PLASMA CD33 LEVEL IS A MARKER OF VIRUS CONTROL POST KICK-AND-KILL CURE INTERVENTION

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BACKGROUND

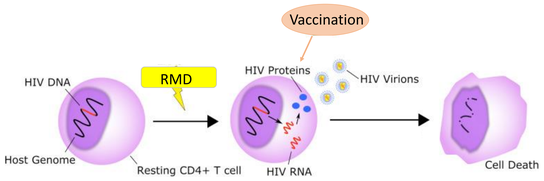
HIV-cure strategies will require the elimination of the latent virus from the body, including the central nervous system (CNS) and agents that can reactivate viral brain reservoir will be needed. The BCN02 HIV-vaccine clinical trial was focused on a "Kick and kill" strategy using T cell vaccination and romidepsin, a LRA with known effects on the CNS.

"kick"

Is based on the administration of the latency reversing agent romidepsin (RMD)

"kill"

Intensification of the immune response through vaccination with a MVA-vectored T-cell vaccine



RMD:

1. A HDACi (histone deacetylase inhibitor)
2. Can reactivate viral replication in-vitro and in-vivo (Datsen George Wei, et al,. 2014)
3. Has shown beneficial effects on the CNS in HIV unrelated diseases (Hongmei Zeng, et al,. 2021)

OBJECTIVE

Study longitudinal inflammatory and neurological plasma proteomes from BCN02 participants to identify biomarkers associated with virus control during monitored antiretroviral pause (MAP).

RESULTS

Plasma proteomes during the BCN02 trial

Neurological and inflammation plasma factors progressively differ during the BCN02 trial, with most accentuated differences during MAP (Fig1). Interestingly, the intervention accentuated protein plasma detection, after RMD administration (49 proteins) and during MAP (76 proteins), 29 of them being in common (Fig2).

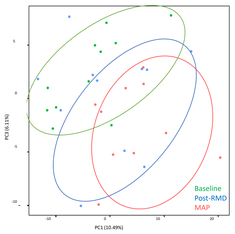


Fig 1. PCA analysis showing segregation of the samples by timepoints

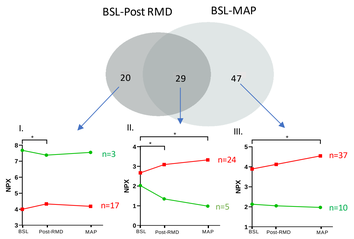


Fig 2. Significant plasma proteins during BCN02 clinical trial

Plasma proteomes discriminate between MAP-C and MAP-NC

Inflammatory and neurological proteins differ between MAP-C and MAP-NC (Fig3).

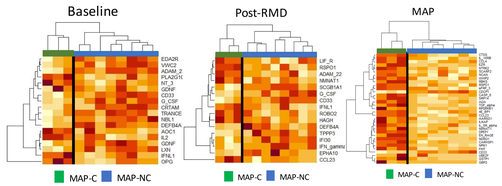


Fig3. Differential plasma proteomes between MAP-C and MAP-NC

CD33 protein levels are associated with viral replication CD33 protein was uniquely increased upon RMD administration and maintained during MAP and allowed to discriminate between MAP-C and MAP-NC (Fig4).

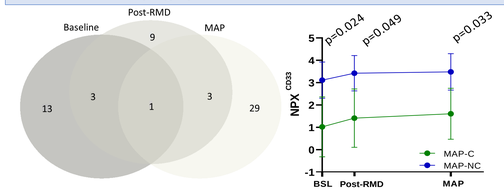


Fig4. CD33 discriminates between MAP-C and MAP-NC and intervention follow-up

CD33 plasma levels were positively associated with viral load and proviral levels in the BCN02 trial (Fig5).

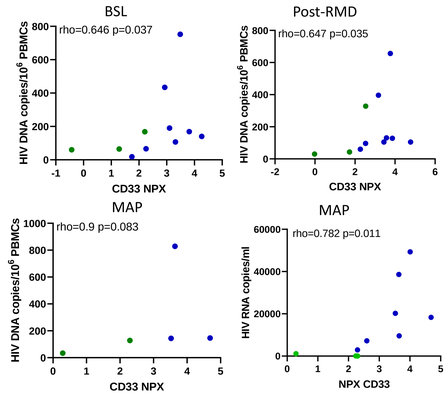


Fig 6. Correlation analysis between CD33 plasma levels and viral parameters

CD33 protein levels in natural infection

Validation untreated HIV infected individuals confirmed the data observed in BCN02 trial, showing higher plasma levels in uncontrolled infection (Fig6) and associations between CD33 and viral load and proviral levels (Fig7).

