Therapeutic Immunization with Autologous DC Pulsed with Autologous Inactivated HIV-1 Infected Apoptotic Cells

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HIV and DC Vaccines

- During seroconversion, there is an equilibrium between viral replication and immune control.
- HIV immune response fail to control virus once viral escape mechanisms prevail
- HAART results in enhancement of CD4 T cell reactivity but not to HIV[1]
- Enhancing host immune response may be a valid strategy to overcome lack of viral control in chronic HIV infection
- Trials of treatment interruption (*auto-vaccination*[2]) fail to enhance viral control[3]
- Strategy of “therapeutic vaccination” to enhance strength and extent of HIV-1 cellular immune response could be effective for viral control

[1]Rinaldo JID 179:329; [2]Ortiz J Clin Invest 104:R13; [3]Carcelain J Virol 75:234

DC loaded with inactivated autologous HIV

- Lu et al. (Nat Med 12/04)
  - 18 untreated HIV infected patients vaccinated with autologous DCs pulsed with AT-2 inactivated, autologous HIV
  - Major Results:
    - HIV RNA $\downarrow$ 80% (median) at day 112
    - $\geq$ 90% suppression in 8/18 at one year
    - Peripheral CD4+ cell count increased significantly from days 28-112 but thereafter returned to baseline
    - HIV-specific IL-2 and γ-IFN-expressing CD4+ increased significantly, peaking at day 112, this increase correlated with viral load decrease.

Autologous Inactivated HIV-DC vaccine

- Garcia et al (JID 5/05)
  - 12 HIV+, ART treated received 5 doses of autologous, heat inactivated HIV pulsed DC at 6 week intervals.
  - Results:
    - ≥ 0.5 log decrease in viral load in 4/12 subjects
    - Significant increase in mean viral load doubling time
    - Increase in CTL (CD8+, granzyme B+) which correlated with increase in viral load doubling time

Phase I/II Evaluation of Therapeutic Immunization with Autologous Dendritic Cells Pulsed with Autologous, Inactivated HIV-1 Infected, Apoptotic Cells

- Hypothesis:

  Therapeutic vaccination with autologous DC pulsed with autologous HIV-infected apoptotic cells will be safe and result in a decrease in the HIV-1 RNA set-point when given to HIV-infected adults

Objectives

- Primary objective:
  - To define the safety and tolerability the ApB Dendritic Cell vaccine in HIV-1 infected adults.
- Secondary objectives:
  - To explore the effect of ApB DC vaccination on the viral load set-point 12 weeks after analytic treatment interruption as compared with the pretreatment baseline.
  - To determine if there are immunologic markers that correlate with vaccine response as measured by a decrease of at least 0.5 $\log_{10}$ copies/mL in HIV-1 RNA from baseline to week 12 post-ATI

Study Design

- Phase I/II, single arm, single site, N=16 evaluable subjects
- Three phases/steps:
  - STEP 1: Pre-vaccination Phase
    - Viral isolation from autologous blood
    - Initiation of ART until plasma HIV-1 RNA <50 cp/mL.
  - STEP 2: Immunization Phase
    - Leukapheresis for autologous DC and PBMC for vaccine production
    - Three doses of ApB DC vaccine at 2 week intervals (~10 million cells /dose SC).
  - STEP 3: ATI and Follow Up Phase
    - Stop ART 6 weeks after 3rd vaccine dose
    - 4th vaccine dose 2 weeks after start of ATI
    - Follow up for total of 48 weeks post-leukapheresis. Re-initiate ART based on clinical criteria or at discretion of investigator or primary care physician.

