# Latency Reversal Induced by CD4+ T cell Recognition of HIV-1 Alternate Reading Frame Proteins

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## Background

- HIV-1 alternate reading frame proteins (ARFPs) have been shown to elicit CD8+ T cell responses, but it is not known whether they are also recognized by CD4+ T cells.
- We hypothesized that ARFP-specific CD4+ T cells were present in PWH on ART and that these cells were more likely to be latently infected.
- We hypothesized that stimulation of ARFP-specific CD4+ T cells with cognate peptides would result in latency reversal.

## Objectives

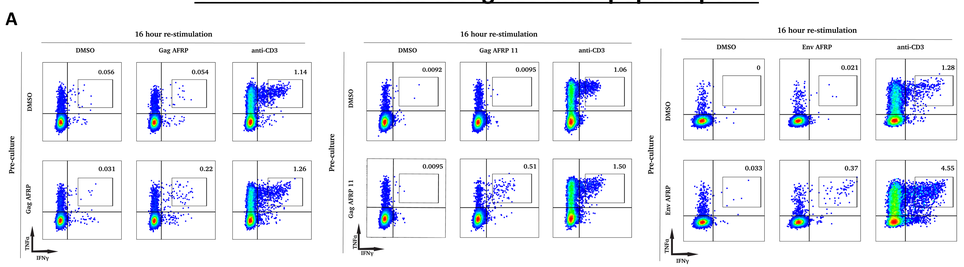
- Determine whether CD4+ T cells recognize ARFP-derived peptides.
- Identify which ARFP peptides elicit the strongest responses.
- Evaluate HLA binding predictions for identified epitopes.
- Investigate whether ARFP peptide stimulation leads to HIV latency reversal.

## Summary

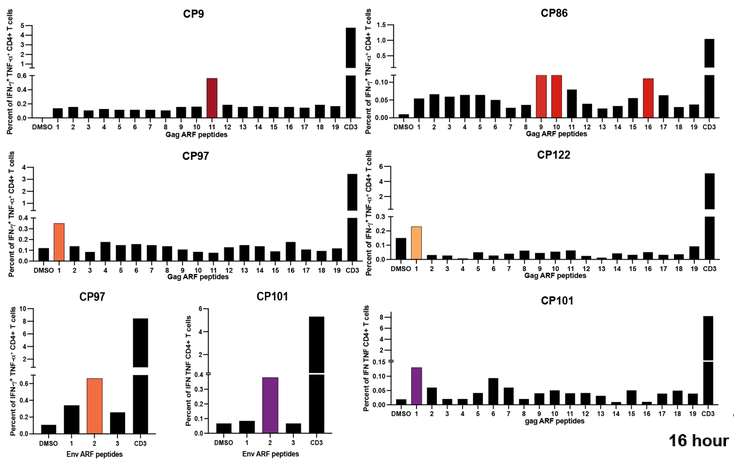

- CD4+ T cells from chronic progressors (CPs) recognize ARFP-derived peptides.
- Gag ARFP peptides were recognized in 7 out of 14 CPs.
- Env ARFP peptides were also immunogenic in 2 out of 14 CPs.
- ARFP peptides induced latency reversal in 3 out 7 CPs.


## Results

### CD4+ T cells from CPs recognize ARFP peptide pools




### Identification of ARFP epitopes



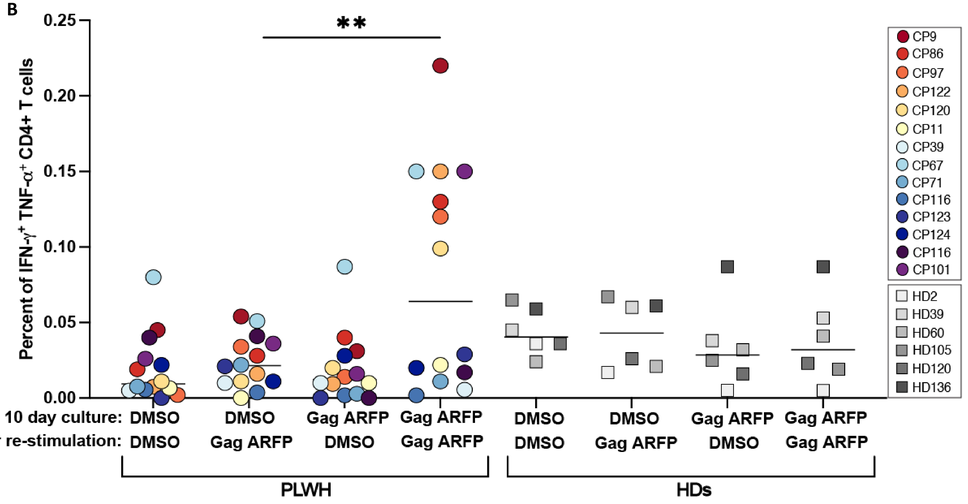




**Figure 2: Functional characterization of CD4+ T cell responses to individual ARFP peptides following in vitro expansion.**
PBMCs from 5 participants were pre-cultured for 10 days with either Gag ARFP or Env ARFP. After expansion, cells were stimulated for 16 hours with DMSO, individual Gag ARFP peptides , individual Env ARFP peptides, or anti-CD3 antibodies and stained for IFN-γ and TNF-α expression. The percentage of IFN-γ+ TNF-α+ CD4+ T cells was measured in the 5 participants. The colored bars represent the most immunogenic peptides.

