

9. Final discussion and conclusions

In 2008, cancer accounted for 13% of all deaths. [1] As medical and chemical science improves, so too should the prognosis and cure rate for these patients. Metastasis is largely responsible for cancer deaths - approximately 30% of patients have clinically detectable metastasis at the time of diagnosis. [156] Therefore therapeutic interventions have to be systemic in nature in order to tackle cancer dissemination. One of the major reasons for the relatively poor prognosis of cancer is resistance to existing treatments and therefore developing agents that are active against MDR cancer cells is a primary objective of contemporary oncology R&D.

With focused efforts worldwide, our understanding of the genotypic and phenotypic biochemical differences between neoplastic and normal cells is increasing. There is therefore much hope that through further research and better understanding, small molecule drugs or biologicals that are “perfectly” selective for a particular cancer can be designed or discovered and utilized to elicit true cures.

The worldwide anticancer market in 2009 was a reputed 50 billion US\$. [157] It is not then surprising that big pharma spends in excess of 25% of its R&D budget on the development of such products. [158] In this time of industry “crisis” and worldwide economic challenges, the pharma industry is following and relying upon a strategy of rational target design to achieve success and return on investment. [159]

Rational drug design involves first identifying a receptor target. Good targets would be critically involved with cell growth or survival and would ideally be highly specific to cancer cells. After a target receptor has been identified: computer modelling, combinatorial chemistry and HTS are tools that can be used to identify structurally complementary small-molecules (ligands) for further pre-clinical development (formulation and *in vivo* proof). In addition, humanized MAb can be genetically engineered with hybridoma technology to specifically target a unique cancer cell antigen of choice, targeting immune responses and directing active delivery of cytotoxins after encapsulation within NDDS. [160]

There should be very good reason behind pursuing a particular TPP and not just random screening. Drug development is costly, even in the academic environment. The industry needs return on research investment to be sustainable. Studies should have a strong translational emphasis and should attempt to bring a product (with benefit) to the patient. Targeting cancer with increasing precision (controlled delivery) through intelligent drug and dosage form design is required to minimize the risk of late stage drug attrition (Figure 9.1.) NDDS development could support the safety and efficacy of practically all new drugs and should therefore be investigated and their assembly optimised. Collaboration between polymer, pharmaceutical and medical science is key to develop and move innovative products along the development path, from the laboratory to the bedside.

Studies in early phases (pre-clinical) should naturally be designed to collect as much relevant scientific information as is possible at the lowest cost, so that decisions pertaining to drug development progression (down the critical path) can be informed. Pre-clinical studies provide more of an opportunity to experiment than do clinical trials in humans. The limitations of pre-clinical (*in vitro* and *in vivo*) models should always be kept in mind. Pertinent and specific questions as to the safety, efficacy and proper functioning of the TPP, considering the shortcomings of the model used, should be asked and answered to reduce failures in later, more expensive development phases.

With respect to the highly morbid and heterogeneous nature (multiple mutations) of cancer, oncological drugs are going to continue to be used in poly-chemotherapeutic regimens, so as to target several action sites (receptors) simultaneously, increasing response and avoiding the development of acquired resistance (extraneous pro cell-survival mechanisms - a hallmark of cancer cells). New chemical entities with novel, potent mechanisms of action and intelligent formulations comprising functional excipients that are affordable to all patients are required.

