in 200 μL IMDM/1% FBS were used for challenging vaccinated or control recipient mice, which resulted in leukemia development in most control recipients between 10 and 14 days post-transfer. Challenged mice were monitored at least once per day for secondary leukemia development as described above.

2.8. Flow cytometric analysis

Tail blood, spleen and bone marrow cells were isolated, and the contributions of BCR-ABL expressing cells to hematopoiesis as

(A)

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Human   VIVHSATGFKQSSKALQRPVASDFEPQGLSEAARWNSKENLLAGPSENDPNLFVALYDFV   73
Mouse   VIVHSATGFKQSSKALQRPVASDFEPQGLSEAARWNSKENLLAGPSENDPNLFVALYDFV   73

Human   74   ASGDNTLSITKGEKLRVLGYNHNGEWCEAQTKNGQGWVPSNYITPVNSLEKHSWYHGPVS   133
Mouse   74   ASGDNTLSITKGEKLRVLGYNHNGEWCEAQTKNGQGWVPSNYITPVNSLEKHSWYHGPVS   133

Human   134   RNAAEYLLSSGINGSFLVRESESSPGQRSISLRYEGRVYHYRINTASDGKLYVSSESRFN   193
Mouse   134   RNAAEYLLSSGINGSFLVRESESSPGQRSISLRYEGRVYHYRINTASDGKLYVSSESRFN   193

Human   194   TLAELVHHSTVADGLITTLHYPAPKRNKPTVYGVSPNYDKWEMERTDITMKHKLGGGQY   253
Mouse   194   TLAELVHHSTVADGLITTLHYPAPKRNKPTIYGVSPNYDKWEMERTDITMKHKLGGGQY   353

Human   254   GEVYEGVWKKYSLTVAVKTLKEDTMEVEEFLKEAAVMKEIKHPNLVQLLGVCTREPPFYI   313
Mouse   254   GEVYEGVWKKYSLTVAVKTLKEDTMEVEEFLKEAAVMKEIKHPNLVQLLGVCTREPPFYI   313

Human   314   ITEFMTYGNLLDYLRECNRQEVNAVVLLYMATQISSAMEYLEKKNFIHRDLAARNCLVGE   373
Mouse   314   ITEFMTYGNLLDYLRECNRQEVSAVVLLYMATQISSAMEYLEKKNFIHRDLAARNCLVGE   373

