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REVIEW

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Nanocrystals: an emerging paradigm for cancer therapeutics

Ayush Patel[1], Krishi Patel[1], Viral Patel[1*], Mithun Singh Rajput[2], Ravish Patel[1] and Amarjitsing Rajput[3]

Abstract

Background Medical fraternity are continuously pitching toward the development of novel mechanisms to combat the menace of cancer and to enhance the efficacy of prevailing molecules. During the drug development phase, majority of new molecular entity pose a threat due to hydrophobic nature, that compromises its bioavailability upon administration. These suboptimal accumulation and low drug loading hampers the clinical translation in cancer therapy.

Main body of abstract Nanotechnology with valuable advantages create possibilities to accelerate the efficacy of treatment. Compared to matrix-based formulations, drug nanocrystals (NCs) with smaller size, high drug loading, high active targeting, extended circulation, great structural stability, tailored dissolution, and being carrier free have sparked a lot of interest in drug delivery. Many hydrophobic drugs were explored as drug NCs such as—doxorubicin, paclitaxel, campothecin and so on. However, premature leakage and clearance by mononuclear phagocytosis system lead to some great obstacles in the clinical applications of drug NCs.

Conclusion In the recent years, strategies leading to surface modification are applied to improve uncontrolled drug release and targeting efficiency to tumor cells. The current review sheds light on various properties of drug nanocrystals, brief insights on its fabricating techniques, approaches for tumor targeting with NCs, and their applications in cancer imaging and therapeutics.

Keywords Nanocrystals, Poorly soluble drugs, Anticancer drugs, Preparation technologies, Progress

Background

The field of medicine faces challenges for effectively addressing the complexities of cancer, a multifaceted group of diseases characterized by uncontrolled growth and spread of abnormal cells. Unlike normal cells, which follow a regulated life cycle, cancer cells disrupt this balance and undergo uncontrolled division, leading to the formation of tumor masses[1]. Many factors contribute to development of cancer, like—genetic predisposition, food habits, lifestyle changes, infection caused by harmful microorganisms, and environmental factors [2]. Cancer has become a global burden with approximately 10 million deaths in the year 2022 [3–5]. The conventional therapies include radiation therapy, chemotherapy or combination therapy, while immuno-therapy, gene therapy, cell therapy, photodynamic therapy, and antibody therapy have also gained momentum, however, there effectiveness is still under debate as it lacks site specificity and low build-up inside the tumor cells [6, 7]. As a consequence, cancer treatment has now turned toward tailoring approaches which are patient specific depending on the profiles and characteristic of the disease. In this era, nanotechnology being a blend of technology, biomedicine and biomaterials, has been growing as a

*Correspondence:
Viral Patel
viral9018@gmail.com
[1] Department of Pharmaceutics, Ramanbhai Patel College of Pharmacy, Charotar University of Science and Technology, Off-Nadiad Petlad Highway, Changa, Anand, Gujarat, India
[2] Department of Pharmacology, Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, India
[3] Department of Pharmaceutics, Bharti Vidyapeeth Deemed University, Poona College of Pharmacy, Erandwane, Pune, Maharashtra 411038, India

plausible approach to overcome the current shortfalls. Of them, NCs have sparked a lot of interest in the field of cancer therapeutics, offering unique advantages over conventional drug delivery systems. These nanoscale particles possess the ability to precisely target tumor tissues, stimulate drug solubility, and enhance therapeutic efficacy, revolutionizing the landscape of cancer treatment. The application of NCs in cancer therapy is driven by their small size, large surface area, and unique physicochemical characteristics. These attributes enable efficient drug loading and controlled release, facilitate targeted drug delivery with minimizing side effects on healthy tissues. They have also shown potential to overcome biological barriers, penetrating deep into tumor tissues by both active and passive pathways, and reaching intracellular targets, thereby maximizing treatment outcomes [8, 9]. In addition to drug delivery, NCs play a pivotal role in cancer imaging techniques. Their unique optical and magnetic properties have transformed cancer imaging, providing high-resolution and sensitive modalities. NCs in the form of semiconductor quantum dots, exhibit size-dependent fluorescence emission and high photo-stability, making them exceptional candidates for precise tumor visualization. Similarly, surface modifications with targeting ligands, NCs can be used as contrast agents for imaging, enabling accurate diagnosis, staging, and real-time monitoring of treatment responses [10, 11]. The integration of NCs with imaging techniques allows for multimodal approaches, facilitating comprehensive cancer diagnosis and personalized treatment regimens. Furthermore, NCs offer a versatile platform for combining different therapeutic agents within a single system, enabling synergistic effects, improved drug ratio, and targeted release. This approach enhances therapeutic efficacy while minimizing systemic toxicity. Further by engineering NCs to respond to external stimuli, controlled drug activation and spatiotemporal release can be achieved, paving the way for treatment tailored to individual patient profiles [12, 13].

Despite their immense potential, the full utilization of NCs in cancer treatment requires addressing challenges associated with formulation stability, scalability, and establishing reliable quality control measures. Thus, the current review comprehensively focuses on unveiling the potential of NCs as targeted delivery for the management of cancer.

Main text

Brief insights on nanocrystals and their fabricating method

NCs are nanometer-sized particles consisting purely of drug substances stabilized using suitable stabilizers [14]. The term "nanocrystals" is specifically used to refer to these crystalline nanoparticles or liquid crystalline nanoparticles. Comparing NCs to other carrier-based nanoparticles (NPs), nanocrystals exhibit a high drug loading which allows for a reduction in dose while still enhancing bioavailability and drug safety. Also, for formulating NCs, there is practically no use of organic solvents, making NCs devoid of residual solvents and solvent associated toxicity concerns. Additionally, improved solubility and dissolution rate will promote the pharmacokinetics and bio-distribution of drugs as suggested by Peltonen et al. [15].

The main excipient in fabricating NCs include a stabilizer. The primary function of a stabilizer is to prevent ostwald ripening or aggregation of intrinsically diminuted drug nanoparticles and to preserve them in nano-crystalline form [16]. The selection of stabilizer and its concentration form one of the critical process parameter [17]. Now-a-days, researchers have a wide list of stabilizer starting from non-ionic, ionic, amphoteric to polymeric. Polymeric stabilizers have gained an edge over the others due to their enhanced wetting and steric stabilization property. These stabilizers impart stability to NCs via electrostatic repulsion, van der Waals forces, steric stabilization, surface coating, reducing interfacial tension, etc., [18].

Production techniques of NCs are categorized as top-down, bottom-up and combination technologies (details briefed in Table 1), subsequent section describes briefly the different sub-types of these technologies explored by researchers across the globe.

Table 1 Different techniques for formulating nanocrystals, including both top-down and bottom-up approaches, as well as combinational technologies [19–22]

Formulation methodology	Formulation techniques	Mechanism of size reduction
Top-down approach technologies	Media milling	Impact and attrition
	High-pressure homogenization	High shear and high pressure
Bottom-up approach technologies	Precipitation or crystallization	Precipitation or controlled crystallization processes
Combinational technologies	Hybrid approaches	High energy driven
	Supercritical fluid technology	Uses super critical fluids

Top-down techniques diminute the drug substances in micron size state to nanometer size. Different approaches include—media milling, high-pressure homogenization (HPH) (diagrammatically described in Fig. 1), and ultrasonication. Media milling, a mechanical technique used for size reduction, has demonstrated considerable efficiency in the production of NCs. This method employs milling media to fragment drug particles into smaller dimensions, milling agent such as beads/coated balls made of—glass, zirconium oxide, chromium, agate or some special polymers for grinding of drug particles and a stabilizer to ensure the stability of formulation. The main principle of media milling includes impact and attrition [23–26]. HPH is another top-down technique used for NC formulation. It involves subjecting the drug suspension to high pressure (ranging from 100 to 2000 bar)/ultra-sonication, forcing it through a narrow nozzle or valve. During this process, the particles undergo high shear stress resulting in generation of nano-sized crystalline particles [27, 28]. Dissocubes, Nanopure and Nanoedge are few patented technologies based on use of HPH [29]. Another approach, utilizes ultra-sonication as a means to reduce the particle size. Probe sonicator is a tool to transmit high and low power ultrasound cycles through the drug suspension. These cycles create alternative low and high pressure respectively in the suspension, causing formation of bubbles in low pressure cycle, which break during high pressure cycle. These results in cavitation effect causing reduction in particle size [30, 31].

