

# The Sydney Blood Bank Cohort of Transfusion-Acquired HIV Recipients:

from vaccine model  
to residual survivors.

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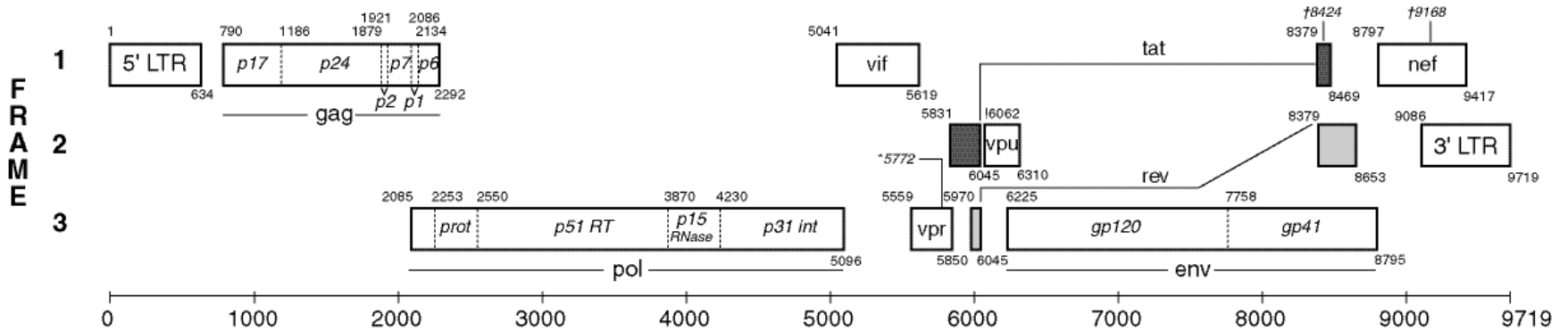
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# History of SBBC research highlights

- ◆ 1981 – 1984: transmission from common donor.
- ◆ 1989: cluster of healthy recipients with common blood donor first identified.
- ◆ 1992: Lancet paper- SBBC with common donor first identified.
- ◆ 1995: genomic deletions in the *nef*/LTR region, confirming SBBC strain has reduced virulence.
- ◆ 1996 – 1998: studies demonstrate immunogenicity but normal immune function- a possible live attenuated vaccine strain?
- ◆ 1999: signs of disease progression in donor and two recipients.
- ◆ 2000 – 2006: genomic studies- SBBC HIV strain evolution.
- ◆ 2004 – 2008: analyses of mechanisms contributing to long term non-progression into the 3<sup>rd</sup> decade of HIV infection.

# HIV-1 genomic map



# **Nef- a major determinant of virulence:**

- ◆ **Downregulation of CD4 expression (early):**
  - reduces multiple infection of target cells.
  - increased efficiency of progeny virus budding.
  - reduced antigen driven signaling via TCR.
- ◆ **Downregulation of CD28 expression:**
  - reduced co-stimulation signals for antigen-specific T cell activation → reduced antiviral effector function.
- ◆ **Downregulation of MHC Class I expression:**
  - evasion of recognition of infected cells by CTL.
- ◆ **Reduced apoptosis:**
  - prolonged shedding of progeny virus.
- ◆ **Nef binding to numerous host cell protein kinases:**
  - hijacked cell signaling → increased virion production.
- ◆ **Interference of the TCR signaling pathway, IL-2R signaling, chemokine production pathways, .....**

**ORIGINAL ARTICLES****Long-term symptomless HIV-1 infection in recipients of blood products from a single donor**

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There have been reported cases of long-term symptomless human immunodeficiency virus type 1 (HIV-1) infection, but it is not clear whether the benign course of infection was due to host, viral, or other unknown factors. During follow-up of subjects with transfusion-acquired HIV-1 infection in New South Wales, Australia, we identified a group of 6 subjects who had been infected through a single common donor. We were therefore able to study the contributions of various factors to the course of infection.

Throughout follow-up (range 6.8–10.1 years after infection), 5 of the recipients and the donor (last follow-up 10.2 years after infection of the first recipient) remained clinically free of symptoms, with normal CD4 cell counts and no p24 antigenaemia. HIV-1 was isolated from only 1 recipient; the isolate did not induce syncytia in a SUPT1 co-culture assay and had a limited in-vitro host range. 1 infected recipient (who had received extensive immunosuppressive treatment for systemic lupus erythematosus) developed *Pneumocystis carinii* pneumonia and died 4.3 years after infection. The frequency of progression to AIDS or a CD4 cell count below  $0.50 \times 10^9/l$  was significantly lower among the 6 subjects with a common donor (1/6) than among 101 other HIV-1-infected transfusion recipients for whom data from 7 years of follow-up were available (94/101;  $p < 0.0001$ ).

These findings suggest that the subjects were infected by a less virulent strain of HIV-1. The identification of this group of subjects should stimulate a search for other similar groups, which will provide important information on the immunopathogenesis of HIV-1 disease.

*Lancet* 1992; **340**: 863–67.

**Introduction**

After primary infection with human immunodeficiency virus type 1 (HIV-1) the risk of severe immunodeficiency and symptoms of disease increases with time.<sup>1–6</sup> The median time to development of acquired immunodeficiency syndrome (AIDS) in HIV-1-infected people is 7–10 years.<sup>1,2,7</sup> However, it is still not clear what proportion of those infected will eventually develop severe HIV-1 disease.

There have been a few reports of people who have been infected for long periods but who have remained symptom-free with a normal absolute number of peripheral CD4 cells.<sup>2,8</sup> It is not clear whether the benign course is due to

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## Cluster of long-term survivors first identified

- ◆ Frequency of progression to AIDS or CD4 T cell count  $<500$  was lower in this cluster (1/6) compared to the rest of the TAHIV cohort (94/101).
- ◆ Virus isolation and culture, along with lack of disease progression, suggested a less virulent HIV strain.

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# Genomic Structure of an Attenuated Quasi Species of HIV-1 from a Blood Transfusion Donor and Recipients

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A blood donor infected with human immunodeficiency virus–type 1 (HIV-1) and a cohort of six blood or blood product recipients infected from this donor remain free of HIV-1–related disease with stable and normal CD4 lymphocyte counts 10 to 14 years after infection. HIV-1 sequences from either virus isolates or patient peripheral blood mononuclear cells had similar deletions in the *nef* gene and in the region of overlap of *nef* and the U3 region of the long terminal repeat (LTR). Full-length sequencing of one isolate genome and amplification of selected HIV-1 genome regions from other cohort members revealed no other abnormalities of obvious functional significance. These data show that survival after HIV infection can be determined by the HIV genome and support the importance of *nef* or the U3 region of the LTR in determining the pathogenicity of HIV-1.

Among people infected with HIV-1 there are some who, even after infection for 10 years or more, remain healthy with no signs

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of clinical progression to acquired immunodeficiency syndrome (AIDS) and have stable, normal CD4 lymphocyte counts (1–5). The explanation for the benign course of HIV-1 infection in such long-term nonprogressors (LTNPs) may be stochastic or related to host or viral factors or a combination of both (6).

In 1989, a review of the registry of individuals in New South Wales with blood transfusion–transmitted HIV infection revealed that 6 years after transfusion two infected recipients and a common donor were asymptomatic, with normal CD4 counts. A total of seven HIV-1–infected recipients of HIV-1–infected blood from the same donor (D36, a sexually active homosexual male who became infected between December 1980 and April 1981) have been found (Table 1) (1, 7) among recipients of components of units donated by D36 between 3 February 1981 and 24 July 1984 (8). The donor and recipients (hereafter referred to as the

- ◆ Deletions in the *nef*/LTR genomic region.
- ◆ *Nef* is a major determinant of virulence.
- ◆ Weakened HIV strain.
- ◆ Has not caused disease.
- ◆ A natural human model for a live attenuated vaccine? Further attenuations?

