Challenging the fundamental conjectures in nanoparticle drug delivery for chemotherapy treatment of solid cancers

Juanjuan Yang, Xiaojin Wang, Bingshun Wang, Kinam Park, Karen Wooley, Shiyi Zhang

School of Biomedical Engineering, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, PR China
Department of Biostatistics, Clinical Research Institute, Shanghai Jiao Tong University School of Medicine, 227 South Chongqing Road, Shanghai 200025, PR China
Weldon School of Biomedical Engineering, and Department of Pharmaceutics, Purdue University, West Lafayette, IN 47907, USA
Departments of Chemistry, Materials Science & Engineering and Chemical Engineering, Texas A&M University, College Station, TX 77843, USA

Abstract

Nanomedicines for cancer treatment have been studied extensively over the last few decades. Yet, only five anticancer nanomedicines have received approvals from the United States Food and Drug Administration (FDA) for treating solid tumors. This drastic mismatch between effort and return calls into question the basic understanding of this field. Various viewpoints on nanomedicines have been presented regarding their potentials and inefficiencies. However, the underlying logics of nanomedicine research and its inadequate translation to the successful use in the clinic have not been thoroughly examined. Tumor-targeted drug delivery was used to understand the shortfalls of the nanomedicine field in general. The concept of tumor-targeted drug delivery by nanomedicine has been based on two conjectures: (i) increased drug delivery to tumors provides better efficacy, and (ii) decreased drug delivery to healthy organs results in fewer side effects. The clinical evidence gathered from the literature indicates that nanomedicines bearing classic chemotherapeutic drugs, such as Dox, cis-Pt, CPT and PTX, have already reached the maximum drug delivery limit to solid tumors in humans. Still, the anticancer efficacy and safety remain unchanged despite the increased tumor accumulation. Thus, it is understandable to see few nanomedicine-based formulations approved by the FDA. The examination of FDA-approved nanomedicine formulations indicates that their approvals were not based on the improved delivery to tumors but mostly on changes in dose-limiting toxicity unique to each drug. This comprehensive analysis of the fundamentals of anticancer nanomedicines is designed to provide an accurate picture of the field's underlying false conjectures, hopefully, thereby accelerating the future clinical translations of many formulations under research.

1. Introduction
2. The two conjectures central to cancer nanomedicine
   2.1. Conjecture 1: Increased drug concentrations in tumors provide better efficacy.
   2.2. Conjecture 2: Decreased drug concentrations in healthy organs result in less general side effects
3. Analysis of the reasons for nanomedicine approval.
   3.1. The development and approval of Doxil®
   3.2. The development and approval of Abraxane®
   3.3. The approval of Onivyde®
4. Future directions

Abbreviations: DOX, doxorubicin; API, active pharmaceutical ingredient; FDA, the U.S. Food and Drug Administration; HIV, human immunodeficiency virus; NP, nanoparticle; NSCLC, non-small cell lung cancer; HPLC, high-performance liquid chromatography; Ref., reference; VAD, vincristine and dexamethasone; LVEF, left ventricular ejection fraction; HFS, hand-foot syndrome.

© 2022 Elsevier B.V. All rights reserved.
1. Introduction

Nanomedicine is defined as the application of nanotechnology to medicine, and it promised a significant impact on the treatment of cancer [1]. Nanomedicine is an umbrella term that encompasses a wide variety of drug delivery systems. One of the most defining characteristics is that hundreds of different formulations are treated similarly, and the success of one formulation is hailed as the success of the whole nanomedicine field while ignoring the failure of many others. The progress made by nanomedicine researchers in the last two decades is breath-taking in terms of the number of publications [2]. According to Web of Science, the number of publications with the term "((nanoparticle or nanomedicine) and (cancer or tumor)) and delivery" during the period of 2012–2022 is 42,825 (search performed on January 5, 2022, Figure S1a). Such extensive publications demonstrate the great enthusiasm of researchers and nanomedicine’s high potential to change the current standard of care in cancer treatment [3,4]. The main reason for this enthusiasm is based on the small animal experiments showing that nanomedicine was better in drug accumulation in tumors, resulting in greater shrinkages in tumor sizes and prolonging animals' survival times than the solution formulation controls [5,6]. It was widely believed that this potential seen in mice could be successfully duplicated in human clinical trials [7]. Contrary to such high expectations and significant investment [8,9], only five nanomedicines have been approved for solid tumor treatment by the FDA [10]. There are another three drugs approved by regulatory agents outside the United States [11,12], Table 1 [13–35] lists the clinically approved nanomedicines. This stunning mismatch of expectation and reality has recently led to extensive debate. Interestingly, four of the five FDA-approved nanomedicines are based on liposomes, which are several decades old. Albumin nanoparticles are nothing more than proteins with small molecules bound in the form of paclitaxel-albumin complexes, i.e., Abraxane. For clinical applications, the simpler formulations are better than complex ones that may be better suited for publication.

In May 2019, the U.S. National Cancer Institute (NCI) announced that it would stop its decade-long program for Centers of Cancer Nanotechnology Excellence (CCNEs), which is considered the beginning of the end of the over exuberance for nanomedicine [8,9]. Recently, dozens of high-profile reviews and comments in this field have discussed the translational problem in the development of cancer nanomedicines [36–38]. For example, only 0.70% of the injected dose (ID) of intravenously administered nanoparticles typically accumulates in tumors, indicating nanomedicine's low delivery efficiency [39]. Another study used physiologically-based pharmacokinetic (PBPK) models to analyze 376 data sets covering studies of nanomedicines published from 2005 to 2018 and found that the median delivery efficiency was 0.76% ID [40]. Although the delivery efficiencies appear to be higher than those in previous findings, they are still relatively low and represent a critical barrier in nanomedicines' clinical translation. It is practically impossible to call this tumor-targeted drug delivery. Other previously published perspectives discussed the questionable enhanced permeability and retention (EPR) effect and heterogeneity of the EPR effect in humans [31,41–43]. A more recent paper discussed three design criteria based on the EPR effect of anticancer nanomedicine and their relations with clinical efficacy and safety [44]. The other perspective published in 2019 claimed that nanomedicine has lost its

<table>
<thead>
<tr>
<th>Name</th>
<th>Nano platform</th>
<th>API</th>
<th>Indications</th>
<th>Year</th>
<th>Approval status</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abraxane®</td>
<td>Albumin NP</td>
<td>Paclitaxel</td>
<td>Breast Cancer [22–23]</td>
<td>2005</td>
<td>Approved by FDA</td>
<td>Celgene</td>
</tr>
<tr>
<td>Mepact®</td>
<td>Liposome</td>
<td>Cytarabine</td>
<td>Multiple Myeloma [16–17]</td>
<td>2005</td>
<td>Approved by FDA</td>
<td>Biopharm</td>
</tr>
<tr>
<td>Abraxane®</td>
<td>Albumin NP</td>
<td>Paclitaxel</td>
<td>Breast Cancer [29]</td>
<td>2007</td>
<td>Approved in Korea</td>
<td>Bioprint</td>
</tr>
<tr>
<td>Mepact®</td>
<td>Liposome</td>
<td>Muramyl Tripeptide Phosphatidyl-Ethanolamine Iminotecan</td>
<td>2000</td>
<td>Approved in Europe</td>
<td>Bioprint</td>
<td></td>
</tr>
<tr>
<td>Onivyde®</td>
<td>Pegylated Liposome</td>
<td>Paclitaxel</td>
<td>Post-Gemcitabine Metastatic Pancreatic Cancer [35]</td>
<td>2015</td>
<td>Approved by FDA</td>
<td>Merrimack</td>
</tr>
</tbody>
</table>
way during the translation process because clinical multifunctional-ity is distinct from traditional nanoscale multifunctionality [45]. Moreover, perspectives have questioned the gap between preclinical studies and clinical trials [46,47], the random acts of cancer nanomedicine [48], and the lack of quantitative data in the field [49]. Regardless of the points chosen, those review articles have opined that the current drug delivery techniques have failed their promise of clinical utility and that new ways of thinking should be adopted [50]. However, none of these articles discussed the fundamental conjectures that nanomedicine relies on and their relationship to their successful approvals. More importantly, a vast majority of those review articles studied data on reported animal results instead of focusing on nanomedicines’ valuable clinical outcomes.