An alternative strategy for developing NCs is through a "bottom-up" approach, where drug substances are precipitated to form nanoparticles with accurate control on particle growth. The critical aspect with this approach is controlling the size of re-crystallized particles, as uncontrolled growth might result in Oswald ripening. Some of the technologies using these approach include- high gravity controlled precipitation, sono-crystallization, liquid jet precipitation, rapid expansion of supercritical solution, super critical anti-solvent, multi-inlet vortex mixing, evaporative precipitation into aqueous solution, etc. All these approaches have their own applications, however, major limitation lies in the use of organic solvents [20, 32, 33]. Some of these techniques have been modified by using supercritical fluids instead of organic solvents. Rapid expansion of supercritical solutions (RESS) and supercritical solvent/anti-solvent (SAS) methods of fabricating drug NC are based on the solubility of drugs in supercritical fluids [34, 35]. Hydrosol® and

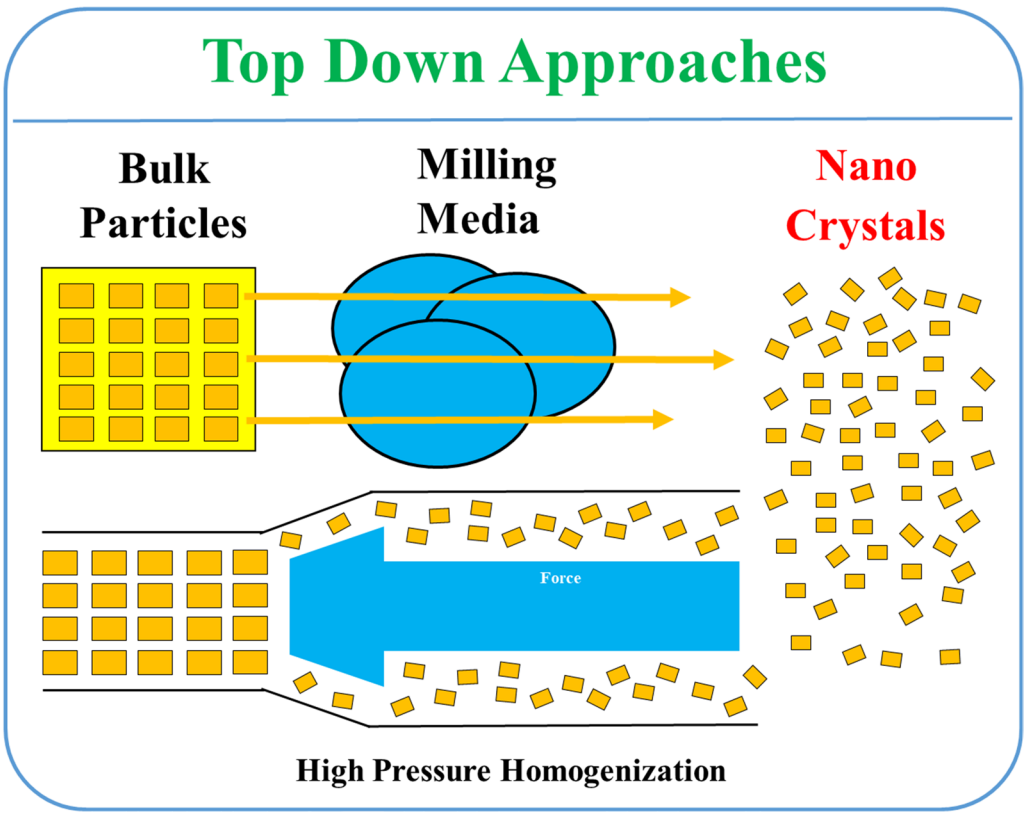

Fig. 1 Top-down approaches for fabrication of nanocrystals

Nanomorph® are the patented technologies to produce drug NCs with size less than 100 nm. An innovative work reported by Han et al., revealed fabrication of paclitaxel nanocrystals (PNC) using a novel approach of evaporation and precipitation technique. The PNC were coated with tannic acid and ferric chloride to enable them for dual therapy—photothermal-chemotherapy. The in-vivo results exhibited mild photothermal activity along with strong tumor inhibition [36]. Through such innovative approach, advancements in the field of cancer treatment are being made, offering the potential for more effective and targeted therapies.

The combination technologies comprise of a two-step process—pre-treatment followed by processing. The pre-process step might be- pre-milling or precipitation, with a high-energy top-down process, such as milling or HPH, while the processing step includes evaporation, lyophilisation, etc. [37]. NANOEDGE®, was developed by Baxter, Inc., USA which involved precipitation of crystals as pre-treatment step and later the processing involved HPH to control the particle size and morphology [38]. More recent advancements have introduced- Nanopure® and SmartCrystal® technology into the market. Later technology integrated high-pressure homogenization with various pre-processes—like H42 (spray-drying pre-process), H69 (precipitation pre-process), H96 (lyophilization pre-process), and CT (media milling pre-process) [39, 40]. Hence, it could be summarized that all the different technologies are efficient enough for fabrication of different and stable NCs.

Role of nanocrystal technology in tumor targeting

Nanotechnology has sparked a lot of possibilities for effective delivery of poorly soluble drugs [41, 42]. They have emerged as a promising tool in the field of cancer therapy, offering unique properties and versatility to revolutionize treatment approaches [8]. One of the key advantage is their ability to serve as carriers for chemotherapy drugs by addressing crucial challenges of poor solubility, limited bioavailability, and systemic toxicity, thereby enhancing drug stability and solubility while enabling their efficient delivery to the tumor site. By modifying the surface of NCs with specific ligands, such as antibodies or peptides, they can be engineered to selectively recognize and bind to cancer cells. These targeted approach facilitates the direct delivery of therapeutic agents to the tumor site while minimizing exposure to healthy tissues [43].

NCs offer opportunities for both passive and active targeting. Passive targeting exploits the enhanced permeability and retention (EPR) effect, leveraging the unique characteristics of tumors to achieve increased drug concentration at the tumor site. This occurs due to the presence of leaky blood vessels and impaired lymphatic drainage in tumor tissues, allowing NCs to passively accumulate. On the other hand, active targeting involves incorporating ligands on the NC surface that can specifically recognize and bind to receptors overexpressed on tumor cells. This active targeting further enhances the accumulation of NCs within the tumor, augmenting treatment effectiveness [15, 44, 45]. The unique features of both pathways have been reflected in Table 2. Furthermore, NCs possess imaging capabilities that enable real-time monitoring of treatment response. Certain types of NCs, like quantum dots, exhibit unique optical properties and emit fluorescent signals when stimulated by light. This characteristic enables non-invasive imaging of tumors, facilitating early detection, precise diagnosis, and evaluation of treatment outcomes [46, 47]. Detailed application of NC-based treatment for targeting different cancers with different delivery mechanism has been discussed in the following sections.

Passive targeting

Passive targeting is a decisive strategy involving advantage of tumor vasculature hyper-permeability, and the immature lymph drainage system, NCs facilitate the accumulation of cytotoxic agents in tumor masses through EPR effect [48, 49]. This effect is particularly advantageous with nanoparticles having the size range of

Table 2 Role of nanocrystals in tumor targeting, along with their description, applications, and unique characteristics

Role	Description	Applications
Passive targeting	Facilitated by the enhanced permeability and retention effect	Imaging and Drug delivery
Active targeting	Surface modified with ligands or targeting molecules that specifically recognize biomarkers found in tumors	Targeted delivery to tumor cells that express specific biomarkers Facilitates precise imaging
Antibody-mediated targeting	Conjugation with antibodies that bind to tumor-specific antigens	Precise delivery and accurate imaging with great selectivity
Surface receptor-mediated	NCs are designed to specifically identify and attach to surface receptors on tumor cells	Facilitates precise receptor targeted drug delivery

20–500 nm. The size of particles has an impact on their interaction with macrophages and uptake by tumor cells [28]. Generally, smaller nanoparticles (20–100 nm) are cleared from the bloodstream at a slower rate (as shown in Fig. 2) [50]. For instance, Zhang et al. developed carrier-free NCs with an average size of 461.6 ± 57.72 nm, which were designed to incorporate indomethacin (IDM) and paclitaxel (PTX) using a one-step method. The concentration of PTX in tumor tissue using IDM/PTX- NCs was 2.21 times higher than that achieved with free PTX, resulting in a higher inhibition rate of 70% compared to 54% for the free PTX group [51]. Similarly, the self-assembled mitoxantrone NCs (MTO NCs) were developed by Mao et al., which showed passive targeting with particle size of 100 nm. The size optimization improved the lymph targeting of MTO NCs while reducing systemic toxicity. Bolaños et al. developed gold NCs coated with albumin, a natural protein that enhances nanoparticle stability and biocompatibility. The albumin coating improved the circulation time of the NCs and facilitated accumulation in tumor tissues. Thus, surface modifications, such as adjusting molecular weight, size, surface hydrophobicity, and surface charge, surface functionalization, etc. play a critical role to enhance circulation bypassing reticuloendothelial system (RES) [52]. Polyethylene glycol (PEG) is the most commonly used biocompatible polymer for surface coating as it extends the circulation time of the NCs in the bloodstream, allowing them to act as depot for the administered drugs until they reach the targeted tumor tissues [53–55]. In a study conducted by Mohammad et al., febuxostat NCs were coated with polylactic-glycolic acid/polyethylene-glycol (PLGA-PEG) using nanoprecipitation method, resulted in potential cytotoxic effect with significant high percentage of apoptotic cells in A549 lung cancer cells, suggesting effectiveness of NCs for targeted delivery [56, 57]. By understanding these studies and harnessing passive targeting strategies with NCs, remarkable advancements can be made in cancer treatment, improving drug delivery, minimizing side effects, and enhancing overall therapeutic efficacy.

