1. Introduction

Cancer, characterized by invasive growth of self-tissue with insensitivity to normal death signals, is a consequence of microevolution - multiple genetic mutations occurring over time to genes intricately involved with the control of cell proliferation. Each year, more than seven million deaths are attributed to malignant tumours. [1] Death ensues as a result of vital organ or system obliteration and/or as a consequence of opportunistic secondary infections (owing to a progressive weakening). [2] Present therapy is multimodal and includes surgical resection, radiotherapy, immunotherapy or chemotherapy. Localised modalities (by nature) have a limited scope of application once the disease has metastasized.

Chemotherapy is most often indicated in the setting of advanced or disseminated disease with an aim to palliate symptoms and prolong life. Numerous other therapeutic modalities may include chemotherapy as an adjuvant. The intent unfortunately, is only curative in limited instances such as acute leukaemia, some types of lymphomas and testicular cancer, accentuating the great need for the development of innovative therapeutic strategies and improved early detection technology.

Contemporary cancer chemotherapy is limited by dose-related systemic toxicity owing to inefficient cytotoxic discernment (specificity) for cancer cells. Differential cytotoxic specificity depends on: cellular characteristics (chemosensitivity) of different transformed and non-transformed cells; the pharmacodynamic actions of administered drug/s at various ratios and concentrations; the relative pharmacokinetic distribution into tumour tissue. Through dynamically and kinetically increasing (controlling by design) the cancer specificity of a pharmacological intervention it is hoped that the vision of a “magic-bullet” as first conceptualised by Paul Ehrlich one hundred years ago can be realised. [3]

Pharmacodynamic sensitivity for cancer cells specifically can be improved through utilization of target-designed drug products or through the use of chemotherapeutic combinations - most notably Fixed-Ratio Drug Combinations (FRDC) that have been optimized \textit{in vitro} for maximal synergistic effect against cancer. A further
obstacle thwarting chemotherapy is the development through somatic evolution of multi-drug resistance (MDR) that renders the cancer cell population progressively more insensitive to the cytotoxic action of chemotherapeutics.

Pharmacokinetic specificity can be improved by loco-regional administration (provided there is significant first pass retention) or through the use of tumour targeting, Nanoparticulate Drug Delivery Systems (NDDS). NDDS are the enabling technology that allows favourable uptake and retention of cytotoxic drugs by disseminated tumours after intravenous administration, thus minimizing systemic exposure (toxicity), affording dose reduction and improving anticancer efficacy. [4]

Riminophenazines have demonstrated potent in vitro and in vivo antineoplastic activity against a broad range of drug resistant tumour cells. The Riminophenazine antiproliferative activity is multi-mechanistic, seemingly inferring resistance against intrinsic drug resistance. Furthermore, Riminophenazines have been shown in vitro to inhibit P-glycoprotein (Pgp), an energy-dependent transmembrane efflux pump that is largely responsible for acquired resistance. Riminophenazine drug products therefore possess huge therapeutic and commercial promise as they could speculatively be included within many chemotherapeutic regimes involving Pgp substrates. It is also hypothesized that Riminophenazines are capable of inhibiting the action of other ATP-binding cassette (ABC) transport proteins in addition to Pgp, thus further warranting the title “broad-spectrum resistance circumventor”.

The scope of this study was the pre-clinical development of Riminophenazines as resistance circumventing, anticancer agents. The pre-clinical studies planned and described were intended to provide data that could justify and support the conduct of clinical trials. This project was more than merely academic - efforts were focused on progressing towards human trials and ultimately an approved pharmaceutical product. As such, the development strategy (founded upon the target product profile) was devised with due consideration to appropriate international regulatory guidelines and to the available resources.
The research question serving to drive and focus this project was: does a synergistic FRDC (including a Riminophenazine and Pgp substrate) encapsulated within a tumour-targeting NDDS result in a significantly improved anticancer effect compared to the standard chemotherapeutic alone.
2. Literature study

2.1. Multidrug resistant (MDR) cancer and circumvention thereof

Nearly half of all patients with cancer suffer from malignancies that are intrinsically resistant to chemotherapeutics. Furthermore, the majority of the remaining half will acquire resistance during the course of their therapy. [5] MDR has been described as the “thorniest obstacle” in developing improved systemic therapies for disseminated cancer. [6] Resistance to various structurally and mechanistically unrelated chemotherapeutics characterizes the MDR phenotype.

