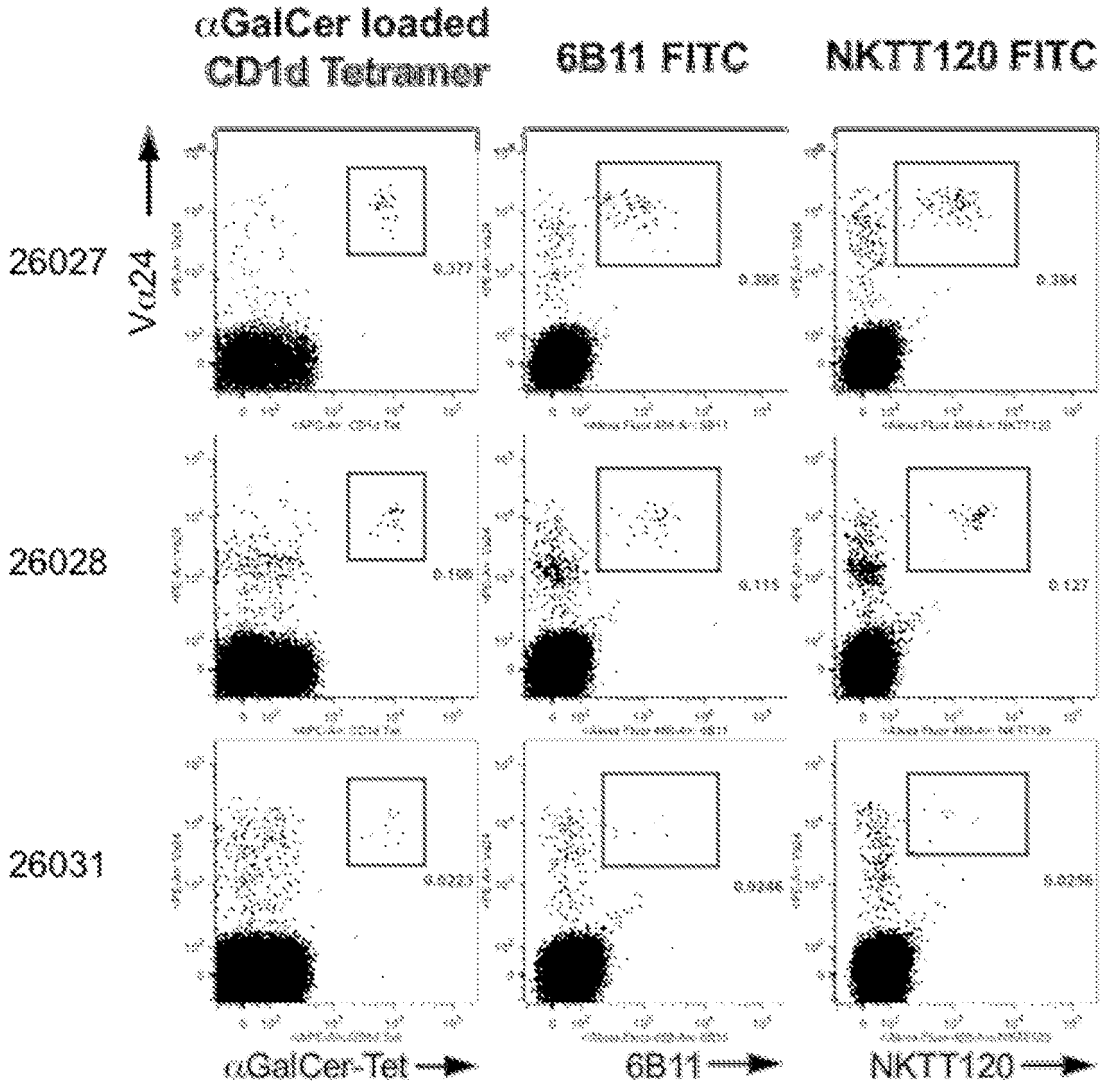
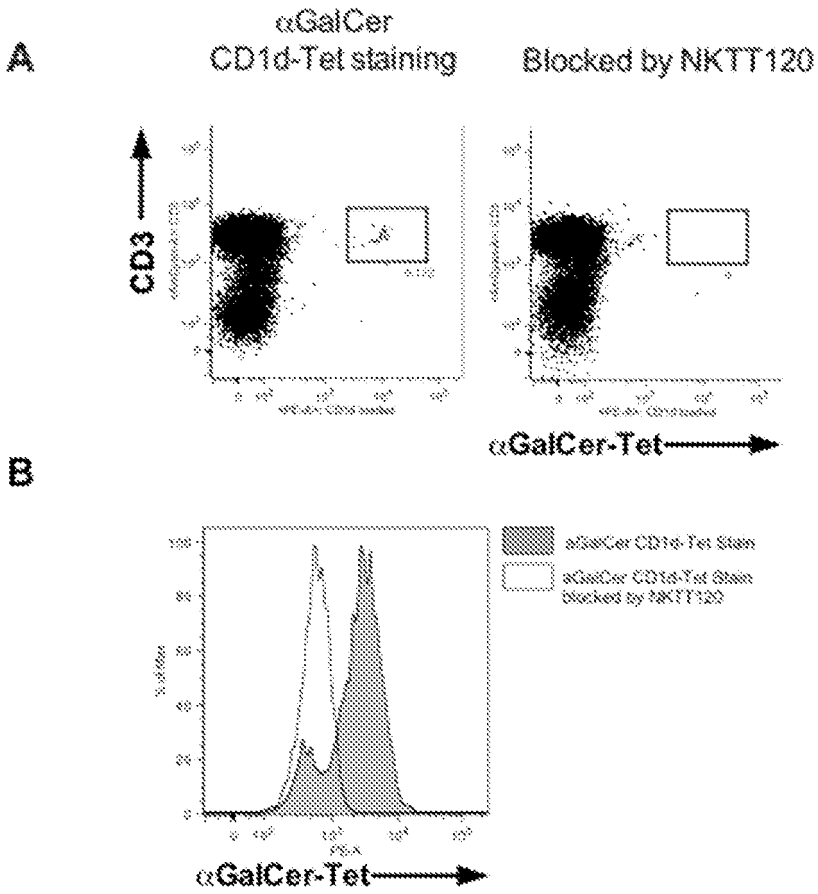
Figure :1 NKTT120 Binding to Human Peripheral Blood iNKT Cells



