Highway to Success—Developing Advanced Polymer Therapeutics

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Polymer therapeutics are advancing as an important class of drugs. Polymers have already demonstrated their value in extending the half-life of proteins. They show great potential as delivery systems for improving the therapeutic index of drugs, via biophysical targeting and more recently with more precision targeting. They are also important for intracellular delivery of nucleic acid based drugs. The same frameworks that have been successfully applied to improve the small molecule drug development can be adopted. This approach together with improved pathophysiological disease knowledge and critical developability considerations, imperative given the size and complexity of polymer therapeutics, provides a structured framework that should improve their clinical translation and exploit their functionality and potential. Progress in understanding the right target, gaining the right tissue and cell exposure, ensuring the right safety, selecting the right patient population is discussed. The right commercial considerations are outlined and the need for a multi-disciplinary approach is emphasized. Crucial developability factors together with scientific and technical advancements to enable pharmaceutical development of a quality robust product are addressed. It is argued that by applying this structured approach to their design and development, polymer therapeutics will continue to grow and develop as important next generation medicines.

1. Introduction

The purpose of many of us working in pharmaceutical research and development whether within the industry or academia is to deliver medicines to improve the lives of patients around the world. There is now a wealth of different modalities being investigated in drug discovery moving beyond more traditional small molecules, extracellular antibodies, and peptides and polymer therapeutics are emerging as an important class. Polymer therapeutics is a term originally devised in the last century[1] to describe those advanced therapeutics, where a polymer plays a functional role in the delivery system rather than polymer-based drug delivery systems that simply entrap a drug for controlled release. It is now often used to describe a multitude of systems including polymeric drugs, polymer-drug conjugates, polymer-protein conjugates, polymeric micelles, polyplexes, and other self-assembling polymer systems for intracellular delivery and increasingly hybrid systems where polymers linked to antibodies or fragments for precision targeting or linked to lipids for hybrid intracellular systems. Polymers as a class have opportunity to be composed of different materials, geometries, and sizes and are amenable to many chemistries to easily attach targeting ligands, thus introduce the possibility of targeting organs, tissues, and individual cells. At the beginning of this century, polymer therapeutics was described as the “dawning era” by one of the pioneers of the field Ruth Duncan[2] following the first successful clinical applications of the first polymer therapeutics; namely polymer-protein conjugates, and promising clinical results arising from trials with polymer-anticancer-drug conjugates. Nearly 15 years later, with 25 commercial polymer therapeutic products, mainly polymer-protein conjugates where the polymer is used to prolong circulation time, Ruth Duncan described the field as “at a cross-roads.” There were large numbers of failed clinical trials, rising number of generics and biosimilars, safety concerns with respect to polyethylene glycol (PEG) immunogenicity and intracellular accumulation of non-biodegradable polymers as well as the hype and lack of focus created by the nanomedicine boom.[3] Indeed many others have also challenged and expressed concern at the lack of progress with nanomedicines of which polymer therapeutics are clearly a large subset.[4] It sparked much heated debate, for instance, to a packed auditorium at the 2019 Annual meeting of the Controlled Release Society in Valencia. So have we now passed those crossroads? How far have we come? Have we found a suitable path forward for improved translation of polymer therapeutics and how do we keep that momentum?

One of the main challenges in drug discovery still remains achieving the correct balance between efficacy and safety and getting the therapeutic index correct. This can be achieved by both drug design and medicinal chemistry approaches to...
change specificity and absorption, distribution, metabolism and elimination (ADME) properties or targeted drug delivery. Polymer therapeutics can play a pivotal role in either shifting the balance between on and off target engagement by changing drug distribution or by providing more targeted drug delivery to tissue or specific cells within those tissues. This spatiotemporal control of the administered drug leads to enhanced therapeutic effects and/or reduced side effects. This is particularly important in oncology for solid tumors where cytotoxic drugs, still pharmacologically.

It is estimated that ≈85% of the human proteome is currently “undruggable,” that is our current drugs cannot modulate proteins pharmacologically.[8] Many of these require novel drug classes that interact at the nucleic acid rather than protein level and therefore mandate efficient intracellular delivery of sensitive nucleic acid based modalities for example oligonucleotides, mRNA, CRISPR Cas9. To maximize the potential of these exciting modalities, successful targeted and efficient intracellular drug delivery approaches will be key and polymer therapeutics have a big role to play here.[9]

Both biophysical targeting, often referred to as the enhanced permeation and retention (EPR) effect[10] and more active targeting approaches where targeting ligands have been added to a polymeric carrier have been explored.[11] Much work to date has focused on oncology for solid tumors where cytotoxic drugs, still the mainstay of cancer care, have little or no therapeutic index. However, interest in using nanomedicines for haematological diseases is growing.[12] Table 1 shows those polymer therapeutics in the clinic that are being employed to improve safety and/or efficacy. More recently it has been established that immunotherapy plays an important part in cancer treatment and polymeric drug delivery and nanomedicine will play an important role. This could be through local delivery or systemic therapies designed to modulate the tumor microenvironment via an EPR effect,[13] “back-packing” T or other immune cells, targeting the lymphatics directly or via targeting the bone marrow and immune training.[14]

Polymer therapeutics also have a big role to play in other diseases; such as many inflammatory conditions which drive many diseases,[15] cardiovascular diseases,[16] kidney diseases,[17] and vaccines.[18] In diabetes, a polyethylene glycoloxenatide (PEX168) is a novel once-weekly subcutaneously administered GLP-1 receptor antagonist which has successfully demonstrated significantly improved glycaemic control in Type II Diabetes patients in a Phase IIIa trial.[19] Local delivery for early disease and pre-malignant disease is growing in importance as early detection and diagnostic approaches improve, polymer therapeutics play an important role as delivery systems for many easily assessable sites such as brain, peritoneum, bladder, and eye where the physiological barriers to accessing tumors are reduced. Whatever the indication, a disease led approach is required and a full pathophysiological understanding of the disease biology, the drug and its target, and the polymer therapeutic is necessary.[20]

Significant progress has been made in understanding what is needed to translate polymer therapeutics aimed at increasing therapeutic index and important knowledge is emerging in the field of intracellular delivery. This review will focus on how learning from small molecule development is being applied to polymer therapeutics and make the case that polymer therapeutics have passed those “crossroads” and highlight areas to ensure polymer therapeutics are well on the way and rapidly becoming an important part of the next generation of therapeutics.

2. Applying Learnings from Small Molecule Drug Development

Polymer therapeutics have been successful as protein conjugates and extending the half-life of proteins in clinical use however developing polymer therapeutics to improve therapeutic index or provide intracellular delivery has been less successful. Similarly attrition rate within pharmaceutical drug development has been high and there has been much criticism,[21] and many companies have now adopted rules or frameworks to try to improve on this attrition and the last few years have seen rises in productivity.[22] Application of AstraZeneca’s “5R Framework” has had a significant impact improving the success rate from the candidate nomination to Phase III. Success rate increases from 4% in 2005–2010 period (below Industry average of 5%) to 19% in 2010–2015 significantly above the industry average following implementation. The “5R Framework” uses five determinants: right target, right tissue, right safety, right patient, and right commercial potential. In summary, the right target means that pre-clinically the candidate drug must achieve target engagement and demonstrate a strong link between the target and disease and predictive biomarkers must be available. In addition, the candidate drug must achieve the appropriate level of drug exposure in the right target tissue with a full knowledge of the pharmacokinetic-pharmacodynamic (PK-PD) properties and drug-drug interactions. To achieve the right safety means that clear safety margins are needed with a detailed understanding of the drug and any metabolites’ toxicity profiles as well as an understanding of secondary pharmacology and target related safety liabilities. Further, there must be a patient selection hypothesis to select the most responsive patients and appropriate biomarkers in place. Finally, the project must target the correct, commercially attractive, patient population.

A similar framework should be applied for all advanced drug delivery strategies to provide structure in design to improve clinical translation especially given the added cost and complexity of the development. Additionally, adoption of this framework should result in the transition toward a more disease-driven design approach for nanomedicines, where rationally selecting the drug, designing delivery system, and understanding the target patient population and disease to maximize therapeutic efficacy is critical.[20,21] In addition a sixth factor, having the right culture, was also identified as a vital factor for project success.[22] The right culture was described as asking the “killer” questions to determine the validity of a hypothesis, demonstrating truth seeking.
behaviors and using quantitative sciences and decision making rather than volume based goals. This effective decision making is particularly important for polymer therapeutics which are different to typical small molecule or antibody discovery programs and have been in the minority in pharmaceutical development. Alternative thinking, skills, ways of working, and a diverse multidisciplinary team are required. Different experiments are required and different questions need answering in research and development (R & D) than a traditional drug discovery and development program and thus this sixth factor is critical and plays an even more important role for successful progression.

Normally in pharmaceutical development formulation optimization and process optimization occurs during clinical development. For a polymer therapeutic, the formulation needs to be optimized and defined at candidate nomination as the polymer is an integral part of the active moiety. The added complexity of many polymer therapeutics makes them more costly than more simple dosage forms and development is more complex as there is less precedence, many of them are nanomaterials and they have also been termed non-biological complex drugs (NBCDs). Keeping an eye on a practical, sustainable, and scalable manufacturing process with the necessary advanced analytical and process controls for complex products will be critical to ensure the quality of the product produced and successful pharmaceutical development. Right “Developability” could be considered a seventh R. This extra cost of development and potential cost of goods

Table 1. Polymer therapeutics in the clinic aimed at either improving safety or efficacy.

| Name/company | Polymer | Drug, class/loading method | Delivery system type/size | Clinical stage/indication |
|--------------|---------|----------------------------|----------------------------|---------------------------|
| AstraZeneca  | AZD0466 | Cabazitaxel, microtubule inhibitor/hydrolytic linker | Micelles polymer metal complex formation, ≈30 nm | Phase II in advanced solid tumors or soft tissue sarcoma NCT03771820 |
| Starpharma  | DEP® | Docetaxel, microtubule inhibitor/hydrolytic linker | Core cross linked micelle, 65 nm | Phase II, platinum resistant ovarian cancer NCT03742713 |
| NanoCarrier  | DEP® | Docetaxel, microtubule inhibitor/hydrolytic linker | Dendrimer conjugate | Phase I/II advanced malignancies in combination EudraCT: 2009-004332-36 |
| DEP® | Cabazitaxel, microtubule inhibitor/hydrolytic linker | Dendrimer conjugate | Phase I/II advanced solid tumors EudraCT: 2017-003424-76 |
| Starpharma  | DEP® | Cabazitaxel, microtubule inhibitor/hydrolytic linker | Dendrimer conjugate | Phase I/II advanced solid tumors EudraCT: 2019-001318-40 |
| Starpharma  | DEP® SN38 | SN38, topoisomerase I inhibitor/hydrolytic linker | Dendrimer conjugate | Phase I advanced haematological or solid tumors NCT04214093 |
| AstraZeneca  | AZD0466 | AZD4320 | ND | ND |
| Starpharma  | AZD2811 nanoparticle | PLA-PEG copolymer | AZD2811 aurora kinase B inhibitor/encapsulated via hydrophobic ion pair | Phase II small cell lung cancer NCT0425391 |
| Nektar therapeutics | NKTR-214 | 6 × 20 kDa PEG chains to form prodrug | Recombinant human IL2, CD122-preferential IL-2 pathway agonist/hydrolytic linker | Phase III metastatic melanoma, NCT03635983, advanced renal carcinoma, NCT03729245, muscle invasive bladder cancer, NCT04209114 |
| Nektar therapeutics | NKTR-255 | Recombinant human IL-15 receptor agonist/stable linker | ND | Phase II non-Hodgkin’s lymphoma and multiple myeloma NCT04136756; head and neck and colorectal cancers NCT04616196 |
| Nektar therapeutics | NKTR-262 | PEG prodrug | TLR agonist | Phase I, dosed intra-tumorally NCT03435640 |
| Polynx | PLX038 | 4 branched 40kDa PEG | Branched PEG conjugate, 15 nm | Phase I solid tumors NCT02468852 |
| CRXL-1 | Cyclodextrin-polyethylene glycol-based polymer | Camtoptokin, topoisomerase I inhibitor/β-eliminative linkers | Sealed paper multiple, interstrand, inclusion complex ≈20–30 nm | Phase II metastatic castrate resistant prostate cancer with enzalutamide NCT03531827 |
| Nektar therapeutics | EP0057 (formerly CRXL-101) | Cyclodextrin-polyethylene glycol-based polymer | Camtoptokin, topoisomerase I & II inhibitor/hydrolytic agent | Sealed paper multiple, interstrand, inclusion complex ≈20–30 nm | Phase I/II in relapsed/refractory small cell lung, bladder, and prostate cancers with olaparib NCT02769962 |
As a result of this site specific PEGylation, NKTR-214 drives invasive bladder, and advanced metastatic renal cell cancers. In contrast, NKTR-358, where the PEGylation is at the lysine residues of the IL-2-IL-2R interface has reduced binding to the IL-2 receptor α-subunit (IL-2Rα) however binding to the IL-2 receptor β-subunit (IL-2Rβ) is barely affected, is in Phase 3 clinical trials in melanoma, muscle invasive bladder, and advanced metastatic renal cell cancers. As a result of this site specific PEGylation, NKTR-214 drives increased proliferation of CD8+ tumor killing memory effector T cells and reduced proliferation of immunosuppressive regulatory T cells (Treg) and greater anti-tumor efficacy when compared to IL-2 itself in preclinical evaluation. In contrast, NKTR-358, designed for the treatment of autoimmune indications, due to its different IL-2 PEGylation site, exhibits reduced affinity for IL-2Rβ while maintaining its affinity for IL-2Rα, enabling preferential activation of Treg with their suppressive activity. NKTR-358 has shown efficacy in a preclinical model of systemic lupus erythematosus and selective induction of Treg in healthy volunteers.