Categorically, MDR is classically associated with the over-expression of ATP binding cassette (ABC) transmembrane efflux pumps, particularly P-glycoprotein (Pgp). Pgp is a 170 kDa transmembrane “permeability” glycoprotein. Among other ABC transporters, multidrug resistance associated protein (MRP) and breast cancer resistant protein (BCRP) are also of clinical relevance. [7] These transmembrane proteins are energy dependent pumps that can efficiently expel various structurally and mechanistically unrelated chemotherapeutics including Epipodophyllotoxins (etoposide), Taxanes (paclitaxel) and Vinca alkaloids (vinblastine) to the extracellular environment (Figure 2.1.) thereby maintaining intracellular concentrations below effective cytotoxic levels. [8] Increased expression of these multidrug resistance proteins is associated with a poor response to treatment and grave prognosis.

Numerous different compounds have been shown to inhibit the efflux activity of Pgp and other ABC transporters thus restoring sensitivity to cytotoxic agents. [9,10] Unfortunately, due in the main to toxicity and poor pharmacokinetic control (altered distribution and thus altered toxicity profile), no such agents (termed chemosensitizers) are yet clinically available. [11] Verapamil and cyclosporin A are two examples of first generation chemosensitizers. Second and third generation chemosensitizers were developed with the aim of achieving more specific Pgp inhibition and fewer systemic pharmacological effects. [7]
Non-classical (non-transporter mediated) MDR involves evasion of cellular death through impairment of biochemical pathways controlling programmed cell death, as well as altered expression of enzymes responsible for intracellular detoxification and repair. Many of these alterations (e.g. inappropriate expression of oncogenes and loss of tumour suppressor gene function) are essential to neoplastic transformation itself. As the dynamics of these changes are complex and ill understood the contemporary strategy against MDR cancer involves the use of chemotherapeutic agents in combination. The rationale being that the MDR phenotype can be overcome (circumvented) through the action of multiple unrelated cytotoxic mechanisms acting in concert. [6]

In addition, non-cellular MDR resistance mechanisms may exist such as increased interstitial fluid pressure that negatively influences a cytotoxic drug’s ability to penetrate deep into solid tumours rendering it ineffective and possibly toxic to healthy tissue. Clearly a targeted, selective approach employing synergistic drug combinations is called for to prevent and circumvent MDR.
2.2. Combination chemotherapy

Combination chemotherapy has been the mainstay of successful (curative) chemotherapy for the last 40 years, [12, 13] so much so, that it is now considered standard practice. [14] A strong correlation exists between the number of agents administered and successful cure rates. [15]

Combination chemotherapy regimens have predominately been developed empirically in late-stage clinical trials. Active single agents have typically been combined on the basis of minimizing the potential for overlapping toxicities (different organ toxicities) and possessing different (hopefully complementary), non-cross-resistant mechanisms of action. [16]

These poly-chemotherapy regimens offer the potential for increased efficacy (greater fractional affect), increased neoplastic specificity, decreased dosage (hence reduction in systemic toxicity) and a broader spectrum of activity against sub-populations present within a heterogeneous tumour cell population which in turn minimises the development of resistance. [12]

Clinically, the drugs are typically administered at their individual maximum tolerated doses. [14] This “more-is-better” approach ignores the possibility of subtle concentration and ratio dependent interactions capable of eliciting synergistic responses. [17, 18] Fixed-ratio drug combinations (FRDC) have recently been proposed as a more rational approach for the combination of drugs. Through \textit{in vitro} optimisation, FRDC hold the promise of capturing maximal synergistic drug interactions thus reducing the total dose required to produce a particular fractional affect.

Were a synergistic FRDC to be administered intravenously in some arbitrary carrier solvent, the two drugs making up the FRDC would be distributed and eliminated independently of one another over time as a consequence of their inherent dissimilar pharmacokinetics. Consequently the optimized fixed-ratio would
not be maintained - moreover, there would not be an element of tumour specific drug delivery.

Ultimately, *in vivo* use of fixed drug ratio dependent synergistic pharmacodynamics is dependent upon the use of delivery systems that can maintain the optimised ratio after administration and selectively deliver the drug combination to the tumour site.

