

1.1 General Introduction and Overall Aim

Delivery of a drug to a specific site within the body is a necessity in improving disease treatment and is an ongoing aim of the pharmaceutical scientist. Through selective accumulation in the pathological site and lower accumulation elsewhere, the drug's therapeutic value is increased. Although this idea was first conceived by Ehrlich for the treatment of syphilis by targeting bacteria in the late 1800's, targeted drug delivery remains a challenge today. Nevertheless, antibody-based targeting systems have been successful. The first antibody drug conjugate, gemtuzumab ozogamicin (Mylotarg[®]), targeting CD33 for the treatment of leukaemia was brought to market in 2000 (Wyeth, 2003).

Since Ringsdorf (1975) proposed a polymer carrier with a targeting ligand as a selective therapy, much work has been concentrated in this field (Fig.1.1). This has seen the first targeted polymer-drug conjugate (PK2, galactose targeted *N*-(2-hydroxypropyl) methacrylamide copolymer (HPMA-Dox)) enter phase I/II clinical trials as anticancer agents (Seymour et al., 2002). Currently a number of targeted drug delivery systems based on antibodies, polymers and liposomes are in clinical trials and a higher number developed pre-clinically (Table 1.1). This study is based upon Ringsdorf's visionary model and the pioneering work of Wu & Wu (1988) who were the first to design a targeted non-viral vector for gene therapy. This employed the covalent linkage of a galactose-terminated protein (asialoorosomuroid) to poly-(L-lysine) (PLL) which was then used to non-covalently condense DNA (Fig.1.1).

With the advent of the human genome project and its completion much hope has been pinned on gene therapy as a means to treat life threatening or debilitating diseases. The use of gene therapy has been embraced by the scientific community, particularly as a means for the treatment of cancer (Edelstein, 2005). Currently, clinical gene therapy trials are using several therapeutic genes for the treatment of cancer. Examples include: the introduction of wild type cell cycle regulatory gene e.g. p53 (Lane, 2004) (now approved by regulatory authorities in China (Gendicine[™]) (Surendran, 2004)); introduction of a foreign enzyme gene e.g. thymidine kinase to activate a drug treatment delivered later, in this case ganciclovir (Mesnil & Yamasaki, 2000); introduction of a cytokine gene e.g. interleukin 2 (Schreiber et al., 1999).

Table 1.1 – Actively targeted drugs

Phase	Carrier (Name)	Target	Ligand	Drug/ treatment	Reference
A	Antibody (Mylotarg [®])	CD33	Recombinant humanised antibody	Calicheamicin/ Acute Myeloid Leukaemia	(Allen & Cullis, 2004, Sievers et al., 2001, Wyeth, 2003)
A	Antibody (Zevalin [®])	CD20	Mouse anti CD20	⁹⁰ Y/ non-Hodgkin Lymphoma	(Ross et al., 2004, Wiseman et al., 2001)
A	Antibody (Bexxar [®])	CD20	Mouse/human chimera anti CD20	¹³¹ I/ non-Hodgkin Lymphoma	(Allen, 2002)
I/II	HPMA Copolymer (PK2)	Asialoglycoprotein receptor	Galactose	Doxorubicin/ Hepatocarcinoma	(Seymour et al., 2002, Duncan, 2002)
I	Antibody	Prostate specific membrane antigen	mAb J591	Radionuclides ⁹⁰ Y, ¹¹¹ In, ¹⁷⁷ Lu/ diagnosis and treatment	(Bander et al., 2003)
I/Pc	Chelating agents, peptides	Folate receptor	Folic acid	Radionuclides, ⁶⁷ Ga, ¹¹¹ In, ^{99m} Tc, ⁶⁶ Ga, ⁶⁴ Cu/ Diagnosis	(Reviewed in Ke et al., 2004)
Pc	PEG-coated liposomes	$\alpha_v\beta_3$ integrin	RGD (cyclic Arg-Gly-Asp)	Doxorubicin/ colon carcinoma	(Schiffelers et al., 2003)
Pc	Poly [lactid acid]	$\alpha_v\beta_3$ integrin	RGD (Gly-Arg-Gly-Asp-Ser)	Poly-lactic microbubble ultrasound contrast agent/ diagnosis	(Lathia et al., 2004)