A polymer therapeutic intended to improve therapeutic index needs to change the distribution of the drug and its safety-efficacy balance. It needs to deliver more drug to the target safely; how much of a change is needed and whether the focus needs to be on more drug specifically delivered to its target tissue or whether the focus needs to be on avoiding toxicities very much depends on the target and the potential on and off target toxicities. The non-existent therapeutic index for many oncology drugs has driven significant drug delivery activity in this area. Cytotoxic drugs kill rapidly dividing cells through interacting with components of their mitotic and or DNA replication pathways but offer little selectivity; thus that challenge is more about whether selectivity can be obtained rather than whether these are the right targets. There are several classes of approved cytotoxics in clinical practice with various mechanisms of action including alkylating agents, antimicrotubule agents, and topoisomerase inhibitors. Many of these are and have been investigated as polymer therapeutics (Table 1 and ref. [29]). Doxorubicin which was conjugated to a copolymer of N-(2-Hydroxypropyl)methacrylamide (HPMA) and was the first synthetic anticancer polymer conjugate in clinical trials. Understanding PK-PD in the target tissue is imperative. Many nanomedicines result in a reduced Cmax and often an increased area under the curve (AUC) or at least a flatter profile. For many cytotoxics the cumulative amount of drug in the tumor is well worth it if new drugs can be enabled and better treatment outcomes realized through more efficient delivery.

2.1. Selecting and Understanding the Right Target for a Polymer Therapeutic

The right target and thus drug for a polymer therapeutic depends on the aim or application. For a polymer therapeutic that consists of a PEG-protein conjugate, the improvements in protein stability, plasma half-life and immunogenicity, overall safety profile, and reduction in frequency of dosing appear to be a robust strategy. This is true for a range of different protein drugs and diseases and there are now over 19 marketed drugs in the United States and significant numbers in clinical development. Progress with polymer-protein conjugates has been recently reviewed. PEGylation at different sites on a protein provides an opportunity to control the selectivity of protein binding to its receptor and resultant pharmacodynamics. For example, a PEGylated IL-2, NKTR-214, where the PEGylation is at the lysine residues of the IL-2-IL-2R interface has reduced binding to the IL-2 receptor α-subunit (IL-2Rα) however binding to the IL-2 receptor β-subunit (IL-2Rβ) is barely affected, is in Phase 3 clinical trials in melanoma, muscle invasive bladder, and advanced metastatic renal cell cancers. As a result of this site specific PEGylation, NKTR-214 drives increased proliferation of CD8+ tumor killing memory effector T cells and reduced proliferation of immunosuppressive regulatory T cells (Treg) and greater anti-tumor efficacy when compared to IL-2 itself in preclinical evaluation. In contrast, NKTR-358, designed for the treatment of autoimmune indications, due to its different IL-2 PEGylation site, exhibits reduced affinity for IL-2Rβ while maintaining its affinity for IL-2Rα, enabling preferential activation of Treg with their suppressive activity. NKTR-358 has shown efficacy in a preclinical model of systemic lupus erythematosus and selective induction of Treg in healthy volunteers.

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Initial efforts with ADCs, focused on very potent cytotoxic payloads with in vitro activity in the picomolar range, several orders of magnitude more potent than marketed cytotoxics, thus precision targeting and site specific drug release are essential. Indeed, the field struggled with a lack of therapeutic index for many years and it is not until more recently with less potent payloads with nanomolar potency, rapidly cleared payloads and better conjugation chemistries, and better distribution understanding that ADCs as a class have progressed. They are now rapidly becoming important agents for oncologists with 4 approvals in the last year (44% of total). For potent drugs with potential for on target toxicities in healthy tissues, it may also be important to deliver specifically to the intended intracellular target with a polymer therapeutic. Adding a targeting ligand to a polymer drug delivery system offers several advantages over ADCs especially with respect to drug loading and flexibility as they offer a more modular design. In addition, ADCs are prone to aggregation and rapid systemic clearance especially as the number of drug molecules increases.

For those intracellular targets, where nucleic acid based drugs are explored to either generate or knockdown a protein, careful consideration to the number of cells needing to be transfected in order to modulate disease is needed. For instance, p53 loss is known as a key driver for many tumors, delivering an mRNA to replace this is possible; however, a large amount of cells within the tumor are likely to be required to express this protein for disease modification which may preclude such an approach. Conversely, for immunotherapy much smaller amounts of protein will be required to stimulate the immune system and afford an effect. Currently there are 15 mRNA targets as immunotherapies in the clinic as well as the recent rapidly developed nanomedicines as coronavirus disease vaccines. Many pre-clinical studies have shown the knock down of kRAS with siRNA, a key and challenging target. However despite success pre-clinically, these disease models are known to be poor in replicating the human disease and few have progressed to the clinic. Understanding the percentage of cells that need to be transfected and the spatio-temporal effect required as a result of knock-down or protein expression to modify the disease is critical for success.

2.1.1. Getting the Right Drug for the Right Target and Right Polymer Therapeutic

As well as ensuring that a polymer therapeutic can deliver a drug to engage with the target to afford disease modification, the drug needs to have the right properties to be either encapsulated within or have chemical groups for conjugation to a polymer. Suitable groups for conjugation include amines, hydroxy groups, thiols, and carboxylic acids which are present in many
drugs. Working with medicinal chemists allows drugs to be designed for delivery as well as target engagement.

The benefits of encapsulating drugs mean that a new chemical is not created and thus chemical development is normally complete and an easier regulatory path possible. Polyesters have been widely employed as parenteral drug delivery systems with a number of poly(lactic-co-glycolic acid) (PLGA) based products on the market. PLA-PEG and PLGA-PEG copolymers have been widely explored as polymeric nanoparticles due to their clinical precedence as drug delivery systems, safety, and biodegradability however it is difficult to encapsulate many drugs with high drug loading, low burst effect, and control their release. A hydrophobic ion pair approach has been applied to formulate nanoparticles with adjustable release rates and higher drug loadings. Various groups have modified the polymer composition and architecture of PEG polyesters to try to improve drug loading and release and make more compatible with typical drug properties.

From a nucleic acid based drug perspective, polymers give lots of flexibility; the first stage of formulation is normally complexation and a resulting condensation which provides protection from degradation until the nucleic acid is internalized in the target cell. Once within the cell endosome escape is still seen as the main blocker and then nucleic acid needs to be released from its carrier; siRNA, double stranded and with ≈20 base pairs is very different to a single stranded mRNA of ≈1000 nucleotides, to mRNA encoding for Cas9 of ≈5000 nucleotides and self-amplifying RNAs which are ≈10 000 nucleotides. Guide RNAs need to be formulated with the mRNA or with the Cas 9 protein for therapeutic gene editing purposes. Plasmid DNA needs to be delivered to the nucleus. For in vivo expression of antibodies, two mRNA, one for the heavy and one for the light chain are often used. The larger the nucleic acid based drug the greater the packaging required and getting the correct affinity and avidity to ensure complexation yet release of the nucleic acid is imperative.

### 2.2. Gaining the Right Exposure in Tissue/Cell

When polymers are used to improve therapeutic index or deliver a nucleic acid, to get the right exposure in the tissue, the nanocarrier needs to accumulate, distribute, and be retained and importantly the drug released there. In this section, factors affecting accumulation, distribution, retention, and drug release and their impact on exposure will be discussed. Most of the focus to date has been on prolonging circulation time to afford greater accumulation in tumors or other tissues via the EPR effect in tumors which has been the basis for most oncology nanomedicine research and development. A special issue of theranostics has recently been published; “The EPR effect and beyond: Strategies to improve tumor targeting and cancer nanomedicine treatment efficacy” collating 24 research articles and reviews discussing different aspects of the EPR effect providing a comprehensive overview of our current understanding and expert perspectives on how to improve the design of nanomedicine formulations for cancer therapy. There has been significant progress in our understanding of tumor targeting and the use of advanced imaging techniques to aid that understanding. Two important learnings that perhaps have been underappreciated in the field are: first the importance of macrophage uptake of nanoparticles and that they, as well as the poor lymphatic drainage are responsible for tumor retention and second the contribution of epithelial transcytosis to tumor accumulation. A similar biophysical targeting concept for macromolecules is also responsible for accumulation in other inflamed tissues, this has been more recently termed ELVIS, extravasation through leaky vasculature and the subsequent inflammatory cell-mediated sequestration, and applicable to various inflammatory diseases. Inflammation is a common feature of many diseases and there is scope for far more application of polymer therapeutics outside oncology.

### 2.2.1. Tissue Accumulation: The Importance of Circulation Time

Prolonged circulation times are needed to allow enough nanoparticles to circulate long enough to afford tissue accumulation either through biophysical means or active targeting; it is predominantly maintaining the initial plasma kinetics of the nano-carrier that drives this where high numbers of nanoparticles/macromolecules are available for extravasation. Briefly, once injected intravenously, nanoparticles interact with blood components, are recognized as foreign material and are opsonized by deposition of proteins allowing them to be phagocytosed and cleared by the organs of the reticuloendothelial system (RES), primarily the liver, spleen and if small enough, kidneys. Generally, a molecular weight greater than ≈40 kDa and size greater than ≈10 nm is required to avoid clearance via the kidneys and a size smaller than ≈200 nm is needed to avoid the Kupffer cells in the liver thus more rapid clearance. PEGylation and use of other polymers minimize protein deposition reducing clearance and enabling longer circulation time and greater tumor accumulation. Understanding this polymer-bio interface is critical in the design of polymer therapeutics. This is illustrated by a study with a fifth generation lysine dendrimer where 50% functional groups were conjugated to polyoxazolines and remaining groups had either amino groups (positive charge) or carboxyl groups (negative charge). Despite the overall zeta potential on both polymers being low, a large difference in plasma clearance and tumor accumulation was observed between the two polymers. The importance of circulation time on tumor exposure is illustrated below for nanocarriers with different circulation times and different release rates using a predictive mathematical model which was developed to understand the disposition of a nanocarrier (Figure 1).

