

cytotoxics and hormone based. Cytotoxins assault DNA at some level, either in its synthesis, replication or processing (Denny, 2001). Cytotoxins can be further split into: alkylating agents e.g. cyclophosphamide; antimetabolites e.g. methotrexate; cytotoxic antibiotics e.g. doxorubicin and vinca alkaloids e.g. taxol (Rang et al., 1996). When tumours are formed from hormone sensitive tissue they may be hormone-dependent and hormone antagonism (e.g. tamoxifen) can be used to delay the tumour growth. The main problems associated with the above mentioned therapies are their lack of specificity for tumour cells leading to non-specific toxicity and the development of resistance. Most small molecules are rapidly distributed throughout the body without selective accumulation in the tumour. They achieve their action due to the increased proliferation of cancer cells but have debilitating side effects and low efficiency. The use of a targeting method to deliver the therapy to cancer cells reduces non-specific action by localising cytotoxic drugs to the tumour.

Genetic therapies offer a new approach to the treatment of cancer. The main focus of gene therapy investigations is for the treatment of cancer (Fig. 1.2). It is considered that toxicity may even be abolished through the use of gene therapy. Indeed, adenovirus carrying p53 (Gendicine[®]) has been approved in China; 64 % of patients treated with radiotherapy and Gendicine[®] showed complete tumour regression compared with three times lower tumour regression in radiotherapy only patients (Surendran, 2004).

1.3 Targeting Therapeutics

Through targeting, scientists have tried to achieve greater success in the treatment of cancer. The word “Targeting” is used in this project to describe methods employed to achieve preferential localisation of a therapeutic in the region of disease and subsequently an increase of the local concentration. It was realised back in the 16th century “Poison is in everything, and no thing is without poison. The dosage makes it either a poison or a remedy” (Paracelsus, 1490-1541). Through targeting the delivery of a drug, and therefore increasing its concentration in the region of pathology, it is suggested that a lower total dose may be administered compared with a non-targeted drug. The lower concentrations at non-pathological sites would decrease the non-specific dose related toxicity of the therapeutic.

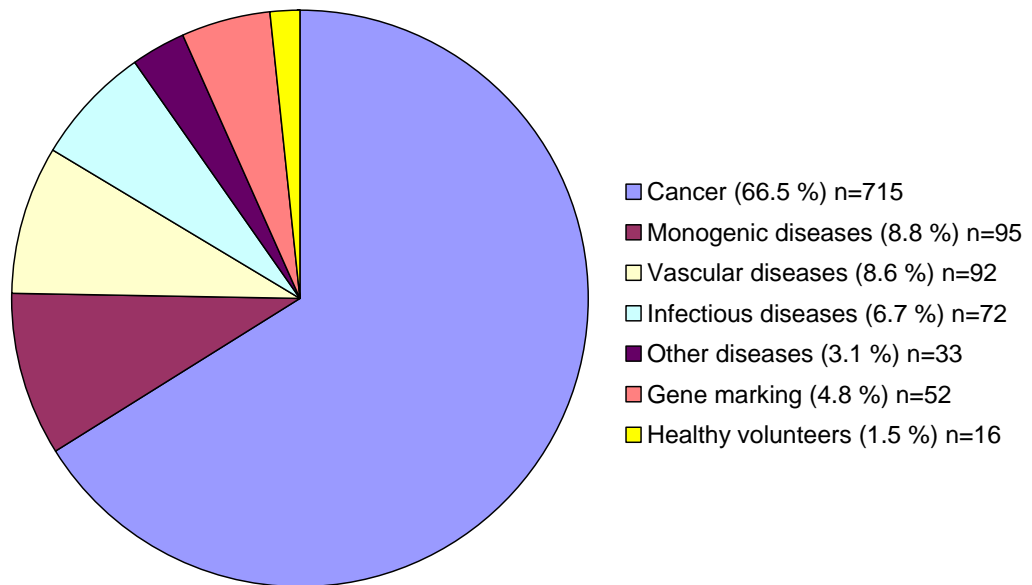


Figure 1.2 - Gene therapy treatment by disease

A chart showing the diseases investigated in gene therapy clinical trials worldwide (adapted from Edelstein, 2005).

It is envisioned that through the use of drug targeting strategies unwanted side-effects, i.e. those seen with many chemotherapies, will be drastically diminished. The targeting of a pathway or enzyme such as selective tyrosine kinase inhibition shows promise, however, it is outside the scope of this project and not considered further (for a review see Ross & Hughes, 2004).