Major Inclusion/Exclusion Criteria

- Inclusion criteria:
  - HIV-infected adults
  - ART naïve
  - CD4≥350 cells/mm^3
  - Plasma HIV RNA 5,000-100,000 copies/mL
  - Acceptable baseline laboratory tests; no chronic HBV or HCV
  - Willingness to interrupt ART for 12 weeks
- Exclusion criteria
  - Prior or current AIDS defining condition
  - Immunosuppressive/immunomodulatory treatment
  - Recent investigational therapy/treatment of any type
  - Pregnancy or breastfeeding
  - Lack of adequate venous access

Statistical Considerations

- Primary Endpoint: safety of autologous HIV-1 ApB DC vaccine as defined by:
  - Two consecutive HIV RNA ≥400 copies/mL following any vaccination (prior to ATI), or
  - Any grade 3 or 4 clinical or laboratory abnormality
- Secondary Endpoints:
  - Mean of $\log_{10}$ HIV RNA at end of ATI (average of week 11 and 12 post-ATI) minus the mean of $\log_{10}$ HIV RNA at the 2 visits preceding ART initiation
  - Immunologic response in ELISPOT defined as 2-fold increase in reactive cells over baseline
- Safety endpoint: with 16 evaluable subjects, the probability of detecting at least 2 subjects with toxicity is 67% if the true rate is 10% and 18% if the true rate is 5%
- Virologic endpoint: the study has >80% power to detect 0.6 $\log_{10}$ difference in means before and after vaccination (estimated sd 0.5, alpha 0.05) using 2-sided Wilcoxon signed rank test

Vaccine Preparation

Virus Isolation

PBMC → Autologous Virus Isolation → Viral supernatant produced

PBMC ⇢ CD8 depleted

Viral supernatant produced ⇢ Checked for p24/infectivity

Viral supernatant produced → **BANKED**

Goal: 25 ng/ml
50-100 ml

CD4 superinfected and UV inactivated ⇢ Apoptotic bodies

DC Culture

Leukapheresis → Lymphocytes Harvested → Apoptotic bodies

Leukapheresis → Monocytes Harvested → Cultured with IL4 and GM-CSF → Immature DCs

Apoptotic bodies + Immature DCs → Co-culture & Maturation → **ApB DC Vaccine**

Pre-clinical vaccine development

- 4 patients, HIV+, not on ART
- Blood collected weekly for viral isolation
- Full-scale vaccine production (n=2)

Isolation of HIV-1 from PBMC

p24 levels in supernatants of CD4+ cell co-cultures (virus isolation)

"Feeder CD4+cells"	HIV-1+ Subjects		
	#1	#2	#3
Allo (normal donor)	38	2.7	7.0
Auto	**>50**	**>50**	**>50**
Auto + CD3/CD28 beads	**>50**	4.0	6.3

Data are in ng/ml as measured in ELISA assays. The supernatants were harvested on day 29

Viral Isolation

Isolation of virus from autologous PBMC

Subject	p24 (ng/ml)	$TCID_{50}$
#1	337	31,623
#2	227	215
#3	39	14,678

Co-culture supernatants were repeatedly tested on various days (d 4-37). The highest titers were obtained on days 10- 19. The p24 levels were measured by ELISA. TCID-50 was measured in a colorimetric assay with an indicator cell line TZM-bl