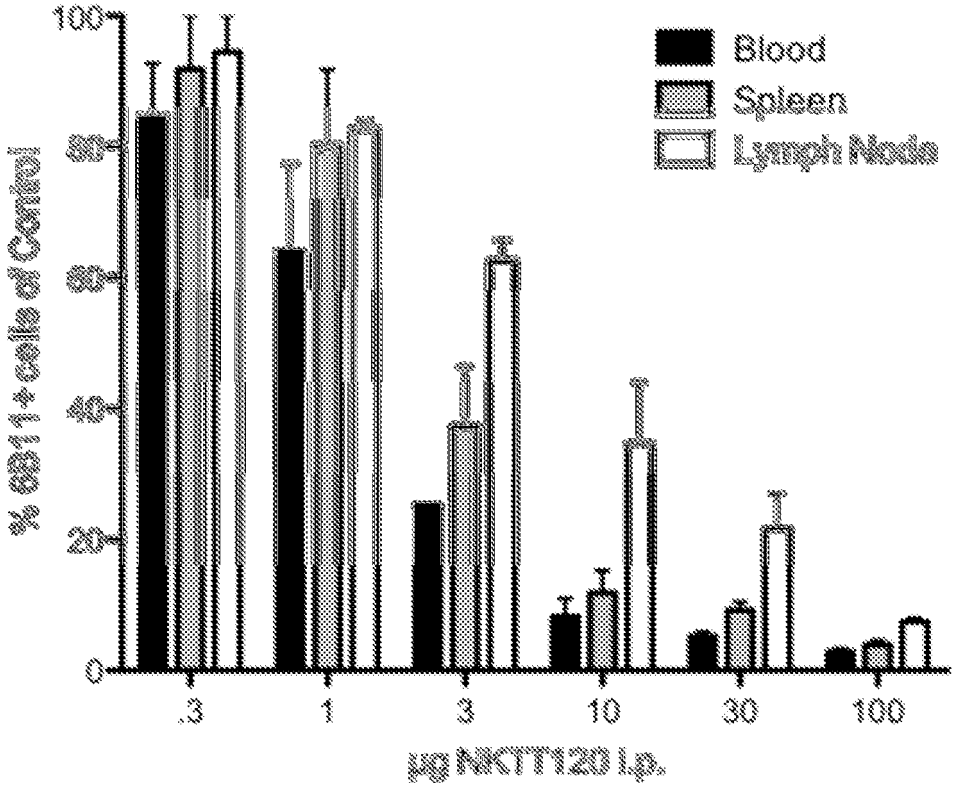
Human whole blood from three donors (26027, 26028, 26031) was stained with α-GalCer-loaded CD1d-tetramer, 6B11-FITC or NKTT120-FITC in conjunction with anti-CD3 and anti-Vα24 mAbs. Red blood cells were subsequently lysed and cells analyzed by flow cytometry. Cells shown are gated on CD3+ lymphocytes.

