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NANOCRYSTAL DRUG DELIVERY CONQUERS THE COMPLICATIONS ASSOCIATED WITH PHARMACOKINETIC EVENTS OF ANTICANCER FORMULATIONS

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ABSTRACT

Nanotechnology has recently gained increased attention for its capability to effectively diagnose and treat various tumors. Nanocarriers have been used to circumvent the problems associated with conventional antitumor drug delivery systems, including their nonspecificity, severe side effects, burst release and damaging the normal cells. Nanocarriers improve the bioavailability and therapeutic efficiency of antitumor drugs, while providing preferential accumulation at the target site. A number of nanocarriers have been developed; however, only a few of them are clinically approved for the delivery of antitumor drugs for their intended actions at the targeted sites. Nano/crystals owing to its ability to modify the physicochemical and biological properties of the drug have gained widespread attention among the research scientists. This review provides comprehensive detail on the associated advantages, challenges, factors affecting physicochemical properties, and optimization parameters about the stability of nanocrystals. In this review, the evolution of nanocrystals is discussed as first-generation simple nanocrystals, second generation nanocrystals within a carrier, and third-generation surface-modified nanocrystals. It also provides a detailed account of various preparation methods and evaluation of surface-modified nanocrystals.

Keywords: Nanocrystals, Nanocarriers, drug delivery, solid lipid nanoparticles

INTRODUCTION

In the last few decades, researchers and pharmaceutical industries have been developing new approaches to overcome the solubility and bioavailability limits observed with poorly soluble drugs. With the advancement of nanotechnology, nanocrystals have emerged as a great potential to overcome these limitations. Nano/crystals owing to its ability to modify the physicochemical and biological properties of the drug have gained widespread attention among the research scientists. This review provides comprehensive detail on the associated advantages, challenges, factors affecting physicochemical properties, and optimization parameters about the stability of nanocrystals. In this review, the evolution of nanocrystals is discussed as first-generation simple nanocrystals, second generation nanocrystals within a carrier, and third-generation surface-modified nanocrystals. It also provides a detailed account of various preparation methods and evaluation of surface-modified nanocrystals. In the proposed "King Design," nanocrystals of the third generation are placed on the top due to their advantage over other nanocarriers like high drug payload, site-specific delivery, improved activity, commercial manufacturing, and easy scale-up. Third generations nanocrystals can provide a novel therapeutic solution for the site-specific, targeted, and efficient delivery for treatment of various acute as well as chronic diseases with high stability and scale-up potential. Drug nanocrystals offer an attractive approach for improving the solubility and dissolution rate of poorly soluble drugs which accounts for nearly 40 % newly discovered drug molecules. Both methods for manufacturing drug nanocrystals have high industrial acceptability for being simple and easy to scale which is evident from the number of approved products available in the market. Ability to modify multiple aspects of dosage form like bioavailability, release pattern and dosage form requirement along with flexibility in choosing final dosage form starting from the tablet, capsule, suspension to parenteral one, have made nanocrystals technology one of the very promising and adaptable technology for dosage form design Nanocrystals, a carrier-free colloidal delivery system in nano-sized range, is an interesting approach for poorly soluble drugs. Nanocrystals provide special features including enhancement of saturation solubility, dissolution velocity and adhesiveness to surface/cell membranes. Several strategies are applied for nanocrystals production including precipitation, milling, high pressure homogenization and combination methods such as Nano- Edge™, Smart Crystal and Precipitation-lyophilization-homogenization (PLH) technology **[1]**.

Special features of nanocrystals to enhance oral bioavailability

Poorly soluble drugs encounter biopharmaceutical delivery problems such as low bioavailability after oral administration, low penetration of the drug into the skin, large injection volume for intravenous (i.v.) administration and undesired side effects after i.v. injection when using traditional formulations. Drug nanocrystals possess outstanding features enabling to overcome the solubility problems including an increase in saturation solubility, an increase in dissolution velocity, and an increased adhesiveness to surface/cell membranes. These features are resulted from transferring of particle size from macro particle to nanodimension that changes their physicochemical properties on the basis of nanotechnology. A detailed description of the physical background of these effects is shown below **[2]**.

1. An increase in saturation solubility (Cs)

In general, saturation solubility is a compound-specific constant, which is depending on physicochemical properties of the compound, dissolution medium and temperature. However, this definition is only valid for drug particles with a minimum particle size in the micrometer range. Furthermore, the saturation solubility is also a function of the crystalline structure (i.e. lattice energy) and particle size. The polymorphic modification with highest energy and lowest melting point leads to the best solubility. Occasionally, homogenization process generates amorphous fraction with high inner energy that contributes to an increased solubility of the substance. For the particle size aspect, the saturation solubility is also a function of particle size when a critical size is below 1e2 mm. The saturation solubility increases with decreasing particle size below 1000 nm. This phenomenon can be explained by the Kelvin and the Ostwald Freundlich equations.

2. Special features of nanocrystals to enhance oral bioavailability

Poorly soluble drugs encounter biopharmaceutical delivery problems such as low bioavailability after oral administration, low penetration of the drug into the skin, large injection volume for intravenous (i.v.) administration and undesired side effects after i.v. injection when using traditional formulations. Drug nanocrystals possess outstanding features enabling to overcome the solubility problems including an increase in saturation solubility, an increase in dissolution velocity, and an increased adhesiveness to surface/cell membranes **[3]**. These features are resulted from transferring of particle size from macro particle to nanodimension that changes their physicochemical properties on the basis of nanotechnology.

3. An increased adhesiveness to surface/cell membranes

Comparing with microparticles, drug nanocrystals have another outstanding feature because they can distinctly increase adhesiveness to surface/cell membranes. An increased adhesiveness of nanomaterials is usually due to an increased contact area of small particles versus large particles (at identical total particle mass). Similar to other nanoparticles, drug nanocrystals show an increased adhesiveness to tissue which lead to an improvement of oral absorption of poorly soluble drugs apart from the increased saturation solubility and dissolution rate **[4-5]**. This aspect will be further discussed in topic "*In vivo* performances of drug nanocrystals in oral administration routes.

Nanocrystal NPs

Nanocrystals are versatile NPs that are used to improve the PK/PD properties of poorly soluble organic or inorganic materials by increasing their bioavailability and solubility **[6-8]**. Nanocrystals possess a narrow, tunable, symmetric emission spectrum and are photochemically stable. They are composed of an optically active core surrounded by a shell that provides a physical barrier against the external environment, making them less sensitive to photo-oxidation or medium changes. Nanocrystal-based drugs are unique because they are composed entirely of drug compound **[9]**. Increased surface area on the nanoscale promotes enhanced dissolution speed and saturation solubility **[10]**. Saturation solubility increases the forces that drive diffusion-based mass transfer through biologic structures, such as the walls of the gastrointestinal tract. However, the oral absorption mechanism for nanocrystal formulations is not fully understood, and their behavior after subcutaneous injection is not fully predictable.4 Solubility issues for a number of drug compounds have been resolved through conversion into nanocrystals, which are marketed for a wide range of indications. Rapamune, granted FDA approval in 2000, was the first milled organic nanocrystal drug. Its active ingredient is sirolimus, a bacterial-derived macrocyclic immunosuppressant used to prevent rejection after transplantation of an organ (particularly a kidney). Rapamune's nanocrystal-based formulation provides poorly soluble sirolimus with a continuous extended-release profile that is well suited for its indication. The milling technology developed by Elan Nanosystems (now Alkermes) that produced Rapamune nanocrystals has proven to be flexible for application to other types of formulations, including oral suspensions, tablets, and intramuscular injections. After the FDA approved Rapamune, the same milling technique was used to produce several other approved nanocrystal drug formulations, such as Tricor (fenofibrate, AbbVie) and Emend (aprepitant, Merck). This milling approach is expected to be applied as a potential solution for the wide range of solubility issues that occur with an estimated 70% to 90% of drug compounds. Inorganic nanocrystal formulations approved by the FDA are limited and include only nanocrystal forms of hydroxyapatite and calcium phosphate for use as bone-graft substitutes. Matinas Biopharma is developing two lipid nanocrystal formulations of antimicrobial agents. MAT2203, a nanoformulation of the antifungal amphotericin B, is undergoing phase 2 trials in patients with chronic candidiasis who are intolerant or refractory to standard non-intravenous treatments.15 MAT2501 is a lipid nanocrystal formulation of amikacin. Conventional formulations of amikacin are associated with neurotoxicity and nephrotoxicity and require careful monitoring. The targeted delivery of amikacin to designated areas by MAT2501 results in a reduction in total dose and an improved safety profile. Matinas Biopharma is waiting for an IND designation from the FDA prior to commencing phase 1 trials with MAT2501 **[11]**.

NANOCRYSTAL PREPARATION METHODS [5]

Several preparation methods devolved today, implemented preparation methods of Nanocrystal formulations can be classified as "bottom up", "top-down", "top down and bottom up" and "spray drying". "Bottom up" technology begins with the molecule; active drug substance is dissolved by adding an organic solvent, and then, solvent is removed by precipitation. "Top down" technology applies dispersing methods by using different types of milling and homogenization techniques. "Top down" technology is more popular than "Bottom up" technology; it is known as "nanosizing". In other words, it is a process which breaks down large crystalline particles into small pieces. In "top down and bottom up" technology, both methods are utilized together. Spray drying is also a method for preparing drug nanocrystals, which is faster and more practical compared to the other methods **[12-14]**.

1. Bottom up

a) Nanoprecipitation.

2. Top down

a) Milling.

b) Homogenization.

3. Top down and Bottom up

4. Spray drying

5. Other Techniques used for the Production of Drug Nanocrystals

a) Rapid expansion from a liquefied-gas solution (RESS)

b) Nanopure XP technology

c) Spray Freezing into Liquid (SFL) technology.

1. Bottom up technology: The principle of this method is based on the dissolution of the active drug substance in an organic solvent which is then added into a nonsolvent (miscible with the organic solvent). In the presence of stabilizers, thereafter, the nanocrystals are precipitated. Basic advantage of the precipitation technique is that it is simple and has a low cost. Also, scale up is simple in this method. It should be kept in mind that several parameters; such as stirring rate, temperature, solvent/ nonsolvent rate, drug concentration, viscosity, type of solvent and stabilizer should be controlled in order to obtain homogenous nanocrystals by this technique **[15]**.

a) Precipitation methods: The drug is dissolved in a solvent and subsequently added to a nonsolvent, leading to the precipitation of finely dispersed drug nanocrystals. One must consider in mind that these nanocrystals need to be stabilized in order when they are not allowed to grow to the micrometer range. In addition, the drug needs to be soluble in at least one solvent, which creates problems for newly developed drugs that are insoluble in both aqueous and organic media. Due to some of these reasons as reported, this technology has not been applied to a product as yet. A solution of the carotenoid, together with a surfactant in digestible oil, is mixed with an appropriate solvent at a specific temperature. To obtain the solution a protective colloid is added. This leads to an O/W two phase system. The carotenoid stabilized by the colloid localizes in the oily phase. After Lyophilisation X-ray analysis shows that approximately 90% of the carotenoid is in an amorphous state **[16]**.

2. Top down technology: "Top-down" technology applies dispersing methods by using different types of milling and homogenization techniques. "Top-down" technology is more popular than "Bottom up" technology; it is known as "nanosizing". In other words, it is a process which breaks down large crystalline particles into small pieces. In "top down and bottom up" technology, both methods are utilized together. Top-down technology can be applied by either homogenization or milling.

a) Milling methods: The classical Nanocrystal® technology uses a bead or a pearl mill to achieve particle size diminution. Ball mills are already known from the first half of the 20th century for the production of ultrafine suspensions. Milling media, dispersion medium (generally water), stabilizer and the drug are charged into the milling chamber. Shear forces of impact, generated by the movement of the milling media, lead to particle size reduction. In contrast to high pressure homogenization, it is a low energy milling technique. Smaller or larger milling pearls are used as milling media. The pearls or balls consist of ceramics (cerium or yttrium stabilized zirconium dioxide), stainless steel, glass or highly cross-linked polystyrene resin-coated beads. Erosion from the milling material during the milling process is a common problem of this technology. To reduce the amount of impurities caused by erosion of the milling media, the milling beads are coated. Another problem is the adherence of product to the inner surface area of the mill. There are two basic milling principles. Either the milling medium is moved by an agitator, or the complete container is moved in a complex movement leading consequently to a movement of the milling media. When one assumes that 76% of the volume of the milling chamber is to be filled with milling material, larger batches are difficult to produce when moving the new container, so mills using agitators are used for large sized mill for large batches. The milling time depends upon many factors such as the surfactant content, hardness of the drug, viscosity, temperature, energy input, size of the milling media. The milling time from about 30 minutes to hours or several days. This new technology is an important particle size reduction which is proven by four FDA-approved drugs using it, which will the subject later in this text **[17]**.

b) Homogenization methods: The Micro fluidizer is a jet stream homogenizer of two fluid streams collide frontally with high velocity (up to1000m/sec) under pressures up to 4000 bar. There is a turbulent flow, high shear forces and particles collide leading to particle diminution to the nanometre range. The high pressure applied and the high streaming velocity of the lipid can also lead to cavitations additionally, contributing to size decreased. To prevent the particle size, stabilization with phospholipids or other surfactants and stabilizers is required. In many cases, 50 to 100 time- consuming passes are necessary for a sufficient particle size reduction.

Piston-gap homogenization in water: Drug nanocrystals can also be produced by high-pressure homogenization using piston gap homogenizers. Depending on the homogenization temperature and the dispersion media, there is a difference between the Disso cubes® technology and the Nanopure® technology. Dispersion medium of the suspensions was water. A piston in a large bore cylinder creates pressure up to 2000 bar. The suspension is pressed through a very narrow ring gap. The gap width is typically in the range of 3- 15 micrometers at pressures between 1500-150 bars.

3. Top down and bottom up technology: In “top down and bottom up” technology, both methods are used together. Nano- Edge® is a product obtained by such a combination technology. Nano-edge technology described the formulation method for poorly water-soluble drugs. It is a useful technology for active ingredients that have high melting points and high noctanol- water partition coefficients. It is based on direct homogenization, micro precipitation, and lipid emulsions. In micro precipitation, the drug first is dissolved in a water-miscible solvent to form a solution. Then, the solution is mixed with a second solvent to form a pre suspension and energy is added to the pre suspension to form particles having an average effective particle size of 400 nm to 2 µ.

4. Spray Drying: One of the preparation methods of nanocrystals is spray drying. This method is usually used for drying of solutions and suspensions. In a conical or cylindrical cyclone, solution droplets are sprayed from top to bottom, dried in the same direction by hot air and spherical particles are obtained. Spraying is made with an atomizer which rapidly rotates and provides scattering of the solution due to centrifugal effect. The solution, at a certain flow rate, is sent to the inner tube with a peristaltic pump, nitrogen or air at a constant pressure is sent to the outer tube. Spraying is provided by a nozzle. Droplets of solution become very small due to spraying; therefore, surface area of the drying matter increases leading to fast drying. Concentration, viscosity, temperature and spray rate of the solution can be adjusted and particle size, fluidity and drying speed can be optimized. The dissolution rate and bioavailability of several drugs, including hydrocortisone, COX-2 Inhibitor (BMS-347070) were improved utilizing this method.

PHARMACOKINETIC EVENTS OF ANTI-CANCER FORMULATIONS

Cancer remains one of the world's most devastating diseases, with more than 10 million new cases every year **[18]**. However, mortality has decreased in the past 2 years **[19]** owing to better understanding of tumour biology and improved diagnostic devices and treatments. Current cancer treatments include surgical intervention, radiation and chemotherapeutic drugs, which often also kill healthy cells and cause toxicity to the patient. Conventional chemotherapeutic agents also do not show targeted action and are distributed non-specifically in the body where they affect both cancerous and normal cells, thereby limiting the dose achievable within the tumour cells and also resulting in suboptimal treatment due to excessive toxicities. Molecular targeting therapy has emerged as one approach to overcome the lack of specificity of conventional chemotherapeutic agents **[20]**. However, the resistance development in cancer cells can dodge the cytotoxicity not only of conventional chemotherapeutics but also of newer molecular targeting therapeutics **[21]**. By using both passive and active targeting strategies, intracellular concentration of drugs in cancer cells can be improved by nanoparticles while avoiding toxicity in normal cells **(Figure 1) [22-23]**. Passive targeting feats the characteristic features of tumour biology that allows nanocarriers to accumulate in a tumour by the enhanced permeability and retention (EPR). Active approaches achieve this by conjugating nanocarriers containing chemotherapeutics with molecules that bind to overexpressed antigens or receptors on the target cells. Nanoparticles offer many advantages as drug carrier systems, there are still many limitations to be solved such as poor oral bioavailability, instability in circulation, inadequate tissue distribution and toxicity. In this reviews provide perspective on the use of nanotechnology as a fundamental tool in cancer research and nanomedicine **[24-25]**. Here we focus on the types and characteristics of nanoparticles, how nanoparticles are being used as drug delivery systems to kill cancer cells more effectively and also to reduce or overcome drug resistance and how nanoparticles will be developed to improve their therapeutic efficacy and functionality in future cancer treatments. Many anticancer drugs, due to their hydrophilicity, have poor drug loading in nanoparticles, which has limited efficient drug delivery. Charge-charge interactions may be effective for improving loading where charges in nanoparticles attract oppositely charged drug molecules. A new strategy, incorporation of charged hydrophobic excipients into nanoparticles followed by drug loading via incubation of nanoparticles in the presence of drug solution, may effectively increase drug loading.

Targeted delivery of nanoparticles

Ideally, for the effectiveness of anticancer drugs in cancer treatment, they should first, after administration, be able to penetrate through the barriers in the body and reach the desired tumour tissues with minimal loss of their volume or activity in the blood circulation. Second, after reaching the desired site, drugs should have the ability to selectively kill tumour cells without affecting normal cells. These two basic approaches are also associated with improvements in patient survival and quality of life by increasing the intracellular concentration of drugs and reducing dose-limiting toxicities simultaneously. Increasingly, nanoparticles seem to have the potential to satisfy both of these requirements for effective drug carrier systems.

Potential Pharmacokinetic Benefits

A common practice in the development of nano drugs is to conjugate or encapsulate a therapeutically active agent to an NP to alter its PK. Nano-pharmaceuticals can overcome some of the limitations of conventional medicines by promoting more desirable PK and distribution, independent of the molecular structure of the active ingredient. They can be designed to enable a medicine to reach previously impervious areas, circulate for longer times to allow greater accumulation, or be targeted toward a disease site. The incorporation of NPs in a pharmaceutical formulation can also alter the concentration-time profile of a drug, enabling its release (and exposure to diseased and/or healthy tissues) in a controlled and sustained manner. Currently, most nano-drugs are previously existing drugs conjugated to NPs to improve PK and/or pharmacodynamic

(PD) properties. In the majority of cases, these drug–NP conjugates use "passive targeting," which involves nonspecific accumulation in diseased tissue, often tumors. However, "active targeting" can be achieved by attaching ligands (e.g., proteins, antibodies, or small molecules) to the surface of the drug–NP conjugate that are designed to attach to receptors on specific cells. Active targeting can result in an increase in intracellular drug accumulation and uptake by the cells of the targeted tissue. Preclinical and clinical studies are necessary to characterize the PK, PD, biodistribution, efficacy, and toxicity of nano-pharmaceuticals to understand how they differ from conventional dosage forms. These studies are needed because drugs formulated with NPs can dramatically alter PK. For example, administration of a 50 mg/m2 dose of liposomal doxorubicin in humans was found to increase the area under the curve (AUC) by 300-fold and reduce clearance 250-fold compared to free drug .

Potential Efficacy Benefits

When formulating nanodrugs, diverse strategies can be applied to improve drug efficacy. These include: exploiting the small size of NPs to circumvent important physiological barriers (the immune system, renal clearance, enzymatic and mechanical degradation, and others); using NPs to entrap drug molecules to protect them from physiologically hostile environments; and/or using surface conjugation to target drugs to specific tissues, enabling higher therapeutic levels at a target site even with the use of lower doses. Nanomaterials also have immunomodulatory effects that might potentially promote or shape the adaptive immune response.8 Some (e.g., polymeric NPs, liposomes, nanoemulsions, and virus-like NPs) are capable of entering antigen presenting cells **[9, 10]**. This ability may potentially permit them to regulate the immune response, for example, by inducing preclinical and clinical studies have shown that nanoformulations can passively enhance tumor accumulation, decreasing normal tissue exposure.

However, the clinical validation of active NP targeting is more limited and not as easily achieved. Another way nano-pharmaceutical formulations can benefit cancer treatment is through the incorporation of drugs into long-circulating NPs that remain active for an extended period of time. Consequently, tumor sites experience longer exposure to the drugs due to the slow rate of drug release from the NP and the retention of the drug-loaded NPs in the vascular compartment.

Potential Safety Benefits (Table 1)

