Cell-Delivered Gene Therapy for HIV

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1. Introduction

Gene therapy involves the transfer of genetic material into cells of an individual to treat an underlying illness either through the expression of advantageous genes or the silencing of disadvantageous ones (Flotte 2007; Kohn and Candotti 2009). Gene therapy has been used successfully to treat several diseases, for example SCID-X1 (Cavazzana-Calvo, Hacein-Bey et al. 2000) and SCID-ADA (Aiuti 2004) and holds out promise as a more general treatment regimen (Flotte 2007). One of the driving forces behind the area of research into the treatment of HIV is the resistance to, and side effects of, the current drugs being used. This development of resistance and the need for continuous and ongoing daily medication have been major shortcomings of conventional highly active antiretroviral therapy (HAART) when employed as a treatment against HIV (Perno, Moyle et al. 2008). An additional driving force behind interest in gene therapy is the potential for a one-off treatment that would continue to work for the life of the individual (Symonds, Johnstone et al. 2010). One can envisage gene therapy as a full or partial replacement for HAART, that may help to overcome issues of viral resistance, co-morbidity and attendant compliance (i.e. daily administration of HAART for life).

While HAART is a systemic form of treatment which provides a substantial level of protection to HIV susceptible cells in the body for many years, it is highly susceptible to the development of a resistant HIV quasispecies, that may selectively expand due to the strong evolutionary pressure exerted by HAART (Perno, Moyle et al. 2008). Whereas HAART-based treatments bathe each cell in some level of drug, gene-therapy results in a polar population dynamic consisting of gene protected and unprotected cells. This is due to the fact that it is neither practical nor possible to have a protective gene against HIV introduced into all cells of the body, but rather only a subset of the total cell population is afforded protection (Symonds, Johnstone et al. 2010). This polar dynamic is predicted to provide additional pressures to the suriviving HIV population (Applegate, Birkett et al. 2010). Cells that might be afforded protection include CD4+ T cells and macrophages, which are known to be targets of HIV infection, as well as other cell populations susceptible to HIV infection.

In this chapter we describe the biological and clinical underpinnings of gene-therapy including the therapeutic genes employed for protection against HIV, delivery methods of

the vectors carrying these protective genes into the cells, expression cassettes and finally the target cells into which the protective genes are introduced. We then estimate the potential in-vivo protective effects of gene-therapy against HIV.

2. Biological and clinical aspects of gene-therapy

In this section we look at the biological and clinical aspects of gene-therapy. Observations associated with natural immunity that may be utilized in gene-therapy against HIV are discussed in section 2.1. Stages of the HIV infection cycle that may be inhibited by gene-therapy, and the various gene therapeutic that may be employed to this aim, are the subject of section 2.2. Various delivery vectors and promoters that can achieve effective delivery and transcription of the protective gene into the cell to be transduced are the subject of section 2.3. The biological underpinnings of the target cell to be transduced with a protective gene, either CD4+ T cells or Hematopoietic Stem Cells (HSC) are discussed in section 2.4. The clinical aspects of collection of cells for transduction via apheresis and associated preparation regimens are discussed in section 2.5. Finally, Section 2.6 is concerned with clinical trials to-date of anti-HIV gene-therapy and results reported therein.

2.1 CCR5 and the 32-nucleotide deletion mutation: A strong case for gene therapy

Recent additional impetus for gene-therapy for HIV is based upon the earlier observation that some individuals do not become infected upon repeated exposure to HIV (Zimmerman, Buckler-White et al. 1997). Studies of these individuals led to the discovery of a mutation in CCR5, an important co-receptor for HIV attachment to target cells prior to infection. Such a mutation was found to confer natural immunity against HIV (Zimmerman, Buckler-White et al. 1997).

The mutation discovered was found to be a 32 nucleotide deletion (CCR5d32) within the CCR5 gene (Zimmerman, Buckler-White et al. 1997). This mutation was observed to be very common among individuals of European background and it has subsequently been determined that of Caucasian individuals, approximately 10% are heterozygous and 1-3% homozygous for this mutation (Dean, Carrington et al. 1996; Liu, Paxton et al. 1996; Samson, Libert et al. 1996; Agrawal, Lu et al. 2004), with the mutation being almost non-existent in all other populations. There has been considerable speculation regarding the origin and purpose of the mutation. It has been shown that the percentage of CCR5d32 mutation occurring in today's population is roughly comparable to that found in samples from individuals of the Bronze Age (approximately 3000 years ago) (Hummel, Schmidt et al. 2005; Hedrick and Verrelli 2006). There is evidence suggesting that smallpox provided a selective advantage for CCR5d32 (Galvani and Slatkin 2003), indicating that there may be other selective advantages associated with the mutation. The mutation does not seem to present any significant disadvantages to the individuals other than an increased risk of West-Nile disease (Glass, McDermott et al. 2006). Such observations led to an interest in mimicking this natural mutation for HIV-infected individuals via genetic manipulation (i.e. transduction) of cells vulnerable to HIV infection. (see below)

It has been noted that the 32 nucleotide deletion results in 31 new amino acids being coded for, resulting in an active CCR5d32 protein. This protein instead of presenting as CCR5 receptors on the cell surface like the wild-type counterpart, CCR5d32 actually binds to and interacts with CXCR4 receptors (Agrawal, Lu et al. 2004), the other major coreceptor for HIV attachment. This provides an additional protection against HIV infection beyond the mere

absence of a functional CCR5 co-receptor, especially when concerning strains capable of utilizing the CXCR4 coreceptor (Agrawal, Lu et al. 2004; Jin, Agrawal et al. 2008). Further evidence towards the beneficial effect of the CCR5d32 protein comes from evidence that a polymorphism in the promoter region in CCR5-/- individuals can affect the protective capabilities of the d32 mutation. It has been demonstrated that an increase in CCR5d32 protein expression will improve resistance to HIV, while decreased CCR5d32 expression reduces the protective effect (Jin, Agrawal et al. 2008).

This CCR5d32 mutation has been successfully utilized in a patient, who suffered from both HIV/AIDS and leukaemia (Hutter, Nowak et al. 2009; Allers, Hutter et al. 2010). This individual, termed the "Berlin patient", had complete ablation of their immune system (to treat the leukemia) before matched allogeneic donor hematopoietic stem cells (HSC) homozygous for the CCR5d32 mutation were transfused into the patient. After one recurrence of leukaemia and a repeat of the treatment (ablation and reconstitution), the patient has had undetectable levels of HIV (and no recurring leukaemia) for more than 3 years without the use of any antiretroviral drugs (Hutter, Nowak et al. 2009; Allers, Hutter et al. 2010). This unique result of "functional" cure of HIV indicates significant potential for the use of gene-therapy to mimic this result by down-regulation of CCR5.

2.2 Choosing a stage of HIV infection cycle to inhibit: Which therapeutic genes hold out promise?

2.2.1 Classes and methods of HIV inhibition

Gene-therapy may be aimed to target various stages of the HIV infection cycle as shown in Figure 1. Class 1 therapy inhibits all steps prior to viral integration into the cellular genome, Class 2 inhibits expression of viral genes and Class 3 inhibits production of new virions once integration and expression has taken place (von Laer, Hasselmann et al. 2006). According to predictions from mathematical modelling, as discussed in section 3.1, Class 1 gene therapies are likely to be the most effective as they inhibit HIV at the first steps, and provide a selective advantage to these cells by avoiding any viral or immunological induced death from infection. Hence many gene therapeutics currently under investigation include components that impair attachment or fusion stages of the viral life-cycle (Symonds, Johnstone et al. 2010).