Figure 2: Blocking of CD1d-tetramer Binding to iNKT Cells by NKTT120



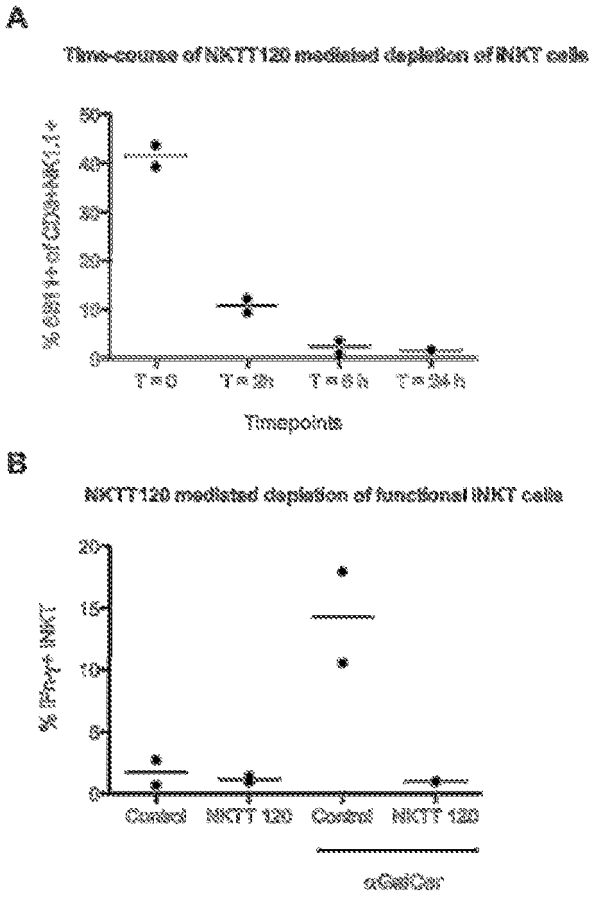
A) Human PBMCs were incubated in presence or absence of 30 µg/ml NKTT120 for 30 minutes and then, without washing, stained with CD3 and α-GalCer-loaded CD1d-tetramer. The presence of the antibody blocks tetramer staining.
B) Human iTCR transfectant (expressing both Vα24-Jα18 and Vβ11 chain of the human iTCR) was incubated in presence or absence of 30 µg/ml NKTT120 for 30 minutes and then, without washing, stained with α-GalCer-loaded CD1d-tetramer. The presence of NKTT120 blocks binding of α-GalCer-loaded CD1d-tetramer.

