Immune reconstitution in HIV-1 infection: the effects of antiretroviral and immune therapy

by

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Doctor of Medicine

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Declarations

Originality
This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgment is made in the text of the thesis, nor does the thesis contain any material that infringes on copyright.

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Statement of Co-authorship
The following people and institutions contributed to the publication of the work undertaken as part of this thesis:

Paper 1
Restoration of HIV-1 specific responses in HIV-1 infected individuals changing from protease to non-nucleoside reverse transcriptase inhibitor containing antiretroviral therapy. *Scand J Immun* 2003; 57:600-7

Authors
Sullivan AK (40% conception, recruitment, laboratory work, analysis and writing manuscript), Burton CT (laboratory), Nelson MR (recruitment), Moyle G
(recruitment), Mandalia S (data analysis), Gotch F (conception and manuscript review) and Gazzard BG (conception and manuscript review) (total 20%), Imami N (40% - conception, laboratory work, analysis and writing manuscript)

Paper 2
Interleukin-2 associated viral breakthroughs induce HIV-1-specific CD4 T-cell responses in patients on HAART. AIDS 2003; 17:628-9

Authors
Sullivan AK (45%-conception, recruitment and clinical management, laboratory work, analysis, manuscript), Hardy GAD (20%-conception, laboratory work), Nelson MR (patient recruitment), Gotch F and Gazzard BG (5%-conception, manuscript review), Imami N (30%-conception, laboratory work, analysis, manuscript)

Paper 3

Authors
Youle M, Emery S, Fisher M, Nelson M, Tavel J, Fosdick L, Janossy G, Loveday C, Sullivan A, Davey R, Johnson M, Lane C. This was a large multi-centre trial. My role was the recruitment and clinical management of the patients at one of the three study centres, and review of the manuscript. However I conceived and managed the nested sub-study, performing all laboratory work and analysis (unpublished)
We the undersigned agree with the above stated “proportion of work undertaken” for each of the above published peer-reviewed manuscripts contributing to this thesis:

Signed: ___________________  ___________________

    Supervisor                      Head of School
    School Of Medicine              School of Medicine
    University of Tasmania          University of Tasmania

Date:____________________

**Ethical conduct**

The research associated with this thesis abides by the International and United Kingdom codes on human and animal experimentation.
Abstract

This thesis presents findings from two randomised, controlled pilot studies, with nested sub-studies, an observational study, a compassionate release programme and a mortality audit. It aims to examine the effects, at a clinical and cellular level, of antiretroviral and immunotherapy in HIV-1 infection. Combination antiretroviral therapy (cART) enables quantitative and a degree of qualitative immune recovery; however this is neither universal nor complete. The first part of the thesis explores the effects of cART on surrogate immune markers (SIM), treatment outcomes, disease progression and death. Significant variations are observed and further reinforced by the mortality audit. I describe for the first time patterns of SIM decline and treatment response from which a model predicting treatment outcome could be developed. This section concludes with an observational study describing a differential effect on immune restoration of different classes of cART. Together this data suggests additional therapeutic interventions will be required to address the current inadequacies of cART to fully restore HIV-1 associated immune deficiencies. Therefore, the second part of the thesis examines the effect of interleukin-2 (IL-2) therapy in different settings; with and without cART, with therapeutic immunisation and in advanced disease. In the absence of cART, IL-2 increased CD4 T-cell counts without adversely affecting viral load or immune activation, potentially delaying the need for cART initiation. The compassionate release programme also demonstrated a ‘delaying’ effect which could be exploited in patients awaiting new therapies. The main therapeutic intervention study involved IL-2 and therapeutic immunisation in the context of cART, and reports novel findings of the acute effects of IL-2, including induction of HIV-1 specific responses. Overall increases were observed in CD4+, CD4+CD28+ and CD4+CD25+ T-cells, the latter being of particular interest as the precise
function of these cells in HIV-1 infection and following IL-2 therapy is still to be fully defined. As a pilot study these findings are preliminary but there is a trend for several effects to be more marked and more sustained in the arm combing all three treatments; suggesting therapeutic potential for combination immunisation and cytokine therapy which is worth pursuing. Despite the fact that the final IL-2 study in this thesis was completed in 2003, the recent publication of two large clinical outcome studies of IL-2, with somewhat unexpected results, makes these findings all the more pertinent today, and may afford some insight into the negative results observed in these large phase three trials. In summary, cART results in incomplete immune reconstitution, which can be enhanced by IL-2 and therapeutic immunisation at a cellular level. The challenge is to determine how, if at all, this can be translated into clinical benefit. Using additional SIM may help in targeting and monitoring therapy; the timing, scheduling and combination of which will be key and warrants further investigation.
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<td>Activation induced cell death</td>
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<td>AIDS</td>
<td>Acquired immunodeficiency syndrome</td>
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<td>APC</td>
<td>Antigen presenting cell</td>
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<td>ARV</td>
<td>Antiretroviral</td>
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<td>ARVT</td>
<td>Antiretroviral therapy</td>
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<td>AUC</td>
<td>Area under the curve</td>
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<td>bDNA</td>
<td>Branched DNA</td>
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<td>CAND</td>
<td>Candida</td>
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<td>cART</td>
<td>Combined antiretroviral therapy</td>
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<td>CCR5</td>
<td>Chemokine receptor 5</td>
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<td>CI</td>
<td>Confidence interval</td>
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<td>CMI</td>
<td>Cell mediated immunity</td>
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<td>CMV</td>
<td>Cytomegalovirus</td>
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<td>CONA</td>
<td>Concanavalin A</td>
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<tr>
<td>cpm</td>
<td>counts per minute</td>
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<td>CTL</td>
<td>Cytotoxic T lymphocyte</td>
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<td>CVL</td>
<td>Community viral load</td>
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<tr>
<td>CWH</td>
<td>Chelsea and Westminster Hospital</td>
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<td>DIR</td>
<td>Discordant immune response</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>DTH</td>
<td>Delayed type hypersensitivity</td>
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<td>DVR</td>
<td>Discordant virological response</td>
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<td>EAP</td>
<td>Expanded access programme</td>
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<td>EDTA</td>
<td>Ethylenediamine tetraacetic acid</td>
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<td>FITC</td>
<td>Fuorescein isothocyanate</td>
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<td>FLU</td>
<td>Influenza A</td>
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<td>Acronym</td>
<td>Definition</td>
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<td>GALT</td>
<td>Gastrointestinal associated lymphoid tissue</td>
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<td>HAR</td>
<td>High affinity receptor</td>
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<tr>
<td>Het</td>
<td>Heterosexual</td>
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<td>HIV-1</td>
<td>Human immunodeficiency virus type 1</td>
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<td>HPA</td>
<td>Health Protection Agency</td>
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<td>HSV</td>
<td>Herpes simplex virus</td>
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<td>HTL</td>
<td>Helper T-lymphocyte</td>
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<td>IAR</td>
<td>Intermediate affinity receptor</td>
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<td>ICL</td>
<td>Imperial College Laboratories</td>
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<td>IDU</td>
<td>Intravenous drug user</td>
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<td>IL-2</td>
<td>Interleukin-2</td>
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<td>IQR</td>
<td>Inter-quartile range</td>
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<td>IRC</td>
<td>Immune Response Corporation</td>
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<td>IRIS</td>
<td>Immune restoration inflammatory syndrome</td>
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<td>KS</td>
<td>Kaposi’s Sarcoma</td>
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<td>LAK</td>
<td>Lymphokine-activated killer cells</td>
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<td>LAR</td>
<td>Low affinity receptor</td>
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<tr>
<td>LCA</td>
<td>Leukocyte common antigen</td>
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<td>LPR</td>
<td>Lymphoproliferative response</td>
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<td>LTFU</td>
<td>Lost to follow up</td>
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<td>LTNP</td>
<td>Long term non-progressors</td>
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<td>MAC</td>
<td>Mycobacterium avium complex</td>
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<td>MFI</td>
<td>Mean fluorescence intensity</td>
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<td>MHC</td>
<td>Major histocompatibility complex</td>
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<td>MIU</td>
<td>Million international units</td>
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<td>MSM</td>
<td>Men who have sex with men</td>
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<td>MTB</td>
<td>Mycobacterium tuberculosis</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>NK</td>
<td>Natural killer cells</td>
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<tr>
<td>NA</td>
<td>Nucleoside analogues</td>
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<tr>
<td>NHL</td>
<td>Non-Hodgkin’s Lymphoma</td>
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<tr>
<td>NNRTI</td>
<td>Non-nucleoside analogues</td>
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<tr>
<td>OI</td>
<td>Opportunistic infections</td>
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<tr>
<td>PBMC</td>
<td>Peripheral Blood Mononuclear Cells</td>
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<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
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<tr>
<td>PCL</td>
<td>Primary cerebral lymphoma</td>
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<td>PCP</td>
<td><em>Pneumocystis carinii</em> pneumonia</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PC5</td>
<td>PE-cyanine 5</td>
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<td>PE</td>
<td>Phycoerythrin</td>
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<tr>
<td>PJP</td>
<td><em>Pneumocystis jirovei</em> pneumonia</td>
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<td>PHI</td>
<td>Primary HIV-1 infection</td>
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<tr>
<td>PI</td>
<td>Protease inhibitor</td>
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<tr>
<td>PPD</td>
<td>Purified protein derivative</td>
</tr>
<tr>
<td>PWM</td>
<td>Pokeweed mitogen</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
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<tr>
<td>QOL</td>
<td>Quality of life</td>
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<tr>
<td>RCT</td>
<td>Randomised control trial</td>
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<tr>
<td>rIL-2</td>
<td>Recombinant human interleukin-2</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SE</td>
<td>Standard error</td>
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<tr>
<td>SI</td>
<td>Stimulation index</td>
</tr>
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<td>sIL-2</td>
<td>Soluble IL-2 receptors</td>
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<td>SIM</td>
<td>Surrogate immune markers</td>
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<td>STI</td>
<td>Structured treatment interruption</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>TCR</td>
<td>T cell receptor</td>
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<td>TF</td>
<td>Treatment failure</td>
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<tr>
<td>TH1</td>
<td>T helper type 1</td>
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<tr>
<td>TH2</td>
<td>T helper type 2</td>
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<td>TRECS</td>
<td>T-receptor excision circles</td>
</tr>
<tr>
<td>Tregs</td>
<td>Regulatory T cells</td>
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<td>TS</td>
<td>Treatment success</td>
</tr>
<tr>
<td>TTOX</td>
<td>Tetanus toxoid</td>
</tr>
<tr>
<td>VL</td>
<td>Viral load</td>
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<tr>
<td>VLBD</td>
<td>Viral load below the level of detection</td>
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<tr>
<td>VZV</td>
<td>Varicella zoster virus</td>
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<td>WB</td>
<td>Western Blot</td>
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### 3.4 Study Two: Restoration of HIV-1 specific responses in patients changing from protease to non-nucleoside reverse transcriptase inhibitor based antiretroviral therapy

### 3.5 Summary

## RESULTS: IL-2 therapy in the context of cART

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### 4.2 Sub-study - Interleukin-2 associated viral breakthroughs induce HIV-1-specific CD4 T-cell responses in patients on cART

### 4.3 Summary

## RESULTS: IL-2 in the absence of fully suppressive cART – does it have a role?

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## REFERENCES

## WORK ACCEPTED FOR PUBLICATION AND PRESENTATIONS
CHAPTER ONE: INTRODUCTION AND BACKGROUND

1.1 Human immunodeficiency virus type 1 (HIV-1) and the immune response

1.1.1 Introduction and epidemiology

The Human immunodeficiency virus type 1 (HIV-1) causes immune dysregulation with hyperactivation and immune exhaustion leading to quantitative and qualitative immune deficiency. Without treatment HIV-1 infection leads to the acquired immunodeficiency syndrome (AIDS) and death. There have been significant advances over the past 30 years of the pandemic in the understanding of HIV-1 immunopathogenesis and in developing effective treatments, in the form of combination antiretroviral therapy (cART), resulting in significant reductions in both the morbidity and mortality associated with HIV-1 (Pallela et al., 1998, Lederman et al., 2000). Although treatment is becoming increasingly available in developing nations, the main treatment benefits to date have been seen in the developed world (UNAIDS). Understanding the mechanism of infection, the subsequent immune damage and the treatment associated reconstitution within the immune system, as well as identifying the persistent uncorrected immunodeficiencies should enable more effective and novel therapeutic interventions in those already infected, and may lead to potential preventative vaccination targets.

In the UK there are estimated to be 83,000 people living with HIV-1 (HPA, 2010). However up to one quarter are unaware of their status, and more than one quarter present with advanced disease (HPA, 2010). Those unaware of their status are therefore unable to access the therapeutic interventions they require and there remains a significant number of patients presenting with advanced immunological deficiency which may not be completely recoverable.
Two types of HIV infect humans - type 1 which accounts for the majority of infections world wide and type 2 which is seen primarily in western Africa and has a more indolent clinical course. HIV-1 is comprised of various clades and subtypes which have specific geographical distribution (Figure 1.1). Clade B predominates in the western world. Such viral variation is likely to have important implications for vaccine development.

Figure 1.1 Global distribution of HIV-1 clades (IAVI report, August 2003)
1.1.2 Virology of HIV-1

HIV-1 is a retrovirus consisting of an outer envelope, a nucleocapsid layer, two identical strands of RNA, viral enzymes and proteins (Figure 1.2). There are 9 viral genes - the structural genes - env, gag, pol, the regulatory genes - rev, nef, tat and the accessory genes - vpr, vpu, vif (Subbramanian et al., 1994, Frankel et al., 1998, Seelamgari A et al., 2004).

![Figure 1.2 Structure of HIV-1 virion](image)

HIV-1 can infect human T-cells, dendritic cells and macrophages. The understanding of the process whereby HIV-1 enters the cell has increased considerably in recent years. The virus enters its target cell by fusing its envelope with the cell membrane, the viral initiating glycoproteins responsible are gp120 and gp41 (Eckert et al., 2001, Markovic et al., 2004). Initially thought to be the primary cell receptor for HIV-1, the CD4 receptor is now considered to play an accessory role to the chemokine receptors CCR5 (on dendritic cells, macrophages and T-cells) and CXCR4 (on activated T-cells) (Moore et al., 1997, Alkhatib et al., 2007). These G protein coupled
receptors interact with gp 120 in the presence of CD4 and determine factors such as viral tropism; CCR5-R5 virus, macrophage-tropic and seen throughout infection and the lymphocyte-tropic CXCR4-X4 virus emerging with disease progression. Their ligands (CCR5: MIP-1α, MIP-1β, RANTES and CXCR4: SDF-1) can block HIV-1 entry and mutations in the receptor genes can afford protection against infection (homozygous 32Δ) or slow disease progression (heterozygous 32Δ) (Blanpain et al., 2002). Viral tropism for these receptors now has implications for treatment with the recent development of receptor antagonists such as maraviroc.

HIV-1 production rate is extremely high, although initially thought to be in the order of $10^9$–$10^{10}$ virions per day (Ho et al., 1995, Perelson et al., 1996, Mohri et al., 1998), subsequent work has downgraded this estimate to $10^3$ virions per day (Walker et al., 1998, Rosenzweig et al., 1998). The HIV-1 virus has a very high level of transcription errors which, when combined with its high replication rate, results in the daily production of multiple mutations, many of which are less fit than wild type or non-viable. Immune escape and subsequently, drug resistance tends to emerge in those patients on therapy (drug pressure) with incomplete viral suppression (Fischer et al., 2010, Delaugerre, 2010).