**Polymers to Increase Plasma Circulation Time:** PEG has been the polymer of choice to prolong exposure for many delivery systems and has been successfully used in this way on a number of clinical products in different nanoparticles including liposomal systems like Doxil, Marquibo, and Ovidyne. The long circulating time of high molecular weight PEG has also been used for drug conjugates and 4- and 8-arm polymer architectures have been employed to increase the drug loading with Nektar Therapeutics taking the lead in this space. For example, etirinotecan pegol, a 4-armed PEG each linked to the prodrug irinotecan extended the plasma half-life of SN38, its active drug from 2 to 50 days in the clinic. Block copolymers of PLA-PEG and PLGA-PEG have also been synthesized and used as polymeric
nanoparticles to afford tumor targeting. Fabricating these nanoparticles with amphiphilic co-polymers containing PEG significantly increases the circulation time and reduces the liver uptake. For example in a pioneering study by Gref et al. 66% PLGA nanoparticles were taken up 5 min post injection in mice while less than 30% of PEGylated nanoparticles were taken up post 5 h. Carefully designed PLA-PEG nanoparticles have reported plasma half-lives of 10 h in mice and 18 h in rat. These prolonged circulation times have translated well across species, from mouse to rat, cynomolgus monkeys, and into clinical studies showing prolonged circulation times at least two orders of magnitude higher for docetaxel delivered in the PLA-PEG nanoparticles over a solvent based docetaxel formulation. PEGylation has also been used to extend circulation time for dendrimers however dendrimer generation, charge, PEG chain length, and density are all important design criteria. Generally, plasma circulation time increases as molecular weight increases. Plasma half-life can be extended from several minutes to over 50 h in mice through PEGylation and careful design with increase in plasma half-life resulting in high tumor accumulation to 15% ID/g tumor. PEGylated fifth generation polylysine dendrimers (DEP Dendrimer, Starpharma) are currently in clinical trials with docetaxel, cabazitaxel, irinotecan, and AZD0466 (Table 1). Alternatives to PEG for prolonging circulation time are poly(2-oxazolines). Serina Therapeutics, Inc. has developed a proprietary drug delivery polymer technology based upon poly(2-oxazolines), and is currently in clinical trials with drugs focusing on CNS disorders; their lead candidate is a once weekly dose subcutaneous dose of POZ-rotigotine. Poly(N-2-hydroxypropyl methylacrylate) (pHPMA) is another type of polymer with low protein adsorption that has been used extensively for drug delivery and was one of the first polymer conjugates to go to the clinic. Second generation systems have been modified to improve circulation time, drug loading, and biodegradability. Primarily by increasing the molecular weight a 3–5-fold improvement in plasma half-life in mice and significantly better tumor efficacy was observed for a range of conjugated cytotoxics over the first generation systems. Core cross linked micelles made from the copolymer PEG-b-pHPMA demonstrated a mean plasma half-life of released docetaxel of 16 h in rat and 39.7 h in human. Synthetic polypeptides are an expanding class of materials which can be modified to provide prolonged circulation. These include polylglutamates as star polymers and their self-assembled systems which both serve to increase molecular weight and improve retention in the circulation. More recently the polypeptoid, polysarcosine, n-methyl glycine has recently been used in a number of formats as an alternative to PEG. When used to as the outer polymer on a fifth generation polysyrline dendrimer plasma half-lives in excess of 50 h were achieved in mice.

The protein corona is known to affect distribution of nanoparticles but literature data on polymer therapeutics is relatively scarce. Adsorption of proteins is driven via attractive intermolecular forces such as Van der Waals, hydrogen bonding, disulfide interactions, electrostatic forces, and hydrophobic forces. Generally larger particles have both a larger surface area and a lower degree of curvature providing more potential binding sites for and a better interaction with proteins. Conversely, for small nanoparticles may completely avoid or have reduced protein adsorption. As well as size, morphology, surface charge, hydrophobicity, and ligands all affect protein adsorption. PEGs in a brush conformation and with densities greater than 7–20 PEG chains/100 nm² make protein interactions more difficult and thus minimize effects on clearance. The importance of density was also highlighted for polysarcosine chains. Recent work has shown that for soft small nanoparticles, polymeric micelles with hydrodynamic radius of 20–30 nm with either PEG, poly(sarcosine) (PSar), or pHPMA as dense hydrophilic shells there was negligible protein binding after incubation in human plasma.

Alternative Approach to Tissue Accumulation: Recently however there is some debate as to whether a more “hit and run” approach is more desirable for managing off target toxicities and rapid clearance by the kidney has benefits for clearance of nanocarriers that are non-biodegradable such as Elucida’s C Dots based on silica particles. This rapid clearance also offers advantages for diagnostic imaging applications and many of the systems used originated from diagnostics. Renally cleared nanocarriers minimize the accumulation of nanoparticles in liver and spleen. To afford greater tumor accumulation for this type of nanoparticle, ligand targeted approaches are required. C-dots have been explored to target a variety of tumor models through active targeting approaches. This delivery strategy will contribute to an improved therapeutic index by the both enhanced efficacy and safety and due to their size and flexibility this “hit and run” approach to targeting can also be explored more with polymeric delivery systems. However, the tumor AUC has been
shown to be significantly reduced via the shorter circulation time[70] (and as illustrated in Figure 1) and thus more potent accurately targeted systems will be required.

Drug delivery to the kidney is important for a number of diseases, and there is renewed interest in delivery to this organ and in particular access the different cell types; glomerular endothelial cells and basement membrane, podocytes, mesangial cells, apical and basolateral proximal tubular cells through both biophysical and active targeting polymeric delivery systems. There is a size-dependent distribution of nanoparticles as a result of various filters and physiological barriers between the kidney and the surrounding tissue or fluids. Nanoparticle shape, surface chemistry, rigidity, and charge all play a role in distribution within the kidney.[72] Polymeric delivery systems with their flexibility for size, architecture/shape will be important class of therapeutics.

Considerations for Intracellular Delivery: For intracellular delivery, different sizes may be optimal but will very much depend on the mechanism of intracellular uptake and often a compromise is needed between the optimal size of tissue accumulation and that for cell uptake. The charge, shape, and rigidity of the polymer and any resultant protein corona will influence cell uptake.[73] Nanoparticles are often taken up via active endocytic mechanisms, normally clathrin or caveolin mediated, or via non-specific cell membrane interactions such as phagocytosis for large particles or pinocytosis for smaller ones.[73] To be taken up by cells, generally there needs to be a membrane wrapping process and the energy barrier for internalization of small particles has been shown to be small. This energy barrier is dependent on type of particle, cell, and ligand density. Generally particles of ≈50 nm in size have tended to show good cell uptake however there are reports that some smaller particles (30 nm) may be too small to drive membrane wrapping.[74] A number of mathematical models have been developed to understand this process and it needs to be elucidated for desired cell type/nanoparticle. The cell uptake mechanism of a polymer therapeutical will affect the subsequent trafficking in the cell[75] and again needs to be understood as this affects drug release and target engagement.

Tumors are heterogeneous and some are poorly vascularized with low or little EPR effect,[76] in such cases immune cells can be exploited to target the tumor microenvironment.[77] For targets in immunotherapy, different approaches can be adopted. Delivering to the lymphatic system enables delivery to the potential sites of antigen presentation and immune cell proliferation either in the lymph nodes and other secondary lymphoid tissue to induce adaptive immune response.[146] Where T cells are the target, delivering via subcutaneous dosing to access T cells directly is liable to be beneficial. Stimulating the innate immune system will demand safe and precise delivery to the tumor microenvironment. Conversely, to target the immunosuppressive cells, namely tumor-associated macrophages, myeloid-derived suppressor cells, tumor-associated neutrophils, and Treg, will be required in the tumor, spleen, blood, and lymph.[78] The flexibility of polymer therapeutics will allow them to deliver immunomodulatory agents specifically to a variety of immune cell types.[79]

Ligand Targeted Systems in Relation to Tissue Accumulation and Right Exposure: Addition of targeting ligands to polymers can allow cellular specificity, improves intracellular delivery and enables tumor retention but biophysical targeting is still required to assess the tumor. Polymeric carriers can be designed to be similar in size to antibodies, nature’s endogenous targeting systems (≈150 kDa and 10–20 nm), which should permit good tissue accumulation and penetration.

Some of the key design features for selecting and designing such a ligand targeted system have been proposed.[80] Some receptors such as transferrin, EGFR, or folate are rapidly internalizing whereas others are more either non-or slowly internalizing or the receptor is rapidly recycled to the cell surface, thus efficient intracellular delivery is precluded. Her-2 is one such receptor however receptor cross-linking through multiple binding sites will trigger internalization more efficiently, trafficking to lysosomes.[81] Viruses are similar in size to many nanoparticles of 30–100 nm and are taken up into cells by attachment of viral spikes to various receptors on host cells to propagate infection. The binding of viruses is often highly specific however, they usually bind with low intrinsic affinity and bind to multiple receptors to enhance their binding avidity, causing translational signal to initiate endocytosis or trigger membrane fusion. There is much to be learned from nature.[82] In terms of ligand density, viruses show a range of spike proteins, 0.01 to 1.73 per 100 nm² a lot less than traditionally explored as ligands on a range of nanoparticles 0.17–83 ligand per 100 nm² of nanoparticle surface and generally this is an optimal ligand density for efficient cell uptake and internalization.[83] Polymeric carriers can exploit this multivalent targeting to afford greater selectivity and more efficient delivery. Adding 2–10 Her-2 affibody peptides to HPMA copolymer chains illustrated improved drug efficacy in vivo where the number of ligands was critical for rapid endocytosis.[84]

However, addition of targeting ligands can result in reduced circulation times and therefore less biophysical targeting via the EPR effect, with a concomitant increase in off target effects. Designing the correct ligand density to balance between “stealthiness” and therefore maintaining a prolonged circulation time and enhanced cellular uptake density is imperative.[85] A compromise will be required and the balance is liable to be ligand dependent and delivery system dependent. For example, the addition of RGD or NRD ligands for targeting tumor vasculature on to small HPMA star polymers (≈10 nm), improved initial tumor accumulation. However, the overall accumulation in tumors was significantly reduced. This was attributed to the ligands increasing the clearance of the star polymers.[85] Similarly, despite targeted nanoparticles using multivalent peptides to target CD138 receptor having much higher binding and cell uptake in vitro, they were inferior in an in vivo melanoma xenograft model than CD38-targeted nanoparticles and non-targeted nanoparticles. As the biodistribution in the clearance organs was similar, it was suggested that binding to healthy circulating lymphocytes upon injection would reduce the number of nanoparticles available for tumor accumulation. These off target effects being an inherent property of the rapidly internalizing CD138 receptor.[86]

Sivram et al. designed an elegant study to understand the impact of scFv PSMA ligand density on cellular uptake and biodistribution of polymeric micelles. They demonstrated that an optimal ligand density is required to effectively accumulate in the tumor and avoid immune cell uptake. They showed the higher the antibody fragment content in the micelle, the greater the interaction with the immune cell population in mouse blood and immune...
Figure 2. Considerations for ligand targeted polymer therapeutics; A) Disease led design integrating drug-target, pathophysiology of disease, ligand receptor interaction and delivery systems, B) An ADME cascade needs to be set up to determine biodistribution, bioanalytical methodologies for total and released drug and PBPK modeling to aid design, C) Balancing factors that may improve targeting with those that may be detrimental D) Example PB-PK model to describe tissue distribution and intracellular uptake and trafficking.

The value of adding a targeting ligand to a nanoparticle decreases as the size of nanocarrier increases particularly where there is a biophysical component such as in tumors or inflamed tissues. For example, when small, short-circulating (10 kDa, ≈7 nm, t_1/2 ≈ 1 h) and larger, longer-circulating (40 kDa, ≈13 nm, t_1/2 ≈ 13 h) riboflavin-targeted branched PEG polymers were compared. The longer circulating larger polymer accumulated in tumors four times more efficiently than the smaller polymer but active targeting did not provide additional accumulation. The riboflavin targeting enhanced the cellular internalization in both tumor models and for both polymer sizes however the 10 kDa polymer had highest uptake in tumor cells and the 40 kDa by tumor associated macrophages.[88] Zhang et al. demonstrated that the protein corona formed was quantitatively altered for three different ligands for the transferrin receptor (two different 1 kDa peptides and transferrin; MW = 77 kDa) on polystyrene nanoparticles. Differences were also observed between in vitro and in vivo corona composition. Ligand size and conformation and resultant corona are important for cellular internalization and exocytosis.[89] An additional challenge with ligands, particularly those of high affinity such as antibodies or antibody fragments is binding site barriers between the ligand and target.

