# LOWER ADCC AFTER 26 WEEKS OF PEG-IFN-α2b AND TWO bNAbs IN OTHERWISE SUPPRESSED HIV-1+ INDIVIDUALS


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## Abstract


**Background:** Previous studies suggested that pegylated interferon α2b (peg-IFN-α2b) and the broadly neutralizing antibodies (bNAbs) 3BNC117 and 10-1074 may contribute to cure-related strategies. We evaluated the effect of a 26-week immunotherapy course with peg-IFN-α2b+bNAbs in the cytotoxic function and activation of natural killer (NK) cell subsets in persons with HIV infection (PWH) that participated in the BEAT 2 study (NCT03588715).

**Methods:** Fourteen PWH receiving suppressive antiretroviral therapy (ART, <50 HIV-1 copies/ml) underwent ART interruption (ATI) while receiving a 26-week immunotherapy course of peg-IFN-α2b+bNAbs. Peripheral blood mononuclear cells (PBMC) were collected prior to ATI/immunotherapy (ART alone, time-point 1), on ART+4 weeks peg-IFN-α2b (time-point 2), and on ATI+26 weeks peg-IFN-α2b+bNAbs (time-point 3). Fresh PBMC were used in $^{51}$Cr release assays for assessment of antibody-dependent cell-mediated cytotoxicity (ADCC) against NK-resistant lymphoblastic target cell line prior and after in vitro stimulation with gp120, and for direct cytotoxicity against MHC-cell null cancer target cell line prior and after in vitro stimulation with IFN-α. Cryopreserved PBMC were used for immunophenotypic characterization by flow cytometry of NK cell subsets and of markers associated with NK activation, inhibition or maturation (e.g. CD38, NKp46, NKG2A, Siglec 7, Siglec 9, CD57). Statistics were performed by JMP 15.

**Results:** Immunotherapy did not affect IFN-α-induced NK direct cytotoxicity but resulted in a decrease in gp120-mediated ADDC. Reduced ADCC was observed together with an increase in the cytokine producing CD56$^{hi}$ and in CD56$^{lo/+}$CD16$^{-}$ % of CD56$^{+}$ NK cells, and a decrease in the cytotoxic CD56$^{lo/+}$CD16$^{+}$ % of CD56$^{+}$, suggesting that decrease in the expression of Fc receptor CD16 on NK could be associated with lower ADCC function. These findings were supported by a negative correlation between ADCC and CD56$^{lo/+}$CD16$^{-}$ % of lymphocytes after IFN-α immunotherapy (end of step 2). Finally, the gp120-induced ADCC decrease was observed together with a decrease in the maturation/cytotoxicity marker CD57 in CD56$^{lo/+}$CD16$^{+}$ NK cells, despite an increase in activation (CD38, NKp46) and inhibition (NKG2A, Siglec 7) markers.

**Conclusion:** In PWH, combined immunotherapy with peg-IFN-α2b+bNAbs resulted in no effect on IFN-α-induced NK direct cytotoxicity and an unexpected decrease in gp120-induced ADCC and in circulating CD16$^{+}$ NK cell subsets.


## Introduction

In our previous studies NCT00594880 **(1-3)** and NCT01935089 **(4)** we showed that pegylated interferon (peg-IFN-α2a and peg-IFN-α2b respectively) can achieve sustained virological control in patients with HIV during antiretroviral therapy interruption (ATI). Pillar-Mendoza et al. **(5)** have demonstrated the effectiveness of combining two broadly neutralizing antibodies (bNabs), 3BNC117 and 10-1074, in maintaining viral suppression during analytical treatment interruption in participants with sensitive virus at baseline. These data suggest that pegylated interferon and the bNAbs 3BNC117 and 10-1074 may contribute to cure-related strategies. We conducted the NCT03588715 study to test whether the combination of bNabs and peg-IFN-α2b, when administered in together with antiretroviral therapy, would have a significant impact on achieving durable suppression off ART together with a reduction of the HIV reservoir. Here we present the results of the evaluation of the effect of a 26-week immunotherapy course with peg-IFN-α2b+bNAbs in the cytotoxic function and activation of natural killer (NK) cell subsets in persons with HIV infection (PWH) that participated in this study.