1.1.3 Immune response to infection

This brief overview is aimed to highlight aspects of the response to infection particularly relevant to this thesis and is in no way meant to represent the entirety or complexity of the immune response.

Immature T lymphocytes, produced by the bone marrow, migrate to the thymus where positive and negative selection and T cell receptor (TCR) rearrangement result in the production of naive T-cells (CD45RA+CD62L+). A
by-product of this process is the production of T receptor excision circles (TRECs) and high levels within a cell are markers of recent thymic migration (Douek et al., 1998). Exposure of the naive T-cell to antigen via antigen presenting cells (APC, e.g. dendritic cells) causes transformation to memory/effector T-cells (CD45RO+).

Infection causes an innate immune response followed by an adaptive immune response, which can be either predominantly type 1 or type 2. Type 1 (TH1) response is largely cell mediated immunity (CMI) and is the immune system’s response to viral and other intracellular infections. CMI requires the interaction of APC, CD4 T-cells and CD8 T-cells and is further characterised by specificity and memory. Cytotoxic T-lymphocytes (CTL) kill the infected cell but there is evidence that in chronic viral infections this also requires virus-specific T-helper cells (Zajac et al., 1998). Type 2 (TH2) response is predominantly an antibody response., also known as the humoral immune response. Both responses are associated with specific cytokine profiles; TH1 predominantly with IL-2, -12 and IFNγ and TH2 with IL-4, -6 and -10. The development of one type of response tends to inhibit the development of the alternative response. This is of particular importance in the context of HIV-1 infection, where with disease progression TH2 response tends to dominate and thereby inhibits the more effective antiviral TH1 response (Clerici et al., 1993).

Viral infections generate a CTL response which inhibits viral replication by direct killing and the release of soluble antiviral factors. Direct killing results from the infected cell releasing granzymes and perforin which, along with antiviral factors, are found in CTL secretory granules. This process results from the APC processing the viral antigen and, in the context of MHC class I, interacting with the CTL TCR (Yang et al., 1996, Wagner et al., 1998).
Additionally soluble antiviral factors are released. In HIV-1 infection these include β chemokines (eg. RANTES, MIP-1α and MIP-1β) which interact with the chemokine receptors and prevent further infection of CD4 T-cells. The phenomenon of class I restriction has important implications for an individual’s ability to mount an effective immune response as it determines the viral peptides presented to the TCR. This impacts on the possibilities for immune manipulation and may well limit its potential as a sustainable therapeutic intervention. In addition to CTL there is generation of virus specific T-helper cells which occurs following antigen presentation by the APC in the context of MHC class II. These T-helper cell responses are essential for the maintenance of CTL. T-help probably consists of a combination of direct cell-cell interaction and the release of cytokines such as IL-2 (Smith et al., 2008).

1.1.4 T-Lymphocyte surface receptors

All these immunological processes occur subsequent to various cell surface receptor/molecule interactions. The presence of specific receptors or molecules on a cell surface identifies a cell’s functions, capabilities and to a certain extent history and previous contacts (Appay et al., 2008). There are numerous surface receptors with a wide variety of functions. Described below are those relevant to this thesis.

1.1.4.1 CD3

The CD3 molecule is expressed on mature T-lymphocytes and some thymocytes. This 16-25 kDa molecule consists of 5 polypeptide chains and is an integral part of the TCR complex. It participates in antigen recognition (MHC I and II bound peptides) and T-cell activation (van Agthoven et al., 1981). Normal ranges quoted vary from approximately 65-78% of peripheral
blood mononuclear cells (PBMC) being CD3 positive (CD3+) to 55-84% (Bisset et al., 2004). In HIV-1 infection signalling across the TCR/CD3 receptor may be affected by the virus (Iafrate et al., 1997).

The presence of CD3 receptors on cells was used in the phenotypic analyses detailed in this thesis to enhance identification of the T-cell population for further analysis.

1.1.4.2 CD4

The CD4 molecule is present on T-cells (approximately 45% of PBMC), thymocytes (80%, co-expressed with CD8 on immature cells) (Micelli et al., 1991), eosinophils, dendritic cells and macrophages (100%, but at a lower density compared to T-cells) (Hannet et al., 1992). This 59 kDa transmembrane glycoprotein is a member of the Ig superfamily. It has an extracellular domain, which with the TCR complex interacts with the MHC class II molecule on the APC (Sakihama et al., 1995), enabling antigen recognition and subsequent T-cell activation (Smith-Garvin et al., 2009). Its intracellular domain causes the activation of the protein tyrosine kinase Lck, initiating transcription and cellular activation (Ryan et al., 1995). The normal range of CD4+ T-cells in the peripheral blood of an uninfected individual is 450-600 cells/µL, 35 - 45% (Imperial College Laboratories (ICL), London 2010), with different ranges given by other authors; 309 -1139 cells/µL, 32.5 – 63.0% (Bisset et al., 2004) and 715–1085 cells/ µL (Autran et al., 1997).

The CD4 molecule is a receptor for the gp120 molecule of HIV-1, binding it with high affinity (Berger et al., 1999), with subsequent down regulation of CD4 expression. MHC-class II dependent binding requires CD4 tetramerisation, whereas only single CD4 molecules are required for gp120 binding. Additionally, nef has been shown to induce endocytosis and degradation of CD4 in lysosomes and to disrupt the association of CD4 with
Lck (Anderson et al., 1993, Rhee et al., 1994, Aiken et al., 1994, Salgheetti et al.,
1995, Iafrate et al., 1997). There is low level expression of CD4 on some
mature, activated CD8+ T-cells peripherally which may explain CD8 T-cell
infection by HIV-1 (Kitchen et al., 1998). CCR5 interaction only requires low
density CD4 expression, such as is found on macrophages, however CXCR4
requires high density, as seen on activated T-cells, and this may explain some
of the viral tropism seen in HIV-1 disease.

In HIV-1 infection the number and percentage of CD4+ T-cells decrease with
time. In the majority of patients effective cART results in an increase in CD4 T-
cells often to within the normal range. Additionally exogenous IL-2 increases
the number of CD4 T-cells.

1.1.4.3 CD8

The CD8 molecule is present on CTL and some NK cells, but at a lower density
(Terry et al., 1990). This molecule consists of an α and a β chain, 32-34 kDa
each. The extracellular domain of the CD8 receptor interacts with the MHC
class I molecule on APC (Salter et al., 1990). Its intracellular domain also
interacts with the protein tyrosine kinase Lck (Anderson et al., 1993), initiating
cellular activation.

The normal range of CD8+ lymphocytes is 190-1210 cells/µl (15-50%) (ICL),
with other reference ranges being 137 – 823 cells/µl, 11.6-38.6% (Bisset et al.,
2004) and 385-715 cells/µL (Autran et al., 1997). The number of CD8+ T-cells
increases in response to any viral infection, including HIV-1. HIV-1 infection
has additionally been shown to down-regulate the expression of CD8 on
infected cells (Appay et al., 2000). The number of CD8 T-cells also declines
with disease progression, albeit at a later stage compared to the decline in
the CD4 T-cell count, and the initiation of cART tends to return the ratio of
these cells towards normal.
1.1.4.4 Naive and memory T-cell surface markers – 45RA, 45RO and 62L

The CD45 molecule, previously known as the leukocyte common antigen (LCA) is expressed as isoforms with different molecular weights. CD45RA is the 220 kDa isoform, incorporating exons A, B and C, and is expressed on a subset of T-cells, B cells and monocytes. The 180 kDa isoform, lacking these exons, is CD45RO, found on 70% CD4+ T-cells, 35% CD8+ T-cells, thymocytes and monocytes (Janeway, 1992, Wills et al., 1999). There is a sub-population of T-cells which co-express both CD45RA and CD45RO (Zola et al., 1992, Prince et al., 1992). CD45 regulates T-cell responses to antigens, influencing activation (De Jong et al., 1991). CD45RA T-cells are also referred to as naïve cells, and CD45RO T-cells as memory or effector cells (Wills et al., 1999) When CD45RA+ T-cells interact with an antigen they become activated, express CD45RO and down regulate CD45RA. These CD45RO+ cells can subsequently respond to recall antigens. CD45RO facilitates CD4 molecule grouping on the T-cell surface, enabling interaction with APC (Janeway, 1992), as this requires CD4 tetramerisation. The proportion of CD45RO+ cells increases with age (Wills et al., 1999). Reversion from CD45RO+ to CD45RA+ has been documented within both the CD8+ and CD4 T-cell populations (Appay et al., 2008).

In HIV-1 infection the CD45RO T-cell population declines throughout the course of the disease. This is reflected in the gradual loss of response to recall antigens and mitogens as would be expected with ongoing loss of effector cells. It correlates well with the observed clinical course. With disease progression and loss of these effector cells individuals become increasingly susceptible to various opportunistic infections, including those to which they had previously mounted an adequate immune response.
cART results in an early rapid increase in CD45RO+ T-cells, a significant proportion of which is probably due to redistribution. This is followed by an increase in CD45RA cells in which thymic contribution probably plays a role (Autran et al., 1997, Pakker et al., 1998), along with peripheral expansion and decreased apoptosis. This is reflected clinically in improved protection against opportunistic infections, permitting the cessation of primary and secondary prophylaxis (El-Sadr et al., 2000), and the occurrence of immune reconstitution inflammatory syndrome (IRIS) (Zegans et al., 1998, Shelburne et al., 2003, Antonelli et al., 2010).

CD62L is an adhesion molecule expressed on the surface of T and B lymphocytes, which can be used in addition to CD45RA to identify the naïve cell pool, as it is expressed on unstimulated cells (Hannet et al., 1992) and interaction with antigen causes down regulation (Springer et al., 1994). 62L binds to the high endothelial venules in the lymph nodes, allowing naïve cell recirculation (Bevilacqua et al., 1993).

Quoted normal ranges for naïve and memory CD4+ and CD8+ T-cells (Bisset et al., 2004):

<table>
<thead>
<tr>
<th>Type</th>
<th>CD4/CD45RA/62L+</th>
<th>CD8/CD45RA/62L+</th>
<th>CD4/CD45RO+</th>
<th>CD8/CD45RO+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naïve</td>
<td>84-761 cells/µL</td>
<td>42-360 cells/µL</td>
<td>247-807 cells/µL</td>
<td>72-377 cells/µL</td>
</tr>
<tr>
<td>Memory</td>
<td></td>
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<td></td>
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</tbody>
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1.1.4.5 Activation markers – HLA-DR and CD38

HLA-DR (class II, 29-33 kDa) is expressed on B-cells, activated T-cells (Hannet et al., 1992) and monocytes. It is involved in the interaction between lymphocytes and macrophages, T and B cells and in cytotoxicity (Sonderstrup et al., 1998).
The hyperactivation of the immune system associated with HIV-1 infection results in a multitude of changes, one of which is an increase in HLA-DR expression (Douek et al., 2009). CD8+HLA-DR+ cells have decreased proliferative capacity (Pantaleo et al., 1990) and the increased levels are associated with T:T presentation leading to a state of anergy (Lombardi et al., 1997). Initiation of cART with subsequent control of viraemia leads to down regulation of HLA-DR.

The normal range of CD4/HLA-DR+ T-cells is 11-55 cells/µL, 0.8-4.4%, and CD8/HLA-DR is 6-108 cells/µL, 0.3-6.4% (Bisset et al., 2004).

The CD38 molecule is 45 kDa and is expressed on the surface of activated T-cells and plasma cells. It is involved in the activation and regulation of T and NK cells (Funaro et al., 1990). As with HLA-DR, high levels of expression reflect hyperactivation of the immune system as occurs in HIV-1 infection. It has been identified as a surrogate marker of disease progression. Initiation of cART results in decreased expression on T-cells.

The normal range of CD4/CD38+ T-cells is 69-547 cells/µL, 6.1-32.2%, and CD8/CD38+ is 13-124 cells/µL, 0.9-7.0% (Bisset et al., 2004).

1.1.4.6 Co-receptor CD28

CD28, a 44 kDa molecule, is expressed on the majority of CD4 T-cells and on 50% of CD8 T-cells (Lenschow et al., 1996). It binds with CD80 and CD86 on APC (Wang et al., 2004, Corthay, 2006), providing the essential signal 2 in T-cell activation. This stabilises the mRNA for IL-2 transcriptional factors, enhances mRNA production and increases cell surface IL-2 receptor expression (Barker et al., 1997). This co-stimulatory signal plays a role in T-cell differentiation and proliferation (Lenschow et al., 1996), maintenance of peripheral tolerance (Schwartz et al., 1996, Chambers et al., 1997) and the prevention of apoptosis. Its deficiency results in T-cell anergy.
There is progressive loss of CD28 expression on T-cells in HIV-1 infection, being most marked in the CD8+ population. This down regulation correlates with disease progression, decline in CD8+ T-cell antiviral activity (Landay et al., 1993, Barker et al., 1997, Imami et al., 1999), and increased viral replication (Haffar et al., 1995).

1.1.4.7 IL-2 receptors

Interleukin-2 receptors are discussed in section three of the Introduction.

1.1.5 Immune response to HIV-1 infection

1.1.5.1 Cell mediated immune response to HIV infection

HIV-1 infection results in both quantitative and qualitative immune dysfunction, characterised by hyper-activation and immune exhaustion (Gea-Banacloche et al., 1999). Understanding the immunopathology of HIV-1 infection and the effects (beneficial and partial) of cART on the immune system will guide identification of potential targets for immune therapy. It would appear with our current therapies viral eradication is not yet possible, largely due to viral latency in long term effector cells, and the next therapeutic breakthrough may be through manipulation of the immune system. The immune system plays a key role in determining the viral set point and disease progression, and examination of immune parameters in different clinical cohorts in regards to disease progression can also inform these potential strategies.

In primary HIV-1 infection (PHI) HIV-specific CD8 CTL and CD4 T-helper lymphocytes (HTL) are produced which initially control viral replication and determine the viral set point (Koup et al., 1994, Borrow et al., 1997, Goulder et al., 1997, Price et al., 1997, Kent et al., 1997). Depletion of CTL prior to SIV infection in macaques causes loss of this initial viral control (Matano et al.,...
The high replicative and mutational rate of HIV-1 results, over time, in viral evolution and the emergence of viral swarms and quasi species able to evade the immune response. Studies suggest that CTL are subsequently deleted in the presence of the resulting persistent high viral load (Moskophidis et al., 1993). This deletion may also represent a qualitative deficiency as well as a quantitative reduction, with persistence of some dysfunctional or anergic cells. (Goulder et al., 1999). The activated CD4 T-cells are prey to this elevated viral load (Schnittman et al., 1990) and increased rates of infection, dysfunction and destruction result, with a degree of HTL dysregulation also occurring prior to the actual decline in numbers of CD4 T-cells. It is this loss of strong HIV-1 specific helper T-cell responses that is the most marked deficiency in advanced disease (Miedema et al., 1994). This loss of HTL function would impair events downstream, including activation of CTL. Another mechanism may be cells undergoing activation-induced cell death in the presence of an high antigen load (Abbas et al., 1996). Therefore with early intervention in PHI we see enhanced HIV-1 specific T-helper cell function (Rosenberg et al., 1997, 1998, Al-Harthi et al., 2000, Oxenius et al., 2000, Malhotra et al., 2000, Deeks et al., 2000). If cART is commenced in chronic infection reduced HIV-specific immunity, especially CTL, is observed (Kalams et al., 1999, Ogg et al., 1999, Markowitz et al., 1999). This is presumably due to lack of ongoing antigenic stimulation required to maintain these responses, and this loss may be accelerated by lack of T-help. This qualitative immune deficiency may also be partly related to IL-2 dysregulation and activation induced anergy. During HIV-1 infection a reduction in the production of IL-2 is observed. This has been shown to occur in the early stages of infection with production becoming more limited during progression of the disease (Gruters et al., 1998, Jin et al., 1999, Schmitz et al., 1999).
1990, Shearer et al., 1992, Barker et al., 1997). It is likely this phenomenon is related to a switch from a TH1 to a TH2 immune response, in which the predominant cytokines change from IL-2 to IL-4 and IL-10 which in turn further inhibit IL-2 production (Clerici et al., 1994, Barker et al., 1997).