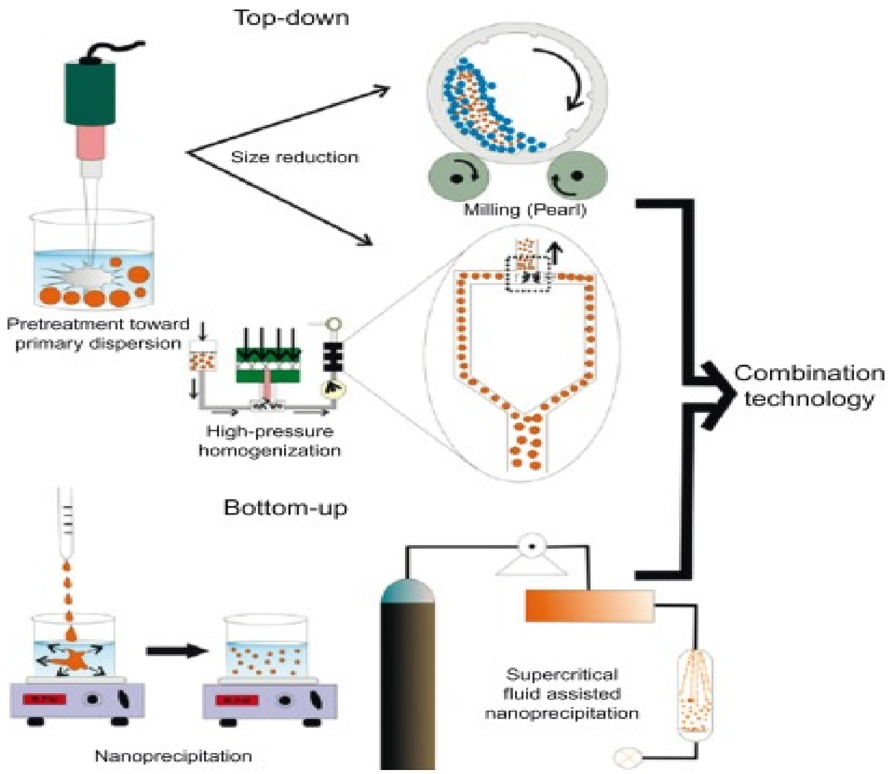

Figure 1: Multifunctional nanocrystals for cancer therapy as a potential nanocarrier

Table 1: FDA Approved Nanocrystals available for Clinical use and Benefits

S. No.	Generic Name	Indication	Benefits
1	Morphine sulfate	Psychostimulant	Greater drug loading and bioavailability
2	Aprepitant	Antiemetic	Greater absorption and bioavailability
3	Dexamethylphenidate HCl	Psychostimulant	Greater drug loading and bioavailability
4	Sirolimus	Immunosuppressant	Greater bioavailability
5	Methylphenidate HCl	Psychostimulant	Greater drug loading and bioavailability
6	Fenofibrate	Hyperlipidemia	Greater bioavailability simplifies administration
7	Megestrol acetate	Antianorexic	Lower dosing
8	Tizanidine HCl	Muscle relaxant	Greater drug loading and bioavailability

The increased drug accumulation in diseased tissue provided by nanoformulations may allow the effective dose of a drug to be reduced, diminishing side effects. It has been observed that typically less than 0.01% of an injected dose of angstrom sized agents accumulates in a target region, compared to 1% to 5% for NPs. Better accumulation, as well as targeted release, can enable dose reduction, which decreases side effects. In fact, the earliest nano-drugs were granted approval by the FDA based on lower toxicity compared with conventional formulation counterparts. Doxil (doxorubicin hydrochloride, Janssen), the first nano formulated drug to gain FDA approval, received an indication for the treatment of Kaposi's sarcoma in patients with human immunodeficiency virus (HIV) in 1995. Although equally effective, its main advantage in comparison to conventionally formulated doxorubicin is considered by many to be reduced cardiotoxicy; however, Doxil has been associated with adverse events related to the nanoformulation, such as palmar-plantar erythrodysesthesia and complement activation-related pseudoallergy-like infusion reactions. More than 20 years after its approval, Doxil is still widely used for its original indication, as well as to treat ovarian and metastatic breast cancer and multiple myeloma. Nanoformulations can also help manage the dose-limiting toxicities

associated with conventional chemotherapeutic agents. Many cancer chemotherapies are hydrophobic and relatively insoluble in aqueous solutions. Therefore, they often require toxic solubilizing agents for parenteral administration (such as polyethoxylated castor oil [Kolliphor EL, BASF Corp.] for paclitaxel). Consequently, these drugs often require dose reduction to manage systemic toxicity, limiting efficacy. There has long been interest in developing delivery systems for these therapies that do not require toxic solubilizing agents, and nanoformulation is viewed as a viable solution to the problems associated with administering poorly water-soluble drugs. For these reasons, nanoformulations of many chemotherapies have been approved and more are in clinical development. Perhaps most notable is Abraxane (nab-paclitaxel, Celgene), a formulation of paclitaxel bound to albumin NPs. Abraxane was approved by the FDA in 2005 for previously treated metastatic breast cancer and has since been granted indications for other cancers.4,5,7 Abraxane is considered to be more tolerable than conventional paclitaxel, which is formulated with Kolliphor EL. The increased tolerance (which is attributed in part to the absence of toxic solvent) allows Abraxane\ to be administered to patients at a considerably higher dose, potentially achieving greater efficacy.

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