Therapeutic delivery can be considered at different levels within the body: system, organ, cell and subcellular compartment (Duncan, 2005) (Fig. 1.3). The areas that are particularly relevant to this project are those of the organ and the cell. However, the intracellular trafficking of non-viral vectors is another important issue. The enhanced permeability and retention effect (EPR) can be considered to exist at the organ level (discussed in Section 1.3.1) and would surely be applicable to the nano-sized particles used in gene therapy, whilst ligand targeted therapy occurs at a cellular level (discussed in Section 1.3.2).

Many researchers have attempted to categorise drug targeting mechanisms. The peptide-targeted delivery developed in this thesis is termed ligand-targeted therapy (LTT) after that described by Allen (2002). According to Schatzlein (2003) targeting strategies fall into two categories; intrinsic and extrinsic (Schatzlein, 2003). Intrinsic targeting refers to the use of pharmacokinetic and/or biodistribution characteristics of a drug or carrier system and extrinsic to the use of a ligand to provide a specific interaction with cell receptors and therefore selective accumulation in the pathology (Schatzlein, 2003). This categorisation is synonymous with passive and active targeting systems respectively (Duncan, 2002, Moghimi et al., 2005, Schatzlein, 2003). LTT would fall into the categories of active or extrinsic targeting. The uptake found due to the EPR effect is considered as passive targeting.

Many intricate and ingenious methods utilising both passive and active targeting have been developed to achieve greater control over site-specific drug release, these are summarised in Table 1.2 but further discussion of their concepts is outside the scope of this thesis. I expect these multi-stage methods, especially those with external control, to find applications in several diseases.

As gene therapy, especially non-viral gene therapy, is such a novel area, the targeting of chemotherapy for the treatment of cancer is first considered. Seymour et al. (2002) found liver targeting and lower toxicity using PK2 (LTT: galactose targeted HPMA-Dox copolymer) compared with free doxorubicin.

