

Akshaya Bio Inc.

Chimigen® Multi-antigen HIV Vaccine

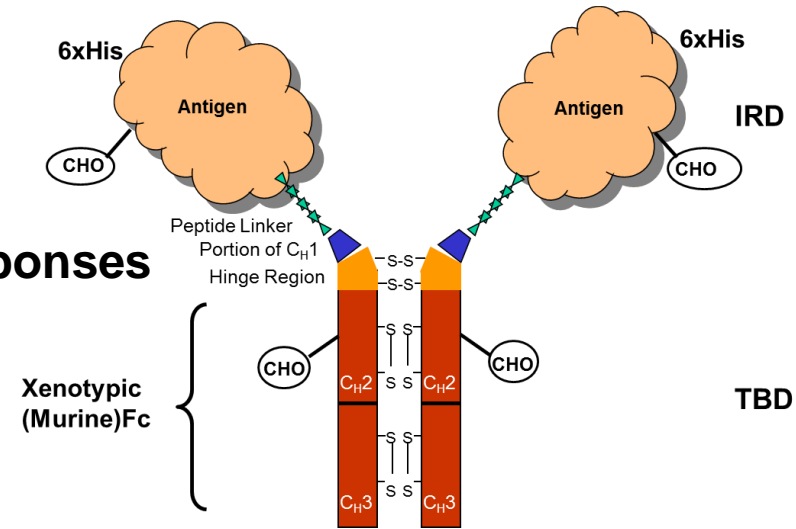
**A Novel Dendritic Cell Receptor-Targeted
Prophylactic/ Early intervention therapeutic HIV Vaccine**

***Rajan George
President & CSO***

**Akshaya Bio Inc.
Edmonton, Alberta
May 2014**

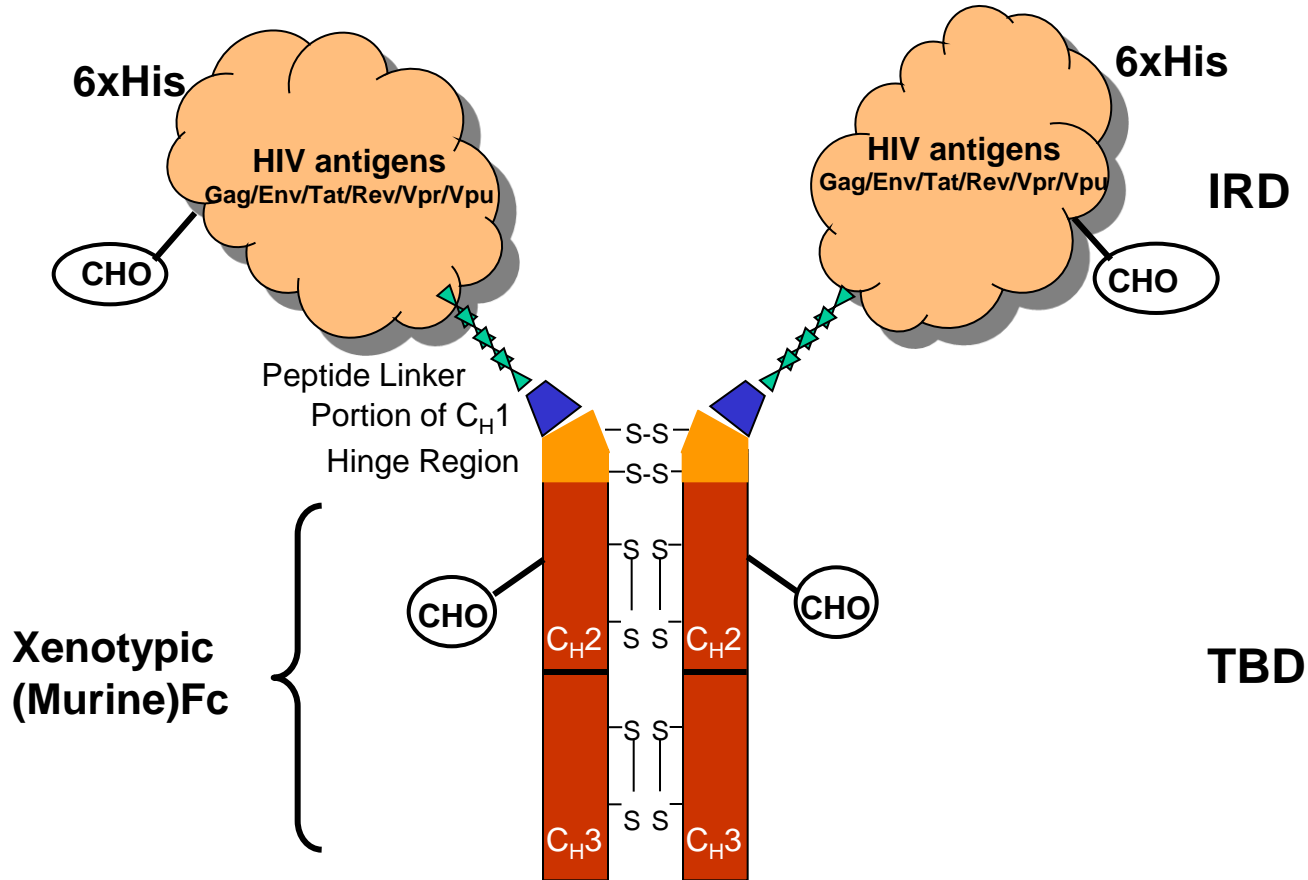
Chimigen® Platform Technology

- **Antigen – Xenotypic Antibody Fc-fusion**
 - High immunogenicity
 - Targets APC receptors
 - Dendritic Cells (Fcγ, Lectin Receptors)
- **Generates Cellular & Humoral immune responses**
 - Insect cell-based production
 - Imparts non-mammalian glycosylation
 - High immunogenicity
- **No added adjuvant**
 - Eliminates many adverse events
 - Eliminates T cell sequestration, dysfunction & deletion
- **Adaptable platform**
 - HBV, HCV, HIV, Alphaviruses, Influenza, Malaria, TB, Cancer ...
- **Potential use for both Prophylactic & Therapeutic Vaccines**
- **Strong IP position: 18 issued patents, over 30 pending applications**



