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# Shrinking the battlefield in cancer therapy: Nanotechnology against cancer stem cells

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ABSTRACT

Cancer remains one of the leading causes of mortality worldwide, presenting a significant healthcare challenge owing to the limited efficacy of current treatments. The application of nanotechnology in cancer treatment leverages the unique optical, magnetic, and electrical attributes of nanomaterials to engineer innovative, targeted therapies. Specifically, manipulating nanomaterials allows for enhanced drug loading efficiency, improved bioavailability, and targeted delivery systems, reducing the non-specific cytotoxic effects characteristic of conventional chemotherapies. Furthermore, recent advances in nanotechnology have demonstrated encouraging results in specifically targeting CSCs, a key development considering the role of these cells in disease recurrence and resistance to treatment. Despite these breakthroughs, the clinical approval rates of nano-drugs have not kept pace with research advances, pointing to existing obstacles that must be addressed. In conclusion, nanotechnology presents a novel, powerful tool in the fight against cancer, particularly in targeting the elusive and treatment-resistant CSCs. This comprehensive review delves into the intricacies of nanotherapy, explicitly targeting cancer stem cells, their markers, and associated signaling pathways.

## 1. Introduction

Cancer is a global health menace that continues to be one of the primary causes of death worldwide. Its incidence is influenced by a myriad of factors, which can broadly be categorized into genetic and environmental. (Laconi et al., 2020; Kaminska et al., 2015; Siegel et al., 2023; Song and Giovannucci, 2016; Vineis and Wild, 2014).

In recent years, an increasing number of studies have stated that cells with stem-cell-like properties may play an indispensable role in oncogenic transformation, and the development of tumors (Wang and Dick, 2005; Koren and Fuchs, 2016; Zhu and Fan, 2018; Ayob and Ramasamy, 2018; Yu et al., 2012) is on the rise. Various other reports also state that voiding the system of stem cells is essential in preventing relapses and achieving long-term remission (Polyak and Hahn, 2006; Jordan, 2005). Advancements in the knowledge of the specifics of cancer stem cells (CSCs) have unveiled the importance of their targeted eradication in treating cancer. Indeed, the numerous drawbacks and challenges posed by conventional cancer therapeutics (especially in developing resistance to drugs) have made way for newer and more advanced technologies that are much required to improvise.

Nanotechnology is one such area that is aimed at utilizing the characteristics of nanomaterials in the diagnosis, prognosis, and treatment of cancers at both cellular and molecular levels. Interestingly, nanomaterials are very similar to biomolecules in terms of microscopic sizes, and therefore their use may be exploited in the engineering of various potentially useful medical applications.

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Contrary to atoms and other macroscopic molecules commonly used as drugs in anticancer therapy, nanoparticles possess a high ratio of volume to surface area and can be engineered to have a variety of compositions, shapes, and surface chemical characteristics (Xia et al., 2009; Peer et al., 2007). Customizing these properties molds nanomaterials into an attractive target for developing a new generation of drug delivery vehicles and diagnostic and imaging probes.

The scope of the current work is to bring to the surface the existing burden of cancer while shedding light on the vitality of nanomedicine in cancer. This review will mainly focus on the application of nanomedicine in targeting CSCs, outline the clinical utilization of such technology, and highlight the current challenges and future implications.

## 2. Cancer epidemiology and burden of the disease

Cancer, a pathological condition characterized by the abnormal growth of cells, is known to be the leading cause of death all over the globe (Bray et al., 2021). It was known to be the most dreaded disease of the 20$^{th}$ century and is becoming increasingly prevalent in the 21st century (Roy and Saikia, 2016) (Fig. 1).

The burden of cancer is rapidly rising in both the aging and growing populations, while the major risk factors are largely linked to socioeconomic development (Omran, 2005; Gansler et al., 2010). According to the most recent GLOBOCAN (2020) statistics for cancer, nearly 19.3 million new cancer cases and 10 million cancer-related deaths were observed worldwide in 2020 (Mattiuzzi and Lippi, 2019). Moreover, although cancer is thought to be a gender-neutral disease, men were found to have a 19 times greater incidence rate compared to women (Mattiuzzi and Lippi, 2019). Among the various classes of cancer, breast cancer was identified as the leading cause of cancer incidence across the globe in 2020. Incidence rates are elevated in counties with a higher development index and are, therefore, much more common in such countries than transitioning countries. These elevated statistics among more developed nations may likely arise due to lifestyle-related and/or access to improved cancer diagnostics and mammographic screening programs in these countries (Mattiuzzi and Lippi, 2019) countries.

Similarly, the incidence and mortality rates of lung cancer are much higher in developed countries than developing or transitioning countries. After breast cancer, lung cancer remains the second most commonly diagnosed cancer world wide[16]. In addition, it is also the leading cause of cancer deaths in the year 2020 (Sung et al., 2021). It is not surprising that lung cancer ranks as the leading cause of cancer-related disease and mortality in men across the globe; however, it ranks third in cancer incidence rates in women, after breast and colorectal cancer (Mattiuzzi and Lippi, 2019; Sung et al., 2021). Evidently, due to its relevance as a tobacco smoking disease, lung cancer is less defined among women (Thun et al., 2012). Moreover, apart from tobacco smoking, in certain East Asian countries, the incidence of lung cancer has also been attributed to exposure to air pollutants and particles generated through the household combustion of solid fuels for cooking and other household uses (Mu et al., 2013; Turner et al., 2020). However, the strict enforcement of tobacco control laws and the promotion of anti-smoking campaigns have led to a decline in tobacco usage in both men and women, as observed in China (Lin et al., 2023) and in various other countries (Flor et al., 2021; Wilson et al., 2012).

Finally, colorectal cancer ranks third as the most frequently occurring cancer globally after breast and lung cancer. In addition, colorectal cancer also ranks as the second leading cause of cancer-related death worldwide (Sung et al., 2021). The highest rates of colorectal cancer incidence have been reported in Europe, with Hungary and Norway ranking first in men and women, respectively. However, the incidence rates of rectal cancer are observed to be exceptionally high in East Asian countries. On the other hand, the lowest rates of both colon and rectal cancer are found in the African continent and across south central Asia.

A general trend has been observed in the rise of colorectal cancer among countries with a high development index (Bray and Soerjomataram, 2015; Fidler et al., 2016). In general, factors like lifestyle, diet, obesity, and the consumption of alcohol, red meat, processed foods, etc., have been strongly associated with colorectal cancer (Siegel et al., 2020; Sullivan et al., 2015). Evidently, in many countries, healthier changes to lifestyle and diet and the adequate implementation of screening programs, have deeply reflected in a decline in colorectal cancer cases (Arnold et al., 2020; Edwards et al., 2010).

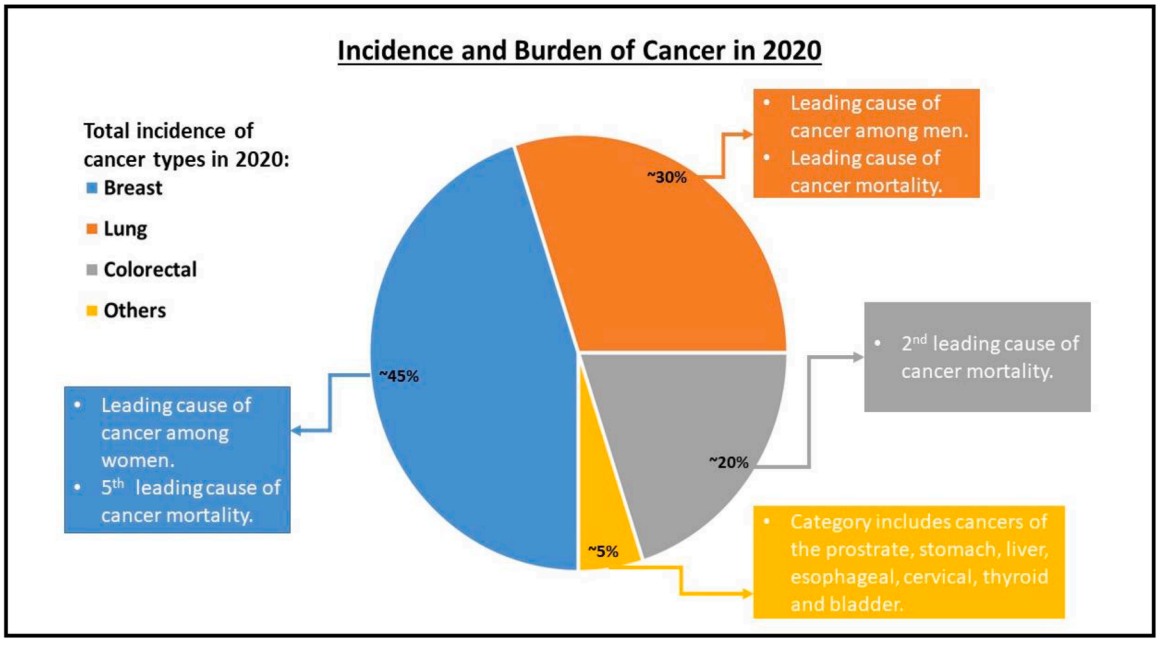


**Fig. 1.** A depiction of the incidence and burden of cancer (Sung et al., 2021). According to the most recent cancer statistics, breast cancer is the leading type of cancer incidence followed by lung and colorectal cancer. Lung cancer tops as the leading cause of cancer mortality followed by colorectal cancer; while breast cancer ranks 5th as a cause of cancer mortality. Breast and lung cancer remain to be the leading causes of gender specific cancers in women and men respectively. (Created with Biorender.com).

