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Review

Polymer therapeutics: Top 10 selling pharmaceuticals — What next?

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ABSTRACT

At the time of the first issue of the Journal of Controlled Release (JCR), polymeric drugs, polymer–drug and protein conjugates and block copolymer micelles carrying bound drugs, i.e. polymer therapeutics, were still regarded as scientific curiosities with little or no prospect of generating practical to use medicines. How this perception has changed. Many major Pharma now have R&D programmes in this area and in 2013 two polymer therapeutics, Copaxone® and Neulasta®, are featured in the Top 10 US pharmaceutical sales list. Although there are a growing number of marketed products (e.g. PEGylated proteins, a PEG-aptamer and oral polymeric sequestrants), and the first follow-on (generic products) are emerging, the first polymer–drug conjugates and block copolymer micelle products (as covalent conjugates) have yet to enter routine clinical use. Industrial familiarity and recent advances in the underpinning scientific disciplines will no doubt accelerate the transfer of polymer therapeutics into clinically useful medicines and imaging agents. This short personal perspective reflects on the current status of polymer therapeutics and the future opportunities to improve their successful translation. It adds to recent and historical reviews that comprehensively document the evolution of the field since JCR was born.

1. Introduction

Last year, in a review written to mark the 25th Anniversary of Advanced Drug Delivery Reviews entitled "Polymer therapeutics-prospects for the 21st century: The end of the beginning" [1] we noted that the field has "… come a long way since its beginnings, and arguably polymer therapeutics have been amongst the most successful first generation nanomedicines (reviewed in [2])". Progress continues with two polymer therapeutics being featured in the US Top 10 selling drugs list for 2013 [3], Neulasta® and Copaxone®, and more products are arriving to market as innovator (new) products (e.g. Lymphoseek® (Tilmanocept), a mannonsylated dextran-based sentinel lymph node imaging agent for melanoma and breast cancer patients [4]), and also into clinical trial as 'follow-on' (generic) products (e.g. PEG-G-CSF (DA-3031) [5]). This short personal perspective adds to past comprehensive reviews that have documented, the evolution of both basic and applied research over the lifetime of JCR (e.g. [6]), the introduction of polymer therapeutics as clinically important medicines [7,8], the challenges they present for clinical development [9], and not least the future opportunities and challenges for commercialisation as medicines, imaging agents and theranostics [1,10]. Despite the above-mentioned successes, the first polymer–drug conjugates, drug conjugated micelles and polymer-based non-viral vectors designed for cytosolic delivery of biopharmaceuticals have yet to enter the market. As we celebrate the 30th birthday of the Journal of Controlled Release (JCR) it is interesting to reflect on the current status and future opportunities to increase translation of current and newly emerging technologies from lab to clinical use.

2. From hypothesis to clinically useful medicines

2.1. JCR: the emergence of polymer therapeutics into clinical use

A glance at the index pages of the first two Issues of JCR (1984) show that by far the primary interest at that time was advanced drug delivery systems/controlled release formulations for human applications with papers describing transdermal patches, vaginal pessaries, a powder dosage form for intranasal administration of insulin, and proposal of sophisticated parenteral delivery systems such as a self-regulating insulin delivery system and polymer matrices containing magnetic beads to trigger drug release. (The latter were way ahead of their time!) In 1984 there was already a rapidly growing interest in design and evaluation of first generation nanomedicines for improved drug targeting, triggered drug release, and improvement of drug passage across biological barriers. The approaches then being investigated included liposomes, polymer-based, and lipidic, nanoparticles, antibody–drug, polymer–drug and polymer–protein conjugates (this history is discussed in [2]). Surprisingly studies involving most of these technologies were not featured in the first issues of JCR (1984). The only exception being two papers of Schacht and colleagues describing the synthesis and

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characterisation of polysaccharide (dextran and inulin)-procainamide conjugates [11], and via our collaboration, their pinocytic uptake by cells *in vitro* [12]. The latter study is particularly notable given the now growing appreciation of the importance of defining cellular and whole body pharmacokinetics of polymer therapeutics [13,14]. Indeed a review on "endocytosis of nanomedicines" [15] is amongst the most cited articles in JCR over the last 5 years!

Natural polymers have been used for millennia as components of herbal remedies, so it would be wrong to suggest that polymer therapeutics per se are "novel", but the rational design of polymer-based therapeutics did begin in earnest in the second half of the 20th century following the arrival of synthetic polymer chemistry (discussed in [1]). Early contributions are worthy of note; polymers as drugs (especially antibacterial agents and immunomodulators) [16–18], radioprotectants [19], polymer–drug [6,20] and polymer–protein conjugates [21,22], and block copolymer micelles [23]. Experience gained with natural and synthetic polymers explored clinically over the last century gave first insights into polymer characteristics important for quality, safety and efficacy (i.e. those factors governing risk-benefit for clinical use). The iron–dextran complexes were first introduced as intravenous (i.v.) iron replacement infusion solutions in the 1940s and the properties (characteristics/safety) of the polymers and oligomers used to stabilise such iron complexes are still widely discussed in terms of features governing clinical safety and efficacy [24].

2.2. Learning from recent clinical successes and failures

During the lifetime of JCR several distinct classes of polymer therapeutics have progressed into first-in-man clinical trials and moreover into routine clinical use (comprehensive lists given in [1,2]). All involve a synthetic (e.g. PEG, HPMA copolymers, crosslinked polyamines), a pseudosynthetic (e.g. polyglutamic acid (PGA), lysine-based dendrimers) or a natural polymer (e.g. dextran, polysialic acid, alginate oligomers) as the core component. Products have been developed for different routes of administration (e.g. oral, intravenous (i.v.), subcutaneous (s.c.), intramuscular (i.m.), topical and intra-vitreal), and for a diversity of clinical applications as drugs, sequestrants or imaging agents. Moreover, products designed as conjugates for drug targeting and/or controlled release can contain a diverse array of therapeutic (or imaging) payloads including low molecular weight drugs (e.g. the anticancer conjugates containing doxorubicin, paclitaxel, and camptothecins), and biopharmaceuticals including peptides or proteins and aptamers/siRNA.