Active targeting

Active tumor-targeted drug delivery is a strategy that involves modifying the surface chemistry of NCs with ligands or molecules such as proteins, nucleic acids, polysaccharides, peptides, and antibodies [58]. In general, two types of targeting are: active targeting to tumor cells

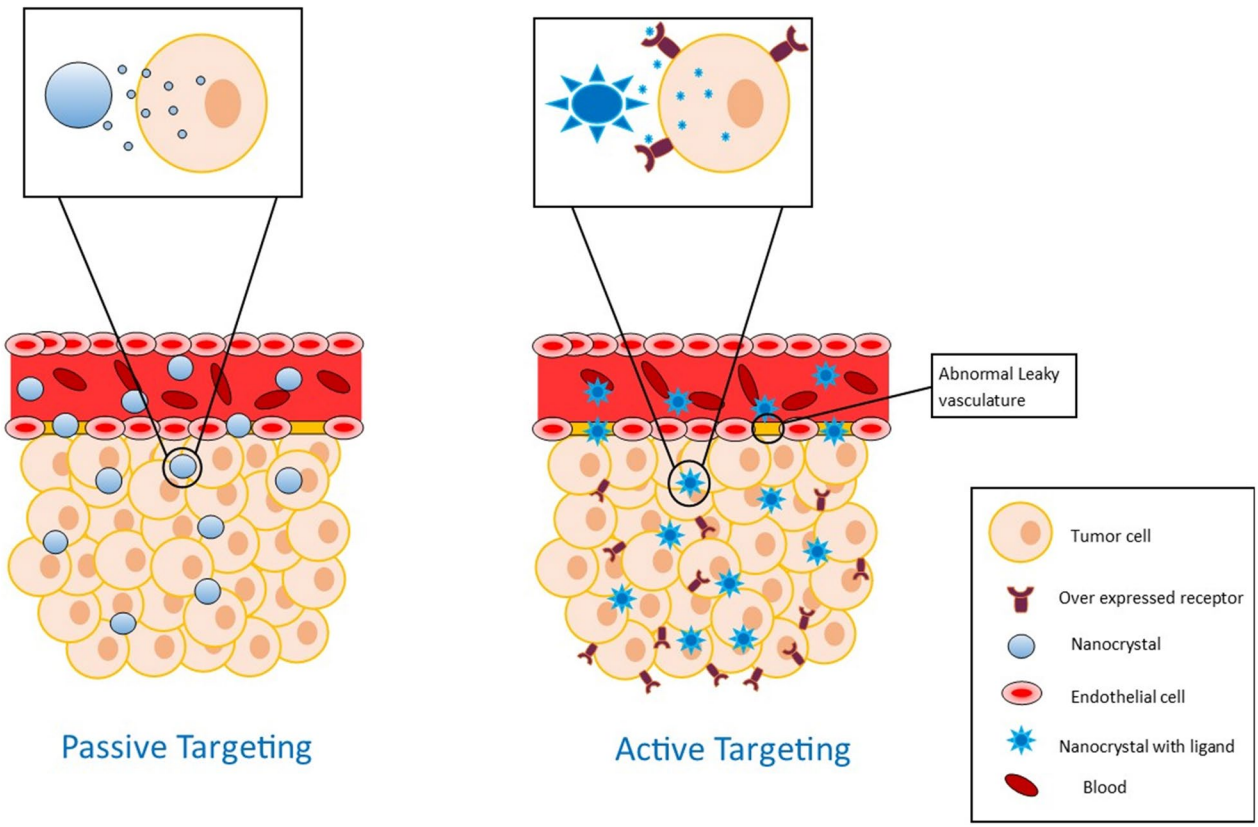

Fig. 2 Active and passive targeting mechanisms

and active targeting to the tumor endothelium. Tumor cell targeting involves the recognition and binding of ligands to receptors that are overexpressed on tumor cells, such as transferrin, folate, epidermal growth factor (EGF), or glycoproteins (as shown in Fig. 2). On the other hand, tumor endothelium targeting aims to target the blood vessels within tumors by recognizing and binding to overexpressed factors like vascular endothelial growth factors (VEGF), αvβ3 integrins, vascular cell adhesion molecule-1 (VCAM-1), or matrix metallo-proteinases (MMP). Different approaches for active targeting include- receptor-mediated targeting, peptide-mediated targeting, antibody-mediated targeting, and aptamer-mediated targeting. Each approach utilizes specific ligands or molecules that facilitate the active recognition and binding of NCs to the desired target, thereby increase drug accumulation while reducing drug distribution in normal tissues, thereby reducing toxicity [59, 60]. In addition, there is a tactic for organelle-specific targeting, such as targeting the cytoplasm, mitochondria, or nucleus. Biomimetic nano-carriers can mimic the surface molecules on cell membranes and exhibit active targeting [61, 62]. A study involved combination of doxorubicin and tumor necrosis factor (TNF) related apoptosis-inducing ligand formulated as NPs and surface coated with P-selectin for targeting overexpressed CD44 over the tumor cells of breast revealed significant reduction in growth of tumor and metastatic nodules [63]. In a study conducted by Danhier et al. [64] authors investigated the active targeting using arginine–glycine–aspartic acid (RGD)-grafted PLGA-NPs loaded with the PTX. This results highlighted the potential of RGD peptides, to enhance drug delivery to tumor endothelium. Similar study was reported by Amreddy et al. [65] on polymeric nanoparticle-mediated gene delivery for lung cancer treatment. These approach illustrated accurate drug biodistribution, minimizing multiple dose administration, enhancing biological barrier penetration, and improving overall treatment safety. Hence, presenting a new frontier in the fight against cancer, offering hope for more effective and personalized therapy.

Antibody-mediated targeting

Antibody-mediated drug targeting has emerged as a highly promising and precise strategy for delivering therapeutic agents directly to tumor cells. It is based on recognizing and binding to cancer-related antigens (carcinoembryonic antigen, fetoprotein, and human chorionic gonadotropin antigen) [66–68]. Antineoplastic immunoconjugates are created by linking monoclonal antibodies (MoAbs) with anticancer drugs through covalent bonds. To implement this conjugation, an inert spacer molecule, is engaged to facilitate the direct attachment of the drugs and antibodies. Several drugs, such as methotrexate, mitomycin C, 5-fluorouridine, maytansinoids, alkaloids, and daunorubicin, have been successfully conjugated with antibodies [69–71]. Zhi et al. [72] conducted a study harnessing antibody-conjugated NCs for the treatment of breast cancer. They synthesized NCs bounded with a potent anticancer drug and modified its surface with antibodies that specifically recognized human epidermal growth factor receptor 2(HER2) receptors. This resulted in targeted approach with increase in drug accumulation within the cancer cells, and minimizing off-target effects [72]. Antibody–drug conjugates like Mylotarg and Zevalin have gained food and drug administration (FDA) approval for the treatment of acute myelogenous leukemia (AML) and non-Hodgkin's lymphoma (NHL), respectively [73]. Moreover, the other side of antibody-mediated drug targeting presents a challenge of potential cross-reactivity with healthy cells that possess surface proteins similar to those present on cancer cells. Additionally, the restricted penetration of large macromolecular antibody-conjugates into solid tumors can hinder their ability to effectively target cancer cells deep within the tumor mass [74]. In-spite of such limitations, this strategy holds a ray of hope at the end of tunnel in enhancing the therapeutic effects of anticancer drugs and minimizing off-target toxicity, ultimately improving patient outcomes.

