Increased CTL Trafficking to LN Induced by hetIL-15 and Induction of Potent CTL Responses to HIV Conserved Elements DNA vaccine as Improved Therapeutic Approaches

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Background: IL-15 stimulates the growth and activation of NK cells and cytotoxic lymphocytes. We have produced heterodimeric IL-15 (hetIL-15), the authentic form of IL-15 found in the circulation, in human cells and demonstrated that administration of hetIL-15 in macaques has improved pharmacokinetics and pharmacodynamics without significant side effects. We have previously reported that therapeutic DNA vaccination together with pDNA encoding hetIL-15 in ART-treated macaques induces T cell responses able to control viremia after ART release.

Methods: We have developed stable human HEK293-derived cell lines producing hetIL-15 cytokine. The heterodimer was purified and tested for pharmacokinetics and pharmacodynamics properties in macaques upon SC administration. Phenotype and functional changes in lymphocyte subsets were monitored by flow cytometry and multiplexed confocal imaging (MCI).

To overcome HIV sequence diversity and to address potential "decoy" epitopes preventing efficacy, we developed DNA vaccines targeting the highly conserved regions (CE) in Gag and vaccine-induced cellular immunity was analyzed.

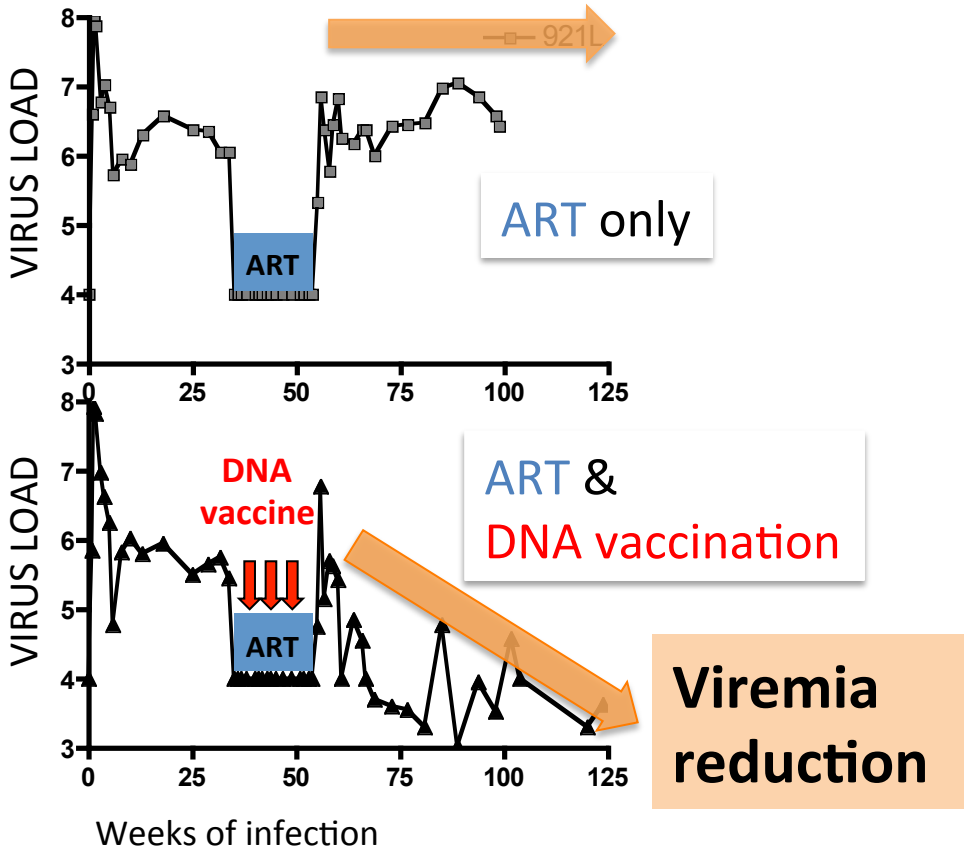
Results: Treatment with hetIL-15 resulted in a significant increase of circulating CD8+ effector T cells and NK cells with activated cytotoxic phenotype (Granzyme$^+$). This expanded T lymphocyte population was also present in secondary lymphoid organs where an increased frequency of Ag-specific effector CD8 T cells could be observed by both flow cytometry and MCI. A subset of CD8 T cells present in lymph nodes expresses CXCR5, indicating ability to migrate into germinal centers where chronically infected CD4$^+$Tfh reside. MCI confirmed their presence in germinal centers and showed that these cells are cytotoxic (GrzmB$^+$) and actively proliferating (Ki67$^+$) in response to hetIL-15.