Furthermore, early in HIV-1 infection there is migration of virus to the gastrointestinal associated lymphoid tissue (GALT) with subsequent infection and marked loss of CD4 T-cells (Brenchley et al., 2004, 2006). This allows translocation of microbial antigens, resulting in generalised immune system activation (Brenchley et al., 2006). This persists in chronic infection as the loss of GALT associated CD4 T-cells is not restored by cART, and thus the associated immune system activation remains uncorrected.

The resulting chronic immune system activation is reflected in high levels of CD4 and CD8 T-cell activation markers (e.g. CD38, HLA-DR) seen in chronic HIV-1 infection (Liu et al., 1997), which correct towards those levels seen in HIV-1 uninfected individuals but do not completely normalise on cART initiation.

The ability of an individual to mount an effective immune response to HIV-1 is likely to be genetically determined to a significant extent (Hill, 1998) and therefore has significant bearing on the prospect of sustainable therapeutic immune system manipulation. This has important clinical ramifications as viral load is a primary determinant of disease progression and, as early as six months post PHI, is highly predictive of subsequent disease progression (Lyles et al., 2000). The resultant level of immune system activation is as important, if not more so, in predicting disease outcome (Liu et al., 1997, Douek et al., 2009). Despite a degree of initial HIV-1 control however, the majority of individuals experience progressive immune dysfunction with resultant immunodeficiency, progressing to AIDS and death. With chronic infection,
the ongoing high viral replication and associated immune activation results in immune exhaustion. The subsequent increase in viral replication is due to both viral and host factors.

In many other viral infections the immune system mounts an effective protective response, sufficient to contain, but not eradicate the virus. However this does not occur in the majority of patients infected by HIV-1. There is a small group of individuals who are able to control the virus and protect their immune system for prolonged periods of time (Deeks et al., 2007). These individuals, known as long term non-progressors (LTNP) are an important source of data regarding those factors critical to viral control. LTNP are defined as individuals infected by HIV-1 for at least 10 years, with a CD4 T-cell count >500 cells/µL and a viral load <1000 copies/ml in the absence of antiretroviral therapy. LTNP account for <1% of individuals infected with HIV-1 but reports indicate that all individuals will eventually progress (Westrop et al., 2009, Blankson, 2010). It is likely that the partially protective immune response these individual mount is genetically determined. Immunologically these individuals have broad, strong virus specific CTLs (Harrer et al., 1996, Gea Banacloche et al., 2000), strong virus specific HTLs (Rosenberg et al., 1997, 2000), high levels of chemokines, low levels of activation markers and, in some cases, neutralising antibodies. In some LTNP strong virus specific CTL responses have been identified 20 years post PHI. In effect the LTNP immune response is characterised not only by its strength but also by its breadth, which is likely to be highly genetically determined (Valdez et al., 2002). The breadth of CTL and HTL better enables the patient to deal with the increasingly diverse HIV viral swarms. LTNP in some cases have shown the presence of a strong response to env and gag proteins (Riviere et al., 1995, Yang et al., 1996, Rosenberg et al., 1997, 2000, Pontesilli et al., 1998, Ogg et
al., 1998, Oxenius et al., 2000, Malhotra et al., 2000) with an associated negative correlation with viral load (Kalams et al., 1999). LTNP have higher levels of β chemokines (Gruters et al., 1990, O'Brien et al., 1998) which have a direct antiviral effect and will protect against cellular infection to a degree. Delineating LTNP’s immune system characteristics has identified possible therapeutic goals for immune manipulation. The vital role of an advantageous genetic profile somewhat dampens the optimism regarding these as targets for immunotherapy with sustainable outcomes. Other host genetic factors such as polymorphisms in chemokine co-receptors are associated with different rates of disease progression (Smith et al., 1997, Martin et al., 1998, O’Brien et al., 1998). HLA alleles also have associations with the rate of disease progression, which is likely to be related to the ability of the host to present different viral epitopes. There are also viral-determined factors which influence disease progression and potentially affect both immune and treatment responses. With progression of disease, viral phenotype switches from non syncitial inducing (NSI) to syncitial inducing (SI) and from CCR5 to CXCR4, with implications for tropism and fitness. Other viral related factors include the nef induced down-regulation of HLA class I expression (Schwartz et al., 1996, Le Gall et al., 1998) reducing the effective immune presentation of antigen. The existence of viral reservoirs is another important factor in the persistence of disease and the present pessimism regarding eradication. Soon after HIV-1 infection a population of CD4 T-cells that harbour HIV-1 in a transcriptionally silent state are generated (Pierson et al., 2000). The virus in these cells is in a post integration latent state and therefore not susceptible to immune recognition. Activation of these cells would expose them to attack but would also allow them to seed other susceptible cells, re-establishing the reservoir.
Several recent studies of IL-2 therapy have addressed the possibility of flushing these reservoirs but this approach has not been shown to be effective to date. Recent drug developments address this viral latency with a view to eradication. It is now recognised these cells are the more likely source of low level viral blips observed clinically, rather than on-going low level viral replication (Kelleher, 2008).

The natural history of HIV-1 infection is therefore one of increasing viral load and diversity causing immune system hyperactivation and thus decreasing T-cell number and function, both CD4 and CD8, through direct killing, activation induced cell death and anergy.

1.1.5.2 Humoral immune response to HIV infection

The other arm of the adaptive immune response to HIV, referred to as the humoral response, results in the generation of HIV specific antibodies (Ab). There are several main Ab categories or processes; binding antibodies (bAb), neutralising antibodies (nAb), antibody-dependent cellular cytotoxicity (ADCC) and complement binding antibody.

Antibodies are immunoglobulins produced by B cells and can be innate or antigen specific. The humoral response is closely interlinked and dependent upon cell mediated immunity; CD4 T-cells are integral to this via essential helper/inducer function; achieved by cell to cell contact and local release of regulator cytokines (e.g. IL-2, IFN-gamma). Antibodies are key components in host defences. They are seen as being of increasing importance in HIV vaccine research and development, given the lack of success to date of vaccines capable of inducing either therapeutic or protective cell mediated immunity to HIV.

HIV Ab are produced within 1-2 weeks of acute infection; they circulate in the blood and are secreted at mucosal surfaces. The kind of activity an
antibody has depends on its isotype; light chain specificity and heavy chain class, and this can also be influenced by the cytokine milieu. The specificity of anti-HIV antibodies evolves over time with gag responses appearing first followed sequentially by nef, rev and finally env, and a stable peripheral B cell repertoire is established over the first year of infection. IgG appears first and is followed by the production of IgA. Following HIV infection B cell dysfunction is seen with polyclonal activation, hypergammaglobulinaemia and impaired primary and secondary responses (innate and adaptive).

**Binding Antibodies**

Binding antibodies (bAbs) may or may not neutralise HIV infectivity but could potentially be protective as suggested by the finding of a degree of protection in a recent Thai vaccine trial (Rerks-Ngarm et al., 2009) where high levels of bAb were seen in vaccinated volunteers, and fewer vaccinated individuals had nAb compared to bAb.

**Neutralising Antibodies**

B cells produce specific Ab in response to HIV which bind to the virus and neutralise it. The neutralising Ab (nAb), usually IgG, are specific for the HIV envelope glycoproteins gp120 and gp41, particularly the CD4 binding region and co-receptor binding site of gp120. Those Ab directed against gp120 tend to be clade specific, whereas gp41 Ab tend to have wider interclade activity. There are both conformational and continuous determinants within envelope, and the response appears to evolve over time. nAb are often only specific for the initial native virus and the loss of cross or broad reactivity is now thought to be more closely related to conformational change in gp120 following sequential binding to CD4 and co-receptors. High antibody titres are required for neutralisation as any protein left unbound can bind to target cells and initiate fusion and thence infection. High levels of nAbs against
common laboratory strains are frequently encountered, however levels of nAb directed against an individual’s own virus are less consistent reflecting a loss of effective reactivity. nAb are able to prevent infection of susceptible cells but are ineffective once infection has occurred.

Antibody Dependent Cellular Cytotoxicity (ADCC or enhancing Antibodies)

Antibodies to viral env can also induce ADCC which is mediated by NK cells and macrophages. HIV specific Ab (IgG1) bind to infected cells which express the viral epitopes gp120 and gp41 on their surface. The Fc region of this bound Ab then binds to NK cells or macrophages via IgGFc or complement receptors, and induces ADCC. Two mechanisms mediate ADCC: perforin or cytolyisin produced by the NK cell bind to the infected cell’s surface membrane resulting in transmembrane channel formation and osmotic death, and apoptosis. With HIV disease progression there is progressive dysfunction of NK cells which, combined with immune escape variants (conformational) that emerge from effective ADCC responses, are likely to result in a decline in ADCC responses in chronic HIV infection. Of note ADCC activity is higher in LTNP, and the potential to induce or restore this activity is a major focus of current vaccine development.

1.1.6 The clinical course of HIV-1 infection and treatment response

HIV-1 infection is characterised in the majority of cases by a prolonged clinically latent state. With disease progression profound immunodeficiency ensues with resultant opportunistic infections (OI) and malignancies and the development of AIDS. The infection is, without treatment, ultimately fatal. This immunodeficiency is, at least in part, reversible with cART and its introduction has led to a dramatic decrease in HIV-1 related morbidity and mortality in the developed world.
In routine clinical care assessment of an individual’s immune function tends to be a combination of clinical observation and measuring surrogate immune markers (SIM) such as CD4 T-cell counts. Quantification of CD4 bearing lymphocytes was the first reliable predictor of progression of HIV-1 infection to AIDS or death, with CD4+ T-cell counts <200 cells/μl blood being one of the definitions for AIDS. The significance of the CD4 T-cell count in HIV-1 disease is of great importance, however the full significance of the measured level is still incompletely understood, as undoubtably both qualitative and quantitative factors are at play. More in depth immunological assessment can be performed, particularly measuring T-cell responses (proliferation, cytokine production etc.) and T cell phenotype, although there is as yet little evidence of direct clinical correlation, nor evidence that manipulation has resultant clinical benefit. The effects of cART, via virological suppression, on the immune system can be observed through phenotypic analyses of cell surface markers. Significant changes have been seen four weeks post treatment (Autran et al., 1997, Bisset et al., 1998), with increases in memory (CD4+CD45RO+) T-cells and decreases in activated lymphocytes (CD4+HLA-DR+). Such measurements may assist the evaluation of the immune reconstitution that ensues post cART, although functionality is also important, some of which can be inferred by phenotypic changes but not completely. Measurement of activated CD8 T-cells (e.g. CD8+CD38+) is a predictor of the development of AIDS and of disease outcome (Liu et al., 1997, Douek et al., 2009).

The clinical course is an indicator of immune decline as different OI and malignancies tend to occur at ‘typical’ CD4 T-cell counts. Another clinical correlate of immune function is to measure delayed type hypersensitivity (DTH) as an indicator of overall immune function, but it is now infrequently
utilised in clinical practice. It monitors mainly T-helper responses and typically decreases with increasing progression of disease. DTH responses have been demonstrated to recover with both cART and IL-2 therapy.

1.2. cART in Chronic HIV-1 Infection

1.2.1 Background

Since the introduction of cART there has been a dramatic decrease in the morbidity and mortality associated with HIV-1 infection for those individuals with access to therapy (Palella et al., 1998, Lederman et al., 2000). Whilst it has undoubtedly saved countless lives, its widespread, long term clinical use has resulted in the initial optimism regarding its success being tempered with concerns regarding side effects, toxicity, and the emergence of resistance. Spiralling costs of life time therapy and the management of associated complications in HIV-1 infected individuals have increasing implications for costs, healthcare capacity and individual health with the continuing improvement in survival of an aging cohort. These factors have combined to encourage investigation into alternative and adjunctive therapies and different ways of administering cART to minimise side effects and costs. Such approaches range from simple switching of therapies to overcome side effects and avoid potential toxicities, to structured treatment interruptions (STI), to the use of novel therapies such as cytokines and therapeutic vaccinations. Successful alternatives to life long, continuous, triple therapy may additionally expand the number of people world-wide able to access therapies. Much interest has been focused on the role of cART in primary HIV-1 infection (PHI) with a view to elimination of the virus or, failing that, influencing the viral set point and hence improving an individual’s prognosis. In several settings this has been attempted with STIs, although data concerning emerging resistance, the possible damage resulting from
increased immune activation and lack of demonstrable benefit, has led to caution regarding this approach. The role of cART in PHI however is outside the scope of this thesis and the focus of this section will be cART in chronic HIV-1 infection. Within this patient group ongoing research is taking place regarding the optimum time to commence therapy, with a shift back towards support for the mantra ‘hit early and hit hard’. This is in recognition of the fact that even fully suppressive cART does not allow complete immune reconstitution and that the prevention of damage may indeed be the correct goal rather than treatment after immune damage has occurred. The long term and as yet ill-defined consequences of chronic generalised hyperactivation of the immune system support this approach. The increased event rate in the SMART study (El-Sadr et al., 2008) in those patients who interrupted treatment lends further credence to starting therapy at diagnosis or with a significantly higher CD4 T-cell count than is currently recommended. Recent evidence also suggests this approach may have significant public health benefit via reduced transmissions through reduction in both individual and community viral load (CVL). The currently recruiting international START study should provide more evidence to guide this decision at an individual level. Current UK guidelines for commencing therapy using SIM as a guide recommend a CD4 T-cell cell cut off of 350 cells/µL (Gazzard et al., 2008).