Surface receptor-mediated targeting

Scientists have made significant progress in enhancing the accuracy and effectiveness of cancer treatments by taking whip-hand of the specificity and binding ability of surface receptors. They have developed nanoparticles that are seasoned with targeting ligands or peptides that attach to the surfaces of cancer cells and enter them through a process called receptor-mediated endocytosis [75]. In a study focused on treatment of prostate cancer, NCs functionalized with ligands specific to the prostate-specific membrane antigen (PSMA) exhibited aberrant binding affinity at receptor site, facilitating enhanced uptake of therapeutic agents, leading to improved anticancer activity [76]. Integrins, a type of cell surface receptors involved in cell attachment and the surrounding matrix, have been targeted for tumor therapy. Peptides and peptidomimetics that bind to integrin αvβ3, expressed on endothelial cells in tumor blood vessels, have shown propitious results. In a study, carbohydrate-based NCs covalently bound to DOX have demonstrated tumor-specific drug targeting [77]. Similarly, folate-mediated targeting has also gained engrossement due to the overexpression of folate receptors on cancer cells. FA-modified stearic acid-grafted chitosan micelles and FOL-PEG-DOX conjugates have exhibited enhanced

intracellular uptake and potent cytotoxic effects in folate receptor-positive cancer cells [78]. Moreover, by utilizing ligands that recognize and bind to cancer-specific surface receptors, personalized treatment plans can be developed based on the characteristics of patients cancer cells, thus maximizing therapeutic outcomes [79]. Researches on NC-based drug therapy are listed in Table 3.

Nevertheless, the development of resistance poses a significant limitation to surface receptor-mediated targeted therapies. Over time, cancer cells can develop resistance to targeted treatments. This resistance can diminish the long-term effectiveness necessitating the exploration of combination therapies or alternative treatment approaches to overcome resistance mechanisms [80].

Pharmacokinetics, bio-distribution, and in-vivo fate of nanocrystals

Understanding the pharmacokinetics, biodistribution, and in-vivo fate of drug-NCs is crucial for their buoyant use in cancer therapeutics. In the case of oral ingestion, NCs enter the gastrointestinal tract (GIT) and pass through the intestinal epithelium to enter the bloodstream. Later upon absorption they undergo metabolism via enzymatic degradation and post-absorption metabolism by liver enzymes [104]. On contrary when injected into the bloodstream, NCs can spread around the body, and their distribution is influenced by factors such as particle size, surface characteristics, and targeting strategies. They undergo biotransformation through processes such as immune recognition, enzymatic degradation, or elimination (described in Fig. 3) [105]. Hence, understanding the in-vivo fate of NCs and the quirky challenges posed by the physiological environment is crucial for their successful application in drug delivery [106, 107].

Oral administration

NCs when administered via oral route enter into the GIT. A part of the NCs are absorbed via villi in the intestine, the remaining amount enters the blood stream while some still get excreted via feces. In the intestinal lumen they form complex with the bile acid, which later go across the mucus layer, epithelial layer and finally reach the blood capillary. In the blood stream, enzymes cause metabolism of the NCs in liver and spleen, a part of it is excreted via kidney and a fraction reaches the target site. In a study, PTX-NCs were administered orally to BALB/c tumor-bearing mice with two doses 80 mg/kg and 60 mg/kg. The tumor volume measured after day 6 of the treatment revealed reduction to one third volume when treated with NCs as compared to free PTX [108]. In another study PTX-NCs were coated with

Table 3 Concise literature on research undertaken with NC-based drug formulations

Drug NCs	Route of administration	Indication	Particle size (nm)/PDI/Zeta potential(mV)	References
Bexarotene	Oral	Breast and prostate cancer	631; 0.33; + 24.6	[81]
5-flurouracil	I.V	Colorectal cancer	69.53 ± 1.14	[82]
Resveratrol	I.P	Ehrlich's ascites tumor	270; 0.31	[83]
Paclitaxel	I.V., I.P., I.T., Oral	All benign tumors	118–397; < 0.3; − 20.87 ~ + 52.5	[84–90]
Docetaxel	I.V., intravaginal	Breast-, head and neck-, stomach-, hormone-resistant prostate cancer	70–526; 0.3 < ; − 20.8 ~ + 10.4	[91–93]
Cabazitaxel	I.V	Breast cancer	110	[47]
Campothecin	I.V	Breast cancer	80–700; 0.128; − 11.4 ~ − 28.5	[94, 95]
Salinomycin	Oral	Colorectal cancer	210 ± 10	[96]
Amoitone B	I.V	Anti-cancer	256.3; 0.206; − 21.52	[97]
Anlotinib	I.V	Hepatocellular carcinoma	200;/; − 30	[98]
Nintedanib	Oral	Non-small cell lung cancer	325; 0.22; + 32.70	[99]
Curcumin	I.V	Inhibits cell proliferation and migration	158–749; 0.156; − 29.1	[100]
Flubendazole	I.P	Lung cancer	253; 0.358; − 30.45	[101]
Carfilzomib	I.V	Breast cancer	270–328; 0.27; − 13.7	[102]
Parthenolide	I.V	Advanced hepatocellular carcinoma	126–208; 0.230; − 11.18	[103]

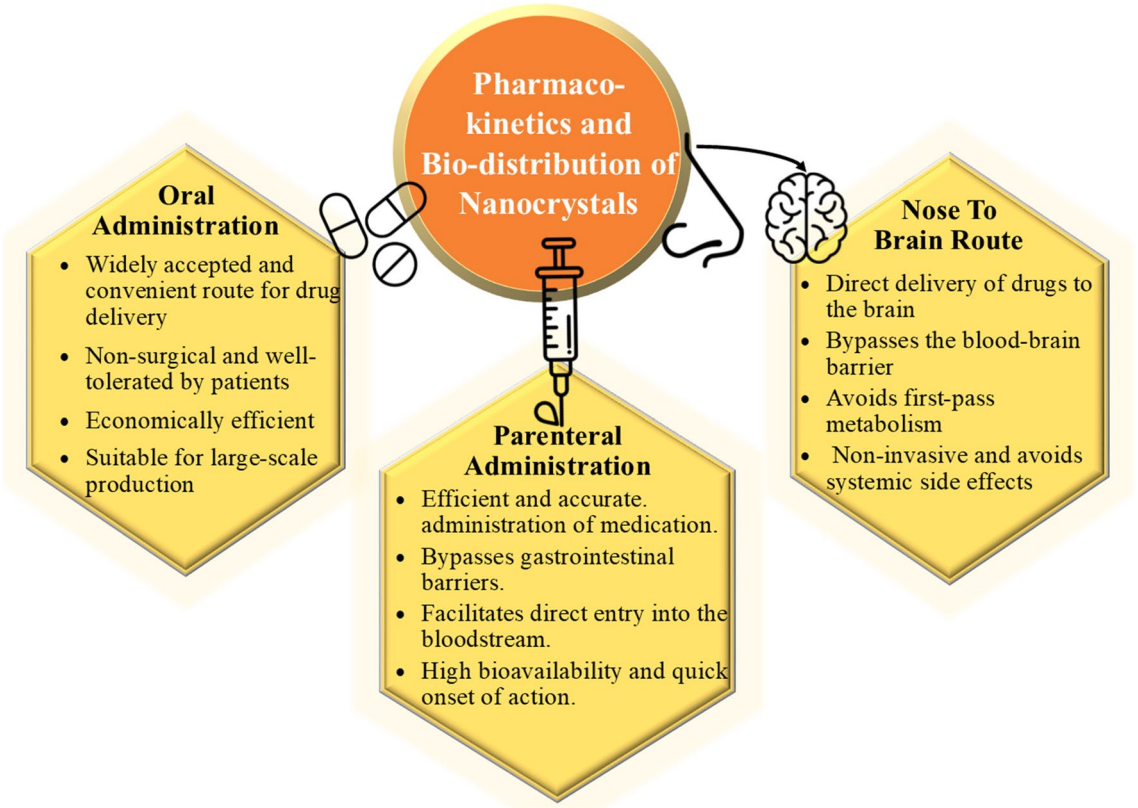

Fig. 3 Pharmacokinetics and bio-distribution of nanocrystals via different routes of administration

N-((2-hydroxy-3-trimethylammonium) propyl) chitosan chloride (HTCC) maintaining the average particle size around 130 nm, were observed to be localized in GIT within 3 h of oral administration. Eventually after 24 h, fluorescence signals showed their preferential accumulation in the tumor. The control group treated with taxol, also inhibited the tumor growth, however, the NCs had long residence time in tumor resulting in remarkable inhibition. The medial survival rates were lengthened even after discontinuation of the treatment [8, 109]. Thereby, such studies have unveiled the path traveled by NC after oral administration.

Parenteral administration

Upon i.v. administration the NCs enter the blood stream and bruit around in the body. During this journey they encounter interactions with different proteins and other elements present in the blood stream. While circulating, they stumble on with the mononuclear phagocytic system (MPS), the larger size NCs are taken up by these MPS present in organs. The smaller size NCs, take a french leave across the MPS and show prolong circulation in the blood. Upon reaching the tumor site, they penetrate through the leaky vasculature of the tumor cells and undergo the EPR effect resulting in accumulation of NCs. Some of NCs enter the lymphatic system via the lymph nodes of the tumor tissue and go efferent into the blood stream. The extended circulation of NCs ensure their extended residence in the tumor, resulting in better efficacy and lower toxicity to healthy tissues [110, 111].