Human   374   NHLVKVADFGLSRLMTGD   391
Mouse   374   NHLVKVADFGLSRLMTGD   391
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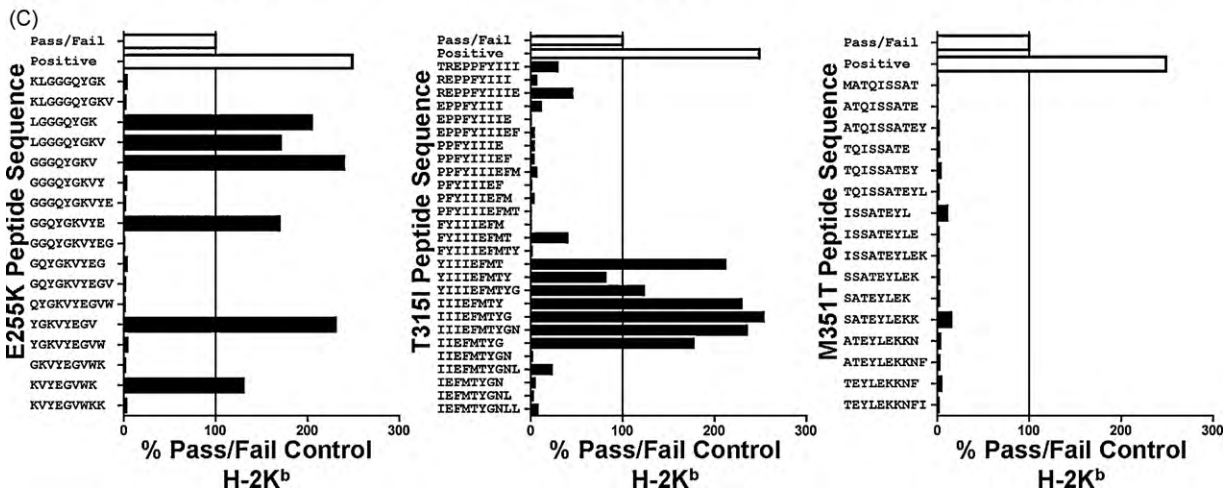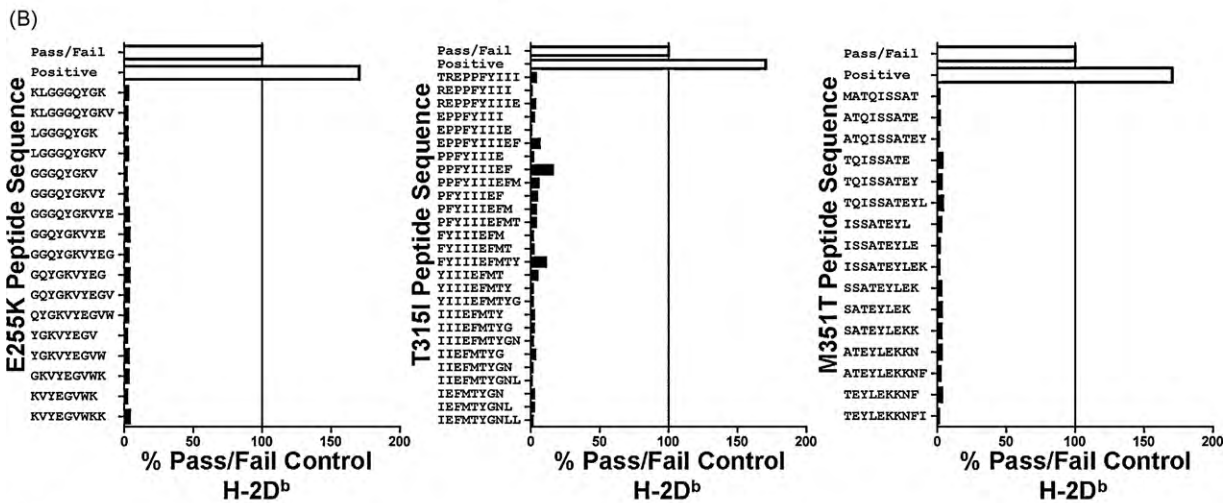

Fig. 1. Six BCR-ABL^{T315I} peptides bound H-2K^b *in vitro*. Partial human (top row) and mouse (bottom row) BCR-ABL amino acid sequences are shown. The BCR and ABL fusion-point (b3a2) is underlined and commonly mutated amino acids that result in IM drug-resistance are bold and underlined. Orthologous differences between human and mouse sequence are in bold. The amino acid sequence expressed by yeast^{T315I} is bracketed (A). Candidate peptides were synthesized in a PEPscreen library® (ProImmune). Binding scores from the *in vitro* MHC class I peptide binding assay (REVEAL®, ProImmune) are represented as percent signal over a weakly binding control peptide (% Pass/Fail Control) for the C57BL/6 alleles H-2D^b (B) and H-2K^b (C).

well as developing leukemias was determined using flow cytometry. Single-cell suspensions of hemolysed bone marrow, spleen or peripheral blood were washed in PBS containing 1% BSA (PBS/1% BSA), and resuspended in PBS/1% BSA plus 5% supernatant from hybridoma cells producing the 2.4G2 monoclonal antibody against the Fc receptor. Cells were stained in 20 μL of antibody for 20 min at room temperature. Cells were washed once with 1 mL of PBS/1% BSA and resuspended in 400 μL of PBS/1%BSA/2 mM EDTA for flow cytometric analysis. Fluorescence was detected with CyAn (DAKO, Carpinteria, CA, USA) or Cell Quanta SC MPL (Beckman Coulter, Allendale, New Jersey, USA) cytometers. PE-conjugated α-H-2L^d/D^b (43-1-2S) and PE-conjugated α-H-2K^d/D^d/K^b (28-14-8) were used for MHC class I expression studies.

2.9. Statistical analysis

Statistical analysis was performed using GraphPad Prizm 4 software. Asterisks represent p values of 2-tailed t-tests. "*" is used for $p < 0.05$, "**" for $p < 0.01$, and "***" for $p < 0.001$. The development of fatal leukemias was plotted on Kaplan/Meier curves, and the statistical significance of yeast^{T315I} therapy was determined using the logrank test.