2.2.1. Polymer conjugates of biopharmaceuticals

Market approval in the early 1990s of the first polymer–protein conjugates (e.g. Zinostatin stimalmer (styrene maleic anhydride neocarzinostatin, SMANCS) in Japan, PEG-adenosine deaminase (Adagen®) and PEG-asparaginase (Oncaspar®)) was a pivotal landmark in the history of polymer therapeutics (discussed in [7]). PEGylation [22] is now an accepted tool, and the composition of biopharmaceutical conjugates is increasingly well-defined (usually a 1:1, PEG: protein/aptamer). Many improved synthetic routes have emerged (current status reviewed in [25]), and products developed for a diverse array of clinical indications, e.g. as antiviral agents, anticancer agents, as an adjunct to chemotherapy, and to treat arthritis, gout and age-related macular degeneration. FDA approval in the early 2000s of two PEG-interferon conjugates (PEG-Intron®; PEG-ASYS®) for s.c. injection to treat chronic hepatitis C gave the field heightened visibility. Their use has subsequently been broadened to other indications with PEG-interferon α-2b (Sylatron™) now approved (2011) as an adjuvant therapy for treatment of high-risk melanoma [26], and a PEG-interferon-β-1a conjugate is currently being tested in Phase III clinical trials as a treatment for multiple sclerosis [27].

PEG conjugation of proteins, peptides and more recently aptamers (Macugen® was the first approved aptamer-based drug, discussed in [28]), is typically undertaken to improve the pharmacokinetic profile (increased plasma half-life, longer absorption profile), and reduce antigenicity and immunogenicity, especially of non-human proteins. The molecular weight of the PEG, site of conjugation and linking chemistry used, together with the clinical indication for use can all influence performance in terms of safety/efficacy. Although both PEG–interferon conjugates are used in combination with ribavirin to treat hepatitis C their composition is very different. PEGASYS® consists of recombinant human alfa-2a interferon conjugated to a single branched PEG of molecular weight ~40,000 g/mol whereas PEG-Intron® contains recombinant human interferon alfa-2b conjugated to a single chain PEG of molecular weight ~12,000 g/mol. The impact of the pharmacokinetic–pharmacodynamic (PKPD) properties of these conjugates on their relative safety and efficacy is still much debated [29]. The PEG-recombinant granulocyte colony-stimulating factor (G-CSF) (Neulasta®) contains ~20,000 g/mol PEG, and it perfectly illustrates the benefit of prolonged circulation compared to the unmodified protein. Neulasta® was approved by the FDA in 2002 for s.c. administration to cancer patients in order to minimise chemotherapy-induced neutropenia. The reduced rate of renal elimination of GCSF by PEGylation enables a single injection per chemotherapy cycle, which is a significant advantage for the patient compared to the ~10 daily injections required when using G-CSF alone (reviewed in [30]).

Two decades of clinical experience with PEG conjugates has generated a significant post-marketing database relating to clinical outcomes. In most cases benefits of PEGylation have been clearly shown to outweigh disadvantages. Moreover, although it was often suggested that cost of manufacture of polymer therapeutics would prohibit their commercialisation, pharmacoeconomic studies have demonstrated the cost-effectiveness of PEGylated products in almost all cases (discussed in [31]). As early PEG conjugates now start to come off patent, their healthcare contribution and commercial success have stimulated an eagerness to enter the market with first "follow-on" products, e.g. Phase II evaluation of DA-3031, a PEGylated G-CSF [5]. There is currently considerable debate as to the regulatory requirements needed to ensure equivalence of quality, safety and efficacy of complex, multi-component "follow-on" nanomedicines in general as they cannot simply be assessed using the classical procedures established to define bioequivalence of low molecular weight generic drugs (discussed in [32,33]). In parallel studies describing the design of more controlled industrial scale manufacturing processes [34], improved purification techniques [34,35] and improved validated analytical methods for conjugate characterisation [36] can be seen.

Safety concerns have however been voiced regarding the use of PEGylation. Intravenous administration of Doxil® (a PEGylated liposomal doxorubicin) can cause infusion reactions, albeit this is in <10% of patients and can be easily managed clinically. Certain PEG–protein conjugates have also demonstrated hypersensitivity reactions, which it has been suggested, is due to induced or pre-existing anti-PEG antibodies. Immunosuppressive strategies were recently proposed to minimise risk of infusion reactions when treating gout in patients using PEG-recombinant porcine uricase given i.v. (Krystexxa®) [37]. Why some patients exhibit infusion reactions while others do not remains unclear. Some argue that the PEG component triggers production of anti-PEG IgM antibodies [38]. However others state that, "most, if not all assays for anti-PEG antibodies are flawed and lack specificity" highlighting the need for "standardisation of the anti-PEG antibody assays and the development of reference sera" [39]. The debate continues, but it is important to remember that the diversity of therapeutic, PEG molecular weight and linking chemistry will all play a part in the product toxicological profile seen in a particular clinical setting.

Certain PEG conjugates have recently displayed unacceptable toxicity which caused termination of clinical trial/use. While PEG–L-asparaginase (Oncaspar®) is now a standard therapy for paediatric acute lymphocytic leukaemia (ALL), in a recent Phase II clinical trial in advanced ovarian cancer patients, PEG–L-asparaginase was very poorly

tolerated and the trial had to be stopped [40]. Differences in route of administration can be important although comparison of allergic reactions in patients receiving i.v. or i.m. PEG–asparaginase found that although the onset was more rapid after i.v. administration, in general the grade of the reaction was similar for both routes [41]. It is important to note that such allergic reactions can be managed clinically for PEG–asparaginase [41]. A PEG conjugate of a novel erythropoietin-stimulating peptide (OMONTYS®, Peginesatide) designed to treat anaemia in haemodialysis patients with chronic kidney disease has recently demonstrated mores serious toxicity. This conjugate contains a lysine-branched PEG chain (molecular weight ~40,000 g/mol) conjugated to a dimeric 21-amino acid peptide via an iminodiacetic acid and β-alanine linker. In pre-approval clinical trials it showed similar activity to human recombinant erythropoietins administered using a more frequent dosing regimen (up to three times per week), and exhibited good safety profile in patients on haemodialysis, although higher rates of adverse cardiovascular events were reported in patients not on dialysis [42]. The product was FDA approved in 2012, but later withdrawn in 2013 due to post-marketing reports (13 cases) of serious hypersensitivity reactions, including anaphylaxis, that was life-threatening or fatal. To note only 0.2% of patients had severe allergic reactions, with a 0.02% rate of fatal anaphylactic reactions [42–44]. These observations were disappointing as this PEG–peptide conjugate was viewed to have many advantages compared to the recombinant erythropoietins currently used, not least the need for less frequent administration, ease of manufacture (no need for recombinant DNA technology) and the reduced cost. Although the mechanism(s) of this unexpected toxicity of OMONTYS® are not yet understood, it is essential to learn whether it relates to specific design features of the product, batch to batch reproducibility/quality of the product, and/or the biological behaviour of the novel peptide designed to stimulate the erythropoietin receptor. If the mechanisms can be understood they may aid improved design of future PEG and other polymer conjugates. Identification of patient biomarkers that cause such PEG hypersensitivity reactions would help to select those patients most likely to gain benefit from polymer therapeutic therapy.

