BNZ-2, a dual specific IL15/IL21 inhibitor, rescues humanized NOG-IL15 transgenic mice from intestinal acute graft vs host disease without disrupting NK and CD8 T cell engraftment

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Abstract

Advances in adoptive transfer immunotherapy have found beneficial effects for Interleukin-15 (IL-15) in the graft versus tumor (GvT) activity of NK and cytotoxic T- cells. However, the benefits of this activity must be weighed against the increased risk of acute graft versus host disease (GvHD) in patients. We propose that BNZ-2, a peptide antagonist of IL-15 and IL-21 signaling, as a therapeutic option for GvHD that may relieve intestinal pathologies while retaining adoptive transfer activity. As a novel model of intestinal inflammatory disease, we have found that humanization with peripheral blood mononuclear cells (PBMCs) of NOG mice with transgenic expression of human IL-15 (NOG-IL15) consistently catalyzes the onset of intestinal GvHD within twenty days of engraftment. To investigate whether BNZ-2 can inhibit IL-15-catalized intestinal GvHD we treated NOG-IL15 mice and assessed the degree of intestinal GvHD and the immunophenotype of engrafted cells following twenty days of humanization with four million unmanipulated PBMCs. BNZ-2 treatment inhibited with equal efficacy to anti-IL-15, the localization of immune cells to the intestinal lamina propria protecting the tissue from inflammation-induced loss of tissue integrity. However, BNZ-2 treatment retained the systemic engraftment of the blood and spleen, including NK and CD8 cytotoxic T-cells. These data support BNZ-2 as a therapeutic candidate for GvHD treatment, describe a new model of intestinal GvHD, and suggest that a therapeutic window exists that separates intestinal GvHD pathologies from the benefits of adoptive cell transfer.

Materials and Methods.

NOG & NOG-IL15 mice

NOG or NOG mice with transgenic ubiquitous expression of human IL-15 at 200pg/mL in blood plasma, Taconic, model number 13683, were used in this study.
Katano I, Nishime C, Ito R, et al. Sci Rep 2017;7:17230.

PBMCs

Human derived peripheral blood mononuclear cells (PBMCs) isolated from healthy donors (HemaCare) were cryopreserved then thawed and injected unmanipulated at $4x10^6$ cells/mouse on day 0 of study.

FITC-Dextran-4000

FITC-Dextran (Sigma) was administered orally on day 20 of the study followed by retro orbital bleed after four hours and measured in the blood plasma to assess tissue permeability.

BNZ-2 & α-IL-15 AB

BNZ-2 formulated for SC injection (CPC Scientific) or anti-IL-15 (R&D Systems, clone 34593) were administered SC or IP, respectively at the doses indicated.
Ciszewski C, Discepolo V, Pacis A, et al. Gastroenterology 2020;158:625-637 e13.
Yokoyama S, Watanabe N, Sato N, et al. Proc Natl Acad Sci U S A. Volume 106, 2009:15849-54.

Flow Cytometry Antibodies

Fluorescence-conjugated antibodies (BioLegend) for human CD8 (301066), human CD45 (368522), human CD4 (317407), human CD56 (362533), mouse CD45 (147707), and human CD3 (317335) were used for flow cytometry experiments.

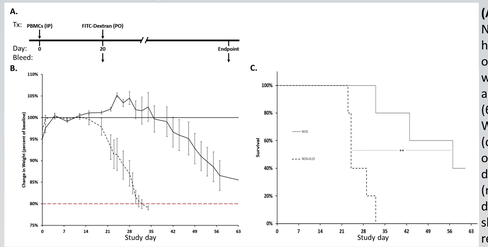
Tissue Histology

Formalin-fixed paraffin-embedded tissues were sectioned, mounted on slides and stained with either H&E or by Immunohistochemistry for CD45 (ThermoFisher, , MA5-13197), CD8 (Cell Signaling, 70306), or CD4 (Abcam, ab133616) to assess tissue histology and cellular engraftment.

