CONTROL OF LEUKEMIA DRIVEN BY THE T315I ESCAPE MUTATION IN BCR-ABL WITH YEAST-BASED TARMOGEN IMMUNOTHERAPY

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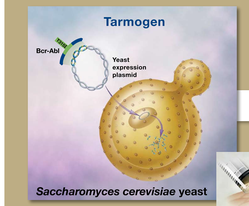
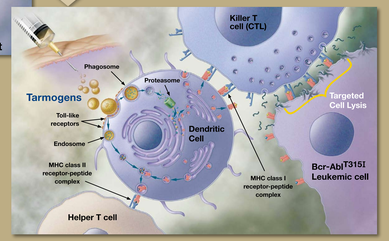


Heat-killed, recombinant *S. cerevisiae* transfected with gene encoding T315I-mutated Bcr-Abl protein (GI-10001).

Uptake of Tarmogens results in:

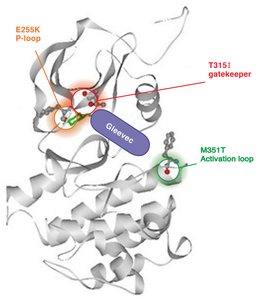
- Activation of dendritic cells
- Presentation of antigens through both MHC class I and II
- Production of antigen specific T helper ($CD4^+$) and CTL ($CD8^+$) cells
- Killing of antigen bearing leukemic cells



Introduction

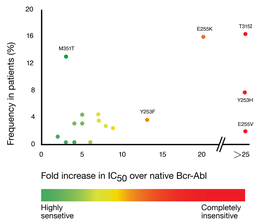
Gleevec imatinib mesylate (imatinib) resistance mutations in Bcr-Abl constitute a significant vulnerability in the clinical treatment of chronic myelogenous leukemia (CML). The most prevalent escape mutation to imatinib, dasatinib and nilotinib is a threonine-to-isoleucine mutation at codon 315 of Bcr-Abl (T315I mutation). The Bcr-Abl^{T315I} epitope is a true tumor-specific mutated antigen.

Figure 1A: Imatinib escape mutants in Bcr-Abl protein structure



*Sergio A Alencar, Julio C Lopes, Andrelly M Jose

Figure 1B: Clinical frequency of imatinib escape



Unlabeled points represent M244V, G250E, Q252H, F311L, F317L, E355G, F359V, V379I, L387M, H396P, and H396R.

*O'Hare, T. et al. Blood 2007; 110:2242-2249

Hypothesis

Immunotherapy targeting the Bcr-Abl^{T315I} imatinib mesylate escape epitope should trigger the specific elimination and control of leukemic cells harboring the T315I mutation. Figure 2 shows the study design wherein leukemic cells driven by Bcr-Abl without (native) or with the T315I mutation (T315I) are transferred to recipients, then the proliferation of leukemic cells and survival is monitored. The immunization regimen, prior to leukemia challenge, is shown below.

Figure 2:

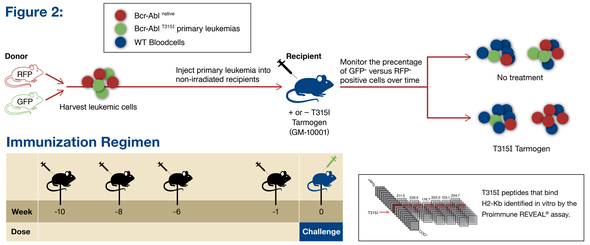


Figure 3A: Immunotherapy targeting Bcr-Abl^{T315I} does not protect from challenge with Bcr-Abl^{native} leukemia

Top: Flow cytometric analysis of B220$^+$ Bcr-Abl^{native} leukemia used for challenge. Bottom: No difference in leukemia-free survival was observed after challenge with Bcr- Abl^{native} leukemia.