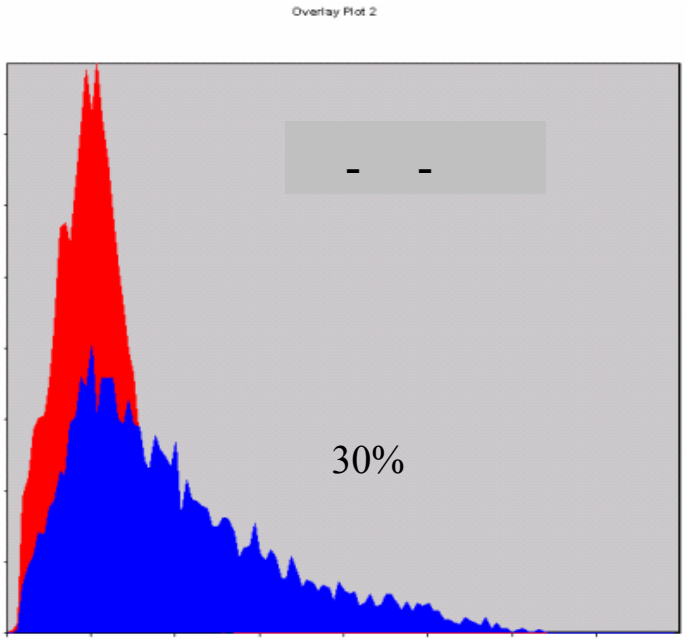
Superinfection of CD4$^+$ T-cells

Superinfection of normal CD4+T cells

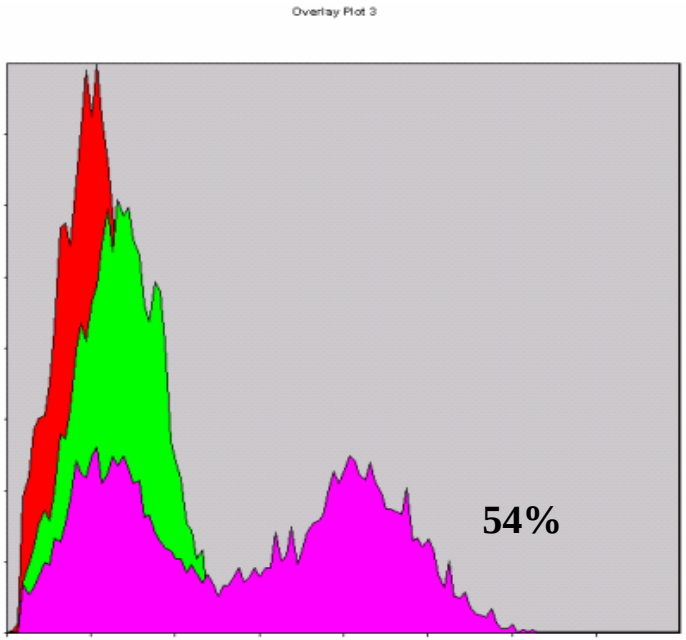
Culture day	p24+cells %	p24 in cells ng/10^5 cells	p24 in sup ng/mL
3	5	0.06	2.0
4	11	0.12	10.8
5	28	0.80	19.7
Pos cont	51	3.60	16.6
Neg cont	0	0	0

% p24+ cells was determined by flow cytometry; cell associated p24 in lysed CD4+cells and in CD4+ cell supernatants by ELISA . Viral sup of Pt. #1 was used for infection of CD4+ T cells (98% purity) separated by positive selection on immunobeads from PBMC of a normal donor.