Contrary to *gag* DNA vaccine, all CE pDNA-vaccinated macaques developed robust cytotoxic T cell responses targeting the conserved epitopes. Interestingly, vaccination with intact Gag efficiently boosted magnitude and breadth of preexisting CE responses indicating a change in epitope hierarchy. The induced T cell responses rapidly disseminated into secondary lymphoid organs and effector mucosal sites.

Conclusion: hetIL-15 treatment in combination with pDNA vaccine targeting the "Achilles' heel" of the virus, i.e., the highly conserved regions (CE), in virus sanctuary areas (germinal centers) is a promising HIV eradication strategy. We are exploring the potential of IL-15 as a viral reservoir reducing agent in ART-treated SIV infected macaques therapeutically vaccinated with plasmid DNA (CE pDNA) encoding conserved elements of the virus.



Therapeutic DNA Vaccination Decreases Viremia



- Potent induction of SIV-specific T cell responses in DNA immunized macaques during ART
  - 1-2% of circulating T cells in the periphery are SIV-specific
- Durable reduction in viremia

von Gegerfelt et al J Virol (2007); Valentin, Vaccine 2010

AIM to improve efficacy of therapeutic vaccination:

1. Use of optimized DNA vaccine to focus the immune response to the highly conserved regions of the virus
2. Combination with hetIL-15 cytokine to enhance immunotherapy

Successful Development of Unique Conserved Element (CE) Immunogens Targeting the Achilles' Heel of HIV

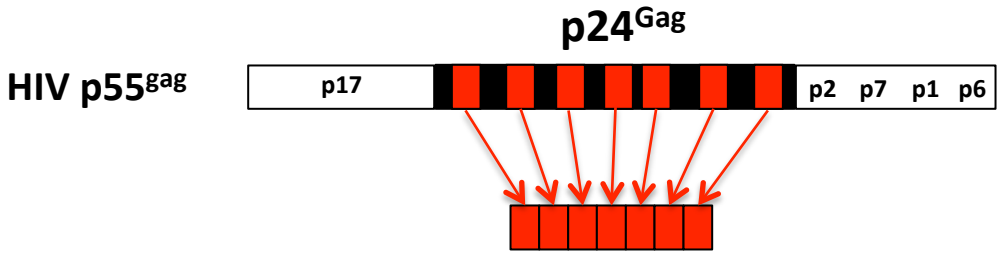
Problem:

HIV **sequence diversity** and potential **"decoy" epitop**es are hurdles in the development of an effective AIDS vaccine

Solution:

Produce **novel immunogens** containing **only conserved immunogenic regions** of HIV proteins,

associated with HIV control

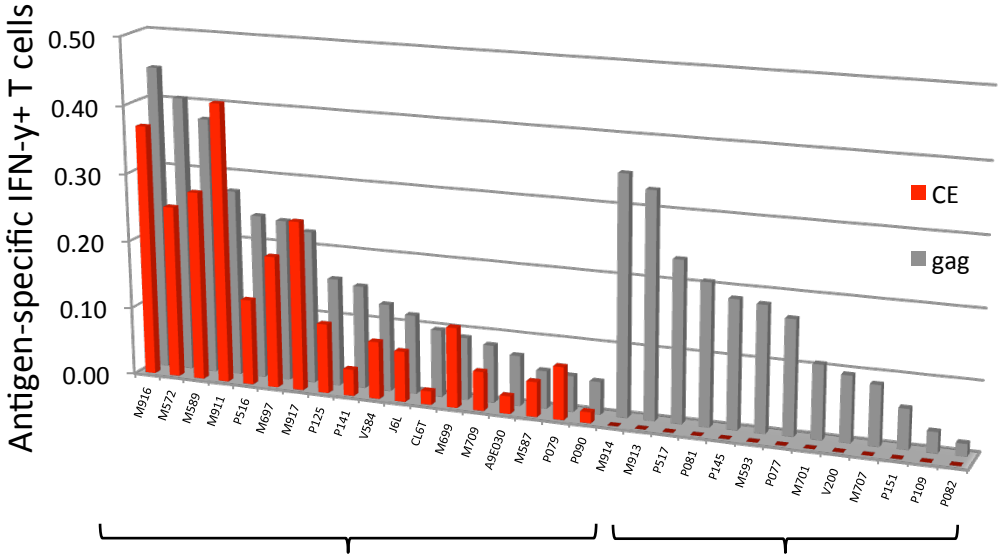


7 highly conserved elements (CE, 12-24 AA)
cover >98% of all known HIV sequences

Kulkarni, PLoS One 8: e60245, 2013;
Kulkarni, PLoS One;9:e86254. 2014



Only ~ 50% of *gag* DNA Vaccinated Macaques Elicit Cellular Responses to the Conserved Regions of Gag



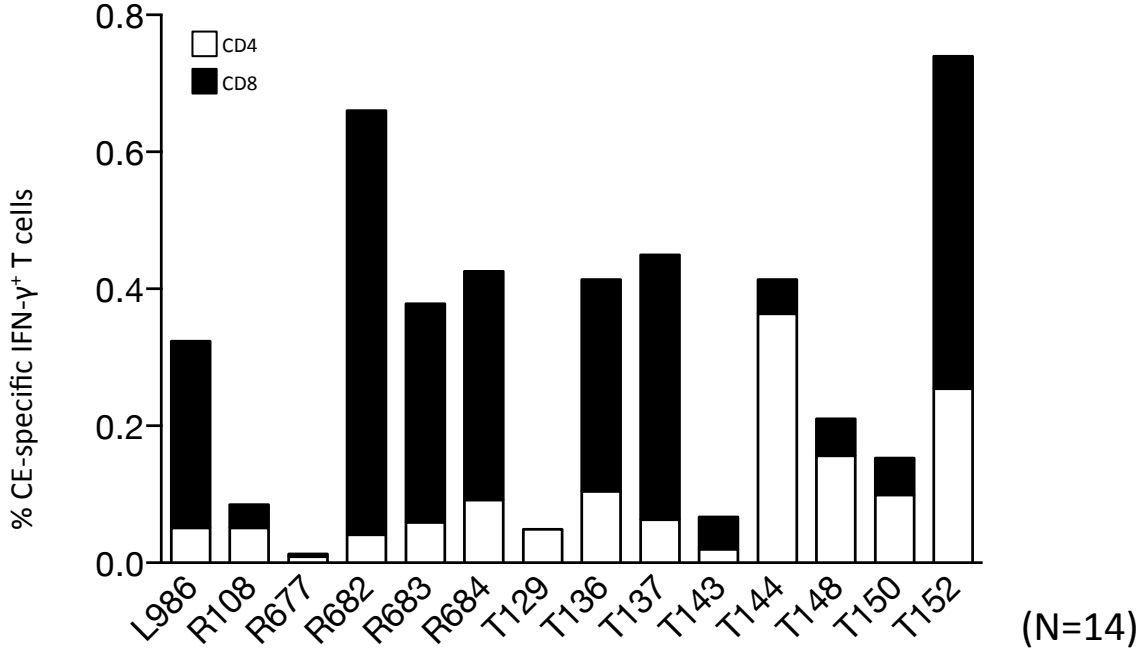
Gag DNA vaccinated macaques **WITH** CE-specific T cell responses (N=18)

Gag DNA vaccinated macaques **WITHOUT** CE-specific T cell responses (N=13)

Similar magnitude of Gag T cell responses in both groups



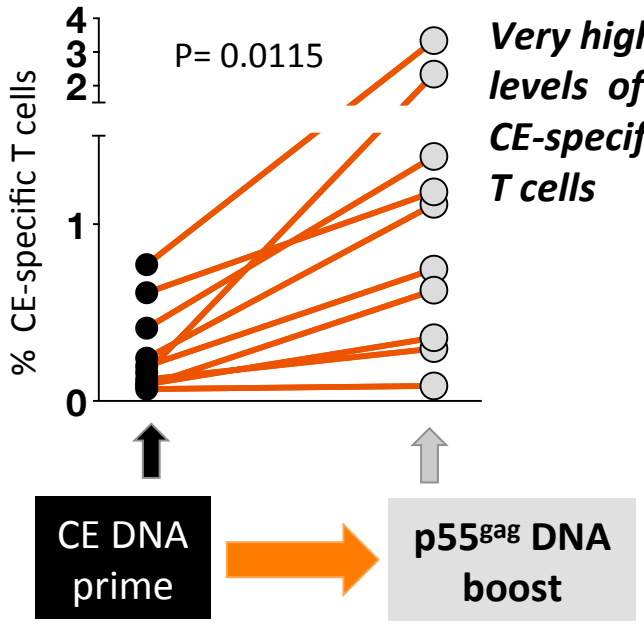
CE DNA Vaccination Elicits T Cell Responses Targeting the Conserved Elements in 100% of Macaques