While all these classes are potential HIV gene therapeutics, practically, the use of multiple therapeutics in combination is likely to be the most effective method. This is analogous to the antiretroviral situation where it does not take long for HIV resistance to emerge against single antiretroviral drugs. These antiretroviral drugs have been shown to be far more effective when used in combination. It is for this reason that gene therapy research has often been focused on the use of multiple gene therapuetics used in conjunction with one-another. As well as the variety of targets being investigated, there is additionally a wide range of methods to achieve inhibition of these targets. The most commonly employed methods to-date include the following:

Antisense (Class 2): Antisense RNA is a synthetic nucleotide sequence that binds to mRNA in order to inhibit its function. This method can be used against a wide range of targets, including the HIV envelope (Levine, Humeau et al. 2006).

Aptamers (Class 2 or 3): Aptamers are single-stranded RNAs or DNAs. They disrupt at the protein level by tightly binding to their target ligand (Que-Gewirth and Sullenger 2007). Aptamers can be used to target a wide array of proteins and as such have potential to be used in multiple settings.
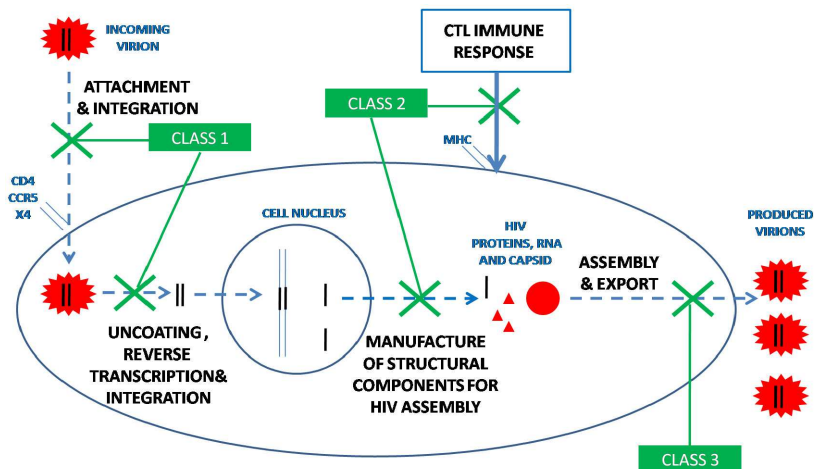
Fig. 1. The three Classes of gene-therapy according to cycle of HIV infection inhibited as defined by von Laer et al(von Laer, Hasselmann et al. 2006; von Laer, Hasselmann et al. 2006). Class 1 inhibits all steps in the infection cycle prior to integration of the HIV RNA into the cellular genome. In particular, Class 1 inhibits either the entry of the HIV virion into the cell (i.e. inhibition of attachment/integration of the HIV virion through the CD4, CCR5 and X4 receptors/coreceptors) or inhibits integration into the cellular genome once a virion has entered the cell (i.e. blocks uncoating, reverse transcription or integration). Class 2 inhibits gene expression and the production of structural components required for the assembly of new HIV virions. Class 2 also results in lower susceptitibliy to cell death through the cytotoxic T lymphocyte (CTL) immune response, as a result of reduced recongnition via the Major Histocompatiblity Complex (MHC). Finally, Class 3 inhibits the assembly and export of virions from the infected cell.

Intracellular Antibodies (Class 1 or 3): Intracellular antibodies, or "intrabodies", are designed to bind to and inactivate target molecules inside host cells (Chen, Bagley et al. 1994). One target which has been used by intrabodies is CCR5, whereby the intrabodies bind to CCR5 and block surface expression (Rossi, June et al. 2007).

Ribozymes (Class 2): Ribozymes are catalytic RNA molecules that have the ability to degrade RNA in a sequence-specific manner (Sun, Wang et al. 1995). When used as anti-HIV agents, they have the potential to target multiple steps, affecting incoming RNA (during infection, in this sense they can act in part as Class1), primary RNA transcripts (from integrated provirus), spliced mRNAs and mature RNA being packaged into virions. These are primarily Class 2 inhibitors and examples are those designed to target the conserved regions of HIV such as the overlapping regions of *vpr* and *tat* reading frames (Mitsuyasu, Merigan et al. 2009). Highly conserved regions are desirable as targets so that sequence specificity is more likely to be maintained.

Short hairpin RNA (Class 1 or 2): Short hairpin RNA is a sequence of RNA that folds back upon itself in a hairpin turn; it can be used to initiate RNA interference and consequently silence gene expression (McIntyre and Fanning 2006). shRNA expression vectors utilise a promoter to drive expression of the shRNA. As an integrated vector, this expression cassette will be passed on to daughter cells, allowing the gene silencing to be maintained *in vivo*. The

shRNA hairpin structure is cleaved by the cellular machinery into siRNA which is then bound to the RNA-induced silencing complex. This complex binds to and cleaves mRNAs which match the siRNA that is bound to it (Hannon and Rossi 2004). The use of siRNA for gene silencing has become a method of choice and can be potentially applied to many targets, including down-regulation of CCR5 that will decrease target cell infectivity by HIV and other host receptors as the removal or impairment of these receptors will render HIV non-infectious (Class 1).

Class	Target Site	Why	Goal	How
1	CCR5	Important co-receptor	Remove/prevent expression of CCR5	Zinc-finger, siRNA
1	CD4	Essential receptor for HIV attachment	Remove/prevent expression of CD4	Zinc-finger, siRNA
1	CXCR4	Important co-receptor	Remove/prevent expression of CXCR4	Zinc-finger, siRNA, ribozyme
1	Membrane Fusion (HIV heptad repeat)	Essential for viral entry	Prevent entry of HIV through host-cell membrane	siRNA
2	Tat	Important for Transcription	Disrupt *tat* gene	Tar decoy, siRNA, ribozyme
2	Rev	Important for virion Translation	Disrupt *rev* gene	siRNA, REV mutants
3	Env, Protease, Helicase	Important for virion maturation	Prevent virion maturation	siRNA, Antisense RNA,
3	Gag	Important for virion assembly	Disrupt gag gene	Ribozyme, siRNA

Table 1. A list of some HIV gene therapy targets, the goals and the mechanics of how they are being explored. This table shows a variety of Class 1, 2 and 3 therapies and the range of approaches against targets.

Fusion Inhibitors (Class 1): One fusion inhibitor which has been researched in detail is the maC46 peptide (C46) (Zahn, Hermann et al. 2008). It inhibits viral fusion by interacting with the N-terminal hydrophobic alpha-helix. This prevents changes essential for membrane fusion of the virus and host cell. This fusion inhibitor has been found to be highly effective at blocking HIV replication (Zahn, Hermann et al. 2008).

Zinc Finger Nucleases (ZFNs) (Class 1 or 2): ZFNs bind to targeted open reading frames. Two juxtaposed ZFN's on DNA results in dimerisation of the endonuclease domains, generating a double-stranded break at the targeted DNA (Porteus and Carroll 2005). The

mutagenic pathways relied on to repair the DNA breaks result in nucleotide mutations at the break-sites, thus permanently disrupting the gene (Porteus and Carroll 2005). While experiments in mice have shown this method to be effective, there is still a risk of non-target directed mutagenesis. Another limitation of this technique is the inability to add protective genes, as only the effective deletion/inactivation of genes can be performed, thus limiting the applications for the use of ZFNs to applications such as inactivation of CCR5 (Class 1).

2.2.2 Strong arguments for gene-therapy based entry inhibition

Class 1 inhibitors generally act at the level of HIV binding to the target cell (von Laer, Hasselmann et al. 2006). It is expected that this would be the most effective as HIV is blocked from entry to the target cell and any subsequent replication steps cannot take place. An important recent contribution to the argument for Class 1 inhibitors is the discovery of the cause of the so-called 'bystander effect' where apparently non HIV infected cells also succumb to HIV pathogenesis (Doitsh, Cavrois et al. 2010). The observation that productively infected cells are not the only contributors to host-cell death has been noted previously, however the cause of this cell death had remained unknown until Doitsh et al discovered abortive/nonproductive HIV infection in host-cells (approximately 95% of infected cells) and the induction of apoptosis in these cells (Doitsh, Cavrois et al. 2010). This "bystander effect" is likely to have contributed to the lack of success of some antiretroviral therapy methods, including a variety of clinical trials whereby HIV infection was only inhibited after HIV entry, as host-induced apoptosis would greatly reduce the effectiveness of treatment. This effect indicates a crucial additional benefit of entry-inhibitors over other classes of antiviral treatment.