The challenge is to develop strategies to design next generation systems, balancing the various competing factors including enhanced cellular uptake through higher ligand density and managing off target toxicities with need for “stealthiness” to avoid rapid immune recognition and clearance. Progress has been made in understanding some fundamental properties in the drive toward more precision targeting and has been reviewed by Mi et al.[90] Attempts to model nanoparticles interactions with biological systems have been carried out. A superselectivity theory has been presented and supported via experimental data. It illustrates that a combination of multiple low-affinity ligands creates on-off association profiles and that a multivalent scaffold saturates the receptors only above a given onset receptor density, while binding does not occur at lower receptor densities.[91] This model has been adapted and refined for a multivalent polymersome designed to minimize unspecific interactions and with more realistic binding energies. It provides a theoretical framework to design targeted systems balancing parameters such as particle size, brush length and density, tether length, affinity, and ligand number. It also shows that the ligand density can be designed to switch the intracellular entry mechanism from endocytosis to transcytosis.[92] Ligand targeted polymeric delivery systems have reached the clinic in oncology applications. In addition, there are substantial pre-clinical investigations in crossing the blood-brain barrier and cardiovascular diseases. Figure 2 proposed workflow to design an actively targeted polymer therapeutic balancing some of these properties.

Hybrid systems are emerging where polymers conjugated with drugs are attached to antibodies to improve the drug to antibody ratio are being explored. Mersana Therapeutics have their dolaflexin platform which utilizes their proprietary biodegradable polyacetal polymer (Fleximer) able to carry multiple drug molecules with their antibodies. XMT-1536 targeting the sodium-dependent phosphate transport protein NaPi2b expressed across a number of tumor types has entered Phase I in patients with tumors likely to express NaPi2b, including ovarian cancer and non small cell lung cancer (NSCLC) (NCT031319628). Mablink Bioscience has their PSarlink technology, integrating short polysarcosine chains into the linker to mask the hydrophobicity of any drugs so providing prolonged circulation. In addition, this provides an increased drug loading capacity (DAR 8 or 16), reduces propensity to aggregate, and provides a more
homogeneous product with good plasma stability and flexibility for different linkers. This has been termed a third generation antibody drug conjugate (ADC).[91]

Number of Particles Affecting Tumor Exposure: In terms of tumor accumulation a recent evaluation has demonstrated that it is the number of particles that are important for avoiding liver and RES uptake for both active and passively studied particles.[94] The liver does not get saturated but can only clear a specific number of particles, so as liver accumulation decreases, tumor accumulation increases. This suggests that at least a trillion particles are important for significant tumor accumulation and those nanomedicines that have been successful in the clinic have particle numbers above this value. In this analysis, the number of nanoparticles contributes more to tumor delivery than size, targeting design, nanoparticle type, or cancer model. Many of the polymer conjugates may well have advantages here. This is liable to have important implications for the clinic in terms of infusion times and rates and dosing schedule, perhaps not until now has it been a consideration for clinicians. In the design of nanomedicines this finding also has important implications for the balance of drug loading with number of particles. Efforts to drive drug loading up are important to minimize the delivery of excipients and the number of vials and doses for the patient and to lower the cost of goods. For a drug with molecular weight of 500 Da, a 20% w/w drug load for a 100 nm polymeric nanoparticle gives $\approx 1 \times 10^{11}$ nanoparticles per mg of drug dosed compared to $\approx 1 \times 10^{10}$ nanoparticles per mg of drug on a fifth generation dendrimer with $\approx 20\%$ loading by weight where 50% of the surface functional groups are conjugated with drug.

2.2.2. Distribution in Tumors

The distribution of nanoparticles in tumors is limited by elevated interstitial fluid pressure which hinders convection and the high viscosity of the extracellular matrix which restricts their diffusion. In terms of tumor distribution, size has been shown to be an important factor with smaller sizes favoring better tumor penetration. A range of different sized polymeric micelles between 30 and 100 nm were evaluated in highly and poorly permeable tumors. It was found that only the 30 nm particles could penetrate the poorly permeable tumors and give efficacy.[95] Using fluorescent quantum dots of 12, 60, and 125 nm and intra vital imaging, the smaller nanoparticles extravasated and diffused furthest from the blood vessel. Using mathematical models and supporting experiments Chauhan et al. showed that smaller nanoparticles (12 nm) have more rapid and uniform tumor penetration than larger nanoparticles (125 nm).[96] Generally nanoparticles have a more heterogenous distribution than free drug within a tumor.[97] However, it has recently been demonstrated that nanoparticle tumor penetration is enhanced and heterogeneity reduced with more nanoparticles reaching the tumor cell population at higher doses.[94] This is in contrast to work from the same group previously showing that intravenously injected nanomedicines remained largely extracellular in tumors, with only 2% of cancer cells being positive for nanoparticles.[98] Despite a high tumor accumulation (18.6% ID/g) only 1.5% of tumor cells in a 4T1 tumor xenograft model were positive for a PEG-b-pHPMA-based core-crosslinked polymeric micelles with hydrodynamic radius of 20–30 nm. The good efficacy achieved with these clinical stage polymeric micelles was therefore attributed to providing a local depot within the tumor microenvironment rather than the polymeric particle accessing the tumor cells.[99] In contrast, more than 15% of immune cells in the tumor microenvironment were found be associated with the micelles and overall, 66% of intracellular micelles were present in CD45+ leukocytes. Similar preferential uptake by immune cells over tumor cells (≥3 times) within the tumor microenvironment was observed for PLA-PEG nanoparticles.[100] This propensity for immune cell uptake offer applications in immunotherapy where modulation of the tumor microenvironment is beneficial. For example, employing cross-linked cycloexextrin nanoparticles[101] and a high drug loaded poly(2-oxazoline) (POZ)-based nanomicellar formulation of TLR 7/8 agonists[102] to target the M2-like pro-tumor macrophages in the tumor microenvironment in order to promote polarization to M1-like anti-tumor macrophages is emerging as a promising immunomodulatory strategy. “Phagocyte hitchhiking” has been reported as an important mechanism for αvβ3-integrin targeted nanoparticles to aid tumor targeting and distribution.[102] Harnessing nanoparticle-immune cell interactions is liable to become an important design feature for polymer therapeutics intended for immunomodulation.

Tumor and likely other tissue distribution is also complicated by transcytosis. Transcytosis has been shown to be an important mechanism of distribution within tumors and should be considered in design of nanomedicines. However, the design rules for transcytosis still remains unclear. The complex process of cell-nanoparticle interactions involves endocytosis, intracellular trafficking, and exocytosis, utilizing several pathways. Understanding endocytosis better will enable better design of nanoparticles to exploit this process and the ability for subcellular targeting.[103] Transcytosis is thought to play an important part in tumor accumulation and distribution in pancreatic ductal adenocarcinoma where the tumor stroma provides a barrier to drug delivery.[104] Studies on tumor spheroids, especially those co-cultured with immune cells or fibroblasts, should inform nanoparticle distribution through tumors; for example, PEGylated particles were shown to distribution better through spheroids than those without PEG.[105] RGD targeted PLA-PEG nanoparticles were shown to be trafficked through spheroids better with a linear rather than cyclic ligand.[106] cRGD-polymeric micelles were shown to cross the vascular barrier through transvascular transport by cRGD-αvβ3 integrin mediated transcytosis and have enhanced cellular uptake in glioblastoma cells.[107] Transcytosis is emerging as an important component of tumor distribution.[108] Distribution through tumors is complex and much is still to be learned. The importance of distribution of the nanoparticles depends very much on the drug payload and whether intracellular delivery is required to improve and enable efficacy, or getting to the tumor and acting as a depot for a small molecule is enough.

2.2.3. Retention in Tumors

Nanoparticles are retained in tumors thus providing enhanced drug residence time in the tumor. This retention has been observed for $\approx 100$ nm PLA-PEG polymeric nanoparticles[97] and...
smaller polymers as observed via mass spectrometry imaging. Interestingly for liposomal systems the tumor Cmax appears to be at 24–48 h however for small polymeric systems which are reported to accumulate and distribute better, the Cmax appears to be later. For example, the tumor Cmax is ≈ 72 h for star copolymers with 9% D/g tumor at ≈ 15%, in excess of 48 h for PEG-b-pHPMA-based polymeric micelles with 18.6% ID/g and 14% of injected dose by 4 days for 15 nm 4-arm branched PEG. The greater accumulation and delayed Cmax is thought to be due to the longer circulation time and potentially larger number of particles giving more time to penetrate the tumor. Beckford Vera et al. following a PET study measured the efflux from tumors of 15 nm PEG conjugates and reported elimination half-life values of 300–400 h, fivefold slower than from other tissues. Tumor concentration was still 10% ID dose 9 days after injection.

2.2.4. Drug Release from Nanocarrier

So far, the progress in understanding the design features required to get the polymer therapeutic to the tissue of interest has been discussed, yet it is the release of drug in that tissue/cell and how it interacts with its intended target that is critical for efficacy. The release rate required will be different for different targets and the degree of target engagement needed for different diseases. Polymer therapeutics aimed at haematological malignancies will benefit from prolonged circulation times in the blood and drug release in the bone marrow, lymph nodes, and spleen. However, for solid tumor indications tumor accumulation, distribution, and retention in primary and metastatic lesions with be required and providing a depot of drug to prolong tumor concentration may be enough.

For polymer therapeutics in clinical development, the release mechanism is diffusion based for encapsulated systems like the PLA-PEG polymeric nanoparticles and via hydrolysis of chemical linkers for polymer conjugates like Starpharma’s DEP dendrimer platform (paclitaxel, cabazitaxel, SN38, and AZD0466), Cristal therapeutics’ CPC634 and Prolynx’s PLX038. Release rates from all these delivery systems are tuneable, either via hydrophobic ion pairing and/or manipulation of polymer molecular weight and composition in the case of the polymeric nanoparticles or via careful chemical design in the case of the linker chemistries attaching the drug to the polymer. Tuning the release has been demonstrated to be imperative for maximizing the therapeutic index advantage. The benefits of hydrolytic linkers are that they are independent of enzymes, are much more consistent across species and potential patient populations and often in an in vitro in vivo correlation (IVIVC) can be made which enables mathematical modeling to be used to help design a suitable release rate. Some linkers rely on a hydroxide-catalyzed β-elimination reaction and multiple linker chemistries have been developed that have cleavage rates determined by the acidity of a C–H bond on the linker which is controlled by electron-withdrawing groups attached to the ionizable C–H. Such linker chemistries can provide a range of release rates with half-lives ranging from hours to months.

To afford greater site specificity, physicochemical and pathological factors in diseased regions can be exploited to increase the specificity of drug delivery. These can be external stimuli such as thermal, light, ultrasound, and magnetic fields or disease or endogenous stimuli such as pH, redox potential, temperature, hypoxia, or enzymes. To date, a combination of poor specificity and heterogeneous distribution has affected the success of many stimuli-responsive delivery systems.

Self-immolative type linkers are increasingly popular choices as stimulus responsive linkers, where a cascade of reactions leads to the drug release. They are already an established linker type with ADCs, where typically an enzymatic cleavage of a peptide or substrates for β-Glucuronidase/β-Galactosidase leads to a 1.6 elimination of drug via a p-aminobenzylalcohol spacer providing high specificity to the target. To date, self-immolative linkers involving chemical triggers via pH changes have tended to be employed in polymer therapeutics.

Targeting the lower pH of the tumor microenvironment, a recognized hallmark of cancer and so called Warburg effect, as a result of glycolysis and lactate production, provides site specificity for the tumor microenvironment across a range of tumor types. For those polymeric systems, designed for intracellular release, the low pH of the endosome provides a site specific trigger. pH sensitive linkers such as hydrazones, imines, acetics, or carbonates have been employed to take advantage of lower tumor and endosomal pH. Hydrazone linkers are popular in the literature however they are typically limited to a handful of drugs for direction conjugation. For example, with anthracyclines doxorubicin and epirubicin as the resulting hydrazones provide useful release at acidic pH in tumors. pH-responsive epirubicin-loaded polymeric micelles (NC6300) have entered Phase II study (NCT03168061) for evaluating the dose, activity, and tolerability in patients with soft tissue sarcoma. Preclinical clinical studies demonstrated that the epirubicin-loaded polymeric micelles reduced epirubicin cardiotoxicity and enhanced efficacy in a hepatocellular carcinoma model and treated breast cancer axillary lymph nodes metastasis through selective accumulation and pH-triggered drug release. Acetals have also been used for a number of drugs, for example, a vinyl ether functionalized block copolymer of PEG-p(acrylic acid) was reacted with paclitaxel to form the acetal linkage. Conversely, drugs can be directly incorporated into the polymer backbone via acetal linkages (polyacetics), for example combining curcumin and diethylstilbestrol into a PEG-based polyacetal for combination treatment of prostate cancer. The β-thiopropionate linkage has also been useful as a pH responsive linkage for camptothecin triggered by mild acidic pH (5-6).

