Successful Direct-Acting Antiviral Therapy (DAA) in HIV/HCV Co-Infected Patients Fails to Restore Circulating Mucosal-Associated Invariant T Cells (MAITs)

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Mucosal-associated invariant T (MAIT) cells are unconventional T lymphocytes characterized by the high expression of CD161 and semi-invariant T cell receptor (TCR) [1] and are restricted by the evolutionarily conserved major histocompatibility complex related molecule, MR1. MAIT cells are abundant in human blood (1-10%), gut (4-10%) and liver (20-40%) [2]. Human MAIT cells react to bacterially infected cells in an MR1-dependent manner and throughout the course of infections they contribute to the host response secreting inflammatory cytokines and accumulating early in infected tissues [3].

MAITs play a crucial role in innate immunity. In chronic viral infections, they are impaired in frequencies and functions and correlate with disease progression [4]. A substantial reduction in MAITs has been described in untreated HIV, which is not restored by cART (combination antiretroviral therapy) [5].

We have read with great interest the article by Hengst et al. that investigated whether MAIT cell recovery occurs upon HCV-clearance in HCV-monoinfected patients (pts) receiving an IFN-free treatment regimen, consisting of sofosbuvir and ribavirin [6]. Interestingly, in contrast to other immune cells, MAIT cells were not reinvigorated following successful HCV-clearance using IFN-free therapy [6]. Little is known about MAITs frequency or function in HIV/HCV co-infected patients and their fate after HCV elimination by **direct-acting antiviral therapy** (DAA).

Here, we would like to add to the discussion by Hengst et al. [6] by sharing our investigation of CD161+MAIT cell frequencies in cART-treated HIV/HCV co-infected patients monitored longitudinally pre-HCV treatment, after unsuccessful pegylated Interferon alpha/ribavirin therapy (peg-IFNα /RBV) and after successful IFN-free treatment. We enrolled 15 HIV+/HCV+ pts [HIV-RNA<40cp/ml; median CD4 527/mmc (IQR 409-780)] and 10 age-matched healthy controls (HC). All patients were initially treated with peg-IFN-based anti-HCV treatment: 9/15 patients (60%) achieved a sustained virologic response (SVR), 6/15 patients (40%) failed to clear HCV infection (non-responders-NR). One/6 pts was not eligible for DAA treatment, 5/6 NR pts started DAA-based therapy (3 PI-based vs 2 with an NS5A inhibitors) and all achieved HCV clearance (Figure 1A).

We measured MAIT frequency (CD161++Va7.2+ CD3 or CD8), activation (CD69), exhaustion (CD39/PD-1), IL18R expression, cytolytic activity (granzyme B/perforin A) (flow cytometry) at baseline (T0), Interferon end-of-treatment (T1) and DAA end-of-treatment (T2) as well as in HC (Figure 1A).

At baseline, HIV/HCV pts displayed substantially contracted total (CD3) and CD8 MAITs as compared to HC (Figure 1B). In chronic infections, CD161 down-regulation was suggested as a mechanism behind MAIT cell depletion [7]; however in our cohort of HIV/HCV patients we failed to detect an increased frequency of Vα7.2+CD161- cells compared to HC (p=0.87; data not shown). All MAIT subsets of HIV/HCV patients showed a trend towards higher CD69 and PD-1 expression (Figure 1C) and a trend towards higher granzyme B expression (p= 0.07), but no differences in CD39, IL-18R and perforin expression were detected between HIV/HCV patients and HC (data not shown).

We next sought to longitudinally investigate the possible peripheral MAIT cell restoration after HCV therapy. Interestingly, following peg-IFN-based therapy (T1) we found no change in total and CD8 MAITs, irrespective of whether SVR was achieved or not (Figure 1D). Likewise, following DAA treatment (T2), despite HCV clearance being reached by all patients, total and CD8 MAITs frequency failed to recover significantly (Figure 1D), always remaining lower than HC. No differences in PD-1 (p=0.316) and CD69-expressing total and CD8+ MAIT cells (p=0.436) were shown in HIV/HCV patients upon HCV clearance (Figure 1E).

In line with data obtained by Hengst et al in HCV mono-infected patients, we hereby describe a profoundly depleted circulating MAIT compartment in cART-treated HIV/HCV co-infected patients that is not restored by successful anti-HCV treatment and HCV virus eradication. We show, in a longitudinal cohort of patients, that in contrast to what is described for other cell populations (e.g. partial recovery of HCV-specific CD8 T cells [8] and NK cells [9]), successful DAA treatment does not restore the impoverished MAIT cell compartment, whose dysfunction appears to be nonreversible. A limitation our study was the short- term follow-up of the patients. Indeed, recent studies

showed that it takes 5-6 years for MAIT cells to expand after birth or after hematopoietic stem cell transplantation [10]. To our knowledge, this is the first report to longitudinally describe MAIT cell frequencies in HCV/HIV co-infection following IFN-free treatment therapy. Future research is needed to dissect long-term follow up and molecular mechanisms governing the homeostasis of circulating and tissue MAIT cells upon viral clearance in HIV/HCV co-infected patients.

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Authors Contribution

ESC and MC designed and performed the experiments, analyzed and interpreted the data, designed the figures, and wrote the manuscript. BVW, LS and EM performed the experiments and helped with analyzing the data. ADB helped with analyzing clinical data. ADM helped with interpreting the results and edited the manuscript. PK and GM conceived and designed the study, interpreted the data and wrote the manuscript.

Conflict of interest:

Authors declare no commercial or financial conflict of interest

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Figure 1. Frequency and function of Vα7.2+CD161++CD3*/CD8+ (MAITs) of HCV/HIV co-infected patients.