One of the resistance mechanisms developed by HIV against the antiretroviral CCR5 antagonists, such as maraviroc, is not just the use of other co-receptors such as CXCR4, it is the use of maraviroc-bound CCR5 receptors (Westby, Smith-Burchnell et al. 2007). This mechanism of resistance would not be available against cells containing a down-regulation, or mutation-mediated deletion of the CCR5 receptor produced by gene therapy. An added bonus of the use of attachment and/or fusion inhibitors is that they do not provide cross-resistance with other treatment methods such as protease and integration inhibitors.

2.3 Vectors, delivery methods and promoters: Delivering the protective gene into the cell

The therapeutic used in gene therapy must be carried within a suitable vector or delivery system; for HIV gene therapy these vectors should generally be capable of integrating into the host cells with minimal risk of generation of replication competent lentivirus or insertional mutagenesis (Wu, Wakefield et al. 2000; Symonds, Johnstone et al. 2010). The vector must also be non-toxic to the host while allowing the expression of the relevant gene(s). There are many techniques and delivery vectors which can be utilized for this purpose. Examples of the most commonly used delivery vectors are shown in Table 2.

Transposon-based delivery systems consist of a synthetic transposon and an associated transposase and work via a cut-and-paste mechanism whereby the transposase recognises the inverted direct sequences in the transposon, and then the transposon is excised and later integrated into a target DNA region (Tamhane and Akkina 2008). They can, for example, be used to carry shRNAs.

Delivery Vector	Advantage	Disadvantage
Transposons	Can provide permanent expression of multiple genes	Potential for insertional mutagenesis
Plasmid DNA Nucleofection	Treatment has been highly effective (Holt, Wang et al. 2010)	Slight increase in apoptosis of HSCs (Holt, Wang et al. 2010)
Murine Leukaemia Virus	Little/no adverse effects (Amado, Mitsuyasu et al. 2004; Macpherson, Boyd et al. 2005)	Can only infect actively replicating cells (Roe, Reynolds et al. 1993) May induce insertional mutagenesis (Symonds, Johnstone et al. 2010)
Adenovirus	Can infect non-replicating cells (Zhang, Sankar et al. 1998)	Innate immune response (Liu and Muruve 2003)
Lentivirus	Can infect non-replicating cells (Zufferey, Dull et al. 1998), Does not effect proliferation or differentiation of HSCs (Gervaix, Schwarz et al. 1997)	Slight risk of insertional mutagenesis (Philippe, Sarkis et al. 2006)
Conditionally replicating virus	Higher transduction efficiency	Risk of mutation/recombination

Table 2. A variety of commonly used delivery vectors and their associated advantages and disadvantages.

Nucleofection of DNA involves directly adding the DNA into the targeted cells by disrupting the cell membrane through electroporation. While this is not an ever-present biological vector as those mentioned above, it is more of an event-based vector which can provide a method of entry either for less entry-capable vectors, or plasmid DNA (Aluigi, Fogli et al. 2006).

Viral delivery vectors are typically made from the backbone of suitable viruses, whereby pathogenic, and (often) replication-mediating genes are removed, and only the essential genes remain (Kootstra and Verma 2003). The therapeutic gene(s) being used is/are then added to the viral backbone. The virus is then able to infect host cells as would its natural counterpart. However without the ability to replicate or express harmful genes. It is used only to integrate into the host genome and allow the therapeutic gene to be active.

One of the main concerns regarding gene therapies is the potential for insertional mutagenesis. This has been shown to occur in SCID-X1 trials (Howe, Mansour et al. 2008) where the insertional mutagenesis led to myeloproliferation/leukemia (Howe, Mansour et al. 2008). While insertional mutagenesis events have occurred in this and a few other gene therapy trials eg CGD (Stein, Ott et al. 2010), they have not occurred in HIV gene therapy trials, and a great deal of effort is undertaken to ensure that this event does not occur.

To ensure efficient transcription of the therapeutic gene, a suitable promoter is required. A promoter is a region of DNA that facilitates the transcription of nearby downstream gene(s), and is essential for the efficient expression of the desired gene(s). The choice of the promoter to be used in gene therapies is highly important, and various promoters have been tested in laboratory studies and clinical trials. Promoters currently in use in HIV gene therapy studies

are quite diverse and include U6 (human derived), T7 (bacteriophage derived), and Ubc (Human ubiquitin c) (Anderson, Banerjea et al. 2003; Boden, Pusch et al. 2003; Weber and Cannon 2007):

There have been studies using different promoters in HIV gene therapy work-up and many have been shown to be effective. However, due to the many different therapeutic genes, their delivery vectors, and the cells targeted for transduction, it is difficult to determine which promoters are the most effective and as such, each needs to be tested.

It has been noted that a highly expressive promoter may not be the ideal candidate, as many highly efficient promoters can have other side-effects. As noted above, of key concern is the trans-activation (insertional mutagenesis) of nearby cellular genes (Weber and Cannon 2007), potentially leading to oncogenic effects by over-expression of important proteins.

2.4 Transduction targets: Which cells should be protected against HIV?

HIV infection is typically characterized by CD4+ T cell infection and depletion. In addition, other cells are also infected by HIV, including macrophages and monocytes and most recently there have been reports of hematopoietic stem cell (HSC) infection (Stanley, Kessler et al. 1992; Carter, Onafuwa-Nuga et al. 2010; Carter, McNamara et al. 2011). In the case of gene therapy for HIV the two most common cell types that have been transduced to date with the therapeutic relevant gene are CD4+ T lymphocytes and HSC. Transduction of these cells is expected to provide the best outcome due to CD4+ T cells being the main targets of HIV infection and the ability of HSC to differentiate into all susceptible cells. In this subsection we discuss the biological aspects of transducing either CD4+ T cells or HSC with a protective gene.
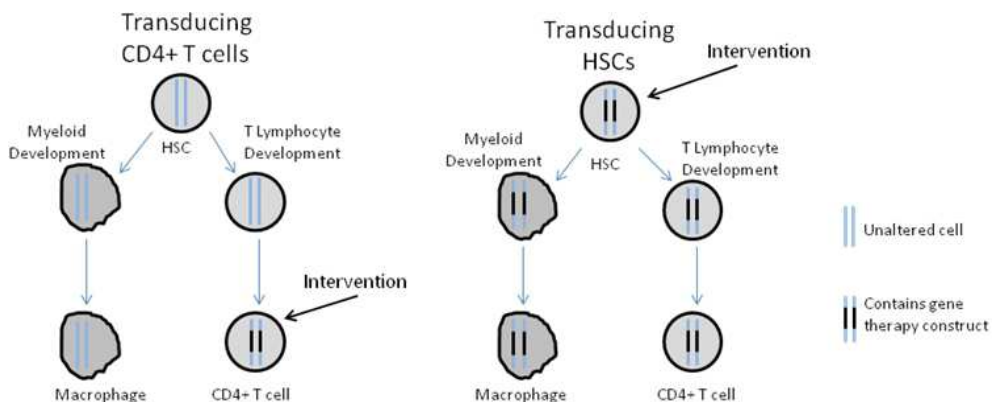

Fig. 2. Two ways of achieving cell populations protected against HIV as a result of either transducing CD4+ T cells or HSC. If the CD4+ T cell population is transduced with a protective gene (left), then protection against HIV is only afforded to CD4+ T cells. If on the other hand HSC are transduced with a protective gene (right), then the protected gene is retained by all cells derived from the HSC via differentiation through the myeloid (e.g macrophage) and lymphoid lineages (e.g CD4+ T cell). The approach of transducing HSC thus provides protection against HIV to a broader class of cells.