Apart from breast, lung, and colorectal cancer ranking as the top three most frequently occurring cancers, other cancers of the prostate, stomach, liver, esophagus, cervix, thyroid, and bladder are also often on the rise across all nations worldwide (Sung et al., 2021). Overall, an estimated 28.4 million new cancer cases are projected to occur in 2040 (Sung et al., 2021). These figures reflect a 47 % increase in the number of cases in 2020, thus depicts the magnitude of the burden of cancer in the near future. Cancer diagnosis and management have come a long way in the last five decades (Roy and Saikia, 2016). In terms of cancer therapy, chemotherapy, radiotherapy, and surgery remain the mainstay of cancer therapeutics.

## 3. Cancer stem cells (CSCs)

Despite effective treatment regimen including chemotherapy and radiotherapy followed by surgical removal of the tumor mass, chemoresistance is a prevailing challenge that contributes to relapse and recurrence in cancer. More specifically, the resistance to chemotherapeutic drugs and recurrence or relapse of cancer may be correlated to the presence of cancer cells with attained stemness properties, commonly known as Cancer Stem Cells (CSCs). In recent years, the relevance of CSCs is becoming significantly important. It is speculated that CSCs are known to originate through the oncogenic transformation of normal stem cells in an organism (Soltysova et al., 2005). Stem cells are defined by their sole characteristics of self-renewal and pluripotency. Interestingly, the identification and targeting of CSCs led to newer implications regarding cancer therapeutics.

CSCs are known to possess excessive capabilities of aberrant differentiation, renewal, tumor initiation, and progression (Ayob and Ramasamy, 2018; Zhou et al., 2023; Yilmaz et al., 2023); therefore, the sustenance of CSCs has been a major factor leading to poor prognosis and survival in cancer (Palomeras et al., 2018). In addition, CSCs are also shown to be a potent inducer of metastasis (Sahai, 2005). Reports suggest that most drugs that target cancer cells mostly fail to eradicate CSCs, thereby aiding tumor recurrence and permitting the perpetuation of cancer (Brasseur et al., 2017; Cho and Clarke, 2008). A particular study reported that a poly(propylene imine) drug delivery system coupled with paclitaxel showed high therapeutic potential in targeting and eliminating ovarian CSCs, leading to more efficient remission (Tefas et al., 2021). The major characteristics of CSCs are depicted in Fig. 2. Furthermore, various other reports suggest that CSCs could potently use numerous signaling pathways for their differentiation and self-renewal. The major pathways involved in CSC growth and maintenance are the Wnt/β-catenin, Notch, and Sonic hedgehog pathways (Essex et al., 2019; Borah et al., 2015; Farnie and Clarke, 2007; Clement et al., 2007). In addition, other cell growth signaling pathways, such as PI3K/AKT, JAK/STAT, mTOR, and NF-κB, are found to be deregulated in CSCs, thus further promoting their characteristics of self-renewal (Yang et al., 2020; Ajani et al., 2015; Kroon et al., 2013).

Though the origin of CSCs still remain unclear, several theories have been proposed. Hypothetically, CSCs are either normal stem cells that are mutationaly changed to be oncogenic or they are cancer cells acquiring stemness within individual tumors of the same origin (Plaks et al., 2015).

CSCs express specific cell surface markers, such as CD9, CD24, CD44, and CD133 (Abbaszadegan et al., 2017). A higher expression of drug efflux mediating transport proteins such as ATP-binding cassette superfamily G member 2 (ABCG2) is also observed in these specialized cells that facilitate drug efflux in cancer cells thereby evading drug effects. (Kukal et al., 2021). Moreover, most CSCs are reported to have a

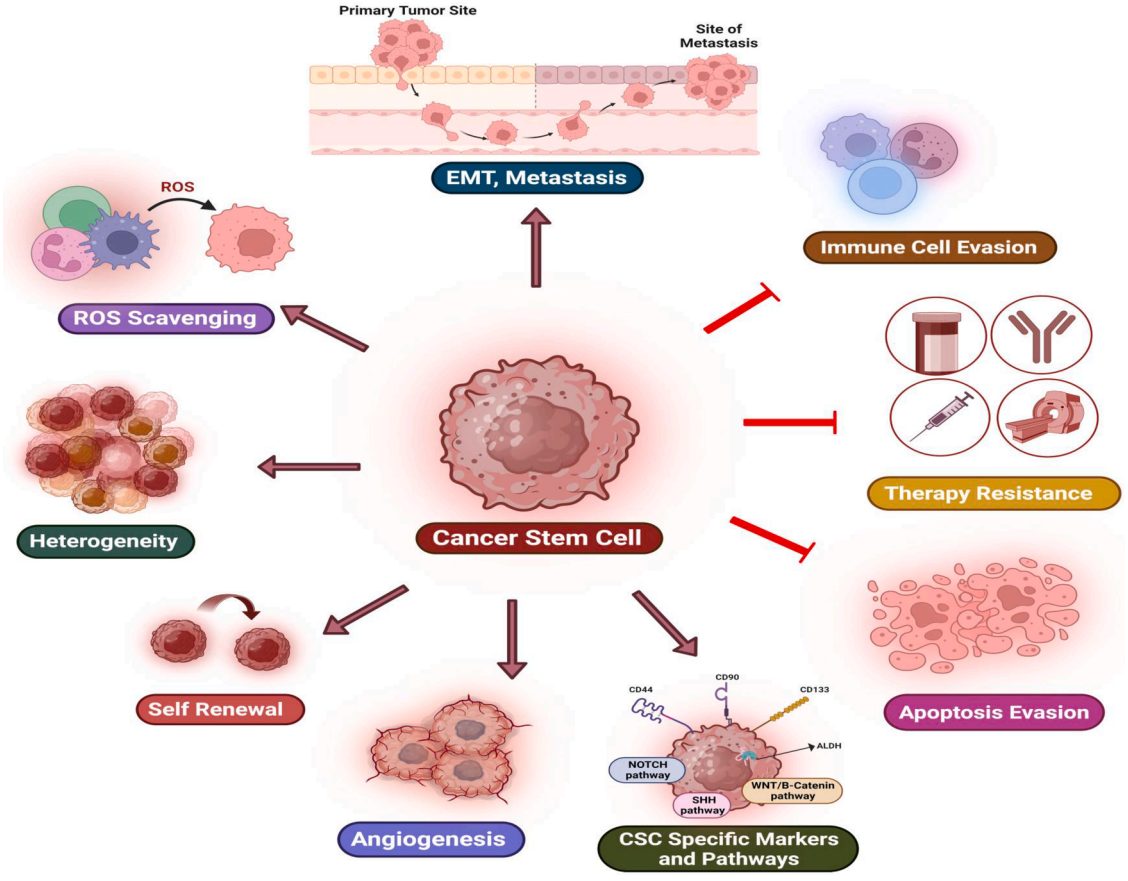


**Fig. 2.** Schematic representation of characteristic features of Cancer Stem Cells. Abbreviations: EMT- Epithelial to mesenchymal transition, ROS: Reactive Oxygen Species. (Created with Biorender.com).

higher expression of a specific marker named aldehyde dehydrogenase (ALDH) (Abbaszadegan et al., 2017). Enhanced ALDH activity also appears to confer resistance to specific chemotherapeutics wherein repors suggest that ALDH-positive breast cancer cells were capable of forming xenograft tumors with as little as 500 CSCs (Abdullah and Chow, 2013). Moreover, a major mechanism by which CSCs exhibit chemoresistance is through an enhanced DNA damage response through hypoxia inducible factor transcription factors (Olcina et al., 2010). Reportedly, the characteristic features of CSCs, such as the expression of CSC-specific markers, vary across different types of cancers (Cho and Clarke, 2008; Quintana et al., 2008). For instance, in breast cancer, CSCs were found to exhibit the expression of CD44+/ESA+/CD24−/ALDH1 markers, however in leukemia, CSC markers like CD34+/CD38−/CD96 were prominently expressed (Wang and Dick, 2005; Hosen et al., 2007). In gastric cancer, CD15/Lewis X, Lgr5/GPR49 are the majorly observed CSC markers. CD27, CD38, CD20/MS4A1, CD19, and CD138Syndecan-1 are identified in CSCs of myeloma (Zhao et al., 2017). A comprehensive understanding of the tumor's CSC-mediated plasticity and aggressiveness is essential for enhancing therapeutic approaches that target and eradicate CSCs (Eun et al., 2017).