The hydrolytic cleavage of a diverse set of molecular structures has been examined for their use pH-sensitive delivery systems by comparing their hydrolysis profiles at pH 5.5 and their relative hydrolysis at pH 5.5 versus pH 7.4. A wide variety of hydrolytic stability profiles were found in the structures commonly used as pH sensitive linkers and a slight modification to the structure could have a profound and surprising effect on stability. This questions the suitability of some of the typical pH sensitive linkers and emphasizes the importance of rational design and understanding structure-reactivity relationship and the need for more differentiating chemistries. Importantly for the design of polymer therapeutics, incorporating these linkers into larger structures reduced their hydrolysis rate but gave similar relative reactivity. The progress in predictive science
on chemical reactivity should aid in future design of polymer therapeutics.

Despite significant work on pH responsive linker chemistries, the pH differential between plasma pH of 7.4 and tumor is often less than 1 and known to be heterogeneous and therefore it is difficult to achieve specificity with conventional small molecule linker chemistries. However, tumor acidosis has been successfully used in the clinic for the detection of tumors with both (pH-low insertion peptides) pHLIP® technology and by tunable pH-sensitive amphiphilic polymeric micelles that generate a fluorescent output at reduced pH. The heterogenous nature of the tumor microenvironment, the intracellular pH together with the speed with which delivery systems are trafficked through the endolysosomal pathways is variable between cell types. This biological variability drives the need for more rapid and sensitive responsive materials.

Polymeric micelles can incorporate various functional groups to detect subtle changes in their environment by modifying their composition and polymer architecture. In a self-assembled polymeric micellar system electrostatic, hydrogen bonding, and hydrophobic interactions are interwoven in a polyvalent structure to create a cooperative and highly responsive system. pH sensitive micelles are often designed to have either ionizable groups or stable linkers at pH 7.4 that are able to rapidly protonate or hydrolyze at mildly acidic pH. A combination of nanoscale cooperativity and phase transition is required to sense and amplify physiological signals in order to improve the therapeutic outcome. Polymers based on a poly(methyl methacrylate) backbone and more recently a biodegradable polycarbonate backbone have been designed where the acute pH sensitivity comes from ionizable tertiary amines with different hydrophobic substituents. In response to a slight reduction in pH, cooperative micelle disassembly occurs and has been demonstrated to provide enhanced tumor targeting and intracellular delivery. This molecular and nanoscale cooperativity has the potential to significantly improve site specific release and thus more precision targeting.

An alternative approach is to design smart superstructures like the pH sensitive PEG-b-poly(2-azepane ethyl methacrylate) (PAEMA) which was conjugated cisplatin-prodrug covalently bound to polyamidoamine dendrimers, which formed clustered nanoparticles of ≈100 nm at physiological pH as a result of the hydrophobic interactions of the unionized PAEMA blocks. At mildly acidic pH, the PAEMA is rapidly protonated and dissociates into positively charged small prodrg particles (<10 nm) enabling improved tumor penetration, cellular uptake, and better efficacy. Glutathione (GSH), is the most abundant biological reducing agent and is present in tumor tissues at a concentration at least fourfold higher than in normal tissues. In addition, GSH is present at concentrations of ≈2–10 mM in the cytosol and cell nucleus, 2–3 orders of magnitude higher than in the blood and extracellular environment. Reduction-responsive polymeric nanoparticles and polymer conjugates have been engineered for tumor-specific drug release have been widely explored. The main design approach is the use of disulfide linkages that undergo rapid cleavage in the presence of reducing environments but stable under oxidative conditions. Polymeric carriers have been designed predominantly using two different strategies either using a disulfide bond in the polymer backbone or employing reduction-sensitive crosslink molecules which can be incorporated in the core or shell of the polymer. This area has been recently reviewed by Monteiro et al. and Quinn et al. However clinical translation of this approach remains a challenge with tumor heterogeneity and the complexities of the materials suggested as the major impediments. The reactive oxygen species, ROS, concentration is raised by 2–3 orders of magnitude in tumor cells with a concentration as high as 100 μM. This higher oxidative condition has driven the development of ROS-responsive polymer conjugates. Combination with other responsive systems such as pH in the tumor microenvironment has also been explored to drive greater site specificity. Enzyme responsive systems have also been explored. Cathepsin B expression is upregulated in many solid tumors and is correlated with an invasive phenotype. The peptides glycine-leucine-phenylalanine-glycine and valine-citrulline are specifically cleavable by cathepsin B and have been used extensively for cleavable linkers in ADCs. Use of enzymatic cleavage mechanisms of release with polymeric systems, however, has been less successful due to steric hindrance effects preventing access of the enzymes. For example, at high conjugation ratios of paclitaxel conjugated to poly(l-glutamic acid) steric hindrance prevented the access of enzymes a slowed the polymer degradation and drug release. Increasing PEG chain length on dendrimers or increasing dendrimer generation increases surface PEG density and thus the steric shielding of peptide linkers, thus reducing drug release of a cathepsin cleavable peptide linker. Reducing stealth or dendrimer size however results in more rapid clearance and thus careful design is required for this type of linker chemistry is to be employed. Matrix metalloproteinases (MMPs) are a major class of extracellular enzymes involved in cancer initiation, progression, and metastasis and thus upregulated in tumors. MMPs are used as a diagnostic and have been explored as therapeutic targets. MMPs are also upregulated in other diseases such as cardiovascular diseases, arthritis, fibrosis, and brain injury and it has been argued that they are more robust as a responsive trigger than both pH and redox potential. Relatively short peptide sequences have been engineered into a range of different polymeric drug delivery systems including dendrimers, polymeric nanoparticles, and polymeric micelles to address site specificity however this type of responsive system is still at the pre-clinical stage.

For nucleic acid based drugs, electrostatic charge has been used to combine with polymers avoiding any covalent chemistries. Here the size of the nucleic acid needs to be considered as the avidity is very different for small double stranded systems, to larger single stranded modified mRNA, ≈900–5000 nucleotides and larger still self-amplifying RNAs (saRNA), ≈10 000 nucleotides, where secondary and tertiary structures will also play a part. For example, comparing siRNA and mRNA using chitosan as the cationic polymer showed that mRNA gave higher avidity and thus higher polyplex stability and thus lower mRNA available for translation and a lower transfection efficiency. As with lipid delivery systems many of the same polymeric delivery systems have been explored for siRNA, mRNA of various sizes, plasmid DNA and now more recently saRNA. Polymer chain length and charge density of poly(ethylene imine) based copolymers were shown to be important for transfection efficiency for
pDNA, mRNA, and saRNA polyplexes and the largest saRNAs having a narrower design space.\[140\] To improve tolerability and delivery of the saRNA, a bioreducible, linear, cationic polymer, poly[N,N′-cystaminebis (acrylamide) co-4-aminobutyro poly

\[141\] mer (pABOL) was engineered to provide larger molecular weight polyamidoamines and showed enhanced delivery both in vitro and in vivo.\[141\] Employing a high throughput design-make-test cycle to understand complexation, particle size, charge, cell toxicity, cell uptake, endosome escape as well as productive uptake will accelerate the design of more efficient intracellular delivery systems to ensure optimal functional delivery of the nucleic acid.\[142\]

2.2.5. Assessing Exposure

A recent re-analysis of the nanoparticle tumor delivery from historical literature studies using classical pharmacokinetic metrics showed that the relative tumor delivery of nanoparticles was \( \approx 100 \)-fold greater, as assessed by the standard \( \text{AUC}_{\text{tumor}} / \text{AUC}_{\text{blood}} \) ratio than by %ID in tumor, in the somewhat provocative paper from the Chan group.\[143\] Polymeric based systems showed a threefold better delivery efficiency than liposomal systems in this study. A more appropriate measure would be the ratio of free drug concentration rather than total drug concentrations particularly with respect to optimizing therapeutic index and minimizing off target effects but obtaining free drug data is extremely challenging. The large, usually greater than 1000-fold difference between total initial concentrations in the plasma to released drug concentrations, particularly, for a slow releasing nanomedicines, mean that accurate separation of free drug from bound or encapsulated drug is technically challenging and prone to errors.\[144\] This is further complicated by labile release mechanisms and challenges with post sample stabilization. Errors from later timepoints or those tissues with lower concentrations are likely to be less. A number of novel bioanalytical techniques have been proposed\[145\] however more methods to solve bioanalytical challenges that can be more readily applied during both design and development of polymer therapeutics are needed. Mathematical modeling should be used more to understand the free drug concentration and aid in both the design and development of nanomedicines.

Physiologically based pharmacokinetic (PB-PK) mathematical modeling is routinely used and critical to understanding the absorption, distribution, metabolism, and accurate free drugs and needs to be adapted and used more in polymer therapeutics design. Mathematical modeling is being explored in nanomedicine design. A range of diverse mathematical modeling techniques including kinetic and coarse grained molecular dynamic simulations to investigate protein corona formation, continuum and hybrid models to look at microvascular transport, discrete models to evaluate intracellular delivery, pharmacokinetic models for distribution and clearance, hybrid models for tumor delivery and pharmacodynamic models to describe efficacy and safety are being explored.\[146\] Measurement of drug concentration at the target site and in the main toxicological organs is challenging yet critical for better design and development of any nanomedicine. Predictive mathematical modeling can play an important role in aiding this design. A predictive mathematical model for whole-body pharmacokinetics and tumor delivery has been developed and via sensitivity analyses has identified the factors that result in low tumor delivery efficiency and high off-target accumulation of nanoparticles. The analyses revealed that nanoparticle size and degradation rate, tumor blood viscosity, tumor vascular fraction, and tumor vascular porosity are the key parameters in governing nanoparticle kinetics in the tumor interstitium.\[147\]

For polymer therapeutics where the drug is attached via a hydrolytic bond an in vitro in vivo correlation of the release rate can often be established. This enables mathematical modeling to be used in the design of a nanomedicine. This is exemplified by the design of dual Bcl-2/Bclxl conjugated dendrimer, AZD0466, where a simple semi-empirical mathematical model was used to design the optimal release rate for improving the therapeutic index.\[133\] An analogous PB-PK model was used to describe the drug delivery properties important for PLA-PEG nanoparticle disposition.\[146\] Non-invasive in vivo imaging permits both the visualization and quantification of the in vivo disposition of nanoparticles and is crucial for a comprehensive understanding of their distribution. The use of image-guided mathematical modeling for pharmacological evaluation of nanomaterials has been recently reviewed by Dogra et al.\[148\] An integrated SPECT/CT imaging-based pharmacokinetics mathematical model to evaluate the disposition of mesoporous silica nanoparticles has been developed.\[149\]

Mass spectrometry imaging (MSI) is a powerful label-free tool that can map the nanocarrier, the drug, and its pharmacodynamic effect in tissues therefore providing critical additional data to deepen mechanistic understanding.\[150\] Quantitative data can then be used to guide the development and parameterization of mathematical models for both descriptive and predictive purposes in nanomedicine design and development. To further understand delivery system distribution within tissues at a cellular level, imaging mass cytometry (IMC) can be employed. This technique enables simultaneous analysis of up to 40 parameters at subcellular resolution in a single tissue section. Many polymeric systems are amenable to facile labeling with metals thus whole body distribution via PET and cellular distribution within specific tissues via IMC can be rapidly provided. Figure 3 shows the granular distribution of a lanthanide labeled polysarcosine star polymer (S-Dend159Tb), with molecular weight 115 kDa, in mouse duodenum tissue following intravenous dosing.\[151\] Integrated molecular imaging and advanced image analysis have the opportunity to make a huge impact in the clinical translation of polymer therapeutics.

Integration and development of mathematical modeling techniques with innovative experimental investigation of polymer therapeutics will aid both the understand structure-activity relationships to inform design and will aid more successful clinical translation.

2.3. Right Safety

For polymer therapeutics, considerations with respect to safety need to include the active drug, the polymer carrier, and its degradation materials, any linkers or ligands as well as the whole delivery system. This is a very different safety assessment to a typical small molecule. A “safe by design” approach to nanomedicines has been proposed for inorganic particles however many of the
principles can be transferred to polymer therapeutics.\textsuperscript{152} For actively targeted systems, the selectivity of the ligand needs to be carefully optimized; here a bioinformatics approach can be used to identify key tissues where the receptor is overexpressed to direct toxicity evaluation.