1.2.2 Classes of antiretroviral drugs

There are currently 7 classes of antiretroviral (ARV) drugs and 22 licensed drugs available in the UK for the treatment of HIV-1 infection (Table 1.1). These drugs act at distinct points in the HIV-1 life cycle (Figure 1.1)
Table 1.1 Antiretroviral therapy licensed in the United Kingdom

<table>
<thead>
<tr>
<th>CLASS</th>
<th>DRUG</th>
<th>Abbreviation</th>
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<tr>
<td>Nucleoside analogues</td>
<td>Zidovudine</td>
<td>AZT</td>
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<tr>
<td></td>
<td>Didanosine</td>
<td>ddI</td>
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<td></td>
<td>Abacavir</td>
<td>ABC</td>
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<td></td>
<td>Stavudine</td>
<td>d4T</td>
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<td></td>
<td>Lamivudine</td>
<td>3TC</td>
</tr>
<tr>
<td></td>
<td>Emicitricibane</td>
<td>FTC</td>
</tr>
<tr>
<td>Nucleotide analogues</td>
<td>Tenofovir</td>
<td>TFV</td>
</tr>
<tr>
<td>Non-nucleoside reverse</td>
<td>Nevirapine</td>
<td>NVP</td>
</tr>
<tr>
<td>transcriptase inhibitors</td>
<td>Efavirenz</td>
<td>EFV</td>
</tr>
<tr>
<td></td>
<td>Etravirine</td>
<td>ETV</td>
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<tr>
<td>Protease inhibitors</td>
<td>Nelfinavir</td>
<td>NFV</td>
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<tr>
<td></td>
<td>Saquinavir (soft/hard</td>
<td>SQV SG/HG</td>
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<td></td>
<td>gel)</td>
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<tr>
<td></td>
<td>Ritonavir</td>
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<td></td>
<td>Indinavir</td>
<td>IDV</td>
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<td></td>
<td>Lopinavir</td>
<td>LPV</td>
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<td></td>
<td>Atazanavir</td>
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<td></td>
<td>Darunavir</td>
<td>DRV</td>
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<td></td>
<td>Fosamprenavir</td>
<td>FPV</td>
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<td></td>
<td>Tipranavir</td>
<td>TPV</td>
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<tr>
<td>Fusion inhibitors</td>
<td>Enfurvitide</td>
<td>T-20</td>
</tr>
<tr>
<td>Entry inhibitors</td>
<td>Maraviroc</td>
<td>MVC</td>
</tr>
<tr>
<td>Integrase inhibitors</td>
<td>Raltegravir</td>
<td>RGV</td>
</tr>
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</table>

Figure 1.3 Sites of action in HIV-1 life cycle of the different classes of antiretroviral drugs (N Imami)
1.2.3 Effects of cART initiation

1.2.3.1 Immune reconstitution

The use of cART in patients with immunosuppression is associated with significant increases in the CD4 T-cell count in the majority of patients (Staszewski S et al., 1999, Gallant JE et al., 2006, Mills AM et al., 2009, Molina JM et al., 2010). Introduced in 1996, initially it was unclear whether this quantitative immune restoration would be accompanied by qualitative immune restoration. Clinical evidence of benefit soon became apparent with cases of resolution of Kaposi’s Sarcoma (KS) and successful discontinuation of prophylactic therapy for opportunistic infections (OI) (Gill et al., 1998, El-Sadr et al., 2000). The CD4 T-cell rise following virological suppression subsequent to cART initiation is due to several mechanisms. Initially there is redistribution and peripheral proliferation (Wei et al., 1995, Autran et al., 1997, Pakker et al., 1998). This redistribution of memory cells from lymphoid tissue follows the cART associated reduction in immune system activation, thus enabling the release of these lymphoid sequestered cells. Peripheral proliferation of existing memory CD4 T-cells also occurs following cART initiation (Wei et al., 1995). In both these circumstances these cells have undergone multiple cell divisions and therefore this initial phase is associated with a decrease in TREC levels due to the dilutional effect of cell division (Wei et al., 1995, Pakker et al., 1998, Mohri et al., 2001, Pido-Lopez et al., 2003). This phase is followed by thymic production of naïve CD4 T-cells (Autran et al., 1997, Bisset et al., 1998, Douek et al., 1998, Pakker et al., 1998, Smith et al., 2000). Thymic damage is observed in chronic HIV infection with hypoplasia and decreased function (Stanley et al., 1993), and a degree of recovery, in terms of size and function, is observed with cART (McCune et al., 1998). Douek and colleagues (Douek et al., 1998) demonstrated increased CD4 T-
cells of thymic origin following the initiation of cART as determined by the presence of TRECs. Autran demonstrated an initial rise in memory CD4 T-cells four weeks after starting cART, followed by a rise in naïve cells of thymic origin (Autran et al., 1997). Therefore the CD4 T-cell count increases due to both thymic dependent de novo production and the thymic independent peripheral mechanisms of proliferation and redistribution.

Improvements in other immunological parameters are also seen, with recovery of some T-cell function. CD4 T-cell proliferative responses to recall antigens can still be detected for a significant period of time in HIV-1 infection (Ballett et al., 1998), however they start to decline as the CD4 T-cell count falls (Giorgi et al., 1987). Initiation of cART results in some restoration of immune function with, for example, recovery of responsiveness to recall antigens and mitogens (Kelleher et al. 1996, Autran et al., 1997, Pakker et al., 1998), however even this recovery is not absolute as some recall responses are not recovered even after 12 months of therapy (Autran et al., 1997). Recovery may be more likely in long term, persistent infections such as HSV and CMV, and less so with short term, intermittent infections and vaccinations such as tetanus (Hardy et al., 2003). Immune restoration inflammatory syndrome (IRIS) is the clinical correlate of this recovery, in which immune recovery results in an individual mounting a response to an already present but previously silent infection due to their profound immunosuppression. This is particularly demonstrated in those infections in which the appearance of disease is primarily due to damage resulting from the immune response to the infection, for example Mycobacterium tuberculosis (MTB) and Mycobacterium avium complex (MAC) (Price et al., 2009).
However HIV-1 specific proliferative responses disappear early in infection (Rosenberg et al., 1997), and whilst they can be maintained by introducing cART in PHI (Rosenberg et al., 1997, Al-Harthi et al., 2000) they do not generally re-appear following the commencement of cART in chronic HIV-1 infection (Autran et al., 1997, Pontesilli et al., 1998). This is likely to be due to several factors; effective cART, by keeping the viral load below the level of detection, reduces the antigenic stimulation provided by the residual virus to such a level that it is ineffective in eliciting an immune response. This is coupled with the lack of complete qualitative immune recovery and persistence of T-cell anergy and subsequent dysfunction; the HIV-1 specific CD4 T-cells may in fact still be present but in an anergic, non-proliferative state (Pitcher et al., 1999, Wilson et al., 2000). Others suggest inappropriate co-stimulation in a suppressive cytokine milieu may be responsible for the lack of proliferation (Meyaard et al., 1993). However some studies have observed a very modest degree of recovery of these specific responses in chronic infection during cART (Kelleher et al., 1996, Li et al., 1998, Angel et al. 1998, 2001, Deeks et al. 2000, Blankson et al., 2001) and we show in Chapter Three some recovery by switching the drug class utilised in the cART regimen, and in Chapter Four following IL-2 therapy. These initial observations set the stage for several studies of structured treatment interruptions (STI), proposing intermittent auto-vaccination via therapy interruptions as a means to stimulate HIV-1 specific responses. To date these have produced mixed and non-sustained results from the immunological recovery point of view. They carry the additional risk of further immune damage, especially in those patients with low nadir CD4 T-cell counts, and the possible emergence of resistant virus. The results of the SMART study (El-Sadr et al., 2008) have made further trials of STI unlikely. Therapeutic vaccination offers a theoretical
alternative as a source of antigenic stimulation without the inherent risks of STI. The challenge will be to do this in such a way as to avoid or minimise generalised immune system activation with all the associated adverse effects.

Not all patients respond optimally to the initiation of cART. There are four possible outcomes in respect to immunological and virological responses to treatment, two concordant and two discordant or disconnected. The definition and time frame of these outcomes varies but frequently used definitions are described here. Treatment success (TS) results in complete suppression of detectable plasma viral replication (currently <50 copies/ml in routine clinical practice, viral load below the level of detection [VLBD]) and an adequate and concordant rise in the CD4 cell count, arbitrarily defined as >50 cell rise over a six month period. In treatment failure (TF) there is ongoing detectable viraemia and a stable or falling CD4 T-cell count. There are also two possible discordant or disconnected outcomes. One is referred to as a discordant immunological response (DIR) in which the CD4 T-cell count fails to rise despite a fully suppressive cART regimen, usually taken as <50 cell rise after 6 months on cART with a VLBDL. The final possible outcome is referred to as a discordant virological response (DVR), in which despite ongoing detectable virus the CD4 T-cell count continues to rise. These phenomena are examined in more detail in Chapter Three.

The commencement of cART results in multiple phenotypic changes within the T-cell sub-populations. CD4 T-cell counts rise. Initially this is attributed to redistribution, followed by peripheral expansion with an increase in predominantly memory/effector cells (CD45RO) for the first few weeks, followed later by an increase in naive cells (CD45RA) (Autran et al., 1997). There is also evidence of cART increasing the thymic output of naive
precursors (Autran et al., 1997, Douek et al., 1998). Along with peripheral expansion and increased thymic production, decreased apoptosis is thought to play a role in the CD4 T-cell count rise (Dieye et al., 2000, Ensoli et al., 2000). The markers of activation such as CD38 and HLA-DR decline reflecting control of viral induced hyper-activation (Autran et al., 1997). cART also results in increased expression of the co-receptor CD28 which is essential for effective T-cell signalling; its lack is associated with T-cell anergy and unresponsiveness. Overall cART tends to promote a return towards normality in the immune system, largely by reducing viral burden and hence limiting direct cell killing, activation induced cell death (AICD) and anergy.

The production of naive T-cells and the limited nature of qualitative immune recovery in respect to HIV-1-specific immunity provides the rationale for therapeutic vaccination in the context of fully suppressive cART.

There is a vast array of other changes that have now been described within the immune system occurring after the initiation of cART, but are outside the scope and focus of this thesis.

1.2.3.2 Clinical

The immunological recovery due to cART is reflected clinically in the improvement of various HIV-1 related conditions and the decrease in the incidence of HIV-1 related clinical events. DTH to common recall antigens reappear or increase, and dermatological conditions such as psoriasis, HPV and HSV infection and seborrhoeic dermatitis can all show improvement. Regression of Kaposi’s sarcoma has been observed and both primary and secondary prophylaxis for OI can safely be stopped. Although still as yet incompletely defined IRIS reflects this cART induced immune recovery, representing the emergence of an effective immune response to an OI.
which due to profound immunosuppression had been silent prior to the initiation of therapy.

There has also been a marked decrease in the incidence of OI occurring following the commencement of cART and its accompanying immunological improvement. In some individuals despite a relative poor CD4 T-cell response there still appears to be a protective effect afforded by the therapy itself, in effect a qualitative improvement despite lack of quantitative advantage. This may be due in part to the effect of decreased immune system hyperactivation by achieving a VLBLD. However, it would appear the strongest predictor of future events is the CD4 T-cell recovery, irrespective of the VL, and this is further explored in Chapter Three.

To further examine the effect of cART on disease outcome and provide the background for this thesis, we undertook a review of mortality in HIV-1 infected patients during the pre-, early- and established cART eras, which is described in Chapter Three.

1.2.4 Side effects, toxicities, adherence and resistance

The different antiretroviral drugs have different side effects, ease of administration and genetic barriers to resistance. Major side effects and toxicities include lipodystrophy, hypersensitivity, lactic acidosis, hepatitis, hyperlipidaemia, diabetes and renal, neurological and cardiovascular disease. More common side effects which also frequently affect a patient’s willingness to continue with therapy include rash, gastrointestinal side effects and sleep and mood disturbances. Adherence to therapy plays a major role in the success or failure of therapy. Although a patient’s health beliefs are thought to be a major influence, pill burden, dose frequency and food restrictions may all influence a patient’s level of adherence. The emergence
of drug resistant virus is a major cause of treatment failure and not infrequently results from a patient’s poor adherence and is a result of ongoing viral replication in the presence of drug pressure. Some ARV however, such as 3TC and some NNRTIs, have a much lower genetic barrier to resistance than others, requiring only one mutation to confer significant resistance.

1.2.5 Limitations
Whilst cART has revolutionised the care of individuals living with HIV-1 there continues to be significant limitations associated with its use. Availability is a key factor, and not only in the developing world, with cost driven waiting lists currently in eight US states (NASTAD, 2010). The degree of quantitative and qualitative immune system recovery is at times sub-optimal. cART is not always fully suppressive with intermittent viral load blips and rebound allowing the emergence of resistant viral strains and re-seeding of latent viral reservoirs. The incomplete recovery of GALT and chronic immune activation results not only in ongoing direct immune system damage but is also linked to several long term complications, including cardiac, renal and metabolic disease. Whilst recognising the revolutionary effect cART has had on the HIV-1 epidemic and individual health, these limitations support the investigation of other treatments and strategies in an attempt to address some of these deficiencies.

1.3 Interleukin-2 in HIV-1 Infection
1.3.1 Introduction
HIV-1 infection is characterised by progressive immune dysregulation and depletion. This is associated with specific immune system defects and their
characterisation has identified possible therapeutic targets for immunotherapy. Abnormalities in the cytokine milieu are a major defect and interleukin-2 (IL-2) related immune dysfunction in particular is a key factor in HIV-1 related immunodeficiency. However early attempts at immune manipulation by cytokine therapy were largely unsuccessful due to the inability to control viral replication, less than optimal route of administration and the limiting dose related side effects. With the advent of cART and effective suppression of viral replication to undetectable levels there has been renewed optimism regarding the ability of immunotherapy to restore the immune system in HIV-1 infected individuals. Disappointingly, recent large clinical trials (Abrams et al., 2009) have failed to show translation of this reconstitution to a clinical benefit in the specific patient groups represented in these studies.

The discussion in this thesis will concentrate on the role of IL-2 in the context of HIV-1 infection. It is highly likely however that just as the immune defects in HIV-1 infection are multifactorial so too will be the solution, and combination immunotherapies are likely to be the way forward.

### 1.3.2 Interleukin-2

Interleukin-2, first described in 1976, was one of the first lymphokines to be identified; it is a major autocrine growth factor that plays a central role in the clonal expansion of activated lymphocytes, by interacting with its specific cell surface receptor (Minami et al, 1993). It was first used clinically in patients with metastatic renal cell carcinoma, its sole licensed indication in the United Kingdom, and malignant melanoma. It was first studied in phase I studies in HIV-1 infection in 1983. There has been a wide range of studies looking at different doses (low, intermediate, high), routes of administration
(subcutaneous (sc), intravenous (IV)), formulations (pegylated (PEG)) and schedules (intermittent with varying cycle length, continuous). Numerous combinations of these alternative strategies have been investigated in patients on no, suboptimal and fully suppressive antiretroviral therapy. It is the most extensively studied immune-based therapy in HIV-1 disease. However, the wide variety of doses and schedules and the failure to date to demonstrate clinical and survival advantage, indicate the uncertainty regarding the optimal therapeutic use of this agent.

1.3.2.1 Basic function

Endogenous IL-2 is a glycoprotein consisting of an 133-amino acid sequence with a molecular weight of 15-18 kiloDaltons, depending on the degree of glycosylation (Eckenberg et al., 2000). It is produced by activated T lymphocytes and NK cells following antigen activation (Gaffen, 1998). It plays an essential role in the immune response, inducing proliferation and differentiation of T lymphocytes, NK cells and B cells and the production of other cytokines, by interacting with its specific surface receptor (Minami et al., 1993). It influences the development and maturation of thymic lymphocytes. It is chemotactic to lymphocytes and facilitates leukocyte trafficking and adhesion to endothelium (Marincola et al., 1994).

IL-2 is produced following antigenic stimulation of T-cells. This IL-2 activates naive T-cells transforming them into effector cells with the ability to secrete IL-2 and function as antigen-specific CTL (Kaplan et al., 1992, Adachi et al., 1996). This increased production of IL-2 by CTL stimulates T cells, B cells and NK cell proliferation (Kaplan et al., 1992, Marincola et al., 1994, Seder et al., 1995, Adachi et al., 1996, Kinter et al., 1996). IL-2 also upregulates the expression of IL-2R on T and B cells in the presence of antigen, and on NK cells with or without antigen. This results in increased production of IFNγ, GM-
CSF and TNFα+β (Kaplan et al., 1992, Marincola et al., 1994). There is some evidence that this upregulation of IL-2R on T-cells may also occur without antigenic stimulation (Sereti et al., 2000), and the functionality of these cells is currently under investigation. IL-2 triggers B cells by cross-linking immunoglobulins resulting in IL-2R expression. The IL-2 resulting from the antigen activated T-cells binds to the receptor causing a switch from membrane to secretor IgM and therefore initiating a primary humoral response (Kaplan et al., 1992).

1.3.2.2 IL-2 receptors

IL-2 receptors exist as both cell membrane bound and soluble forms. It consists of three basic polypeptide sub-units. The different sub-units are expressed on different cells and have differing capacity for intracellular signalling. The sub-units are α (CD25), β (CD122) and γ (CD132). Various combinations of these sub-units result in receptors of differing affinity for IL-2.