### 2.3. Riminophenazines

#### 2.3.1. Background

The name “Riminophenazine” comes from the fact that “R” substitution has occurred at the imino region of the phenazine nucleus. [19]

Clofazimine [3-(4-chloroanilino)-10-(4-chlorophenyl)-2, 10-dihydro-2-(isopropylimino)-phenazine] with empirical formula C$_{27}$H$_{22}$Cl$_{2}$N$_{4}$ (molecular weight of 473.14) (Figure 2.2.), otherwise known as B663 and marketed by Novartis under the trade name of Lamprene® is considered the prototype Riminophenazine. Clofazimine, has a characteristic deep red-orange colour that changes under different pH conditions. [20] B663 is very hydrophobic with a reported log $P$ of 7.48. [21] B663 is a basic drug with a reported pKa value of 8.35. [21]

Clofazimine was originally described as a breakthrough in the treatment of tuberculosis. [22] Currently, clofazimine is primarily used in combination with rifampicin and dapsone for the treatment of *Mycobacterium leprae* (leprosy). [23] Clofazimine is also recommended in the treatment of *Mycobacterium avium* complex associated with AIDS. [24] B663 has broad activity against gram-positive organisms and has also been found to possess immunosuppressive and anti-inflammatory properties. [25] Furthermore, clofazimine is non-myelosuppressive, non-carcinogenic and non-teratogenic. Adverse effects include red-brown skin discoloration and reversible gastrointestinal toxicity. [20] Clofazimine has a safety record of more than 50 years in humans. Riminophenazines are attractive as drug
combination candidates because they have a long history of resistance circumventing actions in both cancer and mycobacterial models.

Clofazimine possesses an impressive multi-mechanistic anticancer action. The mandate driving this pre-clinical R&D project was to further develop (re-position) Riminophenazines as anticancer agents, so as to provide benefit to patients and a competitive advantage in the market by adding MDR circumventing value to standard chemotherapeutics regimes.

![Clofazimine (B663) and TMP-substituted phenazines](image)

**Figure 2.2. Structure of Clofazimine and the lead Tetramethylpiperidine (TMP)-substituted Riminophenazine - B4125, where R₁ and R₂ are 2-Cl positional isomers**

**2.3.2. Clofazimine: Antineoplastic and Chemosensitizing potential**

*In vitro* studies conducted on B663 have served to highlight the ability of Riminophenazines to subvert both intrinsic (innate) and acquired, classical and non-classical MDR cancer thus justifying the label “broad-spectrum”:

Van Rensburg *et al.* [26] reported the *in vitro* cytotoxic activity of clofazimine to be comparable to that of methotrexate, bleomycin and cisplatin (standard chemotherapeutics) against a range of human neoplastic cell cultures including pharynx, cervical, bladder and hepatocellular carcinomas.
Van Rensburg et al. [27] showed clofazimine to uniformly inhibit (in contrast to vinblastine and etoposide, among others) the proliferation of various intrinsically resistant neoplastic cell lines at therapeutically relevant concentrations.

Van Rensburg et al. [28] demonstrated the chemosensitizing action of clofazimine using a P-glycoprotein (Pgp) expressing small cell lung cancer cell line (H69/LX4). Chemosensitivity was restored to a range of Pgp substrates when used in combination with non-toxic concentrations of clofazimine.

Myer and van Rensburg [29] using numerous sub-clones of an erythroleukaemia cell line expressing varying levels of Pgp, again demonstrated the ability of clofazimine to inhibit the efflux action of Pgp.

The anticancer potential of clofazimine has been investigated using several in vivo models of experimental oncology. These studies have served to demonstrate both the anticancer efficacy and safety of clofazimine:

Van Rensburg et al. [30] showed an oral dose of 30 mg/kg/day of clofazimine increased survival time and decreased tumour load in carcinogen induced rodent tumours models. Neither clinical signs of toxicity nor haematological toxicity was observed at 60 mg/kg/day.

Sri-Pathmanathan et al. [31] demonstrated significant reduction in the tumour load of drug resistant human (non-small cell) lung carcinoma xenografted subcutaneously into nude mice. After 22 days of treatment the tumours were approximately one-third the size of the control group. Moreover, neither mortality nor gross toxicity in terms of weight loss was observed at an approximate oral dose of 120 mg/kg using a diet supplemented with B663 (0.5% w/w).

Pourgholami et al. [32] using a rodent syngeneic (Novikoff), orthotopic hepatocellular carcinoma (HCC) model demonstrated regression of tumours after single administration of clofazimine solubilized in Lipiodol delivered via the intra hepatic artery.
Two clinical trials have been done to evaluate the anticancer activity of clofazimine against unresectable and metastatic HCC: Ruff et al. [33] reported an objective response rate of 10%, 43% disease stabilization for up to 20 months and an improved median survival time; Falkson and Falkson, [34] evaluated the combination of clofazimine (600 mg orally for 2 weeks followed by 400 mg daily thereafter) and doxorubicin and reported disease stabilization in 42% and a median survival time of 7 weeks. These results should be considered in the context that unresectable HCC is prognostically extremely poor and notoriously unresponsive to systemic chemotherapy. [35]

2.3.3. Tetramethylpiperidine (TMP)-substituted Riminophenazines

Over the course of the last 40 years, hundreds of analogues have been synthesized allowing for extensive quantitative structure activity relationships (QSAR) to be performed. Modifications have focused on substitution in the imino nitrogen region of the molecule (at position 2 of the phenazine nucleus) along with varying halogenation profiles in the phenyl- and anilino-rings.

