Opinion

Challenges in preclinical to clinical translation for anticancer carrier-mediated agents

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Major advances in carrier-mediated agents (CMAs), which include nanoparticles and conjugates, have revolutionized drug delivery capabilities over the past decade. While providing numerous advantages over their small-molecule counterparts, there is substantial variability in how individual CMA formulations and patient characteristics affect the pharmacology, pharmacokinetics (PK), and pharmacodynamics (PD) (efficacy and toxicity) of these agents. Development or selection of animal models is used to predict the effects within a particular human disease. A breadth of studies have begun to emphasize the importance of preclinical animal models in understanding and evaluating the interaction between CMAs and the immune system and tumor matrix, which ultimately influences CMA PK (clearance and distribution) and PD (efficacy and toxicity). It is fundamental to study representative preclinical tumor models that recapitulate patients with diseases (e.g., cancer) and evaluate the interplay between CMAs and the immune system, including the mononuclear phagocyte system (MPS), chemokines, hormones, and other immune modulators. Furthermore, standard allometric scaling using body weight does not accurately predict drug clearance in humans. Future studies are warranted to better understand the complex pharmacology and interaction of CMA carriers within individual preclinical models and their biological systems, such as the MPS and tumor microenvironment, and their application to allometric scaling across species. © 2016 Wiley Periodicals, Inc.

INTRODUCTION

The number of available carrier-based drug systems has seen exponential growth in the past 20 years. In 2006 alone, nearly 130 nanotechnology-based products were estimated to be undergoing the drug development process worldwide.1 Today there are more than 1600 nanotechnology-based products in the market, and almost 250 nanomedicine products in the market or in clinical trials in 2013, with more emerging at a rapid pace.2 While the number of agents used clinically is still limited, the plethora that are emerging as potential therapeutic agents warrants the need for detailed studies of their unique pharmacology in animal models and in humans.

Several reviews exist that summarize currently available and later stage development of chemotherapeutic carrier-mediated agents (CMAs).3,4 Examples
of various types of CMAs include liposomes, fullerences, carbon tubes, quantum dots, nanoshells, polymers, dendrimers, and conjugates, including antibody–drug conjugates (ADCs). While conventional drugs encounter numerous obstacles in route to their target, CMAs can take advantage of tumor’s leaky vasculature to extravasate into tissue via the enhanced permeability and retention (EPR) effect. This passive targeting in theory exploits the classic features of tumor biology in order to increase exposure of CMA in the tumor. Relative to normal cells, tumor cells may overexpress certain surface antigens. New methods of ‘active targeting’ of CMAs may further improve tumor delivery and activity by allowing the CMA to bind to specific cells in tumors using surface-attached ligands by maximizing the specificity of binding of a targeting agent, such as monoclonal antibodies and ligands. Further unique strategies for drug release from CMAs based on a particular external stimuli, or ‘trigger,’ (such as heat, ultrasound, light, or pH) are also in development.

The disposition of CMAs is dependent upon the carrier and not the therapeutic entity until the drug gets released. This has created a new nomenclature used to describe CMA pharmacokinetics (PK), including: encapsulated (the drug within or bound to the carrier), released (active drug that no longer associates with the carrier), and ‘sum total’ or ‘total’ (encapsulated drug plus released drug). After the drug is released from its carrier, it is pharmacologically active and subject to the same routes of metabolism and clearance as the noncarrier form of the drug. Thus, analytical methods must be performed in order to assess the disposition of encapsulated and released forms of the drug in plasma, tumor, and tissues. Moreover, considerable inter-patient variability exists in the PK and pharmacodynamics (PD) of CMAs, and while the exact factors have not been finalized, it is hypothesized that the mononuclear phagocyte system (MPS) plays a key role.