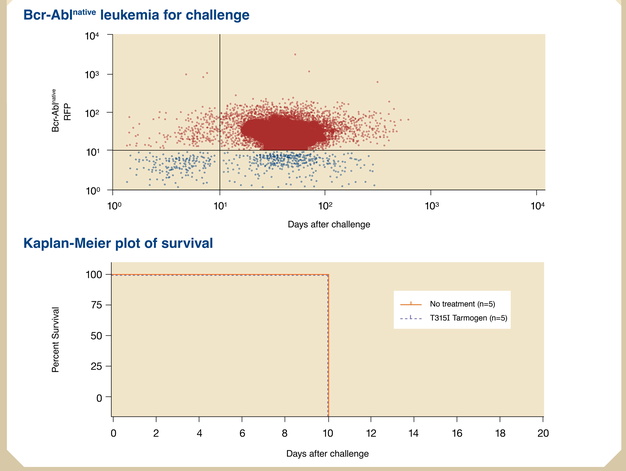


Figure 3B: Administration of T315I Tarmogen selectively eliminated Bcr-Abl^{T315I} leukemia

Mice were not treated or were administered T315I Tarmogen. After challenge with a mixed population of Bcr-Abl^{T315I} leukemia and Bcr-Abl^{native} leukemia (top), Tarmogen-treated mice selectively eliminated the Bcr-Abl^{T315I} leukemic cells but not the Bcr-Abl^{native} leukemic cells (middle and bottom).

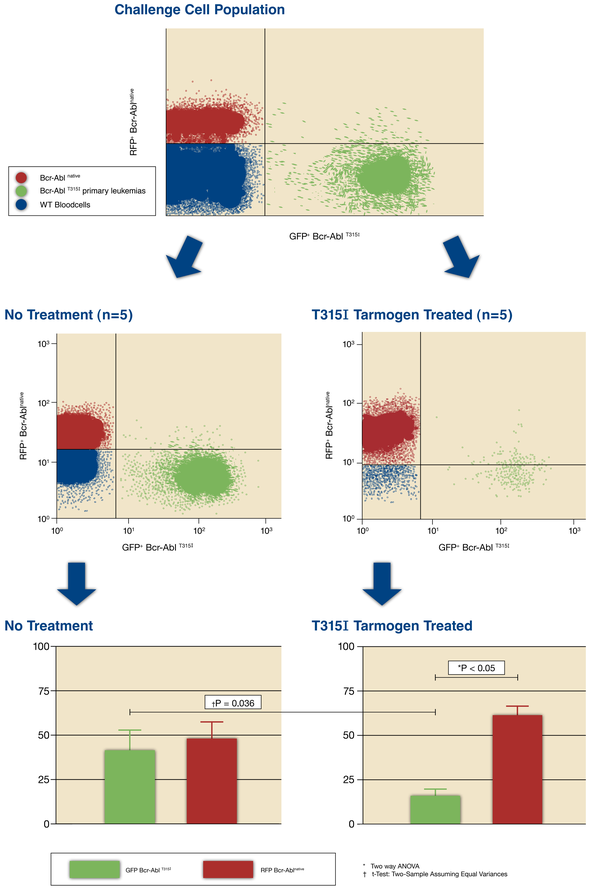
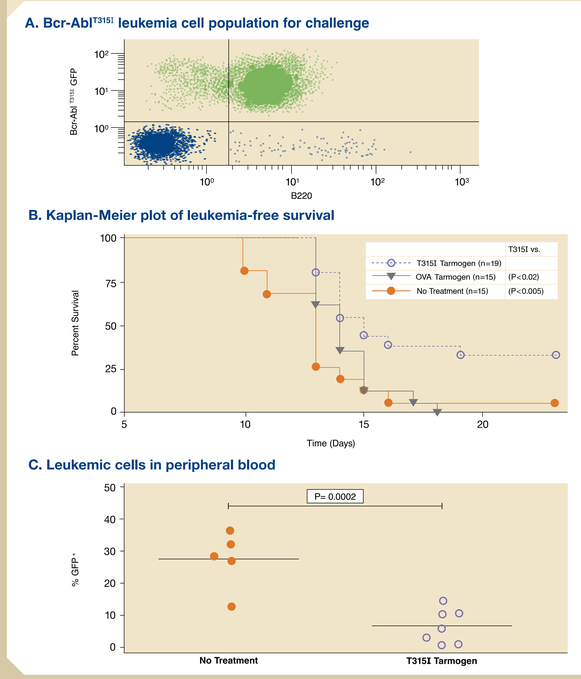


Figure 4: Administration of T315I Tarmogen prolonged leukemia-free survival after challenge with Bcr-Abl^{T315I} leukemia

Top: Flow cytometric analysis of B220$^+$ Bcr-Abl^{T315I} leukemia used for challenge. Middle: T315I Tarmogen treatment significantly extended leukemia-free survival compared either to treatment with a control Tarmogen expressing ovalbumin (OVA) or no treatment. Bottom: A significantly lower percentage of leukemic cells were detected in the peripheral blood of T315I Tarmogen treated mice on day 10.



