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NANOTECHNOLOGY AS CUTTING EDGE TECHNOLOGY FOR SCREENING, DETECTION AND TREATMENT OF COLORECTAL CANCER

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ABSTRACT

Colorectal Cancer (CRC) remains one of the most devastating disease threatening public health, causing high mortality worldwide every year. For decades, chemotherapy has served as the preferred treatment. However, conventional chemotherapeutics can't distinguish cancer cells from normal cells, and damage healthy cells and tissues with evident toxicity. Therefore, it is of central importance to develop efficacious anti-cancer drugs that selectively target cancer cells with low toxicity. Nanomaterials have recently been emerging as attractive pharmacological vehicles for drug delivery, cancer therapy and tumor targeting. The engineered nanomaterials can gain unusual physiochemical characteristics because of their small sizes, surface structure, solubility and shapes. Importantly, nanomaterials can be designed as nanoscale drug carriers to avoid immune clearance by lymphocyte-macrophage system and therefore allow drugs to efficiently target cancer cells. The targeting schemes explored for many of the reported nanoparticles systems suggest the great potential of targeted delivery to revolutionize colorectal cancer treatment. The present article is compilation of cutting edge technologies such as quantum dots, (polylactide-co-glycolide) PLGA nanoshells, dendrimers, iron oxide nanocrystals and gold nanospheres for screening, detection and treatment of CRC.

INTRODUCTION:

CRC is a heterogeneous disease that begins in the colon and the rectum, which are parts of the large intestinal system. The colon is divided into four sections referred to as ascending, transverse, descending and sigmoid colon and the latter is where most CRC arise. The majority of CRC develop slowly from adenomatous polyps or adenomas [1]. The intestinal epithelium has a high turnover rate, which makes it hotspot for malignant transformations such as CRC. Vogelstein first explored the colorectal carcinogenesis model stating that specific genetic events could result in morphological tissue changes [2]. This model has been referenced for more than 20 years and now-a-days several studies suggest that CRC is a consequence of an array of factors, which are not only inherited but also acquired over the life time of the individual. Different molecular markers are associated with CRC development and metastasis, for example the metastatic potential of CRC could be linked to cell adhesion molecules such as cadherins and catenins, that when down regulated could facilitate tumour cell detachment thus mediating the prognosis of this cancer [3]. The risk to develop CRC could

also be linked to environmental, socioeconomic and lifestyle factors. However, making out exactly all the mechanisms and the associations between all these factors is difficult. The research going on the molecular basis of CRC carcinogenesis and how this can be influenced by the lifestyle of different populations around the world, has been invaluable as a preventive strategy. Moreover, the evolving field of molecular pathological epidemiology (MPE) has brought the molecular and population science closer to the medical practice. It combines two traditionally used disciplines that use different approaches to understand disease, which are epidemiology and pathology. This multidisciplinary investigation comprises the understanding of the different aspects involved in the carcinogenesis process such as environmental/ exogenous (e.g.: lifestyle) and endogenous (e.g.: genetic) factors, molecular pathological markers for tumour initiation and progression and response to different types of treatments, which collectively have led to important studies not only in CRC but also in other diseases [4]. An example of an important study associated with MPE research is the one describing that aspirin use is linked to a better prognosis and clinical outcome in *PIK3CA* mutated CRC [5]. Therefore, personalized medicine involving a multidisciplinary team is an important tool, which aims to target cancer therapies based on unique genetic patterns of the tumor [6, 7].

As mortality due to cancer continues to rise, advances in nanotechnology have significantly become an effective approach for achieving efficient drug targeting to tumour tissues by circumventing all the shortcomings of conventional chemotherapy. During the past decade, the importance of polymeric drug delivery systems in oncology has grown exponentially. In this context, poly(lactic-co-glycolic acid) (PLGA) is a widely used polymer for fabricating 'nanoparticles' because of biocompatibility, long-standing track record in biomedical applications and well-documented utility for sustained drug release, and hence has been the centre of focus for developing drug-loaded nanoparticles for cancer therapy. Such PLGA nanoparticles have also been used to develop proteins and peptides for nanomedicine, and nanovaccines, as well as a nanoparticle-based drug- and gene-delivery system for cancer therapy, and nanoantigens and growth factors. These drug-loaded nanoparticles extravagate through the tumor vasculature, delivering their payload into the cells by the enhanced permeability and retention (EPR) effect, thereby increasing their therapeutic effect. Ongoing research about drug-loaded nanoparticles and their delivery by the EPR effect to the tumour tissues has been elucidated in this review with clarity. Rapid advances in cell and molecular biology have allowed us to develop a better understanding of the pathophysiology of various diseases [8]. As mortality due to cancer continues to rise, progress of research in this area requires a new paradigm in the diagnosis and therapy of the disease. Although chemotherapy has an important place in the clinical management of cancer, the molecular complexity associated with drugs and the inaccessibility of most physiological targets have resulted in the development of alternative therapies as an approach to medical intervention [9]. On the one hand, genomics and proteomics research are identifying new tumour-specific molecular targets and, on the other, innovative drug-delivery systems are being designed to guide drugs more precisely to tumour cells and away from sites of toxicity to maintain drugs at a therapeutic concentration over long periods of time[10]. The long-cherished goal of targeting drugs to specific sites in the body, where the pharmacological action is desired, sparing other tissues, has been actively pursued all these years. The concept of 'magic bullets' given by Ehrlich has now seen a metamorphosis to 'magic wands,' in the form of drug-delivery systems [11]. Advances in nanotechnology have significantly impacted the field of therapeutics delivery significantly. The medical application of nanotechnology (i.e., 'nanomedicine') has enormous potential to improve health care, particularly in cancer. The last few decades have hosted a revolution in materials science by the miniaturisation of devices for use as diagnostics, biosensors and imaging agents on the one hand, and, on the other, ever more sophisticated synthetic chemistry is producing nanocarriers for drug delivery. At present, non-invasive imaging approaches, including X-ray-based computer-assisted tomography (CT), positron emission tomography (PET), single-photon emission tomography and magnetic resonance imaging

(MRI), are used as important tools for detection of human cancer [12]. The development of tumor-targeted contrast agents based on a nanoparticle (NP) formulation may offer enhanced sensitivity and specificity for in vivo tumor imaging using currently available clinical imaging modalities. As drug-delivery agents, these multifunctional nanocarriers are capable of targeting cancer cells, delivering and releasing drugs in a regulated manner and detecting cancer cells with enormous specificity and sensitivity. Nano vehicles of the mesoscopic size range of 5–200 nm result in unique interaction with biological systems at the molecular level. As a result of their material composition, these nanocarriers are also capable of self-assembly and maintaining stability and specificity, which are crucial to drug encapsulation and biocompatibility. Therefore, their application as cancer cell-specific delivery vehicles is a significant addition to the currently available armory for cancer therapeutics and imaging [13, 14].The colorectal cancer and the formation of polyp is given in Figure 1 and Figure 2.

Nanotechnology in screening, detection and treatment of CRC

For last few decades, foundations were laid down for nanotechnologies to fabricate diagnostic and therapeutic agents in a more well-organized way [15]. The dream came to reality with the rising numbers of nanodignostics and nanotherapeutics being commercialized and reached to the clinical stage.(16) Several nanotechnological applications have been identified in onchology, including early detection of tumors and the development of treatment approaches that cannot be achieved using the existing conventional technologies.(17)In fact,in certain type of cancers,nanosized particles of diverse shapes and compositions have come out as promising and important new tools for CRC diagnosis,staging and treatment.[18] Early detection of CRC is the key for the prevention and has the potential to impact long term survival of the patient with CRC. Recent advancements in the development of nanoparticles that offered new prospects for the early detection and successful treatment of CRC were discussed in the following Table 1.