Table 1.1 continued – Actively targeted drugs

Phase	Carrier (Name)	Target	Ligand	Drug/ Treatment	Reference
Pc	Liposomes	HER2	mAb	Doxorubicin/ Breast cancer	(Park et al., 2002)
Pc	Poly-L-Glutamic acid	EGFR	mAb	Doxorubicin/ vulvar squamous carcinoma (A431 tumour)	(Vega et al., 2003)
Pc	Palmitoylated glycol chitosan vesicles	Transferrin receptor	Transferrin	Doxorubicin/ vulvar squamous carcinoma (A431), ovarian carcinoma (A2780), prostate carcinoma (PC3)	(Dufes et al., 2004)
Pc	Transferrin	Transferrin receptor	Transferrin	Paclitaxel/ prostate carcinoma (PC3)	(Sahoo et al., 2004)
Pc	Transferrin	Transferrin receptor	Transferrin	Mitomycin C/ Ehrlich ascites carcinoma (EAC), sarcoma 180 (S180)	(Reviewed in Tanaka et al., 2004)
Pc	PEG or HPMA	Bone	Alendronate and aspartic acid peptide	FITC/ model drug	(Wang et al., 2003)

Pc = pre-clinical; A = Approved; mAb = monoclonal antibody; fAb = fragments of monoclonal antibody; EGFR = epidermal growth factor receptor; FITC = fluorescein isothiocyanate