Conclusions

1. Bcr-Abl^{T315I} CML is clinically refractory to 1st (imatnib) and 2nd generation (dasatinib, nilotinib) tyrosine kinase inhibitors.
2. Six Bcr-Abl^{T315I} imatnib escape mutant peptides that bind MHC class I were identified.
3. Tarmogen immunotherapy targeting Bcr-Abl^{T315I} selectively eliminates Bcr-Abl^{T315I} leukemic cells *in vivo.*
4. Tarmogen immunotherapy targeting Bcr-Abl^{T315I} significantly extends survival after challenge with Bcr-Abl^{T315I} leukemia.
5. Immunotherapy may be combined with targeted therapies (e.g. tyrosine kinase inhibitors) for the prevention of cancer drug resistance.

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Abstract

The emergence of targeted therapy-resistance mutations in Bcr-Abl constitutes a significant vulnerability in the clinical treatment of chronic myelogenous leukemia (CML). The most prevalent escape mutation to CML treatment with Gleevec imatinib mesylate (imatinib) or second generation tyrosine kinase inhibitor drugs (dasatinib and nilotinib) is a threonine-to-isoleucine mutation at codon 315 of Bcr-Abl (T315I mutation). The goal of this study was to test whether yeast-based immunotherapy (Tarmogen®) targeting the T315I epitope triggers the specific elimination and control of leukemic cells driven by Bcr-Abl harboring the T315I mutation. Since the Bcr-Abl^{T315I} epitope is a true tumor-specific antigen that is either not present or present at low levels prior to imatinib therapy, tolerance to this epitope should be low. Moreover, the immediate goal of Tarmogen immutherapy in this care is not to eradicate the full leukemic burden, but to work in combination with targeted tyrosine kinase small molecules to prevent the outgrowth of drug-resistant leukemic cells, thereby maintaining the effectiveness of current small molecule tyrosine kinase inhibitor therapies targeting the Bcr-Abl kinase domain.

Prior to testing the immunotherapeutic approach in a mouse leukemia challenge model, preliminary *in silico* and *in vitro* analyses of the feasibility of generating MHC class I T-cell epitopes encompassing the T315I mutation were performed. Overlapping 8-10 amino acid peptides spanning Bcr-Abl^{T315I} were analyzed using MHC-binding peptide prediction algorithms, synthesized and evaluated in an *in vitro* MHC-binding assay. Six candidate T315I H2-Kb binding peptides were identified. Recombinant *S. cerevisiae* baker's yeast were then engineered to express ~300 amino acids of the mouse-specific version (2 amino acids different than human ABL sequence) of T315I-mutated Bcr-Abl (GI-10001 yeast). Mice challenged with 10^5 leukemia cells whose proliferation is driven by Bcr-Abl succumb ~10 days after challenge. Administration of GI-10001 yeast significantly extended survival in two different mouse strains upon challenge with leukemias harboring Bcr-Abl^{T315I} but not wild-type Bcr-Abl^{native}. These results indicate that immune protection was targeted against the single amino acid alteration in the mutated Bcr-Abl protein expressed by the leukemia cells. In confirmation of this result, the peripheral circulation of Bcr-Abl^{T315I} cells was reduced or eliminated in immunized, but not control, animals. Furthermore, after challenge with a mixed population of wild-type Bcr-Abl^{native} and mutated Bcr-Abl^{T315I} leukemias, a more physiologically relevant model, the number of leukemic cells harboring Bcr-Abl^{T315I} was significantly reduced relative to cells expressing wild-type Bcr-Abl^{native} in mice vaccinated with the GI-10001 yeast, but not control yeast. In summary, yeast-based immunotherapy targeting drug resistance or escape mutations represents a powerful approach to extend the clinical effectiveness of targeted cancer therapies.