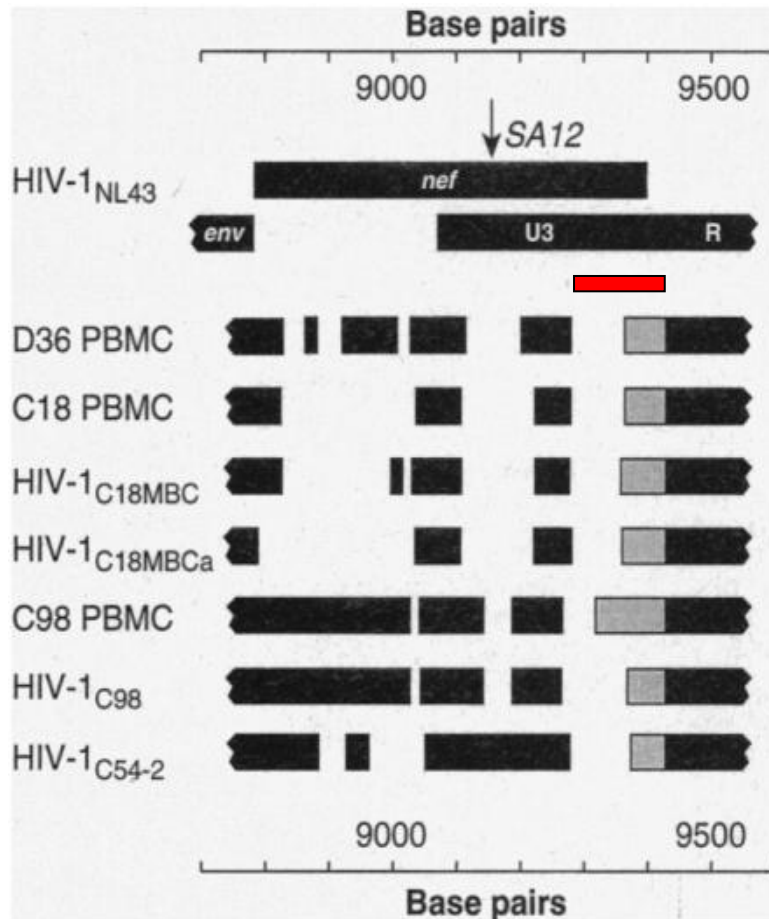
## Vaccine candidate?

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# Signature genomic deletions/duplications of the SBBC $\Delta$ *nef* HIV-1 strain



◆ Ancestral deletion at the 3' end of *nef* in the LTR overlap.

◆ Antibodies from recipients recognise peptides spanning whole *nef* except the common ancestral 3' *nef*/LTR deletion.

◆ Duplications and rearrangements of the NF- $\kappa$ B and SP1 binding sites from the LTR alone region.

◆ Secondary deletions in *nef* and LTR independently evolved in each member.

# Lymphoproliferative immune function in the Sydney Blood Bank Cohort, infected with natural *nef*/long terminal repeat mutants, and in other long-term survivors of transfusion-acquired HIV-1 infection

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and John S. Sullivan

**Objectives:** To assess T-helper cell immune function (proliferation) in members of the Sydney Blood Bank Cohort (SBBC) compared with other individuals with transfusion- and sexually acquired HIV-1 infection and with matched HIV-negative controls.

**Design and methods:** Decreasing CD4 counts and T-helper cell function are associated with disease progression. Peripheral blood mononuclear cells (PBMC) from study subjects were assayed for *in vitro* proliferative responses to HIV-1-derived antigens, recall antigens and alloantigen. T-helper cell function and CD4 counts in members of the SBBC were followed longitudinally.

**Results:** Proliferative responses and CD4 counts from members of the SBBC were similar to or better than those of other transfusion- or sexually-acquired HIV-1-positive long-term non-progressors (LTNP), including the HIV-negative matched SBBC control groups. However, individuals with disease progression had reduced or undetectable proliferative responses to recall antigens but a conserved response to alloantigen; they also had low CD4 counts and low CD4 : CD8 ratios. In the SBBC, these immune parameters were usually stable over time.

**Conclusions:** The unique SBBC with natural *nef*/long terminal repeat deletions in the HIV-1 genome were genuine LTNP without showing signs of disease progression. They appeared to be a group distinct from the tail-end of the normal distribution of disease progression rates, and may remain asymptomatic indefinitely. The SBBC virus may form the basis of a live attenuated immunotherapeutic or immunoprophylactic HIV vaccine.

*AIDS* 1997, 11:1565-1574

## Normal CD4 T cell function in the SBBC compared to progressors in the TAHIV cohort

- ◆ CD4 T cell function comparable to age, sex and transfusion matched controls, and better than other LTNP in the TAHIV cohort.
- ◆ React to HIV proteins.
- ◆ Infection with the *nef*-defective HIV strain had not impacted on CD4 T cell function.

# Infection with attenuated HIV-1 did not induce the same phenotypic changes in lymphocytes as intact HIV-1.

AIDS RESEARCH AND HUMAN RETROVIRUSES  
Volume 15, Number 17, 1999, pp. 1519–1527  
Mary Ann Liebert, Inc.

## Effect of Long-Term Infection with *nef*-Defective Attenuated HIV Type 1 on CD4<sup>+</sup> and CD8<sup>+</sup> T Lymphocytes: Increased CD45RO<sup>+</sup>CD4<sup>+</sup> T Lymphocytes and Limited Activation of CD8<sup>+</sup> T Lymphocytes

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### ABSTRACT

Members of the Sydney Blood Bank Cohort (SBBC) have been infected with an attenuated strain of HIV-1 with a natural *nef*/LTR mutation and have maintained relatively stable CD4<sup>+</sup> T lymphocyte counts for 14–18 years. Flow cytometric analysis was used to examine the phenotype of CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes in these subjects, including the immunologically important naive (CD45RA<sup>+</sup>CD62L<sup>+</sup>), primed (CD45RO<sup>+</sup>), and activated (CD38<sup>+</sup>HLA-DR<sup>+</sup> and CD28<sup>-</sup>) subsets. The median values were compared between the SBBC and control groups, comprising age-, sex-, and transfusion-matched HIV-1-uninfected subjects; transfusion-acquired HIV-1-positive LTNPs; and sexually acquired HIV-1-positive LTNPs. Members of the SBBC not only had normal levels of naive CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes, but had primed CD45RO<sup>+</sup> CD4<sup>+</sup> T lymphocytes at or above normal levels. Furthermore, these primed cells expressed markers suggesting recent exposure to specific antigen. SBBC members exhibited variable activation of CD8<sup>+</sup> T lymphocytes. In particular, SBBC members with undetectable plasma HIV-1 RNA had normal levels of activated CD8<sup>+</sup> T lymphocytes. Therefore, the result of long-term infection with natural *nef*/LTR mutant HIV-1 in these subjects suggests a decreased cytopathic effect of attenuated HIV-1 on susceptible activated CD4<sup>+</sup> T lymphocyte subsets *in vivo*, and minimal activation of CD8<sup>+</sup> T lymphocytes.

- ◆ Memory CD4 T cells susceptible to activation and infection by HIV.
- ◆ Normal levels of memory CD4 T cells in the SBBC.
- ◆ Activated CD38<sup>+</sup> CD8 T cells are induced by HIV replication, but activation was minimal in SBBC.

# A vaccine model- can the attenuated SBBC HIV strain induce sufficient immune responses to protect against challenge with wild-type HIV-1?