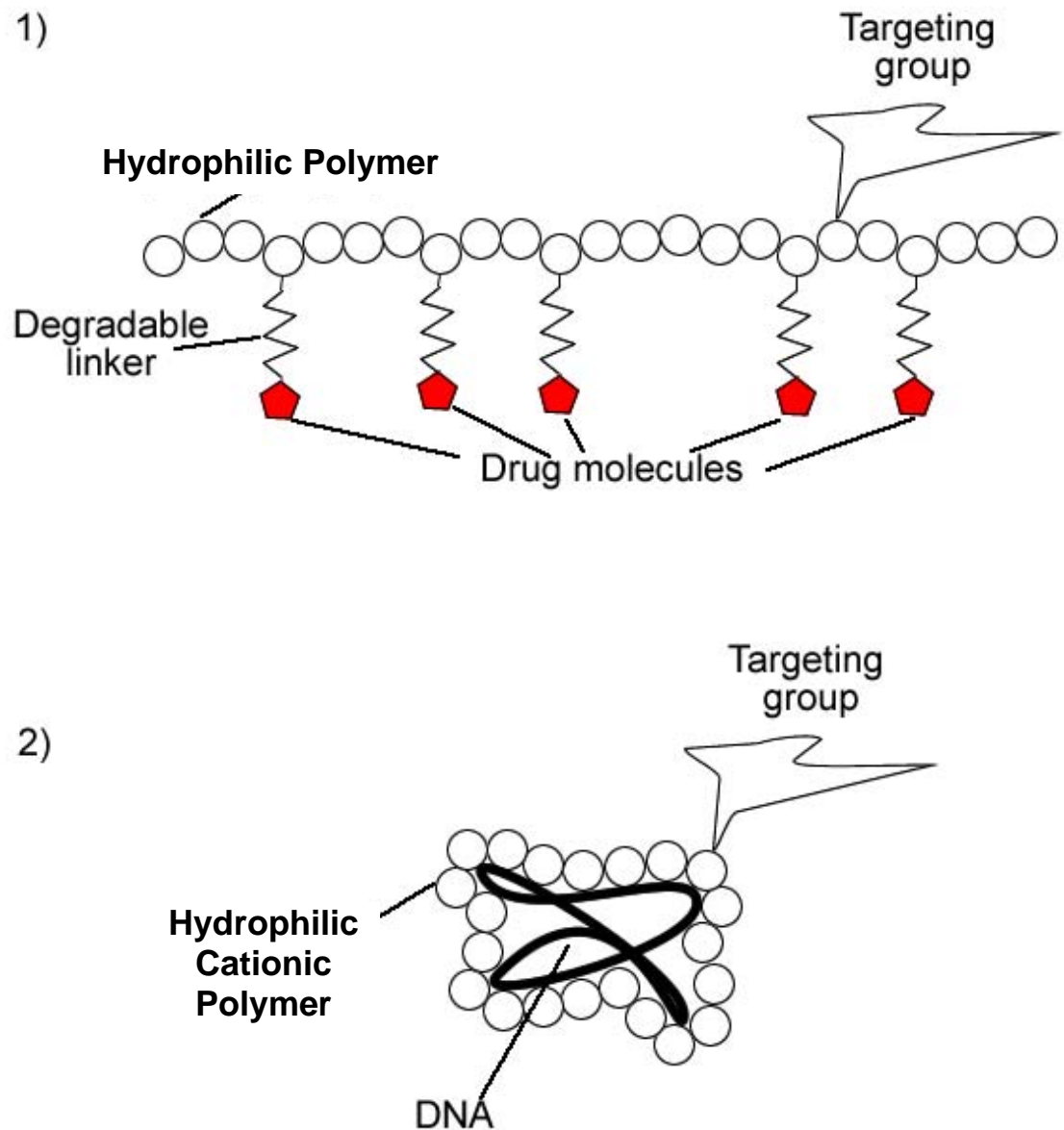


Figure 1.1 – Targeted delivery models

1) Ringsdorf's targeted polymer model (Ringsdorf, 1975), 2) Proposed targeted non-viral gene delivery model.

Current clinical trials are mainly using viral vectors for gene delivery, however it is largely believed that non-viral gene therapy will be safer. The first clinical trial using PEI-mediated gene therapy was published in 2004: poly(ethylenimine) (PEI) complexed diphtheria toxin A-chain plasmid to treat bladder cancer (Ohana et al., 2004).

Targeting specific proteins on the surface of cells for drug delivery requires the engineering of a suitable carrier bearing the appropriate ligand. In targeted drug therapies, carriers such as liposomes (Zhang et al., 2002), antibodies (Ross et al., 2004) and polymers (Duncan, 2003) have been suggested. In this study the focus was on the design of water soluble cationic polymers for targeted gene delivery. The urokinase plasminogen activator receptor (uPAR) is over-expressed in many cancers and it has a high affinity for its ligand (Blasi, 1997).

In this project the development of a peptide targeted non-viral gene delivery system based on biocompatible polymers was investigated and the chosen target was uPAR. From potential polymers, chitosan was chosen as the delivery vector due to its biocompatibility (Corsi et al., 2003, Lee et al., 2001), good transfection efficiency (Thanou & Junginger, 2004), and potential for chemical modification. The following sections of the introduction outline the background to this research starting with the need for targeted cancer therapy.

1.2 The Need for Targeted Therapy of Cancer

According to the world health organisation (WHO) cancer is responsible for six million (12 %) deaths worldwide (WHO, 2005). Cancer Research UK report that cancer has recently overtaken heart disease as the UK's major killer (Cancer Research UK, 2005). With an aging population it is likely that the treatment of cancer will be increasingly important.

In 2001 breast and prostate cancers contributed 26 % to the UK incidence of cancer and 14 % of the mortality (Cancer Research UK, 2005). This large incidence and mortality shows the necessity for improvement in the treatment of these diseases. Both breast and prostate cancers have been reported to over-express uPAR (Blasi, 1997). This is one of the factors leading to the choice of this receptor as a target for non-viral gene delivery. The receptor and its function are described more fully in Section 1.4.

Great advances have been made in the treatment of cancer, but there remains much to accomplish. Current clinical anticancer drugs fall into two general categories: