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## APPENDIX I

### CHEMICAL AGENTS AND INSTRUMENTS

#### A. Chemical substances

Agarose (GIBCO; Grand Island, N.Y. USA)  
Bromphenol blue (Sigma, MO, USA)  
2-Mercaptoethanol (Sigma, MO, USA)  
Diethylpyrocarbonate: DEPC (Sigma, MO, USA)  
dNTPs (Promega, USA)  
Ethanol (C<sub>2</sub>H<sub>5</sub>OH) (Sigma, MO, USA)  
Ethidium bromide (Sigma, MO, USA)  
Ficoll-Hypaque (Robbins scientific, Norway)  
Guanidinium thiocyanate (GTC) (Sigma, MO, USA)  
Isopropanol (Sigma, MO, USA)  
MMLV-Reverse Transcriptase (Promega, USA)  
Oligo dT (Promega, USA)  
Rnasin ribonuclease inhibitor (Promega, USA)  
RPMI 1640 (GIBCO, Grand Island, NY)  
Sarcosyl (Sigma, MO, USA)  
Sodium acetate [CH<sub>3</sub>COONa] (Sigma, MO, USA)  
Sodium citrate (Sigma, MO, USA)  
Phenol/Tris (Amnesco, Solon, Ohio, USA)  
Chloroform (J.T.Baker, USA)  
Isoamyl alcohol (Sigma, MO, USA)  
*Taq* DNA polymerase (Promega, USA)  
Tetrasodium ethylene diamine tetraacetate dihydrate (EDTA)  
[CH<sub>2</sub>N (CH<sub>2</sub>COONa)<sub>2</sub>] .2H<sub>2</sub>O (E.Merch, Darmstadt, W., Germany)

**B. Instruments**

Agarose submarine gel apparatus

Automatic pipette (Gilson, Lyon, France)

Analytical balance (Shimudzu, Kyoto, Japan)

Electrophoresis power supply (Biorad, CA, USA)

Gloves, sterile

Incubator (Forma Scientific, Ohio, USA)

Microcentrifuge (Eppendorf, USA)

Mixer-Vertex-Genic (Scientific industries, N.Y., USA)

Pipette tip

pH meter, Model 10 (Corning, N.Y., USA)

PCR machine GeneAmp PCR System 9600 (Perkin elmer)

Refrigerated centrifuge, Model Centra 7-R (IEC, Boston, MA., USA)

Spectrophotometer (Spectronic Genesys 5, Minton Roy. USA)

Speed Vac Dryer (Savant Holbrook, NY, USA)

UV trans-illuminator (ULTRA-LUM, Carson, California)

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## APPENDIX II

### REAGENTS AND PREPARATIONS

#### 1. Reagents for sample preparation

##### 1.1. Guanidinium thiocyanate (GTC) lysis buffer

###### 1.1.1. 4 M Guanidinium thiocyanate (GTC)

GTC 250 g

Make up to 300 ml with DEPC-H<sub>2</sub>O, dissolve at 65 °C.

###### 1.1.2. 0.75 M Sodium citrate, pH 7

Sodium citrate 3.87 g

DEPC-H<sub>2</sub>O 20 ml

Adjust pH to 7.5

###### 1.1.3. 10% Sarcosyl

Sarcosyl 5 g

Make up to 50 ml with DEPC-H<sub>2</sub>O

##### Stock solution:

Add 17.6 ml 0.75 M sodium citrate, pH 7 and 26.4 ml 10% sarcosyl to 4 M GTC, then store at room temperature for up to 3 months

##### Working solution:

Add 0.18 ml 2-mercaptoethanol (2ME) to 25 ml of stock GTC solution to make working solution (final concentration of 2 ME = 0.1 M)

##### 1.2. 2 M Sodium acetate, pH 4

CH<sub>3</sub>COONa.3H<sub>2</sub>O 6.75 g

H<sub>2</sub>O 20 ml

Adjust pH to 4 using around 4 ml of acetic acid



## 1.3. Water-saturated phenol or Phenol/Tris

Commercially prepared "Buffer-saturated Phenol"

## 1.4. Chloroform/isoamyl alcohol (49:1)

Chloroform	4.9	ml
Isoamyl alcohol	0.1	ml

1.5. Diethylpyrocarbonate (DEPC)-treated H<sub>2</sub>O

Commercially prepared DEPC	1	ml
H <sub>2</sub> O	500	ml

Incubate overnight at 37 °C in waterbath, then autoclave at 121°C for 15 minutes.