2.4.1 Transduction of CD4+ T cells

The use of CD4+ T cells as target cells for HIV gene therapy has been explored in several studies (see section relating to clinical trials). Isolation and transduction of CD4+ T cells is relatively simple. The key advantage of targeting CD4+ T cells is the ease with which they may be accessed. As they largely populate and regularly traffic through peripheral blood, no stimulatory factors are required to mobilize them prior to collection. Conceptually it can be envisaged that the introduction of a protected population of CD4+ T lymphocytes should have impact as these are the cells specifically depleted by HIV infection; the greater the severity of HIV infection the greater the CD4+ T lymphocyte decline.

One such study involving the therapy of CD4+ T cells was performed by Levine in 2006 (Levine, Humeau et al. 2006) whereby peripheral blood CD4+ T cells were harvested from each subject by apheresis. The collected samples were then depleted of CD8+ cells and monocytes, transduced with the gene construct *ex vivo*, activated via CD3 and CD28 costimulation and expanded before being re-infused into the patients. This method of therapy was shown to be both safe in treatment, and effective in delivery of the therapeutic gene (Levine, Humeau et al. 2006; Brunstein, Miller et al. 2011).

Predicted in-vivo dynamics of CD4+ T cell transduction, based on mathematical modelling, are discussed in section 3.2.1.

2.4.2 Transduction of hematopoietic stem cells (HSC)

Due to the range of cells which HIV infects, it is thought to be a significant advantage to transduce HSC, as these cells provide a continuous supply (following differentiation) into a range of immunological cells (monocytes, macrophages, CD4+ T cells, CD8+ cells, dendritic cells, microglial cells) which may thus be protected against HIV infection (Carter and Ehrlich 2008). A delay in the newly 'protected cell' production would be expected, thus delaying the effect of the therapeutic gene(s). However, there can still be a significant production of CD4+ T lymphocytes, the supply of which has been predicted to be a rate of approximately 1.65 cells/μL of blood/day (due to thymic reconstitution) (Murray, Kaufmann et al. 2003). This results in the production of a stable population of protected cells which could impact on CD4+ T cell number and viral load.

While CD4+ T cells (and other cell types common in peripheral blood) can be obtained relatively simply prior to transduction by apheresis from peripheral blood, HSC must first be mobilised from the bone marrow (discussed in detail below). This creates an additional component to the treatment process. Currently the most common method for the mobilisation of HSC is the use of granulocyte colony stimulating factor (G-CSF), a treatment that usually spans 4-5 days before the apheresis of peripheral blood can begin. Predicted in-vivo dynamics of HSC transduced with a protective gene are discussed in section 3.2.2.

2.5 Collection of cells for transduction: Apheresis and treatment methods for optimized and high-volume cell collection

Current gene therapy protocols for HIV require the isolation of the relevant cells to be transduced, generally following apheresis (Symonds, Johnstone et al. 2010). Apheresis is the process of removing mononuclear cells from blood and returning neutrophils, platelets, plasma and red blood cells to the donor, in order to collect more of one particular part of the blood than could be separated from a unit of whole blood. Apheresis allows for the collection of large quantities of cells, and in the case of gene therapies for HIV, total lymphocytes, CD4+ cells, or HSC are the cell types collected.

It is common practice to use a stimulating agent such as G-CSF in order to increase the quantity of HSC in the peripheral blood. The resulting increase in cell numbers in peripheral blood is due to redistribution of cells from other compartments of the body (i.e bone marrow and lymph tissue). The use of G-CSF and other stimulating factors is essential when HSC (largely inhabiting the bone marrow) are to be transduced with the therapeutic gene. Various trials have shown HSC cell counts in peripheral blood increase 20-50-fold over the course of GCSF administration (Lane, Law et al. 1995; Law, Lane et al. 1999; Valgimigli, Rigolin et al. 2005).
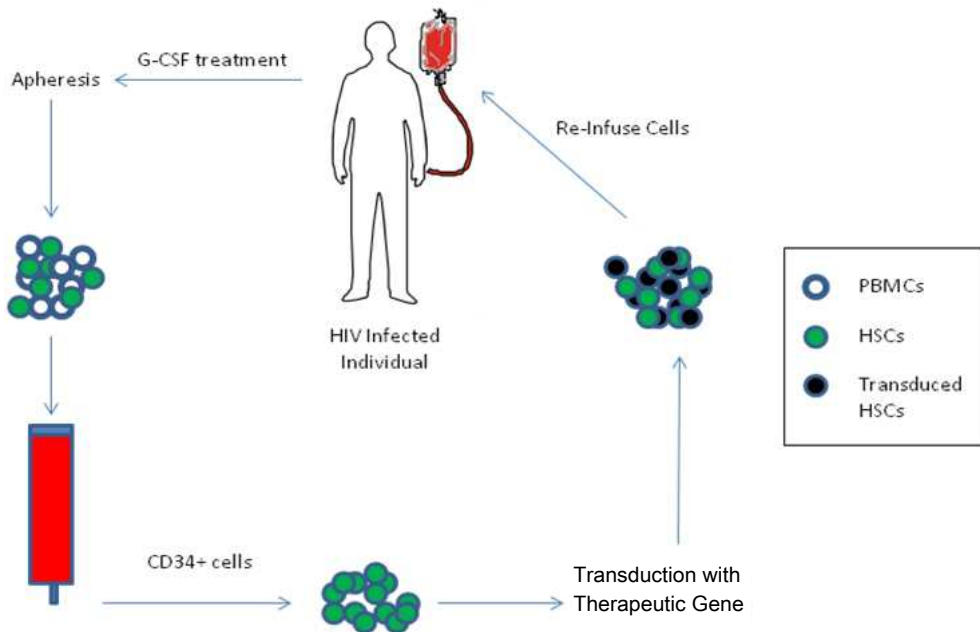

Fig. 3. Illustration of the clinical aspects of therapeutic apheresis (for HSC harvesting), and the subsequent processes of transduction and reinfusion. The HIV infected individual is first administered G-CSF in order to effect mobilization of HSC from bone marrow into peripiheral blood. The mobilized HSC are then collected from peripheral blood and subsequently transduced with a protective gene. The transduced HSC are then reinfused into the patient.

A technique known as myeloablation has been utilized in some clinical trials (before the transduced cell infusion) in order to improve engraftment of the gene-containing cells (Strayer, Akkina et al. 2005). This procedure involves the killing of HSC, thereby reducing the endogenous non-transduced cells, thereby creating more space for the transduced cell population.

2.6 Important studies involving gene-therapy: Promising results and insights
Several mouse studies and clinical trials have been conducted in the area of HIV gene therapy, with several different therapuetic targets.

Target/Mechanism of Action	Construct	Results	Reference
Rev	Inhibitory Rev protein, Rev M10, delivered to CD4+ cells by gold particles	Preferential survival of cells with construct. Limited duration of engraftment.	(Woffendin, Ranga et al. 1996)
Rev	Inhibitory Rev protein, Rev M10, delivered to CD4+ cells by retroviral vector	More persistent engraftment compared with gold particle delivery. No change	(Ranga, Woffendin et al. 1998)
Rev	"Humanized" dominant-negative REV protein (huM10) and nontranslated marker gene (FX) as an internal control in retroviral vector	Gene marking in first months, then low or undetectable except in one patient when viral load increased. No serious adverse events.	(Podsakoff, Engel et al. 2005)
rev/TAR	Trans-dominant rev with or without antisense TAR and control (neo) gene in CD4+ T lymphocytes	Long term survival of cells at low level. Preferential survival of gene-containing cells in a patient with high viral load.	(Morgan, Walker et al. 2005)
RRE decoy	Retroviral-mediated transfer of an RRE decoy gene into bone marrow CD34+ cells	No adverse effects. 2 subjects' cells detected containing both the RRE and LN vectors on the day after cell infusion. All subsequent samples negative for the L-RRE-neo vector. Cells containing the control LN vector detected up to 330 days.	(Kohn, Bauer et al. 1999; Bauer, Selander et al. 2000)
Env antisense	Single infusion of VRX496™, a lentiviral construct encoding an antisense targeting HIV env, in CD4+ T cells	CD4+ counts increased in 4/5 patients, viral loads stable, prolonged engraftment. Well tolerated. Transient vector mobilization. Safe to date.	(Levine, Humeau et al. 2006)
rev/tat ribozyme	tat and tat/rev ribozyme in CD34+cells in autologous CD34+ cells and empty vector backbone in two patient groups with and without ablation	Trial 1 - 3/5 patients showed low-frequency marking of PBMC with ribozyme and vector backbone. Trial 2 – gene marked cells detected after infusion and to one year, and RNA expression detected.	(Michienzi, Castanotto et al. 2003)