JOURNAL OF VIROLOGY, Jan. 1999, p. 436-443  
0022-538X/99/\$04.00+0  
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Vol. 73, No. 1

## Strong Human Immunodeficiency Virus (HIV)-Specific Cytotoxic T-Lymphocyte Activity in Sydney Blood Bank Cohort Patients Infected with *nef*-Defective HIV Type 1

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Received 13 July 1998/Accepted 21 September 1998

Proposals for the use of live attenuated human immunodeficiency virus (HIV) type 1 (HIV-1) as a vaccine candidate in humans have been based on the protection afforded by attenuated simian immunodeficiency virus in the macaque model. Although it is not yet known if this strategy could succeed in humans, a study of the Sydney Blood Bank Cohort (SBBC), infected with an attenuated HIV-1 quasispecies with natural *nef* and *nef*/long terminal repeat deletions for up to 17 years, could provide insights into the long-term immunological consequences of living with an attenuated HIV-1 infection. In this study, HIV-specific cytotoxic T-lymphocyte (CTL) responses in an SBBC donor and six recipients were examined over a 3-year period with enzyme-linked immunospot, tetrameric complex binding, direct CTL lysis, and CTL precursor level techniques. Strong HIV-specific CTL responses were detected in four of seven patients, including one patient with an undetectable viral load. Two of seven patients had weak CTL responses, and in one recipient, no HIV-specific CTLs were detected. High levels of circulating effector and memory HIV-specific CTLs can be maintained for prolonged periods in these patients despite very low viral loads.

- ◆ Vaccine design aimed at inducing broad CTL, recognising multiple conserved epitopes, with cross-clade protection.
- ◆ Sub-unit vaccines had failed to induce protective CMI, could a live attenuated vaccine work?
- ◆ The SBBC strain provided a model of a live strain, with minimal viral replication yet capable of inducing strong CTL that may protect against HIV challenge.

IMMUNOLOGIC AND VIROLOGIC STATUS AFTER 14 TO 18 YEARS  
OF INFECTION WITH AN ATTENUATED STRAIN OF HIV-1

A Report from the Sydney Blood Bank Cohort

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## ABSTRACT

**Background and Methods** The Sydney Blood Bank Cohort consists of a blood donor and eight transfusion recipients who were infected before 1985 with a strain of human immunodeficiency virus type 1 (HIV-1) with a deletion in the region in which the *nef* gene and the long terminal repeat overlap. Two recipients have died since 1994, at 77 and 83 years of age, of causes unrelated to HIV infection; one other recipient, who had systemic lupus erythematosus, died in 1987 at 22 years of age of causes possibly related to HIV. We present longitudinal immunologic and virologic data on the six surviving members and one deceased member of this cohort through September 30, 1998.

**Results** The five surviving recipients remain asymptomatic 14 to 18 years after HIV-1 infection without any antiretroviral therapy; however, the donor commenced therapy in February 1999. In three recipients plasma concentrations of HIV-1 RNA are undetectable (<200 copies per milliliter), and in two of these three the CD4 lymphocyte counts have declined by 9 and 30 cells per cubic millimeter per year ( $P=0.3$  and  $P=0.5$ , respectively). The donor and two other recipients have median plasma concentrations of HIV-1 RNA of 645 to 2850 copies per milliliter; the concentration has increased in the donor ( $P<0.001$ ). The CD4 lymphocyte counts in these three cohort members have declined by 16 to 73 cells per cubic millimeter per year ( $P<0.001$ ). In the recipient who died after 12 years of infection, the median plasma concentration of HIV-1 RNA was 1400 copies per milliliter, with a decline in CD4 lymphocyte counts of 17 cells per cubic millimeter per year ( $P=0.2$ ).

**Conclusions** After prolonged infection with this attenuated strain of HIV-1, there is evidence of immunologic damage in three of the four subjects with detectable plasma HIV-1 RNA. The CD4 lymphocyte counts appear to be stable in the three subjects in whom plasma HIV-1 RNA remains undetectable. (N Engl J Med 1999;340:1715-22.)

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IT is well recognized that host and viral factors modify the rate at which infection with the human immunodeficiency virus type 1 (HIV-1) progresses to the acquired immunodeficiency syndrome (AIDS). Identified host factors include age<sup>1-4</sup> and genetic background.<sup>5-8</sup> The *nef* gene is a major determinant of virulence in primate lentiviruses. Strains of simian immunodeficiency virus (SIV) lacking the *nef* gene have been shown to be less pathogenic in macaques and to replicate less well in vivo than isogenic strains with an intact *nef* open reading frame.<sup>9</sup> Kirchhoff et al. reported a single case of long-term, factor VIII-transmitted, nonprogressive infection due to a strain of HIV-1 with deletions in the proximal *nef* gene and the region in which the *nef* gene and the long terminal repeat (LTR) overlap that were distinct in size and exact position from those of the virus infecting persons in the Sydney Blood Bank Cohort.<sup>10</sup> The Sydney Blood Bank Cohort is a group of HIV-1-infected persons with apparently nonprogressive HIV infection, comprising a blood donor and eight persons who received transfusions of blood products from that donor. The group was first described in 1992.<sup>11</sup> Subsequently, these subjects were shown to be infected with a strain of HIV-1 (subtype B) with a conserved deletion of 150 or more base pairs (bp) in the *nef*-LTR overlap region and with duplications and rearrangements of nuclear factor- $\kappa$ B (NF- $\kappa$ B) and Sp1 transcription factor binding sites in the LTR.<sup>12</sup>

We present a comprehensive analysis of all the longitudinal immunologic and virologic data available on this unique cohort through September 30, 1998.

From the Australian Red Cross Blood Service—New South Wales, Sydney (J.C.L., A.E.G., C.H.R.-G., W.B.D., L.M., J.S.S.); the National Centre in HIV Virology Research and the Macfarlane Burnet Centre for Medical Research, Fairfield, Victoria (J.M., R.B.O., D.I.R., N.J.D.); the National Centre in HIV Epidemiology and Clinical Research, Sydney (L.J.A.); and the Royal Prince Alfred Hospital, Sydney (R.J.G.)—all in Australia. Address reprint requests to Ms. Learmont at the Australian Red Cross Blood Service—NSW, 153 Clarence St., Sydney, NSW 2000, Australia, or at [jlearmont@arcbs.redcross.org.au](mailto:jlearmont@arcbs.redcross.org.au).

# Hopes of a live attenuated HIV vaccine dashed: some of the SBBC are now showing signs of disease progression.

- ◆ Significant CD4 T cell decline in the donor and two recipients.
- ◆ Stable CD4 T cell counts in the 3 recipients without detectable HIV viraemia.
- ◆ Two outcomes of infection with attenuated HIV: slow and non-progression.

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### High frequency of nonprogression 20 years after transfusion-transmitted human immunodeficiency virus type-1 infection

Now into the third decade of the human immunodeficiency virus (HIV) pandemic, genuine long-term nonprogressors (LTNPs) have become a rarity owing to gradual disease progression of most long-term infected individuals. In addition, treatment with antiretrovirals is considered incompatible with the LTNP definition. The introduction of routine quantitative plasma HIV RNA load testing confirmed that increased viral replication was associated with risk of disease progression<sup>1</sup> and hence a virologic parameter was needed for defining true nonprogression. Using a stricter definition, studies by Lefrère and coworkers<sup>2</sup> showed that previous estimates of the proportion of LTNPs in the HIV-infected population (typically 5%) were indeed overestimates. They had 21 from a cohort of 249 classified as LTNPs according to earlier definitions, but after a strict examination of all signs of disease progression. Including virologic parameters, only two could be classed as true nonprogressors. We were therefore interested in a recent update from this group<sup>3</sup> showing that only one individual remained asymptomatic and antiretroviral naïve after 20 years infection, yet was now showing signs of disease progression.

