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**REVIEW ARTICLE**

# Has nanotechnology led to improved therapeutic outcomes?

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**Abstract**

Nanoparticulate drug delivery systems provide new opportunities for solving issues associated with problematic drugs or disease states and have, therefore, created great expectations in the field of drug delivery. This review focuses on the potential benefits of nanoparticles compared with other conventional delivery systems. Several nanoparticulate drug delivery systems have already been marketed or are currently under development and are presented in this review. Results from clinical trials demonstrate that nanoparticulate formulations generally enable superior therapeutic outcomes than compared with standard formulations. Therefore, the implementation of nanotechnology in drug delivery represents an important advance with substantial potential to improve therapeutic effectiveness and increase patient's quality of life.

**Keywords:** nanotechnology, oral delivery, pulmonary delivery, parenteral delivery, poorly soluble drugs, efficacy of nanoparticles, safety of nanoparticles, drug targeting



## Introduction

Over the past few decades, nanoparticulate drug delivery systems have become an area of extensive research as they enable bioavailability improvement of poorly water-soluble compounds as well as targeted delivery of active pharmaceutical ingredients to various tissues and organs[1,2]. Generally, nanoparticles in drug delivery are defined as submicron colloidal particles ranging in size from 10 to 1000 nm[3]. The US Patent and Trademark Office (USPTO), however, defines nanotechnology using a scale from only 1 to 100 nm and slightly larger[4]. Owing to their reduced size, physical and chemical properties of nanoparticles differ significantly from their larger scale counterparts. The main difference is the significantly increased surface-area-to-volume ratio of nanoparticles than compared with microparticles. The enlarged surface area of nanoparticles directly translates into enhanced dissolution rates, which is especially beneficial in the delivery of poorly water-soluble drugs[1]. Additionally, nanoparticles are characterized by increased biologic activity, for example, increased uptake and interaction with biological tissues, as a greater fraction of molecules is located at the surface allowing for more readily interaction with the external environment[5]. Due to their small size, nanoparticles also differ from microparticles in terms of their *in vivo* distribution and ability to target specific tissues. For instance, nanoparticles of appropriate size and surface characteristics are able to extravasate through fenestrated tumor capillaries thereby passively accumulating in tumor tissue[6]. The preferential localization of nanoparticles at the site of interest is beneficial in that it reduces the occurrence of adverse side effects associated with nonspecific drug distribution. Overall, the unique features of nanoparticles have raised great expectations in the field of drug delivery because of their potential to improve clinical efficacy of problematic drug compounds. Compounds that previously could not be formulated due to insolubility or insufficient target specificity have the potential to be efficaciously delivered employing nanotechnology. Besides, nanoparticulate drug delivery systems offer exceptional flexibility as a wide variety of active agents including hydrophilic and hydrophobic drugs, proteins, vaccines, and biological macromolecules may be delivered via numerous routes of administration[7]. Few products have been commercialized yet, but several therapeutic agents are currently under development and expected to enter the market in the near future. It is the purpose of this review to highlight

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benefits of nanoparticulate formulations delivered via the oral, pulmonary, and parenteral route and to discuss the therapeutic outcomes achieved till date.

## Nanoparticles delivered via the oral route

### Benefits of nanoparticles for oral delivery

#### *Dissolution velocity enhancement*

When a drug is administered in form of a solid oral dosage form, it needs to first dissolve in the gastrointestinal (GI) fluids to then be absorbed through the intestinal mucosa into the bloodstream from where it is distributed throughout the body[8]. Consequently, the key factors that determine the rate and extent of oral drug absorption are the aqueous solubility of the drug and its permeability through the intestinal mucosa. The Biopharmaceutics Classification System (BCS) is a scientific framework that classifies drugs based on these two parameters into one of four categories: BCS I: high solubility, high permeability; BCS II: low solubility, high permeability; BCS III: high solubility, low permeability, and BCS IV: low solubility and low permeability[9]. A drug is considered highly permeable when the extent of absorption in humans is determined to be more than 90% of an administered dose, based on mass-balance or in comparison to an intravenous reference dose[10]. Furthermore, the criterion of high solubility is met, if the highest drug dose strength is soluble in 250 mL or less of aqueous media over the pH range of 1–7.5. It is estimated that about 60% of drugs that come directly from chemical synthesis are characterized by poor aqueous solubility (BCS II or BCS IV[11]). While BCS IV compounds often do not enter later stages of drug development due to the unfavorable combination of low solubility and permeability, BCS II drugs may be successfully formulated employing several innovative formulation approaches aimed at improving their solubility/dissolution. Promising formulation strategies, which have emerged include particle size reduction, amorphization of crystalline drug compounds, self-emulsifying drug delivery systems, and solubilization via drug-cyclodextrin inclusion complexes[12]. The conversion of a crystalline drug material into the amorphous form significantly increases the apparent solubility, but at the same time, it raises concerns regarding stability due to increased reactivity and hygroscopicity[13]. Self-emulsifying drug delivery systems, which are mixtures of oils and surfactants and sometimes co-solvents, can be employed for the formulation of highly lipophilic compounds. These systems spontaneously emulsify in the aqueous fluids of the GI tract to produce fine oil-in-water emulsions. Generally, the surfactant accounts for 30–60% (w/w) of the formulation, which increases the risk of undesirable side effects such as GI irritations[14]. The inclusion of poorly soluble drugs into the central cavity of cyclic oligosaccharides (cyclodextrins) is another potential way of increasing solubility. Still, cyclodextrins generally possess low drug inclusion capacity and in addition are limited to drugs that exhibit certain physico-chemical characteristics that allow for complexation[15]. Particle size reduction as means of solubility enhancement has the advantage of being a nonspecific formulation strategy that it is applicable to almost any drug molecule. Exceptions include low-melting point drugs, which can potentially melt during size reduction due to high energy input. As particle size and specific surface area are inversely proportional, a size reduction will lead to an increase in surface area. For example, by reducing the particle size from 50 µm to 500 nm, the specific surface area will increase by a factor of 100. The increase in surface area will result in an enhanced dissolution velocity as described in the Noyes–Whitney equation (Eq. 1):

$$\frac{dM}{dt} = \frac{D \cdot A}{h} \cdot (c_s - c_t) \quad (1)$$

where dM/dt is the dissolution velocity, D the diffusion coefficient, A the surface area, h the diffusion layer thickness, and ($c_s$–$c_t$) the concentration gradient between the diffusion layer and the bulk solution[16]. In addition, studies have shown that the size reduction also results in a decrease in the thickness of the diffusion layer surrounding each particle, a phenomenon that is especially pronounced for particles of less than 5 µm in size[17,18]. According to Eq. 1, this will even further increase the dissolution rate. Lastly, the reduction in particle size also increases the saturation solubility. Generally, the saturation solubility is a constant depending on the compound, the dissolution medium, and the temperature. However, below a critical size of 1–2 µm, the saturation solubility becomes a function of particle size[19]. The saturation solubility (S) increases with decreasing particle radius (r) as described in the Ostwald–Freundlich equation (Eq. 2):

$$S = S_\infty \cdot \exp\left(\frac{2 \cdot \gamma \cdot M}{r \cdot \rho \cdot R \cdot T}\right) \quad (2)$$

where $S_\infty$ is the solubility of an infinitely large crystal, γ is the crystal-medium interfacial tension, M is the molecular weight of the solid, and ρ its density. According to the Noyes–Whitney equation, the dissolution velocity is further enhanced because it is directly proportional to the concentration gradient. On the basis of these facts, particle size reduction has become the method of choice for improving oral bioavailability of drugs that show dissolution-rate limited absorption.

#### *Elimination of food effects*

In the presence of food, drug absorption can generally be reduced, delayed, increased, or remain unchanged[20]. Drugs that exhibit poor aqueous solubility commonly show an incomplete absorption as a result of their poor solubility in the GI fluids. Interestingly, when those drugs are taken with food an increase in absorption can be seen, which is referred to as a positive food effect. Gu and coworkers analyzed clinical data of food effects of 90 marketed compounds and found that 71% of BCS class II drugs showed a positive food effect[21]. This positive food

effect can be attributed to several factors including a delay in gastric emptying, pH alterations, and stimulation of bile secretion and portal blood flow[22]. Food effects can negatively influence therapeutic outcomes especially in the case of drugs that exhibit a narrow therapeutic window. If for example a high-fat meal is required to achieve effective drug levels, subtherapeutic concentrations may be obtained if the patient is taking the medicine without food[23]. Nanoparticulate formulations are able to eliminate positive food effects of poorly soluble compounds and, therefore, have the potential of enhancing therapeutic outcomes by preventing the occurrence of either subtherapeutic or toxic concentrations of the drug[1].

## Marketed products

Particle size reduction of drugs can generally be conducted by employing top-down or bottom-up approaches[24]. Top-down processes consist of particle size reduction of large drug particles into smaller particles using various milling techniques, such as media milling or high-pressure homogenization techniques. In contrast, the bottom-up approach includes different particle formation processes, in which the drug is commonly dissolved in a suitable solvent and then precipitated on addition of an antisolvent in the presence of suitable stabilizers. A number of commercial drug products employing the top-down approach have been approved by the FDA. Specifically, the media milling technique, patented by ELAN under the name Nanocrystal®, has shown great commercial success with already four oral drug products launched till date[25]. Media milling is a type of wet milling, in which the milling chamber is charged with the milling media, the dispersion media, which can be aqueous or nonaqueous, the drug and suitable stabilizers[26]. Particle sizes reported range from 80–400 nm and are a result of high shear forces generated by the impaction of the milling media with drug particles. The stabilizer(s) added to the milling chamber will adsorb to the nanocrystal surface thereby preventing agglomeration and improving wetting of particles. Typically, stable formulations are obtained at weight ratios of drug to stabilizer of 20:1–2:1. The final product after milling is a nanosupension, which can be dried and particles further processed to obtain solid oral dosage forms such as tablets or capsules.

In the following paragraphs, all currently approved Nanocrystal®-based drug products for oral delivery will be discussed in more detail.

### *Rapamune® (Sirolimus)*

Sirolimus, a triene macrolide antibiotic, is one of the most potent immunosuppressive agents for the prevention of graft rejection in organ transplantations[27]. Still, the formulation of sirolimus represents a significant challenge because of the compound's low aqueous solubility of 2.6 μg/mL and high log *P* of greater than 5[28,29]. In 1999, an oral lipid-based solution of sirolimus at a concentration of 1 mg/mL was approved by the FDA[30]. Specifically, sirolimus is solubilized in a phosholipid-based carrier, Phosal®50 PG, and polysorbate 80. The bioavailability of the oral solution is only 14%, which can be mainly attributed to metabolism of sirolimus by intestinal and hepatic cytochrome P 450 3A (CYP3A) enzymes and intestinal P-glycoprotein transport[31]. The influence of a high-fat meal on the systemic absorption of sirolimus after oral administration of the solution was investigated in 22 healthy volunteers[32]. It was found that sirolimus was absorbed more slowly when administered after a high-fat meal than when administered after fasting. The oral bioavailability was only increased to a modest extent of 35%, which was ascribed to a combination of enhanced absorption and inhibition of CYP3A and P-glycoprotein in the intestine. Besides low bioavailability, the solution of sirolimus has several other disadvantages such as unpleasant taste and need for refrigeration. To overcome problems associated with the oral solution, an oral tablet using the Nanocrystal® technology was developed and launched by Wyeth in 2000. This tablet provides patients with more convenient administration and storage than the oral solution. The pharmacokinetic parameters, efficacy, and safety of sirolimus tablet and oral solution for the prevention of renal allograft rejection were evaluated and compared in a multicenter, open-label study involving 447 patients[33]. Pharmacokinetic analysis demonstrated that there were no statistically significant differences for the $AUC_{0-24\ h}$ and oral-dose clearance among the two formulations. It was further noted that intersubject variabilities for the $AUC_{0-24\ h}$ were large for both formulations. Besides, the $t_{max}$ was significantly higher for the tablet when compared with the solution, indicating that sirolimus was more slowly absorbed from the tablet formulation. Furthermore, equivalence of both formulations was demonstrated regarding efficacy failure, defined as a composite of the first occurrence of biopsy-confirmed acute rejection, graft loss, or death in the first 3 months. In addition, no significant differences in patient and graft survival 3, 6, and 12 months after transplantation were observed between the treatment arms. No significant differences in the incidence of adverse events such as infections, malignancies, or death were seen between the tablet and solution treatment group. Overall, the results demonstrated that the sirolimus tablet formulation based on the Nanocrystal® technology is therapeutically equivalent to the oral solution formulation. The tablet formulation, however, provides additional benefits like stability at room temperature and improved palatability.