Figure 3: Depletion of iNKT Cells in Blood Spleen and Lymph Node by NKTT120



Vα24 transgenic Jα18-deficient mice were dosed intraperitoneally with NKTT120 (n=2 per dose) and sacrificed forty-eight hours later. Blood, spleen and lymph node lymphocytes isolated and iNKT cell numbers were subsequently determined by flow cytometry.

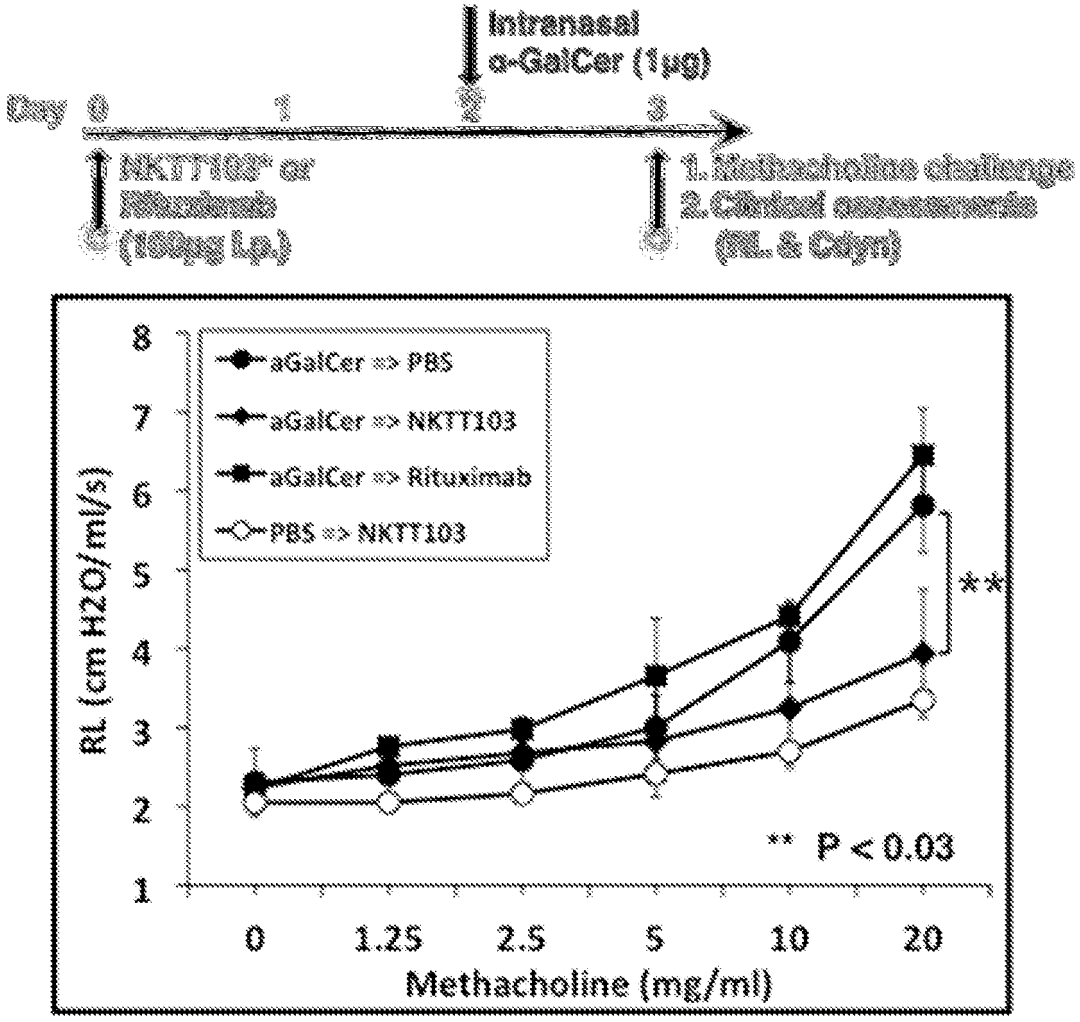
Figure 4: Time Course of iNKT Cell Depletion by NKTT120 in Va24 Transgenic Mice