We report here on six therapy naïve LTNPs that have been infected for around 20 years, with five showing no signs of disease progression. The known infection date for each is shown in Fig. 1. These cases represent more than 4 percent of the original cohort of 137 transfusion recipients infected with HIV-1 in the state of New South Wales, Australia, before HIV screening. Using the LTNP definition from the early 1990s, we identified six individuals who subsequently formed the recipients of the Sydney Blood Bank Cohort (SBBC), each infected with a mutant *nef*HIV-1 strain from a common donor,<sup>4</sup> and seven other LTNPs with wild-type HIV-1 transmitted from different blood donors (Cohort 2). Today, three individuals from each cohort have retained LTNP status (Fig. 1). It could be argued that C122, a man aged 84 years, now shows signs of progression, involving a gradual decline in CD4 T cells, increasing CD8 T cells, and a more recently detectable viral load, which surged to 9000 copies per mL immediately after an influenza immunization in March 2002. Unlike a previous transient increase in viral load, this viremia spike has not resolved spontaneously. Nevertheless, HIV-1 RNA has consistently remained below detectable limits in the other five LTNPs (Fig. 1), apart from one self-resolving spike in C53). Also noteworthy were normal CD4:CD8 ratios in C49 and C13, and ratios of 1:1 in C64, C135, and C53, in contrast to normal HIV-1 infection where an inverted CD4:CD8 ratio is caused by elevated CD8 T cells, consistent with an ongoing CTL response to replicating virus. Three had host genetic factors that favor slow pro-

gression. HLA B57 in C135 and B27 in C13 and C122, whereas only C135 was heterozygous for the CCR5 Δ32 deletion.

These six individuals represent a higher than expected proportion (4.4%) of our original cohort of 137 transfusion recipients that have remained LTNPs after 20 years of infection. Conservatively, if C122 was downgraded to slow progressor status, this still gives 5 of 137 (3.6%) true LTNPs after 20 years. Additionally, if we were to eliminate the SBBC bias of an attenuated HIV-1 strain, removal of all recipients of the SBBC donor leaves 2 of 128 (1.6%) true LTNPs after 20 years of infection, which is still a higher LTNP proportion than the cohort of Lefrère and colleagues.<sup>3</sup> Like Lefrère's study group, however, our LTNP cohort is dwindling. It is likely that death from advancing age or other natural causes will diminish their ranks before disease progresses. Overall, our remaining true LTNPs are scientifically valuable individuals that are the subject of ongoing monitoring and study. The absence of any sign of disease progression in the 5 true LTNPs after 20 years HIV-1 infection suggests that the tail end of the Gaussian distribution of rates of disease progression may be very long indeed.

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### REFERENCES

1. Mellors JW, Rinaldo CR, Gupta P, et al. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* 1996;272:1167-70.
2. Lefrère JJ, Morand-Joubert L, Mariotti M, et al. Even individuals considered as long-term nonprogressors show biological signs of progression after 10 years of human immunodeficiency virus infection. *Blood* 1997;90:1133-40.
3. Lefrère JJ, Morand-Joubert L. Non-progression of HIV infection 20 years after diagnosis. *Transfusion* 2004;44:623-4.
4. Deacon NJ, Tsykin A, Solomon A, et al. Genomic structure of an attenuated quasi species of HIV-1 from a blood transfusion donor and recipients. *Science* 1995;270:988-91. □

## Status of the TAHIV cohort after 20 years.

- ◆ Lefrère et al (1997) reported 21 LTNP in cohort of 249; all but 2 showed some sign of disease progression.
- ◆ Lefrère et al (2004) update: only one remaining LTNP, but now showing signs of disease.
- ◆ We reported 6 from the original 13 retaining LTNP status, one showing signs of disease, 5 others elite LTNP status.
- ◆ 6 LTNP from 137 TAHIV after 20 years  
→ high frequency of non-progression.

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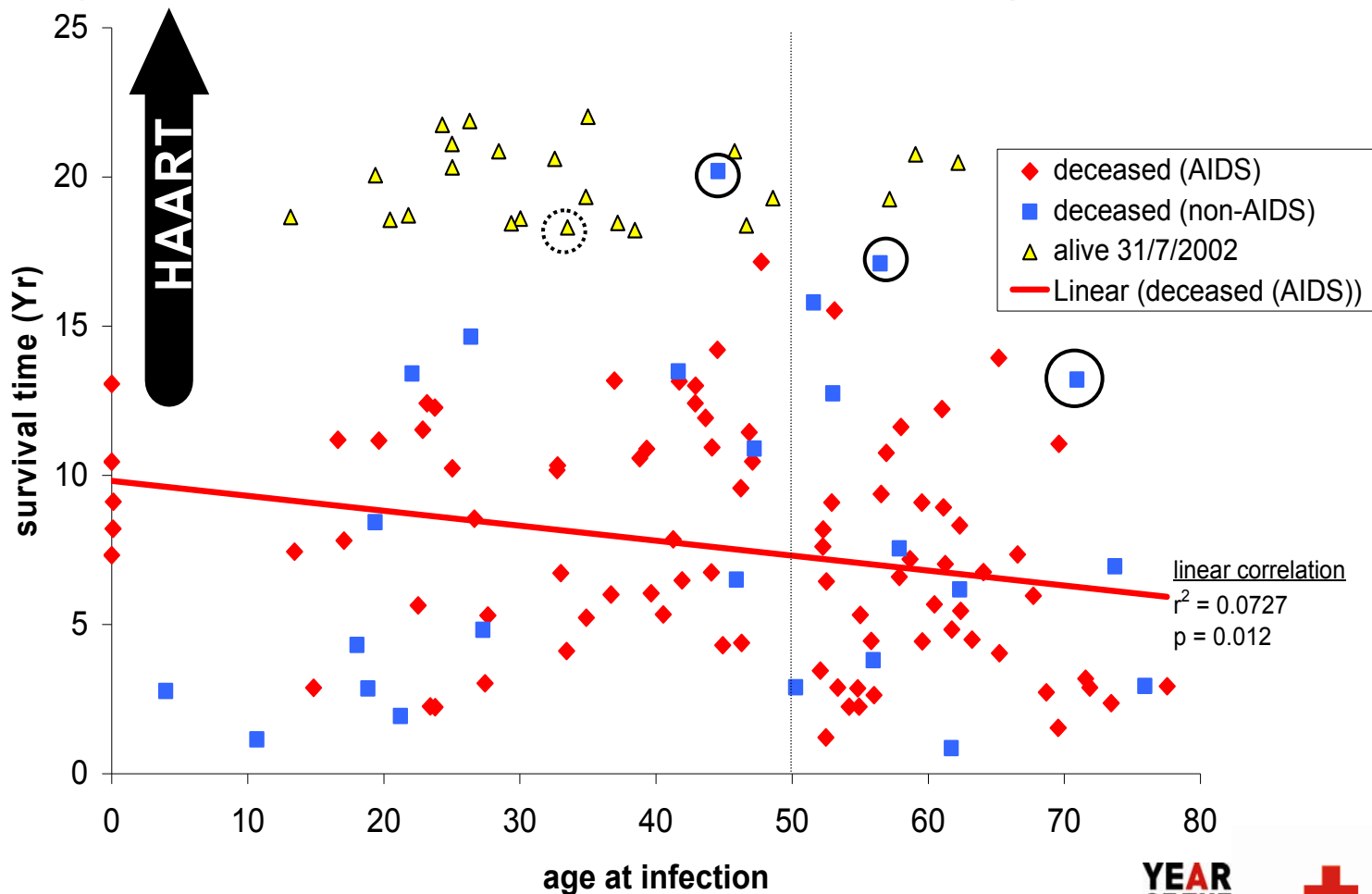
	LTNP status	CD4 cell counts (per $\mu$ L)	HIV-1 viral load (copies/mL)
*C49	dob: 9/6/1954 age: 49 date transfused: 11/6/1984 years HIV-1+: 20.1 ART status: naïve CCR5 genotype: wt / wt HLA: A2,11 B7,60		
*C64	dob: 20/3/1926 age: 76 date transfused: 4/5/1983 years HIV-1+: 21.2 ART status: naïve CCR5 genotype: wt / wt HLA: A2,32 B7,44		
*C135	dob: 23/2/1946 age: 58 date transfused: 20/2/1981 years HIV-1+: 23.4 ART status: naïve CCR5 genotype: Δ32 / wt HLA: A1,33 B50,57		
**C13	dob: 20/5/1946 age: 57 date transfused: 28/10/1984 years HIV-1+: 19.7 ART status: naïve CCR5 genotype: wt / wt HLA: A3,25 B18,27		
**C53	dob: 5/6/1947 age: 56 date transfused: 2/8/1984 years HIV-1+: 19.9 ART status: naïve CCR5 genotype: wt / wt HLA: A2,24 B15,40		
**C122	dob: 14/12/1919 age: 84 date transfused: 9/2/1982 years HIV-1+: 22.4 ART status: naïve CCR5 genotype: wt / wt HLA: A2,31 B27,44		