The uptake and removal of CMAs from the circulation are mediated by both circulating (i.e., monocytes, leukocytes, and dendritic cells) and resident tissue phagocytes (such as Kupffer cells in the liver, dendritic cells in the lymph nodes, macrophages, and B cells in the spleen) of the MPS. Figure 1 illustrates the interaction and clearance of CMA with these immune cells, collectively termed the MPS. Many CMAs have been developed for the purpose of evading rapid clearance from the bloodstream, thereby extending systemic circulation time. Uptake mechanisms may occur through different pathways and are often facilitated by the adsorption of opsonins to the CMA surface and subsequent phagocytosis. However, CMA PK and PD varies between individual patients and can be attributed to many variables, including duration and rate of interaction and overall activity of MPS components. Although this interaction has been documented, it is not known whether CMA uptake occurs through phagocytosis in the tumor, liver, and spleen, ‘capturing’ the CMA as it reaches the organ through the bloodstream, and/or if the circulating MPS cells ‘hijack’ the CMA in the bloodstream and carry it to the site of action, leading to the increased tumor accumulation seen with these agents.

Development or selection of animal models is used to predict the effects within a particular human disease. While no single animal model may exactly replicate responses observed within humans, with the wide availability of preclinical models, now including genetically engineered models, a closer understanding in the choice to use a particular models is required for two primary reasons: (1) to ensure the proper selection of preclinical model(s) that will best reflect the true human disease characteristics and (2) to observe pharmacological differences that occur within preclinical models of presumably similar characteristics.

VARIABILITY IN PATIENTS AND ANIMALS

A major assumption made when testing drugs within various species is that a drug’s PK and PD effects will be similar, though potentially varying by scale. However, we now know that this is not necessarily the case for most drugs, but especially for CMAs. One of the most well-known examples of this variability between species is exemplified with the PK of PEGylated liposomal doxorubicin (PLD). Suzuki et al. showed within dogs that repeated dosing of PLD resulted in increasing clearance from the first to third administration. This accelerated blood clearance (ABC) phenomenon, hypothesized to be due to the creation of anti-PEG immunoglobulins, observed within dogs has also previously been observed in mice, rats, and rhesus monkeys. However, this is not the case in humans. Gabizon et al. reported on the dose- and cycle-dependence of PLD PK within human subjects. This study showed there is a significant increase of dose-normalized AUC values and a correspondingly decrease in clearance and volume of distribution progressing from the first to third cycles. Furthermore, changes in PLD PK were found to be related to the changes in the MPS,
speciﬁcally patient’s pre-cycle monocyte count. This variability within CMA preclinical PK can then potentially compromise the translation to human phase I clinical trial, where preclinical data is compiled to extrapolate starting doses and PK disposition. The difference in PK over cycles in animals and patients is a prime example of some animal models not predicting effects in patients.

Variability in CMA PK disposition has been documented within the literature, both for formulations currently approved and/or in development. One such example is a phase I PK study of S-CKD602, a liposomal formulation of a camptothecin analog, where the goal was to determine maximum tolerated dose (MTD) and the PK of the drug. When comparing S-CKD602 dose to CKD602 AUC (either encapsulated or released), high inter-patient variability in PK disposition was observed with as much as 100-fold at lower doses and 10- to 25-fold near MTD. Factors with the potential to affect CMA PK, including CMA-associated physical, host-associated, and pharmacologic-associated characteristics, have been reviewed previously. However, additional studies are needed to better understand the mechanisms and the factors affecting the complex interaction between the MPS and CMAs and the factors influencing tumor delivery of CMAs.

A recent meta-analysis is the first to directly compare inter-patient PK variability of nine liposomal and small-molecule (SM) anticancer agents from Phase I and II studies between 1966 and 2011. High inter-patient PK variability observed with many.

FIGURE 1 | Clearance of nanoparticles and carrier-mediated agents (CMAs) via the mononuclear phagocyte system (MPS). Small-molecule (SM) anticancer agents undergo a standard route of metabolism and elimination, including enterohepatic recycling and removal through the kidney. CMAs, however, which are engulfed by phagocytes, are contained primarily in compartments such as the spleen, liver, and peripheral blood mononuclear cells. (Reprinted with permission from Refs 15,16. Copyright 2015 Future Medicine Ltd and 2012 John Wiley & Sons, respectively).
anticancer agents makes it difficult to predict a patient’s response to a particular drug, with individual encapsulated drug exposure varying between 20- and 100-fold. Moreover, liposomes often report higher PK variability compared with SM and nonliposomal agents, as agents (e.g., PEGylated liposomal agents) with a lower clearance demonstrated a greater degree of PK variability. Higher reported AUC variability was also observed when samples were obtained up to 14 days compared with only 0–24 h, suggesting limited sample collection to 24 h after administration of a liposomal agent underestimates inter-patient PK variability. This data would suggest that the development of a standardized sampling strategy that may be applied to all CMAs is warranted as a means of reducing inaccurate documentation of PK variability that arises from suboptimal study designs.