It is generally accepted that anticancer nanomedicines’ ultimate goal is to prolong patients’ overall survival (OS) while maintaining a relatively good quality of life instead of slightly increasing the survival time of mice. Thus, clinical trials are the only criterion to know whether the drug works in humans. Therefore, this article examines the fundamental logics or conjectures of anticancer nanomedicine by studying clinical data from human trials that nanomedicine researchers often overlook. The analysis from a clinical perspective provides a rigorous investigation of the fundamentals of anticancer nanomedicine. The clinical outcomes of well-known nanomedicines, such as Doxil®, Abraxane®, and Onivyde®, were also analyzed. The analysis indicates that their successful regulatory approvals were not based on the commonly believed conjectures. The lessons learned from the past and approved nanomedicines also provide research strategies that may guide and accelerate the clinical translation of anticancer nanomedicine.

2. The two conjectures central to cancer nanomedicine

A conjecture is an auxiliary hypothesis that can be tested and assumed to be true if the test works as planned [51]. It is common for scientists, or any individual, to use conjectures to plan a test or start an experiment in rapidly developing fields when resources and information are limited [52]. The research field of nanomedicine has developed rapidly during the last two decades. It is an interdisciplinary subject related to physics, biology, chemistry, and medicine, developing in parallel with these subjects. The frontiers of nanomedicine are the frontiers of several subjects, which need conjectures to carry out their research.

Information on whether or which conjectures are used in nanomedicine studies can be found in the results or conclusions of individual studies. The most widely read and cited publications in the field of anticancer nanomedicine during the last decade were analyzed to best reflect the influence of the highly-cited papers on the research trend of the whole field. The Web of Science database was used to search the terms "((nanoparticle or nanomedicine) and (cancer or tumor)) and delivery" from 2012 to 2022. The 1000 most cited articles were analyzed, and then 325 reviews were excluded. Figure S1a, Methods can be found in Supporting Information Methods Section). The 146 relevant studies using nanoparticles to deliver chemotherapeutic drugs into tumors were analyzed for their research schemes and methods. For each article, the abstract and introduction were reviewed to find a sentence indicating that targeted delivery is an effective way to enhance efficacy and improve safety. Nineteen studies had in their abstract or introduction claiming that “targeted delivery is an effective way to enhance efficacy and safety”. Eleven papers had a sentence claiming that “targeted delivery is an effective way to enhance efficacy”. Seven papers had a sentence claiming that “targeted delivery is an effective way to reduce the general side effects caused by chemotherapy” (Figure S1b). The remaining 109 studies that did not state the relationship between targeted delivery and efficacy or safety directly in their papers were further reviewed for the methods and results. Of the 109 studies, 97 papers studied cancer cellular uptake or biodistribution through confocal microscopy or small animal fluorescence imaging to evaluate delivery efficiency. At the same time, they studied cell viability, tumor volume, or mouse survival time to evaluate therapeutic efficacy and body weight to assess safety profiles. These research schemes discretely provide a link between increased drug accumulation in tumors and enhanced efficacy or safety. Overall, these analyses indicate that better efficacy and safety were related to the increased drug accumu-
mulation in tumors by the investigators. Thus, the survey of the nanomedicine literature has identified two conjectures in anticancer nanomedicine: (i) the increased drug concentrations in tumors provide better efficacy, and (ii) decreased drug concentrations in healthy organs result in less general side effects. (Fig. 1). These two conjectures appear reasonable and self-evident that most nanomedicine developments were focused on increasing drug accumulation in tumors while avoiding healthy tissues.

The developmental goal of nanomedicine is to increase the efficacy and safety of a drug in patients, as the FDA requires for all new drugs [53]. Nanomedicines’ best advantage is their ability to prolong the systemic circulation time in plasma and deliver more drugs to tumors than the solution controls [54]. Nanomedicine delivery systems use various methods to increase drug accumulation in tumors. Different materials, shapes, and sizes are used to realize passive targeting to increase drug concentrations in tumors. Targeting moieties are added to the delivery system to achieve active targeting to avoid the nonspecific accumulation of drug molecules in healthy tissues. The terms “passive targeting” and “active targeting” are misnomers, as targeting is simply a result of delivery by blood circulation and less removal of the nanomedicines than the solution counterparts. “Active targeting” occurs when the ligands on the nanomedicine surface interact with the target cells’ receptors. There are further complications a common oversight in thinking that targeted cancer therapy is somehow like shooting an arrow toward a target, without recognition that transport of nanomedicines is reliant on normal pathways with only an ability to retain or capture the materials is possible, unless there is an ability to “hijack” natural transport mechanisms. When only a few percentages of the administered drug are delivered to the target tumor, the term “targeting” is inappropriate and inaccurate. Researchers are trying to increase drug accumulation in tumors to increase efficacy and reduce general side effects, which is considered the fundamental logic of anticancer nanomedicine. As nanomedicine has failed its promise clinically, many debates have emerged. Recent debates and discussions of anticancer nanomedicine are mostly focused on drug delivery efficiency. The two conjectures are considered the basis of nanomedicine theory and should be carefully examined and tested when more evidence becomes available.

After the two conjectures of anticancer nanomedicine were identified, their falsifiabilities were tested. It was necessary to ensure that they are already well confirmed to provide background knowledge for future research. In the past few decades, the information has been limited with only a small number of clinical trials. Over time, more clinical trials have been registered, and meaningful results have been obtained. Currently, there is enough information to reflect on the status of the conjectures of cancer nanomedicine.

2.1. Conjecture 1: Increased drug concentrations in tumors provide better efficacy

Tumor targeting systems aim to provide better efficacy in humans by achieving a higher accumulation of drugs at the tumor site and, it is then assumed, accumulation of drugs in the tumor and even cancer cells can treat the carcinoma locally. A substantial number of animal experiments have demonstrated the higher accumulation of drugs than controls in various xenograft models. These animal models are not as reliable as clinical studies in representing human conditions. Thus, a better illustration of the clinical conditions requires clinical examples exhibiting nanoparticle accumulation in solid tumors in the human body. This type of study in clinical trials, however, is rare. Unlike preclinical studies, very few clinical trials have taken biopsies or positron emission tomography/ computed tomography images from patients to measure the drug accumulation. Such information was obtained from nanomedicine companies’ websites, the clinical trials of the approved nanomedicines, the registered clinical trials of nanomedicines (https://clinicaltrials.gov), and the literature (Methods can be found in Supporting Information). The resulting trials are listed in Table 2 [55–57].

Overall, nanomedicine demonstrates increased drug concentrations in tumors compared with conventional chemotherapies in humans [58,59]. For instance, Doxil®, PEGylated phospholipid vesicles encapsulating doxorubicin, enhanced the drug deposition in tumors [60]. In phase I studies, the drug levels at tumor sites were 4–16 times greater than those of free doxorubicin in lung cancer, ovarian cancer, and breast cancer patients, or 5.2–11.4 times higher in patients with Kaposi’s sarcoma (KS) [14]. HPMA copolymer-bound drug is another critical examples to demonstrate increased tumor drug concentration. They have made remarkable journey from lab to clinic, and demonstrated great potential over free drugs, especially for its accumulation in tumors [61,62]. For example, PK2 (FCE28069) comprises doxorubicin bound to an N-(2-hydroxypropyl)methacrylamide copolymer (HPMA) via a lysosomally cleavable peptidyl linker with galactosamine to target the hepatocyte asialoglycoprotein receptor [63]. In a Phase I clinical evaluation, PK2 demonstrated selective hepatic delivery in patients with liver cancer and a 12–50 times increase in theoretical tumor drug concentrations compared to free doxorubicin [56,57]. Together with the results of numerous preclinical studies and limited clinical studies, nanomedicine’s EPR effect relative to that of free drugs seems to be a universal physiological phenomenon in several types of tumors.