2.2.2. Polymeric drugs and sequestrants

The search for inherently active synthetic polymeric drugs began in the 1960s with the failed anticancer clinical trials involving the synthetic polyanion DIVEMA (reasons discussed in [7]). It was FDA approval (1996) of the synthetic polypeptide Copaxone® (glatiramer acetate) for treatment of patients with relapsing–remitting multiple sclerosis that produced the important breakthrough in this field (reviewed in [45]). Copaxone® is a complex product being a random copolymer of glutamic acid, lysine, alanine, and tyrosine of average molecular weight ~5000–9000 g/mol. Moreover, it is not understood exactly which amino acid sequence(s) present therein are responsible for pharmacological activity, pleiotropic immunomodulatory properties have been noted [46]. Traditionally Copaxone® is administered daily by s.c. injection, but very recently (2014) FDA approved a more convenient formulation requiring injection only 3 times per week. "Follow-on" glatiramoids are now also on the horizon, and the regulatory requirements needed to ensure equivalent performance of these complex, and very heterogeneous products are under discussion [33].

Another significant success story in this area (reviewed in [47]) was the development of orally administered crosslinked poly(allylamine hydrochloride)-based sequestrants designed to bind and remove phosphate (Renagel®, sevelamer hydrochloride) in end stage renal failure patients, or bile acids in order to lower cholesterol (Welchol®, colesevelam hydrochloride). The perceived disadvantages of Renagel®, including potential for metabolic acidosis and a relatively low affinity and selectivity for phosphate anions, led to subsequent development of Renvela® (sevelamer carbonate). It has an equivalent ability to lower serum phosphorus concentrations, but it does not decrease serum bicarbonate levels thereby bringing potential benefits to certain patients [48]. Welchol® acts as a lipid- and glucose-lowering agent and is thus able to improve glycaemic control in adults with type 2 diabetes mellitus. Although its precise mechanism(s) of action are still being elucidated, Welchol® does show additive cholesterol-lowering effects when used in combination with other lipid-lowering drugs [49].

Other polymeric drugs have so far fared less well. Since the early 2000s multivalent polymers have been explored clinically. For example, Tolevamer (GT160-246), a sodium salt of a high-molecular weight (>400,000 g/mol) styrene sulfonate polymer designed to bind to *Clostridium difficile* toxins A and B was evaluated as an orally administered solution to treat *C. difficile*-induced diarrhoea. Although a Tolevamer potassium–sodium salt formulation was generally safe and well tolerated in healthy volunteers [50] it did not meet its primary endpoint of non-inferiority versus standard antibiotic treatment (vancomycin or metronidazole) in pivotal Phase III trials [51]. A second example is VivaGel®, a 3% (w/w) carbopol gel formulation of the lysine-based dendrimer SPL7013 that displays a broad spectrum of antiviral activity (e.g. against HIV-1, HIV-2, herpes simplex viruses type-1 and human papillomavirus). In pivotal Phase III studies VivaGel® was evaluated as a topical vaginal virocide for the treatment of bacterial vaginosis. Although it showed statistically significant activity, the primary endpoint of clinical cure maintained at 2–3 weeks after the end of treatment was not met [52]. In healthy, sexually abstinent women VivaGel® was safe and well tolerated, without any evidence of systemic toxicity [53], whereas in sexually active young women there was a somewhat higher incidence of low-grade related genital adverse events compared to a placebo gel [54]. Poor patient acceptability of vaginal microbiocide formulations in general illustrates the difficulty of controlling such efficacy trials where poor compliance can compromise the validity of results obtained. However, as this dendrimer does exhibit important antiviral/microbiocidal activity a VivaGel®-coated condom was developed as an alternative strategy, and this product has just been approved for human use in Japan (March, 2014) [55].

Interest in the development of natural as well as synthetic polymers as polymeric drugs continues. One noteworthy example is the alginate oligosaccharide (Oligo-G) being developed as a novel antibacterial agent [56,57]. Oligo-G reduces the viscosity of cystic fibrosis patient sputum allowing improved efficacy of antibiotics, and it is currently in early clinical trials as an inhaled formulation for the treatment of cystic fibrosis. In a first Phase 1 study involving healthy volunteers Oligo-G was well tolerated and all adverse events seen were mild and transient. Pharmacokinetic studies suggested no systemic absorption [58]. This is an interesting example of a polymeric drug being developed for pulmonary administration.

2.2.3. Still awaiting first products: polymer–drug conjugates, block copolymer micelles, polymeric non-viral vectors for cytosolic delivery

Despite the first synthetic polymer–drug conjugate entering clinical trials in 1994, and block copolymer micelles containing covalently bound drugs following some years later, the first products to market in both classes are still awaited. (To note micelles that simply entrap/solubilise drugs non-covalently are not discussed here.) Pivotal clinical trials are ongoing and hopefully this milestone will be achieved soon. The anticancer drug conjugates Opaxio® and Etirinotecan Pegol (NKTR-102) are in advanced Phase III trials. Opaxio® (PGA-paclitaxel) has just finished enrolling patients in a Phase III trial (January 2014) where it has been evaluated as a maintenance therapy in women with advanced ovarian cancer [59]. It has also been studied in other Phase I/II trials e.g. in combination with radiotherapy for treatment of glioblastoma and for advanced head and neck cancer. Etirinotecan Pegol (NKTR-102) is a PEG- irinotecan conjugate undergoing Phase III evaluation in patients with metastatic breast cancer and it has recently passed an interim efficacy checkpoint (January 2014) [60]. The trial is expected to finish patient accrual within 2014 or early 2015. NKTR-102 is also being studied in Phase II trials in ovarian and colorectal cancer.