Target/Mechanism of Action	Construct	Results	Reference
tat/vpr ribozyme	Phase I study: Moloney murine leukaemia retroviral vector encoding a ribozyme vs control LNL6 vector in CD34+ HPSC	de novo production of myeloid and lymphoid cells. Degree of persistence of gene-containing cells dependent on transduced cell dose	(Amado, Mitsuyasu et al. 1999; Amado, Mitsuyasu et al. 2004)
tat/vpr ribozyme	Retroviral vector encoding a ribozyme vs control LNL6 vector to transduce T lymphocytes, predominantly CD4+ T lymphocytes	Safe and feasible procedure. Long-term survival of genetically modified T-lymphocytes.	(Macpherson, Boyd et al. 2005)
tat/vpr ribozyme	Phase II study: Moloney murine leukaemia virus-based, replication-incompetent gamma retroviral vector with gene encoding a ribozyme vs placebo in CD34+ cells	No significant difference mean plasma viral load at primary end-point but lower TWAUC. No safety concerns.	(Mitsuyasu, Merigan et al. 2009)
Fusion inhibitor	Gene encoding membrane anchored peptide C46 fusion inhibitor delivered by retroviral vector in T cells.	Increased CD4. No significant change in viral load (except after treatment change). Modified cells detected at one year. Low level marking. No major toxicity	(van Lunzen, Glaunsinger et al. 2007)
CCR5	CCR5-specific zinc finger nuclease based product, SB-728-T, in autologous CD4+ T cells. Two phase 1 trials with various dosing regimens in different patient groups.	Preliminary data on 1 patient only ZFN-modified cells persisted in circulation and observed in GALT. Suggested delay in return of viral load after structured treatment interruption.	(2009)
Tat/rev, CCR5, TAR decoy	Tat/rev short hairpin RNA, TAR decoy and CCR5 ribozyme expressed from a self-inactivating lentiviral vector transduced in CD34+ cells, along with standard unmanipulated HPCs in 4 patients with HIV and non-Hodgkin's lymphoma	Engraftment by 11 days. Low levels of gene marking observed up to 24 months as was expression of siRNA and CCR	(DiGiusto, Krishnan et al. 2010)

Target/Mechanism of Action	Construct	Results	Reference
Modified T-cell receptor	Autologous infusion of CD4+ and CD8+ T cells modified by CD4ζ in a murine moloney leukaemia virus backbone given +/- IL-2.	Gene-modified cells followed and detected to 12 months with no difference due to IL-2. No significant change in plasma viral load. CD4ζ signal detected in rectal biopsy.	(Mitsuyasu, Anton et al. 2000)

Table 3. (Apapted from Symonds et al (Symonds, Johnstone et al. 2010)): A list of HIV gene therapy clinical trials and their outcomes. Each of these studies vary in their gene therapy target, and method of targeting the specific region.

To date, as shown in Table 3, several different gene therapies have entered Phase 1 clinical trials, (and some into Phase 2) indicating the safety of a range of HIV gene therapeutics including antisense, ribozymes, decoys, intracellular antibodies and zinc fingers targeting CCR5.

3. Protective effects of anti-HIV gene-therapy: Predictions from mathematical modeling

In gene-therapy research, mathematical modelling has been employed to predict the protective effects of anti-HIV gene-therapy (von Laer, Hasselmann et al. 2006; von Laer, Hasselmann et al. 2006). In this section we review current results on mathematical modelling, with respect to predictions of the *in-vivo* anti-HIV protective effects. Mathematical models deal with the complex interactions between gene-therapy, the immune system and HIV infection (Perelson, Essunger et al. 1997). Given the relative sparsity of current clinical trial data of gene-therapy for HIV, and the long time-spans over which predictions are to be made (i.e. over many years) mathematical modelling can provide predictions on the likely *in-vivo* effectiveness of current and future gene-therapies. Modelling work to-date has led to important insights regarding key design factors as well as parameters that should be optimized in order to maximize the effectiveness of therapy (von Laer, Hasselmann et al. 2006; von Laer, Hasselmann et al. 2006). In this section we review current results and key insights.

3.1 Why is Class 1 gene-therapy the most promising approach?

As discussed previously, three different broad stages of the HIV infection cycle may be targeted for inhibition of HIV infection (Figure 1) with the inhibitors referred to as Class 1, 2, and 3 (von Laer, Hasselmann et al. 2006; von Laer, Hasselmann et al. 2006). It is of interest whether inhibiting earlier stages (via Class 1), intermediate stages (via Class 2) or later stages (via Class 3) of the infection cycle might provide maximum effectiveness of the therapy. Is it more desirable to prevent HIV entry and integration into the cellular genome via Class 1, to inhibit the production of structural components for HIV assembly via Class 2, or to inhibit the assembly/export of new HIV virions via Class 3? This question has been addressed by a number of investigators (von Laer, Hasselmann et al. 2006; von Laer, Hasselmann et al. 2006; Applegate, Birkett et al. 2010), subject to a variety of modelling assumptions reflecting differing levels of complexity of the interaction between HIV, gene-therapy and the immune system.

Investigations to-date have demonstrated that Class 1 protection appears to be highly desirable in terms of reducing viral loads and increasing CD4+ T cell counts (von Laer, Hasselmann et al. 2006; Applegate, Birkett et al. 2010; Aviran, Shah et al. 2010). The underlying reason for the superiority of Class 1 therapy (over Class 2 and Class 3) has been attributed to the high selective advantage of the protected cell population conferred by Class 1 inhibition (von Laer, Hasselmann et al. 2006; von Laer, Hasselmann et al. 2006; Applegate, Birkett et al. 2010). Since Class 1 inhibits all steps prior to viral integration into the cellular genome (Figure 1), any cell containing the protective gene is less likely to be infected than a non-protected cell. Consequently, Class 1 promotes the survival and expansion of the protected non-infected cells, whereas the non-protected cells are more prone to infection and selective killing through cytopathic effects associated either with the virus or the CTL immune response (von Laer, Hasselmann et al. 2006; von Laer, Hasselmann et al. 2006).

In contrast to Class 1 agents, Class 2 and Class 3 therapies have been shown to require much higher degrees of inhibition in order to achieve clinically significant effects (von Laer, Hasselmann et al. 2006; von Laer, Hasselmann et al. 2006; Applegate, Birkett et al. 2010). Class 2 inhibits cytopathic effects associated with the viral infection and the CTL immune response (Figure 1). Any infected cell with Class 2 protection is therefore longer-lived and also has a reduced viral production rate compared to an unprotected and infected cell (von Laer, Hasselmann et al. 2006; von Laer, Hasselmann et al. 2006; Applegate, Birkett et al. 2010). Class 2 consequently confers a selective survival advantage to the infected cells containing the protective gene relative to other infected cells, but not to non-infected cells containing the protective gene (as is the case with Class 1). In contrast, Class 3 only inhibits the export of new HIV virions from an infected cell and thus provides minimal selective advantage (von Laer, Hasselmann et al. 2006; von Laer, Hasselmann et al. 2006). Hence Class 1 is the only class that confers a selective survival advantage to non-infected cells containing the protective gene (von Laer, Hasselmann et al. 2006; von Laer, Hasselmann et al. 2006; Applegate, Birkett et al. 2010).