## 4. The rise of nanotechnology in the management of cancer

Insufficient drug delivery systems and rising anti-neoplastic therapy resistance are the main challenges to developing effective cancer medicines. The complexity of tumor pathophysiology, highlighted by their intricate blood and lymphatic vasculature, exacerbates these difficulties even more, drastically reducing the potential advantages of conventional cancer therapy. Additionally, the labyrinthine circulatory network and pathophysiological characteristics of malignant neoplasms significantly reduce the efficacy of conventional oncological therapies. Thus, addressing these impediments is paramount to improving the therapeutic outcome in cancer management (Hejmady et al., 2020).

The tumor microenvironment (TME) effectively interacts with cancer cells at various levels including regulating cell proliferation, evading growth suppression and immune-surveillance, evading apoptosis, regulating angiogenesis and promoting EMT/metastasis (Hanahan and Coussens, 2012). Moreover, the intreractions between CSCs and microenvironment facilitate drug evading mechanisms towards anti-cancer therapies thus elevating disease recurrence (Albini et al., 2015). Plasticity of CSCs refers to their ability to dynamically interconvert between stem-like and non-stem-like states. This plasticity is influenced by various factors within the tumor microenvironment, including hypoxia, acidity, and interactions with stromal cells, immune cells, and extracellular matrix components (Thankamony et al., 2020). Understanding this ability of CSCs within the TME is however crucial for the development of effective therapeutic strategies for several reasons including identifying critical targets such as signaling pathways, designing targeted delivery systems, and in developing comibnation therapies. In fact, it is becoming increasingly clear that the tumor microenvironment greatly influences a wide range of factors that may reduce the effectiveness of treatment. A noteworthy aspect of this challenge is the non-cellular mechanisms of drug resistance that emerge from poorly vascularized regions within the tumor mass. These regions present substantial barriers to successfully transporting chemotherapeutic agents, thus thwarting their optimal delivery. Further complicating the matter is the reduced microvascular pressure within the tumor, which severely impedes drug absorption at the tumor site. This physiological characteristic limits the extent of drug penetration into the tumor, curtailing the therapeutic potential of administered agents. These intricacies of the tumor microenvironment and its vascular dynamics underscore the multifaceted challenges facing effective drug delivery and absorption, highlighting the imperative for innovative strategies in cancer therapeutics (Tredan et al., 2007).

Furthermore, cellular mechanisms of drug resistance pose a significant hurdle in effective cancer treatment. This often materializes as a consequence of tumor cells evading programmed cell death or apoptosis, which is a critical component of the natural defense mechanism against the proliferation of malignant cells. Concurrently, modifications in cellular transport mechanisms, such as the efflux systems mediated by P-glycoprotein, have been recurrently implicated in multi-drug resistance in various cancer types (Halder et al., 2022; Bruno et al., 1987).

Amidst these challenges, an emerging solution resides in the realm of nanotechnology. Nanotechnology may be traditionally defined as the use of nanoparticles specifically within the size range of 5 to 500 nm as target molecular devices in at least one dimension. In particular, previous research attempts to engineer nanotherapeutic agents have cumulated in the development of potent tools like liposomes, nanotubes, polymer nanoparticles,dendrimers, inorganic nanoparticles and other micellar compounds (Ediriwickrema and Saltzman, 2015). Specifically, the association of chemotherapeutic agents with colloidal nanoparticles holds substantial promise for mitigating cellular and non-cellular barriers contributing to drug resistance in cancer. By exploiting the unique properties of these nanoscale entities, we can potentially navigate around the inherent and acquired resistive mechanisms, thereby enhancing the overall therapeutic index of anticancer treatments (McCarthy et al., 2005; Brigger et al., 2002; Davis et al., 2010).Moreover, nanotechnology-based strategies are capable of specifically targeting the complexities of the tumor microenvironment that are key to understanding tumor progression (Mendes et al., 2021).

Furthermore, the microscopic sizes of nanoparticles coupled with their expansive surface area facilitating high-capacity drug loading, render them superior facilitators for enhanced anti-neoplastic therapies (Bedhiafi et al., 2023; Bedhiafi et al., 2023).Examples of nanoparticles leveraged in nano-drug delivery systems extend to a broad range, encompassing metal nanoparticles, nanocapsules, nanoshells, liposomes, and dendrimers. This is further underscored by the extensive body of research validating their potential in cancer therapeutics (Yilmaz et al., 2023). Given these properties, it is indisputable that nanomedicine embodies robust, transformative solutions that are poised to revolutionize the diagnosis, management, and treatment of cancer (Gindy and Prud'homme, 2009).

In particular, advancements in protein engineering, formulation sciences, and drug delivery systems, all forged through the dexterous application of nanotechnology, instill unprecedented optimism in cancer patients. This wave of innovative strategies, powered by the intricate marriage of biotechnology and nano-engineering, continues to invigorate the landscape of cancer therapeutics, offering renewed hope in the ongoing battle against this formidable disease. (Peer et al., 2007).

Several nanomaterials have received FDA approval for use in humans (Peer et al., 2007). The applications of nanomaterials as drug carriers offer the advantage of a considerably enhanced surface area for the coating of antitumor molecules (Kim et al., 2010). In particular, targeting of tumors can be achieved through nanomaterials introduced into the bloodstream which are more efficiently assimilated into the tumor; owing to their improved permeability into the microscopic pores of the vasculature of the early tumor (Hobbs et al., 1998).

More specifically, applying nanomaterials as drug carrier systems could reduce the related adverse effects by inhibiting non-specific uptake into tissues (Prencipe et al., 2009; Alexis et al., 2008; Xu et al., 2023) . In line with this, certain studies have reported that the recombinant human tumor necrosis factor-alpha (TNF-α) was less taken up by healthy tissue when coated on gold nanoparticles than its direct injection. Thus subjects could tolerate much higher doses of the compound, leading to the unleashing of a comparatively enhanced antitumor effect. (Libutti et al., 2010; Visaria et al., 2006). Similarly, yet another study reported that patients could tolerate much higher doses of drugs through nanoparticles with comparatively no side effects (Kim et al., 2004). This is most likely due to the altered pharmacokinetic behavior of the drugs achieved through coupling them with nanomaterials. In addition, nanosystems have also been utilized to reduce toxicity and drug resistance issues (Sun et al., 2023), particularly of conventionally used

cancer drugs like cisplatin (Han et al., 2022; Ghosh et al., 2021) . However, despite the success of nanoparticle-based drug delivery systems, reports have also brought to light their potential cytotoxicity, thus highlighting major safety concerns. The toxic effects are known to be triggered both in the intestines as well as in the bloodstream (Araujo et al., 2015). Moreover, the charge and the surface properties of the nanoparticles are also known to produce detrimental interactions within biological systems (Arora et al., 2012). In addition, the cytotoxic effects of nanoparticles may be further pronounced as the pharmacokinetic properties of the drug are liable to change once incorporated into nanosystems (Baldrick, 2010).

The promise of nanomedicine is to provide sustainable therapeutic options against cancer cells that largely counter the drawbacks of conventional chemotherapy and other theranostic drugs while also offering reliable solutions for the early detection/diagnosis of various classes of cancer (Hejmady et al., 2020). Moreover, nanomedicine can also provide highly potent personalized cancer therapies that could offer synergistic solutions to the existing issues of drug resistance (Bar-Zeev et al., 2017).

Moreover, in light of cancer diagnostics, nanotechnology has also risen as attractive agent for cancer imaging. In particular, nanomaterials could serve as contrast agents for diagnostic imaging (Kim et al., 2010). According to a particular report (Harisinghani et al., 2003), nanomaterials were used in prostate cancer to detect lymph node metastases. Here, owing to their smaller sizes, the nanoparticles could traverse through the lymphatic vessels and enter the lymph nodes, thus ensuring uptake by the resident macrophages. Moreover, according to another report (Enochs et al., 1999), using nanoparticles allowed for identifying tumor margins for extended periods on an MRI, as compared to conventional approaches. This is because nanoparticles can easily enter the tumor (owing to their smaller sizes) and take much longer to diffuse out of the tumor (Perrault et al., 2009). Superparamagnetic iron oxide nanoparticles (SPIONs) have been widely used as MRI contrast agents to enhance the visualization of tumors (Li et al., 2013) . Similarly, gold nanoparticles can be used as contrast agents in CT scans due to their high X-ray attenuation coefficient (Cormode et al., 2014).

On the whole, the extension of nanotechnology into the various strata of cancer management, like therapeutics, diagnostics, and prognostics, serves as sufficient evidence to highlight the technology's robustness in alleviating the disease's burdens. Table 2 summarizes the common applications of nanotechnology in cancer diagnostics and therapeutics and their associated limitations.

## 5. Nanomedicine in targeting cancer stem cells

Nanotechnology has evolved to play a critical role in modern medicine by developing improved therapeutic options compared to conventional treatment methods. Owing to its effectiveness in modulating distribution, as well as target site accumulation of the drug (that allows for the increase of specificity and decrease of toxicity), nanomedicine has been increasingly used for the enhancement of existing therapeutic options, thus eventually leading to an increase in the therapeutic index of the drugs (Garbayo et al., 2020).