In the case of micelle-based products, the Japanese company NanoCarrier Co., Ltd. (in partnership with other companies) has a strong pipeline of polymeric micelle-based anticancer agents [61,62]. Some contain simply entrapped drugs, others have the bioactive linked to the carrier. Most advanced are a paclitaxel-containing micelle (NK105) currently being evaluated in Phase III trials for the treatment of breast cancer and this trial is expected to conclude in 2014, and a platinum-containing micelle (NC-6004) being evaluated in Phase III as a treatment for pancreatic cancer [62].

It is interesting to remember that when JCR was born in 1984 the concept of cytosolic delivery of biopharmaceuticals was yet to come. Despite considerable scepticism that it would ever happen, in 2013 the first gene therapy product (outside China) arrived with EMA approval of Glybera® for the treatment of familial lipoprotein lipase deficiency. Even so the product is authorised for a single treatment in this very rare indication and it uses a viral vector. Launch is not expected until 2014. Since the earliest studies proposing cationic polymers for gene delivery [63,64], a vast number of polymer-based vectors have been explored preclinically for gene, ribozyme, antisense and most recently siRNA delivery. Almost all have failed to reach clinical trials, and the results from those that have, have so far been modest [65]. Intensive efforts to design improved endosomolytic polymers for siRNA delivery has brought a renaissance to this field. Examples of novel polymers recently reported include amphiphilic, biodegradable polypeptide copolymers [66,67] and poly(amido amines) containing bioreducible disulfides [68]. Interestingly many of the currently proposed constructs include ligands for heptocyte targeting e.g. 'dynamic polyconjugates' designed using bioreversible conjugation chemistry [69,70]. The principal challenges for successful cytosolic delivery remain selective delivery to the target/diseased cells *in vivo*, and at the cellular level efficient endosomal escape and cytosolic delivery. Most preclinical studies still rely on *in vitro* and *in vivo* screening of pharmacological endpoints without assessing cellular pharmacokinetics. Quantitation of how much drug actually arrives to the cytosol is almost always missing (discussed in [14]) and this would surely help to improve non-viral vector design.

3. What next? Opportunities and challenges

The 1970s/80s was a golden age of innovation for first generation advanced drug delivery systems/nanomedicines [2]. Considerable scientific progress was made which helped to define critical features of many technologies in terms of their safety and efficacy, bring new routes to synthesis and manufacture, and developed first *in vitro* and *in vivo* screening methods. Given the relatively small community then working in the areas of advanced drug delivery, particularly those developing polymer therapeutics, compared to the vast army developing low molecular weight chemical entities success rates in terms of lab to clinic might be considered comparatively high. Some point out the lengthy timeline involved for transfer of first technologies from lab to product, but it is important to remember that development of the first-in-class agents while gathering the practical know-how for scale-up manufacture, validated characterisation, formulation and also learning the optimum schedule/dose for clinical use is not so easy. Also progress must be put in context of the high drug attrition rates seen for all novel agents. On average ~90–95% entering Phase I still fail to progress to market [71], and despite all efforts to improve this statistic, if anything Phase I failures rose over the last decade [72,73]. This is leading to a continued global effort to improve performance during translation of all drugs [74].

Due to the growing acceptance of polymer therapeutics as clinically important agents, continued search for innovation in big Pharma, and the rapid convergence of interests of many scientific disciplines arising from the popularisation of nanomedicine(s), a new 'golden' era for polymer therapeutics is just beginning [2]. There has been a ten-fold increase in publications relating to the 'pubmed' key words 'polymer therapeutics'/'poly conjugates' since 1984 and many, many more studies are hidden within the 'nano' and other descriptors. Over the last decade new hybrid technologies have emerged, e.g. polymer drug combination therapy, theranostics, complex polymer conjugates assembled into nanoparticles, new polymer chemistries, and compositions trying to capitalise on the unique physico-chemical behaviour of nanomaterials (e.g. including gold rods, quantum dots, etc.). Increased investment in 'nanomedicine' research has brought anticipation of measurable healthcare benefits within a reasonable time frame. Reviewing progress over the last decade shows this hasn't yet happened. Arguably an increasingly small percentage of research investment is generating credible lead candidates (including polymer therapeutics) for clinical development [75]. Why is this? "Cancer nanomedicines: So many papers and so few drugs" [76] and the 'translational gap' for anticancer nanomedicines [77] have recently been discussed. I believe that successful translation relies equally on good science fuelled by creativity and innovation and a strategic vision as to what is needed down the line during clinical development. When recently refereeing our paper [2] one reviewer suggested that the authors should "focus more on the true science of the field and not worry so much about the recounting of history or the lack of a common terminology. In the end, good science will be the only way that this field moves forward". This comment illustrates the challenge, and reminded me of the popular cartoon, Good Science → Then a Miracle Occurs → Benefit to Society. Without stage 1 of course we have nothing, but without an eagerness to embrace the needs of stage 2, successful arrival to the destination will never happen.

Good science and strong scientific method are both essential, but unfortunately the rush to publish often means this is not always implemented. I strongly agree with Weinberg's comment 'hypothesis first' when he was discussing the perceived lack of outcome made against healthcare benefits promised by the Human Genome Project [78], and with De Duve (a founding father of so much that we do in drug delivery; sadly lost in 2013) who said "In conducting your research, observe total rigour and intellectual honesty in the analysis of facts, consider all possible hypotheses, plan your approach to test those hypotheses, … Never conduct research with the aim of proving a theory, but, rather, to invalidate it, if it should be wrong" and moreover he said "… pay special attention to the quality and reliability of the instruments and techniques you use — and to your own ability to handle them" [79]. Recent quantitation of the lack of reproducibility of published preclinical research [80,81] (one study showed that in only 11% of the results in selected 'landmark' papers could the findings be confirmed [80]) was highlighted in respect to the negative impact this has on success of clinical trials. Moreover, it was noted that even when results of studies cannot be reproduced they still generate many secondary publications [80] often creating unhelpful dogma that misdirects research efforts in the field.

