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

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Introduction
Germline missense mutations (exon 17) in BCR-ABL constitute a significant vulnerability in the clinical treatment of chronic myelogenous leukemia (CML). The most prevalent escape mutation in CML is T315I. It results in a dominant-negative mutation at codon 315 of Bcr-Abl (T315I mutation). The Bcr-Abl T315I-epitopes is a novel immunotherapeutic agent.

Figure 1A: Immunogenicity of Tarmogen

Figure 1B: Clinical efficacy of Tarmogen

Hypothesis
Immunotherapy targeting the Bcr-Abl T315I-mutated escape epitope might trigger the specific elimination and control of leukemic cells harboring the T315I mutation. Figure 2 shows the study design where leukemic cells driven by Bcr-Abl T315I mutation with the T315I mutation (T315I mutant) is transferred to recipient, then the proliferation of leukemic cells and survival is monitored. The immunization regimens, prior to leukemia challenge, is driven below.

Figure 2: Immunization Regimens

Challenge Cell Population
Bcr-Abl T315I
Tarmogen
No treatment

B. Kaplan-Meier plot of leukemia-free survival

C. Leukemic cells in peripheral blood

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

Figure 3C: Administration of T315I Tarmogen prolonged leukemia-free survival after challenge with Bcr-Abl T315I leukemia

Conclusions
1. Bcr-Abl T315I-CML is clinically refractory to 1st (imatinib) and 2nd generation (dasatinib, nilotinib) tyrosine kinase inhibitors.
2. The Bcr-Abl T315I-mutant escape mutant peptide (linear B8) can be identified.
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.

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

Top: Flow cytometric analysis of RFP Bcr-Abl T315I leukemia used for challenge. Middle: T315I Tarmogen treated mice selectively eliminated the Bcr-Abl T315I leukemic cells (middle and bottom).

A. Bcr-Abl T315I leukemia cell population for challenge

B. Kaplan-Meier plot of leukemia-free survival

C. Leukemic cells in peripheral blood

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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 immatherapy 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-AblT315I 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 10⁵ 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-AblT315I 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-AblT315I 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-AblT315I leukemias, a more physiologically relevant model, the number of leukemic cells harboring Bcr-AblT315I 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.