It was important to know whether an improved tumor accumulation of nanomedicine resulted in increased efficacy in late-stage clinical studies. Unfortunately, although PK2 exhibits liver targeting ability, only 3 of 31 patients with liver tumors treated with

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Evidence of increased tumor accumulation of nanomedicine compared with conventional chemotherapeutics.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>API</strong></td>
<td><strong>Treatment Arms</strong></td>
</tr>
<tr>
<td>DOX</td>
<td>1. DOX 25 mg/m2 and Doxil® 25 mg/m2 (one patient) 2. DOX 50 mg/m2 and Doxil® 50 mg/m2 (two patients)</td>
</tr>
<tr>
<td>DOX</td>
<td>1. Doxil® (10, 20, 40 mg/m2) 2. DOX (10, 20, 40 mg/m2)</td>
</tr>
<tr>
<td>DOX</td>
<td>1. PK2 20 to 160 mg/m2 (doxorubicin equivalents)</td>
</tr>
</tbody>
</table>
Fig. 2. Meta-analyses of objective response rates (ORRs) comparing nanomedicine with conventional chemotherapy. The ORR difference is first analyzed by API and then subgroup analysis by nano-platform within each API class. Odds ratios and 95% confidence intervals (95% CIs) for clusters of studies comparing ORRs in patients treated with nanomedicine versus those treated with conventional chemotherapy. The solid squares are the point estimates (odds ratios) for the analyses, and the bars on either side of the squares are 95% CIs. The one-sided irinotecan is due to the small number of patients in this subgroup. The vertical dashed line indicating an odds ratio of 1.0 is the line at which treatment was found to have no effect. A risk ratio of more than 1.0 indicates a higher chance of response with nanomedicine than with chemotherapy. The meta-analyses showed that despite there is a significant increase of ORR for Abraxane (P less than 0.0001), nanomedicine as a whole did not significantly improve the ORR compared to chemotherapy (P = 0.08) [Odds ratio (RR) 1.13, 95% CI 1.05–1.21, \( P = 0.08 \)].

Fig. 3. Meta-analyses of the overall survival (OS) of patients in clinical trials comparing nanomedicine with conventional chemotherapy. The OS difference is first analyzed by API and then subgroup analysis by nano-platform within each API class. Hazard ratios and 95% confidence intervals for clusters of studies comparing overall survival in patients treated with nanomedicine versus those treated with conventional chemotherapy. The solid squares are the point estimates (hazard ratios) for the analyses, and the bars on either side of the squares are 95% confidence intervals. The vertical dashed line indicating an odds ratio of 1.0 is the line at which treatment was found to have no effect. A risk ratio of less than 1.0 indicates a lower risk of death with nanomedicine than with chemotherapy. The meta-analyses showed that patients’ overall survival (OS) in both groups was almost the same (hazard ratio = 1.01, 95% CI = (0.94, 1.08), \( P = 0.88 \)).
PK2 exhibited a partial response [56]. The response rate of PK2 (3/31) was not better than that of free doxorubicin [64] or PK1 (6/62), [65] which is a drug-copolymer conjugate without targeting moieties. This example of PK2 represents the failure of a targeting strategy for nanomedicine. After all, targeting can occur after the formulation is delivered to the target site by blood circulation. Thus, the concept of “targeting” is quite different from delivering more drugs into cells where the drug exerts its efficacy. Although Doxil® showed increased tumor drug concentrations in phase I trials, neither Doxil® nor Myocet® showed improved efficacy than free doxorubicin, or even worse (Fig. 2). Prior meta-analyses have already demonstrated lack of clinical efficacy for anticancer nanomedicine [66–68]. We conduct a more comprehensive meta-analysis to evaluate the efficacy advantage in clinical trials of nanomedicine with conventional chemotherapy head-to-head (Figure S2). Methods can be found in SI [23,24,69–89]. Objective response rates (ORRs), progression-free survival (PFS), and overall survival (OS) were analyzed to evaluate the efficacy of nanomedicine and chemotherapy. Subgroup analysis of the 7 doxorubicin trials (including 4 Doxil® trials and 3 Myocet® trials) showed no difference between nanof ormulations and doxorubicin in ORR (odds ratio (OR), 0.93; 95 % confidence interval (CI), 0.85–1.02; p = 0.13) (Fig. 2), PFS (hazard ratio (HR), 1.04; 95 % CI, 0.92–1.18; p = 0.52) (Figure S3) or OS (HR, 1.00; 95 % CI, 0.86–1.16; p = 1.00) (Fig. 3).

The above analysis demonstrates increased tumor drug concentration but no difference in efficacy for Doxil®. Although nanomedicine accumulation in solid tumors was seen for only PK2 and Doxil® because of the very few available clinical trials, a meta-analysis of the efficacy of nanomedicines as a whole was performed. The ORR, PFS, and OS of all nanomedicines in clinical trials were analyzed, comparing the efficacy endpoint of nanomedicine to conventional chemotherapy (Figure S2). The results show no significant difference between nanomedicines and conventional formulations in ORR (OR, 1.12; 95 % CI, 0.99–1.27; p = 0.08) (Fig. 2), PFS (HR, 0.97; 95 % CI, 0.91–1.03; p = 0.36) (Figure S3), or OS (HR, 1.01; 95 % CI, 0.94–1.08; p = 0.99) (Fig. 3). Most cancer nanomedicines failed to achieve the goal of improving efficacy, even with improved drug concentrations in tumors. Overall, these data suggest that drug accumulation at the tumor sites can be improved in humans, but it was not translated into improved therapeutic efficacy.

The above analyses show that an increased concentration of the drug in tumors does not mean an improvement in clinical efficacy, especially in patients’ OS. The effect of drugs on tumor cells probably only relates to one clinical endpoint, that is, the ORR. The ORR is reflected by tumor size shrinkage during a specific period. Therefore, if the drug concentration in a tumor increases, it may affect more tumor cells and result in more significant tumor size shrinkage and better ORR. Nevertheless, the clinical endpoints usually include ORR, PFS, and OS, independent of each other in most cases. There is no one-to-one association among ORR, PFS, and OS [90]. It has been shown in clinical trials that tumor response rates (e.g., size shrinkage) are not accurate predictors of survival benefit [91–93] which means that tumor size shrinkage does not guarantee patient survival clinically. Nevertheless, in animal experiments, the reduction in tumor size and the increase in animal survival are often more closely related. This is the most critical difference between animal experiments and clinical experiments. In general, there is no absolute positive correlation between ORR and OS in clinical trials, and only OS is the gold standard in clinical trials of anticancer drugs. Therefore, it is practically impossible to draw the general conclusion that higher drug concentrations in a tumor provide better efficacy.

2.2. Conjecture 2: Decreased drug concentrations in healthy organs result in less general side effects

The promises of nanomedicine are to enhance the treatment efficacy for tumors and improve the safety profile by reducing toxicity to normal tissues. From the regulatory perspective, safety is just as important as efficacy. In this part, instead of examining the drug accumulation of nanomedicines compared with chemotherapeutics, the increased drug accumulation in tumors was examined compared with that in healthy tissues and its relationship with the clinical outcome in terms of the safety profile (Table 3 [93–99]).