Genetic factors may be associated with the variable disposition of PLD. This was tested by evaluating the plasma and tissue disposition of doxorubicin after administration of PLD at 6 mg/kg IV x1 via tail vein in 23 different male inbred mouse strains and then performing a pharmacogenetics analysis. An approximately 13-fold difference in plasma clearance of PLD was observed among inbred strains. There was a correlation between strain-specific differences in PLD clearance and genetic variation with a genomic region encoding GULP1 (PTB domain containing engulfment adapter 1) protein using haplotype-associated mapping and the efficient mixed-model association algorithms. The results also show Gulp1 expression in adipose tissue was associated with PLD disposition in plasma. These findings suggest the genetic variants may be associated with inter-individual PK differences in CMA clearance.

EFFECTS OF TUMORS ON PK

The physical and biochemical changes caused by tumors have been found to be unique and can affect the PK of CMAs. Physiological factors related to the tumor vasculature, such as heterogeneous blood supply, elevated interstitial pressure, and larger transport distances in the interstitium, have been found to be responsible for the poor localization of macromolecule delivery within tumors. Such abnormalities in the tumor microvasculature can further create hostile microenvironments, hindering anticancer treatments (including chemotherapy and radiation). Current CMA therapies on the market rely on the EPR effect, characterized by hypervascularature, defective vascular architecture, and impaired lymphatic drainage. However, several factors have also been found to influence the EPR effect in tumors, influencing the diffusion and penetration of CMAs: the extent of macrophage tumor infiltration, tumor size, type, and location, the vascular bed and surrounding stroma, tumor fibrosis, and the presence/absence of functional lymphatics.

Tumor exposure and antitumor activity of liposomal CMAs has also been found to be related to the presence of the MPS in tumors. This has been previously demonstrated in mice bearing SKOV-3 human ovarian and A375 human melanoma xenografts receiving S-CKD602. The ratio of S-CKD602 in tumor to plasma was 1.7-fold higher in mice bearing SKOV-3 compared with that of A375. In addition, there was a twofold higher exposure of released drug in tumor in SKOV3 compared with that of A375. Moreover, the staining of MPS cells was 4.5-fold higher in SKOV-3 compared with that of A375. The increased tumor delivery and release of CKD-602 in SKOV-3 compared with A375 xenografts was consistent with the increased staining of MPS cells, suggesting that variability in the MPS may affect the tumor disposition and activity of nanosomal anticancer agents. This also suggests that tumor type plays a role in the PK of CMAs.

Further studies evaluated the role of heterogeneity of the tumor microenvironment in the variability of CMA delivery and efficacy. C3-TAg GEMM mice and Balb/c mice bearing T11 orthotopic tumors, each representing a model of human triple-negative breast cancer, were treated with either SM doxorubicin or PLD. While the encapsulated and released AUC from PLD within plasma was similar between the two models and SM doxorubicin was similar within each tumor. The delivery of doxorubicin (as measured by AUC) was twofold greater within the C3-TAg model versus T11 model (Figure 2(a) and (b)). Accumulation of PLD within the liver was also 1.5-fold higher in the T11 model versus C3-TAg (p = 0.002), though similar within other tested organs. In addition, C3-TAg tumors were shown to be more responsive and have better efficacy to PLD compared with T11 tumors. This data would suggest that the tumor microenvironment and/or tumor cell features within models of similar types of breast cancer affected CMA tumor delivery, but not the SM drug. Differences in tumor chemical signaling to illicit an immune response may lead to the altered CMA PK that has been observed. For instance, the chemokines CCL2 and CCL5 are key mediators for the recruitment of monocytes and macrophages into tissues and tumors. Studies in mice bearing SKOV3
ovarian cancer xenografts have shown that the presence of tumor cells and a higher presence of chemokines (intra-tumoral and in plasma compared to nontumor bearing mice; \( p = 0.001 \) and 0.020, respectively) was significantly associated with increased clearance of PLD compared to nontumor bearing mice \( (p < 0.001) \) (Figure 3(a)–(c)). Increased expression of VEGF-a was also observed after PLD administration, indicating pro-inflammatory cytokine production in response to CMA therapy. A separate study investigating other potential physiologic factors that affected PLD breast