3.1. Strategy: learning lessons, joining the dots, asking the right questions

In my experience there are a number of tactics that help to increase the probability of successful translation:

1. *Strong multidisciplinary teams with a common goal*: Needed at the outset with experts in each of the collaborating sciences, happy to act as equal partners, and strong interdisciplinary leadership. When development progresses as a 'relay race', e.g. from polymer chemist to biologist to pharmaceutical scientist to clinician very likely the baton will fall. During the early research phase an awareness of the final clinical setting and needs during development from laboratory to first in man studies is essential to accelerate progress against a checklist of stop–go points relating to the goal.
2. *Definition of the clinical goal/target product profile*: At the outset it is essential to ask the questions, 'What do we want to make and Why?' This leads to definition of the critical design features for the product bearing in mind the route and frequency of administration

(together with any drug payload this will impact on safety considerations) and the target patient population (e.g. gender/age) and clinical setting for use (at home, in hospital, etc.).

3. *Understanding the current standard-of-care treatment*: Knowledge of the limitations of the current therapeutic regimes (toxicity, efficacy, pharmacokinetics, dose required, etc.), the profile of the products in clinical development, and not least what has been tried before and failed (and why). This is important to allow benchmarking progress for any new technology. It avoids repeating failures, and is essential to ensure that effective patent protection in this now crowded field. Few papers (even in JCR) begin with a few sentences providing such information with details of the new hypothesis with explanation as to why their new technology might be better. Moreover there is a general failure in pharmaceutical science journals to cite clinical studies/position papers in disease-specific journals.
4. *Choosing/designing appropriate polymers* (*safety, practicality of manufacture, formulation, etc.*) *in context of proposed clinical use*: Too many materials are still being proposed for biomedical uses for which they are totally unsuited, even though a decade of 'nanomedicine' research is beginning to alert chemists to the importance of choosing the right material for the right application [82]. Emerging new derivatives of natural polymers and innovative synthetic polymer chemistry are producing a vast array of new exciting materials, but the art is to apply them to the technology development for which they are best suited. This may, or may not be biomedical or even polymer therapeutic applications.
5. *Identifying the critical quality attributes of the polymer therapeutic — as a drug substance and final formulation, i.e. the product*: Performance controlling features may include composition (drug carrying capacity in relation to drug potency, ligand content needed for effective receptor mediated targeting), molecular weight and polydispersity of polymers and size and size distribution of micelles in relation to their control of pharmacokinetics (this will govern safety and efficacy), stability in both the relevant bio-environment and the formulation, and potential effect of macromolecular impurities on performance, etc. For each of the critical quality attributes, an acceptable batch-to-batch variability should be justifiable, even for preclinical studies.
6. *Understanding the requirements to progress from lab to market approval*: It is now easier than ever before to access information regarding the regulatory requirements that must be met in order to progress from laboratory to first in man and then onwards to market entry, e.g. [32] and the reflection papers cited therein, and [83]. For sure basic researchers do not need to be experts in the legal aspects of medicine regulation, but the guidance documents and reflection papers (e.g. the Joint MHLW/EMA Reflection Paper on the development of block copolymer micelle medicinal products [84]) are often short, easy to read, and moreover science-based. They can help a researcher understand the key features of each technology and possible methodology that will help address the important scientific questions during their research and later translation. It is also important to understand the distinction between polymer toxicity/biocompatibility, and the safety of a specific medicinal product, which depends on many other factors, not least safety profile of any drug payload carried and route and frequency of administration (discussed fully in [85]). The regulatory process is a servant of society and undertakes an integrated assessment of quality (put simply, what we have in the bottle), safety and efficacy to ensure proactive management of risk–benefit. The regulatory framework is built to ensure timely introduction of safe and effective medicines for the benefit of all. It is a good learning curve for all young scientists to understand the basic ethics/principles of medicines regulation.

3.2. Scientific advances: opportunities to improve successful translation

Three decades of JCR have seen remarkable scientific advances in all the core disciplines underpinning polymer therapeutics in research and development. Some of the key issues are discussed below in the context of potential to improve translation efficiency.

3.2.1. Unmet medical need: the changing landscape of patients and therapeutics

Although polymer therapeutics are still being developed as anticancer agents, there has been a significant broadening of clinical goal e.g. anti-infective agents, treatments of musculoskeletal diseases, polymer therapeutics for tissue regeneration and repair, and treatments for CNS diseases (reviewed in [1]). Given the future healthcare needs of the ageing population, and the current drive to find improved treatments for diseases of poverty associated with lower income countries this continued diversification is a great opportunity. For all target diseases it is important however to keep in mind the rapid advances being made in clinical practice. As an example in 1975 (when beginning my PhD studies) the outlook for a woman diagnosed with breast cancer in the UK was a 40% chance of survival at 10 years. Today statistics indicate an ~80% chance of survival due to improved diagnosis and therapy. Similarly for prostate cancer the 10-year survival statistics have risen from 20% to ~70% today. Knowledge has grown apace regarding the molecular basis of many diseases, underlying pathophysiology in health and disease, and the pleotropic mechanisms of drug toxicity and resistance. When establishing the rationale for optimum design of a polymer therapeutic it is important to narrow the goal to not only a particular disease, but to a stage of the disease and the ultimate clinical setting proposed or use, and maybe even a patient sub-group if objective biomarkers are known. Design of polymer therapeutics to circumvent drug resistance is also a great opportunity.

3.2.2. Selection of the most appropriate polymer and drug for a chosen application

As shown only a small fraction of the polymer chemistries reported have progressed into clinical trial. The key to success is judicious choice of polymer for a particular route of administration in the context of an unmet medical need. The oral poly(allylamine hydrochloride)-based sequestrants Renagel® and Welchol® and the topical lysine-based dendrimer Vivagel® discussed above are good examples of a thoughtful development plan. (Dendrimers are discussed elsewhere in this issue "R. Duncan, Commentary: Dendrimers: Relationship between structure and biocompatibility *in vitro*, and preliminary studies on the biodistribution of ^{125}I-labelled polyamidoamine dendrimers (2000))".