Statistical Analysis

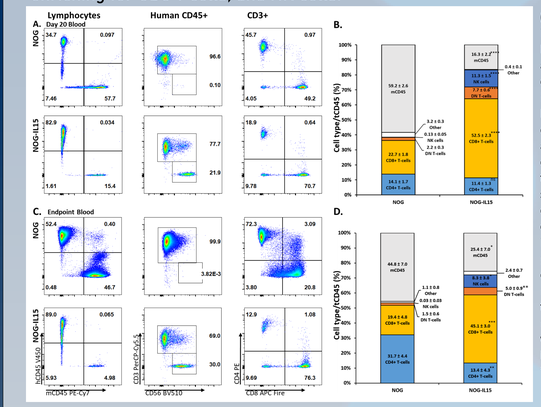
For comparison of NOG to NOG-IL15 a t-test was performed to determine significance. For comparisons between NOG-IL15 treatment groups a one-way ANOVA was performed, and, when appropriate, a post-hoc Dunnett's T-test determined significance and the p-value was reported in the text.

Figure 1. NOG-IL15 mice humanized with PBMCs develop aggressive acute GvHD with increased mortality relative to NOG mice.



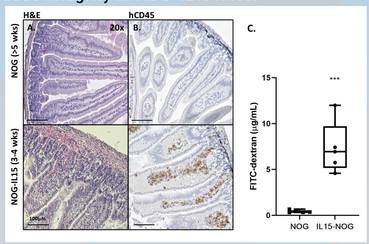
(A) To assess the onset of GvHD in this model, NOG (n=5) and NOG-IL15 (n=5) Mice were humanized with $4x10^6$ unmanipulated PBMCs on day 0. Engraftment and tissue integrity were assessed on day 20 by oral administration of FITC-dextran 4000 (600mg/kg) 4 hours prior to bleeding. **(B)** Weights of NOG (solid line) and NOG-IL15 (dashed line) mice were monitored every other day with the study endpoint being defined by a loss of 20% of baseline weight (red dashed line). **(C)** A Kaplan-Meier curve describes the survival of the NOG vs NOG-IL15 showing that transgenic IL-15 expression reduced survival (Log-rank test, p=0.0045).

Figure 2. Humanization of NOG-IL15 accelerates PBMC engraftment while preferentially enriching for CD8 T cells, and NK cells.



(A) Representative flow cytometry of immunophenotyping to assess Humanization of NOG (upper panels) and NOG-IL15 (lower panels) mice on day 20 found significantly accelerated human cell engraftment in the NOG-IL15 mice. **(B)** Quantification of The cell distribution of immune cells in NOG and NOG-IL15 mice on day 20 found an enrichment in $CD8^+$ over $CD4^+$ T-cells in the IL-15 transgenic. Furthermore, IL-15 supported the engraftment of $CD4^-CD8^-$ double negative (DN) T-cells and NK cells with both cell types nearly absent in the NOG controls. **(C)** Immunophenotyping of mice at the study endpoint found a consistent changes in relative PBMC engraftment. **(D)** While the cellular makeup of NOG-IL15 mice remains similar, mortality correlated with enrichment for $CD4^+$ T-cells in the NOG mice. (T-test, ****p<0.0001, ***p<.001, **p<0.01, *p<0.05)

Figure 3. Intestinal histology reveals tissue degradation and immune cell infiltration of the lamina propria that correlates with a loss of tissue integrity in NOG-IL15 mice.



(A-B) Formalin-fixed paraffin-embedded (FFPE) tissue sections (5μm) from the duodenal segment of the small intestine were stained with either H&E **(A)** or anti-human CD45 **(B)**. Tissue from end-stage GvHD humanized NOG-IL15 mice (lower panels) have clear damage, tissue hemorrhaging, and immune cell infiltration when compared to end-stage GvHD NOG controls (upper panels). **(C)** This damage correlates with day 20 FITC-dextran-4000 tissue permeation detected in the blood 4 hours after oral administration (600mg/kg) (One-way T-test, p=0.0003).

Figure 4. BNZ-2 or anti-IL-15 treatment rescue NOG-IL15 mice from GvHD-related weight loss at day 20 of humanization.

(A) Given the previous data, mice were treated with vehicle (n=7), or the IL-15-targeted therapies, BNZ-2 (50mg/kg, QOD, n=5) and anti-IL-15 neutralizing antibody (100ug, BIW, n=7) starting one day prior to humanization and assessed for engraftment and tissue permeability at day 20. Day 20 was chosen as it provided a loss of tissue integrity as shown by FITC-dextran permeation while avoiding end stage histological deterioration associated with significant wasting disease as well as to avoid attrition due to weight loss. **(B)** Monitoring health and weight every other day found no humanization-associated mortality with observable weight loss becoming apparent in only the vehicle group by the study's end.