Recently, there is considerable interest in eliminating CSCs using nanoformulation-based anticancer therapies wherein nanoparticles are specifically formulated to accurately identify and target CSCs based on their molecular markers (Lyakhovich and Lleonart, 2016). Below are the applications of nanoformulations aimed at the specific targeting of CSC markers and CSC-associated signaling pathways, as evidence of their roles as antitumor mediators (Table 1). Also, Fig. 3 depicts the types, applications, and molecular targets of nanoformulations used to eliminate CSCs.

### 5.1. Nanotherapy targeting CSC markers

#### 5.1.1. CD44-targeted nanotherapy

Cluster of differentiation (CD) refers to certain glycoproteins observed on the tumor cells' surface. As previously mentioned, several antigens belonging to certain CDs have been exclusively identified as molecular markers of CSCs. Recent advancements in nanomedicine have been highly implicated in targeting these markers, especially CD44, CD90, and CD133. CD44 is a cell surface glycoprotein that plays a crucial role in cell-cell and cell-matrix interactions, cell adhesion, and migration. It is highly expressed on the surface of many CSCs and is associated with tumor progression, metastasis, and therapy resistance. Therefore, CD44 is considered a promising target for the selective targeting of CSCs . It is one of the most studied CSC markers that regulate metastasis (Senbanjo and Chellaiah, 2017). The expression of CD44 has been identified in several cancers of the breast (Brown et al., 2011), colon (Su et al., 2011), head and neck (Dubey et al., 2022), ovaries (Martincuks et al., 2020) and pancreas (Al-Hajj et al., 2003). Application of CD44 antibodies via nanoformulations of biodegradable poly [(D, L-lactide co-glycolide)-co-PEG] (PDLGA-co-PEG) polymeric micelles loaded with paclitaxel were reported to elevate the intracellular concentration of the drug and provide effective delivery to the CSCs in breast cancer (Gener et al., 2015). CD44-targeted nanotherapy involves the use of nanoparticles that are functionalized with ligands or antibodies that specifically bind to CD44 expressed on the surface of CSCs. These nanoparticles can be loaded with therapeutic agents, such as chemotherapeutic drugs, siRNAs, or miRNAs, that can inhibit the growth and survival of CSCs. When administered, these nanoparticles selectively bind to CD44-positive CSCs, facilitating the targeted delivery of the therapeutic agents specifically to the CSCs while minimizing off-target effects on normal cells (Xu et al., 2020).

Moreover, magnetic nanoparticles coupled with anti-CD44 antibodies were shown to have an enhanced effect on targeting CSCs in pancreatic and breast cancer (Aires et al., 2016). Yet another study showed that liposomal doxorubicin functionalized with anti-CD44 antibodies effectively increased the circulation time, biodistribution, and efficiency of the drug compared to conventional drug delivery systems (Arabi et al., 2015). In addition, CD44-targeted glutathione-sensitive hyaluronic acid-mercaptopurine prodrug (HA-GS-MP) linked via carbonyl vinyl sulfide was also used in the treatment of acute myeloid leukemia (AML) (Qiu et al., 2017), where the nanoformulation proved to be highly stable and soluble, and thereby attained rapid drug release. Similarly, in colorectal cancer, a recent study reported that SN38 (a chemotherapeutic agent) loaded into human serum albumin (HSA)–hyaluronic acid (HA) nanoparticles (SH/HA NPs) combined with radiotherapy might be a promising therapeutic artifice for colorectal cancer in the near future (Yang et al., 2023).

The impact of CD44-targeted nanotherapy on CSCs includes:

(1) Inhibition of CSC Growth and Survival: The targeted delivery of therapeutic agents to CD44-positive CSCs can lead to the inhibition of key signaling pathways (e.g., Wnt/β-catenin, Notch, Hedgehog) that are essential for the self-renewal and survival of CSCs (Chen et al., 2018).
(2) Induction of Apoptosis: The targeted delivery of chemotherapeutic drugs or siRNAs that induce apoptosis can lead to the selective elimination of CD44-positive CSCs (Vinogradov and Wei, 2012).
(3) Inhibition of CSC Migration and Invasion: CD44 plays a crucial role in the migration and invasion of CSCs. Targeting CD44 with nanoparticles can inhibit these processes, thereby preventing the metastasis of CSCs (Hassn Mesrati et al., 2021).
(4) Sensitization to Other Therapies: Targeting CD44-positive CSCs with nanoparticles can lead to the downregulation of multidrug resistance genes and the sensitization of CSCs to other therapies, such as radiation or chemotherapy (Vinogradov and Wei, 2012).

**Table 1**
List of nanoformulations targeting CSC specific markers and signaling pathways. Abbreviations: DOX: Doxorubicin, DS-PLGA: poly lactic-co-glycolic acid -encapsulated DiSulfuran, GSI DAPT: γ-secretase inhibitors (N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester), HA-CS NP: hyaluronic acid -chitosan nanoparticles, HA-SS-PLGA: hyaluronic acid-cystamine-polylactic-co-glycolic acid, IONP: Iron Oxide Nano Particle, MSN: Mesoporous Silica Particles, NanoHHI: Nanoparticle Encapsulated Hedgehog Pathway Inhibitor HPI-1, PaxSLN: Paclitaxel-encapsulated core-shell nanoparticle, PDLGA-co-PEG: Poly(ethylene glycol)-block-Poly(lactic-co-glycolic) acid, PEI-PGMA: Polyethyleneimine-PolyGlycidal Methacrylate polyethyleneimine.

| Nanoformulations | Target | Type of Cancer | Samples /cell lines (*In vitro/In vivo*) | Significance | Reference |
|---|---|---|---|---|---|
| **CSC Marker-based Nanotherapy** | | | | | |
| Anti-CD44 antibodies + nano-formulations of PDLGA-co-PEG polymeric micelles loaded with paclitaxel. | CD44 | Breast | • MCF7 cell line<br>• MCF7-ALDH1A1/tdTomato<br>• MDA-MB-231<br>• HCT8,<br>• HCT116<br>• HCT116-ALDH1A1/tdTomato<br>• MCF7-ALDH1A1/tdTomato cells | Improved intracellular concentration of the drug and enhanced delivery to CSCs. | Gener et al. (2015) |
| Magnetic nanoparticles + anti-CD44 antibodies | | Breast and Pancreas | • Panc-1<br>• MDA-MB231 cancer cell lines<br>• MCF-10A cell line | Enhanced targeting of CSCs | Aires et al. (2016) |
| PPI drug delivery system + paclitaxel | | Ovarian | • Ascitic fluid with cancer cells<br>• Athymic nu/nu mice | Improved therapeutic elimination of CSCs. | Shah et al. (2013) |
| Nano-formulation of liposomal doxorubicin + anti-CD44 antibodies | | Colon | • C-26 cancer cell line | Increased the circulation time, biodistribution and efficiency of the drug. | Arabi et al. (2015) |
| SN38 loaded with SH/HA nanoparticles + radiotherapy | | Colorectal | • SW620, HT29 cancer cell lines | Promising CSC-targeted therapy. | Yang et al. (2023) |
| Salinomycin + nanoparticles loaded with CD133 aptamers | CD133 | Osteosarcoma | • Saos-2 CD133(+) cancer cell line | Offered high specificity and improved drug efficiency to CD133+ CSCs, resulting in tumor cell death. | Ni et al. (2015) |
| MSNs + anticancer drugs / siRNA | | laryngeal carcinoma | • Hep-2 cells<br>• BALB/c-nu/nu mice | Improvement in therapeutic outcome. | Qi et al. (2016) |
| Nanoencapsulation of curcumin | | Brain | • Embryonal tumor derived lines: DAOY and D283Med<br>• Glioblastoma neurosphere lines: HSR-GBM1 and JHH-GBM14 | Enhanced its anti-tumor effects | Lim et al. (2011) |
| CdSe core nanocrystals (Trifluoperazine + anti-CD90 antibody) | CD90 | Leukemia | • $CD90^{+}$ leukemia CSCs | Enhanced cancer cell death. | Asghari et al. (2019) |
| PaxSLN core-shell nanoparticle | ALDH | Colorectal | • CT26 colorectal carcinoma animal model | Enhanced targeting of ALDH expressing cells. | Dastidar et al. (2019) |
| DS-PLGA Nanoparticles | | Liver | • Huh7<br>• PLC/PRF/5 cancer cell lines | Improve half-life of the drug. | Wang et al. (2017) |
| **CSC-Associated Signalling Pathway-based Nanotherapy** | | | | | |
| Cationic lipid-protamine nanoparticles | Wnt/ β-Catenin Pathway | Melanoma | • Murine BRAF-mutant melanoma cell lines BPD6 and D4M ($BRAF^{V600E}$, $PTEN^{-/-}$, syngeneic with C57BL/6) C57BL/6 mice | Repressed expression of Wnt5a and inhibited tumor growth. | Liu et al. (2018) |
| Nano-formulation of Cromolyn | | Colorectal | • Male Wistar albino rats | Enhanced anticancer effects | Motawi et al. (2017) |
| PEI-PGMA nanoparticles + shRNA | | Human Embryonic Kidney, breast cancer, colon cancer, leukemia | • HEK293<br>• MCF7, and MDA-MB231<br>• COLO205<br>• Jurkat<br>• Brca2/p53 knockout mice,<br>• Apc knockout model (AhCre-ErT $Apc^{fl/fl}$) mice | Effective targeting of c-Myc | Tangudu et al. (2015) |
| Gold nanoshells + anti-FZD7 antibodies | | TNBC | • MDA-MB-231 and MCF-10A | Effective inhibition of cell growth and proliferation. | Riley and Day (2017) |
| IONPs | | Breast | • MDA-MB-231 cancer cell line | Enhanced delivery and inhibit the growth and proliferation of cancer cells. | Miller-Kleinhenz et al. (2018) |
| MSN + GSI DAPT | Notch Signalling Pathway | Breast | • MDA-MB-231 and MCF7 cancer cell lines | Achieved a significant reduction of CSCs. | Mamaeva et al. (2016) |
| Fe3O4@SiO2(FITC)/PEI-FA/Notch-1 shRNA nanoparticles | | | • MDA-MB-231 cancer cell line | Reduced Notch-1 expression and cell growth, and induced cancer cell death. | Yang et al. (2014) |