Quantum dots:

Quantum dots (QDs) are semiconductor nanocrystals that fluoresce on excitation with light and have exceptional optical characteristics such as high brightness, resistance to photo-bleaching, and the ability to emit fluorescence at different wavelengths. Given the optical and chemical advantages of QDs, QD-based nanotechnology is an emerging platform for studying various type of cancers including the CRC. [19-21]. In a recent investigation, researchers qualitatively analyzed the expression level of large external antigen in tissue samples from CRC patients using QD-based immunohistochemistry (QD-IHC) and conventional IHC. [22] Compared with conventional IHC, QD-IHC rendered several noticeable advantages for protein marker quantification. These included higher sensitivity, simpler operation, less human interference and increased capability for simultaneous multifactor analysis, which would lead to more accurate clinical evaluation. Therefore, QD-IHC could be a better substitute for conventional IHC in clinical applications. In addition, unlike the majority of other studies that primarilyfocus on marker detection in tissue sections using QD probes, QD-based immunocytochemistry combined with imaging quantitative analysis for the detection of large external antigen in living cells obtained results that were in agreement with those generated using flow cytometry. This result suggested that this method is for noninvasive molecular analysis of rare CTCs and provided a rationale for expanding the application of QD probes to clinical practice. [23] In another study, a simple and highly sensitive technique was developed to detect aldo-ketoreductase family 1 member B10 (AKR1B10), a prognostic predictor and therapeutic target for CRC in the serum, using QDs with a high-fluorescence quantum yield against photo bleaching and size-controlled luminescence. This immune fluorescence assay to detect serum AKR1B10 using anti-AKR1B10-conjugated CdTe/CdS QDs is a promising approach for the early prediction of CRC. This technique was simple and fast with high sensitivity and specificity. Carbary-Ganz et al. [24].

Developed a technology in which optical coherence tomography/laser-induced fluorescence dual-modality imaging allows for slightly invasive, nondestructive endoscopic visualization of CRC.This strategy enables simultaneous longitudinal tracking of morphological (optical coherence tomography) and biochemical (fluorescence) alterations during CRC development and progression. In the carcinogen-treated mice, QDot655 targeted to vascular endothelial growth factor receptor 2 (QD655-VEGFR2) localized to the colon and offered a considerably high contrast between the diseased and healthy tissue with high sensitivity and specificity ex vivo.[25] In another study, researchers developed an exceptional cell-targeted, paramagnetic-fluorescent double-signal molecular nanoprobe (GdDTPA·BSA@QDs-PcAb) for in vivo magnetic resonance imaging (MRI) diagnosis and subsequent biopsy of CRC. These multipurpose GdDTPA·BSA@QDs-PcAb nanoprobes were synthesized by surface engineering of QDs with DTPA·BSA-$Gd3^{+}$ macromolecule complex under ultrasonication conditions. The resulting GdDTPA·BSA@QDs exhibit excellent colloidal constancy with fine hydrodynamic size in a broad array range of pH values and ionic strengths. They also exhibit much higher transverse relaxivity and longitudinal relaxivity in water than commercial Gd–DTPA solutions. In vivo MRI revealed GdDTPA·BSA@QDs-PcAb to be a promising candidate for use in CRC contrast-enhanced MRI diagnosis. Biodistribution results showed gradual clearance of the nanoprobes from the body via biliary (hepatobiliary) excretion. No obvious in vitro or in vivo toxicity was observed in the MTT assay or toxicity studies, respectively. Based on these experimental evidences, the GdDTPA·BSA@QDs-PcAb nanoprobes show great potential for CRC tumor-targeted MRI and tumor tissue biopsy analysis. Gazouli et al [26] assessed the CRC-targeting ability of bevacizumab-conjugated QDs in vitro and in vivo. In the in vitro studies, immunocytochemical data confirmed strong and specific binding of the QD-bevacizumab complex to the cells. In vivo fluorescence imaging showed improvement of the tumor-specific signal following injection of the QDs. This study successfully detected VEGF-expressing tumors using QDs-bevacizumab nanoprobes in vitro and in vivo, and these consequences represent a significant advancement in VEGF-targeted noninvasive imaging in clinical practice. In another study, Carbary-Ganz et al [27] successfully labeled CRC in vivo using QDs targeted to VEGFR2. QDs with emission centered at 655 nm were bioconjugated to anti-VEGFR2 antibodies through streptavidin/biotin linking. The resulting QD655-VEGFR2 contrast agent was functional in vivo and localized to the colon of azoxymethane-treated mice following lavage and incubation. This QD655-VEGFR2 contrast agent significantly enhanced the contrast between diseased and normal tissues. Specificity was assessed by observing an inappropriate increase in contrast, when labeling colons of azoxymethane-treated mice with QDs bioconjugated to isotype control antibodies and by labeling the colons of saline-treated mice (control). This contrast agent shows great possibility for in vivo endoscopic imaging of the colon. Using the subtractive Cell-SELEX technology under selective conditions, Li et al [28] produced a panel of seven aptamers (Apts) that specifically bind metastatic CRC cells (LoVo cells) and some other metastatic cancer cell lines with high affinity, thereby offering broad-spectrum specific recognition of metastatic cancer cells. This study demonstrated that Apts selected from single Cell-SELEX can be individually functionalized for various purposes according to the biochemical properties of the targets, thereby increasing the advantages of the Cell-SELEX approach. The receptor-targeting Apt W14 was used as a targeted carrier for specific delivery of doxorubicin to target cells in order to remarkably decrease the cytotoxicity of this drug. The nonmembrane receptor-binding Apt W3 conjugated to a QD was used as a molecular probe for targeted imaging of metastatic cancer cell lines, metastatic tumor-bearing tissue sections, and formalin-fixed paraffin-embedded specimens from CRC patients.[29] These two Apts (W14 and W3) may be adopted for combinational diagnostic and therapeutic applications as they target the same cells. In addition, the seven Apts investigated in this study exhibited no cross recognition of their individual targets. This finding suggested that these Apts can be used in combination for multitarget cancer cell imaging and multitarget drug therapy to overcome drug resistance, gaining high detection sensitivity, and attaining good

treatment effectiveness for metastatic cancer. Therefore, QDs are a technological advancement that can revolutionize CRC diagnosis and treatment. As stated earlier, QDs are now widely used for the detection of cancer biomarkers and cancer cell invasion and for focusing on the tumor environment. Furthermore, they represent a new strategy for deeper analysis of CRC tumor heterogeneity, as well as showing promise for the diagnosis and treatment of CRC.

Iron oxide nanocrystals:

Iron oxide NPs have the dual ability to act as both magnetic and photothermal agents and have been approved for human use as MRI contrast agents. They also have excellent biodegradability in vivo, and the iron ions they release upon dissolution can be assimilated by the body through a tightly regulated physiological process.Kuo et al[30] fabricated smart multifunctional magnetic nanovehicles encapsulating anticancer drugs and an antibody-targeting peptide AP-1 (MPVA-AP1). In this study, the magnetic nanovehicles with consistent sizes and dispersed in aqueous solution displayed good hemocompatibility and no toxicity toward L929 fibroblasts, which showed their potential for applications in therapeutics. Using a simple synthesis method, that is, the double-emulsion method, the researchers observed considerable encapsulation of both hydrophilic and hydrophobic low-molecular-weight drugs and protein-like drugs. Furthermore, the antibody-targeting peptide AP-1 was immobilized on the surface of the magnetic nanovehicles, and the immobilization was confirmed through electron spectroscopy. A CRC cell (CT26-IL4Rα) test revealed that the AP-1-bound nanovehicles (MPVA-AP1) exhibited outstanding targeting and selectivity. A stable storage test confirmed near-zero leakage of the encapsulated drugs in the absence of the magnetic stimulus. In contrast, the doxorubicin-loaded nanovehicles ruptured upon high-frequency magnetic field treatment, that is, rapid and accurate controlled release. Moreover, in vivo studies established that the magnetic nanovehicles displayed obvious thermotherapeutic and chemotherapeutic effects. Thus, smart magnetic nanovehicles, such as MPVA-AP1, have significant potential for targeted doses and accurate controlled release in anticancer applications. Esmaelbeygi et al [31] demonstrated the effectiveness of polylactide-co-glycolic acid (PLGA) NPs as a 5-fluorouracil (5-FU) carrier with and without an iron oxide core and hyperthermia at the point of DNA damage in a spheroid culture model of HT-29 colon cancer cell lines by alkaline comet assay. In their study, the cells treated with a combination of hyperthermia and 5-FU or NPs as 5-FU carriers showed more DNA damage than the controls. The degree of DNA damage following the treatment with 5-FU-loaded NPs and hyperthermia was considerably more than that for 5-FU and hyperthermia. Between 5-FU-loaded NPs with and without the iron oxide core, the NPs with the iron oxide core in combination with hyperthermia induced more DNA damage than those without the iron oxide core. As per this study, hyperthermia is a harmful agent, and NPs are efficient delivery vehicles of drugs to CRCs. The iron oxide-filled NPs increased the effect of hyperthermia and could be highly useful in the treatment of CRCs. Yang et al. [32] synthesized magnetic nanoparticles coated with antibodies against carcinoembryonic antigen (CEA) and studied different characteristics like particle size, particle suspension, bioactivity, and the constancy of biomagnetic NPs suspended in liquid. In addition, the characteristics of the CEA molecules in serum were studied, and the assay method used was immunomagnetic reduction. Their results showed that the effects of common materials in serum that interfere with the detection of signals are not vital. The lower limit of detection was 0.21 ng/mL, which is below the clinical threshold of 2.5 ng/mL. The dynamic range for the assay of CEA molecules in serum was 2.5–500 ng/mL. By quantifying serum CEA levels in 24 controls (normal) and 30 CRC patients, the threshold for the serum-CEA concentration for CRC diagnosis was found to be 4.05 ng/mL, which yields a clinical sensitivity of 0.90 and specificity of 0.87In another study, researchers encapsulated the pacli-taxel (PTX) and super-paramagnetic iron oxide (SPIO) inside the core of PEALCa micelles and used these for potential cancer therapy.[33] Drug release study showed that the PTX in the micelles was released faster at pH 5.0 lower than the neutral pH. Cell culture experiments revealed that the PTX-SPIO-PEALCa micelle was successfully internalized by human CRC LoVo cells, and

PTX was likely entrenched within lysosomal compartments. In addition, the accuracy of delivery to LoVo cells was confirmed in vivo by MRI and histological analysis. Furthermore, successful suppression of CRC LoVo cell growth was confirmed. Their observations established that the PTX-SPION-loaded pH-sensitive micelles were a capable MRI-visible drug-release system for CRC therapy. Mannucci et al [34] tested magnetosomes (MNs), magnetic NPs produced by magnetotactic bacteria, to develop novel therapeutic strategies for neoplastic diseases.

They extracted MNs from the gram-negative bacterium, *Magnetospirillum gryphiswaldense*, and tested their interactions with cellular elements and antineoplastic activity both in vitro and in vivo. In vitro studies executed on HT-29 cell cultures established strong uptake of MNs without any cytotoxicity. In vivo experiments were carried out on subcutaneous tumors in mice, and MNs were administered by direct injection into the tumor, and the tumors were monitored by MRI. Histological investigation showed fibrous and necrotic areas close to the MN injection sites in mice subjected to a total thermotherapy procedure. These observations, even though relating to a specific tumor model, might be helpful to further investigate the feasibility and efficacy of protocols based on magnetic fluid hyperthermia. In addition, magnetic nanoparticles naturally produced and extracted from bacteria appear to be appropriate candidates for applications in cancer therapy.

He et al [35] reported the covalent conjugation of lectin on Fe_2O_3@Au core@shell NP (lectin-Fe_2O_3@Au NP) for T2-weighted MRI and X-ray CT dual-modality imaging. [51] The lectin-Fe_2O_3@Au NPs are synthesized by combining lectins with the Fe_2O_3@Au NP surfaces through bifunctional polyethylene glycol (PEG) NHS ester disulfide (NHS-PEG-S-S-PEG-NHS) linkers. After the nonspecific adsorption places on the surface of NP are blocked by thiolated PEG (PEG-SH), the lectin-Fe_2O_3@Au NPs showed exceptional stability in biological medium and imperceptible cytotoxicity. A sequence of both in vitro and in vivo trials performed for assessing the potential of three selected lectins (ConA, RCA, and WGA)-Fe_2O_3@Au NPs revealed that the lectin-Fe_2O_3@Au NPs could be used for dual mode MRI and CT imaging in vitro and also for MRI and CT imaging of CRC in vivo. The obtained results recommend that lectins could be used as tumor targeting ligands in NP-based contrast agents

PLGA nanoparticles/nanocells:

Biodegradable PLGA NPs have been used as carriers for peptides, proteins, nucleotides, vaccines, and drugs [36]. These can help in protecting drug moieties from degradation and consequently ensure sustained release of drugs. NPs have also been extensively studied for their potential use in cancer therapy, particularly for CRC. For instance. [37] curcumin-loaded PLGA NPs for colon delivery were successfully prepared using the emulsion–solvent evaporation technique. The use of the 2^3 factorial design model enabled the development of an optimized curcumin-loaded PLGA-based nanoformulation using minimal raw material in a short time. Studies based on differential scanning calorimetry confirmed the presence of curcumin in an amorphous or disordered crystalline phase of molecular dispersion form or in a solid solution state in the polymer matrix. Fourier transform infrared studies confirmed that no substantial interactions occur between curcumin and PLGA. Transmission electron microscopy images of the optimized blank NPs (B-PNP_9) and curcumin-loaded PLGA NPs (C-PNP_9) revealed that the NPs were discrete, nonaggregated, and spherical in shape and displayed good size distribution. The smaller size, sustained release, better colloidal stability in synthetic gastrointestinal fluids, long-term stability, and a considerably higher cellular uptake in HT-29 cells as compared with those of pure curcumin solution indicate that the optimized curcumin-loaded PLGA NP formulation (C-PNP_9) has high potential as an initial platform for the further development of an efficient oral targeted drug-delivery system to the colon, especially if it is further functionalized with a mucoadhesive polymer or a specific targeting ligand.

In another study, researchers prepared chitosan polymeric NPs by the solvent evaporation emulsification method, with varied ratios of polymer. They concluded that the NPs prepared by solvent evaporation emulsification using the polymer at the optimum ratio