Dendritic Cell Receptor-Targeted Vaccines

Chimigen® HIV Vaccine



Multiple HIV Antigen Targets

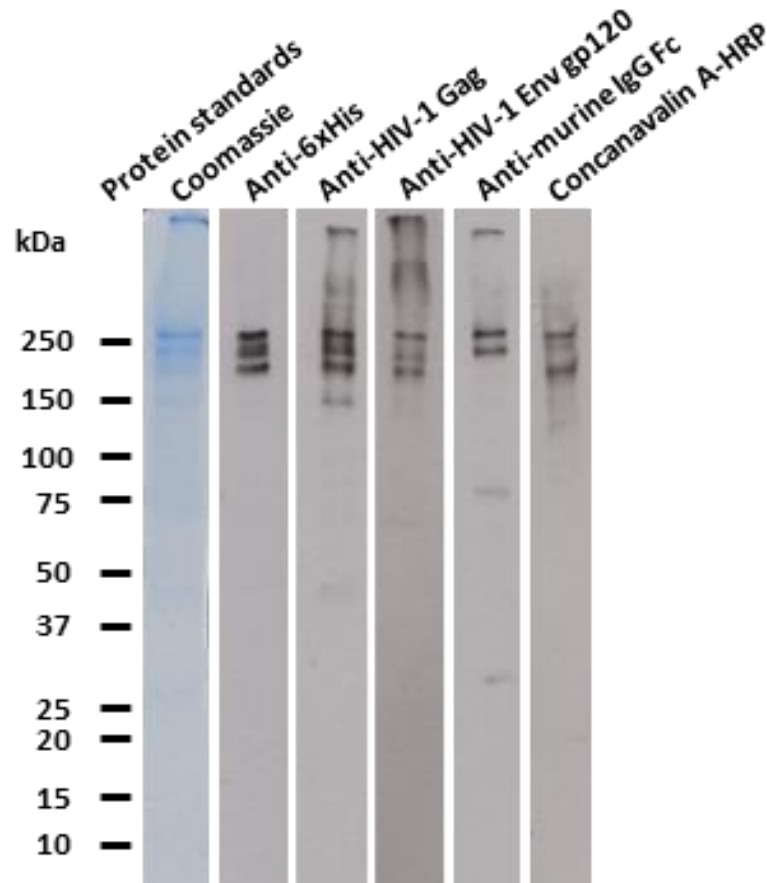
Chimigen® HIV Vaccine

Cloning and Expression

- **Cloning**
 - HIV antigens chemically synthesized (GenScript)
 - Codon optimized for expression in Sf9 insect cells
 - Baculovirus produced using Bac-to-Bac (Life Technologies)
- **Expression**
 - Sf9 Insect Cells, Wavebag Bioreactor (5L scale) (*GE Healthcare*)
- **Purification**
 - Two-step purification from infected Sf9 cell pellet
 - First step- Protein A affinity with MabSelect Xtra™ (*GE*)
 - Second step- Ni Affinity with HisTrap™ FF (*GE*)

Chimigen® HIV Vaccine

Biochemical Characterization



Chimigen® HIV Vaccine is purified intact, and is glycosylated

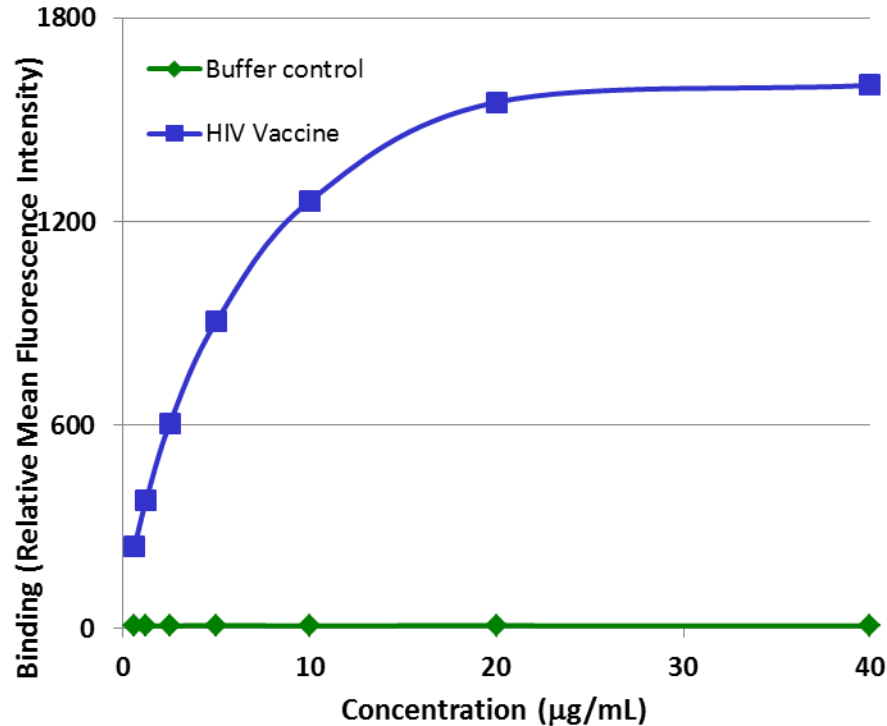
Chimigen[®] HIV Vaccine

Immune Responses, *ex vivo*

- Binding to Dendritic Cells
- Dendritic Cell / T Cell Antigen Presentation Assay (APA) *ex vivo* Human Peripheral Blood Mononuclear Cells (PBMCs)
 - T cell immune responses
 - T cell proliferation (CFSE staining)
 - Th1 cytokine production (IFN- γ , TNF- α)
 - CTL activity CD8+ T cells
 - Granzyme B & Perforin production
 - T cell antigen-specificity responses
 - Peptide pools (Env, Gag: accessory proteins)
- B Cell Differentiation Assay

Chimigen® HIV Vaccine

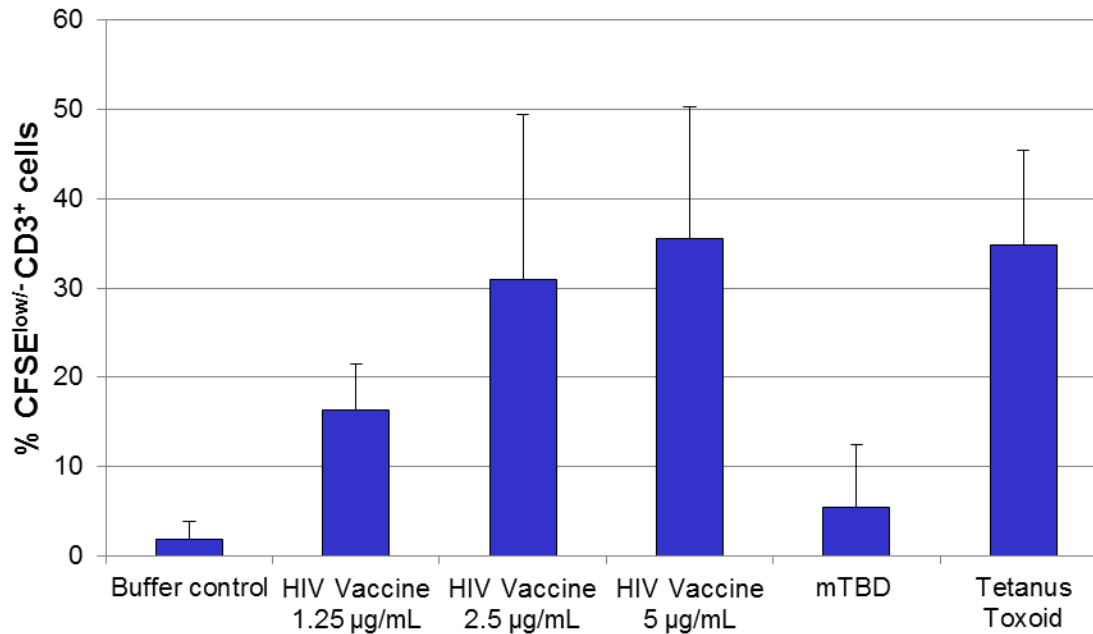
Binds to Immature Dendritic Cells



The Chimigen® HIV Vaccine binds to cultured immature DCs in a saturable and dose-dependent manner

Chimigen® HIV Vaccine

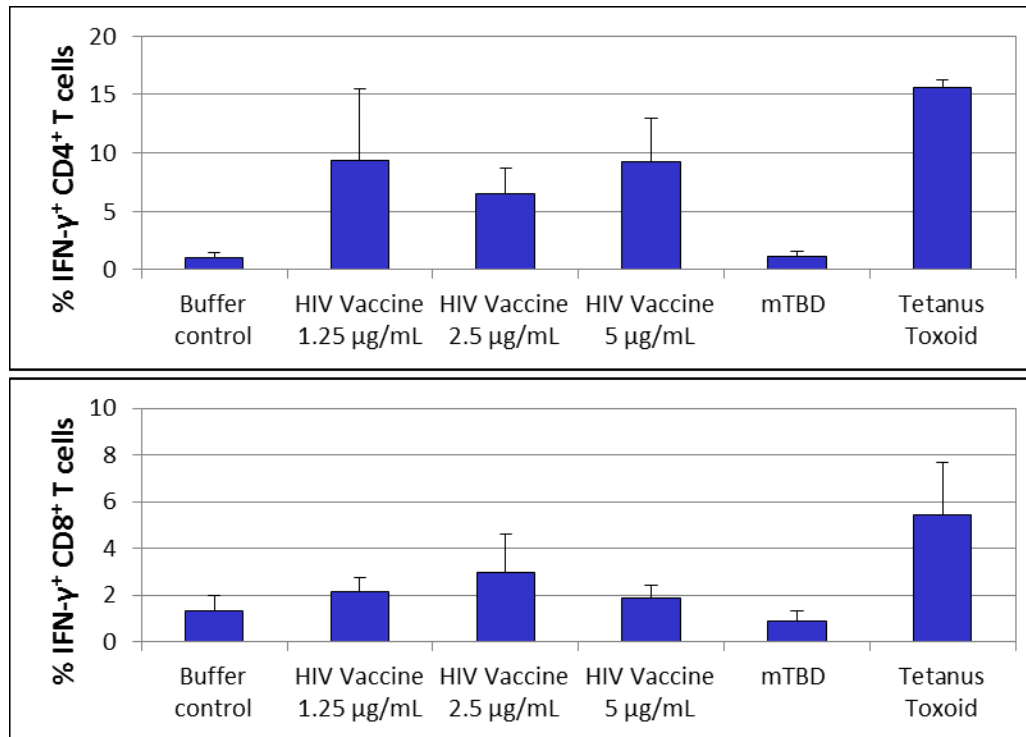
Induces T cell proliferation (One Round of Antigen Presentation)



