

In a similar approach we hypothesise that peptides derived from the uPA ATF binding region may have ligand properties to uPAR. Therefore, the aim of this project was to use the u7 peptide, shown by Drapkin et al. (2000) to increase adenoviral uptake, and the u11 peptide, identified from the binding region determined by Appella et al. (1987), to target the over-expressed uPAR on cancer cells and hijack its entry into the cell as a means to internalise a non-viral gene therapy.

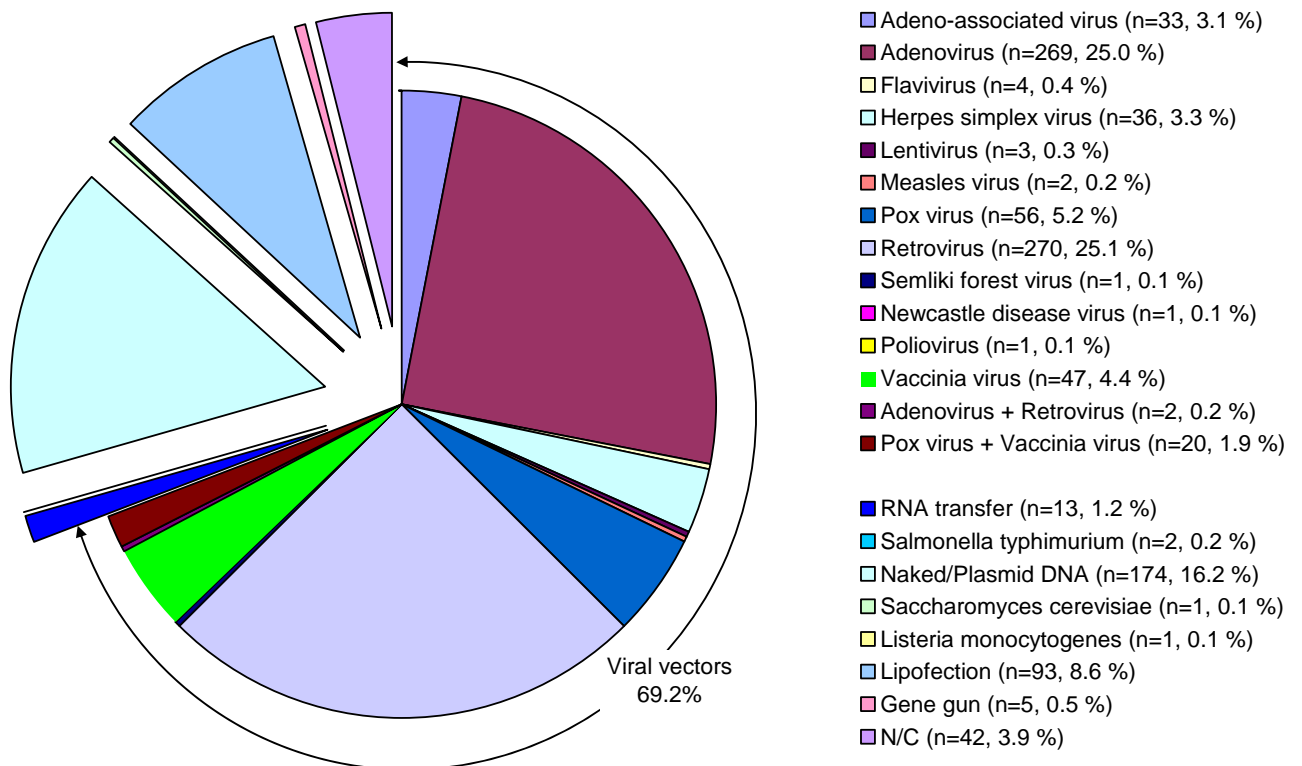
In the study by Drapkin et al. (2000) adenovirus was modified with bifunctional PEG and the u7 peptide conjugated. These surface modified adenoviruses were applied to the surface of excised human airway epithelia and  $\beta$ -galactosidase expression was found to be 10-fold higher than adenovirus coated with PEG or adenovirus coated with PEG and a mutated u7 peptide (Drapkin et al., 2000). The u11 sequence was proposed by Appella et al. (1987) as essential to the binding specificity of uPA whereas the u7 sequence has homology with the EGF growth factor domain (Appella et al., 1987).

