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# P17-21. Using ubiquitin fusion to augment CD8+ T cell immune responses against HIV-1 antigens

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## Background

Development, evaluation and optimisation of HIV-1 genetic vaccine components to generate a large pool of CD8+ T cell memory cells recognising multiple cytotoxic lymphocyte (CTL) epitopes is one of the primary strategies in anti-HIV-1 vaccination protocols. This project, as part of the Patterson VDC/CAVD consortium http://www.cavd.org, aims to develop viral 'stealth'/non-viral vector vaccines against HIV-1 by targeting skin dendritic cells (DC).

We aimed to induce immunity to HIV-1 infection by developing a cohort of vectors in which HIV-1 and SIV vaccine components have been engineered and modified to stimulate broader CTL responses through genetic fragmentation and ubiquitination.

## Methods

Full-size codon optimised HIV-1 and SIV gag genes were fused to mono- or tetra-ubiquitin (Ub) sequences and tested on DC and non-DC cell lines and compared to their native, non-ubiquitinated forms. Recombinant adenoviral vectors (rAds) were produced and used in these experiments. In parallel, we tested the stability of the Ub sequences within the context of rAd vector development. We subsequently constructed rAds carrying genetically fragmented ubiquitinated versions for both HIV and SIV gag genes in an attempt to reduce antigenic competition and alter epitope dominance.

## Results

Ubiquitin fusion resulted in efficient proteasomal targeting of Gag compared to the degradation rate in its absence, as shown by experiments involving the proteasomal blocking agent MG132. The tetra-Ub sequence was shown to be unstable during rAd vector propagation whereas mono-Ub versions of antigens were efficiently created and currently undergoing extensive *in vitro* and *in vivo* testing.

## Conclusion

We are extending our studies using an *in vitro* CTL epitope-mapping system employing human monocyte-derived DC from healthy and HIV-1+ individuals alongside mouse *in vivo* studies in order to investigate the effect of antigen ubiquitination and fragmentation on the quality of the generated immune response. Additionally, the SIV constructs will be tested in a non-human primate challenge model (cynomolgus macaque).