Induces CD3⁺ (CD4⁺ and CD8⁺) T cell activation and expansion *ex vivo* following the first exposure of naïve T cells to vaccine-pulsed mature DCs

Chimigen® HIV Vaccine

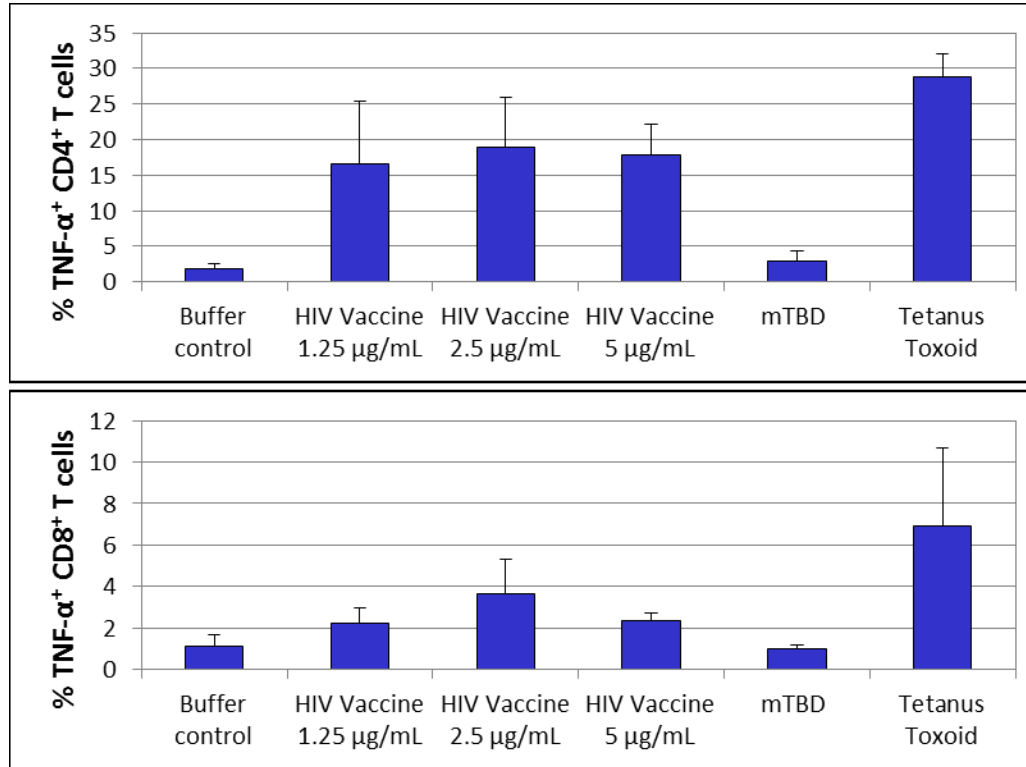
Induces IFN- γ Production in T cells (Two Rounds of Antigen Presentation)



A second exposure of T cells to vaccine-pulsed mature DCs resulted in the enhanced expression of IFN- γ both CD4⁺ and CD8⁺ T cells

Chimigen® HIV Vaccine

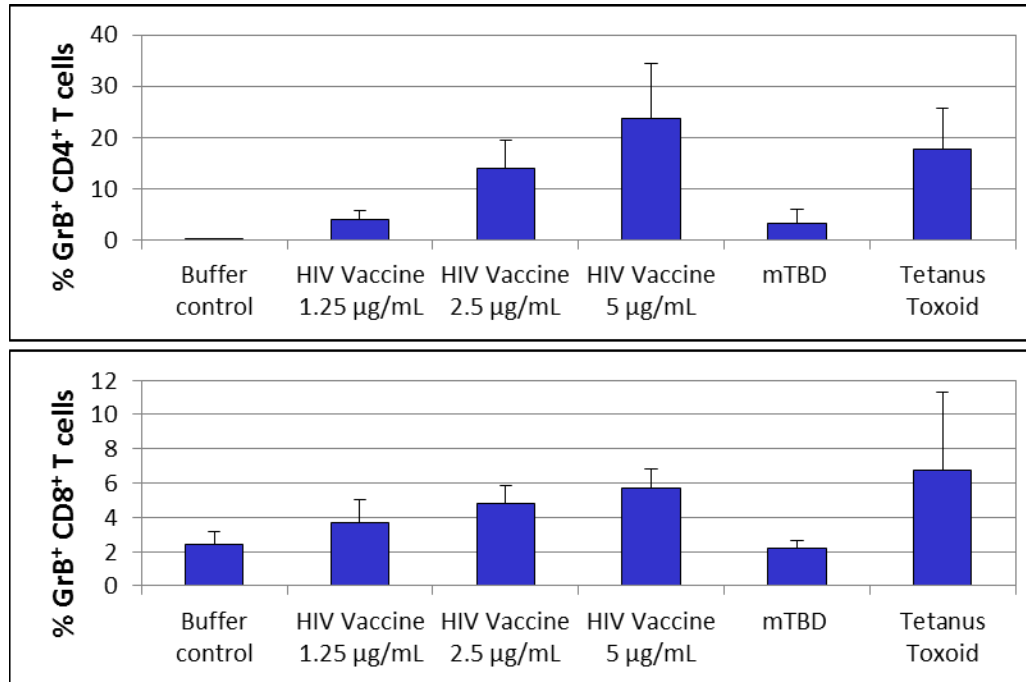
Induces the production of TNF- α in T cells (Two Rounds of Antigen Presentation)



A second exposure of T cells to vaccine-pulsed mature DCs resulted in the enhanced expression of TNF- α in both CD4+ and CD8+ T cells