The α chain, CD25 (IL-2Rα (p55)) is expressed on activated T and B cells and macrophages, and has low affinity for IL-2 (low affinity receptor (LAR)) (Thèze et al., 1996). It plays no role in either IL-2 internalization or signalling (Minami et al., 1993), but facilitates IL-2 binding. It binds IL-2 with a Kd of 10⁻⁸ mmol/L with a dissociation time of seconds.

The β chain, CD122 (IL-2Rβ (p75)) is located on NK cells and activated T-cells. Its intracellular domain consists of three regions, serine-rich, acidic and proline-rich, which are responsible for various intracellular interactions and signals.

The γ chain, CD132 (IL-2Rγ) is expressed on NK and T and B cells and is a member of the cytokine receptor superfamily. It is important for receptor-mediated internalization of IL-2.
Heterodimerization of the β and γ chain results in the formation of the intermediate affinity receptor (IAR), IL-2R βγ (p75/64) which has a Kd of 10⁻⁹ mmol/L and dissociation time of 45 minutes.

Once expressed, CD25 associates with IL-2Rβ chain (CD122) and common γ chain (CD132), which are constitutively expressed on the surface of resting T cells, forming a high affinity receptor (HAR, IL-2Rαβγ (p55/75/64)). This receptor has a Kd of 10⁻¹¹ M and a dissociation time of 50 minutes (Thèze et al., 1996).

T-cells express α, β and γ subunits and can express high affinity receptors when activated. Activated monocytes only express low affinity receptors.

NK cells are the only cells to constitutively express IL-2R. Those which are CD56+brightCD16- (10% of all NK cells) express both IAR and HAR. When the IAR is exposed to IL-2 for 4-6 hours activation of NK cytotoxicity occurs. IL-2 stimulation of HAR for 4-5 days activates NK cells to generate lymphokine-activated killer cells (LAK) ex vivò (Brenner et al., 1991, Kaplan et al., 1992).

NK cells also mediate ADCC in HIV-1 infection. HIV antibodies bind to NK receptors resulting in lysis by NK cells. Complement mediated NK lysis is increased by IL-2.

1.3.2.3 IL-2 related defects in HIV-1 infection

Abnormalities in IL-2 production, function and receptor expression are seen in HIV-1 infection. The TH1 to TH2 shift occurring with disease progression alters the predominant cytokine profile and the high apoptotic rates result in a high level of cell destruction.

1.3.2.3.1 Decreased IL-2 production

With HIV-1 disease progression there is decreased IL-2 production and decreased T-cell proliferation (Winkelstein et al., 1989, Clerici et al., 1993, Lederman et al., 1995, Kinter et al., 1996, Clerici et al., 1996). As previously
described, with disease progression there is a shift in the immune response from a TH1 to a TH2 response. The production of IL-2 therefore declines and is in fact inhibited by the TH2 cytokine milieu (Clerici et al., 1993, 1996, Barker et al., 1995, Vyarkaman et al., 1995). In addition, it may be that the decrease in IL-2 levels is in part caused by the cross-linking of antibodies to env proteins of HIV-1 to IL-2 (Bost et al., 1988). Furthermore, levels of soluble IL-2 receptors (sIL-2R) are increased in HIV infection (Winkelstein et al., 1989, Scott-Alzara et al., 1991, Poli et al., 1992, Vyarkaman et al., 1995). This increase is usually correlated with a decreased CD4 T-cell count and decreased levels of serum IL-2. The decrease seen in free circulating IL-2 may therefore be due to free IL-2 binding to receptors released from lysed T-cells. Several of HIV-1’s 9 regulatory and structural genes influence immune function by directly affecting IL-2 production and function; tat, rev and nef inhibit IL-2 production at the transcriptional level. (Westendorp et al., 1994, Vyarkaman et al., 1995, Puri et al., 1995).

1.3.2.3.2 Decreased IL-2R expression

CD25 expression is reduced early in HIV-1 infection with shedding of the protein (Honda et al., 1987). Tat, rev and nef downregulate some IL-2R (Westendorp et al., 1994, Vyarkaman et al., 1995, Puri et al., 1995), but upregulation of CD122 on CD8+ T cells but not on CD4+ cells (Johnson et al., 1997) is seen.

1.3.2.3.3. Apoptosis

Apoptosis is a normal physiological process whereby cells undergo programmed cell death. In the immune system it is responsible for clonal selection and cell mediated cytotoxicity (Meyaard et al., 1991). It is influenced by many factors, including cytokines. When CD4 T-cells are stimulated by antigen they produce IL-2 which results in clonal expansion.
However with repeated exposure they gradually lose the ability to secrete IL-2. This leads to activation-induced cell death (Smith et al., 1988, Meyaard et al., 1991, Adachi et al., 1996). With T-cell maturation there is increased expression of Fas antigen and gradual loss of expression of Bcl-2 antigen (Adachi et al., 1996). Fas is a marker for increased susceptibility to apoptosis and correlates with disease progression in HIV-1 infection (Miyawaki et al., 1992).

In HIV-1 infection marked apoptosis is seen in advanced disease (Pandolfi et al., 1995, Kinter et al., 1996, Clerici et al., 1996) as are low levels of endogeneous IL-2 (Kaplan et al., 1992). The addition of IL-2 in vitro blocks spontaneous apoptosis, reducing the down regulation of Bcl-2 expression and therefore increasing cellular survival without inducing cellular division (Adachi et al., 1996). In vitro, withdrawing IL-2 from gp120 primed lymphocytes results in increased levels of apoptosis (Radrizzani et al., 1995, Clerici et al., 1996).

Additionally, it has been shown that the addition of IL-2 can rescue antigen specific T-cells from radiation induced apoptosis (Mor et al., 1996). This suggests that IL-2 increases the number of CD4 T-cells by preventing apoptosis, with the expansion of existing clones rather than new clones, accounting for the increased CD4 T-cell count (Tepler et al., 1993, Wood et al., 1993, Kovacs et al., 1995, 1996, Kinter et al., 1995, Adachi et al., 1996, Jacobson et al., 1996). This is consistent with clinical studies of IL-2 showing the expansion of pre-existing (mainly memory) CD4 T-cells (Kovacs et al., 1995, Jacobson et al., 1996, Kovacs et al., 1996). The in vitro effect of IL-2 on lymphocyte apoptosis was seen to a greater degree in those with a CD4 T-cell count >200 cells/μL (Adachi et al., 1996).
1.3.2.4 Exogenous IL-2 in HIV-1 infection

Aldesleukin (Proleukin, Chiron, Emeryville, USA) is currently licensed in the United Kingdom for use in patients with metastatic renal cancer. IL-2 therapy was added to the French HIV treatment guidelines for individuals with low CD4 T-cells, however access to IL-2 therapy was halted in 2007. IL-2 is currently being used in the UK in HIV-1 infection almost exclusively within the context of clinical trials. It is on occasion being used as ‘off-license’ therapy for HIV-1 infection in the UK.

1.3.2.4.1 Basic structure, pharmacokinetics and function

Aldesleukin is genetically engineered, *Escherichia coli*-expressed recombinant human interleukin-2 (rIL-2) (Chiron). Structurally different from endogenous IL-2, aldesleukin is not glycosylated, lacks a N-terminal alanine residue and at amino acid position 125 the cysteine is replaced by serine. However it is essentially pharmacologically the same as the endogenous cytokine (Doyle, 1985).

The pharmacokinetics of IL-2 is two compartmental and it is rapidly distributed into interstitial fluid. Its distribution half life is 6-20 minutes and its elimination half life is 85 minutes. These are independent of dose and are similar for both intravenous and subcutaneous administration. The mean peak serum concentration is dose dependent and steady state is arrived at in two hours. IL-2 is metabolised by the kidneys.

In patients with metastatic renal carcinoma aldesleukin therapy results in an increase in the levels of sIL-2R. This is associated with increased macrophage activity (Lissoni et al., 1991) and may be indicative of increased immune reactivity (Farace et al., 1995). Increased sIL-2R correlates with increased proliferation of peripheral blood CD25+ T-cells (Hanninen et al., 1991).
Exogenous IL-2 administration results in activation and proliferation of cytotoxic T lymphocytes and NK and LAK cells. Some effects appear dose and schedule-dependent. A transient acute eosinopaenia and lymphocytopenia, seen immediately after administration, is followed by a rebound increase in the number of both; peaking 1-3 weeks after the start of therapy. The number and activity of the circulating NK cells increase dependent on dose and schedule. A transient increase in both IFNγ and TNFα is seen 1-4 hours after administration. There is also an increase in the serum levels of other endogenous interleukins 5, 6, 8 and 10. Production of antibodies specific to rIL-2 has been observed frequently in patients with renal carcinoma, with up to 90% of patients developing antibodies (Whittington et al., 1993, Hanninen et al., 1993), but neutralizing activity is rare (5%) (Hanninen et al., 1991); these patients additionally have lower sIL-2R levels. The clinical significance of these antibodies is as yet undetermined.

1.3.2.4.2 Mechanism of action in HIV-1 infection

IL-2 therapy in HIV-1 infection results in a significant rise in CD4 T-cells. Although still a topic of considerable debate three mechanisms appear to be involved; increased thymic production, peripheral polyclonal T-cell proliferation, with expansion of both memory and naive phenotypes, and reduced apoptosis. Several studies have postulated a direct effect of recombinant IL-2 on the thymus (Plum et al., 1987, Reya et al., 1998), increasing de novo production and maintaining a milieu conducive to support thymocyte growth and selection. Carcelain and colleagues demonstrated an IL-2 associated increase in the levels of TRECS indicating an increase in thymic production of naive cells (Carcelain et al., 2003). Additionally, increased CD4 T-cell proliferation accounts for some of the CD4 T-cell expansion observed with IL-2 therapy (Caggiari et al., 2001, Natarajan
et al., 2002. Pido-Lopez et al., 2003). Despite an immediate initial mean increase in T-cell death due to an increased apoptotic rate of both CD4 and CD8 T-cells (Sereti et al., 2001), the apoptotic rate subsequently declines (Caggiari et al., 2000), resulting in an increase in CD4 T-cells. Kovacs and colleagues have also demonstrated that IL-2 therapy induces significant prolongation of CD4 T-cell survival (Kovacs et al., 2005).

This expansion of CD4 T-cells is associated with selective induction of the α chain of the IL-2 receptor (CD25) on CD4 T-lymphocytes (Sereti et al., 2000). CD4+CD25+ T-cells are also known as regulatory T-cells or Tregs, as they play a key immuno-regulatory role. However those resulting from IL-2 therapy may be qualitatively different to those resulting from antigenic-stimulated expansion (Sereti et al., 2000, 2002), being less anergic. IL-2 receptors of high (αβγ) and intermediate affinity (βγ) are up-regulated differentially on CD4 and CD8 T-cells by exogenous IL-2 administration.

This quantitative immunological improvement is accompanied by at least a degree of qualitative improvement. IL-2 results in an increase in delayed type hypersensitivity reactions to such antigens as tetanus toxoid and tuberculin (Carr et al., 1998). Exogenous IL-2 has been shown to increase recall antigen specific CD4 T-cell proliferation (Davey et al., 2000, Levy et al., 2003). However the timing of cytokine therapy in relation to antigenic stimulation and recovery of these responses may be crucial (Blattman et al., 2003, Hardy et al., 2004). Re-exposure to HIV-1 antigens in the presence of IL-2 has been shown to induce HIV-1 specific responses, although sustainability has yet to be demonstrated. However, the clinical benefit of such apparent immune reconstitution remains unknown.
In the context of HIV-1 infection, IL-2 has well described effects on NK cells, humoral immune responses and other cytokines such as IFNγ but these are outside the scope of this thesis.

1.3.2.4.3 Side effects

The side effects of exogenous IL-2 therapy at current doses are predictable and in the majority of cases preventable or controllable by either dose reduction or the administration of prophylactic medication. Earlier trials in HIV-1 infected individuals were limited by the serious, potentially life threatening side effects associated with the high doses of intravenous IL-2 being administered. The majority of side effects are related to the dose and route of administration. There are a wide variety of side effects including pulmonary oedema, cardiac, gastro-intestinal, hepatic and renal dysfunction. The majority are transient and resolve with treatment cessation. The most frequently experienced side effects are flu-like symptoms with chills, fever and malaise, occurring in almost all patients, although rarely to the extent requiring therapy cessation. Fever and chills have been reported as occurring to some degree in up to 100%, although only 5% experienced grade 4 symptoms (Buter et al., 1993). Skin reactions with erythema and pruritis are also common. Inflammation and induration at the injection site are also almost universal and resolve spontaneously over weeks to months. Grade 2-3 local reactions have been reported in 33-100% of patients (Lissoni et al., 1992, Buter et al., 1993, Angevin et al., 1995, Tourani et al., 1996). This can be reduced by site rotation and vigorous rubbing post injection to aid dispersal.

Potentially more serious adverse events include capillary leak syndrome resulting in hypotension and pulmonary and peripheral oedema. This is more commonly associated with high dose intravenous administration (60% mild,
grade 1-2) and is infrequently seen with subcutaneous administration at current doses (0-26%) (Buter et al., 1993).

Other side effects include renal dysfunction, haematological abnormalities (thrombocytopenia, neutropenia), nausea, vomiting and diarrhoea. Hypothyroidism is the major endocrine complication with anti-thyroid antibodies detected in 50%. Thyroid dysfunction is usually temporary and required thyroid substitution in 15% in two trials of patients with renal carcinoma (Buter et al., 1993 Angevin et al., 1995). It has been observed in 10% of patients receiving IL-2 for HIV-1 infection.

Neurolgical and psychiatric disturbances with moderate to severe mental state changes are common and sometimes treatment limiting. Mood depression is not infrequently described, often days to weeks after cycle completion. Musculoskeletal disorders are transient and tend to resolve spontaneously.

As with all drug regimens, acceptability of a treatment takes into account the relationship between the therapeutic benefits to the patient (either real or perceived) and the incidence and tolerability of adverse events. This is of particular relevance when IL-2 is administered to HIV-1 infected individuals with high baseline CD4 T-cell counts.

1.3.2.4.4 Clinical trials

The past 15 years have resulted in a wealth of IL-2 clinical trials showing improvement in SIM and exploring various therapeutic strategies to determine the optimum regimen. Large studies recently conducted were designed to assess whether this gained wisdom translates into improved survival benefit for the individual infected with HIV-1. The major studies are summarised in Table 1.2.
Early trials were hampered by inadequately controlled viraemia and dose limiting side effects. They did, however, demonstrate sufficient benefit to encourage further research into optimum dose, treatment schedules, drug formulation and the method of administration. Trials based on continuous, high dose intravenous IL-2 infusions, limited by serious life threatening toxicities have evolved into subcutaneous, low and intermediate IL-2 dose trials, administered in an outpatient setting, with side effects ameliorated with dose manipulation and prophylactic medication. The resulting data demonstrated SIM benefit at all stages of disease, both with and without cART and without adversely affecting plasma viraemia. Subcutaneous IL-2 has been found to be superior to continuous intravenous and pegylated IL-2 in terms of efficacy balanced against serious side effects and the effect on patients’ quality of life (QOL).