represents an approach for potentially successful delivery of the active pharmaceutical element to the colorectal tumors. [38] Total health centre complex N-38 is a highly effective drug against many cancers, as it contains pharmaceutically active ingredients. However, the development of an optimal delivery system for SN-38 is challenging, owing to its low solubility and the presence of a labile lactone ring. Essa et al [39] used SN-38 encapsulated in poly(D,L-lactide-co-glycolide) NPs in their study to improve its cellular uptake, solubility, and stability. SN-38-loaded NPs prepared by the spontaneous emulsification solvent diffusion method have the ability to protect the active lactone ring of SN-38 against degradation under physiological conditions. They used the COLO-205 (colorectal adenocarcinoma) cell line to assess the effects of NPs on cytotoxicity and cellular uptake and found significantly decreased cell proliferation and apoptosis. Their results suggest that these SN-38-loaded NPs are a potentially desirable drug-delivery system to treat CRC. Polymeric NPs are known to assist the intracellular uptake of drugs to enhance their effectiveness, with lowest bioreactivity. In a study, researchers assessed cellular uptake and trafficking of PLGA NPs and chitosan (Chi)-covered PLGA NPs in human colorectal adenocarcinoma cells (COLO-205). [40] Neither Chi-PLGA nor PLGA NPs were toxic to the assessed cells at concentrations of up to 2,500 μg/mL. This study offered novel insights into the interaction of NPs with target cells, sustaining the use of NPs as new drug-delivery systems in cancer therapy. In another study, Tang et al [41] prepared NPs loaded with 5-FU and investigated the feature of NPs and their role in peritoneal metastasis nodule pattern in colon cancer. They successfully synthesized antitumor NPs loaded with 5-FU, which had inhibitory effects on the CRC cell line and peritoneal disseminations in a nude mouse model. This might represent a new antitumor preparation for peritoneal metastases, with intense clinical applications. In a study, researchers proposed the use of PLGA-based polymeric oil-core nanocapsules (NCs) for curcumin loading and delivery to CRC in mice after systemic injection. [42] Their findings specify that castor oil-core PLGA-based NCs provide elevated drug-loading efficiency and that the curcumin-loaded NCs are more effective against CT26 cells than the free drug and apply therapeutic activity in vitro, leading to apoptosis and blockade of the cell cycle. Additionally, the formulated NCs have a better half-life in blood circulation than that of the non-PEGylated NCs and accumulate in the subcutaneous CT26 tumors in mice after complete administration. The obtained results are established by optical and single-photon emission CT/CT imaging. In addition, in vivo growth delay experiments were performed, and significantly smaller tumor volumes were observed than those in animals injected with empty NCs. This study demonstrates an immense potential of the formulated NC in treating CRC. [42] To enhance the effectiveness of drug delivery, active targeted nanotechnology-based drug-delivery systems are receiving significant consideration, as they have the ability to minimize the side effects, reduce the toxicity, and elevate the effectiveness of anticancer treatment. For instance, Li et al [43] synthesized curcumin-loaded lipid-polymer-lecithin hybrid NPs (CUR-NPs) and functionalized them with RNA Apts against EpCAM for targeted delivery to colorectal adenocarcinoma cells. Their results demonstrated that the EpCAM Apt-functionalized CUR-NPs showed improved targeting and delivery of CUR to CRC cells.

Dendrimers:

Dendrimers are three-dimensional macromolecular structures that originate from a central core molecule and are bounded by the consecutive addition of branching layers. [44] These structures show a high degree of molecular consistency, a fine molecular weight distribution, tunable size, in addition to shaping characteristics, along with multivalency. [45] Altogether, these physicochemical properties with progressions in design of biodegradable backbones have conferred numerous applications to dendrimers in the development of nanopharmaceuticals. [46] Xie et al [47-49] hypothesized that the dual antibody conjugates may confer the advantage of capturing CTCs purposely as opposed by their single antibody counterparts.They established that the surface-functionalized dendrimers can be consecutively covered with two antibodies directed to surface biomarkers (EpCAM and

Slex) of human colorectal CTCs. The dual antibody-coated dendrimers demonstrate a considerably improved specificity toward capturing CTCs in the presence of interfering blood cells and in both patient blood and nude mice administered the labeled CTCs compared to their single antibody-coated counterparts. In addition, the dual antibody-coated conjugates down-regulate the captured CTCs. This study provided the first theoretical confirmation of two antibodies that could be biocompatible conjugated to a nanomaterial to capture and down-regulate CTCs in vivo with the improved specificity. Furthermore, these findings provided the foundation for designing effective and safe nanomedicines for capturing and restraining CTCs and, for the first time, provided conceptual evidence for a new strategy for the prevention of cancer metastasis. Xie et al in another study reported a successful approach to specifically bind and capture HT29 cells by means of several Sialyl Lewis X antibodies (aSlex)-conjugated Poly(amidoamine) dendrimers. [49] The conjugation was characterized by ultraviolet, atomic force microscope, and fluorescence measurements. The capture and regulation of colon cancer cells by the aSlex-coated dendrimer conjugate were examined by microscopy and flow cytometry. The conjugate showed improved capture of HT29 cells in a concentration-dependent manner, and the utmost capture competence was attained within 1 hour of exposure. The G6-5aSlex-FITC conjugate proved a better capture efficiency than FITC-G6-COOH-5aSlex conjugate. In addition, the G6-5aSlex-FITC conjugate particularly captured HT29 cells, even after the target HT29 cells were diluted with the interfering cells to a low concentration. The capture resulted in a concentration-dependent control of cell activity. Consequently, the aSlex-coated dendrimer conjugate displayed immense potential in capturing and restraining colorectal CTCs in blood. [50] Moreover, the study on regulation mechanism proved that the viability of captured CTCs declined in a modest concentration-dependent manner. This improved cell capture and regulation ability of conjugates allowed the design of new functionalized nanomaterials to capture and restrain CTCs in blood.

A sequence of telodendrimers, suspended with linear PEG-blocking dendritic oligomer of cholic acid and vitamin E, designed with architectures optimized for competent delivery of gambogic acid (GA) and other natural anticancer compounds have also been reported previously.[50] Two of the telodendrimers with segregated cholic acid and vitamin E domains self-assembled into cylindrical or spherical NPs after being filled with GA as observed under TEM, which correlated with the dynamic light scattering analysis of sub-30 nm particle sizes. A high GA loading ability and constant drug release were obtained with the optimized telodendrimers. These novel nanoformulations of GA were established to display similar in vitro cytotoxic activity against colon cancer cells as the free drug. Near-infrared fluorescence small animal imaging showed favored accumulation of GA-loaded NPs into tumor tissue. Optimized nanoformulation of GA accomplished superior antitumor effectiveness compared with GA-Cremophor EL formulation at corresponding doses in HT-29 human colon cancer xenograft mouse models. Therefore, the moderate unfavorable effects linked with this natural compound and the improved anticancer effects via tumor-targeted telodendrimer delivery make this optimized GA nanoformulation a capable substitute to the traditional chemotherapy in colon cancer treatment.In 2014, researchers reported a new platform that was developed to re-engineer nanoscale dendrimers for the capture of CTCs in blood and to facilitate the adhesion of interfering CTCs to vascular endothelial bed to form micrometastatic foci. These nanoscale dendrimers were accommodated with dual antibodies to target two surface biomarkers of colorectal CTCs. The dual antibody conjugates were capable to particularly recognize and bind CTCs and reasonably down-regulate the activity of the captured CTCs by arresting cells in the S phase. The associated adhesion assay showed that the dual antibody conjugates obstructed the hetero-adhesion of CTCs to fibronectin (Fn)-coated substrates and human umbilical vein endothelial cells. Moreover, dual antibody conjugates demonstrated improved specificity and competence in restraining CTCs in vitro and in vivo in contrast to their single antibody counterparts.

These observations revealed an innovative means of effectively preventing cancer metastatic initiation by binding and restraining CTCs and inhibiting their hetero-adhesion to blood vessels, but not by traditional cytotoxic-killing of cancer cells.