**FIGURE 2** | Concentration versus time profiles of doxorubicin after administration of PLD and nonliposomal doxorubicin (NL-doxo) at 6 mg/kg IV \( \times 1 \) via tail vein in tumor (a) and liver (b) in basal-like C3-TAg and claudin-low T11 breast tumor models. Sum total (encapsulated + released) samples \( (n = 3 \) mice at each time point) were obtained at 0.083, 0.5, 1, 3, 6, 24, 48, 72, and 96 h following PLD administration. \( P < 0.05 \) (AUC\(_{0-96\text{h}}\) in the C3-TAg model versus AUC\(_{0-96\text{h}}\) in the T11 model). (Reprinted with permission from Ref 9. Copyright 2014 American Association of Cancer Research).

**FIGURE 3** | PLD PKs in plasma and tissues and profiling of CCL2 and CCL5 in nontumor bearing (NT) mice and mice-beariing SKOV3 orthotopic ovarian cancer xenografts after administration of PLD at 6 mg/kg IV \( \times 1 \) via tail vein. CCL2 (a) and CCL5 (b) concentration versus time profiles in plasma and tumors following PLD administration. Encapsulated and released doxorubicin exposure in plasma and sum total doxorubicin exposure in the liver and spleen (c). Data represents mean \pm SEM. \( (n = 3) \). Plasma chemokine AUC\(_{0-96\text{h}}\) and its association with PLD PKs in patients with EOC. Plasma encapsulated doxorubicin exposure positively correlated with CCL2 AUC (d) \( (p = 0.017) \) and CCL5 AUC (e) \( (p = 0.009) \); however, no association was observed in patients treated with PLD plus carboplatin \( (p = 0.05, \) data not shown). \( R^2 \) and \( p \)-values are calculated using linear regression followed by adjustment for multiple comparisons using Holm test. (Reprinted with permission from Ref 30. Copyright 2015 Elsevier Inc).
cancer tumor PK revealed that, compared to C3-TAg tumors, T11 tumors showed significantly higher expression of CCL2 and VEGF-a, greater vascular quantity, and decreased expression of VEGF-c ($p < 0.05$). Studies have also reported differences in the clearance of nanoparticles based on T-helper cell and macrophage status. This is thought to occur due to inflammatory chemokine’s effects on T-helper cell polarization (Th2 over Th1), leading to functional changes in polarized macrophages (M2 over M1). Similar to other studies, CMA clearance was slower in Th1-prone mice versus Th2-prone mice, where Th2 polarization induces an anti-inflammatory M2 phenotype. In addition, the bidirectional interaction between PLD and chemokines has been reported in patients with recurrent epithelial ovarian cancer (EOC). The overall exposure of encapsulated PLD within plasma demonstrated a positive correlation ($R^2 = 0.79, \ p = 0.017$) with total exposure (as measured by AUC) of CCL2 and CCL5 in plasma (Figure 3(d) and (e)), suggesting that PLD induces chemokine secretion from immune cells within blood and tissues which highlights the bidirectional interaction between liposomal agents and the MPS. These studies demonstrate the important role and bidirectional interaction between the immune system responses, the relationship of tumor vasculature, and alterations in response to a tumor’s environment can affect CMA disposition.

In an effort to increase the tumor delivery of both CMAs and SM anticancer agents, a number of different pretreatment methods have been studied. Jain et al. attempted to increase the penetration of antibodies and viral nanoparticles into tumors by pretreating tumors with collagenase, a matrix degrading enzyme. Pretreatment with collagenase resulted in a two- to threefold increase in the penetration of antibodies and viral nanoparticles into tumor over noncollagenase-treated tumors. Similarly, the hormone relaxin has been studied to increase the delivery of antibodies and macromolecules to tumor. Relaxin induces the production of matrix degrading enzymes, which causes changes in the structure of collagen. Pretreatment of tumors with relaxin resulted in a two- to threefold increase in the tumor delivery of antibodies and macromolecules over tumors that have not been pretreated with relaxin. Also the priming of tumors with low-dose IV administration of traditional chemotherapy drugs prior to the administration of liposomal formulations to increase liposomal tumor delivery has been evaluated. Pretreatment with paclitaxel 48 h prior to administration of PLD increased the tumor exposure of PLD by 1.4-fold over the administration of PLD alone. However, this study did not evaluate the effects of paclitaxel on the systemic/plasma PK of PLD which may confound the results.