Diving into the fate, NCs are recognized and rapidly taken up by the phagocytic cells of macrophages. At this time, they are also labeled by opsonins, and immunoglobulins. They will be docked onto the receptors present on the macrophages, monocytes, and neutrophils, causing catastrophe of NCs via phagocytosis. The in-vivo fate is influenced by many factors- particle size, surface charge, surface hydrophilicity/hydrophobicity, morphology, dissolution rate, as well as concentration. Among these factors surface morphology influence the most [112]. Many anticancer drugs are delivered in the form of NCs—methoxyestradiol (2-ME), puerarin, and oridonin via i.v. injection [113, 114]. In a recent study consisting of PTX and campothecin NCs stabilized using pluronic-F127, significant inhibition of growth of human cancer and murine breast cancer, was observed [115, 116]. Similarly, PTX-NCs having nearly spheroid

shape and hydrodynamic diameter of 419.9 ± 80.9 nm, were surface coated with polydopamine to form a reaction platform for effective PEGylation and RGD peptide conjugation. Cellular uptake and growth inhibition studies over A549 lung cells demonstrated superior activity over non-PEGylated NCs. The results suggest intratumor accumulation and retarded tumor growth, giving a hope toward coating being effective in functionalization of NCs for enhanced anti-cancer activity [117]. In another study, tocopheryl polyethylene glycol succinate (TPGS) stabilized PTX-NCs showed better therapeutic effect in taxol-resistant ovarian cancer both in-vivo and in-vitro [118]. Similarly, campothecin NCs were studied over lung metastasis of MDA-MB-231 and MCF-7 tumors. The results supported with the confocal images of the tumor sections revealed a better inhibition of tumor metastasis and apoptosis with NCs compared to campothecin salt solution and control group [116]. Such study reports supply glimpse of information depicting the in-vivo fate of NCs upon parenteral administration.

Applications of NC in cancer diagnostics and bio-imaging

NCs have also garnered significant attention in the field of bio-imaging offering promising prospects toward unambiguous detection. Cellulose NCs (CNCs) conjugated with fluorescent agents (rhodamine-B-isothiocyanate and FITC) have been explored for therapeutic imaging of tumors by measuring the acoustic signals. Similarly, non-conjugated CNCs were used for photoacoustic imaging of ovarian cancer in mouse models. The peak photoacoustic signal was reported at 700 nm with doses below 1.2 mg/mL in carcinomatous cells. For efficient bioimaging the NCs were conjugated fluorescent agent to passively trigger cell internalization with insignificant toxicity [119]. Mahmoud et al. proved the capability of fluorescent CNCs to penetrate the cancerous cells without a sign of toxicity and no effect on integrity of cell membrane. They also provided evidence of intake of positively charged CNC-RBITCs within the membranes of Spodoptera frugiperda (Sf9) and human embryonic kidney 293 (HEK 293) cells, while CNC-FITC were not taken-up owing to their negative charge. These reports suggest the relevance of surface charge of NCs with their uptake [120]. Magnetic-NCs in the form of magnetic probes have opened new avenues for bio-imaging with its signal enhancement capabilities. Although magnetic resonance imagining (MRI) is widely accepted diagnostic tool, its non-invasive nature, tomographic compatibilities and low-signal sensitivity hinders its applications. Iron-oxide-based magnetic NCs including superparamagnetic iron oxide (SPIO) are being used for enhancing the signal for better tomographic imaging. Recently, a water soluble magnetic iron-oxide NCs was fabricated with tunable MR signaling effect. The magnetic NC probes were successful in diagnosis of breast cancer tested over SK-BK-3 cell lines. Similar study was tested in breast cancer induce mice and the results suggest that the magnetic NC-antibody probe was able to signal the diagnosis of cancer with magnetic-field of 1.5 and 9.4 T. At higher magnetic-field Yong et al. [121] reported the detailed monitoring of cancer cells with complex vasculature and tumor tissues, thus suggesting the enlightening potential of magnetic NCs for diagnostics purposes.

Gold-NCs with different size and shapes have potential to extend the resonance from visible to near infrared range where light can infiltrate into the tissues. At resonance wavelength gold can absorb and scatter the light, this property enables a distinct color to gold which is sensitive to be detected by naked eye, making gold NCs excellent for biomedical diagnostics. Typically, such gold NCs have been extensively used for immunogenic strip test. The surface electrons on the gold NCs can amplify Raman scattering signals, thus AuNCs with immune probes/antibodies significantly enhance sensitivity and contrast of detection which are stable with repetitive analysis, making it an important candidate as imaging agent for cancer cell identification and diagnosis [122]. In a study conducted with fabrication of Eu/Gd codoping HAP-NCs using co-precipitation technique possess a luminescence property, which enabled successful cell labeling and in-vivo imaging [123]. In addition, flow of magnetic-NCs within the body can be controlled by external magnetic field, making it more convenient to tailor it toward specific and targeted imagining. Such smart use of NCs has shown tremendous applications in the field of diagnostics and effective imaging [124].

Beyond diagnostics, NCs may also be used in cancer biomarker detection and liquid biopsies. NCs-biosensors detect cancer-specific biomarkers with high sensitivity and specificity, allowing for early cancer detection**.** These biosensors can detect and analyze biomarkers from blood samples such as circulating tumor cells, exosomes, and cell-free deoxyribonucleic acid (DNA), allowing for non-invasive monitoring of tumor growth and therapy response [125]. These aid of NCs in the development of liquid biopsy technologies, will revolutionize cancer detection.

Metallic nanocrystals for cancer theranostics

Metallic-NCs are evolving in the field of theranostics [126]. Metallic-nanoparticles can be modified in terms of size and shape, which enables precise control of their physical and chemical characteristics, such as magnetic behavior, surface plasmon resonance, and catalytic activity [127]. Additionally, the possibility of surface functionalization makes metallic-NCs flexible platforms

for targeted therapy, real-time imaging, and treatment response monitoring. The majority of chemotherapeutic drugs distribute throughout the body, causing poor patient compliance, general toxicity, and even treatment cessation. This makes it difficult to deliver therapeutic agents to tumor cells with precision. Moreover, metal nanoparticles are superior to other nanoparticles because of their innate anticancer activity, which eliminates the need for additional carriers. Additionally, they are quickly eliminated from the body and are biocompatible in nature. Metal nanoparticles can be used to encapsulate or conjugate medicinal molecules, making them a viable alternative to other delivery systems [128, 129]. Some of the research related to applications of metallic NCs are discussed in subsequent sections. Table 4 summarizes studies where metallic nanoparticles loaded with anticancer agent have been used for cancer treatment.

Iron oxide nanocrystals

Fe-nanoparticles have earned importance for both therapeutic and diagnostic applications. These metal oxides can be functionalized to act as carriers for anticancer agents or imaging agents by conjugating to their surface or embedding them in a polymeric matrix. Additionally, they can also be employed as contrast agents for imaging by MRI [130, 131]. Fe-NCs were mostly synthesized using a principles of green chemistry. The scientist Mathur et al., produced water soluble Fe_3O_4-NCs by reducing colloidal iron hydroxide with green tea polyphenols (epigallocatechin gallate and epicatechin). The NCs, displayed size range of 2.5–6 nm with high degree of crystallinity. These green teas coated superparamagnetic Fe-NCs (SPIONs) served as negative contrast agent both in-vitro and in-vivo over primary macrophages and colon cancer cells-CT26, showing high uptake efficiency. Thus, SPIONs with promising transport and uptake characteristics can be explored further for multimodal imaging and therapeutic applications [132]. An in-vivo study conducted with SPION coated with phospholipid and PEG revealed substantial influence on bio-distribution and bio-clearance of bio-degradation products, suggesting their safety in cellular environments of blood and organs [133]. SPIONs have showed a great magnetic moment under the influence of static external magnetic field. These property enables them to be used as contrast agents in imaging purpose-MRI, to improve tumor visualization and identification with low poly-dispersity, and functionalized to serve as carriers for the delivery of specific drugs. Beyond imaging and medication delivery, Fe^{+2}-NCs can be used in photo-thermal therapy to absorb light energy and turn it into heat, causing hyperthermia and subsequently killing tumor cells. Additionally, through Fenton or Fenton-like reactions, Fe^{+2}-NCs have the capacity to produce reactive oxygen species (ROS), which cause cytotoxicity and destroy cancer cells. Ma et al., proposed conjugating folic acid to the surface of SPION-loaded polymeric nanoparticles in order to create SPION-based MRI contrast agents with great selectivity to cancer cells. These nanoparticles showed improved MRI efficacy in comparison to a commercial contrast agent and showed selectivity to MCF-7 and SPC-A-1 cells [134]. Another study described the use of MRI-based MUC1-expressing ovarian cancer detection utilizing C595 monoclonal antibody-conjugated SPIONs. With no in-vivo toxicity, the nano-conjugate showed considerable tumor accumulation, detection specificity, and potential anti-ovarian cancer efficacy [135].