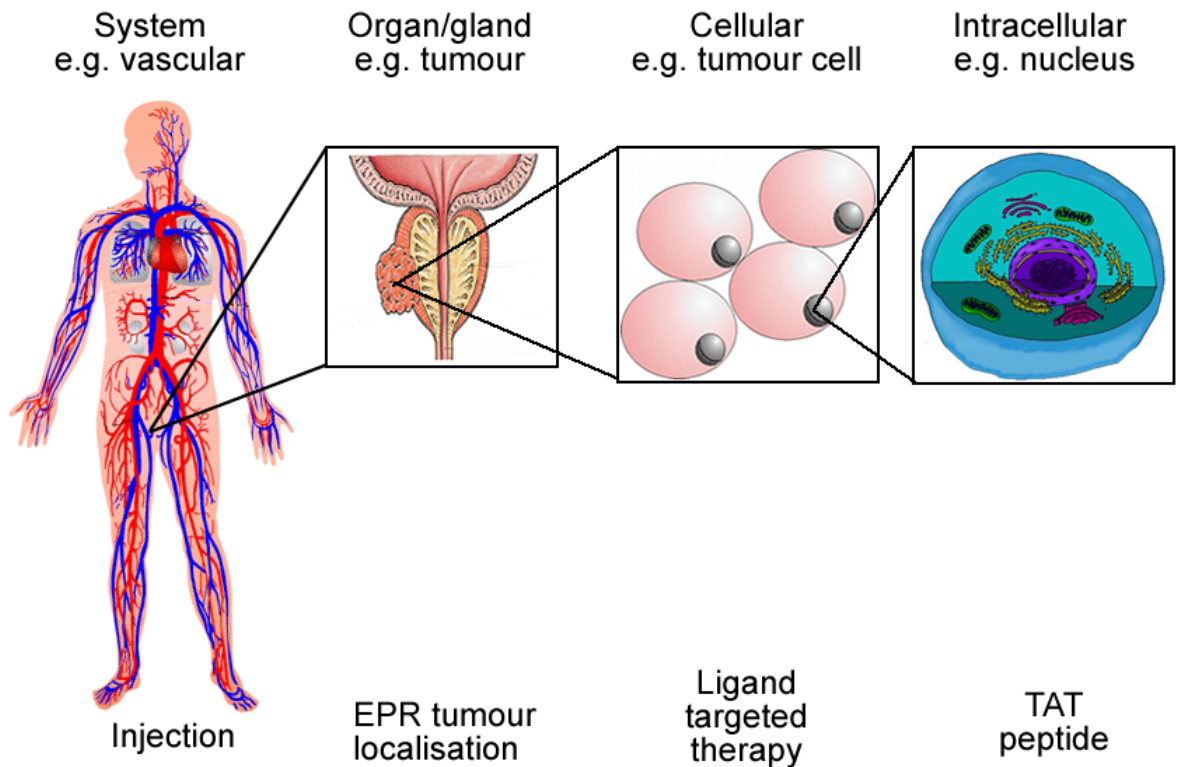


Figure 1.3 - Levels of targeting within the body

Targeting is considered to occur at the levels outlined above the diagrams. Below the diagrams are examples of targeting mechanisms used at each level (Diagrams adapted from Familydoctor, 2005, Harcourtschool, 2005, Talktransplant, 2005).

Table 1.2 – Receptors being targeted in cancer therapy

Target	Function	Reference
$\alpha_v\beta_3$ integrin	Cellular adhesion, over-expressed in angiogenic blood vessels	(Anwer et al., 2004)
Aminopeptidase N (CD13)	Membrane bound metalloprotease, over-expressed in angiogenic blood vessels	(Arap et al., 1998)
Folate receptor	Folate uptake, over-expressed in several cancer cells due to requirement for DNA replication	(Russell-Jones et al., 2004)
Transferrin receptor	Iron (transferrin) uptake, expressed in blood brain barrier, used in cancer targeted therapies	(Wagner et al., 1990)
Asialoglycoprotein receptor	Removes partially deglycosylated proteins from circulation, expressed in hepatocytes.	(Nishikawa et al., 2000)
Vasculature endothelial growth factor receptor (VEGFR)	Growth factor receptor over-expressed in angiogenic blood vessels	(Spooner et al., 2003)
Epidermal growth factor receptor (EGFR)	Growth factor receptor over-expressed in angiogenic blood vessels	(El-Rayes & LoRusso, 2004)

Nevertheless, the maximum tolerated dose was lower than that of the passively targeted HPMA-Dox (PK1). There was an accumulation of 15-20 % of the total dose in the liver as opposed to a general body distribution with PK1 (Seymour et al., 2002). The source of toxicity observed in PK2 over PK1 was not clear. Table 1.1 gave an overview of the current research on receptor targeting for drug delivery.

The antibody (anti-CD33)-calicheamicin (Mylotarg[®]) showed no cardiac or cerebellar toxicity, but grade 4 neutropenia and thrombocytopenia was observed (Sievers et al., 2001). It was however predicted that this type of toxicity may occur due to the targeting of CD33 which is also present on normal maturing haematopoietic progenitor cells (Sievers et al., 2001). Calicheamicin, the active component of Mylotarg[®], does not appear to be used as a single drug entity. Therefore it is not possible to make a comparison to the single drug efficacy. From these studies it can be seen that targeting has a role to play in the arsenal against cancer but more specific targets should be sought to improve therapy.

A reduction of the side-effects of a gene therapy cannot be truly considered as this is a new field. The therapeutic gene could be engineered in such a way that no toxicity is expected and it may be the vector rather than the gene/protein which is toxic. In non-viral gene therapy targeting is used to overcome the cell membrane barrier and increase uptake, through receptor mediated endocytosis (RME), of the polyplex. This is addressed in Section 1.5.3.

1.3.1 Passive Targeting of Drugs and/or Carriers

It was reported in 1984 (Maeda et al., 1984) that macromolecules can accumulate selectively in tumour tissues. Later it was observed that the polymer-protein conjugate styrene-maleic acid neocarzinostatin (SMANCS) selectively accumulated in mouse solid tumours (Matsumura et al., 1987). To explain this phenomenon Maeda and Matsumura introduced the term “enhanced permeability and retention (EPR) effect” (Matsumura et al., 1987). Neovascular tissue angiogenically formed by the tumour is disorganised, meaning macromolecules can extravasate into the interstitial space in a tumour (Dvorak et al., 1988). Neovascular tissue is formed by the tumour due to its increased nutrient requirements (Folkman & Shing, 1992, Satchi-Fainaro et al., 2004). The epithelium of these new blood vessels is disordered and therefore more permeable than normal vasculature (Maeda et al., 2000). Although now accepted, the EPR effect