



ASX Announcement (332)
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Identification of a Novel Immune Mechanism for Suppression of HIV Replication
Immunological Testing from VIR201 Australian Trial at Prestigious International AIDS Society Meeting – Cape Town

Virax Holdings Limited (ASX:VHL) announced today that VIR201 HIV vaccine clearly demonstrates an immune response confirming the results of viral load suppression from the Phase I/IIa clinical trial completed in Australia. This data has been presented by a group of Virax collaborators led by Professor Martyn French from Royal Perth Hospital at the prestigious International AIDS Society conference in Cape Town, South Africa. The presentation details the immune system's antibody response to the VIR201 vaccine and is the outcome of additional immunological testing of clinical samples from the Australian trials of VIR201.

Virax's Chief Executive Officer, Dr. Larry Ward stated, "This is an extremely significant milestone for the development of VIR201. The Australian trials of VIR201 demonstrated that the vaccine suppressed the levels of HIV virus in infected individuals. The paper documents clinical data of an immune readout potentiated by VIR201 that correlates with the viral load suppression. This data fills a gap in the results from the Australian trials. In order to further build on this data we will be using the antibody isotyping assays as major immune read-out in our ongoing trial in South Africa".

The data demonstrated that VIR201 vaccination induced an IgG2 antibody response against the p24 antigen encoded in the vaccine. Such an antibody response was not observed in those participants receiving a placebo injection. This data was statistically significant ($p=0.014$). In addition VIR201 induced an IgG1 immune response against p24 relative to placebo ($p=0.018$) and interestingly also an IgG1 response against gp41 ($p=0.066$), an antigen not encoded for in VIR201. A VIR201 induced IgG2 response against gp41 was however not observed. Importantly vaccine-induced IgG2 antibodies to a vaccine-encoded antigen (p24) were associated with lower HIV replication and could provide the mechanism as to how VIR201 suppressed viral load in the Australian trials.

Professor French and co-workers noted that because of recent high profile failures in the preventative vaccine arena it was important that new strategies for producing immune responses other than the purely T cell-based HIV vaccines approaches are pursued. The mechanism of action described here namely antibody isotype switching (IgG1 to IgG2) is such a novel mechanism of action.

Further details of the paper will be available on the Virax website.

This data further enhances Virax's approach to developing VIR201 as product that potentially defers the introduction to anti-retroviral therapies for patients. Key benefits include the reduction of adverse side effects, increased patient compliance (particularly in the developing world) and a health economic benefit given the annual cost of anti-retroviral treatments of approximately USD 15,000 per patient. The market for HIV medications is large and growing - projected to increase from USD 9.3 billion in 2007 to USD 15.1 billion in 2017 (Source: Datamonitor). A small participation in this market would bring significant benefits to Virax.

About VIR201

The HIV immunotherapeutic vaccine VIR201 utilises Virax's patented Co-X-Gene™ technology. VIR201 has previously been tested in two Australian Phase I/IIa trials where it was shown to be safe and well tolerated and possessed the ability to suppress viral load in patients undergoing antiretroviral treatment in the context of a treatment interruption. This ability to suppress HIV viral load marks VIR201 as one of the most advanced therapeutic vaccines for HIV currently undergoing clinical testing.

Virax which is developing VIR201 for application in both developed and developing world settings is currently undertaking a clinical trial of VIR201 in southern Africa. The South African trial design differs from the previous Australian trials of VIR201 in that it utilises an increased dose of a more highly purified VIR201 vaccine and includes both ART treatment naïve and experienced participants.

A major aim of this trial is to compare the immune responses to VIR201 in both patient populations. This trial data will contribute to the identification of the appropriate time and conditions to vaccinate patients so as to promote an immune response that can reduce the levels of HIV in infected individuals. It is postulated that the higher vaccine dose will promote a stronger immune response and have a yet more significant effect on HIV viral load.

Additional information about Virax

Virax, based in Melbourne, Australia, is a biopharmaceutical company engaged in the discovery and development of novel immunotherapeutic products for the treatment of chronic infectious diseases and cancer. The Company's lead product, VIR201, a HIV/AIDS immunotherapeutic (therapeutic vaccine), has been tested successfully in two clinical trials in Australia. A further Trial of VIR201 has commenced in South Africa. Funding for the Southern African Trial is via contributions from a consortium of global and Southern African resource companies led by BHP Billiton.