3. Results

3.1. BCR-ABL^{T315I} peptides bind MHC class I

The BCR-ABL^{E255K}, BCR-ABL^{T315I} and BCR-ABL^{M351T} mutations are the three most common BCR-ABL mutations conferring resistance to IM found in treated patients (Fig. 1a) [40]. Prior to testing *in vivo*, overlapping 8–10 amino acid peptides spanning the drug-resistance mutations BCR-ABL^{E255K}, BCR-ABL^{T315I} and BCR-ABL^{M351T} (Fig. 1a) were analyzed using MHC-binding peptide prediction algorithms to identify peptides able to be presented to CD8$^+$ T cells (Supplementary Fig. 1). Mutant epitopes with sufficient binding affinity to be detected were identified in an *in vitro* MHC class I binding assay (Proimmune, REVEAL®). Both C57BL/6 MHC class I alleles were tested. None of the peptides were found to bind H-2D^b (Fig. 1b). Six BCR-ABL^{E255K} peptides and six BCR-ABL^{T315I} peptides were found to bind the H-2K^b MHC class I allele (Fig. 1c), while none of the BCR-ABL^{M351T} peptides were of sufficient binding affinity in this assay to be scored positively. When H-2K^b binding BCR-ABL^{T315I} peptides were graphed, they exhibited a bell-shaped normal Gaussian distribution with an R^2 of 0.8364 (Supplementary Fig. 2) indicating that of the T351I peptides tested, those with an asparagine carboxy terminal cleavage site were optimal and deviations from this position impaired peptide binding. Computer modeling simulations were consistent with binding of T315I peptides IIIEFMTY and YIIIEFMTY to the H-2K^b allele (Fig. 2a), with the 315I mutant amino acid facing the TCR in P3 or P4 respectively (Fig. 2b). These results indicated that peptides framing the BCR-ABL^{T315I} mutation were likely to be presented to CD8 T cells by activated DCs in the C57BL/6 mouse strain. The clinically significant BCR-ABL^{T315I} mutation was pursued in the remaining experiments, given that this common mutation confers resistance to all approved BCR-ABL kinase inhibitors.

3.2. Primary leukemia challenge into immunocompetent recipients

A model system for testing the immunogenicity of the T315I mutation was developed to be compatible with BCR-ABL$^+$ leukemogenesis, a full vaccination course and an intact immune system. Murine BCR-ABL$^+$ leukemias were produced by retroviral transduction of the full length p210 BCR-ABL gene into donor bone marrow stem and progenitor cells followed by a sublethal irradiation and