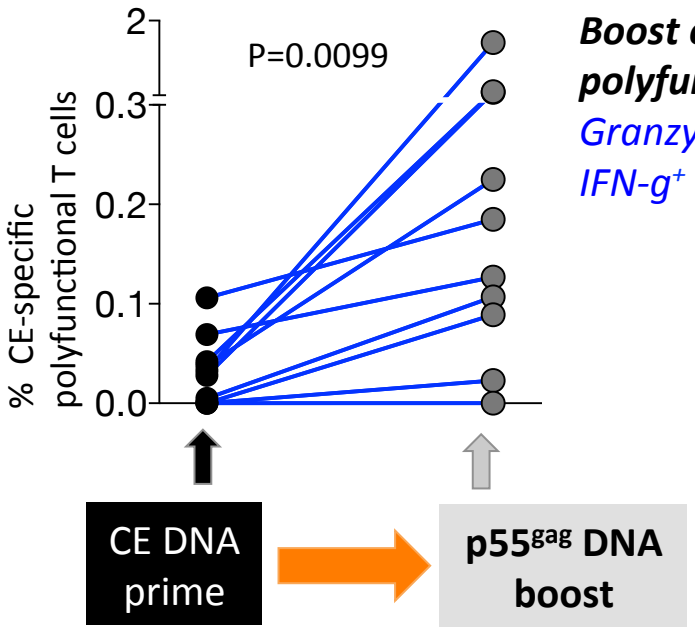


CE DNA vaccine focuses the immune responses to the highly conserved regions of HIV

- Potent cytotoxic T cell responses
- Induces long-lasting antigen-specific memory responses (> 8 years)
- Can be administered repeatedly

Efficient Boosting of CE-specific Cellular Responses by Full-length p55^{gag}

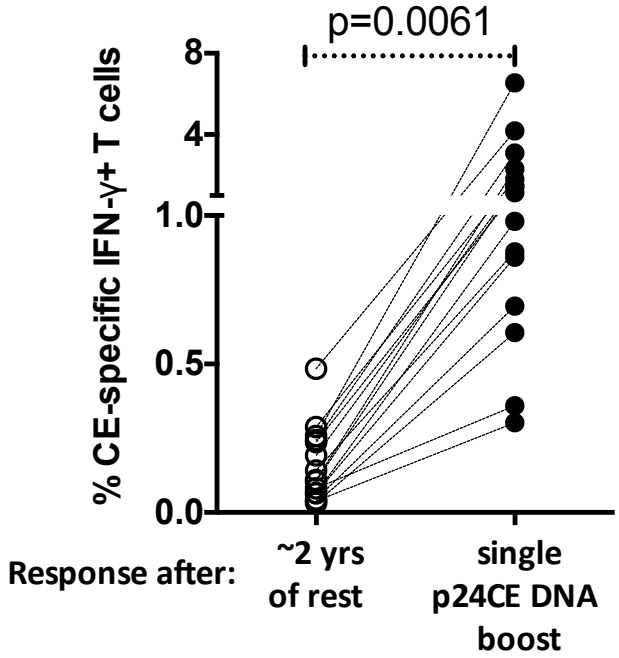




CE epitopes within the p55^{gag} protein are processed and presented to the immune system

- are able to potently augment pre-existing immune responses
- but are impaired in eliciting *de novo* responses, because other epitopes within Gag focus the immune responses away from the CE