The growing appreciation of the disadvantages of non-biodegradable polymers (discussed in [1,86]) and the need for more precisely defined products is prompting increased interest in biodegradable polymers and the use of recombinant techniques to prepare conjugates. Polysialyated erythropoietin (ErepoXen®) is currently in Phase III clinical studies as a treatment for chronic anaemia [87]. PASylation, which uses recombinant techniques to attach a polypeptide composed of proline, alanine, and/or serine to a protein or peptide, is being explored as an alternative to PEGylation [88]. Increasing awareness of polymer-induced cellular vacuolation due to lysosomal accumulation of non-biodegradable is also prompting design conjugates that minimise this risk [89].

There is also an opportunity for greater vision when selecting the drug payload. In the early 1980s when we chose daunomycin and doxorubicin to synthesise anticancer HPMA polymer–drug conjugates [8] these anthracyclines were newly approved drugs. So many studies still use 'old drugs' as the bioactive component. Given the now huge collection of successful and failed modern medicines, including low molecular weight drugs targeted to specific pathways/pharmacological receptors, novel biopharmaceuticals and innovative drug combinations, there are great opportunities to design really novel constructs that would generate more interesting candidates for clinical trial. Recent papers in JCR exemplifying diversification include the peptide–hyaluronan conjugates designed as to treat autoimmune encephalomyelitis [90], PEG–RAGE peptide conjugates designed to prevent transthyretin aggregate-induced cytotoxicity in familial amyloidotic polyneuropathy

[91], and an HPMA copolymer-based anticancer combination therapy that includes an HPMA–cyclopamine conjugate designed to cause preferential toxicity to cancer stem/progenitor cells [92].

3.2.3. Characterisation and analytical tools

Polymer chemistry presents specific challenges for controlled synthesis and control of batch-to-batch reproducibility [1,93]. Nevertheless all too frequently there is still inadequate detail of characterisation given in research papers where biological properties of polymer therapeutics are assessed. This is a recipe for the poor preclinical research reproducibility as mentioned above [80,81]. Factors that impact on biological properties include molecular weight and polydispersity, micelle size/size distribution, and conjugate/micelle stability both in storage and under the assay conditions. For conjugates the distribution of drug, targeting ligand and/or imaging agent loading across the molecular weight range present, and not least tendency to form unimolecular micelles (typical of hydrophilic polymers carrying hydrophobic drug payloads) or intramolecular aggregates is also important. For constructs carrying drugs, contaminating free drug content (always present to the limit of detection of the assay used to measure it) can have a significant impact on results obtained, particularly in *in vitro* assays. Attempts are being made to better define these complex issues, for example, quantitative methods able to assess homogeneity of drug and ligand distribution in preparations of PAMAM dendrimers have been described [94,95], and the effect of aggregation of HPMA folate conjugates on folate receptor mediated uptake [96].

Increasingly sophisticated analytical techniques (including those more usually used in soft matter research) such as small-angle neutron scattering (SANS), 2D ^{1}H NOESY and TOCSY nuclear magnetic resonance (NMR), and pulsed gradient NMR etc. are being used to ascertain the fundamental solution properties of polymer–drug [97] and PEG–protein conjugates [98]. In turn this aids more meaningful definition of structure activity relationships in complex biological environments. New analytical tools can also assist characterisation of complex polymer therapeutics destined for clinical trial (discussed in [1,2,9]). Introduction of a quality-by-design approach and related analytical methods into pharmaceutical development [99–101] and development of new tools for manufacturing process control that are applicable to complex drugs including polymer therapeutics will certainly afford the opportunity to improve successful translation [35,36].

3.2.4. Clinically relevant preclinical models

Continuing efforts strive to make clinically relevant preclinical models for evaluation of all drugs, with primary human tissue validated for biomarkers of safety, efficacy, and drug resistance being the cornerstone of these efforts. Although polymer therapeutic research can often benefit such *in vitro* and *in vivo* models, they are often poorly characterised in respect of the specific biomarkers related to the proposed mechanism of action of the specific polymer therapeutic under investigation.

Things are changing. One example, which can serve as a lesson for other models to be developed to establish mechanistically based PKPD for other therapeutic areas, is the improving characterisation of *in vivo* anticancer models. The growing interest in big Pharma in antibody–drug conjugates (ADC) [102] and other nanomedicines is helping to push this agenda. It was recently noted that "… designing an ADC is more complex than a simple meccano game, requiring thoughtful combination of antibody, linker, and drugs in the context of a target and a defined cancer indication" [103]. These comments apply equally well to polymer/micelle–drug conjugates. Presence of the pharmacological target receptor in the tumour tissue is essential, but numerous other factors ultimately control performance of anticancer polymer therapeutics. At the cellular level these often include (i) effective endocytic internalisation, (ii) appropriate intracellular trafficking to endosomes or lysosomes and exposure to conditions that trigger drug release (e.g. lysosomal enzymes such as cathepsin B or low pH), and (iii) if designed for receptor-mediated targeting the number of receptors per cell and their internalisation, recycling and turnover rate are important (all influence concentration/dose-dependency and impact clinical protocol design). *In vivo*, the pharmacokinetic considerations include the ability to (i) avoid rapid RES clearance, (ii) escape rapid renal elimination (iii) avoid accumulation in potential sites of off-target toxicity, and not least (iv) demonstrate selective tumour targeting. Increased permeability of angiogenic tumour vasculature is essential for passive targeting by the EPR effect. Receptor-mediated targeting requires homogeneity of receptor expression on tumour cells, and conjugate ability to penetrate the intra-tumoural extracellular matrix bypassing the binding site barrier. ADME (absorption, distribution, metabolism, excretion) of polymer therapeutics has recently been reviewed [13].

The differences in the cellular pharmacokinetics of potent low molecular weight cytotoxic drugs and their respective polymer–drug conjugates means that *in vitro* cytotoxicity tests comparing IC_{50} values have little value when selecting lead candidates (discussed in [8]). Thus well-characterised *in vivo* tumour models that enable mechanistically-based PKPD evaluation are an essential tool. We used two probes (Evans Blue and HPMA copolymer–doxorubicin) in a panel of murine and human xenograft tumours to define the effect of tumour type and tumour size on EPR-mediated tumour localisation [104]. Using the drug conjugate we could also measure cathepsin B-mediated drug release rate. (The lysosomal enzyme cathepsin B is often the trigger used to release drug intracellularly e.g. by linker degradation in polymer–drug conjugates and ADCs, and PGA backbone degradation in conjugates such as Opaxio®.) The differences observed were significant with an ~12-fold variation in EPR and a ~200-fold variation drug release rate. Tumour size-dependency was seen in some tumour models but not others [104]. Novel cathepsin B-activatable fluorescent probes are now affording new opportunities for functional monitoring of tumour cathepsin B levels *in vivo* [105,106]. An HPMA copolymer conjugate containing both PTX/SQ-Cy5 (a self-quenched near-infrared fluorescence probe) and paclitaxel designed as a polymer-based theranostic has recently demonstrated *in vivo* ability to provide real-time deep tissue imaging of cathepsin B activity that correlates with drug delivery [105].