Vaccine, Melanie R Bui, Fig. 2.
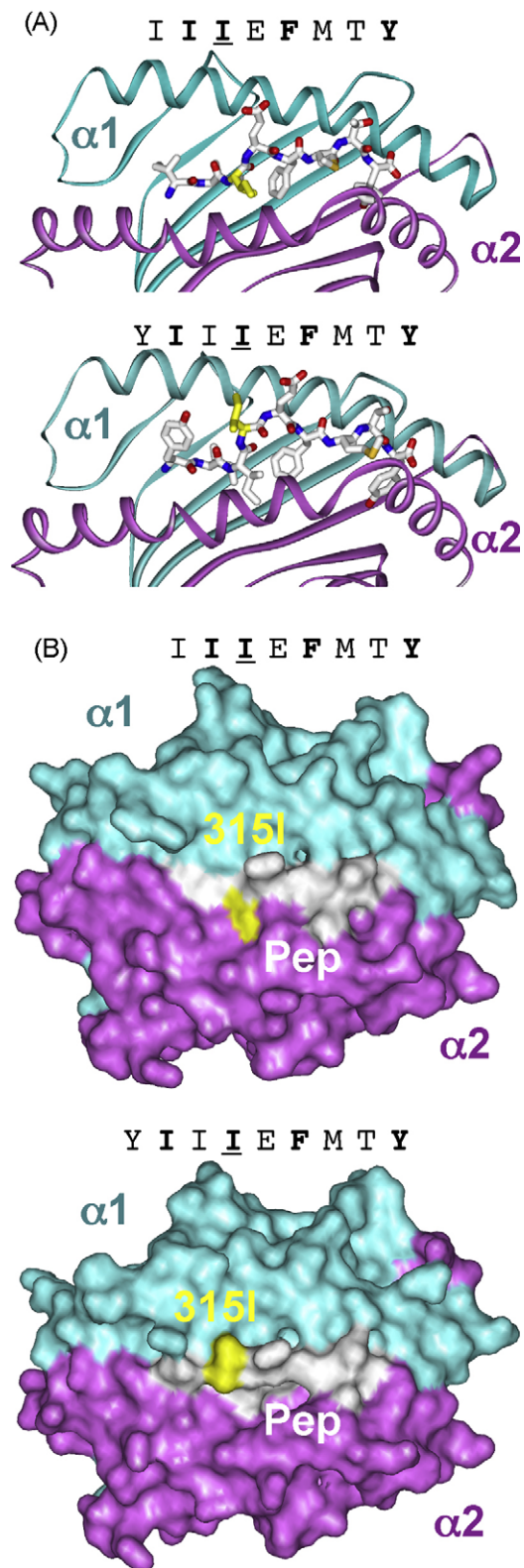
Fig. 2. Computer modeling simulations represent the T315I peptides IIIEFMTY and YIIIEFMTY binding to the H-2K^b MHC class I allele. The peptide is in 'ball-and-stick' format where carbon atom are gray, nitrogen atoms are blue, and oxygen atoms are red (A). Side view (A) and surface view (B) are shown. The mutant isoleucine residue is yellow, the peptide is white, the MHC α1 helix is cyan and the MHC α2 helix is shown in magenta.
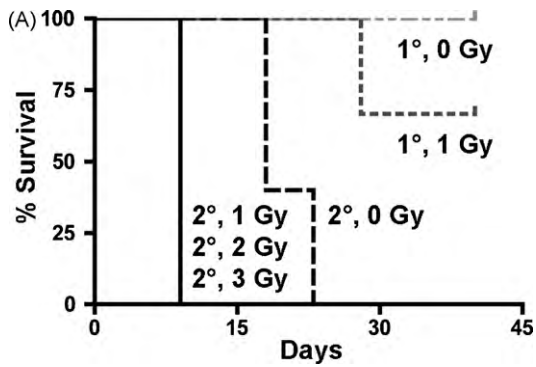
Fig. 3. Primary leukemia can be transferred into non-irradiated mice, allowing vaccination of recipient mice with Tarmogen® yeast[T315I]. Primary (1°) leukemias could not be initiated in unconditioned recipient mice (0 Gy), and induced leukemias with low penetrance in 1 Gy conditioned recipients. Transfer of established primary BCR-ABL leukemias successfully caused secondary (2°) leukemias. Recipient mice conditioned with 0, 1, 2 or 3 Gy radiation all developed 2° leukemia on after transfer of 1° BCR-ABL leukemia.
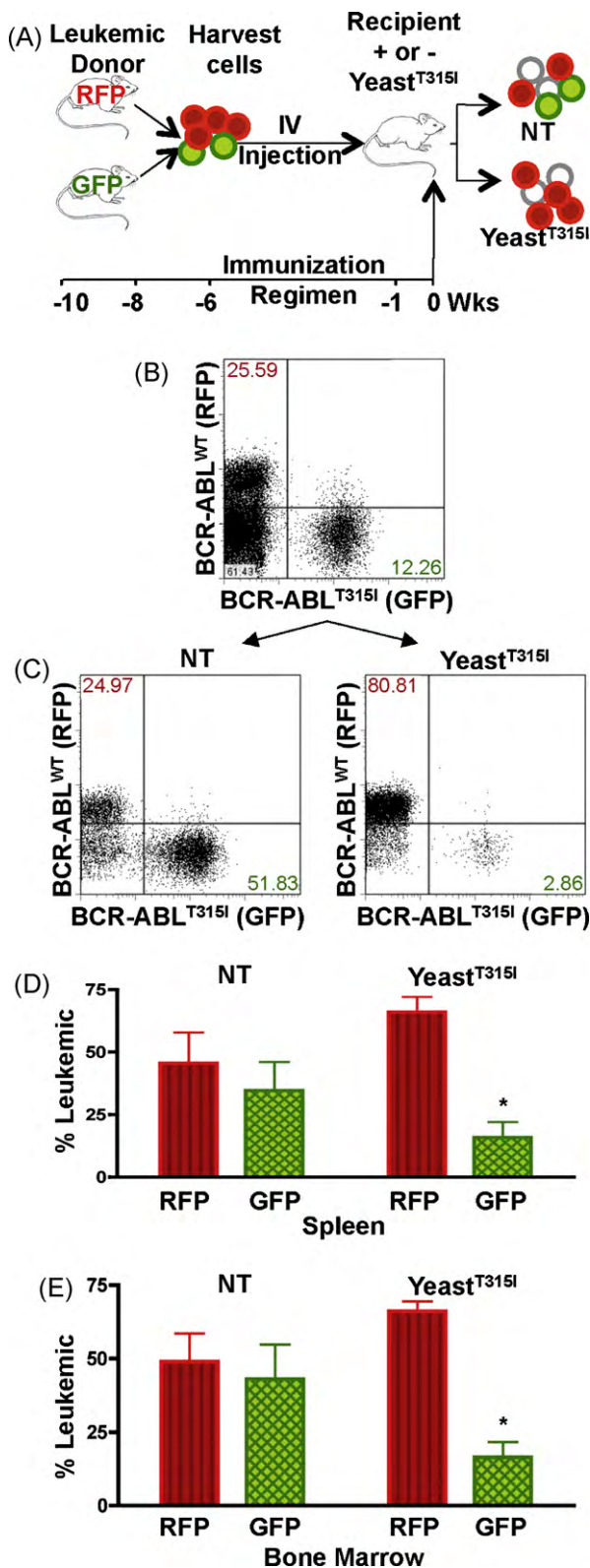
subsequent bone marrow transplant [41]. Murine recipients of *BCR-ABL* transduced stem and progenitor cells developed BCR-ABL primary leukemias. Since immune system disruptions would interfere with vaccine experiments, we tested whether leukemias could be transferred into unconditioned recipients. Consistent with previous reports, our primary leukemias could not be initiated in unconditioned recipient mice [42,43]. However, transfer of some established primary leukemias consistently caused fully penetrant secondary leukemias in unconditioned recipient mice (Fig. 3a). Not