A. Vα24 transgenic mice (n=2 per group) were injected intraperitoneally with 100 µg of NKTT120 and sacrificed at indicated time points. Gating on splenic CD3+NK1.1+ iNKT cells, the percentage of 6B11 binding cells was assessed.

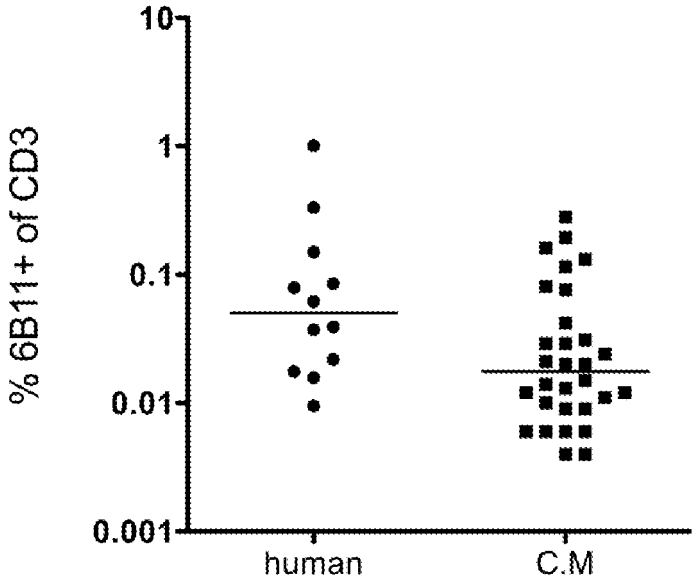
B. Vα24 transgenic mice (n=2 per group) were injected with 100 µg NKTT120. A control group did not receive NKTT120. Forty-eight hours later, separate NKTT120-treated mice and control mice were injected with 2 µg α-GalCer. Two hours after α-GalCer injection, mice were sacrificed and splenic CD3+NK1.1+ iNKT cells were assayed for intracellular IFNγ by flow cytometry.

Figure 5: Pharmacologic Depletion of iNKT Cells in Vα24 Transgenic Mice Abrogates α-GalCer-Mediated iNKT Cell-dependent AHR