Chimigen® HIV Vaccine

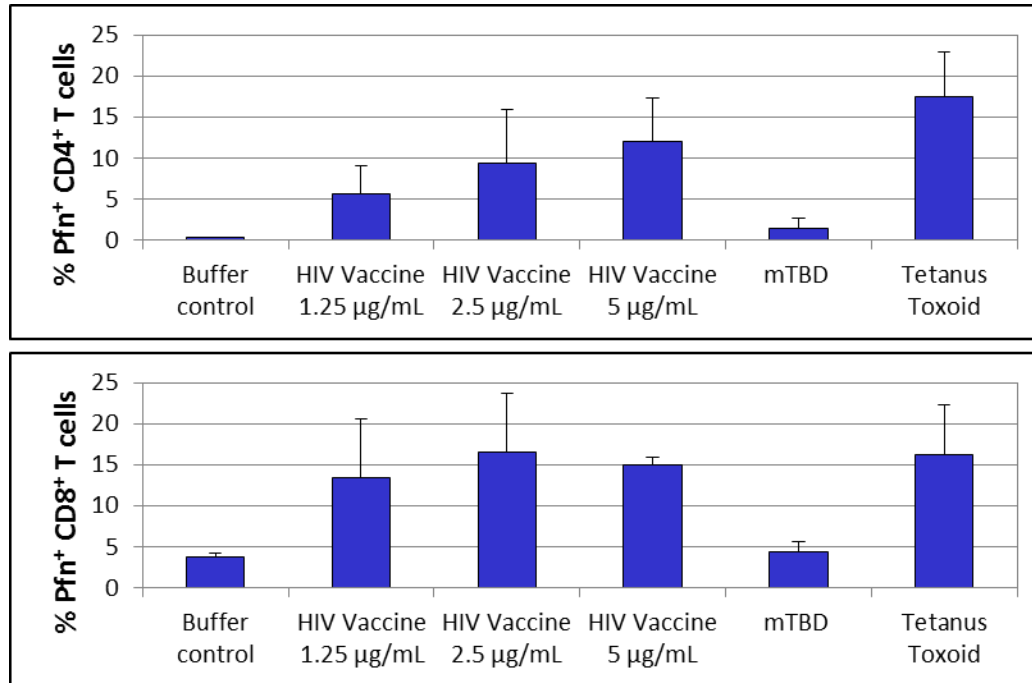
Induces the production of Granzyme B in T cells (Two Rounds of Antigen Presentation)



**A second exposure of T cells to vaccine-pulsed mature DCs
Increased the expression of Granzyme B in both CD4⁺ and CD8⁺ T cells**

Chimigen® HIV Vaccine

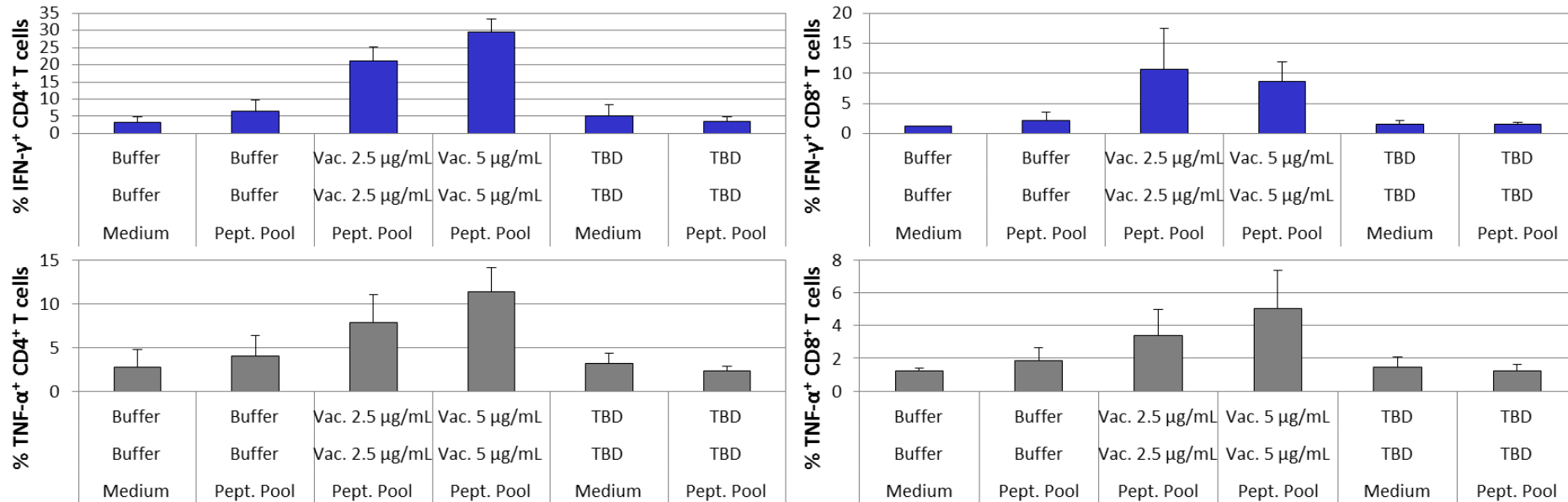
Induces the production of perforin in T cells (Two Rounds of Antigen Presentation)



A second exposure of T cells to vaccine-pulsed mature DCs resulted in the enhanced expression of Perforin in both CD4⁺ and CD8⁺ T cells

Chimigen® HIV Vaccine

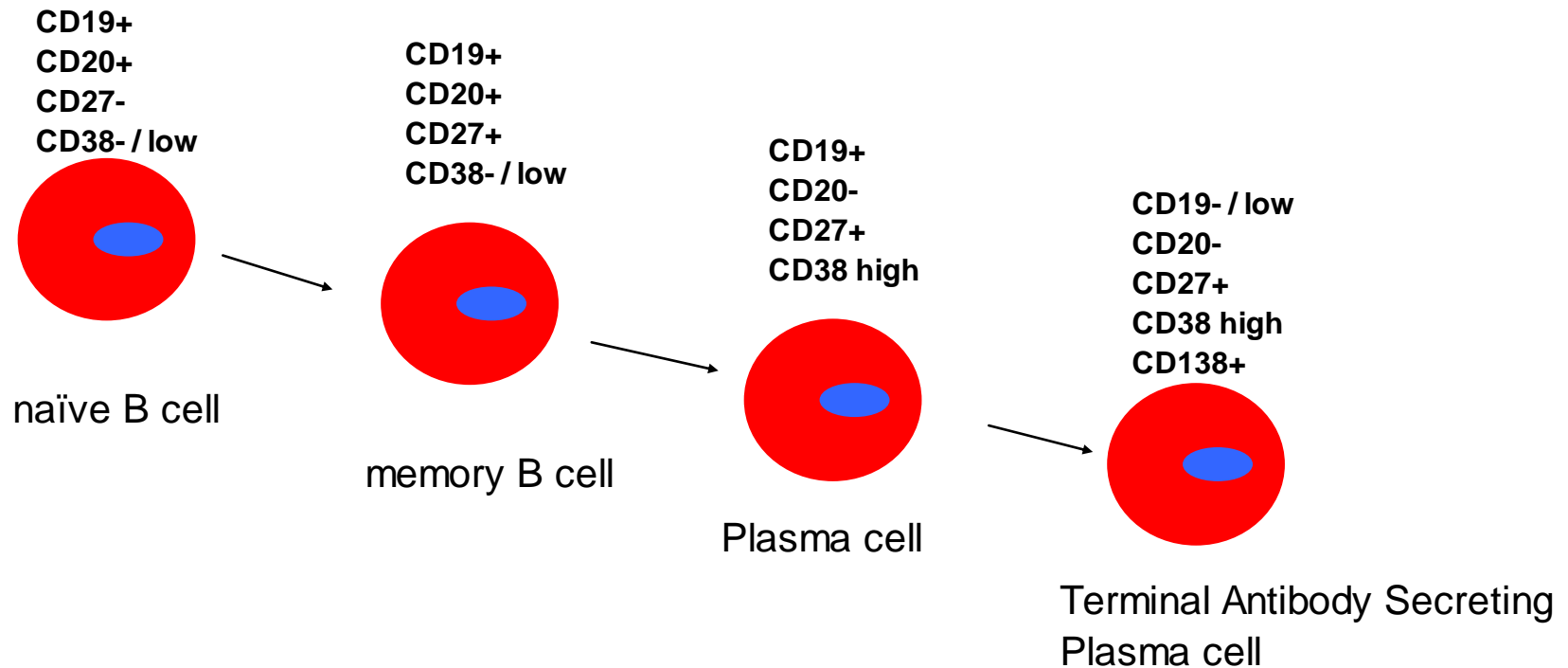
Induces HIV antigen-specific T cell responses (Three Rounds of Antigen Presentation)



Vaccine-primed T cells re-stimulated with a HIV peptide pool showed an increase in CD4+ and CD8+ T cell IFN-γ and TNF-α production