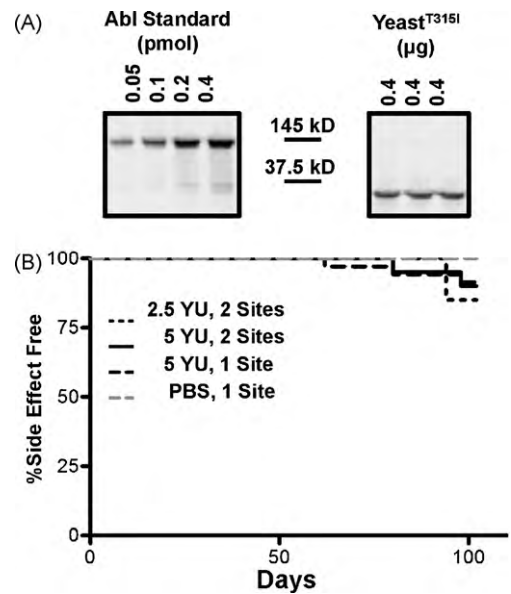
Fig. 4. Injection of *S. cerevisiae* expressing the ABL[T315I] target antigen results in minimal side-effects in mice. Target antigen Abl[T315I] was recombinantly expressed in *S. cerevisiae* as shown by western blot (yeast[T315I]). Wild-type Abl protein was loaded in lanes 1–4 at 0.05, 0.1, 0.2 and 0.4 pmol respectively. A triplicate load of 0.4 μg of yeast[T315I] vaccine from the same blot with the same exposure is shown on the right (A). Subcutaneous vaccination with yeast[T315I] caused mild and preventable pruritic lesions in C57BL/6 mice (B).
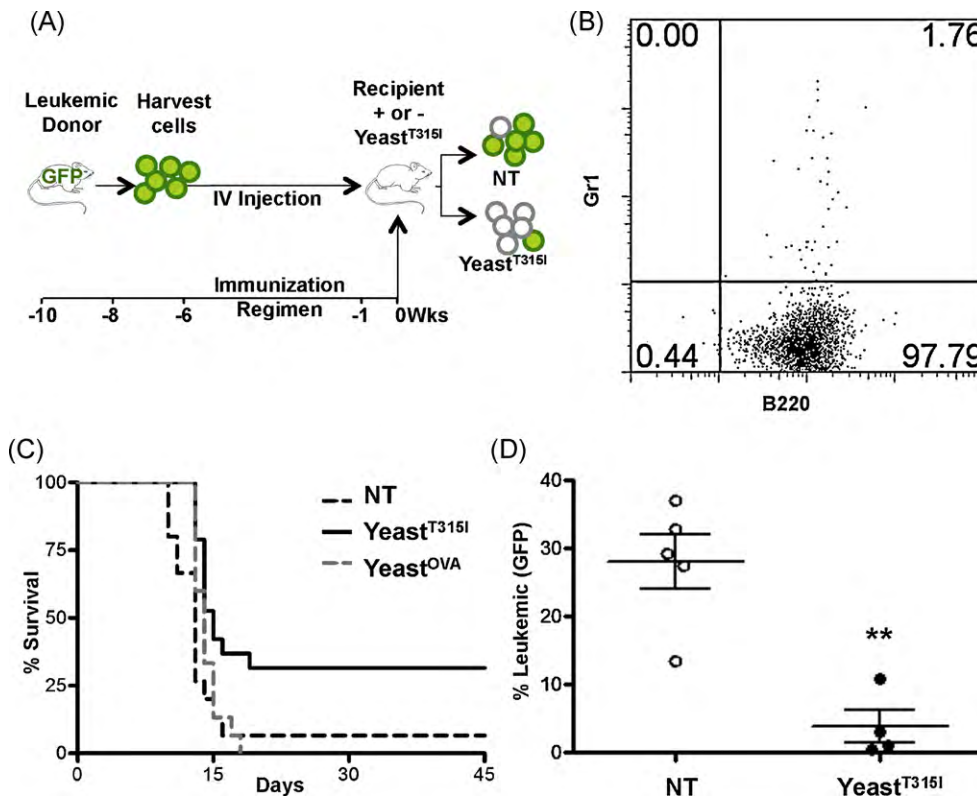
Fig. 5. Vaccination against BCR-ABL[T315I] extended leukemia free survival after challenge with BCR-ABL[T315I]. Schema of the vaccination schedule and BCR-ABL disease in mice. C57BL/6 mice were injected s.c. with PBS, 10 YU yeast[OVA] or 10 YU yeast[T315I] at −10, −8, −6, and −1 weeks before challenge. Primary leukemias with BCR-ABL[T315I] co-expressing GFP were generated as described in Section 2 and harvested. BCR-ABL[T315I] primary leukemic cells were injected intravenously into control (NT = no treatment) or yeast[T315I] vaccinated recipient mice. Secondary leukemia development was then monitored by flow cytometry (A). Primary BCR-ABL[T315I] leukemias used for challenge were Gr-1^{neg} and B220[+] as shown by flow cytometry (gated on GFP[+]) (B). Kaplan Meier curve with data from three independent experiments is shown. Treatment group sizes were 19, 15, and 15 mice for the no treatment, yeast[T315I], and yeast[OVA] groups respectively (C). Leukemic burden in the peripheral blood on day 10 post-challenge is shown from one representative experiment (D).