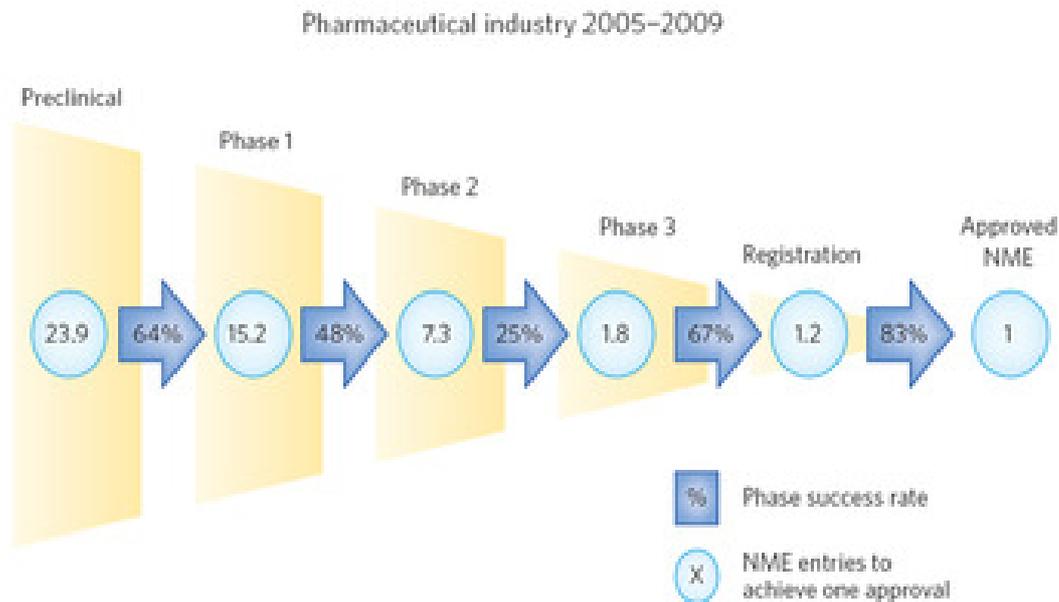


Figure 9.1. Success rate of Pharmaceutical R&D [156]
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Potent pharmacodynamics does not necessarily imply efficient pharmacokinetics nor make formulation any easier for a particular drug. The key to achieving better therapeutic responses may lie in utilizing already available (off patent) drugs more effectively (in combination) rather than in developing new drugs. [45] Many innovative approaches have been used towards more selectively delivering cytotoxic drugs to tumorous tissue, thus increasing the drug dose (at tumour site) and reducing systemic exposure.

As has been investigated in this study, a pragmatic approach to improve therapeutic responses without the discovery or design of novel drugs is to rationally combine registered drugs with complimentary action in fixed-ratios to obtain maximal synergy. As numerous drug combinations are possible, *in vitro* studies are the only ethical means of optimizing synergistic effects, prior to *in vivo* studies where the enhanced efficacy effects and safety profile can be confirmed.

To fully realize the potential of synergistic FRDC, the specified drug ratio must be maintained *in vivo* and delivered (targeted) to the tumour tissue. NDDS represent an effective way to selectively deliver multiple drugs to the tumour site in specified synergistic combination ratios. The integrity of the delivery system *in vivo* as reflected by maintenance of the loaded drug-drug ratio as well as by prolonged systemic circulation is pre-requisite in order to attain effective passive tumour targeting.

It was hypothesized that through using synergistic drug combinations selectively delivered through targeting NDDS, specificity for cancer can be improved both pharmacodynamically and pharmacokinetically over currently used chemotherapeutics.

To restate (*Pre-clinical development plan, page 27*), the long term aims of this study were to:

Phase 1. Identify the lead synergistic FRDC *in vitro*

Phase 2. Assemble and characterize a novel NDDS that (co)-encapsulates the optimised FRDC

Phase 3. Evaluate the *in vivo* safety, efficacy and pharmacokinetic functionality of the FRDC-NDDS

In this study, the *in vitro* combination potential of three commonly used SC in combination with Rimirinophenazines was assessed. Rimirinophenazines were shown *in vitro* to act in quantifiable synergy with ETOP, PTX and VIN. Any of these three Pgp substrates that are now off patent can stand to benefit greatly from re-formulation in combination with either B663 or its more hydrophilic TMP derivative B4125. In addition, B663 at non-toxic concentrations was shown to improve the activity of DOX using a MRP expressing neoplastic cell culture, thus validating the label of – broad-spectrum resistance circumventor.

In terms of re-positioning Rimirinophenazines as anticancer drugs: after *in vitro* studies the already registered Rimirinophenazine, clofazimine (B663) was combined

with the widely used SC, PTX for further pre-clinical development (formulation and *in vivo* experiments).

Using the thin film hydration method, PTX and B663 were successfully and stably encapsulated at a synergistic molar ratio of 1:4.5 (PTX:B663) within a mixed lipopolymeric micellular system called Riminocelles™. As Riminocelles was shown to be of a good particle size for passive tumour accumulation (~ 200 nm) and possessed adequate electrostatic stability, the formulation was evaluated using *in vivo* models of experimental toxicity and oncology.

An acute toxicokinetic study (14 day observation period) and a 21 day GLP repeat dose toxicity study in mice has shown PTX-Riminocelles to be well tolerated and non-toxic at clinically used PTX dosages in contrast to the current formulation Taxol, that incurred statistically significant ($P < 0.5$) weight loss after 14 days.