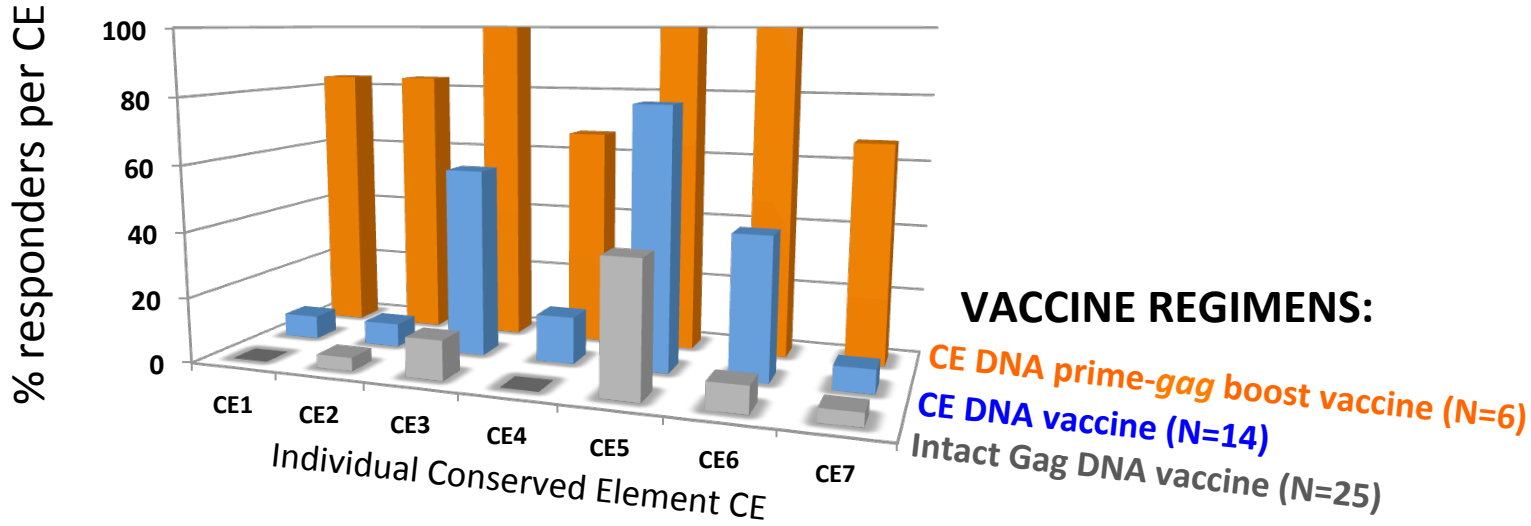
CE DNA Vaccine Induces Long-Lasting Memory T Cell Responses that Are Rapidly Boosted Upon a Single CE DNA Vaccination after 2 Years of Rest



N=15

- Persistence of CE-specific responses over the ~1.5-2 years of rest
- Range ~0.05-0.5% CE-specific T cells
- Robust increase of CE-specific responses up to ~7% of T cells

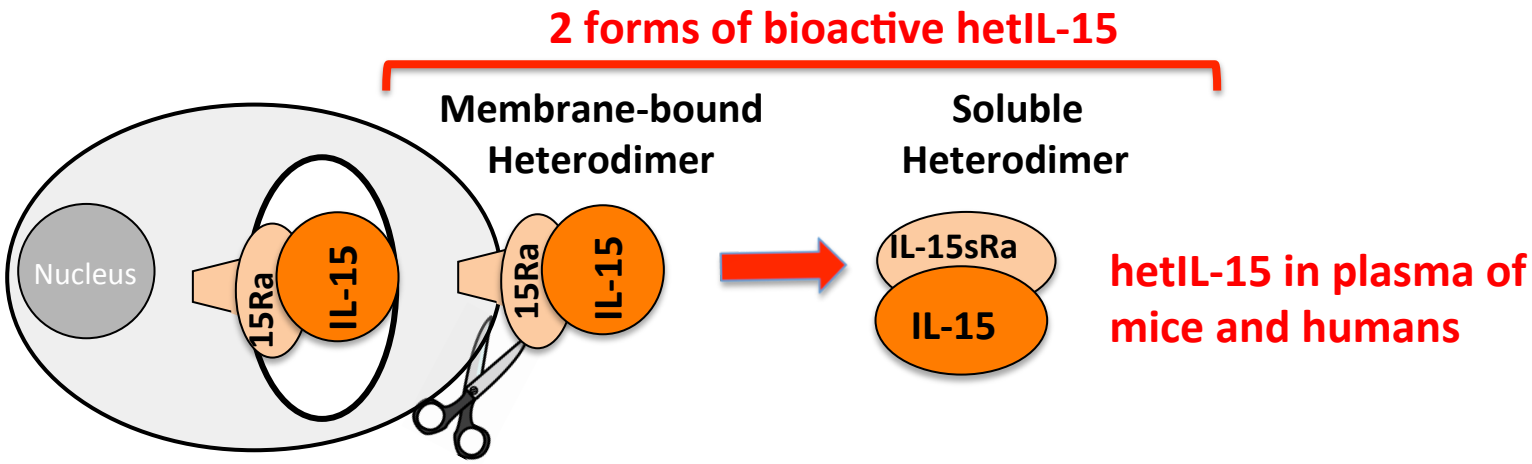
Optimized Conserved Element DNA Vaccine Regimen Maximizes Breadth and Magnitude of Cellular Immune Responses