(*continued on next page*)

**Table 1** (*continued*)

| Nanoformulations | Target | Type of Cancer | Samples /cell lines (*In vitro/In vivo*) | Significance | Reference |
|---|---|---|---|---|---|
| DOX-miR-34a co-loaded HA-CS nanoparticles | | TNBC | • MDA-MB-231 cancer cell line | targeted modulation of the notch signaling pathway resulting in tumor cell death. | Deng et al. (2014) |
| Anthothecol-encapsulated PLGA nanoparticles | Hedgehog Signalling Pathway | Pancreas | • AsPC-1, Mia-Paca-2 and PANC-1 cancer cell lines | Inhibition of pancreatic CSC. | Verma et al. (2015) |
| α-Mangostin-loaded PLGA nanoparticles | | Pancreas | • Transgenic (Kras(G12D), and Kras(G12D)/tp53R270H) mice | | Verma et al. (2016) |
| Long-circulating GlaB-loaded oil-cored polymeric nanocapsules | | Ovarian cancer, Pancreatic cancer and glioma | • SKOV3<br>• PANC1<br>• GL261<br>cancer cell lines | Offered superior stability, cytotoxicity and bio-distribution of the drug. | Ingallina et al. (2017) |
| HA-SS-PLGA nanoparticles + doxorubicin and cyclopamine | | Breast | • MDA-MB-231<br>• MCF-7<br>Cancer cell lines | Enhanced drug efficacy | Hu et al. (2015) |
| Cyclopamine-paclitaxel polymer nanoparticles | | Pancreas | • Orthotopic MiaPaca-2-luc pancreatic xenograft model<br>• Orthotopic PDX models<br>• KPC-Luc transgenic mouse model | Inhibition of hedgehog signalling and enhanced therapeutic activity. | Zhao et al. (2018) |
| NanoHHI | | Liver | • Tumor specimens from HCC patients | Decreased growth and metastasis | Xu et al. (2012) |
| NanoHHI + gemcitabine | | Pancreas | • Pa03C<br>• HEK293 cancer cell lines | Inhibition of tumor growth and proliferation. | Chenna et al. (2012) |
| Polyethylenimine-Spherical nucleic acid nanoparticles | | Brain | • U87-MG cancer cell line | Reduced chemoresistance and stemness properties. | Melamed et al. (2018) |

#### 5.1.2. *CD133-targeted nanotherapy*

CD133 is a transmembrane glycoprotein that is a universal marker of CSCs across various types of cancers (Glumac and LeBeau, 2018; Taib et al., 2023). CD133-targeted nanoparticles are designed to selectively target and deliver therapeutic agents to cancer cells expressing the CD133 antigen on their surface. This is typically achieved by functionalizing the surface of nanoparticles with antibodies, peptides, or small molecules that have a high affinity for CD133. The specificity of these targeting ligands for CD133 is crucial for achieving selective targeting of CD133-expressing CSCs (Tian et al., 2022). Targeting CD133 for effective drug delivery has been successful in osteosarcoma, wherein a study reported that Poly (lactic-co-glycolic acid) nanoparticles loaded with CD133 aptamers successfully delivered salinomycin with high specificity to CD133 expressing CSCs, resulting in tumor cell death (Ni et al., 2015). Further, according to another study, mesoporous silica nanoparticles (MSNs) were utilized to deliver both anticancer drugs, specifically cisplatin, and short interfering RNA (siRNA) targeting the mRNA of CD133, into CD133+ cancer cells, resulting in a significant improvement in the therapeutic outcome of laryngeal carcinoma (Qi et al., 2016). Specifically, MSNs were used to deliver SiRNAs against ABCG2 transport protein. Moreover, curcumin, a natural compound that possesses potent anticancer activities as evidenced in several groups of cancers (Si et al., 2023; Mishra et al., 2023; Dytrych et al., 2023), was also highly efficient in inhibiting the growth of CD133-expressing cells (Zhou et al., 2023). Therefore, studies have attempted the nano-encapsulation of curcumin to enhance its anti-tumor effects further. Such formulations are made by combining N-isopropyl acrylamide, vinylpyrrolidone, and acrylic acid in a molar ratio of 60:20:20 to encapsulate curcumin; thereby leading to the generation of "nano curcumin", which is found to be effective in targeting CSCs in the brain (Li and Zhang, 2014; Lim et al., 2011).

In a recent study, a 15-nucleotide base-pair CD133 aptamer targeting glycoprotein antigen CD133/AC133 in Glioblastoma stem cells was conjugated to gold-PEG-CB-839 to target CD133+ stem cells. It has been reported that the high affinity of gold nanoparticles to thiol groups helped in effective penetration of the blood brain barrier and biocompatibility. Moreover, the study also showed that this approach ensured precise drug delivery to CSCs (Poonaki et al., 2022) . In an earlier study, Swaminathan et al reported that polymeric nanoparticles loaded with paclitaxel and surface functionalized with anti-CD133 antibody demonstrated efficient inhibition of tumor-regrowth in vivo (Swaminathan et al., 2013).

However, there are several challenges and potential off-target effects associated with CD133-targeted nanotherapy:

(1) Heterogeneity of CD133 Expression: CD133 is not uniformly expressed on all CSCs and its expression can vary between different types of cancer and even within the same tumor. This heterogeneity can result in incomplete targeting of CSCs and may leave some CSCs unaffected by the therapy.
(2) Non-Specific Uptake: Nanoparticles can be taken up by cells via non-specific endocytosis, leading to the uptake of nanoparticles by non-targeted cells. This non-specific uptake can result in off-target effects and toxicity to normal cells.
(3) Expression of CD133 on Normal Cells: CD133 is also expressed on the surface of some normal stem cells. Targeting CD133 may therefore affect normal stem cells and lead to toxicity in non-tumor tissues.
(4) Development of Resistance: CSCs may develop resistance to CD133-targeted nanotherapy by downregulating the expression of CD133 or by activating alternative signaling pathways that promote their survival.

To address these challenges and minimize off-target effects, several strategies can be employed:

(1) Combination Targeting: Combining CD133-targeting with other CSC markers or signaling pathways can help achieve more comprehensive targeting of CSCs and minimize the risk of resistance development.
(2) Optimization of Nanoparticle Properties: The size, shape, and surface properties of nanoparticles can be optimized to minimize non-specific uptake and maximize selective targeting.

**Table 2**
Application of nanoparticles in cancer therapy and diagnostics and their associated limitations.

| Nanoparticle Type | Applications | Drawbacks |
|---|---|---|
| | Cancer Diagnostics | |
| Nanoshells | Nanoshells facilitate the conversion of electrical energy to light energy thus enabling diagnostic imaging that is devoid of the heavy-metal toxicity issues Nunes et al. (2019). | The application of nanoshells are limited by their large sizes Nunes et al. (2019). |
| Magnetic nanoparticles (MNPs) | Nebulization of MNPs along with Epidermal growth factor receptor (EGFR) have been used to detect the epidermal growth protein, that is commonly associated with non-small cell lung cancer (NSCLC) Murase et al. (2015). | High costs and potential safety issues Ulbrich et al. (2016). |
| Colloidal Gold Nanoparticles | Colloidal gold nanoparticles act as efficient contrast agents, providing enhanced diagnostic imaging owing to the permeability tension effect in tumor tissues Fu et al. (2018). | - |
| Near Infrared (NIR) Quantum Dots | NIR Quantum dots are capable of emitting fluorescence in the near-infrared spectrum in order to enhance the tracking of diagnostic devices used in solid cancers Parungo et al. (2004), Gao et al. (2004), Dubertret et al. (2002). | Low tissue penetration and lacks effective quantification Aswathy et al. (2010). |
| | Cancer Therapeutics | |
| Polymeric Micelles | Polymeric micelles act as superior cancer drug carriers due to their smaller volumes and strong thermodynamic stability that allows for enhanced endothelial permeability, while also minimizing kidney rejection Savic et al. (2003). | Low stability in the bloodstream Ghezzi et al. (2021). |
| Liposomes | Liposomes are known to enhance the delivery of hydrophobic drugs into the intracellular spaces of tumors Bozzuto and Molinari (2015). | Costly methods of formulation and assembly Ghezzi et al. (2021). |
| Carbon Nanotubes | Carbon nanotubes as cancer drug carrier are particularly advantageous due to their light absorbing properties that enable them to heat up, thus allowing better penetration into tumors Burlaka et al. (2010). | Cytotoxicity and safety issues Batra et al. (2022). |

(3) Use of Alternative Targeting Ligands: Developing alternative targeting ligands with higher specificity and affinity for CD133 can help improve the selectivity of CD133-targeted nanoparticles.