In 15 randomised controlled trials of IL-2 therapy in HIV-1 infected patients on ARVT several key factors have now emerged regarding the dosing regimen of IL-2. It appears at present the optimal dose of IL-2 is 7.5 MU bd (Davey et al., 1999). Five day treatment courses at 8 week intervals result in adequate efficacy but with reduced toxicity and avoiding tachyphylaxis (Miller et al., 2001). Additionally greater CD4 T-cell expansion is seen in those patients with a higher baseline CD4 cell count (Davey et al., 1997). The pegylated formulation of IL-2 was found to be inferior in terms of efficacy and similar in terms of side effects (Carr et al.1998, Levy et al. 1999). The majority of these trials are of IL-2 plus ARVT vs ARVT alone, however importantly this does not in all cases equate to virologically effective cART, and in many instances involves mono and dual ARVT.

In a meta-analysis, Emery and colleagues showed in three RCT a decrease in the HIV-1 RNA in 157 patients over 28-31 weeks, maintenance of the CD4 T-
cell rise, and the possibility that there may be decreased risk of disease progression or death in the IL-2 treated patients (Emery et al., 2000). One other RCT has shown a slight decrease in viral load (Davey et al., 2000). The Vanguard study, as discussed in Chapter Five, showed no deleterious effect on viral load in patients receiving IL-2 therapy in the absence of cART (Youle et al., 2006). Demonstration of the clinical benefit of IL-2 therapy remains an important goal, and two large international, phase three, randomised, controlled, clinical end point studies of IL-2 in HIV-1 positive patients have recently been reported; ESPRIT and SILCAAT (Abrams D et al., 2009). The Evaluation of Subcutaneous Proleukin in a Randomised International Trial (ESPRIT) was a six year study of 4111 patients with CD4 T-cell counts \( \geq 300 \text{ cells/\mu L} \). The Study of IL-2 with Low CD4 Counts on Active Anti-HIV Therapy (SILCAAT) was a 4 year study of 1695 patients with CD4 T-cell count range of 50-299 cells/\mu L. All patients were on cART, although importantly an undetectable VL and minimum CD4 T-cell nadir were not entry criteria. IL-2 cycles were administered for 5 days every 8 weeks; in EPSRIT the dose was 7.5 MIU bd for three cycles and in SILCAAT 4.5 MIU bd for 6 cycles. Additional maintenance cycles were recommended. The main outcome of the study showed that despite a significant and sustained CD4 T-cell increase, no clinical benefit was seen as measured by opportunistic disease or death. In fact, those patients receiving IL-2 did worse in terms of adverse events. This was an unexpected result considering the weight given to the absolute CD4 T-cell count as a reliable SIM, predictive of prognosis. There are many possible explanations for these results. It may be the resultant expanded CD4 T-cell population are in some way defective or poorly functioning, and some evidence exist for this with reported differences observed in the CD4+CD25+
T-cells resulting from IL-2 therapy as compared to ‘natural’ antigenic stimulation (Sereti et al., 2000). Patient selection may also have influenced the outcome as, with no minimum nadir, the potential effector memory T-cell repertoire available for expansion by IL-2 would have been reduced, limiting the potential to protect against OI. Incomplete viral suppression, seen in 20% at enrolment, may also have contributed a dampening effect of any potential IL-2 benefit due to the increased levels of immune activation. In contrast to these explanations it may actually be that we are measuring the wrong SIM, and using that to inadequately drive maintenance therapy; to optimise IL-2 therapy we may need to look elsewhere for alternative or additional SIM, for e.g. activation markers, Tregs. Alternatively, it may be that the absolute CD4 T-cell count is merely a surrogate marker for some other immune function which IL-2 does not correct. Finally, it may be that IL-2 does not produce sufficient qualitative immune reconstitution to impact on what is the key outcome of any therapy; disease progression and patient survival. It would, however, be premature to abandon this therapy as the situation currently has several parallels to the early days of mono- and dual-antiretroviral therapy. We still have an incomplete understanding of the pathophysiological mechanisms involved and, as in the past, combination immunotherapy may yield superior benefits as it did with combination ARVT.
Table 1.2: Summary of IL-2 clinical trials

<table>
<thead>
<tr>
<th>Author, year RCT status</th>
<th>n=</th>
<th>IL-2 dose and route</th>
<th>IL-2 regimen Days/Week/Cycle number</th>
<th>ARVT</th>
<th>CD4 T-cell count (cells/µL)</th>
<th>Viral load (copies/ml)</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kovacs, 1995</td>
<td>23</td>
<td>8-18 MIU IVI od with dose escalation</td>
<td>5D/8W 7-25 monthly intervals</td>
<td>yes, not cART</td>
<td>&gt;200</td>
<td>&lt;200</td>
<td>60% of patients with CD4&gt;200 had a CD4 T-cell count rise ≥ 50%, cells were polyclonal with similar Vβ analysis as pre-IL-2 distribution (expansion of the existing repertoire and prolonged survival by decreased apoptosis). Decreased CD8 T-cell activation (HLA-DR, CD38). Progressive increase in CD25+ cells; associated with a higher CD4 cell count. Stable VL overall, peri-cycle increases seen.</td>
</tr>
<tr>
<td>Kovacs, 1996</td>
<td>60</td>
<td>18 MIU IVI daily</td>
<td>5D/8W/6C then CD4 driven</td>
<td>1 or 2 NA</td>
<td>&gt;200</td>
<td>&lt;200</td>
<td>Increased VL. No change in CD4.</td>
</tr>
<tr>
<td>Jacobson, 1996</td>
<td>16</td>
<td>62.5-250k MIU/m² daily for 6M</td>
<td>daily</td>
<td>≥1M not cART</td>
<td>200-500</td>
<td>Increase in CD4, no change in VL. DTH to common recall antigens reappeared.</td>
<td></td>
</tr>
<tr>
<td>Davey, 1997</td>
<td>18</td>
<td>1.5 or 7.5 MIU bd sc dose escalation</td>
<td>5D/4 or 8 W/3C</td>
<td></td>
<td>&gt;200</td>
<td></td>
<td>44% had &gt;200 increase in CD4 T-cell count, further 33% had up to 200 increase. Greatest effect in those with higher baseline CD4. Transient (1 month) rises in VL</td>
</tr>
<tr>
<td>Carr, 1998</td>
<td>115</td>
<td>A -12 MIU CIVI B - 0.5 MIU sc (PEG)</td>
<td>A - 5D/8W B- day 1 and 3/8W</td>
<td>&gt;2 M</td>
<td>200-500</td>
<td>Group A - increased CD4 T-cell count (greater increase at lower VL) and DTH, decreased CD8+HLA-DR, VL unchanged</td>
<td></td>
</tr>
<tr>
<td>Study &amp; Year</td>
<td>Participants</td>
<td>Treatment</td>
<td>Duration</td>
<td>Additional Details</td>
<td></td>
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<tr>
<td>Hengge, 1998</td>
<td>64</td>
<td>4.5 MIU bd</td>
<td>5D/6W or CD4 driven</td>
<td>cART</td>
<td>Increased CD4, decreased VL, activation and CD25, increase in DTH, less clinical skin related diagnoses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Levy, 1999</td>
<td>94</td>
<td>A-12 MIU IVI B-6 MIU/m² sc C-2 MIU/m² PEG IVI</td>
<td>5D/8W/7C</td>
<td>naïve, started dual ARVT</td>
<td>250-500</td>
<td>Increases in naïve and memory CD4 T-cell count (IV and sc had greater effect of PEG), CD28+ and LPR (mitogen and recall). VL stable</td>
<td></td>
</tr>
<tr>
<td>Arno, 1999 RCT</td>
<td>25</td>
<td>3 MIU sc</td>
<td>5D/4W/6C</td>
<td>stable, effective 6M</td>
<td>&lt;250</td>
<td>Increase in CD4 T-cell count, initially memory then naïve</td>
<td></td>
</tr>
<tr>
<td>Davey, 1999</td>
<td>49</td>
<td>1.5 or 7.5 MIU bd sc</td>
<td>5D/4 or 8W</td>
<td>≥6W 1, 2 or 3 drugs</td>
<td>≥500</td>
<td>Increased CD4 T-cell and %, dose dependent</td>
<td></td>
</tr>
<tr>
<td>Lalezari, 2000 RCT</td>
<td>115</td>
<td>1.2 MIU/m²/day sc</td>
<td>daily</td>
<td>cART</td>
<td>&lt;300</td>
<td>Increase in CD4 % but not absolute count, with preferential expansion of naïve cells. Increase NK cells. Stable VL. Trend to less treatment changes</td>
<td></td>
</tr>
<tr>
<td>Emery, 2000</td>
<td>218</td>
<td>Metanalysis 3 RCT</td>
<td>Metanalysis 3 RCT</td>
<td>cART</td>
<td>&gt;350</td>
<td>Increase in CD4; dose related, decreased VL. Trend to improved clinical outcomes</td>
<td></td>
</tr>
<tr>
<td>Losso, 2000 RCT</td>
<td>73</td>
<td>1.5/4.5/7.5 bd sc</td>
<td>5D/8W/6C</td>
<td>cART</td>
<td>&gt;350</td>
<td>Increase CD4 for 4.5/7.5. Stable VL</td>
<td></td>
</tr>
<tr>
<td>Davey, 2000 RCT</td>
<td>82</td>
<td>7.5 MIU bd sc</td>
<td>5D/8W</td>
<td>cART</td>
<td>&gt;350</td>
<td>Increase in CD4 T-cell count and %, decrease in VL</td>
<td></td>
</tr>
<tr>
<td>Ruxrungtham, 2000 RCT</td>
<td>72</td>
<td>3 doses</td>
<td>24W</td>
<td>ARVYT</td>
<td>≥350</td>
<td>Increase in CD4, Stable VL</td>
<td></td>
</tr>
<tr>
<td>Tambussi, 2001 RCT</td>
<td>61</td>
<td>CIV or 7.5 or 3 MIU bd sc</td>
<td>5D/8W</td>
<td>ARVT</td>
<td>no requirement</td>
<td>Increase CD4 stable VL and proviral DNA</td>
<td></td>
</tr>
<tr>
<td>Miller, 2001 RCT</td>
<td>22</td>
<td>4.5 MIU bd</td>
<td>5D/8W/4C or CD4 driven</td>
<td>cART</td>
<td>&gt;200</td>
<td>Increased CD4, no difference b/w groups. Decrease VL</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Strategy</td>
<td>Duration</td>
<td>CD4 Increase</td>
<td>Other Observations</td>
<td></td>
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<tr>
<td>Marchetti, 2002 RCT</td>
<td>22</td>
<td>low dose</td>
<td>12M cART</td>
<td>&lt;200</td>
<td>Increase CD4 count, increase naïve cells, decrease clinical events, stable VL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kattlama, 2002 RCT</td>
<td>72</td>
<td>4.5MIU bd</td>
<td>5D/6W/4C</td>
<td>cART 25-200</td>
<td>Increase CD4, more achieving CD4&gt;200, stable VL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abrams, 2002 RCT</td>
<td>511</td>
<td>4.5 or 7.5 MIU bd sc</td>
<td>5D/8W then CD4 driven</td>
<td>cART &gt;300</td>
<td>Increase in CD4, no effect on VL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>deBoer, 2003 RCT</td>
<td>81</td>
<td>12MIU CIV</td>
<td>3,4 or 5D/8W/6C</td>
<td>cART 100-300</td>
<td>Increase in CD4, varied with IL-2 duration, no effect on VL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Levy, 2003 RCT</td>
<td>118</td>
<td>5MIU bd</td>
<td>5D/4W/3C then 8W/7C</td>
<td>cART 200-550</td>
<td>Increase in CD4, no effect on VL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vogler, 2004 RCT</td>
<td>115</td>
<td>1MIU od sc</td>
<td>24W</td>
<td>1,2 or 3 drugs 300-700</td>
<td>Did not prevent CD4 decline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marchetti, 2004 RCT</td>
<td>15</td>
<td>3C</td>
<td>cART</td>
<td></td>
<td>Increase in CD4 count, proliferation and TRECS of CD8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Youle, 2006 RCT</td>
<td>36</td>
<td>4.5 or 7.5 MIU bd sc</td>
<td>5D/8W/3C</td>
<td>none &gt;350</td>
<td>Dose dependent increase in CD4 T-cell count. Stable VL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitsuyasu, 2007</td>
<td>204</td>
<td>CIV or sc</td>
<td>5D/8W</td>
<td>commenced cART 50-350</td>
<td>VL&lt;5000 Increase CD4. Stable VL. Fewer AIDS diagnoses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Durier, 2007 RCT</td>
<td>131</td>
<td>bd sc</td>
<td>5D/CD4 driven</td>
<td>pre and post cART era</td>
<td>Increase in CD4, decrease in VL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arudino, 2004 metaanalysis</td>
<td>218</td>
<td>1.5/4.5/7.5MIU bd sc</td>
<td>5D/8W/3C then CD4 driven</td>
<td>cART &gt;350</td>
<td>Increase in CD4, dose related</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molina, 2009 RCT</td>
<td>130</td>
<td></td>
<td>no ARVT</td>
<td>300-500</td>
<td>Fewer reached negative endpoints, delaying initiation of cART</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESPRIT, 2009 RCT, Abrams</td>
<td>4111</td>
<td>7.5 MIU sc bd</td>
<td>5D/8W/3C</td>
<td>cART &gt;300</td>
<td>Increase in CD4 T-cell count. No clinical benefit. Increase in grade 4 clinical events</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SILCAAT, 2009 RCT, Abrams</td>
<td>1695</td>
<td>4.5 MIU sc bd</td>
<td>SD/8W/6C</td>
<td>cART</td>
<td>50-200</td>
<td>≤10,000</td>
<td>Increase in CD4 T-cell count. No clinical benefit</td>
</tr>
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<tr>
<td>MIU</td>
<td>million international units</td>
<td>DTH</td>
<td>delayed type hypersensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVI</td>
<td>intravenous infusion</td>
<td>od/bd</td>
<td>once/twice daily</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days/Week/Number</td>
<td>cycle length/cycle interval/number of cycles</td>
<td>sc</td>
<td>subcutaneous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D/W/C</td>
<td>days/weeks/cycle number</td>
<td>LPR</td>
<td>lymphoproliferative responses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEG</td>
<td>pegylated</td>
<td>ARVT</td>
<td>antiretroviral therapy</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
Can IL-2 flush viral reservoirs?

HIV-1 persists as latent, replication competent, integrated virus, despite long term effective cART (Finzi et al., 1997, Wong et al., 1997). These infected resting cells produce infectious virions when the cell is activated. The premise therefore is that IL-2 will activate the resting cell, producing infectious virions, but in the presence of cART this will not result in other cells becoming infected. One study which looked at resting, latently infected CD4 T-cells, found a lower frequency of infectious virus in a group of IL-2 treated patients on cART compared in a non-randomised fashion to patients on cART alone (Chun et al., 1999). However, virological relapse occurred in all patients who have subsequently stopped cART in this and other studies, indicating eradication had not occurred (Davey et al., 1999, Fraser et al., 2000, Lafeuniaede et al., 2001). In addition as only one in a million PBMCs are latently infected it would require a significant level of activation of all cells to purge the whole reservoir; this degree of immune activation is likely to be detrimental to the host, and toxicity would be a major limiting factor (Fraser et al., 2000).

IL-2 however may be useful in promoting control if eradication is not possible. There are three basic observations that underpin this possibility. The central defect in HIV-1 infection is a lack of virus specific T-helper cell response and the main cytokine produced by these cells is IL-2. In chronic infection there are likely to be virus-specific T-helper cells that are not functioning properly (i.e. anergic) because of IL-2 deficiency. cART results in the production of new naive cells which due to cART induced lack of antigen (i.e. VLBLD) may not change to HIV-1-specific effectors. Therefore IL-2 by stimulating viral antigen from viral reservoirs, may provide this required antigenic stimulus in a
clinically safe way, and may permit reversal of the anergy of the T-helper cells.