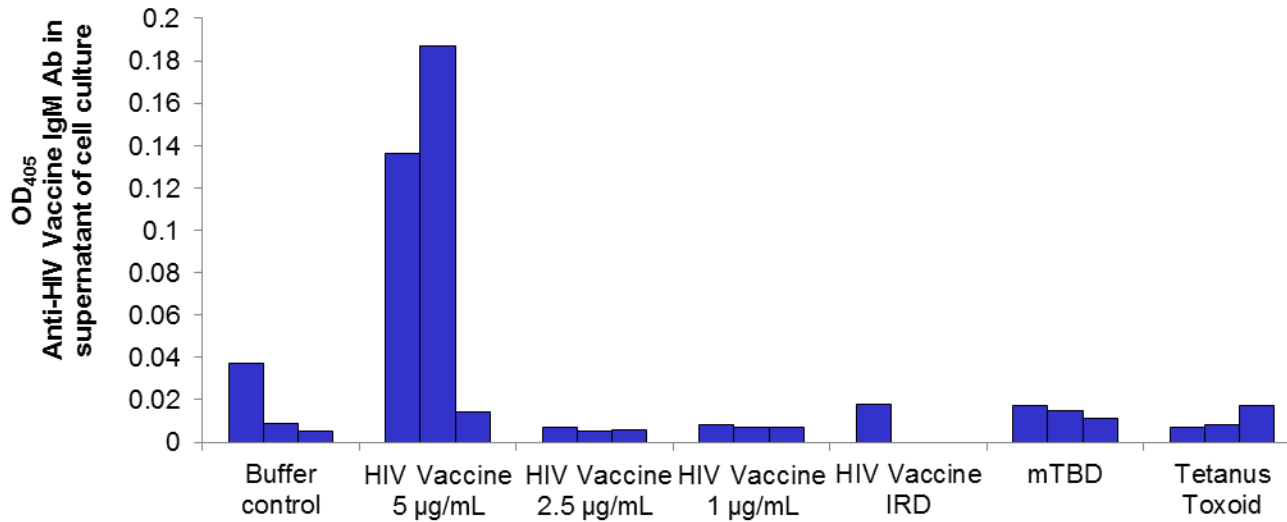
Chimigen[®] HIV Vaccine Immune Responses

B cell Differentiation



Chimigen® HIV Vaccine

Induces the production of antigen-specific
IgM antibody



Chimigen® HIV Vaccine: Summary

- **Chimigen® HIV Multi-antigen Vaccine was designed & cloned**
 - Prophylactic/Therapeutic applications
- **Vaccine**
 - Produced in Sf9 insect cells & purified
 - Immune response *ex vivo*, using human DC/T cells
 - Immune response *in vivo, in rats*, currently in progress
- **Chimigen® HIV Vaccine, in human DC/T cell assays *ex vivo***
 - Binds to immature DCs and induces both T cell and B cell responses
 - Induces
 - Activation and proliferation of CD8⁺ and CD4⁺ T cells
 - Production of IFN- γ and TNF- α in CD8⁺ and CD4⁺ T cells
 - Production of GrB⁺, Pfn⁺ CD8⁺ and CD4⁺ T cells
 - T cell responses are HIV antigen-specific
 - Production of antigen-specific antibodies (IgM)
- **Chimigen® HIV Multi-antigen Vaccine shows excellent potential for further development**

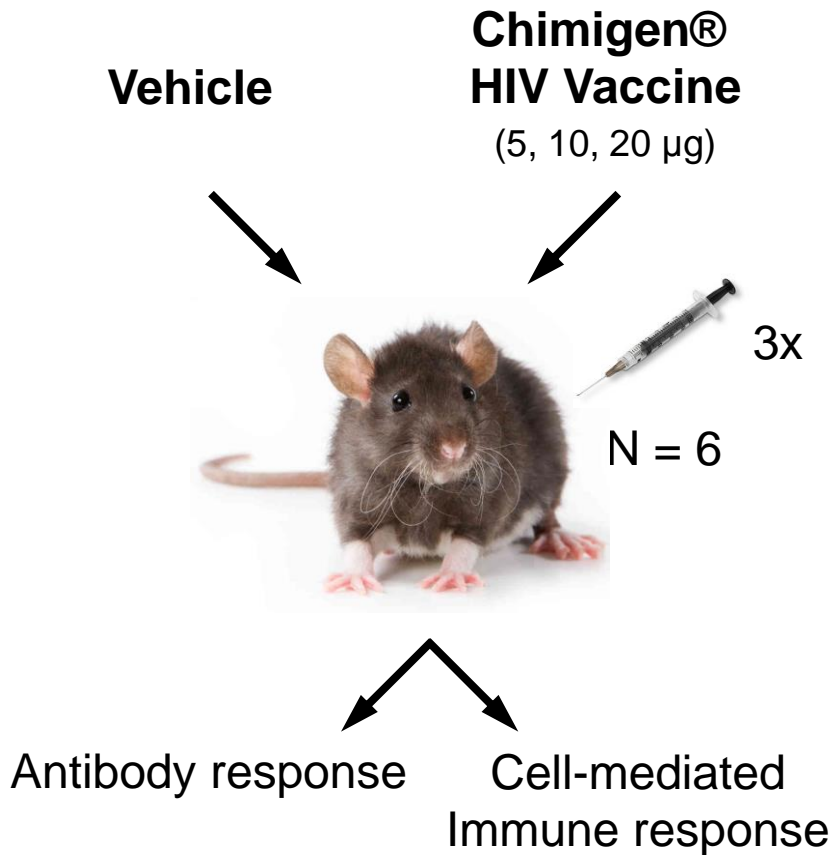
Chimigen[®] HIV Vaccine

Immune Responses, *in vivo*, (Rats)

- Evaluation of antigen-specific T and B cell responses following vaccination
 - IFN- γ , IL-4, TNF- α secretion
 - Antigen-specific antibodies
 - Immune response specificity for recombinant Gag, Env, Tat,-Rev-Vpr-Vpu (TRVV), and murine IgG1 Fc (mTBD)
 - VIDO/Intervac (University of Saskatchewan)