## Subjects, Materials & Methods

### Study participants

A total of 14 PWH participated in the study. All participants were adults living with HIV and well-controlled viral replication with antiretroviral therapy (ART) for more than 6 months, with a current CD4$^{+}$ T cell count ≥450 cells/mm$^{3}$. Participants underwent ART interruption (ATI) while receiving a 26-week immunotherapy course of peg-IFN-α2b+bNAbs.

### Methods

Peripheral blood mononuclear cells (PBMC) were collected prior to ATI/immunotherapy (ART alone, time-point 1), on ART+4 weeks peg-IFN-α2b (time-point 2), and on ATI+26 weeks peg-IFN-α2b+bNAbs (time-point 3) (**Figure 1**). Assays performed:

Antibody-dependent cell-mediated cytotoxicity (ADCC)

Fresh PBMC were used in $^{51}$Cr release assays for ADCC against NK-resistant lymphoblastic target cell line prior and after in vitro stimulation with gp120. Percent lysis was determined by the following formula: [(experimental counts-spontaneous released counts)/(total counts-spontaneous released counts)] x 100. Results were expressed as area under the curve (AUC) for effector to target (E:T) ratios of 50:1, 25:1, 12.5:1 and 6.25:1 for both constitutive and gp120-induced ADDC. Data were normalized by number of NK.

Direct cytotoxicity

Fresh PBMC were used in $^{51}$Cr release assays for direct cytotoxicity against MHC-cell null cancer target cell line prior and after in vitro stimulation with IFN-α. Percent lysis was determined by the following formula: [(experimental counts-spontaneous released counts)/(total counts-spontaneous released counts)] x 100. Results were expressed as area under the curve (AUC) for effector to target (E:T) ratios of 50:1, 25:1, 12.5:1 and 6.25:1 for both constitutive and in vitro IFN-α-induced NK function. Data were normalized by number of NK.

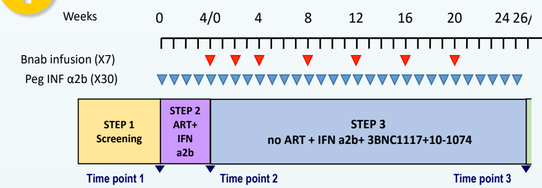
Immunophenotypic characterization by flow cytometry

Cryopreserved PBMC were used for immunophenotypic characterization by flow cytometry of NK cell subsets and of markers associated with NK activation, inhibition or maturation (e.g. CD38, NKp46, NKG2A, Siglec 7, Siglec 9, CD57). Results were expressed as mean fluorescent intensity (MFI), and percent (%) positive.

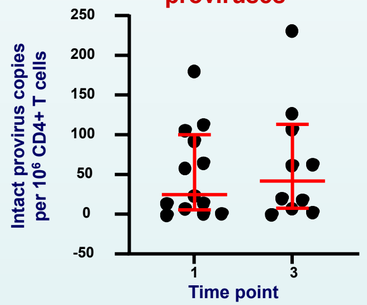
### Statistical analysis

Data were described as medians, 25$^{th}$ and 75$^{th}$ percentiles. Variable distributions were analyzed for normality using the Shapiro-Wilk W test (p>0.05). Depending on data distribution, between time points comparisons were performed using non-parametric Wilcoxon Sign-Rank test or paired t-tests. P-values <0.05 were considered statistically significant. All statistics were performed with JMP Pro15 (SAS Institute, Cary, NC).