Collectively therefore, modelling work to-date implies that Class 1 is essential due to the selective survival advantage conferred to the protected and non-infected cells. Still, it is important to note that augmenting Class 1 with Class 2 and/or Class 3 protection might further increase the effectiveness of therapy (von Laer, Hasselmann et al. 2006; von Laer, Hasselmann et al. 2006; Applegate, Birkett et al. 2010). Recent findings relating to the "Bystander Effect", as discussed previously in section 2.2.2, have lent further support to arguments relating to Class 1 inhibition (Doitsh, Cavrois et al. 2010), as abortive infections (HIV virion enters the cell, but does not integrate into cellular genome) comprise 95% of all cell death resulting from HIV infection.

3.2 Two different transduction approaches: To transduce CD4+ T cells or HSC with a protective gene?

As discussed previously in section 2.4, it is possible to either transduce CD4+ T cells with a protective gene (for an immediately protected population of CD4+ T cells) or to transduce HSC, that provide protection to CD4+ T cells following differentiation through the lymphoid line and to monocyte/macrophages following differentiation throught the myeloid line. While the relative merits of each approach have attracted substantial interest, the long-term quantitative advantages and disadvantages of each approach in the clinical

setting remain to be elucidated. Consequently, investigators have turned to predictions from mathematical modelling in order to shed light on the in-vivo dynamics of the two approaches. In this section, we review the predictions from such modelling work to-date.

3.2.1 Transducing CD4+ T cells with a protective gene: Can we achieve establishment of a sufficiently large and sufficiently "receptor-diverse" CD4+ T cell population that is protected against HIV?

Expansion of numbers of CD4+ T cells containing a protective gene is subject to the rate-limiting step of homeostatic cell division and proliferation (von Laer, Hasselmann et al. 2006). Thus it is important to determine how quickly a substantial CD4+ T cell population could expand from a small initial fraction of protected cells. Such considerations are motivated by the fact that it is currently feasible and practical to transduce only a portion of the total CD4+ T cell population (Dropulic and June 2006; von Laer, Baum et al. 2009), so that expansion of the protected CD4+ T cell population will have to rely on in vivo mechanisms.

While modelling has shown that a small fraction of initially transduced cells could potentially result in significant expansion of the protected CD4+ T cell population, reductions of viral load, and also a delay in the onset of AIDS (Lund, Lund et al. 1997; Leonard and Schaffer 2006; von Laer, Hasselmann et al. 2006; Aviran, Shah et al. 2010), most of these models have assumed a strong feedback mechanism upregulating cellular proliferation when numbers fall below a normal level. Whereas such homeostatic mechanisms are believed to contribute to the maintenance of T cell numbers in healthy individuals (Khaled and Durum 2002), the speed with which they occur is likely to be significantly slower in practice. Current clinical trials have not produced CD4+ T cell expansions at rates as fast as predicted by mathematical modelling (Dropulic and June 2006; von Laer, Baum et al. 2009).

Current estimates of T lymphocyte division put the normal rate at approximately 1 division every 3.5 years for naive T cells and 1 division every 22 weeks for memory T cells (McLean and Michie 1995). If the transduced CD4+ T cells are to expand in vivo, then such time-scales should provide an indication of the slow nature of any in vivo expansion of the transduced CD4+ T cell population unless driven by strong selective pressure by HIV.

More realistic upper bounds on rates of CD4+ T cell expansion in-vivo under gene-therapy may be obtained by consideration of CD4+ T cell reconstitution on HAART (Byakwaga, Murray et al. 2009). Reconstitution of the CD4+ T cell population under HAART appears relatively slow with average increases of approximately 300 cells/μL observed after about 6 years (Byakwaga, Murray et al. 2009). Given that reconstitution on HAART usually only takes place under complete viral suppression (as opposed to gene-therapy where a measurable viral population may be present), it appears likely that the expansion rates of the protected CD4+ T cell population under gene-therapy may be substantially slower. Unlike the situation with HAART high viral levels may be preferable in early stages of gene therapy to act as a driving force for the expansion of a protected CD4+ T cell population via a selective mechanism.

Several additional factors might further inhibit the expansion of the protected CD4+ T cell population in-vivo. First, unless sufficient selective survival advantage is conferred to the protected CD4+ T cell population, the protected cell population might not expand

substantially in-vivo. Second, the transduced CD4+ T-cells might have increased death rates or decreased proliferative ability due to interference of the protective gene with normal cell functionality (Dropulic and June 2006; von Laer, Baum et al. 2009; Tayi, Bowen et al. 2010). Third, the unprotected de-novo CD4+ T-cells exported from the thymus might effectively dilute the transduced CD4+ T-cells in the periphery (Aviran, Shah et al. 2010). This latter problem may potentially be addressed by subsequent "booster" treatments involving repeated infusions of transduced CD4+ T cells or by also using HSC.

An additional disadvantage associated with the direct transduction of CD4+ T cells is that peripheral expansion of their number does not necessarily correspond to an equivalent expansion in the T cell repertoire (Nikolich-Zugich, Slifka et al. 2004; Allen, Turner et al. 2011; Wiegers, Kaufmann et al. 2011). This is important as any resulting "gaps" in the T cell repertoire may result in increased probability of immune system evasion by pathogens and consequently in increased risk of infection or morbidity (Nikolich-Zugich, Slifka et al. 2004; Allen, Turner et al. 2011; Wiegers, Kaufmann et al. 2011).

Hence although direct transduction of CD4+ T cells results in a faster appearance in peripheral blood of a protected component of this susceptible population, there may be disadvantages in that these may not provide a diverse immune response and other cell populations will not protected.

3.2.2 Transducing HSC with a protective gene: Increasing T cell receptor repertoire and broadening class of protected cells.

An alternative to transducing CD4+ T cells directly is to instead transduce HSC. In this case, the production of de-novo CD4+ T cells containing the protective gene occurs as a result of HSC differentiation through the lymphoid line and subsequent export from the thymus (Symonds, Johnstone et al. 2010).
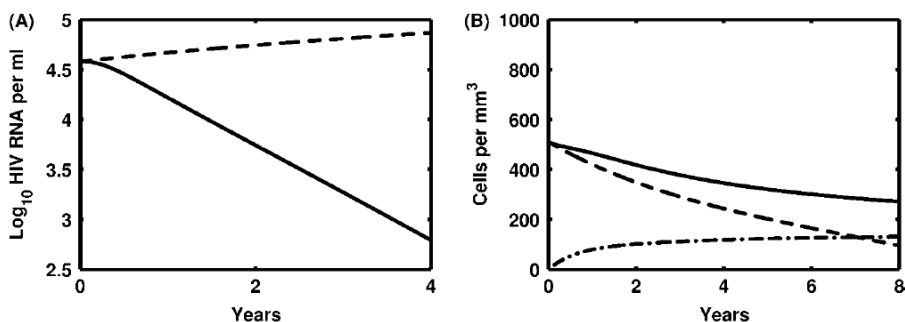

Fig. 4. Modelling predictions by Murray et al.(Murray, Fanning et al. 2009) regarding comparison of the scenario that 20% of all HSC in the bone marrow are transduced with a tat-vpr specific anti-HIV ribozyme (OZ1) versus the scenario that no gene-therapy treatment is received. Reproduced with permission from Murray et al.(Murray, Fanning et al. 2009). The patient was assumed HAART-naive. The time-scale on the horizontal ordinate denotes the time since receiving gene-therapy at year 0. (A) Treatment with OZ1, $\log_{10}$ HIV RNA copies/ml (solid line); No treatment, $\log_{10}$ HIV RNA copies/ml (dashed line). (B) Treatment with OZ1, total CD4+ T lymphocytes/mm³ (solid line), OZ1+CD4+ T lymphocytes/mm³ (dash–dot line); No treatment , total CD4+ T lymphocytes/mm³ (dashed line).