Chimigen[®] HIV Vaccine

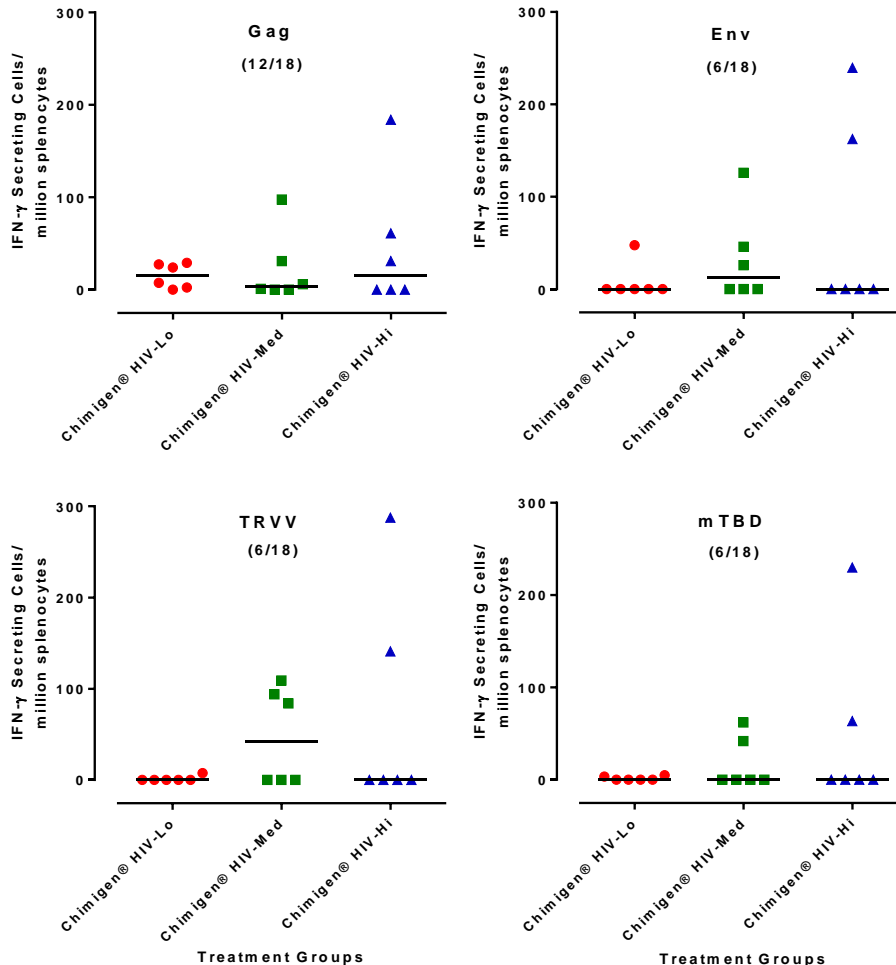
Study Design



- Evaluate immunogenicity of the Chimigen[®] HIV Vaccine
- Female Sprague Dawley rats (3-4 weeks) divided into groups of 6
- Animals vaccinated subcutaneously three times (0, 4, 8 weeks)
- Chimigen[®] Vaccine dose: 5, 10, or 20 µg
- Serum was sampled every two weeks to assay for antigen-specific **antibody responses** to individual HIV antigens and the mTBD
- Splenocytes were isolated after the third immunization to assay for antigen-specific **T cell responses**

Chimigen[®] HIV Vaccine

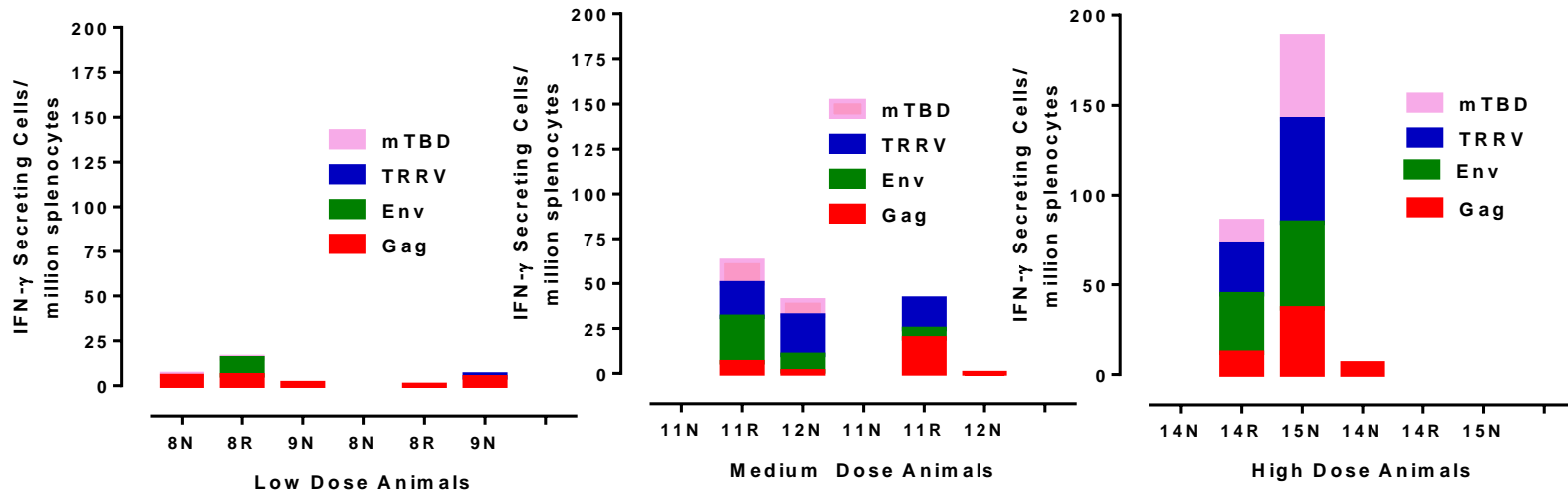
Vaccine antigen-specific IFN- γ secretion



- Splenocytes isolated after the third vaccination
- IFN- γ release in response to re-stimulation with individual HIV antigens
- Animals within each group showed different levels of recall response to antigens
- The Chimigen[®] HIV Vaccine is capable of inducing antigen-specific Th1 immune response

Chimigen[®] HIV Vaccine

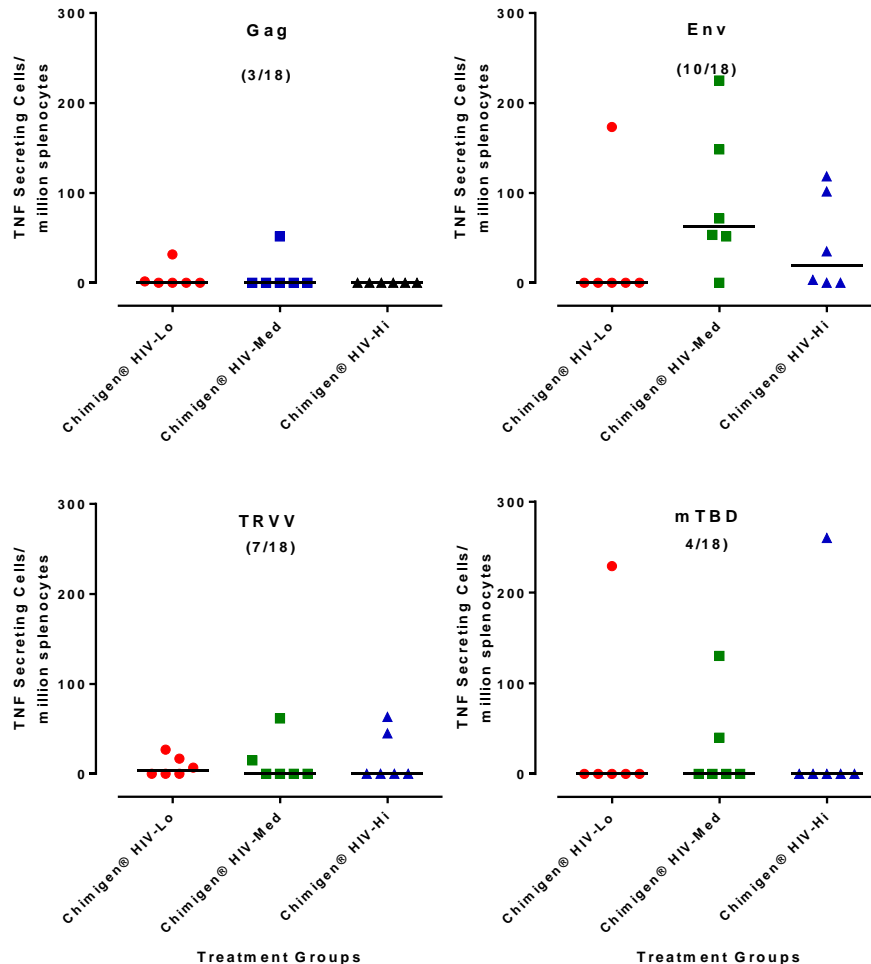
Vaccine antigen-specific IFN- γ secretion



The Chimigen[®] HIV Vaccine induces IFN- γ production in T cells in a dose-dependent manner