1.6 DEPC-H<sub>2</sub>O + RNase inhibitor

RNase inhibitor.	0.5	µl
DEPC-H <sub>2</sub> O	20.0	µl

Make sufficient volume for samples to be resuspended plus a little extra, prepare immediately before use.

## 2. Reagents for agarose gel electrophoresis

## 2.1. 50X Tris-acetate buffer (TAE)

Tris-base	242.0	g
Glacial acetic acid	57.1	ml
0.5 M EDTA pH 8.0	100.0	ml

Adjust the volume to 1 liter with deionized distilled water and sterilize by autoclaving at 121 °C for 15 min

## 2.2. 10 mg/ml Ethidium bromide

Ethidium bromide	1	g
DDW	100	ml

Stir on a magnetic stirrer for several hours to ensure that dye has dissolved. Wrap the container in aluminium foil or transfer to a dark bottle and store at 4 °C

### 2.3. 1.5% Agarose gel

Agarose ultrapure	0.3	g
1X TAE	20.0	ml
10 mg/ml ethidium bromide	1.0	µl



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## APPENDIX III

### INCLUSION AND EXCLUSION CRITERIA

#### 1. Inclusion criteria

Patients must fulfil all of the following inclusion criteria to be eligible for therapy,

- a) documented HIV-1 infection (positive HIV-ELISA and confirmatory test by one of several methods).
- b) minimum age of 18 years.
- c) a single CD4+ cell count of at least 350 cells/mm<sup>3</sup> within 30 days prior to commencement of study therapy.
- d) patients must receive antiretroviral therapy for at least 7 days prior to receipt of s.c. IL-2 therapy.
- e) the following clinical laboratory parameters must be observed within 30 days of commencement of study therapy:
  - i. serum AST  $\leq$  4.9 times upper limit of normal range (ULN) for the study site.
  - ii. serum bilirubin  $\leq$  2X ULN
  - iii. serum creatinine  $\leq$  2.0 gm/dl
  - iv. granulocyte count  $\geq$  1000 cells/mm<sup>3</sup>
  - v. hemoglobin  $\geq$  11.0 g/dl
  - vi. platelet count  $\geq$  50000 cells/mm<sup>3</sup>
- f) a Karnofsky Performance Status  $\geq$  80
- g) women of childbearing potential must provide a negative serum or urine pregnancy test within 14 days prior to randomization.
- h) men and women should employ best available barrier contraception at all times.

- i) patients should provide written informed consent that conforms to local, national and international requirements for protection of human subjects in medical research.

## **2. Exclusion criteria**

- a) prior IL-2 therapy.
- b) concurrent malignancy other than mucocutaneous Kaposi's sarcoma or malignancy treated within the past 5 years.
- c) any concurrent or history of AIDS defining illness.
- d) use of systemic corticosteroids, chemotherapy or experimental cytotoxic drugs within four weeks prior to study therapy.
- e) use of any agent approved or experimental with clinically significant immunomodulatory effects.
- f) any CNS abnormality that requires treatment with anti seizure medication.
- g) patients with current or historical Crohn's disease, psoriasis or other autoimmune/inflammatory diseases with potentially life threatening complications.
- h) pregnant or lactating women.
- i) occasional or habitual use of recreational drugs/alcohol that in the opinion of the principle investigator would affect patient safety and/or compliance.
- j) patients with any serious psychiatric, medical and/or cognitive disturbance or illness that in the opinion of the principle investigator may affect safety, compliance or ability to provide written informed consent.

## BIOGRAPHY

Miss Chalinthorn Sinthuwattanawibool was born on October 19, 1972 in Kanchanaburi, Thailand. She graduated with the Bachelor degree of Science in Medical Technology from the Faculty of Medical Technology, Chiang Mai University in 1994. In 1994, she worked as a medical technologist at Thai Otsuka Pharmaceutical Co., Ltd.



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