Other NPs used in the detection and treatment of CRC

Encapsulating chemotherapy drugs within targeted nano-delivery systems is a capable strategy to undertake cancer and avoid the adverse influences of conventional treatment. In the last 10 years, although numerous nanocarriers with diverse characteristics have been assessed, polypeptide-based copolymers have involved substantial consideration for their biocompatibility, controlled and slow biodegradability, and low toxicity. For example, researchers have formulated, characterized, and assessed poly (trimethylene carbonate)–block–poly(L-glutamic acid) derived polymersomes, which were targeted to the EGFR, loaded with plitidepsin, and tested for specificity and efficacy in HT29 and LS174T CRC cell lines. [80] A systematic in vitro cytotoxicity assessment of the unloaded polymersomes was carried out to determine viability, cell membrane asymmetry, biocompatibility check, and reactive oxygen species levels. These cytotoxicity assays established a fine biocompatibility for plitidepsin-unloaded polymersomes. The cellular uptake and cytotoxic effect of EGFR-targeted plitidepsin-loaded polymersomes specified that the CRC cell lines were more sensitive to anti-EGFR drug-loaded than untargeted drug-loaded polymersomes. Additionally, the use of untargeted polymersomes reduced plitidepsin cytotoxicity and cellular uptake, indicating that the use of this targeted nanocarrier in both cell lines is a capable strategy to undertake CRC and avoid unwanted side effects of the normal treatment. Gold nanoshells or gold nanospheres have been studied over the past decade because of their inherent localized surface plasmon resonance. Altering the core size, gold shell thickness, and composition of the particles, the plasmomic resonance of gold nanospheres can have an absorbance ranging from the visible region of the spectrum to the near-infrared region. Water and naturally occurring fluorochromes have the least absorption coefficient in the near-infrared region; consequently, light can penetrate deeper into the tissues. Gold nanospheres can also be conjugated with imaging reporters and carry drug payloads, genes, and other chemotherapeutic agents for theranostic applications. Although they can passively accumulate in tumors, gold nanospheres can be functionalized with active targeting ligands such as antibodies, Apts, and peptides to increase the specific binding of the particle to required targets. [51] In another study, researchers described new near infrared fluorescent proteinoid-poly (L-lactic acid) (PLLA) NPs preparation.[83] Here they prepared a P (EF-PLLA) random copolymer by thermal copolymerization ofL-glutamic acid (E) with L-phenylalanine (F) and PLLA. [52] Under optimal conditions, this proteinoid-PLLA copolymer can self-assemble into hollow nanosized particles and was used to encapsulate the NIR dye, indocyanine green. The encapsulation process increased the photostability of the dye, and these near-infrared fluorescent NPs were steady and nontoxic. There was no detectable leakage of the near-infrared dye from these NPs into phosphate-buffered saline containing human serum albumin. Tumor-targeting ligands, such as peanut agglutinin and anti-CEA antibodies, were covalently conjugated to the surface of the near-infrared fluorescent P (EF-PLLA) NPs, thereby increasing the detectable fluorescent signal from tumors with the corresponding up-regulated receptors. Specific colon tumor detection by the near-infrared fluorescent P (EF-PLLA) NPs has been demonstrated in a chicken embryo.

Combinatorial nanomedicines for CRC therapy

Nanomedicine has an incredible potential for revolutionizing the therapeutics and diagnostics under the premise of developing ingenious nanodevices. Researchers reported multidrug delivery using NPs to mediate resistance in relapsing cancers and to improve cancer treatment efficacy. [53-55] Recently, a research group focused on an effective strategy to improve the efficacy of 5-FU-assisted chemotherapy against colon cancer. This has been managed by combinatorial strategy in which CRC was used in combination with 5-

FU. The potential of both the drugs was enhanced by nanoencapsulation, in which a nontoxic polymeric carrier system "thiolated chitosan" was used. In this study, the enhanced anticancer effects of combinatorial nanomedicine are advantageous in terms of decrease in the dosage of 5-FU, thereby enhancing the chemotherapeutic efficacy and patient compliance of CRC cases. The nanosized drug-encapsulated systems are advantageous in terms of passive targeting, which helps in the retention of more drug-loaded NPs into tumors through enhanced permeability and retention effect. These exciting strategies have served as a base for the next phase of cancer nanomedicine in the clinic. [56-57]The overview on successful cancer targeting and cancer therapy in vitro and in vivo using different antineoplastic drugs are given in Table 2

Dexamethasone (a glucocorticoid): It acts as an antineoplastic agent having both anti-proliferative and anti-inflammatory effects. PLGA-based NPs have been formulated to encapsulate dexamethasone, with an intracellular site of action. The drug binds to the cytoplasmic receptors, and the subsequent drug–receptor complex is transported to the nucleus, resulting in the expression of certain genes that control cell proliferation. These drug-loaded NP formulations that released higher doses of drug for a prolonged period of time completely suppressed proliferation of VSMCs as also studied by Panyamet al [58]. Butoescu et al. and Patil et al. have also shown the enhanced in vivo efficacy of drug-loaded nanocarriers for the local treatment of arthritis and angiogenesis, respectively. [59]

Paclitaxel: It is a product of taxane plant that promotes the stabilisation of tubulin polymerisation. The microtubule formed in the presence of paclitaxel is exceptionally stable and dysfunctional; consequently, cell cycle arrest at the G2/M phase and inhibition of mitosis occurs after paclitaxel treatment. Paclitaxel has been shown to exhibit significant activity against a variety of solid tumours, including breast cancer, advanced ovarian carcinoma, lung and head and neck carcinomas and acute leukaemias. However, the success of its clinical application is mainly restricted due its low therapeutic effect and low solubility in water as well as in many other pharmaceutical solvents acceptable for intravascular (i.v) administration. Presently, the only available formulation for clinical use consists of a solution of paclitaxel (6 mg ml−1) in a Cremophor EL with dehydrated alcohol at a 50:50 volume/volume (v/v) ratio. However, this vehicle has been associated with severe hypersensitivity reactions and incompatibility upon intravenous administration. PLGA NPs encapsulated with paclitaxel were prepared as an answer to the shortcomings of the native drug and its in vitro efficacy was tested on different tumours. In a recent study, PLGA NPs were prepared using a quaternary ammonium salt, didodecyl dimethylammonium bromide (DMAB), and their utility was checked to deliver paclitaxel by the oral route to treat chemical-induced breast cancer in rats. The development of drug resistance is also another major obstacle to the success of cancer chemotherapy. To counteract this, NP-mediated simultaneous and targeted delivery of paclitaxel and tariquidar (inhibitor of P-glycoprotein) to tumour drug resistance has been studied by Patil et al. [60]. The in vitro antitumoural activity of a developed PLGA NP formulation incorporating paclitaxel has been also been assessed on a human adenocarcinoma cell line (HT-29) and a human small-cell lung cancer cell line (NCI-H69 SCLC) and compared with the in vitro antitumoural activity of the commercial formulation by Feng et al. and Fonseca et al., respectively[61,62]. Similarly, Gao et al. demonstrated higher in vitro cytotoxicity of paclitaxel-loaded methoxy poly (ethylene glycol)-poly(lactic-co-glycolic acid) (PEG-PLGA) NPs over native paclitaxel on human laryngeal cancer Hep-2 cells. The in vivo efficacy of paclitaxel-loaded polyethylene glycol (PEG) ylated PLGA-based NPs was accessed on transplantable liver tumour (TLT) implanted in the gastrocnemius muscle in the posterior leg of 8-week-old male NMRI mice by Danhier et al.,[63] and their studies proved that paclitaxel-loaded NPs inhibited tumour growth more efficiently than native drug. Better in vitro and in vivo efficacy of paclitaxel-loaded nanocarriers was also confirmed by Jin et al. using breast carcinoma (MCF-7) and carcinoma cervicis (HeLa) cell lines. Based on these results, it was concluded that paclitaxel incorporated in PLGA NPs might be considered as a promising system to eradicate hypoxic tumour cells .Zhao and

Feng[64] studied the in vitro viability of the MCF-7 human breast cancer cell line using paclitaxelloaded NPs with vitamin E TPGS as emulsifier. They also demonstrated that such a nanoparticulate formulation could enhance the oral bioavailability of the drug up to 10 times, which resulted in around ninefold higher therapeutic effect than native drug. Recently, the use of submicron/nanoscale PLGA implants to deliver paclitaxel to intracranial glioblastoma inmice was studied by Ranganath et al. Their studies showed that the nanoscale implants were able to demonstrate optimal paclitaxel pharmacokinetics in BALB/c nude mice with intracranial human glioblastoma (U87 MG-luc2) with significant tumour inhibition in the xenograft model and, hence, could be potentially useful to treat highly recurrent glioblastomas. Moreover, Ong et al.[65] delivered paclitaxel-loaded PLGA foams and used it in postsurgical chemotherapy against glioblastoma multiforme.