P24 Expression on CD4 cells after Superinfection



Subject Q -05 -131 p24 PE intracellular staining in CD4+ cells



Negative and Positive Controls for p24 PE Staining

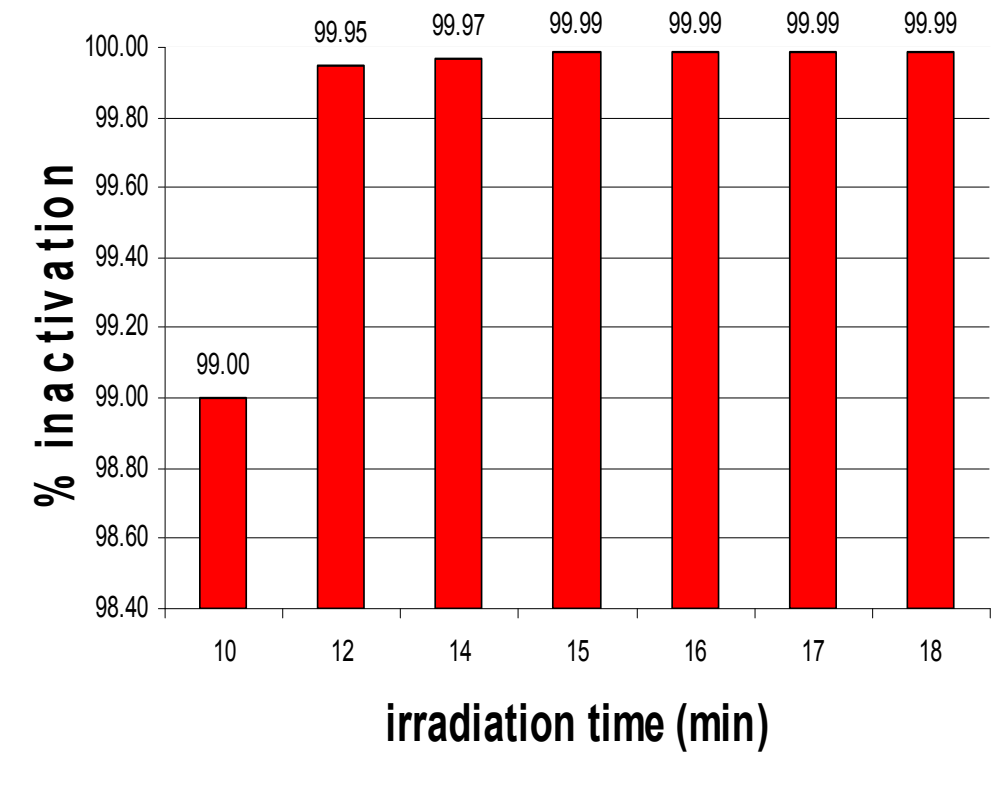
Superinfection of autologous CD4+ T cells

Superinfection of CD4+T cells of Pt # 3 (autologous system)

Culture day	p24+cells %	p24 in cells ng/10^5 cells	p24 in sup ng/mL	$TCID_{50}$ per mL
_4	25	0.11	0.10	3,162
5	18	0.08	0.02	10,000
Pos cont	52	0.03	0.09	3,162
Neg cont	0	0	0	0