- Gag DNA vaccine elicits responses to few CE
- Conserved Element DNA vaccine induces broad CTL responses to subdominant conserved regions
- CE prime-*gag* DNA boost vaccine regimen induces broadest responses

➢ *Translation to clinic in 2016 supported by NIAID and HVTN*

IL-15 Is A Heterodimeric Cytokine (hetIL-15)



- Co-expression of IL-15 and IL-15Rα produces a stable heterodimer
  - IL-15 and IL-15Rα chains stabilize each other
  - Essential for cytokine production *in vivo* **and for large scale production in bioreactors**
- IL-15 single chain **is unstable**, **not found *in vivo***

Bergamaschi, J Biol Chem. 2008; J Immunol. 2009
Bergamaschi, Blood 2012; Chertova, J.Biol Chem 2013

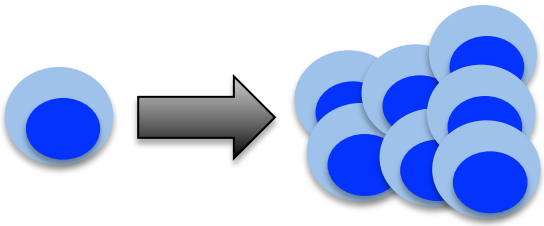
Discovery of hetIL-15 Regulation of Expression Facilitated Bioproduction And Clinical Development



Clinical Development of hetIL-15: phase I (first-in-man) clinical trial (NCT02452268) in adults with metastatic cancer at the NIH Clinical Center

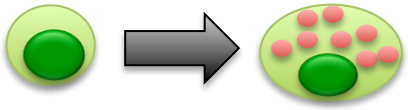
Kevin Conlon, Thomas Waldmann & George Pavlakis
National Cancer Institute

Functions of hetIL-15



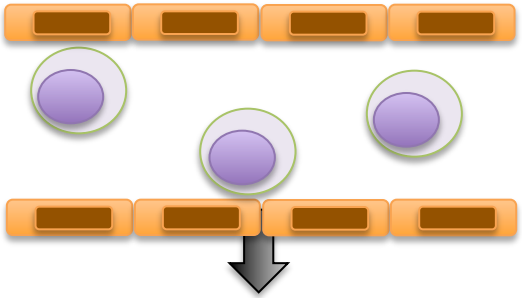
Lymphocyte Expansion and Differentiation