all primary BCR-ABL leukemias produced a secondary leukemia in non-irradiated recipient mice (unpublished observations). Therefore, only fully penetrant primary leukemias were used in the following studies.

3.3. Vaccination with yeast^{T315I} significantly extends leukemia-free survival

Recombinant *S. cerevisiae* Tarmogen® yeast were engineered to express a mutated ABL^{T315I} polypeptide (yeast^{T315I}, Fig. 4a). Tarmogen® yeast vaccination caused mild injection site reactions, in the form of pruritis and resolvable skin lesions, in a subset of C57BL/6 mice (Fig. 4b). No side-effects were observed in the BALB/c background (data not shown).

For vaccine efficacy experiments, primary leukemias co-expressing BCR-ABL^{T315I} and GFP were generated in C57BL/6 mice and harvested as described in Section 2. Vaccinated and control mice were challenged with a homogeneous population of Gr-1^{neg}, B220$^+$, BCR-ABL^{T315I} leukemia (Fig. 5a and b). Leukemia development was monitored by flow cytometric analysis of the peripheral blood. Animals succumbed to the leukemia, typically evidenced by hind limb paralysis just prior to sacrifice, within 10–25 days after challenge. The development of secondary leukemias was confirmed upon autopsy with complete blood counts together with flow cytometry for GFP and hematopoietic lineage markers in the spleen and bone marrow (data not shown).