**THE MPS IN CLINICAL AND PRECLINICAL MODELS**

Understanding of the biomolecular interactions of nanomedicines and the MPS is critical for optimal development of CMAs and better prediction of efficacy of CMA-based therapy. However, there are limited preclinical and clinical data to understand the mechanisms for highly variable disposition of nanomedicines in relation to the MPS interaction and related immune responses.

To evaluate the effects of tumor-associated macrophages (TAMs) on the delivery of PLD and characterize the interaction of PLD within similar tumor models, C3-TAg tumors and T11 tumors from the PK studies described above were stained for F4/80, a glycoprotein expressed by mature tissue macrophages. There was no significant difference in the baseline level of TAMs in any subregions between these two breast cancer models. However, after PLD administration, a nadir in TAMs occurs at 24 h after PLD followed by TAM infiltration in both models. The percentage decrease at nadir in TAMs localized in the viable tumors being greater in T11 compared with that of C3-TAg (37.2 versus 6.6%, $p < 0.05$).

Consistent with high variation observed in the encapsulated doxorubicin within PK studies of 23 inbred mouse strains, there was a notable difference in release of doxorubicin from liposome carriers among mouse strains. These laboratory mice can serve as valuable experimental tools to model the phenotypic variation and identify the genes implicated, but other biological variation may exist that may impact the MPS. However, it is not known whether variability in the monocyte levels in blood contributes to differential PK between these models. The percentage of monocytes within whole blood in the same 23 inbred mouse strains not only differed between strains, but also based on gender. While these differences in monocyte count exist, when compared against PLD clearance, there was only a minor association between monocyte count and PLD PK across the different inbred strains.

However, studies where evaluation of individual circulating monocytes (MO) is utilized as a means to predict CMA clearance could present a different result than using population-based monocyte counts.
The first report of reduced CMA clearance being associated with the percentage decrease of monocytes was shown in a phase I/II study of S-CKD602. In all patients ($R^2 = 0.56$), and those below 60 years of age ($R^2 = 0.82$), a linear relationship was observed between the percentage decrease in monocytes and S-CKD602 clearance. A second phase I/II study evaluating the PK of PLD in patients with solid tumors or Kaposi’s sarcoma demonstrated similar results. This study demonstrated the association between a reduction in pre-cycle monocyte count and decreased PLD clearance from cycles 1 to 3 of therapy ($p = 0.09$). A recent pilot study further assessed the monocyte subpopulations before PLD administration in a small patient population ($n = 9$). Inter-individual variability in monocyte subpopulations was observed, representing three distinct populations with different immunological responses: classical (CD14++ CD16--), non-classical (CD14+ CD16++), and intermediate (CD14++ CD16+). While a majority displayed similar characteristics to healthy populations, two patients had lower populations of nonclassical monocytes (3.5 and 4.5 versus 10.9%). Two others showed a decreased proportion of classical monocytes and a higher level of nonclassical and intermediate monocytes. These studies highlight that the MPS plays an important role in the distribution of liposomes, and potentially other CMAs.

Another recent study demonstrated the use of phenotypic probes that measured MPS function measuring MO phagocytosis and reactive oxygen species (ROS) in mice, rats, dogs, and patients with refractory solid tumors treated with various PEGylated-liposomal anticancer CMAs. In peripheral whole blood MO across species, a positive association was observed between phagocytosis activity and clearance of PLD ($R^2 = 0.95$), S-CKD602 ($R^2 = 0.99$), and SPI-077 ($R^2 = 0.73$) (Figure 4). A similar trend was observed when comparing baseline ROS production (without stimulus) with clearance of each agent ($R^2 = 0.77$, 0.77, and 0.66, respectively). These findings suggest that probes of MPS function may better help predict PEGylated liposomal CMA clearance across species compared to other variables. Furthermore, in patients with EOC, associations were observed between PLD clearance and phagocytosis ($R^2 = 0.43$, $p = 0.04$) and ROS production ($R^2 = 0.61$, $p = 0.008$) in blood MOs, suggesting probes of MPS function help predict PLD clearance in patients with EOC. While the phenotyping of MPS probes could provide reduced treatment toxicity while maintaining therapeutic efficacy, further large-scale clinical trials are still needed to support MPS-based dose adjustments.