Transducing HSC with a protective gene has two distinct advantages. First, the export of protected de-novo CD4+ T cells from the thymus results in a diversification of the T cell receptor repertoire (Allen, Turner et al. 2011; Wiegers, Kaufmann et al. 2011). Consequently the expanded CD4+ T cell population containing the protective gene exhibits more "extensive" TCR coverage over time, reducing the risk that pathogens might evade the immune response (Nikolich-Zugich, Slifka et al. 2004; Allen, Turner et al. 2011; Wiegers, Kaufmann et al. 2011). Secondly, HSC differentiate into a broad range of cells (besides CD4+ T cells), including macrophages that are susceptible to HIV infection and that may represent important latent HIV reservoirs (Chun, Carruth et al. 1997; Chun, Stuyver et al. 1997; Crowe and Sonza 2000). Consequently, HSC transduction provides protection against HIV to a broader class of cells than just CD4+ T cells.

The transduction of HSC does not immediately provide a protected population of CD4+ T cells in the periphery, but rather the protected CD4+ T cell population is established relatively slowly as HSC differentiate and are exported from the thymus (Symonds, Johnstone et al. 2010). Thymic production of CD4+ T cells has been estimated at approximately 1.65 cells/µL/day (Murray, Kaufmann et al. 2003) in peripheral blood. Assuming that a percentage P of total HSC in the bone marrow is transduced, then one would correspondingly expect that CD4+ T cells containing the protective gene would be exported at a rate of 1.65 x P cells/µL/day from the thymus (Murray, Fanning et al. 2009). Such numbers provide estimates of rates at which the establishment of a protected CD4+ T cell population might take place in-vivo in peripheral blood.

Achieving high engraftment efficiencies of HSC in the bone marrow is important. While a number of clinical trials in which HSC were transduced reported indications of clinical effect against HIV (Symonds, Johnstone et al. 2010), engraftment percentages in the bone marrow have been relatively low (Dropulic and June 2006; Mitsuyasu, Merigan et al. 2009; von Laer, Baum et al. 2009; Symonds, Johnstone et al. 2010). Such results underscore the need for more effective methods of cell harvesting, transduction and homing, that achieve higher engraftment efficiencies. Increased engraftment percentages should lead to more substantial clinical effects in terms of protection against HIV (Mitsuyasu, Merigan et al. 2009; Murray, Fanning et al. 2009).

Despite relatively low engraftment efficiencies to-date, it is of practical interest for future research directions to determine what engraftment percentages might suffice for clinically meaningful effects of the therapy. This question was addressed in recent modelling work (Murray, Fanning et al. 2009), that considered HSC transduction with a *tat-vpr* specific anti-HIV ribozyme (OZ1) employed in a recent phase 2 clinical trial (Mitsuyasu, Merigan et al. 2009). Under the assumption that 20% of all HSC in the bone marrow are transduced (i.e. engraftment percentage P = 20%), and that correspondingly 20% of CD4+ T cells exported from the thymus contain the protective gene, the modelling predicted reductions of 0.5 log_{10} in viral load for a HAART-naive individual after 1 year (Figure 4 A). Benefits in terms of forestalment of onset of AIDS at 8 years post-infection were also estimated (Figure 4 B). Slighly less pronounced effects were observed for patients that were concurrently enrolled on HAART (Murray, Fanning et al. 2009). Such results are encouraging and indicate that relatively modest engraftment percentages could achieve a clinically relevant effect. Consequently full bone marrow ablation may be unnecessary.

3.3 Resistance development under gene-therapy: How does it differ from HAART?
Systemic antiretroviral therapy bathes each cell in some level of the drugs being used depending on the penetration of the individual drugs to that region of the body, their

concentration, pharmacokinetics and timing between dosages (Abdel-Rahman and Kauffman 2004). The clinical management of the combinations of drugs used in a regimen is an important part of successful treatment through suppressing the development of drug resistance. Early in the development of antiretroviral drugs there were few agents available and by necessity these were applied as monotherapy leading to the failure of these and subsequent drugs from the same class. Current HAART regimens involve three drugs from at least 2 drug classes to limit the likelihood that mutations in the HIV quasispecies will be present prior to the commencement of therapy or will develop subsequently.
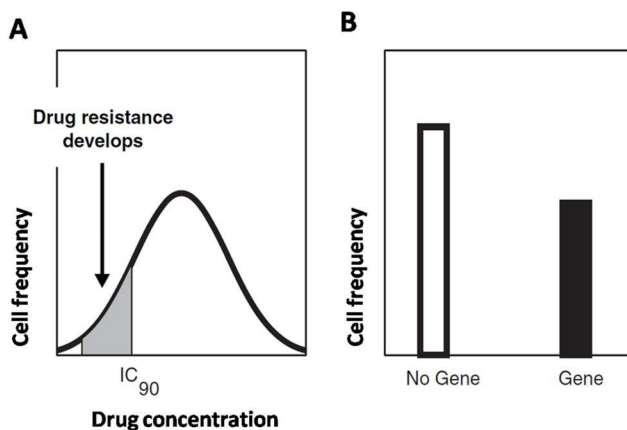
Fig. 5. Illustration of principles behind selection pressures driving the development of resistance with antiretroviral therapy and with gene-therapy. Adapted with permission from Applegate et al.(Applegate, Birkett et al. 2010). (A) The horizontal ordinate denotes the concentration of antiretroviral drug received, and the vertical ordinate denotes the frequency of cells receiving the antiretroviral drug concentration. The selection pressure driving resistance in systemic antiretroviral therapy results from bathing each cell in some drug concentration. This provides a "continuous spectrum" for selection of HIV escape mutants, since many cells will receive suboptimal drug concentrations allowing viral replication and the preferential development of drug-resistant strains (as shown by shaded region indicated by the arrow). (B) The horizontal ordinate splits the cell population into two parts of either having a protective gene or not. The vertical ordinate denotes the frequency of cells containing the gene and not containing the gene. The bipartite distribution of protected and unprotected results provides a different selection environment whereby sufficient wild-type replication takes place in the non-protected population (i.e. no gene), thus mitigating the escape of viral mutants.

Similar concerns exist for the development of resistance to HIV gene therapy (Leonard and Schaffer 2006; Applegate, Birkett et al. 2010). The quasispecies nature of HIV and its high mutation rate imply the existence of every single mutation to any agent prior to the start of therapy. If there is sufficient viral replication under therapy, even for a reasonably short period, there is the chance that these singly resistant clones will evolve into variants with additional mutations and that are highly resistant to therapy. HIV gene therapy seems to fall into the classification of approaches that lend themselves to the development of resistance: not all cells will contain the therapy and so there will be considerable viral replication, and

this will be even more evident for gene therapy delivered to HSC since it will take some time for the protected CD4+ T cells to mature from the HSC and appear in the periphery (Applegate, Birkett et al. 2010). However there is a considerable difference between gene therapy and a systemic treatment that is not suitably suppressive.

Unless myeloablation is conducted to eliminate endogenous non-gene containing HSC and T cells, it is expected that there will always be a sizeable proportion of HSC and CD4+ T cells that do not contain the therapy. Achieving 20% gene transduced HSC without ablation may be an upper bound (this remains to be tested). Similar limitations exist for those trials that instead transduce peripheral CD4+ T cells. At any time there is an estimated 2% of total T cells in peripheral blood and not all T cells are likely to traffic to this compartment. So large-scale apheresis of CD4+ T cells from peripheral blood will remove, and be able to infuse, only a fraction of their total.

Hence gene therapy will partition HIV-susceptible cells into a bipartite population: those containing the therapy and those that do not. This 0-1 distribution is very different from the continuous distribution of drug concentration within cells for an individual receiving antiretroviral therapy (Figure 5). In this situation gene therapy is less likely to lead to the development of resistance (Applegate, Birkett et al. 2010). However there is a trade-off in that it is also less suppressive for the same reason. As cells containing gene therapy become more widespread in the body of an infected individual they will exert more pressure on the virus and select for resistance (Leonard and Schaffer 2006). For this reason the same general principles that apply to antiretroviral therapy are also valid in this context. Gene therapy should target multiple viral and cellular mechanisms.