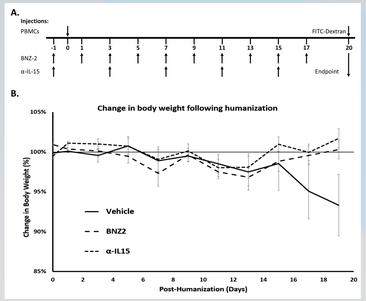
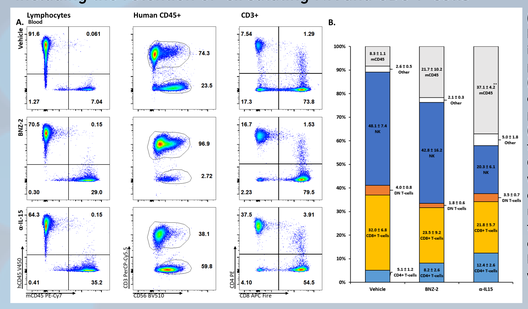


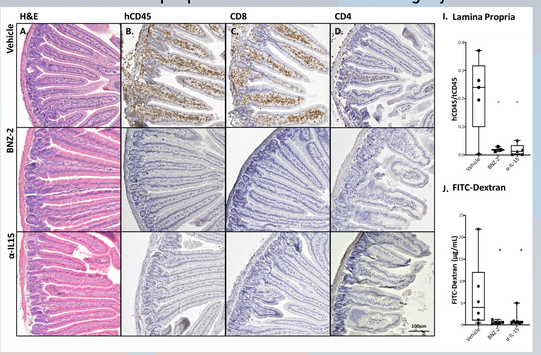
Figure 5. Treatment with BNZ-2 or anti-IL-15 has nominal effects on PBMC engraftment, including the retention of circulating NK and CD8 T-cells



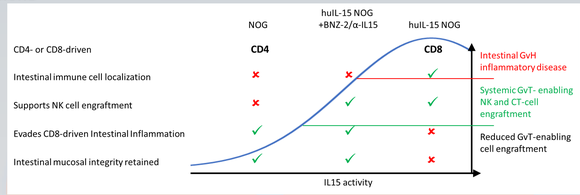
(A) While immunophenotyping of blood on day 20 found little difference in engraftment from vehicle (upper panels) to BNZ-2 (middle), αIL-15 AB (lower) treatment decreased the rate of engraftment (One-way ANOVA, p=0.0052) **(B)** The trend of both BNZ-2 (n=5) and aIL-15 (n=7) treatments was to reduce the relative engraftment of $CD8^+$ T-cells, NK cells, and DN T-cells, while increasing $CD4^+$ T-cells when compared to vehicle (n=6). However, neither treatment significantly reduced the IL-15-dependent NK cell, $CD8^+$ T-cell, or DN T-cell engraftment of the blood (One-way ANOVA) relative to vehicle treatment.

Figure 6. BNZ-2 or anti-IL-15 AB treatments reduced GvHD-associated inflammation, blocked engraftment of the intestinal lamina propria and retained tissue integrity.

(A) Day 20 histology of Vehicle (upper panels), BNZ-2 (middle) and α-IL15 AB (lower) duodenal tissues show limited disease pathology for all groups. **(B)** Despite the limited inhibition of blood engraftment, human CD45 tissue staining found that BNZ-2 and α-IL15 ablated immune cell localization to the intestine. **(C-D)** These cells are primarily $CD8^+$ cytotoxic T-cells (C) rather than $CD4^+$ T-cells (D). **(E)** Flow cytometry confirms reduced immune cell localization to the lamina propria in BNZ-2 (p=0.0280) and α-IL15 AB (p=0.0119) treated mice relative to vehicle. (F) These data correspond to improved tissue integrity assessed by FITC-dextran 4000 permeability in BNZ-2 (p=0.0381) and α-IL15 (p=0.0429) treated mice.



Conclusions



In this study we show that transgenic IL-15 expression in a NOG GvHD model system drives a rapid and distinct intestinal pathology that corresponds to CD8 CTL rather than CD4 enrichment in the periphery. While these animals support NK cell engraftment systemically they have significant localization of immune cells to the intestinal lamina propria and a loss of mucosal tissue integrity by 20-days post-humanization. Interestingly, therapeutic doses of BNZ-2 and anti-IL-15 retain NK and CD8 CTL engraftment in the periphery while attenuating the intestinal localization, pathology and retains tissue integrity. These data suggest that there is a therapeutic window that protects patients from intestinal GvHD while supporting the engraftment of GvT cell types such as NK cells.