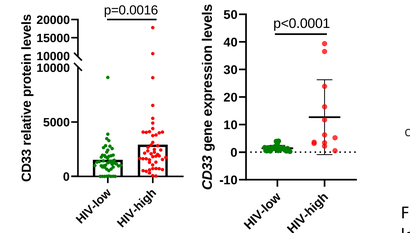


Fig 6. CD33 levels in natural untreated HIV-infection

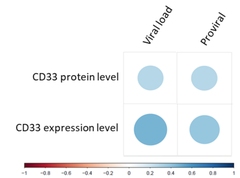


Fig 7. Correlogram plot between levels of CD33 in plasma and PBMCs and viral parameters.

In vitro experiments using PHA-blasts and monocyte-derived macrophages (MDMs: Anti-CD33 antibody reduces HIV replication and total HIV DNA levels in a dose-dependent manner (Fig8).

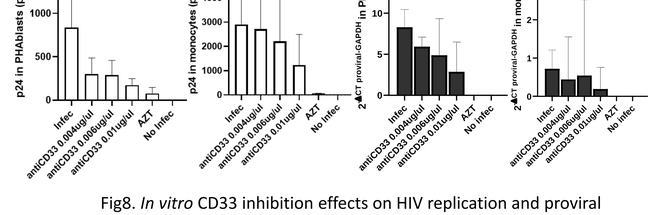
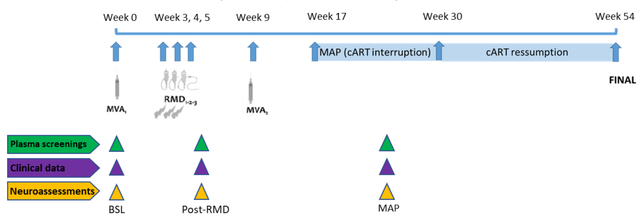


Fig8. *In vitro* CD33 inhibition effects on HIV replication and proviral

METHODS

- **The BCN02 clinical trial** was based on 3 RMD infusions and 2 MVA T cell vaccinations, and included a monitored antiretroviral pause (MAP) for up to 32 weeks.



Plasma samples from 11 BCN02 participants that were classified according to time to virus-rebound in MAP: 8 MAP-NC that did not control viral replication (viral rebound <4weeks) and 3 MAP-C that control virus replication during all the MAP phase (>32weeks) to below 2,000 copies.

- **Proximity Extension Assay** (Olink®) based on 92-plex cytokine panels for *Inflammation*, *Neurology* and *Neuro-Exploratory;* levels are expressed as relative plasma levels (Normalized Protein eXpression, NPX).

- **Validation cohorts:** plasma and dry pellet PBMCs samples from HIV infected individuals without ART treatment with different control of viral replication (47 HIV-high, >50.000 HIV-RNA copies/ml; and 49 HIV-low, <10.000 HIV-RNA copies/ml) to evaluate the CD33 plasma levels and gene expression by RT-PCR.

- **HIV replication in PHA-blasts and monocyte-derived macrophages (MDMs):** PHA-blasts and MDMs were infected with/without HIV NL4-3 and BAL strains (MOI=0,01), cultured during 3 (PHA blasts) and 4 days (MDMs), respectively, in different conditions:

a. Non infected
b. Infected
c. Infected with AZT (1ug/ml)
d. Infected with anti-CD33 0.004ug/ul, 0.006ug/ul and 0.01ug/ul

Supernatant p24 quantification by ELISA (Innogenetics) and proviral quantification by PCR were performed.

- **Data analysis**: Principal component analysis (PCA), heatmaps and venn diagrams were carried out using R software. Differences in CD33 between groups were analyzed using the Mann-Whitney test. Spearman's rank test was applied for the correlation analysis. For in vitro experiments analysis, ANOVA test was used to analyze differences between conditions. Statistical significance was set at $p<0.05$.

CONCLUSIONS

- ❑ **Plasma proteomes changed longitudinally along the BCN02 clinical trial. Specially, inflammatory and neurological proteins differed markedly between MAP-C and MAP-NC.**
- ❑ **CD33 (Siglec-3) protein is a marker for uncontrolled viral infection in the MAP phase of BCN02 clinical and in untreated chronic infection.**
- ❑ **In vitro experiments with anti-CD33 reduces HIV replication and proviral levels, indicating that CD33 is required for effective virus propagation.**

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