Chimigen[®] HIV Vaccine

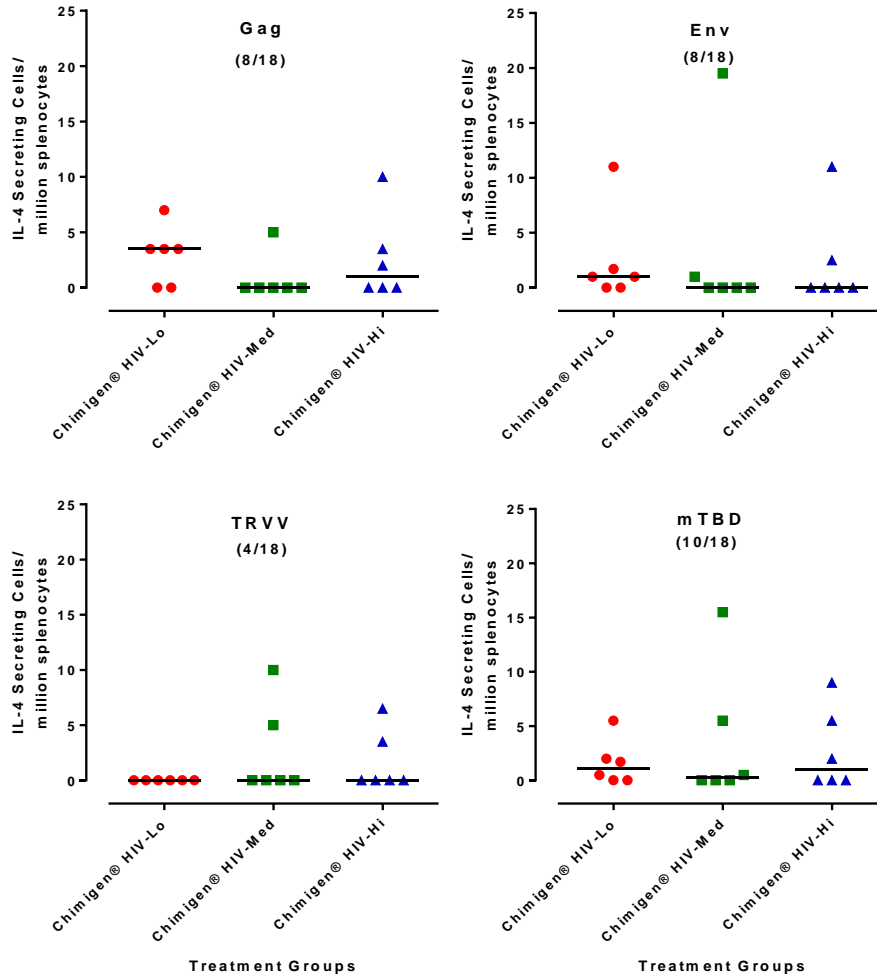
Vaccine antigen-specific TNF- α secretion



- Splenocytes isolated after the third vaccination
- Measured TNF- α release in response to re-stimulation with individual HIV antigens
- Animals within each group showed different levels of recall response to antigens
- Chimigen[®] HIV Vaccine induces antigen-specific Th1 immune response

Chimigen® HIV Vaccine

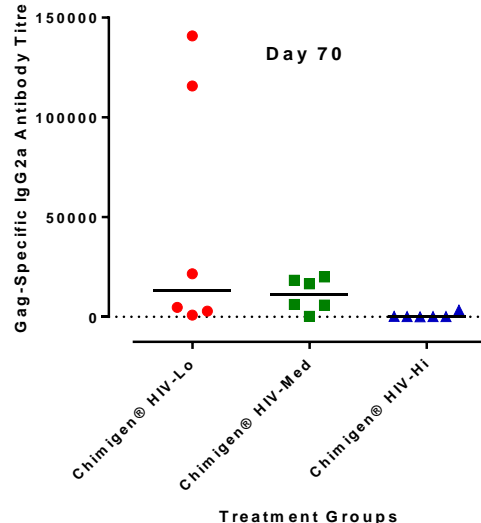
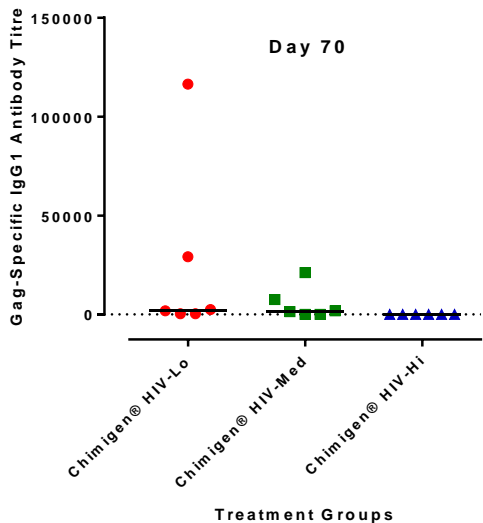
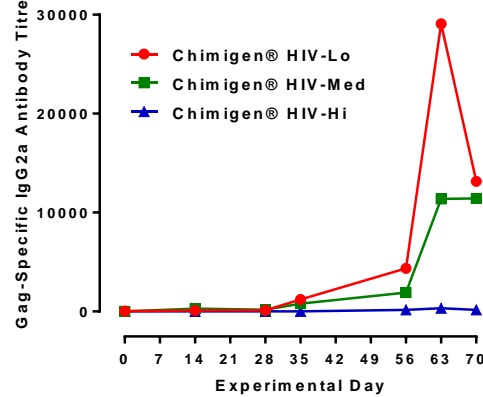
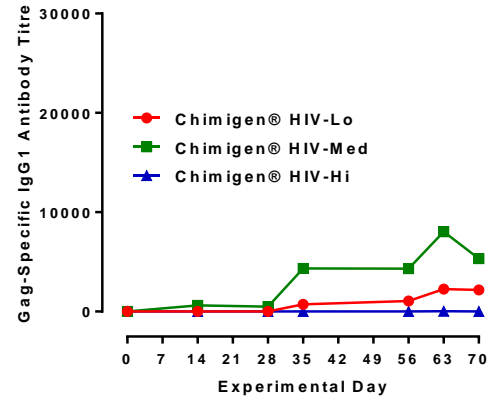
Vaccine antigen-specific IL-4 secretion



- Splenocytes isolated after the third vaccination
- Measured IL-4 release in response to re-stimulation with individual HIV antigens
- Animals within each group showed different levels of recall response to antigens
- The Chimigen® HIV Vaccine is capable of inducing an antigen-specific Th2 immune response

Chimigen® HIV Vaccine

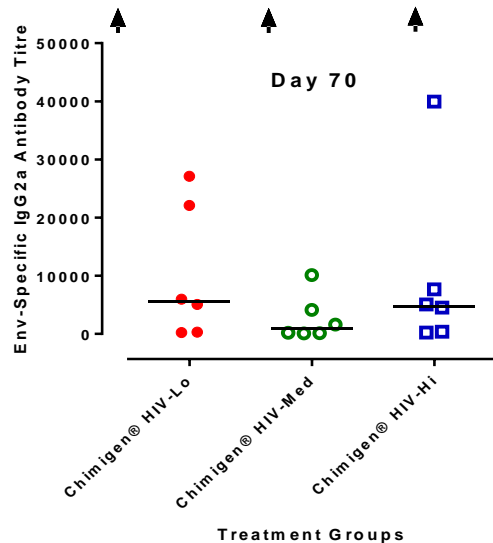
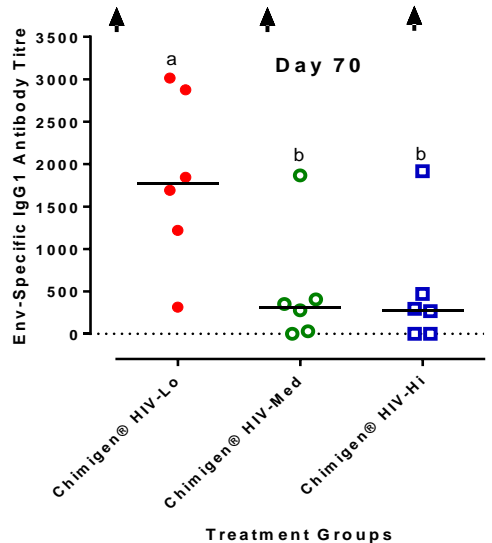
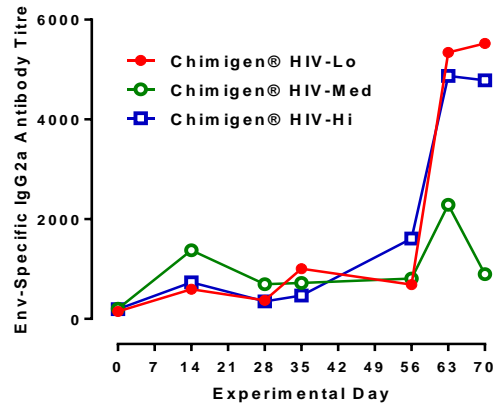
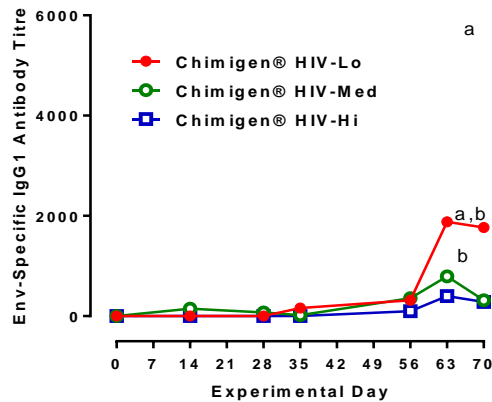
Antibody responses specific to Gag component