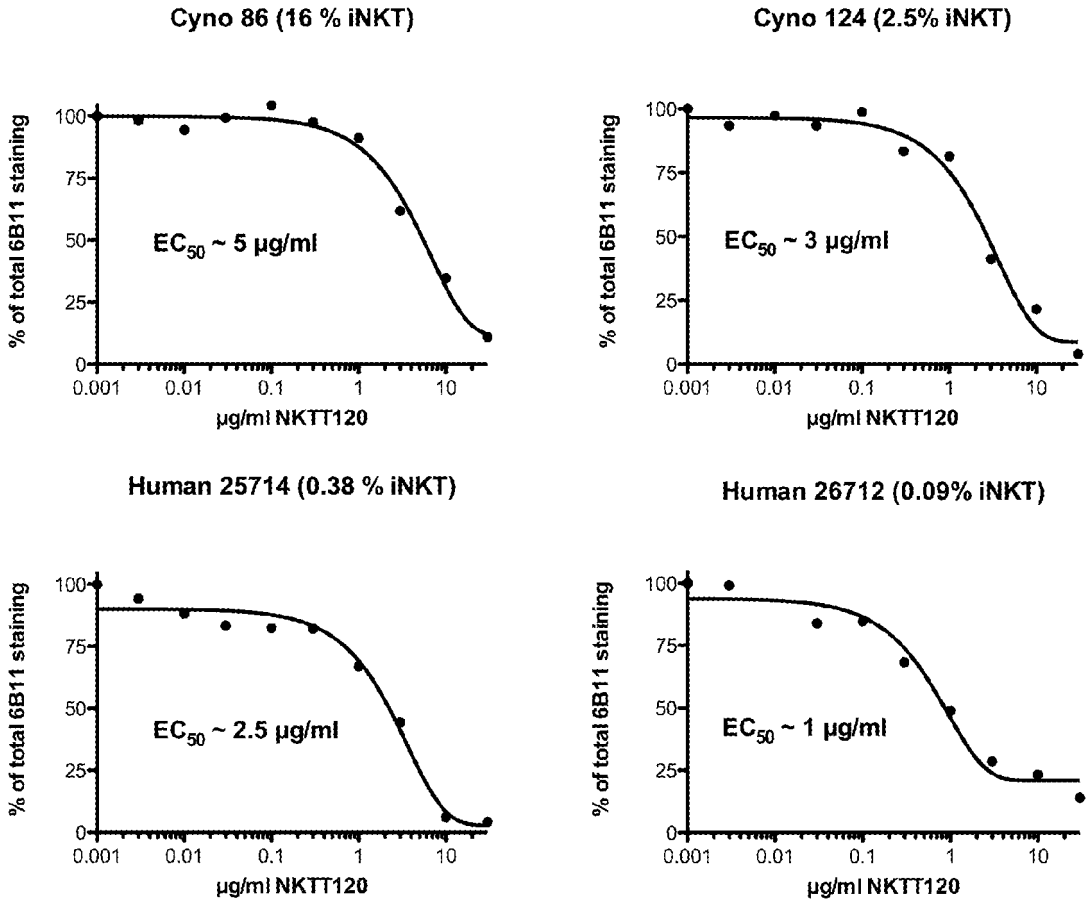
Vα24 transgenic mice, backcrossed to BALB/c n=6 (n=3 per group), were injected intraperitoneally with 100 μg of NKTT103 or Rituximab. Forty-eight hours later, mice were challenged with 1 μg of α-GalCer. After an additional 24 hours, mice were challenged with methacoline and lung resistance (RL) and dynamic compliance (Cdyn, not shown) were assessed. Rituximab was used as a negative isotype control.

Figure 6: Comparison of Human and Cynomolgus Monkey iNKT Cell Frequencies



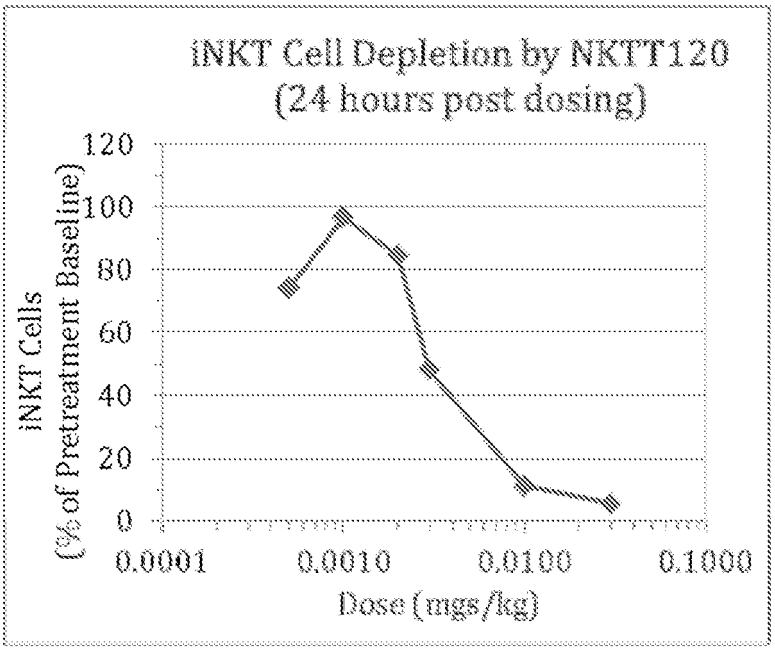
Cynomolgus monkey frequency range of iNKT cells, as determined by CD3, anti-Vα24 (Clone C15) and 6B11 binding, in peripheral blood is strikingly comparable to that found in human subjects.