Tetramethylpiperidine substitution (Figure 2.2.) at the imino nitrogen position in comparison to the isopropyl group found in clofazimine has been shown in vitro to incur superior direct cytotoxicity against several intrinsically resistant neoplastic cell lines. [35] In addition they have been shown to possess greater chemosensitization activity using various Pgp expressing cell lines. [25, 37, 38]

These Riminophenazines thus possess the potential for inclusion in several chemotherapeutic regimes. B4125 has been identified from a review of the structure activity relationships (Appendix B) as possessing the best neoplastic-specific cytotoxicity.
2.3.4. Pharmacodynamics: Anticancer multi-mechanism

As Riminophenazines possess a multi-mechanistic cytotoxic action, they are of use against non-classical MDR. They are thought to be active at the plasma membrane, the mitochondrial and the nuclear level.

The cytotoxic action at the level of the plasma membrane is attributed to enhancement of phospholipase A$_2$ (PLA$_2$) activity. This in turn, leads to increased levels of lysophospholipids, which are potent detergents and membrane destabilizing agents. Lysophospholipids activate NADPH-oxidase leading to the production of cytotoxic oxidants by phagocytes. Furthermore the membrane associated enzyme Na$^+$, K$^+$ ATPase is inactivated by lysophosphatidylcholine. This has significant antineoplastic consequences, as this enzyme is essential for cellular proliferation. Inhibition of this enzyme along with perturbations to the membrane lipid environment may be responsible for the observed inhibition of Pgp efflux activity. [25]

Riminophenazines have been shown [39] to act as artificial electron acceptors competing with cytochrome oxidase thus inhibiting energy-yielding reactions of the respiratory chain. This in turn is thought to inhibit down-stream energy requiring cellular processes including ABC pumps.

Morrison and Marley [21] demonstrated that Riminophenazines are capable of forming stable complexes with DNA through binding along guanine sequence regions associated with the minor groove resulting in loss of DNA template function.
2.4. Refinements in the pharmacokinetic usage of anticancer drugs

2.4.1. Loco-regional chemotherapy

Although systemic chemotherapy has proved effective in certain haematological malignancies (Acute myelogenous leukaemia) and a few solid tumours (notably testicular cancer), to date there is relatively poor efficacy against most systemic therapies (due to a lack of targeting) and the vast majority of disseminated cancers remain incurable.

Since Klopp et al. [40] first attempted intra-arterial chemotherapy in 1950 there has been a growing interest in delivering cytotoxic agents locally into the region of tumour growth via the artery supplying the region. The rationale being to expose the tumour to higher drug concentrations (above the maximum tolerated dose) thus producing a greater fractional tumour cell kill whilst limiting the side effects as systemic exposure is reduced. Regional delivery is thus an approach used to increase the exposure of cancer cells to drug/s beyond what can be achieved safely through systemic drug delivery. [41]

Regional chemotherapy can be divided into two categories: third space regional compartment therapy (e.g. cerebrospinal fluid space, peritoneal cavity, pleural space etc.) and intra-arterial infusion into an afferent artery feeding the tumour containing organ or body region.

Regional delivery of chemotherapeutics to tumours has a pharmacokinetic advantage over conventional systemic routes and offers significant benefit to patients. [42] Regional administration may increase exposure by 100-1000 fold compared to systemic administration. [43] The theoretical pharmacokinetic principles underlying the advantage offered by regional administration have been formally defined by Eckman et al. [44] This advantage is limited by how much drug is retained by the tumour after the “first pass” and for this reason the use of oil based vehicles as well as arterial occluding, embolizing agents of various forms
(e.g. starch microspheres) that act to prolong the transit time of the drugs within the tumour interstitium have great scope. [45]

The relative ease with which tumour blood supply can be accessed with high reproducibility and acceptable low complication rates is attributed to advances in surgical technique along with improved skills in interventional radiology, particularly the development steerable guide wires under fluoroscopic imaging guidance. Many of the smaller afferent arteries feeding prominent tumour types can now be routinely catheterized supra-selectively via percutaneous angiography using the Seldinger technique. [46] Isolated perfusion techniques, whereby artificial circuits are created removing the drug prior to entry into the systemic compartment, offer further benefit to the patient [46] all be it at the expense of time, money and added risk.