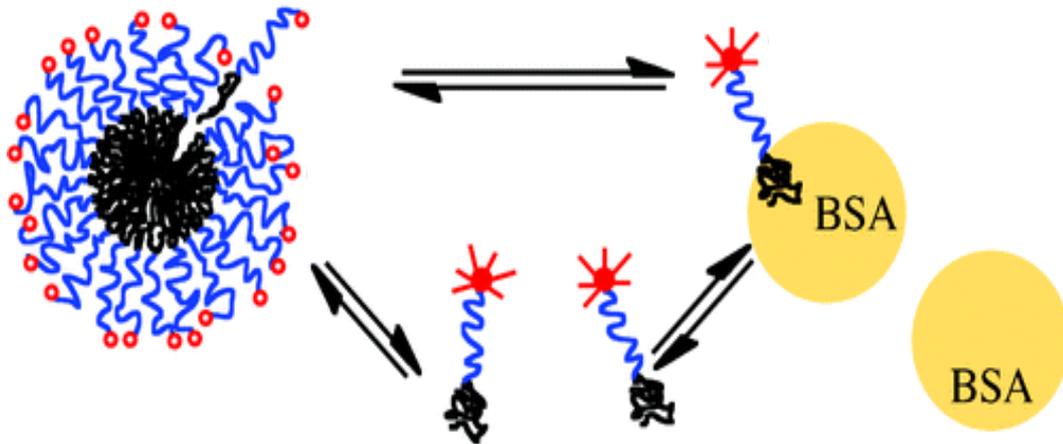
HCT-15 cells are particularly drug resistant human colorectal adenocarcinoma, for which surgery is the primary modality with curative intent and PTX (a substrate of Pgp) would not be a front line drug. This intrinsic Pgp expression model was used first for *in vitro* confirmatory studies before conducting GLP repeat dose toxicity and efficacy studies in nude mice implanted subcutaneously as a proof of concept of improved efficacy.

The FRDC (1:5, PTX:B663) of Riminocelles showed a 72% improvement *in vitro* (IC_{50} value) and clearly a superior benefit using *in vivo* models of experimental oncology with 12.5% of the QDx7 Riminocelles treatment group surviving 31.3% longer than both the untreated control group and the Q7Dx2 Taxol treatment group. The most outstanding result of this study was that at an equivalent PTX dose, Riminocelles was statistically ($P < 0.05$) more efficacious and less toxic than Taxol in a relevant Pgp drug resistant model. This illustrates the benefit of the drug combination and serves to suggest that PTX-Riminocelles could be used with benefit in patients with refractory ovarian, breast or lung cancers who may traditionally be treated with Taxol.

Although *in vitro* drug retention studies under simulated sink conditions demonstrated adequate drug retention within the delivery system over time, the pharmacokinetic study conducted in healthy mice served to unveil the shortcomings of the delivery system *in vivo*. The results attained (particularly the initial lack of control over the drug ratio between PTX and B663 in plasma within the first 30 min) and supported by recent thermodynamic revelations in literature, [155] indicate that although Riminocelles (simple DSPE PEG 2000 micelles) can endure huge dilution, their integrity is not maintained long in plasma due to rapid adsorption onto the highly abundant albumin which possess strong affinity for the hydrophobic acyl chains of phospholipids. [155]

In vivo, the amphiphiles (DSPE-PEG and PC) making up Riminocelles would exist in a dynamic equilibrium in either micellar, monomeric or albumin bound states (Figure 9.2). Due to the prevalence of albumin in plasma and insufficient forces maintaining aggregation, equilibrium strongly favours the albumin bound state and micelle break up would therefore occur rapidly, in contrast to what has been previously thought and reported [101, 102, 103, 108, 109]. This discrepancy and erroneous consensus about DSPE-PEG based micelle stability (lasting for more than a decade) has been explained by targeting that occurs quickly prior to micelle disassemble [155].

Taking all the collected data together, the efficacy and the pharmacokinetic study results indicate that passive tumour accumulation was not satisfactorily achieved and that the greater anticancer effect (relative to Taxol) observed is due to the drug combination rather than due to enhanced tumour delivery. For this reason (*Stage III. Checkpoint, page. 28*), further repeat dose toxicity studies in a second (non-rodent) animal species in preparation for clinical trials is not warranted without first making improvements to the Riminocelles formulation with special attention to the *in vivo* stability and establishing efficacy in additional experimental (PTX relevant) models of drug resistant cancer. Future micelle development using lipopolymers should therefore include an *in vitro* assessment of stability in plasma or albumin rich solutions.