Figure 7: Comparison of Saturation binding to Human and Cynomolgus Monkey iNKT Cells.



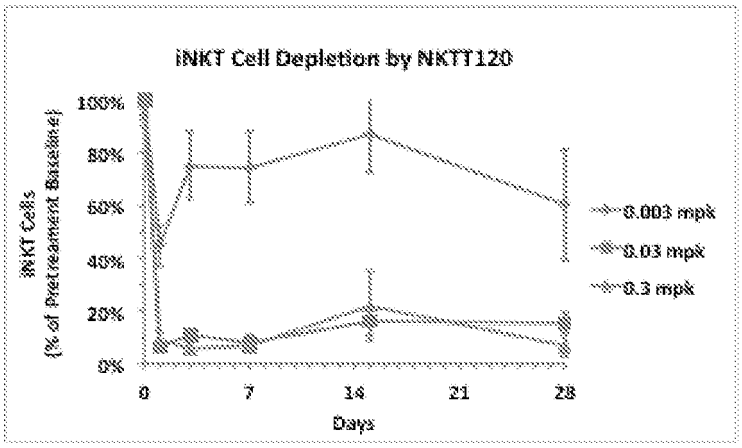
Cynomolgus monkey (NHP) and human iNKT cells demonstrate similar saturation binding of NKTT120 in a direct competitive binding assay. Cynomolgus monkey or human whole blood was incubated with indicated concentrations of NKTT120 and then stained with fluorochrome-labeled 6B11 to assess saturation binding of iNKT cells. Human samples were purchased from Research Blood Components, Inc.

Figure 8: iNKT Cell Depletion by NKTT120 24 Hours After Dosing



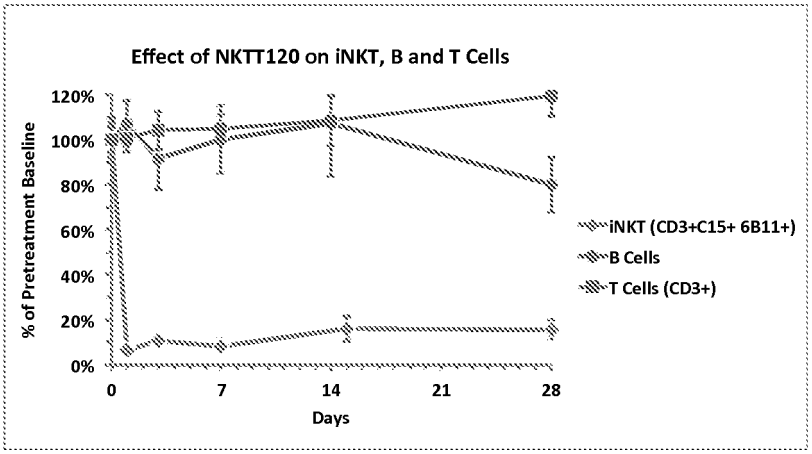
Animals were treated with a single dose of NKTT120 and monitored for seven days. Data show a dose-dependent decrease in iNKT cells 24 hours after treatment (n=1 per dose). The pretreatment baseline iNKT levels in this cohort of animals ranged from 0.12% - 1.13% of CD3+ cells. Complete depletion of iNKT cells was seen at 24 hours following dosing with the 0.03 mg/kg dose.