% p24+ cells was determined by flow cytometry; cell associated p24 in lysed CD4+cells and in CD4+ cell supernatants by ELISA . Viral sup of Pt. # 3 was used for infection of CD4+ T cells (95% purity) separated by positive selection on immunobeads from PBMC of Pt # 3.

Psoralen/UV inactivation of HIV-1 (Cell associated HIV IIIb)



Irradiation time	TCID-50
0	1,000,000
10	10,0000
12	468
14	234
15	32
16	23
17	9
18	6

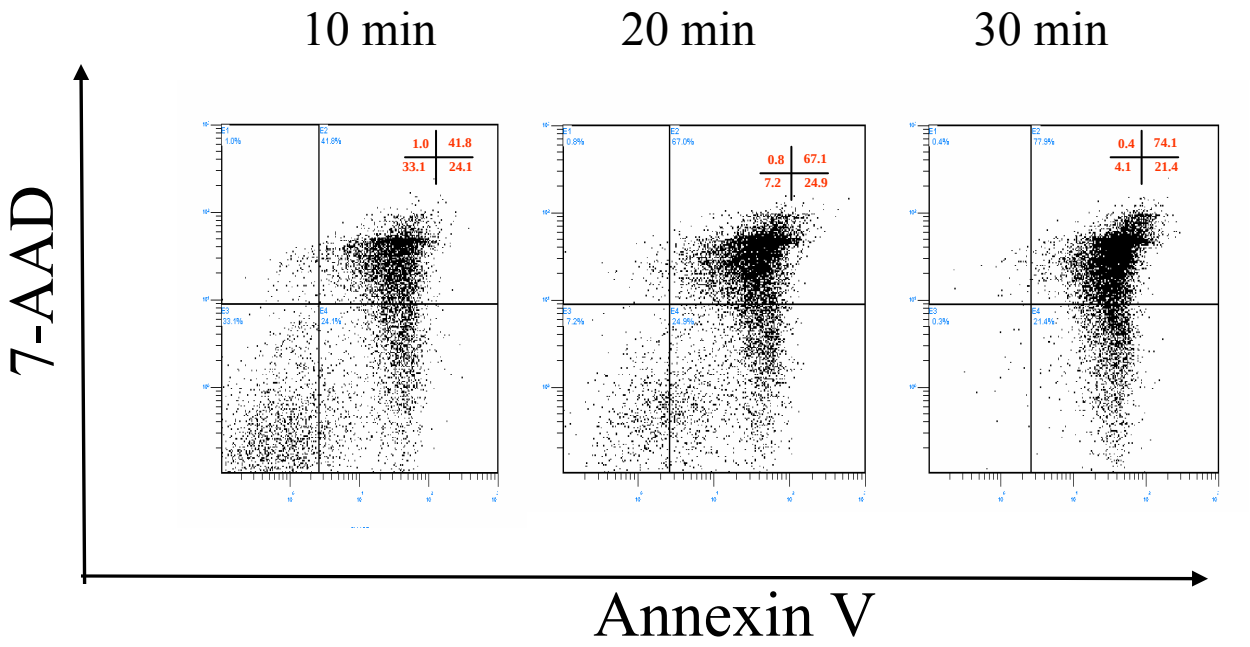
Psoralen/UV inactivation of autologous HIV-1