- IgG1 and IgG2a antibody titres against Gag increased after the second and third immunizations
- Titres of Gag-specific IgG2a antibody are higher than those of IgG1 after the third immunization with the Chimigen® HIV Vaccine, indicative of a Th1 immune response
- The Chimigen® HIV Vaccine induced Gag-specific antibody responses

Chimigen® HIV Vaccine

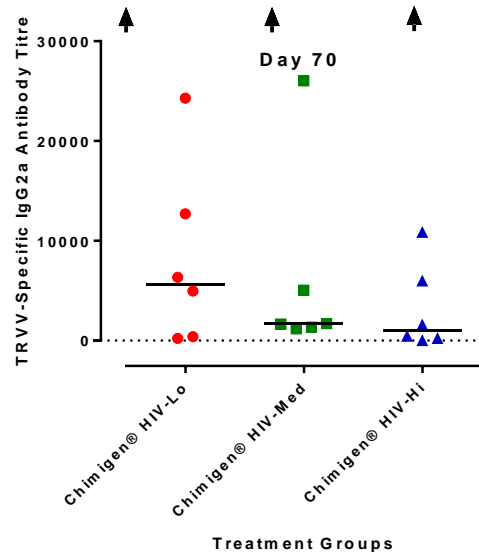
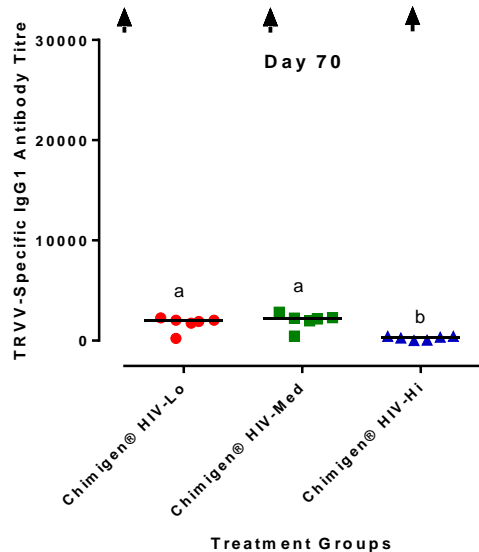
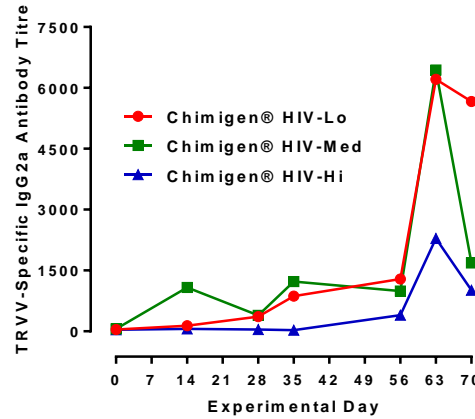
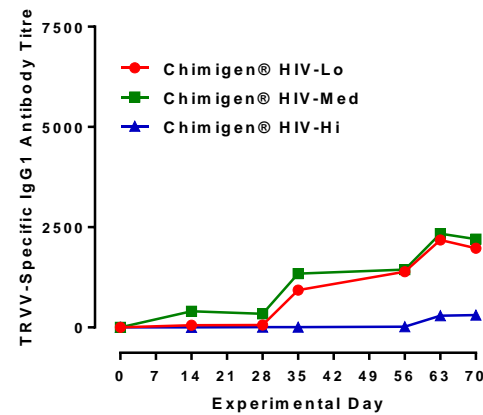
Antibody responses specific to Env



- IgG1 and IgG2a antibody titres against Env increased after the second and third immunizations
- Titres of Env-specific IgG2a antibody are significantly higher than those of IgG1 after the third immunization with the Chimigen® HIV Vaccine, indicative of a **Th1 immune response**
- The Chimigen® HIV Vaccine induced Env-specific antibody responses

Chimigen® HIV Vaccine

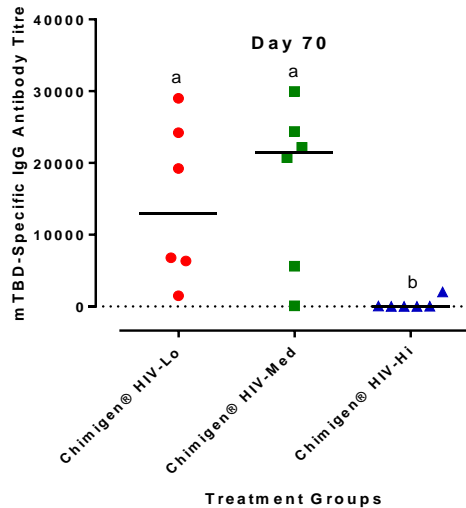
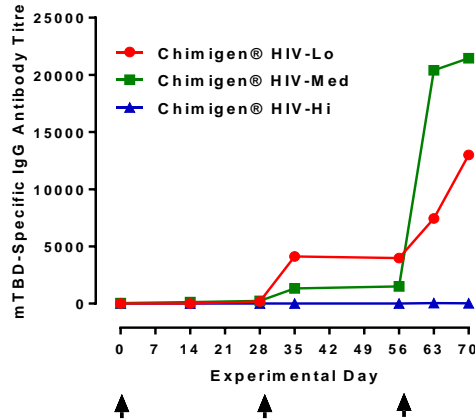
Antibody responses specific to Tat-Rev-Vpr-Vpu



- IgG1 and IgG2a antibody titres against TRVV increased after the second and third immunizations
- Titres of TRVV-specific IgG2a antibody are higher than those of IgG1 after the third immunization with the Chimigen® HIV Vaccine, indicative of a **Th1 immune response**
- Chimigen® HIV Vaccine induced TRVV-specific antibody responses

Chimigen[®] HIV Vaccine

Antibody responses specific to mTBD component



- Total IgG antibody titres against mTBD increased after the third immunization
- Response was not dose-dependent

Chimigen® HIV Vaccine

Summary

- Chimigen® HIV Vaccine is **immunogenic**, not all animals responded
- Chimigen® HIV Vaccine is capable of inducing an **antibody** response
 - Response is **specific** for the vaccine antigens
 - Moderate to significant response following the third immunization
 - Induced higher antigen-specific IgG2a antibody titres relative to IgG1, which is indicative of a **Th1 immune response**
- Chimigen® HIV Vaccine induced a **dose-dependent Th1 immune response**
 - **Specific** for Chimigen HIV Vaccine component antigens
 - Moderate to significant response following the third immunization
- Effective at low doses
- No added **adjuvant**
- Chimigen® HIV Vaccine is **very effective** for the induction of a systemic cell-mediated immune response

Akshaya Bio Inc.

Thank you.....

CHTD, NRC-IRAP & AITF