For those drugs where therapeutic index is the goal, improving safety is critical. Generally, minimizing the Cmax and drug plasma profile improves both on-target and off-target toxicities. Figure 1 shows the predicted drug plasma profile for different circulation times and release profiles. For example, one of the key on-target toxicities for the dual Bcl-2/BclxL inhibitor, AZD4320 was cardiovascular toxicity. We designed a dendrimer-based conjugate to minimize this whilst maintaining efficacy, which enabled a 25-fold reduction in plasma concentration for similar efficacy in the rat and an 18-fold improvement in therapeutic index in dog based on dose. Employing a dendrimer-drug conjugate also had that benefit of reducing thrombocytopenia, one of the key toxicities for the Bcl-2 family of inhibitors.\textsuperscript{151} The magnitude of the safety improvement required is dependent on the cargo and its on and off target toxicities. Traditionally cytotoxics have been dosed to MTD however with improved delivery to the tumor and better efficacy this should no longer be needed. The amount of free drug present in product should be managed by the specification with a tight specification being required for drugs with toxicity concerns. Toxicity studies should be designed to ensure free drug concentration expected at the end of the shelf life and infusion is suitably qualified during initial good laboratory practice (GLP) toxicity studies.

By changing the drug’s distribution and employing functional polymers, different toxicities of the drug may well be observed. For instance, for Doxil, although cardiac toxicity can be managed by virtue of the liposomal formulation, hand and foot syndrome is thought due to being prolonged circulation and release of doxorubicin from liposomes in the periphery.\textsuperscript{151} Skin toxicity has also been observed as the DLT of the docetaxel polymeric micelle, CPC634, with those patients with a higher plasma AUC for total docetaxel experiencing skin toxicity. This has been attributed to prolonged systemic exposure and different biodistribution of docetaxel released by CPC634.\textsuperscript{43} It is likely that such skin toxicities could present as DLT for other polymer therapeutics with prolonged circulation times. A compromise is needed between prolonged circulation leading to higher tumor accumulation versus higher propensity for off target effects and nonspecific uptake by tissues.

As well as managing the toxicity of the drug, the polymer tolerability needs to be considered. Many polymers are biodegradable or are small enough to be excreted via the kidneys.\textsuperscript{154} Larger structures, such as hyper-branched polymers and self-assembled systems should have labile bonds which can be degraded to low molecular weight sub-units that can be renally excreted. The advantage of polymers as a biomaterial delivery system is that they can be built up from natural materials such as lactic acid, lysine, glutamine and they can be designed to be biodegradable and of low immunogenicity.

There has been much debate on the safety of PEG, following its extensive use with proteins, generally where the molecular weight is below 40kDa to enable some renal elimination due to concerns of accumulation.\textsuperscript{44} PEG has been employed as it was considered largely inert and a review of available data from PEGylated biopharmaceuticals indicates that toxicological effects came from the active moiety in the drug substance rather than the PEG moiety.\textsuperscript{155} However, recent data both in
experimental and clinical research with different PEGylated drugs reveal the rise of anti-PEG IgM and IgG both in animal models and in patients. These anti-drug antibodies may cause adverse immune effects such as hypersensitivity reactions which can sometimes lead to anaphylactic shock and even death or cause an acceleration of the blood clearance (known as the ABC phenomenon), resulting in efficacy loss. This phenomenon has been recently reviewed for PEG containing nanomedicines and PEGylated proteins and depends on the structure of the polymer, end groups, linker, and nature of drug and nanocarrier.156

Nanoparticles are cleared by organs of the RES; the liver is by far the largest RES organ and takes up a significant portion of administered nanoparticles (30–99%) acting as a biological filtration system.157 The balance between those nanoparticles going to the spleen, liver, and lung is different and is dependent on surface chemistry, shape, size, and charge. This selective tissue tropism has been nicely demonstrated for lipid nanoparticles.158 Biodistribution studies are critical to understand tissue distribution and exposure over time and enable focused histopathology and clinical chemistry on those tissues with greater accumulation.

Within the liver, hepatocytes which make up of 70–80% of cells, favor positively charged particles, and liver sinusoidal endothelial cell (LSEC) and Kupffer cells (KCs) negatively charged particles due to electrostatic interactions with scavenger receptors.157 Residual amino groups on a poly(2-methyl-2-oxazoline) modified lysine dendrimers led to more uptake in hepatocytes compared to the professional scavenger cells, KCs, and LSECs whereas residual carboxylic acid groups had less overall liver uptake and less preference for hepatocyte uptake allowing prolonged blood circulation.151 This enhanced understanding of distribution within the liver is important if the associated drug has liver toxicity liabilities and if changes to the delivery system in circulation due to drug release from linker or disassembly of polymeric micelles lead to greater uptake/liver scavenging. Hepatocytes remove foreign substances and particulates by endocytosis, followed by their enzymatic breakdown and excretion into the bile via the biliary system and ultimately into the small intestine and feces. This process takes days to weeks.159

Phagocytic cells in blood and tissues clear polymers from the body. The phagocytic cells include; blood-circulating monocytes, hepatic Kupffer cells and LSECs, splenic red pulp, and marginal zone macrophages as well as bone marrow perisinal macrophages. Cellular vacuolation can be seen microscopically particularly in macrophages in a number of tissues. In a review of the data collected from pre-clinical toxicity studies of PEGylated biopharmaceuticals, the extent of vacuolation depended on physical characteristics, the molecular backbone, the dose, the drug target/pharmacology, and duration of exposure of the PEG product.160 In addition, PEG-related vacuolation is not associated with demonstrable cell and tissue damage or dysfunction and is reversible with sufficient duration of drug-free periods. A cross-industry group has published a “points to consider” paper to aid in designing studies and interpreting data from toxicity and safety studies intended to support regulatory submissions.160

This learning and guidance should be invaluable to design and interpret safety studies for polymer therapeutics.

Biodegradable polymers that degrade when phagocytosed have advantages over non-biodegradable nanoparticles that can remain within the cell and be sequestered in the spleen and liver for more than 6 months however the degradation pathway and time should be understood. Ouyang et al. showed for PEGylated gold particles that liver accumulation and Kupffer cells uptake decreased as the number of nanoparticles increased to a threshold single dose of 1 trillion nanoparticles.194 At doses above this a minor proportion of nanoparticles accumulated in hepatocytes suggesting that hepatocytes served as a liver accumulation reservoir once Kupffer cells are saturated. The liver in rat is ≈6% body weight in comparison to ≈2% in humans and this is liable to be different for different types of nanoparticles but suggests an optimal number may be ideal from both efficacy and avoiding excess polymer within the body. A similar cellular biodistribution was observed for PEG-b-pHPMA-based core-crosslinked polymeric micelles that were located within macrophages and Kupffer cells in the liver and macrophages in the spleen, but not hepatocytes or splenocytes.160 Similarly for a dendrimer based PSar star polymer, co-localization of the polymer with macrophages of the red pulp of spleen and within the liver was observed (Figure 3). Employing advanced imaging techniques can further aid understanding of tissue and cellular distribution and thus safety liabilities especially when toxicological markers can also be employed.

Even though PEG and other polymers have been used to minimize protein deposition and thus prolong clearance, they do not avoid complement activation.161 The complement system, is an integral component of the innate immunity and uncontrolled complement activation is undesirable and can contribute to disease pathogenesis such as tumor growth. PEG conformation and spacing can be modified to avoid protein deposition and it has been shown that PEG chains need to be less than 1 nm apart.161 An alternative approach explored to minimize complement activation is by using surface pairing of long PEG chains with shorter chains which both reduce protein binding to the nano surface, but can also change the configuration of longer PEG chains.162 Poly(2-oxazoline)s have avoided complement activation and circulate for prolonged periods of time in rodent models however, in human blood these nanoparticles rapidly trigger complement activation through C1q binding and undergo C3b opsonization, the latter making them prone to rapid ingestion by human macrophages.163 The design of more biocompatible super-hydrophilic species that confer universal protein-repelling properties is still required.

Infusion reactions occur with many nanomedicines resulting in the need for systemic administration of immunosuppressive, anti-pyretic, and anti-inflammatory medications before and/or during the infusion. The mechanisms behind these immune-mediated side effects that mainly occur within minutes to hours post intravenous administration are complex and need to be understood to help aid smooth transition of polymer therapeutics to the clinic. A roadmap to fill some of the gaps in knowledge has been presented.164 Physicochemical attributes of polymer therapeutics that effect the complement, cytokines, macrophages, platelets need to be understood and great harmonization of methods, models, and biomarkers for predicting IRs in patients is required. An in vitro assay cascade with standardized protocols to assess binding and internalization of nanomaterials, blood contact properties such as coagulation, plasma protein binding, hemolysis, platelet aggregation, and immunotoxicity is available.
from both the Nanoparticle Characterization Lab (NCL) and the EUNCL European nanoparticle characterisation laboratory.[165] There is considerable amount of effort and publications in this field that should help next generations of polymer therapeutics.

Progress with investigative toxicology has contributed to a shift in pharmaceutical toxicology, from a descriptive to an evidence-based, mechanistic discipline. One area of growth is humanized in vitro test systems or “organ on a chip” which should be explored more with targeted systems to understand the impact of both temporal and responsive release in systems mimicking healthy and diseased tissue. Together with in silico and in vitro systems these can be used to design future polymer therapeutics.[166]

2.4. Right Patient

Disease understanding is critical for any polymer therapeutic approach. Cancer is over 200 very different diseases and it is imperative for the successful translation of nanomedicines that the design is disease led rather than formulation led.[20] The tumor phenotype is very different across tumor types as well as heterogeneous within a tumor type. There appears reticence to adopt, and no precedence for a selection tool for nanomedicines based on nanotechnology, and neither biomarkers nor companion diagnostics have been employed routinely in clinical practice. Conversely, patient selection is common practice now in oncology agnosticshave been employed routinely in clinical practice. Combined with computed tomography (CT) the tumor accumulation of $^{64}$Cu-labeled HER2-targeted liposomal doxorubicin was evaluated in HER2-positive metastatic breast cancer patients with a number of metastasis. Higher tumor accumulation levels correlated with more favorable therapeutic outcomes.[172] Beckford Vera et al. have published a preclinical study using using $^{89}$Zr labeled PET conjugates of similar size and charge to their SN38 clinical candidate, PLX038 suggesting that these labeled conjugates could be used as effective diagnostic to identify tumors susceptible to PLX038 and other similar size nanocarriers.[110] A $^{89}$Zr labeled PET with the polymeric micelle CPC 634 study revealed enhanced and prolonged tumor accumulation in humans with uptake in 43% of evaluable tumor lesions (16/37). Again this radiolabeled CPC-6634 offers the opportunity to aid patient selection.[173]

Clinically, imaging is emerging as a promising tool for understanding nanomedicine distribution in patients and will aid design of the next generation of polymer therapeutics. However, due to the need for dedicated radiolabeled formulations and additional hospital visits, it is unlikely to be adopted universally for patient stratification in the future. Analysis and quantification of drug concentration in tumor biopsies from patients should be possible with MSI. Combining histological analysis with MSI, will allow nanoparticle and potentially drug distribution to be correlated with features of the tumor which affect nanoparticle uptake and distribution as well as PD markers. Understanding these features should allow patients to be selected who might benefit more from nanomedicines.

In terms of getting the right dose to the patient, it has now been shown in rat that particle number is important for a diverse set of nanomedicines, and splitting a dose will have a different tumor accumulation due to uptake via liver than a single dose.[94,174] The effect of infusion time on polymer therapeutic kinetics in human has yet to be established but it is likely that the rate of input to the liver is important. Therefore, infusion times should be kept constant during clinical investigation if possible and the number of particles kept above the trillion threshold. The same study demonstrated that dose reduced heterogeneity of tumor accumulation, again important for improved patient outcome. Patient age, gender, type of cancer, function of monocytes, and weight of patients with cancer have been previously reported to affect nanoparticle pharmacokinetics.[175] A tumor-compartment bearing PB-PK model has recently been used to investigate the effects of nanoparticle properties, tumor variables, physiological differences, and RES sequestration on tumor delivery, and excretion of nanoparticles. This model provides important insight and mechanistic information on physiological and pathophysiological conditions that could affect tumor delivery and inform patient selection, however it does not address drug release.[147]

Previously, it has been suggested that the variability in peptidic enzymes has contributed to the failure of systems relying on enzymatic degradation for their action. Poly(1-glutamic acid)
conjugated to paclitaxel via an ester linkage reached Phase III clinical trials in NSCLC patients however results were disappointing although greater activity was observed in female patients. Paclitaxel release from the polymer was via both the hydrolysis of the ester linker and the enzymatic degradation of polyglutamate backbone. Cathepsin B and estrogen concentrations are important for the release of paclitaxel from the polyglutamate and these are heterogeneous across patient populations. A trial co-administering transdermal oestrogen to prostate cancer patients was carried out in an attempt to improve cathepsin concentration however there was still a lack of significant activity.\cite{136} Such results further emphasize the importance of patient selection and stratification in the design of clinical trials.