CD133-targeted nanotherapy has shown promising results in various preclinical studies. For example, nanoparticles functionalized with anti-CD133 antibodies and loaded with chemotherapeutic drugs have been shown to selectively target CD133-positive cancer stem cells (CSCs) and inhibit tumor growth in mouse models of liver cancer and glioblastoma (Poonaki et al., 2022) . Additionally, CD133-targeted liposomes encapsulating siRNA have been shown to effectively silence target genes in CD133-positive CSCs and inhibit tumor growth in mouse models of colorectal and liver cancer (Wang et al., 2020).

However, the translation of CD133-targeted nanotherapy from preclinical studies to clinical trials has been challenging due to several factors:

(1) Heterogeneity of CD133 Expression: The heterogeneous expression of CD133 among different types of cancer and even within the same tumor makes it difficult to predict the efficacy of CD133-targeted nanotherapy in a clinical setting.
(2) Off-Target Effects: The expression of CD133 on normal stem cells raises concerns about potential off-target effects and toxicity in non-tumor tissues.
(3) Development of Resistance: The potential for CSCs to develop resistance to CD133-targeted nanotherapy by downregulating CD133 expression or activating alternative signaling pathways is a significant concern for the long-term efficacy of this approach.

To our knowledge, there are currently no clinical trials specifically evaluating the efficacy and safety of CD133-targeted nanotherapy. However, several clinical trials have investigated the efficacy and safety of therapies targeting CD133-positive CSCs using other approaches, such as CAR-T cells or monoclonal antibodies. These studies have shown some promising results, but also highlight the challenges associated with targeting CD133-positive CSCs.

#### *5.1.3. CD90-targeted nanotherapy*

CD90 is a glycosylphosphatidylinositol-anchored glycoprotein observed in leukocytes and several CSCs, including breast and liver. Cells with CD90 expression are confirmed to possess elevated tumorigenicity and metastatic ability (Yang et al., 2008; Yang et al., 2008). A particular report has addressed the use of CD90 as a potential therapeutic target in cancer (Bakalova et al., 2004), advocating that photosensitizer trifluoperazine loaded with anti-CD90 antibody-mediated water-soluble CdSe core nanocrystals showed effective cell death in leukemic cells.

#### *5.1.4. ALDH-targeted nanotherapy*

ALDH is a molecular marker of CSCs that belongs to a family of detoxifying enzymes (Li et al., 2015; Chen et al., 2014). Overexpression of ALDH is observed in many cancers, such as ovarian cervical, lung, melanoma, and breast cancer (Therachiyil et al., 2022; Shao et al., 2014; Liu and Zheng, 2013; Vira et al., 2012; Sher et al., 2022)). A particular study (Dastidar et al., 2019) reported the development of a novel nanoformulation of paclitaxel-encapsulatedcore–shell nanoparticle of cetyl alcohol (PaxSLN) that could target aldehyde ALDH overexpressing cancer cells after oral administration. Disulfiram is a specific (DS) inhibitor of ALDH with proven cytotoxic effects on a wide range of CSCs of the colon, breast, and glioblastoma (Hothi et al., 2012; Liu et al., 2012; Yip et al., 2011). Disulfiram irreversibly inhibits ALDH2 by carbamylation of the catalytic Cys302 residue and thereby prevents acetaldehyde metabolism. This in turn increases circulating acetaldehyde levels in the cells, exerting a toxic effect to CSCs (Lipsky et al., 2001) .

However, a major drawback of DS is its short half-life in the bloodstream. A nanoformulation created through the encapsulation of DS in poly(lactic-co-glycolic acid) (PLGA) nanoparticles (DS-PLGA) was proven to improve its half-life in the bloodstream. This formulation also showed synergistic cytotoxicity with 5-fluorouracil and sorafenib in liver cancer stem-like cells (Wang et al., 2017).

#### *5.1.5. Low-density lipoprotein receptor (LDL-R) -targeted nanotherapy*

LDL receptor regulates the endocytosis of cholesterol-rich LDL and helps maintain the plasma level of LDL. In the context of cancer, LDL-R is observed to have a differential expression in different cancers. Report from Yang et al states that oxidised LDL could promote stemness in bladder cancer with hypercholesterolemia (Yang et al., 2021) . In this regard, a recent report showed targeting LDL-R via nanoparticle conjugated with ligands to facilitate effective drug delivery to CSCs in colon, liver and kidney cancer cells. The group successfully conjugated PCSK9

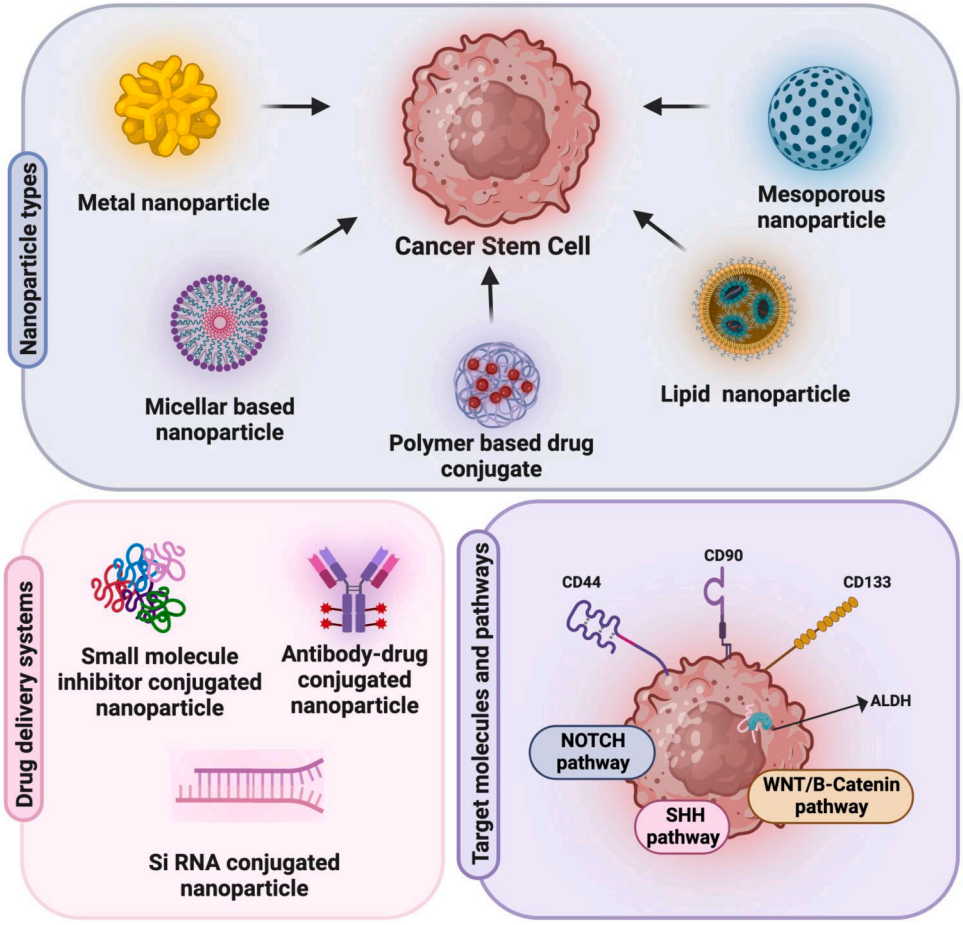


**Fig. 3.** Types and applications of nanomedicine in targeting cancer stem cells Created with Biorender.com).

(an LDL -R ligand) with liposomes to deliver chemotherapeutic drug paclitaxel (PTX) to cancer cells. They found that conjugated liposomes had a significantly higher growth inhibition than the unconjugated (control) liposomes in colorectal cancer cell line HCT116 (Charbe et al., 2022). Moreovere, the study confirms that this novel PCSK9 conjugated liposomes has a potent and precise *in vitro* anticancer activity could be a a promising targeted delivery system for cancer treatment.