The application of regional chemotherapy has provided local remission in a number of tumour locations, including but not exclusive to brain, breast, head and neck and liver. As these studies have typically included patients with advanced, disseminated disease, the survival outcome has been determined by the extent and severity of metastatic disease. Viewed from such a point, other local treatment options - surgery and radiotherapy are considered more appropriate, less expensive and less toxic than regional chemotherapy.

Regional chemotherapy can be viewed as a local ablative technique paralleling surgery and radiation therapy but as it is based on molecular discernment, it holds the promise of greater specificity between cancerous and normal cells. [43] It must be remembered that ablation of disseminated disease is the real issue and the required outcome.

Advances in interventional radiology and the development of reliable, implantable drug delivery devices have made regional chemotherapy a rational endeavour to be combined (neo)-adjuvantly with other modalities to obtain greater therapeutic responses.
2.4.2. The Enhanced Permeability and Retention (EPR) effect

The enhanced permeability and retention effect (Figure 2.3.) of macromolecules and lipid-based particles is a general characteristic of viable and rapidly growing solid tumours. The increased vascular permeability underlying this effect is often considered the other side of the angiogenesis coin. [48] The EPR effect has been described both as the “gold standard” [48] and as the “royal gateway” in the design of new anticancer agents. [49]

Selective, passive targeting via the tumour vasculature is possible as result of extensive production of vascular permeability mediators including bradykinin, nitric oxide, vascular endothelial growth factor, peroxynitrite, prostaglandins and matrix metalloproteinases that promote angiogenesis or facilitate extravasation. Defective tumour vasculature that lack a continuous smooth muscle layer and contain gaps in endothelial cell-cell junctions further contribute towards permeability. This results in leakage of colloidal blood plasma components such as polymer conjugates and nanoassemblies (typically between 10 and 500 nm, dependent upon model) into the tumour tissue. [50]

Figure 2.3. Depiction of the EPR effect displaying the leakage of nanoparticles through porous neoplastic endothelium. Modified from [47] (Used with permission)
Impaired lymphatic drainage and slow venous return ensure that colloidal drug carriers are retained within the tumour interstitium for long periods. Thus favourable distribution (akin to passive targeting) can be achieved which increases drug levels at the tumour site and reduces systemic exposure. [49]

The EPR effect and consequent passive targeting of colloidal drug carriers is dependent upon the molecular weight of the carrier being above the renal clearance threshold (>40 kDa) and very crucially that it possesses a lengthy plasma half live (>6 h). [48, 49] Passive accumulation of colloidal particles through exploitation of the EPR effect has been compared to the “magic bullet” concept put forward by Paul Ehrlich. [51]

It is well established that the ability of a NDDS to passively target tumours is dependent upon extended circulating properties and adequate particle size for optimal extravasation in the poorly formed tumour vasculature. A particle size of 50-200 nm is reported to be optimal. Colloidal particles smaller than 50 nm are thought to be eliminated by renal filtration or lost by non-specific extravasation into liver or bone marrow sinus endothelia. Particles larger than 200 nm may exceed the cut off size to traverse tumour vasculature. Splenic filtration may also rapidly clear such large particles. [52]

Kommareddy et al. [53] has stated that depending on the anatomical region and particular tissue model the pore size in discontinuous endothelium ranges from 100 -780 nm with a mean of approximately 400 nm.

2.4.3. Lipiodol Ultra-Fluid

Lipiodol is an iodinated (480 mg iodine/ml) radio-opaque derivative of poppy seed oil, composed of ethyl esters of linoleic (73%), oleic (14%), palmitic (9%) and stearic acids (3%). [54] Lipiodol is used as a contrast agent in certain radiological investigations including lymphography, hysterosalpingography and sialography. [55]
Lipiodol has therapeutic potential as a targeting carrier of chemotherapeutics and/or radioisotopes. After intra-arterial injection into respective tumour feeder arteries, Lipiodol has been found through X-ray and CT imaging to remain selectively (for periods in excess of 2 months), within tumour tissue of the liver, lung, pancreas, gallbladder [56], bronchus, kidney [57], breast [58], bladder [59] and others. [60]

Lipiodol has been found to effectively solubilize clinically relevant concentrations of various drugs including: 5-fluorouracil, doxorubicin and mitomycin C [60], epirubicin [58] and paclitaxel. [62]