Only seven trials, mainly trials of Doxil®, have demonstrated preferential accumulation of nanomedicines in tumors rather than surrounding healthy tissues. The first report of PEGylated liposome

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Typical examples of nanomedicines with greater accumulation in the tumor than in the surrounding healthy tissue.</th>
</tr>
</thead>
<tbody>
<tr>
<td>API</td>
<td>Treatment arms</td>
</tr>
<tr>
<td>DOX</td>
<td>1. Patient 1 Doxil® total dose 670 mg/m² (plus 100 mg/ m² of free DOX) 2. Patient 2 Doxil® total cumulative dose 885 mg/m²</td>
</tr>
<tr>
<td>DOX</td>
<td>1. Doxil® 25 mg/m² on day 1 and day 21 and radiotherapy with a total dose of 45 Gy</td>
</tr>
<tr>
<td>DOX</td>
<td>1. Doxil® 25 mg/m² every-two weeks and radiotherapy with a total dose of 70 Gy</td>
</tr>
<tr>
<td>DOX</td>
<td>1. Doxil® 40 mg/m² and cisplatin 60 mg/m² every 21 days</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>1. Lipoplatin: a single dose of 100 mg/m²</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>1. Paclitaxel oleate associated with the nanoeulsion: 60 mg total lipid mass and 12 mg of paclitaxel oleate, at a volume of 2 mL</td>
</tr>
<tr>
<td>Camptothecin</td>
<td>1. CRLX101 15 mg/m²</td>
</tr>
</tbody>
</table>
(Doxil®) accumulation in tumor tissues other than healthy tissues was in 1999 by Zvi Symon [93]. Bone tumor fragments were obtained during the surgery of two patients, and the concentration of liposomal doxorubicin in the bone tumor fragments was 10-fold larger than that in the tumor-free muscle. In a separate study, Koukourakis et al. investigated the relative accumulation of technetium-99 m diethyleneaminopentaetic acid (99mTc-DTPA) radiolabeled with Doxil® in 10 patients with metastatic brain tumors and five patients with brain glioblastoma undergoing radiotherapy [94]. In glioblastomas, radiolabeled Doxil® accumulation was 13–19 times higher than that in the normal brain. For metastatic lesions, the drug concentration was 7–13 times higher than that in the normal brain [94]. Another study conducted by the same research group investigated the concurrent use of radiotherapy with Doxil® in a cohort of 7 patients with locally advanced or recurrent sarcoma. Scintigraphic imaging with Caelyx®–99mTc-DTPA showed a 2.89 ± 0.9-time higher intratumoral drug accumulation than that in the surrounding healthy tissue [95]. After that, in a phase II trial evaluating Doxil® accumulation in tumors, the accumulation of Doxil® in tumors vs soft tissues was 1.63–4.7 times higher, [96] indicating increased levels of Doxil® uptake in tumors compared with surrounding soft tissues. These three studies indicate that Doxil® can achieve accumulation in tumors rather than in surrounding tissues.

A similar phenomenon was observed in patients treated with lipoplatin, a liposomal formulation of cisplatin coated with poly(ethylene glycol) [100,101]. In 2005, Teni et al. reported a study demonstrating the preferential tumor uptake of lipoplatin in 4 colon cancer patients [97]. After systemic lipoplatin infusion, platinum levels were measured directly from specimens of the excised tumors and normal tissues. The results showed that the total platinum levels were on average 10–50 times higher in malignant tissue than in adjacent normal tissue specimens. In particular, metastatic colon tumors exhibit the most effective drug targeting, with an accumulation up to 200-fold higher in metastatic colon tumors than in normal colon tissues. These results demonstrate that lipoplatin can preferentially accumulate in malignant tissues of both primary and metastatic tumors after systemic intravenous infusion in patients.

Nanoemulsion paclitaxel is another example [98]. Paclitaxel oleate is associated with a cholesterol-rich nanoemulsion. It was previously reported that the particles of a cholesterol-rich nanoemulsion show a more than 5-time greater uptake in breast carcinoma than in normal contralateral mammary tissue in patients [102]. In patients with ovarian carcinomas, the nanoemulsion showed a roughly 8-fold greater concentration than that in the contralateral normal ovaries [103]. In a study evaluating the tumoral uptake of paclitaxel associated with nanoemulsions, paclitaxel oleate was labeled with [3H] and associated with [14C]-cholesterol oleate-nanoemulsion to measure the drug concentration in the tumor tissue. The uptake of both cholesterol oleate and paclitaxel oleate in tumoral tissue was 2.4 times greater than that in normal tissues, suggesting that the nanoemulsion drug preferentially concentrated in the tumor site.

Camptothecin (CPT) is also being delivered as a payload in nanoparticle-based therapeutics [99,104]. CRLX101, an intact polymer-drug nanoparticle, is administered to patients with solid tumors [99]. Overall, CPT fluorescence was detected in all nine post-treatment tumors, while no definitive CPT fluorescent signal was observed in any adjacent nontumor biopsy specimens. These data demonstrate preferential tumor vs normal tissue uptake of CRLX101.

Doxil® lipoplatin, paclitaxel nanoemulsion, and CRLX101 all demonstrate increased tumor drug concentrations compared to the surrounding normal tissue. These studies indicate that nanoformulation cancer therapeutics can effectively accumulate in solid tumors, not only when compared with free drugs but also when compared with surrounding healthy tissues. These studies provide evidence of fewer drugs in healthy organs than in the tumor site for the above four nanomedicines in a few patients with specific cancer types.

These examples indicate the preferential accumulation of nanomedicine in tumor sites rather than in healthy tissues. Testing the conjecture of cancer nanomedicine requires analysis of the safety profile of nanomedicines compared with small compound drugs in the clinic. Another analysis was performed of clinical trials comparing the adverse events of the two arms of nanoformulation drugs and conventional drugs [23,24,69–89]. The above-mentioned randomized controlled clinical trials comparing nanomedicine and conventional chemotherapy were reviewed to determine the incidence of reported adverse events among patients with solid tumors.

Each study provided the number of patients who had side effects reported in the literature and the number of participants in the experimental and the control groups. The information was used to calculate the incidence rate of each side effect. The incidence rate of each side effect in the experimental group was calculated by dividing the number of patients who had side effects by the number of participants in the experimental group. The incidence rate of each side effect in the control group was calculated similarly. If the corresponding side effect was not reported in the article, it was not included in the counting process.

Overall, the rate of adverse events from any cause was 92.57 % in the chemotherapy group and 91.25 % in the nanomedicine group (Fig. 4). The rate of overall grade greater than 3 adverse events was 36.73 % in the chemotherapy group and 46.37 % in the nanomedicine group. The most common adverse event in the two groups was alopecia, and the incidence rate of alopecia was 75.15 % in the chemotherapy group and 65.17 % in the nanomedicine group. However, nanomedicine showed increased risks for patients to develop other skin toxicities such as hand-foot syndrome (HFS) (chemotherapy vs nanomedicine: 10.16 % vs 34.86 %) and rash (chemotherapy vs nanomedicine: 16.07 % vs 24.16 %). There is no evidence to show that overall adverse events are decreased by reducing drug concentrations in normal tissues.