**WHAT IS THE MOST APPROPRIATE MODEL?**

Being able to predict PK parameters in humans based on extrapolated data from animal models is an essential step in the drug development process. The
ability to generalize preclinical data in order to predict the safety and efficacy of new compounds in humans is often viewed with skepticism, but it is thought to provide accurate data when selecting the appropriate PK parameters.\textsuperscript{41} The two most commonly selected preclinical models used in toxicology and toxicokinetic studies of SMs and CMAs are rats and dogs. From an evolutionary standpoint, all mammals are quite similar, but differ in terms of their environmental adaptations.\textsuperscript{44} Due to the similarities in biochemistry, anatomy, and physiology with other mammals, the most significant difference between models is their size.\textsuperscript{41}

Allometric scaling and physiologically based pharmacokinetic modeling are the most commonly used and recommended strategies for predicting PK parameters in humans.\textsuperscript{41} However, biology is rarely this simple, exemplified in the previous discussion surrounding the differences in AUC observed after repeated dosing in dogs and humans.\textsuperscript{18,22} While using allometric scaling to predict clearance in humans has often been found to be ineffective, it is one of few methods available to estimate starting dose in phase I clinical trials, widely used and accepted by the Food and Drug Administration.\textsuperscript{42}

However, CMAs have not been widely evaluated in such a manner, with the first study to evaluate the application of allometric scaling for PEGylated liposomal and CMA anticancer drugs being reported in 2011.\textsuperscript{41} Three preclinical species (mice, rats, and dogs) were evaluated using published S-CKD602, Doxil, and SPI-077 data compared against various physiological factors to determine the best variables and animal models to use in order to predict PK disposition in humans.\textsuperscript{41} Using standard allometric scaling, clearance of all three PEGylated liposomal agents correlated with body weight as well as MPS-associated variables, including: liver weight, spleen weight, spleen blood flow, liver blood flow, and monocyte count. The strongest correlation coefficients observed when scaled by total monocyte count ($R^2 = 0.954, 0.989,$ and 0.933, for S-CKD602, Doxil, and SPI-077, respectively).\textsuperscript{41} This correlation slightly improved when accounting for maximum life-span potential.

However, when concentration versus time data were scaled using standard scaling equations to predict drug clearance in humans using body weight, results did not scale well, resulting in a 390, 696, and 262\% difference between predicted and actual clearances for S-CKD602, Doxil, and SPI-077, respectively.\textsuperscript{41} The correlation was shown to improve when using MPS-associated variables as opposed to body weight, further demonstrating the most accurate prediction of PLD clearance using monocyte count, with –20.4, 186, and –78.2\% difference from observed clearances, respectively.\textsuperscript{41} However, this study demonstrated that PEGylated liposomal formulations did not scale well using conventional allometric scaling methods, other nanoparticle agents, such as gold-TNF nanoparticles, do scale well using these methods, suggesting that scaling methods should differ depending on formulation characteristics (e.g., size, charge) and requires further investigation.

The lack of proper scaling can further be compounded when attempting to determine CMA agent starting doses in phase I clinical trials. While the optimal species remains unclear, the maximum recommended starting dose (MRSD) is determined based on the no observed adverse effect levels (NOAELs) in the tested animal species, including the most sensitive species.\textsuperscript{44} A recent review evaluated the design, progression, and outcomes of phase I studies of CMAs compared with SM anticancer agents in patients with advanced solid tumors, including: the basis for starting dose, number of dose escalations, number of patients enrolled, and the ratio of MTD to starting dose.\textsuperscript{44} Of greatest impact, the average ratio of MTD to starting dose in these studies were significantly greater and more variable for CMAs compared to SMs (13.9 ± 10.8 versus 2.1 ± 1.1, \(p = 0.005\)).\textsuperscript{44} The number of dosing levels to reach MTD was also significantly greater for CMAs (7.3 ± 2.9) compared to their SM (4.1 ± 1.5) counterparts (\(p = 0.008\)).\textsuperscript{44} With studies utilizing the conventional 3 + 3 modified Fibonacci design, this could lead to greater than a $400,000 difference (nearly 2-fold difference) in estimated total patient cost associated with a phase I clinical trial for a SM drug versus a CMA.\textsuperscript{44} These results point out current inefficient model selection in preclinical studies that can affect phase I studies of CMAs relative to their SM counterparts.