Vincristine sulphate: (VCR) is an effective chemotherapeutic agent, which has been used extensively for the treatment of various cancers, including AIDS.[66] Unfortunately, many tumour cells are not sensitive to VCR because of efflux from the tumour cells mediated by P-glycoprotein and associated proteins. The rationale behind the association of drugs with colloidal carriers against drug resistance comes from the fact that P-glycoprotein probably recognises the drug to be effluxed out of the tumoural cell only when this drug is present in the plasma membrane, and not when it is located in the cytoplasm or lysosomes, after endocytosis. As a drug-loaded NP is mostly present in the endolysosomal complex after internalisation by cells, it probably escapes the P-glycoprotein pump. Based on the optimal parameters, it was found that vincristine-loaded PLGA NPs could be formulated with expectable properties by combining the o/w emulsion–solvent evaporation method and the salting-out method. This study also showed that two hydrophilic low-molecular-weight drugs, VCR and verapamil (VRP), a chemosensitiser, could be simultaneously entrapped into PLGA NPs, with a relatively high entrapment efficiency of 55.35±4.22% for VCR and 69.47±5.34% for VRP, respectively, in small-sized particles of 100 nm. Moreover, their studies showed that PLGA NPs simultaneously loaded with an anticancer drug and a chemosensitiser might be the formulation with the most potential in the treatment of drug-resistant cancers in vivo[67,68].

Curcumin: It is derived from the common food spice turmeric, has been used for centuries as a remedy for many disorders including neurodegenerative, cardiovascular, pulmonary, autoimmune and neoplastic diseases where the process of inflammation plays an important role in the aetiology of the disease. [69] Scientific research over the past decade has shown the compound to possess preventive and therapeutic value against wide variety of diseases including cancer. Despite its promising pharmacological activity, slow oral bioavailability of curcumin has remained as a major hurdle, which needs to be overcome to enhance the therapeutic efficacy of this potent anticancer compound70. Nanotechnology-based carriers (PLGA NPs) emerged as a new hope in curcumin delivery to tumour sites. Curcumin-loaded PLGA NPs were prepared by the emulsion diffusion evaporation method using different stabilizers such as cetyl trimethylammonium bromide (CTAB) or PVA or PEG-5000. The present comprehensible data certainly testifies a well-established product profile, opening up new avenues for the miracle molecule curcumin on PLGA due to higher cellular uptake and increased in vitro bioactivity and superior in vivo bioavailability in comparison to native curcumin. Yallapu et al., in their studies, proved the enhanced therapeutic efficacy of curcumin-encapsulated PLGA NPs against metastatic ovarian and breast cancer cells [70]. Similarly, curcumin-loaded PLGA nanospheres were used as an adjuvant therapy for clinical application in prostate cancer by Mukerjee and Vishwanatha [71]. Superior in vivo bioavailability of curcumin-loaded PLGA NPs was observed in BALB/c mice by Anand et al [72]. Their results clearly indicate that serum levels of curcumin were almost twice as high in the case of curcumin-loaded NPs when compared with native curcumin. In addition, the half-life of curcumin-loaded NP was substantially longer than that of curcumin, proving that, in animals, curcumin-loaded NP was more bioavailable and has a substantially longer half-life, which was in agreement with Bisht et al[73]. Another independent study

conducted by Shahani et al. proved the marked anticancer efficacy of curcumin microparticles in a nude mice xenograft model [74].

Camptothecin (CPT): selectively inhibits mammalian topoisomerase I, a DNA replication enzyme overexpressed in a wide range of tumours, including advanced human colon, ovarian and oesophageal carcinomas. CPT exhibits antitumourigenic effects by trapping topoisomerase-I with DNA in topoisomerase I-cleavage complexes. Drug-loaded NPs were prepared by the nanoprecipitationmethod and examined for particle characteristics and in vitro release in phosphate-buffered saline. Prepared NPs described were considered potentially useful in both stabilizing and delivering 9-nitrocamptothecin (a modified form of CPT), thus enhancing the efficacy of this drug for cancer treatment. PLGA microspheres containing various CPT loadingswere prepared and characterised, and the cytotoxicity of these microspheres was then evaluated on B16 melanoma cells by Tong et al. [75] Their studies indicated the superior antiproliferative activity of drug-loaded microspheres for cancer therapy. Similarly, Mallery et al. [76] verified the higher potency of 10-hydroxycamptothecin, delivered from locally injectable PLGAmicrospheres in a murine human oral squamous cell carcinoma regression model. Their studies showed that PLGA microspheres were not only capable of maintaining higher intratumour concentrations of drug relative to local bolus and intra-peritoneal routes but also significantly reduced tumour volume in comparison to native drug. The in vitro and in vivo antitumour characteristics ofmPEG-PLGANPs containing CPT have been examined by Miura et al.[77] After intravenous administration in rats, CPT-loaded NPs showed longer plasma retention than CPT solution with high and long tumour localisation. In both single and double administration to mice bearing sarcoma solid tumour, CPTloaded NPs were much more effective than CPT solution; in particular, the tumour disappeared completely in three of the four mice after double administration of CPT-loaded NPs.

Doxorubicin: It is an anthracycline antibiotic and one of the most widely used anticancer agents, shows high antitumour activity. However, its therapeutic effects are limited due to its dose– dependent cardiotoxicity and myelosuppression [78]. Numerous publications have indicated improved anticancer activity for liposome-associated doxorubicin, including its metastatic inhibition activity. However, the two main factors that have limited the development of liposomes are the instability of the drug in solution within the vesicles and the rapid leakage of compounds across the phospholipidic bilayer. Under optimal conditions, the drug carried within the liposomal aqueous space should leak at a sufficient rate to become bioavailable on arrival at the tumour; however, rapid leakage before reaching the tumour site makes the drug vulnerable to the metabolic enzymes present in the body, leading to their inactivation in the plasma. In this regard, PLGA NPs containing doxorubicin (DOX) were successfully formulated, characterised and evaluated in vitro [79]. These NPs promise to be an effective system for the targeted and controlled release of doxorubicin with reduced systemic toxicity, increased therapeutic efficiency and increased patient compliance. Moreover, multifunctional PLGA NPs for combined doxorubicin and photothermal treatments were studied by Park et al. to deliver both drug and heat simultaneously to a selected tumourigenic region. Studies also showed that doxorubicin, when conjugated chemically to a terminal end group of PLGA by an ester linkage and then formulated into NPs, showed increased uptake by a human hepatocellular liver carcinoma (HepG2) cell line with a slightly lower half-maximal inhibitory concentration (IC50) than free doxorubicin. An in vivo antitumour activity assay also showed that a single injection of the NPs had comparable activity to that of free doxorubicin administered by daily injection. The in vivo pharmacokinetics, toxicity and the blood persistence properties of DOX-loaded NPs were evaluated in female Sprague–Dawley (SD) rats by Kalaria et al.[80] The results confirmed that NPs showed promise in improving the oral bioavailability of doxorubicin and reduced cardiotoxicity, though the tissue distribution of these particles remained to be investigated. The feasibility of drug delivery to the brain using the surfactant-coated DOX-loaded PLGA NPs was investigated by Gelperina et al. [81, 82] where binding of doxorubicin to the surfactantcoated PLGA NPs enabled a high antitumour effect

against an intracranial 101/8 glioblastoma in rats[83-85].