Figure 9: iNKT Cell Depletion by NKTT120 in Cynomolgus Monkeys



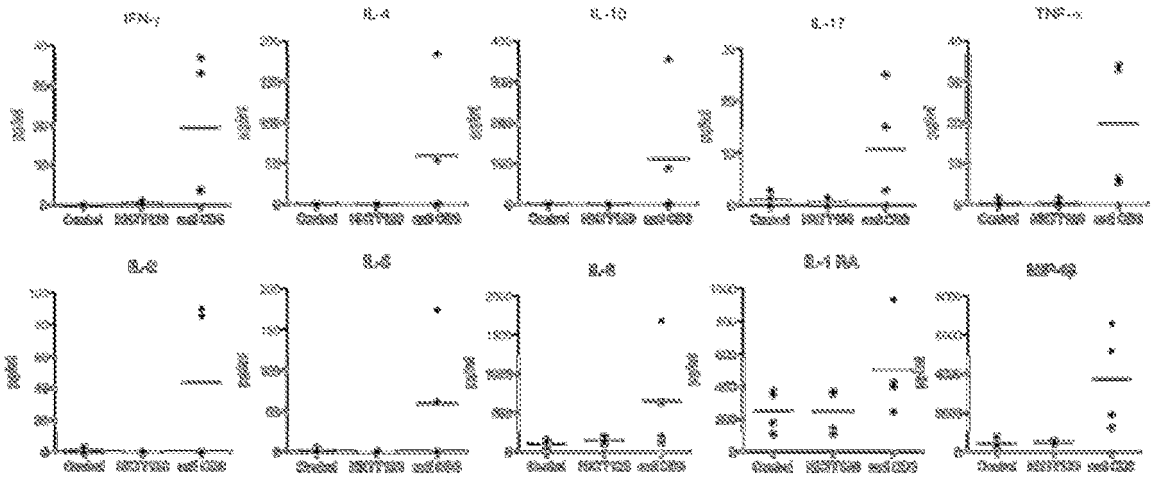
NKTT120 dose response of the depletion of iNKT cells in cynomolgus monkeys.

Figure 10: Effect of NKTT120 on iNKT, B and T Cells



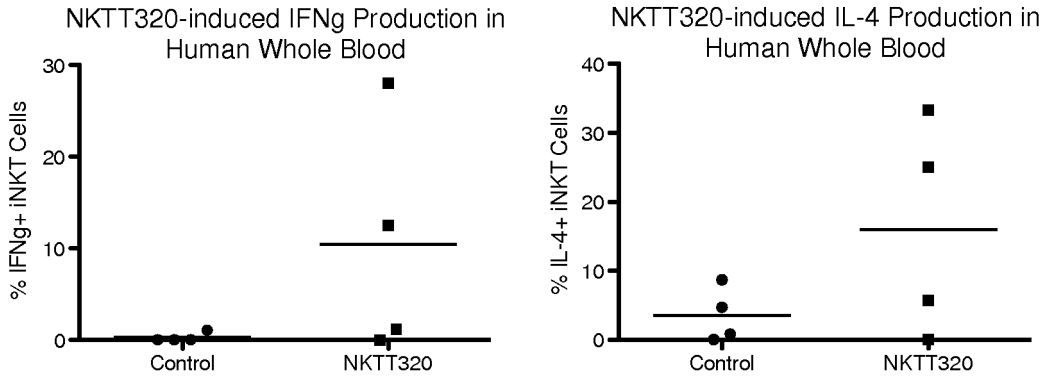
NKTT120 (0.03 mg/kg) treatment has no significant effect on B cells and other T cells.

Figure 11: Effect of NKTT120 Cytokine Levels in Human Whole Blood



Cytokine levels in untreated, NKTT120- and anti-CD3-treated whole blood samples from healthy donors (n=4). Only results that differed from control levels are shown. Blood samples from healthy donors were purchased from Research Blood Components, Inc.

Figure 12


NKTT320-induced IFNg Production in Human Whole Blood
% IFNg+ iNKT Cells
30
20
10
0
Control
NKTT320
NKTT320-induced IL-4 Production in Human Whole Blood
% IL-4+ iNKT Cells
40
30
20
10
0
Control
NKTT320