### 5.2. Nanotherapy targeting CSC-associated signaling pathways

#### 5.2.1. Wnt/β-catenin pathway

Wnt signaling is a key pathway involved in cellular development and stemness and is aberrantly upregulated in several cancers like pancreatic ductal carcinoma, breast, colon, and others (Zhan et al., 2017; Nasrolahi et al., 2023; Ram Makena et al., 2019). As a major pathway involved in conferring cellular properties of stemness, chemoresistance and metastasis, resulting in poor patient outcomes, targeting the Wnt pathway remains a promising strategy in combating tumorigenesis (Valcourt et al., 2020). The molecular inhibitors of the Wnt pathway may be naturally occurring compounds (Liu et al., 2018) or synthetic agents (Bhattacharyya et al., 2017; Motawi et al., 2017). Several studies have shown that the conjugation of Wnt inhibitors with nanoparticles successfully inhibits the pathway, thereby unleashing an anti-proliferation effect on the cancer cells. A particular study demonstrated cationic lipid-protamine nanoparticles that delivered plasmid DNA encoding a trap protein capable of binding Wnt5a, thereby repressing its expression and inhibiting tumor growth in melanoma. This study confirms that Wnt5a trapping resulted in remodeling the fibrotic immunosuppressive TME, and facilitated T cell infiltration, thereby offering a promising platform for the treatment of desmoplastic BRAF-mutant melanoma. This is especially true when combined with a commonly accepted chemotherapy that can further lead to preparation of a tumor specific vaccine (Liu et al., 2018).

Moreover, Niclosamide (NIC) and cromolyn are synthetic Wnt inhibitors that have been successfully incorporated into nanoparticle carriers, and they act by promoting Frizzled internalization, and thereby block wnt pathway (Chen et al., 2009). In a study, the group successfully developed and preclinically tested NIC-loaded polypeptide nanoparticles that were synthesized by conjugating NIC to recombinant chimeric polypeptides (CPs) to generate CP-NIC, that behaved as a pro-drug to considerably increased the plasma exposure of NIC, thereby increasing the effect of the inhibitor. Furthermore, nanoformulating NIC with polypeptides to overcome its low solubility and bioavailability has effectively inhibited β -catenin levels in the cytosol . Moreover, a Cromolyn nanoparticle based-formulation has also been shown to have an anticancer effect in rats with induced colorectal cancer (Motawi et al., 2017). The study showed that cromolyn chitosan nanoparticles (CCSNPs) sustained drug release pattern over 48 h significantly reduced tumor-signaling molecules. Conclusively, CCSNOs ameliorated the whole tumor pathophysiology, enhanced the drug effect and could provide a better and novel approach for colon cancer treatment (Motawi et al., 2017).

Using nanoparticles in conjugation with siRNA or microRNA (miRNA) are also promising approach against targeting the Wnt pathway, as β -catenin, the major protein involved in the pathway, is practically undruggable. According to a particular report, β-catenin inhibition can be achieved using lipid nanoparticles (LNPs) containing dicer substrate siRNA (DsiRNA) targeting CTNNB1, the gene that encodes β-catenin (Ganesh et al., 2016).

Gold nanoshells are reported to be effective in delivering Frizzled7

(FZD7) antibodies and inhibiting cell growth and proliferation in triple-negative breast cancer (TNBC) (Riley and Day, 2017). The use of peptide nanoparticle compounds has also been demonstrated against Wnt signaling using ultra-small magnetic iron oxide nanoparticles (IONPs) coated with peptides that target both Wnt/LRP5/6 and uPA receptors. These were shown to successfully deliver, inhibiting the growth and proliferation of breast cancer cells (Miller-Kleinhenz et al., 2018).

#### 5.2.2. Notch signaling pathway

The Notch signaling pathway is associated with pro-survival genes that regulate self-renewal and proliferation of cancer cells and determine stem cell fate (Mumm and Kopan, 2000). As a frequently deregulated pathway in cancer, Notch hyperactivation is almost always associated with carcinogenesis, thus highlighting it as a useful therapeutic target. Notch signaling is crucial for stem and progenitor cell functions in several tissues (Matsui, 2016). Moreover, Notch reduces the expression of PTEN, a tumor suppressor gene, and enhances the expression of c-Myc oncogene, thereby increasing the occurrence of cancer (Hill and Wu, 2009). Out of the several inhibitors of notch that have been extensively studied, gamma-secretase inhibitors (GSIs) that block the cleavage of the intracellular domain of notch (thus preventing its translocation to the nucleus) have been broadly investigated (Espinoza and Miele, 2013). However, as the oral administration of GSI was found to be linked to several severe gastrointestinal problems, its coating on nanoparticles considerably alleviated such issues. Interestingly, a particular study (Mamaeva et al., 2016) attempted the development of MSN to deliver a GSI, namely, N- [N-(3, 5-Difluorophenacetyl)-L-alanyl]-S-phenyl glycine t-butyl Ester (DAPT) into breast cancer cell lines MDA-MB-231.

Consequently, this study thereby reported a significant reduction in the number of CSCs. In addition, polylactic acid-based nanoparticles have also been used to deliver DAPT, in combination with the EGFR-inhibitor erlotinib, to TNBC cells (Wan et al., 2019). A nano-formulation of shRNAs targeting Notch-1 has also been developed using iron oxide-silica nanoparticles for use in TNBC. This study reported that the nanoparticles successfully reduced Notch-1 expression and cell growth while also triggering cell death (Yang et al., 2014). In addition, using chitosan-based nanoparticles coupled with miRNA (miR-34) has also been demonstrated to influence the Notch signaling pathway, resulting in cell death in TNBC (Deng et al., 2014). These studies indicate that notch signaling could potentially serve as a huge target to inhibit tumor growth and chemoresistance in cancer cells.

Targeting Notch signaling pathway has a widely accepted remarkable effect in cancer therapy owing to its potential in inhibiting cell proliferation and inducing apoptotic cell death. Much of theCSC inhibiting effects of inhibiting notch pathway could be attributed to the extensive crosstalk between Notch and other cellular signaling pathways crucial for growth and development of cancer cells including Ras, Akt and NF-κB. Combination of Notch inhbitors with chemotherapeutic drugs have found to have a surprising anticancer effect in many cancers. Though effective, some of the Notch inhibitors when tested at clinical trilas have found to have gastrointestinal toxicity. Moreover, low specificity of gamma-secretase inhibitors are also observed, though they are already used in clinical trials. Apart from the side effects and limited existing options for Notch inhibitors, there is growing optimism that Notch inhibtion could be a batter approach in the future therapy options in cancer (Purow, 2012).

The Notch signaling pathway interacts with various other signaling pathways, and its inhibition can have a broad range of effects on cellular processes. Some of the key pathways that interact with Notch signaling include:

(1) Wnt/β-Catenin Pathway: The Notch and Wnt pathways are interconnected and can have both synergistic and antagonistic interactions. For example, Notch signaling can inhibit the Wnt pathway by promoting the degradation of β-catenin, while Wnt signaling can inhibit the expression of Notch target genes.
(2) Hedgehog Pathway: The Notch and Hedgehog pathways also have complex interactions. In some contexts, Notch signaling can inhibit Hedgehog signaling, while in others, it can promote Hedgehog pathway activation.
(3) TGF-β/Smad Pathway: Notch signaling can interact with the TGF-β pathway by regulating the expression of TGF-β ligands and receptors, as well as by modulating the activity of Smad proteins.
(4) MAPK/ERK Pathway: Notch signaling can modulate the activity of the MAPK/ERK pathway by regulating the expression of MAPK pathway components and by interacting with Ras and Raf proteins.
(5) PI3K/Akt/mTOR Pathway: Notch signaling can activate the PI3K/Akt/mTOR pathway by upregulating the expression of PI3K and Akt, and by promoting the phosphorylation of mTOR.

Inhibition of the Notch signaling pathway can have several potential negative impacts on these interacting pathways:

(1) Wnt/β-Catenin Pathway: Inhibition of Notch signaling can lead to the activation of the Wnt pathway, which may promote tumorigenesis and metastasis in certain contexts.
(2) Hedgehog Pathway: Inhibition of Notch signaling can lead to the activation of the Hedgehog pathway, which may promote cell proliferation and survival.
(3) TGF-β/Smad Pathway: Inhibition of Notch signaling can lead to the dysregulation of TGF-β signaling, which may result in impaired cell differentiation and tissue homeostasis.
(4) MAPK/ERK Pathway: Inhibition of Notch signaling can lead to the downregulation of the MAPK/ERK pathway, which may impair cell proliferation, survival, and migration.
(5) PI3K/Akt/mTOR Pathway: Inhibition of Notch signaling can lead to the downregulation of the PI3K/Akt/mTOR pathway, which may impair cell metabolism and survival.

In summary, the Notch signaling pathway interacts with various other signaling pathways, and its inhibition can have a broad range of effects on cellular processes. It is important to consider these interactions and potential negative impacts when targeting the Notch pathway for cancer therapy.