These data indicate that reducing normal tissue accumulation does not necessarily reduce the incidence rate or severity of adverse effects. This observation is contradictory to what is often seen in preclinical studies. In animal experiments, most studies have reported that the use of nanomedicines successfully decreases the drug concentration in normal tissues, and the maximum tolerated dose (MTD) of nanomedicine is increased compared with conventional chemotherapy. This mismatch may be due to the difference in MTD between mice and humans. In preclinical studies, the MTD of a mouse is the dosage that causes the body-weight loss of greater than 15 %, or its activity, appearance, and body condition start to show moderate deviation from normal conditions [105,106]. This mouse MTD reflects severe whole-body systemic toxicities, most likely coming from critical organs such as the brain, heart, liver, spleen, kidney, or lung. On the other hand, the clinical MTD of a patient is the highest dose of a drug that does not cause unacceptable adverse effects [107], which is often restricted to one or two organs. The toxicity that limits the use of higher doses is often called dose-limiting toxicity (DLT). When a nanomedicine is formulated as an approved or clinically tested drug, clinical MTDs should be carefully evaluated in clinical studies instead of examining the overall preclinical MTD.

The observation that reduced normal tissue accumulation is irrelevant to the reduced incidence rate or severity degree of adverse effects in the clinic suggests that nanomedicines only change the DLT of conventional chemotherapy. When the incidence rate or severity degree of DLT decreases, the incidence rate
or severity degree of another side effect may increase to become a new DLT because a higher dose of the drug would be allowed. For example, Doxil® successfully mitigates doxorubicin’s cardiotoxicity, but Doxil® treatment is still limited by HFS incidence [71]. Abraxane successfully reduced the incidence of hypersensitivity during injection, but patients are still bothered with neuropathy when given a higher dose of paclitaxel. Another reason is that nanomedicine-induced drug distribution changes are insufficient to provide meaningful clinical improvements for reducing general side effects. As an example of a free drug, according to a study investigating the tumor deposition of doxorubicin, the doxorubicin concentration at the tumor site was 0.055–0.819 μg/g when injected at a dose of 25 mg/m² [57]. Under the assumption that the average body area is 1.6 m² and the average tumor weight is 100 g, which is estimated from a median tumor size of 5.5 cm [108], the proportion of free doxorubicin in tumors is 0.013–0.2%. Even if the drug concentration is increased 10-fold through delivery by nanomedicine, the proportion of nanomedicine in tumors is less than 2% per injected dose [39,40]. This means that more than 98% of the drug is still distributed in normal tissues. This change in distribution is far from enough to lower the side effects of drugs on normal tissues significantly. Therefore, nanomedicine can only alter DLT rather than reduce overall side effects.

3. Analysis of the reasons for nanomedicine approval

The above analysis demonstrates the fallacy of anticancer nanomedicine’s conjectures, which makes regulatory approval of nanomedicine difficult. Understanding the reasons for the FDA approval of nanomedicines will provide a pathway for developing more FDA-approved nanomedicines. This section investigates the logistics of three typical nanomedicines for their research and development process that led to successful FDA approvals. This analysis of past successes may serve as a useful guide for developing the next-generation nanomedicines.

To date, five anticancer nanomedicines have been approved by the FDA for solid tumors. Four of them are liposomal nanomedicines, and one is albumin-bound paclitaxel. The three representative examples we discuss here are Doxil®, Abraxane®, and Onivyde®. Doxil® is the first approved nanomedicine. Abraxane® is approved as a unique formulation of albumin-bound paclitaxel and is still the best seller of anticancer nanomedicine. Onivyde® is the most recently approved nanomedicine after ten years of no approved products in the field of anticancer nanomedicine. Evaluation of the development history of the three formulations found that their FDA approvals were not based on the conjectures as mentioned earlier. The successful approval of each nanomedicine had a unique reason.

3.1. The development and approval of Doxil®

Doxil®, named Caelyx® outside of the US, was the first FDA-approved nanosized drug carrier formulation, and its approval is a typical example representative of the approval of cancer nanomedicine [109]. The active pharmaceutical ingredient of Doxil® is doxorubicin, which is the most widely studied chemotherapeutic drug in cancer treatment and anticancer nanomedicine [110]. According to the NCI, doxorubicin is a chemotherapy agent widely used to treat 14 different malignancies, including hematological malignancies and solid tumors [111]. However, this general use is most affected by one accumulated adverse event, cardiotoxicity, which is the DLT of doxorubicin [112]. The most common symptoms are cardiomyopathy and congestive heart failure, which can sometimes be fatal [113,114]. Patients often have to give up the treatment due to severe cardiotoxicity when an accumulated dose of doxorubicin reaches 550 mg/m² [115]. The combination of the widespread use and accumulated DLT of doxorubicin makes it...
appealing for liposome companies to develop a better version of doxorubicin to overcome this side effect [109].

To address the accumulating cardiotoxicity of doxorubicin, the ‘first generation’ liposome doxorubicin, also referred to as OLV-DOX, was developed. It is negatively charged, with doxorubicin-loaded by medium-size oligolamellar liposomes (OLVs) (Fig. 5a).

However, the first clinical trial using OLV-DOX did not justify further clinical development [116,117]. There were two limitations of OLV-DOX. First, cardiotoxicity remained, resulting from significant drug leakage from liposomes. Second, OLV-DOX was quickly cleared by the reticuloendothelial system (RES) (or mononuclear phagocyte system, MPS), which may affect its therapeutic efficacy.

An analysis of the shortcomings of OLV-DOX revealed that the next generation of the liposomal doxorubicin product should contain the following features. First, doxorubicin should be actively loaded into the liposome by precipitation or crystallization to

Table 4
Representative clinical trials of Doxil® to demonstrate changes in dose-limiting toxicity. Review in [122]. *: Significant change; ns: not significant.

<table>
<thead>
<tr>
<th>Treatment arms</th>
<th>Patient #</th>
<th>Efficacy</th>
<th>Cardiovascular safety</th>
<th>Hematological and skin toxicities</th>
<th>Indication</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxil® versus DOX</td>
<td>95</td>
<td>ORR 10 % versus 9 %</td>
<td>PFS 65 versus 82 d</td>
<td>OS 320 versus 246 d</td>
<td>Neutropenia 38 % versus 88 %* HFS 51 % versus 0 %* Stomatitis 55 % versus 51 %*</td>
<td>Soft Tissue Sarcoma</td>
</tr>
<tr>
<td>VAD Doxil® versus VAD DOX</td>
<td>259</td>
<td>ORR 61.3 % versus 61.4 %</td>
<td>PFS 24.30 versus 23.93 m</td>
<td>OS was similar</td>
<td>Neutropenia 15 % versus 20 % ns HFS 13 % versus 2 %* Mucositis 15 % versus 7 % ns</td>
<td>Multiple Myeloma Dimopoulos et al. [70]</td>
</tr>
<tr>
<td>Doxil® 50 mg/m² /4 weeks versus DOX 60 mg/m² /3 weeks</td>
<td>509</td>
<td>ORR 33 % versus 38 %</td>
<td>PFS 6.9 versus 7.8 m</td>
<td>OS 21 versus 22 m</td>
<td>Neutropenia 4 % versus 10 %* HFS 48 % versus 2 %* Mucositis 3 % versus 15 %*</td>
<td>Metastatic Breast Cancer O’Brien et al. [71]</td>
</tr>
<tr>
<td>VAD Doxil® versus VAD DOX</td>
<td>192</td>
<td>ORR 44 % versus 41 %</td>
<td>PFS and OS were similar</td>
<td>–2.3 % versus –4.5 % ns (As change in LVEF)</td>
<td>Neutropenia 18 % versus 30 %* HFS 25 % versus 1 %* Stomatitis 29 % versus 21 %*</td>
<td>Multiple Myeloma Rifkin et al. [72]</td>
</tr>
<tr>
<td>(Doxil® 30 mg/m² + Docetaxel 60 mg/m²)/3 weeks versus Docetaxel 75 mg/m²</td>
<td>751</td>
<td>ORR 35 % versus 26 %</td>
<td>PFS 9.8 versus 7.0 m</td>
<td>OS was similar</td>
<td>Neutropenia 66 % versus 65 % ns HFS 61 % versus 1 %* Mucositis/stomatitis 52 % versus 14 %*</td>
<td>Metastatic Breast Cancer Sparano et al. [121]</td>
</tr>
</tbody>
</table>

Fig. 5. Structure and clinical trials of Doxil®. (a) A graphical representation of Doxil® PEGylated nano (less than100 nm) unilamellar liposome. (b) Cryo-TEM of DOXG, which is doxorubicin glucuronate remotely loaded on pegylated nanoliposomes. DOXG does not present intraliposomal drug crystals, while Doxil® shows intraliposomal precipitation to avoid doxorubicin leakage from Doxil®. For more details on DOXG, see [109]. (d) Doxorubicin levels in patients’ tumor biopsies, comparing free doxorubicin and Doxil®. (e) Overall survival after treatment with vincristine, doxorubicin, and dexamethasone (VAD bolus) or VAD with liposomal doxorubicin (VAD Doxil®). (f) Overall survival of patients with breast cancer comparing Doxil® with doxorubicin. Reproduced from reference. [70,71,109].
avoid cardiotoxicity caused by drug leakage from circulating liposomes. Second, the liposome should be composed of high-
T<sub>ms</sub> liposomes to demonstrate a highly prolonged plasma circulation time to enable the slow release of doxorubicin. The amount of the drug released from the nanoparticle at the targeted site is another important factor that could affect the effective treating dose. If the drug is delivered to the target site but not released, there is no way for the drug to have any influence on the therapeutic efficacy. Therefore, the oligomannose liposome of OLV-DOX with low-
T<sub>ms</sub> (fluid) phospholipids is changed into a PEGylated liposome of Doxil<sup>®</sup> with high-
T<sub>ms</sub> phospholipids to increase the circulation time and remote loading of doxorubicin. The size of the original liposome was reduced to the nanoscale to enable liver leakage and avoid RES (or MPS). Most importantly, doxorubicin is loaded into a more compact double-layer liposome by crystallization (Fig. 5d) to avoid drug leakage during circulation, resulting in reduced cardiotoxicity.

The first clinical trial of PEGylated liposome doxorubicin (Doxil<sup>®</sup>) showed an increased circulating time of doxorubicin and avoidance of the RES of liposomes in humans after intravenous administration, which remedies the defects of the first-generation OLV-DOX<sup>®</sup> (Fig. 5d). Since then, a large number of clinical studies have been conducted in various cancers. These studies demonstrated dramatically decreased cardiotoxicity from doxorubicin and a few other toxicities. Despite this, the efficacy remained the same (Fig. 5e and 5f), and the overall mucocutaneous toxicity was increased compared with doxorubicin (Table 4). HFS is mainly recognized as a new DLT that is not well known by academia but significantly bothers patients and brings much trouble to their lives [118,119]. From this clinical evidence, not just the preclinical results, we can conclude that liposome technology can enhance tumor accumulation and prolong circulation time in plasma. Most importantly, liposomes avoid heart uptake when delivering doxorubicin, which has severe cardiotoxicity. At the same time, nanometer-sized liposomes may deposit in the mucosa, leading to substantial mucocutaneous toxicity [120–122].

In summary, the development of Doxil<sup>®</sup> involved a process of targeting to overcome the DLT of doxorubicin. Researchers focused on the dose-limiting cardiotoxicity of doxorubicin and developed first-generation OLV-DOX, the use of which was limited by cardiotoxicity mainly due to the fast release of doxorubicin. The structure of the liposome layer was optimized in Doxil<sup>®</sup> to prolong its circulation time and slow its drug release into the blood. For patients, Doxil<sup>®</sup> changed the DLT from a lethal side effect, cardiotoxicity, into another painful and troublesome adverse effect. HFS. This change in DLT is the critical factor in the successful approval of Doxil<sup>®</sup>. For Doxil<sup>®</sup>, the right drug to be carried was chosen, and doxorubicin is a critical factor in the success of Doxil<sup>®</sup>. The liposome company also tried to carry other drugs using liposomes, such as paclitaxel or cisplatin, but few of them became drug products on the market. This choice of API was significant for the success of Doxil<sup>®</sup>.

3.2. The development and approval of Abraxane®

Abraxane<sup>®</sup>, developed by Abraxis Bioscience, was approved by the FDA and the European Medicines Agency (EMA) in 2005 and 2008 [123,124]. It utilizes serum protein albumin to solubilize and carry paclitaxel in circulation [125]. Paclitaxel was discovered in the early 1960s and had a significant impact on clinical cancer therapies. However, formulating paclitaxel for systemic administration proved to be a tough challenge because of its poor water solubility (Fig. 6a). The first-generation drug formulation for paclitaxel is Taxol<sup>®</sup>, which was the best-selling cancer drug ever manufactured by 2000 [126,127]. Cremophor EL was used to formulate the drug to increase its solubility. However, the use of Cremophor EL is responsible for many of the side effects observed with solvent-based paclitaxel (sb-paclitaxel or Taxol<sup>®</sup>), including severe acute hypersensitivity reactions during the infusion and hypersensitivity-induced neutropenia [128].

Abraxane<sup>®</sup> (nab-paclitaxel) was developed to avoid the toxicities typically associated with Cremophor EL in sb-paclitaxel (Taxol<sup>®</sup>) [129]. It is formulated with human serum albumin to benefit from the absence of Cremophor EL, thereby facilitating dissolution and reducing the risk of acute hypersensitivity reactions during infusion by Cremophor EL [23,130]. This reduction in toxicity allows Abraxane<sup>®</sup> to be more aggressively dosed than Taxol<sup>®</sup>. The recommended paclitaxel dosing for Taxol<sup>®</sup> is 175 mg/m<sup>2</sup>, compared to 260 mg/m<sup>2</sup> for Abraxane<sup>®</sup>, which is an approximately 50% increase in the paclitaxel dose [131]. Due to this dose increase, Abraxane<sup>®</sup> does seem to have efficacy gains over Taxol<sup>®</sup> in comparing the ORR and OS in several clinical studies (Fig. 6b) [23]. The avoidance of Cremophor EL also allows Abraxane<sup>®</sup> to be administered in 30 mins vs Taxol<sup>®</sup> in 3 h and without premedication to mitigate acute side effects. It is the increased MTD that guaranteed the approval of Abraxane<sup>®</sup> by the FDA.

The albumin platform is a versatile protein carrier platform for increasing drug solubility and improving the pharmacokinetic profile of Cremophor EL formulation-based drugs. It is the most abundant plasma protein (35–50 g/L human serum) with a molecular weight of 66.5 kDa [132]. Similar to liposome technology, albumin-bound technology is used for other drug delivery applications. For example, a methotrexate–albumin conjugate [133] and an albumin-binding prodrug of doxorubicin have been evaluated clinically [134], but neither increased efficacy nor improved safety profile have been obtained, and no future development is launched.

**Fig. 6.** (a) Structural formula of paclitaxel and (b) Representative results from clinical trials of Abraxane<sup>®</sup>. (p = 0.024) Reproduced from reference [23].
for these two drugs. The significance of albumin-bound technology is that it can increase the drug loading capacity without using additional solvents, reducing the hypersensitivity reaction during intravenous injection caused by solvents. If the drugs do not cause hypersensitivity or have good solubility, the use of albumin becomes less meaningful, such as methotrexate prodrugs or doxorubicin prodrugs.

In summary, albumin technology perfectly adapts to the unmet clinical needs of paclitaxel drugs, hypersensitivity. It is an excellent example of using the right technology to solve an unmet clinical need. The successful approval of Abraxane® could not be achieved without the proper choice of paclitaxel as its API or if its development did not solve the problem of hypersensitivity caused by the solvent. This is the lesson we can learn from the development of Abraxane®.