Similar, Fe-NCs were developed by group of researcher lead by Vellingiri et al., using goat blood as bioprecursor. The γ-Fe_2O_3-NCs were coated with PEG and combined with doxorubicin with 60–70 nm size for targeting lung cancer cells. The morphological changes, cytotoxicity, and intracellular presence of iron was observed to be dose dependent within the investigational cells. Moreover, the

Table 4 Types of metallic nanocrystals used for cancer theranostic agents, including the metals used, their properties, synthesis methods, and applications

Nanocrystals type	Properties	Method for synthesis	Applications
Iron nanocrystals (Fe-NCs)	Magnetism, biocompatibility, and superparamagnetic characteristics. Magnetite (Fe_3O_4) and maghemite (–Fe_2O_3) are two examples	Thermal decomposition, co-precipitation, hydrothermal synthesis, Microemulsion method, Iron Sono chemical synthesis	Drug delivery, magnetic hyperthermia, and MRI
Zinc nanocrystals (Zn-NCs)	Luminescent qualities and biocompatibility	Precipitation, Wet-chemical synthesis, Solid-state pyrolytic method, Sol–gel method	Fluorescence imaging and therapeutic delivery
Gold nanocrystals (Au-NCs)	Optical properties and biocompatibility	Turkevich method, Perrault method, Block copolymer- mediated synthesis, Brust method	Drug delivery, imaging, and biosensing by photothermal treatment
Silver nanocrystals (Ag-NCs)	Strong antibacterial activity and plasmonic characteristics	Chemical reduction, Physical synthesis, Biological synthesis	Imaging, photothermal treatment, and antimicrobial applications

presence of DOX within the nuclei reveals the pathway toward nuclear chemotherapy. In-vivo studies in tumor bearing mice suggest suppression of carcinogenesis while there was no obvious toxicity in healthy organs. Such studies reflect the potential of natural sources to be developed into nanocarriers as future of targeted nuclear treatment for cancer [136]. A very similar study, with Fe_2O_3-NC synthesized from goat blood using chemical reduction method, demonstrated significant cytotoxicity and active transport to cell nucleus. Further functional proteomic analysis implies the cancer cell proliferation is targeted by Fe_2O_3-NC, providing new insights for nuclear targeted cancer treatment [137]. PEG-capped Fe-NCs with different morphologies (spheres, polymorphs and wires) demonstrated strong ferromagnetic behaviour and hysteresis losses, considering hyperthermia effect with temperature rising up to 42.6 °C for medical hyperthermia applications [138]. Cheon et al., fabricated a probe system which controlled the size, magnetism and induced nuclear spin relaxation in Fe_3O_4-NC model. The Fe_3O_4-NCs were conjugated with Herceptin. This system of NC-antibody probe was used for diagnosis of breast cancer under MRI [126]. For precise delivery, a tumor microenvironment responsive nano-system was designed by decorating Fe_3O_4-NCs with methoxypolyethene glycol (mPEG) and trans-activator of transcription (TAT). These decorated NCs were utilized to facilitate MRI, tumor magnetic hyperthermia (MHT) and mild heat-mediated immune stimulation. The cleavage of mPEG strongly inhibited the growth of tumor cells. In-vivo experiments with CT26 tumor-bearing mice showed 85.5% tumor inhibition rate and induced a magnetic hyperthermia–immune synergistic therapy, together with no obvious anticancer agent this study demonstrated a promising approach for delivering nanomedicine using MR imaging-guided tumor-targeting MHT [139].

Lin et al. created a Fe^{+2} nanocomposite for MRI-visible delivery of small interfering (si)RNA. The nanocomposite displayed equivalent silencing efficacy to Lipofectamine 2000, a commercial transfection agent, and successfully silenced the target mRNA, decreasing the expression of P-glycoprotein (P-gp). Under an MRI scanner, the transfected cells displayed a noticeable contrast enhancement, suggesting a way to tackle multi-drug resistant in cancer cells**.** Commercially, two members of SPION family, Ferumoxides (Endorem®–Europe, Feridex® in the USA and Japan) and Ferucarbotran (Resovist®–Europe and Japan) are approved for intravenous use. These SPIONs are coated with dextran and carboxy-dextran respectively. In addition, Nanotherm consisting of superparamagnetic iron oxide coated with amino silane is approved by USFDA and EMA for the treatment of Glioblastoma, prostate, and pancreatic Cancer [140].

Zinc nanocrystals (Zn-NCs)

Zn-nanomaterials have gained popularity specifically for therapeutic drug delivery, stimuli-responsive targeting, and also for diagnostic purposes. Nanoparticles composed of zinc oxide (ZnO) have received a lot of interest due to their possible use in the treatment of cancer. These nanoparticles have unique properties including photoluminescence for biosensing and characteristics of wide band-gap semiconductors that encourage the production of ROS [141]**.** Nanosized ZnO produces numerous electron–hole pairs even in the absence of UV radiation because of crystal defects, which leads to an increase in the formation of ROS. ZnO's band gap enables electrons and holes to interact with oxygen and hydroxyl ions, generating superoxide and hydroxyl radicals, respectively. Their innate propensity for cytotoxicity against cancer cells results from their capacity to trigger the production of ROS, which causes cell death [142]. Additionally, ZnO nanoparticles have been explored for imaging purposes, enabling optical imaging of particular cancer cell receptors and acting as multimodal imaging agents for cancer detection [143].

Amino-propyl functionalized ZnO-NCs exhibited hexagonal wurtzite crystalline structure with + 22 mV zeta potential. During preliminary phase study with these NCs, it was discovered that treatment of 3 times/day cause in highest toxicity rates. Further, the viability assay confirmed pro-apoptotic stimulus and necrosis. The study concluded three probable mechanism of cell death—bubble cavitation, nanoscalpel effect and electric change imbalance, thus opening new avenues for application of ZnO-NCs as effective tool for cancer treatment [144]. Cauda et al., synthesized ZnO-NCs using very efficient microwave-assisted solvo-thermal method, having spherical morphology with 20 nm size. To evaluate the cytotoxicity and cell internalization over cancerous human cell lines, the ZnO-NCs were functionalized with amine. Surface functionalization with amine enables labelling with fluorescent dyes which could be detected with flow cytometry. Also, water-soluble-tetrazolium-salt1 assay was used to quantify cell viability. The results infer significant reduction in human-epithelial cancer cell viability at dose of 10 μg/mL. Cellular internalization was observed with fluorescent signals suggesting visible internalization of ZnO-NCs [145]. Coating with lipids shields the ZnO-NCs, preventing self-aggregation, premature degradation, and promote cellular uptake in HeLa cancer cells with reduced cytotoxicity. Such results signifying the importance of multifunctional ZnO-NCs for therapeutic and bio-imaging purpose [146]. In another study, membrane destabilization occurs which allows pristine ZnO-NCs to enter cytoplasm and elicit cytotoxic response within the tumor cells [147]. Nair et al. studied

the dissolution of ZnO-NCs and reported that the NCs undergo rapid dissolution in acidic pH 5–6 causing ROS stress, depolarization and superoxide formation in mitochondria, and apoptosis. This elucidates toxic mechanism of ZnO-NCs in destabilization of cancer cells using its own hostile acidic environment [148]. A novel work was carried out by Prasad et al., to enhance PDT of Chlorine, using excitation created by second harmonic (SH) light generated by ZnO-NC, where they are internalized through endocytosis mediated by folate receptors. Here, the SH light was generated by in-situ presence of ZnO-NCs, which caused activation of photons leading to cell death (apoptosis and necrosis) in the cytoplasm of cancer cells. Such studies provide insight of ZnO-NCs as powerful tool for developing phototherapy selectively targeting specific organelle [149]. For the therapeutic purpose, researchers developed ZnO-nanoparticles loaded with DOX. This co-administration caused synergistic effect and enhanced cytotoxicity [150]. Additionally, ZnO can be used for biosensing, which enables the early identification of malignancy [151]. Moreover, the effect of zinc nanostructures on living cells, in particular cancer cells, is still under debate.

Gold nanoparticles (Au-NCs)

Over the period of time, novel delivery carriers resulted in reduction in dose dependent toxicity in comparison to conventional products. These lead to increase in research on exploring metallic materials for targeted delivery and diagnosis. Au-NC are aggregates of Au atoms ranging between 10 to 400 nm in size. The amount of gold, material of inorganic substrates, pH of medium, temperature and mechanical forces used during the synthesis governs the shaper of Au-NCs. The Au-NCs when conjugated with stimuli-responsive polymers, are found to be used for targeted delivery to specific organs of human body [152]. Various reports have successfully loaded anticancer drugs—campothecin, thymectacin, busulfan and cyclosporine using Au-NCs [8].

A study to treat metastatic breast cancer was carried out by Wang et al., using DOX decorated Au-nanorods. With irradiation of near-infrared light over these decorated Au-nanorods, caused increase in temperature along with release of DOX within the tumor as Au-NCs show a sharp absorption peak around 500–550 nm due to excitation of surface plasmons. These combination was observed to be more toxic in 4T1 breast cancer cells [153, 154]. A similar study was conducted over 4T1 breast cancer cells with Au-NCs loaded with 10-hydroxycampothecin. The AuNCs possessed average size of 130 nm with 75% drug loading, when administered i.v, showed a sustain drug release pattern and enhanced cytotoxicity [155]. A study conducted by Shen et al., revealed the use of Au-NCs for inhibition of retinal angiogenesis. The Au-NCs with average size of 26.2 nm and potential of 24.9 mV, were able to inhibit the cell proliferation to an extent of 50–72% and 54–83% inhibition of cell migration at concentration 10 µg/mL and 20 µg/mL, respectively [156]. Li et al., carried out a study to test Au-NCs as potential antiangiogenic agents. In this investigation, Au-NCs were used to target human recombinant endostatin (angiogenesis inhibitor). The results showed promising effects with tumor vascular normalization and strengthened blood vessel [157]. Au-NCs fabricated in conjugation with hydroxylcampothecin and polydopamine, when injected i.v. into the mice, showed accumulation and cytotoxicity in the tumor [158].

One of the major benefit of Au-NCs is its traceability with MRI. Enhanced light scattering and absorption caused by surface plasmon resonance allows them to act as imaging probes. It can accumulate within the tumor site along with drugs. In a study performed by Chandra et al., Au-NCs stabilized using gum arabic was successfully explored as X-ray agent for imaging the tumors of brain in mice and dog models. The treatment was followed as 5 consecutive intratumoral injections GA-AuNCs, later threshold accumulation was attained in 5 h showing complete saturation. The CT scan images revealed the retention of GA-AuNCs in the tumor site along with no clinical changes and cellular toxicity. Further, a pilot study was performed in male dog with thyroid carcinoma and osteosarcoma. Intratumoral injections were administered to the dog, post 3 weeks of treatment, the dog was euthanized and necropsy was performed. The results revealed no toxicity in vital organs, signifying use of GA-AuNCs as diagnostic agent [159]. An augmented approach was adopted by Menon et al., for synthesizing Au-NCs using garlic as precursor. The Au-NCs were suspended in garlic extract in presence of chloroauric acid with constant stirring under elevated temperature. The end point of coating was the change in color of solution from pale yellow to purple-red. The cytotoxicity was performed using MCF-7 human breast cells and L929 mouse fibroblast cells at different concentrations, observations after 24 h of incubation revealed no cytotoxic effect. Thus, it could be concluded that garlic extract did not render any cytotoxic reaction, also it formed a stable shealth around the Au-NCs, serving immense applications in imaging diagnostics [160]. Such studies reflect the potential benefits of Au-NCs which in future can be explored for tumor imaging and diagnosis.

Silver nanoparticles (Ag-NCs)

In the past decade, a prominent focus on translational research for introducing materials with nano-metric size range for cancer therapy has gained momentum.

As a consequence, to date, several biomaterials including metal-based nanostructures have been explored as treatment modalities in various trials conducted to overcome cancer. Among metals, silver (Ag) has a significant anti-microbial property and a unique mode to induce cell death. Ag-nanoparticles unveils tremendous scientific data to show its possible application as anticancer agent, its high efficacy and safety [161]. Following their uptake, these Ag-nanoparticles (AgNPs) are taken up by endocytosis and within the endosomes they undergo lysosomal fusion in the organelle. The acidic environment of lysosome cause release of Ag-ions, which create unbalance cellular homeostatis leading to apoptotic cell death. Such mechanism is referred as Trojan horse. It is rightly said that to exploit the nano nature of metallic material, they are applied along with cytotoxic drugs, however, despite of favorable features Ag appears to be toxic to healthy tissues. Hence, a careful monitoring of Ag accumulation into the cancerous cells needs to be ensured. To achieve this multiple methods—active and passive targeting have already been explored for developing Ag-based nanocrystals (AgNCs). Several research groups have demonstrated the cross-talks between the cancer cells and AgNCs [162].

According to Muhammad et al., AgNCs functionalized with PTX boost the anticancer activity in human cells. The PTX-NCs were surfaced with polydopamine, later the AgNPs and tumor targeting peptide NR1 was decorated onto the PDA. This grafted Ag-PTX-NC system dramatically enhanced the cellular absorption during in-vitro anticancer models. Additionally, the Ag-PTX-NCs showed synergistic influence causing cell membrane lysis, nucleus damage, mitochondrial dysfunction, ROS over production, and breakage of DNA. Such observations linked the potential application of NR1/Ag-NC decorated PTX for targeted therapy of anticancer drugs [163]. Liang et al., demonstrated the anticancer effect of camptothecin (CPT)/Ag-NCs. Silver exhibit excellent inhibitory effect on drug resistance related P-glycoprotein (Pgp) while CPT has proven data of being cytotoxic. The combination of both CPT/Ag-NCs was able to bypass the Pgp recognition resulting in indiscriminate cytotoxicity. In addition, the drastic release of CPT into the tumor triggered by cleavage of Ag-ions in acidic microenvironment led to chromatin structure breakage, DNA damage and apoptosis. Nevertheless, such exploration of molecular events needs support of in-vivo studies [164]. Kim et al. investigated the toxic effect of synthesized crystalline AgNPs on F9 cells. They identified the dose-dependent toxic effect of AgNPs was linked with leakage of lactate dehydrogenase, ROS, and mitochondrial dysfunctioning. At high concentration DNA fragmentation, neuronal differentiation, increased expression of apoptotic genes, and decreased expression of anti-apoptotic genes was reported. The results support the use of crystalline AgNPs for differentiation therapy in amalgamation with chemotherapeutic agents [165]. In a report published by Paul et al., the crystalline AgNPs were biosynthesized using ethanolic leaf powder extract of *Premna serratifolia* L. and possessed 22.97 nm size. Anticancer effect of the synthesized AgNPs was evaluated on Swiss albino mice induced with liver cancer. The results revealed AgNPs were non-toxic with protective effects of other organs while they sustained control over the cancer progression. Such studies open up ways toward cost-effective economic alternatives for anticancer therapy, however certain understanding of molecular mechanism restrain the use of synthesized Ag-NPs of *P. serratifolia* [166]. Nima et al. developed Ag–Au nanorods functionalized with certain molecules and antibodies improved photothermal contrast and surface-enhanced Raman scattering (SERS) for the diagnosis of breast cancer [167]. Thus, AgNCs embark great potential to be explored for diagnostic and therapeutic purposes. A brief list of metal based drug delivery explored for cancer therapeutics is reported in Table 5.

Regulatory challenges toward formulation of drug NCs

In addition to opportunities and challenges, hurdles pertaining to approval of NCs by the regulatory bodies are one of the major obstacle in its commercialization. A bridge needs to be build-up with validation, toxicity, reproducibility and stability of NCs produced in academic setting to enable collaboration with industries. A helping hand with adopting minimal standards for conducting pre-clinical studies was offered by US-FDA and European medical agency (EMA), to promote clinical translation of academic-industry collaboration. On the other hand, following good manufacturing practice (GMP) becomes crucial while demonstrating the promise of technology for effective drug delivery and diagnostics. However, following GLP might increase the overall cost, but with previously demonstrated proof of concept, the collaborators may seek funding from different organization for the said purpose. Pre-clinical studies might also need placebo controlled treatment regimens for appropriate evaluation of safety and efficacy. In these context, FDA has initiated the Nanotechnology Regulatory Science Research Plan. The plan aims to address the gap in scientific knowledge required to make regulatory assessment of NCs hassle free. The plan includes major criteria- physio-chemical characterization, preclinical models, risk characterization, risk assessment, and risk communication open for discussion with collaborators [177]. A fruitful outcome of the plan is the establishment of Nanotechnology Characterization Lab, that performs

Table 5 Brief list of recent metallic based drug delivery explored for cancer therapeutics

Metal	Conjugating drug	Cell lines	IC_{50} value	Proposed MOA	Reference
Gold	Docetaxel	Lung cancer cell line (H520)	25 µm	Cell disruption and apoptosis	[168]
Silver	Epirubicin	HepG2 cells	1.92 µg/mL	–	[169]
Copper	Paclitaxel	Drug-resistant prostate cancer cell	85, 172, and 193 nM	Inhibition of proteasome/poly-Ub protein degradation pathway by targeting the NPL4 protein	[170]
Gold and iron	Doxorubicin	HeLa cancer cells	2.3 µg/mL	Cytoplasmic internalization and cell death	[171]
Silver	Methotrexate	Breast cancer cells MCF-7	258.6 µg/mL	Blocks the synthesis to tetrahydrofolate	[172]
Silver	Acety-lshikonin and beta-dimethyl-acrylshikonin	Human chronic myeloid leukaemia	100 nM	ROS induced toxicity	[173]
Gold	Doxorubicin	Glioma carcinoma cell line (LN 229)	4 µg/mL	DNA intercalation and cell death	[174]

characterization of the different nanoparticles received from government funded research labs, academia, and industry. These platform integrates academic-industry collaborations to gather relevant data for filing Investigational New Drug application. Thus, such resource utilization enables academic-industry collaborations for effective translation of the NC-based therapeutics established by academic to reach market [178].