$^{131}$I-Lipiodol has been developed as an internal radiation therapeutic agent [63] and is reputed to be capable of delivering an ablation dose of beta radiation resulting in high treatment response rates. [64]

Selective accumulation and prolonged retention after intra-arterial injection is attributed to first pass extraction (presumable due to the EPR effect). [48, 54] In addition, hepatoma cells have been shown to rapidly take up large quantities of Lipiodol through endocytosis and exhibit prolonged intracellular retention facilitating the activation of intracellular mechanisms of death induction. [65] This demonstrates that through intracellular trapping, Lipiodol and similar oily preparations may be an effective tool used to overcome high interstitial fluid (hydrostatic) pressure and serve as a reservoir of drugs for sustained release. As eluded to earlier, Lipiodol has been reported to offer a marked embolic effect thus effectively prolonging drug residence time within the tumour. [66]

As such, several benefits can be attained through implementation of such a targeted chemo-embolization strategy - notably, high cytotoxic specificity for cancer cells, prolonged action and imaging capabilities. However, as loco-regional administration is a requirement for such potent specificity, this approach is limited in the same way as surgery in that it is local and hence cannot find occult metastasis. The preparation needs to be distributed systemically and should passively accumulate or preferable actively target disseminated tumours.
So as to overcome the complications/limitations implicit in administration of an oil IV (pulmonary oil embolization), efforts have been made to encapsulate Lipiodol within nanostructures with controlled droplet size. Recently, Bae et al. [67] prepared and characterized a covalently cross-linked nanocapsule, consisting of an inner Lipiodol phase surrounded by a Pluronic (PEO-PPO-PEO)/polyethylene (PEG) shell layer (corona). Paclitaxel was effectively solubilized within the inner Lipiodol phase.

Similarly, Ho Kong et al. [68] developed a Lipiodol encapsulated Pluronic (PEO-PPO-PEO)/PEG cross-linked nanocapsule. Results from this study suggest that such a nanoreservoir could efficiently deliver lipophilic therapeutic agents to cancerous tissue. In addition, owing to the high iodine concentration, the Lipiodol filled nanocapsule was shown to have effective X-ray attenuation properties. However, the cross-linked nature of the nanocapsule may impair effective release of drugs at the tumour site.

To the extent of the author’s literature search, a Lipiodol loaded diacyl-lipid nanoemulsion has not been reported to date.

2.4.4. Intelligent formulations: Nanoparticulate drug delivery systems

Drugs are inanimate, exogenous chemical entities that have affinity for and intrinsic activity upon a receptor that can then elicit a biological response. The chemical structure of a drug dictates its pharmacological utility (both dynamic and kinetic). The physicochemical properties of a drug determines to a large extent, the ability of a drug to be adequately absorbed and distributed to the intended biophase. [69]

With the advent of combinatorial chemistry, computational *in silico* modelling, genomics and proteomics (supplying targets) there is an abundance of novel chemical entities that are emerging as drug candidates. Invariable *in vitro* assays are the means by which a particular target or suspected activity is determined (validated) using multi-well bioassays. As such, molecules with potent pharmacodynamics are discovered in high throughput screening (HTS) without
much consideration for their pharmacokinetic properties. Possessing impressive pharmacodynamics does not mean impressive pharmacokinetics.

Due to the ability to traverse membranes, the majority of the most potent anticancer agents discovered are hydrophobic in nature and are plagued by poor pharmacokinetic profiles, which requires higher doses for efficacy but also contributes towards toxicity and adverse effects. Many of these potent drugs are not easily formulated using conventional strategies. The lack of adequate aqueous solubility relative to the required therapeutic dose is the leading problem encountered for drugs intended for IV administration. [70] The formulation of new drugs has often been perceived as the bottleneck in the development of anticancer drugs. [71]

It is therefore not surprising that pharmaceutical companies often employ physicochemical screens and predictive software to eliminate compounds with undesirable physicochemical (implying pharmacokinetic) properties so as to decrease the attrition rate later in the pipeline thus reducing overall costs and speeding up the time to market of a new drug product. [72]

Formulation is an important and often underestimated aspect in the development of chemical entities as effective drug products. All drugs need to be formulated with excipients in a manner conducive to their intended route of administration. A solution must be found considering the particular physicochemical parameters of the drug and the therapeutic intent. As all living cells in vivo have a continuous blood supply in order to sustain life, the blood (circulatory system) presents a route by which targeting particulate (colloidal) drug products can be delivered. Parenteral dosage form excipients should serve as more than just a biocompatible vehicle for solubilising active compounds. There is an opportunity to impart “intelligence” through the use of a delivery system comprising functional excipients controlling distribution and release characteristics. [73]