3.3. The approval of Onivyde®

Onivyde®, also known as MM-398 or PEP02, has been designed and developed as a liposomal formulation of irinotecan [135]. Unlike Doxil® or Abraxane®, the approval of Onivyde® was not because of improved efficacy or a changed safety profile when compared head-to-head with free drugs in clinical trials [78]. Onivyde® is approved as a combination therapy with 5-fluorouracil (5-FU) and leucovorin for only one indication, namely, metastatic pancreatic cancer, as second-line therapy after treatment with gemcitabine-based chemotherapy [136,137].

In a mouse xenograft model of human colon carcinoma, nano-irinotecan could achieve higher intratumoral levels of the prodrug and its active metabolite SN-38 than free irinotecan. Liposome-irinotecan administered at doses 5-fold lower than free irinotecan achieved superior antitumor activity, demonstrating the great potential of nano-irinotecan to improve efficacy and safety [138]. To translate the preclinical superiority of nano-irinotecan into clinical trials, phase II trials of Onivyde® were carried out as head-to-head experiments with free irinotecan [80]. The OS of the Onivyde® group was not improved compared with that of the irinotecan group [Fig. 7a] [139]. The phase III trial was designed with three arms of drug combinations. In phase III NAPOLI-1 study, 417 patients with progression of pancreatic cancer after gemcitabine-based treatment were randomized. Patients received Onivyde® monotherapy (n = 151), folinic acid and 5-FU (FF) (n = 149), or a combination of liposomal irinotecan and FF (n = 117) [35]. There was no difference in OS between the FF and Onivyde® monotherapy arms (HR = 0.99, p = 0.94) (Fig. 7b). However, this trial demonstrated a significant improvement in the median OS of 6.1 months in the combination therapy arm compared to 4.2 months in the FF arm (HR = 0.67, 95% CI = 0.49–0.92, p = 0.012) (Fig. 7c). The above findings led to US FDA approval in October 2015 for liposomal irinotecan (Onivyde®) combined with 5-FU and leucovorin to treat patients with metastatic pancreatic cancer previously treated with gemcitabine-based chemotherapy.

Unlike Doxil® or Abraxane®, the phase III trial leading to FDA approval did not compare irinotecan to liposome irinotecan head-to-head. Although the preclinical study demonstrated the great potential of liposome-irinotecan to improve efficacy and safety, the phase II trial failed to show an increased benefit of efficacy or safety for liposome-irinotecan compared with conventional irinotecan. The phase III trial demonstrated a prolonged survival time but increased incidence rates of almost every side effect when liposome irinotecan plus fluorouracil and folinic acid were compared with fluorouracil plus folinic acid. The successful approval of Onivyde® cannot be credited to enhanced efficacy or safety compared with free irinotecan but to the right choice of cancer type and drug combination therapy.

4. Future directions

Much attention, time, and resources have been channeled toward creating nanomedicines, but clinical progress remains limited. The progress of science is driven by conjectures but also limited by them. Researchers need conjectures as a logical basis to guide the design of experiments, and conjectures need to be examined by obtained results. In the field of anticancer nanomedicine, most studies are carried out based on two conjectures: (i) increased drug concentrations in tumors provide better efficacy, and (ii) decreased drug concentrations in healthy organs result in fewer side effects. These two conjectures appeared to be verified by animal experiments, but not clinical results. As clinical data showed, the conjectures based on the small animal experiments could not be translated to clinical studies. We should reevaluate the current conjectures and try to find alternative assumptions or modify the current conjectures to establish a new theoretical framework to guide our future experiments. Here are some suggestions that can help to guide our future experiments which may lead to clinical translation.

4.1. How to increase clinical efficacy?

4.1.1. Choose a suitable API according to the clinical need

A drug carrier is just a tool for the delivery of a drug or drugs. From the above analysis of anticancer nanomedicine conjectures, we can see that although nanoparticles could accumulate in
human tumors, they did not improve the efficacy and safety of patients. Possible reasons for nanomedicine’s poor clinical outcome could be that the API it carries cannot improve the ORR or OS by increasing its concentration in tumors, and an improved ORR in patients cannot translate into patient survival benefits. For example, clinical evidence demonstrates the increased tumor concentration of PK2 in liver tumors in patients but has shown poor clinical outcomes [56,140]. In another example, a phase III trial compared the response rates of 60 and 240 mg toremifene doses with 40 mg of tamoxifen, and there were no statistically significant differences between the 40 mg of tamoxifen group and the 60 and 240 mg toremifene dose groups [141]. These results imply that the ORR for some drugs may not increase by improving drug concentrations in tumor.

Tumor response rates (e.g., size shrinkage) have shown to be inaccurate predictors of survival benefit for some drugs, and the shrinkage of tumor size does not guarantee patient survival clinically [91,92]. Different drugs in different settings of anticancer treatment have different results due to their unique correlation of ORR or OS with tumor drug concentration. If the increase of the OS of patients is intended, efforts should be made to confirm that the APIs delivered can improve the OS by increased tumor accumulation. Doxorubicin is not one of these drugs, even though it is the most widely used API for preclinical studies in nanomedicine. Doxil® successfully mitigates the cardiotoxicity of doxorubicin by avoiding its uptake by cardiac cells, then gets approved. At the same time, the drug concentration in tumors is improved. However, it fails to enable an increase in efficacy and safety by increasing drug concentrations in tumors. The API used in those cases may not be the best one for nanocarrier delivery to increase efficacy and reduce side effects.

It is vital to identify a suitable API for obtaining the desired outcome instead of choosing APIs convenient for obtaining intended results from small animal research. Doxorubicin has been widespread for nanomedicine research mainly because it is easy to load into nanoparticles, and its fluorescence property is very convenient for characterization. Thus, its wide use is based on convenience, not for translational reasons. A successful clinical example of choosing a suitable API is the development of antibody-drug conjugate (ADC) drugs [142], whose drug design strategy is very similar to that of nanomedicine. Both ADCs and nanomedicines use targeting strategies to increase drug concentrations in tumors, but ADC drugs have produced more potential drugs for clinical use than nanomedicines in a shorter period of time. The first ADC drug approved is in 2000. After 22 years of development, the ADC drug development has undergone 3 generation of drug developments: first generation: using mouse monoclonal antibody; second generation: using human-sourced monoclonal antibody; third generation: using site-specific monoclonal antibody [142]. During the 20 years of development of ADC drugs, there are already 5 ADC drugs for solid tumors approved by FDA, and more than 700 clinical trials in research. (https://clinicaltrials.gov, search “antibody drug conjugate”). In comparison, the first anticancer nanomedicine approved is in 1995. There are also 5 drugs approved by FDA, but mainly approved before the year of 2000. After 27 years of development of anticancer nanomedicine development, there are only 25 clinical trials in research (https://clinicaltrials.gov, search “antibody drug conjugate”). Unlike nanomedicine, ADC drugs often select highly potent molecules with significant toxicity as APIs themselves [143]. The application of these APIs alone is mostly limited by their very narrow therapeutic windows. Through the sufficient enrichment of drugs in tumors, a therapeutic window is realized so that these ADC drugs can eventually be applied to treat certain types of cancers. The success of ADC development is the combination of the ADC delivery strategy and the selection of highly toxic drugs.

In general, drug properties need to be fully characterized, ranging from physicochemical properties (such as water-solubility, octanol–water partition coefficient, and half-maximal inhibitory concentration) to pharmacokinetic properties (including pharmacodynamics, efficacy, and adverse effects) in animals and humans. We need to cast more strict control on manufacturing. Biodegradability of the carriers and kinetics of drug release should be fully studied before entering clinic. We also need to evaluate whether a selected drug can be assisted with the advantage of nanoparticles to increase clinical efficacy and reduce side effects for patients. For drug delivery systems, other than target the tumor site itself, it makes more sense to target biomarkers. The focus should be on the drug, not just the delivery system.