## Virological control in 5/6 LTNP predictive of on-going non-progression

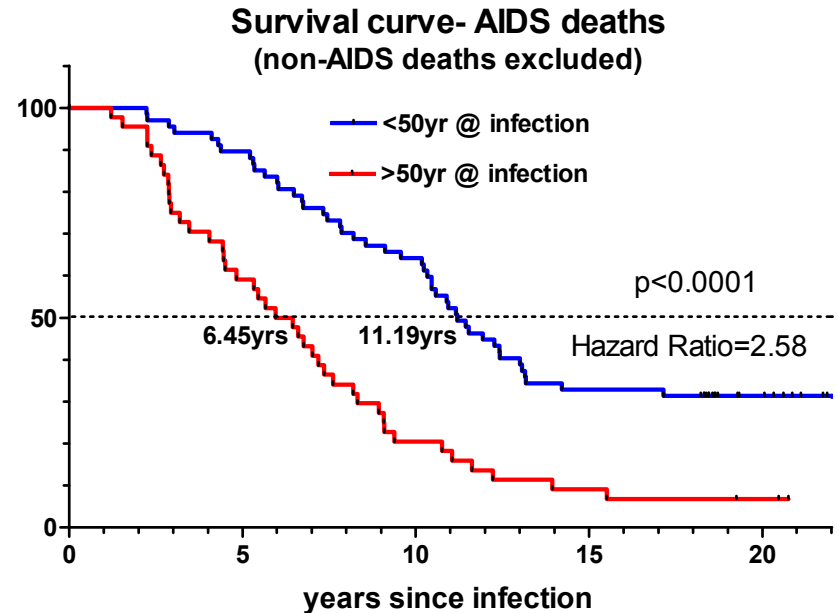
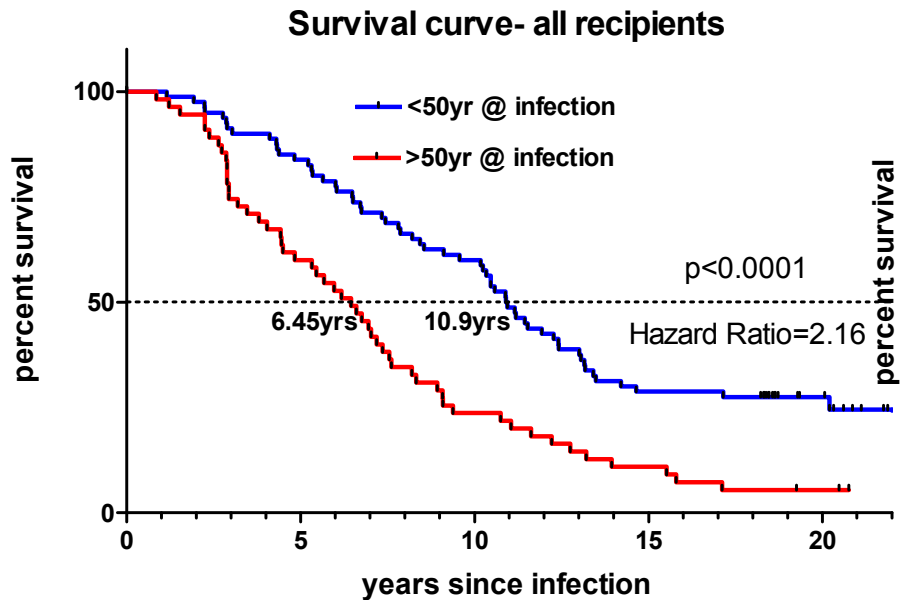
Survival advantage factors:

- ◆ 3 surviving SBBC recipients: *nef*-defective virus, one also had HLA and coreceptor polymorphisms.
- ◆ 3 cohort 2 recipients: no viral genetic factors, 2 had HLA polymorphisms.
- ◆ Classic signs of early disease progression in elderly recipient C122, predictive of symptomatic HIV disease.

# Survival at 20 years post infection depends on antiretroviral therapy; genetic or immune factors may also contribute to non-progressive disease.



# Survival analysis- age at infection



# Two patterns of HIV disease in the SBBC: Slow progressors and elite non-progressors. Why?

- ◆ **Viral factors- any evidence of reversion toward pathogenic HIV genotypes?**
- ◆ **Host genetic polymorphisms and antiviral immune responses.**

# Independent viral quasispecies evolution in each SBBC member after infection: -convergent evolution to a minimal *nef*. -divergent evolution in pathogenicity.

JOURNAL OF VIROLOGY, Jan. 2006, p. 1047–1052  
0022-538X/06/\$08.00+0 doi:10.1128/JVI.80.2.1047-1052.2006  
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Vol. 80, No. 2

## Longitudinal Analysis of Human Immunodeficiency Virus Type 1 *nef*/Long Terminal Repeat Sequences in a Cohort of Long-Term Survivors Infected from a Single Source

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and Paul R. Gorry<sup>1,4\*</sup>

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*Monash University, Melbourne, Victoria, Australia*<sup>4</sup>

Received 13 October 2005/Accepted 25 October 2005

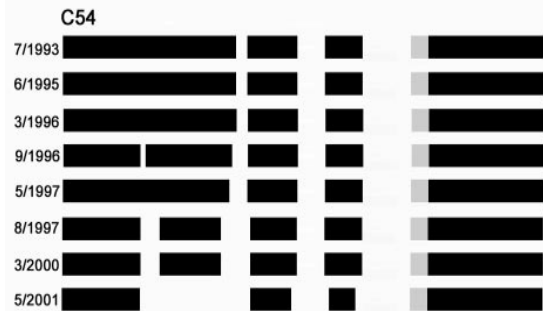
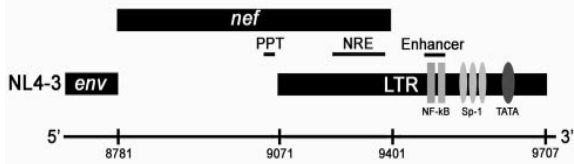
We studied the evolution of human immunodeficiency virus type 1 (HIV-1) in a cohort of long-term survivors infected with an attenuated strain of HIV-1 acquired from a single source. Although the cohort members experienced differing clinical courses, we demonstrate similar evolution of HIV-1 *nef*/long-terminal repeat (LTR) sequences, characterized by progressive sequence deletions tending toward a minimal *nef*/LTR structure that retains only sequence elements required for viral replication. The *in vivo* pathogenicity of attenuated HIV-1 is therefore dictated by viral and/or host factors other than those that impose a unidirectional selection pressure on the *nef*/LTR region of the HIV-1 genome.