In the past, the conventional means by which sparingly water-soluble drugs were formulated for parenteral administration included the use of low molecular weight
surfactants, pH adjustment, water miscible co-solvents and complexation. [72] Although effective at solubilising various drugs, these techniques suffer from several drawbacks including the risk of in vivo precipitation upon administration resulting in thrombophlebitis as well as causing allergic reactions. [74]

Recently, various nanoparticulate, colloidal drug carrier systems have been proposed as a solution to overcome poor solubility, low stability and toxicity issues. The advancement of polymer and material science has provided new opportunities for levels of pharmacokinetic intelligence to be added to dosage forms by design. There is currently much interest in the development of novel NDDS. Such new formulations may allow for the usable life of older drugs to be extended. [75]

High molecular weight surface active compounds (surfactants/amphiphiles) that possess both a hydrophilic and a hydrophobic portion in the same molecule are capable of forming a variety of assembles at different weight percentages to service various administration (application) objectives.

The design of any cancer targeting drug delivery system should exploit at a minimum the EPR effect. The discovery that amphiphiles with hydrophilic polymeric domains (such as PEG and PVP) prevent opsonin binding through providing a steric barrier resulting in evasion of the mononuclear phagocyte system (MPS) has catapulted NDDS from vision to clinically reality. The term “stealth” has been used in contemporary literature to describe long circulating, immune evading, sterically stabilized/PEGylated nanoparticles. [76]

Over and above the EPR effect, there are several other tumour characteristics that can be exploited during drug product design. Active targeting through ligand and monoclonal antibody (MAb), e.g. Folate and 2C5 conjugation [77] to the PEG distal terminus have been shown to add further tumour selectivity. Active triggering mechanisms specifically at the tumour site add another dimension of focused drug delivery. Active triggering mechanisms are dependent on exploiting microenvironmental differences between normal and neoplastic cells and designing the nanoassembly to disassemble accordingly. Such triggering
mechanisms include enzymatic, ultrasonic, temperature and pH induced disassembly. [76]

A vast array of nanoparticulate structures have been assembled from various amphiphilic copolymers including but not exclusive to micelles, liposomes, nanospheres, nanocapsules, polymersomes, niosomes, solid lipid nanoparticles, nanoemulsions and microemulsions. This has led to much of the terminology been used interchangeable and at times erroneously. A recent review has attempted to more clearly classify the different nanoparticulate forms and clarify the terminology through an overview of the major features and factors that influence formation of the different structures. [78] This diversity in structure demonstrates the versatility of techniques to encapsulate compounds of different physicochemical properties within segregated compartments/cores of similar polarity for a variety of different applications.

Micelles are a binary system of water and amphiphile (surfactant) molecules. Micelles can be subdivided as either low molecular weight surfactant (detergent) or polymeric high molecular weight. [79] Polymeric amphiphiles possess lower Critical Micelle Concentration (CMC) values (the surfactant concentration above which micelles form) and are consequently more stable upon dilution. Mixed micellar systems with co-surfactant are common to aid either functionality or stability. Micelles have a hydrophobic core that lends itself to encapsulation of multiple lipophilic drugs. Encapsulation of drugs within the hydrophobic core of micelles by both thin film hydration and dialysis methods have been described [80]. The thin film hydration method is thought preferable in terms of optimisation of drug loading capacity.
2.5. Pre-clinical development of anticancer drug products: Regulatory perspectives

Pre-clinical development encompasses all the activities required (deemed necessary to ensure safety) before a new chemical entity can enter into human trials. The goal of pre-clinical studies is to provide accurate, reliable and timely data that will be used to justify the conduct of clinical trials in humans. It therefore follows that a drug development project must be undertaken with due consideration to appropriate regulatory guidelines.

These guidance documents are not intended to establish legally enforceable requirements, but rather reflect the current thinking of the respective agencies. The most scientifically sound and ethically correct approach for the particular “Target Product Profile” (TPP) under development should be adopted. Agent-directed, pre-clinical studies should be designed so as to support the conduct of clinical studies that may follow [81].

Pre-clinical modelling is aimed a sparing time and resources. The principle outcome is to treat humans. Assumptions are made that pre-clinical data is predictive of activity in humans - therapeutic index (efficacy vs. toxicity). The value of any model is thus based on its ability to be predictive of clinical responses.