Transgene (Eurolist Paris: FR0005175080) has a License Agreement with Virax for access to Co-X-Gene™ technology for use in two of Transgene's immunotherapeutic products. These are TG4001 - a treatment for pathologies relating to human papilloma virus (HPV) infection that can lead to cervical cancer - and TG4010 - a treatment for non-small cell lung cancer (NSCLC). This was reported to the ASX in the Company's announcement of March 2007 and is referred to as the "Transgene Sub-licence". TG4001 is in advanced development with one completed Phase II trial showing promising safety and efficacy. An additional Phase II trial is planned so as to optimise the product profile. Transgene has licensed TG4001 to the pharmaceutical company Roche for treatment of HPV related pathologies. Successful Phase IIb testing of TG4010 in NSCLC has been achieved. Transgene reported that the FDA had supported the approach for further development of TG4010 in Phase III trials and that it is finalising discussions with potential partners to complete the last stages of clinical development and bring TG4010 to the market. The Company would benefit from any payments to Transgene upon completion of such an agreement together with future milestone payments and royalties.

Additional information about Virax is available at www.virax.com.au

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Vaccine-induced IgG2 antibodies to HIV antigens are associated with partial control of HIV replication

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Background

In the light of recent 'T cell-based HIV vaccine' failures, strategies for augmenting 'protective' immune responses against HIV antigens have to be rethought. We have previously demonstrated that vaccination of HIV-infected patients receiving cART with a recombinant DNA construct encoding genes for HIV gag-pol, human interferon- γ (IFN- γ) and a fowlpox virus vector was associated with partial suppression of HIV replication after cART was ceased (1). However, IFN- γ ELISPOT responses to vaccine-encoded antigens were not increased (2), suggesting that inclusion of the IFN- γ gene in the vaccine had a beneficial effect but not by enhancing effector T cell responses. As IFN- γ augments the production of IgG2 in humans and IgG2 antibodies might have a beneficial effect in humans with HIV infection and animals with other lentiviral infections, we have tested the hypothesis that the IFN- γ enhanced IgG2 antibody responses to the HIV antigens in the vaccine.

Methods

Plasma samples from weeks 0, 9 and 20 after ceasing ART were available from patients who received the full construct (FC) of the vaccine (n=9), a partial construct (PC) of the vaccine that did not include the IFN- γ gene (n=6) or a placebo (n=8). An *a priori* decision to combine data from the PC and control groups (n=14) was made based on the previously published data (1).

IgG2 and IgG1 antibodies to p24 and gp41

Plasma samples were diluted 1:10 in 1% skim milk/TBS and added to HIV-1 nitrocellulose blot strips (National Serology Reference Laboratory, Victoria, Australia). After incubation, the strips were blocked with 3% skim milk/TBS, washed and incubated with alkaline phosphatase-conjugated murine antibodies to human IgG1 or IgG2 (Invitrogen, Carlsbad, CA). After washing, BCIP/NBT substrate (Sigma, St Louis, MO) was added and the strips were washed with 70% ethanol and air-dried. High-resolution images of each strip were captured using a flat-bed scanner and the density of bands corresponding to p24 and gp41 proteins was determined using the Quantity One program (Bio-Rad, Hercules, CA).

Results

At week 0, plasma levels of IgG2 antibodies to p24 were higher in the FC group than the PC/control group (Fig. 1A). In contrast, IgG2 antibodies to gp41 did not differ between the two groups (Fig. 1B). Plasma levels of IgG1 antibodies to both p24 and gp41 were also higher in the FC group than the PC/control group (Figs. 1C and D) and IgG2 anti-p24 correlated with IgG1 anti-p24 (Fig. 1E) and anti-gp41 (Fig. 1F).

To determine if plasma levels of IgG2 antibodies to p24 at week 0 might affect HIV replication after ceasing ART, antibody levels for each group were divided into tertiles and the time-weighted increase in plasma HIV RNA level over 20 weeks was compared for each tertile. As shown in table 1, the increase in plasma HIV RNA levels was lowest in patients who belonged to the highest tertile of IgG2 anti-p24. A similar association was also observed for IgG1 anti-gp41 (not encoded by the vaccine). Adjusted analyses were attempted but could not further delineate the independent effects of IgG2 anti-p24 and IgG1 anti-gp41 on plasma HIV RNA levels.

Results

Fig 1. IgG2 and IgG1 antibodies to p24 and gp41 at week 0. Antibody levels are expressed as band densities.