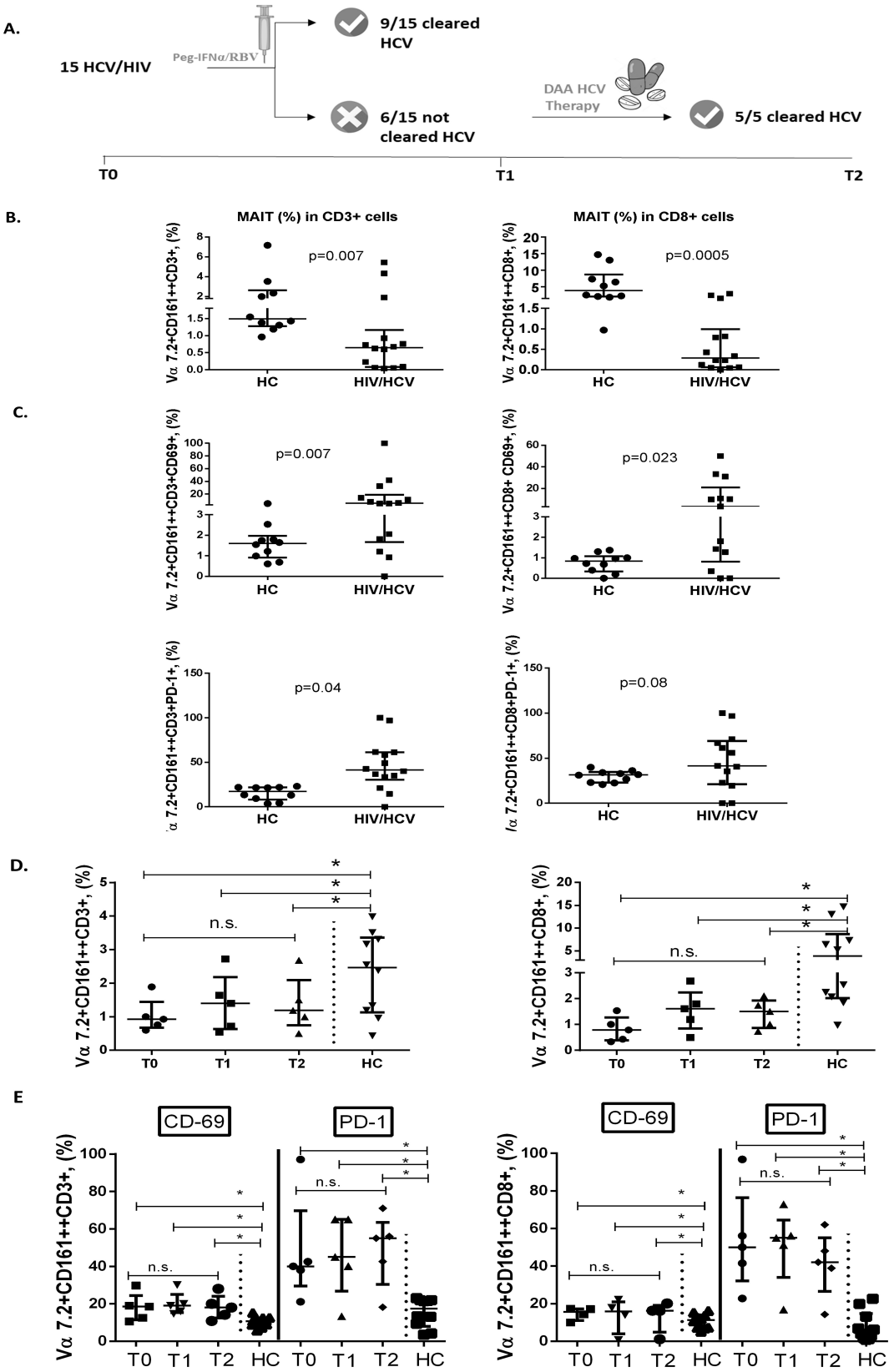

Figure 1. Frequency and function of Vα7.2+CD161++CD3+/CD8+ (MAITs) of HCV/HIV co-infected patients (pts).

A) Study Design. 15 HIV/HCV+ cART-treated pts were enrolled in the study. All patients were treated with pegylated Interferon alpha/ribavirin **(peg-IFNα /RBV)**: 9/15 patients (60%) achieved a sustained virologic response (SVR), 6/15 patients (40%) failed to clear HCV infection (non-responders-NR). Samples were collected within 24 weeks after treatment discontinuation. One/6 pts was not eligible for DAA treatment, 5/6 NR pts started **(DAA)** -based therapy (ombitasvir/paritaprevir/ritonavir/dasabuvir/RBV, simeprevir/sofosbuvir (SOF), daclatasvir/SOF or ledipasvir/SOF/RBV). Samples were collected 12 weeks after end of treatment (SVR12) **B)** Compared to healthy individuals (HC), virally-infected patients displayed a significant lower proportion of peripheral MAIT cells (gated on live, CD3+ or CD3+CD8+ CD161+Va7.2+). **C)** CD8+ and CD3+ MAIT of co-infected patients tended to be more exhausted/activated when compared to HC. **D)** No change in total or CD8 MAITs were shown after both Interferon-based and Interferon-free HCV therapy. Baseline (T0), Interferon end-of-treatment (T1), DAA end-of-treatment (T2). **E)** No changes in CD69+ and PD-1+ total and CD8+ MAITs were shown after both Interferon-based and Interferon-free HCV therapy; The different groups of patients and the different time points were compared using Kruskal-Wallis or Wilcoxon matched pairs test as appropriate *P values <0.05 were considered statistically significant. Data were analyzed with GraphPad 5 Prism