**YEAR  
OF THE  
BLOOD  
DONOR**  
2009

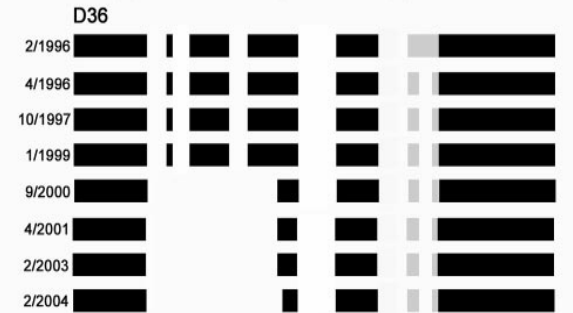
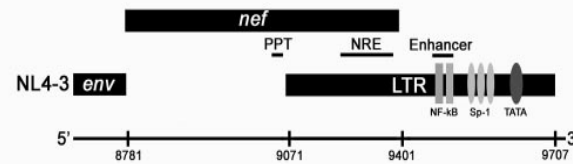


Australian Red Cross  
**BLOOD SERVICE**

### A SBBC Long Tern Nonprogressors



### B SBBC Slow Progressors



## Evolution of SBBC *nef*/LTR sequence deletions

- ◆ Stable highly evolved strain in C49, not associated with increased pathogenicity.
- ◆ Gradual evolution toward stable strain in other LTNP (C54, C64).
- ◆ Reversion toward increased pathogenicity in slow progressors not related to *nef*. Enhanced transcriptional activity in LTR clones in D36, and independent evolution of *env* (tropism) in D36, C98.
- ◆ HIV can evolve under low replication rates.

# Two patterns of HIV disease in the SBBC: Slow progressors and elite non-progressors. Why?

- ◆ Viral factors- any evidence of reversion toward pathogenic HIV genotypes?
- ◆ Host genetic polymorphisms and antiviral immune responses.

Research

Open Access

## Mechanisms of HIV non-progression; robust and sustained CD4+ T-cell proliferative responses to p24 antigen correlate with control of viraemia and lack of disease progression after long-term transfusion-acquired HIV-1 infection

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Published: 11 December 2008

Received: 24 September 2008

*Retrovirology* 2008, 5:112 doi:10.1186/1742-4690-5-112

Accepted: 11 December 2008

This article is available from: <http://www.retrovirology.com/content/5/1/112>

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# Genetic factors: survival advantage

- ◆ LTNP identified 1994: SBBC n=6, cohort 2 n=7, total 13.

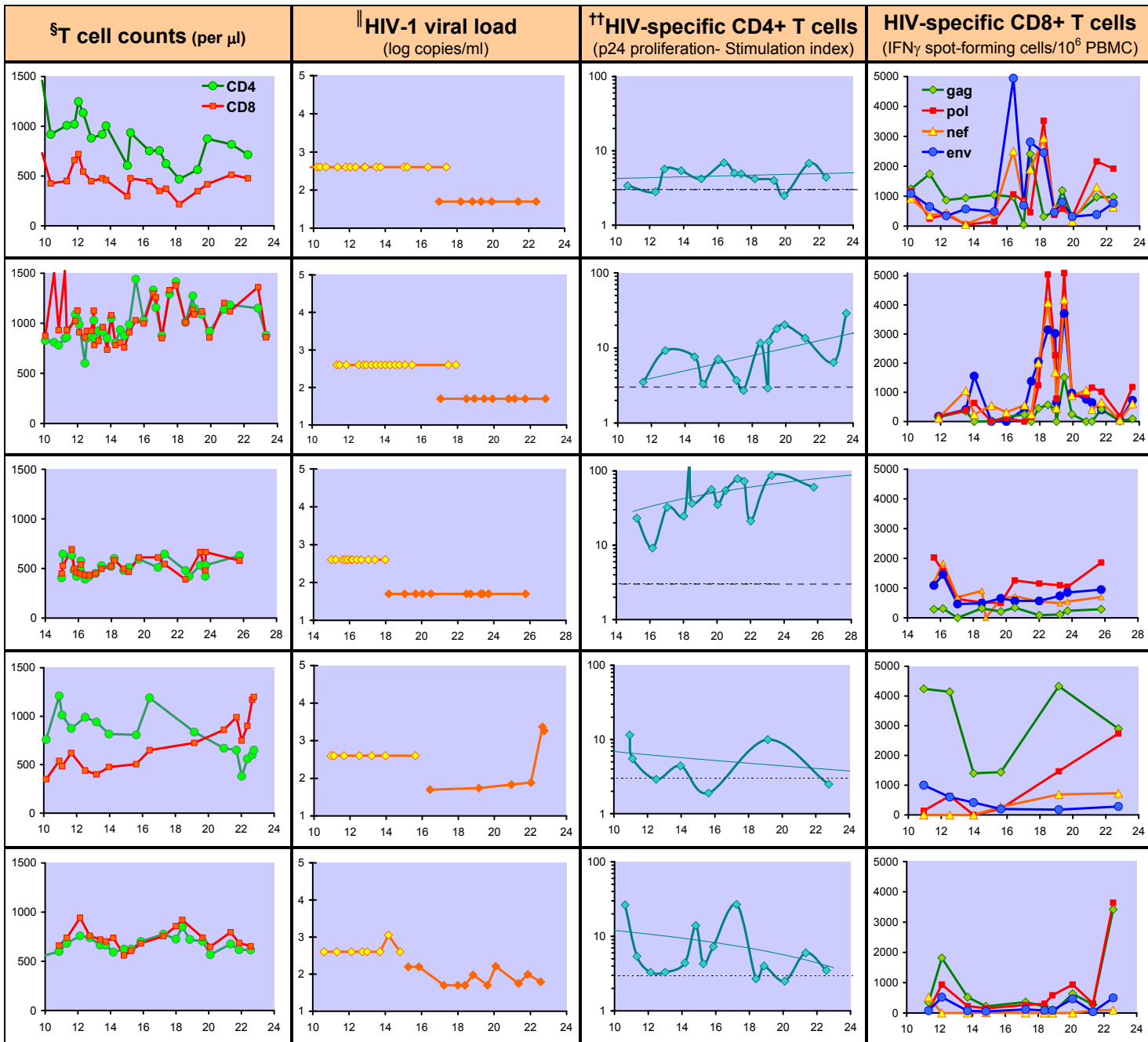
## Polymorphisms conferring a survival advantage:

- ◆ Viral attenuation:  $\Delta$  *nef*: 6/13 (SBBC only).
- ◆ Chemokine receptor genotype (CCR5-  $\Delta$  32 and/or CCR2-64I): 3/13.
- ◆ HLA polymorphisms (B27 or B57): 5/13.
- ◆ Toll-like receptor polymorphisms (TLR2-753 or TLR4-299/399): 3/13.
- ◆ At least one genetic survival advantage: 12/13.