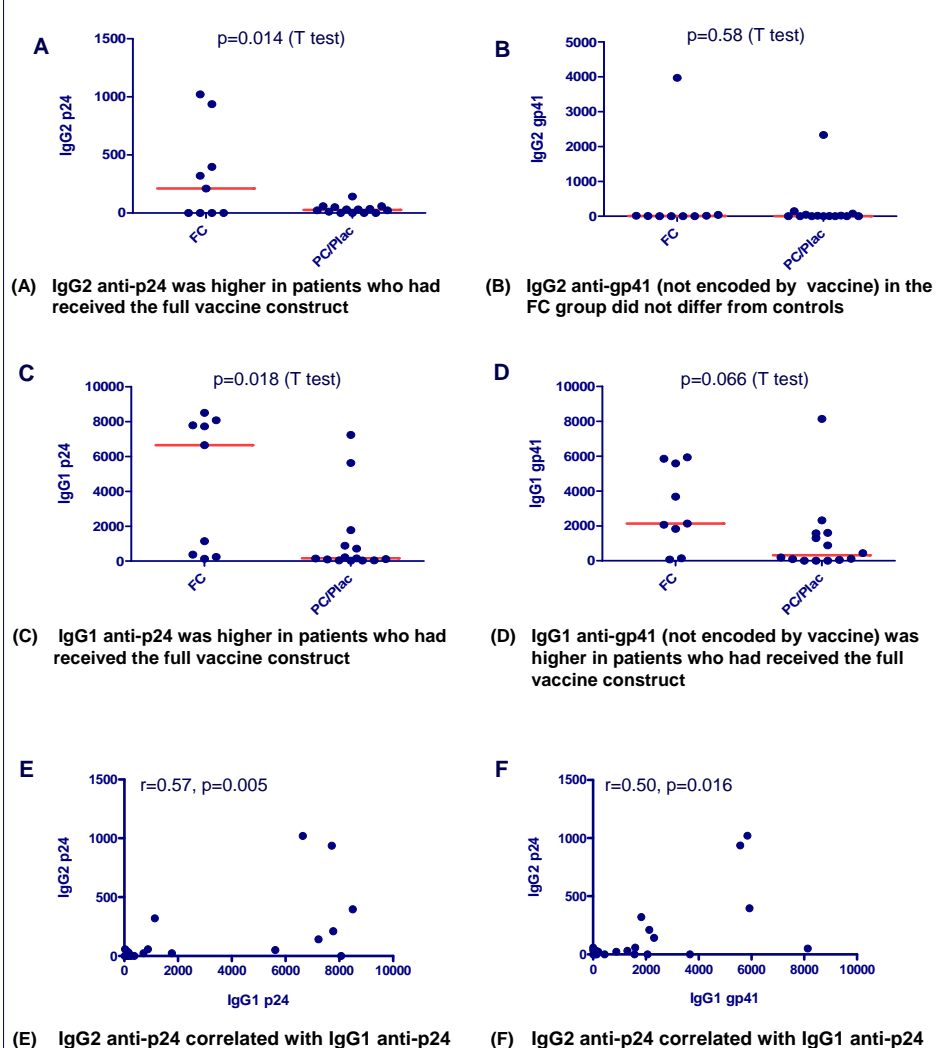


Table1. Time-weighted increase in log HIV RNA by HIV antibody tertiles at week 0

Tertile	IgG2 anti-p24	IgG2 anti-gp41	IgG1 anti-p24	IgG1 anti-gp41
Low	1.45	1.64	1.55	1.53
Medium	2.01	0.88	1.66	1.92
High	0.86	1.55	1.07	0.85
P*	0.056	0.265	0.294	0.059

* Overall test of heterogeneity (Kruskal-Wallis)

Conclusions

• Despite the small number of patients studied, the findings are broadly consistent with our hypothesis that vaccine-induced IgG2 antibodies to a vaccine-encoded antigen (p24) were associated with lower HIV replication after ceasing cART.

• An additional effect of IgG1 anti-p24 or anti-gp41 could not be excluded, but antibody 'isotype switching' to IgG2 was only observed for anti-p24.

• We suggest that induction of IgG2 antibodies to HIV antigens should be investigated as a strategy for controlling HIV replication.

References

- Emery S et al. Influence of IFN γ co-expression on the safety and antiviral efficacy of recombinant fowlpox virus HIV therapeutic vaccines following interruption of antiretroviral therapy. *Human Vaccines* 2007;3:260-67.
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