An internationally-harmonised document, ICH S9 [82], was recently released (29 October 2009) for final review (Step 4) by the respective regional agencies: USA - Food and Drug Administration (FDA); Europe - European Agency for the Evaluation of Medicinal Products (EMEA); Japan - Ministry of Health, Labour and Welfare (MHLW) before adoption. It is important to realize that ICH S9 is intended to enhance and provide clarity [83] to the earlier guidelines implying DeGeorge et al. [84]; CPMP/SWP/997/96 [85] and Nakae et al. [86] representing the recommendations of the FDA, EMEA and MHWL respectively. These documents are unique in that most guidelines, including those of the local (South African) regulatory authority - the Medicines Control Council (MCC), either explicitly or implicitly exclude cancer therapies from their recommendations. [87]
ICH S9

The objectives of the ICH S9 document are to: Facilitate and accelerate the development of anticancer pharmaceuticals and to protect patients from unnecessary adverse effects.

Because malignant tumours are life threatening and because the death rate from these diseases is high and existing therapies have limited effectiveness, the pre-clinical evaluation of anticancer drug products is often abbreviated. Pre-clinical evaluations are conducted to: identify the pharmacological properties of a pharmaceutical; establish a safe initial dose level for the first human exposure and to understand the toxicological profile of a pharmaceutical.

The active pharmaceutical substance used in pre-clinical studies should be well characterized and should adequately represent the drug product (final dosage form) to be used in clinical trials. Concerning the required pharmacological studies, appropriate models should be selected based upon both the target and mechanism of action, but the pharmaceutical need not be studied using the same tumour types intended for clinical evaluation. These studies can serve to: provide non-clinical proof of principle; guide schedules and dose escalation schemes; provide information for selection of test species; aid in start dose selection and selection of investigational biomarkers (where appropriate) and justify pharmaceutical combination.

An assessment of the pharmaceuticals effect on vital organ functions should be made as part of a general toxicology test. However, in the absence of specific risk these studies will not be called for to support clinical trials nor marketing.

The evaluation of limited pharmacokinetic parameters can facilitate dose selection, schedule evaluation and escalation schemes during first in human studies. As the toxicity of a drug can be greatly influenced by schedule (dosing regimen), an approximation of the clinical schedule should be evaluated using usually both rodent and non-rodents. The reversibility of any observed toxicity should be investigated. Reproductive toxicology, genotoxicity and carcinogenicity
studies are not considered essential to support clinical trials intended for the treatment of patients with advanced cancer.

One of the primary goals of pre-clinical testing is to identify a safe dose that is reasonably expected to have pharmacological effects. Common practice is to set the start dose at 1/10 of STD10 (severely toxic dose in 10% of animals) in rodents. If the non-rodent is thought to be a more appropriate animal model, then 1/6 of a highest non-severely toxic dose (HNSTD) is to be the start dose in humans.

In general, the highest dose or exposure tested pre-clinically does not limit the dose escalation or highest dose investigated in a clinical trial. In Phase 1 clinical trials, treatment can continue according to the patient’s response. A new toxicology study is not called for to support continued treatment beyond the duration of the prior completed toxicology studies.

Concerning drug combinations, data should be available that supports the rationale prior to starting clinical trials. Concerning conjugated products the safety of the conjugated material is the primary concern - stability of the conjugate in the test species and human plasma should be provided. A complete evaluation of drug delivery systems is not warranted if the unencapsulated Active Pharmaceutical Ingredients (API) have been well characterized. [82]

ICH S9 is to be read in conjunction with other pertinent guidelines: Regulatory requirements for the pre-clinical (non-clinical) safety studies required to support the conduct of human clinical trials is addressed by the ICH M3R2 [88]. In ICH M3R2 it is stated that only 1 GLP toxicity test, either acute or chronic is required for progression to clinical studies. Repeat dose toxicity studies mimicking the proposed clinical schedule and route of administration in two species (1 being non-rodent) will generally support any clinical trial up to an equivalent duration.

Although not an official FDA guideline, DeGeorge et al. [84] was authored by senior FDA employees and has directed much of contemporary thinking. [81] The primary aims of safety studies have been highlighted as: determine a safe starting
dose for subsequent clinical trials that is both reasonably safe and allows for possible clinical benefit to patient; identify organs of toxicity and the reversibility thereof. The use of specialized delivery systems and chemosensitizers is further discussed and may necessitate additional pre-clinical evaluation beyond that of conventional cytotoxic drugs. The EMEA [85] advocates pre-clinical investigation of pharmacodynamic, kinetic and toxicological interactions of drug combination products before clinical trials.