A part from these, a significant consideration must also be given to selection of demographics for performing clinical trials. Recently, a phase 2 clinical study failed to achieve its primary end point for treating lung cancer, and prostate cancer using BIND-014 (PSMA-targeted docetaxel nanocrystals). This results reflect the need for designing clinical trials, considering selection of patients based on EPR, tumor heterogeneity, presence of target receptors, ability of NCs to bind to receptors, and the simultaneous need for diagnostics during the study are some of the crucial parameters for achieving better outcomes [175]. Another NC-based product called as Panzem Nanocrystal Colloidal Dispersion is currently under phase 2 clinical trials, being explored for its potential effect on prostrate and ovarian cancer. Some of the NC-based formulation approved for commercialization by FDA are listed in Table 6. A pioneering effort in this direction was adopted by Merrimack Pharmaceuticals, to

Table 6 Drug NCs with approval of commercialization

Drug	Product	Method of manufacturing	Route	Indication
Cabotegravir/rilpivirine	Cabenuva	Media milling	I.M	AIDS
Meloxicam	Anjeso	Media milling	I.V	Analgesics
Aripiprazole Auroxil	Aristada	High pressure homogenization	I.M	Schizophrenia
Paliperidone Palmitate	Invega Trinza	HPH	I.M	Schizophrenia
Dantrolene sodium	Ryanodex	Media milling	I.V	Malignant hyperthermia
Nepafenac	Ilevro	Media milling	Drops	Analgesic and anti-inflammatory
Theophylline	Theodur	Media milling	Oral	Bronchiectasis
Naproxen sodium	Naprelan	Media milling	Oral	Anti-inflammatory
Fenofibrate	Triglide	HPH	Oral	Reduce Cholesterol/Triglycerides
Megestrol acetate	Megace ES	We media milling	oral	Appetite stimulation
Cannabinoid	Cesamet	Co-precipitation	Oral	Nausea and vomiting
Aprepitant	Emend	Wet media milling	Oral	Antiemetic
Tizanidine	Zanaflex	Wet media milling	Oral	Muscle relaxant
Diltiazem	Herbesser	Media milling	Oral	Angina
Morphine sulfate	Avinza	Media milling	Oral	analgesia
Methyl phenidate	Ritalin Focalin	Media milling	Oral	Attention Deficit Hyperactivity Disorder
Sirolimus	Rapamune	We media milling	Oral	Immune suppression

determine the accumulation of ferumoxytol iron nanoparticles (FMX) using quantitative MRI and may predict the response to nanoliposomal-irinotecan. The study concluded that the tumors with high accumulation of FMX were more responsive to nano-liposomes [176]. This study was based on simultaneous quantitative estimation using MRI, and we believe that such initiatives along with pre-selection of patients will turn out to be a bridging bench-bed gap. Thereby promoting the commercialization of highly potent NCs with detailed safety and efficacy profiles along with backbone of clinical data.

Conclusion and future prospects

Delivery of BCS class II and IV is challenging yet a promising pitch for research. NC-based drug delivery has emerged with incomparable drug loading over other carrier-based drug delivery systems for poorly soluble drug substances. Over the last two decades, NC technology have shown useful and prevailing role in the treatment of cancer providing improved drug targeting and delivery. Current research on these drug substances deals with strategies to improve their absorption and selective delivery to tumor cells, leading to emergence of surface functionalization and usage of novel excipients. To add cherry on the top, their functionalization with targeting ligands offers excellent dynamism to control the growth of tumors. With the on-going research, different new techniques have also evolved for the production of NCs. These techniques involve combination of top-down and bottom-up approaches to effectively rationalize their advantages. Nevertheless, the clinical efficacy of drug NCs depends on number of factors. The present review highlighted multifaceted applications of different types of NCs in the management of cancer. Metal based NCs have offered simultaneous application in diagnostic and therapy with targeted drug delivery, thereby revolutionizing cancer management and therapy. The drug-metal NC complex enable tracking within the body owing to their interaction with light, thus offer an efficient therapy in addition to chemotherapy. Despite these facts, their clinical translation and market authorization is challenging due to certain bottlenecks. Toxic effects are possible due to their tiny size and large surface area which boosts reactivity with the biological targets. Numerous problems with respect to long-term sustainability and safety of nanoparticles has always remained unresolved. Inflammation, genotoxicity, and organelle failure in cells are few dose dependent toxic effects of nanocrystalline particles. Also, the activation of oxidative enzymes results in increase in concentration of free radicals and in-turn increase oxidative stress and cell damage. Moreover, they have a great potential if the risks and toxicity are controlled from production to treatment. Certain ways including coating with polymers and with the concept of green synthesis, a relatively safe way for the use of metallic nanoparticles can be en-routed.

As far as biological challenges are concerned, the bridge between the disease pathology and human heterogeneity is required. Also, the physicochemical properties of drug NCs should primarily focus on overcoming biological barriers to achieve target and reduce build-up in nonspecific organs. Unfortunately, lack of attention toward these aspects, cause failure observed during the translation of promising NCs in clinical trials. These factors could be deterrent for pharmaceutical industries for investing on commercialization of NC-based products. To cope with this challenge, there is a dire need for inclusive evaluation of preclinical data with special emphasis on efficacy, safety, pharmacokinetics (ADME profile), targeting efficiency and stability with appropriate tumor induced animal models. Also, reproducibility of the results must be validated using different animal models, such practise will boost the reliability toward the anticancer therapy. For most of commercialized products, EPR-mediated accumulation has been reported in most of the animal models. Moreover, the tumors are heterogeneous and may possess inter and intra patient variability. With NC-based products, the amount of cellular uptake and drug release kinetics inside the target sites can be tailored, moving away from the traditional concept of one-size-fits all.

Although most of the research about drug NCs on cancer therapy are still in preclinical development, the motivation arises from those products which have reached the market, with enhanced circulation time, site specific cellular uptake, long retention in tumor environment and reduction in dose-dependent toxicity. Also, decorated NCs with targeting ligands have generated a ray of hope for cancer therapy. Even, NCs can be labeled with dyes/contrast agents to further augment visualization and theranostic applications. In nutshell, NC technology has profound opportunities to mitigate cancer therapy and with gradual maturation of drug NCs, commercialization of NC-based products will emerge like a boon for cancer therapeutics.

Abbreviations

NCs	Nanocrystals
HPH	High-pressure homogenization
RESS	Rapid expansion of supercritical solutions
PNC	Paclitaxel nanocrystals
EPR	Enhanced permeability and retention
PTX	Paclitaxel
MTO NCs	Mitoxantrone nanocrystals
RES	Reticuloendothelial system
PEG	Polyethylene glycol
PLGA-PEG	Polylactide-co-glycolide-Polyethylene glycol
MMP	Matrix metallo-proteinases

TNF	Tumor necrosis factor
PSMA	Prostate-specific membrane antigen
FA	Folic acid
I.V.	Intravenous
GIT	Gastrointestinal tract
MPS	Mononuclear phagocytic system
TPGS	D-a-tocopheryl polyethylene glycol 1000 succinate
CNCs	Cellulose nanocrystals
FITC	Fluorescein isothiocyanate
RBITC	Rhodamine-B-isothiocyanate
MRI	Magnetic resonance imaging
AuNCs	Gold nanocrystals
DNA	Deoxy ribonucleic acid
Fe-NCs	Iron nanocrystals
Zn-NCs	Zinc nanocrystals
Ag-NCs	Silver nanocrystals
SPIONs	Superparamagnetic iron nanocrystals
ROS	Reactive oxygen species
DOX	Doxorubicin
mPEG	Methoxypolyethene glycol
TAT	Trans-activator of transcription
UV	Ultra violet
ZnO	Zinc oxide
Pgp	P-glycoprotein
CPT	Camptothecin
US-FDA	United States Food and Drug Administration
EMA	European medical agency
GMP	Good manufacturing practice
BCS	Biopharmaceutical classification system
MOA	Mechanism of action

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