Identification of predictive clinical markers of nanomedicine pharmacokinetic variability in patient populations of interest will be required to ensure successful translation of polymer therapeutics.

The current mRNA vaccines and mainly using lipid nanoparticles as the delivery system and have had an extremely rapid translation to the clinic and commercialization. However, the requirement for frozen storage to achieve an adequate shelf-life limits their use as a global vaccine. Polymer therapeutics have potential to offer both ambient storage and simpler processing and more flexibility for larger nucleic acid moieties.

2.5. Right Commercial

Currently, advanced drug delivery systems are more expensive to develop than traditional less complex tablets and simple injections; however, as delivery scientists it is important we improve processes and characterization to help drive both development costs and costs of goods down. In addition, we need to continue to increase our understanding to reduce attrition rates down there for improving R & D productivity. The delivery system needs to be fixed much earlier for polymer therapeutic and normally in the Discovery phase and prior to GLP toxicity. For many nucleic acid based drugs, primarily due to the very poor efficiency of endosomal escape following intracellular delivery, is low, typically 1–2% of the dose and thus bioavailability is very poor.\cite{136} For some of the most expensive drugs to manufacture this is extremely inefficient and provides lots of scope for delivery scientists to improve delivery and return on investment. Currently even PEG 2000, a well-known and used polymer, is expensive when needing to source to good manufacturing practice (GMP) quality and a tight specification. Having polymers that can be manufactured and controlled and formulated easily will improve development costs and costs of goods. In terms of characterization of polymer therapeutics, there is often a desire by measurement scientists to want to use the specialist techniques to measure everything. This is often desirable to improve fundamental understanding but during development it is important that a risk based approach is taken to determining critical quality attributes (CQAs) to ensure development costs are minimized and polymer therapeutics do not get a label of being “expensive” which will make it difficult to bring future projects into a portfolio. As patient stratification increases, the patient populations receiving medicines will decrease and thus development costs and costs of goods need to be reduced and smarter development strategies adopted to ensure return on investment.

Frozen and even cold supply chains as well as precluding global accessibility are expensive so the ability to provide a lyophilized final product that is capable of being stored at ambient temperatures provides considerable advantages.

Overall, although managing development costs and risk is important, it is the strength of the clinical benefit and better outcome in disease treatment and/or a large reduction in the costs managing adverse events, be it need for additional drugs or less hospital treatment time that will drive the adoption of new polymer therapeutics.

2.6. Right Culture

The pharmaceutical research and development of polymer therapeutics are different to small molecules and in order to successfully translate them into clinical products different thinking and skills are required. It is important to design the polymer therapeutic with the end disease in mind, thoroughly understand the pathophysiology of the disease, the drug and target and level of target engagement needed, and the delivery technology. Design is important to demonstrate the concept but with a line of sight to both clinical and commercial development to ensure a successful product can be manufactured and meet regulatory standards. Clear go/no go criteria should be set at each stage and questions asked to fully test the hypothesis. Many of those working in pharmaceutical development are focused on small molecules where the delivery system is fixed following early clinical development and factors affect rate and extent of absorption are considered. For a polymer therapeutic, more detailed understanding of the ADME properties is required and a drug delivery scientist should play a more central role in the design and development. This requires a range of multi-disciplinary skills including mathematical modeling and novel thinking and approaches to understand the CQAs affecting in vivo performance. Doing things differently takes courage, risk, drive, and a certain amount of resilience, especially in a large Pharma culture. The more available precedence for the development of advanced drug delivery systems the easier the pathway in the future.

3. Developability

Any advanced delivery system needs to be developed thus the Chemistry and Manufacturing Controls (CMC) needs to be put in place to do this and the quality and in vivo performance of the product needs to be consistent for clinical trials and robust enough for global supply for a number of years. A good prototype, that is at ideally qualitatively and quantitatively representative of the final product needs to be identified in the discovery phase and with early definition of the CQAs affecting in vivo performance. Therefore, those critical properties of the polymer therapeutics affecting both the safety and efficacy need to be fully understood and characterized. Defining a target product profile early for any polymer therapeutic is critical and this should allow a quality by design (QbD) approach\cite{177} to the design of polymer therapeutics. Once the likely CQAs are identified, the critical material, process, and formulation attributes that in turn affect the CQAs and
in vivo performance should be identified and monitored as part of the design and during development. Pharmaceutical development of complex products can be costly and challenging and there it is worth remembering Albert Einstein’s famous scientific quote when designing an advanced delivery system “Everything should be made as simple as possible, but not simpler.”

3.1. Polymer Chemistry Progress

The versatility of polymer chemistry in terms of properties, architectures, and functionalities enables unique and bespoke materials to be synthesized with the desired properties as advanced drug delivery systems. However, by nature such macromolecules suffer from heterogeneity leading to challenges in controlling their quality. Polymer chemistry has come a long way since its first use over 100 years ago. All three traditional classes of polymer architectures have been applied as polymer therapeutics, linear, cross-linked, and branched. Recent advances in polymer chemistry in a number of key areas have overcome many of the challenges previously seen as concerns/blockers for their large-scale pharmaceutical development.

Controlling polydispersity is a critical material attribute and important for processability and reproducibility of a final product. Significant improvements have been made in the control of polymerization. For example, with the introduction of controlled radical polymerization using reversible-deactivation radical polymerization techniques including reverse addition fragment chain transfer (RAFT), atom transfer radical polymerization (ATRP), and nitroxide-mediated radical polymerization means a polymer like HPMA can be synthesized with lower molar mass dispersity (e.g., 1.1–1.2). Improvements for the cationic ring opening polymerization of polyepoxides including poly(2-oxazoline)s have enabled high molar mass polymers with low PDI.s. Polyamino acids have also seen major recent innovation for producing well defined polymers.

Dendrimers, highly branched, yet precise and discrete macromolecules offer a broad range of functionality and are being used increasingly as either drug themselves (e.g., Vivagel, Starpharma), gene delivery vehicles or as scaffolds for creating larger star polymers. They provide a means of overcoming polymer heterogeneity seen with other architectures. Originally their branching units, or generations, radiating from an initiating core unit either through divergent or convergent methodologies, were added stepwise, affording high synthetic precision and low dispersity. Many perceive complexity in their manufacture as a disadvantage, and defects in the dendritic architecture have been observed, especially at high generation/functionality. Using a convergent approach by starting with the periphery of the molecule as dendritic fragments and coupling to a core molecule requires fewer chemical reactions, offers further synthetic flexibility, and potential for increased purity for higher generation materials. However, this method often leads to more complicated purification process and can be limited by steric crowding.

Some of the challenges of early drug conjugates, especially linear polymers such as PEG, was drug load. Progress in supramolecular chemistry and self-assembled systems have addressed drug loading as well as offering versatility and more precise polymeric materials. Initially amphiphilic copolymers were produced providing a hydrophobic core such as polyesters and poly(amo) esters. Despite clinical success, the polyesters have limited functionality and more suited to more hydrophobic drugs and still require complex formulations to improve drug loading and tuneability of release. Polymersomes, stable polymeric vesicles of amphiphilic block copolymers have more advantages as they enable loading of both hydrophilic and hydrophobic drugs either in their core or shell and potential for surface modification.

Conjugating drugs to amphiphilic polymers to provide polymeric micelles improved the drug loading and aided control of release though linker chemistries and there are a number of poly(amo) esters in clinical development (Table 1). However, uncontrolled conjugation leads to greater heterogeneity and polydisperse polymer chains with varied drug loadings and sites of modification. Greater control is required to ensure a robust scalable product and new more precise synthetic methodologies such as drug-initiated living in situ polymerization and living polymerization of prodrug monomers have been developed and employed for the regioselective and chemoselective incorporation of drugs into synthetically precise polymeric nanoparticles at nearly quantitative loading efficiencies.

However, to achieve targeting, micelles need to be structurally stable upon injection and above their critical micelle concentration particularly upon the large dilution experienced in human (~1: 150–175). In addition, instability can occur in biological fluids at a concentration significantly above the critical micelle concentration causing dissociation and premature drug release. These concerns have led to the development of stabilized core or core cross-linked systems either through physical interactions such as π–π stacking, hydrogen bonding, host-guest complexation, stereocomplexation, and coordination interaction or chemical cross-linking via free radical polymerization, disulfide, and hydrazone bonding and click chemistries. Employing linker chemistries developed from increased pathophysiological understanding of disease offers the ability to employ more stimuli responsive systems. Combining modern responsive linker chemistries with supramolecular polymer chemistry and high order self-assembled structures is leading to far more precise nanocarriers and thus great site specificity.

Full characterization of the polymers used is essential and alongside the chemistry, the analytical techniques and understanding have developed. Automation of many core analytical techniques has made the polymer scientists’ life easier, improving throughput and iterative design. Improvements and progress with advanced analytical techniques have all contributed to significantly better polymer characterization. Newer developments in asymmetric flow-field flow fractionation (AF4) have resulted in far better macromolecular separation. Advances in size exclusion detector technology have brought huge improvements in sensitivity with quadrupole detector array means rapid and data rich acquisition, absolute molecular weight (Mw) and distribution, intrinsic viscosity (IntrVw) and distribution, hydrodynamic radius in solution (Rhw) and distribution, Mark-Houwink coefficient (polymer-solvent interaction). Principle component analysis can be applied to look for independent variability between batches. The application of interferometric scattering microscopy to polymer science introduces an orthogonal technique for molar mass determination. Some of these techniques have been adapted...
for in situ, in-line or online analysis of polymerizations allowing for rapid feedback of reaction progress. Further to this, Process Analytical Technology (PAT), commonly used in manufacture of pharmaceuticals, applied to polymerization monitoring can play an important role in the design phase as well as large scale synthesis of the polymers used in drug delivery systems. In terms of scale up, PAT technologies to aid polymerization monitoring via online analysis using Fourier-transform infrared spectroscopy, RAMAN or UV-visible spectroscopy will give added process control and provide instant feedback for any correction. This will enable the reaction progress to be closely followed and stopped when complete so optimizing process efficiencies and reducing manufacture time and costs. The combination of flow chemistries with PAT and other online analyses such as dynamic light scattering (DLS) and NMR are rapidly emerging and will further lead to more efficient scale up and processing. The use of automated flow with machine learning for polymer synthesis and nanoparticle formulation and adoption of AI is advancing.\[190\]

Purity and control of impurities in polymers are imperative. Improvements in purification techniques including fractional separations with size exclusion chromatography and ultrafiltration/diafiltration processes have all contributed to improved polymer quality. Creative ways to process in non-aqueous conditions are required for hydrolytically cleavable linkers. Hybrid delivery systems with bioengineered ligands present challenges during purification to ensure no degradation or loss of functionality.

An important and often overlooked consideration for polymers is the environmental impact for those that cannot be easily degraded following elimination from the body and suggests that systems should be inherently designed to degrade into safe metabolites or bio-reusable building blocks. With environmental impact in mind, the source of materials, building blocks, and monomers should also be considered. In place of monomers derived from petrochemical origins, renewable materials should be at the forefront of polymer design and reassuringly significant gains have been achieved in the development of polymers such as polyamino acids, polysaccharides, and naturally derived metabolites or bio-reusable building blocks. With environmen-

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tal impact in mind, the source of materials, building blocks, and monomers should also be consid
manufacturing process. However, significant work has been done to control and develop a robust manufacturing process via a quality by design approach.[194] Work has also been focused on nano-precipitation techniques including using microfluidic technology to fabricate nanoparticles[41,195] but to date despite modification of polymers to improve encapsulation[41] have not been able to match the target product profile. Nano-precipitation is potentially a much simpler process; however, fluid dynamics needs to be carefully controlled to achieve homogeneous nanoparticles reproducibly. The type of flow, turbulent, transition, laminar, or the lateral mixing between polymer solution and aqueous phase strongly affect the kinetics of phase separation, the particle nucleation and growth, and potentially aggregation. Microfluidic technology provides many advantages due to the high precision of the mixing regimes and the ability to control fluids involved in the formulation process.[196] With more bespoke design of microfluidic chips, more core shell type nanoparticles can be produced which is necessary for adequate control of release. However, most microfluidic methods still face challenges in scaling up and a parallelization approach is often required to achieve the same throughput as conventional methods for large batch production.