Endocytic uptake is a common characteristic of all polymer therapeutics, that in many cases is pivotal for efficacy, and in all cases can influence the safety profile. It was De Duve and colleagues who gave us the first analytical tools (subcellular fractionation and isolated of lysosomal enzymes) and the concepts of lysosomotropic delivery that underpin so much of what we do today [107,108]. Elsewhere [14] we have comprehensively reviewed the current opportunities and challenges relating to use of endocytosis and intracellular trafficking as gateways for nanomedicine delivery. Some of the key points made are however worthy of repeating here. Endocytosis and trafficking are cell type dependent and thus it is impossible to generalise structure–activity relationships (e.g. effects of charge, molecular weight, size, shape etc.) regarding uptake and trafficking from one cell type to another. Endocytosis and trafficking are often dysregulated in disease, e.g. in cancer [109]. This can alter internalisation rate and impart resistance to macromolecular drugs. There is increasing awareness of the central role played by lysosomal membrane proteins as a controller of cellular function in health and disease [110], and impaired lysosome function has been implicated as a central player in an increasing number of diseases e.g. Parkinson's disease [111]. Accumulation of natural products, e.g. as occurs in lysosomal storage diseases, or indeed the vector used for drug delivery in lysosomes can change intra-vesicle pH and disrupt trafficking pathways. Consequently this may limit efficient access of the proposed endosomotropic/lysosomotropic delivery systems (reviewed in [14]). *In vitro* analytical methods being used to document endocytosis are also being refined to become more quantitative and more relevant to the *in vivo* situation. It is clear that cell culture conditions (e.g. when cells are grown under flow, or in a 3D matrix, etc.) influence the rate of endocytosis measured in a particular cell type. Although fluorescence microscopy techniques are widely used to visualise cellular uptake and trafficking, improved quantitation of

absolute rates of uptake and intracellular fate localisation is improving the definition of the structure–activity relationships, so important to improve the design/performance of polymer therapeutics (reviewed in [14] with examples).

Quantitative definition of pharmacokinetic aspects of drug targeting and controlled release *in vivo* is also of pivotal importance. Many early studies involving polymer–drug conjugates developed HPLC and radiolabelling techniques able to quantify preclinical and clinical pharmacokinetics (reviewed in [9,112]). The first gamma camera probes were also described and used for preclinical and clinical imaging [112]. Over many years there has been a trend towards use of fluorescence to monitor fate *in vivo*. Although this is a useful tool to enable visualisation of local tissue distribution (tumour penetration), the lack of ability to quantitate whole body tissue distribution over time, the routes of elimination and/or determine mass balance of fate has been a retrograde step. These are the key issues, together with drug release rate in the circulation and target tissues, that underpin polymer therapeutic design and optimisation. Due to interest in translation of emerging nanomedicines and ADC, quantitation of pharmacokinetics is seeing a revival. Lessons can be learnt from methods (including modelling) used to quantitate the fate of ADC [113], and to monitor full ADME of specific ADC conjugates that have entered clinical use [114]. The growing database of preclinical pharmacokinetic studies is for the first time enabling comparison of the parameters measured in different species for nanomedicines [115].

Finally it is important to note the increasing opportunity to compare preclinical results (pharmacokinetics, toxicity and efficacy) with clinical trial observations for polymer therapeutics (e.g. [77,90,116]). This can only help to identify the most appropriate models/species for lead candidate selection. HPMA copolymer-anthracycline (FCE28068, FCE28069) and -platinate (AP5280, AP5346) conjugates (reviewed in [9]), and the linear, cyclodextrin-polyethylene glycol (CD-PEG) co-polymer conjugates that self-assemble into nanoparticles (CRLX101 formerly IT-101) [116] showed a good preclinical–clinical correlation in terms of pharmacokinetics and the reduced toxicity of the drugs carried. There was also some evidence of antitumour activity in the Phase I//II patients enrolled in these studies.

3.2.5. Improving clinical trial design using relevant patient biomarkers and companion diagnostics

Failure of drugs in clinical development is typically ascribed to lack of efficacy, unacceptable toxicity, issues relating to pharmacokinetics/bioavailability, or other strategic, commercial and financial issues [71,72]. Ability to reproducibly manufacture a product of adequate quality, with a justified specification in terms of efficacy and safety, is also essential. Successful translation of polymer therapeutics will undoubtedly certainly benefit from the ongoing modernisation of the pharmaceutical development process (target validation, biomarkers for patient individualisation of therapy, systems pharmacology and toxicology, etc.) and advances in 'regulatory science', a new approach ensuring scientific state of art is integrated into all aspects of medicine regulation [74].

Pharmacological target validation has always been a primary objective of preclinical and early clinical trials when developing low molecular weight chemical entities. However, the advent of macromolecular biopharmaceuticals and nanomedicines is increasing awareness of the importance of verifying pharmacokinetic parameters that will govern efficacy and safety. The need for a more physiologically-based view of pharmacokinetics [117] and the benefits of mechanistic PKPD modelling when translating ADCs from lab to clinic [118] have become ever more evident. Tools to aid better selection of those patients who are most likely to respond favourably to nanomedicines in clinical trials, are on the horizon. For example, a recent study investigating inter-patient variability in the pharmacokinetics of Doxil® (now a large database) identified factors (e.g. age, gender, and monocyte counts) that appeared to correlate with Doxil® clearance; it is known that this influences drug performance [119]. If verified, such biomarkers would in future allow patient individualisation of Doxil® therapy.