NK, CD8, effector CD4, γδ T cells



Enhanced Activation, Cytotoxicity

Increased expression of granzymes, perforin
Enhanced ADCC



Migration

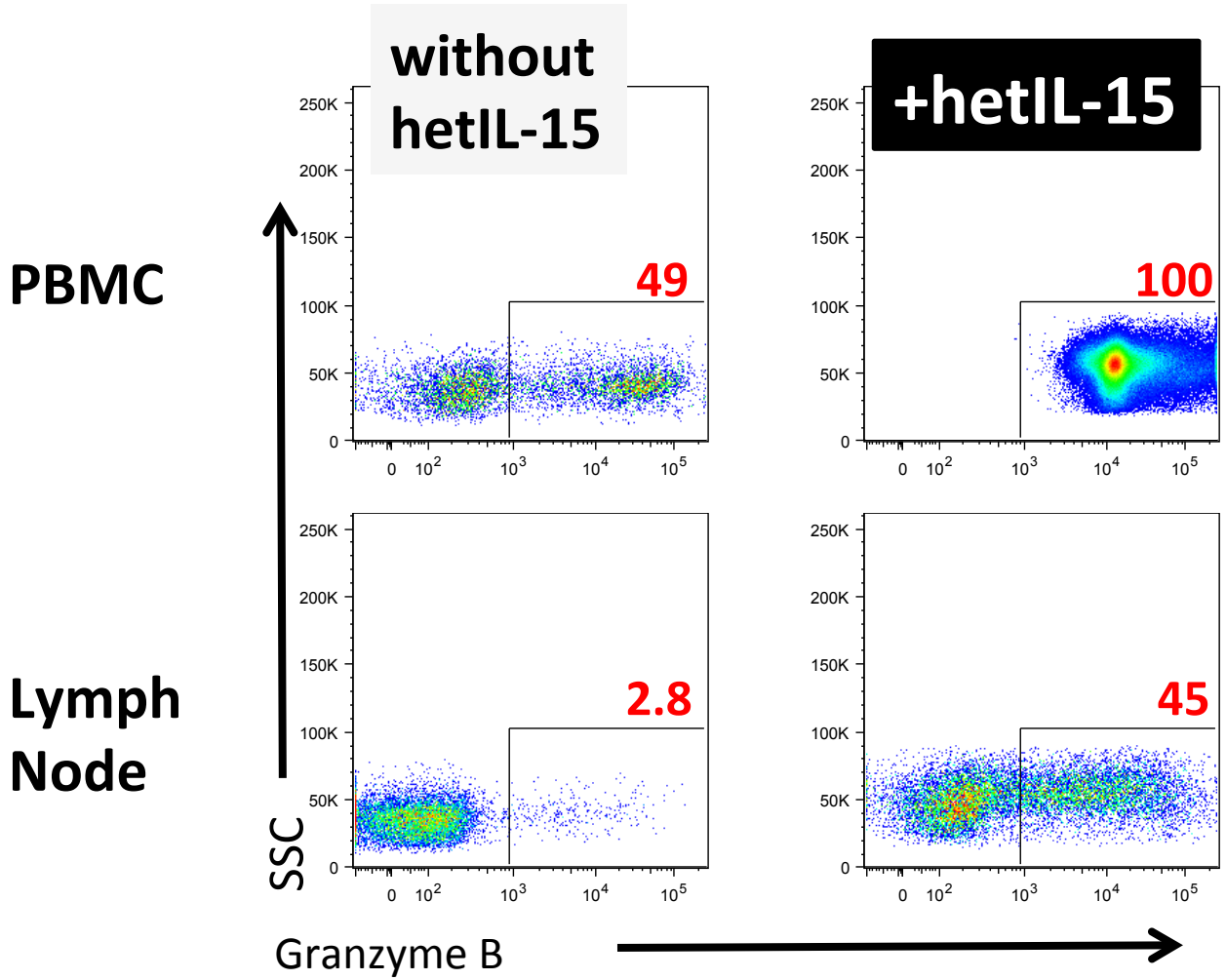
Rapid extravasation of lymphocytes to tissues

- Increases CD8 Effectors in tumors

hetIL-15: A Unique "PUSH" Mechanism For Cancer and AIDS Immunotherapy and Vaccines



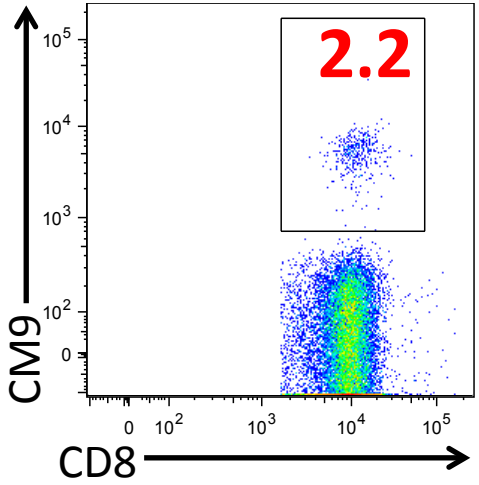
hetIL-15 Triggers a Cytotoxic Commitment (increased Granzyme B) in CD8$^+$, CD4$^+$, and NK Cells in Tissues, Including Lymph Nodes



Towards HIV Eradication: Therapeutic CE DNA Vaccination in Combination With hetIL-15 Administration to Eliminate the Reservoir

DNA vaccine induces T cell responses that disseminate efficiently to the lymph nodes

+hetIL-15 Increases CD8 cells



Conclusions

- hetIL-15 administration increases number of effector CD8 cells systemically
- Conserved element (CE) DNA vaccination targets the 'Achilles' heel of the virus, i.e., the highly conserved regions
- Combination of CE/*gag* DNA vaccination and hetIL-15 treatment is a promising regimen strengthen the immune response during ART