While these studies were ultimately unable to determine an ideal preclinical model, of the model species tested, dogs were consistently an outlier, demonstrating increased clearance and sensitivity of PEGylated agents compared to other species.\textsuperscript{41,44} These studies also provided preliminary evidence that factors associated with the MPS, such as monocyte count, may improve the prediction of drug clearance within humans.\textsuperscript{41} However, there may be considerable differences across species in terms of MPS activity or function, requiring further research to identify suitable models. While the MPS has not been comprehensively evaluated in animal models (e.g., nonhuman primates), it is known that dogs exhibit marked differences in red blood cells compared to other species.\textsuperscript{41} As clinical phase I studies use data from the
most sensitive preclinical species to determine the starting dose in a phase I clinical trial, dogs may not be an appropriate model for toxicology and PK studies of liposomal and nanoparticle agents. Furthermore, the results observed in patient-derived xenografts may differ depending on the mouse model utilized (e.g., nude versus SCID versus NSG mice), though this has not yet been evaluated.

**CONCLUSIONS**

Studies have begun to emphasize the importance of preclinical animal models in understanding and evaluating the interaction between CMAs and the immune system, including the MPS, which ultimately influences CMA PK (clearance and distribution) and PD (efficacy and toxicity). It is fundamental to study representative preclinical tumor models that

**FIGURE 5** Bi-directional interaction between nanoparticles and MPS. There is a growing body of evidence to indicate that factors such as age, body composition, gender, cellular function, and concomitant medications contribute to the variability in the PK/PD of CMAs in patients. All these factors operate at the level of the MPS and have the ability to up- or down-regulate its function, thereby altering CMA clearance and AUC/exposure. This ultimately leads to either reduced or greater efficacy and/or toxicity. The predicament of this clinical manifestation lies in the fact that the target response must balance efficacy and toxicity, and this ideal CMA exposure needs to be elucidated through further research and determination of proper preclinical model(s).
recapitulate patients with solid tumors (xenografts versus GEMM) and other malignancies and evaluate the interplay between CMAs and the immune system, including the MPS, chemokines, hormones, and other immune modulators (Figure 5(a)). Furthermore, the growth in development and characterization of biomimetic models could offer clinical insight in more realistic preclinical tissue environments compared to conventional tissue culture. It will be essential to take into account the tumor types and the tumor microenvironment, including phenotyping of the MPS and chemokine network, when evaluating CMAs for cancer therapy and translating into humans from preclinical models. This review further demonstrates that a general relationship exists between MPS-associated variables and the clearance of CMA anticancer agents, but standard allometric scaling methods did not accurately predict drug clearance in humans. However, the focus of this article is on CMA formulations that are currently available on the market and/or within clinical trials as anticancer treatments as the goal of this review was to highlight translational issues of CMAs. As such, other nanoparticles like nanoscale metals or nanoshells have not been addressed and their characteristics may differ from liposomal CMAs.

Most current findings were related to liposome/PEGylated liposomes compared to other CMA formulations due to current market availability, and thus there is a need for further comparison between other CMA subtypes and/or formulations is warranted. Future studies also need to be performed to determine whether similar relationships between chemokines and other immune modulators exists for these other CMA formulations. However, future studies could be complicated by the fact that certain models (e.g., GEMMs) may not yet exist, underlying the importance of beginning to characterize and understand the unique signaling occurring within individual models and its potential effects on PK and PD of CMAs (Figure 5(b)). Further studies are also required to evaluate whether the clearance of CMAs can be scaled across species using other measurements of the MPS or other factors to better predict efficacy and toxicity. In addition, additional studies and reviews on the interplay between nanoparticles and the immune-suppressed tumor microenvironment are warranted.

FURTHER READING

REFERENCES