Mathematical modelling of gene therapy delivered to HSC that targets multiple mechanisms with reasonable efficacy and where the resistant virus is also less fit than wild-type determined that this therapy will reduce virus and maintain a viable T cell population for extended periods without the expansion of resistant virus (Applegate, Birkett et al. 2010). However there were important qualifications to the extent of this success. Primarily the gene therapy needs to be Class 1 and inhibit infection of cells. Additionally the likelihood of resistance to a particular component of the therapy and the fitness cost that incurs will also contribute to the speed at which virus overcomes the therapy.

3.4 Future perspectives: What can we expect from gene-therapy against HIV?

Gene therapy holds out high promise as an effective therapy against HIV. The definition of success of gene-therapy treatment may vary, depending on a variety of circumstances. As discussed in Section 2.1, an obvious success would be one similar to that of the Berlin Patient whereby an individual would be completely and sustainably cured of HIV and have their immune system restored to "normal" levels. It is however important to provide more practical goals, as it is not likely that a "cure" will be achieved with all patients, and as such, more "modest" goals might be more practical and more realistic. Removing the need for an individual to be on HAART would be defined as success, as this can save the individual from life-long drug regimens often with considerable side-effects (Yeni 2006). Another "successful" outcome might consists in preservation of immune system functionality depite the presence of measurable viral loads, as observed during SIV infection in its natural hosts (Liovat, Jacquelin et al. 2009; Pandrea, Silvestri et al. 2009).

As discussed in section 3.2.2 above, predictions from mathematical modelling indicate that full ablation of the immune system need not be necessary in order for clinically significant

effects to be observed (Murray, Fanning et al. 2009). Assuming HSC engraftment percentages of about 20%, it has been predicted that substantial viral control may be achieved and CD4+ T cell counts maintained above the critical limit of 200 cells/µL (Murray, Fanning et al. 2009). The major challenge to achieving substantial clinical effect thus relates to achieving sufficient engraftment percentages.

Gene therapy will have varying degrees of effectiveness depending on the circumstances of the individual. The length of time for which an individual has been infected with HIV is an important factor to consider when providing gene therapy treatment. Due to the tropism of HIV in an infected individual changing over the duration of infection from CCR5-tropic to CXCR4-tropic, any treatment targeting CCR5 would be best used on patients in fairly early-phase infection (Mosier 2009). For gene-therapy aiming to transduce HSC, it also appears reasonable to expect that the therapeutic effects in younger patients will be more pronounced due to their greater rates of thymic activity (Pido-Lopez, Imami et al. 2001). Futhermore, patients on HAART, patients for whom available antiretroviral therapies have been exhausted and patients suffering severe HAART-associated side-effects should also benefit, given that gene-therapy provides an alternative layer of protection via cell-mediated immunity in addition to antiretroviral therapies (Symonds, Johnstone et al. 2010).

Finally, as discussed in section 3.2, both HSC and CD4+ T cells represent feasible targets for transduction. While CD4+ T cell transduction may suffer from limitations due to a restricted T cell receptor repertoire and not protecting other susceptible cell population, it will however provide an immediate protected population. Conversely HSC are limited by the degree of thymic production and bone marrow engraftment, yet have the potential to generate a long-lasting array of HIV protected immune cells. Thus it appears that most effective therapies might employ a combination of these two approaches in order to provide optimum protection, possibly employing infusions of transduced cells.

4. Conclusion

In this chapter we discussed the current biological underpinnings of gene-therapy against HIV, as well as predictions from mathematical modelling of the clinical effects achievable through gene-therapy.

We discussed the various biological and clinical aspects relating to HIV gene therapy. An indication of the possible effectiveness of gene therapy was provided in terms of the naturally occurring mutation, CCR5d32, which provides extremely high levels of resistance against HIV infection. Most importantly however, the utilisation of this mutation in a bone marrow transplant, ridding an individual of any measurable HIV levels, indicates the capability of using gene therapy to functionally "cure" people of HIV. An assessment of the target areas of HIV gene therapy was conducted, indicating not only the possibility, but also a clear need to target multiple aspects of HIV infection (favourably entry stage), in order to prevent the emergence of resistance. The various options for delivery methods were discussed, indicating a range of techniques by which to introduce the therapeutic gene. Each of these methods exhibit their own advantages and disadvantages, however all are valid options in a variety of situations, with lentiviral vectors showing some of the most promise. The options for the ideal cell-type to target were discussed, indicating validity of using either CD4+ T cells for their immediate effect or HSC for the more sustained and broad spectrum protection. However it is also critical to consider the combination of these as an option in gene therapy. The aspects and priniciples of apheresis, ablation, and G-CSF-

induced mobilisation were discussed, indicating their role in treatments. With mobilisation being crucial for the efficient transduction of HSC, and ablation of non-tranduced cells having the potential to provide a significant proportional increase in the amount of protected cells, both are critical when designing treatment regimens. Finally, clinical trials whereby HIV gene therapy has been conducted, and the outcomes of these trials were summarised, highlighting the high safety levels associated with gene therapuetics. Due to the observed high safety in these studies, with the promise of reasonable levels of efficacy and a proof of concept (in the Berlin Patient), HIV gene therapies are a very promising area of HIV research.

In the final sections we discussed predictions obtained from mathematical modelling regarding the in-vivo effectiveness of gene-therapy. We outlined why HIV virion entry inhibition via Class 1 gene-therapy has been shown to be essential in terms of achieving clinically meaningful effects. We explained how the selective survival advantage conferred to non-infected cells containing the Class 1 protective gene is the key factor contributing to the success of Class 1 therapy. We saw that transduction of CD4+ T cells provides an immediately protected CD4+ T cell population, but that in-vivo expansion of the protected cells may be a slow process and does not result in increased T cell receptor diversity in the expanded population. In contrast, transduction of HSC results in higher T cell receptor diversity, and in protection of a broader range of cells than solely CD4+ T cells. We also discussed the differences in viral resistance development under HAART and under gene-therapy. While HAART bathes each cell in some drug concentration, resulting in suboptimal dosages for many cells and consequent promotion in escape of viral mutants, gene-therapy partitions the cell population into protected (contains gene) and unprotected (does not contain gene) cell populations. We outlined how this bi-partite distribution promotes the expansion of a cell population protected against HIV, while at the same time mitigating risks of viral mutation escape as a result of sufficient wild-type viral replication in the non-protected cell population. Finally, we discussed future perspectives outlining how gene-therapy promises to achieve sufficient preservation of immune system functionality (without HAART-associated toxicity and non-adherence issues) resulting in forestallement of AIDS and thereby achieving similar effects as observed during SIV infection in its natural hosts. We also outlined how gene-based therapies may be employed in conjunction or disjunction with HAART depending on individual patient circumstances and viral tropism in the infected individual.

In conclusion, based on the clinical results and mathematical modeling work to-date, further clinical investigation of gene-therapy is more than justified, as gene-therapy holds high promise in terms of controlling HIV infection, preserving immune system functionality, and prevention of the onset of AIDS.

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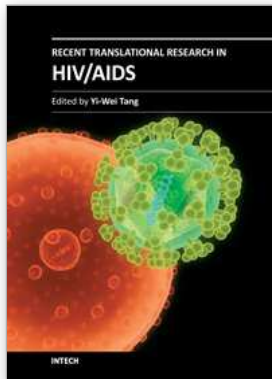
Recent Translational Research in HIV/AIDS

Edited by Prof. Yi-Wei Tang

The collective efforts of HIV/AIDS research scientists from over 16 countries in the world are included in the book. This 27-chapter Open Access book well covers HIV/AIDS translational researches on pathogenesis, diagnosis, treatment, prevention, and also those beyond conventional fields. These are by no means inclusive, but they do offer a good foundation for the development of clinical patient care. The translational model forms the basis for progressing HIV/AIDS clinical research. When linked to the care of the patients, translational researches should result in a direct benefit for HIV/AIDS patients.

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