After endocytosis uPAR has been shown to be recycled to the membrane without degradation in human monocyte-like U937 cells and in murine LB6 clone 19 cells, a mouse cell line transfected to overexpress the human uPAR (Nykjaer et al., 1997). The endocytosis in U937 cells is an interesting case both because this is the most widely studied cell line with regards to uPAR and because there is a fusion between the mixed-lineage leukaemia translocated to 10 gene and the gene coding for phosphatidylinositol-binding clathrin assembly protein (Cottier et al., 2004). This may mean that this pathway is aberrant in this cell line (Cottier et al., 2004). Through a different endocytic mechanism uPAR has been shown to associate with the cation-independent mannose 6-phosphate insulin-like growth factor-II receptor independent of uPA for endocytosis and lysosomal degradation in human fibroblasts (Nykjaer et al., 1998).

1.4.4 Targeting uPAR

Clearly, uPAR presents a new and interesting target that has the potential to promote tumour-specific drug delivery. Indeed, a German company, Wilex, has several small molecule and antibody products aimed at the urokinase system in clinical trials (Wilex, 2005). These include a small molecule serine protease inhibitor (WX-UK1) in two Phase 1b clinical trials (Wilex, 2005). Wilex are also testing an orally available small molecule serine protease inhibitor (WX-671) to block metastasis and tumour growth in Phase I clinical trials (Wilex, 2005). Small molecule uPA inhibitors are being developed, as are ligands for soluble uPAR (Wilex, 2005).

An alternative approach has been to target uPAR using a diphtheria toxin/urokinase fusion protein (Ramage et al., 2003, Vallera et al., 2002). A recombinant fusion protein of the amino terminal fragment of uPA to diphtheria toxin (DTAT) was made (Vallera et al., 2002). An *in vitro* assay using human glioblastoma cells (U118MG) showed that DTAT toxicity had an IC_{50} <1 nM. *In vivo* DTAT regressed U118MG tumours in mice (Vallera et al., 2002). DTAT toxicity, tested against acute myeloid leukaemia (AML) cells expressing uPAR *in vitro*, correlated with uPAR expression (Ramage et al., 2003). Fusion proteins of ATF with pseudomonas exotoxin have also been made, these were shown to be endocytosed without the α_2-macroglobulin receptor with cytotoxicity being retained; IC_{50} = 2.8 pM in MCF7 cells (Rajagopal & Kreitman, 2000). So far, few studies have used uPAR as a target to improve gene therapy. Some of the advantages/disadvantages of targeting uPAR that have been identified are in Table 1.4.

Table 1.4 Summary of avantages and disadvantages of uPAR as a target

Potential Advantages	Potential Disadvantages
Over-expressed in many solid tumours	uPA is also over-expressed – increasing receptor competition
Endocytosed in conjunction with many membrane receptors – downregulation of one endocytic partner may not prevent endocytosis	Different pathways of receptor endocytosis – less control over fate of therapeutic
High affinity ligand – uPA has a 0.1-20 nM affinity for uPAR	uPAR can be cleaved from the membrane – therapeutic may bind cleaved uPAR in the circulation
Novel target – may succeed where other therapies have failed	uPAR expression on normal cells – would lead to uptake of therapeutic in non-cancerous cells

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 Apella 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 β-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).