Etoposide: It is an anticancer agent used in the treatment of a variety of malignancies, including malignant lymphomas. It acts by inhibition of topoisomerase-II and activation of oxidation–reduction reactions to produce derivatives that bind directly to DNA and cause DNA damage. The effective chemotherapy of tumours depends on continuous exposure to anticancer agents for prolonged periods. Etoposide has a short biological half-life (3.6 hour), and although intra-peritoneal injection would result in initial high local tumour concentrations, prolonged exposure of tumour cells may not be possible. It is envisaged that intra-peritoneal delivery of etoposide through NPs would be an improved approach for effectual treatment of peritoneal tumours. In this perspective, etoposide- loaded NPs were prepared applying nanoprecipitation and emulsion–solvent evaporation techniques using PLGA in the presence of Pluronic F68 by Reddy et al. The methods produced NPs with good entrapment efficiency of around 80% with sustained release of the drug up to 48 h. [86] Moreover, etoposide-loaded PLGA nanoparticulate formulations were radio labelled, and their biodistribution and pharmacokinetics were studied after I.V. administration in healthy mice and rabbits, respectively, by Snehalatha et al. [87]

Rapamycin :- It serves as promising new drugs that can be used to inhibit the growth of breast cancer cells efficiently by alternative mechanism. Clinically, rapamycin analogues with improved stability and pharmacological properties have been well tolerated by patients in Phase I trials, and these agents have shown a promising antitumour effect in breast cancer. However, despite the potency of rapamycin in preclinical studies, the clinical development of rapamycin floundered due to its poor solubility in water (2.6 μg ml^{-1}) no tumour tissue specificity, low bioavailability and dose limiting toxicity. Nowadays, nanoparticulate drug-delivery systems are being developed to deliver smaller doses of rapamycin in an effective formwith a controlled drug distributionwithin the body to treat different diseases. Recently, the therapeutic utility of rapamycin has been optimised by developing efficient delivery system for the drug, that is, nanoscale delivery vehicles (such as PLGA NPs), which are capable of controlled release of the drug, thereby enhancing its suppressive activity on dendritic cells by altering their maturation profile[88]. The effect of rapamycinloaded PLGA NPs on the proliferation, distribution in cell cycle and expression of p27 protein in human umbilical arterial vascular smooth muscle cell (HUASMC) in in vitro conditions was also investigated by Miao et al [89]. Furthermore, it was found that rapamycin-loaded PLGA NPs improved the in vivo
retention and uptake of NPs in the arterial walls for efficient localisation of the therapeutic agents at the restenosis site. [90] Recently, our group tested the efficacy of such rapamycin-loaded NPs in breast cancer cells, suggesting that such drug-loaded NPs can also be used for treating a wide spectrum of cancers in the near future. [91]

5-Fluorouracil:-It is a pyrimidine analog that is an antineoplastic antimotabolite.It interferes with DNA synthesis by blocking the thymidylate synthetase which converts deoxyuridylic acid to thymidylic acid.Tumalla et al [92] prepared chitosan nanoparticles containing 5-FU by solvent emulsification evaporation technique. Different ratios of drug: polymer (1:1, 1:2, 1:3, 1:4) was selected to optimize and select the best one and also to observe the effect of polymer on the formulation. It can be concluded from the study that 5-FU was successfully encapsulated in chitosan polymer by solvent evaporation emulsification method. 5-FU chitosan nanoparticles (drug: polymer ratio of 1:3) yielded more entrapment efficiency, drug content and cumulative drug release when compared to the other polymeric nanoparticles with different polymer ratios. *In vitro* release revealed that 5-FU E1 nanoparticles released drug after 4 h once it enters intestinal fluid therefore protecting the drug release in the gastric environment and enhancing its drug release at colonic region that fulfilled the objective. It can be concluded that the formulated nanoparticles improved localization of the drug at the colon area and also achieved sustained release over a prolonged period of 24 h. This showed decreased toxicity to healthy cells as more amount of drug was localized in the colon area. These changes made the patient more compliant by decreasing the dosing frequency and dose that can be administered. So it can be

concluded that nanoparticles prepared by this method using the same polymer with the optimized ratio can represent as potential drug delivery approach for effective delivery of the active pharmaceutical ingredient to the colorectal tumors.

Capecitabine:- Capecitabine is a tumour selective fluoropyrimidine carbamate.CAP is widely used alone or in combination regimens in thetreatment of CRC. For example, CAP/oxaliplatin highly effective for treating CRC. AlthoughCAP presents a promising drug for CRC therapy, the short drug elimination half-life with 0.5–1 h requires relatively high dose with 150 mg/m^2 twice per day. The commonest overdosage toxicities include bone-marrow depression, cardiotoxicity,diarrhea, nausea and vomiting steatites, dermatitis.Patel et al (93)formulated and evaluated alginate-pectin microbeads of cap and founded that an *in-vitro* wash off test indicated 70% mucoadhesion by the beads. *In-vitro* dissolution studies of beads loaded into enteric coated capsules revealed negligible release in simulated gastric as well as intestinal fluid, followed by 49.23% release in simulated colonic fluid, in 4 hours. It revealed that colorectal cancer can be curable if detected in the early stage. Researchers are looking for ways to improve the accuracy of colonoscopy and to detect colorectal cancer even earlier than is currently possible. Abnormalities detected at the earliest stages would be much less likely to have spread by the time they are found. Nanotechnology, the branch of engineering that deals with the manipulation of individual atoms and molecules, has the potential to help identify cancerous or precancerous cells well before a visible growth has formed and to deliver cancer-killing drugs directly to the cancerous cells.In one application of this approach, the Center of Cancer Nanotechnology Excellence at Stanford University, part of the National Cancer Institute's (NCI) Alliance for Nanotechnology in Cancer, has developed a system in which gold nanoparticles are used to seek out and bind to cancer cells. When light shined from a device that is inserted into a standard endoscope (for example, a colonoscope) reaches cancer cells that have bound the gold nanoparticles, they stand out from the normal cells and can be removed.

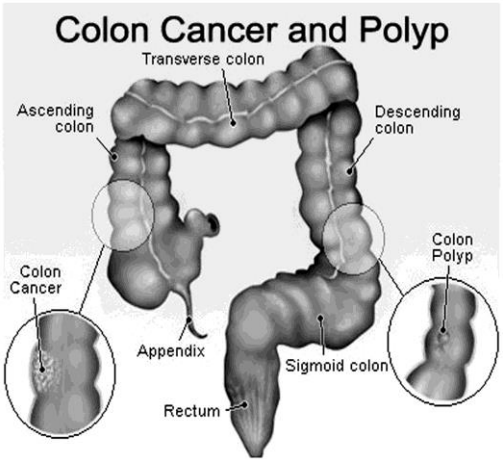

Fig 1: Colon cancer and development

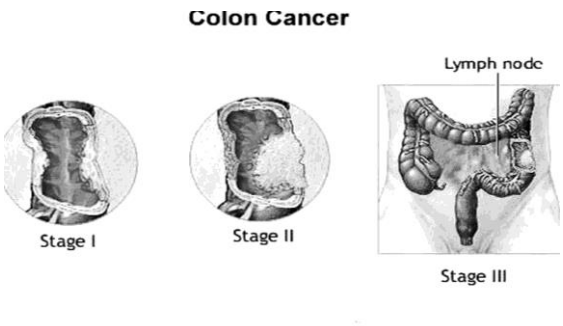

Figure 2 Different stages of colorectal cancer

Future Prospectives:

Nanoscience applied to cancer research is proving to be a critical and encouraging approach for the eventual elimination or at least chronic control of cancer. Nanotechnology has been making a significant impact on cancer diagnosis and therapeutic management in revolutionary ways. Controlled release drug delivery can considerably increase the therapeutic consequence of a drug. The striking properties of nanomedicines include their capability to controlled release of drugs, targeting of definite tissues, and biocompatibility. Because of their minute size, NPs can be taken up very efficiently by cells forming a constant nanocomplex, thereby defending it from nuclease degradation and allowing successful delivery to the tumor site. In spite of the challenges restricting its application, it is promising that nanomedicine in future would play a critical role in the detection and treatment of human CRC, and also in the improvement of normal human physiology.