Administration of yeast^{T315I} significantly extended survival in C57BL/6 mice upon challenge with BCR-ABL^{T315I} expressing leukemias, compared to untreated ($p = 0.0037$) and yeast^{OVA}-treated ($p = 0.0104$) controls (Fig. 5c). Vaccination with yeast^{OVA} resulted in a significant increase in peripheral blood CD8$^+$ T cells specific for the OVA peptide, SIINFEKL, presented on H-2K^b (data not shown). Surviving yeast^{T315I} treated mice had no detectable leukemia upon autopsy even 2 months post-challenge (data not shown). In confirmation of this result, the peripheral circulation of BCR-ABL^{T315I} cells at day 10 after challenge was reduced or eliminated in immunized, but not control, C57BL/6 animals ($p < 0.0018$) (Fig. 5d). These results highlighted the T315I mutation-selective immune response that was elicited by administration of yeast^{T315I}.

Antigen specific anti-leukemic responses were reproduced with several secondary leukemias. In one case, however extended leukemia-free survival of vaccinated mice was not observed (Supplementary Fig. 3a). Nonetheless, even for this leukemia, yeast^{T315I} vaccinated mice had a reduced leukemic burden in the peripheral blood early in the course of the disease (Supplementary Fig. 3b).

A common mechanism of immune evasion by cancer cells is MHC class I downregulation. Therefore, we confirmed that the primary leukemias used for challenge expressed MHC class I and that they responded normally to IFN-α by up-regulating expression on the cell surface (Supplementary Fig. 4a and b). Additionally, H-2K^b expression was assessed on secondary leukemias from moribund vaccinated and control mice. No significant difference was found between these groups, suggesting that immune evasion via MHC class I loss could not account for leukemic progression in some of the vaccinated mice (Supplementary Fig. 5).

Fig. 6. Vaccination against BCR-ABL^{T315I} selectively reduced the BCR-ABL^{T315I} leukemia burden after challenge with a mixed population of BCR-ABL^{WT} and BCR-ABL^{T315I} primary leukemic cells. Mice were injected s.c. with 10 YU yeast^{T315I} at −10, −8, −6, and −1 weeks before challenge. Primary leukemias with either BCR-ABL^{WT} co-expressing RFP or BCR-ABL^{T315I} co-expressing GFP were generated as described in Section 2 and harvested. A combination of BCR-ABL^{WT} and BCR-ABL^{T315I} primary leukemic cells were injected i.v. into control or yeast^{T315I} vaccinated recipient mice. Secondary leukemia development was then monitored by flow cytometry (A). Flow cytometric dot plot of BCR-ABL^{WT} co-expressing RFP (Y-axis, 63%), and BCR-ABL^{T315I} co-expressing GFP (X-axis, 37%) primary leukemic cells used for challenge

(B); non-leukemic cells are evident in the lower left quadrant. The representation of BCR-ABL^{WT} and BCR-ABL^{T315I} leukemic cells in the spleens and bone marrow of morbid mice was determined by flow cytometric analysis for control or yeast^{T315I} treated mice. Dot plots of spleen cells from one representative mouse of each group are shown (C). Percentages of BCR-ABL^{WT} and BCR-ABL^{T315I} leukemia cells were plotted from all challenged mice (gated on PI^{neg}, RFP$^+$ or PI^{neg}, GFP$^+$) in the spleen (D) and bone marrow (E).

3.4. Vaccination with yeast^T315I selectively eliminates BCR-ABL^T315I cells in the presence of wild-type BCR-ABL leukemia cells

In order to better reflect the development of clinical drug resistance, where a subpopulation of leukemia cells express the drug-resistance mutation, vaccinated and control BALB/c mice were challenged with a mixed population of 63% wild-type BCR-ABL (BCR-ABL^{WT}) and 37% drug-resistant BCR-ABL^{T315I} leukemias (Fig. 6a and b). As BALB/c mice express different MHC Class I molecules as compared to C57BL/6 mice, these experiments also address whether the T315I containing peptide can be presented by other MHC haplotypes. Primary leukemias were Gr-1^{neg} and B220$^+$ (data not shown). Upon leukemia development in recipient mice, the number of leukemic cells harboring BCR-ABL^{T315I} was significantly reduced ($p < 0.05$) relative to cells expressing BCR-ABL^{WT} in the spleen and bone marrow of mice administered the yeast^{T315I} vaccine. Sixty-eight percent antigen-specific elimination of leukemic cells was observed in the spleen (Fig. 6c and d) and 71.88% specific lysis in the bone marrow (Fig. 6e). These results indicate that immune protection was targeted against the single amino acid alteration in the mutated BCR-ABL^{T315I} protein expressed by the leukemia cells. Additionally, given that vaccination with yeast^{T315I} resulted in the specific elimination of BCR-ABL^{T315I}-expressing leukemic cells, we can infer that peptides spanning T315I can also be presented on BALB/c MHC alleles (of the d haplotype).