**Figure 1: Functional characterization of antigen-specific CD4+ T cell responses following in vitro expansion**.
(A) PBMCs from two participants (CP9 & CP97) were pre-cultured for 10 days with either DMSO (top row) or Gag ARFP & Gag ARFP 11 (left & middle bottom row) or Env ARFP peptides (right bottom row). After expansion, cells were stimulated for 16 hours with DMSO, Gag ARFP peptides, Gag ARFP 11 peptides, Env ARFP peptides or anti-CD3 antibodies and stained for IFN-γ and TNF-α expression. The percentages in each quadrant indicate the proportion of cells expressing the respective cytokines.
(B) The percentage of IFN-γ+ TNF-α+ CD4+ T cells was measured in 20 participants (14 CPs & 6 HDs) after a 10-day pre-culture and 16-hour restimulation with Gag ARFP peptide pool, with DMSO as the negative control. **p<0.01

### Immunogenic ARFP peptides are predicted to bind to CP MHC II alleles

| Participant | Peptide name | Peptide Sequence | HLA allele | Predicted MHC-II allele (IEDB analysis) |
|---|---|---|---|---|
| CP9 | Gag ARFP 11 | ANSPTRRELQVWGRDNN | DRB1*07:01; DRB1*11:02; DRB3*02:02; DRB4*01:03; DQA1*02:01/DQB1*02:02; DQA1*05:05/DQB1*03:01; DPA1*01:03/DPB1*04:01; DPA1*03:01/ DPB1*105:01 | DRB1*11:02; DRB4*01:03; |
| CP86 | Gag ARFP 9; Gag ARFP 10; Gag ARFP 16 | FFREDLAFPQGKAREFS; KAREFSSEQTRANSPTR; IIRRSHPTRFKHHAKHS | DRB1*11:02; DRB1*15:03; DRB3*02:02; DRB5*01:01; DQA1*05:05/DQB1*03:19; DQA1*01:02/DQB1*06:02; DPA1*01:03/DPB1*01:01; DPA1*02:01/DPB1*02:01 | DRB3*02:02; DRB5*01:01; DPA1*01:03/DPB1*01:01; DPA1*02:01/DPB1*02:01 |
| CP97 | Gag ARFP 1; Env ARFP 2 | MGKSSRREGFQPRSDTH; FVHQMLKHMIQRYIMGF | DRB1*13:02; DRB3*03:01; DQA1*01:02/DQB1*06:04; DQA1*01:02/DQB1*06:09; DPA1*01:03/DPB1*02:01; DPA1*01:03/DPB1*03:01 | DPA1*01:03/DPB1*03:01 |
| CP101 | Gag ARFP 1, Env ARFP 2 | MGKSSRREGFQPRSDTH; FVHQMLKHMIQRYIMGF | DRB1*12:01; DRB1*13:02; DRB3*01:01; DRB3*03:01; DQA1*01:02/DQB1*05:01; DQA1*01:05/DQB1*05:01; DPA1*01:03/DPB1*02:01; DPA1*02:12/DPB1*85:01 | DRB1*12:01; DRB1*13:02; DPA1*01:03/DPB1*02:01; DPA1*01:05/DPB1*05:01 |
| CP122 | Gag ARFP 1 | MGKSSRREGFQPRSDTH | DRB1*12:01; DRB1*13:01; DRB3*02:02; DRB3*01:01; DQA1*05:05/DQB1*03:01; DQA1*01:03/DQB1*06:03; DPA1*01:03/DPB1*02:01; DPA1*03:01/DPB1*105:01 | DPA1*01:03/DPB1*02:01 |

**Table 1: Class II HLA alleles of CPs who responded to ARFP peptides**

### Reversal of latency by ARFPs in some CPs

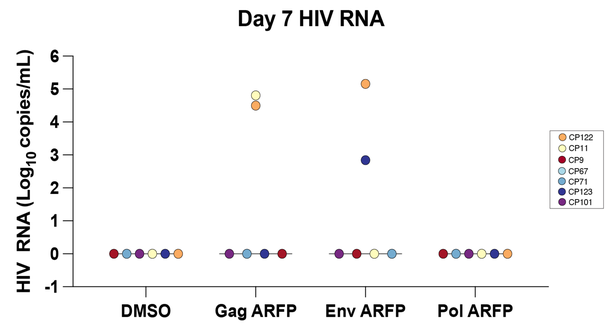



**Figure 3: Quantification of HIV RNA levels in culture supernatant after AFRP peptide stimulation**. PBMCs from 5 participants were cultured with either Gag ARFP or Env ARFP or Pol ARFP. On day 7, the HIV RNA levels were measured in 7 CPs.

## Acknowledgments

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