In the context of anticancer polymer–drug conjugates, during the 1980s we developed gamma camera imaging conjugates corresponding to HPMA copolymer–doxorubicin (FCE28068) and HPMA copolymer–doxorubicin-gal (FCE28069) [120] that we transferred into Phase I/II clinical trials for gamma camera/SPECT imaging. The goal was the verification of tumour localisation of FCE28068 by the EPR effect, and to aid the dosing protocol for FCE28069 where receptor-saturation during dose escalation of this gal-targeted anthracycline conjugate was a possibility. This approach was ahead of its time, as the importance of investing in 'companion diagnostics' (*in vitro* diagnostic devices and patient imaging agents) has only recently become valued [121,122]. It has long been known that different tumour types (and stages of development) display differences in angiogenesis and vascular permeability. Thus for any anticancer nanomedicine relying on EPR mediated targeting as a primary mechanism of action, confirmation of tumour vascular permeability by patient imaging is scientifically, and ethically, essential prior to selection for such a therapy. The routes to optimum imaging probes to implement this strategy are currently under discussion [123]. Indeed companion imaging agents for receptor verification are already in clinical development in other settings, e.g. the folate-receptor-targeted therapeutic (Vintafolide®) and its companion SPECT imaging agent ^{99}mTc-Etarfolatide® [124]. In pharmaceutical development generally, *in vitro* diagnostic devices have established an important role in verification of the pharmacological target, and gene profiling to assess therapeutic risk–benefit, patient selection for therapy and therapy monitoring. They have increased the efficiency of clinical development and brought patient individualisation of therapy, indeed recently approved ADCs (2013) was swiftly followed by FDA approval of the corresponding *in vitro* companion diagnostic tools. There is considerable scope to investigate tailored *in vitro* diagnostics for more specific application to mechanisms relating to polymer therapeutics. For example, verification of biomarkers that relate to mechanisms of drug release (e.g. cathepsin B levels in patient derived tumour biopsys), or biomarkers of dysregulation of endocytic function. Such tools coupled with more sophisticated, clinically applicable, polymer-based *in vivo* imaging probes including theranostics [105] and polymer-based PET imaging agents [125,126] will play a very important role in future in enhancing success of polymer therapeutics during clinical development.

The global regulatory framework is also quickly evolving to reduce the time frame for transfer of innovative therapeutics to patients. The FDA 'breakthrough therapy' scheme [127] is one example, defined to include agents where the "preliminary clinical evidence indicates that the drug may demonstrate substantial improvement over existing therapies on one or more clinically significant endpoints, such as substantial treatment effects observed early in clinical development". In Europe new mechanisms to speed up clinical trial authorisation, and a move towards transparency of clinical trial results will soon be in place, with considerable potential to accelerate the progress of innovative medicines [128,129]. Finally it is important to stress the diversity of biomedical applications proposed for natural and synthetic polymers (e.g. biomaterials, pharmaceutical containers and administration devices, pharmaceutical excipients and medicinal products). Awareness of the regulatory requirements for the specific application proposed, including the differences between device, medicinal product and combination therapy, is essential if the goal to progress from a nice publication into a real candidate for clinical development is to increase (discussed in [2]).

4. Terminology and communication: does it really matter what we call things?

Modern electronic databases coupled with journal (including JCR) 'key words' are a great tool to join the dots of the history of science, and find and share results describing efforts to improve key design

features in specific classes of technology. 'Key words' in this field have changed significantly over the last 3 decades. Is terminology important? In basic research perhaps not, although common terms with different meanings sometimes make communication tricky across multidisciplinary teams, e.g. EPR, is it tumour vascular permeability? Or electron paramagnetic resonance? For a legally binding regulatory process, which must avoid misunderstanding at all costs, the answer is clearly yes [2,83]. During preclinical development of HPMA copolymer–anticancer drug conjugates into Phase I/II trials, colleagues from the regulatory department in Farmitalia Carlo Erba, Milan, pointed out that polymer–drug conjugates are not well-described as 'drug delivery systems' or 'formulations' as this confuses them with technologies that simply entrap a bioactive. Thus in 1994 when establishing the Centre for Polymer Therapeutics at the London School of Pharmacy, and the descriptor "*polymer therapeutics*" defined to better represent the novel medicines we were trying to develop [7,130].

Polymer therapeutics are complex, multicomponent and also nano-sized constructs, so in this new 'nano' era, they are also viewed by some as "nanomedicines" [2], falling within definitions such as: "Nanomedicines are purpose designed, often using multiple components, and all have at least one dimension in the nano-size range" [32]. It is questionable whether the overarching terms 'nanomedicine' or indeed 'nanoparticle' which has become popularised to include many classes of well-established nano-sized drug delivery systems, e.g. liposomes, micelles, polymeric and lipid nanoparticles, are helping to highlight the product critical features that will govern best clinical performance. In conclusion the most important for a scientific researcher and regulator alike is to understand the critical design features of a specific product and understand how they impact on safety and efficacy, using terminology that allows meaningful communication with others.

5. Conclusions

During the lifetime of JCR a growing number of polymer therapeutics progressed from concept to clinically important medicines. With the healthy pipeline currently in clinical development, and innovative materials and the new concepts continuing to emerge the future looks bright. Moreover, continuing advances in basic and regulatory science provide an ideal platform to enhance the probability of successful translation. JCR has proved an important forum to share ideas and research results in this field. Its strength has always been the remit to publish high-quality interdisciplinary research covering all aspects of drug targeting and controlled release relevant to academia and industry. Its breadth of scope is illustrated by the papers that we have published over the years, from the fundamental study on endocytosis in issue one [12], to the preclinical studies of the HPMA copolymer anthracycline conjugates [131] undertaken in industry when developing them into early clinical trial, to more recent papers describing (i) subcellular fractionation methods used to quantitate cytosolic access of endosomolytic poly(amido amine)s [132], and (ii) first *in vivo* proof of concept for a bioresponsive dextrin-rhEGF designed to promote wound repair [133]. Long may JCR continue to support our scientific community as *the* forum for exchange of ideas during development of innovative medicines designed for drug targeting and controlled release!

Finally, perhaps the most important of all, amongst De Duve's recommendations to young scientists mentioned earlier [79], he said "… enjoy it, science is fun" and "Choose your mentors well. Good research is not learned in books, but at the bench, like the crafts in the Middle Ages, under the supervision of a master." I certainly agree for it is the coming generation of scientists that will develop next generation polymer therapeutics for the 21st century!

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