## Polymorphisms associated with disease progression:

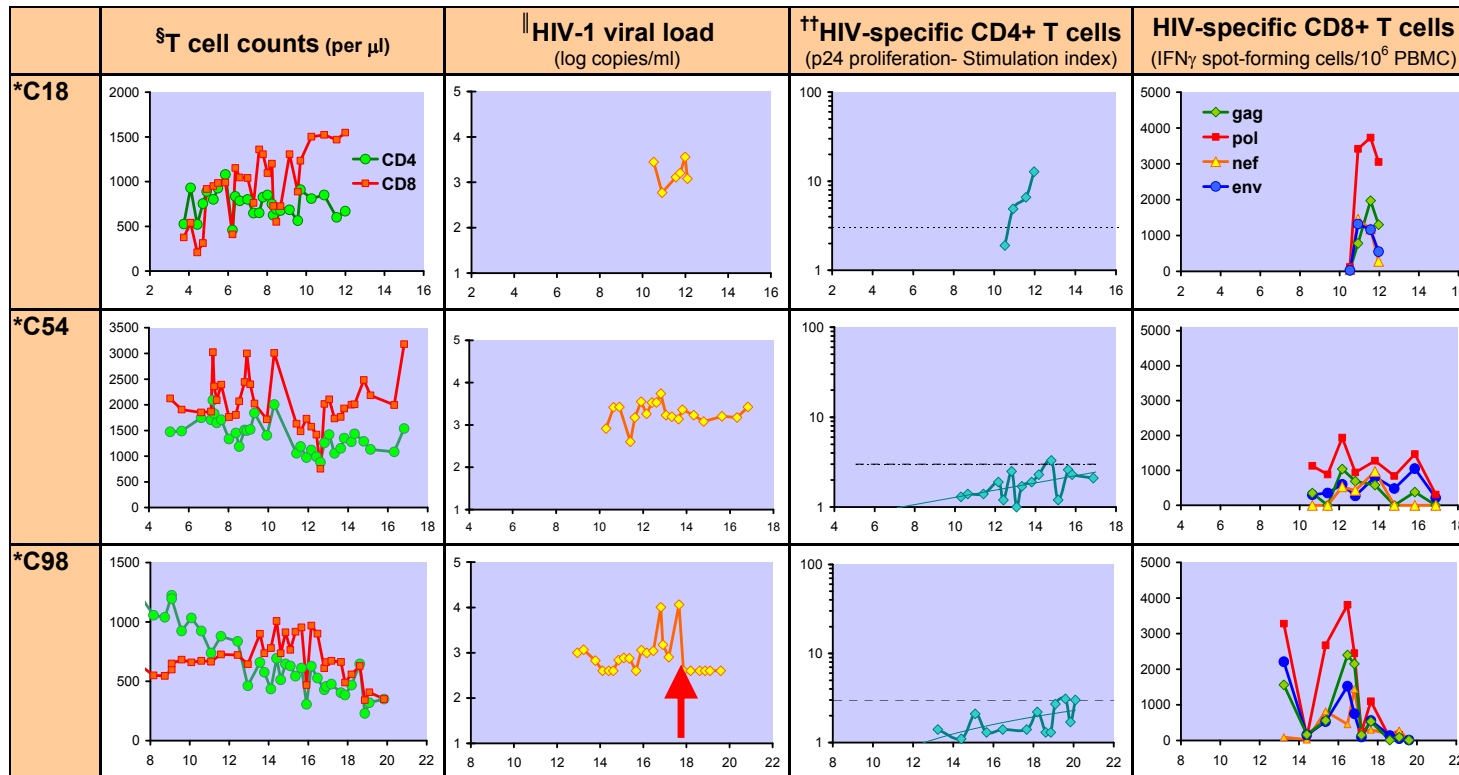
- ◆ Fc  $\gamma$  RIIa-R/R: 5/13 (all have since lost non-progressor status).