#### 5.2.3. Hedgehog (Hh) signaling pathway

The Hh pathway is involved mainly in cell growth and morphogenesis. Moreover, this signal transduction pathway regulates embryonic development and plays a role in the maintenance of stem cells and tissue repair (Ruiz i Altaba et al., 2002). Hh signaling has been widely associated in CSC function and maintenance (Cochrane et al., 2015) . Activation of this pathway is initiated upon ligand binding to Patched 1, which drives an intracellular signaling cascade, ultimately activating three Gli transcription factors. These factors regulate several genes involved in cell proliferation, inhibition of apoptosis, metastasis, invasion, angiogenesis, and cancer stemness (Melamed et al., 2018; Rubin and de Sauvage, 2006; Pasca di Magliano and Hebrok, 2003). Aberrant activation of Hh signaling is observed in several cancers, including those of the breast, ovary, blood, prostate, and others (Kiesslich et al., 2012; Gonnissen et al., 2013; Bhateja et al., 2019).

The inhibition of Hh pathway members via antibodies, small molecules, and RNA interference has paved its way into clinical trials and are also approved as cancer treatment (Kiesslich et al., 2012). However, the poor bioavailability and resistance to these therapies have invoked the development of novel nanoparticle-based delivery systems. In this regard, antothecol-loaded PLGA nanoparticles were observed to reduce cell proliferation and induce apoptosis in pancreatic CSCs by inhibiting Gli-DNA binding (Verma et al., 2015). Moreover, according to another report, α-Mangostin-loaded PLGA nanoparticles were able to target

pancreatic CSC by disrupting Gli-DNA binding (Verma et al., 2016). Similarly, another study (Ingallina et al., 2017) also reported that glabrescione B conjugated with PLGA nanoparticles was effective in eradicating CSCs in solid tumors. In addition, the stability, *in-vitro* cytotoxicity and *in-vivo* bio-distribution of the drug were found to be comparatively much superior in the nanoformulation (Ingallina et al., 2017).

Cyclopamine, a natural inhibitor of the Hh signaling pathway, is reported to have poor solubility and stability and is often linked to the occurrence of several side effects. However, the development of a nanoformulation using polymeric nanoparticles in combination with doxorubicin was found to be effective in a breast cancer model (Hu et al., 2015). Similarly, another study brought about the beneficial effect of combination therapy by using cyclopamine-paclitaxel polymer nanoparticles in mice models of pancreatic and prostate cancer (Zhao et al., 2018; Yang et al., 2017). Moreover, PLGA nanoparticles formulated with the Gli1/2 antagonist, hedgehog pathway inhibitor-1 (HPI-1), are reported to impede the growth and metastasis of hepatocellular carcinoma in mice. In addition, this nanoformulation was also effective against CD133 expressing CSCs in liver cancers (Xu et al., 2012). Moreover, when combined with the anticancer drug gemcitabine, the nanoformulation successfully inhibited the growth of pancreatic cancer xenografts (Chenna et al., 2012). CSC death was also achieved by utilizing the Gli1 inhibitor GANT61 on PLGA nanoparticles, as reported by a particular study (Borah et al., 2017) demonstrating the destruction of cancer cells through the modulation of the Hh Pathway.

RNA interference using siRNAs is also extensively studied in Hh signaling inhibition, wherein most reports focus on inhibiting GLI proteins. In a particular report, Melamed et al. produced spherical nucleic acids wrapped in polyethyleneimine by conjugating GLI1 siRNA to gold nanoparticles. This nanoformulation successfully inhibited GLI1 and its downstream effectors (Melamed et al., 2018). Moreover, the use of a non-viral gene delivery vector has also been demonstrated in conjugation with a nanoparticle platform that further enhanced its efficacy. Here, the nano-drug delivery system was developed through the conjugation of siGLI1 with 1,2-dioleoyl-3-triethylammonium-propane (DOTAP)-conjugated methoxy-PEG-poly(lactide) copolymer that was able to induce cell death and inhibit cell growth of subcutaneous glioblastoma xenografts *in-vivo* (Zhou et al., 2018).

**Challenges associated with targeting CSCs in cancer treatment**

Targeting Cancer Stem Cells (CSCs) in cancer treatment is a promising strategy to enhance treatment efficacy and sensitize cells to therapy. However, this approach presents several challenges that need to be addressed to maximize its therapeutic potential.

(1) Heterogeneity of CSCs: CSCs are highly heterogeneous, both within and between tumors. This heterogeneity is reflected in the expression of surface markers, genetic mutations, and signaling pathways, which can vary significantly among CSCs from different tumors (inter-tumoral heterogeneity) or even within the same tumor (intra-tumoral heterogeneity) (Botchkina et al., 2009). This heterogeneity can interfere with the selective targeting of specific CSC sub-populations and may also affect signaling pathways or the tumor microenvironment that might not be the real targets for cancer treatment. Additionally, this heterogeneity can lead to the development of resistance to targeted therapies, as sub-populations of CSCs that are not effectively targeted may survive and contribute to tumor recurrence.
(2) Complexity of the Tumor Microenvironment: The tumor microenvironment is a complex network of various cell types, extracellular matrix components, and signaling molecules that can influence the behavior of CSCs. For example, interactions with stromal cells, immune cells, or extracellular matrix components can promote the survival, self-renewal, and invasion capabilities of CSCs. Additionally, the tumor microenvironment can also influence the accessibility of therapeutic agents to CSCs, thereby affecting the efficacy of targeted therapies.
(3) Molecular Drivers of CSCs: CSCs are regulated by multiple molecular drivers that contribute to their self-renewal, differentiation, and survival. These molecular drivers can include signaling pathways (e.g., Wnt/β-catenin, Notch, Sonic hedgehog), transcription factors (e.g., Sox2, Oct4, Nanog), and epigenetic regulators (e.g., histone deacetylases, DNA methyltransferases). Targeting these molecular drivers is crucial for the effective inhibition of CSCs. However, given the complexity and redundancy of these regulatory networks, it is often challenging to identify and target the key drivers that are essential for CSC maintenance and function.
(4) Combination Therapies: Given the challenges associated with targeting CSCs, a more effective approach may be to combine therapies that target the molecular drivers of CSCs with standard chemotherapeutic treatments. This combination approach can help overcome the limitations of single-agent therapies, enhance the efficacy of treatment, and minimize the risk of resistance development (Turdo et al., 2019).

In summary, targeting CSCs in cancer treatment presents several challenges related to the heterogeneity of CSCs, the complexity of the tumor microenvironment, and the identification and targeting of key molecular drivers. Addressing these challenges will be crucial for the development of more effective therapeutic approaches that specifically target CSCs and yield better therapy outcomes.

## 6. Conclusion and future perspective

In conclusion, our understanding of cancer and its mechanisms has come a long way, yet the disease remains a formidable adversary, partly due to the limitations of existing treatment modalities. Conventional chemotherapy, the standard tool in our armamentarium, has been mired with issues such as cytotoxicity, lack of selectivity, and induction of multi-drug resistance. These limitations inevitably give rise to an urgent need for alternative therapeutic strategies. The application of nanotechnology in cancer treatment has begun to fulfill this need, offering promising breakthroughs and bringing a new level of sophistication to the treatment landscape.

Nanomaterials, with their unique properties, have demonstrated immense potential for enhancing the delivery of anticancer drugs to the target cells, reducing off-target effects, and specifically targeting CSCs. Clinical studies exploring these nanoformulations have shown promising results, highlighting their potential to become a cornerstone in cancer therapeutics. Yet, despite these strides, the approval of nanodrugs has remained relatively low, reminding us of the challenges that persist. As we advance, researchers and clinicians alike must channel their efforts towards mitigating these challenges. To increase the success rate of nano-drug approval, the focus needs to be on reducing the toxicity of nanomaterials, refining their permeability and retention for optimal drug delivery, and minimizing the protein corona's shielding effect.

Moreover, there is a need to deepen our understanding of cancer stem cell biology and their interactions with nanomaterials, as this can pave the way for developing more specific and effective nano-based therapeutic strategies. In addition, expanding clinical trials of nanotherapeutics, including those targeting CSCs, is crucial for validating their safety and efficacy in a diverse population. The promising future of nanotechnology in cancer treatment is in sight, yet it demands concerted efforts and relentless research. With the necessary commitment and resource allocation, we believe that nanotechnology could significantly change the prognosis for patients battling this deadly disease, truly revolutionizing the field of oncology.

## Ethical approval

he study does not require ethical approval

## Consent to participate

Not applicable

## Consent for publication

Not applicable

## Availability of data and material

Not applicable

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**Queenie Fernandes:** Writing – original draft, Project administration. **Lubna Therachiyil:** Conceptualization, Writing – original draft. **Abdul Q. Khan:** Conceptualization, Writing – original draft. **Takwa Bedhiafi:** Writing – review & editing. **Hesham M Korashy:** Writing – review & editing. **Ajaz A. Bhat:** Conceptualization, Writing – review & editing. **Shahab Uddin:** Supervision, Writing – review & editing, Writing – original draft.

## Declaration of Competing Interest

The authors declare no conflict of interest

## Data availability

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