4.1.2. Use drug-resistant tumor animal models instead of conventional animal tumor models.

The translational ability from preclinical models to clinical trials is critical for anticancer nanomedicine. Most studies using xenograft models exhibit a promising efficacy in preclinical experiments, which has not been extended to clinical trials [144]. If the results in mouse studies are not reproduced in humans, the small animal model needs to be changed. Generally speaking, animal models can only feature or mimic one aspect of a human disease or the human body. The principle of choosing the animal model depends heavily on the goal of the overall purpose of the study. Whether attempting to increase therapeutic efficacy or reduce side effects, nanomedicine’s developmental goal is to bring benefits for patients, enhance the quality of life, and reduce the cost of healthcare. This nanomedicine design goal requires creating a tailored solution to address a specific bedside problem in the clinic. Therefore, the design of animal experiments should fit with the clinical situation, rather than using any conventional animal models. For example, clinical trials often recruit second-line or third-line patients who have been treated heavily with other treatments. The previous treatment, age, molecular features, and genotype of patients [145,146] could be factors that affect the final clinical outcome, which should be taken into account in preclinical stages to build a more mimetic animal model [147,148]. Instead of using conventional xenograft models, a drug-resistant model can be more mimetic for the study of anti-cancer drug development researches.

4.1.3. Nanomedicines in combination with immunotherapy.

The immune-oncology aspect had been ignored by the nanomedicine field for a long time. The recent trend in the treatment of cancer is now moving from a traditional chemotherapy towards cancer immunotherapy and combination therapy [149]. Immunotherapy treats diseases by inducing, enhancing, or suppressing an immune response. For example, cancer vaccines can be designed to combine Toll-like receptor (TLR) agonists and antigen-carrying lipid nanocapsules to induce immune response and help the immune system to effectively destroy cancer cells [150]. The newer strategies include T cell-mediated therapy and immune checkpoint inhibitors, which are already being clinically applied [151,152]. The nanoparticle-based anticancer immunotherapies seem to be effective, in that they bring in synergistic effects and improved immune response towards tumor eradication [153]. It is important to point out that, the combination of immunotherapy and chemotherapy has been proved to have better efficacies [154]. When chemotherapy is involved, the above two conjectures should be taken into account carefully. More importantly, when chemotherapy is not used in combination with immunotherapy, it is also necessary to keep alert that whether the phenomenon observed in animals can be translated to humans.
4.2. How to reduce side effects?

From the results of our meta-analysis, we can conclude that nanomedicine cannot eliminate general side effects systemically. In fact, the side effects generated by nanomedicine is not related to small Mw drugs, whether from the amount of the incidence or the severity. From our case study report, we found that the most valuable advantage of the approved nanomedicine is the distribution change of the drugs, resulting in reducing of the dose-limiting toxicity. The change of the dose-limiting toxicity makes the nanomedicine to be approved. In the meantime, new dose-limiting toxicity generated with nanomedicine. Nanomedicine itself cannot reducing the general side effects as a whole. The current problem with the nanomedicine research is that, the researchers focus overly on reducing the general side effects, but overlook the dose-limiting toxicity of the small Mw drug itself. And they forgot to ask, whether the biodistribution of nanomedicine is related to the dose-limiting toxicity.

4.2.1. Build specific animal models to reducing dose-limiting side effects.

If the goal of a formulation is to reduce side effects in patients, the specific problem needs to be clearly defined first. For example, the main DLTs of paclitaxel drugs are hypersensitivity reactions and neuropathy. Therefore, the development of paclitaxel-based nanomedicine should pay close attention to these two side effects. However, in preclinical animal experiments evaluating paclitaxel nanomedicine, very few studies have used suitable animal models to simulate either the hypersensitivity or neuropathy caused by the paclitaxel solution. Instead, the animal model used in most animal experiments simulates bone marrow suppression, which is not the intent-to-solve clinical need for this group of patients. Without the appropriate animal model, any data showing decreased general side effects may not be translatable in clinical trials.

For decreasing the side effect of a formulation, the selection of a small animal model should take animal age, species, dosage, and the dosing time course into consideration. For example, if reducing the cardiotoxicity of doxorubicin is the goal, then a suitable cardiotoxicity animal model needs to be identified first. Clinically, cardiotoxicity is cumulative toxicity that occurs most frequently after 4 to 6 months of dosing. Theoretically, to mimic accumulative chronic doxorubicin administration, doxorubicin should be administered for an extended period, not just a few weeks, as often done in small animal studies. This plan takes a substantial amount of time, and few research groups can afford to do that. Better animal models take a shorter time, e.g., four weeks, to mimic chronic cardiotoxicity with a cumulative dose of 20 mg/kg [155,156]. However, very few studies have used this model for nanomedicine research when planning to reduce the cardiotoxicity of doxorubicin. If animal models are used to simulate side effects, a more sophisticated model may be needed to mimic the human condition more accurately.

The current preclinical models are incapable of predicting the clinical efficacy or side effects of anticancer nanomedicine. Even so, conventional small animal models continue to be the first choice, citing the reason that there are no other models available. Such reasoning, however, is the same as analyzing the data only to see the expected results and ignoring other contradictory results. It is the time to re-evaluate the xenograft mouse model and find alternative experimental methods that can accurately represent the efficacy and safety profile of nanomedicine in humans. It is the time to understand that each formulation is unique, and simply labeling widely different formulations collectively “nanomedicines” is inaccurate. Some nanomedicines may be clinically effective, but that does not mean other nanomedicines will also be effective. Instead of using the generic term nanomedicine, each formulation needs to be identified uniquely, as many conventional low molar mass drugs are identified by their chemical structures and properties. Nobody calls doxorubicin and paclitaxel collectively “molecular medicine” or “subnanomedicine”.

5. Conclusions

The survey of the nanomedicine literature from the past one-to-two decades identifies two conjectures in anticancer small molecule-based nanomedicine: (i) increased drug concentrations in tumors provide better efficacy, and (ii) decreased drug concentrations in healthy organs result in fewer side effects. However, these two conjectures have proven to be fallacies from the results of currently available clinical trials with classic chemotherapeutic drugs, such as Dox, cisPt, CPT and PTX, and the analyses of the approved nanomedicines bearing those chemotherapeutic agents cannot confirm these two conjectures. Future re-analysis is still needed when more clinical data with success stories became available. Even though these two conjectures are not taken alone as frequently as before, they are still used a lot when chemotherapy is combined with other therapeutics. Therefore, at this critical moment, a new theoretical framework is urgently needed to explore new strategies that further improve safety and efficacy.

Nanomedicine development should be based on the benefits of patients. It is crucial to encourage drug delivery scientists to ‘begin with the end in mind’ and focus on a specific goal of anticancer nanomedicine development. Success in anticancer nanomedicine development cannot be achieved based on a single factor. Multiple factors, such as a specific clinical problem, a suitable drug carrier, a suitable choice of API, and a good animal model, can be used as a new evaluation system. All these factors contribute to the success of anticancer nanomedicine and ensure that preclinical studies are translatable and have practical applications. The abuse of the term “nanomedicine” should stop now so that the next generation of drug delivery scientists are unbound to exploring their endless imagination. Finally, whatever the next generation of nanoscopic drug delivery systems may be, they must be designed holistically, taking into account the disease and disease biology, and also the composition, structure and long-term, potentially adverse effects of the drug delivery platform itself.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This work was in part supported by the Youth Thousand-Talents Program of China, start-up grants from Shanghai Jiao Tong University (WF220408211). This work was also supported by the grants from the State Key Laboratory of Oncogenes and Related Genes (90-17-02) and from the Interdisciplinary Program of Shanghai Jiao Tong University (YG2017MS18).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.addr.2022.114525.