In summary IL-2 therapy induces many restorative immunological effects, both quantitative and qualitative. Translation to clinical benefit has yet to be observed. It is likely additional therapeutic immune manipulation and possibly alternative SIM drivers of therapies will need to be identified to realise the promise of IL-2 therapy.

1.4 Therapeutic Immunisation in HIV-1 Infection

1.4.1 Background

Expert opinion suggests that an effective preventative vaccine against HIV-1 infection will require both cellular and humoral immunity to achieve protection (Mooij et al., 2001). However, which T-cell responses are critical for protection remain unanswered, furthermore the ability of a vaccine to generate neutralising antibodies remains elusive (MacMichael A, 2006). The added challenge will be to devise a vaccine with sufficient breadth to deal with the different HIV-1 clades encountered around the world. Vaccines can additionally be used therapeutically in those already infected to induce or augment an individual’s response to infection, as first proposed by Salk in 1987 (Salk et al., 1987).

Detailed immunological profiling of LTNP has identified various parameters conducive to delayed progression of HIV-1 infection (Deeks et al., 2007). This provides targets for immune manipulation in an attempt to recreate this milieu in HIV-1 infected individuals. The advent of cART has created an environment more permissive to achieving these aims. Prior to the introduction of cART and effective plasma viral suppression, vaccination-induced activation of T-cells provided targets for the virus present in the
plasma. Effective cART has, by viral suppression, minimised this threat, and enhanced its potential as a therapeutic agent.

Initial therapeutic vaccine studies focused on attempts to induce blocking antibodies. Although successful in animal models, trials of agents such as recombinant gp 120 immunogen have proved less successful in humans infected with HIV-1, with no demonstrable clinical benefit (Salk et al., 1987, Ross et al., 2010).

1.4.2 Remune: HIV-1 Immunogen

Remune is one therapeutic vaccine candidate which initially appeared promising, although subsequent clinical data has somewhat dampened the early enthusiasm. As is often the case, favourable immune profiles induced by the immunogen have so far failed to translate into appreciable clinical benefit. Remune (Immune Response Corp. (IRC), San Diego CA) is a whole, inactivated, gp120 depleted immunogen suspended in incomplete Freund’s adjuvant (IFA) (Moss et al. 1994, Trauger et al., 1994). It is based on clade A env and clade G gag from the recombined Zairian primary isolate HZ-321 and grown in the HuT-78 cell line (Moss et al., 1998). It has been administered to more than 3000 trial patients, and appears to be safe and well tolerated (Levine et al., 1996, Churdboonchart et al., 2000, Chantratita et al., 2004). It is administered at doses of 50-400µg total protein, by intramuscular injection at twelve week intervals. Typical reported side effects include flu-like symptoms and local injection site reactions. Several studies have demonstrated improvement in various immunological parameters, including a beneficial effect on CD4 T-cell count and viral load (Turner et al., 2001, Sukeepaisarncharoen et al., 2001, Chantratita et al., 2004). Its use is associated with enhancement of HIV-1 specific delayed type hypersensitivity
(DTH) responses and lymphocyte proliferative responses (LPR) (Valentine et al., 1996, Churdboonchart et al., 2000, Turner et al., 2001, Robbins et al., 2003). It increases levels of IFNγ (TH1 cytokine) and β chemokines (Moss et al., 1997, Valentine et al., 1998). It has been shown to augment cell mediated immune (CMI) responses to autologous virus and increased antibody reactivity has been described; both strength and breadth of Western Blot (WB) reactivity (Churdboonchart et al., 2000).

The first clinical study of Remune in 1994 was a phase one, dose ranging study in ARV naïve HIV-1 infected individuals which showed improved HIV-1 specific DTH and antibody response (Turner et al., 1994). A subsequent study (Levine et al., 1996) demonstrated improved DTH in approximately 50% of the 25 patients, who then went on to have a decreased rate of clinical events and death. This association was potentially biased by the possibly more conserved immune function of those individuals in the more reactive group. An IRC study in 15 patients, demonstrated increases in IFNγ, β chemokines and LPR to HIV-1 specific antigens (Moss et al., 1997). A Thai study of 29 ARV naive individuals, 28 infected with clade E, also showed improvement in WB reactivity in those receiving Remune. A subsequent larger, double-blinded, adjuvant-controlled trial demonstrated increases in CD4 T-cell counts and HIV-1-specific immunogenicity (DTH and WB) (Churdboonchart et al., 2000). Other Thai studies have shown stabilisation of body weight, viral load and CD4 T-cell percentage (Sukeepaisarncharoen et al., 2001, Chantratita et al., 2004). Valentine and colleagues reported increased levels of gag-specific T-helper-cell responses (Valentine et al., 1996) and β chemokines (Valentine et al., 1998)) comparable to that seen in LTNP, along with a greater proportion of individuals achieving viral loads BLD in those receiving Remune. Others
have also observed increases in HIV-1 specific CD4 T-cell proliferative responses (Moss et al., 2000, Robbins et al., 2003).

The largest study to date is a phase three, multicentre, double-blind, placebo controlled, randomised, clinical endpoint trial in 2527 ARV T naïve HIV-1 infected individuals (Kahn et al., 2000). HIV-1 progression-free survival was the primary endpoint with secondary endpoints being overall survival, HIV RNA, CD4 T-cell count and percentage, body weight and immunogenicity. The only difference observed was an increase in the mean CD4 T-cell count, and no difference in clinical outcomes was observed. The publication of these results was controversial due to author disagreement with the sponsoring company regarding analysis and interpretation of the results. Results from a prospectively identified sub-group (n=252) by Moss and colleagues (IRC) revealed the vaccinated group had a significantly greater decline in plasma viral RNA, both in the speed of decline and the percentage of individuals becoming undetectable, and enhanced lymphoproliferative response (LPR) to HIV-1 specific antigens (Moss et al., 2000).

Remune does appear to have potentially beneficial effects. Whether this translates to clinical advantage remains to be seen. Enhancement of the effects observed to date may also be of benefit. We therefore included Remune in the study described in Chapter Four of this thesis to observe its effect on T-cell phenotype and whether these were further influenced by IL-2 immunotherapy.
CHAPTER TWO: MATERIALS AND METHODS

2.1 Study populations and ethical approval

Participants were HIV-1 infected patients attending the St. Stephen’s Centre, Chelsea and Westminster Hospital. Different study populations were used for each study. Details specific to each study relating to participant characteristics are contained in the relevant chapter. Where required ethical approval was obtained for each study and all patients gave written informed consent.

2.2 Blood samples

All procedures were carried out according to health and safety regulations. Blood samples were collected at different time points for the different studies and are detailed in the relevant chapter. Peripheral venous blood was collected into sodium heparin and ethylenediamine tetraacetic acid (EDTA) containing vacutainer tubes (Becton Dickinson, Oxford, UK). Heparinised blood was used for lymphoproliferative, Cr$^{51}$ release and ELISPOT assays. EDTA preserved blood was used for flow cytometric and viral RNA and proviral DNA analysis.

2.3 Containment Level III (Cat III) training

All experiments involving HIV-1+ blood were performed in the Containment Level 3 Suite in accordance with the code of practice and the guidelines recommended by the Advisory Committee on Dangerous Pathogens (ACDP). Briefly, the Cat III training involved 30 hours of observation followed by 30 hours of supervised work, after which authorization to work in the Cat III suite was granted upon successful completion of an assessment.
2.4 Plasma separation

Plasma was separated by gravity or centrifugation for 10 minutes at 1800 rpm, aliquoted into 1/5ml skirted tubes (Elkay, Costello, Ireland) and stored at –80°C. The cell fraction was saved for the separation of peripheral blood mononuclear cells (PBMC).

2.5 Peripheral blood mononuclear cell (PBMC) separation from whole blood

The plasma volume removed was replaced with equal volume of RPMI 1640 medium (Sigma, Poole, UK) supplemented with 100IU/ml penicillin, 100µg/ml streptomyacin and 2mM L-glutamine (all Sigma). The cells were layered over 20mls of Histopaque (Sigma) and centrifuged at 2200 rpm for 20 minutes without brake. The interface, PBMC, was collected, transferred into sterile falcon tubes and diluted with sterile phosphate buffered saline (PBS) (Sigma), after which cells were washed at 1800 rpm for 10 minutes. The cells were counted (see 2.5.2) and aliquoted as required for functional and phenotypic assays. Remaining PBMC were frozen and stored in liquid nitrogen (see 2.6).

2.5.1 Cell counts and viability

The KOVA method was used for enumeration and determination of PBMC viability. This involved the transfer of a 50μl aliquot of cells into an eppendorf tube and the addition of 50μl of 0.4% Trypan blue solution (Sigma). After cells were mixed by pipetting, approximately 10μl was placed on a KOVA Glastic® slide (Hycor Biomedical Inc, Edinburgh, UK). The slide was placed on an inverted microscope and the PBMCs were counted using the x40 lens. The number (n) of cells in 9 small diagonal squares were counted and multiplied by 2 (Trypan blue dilution factor 1:1). This number was multiplied by 10⁴ (chamber factor) to get the number of cells per ml in the cell suspension. A
final multiplication by 50 (volume) gave the total cell yield in the 50ml falcon tube. Thus, total cell count = n x 2 x 10^4 x 50. Cells were then centrifuged at 1500rpm for 10 minutes with brakes set to 9. Once the supernatant was discarded the cell pellet was resuspended at 10^6 cells per ml in sterile PBS. This was then used for functional assays (LPR as detailed below) and phenotypic analysis (flow cytometry, as detailed below).

2.6 Cryopreservation and thawing of PBMC

PBMC were resuspended using 2 mls of cold (4°C) foetal calf serum (Sigma) supplemented with 10% dimethyl sulfoxide (Sigma). One ml was aliquoted into cryotubes (Fisher Scientific, Loughborough, UK) on ice. These were placed in a Naglene ‘Mr Frosty’ box (Fisher Scientific) and stored at –80°C for 24 hours. After 24 hours the vials were moved on dry ice into liquid nitrogen storage.

Thawing was performed as rapidly as possible. Cells were conveyed on dry ice and thawed and resuspended in 50mls of cold phosphate buffered saline (PBS, Sigma). They were spun immediately at 1800rpm at 4°C for 10 minutes. Cells were then washed twice in PBS.

2.7 Lymphocyte subset quantification

The Epics XL-MCL (Beckman Coulter) was used for four colour flow cytometric analysis of total CD3⁺, CD4⁺, CD8⁺, CD16⁺/CD56⁺ (NK cells) and CD19⁺ (B cells) lymphocytes, using whole blood. Murine monoclonal antibodies (mAbs) used were anti-human CD3, CD4, CD8, CD16/CD56 and CD19 (TetraOne, Beckman Coulter, High Wycombe, UK). These tests were conducted by the clinical laboratory staff at Chelsea and Westminster Hospital.
2.8 Plasma viral RNA assay

Plasma HIV-1 RNA copy levels were measured using the Quantiplex HIV RNA 3.0 Chiron branched DNA (bDNA) assay with a detection limit of 50 copies/ml (Chiron Diagnostics, Halstead, UK). These tests were conducted by the clinical laboratory staff at Chelsea and Westminster Hospital.

2.9 Immunophenotyping: Flow cytometric analysis

PBMC were processed either as fresh samples or as batched cryopreserved samples (see 2.6). Optimal volumes of antibodies were used as assessed independently by Imami, Sullivan and Burton (London, UK) and Autran and colleagues (Paris, France) as part of the INITIO study (Yenni et al., 2006). 10 µl of FITC and PE conjugated mAbs and 5 µl for PE-Cy5 conjugated mAbs (Table 2.1) were incubated with 100 µl of EDTA sample (1×10⁶ cells) for 30 minutes on ice in a darkened CAT III hood. PBMC were washed with PBS at 1500 rpm for 5 minutes and resuspended in 500 µl of 2% paraformaldehyde in PBS. Samples were analysed within 24 hours of preparation (refrigerated and covered if not acquired immediately). All reagents were kept in the dark at 4°C.

Three colour flow cytometry acquisition and analysis was performed on FACSCalibur with Cell Quest software (both Becton Dickinson). A combination of forward and side scatter density plots and CD3+ plots were used for gating. Two further gates were set around the CD4+ and CD8+ T-cell populations. 10,000 gated events were collected. Analysis was performed using logical gating in Cell Quest software. Conjugated isotype matched controls were used to set compensation and voltage.
Table 2.1  Murine monoclonal antibodies used for three colour flow cytometric analyses (Beckman Coulter, Marseilles, France).

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FITC - fluorescein isothiocyanate, PE - phycoerythrin, PC5 - PE-cyanine 5.

2.10 Proliferation assays

Proliferation assays were set up using a standardised protocol. Recall antigens, mitogens and cytokines were suspended in 10% AB plasma/RPMI (Sigma), at the concentrations below. 100μL was added to 96 well round bottomed microtiter plates (Greiner Laboritechnik, Stonehowe, UK). Each antigen was tested in triplicate, and two triplicates of TCM were set up as negative controls. Freshly separated PBMC were added to each well giving a final concentration of 10^5 cells/well. Antigens and mitogens used at concentrations as listed (Imami et al., 1999):

- Baculovirus-derived recombinant p24, EVA no.620, 10μg/ml
- Baculovirus-derived recombinant gp120, EVA no 646, 10μg/ml
- E.coli- derived recombinant nef, EVA no 650, 10μg/ml
- Influenza A, Central Public Health Lab (CPHL), 1/40
- Herpes simplex virus, CPHL, 1/40
Varicela Zoster, CPHL, 1/40
Cytomegalovirus, Behring, 1/40
Toxoplasma antigen, Behring, 10μg/ml
Purified protein derivative, Sigma, 10μg/ml
*Candida albican* NIBSC
Concanavlin A, Sigma, 10μg/ml
Pokeweed mitogen, Sigma, 10μg/ml
Recombinant IL-2, Boehringer, 20 and 100 U/ml

Plates were incubated for 5 days at 37°C and 5%CO₂. On day 5, 100 μl of supernatant was collected and stored at -20°C for subsequent cytokine measurement. Each well was then pulsed with 1 μCi ³H-methyl thymidine (³H-TdR; Amersham International, Amersham, UK) and 16 hours later cells were harvested onto glass fibre filtermats (Wallac Oy, Turka, Finland).

Proliferation as measured by beta particle emission from ³H-TdR incorporation was evaluated by liquid scintillation spectroscopy using a 1205 Betaplate counter (Wallac) (Imami et al. 1994). Results are expressed as stimulation index (SI) and as mean counts per minute (cpm) for triplicate cultures, with percentage error of the mean <15%. The SI was calculated by dividing the experimental beta particle cpm by the background cpm. A positive response is defined as a SI of five or more. Control wells, for calculation of background activity, contained PBMC only.

Recombinant HIV-1 antigens were obtained from Medical Research Council Centralised Facility for AIDS Reagents (NIBSC, Potters Bar, UK).

Performed by Dr Nesrina Imami and AKS.
2.11 Measurement of IL-2 and IL-4 production

Supernatant (50 µl) from proliferative cultures was transferred to a pair of 96-well round bottomed plates as triplicates for measurement of IL-2 and IL-4 using indicator cell lines CTLL-2 (European Collection of Animal Cell Culture, Salisbury, UK; ECACC) and CT.h4S (a generous gift of W. Paul, Bethesda, MD). 50 µL CTLL-2 at a concentration of $10^3$ cells/well or CT.h4S at a concentration of $5 \times 10^3$ cells/well was added giving a final volume of 100 µL. After 24 h wells were pulsed with $^{3}$H-TdR and harvested as described above (Imami et al., 1994, Fessel et al., 2000).