A final step to formulation is liable to be sterilization process; and the majority of polymer therapeutics will need to be prepared aseptically and sterilized through 0.2 μm filters. Alternative means of sterilization will be beneficial and supercritical carbon dioxide technology offers a sustainable solution for many biomaterials.[197]

### 3.4. Control of Impurities

Impurities in polymer therapeutics need to be managed as with any other product however, currently there is no separate frame-work for the control and qualification of impurities in polymeric therapeutics per se. Impurities in polymeric systems can be divided into three major categories, i) impurities and degradation products related to the drug ii) small molecule impurities in the polymer, and iii) related polymeric substances.

For a polymer therapeutic where the drug is a small molecule ICH guidelines Q3A “Impurities in new drug substances”[198] and Q3B “Impurities in new drug products”[199] are applicable. Similarly ICH guidelines Q3C and Q3D should be referred to for residual solvents and elemental impurities, respectively.[200] However, where the active is a nucleic acid, due to their size and nature they fall between guidelines for small molecules and biologics and neither guidelines are relevant. Two position papers published by Oligonucleotide Safety Working Group[201] and Japanese Pharmaceutical Manufacturing Association Quality Task Force[202] provide scientific advice on the control and qualification of product related impurities in oligonucleotide therapeutics. They share a consistent view on reporting, identification, and qualification thresholds. The proposed control strategy for impurities is widely used in the industry for therapeutic oligonucleotides. These recommendations are also considered appropriate for polymeric therapeutics containing oligonucleotides. Similarly, there is no specific guidance on impurities for mRNA-based therapeutics and a pragmatic approach needs to be presented. Typical impurities in mRNA include shorter and longer RNA fragments and impurities where the Poly A tail and end Cap are missing. Residual plasmid DNA template, and residual proteins from the manufacturing process can also be present.

Small molecule impurities in polymers can include unreacted starting materials, by-products, degradants, and residual solvents. These impurities can react with the drug with potential to impact product safety and performance. For example, reactive impurities such as residual peroxides from synthesis,
aldehydes, and organic acids through oxidative degradation are widely reported.\textsuperscript{[203]} Oxidation of PEG is mediated by trace metals and peroxides and the resulting impurities can react with the drug.\textsuperscript{[204]} The formation of impurities is dependent on the manufacturing process and the storage temperature, the exposure to air/oxygen, and light.

Polymeric products and excipients are often composed of large number of closely related components. The nature of polymer chemistry is that a distribution of chain lengths will be produced and depending on the synthetic methods used will also give rise to structurally similar impurities resulting from events that occur during the polymerization; for example, chain transfer or termination events, cross-linking, unwanted chain end group formation and incomplete branching. These could impact the CQAs and thus both safety and in vivo product performance. Analysis of such impurities is difficult owing to the similarity in properties of the impurity to the bulk materials and advanced characterization techniques are required.\textsuperscript{[205]} For example, with methoxy-PEG, it is well known that diol impurities form as a result of the trace amounts of water in the polymerization.\textsuperscript{[106]} Whilst for some applications this may not be problematic, if further modification of this polymer is required to create diblock copolymers then inevitably unwanted triblock copolymer impurities result and may impact on the performance of the final product.

For polymer-drug conjugates and ligand conjugated systems, often the conjugation is the last step of a synthesis and therefore residual drug, ligand, linker, reagents, catalysts used for the conjugation have to be stringently removed to avoid impurities in the final product.

3.5. Advanced Analytical Characterization

The unique properties of the polymer therapeutics, such as high molecular weight, structural complexity, polydispersity, high surface to volume ratio, functionality, and controlled release of the drug that are critical to their therapeutic outcome, are also responsible for their manufacturing and characterization complexity. A thorough understanding of the physicochemical properties and structural characteristics of the formulations is essential for both the design and clinical development of these macromolecules. Access to a suite of advanced characterization methods is essential for developing the process understanding and a robust control strategy needed to ensure consistency and quality throughout the lifespan of the polymer therapeutic.

Over the last decade, technological breakthroughs in measurement sciences and the development of advanced data processing tools mean it is now possible to better characterize these complex macromolecular systems. This has led to a far greater understanding of their physicochemical properties and understanding the bio-nano interface. Accessibility to these technologies has also significantly improved. Some of these advanced tools that were hard to access previously are now widely available both in the pharmaceutical industry and research centers around the world. In this regard, two large groups, the US National Cancer Institute Nanotechnology Characterization Laboratory\textsuperscript{[165b]} and the European Nanomedicine Characterization Laboratory\textsuperscript{[165a]} have played a crucial role in providing access to expertise, infrastructure, and protocols offering a wide set of physical, chemical, in vitro and in vivo biological testing. In addition, work is on-going to provide international standards and reference materials that facilitate efficient regulatory evaluation and reliable manufacturing as well as to rapidly apply emerging innovation in measurement and imaging science to polymer therapeutics.\textsuperscript{[207]}

Lack of reproducible data and experimental details has been blamed for lack of progress in the application of nanotechnology and these protocols and standards will go a long way to help understand both the physicochemical characterization of the polymer itself and biological characterization of the resultant polymer therapeutic.\textsuperscript{[208]} Greater adoption of protocols should improve reproducibility, enable quantitative comparison of bio-nano materials and facilitate meta analyses and in silico modeling to help design innovative systems that can be rapidly translated into medicines. Since polymeric therapeutics cover a range of constructs, their physicochemical properties can vary significantly, therefore, characterization requirements for a given polymeric drug greatly depend on the nature of the construct, manufacturing process, and the associated CQAs. Considering challenges associated with characterizing these complex systems, a smart combination of multiple complementary approaches is needed to fully analyze these drugs.

A detailed summary of the characterization techniques and the corresponding physicochemical parameters of the nanoparticles have been discussed in detail by Mourdikoudis et al.\textsuperscript{[209]} Some of the techniques that have evolved in characterization of polymeric delivery systems include advanced use of DLS, nanoparticle tracking analysis, small-angle x-ray scattering, small-angle neutron scattering, Taylor dispersion analysis, analytical ultracentrifugation, transmission electron microscopy, scanning electron microscopy, size exclusion chromatography coupled with triple detection array, AF4, and atomic force microscopy.

3.6. Regulatory Considerations

Regulatory needs for polymer therapeutics are not fully covered by ICH guidelines for small molecules and biologics. This is because small molecules guidelines deal with well-defined chemical structures that are synthesized using chemical starting materials and chemical processes and can be fully characterized by the physicochemical analytical means. In contrast, polymer therapeutics although also synthesized by chemical processes are large, complex, and heterogeneous. Biologics guidelines are also not directly applicable because unlike polymer drugs, these guidelines deal with complex heterogeneous structures such as proteins that are derived from living organisms rather than chemical processes.\textsuperscript{[210]} In order to bridge this gap, the concept of NBCD has been proposed.\textsuperscript{[211]} The NBCD is defined as a non-biological drug product, where the active substance is not a homo-molecular structure, but consists of different structures that cannot be isolated and fully characterized by physicochemical analysis and where the clinical meaning of the differences is not known. The composition, quality, and in vivo performance of NBCDs are highly dependent on the consistent, tightly controlled manufacturing processes of drug substance and drug product. The term “the process defines the product” has been used
emphasizing the need for robust process engineering. The regulatory approach for NBCD takes into consideration the specific characteristics of the drug and formulation and the resulting critical attributes to achieve their desired quality, safety, and efficacy. In 2009, a cross industry NBCD working group was established to align on science-based approval and post-approval standards for NBCDs, to promote discussion on the safety, efficacy, and quality of these drugs.\textsuperscript{212} The working group aims to influence and help shape the regulatory landscape through a science-based approach for the approval of NBCD products and has published a large number of papers on this subject.

The FDA have emphasized that drug products containing nanomaterials must meet the same standards for quality, safety, and efficacy as for drug products not containing nanomaterials. The regulatory review of these products follows the existing regulatory pathway. It is recognized however, that unique properties that arise from the small size and large surface area of nanomaterials as well as added functionality lead to additional requirements for physicochemical testing and manufacturing controls. A comprehensive overview on the landscape of nanotechnology application in medicine taken from the analysis of data from more than 350 products submitted to the FDA has been published. Some key trends were identified including an increased number of submissions with nanomaterials generally and an increase in durable nanomaterials. This has triggered research into their long-term effects post administration. Further trends are also being monitored.\textsuperscript{213}

In order to guide the developers of innovative and generic drug products that contain nanomaterials the FDA issued the draft guidance for industry “Drug Products, Including Biological Products, that Contain Nanomaterials.”\textsuperscript{214} The guidance reflects FDA’s current thinking on this topic. Due to the large number of diverse and structurally different nanomaterials, the guidance provides the general principles rather than product specific detailed instructions and proposes a risk-based approach focusing on risk factors. Over the last few years, there have been significant efforts to harmonize the global regulatory requirements for complex products. This was followed up with an (Association of American Pharmaceutical Sciences AAAPS) workshop on the appropriate regulatory pathway for approval of drug products containing nanomaterials, and how to determine CQAs for nanomaterials. Some of the recommendations where the need to learn more from biologics, which is increasingly important as hybrid delivery systems evolve, more science needs to be carried out and disseminated on determining CQAs and more global alignment on protocols and regulatory pathways.\textsuperscript{215} In 2019, Joint Research Centre and the Global Coalition for Regulatory Science Research (GCRSR) organized a Global Summit “Regulatory Science 2019, Nanotechnology and Nanoplastics.” GCRSR is an international coalition that aims to facilitate education, scientific training, and scientific exchanges in the field of regulatory science. The summit provided an opportunity for collaboration and discussion on the regulatory policies and practices for nanomaterials and nanoplastics technologies. Over 200 scientists from 36 countries representing regulatory agencies, academia, and industry, discussed global regulatory science perspectives on medical products, food, and standards related to nanotechnology. Five major recommendations came from the summit; the need for i) development/standardization of the most needed methods and reference materials for the regulatory assessment of nanomedicines, ii) adaptation of methods/standards from other science areas iii) robust testing strategy iv) implementation of quality-by-design approach iv) early dialogue with regulatory agencies v) knowledge and experience sharing. In addition, the need for a risk based approach to identify the CQAs of the product, to undertake characterization and analysis using complementary orthogonal techniques, and develop validated in vitro and in vivo models to ensure regulatory alignment was emphasized.\textsuperscript{216}
4. Conclusions

Applying a structured approach to polymer therapeutics should enable better design and clinical translation. By adapting the 5 R’s to include right developability and considering pharmaceutical development in the design phase should enable more successful translation. Figure 5 summarizes the progress against each of the criteria for small molecules and nucleic acid based drugs. It argues that the understanding of key factors affecting successful design is maturing for polymer therapeutics based on small molecules for biophysical targeting and understanding is rapidly increasing for more precision targeting. In contrast, polymer therapeutics are still emerging as a delivery technology for nucleic acid based drugs but show huge potential. This approach to design and developing polymer therapeutics has built on that successfully introduced into AstraZeneca and has guided some of the design and development of our internal polymer therapeutics, currently progressing through clinical development and increasing the efficacy or safety of small molecule cancer therapeutics and enabling their development. We and others are also exploring applications in more precision targeting and intracellular delivery of the rapidly emerging nucleic acid based drugs that are in desperate need of more efficient and safe delivery. Polymer therapeutics has definitely passed the cross roads and is on the highway to success. However to ensure this trajectory it is important that design thinking is applied early to ensure an optimal clinical and pharmaceutical development path. Advanced imaging, mathematical modeling, investigational toxicology and patient stratification as well as modern and advanced pharmaceutical manufacturing and process technologies and analytical characterisation are needed together with effective multi-disciplinary collaboration.

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Conflict of Interest

The authors are employees and shareholders of AstraZeneca.

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clinical translation, drug delivery, pharmaceutical development, polymer drug-conjugates, polymer therapeutics, polymeric micelles, self-assembled nanoparticles

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