### *Emend® (Aprepitant)*

Aprepitant is a highly selective antagonist of the neurokin-1 receptor[34]. Studies have shown that aprepitant combined with a 5-hydroxytryptamine$_3$ receptor antagonist and dexamethasone effectively prevents highly emetogenic chemotherapy-induced nausea and vomiting[35].

Aprepitant is a basic compound with a p$K_a$ value of 9.7[36]. The aqueous solubility of its thermodynamically stable polymorphic form I varies from 3 to 7 μg/mL in

the pH range of 2–10, while a log *P* value of 4.8 indicates high lipophilicity. During phase I and IIa clinical trials, significant positive food effects were seen with tablet formulations containing micronized aprepitant particles at a dose of 100 mg. Particularly, a threefold increase in exposure was observed under fed conditions. Data also suggested that high doses of aprepitant would be necessary to achieve efficacious drug levels. To guide formulation development, a beagle dog model was employed and the influence of particle size and food on the absorption of aprepitant was investigated[36]. Specifically, suspensions of alpine-milled (mean particle size 5.49 μm), jet-milled (mean particle size 1.80 μm), wet-milled (mean particle size 0.48 μm), and Nanocrystal® media milled (mean particle size 0.12 μm) aprepitant were dosed orally. The decrease in particle size resulted in a significant increase in oral absorption: a fourfold increase in AUC was observed for the media-milled suspension compared with the alpine-milled suspension. Additionally, the alpine-milled suspensions exhibited a significant food effect, while a food effect was completely eliminated for the media-milled suspension. On the basis of the results, aprepitant was formulated using the Nanocrystal® technology. The nanosuspension obtained by media-milling is converted into beads by applying a colloidal coating dispersion in a Wurster-column coating process followed by filling into hard-gelatin capsules[37]. The product was launched by Merck in April 2003 and is available in two dose strengths, 80 and 125 mg. Phase III clinical studies showed that the addition of aprepitant capsules to a standard regimen of ondansetron and dexamethasone improved the control of chemotherapy-induced nausea and vomiting associated with highly emetogenic cisplatin-based chemotherapy throughout the acute and delayed phases[35]. In general, the aprepitant regimen was well tolerated, with adverse events comparable with those observed with the standard regimen. Overall, aprepitant can greatly enhance supportive care of patients with cancer. A study by Poli-Bigelli reported that 74.7% of patients showed minimal or no impact of chemotherapy-induced nausea and vomiting on daily life, compared with 63.5% for patients on standard therapy[38].

### *Tricor® (Fenofibrate)*

Fenofibrate is a third-generation fibric acid derivative indicated for the treatment of hypercholesterolemia, combined dyslipidemia, remnant hyperlipidemia, hypertriglyceridemia, and mixed hyperlipemia[39]. Several fenofibrate products have been marketed since its discovery in 1975, among them a micronized capsule formulation (TriCor®; 67 and 200 mg) in 1998 and a micro-coated tablet formulation (TriCor®; 54 and 160 mg) in 2001[40,41]. The latter was developed to improve low fenofibrate bioavailability and high intersubject variability associated with the micronized capsule formulation. Particularly, a suspension containing micronised fenofibrate and the hydrophilic polymer polyvinylpyrrolidone was directly coated onto an inert hydrosoluble excipient core[42,43]. Even though, this tablet formulation demonstrated enhanced bioavailability combined with less variability in the maximum plasma concentration and extent of absorption between the fed and the fasted state, a positive food effect could not completely be eliminated[41]. In fact, bioavailability of the tablet formulation was increased by 35% when taken together with food compared with the fasted state[44]. Therefore, fenofibrate tablets had to be taken with food. Since it cannot be expected that patients strictly adhere to the guidelines, inconsistent and suboptimal exposure is likely to occur with the tablet formulation. In November 2004, a new fenofibrate tablet formulation based on the Nanocrystal® technology was introduced in the United States to replace the micro-coated tablet formulation. The absorption of the new formulation (TriCor®; 48 and 145 mg) is not dependent on food and, therefore, results in more consistent blood concentrations. A study conducted by Maciejewski and Hilleman compared the effectiveness of the 145-mg nanocrystalline tablet formulation with the micro-coated 160-mg tablet formulation in patients with coronary heart disease and dyslipidemia[44]. Patients were treated for a minimum of 6 months with fenofibrate 160 mg/day and were then switched to a minimum of 3-months treatment with fenofibrate 145 mg/day. Statistically significant reductions of 4.6% for mean triglycerides and 2.3% for low-density lipoprotein cholesterol (LDL) were observed after switching from fenofibrate 160 mg/day to fenofibrate 145 mg/day. Although no significant changes in the level of high-density lipoprotein cholesterol were noticed, the improvements in LDL and triglycerides indicate that nanoparticulate fenofibrate is more effective for treating combined dyslipidemia. Additionally, the elimination of a positive food effect for the nanoparticulate fenofibrate simplifies administration of the dosage form thereby greatly increasing patient compliance.

### *Megace®ES (Megestrol acetate)*

Megestrol acetate is a synthetic derivative of naturally occurring progesterone[45]. The drug is an effective appetite stimulant and, therefore, commonly prescribed to treat anorexia–cachexia syndrome associated with underlying diseases such as AIDS and cancer[46]. Megestrol acetate is characterized as a BCS II drug with a low aqueous solubility of 2 μg/mL and high intestinal permeability[47]. An oral suspension (Megace®) containing the drug in micronized form at a concentration of 40 mg/mL was first approved by the FDA in 1993. The initial adult dose recommended is 800 mg/mL requiring a large volume of 20 mL to be administered per day. In addition, the highly viscous character of the suspension results in relatively long residence times in the mouth or tubing. Therefore, the suspension is not well accepted by patients, particularly those depending on feeding tubes[48]. Administration of megestrol acetate suspension also results in highly variable levels of systemic exposure. A pharmacokinetic study in patients infected with HIV who were administered a single oral dose of 800 mg for

21 days reported a high degree of interpatient variability in megestrol acetate pharmacokinetic parameters with an eightfold and fivefold range in the rate and extent of absorption, respectively[49]. To increase bioavailability and decrease interpatient variability as well as suspension viscosity, an oral nanocrystal suspension was developed. Megace®ES, a more concentrated suspension containing 125 mg megestrol acetate per mL, was approved by the FDA in 2005. Due to the increase in drug concentration, the volume to be administered was reduced by a factor of four. Megace®ES is also 16 times less viscous (10 cP versus 163 cP) than the original Megace® formulation and, therefore, it is easier to consume, which will potentially increase patient compliance. The efficacy of Megace®ES oral suspension for treating anorexia–cachexia syndrome in patients with AIDS has been demonstrated in placebo-controlled, randomized efficacy trials. Megace®ES effectively stimulated appetite in nine of 10 patients and increased mean weight gain by 10.7 pounds versus placebo in 12 weeks[50].

In addition, a study conducted by Deschamps et al. investigated the pharmacokinetic properties of Megace®ES and Megace® under fed and fasted conditions in healthy male volunteers[51]. For the Megace®, oral suspension the AUC and $C_{max}$ in the fed state were increased by 52% and 86%, respectively, relative to the fasted state. In contrast, AUC and $C_{max}$ of Megace®ES in the fed state were only increased by 26% and 30%, respectively. Overall, AUC in the fed state was comparable for Megace® and Megace®ES. However, in the fasting state, Megace®ES resulted in significantly enhanced bioavailability. The results clearly indicate that Megace®ES is the preferred formulation for treating patients with anorexia–cachexia.

## Nanoparticles delivered via the pulmonary route

### Characteristics and benefits of nanoparticles for pulmonary delivery

The lung represents an attractive target for the delivery of drugs due to its unique anatomy and physiology. The respiratory system consists of two regions: the conducting airway, including trachea, bronchi, and bronchioles, and the respiratory region, comprising the respiratory bronchioles, alveolar ducts, and alveoli[52].

The morphology and clearance mechanisms of these two regions differ considerably. While the conducting airways are characterized by a lower surface area and regional blood flow, the alveolar region of the lung features a large absorptive surface area and rich vascularization. Furthermore, clearance of foreign particles in the conducting airways is facilitated by entrapment in secreted mucus and transport toward the oropharynx by the continuous beating of the cilia of the respiratory epithelium (mucociliary escalator). In contrast, particles deposited in the alveolar region are primarily cleared from the lung by uptake into alveolar macrophages[53].

Inhalation of drugs through the respiratory tract can be utilized for both locally and systemically acting drugs. Local delivery of drugs to the lungs is attractive for many different diseases like asthma, pulmonary infections, or lung cancer as it directs the drug to the site of interest thereby achieving high local concentrations while minimizing systemic exposure. In addition, the alveolar region of the lung presents an excellent target for systemic delivery of drugs because of its large surface area of approximately 75–140 $m^2$ [54, 55]. Besides, the alveolar epithelium is extremely thin offering a very short airway-blood path that enables fast absorption. Pulmonary delivery of drug compounds also avoids first-pass metabolism and, therefore, offers the potential for higher systemic levels than would be achieved after oral delivery. Finally, pulmonary delivery represents a noninvasive delivery route for peptides and proteins that are normally administered via the parenteral route.

Drugs intended for local or systemic pulmonary delivery are generally administered in form of an aerosol that may be generated by employing metered dose inhalers, nebulizers, or dry powder inhalers. The deposition of aerosol particles within the respiratory tract is influenced by several factors including particle characteristics (e.g. particle size, density, hygroscopicity, shape, and electrical charge), the patient's breathing pattern (e.g. flow rate and ventilation volume), and lung morphology[56]. Particle size is one of the most important characteristics as it influences both, the total amount of inhaled particles and the site of deposition in the respiratory tract. Generally, particles will deposit by three different mechanisms: inertial impaction, gravitational sedimentation, and Brownian diffusion[57]. Impaction is the major deposition mechanism for particles with mass median aerodynamic diameters (MMAD) greater than 5 μm and primarily occurs in the upper airways and near-bronchial branching points[58]. It can be further increased by high inspiratory flow rates. Particles deposited by inertial impaction will subsequently be swallowed and do, therefore, not provide any therapeutic response. Particles with MMADs between 1 and 5 μm will preferentially be deposited by gravitational sedimentation in small airways and respiratory bronchioles, while particles with MMADs below 500 nm are mostly confined to the alveoli. Particles of below 1 μm are, however, to the most part not deposited in the airways but rather exhaled after inspiration. This has led to a general MMAD recommendation of 1–3 μm for optimal deep lung delivery[59]. Interestingly, ultrafine particles of less than 100 nm are also efficiently deposited in the deep lungs[60]. A study conducted in healthy adults demonstrated high deposition rates of ultrafine particles with the total deposition fraction increasing with decreasing particle size from from 100 nm to 40 nm and increasing respiratory time[60]. However, the delivery of ultrafine particles is limited by several factors such as lack of appropriate formulation techniques and restriction of the dose that can be delivered[61]. To ensure efficient deposition of nanoparticles in

the deep lungs, "Trojan" particles have been suggested as an alternative[62]. Specifically, nanoparticles are formulated into larger porous structures either completely composed of nanoparticles or existing of nanoparticles and suitable carrier materials such as sugars or phospholipids. Once the micron-sized particles are effectively deposited in the lungs, they will disassociate to release the nanoparticles. The influence of different formulation parameters on the properties of these delivery systems has been widely explored; however, *in vivo* performance has yet to be evaluated[63–65].

Once particles have deposited in the respiratory system, they are subjected to clearance mechanisms. Particles that are deposited onto the surface of the ciliated upper bronchial area are normally removed by the mucociliary escalator within 24 hr[66]. It has been reported that nanoparticles may overcome mucociliary clearance due to rapid displacement to the airway epithelium[67]. In addition, particles deposited in the alveolar region are commonly removed through phagocytosis by alveolar macrophages. The process of phagocytosis is, however, dependent on a variety of factors including particle characteristics such as stiffness, coating, and size[68]. Results from few studies indicate that uptake of inhaled nanoparticles by macrophages is insufficient and occurs rather sporadic and unintentional[69–71].

The combination of reduced phagocytotic and mucociliary clearance of nanoparticles provides the benefit of extended residence time of the drug at the site of action. In addition, nanoparticles facilitate a rapid onset of action, resulting from fast dissolution of the drug.

## Potential applications of nanoparticles in pulmonary delivery

### *Pulmonary mycosis*

Nanoparticles containing antifungals have been actively researched as inhaled therapeutic carriers for the prophylaxis and treatment of pulmonary mycoses. Aerosolized nanostructured formulations of itraconazole have shown promising results for the prevention of invasive pulmonary aspergillosis, a severe infection caused by *Aspergillus* ssp[72]. Itraconazole is a poorly water-soluble compound that is commercially available for oral administration in form of capsules or an oral solution. The use of itraconazole is, however, challenged by the low and erratic bioavailability of the capsule formulation, considerable GI side effects associated with the oral solution, and numerous drug interactions due to systemic exposure[73]. A study by Vaughn et al. compared the lung and serum concentrations in mice following oral and pulmonary dosing of amorphous nanoparticulate itraconazole compositions as well as the commercially available oral solution[74]. High and sustained lung tissue concentrations were obtained after inhalation of nanoparticulate itraconazole. Particularly, pulmonary nanostrucutred itraconazole achieved significantly greater (>10-fold) lung tissue concentrations compared with the oral solution, while serum levels were maintained above the minimum lethal concentration of *Aspergillus fumigatus.* Furthermore, *in vivo* efficacy in mice infected with *Aspergillus fumigatus* was demonstrated: nanostructured formulations of itraconazole significantly improved survival rate relative to the commercial itraconazole oral solution[72]. An additional study evaluated the safety of pulmonary nanostrucutred itraconazole in mice as assessed in changes in pulmonary histology. It was demonstrated that aerosolized administration of nanostructured itraconazole is safe with no evidence of bronchiolar, peribronchiolar, or perivascular inflammation observed in mouse lungs by histology[75].

### *Lung cancer*

Nanoparticles have also been suggested for local lung delivery of anticancer drugs. The efficacy of chemotherapy in lung cancer is mostly insufficient due to rapid development of cancer cell resistance during treatment[76]. As a consequence, higher doses of toxic anticancer drugs have to be administered, thereby increasing the risk of adverse side effects in other healthy tissues of the body. Localized delivery by inhalation increases accumulation in lung tumor cells and reduces systemic drug exposure and subsequent distribution into other organs. Nanoparticles are especially suitable for drug delivery to tumor cells since they selectively accumulate in the leaky tumor vasculature[77]. Hitzman et al. determined the feasibility of delivering 5-fluorouracil in form of lipid-coated nanoparticles to hamsters for use in lung cancer chemotherapy[78].The drug is currently being administered intravenously with only a small fraction reaching the site of action. Nanoparticles investigated were coated with a mixture of tripalmitin and cetylalcohol to sustain drug release. Delivery of these particles via inhalation to hamsters resulted in efficacious and sustained concentrations of 5-fluorouracil in expected tumor sites. The drug serum level was nearly 1000 times lower than the level in the lung demonstrating the effectiveness of local delivery.

Local lung delivery of nanoparticles loaded with doxorubicin has also been studied[79]. Particularly, doxorubicin nanoparticles were incorporated into inhalable lactose carrier particles using a spray-freeze-drying technique. Cytotoxic effects of particles were evaluated in lung cancer cell lines and compared with free doxorubicin. Doxorubicin nanoparticles demonstrated higher cytotoxicity compared with free drug possibly due to the fact that nanoparticles were readily internalized into cells by endocytosis, while free drug entered the cells through passive diffusion. Both, inhaled 5-fluorouracil and doxorubicin (Resmycin™) have been tested in form of aerosolized drug solutions in clinical trials in patients with malignant diseases in the lung[80,81]. No clinical studies have been conducted involving nanoparticulate doxorubicin and 5-fluorouracil yet. However, *in vitro* data as well as *in vivo* data from animal models suggest that nanoparticulate systems may act superior to the free drug.

### *Asthma*

Pulmonary delivery of budesonide in form of a nanocrystal suspension has been suggested for the treatment of

steroid-responsive pulmonary diseases such as asthma[82]. Budesonide is a poorly water-soluble compound that is available as a suspension for inhalation (Pulmicort Respules) with a nebulizer. The particle size of suspended particles is approximately 4.4 μm. The nanosuspension of budesonide that was suggested as an alternative was prepared employing the Nanocrystal® technology with particles in the range of 75–300 nm. The safety, delivery, and pharmacokinetics of nebulized nanobudesonide and Pulmicort Respules was evaluated in a randomized, double-blind, single-dose crossover study involving 16 healthy volunteers. Overall, both nebulized formulations were well tolerated. Nanobudesonide and Pulmicort Respules resulted in similar AUCs, suggesting a similar extent of pulmonary absorption. However, a higher $C_{max}$ was seen with nanobudesonide and in addition the $t_{max}$ was significantly smaller. Finally, nebulization of nanobudesonide resulted in significantly shorter nebulization times compared with Pulmicort Respules, which is advantageous with respect to patient compliance.

### *Pulmonary tuberculosis*

Nanoparticles in pulmonary drug delivery can also be useful in targeting specific cell types such as alveolar macrophages[83]. Uptake into these macrophages can be controlled by manipulating particle size and surface characteristics[84]. Alveolar macrophage targeting using nanoparticulate carriers has been specifically investigated for the treatment of pulmonary tuberculosis. The infection is caused by *Mycobacterium tuberculosis,* which is spread via airborne dissemination. Once inhaled, the pathogen reaches the alveoli where it is taken up by alveolar macrophages[85]. Pulmonary delivery of antitubercular drugs like isoniazid, rifampicin, and pyrazinamide ensures effective drug levels directly at the site of infection thereby decreasing systemic side effects and bypassing first-pass metabolism. The effectiveness of nanoparticulate pulmonary drug delivery systems in treating *Mycobacterium tuberculosis* has been demonstrated for different carrier materials in animal models. Pandey et al. investigated the suitability of poly (D,L-lactide-co-glycolide) (PLG) nanoparticles encapsulating isoniazid, rifampicin, and pyrazinamide for nebulization[86]. The majority of drug-loaded PLG-nanoparticles were in the size range of 186–290 nm, while the MMAD of nebulized particles was determined to be 1.88 μm favoring broncho-alveolar lung delivery. Single nebulization to guinea pigs resulted in sustained therapeutic drug levels in plasma and lungs. Relative bioavailability compared with oral administration was enhanced by factor 12.7, 32.8, and 14.7 for rifampicin, isoniazid, and pyrazinamide, respectively. Upon pulmonary administration of drug-loaded nanoparticles to guinea pigs infected with *Mycobacterium tuberculosis,* no tubercle bacilli were detected in the lungs after five doses of treatment. In contrast, 46 daily doses of orally administered drug were required to obtain an equivalent therapeutic benefit. A study conducted by Sharma et al. investigated the influence of a lectin coating on the dose frequency of antitubercular drugs encapsulated into PLG-nanoparticles[87]. The lectin coating was chosen based on the assumption that interactions of lectin with its receptors on the alveolar epithelium could potentially sustain drug release. In fact, after pulmonary administration of coated and uncoated PLG-nanoparticles, rifampicin was detectable in plasma for 13–14 and 4–6 days, respectively, indicating effective binding of lectin to the alveolar epithelium. Besides biodegradable polymers like PLGA and PLG, lipid materials have been investigated as nanocarrier materials for treatment of pulmonary tuberculosis. Particularly, solid lipid nanoparticles (SLN) have shown promising therapeutic potential[88]. SLN exhibit several advantages over polymeric nanoparticles such as higher tolerability and faster *in vivo* degradation[89,90]. However, research related to pulmonary delivery of SLN is still in its infancy and their full potential has yet to be explored[91]. Overall, nanoparticulate carriers for pulmonary delivery of antitubercular drugs have demonstrated the potential to effectively target alveolar macrophages thereby minimizing toxic side effects and dosing frequency. These, in turn, are key factors for improving therapeutic outcomes as well as patient compliance.

### *Insulin-dependent diabetes mellitus*

Nanoparticles for systemic delivery have the potential to sustain drug release in the lung and accordingly systemic circulation as shown for insulin. Kawashima and coworkers prepared biodegradable PLGA-nanospheres with mean diameters of 400 nm by a modified emulsion solvent diffusion method[92]. An aqueous dispersion of insulin-loaded-PLGA nanospheres and an insulin solution serving as a control were nebulized into the trachea of fasted guinea pigs for 20 min. The PLGA-nanosphere dispersion significantly reduced blood glucose levels over a prolonged period of 48 hr compared with only 6 hr for the insulin solution. In addition, Zhang et al. investigated the suitability of insulin-loaded polybutylcyanoacrylate nanoparticles for systemic lung delivery[93]. The nanoparticle dispersion was given intratracheally to rats and their hypoglycemic effect compared with an insulin solution administered either intratracheally or subcutaneously by bolus injection. It was shown that the hypoglycemic effect of insulin-loaded nanoparticles lasted much longer than in the case of the insulin solution. However, results indicated that bioavailability of insulin-loaded nanoparticles by intratracheal delivery was lower than by subcutaneous administration, with a relative bioavailability of insulin-loaded nanoparticles of 57.2% compared with subcutaneous administration of the solution.

In both cases, the polymeric nanocarrier materials sustained insulin release reducing glucose blood levels over a prolonged time compared with a pulmonary-delivered insulin solution. Overall, pulmonary delivery of insulin nanoparticles has the potential to increase patient compliance by reducing dose frequency. Additionally, it represents a more patient-friendly noninvasive form of administration.

#### *Concerns regarding nanoparticles in pulmonary delivery*

The high surface area of nanoparticles, which makes them so attractive for drug delivery, also raises concerns regarding potential toxicity since it is chemically more reactive. As the respiratory tract is the primary entrance way for nanoparticles into the human body, numerous studies have studied the potential of nanosized-airborne pollutants to cause adverse health effects. These studies have reported the occurrence of inflammatory reactions as well as carcinogenicity[94,95]. Early studies in rats showed that ultrafine $TiO_2$ particles (below 50 nm) have a significantly greater pulmonary inflammatory potency than larger $TiO_2$ particles (~200 nm[96]). Data suggest that ultrafine particles due to their high surface area lead to oxidative stress and calcium changes in alveolar macrophages and epithelial cells thereby activating cells for inflammation[97,98]. Furthermore, studies have shown that poorly soluble inhaled nanoparticles increase the incidence of lung tumors in rats[99,100]. It was hypothesized that inflammation and inflammatory cell-derived oxidants as induced by high doses of insoluble nanoparticles initiate and promote neoplastic transformation of epithelial cells[101]. However, it is important to note that most studies have investigated inorganic and insoluble materials like $TiO_2$, while carrier materials for drug delivery are commonly of soluble or biodegradable and organic nature. On the basis of that, physiological responses caused by therapeutic materials are expected to notably differ from those originated by insoluble materials[83]. Concerns regarding the toxicity of nanoparticles also stem from the fact that they can be taken up by the epithelium gaining access to the systemic circulation and extrapulmonary organs causing undesirable side effects[102]. Overall, the influence of key factors such as size, persistence, solubility, and charge has to be further investigated to understand nanoparticles full impact on the body.

## Nanoparticles delivered via the intravenous route

### Benefits of nanoparticles delivered intravenously

In the case of intravenous administration, the medication is directly injected into a patient's vein by means of a small-volume bolus injection (<50 mL) or an infusion (50–1000 mL[103]). Since the drug is directly delivered to the bloodstream, a rapid onset of action is achieved, which is often vital in emergency situations such as cardiac arrest or status epilepticus[104]. Besides, intravenous administration may be employed for drugs that show poor absorption from the GI tract or undergo extensive first-pass metabolism after oral administration.

To ensure safe intravenous therapy, formulations have to be sterile and free from pyrogens and visible particulate matter[105]. In addition, pH and tonicity of intravenous formulations should preferably be matched to physiological values to avoid pain and tissue irritation. As water is the principal constituent of blood, intravenous formulations are ideally formulated as aqueous solutions to maximize *in vivo* tolerability. However, poorly water-soluble drugs intended for intravenous administration often need to be solubilized with large amounts of organic cosolvents and surfactants or cyclodextrins, which may cause undesirable side effects[1]. Furthermore, dilution of the solubilized formulation in the bloodstream may result in precipitation of the drug, which in turn may induce inflammation of the vein wall[106].

Intravenous delivery of poorly water-soluble drugs in form of aqueous suspensions is a viable alternative that avoids the use of harsh solubilizing excipients. The formulation of suspensions for intravenous administration, however, requires the particle size to be reduced to the submicron range (nanosuspensions) to prevent vascular occlusion[107]. The use of drug nanosuspensions also ensures conformance with the limits specified by the USP regarding particulate matter in injections, which requires that the average number of particles present in the units tested does not exceed 3000 per container equal to or greater than 10 μm and 300 per container equal to or greater than 25 μm when the microscopic particle count test is applied[108]. As pointed out earlier, the use of nanosuspensions is also beneficial in that it goes along with a substantial increase in the dissolution rate. Besides, nanosuspensions are favored when low-potency drugs are formulated since they offer higher mass per volume loadings[109]. Also, nanosuspensions are generally preferred over solutions as drugs are more chemically stable in the solid-state than in the dissolved-state. Finally, administering drugs in form of nanoparticles enables targeted delivery due to their particulate nature and the possibility of surface modification. Targeted delivery of nanoparticles via intravenous administration has been especially explored to treat various types of cancer.

### Nanoparticles in cancer therapy

#### *Size and surface characteristics*

The size and surface characteristics of intravenously administered nanoparticles are key factors determining their circulation time and target specificity. To stay in the systemic circulation for a sufficient time, nanoparticles need to escape the reticuloendothelial system (RES), which is predominantly distributed in liver, lung, spleen, and bone marrow[110]. Surface modification with water-soluble polymers such as PEG has shown to reduce RES uptake of nanoparticles by minimizing interactions between nanoparticles and opsonin molecules[111]. In addition, smaller particles (~100 nm) escape the RES more easily. The tumor vasculature is known to be leaky with junctions between the epithelial cells ranging from 100 to 600 nm depending on the type of tumor[112]. Therefore, the optimal size of nanoparticles is thought to be between 10 and 100 nm.

#### *Passive targeting*

Nanoparticles that exhibit the size and surface features described above will passively accumulate at the tumor site. Accumulation in healthy tissue is theoretically

restricted by the fact that normal vasculature has much tighter junctions of less than 10 nm[110]. Besides increased permeability, tumor vessels are also characterized by a less effective lymphatic drainage system so that drugs are retained in the interstitium for prolonged times.

### *Active targeting*

Nanoparticles offer the additional benefit that their surface can be easily modified, for example, through the addition of targeting ligands[113]. Targeting ligands in cancer research commonly include peptides, proteins, and antibodies all of which are added to facilitate binding to receptors that are over-expressed in tumor cells. Since they enable selective uptake into tumor cells, they have become an active area of research. Promising results have been demonstrated with biodegradable poly (d,l-lactic-co-glycolic acid)-block-poly (ethylene glycol) (PLGA-b-PEG) nanoparticles loaded with doxetaxel that were surface-targeted to the extracellular domain of a prostate-specific membrane antigen by conjugation of an RNA aptamer[114]. *In vitro* cytotoxicity tested in prostate epithelial cells demonstrated that nanoparticles carrying the RNA aptamer were significantly more cytotoxic as compared with control nanoparticles that were missing the targeting ligand. The aptamer-nanoparticles also exhibited remarkable efficacy and reduced toxicity in xenograft mice. After a single intratumoral injection, complete tumor reduction was observed in five of seven mice and 100% of animals survived the 109-day study. In contrast, only two of seven mice that were administered nanoparticles without the targeting aptamer showed complete tumor reduction and only 57% survived the study.

*In vivo* biodistribution studies comparing targeted and nontargeted nanoparticles in a murine model demonstrated that the enhanced efficacy of targeted nanoparticles is not necessarily a result of increased tumor accumulation[115]. More generally, targeting ligands increase cellular internalization without affecting overall tumor uptake. This highlights the importance of the physicochemical properties of the nanoparticle carrier material itself as it will largely determine the overall biodistribution and pharmacokinetics of the delivery system.

### *Multidrug resistance*

Resistance of tumor cells to a variety of anticancer drugs, for example, through P-glycoprotein-mediated efflux, represents a major challenge in achieving clinical efficacy. Results from different studies suggest that nanoparticulate delivery systems are able to circumvent efflux through transmembrane P-glycoprotein[116,117]. One suggestion is that since nanoparticles enter the cell via endocytosis, they avoid recognition by P-glycoprotein reaching high intracellular concentrations. However, there is no ultimate verification that nanoparticles are able to evade P-glycoprotein-mediated efflux in humans, even though clinical data points to it[113].

## Marketed product

### *Abraxane® (Nanoparticle albumin-bound (nab™)-paclitaxel)*

Paclitaxel is a mitotic inhibitor that has a wide spectrum of antitumor activity when used as monotherapy or in combination chemotherapy regimens[118]. The poorly water-soluble molecule was first formulated in form of a nonaqueous solution for intravenous infusion (Taxol®). In this solution, paclitaxel is solubilized in a mixture of polyoxyethylated castor oil (Cremphor®EL) and ethanol. The formulation can cause considerable side effects, particularly neuropathy and hypersensitivity reactions, both of which are linked to the use of Cremephor®EL. To prevent the occurrence of hypersensitivity reactions, premedication with steroids and antihistamines is required. To overcome the problems associated with the solution, a nanoparticulate paclitaxel formulation (Abraxane®) was developed. The nanoparticles are composed of a hydrophobic drug core surrounded by a hydrophilic albumin coating. The particles are manufactured by forming an oil-in-water emulsion of a water-immiscible solvent containing paclitaxel and an aqueous phase containing albumin. This emulsion is high-pressure homogenized to reduce the particle size and cross-link the albumin to stabilize particles[119]. Finally, the solvent is evaporated to yield a colloidal dispersion containing albumin-coated nanoparticles of mean diameters of 130 nm. The small particle size allows for sterile filtration, an important feature, since the dispersion cannot be sterilized by conventional means such as autoclaving due to the high amount of albumin present. In addition, albumin may serve as a substrate for microbial growth; therefore, the formulation is stored in lyophilized form and has to be reconstituted before infusion[120]. Particularly, nanoparticle albumin-bound paclitaxel (nab–paclitaxel) is reconstituted in normal saline at concentrations of 2–10 mg/mL compared with 0.3–1.2 mg/mL for the Cremophor®EL containing solution. Therefore, the volume to be administered and consequently the infusion time could be significantly reduced[118].

Nab–paclitaxel can leave the circulation through leaky tumor microvasculature and accumulate in the interstitium. In addition, the albumin shell facilitates interaction with receptors on endothelial cells (gp60) and in the tumor interstitium (SPARC). Specifically, albumin-gp60 binding leads to endothelial transcytosis of nab-paclitaxel into the extravascular space, where SPARC, which is overexpressed in many types of cancer, entraps the albumin in tumor cells resulting in high intratumoral accumulation[121]. In fact, mice bearing human tumor xenografts treated with nab–paclitaxel showed a paclitaxel area under the curve that was 33% higher than for Cremophor® EL-based paclitaxel, indicating more effective intratumoral accumulation[122]. It was also determined that the maximum-tolerated dose is 300 mg/m$^2$, which is about 70% higher than for the Cremophor®EL-based solution of paclitaxel.

Furthermore, phase I clinical trials demonstrated that paclitaxel $C_{max}$ and area under the curve values

increase linearly over a dose range of 135–300 mg/m$^2$ [118]. This represents a huge advantage over the Cremophor®EL-paclitaxel formulation, which exhibits parabolic pharmacokinetics causing an unpredictable relationship between dose, efficacy, and risk of toxicity[123]. The nonlinear pharmacokinetics has been related to the formation of Cremophor®EL micelles in the plasma, which entrap paclitaxel, sequestering it in the plasma[124].

A phase III clinical trial involving 454 patients with metastatic breast cancer demonstrated that nab–paclitaxel administered at a dose of 260 mg/m$^2$ resulted in significantly higher response rates (33%) compared with CremophorEL®-based paclitaxel administered at a dose of 175 mg/m$^2$ (19%[125]). Despite the higher dose of nab–paclitaxel that was delivered, patients treated experienced significantly less neutropenia than patients treated with CremophorEL®-based paclitaxel. The incidence of hypersensitivity reactions (any grade) was low for nab–paclitaxel (<1%) despite the absence of premedication.

In summary, nab–paclitaxel overcomes several limitations of the Cremophor®EL-based paclitaxel. It clearly represents an important advance in the treatment of patients with metastatic breast cancer offering significantly improved efficacy without compromising patient safety and quality of life.

## Conclusions

Nanoparticulate drug delivery has clearly provided new opportunities for solving issues associated with problematic drugs or disease states. Great commercial success has been achieved in the area of oral drug delivery either by formulation of new water-insoluble drugs or reformulation of established therapeutic agents. Results from clinical trials demonstrate that nanoparticulate formulations generally enable superior therapeutic outcomes than compared with standard formulations. Even in the case where nanoparticulate drug delivery systems have shown no superiority but rather equivalence regarding their efficacy; they commonly offer additional benefits such as improved stability or simplified administration thereby improving patient compliance and consequently therapeutic responses.

Despite significant accomplishments in the area of nanoparticulate drug delivery, some challenges still persist. Particularly, concerns regarding the safety of nanoparticles represent a considerable barrier toward successful establishment of nanoparticulates in the field of drug delivery. To make nanoparticulate drug delivery systems available for a wide variety of disease states and delivery routes, their full biological impact on the human body has to be more closely explored.

Overall, the implementation of nanotechnology in drug delivery represents an important advance with substantial potential to improve therapeutic outcomes and increase patient's quality of life.

## Declaration of interest

The authors report no conflict of interest.

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