## **1.5 Cancer Gene Therapy**

Introducing a gene to correct a defect in the genetic makeup of a cell is an attractive strategy for the treatment of many diseases including cancer. The completion of the human genome project gives us a plethora of information from which we can source targets and design therapies against them (Venter et al., 2001). With increasing knowledge, the methods employed in gene therapy are almost as varied as the diseases under attack. These include: DNA immunisation (Toda et al., 1998), GDEPT/VDEPT (Chung-Faye et al., 2001, Martiniello-Wilks et al., 2004), restoration of a cell checkpoint protein (Dolivet et al., 2002), cytokine introduction, inhibition of tumour angiogenesis, gene silencing/antisense (Brooks, 2002, Lattime & Gerson, 1999). Through the last decade the main challenge in the above mentioned therapies has been difficulties in achieving successful delivery of the genetic material through the circulation to the target tissue and then to the correct compartment of the target cell.

### ***1.5.1 Viral Vectors in Gene Therapy***

The majority of gene therapy that has progressed to clinical trials is viral (69.2 %; Fig. 1.10; (Edelstein, 2005)) with polymeric delivery agents having just arrived at the clinical setting (Ohana et al., 2004).



**Figure 1.10 - Gene therapy clinical trial vectors**

Analysis of gene delivery vectors in clinical trials worldwide (adapted from Edelstein, 2005)

Viral vectors include: adenovirus (St George, 2003), adeno-associated virus (Porteus et al., 2003), lentivirus (Rubinson et al., 2003) and retrovirus (Kafienah et al., 2003). However, with the development of leukaemia in two of the ten children treated in the Cavazzana-Calvo (Cavazzana-Calvo et al., 2000) trial treating children with X-linked severe combined immunodeficiency (X-SCID) there is a great deal of concern (Check, 2003, Kohn et al., 2003, Lemoine, 2002). This has not stopped the Chinese approval of Gendicine<sup>®</sup>, a replication-deficient adenoviral vector carrying the p53 gene (Surendran, 2004). A similar viral carrier is in late stage clinical trials in the USA (Lane, 2004).

Viral gene therapy is characterised by high efficiency of gene transfer and possible long-term gene incorporation, although it is thought that this incorporation into actively replicating genes is responsible for unwanted gene expression i.e. insertional mutagenesis such as the leukaemia found in X-SCID children (Check, 2003).

Targeting, or re-targeting, has not been ignored by those pursuing viral gene delivery and re-targeting of viral vectors has been attempted with some success (Buning et al., 2003, Drapkin et al., 2000, Wickham, 2003). However, Reddy et al. (2001) attempted to re-target retrovirus and adenovirus particles to the folate receptor, they found uptake, but no gene expression (Reddy et al., 2001). This may have been due to the virus being unable to escape the cell compartment into which it was internalised. Parker et al. (2005) successfully re-targeted HPMA-coated adenovirus with a peptide (SIGYPLP) to achieve efficient and specific uptake in HUVEC.

Many problems exist in the re-targeting of viral vectors as they have endogenous ligands in their viral coat. Adenoviral attachment is governed by coxsackievirus and adenovirus receptor (CAR), this and other attachment ligands must be removed and the 'new' targeting ligand expressed (Wickham, 2003). In this recombinant approach the targeting molecule has successfully been expressed but often results in low viral titer (Wickham, 2003).

The main problems with viral vectors are their immunogenicity, limited gene carrying capacity, manufacturing difficulties and the possibility of integration into an active gene. These factors have prompted a wider exploration of non-viral gene delivery systems (Fig. 1.11).