## 1 Study schema



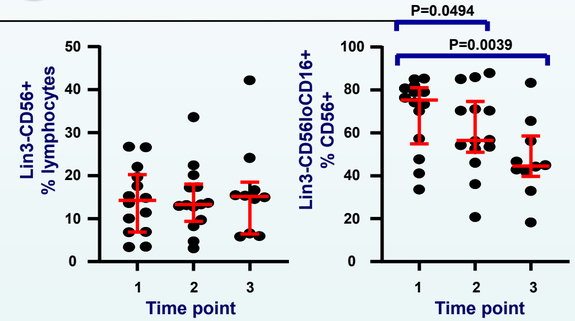

## 2 No effect on intact HIV proviruses



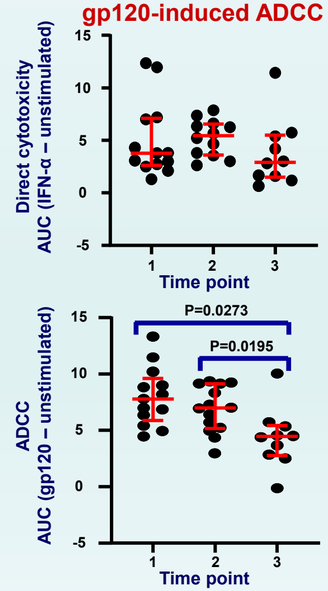

## 3 Decrease in NK of maturation/cytotoxicity markers in contrast to increase in activation markers

| Variable | Function | Time point 1 Median (25-75 IQR) | Time point 3 Median (25-75 IQR) | P |
|---|---|---|---|---|
| CD57+/CD56lo/CD16+ % of CD56lo/CD16+ | Maturation/cytotoxicity marker | 77.98 (70.55-85.05) | 61.49 (48.2-72.6) | 0.0098 |
| CD38+/CD56lo/CD16+ % of CD56lo/CD16+ | Activation marker | 71.73 (64.59-87.47) | 97.66 (95.7-99.4) | 0.0039 |
| MFI of CD38 on CD56lo/CD16+ | Activation marker | 1817 (1322-3732.5) | 10012.5 (6992-12109.25) | 0.0195 |
| NKp46+/CD56lo/CD16+ % of CD56lo/CD16+ | Activation marker | 79.97 (64.27-85.75) | 91.61 (82.77-95.39) | 0.0371 |
| MFI of NKp46 on CD56lo/CD16+ | Activation marker | 935 (579.75-1082.25) | 1535.5 (1191.75-2478) | 0.0098 |
| NKG2A+/CD56lo/CD16+ % of CD56lo/CD16+ | Inhibition marker | 28.67 (15.38-45.22) | 44.36 (37.87-62.11) | 0.0059 |
| MFI of NKG2A on CD56lo/CD16+ | Inhibition marker | 117.5 (89.47-179.5) | 158 (143-230.75) | 0.0137 |
| MFI of Siglec7 on CD56lo/CD16+ | Inhibition marker | 3736.5 (1868.25-6089.25) | 3058.5 (1584.75-3912) | 0.0371 |
| Siglec9+/CD56lo/CD16+ % of CD56lo/CD16+ | Inhibition marker | 31.84 (26.11-43.27) | 14.33 (6.42-22.29) | 0.002 |
| MFI of Siglec9 on CD56lo/CD16+ | Inhibition marker | 131.5 (102.17-196.5) | 58.4 (50.42-77.52) | 0.002 |

## 4 No effect on total NK in contrast to a decrease in CD16+ NK cell subsets




## 5 No effect on IFN-α-induced NK direct cytotoxicity in contrast to a decrease in gp120-induced ADCC




## Conclusions

In PWH, combined immunotherapy with peg-IFN-α2b+bNAbs on ART resulted in:

- No effect in HIV proviruses levels
- Increase in NK activation
- Decrease in maturation/cytotoxicity markers
- Reduction of NK functionality as shown by:
  - the decrease in circulating CD16$^{+}$ NK cell subsets
  - the decrease in gp120-induced ADCC
  - the lack of effect on IFN-α-induced NK direct cytotoxicity

## Interpretation

The cause for the reduced NK functionality was clearly associated with the presence of immunotherapy before bNAb infusions. It remains to be determined if other preclinical immunotherapy strategies administered on ART and associated with IFN-α induction could also result in a reductions of NK ADCC function.

## References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5. Mendoza P, Gruell H, Nogueira L, et al. Nature 2018;561(7724):479-484.


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