4. Discussion

The dramatic improvement in CML treatment with targeted therapies such as IM has been accompanied by the development of drug-resistant escape mutations, much like that found upon small molecule drug inhibition of viral replication. Rational prevention of drug resistance has the potential to prolong the efficacy of available drugs and improve clinical outcomes. Thus, similar to HAART for control of HIV infection, effective cancer therapy may necessitate innovative combination treatments. Experiments described here address the problem of drug escape mutations using an immune-mediated approach that may complement IM, dasatinib, or nilotinib therapy. Mutation-selective immune responses targeting BCR-ABL^{E255K}, BCR-ABL^{T315I} and BCR-ABL^{M351T} could eliminate drug-resistant leukemia clones when they are clinically silent. Thus, the incidence of relapse due to drug-resistance may decrease if leukemia patients taking IM or one of its derivatives are effectively immunized against the BCR-ABL^{T315I} antigen or other drug escape epitopes. In this study, we have demonstrated that immunization with Tarmogen® yeast^{T315I} activates protective immunity against challenge with BCR-ABL^{T315I} expressing leukemia.

By transferring fully penetrant leukemias into vaccinated mice, we were able to assess the efficacy of yeast^{T315I} vaccination, but our experimental design was constrained by the lack of a transferrable chronic phase model with slow kinetics. Thus, our experiments tested vaccine efficacy using an aggressive blast crisis leukemic challenge, for which immunotherapy post-leukemic challenge is not feasible. The immune response induced by yeast^{T315I} vaccination had only a median of 13.5 days to eliminate a rapidly expanding, aggressive leukemic population, as opposed to a clinical setting where an immune response could have years over which to either prevent emergence or to eliminate the minimal residual disease found in CML patients undergoing IM therapy. The 32% long-term leukemia-free survival observed in yeast^{T315I} vaccinated mice (Fig. 5c) is striking when considering the aggressiveness of the blast crisis leukemia model and may be even more effective against the more indolent chronic phase disease observed in the setting of IM treated CML.

Patients developing IM drug-resistance start out either with no resistant variants prior to IM treatment or with a low frequency drug-resistant population that expands over time while under IM selection. We attempted to more accurately model the latter situation by challenging with a mixed population of drug-susceptible and drug-resistant leukemias. Additionally, we were interested in whether or not we could see antigen specific elimination of BCR-ABL^{T315I} leukemia cells in a strain other than C57BL/6. Therefore, we challenged vaccinated and control BALB/c mice with a heterogeneous population of BCR-ABL and BCR-ABL^{T315I} leukemia cells. Yeast^{T315I} vaccinated BALB/c mice were able to selectively reduce the BCR-ABL^{T315I} cells in the presence of BCR-ABL cells suggesting that the single amino acid BCR-ABL^{T315I} mutation is antigenic in the BALB/c background as well as in the C57BL/6 strain. These results highlight two independent lines of evidence from these studies for the activation of mutation-selective immunity in this murine leukemia model, with two different mouse strains. The sum of the results reflect the potential for BCR-ABL$^+$ leukemia treatment benefit following immunization of Tarmogen® yeast^{T315I} in a diverse population of subjects.

To our knowledge, the BCR-ABL^{T315I} tumor specific antigen is the first cancer drug-resistance antigen to be targeted by vaccination. We have shown that several peptides bearing the BCR-ABL^{T315I} point mutation bind H-2K^b in vitro, likely, in such a way as to present the mutant isoleucine facing a CD8$^+$ T cell receptor. We have expressed the BCR-ABL^{T315I} antigen in a safe and immunostimulatory yeast vaccine vector [39], and have shown antigen specific reductions in leukemia cells in the peripheral blood. Moreover, vaccination increased leukemia-free survival after challenge with BCR-ABL^{T315I} leukemia. In addition, yeast^{T315I} vaccination successfully reduced the drug-resistant leukemic burden in two mouse strains. In summary, yeast^{T315I} vaccination represents a novel approach against the emergence of IM resistance in BCR-ABL$^+$ leukemias and potentially could be used to prevent the emergence of resistance to other selective cancer therapies.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.vaccine.2010.06.085.

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