# Surviving non-progressors

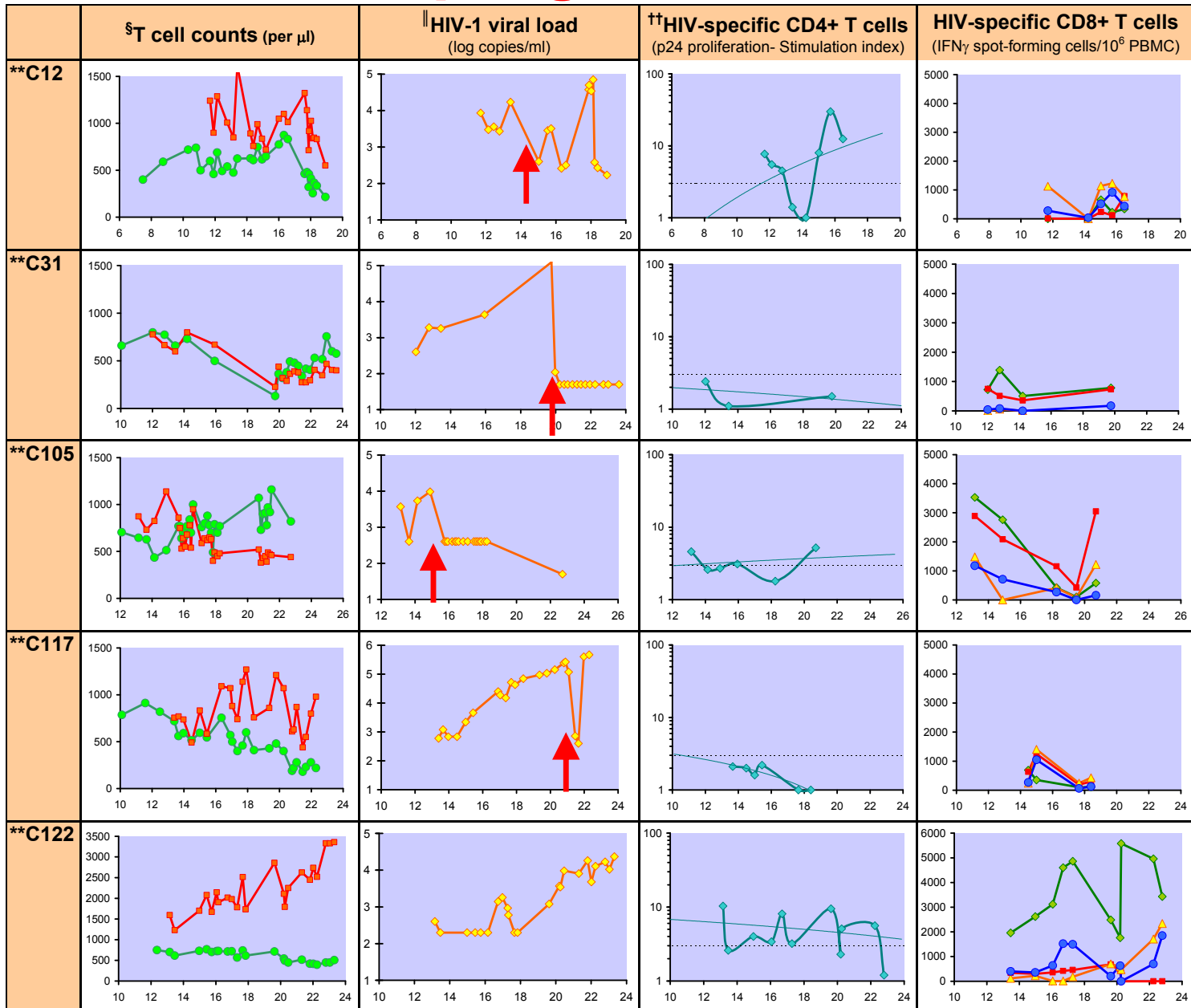


# Loss of non-progressor status- SBBC

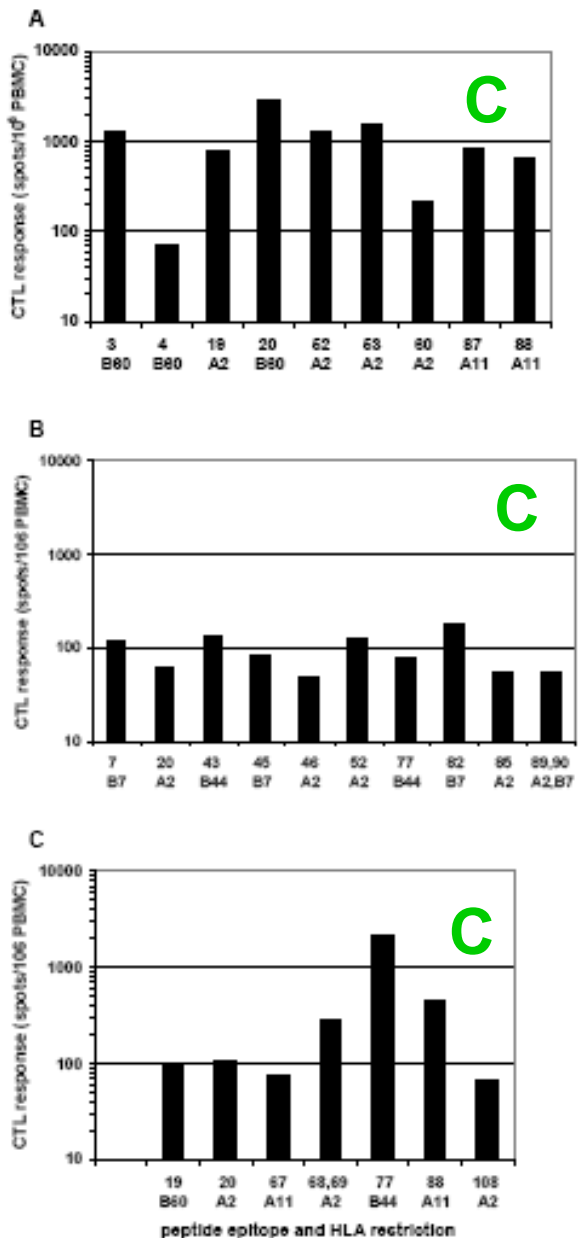
- ◆ All had detectable HIV viraemia.
- ◆ Two died before disease progression.
- ◆ Disease progression in C98, requiring ART (arrow).



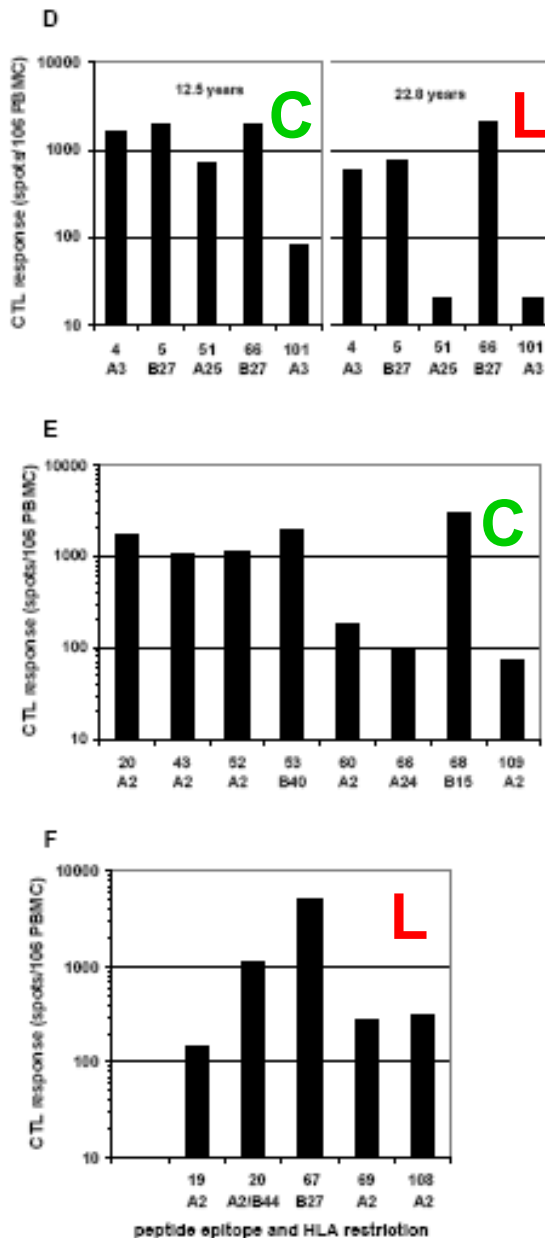
# Loss of non-progressor status- cohort 2



# SBBC



# Cohort 2



**Breadth of Gag CTL response associated with HIV control.**

**Note: dominant CTL response in SBBC against HIV Pol targets.**

**Viral escape from Gag CTL?**

# Viral escape from immunodominant CTL

- ◆ The immunodominant HLA-B27 Gag epitope is associated with control of viraemia in LTNP, but emergence of a two-step late escape mutant precede disease progression (Kelleher et al, JEM 193:375-85).
- ◆ Viral escape in only 1/3 HLA-B27 individuals with increasing VL. Escape mutants detected in earliest specimen, proliferative response was also negative from the outset.
- Other factors? Decline in p24 proliferative response in C13, C122. →CTL less effective in controlling viral replication.

	260										272		
consensus clade B	E	I	Y	K	R	W	I	I	L	G	L	N	K
C13 plasma 31/7/07													
C117 plasma 22/3/95	D				G								
C117 plasma 26/6/96													
C117 plasma 29/10/96	D				G								
C117 plasma 23/6/99	D				G								
C117 plasma 22/3/00	D				G								
C122 plasma 18/5/99													
C122 plasma 3/5/04													
C122 PBMC 3/5/04													

# Neutralising antibodies and viral replication

JOURNAL OF VIROLOGY, Sept. 2007, p. 9268–9278  
0022-538X/07/\$08.00+0 doi:10.1128/JVI.00650-07

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Vol. 81, No. 17

## Viral Phenotypes and Antibody Responses in Long-Term Survivors Infected with Attenuated Human Immunodeficiency Virus Type 1 Containing Deletions in the *nef* and Long Terminal Repeat Regions<sup>▽</sup>

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Received 27 March 2007/Accepted 6 June 2007

The Sydney Blood Bank Cohort (SBBC) consists of eight blood transfusion recipients infected with *nef*-attenuated human immunodeficiency virus type 1 (HIV-1) acquired from a single donor. Here, we show that viral phenotypes and antibody responses differ considerably between individual cohort members, despite the single source of infection. Replication of isolated virus varied from barely detectable to similar to that of the wild-type virus, and virus isolated from five SBBC members showed coreceptor usage signatures unique to each individual. Higher viral loads and stronger neutralizing antibody responses were associated with better-replicating viral strains, and detectable viral replication was essential for the development of strong and sustained humoral immune responses. Despite the presence of strong neutralizing antibodies in a number of SBBC members, disease progression was not prevented, and each cohort member studied displayed a unique outcome of infection with *nef*-attenuated HIV-1.

- ◆ NAb titres inversely proportional to VL in regular LTNP.
- ◆ SBBC: NABs directly proportional to VL, but weaker than other LTNP.
- ◆ Strong NABs required replicating virus, but contributed to control of viraemia.
- ◆ Strong NABs did not prevent disease progression in some members of the SBBC.

# Mechanisms of non-progression

- ◆ Viral attenuation: infection with  $\Delta$  *nef*-LTR HIV-1 strain established non-progressive disease course in SBBC.
- ◆ Host genetic factors: contributed to non-progression beyond the first decade.
- ◆ Proliferative response to Gag-p24 protein: associated with control of viraemia and non-progression. Response to p24 was absent or declined in those that progressed to HIV disease.
- ◆ Anti viral CTL: control of viraemia into and beyond the second decade, but only when combined with CD4 T cell proliferative responses to p24. Breadth of Gag CTL response important; restricted CTL ultimately failed to prevent late disease progression in the absence of p24 responses.
- ◆ Neutralising antibodies: replicating HIV required to generate strong Neut Abs, but these did not prevent disease progression.

# **Conclusions: prolonged non-progression**

- ▶ **Proliferative CD4 T cell response against HIV p24 was the single over riding mechanism independently associated with ongoing functional control over HIV replication and non-progression to AIDS in the NSW TA-HIV cohort.**
- ◆ **Ongoing viral evolution remains the primary concern:**
  - **Is viral turnover in the 3 surviving SBBC below an a threshold that allows evolution toward increased pathogenesis, or escape from immune control?**
  - **Low but detectable viraemia in the two Cohort 2 LTNP may facilitate eventual viral escape or erosion of immune control.**
- ◆ **Future of the SBBC; the last recipients standing? Is viral attenuation the final arbiter of non-progression?**