Performed by Dr Nesrina Imami and AKS.

2.12 Latent proviral DNA

HIV-1 proviral DNA was measured utilising spectrophotometric PCR methodology (Hardy et al., 1999) with an analytic sensitivity of 10 copies/µg of total cellular DNA.

Performed by Dr Nesrina Imami, Dr Antonio Pires, Dr Catherine Burton and AKS.

2.13 Statistical analysis

The statistical anlaysis used for each study is detailed in the relevant chapter.

All statistical analyses were conducted in collaboration with the onsite statistician, Dr Sundhiya Mandhalia.
These chapters have been removed for copyright or proprietary reasons.
The introduction and subsequent refinement of antiretroviral therapy for HIV-1 infection has undoubtedly revolutionised the care of individuals infected with HIV-1 worldwide. Through viral suppression it allows immune recovery, both quantitative and qualitative (Kelleher et al. 1996, Autran et al., 1997, Staszewski S et al., 1999, Pakker et al., 1998, Gallant JE et al., 2006, Mills AM et al., 2009, Molina JM et al., 2010). However this restoration is not complete and there is evidence that once lost, some facets of immune function cannot be restored utilising current therapeutic strategies (Autran et al., 1997, Hardy et al., 1999). In my thesis I have endeavoured to explore the clinical outcomes of cART, examining both its beneficial effects and residual deficiencies, and thereby set the scene for a more in-depth exploration of immunological therapies which may address such therapeutic inadequacies.

The mortality audit and discordant CD4 response study (Chapter Three) demonstrate significant benefit from cART; they also highlight the ongoing issue of incomplete qualitative immune recovery on cART. Although mortality has declined dramatically with the introduction of effective ARVT (Pallela et al., 1998, Lederman et al., 2000) and many patients dying with HIV-1 in the UK do so due to late diagnosis and delayed treatment initiation (Lucas et al., 2008), there remains a significant number who are still dying from conditions, such as lymphoma and other cancers, related to incomplete immune recovery despite fully suppressive cART (Lewden et al., 2007). This audit collected data on a large number of patients, attending two different UK centres over a long period. It demonstrated a fall in the crude all cause mortality rate from 7.3 to 2.6%, and a rise in the median CD4 T-cell count at death from 20 to 116 cells/µL blood. As with all retrospective audits however
it has its limitations, mainly related to data capture through inadequate documentation. I addressed these concerns by widening the data sources to include physician interviews, hospice and other hospitals’ records and coroner’s records as well as our local hospital records. Whilst I believe this maximised the accuracy regarding causes of death, the details of cART, in particular previous combination history and reasons for therapy change and cessation is less robust.

The discordant CD4 response cohort data demonstrates that not every individual realises the ideal treatment response. The most pertinent finding within this study is that an adequate CD4 T-cell rise is a clear predictor in terms of clinical outcome, irrespective of virological suppression. The findings add support to the theory that ongoing immune activation plays a role in those patients experiencing a discordant immunological response; with inadequate CD4 T-cell recovery despite an apparently fully suppressed viral load (Garcia et al., 2000, Leng et al., 2001, Anthony et al., 2003). This may be due to several factors. There may be ongoing low level viral replication which is not captured with routine VL monitoring due to current test sensitivity and the timing of sampling. Viral production from latently infected cells may be causing low level immune activation with the associated sequelae and reseeding of resting T-cells (Kelleher, 2008). Additionally antigenic stimulation from translocation of foreign proteins across the gut mucosa may play a key role in the generalised immune activation, with HIV-1 in fact playing more of a bystander role (Douek et al., 2009). It is thought that GALT is significantly depleted relatively early in HIV-1 infection (Brenchley et al., 2004), and this permits the passage of foreign proteins resulting in immune hyperactivation (Brenchley et al., 2006). Hyperactivation as measured by CD8+CD38+ is a prognostic indicator (Liu et al., 1997, Douek et al., 2009), and could
potentially be used as an extra SIM in addition to the CD4 T-cell count to predict treatment response, and also possibly to identify patients who may be suitable for additional therapeutic interventions; whether this be intensification of cART or immune therapies such as IL-2, IL-7 (Sereti et al., 2009) or immunosuppressants such as hydroxychloroquine (Richman et al., 2009). It is of interest that further support is afforded within the IL-2 Remune study where those patients who failed to achieve the CD4 T-cell requirement of 300 cells/μL blood for randomisation had higher mean baseline CD8+CD38+ T-cells and markedly less decline over the 16 weeks of cART with higher week 16 levels of activation as measured by this SIM. We decided this cut-off on the basis of other work suggesting better IL-2 responses in those with higher CD4 T-cell counts (Davey et al., 1997), and we may therefore have inadvertently excluded the very patients who may have demonstrated benefit. This adds further support to the use of additional or alternative SIM in initiating and monitoring immune therapy. The association I describe between the slope of the pre-cART CD4 T-cell decline and subsequent CD4 T-cell rise, not previously reported, could be used to produce a model for predicting cART outcome, and may even be of use in deciding the initial cART regimen or early interventions to address inadequate recovery. The effect on clinical outcomes such as disease progression and death of an adequate rise in the CD4 T-cell count regardless of the cART mediated effect on VL suggests measures which increase CD4 T-cell counts, possibly regardless of the effect on VL, may prove to be beneficial. This study included over one thousand patients recruited over six years and followed over a two year period, however as a retrospective cohort study it has inherent limitations, including potential bias and missing data. The statistical method employed (Chapter Three, 3.3.3) was chosen to reduce these
limitations as much as possible within this study design. The fact that all patients attended one treatment centre may affect some of these findings being applied to other settings. Although National prescribing guidelines are adhered to at this centre, individual physicians may respond differently to the different treatment outcome scenarios, and within different time frames. The other main limitation of this and other similar studies (Picketty et al., 1998, Perrin et al., 1998, Barreiro et al., 1999, Grabar et al., 2000) is a lack of uniform definitions. Additionally duration of follow-up and the inclusion of non-ART naïve patients in other studies make cross study comparisons difficult. However I believe given a difference was observed in clinical outcomes using our relatively generous definitions and that this difference was observed at 12 months, supports the definitions and time periods I selected. Building on these findings, possible future studies would clearly include longer term follow-up of this particular cohort, as well as studies to explore the potential of other SIM both as predictive and monitoring measures. Intervention studies would explore whether cART manipulation and/or immune therapies may influence discordant responses and whether this translates to clinical benefit.

The above study did not demonstrate any difference between the two classes of ARVT when only boosted PI therapy (now standard of care) was considered. There is however some in vitro data of a differential effect of NNRTI and PI on immune cell function (Andre et al., 1998, Chavan et al., 2001). The aim of the next study (Chapter Three, section 3.4) therefore was to see if a switch in therapy, already planned for a clinical indication, impacted on the individual’s HIV-1 specific responses, as well as recall antigen responses and IL-2 responsiveness. We did find an improvement in all three parameters including HIV-1 specific responses following a switch to a NNRTI
based regimen. Unlike similar recovery in other studies (Gotch et al., 1999) the responses were sustained out to 24 weeks. Although HIV-1 specific responses are recognised as advantageous in, for example LTNP and elite controllers (Westrop et al., 2009), it is not known whether their restoration translates to clinical advantage. Although intuitively one would assume this to be the case it would need to be demonstrated before it could be advocated as a therapeutic strategy (Imami et al., 2007). Demonstrating this in a larger cohort and over a prolonged period would be a significant undertaking and pragmatically of unclear benefit as NNRTIs are currently the first line recommended ARVT in the UK (Gazzard et al., 2008). A smaller more realistic study would be to examine any potential association between this possible recovery of HIV-1 specific responses with improved control of low level viral replication and production and resultant improvement in immune activation markers. If these findings are borne out in larger studies, it may well have implications for future trials of immune therapy, and potentially the findings of IL-2 studies to date.

On this background I then carried out several further studies to examine in more detail the effect of cART, IL-2 and Remune on the immune system in different therapeutic settings. The aims of this work were to examine whether, in addition to confirming the findings of other researchers in respect to IL-2 and standard SIM, we could identify any new effects, particularly in relation to the novel immune therapeutic strategy we employed in the IL-2 Remune study. I was also interested to see if we could define alternative or additional SIM to drive IL-2 scheduling and monitor its effect. Furthermore I wanted to explore possible benefits in those on no cART and in those with few treatment options at extreme risk of clinical progression. In summary, the studies found a beneficial effect on several parameters which translate to a degree of
immune recovery. The side effects were tolerable for the majority of patients. Given the fact that these were all pilot studies or compassionate release programmes, it is not possible to draw firm conclusions due to the small number of patients with resulting wide confidence intervals, however several interesting findings were observed. IL-2 causes a return towards normalisation of absolute CD4 and CD8 T-cells and their ratio in the majority of patients. We also observed a decrease in T-cell activation in patients who received IL-2. These changes were of a greater magnitude and more sustained in those patients receiving Remune in addition to IL-2. The rise in CD4 T-cells would be expected to translate to clinical benefit but in the large clinical outcome trials to date (Abrams et al., 2009), this does not appear to be the case. There are several possible explanations for this. It may be the CD4 T-cells associated with IL-2 therapy are in some way different to those associated with cART and do not confer benefit, as suggested by Sereti and colleagues (Sereti et al., 2000). IL-2 may induce some other effect which we are not capturing which negates the potential benefit of the elevated CD4 T-cell count. It may also be that we are using the incorrect SIM to drive repeated cycles and are therefore not maximising the potential therapeutic benefit of IL-2 therapy. Patient selection may have reduced any potential beneficial effects of IL-2 therapy by not mandating a high CD4 cell nadir and effective virological control. It may also be that IL-2 therapy should be more targeted to certain patients who may benefit more, due to specific characteristics, for example ongoing immune activation or a discordant immune response in relation to CD4 T-cell recovery. A novel finding in the Remune IL2 study of particular interest is the viral load blips associated with the IL-2 cycles which resulted in recovery of HIV-1 specific responses, some of which were sustained. This is in contrast to some of the STI studies and suggests a
synergistic action between IL-2 and low level viraemia. Despite associated IL-2 cycle related activation, this did not result in a sustained hyperactivation, and in fact there was less activation of CD8 T-cells in those patients who had received IL-2 and Remune. We also observed significant increases in CD25 expression. This surface receptor is a marker for both activated T cells and Tregs; cells which play a key role in immunomodulation and control of the immune response. One limitation of this observation was that we did not include other markers to better characterise these cells which were not available at the commencement of this study. Some authors believe IL-2 induced Tregs are less immunosuppressive than those stimulated by ‘natural’ antigen (Sereti et al., 2000). However we did not find evidence to support this overall, as in the IL-2 Remune study we observed both a sustained increase in CD4+CD25+ T-cells and a reduction in immune activation. However it may be this lack of suppressive function is reflected in the peri-cycle increase in activation and HIV-1 specific responses; not being suppressed by the cycle associated rise in Tregs. The subsequent stimulation with Remune may then have influenced these cells to behave in a more typical fashion. This however is highly speculative and these observations can only be considered an association not causation. The observations however are of sufficient interest and novelty to warrant further investigation. In several analyses it did appear the observed differences between the two groups were being driven by the patients who received both IL-2 and Remune and in some instances the responses were more sustained in this arm. This was observed as described above for CD4 and CD8 T-cell counts and CD8 activation and also for the co-receptor CD28 expression. The Remune was administered with the first IL-2 cycle and then at three time points subsequent to the final cycle of IL-2 and it may be there is a synergistic
effect, with IL-2 priming the immune system to be more receptive to therapeutic immunisation. It is of interest to note the change in CD4 T-cells was more marked and sustained in the memory/effector subset. The lack of any real difference in DTH as measured clinically again raises the question of translation to clinical benefit. However DTH is a relatively insensitive measure and the scheduling we employed for IL-2 and Remune administration may have influenced this to a degree.

These studies were exploratory pilot studies and a compassionate release programme and as such were not powered to detect a significant difference between the groups. However the IL-2 Remune study was a randomised controlled study and this should address some of the potential biases. As with all IL-2 studies the side effect profile of IL-2 does not permit placebo arms. Although clearly limited by the small numbers the novel approach to the administration of immune therapy resulted in several novel, interesting observations and raises several questions which should be further explored in larger studies (Downey et al., 2010).

In conclusion, cART contributes significantly to immune reconstitution but it is incomplete. IL-2 and therapeutic immunisation result in enhancement of the immune recovery seen with cART, at least at a cellular level. The challenge is to determine how this can be translated to clinical benefit. Using additional SIMD, such as CD38, CD28 and CD25, may help in determining those who may benefit most, as well as monitoring treatment response and driving maintenance therapy. The timing and schedule employed will be key and warrants further investigation. It is likely however, as with ARVT, immunotherapy will require a similar approach with a combination of different biological therapies acting via various different mechanisms and targeting distinct components of the immune system to produce a variety of
changes to better normalise immune function. This could potentially consist of cytokines (for e.g. IL-2, IL-7, GM-CSF), therapeutic immunisation, ideally inducing both HIV specific responses and neutralising antibodies, and immuno-modulators such as hydroxychloroquine. Further research is required and although demonstrating clinical benefit is essential to underpin any therapeutic recommendation, the recent negative clinical trial results should not lead to the abandonment of IL-2 as a potential HIV -1 therapy; it may be that similar to zidovudine being used as monotherapy in the eighties, we haven’t got it wrong; we just haven’t quite got it right.
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CHAPTER EIGHT WORK ACCEPTED FOR PUBLICATION AND PRESENTATIONS

The first section relates to work included in the thesis; published papers are in Appendix. The second section is additional research I have carried out in collaboration with colleagues as part of the studies included in my thesis.

Section One
Papers and letters published in peer review journals


Reviews
Imami N, Sullivan AK, Gotch FM. Immunomodulation in HIV-1 infection in the HAART era. J HIV Therapy 2001; 6:77-84


Peer reviewed abstract presentations
Sullivan AK, Mandalia S, Nelson MR, Gazzard BG. Discordant immunological and virological responses to HAART in ARV naïve HIV infected individuals. IAS, Rio de Janeiro, July 2005, WePe12.2CO1


7th Annual Meeting of the British HIV Association, Brighton April 2001


2. Sullivan AK, Pires A, Hardy GAD, Gotch FM, Gazzard BG, Imami N. Phenotypic T cell changes in HIV-1 infected individuals receiving highly active antiretroviral therapy. [abstract P204]

3. Sullivan AK, Nelson MR, Pozniak AL, Gazzard BG. Interleukin-2 therapy in HIV-1 infected individuals with late stage disease and failing antiretroviral therapy. [abstract P200]

5th International Congress on Drug therapy in HIV Infection, Glasgow October 2000


13th International AIDS Conference, Durban July 2000


6th Annual Meeting of the British HIV Association, Edinburgh March 2000


Section Two


8th CROI, Chicago USA, Feb 2001


2. G Hardy, N Imami, A Sullivan, J Wilson, C Burton, R Moss, B Gazzard, F Gotch. Effects of combined treatment with IL-2 and an inactivated gp120 depleted HIV-1 immunogen (remune) on immune reconstitution in HAART treated HIV-1 infected individuals.
BSI/BSACI Congress, 1999
Interleukin-2-associated viral breakthroughs induce HIV-1-specific CD4 T cell responses in patients on fully suppressive highly active antiretroviral therapy
Sullivan, Ann K; Hardy, Gareth AD; Nelson, Mark R; Gotch, Frances; Gazzard, Brian G; Imami, Nesrina

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