CD4+ T-cell apoptosis and HIV-1 inactivation by psoralen and UVB (Pt. # 3)

	$TCID_{50}$
Untreated cells	14,678
Treated cells	3
Positive con (not treated)	3,162

CD4+ T cells of Pt.# 3 were superinfected with the autologous virus (HIV-1+ supernatant)
and on day 5, they were treated with psoralen (20ug/mL)
and UVB ($3mW/m^2$) for 30 min.

Effects of UVB/psoralen treatment on CD4+ T-cell viability: 30 min of treatment is sufficient to induce apoptosis (Annexin+/7-AAD+)



Leukapheresis Product

	Cell counts	Purity (%)
Initial WBC count	3.9×10^{10}	
ELUTRA™ fraction 2 ($CD45^+CD14^-$)	1.2×10^{10}	99
ELUTRA™ fraction 5 ($CD45^+CD14^+$)	5.6×10^{9}	77
Post-CliniMACS $CD4^+CD8^-$ T cells	9.8×10^{8}	95

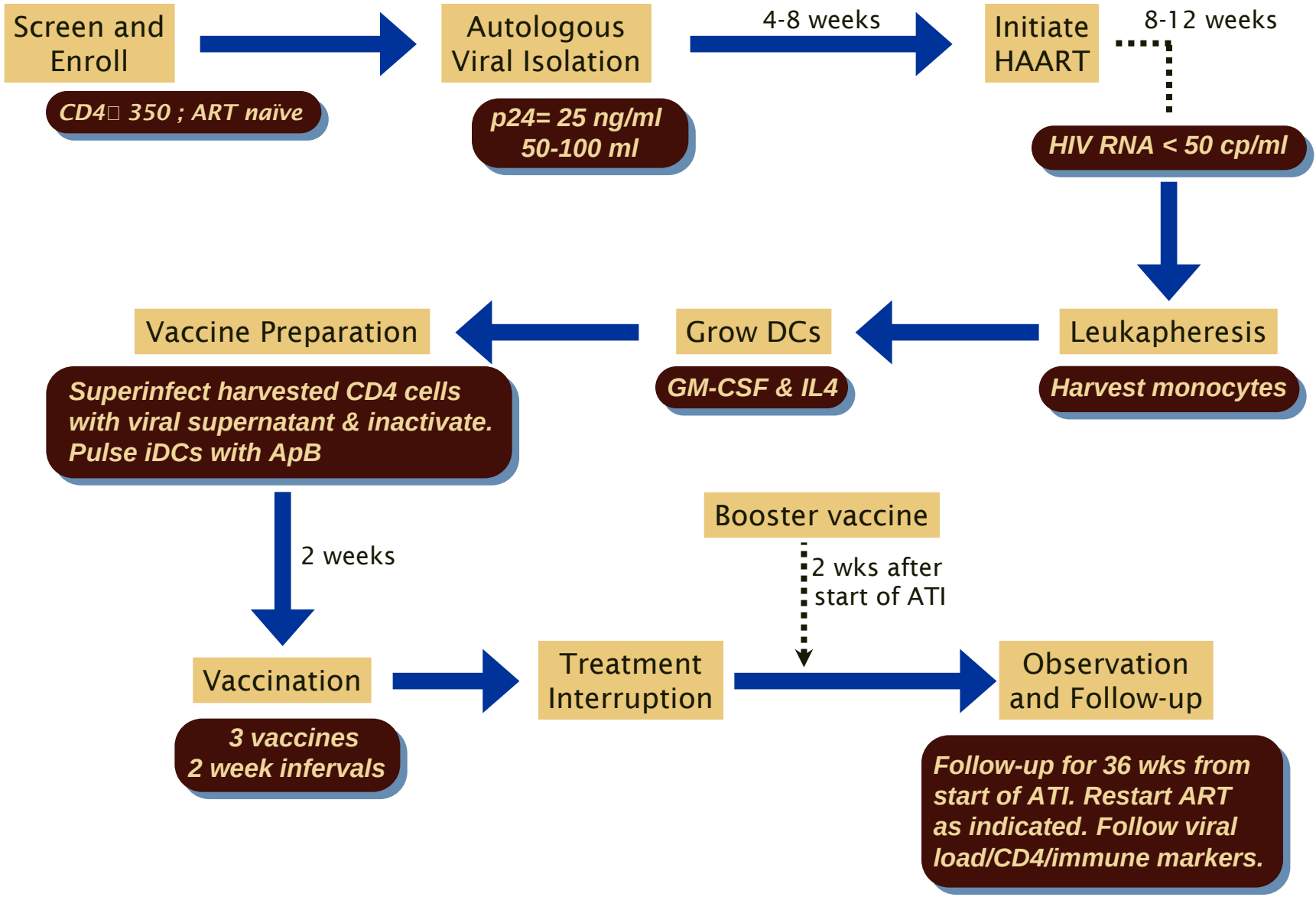
Characteristics of DC generated from monocytes (HIV-1$^+$ subject)[b]

Phenotype:

Marker	iDC	αDC-1 alone % positive[a]	αDC-1 + ApB
HLA-DR	86	94	97
CD80	50	94	93
CD83	4	60	49
CD86	19	86	91
CD11c	97	96	96
CD40	86	96	97
CCR7	10	45	42

Recovery: 57%
Viability: 87% after co-incubation with apoptotic CD4+CD8- T cells
Sterility (14 d); mycoplasma, endotoxin: negative
IL-12 production: 473 pg/mL
TCID-50/mL: unteated CD4+CD8- T cells = 1,467,799
mDC + ApB = 3
Stability: 4 h at room temperature

Study Schema



Study Status

- Total no. of patients screened = 7
- Total enrolled = 3
- Screened out = 3
- Pending results = 1
- Successful viral isolation = 3/3
- Subject 1 now on 3rd month of ART; HIV RNA <50 copies; Leukopheresis mid-June
- Subjects 2 & 3 on ART

Viral Isolation of Subject 1

	Day 4	*Day 7*	*Day 13*	*Day 21*	*Day 30*
p24 ng/ml	0.2	87.7	450.3	590.0	274.4

The supernatants from culture day 7 onwards were combined and tested for p24 by ELISA and the TCID-50 was determined.

- Supernatant volume = 89 ml
- p24 ELISA = 173.9 ng/ml
- TCID-50 = 31,623

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