Table 1: Novel nanoparticles for colorectal cancer targeting

Sl no	Formulation	Result	References
1	**Quantum dots**	In a recent investigation, researchers qualitatively analyzed the expression level of large external antigen in tissue samples from CRC patients using QD-based immunohistochemistry (QD-IHC) and conventional IHC.	**22**
2	**Iron oxide nanocrystals**	Kuo et al[30] fabricated smart multifunctional magnetic nanovehicles encapsulating anticancer drugs and an antibody-targeting peptide AP-1 (MPVA-AP1).[39] In this study, the magnetic nanovehicles with consistent sizes and dispersed in aqueous solution displayed good hemocompatibility and no toxicity toward L929 fibroblasts, which showed their potential for applications in therapeutics.	**30**
3	**PLGA nanoparticles/nanocells**	In a study, researchers proposed the use of PLGA-based polymeric oil-core nanocapsules (NCs) for curcumin loading and delivery to CRC in mice after systemic injection. [42] Their findings specify that castor oil-core PLGA-based NCs provide elevated drug-loading efficiency and that the curcumin-loaded NCs are more effective against CT26 cells than the free drug and apply therapeutic activity in vitro, leading to apoptosis and blockade of the cell cycle	**42**
4	**Dendrimers**	Xie et al[47-49] hypothesized that the dual antibody conjugates may confer the advantage of capturing CTCs purposely as opposed by their single antibody counterparts.They established that the surface-functionalized dendrimers can be consecutively covered with two antibodies directed to surface biomarkers (EpCAM and Slex) of human colorectal CTCs. The dual antibody-coated dendrimers demonstrate a considerably improved specificity toward capturing CTCs in the presence of interfering blood cells and in both patient blood and nude mice administered the labeled CTCs compared to their single antibody-coated counterparts. In addition, the dual antibody-coated conjugates down-regulate the captured CTCs	**47-49**
5	**Gold nanospheres**	Gold nanospheres can also be conjugated with imaging reporters and carry drug payloads, genes, and other chemotherapeutic agents for theranostic applications. Although they can passively accumulate in tumors, gold nanospheres can be functionalized with active targeting ligands such as antibodies, Apts, and peptides to increase the specific binding of the particle to required targets	**51**
6	**Combinatorial nanomedicines**	A research group focused on an effective strategy to improve the efficacy of 5-FU-assisted chemotherapy against colon cancer. This has been managed by combinatorial strategy in which CRC was used in combination with 5-FU. The potential of both the drugs was enhanced by nanoencapsulation, in which a nontoxic polymeric carrier system "thiolated chitosan" was used. In this study, the enhanced anticancer effects of combinatorial nanomedicine are advantageous in terms of decrease in the dosage of 5-FU, thereby enhancing the chemotherapeutic efficacy and patient compliance of CRC cases	**[53-54,56-57]**

Table 2- Critique on successful cancer targeting and cancer therapy *in vitro and in vivo* using various anticancer drugs.

Drug	Major target organales	*In vitro* application	*In vivo* application	Reference No
1.Dexamithasone	Cytoplasmic receptors	Effective suppression proliferation of vascular smooth muscle cells by drug loaded NPs	Enhanced *in vivo* efficacy of drug loaded nanocarriers for the local treatment of arthritis and angiogenesis was studied using these NPs	[58-59]
2.Paclitaxel	Microtubules	Efficacy of paclitaxel mediated NP delivery was tested on human small cell lung cancer (NCI-H69 SCLC), human adenocarcinoma (HT-29), human laryngeal cancer (Hep-2), breast carcinoma(MCF-7) and carcinoma cervicis (HeLa) cell lines.	In vivo efficacy of paclitaxel-loaded nanoparticles was Accessed on transplantable liver tumor in male NMRImice and in model glioblastoma tumors	[60-65]
3.Vincristine sulphate	Tubulin	PLGA nanoparticles simultaneously loaded with vincristine sulfate and verapamil hydrochloride for cancer therapy.	Moreover studies also showed that PLGA NPs simultaneously loaded with anticancer drug and chemo sensitizer might be the most potential formulation in the treatment of drug resistant cancers in vivo	[66-68]
4.Curcumine	Cytoplasmic proteins	Nanoparticle encapsulation improves oral bioavailability of curcumin and was effective against metastatic ovarian , breast cancer and prostate cancer cells	In vivo bioavailability of curcumin-loaded PLGA nanoparticles was performed in Balb/c mice. Another independent study proved the marked anticancer efficacy of curcumin microparticles in nude mice bearing MDA-MB-231 xenografts	[69-74]

5.Camptothecin	Topo I	Efficacy of PLGA NPs were investigated on B16 melanoma cells murine and human oral squamous cell carcinoma	The in vitro antitumor characteristics of methoxy poly (ethylene glycol)-PLGA nanoparticles containing camptothecin have been examined in mice bearing sarcoma solid tumor. Doxorubicin Topo II Multifunctional PLGA nanoparticles were used against MDA-MB-231 breast cancer cells and against human hepatocellular liver carcinoma (HepG2) cell line In vivo pharmacokinetics of DOX loaded NPs was evaluated in female SD and in intracranial 101/8 glioblastoma in rats	[75-77]
6.Doxorubicin	Topo II	PLGA nanoparticles were used against MDA-MB-231 breast cancer cells and against human hepatocellular liver carcinoma (HepG2) cell line	In vivo pharmacokinetics of DOX loaded NPs was evaluated in female SD and in intracranial 101/8 glioblastoma in rats	[78-85]
7.Etoposide	Topo II	Drug was loaded in PLGA in presence of Pluronic F68 for efficient cancer therapy	Etoposide loaded PLGA nanoparticulate formulations were radio labeled and their biodistribution and pharmacokinetics were studied after intravenous administration in healthy mice and rabbits	[86-87]
8.Rapamycin	Tyrosine Kinase	Enhanced suppressive activity on dendritic cells , vascular smooth muscle cell (HUASMC) and MCF 7 breast cancer was investigated using these Np	Improved in vivo retention and uptake of nanoparticles in the arterial walls for efficient localization of the therapeutic agents in rest enosis site.	[88-91]
9. 5-Fluorouracil	Thymidylate synthetase	*In vitro* drug release studies were done using dialysis bag technique using simulated fluids at	It can be concluded that nanoparticles prepared by this method using the same polymer with the optimized ratio can	[92]

		various pH (1.2, 4.5, 7.5, 7.0) to mimic the GIT tract. 5-FU nanoparticles with drug: polymer ratio of 1:2 and 1:3 has shown better particle size (149 ± 1.28 nm and 138 ± 1.01 nm respectively), entrapment efficiency (48.12 ± 0.08% and 69.18 ± 1.89 respectively).	represent as potential drug delivery approach for effective delivery of the active pharmaceutical ingredient to the colorectal tumors.	
10.Capecitabine	Thymidylate synthetase	An *in-vitro* wash off test indicated 70% mucoadhesion by the beads. *In-vitro* dissolution studies of beads loaded into enteric coated capsules revealed negligible release in simulated gastric as well as intestinal fluid, followed by 49.23% release in simulated colonic fluid, in 4 hours..	In conclusion, the formulated beads showed colon specific controlled release properties and thus could prove to be effective for colon cancer treatment.	[93]

CONCLUSION:

Recently, there has been an increased focus on developing novel drug delivery systems, targeted therapies, and medical devices, including the use of nanotechnology and nanomaterials. This focus is translating to increase in vitro diagnostics through a number of submissions for drug products and medical devices to the Food and Drug Administration. Although subject to the similar regulatory principles and pathways as any other drug or device, the unique properties that arise from the small-sized and large surface area of nanomaterials may lead to further scientific considerations when following the present guidelines and practices of the Food and Drug Administration. Nanotechnology may enable medical products to develop beyond a single mode of action into multifunctional platforms performing several functions such as nanotheranostics that combines therapeutics with diagnostics. Researchers from around the globe are actively incorporating nanotechnology in CRC treatment. Preclinical characterization of nanomaterials has shown considerable advancement over the last decade. Methods are being developed and optimized continually in order to meet the needs of the evolving complexity of nanomedicines. Detailed NP surface characterization, predictive immunotoxicity assays, and quantitative evaluation of the encapsulated versus free drug fractions highlight the growth of this field. The pursuit for the development of new methods and conducting research directed at understanding the nano–bio interface will uncover additional relationships between NP structure and biological activity. This information will be invaluable in devising new strategies for using nanotechnology to improve upon the existing pharmaceuticals and deliver novel therapies in future.

Competing Interests

The author declares that there are no competing interests regarding the publication of this paper.

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