**Figure 9.2. Thermodynamic instability of DSPE PEG micelles in the presence of bovine serum albumin (BSA). [155]
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Although the developed Riminocelle formulation did not function as desired *in vivo* (i.e. passively target cancer); it must be stressed at this stage that all the components, including the two drugs and the mixture of surfactants used to assemble Riminocelles are already approved and registered individually for medicinal use and therefore, in principal accelerated development and clinical usage is feasible particularly considering that Riminocelles has been shown to outperform Taxol in terms of both efficacy and safety in a mouse study.

A second NDDS, a nanoemulsion formulation called RiminoPLUS™ imaging was successfully developed that entraps Lipiodol contrast agent at its core and is thus thought to enable CT imaging capabilities after passive tumour accumulation following either loco-regional (intra-arterial) or IV administration. The aqueous titration method, aided by ternary phase diagrams (made simple through the application of an Excel spreadsheet) was used in conjunction with the input of ultrasonic energy at predetermined points to assemble monodisperse

nanoemulsions of ~100 nm in diameter. Although not taken further into *in vivo* studies due to poor zeta potential and stability of only a week (therefore not a finished product), further studies are warranted to evaluate the *in vivo* imaging ability of this system. The simplified protocol using automated titration calculations following the respective tie lines of a ternary phase diagram will be useful for the design of future such systems.

Prior to additional *in vivo* investigations, the activation barrier for desorption (disassembly of NDDS) needs to be increased to provide the required stability for prolonged systemic circulation thus fulfilling the passive targeting prerequisite. [155] Future possible improvements to the formulations may include: the use of exogenous polymeric amphiphiles for which albumin has no affinity [161]; covalent cross-linking of the outer corona with PEG [67, 68] although this may impede release at the target site; strengthening the hydrophobic forces holding the micelles together by the addition of cholesterol whose structuring effect has been shown to reduce albumin induced disassembly [128]; inclusion of a interwoven polymeric/protein scaffold to provide increased hydrophobic, electrostatic and steric stability as well as target recognition as in the case of lipoproteins, that have evolved over millennia as an effective way to transport and selectively distribute lipid substances and hydrophobic compounds throughout the body. It is therefore not surprising that lipoproteins have been reconstituted to deliver drugs. [162] Future NDDS will greatly benefit from adding targeting ligands (selective for cancer specific receptors).

To date, only a few nanoparticulate formulations, e.g. Doxil (Liposomal DOX) and Abraxane (Albumin bound PTX) have successfully entered the market as nanoparticulate re-formulations of standard chemotherapeutics. The pharmaceutical development of many novel PTX nanoparticulate re-formulations with improved *in vivo* safety and efficacy performance have stalled after the initial pre-clinical studies or in early clinical trial phases (e.g. LEP-ETU, Genexol-PM, PGG-PTX, Nanotax and NK105). These novel investigational products, that like Riminocelles, provide verifiable benefit over Taxol are confronted with a translational challenge and the question – why is Taxol still used clinically to treat

patients when new and improved, cremophor-free formulations exist? The challenge is therefore to develop a significantly better drug that is more affordable so as to compete in the market. The development of FRDC formulations (in particular combinations of already approved drugs with synergistic interactions and broad action against diverse resistance mechanisms) represents substantial improvement justifying further development and its use over that of Taxol.

To proceed to clinical trials, a competitive advantage would need to be offered to the extent that the product will not be eclipsed by another new product entering the market. The need to avoid an expensive exercise in redundancy must be considered. It would therefore be prudent to provide evidence of the proper functioning (selective delivery) of the delivery system and superior efficacy in diverse models of experimental oncology. Only then can the expense of a second species repeat dose toxicity test and clinical trials in humans be scientifically justified

The results of this study serve to highlight the great potential of *in vitro* optimized synergistic FRDC against MDR cancer. Lipopolymeric micelles are an effective way to formulate multiple hydrophobic drugs for intravenous administration and present a means by which disseminated cancer could be targeted; provided that the delivery system possess the prerequisite *in vivo* stability and surface attributes.

Therefore, future recommendations are that improved NDDS with greater *in vivo* stability and functionality should be developed using advanced bio-polymeric materials and that the synergistic FRDC concept should be expanded upon to include several different drugs and combinations thereof that possess proprietary value. Novel binary (micelles) and ternary (nanoemulsions) NDDS could be developed using the methods outlined in this study. The emphasis of future projects should be further stressed on translating the research into clinical applications. Through applying the FRDC-NDDS concept with more refinement, improved drug products comprising synergistic combinations of pre-registered drugs could be developed quickly to the benefit of patients.